DISSERTATION

MICROBIOME-MEDIATED PHOSPHORUS ACQUISITION IN WILD AND DOMESTICATED TOMATOES

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2025

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ABSTRACT

MICROBIOME-MEDIATED PHOSPHORUS ACQUISITION IN WILD AND DOMESTICATED TOMATOES

Phosphorus (P) is a plant-essential mineral, and because of soil retention reactions, is often sparingly available. To address this limited availability, P fertilizers that are derived from finite phosphate rock are necessarily applied. As a result, modern cultivars are adapted to grow with high fertility. Conversely, wild crops grow in terrestrial soils with poor nutrition. However, it remains unclear how domestication altered the soil microbiome related to P recovery in certain crops and how soil microbiomes of wild crops respond to exogenous P. Here, we use tomato as a model crop because of its global importance, rapid growth, and morphological variation.

To begin to address these unknowns, we investigated how P deficiency affected microbial associations at different stages of domestication and breeding: wild, traditional (developed circa 1900), and modern (developed circa 2020). We determined that although modern and traditional tomato were minimally affected by the P deficit, as showcased through their higher shoot P concentrations and relative biomass, wild tomatoes promoted associations with P solubilizing bacteria. There was variation within wild tomatoes, with *Solanum pennellii* associating with P solubilizing bacteria greater than other accessions. Conversely, traditional and modern tomatoes responded similarly to P deficiency in their growth and microbial associations.

We subsequently investigated the responsiveness to P by these bacteriomes. Using the data culled from the first experiment, we explored the degree to which the microbiome of wild, traditional, and modern tomato responded to exogenous P application. By examining the

microbial co-occurrence networks of P depleted soils, we determined that rhizosphere microbiome complexity increased progressively along a domestication gradient. However, upon P resupply, these differences diminished, and the wild tomato rhizosphere was as complex as the domesticated tomatoes. These results suggest that microbiome of wild tomato is highly responsive to fertilization.

Finally, we explored how a wild and modern tomato representative responded to applied P. Because all tested modern and traditional tomatoes showed similar responses in the prior experiments, a single modern representative (*S. lycopersicum* 'Quali T 27') was selected for this investigation. *Solanum pennellii* was selected as the wild representative because of its strong ability to associate with its soil microbial community. We determined that wild tomato maintained higher soil P values compared to modern while also promoting microbial fluctuations, microbial biomass, and P solubilizer relative abundance. This microbially-informed strategy by wild tomato is likely a result of its root exudate profile. Wild tomato exuded trehalose and glycerol compounds, which were shown to promote bacterial P solubilizing activity. In a separate trial, we grew modern tomato in soils conditioned with a modern tomato or a wild tomato. Biomass was greater for those tomatoes planted in soils with an initial wild plant. However, this difference may be a result of residual P by wild tomato because the modern tomato was unable to maintain the microbiome cultivated by the wild tomato.

Taken together, these findings underscore how domestication has attenuated root-microbe interactions and responsiveness related to P. By elucidating this divergence in wild and modern tomatoes, we can begin to design methods to reincorporate traits lost to domestication, thereby promoting soil P cycling while reducing exogenous inputs of nonrenewable P fertilizers.

ACKNOWLEDGEMENTS

I'd like to take this opportunity to recognize the support of some wonderful people. I am deeply thankful for the endless support of my major professor, Dr. Jorge Vivanco. I'd like to express my sincere appreciation to Dr. Dan Manter for his advice and guidance. My gratitude is extended to the rest of my committee, Drs. Jessica Davis and Bill Bauerle. I would also like to thank Dr. Jessica Chitwood-Brown, who advised me as I was beginning my degree. Many thanks to the Sakata family whose financial support helped me conduct my research.

My deepest thanks are extended to Dr. Antisar Afkairin, an incredible colleague and friend on whom I have heavily relied. I would like to thank everyone else in the Vivanco lab with whom I've had the pleasure of interacting: Emma Byerly, Carley Rohrbaugh, Dr. Derek Newberger, Dr. Hugo Pantigoso, and Dr. Samira Valdez. I would also like to thank the wonderful researchers in other labs that helped me to complete my experiments: Dr. Ali Hamm, Tim Creed, Cassidy Buchanan, Dr. Nathalie Munoz, Dr. Kent Bloodsworth, and Dr. Vimal Balasubramanian.

Finally, I would like to thank Erica Tardiff as well as Iain, Trini, Katie, and Daniele Dixon for their personal support and kind words.

DEDICATION

To my parents, Iain and Trini.

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CHAPTER 1 – ASSESSING THE DIVERGENT SOIL-PHOSPHORUS RECOVERY STRATEGIES IN WILD AND DOMESTICATED CROPS[1]

Abstract

Plant-essential phosphorus (P) is a sparingly available mineral in soils. Phosphorus fertilizers—produced by the transformation of insoluble to soluble phosphates—are thus applied to agroecosystems. With advancements in commercial agriculture, crops have been increasingly adapted to grow in fertile environments. Wild crop relatives, however, are adapted to grow in unfertilized soils. In response to these two conditions of P bioavailability (fertilized agroecosystems and unfertilized natural soils), domesticated crops and wild species employ different strategies to grow and develop. It is essential to understand strategies related to P acquisition that may have been lost to domestication, and here we present, for the first time, that across species, modern cultivars engage in physical (i.e., root morphological) mechanisms while their wild relatives promote ecological (i.e., root-microbial) mechanisms. Domesticated crops showcase shallower root system architecture and engage in topsoil foraging to acquire P from the nutrient-stratified environments common to fertilized agroecosystems. Wild species associate with P-cycling bacteria and AM fungi. This divergence in P recovery strategies is a novel delineation of current research that has implications for enhancing agricultural sustainability. By identifying the traits related to P recovery that have been lost to domestication, we can strengthen the P recovery responses by modern crops and reduce P fertilization.

[1] This research was originally published in *Plants: Dixon, M.M. and Vivanco, J.M., 2025. Assessing the Divergent Soil Phosphorus Recovery Strategies in Domesticated and Wild Crops. Plants, 14(15), 2296.*

Introduction

Crop domestication has independently occurred as far back as 12,000 years ago (YA) to as recent as 3000 YA[1,2]. Some of the first crops to be domesticated by early humans were cereals, with wheat (*Triticum aestivum*) being domesticated in Turkey and Syria approximately 10,000 YA[3], barley (*Hordeum vulgare*) in the areas surrounding Iran, India, and Tibet 8000 YA[4], and lentils (*Lens culinaris*) in Southwest Asia between 8000 and 10,000 YA[5]. The outcomes of these domestication events have allowed for increased production and for the incidence of hunger to be reduced[6].

However, crop domestication has also reduced the effective population size of a given crop, as exemplified through domestication bottlenecks[7,8]. For example, only approximately 2% of the ancestral wild population of soybean (*Glycine max*) is estimated to have been used to domesticate early landraces[9]. Similarly, when early tomato (*Solanum lycopersicum*) was transported from Mesoamerica to Europe, there was concomitant sharp decrease in diversity[10]. Continued selection and migration events further diminished tomato genetic diversity[10]. This reduction poses a challenge for production and breeding because of changing environmental conditions and increasing consumer demand paired with a declining pool of genetic information for developing new cultivars[11]. Thus, crop breeders often rely on introgressions from wild relatives to introduce beneficial genes into modern germplasm[12].

Wild crops provide an opportunity to increase abiotic stress resistance in modern crops[12]. In ancient terrestrial ecosystems, phosphorus (P) is limited because of factors such as erosion, weathering, leaching, and low parent material[13,14]. Erosion and weathering cause a gradual disappearance of phosphates from terrestrial soil systems that do not receive regular nutrient additions. In addition to P deficiency, nitrogen (N) is also often deficient and limits production.

For plants growing in these older terrestrial ecosystems, low concentrations of N and P are the primary limitations for growth and development[14]. Wild crop relatives are adapted to grow in these nonagricultural soils that have low fertility and thus grow slowly[15]. Conversely, faster growing, cultivated crops are adapted to agricultural soils with high fertility and thus necessitate human intervention for survival[16]. Exogenous fertilizers, for example, are required for modern crops to grow and develop without showcasing symptoms of nutrient deficiency. Approximately 90% of current P fertilization is derived from phosphate rock, a finite mineral[17]. Because P is often the limiting factor for sustaining healthy plant growth, P fertilizers have historically been applied to agroecosystems. With changing agricultural practices from events like the Green Revolution, mining of phosphate rock and global P fertilizer applications have starkly increased[18,19]. In 2022, 42 million Tg P_2O_5 were applied globally for agricultural use[20]. This reliance on phosphate rock is notable because the average quality of phosphate rock is decreasing as higher quality reserves are preferentially mined, and current models predict that demand for phosphate rock will continue to increase[21].

Further, although current estimates of total soil P concentrations are above crop requirements (100–3000 $mg \cdot kg^{-1}$), most soil P is not readily available for plant uptake[22,23]. Total estimates can vary with environmental conditions, but on average, only 9% of total soil P is in an available form[24]. When the pool of available P is less than the level required to sustain healthy plant growth, plants must employ strategies to acquire P from sparingly available pools. It has been highly documented that the types of strategies employed by plants varies with domestication[25-28]. However, a synthesis of this domestication effect for P recovery has not been performed. Here, were explore current literature to elucidate the divergent strategies employed by cultivated crops and their wild relatives to acquire P, including physical and ecological

mechanisms. Physical mechanisms are defined those involving root system architecture while ecological mechanisms involve root-microbe associations.

Phosphorus in soil and uptake by plant roots

Highly efficient P uptake and transport is important because while P is the 11th most abundant element in the Earth's crust, the pool of bioavailable P tends to be poor[21]. Depending on the pH of the soil system, bioavailable inorganic orthophosphate exists in different forms: PO_4^{3-} between pH 10.0 and 14, HPO_4^{2-} between pH 7.2 and 12.1, and $H_2PO_4^-$ below pH 7.2[29,30]. Dihydrogen phosphate ($H_2PO_4^-$) is the primary form of orthophosphate favored by plants because the maximum uptake of P tends to occurs in the pH range for which this form is dominant (below 7.2)[31] (Figure 1-1). Inorganic orthophosphate is labile and therefore readily undergoes transformations in the soil. Orthophosphate can be immobilized into organic forms, adsorbed to mineral surfaces, or precipitated into secondary minerals (Figure 1-1). Secondary P minerals, such as calcium (Ca)-, magnesium (Mg)-, aluminum (Al)- and iron (Fe)-phosphates, are sparingly soluble, and their dissociation depends on soil pH. Iron- and Al-phosphate solubility tends to increase with increasing pH, while Ca- and Mg-phosphate solubility tends to increase with decreasing pH[32] (Figure 1-1). Primary P minerals (e.g., apatite and strengite) are stable and release P slowly through weathering activity[32]. Through the activity of root exudation, microbial activity, or shifts in root architecture, the solubility of bound-P may increase and become plant available.

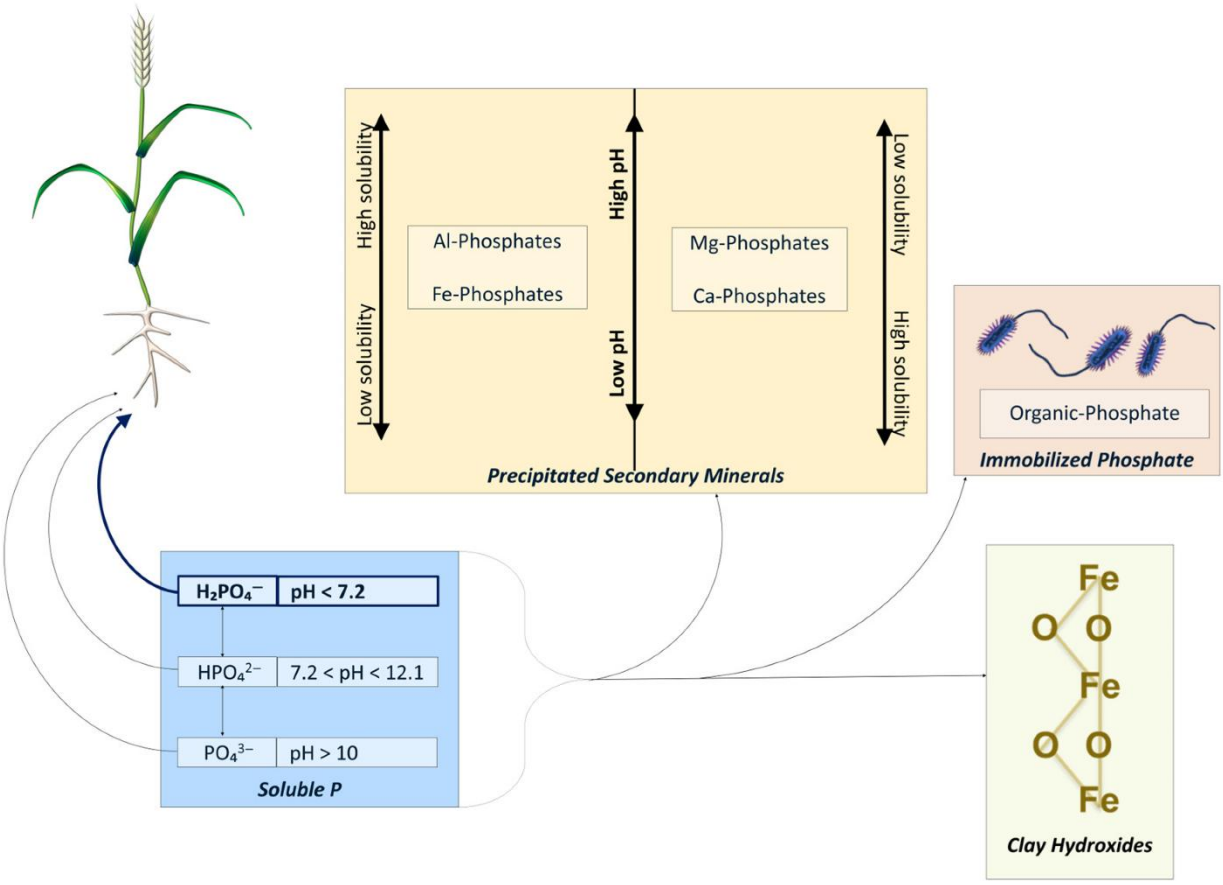

Figure 1-1 . Common forms of loss of soluble phosphorus (P) from soil solution. The form of soluble P varies with pH (blue box). Soluble P is plant available and is taken up by roots. H2PO4− is the predominant form of phosphate taken up by plant roots (bolded text in blue box). Soluble P can react with cations to form precipitated secondary minerals (dark yellow box). The solubility of the P forms varies with pH (indicated by the arrows in the dark yellow box). Soluble P can also be immobilized to organic forms (red-orange box) or fixed to clay matrices (light yellow box). Figure generated by the authors with information culled from sources within the article[29-32].

Phosphorus (P) constitutes approximately 0.1–0.5 % plant dry matter and is an essential plant macronutrient[30,31]. It is a component of nucleic acids and the phospholipid membrane of cells[33]. The primary function of P is as a constituent of adenosine diphosphate (ADP) and adenosine triphosphate (ATP), making it necessary for energy storage and transfer[30,31]. When orthophosphate is cleaved from ADP or ATP, chemical energy is released. ATP is necessary for plant development, as it is a regulator of membrane transport, photosynthesis, and protein biosynthesis. Therefore, when in P deficient conditions, plants show stunted growth[30]. The co-

limitation of P and N commonly occurs, and there are strong interactions between the P and N in their signaling pathways[34]. When plants are deficient in P, there is a concomitant decrease in plant N concentration[35]. Further, a component of the Phosphate Starvation Response pathway works in conjunction with the Nitrogen Limitation Adaptation genes to induce responses to P deficiency[34]. Thus, N and P uptake are tightly interconnected and contribute to healthy plant growth.

