Microbiome

RESEARCH

Open Access

Quality traits drive the enrichment of *Massilia* in the rhizosphere to improve soybean oil content

Qin Han[1,2†], Guanghui Zhu[1†], Hongmei Qiu[3†], Mingbo Li[1], Jiaming Zhang[1], Xinying Wu[1], Renhao Xiao[1], Yan Zhang[1], Wei Yang[1], Bing Tian[1], Lanxi Xu[1], Jiayang Zhou[1], Yutong Li[1], Yueqiang Wang[3*], Yang Bai[4*] and Xia Li[1,3*]

Abstract

Background Soybean seeds are rich in protein and oil. The selection of varieties that produce high-quality seeds has been one of the priorities of soybean breeding programs. However, the influence of improved seed quality on the rhizosphere microbiota and whether the microbiota is involved in determining seed quality are still unclear. Here, we analyzed the structures of the rhizospheric bacterial communities of 100 soybean varieties, including 53 landraces and 47 modern cultivars, and evaluated the interactions between seed quality traits and rhizospheric bacteria.

Results We found that rhizospheric bacterial structures differed between landraces and cultivars and that this difference was directly related to their oil content. Seven bacterial families (*Sphingomonadaceae*, *Gemmatimonadaceae*, *Nocardioidaceae*, *Xanthobacteraceae*, *Chitinophagaceae*, *Oxalobacteraceae*, and *Streptomycetaceae*) were obviously enriched in the rhizospheres of the high-oil cultivars. Among them, *Oxalobacteraceae* (*Massilia*) was assembled specifically by the root exudates of high-oil cultivars and was associated with the phenolic acids and flavonoids in plant phenylpropanoid biosynthetic pathways. Furthermore, we showed that *Massilia* affected auxin signaling or interfered with active oxygen-related metabolism. In addition, *Massilia* activated glycolysis pathway, thereby promoting seed oil accumulation.

Conclusions These results provide a solid theoretical basis for the breeding of revolutionary soybean cultivars with desired seed quality and optimal microbiomes and the development of new cultivation strategies for increasing the oil content of seeds.

Keywords Improvements in host quality traits, Oil content, Rhizosphere microbiota, Root exudates, Phenylpropanoid biosynthetic pathways, Glycolysis pathway

†Qin Han, Guanghui Zhu and Hongmei Qiu contributed equally to this work.

*Correspondence:
Yueqiang Wang
82516942@qq.com
Yang Bai
ybai@genetics.ac.cn
Xia Li
xli@mail.hzau.edu.cn
Full list of author information is available at the end of the article

Background

Soybean (*Glycine max* (L.) Merr.) is one of the world's most important oilseed crops, accounting for more than 50% of the total edible oils consumed globally, and is also the principal source of vegetable protein worldwide [1, 2]. Because of the growing demand for oil in the global oilseed market, the development of high-oil soybean cultivars has been a top priority in soybean breeding programs. The majority of soybean varieties before the 1950s were landraces, and a small number of modern cultivars were released from the 1960s to the 1980s, while most varieties since the 1990s are modern cultivars, with a continuous increase in oil content (Figure S1). To date, many improved cultivars with high yield and high oil quality have been developed through breeding programs but the gap between the current supply and the ever-increasing demand remains significant [3, 4]. The identification of the factors influencing seed oil quality traits is essential for the improvement of soybean quality. Recent studies have shown that plant domestication and genetic improvement of agricultural traits, such as stress tolerance and disease resistance, alter the structures and functions of rhizosphere microbial communities [5–13]. Whether breeding for crop seed quality affects the rhizospheric microbiota and the contribution of the rhizospheric microbiota to seed quality remain unclear.

Soil is the habitat for plants and associated microorganisms and is the medium through which they interact. To some extent, host plants drive the selection of microorganisms by depositing specific secretions (root exudates) at the soil-root interface [14, 15]. Root exudates are a mixture of soluble organic substances, including sugars, amino acids, organic acids, fatty acids, proteins, and other secondary metabolites, and can act as nutrients, antimicrobial substances, and chemotaxis or signaling molecules that coordinate shifts in rhizosphere microbial composition [16–21]. The composition and quantity of plant root exudates are species- or cultivar-specific and vary significantly throughout the life cycle and in response to changing environments [22–25]. These changes place selection pressure on the rhizosphere microbiome. The MYB72-dependent antimicrobial coumarin scopoletin and indole-derived benzoxazinoid compounds act as dominant metabolites in *Arabidopsis thaliana* and maize (*Zea mays* L.) and are involved in host iron absorption and insect pest/pathogen resistance, and changes in their levels affect the assembly of their rhizosphere microbial community [26–28]. The interplay between host traits (flowering time, plant disease resistance, rhizosheath formation), root exudates, and the microbiota has been widely reported [29–31]. Therefore, it is presumed that changes in plant root exudates are accompanied by changes in host traits that mediate changes in microbial structure.

Microbial colonization in the plant rhizosphere can endow the host with various biological functions. For example, nitrogen-fixing rhizobia can form symbiotic nodules to provide a nitrogen source for the growth of leguminous hosts, which represents the highest level of mutual benefit between host plants and rhizosphere bacteria [32, 33]. Many *Pseudomonas* and *Bacillus* species can secrete siderophores and antibacterial substances to provide iron nutrition for plants and confer resistance to pathogenic bacteria [34–36]. Recently, increasing evidence has demonstrated the regulatory functions of the microbiota and the community's effect on plant development and adaptation to environments. Root-enriched synthetic communities in *indica* rice were recently found to improve the use efficiency of organic nitrogen [37]. Furthermore, a single bacterial genus (*Variovorax*) can completely reverse the severe inhibition of root growth caused by the complex microbial community through auxin degradation [38]. The microbiota and root endodermis can support plant mineral nutrient homeostasis through the inhibition of the abscisic acid signaling pathway [39]. These results indicate that rhizosphere microorganisms have evolved functions that strongly influence plant growth and development. It remains unknown whether and how the microbiota affects seed oil content and quality.

Here, we first investigated the effects of soybean cultivar type and seed oil content on the rhizosphere bacterial community in the field via 16S metabarcoding sequencing. We subsequently elucidated the selective effect of high-oil cultivars on soil bacteria by directly treating the soil with root exudates. A correlation between *Massilia* and substances in the phenylpropanoid metabolic pathway was found via the integration of metabolomics and 16S sequencing analysis. Finally, through in vitro growth testing and pot experiments, we confirmed that substances in the phenylpropanoid metabolic pathway could regulate the growth of *Massilia*, which in turn increased the seed oil content by affecting plant glycolysis pathways. Our findings reveal the mechanism driving the effects of seed quality traits on the rhizospheric bacterial community and provide a new approach for improving the soybean oil content.

Materials and methods

Plant species, growth, and sample collection

A total of 100 soybean accessions were selected for this study, including 53 landraces and 47 modern cultivars that were released from 1949 to 2010 in the major soybean-producing area of Northeast China (Jilin, Liaoning, and Heilongjiang, Table S1). The experiments

were conducted at Gongzhuling, Jilin Academy of Agricultural Sciences (43°51′ N, 124°81′ E). The soil was a typical black soil with a high organic matter content (pH, 7.02; organic matter, 20.6 mg/kg; total N, 2017.3 mg/kg; available P, 513.07 mg/kg; and available K, 1.95 mg/kg). Seeds of each accession ($n=10$ per accession) were sown in the soil. Rhizosphere soil was collected from 100 soybean plants at the pod-filling stage as described by Mendes et al. [40]. The plants were manually uprooted from the soil, and the roots were shaken to remove loose soil. The remaining attached soil was subsequently collected as rhizosphere soil. Unplanted soil samples were used as bulk soil. For each variety, three replicates of 3 to 4 plants each were examined.

16S rRNA gene sample preparation, sequencing, and analysis

In total, 306 samples (300 rhizosphere soil and 6 bulk soil samples) were used for sequencing. Microbial DNA was extracted using an E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA). The V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using the primers 515F and 806R. PCR was performed as follows: 3 min at 95 ℃; 27 cycles of 30 s at 95 ℃, 30 s at 55 ℃, 45 s at 72 ℃, and 72 ℃ for 10 min. The reactions were performed in triplicate in 20-μL mixtures containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. The products were extracted from 2% agarose gels, purified via an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified via QuantiFluor™-ST (Promega, Madison, WI, USA) according to the manufacturers' protocols. The amplicons were pooled at equimolar concentrations and paired-end sequenced (2 × 300) on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to standard protocols at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 7.1; http://drive5.com/uparse/); chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA sequence was analyzed via the RDP classifier algorithm (http://rdp.cme.msu.edu/) against the Silva 138/16S_bacteria database with a confidence threshold of 70%. The Chao1 and Shannon alpha-diversity indices were calculated via Mothur software (Schloss). Constrained principal coordinate analysis (PCoA) was performed at the genus level via the R package amplicon [41, 42].

Root exudate collection

Six varieties were selected on the basis of their differences in oil content and abundance of rhizosphere bacteria, including three high-oil varieties (Hefeng 52: 23.49%, Gongye03-5570: 21.9%, and Suinong14: 21.86%) and three low-oil varieties (Baiqixiaojinhuang: 18.61%, Baike: 17.77%, and Jilinchalihua: 14.17%). The root exudates of the six varieties were collected according to the methods of Liu et al. (2014) and Wang et al. (2022), with several modifications. Chlorine gas-sterilized seeds of six soybean varieties were planted in pots (13 × 13 cm) containing sterilized vermiculite, watered alternating with deionized water and Broughton and Dilworth (BD) nutrient solution, and placed in a greenhouse under long-day conditions (16 h photoperiod, 28 ℃/20 ℃, day/night). Fifteen-day-old seedlings were rinsed several times with deionized water and then transplanted to a 250-mL flask (six seedlings per flask) with 100 mL of sterile distilled water. For each treatment, there were three replicates. After 12 h of overnight cultivation, the plants were transferred to new flasks with 100 mL of sterile water for another 24 h. Then, the root exudate solution was collected and centrifuged at 4500 r/min at 4 ℃ for 10 min. The supernatant solution was transferred to a polyethylene tube and stored at − 80 ℃ until further use.

