

1 **Disentangling the genetic basis of rhizosphere microbiome assembly in tomato**

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15 **Abstract (word count 150)**

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17 Microbiomes play a pivotal role in plant growth and health, but the genetic factors involved in
18 microbiome assembly remain largely elusive. Here, 16S amplicon and metagenomic features of
19 the rhizosphere microbiome were mapped as quantitative traits of a recombinant inbred line
20 population of a cross between wild and domesticated tomato. Gene content analysis of prioritized
21 tomato QTLs suggested a genetic basis for differential recruitment of various rhizobacterial
22 lineages, including a *Streptomyces*-associated 6.31-Mbp region harboring tomato domestication
23 sweeps and encoding, among others, the iron regulator FIT and the aquaporin SITIP2.3. Within
24 metagenome-assembled genomes of the rhizobacterial lineages *Streptomyces* and *Cellvibrio*, we
25 identified microbial genes involved in metabolism of plant polysaccharides, iron, sulfur, trehalose,
26 and vitamins, whose genetic variation associated with either modern or wild tomato QTLs.
27 Integrating ‘microbiomics’ and quantitative plant genetics pinpointed putative plant and reciprocal
28 microbial traits underlying microbiome assembly, thereby providing the first step towards plant-
29 microbiome breeding programs.

30 **1. Main**

31 Root and shoot microbiomes are fundamental to plant growth and plant tolerance to (a)biotic stress
32 factors. The outcome of these beneficial interactions is the emergence of specific microbiome-
33 associated phenotypes (MAPs)¹, such as drought resilience², disease resistance³, development⁴ and
34 heterosis (i.e. hybrid vigor)⁵. The microbes inhabiting the surface or internal tissues of plant roots
35 are selectively nurtured by diverse plant-derived compounds in the form of primary and secondary
36 metabolites^{6,7}. Microbes reciprocate by supporting plant growth and producing metabolites that
37 mediate processes such as nutrient acquisition and pathogen suppression^{8,9}. Developing a blueprint
38 of the genetic architecture for this ‘chemical dialogue’ and how these interactions lead to specific
39 MAPs is a one of the key focal points in current plant microbiome research. The promise is that
40 these genomic and chemical blueprints can be integrated into microbiome breeding programs for

41 a new generation of crops that can rely, in part, on specific members of the microbiome for stress
42 protection, enhanced growth and higher yields¹⁰.

43 Selective breeding for yield-related traits has left a considerable impact on the taxonomic
44 and functional composition of modern crop microbiomes^{11,12}. Wild plant relatives represent a
45 ‘living library’ of diverse genetic traits that may have been lost during domestication¹³. For
46 example, recombinant inbred lines (RILs) of crosses between wild tomato relatives and modern
47 tomato cultivars have been used to identify genetic loci controlling important agronomic traits,
48 including tolerance to abiotic¹⁴ and biotic stress¹⁵, as well as nutritional quality and flavor
49 profiles¹⁶. To date, microbiome traits are not yet considered for breeding purposes, except for
50 specific quantitative MAPs such as the number of nodules in legume-rhizobia symbioses¹⁷.
51 However, technological advances in sequencing now make it feasible to treat microbiomes as
52 quantitative traits for selection. This approach has been adopted for the phyllosphere microbiome
53 and, recently, for the *Arabidopsis* and sorghum rhizosphere microbiomes^{18,19}. For most plant
54 species, however, investigations leveraging diverse plant populations to map microbiome
55 Quantitative Trait Loci (QTL) are still at their infancy^{20,19,18}. In these recent studies, the
56 microbiomes were characterized by amplicon sequencing to detect loci involved in alpha and beta
57 diversity as well as individual OTU abundances²¹. These studies provide strong evidence that
58 microbiome recruitment has a genetic component, but the functional nature of the corresponding
59 plant-microbe interactions cannot be elucidated from amplicon data. Hence, functional genomic
60 features of the microbiome as well as intraspecific diversity within microbial species have not yet
61 been taken into account²².

62 Here, we used both amplicon and shotgun metagenome sequencing to generate taxonomic
63 as well as functional microbiome features as quantitative traits. Using an extensive recombinant
64 inbred line (RIL) population of a cross between modern *Solanum lycopersicum* var. MoneyMaker
65 and wild *Solanum pimpinellifolium*²³, we were able to identify reciprocal associations between
66 specific plant and microbiome traits and to infer putative mechanisms for rhizosphere microbiome
67 assembly. While both wild and modern alleles were identified, the large number of QTLs linked
68 to modern alleles suggests that domestication has had a significant impact on rhizosphere
69 microbiome assembly. The plant traits identified were related to growth, stress, amino acid
70 metabolism, iron and water acquisition, hormonal responses, and terpene biosynthesis, whereas
71 the microbial traits were related to metabolism of plant cell wall polysaccharides, vitamins, sulfur,
72 and iron. Furthermore, we show that amplicon-based approaches allow detection of QTLs for rarer
73 microbial taxa, whereas shotgun metagenomics allowed mapping to smaller and thus more defined
74 plant genomic regions. Together, these results demonstrate the power of an integrated approach to
75 disentangle and prioritize specific genomic regions and genes in both plants and microbes
76 associated with microbiome assembly.

77 **2. Results**

78 ***2.1 Baseline analyses of the tomato Recombinant Inbred Line population***

79 Prior to detailed metagenome analyses of the microbiome of the tomato RIL population, we first
80 investigated whether QTLs previously identified in the same RIL population under sterile *in vitro*
81 conditions could be replicated in our experiment conducted under greenhouse conditions with a
82 commercial tomato greenhouse soil (Figure 1A and B, Supplemental table 1)²⁴. We identified
83 QTLs for Shoot Dry Weight (SDW) coinciding with a QTL identified previously on chr9²⁴.

84 Similarly, we identified QTLs for Rhizosphere Mass (RM), defined here as a the total mass of the
 85 roots with tightly adhering soil, which coincide with root trait QTLs previously identified for
 86 lateral root number, fresh and dry shoot weight, lateral root density per branched zone and total
 87 root size (Figure 1B)²⁴. An analysis of variance (ANOVA) yielded significant variation in SDW
 88 based on the additivity of alleles linked to SDW (zero, one or two alleles) ($F(2, 186) = 16.02$, $p =$
 89 3.76×10^{-7}) (Figure 1C and 1D). A post hoc Tukey test further demonstrated significant differences
 90 between all pairwise comparisons ($p < 0.05$). For RM, an ANOVA yielded a significant difference
 91 ($F(2, 186) = 16.02$, $p = 3.76 \times 10^{-7}$); a post hoc Tukey test demonstrated a statistically significant
 92 difference only between presence of either one or two alleles ($p < 0.05$), but did not support
 93 additivity ($p = 0.15$) (Figure 1E and 1F). Collectively, our results confirm and extend earlier work
 94 conducted on the same tomato RIL population *in vitro*²⁴, providing a solid basis for QTL mapping
 95 of taxonomic and genomic features of the rhizosphere microbiome.
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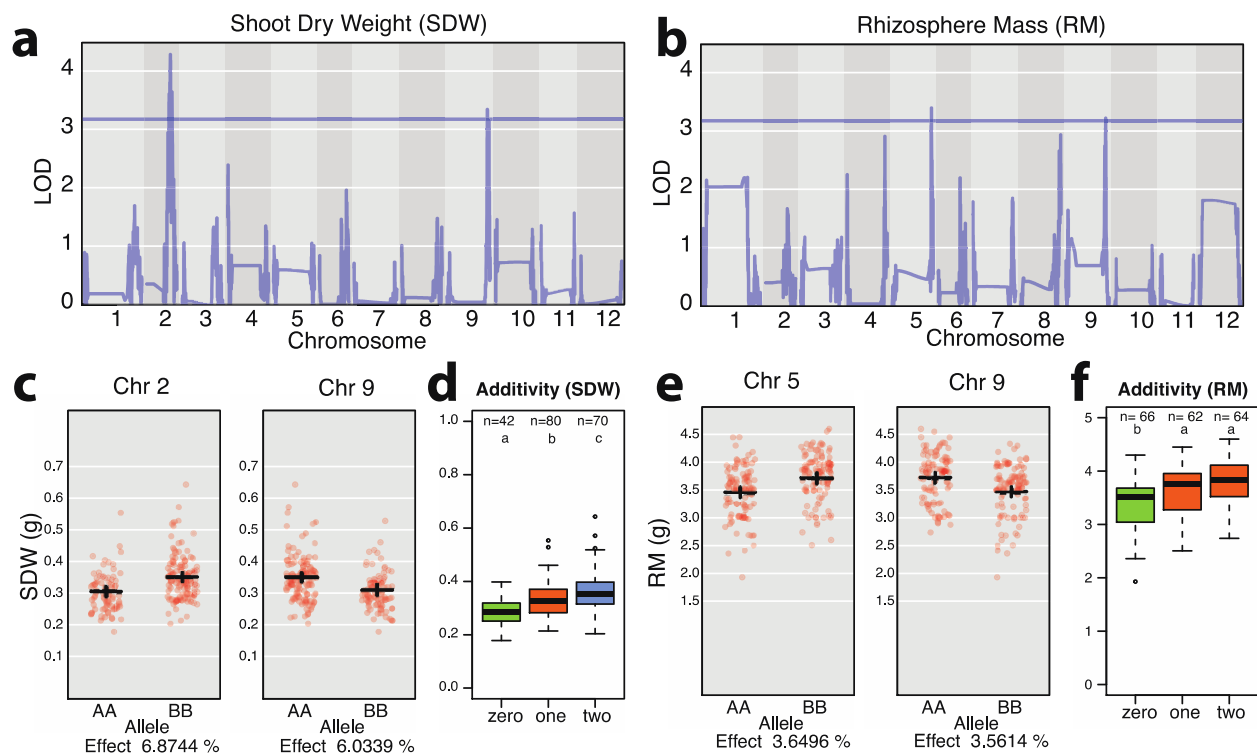


Figure 1: Identification of shoot dry weight (SDW) and rhizosphere mass (RM) QTLs in the recombinant inbred line (RIL) population of tomato. (a) QTLs identified for SDW on chromosome 9 position 63.63719184 and on chromosome 2 position 42.7291229, coinciding with a QTL identified previously (chromosome 9 position 62.897108) by Khan et al 2012. (b) QTL of RM on chromosome 5 position 62.00574891, and chromosome 9 position 62.71397636, which coincide with root trait QTLs previously identified by Khan et al 2012 for lateral root number chromosome 5 position 53.4-86.1, and several on chromosome 9, including fresh and dry shoot weight, (chromosome 9 position 81.3-95.3), lateral root density per branched zone (chromosome 9 position 33.8-88.7), and total root size (chromosome 9 position 39.4-75.1). (c) Scatter plots showing the distribution of SDW measurements on chromosome 2 position 42.7291229 and chromosome 9 position 63.63719184 for both modern (AA) and wild (BB) tomato alleles. (d) Significant additivity of tomato alleles for shoot dry weight ($p < 0.05$); n of 42, 80 and 70 for tomato plants containing neither allele (labeled zero), either BB allele on chromosome 2, or AA on chromosome 9 (labeled one), or both AA and BB alleles (labeled two), respectively. (e) Scatter plots showing the distribution of RM measurements on chromosome 5 (pos 62.00574891), and chromosome 9 (pos 62.71397636) for both modern (AA) and wild (BB) alleles. (f) No additivity of alleles was observed for RM.