Unlike the higher concentrations in plant shoot tissue, the concentration of P in soil solution is low, often ranging from 0.001 to 1 mM[30,31]. Further, soil P is immobile, only moving an average of 0.13 mm per day[30]. However, it is important to note that this diffusion coefficient is affected by soil type and water availability; water increases P diffusion, and this effect is increased for lighter-textured compared to clayey soils[36]. Because of this immobility in the soil, plants take up phosphate from the soil surrounding root at a faster rate than phosphate in bulk can translocate to the root zone[37]. Thus, a zone of depletion of available P commonly forms in the rhizosphere[37]. Because of the low concentration and poor mobility of phosphate in soil solution, plants rely on high-affinity root phosphate transporters to uptake phosphate across a steep chemical potential gradient[31]. Phosphorus uptake is an energy-mediated process and requires active symport with H^{+}[32]. There are five phosphate transporter families that moderate P uptake and translocation across different locations within the plant: PHT1 in the plasma membrane drives soil P uptake, PHT2 in the chloroplast inner plastid membrane moderates P translocation, PHT3 in the mitochondrial membrane regulates P distribution, PHT4 in the Golgi apparatus regulates cytosol P transport, and PHT5 in the vacuole regulate vacuolar P transport [38].

Physical mechanisms of soil P recovery

Here, we define physical mechanisms as those associated with root architecture. Phosphorus is soil-immobile, so there is a heterogenous distribution of P in soil systems[39]. As plants take up P, they create a concentration gradient around the root[40]. As P diffuses through the soil, plant roots intercept this P and take it up through their root system. Because a major method of soil P acquisition is root interception, proper root development to exploit a given volume of soil is essential[41]. Thus, the production of adventitious and ageotropic roots is an advantageous method to scavenge potential pools of soil P[39]. As described in their work with bean (*Phaseolus vulgaris*), Lynch and Brown[42] determined that overarching P efficiency was largely determined by the plasticity in root gravitropism and adventitious root growth. The authors contended that plasticity in root architectural traits was quantitively inherited and can be putatively used in marker aided breeding strategies[42]. Thus, these physical mechanisms of soil P recovery can be used in breeding programs to further enhance the P acquisition efficiency of modern crops. Although both wild and domesticated crops engage in these physical shifts in response to low P, there is a marked difference in the degree to which wild and domesticated crops change their root architecture in response to deplete P conditions.

Topsoil foraging

Root plasticity expressed through topsoil foraging is a valuable trait which can allow for better exploration of shallow soil horizons[42,43]. Compared to deep soil horizons, surface horizons have greater P concentrations across a range of cropping systems and soil types[24]. This stratification of P is often present in agroecosystems because of manure and fertilizer application, both of which increase soil P concentrations in the top 5 cm of soil over time[44]. Therefore, when P is deficient, there is often a reduction in primary root growth in plants[43]. It is important to consider, however,

that this reduction in primary root growth because of P deficiency occurs when P is the most limiting nutrient. Root architectural responses have also been noted for deficiencies of other nutrients such as N and K[45]. The resultant shallower root system can then better exploit a given volume of soil for soil nutrients. This strategy of topsoil foraging has been noted in several horticultural and agronomic crops. Genotypes of maize (*Zea mays*)[46] and bean[42] with shallow root systems have been shown to accumulate more P from soils and express better growth than those genotypes with deeper root systems.

Because of agricultural innovations (e.g., Green Revolution, fertigation), modern agricultural systems have become more exposed to increased total amounts and incidents of fertilization[18,47,48]. Over time, this increased fertilization causes an accumulation of nutrients in the soil surface and therefore a stratification of soil P[44]. Applications of organic material such as compost and manure can also increase the P stratification[49,50]. Current crop cultivars are adapted to grow in these highly fertile and P-stratified environments. Long-term fertilization over the course of centuries may therefore have altered the growth habit of modern crops to promote root growth along the topsoil horizon. For example, Zhao et al.[51] screened over 300 accessions of soybean across a domestication gradient (wild, semi-wild, cultivated) and examined their spatial root configuration under P deficiency. The authors found that under low P, there was a clear evolutionary pathway in root architecture: from deep to shallow root systems[51]. Cultivated soybean had shallow roots, the wild had deep roots, and the semi-wild showed an intermediate root system[51]. The shallow roots system of cultivated soybean allowed it to better acquire

nutrients in the topsoil, and modern soybean therefore had greater P efficiency than its wild ancestor.

This trend of root topsoil foraging promotion along a domestication gradient continues for other horticultural and agronomic crops. Akman[52] conducted a study exploring the P efficiency-related root traits of domesticated and wild wheat. In this study, great genotypic diversity was identified, and ultimately, modern wheat varieties were shown to distribute their root biomass along the topsoil region of the soil profile more than wild wheat varieties[52]. Moreover, in their recent comprehensive assessment of P-stressed modern and wild tomato, Demirer et al.[53] illustrated the divergence in primary root elongation. The authors determined that modern tomato showed marked reductions in primary root elongation when P levels were reduced. However, in wild tomato, there was no reduction in primary root growth[53]. Thus, topsoil foraging was more apparent in the modern tomato accession compared to the wild tomato accession. Furthermore, as demonstrated by Rahman et al.[54], domesticated and wild lettuce (*Lactuca sativa*) showed different root architectural traits when exposed to P deficiency. The domesticated variety expressed shallow root growth and enhanced topsoil foraging, whereas wild lettuce showed greater taproot elongation and greater total taproot depth[54]. Therefore, when examining crops across a domestication gradient, there is evidence that domesticated accessions tend to showcase a strategy of reduced primary root growth and enhanced topsoil foraging (Figure 1-2A).

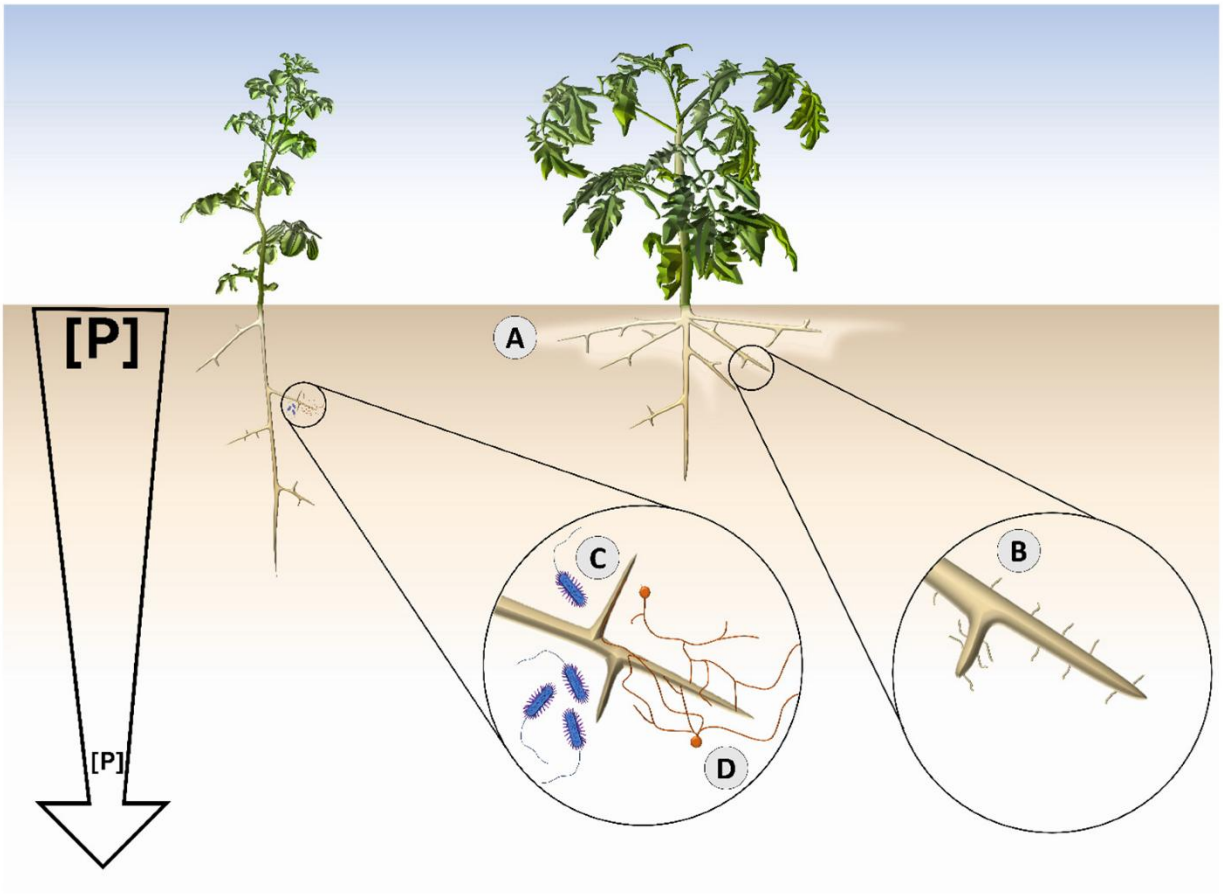

Figure 1-2. Prototypical methods of soil phosphorus (P) acquisition in P deplete conditions in wild (left) and modern (right) crops. Relative soil P concentration is illustrated with the arrow on the left (topsoil with high concentration and deeper soil with low concentration). To recover P in low-P soils, modern crops tend to exploit topsoil horizons which have a greater abundance of available P compared to lower soil horizons (A) and show responsive root hair growth that increases in length with decreasing concentration of P (B). Wild crop relatives show strong responsive associations with soil P solubilizing bacteria (C) and with arbuscular mycorrhizal fungi (D). Figure generated by the authors using information culled from sources cited within this article[18,26-28,44,47-79].

However, this pattern of decreased primary root length is not as apparent when examining cultivated varieties across time. Ning et al.[80] determined that for maize, newly developed cultivars were shown to possess deeper roots and more root length than previous varieties when they reached the post-silking stage of development. However, prior to maturity and at silking, the newer maize varieties had the same root length as the older varieties. Ultimately, across horticultural and agronomic vegetable crops, there appears to be a trend

toward exploiting the topsoil horizon to acquire nutrients as crops progress with domestication, even if select newer cultivars do not follow this trend.

Nutrient-stratified soil environments that are common in agroecosystems provide the ideal environment for the strategy of topsoil foraging to be effective. Conversely, wild crop relatives—which grow in less stratified and more homogeneous natural soils—are not adapted to nutrient stratification and are not equipped to use topsoil foraging as means to recover soil P (Figure 1-2A). Rubio et al.[55] demonstrated that plants grown in soils with a uniform distribution of P, regardless of concentration, did not benefit from having shallow root systems. Thus, modern crops that have been bred and developed over many generations may be adapted to these nutrient-stratified soils and are therefore better equipped to use topsoil foraging.

Shifts in the production of root types and density

Plants are sessile organisms, so a necessary strategy to adapt to abiotic stressors (e.g., P deficiency) is to adjust their root spatial configuration[81]. When exposed to mechanical impedance and other stressors, plants may promote lateral rooting by initiating a primordium in the pericycle cells below the root apical meristem[81]. Like topsoil foraging, increasing lateral root density allows for improved P uptake from soil systems as a result of more intensive exploration of soil[82]. Lateral roots are regulated by P availability and play an important role in P solubilization and acquisition because the lateral roots requires less P and biomass investment in development[83]. As shown in greenhouse and field trials with maize, the accessions with greater lateral root density (IBM79, IBM295, IBM301, IBM321) were more efficient in accumulating P[82]. Because of the nutrient stratification that occurs in agroecological soils, lateral rooting may have been positively affected by domestication.

Modern maize and wild teosinte have been shown to display markedly different root structures when grown in low P, likely as a result of different P conditions experienced by wild teosinte and modern maize. For example, domesticated maize readily produced lateral seminal roots whereas wild teosinte did not[26]. This disparity is significant because approximately one third of the P acquired by maize during its life history is acquired through its seminal roots, and thus, domesticated maize is more adapted to acquire P than wild[26]. It has been proposed that the genes for seminal root and lateral root development in modern maize were indirectly selected, but the benefit from these genes is apparent in P acquisition[56]. However, it is important to note that while possessing fewer seminal roots, wild teosinte has an abundance of very fine roots (i.e., <0.03 mm diameter), thus increasing its total surface area to volume ratio[84]. This finding is valuable because root hairs are responsible for up to 50 % of total root P uptake[85]. Wild teosinte is adapted to environments with poor P bioavailability, and it may therefore be possible that teosinte utilizes root hairs to acquire soil P.

Although domesticated maize showed greater lateral rooting but less root hair growth compared to wild[26,84], domesticated tomato showed both increased lateral rooting and root hair growth relative to wild[53]. In low P, modern tomato increased lateral rooting and root hair elongation[53] (Figure 1-2B). Wild tomato was insensitive and maintained consistent lateral rooting and root hair length regardless of P level[53]. Further, greater lateral rooting has been observed in domesticated lettuce rather than wild lettuce[86]. Thus, across different crop species, there may be improvements in rooting strategies to quickly and efficaciously acquire P from fertile soil systems common in agricultural systems, such as through investment in lateral rooting structures.

Selective partitioning of biomass and phosphorus

One method to maximize the potential for root interception of soil P is to preferentially promote root biomass and thereby increase the root-to-shoot ratio (R:S)[39]. At the expense of shoot growth, plants tend to allocate energy and resources into root growth[39,87]. This pattern of selectively partitioning biomass to root tissue rather than shoot tissue in response to P deficiency has been observed in many species such as lantana[87], maize[88], rice[89], and clover[90]. The promotion of R:S is moderated by the P status of the soil. Under conditions of deficient P, sucrolytic activity is largely inhibited in roots, resulting in a greater hexose-to-sucrose ratio, root biomass accumulation, and stable sucrose utilization in roots[88]. Shoot growth is therefore inhibited and carbon is allocated to root tissue[91].

Although there tends to be a relationship between R:S and external P levels, there is variability and inconsistency in reported R:S responses for wild crops and their domesticated counterparts. Araújo et al.[92], across two experiments and two P levels (20 and 80 mg P·kg−1 soil), assessed the P efficiency traits of appx. 30 genotypes of common bean. The authors found that in both experiments, regardless of P level, cultivated common bean showed greater R:S than wild varieties[92]. However, certain domesticated agronomic crops show diminished R:S compared to their wild ancestors. Cultivated wheat varieties tend to partition less biomass to their root tissue compared to wild varieties[57]. Similarly, wild teosinte has been shown to have a narrower R:S compared to cultivated maize[84]. These differing responses to P deficiency among species of wild and domesticated crops indicate that the variability in R:S may be genotypic rather than a domestication-driven change.