Treatment of soil with root exudates and 16S rRNA gene sequencing

One gram of fresh unplanted soil sample was transferred into a 5-mL centrifuge tube with 2 mL of filtration sterilized root exudates or sterile distilled water (control). After incubation for 24 h at 28 ℃ with shaking at 180 r/min, the soil samples were collected by centrifugation in a refrigerated centrifuge at 10,000 r/min for 5 min and stored at − 80 ℃ (Figure S2). Each treatment had three replicates. In total, 21 samples were used for sequencing according to standard protocols at Personal Biotechnology Co., Ltd. (Shanghai, China). The V3–V4 target region of the 16S rRNA gene fragments was amplified and sequenced on an Illumina NovaSeq PE250 sequencing platform (Illumina, San Diego, USA). Sequence data analyses were performed mainly via the QIIME 2 2019.4 and R packages (v3.2.0). The Chao1, observed species, and Shannon alpha-diversity indices were calculated via the non-singleton amplicon sequence variant (ASV) table in QIIME 2 and visualized as box plots. β-Diversity (between-sample diversity) was estimated by the Bray–Curtis distance and the differentiation of microbiota structure among groups was assessed via permutational multivariate analysis of variance (PERMANOVA) in QIIME 2.

Targeted metabolomics study

The root exudates were freeze-dried, dissolved in 1 mL of 70% methanol with an internal standard, vortexed for 3 min, and centrifuged (12,000 r/min, 4 ℃) for 10 min. The supernatant was filtered with a microporous filter membrane (0.22 μm) and stored in a sample flask. The extract samples were examined via a UPLC–ESI–MS/MS system (UPLC, SHIMADZU Nexera X2, www.shimadzu.com.cn/; MS, Applied Biosystems 4500 Q TRAP, www.appliedbiosystems.com.cn/) according to standard protocols at MetWare Biotechnology Co., Ltd. (Wuhan, China)). Principal component analysis (PCA) was performed via the statistics function prcomp within R (www.r-project.org). The hierarchical cluster analysis (HCA) results of the samples and metabolites are presented as heatmaps with dendrograms. Significantly differentially abundant metabolites between groups were determined by VIP ≥ 1 and absolute Log2FC (fold change) ≥ 1. VIP values were extracted from the OPLS-DA results, which also contain score plots and permutation plots, generated via the R package MetaboAnalystR. The data were log transformed (log2) and mean-centered before OPLS-DA. To avoid overfitting, a permutation test (200 permutations) was performed. The identified metabolites were annotated via the KEGG Compound database (http://www.kegg.jp/kegg/compound/), and the annotated metabolites were then mapped to the KEGG Pathway database (http://www.kegg.jp/kegg/pathway.html).

Microbe isolation and in vitro growth detection

To isolate *Massilia*, bacteria showing positive responses to root exudates of the high-oil cultivars, we treated the soil with root exudates from Hefeng52 as described above; and gradient dilutions were prepared and plated on minimal medium (MM; 0.5 g of glucose, 0.5 g of polypeptone, 0.5 g of monosodium glutamate, 0.5 g of yeast extract, 0.44 g of K_2HPO_4, 0.1 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4$; pH 7.0). Representative clones based on morphology and color were picked, purified, and stored at − 80 ℃ in MM containing 20% glycerol until further use. The classification of the isolates at the genus level was performed via 16S rRNA gene sequencing with the 27F and 1492R primers.

To assess the effects of the candidate metabolites on *Massilia*, the tested strains were inoculated in 5 mL of MM and incubated overnight at 28 ℃ with shaking at 180 rpm. The OD of the bacterial cultures was adjusted to 0.1 at 600 nm, and then the cultures were subjected to tenfold serial dilution (10^{-1}–10^{-4}) in ddH_2O and spotted (1 μL) onto M9 minimal salt medium agar supplemented with candidate metabolites, glucose or dimethyl sulfoxide (DMSO) as a control. Stock solutions (50 mM) of the metabolites were prepared by dissolving each into DMSO and subsequently performing filtration sterilization. Each treatment included three replicates. The plates were incubated at 28 ℃ for 36–48 h and photographed. Additionally, growth curves were generated for 1/2 MM with different concentrations of candidate metabolites. A 10 μL suspension of bacteria (OD_{600} = 0.1) was inoculated into a 2-mL centrifuge tube containing 800 μL of liquid 1/2 MM medium with candidate metabolites or DMSO (control). A 96-well plate reader was used to measure the change in the OD_{600} at the indicated time points. Each assay was carried out in triplicate.

Seed oil content and fatty acid analysis

Chlorine gas-sterilized seeds (Jilinchalihua and Hefeng52) were planted in pots (15 × 15 cm) containing a mixture of soil and vermiculite (volume:volume = 3:1) and grown for 4 days. The plants were then inoculated with a 30-mL suspension of mixed *Massilia* (OD_{600} = 0.1), with the same volume of water used as the control. The plants were grown under a 16-h photoperiod at 28 ℃/20 ℃, day/night. Normal water-fertilizer treatment was performed during the growth period, and the same concentration of bacterial suspension was inoculated again during the flowering period. After maturity, the oil content in the *Massilia*-treated and untreated plant seeds was measured according to the method described by Wang et al. [43]. The seeds were ground in hexane to extract the lipids. After incubation at 37 ℃ for 5 h, the tube was then centrifuged at 11,000 r/min for 10 min at room temperature. The supernatant was evaporated under vacuum in a preweighed 1.5 mL centrifuge tube until a constant weight was reached. This procedure was repeated twice. The tube was then weighed again. The seed lipid content was determined by dividing the amount of lipid in the tube by the seed weight, after which the seed fatty acid content was measured as described by Tang et al. [44].

Detection of plant height, root length, and nitrogen content

Chlorine gas-sterilized seeds (Jilinchalihua) were germinated on sterile water agar for 3 days. The plants were subsequently transplanted to vermiculite with *Massilia* M16 or M117 suspensions (OD_{600} = 0.1) in pots (13 × 13 cm) to ensure one seedling per pot. The vermiculite with sterile water was used as a control. The plants were grown under a 16-h photoperiod at 28 ℃/20 ℃, day/night, and watered with sterilized H_2O as needed. After 15 days of growth, the seedlings were treated with nitrogen-free (NF), low-nitrogen (LN, 0.25 mM), and normal-nitrogen (NN, 7.5 mM) BD nutrient solutions. Each treatment included fifteen seedlings. After growing for 15 days, the plants were sampled to determine their height and length. The samples were subsequently

divided into 2 parts: one part was dried at 105 ℃ for half an hour and then at 70 ℃ until a constant weight was reached for nitrogen content determination via the Kjeldahl method [45], and the other part was stored at – 80 ℃ for transcriptome sequencing.

RNA extraction and RNA-seq analysis

Total RNA was extracted from roots and leaves via TRIzol® Reagent (plant RNA purification reagent for plant tissue) according to the manufacturer's instructions (Invitrogen), and genomic DNA was removed via DNase I (TaKaRa). RNA degradation and contamination were monitored on 1% agarose gels. RNA purification, reverse transcription, and library construction and sequencing were performed at Shanghai Majorbio Biopharm Biotechnology Co., Ltd. (Shanghai, China) according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Clear reads obtained after trimming the adapter sequence, removing low-quality bases, and filtering short reads were used for subsequent analysis. Then, the clean reads were separately aligned to the *Glycine max* Wm82. a2. v1 reference genome in orientation mode via HISAT2 (http://ccb.jhu.edu/software/hisat2/index.shtml) software. DEGs (differentially expressed genes) between two different treatments were identified via DESeq2, and the expression level of each gene was calculated according to the transcripts per million reads (TPM) method. Genes that presented at least a twofold change in expression and an FDR ≤ 0.05 were considered DEGs. RSEM (http://deweylab.biostat.wisc.edu/rsem/) was used to quantify gene abundances. Gene Ontology (GO, http://www.geneontology.org) and Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/) enrichment analyses were carried out via GOATOOLS (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do) [46].

Quantitative real-time PCR

Total RNA from roots and leaves was extracted via TRIzol reagent, quantified using a NanoDrop 2000 (Thermo Fisher Scientific), and reverse-transcribed to cDNA via Hifair RII 1st Strand cDNA Synthesis SuperMix (YEASEN, CA, USA) according to the manufacturer's instructions. qRT-PCR was performed with the SYBR Green PCR master mix (YEASEN, CA, USA) on a CFX96 real-time PCR detection system (Bio-Rad). Each 10 μL reaction mixture was composed of 5 μL of SYBR Green (Bio-Rad), 0.5 μL of 10 μM primers, 3 μL of deionized H_2O, and 1 μL of cDNA. The qPCR cycling conditions were as follows: initial denaturation for 30 s at 95 ℃, followed by 40 cycles of denaturation at 95 ℃ for 10 s, and annealing at 60 ℃ for 45 s. The transcript level was calculated via the standard curve method from duplicate data, with the soybean *GmELF1b* (Glyma.02G276600) gene used as the internal control. The analyzed genes and corresponding primers used in this study are listed in Table S1.

