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Microbial footprints of tomato domestication

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Microbial footprints of tomato domestication

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NIOO
NEDERLANDS INSTITUUT VOOR ECOLOGIE (NIOO-KNAW)
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Microbial footprints of tomato domestication

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“I will start with small things”

— Vincent van Gogh, letter to Theo van Gogh. The Hague, August 5, 1882

Table of Contents

Chapter 1	General Introduction and Thesis Outline	9
Chapter 2	Unveiling diversity and adaptations of the wild tomato microbiome in their center of origin in the Ecuadorian Andes	39
Chapter 3	Changes in taxonomic diversity and functional traits of the tomato root microbiome along a domestication trajectory	83
Chapter 4	Microbiome-mediated tolerance of wild tomato to the invasive insect *Prodiplosis longifila*	131
Chapter 5	Disentangling the genetic basis of rhizosphere microbiome assembly in tomato	165
Chapter 6	General Discussion	201
References		213
Summaries		253
	Summary	255
	Samenvatting	257
	Resumen	259
Curriculum vitae		263
List of publications		267
Acknowledgements		271

Chapter 1

General Introduction and Thesis Outline

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Abstract

Plant domestication and breeding not only resulted in multiple phenotypic changes but also impacted the agricultural ecosystems in which our current crops are cultivated. Most crops to date rely on the extensive use of fertilizers and pesticides to support crop growth and health. To minimize the environmental impact of these management practices, the plant microbiome has gained renewed attention as a large yet untapped resource of microorganisms with beneficial effects on plant growth and health. In the past decade, it has become evident that the microbiome of plants plays a key role in nutrient acquisition, plant development, and tolerance to diverse abiotic and biotic stresses. Here, we review past and present knowledge of the microbiome of tomato as a model for unraveling the functional potential of plant microbiomes, the impact of domestication, and the underlying genetics of microbiome assembly and activity. We also provide perspectives on how this knowledge can be adopted to enhance crop productivity and strengthen the sustainability of agricultural management practices.

***Keywords*:** domestication, microbe-assisted breeding, microbial functionality, microbiome composition, production, tomato

General Introduction

Tomato is among the most valuable vegetables worldwide, with an estimated production of approximately 190 million tons, covering over five million hectares (FAO, 2020, 2021; Klee & Resende, 2020). Asia is the continent with the highest tomato production, accounting for 62.6% of the total tomato production, followed by the Americas (13.1%), Europe (12.2%), Africa (11.9%), and Oceania (0.2%) (FAO, 2020). Tomato is a source of carotenoids, vitamins, glycoalkaloids, and other metabolites with antioxidant, anti-inflammatory and anti-mutagenic activities (Chaudhary et al., 2018). Tomatoes are also a popular ingredient in several culinary traditions, which has triggered extensive breeding programs to develop an impressive variety of tomato cultivars with a multitude of flavors, colors, and shapes (Domínguez et al., 2020).

Domestication and subsequent breeding not only resulted in multiple phenotypic changes but also impacted the natural and agricultural ecosystems in which tomatoes are cultivated. These include the extensive use of fertilizers and pesticides to support tomato growth and health. For example, to control pests, diseases, and weeds in open-field tomatoes, chemicals have been extensively used due to their low cost and high control efficiency (Desneux et al., 2022). In addition, distinct pesticide classes are used in different countries, such as organophosphates, organochlorines, carbamates, triazines, pyrethroids, dithiocarbamates, benzimidazole, chloronitriles, liquid copper fungicides, and herbicides (Köhler & Triebskorn, 2013). Hence, alternative strategies are needed for sustainable pest and disease control. In addition, strategies for improving crop resilience to stresses posed by climate change, such as extreme temperatures, drought, and salinity, as well as soil degradation, will be essential. This has led to a renewed attention to the plant microbiome as a large yet untapped resource of microorganisms with beneficial effects on plant growth and health. For example, inoculating the tomato rhizosphere with microbial consortia can offer various beneficial functions to the plant, such as enhanced nutrient uptake efficiency and protection against pathogens (Gu et al., 2022; Hu et al., 2016; Schmidt et al., 2019). This scenario highlights the functional potential of the tomato microbiome as an integral component of a novel sustainable strategy of crop production. In this context, research programs have been initiated to investigate the impact of domestication on the tomato microbiome composition and functions (Alsharif et al., 2020; Barajas et al., 2020; Carrillo et al., 2019; Cordovez et al., 2021; Lee Díaz et al., 2022; Martínez-Romero et al., 2020; Oyserman et al., 2022). Central questions in these research programs include the following: (i) What is the impact of domestication on the taxonomic and functional diversity of the seed, root, and shoot microbiomes? (ii) What is the genetic basis of plant microbiome assembly and functioning? (iii) Which plant and microbiome functions were lost during domestication and can be reinstated to control (a)biotic stresses such as drought, salinity, nutrient

deficiency, diseases, and pests? Enhancing beneficial plant–microbe interactions can provide a substantial contribution to food security in the face of global climate change, land-use intensification, and increased (a)biotic stresses in agricultural production systems (Cordovez et al., 2019; Finkel et al., 2017; Porter et al., 2020; Porter & Sachs, 2020).

Tomato is an ideal plant species for studying the impact of domestication on microbiome assembly and functioning. Several compelling factors make tomato a suitable choice for such investigations. First, tomato plants have a relatively short life cycle, facilitating timely research and observation. Additionally, tomato possesses a diverse range of genetic resources, including multiple varieties and wild relatives, which offer ample opportunities for comparative analysis. Moreover, the availability of tools for genetic modification, such as CRISPR-Cas genome editing, further enhances the experimental capabilities for studying tomato–microbiome interactions. The knowledge of tomato chemistry, as well as access to native soils in the centers of origin, provides a valuable context for investigating the impact of domestication on microbiome assembly and functions. Lastly, the economic importance of tomato in both food production and research makes it a highly relevant model plant.

Here, we provide a comprehensive review of the tomato plant, including its taxonomy and origin, as well as the current knowledge of the taxonomic and functional diversity of its microbiome. We focus on bacterial communities, but whenever possible, we also highlight research on fungal communities associated with tomato. We specifically explore how the microbiome is modulated by the tomato genotype and environmental factors and how domestication affected microbiome assembly. Our aim is to provide an integrated perspective of the diversity of the tomato microbiome and its potential contribution to sustainable tomato production.

Taxonomy and geographic distribution of tomato

Botanical description

Most tomatoes are considered annual herbs, as they cannot withstand prolonged dry or cold seasons (Peralta & Spooner, 2000; Waheed et al., 2019). However, biennial and perennial members can be found in areas with favorable environmental conditions, allowing for a woody secondary growth with new shoots and adventitious roots with a lignified base (Peralta & Spooner, 2000). Tomato has a woody, hairy, and coarse stem, which first grows vertically and later becomes decumbent because of the weight of the branches. Generally, wild tomato plants show an indeterminate growth of several meters, whereas domesticated tomatoes have a determinate growth (Peralta & Spooner,

2000). The leaves have an imparipinnate arrangement comprising two to six opposite leaflet pairs and a terminal leaflet, all covered with glandular trichomes (Peralta & Spooner, 2000). Tomato flowers are arranged in a scorpioid cyme inflorescence with 6 to 12 flowers (Peralta et al., 2008). Fruits are fleshy, globose or ovoid, and generally bilocular in wild species or multilocular berries in cultivated varieties. Fruits typically contain 50 to 200 seeds per locule, which are covered with a jelly substance to avoid immediate germination (Peralta et al., 2008). The typical fruit color is red due to the accumulation of the pigment lycopene during fruit ripening (Howe & Smallwood, 1982; Zhong et al., 2013), whereas some varieties and wild species are yellow, orange, green, or purple due to the presence of carotenoids or anthocyanin (Peralta & Spooner, 2000; Waheed et al., 2019).

Taxonomy

Tomatoes belong to the section Lycopersicon of the genus *Solanum* of the family Solanaceae, which includes 13 species, all native to western South America (Knapp & Peralta, 2016; Waheed et al., 2019). Although other tomato relatives are found in the sections Lycopersicoides and Juglandifolia (Peralta et al., 2008), *Solanum pimpinellifolium* is considered the closest wild relative of the domesticated tomato *S. lycopersicum* (The Tomato Genome Consortium, 2012) (Figure 1). In some botanical books, tomatoes are referred to as nightshades. The term “nightshade” is derived from the fact that some members of the Solanaceae family thrive in shady environments, whereas others bloom at night. Additionally, the presence of psychoactive alkaloids in Solanaceae plants may have contributed to the name “nightshade” (Lee, 2006).

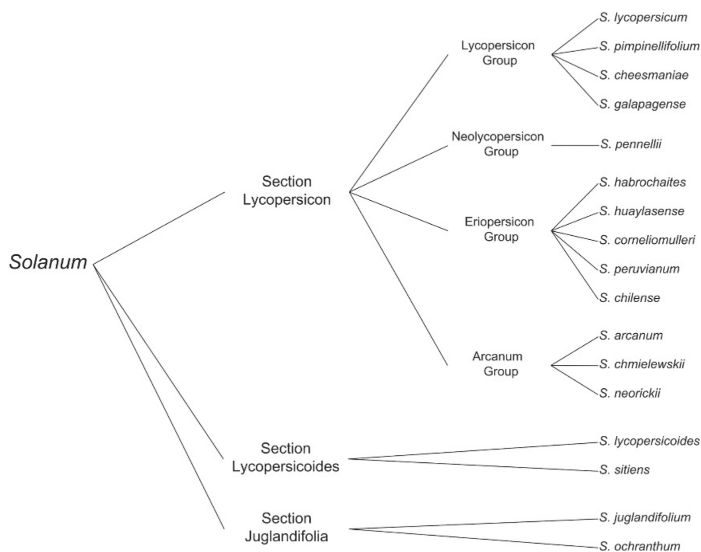

Figure 1. Taxonomy of tomato and its wild relatives (based on Peralta et al., 2008).

Native habitat

The *Solanum* species in sections Lycopersicon and Lycopersicoides are all wild species and native to western South America, spread from Ecuador (including Galápagos) to northern Chile (Knapp & Peralta, 2016). They are found in a wide range of habitats and conditions but prefer warm and dry climates in the region of the western Andes, including coastal areas, mountain slopes, and valleys (Kimura & Sinha, 2008; Peralta et al., 2008; Waheed et al., 2019). Some wild tomato populations, such as *S. chilense*, *S. habrochaites*, *S. pennellii*, *S. peruvianum*, and *S. arcanum*, occur in semi-highlands (5-30°S latitude) called "lomas" (i.e., hills) situated along the Pacific coast of Peru and Northern Chile. These lomas are areas of "fog-watered" vegetation in the Andes. The presence of this seasonal fog from September to December creates a microclimate with enough moisture to allow for flowering. During El Niño, this region experiences high rainfall, resulting in better growth, mass flowering, and high seed dispersal due to water runoff (Peralta et al., 2008). Nevertheless, *S. pimpinellifolium* populates coastal habitats or river valleys in the Low Andes region and has a wider distribution compared with the other wild species found in the lomas region (Peralta & Spooner, 2000, 2000; Ramón, 2008). Two species exclusive to the Galápagos Islands are *S. cheesmaniae*, found at various elevations, and *S. galapagense*, found in lower elevation habitats, typically on lava flows and affected by the Pacific Ocean spray (Peralta et al., 2008). This robustness to harsh environmental conditions, especially low relative humidity, is typical for the wild tomato species of the Lycopersicon group. Domesticated tomato populations can also survive in semidry conditions, but their ability to persist in the wild for many generations is limited (Peralta et al., 2008).

Center of domestication and diversification

Early domestication

Speciation between *S. pimpinellifolium* and *S. lycopersicum* var. *cerasiforme* occurred in South America 78,000 years ago without human intervention (Klee & Resende, 2020; Razifard et al., 2020). The domestication of tomato is thought to have started in Ecuador from *S. lycopersicum* var. *cerasiforme* by the agricultural culture Mayo-Chinchipe around 3,000 to 2,000 BCE (Blanca & Cañizares, 2021). Interestingly, the same culture was responsible for the domestication of cacao in the Andean region called the Low Andes (Zarrillo et al., 2018). This region in the south of Ecuador is considered a biodiversity hotspot, as it is characterized by a depression in the height of the Andes, with lower mountain ranges connecting the Pacific Coast to the Amazon rainforest, as well as the Northern Andes to the Central Andes (Ramón, 2008). The unique topography of this region is believed to have facilitated interactions between several cultures in both East-West and North-South directions and at various altitudes (Valdez, 2008),

which eventually marked the tomato's "journey" from South America to Mesoamerica (Klee & Resende, 2020; Figure 2).

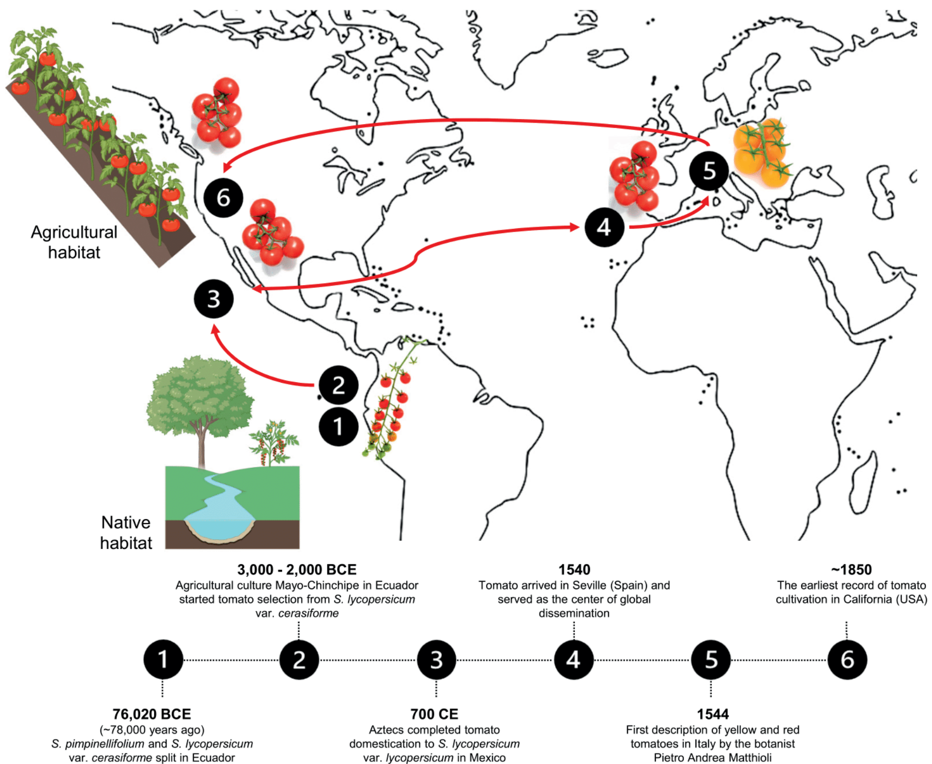

Figure 2. Historical view of the worldwide journey of tomato. Habitat figures were designed with BioRender.com.

S. lycopersicum var. *cerasiforme* underwent selection by humans for fruit traits (Blanca & Cañizares, 2021; Klee & Resende, 2020) and was grown on the high rainfall slopes of the Eastern Andes (Rick, 1983). When the ancestors of wild and early domesticated tomato arrived in Central America, they probably lost or reduced some of their original traits, including fruit size, sugar content, and floral display. This transformation likely occurred in Mexico with the fully domesticated tomato *S. lycopersicum* var. *lycopersicum* dating back to approximately 700 CE (Bai & Lindhout, 2007; Blanca et al., 2015; Jenkins, 1948; Keoke & Porterfield, 2009; Klee & Resende, 2020; Razifard et al., 2020). Interestingly, it was only in Mexico that this new crop received the name "tomato." This term was derived from the Nahuatl (Aztec language) word *xītomatl* (*xī*: peeled/skinned; *toma*: round fat thing; and *atl*: water, aqueous) to differentiate the fruit of *S. lycopersicum* from the husk tomatoes *Physalis philadelphica* and *P. ixocarpa* ("tomatl"

in Nahuatl language), which were also cultivated by Aztec families in small orchards known as "milpas" (Bowles, 2018; Díez & Nuez, 2008; Gran Diccionario Náhuatl, 2012; Jenkins, 1948; Long, 2022; Mediavilla, 2020; Wood, 2000).

Tomato distribution worldwide

Following contact with Indigenous communities in Mesoamerica, the Spanish conquerors were the first to bring the tomato to Europe in the 16th century (Díez & Nuez, 2008). These tomatoes first arrived in Seville (Spain), from where tomato quickly distributed throughout the country. The Spanish and Italians were the first to accept the use of tomato for consumption in a similar manner as Aztec culture, for instance, fried with oil, salt, and pepper (Caramante et al., 2021; Long, 2022; Peralta et al., 2008), unlike the French and North Europeans, who used tomatoes as ornamental plants, as they believed tomatoes to be toxic due to their similarity to poisonous European members of the Solanaceae family (Díez & Nuez, 2008; OECD, 2017).

In 1544, the botanist Pietro Andrea Matthioli first described the tomatoes that arrived in Italy as a flattened and segmented "mele rose" (variety of apple) that were green at first and ripened to a golden color. Later in 1554, Matthioli mentioned the red coloration in tomato and called tomatoes "pomi d'oro" ("golden apples"), which later became "pomodoro", the modern Italian word for tomato (McCue, 1952; Peralta et al., 2008). This revealed that the first tomatoes that arrived on the European continent had undergone a complete domestication process (big size) and that yellow varieties were initially more popular than the red ones (Peralta et al., 2008). Then, tomatoes were subjected to further domestication and bred into different varieties for human consumption (larger fruits, better flavor, higher yields) and compatible with new agricultural systems. Thus, Spain and Italy are acknowledged as secondary centers for tomato diversification and are resources of tomato landraces (Caramante et al., 2021; Cebolla-Cornejo et al., 2013; Lázaro, 2018).

Eventually, tomato was spread globally via commercial trading routes (Caramante et al., 2021; Díez & Nuez, 2008). For instance, in the 18th century, tomato plants were brought back to the Americas at commercial harbors on the eastern North American coast by European colonists (Díez & Nuez, 2008), where tomato was also registered first as an ornamental plant and only later, by the end of the 19th and beginning of the 20th century, was accepted as edible (Rick, 1978). The earliest record of tomato cultivation in the United States is in 1850 in San Diego, but only in 1867 did the United States start the production of commercial varieties (Díez & Nuez, 2008; Stevens & Rick, 1986).

Genetic resources

DNA-based analyses have demonstrated that domesticated tomatoes underwent a significant genetic bottleneck during their journey from South America to Central America and Europe. Compared with their wild relatives, domesticated tomatoes show less than 5% of genetic variation among them (Bai & Lindhout, 2007; Razifard et al., 2020). The tomato fruit enlargement and other improved agronomic traits were the result of genomic sweeps in two rounds, during the transition from the wild species *S. pimpinellifolium* to *S. lycopersicum* var. *cerasiforme* and then further improvement to the modern tomato *S. lycopersicum* (Lin et al., 2014). To preserve the genetic diversity of tomatoes, germplasm resources are essential. Pioneering work by Charles M. Rick led to a collection of thousands of wild tomato accessions from their natural habitats in the Andes and the Galapagos Islands. The Tomato Genetics Resource Center in Davis, California, maintains this tomato collection, as well as monogenic mutants. In the Netherlands, the Botanical and Experimental Garden maintains the most extensive ex situ non-tuberous Solanaceae species collection in the world (Bai & Lindhout, 2007; Barendse & van der Weerden, 1997). Additionally, the Solanaceae Genome Network (https://solgenomics.sgn.cornell.edu/) provides genomic information for the Solanaceae species, including tomato (*S. lycopersicum*), potato (*S. tuberosum*), eggplant (*S. melongena*), and pepper (*Capsicum annuum*) (Mueller et al., 2005). Remarkably, wild accessions with inferior phenotypes have been found to be a source of alleles that, when incorporated into cultivated backgrounds, lead to favorable phenotypes such as disease resistance and higher soluble solid content (Lin et al., 2014). Therefore, native germplasm and seed banks harbor large genetic potential for cultivated germplasm (Barone et al., 2008). Lastly, the significant contribution of the Andean and Mesoamerican regions in the early stages of tomato domestication should be recognized, as rural areas continue to harbor populations of wild tomatoes, landraces, and heirloom domesticated tomato varieties. These resources represent a valuable gene pool, in addition to existing seed banks, for new discoveries and modern breeding strategies (Bai & Lindhout, 2007; OECD, 2017; Rick, 1983). Hence, collaboration of governmental, nongovernmental, academic, and private-sector interest groups is essential to enhancing the generation of information and ensuring the proper management and conservation of tomato genetic resources (Bretting, 2018; Tanksley & McCouch, 1997).

Diversity of the tomato microbiome

Factors influencing microbiome assembly

The microbiome of plants plays an important role in development, growth, and health (Cordovez et al., 2019; Oyserman et al., 2018; Trivedi et al., 2020). The assembly of the microbiome and expression of specific microbial functional traits is affected by multiple abiotic and biotic factors (Dastogeer et al., 2020; Philippot et al., 2013; Trivedi et al., 2020; Turner et al., 2013). Here, we review current knowledge of host and environmental factors that influence tomato microbiome assembly and functions (Table 1).

Table 1. Microbial composition in tomato according to plant compartments.

Compartment	Microbial composition	Reference
Rhizosphere	Bacillaceae, Streptomycetaceae, Comamonadaceae, Oxalobacteriaceae, Mycobacteriaceae, Alphaproteobacteria, Burkholderiales, Pseudomonadaceae	Allard et al., 2016
Rhizosphere	Xanthomonadales, Nitrosomonadales, Myxococcales, Rhizobiales, Burkholderiales, Sphingobacteriales, Cytophagales, Acidobacteria Subgroup 4, Acidobacteria Subgroup 6	Cheng et al., 2020
Rhizosphere	Bacteria: *Paenibacillus, Bacillus, Patulibacter* and members of Gemmatimonadetes, Acidobacteria, Deltaproteobacteria Fungi: *Emmonsia, Alternaria, Cladosporium, Acremonium,* no identified Basidiomycota, *Conocybe, Hohenbuehelia* and *Rhodotorula*	Cordero-Ramírez et al., 2012
Rhizosphere	*Acinetobacter, Pseudomonas, Ensifer, Rhizobium*	Dong et al., 2019
Rhizosphere	Bacteria: *Nitrospira, Reyranella, Lactobacillus, Fibriiomonas, Rhodopila, Methylovorus*, *Dongia* Fungi: *Oidiodendron*, *Talaromyces*	Fuentes et al., 2020
Rhizosphere	Sphingomonadaceae, Micrococcaceae, Microbacteriaceae, Streptomycetaceae, Acidobacteria Subgroup 4	Lee et al., 2016
Rhizosphere	Bacteria: *Sphingobium, Sphingomonas, Microbacterium, Arthrobacter, Afipia, Leifsonia, Luteimonas* Fungi: *Hyphodiscus, Aspergillus, Trichoderma, Chrysosporium, Oidiodendron*	Lee et al., 2019
Rhizosphere	*Fusarium, Gibellulopsis, Penicillium, Phoma, Pyrenochaetopsis, Sarocladium*, *Trichoderma*	Poli et al., 2016
Rhizosphere	*Aspergillus, Penicillium, Trichoderma*, *Fusarium*	Tyagi and Tyagi, 2016
Rhizosphere	Soil Crenarchaeota Group (Thaumarchaeota), *Methanosarcina* (Euryarchaeota), *Methanoculleus* (Euryarchaeota)	Taffner et al., 2020
Phyllosphere	Flower: Xanthomonadaceae, Pseudomonadaceae, Microbacteriaceae, Enterobacteriaceae Fruit: Rhizobiaceae, Sphingomonadaceae, Microbacteriaceae, Pseudomonadaceae, Xanthomonadaceae	Allard et al., 2016
Phyllosphere	Leaf and stem: *Acinetobacter, Enterobacter, Pseudomonas*	Dong et al., 2019

Compartment	Microbial composition	Reference
Phyllosphere	Leaf: Bacteria: *Pantoea, Pseudomonas, Spingomonas, Xanthomonas,* Enterobacteriaceae, *Exiguobacterium, Bacillus* Fungi: Basidiomycota: *Rhodosporidiobolus, Filobasidium, Sporobolomyces, Tremellales;* Ascomycota: *Cladosporium*	Morella et al., 2020
Phyllosphere	Leaf: *Pseudomonas, Erwinia, Sphingomonas*	Ottesen et al., 2016
Phyllosphere	Leaf: Bacteria: *Pseudomonas, Sphingomonas, Methylobacterium, Variovorax, Rathayibacter, Cultibacterium, Microbacterium, Bacillus* Fungi: Basidiomycota: *Erythrobasidium, Symmetrospora, Filobasidium, Vishniacozyma, Cryptococcus*; Ascomycota: *Davidiella tassiana, Aureobasidium pullulans, Phoma medicaginis, Ophiosphaerella, Cladosporium, Alternaria*	Runge et al., 2022
Phyllosphere	Leaf: Bacteria: S*phingomonas, Methylobacterium, Pseudomonas, Deinococcus* Fungi: *Cladosporium, Dioszegia, Moesziomyces, Hannaella*	Toju et al., 2019
Phyllosphere	Leaf trichomes: *Bacillus, Deinococcus, Acinetobacter, Paracoccus, Sphingomonas, Massilia, Caulobacter, Capnocytophaga, Pseudomonas, Pedobacter, Luteimonas*	Kusstatscher et al., 2020
Endosphere	Root and leaf: *Acinetobacter*, *Enterobacter*, *Pseudomonas* Stem: *Acinetobacter*, *Enterobacter*, *Pseudomonas, Pantoea* Fruit: *Enterobacter, Tatumella, Acinetobacter, Weissella* Seed: *Enterobacter, Lachnospiraceae, Bacteroides*	Dong et al., 2019
Endosphere	Root: Deinococcacea, Enterobacteriaceae, Bacillaceae, Chromobacteriaceae, Halomonadaceae	French et al., 2019
Endosphere	Root: Bacteria: *Enterobacter, Acidovorax, Variovorax, Pseudomonas, Rhizobium, Streptomyces* Fungi: *Alternaria, Colletotrichum*	Lee et al., 2019
Endosphere	Leaf: *Methylobacterium radiotolerans*, *Shinella* sp., *Achromobacter xylosoxidans* Root: *Burkholderia cepacia, Pseudomonas* sp., *Sphingobium herbicidovorans, Rhizobium radiobacter*	Longoria-Espinoza et al., 2020
Endosphere	Leaf: *Pseudomonas, Propionibacterium, Streptococcus, Shinell*a. *Clavibacter* Root: *Curtobacterium, Clavibacter, Staphylococcus, Streptococcus, Acinetobacter*, *Pseudomonas*	López et al., 2020
Endosphere	Seed: *Bacillus aryabhattai, Bacillus nakamurai, Ralstonia pickettii, Stenotrophomonas maltophilia, Stenotrophomonas pavanii*	Bergna et al., 2018

Abiotic factors

Among the abiotic factors, soil physicochemical properties are the strongest determinant of the diversity of microbial communities (Bandyopadhyay et al., 2017; Jeanbille et al., 2016). Soil serves as the primary source of microorganisms, and its properties impact soil microbial diversity (Kandasamy et al., 2021; Lee et al., 2019; Philippot et al., 2013). Hence, agricultural practices such as the conversion of native habitats to crop production systems may have led to the loss of soil microbial diversity due to the external inputs of synthetic fertilizers and pesticides (Cordovez et al., 2019; Matson et al., 1997; Rodrigues et al., 2013; Schmidt et al., 2019). In the past, anthrosol (anthropogenic soil) was formed by farmers through organic soil amendments and management practices to improve soil fertility and plant productivity. These prac-

tices presumably also selected and enhanced populations of beneficial soil microbiomes (Martínez-Romero et al., 2020). The earliest recorded method of tomato cultivation in Mesoamerica was a farming system called "milpa," a polyculture association that included maize (*Zea mays*), beans (*Phaseolus* spp.), squash (*Cucurbita* spp.), and other species such as chili pepper, husk tomato, and tomato (Casas, 2001; Díez & Nuez, 2008; Long, 2022). Tomato underwent the same intensive selection process exerted by a habitat change as other useful weeds; that is, plants were collected from natural populations and protected within cultivation systems (Casas, 2001).

With the latest advances in agriculture technology, plants can now be grown under varying environmental and cultural conditions. These range from traditional open-field farms and small-scale gardens to large-scale production by the agroindustry. Innovative techniques include acclimatized greenhouses, plastic covers (Moreno & Moreno, 2008), hydroponics, and fertirrigation, as well as agroecology in alternative production systems (OECD, 2017).

Conventional management practices of tomato use mechanical structures, devices, and synthetic chemicals to condition the growing space, optimize the yield, and maintain the fruit's organoleptic traits along the food chain (Aghdam et al., 2012; Distefano et al., 2022; Garza Arizpe & Molina Velázquez, 2008; Islam et al., 2017; Lima et al., 2022; López Marín, 2017; Nicola et al., 2009; Rodrigues & Furlong, 2022).

Considering these large-scale changes in soil and crop management practices, the rhizosphere microbiome assembly of tomato is greatly affected. For example, field soils harbor more diverse bacterial communities than hydroponic systems do (Cheng et al., 2020; Chialva et al., 2018; Thomas et al., 2023; Vargas et al., 2021). More specifically, soil largely contributed to microbiome assembly in the tomato rhizosphere than tomato genotypes because they did not show statistically significant differences in bacterial diversity when grown in the same soil. Tomato rhizobacterial communities differed according to the soil source and organic matter; phosphorous, potassium, and manganese were the main soil properties that impacted rhizobacterial assembly (Cheng et al., 2020). The effects of the management practices on soil are indirect, as they impact soil properties as well as plant physiological traits that concomitantly alter the taxonomic and functional composition of the microbiome (Cai et al., 2017; Saleem et al., 2018; Schmidt et al., 2019). Hence, research focused on improving soil physicochemical properties on a large scale will be pivotal in promoting beneficial microbe–tomato interactions.

Biotic factors

Several studies have shown that the host genotype plays a relatively minor role (<10%) in microbiome assembly (French et al., 2020; Gutierrez & Grillo, 2022; Tabrett & Horton, 2020) and can decrease over time (Morella et al., 2020). In fact, human interventions have diminished the genetic diversity of domesticated crops and drastically changed plant morphology, physiology, and immune response (Chen et al., 2015; Doebley et al., 2006). Hence, domesticated tomatoes, as well as other plant species, have become more reliant on nutrients, water, and protective measures against biotic stresses (Raaijmakers & Kiers, 2022). Different tomato traits have changed drastically during domestication, including the shift from wild allogamous species to strictly autogamous commercial tomato cultivars (Razali et al., 2018; Rick, 1978; Taylor, 1986). This artificial selection has led to an increased fruit yield in domesticated varieties and enhanced disease, pest, and abiotic stress resistance (Stevens & Rick, 1986) but also reduced outcrossing and narrowed the genetic variation within tomatoes (Peralta et al., 2008). Both the native tomato *S. pimpinellifolium* and the landrace *S. lycopersicum* var. *cerasiforme* show higher genetic diversity compared with populations of the domesticated tomato *S. lycopersicum* var. *lycopersicum.* The latter displays higher frequencies of six loci linked to fruit traits, highlighting intense selection pressure for fruit weight and shape in modern tomato varieties (Blanca et al., 2015). The observed selective pressure may explain why different genotypes can exhibit varying degrees of microbiome assembly. For example, transgenic tomatoes deficient in salicylic acid (SA) or ethylene (ET) signaling showed significantly less alpha diversity in the root endosphere microbiome than the parental tomato lines, indicating that the tomato immune system affects microbiome assembly (French et al., 2019). In another study, Cordovez et al. (2021) highlighted the impact domestication and breeding can have on tomato microbiome assembly. They found that the bacterial alpha diversity decreased and beta diversity increased over time in wild and domesticated tomato in successive growth cycles, indicating that the host plant increased its selective pressure on microbiome composition, resulting in a more dissimilar microbiome between tomato genotypes. Sillo et al. (2022) found that two different domesticated *S. lycopersicum* cultivars showed changes in their microbiome composition under medium water stress in field conditions, with one of these cultivars displaying a decrease in Bacteroidetes and an increase in Firmicutes phyla in the rhizosphere.

Regarding genetics involved in microbiome assembly, Oyserman et al. (2022) found specific genomic regions in tomato associated with specific taxa and genes in rhizosphere bacteria. They observed associations between plant growth, stress, amino acid metabolism, iron and water acquisition, hormonal responses, and terpene biosynthesis and microbial traits such as metabolism of plant cell wall polysaccharides, vitamins, sulfur, and iron. Particularly, *Cellvibrio* and *Streptomyces* were shown to be differential

root colonizers of wild and modern tomato, respectively. Furthermore, these bacterial taxa harbored functions related to iron acquisition and carbohydrate metabolism that may allow them to profit from tomato rhizodeposition (Oyserman et al., 2022).

To date, domestication-associated changes in physiological and morphological traits such as root exudation and root architecture have been largely understudied. Specifically, more emphasis is given to the impact of root exudates (Gutierrez & Grillo, 2022) than root volatile emissions (Lee Díaz et al., 2022). Root exudation is affected by both plant host factors, such as genetics and developmental stages, and environmental conditions, such as water and nutrient availability (Hale et al., 1971). For instance, the composition of tomato root exudates varies during plant development, with amino acids, organic acids, and sugars being more prevalent in tomato roots compared with the fruiting stage (Vančura & Hovadík, 1965). This exudation profile in tomato roots likely attracts bacteria specialized in carbohydrate metabolism and conversion of sugars or toxic carbonyl compounds, enabling them to thrive in the root environment over time (Levy et al., 2018). For example, *Pseudomonas*, whose *mdh* operon encodes malate dehydrogenase, depends on organic acids from tomato root exudates for efficient root colonization (Lugtenberg et al., 2001).

In addition, stress by herbivorous insects as well as pathogens induces changes in the root exudation profile (Yi et al., 2011). In this context, the "cry for help" hypothesis has been coined and is defined as the mechanism by which plants change their root exudate chemistry to recruit or activate beneficial members of the microbiome, enabling plants to adapt to biotic stress (Berendsen et al., 2012; Rizaludin et al., 2021; Rolfe et al., 2019). The "cry for help" was first addressed by Rudrappa et al. (2008), who evidenced that *Pseudomonas syringae* pv. *tomato* elicited the secretion of L-malic acid from *Arabidopsis* roots to promote the recruitment of *Bacillus subtilis* and suppress subsequent pathogen attack. Moreover, recent studies showed significant differences in root volatile emissions between wild and domesticated tomatoes under leaf herbivory stress (Lee Díaz et al., 2022). More specifically, the wild tomato *S. pimpinellifolium* showed the largest change in root volatilome between *Spodoptera exigua*-stressed plants and the control. This and other studies highlight the importance of adopting a holistic view on microbiome assembly. Furthermore, metabolomics studies of water-soluble and volatile root compounds of wild and domesticated tomatoes, as well as transcriptomics of recruited microbiomes under stress conditions, are needed to understand the mechanisms involved in selective colonization and improved plant health.

Rhizosphere microbiome

The rhizosphere is the narrow zone of soil that is influenced by the presence and activity of living roots (Berendsen et al., 2012). The tomato rhizosphere microbiome composi-

tion is determined largely by the soil type. For example, Cheng et al. (2020) found no differences in the rhizosphere microbiome composition among 11 tomato genotypes grown in lawn soil collected from Fujian Normal University in Fuzhou, China. In this study, the tomato rhizosphere microbiome was defined by Proteobacteria (34%), Bacteroidetes (16%), and Acidobacteria (15%) as the most abundant phyla. However, when sowing a single tomato genotype (*S. lycopersicum* var. Meiguodahong 168) in seven different soils and substrates, they found that the rhizosphere microbiome differed greatly among soil sources (Cheng et al., 2020). Dong et al. (2019) showed that Proteobacteria even constituted more than 80% of the phyla detected in the tomato rhizosphere. Studies on the tomato rhizosphere microbiome in Mexico, the center of domestication, revealed that the rhizosphere microbiome of tomato grown in soil from this area differs from that of other studies. Members of the Firmicutes (45%), Proteobacteria (15%), Gemmatimonadetes (13%), Actinobacteria (11%), and Acidobacteria (10%) phyla were the most abundant bacterial taxa in a domesticated tomato grown under field conditions in Mexico (Cordero-Ramírez et al., 2012). When considering that over 30% of the world's tomato production occurs in greenhouses, Lee et al. (2016) found that the most abundant phyla in the rhizosphere microbiome of greenhouse tomatoes were Proteobacteria (78%, dominated by Alphaproteobacteria), Actinobacteria (9%), Bacteroidetes (4%), Acidobacteria (3%), and Planctomycetes (1%). Kim et al. (2006) found that Proteobacteria (53%) and Cytophaga–Flavobacterium–Bacteroides (35%) were the dominant phyla in the tomato rhizosphere under greenhouse cultivation. On the other hand, Resendiz-Nava et al. (2023) found bacterial differences in the tomato rhizosphere under a soilless culture system. They showed that Flavobacteriaceae and Rhodobacteraceae were more abundant in an organic fertilization regime, whereas Streptomycetaceae, Caulobacteraceae, and Chitinophagaceae were more abundant in the conventional fertilization regime.

In contrast to bacterial communities, less research has been conducted on fungal communities in the tomato rhizosphere. Nonetheless, soil was shown to be the main factor in shaping the mycobiome composition of the tomato rhizosphere (Poli et al., 2016). Following a culture-based approach, Cordero-Ramírez et al. (2012) and Poli et al. (2016) showed that most of the genera isolated belonged to the Ascomycota. There is, to our knowledge, no conclusive evidence supporting the occurrence of mycorrhizal fungi in significantly high numbers in the tomato rhizosphere, and their presence seems to be influenced by the plant genotype. Fuentes et al. (2020) showed a differential recruitment of members of the Glomeromycota by two native plants species from the Atacama Desert, *S. chilense* and *Billardiera scandens.* Although the tomato plants were not able to recruit Glomeromycota under natural conditions, they can respond better to symbiotic interaction when mycorrhizal fungi are inoculated. There is also a limited number of studies on the archaeal members of the tomato microbiome. In a study

by Taffner et al. (2020), members of the Thaumarchaeota (60.7%) and Euryarchaeota phyla (*Methanosarcina*: 12.6%, *Methanoculleus*: 3.4%) composed the tomato rhizosphere microbiome.

Phyllosphere microbiome

The phyllosphere, the aboveground plant compartment, including leaves, stems, blossoms, and fruits surfaces (Allard et al., 2016), is a microbial habitat strongly influenced by environmental conditions (Dong et al., 2019; Mechan Llontop et al., 2021; Morella et al., 2020; Müller & Ruppel, 2014; Ottesen et al., 2016). Proteobacteria dominate the tomato phyllosphere, such as leaves, stem, flowers, and ripe fruits, under both greenhouse and field conditions (Allard et al., 2016; Dong et al., 2019; Morella et al., 2020; Ottesen et al., 2016). A recent study on the leaves of wild tomatoes collected in their native habitat (Lima, Peru) revealed more bacterial diversity in *S. peruvianum* and *S. pimpinellifolium* compared with *S. habrochaites* and *S. corneliomulleri* (Runge et al., 2022). In addition, the genotype effect on microbiome composition was observed in *S. habrochaites* and *S. corneliomulleri* but not in *S. peruvianum* and *S. pimpinellifolium*. Also in this study, Proteobacteria were the most abundant in *S. habrochaites* and *S. corneliomulleri*, whereas Actinobacteria and Firmicutes were more abundant in the phyllosphere of *S. peruvianum* and *S. pimpinellifolium* (Runge et al., 2022). The fungal composition in the wild tomato phyllosphere was dominated by Basidiomycota (yeasts) and Ascomycota. Intriguingly, the native habitats assembled a more diversified community in the phyllosphere than in crops grown under greenhouse or field conditions (Morella et al., 2020; Runge et al., 2022). Moreover, Kusstatscher et al. (2020) found differences in the bacterial composition in tomato leaf trichomes between *S. habrochaites* LA1777 and *S. lycopersicum* LA4024. *S. habrochaites* harbored richer bacterial communities than did *S. lycopersicum*. Trichomes of *S. habrochaites* were significantly enriched in bacterial classes Alphaproteobacteria and Bacilli, whereas *S. lycopersicum* trichomes were dominated by Gammaproteobacteria and Bacteroidia.

Endosphere microbiome

The endosphere refers to the compartment inside plant tissue that is characterized by a distinct microbial assemblage and lower diversity than in the external compartments, such as the rhizosphere (French et al., 2020; Lee et al., 2019). López et al. (2020) studied the endosphere microbiome composition of healthy and diseased roots and leaves of tomato plants under greenhouse conditions. They discovered that the most prevalent classes in healthy roots were Actinobacteria (23.30%), Bacilli (15.02%), and Gammaproteobacteria (12.58%), whereas in diseased roots, Actinobacteria was reduced to 7.43% but Bacilli (17.58%) and Gammaproteobacteria (28.20%) increased. On the other hand, the endosphere of healthy leaves primarily harbored Proteobacteria (32.86%) and Actinobacteria (15.73%), in contrast with symptomatic leaves

that showed an increase of Actinobacteria (58.28%) and a decrease of Proteobacteria (25.72%). French et al. (2020) found that the tomato root endosphere was dominated by Proteobacteria (Gammaproteobacteria: 46.6%, Alphaproteobacteria: 22.5%, and Deltaproteobacteria: 3.4%), Actinobacteria (15.0%), and Firmicutes (6.8%) in eight tomato genotypes. Nevertheless, the root endosphere did not show a clustering pattern between wild *S. pimpinellifolium* and domesticated tomato *S. lycopersicum*, and Bacillaceae and Rhizobiaceae where the high-frequency endosphere colonizers (French et al., 2020). Likewise, Dong et al. (2019) and Longoria-Espinoza et al. (2020) showed that the endophytic community of root, shoot, leaf, fruit, and seed in tomato was characterized by the phylum Proteobacteria. For the endophytic fungal community, particularly in roots, Ascomycota was the major phylum of tomato roots (93.9%), followed by Basidiomycota (3.5%) and Zygomycota (0.2%) (Lee et al., 2019).

Seed microbiome

Microorganisms can be vertically transmitted from seeds over plant generations (Berg & Raaijmakers, 2018). A meta-analysis of the seed microbiome of 50 plant species revealed dominance of Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes and fungal classes Dothideomycetes, Sordariomycetes, and Tremellomycetes (Simonin et al., 2022). The core taxa included *Pantoea agglomerans*, *Pseudomonas viridiflava*, *Pseudomonas fluorescens*, *Rhizobium* spp., *Cladosporium perangustum*, and *Alternaria* spp. (Simonin et al., 2022). In addition, Bergna et al. (2018) found that seeds of *S. lycopersicum* (cultivars Moneymaker and Hildares F1) were selective for a few dominant bacterial taxa, such as Burkholderiaceae (19%), Pseudomonadaceae (7%), and Comamonadaceae (6%), which showed a continuous turnover after one generation. Similarly, Taffner et al. (2020) found that the seed endosphere of *S. lycopersicum* (Moneymaker and Hildares F1) was dominated by Thaumarchaeota and Euryarchaeota. Nevertheless, the archaeal diversity was low, and it exhibited a decline in the subsequent generation.

Functions of the tomato microbiome

The microbiome provides numerous life-support functions for plant growth and health. In tomato, a range of functions have been reported as well, including nutrient acquisition and tolerance to abiotic and biotic stresses.

Nutrition acquisition

Nitrogen (N), phosphorus (P), potassium (K), and iron (Fe) are essential elements for tomato development. Plant-associated microorganisms can improve plant nutrient uptake and, consequently, the overall health and productivity of plant systems through

different mechanisms. Below, we review the main microbial functions that facilitate nutritional absorption in tomato.

Nitrogen fixation

Nitrogen can be abundant in soil, but plants require it in a usable form, such as ammonium (NH_4^+) or nitrate (NO_3^-). The process of converting N_2 from the surrounding atmosphere to ammonium is known as nitrogen fixation (Singh et al., 2019). This process is carried out by specialized bacteria, either symbiotic bacteria such as *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Frankia* or nonsymbiotic bacteria such as *Anabaena*, *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, *Nostoc*, and *Pseudomonas* (Prasad et al., 2019). Mohandas (1988) reported that *Azospirillum* present in the rhizoplane and endosphere of tomato was capable of fixing nitrogen under field conditions. Caballero-Mellado et al. (2007) found N_2-fixing *Burkholderia* species (*B. unamae*, *B. xenovorans*, *B. tropica*, and *B. kururiensis*) in the tomato rhizoplane in field crops in Mexico. Furthermore, Masood et al. (2020) determined that the addition of nitrogen into the soil activated the nitrogen fixation by *Bacillus pumilus* and increased nutrient uptake and tomato growth. Similarly, inoculating tomato plants with rhizobia (*Rhizobium etli* CE-3, *R. leguminosarum* SCR, and *R. leguminosarum* Semia-4088) promoted plant growth, and *R. etli* significantly increased tomato growth, NPK foliar content, and yield under field conditions compared with uninoculated plants, although the conclusive role of nitrogen fixation by the introduced rhizobia in growth promotion was not experimentally validated (Toledo Cabrera, 2021).

Phosphate solubilization

Phosphate-solubilizing bacteria release low molecular weight organic acids, such as gluconic and ketogluconic acid, that make inorganic phosphate available to the plant in the form of $H_2PO_4^-$ and HPO_4^{-2} ions (Goldstein, 1995). These bacteria can be found across various genera, including *Arthrobacter, Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Mesorhizobium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, *Rhodococcus*, and *Serratia* (Prasad et al., 2019). Under greenhouse conditions, Zhang et al. (2017) demonstrated that phosphate-solubilizing *Acinetobacter* (Gammaproteobacteria) and *Ochrobactrum* (Alphaproteobacteria), isolated from mushroom residues, promoted tomato growth. Similarly, Tchakounté et al. (2020) inoculated *Arthrobacter* and *Bacillus* on tomato plants, which increased phosphorous uptake in both high and low salt concentrations and promoted plant growth. Nassal et al. (2018) found significantly increased microbial phosphatase activity in the rhizosphere of tomato plants and plant growth promotion by *Pseudomonas* sp. strain RU47.

Iron acquisition

Iron is an element typically abundant in soil in an insoluble form and therefore not accessible to the plant. Thus, rhizobacteria play a role in solubilizing or chelating iron from complex organic or inorganic forms via the production of siderophores. The main types of siderophores produced by microbes are pseudobactins, pyoverdins, and pyochelins (Kannojia et al., 2019). Radzki et al. (2013) found that the siderophore produced by *Chryseobacterium* strain C138 increased plant growth, chlorophyl, and iron content in iron-starved tomato plants as compared with the uninoculated control plants. Similarly, Abbamondi et al. (2016) reported that *Pseudomonas*, *Rhizobium*, *Rhodococcus*, and *Agrobacterium* isolates from the endospheres of different tomato cultivars produced siderophores and promoted root hair formation in *Arabidopsis thaliana.* In a related study, Karuppiah et al. (2022) identified the rhizobacterium *Pseudomonas stutzeri* KRP8 as the highest siderophore producer, which enhanced the bioavailability of iron, improved plant tolerance to heavy metals, and promoted plant growth.

Enhanced tolerance to biotic and abiotic stresses

Regarding the plant health status, eubiosis refers to a balanced microbiome that provides beneficial effects to the host, whereas dysbiosis refers to a disturbed microbiome that causes deleterious effects on plant growth and health (Lee et al., 2021). For instance, it has been found that dysbiotic leaves (growth defects, leaf chlorosis and necrosis) of wild tomato exhibited lower bacterial richness (Runge et al., 2022). Many studies have indicated that specific single bacterial or fungal strains can provide protection to tomato plants against bacterial, fungal, and oomycete pathogens, as well insect pests, both below- and aboveground. These include *Pseudomonas* (García-Villaraco et al., 2021; Mohammed et al., 2020), *Bacillus* (Janahiraman et al., 2016; Zhou et al., 2021), and *Streptomyces* (Le et al., 2021; Ling et al., 2020), bacterial genera well known for the production of diverse antimicrobial compounds such as 2,4-diacetylphloroglucinol, amphisin, hydrogen cyanide, phenazine, oomycin A, tropolone, pyoluteorin, tensin, pyrrolnitrin, cyclic lipopeptides, kanosamine, oligomycin A, xanthobaccin, and zwittermycin. Additionally, *Pseudomonas, Streptomyces*, and *Bacillus* can counteract phytopathogens through production of fungal cell wall–degrading enzymes, such as chitinase or β-1,3-glucanase (Singh et al., 2019). Moreover, these and other bacterial genera were shown to induce systemic resistance in tomato against leaf pathogens such as *Botrytis cinerea* (Audenaert et al., 2002) and *Phytophthora infestans* (Tran et al., 2007). Taking a microbial community approach, Hu et al. (2016) showed that the suppression of *Ralstonia solanacearum* in tomato was dependent on the diversity of a *Pseudomonas* consortium. Subsequently, the study by Lee et al. (2021) revealed that *Ralstonia solanacearum* infections in tomato led to a decline in Firmicutes and Actinobacteria in the rhizosphere. Furthermore, they showed that treatment of the rhizosphere with the antibiotic vancomycin mimicked this dysbiotic tomato phenotype. However, when a

synthetic bacterial community (SynCom) was introduced, consisting of four isolates of Actinobacteria (*Brevibacterium frigoritolerans* HRS1) and Firmicutes (*Bacillus niacini* HRS2, *Solibacillus silvestris* HRS3, *Bacillus luciferensis* HRS4), disease protection of tomato against *R. solanacearum* was restored. The SynCom did not exhibit direct antagonism against the pathogen; therefore, they proposed that induced systemic resistance was the most likely mechanism of protection. This was supported by additional results showing that the SynCom upregulated the expression of jasmonic acid and SA signaling marker genes, such as *Pin2*, *AOS*, *LoxD*, *PR-P6*, *NPR1*, and *PR1a*, but did not activate the expression of ET and abscisic acid (ABA) signaling genes (Lee et al., 2021). Inoculation of *Aspergillus niger*, *A. flavus*, *Mucor circinelloides*, and *Penicillium oxalicum* on tomato (cv. Castlerock II PVP) reduced the disease severity of *Fusarium* wilt (Attia et al., 2022). Among these fungal species, *A. niger* showed the highest protection rate, whereas *M. circinelloides* showed the strongest increase in chlorophyll content, as well as in total soluble proteins and carbohydrates. In addition, growth promotion was observed on inoculation of tomato plants with *P. oxalicum*.

Salinization is one of the major stressors in agriculture systems of arid and semiarid regions, mainly due to the saline water used for irrigation and inadequate drainage. Salt stress causes metabolism imbalances induced by ion toxicity and water deficit due to salt accumulation in plant cells. Salinity leads to the formation of reactive oxygen species (ROS), such as superoxide (O^{2-}), singlet oxygen (O_2), hydroxyl (OH^-), and hydrogen peroxide (H_2O_2), resulting in redox imbalance and oxidation of cell membranes. Hence, an antioxidant enzyme system is activated during stress conditions, including H_2O_2 scavengers such as catalase, peroxidase, ascorbate peroxidase, glutathione peroxidase, glutathione S-transferases, glutathione reductase, superoxide dismutase, peroxiredoxin, scorbate, glutathione, α-tocopherol, and flavonoids (Bharti & Barnawal, 2019). Despite these adverse effects, salinity has also been reported to have a positive impact on the fruit quality of tomato landraces in comparison with commercial cultivars such as Moneymaker (Massaretto et al., 2018). Rhizobacteria such as *Pseudomonas*, *Azospirillum*, *Burkholderia*, *Arthrobacter*, *Bacillus*, *Enterobacter*, and *Azotobacter* can enhance the ability of crops to tolerate high levels of salt. These bacteria achieve this by producing antioxidants, including catalase and other ROS-scavenging enzymes (Bharti & Barnawal, 2019). For example, a synthetic community of five bacterial strains isolated from the root of *Indigofera argentea*, composed of *Massilia* sp. SA087, *Enterobacter* sp. SA187, *Ensifer* sp. SA403, *Bacillus* sp. SA436, and *Streptomyces* sp. SA444, successfully protected tomato plants against high salt stress (Schmitz et al., 2022). In other studies, *Enterobacter* sp. EJ01, *Achromobacter piechaudii* (Mayak et al., 2004), and *Pseudomonas mendocina* (Sadrnia et al., 2011) were found to promote growth and increase salt stress resistance in tomato plants through multiple mechanisms, such as the rapid upregu-

lation of salt stress–responsive genes and enhancement of ROS-scavenging activities (Kim et al., 2014; Mayak et al., 2004; Sadrnia et al., 2011).

When plants are subjected to salt stress, it triggers the overproduction of ET, which inhibits plant growth and development. However, certain rhizobacteria can produce ACC deaminase, an enzyme that cleaves ACC to α-ketobutyrate and ammonia, thereby decreasing the levels of ET in the host plant (Bharti & Barnawal, 2019). Among these rhizobacteria are *Pseudomonas fluorescens*, *P. aeruginosa*, and *P. stutzeri*, which were isolated from the tomato rhizosphere and enhanced salinity tolerance and promoted growth in tomato plants through ACC deaminase activity and phytohormones production (Tank & Saraf, 2010). Similarly, *P. putida* UW4 was observed to upregulate the expression of Toc GTPases genes, which are involved in facilitating the import of stress-responsive proteins into chloroplasts, resulting in increased salt stress tolerance in tomato plants (Yan et al., 2014).

With water scarcity posing a threat to global food security, one promising option is the use of rhizobacteria to promote the formation of lateral roots and increasing the length of primary roots, thus improving water and nutrient search and uptake. For example, Shintu and Jayaram (2015) showed that tomato inoculated with *Bacillus polymyxa* increased plant growth and yield under drought conditions compared with uninoculated plants. In another study, although the efficiency of the co-inoculation of *Rhizophagus irregularis* and *Variovorax paradoxus* 5C-2 in tomato recombinant inbred lines (RILs) under drought stress was variable, Calvo-Polanco et al. (2016) found that these inoculants positively impacted drought tolerance in RIL66, which was highly responsive to the inoculation and increased dry biomass, CO_2-assimilation rate, root hydraulic conductivity, and decreased proline accumulation under drought conditions. Additionally, different rhizobacteria, including *Alcaligenes*, *Azospirillum*, *Azotobacter*, *Bradyrhizobium*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Mycobacterium*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Xanthomonas*, produce the phytohormone indole-3-acetic acid, which promotes root growth and enhances water and nutrient uptake (Barnawal et al., 2019). Also, the production of the phytohormone ABA may induce stomatal closure, thereby reducing water loss upon drought stress (Porcel et al., 2014). For example, Brilli et al. (2019) showed that in tomato roots inoculated with *Pseudomonas chlororaphis* subsp. *aureofaciens* strain M71, the leaf hormonal content of ABA and indole-3-acetic acid increased, resulting in drought tolerance through improved water use efficiency (Brilli et al., 2019). The study by Flemer et al. (2022) revealed that salinity stress in combination with *Verticillum dahliae* inoculation reduced the alpha diversity and significantly decreased the abundance of Deltaproteobacteria, Firmicutes, Planctomycetes, Chlamydiae, and Verrucomicrobia, whereas salinity stress alone increased bacterial phyla Proteobacteria and Bacteriodetes. Furthermore, isolated root endophytes, such

as *Microbacterium* from *V. dahliae*-infected plants and *Paenibacillus* from *Fusarium oxysporum* f. sp. *lycopersici*-infected plants, displayed salt and drought tolerance in vitro. These findings suggest that the tomato root endosphere was enriched by beneficial microorganisms that can mitigate the (a)biotic stress (Flemer et al., 2022).

Inoculation of tomato plants with arbuscular mycorrhizal fungi was shown to alleviate abiotic stress (Chandrasekaran et al., 2021). For instance, Leventis et al. (2021) found that tomato (cultivar EVIA F1) inoculated with the arbuscular mycorrhizal fungi *Funneliformis mosseae* and *Rhizophagus irregularis* displayed efficient drought stress mitigation and increased nutrient uptake when compared with the fully watered nonmycorrhizal controls. Ronga et al. (2019) also found that tomato plants inoculated with *Funneliformis mosseae* had higher leaf chlorophyll content, nitrogen balance index, and water use efficiency under drought conditions than *Rhizophagus intraradices* on the three tomato genotypes tested (Pearson, Everton, and H3402). Furthermore, Morsy et al. (2020) found fungal endophytes, such as *Ampelomyces* spp. and *Penicillium chrysogenum*, to mitigate drought and salinity stress, respectively. Halo et al. (2020) showed that the endophytic fungus *Talaromyces omanensis* conferred drought tolerance in tomato variety Platinum, as indicated by phloem and cortex thickness, shoot dry weight, root length, number of flowers, and fruit weight, as well as higher concentrations of gibberellic acid than control plants. These studies exemplify the enormous potential of fungal communities to enhance the stress resilience of tomato plants.

Breeding for beneficial microbiome–plant interactions

Restoring beneficial microbe–plant interactions in modern crops requires an interdisciplinary effort of breeders and scientists, with a focus on disentangling the complexity of these interactions (Oyserman et al., 2022; Wissuwa et al., 2008). Furthermore, when implementing microbial inoculants in agricultural systems, it is important to consider the coevolutionary trajectory of plant microbiome assemblage during the genetic improvement process to ensure persistence of beneficial microbes throughout the cropping season (Cordovez et al., 2019). Because rapid environmental changes can disrupt mutualistic relationships, finding the optimal match between the microorganism and plant genotype is crucial for rapid adaptation to new environmental conditions (O'Brien et al., 2021; Oyserman et al., 2021) Therefore, modification of the plant-associated microbiome should be viewed as a complementary strategy for developing a new and sustainable agriculture (Martínez-Romero et al., 2020).

Microbial inoculation

The integration of microbial inoculants into modern agricultural systems has revitalized interest in and applicability to microbiome engineering approaches. Thus, the addition of biofertilizers and inoculants to enhance plant fitness is a promising strategy, particularly in degraded agroecosystems impacted by the overuse of synthetic fertilizers and pesticides (Bandyopadhyay et al., 2017). The functional characterization of beneficial microorganisms and microbial communities, by both 'omics technologies and classical in vitro validation assays, is harnessed to develop SynComs, which are groups of three or more keystone microbial isolates that complement each other to enhance plant productivity (Bandyopadhyay et al., 2017; Mendes et al., 2011; van der Heijden & Hartmann, 2016). This strategy has been proposed to "artificially" engineer the plant microbiome (Marín et al., 2021; Vorholt et al., 2017). In this context, a meta-analysis of the application of microbial inoculants showed that crop yield was enhanced due to nutrient availability provided by the application of five bacterial genera: *Pseudomonas*, *Bacillus*, *Enterobacter*, *Burkholderia*, and *Rhizobium* (Li et al., 2022). The impact of fungal inoculants on tomato growth and health has also received attention, albeit less than bacterial inoculants. For example, the inoculation of tomato seedlings with the arbuscular mycorrhizal fungus *Funeliformis mosseae* led to modifications in the root architecture, such as shorter root length and greater root branching, as well as increased leaf area when compared with noninoculated plants (Cesaro et al., 2020). Moreover, *Rhizophagus irregularis* significantly improved, under drought stress, water use efficiency and enhanced the leaf area and nutritional status of tomato (Hart et al., 2015; Leventis et al., 2021; Volpe et al., 2018). *Trichoderma* species have also been widely applied in agriculture, and their products have demonstrated efficiency as biofertilizers and biocontrol agents against various pathogens in tomato (Natsiopoulos et al., 2022; Ye et al., 2020).