Ecological mechanisms of soil P recovery

Ecological mechanisms are defined here as those that are involved in the interactions between plants and their surrounding microbes in the rhizosphere. The rhizosphere is the compartment of

soil that is influenced by the root[93]. It is a highly active region comprising diverse microbiota that cohabitate and interact with the host plant[94]. Co-evolved root-associated microbial communities influence the ability of the plant host to tolerate environmental stressors, such as nutrient deprivation[5]]. For example, P-solubilizing bacteria transform recalcitrant P to plant-available forms[58] and arbuscular mycorrhizal fungi that help to better exploit a given volume of soil[59] (Figure 1-2D). These root-microbial associations are essential for plant survival, and it is recognized that plants themselves are not standalone entities, but rather a holobiont of the plant host and its associated microbial communities[96].

Rhizosphere microbial composition can change with environmental conditions and host plant genotype, and the rhizosphere microbiomes of wild crop relatives are often shown to be more sensitive to environmental changes than domesticated varieties[60,61]. These highly responsive microbiomes found in wild crop relatives often vary in composition from the microbiomes of modern crops[28,62,63,64,65]. These shifting microbial communities provide insights into P acquisition strategies and capabilities of the host plant as a result of crop domestication.

Alterations in the rhizosphere bacteriome

There are compositional differences between the rhizosphere bacteriome of domesticated and wild crops: wild being enriched in Bacteroidetes and domesticated being enriched in Actinobacteria and Proteobacteria[66,97]. Some of the species in these phyla are known to solubilize and mineralize soil P. Phosphorus solubilizing and mineralizing bacteria are able to convert sparingly soluble P into bioavailable forms, principally through the production of extracellular hormones[98]. Fertilizers can affect soil bacterial populations[99], and while the population of some P-solubilizing microbes attenuate in high P soils that are common in agricultural conditions,

others maintain their populations in high and low P[100]. These P-solubilizing bacteria can be used as biofertilizers because of their potential to markedly improve P uptake by roots[98,101].

Though both P-solubilizing and P-mineralizing bacteria transform P from sparingly soluble to soluble forms, these two bacterial groups target separate pools of soil P. Phosphorus-mineralizing bacteria target organic P[101]. To mineralize organic sources, P-mineralizing bacteria harbor a member of the pho regulon, a phosphate regulation mechanism involving enzyme and phosphate transporter activity[102]. The most common enzymes capable of mineralizing organic P through the activity of hydrolyzing phosphomonoesters phosphodiesters include alkaline phosphatase (*phoA*), phospholipases (*phoD*), phosphodiesterase (*GlPQ*), and phytase (*PhyC*)[102,103]. The *phoD* gene is highly abundant in soil bacterial populations relative to the abundance of other phosphate-mineralizing enzyme families[104]. This gene is activated by calcium and can effectively hydrolyze both phosphomonoesters and phosphodiesters[104].

Conversely, P solubilizing bacteria do not target phosphoesters, but rather obtain P from poorly soluble precipitated secondary minerals, typically through gluconic acid exudation[105] (Figure 1-3). For gluconic acid to be produced, the glucose dehydrogenase enzyme and corresponding cofactor, pyrroloquinoline quinone (PQQ) are needed[105] (Figure 1-3). For PQQ

biosynthesis, the pyrroloquinoline quinone synthase C is required, which is encoded by the pqqC gene[105], and thus, P-solubilizing bacteria contain *pqqC* (Figure 1-3).

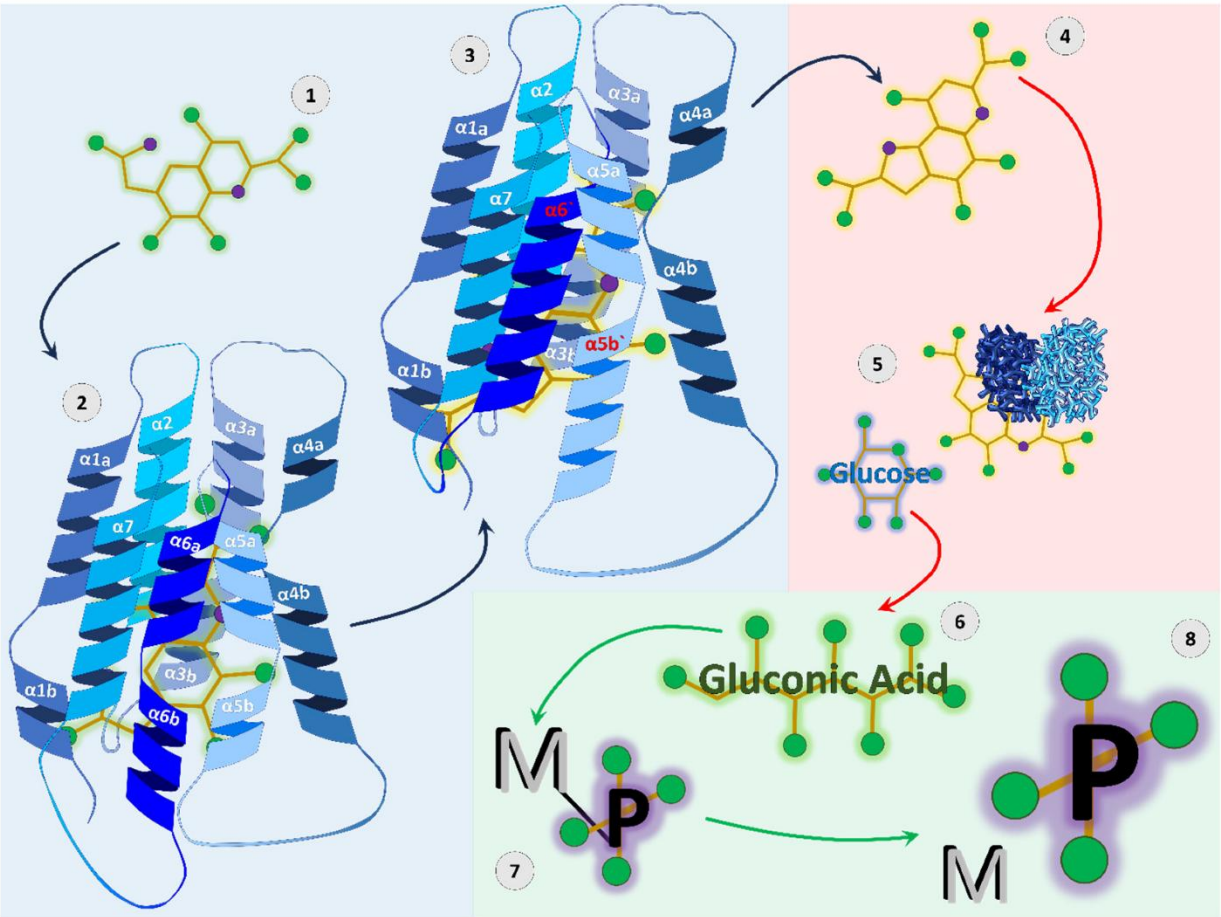

Figure 1-3. Mechanism of phosphorus (P) solubilization by the pqqC gene. The pqqC gene regulates soil P solubilization through biosynthesis of pyrroloquinoline quinone (PQQ) (blue background), subsequent catalyzation of gluconic acid (red background), and dissociation of metal-phosphate compounds (green background). An intermediate of PQQ enters the 7-helix structure of pqqC (steps 1 and 2). Once in the reaction matrix, pqqC undergoes conformational change at the α5b and α6 helices (step 3). PQQ is then released from the reaction matrix (step 4). PQQ functions as a cofactor to glucose dehydrogenase and catalyzes the reaction to form gluconic acid initially from glucose (steps 5 and 6). The acidification and reductant potential of gluconic acid allows it to react with metal-phosphate compounds (step 7). The metal-phosphate compounds dissociate, and bioavailable phosphate is released into the soil solution (step 8). Figure generated by the authors from information cited within this article[98,105].

It has been proposed that wild crop ancestors form stronger associations with the members of their rhizosphere microbiome than modern crops[66,67,68]. In wheat, for example, the functional diversity of the wild ancestor is greater than domesticated relatives[106]. This type of heightened diversity is beneficial because functional traits (e.g., hormone balance, nutrient

cycling) promote plant fitness[69]. These microbial differences are further illustrated in the diverging populations of wheat-associated P-cycling bacteria. Wild wheat is more strongly associated with P-decomposing bacteria (targets organic P), while domesticated wheat promotes P-solubilizing bacteria (targets inorganic P)[62]. Agricultural soils tend to have low organic matter compared to native ecosystems, such as grasslands or forests[107]. Thus, the prevalence of these two separate P-cycling bacterial groups in wheat may be a result of the soil environmental factors by which wild and domesticated wheat have evolved.

Further, in tomato, wild relatives were shown to accumulate an abundance of P-solubilizing and P-decomposing bacteria in low-P soil when compared to cultivated tomato[67,68]. These bacteria were also responsive; when P fertilizer was applied, the co-occurrence network complexity increased only in wild tomato, not domesticated[61]. Moreover, Cyanobacteria members have been shown to be enriched in the wild tomato rhizosphere[67], and bacteria in this phylum are important for promoting P cycling[70]. When Cyanobacteria was applied to soils, water-soluble P and Olsen-extractable P increased over a period of seven weeks[71]. Another notable phylum is Bacteroidetes, which tends to be enriched in the rhizospheres of wild crop relatives[57,66]. Bacteroidetes members have marked capacity to break down complex organic compounds, of which many root exudates are composed[57]. Thus, it is possible that this phylum is reliant on root exudates, and therefore, a more plant-responsive microbiome may be present in wild crops.

Although root–bacterial associations may have attenuated post-domestication (Figure 1-2C), the beneficial bacteria that colonize wild crop relatives are still highly useful for modern cultivars. In potato, it has been shown that inoculation of PSB isolated from its wild progenitor resulted in a significant improvement in biomass and shoot P concentration[108]. Similarly, in rice,

P solubilizing bacteria isolated from a wild ancestor was used as an inoculant for modern rice, and researchers found that inoculations improved growth[109].

Associations with arbuscular mycorrhizal fungi

Arbuscular mycorrhizal (AM) fungal symbiosis is estimated to have originated 450 million years ago, and approximately 80 % of terrestrial plants form symbiosis with AM fungi[63,110,111], but it has been only in the past 30 years that we have begun to understand the underlying mechanisms of AM fungal symbiosis[63]. There are two main groups of mycorrhizal fungi: aseptate (meaning hyphae are present without separating cell walls, e.g., Glomeromycota) and septate (meaning hyphae are separated by cell walls, e.g., Basidiomycota)[112]. AM fungi are endomycorrhizal fungi because they colonize the intracellular space, rather than the intercellular space and root tip (i.e., ectomycorrhizal fungi (EM fungi))[112]. While EM fungi are prominent in forest systems, AM fungi are the dominant form of symbiosis on a broader scale[112].

Like high-affinity root phosphate transporters, there are also AM fungi phosphate transporters. The high-affinity (18 μM Km) transporter, GvPT (identified from *Glomus versiforme*) is expressed in the extraradical mycelia, thus indicating its active selection of phosphate from the soil solution. AM fungal transporter utilize either H^+ (PHO84) or Na^+ (PHO89) symport, at which point the phosphate enters the arbuscule branch[113]. This process is important because, even though it can be energetically expensive for plants, the colonization of roots by AM fungi can markedly increase P uptake, especially in low-P soils[114].

However, this energetic-nutrient tradeoff does not always net benefit the plant. The carbon cost of AM fungal symbiosis, concomitant with the typical high fertility levels of agricultural soils, indicates that symbiosis with AM fungi in modern plant hosts may be less

beneficial for the host plant[63,72,73] (Figure 1-2D). Current cultivation methods involving high pesticide use, nutrient input, and fallowing further exacerbate AM symbiosis imbalance[63]. While domesticated crops have been shown to benefit from AM fungal colonization, this benefit tends to occur only under P deficiency. For example, Martín-Robles et al.[73] grew 27 varieties of domesticated and wild herbaceous crops representing major families (e.g., Fabaceae, Cucurbitaceae, Poaceae). Upon inoculation of AM fungi in both high P and low P conditions, the representative wild progenitors showed clear benefit, with varieties either increasing biomass or P uptake. Conversely, the domesticated crops only showed increased production when AM fungi were inoculated at an initially deficient concentration of P[73]. Similarly, wild rice is more readily colonized by AM fungi[63]. Even when associating with AM fungi, domesticated rice shows reduced activity of the pathway for mycorrhizal P acquisition[27]. Further, in a field study with wild and cultivated soybean, there was AM fungal symbiosis present in wild soybean, particularly with an enrichment in *Paraglomus*, but no identifiable symbiosis in cultivated varieties[74]. Wild ancestors of sunflower (*Helianthus annuus*), barley, and wheat also form symbiosis with AM fungi more readily than domesticated counterparts[75-77].

This pattern of attenuating reliance on AM fungal associations with time continues even after domestication, as shown by wild compared to modern wheat. Varieties of wheat released prior to 1950 show greater dependence on AM fungal symbiosis compared to modern varieties[57,115]. This result indicates that there was apparent AM fungal colonization on wheat varieties released both prior and subsequent to 1950. However, the benefit (through increased P uptake and biomass) was more present in the varieties predating 1950. However, a wheat variety released in the 2000s shows enrichment of Glomeromycota in the rhizosphere, but a weakened association of fungi in general[116]. Therefore, although modern accessions are able to respond to

AM fungal associations, especially in low P, their capability to do so at high P levels is diminished compared to wild relatives.

Associations with non-AM fungi

Fungal populations are essential to soi health, and fungal richness has been positively related to ecosystem multifunctionality[99,117]. Thus, plants may form associations with both AM- and non-AM fungi as means to acquire soil P[118,119]. For instance, applications of varied fungal isolates to wheat promoted not just bioavailable P concentrations in soils, but also P uptake and plant growth by wheat[120]. In rice, fungal associations have been shown to help promote soil P mobilization in conditions of low P[119]. Further, *Rhizopogon luteolus*, an EM fungus that predominantly colonizes pine tree roots, produces an abundance of extracellular acid phosphatase which targets organic forms of recalcitrant P[118].

Like the diminishing associations with AM fungi, there may also be decreased associations with other fungal populations post-domestication. In wheat, for example, the relative abundance of bacterial and fungal populations changed with domestication[62]. There was a smaller proportionate composition of fungi compared to bacteria in domesticated wheat compared to wild[62]. Wild wheat has also been shown to accumulate more Ascomycota[78], a phylum that is known to harbor many different P solubilizing fungal species[79]. Similarly, wild legumes harbor greater diversity of fungal species in the rhizosphere compared to their cultivated counterparts[28]. The fungi associated with wild soybean also possessed more diverse putative beneficial functions compared to cultivated soybean, which harbored fungi capable of organic matter and cellulose decomposition[64]. Wild and cultivated soybean have been shown to recruit different fungi in their rhizosphere, with wild soybean soils being enriched in genera such as Ascomycota and Basidiomycota and cultivated being enriched in *Funineliformis* and

Rhizophagus[64,121]. Ultimately, there has been a shift in the composition of fungal populations in the rhizosphere of wild and modern crops, with an apparent reduction in functionality and diversity in domestication varieties.