Statistical analysis

Graphical representations were generated with GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Spearman correlations between metabolites and bacterial taxa were calculated via R ($r > 0.8$). A false discovery rate-corrected P value < 0.05 was considered indicative of statistical significance. The means and standard deviations of the data were calculated. The Wilcoxon rank-sum test was performed to compare the alpha diversity of different varieties.

Results

Improvements in host quality traits affect the soybean rhizosphere microbiota

To investigate whether genetic improvement influences the soybean rhizosphere microbiota, 100 soybean accessions from a historical collection of varieties obtained during soybean breeding were selected, including 53 Chinese landraces and 47 modern cultivars (Table S1). Across all the samples, we obtained 113,899,988 high-quality sequences (average read length = 436 bp), with a median of 36,861 sequences per sample (ranging from 30,015 to 44,952 sequences per sample). The rarefaction curves of all the samples based on the number of OTUs are shown in Figure S3. The sequencing data were rarefied to the lowest number of sequences (15,591) observed in a single sample, and 9065 bacterial OTUs were identified (Table S2).

First, to determine whether the rhizosphere bacterial composition differed between modern cultivars and landraces, we conducted constrained PCoA on the basis of Bray–Curtis phylogenetic distances. The analysis results revealed a clear clustering pattern was observed between the modern cultivars and the landraces (Fig. 1a). Subsequently, we selected eight high-oil (HO) and eight low-oil (LO) cultivars from among the modern cultivars and landraces, respectively. Similarly, the rhizosphere samples of HO and LO cultivars showed greater separation at the genus level (Fig. 1b). PERMANOVA based on the Bray–Curtis distances further confirmed the significant differences in the microbial communities between the high- and low-oil soybean cultivars ($P = 0.002$; Table S3). These results indicated that a higher oil content in a variety may lead to a shift in the rhizosphere bacterial community. Finally, linear discriminant analysis (LDA; log score threshold > 2.5 and $P < 0.05$) revealed that 17 families were enriched in the high-oil varieties, with 7 bacterial families, *Sphingomonadaceae*, *Gemmatimonadaceae*,

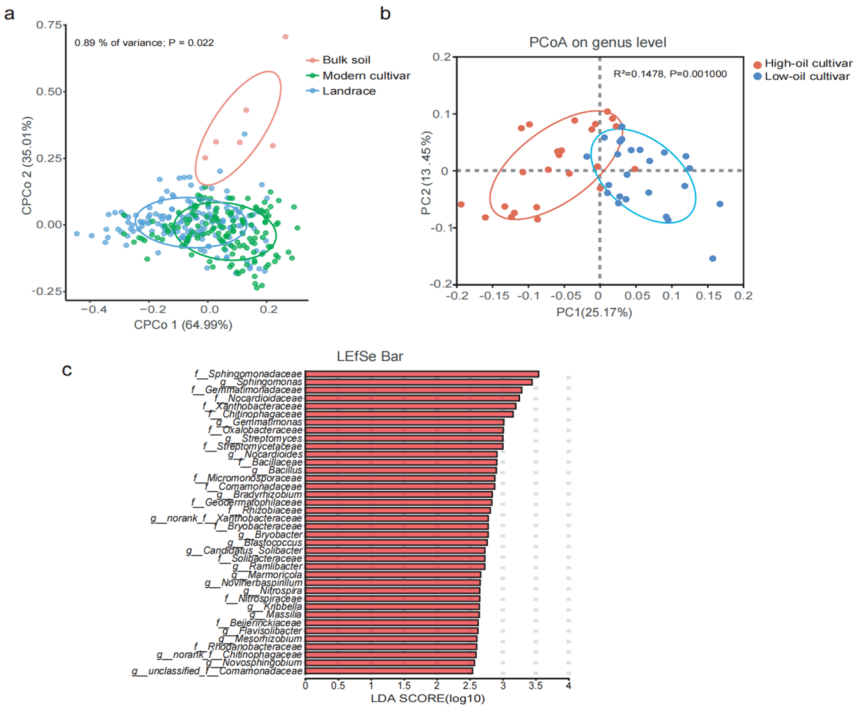

Fig. 1 Effects of soybean cultivar type and seed oil content on the rhizosphere bacterial community. **a** Constrained PCoA plot of the Bray–Curtis distances between samples. $n = 306$. **b** PCoA based on Bray–Curtis distances of the rhizosphere bacteria of eight high-oil and eight low-oil cultivars; $n = 48$. Clustering significance was determined via Adonis (Pr (> F) = 0.001). **c** Major families and genera with significant differences between high- and low-oil cultivars; the Wilcoxon rank-sum test was used to evaluate the significance of differences between the groups ($n = 48$)

Nocardioidaceae, *Xanthobacteraceae*, *Chitinophagaceae*, *Oxalobacteraceae*, and *Streptomycetaceae*, having LDA scores greater than 3. At the genus level, 21 genera were enriched in the rhizospheres of the high-oil varieties, with 3 genera, *Sphingomonas*, *Gemmatimonas*, and *Streptomyces,* having LDA scores greater than 3 (Fig. 1c).

Oxalobacteraceae (*Massilia* sp.) is enriched by the root exudates of high-oil cultivars

As the main bridge between plant traits and the soil microbiota, root exudates play a crucial role in the establishment of the rhizosphere microbial community. To test the response of the soil bacterial community to root exudates from HO and LO cultivars, we collected the root exudates of six soybean cultivars with different oil contents via a hydroponic system and used them to treat the soil samples in vitro. At 24 h after root exudate treatment, we performed 16S ribosomal RNA sequencing (V3–V4) and diversity analysis at the ASV level. NovaSeq sequencing of 21 samples yielded 1,821,716 (average of 86,748) high-quality effective sequences. All samples were rarefied to the same sequences on the basis of 95% of the lowest sequence number using the "qiime feature-table rarefy" function to ensure the same depth of sequencing across the samples (Figure S4), and 46,626 ASVs were obtained. The general features of the high-throughput sequencing results as well as the alpha-diversity indices are shown in Table S4. The results revealed that the Chao1 index did not significantly differ among the treatments. The Shannon index in the root exudate treatments was somewhat lower than that in the water control treatment (Fig. 2a), indicating that root exudates may reduce the diversity of the soil microbiota.

Next, we performed a PCoA based on Bray–Curtis distances and found that the samples treated with root exudates were obviously separated; the soil samples treated with the root exudates of Hefeng52 (23.49%), Gongye03-5570 (21.9%), Suinong14 (21.86%), Baiqixiaojinhuang (18.61%) and Baike (17.77%) were obviously separated from those treated with the root exudates of Jilinchalihua (14.17%) and H_2O (Fig. 2b). PERMANOVA at the same distances revealed that the microbial community structures in these treatments were significantly different (Table S5). Among the 16 changed bacterial families (one with low abundance was not counted) under field conditions, only the relative abundance of *Oxalobacteraceae* significantly increased after root exudate treatment of two high-oil cultivars (Gongye30-5570 and Hefeng52). The relative abundance of *Comamonadaceae* increased after treatment with root exudates from all six cultivars, but

the variation amplitude between cultivars was relatively small. The relative abundances of the remaining genera decreased to varying degrees after treatment with root exudates from the high-oil cultivars, with those of *Sphingomonadaceae* and *Gemmatimonadaceae* decreasing the most (Fig. 2c). Similarly, at the genus level, only the abundance of *Massilia* (belonging to the *Oxalobacteraceae*) significantly increased after treatment with root exudates from the two high-oil cultivars (Fig. 2c). These results suggested that *Massilia* responded positively to the root exudates of high-oil cultivars, again indicating a correlation between *Massilia* and high-oil cultivars.

The phenylpropanoid biosynthesis pathway is related to *Massilia* enrichment

A total of 632 metabolites were detected, including 100 lipids, 86 phenolic acids, 78 organic acids, 64 amino acids and derivatives, 55 flavonoids, 51 nucleotides and derivatives, 39 alkaloids, 35 terpenoids, 18 lignans and coumarins, 7 tannins and 89 others (Table S6). The heatmap results revealed that the contents of the top 50 detected substances varied among the six cultivars (Fig. 3a), and the PCA results revealed that the metabolite profiles of the root exudates of different cultivars were separate, except for Gongye03-5570, which overlapped slightly with Hefeng 52 and Baiqixiaojinhuang (Fig. 3b). These results indicated the existence of different metabolic components in the root exudates of the six cultivars. We subsequently conducted a correlation analysis between metabolite contents and the abundance of *Massilia*. The results revealed that 68 metabolites were positively correlated with *Massilia* ($P<0.05$, $r>7$), suggesting that these metabolites might be related to the increase in *Massilia* abundance (Fig. 3c, Table S7). Among them, the correlations of 8 substances with *Massilia* were greater than 0.8, namely, 4-hydroxybenzaldehyde, 4-O-glucosyl-4-hydroxybenzoic acid, piceatannol-3′-O-glucoside, licoflavonol, 2-propylsuccinic acid, isolicoflavonol, licochalcone D, and N-acetyl-L-aspartic acid. Interestingly, further analysis of the metabolomics data revealed that related substances, such as p-coumaric acid (p-CA, $r=0.68$), 4-hydroxybenzaldehyde (4-HBZ, $r=0.83$), 4-hydroxybenzoic acid (4-HBA, $r=0.66$), isoliquiritigenin (ILIG, $r=0.73$), liquiritigenin (LIG, $r=0.72$) and daidzein (DAZ, $r=0.64$), are involved in the phenylpropanoid biosynthesis pathway [47].