If plants and their associated microorganisms have coevolved for over hundreds of millions of years, then wild plants in their native ecosystems may still harbor ancient mutualistic plant–microbe associations, such as potential phytostimulants and biocontrol agents (Pérez-Jaramillo et al., 2016). Additionally, plants that grow in marginal soils and extreme conditions (drought, salt, heat, cold) similar to their natural habitats have established robust associations with microorganisms that enable them to survive these harsh environmental conditions (Barajas et al., 2020). Therefore, retrieving, identifying, and characterizing these microorganisms that associate with wild plants is essential to enhancing their applications in modern agriculture (Gutierrez & Grillo, 2022; Pérez-Jaramillo et al., 2018). Also, natural disease-suppressive soils remain a valuable resource for identifying individual species or consortia of microorganisms that counteract pathogen establishment or prevent disease in a determined crop species (Gómez Expósito et al., 2017). In this regard, a suppressive soil against *Fusarium oxysporum* f. sp. *lycopersici*,

from the Rosta region in Italy, elicited an alert status in tomato plant by showing up-regulation of phenylpropanoid metabolism genes and other defense responses in the presence of the fungus, as well as the expression of marker genes for plant–microbe interactions, when compared with disinfected soil and artificial substrate (Chialva et al., 2018). The selection of specific rhizobacteria from tomatoes, mainly belonging to the Firmicutes phylum, inhibited the growth of "helper" bacteria that stimulate the growth of the pathogen *Ralstonia solanacearum* and effectively reduced the density and incidence of the pathogen (Li et al., 2022).

Tomato breeding strategies

The process of domesticating plants has resulted in genetic erosion due to the loss of certain plant traits found in wild relatives (Priyadarshan, 2019). From the 1950s to the early 1980s, the tomato industry demanded the development of specific plant traits to meet market demands, which resulted in phenotypic selection (Foolad & Panthee, 2012). Classical breeding techniques were used to achieve phenotypic traits such as fruit morphology, flowers with inserted stigmas (strict autogamy), and high yield, whereas modern plant breeding was required for resistance to diseases and pests (Caramante et al., 2021; Ercolano et al., 2020; Fentik, 2017; Rick, 1950). To advance tomato genetics, both classical and modern breeding methods have been utilized. Classical breeding techniques include mass selection and pedigree methods, whereas modern methods include hybridization, backcrossing, and transgenesis (Fentik, 2017). Mass selection involves repeatedly planting several phenotypically superior lines over several years until desired traits are achieved. In the pedigree method, a controlled cross is performed, followed by several successive generations of a single selected plant. This strategy, which spans several generations (from F2 to F6), aims to develop a new variety from a single cross. Each generation must be grown in the same environment for genetic differences to be expressed, and selected traits are examined among individual plants in the early generations (Fentik, 2017).

Phenotypic selection has limitations, such as availability of screening environments, reduced response to low heritable traits, and the need for large populations and land areas for experimentation. In response, hybridization has been used to boost the heterosis potential of tomato (Foolad & Panthee, 2012). Hybridization improves traits such as earliness, total yield, resistance attributes, adaptability, and external appearance in the first F1 cultivar compared with the parental cultivars. Moreover, superior performance of hybrids has been attributed to the nullification of undesirable gene effects, such as low pollen fertility, poor fruit set, or necrosis due to heat stress, because these genes are in the heterozygous state in F1 hybrids (Cheema & Dhaliwal, 2004). In the same way, introgressive hybridization, also known as introgression, has enabled interspecific gene transfer. For example, disease resistance, a desirable trait in tomato cultivation, can

be readily inherited from wild species through introgression into tomato inbred lines with good horticultural traits (Díez & Nuez, 2008; Scott et al., 2013). These hybrids contain chromosome segments from a wild tomato crossed into the background of a modern domesticated tomato, making them useful for quantitative trait locus (QTL) mapping and gene identification studies using molecular markers (Martínez-Romero et al., 2020; OECD, 2017). However, developing some tomato phenotypes, such as insect resistance, is challenging because it requires the introgression of multiple "wild" QTLs, the combination of genes, and the development of numerous molecular markers associated with pest resistance (Kortbeek et al., 2021; Zeist et al., 2018). Nevertheless, efforts are underway to stack several desirable traits in one specific genotype. For example, developing tomato varieties with heat tolerance is a long-term research goal, as higher heat-stress sensitivity can reduce pollen viability. Although breeding for improved tomato flavor by increasing sugar and acid contents in fruits is possible, breeding for relating flavor, aroma, and volatiles to specific genes and molecular markers remains a challenge (Fentik, 2017; Tieman et al., 2017).

Marker-assisted selection has greatly advanced the study of tomato genetics by using molecular markers to select for desirable traits in crops. This technology helps in the identification of QTLs in tomato for improvement purposes, such as hybridity tests prior to hybrid seed sales, yield, fruit quality, and resistance to biotic and abiotic stresses (Azzi et al., 2015; Fentik, 2017; Foolad & Panthee, 2012; Scott et al., 2013). A range of molecular markers have been developed and used in tomato breeding, including restriction fragment length polymorphisms, simple sequence repeats, cleaved amplified polymorphic sequences, amplified fragment length polymorphisms, and single nucleotide polymorphisms. Recently, advanced molecular breeding strategies such as advanced backcross QTL mapping have been developed. Advanced backcross QTL mapping identifies the transference of favorable QTL alleles from wild or non-adapted to cultivated germplasm, and once the QTL alleles have been detected in segregating populations (BC_2 or BC_3), near isogenic lines or introgression lines can be developed and phenotyped to confirm the QTL effect and subsequently be used in the develop of an improved tomato variety (Barone et al., 2008; Grandillo et al., 2013). Whereas it is relatively easy to identify traits controlled by individual genes, identifying QTL regions is more complex and requires the development of sophisticated populations and genetic marker analysis over many generations to validate the QTLs for plant breeding (Ganal, 2013).

QTL analysis is referred to here as associated genetic studies that involve the identification of QTLs using several molecular markers in unrelated individuals with defined phenotypes. According to the marker variation observed, it is inferred which markers show significant associations with a trait of interest. On the other hand, single-nucle-

otide polymorphisms are not inherited as individual segregating units but as a small proportion within a genomic region called a haplotype and can be considered alleles at a given locus. Haplotypes can extend over smaller or larger genomic regions. The extent of the conserved haplotypes, also known as linkage disequilibrium, varies depending on the analyzed population, and its frequency of genetic recombination in the respective region can be estimated. Genome-wide association studies (GWASs) involve the identification of single-nucleotide polymorphisms, haplotype ranges, and linkage disequilibrium in many individuals influencing complex traits, such as a disease factor. Thereby, GWASs can reveal gene function, chromosomal location, and other available information (Ganal, 2013). Furthermore, GWASs can help to estimate the heritability of microbiome traits and predict the response of plant microbiomes to natural and artificial selection with high reliability (Gutierrez & Grillo, 2022).

Mutational approaches have also been developed to investigate the genetic and molecular bases of agronomic traits in tomato, such as TILLING (Targeting Induced Local Lesions In Genomes) (Azzi et al., 2015). Other methods include functional analyses of candidate genes through transgenesis on tomato plants using *Agrobacterium tumefaciens* (Sharma et al., 2009) gene repression through virus-induced gene silencing, and genetic edition via clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 mutagenesis system (Brooks et al., 2014).

To improve the microbiome of plants, it is essential to consider traits that are involved in recruiting and activating beneficial microbial communities (Oyserman et al., 2018, 2021; Pérez-Jaramillo et al., 2016). Quantitative profiling of microbiome-associated plant phenotypes, such as nutrient acquisition and pathogen protection, can be used to determine the association of a particular plant phenotype with a subset of the microbiome and to validate the compatibility between microbial consortia and the host (Oyserman et al., 2018). This approach can be complemented by assisted genotype or microenvironment modification for desired plant phenotypes (Cordovez et al., 2019; Oyserman et al., 2021; Vorholt et al., 2017). Root architecture and exudates are key factors in shaping belowground plant–microbiome interactions. Therefore, future breeding programs should aim to improve the microbiome based on these traits (Gutierrez & Grillo, 2022; Saleem et al., 2018). However, the use of microorganisms from native habitats may be challenging in domesticated crops due to modifications in root architecture, exudation, and plant defense mechanisms (Martínez-Romero et al., 2020). To develop more symbiotically robust cultivars, breeding strategies such as targeted introgression, hybridization, and gene editing could enhance symbiosis function in crops (Porter & Sachs, 2020). For example, studies have demonstrated microbiome selection by genomic regions in tomato. Smith et al. (1999) found significant phenotypic variation for *Pythium torulosum* disease suppression by *B. cereus* UW85

in a tomato RIL population (*S. lycopersicum* [cultivar UC204 C] × *S. cheesmanii* [LA 483]). Three QTLs were identified as contributors to microbial biocontrol by UW85, two from modern varieties and one from the wild parent, suggesting that the disease suppression was supported by biocontrol agent's ability to colonize the host and that the wild parent contributed with an allele at one of the three loci. Similarly, Oyserman et al. (2022) found that the 6.31 Mbp region in the tomato genome, which encodes the iron regulator FIT and the water channel aquaporin SlTIP2.3, was associated with differential recruitment of *Streptomyces* in the rhizosphere. Also, the genetic variation of bacterial genes in *Streptomyces* and *Cellvibrio*, involved in the metabolism of plant polysaccharides, iron, sulfur, trehalose, and vitamins, was associated with specific tomato QTLs.

Concluding remarks

The study of the tomato microbiome represents a vital avenue of research with significant implications for enhancing both agricultural productivity and sustainability. Researchers have dedicated their efforts to unravelling the intricate plant–microbiome relationship. The unique tomato plant attributes make it an excellent model for studying various aspects of microbiome research and developing new strategies for optimizing crop management, promoting plant health, and ensuring a resilient food supply in the face of a changing climate. Microbiome studies have traditionally focused on bacterial communities, but there is growing recognition of the importance of fungal communities (mycobiome) for improving the growth and health of crops, including tomatoes. Furthermore, the interplay between bacterial and fungal communities can have a profound impact on plant phenotypes, making it important for further microbiome research to consider both microbial kingdoms. Methods of studying the effects of domestication on the tomato microbiome include trait selection and innovative crop management practices. By exploring these aspects, we can gain valuable insights into establishing persistent mutualistic interactions between the plant and its associated microbiome. This knowledge will enable the optimization of agricultural practices to maximize tomato yield and quality. Furthermore, by using modern breeding tools such as GWASs, marker-assisted selection, CRISPR, and interspecific crosses, it is possible identify the genetics of wild and modern tomatoes to optimize the microbiome–plant mutualistic interactions into modern agroecosystems.

The novel approach of profiling tomato wild microbiomes in their centers of origin presents an exciting opportunity for revitalizing the fitness of cultivated tomato plants. By harnessing beneficial traits inherent in wild microbiomes and transferring them to modern cultivated varieties, we can develop effective strategies to counteract pests,

diseases, and environmental stresses that currently limit tomato productivity. Furthermore, investigating chemical signals in wild and domesticated tomatoes, such as root exudates and volatiles, offers a means of manipulating and controlling the microbiome composition. This knowledge can be leveraged to effectively engineer plants associated with microbial communities that promote plant growth, suppress pathogens, and enhance stress tolerance.

On the microbial front, the emerging field of microbial genomics provides a deeper understanding of the genetic mechanisms underlying the adaptation of microorganisms to their tomato hosts. By unraveling the genomic landscape of these interactions, targeted interventions and microbiome-based strategies can be developed to enhance tomato productivity and resilience. By addressing these areas of focus, we can advance our understanding of the tomato microbiome and harness its potential to improve plant health, increase productivity, and promote sustainable agricultural practices. This multidisciplinary approach presents a promising future for tomato cultivation and expands the field of microbiome research.

Thesis aim and outline

The overall aim of my PhD thesis was to decipher the impact of tomato domestication on rhizosphere microbiome assembly. More specifically, the objectives were to:

- explore the composition and functional potential of the native rhizosphere microbiome of wild tomato in its center of origin.
- investigate the impact of habitat and tomato genotype domestication on rhizosphere microbiome assembly.
- understand the functional role of the native soil microbiome in tolerance of wild and domesticated tomato to insect herbivory.
- disentangle microbial and plant genetic traits associated with tomato rhizosphere microbiome assembly.

To this end, the taxonomic and functional diversity of the tomato rhizosphere microbiome was investigated both in the tomato's center of origin in the Ecuadorian Andes and in controlled greenhouse assays with agricultural and native soils. Furthermore, the genetic basis for tomato rhizosphere microbiome assembly was investigated by using a recombinant inbred line (RIL) population of a cross between a wild and domesticated tomato species. Overall, the findings obtained in this thesis significantly increased our knowledge about the diversity and functions of the rhizosphere microbiome of wild

crop relatives in their native habitats and pinpointed specific microbiome members that may enhance resilience of the domesticated tomato cultivars to (a)biotic stressors.

Chapter 2 of this thesis explores the taxonomic and functional diversity of the rhizosphere microbiome of wild tomato in its center of origin in Ecuador. Here, the taxonomic composition of the bacterial and fungal rhizosphere microbiome of wild tomatoes grown in the Ecuadorian Andes was characterized by amplicon sequencing and the functional potential of this community was investigated by shotgun metagenome sequencing. We showed that in three different wild tomato populations growing in different soils with different microbiomes in the Ecuadorean Andes, wild tomatoes were able to recruit similar bacterial communities dominated by Enterobacteriaceae. These bacterial taxa showed features related to chemotaxis and motility, as well as phytohormone and antibiotic production, which likely conferred high competitiveness in the rhizosphere of wild tomato. **Chapter 3** describes the differences in the taxonomic composition and functional potential of the bacterial rhizosphere microbiome of different wild and domesticated tomato genotypes grown under controlled conditions in Ecuadorian native and agricultural soils as well as Dutch greenhouse soil. We found microbial composition significantly different along the tomato domestication trajectory, with habitat domestication having a major contribution on microbiome assembly. **Chapter 4** revealed that the soil microbiome plays a critical role in tolerance of wild tomatoes for leaf damage caused by the endemic insect *Prodiplosis longifila.* We found significant associations between specific members and functional traits of the tomato microbiome and tolerance to insect damage. We further showed that depletion of *Actinoplanes* from the rhizosphere microbiome by soil sterilization correlated with increased leaf damage in wild tomato by *P. longifila.* Functions of *Actinoplanes* associated with this phenotype encompassed motility, chorismate and secondary metabolite production. **Chapter 5** addresses the genetic basis in rhizosphere microbiome assembly of tomato. To disentangle the microbial and plant genetic traits associated with microbiome assembly, we studied the taxonomic and metagenomic diversity of the rhizosphere microbiome of a tomato RIL population (N = 100) from a cross between domesticated tomato *Solanum lycopersicum* and wild tomato *Solanum pimpinellifolium*. These analyses revealed reciprocal features associated between specific QTLs of wild and domesticated tomato with key taxa *Cellvibrio* and *Streptomyces* functionality, such as genes involved in metabolism of plant polysaccharides, iron, sulfur, trehalose, and vitamins. **Chapter 6** summarizes the most important findings of this thesis, connecting the results of the different experimental chapters on the impact of tomato domestication on rhizosphere microbiome assembly. I also provide an outlook on potential future research directions, highlighting how incorporating microbial features into management practices could enhance sustainability in modern agriculture.

Chapter 2

Unveiling diversity and adaptations of the wild tomato microbiome in their center of origin in the Ecuadorian Andes

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Abstract

Microbiome assembly has been studied for many plant species and is recognized as a key driver of plant growth and plant tolerance to (a)biotic stresses. To date, assembly of the tomato rhizosphere microbiome has been investigated primarily for commercial varieties and field soils subjected to agricultural management practices, whereas the microbiome of wild tomato genotypes in their native habitats remains largely unexplored. This research focused on distinct populations of *Solanum pimpinellifolium* in three natural habitats in the Ecuadorian Andes to identify the taxonomic and functional diversity of their rhizosphere microbiome. The results showed that, despite genotypic differences among the wild tomato populations, different soil types and soil microbiome compositions, the rhizosphere microbiome showed strikingly compositional similarity across the three habitats. Proteobacteria, in particular taxa classified as Enterobacteriaceae, and specific unclassified fungal taxa were highly represented in the rhizosphere of *S. pimpinellifolum*. Metagenomic analyses suggested that the prevalence of Enterobacteriaceae on wild tomato roots may be explained by several traits, in particular nutrient competition, motility, iron acquisition, membrane transport, stress response, and plant hormone biosynthesis. These results reveal a conserved microbiome signature associated with wild tomato rhizosphere in their center of origin. Just as the genomes of wild crop ancestors provide a valuable source of beneficial traits for breeding cultivated varieties, exploring their microbiome in native environments could uncover microbial taxa and traits that similarly contribute to crop growth and health.

Keywords: rhizosphere microbiome, *S. pimpinellifolium*, center-of-origin, Enterobacteriaceae, metagenomics

Introduction

Tomato holds significant importance for global food security and human health because of its high nutritional value. The incredible domestication journey of tomato through both space and time has left an impact on its genome and microbiome (Blanca et al., 2015; Oyserman et al., 2022; Razifard et al., 2020; Sarango Flores et al., 2023). Hence, the genome of wild tomatoes has been used as valuable source of plant genetic traits for crop improvement strategies (Bai & Lindhout, 2007; Gao et al., 2019; Grandillo et al., 2011; Mata-Nicolás et al., 2020; Wang et al., 2020). Similarly, wild tomato growing in its native habitat is a largely unexplored resource for the discovery of beneficial microorganisms that were depleted or lost during domestication. Native habitats can serve as repositories of a high microbial diversity, potentially reflecting long-term associations linked to the plant's ecological niche and adaptation to specific, even harsh, environmental conditions. However, such beneficial native associations may have become diminished or unnecessary under modern agricultural settings (Barajas et al., 2020). This is the case with the wild tomato, *Solanum pimpinellifolium,* the closest relative to domesticated tomato, which grows naturally in the semi-arid regions of southern Ecuador, where it has adapted to high daytime temperatures, water scarcity, and other (a)biotic stresses (Blanca et al., 2012; Peralta et al., 2008; Ramírez-Ojeda et al., 2021; Razifard et al., 2020). Hence, microbial communities that co-evolved associations under such native selective conditions with wild tomatoes may play pivotal roles for the plants' survival (Pérez-Jaramillo et al., 2016; Wallenstein, 2017).

Consequently, there is a growing interest in unraveling the effects of plant domestication on microbiome assembly in the rhizosphere, the narrow zone of soil surrounding and influenced by the plant roots (Alsharif et al., 2020; Berg et al., 2014; Schmidt et al., 2019; White et al., 2017). The rhizosphere microbiome plays an important role in nutrient cycling and plant health (Chepsergon & Moleleki, 2023; Cordovez et al., 2019; Hayat et al., 2010; Trivedi et al., 2020). Therefore, understanding the taxonomic and functional diversity of the rhizosphere microbiome of wild tomatoes in their native habitats will be essential to pinpoint microbial traits that co-evolved with its host and that may have been depleted or lost through domestication and subsequent breeding. In turn, these 'missing' microorganisms or their beneficial traits could be a valuable resource for making domesticated tomato more resilient to (a)biotic stresses.

Here, we collected rhizosphere samples of three populations of wild tomato *S. pimpinellifolium* growing naturally in the Andean Mountain range in southern Ecuador, an area that is part of the species' center of origin. We reveal that these wild tomatoes were able to assemble taxonomically similar rhizosphere bacteriomes despite growing in different regions, varying soil types with variable microbiomes, even for phylogenetically different wild tomato populations. In particular, Enterobacteriaceae dominated the rhizosphere bacteriome of *S. pimpinellifolium*. Results of the metagenomic analyses further suggest that this dominance may be due to specific traits associated with high competitivity and colonization efficiency. Collectively, these findings improve our fundamental knowledge about the root microbiome of wild crop relatives grown in their native habitats and pinpoint specific microbiome members that may enhance resilience of the domesticated crop cultivars to (a)biotic stress factors.

Results

Genetic diversity and genomic relationships of wild tomato populations

DArT-SNP genotyping of the 37 tomato samples, including 34 wild tomato plants, two accessions of *S. pimpinellifolium* (LPI and SPI), and one accession of domesticated *S. lycopersicum* cv. Moneymaker (MON), resulted in five genotypic clusters consistent with the three sampling sites (Calvas, Paltas, Zapotillo – three clusters), LPI and SPI accessions (one cluster) and MON accession (one cluster) (Figure 1a). Furthermore, the genetic diversity of the 34 wild tomato samples was significantly different among the three sites (PERMANOVA, $R^2 = 0.4790$, $p = 1e\text{-}4$).

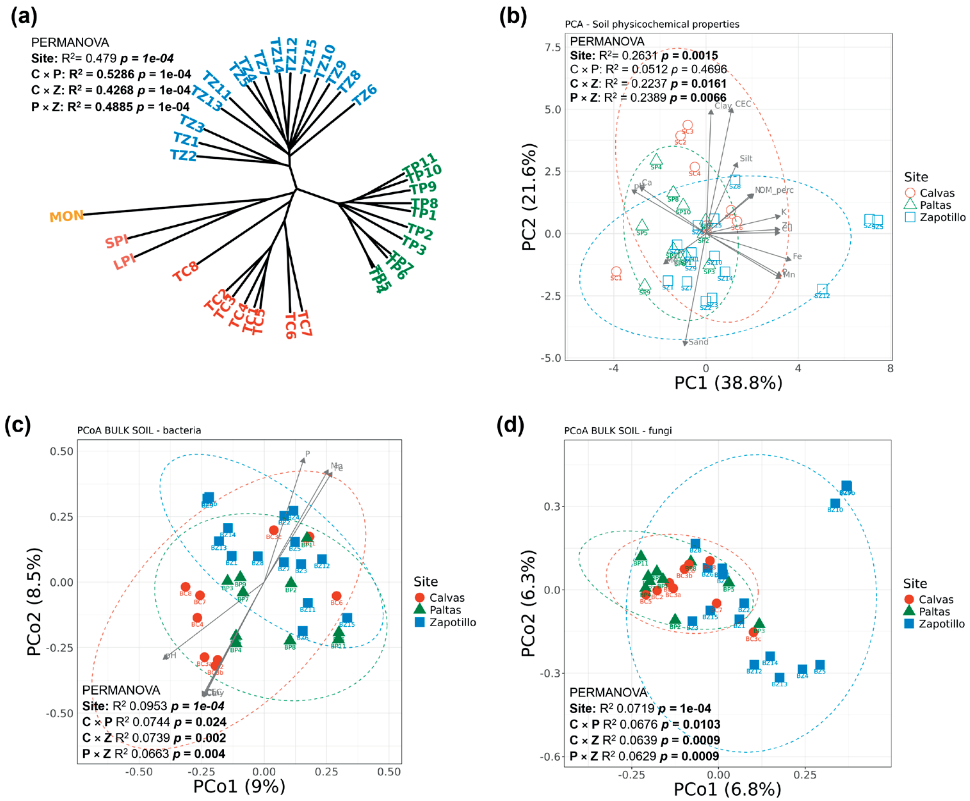

Figure 1. Genetic diversity of wild tomato plants, variation in soil properties and soil microbial communities across sampling sites. (a) Genetic diversity of 34 wild tomato samples collected from Calvas (TC), Paltas (TP), and Zapotillo (TZ) based on DArT SNP analysis; the wild tomato accessions *S. pimpinellifolium* (SPI, LPI) and the domesticated tomato *S. lycopersicum* cv. Moneymaker (MON) are included as references **(b)** Principal Component Analysis (PCA) of soil physicochemical properties based on the Euclidean distance across the three sampling sites; **(c)** Principal Coordinates Analysis (PCoA) of bulk soil bacterial communities based on the Bray–Curtis distance between the three sampling sites and their correlation with soil physicochemical properties; **(d)** PCoA of bulk soil fungal communities based on the Bray–Curtis distance between the three sampling sites. Labels BC, BP and BZ refer to bulk soil from Calvas, Paltas, Zapotillo sites, respectively. For each pairwise comparison between sampling sites (C, P, Z), statistically significant differences based on PERMANOVA are indicated.

Soil physicochemical properties and bulk soil microbiome composition

The physicochemical properties of the native soils were significantly different between the sampling sites (PERMANOVA, $R^2 = 0.2631$, $p = 0.0015$). However, soils from Calvas and Paltas showed similar physicochemical properties ($p = 0.4696$). The first principal component showed that soils from Calvas and Paltas were characterized by higher pH, Ca and Mg, while Zapotillo soils were characterized by higher content of

Fe, Mn, P, K, Cu and Zn. The second principal component was represented mainly by soil texture, with soils from Calvas and Paltas, in general, characterized by higher clay, silt and CEC values whereas soils from Zapotillo were characterized by higher content of sand (Figure 1b; Supplementary Table S3).

No statistically significant differences were found for the alpha diversity (Shannon index) of the bacterial communities in the soils from the different sites (ANOVA, p = 0.865), but significant differences were found for the fungal communities (ANOVA, p = 0.0347). More specifically, a lower alpha diversity was found for the fungal communities in soils from Paltas compared to soils from Calvas and Zapotillo. Simpson index confirmed a similar trend for bacterial communities (ANOVA, p = 0.983) and fungal communities (ANOVA, p = 0.0033), with all sites showing relatively comparable values, indicating similar community dominance (Supplementary Figure S5; Supplementary Table S4). The beta diversity was significantly different among the sites for both bacterial (PERMANOVA, p = 1e-4, R^2 = 0.0953; Figure 1c) and fungal communities (PERMANOVA, p = 1e-4, R^2 = 0.072; Figure 1d; Supplementary Table S5). In addition, significant relationships were found between physicochemical soil properties and bacterial diversity in native soils (Mantel test, ρ = 0.1973, p = 0.0256), but not between the soil properties and the fungal community diversity (Mantel test, ρ = 0.0181, p = 0.4081). Seven out of 15 physicochemical soil properties correlated with the bacterial community distribution in the different native soils. These included Ca (R^2 = 0.35), clay (R^2 = 0.30), CEC (R^2 = 0.29) and pH (R^2 = 0.30) for soils from Calvas and Paltas, and Fe (R^2 = 0.33), Mn (R^2 = 0.24) and P (R^2 = 0.24) for soils from Zapotillo (Figure 1c).

Wild tomatoes assemble similar rhizosphere bacteriomes across native sites

Bacterial communities differed between bulk soil and rhizosphere (PERMANOVA, p = 1e-4, R^2 = 0.094) (Supplementary Figure S6a; Supplementary Table S5). The alpha diversity of the bacterial community of the rhizosphere of wild tomato, exemplified by the Shannon (ANOVA, p = 0.0526) and Simpson (ANOVA, p = 0.545) indices, was similar for each of the three sampling sites (Figure 2a; Supplementary Table S4). Despite significant differences between the bulk soil bacterial communities of the three sampling sites, wild tomato rhizosphere bacterial communities were mostly similar across the sites based on Bray–Curtis distance, except for the rhizosphere bacteriomes of wild tomatoes from Paltas (P) and Zapotillo (Z) (PERMANOVA, p = 0.0183, R^2 = 0.074) (Figure 2b; Supplementary Table S5). For the three sites combined, no significant relationships were found between the wild tomato genetic diversity and rhizobacterial abundance (Mantel test, ρ = -0.1202, p = 0.9184) nor between physicochemical soil properties and rhizobacterial abundance (Mantel test, ρ = 0.0582, p = 0.2798). For

the sampling sites separately, significant correlations were found between specific soil properties and rhizobacterial community composition for Paltas (Ca: R^2 = 0.23, p = 0.0478) and for Zapotillo (Cu: R^2 = 0.30, p = 0.0046) (Supplementary Figure S7).

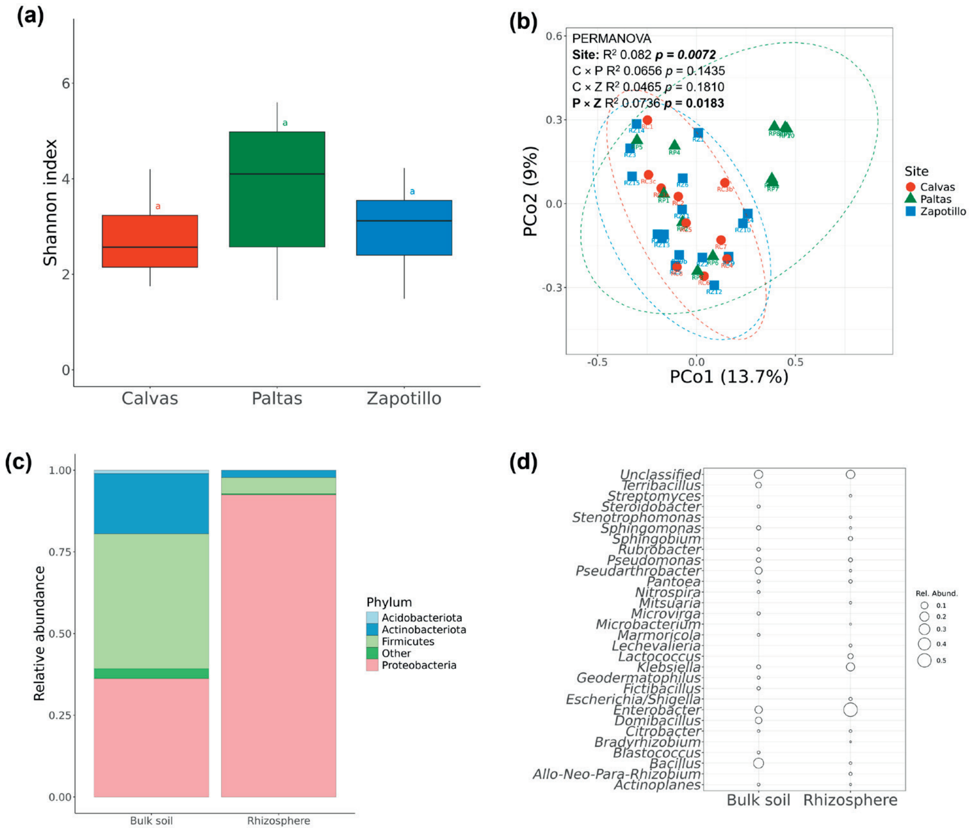

Figure 2. Bacterial diversity in wild tomato rhizosphere. (a) Alpha diversity of the bacterial communities in wild tomato rhizosphere based on Shannon diversity index according to each sampling site (Calvas n = 8; Paltas n = 11; Zapotillo n = 15); no significant differences were found by Tukey HSD test $p < 0.05$; **(b)** Principal Coordinates Analysis (PCoA) of bacterial communities based on the Bray–Curtis distance between rhizospheres of the three sampling sites; for each pairwise comparison between sampling sites statistically significant differences based on PERMANOVA are indicated; **(c)** Relative abundance of bacterial phyla in bulk soil and rhizosphere of wild tomato; "Other" category corresponds to grouped phyla with relative abundance < 0.01; **(d)** Relative abundance of highest abundant (top 20) genera found in bulk soil and rhizosphere of wild tomato. ASVs with significant differential abundance between bulk soil and rhizosphere were grouped according their phylum or genera and plotted as stacked bar and bubble charts, respectively.

Differential abundance analysis of the bacterial community composition of the pooled bulk soils and tomato rhizosphere samples identified 197 ASVs as significantly different, i.e., 111 were more abundant in bulk soil and 86 more abundant in the tomato rhizosphere. In bulk soil, the majority of the ASVs belonged to the Firmicutes (41%), Proteobacteria (36%) and Actinobacteriota (18%). In the rhizosphere of wild tomato, abundant ASVs mainly belonged to Proteobacteria (92%), Firmicutes (5%) and Actinobacteriota (2%) (Figure 2c). At the genus level, the rhizosphere of wild tomatoes was characterized by a higher relative abundance of *Enterobacter, Klebsiella, Sphingobium, Escherichia/Shigella, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium, Lactococcus,* and *Lechevalieria* (Figure 2d).

Wild tomatoes assemble distinct rhizosphere mycobiomes across native sites

The rhizosphere mycobiome was significantly different between Paltas and the other two sampling sites, Calvas and Zapotillo, based on the Shannon (ANOVA, $p = 0.0094$) and Simpson (ANOVA, $p = 0.0003$) diversity indices (Figure 3a; Supplementary Table S4). Both indices showed lower values at Paltas, indicating higher community dominance by a few ASVs compared to Calvas and Zapotillo. The beta diversity of fungal communities differed between bulk soil and rhizosphere (PERMANOVA, $R^2 = 0.098$, $p = 1e\text{-}4$) (Supplementary Figure S6b), as well as between the three sampling sites (PERMANOVA, $R^2 = 0.1134$, $p = 1e\text{-}4$) (Figure 3b; Supplementary Table S5). Furthermore, a significant relationship was observed between the tomato genetic diversity and rhizosphere fungal abundance (Mantel test, $\rho = 0.1936$, $p = 0.0093$), but not between the physicochemical soil properties and rhizosphere fungal abundance (Mantel test, $\rho = -0.0217$, $p = 0.4665$).

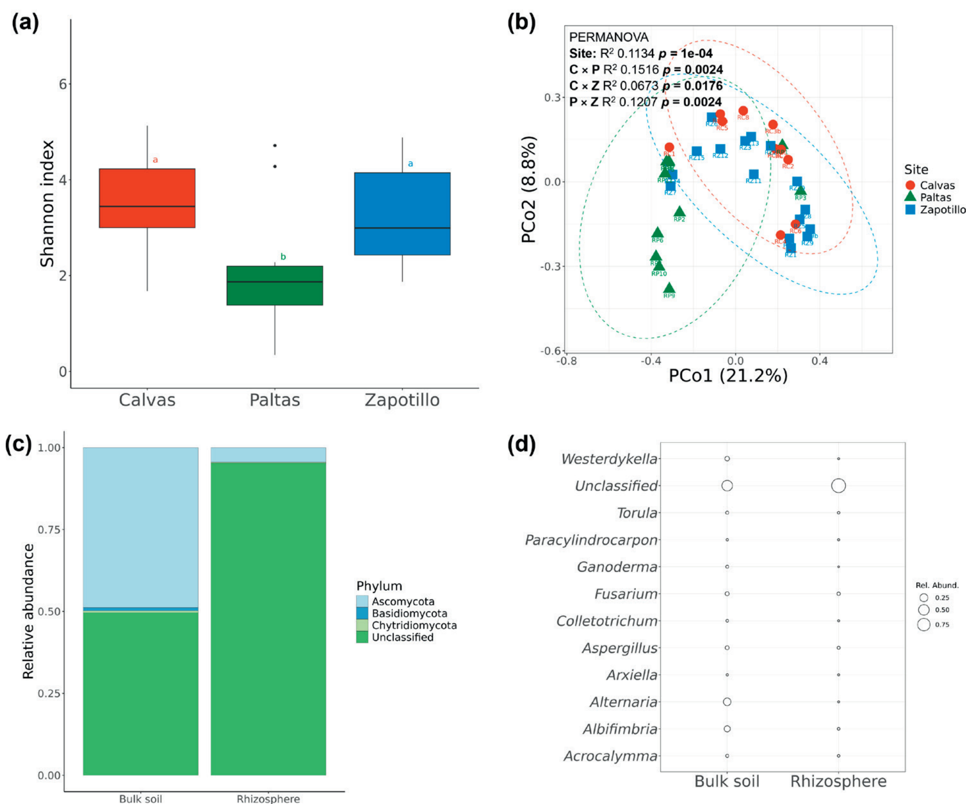

Figure 3. Fungal diversity in wild tomato rhizosphere. **(a)** Alpha diversity of the fungal communities in wild tomato rhizosphere based on Shannon diversity index according to each sampling site (Calvas n = 8; Paltas n = 11; Zapotillo n = 15); different letters above boxplots show significant difference by Tukey HSD test ($p < 0.05$); **(b)** Principal Coordinates Analysis (PCoA) of fungal communities based on the Bray–Curtis distance between rhizospheres of the three sampling sites; for each pairwise comparison between sampling sites statistically significant differences based on PERMANOVA are indicated; **(c)** Relative abundance of fungal phyla in bulk soil and rhizosphere of wild tomato; **(d)** Relative abundance of major fungal genera in bulk soil and rhizosphere of wild tomato. ASVs with significant differential abundance between bulk soil and rhizosphere were grouped according their phylum or genera and plotted as stacked bar and bubble charts, respectively.

Differential abundance analysis of the fungal community composition of the pooled bulk soils and tomato rhizosphere samples revealed 18 ASVs significantly different, 6 ASVs for the bulk soil and 12 for the tomato rhizosphere. The more abundant ASVs in the bulk soil were unclassified fungi (49.6%), Ascomycota (48.8%), Basidiomycota (1%) and Chytridiomycota (0.6%) phyla, while in the wild tomato rhizosphere mycobiome, the unclassified fungi (95.3%) were further enriched, whereas the relative abundance of the Ascomycota (4.5%) and Chytridiomycota (0.2%) was reduced as compared to the bulk soil (Figure 3c). ASVs of abundant classified fungi in the rhizosphere were taxonomically delineated as *Fusarium* and *Aspergillus* (Figure 3d).

Functional diversity of the wild tomato rhizosphere bacteriome

Analyzing the significantly enriched rhizosphere bacterial ASVs for each sampling site, two ASVs were found to be conserved in the wild tomato rhizosphere across all three sites; these ASVs were taxonomically assigned as Enterobacteriaceae (ASV13; 2.41% relative abundance) and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (ASV198; 0.12% relative abundance) (Supplementary Figure S8 and S9). To gain insights into the functional diversity of the wild tomato rhizosphere, particularly regarding these two ASVs, we conducted a shotgun sequence analysis of 24 rhizosphere DNA samples (7 from Calvas; 8 from Paltas and 9 from Zapotillo). This analysis resulted in four metagenome-assembled genomes (MAGs) belonging to the major rhizosphere enriched phyla in the 16S amplicon data (Figure 2c), two of them were assigned to the Enterobacteriaceae family (Proteobacteria, bin 074 and bin 136), one bin to the genus *Lactiplantibacillus* (Firmicutes, bin 310) and one to the Micrococcaceae (Actinobacteriota, bin 296) (Figure 4). Unfortunately, no high-quality bins assigned as *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* could be assembled (Supplementary Table S6).

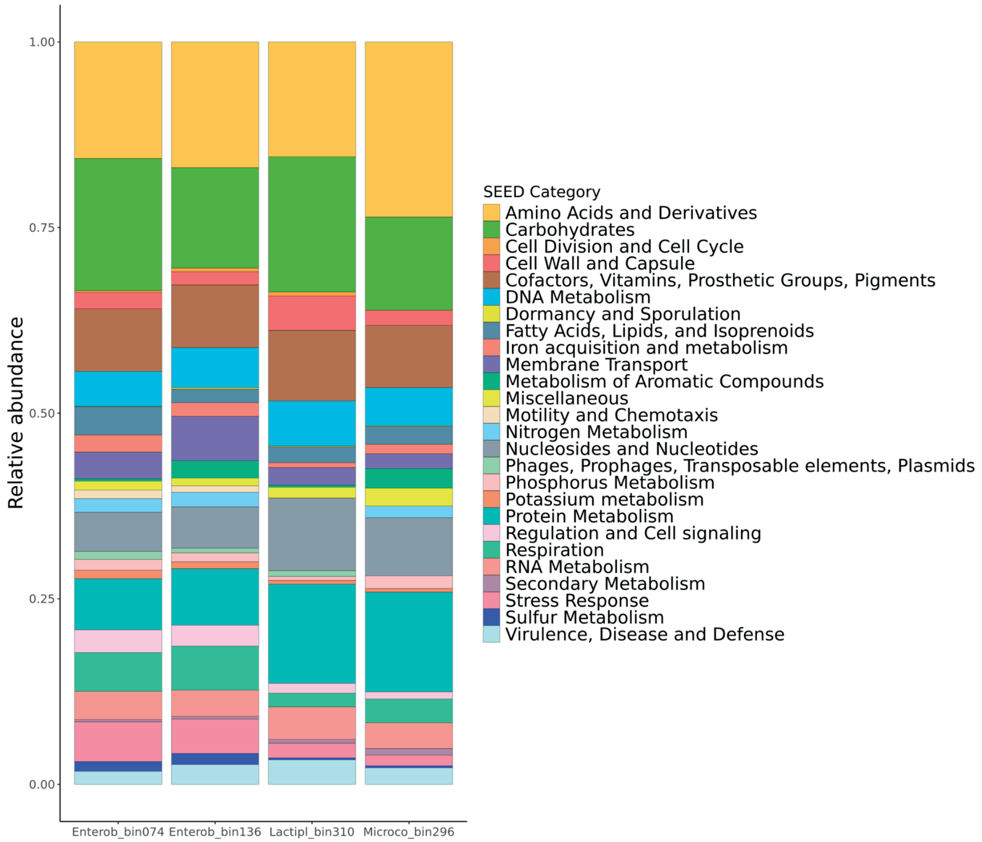

Figure 4. Annotation of bacterial metagenome-assembled genomes. Relative abundance of SEED categories annotated by RAST (Rapid Annotation using Subsystems Technology) from MAGs of Enterobacteriaceae (bins 074 and 136), *Lactiplantibacillus* (bin 310), and Micrococcaceae (bin 296) found in wild tomato rhizosphere. Protein encoding genes were grouped according their SEED categories and plotted as stacked bar charts.

Using the SEED subsystems database, the RAST server identified in the Enterobacteriaceae MAGs the highest number of protein-encoding genes (bin 074: 1307; bin 136: 1418), *Lactiplantibacillus* (bin 310: 757) and Micrococcaceae (bin 296: 1035 genes) (Supplementary Table S7). These four MAGs mostly included genes associated with SEED categories involved in the synthesis of amino acid and derivatives; carbohydrates; cofactors, vitamins, prosthetic groups, pigments; DNA metabolism; nucleosides and nucleotides; protein metabolism; RNA metabolism; virulence, diseases and defense (Figure 4). In addition, the Enterobacteriaceae MAGs were characterized by a relatively high number of annotated genes associated with iron acquisition and metabolism, membrane transport, nitrogen metabolism, phosphorus metabolism, potassium metabolism, regulation and cell signaling, respiration, stress response, and sulfur metabolism. Furthermore, these MAGs harbored genes involved in motility and chemotaxis. *Lactiplantibacillus* bin 310 included mostly genes associated with cell wall and capsular polysaccharides and Micrococcaceae bin 296 included genes associated with metabolism or aromatic compounds and secondary metabolism (Table 1). Biosynthesis of the plant hormone auxin was a common secondary metabolism trait identified for all four MAGs representing the Enterobacteriaceae, *Lactiplantibacillus* and Micrococcaceae (Table 1).

Table 1. Main SEED subsystems found in MAGs of Enterobacteriaceae (bins 074 and 136), *Lactiplantibacillus* (bin 310), and Micrococcaceae (bin 296) associated with wild tomato *S. pimpinellifolium* rhizosphere.

Bacteria	Category	Subcategory	Subsystem	No. of genes
Enterobacteriaceae bin 074 and bin 136	Motility and Chemotaxis	Flagellar motility in Prokaryota	Flagellar motility	12
	Iron acquisition and metabolism	Iron acquisition and metabolism - no subcategory	Encapsulating protein for DyP-type peroxidase and ferritin-like protein oligomers	2
			Hemin transport system	5
		Siderophores	Siderophore Aerobactin	11
			Siderophore Enterobactin	14
	Membrane Transport	Protein secretion system, Type I	Type I secretion system for aggregation	4
		Protein and nucleoprotein secretion system, Type IV	IncF Conjugal Transfer System	21
			Conjugative transfer	21
			Type IV pilus	10
		Protein secretion system, Type V	Two partner secretion pathway (TPS)	2
		Protein secretion system, Type VII (Chaperone/ Usher pathway, CU)	sigma-Fimbriae	5
			Type 1 pili (mannose-sensitive fimbriae, gamma-fimbriae)	2
	Stress Response	Osmotic stress	Osmoprotectant ABC transporter YehZYXW of Enterobacteriales	4
			Synthesis of osmoregulated periplasmic glucans	4
		Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	3
			Glutathione: Non-redox reactions	10
			Glutathione: Redox cycle	5
			Glutathionylspermidine and Trypanothione	2
	Sulfur Metabolism	Inorganic sulfur assimilation	Inorganic Sulfur Assimilation	16
		Organic sulfur assimilation	Alkanesulfonate assimilation	3
		Sulfur Metabolism - no subcategory	Galactosylceramide and Sulfatide metabolism	1
	Secondary Metabolism	Plant Hormones	Auxin biosynthesis	5
Lactiplantibacillus bin 310	Cell Wall and Capsule	Capsular and extracellular polysaccharides	Sialic Acid Metabolism	11
		Gram-Positive cell wall components	D-Alanyl Lipoteichoic Acid Biosynthesis	3
	Secondary Metabolism	Plant Hormones	Auxin biosynthesis	4

Table 1. Main SEED subsystems found in MAGs of Enterobacteriaceae (bins 074 and 136), *Lactiplantibacillus* (bin 310), and Micrococcaceae (bin 296) associated with wild tomato *S. pimpinellifolium* rhizosphere. (*continued*)

Bacteria	Category	Subcategory	Subsystem	No. of genes
Micrococcaceae bin 296	Metabolism of Aromatic Compounds	Metabolism of central aromatic intermediates	Protocatechuate branch of beta-ketoadipate pathway	9
		Metabolism of central aromatic intermediates	Central meta-cleavage pathway of aromatic compound degradation	4
	Secondary Metabolism	Hydrocarbons	Alkane synthesis in bacteria 2	4
		Plant Hormones	Auxin biosynthesis	5

Next, mining of the MAGs for biosynthetic gene clusters (BGC) using antiSMASH revealed BGCs for carotenoids (terpene), dichrysobactin (NRP) and aerobactin (siderophore) in the Enterobacteriaceae MAGs. For the *Lactiplantibacillus* bin 310, seven BGCs were predicted with no or low similarities (<50%) to known metabolites. Also, for Micrococcaceae bin 296, a BGC with 100% similarity to a carotenoid (terpene) BGC was found (Table 2; Supplementary Figure S10–S13).

Table 2. Biosynthetic gene clusters (BGCs) predicted by antiSMASH bioinformatics tool from MAGs of Enterobacteriaceae (bins 074 and 136), *Lactiplantibacillus* (bin 310), and Micrococcaceae (bin 296) highly associated with wild tomato *S. pimpinellifolium* rhizosphere.

Bin ID	Length (nt)	antiSMASH type predictor	Secondary metabolite	Similarity	Reference in MiBIG database	Organism in MiBIG database
Enterobacteriaceae bin 074	23,579	Terpene	Carotenoid	100%	BGC0000642	Enterobacteriaceae bacterium DC413
Enterobacteriaceae bin 136	19,062	Terpene	Carotenoid	100%	BGC0000640	Enterobacteriaceae bacterium DC404
Enterobacteriaceae bin 136	14,409	NI-siderophore	Aerobactin	100%	BGC0001499	*Pantoea ananatis*
Enterobacteriaceae bin 136	39,218	NRP-metallopore, NRPS-like	Trichrysobactin, cyclic trichrysobactin, chrysobactin, dichrysobactin	84%	BGC0002414	*Dickeya chrysanthemi*
Enterobacteriaceae bin 136	43,855	NRPS-like	Minimycin	60%	BGC0002295	*Streptomyces hygroscopicus*
Lactiplantibacillus bin 310	26,778	NRPS	Mutanocyclin, leuvalin, tyrvalin	46%	BGC0002287	*Streptococcus mutans*
Micrococcaceae bin 296	17,892	Terpene	Carotenoid	100%	BGC0000633	*Streptomyces avermitilis*

Discussion

To enhance our understanding of the diversity and microbial assembly in the rhizosphere of wild tomatoes in their center of origin in Ecuador, we analyzed the microbiome of bulk and rhizosphere soils from *S. pimpinellifolium* plants at flowering and fruiting developmental stage. These plants were growing naturally at three different sites in Loja province, southern Ecuador (Calvas, Paltas and Zapotillo). Our results showed different soil properties among these sites, with soils from Zapotillo displaying higher content of Fe, Mn, P, K, Cu, Zn and sand than soils from Calvas and Paltas. Wild *S. pimpinellifolium* typically grow in warm and dry climates of the Western Andes (Kimura & Sinha, 2008; Peralta et al., 2008; Ramírez-Ojeda et al., 2021; Waheed et al., 2019). In Ecuador, *S. pimpinellifolium* occurs at high density in river valleys in semi-arid habitats of the 'Low Andes' region, where a natural depression in the Andes mountains occurs (Blanca et al., 2012; Peralta & Spooner, 2000; Ramón, 2008; Zuriaga et al., 2009). We sampled a total of 34 wild tomato individuals which were distributed mainly in places with temperatures around 30 ºC (Calvas: 29.8, Paltas 30.4, and Zapotillo 31.1 ºC) and dry environment with on average a relative humidity of only 7% (Calvas: 7.5, Paltas 6.6, and Zapotillo 6.1%) recorded during the field work.

The DArT genotyping results showed that the genetic diversity of the wild tomatoes corresponded to the sampling sites, suggesting limited spread of and gene flow between the wild tomato populations and adaptations to the abiotic factors prevalent in these geographically separated sites (Caicedo & Schaal, 2004; Kahlon et al., 2020; Mata-Nicolás et al., 2020). Particularly, climatic adaptation to temperature and precipitation has been proposed earlier for the genetic divergence of *S. pimpinellifolium* among regions (Lin et al., 2020; Zuriaga et al., 2009).

Microbiome analyses of the soils revealed that the bacterial and fungal community compositions were different among the native soil sites. Overall, soil bacteriomes mainly consisted of members of Firmicutes, Proteobacteria and Actinobacteriota, while soil mycobiomes included Ascomycota, Unclassified fungi and Basidiomycota. Similar microbiome composition profiles were described by Lee et al. (2019) in different locations of cultivated tomatoes in South Korea. However, we found that only bulk soil bacterial community variation correlated with soil physicochemical properties, especially with pH, CEC, clay, Ca, P, Mn and Fe (Figure 1c). Soil texture provides microenvironments and determines water and nutrient retention (Kandasamy et al., 2021). For example, higher pH, CEC and clay content can explain a base saturation associated with higher soil fertility due to the readily available nutrients (Zheng et al., 2019). Furthermore, a correlation between soil texture and phosphorus content has been reported, where organic phosphorus forms were predominant in sandy soils and inorganic forms in finer

textured soils (Ducousso-Détrez et al., 2022). With reference to nutrients, Ca content can alter the bacterial composition of the soil by increasing Firmicutes and Actinobacteriota and decreasing Gammaproteobacteria (Shabtai et al., 2023), as observed in the bulk soil from Paltas and Zapotillo (Supplementary Figure S6c).

Contrary to the soil bacteriome, the soil mycobiome diversity could not be explained by prevailing soil physicochemical properties. Previous studies have shown that edaphic parameters are poor predictors of the fungal diversity, whereas climatic variables such as aridity, precipitation, temperature, as well as plant cover or litter accumulation, are stronger predictors of fungal diversity (Egidi et al., 2019; Kazerooni et al., 2017; Liu et al., 2020; Maul et al., 2014; Wu et al., 2008; Wubet et al., 2012). Fungal phyla, such as Ascomycota, along with a significant proportion of unclassified fungi according to the UNITE database, were highly abundant in the native soils, including genera like *Alternaria*, *Albifimbria* and *Westerdykella* (Figure 3d). The presence of a distinct group of 'unclassified fungi' highlights the limitations of current fungal databases and suggests that several fungal species in Southern Ecuador remain undiscovered or uncharacterized. Furthermore, this underscores our limited understanding of the soil mycobiome of this region, which represents an opportunity for future research. This composition as well as the high abundance of unidentified fungal taxa at phylum and genus levels were consistent with previous studies in arid environments (Murgia et al., 2018; Vikram et al., 2023). Ascomycota phylum dominates soils globally, which is related with the versatile trophic capabilities for resource utilization, competition and stress tolerance (Egidi et al., 2019). For example, members of this phylum include dark septate fungi characterized by their melanin pigment, which allows survival in arid conditions and confers a competitive advantage over other fungi lacking these adaptations (Challacombe et al., 2019). Basidiomycota is a diverse fungal group that includes mushrooms, smuts, rusts and yeasts, whose main contribution to the soil ecosystem is lignocellulose decomposition from wood and leaf litter (He & Zhao, 2021; Šnajdr et al., 2011). Fungi belonging to the Chytridiomycota possess motile zoospores propelled by a posterior flagellum. Due to their smaller size, their rhizoid can attach to various substrates, including hard and resistant solids as sand or pollen grains. Additionally, Chytridiomycota efficiently digest cellulose, chitin and protein from soil organic matter and can respond to drought by forming desiccation-resistant sporangia as defense mechanisms (Freeman et al., 2009; Hanrahan-Tan et al., 2023).

Rhizosphere bacteriome assembly of the wild tomato *S. pimpinellifolium* at flowering and fruiting developmental stages revealed not significantly different alpha and beta diversities among sampled sites. Moreover, the variation of the rhizobacterial composition could not be explained by the measured physicochemical soil properties nor by genotype diversity. These results suggest that *S. pimpinellifolium* as a host exerts selective pres-

sure to orchestrate its bacterial community composition within its native habitat (R^2 = 0.082; Figure 2b), despite significant differences in soil types and soil bacteriomes. We further found that the persistent enriched bacteria ASVs in rhizosphere samples were Enterobacteriaceae and *Rhizobium* (Figure 2d, Supplementary Figure S6e, S8 and S9). On the other hand, when we analyzed the samples from Paltas and Zapotillo separately, we discovered some interesting differences. Although the overall difference was only slightly significant, the variation in the rhizobacterial composition between these two sites could be attributed to the higher Ca content in the soil sampled from Paltas site (Supplementary Figure S7). This finding highlights the importance of site-specific environmental factors in microbial assembly. For example, Ca can modulate plant-microbe interactions and plant responses to abiotic stresses such as salt, drought, heat, and cold, by mediating signaling pathways that activate defense-related gene expression and phytohormone synthesis (Agaras et al., 2023). Additionally, an increase of Firmicutes and Actinobacteriota in response to higher Ca content has been observed in incubated soils with leaf litter (Shabtai et al., 2023). Furthermore, the type and concentration of calcium salts has been identified as an important factor to enhance Actinobacteriota's cultivation efficiency (Fang et al., 2017).

Fungal communities associated with the wild tomato rhizosphere showed a similar pattern as observed for the rhizosphere bacteriome (Figure 3c; Supplementary Figure S6d), displaying a group of fungi strongly associated with *S. pimpinellifolium* rhizosphere in its native habitat. Differential abundant ASVs assigned as *Fusarium* and *Aspergillus* genera were observed to be more abundant in the wild tomato rhizosphere (Figure 3d; Supplementary Figure S6f). These genera have been abundantly identified also through culturing approaches in tomato rhizosphere (Poli et al., 2016; Tyagi & Tyagi, 2016). The genus *Fusarium* includes well-known pathogenic species, which cause wilt, root necrosis or rot root in tomato (Carmona et al., 2020). During the sampling phase in the field, none of the sampled plants showed symptoms and no wilting was observed. Therefore, it is well possible that *Fusarium* found by amplicon sequencing represents nonpathogenic strains colonizing wild tomato rhizosphere. Nonpathogenic *Fusarium* strains regulate induced systemic resistance (ISR) genes and can elicit ethylene and nitric oxide production that stimulate formation of root hairs (Aparicio et al., 2023; de Lamo et al., 2021; Fuchs et al., 1997; Veloso & Díaz, 2012). Also, *Aspergillus* has been found as a dominant fungus in the tomato rhizosphere (Chen et al., 2022; Gherbawy & Abdelzaher, 1999; Kazerooni et al., 2017) and may serve as a plant growth promoter due to its capacity to produce hydrogen cyanide (HCN), indole-acetic acid (IAA) and siderophores, among other plant beneficial functions (Adedayo & Babalola, 2023; Daigham et al., 2023; Yoo et al., 2018). These results suggest that *S. pimpinellifolium* may also benefit from their native mycobiome, although isolation, genomic

analyses and functional validation with cultured isolates will be needed to support these hypotheses.

In the metagenomic analysis, four high-quality bins were identified representing enriched bacterial genera from the predominant phyla found in the wild tomato rhizosphere, including Proteobacteria, Firmicutes and Actinobacteriota (Figure 2c and 4; Supplementary Table S6). Analyses of the metagenome assembled genomes of the Enterobacteriaceae (bins 074 and 136) revealed functions related to motility and chemotaxis. These bacteria possess flagella for chemotaxis-oriented motility, which in turn enhances their ability to colonize roots. This motility advantage could make Enterobacteriaceae efficient rhizocompetitors (Chepsergon & Moleleki, 2023; Feng et al., 2021; Gao et al., 2016; Knights et al., 2021; López et al., 2023; Lucero et al., 2020). Additionally, secretion systems found in Enterobacteriaceae bins may further enhance their colonization ability in tomato rhizosphere. For example, Type I and V secretion systems, are the 'simplest' secretion bacterial systems (Wells & Henderson, 2013). Type I transports proteins like toxins and adhesins directly from the cytoplasm to the extracellular environment, playing roles in biofilm formation and virulence (Spitz et al., 2019; Tchagang et al., 2018; Wells & Henderson, 2013). While, Type V, particularly the Two-partner secretion system (TPS), facilitates adhesion to specific host cell structures through surface glycans (Leo et al., 2012; Meuskens et al., 2019; Rojas et al., 2002; Wells & Henderson, 2013). As well as, Type IV are involved in the in the biogenesis of conjugative pili for DNA transfer and the delivery of effector proteins or toxins (Costa et al., 2023; Craig et al., 2019), whereas Type VII secretions systems are essential for bacterial adherence to surfaces, since these systems include Type 1 pili that bind to mannose residues on plant tissues (Busch & Waksman, 2012; Gahlot et al., 2023; Geibel & Waksman, 2014) Thus, Type IV and VII facilitate direct interactions between bacteria and host cells (Wallden et al., 2010; Zechner et al., 2012).

For nutrient acquisition, Enterobacteriacea MAGs harbor several gene clusters involved in iron metabolism, including genes for encapsulins which are protein compartments that play roles in iron storage, oxidative stress resistance (Giessen, 2022), and hemin transport systems for acquiring iron from organic sources (Kalidasan et al., 2018; Mimmo et al., 2014). Enterobacteriaceae bins also contained genes related to the production of siderophores, such as enterobactin and aerobactin, which chelate ferric iron (Fe^{3+}) and enhance iron uptake (Ahmed & Holmström, 2014; Timofeeva et al., 2022). Enterobactin, has a higher affinity for iron compared to aerobactin and pyoverdine, and therefore may increase competitiveness in interactions with neighboring rhizosphere microbes (Chepsergon & Moleleki, 2023; Loper & Buyer, 1991; Loper & Henkels, 1999). Enterobacteriaceae MAGs also showed key stress resistance features, such as osmotic stress resistance via osmoprotectant ABC transporters, such as YehZYXW, and

the synthesis of osmoregulated periplasmic glucans, which help regulate intracellular osmolarity and maintain cell homeostasis (Agrawal et al., 2020; Bontemps-Gallo et al., 2017; Frossard et al., 2012; Herrou et al., 2017). Oxidative stress is mitigated by systems involved in glutathione metabolism, which are crucial for redox balance and protection against oxidative damage (Imlay, 2015; Lin et al., 2015; Zhang et al., 2005). In addition, periplasmic stress responses in Enterobacteriaceae included genes that assist in the folding and degradation of outer-membrane proteins, essential for bacterial growth under high-temperature conditions (Dai et al., 2015; Raivio & Silhavy, 2001).

Only one high-quality metagenome assembled Firmicutes, another rhizosphere enriched lineage, was identified (Bin 310, representing *Lactiplantibacillus,* Supplementary Table S6). This *Lactiplantibacillus* MAG exhibited genes related to cell wall and capsule functions, specifically D-alanyl/lipoteichoic acid biosynthesis and sialic acid metabolism. Teichoic acids, comprising 30-70% of the Gram-positive cell wall, are vital for ion homeostasis, envelope assembly, flexibility and permeability, and aggregation (Heaton & Neuhaus, 1994; Poyart et al., 2001; Saar-Dover et al., 2012; Schneewind & Missiakas, 2016). Furthermore, D-alanylated teichoic acids in *Lactobacillus plantarum* optimize nutrient uptake through intestinal peptidase activity in *Drosophila* (Matos et al., 2017). Sialic acid, found in bacterial capsules, may assist bacteria to water conservation and competitive adhesion ability in arid conditions (Li & Chen, 2012; Lu et al., 2021; Sakellaris et al., 1988; Vimr et al., 2004). As sialic acids are not present in plants or Archaea (Traving & Schauer, 1998; Vimr et al., 2004), it is likely *Lactiplantibacillus* may depend on other bacteria or fungi inhabiting the wild tomato rhizosphere as sources of this compound.