Conclusions

Phosphorus (P) is a plant-essential nutrient that showcases poor soil mobility and bioavailability. Wild crop relatives are adapted to grow in unfertilized terrestrial soils, while domesticated cultivars grow in fertile P-stratified agroecosystems. When the pool of bioavailable orthophosphate is low, plants must acquire P from sparingly available sources to grow and develop. While the degree to which P acquisition strategies are employed varies with soil edaphic factors, it also shifts with the host plant at both a genotypic and domestication level. Here, we synthesized evidence from the current literature and showed that there are consistent differences in how domesticated and wild crops interact with the soil to obtain sparingly available P. Modern cultivars rely on direct physical mechanisms to recover soil P and optimize their root architecture to engage in topsoil foraging. This strategy is beneficial to modern crops as agroecosystems tend to showcase nutrient-stratified environments from long-term fertilization. Although modern crops show advantageous root structure, their dialogue with microbial partners is diminished compared to wild species. Across species, wild crop relatives emphasize an ecological approach to form more intimate associations with bacterial and fungal communities. This novel conclusion that wild relatives consistently promote an ecological response while domesticated crops engage in physical mechanisms to acquire soil P provides a valuable insight into how we can promote soil P cycling in modern cultivars. By understanding what mechanisms were lost to domestication, we can begin to develop methods to reincorporate these strategies and reduce exogenous P inputs in agricultural systems.

Contextualizing this literature review within the scope of this dissertation[2]

This literature review presented above delineates how the response to phosphorus (P) deficiency varies with the domestication status of the plant. Wild crops tend to promote microbial-facilitated P acquisition, while domesticated cultivars optimize root system architecture to maximize root intercept with soil P. Understanding these differences is important to enhance agricultural sustainability because phosphate rock, from which P fertilizers are mined, is finite in supply and the overaccumulation of legacy P in soils causes environmental degradation via eutrophication. Thus, elucidating the mechanisms of soil P recovery that have attenuated upon domestication allows for the opportunity to expand P acquisition in modern crops. Doing so would reduce the total need for exogenous P fertilizer application.

The following experimental chapters explore this above concept by investigating the varied P acquisition strategies employed by wild, traditional, and modern representatives of tomato. These subsequent chapters identify marked differences in the P recovery of wild and domesticated tomato in conditions of both P deficiency and P sufficiency. The results from the experimental chapters are in agreement with the findings by other researchers outlined in this review (i.e., wild tomato is microbially responsive and domesticated tomato efficiently exploits soil) and build upon these findings to provide novel knowledge. In the first experiment, we do this by exploring not just domestication-level effects on P recovery, but also subsequent breeding efforts. We discovered that breeding events like the Green Revolution did not further impact microbial associations related to P acquisition relative to the event of domestication. In the second experiment, we provided novel insights into how the microbiome of these tested tomato

[2] This section, "Contextualizing this literature review within the scope of this dissertation" is an addendum added to this dissertation, and is not a part of the published version of this article.

accessions respond to P additions. We discovered that the wild tomato rhizosphere, which was previously shown to be comprised of an abundance of P solubilizing bacteria, was highly responsive to P application, whereas the domesticated varieties had a relatively unresponsive microbiome. The final experimental chapter provides further novel insight into the divergent mechanisms of P recovery by wild and domesticated tomato. Here, we discover heightened soil P levels in wild tomato that are likely driven by the activity of wild tomato root exudates and its root-associated microbiota.

Ultimately, we use the framework of knowledge delineated in the above literature review describing the diverging P acquisition traits of wild and domesticated crops as a foundation for research. The following experiments use this knowledge to make new discoveries regarding plant P uptake and acquisition in wild and domesticated tomato. We contribute to scientific knowledge that wild tomato, particularly *Solanum pennellii*, forms unique rhizosphere associations though its root exudate activity that allow it to grow and take up P. Conversely, while the modern tomato representatives form less intimate microbial associations, it showcases greater overall tolerance to nutrient depletion. Together, these results inform on the ways by which domestication has attenuated biological associations between plants and their soil microbial communities and provides insight into how we can use these strategies by wild tomato to improve sustainable nutrient management in agroecosystems.

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CHAPTER 2 – TOMATO DOMESTICATION RATHER THAN SUBSEQUENT BREEDING EVENTS REDUCES MICROBIAL ASSOCIATIONS RELATED TO PHOSPHORUS RECOVERY[3]

Abstract

Legacy phosphorus (P) is a reservoir of sparingly available P, and its recovery could enhance sustainable use of nonrenewable mineral fertilizers. Domestication has affected P acquisition, but it is unknown if subsequent breeding efforts, like the Green Revolution (GR), had a similar effect. We examined how domestication and breeding events altered P acquisition by growing wild, traditional (pre-GR), and modern (post-GR) tomato in soil with legacy P but low bioavailable P. Wild tomatoes, particularly accession LA0716 (*Solanum pennellii*), heavily cultured rhizosphere P solubilizers, suggesting reliance on microbial associations to acquire P. Wild tomato also had a greater abundance of other putatively beneficial bacteria, including those that produce chelating agents and antibiotic compounds. Although wild tomatoes had a high abundance of these P solubilizers, they had lower relative biomass and greater P stress factor than traditional or modern tomato. Compared to wild tomato, domesticated tomato was more tolerant to P deficiency, and both cultivated groups had a similar rhizosphere bacterial community composition. Ultimately, this study suggests that while domestication changed tomato P recovery by reducing microbial associations, subsequent breeding processes have not

[3] This research was originally published in *Scientific Reports: Dixon, M.M., Afkairin, A., Davis, J.G., Chitwood-Brown, J., Buchanan, C.M., Ippolito, J.A., Manter, D.K. and Vivanco, J.M., 2024. Tomato domestication rather than subsequent breeding events reduces microbial associations related to phosphorus recovery. Scientific Reports, 14(1), p.9934.*

further impacted microbial P acquisition mechanisms. Selecting microbial P-related traits that diminished with domestication may therefore increase legacy P solubilization.

Introduction

Phosphorus (P), a plant-essential nutrient, undergoes soil processes, such as immobilization, precipitation, and adsorption, that reduce bioavailability[1]. Thus, to meet crop demand, modern cropping systems often rely on high applications of synthetic P fertilizers, which are sourced from nonrenewable rock phosphate[1,2]. Further, soil reactions that reduce bioavailability result in soil P accumulation in agroecosystems[2]. This growing pool of sparingly available legacy P provides a potential reservoir of nutrients which can offset inorganic P fertilization and reduce environmental risks associated with over fertilization, such as eutrophication[3]. Therefore, there is a need to improve P acquisition from legacy P pools.

Legacy P is sparingly available, but plant-microbe interactions promote P solubilization[4]. Exudation of root-derived secondary metabolites regulate these interactions, resulting in transformation of P from unavailable to bioavailable forms[5,6]. Root exudation may directly solubilize P or promote P-solubilizing microbial growth[7,8]. The ability of plants to form associations with P-solubilizing microbes varies between and within species[9-11]. Thus, strategies to enhance root-associated microbial interactions for the purpose of P solubilization are needed to reduce reliance on nonrenewable P fertilizers.

Root-associated microbiomes are impacted by numerous factors including exogenous P supply, soil properties, types of fertilization, plant development, and genotype[12-16]. For instance, tomato (*Solanum* spp.) rhizosphere bacterial communities vary between wild and cultivated groups[17,18]. Under fully fertilized conditions, groups of beneficial bacteria changed in abundance in the rhizosphere of successively planted tomato, in which wild tomato enriched *Rhizobium* and

Massilia growth, and modern varieties enriched *Pseudomonas*[19]. Separation of the microbial communities may be due to changes in long-term plant breeding goals and agricultural practices which have, over time, impacted soil fertility and the rhizosphere microbiome[20-22].

At domestication, desired traits were selected which were controlled by a limited number of alleles, resulting in a reduction in genetic diversity[23]. As breeding programs advanced, particularly during the Green Revolution (GR), cropping programs became reliant on increased use of inorganic fertilizers[24]. The Green Revolution resulted in high-yielding crops and intensive fertilization regimens which inadvertently affected rhizosphere community composition in crops such as wheat[22,25]. It is unknown if root-microbial associations related to nutrient solubilization are varied between pre-GR (traditional) and post-GR (modern) varieties of crops and how these abilities compare to wild crop relatives. Comparisons between wild, traditional, and modern varieties are therefore needed to discern differences in P acquisition.

To determine how two key plant breeding events (domestication and the GR) influenced P acquisition, representative wild, traditional, and modern tomato varieties were grown in sufficient and insufficient P conditions. Those varieties were grown in soil with low bioavailable P with the purpose of testing the ability of the plants to induce microbial associations related to P solubilization of legacy P. The overarching objective of this study was to examine soil bacteria and tomato domestication group interactions that relate to soil P solubilization and decomposition. It was hypothesized that tolerance to P deficiency and P solubilizing bacterial associations would differ among domestication groups. Ultimately, the varied performances of each domestication group in response to P deficiency elucidated how influential domestication and breeding are regarding rhizosphere P acquisition strategies.

Materials and Methods

Plant material selection

Twelve accessions were selected that represent tomato (*Solanum* spp.) across three domestication groups: modern, traditional, and wild (Supplemental Table 2-1). Modern tomato was defined as commercial cultivars grown currently ('Bobcat', 'Quali T') and experimental lines for incorporation into the commercial tomato market (Line 1, Line 2). Modern tomato accessions were donated by Syngenta (Delaware, United States) and are determinate, processing tomatoes grown in the San Joaquin Valley in California. Traditional tomato were commercial varieties developed before the Green Revolution and these seeds were retrieved from the W. Atlee Burpee Co. ('Rutgers' and 'B Pink') and Victory Seed Co. ('Matchless' and 'Marglobe'). The traditional varieties used are semi-determinate to indeterminate and are grown for fresh market. Wild tomato accessions were obtained from the Tomato Genetics Resources Center (TGRC) at the University of California Davis. The wild tomato species used included *Solanum pennellii* (LA0716), *S. pimpinellifolium* (LA1580), and *S. lycopersicum* var. *cerasiforme* (LA1519, LA1698). *S. pennellii* is adapted to grow in dry and rocky conditions with poor nutrient availability[26], and crosses of this species into cultivated tomato have led to improved abiotic stress tolerance[27]. *S. pimpinellifolium* is the wild progenitor of cultivated tomato and is a valuable resource in tomato breeding due to its stress tolerance[28]. *S. cerasiforme* originated from *S. pimpinellifolium* and migrated to Mesoamerica which may have resulted in reduced polymorphisms and heterozygosity[29]

Soil Collection

To induce P scavenging responses from plants, soils were sought out that had low bioavailable P and a total P concentration that would adequately supply P to plants if P were in an available form. Soil with this characteristic was identified at the Colorado State University (CSU) Agricultural Research, Development, and Education Center (ARDEC) in Fort Collins, CO. Soil was collected at a depth of 5-20 cm and was subsequently mixed with sand (Quikrete Play Sand, Georgia, United States) at a 1:1 by volume ratio to further dilute the P concentration. Water holding capacity was determined following the method of Afkairin et al.[30]. Bioavailable P was measured using the Olsen-P bicarbonate extraction method[31]. The diluted substrate had a low Olsen extractable P concentration (2.6 mg/kg) and greater total P concentration (371 mg/kg) (Table 2-1). The physical and chemical characteristics were analyzed by Ward Laboratories (Kearney, NE) (Table 2-1).

Table 2-1. Physical and chemical characteristics of soil and sand substrate. Agricultural soil was mixed at a 1:1 by volume ratio with sand.

Substrate Characteristic	Value
Soil pH	8.4
Soluble Salts (mmho/cm)	0.47
Water Holding Capacity (%)	12.0
Organic Matter LOI (%)	0.9
Sum of Cations (me/100g)	19.3
Nitrate-N (mg/kg)	3.9
Potassium (mg/kg)	130
Calcium (mg/kg)	3274
Magnesium (mg/kg)	285
Sodium (mg/kg)	58
Zinc (mg/kg)	1.04
Iron (mg/kg)	8.3
Manganese (mg/kg)	3.2
Copper (mg/kg)	0.38
Olsen-Phosphorus (mg/kg)	2.6
Total Phosphorus (mg/kg)	371

Greenhouse Study Conditions

Tomato seeds were surface sterilized with 3 % sodium hypochlorite for 30 minutes followed by five rinses of sterile distilled water, as recommended by the TGRC to readily germinate cultivated and related tomato seeds. The seeds were subsequently placed on moistened filter paper until radicle emergence. At radicle emergence, emerged seedlings were transplanted to a commercial potting mix comprised of sphagnum peat moss, pine bark, and perlite (PromixBK, Québec, Canada) until cotyledons fully expanded. After cotyledon expansion, seedlings were transplanted to pots (117 cm^2 surface area, 13 cm height) filled with a 1:1 by volume mixture of sand and soil.

Seedlings were left to establish in the soil and sand mixture for seven days before fertilization. There were two fertilizer treatments: a treatment fertilized to P sufficiency (high P) and an unfertilized treatment (low P). Because the soil mixture was deficient in nitrogen (N) (3.9 mg/kg) (Table 3), Environmentally Smart Nitrogen (ESN) (44-0-0) was applied at a rate equivalent to 118 kg/ha N to all treatments. Triple superphosphate (TSP) (0-46-0) was applied to plants at rates equivalent to 163 kg/ha P_2O_5 in the P sufficiency treatment. The rates of N and P fertilization were designed for optimum tomato yield based on preliminary soil tests (Ward Laboratories). Plants were arranged in a completely randomized design as determined by a research randomizer software[32]. There were ten replicates per treatment for a total of 240 plants.

Tomato plants were grown from June to August 2022 in a greenhouse at Colorado State University (CSU), Fort Collins, CO (40.572, -105.081). Temperatures ranged from 18 to 21 ºC during the day and 17 to 20 ºC during the night, with a photoperiod of 16 hr.

Biomass and Soil Sampling

Each pot represents an experimental unit. Therefore, for biomass and soil collection, one sample of shoot biomass, root biomass, bulk soil, and rhizosphere soil was collected per pot (N=240). Because plant interactions with microbial inoculants change at the flowering stage[33], samples were harvested during the vegetative phase so as not to conflate observed differences in the rhizosphere microbiome being caused by growth stage as opposed to genotype. To ensure all 12 varieties of tomato were harvested at the same developmental stage, harvest occurred at the end of the vegetative stage (8 weeks), and if any flowers showed signs of developing, they were removed. Plants were removed from pots and gently shaken to remove any loose soil. Soil that did not adhere to the roots was considered bulk soil and was collected for nutrient analysis. Remaining soil adhering to the roots was considered rhizosphere soil[32] and was removed from the root by gently scraping roots with surface sterilized (70% EtOH) polypropylene spatulas. Rhizosphere soil was collected in a sterile 15 mL falcon tube and stored in a -80 ºC freezer until DNA extraction.

After rhizosphere soil was collected, the roots were washed with water to remove any remaining soil. Roots were separated from shoots, and both were placed in a drying oven set at 70 ºC for 4 days. Dry root and shoot biomass were recorded. To account for natural variation in biomass accumulation among tomato genotypes, relative dry mass (RDM) and P stress factor (PSF) were calculated following the method of Bera et al. [34]:

$$RDM\ (\%) = \frac{DM_{P-}}{DM_{P+}} \times 100$$

$$PSF\ (\%) = \frac{DM_{P+} - DM_{P-}}{DM_{P-}}$$

where "DM_{P-}" is dry mass in the unfertilized treatment, and "DM_{P+}" is dry mass in the fertilized treatment. Expressing measurements in relative terms allowed for screening of P-efficiency traits for many genotypes which may express broad morphological variation naturally. Thus, a genotype with high RDM and low PSF would suggest that it is capable of tolerating P stress.