To ascertain whether *Massilia* was directly affected by these metabolites, we isolated and purified more than 50 strains of bacteria from soil treated with the root exudates of the high-oil cultivar Hefeng52, of which 4 strains (M10, M16, M22, and M117; Table S8) were identified as belonging to the genus *Massilia* via 16S full-length sequencing. In the M9 minimum salt medium, all substances except for ILIG, which had no effect on the growth of M22, significantly promoted the growth of the four *Massilia* strains to different degrees, with DMSO and glucose used as solvents and substance controls, respectively (Fig. 3d). This result indicated that the above substances provide a carbon source for the growth of *Massilia*. Moreover, bacterial growth (OD_{600}) was monitored in the presence of the 6 abovementioned substances. The results revealed that a low concentration (50 μM) of 4-HBZ, which had the highest correlation, promoted the growth of *Massilia* M117 and M16, whereas a high concentration (200 μM) of 4-HBZ had a weak inhibitory effect on growth (Fig. 3e). Similarly, p-CA promoted the growth of M117 and M22 to a small extent, whereas HBA had no effect on the growth of the four strains (Figure S5). Among the three flavonoids, DZA slightly promoted the growth of M117 and M16 but had no significant effect on the remaining two strains of bacteria (Fig. 3e). LIG and ILIG had inhibitory effects on the growth of four bacterial strains, with ILIG having a stronger inhibitory ability, but its ability to inhibit M117 was weaker than that of the other three strains (Figure S5). These results suggested that the phenylpropanoid biosynthesis pathway may participate in regulating the abundance of *Massilia*.

Massilia increases the content of oil and fatty acids in soybean seeds

To explore whether *Massilia* plays a role in the accumulation of seed oil, we planted one low-oil cultivar (Jilinchalihua) and one high-oil cultivar (Hefeng52) in a greenhouse under normal water and fertilizer conditions and treated them with the four mixed *Massilia* suspensions twice, at the seedling and flowering stages. The mixed *Massilia* treatments led to a significant increase in the seed weight of the low-oil cultivar Jilinchalihua but not the high-oil cultivar Hefeng52 (Fig. 4a). Intriguingly, we found that the *Massilia* treatments significantly

(See figure on next page.)

Fig. 2 Effects of the root exudates of different oil-content cultivars on soil microorganisms. **a** Shannon index measurement for bacterial communities from water and different root exudate treatments. The values are the means ± SDs ($n=3$). Different letters represent significant differences at $P<0.05$, as determined via ANOVA with the Student–Newman–Keuls test. **b** PCoA based on Bray–Curtis distances of the microbial community of water and different root exudate treatments ($n=3$). **c** The relative abundances of the top 16 family taxa and the top 15 genera after treatment with the different varieties of root exudates. The values are the means ± SDs ($n=3$). Different letters represent significant differences at $P<0.05$, as determined via ANOVA with the Student–Newman–Keuls test

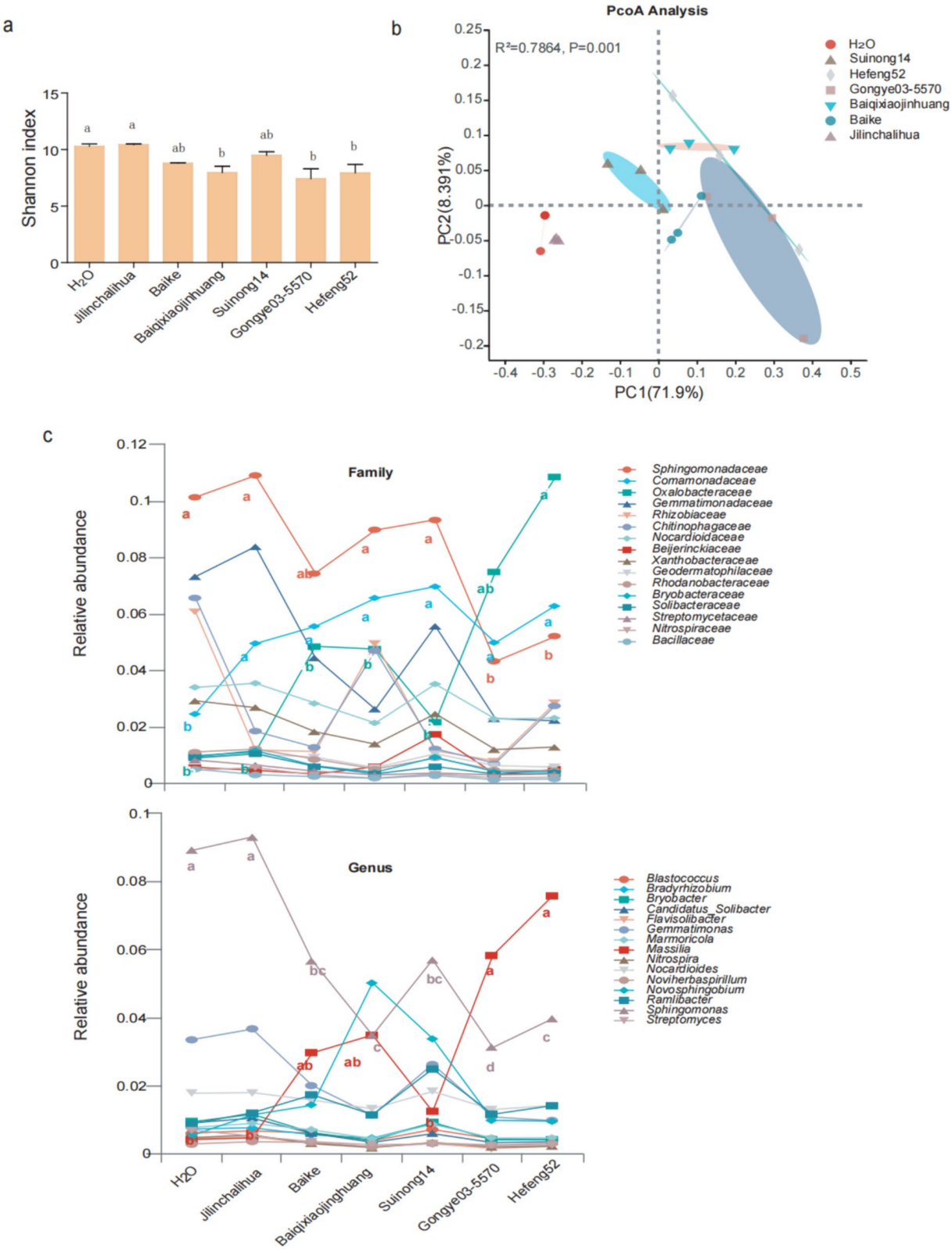

Fig. 2 (See legend on previous page.)

increased the oil content of the seeds of both cultivars (14.9% for Jilinchalihua and 15.7% for Hefeng52) but had no effect on the protein content (Fig. 4a). We subsequently measured the contents of 19 major fatty acids via gas chromatography (GC). In Jilinchalihua, 19 types of fatty acids increased to varying degrees in abundance after *Massilia* treatment; among them, the level of C18:2n6c increased the most, followed by C18:1n9t, C20:0 and C22:0 (Fig. 4b). In contrast, in Hefeng52, the contents of these fatty acids did not change significantly after treatment with *Massilia* (Figure S6), implying that the proportion of fatty acids in the cultivar Hefeng52 may have reached saturation.

Massilia activates the auxin signaling and glycolysis metabolism pathways

As rhizosphere bacteria, *Massilia* species were reported to promote shoot growth and nitrogen accumulation in the maize lateral root-defective mutant *lrt1* under nitrogen deprivation [21]. We selected *Massilia* M16 and M117 to treat soybean separately under different nitrogen conditions. These two strains were the closest relatives of *Massilia* ASV 84777 and ASV 70281 according to the phylogenetic tree based on the 16S rRNA gene (Figure S7). Similarly, they increased soybean plant height and leaf nitrogen accumulation under LN conditions (Fig. 5a). In comparison, the leaf nitrogen content of the plants treated with strain M117 significantly increased under both LN and NN conditions compared with those of the control and M16 (Fig. 5a). In addition, we found that the presence of *Massilia* promoted root length under NF, LN, and even NN conditions (Fig. 5a). To understand how *Massilia* affects the seed oil content and plant growth, we analyzed the transcriptomes of the roots and leaves of the above plant materials treated with M16 and M117 under different levels of nitrogen. PCA revealed that the gene expression patterns in the roots were distinct among the different nitrogen treatments, whereas, in the leaves, the gene expression patterns under the NF and LN conditions were similar, with significant differences compared with those under the NN conditions (Figure S8). This pattern suggested that *Massilia* had the greatest effect on plants under NN conditions relative to the other two nitrogen conditions. The differentially expressed genes (DEGs) between the treatment (M16 or M117) and control samples under the three nitrogen conditions were identified via false discovery rate (FDR < 0.05, and fold change > 2) analysis. In total, 3081 and 6069 significant DEGs were identified in the roots and leaves, respectively.