In the same way, bin 296, linked to Micrococcaceae, was the only one Actinobacteriota family MAG (Supplementary Table S6), harboring genes primarily involved in the metabolism of aromatic compounds through the protocatechuate branch and central meta-cleavage pathway. These pathways include aromatic compound and lignin degradation products like protocatechuic acid and catechol, which are crucial for energy, adaptation to suboptimal growth conditions, and protection against toxicity of aromatic compounds (Díaz et al., 2013; Doron et al., 2023; Huccetogullari et al., 2019; Zhu et al., 2018). Additionally, bin 296 included genes for alkane synthesis from fatty acids, such as those coding for 3-oxoacyl-ACP synthase III and haloalkane dehalogenase-like proteins (Geng et al., 2023; Wang & Lu, 2013). While the precise function of alkane synthesis in bacteria is not fully understood, it is hypothesized that this process may contribute in protecting against temperature fluctuations or dehydration (Carro et al., 2022; Geng et al., 2023).

It is noteworthy that all four analyzed MAGs harbored the genes for auxin biosynthesis, including anthranilate phosphoribosyltransferase, phosphoribosylanthranilate isomerase, and tryptophan synthase. Additionally, the presence of monoamine oxidase suggests involvement in tryptophan degradation for auxin synthesis (Parthasarathy et al., 2018; Patten & Glick, 2002). Auxin, particularly indole-3-acetic acid (IAA), is critical for plant growth and development, influencing cell enlargement, tissue differentiation, and root modification, by promoting the formation of root hairs and lateral roots, which enhances nutrient and water uptake and overall plant health (Chieb & Gachomo, 2023; Glick, 2014; Spaepen et al., 2007; Spaepen & Vanderleyden, 2011; Vacheron et al., 2013).

In the context of secondary metabolism, selected MAGs associated with the wild tomato rhizosphere in its native habitat were analyzed using bacterial antiSMASH for biosynthetic gene clusters (BGCs) annotation (Table 2; Supplementary Figure S9–S12). BGCs are groups of genes that are physically clustered and encode pathways for the biosynthesis of specialized metabolites in bacteria, fungi, and plants (Medema et al., 2015). Our analysis revealed that bins from Enterobacteriaceae and Micrococcaceae contain BGCs associated with carotenoid production. Carotenoids in soil and rhizosphere might can serve as precursors for abscisic acid (ABA), a key phytohormone involved in regulating water use during drought conditions and influencing root growth (Fiodor et al., 2021; Fischer et al., 2011; Swapnil et al., 2021). Additionally, carotenoids are known for their role in oxidative stress response, either by scavenging reactive oxygen species (ROS) or by reinforcing cell membranes to prevent oxidative damage (Avalos et al., 2022). Furthermore, non-ribosomal peptide synthase (NRPS) related BGCs were annotated in Enterobacteriaceae bin 136 and *Lactiplantibacillus* bin 310. In Enterobacteriaceae, we identified BGCs for trichrysobactin/cyclic trichrysobactin/chrysobactin/dichrysobactin and aerobactin. The production of these siderophores is critical for coping with fluctuations in iron availability (Franza & Expert, 2013; Persmark et al., 1989; Sandy & Butler, 2011). The presence of multiple iron uptake systems provides a competitive advantage for colonizing hosts (Franza & Expert, 2013). In *Lactiplantibacillus* bin 310, the NRPS-related BGCs with the highest similarity to known compounds were those associated with mutanocyclin/leuvalin/tyrvalin. Although the exact functions of these compounds remain unclear, they are hypothesized to play roles in host–microbiome interactions, potentially through regulation of electron transfer process and biofilm formation (Barber & Zhang, 2021; Hao et al., 2019).

This study provides a comprehensive analysis of the rhizosphere microbiome composition of wild tomato *S. pimpinellifolium* growing in its native habitat. Our findings revealed that despite variability among the wild tomato genotypes, physicochemical soil properties, and soil microbiomes, similar bacterial communities were assembled in

the rhizosphere while exhibiting distinct fungal communities. The amplicon sequencing approach highlighted a strong and consistent association of Enterobacteriaceae and unknown fungi with the wild tomato rhizosphere across all sampled sites. This consistent predominance underscores the specialized roles of these microorganisms may play in the rhizosphere, potentially enhancing growth and resilience of the wild tomato plants in the harsh native environment. Furthermore, metagenomic analysis of bacterial bins from the Enterobacteriaceae family pinpointed specific features that may contribute to their robust association with the wild tomato rhizosphere. These features may facilitate the establishment of specialized functions that are crucial for the survival and functionality of the host–microbiome interaction in its native habitat. This aligns with findings that Enterobacteriaceae can thrive under water stress, potentially offering the plant enhanced resilience in dry areas (Ayangbenro & Babalola, 2021; Gamalero et al., 2004; Kasotia & Kumar Choudhary, 2014; Muñoz et al., 2020; Ortega-Ortega et al., 2024; Pérez-Rodriguez et al., 2020). These observations highlight the importance of regional studies in understanding plant-microbe interactions and generating various exciting hypotheses on the functional roles of specific microbial members of the microbiome of wild crop relatives grown in their native habitats. Isolation, genomic and extensive metabolic characterization of these conserved rhizobacterial taxa will be needed to allow functional validation of their role in growth and stress tolerance of wild tomatoes in their native habitats.

Materials and Methods

Fieldwork and sampling

A total of 34 plants of the wild tomato *S. pimpinellifolium* were collected in Loja province, southern Ecuador, where the most abundant population of wild tomatoes is found (Morales Palacio et al., 2014); 8 from Calvas, 11 from Paltas, and 15 from Zapotillo. In total, 33 bulk soil samples, 34 rhizosphere soil samples and 34 leaf samples were collected. One bulk soil sample could not be taken due to the plant's roots growing within rock fissures, making the soil inaccessible. Flowering and/or fruit-baring tomato plants of natural *S. pimpinellifolium* populations in the sites Calvas, Paltas and Zapotillo in the Loja province were sampled. The samples were found along the Calvas site, which had the highest altitude ranging from 1,365 to 1,196 meters above sea level (masl), followed by Paltas which ranges from 1,434 to 666 masl and, Zapotillo, with the lowest altitude from 271 to 158 masl (Figure 5; Supplementary Figure S1).

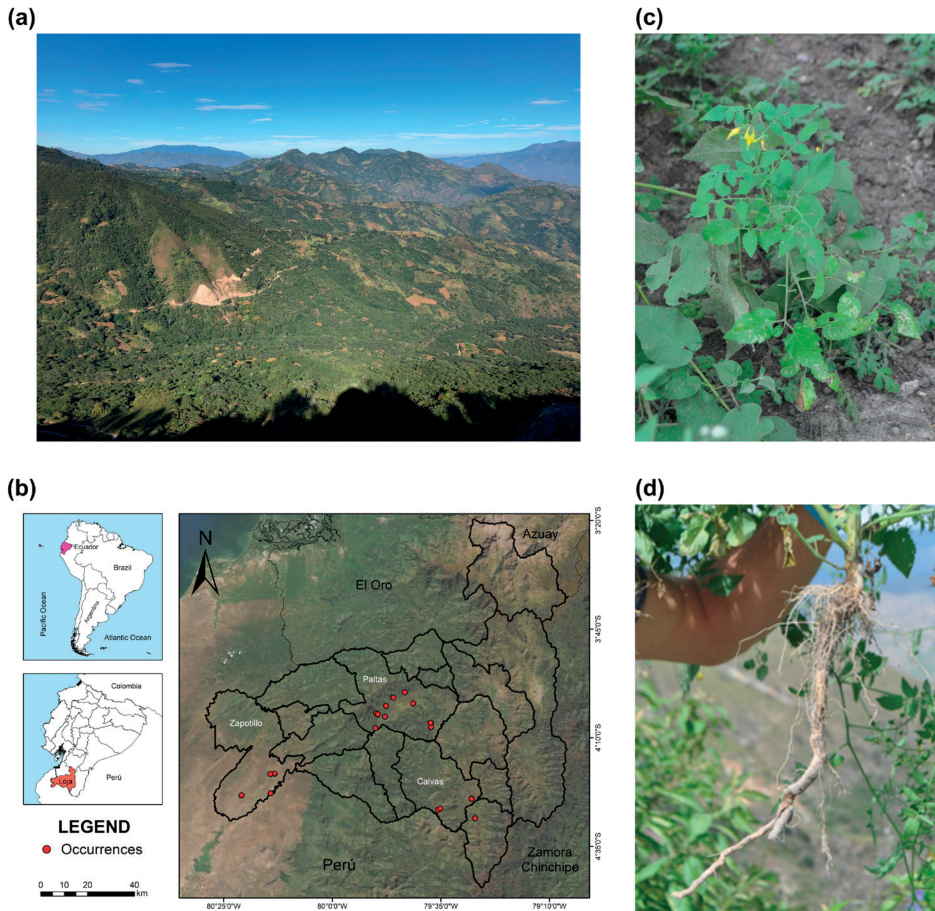

Figure 5. Sampling of wild tomato *Solanum pimpinellifolium* in their native habitat. **(a)** Landscape of native habitat of wild tomatoes in Loja, Ecuador; **(b)** Map of sampling sites of wild tomato native populations; **(c)** *S. pimpinellifolium* at flowering stage; **(d)** *S. pimpinellifolium* roots sampled for microbiome analysis.

The sampling expedition of wild tomatoes was organized in June 2019, when plants were at flowering or fruiting stage. In the field, the areas for sampling were defined by natural barriers such as rivers and springs. The wild tomatoes were found growing at a variety of locations, for instance, next to fences, among farm crops (e.g. corn, common bean, cassava, intercropping), close to the rivers or springs, inside shrubby vegetation, etc. (Supplementary Figure S2–S4). Moreover, wild tomatoes were found with the help of local people living close to the selected sampling sites. Field samples were collected under sterile conditions using disposable gloves and 75% (v/v) ethanol. In addition,

field site information was registered including geographic coordinates, altitude, local temperature, relative humidity in the shade, and the plant developmental stage.

For rhizosphere sampling, using a surface-sterilized steel digging bar, the soil around the tomato plant was dug up from 20-30 cm depth to uncover the root system. The roots were shaken vigorously to remove the loose soil particles as much as possible. Roots with tightly attached soil were cut into 2-3 cm segments and collected into a 50 ml-tube, mixed with 4 ml of LifeGuard® Soil Preservation Solution (Qiagen, USA). Samples were stored in a cooler box with ice gel blocks for transportation to the laboratory. For bulk soil sampling, 4 g of soil were collected at 1 m distance from the sampled individual with no visual presence of roots, and placed into a 15 ml-tube and mixed with 4 ml of LifeGuard® Soil Preservation Solution. After rhizosphere sampling, 1 kg of the soil from the same place where the plant grew was collected in a zip-lock bag for physicochemical analysis. Furthermore, three fully expanded young leaves were cut with 75% ethanol-sterilized scissors from the top of each sampled plant and placed into a paper bag for later plant genotyping at Diversity Arrays Technology Pty Ltd (Bruce, Australia).

Soil physicochemical analysis

Back at the laboratory, 33 soil samples were air dried and sieved (2 mm diameter sieve mesh) and sent to Agrocalidad Laboratory facilities (Tumbaco, Ecuador) for standard physicochemical analysis. Soil physicochemical data were normalized by log transformation [log10(x+1)] to calculate their Euclidean distance and perform permutational analysis of variance (PERMANOVA, 9,999 permutations $p < 0.05$) using vegan R package (Oksanen et al., 2020) among sites, and perform a principal components analysis (PCA, *prcomp* function).

Soil and rhizosphere DNA isolation and amplicon and metagenome sequencing

The rhizosphere samples were pre-processed by pulsing the vortex at maximum speed multiple times, sequentially, to effectively dislodge and transfer as much soil as possible from the roots into the LifeGuard® Soil Preservation Solution. Then the roots were removed from the tubes and the rhizosphere soil suspension was transferred by pipetting with a cut pipette tip into 15 ml-tubes and stored at -20 ºC until DNA extraction. The rhizosphere and bulk soil samples were prepared by pelleting 0.5 g of soil by centrifuging aliquots (1.8 ml) of soil suspension at 10,000 × *g* for 1 min, and discarding the supernatant of LifeGuard® Soil Preservation Solution. The Qiagen DNeasy® PowerSoil® Kit was used to isolate the genomic DNA according to the manufacture's kit protocol. DNA samples were sent to BaseClear (Leiden, The Netherlands) for amplicon library preparation and subsequent sequencing of the V3-V4 regions of

the 16S rRNA gene using the universal bacterial primers 341F (CCTACGGGNGGC-WGCAG) and 805R (GACTACHVGGGTATCTAATCC), while the primers ITS3F (GCATCGATGAAGAACGCAGC) and ITS4R (TCCTCCGCTTATTGATATGC) were used to sequence the ITS2 region. Paired-end sequence reads (2 × 250 bp) were generated using the Illumina MiSeq platform, performed under accreditation according to the scope of BaseClear B.V. (L457; NEN-EN-ISO/IEC 17025). Shotgun sequencing was performed on 24 rhizosphere DNA samples to generate paired-end sequences with the length of 150 bp per read using the NovaSeq platform according to the scope of BaseClear B.V. (L457; NEN-EN-ISO/IEC 17025).

Plant DNA isolation and DArT-SNP genotyping

Dry leaflet samples of 34 individuals of the native tomato populations were used for genotyping. Three seed bank accessions of *S. pimpinellifolium* (CGN14498 and CGN23957, identified as LPI and SPI, respectively) and the domesticated tomato *S. lycopersicum* cv. Moneymaker (CGN14330, identified as MON) were included for comparative purpose (Supplementary Table S1). Dry leaflets were pulverized using the Qiagen TissueLyser II Bead Mill and 50 mg of leaf powder was send to Diversity Arrays Technology (DArT, Bruce, Australia) for plant DNA extraction and tomato genotyping using the DArTseq service. The presence/absence data of 12,745 single-nucleotide polymorphisms (SNPs) from the 34 tomato samples, two *S. pimpinellifolium* accessions and one *S. lycopersicum* cv. Moneymaker (37 tomato genotypes in total) were used to perform the hierarchical clustering using the Jaccard distance in the vegan package; a dendrogram plot was generated using ape package (Paradis et al., 2023). For the permutational analysis of variance (PERMANOVA, 9,999 permutations $p < 0.05$), 11,923 SNPs from 34 tomato samples were used in the vegan R package (Oksanen et al., 2020) to analyze the genetic diversity among sites.

Amplicon sequence analysis

The compressed sequence reads in FASTQ format were processed by the DADA2 v1.16.0 pipeline (Callahan et al., 2016) in RStudio environment (RStudio Team, 2020) to obtain the abundance and taxonomy tables. The taxonomy assignment for bacteria was performed with the SILVA ribosomal RNA gene reference database (v138) (Quast et al., 2013), while the UNITE database (version 8.2) was used for fungal taxonomy assignment (Nilsson et al., 2019).

A total of 1,744,884 of high-quality bacterial sequences were obtained, with a mean of 22,660 sequences per sample. For fungi, a total of 2,378,471 sequences were obtained, averaging 30,889 sequences per sample (Supplementary Table S2). ASVs assigned to Archaea, Mitochondria, Chloroplast and non-fungal Eukaryota were removed prior to downstream analyses. Finally, after taxonomic assignment, 18,452 ASVs were identi-

fied as bacterial and 14,241 as fungal ASVs. The statistical analysis was performed in RStudio environment and R software version 4.3.1 (R Core Team, 2023). R packages such as tidyverse (Wickham et al., 2019), vegan (Oksanen et al., 2020), phyloseq (McMurdie & Holmes, 2013), metagenomeSeq (Paulson et al., 2013) and ggplot2 (Wickham, 2016) were used for alpha diversity (ANOVA, Tukey HSD post hoc test), beta diversity (Bray–Curtis distance, PERMANOVA with 9,999 permutations), and differential abundance analyses. The abundance data were normalized by CSS (Cumulative Sum Scaling) before all analyses. To examine the relationship between wild tomato genetic diversity (Jaccard) and microbiome (Bray–Curtis) distance, as well as between physicochemical soil properties (Euclidean) distance and microbiome (Bray–Curtis) distance, both in bacterial and fungal communities, Mantel tests were performed on Spearman correlations. Moreover, the function *envfit* from the vegan package was used to determine the physicochemical soil properties related to the microbiome distribution (bacteria or fungi). Principal Coordinates Analysis (PCoA) was done with the vegan package using the *cmdscale* function and the Bray–Curtis distance calculated previously. Differential abundance analysis was performed using the metagenomeSeq package. Microbiome data (ASV abundances) were normalized using CSS normalization. Low-abundance ASVs were filtered based on the effective sample size with the *calculateEffectiveSamples* function. The model "~Soil_type" was defined to test for differential abundance of ASVs between soil types (bulk soil vs. rhizosphere) using the *fitZig* function. After identifying differentially abundant ASVs, a new dataset was created containing those ASVs with significant log(2) fold change (adjusted $p < 0.05$). Additionally, the taxonomy of these ASVs was included for further interpretation and result visualization. This procedure was also applied to determine the shared rhizosphere bacterial ASVs between sites. In this case, bulk soil and rhizosphere data were analyzed by site, and significantly abundant ASVs in each rhizosphere were separated into a new dataset for comparison. An UpSet plot to determine the ASVs shared between sites was generated by UpSetR software (Lex et al., 2014).

Metagenome data analysis

The paired-end sequence read libraries in FASTQ format were processed using SqueezeMeta v1.5.1 (Tamames & Puente-Sánchez, 2019). Co-assembly was done using Megahit (Li et al., 2015). Contig statistics were calculated using PRINSEQ (Schmieder & Edwards, 2011), then redundant contigs were removed using CD-HIT (Li & Godzik, 2006) and contigs were merged using Minimus2 (Treangen et al., 2011). Furthermore, RNAs were predicted using Barrnap (Seemann, 2014) and 16S rRNA sequences were taxonomically classified using the RDP classifier (Wang et al., 2007). tRNA/tmRNA sequences were predicted using ARAGORN (Laslett & Canback, 2004), while ORFs were predicted using Prodigal (Hyatt et al., 2010). Similarity searches for GenBank (Clark et al., 2016), EggNOG (Huerta-Cepas et al., 2016) and

KEGG (Kanehisa & Goto, 2000) were done using Diamond (Buchfink et al., 2015). Read mapping against contigs was performed using Bowtie2 (Langmead & Salzberg, 2012). Additionally, binning was done using MaxBin2 (Wu et al., 2016) and Metabat2 (Kang et al., 2019). These binning results were combined using DAS Tool (Sieber et al., 2018) to obtain refined bins.

Relevant information from the metagenomics data (ORF, contig and bin annotations, aggregated taxonomic and functional features) was exported into tables by running the SqueezeMeta utility script *sqm2tables.py* (Tamames & Puente-Sánchez, 2019) to facilitate data handling for further analyses.

Genome bins were qualitatively assessed by CheckM (Parks et al., 2015). Afterwards, two high quality bin files belonging to the Enterobacteriaceae family, one to *Lactiplantibacillus* and one to Micrococcaceae were selected and submitted to the RAST server (Rapid Annotation using Subsystems Technology) (Aziz et al., 2008) to annotate their functional genes. Also, the bin files were submitted to the bacterial antiSMASH software (Antibiotics & Secondary Metabolite Analysis Shell) version 7 (Blin et al., 2023) to identify biosynthetic gene clusters and predicted metabolites.

A schematic overview of the methodological workflow used in this chapter is illustrated in Figure 6.

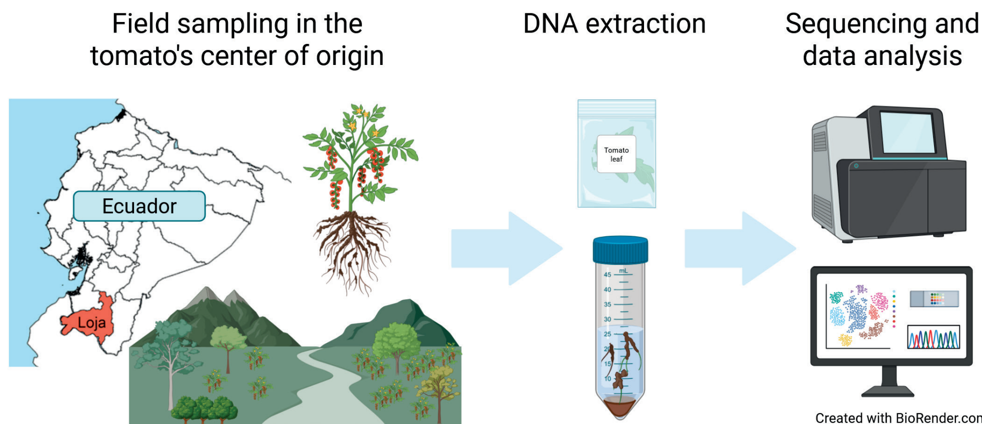

Figure 6. Methodological workflow from field sampling to data analysis. Rhizosphere samples of wild tomato *Solanum pimpinellifolium* were collected in Ecuador, including bulk soil, rhizosphere soil, and leaf material, to study the rhizosphere microbiome in the tomato's native habitat.

Acknowledgements

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Author contributions

SSF: performed the fieldwork, analyzed the data, wrote and edited the manuscript; VC: reviewed and edited the manuscript and advised on data analyses; LMGA: managed the metagenome processing and reviewed the manuscript; ALR: provided laboratory facilities and support; PVTH: provided logistic support, organized research permits, co-coordinated the research in Ecuador, and reviewed the manuscript JMR: reviewed and edited the manuscript, initiated and co-coordinated the research; BOO: performed the fieldwork, assisted logistic preparation of DNA samples, was involved in amplicon processing, statistical analyses, and reviewed the manuscript.

Data availability

The 16S and ITS amplicons and shotgun metagenomics sequencing data have been deposited in the European Nucleotide Archive (ENA) database under the project accession number PRJEB82447. Further inquiries can be directed to the corresponding author Pieter van 't Hof at Universidad San Francisco de Quito (pvanthof@usfq.edu.ec).

Declarations

Competing interests

The authors declare no competing interests.

Conflict of interest

The authors declare no conflict of interest.

Plant material collection permits

This study was carried out under the Genetic Resource Permit numbers MAE-DNB-CM-2018-0085 and MAATE-DNB-CM-2022-0271, issued by the Ecuadorian Ministry of Environment to USFQ. During field sampling, we collected only the minimum number of *S. pimpinellifolium* specimens necessary to achieve the objectives of this research. We prioritized the collection of post-reproductive individuals (flowering and/or fruit-baring tomato plants) to help preserve natural wild tomato populations at the sampling sites. The formal identification of the plant material was carried out in situ by Stalin Sarango Flores and Ben O. Oyserman. No voucher specimens were collected.

Supplementary material

Supplementary Figures

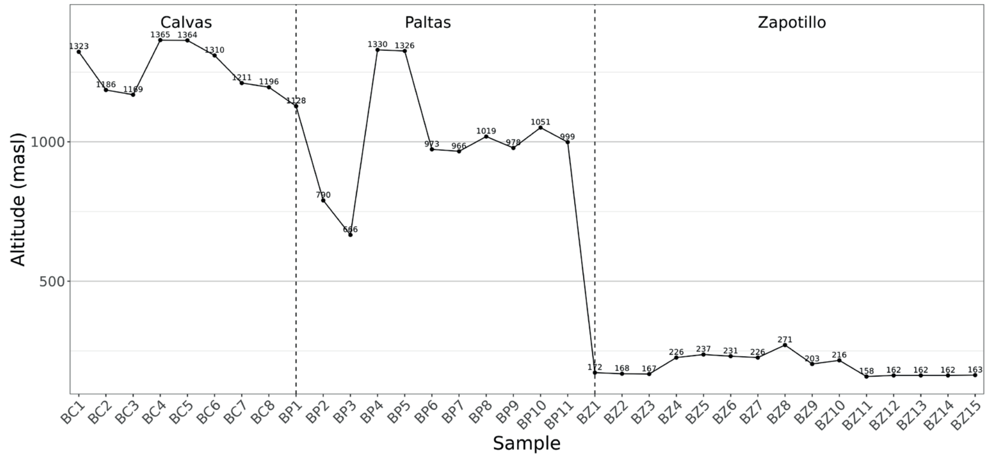


Figure S1. Elevation in meters above sea level (masl) of the wild tomato sites sampled in Loja, Ecuador.

Figure S2. Eight *S. pimpinellifolium* sampled in Calvas (Loja, Ecuador).

Figure S3. Eleven *S. pimpinellifolium* sampled in Paltas (Loja, Ecuador).

Figure S4. Fifteen *S. pimpinellifolium* sampled in Zapotillo (Loja, Ecuador).

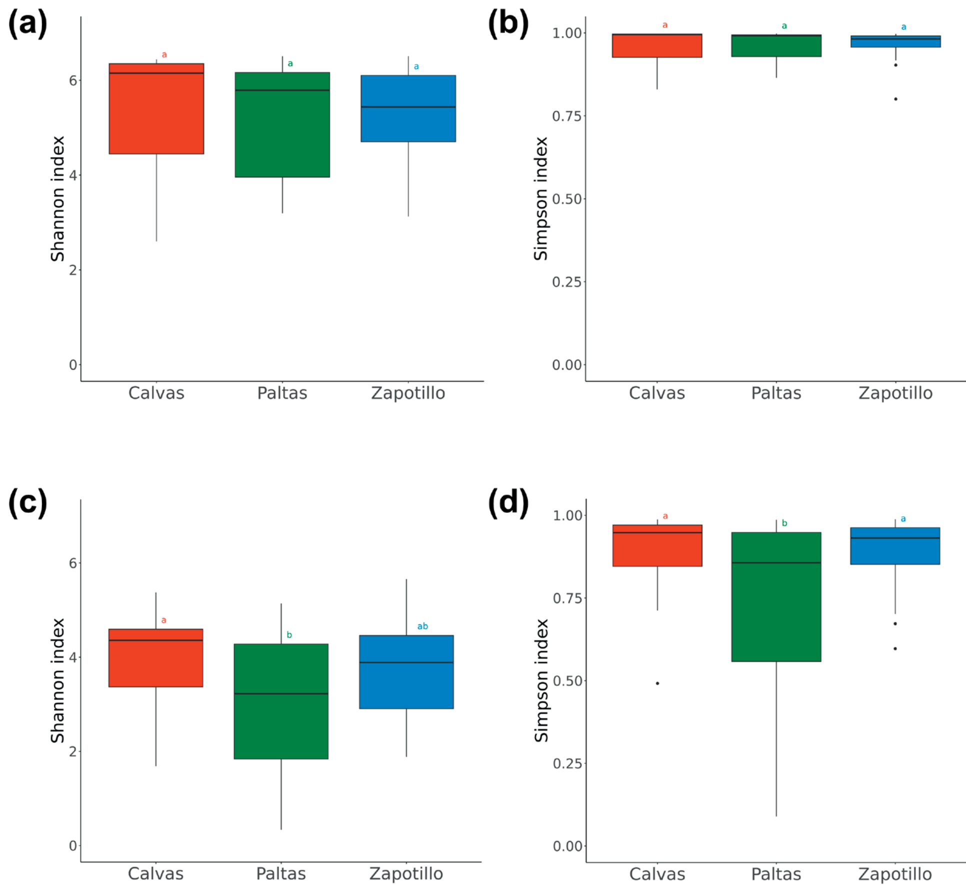


Figure S5. Alpha diversity in bulk soil samples. Shannon **(a)** and Simpson diversity index **(b)** of bacterial communities. Shannon **(c)** and Simpson diversity index **(d)** of fungal communities.

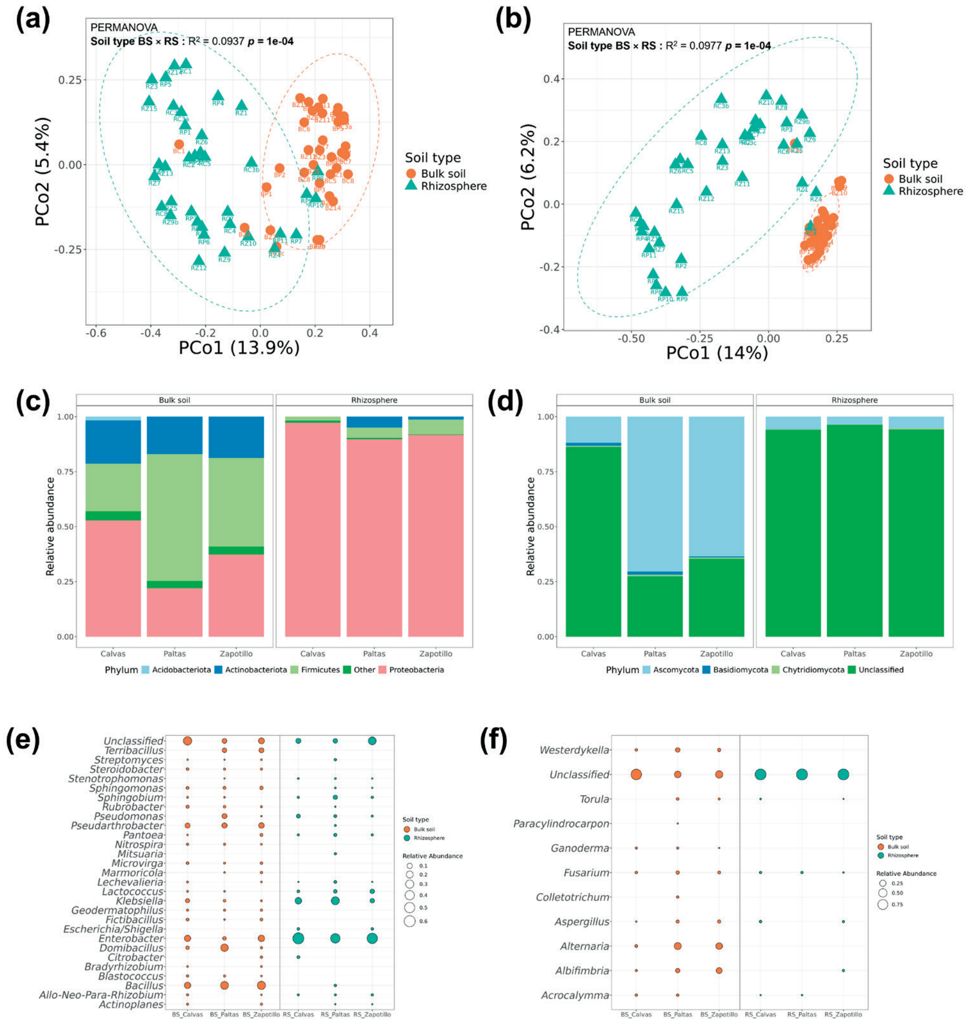


Figure S6. PCoA of **(a)** bacterial and **(b)** fungal communities of bulk soil and rhizosphere samples of *S. pimpinellifolium* in its native habitat; profile of bacteria. Relative abundance of bacterial **(c)** and fungal **(d)** phyla in bulk and rhizosphere soil of wild tomato *S. pimpinellifolium* in three sites of its native habitat. Relative abundance of bacterial **(e)** and fungal **(f)** genera in bulk and rhizosphere soil of wild tomato *S. pimpinellifolium* in three sites of its native habitat (Loja, Ecuador).

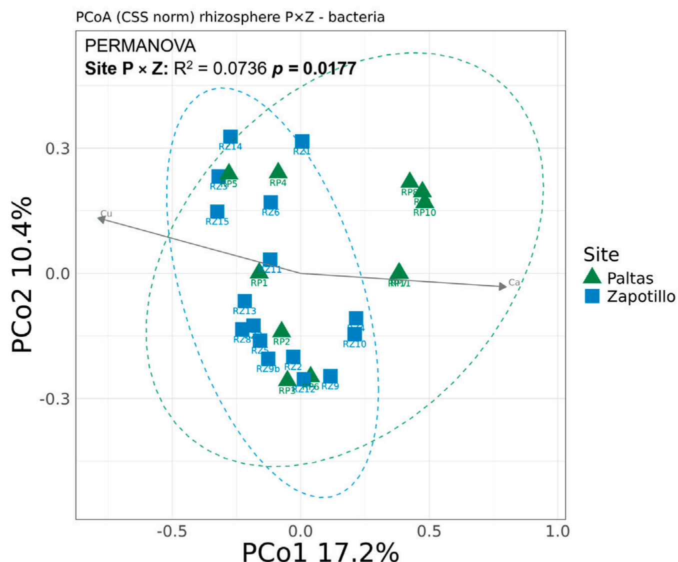


Figure S7. PCoA of rhizosphere bacterial communities from Paltas and Zapotillo samples with significant related soil properties.

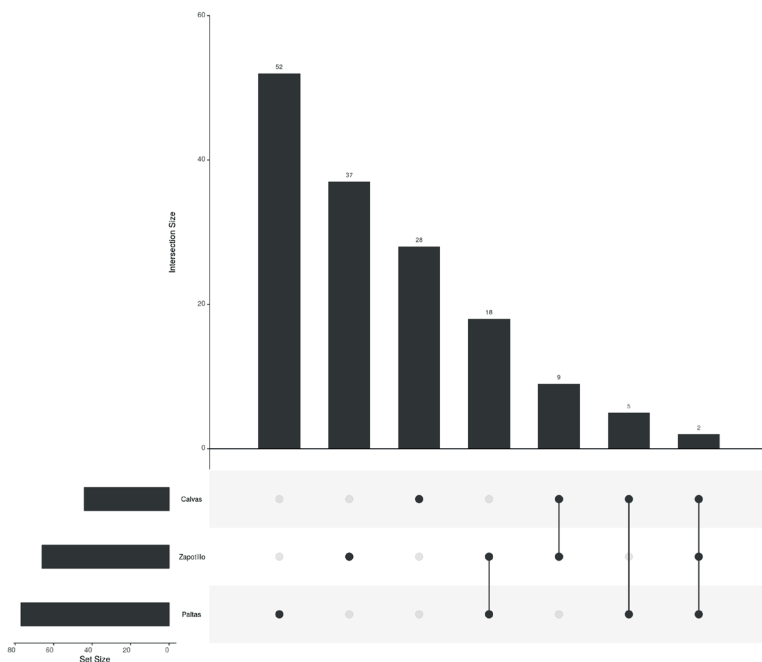


Figure S8. Number of bacterial ASVs shared among wild tomato rhizosphere samples (upset plot generated by UpSetR software (Lex et al., 2014)).

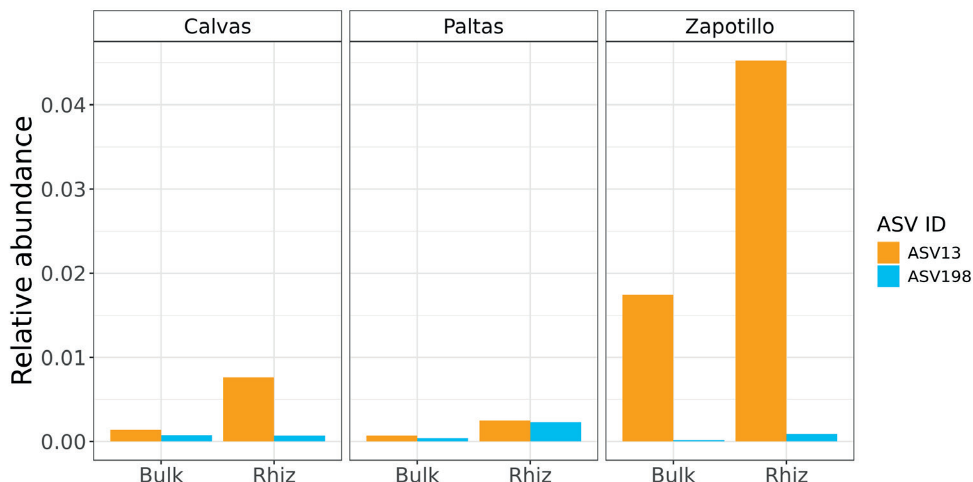
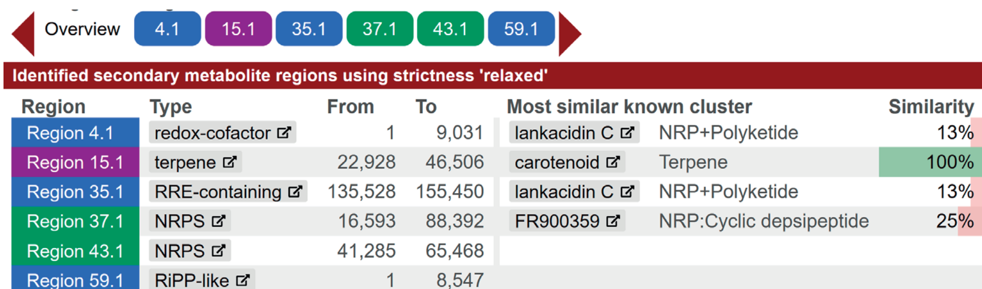


Figure S9. Relative abundance of the two ASVs (ASV13 and ASV198) shared among wild tomato rhizosphere samples.

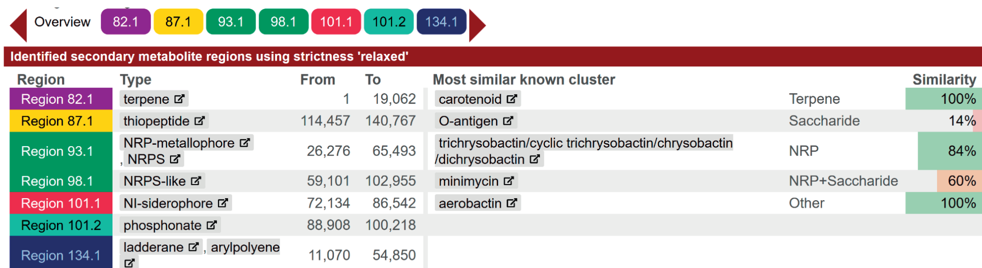


Overview | 4.1 | 15.1 | 35.1 | 37.1 | 43.1 | 59.1

Identified secondary metabolite regions using strictness 'relaxed'

Region	Type	From	To	Most similar known cluster		Similarity
Region 4.1	redox-cofactor	1	9,031	lankacidin C	NRP+Polyketide	13%
Region 15.1	terpene	22,928	46,506	carotenoid	Terpene	100%
Region 35.1	RRE-containing	135,528	155,450	lankacidin C	NRP+Polyketide	13%
Region 37.1	NRPS	16,593	88,392	FR900359	NRP:Cyclic depsipeptide	25%
Region 43.1	NRPS	41,285	65,468			
Region 59.1	RiPP-like	1	8,547			

Figure S10. Overview of BGCs found in Enterobacteriacea bin 074 by bacterial antiSMASH software (Blin et al., 2023).



Overview | 82.1 | 87.1 | 93.1 | 98.1 | 101.1 | 101.2 | 134.1

Identified secondary metabolite regions using strictness 'relaxed'

Region	Type	From	To	Most similar known cluster		Similarity
Region 82.1	terpene	1	19,062	carotenoid	Terpene	100%
Region 87.1	thiopeptide	114,457	140,767	O-antigen	Saccharide	14%
Region 93.1	NRP-metallophore, NRPS	26,276	65,493	trichrysobactin/cyclic trichrysobactin/chrysobactin/dichrysobactin	NRP	84%
Region 98.1	NRPS-like	59,101	102,955	minimycin	NRP+Saccharide	60%
Region 101.1	NI-siderophore	72,134	86,542	aerobactin	Other	100%
Region 101.2	phosphonate	88,908	100,218			
Region 134.1	ladderane, arylpolyene	11,070	54,850			

Figure S11. Overview of BGCs found in Enterobacteriacea bin 136 by antiSMASH software (Blin et al., 2023).

Overview 12.1 19.1 24.1 26.1 33.1 37.1 51.1

Identified secondary metabolite regions using strictness 'relaxed'

Region	Type	From	To	Most similar known cluster		Similarity
Region 12.1	T3PKS	511,783	552,952			
Region 19.1	NRPS	11,746	38,523	mutanocyclin/leuvalin/tyrvalin	NRP	46%
Region 24.1	cyclic-lactone-autoinducer	114,616	135,321			
Region 26.1	RiPP-like	22,938	35,088			
Region 33.1	NRPS, NRPS-like	9,760	69,443			
Region 37.1	terpene	955	20,601			
Region 51.1	NRPS	1	11,926	mutanocyclin/leuvalin/tyrvalin	NRP	30%

Figure S12. Overview of BGCs found in *Lactiplantibacillus* bin 310 by antiSMASH software (Blin et al., 2023).

Overview 6.1 13.1 29.1 33.1 54.1

Identified secondary metabolite regions using strictness 'relaxed'

Region	Type	From	To	Most similar known cluster		Similarity
Region 6.1	terpene	64,916	85,854	carotenoid	Terpene	37%
Region 13.1	terpene	1	17,892	carotenoid	Terpene	100%
Region 29.1	betalactone	368	26,286	microansamycin	Polyketide	7%
Region 33.1	NAPAA	1,207	35,076	stenothricin	NRP:Cyclic depsipeptide	27%
Region 54.1	RiPP-like	1	6,403			

Figure S13. Overview of BGCs found in Micrococcaceae bin 296 by antiSMASH software (Blin et al., 2023).

Supplementary Tables

Table S1. Tomato accessions used for DArT genotyping.

Code	Species	Accession number	Origin	Passport
LPI	*Solanum pimpinellifolium*	CGN14498	NA	https://cgngenis.wur.nl/accessiondetails/CGN14498
SPI	*Solanum pimpinellifolium*	CGN23957	Perú	https://cgngenis.wur.nl/accessiondetails/CGN23957
MON	*Solanum lycopersicum* cv. Moneymaker	CGN14330	Netherlands	https://cgngenis.wur.nl/accessiondetails/CGN14330

Table S2. Summary of amplicon 16S rRNA and ITS gene sequencing data processed by DADA2.

Microbiome	Reads	input	filtered	denoisedF	denoisedR	merged	nonchim
Bacteria	Sum	3387598	2701258	2395876	2556849	2005891	1744884
Bacteria	Mean	43994,78	35081,27	31115,27	33205,83	26050,53	22660,83
Bacteria	Standard deviation	7994,184	6333,351	6103,57	6040,226	7072,529	6017,851
Fungi	Sum	3197507	2523736	2488861	2494709	2429798	2378471
Fungi	Mean	41526,06	32775,79	32322,87	32398,82	31555,82	30889,23
Fungi	Standard deviation	10103,63	8136,397	7986,121	8018,784	7847,328	7673,505

Table S3. Loadings of physicochemical soil properties in the PCA of samples collected in the field.

Soil property	PC1	PC2
pH	-0.32690082	0.173941718
OM_perc	0.19913377	0.160461290
N	0.20629872	0.159826780
P	0.32611163	-0.169426865
K	0.32131938	0.071346178
Ca	-0.28841770	0.189643697
Mg	-0.18305469	-0.122120575
Fe	0.36847612	-0.106984058
Mn	0.32365041	-0.180349710
Cu	0.31950928	0.004014556
Zn	0.31978313	0.016860996
CEC	0.11150202	0.505880556
Sand	-0.09269184	-0.451531797
Silt	0.13514370	0.285787536
Clay	0.02176522	0.498031440

Table S4. Mean of alpha diversity indexes of the bacterial and fungal communities of bulk soil and wild tomato rhizosphere.

Microbiome	Soil	Site	No. samples	Shannon index	Tukey_Shannon	Simpson index	Tukey_Simpson
Bacteria	Bulk soil	NA	34	5,27 ±1,20	a	0,959 ±0,0536	a
Bacteria	Rhizosphere	NA	34	3,15 ±1,11	b	0,864 ±0,101	b
Bacteria	Bulk soil	Calvas	8	5,42 ±1,41	a	0,957 ±0,0645	a
Bacteria	Bulk soil	Paltas	11	5,11 ±1,29	a	0,958 ±0,0485	a
Bacteria	Bulk soil	Zapotillo	15	5,26 ±1,05	a	0,961 ±0,0529	a
Bacteria	Rhizosphere	Calvas	8	2,74 ±0,81	a	0,849 ±0,0888	a
Bacteria	Rhizosphere	Paltas	11	3,84 ±1,47	a	0,893 ±0,136	a
Bacteria	Rhizosphere	Zapotillo	15	2,94 ±0,79	a	0,855 ±0,0818	a
Fungi	Bulk soil	NA	34	4,19 ±0,85	a	0,931 ±0,0827	a
Fungi	Rhizosphere	NA	34	3,00 ±1,24	b	0,763 ±0,218	b
Fungi	Bulk soil	Calvas	8	3,97 ±1,06	a	0,892 ±0,129	a
Fungi	Bulk soil	Paltas	11	3,01 ±1,49	b	0,726 ±0,277	b
Fungi	Bulk soil	Zapotillo	15	3,75 ±1,04	ab	0,892 ±0,102	a
Fungi	Rhizosphere	Calvas	8	3,57 ±1,06	a	0,841 ±0,862	a
Fungi	Rhizosphere	Paltas	11	2,10 ±1,33	b	0,558 ±0,573	b
Fungi	Rhizosphere	Zapotillo	15	3,28 ±0,99	a	0,854 ±0,86	a

Note. Different letters in the same column indicate significant differences (ANOVA, Tukey HSD test $p < 0.05$) among the samples from same Microbiome and Soil

Table S5. Summary of beta-diversity (Multivariate homogeneity of group dispersions (Betadisper) and PERMANOVA) of the bacterial and fungal communities of bulk soil and wild tomato rhizosphere

Microbiome	Soil	Comparison	F (Betadisper test)	p-value (Betadisper test)	R^2 (PERMANOVA test)	p-value (PERMANOVA test)
Bacteria	Bulk & Rhizosphere	Bulk vs. Rhizosphere	6,1284	0,0142	0,0937	1,00E-04
Bacteria	Bulk soil	Site	0,3189	0,7292	0,0953	1,00E-04
Bacteria	Bulk soil	Calvas vs. Paltas	NA	0,6552	0,0744	0,024
Bacteria	Bulk soil	Calvas vs. Zapotillo	NA	0,4701	0,0739	0,002
Bacteria	Bulk soil	Paltas vs. Zapotillo	NA	0,7961	0,0663	0,004
Bacteria	Rhizosphere	Site	1,4695	0,2443	0,082	0,0072
Bacteria	Rhizosphere	Calvas vs. Paltas	NA	0,5357	0,0656	0,1432
Bacteria	Rhizosphere	Calvas vs. Zapotillo	NA	0,3459	0,0465	0,181
Bacteria	Rhizosphere	Paltas vs. Zapotillo	NA	0,1231	0,0736	0,0183
Fungi	Bulk & Rhizosphere	Bulk vs. Rhizosphere	27,024	1,90E-06	0,0977	1,00E-04
Fungi	Bulk soil	Site	0,032	0,9685	0,0719	1,00E-04
Fungi	Bulk soil	Calvas vs. Paltas	NA	0,9114	0,0676	0,0103
Fungi	Bulk soil	Calvas vs. Zapotillo	NA	0,9044	0,0639	0,0009
Fungi	Bulk soil	Paltas vs. Zapotillo	NA	0,7929	0,0629	0,0009
Fungi	Rhizosphere	Site	5,3097	0,0085	0,1134	1,00E-04
Fungi	Rhizosphere	Calvas vs. Paltas	NA	0,1061	0,1516	0,0024
Fungi	Rhizosphere	Calvas vs. Zapotillo	NA	0,1917	0,0673	0,0176
Fungi	Rhizosphere	Paltas vs. Zapotillo	NA	0,0067	0,1207	0,0024

Table S6. High quality-bins assembled by SqueezeMeta.

Bin_ID	Marker_lineage	Completeness	Contamination	Method	Kingdom	Phylum	Class	Order	Family	Genus	Species	Length	GC_perc	Num_contigs	Sum_TPM
maxbin.001.fasta.contigs	k__Bacteria (UID203)	73.43	1.72	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	Serratia	NA	5,054,184	58.96	77	453,510
maxbin.003.fasta.contigs	f__Enterobacteriaceae (UID5066)	91.9	1.27	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	Serratia	NA	4,618,338	58.91	67	201,547
maxbin.040.fasta.contigs	f__Enterobacteriaceae (UID5066)	96.81	3.84	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	Serratia	NA	5,126,743	59.6	64	140,092
maxbin.074.fasta.contigs	f__Enterobacteriaceae (UID5121)	83.94	3.65	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	NA	NA	4,167,963	56.24	68	61,816
maxbin.107.fasta.contigs	f__Enterobacteriaceae (UID5121)	87.37	2.62	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Klebsiella	NA	5,376,945	55.13	88	17,393
maxbin.114.fasta.contigs	f__Enterobacteriaceae (UID5054)	97.8	9.32	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae	Pantoea	NA	5,254,858	56.27	487	105,954
maxbin.136.fasta.contigs	f__Enterobacteriaceae (UID5054)	94.04	2.92	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	NA	NA	4,897,259	53.15	150	17,736
maxbin.156.fasta.contigs	o__Lactobacillales (UID544)	97	9.38	DAS	Bacteria	NA	NA	NA	NA	NA	NA	4,490,188	38.87	564	29,617
maxbin.180.fasta.contigs	f__Moraxellaceae (UID4680)	99.32	5.14	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Moraxellales	Moraxellaceae	Acinetobacter	NA	4,097,206	38.26	106	63,112
maxbin.183.fasta.contigs	f__Enterobacteriaceae (UID5054)	84.61	5.08	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	NA	NA	NA	4,652,845	55.77	765	8,731
maxbin.195.fasta.contigs	c__Alphaproteobacteria (UID3305)	78.1	3.92	DAS	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	NA	2,243,473	62.35	594	38,660

Table S6. High quality-bins assembled by SqueezeMeta. (*continued*)

Bin_ID	Marker_lineage	Completeness	Contamination	Method	Kingdom	Phylum	Class	Order	Family	Genus	Species	Length	GC_perc	Num_contigs	Sum_TPM
maxbin.197.fasta.contigs	f__Enterobacteriaceae (UID5054)	99.01	6.83	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	NA	NA	NA	4,970,158	54.99	74	86,504
maxbin.198.fasta.contigs	f__Enterobacteriaceae (UID5124)	80.4	5.53	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Citrobacter	NA	4,086,740	52.24	149	120,171
maxbin.200.fasta.contigs	f__Enterobacteriaceae (UID5054)	91.77	0.52	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae	Pantoea	NA	4,228,610	55.02	42	37,409
maxbin.296.fasta.contigs	f__Micrococcaceae (UID1623)	97.06	1.61	DAS	Bacteria	Actinobacteria	Actinomycetia	Micrococcales	Micrococcaceae	NA	NA	4,460,542	59.84	206	11,011
maxbin.297.fasta.contigs	k__Bacteria (UID203)	72.81	4.39	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	NA	5,453,927	62.61	202	40,638
maxbin.310.fasta.contigs	o__Lactobacillales (UID462)	99.07	4.09	DAS	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactiplantibacillus	s_L. plantarum	3,468,265	44.09	52	183,939
metabat2.153.fa.contigs	f__Xanthomonadaceae (UID4214)	82.94	1.65	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter	NA	3,622,031	70.96	262	25,755
metabat2.77.fa.contigs	o__Burkholderiales (UID4000)	79.37	2.36	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales		Mitsuaria	NA	4,436,174	69.73	441	53,367

Table S7. Annotated protein encoding genes by RAST server using the SEED Subsystem from bacterial assembled bins of wild tomato rhizosphere.

<table>
<tr><th>bin_name</th><th>Category</th><th>Subcategory</th><th>Subsystem</th><th>Role</th><th>Features</th></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Glutathione-regulated potassium-efflux system and associated functions</td><td>Glutathione-regulated potassium-efflux system ancillary protein KefG</td><td>fig|6666666.1083231.peg.125</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Glutathione-regulated potassium-efflux system and associated functions</td><td>Glutathione-regulated potassium-efflux system protein KefB</td><td>fig|6666666.1083231.peg.124</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Glutathione-regulated potassium-efflux system and associated functions</td><td>Glutathione-regulated potassium-efflux system ancillary protein KefF</td><td>fig|6666666.1083231.peg.901</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Potassium homeostasis</td><td>Potassium-transporting ATPase A chain (EC 3.6.3.12) (TC 3.A.3.7.1)</td><td>fig|6666666.1083231.peg.1853</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Potassium homeostasis</td><td>Glutathione-regulated potassium-efflux system protein KefC</td><td>fig|6666666.1083231.peg.902</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Potassium homeostasis</td><td>Glutathione-regulated potassium-efflux system ancillary protein KefG</td><td>fig|6666666.1083231.peg.125</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Potassium homeostasis</td><td>Large-conductance mechanosensitive channel</td><td>fig|6666666.1083231.peg.749</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Potassium homeostasis</td><td>Potassium-transporting ATPase B chain (EC 3.6.3.12) (TC 3.A.3.7.1)</td><td>fig|6666666.1083231.peg.1852</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Potassium homeostasis</td><td>Glutathione-regulated potassium-efflux system ancillary protein KefF</td><td>fig|6666666.1083231.peg.901</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Potassium homeostasis</td><td>Kup system potassium uptake protein</td><td>fig|6666666.1083231.peg.4210</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Potassium homeostasis</td><td>FKBP-type peptidyl-prolyl cis-trans isomerase SlyD (EC 5.2.1.8)</td><td>fig|6666666.1083231.peg.122</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Potassium homeostasis</td><td>FKBP-type peptidyl-prolyl cis-trans isomerase FkpA precursor (EC 5.2.1.8)</td><td>fig|6666666.1083231.peg.120</td></tr>
<tr><td>Enterobacteriaceae_bin136</td><td>Potassium metabolism</td><td>Potassium metabolism - no subcategory</td><td>Potassium homeostasis</td><td>Potassium-transporting ATPase C chain (EC 3.6.3.12) (TC 3.A.3.7.1)</td><td>fig|6666666.1083231.peg.1851</td></tr>
</table>

Table S7. Annotated protein encoding genes by RAST server using the SEED Subsystem from bacterial assembled bins of wild tomato rhizosphere. (*continued*)

bin_name	Category	Subcategory	Subsystem	Role	Features
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	RNA processing and degradation, bacterial	Ribonuclease E inhibitor RraA	fig|6666666.1083231.peg.4052
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	RNA processing and degradation, bacterial	3'-to-5' oligoribonuclease (orn)	fig|6666666.1083231.peg.368
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	RNA processing and degradation, bacterial	3'-to-5' exoribonuclease RNase R	fig|6666666.1083231.peg.354
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	RNA processing and degradation, bacterial	Ribonuclease III (EC 3.1.26.3)	fig|6666666.1083231.peg.4769
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	RNA processing and degradation, bacterial	Ribonuclease I precursor (EC 3.1.27.6)	fig|6666666.1083231.peg.1797
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	RNA processing and degradation, bacterial	Ribonuclease E (EC 3.1.26.12)	fig|6666666.1083231.peg.4809
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	RNA processing and degradation, bacterial	Ribonuclease E inhibitor RraB	fig|6666666.1083231.peg.2009
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	RNA processing and degradation, bacterial	Exoribonuclease II (EC 3.1.13.1)	fig|6666666.1083231.peg.3902
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	Ribonuclease H	Ribonuclease HI (EC 3.1.26.4)	fig|6666666.1083231.peg.4179
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	Ribonuclease H	Ribonuclease HII (EC 3.1.26.4)	fig|6666666.1083231.peg.1045
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	RNA 3'-terminal phosphate cyclase	RNA-2',3'-PO4:RNA-5'-OH ligase	fig|6666666.1083231.peg.1912
Enterobacteriaceae_bin136	RNA Metabolism	RNA processing and modification	RNA 3'-terminal phosphate cyclase	Transcriptional regulatory protein RtcR	fig|6666666.1083231.peg.1911

Note. The full Table S7 is available online at https://doi.org/10.5281/zenodo.15725075

Chapter 3

Changes in taxonomic diversity and functional traits of the tomato root microbiome along a domestication trajectory

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Abstract

Domestication and breeding have substantially altered the genetic and phenotypic traits of multiple plant species. To date, however, the impact of domestication on the taxonomic and functional diversity of microorganisms colonizing plant tissues remains largely unexplored for the majority of plant species. Here, we examined the bacterial microbiome associated with roots of eight wild and domesticated tomato genotypes grown in three distinct soil management gradients, ranging from native and agricultural soils from the tomato's center of origin in Ecuador to a soil from a tomato greenhouse in The Netherlands. Our findings revealed a higher taxonomic diversity in Ecuadorean agricultural soils than in native and greenhouse soils. Soil was the primary factor governing tomato microbiome assembly, followed by the root compartment and plant genotype. Root microbiomes of tomato grown in native Ecuadorian soils had a higher abundance in Acidobacteriota, Proteobacteria, Bacteroidota, Chloroflexi, and Myxococcota, whereas Actinobacteriota, Cyanobacteria, Firmicutes, and Patescibacteria dominated the root microbiome of tomato grown in agricultural and greenhouse soils. Also, we found significant differences in the root microbiome of the different tomato genotypes, with enhanced abundance in Bacteroidota, Proteobacteria and Chloroflexi on roots of wild tomato genotypes, and Actinobacteriota, Firmicutes and Cyanobacteria on roots of domesticated tomato genotypes. Representative metagenome assembled genomes (MAGs) of bacterial taxa of wild tomato genotypes grown in native Ecuadorean soil were enriched in genes associated with motility and chemotaxis, carbon metabolism, and stress response, while MAGs from the microbiome of domesticated tomatoes grown in agricultural and greenhouse soils were enriched for genes involved in metabolism of nitrogen, iron, amino acids and vitamins. This study highlights the pivotal role of habitat domestication and genetic changes of tomato in microbiome assembly, with alterations in the abundance of functional microbial traits associated with plant growth and health.

Introduction

Plant domestication represents an evolutionary process where wild ancestral species were adapted to human needs, ultimately leading to our modern-day crop cultivars (Purugganan & Fuller, 2009). For most crops, this process was targeted at desirable traits such as larger fruit size, palatability, nutritional quality, reproductive timing or stress resistance (Bergougnoux, 2014). While favoring certain alleles during this process of artificial selection and breeding for these desired traits, many modern crop cultivars show a reduced genetic diversity as other (overlooked) genes were lost (Fernie & Yan, 2019). This phenomenon is referred to as the "domestication syndrome" and was first described for cereals in the 1970s by Harlan et al. (1973). The domestication syndrome not only comprises genetic changes caused by human artificial selection but also unintentional effects arising from agricultural practices to grow the domesticated crops, also referred to as habitat domestication (Barnes et al., 2024; Fernie & Yan, 2019; Soldan et al., 2021). Thus, domesticated species are notably different from their wild relatives, with altered morphology, physiology and ecological interactions, including those affecting microbiome assembly (Barnes et al., 2024; Cordovez et al., 2019; Hassani et al., 2020; Martínez-Romero et al., 2020; Sarango Flores et al., 2023).

Plant-associated microbiomes play essential roles in plant growth and health due to their contribution to nutrient acquisition and stress tolerance, thereby supporting and expanding the host plant's functional capabilities (Adedayo et al., 2023; Ling et al., 2022; Pérez-Rodriguez et al., 2020; Santhanam et al., 2015; Shivega & Aldrich-Wolfe, 2017; Zuluaga et al., 2021). Several factors influence the assembly of the plant microbiome, including abiotic factors (e.g. soil types, climatic conditions, agricultural management practices), plant genotype, and the diversity of local microbial pools in the surrounding habitat (Abdullaeva et al., 2022; Berg & Smalla, 2009; Hewitt et al., 2023; Ofek-Lalzar et al., 2014). Traits such as root architecture, exudate composition and nutritional requirements in domesticated plants significantly affect the composition and diversity of their microbiomes, which in turn may affect their functional benefits to the plant host (Abbamondi et al., 2016; Dennis et al., 2010; Gutierrez & Grillo, 2022; Soldan et al., 2021). Plants recruit specific microbial taxa by specific constituents in root exudates that act as chemical signals and orchestrate the surrounding microbial communities to take advantage of the outsourced functions (Barnes et al., 2024; Nakayasu et al., 2023; Walker et al., 2003; Wen et al., 2023). Furthermore, prevailing soil types, changing climatic conditions and agricultural management practices, also modulate plant microbiome assembly (Cao et al., 2024; Flemer et al., 2022; Wang et al., 2022; Xue et al., 2018). For instance, domesticated plants rely on fertilizers and pesticides and much less on their microbial alliances, which may also limit crop adaptability to future adverse environmental conditions such as drought. This network of

different factors driving microbiome assembly creates complex dynamics that can vary across plant genotypes and growth conditions (Mo et al., 2024; Pérez-Jaramillo et al., 2018; Soldan et al., 2021).