Nutrient Analysis

Bulk soil was air-dried for four days and subsequently processed through a 2 mm sieve. Bulk soil Olsen-P content, representing bioavailable P, was measured[31]. Individual shoot samples were ground to a fine powder and subsequently underwent a concentrated nitric acid digest following the method of Ippolito and Barbarick[35]. Total P concentration in tomato shoots were analyzed using inductively coupled plasma-atomic emission spectroscopy.

DNA Extraction and 16S rRNA Amplicon Sequencing

Rhizosphere soil samples were weighed to 0.25 g and total genomic DNA was extracted from these samples using the DNeasy Power Soil DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration (ng/µL) was quantified using a Qubit Fluorometer (Thermo Scientific, Illinois, United States).

Following DNA extraction, the bacterial 16S ribosomal RNA (rRNA) gene was amplified via polymerase chain reaction (PCR). DNA extracts were diluted at a rate of 1:20, as determined by the Qubit concentrations, with nuclease-free water. For every 4 µL of diluted DNA, 36 µL of master reaction mix was added, which contained 20 µL of Phusion HSII master mix (Thermo Scientific, Illinois, United States), 14.4 µL of nuclease free water, 0.8 µL of forward primer (27F Bacterial Mn, 5' – TTTCTGTTGGTGCTGATATTGC AGRGTTYGATYMTGGCTCAG – 3'), and 0.8 µL of reverse primer (1492 Universal Mn, 5' – ACTTGCCTGTCGCTCTATCTTC

TACCTTGTTACGACTT – 3'). This mixture was amplified under the following conditions: 98 °C for 30 s (1 cycle); 98 °C for 15 s, 50 °C for 15 s, 72 °C for 60 s (25 cycles); and 72 °C for 5 min (1 cycle).

The PCR products were removed from the thermocycler and were purified using paramagnetic beads (AMPure XP beads, Beckman Coulter, Brea, CA). Briefly, the paramagnetic beads selectively bound to the nucleic acids in our sample, and the adhered DNA was rinsed for 30 s twice in 70 % ethanol and eluted. The DNA in the purified PCR products was quantified fluorometrically and then diluted with nuclease-free water to reach a DNA concentration of 4 ng/µL. This diluted and purified PCR product was barcoded using 1 µL of sample-specific barcode per sample from the PCR Barcoding Expansion kit (Oxford Nanopore, Oxford, United Kingdom). In a new PCR plate, the 1 µL of sample-specific barcode, and 5 µL of the purified PCR product were added to 25 µL of Phusion HSII master mix and 19 µL of nuclease-free water. This mixture was amplified in a thermocycler under the following conditions: 98 °C for 30 s (1 cycle); 98 °C for 15 s, 62 °C for 15 s, 72 °C for 60 s (15 cycles); and 72 °C for 5 min (1 cycle).

PCR products were volumetrically pooled and were again purified using paramagnetic beads. The samples underwent ligation using the Ligation Sequencing Kit V14 (SQK-LSK114) (Oxford Nanopore Technologies, Oxford, United Kingdom) following manufacturer's instructions. DNA was quantified and adjusted to 20 ng/µL DNA. To prepare the minION flow cell (Oxford Nanopore Technologies, Oxford, United Kingdom) for sequencing, air was removed with a pipette and the flow cell was primed with a flush buffer. The library (50 mM) was added to the sample port in the flow cell. Sequencing data were collected over a period of 48 h using MinKNOW (Oxford Nanopore Technologies, Oxford, United Kingdom). Resulting signal data were processed (base call, demultiplex) through Guppy basecaller and were filtered to a 70 q

score. Taxonomic abundance profiles were generated using Emu, a microbial profiling software[36]. Data were then filtered to retain samples with greater than 10,000 reads.

KEGG orthologs that were used to determine predictive functions were identified using PICRUSt2[37] to map the proportion of the microbial community to the gene of interest (Supplemental Table 2-1). Phosphorus decomposing bacteria were those that have been identified to have at least one of the following genes: *appA* (acid phosphatase), *phoA* (alkaline phosphatase), *phoD* (alkaline phosphatase D), or *phoN* (class A acid phosphatase). Phosphorus solubilizing bacteria were those that possessed the *pqqC* gene (pyrroloquiniline-quinone synthase).

Statistical Analysis

RStudio version 4.1.2 was used for all statistical analyses. A nested analysis of variance (ANOVA), Y ~ Fertilization*Domestication /Variety, was used to determine differences in Y (PSF, shoot P uptake, Olsen-P, P solubilizer abundance, P decomposer abundance) at an $\alpha = 0.05$. "Fertilization" indicates the fertilizer treatment (unfertilized, fertilized to P sufficiency). "Variety" represents the 12 tomato accessions (LA0716, LA1580, LA1519, LA1698, 'B Pink', 'Marglobe', 'Matchless', 'Rutgers', 'Quali T', 'Bobcat', Line1, Line 2), and "Domestication" denotes the 3 domestication groups (wild, traditional, modern). Fertilization was incorporated into the calculation of relative shoot biomass, relative root biomass, and difference in shoot P, so "Fertilization" was removed from models testing those metrics. According to the residuals-leverage plot, there appeared to be an outlier present in the RDM and Olsen-P models. Using Studentized residuals, a Bonferroni outlier test determined if there was a mean-shift outlier present in these data. Replicate 8 of accession LA1580 was removed from the RDM calculation (Bonferroni-adjusted $p = 3.5\times10^{-40}$) and replicate 8 of fertilized accession LA0716 was removed

from the Olsen-P calculation (Bonferroni-adjusted $p = 5.8\times10^{-122}$). All the above analyses used a Tukey HSD test for mean comparison at an $\alpha = 0.05$.

A permutational analysis of variance (PERMANOVA) was run for each domestication group to examine differences in microbial community structure (Distance ~ Fertilization*Variety). "Distance" refers to the Bray-Curtis dissimilarity, which is a method to quantify distance and ordinate samples onto axes by their relation to reference points[38]. This model was run for each domestication group (wild, traditional, modern). To examine effects across all tested accessions, a nested PERMANVOA was used (Distance ~ Fertilization*Domestication /Variety). A pairwise Adonis test using a false discovery rate (FDR) p value adjustment was used for post-hoc testing at an $\alpha = 0.05$.

A differential abundance (DA) analysis was run using microbiomeMarker, an R/Bioconductor package[39], in which a Wald test was used to compare the log-fold change in bacterial abundance among domestication groups in a negative binomial distribution. An FDR p adjustment method was used to determine significant differences at an $\alpha = 0.05$. Counts were normalized using the relative log expression which used the abundance mean of all samples as reference for scaling. To assess predive functions of rhizosphere bacteria in unfertilized soil, data from PICRUSt2 was explored. PICRUSt2 accurately and significantly correlates gene abundances with those values produced from gene-specific primers, such as those for the *acdS* gene[40]. However, these functions that are linked to bacteria are solely predictive[41]. To understand the different predictive functional profiles in the microbiome, a one-way ANOVA (Abundance ~ Domestication) was run on the relative abundance data of bacteria possessing targeted genes. Data for fertilized soil were partialled out. Some residual plots for bacterial abundance were not normally distributed, so for those genes (*hcnA, budA, budC, E3.2.1.6, ISC, AcP,3PH, 4PH, phlD,*

ituA, fenA, srfAA, rifM, phzE, mbtI, entA, pchB, E3.2.1.21, appA, phoN, nifH, nifD, nifK, nrfA, nirA, NIT-6, nirK, nirS, norB, norC), data were log-transformed. A Tukey HSD test was used for post-hoc testing at $\alpha = 0.05$, and p values were adjusted using an FDR at $\alpha = 0.05$.

Results

Divergent Plant Growth in P Deficiency Across a Domestication Gradient

Domestication ($F=7.11$, $p=0.001$) and variety ($F=2.39$, $p=0.02$) significantly changed relative shoot biomass (Figure 2-1A). Traditional varieties, which were developed around the start of the 1900s (Supplemental Table 2-2), and modern varieties, which were developed after the 2000s (Supplemental Table 2-1), had a greater relative shoot biomass as compared to wild varieties (Figure 2-1A). LA1519, 'Brandywine Pink' (henceforth "B Pink"), 'Rutgers', and 'Quali T 27' (henceforth "Quali T") accumulated more relative shoot biomass than LA0716. Modern and traditional tomatoes had significantly greater relative root biomass than wild tomato (Figure 2-1B) ($F=13.01$, $p=8.95\times10^{-6}$). However, individual varieties did not differ in relative root biomass accumulation (Figure 2-1B) ($F=0.91$, $p=0.52$). Wild tomato also significantly differed from the cultivated varieties regarding its P stress factor (PSF) ($F=7.32$, $p=0.001$). This metric evaluates the degree to which a certain genotype is negatively affected by P deficiency. Wild tomato had a greater PSF than modern or traditional tomato (Figure 2-1C). Phosphorus stress factor also significantly varied among varieties ($F=2.58$, $p=0.01$). Accession LA1580 had a greater PSF than any other tested variety except for accession LA0716 (Figure 2-1C). Visually, stunted growth resulting from P deficiency were minimally observed in modern and traditional tomato as compared to the wild varieties (Figure 2-1D).

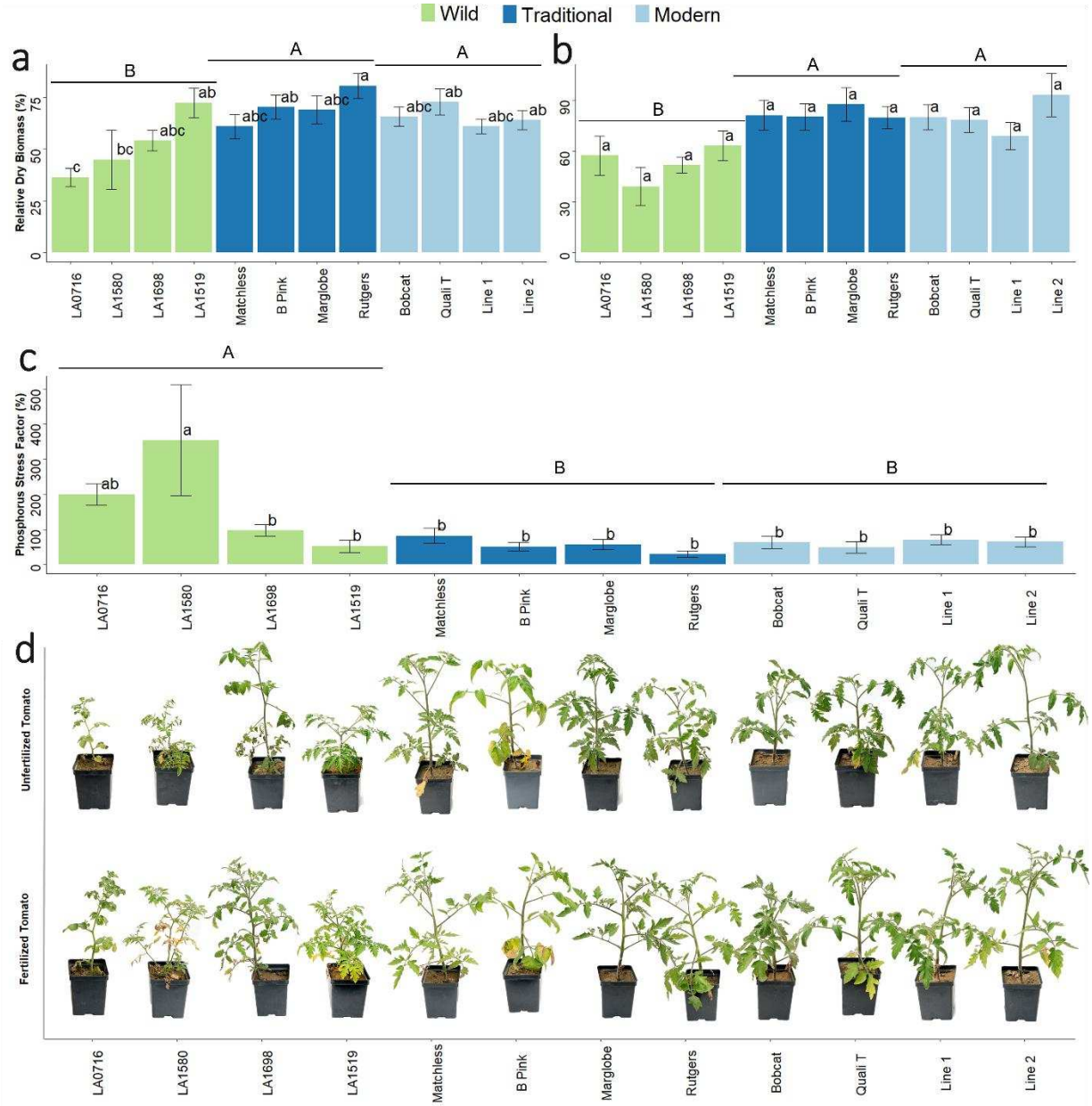

Figure 2-1. Phosphorus deficiency effects on tomato growth.
a *Relative dry shoot biomass. An ANOVA showed differences in for the Domestication main effect (df=2, F=7.107, p=0.001) and for the Domestication-Variety interaction (df=9, F=2.385, p=0.017).*
b *Relative dry root biomass. An ANOVA showed differences in for the Domestication main effect (df=2, F=5.31, p=0.006) and for the Domestication-Variety interaction (df=9, F=2.385, p=0.017).*
c *Phosphorus stress factor. An ANOVA showed differences in for the Domestication main effect (df=2, F=7.318, p=0.001) and for the Domestication-Variety interaction (df=9, F=2.58, p=0.010).*
d *Representative samples of each accession. The top row shows tomatoes grown in the low phosphorus treatment, and the bottom row shows tomatoes grown in the sufficient phosphorus treatment.*
Different colored bars indicate the level of domestication: wild (green), traditional (dark blue), and modern (light blue). An analysis of variance (ANOVA) was performed with a Tukey HSD test for means comparison. Different lowercase letters represent significant differences ($p < 0.05$) in tomato genotype. Different uppercase letters represent significant differences ($p < 0.05$) in tomato domestication group. Treatments sharing a common letter are not significantly different. Presented as mean ± SEM.

Soil Phosphorus Solubilization Changes with Tomato Domestication

P uptake (shoot mg P) showed a significant interaction effect between fertilization treatment, domestication group, and tomato variety ($F=5.3.57$, $p=4.00\times10^{-6}$). In fertilized soil, traditional tomato had the greatest P uptake, followed by modern tomato, and wild tomato (Figure 2-2A). 'Rutgers' and 'Marglobe' had greater P uptake compared to 'B Pink', Line 1, and every wild tomato variety (Figure 2-2B). In unfertilized soil, wild tomato had less P uptake than traditional and modern tomato, and Line 1 had a greater P uptake than LA0716. Phosphorus uptake difference from the fertilized treatment to the unfertilized treatment varied because of domestication ($F=5.31$, $p=0.006$) and variety ($F=2.92$, $p=0.005$) (Figure 2-2B).