Next, we performed Gene Ontology (GO) enrichment analysis of the upregulated and downregulated DEGs between the M117 or M16 and CK treatments. Among the upregulated DEGs in the M117 and CK treatments, only a few GO terms (among the top 50) were enriched, mainly under LN and NN conditions, and they were related to ion transport and plant-type cell wall organization or biogenesis (Fig. 5b). Similar results were observed between the M16 and CK treatments; moreover, "photosynthesis"-related GO terms were enriched under NN conditions (Figure S9). Further gene expression analyses revealed that the genes related to auxin biosynthesis and signaling pathways (*YUCCA3*, *AUX1*, *IAA28*, *IAA20*, *PIF4*, *PIN2*, and *SAU76*) and amino acid transport and metabolism (*NPF3.1*, *ASP5*, *fadM*, *AMP1*, and *ASRGL1*) were significantly increased in M16- and M117-treated roots under LN conditions, whereas the genes associated with nitrogen/amino acid transport and metabolism (*NIT1*, *AAP7*, and *ASP5*) and inorganic ion transport and metabolism (*FRO4*, *CTR1*, *ZIP1* and *YSL3*) were significantly increased in M16- and M117-treated roots under NN conditions (Fig. 5c, Table S9). Under NN conditions, the M16 and M117 treatments also upregulated the expression of auxin-related genes (*AUX1*, *IAA28*, *IAA29*, and *GH3.1*) in the leaves (Fig. 5c, Table S10). These results suggested that *Massilia* induced the expression of auxin- and nutrient absorption-related genes to promote plant growth. However, the expression levels of these genes were greater in plants treated with M117 than in those treated with M16, which was consistent with their growth phenotypes (nitrogen content) under LN or NN conditions.

Notably, we found that *GLGC*, *PYK*, *DGLB*, *DAD1*, *ERLL3, SSG1*, and *SPSA3,* which are related to "lipid transport and metabolism", "glycolysis" and "sucrose

(See figure on next page.)

Fig. 3 Relationships between root exudate compounds and microbial taxa. **a** Heatmap of the identified metabolites (top 50) in the root exudates of the six cultivars. The color indicates the level of metabolite accumulation, from low (blue) to high (red). **b** PCA of the metabolite accumulation levels in the root exudates of the six cultivars. **c** Spearman correlations ($p < 0.05$) between metabolites and *Massilia* were visualized in Cytoscape_v3.7.1. The edge color represents positive (red) and negative (blue) correlations. The other different colors of the nodes (square) indicate the corresponding metabolites. Different colors represent different substance categories. **d** Growth of *Massilia* isolates (M10, M16, M22, and M117) on M9 medium with metabolites in the phenylpropanoid biosynthesis pathway. DMSO, dimethyl sulfoxide; GLU, glucose; ILIG, isoliquiritigenin; DAZ, daidzein; p-CA, p-coumaric acid; 4-HBZ, 4-hydroxybenzaldehyde; 4-HBA, 4-hydroxybenzoic acid. The results represent one of three replicates with similar results. **e** Effects of different concentrations of 4-HBZ and DAZ on the growth of *Massilia* isolated (M10, M16, M22, and M117) within 36 h. Statistical analyses were performed via Mann–Whitney nonparametric tests and significance is denoted by asterisks, where * indicates $P < 0.05$. The data are presented as the median value ± SD ($n = 3$)

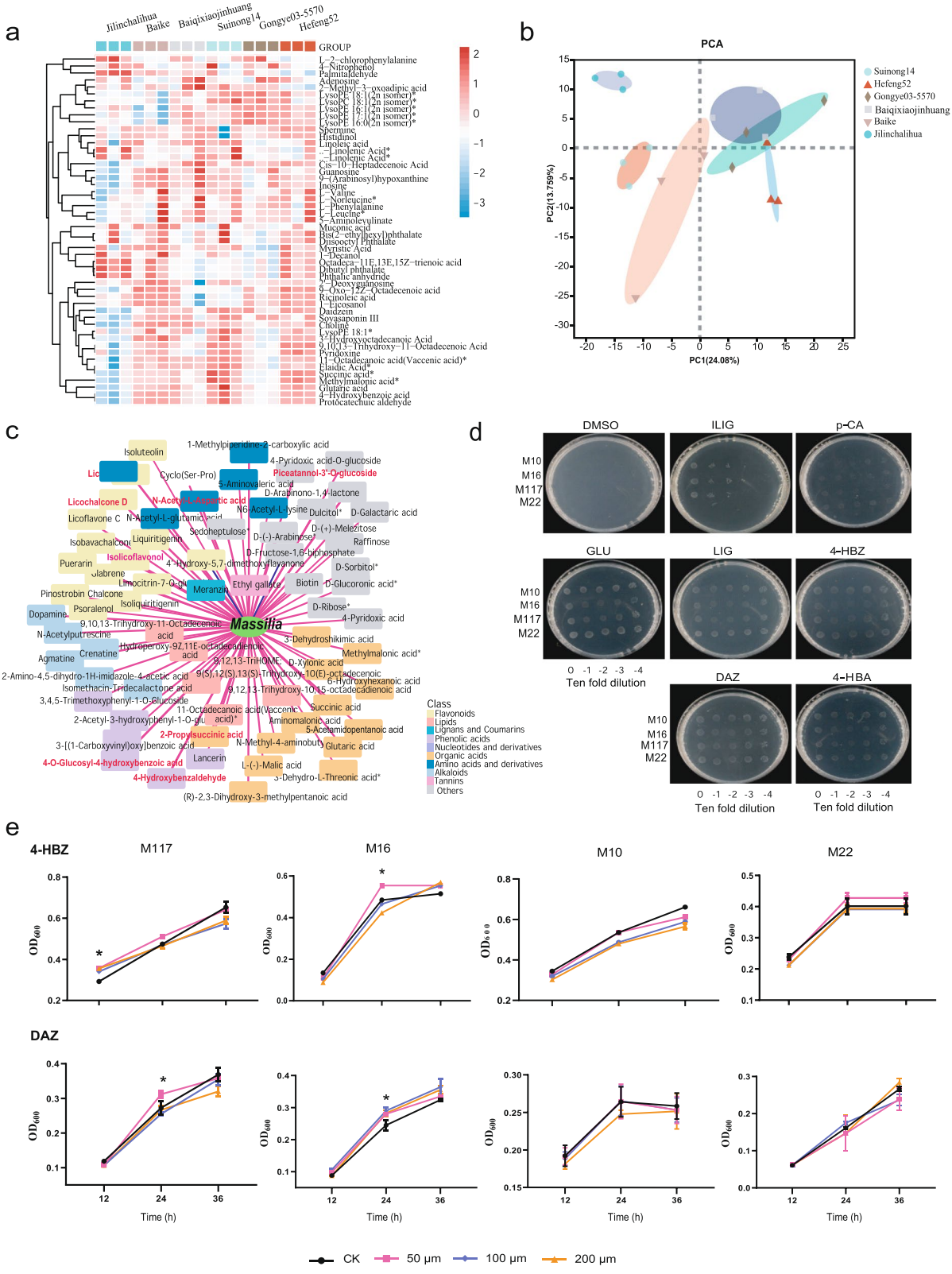

Fig. 3 (See legend on previous page.)

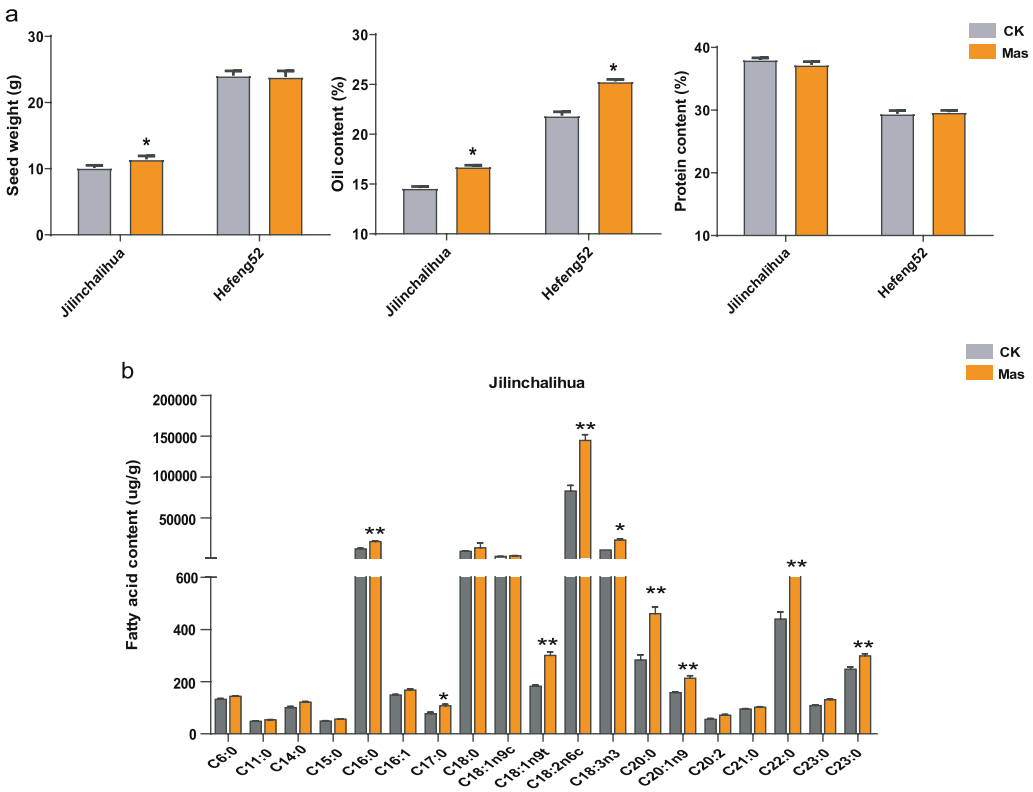

Fig. 4 Effects of *Massilia* on soybean quality and fatty acid content. **a** Seed weight and protein and oil contents in Jilinchalihua and Hefeng52 after *Massilia* treatment. The values are the means ± SDs ($n = 3$). **b** Content of FA species in Jilinchalihua seeds after *Massilia* treatment. The values are the means ± SDs ($n = 3$). Statistical analyses were performed via Mann–Whitney nonparametric tests and significance is denoted by asterisks, where * indicates $P < 0.05$. CK, control; Mas, *Massilia* treatment