Tomato (*Solanum lycopersicum* L.) is an iconic example of plant domestication, which started in the center of origin in the Andean region in South America (Knapp & Peralta, 2016; Razifard et al., 2020). The domestication journey of tomato began with the wild tomato relative *S. pimpinellifolium,* which provided the early traits selected and crossed by indigenous people into the landrace *S. lycopersicum* var. *cerasiforme.* This variety eventually spread beyond its center of origin and continued its domestication process in Central America (Blanca et al., 2012, 2022; Razifard et al., 2020). This process has continued for thousands of years outside of the native tomato habitat, to meet regional phenotypic preferences and cultivation management practices worldwide, which in turn resulted in many modern cultivated tomato varieties of *S. lycopersicum* (Mata-Nicolás et al., 2020; Sarango Flores et al., 2023; Sim et al., 2011).

Examples of notable phenotypic changes in domesticated tomato cultivars include larger fruits with a reduced seed number, uniform shapes and colors, as well as specific metabolic pathways to prioritize sweetness (or reduce bitterness) that are linked to changes in secondary metabolite composition (Bai & Lindhout, 2007; Bergougnoux, 2014). As a consequence of the domestication syndrome, modern cultivated tomatoes suffered a genetic bottleneck which caused a narrowed gene pool compared to their closest relatives, making them more vulnerable to pests, diseases and abiotic stresses (Aflitos et al., 2014; Gao et al., 2019; Kahlon et al., 2020). This loss of genetic variation may also have reduced the modern tomato's ability to effectively select for and interact with the microbial communities surrounding the plants tissues, which in turn can reflect on plant performance (Chen et al., 2022; Huang et al., 2022; Malacrinò et al., 2022; Nerva et al., 2022; Yue et al., 2023). This was exemplified in our previous elaborate analysis of the microbiome of a tomato recombinant inbred line (RIL) population by Oyserman et al. (2022), which revealed significant differences in microbiome assembly across 100 tomato lines and identified specific genetic associations between loci in the tomato genome and in the microbiome metagenome. Additionally, work by Smulders et al. (2021) suggested that tomato wild relatives establish more beneficial interactions with their rhizosphere microbiome than domesticated cultivars.

Building on the findings of Chapter 2, which explored the rhizosphere microbiome of wild tomato *Solanum pimpinellifolium* in its native Andean habitats, we identified a conserved microbial signature dominated by Proteobacteria. While these results revealed how wild tomatoes interact with their native microbiome, they did not address how tomato domestication process and agricultural management may have impacted plant-microbiome associations. To further explore the taxonomic and functional changes in tomato rhizosphere and endosphere microbiomes, we cultivated eight tomato genotypes, representing different stages of the tomato domestication trajectory, in native and agricultural soils from the center of origin in the South of Ecuador, and in a contrasting greenhouse production soil from The Netherlands. In this chapter, we analyzed the bacterial community composition of the rhizosphere and root endosphere by 16S rRNA gene amplicon sequencing and by shotgun metagenomics. We hypothesized that habitat domestication has the largest impact on microbiome assembly, influencing both the taxonomic and functional diversity of the root microbiome.

Results

Soil and genotype drive the taxonomic diversity of the tomato rhizosphere microbiome

No significant differences (Kruskal-Wallis test, $p > 0.05$) were found in alpha diversity, represented by Shannon's diversity index, for the rhizosphere bacterial communities from the tomato genotypes grown on the different soil types (Figure 1). However, the beta diversity revealed significant differences among soil origin (PERMANOVA, $p = 0.0001$), soil type (PERMANOVA, $p = 0.0001$) and tomato domestication degree (PERMANOVA, $p = 0.024$) (Figure 2a). Soil origin and type explained 64% and 39% of the total variability in the rhizobacterial community composition, respectively.

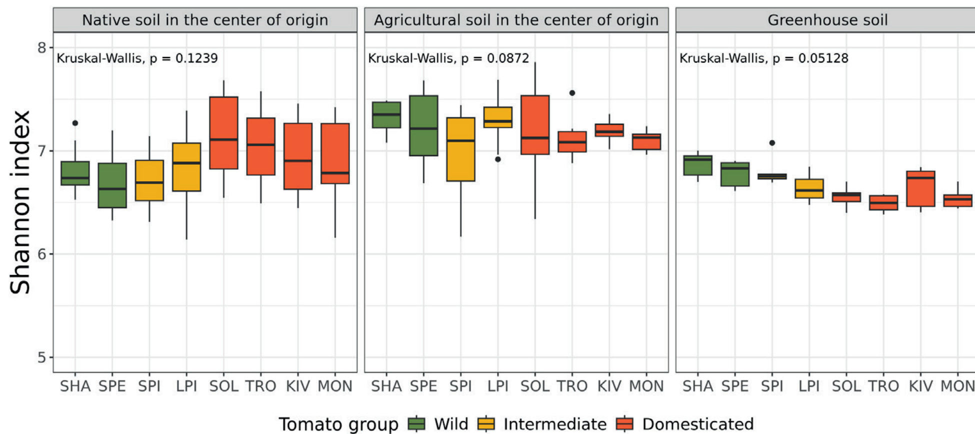

Figure 1. Shannon diversity index of the rhizosphere microbiome of tomato plants grown in Ecuadorian native and agricultural soils, and Dutch greenhouse soil. Boxplots show no significant difference (Kruskal-Wallis test, p < 0.05, *n* = 10) of Shannon diversity index between tomato genotypes. SHA: *S. habrochaites;* SPE: *S. peruvianum;* SPI and LPI: *S. pimpinellifolium;* and tomato varieties of *S. lycopersicum:* SOL: Solarino; TRO: Trovanzo; KIV: Kivu; MON: Moneymaker.

More detailed analysis by soil origin showed significant effects of the eight different tomato genotypes, ranging from 37% of the total variation in Ceiba and Limones native soils from the center of origin, 37-38% in the Ceiba and Limones agricultural soils from the center of origin, and 44% in the Dutch greenhouse production soil (Figure 2b–f). Significant differences were not observed between the wild and intermediate groups but were significant between the four intermediate-wild and four domesticated tomato genotypes (Adonis test, $p < 0.05$, Supplementary Table S4).

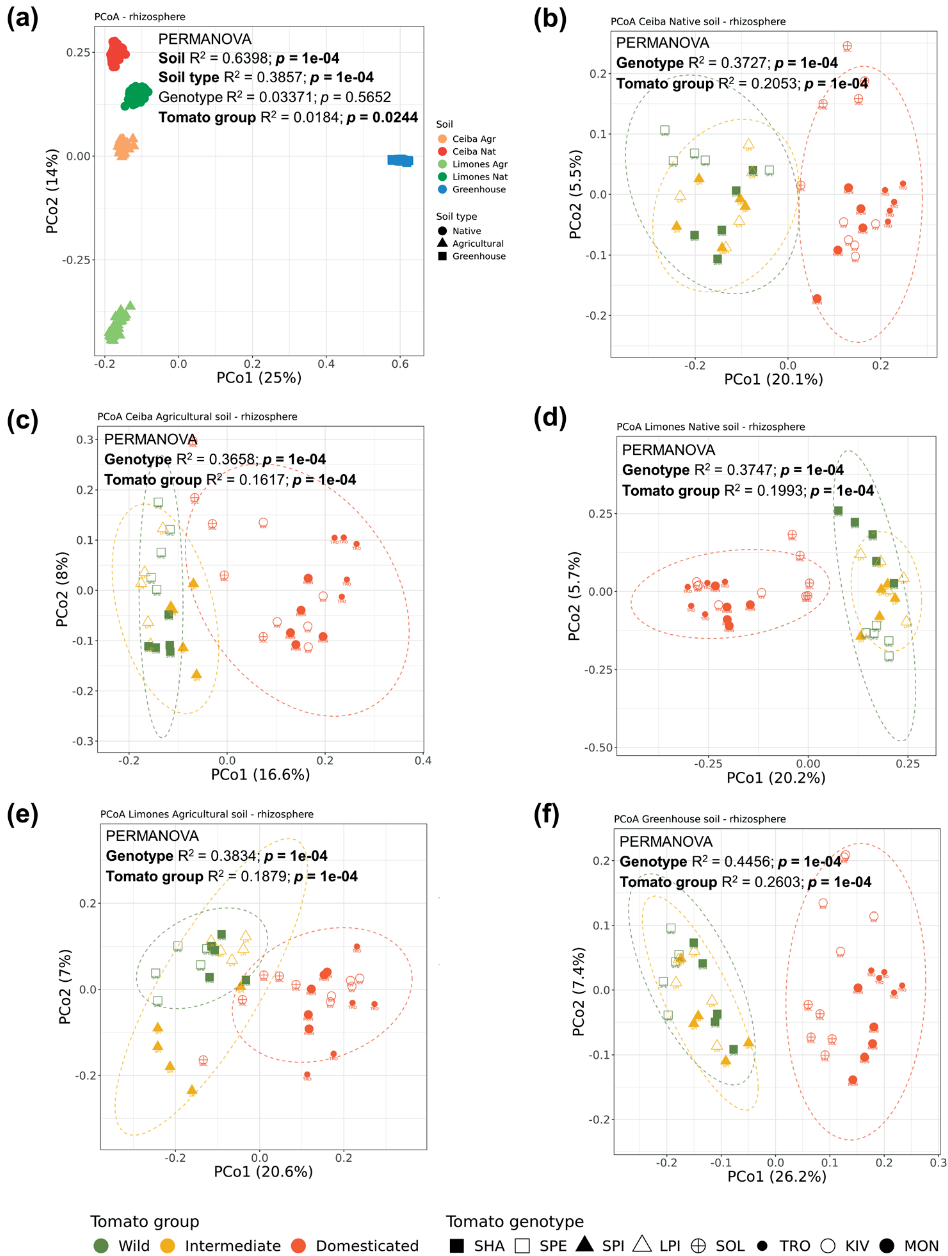

Figure 2. Rhizosphere bacterial community structure of different tomato genotypes grown in Ecuadorian native and agricultural soils, and Dutch greenhouse soil. Principal Coordinate Analysis (PCoA) of rhizosphere bacterial communities of wild (n = 2), intermediate (n = 2) and domesticated (n = 4) tomato genotypes **(a)** PCoA by soil type; **(b) – (f)** PCoA by tomato genotype per soil type. (Agr = agricultural soil, Nat = native soil).

Subsequent differential abundance analysis revealed that soil type impacted the differential enrichment and depletion of ASVs in the rhizosphere of tomato. We first compared ASV abundance in the rhizosphere of tomatoes grown in native and agricultural soils from the South Andes in Ecuador. In native soils, a total of 490 ASVs were significantly more abundant in wild-intermediate tomato genotypes (cluster C3) and 1028 ASVs in the four domesticated tomato genotypes (cluster C4); in the agricultural soils, 1279 ASVs were significantly more abundant in wild-intermediate tomato genotypes (cluster C1) and 1121 ASVs in domesticated tomato genotypes (cluster C2) (Figure 3a). We next compared the differential abundance in the rhizosphere of tomato between the Ecuadorean agricultural soils and the Dutch greenhouse soil. In the Ecuadorean agricultural soils, 1286 ASVs were significantly more abundant in wild-intermediate tomato genotypes (cluster C5), and 2296 ASVs in domesticated tomato genotypes (cluster C4). In the Dutch greenhouse soil, 1148 ASVs showed higher abundance in wild and intermediate tomatoes (cluster C3), but only 109 ASVs were more abundant in domesticated tomatoes (cluster C2) (Figure 3b).

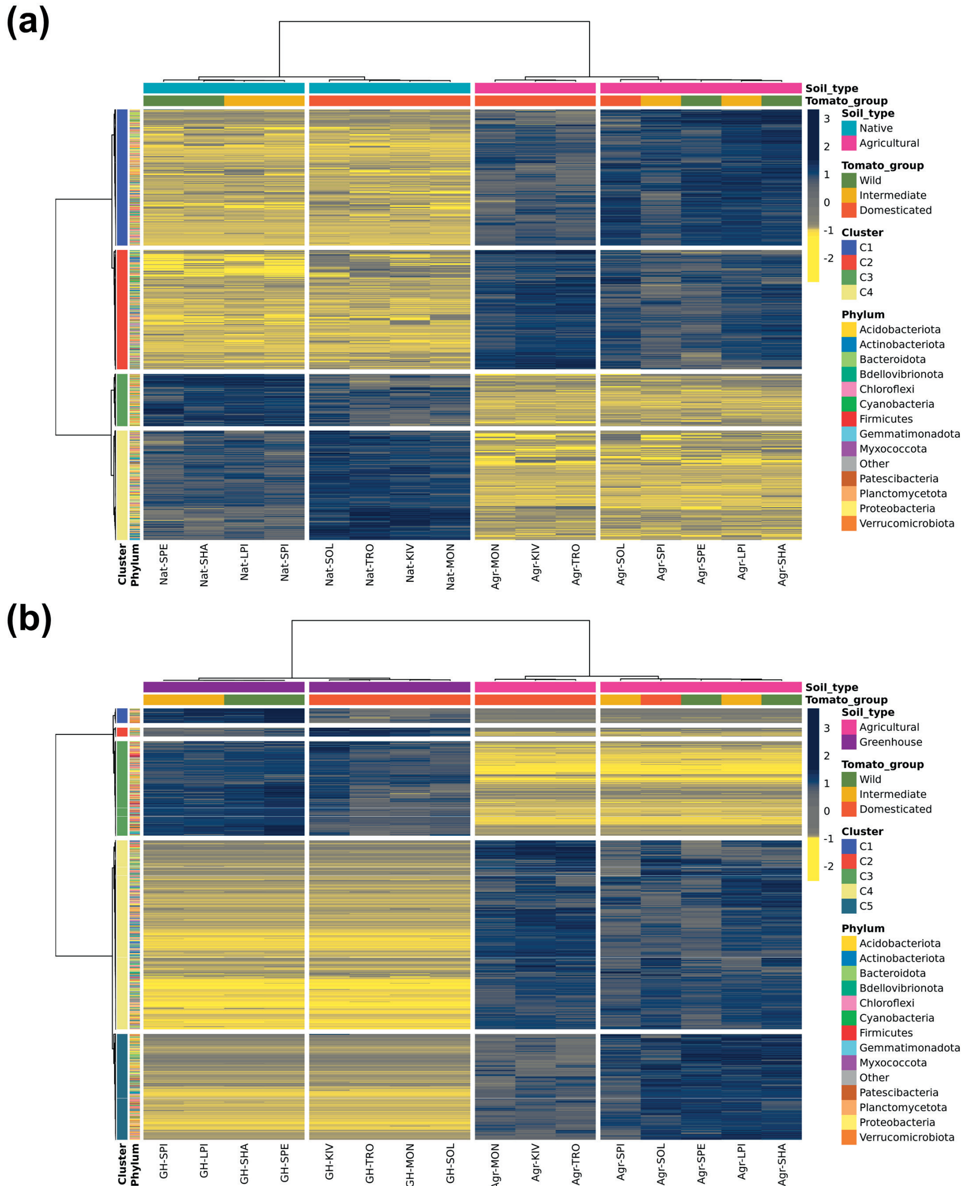

Figure 3. Differential abundance of the tomato rhizosphere amplicon sequence variants (ASVs) in pairwise comparisons of different soil types. **(a)** Heatmap of ASVs in the rhizosphere of tomatoes grown in Ecuadorian native and agricultural soils; clusters C1 = 1279; C2 = 1121; C3 = 490; C4 = 1028 ASVs. **(b)** Heatmap of ASVs in the rhizosphere of tomatoes grown in Ecuadorian agricultural and Dutch greenhouse soils; clusters C1 = 178; C2 = 109; C3 = 1148; C4 = 2296; C5 = 1286 ASVs. Significant ASVs identified from the DESeq differential abundance analysis were hierarchically clustered using Ward's method as the clustering algorithm. SHA: *S. habrochaites;* SPE: *S. peruvianum;* SPI and LPI: *S. pimpinellifolium;* and tomato varieties of *S. lycopersicum:* SOL: Solarino; TRO: Trovanzo; KIV: Kivu; MON: Moneymaker.

By grouping the significantly abundant rhizosphere ASVs from each cluster at phylum and genus level (Figure 4), we observed that the rhizosphere of tomato plants grown in soils from the center of origin, i.e. native and agricultural soils, had a higher relative abundance of Actinobacteriota, Bacteroidota, Cyanobacteria and Proteobacteria when compared with tomato plants grown in the Dutch greenhouse soil, which were more abundant in Chloroflexi, Firmicutes, Gemmatimonadota, Myxococcota, Patescibacteria and Planctomycetota (Figure 4a). At genus level, the rhizosphere of tomatoes grown in native soils from the center of origin had a higher relative abundance of *Brevundimonas, Cellvibrio, Dyadobacter, Ohtaekwangia, Pseudomonas, Rhizobacter and Sphingomonas*. In contrast, tomatoes grown in agricultural soils had an increased relative abundance of *Actinoplanes, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium, Massilia, Nostoc* and *Streptomyces*, whereas tomatoes grown in the Dutch greenhouse soil showed greater abundance of *Bacillus, Devosia, Edaphobaculum, Mesorhizobium, Paenibacillus* and *Sericytochromatia.*

The tomato domestication degree was also linked to changes in specific rhizobacterial taxa. For instance, in native soils, wild and intermediate tomato genotypes had higher relative abundances of *Chitinophaga, Dyadobacter, Ohtaekwangia* (Bacteroidota) as well as *Cellvibrio* and *Rhizobacter* (Proteobacteria). In contrast, *Nocardioides* (Actinobacteriota) were enriched in the rhizosphere of domesticated tomato genotypes grown in the Ecuadorean native soils. In the two Ecuadorean agricultural soils, wild and intermediate tomato genotypes showed higher relative abundance of *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (Proteobacteria) whereas domesticated tomatoes harbored more *Actinoplanes* (Actinobacteriota). In the Dutch greenhouse soil, differences among tomato genotypes were observed in *Bacillus* and *Paenibacillus* (Firmicutes), which were more abundant in wild and intermediate tomatoes, while *Devosia* (Proteobacteria), *Mesorhizobium* (Proteobacteria) and *Sericytochromatia* (Cyanobacteria) were more abundant in domesticated tomatoes. In all three soil types, *Pseudomonas* and *Massilia* had higher abundance in wild-intermediate tomatoes, whereas members of the genera *Sphingomonas* (Proteobacteria) and *Streptomyces* (Actinobacteriota) showed greater relative abundance in domesticated tomatoes in all five soils (Figure 4b).

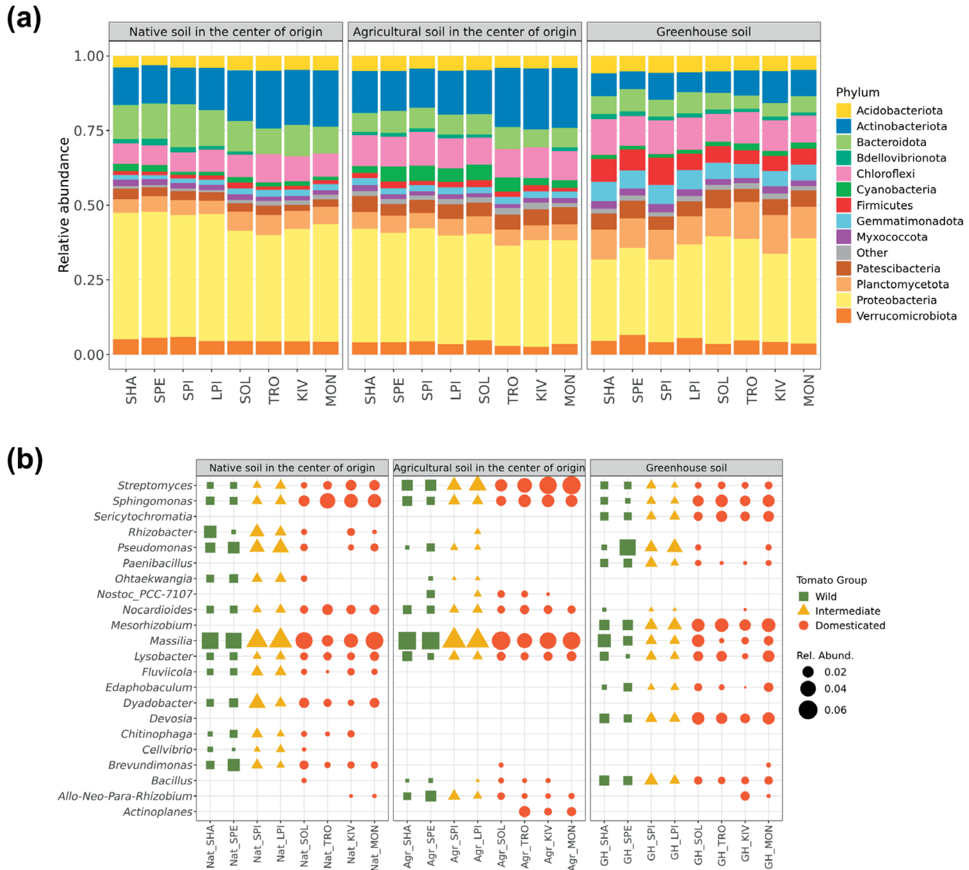

Figure 4. Rhizosphere bacterial community composition of tomato genotypes grown in native and agricultural soils. **(a)** Relative abundance of bacterial phyla in rhizosphere of different tomato genotypes grown in Ecuadorian native and agricultural soils and Dutch greenhouse soil; "Other" category corresponds to grouped phyla with relative abundance < 0.01; **(b)** Relative abundance of most abundant genera found in rhizosphere of different tomato genotypes. ASVs with significant differential abundance between soils and tomato genotypes were grouped according to their phylum or genus level and plotted as stacked bar and bubble charts, respectively. SHA: *S. habrochaites;* SPE: *S. peruvianum;* SPI and LPI: *S. pimpinellifolium;* and tomato varieties of *S. lycopersicum:* SOL: Solarino; TRO: Trovanzo; KIV: Kivu; MON: Moneymaker.

Effects of domestication on functional traits of the tomato rhizosphere microbiome

Following shotgun metagenomics of the rhizosphere samples, 127 MAGs (high quality bins completeness >70%, contamination <10%) were obtained. Of these, 12 MAGs with the highest quality were selected for functional analysis based on their taxonomic consistency with amplicon sequencing results. Thus, functions annotated by SEED Subsystems from selected MAGs related to habitat and the domestication degree of the tomato genotypes revealed significant shifts in microbial functionality. For example, MAGs taxonomically delineated as Bacteroidota (*Chitinophaga, Fluviicola*) or Proteobacteria (*Cellvibrio, Pseudomonas, Rhizobacter* and Rhizobiaceae) were more abundant in wild-intermediate tomatoes in native and agricultural soils, showed a higher abundance of genes associated with motility and chemotaxis, as well as metabolism of carbohydrates and stress response. On the other hand, MAGs taxonomically delineated as Micromonosporaceae, Bacillaceae, Cyanobacteria, *Sphingomonas* and *Streptomyces,* representative taxa of domesticated tomatoes grown in agricultural and greenhouse soils, exhibited a higher number of genes associated with iron acquisition and metabolism of nitrogen, amino acids and derivatives; and cofactors, vitamins, prosthetic groups, pigments (Figure 5).

Figure 5. Heatmap of SEED Subsystems with the highest gene abundance (≥ 5 genes) in rhizosphere bacterial MAGs selected from tomato genotypes across different soil types. The number of protein-encoding genes present in each Subsystem per MAG is shown within the cells. *Chitinophaga*_bin783, *Fluviicola*_bin952, *Rhizobacter*_bin515 and *Cellvibrio*_bin319 belong to wild-intermediate tomatoes grown in native soils; Rhizobiaceae_bin844 belongs to wild-intermediate tomatoes grown in agricultural soils; *Pseudomonas*_bin721 belongs to wild-intermediate tomatoes grown in any soil type. Micromonosporaceae_bin929 and Cyanobacteria_bin643 were representatives of domesticated tomatoes in agricultural soils; Bacillaceae_bin96 and Sericytochromatia_bin549 correspond to domesticated tomatoes grown in greenhouse soil. *Sphingomonas*_bin651 and *Streptomyces*_bin494 belong to domesticated tomatoes grown in any soil type.

Tomato endosphere microbiome assembly is soil type dependent

The bacterial communities detected in the root endosphere displayed a similar Shannon diversity index across tomato genotypes and soils, differing only among the eight tomato genotypes grown in native Ecuadorean soil (Kruskal-Wallis test, $p = 0.0044$). A higher diversity index was observed for domesticated tomato genotypes compared to wild-intermediate tomato genotypes. Interestingly, a same yet statistically insignificant trend was observed for agricultural and greenhouse soils, i.e. a minor yet progressive increase of bacterial alpha diversity along the tomato domestication trajectory (Figure 6).

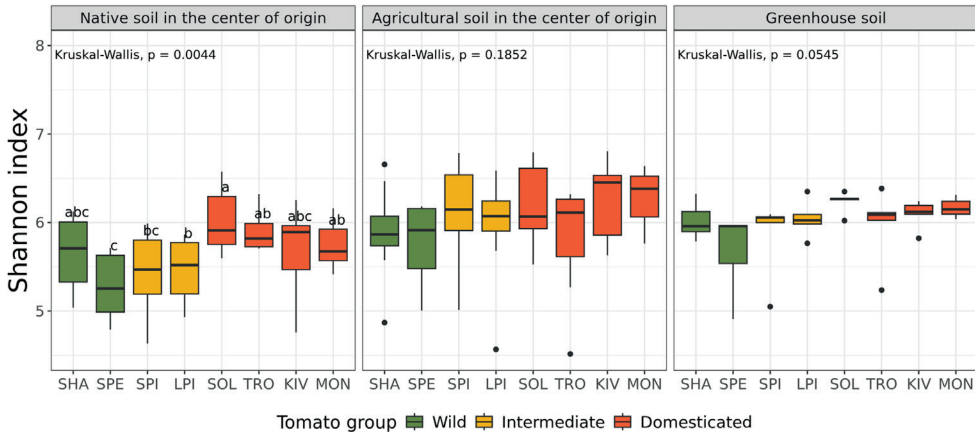

Figure 6. Shannon diversity index in tomato root endosphere grown in Ecuadorian native and agricultural soils and Dutch greenhouse soil. Different letters above boxplots show significant difference (Wilcoxon test, $p < 0.05$, $n = 10$) of Shannon diversity index between tomato genotypes. SHA: *S. habrochaites;* SPE: *S. peruvianum;* SPI and LPI: *S. pimpinellifolium;* and tomato varieties of *S. lycopersicum:* SOL: Solarino; TRO: Trovanzo; KIV: Kivu; MON: Moneymaker.

Overall, PCoA of the root endosphere showed significant differences between soil origin and soil types (PERMANOVA, $p = 0.0001$), but surprisingly there were no significant differences between tomato genotypes or domestication groups (PERMANOVA, $p > 0.05$) (Figure 7a). When the tomato endosphere microbiome composition was analyzed per soil type (i.e. native, agricultural, greenhouse), significant differences were observed between tomato genotypes and domestication degree. This variation observed in the PCoA plot appeared to be primarily driven by the broader dispersion of wild tomato genotypes across the different soils. Although some dispersion is observed in domesticated tomato genotypes, wild genotypes exhibited greater variability. This variation likely contributed to the significant differences found between domesticated tomatoes and both intermediate and wild tomato genotypes (Adonis test, $p < 0.05$, Supplementary Table S4). Specifically, tomato genotype showed 21% of the total variation in

Ceiba agricultural and native soils and in Limones native soil, while 22% was observed in Limones agricultural and greenhouse soils. The domestication effect, i.e. variation in endosphere microbiome composition between wild, intermediate and domesticated tomato groups, showed 7% of the total variation per soil. This percentage of variation represents a marked decrease compared to the variation observed in the rhizosphere microbiome of 20% (Figure 7b–f).

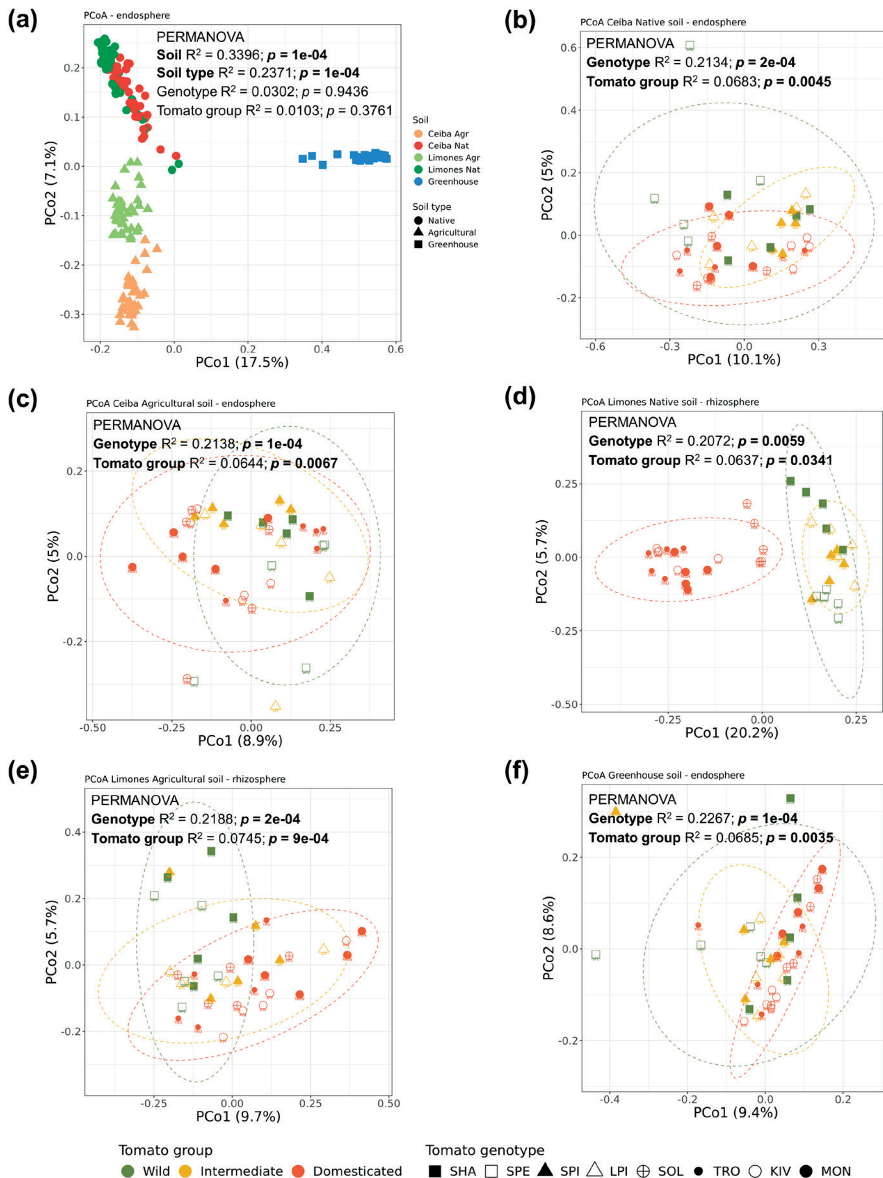

Figure 7. Principal Coordinate Analysis (PCoA) of bacterial communities in tomato root endosphere grown in different soil types. **(a)** PCoA by soil type and **(b) – (f)** PCoA by tomato genotype in each soil.

Furthermore, the differential abundance analysis of bacterial ASVs by DESeq (Wald test) through pairwise comparison between native and agricultural soils revealed no significant differences between the tomato domestication groups. The observed ASV clusters (Figure 8a) also suggest that the differences were mainly attributed to the soil type in which the tomato genotypes were grown. Similar results were found when agricultural and greenhouse soils were compared (Figure 8b).

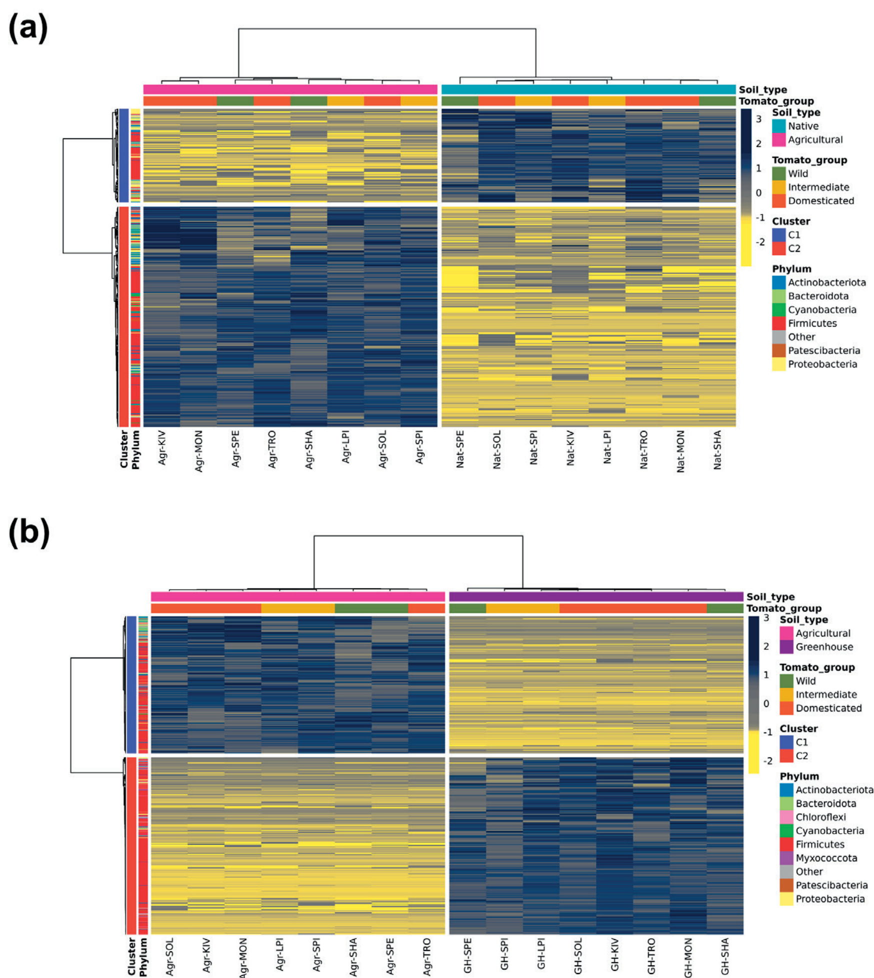

Figure 8. Differential abundance of the tomato root endosphere amplicon sequence variants (ASVs) in pairwise comparisons of different soil types. **(a)** Heatmap of ASVs in the endosphere of tomatoes grown in Ecuadorian native and agricultural soils; clusters C1 = 129; C2 = 304 ASVs. **(b)** Heatmap of ASVs in the endosphere of tomatoes grown in Ecuadorian agricultural soils and Dutch greenhouse soil; clusters C1 = 466; C2 = 602 ASVs. Significant ASVs identified from the DESeq differential abundance analysis were hierarchically clustered using Ward's method as the clustering algorithm. SHA: *S. habrochaites*; SPE*: S. peruvianum*; SPI and LPI: *S. pimpinellifolium*; and tomato varieties of *S. lycopersicum*: SOL: Solarino; TRO: Trovanzo; KIV: Kivu; MON: Moneymaker.

The significantly different ASV clusters in the tomato root endosphere from native and agricultural Ecuadorean soils showed increased abundance of the phyla Actinobacteriota, Bacterioidota, Cyanobacteria, Firmicutes and Proteobacteria. In contrast, Actinobacteriota and Firmicutes were predominantly more abundant in the endosphere of tomato plants grown in Dutch greenhouse soil (Figure 9a). At genus level, the endosphere microbiome of tomato plants grown in native soils showed higher abundance of *Ammoniphilus, Bacillus, Domibacillus, Fictibacillus, Lysinibacillus, Pseudomonas* and *Rhizobacter*, than that of plants grown in agricultural and greenhouse soils. On the other hand, agricultural and greenhouse soils, i.e. managed soils, showed higher abundance of *Clostridium, Cohnella, Massilia, Paenibacillus, Streptomyces* and *Tumebacillus.* Particularly, greenhouse soil increased the abundance of *Tumebacillus, Paenibacillus* and *Cohnella* in tomato endosphere. Although soil was shown to be the main driver of the root endosphere microbiome composition, domestication appeared to impact the abundance of *Actinoplanes, Cohnella, Massilia* and *Streptomyces,* which had higher abundance in the root endosphere of domesticated than of wild-intermediate tomato genotypes, especially when grown in agricultural soil (Figure 9b). Considering the substantial interference of plant DNA in shotgun metagenomic sequencing of endosphere samples, no data on functional genes and traits of the endophytic tomato microbiome were obtained.

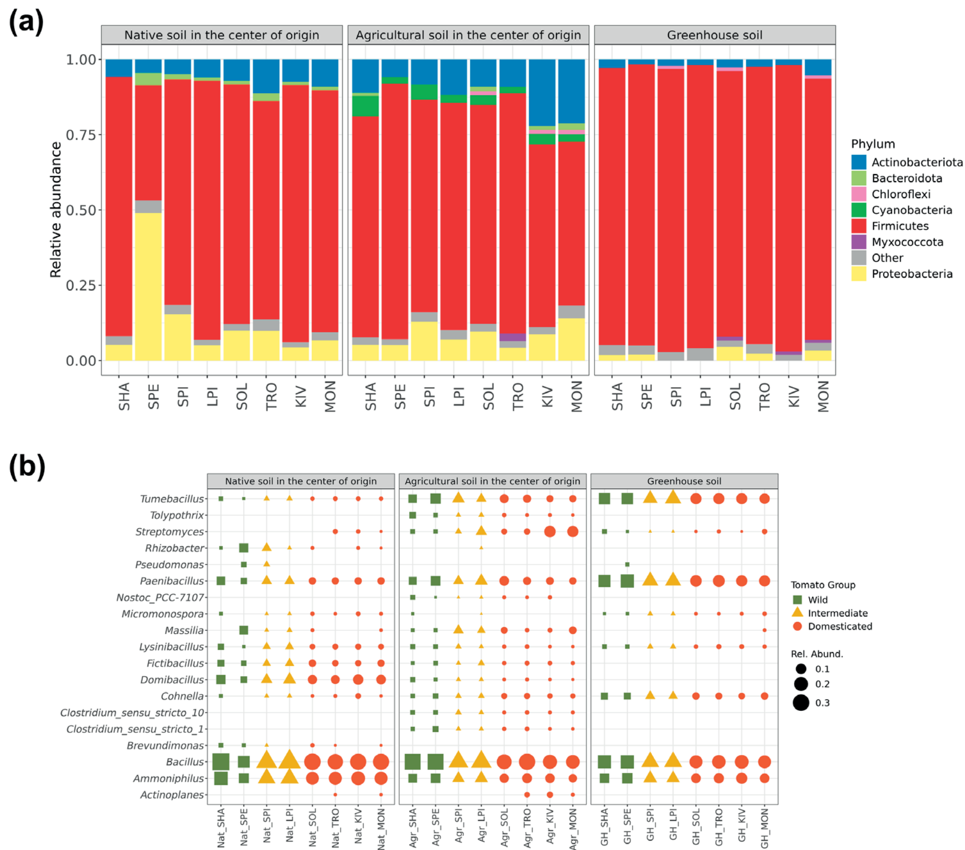

Figure 9. Root endosphere bacterial community composition at phylum and genus level of tomato genotypes in different soil types. **(a)** Relative abundance of bacterial phyla in root endosphere of different tomato genotypes grown in Ecuadorian native and agricultural soils and Dutch greenhouse soil; "Other" category corresponds to grouped phyla with relative abundance < 0.01; **(b)** Relative abundance of highest abundant genera found in root endosphere of different tomato genotypes. ASVs with significant differential abundance between soils and tomato genotypes were grouped according to their phylum or genus level and plotted as stacked bar and bubble charts, respectively. SHA: *S. habrochaites*; SPE: *S. peruvianum*; SPI and LPI: *S. pimpinellifolium*; and tomato varieties of *S. lycopersicum*: SOL: Solarino; TRO: Trovanzo; KIV: Kivu; MON: Moneymaker.

Discussion

Domestication not only evolved specific traits of wild plant species to favor human needs, it also led to substantial changes in their habitats, moving from environmentally harsh, native ecosystems to heavily managed agricultural soils. Additionally, human migration and extensive exchanges of plant germplasm worldwide have further contributed to the vast genetic variation seen in modern tomatoes (Blanca et al., 2022). Agricultural environments can also deplete putative plant growth-promoting rhizobacteria in modern crop accessions (Reid et al., 2024). In this study, we used tomato as a 'model' plant to decipher the impact of domestication on microbiome assembly. To this end, we selected different genotypes and soils, including wild and domesticated tomato genotypes and native and managed soils from the center of origin (Ecuador), as well as greenhouse soil from the center of production (Netherlands) (Supplementary material Tables S1, S2). In this study, we used the same two parental tomato lines (*S. pimpinellifolium* and *S. lycopersicum* cv. Moneymaker) and greenhouse tomato soil as used in our earlier study on microbiome assembly of a tomato RIL population with approximately 100 genotypes (Oyserman et al., 2022). It should be noted that the number of tomato genotypes (n = 8) and soils (n = 5) used in this study is limited to make solid conclusions on the impact of domestication on microbiome assembly. However, the results obtained can serve as a stepping stone for more elaborate screenings and to generate hypotheses that can be experimentally validated on larger sets of plant genotypes and soils.

Our findings revealed that selective filtering of the soil microbiome was stronger in the tomato rhizosphere than in the endosphere (Figures 2, 7). Significant variations in rhizosphere microbiome composition were observed per soil, with wild-intermediate tomato genotypes clustering separately from the domesticated tomatoes (Figure 2). This was further supported by the differential abundance analysis which revealed a similar clustering pattern of the ASVs and whose separation was driven by soil type and the degree of tomato domestication (Figure 3). Soil plays a predominant role in plant microbiome assembly due to its properties, especially nutrient content and the diversity of the microbial 'seed bank' (Cordovez et al., 2019; Ling et al., 2022; Philippot et al., 2024). Particularly, soil amendments such as organic matter, N, P and K, have been shown in several studies to considerably alter the tomato bacterial community assembly in the rhizosphere (Cheng et al., 2020; Dixon et al., 2024; Garcia et al., 2024; Naumova et al., 2022; Zhang et al., 2022). On the other hand, plant genotype can act as a microbiome filter due to the quantitative and/or qualitative differences in root exudation composition, which selects for and enhances the proliferation of specific taxa within the microbiome, as reported in previous studies (French et al., 2020; Poudel et al., 2019). Our results suggest that the differentiation in rhizosphere microbiome

assembly occurred later in the domestication trajectory, likely during breeding and genetic improvement. Based on this, further examination of the differences in tomato root exudate composition per tomato genotype and the effect of soil amendments on the rhizosphere microbiome will be needed to decipher genotype-specific signatures in rhizosphere microbiome assembly.

When summarizing the impact of tomato and habitat domestication on rhizosphere assembly (Figure 4), our results show that: i) Bacteroidota and Proteobacteria had a higher relative abundance in the rhizosphere microbiome of tomatoes grown in native Ecuadorian soils, ii) Actinobacteriota and Cyanobacteria were more abundant in the rhizosphere microbiome of tomatoes grown in Ecuadorean agricultural soils, and iii) Firmicutes were more abundant in the rhizosphere of tomatoes grown in the Dutch greenhouse soil. At genus level, *Chitinophaga, Dyadobacter, Fluviicola, Ohtaekwangia* (Bacteroidota), as well as *Brevundimonas, Cellvibrio* and *Rhizobacter* (Proteobacteria) were more abundant in wild-intermediate tomato genotypes, while *Lysobacter* (Proteobacteria) and *Nocardioides* (Actinobacteriota) were more abundant in domesticated tomato genotypes grown in native soil. In contrast, in agricultural soil, wild-intermediate tomatoes were more abundant in *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (Proteobacteria), while domesticated tomatoes showed increased genera such as *Actinoplanes, Nocardioides* (Actinobacteriota) and *Nostoc* (Cyanobacteria). For greenhouse soil, *Bacillus, Paenibacillus* (Firmicutes) were significantly more abundant in wild-intermediate tomatoes, while *Sericytochromatia* (Cyanobacteria) was more abundant in domesticated tomato genotypes. Interestingly, across all soils, *Pseudomonas* and *Massilia* (Proteobacteria) were more abundant in wild-intermediate tomato genotypes, while *Streptomyces* (Actinobacteriota) and *Sphingomonas* (Proteobacteria) progressively increased along the tomato domestication trajectory (Figure 4).

Several *Pseudomonas* species are known as plant growth-promoting rhizobacteria (PGPR) due their versatile abilities in root colonization, nutrient acquisition, antioxidant activities, pathogen suppression, and stress adaptation (Alattas et al., 2024; Mekureyaw et al., 2022; Sah et al., 2021; Zboralski & Filion, 2020). *Massilia* exhibits copiotrophic behavior and effective attachment to biological surfaces, including fungal hyphae, filamentous algae and Cyanobacteria (Ofek et al., 2012; Salomon et al., 2003; Scheublin et al., 2010), which can make it a successful root colonizer. In our earlier studies, *Massilia* together with *Rhizobium* was also abundant in successively planted wild tomato (Cordovez et al., 2021). Whether these beneficial traits are also prominent for the *Pseudomonas* and *Massilia* species we detected in wild-intermediate tomato genotypes remains to be further investigated.

Our results on the enrichment of *Streptomyces* in the rhizosphere of domesticated tomatoes are consistent with findings from other studies. First and foremost, our results confirm and extend the results of our earlier study on the microbiome of a tomato RIL population, conducted with the same Dutch greenhouse soil and the same parental lines *S. pimpinellifolium* and *S. lycopersicum* cv. Moneymaker (Oyserman et al., 2022). We observed similar patterns of abundance of specific rhizosphere bacteria, especially the significant abundance of *Streptomyces* in the rhizosphere of domesticated tomatoes in any soil type. In our previous study, the increased abundance of *Streptomyces* was associated with a 'modern allele' on chromosome 6 (Oyserman et al., 2022). Also in other studies, similar patterns were reported. For example, Allard (2016) observed higher abundance of members of the Streptomycetaceae family in domesticated tomato (*S. lycopersicum* BHN602) grown in greenhouse conditions. Similarly, Dixon et al. (2024) found that modern (ca. 2020) and traditional tomatoes (ca. 1900) had a greater relative abundance of Actinobacteriota than wild tomatoes, especially in P-fertilized soil. In addition, Chen et al. (2022) demonstrated differential recruitment of rhizobacteria based on tomato fruit color phenotype, with Actinobacteriota being more abundant in tomatoes with yellow fruit compared to those with red fruit. Furthermore, Actinobacteriota can be competitive for root exudates and micronutrients, such as iron, as well as for antibiotic production (Nazari et al., 2023; Oyserman et al., 2022; Pérez-Jaramillo et al., 2016, 2018; Zhao et al., 2018), which may facilitate the successful colonization of *Streptomyces* in domesticated tomato rhizosphere. The observed enrichment of *Sphingomonas* in the rhizosphere of domesticated tomatoes is also confirms and extends the study by Lee et al. (2019) who proposed *Sphingomonas* as one of the indicators of the rhizosphere of domesticated tomato genotypes sampled from different greenhouses. Interestingly, we also observed an increase in Cyanobacteria genera, such as *Nostoc* and *Sericytochromatia* in domesticated tomato genotypes grown in agricultural and in greenhouse soils, respectively. This suggests that these bacterial genera are likely responding to nutrient availability, particularly N and P (Xu et al., 2017), and possibly to the reduced use of organic fertilization in agricultural soils, as observed by Zou et al. (2024).

Our profiling of the microbial traits of key tomato rhizosphere bacteria, in relation to genotype and habitat domestication, provided a better understanding of the interactions between these bacteria, their different tomato hosts, and the soil types (Figure 5; Supplementary Table S6). Unfortunately, we did not find MAGs assigned as *Massilia* or the Oxalobacteraceae family, which were abundant taxa in the rhizosphere of wild tomatoes across the soil types tested. We found a higher number of genes associated with the SEED subsystems Motility and Chemotaxis in rhizosphere MAGs of wild tomato genotypes grown in native soil and taxonomically delineated as Bacteroidetes, Cellvibrionales, Pseudomonadales and Rhizobiales. This observation aligns with find-

ings by Sun et al. (2021), who found that the root microbiome of wild rice accessions exhibited a greater abundance of bacterial chemotaxis genes than the microbiomes of their domesticated counterparts. Moreover, abundance of these bacterial taxonomic lineages was associated by Yin et al. (2020) with plant protection against pathogens. They found a higher abundance of Bacteroidetes, Pseudomonadales and Rhizobiales in tomato cultivars that resisted bacterial canker caused by *Clavibacter michiganensis*. In addition, the higher number of genes associated with flagellar motility observed in these MAGs, particularly in *Pseudomonas, Cellvibrio* and Rhizobiacea, can be linked to enhanced rhizosphere colonization and increased carbohydrate metabolism as revealed in other studies (Barajas et al., 2020; de Weert et al., 2002; Liu et al., 2024; Ramoneda et al., 2024; Zuluaga et al., 2021). Moreover, carbohydrate metabolism also showed a higher number of genes in MAGs associated with the microbiomes of the wild tomato genotypes, consistent with findings by Zboralski & Filion (2020). Additionally, the higher abundance of microbial functions associated with stress response found here may align with the observation that the wild tomato rhizosphere has a greater capacity to adapt to environmental challenges, such as oxidative or osmotic stress, as reported in several studies (Alzate Zuluaga et al., 2021; Anjum et al., 2025; Schmitz et al., 2022).

MAGs associated with the rhizosphere of domesticated tomato genotypes, especially in agricultural and greenhouse soils, exhibited a higher abundance of genes involved in amino acid metabolism, cofactors and vitamins, as well as genes related to N, P and Fe metabolism. Specifically, the increased abundance of genes related to ammonification, allantoin utilization, and siderophore biosynthesis, suggests that the microbiome associated with domesticated tomatoes may respond to fertilizer inputs with both macro- and micronutrients, as indicated in other studies (Adedayo et al., 2022; Dixon et al., 2024; Garcia et al., 2024; Smulders et al., 2021; Terra et al., 2021; Zhang et al., 2022).

With respect to the endosphere tomato microbiome, soil type was the primary determinant of the community assembly (Figures 8, 9). This effect was particularly pronounced in the phylum Firmicutes with *Bacillus* remaining consistent across soils, while *Ammoniphilus, Domibacillus, Fictibacillus* and *Lysinibacillus* showed a progressive decline in abundance from Ecuadorean native to Dutch greenhouse soil. Conversely, *Cohnella, Paenibacillus* and *Tumebacillus* exhibited a progressive increase in abundance from Ecuadorean native to Dutch greenhouse soil. Collectively, these results suggest that soil management influenced microbiome assembly of the tomato root endosphere. Other taxa exhibited genotype-specific differences for Ecuadorean native and agricultural soils. For example, *Brevundimonas, Pseudomonas, Rhizobacter* (Proteobacteria) were more abundant in the endosphere of wild-intermediate tomatoes grown in native soils, while *Actinoplanes, Streptomyces* (Actinobacteriota) and *Tolypotrix* (Cyanobacteria) were more abundant in the endosphere of domesticated tomatoes grown in agricultural soil.

Further investigation is needed to understand how root exudates and soil management practices influence the selective enrichment of these endosphere taxa and their ability to withstand plant defense responses, as well as to adapt to the specific environmental conditions associated with different tomato genotypes and soil types.

In conclusion, our results highlight the significant impact of the tomato domestication process on its microbiome, with habitat domestication playing a pronounced role in root microbiome assembly. Our results suggest that the major differentiation in rhizosphere microbiome assembly occurred later in the domestication process, i.e. during the breeding and genetic improvement stages. Additionally, we observed that wild tomatoes grown in native soils exert strong selective pressure on the rhizosphere microbiome, favoring bacteria enriched in genes associated with motility, chemotaxis and stress response. In contrast, domesticated tomatoes establish microbiome associations with bacteria that exhibit functional adaptations to agricultural and greenhouse environments, presumably fertilizer inputs. Our study emphasizes the importance of understanding microbiome shifts driven by both genotype and habitat domestication. These insights are essential for integrating microbiome management strategies into tomato cultivation and considering the microbiome as a complementary feature in breeding programs. Future research should focus on the identity and role of specific root exudates in microbial assembly for both wild and domesticated tomatoes. Additionally, the effects of soil amendments (organic and synthetic fertilizers) and other agricultural practices on tomato genotypes should be further explored. It is also important to assess the phenotypic response of tomatoes to rhizobacterial inoculation, specifically in terms of plant growth, resistance to (a)biotic stresses, and yield, with an emphasis on the genetic background of different tomato cultivars to maximize the efficiency of microbial interventions. Such studies could provide valuable insights to promote sustainable farming practices through the integration of agricultural techniques with microbial management.

Materials and Methods

Greenhouse experiment

Native and agricultural soils from the tomato's center of origin were collected in June 2021 from two locations in the province of Loja in Southern Ecuador (Ceiba 4°18'07.6"S, 80°13'16.7"W and Limones 4°23'09.2"S, 80°20'50.7"W, Zapotillo, Loja, Ecuador). Native soils were collected from a natural vegetation close to the agricultural locations. Agricultural soil from Ceiba was sampled from a field that was cultivated with onion (vegetative growth stage), whereas the agricultural soil in Limones was sampled from a local rice field (grain filling stage). All four soils were air dried at room

temperature and subsequently sieved (2 mm diameter sieve mesh) and shipped to the greenhouse facility at the Netherlands Institute of Ecology (NIOO-KNAW) in Wageningen, The Netherlands in compliance to the permit approved by the Ecuadorian Ministry of Environment. A Dutch greenhouse used for tomato seed production in South-Holland, (51°57'47"N, 4°12'16"E, collected in June 2017), provided the greenhouse soil from the center of production (Supplementary material Table S1). The five soils of interest were coded as follows: Ceiba native (CN), Ceiba agricultural (CA), Limones native (LN), Limones agricultural (LA) and, Dutch greenhouse soil (GH) (Figure 10).

A total of 8 tomato genotypes were selected and included in this experiment, and given the following abbreviations: two wild tomato species *S. peruvianum* (SPE), and *S. habrochaites* (SHA); two accessions of the closest ancestor *S. pimpinellifolium* (LPI and SPI); and four modern *S. lycopersicum* tomato varieties Solarino (SOL), Trovanzo (TRO), Kivu (KIV) and Moneymaker (MON) (Supplementary material Table S2).

To facilitate the analysis of soils and tomato genotypes, they were grouped in soil types and tomato domestication degree. Hence, Ceiba and Limones native soils were catalogued as "native soil", and Ceiba and Limones agricultural soils were named as "agricultural soil". Also, tomatoes *S. peruvianum* and *S. habrochaites* were considered as "wild", the two *S. pimpinellifolium* accessions were named as "intermediate", and varieties of *S. lycopersicum* (Solarino, Trovanzo, Kivu and Moneymaker) were termed as "domesticated" tomatoes (Figure 10).

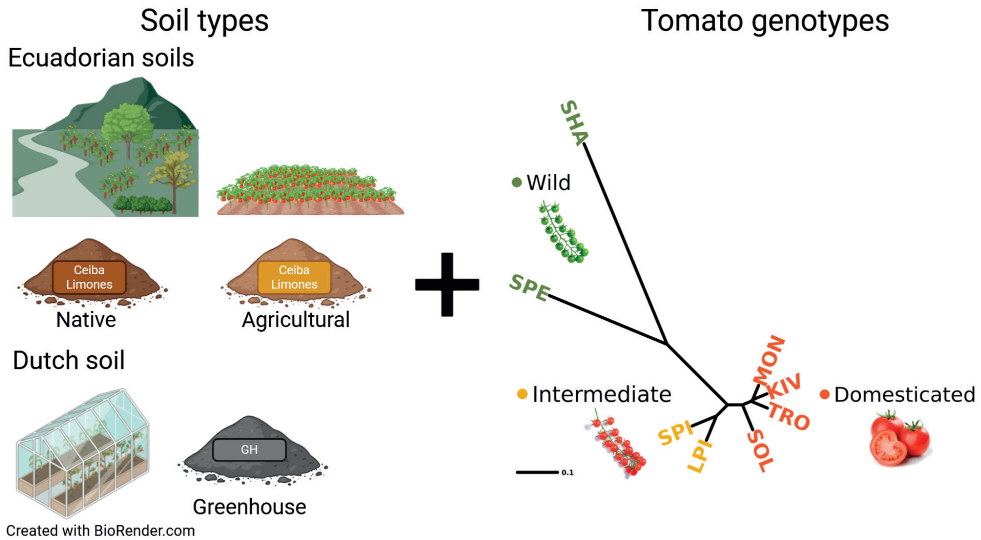

Figure 10. Soil origin (left panel) and genetic diversity of tomato genotypes (right panel) used in the experiment. Genetic diversity of the eight selected tomato genotypes was based on DArT genotyping data (17,855 SNP). The scale bar indicates the Jaccard distance to measure the dissimilarity between tomato genotypes based on SNP presence/absence. Hierarchical clustering was performed by unweighted pair group method with arithmetic mean (UPGMA). Tomato genotypes were grouped according to the domestication trajectory in wild, intermediate, and domesticated tomatoes. SHA: *S. habrochaites;* SPE: *S. peruvianum;* SPI and LPI: *S. pimpinellifolium;* and tomato varieties of *S. lycopersicum:* SOL: Solarino; TRO: Trovanzo; KIV: Kivu; MON: Moneymaker.

Prior to the greenhouse experiment, the soil samples were conditioned during seven days by adding 20% (v/w) of sterile demi-water to the soil bags. The bags were then kept under greenhouse conditions in the shade (25 °C day; 18 °C night). After the conditioning phase, 5% (w/w) of each soil was mixed with 95% moistened sterile fine sand (0.4–0.8 mm), and 500 g of the mixture was transferred into polyethylene pots (10 × 10 × 11 cm).

Tomato seeds were surface sterilized by adding 5 ml of 80% ethanol and shaking for 2 min. The ethanol was then removed, and 5 ml of 1.5% (v/v) sodium hypochlorite was added. The seeds were shaken for 10 min, after which the solution was discarded. To remove any residual solution, seeds were rinsed five times with 5 ml of sterile demi-water, vortexing for 2 min during each cycle and discarding the water after each rinse. Following the sterilization, seeds were placed on a wet filter paper in a Petri dish containing 5 ml of sterile demi-water and incubated at 25 °C for three days. One pre-germinated tomato seed (radicle length ~1 cm) was sown in the center of the pot. Pots were covered with a plastic film for three days to maintain the humidity until the cotyledons emerged. The experiment consisted of eight tomato genotypes (SPE, SHA, SPI, LPI,

SOL, TRO, KIV, MON) and five soils (four Ecuadorian soils: CA, LA, CN, LN, and one Dutch tomato greenhouse soil GH), with a total of 239 pots [5 soils × 8 tomatoes × 5 replicates + 24 control (8 tomatoes in sand × 3 replicates) + 15 bulk soil (5 soil-sand mix × 3 replicates)] randomly distributed.

Plants were grown under greenhouse conditions set at 25 °C/18 °C (±1 °C) day/night; with 16 h light and 60% relative humidity. The watering regime was adjusted to the plant's requirements during the growth period, starting with 5 ml of sterile demi-water per day and gradually increasing to 100 ml per day. Additionally, a 50% Hoagland nutrient solution was applied twice per week throughout the growth period (Supplementary material Table S3).

Rhizosphere sampling

Plants were sampled at their 6th true leaf stage (25–35 days after planting the germinated seeds). The shoots were cut and the root was carefully removed from the pot. Loosely attached soil was shaken off, leaving the tightly attached soil (i.e. rhizosphere soil). A 5 g sample of each root system and 5 g of each bulk soil sample were collected in 15 ml-tubes, immediately frozen in liquid nitrogen and stored at -80 ºC until further processing.

Before DNA extraction, samples were defrosted at 4 ºC and 5 ml of sterile demi-water was added and vortexed at maximum speed to remove the soil particles attached to the roots (rhizosphere). The roots were transferred to a new 15 ml-tube. Both rhizosphere soil suspension and frozen bulk soil samples were freeze-dried to remove the excess water. The roots were surface sterilized by vortexing with 5 ml of 75% ethanol at medium speed (~2000 rpm) for 2 min. The ethanol solution was discarded and 5 ml of 1.5% (v/v) sodium hypochlorite were added. The roots were vortexed at medium speed for 5 min and after that, the hypochlorite solution was removed, followed by addition of 5 ml of sterile demi-water. Tubes were vortexed for 2 min, after which the water was carefully discarded. This washing process was repeated five times. Finally, the surface-sterilized roots were freeze dried to remove any remaining water.