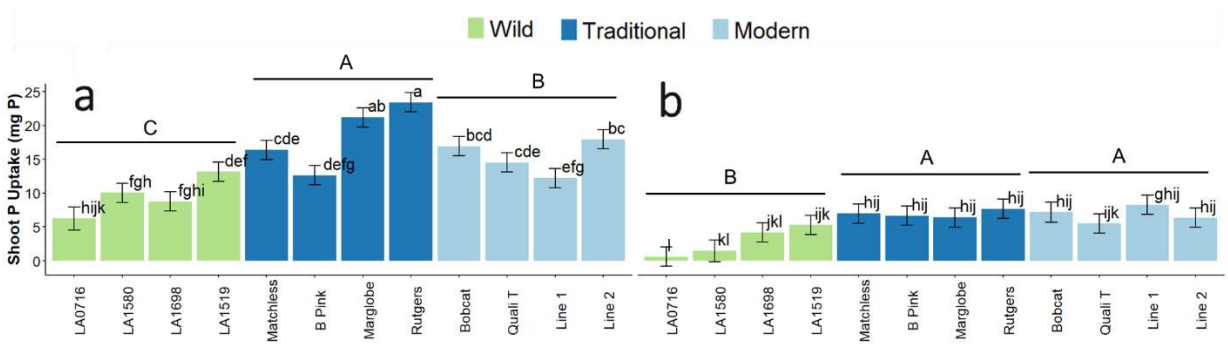

Figure 2-2. Plant phosphorus (P) uptake (shoot mg P) in tomato across a domestication gradient. ***a*** *Shoot P uptake (mg) in the fertilized soil. Presented as mean ± SEM.* ***b*** *Shoot P uptake (mg) in unfertilized soil. Presented as mean ± SEM. Different colored bars indicate the level of domestication: wild (green), traditional (dark blue), and modern (light blue). An analysis of variance (ANOVA) was performed with a Tukey HSD test for means comparison. Different lowercase letters represent significant differences ($p < 0.05$) in tomato genotype. Different uppercase letters represent significant differences ($p < 0.05$) in tomato domestication group. Treatments sharing a common letter are not significantly different. An ANVOA showed differences in shoot P uptake for the Fertilization-Domestication interaction ($df=2$, $F=5.223$, $p=0.006$) and for the Fertilization-Domestication-Variety interaction ($df=18$, $F=3.574$, $p=4.00\times10^{-6}$).*

There was a significant interaction effect in Olsen-P levels as a function of fertilization, tomato domestication, and variety ($F=5.00$, $p=2.17\times10^{-9}$). In fertilized soil, tomato domestication groups did not significantly vary in soil Olsen-P levels. Accession LA0716 had greater Olsen-P concentration than every accession except 'B Pink' in fertilized soil (Figure 2-3A). Regarding

soil P levels in unfertilized soil, Olsen-P concentration was higher in traditional compared to modern tomato, and in accession 'Rutgers' compared to Line 2 (Figure 2-3B).

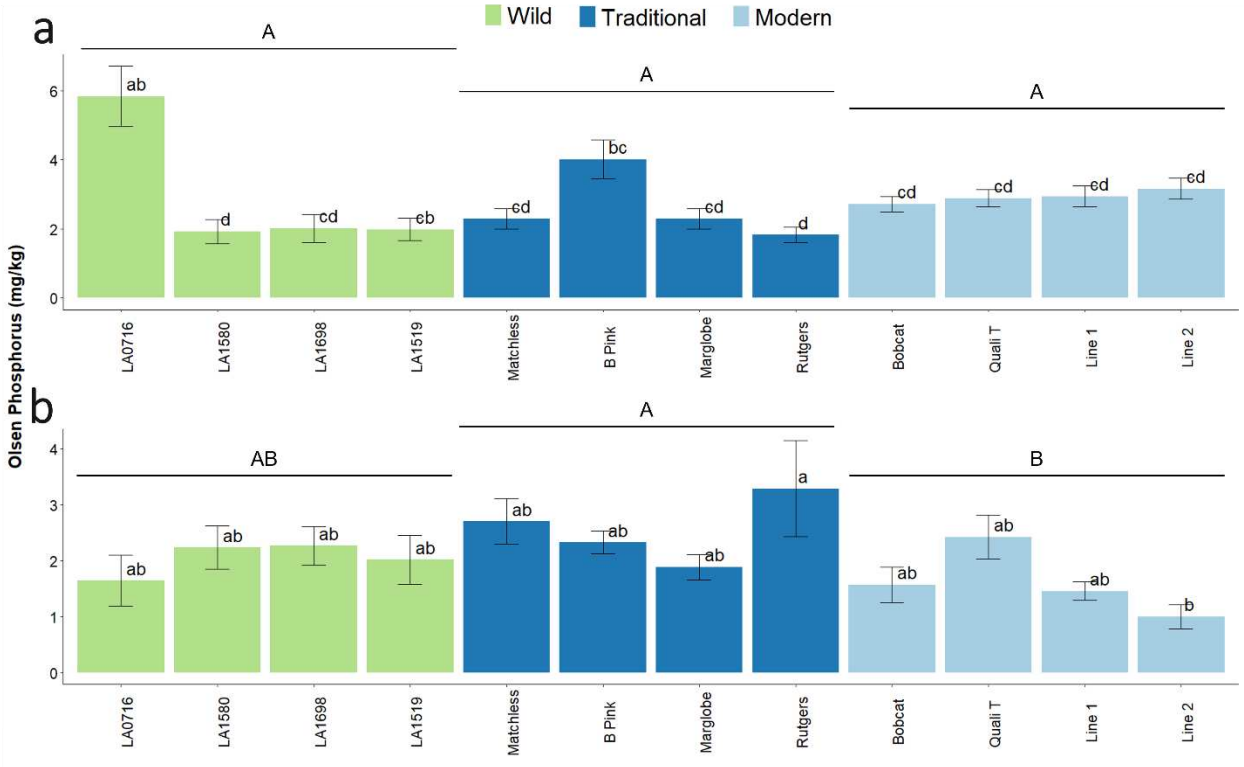

Figure 2-3. Bulk soil Olsen phosphorus concentration (mg/kg) in tomato across a domestication gradient.
***a** Olsen phosphorus concentration in fertilized soil. Presented as mean ± SEM.*
***b** Olsen phosphorus concentration in unfertilized soil. Presented as mean ± SEM.*
An analysis of variance (ANOVA) was performed with a Tukey HSD test for means comparison. While fertilization increased the Olsen-P concentration ($p < 0.05$), there was no significant difference in Olsen P concentration because of domestication or variety ($p > 0.05$). Treatments sharing a common letter are not significantly different.
Different colored bars indicate the level of domestication: wild (green), traditional (dark blue), and modern (light blue). An analysis of variance (ANOVA) was performed with a Tukey HSD test for means comparison. Different lowercase letters represent significant differences ($p < 0.05$) in tomato genotype. Different uppercase letters represent significant differences ($p < 0.05$) in tomato domestication group. Treatments sharing a common letter are not significantly different. An ANVOA showed differences in shoot P uptake for the Fertilization-Domestication interaction ($df=2$, $F=5.073$, $p=0.007$) and for the Fertilization-Domestication-Variety interaction ($df=18$, $F=4.996$, $p=2.17\times10^{-9}$).

Changes in Microbial Community Structure Among Domestication Groups

The rhizosphere microbial community structure across all domestication groups changed with domestication ($F=2.23$, $p=0.004$), fertilization ($F=18.19$, $p=0.001$), and variety ($F=51$, $p=0.002$) (Supplemental Figure 2-1). Pairwise adonis testing indicated that wild tomato had a significantly

different community structure than modern tomato (p_{adj}=0.015) and traditional (p_{adj}=0.03) tomato. However, the community structure did not statistically differ between traditional tomato and modern tomato (p_{adj}=0.64). Accession LA0716 had a significantly different rhizosphere community structure than most tested accessions (LA1580, LA1519, 'B Pink', 'Rutgers', 'Bobcat', 'Quali T', Line 1, Line 2) (Supplemental Figure 2-1).

Within each domestication group, the bacterial community structure significantly changed with fertilization as explained by the distance-based redundancy analysis (db-RDA), with 15 %, 12.3 %, and 12.3 % variation explained by the varietal and fertilization factors for wild (F=6.45, p=0.001), traditional (F=6.05, p=0.001), and modern (F=7.55, p=0.001) tomato, respectively (Figure 2-4). The first two axes of the db-RDAs explained 28.8 %, 19.6 %, and 18.7 % of the variance explained by a PCoA for wild, traditional, and modern tomato, respectively (Supplemental Figure 2-2). However, within the wild tomato rhizosphere, the bacterial community structure varied not only with the fertilization treatment, but also by tomato variety (F=2.17, p=0.001) (Figure 2-4a). Accession LA0716 had a different microbial community structure than did every other wild tomato accession (Figure 2-4A). Tomato variety did not change the bacteria community composition within the traditional (F=1.12, p=0.29) (Figure 2-4B) or modern (F=1.06, p=0.34) groups (Figure 2-4C). Although beta diversity – as measured by bacterial community composition – varied significantly with tomato domestication groups, Shannon's alpha diversity did not significantly change as a function of tomato domestication (F=0.67, p=0.51) (Supplemental Figure 2-3). This measure of alpha diversity only changed with

fertilization; fertilized soil increased Shannon's diversity compared to unfertilized soil (F=6.53, p=0.01) (Supplemental Figure 2-3).

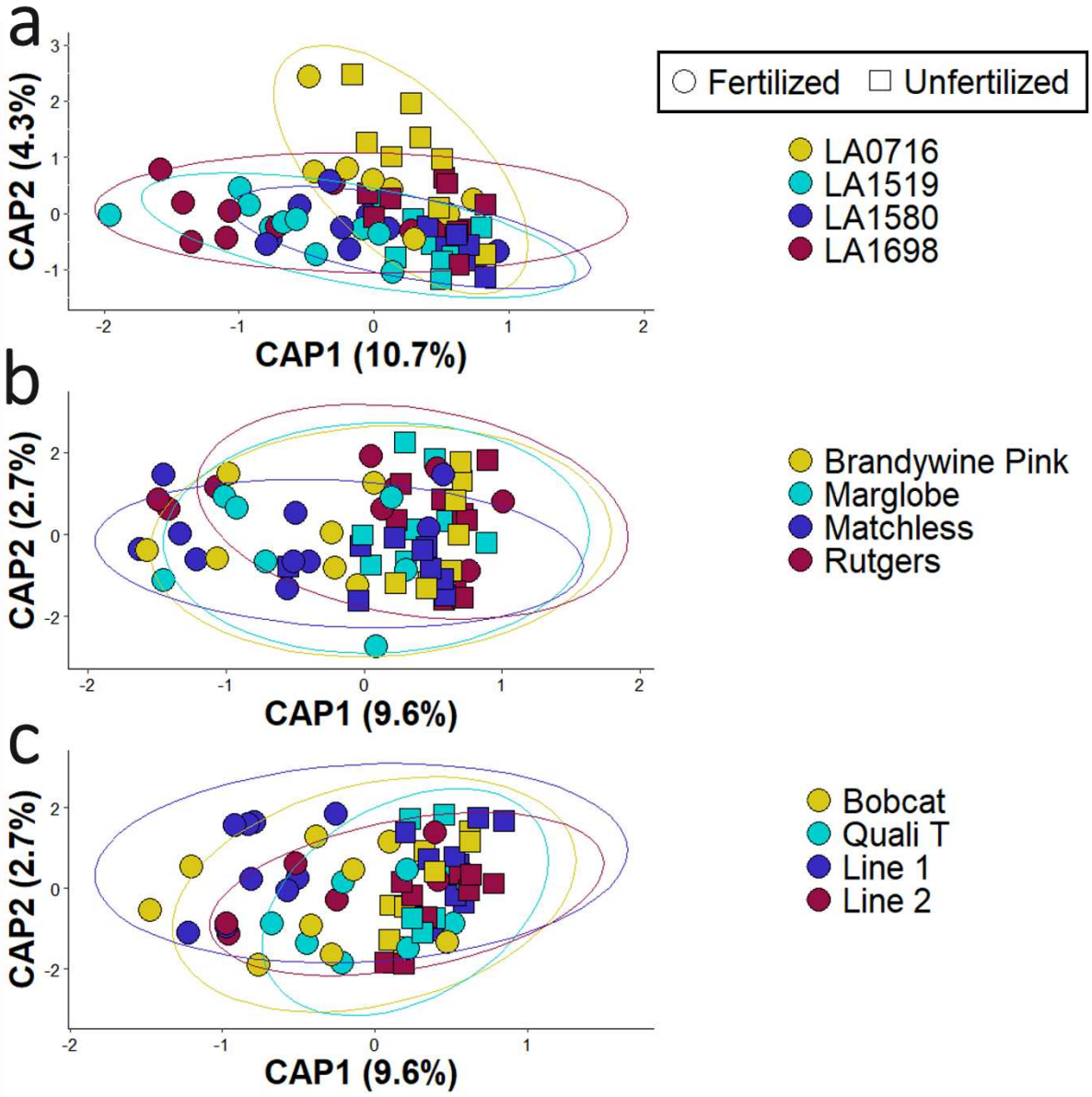

Figure 2-4. Distance-based redundancy analysis (db-RDA) showing clustering based on Bray-Curtis dissimilarity of the bacterial community structure in the rhizosphere of different tomato domestication groups.
a *Wild tomato showed separation based on fertilization treatment (R^2= 0.082, df=1, F=6.449, p=0.001) and variety (R^2= 0.083, df=3, F=2.170, p=0.001).*
b *Traditional tomato separated with fertilizer treatment (R^2= 0.084, df=1, F=6.054, p=0.001) but not variety (R^2= 0.047, df=3, F=1.125, p=0.289).*
c *Modern tomato separated with fertilizer treatment (R^2= 0.108, df=1, F=7.5488, p=0.001) but not variety (R^2= 0.045, df=3, F=1.06, p=0.34).*

The ellipses and colors indicate the different tomato varieties: LA0716, 'Brandywine Pink', 'Bobcat' (yellow); LA1519, 'Marglobe', 'Quali T' (light blue); LA1580, 'Matchless', Line 1 (dark blue); LA1698, 'Rutgers', V8053 (red). The shapes represent the fertilization treatment: fertilized (circle) and unfertilized (square).

A differential abundance analysis revealed differences in rhizosphere bacterial abundance among domestication groups as assessed by the log-2-fold change in abundance data. Three species [(*Rhizorhapis suberifaciens* ($p_{adj}=8.54\times10^{-30}$), *Microcoleus* sp. PCC 7113 ($p_{adj}=0.01$), *Rhizobacter gummiphilus* ($p_{adj}=0.001$)] were more abundant in the wild tomato rhizosphere compared to the traditional tomato rhizosphere (Table 2-2). According to PICRUSt2 analysis, two of these bacteria more abundant in the wild group were putative P decomposers (*Rhizorhapis suberifaciens, Rhizobacter gummiphilus*). Ten species changed in abundance between the wild and modern tomato groups. Of these ten species, six were enriched in the modern tomato rhizosphere [*Longimicrobium terrae* ($p_{adj}=0.02$), *Nitrospira moscoviensis* ($p_{adj}=0.03$), *Brevitalea aridisoli* ($p_{adj}=0.05$), *Brevitalea deliciosa* ($p_{adj}=0.03$), *Nitrospira japonica* ($p_{adj}=0.007$), *Vicinamibacter silvestris* ($p_{adj}=0.03$)] and four were enriched in the wild tomato rhizosphere [[*Polyangium*] *brachysporum* ($p_{adj}=0.04$), *Geitlerinema* sp. PCC 7407 ($p_{adj}=0.04$), *Leptolyngbya* sp. O-77 ($p_{adj}=0.04$), *Rhizobacter gummiphilus* ($p_{adj}=7.33\times10^{-7}$) (Table 2-2). Of these ten differentially abundant species, four were identified through PICRUSt2 to be P decomposers (*Nitrospira moscoviensis, Nitrospira japonica,* [*Polyangium*] *brachysporum, Rhizobacter gummiphilus*) and five were P solubilizers (*Nitrospira moscoviensis, Nitrospira japonica,* [*Polyangium*] *brachysporum, Rhizobacter gummiphilus, Vicinamibacter silvestris*). There were no differences when comparing the bacterial counts of traditional and modern tomato.