97 2.2 Taxonomic microbiome features as quantitative traits

98 To investigate molecular features of the microbiome as quantitative traits, we conducted 16S
99 amplicon sequencing of 225 rhizosphere samples, including unplanted bulk soil, parental tomato
100 genotypes, and all 96 RIL accessions in duplicate (Supplemental table 2-5, BioProject ID
101 PRJNA787039). We observed a separation between the microbiomes of rhizosphere and bulk soil,
102 between the microbiomes of the two parental tomato genotypes and the RIL accession
103 microbiomes (Figure 2A). To limit multiple testing and to focus on common microbiome features
104 with sufficient coverage across all accessions, we prioritized the rhizosphere-enriched amplicon
105 sequence variants (ASVs) to those present in 50% or more of the RIL accessions (Figure 2B). A
106 QTL analysis with these prioritized ASVs was run with R/qt12²⁵ using a high-density tomato
107 genotype map²⁶, harvest date, post-harvest total bulk soil mass, RM, number of leaves at harvest
108 and SDW as co-variates.

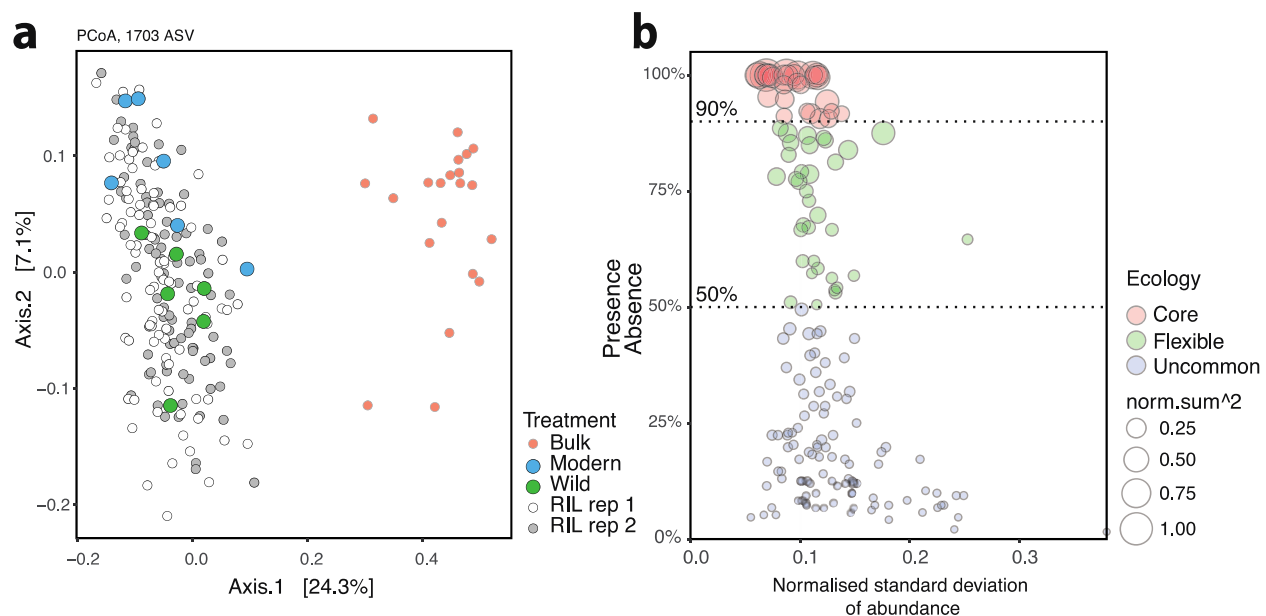


Figure 2: PCoA analysis of the 16S rRNA amplicon data obtained for the microbiomes of bulk soil and the rhizosphere of modern and wild tomatoes and their recombinant inbred line (RIL) population. (a) PCoA analysis of amplicon sequence variants (ASVs) demonstrating a separation between the bulk soil and rhizosphere microbiomes. The rhizosphere microbiome of the 96 RIL accessions distributed around those of the wild and modern rhizosphere microbiomes. Separation between the two replicate RIL populations was not observed. (b) To limit multiple testing, a QTL analysis was conducted only on ASVs that were observed in more than 50% of the RIL accessions.

109 We identified 48 QTL peaks, across 45 distinct loci, significantly associated with 33 ASVs
110 (Supplemental table 6). Our logarithm of the odds (LOD) thresholds for significance had been
111 determined by pooled permutations from all ASVs to attain a genome-wide threshold of P 0.05
112 (LOD 3.35) and P 0.2 (LOD 2.64). Of the significant QTLs, 16 were more abundant in a wild
113 tomato allele and 32 in a modern tomato allele. The QTLs on chromosomes 11, 10, 8 and 2 were
114 all linked to 'modern' alleles; the sole QTL on chromosome 7 was linked to a 'wild' tomato allele.
115 All other chromosomes contained a mix of QTLs linked to either modern or wild alleles (Figure
116 3A). While many rhizobacterial lineages were linked to a single QTL (14 taxa out of 25), others
117 were linked to two or more QTLs (7 and 4 taxa, respectively) (Figure 3B). Of the lineages with
118 multiple QTLs, most were linked only to modern tomato alleles. One salient exception was
119

120 *Methylophilaceae*, with increased abundance linked to a total of 9 QTLs, from both wild and
 121 modern alleles, and distributed across chromosomes 3 (modern, x2), 4 (modern), 7 (wild), 11
 122 (modern x2) and 12 (wild x3) (Figure 3D). Another salient feature of the QTL analysis was the
 123 hotspot for microbiome assembly identified on chromosome 11, including ASVs from
 124 *Adhaeribacter*, *Caulobacter*, *Devosia*, Rhizobiaceae, *Massilia* and *Methylophilaceae* (Figure 3D).

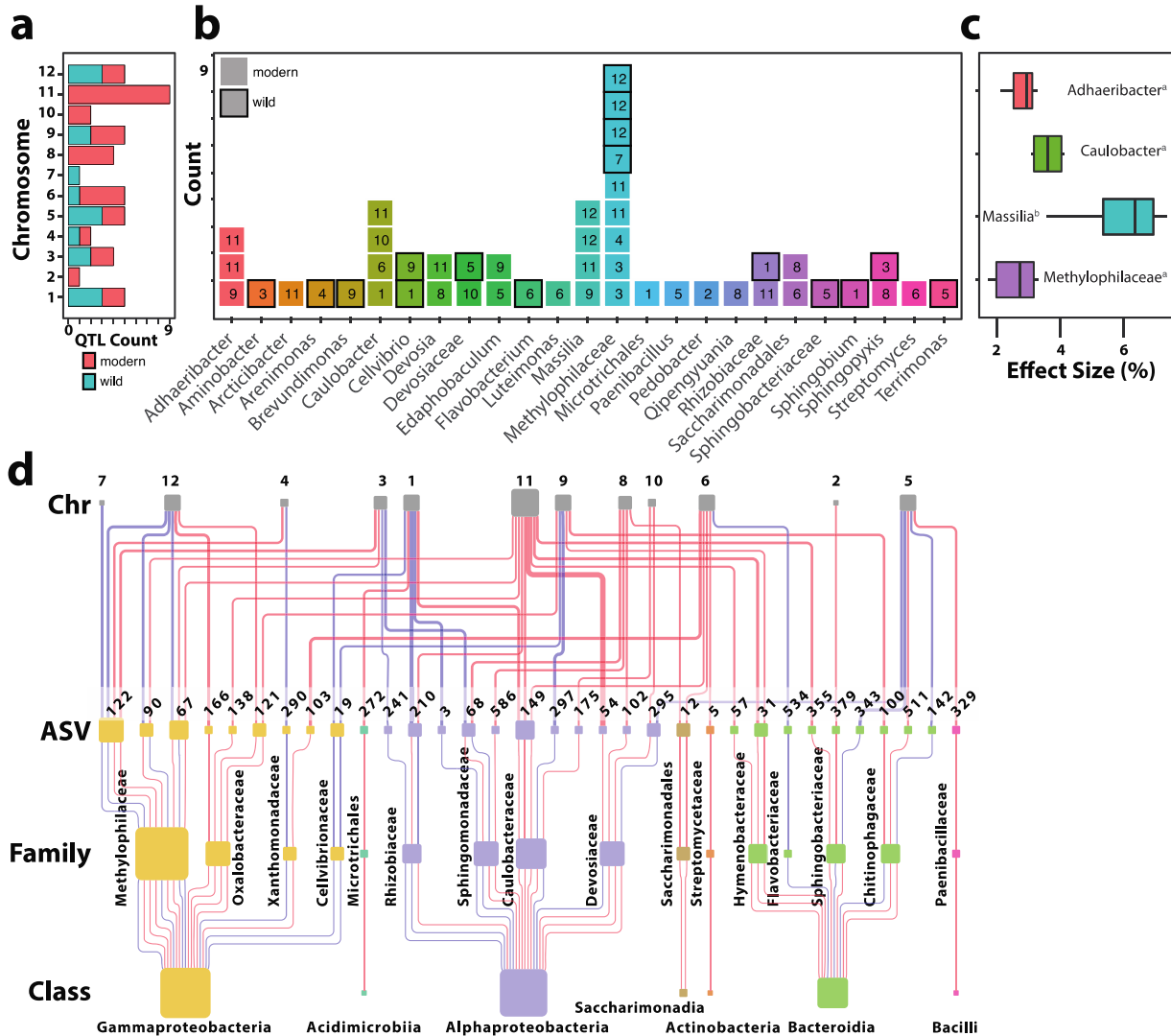


Figure 3: Association between 16S rRNA amplicon sequence variants (ASVs) and tomato QTLs (a) A color-coded summary of the number of 16S rRNA QTLs identified per chromosome of wild and modern tomato alleles. (b) A summary of the number of 16S rRNA QTLs linked to bacterial taxonomies, with the chromosome number of each QTL represented within each square. The presence and absence of dark borders around each square are used to indicate a QTL linked to higher abundance for a wild allele and modern allele, respectively. (c) Effect size for four rhizobacterial lineages with 3 or more QTLs. (d) Hierarchical network depicting the 16S rRNA QTLs identified in this study. From top to bottom: the first row represents tomato chromosomes (Chr), which are linked to specific ASVs in the next row, which taxonomically belong to different families and classes of bacteria in subsequent rows. The size of the chromosome nodes is weighted by the number of outbound edges. The ASV, family, and class node sizes are weighted by the number of in-bound edges. A complex network emerges, whereby the abundance of individual ASVs, at different taxonomic levels, is determined by a network of interactions of multiple tomato alleles from both modern and wild origin.