metabolism", respectively, were also significantly upregulated in roots after the M16 and M117 treatments under LN conditions (Fig. 5c, Table S9), whereas *ACSL*, *WSD1*, *PFKA*, and *PYK*, which are involved in "lipid metabolism" and "glycolysis", were significantly upregulated in roots after *Massilia* treatment under NN conditions (Fig. 5c, Table S9). In leaves, a "lipid metabolic" gene (*DAGL*) and "starch and sucrose metabolic" genes (*OSTB* and *GLGC*) were induced by *Massilia* under LN conditions (Fig. 5c, Table S10), and more "lipid metabolic" genes (*WSD1*, *DAGL*, *PLD1_2*, *FAB2*, and *ACSL)* and "glycolytic process (pyruvate metabolism)" genes (*PDC*, *ADH1*, *GAPDH*, *PYK*, and *PFKA*) were induced by *Massilia* under NN conditions (Fig. 5c, Table S10). Glycolysis has been previously shown to be the key pathway for oil production in oil crops [48]. Further qRT-PCR analysis confirmed the strong upregulation of pyruvate kinase (*PYK*) and pyruvate decarboxylase (*PDC*) in the leaves after M117 treatment (Fig. 5d). Thus, we speculated that the presence of *Massilia* may increase the oil content in soybean by affecting the glycolysis pathway.

For the downregulated DEGs, the GO terms enriched in roots were plant-type cell wall organization, polysaccharide binding, beta-glucosidase activity and jasmonic acid metabolic process, whereas the GO terms enriched in leaves were plant-type cell wall organization or biogenesis under LN conditions. Under NN conditions, the GO terms enriched in the roots were related mainly to "lignin metabolic process" and "metal ion transport", whereas the GO terms enriched in the leaves were few and involved mainly "ion transport". Interestingly, we found that active oxygen-related metabolic processes (hydrogen peroxide metabolic process and peroxidase activity) were enriched in roots under all nitrogen conditions (Fig. 5b), indicating that these responses induced by *Massilia* were independent of external nitrogen conditions. The main genes involved were a respiratory burst oxidase homologous protein-encoding gene (*BRBOH*), cytochrome P450 genes (*CYP84A1*, *CYP71D8*, and *CYP78A3*), and multiple peroxidase genes (including 6, 9, and 11 genes under WN, LN, and NN conditions, respectively) (Tables S9). These results suggested that *Massilia* may also interfere with cell wall-related and active oxygen-related metabolism. The cell wall and active oxygen metabolism are closely related to plant root growth and development [49, 50], but whether these processes participate in the regulation of the oil content in

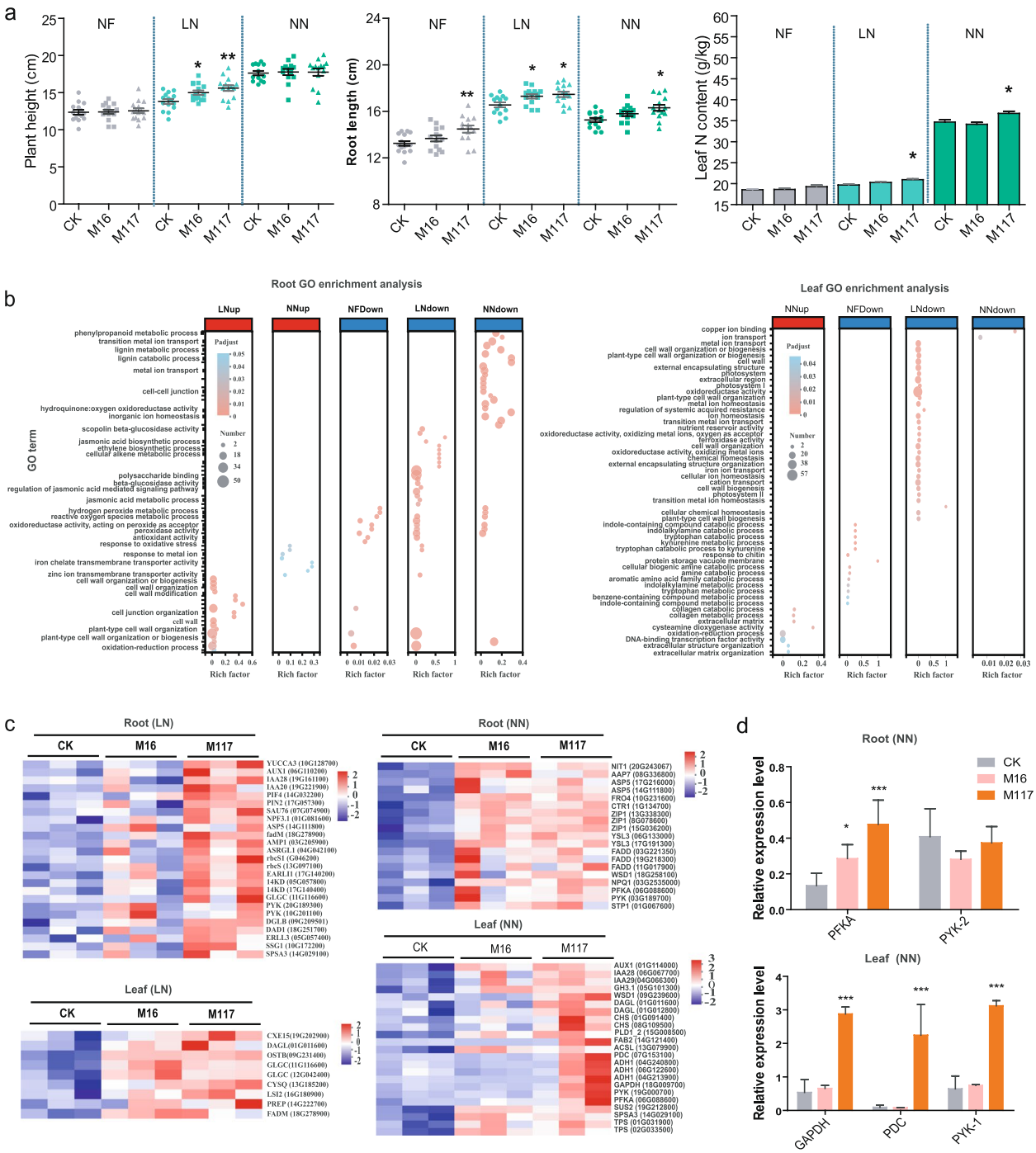

Fig. 5 *Massilia* affected plant growth and the glycolytic pathway. **a** Plant height and N content in the roots and leaves of plant under NF, LN and NN conditions after *Massilia* treatment. **b** GO enrichment analysis of DEGs in roots and leaves between the treatment (M117) and control samples under three nitrogen conditions. **c** Heatmap of DEGs related to ion transport, plant-type cell wall organization or the biogenesis auxin pathway, amino acid transport and metabolism in roots or leaves, and lipid transport and metabolism, glycolysis, and starch and sucrose metabolism identified via RNA-Seq analysis. **d** Quantitative RT–PCR measurements of glycolysis-related gene expression levels. Mean ± SD, $n = 3$ and with 3 technical replicates. Statistical analyses were performed via Mann–Whitney nonparametric tests, and significance is denoted by asterisks, where * indicates $P < 0.05$ and *** indicates $P < 0.001$. NF: nitrogen-free, LN: low-nitrogen; NN: normal-nitrogen

seeds is unclear. Together, these results show that *Massilia* may promote plant growth and improve seed oil quality by interfering with reactive oxygen species metabolism and inducing glycolytic metabolism pathways.

Discussion

The genetic breeding of crops with improved quality and higher yield has been a top priority in the past century. Compelling evidence has indicated that hosts, symbionts, and their associated hologenomes function as single biological entities in crop trait evolution and that plants and rhizosphere microbiomes have coevolved and been coselected [49, 50]. Indeed, host evolution and genetic improvements in agricultural traits, ranging from plant growth and stress tolerance to yield, affect the composition of microbial communities in many crop species [9, 51–55]. However, it has not yet been determined whether the breeding of seed-quality traits influences the microbiota of the plant rhizosphere. Here, we provide evidence that the genetic improvement of seed quality traits (seed oil content) has significant effects on the rhizosphere bacteria of soybeans. First, our results revealed differences in the diversity and composition of rhizosphere-bacteriomes between landraces and modern cultivars of soybean (Fig. 1a). The oil content of the soybean cultivars was subsequently associated with changes in the rhizosphere bacteria, including *Sphingomonadaceae*, *Gemmatimonadaceae*, *Nocardioidaceae*, *Xanthobacteraceae*, *Chitinophagaceae*, *Oxalobacteraceae*, and *Streptomycetaceae* (Fig. 1c). These results suggested that soybean cultivars with different oil contents harbor distinct microbial assemblages. Together, these data suggest that soybean host quality trait-linked genetic improvement affects the rhizosphere bacterial community structure. Thus, our findings establish the associations between crop seed quality traits and rhizosphere microbial composition and broaden our understanding of crop agricultural trait–microbial interactions.