Table 2-2. Differentially abundant bacteria species enriched in different tomato domestication groups. A Wald test was used to determine differences. "Taxa" denotes the bacteria species that had a significantly greater abundance in the treatment represented in the "Enriched Group" column compared to the "Contrast" column. "Log Fold Change" shows the log-2-fold change in abundance between the "Enriched Group" and "Contrast". The p-value was adjusted using a false discovery rate (FDR) at 0.05. KEGG orthologs were used to determine the predicted function indicated in the "Predicted Phosphorus Function". Based on PICRUSt2, bacteria classified into the "Solubilization" group were predicted to contain the pqqC gene (KEGG Ortholog K06137), and those in the "Decomposition" group were predicted to contain the phoA (KEGG Ortholog

K01077), phoD (KEGG Ortholog K01113), phoN (KEGG Ortholog K09474), PHO (KEGG Ortholog K01078), or appA (KEGG Ortholog K01093) gene.

Taxa	Predicted Phosphorus Function	Enriched Group	Contrast	Log Fold Change	Adjusted p value
Rhizobacter gummiphilus	Decomposition	Wild	Traditional	1.126	1.29×10^{-3}
	Solubilization	Wild	Modern	1.398	7.33×10^{-7}
Rhizohapis suberfaciens	Decomposition	Wild	Traditional	32.740	8.54×10^{-30}
Microcoleus sp. *PCC 7113*	NA	Wild	Traditional	1.789	1.45×10^{-2}
[Polyangium] brachysporum	DecompositionSolubilization	Wild	Modern	0.310	4.05×10^{-2}
Geitlerinema sp. PCC 7407	NA	Wild	Modern	1.117	4.05×10^{-2}
Leptolyngbya sp. O-77	NA	Wild	Modern	1.259	4.31×10^{-2}
Nitrospira japonica	DecompositionSolubilization	Modern	Wild	0.369	6.69×10^{-3}
Longimicrobium terrae	NA	Modern	Wild	0.5602	1.70×10^{-2}
Nitrospira moscoviensis	DecompositionSolubilization	Modern	Wild	0.476	2.65×10^{-2}
Vicinamibacter silvestris	Solubilization	Modern	Wild	0.356	3.36×10^{-2}
Brevitalea deliciosa	NA	Modern	Wild	0.381	3.36×10^{-2}
Brevitalea aridisoli	NA	Modern	Wild	0.385	4.55×10^{-2}

Phosphorus-solubilizing and P-decomposing bacteria functional relative abundance (i.e., proportionate composition of PICRUSt2-determined P-solubilizing and P-decomposing bacteria to the whole bacterial population) were examined to further elucidate differences in microbial composition because of domestication. There was a significant interaction effect between fertilization, tomato domestication group, and variety ($F=2.94$, $p=1.1\times10^{-4}$). The wild tomato rhizosphere had a greater relative abundance of PICRUSt2-determined P solubilizing bacteria

compared to traditional and modern tomatoes in unfertilized soil (Figure 2-5A). Accession LA0716 had a greater relative abundance of P solubilizers compared to any other accession (Figure 2-5A). While domestication did not change the relative abundance of P decomposing bacteria in the rhizosphere (F=0.10, p=0.91), tomato variety did have an effect (F=2.18, p=0.005) (Figure 2-5B). Phosphorus decomposer relative abundance was higher in the rhizosphere of accession LA0716 compared to accession LA1698 and 'B Pink' (Figure 2-5B). However, when fertilizer was applied, these differences diminished. In fertilized soil, neither domestication nor tomato variety changed the P solubilizer (Supplemental Figure 2-4) or decomposer (Supplemental Figure 2-4) relative abundance.

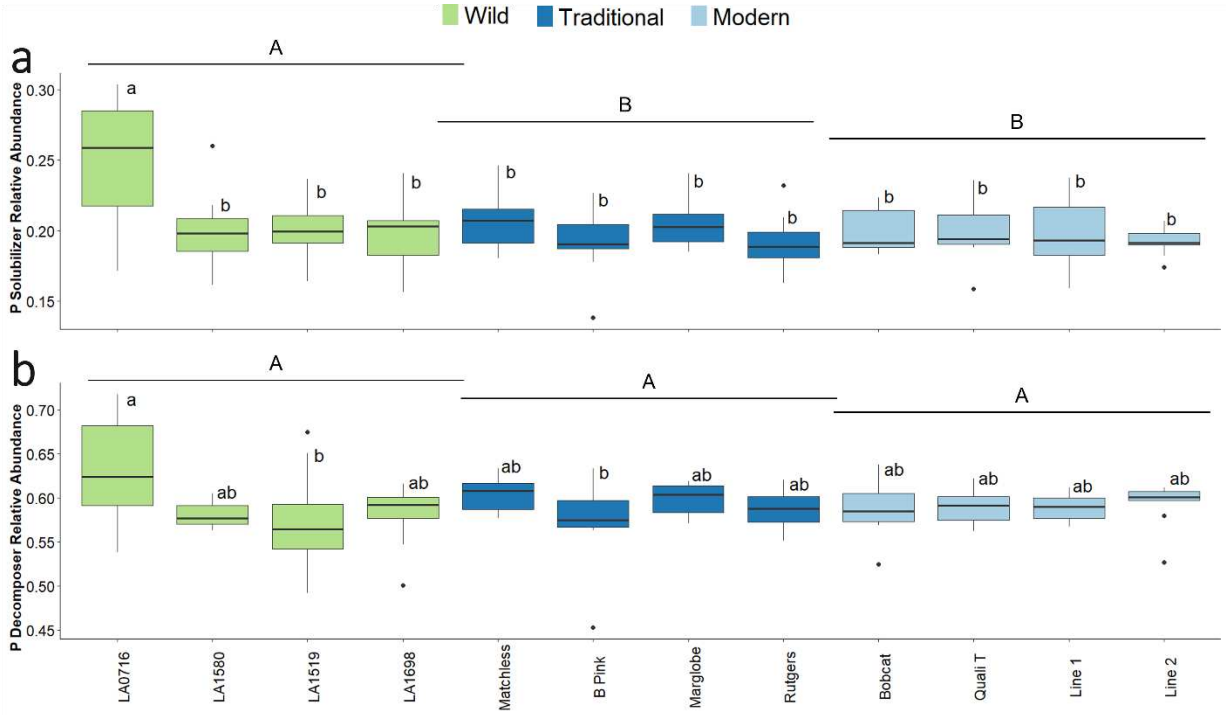

Figure 2-5. Phosphorus (P) solubilizing and P decomposing bacteria relative abundance in unfertilized soil. Different colored bars indicate the domestication level: wild (green), traditional (dark blue), and modern (light blue). An ANOVA was run with a Tukey HSD test for post-hoc comparison. Different lowercase letters denote significant differences ($p < 0.05$) for the different varieties. Different uppercase letters denote significant differences ($p < 0.05$) for the different domestication groups. Treatments sharing a common letter are not significantly different. ***A*** *P solubilizing bacteria in unfertilized soil. An ANVOA showed differences in shoot P uptake for Domestication main effect ($df=2$, $F=7.25$, $p=8.98\times10^{-4}$), Fertilization main effect ($df=1$, $F=36.47$, $p=6.78\times10^{-9}$), the Fertilization-Domestication-Variety interaction ($df=18$, $F=2.936$, $p=1.0\times10^{-4}$), but not the Fertilization-Domestication interaction ($df=2$, $F=0.114$, $p=0.893$).* ***b*** *P decomposing bacteria in unfertilized soil. An ANVOA showed differences in shoot P uptake for Fertilization main effect ($df=1$, $F=20.306$, $p=1.09\times10^{-5}$), the Fertilization-*

Domestication-Variety interaction (df=18, F=2.178, p=0.005), but not the Fertilization-Domestication interaction (df=2, F=0.202, p=0.817) or Domestication main effect (df=2, F=0.097, p=0.908).

Crop Domestication Altered Rhizosphere Bacteria Functions and Community Dynamics

We ran a comprehensive analysis of PICRUSt2 data to elucidate the genes that may be responsible for the interactions occurring between the tested tomatoes and rhizosphere bacteria in unfertilized soil. We found that some bacteria did not change in abundance among the different domestication groups (Supplemental Figure 2-2). Other bacteria potentially possessing enzymes with different functions (e.g., antibiotic, chelation, P decomposition) were significantly more highly associated with wild tomato roots as compared to modern or traditional tomato (Table 2-3). These bacteria contain genes that can encode for enzymes that are chelating agents (glycine dehydrogenase, salicylate synthetase), have antifungal (phloroglucinol synthase, iturin family lipopeptide synthetase D) and antibiotic (AHBA synthesis associated protein, 2-amino-4-deoxychorismate synthase) properties, decompose nitrogen (N) (urease alpha subunit), reduce nitrate (nitrite reductase), and promote denitrification (nitrate reductase, nitric oxide reductase) (Table 2-3). Only one predicted enzyme, isochorismatase, was in lower abundance in the rhizosphere of wild tomato (Table 2-3). Rather, isochorismatase-harboring bacteria were more highly associated in the rhizosphere of traditional tomato compared to wild or modern tomato (Table 2-3). There were also predicted gene functions that did not change in abundance as a function of domestication (biocontrol, root growth, chelation, carbon decomposition, denitrification, P decomposition, P solubilization, N fixation) (Supplemental Table 2-3).

Table 2-3. Varied relative abundance of bacteria with predictive functions among tomatoes across a domestication gradient in unfertilized soil. Bacterial relative abundance (presented as mean ± SEM) in unfertilized soil is shown for each tomato domestication group (Modern, Traditional, Wild). An ANOVA (F and FDR-adjusted p values presented) with Tukey HSD at α = 0.05 was used to determine differences in the relative abundance of rhizosphere bacteria as a function of tomato domestication group. Different letters within each row denote significant differences in the relative abundance of the bacteria with corresponding tested gene. For data that did not have normally distributed residuals, a log-transformation was run (denoted as

"L" in the F-value column). "Function" represents the predictive function for the bacteria with the corresponding gene listed in the "Enzyme (Gene)" column. This table shows the tested genes that were significantly different (Table S2 contains the tested genes that were non-significantly different).

Enzyme (Gene)	Function (KEGG Ortholog)	F	Adj. p	Relative Abundance		
				Modern	Traditional	Wild
Glycine dehydrogenase (*hcnA*)	Chelation[42] (K10814)	6.58[L]	0.027	0.053 ± 0.001 b	0.054 ± 0.002 b	0.067 ± 0.004 a
Phloroglucinol synthase (*phlD*)	Antifungal[43] (K15431)	6.04[L]	0.040	0.053 ± 0.001 b	0.054 ± 0.002 b	0.066 ± 0.004 a
Iturin family lipopeptide synthetase A (*ituA*)	Antifungal[44] (K15661)	4.82[L]	0.045	0.055 ± 0.001 b	0.055 ± 0.002 b	0.065 ± 0.004 a
Fengycin family lipopeptide synthetase D (*fenA*)	Antifungal[45] (K15667)	4.70[L]	0.045	0.065 ± 0.001 b	0.065 ± 0.002 b	0.075 ± 0.004 a
AHBA synthesis associated protein (*rifM*)	Antibiotic[46] (K16017)	4.71[L]	0.045	0.056 ± 0.001 b	0.056 ± 0.002 b	0.068 ± 0.004 a
2-amino-4-deoxychorismate synthase (*phzE*)	Antibiotic[47] (K13063)	5.38[L]	0.045	0.063 ± 0.001 b	0.064 ± 0.002 b	0.076 ± 0.005 a
Salicylate synthetase (*mbtI*)	Chelation[48] (K04781)	4.98[L]	0.045	0.057 ± 0.001 b	0.057 ± 0.002 b	0.067 ± 0.004 a
Isochorismatase (*entB*)	Chelation (EC.3.3.2.1_1)	7.95	0.027	0.029 ± 0.009 b	0.033 ± 0.001 a	0.028 ± 0.001 b
Urease alpha subunit (*ureC*)	Nitrogen Decomposition [49] (K01428)	5.31	0.045	0.376 ± 0.005 b	0.383 ± 0.007 b	0.413 ± 0.013 a
Acid phosphatase (*phoN*)	Phosphorus Decomposition [49] (K09474)	6.65[L]	0.035	0.071 ± 0.005 b	0.071 ± 0.002 b	0.087 ± 0.005 a
Nitrite reductase (NAD(P)H) (*NIT-6*)	Assimilatory Nitrate Reduction[50] (K00366)	4.65[L]	0.045	0.053 ± 0.001 b	0.053 ± 0.002 b	0.064 ± 0.004 a
Nitrite reductase (NO forming) (*nirS*)	Denitrification[5 1] (K15864)	5.85[L]	0.042	0.057 ± 0.001 b	0.058 ± 0.002 b	0.071 ± 0.004 a
Nitric oxide reductase (*norC*)	Denitrification[5 2] (K02305)	4.68[L]	0.045	0.083 ± 0.002 b	0.084 ± 0.003 b	0.098 ± 0.005 a

Discussion

Rock phosphate, from which phosphorus (P) fertilizers are mined, is a nonrenewable resource. Thus, efforts are needed to enhance P acquisition and recovery. Phosphorus stress responses vary not only among species, but also among different genotypes of the same species. Genotypic variation may, in part, result from domestication, as exemplified by bean (*Phaseolus vulgaris*)[53] and maize (*Zea mays*)[54]. Araujo et al.[53] found that wild beans accumulated less biomass than cultivated beans in soils with slightly less than optimal P levels (20 mg/ kg P). Further, Perkins and Lynch[54] identified that a greater number of embryonic roots in domesticated maize compared

to wild teosinte contributed to increased P acquisition in domesticated maize. However, studies investigating P acquisition-related traits at different stages of breeding, not just domestication, are lacking. Here, we examined the P acquisition response of wild, traditional, and modern tomato varieties by observing the effects of low P on plant growth and rhizosphere microbial community composition. We found that domestication altered tomato P acquisition traits, but among the traditional and modern varieties, P acquisition responses were similar.