Soil and root DNA isolation and sequencing

The rhizosphere soil samples were prepared by weighing 0.5 g of freeze-dried soil. For roots, 30 mg of freeze-dried roots were transferred into 2 ml-microtubes and the roots were homogenized with three metal beads (⌀ ⅛ inch) in the TissueLyser (Qiagen) at maximum speed (30 Hz, 1800 oscillations per minute) for 2 min. The resulting root powder was used for endosphere DNA isolation.

The Qiagen DNeasy® PowerSoil® Pro Kit was used to extract genomic DNA from soil and roots according to the manufacture's protocol. DNA samples were sent to Genome Québec (Canada) for amplicon library preparation and subsequent sequencing of the V3-V4 regions of the 16S rRNA gene using the universal bacterial primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). Paired-end sequence reads (300 bp length) were generated using the Illumina NovaSeqX Plus platform. In addition, 24 rhizosphere samples corresponding to two genotypes (LPI and MON) grown in three soils (LA, LN and GH) representing four replicates were subjected to library preparation and shotgun sequencing by Novogene Co, Ltd. (Canada) to provide pair-end reads of 150 bp by Illumina NovaSeq 6000.

Amplicon data analysis

The compressed sequence reads contained in FASTQ format files were processed by the DADA2 v1.16.0 pipeline (Callahan et al., 2016) in RStudio environment (RStudio Team, 2020) to obtain the ASV abundance and taxonomy tables. The modeling of error rates associated with the sequencing process was adjusted to appropriately process NovaSeq data using the DADA2 pipeline, as suggested by Holland-Moritz (2021). The SILVA 16S ribosomal RNA gene reference database (v138) (Quast et al., 2013) was used for bacterial taxonomy assignment. ASVs present in control samples, i.e., plants grown in pure sand, were filtered out from all the samples before the analysis. The statistical analyses were performed using R software version 4.3.1 (R Core Team, 2023). Packages such as vegan (Oksanen et al., 2020), phyloseq (McMurdie & Holmes, 2013), DESeq2 (Love et al., 2014) and ggplot2 (Wickham, 2016) were used for alpha diversity, beta diversity (Bray–Curtis distance, PERMANOVA with 9,999 permutations), and differential abundance analyses, while the tidyverse package (Wickham et al., 2019) was used for formatting and visualization. Significant differences in the Shannon diversity index were determined by Kruskal-Wallis test and Wilcoxon post hoc test, while differences of beta diversity between tomato groups in pairwise comparisons were evaluated using the Adonis test. The abundance data were normalized by CSS (Cumulative Sum Scaling) for Principal Coordinates Analysis (PCoA) which was performed with the *cmdscale* function from the vegan package and the Bray–Curtis distance calculated previously. Differential abundance analyses were performed using the model "Soil_class + Tom_gen", specified in the design formula of the *DESeq* function, where Soil_class refers to the soil type, i.e. Native, Agricultural, or Greenhouse and Tom_gen represents the tomato genotype (eight genotypes). Pairwise contrasts were tested between Native vs. Agricultural soil, and Agricultural vs. Greenhouse soil. To account for varying sequencing depths, size factors were estimated using a geometric mean approach from the counts data. ASVs resulted from differential abundance analysis were visualized in a heatmap using the R package pheatmap (Kolde, 2018). Clusters

were identified through hierarchical clustering, using correlation as the distance metric and Ward's method for the clustering algorithm.

Metagenome data analysis

To gain knowledge about the potential functionality of specific members of the rhizosphere tomato microbiome, bins were produced to be annotated further. First co-assembly of all samples was done using Megahit (Li et al., 2015). Read mapping against contigs was performed using Bowtie2 (Langmead & Salzberg, 2012) and binning was done using MaxBin2 (Wu et al., 2016) and Metabat2 (Kang et al., 2019). Results from both binning approaches were processed with DAS Tool to obtain refined bins (Sieber et al., 2018). Bins were taxonomically annotated using GTDB-tk (v2.4.0) (Chaumeil et al., 2020).

The quality of the resulting bins was assessed using CheckM (Parks et al., 2015). Afterwards, 11 out of 127 bins with completeness higher than 70% and contamination less than 10%, were considered representative metagenome-assembled genomes (MAGs) of the bacteria associated with tomato and soils, consistent with the amplicon results (Supplementary Table S5). These MAGs were submitted to the RAST server (Rapid Annotation using Subsystems Technology) (Aziz et al., 2008) for annotation of functional genes (Supplementary Table S6).

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Author contributions

SSF: methodology, investigation, data curation; writing - original draft, review & editing; VC: supervision, writing - review & editing; data curation; BOO: review & editing; LMAG: metagenomic data analysis; review & editing; NS: conceptualization; methodology; supervision; data curation; JMR: conceptualization; supervision; funding acquisition, writing - review & editing; PVTH: supervision; review & editing. All authors contributed critically to the drafts and gave final approval for publication.

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Plant research permit

This study was carried out under the Genetic Resource Permit N° MAE-DNB-CM-2018-0085, issued by the Ministry of Environment of Ecuador to USFQ.

Supplementary material

Supplementary Figures

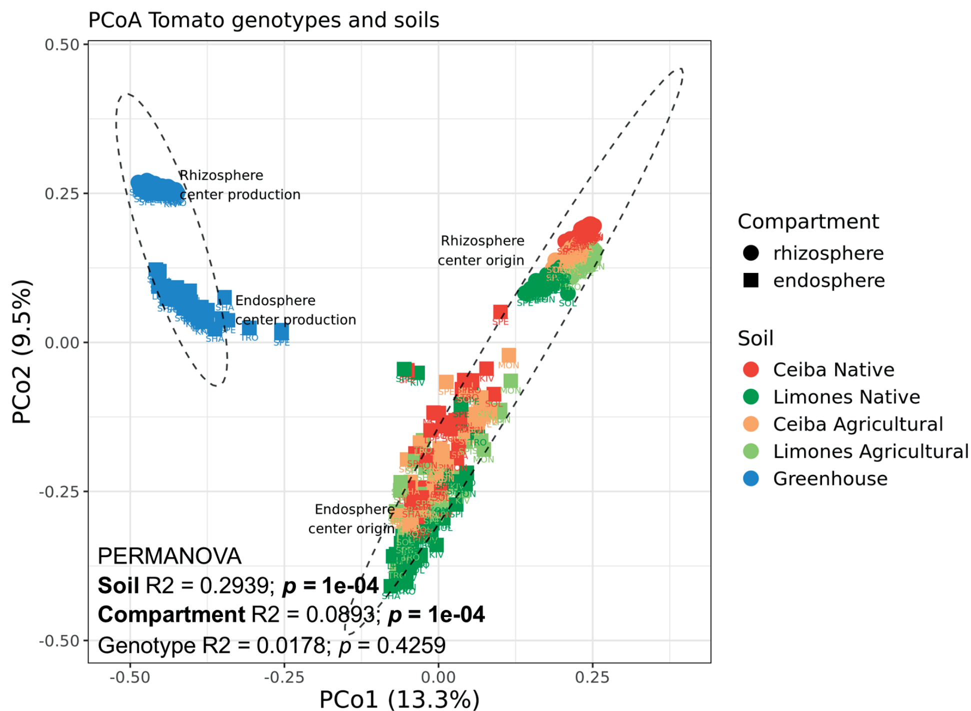

Figure S1. Bacterial community structure of tomato genotypes grown in native, agricultural and greenhouse soils. Principal Coordinate Analysis (PCoA) of rhizosphere bacterial communities showing distinct separation between soils from center of origin and the center of production, as well as differentiation of bacterial communities between tomato rhizosphere and endosphere.

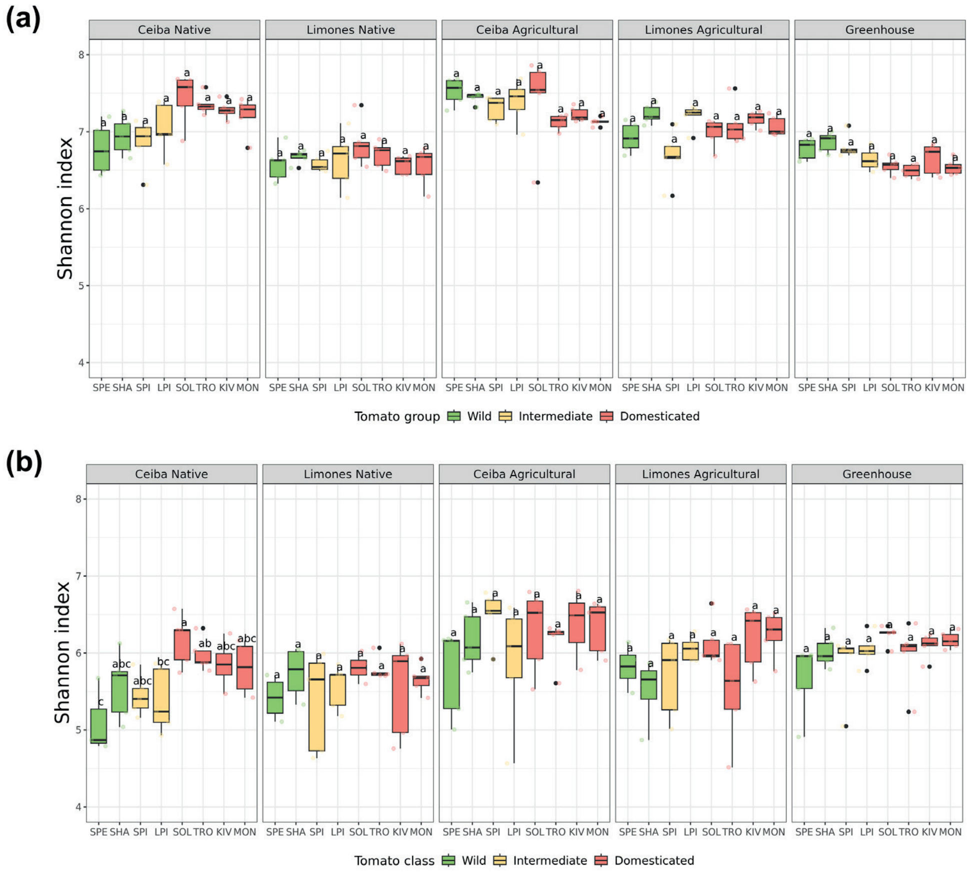

Figure S2. Shannon diversity index in tomato **(a)** rhizosphere and **(b)** endosphere grown in native, agricultural and greenhouse soils. Different letters above boxplots show significant difference (Wilcoxon test $p < 0.05$) of Shannon diversity index between tomato genotypes ($n = 5$). SPE: *S. peruvianum;* SHA: *S. habrochaites;* SPI and LPI: *S. pimpinellifolium;* and tomato varieties of *S. lycopersicum:* SOL: Solarino; TRO: Trovanzo; KIV: Kivu; MON: Moneymaker.

Supplementary Tables

Table S1. Physicochemical properties of soils employed in the experiment.

Soil	pH	OM (g/kg)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	CIC (cmol/kg)	Sand (%)	Silt (%)	Clay (%)	Texture
Ceiba_Agr	7,46	13,10	700,00	19,10	50,80	2957,90	473,00	78,90	6,48	4,28	2,30	15,62	38	36	26	Loam
Ceiba_Nat	7,29	15,10	800,00	29,60	62,60	3266,50	555,70	29,60	5,48	2,56	2,29	16,01	24	50	26	Silt loam
Limones_Agr	7,19	6,20	300,00	66,80	160,30	2154,30	340,50	56,80	6,07	3,67	3,29	9,24	52	32	16	Sandy loam
Limones_Nat	7,83	2,90	100,00	47,70	215,10	2709,40	345,30	50,50	2,97	2,17	2,06	7,58	68	18	14	Sandy loam
Green-house	7,10	24,00	1040,00	15,60	309,00	156,31	196,00	2,01	1,88	0,04	0,39	7,90	85	8	3	Loamy sand

Table S2. Tomato accessions used for DArT genotyping.

Code	Species	Accession number	Origin	Passport
SPE	*Solanum peruvianum*	CGN23955	Perú	https://cgngenis.wur.nl/accessiondetails/CGN23955
SHA	*Solanum habrochaites*	CGN15792	Ecuador	https://cgngenis.wur.nl/accessiondetails/CGN15792
LPI	*Solanum pimpinellifolium*	CGN14498	NA	https://cgngenis.wur.nl/accessiondetails/CGN14498
SPI	*Solanum pimpinellifolium*	CGN23957	Perú	https://cgngenis.wur.nl/accessiondetails/CGN23957
MON	*Solanum lycopersicum* cv Money-maker	CGN14330	Netherlands	https://cgngenis.wur.nl/accessiondetails/CGN14330
KIV	*Solanum lycopersicum* cv Kivu	NA	© Rijk Zwaan	https://www.rijkzwaanusa.com/tomato/KIVU-RZ-F1-72-629-prdSL11187-ctgCrops.tomato
TRO	*Solanum lycopersicum* cv Trovanzo	NA	© Rijk Zwaan	https://rijkzwaan.it/pomodoro/TROVANZO-RZ-F1-72-762-prdSL11225-ctgCrops.tomato
SOL	*Solanum lycopersicum* cv Solarino	NA	© Rijk Zwaan	https://www.rijkzwaan.de/tomate/SOLARINO-F1-72-150-prdSL11078-ctgCrops.tomato

Table S3. Hoagland solution composition.

Hoagland solution stock	**M (g/mol)**	**Molarity**	**g/l Stock**	**1 liter 1/2X**
Macroelements:				
Calcium nitrate $Ca(NO_3)_2 \cdot 4H_2O$	236.16	0.5 M	118.08	5 ml
Potassium nitrate KNO_3	101.11	1.0 M	101.11	2.5 ml
Potassium phosphate KH_2PO_4	136.09	1.0 M	136.09	0.5 ml
Magnesium sulfate $MgSO_4 \cdot 7H_2O$	246.47	0.5 M	123.24	2 ml
Microelements:				1 ml
Boric acid H_3BO_3	61.83	46.3 mM	2.86	
Manganese chloride $MnCl_2 \cdot 4H_2O$	197.91	9.1 mM	1.81	
Zinc sulfate $ZnSO_4 \cdot 7H_2O$	287.54	0.77 mM	0.22	
Copper sulfate $CuSO_4 \cdot 5H_2O$	249.68	0.32 mM	0.08	
Sodium molybdate $Na_2MoO_4 \cdot 2H_2O$	241.95	0.52 mM	0.126	
EDTA ferric salt $C_{10}H_{12}FeN_2NaO_8 \cdot 3H_2O$	421.1	98.6 mM	41.52	

Note. For preparing 1000 ml of Hoagland solution stock: Dissolve the components (g/l Stock) in 1000 ml of sterile demi water and store at room temperature in the shade. Stock solution of microelements can be prepared in 500 ml in an amber bottle or covered with aluminum foil to protect it against light.

For preparing 1000 ml of Hoagland 1/2X concentration: Autoclave 989 ml of demi water and add the volume of each Hoagland component by filtration (Whatman cellulose syringe filter 0,2 µm).

Table S4. Pairwise analysis by Adonis test in tomato rhizosphere and endosphere.

Plant Compartment	Soil	Pairs	R^2	p.adj	sig.
Rhizosphere	Ceiba_Agr	W × I	0,0653	0,0615	
Rhizosphere	Ceiba_Agr	W × D	0,1366	0,00015	*
Rhizosphere	Ceiba_Agr	I × D	0,1283	0,00015	*
Rhizosphere	Ceiba_Nat	W × I	0,0633	0,0742	
Rhizosphere	Ceiba_Nat	W × D	0,1818	0,00015	*
Rhizosphere	Ceiba_Nat	I × D	0,1737	0,00015	*
Rhizosphere	Limones_Agr	W × I	0,0698	0,0442	*
Rhizosphere	Limones_Agr	W × D	0,1693	0,00015	*
Rhizosphere	Limones_Agr	I × D	0,1491	0,00015	*
Rhizosphere	Limones_Nat	W × I	0,0662	0,0605	
Rhizosphere	Limones_Nat	W × D	0,1554	0,00015	*
Rhizosphere	Limones_Nat	I × D	0,1799	0,00015	*
Rhizosphere	Greenhouse	W × I	0,0608	0,1914	
Rhizosphere	Greenhouse	W × D	0,2344	0,00015	*
Rhizosphere	Greenhouse	I × D	0,2104	0,00015	*
Endosphere	Ceiba_Agr	W × I	0,0558	0,1893	
Endosphere	Ceiba_Agr	W × D	0,0509	0,0123	*
Endosphere	Ceiba_Agr	I × D	0,0403	0,12	
Endosphere	Ceiba_Nat	W × I	0,064	0,0506	
Endosphere	Ceiba_Nat	W × D	0,0468	0,0306	*
Endosphere	Ceiba_Nat	I × D	0,0485	0,0306	*
Endosphere	Limones_Agr	W × I	0,0585	0,0966	
Endosphere	Limones_Agr	W × D	0,0631	0,0021	*
Endosphere	Limones_Agr	I × D	0,0458	0,0461	*
Endosphere	Limones_Nat	W × I	0,0583	0,1749	
Endosphere	Limones_Nat	W × D	0,0506	0,0663	
Endosphere	Limones_Nat	I × D	0,0381	0,1749	
Endosphere	Greenhouse	W × I	0,0542	0,3477	
Endosphere	Greenhouse	W × D	0,0545	0,0108	*
Endosphere	Greenhouse	I × D	0,0459	0,0402	*

Table S5. High quality-bins assembled from rhizosphere tomato grown in different soils

Bin_Id	Marker_lineage	Completeness	Contamination	Kingdom	Phylum	Class	Order	Family	Genus	Species
bin.555	p__Bacteroidetes (UID2605)	99.87	0.48	Bacteria	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae		
bin.732	o__Cytophagales (UID2936)	99.7	1.07	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Spirosomaceae	Emticicia	
bin.204	p__Bacteroidetes (UID2605)	99.52	1.9	Bacteria	Bacteroidota	Bacteroidia	AKYH767	b-17BO		
bin.895	p__Bacteroidetes (UID2591)	99.51	2.4	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae		
bin.601	o__Cytophagales (UID2936)	99.03	5.7	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Cyclobacteriaceae		
bin.795	f__Xanthomonadaceae (UID4214)	98.85	6.98	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Dyella	
bin.952	k__Bacteria (UID2569)	98.76	0	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Crocinitomicaceae	Fluviicola	
bin.745	o__Actinomycetales (UID1808)	98.54	3.73	Bacteria	Actinobacteriota	Actinobacteria	Mycobacteriales	Mycobacteriaceae	Mycolicibacterium	
bin.518	o__Sphingomonadales (UID3310)	98.3	4.66	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis	
bin.714	k__Bacteria (UID203)	98.28	9.87	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella	
bin.747	k__Bacteria (UID3187)	98.16	0.89	Bacteria	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	Bdellovibrionaceae	Ga0074139	
bin.224	o__Actinomycetales (UID2012)	97.82	8.06	Bacteria	Actinobacteriota	Actinobacteria	Mycobacteriales	Pseudonocardiaceae	Pseudonocardia	
bin.572	p__Bacteroidetes (UID2605)	97.6	1.94	Bacteria	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae		

Table S5. High quality-bins assembled from rhizosphere tomato grown in different soils (*continued*)

Bin_Id	Marker_lineage	Completeness	Contamination	Kingdom	Phylum	Class	Order	Family	Genus	Species
bin.53	k__Bacteria (UID2569)	97.57	1.4	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Crocinitomicaceae	Fluviicola	
bin.643	p__Cyanobacteria (UID2182)	97.56	1.66	Bacteria	Cyanobacteria	Cyanobacteriia	Elainellales	Elainellaceae	Leptolyngbya_A	
bin.761	o__Sphingomonadales (UID3310)	97.4	6.14	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis	
bin.929	o__Actinomycetales (UID1696)	97.25	2.54	Bacteria	Actinobacteriota	Actinobacteria	Mycobacteriales	Micromonosporaceae		
bin.918	p__Bacteroidetes (UID2605)	96.87	1.22	Bacteria	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	
bin.215	k__Bacteria (UID3187)	96.37	2.68	Bacteria	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	Bdellovibrionaceae	21-14-0-10-47-8	
bin.296	c__Deltaproteobacteria (UID3216)	96.13	6.45	Bacteria	Myxococcota	Polyangia	Polyangiales	Sandaracinaceae		
bin.111	p__Actinobacteria (UID1454)	96.07	1.95	Bacteria	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae		
bin.690	k__Bacteria (UID2982)	95.95	5.41	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	AV2		
bin.890	c__Gammaproteobacteria (UID4202)	95.86	6.02	Bacteria	Proteobacteria	Gammaproteobacteria	Nevskiales	Nevskiaceae	Fontimonas	
bin.721	o__Pseudomonadales (UID4488)	95.73	5.18	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas_E	
bin.485	p__Bacteroidetes (UID2591)	95.71	7.51	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Taibaiella_B	
bin.188	o__Burkholderiales (UID4000)	95.53	7.18	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Herminiimonas	

Table S5. High quality-bins assembled from rhizosphere tomato grown in different soils (*continued*)

Bin_Id	Marker_lineage	Completeness	Contamination	Kingdom	Phylum	Class	Order	Family	Genus	Species
bin.591	k__Bacteria (UID3187)	95.5	1.8	Bacteria	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio_A	
bin.844	o__Rhizobiales (UID3450)	95.41	2.21	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Chelativorans	Chelativorans sp000014245
bin.257	k__Bacteria (UID3187)	95.39	5.73	Bacteria	Acidobacteriota	Thermoanaerobaculia	Gp7-AA8	Gp7-AA8	QHVT01	
bin.324	k__Bacteria (UID2982)	95.35	2.86	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	Haloferula	
bin.633	k__Bacteria (UID2569)	95.26	1.62	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Crocinitomicaceae	Fluviicola	
bin.221	k__Bacteria (UID3187)	94.74	1.26	Bacteria	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	Bdellovibrionaceae	21-14-0-10-47-8	
bin.441	c__Deltaproteobacteria (UID3216)	94.62	3.39	Bacteria	Myxococcota	Myxococcia	Myxococcales	Myxococcaceae		
bin.177	k__Bacteria (UID3187)	94.12	0.89	Bacteria	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	Bdellovibrionaceae	21-14-0-10-47-8	
bin.329	o__Sphingomonadales (UID3310)	93.95	6.7	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	
bin.117	k__Bacteria (UID3187)	93.9	2.41	Bacteria	Bdellovibrionota	Bacteriovoracia	Bacteriovoracales	Bacteriovoracaceae	Bacteriovorax	
bin.330	k__Bacteria (UID3187)	93.87	9.54	Bacteria	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	Luteitalea	
bin.838	c__Betaproteobacteria (UID3959)	93.69	5.35	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	SG8-41		
bin.664	o__Burkholderiales (UID4002)	93.57	4.3	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Herminiimonas	

Table S5. High quality-bins assembled from rhizosphere tomato grown in different soils (*continued*)

Bin_Id	Marker_lineage	Completeness	Contamination	Kingdom	Phylum	Class	Order	Family	Genus	Species
bin.273	k__Bacteria (UID2982)	93.34	5.14	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Opitutales	Opitutaceae	Didemnitutus	
bin.737	c__Deltaproteobacteria (UID3216)	93.12	3.25	Bacteria	Myxococcota	Polyangia	Polyangiales			
bin.319	c__Gammaproteobacteria (UID4444)	92.98	2.39	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Cellvibrionaceae	Cellvibrio	
bin.464	p__Bacteroidetes (UID2591)	92.61	6.03	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Taibaiella_B	
bin.515	o__Burkholderiales (UID4000)	92.5	5.06	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Rhizobacter	
bin.302	p__Bacteroidetes (UID2605)	92.49	1.67	Bacteria	Bacteroidota	Bacteroidia	AKYH767	b-17BO		
bin.861	g__Burkholderia (UID4006)	92.23	0.31	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Paraburkholderia	Paraburkholderia sp000148685
bin.427	f__Micrococcaceae (UID1623)	92.16	4.38	Bacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Micrococcaceae	Sinomonas	
bin.647	c__Gammaproteobacteria (UID4266)	92.16	1.75	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella_C	
bin.606	o__Burkholderiales (UID4000)	91.79	2.29	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Noviherbaspirillum	
bin.607	c__Betaproteobacteria (UID3959)	91.71	5.08	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	SG8-41	PLOWO2-02-64-14	
bin.7	p__Bacteroidetes (UID2591)	91.71	3.2	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Flavisolibacter	
bin.124	o__Burkholderiales (UID4000)	91.49	6.51	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Noviherbaspirillum	

Table S5. High quality-bins assembled from rhizosphere tomato grown in different soils (*continued*)

Bin_Id	Marker_lineage	Com-plete-ness	Contami-nation	King-dom	Phylum	Class	Order	Family	Genus	Species
bin.868	p__Proteobacteria (UID3887)	91.48	8.95	Bacteria	Proteobac-teria	Gammaproteo-bacteria	Burkhold-eriales	Burkholderia-ceae	Lautropia	
bin.373	c__Alphaproteobac-teria (UID3422)	90.57	3.32	Bacteria	Proteobac-teria	Alphaproteo-bacteria	Caulobacte-rales	Caulobactera-ceae	Asticcacaulis	
bin.548	o__Burkholderiales (UID4000)	90.38	4.35	Bacteria	Proteobac-teria	Gammaproteo-bacteria	Burkhold-eriales	Burkholderia-ceae	Noviherbaspirillum	
bin.915	c__Gammaproteo-bacteria (UID4445)	90.04	5.96	Bacteria	Proteobac-teria	Gammaproteo-bacteria	Pseudomonad-ales	Pseudo-monadaceae	Pseudomonas_A	Pseudomonas_A stutzeri_AI
bin.808	f__Micrococcaceae (UID1623)	89.55	2.18	Bacteria	Actinobacte-riota	Actinobacteria	Actinomyce-tales	Micrococcaceae	Arthrobacter_D	Arthrobacter_D sp001428435
bin.472	o__Burkholderiales (UID4002)	89.5	6.46	Bacteria	Proteobac-teria	Gammaproteo-bacteria	Burkhold-eriales	Burkholderia-ceae	Noviherbaspirillum	
bin.96	f__Bacillaceae (UID830)	89.34	4.14	Bacteria	Firmicutes	Bacilli	Bacillales	Anoxybacil-laceae	Parageobacillus	
bin.494	g__Streptomyces (UID2052)	89.32	2.72	Bacteria	Actinobacte-riota	Actinobacteria	Streptomyce-tales	Streptomyce-taceae	Streptomyces	
bin.734	o__Rhizobiales (UID3450)	89.14	2.98	Bacteria	Proteobac-teria	Alphaproteo-bacteria	Rhizobiales	Rhizobiaceae	UBA1059	
bin.716	c__Gammaproteo-bacteria (UID4266)	89.1	0.81	Bacteria	Proteobac-teria	Gammaproteo-bacteria	Legionellales	Legionellaceae	Legionella	
bin.610	o__Burkholderiales (UID4002)	88.51	5.86	Bacteria	Proteobac-teria	Gammaproteo-bacteria	Burkhold-eriales	Burkholderia-ceae	Herbaspirillum	Herbaspirillum aquaticum
bin.827	c__Betaproteobacte-ria (UID3888)	88.47	8.33	Bacteria	Proteobac-teria	Gammaproteo-bacteria	Burkhold-eriales	Methylophi-laceae		
bin.577	c__Alphaproteobac-teria (UID3422)	88.27	3.8	Bacteria	Proteobac-teria	Alphaproteo-bacteria	Caulobacte-rales	Caulobactera-ceae	Asticcacaulis	

Table S5. High quality-bins assembled from rhizosphere tomato grown in different soils (*continued*)

Bin_Id	Marker_lineage	Completeness	Contamination	Kingdom	Phylum	Class	Order	Family	Genus	Species
bin.628	k__Bacteria (UID3187)	88.24	3.3	Bacteria	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	Bdellovibrionaceae	Ga0074137	
bin.499	o__Burkholderiales (UID4000)	87.98	4.95	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Noviherbaspirillum	
bin.505	k__Bacteria (UID3187)	87.89	5.13	Bacteria	Acidobacteriota	Blastocatellia	Pyrinomonadales	Pyrinomonadaceae	OLB17	
bin.248	o__Burkholderiales (UID4001)	86.66	4.57	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Paraburkholderia_E	
bin.280	k__Bacteria (UID2982)	86.36	1.5	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	Haloferula	
bin.445	c__Betaproteobacteria (UID3888)	86.09	3.75	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Methylophilaceae		
bin.783	p__Bacteroidetes (UID2591)	85.47	2.02	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Chitinophaga	
bin.113	o__Rhizobiales (UID3449)	85.45	4.99	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Pseudorhizobium	
bin.8	k__Bacteria (UID2982)	84.5	1.36	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	Haloferula	
bin.777	k__Bacteria (UID203)	84.48	6.03	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Shinella	
bin.898	p__Bacteroidetes (UID2591)	84.44	2.4	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Niastella	
bin.625	f__Micrococcaceae (UID1623)	84.19	3.11	Bacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter_D	Arthrobacter_D subterraneus
bin.780	o__Actinomycetales (UID1663)	84.06	6.49	Bacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Dermatophilaceae	Pedococcus	

Table S5. High quality-bins assembled from rhizosphere tomato grown in different soils (*continued*)

Bin_Id	Marker_lineage	Completeness	Contamination	Kingdom	Phylum	Class	Order	Family	Genus	Species
bin.854	g__Ensifer (UID3566)	84	4.67	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Sinorhizobium	Sinorhizobium meliloti_A
bin.651	c__Alphaproteobacteria (UID3305)	83.77	7.47	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas_A	
bin.225	o__Burkholderiales (UID4000)	83.32	8.76	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Rhizobacter	
bin.725	k__Bacteria (UID2565)	83.26	1.7	Bacteria	Planctomycetota	Phycisphaerae	Phycisphaerales	SM1A02		
bin.537	k__Bacteria (UID2570)	83.07	3.67	Bacteria	Bacteroidota	Rhodothermia	Rhodothermales	Rhodothermaceae		
bin.434	c__Alphaproteobacteria (UID3305)	82.63	5.25	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas_A	
bin.581	o__Cytophagales (UID2936)	82.62	1.99	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Cyclobacteriaceae		
bin.95	p__Cyanobacteria (UID2182)	82.23	0.79	Bacteria	Cyanobacteria	Cyanobacteriia	Neosynechococcales			
bin.383	c__Betaproteobacteria (UID3959)	82.01	1.03	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae	Nitrosospira	
bin.402	k__Bacteria (UID1452)	81.91	3.72	Bacteria	Chloroflexota	Chloroflexia	54-19			
bin.147	k__Bacteria (UID2982)	81.74	7.77	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Opitutales	Opitutaceae	IMCC26134_A	
bin.500	o__Rhizobiales (UID3447)	81.47	4.95	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Anderseniellaceae	QKVK01	
bin.713	k__Bacteria (UID3187)	81.41	2.61	Bacteria	Acidobacteriota	Blastocatellia	Pyrinomonadales	Pyrinomonadaceae	OLB17	

Table S5. High quality-bins assembled from rhizosphere tomato grown in different soils (*continued*)

Bin_Id	Marker_lineage	Completeness	Contamination	Kingdom	Phylum	Class	Order	Family	Genus	Species
bin.634	k__Bacteria (UID203)	81.4	3.45	Bacteria	Patescibacteria	Paceibacteria	UBA9983_A	UBA2163	C7867-001	
bin.14	p__Euryarchaeota (UID3)	81.36	2.67	NA	NA	NA	NA	NA	NA	NA
bin.708	k__Bacteria (UID2495)	81.29	8.79	Bacteria	Gemmatimonadota	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae		
bin.585	p__Cyanobacteria (UID2182)	81.24	1.57	Bacteria	Cyanobacteria	Cyanobacteriia	Neosynechococcales			
bin.35	c__Alphaproteobacteria (UID3305)	81.05	6.81	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas_F	
bin.202	k__Bacteria (UID203)	80.88	5.33	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	UBA4665		
bin.843	p__Actinobacteria (UID1454)	80.85	7.98	Bacteria	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae		
bin.560	o__Burkholderiales (UID4001)	80.7	0.91	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Paraburkholderia_E	
bin.602	p__Euryarchaeota (UID3)	80.49	3.64	NA	NA	NA	NA	NA	NA	NA
bin.84	p__Actinobacteria (UID1454)	79.31	5.03	Bacteria	Actinobacteriota	Thermoleophilia	20CM-4-69-9			
bin.759	k__Bacteria (UID203)	79.28	0	Bacteria	Patescibacteria	Paceibacteria	UBA9983_A	UBA1006		
bin.462	o__Sphingomonadales (UID3310)	79.04	6	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	
bin.549	k__Bacteria (UID1453)	78.89	4.91	Bacteria	Cyanobacteria	Sericytochromatia				

Table S5. High quality-bins assembled from rhizosphere tomato grown in different soils (*continued*)

Bin_Id	Marker_lineage	Completeness	Contamination	Kingdom	Phylum	Class	Order	Family	Genus	Species
bin.688	k__Bacteria (UID2495)	78.61	3.3	Bacteria	Gemmatimonadota	Gemmatimonadetes	Gemmatimonadales	GWC2-71-9		
bin.449	k__Bacteria (UID203)	77.01	1.72	Bacteria	Patescibacteria	Paceibacteria	UBA9983_A	UBA2163	C7867-001	
bin.416	k__Bacteria (UID203)	76.72	0.86	Bacteria	Bacteroidota	Bacteroidia	AKYH767	Palsa-965	GCA-2737665	
bin.604	k__Bacteria (UID3187)	75.33	2.94	Bacteria	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	Bdellovibrionaceae	21-14-0-10-47-8	
bin.180	k__Bacteria (UID2982)	75.1	2.7	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae		
bin.355	o__Burkholderiales (UID4000)	74.96	7.97	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Vitreoscilla_A	
bin.419	k__Bacteria (UID3187)	74.6	4.35	Bacteria	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	Bdellovibrionaceae		
bin.640	k__Bacteria (UID203)	74.57	9.48	Bacteria	Actinobacteriota	Actinobacteria	Frankiales	Frankiaceae		
bin.769	k__Bacteria (UID203)	74.22	7.99	Bacteria	Gemmatimonadota	Gemmatimonadetes				
bin.276	k__Bacteria (UID2982)	74.19	0.04	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales			
bin.652	k__Bacteria (UID203)	74.14	6.9	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Dyella	
bin.368	c__Betaproteobacteria (UID3888)	73.87	5.85	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Leeiaceae	Leeia	
bin.934	k__Bacteria (UID2495)	73.08	6.66	Bacteria	Gemmatimonadota	Gemmatimonadetes	Gemmatimonadales	GWC2-71-9		

Table S5. High quality-bins assembled from rhizosphere tomato grown in different soils (*continued*)

Bin_Id	Marker_lineage	Completeness	Contamination	Kingdom	Phylum	Class	Order	Family	Genus	Species
bin.770	o__Rhizobiales (UID3654)	72.31	3.92	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	
bin.110	k__Bacteria (UID203)	71.9	2.59	Bacteria	Bacteroidota	Bacteroidia	AKYH767	Palsa-965	GCA-2737665	
bin.209	k__Bacteria (UID203)	71.9	9.48	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Dyella	
bin.803	k__Bacteria (UID2495)	71.52	0	Bacteria	Patescibacteria	Paceibacteria	UBA9983_A	UBA5272	UBA11704	
bin.163	k__Bacteria (UID3187)	70.86	4.97	Bacteria	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	UBA1609		
bin.125	k__Bacteria (UID203)	70.33	1.75	Bacteria	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	Bdellovibrionaceae	UBA2316	
bin.297	o__Cytophagales (UID2936)	70.2	3.92	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Cytophagaceae		
bin.437	k__Bacteria (UID203)	70.19	1.72	Bacteria	Patescibacteria	Paceibacteria	UBA9983_A	UBA2163	1-14-0-10-47-16	
bin.469	k__Bacteria (UID203)	70.17	3.45	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Vitreoscilla_A	
bin.848	k__Bacteria (UID203)	70	1.72	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Roseateles	

Table S6. Annotated protein encoding genes by RAST server using the SEED Subsystem database from bacterial assembled bins from different tomato genotypes grown in different soils.

<table>
<tr><th>bin_name</th><th>Category</th><th>Subcategory</th><th>Subsystem</th><th>Role</th><th>Features</th></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrifying reductase gene clusters</td><td>Nitrous oxide reductase maturation protein NosF (ATPase)</td><td>fig|6666666.1425568.peg.2979</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrifying reductase gene clusters</td><td>Copper-containing nitrite reductase (EC 1.7.2.1)</td><td>fig|6666666.1425568.peg.2169</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrifying reductase gene clusters</td><td>Nitrous oxide reductase maturation trans-membrane protein NosY</td><td>fig|6666666.1425568.peg.2978</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrifying reductase gene clusters</td><td>Nitrous oxide reductase maturation protein NosD</td><td>fig|6666666.1425568.peg.2980</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrifying reductase gene clusters</td><td>Nitrous-oxide reductase (EC 1.7.99.6)</td><td>fig|6666666.1425568.peg.2982</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrification</td><td>Copper-containing nitrite reductase (EC 1.7.2.1)</td><td>fig|6666666.1425568.peg.2169</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrification</td><td>Nitrous oxide reductase maturation protein NosF (ATPase)</td><td>fig|6666666.1425568.peg.2979</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrification</td><td>Nitrous oxide reductase maturation protein NosD</td><td>fig|6666666.1425568.peg.2980</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrification</td><td>Nitrous-oxide reductase (EC 1.7.99.6)</td><td>fig|6666666.1425568.peg.2982</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrification</td><td>Nitrous oxide reductase maturation trans-membrane protein NosY</td><td>fig|6666666.1425568.peg.2978</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Denitrification</td><td>Denitrification</td><td>Nitric-oxide reductase (EC 1.7.99.7), quinol-dependent</td><td>fig|6666666.1425568.peg.1512</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Nitrogen Metabolism - no subcategory</td><td>Ammonia assimilation</td><td>Glutamine synthetase type III, GlnN (EC 6.3.1.2)</td><td>fig|6666666.1425568.peg.4262</td></tr>
<tr><td>Chitinopha-ga_bin783</td><td>Nitrogen Metabolism</td><td>Nitrogen Metabolism - no subcategory</td><td>Ammonia assimilation</td><td>Glutamate synthase [NADPH] large chain (EC 1.4.1.13)</td><td>fig|6666666.1425568.peg.1009</td></tr>
</table>

Table S6. Annotated protein encoding genes by RAST server using the SEED Subsystem database from bacterial assembled bins from different tomato genotypes grown in different soils. (*continued*)

bin_name	Category	Subcategory	Subsystem	Role	Features
Chitinophaga_bin783	Nitrogen Metabolism	Nitrogen Metabolism - no subcategory	Ammonia assimilation	Glutamate synthase [NADPH] small chain (EC 1.4.1.13)	fig|6666666.1425568.peg.1008
Chitinophaga_bin783	Nitrogen Metabolism	Nitrogen Metabolism - no subcategory	Ammonia assimilation	Ammonium transporter	fig|6666666.1425568.peg.4388
Chitinophaga_bin783	Nitrogen Metabolism	Nitrogen Metabolism - no subcategory	Ammonia assimilation	Ferredoxin-dependent glutamate synthase (EC 1.4.7.1)	fig|6666666.1425568.peg.242
Chitinophaga_bin783	Nitrogen Metabolism	Nitrogen Metabolism - no subcategory	Nitrosative stress	Nitric-oxide reductase (EC 1.7.99.7), quinol-dependent	fig|6666666.1425568.peg.1512
Chitinophaga_bin783	Potassium metabolism	Potassium metabolism - no subcategory	Potassium homeostasis	FKBP-type peptidyl-prolyl cis-trans isomerase SlyD (EC 5.2.1.8)	fig|6666666.1425568.peg.838
Chitinophaga_bin783	Potassium metabolism	Potassium metabolism - no subcategory	Potassium homeostasis	Potassium-transporting ATPase C chain (EC 3.6.3.12) (TC 3.A.3.7.1)	fig|6666666.1425568.peg.2163
Chitinophaga_bin783	Potassium metabolism	Potassium metabolism - no subcategory	Potassium homeostasis	Potassium efflux system KefA protein	fig|6666666.1425568.peg.3042
Chitinophaga_bin783	Potassium metabolism	Potassium metabolism - no subcategory	Potassium homeostasis	Potassium-transporting ATPase B chain (EC 3.6.3.12) (TC 3.A.3.7.1)	fig|6666666.1425568.peg.2162
Chitinophaga_bin783	Potassium metabolism	Potassium metabolism - no subcategory	Potassium homeostasis	Potassium-transporting ATPase A chain (EC 3.6.3.12) (TC 3.A.3.7.1)	fig|6666666.1425568.peg.1077
Chitinophaga_bin783	Potassium metabolism	Potassium metabolism - no subcategory	Potassium homeostasis	Large-conductance mechanosensitive channel	fig|6666666.1425568.peg.1787
Chitinophaga_bin783	Potassium metabolism	Potassium metabolism - no subcategory	Potassium homeostasis	POTASSIUM/PROTON ANTIPORTER ROSB	fig|6666666.1425568.peg.398
Chitinophaga_bin783	Protein Metabolism	Protein degradation	Serine endopeptidase (EC 3.4.21.-)	Prolyl endopeptidase (EC 3.4.21.26)	fig|6666666.1425568.peg.446, fig|6666666.1425568.peg.1370
Chitinophaga_bin783	Nitrogen Metabolism	Denitrification	Denitrifying reductase gene clusters	Nitrous oxide reductase maturation protein NosF (ATPase)	fig|6666666.1425568.peg.2979

Note. The full Table S6 is available online at https://doi.org/10.5281/zenodo.15725106

Chapter 4

Microbiome-mediated tolerance of wild tomato to the invasive insect *Prodiplosis longifila*

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Abstract

Plant roots are colonized by diverse communities of microorganisms that can affect plant growth and enhance plant tolerance to (a)biotic stresses. Here, we investigated the role of the indigenous soil microbiome in tolerance of tomato to the invasive sap-sucking insect *Prodiplosis longifila* (Diptera: Cecidomyiidae). Native and agricultural soils were sampled from the Andean region in Southern Ecuador and tested, in greenhouse bioassays, for leaf tissue damage caused by *P. longifila* on domesticated *Solanum lycopersicum* cv. Moneymaker and wild tomato *S. pimpinellifolium*. We observed no significant differences in insect damage between domesticated and wild tomatoes grown in live native or agricultural soils. However, when grown in sterilized native and agricultural soils, wild tomato was more severely affected by the insect than the domesticated tomato. Subsequent microbiome analyses revealed that soil sterilization impacted overall rhizobacterial diversity and abundance in the wild tomato. Particularly, the abundance of *Actinoplanes* was reduced upon sterilization which significantly correlated with loss of insect tolerance. Metagenome analyses and subsequent genome assembly of Micromonosporaceae (*Actinoplanes* family) suggested a putative association between motility, chemotaxis, membrane transport, chorismate and lanthipeptide biosynthesis and insect tolerance. Collectively, these results indicate that wild tomato *S. pimpinellifolium,* in contrast to domesticated *S. lycopersicum,* relies on specific members of the root-associated microbiome for protection against the endemic insect *P. longifila*.

Keywords: center of origin, indigenous microbiome, *Prodiplosis* tolerance, rhizosphere *Actinoplanes*, tomato

Introduction

Root-associated microorganisms contribute to plant growth and health via the production of hormones, enhanced nutrient acquisition and protection from pathogens and insect pests (Carrillo et al., 2019; Choi et al., 2020; Lee et al., 2021; Meshram & Adhikari, 2024; Pineda et al., 2017; Smulders et al., 2021; Tronson et al., 2022). Most of the current knowledge on how soil- and root-associated microorganisms impact aboveground insect herbivory arises from single microorganisms and modern crop cultivars grown under controlled conditions in greenhouse soils (Friman et al., 2021; Rashid & Chung, 2017; van de Mortel et al., 2012). However, research on how root-associated microbiomes of wild crop relatives in native habitats impacts plant fitness, including tolerance to insects, remains largely unknown. In this context, it has been postulated that wild crop relatives and their native microbiomes have co-evolved to withstand (a)biotic stresses (Barajas et al., 2020; Pérez-Jaramillo et al., 2016; Wallenstein, 2017). In contrast, environmental disturbances within agricultural systems combined with genetic modifications by plant breeding may have led to the loss of beneficial interactions between domesticated plants and their microbiome (Cordovez et al., 2019; O'Brien et al., 2021; Oyserman et al., 2021). To begin to understand the role of the soil and root microbiome in protection of wild crop relatives against (a)biotic stresses, we sampled native and agricultural soils from the Andean region in Southern Ecuador and tested these soils in greenhouse bioassays with domesticated *Solanum lycopersicum* and wild tomato *S. pimpinellifolium* for leaf tissue damage caused by the insect *Prodiplosis longifila* Gagné (Diptera: Cecidomyiidae) (Figure 1). This sap-sucking invasive insect is an economically important pest of tomato and other solanaceous crops in South America (Duque-Gamboa et al., 2018; Hernandez et al., 2015; Valarezo Cely et al., 2003; Velasco-Cuervo et al., 2016) and designated a priority for Pest Risk Analysis (PRA) by the EPPO Panel on Phytosanitary measures (EPPO, 2017). It can cause up to 100% loss of tomato in Colombia and up to 60% loss in Ecuador (Constante Tubay et al., 2023; Valarezo Cely et al., 2003).

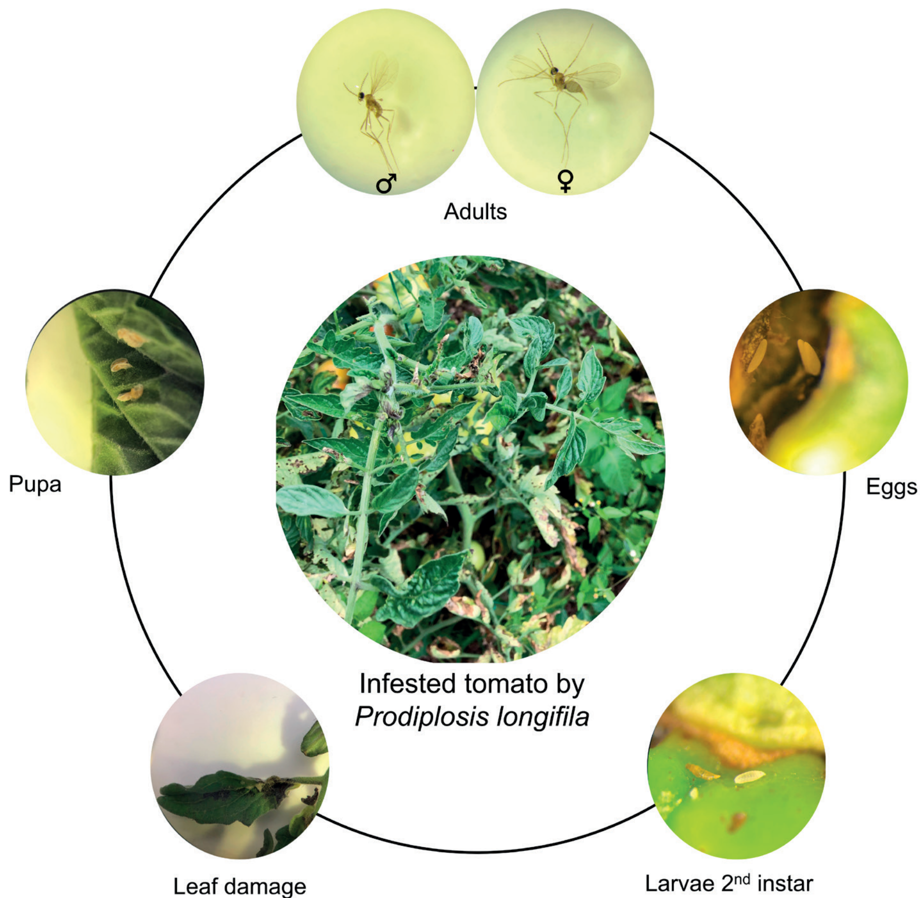

Figure 1. *Prodiplosis longifila* (Diptera: Cecidomyiidae) life cycle (Pictures by SSF). In the center panel, severe damage of tomato (*S. lycopersicum* cv. Elpida) field crop grown in Loja, Ecuador. In the outer panel, a summary of the life cycle. Larve 2nd instar were used to infest greenhouse grown tomato and leaf damage was assessed 7 days after infestation (bottom two panels).

Chapters 2 and 3 demonstrated how the tomato root microbiome is assembled by native habitats, domestication and agricultural management, revealing both conserved microbial signatures and functional shifts across soils and genotypes. Building on these results, Chapter 4 investigates the ecological consequences of microbiome variation by focusing on plant–insect interactions. Specifically, we assessed the role of the native rhizosphere microbiome in protecting tomato against the sap-feeding insect *Prodiplosis longifila*.

To evaluate the protective potential of the tomato microbiome against this insect pest, we 1) assessed the role of the microbiome of native and agricultural soils on tomato leaf damage by *P. longifila;* 2) performed a comparative analyses of the rhizosphere

microbiome composition of wild tomato *S. pimpinellifolium* and domesticated tomato *S. lycopersicum*; and, 3) conducted metagenomics on soils with contrasting phenotypes to identify putative microbial traits associated with protection of wild tomato against *P. longifila.* We found that microbiome disruption by soil sterilization caused a significant increase of leaf damage by *P. longifila* in wild tomato grown in native and agricultural soils, but not in domesticated tomato. The taxonomic diversity of the tomato rhizosphere bacterial microbiome was different between native and agricultural soil, but no substantial differences in taxonomic composition were observed between domesticated and wild tomato. Interestingly, we found a significant negative correlation between the relative abundance of several rhizobacterial taxa, in particular *Actinoplanes,* and insect leaf damage in wild tomato. Subsequent metagenome analyses pinpointed putative traits of *Actinoplanes,* including motility, chemotaxis, membrane transport, and secondary metabolism, that significantly correlated with insect tolerance.

Results

Impact of tomato genotype and soil condition on insect leaf damage

Insect larvae caused significant leaf damage among the treatments (ANOVA, Soil * Condition, p = 0.04043). Both the domesticated tomato *S. lycopersicum* cv. Moneymaker and wild tomato *S. pimpinellifolium* showed low levels of insect leaf damage when grown in agricultural (4.70% and 4.39%, respectively) or native soils (3.48% and 6.98%, respectively) (Figure 2). When the agricultural and native soils were sterilized, the domesticated tomato cultivar remained tolerant to insect leaf damage. In contrast, significantly more insect leaf damage was observed when the wild tomato was grown in sterilized agricultural and native soils, compared with the respective live soils (Figure 2). Insect leaf damage on wild tomato increased to an average of 16.5% and 15.0% in agricultural and native soils, respectively. In contrast, no significant change was observed for insect leaf damage on the modern tomato cultivar grown in sterilized agricultural and native soils. These results indicate that the wild tomato, in contrast to the domesticated tomato, relies on the soil (micro)biome for protection against *P. longifila*.

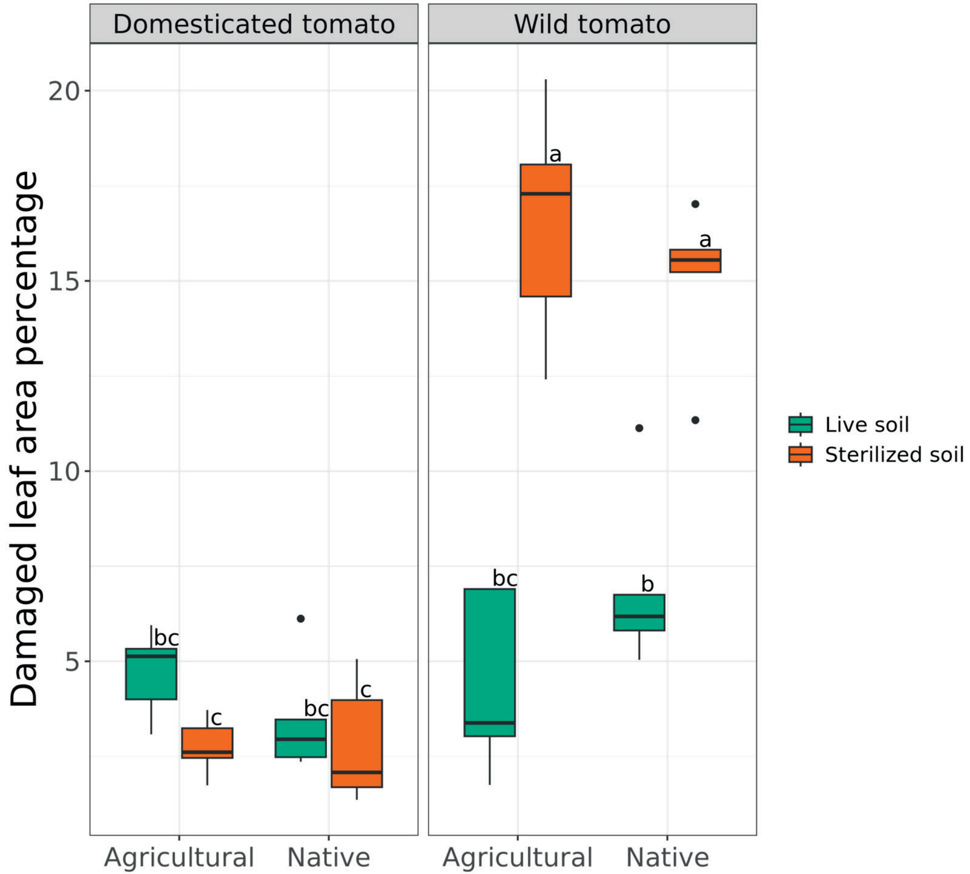

Figure 2. Leaf damage by *Prodiplosis longifila* on domesticated tomato *S. lycopersicum* cv. Moneymaker and wild tomato *S. pimpinellifolium* grown for 41 days in live and sterilized native and agricultural soils. Bars represent the standard deviations of the mean of five replicates. Significant differences in percentage of insect leaf damage between the domesticated and wild tomato plants grown in two soils are indicated by different letters (Tukey HSD post hoc test $p < 0.05$).

Effect of soil sterilization on tomato rhizosphere microbiome composition

Alpha diversity of rhizosphere bacterial communities was significantly higher for tomato plants grown in the live soil as compared to plants grown in the sterilized soils (ANOVA, p = 9.9e-13). This was reflected in higher Shannon indices for tomato plants grown in live soils as compared to sterilized soils (Figure 3a). Significant differences in beta diversity were found between agricultural and native soils (PERMANOVA, p = 0.0035), however, no differences were found between the wild and domesticated tomato in overall composition of the rhizobacterial community (Figure 3b).

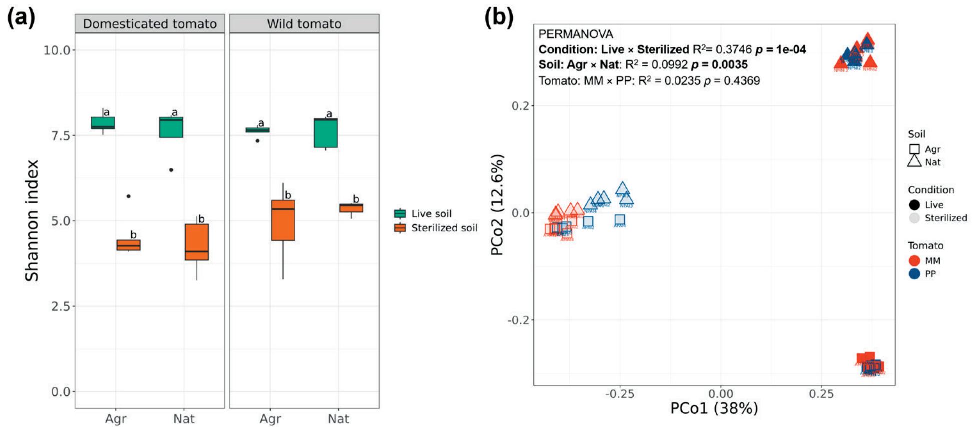

Figure 3. **(a)** Shannon diversity index of bacterial communities in the rhizosphere of domesticated *S. lycopersicum* cv. Moneymaker and of wild tomato *S. pimpinellifolium* grown in live and sterilized native and agricultural soils. Boxplots represent the mean of five replicates. Significant differences between soils and tomato genotypes are indicated by different letters (Tukey HSD post hoc test $p < 0.05$); **(b)** PCoA of bacterial rhizosphere of domesticated *S. lycopersicum* cv. Moneymaker (MM) and wild *S. pimpinellifolium* (PP) tomato infested by *Prodiplosis longifila* in different soil type (Agr: agricultural; Nat: native) and soil condition (Live; Sterilized).

Changes in wild tomato rhizosphere composition by soil sterilization correlate with leaf damage

To identify correlations between changes in the rhizobacterial community composition and the level of leaf damage caused by *P. longifila*, amplicon data of the wild tomato grown in live or sterilized native and agricultural soils were subjected to differential abundance analysis. This analysis showed that 404 ASVs were shared between live agricultural and native soils, whereas 209 ASVs were shared between sterilized agricultural and native soils (Supplementary Figure S2). We observed an impact of soil sterilization on the relative abundance of specific members of the phyla Actinobacteriota, Acidobacteriota, Chloroflexi, Cyanobacteria, Gemmatimonadota and Myxococcota (Figure 4a). More specifically, the genera *Actinoplanes, Sphingomonas, RB41, Geodermatophilus* and *MND1* had a higher relative abundance in live soils (i.e. lower in sterilized soils), while the abundance of *Nocardioides, Methylobacterium, Pseudomonas, Paenarthrobacter* and *Pseudoarthrobacter* was higher in sterilized soils (Figure 4b).

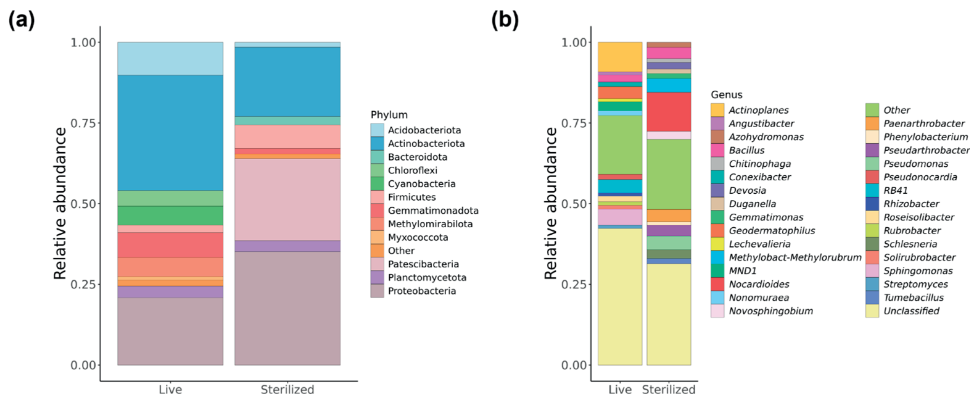

Figure 4. Composition of bacterial phyla **(a)** and genera **(b)** in the rhizosphere of wild tomato *S. pimpinellifolium* in live and sterilized soil conditions; significant differential ASVs shared among live and sterilized agricultural and native soils were grouped according to their phylum or genus and plotted as stacked bar charts; "Other" category corresponds to grouped phyla or genera with relative abundance < 0.01.

To investigate the relationships between insect leaf damage and the abundance of specific rhizobacterial taxa, we performed Spearman correlation analysis and a more stringent analysis based on linear regression modelling. Spearman correlation analysis showed a significant correlation between rhizobacterial abundance and insect leaf damage (ρ Spearman $\geq$ 0.64; Supplementary Figure S3). Further analysis using linear regression modelling predicted significant negative correlations between insect leaf damage and the relative abundance of *Actinoplanes* (R^2 = 0.7677), *MND1* (R^2 =0.6786), *RB41* (R^2 = 0.6748), and *Geodermatophilus* (R^2 = 0.6668). Positive correlations were predicted for *Nocardioides* (R^2 = 0.6982), *Paenarthrobacter* (R^2 = 0.499), *Methylobacterium* (R^2 = 0.462), and *Pseudomonas* (R^2 = 0.6278) (Figure 5). No significant correlations were found between *Sphingomonas* (Figure 5e) and *Pseudoarthrobacter* (Figure 5i) abundances and the proportion of insect leaf damage. Collectively these correlations suggest that a higher relative abundance of *Actinoplanes* and some other genera was associated with lower levels of insect damage, while a higher relative abundance of *Nocardioides* and some other bacterial genera was associated with higher levels of insect damage (Figure 4 and 5).