125 The effect size of the 48 QTLs on ASV relative abundance ranged from 1.3 to 17%, with
 126 an average effect size of approximately 5%, comparable to the effects seen for SDW and RM
 127 (Figure 1C and E). The largest effect was a single modern QTL for an ASV in the genus

128 *Qipengyuania* (17%), and a second modern QTL in *Edaphobaculum* (10%). No statistical
129 difference was found between modern and wild alleles on their effect size ($p = 0.78$, two-tailed t-
130 test). For those lineages with sufficient representation at the class level (Bacteroidia,
131 Alphaproteobacteria, and Gammaproteobacteria), there was no statistically significant difference
132 between effect size ($F(3, 16) = 0.072$, $p = 0.974$). However, an ANOVA on the positive effect size
133 at genus level demonstrated significant differences between lineages ($F(3, 16) = 12.94$, $p = 1.15$
134 $e-04$). A post hoc Tukey test demonstrated QTLs for *Massilia* with a larger positive effect size
135 than other lineages with sufficient sample size for comparison (Figure 3C). Together, the amplicon
136 analysis provided a broad picture, suggesting that microbiome assembly is a complex trait
137 governed by a combination of multiple loci, some being ASV specific, some being pleiotropic to
138 different ASVs and with heterogenous effect sizes (Figure 3D). While positive effects were
139 identified linked to both wild and modern alleles, the large number of QTLs linked to modern
140 alleles, suggests domestication has had a significant impact on rhizosphere microbiome assembly.
141

142 **2.3 Functional microbiome features as quantitative traits**

143 To understand the functional traits associated with rhizosphere microbiome assembly, we
144 generated shotgun metagenomes for each accession in the tomato RIL population (96 total), as
145 well as six samples of the modern tomato parent, five samples of the wild tomato parent and seven
146 bulk soil samples (BioProject ID PRJNA789467). After pre-processing, assembly, back-mapping,
147 CSS normalization and binning, QTL mapping was conducted for the rhizosphere enriched contig
148 and bin abundances. Binning was done using Metabat2 (version 2:2.15)²⁷ and genomic quality of
149 the output was evaluated by CheckM²⁸ (Supplemental Table 7). The bins and assembled contigs
150 larger than 10kb can be found on Open Science Framework (<https://osf.io/f45ek/>). All contigs of
151 10kb and larger were taxonomically assigned using Kraken²⁹ (Supplemental Table 8). With nearly
152 40 million contigs being assembled, we took numerous prioritization steps to reduce the effects of
153 multiple testing. Only rhizosphere-enriched contigs larger than 10kb and with a rhizosphere
154 enrichment greater than 4-fold were selected resulting in 1249 contigs. Only bins with greater than
155 90% completion and less than 5% contamination were mapped (33 out of 588 bins). As with the
156 ASVs, harvest date, bulk soil mass, rhizosphere mass (RM), number of leaves at harvest, and SDW
157 were used as co-variates in QTL mapping (supplemental table 11 and 12, respectively).

158 We identified 7 significant bin QTLs ($LOD > 3.40$, $P < 0.05$) (Supplemental table 9)
159 including *Streptomyces* bin 72 associated with a modern allele on tomato chromosomes 6 and 11.
160 For the contigs, a total of 717 QTLs at 26 unique positions on chromosomes 1, 4, 5, 6, 9 and 11
161 were identified (Supplemental table 10), corresponding to 476 metagenomic contigs from 10
162 different genera ($LOD > 3.47$, $P < 0.05$). The largest number of contig QTLs belonged to the
163 *Streptomyces*, *Cellvibrio* and *Sphingopyxis* lineages (Figure 4A). The *Streptomyces* contigs
164 mapped to QTLs on tomato chromosomes 4 (46 contigs, wild tomato), 6 (190 contigs, modern
165 tomato) and 11 (257 contigs, modern tomato), with a subset of contigs mapping to two or all three
166 of these positions (Figure 4B). These findings corroborate and expand upon the *Streptomyces* QTL
167 identified on chromosome 6 using our 16S amplicon data, as well as that of the bin QTLs identified
168 on chromosomes 6 and 11. The *Cellvibrio* contigs mapped to chromosome 1 (42 contigs, wild)
169 and chromosome 9 (94 contigs, wild), again corroborating the findings from our 16S amplicon
170 analysis described above. In contrast, the *Sphingopyxis* QTLs identified on chromosome 5 (24
171 contigs, wild) and 9 (49 contigs, modern) did not correspond to the QTLs identified on
172 chromosomes 8 and 3 in the 16S amplicon analysis. Interestingly, 4 contigs for *Devosia* also
173 corroborated the results of the 16S QTL analysis. The effect sizes ranged from 9 to 21 % and were

174 significantly different ($F(14, 702) = 530.9$ $p < 2e-16$) between QTL and lineages (Figure 4C).

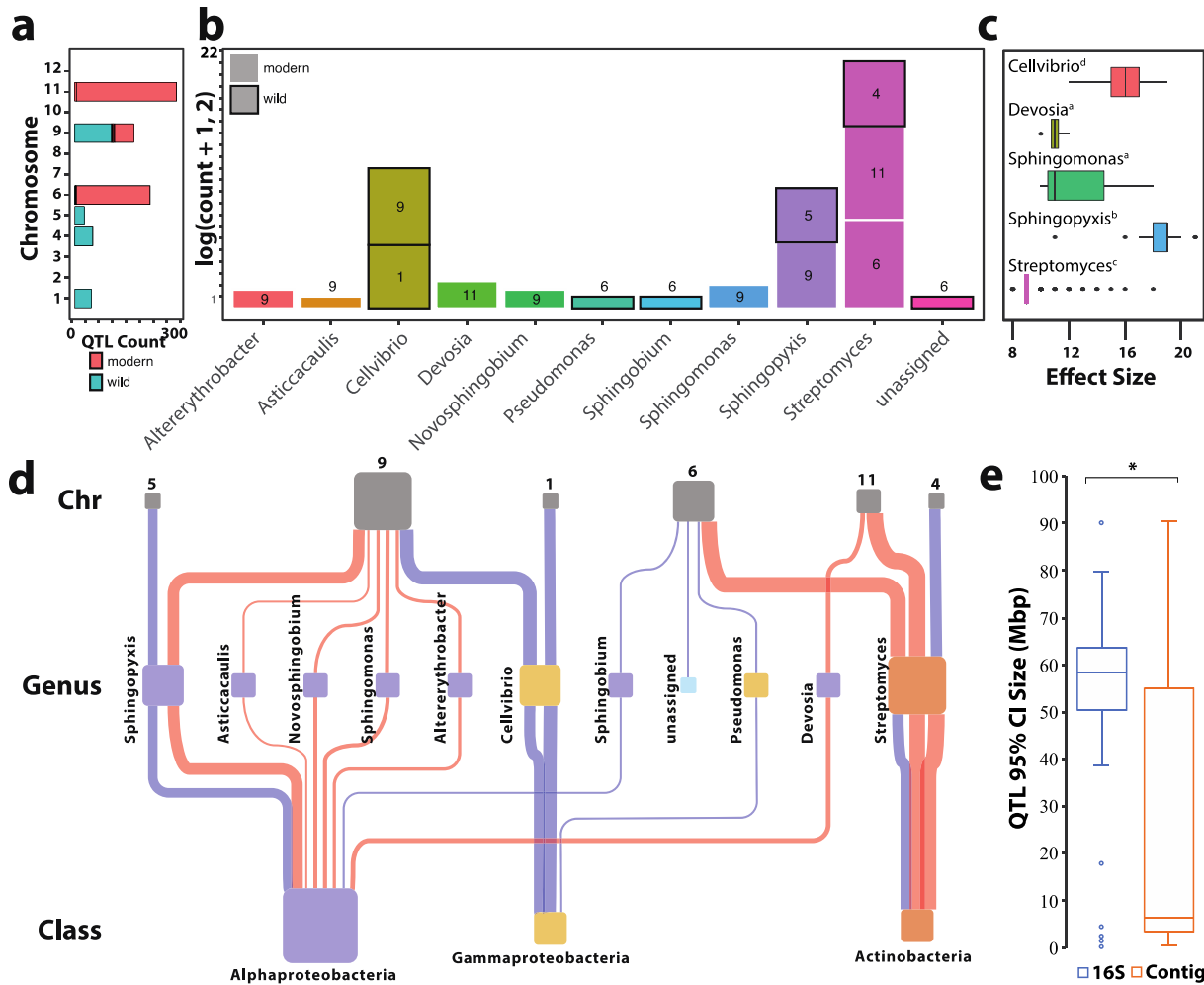


Figure 4: Association between metagenomic contigs of the rhizosphere microbiome and tomato QTLs (a) A color coded summary of the number of contig QTLs identified per chromosome to wild and modern alleles. (b) A summary of the number of contig QTLs found by taxonomies, with the chromosome of each QTL represented within each square. The presence and absence of dark borders around each square are used to indicate a QTL linked to higher abundance for a wild allele and modern allele, respectively. (c) The effect sizes for each lineage were significantly different as indicated by letters ($F(14, 702) = 530.9$ $p < 2e-16$) (d) A hierarchically structured network depicting the contig QTLs identified in this study. From top to bottom are the tomato chromosomes (Chr), which are associated with specific metagenomic contigs and taxonomically linked to different families and classes of bacteria. The size of the chromosome nodes is weighted by the number of outbound edges. The ASV, family, and class node sizes are weighted by the number of in-bound edges. (e) Comparison between the size of the QTL regions identified based on 16S amplicon data and based on metagenomic contigs. The 95% confidence interval of contig QTLs was significantly smaller than the 95% confidence interval of 16S rRNA QTLs (two-sided t.test, $p = 3.32E-09$).