These findings raise the question of how seed quality affects the rhizosphere bacterial community. It has been shown that plants are able to modulate the associated microbiota through the secretion of metabolites, such as triterpenes, coumarins and benzoxazinoids, in the roots or rhizosphere of *Arabidopsis* and maize to adapt to stress conditions [27, 56–58]. Seed oil content and quality are regulated by the spatial and temporal influx of metabolites from plants [59]. It is conceivable that the seed oil trait is closely related to plant metabolism. Indeed, the composition of the bacterial community clearly changed when the soils were treated with the root exudates of the soybean cultivars whose seed quality varied (Fig. 2b). However, among the above quality-related taxa, only *Oxalobacteraceae* (*Massilia*) presented a positive response to the root exudates of the high-oil cultivars at the community level (Fig. 2c). *Massilla* species are members of fast-growing Proteobacteria in the rhizosphere and roots of many plants [60–63], and they have an advantage in utilizing plant-derived compounds [64]. Therefore, we speculated that high-seed-oil soybean varieties may be selected for *Massilia* in their rhizosphere through the secretion of certain root exudates. Our further LC–MS-based metabolomics and plate growth experiments revealed that phenolic acids and flavonoids in soybean root exudates were related to the enrichment of *Massilia* (Fig. 3). Thus, phenolic acids and flavonoids in root exudates play crucial roles in soybean host plant-*Massilia* interactions. Recently, root-derived flavones have been shown to promote the enrichment of *Oxalobacteraceae* in the rhizosphere of maize, which in turn promotes maize growth and nitrogen acquisition under nitrogen deprivation [21]. Here, we found that *Massilia* also promoted the growth of soybean plants under low-nitrogen conditions (Fig. 5a). Most importantly, *Massilia* significantly increased the seed oil content of both the high- and low-oil cultivars (Fig. 4a). To our knowledge, this is the first report of rhizosphere bacteria that are capable of affecting the seed quality traits of host plants.

Massilia species colonize the rhizosphere of various host plant species and interact with host plants through multiple means, such as by solubilizing recalcitrant phosphate sources, producing the agents violacein and cellulose, and degrading polycystic aromatic hydrocarbons [65, 66]. Our transcriptome analysis results revealed that *Massilia* had a significant effect on genome-wide gene expression in soybean plants. The altered genes are involved in many biological processes, such as plant growth regulation, cell wall organization and biogenesis, and hydrogen peroxide metabolism, suggesting that *Massilia* influences many aspects of plant growth and metabolism. Notably, we found that genes related to glycolysis, especially the pyruvate kinase *PYK* and pyruvate decarboxylase *PDC*, were significantly upregulated in the leaves (Fig. 5b–d). In *Arabidopsis*, pyruvate kinase catalyzes a crucial step in the conversion of photosynthate to oil, and disruption of the enzyme-encoding gene caused a 60% reduction in the seed oil content [48]. The loss of the pyruvate transporter also led to a decrease in the seed oil content in *Brassica napus* [43]. These results demonstrated that pyruvate metabolism plays an important role in the accumulation of seed oil. The fact that *Massilia* upregulates pyruvate decarboxylase genes and other genes in the glycolytic pathway suggested that *Massilia* may promote oil production by enhancing glycolysis in soybeans.

Conclusion

Our findings reveal that genetic breeding of seed quality traits alters the rhizosphere bacterial community of soybeans. An increase in the oil content of soybean plants can lead to increased production and secretion of phenolic acids and flavonoids to modulate the abundance of microbes, thereby shaping the structure of the rhizosphere bacterial community. Among these microbes, *Massilia* can be assembled in the rhizosphere of high-oil cultivars and subsequently activate the glycolysis pathway to increase the oil content of plants. These findings establish a link between the genetic improvement of seed quality traits and rhizosphere microbial taxa and advance our understanding of complex plant–microbe interactions. The results of this research will help us better manage the rhizosphere microbial community and design ideal microbial consortia to achieve high crop quality.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-024-01933-7.

Supplementary Material 1.

Supplementary Material 2.

Authors' contributions

Q.H. and X.L. designed research; Q.H.M. and Y.Q.W. collected soybean materials and plant materials; Q.H., G.H.Z., M.B.L., H.M.Q., J.M.Z., X.Y.W., R.H.X., Y.Z., W.Y., B.T., L.X.X., J.Y.Z., Y.L., Y.Q.W., and Y.B. performed the research and analyzed data; and Q.H., G.H.Z., Y.B., and X.L. wrote the main manuscript text. All authors read and approved the final manuscript.

Funding

This research was supported by the National Key Research and Development Program of China (2023YFD1200600; 2023YFA0800800), the Foundation of Hubei Hongshan Laboratory (2021hszd015), the Science and Technology Major Projects of Hubei Province (2023BBA002), the Knowledge Innovation Program of Wuhan-Basi Research (2023020201010126) and the Fundamental Research Funds for the Central Universities (2662021JC010).

Data availability

Raw sequencing data were deposited in the Genome Sequence Archive at the China National Genomics Data Center under the accession number PRJCA016574. Other data supporting the findings of the present study are available within the paper and in the Supplementary Information.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]National Key Laboratory of Crop Genetic Improvement, Hubei Hongshan Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, People's Republic of China. [2]Laboratory of Risk Assessment for Oilseeds Products (Wuhan), Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430061, People's Republic of China. [3]Jilin Academy of Agricultural Sciences / National Engineering Research Center for Soybean, Changchun, Jilin 130033, People's Republic of China. [4]State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, The Innovative Academy of Seed Design, Chinese Academy of Sciences, Beijing 100101, People's Republic of China.

Received: 29 October 2023 Accepted: 13 September 2024
Published: 31 October 2024

References

1. Kim E, Hwang S, Lee I. SoyNet: a database of co-functional networks for soybean *Glycine max*. Nucleic Acids Res. 2017;45(D1):D1082–9.
2. Deng S, Caddell DF, Xu G, Dahlen L, Washington L, Yang J, et al. Genome wide association study reveals plant loci controlling heritability of the rhizosphere microbiome. ISME J. 2021;15(11):3181–94.
3. Eskandari M, Cober ER, Rajcan I. Genetic control of soybean seed oil: II. QTL and genes that increase oil concentration without decreasing protein or with increased seed yield. Theor Appl Genet. 2013;126(6):1677–87.
4. Chaudhary J, Patil GB, Sonah H, Deshmukh RK, Vuong TD, Valliyodan B, et al. Expanding omics resources for improvement of soybean seed composition traits. Front Plant Sci. 2015;6:1021.
5. Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, et al. Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe. 2015;17(3):392–403.
6. Leff JW, Lynch RC, Kane NC, Fierer N. Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower. Helianthus annuus New Phytol. 2017;214(1):412–23.
7. Pérez-Jaramillo JE, Carrión VJ, Bosse M, Ferrão LFV, de Hollander M, Garcia AAF, et al. Linking rhizosphere microbiome composition of wild and domesticated *Phaseolus vulgaris* to genotypic and root phenotypic traits. ISME J. 2017;11(10):2244–57.
8. Pérez-Jaramillo JE, Carrión VJ, de Hollander M, Raaijmakers JM. The wild side of plant microbiomes. Microbiome. 2018;6(1):143.
9. Mendes LW, Mendes R, Raaijmakers JM, Tsai SM. Breeding for soil-borne pathogen resistance impacts active rhizosphere microbiome of common bean. ISME J. 2018;12(12):3038–42.
10. Chen S, Waghmode TR, Sun R, Kuramae EE, Hu C, Liu B. Root-associated microbiomes of wheat under the combined effect of plant development and nitrogen fertilization. Microbiome. 2019;7(1):136.
11. Brown SP, Grillo MA, Podowski JC, Heath KD. Correction to: Soil origin and plant genotype structure distinct microbiome compartments in the model legume *Medicago truncatula*. Microbiome. 2021;9(1):105.
12. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. Plant–microbiome interactions: from community assembly to plant health. Nat Rev Microbiol. 2020;18(11):607–21.
13. Jackrel SL, Yang JW, Schmidt KC, Denef VJ. Host specificity of microbiome assembly and its fitness effects in phytoplankton. ISME J. 2021;15(3):774–88.
14. Hartmann A, Schmid M, Tuinen DV, Berg G. Plant-driven selection of microbes. Plant Soil. 2009;321(1–2):235–57.
15. Marasco R, Mosqueira MJ, Fusi M, Ramond JB, Merlino G, Booth JM, et al. Rhizosheath microbial community assembly of sympatric desert speargrasses is independent of the plant host. Microbiome. 2018;6(1):215.
16. Bressan M, Roncato MA, Bellvert F, Comte G, Haichar FEZ, Achouak W, et al. Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. ISME J. 2009;3(11):1243.
17. Sasse J, Martinoia E, Northen T. Feed your friends: do plant exudates shape the root microbiome. Trends Plant Sci. 2018;23(1):25–41.
18. Zwetsloot MJ, Kessler A, Bauerle TL. Phenolic root exudate and tissue compounds vary widely among temperate forest tree species and have contrasting effects on soil microbial respiration. New Phytol. 2018;218(2):530–41.
19. Koprivova A, Schuck S, Jacoby RP, Klinkhammer I, Welter B, Leson L, et al. Root-specific camalexin biosynthesis controls the plant growth-promoting effects of multiple bacterial strains. Proc Natl Acad Sci U S A. 2019;116(31):15735–44.
20. Zhalnina K, Louie KB, Hao Z, Mansoori N, da Rocha UN, Shi S, et al. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nat Microbiol. 2018;3(4):470–80.