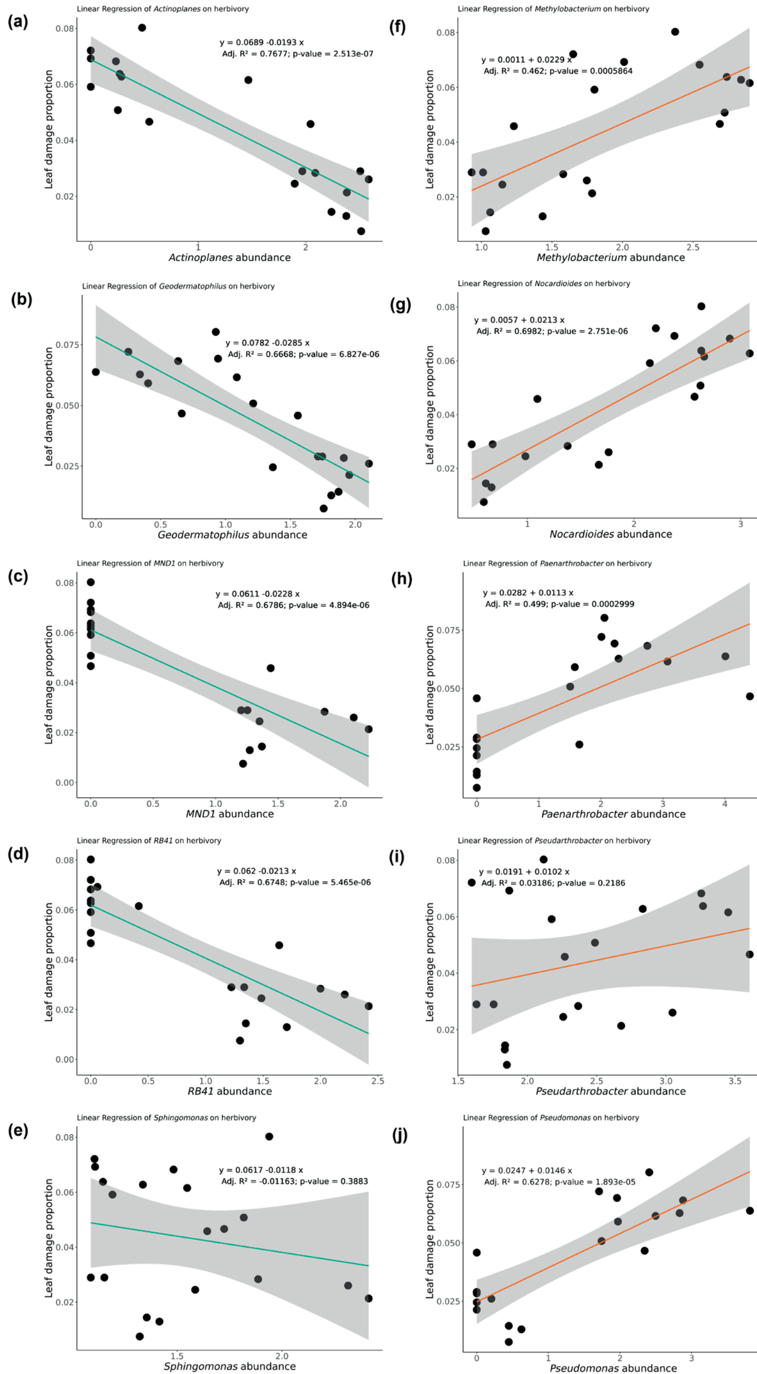

Figure 5. Linear regression models describing the relationship between the abundance of **(a)** *Actinoplanes*, **(b)** *Geodermatophilus*, **(c)** *MND1*, **(d)** *RB41*, **(e)** *Sphingomonas*, **(f)** *Methylobacterium*, **(g)** *Nocardioides*, **(h)** *Paenarthrobacter*, **(i)** *Pseudarthrobacter*, and **(j)** *Pseudomonas* and leaf damage. Leaf damage percentage and absolute abundance data (n = 10) were log10(x+1) transformed.

Functional features of key rhizobacterial genera associated with leaf damage

To get preliminary insight into the functional traits of the two bacterial genera *Actinoplanes* and *Nocardioides*, taxa that negatively or positively correlated to insect damage, respectively, we performed shotgun sequencing with a pooled set of rhizosphere soil samples (five replicates of each treatment, each consisting of eight pooled samples). Two MAGs (high quality bins completeness >70%, contamination <10%) were classified as Micromonosporaceae (*Actinoplanes* family) (bin 580, completeness 88.42%, contamination 2.59%) and *Nocardioides* (bin 2.78, completeness 72.60%, contamination 5.66%) (Supplementary Table S1). To investigate their functional traits, the MAGs were annotated using RAST (Supplementary Table S2) and antiSMASH. '*Actinoplanes* bin 580' showed higher number of protein-encoding genes (883) than '*Nocardioides* bin 2.78' (715). '*Actinoplanes* bin 580' was characterized by motility & chemotaxis and secondary metabolism SEED categories, which were absent in '*Nocardioides* bin 2.78'. Moreover, '*Actinoplanes* bin 580' showed higher number of genes involved in membrane transport; nitrogen metabolism; protein metabolism; secondary metabolism; sulfur metabolism; and virulence, disease and defense SEED categories. In contrast, '*Nocardioides* bin 2.78' showed more genes related with amino acids and derivatives; carbohydrates; cofactors, vitamins, prosthetic groups, pigments; fatty acids, lipids and isoprenoids; metabolism of aromatic compounds; phosphorus metabolism; and stress response SEED categories (Figure 6).

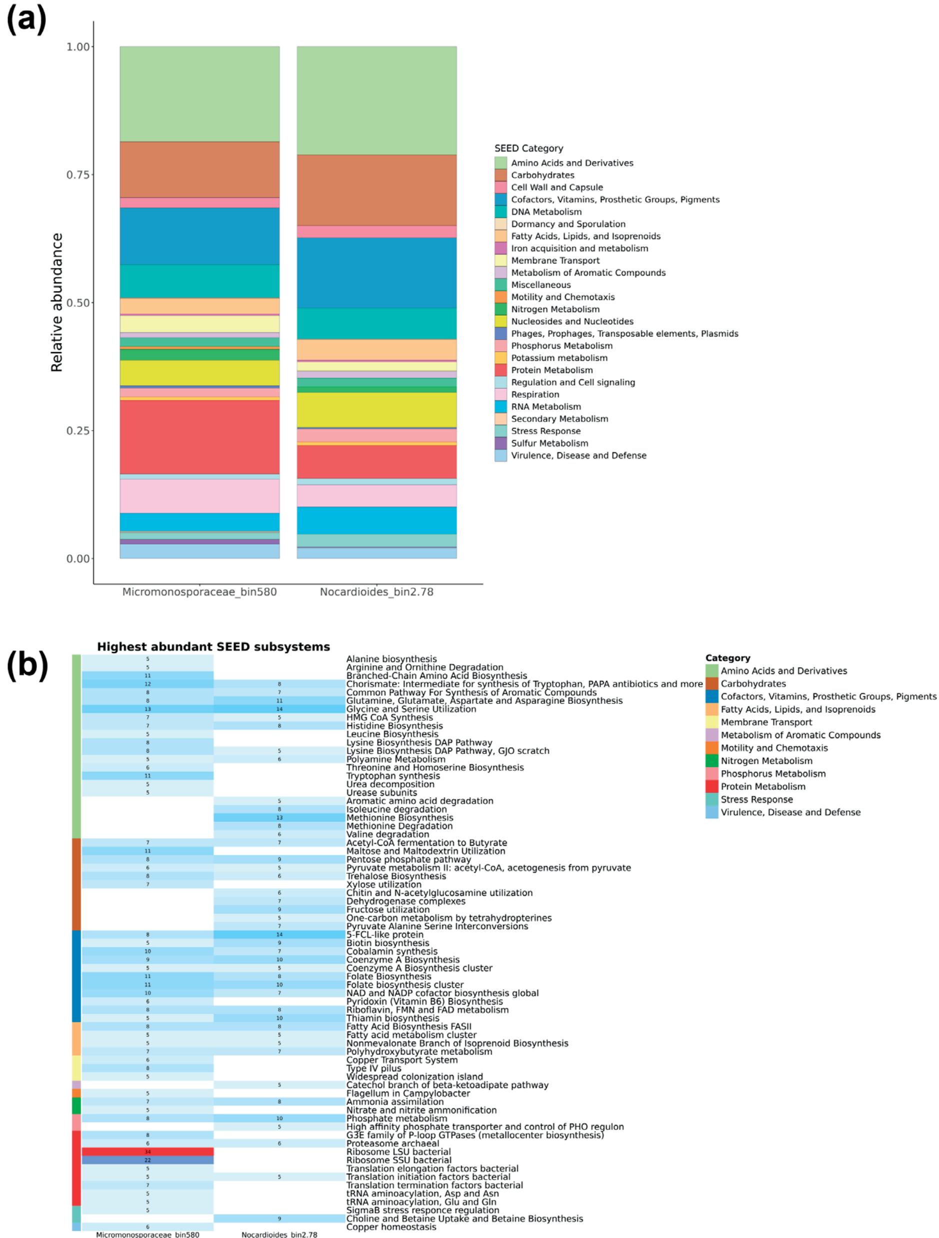

Figure 6. (a) Relative abundance of SEED Categories annotated by RAST (Rapid Annotation using Subsystems Technology) of two bacterial genera *Actinoplanes* and *Nocardioides* from the rhizosphere of wild tomato *S. pimpinellifolium* that showed a negative and positive correlation, respectively, with *Prodiplosis longifila* leaf damage; the relative abundance of protein encoding genes were grouped in their respective SEED Categories and plotted as stacked bar charts; **(b)** Heatmap of main SEED Subsystems showing the number of protein encoding genes.

A total of 11 BGCs were identified for *Actinoplanes* bin 580 and 1 BGC for *Nocardioides* bin 2.78. From these, 3 BGCs of '*Actinoplanes* bin 580' were annotated as a type III polyketide synthase biosynthetic gene cluster predicted to encode loseolamycins A1 and A2, aminopolycarboxylic-acid biosynthetic gene cluster of the siderophore ethylenediaminesuccinic acid hydroxyarginine (EDHA) and the terpene isorenieratene, albeit with low similarity. For '*Nocardioides* bin 2.78', no specific BGCs could be annotated by antiSMASH (Table 1; Supplementary Figure S4 and S5).

Table 1. Biosynthetic gene clusters (BGCs) predicted by antiSMASH bioinformatics tool from bins of Micromonosporaceae bins 580 and *Nocardioides* bin 2.78 enriched in the rhizosphere of the wild tomato *S. pimpinellifolium* infested by *Prodiplosis longifila* in live and sterilized soil, respectively.

Bin ID	Length (nt)	BGC type	Secondary metabolite	Similarity	Reference in MiBIG database	Organism in MiBIG database
Actinoplanes bin 580	23,195	T3PKS	Loseolamycin A1, Loseolamycin A2	64%	BGC0002362	*Micromonospora endolithica*
Actinoplanes bin 580	13,418	Aminopolycarboxylic-acid	EDHA	88%	BGC0002568	*Streptomyces scabiei* 87.22
Actinoplanes bin 580	10,650	Terpene	Isorenieratene	25%	BGC0000664	*Streptomyces griseus* subsp. *griseus* NBRC 13350
Nocardioides bin 2.78	2,627	RRE-containing	NA	NA	NA	NA

Discussion

It has been postulated that wild crop relatives and their native microbiomes have co-evolved to withstand (a)biotic stresses (Barajas et al., 2020; Pérez-Jaramillo et al., 2016; Wallenstein, 2017). In contrast, environmental disturbances within agricultural systems combined with genetic modifications by plant breeding may have led to the loss of beneficial interactions between domesticated plants and their microbiota (Cordovez et al., 2019; O'Brien et al., 2021; Oyserman et al., 2021). In our study, we observed no significant differences in leaf damage by the sap-sucking invasive insect *P. longifila* on both domesticated and wild tomatoes grown in live native or agricultural soils. However, microbiome disruption by soil sterilization caused a significant increase of insect leaf damage in wild tomato grown in native and agricultural soils, but not in domesticated tomato. These results suggest that wild tomato *S. pimpinellifolium,* in contrast to domesticated *S. lycopersicum,* relies on specific members of the root-associated microbiome for protection against the invasive insect *P. longifila* aboveground. It also suggests that the domesticated tomato has an intrinsic resistance to the invasive insect, that

operates largely independently from the soil microbiome. Previous study by Carrillo et al. (2019) found that tomato's resistance to herbivory by *Manduca sexta* (Lepidoptera: Sphingidae) was not dependent on tomato domestication or soil type. However, they observed that the wild tomato *S. pimpinellifolium,* grown consecutively in the same soil, i.e. 'tomato soil', attracted more parasitoid *Cotesia congregate* (Hymenoptera: Braconidae) compared to the domesticated tomato *S. lycopersicum*. In our experimental design under controlled greenhouse conditions, the attraction of parasitoids did not play a role. Most likely, insect tolerance in our experiments is due to root colonization and priming the plants' defense mechanisms (Pieterse et al., 2014) to resist insect attack.

Subsequent analyses showed that heat sterilization of the soils led to a significant increase in leaf damage of wild tomato when grown in both agricultural and native soils (Figure 2). Soil sterilization significantly reduced the alpha-diversity of the bacterial community in the rhizosphere of tomato grown in native and agricultural soils (Figure 3a). The differences observed in the rhizosphere microbiome composition were primarily due to the soil type (agricultural, native) and soil condition (live, sterilized), rather than the tomato genotype (Figure 3b; Supplementary Figure S1). This is consistent with previous studies indicating that soil exerts a stronger effect on rhizosphere assembly than plant host genotype (Cheng et al., 2020; French et al., 2020; Kandasamy et al., 2021; Smulders et al., 2021). Although rhizosphere microbiome assembly was similar for both tomato genotypes, the microbiome changes in the rhizosphere of wild tomato grown in sterilized soil suggested a putative role of the rhizosphere microbiome to minimize leaf damage by *P. longifila*. A comparable effect was found in *S. pimpinellifolium* infested by the aphid *Macrosiphum euphorbiae*, whose degree of infestation was influenced by the soil microbiome. Specifically, higher microbial diversity reduced aphid infestation (French et al., 2021). Moreover, negative impact on insect performance or less damage on plants grown in intact native microbiome has been previously described by Badri et al. (2013) for *Arabidopsis thaliana* and by Hubbard et al. (2019) for the crucifer *Boechera stricta*.

Differential abundance analyses further revealed that the abundance of several bacterial phyla was affected by soil sterilization, such as Actinobacteriota, Acidobacteriota, Chloroflexi, Cyanobacteria, Gemmatimonadota and Myxococcota (Figure 4a). Members of the phyla Actinobacteriota and Cyanobacteria have been investigated for the production of insecticidal compounds (Berry et al., 2008; Kaur et al., 2014; Nassar et al., 1999; Sharanappa et al., 2023; Silva et al., 2022). More specifically, *Streptomyces* spp. have been reported as a source of insecticides with potent larvicidal activity (Amelia-Yap et al., 2022, 2023; Balakrishnan et al., 2017). Here we found *Actinoplanes, Sphingomonas, RB41, Geodermatophilus* and *MND1* to be more abundant in the rhizosphere of wild tomato grown in live soil, while *Nocardioides, Methylobacterium,*

Pseudomonas, Paenarthrobacter and *Pseudoarthrobacter* were more abundant in the rhizosphere of tomato grown in sterilized soil (Figure 4b). With the exception of *Sphingomonas* and *Pseudoarthrobacter*, these bacterial genera showed a significant positive or negative correlation with the proportion of leaf damage caused by *P. longifila* (Figure 5). Interestingly, the abundance of *Actinoplanes* taxa was negatively correlated with leaf damage, whereas the abundance of *Nocardioides* taxa was positively correlated (Figure 5a and 5g). *Actinoplanes* spp. have been studied for their potential as insecticidal agents against other dipteran species such as *Drosophila melanogaster* (Diptera: Drosophilidae) (Al-Kaabi, 2004) and *Aedes aegyptii* (Diptera: Culicidae) (Balakrishnan et al., 2017; Hozzein, 2017). Nevertheless, these studies primarily focused on the activity of these microbial compounds applied directly onto the insects, rather than harnessing the ability of specific microorganisms to activate plant defense mechanisms (Choi et al., 2020; Lee et al., 2021; Ling et al., 2022; Pineda et al., 2017, 2020). Additional experiments, involving isolation of *Actinoplanes* and *Nocardioides* spp., insect bioassays and mechanistic analyses, will be needed to validate the putative roles of these two and possibly other rhizobacterial genera in protection of wild tomato against *P. longifila*. Extensive attempts to isolate *Actinoplanes* from wild tomato rhizosphere have not yet been successful.

Nevertheless, our analysis of the metagenome-assembled genome (MAG) *Actinoplanes* 580 did provide some first insights into the functional features that may be associated with wild tomato's tolerance to *P. longifila* (Figure 6b; Supplementary Table S2). Among these, motility & chemotaxis SEED subsystem was characterized by flagellar functions such as genes encoding synthesis of peptidoglycan (FtsI) and flagellin (FlaA, FlgB, FlgC and FliE). These genes are involved in activating bacterial division and assembling the protein components of the flagellum (Jang et al., 2016; Nedeljković et al., 2021; Tsang & Bernhardt, 2015). Flagella are required for bacterial motility and colonization and adherence to host surfaces (Guerry, 2007; Palleroni, 1976; Uchida et al., 2011). Flagella and pili are distinctive features of *Actinoplanes* zoospores (Kimura et al., 2019; Uchida et al., 2011). Particularly, pilus genes found in 'bin 580', such as PilB, PilC, PilM and PilT, may allow *Actinoplanes* zoospores to colonize and adhere to the tomato root surface (Kimura et al., 2019). Complementarily, within 'bin 580', genes of the widespread colonization island subsystem included tight adherence (TAD) genes, such as TadA, TadB, TadC and TadZ, and the pilin-encoding gene Flp, suggest *Actinoplanes*' capacity to colonize host tissues and surfaces of several environmental habitats (Planet et al., 2003). Furthermore, motility features have been described to be related with bacterial niche differentiation in high carbon availability as the rhizosphere (Ramoneda et al., 2024; Wu et al., 2023). Moreover, the presence of genes encoding lanthionine synthetases (LanB, LanL) of the secondary metabolism SEED category, suggest the potential for the biosynthesis of lanthipeptides, which have demonstrated antimicrobial

activity against Gram-positive bacteria by disrupting cell wall biosynthesis (Li et al., 2021; van Staden et al., 2021) as well as immunomodulatory activity (Ramírez-Rendón et al., 2023). Additionally, lanthipeptides can exert a regulatory role for aerial hyphae formation with limited antimicrobial activity in some actinobacteria (Holtsmark et al., 2008; Kodani et al., 2005). Furthermore, genes encoding amino acids and derivatives in 'bin 580' suggest the biosynthesis of chorismate, which is precursor of several secondary metabolites such as aromatic compounds, including tryptophan, involved in electron transport, signaling communication, plant defense and wound response (Corpas et al., 2021; Macheroux et al., 1999; Mishra & Baek, 2021). In particular, features of the biosynthesis of chorismate and aromatic amino acids may modulate plant defense response against *P. longifila,* which has been demonstrated in other studies on induced resistance against bacterial pathogens and phloem-feeding insects (Assis et al., 2021; Blundell et al., 2020; Shi et al., 2016). Last but not least, we also detected a putative cluster for the biosynthesis of the terpene isorenieratene, which is characteristic for certain Actinobacteria to cope with oxidative stress (Benaud et al., 2021; Krügel et al., 1999). The antiSMASH results also suggest that *Actinoplanes* harbors various underexploited natural products. These findings further emphasize the importance to isolate *Actinoplanes* spp., test their biosynthetic potential and determine the putative role of these metabolites in protection of wild tomato against *P. longifila.*

In conclusion, the results from this study highlight the potential of exploring center-of-origin soils that harbor viable populations of close relatives of economically important modern crops. Such soils may serve as valuable microbial reservoirs of tolerance to various pests and diseases. This is a promising area of research, particularly in understanding how belowground microbial communities, in conjunction with specific plant genotypes, can potentially promote tolerance to aboveground insect pests. Therefore, the integration of genomic approaches, involving the validation of key microbial taxa, along with the assessments of plant metabolites and defense hormones, will be essential to establish and validate the mechanistic links between microbial communities and plant defense pathways. Clarifying these dynamics and the modes of action of these 'ancestral microbiota' (Raaijmakers & Kiers, 2022) could pave the way for designing innovative agricultural practices that harness natural soil microbiomes to improve crop resilience.

Materials and Methods

Soil sampling and processing

Agricultural and native soils were collected in Zapotillo (Loja province, Ecuador). Agricultural soil was collected from a recently harvested corn farm close to the Cata-

mayo river (El Coco site, 4°22'44.0"S, 80°14'11.8"W), while native soil was collected from the right bank of the Alamor river (La Ceiba site, 4°18'07.6"S, 80°13'16.7"W) from a patch with natural vegetation mainly consisting of: *Prosopis juliflora* (Fabaceae), *Croton wagneri* (Euphorbiaceae), *Ipomoea purpurea* (Convolvulaceae), *Cromolaena* sp. (Asteraceae), *Parthenium hysterophorus* (Asteraceae), *Hyptis* sp. (Lamiaceae), and wild tomato *S. pimpinellifolium* (Solanaceae). Soils were transported to the greenhouse of the National University of Loja, air-dried for seven days at room temperature, sieved (2-mm-diameter mesh) to remove stones and plant debris, and stored at room temperature in the shade until further processing.

Experimental design

Domesticated tomato *Solanum lycopersicum* cv. Moneymaker and its close wild relative *S. pimpinellifolium* were grown in native and agricultural soils. In order to enrich the soil with rhizosphere microorganisms, live soils passed a 'microbial activation' phase which consisted of growing tomatoes in the agricultural and native soils for 30 days prior to the experiment. Afterwards, the complete plants and roots were removed from each pot, and half of the soil volume was subjected to heat sterilization (1.5 atm and 121 ºC, 1 h, for two consecutive times with 24 h in between) whereas the other half of the activated soil remained untreated. Tomato seeds were surface sterilized with 5 ml of ethanol 80% (v/v) and vortexed for 2 min. Then, the ethanol was removed and 5 ml of sodium hypochlorite 1.5% (v/v) was added, vortexed for 10 min and discarded. Finally, five washing cycles were done with 5-ml volumes of sterile demi water, vortexing for 2 min and removal of the water. The seeds were then placed on a wet filter paper in a Petri dish containing 5 ml of sterile demi water and incubated at 25 ºC for two days. Before sowing the seeds, each soil type was moistened by adding 10% (v/w) of water and distributed in pots (350 g per pot). In each pot, three pre-germinated seeds (radicle length ~1 cm) were sown at 2-cm depth and after the seedlings emerged, two were removed to obtain one single seedling per pot. This allowed us to have seedlings with uniform sizes across the different treatments. The experiment consisted of 2 tomato genotypes × 2 soil types (agricultural, native) × 2 soil conditions (live, sterilized) × 5 replicates, for a total of 40 experimental units, which were organized in a completely randomized design. Considering the differences in growth between domesticated and wild tomatoes, watering was done with tap water on a total pot weight basis. Plants were grown for 34 days under greenhouse conditions (temperature day/night: 25/22 ºC; relative humidity 60%; 12 h natural light) until the 4th true-leaf developmental stage.

Insect infestation

The insect *Prodiplosis longifilia* Gagné (Diptera: Cecidomyiidae) was used to infest tomato leaves. Infested leaves with *P. longifila* larvae were collected from a tomato field

(*S. lycopersicum* cv. Elpida) at fruiting stage in Catamayo (Loja, Ecuador, 3°54'51.4"S, 79°19'45.8"W) and transported in a cooler to the greenhouse facilities at the National University of Loja (Ecuador). Ten second-instar larvae were carefully transferred to the upper leaves of 34-day-old plants using a fine paint brush. Each infested plant was examined for insect damage at 7 dpi (days post-infestation). To this end, plants were harvested one week after infestation (41 days after sowing) by removing the entire plant from the pot. The shoots were cut at the root collar region, the leaves were detached and spread on paper and photographed with a ruler for scaling and assessing insect damage. Leaf damage by *P. longifila* larvae was quantified with ImageJ (Schindelin et al., 2015). This software associates the number of pixels in the pictures with the real scale or ruler to measure the area (mm scale was used). The total leaf area and leaf damage percentages were determined for each plant. The leaf damage percentages were subjected to analysis of variance (ANOVA) with a linear model using the function *lm* in RStudio environment (R 4.3.1) (R Core Team, 2023). The packages dplyr (Wickham et al., 2023) and ggplot2 (Wickham, 2016) were used for data structuring and plotting.

DNA extraction from the tomato rhizosphere for microbiome profiling

Following the harvest of the 41-day-old tomato plants, roots were shaken vigorously to remove loosely attached soil. The root system was then placed into a 15 ml-tube with 4 ml of LifeGuard® Soil Preservation Solution and stored at 4 ºC. In the Laboratory of Biotechnology, National University of Loja, the rhizosphere samples were split into three subsamples with 1 g of soil and 1 ml of LifeGuard® Soil Preservation Solution and placed into a 2 ml-microtube and stored at -20 ºC until DNA extraction. To extract rhizosphere DNA, the 2-ml microtubes were centrifuged at 10,000 × *g* for 1 min, and the supernatant of LifeGuard® Soil Preservation Solution was discarded. The Qiagen DNeasy® PowerSoil® Kit (Qiagen, USA) was used to isolate the genomic DNA of the remaining soil pellet according to the manufacture's kit protocol. DNA samples were sent to Genome Québec (Canada) for amplicon library preparation and subsequent sequencing of the V3-V4 regions of the 16S rRNA gene using the universal bacterial primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATC-TAATCC). Paired-end sequence reads (300 bp length) were generated using the Illumina NovaSeqX Plus platform. Additionally, shotgun sequencing was performed on eight pooled rhizosphere samples: DNA of five replicates from each treatment were pooled and 20 µl (concentration 20 ng/µl) aliquots were lyophilized and subjected to library preparation and shotgun sequencing to provide paired-end reads of 150 bp by Illumina NovaSeq 6000.

Amplicon data analysis

The compressed sequence reads contained in FASTQ format files were processed by the DADA2 v1.16.0 pipeline (Callahan et al., 2016) in RStudio environment (RStudio Team, 2020) to obtain the ASV abundance and taxonomy tables. The modeling of error rates associated with the sequencing process was adjusted to appropriately process NovaSeq data using the DADA2 pipeline, as previously suggested by Holland-Moritz (2021). The SILVA 16S ribosomal RNA gene reference database (v138) (Quast et al., 2013) was used for bacterial taxonomy assignment. The statistical analyses were performed in RStudio environment and R software version 4.3.1 (R Core Team, 2023). Packages such as vegan (Oksanen et al., 2020), phyloseq (McMurdie & Holmes, 2013), metagenomeSeq (Paulson et al., 2013) and ggplot2 (Wickham, 2016) were used for alpha diversity (ANOVA, Tukey HSD post hoc test), beta diversity (Bray–Curtis distance, PERMANOVA with 9,999 permutations), and differential abundance analyses, while the tidyverse package (Wickham et al., 2019) was used for formatting and visualization. The abundance data were normalized by CSS (Cumulative Sum Scaling) before further analysis. Principal Coordinates Analysis (PCoA) was done using the *cmdscale* function from the vegan package and the Bray–Curtis distance calculated previously. An UpSet plot to determine the shared ASVs among soils was generated by UpSetR software (Lex et al., 2014). Absolute abundance values of bacteria were used in a regression model to analyze their correlation with leaf insect damage percentages.

Metagenome data analysis

The compressed paired-end FASTQ files were processed using SqueezeMeta v1.5.1 (Tamames & Puente-Sánchez, 2019). Co-assembly was done using Megahit (Li et al., 2015). 16S RNAs were predicted with Barrnap (Seemann, 2014) and taxonomically classified using the RDP classifier (Wang et al., 2007). While tRNA/tmRNA sequences were predicted using ARAGORN (Laslett & Canback, 2004). ORFs were predicted using Prodigal (Hyatt et al., 2010). Similarity searches for GenBank (Clark et al., 2016), EggNOG (Huerta-Cepas et al., 2016), KEGG (Kanehisa & Goto, 2000), were done using Diamond (Buchfink et al., 2015). Read mapping against contigs was performed using Bowtie2 (Langmead & Salzberg, 2012) and binning was done using MaxBin2 (Wu et al., 2016) and Metabat2 (Kang et al., 2019). Results from both binning approaches were combined using DAS Tool to obtain refined bins (Sieber et al., 2018). Relevant information from the metagenomics data (ORF, contig and bin annotations, aggregated taxonomic and functional features) was exported into tables by running the SqueezeMeta utility script *sqm2tables.py* (Tamames & Puente-Sánchez, 2019) to facilitate the metadata handling for further analysis.

Bin completeness and contamination were computed using CheckM (Parks et al., 2015). Based on this, and a quality threshold choosing only bins with completeness

higher than 70% and contamination less than 10%, 69 bins were selected (Supplementary Table S1). High-quality bins of unclassified Micromonosporaceae and classified as *Nocardioides* were selected to submit to RAST server (Rapid Annotation using Subsystems Technology) (Aziz et al., 2008) for functional annotation. Also, the bin files were submitted to antiSMASH software (Antibiotics & Secondary Metabolite Analysis Shell) version 7 (Blin et al., 2023) to annotate BGCs (biosynthetic gene clusters) involved in secondary metabolite production.

Figure 7 clarifies the methodological design followed in this chapter:

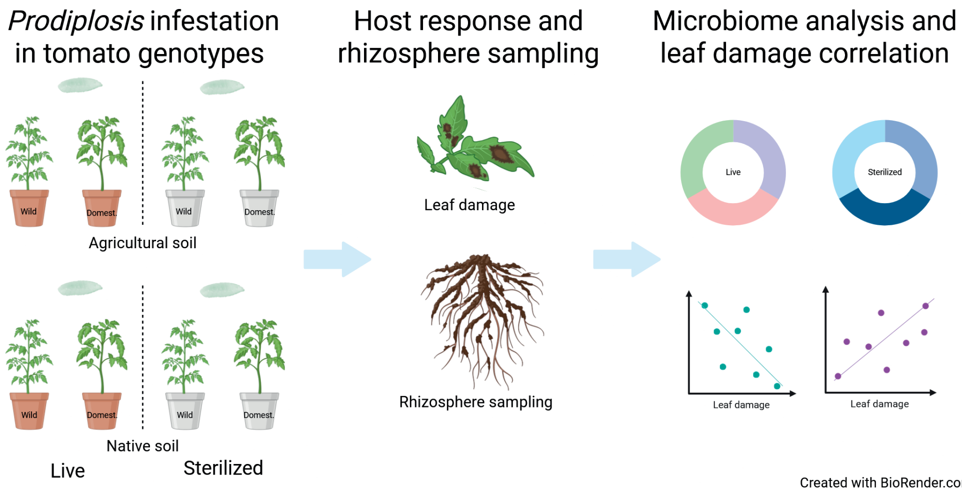

Figure 7. Experimental workflow illustrating the setup of *Prodiplosis longifila* larval infestation in tomato genotypes grown in agricultural and native soils under live or sterilized conditions. Host response was assessed by measuring leaf damage, and rhizosphere samples were collected for microbiome profiling. Microbiome composition was analyzed, and correlations between specific microbiome rhizosphere members and leaf damage were subsequently performed.

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Author contributions

SSF: methodology, investigation, data curation; writing - original draft, review & editing; VC: writing - review & editing; data curation; BOO: investigation, data analysis; writing - review & editing; LMAG: data curation; visualization; review & editing; NS: data curation; JMR: conceptualization; supervision; funding acquisition, writing - review & editing; PVTH: supervision; review & editing. All authors contributed critically to the drafts and gave final approval for publication.

Data availability

Raw 16S and shotgun metagenomics sequences were submitted to the European Nucleotide Archive (ENA) under the project accession number PRJEB82448.

Conflict of interest

No conflict of interest declared

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Plant research permit

This study was carried out under the Genetic Resource Permit N° MAE-DNB-CM-2018-0085, issued by the Ministry of Environment of Ecuador to USFQ.

Supplementary material

Supplementary Figures

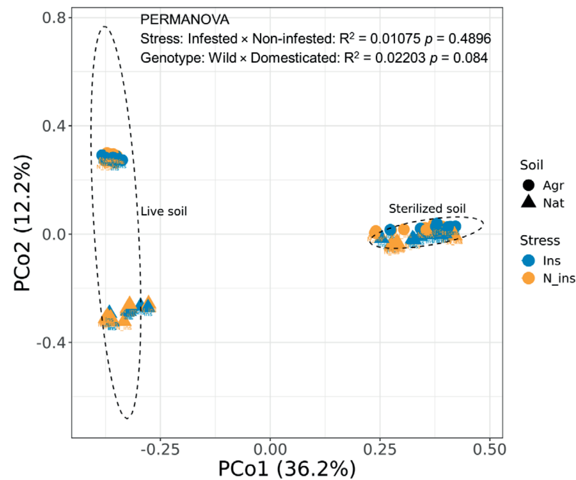


Figure S1. PCoA of bacterial communities in the rhizosphere of domesticated *S. lycopersicum* cv. Moneymaker and wild *S. pimpinellifolium* tomato. The analysis constrasts infested (Ins) and non-infested (N_ins) plants by *Prodiplosis longifila* considering different soil types (Agr: agricultural vs. Nat: native) and soil conditions (Live vs. Sterilized).

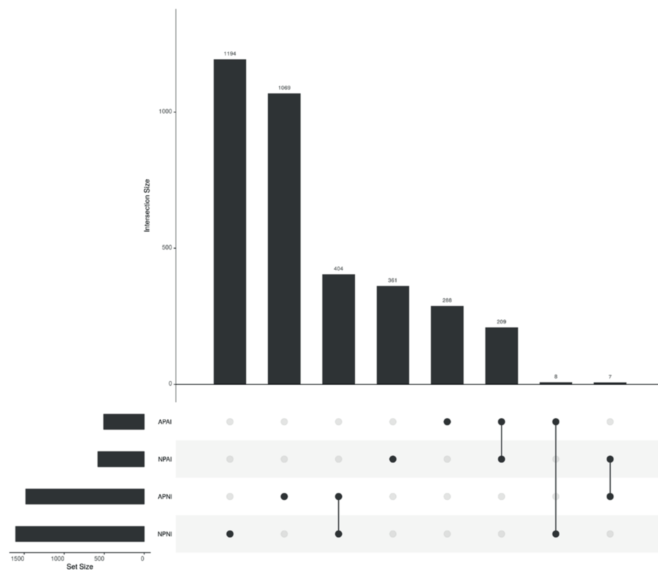


Figure S2. Number of bacterial ASVs shared among wild tomato *S. pimpinellifolium* rhizosphere samples. APAI: sterilized agricultural soil; NPAI: sterilized native soil; APNI: live agricultural soil; NPNI: live native soil. Upset plot generated by UpSetR software https://gehlenborglab.shinyapps.io/upsetr/ (Lex et al., 2014).

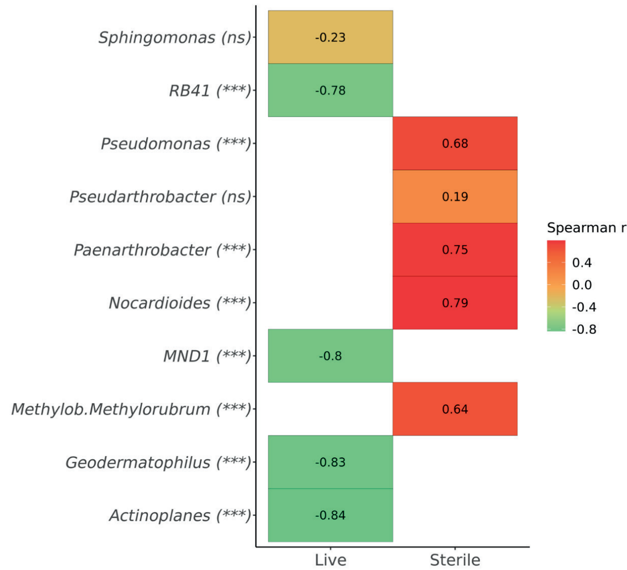
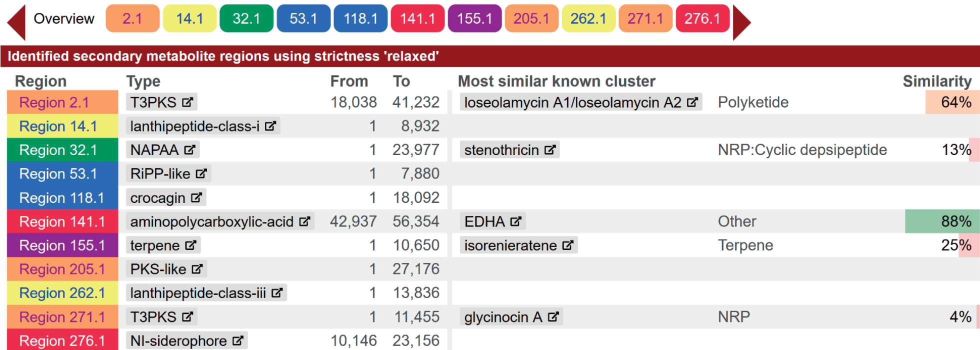


Figure S3. Heatmap of Spearman correlation coefficients between highest abundant rhizosphere bacteria genera in live and sterile soil and leaf damage proportion of *Solanum pimpinellifolium* infested by *Prodiplosis longifila.* Significance of the correlation is shown in parenthesis ($p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***, ns: not significant).

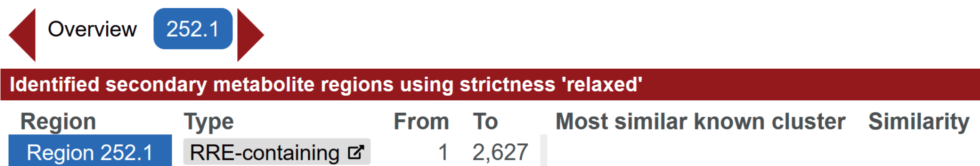


Overview 2.1 14.1 32.1 53.1 118.1 141.1 155.1 205.1 262.1 271.1 276.1

Identified secondary metabolite regions using strictness 'relaxed'

Region	Type	From	To	Most similar known cluster		Similarity
Region 2.1	T3PKS	18,038	41,232	loseolamycin A1/loseolamycin A2	Polyketide	64%
Region 14.1	lanthipeptide-class-i	1	8,932			
Region 32.1	NAPAA	1	23,977	stenothricin	NRP:Cyclic depsipeptide	13%
Region 53.1	RiPP-like	1	7,880			
Region 118.1	crocagin	1	18,092			
Region 141.1	aminopolycarboxylic-acid	42,937	56,354	EDHA	Other	88%
Region 155.1	terpene	1	10,650	isorenieratene	Terpene	25%
Region 205.1	PKS-like	1	27,176			
Region 262.1	lanthipeptide-class-iii	1	13,836			
Region 271.1	T3PKS	1	11,455	glycinocin A	NRP	4%
Region 276.1	NI-siderophore	10,146	23,156			

Figure S4. Overview of BGCs found in Micromonosporaceae bin 580 (*Actinoplanes* family) by bacterial antiSMASH software (Blin et al., 2023).



Overview 252.1

Identified secondary metabolite regions using strictness 'relaxed'

Region	Type	From	To	Most similar known cluster	Similarity
Region 252.1	RRE-containing	1	2,627		

Figure S5. Overview of BGCs found in *Nocardioides* bin 2.78 by bacterial antiSMASH software (Blin et al., 2023).

Supplementary Tables

Table S1. High quality-bins assembled by SqueezeMeta from rhizosphere tomato soil infested by *Prodiplosis longifila*.

Bin_ID	Marker_lineage	Completeness	Contamination	Method	Kingdom	Phylum	Class	Order	Family	Genus	Species	Length	GC_perc	Num_contigs	Sum_TPM_live	Sum_TPM_sterile
maxbin.003.fasta.contigs	p__Bacteroidetes (UID2591)	96.55	4.06	DAS	Bacteria	Bacteroidetes	Chitinophagia	Chitinophagales	Chitinophagaceae	NA	NA	4,923,528	43.67	129	457.062	4150.131
maxbin.004.fasta.contigs	f__Burkholderiaceae (UID4003)	90.15	4.55	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Ralstonia	NA	5,392,280	63.79	231	330.483	426.983
maxbin.006.fasta.contigs	k__Bacteria (UID2565)	80.83	2.55	DAS	Bacteria	Planctomycetes	NA	NA	NA	NA	NA	7,911,020	57.54	209	252.583	25727.007
maxbin.009.fasta.contigs	c__Alphaproteobacteria (UID3422)	75.98	9.96	DAS	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	NA	4,588,661	69.98	510	1140.756	11075.45
maxbin.012.fasta.contigs	k__Bacteria (UID2495)	76.86	2	DAS	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA	NA	3,142,557	63.65	58	280.254	2585.52
maxbin.016.fasta.contigs	c__Gammaproteobacteria (UID4444)	91.66	9.05	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	NA	NA	NA	NA	4,526,198	43.53	773	147.539	9452.228
maxbin.044.fasta.contigs	k__Bacteria (UID2982)	81.01	3.9	DAS	Bacteria	Verrucomicrobia	NA	NA	NA	NA	NA	6,768,386	60.92	1067	778.821	6882.065
maxbin.049.fasta.contigs	k__Bacteria (UID2982)	79.64	8.97	DAS	Bacteria	Verrucomicrobia	NA	NA	NA	NA	NA	5,301,187	51.66	245	259.691	5832.157
maxbin.097.fasta.contigs	c__Betaproteobacteria (UID3959)	94.15	5.89	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	NA	NA	NA	3,767,025	60.82	188	579.976	5384.284
maxbin.104.fasta.contigs	c__Alphaproteobacteria (UID3305)	83.62	4.59	DAS	Bacteria	Proteobacteria	Alphaproteobacteria	NA	NA	NA	NA	3,677,311	62.55	134	784.742	4135.142
maxbin.145.fasta.contigs	p__Bacteroidetes (UID2591)	84.89	5.57	DAS	Bacteria	Bacteroidetes	Chitinophagia	Chitinophagales	NA	NA	NA	3,707,379	43.52	1076	220.066	6235.133

Table S1. High quality-bins assembled by SqueezeMeta from rhizosphere tomato soil infested by *Prodiplosis longifila*. (*continued*)

Bin_ID	Marker_lineage	Completeness	Contamination	Method	Kingdom	Phylum	Class	Order	Family	Genus	Species	Length	GC_perc	Num_contigs	Sum_TPM_live	Sum_TPM_sterile
maxbin.188.fasta.contigs	f__Halobacteriaceae (UID85)	94.73	2.93	DAS	Archaea	Euryarchaeota	Halobacteria	Natrialbales	Natrialbaceae	NA	NA	4,061,149	66.71	360	3775.17	3205.368
maxbin.218.fasta.contigs	k__Bacteria (UID2982)	81.64	7.98	DAS	Bacteria	Verrucomicrobia	NA	NA	NA	NA	NA	5,483,094	51.65	603	434.351	11558.335
maxbin.247.fasta.contigs	k__Bacteria (UID2570)	91.8	2.73	DAS	Bacteria	NA	NA	NA	NA	NA	NA	5,275,354	61.75	544	278.328	7867.893
maxbin.360.fasta.contigs	k__Bacteria (UID203)	76.03	0	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	NA	NA	NA	2,285,668	70.25	115	1561.343	7483.135
maxbin.371.fasta.contigs	p__Bacteroidetes (UID2591)	99.01	0.25	DAS	Bacteria	Bacteroidetes	Chitinophagia	Chitinophagales	Chitinophagaceae	Chitinophaga	Chitinophaga sancti	5,853,332	42.46	132	272.215	7062.444
maxbin.414.fasta.contigs	o__Burkholderiales (UID4002)	89.92	9.74	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	6,142,809	62.63	460	4255.204	906.804
maxbin.415.fasta.contigs	p__Bacteroidetes (UID2605)	95	3.65	DAS	Bacteria	Bacteroidetes	NA	NA	NA	NA	NA	4,687,430	45.72	663	235.57	5912.872
maxbin.432.fasta_sub.contigs	k__Bacteria (UID203)	83.54	8.23	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	NA	NA	NA	2,798,466	64.76	197	342.66	198.749
maxbin.464.fasta.contigs	p__Bacteroidetes (UID2591)	92.86	4.2	DAS	Bacteria	Bacteroidetes	Chitinophagia	Chitinophagales	Chitinophagaceae	NA	NA	7,536,217	47.12	386	1033.659	6339.271
maxbin.468.fasta.contigs	p__Bacteroidetes (UID2591)	95.76	5.62	DAS	Bacteria	Bacteroidetes	Chitinophagia	Chitinophagales	Chitinophagaceae	NA	NA	4,932,603	40.25	609	434.125	5474.293
maxbin.472.fasta_sub.contigs	f__Micrococcaceae (UID1631)	87.84	4.74	DAS	Bacteria	Actinobacteria	Actinomycetia	Micrococcales	Micrococcaceae	NA	NA	3,806,130	66.71	943	5044.113	11672.052

Table S1. High quality-bins assembled by SqueezeMeta from rhizosphere tomato soil infested by *Prodiplosis longifila*. (*continued*)

Bin_ID	Marker_lineage	Completeness	Contamination	Method	Kingdom	Phylum	Class	Order	Family	Genus	Species	Length	GC_perc	Num_contigs	Sum_TPM_live	Sum_TPM_sterile
maxbin.476.fasta_sub.contigs	k__Bacteria (UID2982)	78.41	8.11	DAS	Bacteria	Verrucomicrobia	NA	NA	NA	NA	NA	6,669,823	61.47	1084	503.843	4178.324
maxbin.489.fasta.contigs	k__Bacteria (UID203)	100	9.69	DAS		NA	NA	NA	NA	NA	NA	7,097,239	36.7	331	133.742	10946.075
maxbin.508.fasta.contigs	c__Alphaproteobacteria (UID3305)	76.73	6.79	DAS	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	NA	2,056,600	64.38	622	20498.528	450.123
maxbin.515.fasta.contigs	k__Bacteria (UID203)	75.71	7.55	DAS	Bacteria	NA	NA	NA	NA	NA	NA	1,664,664	47.24	268	241.725	4556.406
maxbin.527.fasta.contigs	k__Bacteria (UID2982)	86.54	3.38	DAS	Bacteria	NA	NA	NA	NA	NA	NA	1,830,959	43.94	213	73,452	3544,979
maxbin.531.fasta.contigs	k__Bacteria (UID2495)	92.28	2.37	DAS	Bacteria	Spirochaetes	Spirochaetia	Leptospirales	Leptospiraceae	Leptospira	NA	3,935,131	40.24	1188	152,639	3064,683
maxbin.568.fasta.contigs	o__Cytophagales (UID2936)	93.71	3.63	DAS	Bacteria	Bacteroidetes	NA	NA	NA	NA	NA	5,298,703	45.68	456	683,266	4101,769
maxbin.580.fasta.contigs	o__Actinomycetales (UID1696)	88.42	2.59	DAS	Bacteria	Actinobacteria	Actinomycetia	Micromonosporales	Micromonosporaceae	NA	NA	4,880,819	70.03	320	67231,883	263,036
maxbin.587.fasta.contigs	k__Bacteria (UID2982)	88.4	7.25	DAS	Bacteria	Verrucomicrobia	NA	NA	NA	NA	NA	5,712,602	52.19	1052	393,692	184,614
maxbin.605.fasta.contigs	f__Enterobacteriaceae (UID5124)	96.22	2.01	DAS	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	NA	NA	4,609,621	56.05	334	91,803	4388,719
maxbin.658.fasta.contigs	k__Bacteria (UID2495)	93.13	7.89	DAS	Bacteria	Gemmatimonadetes	NA	NA	NA	NA	NA	3,353,775	59.82	462	12963,416	14,967
maxbin.673.fasta.contigs	p__Cyanobacteria (UID2189)	95.54	9.63	DAS	Bacteria	Cyanobacteria	NA	NA	NA	NA	NA	9,710,549	46.33	1710	2511,263	4,375
maxbin.683.fasta.contigs	k__Archaea (UID2)	90.78	3.88	DAS	Archaea	Thaumarchaeota	NA	NA	NA	NA	NA	1,783,922	50.62	413	30006,247	13,96

Table S1. High quality-bins assembled by SqueezeMeta from rhizosphere tomato soil infested by *Prodiplosis longifila*. (*continued*)

Bin_ID	Marker_lineage	Completeness	Contamination	Method	Kingdom	Phylum	Class	Order	Family	Genus	Species	Length	GC_perc	Num_contigs	Sum_TPM_live	Sum_TPM_sterile
metabat2.1.fa.contigs	o__Sphingomonadales (UID3310)	70.52	0.69	DAS	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	NA	2,289,747	67.64	129	372,298	5678,63
metabat2.135.fa.contigs	o__Burkholderiales (UID4002)	71.97	1.91	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	4,368,555	59.27	608	6516,666	145,848
metabat2.136.fa_sub.contigs	o__Cytophagales (UID2936)	83.05	1.04	DAS	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	NA	NA	NA	5,256,850	48.07	277	787,309	1005,646
metabat2.157.fa.contigs	k__Bacteria (UID203)	78.09	5.17	DAS	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	NA	NA	4,236,708	60.27	496	281,688	214,139
metabat2.158.fa_sub.contigs	k__Bacteria (UID3187)	81.42	8.55	DAS	Bacteria	Acidobacteria	NA	NA	NA	NA	NA	3,907,414	66.84	543	1878,565	2023,782
metabat2.159.fa.contigs	o__Cytophagales (UID2936)	85.85	1.49	DAS	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	NA	NA	NA	5,162,706	46.72	542	781,227	129,77
metabat2.18.fa.contigs	p__Cyanobacteria (UID2182)	96.7	1.18	DAS	Bacteria	Cyanobacteria	NA	NA	NA	NA	NA	5,852,500	47.66	304	8101,354	4,371
metabat2.190.fa.contigs	p__Bacteroidetes (UID2591)	75.52	2.22	DAS	Bacteria	Bacteroidetes	Chitinophagia	Chitinophagales	Chitinophagaceae	NA	NA	3,332,901	56.47	461	366,619	4627,984
metabat2.204.fa.contigs	k__Bacteria (UID2495)	70.16	4.4	DAS	Bacteria	Gemmatimonadetes	NA	NA	NA	NA	NA	2,822,695	64	414	491,866	1060,357
metabat2.206.fa_sub.contigs	k__Bacteria (UID1453)	80.57	2.85	DAS	Bacteria	Actinobacteria	NA	NA	NA	NA	NA	3,174,811	69.85	234	794,576	2570,79
metabat2.21.fa.contigs	o__Bacillales (UID828)	87.43	2.54	DAS	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Priestia	NA	3,754,103	38.34	598	1972,028	2861,669
metabat2.232.fa_sub.contigs	f__Burkholderiaceae (UID4003)	92.79	2.87	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	NA	5,740,917	66.3	428	480,622	5670,952

Table S1. High quality-bins assembled by SqueezeMeta from rhizosphere tomato soil infested by *Prodiplosis longifila*. (*continued*)

Bin_ID	Marker_lineage	Completeness	Contamination	Method	Kingdom	Phylum	Class	Order	Family	Genus	Species	Length	GC_perc	Num_contigs	Sum_TPM_live	Sum_TPM_sterile
metabat2.239.fa.contigs	c__Alphaproteobacteria (UID3422)	81.08	3.01	DAS	Bacteria	Proteobacteria	Alphaproteobacteria	NA	NA	NA	NA	2,983,850	62.33	436	514,32	1186,826
metabat2.24.fa.contigs	k__Bacteria (UID1453)	74.03	3.78	DAS	Bacteria	Actinobacteria	Acidimicrobiia	NA	NA	NA	NA	2,901,287	71.54	398	1179,018	2732,154
metabat2.262.fa.contigs	k__Bacteria (UID203)	78.45	0.86	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	NA	NA	NA	3,011,244	70.62	284	9017,283	4584,655
metabat2.273.fa.contigs	c__Alphaproteobacteria (UID3305)	85.94	1.52	DAS	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	NA	NA	NA	2,716,380	59.39	90	101,235	2276,446
metabat2.274.fa_sub.contigs	p__Bacteroidetes (UID2591)	97.37	0.74	DAS	Bacteria	Bacteroidetes	Chitinophagia	Chitinophagales	Chitinophagaceae	Chitinophaga	Chitinophaga sancti	6,896,030	45.02	233	83,436	9557,47
metabat2.277.fa_sub.contigs	c__Alphaproteobacteria (UID3305)	78.98	5.71	DAS	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	NA	2,037,372	67.47	351	925,647	925,536
metabat2.288.fa.contigs	k__Archaea (UID2)	79.13	5.34	DAS	Archaea	Thaumarchaeota	NA	NA	NA	NA	NA	1,403,343	35.85	186	8392,975	4,695
metabat2.304.fa.contigs	c__Alphaproteobacteria (UID3305)	76.63	4.5	DAS	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	NA	1,763,881	64.57	238	1393,536	3031,039
metabat2.305.fa.contigs	p__Cyanobacteria (UID2189)	86.44	2.01	DAS	Bacteria	Cyanobacteria	NA	NA	NA	NA	NA	7,075,742	46.75	349	14366,768	2,988
metabat2.353.fa_sub.contigs	f__Micrococcaceae (UID1631)	95.18	1.16	DAS	Bacteria	Actinobacteria	Actinomycetia	Micrococcales	Micrococcaceae	NA	NA	4,100,067	63.68	122	361,341	9011,369
metabat2.358.fa.contigs	c__Betaproteobacteria (UID3959)	74.92	3.4	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Bordetella	Bordetella bronchialis	4,349,268	67.69	821	441,811	3314,553

Table S1. High quality-bins assembled by SqueezeMeta from rhizosphere tomato soil infested by *Prodiplosis longifila*. (*continued*)

Bin_ID	Marker_lineage	Completeness	Contamination	Method	Kingdom	Phylum	Class	Order	Family	Genus	Species	Length	GC_perc	Num_contigs	Sum_TPM_live	Sum_TPM_sterile
metabat2.37.fa.contigs	c__Betaproteobacteria (UID3959)	72.73	1.24	DAS	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	3,062,315	61.47	175	535,807	2006,992
metabat2.38.fa.contigs	c__Betaproteobacteria (UID3959)	75.22	1.31	DAS	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	3,865,322	58.63	592	354,853	2908,733
metabat2.384.fa.contigs	o__Burkholderiales (UID4000)	83.95	1.79	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Variovorax	NA	4,889,619	70.84	462	1449,913	11890,59
metabat2.390.fa.contigs	c__Deltaproteobacteria (UID3216)	74.87	3.46	DAS	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	NA	NA	NA	6,426,917	65.86	349	1506,687	4559,171
metabat2.45.fa_sub.contigs	k__Bacteria (UID203)	74.29	3.45	DAS	Bacteria	Bacteroidetes	NA	NA	NA	NA	NA	3,021,302	36.75	349	3908,152	1225,417
metabat2.48.fa_sub.contigs	p__Bacteroidetes (UID2591)	81.4	1.97	DAS	Bacteria	Bacteroidetes	Chitinophagia	Chitinophagales	Chitinophagaceae	Niastella	NA	6,436,456	44.26	476	1161,871	6493,219
metabat2.59.fa_sub.contigs	o__Burkholderiales (UID4000)	78.63	3.68	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Variovorax	NA	4,352,969	68.35	638	1021,628	4187,495
metabat2.70.fa.contigs	o__Burkholderiales (UID4000)	84.41	3.13	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	NA	NA	NA	3,699,209	69.39	417	1871,94	3296,182
metabat2.78.fa_sub.contigs	o__Actinomycetales (UID1697)	72.6	5.66	DAS	Bacteria	Actinobacteria	Actinomycetia	Propionibacteriales	Nocardioidaceae	Nocardioides	NA	3,223,870	72.41	385	1566,67	9182,806
metabat2.95.fa.contigs	k__Bacteria (UID2982)	77.45	2.49	DAS	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	NA	NA	NA	4,526,054	61.25	692	319,887	3374,055
metabat2.97.fa.contigs	o__Burkholderiales (UID4000)	75.29	2.6	DAS	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	2,332,583	69.16	339	3957,81	3068,633

Table S2. Annotated protein encoding genes by RAST server using the SEED Subsystem database from bacterial assembled bins of tomato rhizosphere

bin_name	Category	Subcategory	Subsystem	Role	Features
Micro-monospora-ceae_bin580	Phages, Prophages, Transposable elements, Plasmids	Phages, Prophages	Phage packaging machinery	Phage terminase, large subunit	fig|6666666.1083232.peg.2626, fig|6666666.1083232.peg.4401
Micro-monospora-ceae_bin580	Phages, Prophages, Transposable elements, Plasmids	Phages, Prophages	Phage lysis modules	Phage holin	fig|6666666.1083232.peg.1045
Micro-monospora-ceae_bin580	Phages, Prophages, Transposable elements, Plasmids	Phages, Prophages	Phage lysis modules	Phage endolysin	fig|6666666.1083232.peg.653, fig|6666666.1083232.peg.3139
Micro-monospora-ceae_bin580	Phages, Prophages, Transposable elements, Plasmids	Phages, Prophages	Phage lysis modules	Phage lysin, 1,4-beta-N-acetylmuramidase (EC 3.2.1.17) or lysozyme	fig|6666666.1083232.peg.243
Micro-monospora-ceae_bin580	Iron acquisition and metabolism	Iron acquisition and metabolism - no subcategory	Ferrous iron transporter EfeUOB, low-pH-induced	Ferrous iron transport peroxidase EfeB	fig|6666666.1083232.peg.3765
Micro-monospora-ceae_bin580	Iron acquisition and metabolism	Iron acquisition and metabolism - no subcategory	Ferrous iron transporter EfeUOB, low-pH-induced	Ferrous iron transport periplasmic protein EfeO, contains peptidase-M75 domain and (frequently) cupredoxin-like domain	fig|6666666.1083232.peg.3766
Micro-monospora-ceae_bin580	Iron acquisition and metabolism	Iron acquisition and metabolism - no subcategory	Ferrous iron transporter EfeUOB, low-pH-induced	Ferrous iron transport permease EfeU	fig|6666666.1083232.peg.3767
Micro-monospora-ceae_bin580	Dormancy and Sporulation	Dormancy and Sporulation - no subcategory	Sporulation-associated proteins with broader functions	Peptidyl-tRNA hydrolase (EC 3.1.1.29)	fig|6666666.1083232.peg.1609
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory dehydrogenases 1	NADH dehydrogenase (EC 1.6.99.3)	fig|6666666.1083232.peg.1191, fig|6666666.1083232.peg.2346, fig|6666666.1083232.peg.2614, fig|6666666.1083232.peg.4063

Table S2. Annotated protein encoding genes by RAST server using the SEED Subsystem database from bacterial assembled bins of tomato rhizosphere (*continued*)

bin_name	Category	Subcategory	Subsystem	Role	Features
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain E (EC 1.6.5.3)	fig|6666666.1083232.peg.4653
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH ubiquinone oxidoreductase chain A (EC 1.6.5.3)	fig|6666666.1083232.peg.1691, fig|6666666.1083232.peg.3401, fig|6666666.1083232.peg.4657
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain C (EC 1.6.5.3)	fig|6666666.1083232.peg.3158, fig|6666666.1083232.peg.3398, fig|6666666.1083232.peg.4655
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain H (EC 1.6.5.3)	fig|6666666.1083232.peg.3397, fig|6666666.1083232.peg.4650
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain J (EC 1.6.5.3)	fig|6666666.1083232.peg.4648
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain F (EC 1.6.5.3)	fig|6666666.1083232.peg.4652
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain N (EC 1.6.5.3)	fig|6666666.1083232.peg.3391, fig|6666666.1083232.peg.4644
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain M (EC 1.6.5.3)	fig|6666666.1083232.peg.3392, fig|6666666.1083232.peg.4645
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain K (EC 1.6.5.3)	fig|6666666.1083232.peg.3394, fig|6666666.1083232.peg.4647

Table S2. Annotated protein encoding genes by RAST server using the SEED Subsystem database from bacterial assembled bins of tomato rhizosphere (*continued*)

bin_name	Category	Subcategory	Subsystem	Role	Features
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain B (EC 1.6.5.3)	fig|6666666.1083232.peg.3157, fig|6666666.1083232.peg.3400, fig|6666666.1083232.peg.4656
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain I (EC 1.6.5.3)	fig|6666666.1083232.peg.3396, fig|6666666.1083232.peg.4649
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NAD(P)H-quinone oxidoreductase chain J (EC 1.6.5.2)	fig|6666666.1083232.peg.3395
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain L (EC 1.6.5.3)	fig|6666666.1083232.peg.3393, fig|6666666.1083232.peg.4646
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain D (EC 1.6.5.3)	fig|6666666.1083232.peg.2571, fig|6666666.1083232.peg.3159, fig|6666666.1083232.peg.4654
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	Respiratory Com-plex I	NADH-ubiquinone oxidoreductase chain G (EC 1.6.5.3)	fig|6666666.1083232.peg.4651
Micro-monospora-ceae_bin580	Respiration	Electron donating reactions	NADH ubiquinone oxidoreductase	NADH-ubiquinone oxidoreductase chain M (EC 1.6.5.3)	fig|6666666.1083232.peg.3392, fig|6666666.1083232.peg.4645
Micro-monospora-ceae_bin580	Phages, Prophages, Transposable ele-ments, Plasmids	Phages, Prophages	Phage packaging machinery	Phage terminase, large subunit	fig|6666666.1083232.peg.2626, fig|6666666.1083232.peg.4401

Note. The full Table S2 is available online at https://doi.org/10.5281/zenodo.15725118

Chapter 5

Disentangling the genetic basis of rhizosphere microbiome assembly in tomato

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Abstract

Microbiomes play a pivotal role in plant growth and health, but the genetic factors involved in microbiome assembly remain largely elusive. Here, we map the molecular features of the rhizosphere microbiome as quantitative traits of a diverse hybrid population of wild and domesticated tomato. Gene content analysis of prioritized tomato quantitative trait loci suggests a genetic basis for differential recruitment of various rhizobacterial lineages, including a *Streptomyces*-associated 6.31 Mbp region harboring tomato domestication sweeps and encoding, among others, the iron regulator FIT and the water channel aquaporin SlTIP2.3. Within metagenome-assembled genomes of root-associated *Streptomyces* and *Cellvibrio*, we identify bacterial genes involved in metabolism of plant polysaccharides, iron, sulfur, trehalose, and vitamins, whose genetic variation associates with specific tomato QTLs. By integrating 'microbiomics' and quantitative plant genetics, we pinpoint putative plant and reciprocal rhizobacterial traits underlying microbiome assembly, thereby providing a first step towards plant-microbiome breeding programs.