175 Interestingly, as with the 16S amplicon analysis, some of the highest LOD scores were for contigs
 176 belonging to *Devosia*. Also, the effect size of the *Sphingopyxis* contigs was large ($\pm 20\%$ on
 177 average), above 15% for *Cellvibrio*, and approximately 10% for *Streptomyces*. The average QTL
 178 region was 51.59 Mbps for the 16S amplicon sequences and 26.64 Mbps for the metagenomic
 179 contigs (two-sided t.test, $p = 3.32E-09$) (Figure 4E). A more striking contrast was observed in the
 180 difference between the median size of amplicon and contig QTL regions which were 58.56 Mbp
 181 and only 6.47 Mbp, respectively. In summary, while many more taxa were identified in the
 182 amplicon-based QTL analysis, the metagenome-based QTL analysis provided QTLs with much
 183 smaller confidence intervals (Figure 4E).

184 2.4 Amplicon-based bulk segregant analysis of *Streptomyces* and *Cellvibrio* abundance

185 The two most abundant rhizosphere taxa with replicated patterns for amplicon and metagenome-
 186 based QTLs were *Streptomyces* and *Cellvibrio*. Therefore, we sought to provide additional
 187 independent support for these QTLs using a bulk segregant analysis of an independent population
 188 of parental and RIL genotypes (Supplemental_Table_11). In particular, we tested the previously
 189 identified amplicon-based QTLs associated with higher *Cellvibrio* abundance at markers 464 and
 190 3142 on chromosomes 1 and 9, respectively with higher *Streptomyces* abundance at marker 2274
 191 on chromosome 6 (Figure 5). In each case, ANOVA showed a statistical difference between
 192 genotypes and bulk soil, respectively ($F(4, 396) = 21.56, p = 4.16 \times 10^{-16}$), ($F(4, 396) = 18.43, p =$
 193 6.68×10^{-14}), ($F(4, 396) = 8.423, p = 1.57 \times 10^{-6}$). A post hoc Tukey test supported the conclusion that
 194 wild allele at markers 464 and 3142 on chromosomes 1 and 9, respectively, are indeed associated
 195 with increased abundance *Cellvibrio* ($p = 3.913 \times 10^{-4}$, and $p = 0.08$ respectively), while the modern
 196 allele at markers 2274 on chromosome 6 was significantly associated with increased abundance of
 197 *Streptomyces* ($p = 1.152 \times 10^{-4}$).

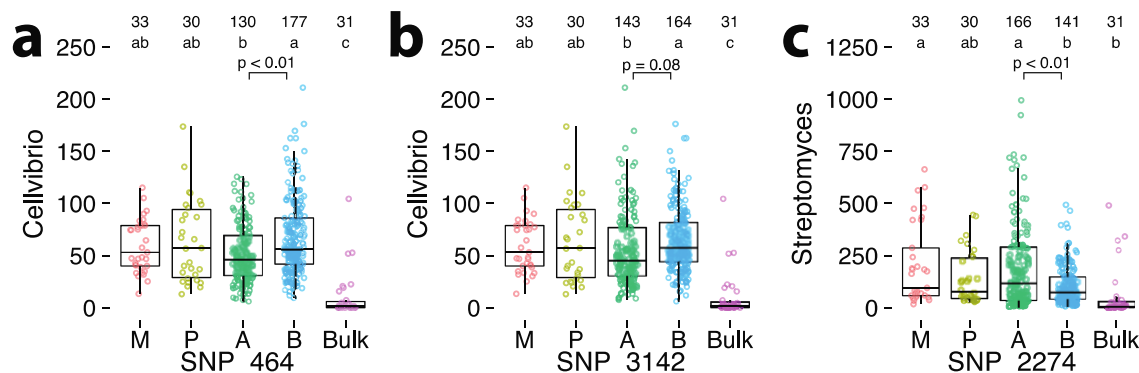


Figure 5: Validation of *Cellvibrio* and *Streptomyces* 16S rRNA QTLs with bulk segregant analysis in an independent experiment with modern, wild and 77 RIL accessions (see Supplemental table 13). The number of replicates for each treatment is detailed in the top row of each panel. The number of replicates within the RIL population are represented by either an A (modern) or B (wild) allele, which depends on the marker in question. The row below represents the statistical group based on Tukey's HSD; a different letter indicates a statistically significant difference. (a) The relative abundances of *Cellvibrio* 16S rRNA in bulk soil, modern, wild, and RIL accessions at SNP marker position 464 on chromosome 1. At this position, 32 and 45 RIL accessions with modern and wild alleles were used (130 and 177 samples with biological replication respectively). (b) Similarly, for SNP marker 3142 on chromosome 9, there were a total of 35 and 42 RIL accessions with modern and wild alleles, (143 and 164 samples with biological replication respectively). (c) The relative abundances of *Streptomyces* 16S rRNA and sequences in bulk soil, modern, wild, and RIL accessions at SNP marker 2274 on chromosome 6. There was a total of 42 and 35 RIL accessions with modern and wild alleles, (166 and 141 samples with biological replication, respectively).

198

199 2.5 Host genetics and rhizosphere microbiome assembly

200 A subset of 5 regions consistent across both the amplicon and metagenome-based analyses were
 201 prioritized with an average size of 2.68 Mbps (Supplemental Table 12). These included positions
 202 on chromosome 1 (positions 87.36 - 90.49 Mbps), chromosome 9 (pos 62.03 - 63.32 Mbps),
 203 chromosome 5 (pos 61.54 - 63.38), chromosome 6 (pos 33.99 - 40.3 Mbps) and chromosome 11
 204 (pos 53.06 - 53.89 Mbps). In total, 1359 genes were identified in these regions. Potential candidate
 205 genes with root-specific transcriptional patterns, defined as a 4 fold increase in the roots compared
 206 to leaf samples, were further prioritized using a publicly available RNAseq dataset³⁰. Based on
 207 this analysis, a subset of 192 root specific genes were identified (Supplemental table 13). A total

208 of 98 root specific genes were linked to *Streptomyces* on chromosome 6 (84 genes) and 11 (14
 209 genes) (Figure 6). Intriguingly, 61 of these genes were found in regions previously identified to be
 210 subjected to selective sweeps related to tomato domestication as well as to subsequent sweeps
 211 related to improvements in fruit quality³¹(Supplemental Figure 1).

212 Two of the most salient genes in this list included genes with high transcription in the roots;
 213 an aquaporin and a Fer-like iron deficiency-induced transcription factor (FIT). The aquaporin
 214 (SITIP2.3) is one of eleven tonoplast intrinsic proteins in the tomato genome³² and has the highest
 215 fold change in the roots compared to all other organs³³. The FIT gene is a bHLH transcriptional
 216 regulator controlling iron homeostasis in tomato^{34,35}. Other genes of interest on chromosome 6
 217 include a glycine rich protein, a receptor like kinase known to be upregulated during drought³⁶,
 218 alcohol dehydrogenase, numerous phosphatases, expansins, ethylene-responsive transcription
 219 factors, gibberellin receptors, aminocyclopropane-1-carboxylate oxidase (ACO), an enzyme
 220 involved in the last step of ethylene biosynthesis, and finally, alpha-humulene and (-)-(E)-beta-
 221 caryophyllene, a known tomato terpene and signaling molecule in tomato^{37,38} and also acting as a
 222 volatile in microbiome assembly³⁹. Root specific genes involved in carbohydrate, protein and
 223 amino metabolism were also identified, including trypsin-alpha amylase inhibitor, prolyl 4-
 224 hydroxylase, polygalacturonase, trehalose phosphatase, glycogenin, xyloglucan fucosyltransferase
 225 and a metallocoxy-peptidase inhibitor, spermidine synthase, acetolactate synthases, alanine
 226 aminotransferase, and an amino acid permease. On chromosome 11, a ferredoxin, an aluminum
 227 activated malate transporter⁴⁰ and a cluster of various acetyltransferases and a sulfotransferase
 228 were identified.

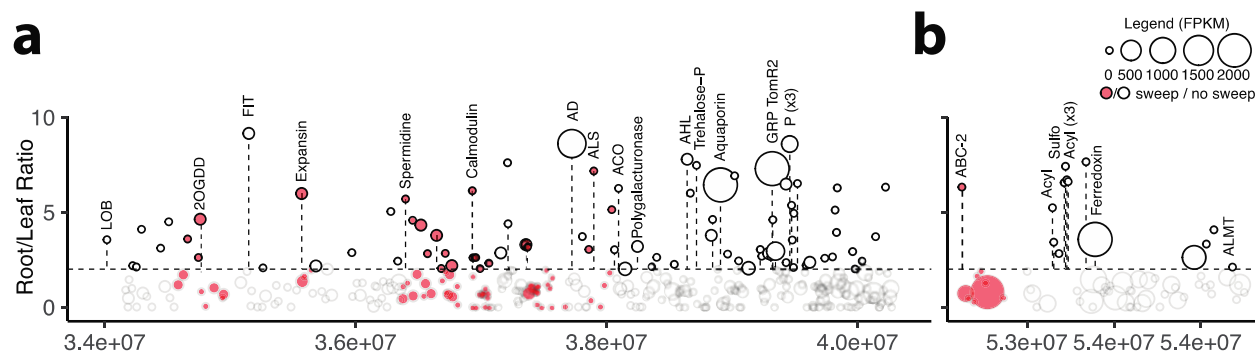


Figure 6: Prioritized regions of the *Streptomyces*-associated QTLs on tomato chromosomes 6 and 11 overlaying previously published data³⁰ on root-specific gene expression and genetic sweeps due to domestication³¹ (in red). Within each region, the \log_2 ratio gene expression patterns from leaf and root materials were calculated and those with a \log_2 greater than 2, as delineated by the dotted line, were further prioritized. The \log_2 root transcript abundances are depicted by the size of the bubble. **a)** The 6.31 Mbp region on chromosome 6, position 33.99-40.3 Mbps. Abbreviations of highlighted genes: LOB - LOB domain protein 4, 2OGDD - 2-Oxoglutarate-dependent dioxygenases, FIT - FIT (Fer-like iron deficiency-induced transcription factor), Spermidine - Spermidine synthase, AD - Alcohol dehydrogenase 2, ALS - Acetolactate synthase, ACO - 1-aminocyclopropane-1-carboxylate oxidase, Polygalacturonase, AHL - AT-hook motif nuclear-localized protein, Trehalose-P - Trehalose 6-phosphate phosphatase, Aquaporin - Tonoplast intrinsic protein 23 / Aquaporin, GPR TomR2 - Glycine-rich protein TomR2, P - Acid phosphatase (x3). **b)** The 0.83 Mbp region on chromosome 11, position 53.06-54.89 Mbps. Abbreviations of highlighted genes: ABC-2 - ABC-2 type transporter, Acyl - Acyltransferase (x4), Sulfo - Sulfotransferase, ALMT- Aluminum-activated malate transporter.