21. Yu P, He X, Baer M, Beirinckx S, Tian T, Moya YAT, et al. Plant flavones enrich rhizosphere *Oxalobacteraceae* to improve maize performance under nitrogen deprivation. Nat Plants. 2021;7(4):481–99.
22. Mark GL, Dow JM, Kiely PD, Higgins H, Haynes J, Baysse C, et al. Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. Proc Natl Acad Sci U S A. 2005;102(48):17454–9.
23. Micallef SA, Shiaris MP, Colón-Carmona A. Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. J Exp Bot. 2009;60(6):1729–42.
24. Haichar FZ, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J, et al. Plant host habitat and root exudates shape soil bacterial community structure. ISME J. 2008;2(12):1221–30.
25. de Vries FT, Williams A, Stringer F, Willcocks R, McEwing R, Langridge H, et al. Changes in root-exudate-induced respiration reveal a novel mechanism through which drought affects ecosystem carbon cycling. New Phytol. 2019;224(1):132–45.
26. Stringlis IA, Yu K, Feussner K, de Jonge R, Van Bentum S, Van Verk MC, et al. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. Proc Natl Acad Sci U S A. 2018;115(22):E5213–22.
27. Kudjordjie EN, Sapkota R, Steffensen SK, Fomsgaard IS, Nicolaisen M. Maize synthesized benzoxazinoids affect the host associated microbiome. Microbiome. 2019;7(1):59.
28. Cadot S, Guan H, Bigalke M, Walser JC, Jander G, Erb M, et al. Specific and conserved patterns of microbiota-structuring by maize benzoxazinoids in the field. Microbiome. 2021;9(1):103.
29. Lu T, Ke M, Lavoie M, Jin Y, Fan X, Zhang Z, et al. Rhizosphere microorganisms can influence the timing of plant flowering. Microbiome. 2018;6(1):231.
30. Yang K, Fu R, Feng H, Jiang G, Finkel O, Sun T, et al. RIN enhances plant disease resistance via root exudate-mediated assembly of disease-suppressive rhizosphere microbiota. Mol Plant. 2023;16(9):1379–95.
31. Alahmad A, Harir M, Fochesato S, Tulumello J, Walker A, Barakat M, et al. Unraveling the interplay between root exudates, microbiota, and rhizosheath formation in pearl millet. Microbiome. 2024;12(1):1.
32. Wan ET. Symbiosis between rhizobia and legumes. In: ecology and evolution of rhizobia. Singapore: Springer; 2019. p. 3–19.
33. Roy S, Liu W, Nandety RS, Crook A, Mysore KS, Pislariu CI, et al. Celebrating 20 years of genetic discoveries in legume nodulation and symbiotic nitrogen fixation. Plant Cell. 2020;32(1):15–41.
34. Han Q, Wu F, Wang X, Qi H, Shi L, Ren A, et al. The bacterial lipopeptide iturins induce *Verticillium dahliae* cell death by affecting fungal signaling pathways and mediate plant defense responses involved in PAMP-triggered immunity. Environ Microbiol. 2015;17(4):1166–88.
35. Andrić S, Rigolet A, Argüelles Arias A, Steels S, Hoff G, Balleux G, et al. Plant-associated *Bacillus* mobilizes its secondary metabolites upon perception of the siderophore pyochelin produced by a *Pseudomonas* competitor. ISME J. 2023;17(2):263–75.
36. Bhat BA, Tariq L, Nissar S, Islam ST, Islam SU, Islam SU, et al. The role of plant-associated rhizobacteria in plant growth, biocontrol and abiotic stress management. J Appl Microbiol. 2022;133(5):2717–41.
37. Zhang J, Liu YX, Zhang N, Hu B, Jin T, Jin T, et al. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. Nat Biotechnol. 2019;37(6):676–84.
38. Finkel OM, Salas-González I, Castrillo G, Conway JM, Law TF, Teixeira PJPL, et al. A single bacterial genus maintains root growth in a complex microbiome. Nature. 2020;587(7832):103–8.
39. Salas-González I, Reyt G, Flis P, Custódio V, Custódio V, Bakhoum N, et al. Coordination between microbiota and root endodermis supports plant mineral nutrient homeostasis. Science. 2021;371(6525):eabd0695.
40. Mendes LW, Kuramae EE, Navarrete AA, van Veen JA, Tsai SM. Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J. 2014;8(8):1577–87.
41. Zgadzaj R, Garrido-Oter R, Jensen DB, Koprivova A, Schulze-Lefert P, Radutoiu S. Root nodule symbiosis in *Lotus japonicus* drives the establishment of distinctive rhizosphere, root, and nodule bacterial communities. Proc Natl Acad Sci U S A. 2016;113(49):E7996-8005.
42. Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, et al. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci U S A. 2015;112(8):E911–20.
43. Wang HW, Zhang B, Hao YJ, Huang J, Huang J, Liao Y, et al. The soybean Dof-type transcription factor genes, *GmDof4* and *GmDof11*, enhance lipid content in the seeds of transgenic *Arabidopsis* plants. Plant J. 2007;52(4):716–29.
44. Tang S, Guo N, Tang Q, Peng F, Liu Y, Xia H, et al. Pyruvate transporter *BnaBASS2* impacts seed oil accumulation in *Brassica napus*. Plant Biotechnol J. 2022;20(12):2406–17.
45. Wang Y, Wang D, Shi P, Omasa K. Estimating rice chlorophyll content and leaf nitrogen concentration with a digital still color camera under natural light. Plant Methods. 2014;10(1): 36.
46. Xie T, Chen X, Guo T, Rong H, Chen Z, Sun Q, et al. Targeted knockout of *BnTT2* homologues for Yellow-Seeded *Brassica napus* with reduced flavonoids and improved fatty acid composition. J Agric Food Chem. 2020;68(20):5676–90.
47. Gholami A, De Geyter N, Pollier J, Goormachtig S, Goossens A. Natural product biosynthesis in *Medicago* species. Nat Prod Rep. 2014;31(3):356–80.
48. Andre C, Froehlich JE, Moll MR, Benning C. A Heteromeric plastidic pyruvate kinase complex involved in seed oil biosynthesis in *Arabidopsis*. Plant Cell. 2007;19(6):2006–22.
49. Vaahtera L, Schulz J, Hamann T. Cell wall integrity maintenance during plant development and interaction with the environment. Nat Plants. 2019;5(9):924–32.
50. Martin RE, Postiglione AE, Muday GK. Reactive oxygen species function as signaling molecules in controlling plant development and hormonal responses. Curr Opin Plant Biol. 2022;69: 102293.
51. Rosenberg E, Zilber-Rosenberg I. The hologenome concept of evolution after 10 years. Microbiome. 2018;6(1):78.
52. Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. The importance of the microbiome of the plant holobiont. New Phytol. 2015;206(4):1196–206.
53. Chaparro JM, Badri DV, Vivanco JM. Rhizosphere microbiome assemblage is affected by plant development. ISME J. 2014;8(4):790–803.
54. Favela A, Bohn MO, Kent AD. Maize germplasm chronosequence shows crop breeding history impacts recruitment of the rhizosphere microbiome. ISME J. 2021;15(8):2454–64.
55. Liu A, Ku YS, Contador CA, Lam HM. The impacts of domestication and agricultural practices on legume nutrient acquisition through symbiosis with rhizobia and arbuscular mycorrhizal fungi. Front Genet. 2020;11: 583954.
56. Huang AC, Jiang T, Liu YX, Bai YC, Reed J, Qu B, et al. A specialized metabolic network selectively modulates *Arabidopsis* root microbiota. Science. 2019;364(6440):eaau6389.
57. Voges MJEEE, Bai Y, Schulze-Lefert P, Sattely ES. Plant-derived coumarins shape the composition of an *Arabidopsis* synthetic root microbiome. Proc Natl Acad Sci U S A. 2019;116(25):12558–65.
58. Harbort CJ, Hashimoto M, Inoue H, Niu Y, Guan R, Rombolà AD, et al. Root-secreted coumarins and the microbiota interact to improve iron nutrition in *Arabidopsis*. Cell Host Microbe. 2020;28(6):825-37.e6.
59. Han X, Zhang YW, Liu JY, Zuo JF, Zhang ZC, Guo L, et al. 4D genetic networks reveal the genetic basis of metabolites and seed oil-related traits in 398 soybean RILs. Biotechnol Biofuels Bioprod. 2022;15(1):92.
60. Li C, Cao P, Du C, Zhang X, Bing H, Bing H, et al. *Massilia rhizosphaerae* sp. nov., a rice-associated rhizobacterium with antibacterial activity against *Ralstonia solanacearum*. Int J Syst Evol Microbiol. 2021;71(9).
61. Raths R, Peta V, Bücking H. *Massilia arenosa* sp. nov., isolated from the soil of a cultivated maize field. Int J Syst Evol Microbiol. 2020;70(6):3912–20.
62. Huq MA, Ma J, Srinivasan S, Parvez MAK, Parvez MAK, Naserkheil M, et al. *Massilia agrisoli* sp. nov., isolated from rhizospheric soil of banana. Int J Syst Evol Microbiol. 2023;73(5).
63. Ofek M, Hadar Y, Minz D. Ecology of root colonizing *Massilia (Oxalobacteraceae)*. PLoS One. 2012;7(7):e40117.
64. Tkacz A, Cheema J, Chandra G, Grant A, Poole PS. Stability and succession of the rhizosphere microbiota depends upon plant type and soil composition. ISME J. 2015;9(11):2349–59.
65. Du C, Li C, Cao P, Li T, Li T, Wang X, et al. *Massilia cellulosiltytica* sp. nov., a novel cellulose-degrading bacterium isolated from rhizosphere soil of rice (*Oryza sativa* L.) and its whole genome analysis. Antonie van Leeuwenhoek. 2021;114(10):1529–40.
66. Sedláček I, Holochová P, Busse HJ, Koublová V, Králová S, Švec P, et al. Characterisation of waterborne psychrophilic *Massilia* isolates with violacein production and description of *Massilia antarctica* sp. nov. Microorganisms. 2022;10(4):704.

Publisher's Note