Introduction

Root and shoot microbiomes are fundamental to plant growth and plant tolerance to (a)biotic stress factors. The outcome of these beneficial interactions is the emergence of specific microbiome-associated phenotypes (MAPs) (Oyserman et al., 2018), such as drought resilience (Marasco et al., 2012), disease resistance (Carrión, 2019), development (Finkel, 2020), and heterosis (i.e., hybrid vigor) (Wagner, 2021). The microbes inhabiting the surface or internal tissues of plant roots are selectively nurtured by diverse plant-derived compounds in the form of primary and secondary metabolites (Canarini et al., 2019; Sasse et al., 2018). Microbes reciprocate by supporting plant growth and producing metabolites that mediate processes such as nutrient acquisition and pathogen suppression (Crowley, 2006; Tracanna et al., 2021). Developing a blueprint of the genetic architecture for this 'chemical dialog' and how these interactions lead to specific MAPs is one of the key focal points in current plant microbiome research. The promise is that these genomic and chemical blueprints can be integrated into crop breeding programs for a new generation of 'microbiome-assisted' crops that can rely, at least in part, on specific members of the microbiome for stress protection, enhanced growth, and higher yields (Oyserman et al., 2021).

Selective breeding for yield-related traits has left a considerable impact on the taxonomic and functional composition of modern crop microbiomes (Favela et al., 2021; Pérez-Jaramillo et al., 2018). Wild plant relatives represent a 'living library' of diverse genetic traits that may have been lost during domestication (Gruber, 2017). For example, recombinant inbred lines (RILs) of crosses between wild tomato relatives and modern tomato cultivars have been used to identify genetic loci controlling important agronomic traits, including tolerance to abiotic (Lopez-Delacalle, 2019) and biotic stress (Vosman, 2019), as well as nutritional quality and flavor profiles (Liu, 2016). To date, microbiome traits are not yet considered for breeding purposes, except for specific quantitative MAPs such as the number of nodules in legume-rhizobia symbioses (Pereira et al., 1993). However, technological advances in sequencing now make it feasible to treat microbiomes as quantitative traits for selection. Quantitative approaches to map the microbiome as a phenotype have been adopted to investigate the phyllosphere microbiome and, recently, for the *Arabidopsis* and sorghum rhizosphere microbiomes (Bergelson et al., 2019; Deng, 2021). However, actualizing microbiome features into breeding programs at a scale for crop improvement has not yet been realized. In fact, for most plant species, investigations leveraging diverse plant populations to map microbiome-associated quantitative trait loci (QTL) are still in their infancy (Bergelson et al., 2019; Deng, 2021; Wallace et al., 2018). In these recent studies, the microbiomes were characterized by amplicon sequencing to detect loci involved in alpha and beta diversity as well as individual OTU abundances (Bergelson et al., 2021). These studies

provide strong evidence that microbiome recruitment has a genetic component, but the functional nature of the corresponding plant–microbe interactions cannot be reliably elucidated from amplicon data. Hence, functional genomic features of the microbiome, as well as intraspecific diversity within microbial species, have not yet been taken into account in QTL analyses (Olm, 2021).

While Chapters 2–4 focused on ecological and functional aspects of tomato–microbiome interactions, they did not address the plant genetic factors underlying microbiome assembly. In this chapter, we take a complementary approach by integrating microbiome profiling with quantitative plant genetics to uncover the genetic basis of rhizosphere microbiome assembly in tomato.

Here, we use both amplicon and shotgun metagenome sequencing to generate taxonomic as well as functional microbiome features as quantitative traits. Using an extensive RIL population of a cross between modern *Solanum lycopersicum* var. Moneymaker and wild *Solanum pimpinellifolium* (Voorrips et al., 2000), we identify reciprocal associations between specific plant and microbiome traits and infer putative mechanisms for rhizosphere microbiome assembly. Using the modern allele as a reference, we find QTLs for numerous taxonomic and metagenomic features of the microbiome with both positive and negative effects. We observe more positive effects related to increases in microbiome feature abundance for the modern reference allele compared to the wild reference allele, suggesting that domestication has had a significant impact on rhizosphere microbiome assembly. We identify plant traits related to growth, stress, amino acid metabolism, iron and water acquisition, hormonal responses, and terpene biosynthesis, whereas the microbial traits we identify are related to the metabolism of plant cell wall polysaccharides, vitamins, sulfur, and iron. Furthermore, we show that amplicon-based approaches allow detection of QTLs for rarer microbial taxa, whereas shotgun metagenomics allowed mapping to smaller and thus more defined plant genomic regions. Together, these results demonstrate the power of an integrated approach to disentangle and prioritize specific genomic regions and genes in both plants and microbes associated with microbiome assembly.

Results

Baseline analyses of the tomato recombinant inbred line population

Prior to detailed metagenome analyses of the microbiome of the tomato RIL population, we first investigated whether QTLs previously identified in the same RIL population under sterile in vitro conditions could be replicated in our experiment conducted under greenhouse conditions with a commercial tomato greenhouse soil (Figure 1a, b and Supplementary Data 1) (Khan, 2012). We identified QTLs for shoot dry weight (SDW) coinciding with a QTL identified previously on chromosome 9 (Khan, 2012). Similarly, we identified QTLs for rhizosphere mass (RM), defined here as the total mass of the roots with tightly adhering soil, which coincides with root trait QTLs previously identified for lateral root number, fresh and dry shoot weight, lateral root density per branched zone and total root size (Figure 1b) (Khan, 2012). An analysis of variance (ANOVA) yielded significant variation in SDW based on the additivity of alleles linked to SDW (zero, one, or two alleles) ($F(2, 186) = 16.02$, $p = 3.76$ e–07) (Figure 1c, d). A post hoc Tukey test further demonstrated significant differences between all pairwise comparisons ($p < 0.05$). For RM, an ANOVA yielded a significant difference ($F(2, 186) = 16.02$, $p = 3.76$ e–07); a post hoc Tukey test demonstrated a statistically significant difference only between the presence of either one or two alleles ($p < 0.05$), but did not support additivity ($p = 0.15$) (Figure 1e, f). Collectively, our results confirm and extend earlier work conducted on the same tomato RIL population in vitro (Khan, 2012), providing a solid basis for QTL mapping of taxonomic and genomic features of the rhizosphere microbiome.

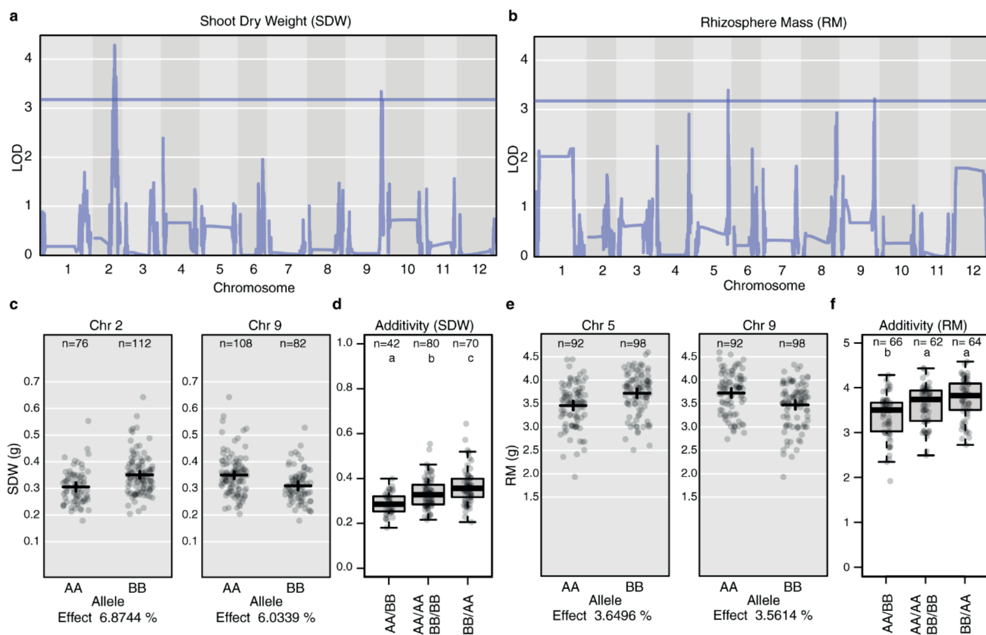

Figure 1. Replication of shoot dry weight and rhizosphere mass QTLs from previous studies. a QTLs identified for SDW on chromosome 9 position 63.63719184 and chromosome 2 position 42.7291229, coinciding with a QTL identified previously (chromosome 9 position 62.897108) by Khan et al 2012. **b** QTL of RM on chromosome 5 position 62.00574891, and chromosome 9 position 62.71397636, which coincide with root trait QTLs previously identified 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) from Khan et al 2021. **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) alleles. For the QTL on Chromosome 2, $n = 76$ and 112 biologically independent samples for AA and BB respectively. For the QTL on Chromosome 9, $n = 106$ and 82 biologically independent samples for AA and BB respectively. In addition to the scatter plot, data are presented as mean values +/– two times the SEM. **d** Significant additivity of alleles for SDW ($p < 0.05$); n of 42, 80, and 70 for biologically independent plants containing neither allele (AA/BB), either BB allele on chromosome 2, or AA on chromosome 9 (AA/AA or BB/BB), or both AA and BB alleles (BB/AA) respectively. In addition to the scatter plot, data are presented with boxplots representing the median value, the interquartile range, and whiskers representing the minimal and maximal values excluding points greater than 1.5 times the interquartile range. **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. For the QTL on Chromosome 5, $n = 92$ and 98 biologically independent samples for AA and BB respectively. For the QTL on Chromosome 9, $n = 92$ and 98 biologically independent samples for AA and BB respectively. In addition to the scatter plot, data are presented as mean values +/– two times the SEM. **f** No additivity of alleles was observed for RM. In addition to the scatter plot, data are presented with boxplots representing the median value, the interquartile range, and whiskers representing the minimal and maximal values excluding points greater than 1.5 times the interquartile range. Source data are provided as a Source Data file.

Taxonomic microbiome features as quantitative traits

To investigate molecular features of the microbiome as quantitative traits, we conducted 16S rRNA gene amplicon sequencing of 225 rhizosphere samples, including unplanted bulk soil, parental tomato genotypes, and all 96 RIL accessions in duplicate (BioProject ID PRJNA787039). We observed separation between the microbiomes of rhizosphere and bulk soil, between the microbiomes of the two parental tomato genotypes, and the RIL accession microbiomes (Figure 2a). To limit multiple testing and to focus on common microbiome features with sufficient coverage across all accessions, we prioritized the rhizosphere-enriched amplicon sequence variants (ASVs) to those present in 50% or more of the RIL accessions (Figure 2b). A QTL analysis with these prioritized ASVs was run with R/qtl2 (Broman, 2019) using a high-density tomato genotype map (Sterken et al., 2023), harvest date, post-harvest total bulk soil mass, RM, number of leaves at harvest, and SDW as covariates.

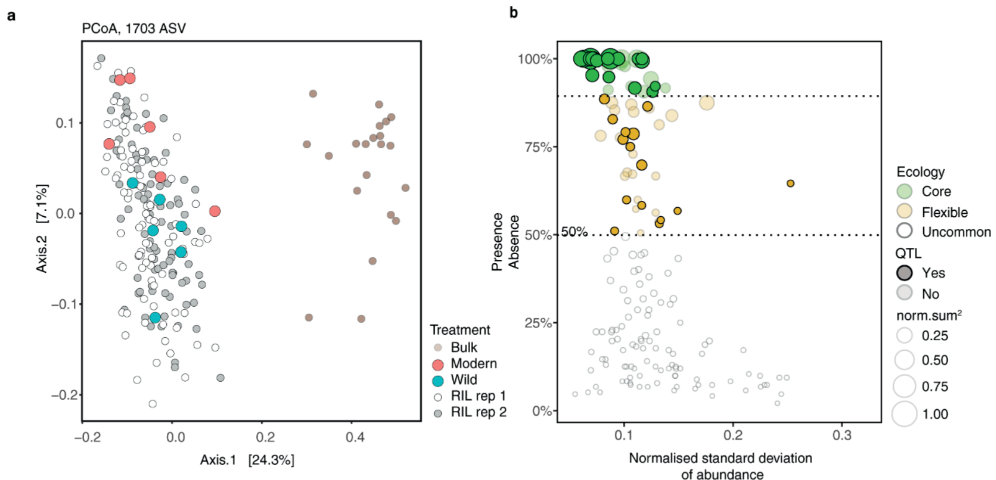

Figure 2. The 16S rRNA microbiomes of the bulk soils, modern and wild tomatoes, and RIL population. a A PCoA analysis of ASVs demonstrating a separation between the bulk soil and rhizosphere microbiomes. The rhizosphere of RIL accessions distribute around the wild and modern rhizospheres. Separation between the two replicate RIL populations was not observed. **b** To limit multiple testing, a QTL analysis was conducted only on ASV that were observed in over 50% of accessions. The 33 ASV that are subsequently found with QTLs are shown with full opacity. All other ASV are partially transparent. Source data are provided as a Source Data file.

We identified 48 QTL peaks, across 45 distinct loci, significantly associated with 33 ASVs (Supplementary Data 6). Our logarithm of the odds (LOD) thresholds for significance had been determined by pooled permutations from all ASVs to attain a genome-wide threshold of *P* 0.05 (LOD 3.35) and *P* 0.2 (LOD 2.64). The modern allele was set at reference, such that negative effects were relatively more associated with the wild allele and positive effects with the modern allele. Of the significant QTLs, 16 were microbiome features less abundant compared to the reference allele, whereas 32 were microbiome features more abundant in presence of the modern reference allele. The QTLs on chromosomes 11, 10, 8, and 2 were associated with increases in abundance in presence of the modern reference allele. In contrast, the sole QTL on chromosome 7 was negative relative to the reference. All other chromosomes contained a mix of QTLs with positive and negative effects on ASV abundance relative to the reference allele (Figure 3a). While many rhizobacterial lineages were linked to a single QTL (14 out of 25 unique taxonomies), others were linked to two or more QTLs (7 and 4 taxa, respectively) (Figure 3b). Of the lineages with multiple QTLs, most were positive relative to the reference allele. One salient exception was *Methylophilaceae*, with a total of 9 QTLs that were both positive and negative relative to the reference and distributed across chromosomes 3 (positive, ×2), 4 (positive), 7 (negative), 11 (positive ×2), and 12 (negative ×3) (Figure 3c). Another salient feature of the QTL analysis was the hotspot for microbiome assembly identified on chromosome 11, including a significant linkage with ASVs from *Adhaeribacter, Caulobacter, Devosia*, Rhizobiaceae, *Massilia,* and *Methylophilaceae* (Figure 3c).

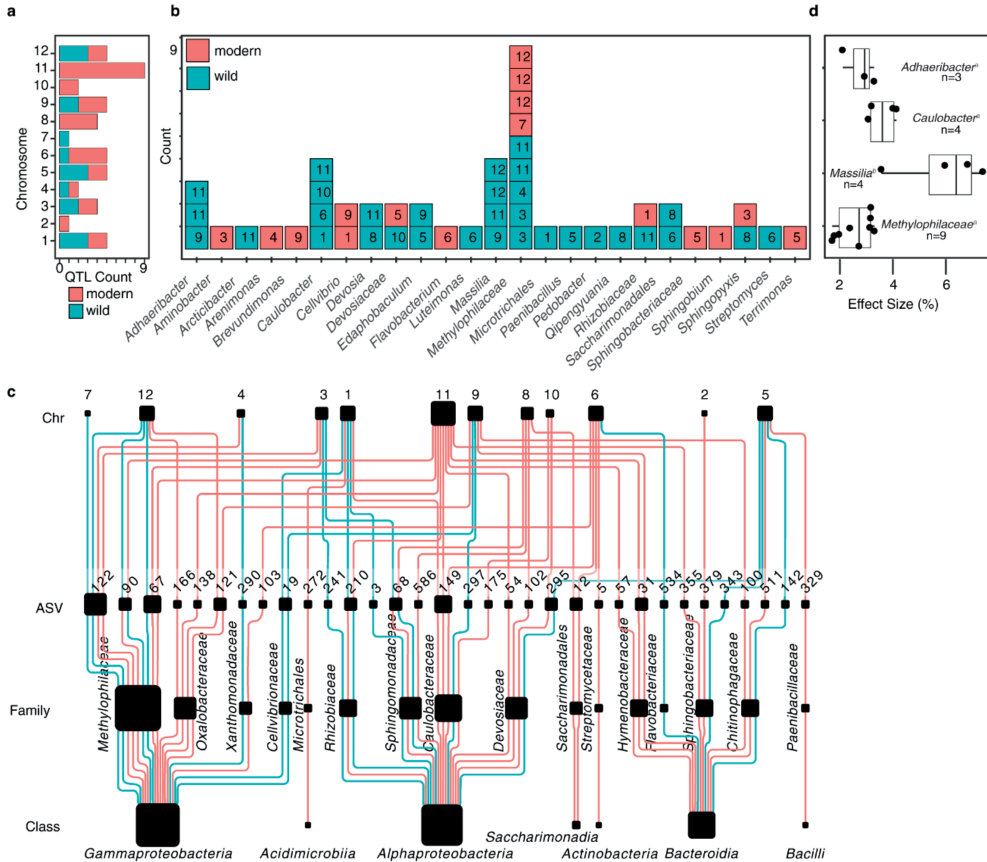

Figure 3. The 16S rRNA QTLs. a A color coded summary of the number of 16S rRNA QTLs identified per chromosome to wild and modern alleles. **b** A summary of the number of 16S rRNA QTLs found by taxonomies, with the chromosome of each QTL represented within each square. The presence and absence of dark borders around each square are used to indicate a QTL linked to higher abundance for a wild allele and modern allele respectively. **c** A hierarchically structured network depicting the 16S rRNA QTLs identified in this study. From the top to bottom: the nodes in the first row represent tomato chromosomes, which are linked to specific ASV in the next row, which are linked 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. The edges are color coded based on negative effect relative to the modern reference (e.g., wild allele), and positive effect relative to the modern reference (e.g., modern allele). The abundance of individual ASV, and at different taxonomic levels, is determined through a complex interaction of multiple alleles from both modern and wild origin. **d** A statistical analysis of the four lineages with 3 or greater QTLs shows that the absolute value of effect size for different lineages is different. Specifically, we find that the effect size for ASV within *Massila* ($n = 4$) was significantly larger than for the other lineages (*Adhaeribacter*, $n = 3$; *Caulobacter*, $n = 4$; *Methylophylaceae*, $n = 9$). The effect size was calculated as the percent change relative to the mean CSS abundance for each ASV. In addition to the scatter plot, data are presented with boxplots representing the median value, the interquartile range, and whiskers representing the minimal and maximal values excluding points greater than 1.5 times the interquartile range. Source data are provided as a Source Data file.

In addition to individual ASVs, we investigated diversity metrics as quantitative traits using Shannon index and principal coordinate analysis (PCoA) with Bray–Curtis dissimilarity. For each approach, we calculated diversity statistics first using all ASVs with a relative abundance greater than the effective samples size (Paulson et al., 2013), and second using the rhizosphere-enriched ASVs present in 50% or more of the RIL accessions. For the Shannon index, LOD thresholds for significance were determined by permutations to attain a genome-wide threshold of *P* 0.05 (LOD 3.27) and *P* 0.2 (LOD 2.63). Two QTLs were identified on chromosomes 1 and 3 (Supplementary Figures 1 and 2) using all, and prioritized, ASVs to calculate Shannon Diversity respectively. Of note, the QTL on chromosome 1 overlaps with the confidence interval of the *Cellvibrio* QTL highlighted later in the results section. For the PCoA, the first two components were mapped as quantitative traits. A LOD threshold for significance was determined by permutations to attain a genome-wide threshold of *P* 0.05 (LOD 3.41) and *P* 0.2 (LOD 2.71). A single QTL was identified on chromosome 6 in the same position as the QTL identified previously for *Streptomyces* ASV 5 (Supplementary Figure 3). Of further interest is that all diversity metric QTLs were negative relative to the reference. Thus, while genetic changes during domestication may have made some ASVs more or less abundant, these genetic changes also impacted overall diversity. Given the non-independence of sequencing-based microbiome features, we suggest caution in interpreting the results of using diversity metrics as microbiome features.

Effect size is an important factor when mapping the genetic architecture of quantitative traits. While some QTLs have large effect sizes, many small effect QTLs may explain a large proportion of trait variation (Lorenz & Cohen, 2012). To date, there is little understanding of the distribution of the effect sizes of QTLs for microbiome features. Here we show that the absolute values of the effect sizes of the 48 QTLs on ASV relative abundance ranged from 1.3 to 17%, with an average effect size of approximately 5%, comparable to the effects seen for SDW and RM (Figure 1c, e). The largest QTL effects were positive for an ASV in the genus *Qipengyuania* (17%), and an ASV in *Edaphobaculum* (10%). However, no statistical difference was found between the absolute value of positive and negative effect sizes ($p = 0.78$, two-tailed *t*-test). Furthermore, for those lineages with sufficient representation at the class level (Bacteroidia, Alphaproteobacteria, and Gammaproteobacteria), there was no statistically significant difference between effect size ($F(3, 16) = 0.072$, $p = 0.974$). However, an ANOVA on the positive effect size at genus level demonstrated significant differences between lineages ($F(3, 16) = 12.94$, $p = 1.15$ e–04). A post hoc Tukey test demonstrated QTLs for *Massilia* with a larger positive effect size than other lineages with sufficient sample size for comparison (Figure 3d). Collectively, our amplicon analysis provided a broad picture, suggesting that the assembly of bacteria in the tomato rhizosphere is a complex trait governed by a combination of multiple loci, some being ASV specific, some being pleiotropic for dif-

ferent ASVs, and with heterogenous effect sizes on ASV abundance (Figure 3d). While QTLs were identified with both positive and negative effects relative to the reference modern allele, the large number of positive effects suggests domestication impacted rhizosphere microbiome assembly.

Functional microbiome features as quantitative traits

To understand the functional traits associated with rhizosphere microbiome assembly, we generated shotgun metagenomes for the rhizosphere microbiome of each accession in the tomato RIL population (96 total), as well as six samples of the modern tomato parent, five samples of the wild tomato parent and seven bulk soil samples (BioProject ID PRJNA789467). After pre-processing, a co-assembly strategy using all metagenomes was implemented (see Supplementary Methods section 4.2.2 for more detail). Subsequently, bin and contig abundances were determined by read depth using CSS normalization, a computational method to adjust for compositional bias (Paulson et al., 2013). QTL mapping was conducted for the rhizosphere-enriched contig and bin abundances. A PCoA analysis of the contigs demonstrated separation between the bulk soil and RIL rhizosphere microbiomes (Supplementary Figure 9). Binning was done using Metabat2 (version 2:2.15) (Kang et al., 2015) and genomic quality of the output was evaluated by CheckM (Parks et al., 2015) (Supplementary Data 7). The bins and assembled contigs larger than 10 kb are publicly available (https://doi.org/10.5281/zenodo.6561541). All contigs of 10 kb and larger were taxonomically assigned using Kraken (Wood et al., 2019) (Supplementary Data 8). With nearly 40 million contigs being assembled, the effects of multiple testing were reduced by prioritizing rhizosphere-enriched contigs (relative to the bulk soil) which were larger than 10 kb and with an enrichment greater than 4-fold. After these stringent prioritization steps, 1249 contigs were remaining. The functional potential of these rhizosphere-enriched contigs represented 8.3% of protein clusters identified in all contigs greater than 10 kb by MMseqs2 using a 50% protein identity threshold (Steinegger & Söding, 2017). Approximately 25% of all proteins were contained within these clusters, suggesting that a considerable fraction of functional diversity was maintained during the prioritization. Only bins with greater than 90% completion and less than 5% contamination were mapped (33 out of 588 bins). As with the ASVs, harvest date, bulk soil mass, RM, number of leaves at harvest, and SDW were used as covariates in QTL mapping.

We identified 7 significant bin QTLs (LOD > 3.40, $P < 0.05$) (Supplementary Data 9) including *Streptomyces* bin 72 with a positive effect on tomato chromosomes 6 and 11. For the contigs, a total of 717 QTLs at 26 unique positions on tomato chromosomes 1, 4, 5, 6, 9, and 11 were identified (Supplementary Data 10), corresponding to 476 metagenomic contigs from 10 different genera (LOD > 3.47, $P < 0.05$). The largest number of contig QTLs were linked to the *Streptomyces*, *Cellvibrio,* and *Sphingopyxis*

lineages (Figure 4a). The *Streptomyces* contigs mapped to QTLs on tomato chromosomes 4 (46 contigs, negative), 6 (190 contigs, positive), and 11 (257 contigs, positive), with a subset of contigs mapping to two or all three of these positions (Figure 4b). These findings corroborate and expand upon the *Streptomyces* QTL identified on chromosome 6 using our 16S rRNA gene amplicon data, as well as that of the bin QTLs identified on chromosomes 6 and 11. The *Cellvibrio* contigs mapped to chromosome 1 (42 contigs, negative) and chromosome 9 (94 contigs, negative), again corroborating the findings from our 16S rRNA gene amplicon analysis described above. In contrast, the *Sphingopyxis* QTLs identified on chromosome 5 (24 contigs, negative) and 9 (49 contigs, positive) did not correspond to the QTLs identified on chromosomes 8 and 3 in the 16S rRNA gene amplicon analysis. Four contigs for *Devosia* also corroborated the results of the 16S QTL analysis. The effect sizes ranged from 9 to 21% and were significantly different ($F(14, 702) = 530.9$ $p < 2e{-}16$) between QTL and lineages (Figure 4c). As with the 16S rRNA amplicon analysis, some of the highest LOD scores were for *Devosia*. Also, the effect size of the *Sphingopyxis* contigs was large (±20% on average), above 15% for *Cellvibrio*, and approximately 10% for *Streptomyces*. The average QTL region was 51.59 Mbps for the 16S rRNA gene amplicon sequences and 26.64 Mbps for the metagenomic contigs (two-sided *t*-test, $p = 3.32E{-}09$) (Figure 4e). A more striking contrast was observed in the difference between the median size of amplicon and contig QTL regions which were 58.56 Mbp and only 6.47 Mbp, respectively. In summary, while many more taxa were identified in the amplicon-based QTL analysis, the metagenome-based QTL analysis provided QTLs with much smaller confidence intervals (Figure 4e).

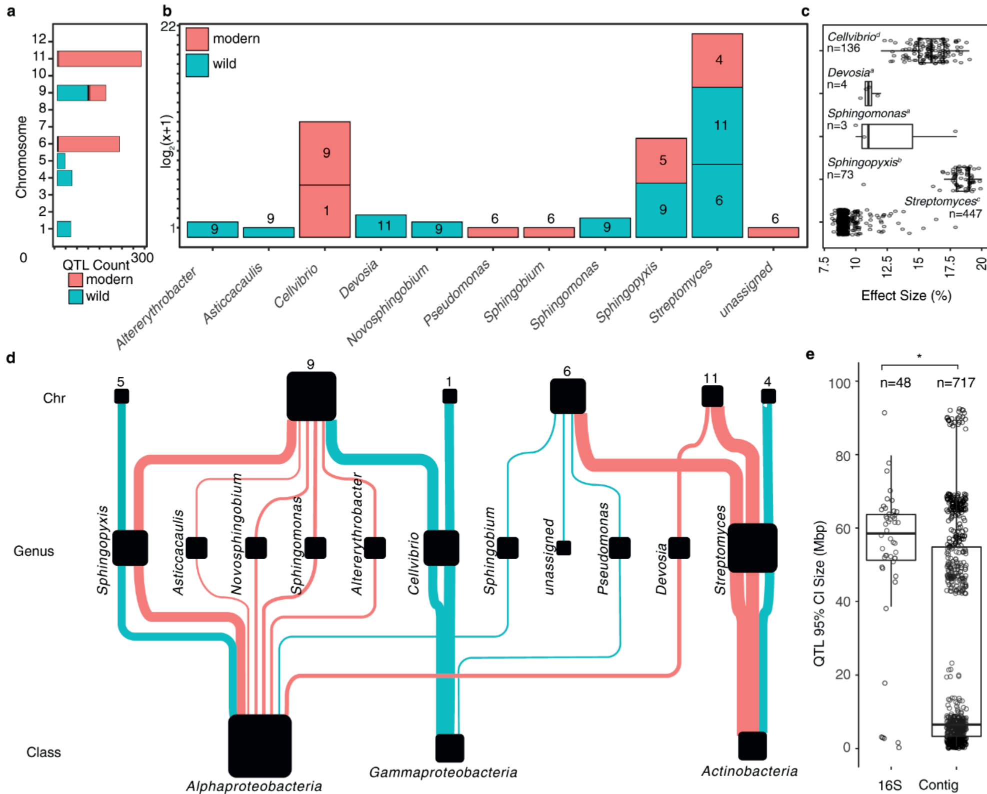

Figure 4. The contig QTLs. a A color coded summary of the number of contig QTLs identified per chromosome to wild and modern alleles. **b** A summary of the number of contig QTLs found by taxonomies, with the chromosome of each QTL represented within each square. The presence and absence of dark borders 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 contigs from each lineage *Cellvibrio* ($n = 136$), *Devosia* ($n = 4$), *Sphingomonas* ($n = 3$), *Sphingopyxis* ($n = 73$) and *Streptomyces* ($n = 447$) were significantly different as indicated by letters ($F(14, 702) = 530.9$ $p < 2e{-}16$). In addition to the scatter plot, data are presented with boxplots representing the median value, the interquartile range, and whiskers representing the minimal and maximal values excluding points greater than 1.5 times the interquartile range. **d** A hierarchically structured network depicting the contig rRNA QTLs identified in this study. From the top to bottom rows are the tomato chromosomes, which are linked to specific contigs, which are 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. In addition to the scatter plot, data are presented with boxplots representing the median value, the interquartile range, and whiskers representing the minimal and maximal values excluding points greater than 1.5 times the interquartile range. **e** When comparing the 95% confidence interval of 16S rRNA amplicon QTLs ($n = 48$) and contig QTLs ($n = 717$), the 95% confidence interval of contig QTLs was significantly smaller (two-sided *t*-test, $p = 3.32E{-}09$). In addition to the scatter plot, data are presented with boxplots representing the median value, the interquartile range, and whiskers representing the minimal and maximal values excluding points greater than 1.5 times the interquartile range. Source data are provided as a Source Data file.

Amplicon-based bulk segregant analysis of Streptomyces and Cellvibrio abundance

The two most abundant rhizosphere taxa with replicated patterns for amplicon and metagenome-based QTLs were *Streptomyces* and *Cellvibrio*. Therefore, we sought to provide additional independent support for these QTLs using a bulk segregant analysis of an independent population of parental and RIL genotypes (Supplementary Data 11). In particular, we tested the previously identified amplicon-based QTLs associated with higher *Cellvibrio* abundance at markers 464 and 3142 on chromosomes 1 and 9, respectively with higher *Streptomyces* abundance at marker 2274 on chromosome 6 (Figure 5). In each case, ANOVA showed a statistical difference between genotypes and bulk soil, respectively ($F(4, 396) = 21.56$, $p = 4.16$ e−16), ($F(4, 396) = 18.43$, $p = 6.68$ e−14), ($F(4, 396) = 8.423$, $p = 1.57$ e−06). A post hoc Tukey HSD test supported the conclusion that wild allele at markers 464 and 3142 on chromosomes 1 and 9, respectively, are indeed associated with increased abundance *Cellvibrio* ($p = 3.913$ e−04, and $p = 0.08$, respectively), while the modern allele at markers 2274 on chromosome 6 was significantly associated with increased abundance of *Streptomyces* ($p = 1.152$ e−04).

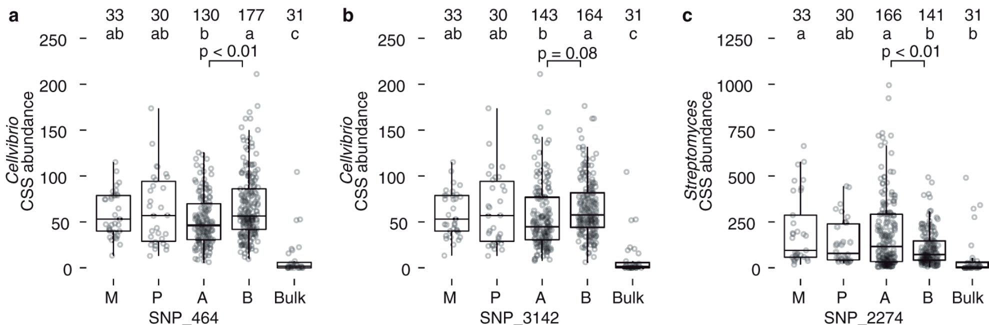

Figure 5. Validation of *Cellvibrio* and *Streptomyces* 16S rRNA QTLs with bulk segregant analysis. A total of 77 RIL accessions were grown with approximately four biological replicates per accession, as well as 33 modern, 30 wild and 31 bulk samples (see Supplementary Data 13). The number of replicates representing for each treatment is detailed in the top row of each panel. The number of replicates within the RIL population is 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. In addition to the scatter plot, data are presented with boxplots representing the median value, the interquartile range, and whiskers representing the minimal and maximal values excluding points greater than 1.5 times the interquartile range. **a** The CSS normalized abundances of *Cellvibrio* 16S rRNA in bulk soil (B), modern (M), wild (W), and RIL accessions at marker position 464 on chromosome 1. At this position, 32 and 45 RIL accessions with modern (A) and wild alleles (B) were used (130 and 177 samples with biological replication respectively). ANOVA showed a statistical difference between genotypes and bulk soil ($F(4, 396) = 21.56$, $p = 4.16$ e−16), A post hoc Tukey test supported the conclusion that wild allele at markers 464 associated with increased abundance *Cellvibrio* ($p = 3.913$ e−04). **b** Similarly, for marker 3142 on chromosome 9, there were a total of 35 and 42 RIL accessions with modern (A) and wild alleles (B), (143 and 164 samples with biological replication respectively). ANOVA showed a statistical difference between genotypes and bulk soil (F(4, 396)

= 18.43, $p = 6.68$ e−14), A post hoc Tukey HSD test supported the conclusion that wild allele at markers 464 associated with increased abundance *Cellvibrio* ($p = 0.08$). **c** The normalized CSS abundances of *Streptomyces* 16S rRNA and sequences in bulk soil (B), modern (M), wild (W), and RIL accessions at marker 2274 on chromosome 6. There was a total of 42 and 35 RIL accessions with modern (A) and wild alleles (B), (166 and 141 samples with biological replication respectively). ANOVA showed a statistical difference between genotypes and bulk soil (F(4, 396) = 8.423, $p = 1.57$ e−06), A post hoc Tukey HSD test supported the conclusion that wild allele at markers 464 associated with increased abundance *Streptomyces* ($p = 1.152$ e−04). Source data are provided as a Source Data file.

Host genetics and rhizosphere microbiome assembly

A subset of 5 regions consistent across both the amplicon and metagenome-based analyses were prioritized with an average size of 2.68 Mbps (Supplementary Data 12). These included positions on chromosome 1 (positions 87.36–90.49 Mbps), chromosome 9 (pos 62.03–63.32 Mbps), chromosome 5 (pos 61.54–63.38), chromosome 6 (pos 33.99–40.3 Mbps), and chromosome 11 (pos 53.06–53.89 Mbps). In total, 1359 genes were identified in these regions. Potential candidate genes with root-specific transcriptional patterns, defined as a 4 fold increase in the roots compared to leaf samples, were further prioritized using a publicly available RNA-seq dataset (The Tomato Genome Consortium, 2012). Based on this analysis, a subset of 192 root specific plant genes were identified (Supplementary Data 13). A total of 98 root specific plant genes were linked to *Streptomyces* on chromosome 6 (84 genes) and 11 (14 genes) (Figure 6). Intriguingly, 61 of these genes were found in regions previously identified to be subjected to selective sweeps, regions of fixed low genetic diversity, related to tomato domestication as well as to subsequent sweeps related to improvements in fruit quality (Lin et al., 2014) (Supplementary Figure 4). While it remains unclear whether the relationship between selective sweeps and changes in microbial feature abundance is causal or coincidental; here we reveal a genomic signature that the domestication process impacted alleles involved in microbiome assembly.

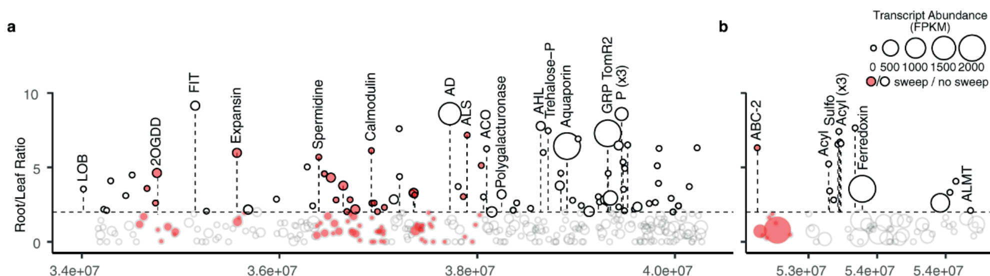

Figure 6. The prioritized regions of the *Streptomyces* QTL on chromosomes 6 and 11 overlaying previously reported data on transcript expression and genetic sweeps due to domestication. Within each region, the $\log_2$ ratio gene expression patterns from leaf and root materials were calculated and those with a $\log_2$ greater than 2, as delineated by the dotted line, were further prioritized. The $\log_2$ root transcript abundances (fragments per kilobase of exon per million mapped fragments, FPKM) are depicted by the size of the bubble. Previously reported genetic sweeps are indicated in red. **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 (×3). **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 (×4), Sulfo–Sulfotransferase, ALMT- Aluminum-activated malate transporter. Source data are provided as a Source Data file.

Two of the most salient genes in this list included genes with high transcription in the roots; an aquaporin and a Fer-like iron deficiency-induced transcription factor (FIT). The aquaporin (SlTIP2.3) has the highest fold change of all tonoplast intrinsic proteins in tomato roots as compared to all other organs (Steinegger & Söding, 2017; The Tomato Genome Consortium, 2012), while the FIT gene is a bHLH transcriptional regulator controlling iron homeostasis in tomato (Lin et al., 2014; Ling et al., 2002). Other genes within this region on chromosome 6 include a glycine rich protein, a receptor-like kinase known to be upregulated during drought (Morcillo, 2021), alcohol dehydrogenase, numerous phosphatases, expansins, ethylene-responsive transcription factors, gibberellin receptors, aminocyclopropane-1-carboxylate oxidase (ACO), an enzyme involved in the last step of ethylene biosynthesis, and finally, alpha-humulene and (-)-(E)-beta-caryophyllene, a known tomato terpene and signaling molecule in tomato (Kong et al., 2021; Zhou & Pichersky, 2020) and also acting as a volatile in microbiome assembly (Huang, 2012). Root specific genes involved in carbohydrate, protein, and amino metabolism were also identified, including trypsin-alpha amylase inhibitor, prolyl 4-hydroxylase, polygalacturonase, trehalose phosphatase, glycogenin, xyloglucan fucosyltransferase, and a metallocarboxypeptidase inhibitor, spermidine synthase, acetolactate synthases, alanine aminotransferase, and an amino acid permease. On chromosome 11, a ferrodoxin, an aluminum-activated malate transporter (Sweeney

et al., 2017), and a cluster of various acetyltransferases and a sulfotransferase were identified. An aluminum-activated malate transporter was also identified in the QTL region on chromosome 6, which has been linked to increased malate accumulation in both fruit and roots (Ye, 2017).

A total of 57 root specific genes were identified in the QTL regions on chromosome 1 and 9 linked to *Cellvibrio*. These include a cytochrome p450 involved in coumarin synthesis, numerous extensins, phosphatases, respiratory burst oxidase-like protein, iron chelator nicotianamine synthase (Nozoye, 2018; Safdarian et al., 2019), and on chromosome 11 phenazine biosynthesis. On chromosome 5, 37 root specific genes were identified including multiple peroxidases, glutamine synthetase, rhamnogalacturonate lyase, pectinesterase, metacaspase, and trehalose-phosphatase. Furthermore, numerous ethylene responsive transcription factors and receptor-like kinases were observed. The QTL on chromosome 1 contains genome-wide sweeps associated with the initial tomato domestication and subsequent improvements of fruit quality traits, suggesting that one or both of these events were connected to or act as a 'side effect' on the decreased abundance of *Cellvibrio* in the tomato rhizosphere.

Illuminating metagenomic traits in Cellvibrio and Streptomyces

To further investigate the potential functional importance of the 476 rhizosphere-enriched metagenomic contigs mapped as QTLs, we performed a deeper analysis into their functional gene content (Supplementary Data 14, 15, and 16). An antiSMASH (Blin, 2021) analysis identified 30 biosynthetic gene clusters (BGCs) across these contigs. These BGCs largely originated from contigs taxonomically assigned to *Cellvibrio* and *Streptomyces*. They included several gene clusters potentially associated with root colonization, such as two melanin BGCs (c00216, NODE_5919; c00255, NODE_7250) from *Streptomyces* (which have been positively associated with colonization (Chewning, 2019)) and a *Cellvibrio* aryl polyene BGC (c00185, NODE_4941), which is thought to protect bacteria against reactive oxygen species generated during immune responses of the host plant (Schöner, 2016). The contigs also contained gene clusters potentially beneficial to the host, such as BGCs encoding iron-scavenging siderophores, which have been associated with disease suppression in tomato (Gu, 2020); specifically, homologs of coelichelin and desferrioxamine BGCs from streptomycetes were found (c00269, NODE_7969, and c00122, NODE_3362), three IucA/IucC-like putative siderophore synthetase gene clusters (c00106, NODE_2973; c00041, NODE_1131; c00238, NODE_6661), as well as a *Cellvibrio* NRPS-PKS gene cluster (c00001, NODE_101) most likely encoding the production of a siderophore based on the presence of a TonB-dependent siderophore receptor-encoding gene as well as a putative *tauD*-like siderophore amino acid β-hydroxylase-encoding gene (Crits-Christoph et al., 2021). The *Cellvibrio* contigs also contain several genes relevant for carbohydrate

catabolism. For example, homologs of *xyl31a* (B2R_23365) and *bgl35a* (B2R_06825-06826) were detected (with 78%, 79 and 65% amino acid identity, respectively), genes that have been shown to be responsible for utilization of the abundant plant cell wall polysaccharide xyloglucan in *Cellvibrio japonicus* (Attia, 2018). In addition, a possible homolog of the β-glucosidase gene *bgl3D* (Nelson, 2017) (B2R_26663), involved in xyloglucan utilization, was also identified, having high similarity to *bgl3D* from *Cellvibrio japonicus* (64% amino acid identity). Also, putative cellulose-hydrolizing enzymes were detected, such as a homolog (B2R_21082) of the cellobiohydrolase *cel6A* from *Cellvibrio japonicus* (Gardner, 2014) encoded in a complex locus of nine carbohydrate-acting enzymes annotated on this contig (NODE_5090) by DBCAN (Yin, 2012) (Supplementary Data 14). Collectively, these results point to a possible role of microbial traits related to iron acquisition and metabolism of plant polysaccharides in tomato rhizosphere microbiome assembly.

Contigs of the metagenome-assembled genome (MAG) associated with *Streptomyces* ASV5 (the key taxon associated with tomato QTLs described above) contained a multitude of functional genes potentially relevant for host-microbe interactions. Taxonomically, the ASV5 MAG was most closely related to a clade of streptomycetes that includes type strains of species such as *arenae*, *flavovariabilis*, *variegatus*, and *chartreusis*. To understand how tomato might differentially recruit ASV5 streptomycetes, we analyzed the MAG for genes and gene clusters potentially involved in colonization. Intriguingly, we found contigs to be rich in genes associated with plant cell wall degradation. In particular, we identified a family 6 glycosyl hydrolases (B2R_10154) of which the glycosyl hydrolase domain has 84% amino acid identity to that of the SACTE_0237 protein that was recently shown to be essential for the high cellulolytic activity of *Streptomyces* sp. SirexAA-E (Wood et al., 2019). Additionally, we detected a homolog (82% amino acid identity) of *Streptomyces reticuli* avicelase, a well-studied cellulase enzyme that degrades cellulose into cellobiose (Schrempf & Walter, 1995) (B2R_29198). Larger gene clusters associated with degradation of plant cell wall materials were also found. These included an 8 kb gene cluster coding for multiple pectate lyases and pectinesterases (B2R_31553-31558), and an 8 kb gene cluster encoding a family 43 glycosyl hydrolase, a pectate lyase L, a rhamnogalacturonan acetylesterase RhgT, a GDSL-like lipase/acylhydrolase, a family 53 glycosyl hydrolase, and an endoglucanase A (B2R_15915-15920). Together, these findings suggest that ASV5 *Streptomyces* has the capacity to effectively process complex organic materials shed by plant roots during growth. These results are in line with a recent study on plant-associated streptomycetes that indicated that their colonization success appears to be associated with the ability to utilize complex organic material of plant roots (Worsley, 2021).

Root exudates also play a key role in the recruitment of microbes. Prominent sugar components of tomato root exudates are glucose, but also xylose and fructose (Kamilova, 2006). The *Streptomyces* MAG contains *xylA* and *xylB* genes (B2R_19014, B2R_19013) and a putative *xylFGH* import system (B2R_29274, B2R_23438, B2R_23439) facilitating xylose metabolism. Similarly, a *frcBCA* import system was identified in the genome (B2R_17966- B2R_17968) as well as a glucose permease (B2R_32780) with 91,5% amino acid identity to *glcP1* SCO5578 of *Streptomyces coelicolor* A3(2) (Bentley, 2002). Other genes putatively involved in root exudate catabolism were also found in the ASV5 MAG, such as sarcosine oxidase (*soxBAG*, B2R_20550-20551, and B2R_21105), which has been shown to be upregulated in the presence of root exudates of various plants (Chaparro, 2013; Matilla et al., 2007).

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

Genomic structure in Cellvibrio and Streptomyces provides insights into adaptations for differential recruitment

Bacterial populations often contain significant genomic heterogeneity. This heterogeneity may be associated with differential recruitment through altered nutrient preferences or host colonization mechanisms. The use of metagenomics enabled us to investigate the population structure within each rhizobacterial lineage and identify intraspecific differences. To do so, we first identified a unique set of 697,731 microbiome Single Nucleotide Variants (SNVs) in a subset of parental and bulk metagenomes using InStrain (Olm, 2021). A set of 15,026 SNVs enriched in either the wild or modern tomato rhizosphere were selected and the abundance of each allele at each SNV was calculated. Using these abundances, QTL mapping was performed using R/qtl2 as described in the methods. A total of 3,357 QTL peaks were identified (LOD > 3.01, $P < 0.05$), to 1229 independent loci. A total of 1354 QTL with positive effects and 2,001 QTL with negative effects were identified, derived from 2,898 unique SNVs, and corresponding to 810 and 1068 unique rhizobacterial genes respectively (Supplementary Data 17).

We investigated the 103 *Streptomyces* SNV QTLs at 94 unique positions within annotated genes whose mapping coincided with the previously identified QTLs for

Streptomyces contigs to tomato chromosomes 4, 6, and 11 (Supplementary Data 17). Numerous *Streptomyces* SNVs were associated positively with the reference tomato alleles on chromosomes 6 and 11. In particular, alpha-galactosidase (B2R_16136) and arabinose import (B2R_29105) had the highest LOD and smallest overlapping confidence intervals with chromosomes 6 and 11 (Figure 7). Indeed, many SNVs in genes involved in the degradation of xylan (Polizeli, 2005), one of the most dominant non-cellulosic polysaccharides in plant cell-walls (Mellerowicz & Gorshkova, 2012), as well as carbohydrate and protein metabolism were associated positively to QTL on chromosomes 6 and 11, including xyloglucanase Xgh74A (B2R_10589), alpha-xylosidase (B2R_23763), endo-1,4-beta-xylanase (B2R_20609), extracellular exo-alpha-L-arabinofuranosidase (B2R_20608), multiple protease HtpX (B2R_19218), cutinase (B2R_19356), and putative ABC transporter substrate-binding protein YesO (B2R_09821) which has been implicated in the transport of plant cell wall pectin-derived oligosaccharides (Sugiura, 2020). A *Streptomyces* SNV in acetolactate synthase (B2R_28001) was associated positively to QTL on tomato chromosome 6 where a plant acetolactate synthase was located. Similarly, multiple SNVs in *Streptomyces* genes involved in putrescine transportation (B2R_25489) were associated positively to QTL on tomato chromosomes 6 and 11, which contain genes for spermine synthase, suggesting a possible metabolic cross-feeding from plant to microbe. A majority of these SNVs were synonymous having no effect on the produced amino acid sequence. However, some were non-synonymous, resulting in an altered amino acid sequence, including the histidine decarboxylase SNV (B2R_16511) mapping to both tomato chromosomes 6 and 11 (Figure 7). *Streptomyces* SNVs that were associated negatively with the QTL on tomato chromosome 4 included an antibiotic resistance gene (daunorubicin/doxorubicin, B2R_28992) and maltooligosyl trehalose synthase (B2R_07820) among others.

Figure 7. The SNP QTLs identified in the *Streptomyces* contigs mapping to the previously identified positions on chromosomes 4, 6, and 11. The figure depicts various features of both the QTL analysis and the SNP. In particular, the edge sizes are relative to the LOD score, and edge color is coded by modern and wild. SNPs are represented by square nodes. Those with confidence intervals <10 Mbp are shaded in dark. Non-synonymous SNPs have a thick border edge. Annotations are provided next to the genes. Source data are provided as a Source Data file.

Similarly, we investigated the 324 *Cellvibrio* SNV QTLs within annotated genes whose mapping coincided with the previously identified *Cellvibrio* contig QTLs to chromosomes 1 and 9. Again, numerous SNV QTLs were identified in genes were related to sugar catabolism, including a gene encoding an extracellular exo-alpha-(1->5)-L-arabinofuranosidase (B2R_16093), fructose import FruK (B2R_22268), a cellulase/esterase-encoding *celE* homolog (B2R_11067), and genes involved in malate (B2R_18213), mannonate (B2R_14081), xyloglucan (B2R_10668) and xylulose (B2R_22179) metabolism. Furthermore, many additional SNV QTL were identified in genes related to vitamin and cofactor metabolism as well as sulfur and iron metabolism. In particular, these included genes for a phosphoadenosine phosphosulfate reductase (B2R_15720), vitamin B12 transporter BtuB (10 different genes, see Supplementary Data 17), a siroheme synthase (B2R_24033), a pyridoxal phosphate homeostasis protein (B2R_17481), a heme chaperone HemW (B2R_12751), a hemin

transport system permease protein HmuU (B2R_09175), a Fe(2+) transporter FeoB (B2R_19968), a biotin synthase (B2R_30007), a catecholate siderophore receptor Fiu (B2R_17486), and a Fe(3+) dicitrate transport ATP-binding protein Fec (B2R_09176) (Supplementary Data 17). Taken together, this analysis suggests that a shotgun metagenomic approach integrated with quantitative plant genetics can be instrumental in a high-throughput manner to discover putative reciprocal genetic links between plant and microbial metabolisms, such as those identified here for polysaccharides, trehalose, iron, vitamin, amino acid, and polyamine metabolism.

Discussion

Breeding for microbiome-assisted crops is a daunting task, encompassing ecological, evolutionary, and cultural processes. What constitutes a desirable trait for selection is context-dependent and differs between societies, crops, and locations (Meyer et al., 2012). As society grapples with modern challenges such as a rapidly changing environment, water scarcity and land degradation, it is becoming increasingly clear that a new era of trait selection is needed with increased focus on sustainability and microbiome interactions (Beilsmith, 2019; Busby, 2017; Gopal & Gupta, 2016; Wille et al., 2019). In this regard, it is also time to reckon with the consequences of historic yield-centric trait selection and accompanying genomic sweeps (Lin et al., 2014), especially with regards to plant–microbe interactions (Figure 8a, b). Current approaches to investigating the genomic architecture determining microbiome assembly rely primarily on mutational studies in known genes and pathways. More recently, studies leveraging the natural variation within plant populations have been used to conduct GWA and QTL of the leaf (Horton, 2014; Wallace et al., 2018) and rhizosphere (Deng, 2021). To date, the microbiome has been primarily characterized through amplicon sequencing, thereby providing limited functional resolution of microbiome structure. Increasing the resolution of phenotyping of quantitative traits has been shown to improve the precision and detection of QTLs (Sideli, 2020). Thus, integrating microbial genomics into microbiome QTL analysis plays a dual purpose; increasing the ecological resolution with which microbial traits may be mapped (e.g., at a community and population level, Figure 8c), and second, affording the identification of the reciprocal microbial adaptations that drive plant–microbe interactions (e.g., by using SNVs a microbiome features). In this investigation, we addressed these challenges by integrating amplicon and shotgun metagenome sequencing to identify microbiome QTLs for the tomato rhizosphere.

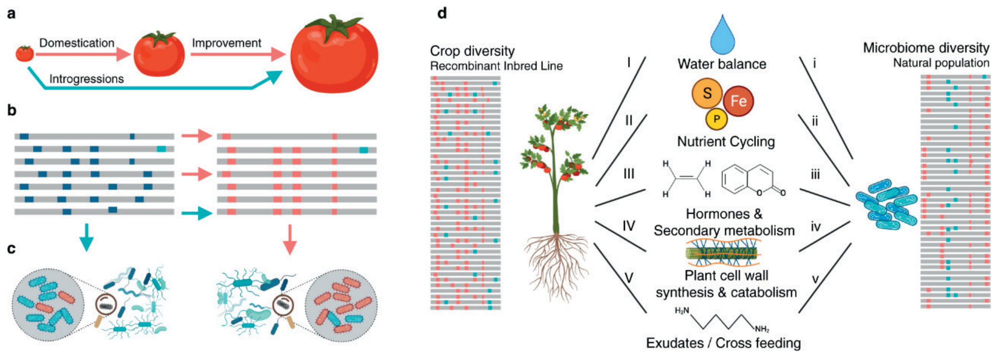

Figure 8. Disentangling the genetic basis of rhizosphere microbiome assembly. a The initial domestication, subsequent crop improvements, and introgression wild tomato traits to modern cultivars. **b** While domestication significantly decreased the allelic diversity of modern tomato cultivars, introgressions of allelic diversity from wild relatives has left a genomic signature. **c** Here we identify QTLs associated with changes in microbiome composition at both the community level, but also within individual populations (e.g., *Streptomyces* and *Cellvibrio*). We show that these QTL overlapping previously identified selective sweeps associated with domestication. **d** By identifying plant QTLs regions using population features of the microbiome (SNVs), it is possible to identify the reciprocal functional adaptations that may link plant and microbe (represented by capital and lower-case letters respectively). These functions may interact directly, or indirectly via the environment. For example, related to water balance (I, i), we identified plant aquaporin and both plant/microbe trehalose metabolism. Selection for altered host water usage may alter the water balance in the soil and associated repercussions on microbiome structure. Similarly, numerous plant and microbial genes related to nutrient cycling (II, ii) involving iron, sulfur, vitamin, and phosphorus acquisition were identified. Plant signaling and hormone genes (III) identified in QTL regions included 1-aminocyclopropane-1-carboxylic acid oxidase, alpha-humulene/beta-caryophyllene synthase, and a p450 involved to coumarin synthesis. Furthermore, plant cell wall metabolism (IV, iv) including expansins, extensins, pectinesterase were linked to microbial genes involved in plant cell wall plant polysaccharides catabolism, cellobiohydrolase glycosyl hydrolases, xylose, sarcosine oxidase, L-arabinofuranosidase, fructose import, a cellulase/esterase, and xyloglucan metabolism. Finally, genes related to exudation and possible cross feeding (V, v) included plant genes such as aluminum-activated malate transporter, polyamine, glutamine, and acetolactate synthetase, and microbial functions related to malate, mannonate, polyamine, and acetolactate metabolism. Created with BioRender.com.

One major difference between the amplicon and contig QTL analysis is the number of lineages for which QTLs were identified. Amplicon-based sequencing, which captures more rare taxa per unit sequencing, provided a broader taxonomic picture and was able to capture QTLs of both abundant and relatively rare rhizobacterial lineages. In contrast, the majority of contig QTLs mapped to the most predominant lineages yet failed to identify QTLs for more rare lineages. Nevertheless, besides the fact that the shotgun-based approach provided functional insights into the associated bacterial taxa, the size of the 95% confidence interval of the QTL region was significantly smaller using contig QTLs, with a median size of just 6.47 Mbp compared to 58.56 Mbp for the amplicon-based QTL regions. Furthermore, for *Streptomyces*, the number of unique

QTLs identified was greater in the contig-based approach. Thus, we identified a trade-off between amplicon and shotgun-based technologies, whereby amplicon sequencing provides a deeper view into broad community structure, whereas shotgun-based approaches provided a more nuanced picture. In particular, the smaller regions identified by our contig-based metagenome mapping provided considerably more functional insights as it enabled us to analyze the genomic content contained in the regions linked to *Cellvibrio* and *Streptomyces*. It is possible that less stringent prioritization steps could be used to increase the number of metagenomic features identified, but this may also increase the false discovery rate. It should be noted that a limitation of the approaches taken is that both amplicon and shotgun-based approaches produce non-independent measurements. Here we use CSS normalization, one of the top performing computational approaches to address compositional bias (Lloréns-Rico et al., 2021). Nevertheless, future approaches that provide community level absolute ASV abundances will further minimize compositionality of the microbiome data and likely perform better when mapping microbiome features as QTLs. Extending these studies to the endophytic compartment and including metatranscriptome analyses may also further improve the identification of microbiome features, provided that the endophytic microbiome can be separated well from the plant cells to obtain sufficient sequencing depth.