229 A total of 57 root specific genes were identified in the QTL regions on chromosome 1 and
 230 9 linked to *Cellvibrio*. These include a cytochrome p450 involved in coumarin synthesis, numerous
 231 expansins, phosphatases, respiratory burst oxidase-like protein, iron chelator nicotianamine
 232 synthase^{41,42} and on chromosome 11 phenazine biosynthesis. On chromosome 5, 37 root specific
 233 genes were identified including multiple peroxidases, glutamine synthetase, rhamnogalacturonate
 234 lyase, pectinesterase, metacaspase and trehalose-phosphatase. Furthermore, numerous ethylene
 235 responsive transcription factors and receptor like kinases were observed. The QTL on chromosome

236 1 contains genome-wide sweeps related to the initial tomato domestication and subsequent
237 improvements of fruit quality traits, suggesting that one or both of these events was linked to a
238 decreased abundance of root-associated *Cellvibrio*.

239 **2.6 Illuminating metagenomic traits in *Cellvibrio* and *Streptomyces***

240 To further investigate the potential functional importance of the 476 rhizosphere-enriched
241 metagenomic contigs mapped as QTLs, we performed a deeper analysis into their functional gene
242 content (Supplemental Table 14-16). An antiSMASH⁴³ analysis identified 30 biosynthetic gene
243 clusters (BGCs) across these contigs. These BGCs largely originated from contigs taxonomically
244 assigned to *Cellvibrio* and *Streptomyces*. They included several gene clusters potentially
245 associated with root colonization, such as two melanin BGCs (c00216, NODE_5919; c00255,
246 NODE_7250) from *Streptomyces* (which have been positively associated with colonization⁴⁴) and
247 a *Cellvibrio* aryl polyene BGC (c00185, NODE_4941), which is thought to protect bacteria against
248 reactive oxygen species generated during immune responses of the host plant⁴⁵. The contigs also
249 contained gene clusters potentially beneficial to the host, such as BGCs encoding iron-scavenging
250 siderophores, which have been associated with disease suppression in tomato⁴⁶; specifically,
251 homologues of coelichelin and desferrioxamine BGCs from streptomycetes were found (c00269,
252 NODE_7969 and c00122, NODE_3362), three IucA/IucC-like putative siderophore synthetase
253 gene clusters (c00106, NODE_2973; c00041, NODE_1131; c00238, NODE_6661), as well as a
254 *Cellvibrio* NRPS-PKS gene cluster (c00001, NODE_101) most likely encoding the production of
255 a siderophore based on the presence of a TonB-dependent siderophore receptor-encoding gene as
256 well as a putative *tauD*-like siderophore amino acid β -hydroxylase-encoding gene⁴⁷. The
257 *Cellvibrio* contigs also contain several genes relevant for carbohydrate catabolism. For example,
258 homologs of *xyl31a* (B2R_23365) and *bgl35a* (B2R_06825-06826) were detected (with 78%, 79%
259 and 65% amino acid identity, respectively), genes that have been shown to be responsible for
260 utilization of the abundant plant cell wall polysaccharide xyloglucan in *Cellvibrio japonicus*⁴⁸. In
261 addition, a possible homologue of the β -glucosidase gene *bgl3D*⁴⁹ (B2R_26663), involved in
262 xyloglucan utilization, was also identified, having high similarity to *bgl3D* from *Cellvibrio*
263 *japonicus* (64% amino acid identity). Also, putative cellulose-hydrolyzing enzymes were detected,
264 such as a homologue (B2R_21082) of the cellobiohydrolase *cel6A* from *Cellvibrio japonicus*⁵⁰
265 encoded in a complex locus of nine carbohydrate-acting enzymes annotated on this contig
266 (NODE_5090) by DBCAN⁵¹ (Supplemental Table 14). Collectively, these results point to a
267 possible role of microbial traits related to iron acquisition and metabolism of plant polysaccharides
268 in tomato rhizosphere microbiome assembly.

269
270 Contigs of the metagenome-assembled genome (MAG) associated with *Streptomyces* ASV5 (the
271 key taxon associated with tomato QTLs described above) contained a multitude of functional genes
272 potentially relevant for host-microbe interactions. Taxonomically, the ASV5 MAG was most
273 closely related to a clade of streptomycetes that includes type strains of species such as *arenae*,
274 *flavovariabilis*, *variegatus*, and *chartreusis*. To understand how tomato might differentially recruit
275 ASV5 streptomycetes, we analyzed the MAG for genes and gene clusters potentially involved in
276 colonization. Intriguingly, we found contigs to be rich in genes associated with plant cell wall
277 degradation. In particular, we identified a family 6 glycosyl hydrolases (B2R_10154) of which the
278 glycosyl hydrolase domain has 84% amino acid identity to that of the SACTE_0237 protein that
279 was recently shown to be essential for the high cellulolytic activity of *Streptomyces* sp. SirexAA-
280 E³¹. Additionally, we detected a homologue (82% amino acid identity) of *Streptomyces reticulii*

281 avicelase, a well-studied cellulase enzyme that degrades cellulose into cellobiose⁵² (B2R_29198).
282 Larger gene clusters associated with degradation of plant cell wall materials were also found.
283 These included an 8-kb gene cluster coding for multiple pectate lyases and pectinesterases
284 (B2R_31553-31558), and an 8-kb gene cluster encoding a family 43 glycosyl hydrolase, a pectate
285 lyase L, a rhamnogalacturonan acetyltransferase RhgT, a GDSL-like lipase/acylhydrolase, a family
286 53 glycosyl hydrolase, and an endoglucanase A (B2R_15915-15920). Together, these findings
287 suggest that ASV5 *Streptomyces* has the capacity to effectively process complex organic materials
288 shed by plant roots during growth. These results are in line with a recent study on plant-associated
289 streptomycetes that indicated that their colonization success appears to be associated with the
290 ability to utilize complex organic material of plant roots⁵³.

291
292 Root exudates also play a key role in recruitment of microbes. Prominent sugar components of
293 tomato root exudates are glucose, but also xylose and fructose⁵⁴. The *Streptomyces* MAG contains
294 *xylA* and *xylB* genes (B2R_19014, B2R_19013) and a putative *xylFGH* import system
295 (B2R_29274, B2R_23438, B2R_23439) facilitating xylose catabolization. Similarly, a *frcBCA*
296 import system was identified in the genome (B2R_17966- B2R_17968) as well as a glucose
297 permease (B2R_32780) with 91,5% amino acid identity to *glcP1* SCO5578 of *Streptomyces*
298 *coelicolor* A3(2)⁵⁵. Other genes putatively involved in root exudate catabolism were also found in
299 the ASV5 MAG, such as sarcosine oxidase (*soxBAG*, B2R_20550- 20551 and B2R_21105), which
300 has been shown to be upregulated in the presence of root exudates of various plants^{56,57}.

301
302 In summary, the *Cellvibrio* and *Streptomyces* contigs encoded a range of functions that likely allow
303 them to profit from tomato root exudates as well as complex organic material shed from growing
304 tomato roots. How these plant traits differ between wild and domesticated tomatoes and if/how
305 these influence differential colonization of roots of wild and domesticated tomato lines by these
306 two bacterial lineages will require detailed comparative metabolomic analyses of the root exudates
307 of both tomato lines as well as isolation of the corresponding *Cellvibrio* and *Streptomyces* ASVs,
308 analysis of their substrate utilization spectrum followed by site-directed mutagenesis of the
309 candidate genes, root colonization assays and *in situ* localization studies.

310 ***2.7 Genomic structure in Cellvibrio and Streptomyces provides insights into adaptations for*** 311 ***differential recruitment.***

312 Bacterial populations often contain significant genomic heterogeneity. This heterogeneity may be
313 associated with differential recruitment through altered nutrient preferences or host colonization
314 mechanisms. The use of metagenomics enabled us to investigate the population structure within
315 each rhizobacterial lineage and identify intraspecific differences. To do so, we first identified a
316 unique set of 697,731 microbiome SNPs in a subset of parental and bulk metagenomes using
317 InStrain²². A set of 15,026 SNPs enriched in either the wild or modern tomato rhizosphere were
318 selected and the abundance of each allele at each SNP was calculated. Using these abundances,
319 QTL mapping was performed using R/qtl2 as described in the methods. A total of 3,357 QTL
320 peaks were identified (LOD > 3.01, P < 0.05), to 1229 independent loci. A total of 1,354 QTL
321 peaks were more abundantly associated to a modern, and 2,001 to a wild plant allele, derived from
322 2,898 unique SNPs, and corresponding to 810 and 1,068 unique rhizobacterial genes respectively
323 (Supplemental Table 17).

324 We investigated the 103 *Streptomyces* SNP QTLs at 94 unique positions within annotated
 325 genes whose mapping coincided with the previously identified *Streptomyces* contig QTLs to
 326 chromosomes 4, 6 and 11 (Supplemental Table 17). Numerous SNPs were associated with a higher
 327 abundance to the modern tomato alleles on chromosome 6 and 11. In particular, alpha-
 328 galactosidase (B2R_16136) and arabinose import (B2R_29105) had the highest LOD and smallest
 329 overlapping confidence intervals with chromosomes 6 and 11 (Figure 7). Indeed, many SNPs in
 330 genes involved in the degradation of xylan⁵⁸, one of the most dominant non-cellulosic
 331 polysaccharides in plant cell-walls⁵⁹, as well as carbohydrate and protein metabolism were linked
 332 to chromosomes 6 and 11, including xyloglucanase Xgh74A (B2R_10589), alpha-xylosidase
 333 (B2R_23763), endo-1,4-beta-xylanase (B2R_20609), extracellular exo-alpha-L-
 334 arabinofuranosidase (B2R_20608), multiple protease HtpX (B2R_19218), cutinase (B2R_19356),
 335 and putative ABC transporter substrate-binding protein YesO (B2R_09821) which has been
 336 implicated in the transport of plant cell wall pectin-derived oligosaccharides⁶⁰. A *Streptomyces*
 337 SNP in acetolactate synthase (B2R_28001) was linked to chromosome 6 where a plant acetolactate
 338 synthase was located. Similarly, multiple SNPs in *Streptomyces* genes involved in putrescine
 339 transportation (B2R_25489) were linked to chromosomes 6 and 11, which contain genes for
 340 spermine synthase, suggesting a possible metabolic cross-feeding from plant to microbe. A
 341 majority of these SNPs were synonymous. However, some were non-synonymous, including the
 342 histidine decarboxylase SNP (B2R_16511) mapping to both chromosomes 6 and 11 (Figure 7).
 343 *Streptomyces* SNPs that were more abundantly associated with the wild tomato allele on
 344 chromosome 4 included an antibiotic resistance gene (daunorubicin/doxorubicin, B2R_28992) and
 345 maltooligosyl trehalose synthase (B2R_07820) among others.

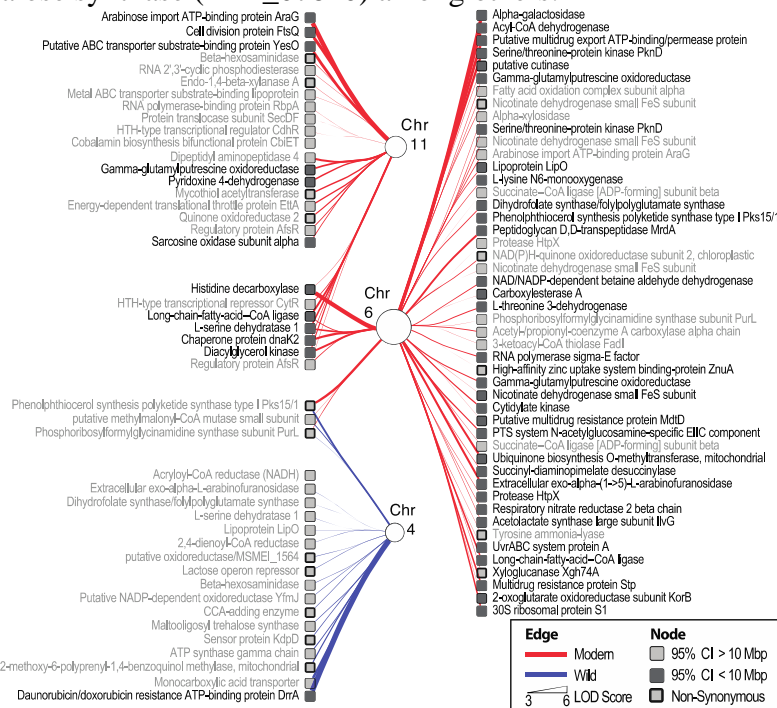


Figure 7: 94 unique SNP within annotated genes on the *Streptomyces* contigs that mapped to QTL positions on tomato chromosomes 4, 6 and 11. The figure depicts various features of both the QTL analysis and the SNPs. In particular, the circular nodes represent the tomato chromosomes 4,6 and 11. The size of these nodes is relative to the number of outgoing edges, which represent individual QTLs. The width of edges is relative to the LOD score and are color coded depending on whether a QTL is linked to increased abundance in the modern or wild allele. The *Streptomyces* SNPs, which were the microbial molecular features mapped as QTLs, are represented by square nodes with annotations alongside. Those SNPs with confidence intervals <10 Mbp are shaded in dark. Non-synonymous SNPs have a thick border edge.

346

347 Similarly, we investigated the 324 *Cellvibrio* SNP QTLs within annotated genes whose
348 mapping coincided with the previously identified *Cellvibrio* contig QTLs to chr. 1 and 9. Again,
349 numerous SNV QTLs were identified in genes were related to sugar catabolism, including a gene
350 encoding an extracellular exo-alpha-(1->5)-L-arabinofuranosidase (B2R_16093), fructose import
351 FruK (B2R_22268), a cellulase/esterase-encoding *celE* homologue (B2R_11067), and genes
352 involved in malate (B2R_18213), mannonate (B2R_14081), xyloglucan (B2R_10668) and
353 xylulose (B2R_22179) metabolism. Furthermore, many additional SNP QTL were identified in
354 genes related to vitamin and cofactor metabolism as well as sulfur and iron metabolism. In
355 particular, these included genes for a phosphoadenosine phosphosulfate reductase (B2R_15720),
356 vitamin B12 transporter BtuB (10 different genes, see Supplemental Table 17), a siroheme
357 synthase (B2R_24033), a pyridoxal phosphate homeostasis protein (B2R_17481), a heme
358 chaperone HemW (B2R_12751), a hemin transport system permease protein HmuU (B2R_09175),
359 a Fe(2+) transporter FeoB (B2R_19968), a biotin synthase (B2R_30007), a catecholate
360 siderophore receptor Fiu (B2R_17486), and a Fe(3+) dicitrate transport ATP-binding protein Fec
361 (B2R_09176) (Supplemental Table 17). Taken together, this analysis suggests that a shotgun
362 metagenomic approach integrated with quantitative plant genetics can be instrumental in a high-
363 throughput manner to discover the reciprocal genetic links between plant and microbial
364 metabolisms, such as those identified here for polysaccharides, trehalose, iron, vitamin, amino
365 acid, and polyamine metabolism.

366 3. Discussion

367 Breeding for microbiome-assisted crops is a complex phenomenon encompassing ecological,
368 evolutionary, and cultural processes. What constitutes a desirable trait for selection is context-
369 dependent and differs between societies, crops and locations⁶¹. As society grapples with modern
370 challenges such as a rapidly changing environment, water scarcity and land degradation, it is
371 becoming increasingly clear that a new era of trait selection is needed with increased focus on
372 sustainability and microbiome interactions⁶²⁻⁶⁵. In this regard, it is also time to reckon with the
373 consequences of historic yield-centric trait selection and accompanying genomic sweeps³¹,
374 especially with regards to plant-microbe interactions. Current approaches to investigating the
375 genomic architecture determining microbiome assembly rely primarily on mutational studies in
376 known genes and pathways. More recently, studies leveraging the natural variation within plant
377 populations have been used to conduct GWA and QTL of the leaf^{66,20} and rhizosphere¹⁸. To date,
378 the microbiome has been primarily characterized through amplicon sequencing, thereby providing
379 limited functional resolution of microbiome structure. Increasing the resolution of phenotyping of
380 quantitative traits has been shown to improve the precision and detection of QTLs⁶⁷. Thus,
381 integrating microbial genomics into microbiome QTL analysis plays dual purpose; increasing the
382 ecological resolution with which microbial traits may be mapped, and second, affording the
383 identification of the reciprocal microbial adaptations that drive plant-microbe interactions. In this
384 investigation, we addressed these challenges by integrating amplicon and shotgun metagenome
385 sequencing to identify microbiome QTLs.

386 One major difference between the amplicon and contig QTL analysis is the number of
387 lineages for which QTLs were identified. In this regard, amplicon-based sequencing provided a
388 broader picture and was able to capture QTLs of both abundant and relatively rare rhizobacterial
389 lineages. In contrast, the majority of contig QTLs mapped to the most predominant lineages, yet
390 failed to identify QTLs for more rare lineages. Nevertheless, besides the fact that the shotgun-

391 based approach provided functional insights into the associated bacterial taxa, the size of the 95%
392 confidence interval of the QTL region was significantly smaller using contig QTLs, with a median
393 size of just 6.47 Mbp compared to 58.56 Mbp for the amplicon-based QTL regions. Furthermore,
394 for *Streptomyces*, the number of unique QTLs identified was greater in the contig-based approach.
395 Thus, we identified a trade-off between amplicon and shotgun-based technologies, whereby
396 amplicon sequencing provides a deeper view into broad community structure, whereas shotgun-
397 based approaches provided a more nuanced picture. In particular, the smaller regions identified by
398 our contig-based metagenome mapping provided considerably more functional insights as it
399 enabled us to analyze the genomic content contained in the regions linked to *Cellvibrio* and
400 *Streptomyces*.

401 The increased QTL mapping resolution provided by shotgun-based phenotyping of the
402 microbiome combined with SNP analysis provided a novel approach to leverage both the host
403 diversity of the RIL and the natural microbiome population diversity to disentangle the reciprocal
404 genomic adaptations between plants and natural microbiomes. For example, understanding the
405 driving forces driving the abundances of rhizospheric *Streptomyces* is of increasing interest and
406 has been linked to both iron⁶⁸ and water limitations⁵³. Here, we pinpointed the genetic basis for
407 these interactions among the short list of highly expressed root-specific tomato genes linked
408 positively to *Streptomyces* abundance including both aquaporin and FIT. More specifically, the
409 aquaporin (SITIP2.3) has the highest fold change in the roots of all tonoplast intrinsic proteins in
410 the tomato genome^{32,33}, while the FIT gene has been shown to largely control iron homeostasis in
411 tomato^{34,35}.

412 In addition to these high priority genes, many other key genes were identified in these
413 regions. Those previously shown to contribute to microbiome assembly included 1-
414 aminocyclopropane-1-carboxylate oxidase, which plays a central role in plant regulation of various
415 processes including bacterial colonization and root elongation⁶⁹ and alpha-humulene/(-)-(E)-beta-
416 caryophyllene synthase, a terpene known to modify microbiome structure³⁹. In addition, numerous
417 genes related to growth, development and cell wall loosening⁷⁰ known to be involved in microbial
418 colonization⁷¹ and aluminum-activated malate transporter, which has been linked to microbiome
419 mediated abiotic stress tolerance⁴⁰.