The increased QTL mapping resolution provided by shotgun-based phenotyping of the microbiome combined with SNV analysis provided an approach to leverage both the host diversity of the RIL and the natural microbiome population diversity to disentangle the reciprocal genomic adaptions between plants and natural microbiomes (Figure 8d). For example, understanding the forces driving the abundances of rhizospheric *Streptomyces* is of increasing interest and has been linked to both iron (Xu, 2021) and water limitations (Worsley, 2021). Here, we pinpointed the genetic basis for these interactions among the short list of highly expressed root-specific tomato genes linked positively to *Streptomyces* abundance including both aquaporin and FIT. More specifically, the aquaporin (SlTIP2.3) has the highest fold change of all tonoplast intrinsic proteins in the tomato genome in the roots when compared to all other organs (Reuscher, 2013; Sade, 2009), while the FIT gene has been shown to largely control iron homeostasis in tomato (Ling et al., 2002; Schwarz & Bauer, 2020). Future experiments will focus on functional validation by, among others, transcriptome analyses and site-directed mutagenesis of the microbial and plant genes identified.

In addition to these high priority genes, many other key genes were identified in these regions. Those previously shown to contribute to microbiome assembly included 1-aminocyclopropane-1-carboxylate oxidase, which plays a central role in plant regulation of various processes including bacterial colonization and root elongation (Nascimento et al., 2018) and alpha-humulene/(-)-(E)-beta-caryophyllene synthase, a terpene

known to modify microbiome structure (Huang, 2012). In addition, numerous genes related to growth, development, and cell wall loosening (Cosgrove, 2016) known to be involved in microbial colonization (Cosgrove, 2017) and aluminum-activated malate transporter, which has been linked to microbiome-mediated abiotic stress tolerance (Sweeney et al., 2017) and selected during tomato domestication resulting in high malate content in both fruit and roots (Ye, 2017). Both low-malate and high-malate haplotypes have been identified in tomato (Ye, 2017), which may form the basis of future studies investigating the role of malate exudation in microbiome assembly.

The historic impact of domestication on genomic regions linked to microbiome assembly is also apparent (Figure 6, Supplementary Data 14, and Supplementary Figure 4). However, the processes and consequences of these sweeps, and possible subsequent recombination events on microbiome assembly remain unclear. In particular, the discontinuity of sweeps in microbiome QTL regions suggests that evolutionary pressure for recombination of key (microbiome associated) traits, such as iron homeostasis and water transport, may have acted against selective sweeps. The approach developed here provides the means to illuminate such complex eco-evolutionary questions, forming the basis of integrating the microbiome into the classic genotype by environment model of host phenotype (Oyserman et al., 2021).

From the microbial perspective, the increased resolution in QTL analysis afforded by our shotgun-based approach also provided a window into the host-specific bacterial adaptations to wild and modern alleles. In particular, the SNV QTL analysis demonstrated that genes related to the degradation of various plant-associated polysaccharides in *Streptomyces* were associated positively with the modern reference allele. Many other functions were identified in both plant and microbe, such as trehalose metabolism, polyamine metabolism, and acetolactate synthase, suggesting either a direct link through cross-feeding (Smith et al., 2019) or signaling (Lunn et al., 2014), or perhaps shared ecological pressures. While the microbial adaptations related to polysaccharides (Beauregard et al., 2013), vitamins (Streit, 1996) and iron metabolism (Gu, 2020; Xu, 2021) are well documented in relation to plant colonization, here we demonstrate that the reciprocal adaptations that drive plant–microbe interactions can be investigated simultaneously to uncover their genetic architecture in both host and microbiome (Figure 8d). From a societal context, linking quantitative genetics with community level microbiome data provides us a tool to understand the complex genotype, environment, microbiome, and management interactions that shape our agroecosystems structure and function. Armed with these tools and molecular insights, we can begin to re-envision the agroecosystem; targeting QTLs for improved plant–microbe interactions, identifying ‘missing microbes’ or functions lost during the domestication process, or pinpointing the molecules that drive these interactions.

Materials and Methods

An F8 RIL population derived from the parental lines *Solanum lycopersicum* cv. Moneymaker (modern) and *Solanum pimpinellifolium* L. accession CGN14498 (wild) consisting of 100 lines were used for this study (Voorrips et al., 2000). A high density map produced from this population was used to map QTLs (Sterken et al., 2023).

Growth conditions for RIL

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

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

Harvesting and processing of plant materials

All plants had between 5 and 7 true leaves at harvest (Supplementary Data 1). Plants were gently removed from the pot and roots and were vigorously shaken. Soil that remained attached to the roots after this stage was considered the rhizosphere. The

remaining bulk soil and rhizosphere (plus roots) fractions were weighed. The root and attached rhizosphere fraction were treated with 4 mL of lifeguard, vortexed, and sonicated. Roots were then removed. The remaining rhizosphere sample was then stored in LifeGuard Soil Preservation Solution (Qiagen) at −20 °C until DNA extraction.

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

rRNA amplicon sequence processing

All DNA was sent to BaseClear (Leiden, The Netherlands) for 16S rRNA gene 300 bp paired-end amplicon sequencing (MiSeq platform). MiSeq primers targeted the V3-V4 region of Bacteria: 341FCCTACGGGNGGCWGCAG, 805RGACTACHVGGG-TATCTAATCC. In total, 20,542,135 16S rRNA gene amplicon read pairs over 225 samples were generated. The raw reads were processed using the DADA2 workflow (v1.14.1) to produce amplicon sequence variants (ASV) and to assign taxonomy based on the Silva database version 138 (Callahan et al., 2016; Quast et al., 2013) (Supplementary Data 2). ASVs tagged as non-bacterial, chloroplast, or mitochondria were removed. Next, ASV counts were normalized using the cumulative sum scaling (CSS) (Supplementary Data 3), which has been shown to be one of the most effective computational transformation techniques (Lloréns-Rico et al., 2021), and filtered based on the effective sample size using the metagenomeSeq package (v1.28.2) (Paulson et al., 2013). Differential abundances between rhizosphere and bulk soil were determined using the eBayes function from the limma package. Enriched rhizosphere ASVs with a greater than log(2) fold change in abundance were analyzed based on their presence and absence, standard deviation and mean values. Using these statistics, stochastic ASVs (<50% of samples) were removed from further analysis (Supplementary Data 4). All ASV sequences may be found in Supplementary Data 5. The remaining microbiome features were then mapped as QTLs as described subsequently. To investigate diversity metrics as quantitative traits, the Shannon diversity of each sample was calculated using all ASV after filtering based on the effective sample size using the metagenomeSeq

package (v1.28.2) (Paulson et al., 2013), and using all ASV in greater than 50% of samples (Supplementary Data 21). Similarly, a PCoA analysis using Bray Curtis distances was conducted, and the values for principal components axis 1 and 2 were extracted (Supplementary Data 22). Both calculations were done in phyloseq version 1.34.0 (McMurdie & Holmes, 2013). These diversity-based microbiome features were then mapped as QTLs as described subsequently.

Metagenomics analysis

For the one set of replicates for each accession, paired-end sequence read libraries were generated in the length of 150 bp per read on NovaSeq paired-end platform by Base-Clear B.V. Demultiplexing was performed before the following analysis. It is computationally expensive to assemble the 114 read libraries all at once. Therefore, a strategy of (merging) partial assemblies was undertaken. Two assemblers were used to create the assembled contigs, namely SPAdes (version 3.13.2) (Bankevich, 2012) and MEGAHIT (version 1.2.9) (Li, 2016). Assembly quality was assessed by running MultiQC (version 1.8) (Ewels et al., 2016) with Quast Module (Mikheenko et al., 2018) (Supplementary Figure 5). First, 6 modern parents, 5 wild parents, and 1 bulk soil sample were co-assembled via SPAdes with the metagenomic mode and parameter of -k 21,33,55,99, generating the first assembly (A1). Subsequently, a second assembly (A2) was done using the unmapped reads from the remaining metagenomes using MEGAHIT with the parameter of --k-list 27,33,55,77,99. The third assembly (A3) was performed similarly as A2, however, included the unmapped reads, ambiguously mapped reads, and mapped reads with a low mapping quality score (MapQ < 20) (Supplementary Data 18). Read mapping was done with BWA-MEM with default settings (H. Li, 2013) and SAMtools was used to convert the resulting SAM files into sorted and indexed BAM files (version 1.10). Extraction of these reads was conducted by samtools bam2fq. Redundancy between assemblies was evaluated by alignment to A1 via nucmer package of MUMmer with --maxmatch option (version:4.0.0) (Marçais, 2018).

Firstly, 111.5 Gbp of reads from the parental samples were assembled, labeled as A1, and yielded a total assembly length of 8.6 Gbp with the largest contig of 933.0 kilobase pairs (Kbp). After aligning the reads from RIL samples to A1, unmapped reads, ambiguously mapped reads, and mapped reads with a low mapping quality score (MapQ < 20) were retrieved and assembled, yielding the second and third assembly (A2 and A3). Specifically, A2 stemmed from solely the unmapped reads while A3 included the ambiguously mapped reads and mapped reads with MapQ < 20 in addition to the unmapped reads. A2 and A3 produced a total assembly length of 9.6 Gbp and 14.0 Gbp, with the largest contig of 56.2 and 86.3 Kbp respectively. There were 1.2, 2.0, and 2.8 million contigs with the length over 1 Kb for A1, A2, and A3 respectively. In particular, 912 contigs in A1 were greater or equal to 50 Kbp whereas 1 or 2 such

large contigs were successfully assembled in A2 or A3. The detailed assembly statistics is given in Supplementary Data 18 and the numbers of contigs with different ranges of length for each assembly are presented in Supplementary Figure 5.

The sequence similarities of the contigs in each assembly (≥1 Kbp) were compared using the nucmer package in MUMer. No contigs in A2 were reported to share an overlapped region with A1, therefore contigs in A1 and A2 could be merged directly. When A3 was aligned to A1, 1.1% of the total length (≥1 Kbp) of A3 was reported to be overlapped with A1, however, only 18 contigs from A3 were 100% identical to regions in larger contigs in A1. The sensitivity of filtering the overlapping contigs was evaluated by a benchmarking test using a random RIL sample to calculate the mapping rates (Supplementary Figure 6). 83.4% reads were mapped to A1 + A3 at MapQ ≥ 20 without filtering. Excluding the contigs from A3 that were completely and identically covered by A1, the mapping rate was nearly the same as the one without filtering. Nevertheless, the removal of all aligned contigs in A3 resulted in a slight drop of mapping rate to 82.6%. To conclude, the final assembly was determined as A1 + A3 with the 18 redundant contigs from A3 removed.

To assess the overall assembly quality and quantify the abundance of contigs among all samples, metagenomic reads were mapped to A1, A1 + A2, and A1 + A3 (deduplicated) respectively. Afterwards, the mapping rates were calculated for the mapped reads with MapQ > 20 in each sample. As shown in Supplementary Figure 7, approximately 70% reads among rhizosphere samples could be mapped to A1, while the mapping rates were 55 to 65% in the bulk soil samples. With the unmapped reads assembled and added to A1, the mapping rates for A1 + A2 increased by 10%. The read recruitment was further improved by assembling and adding ambiguously mapped reads and mapped reads with low MapQ in the final assembly (A1 + A3). A1, as well as de-replicated A3, were merged to acquire the final assembly. All the 'contigs' mentioned below are referring to the contigs in this final assembly.

Binning of metagenomic contigs

Metabat2 (version 2:2.15) (Kang et al., 2019) was used for assigning the contigs into genomic bins. Based on tetra-nucleotide frequency and abundance scores, 588 genomic bins were generated. Afterwards, genomic quality of those genomes was evaluated by CheckM (version: 1.1.1) (Parks et al., 2015) with the command "checkm linage_wf" (Supplementary Data 8). The 33 genomes displaying the completeness larger than 90% and contamination smaller than 5% were used for further study as quantitative traits.

Making phenotype files based on contig depth

Read counts for each position on the assembled contigs were acquired using bedtools genomecov (version: 2.29.2) (Quinlan & Hall, 2010). A custom Python script was applied to calculate the average depth (defined as the number of total mapped reads divided by contig length) and coverage (defined as the number of covered base pairs divided by contig length) of every contig. Furthermore, the average abundance of contigs assigned into a bin was calculated for the high-quality genomic bins detected by CheckM (Parks et al., 2015).

Feature selection

Average depths of the contigs were first normalized using the CSS and filtered based on the effective sample size using metagenomeSeq package (v1.28.2) (Paulson et al., 2013). Differential abundance analysis was performed by moderated *t*-tests between groups using the makeContrasts and eBayes commands retrieved from the R package Limma (v.3.22.7) (Ritchie, 2015). Obtained *P*-values were adjusted using the Benjamini–Hochberg correction method. Differences in the abundance of contigs between groups were considered significant when adjusted *P*-values were lower than 0.01 (Supplementary Data 19).

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

Taxonomic and functional annotation of the metagenome

Taxonomic classifications were assigned to the contigs in the final assembly using Kraken2 (version: 2.0.8) (Wood et al., 2019) based on exact *k*-mer matches. A custom Kraken2 database was built to contain *RefSeq* complete genomes/proteins of archaea, bacteria, viral, fungi, and protozoa. Univec_Core was also included in the custom database (20200308). Using the Kraken2 standard output, a python script based on TaxonKit (Shen & Ren, 2021) was utilized to add full taxonomic names to each contig in the format of tab-delimited table. 76.22% of the contigs > 1 kb were classified. Among the contigs >10 kb, up to 99.44% contigs were classified. Prokaryotic microbial genes were predicted by Prodigal (version: 2.6.3) (Hyatt et al., 2010) with metagenom-

ics mode. 10,246,55 genes were predicted from contigs > 1 kb. Open reading frames (ORFs) on contigs >10 kb were annotated by prokka (v1.14.5) and the *Streptomyces* ASV5 bin (MAG.72) was further annotated by DRAM (v1.2.0) integrating UniRef, Pfam, dbCAN and KEGG databases (Shaffer, 2020). To assess the impact of the prioritization on the functional representation of the metagenome, we identified the fraction of protein clusters represented in the rhizosphere-enriched contigs compared to the rest of the contigs greater than 10 kb. First, Prodigal was used in metagenomics mode to predict genes in the metagenomic assembly with contigs longer than 10 kbp. Next, MMSeqs2 was used to cluster the protein sequences based on 70% similarity and based on 50% similarity, and with or without partial predicted genes (Steinegger & Söding, 2017). To calculate the number of clusters that contained proteins encoded in rhizosphere-enriched contigs, the clusters were searched for the presence of protein IDs of the 1249 rhizosphere-enriched contigs. In total, approximately 8.3% of protein clusters contained genes from the rhizosphere-enriched contigs. In addition to proteins contained on rhizosphere-enriched contigs, these clusters contained approximately 25% of all proteins encoded in contigs larger than 10 kb (Supplementary Data 20).

Single nucleotide variant analysis

To investigate strain level QTLs, we mapped single nucleotide variants (SNVs) identified using inStrain on the 1249 rhizosphere-enriched contigs. A total of 555, 382, and 535,432 SNVs were identified in the modern and wild parental metagenomes respectively. Of these, 162,299 and 142,349 SNVs were unique to each dataset respectively, as they either contained only reference alleles or did not exceed the inStrain SNV calling thresholds. For each unique SNV locus, coverage in the other dataset was determined using SAMtools depth after read filtering with settings comparable to inStrain and was considered identical to the reference allele frequency. Including the unique SNVs, this resulted in a final set of 697,731 SNVs. To select SNVs that showed differential reference allele frequencies between MM and P, first the difference in reference allele frequency (MM–P) was calculated per SNV. From the distribution of all SNVs, the 95% confidence interval (CI) was determined to select the 5% (30,911) most different SNVs (Supplementary Figure 8). SNVs were further selected using a Fisher's exact test based on the allele read count differences between MM and P. *P*-values were sorted, and a final selection of 15,026 differentially abundant SNVs distributed over 1037 contigs was obtained using a Benjamini-Hochberg false discovery rate (FDR) correction of 0.01. SNV allele read counts were extracted from the RIL dataset using the pysam Python package after filtering with settings comparable to inStrain.

Quantitative trait locus analysis

The QTL analysis linking selected amplicon, contig, bin, and SNV features with plant loci was performed using the R package R/qtl2 (Broman, 2019). Pseudomarkers were

added to the genetic map to increase resolution, with a step distance of 1 Mbp between the markers and pseudomarkers. Plant genome probabilities were calculated using the genetic map with pseudomarkers, plant loci cross data, and error probability of 1E-4. Plant locus kinship matrix was calculated as proportion of shared alleles using conditional allele probabilities of all plant chromosomes, which were calculated from the plant genome probabilities. A genome scan using a single-QTL model using a linear mixed model was performed on the SNV allele read counts as phenotypes, plant genotype probabilities as input variables and as covariates the number of leaves, harvest day, rhizosphere soil weight (g), soil starting weight (g) and plant dry weight (g). The LOD score was determined for each plant locus SNV allele combination. A permutation test using randomized data was performed with 1000 permutations to assess the distribution of the LOD scores. The 95% quantile was used as threshold for the selection of LOD peaks, as well as a $P = 0.95$ Bayes credible interval probability.

Independent validation of QTLs through bulk segregant analysis

To validate the QTLs, 33 *Solanum lycopersicum* cv. Moneymaker (modern), 30 *Solanum pimpinellifolium* L. accession CGN14498, and 77 RIL accessions (with replicates of 4 each) were grown and their microbiomes characterized through 16S rRNA gene amplicon sequencing. Parental lines and RIL accessions were germinated in pots filled with 300 g agricultural soil. For each accession, were planted with six plants per replicate pot. The plants were arranged randomly in the growth chamber (25 °C, 16 h daylight) and watered every day. Bulk soil samples without plants were used as controls ($N = 31$).

Rhizospheric soil was collected according to standard methods (Lundberg et al., 2012). In order to synchronize the developmental stage, the plants were harvested after 21 days, or when the 3rd trifoliate leaf was reached. The soil loosely attached to the roots was removed and the entire root system was transferred to a 15 mL tube containing 5 mL LifeGuard Soil Preservation Solution (MoBio Laboratories). The tubes were vigorously vortexed and sonicated. Subsequently, the roots were removed and at least 1 g (wet weight) of rhizospheric soil was recovered per sample for DNA extraction. For the bulk soil samples, approximately 1 g of soil was collected and mixed with 5 mL of LifeGuard solution.

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

The extracted samples were used for amplification and sequencing of the 16S rRNA gene, targeting the variable V3–V4 (Forward Primer: 5′-CCTACGGGNGGCWGCAG-3′ Reverse Primer: 5′-GACTACHVGGGTATACTAATCC-3′) resulting in amplicons of approximately ~460 bp. Dual indices and Illumina sequencing adapters using the Nextera XT Index Kit were attached to the V3–V4 amplicons. Subsequently, library quantification, normalization, and pooling were performed and MiSeq v3 reagent kits were used to finally load the samples for MiSeq sequencing. For more info please refer to the guidelines of Illumina MiSeq System. The RDP extension to PANDASeq (Masella et al., 2012), named Assembler (Cole, 2014), was used to merge paired-end reads with a minimum overlap of 10 bp and at least a Phred score of 25. Primer sequences were removed from the per sample FASTQ files using Flexbar version 2.5 (Dodt et al., 2012). Reads were processed as before except the Silva version 132 was used for taxonomic classification (Quast et al., 2013).

Figure 9 summarizes the methodological workflow followed in this chapter to investigate the genetic basis of tomato rhizosphere microbiome assembly:

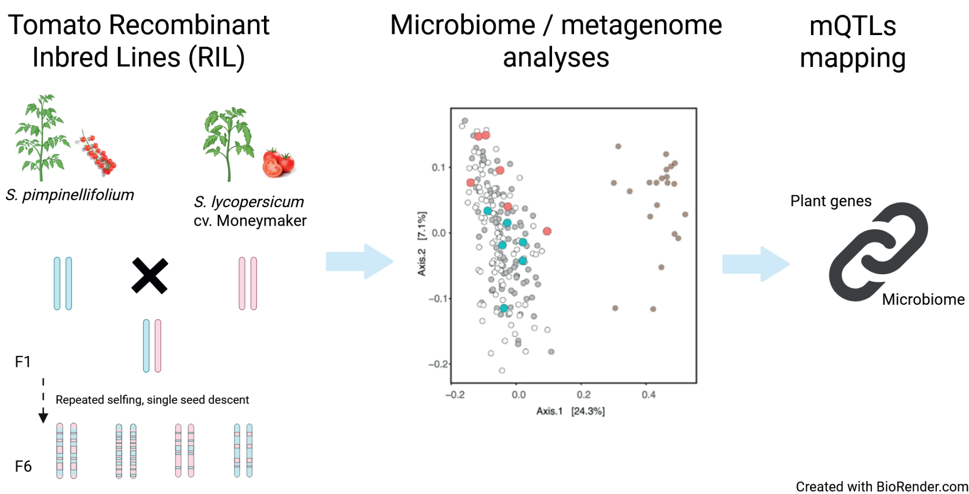

Figure 9. Methodological workflow for identifying plant QTLs of rhizosphere microbiome composition. Recombinant inbred lines (RILs) derived from a cross between *S. pimpinellifolium* and *S. lycopersicum* cv. Moneymaker were used to study tomato genetic effects on rhizosphere microbiome assembly. Taxonomic and metagenome analyses were performed to characterize microbiome composition and functional profiles. Microbiome quantitative trait loci (mQTL) identified association between plant genomic regions and shifts in microbiome features, linking tomato genetic regions with specific microbial taxa and functions.

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Author contributions

The study was conceived and designed by B.O.O., V.J.C., W.Li, M.H.M., and J.M.R. The greenhouse experimentation and lab work were conducted by B.O.O., S.S.F., V.C., V.J.C., and A.N. Contributions to data analysis came from B.O.O., T.G., X.P., E.v.d.W., W.Lo, L.P., N.S., A.K., V.C., V.J.C., B.L.S., M.H.M., J.N.P., and M.M. The manuscript was drafted by B.O.O., B.L.S., M.H.M., and J.M.R. All authors contributed to the revision and agreed upon the final draft.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-30849-9.

Data availability

The 16S amplicons and shotgun metagenomics sequencing data have been deposited in the NCBI database under BioProject ID PRJNA787039 and PRJNA789467, respectively. Metagenome assembled genomes are available at Zenodo [https://doi.org/10.5281/zenodo.6561541]. The Silva database was used to assign taxonomy to 16S rRNA amplicon sequences [https://www.arb-silva.de/download/archive/]. A custom database was used to assign taxonomy for Kraken. Due to size limitation, this database

is available upon request (please contact J.M.R. at j.raaijmakers@nioo.knaw.nl and expect 2 weeks of processing time). Source data are provided with this paper.

Code availability

The code used in the analysis can be found at Zenodo [https://doi.org/10.5281/zenodo.6561541].

Chapter 6

General Discussion

Plant domestication is an evolutionary process driven by both intentional and unintentional human influences (Purugganan, 2022). Early domestication involved the conscious selection of desirable plant traits, such as larger fruit or more vigorous growth. At the same time, changes in cultivation practices and environmental conditions, also referred to as habitat domestication, exerted selective pressures similar to natural selection, shaping domesticated plants in unintended ways (Soldan et al., 2021). These combined forces, human intervention and the environmental context, have influenced both plant traits and their interactions with their surrounding ecosystem, including the microbiome (Pérez-Jaramillo et al., 2016; Mo et al., 2024). The plant-associated microbiome plays a key role in supporting the host plant via nutrient acquisition and stress tolerance (Santhanam et al., 2015; Pérez-Rodriguez et al., 2020; Adedayo et al., 2023). In current conventional agriculture, domesticated crops are highly dependent on fertilizers and pesticides, which can either replace or deplete microbial functions (Pérez-Jaramillo et al., 2018; Soldan et al., 2021). Moreover, the loss of genetic variation in domesticated cultivars is thought to have disrupted the effective communication with their microbiome, as supported by several studies (Chen et al., 2022; Huang et al., 2022; Nerva et al., 2022; Yue et al., 2023). Consequently, beneficial microbial associations may have been significantly affected during the long trajectory of plant domestication (Smulders et al., 2021; Oyserman et al., 2022). Deciphering how plant domestication impacted the assembly and functionality of the microbiome remains a serious challenge. Addressing this knowledge gap is essential for translating microbiome research into practical strategies for sustainable crop production in a world of increasing human population, climate change, and intensified (a)biotic stresses in degraded agro-ecosystems.

My PhD thesis examined the central question if and how tomato domestication has left discernible imprints on the rhizosphere microbiome. By integrating a plethora of ecological, genetic and microbiological approaches, this thesis explores how plant and habitat domestication impacted the assembly and functionality of the tomato rhizosphere microbiome, from its closest ancestor in the center of origin region to cultivated tomatoes in a center of production (The Netherlands). Tomato was selected as the model plant for this study due to various relevant reasons: it is an economically important crop worldwide, has a relatively short life cycle, and offers extensive genetic resources, including seedbank accessions, commercial varieties, hybrids and recombinant inbred lines (RILs) derived from crosses between wild tomato species and their domesticated counterparts. Additionally, the inclusion of both native and agricultural soils from the tomato's center of origin in Southern Ecuador provided a unique opportunity to assess environmental influences on microbiome dynamics across multiple dimensions, including biogeography, tomato genotype, soil context and local/native plant-insect interactions (Figure 1). To accomplish this, we first characterized the rhi-

zosphere microbiome of the wild tomato relative *Solanum pimpinellifolium* in its native habitat in the province of Loja, Ecuador (**Chapter 2**). As a follow-up, we examined the changes in taxonomic and functional traits of the rhizosphere microbiome associated with eight tomato genotypes, both wild and domesticated, grown in a controlled greenhouse setting with native and agricultural Ecuadorian soils —representing the center of origin— as well as a Dutch greenhouse soil —representing a center of tomato production (**Chapter 3**). Next, we investigated whether the native soil microbiome enhances tomato tolerance to the invasive insect pest *Prodiplosis longifila* (**Chapter 4**). Finally, we identified key plant and rhizobacterial genetic traits associated with the assembly of the tomato rhizosphere microbiome (**Chapter 5**).

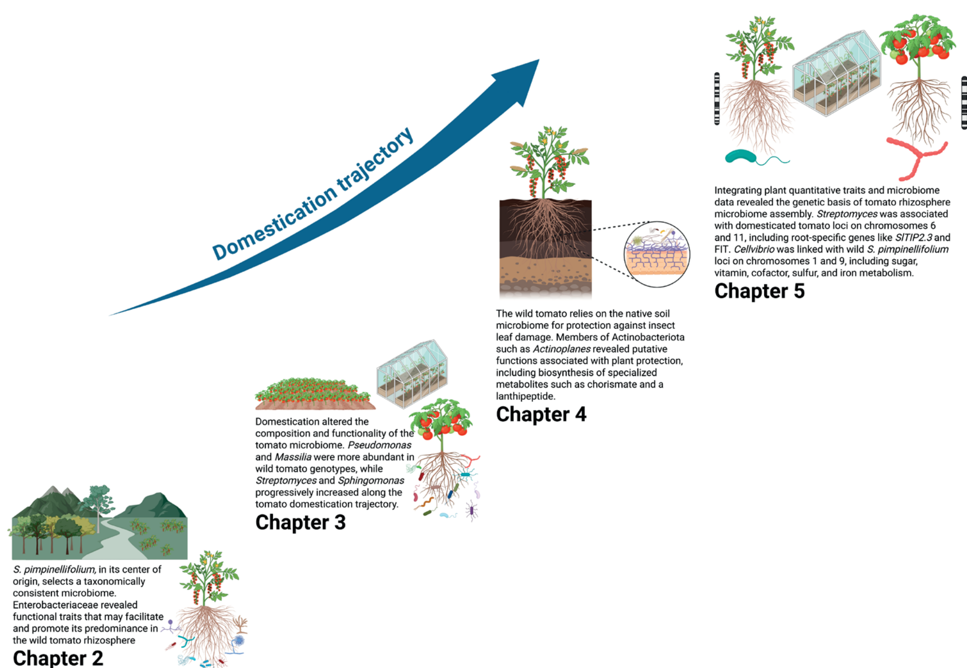

Figure 1. Visual summary of the main findings of this thesis. Created with BioRender.com.

Assembly of wild tomato rhizosphere microbiome in the center of origin

Wild relatives of modern crops have served as a genetic source for past and present crop breeding (Bai & Lindhout, 2007; Grandillo et al., 2011; Gao et al., 2019; Mata-Nicolás et al., 2020; Wang et al., 2020). Despite the knowledge that members of the plant-associated microbiome play a fundamental role in plant growth and health, studies

profiling indigenous microbiomes of wild tomato populations growing in their native habitats still are scarce (Cordero-Ramírez et al., 2012; Fuentes et al., 2020; Runge et al., 2022). The exploration of microbiomes of wild tomato relatives in their native habitats will allow us to understand the natural microbial interactions that could be of interest for the design of novel sustainable agriculture management practices (Wallenstein, 2017; Barajas et al., 2020). Our results (**Chapter 2**) revealed that *S. pimpinellifolium,* growing in its center of origin, selects a taxonomically consistent microbiome, despite a locally diverse physical, chemical, and soil microbiome background. These results suggest that the wild tomato ancestor in its native habitat drives a deterministic assembly of the rhizosphere bacteriome dominated by Enterobacteriaceae and *Rhizobium.* Further examination of the metagenome assembled genomes (MAGs) of Enterobacteriaceae revealed functional traits that may facilitate and promote its predominance in the tomato rhizosphere, such as motility, chemotaxis and antimicrobial compounds production. For instance, type IV secretion pili (Craig et al., 2019; Costa et al., 2023), iron acquisition via siderophore production (Ahmed & Holmström, 2014; Timofeeva et al., 2022), as well as stress resistance features for osmotic (Frossard et al., 2012; Herrou et al., 2017) and high-temperature conditions (Raivio & Silhavy, 2001; Dai et al., 2015), may facilitate direct interactions between bacteria and the host (Wallden et al., 2010; Zechner et al., 2012), and may increase competitiveness in interactions with neighboring rhizosphere microbes (Loper & Buyer, 1991; Loper & Henkels, 1999; Chepsergon & Moleleki, 2023).

Our findings show how core taxa of the rhizosphere bacteriome, such as members of Enterobacteriaceae, can thrive in the harsh native environments where wild tomatoes grow naturally. Metagenomic analyses revealed bacterial traits that may contribute to adaptation to these environmental conditions. Furthermore, our observations highlight the importance of conducting local studies in regions from where commercial cultivars originated, as these are crucial for understanding plant-microbe interactions in an evolutionary context. Regarding the wild tomato's rhizosphere mycobiome, our results indicated a high proportion of unclassified fungi as well as fungi classified as *Fusarium* and *Aspergillus,* which are known either for their pathogenicity or for their beneficial effects on tomato plants via induced systemic resistance (ISR) (Fuchs et al., 1997; Veloso & Díaz, 2012) and plant growth promotion (Adedayo et al., 2023; Daigham et al., 2023). The presence of numerous unclassified fungal taxa in this native wild tomato habitat points to a largely unexplored soil mycobiome. Altogether, this underscores the necessity of identifying potentially valuable microbial candidates for functional validation assays, to assess whether traits beneficial in native ecosystems could support modern tomato cultivation in overcoming biotic and abiotic stresses.

Genotype and habitat domestication altered the composition and functionality of the tomato microbiome

Tomato domestication resulted in multiple phenotypic changes to satisfy human needs (Purugganan & Fuller, 2009). However, the transition from the original native habitats to present-day agricultural production systems not only impacted tomato traits but also unintentionally affected the assembly of their associated (beneficial) microbiome (Fernie & Yan, 2019; Mo et al., 2024). In **Chapter 3,** we tested eight tomato genotypes as representatives of different stages of the domestication trajectory, in combination with native and agricultural soils from the tomato's center of origin (Ecuador) and from one of the current centers of production (The Netherlands). Although our study involved a limited number of tomato genotypes (n = 8) and soil types (n = 5), it provided a first comprehensive screening of the putative impact of domestication on microbiome assembly. Moreover, it offers a basis for generating new hypotheses that can be tested in a broader range of tomato genotypes and soils. We observed differences in the compositions of the rhizosphere microbiome between wild and domesticated tomatoes. These differences were largely determined by the soil type in which the tomato genotypes were grown (native, agricultural, or greenhouse soils). Furthermore, our results suggest that variations in microbiome assembly emerged later in the tomato domestication process, as genotypes classified as wild and intermediate showed comparable rhizosphere microbiome compositions, but different from those of domesticated tomato genotypes.

On one hand, we found that wild tomatoes in native soils associated with specific bacterial genera, such as *Brevundimonas, Cellvibrio* and *Rhizobacter* (Proteobacteria) and *Chitinophaga, Dyadobacter, Fluviicola,* and *Ohtaekwangia* (Bacteroidota), which are known for various functional traits like motility, chemotaxis, and nutrient cycling. On the other hand, the rhizosphere microbiome of domesticated tomatoes grown in agricultural and greenhouse soils typically associated with Actinobacteria (*Actinoplanes, Nocardioides*), Cyanobacteria (*Nostoc, Sericytochromatia*), and Firmicutes (*Bacillus, Paenibacillus*), exhibiting functional traits exemplifying adaptation to modern agricultural management practices, such as the application of fertilizers. Results of our greenhouse experiment further showed that there was a microbial signature for wild and domesticated tomatoes across all soils: *Pseudomonas* and *Massilia* (Proteobacteria) were more abundant in wild-intermediate tomato genotypes, while *Streptomyces* (Actinobacteriota) and *Sphingomonas* (Proteobacteria) progressively increased along the tomato domestication trajectory. The same abundance pattern of *Streptomyces* in domesticated tomato *S. lycopersicum* was observed in the rhizosphere microbiome analysis of the recombinant inbred line (RIL) population of a cross between wild *S. pimpinellifolium* and domesticated *S. lycopersicum* (**Chapter 5**).

Future studies could further build on the microbiome results described here by integrating root exudation profiling and metatranscriptomics to better capture the dynamic nature and underlying mechanisms of selection in tomato microbiome assembly and functioning. To this end, biobanking wild tomato rhizosphere microbiomes is needed for designing targeted bioassays and to monitor the phenotypic effects of compatible host-microbiome combinations *in vivo*. Phenotypic assessment of plant traits such as growth, nutrient uptake and stress tolerance in response to isolated microbial genera can then provide valuable mechanistic insights.

The wild tomato relies on the soil microbiome for protection against insect leaf damage

Wild crop relatives and their native microbiomes have co-evolved for millions of years to withstand local (a)biotic stresses (Pérez-Jaramillo et al., 2016; Wallenstein, 2017; Barajas et al., 2020). However, plant domestication and environmental manipulation for crop cultivation may have caused the loss of beneficial interactions or an adequate communication between domesticated plants and the soil microbiome (Cordovez et al., 2019; O'Brien et al., 2021; Oyserman et al., 2021). In **Chapter 4**, we studied the impact of the native soil microbiome on leaf damage caused by the local sap-sucking insect *Prodiplosis longifila* on the wild tomato ancestor *S. pimpinellifolium* and the cultivated tomato *S. lycopersicum*. The results of our experiment conducted under greenhouse conditions showed that the wild tomato species relies on the soil microbiome for protection against the leaf insect, whereas the modern tomato did not. The results suggested a putative functional role of specific microbial taxa, such as Actinobacteriota and Cyanobacteria, in mediating defense responses against this endemic leaf insect. Specifically, *Actinoplanes* showed a reduced abundance in the rhizosphere of wild tomato plants grown in sterilized soils with higher levels of insect leaf damage aboveground. Our findings support the hypothesis that wild tomato relies on their native soil microbiome, presumably on members of Actinobacteriota such as *Actinoplanes,* to protect itself from leaf damage caused by *P. longifila*. The *Actinoplanes* MAG (metagenome-assembled genome) revealed putative functions associated with plant protection, including genes for motility, chemotaxis, membrane transport, and the biosynthesis of specialized metabolites such as chorismate and a lanthipeptide. To further validate and expand these findings, future experiments should involve isolation and reintroduction of *Actinoplanes* as a potentially beneficial microbial candidate into sterilized soils to directly test its ability to provide insect tolerance. In addition, testing a much broader range of tomato genotypes would help to determine if microbiome-mediated insect tolerance is a conserved trait or genotype-specific. It also remains to be investigated whether the protective effect of the native microbiome extends to other tomato insect

species. If members of Actinobacteriota can confer broader insect tolerance, this could open new avenues for sustainable pest management practices that harness native microbiomes. Although extrapolating these findings to field conditions presents challenges, such as managing environmental variability and soil heterogeneity, our study highlights the potential of native rhizosphere microbial genera as a complementary strategy for pest control in modern agricultural systems.

Integrating plant quantitative traits and microbiome data revealed the genetic basis of tomato rhizosphere microbiome assembly

Microbiomes play a crucial role in plant growth and health and hold great promise for crop breeding (Oyserman et al., 2018; Carrión, 2019; Finkel, 2020). Amplicon sequencing-based studies have previously shown that microbiome assembly by plants has a significant genetic basis (Pérez-Jaramillo et al., 2018; Favela et al., 2021). Moreover, with the latest technological advances, it is now feasible to treat microbiomes as "quantitative traits" of selection (Pereira et al., 1993; Bergelson et al., 2019; Deng, 2021). This fundamental knowledge paves the way for connecting microbial genes and pathways with specific genetic regions (QTLs) in the tomato genome. In **Chapter 5**, we analyzed the rhizosphere microbiome of an extensive RIL population (N = ± 100) derived from a cross between the modern tomato *S. lycopersicum* and the wild tomato *S. pimpinellifolium* (Voorrips et al., 2000), allowing us to identify potential plant and reciprocal rhizobacterial traits underlying microbiome assembly. We found the domesticated tomato was associated with increased overall microbiome abundance compared to its wild relative. Specifically, bacterial genera such as *Altererythrobacter, Asticcacaulis, Devosia, Novosphingobium, Sphingomonas,* and *Streptomyces* were selectively enriched by the domesticated tomato, whereas *Cellvibrio, Sphingopyxis, Sphingobium* were preferentially enriched in the wild relative tomato rhizosphere. Our results showed that the abundance of *Streptomyces* was strongly associated with genetic regions of the domesticated tomato located on chromosome 6 and 11. These regions included root-specific tomato genes such as aquaporin (SlTIP2.3) and the iron transcriptional regulator FIT. On the other hand, the abundance of *Cellvibrio* was linked with genetic regions on chromosomes 1 and 9 of the wild *S. pimpinellifolium,* harboring genes involved in sugar catabolism (arabinofuranosidase, fructose, cellulase/esterase, malate, mannonate, xyloglucan and xylulose), vitamin and cofactor metabolism as well as sulfur and iron metabolism. On the microbial side, these analyses revealed traits associated with various pathways, including plant polysaccharide degradation, trehalose metabolism, polyamine metabolism, acetolactate synthase activity, as well as vitamin and iron metabolism. Although the functional importance of these plant and microbial

traits in assembly and functioning need to be experimentally validated, these findings pinpointed, for the first time, potential targets for microbiome-based plant breeding, also referred to as *M* breeding (Cernava, 2024). Experimental validation will include manipulating microbial communities in controlled assays to test the specific influence of candidate QTLs on microbiome composition and functionality. For instance, the putative role of aquaporin and FIT in *Streptomyces* tomato root colonization can be investigated using transgenic lines of domesticated tomato with site-directed mutations of specific genes. In the same way, experimental validation of genes linked to sugar and vitamin metabolism in *S. pimpinellifolium* could provide insights on how these pathways drive *Cellvibrio* recruitment, offering valuable targets for enhancing microbial resilience or productivity in domesticated tomatoes. By validating these findings also in field trials, we can move one step closer to harnessing the potential of the rhizosphere microbiome to improve tomato cultivation by optimizing yield stability under changing environmental conditions.

Concluding remarks and future outlook

This thesis highlighted the intricate and reciprocal effects of plant and habitat domestication on tomato rhizosphere microbiome assembly. While domestication has shaped beneficial phenotypes in crops, it has unintentionally altered their interactions with microbes. As a result, key plant-microbe associations may have been depleted or lost, which has implications for the adaptability and resilience of modern cultivars to changing environmental conditions. Our results revealed that domestication has profoundly influenced the taxonomic and functional diversity of the tomato's rhizosphere microbiome. The obtained results underscore the important role that microbial communities may play in the adaptation and resilience of plants like tomato, specifically when comparing native habitats to modern agricultural conditions.

The concept of microbiome rewilding (Raaijmakers & Kiers, 2022) presents a promising road map for restoring plant-microbe interactions by reinstating key plant-microbe partnerships that may have been lost during domestication. This approach follows a four-step roadmap: 1) grow wild relatives and domesticated crops in native and agricultural soils to identify key changes in the ancestral microbiome; 2) transplant the ancestral microbiome onto domesticated crops in agricultural soils to validate their beneficial impact on specific phenotypes, particularly tolerance to biotic and/or abiotic stresses; 3) identify plant genetic loci associated with the recruitment and functioning of beneficial ancestral microbiome members; and 4) identify key molecules in the root or shoot exudates that are associated with the recruitment or activation of the beneficial ancestral microbiome members.

In this thesis, we took the first steps of this roadmap by identifying key microbial taxa in the native habitat of wild tomato, as well as the differences in microbiome assembly between wild and domesticated tomato genotypes. These comparisons highlighted how the process of domestication has shaped the tomato microbiomes from their natural center of origin to modern production sites. Specifically, we explored microbial associations in both native and agricultural soils, including a contrasting soil from a greenhouse-intensive tomato production system, located far from the tomato's center of origin. By doing so, we reflected on the journey of domestication, going from wild habitats to present-day breeding strategies, and aimed at understanding and harnessing the microbial functions that influence plant traits. Additionally, we explored the protective function of the native soil microbiome in insect resistance of wild tomato. We also identified specific tomato loci associated with microbiome assembly, providing a foundation for future *M* breeding efforts to reinstate beneficial ancestral microbiome members into modern crops.

We demonstrated that the wild tomato ancestor *Solanum pimpinellifolium* recruits a consistent and functionally specialized rhizosphere microbiome in its center of origin. The host filtering effect observed under natural conditions in Ecuador emphasized that wild tomato selectively enriches for microorganisms linked to motility, siderophore production and stress tolerance. These traits likely evolved to help the plant tolerate the harsh and unpredictable conditions of its native habitat. It is possible that these microbial associations contribute to the plant's ability to persist through extreme environmental stress, such as prolonged droughts, supporting the ecological role of regional plant-microbe co-evolution. However, this remains a hypothesis for further investigation, as the microbiome's role in supporting plant survival under such extreme conditions was not directly tested. Furthermore, our results revealed that plant domestication has significantly altered the tomato microbiome, with the influence of soil habitat amplifying these effects. We found that microbiome divergence became especially pronounced in later stages of domestication. We observed selection for microbial genera adapted to extensively managed soils and agronomic inputs in domesticated tomatoes, while the wild tomato genotypes were associated with microbial features related to stress resilience and nutrient cycling.

At the functional level, we explored the protective role of the native soil microbiome in enhancing insect resistance in wild tomato. Notably, we identified members of the genus *Actinoplanes,* which were depleted in sterilized soil but correlated with reduced leaf damage in soils containing a native microbiome. These bacteria, which exhibited traits related to motility, membrane transport, and secondary metabolism, suggesting a critical role in mediating plant resistance to insect attack. Its presumed role in plant defense makes *Actinoplanes* a potential candidate for enhancing plant resilience. Simi-

larly, we identified *Streptomyces* and *Cellvibrio* as candidates to further investigate the metabolic cross-talk in the tomato rhizosphere.

The results presented in my PhD thesis raise important questions concerning the functional implications of microbiome shifts throughout the domestication trajectory, particularly in the context of the challenges faced in modern agriculture. Central questions include: i) what key plant and microbial traits were lost during domestication, and can they be restored?; ii) how can microbiomes be harnessed to reduce dependence on chemical agricultural inputs such as fertilizers and pesticides?; iii) how can beneficial microbes be effectively integrated into crop breeding and agricultural management practices?; and, iv) how can our knowledge of the genetic basis of microbiome assembly be used in *M* breeding strategies to develop climate-resilient crops? Addressing these questions will require robust functional validation under diverse environmental conditions. Future research will focus on targeted greenhouse assays to test the effects of key microbial taxa, including *Streptomyces, Bacillus, Sphingomonas,* Cyanobacteria, *Actinoplanes, Cellvibrio, Enterobacter, Pseudomonas, Rhizobium,* and *Massilia,* on drought tolerance, nutrient use efficiency and biotic stress resilience. Additionally, high-throughput phenotyping and metabolomic profiling needs to be employed to track colonization dynamics by and host physiological responses to these key microbial genera.

In summary, the work described in this thesis contributes to broader discussions on the future of sustainable agriculture, offering insights into the role of the rhizosphere microbiome in shaping crop resilience. By integrating microbiome-compatible traits into breeding programs, we have the opportunity to restore critical microbial functions that may have been lost during domestication, while simultaneously optimizing crop yield, uniformity in plant growth or fruit quality, and resilience to (a)biotic stresses. With its rich history of domestication and intricate mechanisms of plant-microbe interactions, the tomato system serves an ideal model for advancing these efforts. As we face the challenges of climate change, resource scarcity, and the growing need for global food security, this research paves the way for innovative strategies in crop breeding that leverage the power of microbial partnerships. By identifying key genetic loci and microbiome traits that drive plant performance, we can adopt a promising strategy to expand our agricultural toolkit and meet the needs of future generations.

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Summaries

Summary

Plant domestication and breeding have substantially changed the genetic and phenotypic traits of crop species. However, the impact of domestication on the taxonomic and functional diversity of microorganisms associated with plant tissues is largely unknown. The work presented in this thesis focuses on tomato and how its domestication has shaped the diversity, structure, and functional potential of the root-associated microbiome. By examining the intricate relationships between tomato genotype, soil environment, and microbial community composition, we aim to uncover the ecological and genetic mechanisms that govern plant-microbe interactions along the domestication trajectory.

Field exploration in the native habitat of wild tomato *Solanum pimpinellifolium* in Southern Ecuador revealed a deterministic rhizosphere microbiome assembly dominated by Enterobacteriaceae and *Rhizobium*, as well as the fungal taxa *Fusarium* and *Aspergillus*. Metagenomic analysis revealed that these bacterial taxa possess traits such as motility, chemotaxis, siderophore production, and stress resistance, that may contribute to their successful establishment in the wild tomato rhizosphere under the challenging environmental conditions of their native habitat. Building on these results, controlled experiments with wild and domesticated tomato genotypes grown in Ecuadorian native and agricultural soils from the center of origin and greenhouse soil from a tomato's center of production, showed that both plant genotype and soil type shaped tomato rhizosphere microbiome composition. While the wild tomato genotypes retained similarities in microbiome composition associated with their native soil environments, the rhizosphere microbiome composition of domesticated tomato aligned with managed soil conditions. These shifts in microbiome composition suggest that domestication may unintentionally have led to a loss of beneficial microbes and microbial functions, such as motility, chemotaxis and stress response.

Further exploration revealed specific functional consequences of these changes. The results showed that in presence of its native soil microbiome, the wild tomato is better protected against herbivory by the endemic insect *Prodiplosis longifila*. Tomato plants grown in sterilized native and agricultural soils showed increased leaf damage, which was correlated with a reduced relative abundance of Actinobacteriota, particularly members of the *Actinoplanes* genus. Functional analysis of this genus revealed traits potentially involved in plant growth and stress tolerance, in particular secondary metabolite production and nutrient transport. These findings support the hypothesis that wild tomato, in contrast to its domesticated counterpart, relies on microbial partners in its native habitat for defense against herbivores. To uncover the plant's role in shaping these microbial relationships, we explored the genetic basis of microbiome assembly

using a recombinant inbred line (RIL) population derived from a cross between wild *Solanum pimpinellifolium* and domesticated tomato *S. lycopersicum* var. Moneymaker. By integrating 'microbiomics' and quantitative plant genetics, we identified genomic regions linked to the differential recruitment of *Cellvibrio* and *Streptomyces* by wild and domesticated tomato, respectively. These associations were reciprocally associated with plant traits related to nutrient uptake, stress tolerance, and metabolic functions, while corresponding microbial traits included carbon utilization, vitamin synthesis and iron acquisition.

Collectively, the results of this thesis show that domestication has reshaped not only plants traits but also the rhizosphere microbiome, changing diversity and functional traits associated with plant stress resilience. These insights highlight the importance of integrating microbiome research into plant breeding strategies, reinstating beneficial members of native microbial communities for more resilient and sustainable agricultural ecosystems.

Samenvatting

Domesticatie en veredeling van wilde planten hebben de genetische en fenotypische kenmerken van landbouwgewassen aanzienlijk veranderd. De impact van dit domesticatie proces op de taxonomische en functionele diversiteit van micro-organismen die zich associëren met verschillende plantenweefsels is echter nog grotendeels onbekend. Het onderzoek in dit proefschrift richt zich op domesticatie van tomaten en hoe dit proces de diversiteit, de structuur, en het functionele potentieel van het rhizosfeer microbioom heeft gevormd. Door de complexe relaties tussen genetisch diverse tomaten, het bodemtype, en de samenstelling van microbiële gemeenschappen te onderzoeken, hebben we zowel ecologische als genetische mechanismen onderzocht die de interacties tussen de tomatenplant en het microbioom tijdens het domesticatie-traject hebben beïnvloed.

De resultaten van het veldonderzoek in het oorsprongsgebied van de wilde tomaat *Solanum pimpinellifolium* in het Zuiden van Ecuador bracht een specifieke samenstelling van het rhizosfeer microbioom aan het licht, dat werd gedomineerd door Enterobacteriaceae en *Rhizobium* bacteriën, evenals de schimmeltaxa *Fusarium* en *Aspergillus*. De analyse van het metagenoom toonde verder aan dat deze bacteriële taxa eigenschappen bezitten zoals beweeglijkheid, chemotaxis, de productie van sideroforen, en stressbestendigheid, die mogelijk bijdragen aan hun succesvolle aanwezigheid in de rhizosfeer van deze wilde tomatensoort in hun natuurlijke habitat. Gecontroleerde kasexperimenten met zowel wilde als gedomesticeerde tomatensoorten, die groeiden in natuurlijke grond en landbouwgrond uit het oorsprongsgebied van de wilde tomaat alsook in Nederlandse kasgrond, toonden vervolgens aan dat zowel het tomaten-genotype als het bodemtype de samenstelling van het rhizosfeer microbioom beïnvloedden. Hoewel de microbioomsamenstelling van wilde tomaten-genotypen overeenkwam wanneer ze gekweekt werden in gronden uit hun natuurlijke oorsprongsgebied, kwam het rhizosfeer microbioom van de gedomesticeerde tomaat meer overeen met die gekweekt onder gecontroleerde omstandigheden in de Nederlandse kasgrond. Deze veranderingen in de samenstelling van het microbioom suggereren dat domesticatie onbedoeld kan hebben geleid tot een vermindering van nuttige micro-organismen en hun microbiële functies, zoals beweeglijkheid, chemotaxis en hun stressbestendigheid.

Ons vervolgonderzoek toonde aan dat de wilde tomaat, beter beschermd werd tegen vraat door het endemische insect *Prodiplosis longifila* in aanwezigheid van zijn oorspronkelijke bodemmicrobioom. Tomatenplanten die gekweekt werden op gesteriliseerde natuurlijke grond en landbouwgrond vertoonden meer bladschade, wat gecorreleerd werd aan een verminderde relatieve aanwezigheid van Actinobacteriota, met name leden van het geslacht *Actinoplanes*. Een functionele analyse van *Actinoplanes* onthulde eigen-

Summaries

schappen die mogelijk betrokken zijn bij plantengroei en stresstolerantie, met name de productie van secundaire metabolieten en de acquisitie van specifieke nutriënten. Deze resultaten ondersteunen de hypothese dat de wilde tomaat in haar natuurlijke leefomgeving, in tegenstelling tot gedomesticeerde familieleden, afhankelijk is van het rhizosfeer microbioom voor een adequate verdediging tegen herbivoren zoals *Prodiplosis longifila*. Om meer te weten te komen over de rol van de plant bij het aangaan van deze microbiële relaties, onderzochten we de genetische basis van de assemblage van het rhizosfeer microbioom door gebruik te maken van recombinante inteelt lijnen (RILs) die afkomstig waren van een kruising tussen ouderplanten van de wilde tomaat *S. pimpinellifolium* en de gedomesticeerde tomaat *S. Lycopersicum* var. Moneymaker. Door microbioom analyses en kwantitatieve plantengenetica te integreren, identificeerden we diverse regio's in het tomatengenoom die verband houden met de differentiële rekrutering van specifieke bacteriesoorten, met name *Cellvibrio* en *Streptomyces* door respectievelijk wilde en gedomesticeerde tomaten. Deze waren wederzijds geassocieerd met planteneigenschappen zoals nutriëntenopname, stresstolerantie en ijzer metabolisme, en microbiële functies zoals koolstofgebruik, de aanmaak van vitamines en ijzeropname.

Gezamenlijk laten de resultaten van dit proefschrift zien dat domesticatie niet alleen de eigenschappen van planten heeft veranderd, maar ook het microbioom van de rhizosfeer van deze planten, waardoor de diversiteit en de functionele eigenschappen die verband houden met de stressbestendigheid van planten zijn veranderd. Deze inzichten benadrukken het belang van de integratie van microbioomonderzoek in de huidige plantenveredelingsstrategieën, waardoor nuttige micro-organismen die ontdekt worden in de microbiomen uit oorsprongsgebieden van de voorouders van onze huidige landbouwgewassen kunnen bijdragen aan het herstel van veerkrachtigere en duurzamere landbouwecosystemen.

Resumen

La domesticación y mejoramiento de las plantas han modificado sustancialmente los rasgos genéticos y fenotípicos de las especies cultivadas. Sin embargo, el impacto de la domesticación sobre la diversidad taxonómica y funcional de los microorganismos asociados a los tejidos vegetales sigue siendo en gran medida desconocido. El trabajo presentado en esta tesis se centra en el tomate y en cómo su domesticación ha moldeado la diversidad, estructura y potencial funcional del microbioma asociado a sus raíces. Al examinar las complejas relaciones entre el genotipo del tomate, el ambiente del suelo y la composición de la comunidad microbiana, buscamos descubrir los mecanismos ecológicos y genéticos que regulan las interacciones entre plantas y microorganismos a lo largo de la trayectoria de la domesticación.

La exploración de campo en el hábitat nativo del tomate silvestre *Solanum pimpinellifolium* en el sur de Ecuador reveló un ensamblaje determinístico del microbioma de la rizosfera, dominado por Enterobacteriaceae y *Rhizobium,* así como por los hongos *Fusarium* y *Aspergillus.* El análisis metagenómico reveló que estos taxones bacterianos poseen características como motilidad, quimiotaxis, producción de sideróforos y resistencia al estrés, que podrían contribuir a su exitoso establecimiento en la rizosfera del tomate silvestre bajo las difíciles condiciones ambientales de su hábitat nativo. A partir de estos resultados, experimentos controlados con genotipos silvestres y domesticados de tomate cultivados en suelos nativos y agrícolas de Ecuador, el centro de origen, así como en suelo de invernadero procedente del centro de producción de tomate, demostraron que tanto el genotipo de la planta como el tipo de suelo determinaron la composición del microbioma de la rizosfera del tomate. Mientras que los genotipos silvestres conservaron similitudes en la composición del microbioma asociadas a las condiciones de sus suelos nativos, la composición del microbioma de la rizosfera del tomate domesticado se alineó con condiciones de suelos agrícolas. Estos cambios en la composición del microbioma sugieren que la domesticación puede haber provocado, de forma no intencionada, la pérdida de microorganismos y de funciones microbianas benéficas, como la motilidad, quimiotaxis y la respuesta al estrés.

Un análisis más profundo reveló consecuencias funcionales específicas de estos cambios. Los resultados mostraron que, en presencia de su microbioma nativo del suelo, el tomate silvestre está mejor protegido contra la herbivoría por el insecto endémico *Prodiplosis longifila.* Las plantas de tomate cultivadas en suelos nativos y agrícolas esterilizados mostraron mayor daño foliar, el cual se correlacionó con una menor abundancia relativa de Actinobacteriota, en particular de miembros del género *Actinoplanes.* El análisis funcional de este género reveló características potencialmente implicadas en el crecimiento vegetal y la tolerancia al estrés, en especial la producción de metabolitos

secundarios y transporte de nutrientes. Estos hallazgos respaldan la hipótesis de que el tomate silvestre, a diferencia de su contraparte domesticada, depende de socios microbianos en su hábitat nativo para defenderse de los herbívoros. Para descubrir el papel de la planta en el establecimiento de estas relaciones microbianas, se investigaron las bases genéticas del ensamblaje del microbioma usando una población de líneas endogámicas recombinantes (RIL) derivadas del cruce entre el tomate silvestre *Solanum pimpinellifolium* y el tomate domesticado *S. lycopersicum* var. Moneymaker. Mediante la integración de "microbiómica" con genética cuantitativa vegetal, se identificó regiones genómicas asociadas al reclutamiento diferencial de *Cellvibrio* y *Streptomyces* por parte del tomate silvestre y domesticado, respectivamente. Estas asociaciones se relacionaron recíprocamente con rasgos vegetales vinculados a la absorción de nutrientes, la tolerancia al estrés y funciones metabólicas, mientras que los rasgos microbianos correspondientes incluyeron la utilización de carbono, la síntesis de vitaminas y la adquisición de hierro.

En conjunto, los resultados de esta tesis demuestran que la domesticación ha transformado no solo los rasgos de las plantas, sino también el microbioma de la rizosfera, alterando su diversidad y características funcionales asociadas a la resiliencia frente al estrés de las plantas. Estos hallazgos destacan la importancia de integrar la investigación sobre microbiomas en las estrategias de mejoramiento vegetal, con el fin de reincorporar miembros benéficos de las comunidades microbianas nativas y avanzar hacia ecosistemas agrícolas más resilientes y sostenibles.

Curriculum Vitae

Curriculum vitae

Stalin Wladimir Sarango Flores was born on April 23, 1986, in Loja, Ecuador. In 2011, he completed his undergraduate studies in Agronomy at the National University of Loja. In 2015, he obtained his master's degree in Agricultural Microbiology at the University of São Paulo (Brazil), where he developed his thesis under the supervision of Dr. Rodrigo Mendes at EMBRAPA-Environment (Brazilian Agricultural Research Corporation). This work allowed him to deepen his understanding of plant–microbe interactions and the role of beneficial microorganisms in agriculture. After completing his master's degree, he returned to Ecuador, where he expanded his experience in teaching and research. He later joined the research group of Dr. Pieter van 't Hof at Universidad San Francisco de Quito (USFQ), where he carried out experiments and data analyses involving commercial tomato seeds and natural soils from northern Ecuador. In 2018, he was awarded a PhD scholarship from the Ecuadorian National Secretariat of Higher Education, Science, Technology and Innovation (SENESCYT), and in 2019 began his doctoral research under the supervision of Prof. Dr. Jos M. Raaijmakers at the Netherlands Institute of Ecology (NIOO-KNAW) and the Institute of Biology at Leiden University. Later, he joined to the MiCRop project (www.microp.org). The findings of this work are presented in this thesis, entitled "*Microbial footprints of tomato domestication*". After the completion of his doctoral studies, he returned to Ecuador, where he has continued collaborating on academic and research activities. His motivation as a researcher lies in improving sustainable agriculture through the study and application of beneficial microorganisms. He strongly believes in the power of collaboration, knowledge sharing, and the integration of science with local practices to promote agricultural development, especially in rural areas where scientific knowledge can have a transformative impact on communities and the environment.

List of Publications

List of publications

Sarango Flores, S., Cordovez, V., Oyserman, B.O., Arias Giraldo, L.M, Stopnisek, N., Raaijmakers, J.M., van 't Hof, P. (2025). Microbiome-mediate resistance of wild tomato to the invasive insect *Prodiplosis longifila*. *Environmental Microbiology Reports, 17*(5). https://doi.org/10.1111/1758-2229.70190

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