420 The historic impact of domestication on genomic regions linked to microbiome assembly
421 is also apparent (Figure 6, Supplemental Table 14, and Supplemental Figure 1). However, the
422 processes and consequences of these sweeps, and possible subsequent recombination events on
423 microbiome assembly remain unclear. In particular, the discontinuity of sweeps in microbiome
424 QTL regions suggests that evolutionary pressure for recombination of key (microbiome
425 associated) traits, such as iron homeostasis and water transport, may have acted against selective
426 sweeps. The results obtained here provide the means to illuminate such complex eco-evolutionary
427 questions, forming the basis of integrating the microbiome into the classic genotype by
428 environment model of host phenotype¹⁰.

429 From the microbial perspective, the increased resolution in QTL analysis afforded by our
430 shotgun-based approach also provided a window into the host-specific bacterial adaptations to wild
431 and modern alleles. In particular, the SNP QTL analysis demonstrated that genes related to the
432 degradation of various plant-associated polysaccharides in *Streptomyces* were highly associated
433 with various modern tomato alleles. It should be further noted, that many other functions were
434 identified in both plant and microbe, such as trehalose metabolism, polyamine metabolism and
435 acetolactate synthase, suggesting either a direct link through cross-feeding⁷² or signaling⁷³, or
436 perhaps shared ecological pressures. While the microbial adaptations related to polysaccharides⁷⁴,

437 vitamins⁷⁵ and iron metabolism^{46,68} are well documented in relation to plant colonization, here we
438 demonstrate for the first time that the reciprocal adaptations that drive plant-microbe interactions
439 can be investigated simultaneously to uncover their genetic architecture in both host and
440 microbiome.

441 **4. Methods**

442 **4.1 Greenhouse and Lab work**

443 *4.1.1 Recombinant inbred line population*

444 100 lines of an F8 recombinant inbred line (RIL) population derived from the parental lines
445 *Solanum lycopersicum* cv. MoneyMaker (Modern) and *Solanum pimpinellifolium* L. accession
446 CGN14498 (Wild) were used²³. A high density map produced from this population was used to
447 map QTLs²⁶.

448 *4.1.2 Growth conditions for RIL*

449 The soil was collected in June 2017 from a tomato greenhouse in South-Holland, The Netherlands
450 (51°57'47"N 4°12'16"E). The soil was sieved, air dried, and stored at room temperature until use
451 in 2019. Before the beginning of the experiment, soil moisture was adjusted to 20% water by
452 volume using deionized water. All soil was homogenized by thorough mixing and allowed to sit,
453 covered by a breathable cloth, in the greenhouse for one week prior to potting. The soil was then
454 homogenized once again and then potted. Each pot was weighed to ensure all pots were 175g±0.5
455 (wet weight). Duplicate pots for each accession were planted, as well as 6 replicates of each
456 modern and wild parental accession, and 8 bulk soil pots that were left unseeded. Each replicate
457 was prepared simultaneously. Planting was done separately representing biological replicates.

458
459 In each pot, 3 seeds were planted in a triangular pattern to ensure the germination success for all
460 pots. The first seedling to emerge in each pot was retained and others were removed after
461 germination. All pots were randomly distributed in trays containing approximately 10 plants.
462 Throughout growth, careful attention was given to randomize the distribution of plants. First, tray
463 location and orientation with relation to each other were randomized on a nearly daily basis. In
464 addition, the distribution of plants within trays was randomized three times during growth. All pots
465 were kept covered until germination, which was scored daily. After germination, plants were
466 visually monitored and watered at the same rates. To minimize the impact of environmental
467 differences between pots on microbiome composition, the watering regime for all plants was
468 standardized and leaks from the bottom of the pot and overflows were completely prevented. To
469 achieve this, a minimal volume (2.5 mL to 5.0 mL) of water was used at each watering. This
470 strategy was successful as washout was never observed. Moisture content was measured by
471 weighing the pots at the middle and end of the experiment to ensure all pots had similar moisture
472 contents.

473 *4.1.3 Harvesting and processing of plant materials*

474 All plants had between 5-7 true leaves at harvest (Supplemental Table 1). Plants were gently
475 removed from the pot and roots and were vigorously shaken. Soil that remained attached to the

476 roots after this stage was considered the rhizosphere. The remaining bulk soil and rhizosphere
477 (plus roots) fractions were weighed. The root and attached rhizosphere fraction were treated with
478 4 mL of lifeguard, vortexed and sonicated. Roots were then removed. The remaining rhizosphere
479 sample was then stored in LifeGuard Soil Preservation Solution (Qiagen) at -20 °C until DNA
480 extraction.

481 The dry weight of shoots was measured after drying at 60°C. The dry weight of the bulk
482 soil was measured after storing at room temperature in open paper bags for 1 month. The DNA
483 was extracted using the DNeasy PowerSoil extraction kit (Qiagen). The protocol was optimized
484 for the soil in the following manner: each sample was vortexed and then a volume of approximately
485 1.5 mL was transferred into 2 mL tubes. This subsample was centrifuged at 10,000g for 30 seconds
486 such that a pellet was formed. The supernatant was removed, and a new subsample was transferred,
487 and centrifuged until the total volume of the original sample, without sand, had been transferred
488 to the 2 mL tubes. The resulting pellet was recalcitrant to disruption through bead beating, and
489 therefore was physically disrupted by a pipette tip before proceeding with DNA extraction
490 protocol. In test samples, DNA extractions from the sand fraction yielding no, or marginal levels
491 of DNA.

492 **4.2 Amplicon and shotgun metagenomics analysis**

493 *4.2.1 rRNA amplicon sequence processing*

494 All DNA was sent to BaseClear (Leiden, The Netherlands) for both 16S and 18S 300 bp paired-
495 end amplicon sequencing (MiSeq platform). MiSeq primers targeted the V3-V4 region of Bacteria:
496 341F CCTACGGGNGGCWGCAG, 805R GACTACHVGGGTATCTAATCC. In total,
497 20,542,135 16S read pairs over 225 samples were generated. The raw reads were processed using
498 the DADA2 workflow (v1.14.1) to produce amplicon sequence variants (ASV) and to assign
499 taxonomy⁷⁶. ASVs tagged as non-bacterial, chloroplast, or mitochondria were removed. Next,
500 ASV counts were normalized using the cumulative sum scaling (CSS) and filtered based on the
501 effective sample size using the metagenomeSeq package (v1.28.2)⁷⁷. Differential abundances
502 between rhizosphere and bulk soil were determined using the eBayes function from the limma
503 package. Enriched rhizosphere ASVs with a greater than log(2) fold change in abundance were
504 analyzed based on their presence and absence, standard deviation and mean values. Using these
505 statistics, stochastic ASVs (<50% of samples) were removed from further analysis.

506 *4.2.2 Metagenomics analysis*

507 For the one set of replicates for each accession, paired-end sequence read libraries were generated
508 in the length of 150 bp per read on NovaSeq paired-end platform by BaseClear B.V.
509 Demultiplexing was performed before the following analysis. It is computationally expensive to
510 assemble the 114 read libraries all at once. Therefore, a strategy of (merging) partial assemblies
511 was undertaken. Two assemblers were used to create the assembled contigs, namely SPAdes
512 (version 3.13.2)⁷⁸ and MEGAHIT (version 1.2.9)⁷⁹. Assembly quality was assessed by running
513 MultiQC (version 1.8)⁸⁰ with Quast Module⁸¹(Supplemental Figure 2). First, 6 modern parents, 5
514 wild parents and 1 bulk soil sample were co-assembled via SPAdes with the metagenomic mode
515 and parameter of -k 21,33,55,99, generating the first assembly (A1). Subsequently, a second
516 assembly (A2) was done using the unmapped reads from the remaining metagenomes using
517 MEGAHIT with the parameter of --k-list 27,33,55,77,99. The third assembly (A3) was performed

518 similarly as A2, however included the unmapped reads, ambiguously mapped reads, and mapped
519 reads with a low mapping quality score (MapQ < 20) (Supplemental Table 18). Read mapping was
520 done with BWA-MEM with default settings⁸² and SAMtools was used to convert resulting SAM
521 files into sorted and indexed BAM files (version 1.10). Extraction of these reads were conducted
522 by samtools bam2fq. Redundancy between assemblies was evaluated by alignment to A1 via
523 nucmer package of MUMmer with --maxmatch option (version:4.0.0)⁸³.

524
525 Firstly, 111.5 Gbp of reads from the parental samples were assembled, labelled as A1 and
526 yielded a total assembly length of 8.6 Gbp with the largest contig of 933.0 kilobase pairs (Kbp).
527 After aligning the reads from RIL samples to A1, unmapped reads, ambiguously mapped reads,
528 and mapped reads with a low mapping quality score (MapQ < 20) were retrieved and assembled,
529 yielding the second and third assembly (A2 and A3). Specifically, A2 stemmed from solely the
530 unmapped reads while A3 included the ambiguously mapped reads and mapped reads with MapQ
531 < 20 in addition to the unmapped reads. A2 and A3 produced a total assembly length of 9.6 Gbp
532 and 14.0 Gbp, with the largest contig of 56.2 Kbp and 86.3 Kbp respectively. There were 1.2, 2.0
533 and 2.8 million contigs with the length over 1 Kb for A1, A2 and A3 respectively. In particular,
534 912 contigs in A1 were greater or equal to 50 Kbp whereas 1 or 2 such large contigs were
535 successfully assembled in A2 or A3. The detailed assembly statistics is given in Supplemental
536 Table 18 and the numbers of contigs with different ranges of length for each assembly are
537 presented in Supplemental Figure 2.

538
539 The sequence similarities of the contigs in each assembly (≥ 1 Kbp) were compared using
540 the nucmer package in MUMer. No contigs in A2 were reported to share an overlapped region
541 with A1, therefore contigs in A1 and A2 could be merged directly. When A3 was aligned to A1,
542 1.1% of the total length (≥ 1 Kbp) of A3 was reported to be overlapped with A1, however, only 18
543 contigs from A3 were 100% identical to regions in larger contigs in A1. The sensitivity of filtering
544 the overlapping contigs was evaluated by a benchmarking test using a random RIL sample to
545 calculate the mapping rates (Supplemental Figure 3). 83.4% reads were mapped to A1+A3 at
546 MapQ ≥ 20 without filtering. Excluding the contigs from A3 that were completely and identically
547 covered by A1, the mapping rate was nearly the same as the one without filtering. Nevertheless,
548 the removal of all aligned contigs in A3 resulted in a slight drop of mapping rate to 82.6%. To
549 conclude, the final assembly was determined as A1+A3 with the 18 redundant contigs from A3
550 removed.

551
552 To assess the overall assembly quality and quantify the abundance of contigs among all
553 samples, metagenomic reads were mapped to A1, A1+A2 and A1+A3 (deduplicated) respectively.
554 Afterwards, the mapping rates were calculated for the mapped reads with MapQ > 20 in each
555 sample. As shown in Supplemental Figure 4, approximately 70% reads among rhizosphere
556 samples could be mapped to A1, while the mapping rates were 55% to 65% in the bulk soil
557 samples. With the unmapped reads assembled and added to A1, the mapping rates for A1+A2
558 increased by 10%. The read recruitment was further improved by assembling and adding
559 ambiguously mapped reads and mapped reads with low MapQ in the final assembly (A1+A3). A1
560 as well as de-replicated A3 were merged to acquire the final assembly. All the 'contigs' mentioned
561 below are referring to the contigs in this final assembly.

562 4.2.3 Binning of metagenomic contigs

563 Metabat2 (version 2:2.15)⁸⁴ was used for assigning the contigs into genomic bins. Based on tetra-
564 nucleotide frequency and abundance scores, 588 genomic genomics bins were generated.
565 Afterwards, genomic quality of those genomes was evaluated by CheckM (version: 1.1.1)²⁸ with
566 the command “checkm lineage_wf” (Supplemental Table 9). The 33 genomes displaying the
567 completeness larger than 90% and contamination smaller than 5% were used for further study as
568 quantitative traits.

569 4.2.4 Making phenotype files based on contig depth

570 Read counts for each position on the assembled contigs were acquired using bedtools genomcov
571 (version: 2.29.2)⁸⁵. A custom Python script was applied to calculate the average depth (defined as
572 the number of total mapped reads divided by contig length) and coverage (defined as the number
573 of covered base pairs divided by contig length) of every contig. Furthermore, the average
574 abundance of contigs assigned into a bin was calculated for the high-quality genomic bins detected
575 by CheckM²⁸.

576 4.2.5 Feature selection

577 Average depths of the contigs were first normalized using the cumulative sum scaling (CSS) and
578 filtered based on the effective sample size using metagenomeSeq package (v1.28.2)⁷⁷. Differential
579 abundance analysis was performed by moderated t-tests between groups using the makeContrasts
580 and eBayes commands retrieved from the R package Limma (v.3.22.7)⁸⁶. Obtained P-values were
581 adjusted using the Benjamini–Hochberg correction method. Differences in the abundance of
582 contigs between groups were considered significant when adjusted P-values were lower than 0.01
583 (Supplemental Table 19).

584
585 In either comparison, the contigs that were significantly enriched in the rhizosphere were
586 gathered and regarded as the statistically rhizosphere-enriched contigs after removing the
587 replicated ones. To perform QTL analysis for the abundance of these enriched rhizosphere contigs,
588 only the contigs with biological meanings were kept, i.e. the log (2) fold-change of mean values
589 for the normalized abundances of RIL and bulk samples should be greater than 2, and the contig
590 should be in enough depth with at least the mean value of a group larger than 1. This selection step
591 resulted in 1249 rhizosphere-enriched contigs in the end. The statistics of the filtered normalized
592 abundance were further inspected based on the presence and absence of contigs, standard deviation
593 and mean values of the counts.

594 4.2.6 Taxonomic and functional annotation of the metagenome

595 Taxonomic classifications were assigned to the contigs in the final assembly using Kraken2
596 (version: 2.0.8)²⁹ based on exact k-mer matches. A custom Kraken2 database was built to contain
597 RefSeq complete genomes/proteins of archaea, bacteria, viral, fungi and protozoa. Univec_Core
598 was also included in the custom database (20200308). Using the Kraken2 standard output, a python
599 script based on TaxonKit⁸⁷ was utilized to add full taxonomic names to each contig in the format
600 of tab-delimited table. 76.22% of the contigs > 1kb were classified. Among the contigs > 10kb, up
601 to 99.44% contigs were classified. Prokaryotic microbial genes were predicted by Prodigal
602 (version: 2.6.3)⁸⁸ with metagenomics mode. 10,246,55 genes were predicted from contigs > 1kb
603 (Supplemental_Table_8). Open reading frames (ORFs) on contigs >10kb were annotated by

604 prokka (v1.14.5) and the *Streptomyces* ASV5 bin (MAG.72) was further annotated by DRAM
605 (v1.2.0) integrating UniRef, Pfam, dbCAN and KEGG databases⁸⁹.

606 4.2.7 Single Nucleotide Variant analysis

607 To investigate strain level QTLs, we mapped Single Nucleotide Variants (SNV) identified using
608 inStrain on the 1249 contig enriched genomes. A total of 555,382 and 535,432 SNPs were
609 identified in the modern and wild parental metagenomes respectively. Of these, 162,299 and
610 142,349 SNPs were unique to each dataset respectively, as they either contained only reference
611 alleles or did not exceed the inStrain SNP calling thresholds. For each unique SNP locus, coverage
612 in the other dataset was determined using SAMtools depth after read filtering with settings
613 comparable to inStrain, and was considered identical to the reference allele frequency. Including
614 the unique SNPs, this resulted in a final set of 697,731 SNPs. To select SNPs that showed
615 differential reference allele frequencies between MM and P, first the difference in reference allele
616 frequency (MM – P) was calculated per SNP. From the distribution of all SNPs, the 95%
617 confidence interval (CI) was determined to select the 5% (30,911) most different SNPs
618 (Supplemental Figure 5). SNPs were further selected using a Fisher's exact test based on the allele
619 read count differences between MM and P. P-values were sorted, and a final selection of 15,026
620 differentially abundant SNPs distributed over 1,037 contigs was obtained using a Benjamini-
621 Hochberg false discovery rate (FDR) correction of 0.01. SNV allele read counts were extracted
622 from the RIL dataset using the pysam Python package after filtering with settings comparable to
623 inStrain.

624 4.2.8 Quantitative Trait Locus Analysis

625 The QTL analysis linking selected amplicon, contig, bin, and SNV features with plant loci was
626 performed using the R package R/qlt2²⁵. Pseudomarkers were added to the genetic map to increase
627 resolution, with a step distance of 1 Mbp between the markers and pseudomarkers. Plant genome
628 probabilities were calculated using the genetic map with pseudomarkers, plant loci cross data and
629 error probability of 1E-4. Plant locus kinship matrix was calculated as proportion of shared alleles
630 using conditional allele probabilities of all plant chromosomes, which were calculated from the
631 plant genome probabilities. A genome scan using a single-QTL model using a linear mixed model
632 was performed on the SNP allele read counts as phenotypes, plant genotype probabilities as input
633 variables and as covariates the number of leaves, harvest day, rhizosphere soil weight (g), soil
634 starting weight (g) and plant dry weight (g). The log₁₀ likelihood (LOD) score was determined for
635 each plant locus SNP allele combination. A permutation test was performed with 1000
636 permutations to assess the distribution of the LOD scores. The 95% quantile was used as threshold
637 for the selection of LOD peaks, as well as a P = 0.95 Bayes credible interval probability.

638 4.3 Independent validation of QTLs through bulk segregant analysis

639 To validate the QTLs, 33 *Solanum lycopersicum* cv. Moneymaker (Modern), 30 *Solanum*
640 *pimpinellifolium* L. accession CGN14498, and 77 RIL accessions (with replicates of 4 each) were
641 grown and their microbiomes characterized through 16S sequencing. Parental lines and RIL
642 accessions were germinated in pots filled with 300 g agricultural soil. For each accession, were
643 planted with six plants per replicate pot. The plants were arranged randomly in the growth chamber

644 (25°C, 16h daylight) and watered every day. Bulk soil samples without plants were used as controls
645 (N = 31).

646
647 Rhizospheric soil was collected according to standard methods⁹⁰. In order to synchronize the
648 developmental stage, the plants were harvested after 21 days, or when the 3rd trifoliolate leaf was
649 reached. The soil loosely attached to the roots was removed and the entire root system was
650 transferred to a 15 mL tube containing 5 mL LifeGuard Soil Preservation Solution (MoBio
651 Laboratories). The tubes were vigorously vortexed and sonicated. Subsequently, the roots were
652 removed and at least 1 g (wet weight) of rhizospheric soil was recovered per sample for RNA
653 extraction. For the bulk soil samples, approximately 1 g of soil was collected and mixed with 5
654 mL of LifeGuard solution.

655
656 To extract rhizospheric DNA, PowerSoil Total DNA/RNA Isolation Kit (MoBio Laboratories,
657 Inc., USA) was used in accordance with manufacturer's instruction. Rhizospheric DNA was
658 obtained using RNA PowerSoil DNA Elution Accessory Kit (MoBio Laboratories, Inc. USA). The
659 quantity and quality of the obtained DNA was checked by ND1000 spectrophotometer (NanoDrop
660 Technologies, Wilmington, DE, USA) and Qubit 2.0 fluorometer (ThermoFisher Scientific, USA).
661 DNA samples were stored at -20°C until further use.

662
663 The extracted samples were used for amplification and sequencing of the 16S rRNA, targeting the
664 variable V3-V4 (Forward Primer= 5'CCTACGGGNGGCWGCAG-3' Reverse Primer= 5'-
665 GACTACHVGGGTATACTAATCC-3') resulting in amplicons of approximately ~460 bp. Dual
666 indices and Illumina sequencing adapters using the Nextera XT Index Kit were attached to the V3-
667 V4 amplicons. Subsequently, library quantification, normalization and pooling were performed
668 and MiSeq v3 reagent kits were used to finally load the samples for MiSeq sequencing. For more
669 info please refer to the guidelines of Illumina MiSeq System. The RDP extension to PANDASeq⁹¹,
670 named Assembler⁹², was used to merge paired-end reads with a minimum overlap of 10 bp and at
671 least a Phred score of 25. Primer sequences were removed from the per sample FASTQ files using
672 Flexbar version 2.5⁹³.

673 **5. Data availability**

674 The sequencing data generated in this study are available under ID BioProject ID PRJNA787039
675 (16S amplicons) and PRJNA789467 (shotgun metagenomics). Bacterial ASV reference
676 sequences, and metagenome assembled genomes are available at <https://osf.io/f45ek/>.

677

678 **6. Code availability**

679 The code used in the analysis can be found at <https://osf.io/f45ek/>.

680 **7. Author contributions and acknowledgements**

681 The study was conceived and designed by BOO, VJC, WLi, MHM and JMR. The greenhouse
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