Wild tomatoes may have relied on microbial associations to acquire P and cultured a unique soil microbiome (Supplemental Figure 2-1). Bacterial community composition varied with fertilization for all types of tomato, but for wild tomato, there were compositional differences as a result of variety (Figure 2-4). Approximately 10 % of the total variation in bacterial community structure was explained by fertilization and approximately 3-4 % of the variation was explained by the tomato variety grown, suggesting that tomato genotype and P fertilization have small but significant effect on the soil microbiome. Across all tomato varieties in our study, accession LA0716 associated with distinct soil bacteria (Supplemental Figure 2-1) and accumulated a greater relative abundance of possible P solubilizing bacteria (Figure 2-5). For example, *Rhizobacter gummiphilus*, which possesses the *phoD* gene that contributes to organic-P decomposition, was in greater abundance in the LA0716 rhizosphere. This accession has recently been shown to exude elevated amounts of acid phosphatase in response to P deficiency[26]. Increased phosphatase activity increases organic P decomposition, thereby increasing suitable conditions for soil microbes. Thus, heightened phosphatase exudation of LA0716 may further promote growth of *phoD*-harboring bacteria. Further, LA0716 also proliferates root hair growth in response to P deficiency[26], and increased root hair growth is associated with greater root exudation rates and microbial diversity[55]. By forming associations

with *phoD*-harboring bacteria, LA0716 prioritizes the strategy of decomposing sparingly available soil P pools. LA0716 is partially insensitive to levels of bioavailable phosphate, as outlined by Demirer et al. [26], which may explain why this accession uniquely responded to low-P stress compared to not only modern and traditional tomato, but also to other accessions of wild tomato.

As a group, wild tomato accessions had a greater relative abundance of P-solubilizing bacteria (Figure 2-5A), even though not every predicted P cycling function was in greater abundance in the wild tomato group (Table 2-3). This finding is in agreement with Tang et al. [56] who found that phosphatase exudation was greater in wild compared to domesticated barley. The interactions between roots and soil microbes that facilitate nutrient cycling may be more present in wild tomato and diminished with domestication. Therefore, we suggest that the methods of P recovery employed by tomatoes diverged at the initial event of domestication, but not after subsequent breeding and selection events. Wild tomato appears to rely on microbial P decomposers, but this reliance is not present in the modern and traditional tomato varieties which are more tolerant to P deficiency. However, these bacteria were identified using the predictive tool, PICRUSt2, which uses marker genes to map functions to microbial communities. Thus, although there is no direct confirmation of the functional capabilities of the selected microbes in this study, we can infer the functions which may be associated with bacterial taxa[41].

Our results indicate that wild tomato form associations with beneficial microbes, compared to either of the domesticated groups, as supported by wild tomato accumulating a high abundance of bacteria with the P-solubilizing, *pqqC* gene. Numerous studies have delineated the benefits to plant growth by P-solubilizing bacteria as a result of their organic acid production[57], auxin production[58], pathogen suppression[59], and ability to dissociate calcium-phosphate bonds[60].

Thus, it is possible that increasing the relative abundance of possible P solubilizing bacteria results in improved plant growth. Our tested wild tomato accessions showcased a high abundance of putatively beneficial bacteria, such as cyanobacteria members (*Geitlerinema* sp. PCC 7407, *Leptolyngbya* sp. O-77, *Microcoleus* sp. PCC 7113), which further supports our hypothesis that wild tomato relies heavily on microbial P scavenging strategies to grow and develop with low P. Afkairin et al.[30] found that in low-P soil, cyanobacteria additions increased the pool of soluble P across a seven week period; our experiment took place in a similar soil and over a similar time frame. Thus, the presence of these species may increase the pool of bioavailable P. The finding that wild tomato forms more associations with beneficial bacteria (as compared to traditional or modern tomato) is in congruence with past research focusing on the effects of domestication and symbiosis with plant growth promoting soil bacteria. Symbiosis with beneficial, nodule-forming bacteria (*Rhizobium leguminosarum*), for instance, had a greater ability to cause nodulation in wild legumes (*Vicia* spp., *Lathyrus* spp.) compared to cultivated relatives (*Pisum sativum*, *Vicia faba*) [61]. This finding is also supported by Kovacs et al. [17], who found that wild tomato had greater rhizosphere phospholipid fatty acid content than cultivated tomato, which would suggest a greater absolute microbial abundance in the wild tomato rhizosphere.

This study showed that wild tomato, in addition to interacting with P-solubilizers, highly associated with many other PICRUSt2-predicted beneficial rhizosphere bacteria (Table 2-3). Wild tomato, for example, had a greater abundance of bacteria that produce siderophores and hydrogen-cyanide (HCN) in unfertilized soil compared to modern and traditional tomato (Table 2-3). Siderophores and HCN are chelating agents, and bacteria that produce these compounds have been identified to help enhance P solubility in soil systems[42,62]. Thus, the tested accessions

of wild tomato may strategize soil P recovery by forming associations with bacteria that target P solubilization through diverse mechanisms (i.e., Fe-P chelation, Ca-P solubilization, organic-P decomposition). Many of the bacteria that were in greater abundances in the wild tomato rhizosphere compared to the traditional and modern tomato rhizosphere were predicted to be involved in biocontrol (i.e., produce enzymes with antibiotic and antifungal properties) (Table 2-3). Production of antifungal and antibacterial properties helps to support plant growth indirectly by mitigating or preventing negative effects of phytopathogens[63]. Therefore, the ability of wild tomato to strengthen associations with diverse soil bacteria supports the conclusion that reliance on microbial associations has diminished at domestication but has not been further affected. This conclusion is supported by Cordovez et al. [19] who investigated variation between wild and domesticated crop rhizosphere microbiomes. They found that not only are there differences in the soil microbiome of wild and cultivated tomato, but these differences are also amplified across planting cycles[19]. Yet, this trend may not be true across all crop species. Brisson et al.[64] found that a wild accession and a cultivated accession of maize both harbored phosphate solubilizing bacteria in the rhizosphere and had a similar root exudate response to P deficiency. However, because the researchers tested only one accession of each domestication group[64], and because maize has high genetic diversity[65], it is difficult to conclude whether or not this trait has been conserved through domestication and breeding. This contrasting result may also suggest that there is variation among different species in P acquisition as a function of domestication.

Wild tomato had a greater abundance of PICRUSt2-predicted P solubilizers, P decomposers, and chelating agent producers than domesticated counterparts, which may be explained by changes in root exudate profiles that occur with domestication. A recent study by Sun et al.[66] illustrated how the varied root exudate profiles in wild and domesticated rice (*Oryza*

spp.) caused a shift in bacterial chemotaxis systems in the rhizosphere and concluded that wild rice has co-evolved with microbes to adapt to harsher environments. Similarly, in our studies the exudate profile of tomato may explain how it interacts with the rhizosphere microbiome. In their study on root exudates of P deficient crops, Neumann and Römheld[67] found that hydroponic-grown tomato (cv. Moneymaker) highly exudes protons when in low P conditions, reducing the pH from neutral (7.0-7.5) to acidic (4.7). The 'Moneymaker' cultivar is a domesticated variety of tomato, and it is possible that wild tomato may not be exuding protons to the same degree as domesticated tomato. In calcareous soils, rhizosphere pH has been shown to increase near wild plants[68]. Therefore, because rhizosphere acidification through proton exudation may increase bioavailable P levels[69], traditional and modern tomato varieties may be prioritizing root exudation as a strategy to recover P from soils. Because root exudates impose strong selection power on the development of the microbiome[70], the varied root exudate profiles of the different tomato domestication groups in our study may be, in part, responsible for the changes observed in the microbial community composition. Therefore, exploration of root exudation provides possible avenues for future studies on the interactions between plant breeding events and the soil microbiome.

The possible changed exudate profiles may have caused a different microbial community composition in our tested accessions. Although the rhizosphere microbiome contained a high relative abundance of PICRUSt2-inferred beneficial bacteria in the wild tomato soil, modern tomato had a greater abundance of certain beneficial microbes. When comparing modern to wild tomato, modern tomato had a greater abundance of *Nitrospira moscoviensis* (Figure 2-5B), which facilitates carbon-P and nitrogen-P conversions[71]. However, when comparing modern to traditional tomato, there were no differences in the community structure or specific bacterial

species abundances. In our studies, modern and traditional tomatoes had similar responses to P deficiency, with the exception of Olsen-P. Modern tomato had less Olsen-P than traditional tomato in the low P treatment (Figure 2-3B). However, Recena et al. [72] showed that in low P soil, Olsen-P may not accurately predict plant P uptake. Therefore, although Olsen P varied with these two domesticated groups, this result on its own may not indicate that they differ in P efficiency or in their strategies to acquire P. Both traditional and modern tomatoes had greater relative shoot biomass (Figure 2-1A), relative root biomass (Figure 2-1B), lower P stress factor (PSF) (Figure 2-1C), and greater shoot P uptake (Figure 2-2A) than wild tomato. These differences illustrate the varied magnitude of impact P deficiency had on each tested domestication group. PSF was greater in wild compared to traditional or modern tomato (Figure 2-3C), which suggests that wild tomato may have less of an ability to efficiently acquire or internally utilize P[73], and therefore, cultivated tomatoes are more tolerant to P stress than wild tomato. This varied degree of tolerance in the tested domestication groups regardless of the rhizosphere microbial composition may be a result of crop improvements at domestication. For example, in a recent study of domesticated and wild tomato, it was found that the functions of soil bacteria in domesticated tomato were more highly affiliated with xenobiotic biodegradation and metabolism than wild tomato[74]. Therefore, although wild tomato may be accumulating a high concentration of P solubilizing bacteria – leading to potentially more bioavailable P – domesticated tomato harbor bacteria carrying out other plant-beneficial functions that may promote growth in nutrient-deplete conditions. These observed variations indicate that while reliance on microbial associations may be lessened at the point of domestication, domesticated varieties employ other strategies of soil P recovery.

Both cultivated groups had fewer PICRUSt2-predicted P solubilizers in the rhizosphere of low P soil (Figure 2-5A), suggesting that domesticated tomato may be utilizing intrinsic P acquisition strategies that are different from those employed by wild tomato. These strategies may include efficient translocation of P within plant tissue, as shown by P-utilization efficient bean[75] and lettuce[76]. Further, the larger root systems may lead to greater soil aeration and root exudation rates[55]. It is also possible that our tested modern and traditional tomatoes may be efficient in internal P sensing mechanisms. For example, regardless of P acquisition efficiency, *Banksia* spp.is highly P efficient because of its ability to effectively remobilize P from older and senescing tissue[77]. Rather than relying on *pqqC*-harboring bacteria, domesticated tomato may be associating with other beneficial bacterial symbionts. For example, modern tomato had a greater abundance of the PICRUSt2-predicted P decomposer, *Nitrospira japonica* (Table 2-2). *Nitrospira,* a genus of bacteria involved in P cycling from its enzymatic activity and its ability to alter N and C soil content[78], has been shown to be present in the rhizosphere of domesticated tomato and is abundant near roots when resources are scarce[79]. Although this present study did not investigate symbiosis with arbuscular mycorrhizal (AM) fungi, this strategy is likely not what is enhancing low-P tolerance of our modern and traditional varieties. As shown by Bryla et al.[80], cultivated tomato (*Solanum lycopersicum* 'large cherry') is less responsive to colonization by AM fungi as compared to wild tomato (*S. lycopersicum* var. *cerasiforme*). Further, when P is supplied, domesticated crops decrease symbiosis with AM fungi to a greater degree than wild relatives[81].

The rhizosphere microbiome was similar for traditional and modern tomato. Of the cultivated tomato groups, there were no differences in predicted P solubilizer or P decomposer abundance (Figure 2-5, Supplemental Figure 2-4), and a differential abundance analysis showed

that there were no bacterial species that changed in abundance between traditional and modern tomato. The tested traditional tomato accessions were developed and grown before the Green Revolution (late 1800s to early 1900s), whereas the modern tomato accessions are either grown today or are experimental lines for incorporation into future tomato markets. Overarching tomato breeding goals do not include nutrient use efficiency, but rather involve increasing productivity, disease resistance, and fruit health value[82]. Complex traits, such as low P tolerance, were likely indirectly selected through the tomato breeding process. Thus, cultivated tomato varieties respond similarly to a P deficit regardless of being developed in the 1800s or 2000s. However, because this study took place over a short time (approximately three months), the changes observed may not truly reflect the changes that may have occurred during the entirety of plant domestication or across a plant's reproductive development. Therefore, future studies would benefit from identifying changes in the rhizosphere across a tomato's growth stages.

In summary, our results suggest that wild tomatoes associate more preferably with bacteria that have beneficial functions, perhaps due to a greater reliance on microbes for P acquisition. The differences seen in wild tomatoes compared to the cultivated relatives indicate that there are changes in P acquisition efficiency due to domestication. Our results suggest that wild tomatoes, especially accession LA0716, rely heavily on associations with beneficial P bacteria to grow and develop under P deficiency. Conversely, modern and traditional tomato may be using strategies separate from microbial symbiosis to mitigate P deficiency symptoms. Although the tested domesticated tomato varieties were more tolerant to P stress, they were still negatively impacted by P deficiency as shown by reduced biomass and shoot P uptake. Future studies may be able to increase P acquisition efficiency by combining the divergent P acquisition methods observed here between cultivated and wild tomato and emphasizing those strategies

diminished at domestication. Ultimately, the approach used in this study could potentially be applied to other crops to further reduce our reliance on non-renewable rock phosphate.

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CHAPTER 3 - RHIZOSPHERE MICROBIOME CO-OCCURRENCE NETWORK ANALYSIS ACROSS A TOMATO DOMESTICATION GRADIENT[4]

Abstract

When plant available phosphorus (P) is lost from soil solution, it often accumulates in the soil as a pool of unavailable legacy P. To acquire legacy P, plants employ recovery strategies, such as forming associations with soil microbes. However, the degree to which plants rely on microbial associations for this purpose varies with crop domestication and subsequent breeding. Here, by generating microbial co-occurrence networks, we sought to explore rhizosphere bacterial interactions in low-P conditions and how they change with tomato domestication and breeding. We grew wild tomato, traditional tomato (developed circa 1900), and modern tomato (developed circa 2020) in high-P and low-P soil throughout their vegetative developmental stage. Co-occurrence network analysis revealed that as tomato progressed along stages of domestication, the rhizosphere microbiome increased in complexity in a P deficit. However, with the addition of P fertilizer, the wild tomato group became more complex, surpassing the complexity of traditional and modern tomato, suggesting a high degree of responsiveness in the rhizosphere microbiome to P fertilizer by wild tomato relatives. By illustrating these changing patterns of network complexity in the tomato rhizosphere microbiome, we can further understand how plant domestication and breeding has shaped plant-microbe interactions.

Introduction

[4] This research was originally published in *Microorganisms.* Dixon, M.M., Afkairin, A., Manter, D.K. and Vivanco, J., 2024. Rhizosphere Microbiome Co-Occurrence Network Analysis across a Tomato Domestication Gradient. *Microorganisms, 12*(9), p.1756.

Phosphorous fertilizer is mined from nonrenewable rock phosphate which may deplete in reserves within a century[1]. Thus, it is important to elucidate mechanisms of soil P transfer (from unavailable to available forms) to reduce the application of fertilizer sourced from rock phosphate. Beneficial soil microbes, for example, can increase soil P bioavailability by direct solubilization of precipitated phosphates and exuding extra-cellular enzymes such as phosphatases[2,3]. An important extracellular enzyme is alkaline phosphatase, which hydrolyzes organophosphate esters and makes recalcitrant forms of organic soil P plant-available[2]. Bacteria that produce alkaline phosphatase improve P absorption by plant roots[4], and genes that encode for alkaline phosphatase are widespread across prokaryotic soil phyla[2]. Some soil bacteria also contain a gene expressing pyrroloquinoline quinone synthase (*pqqC*), and these bacteria are capable of soil phosphate solubilization[5]. The *pqqC* gene plays a role in the synthesis of the co-factor, pyrroloquinoline quinone[6], which has several beneficial functions including plant growth promotion, systemic plant defense induction, and chelation of complexed phosphates[5].

A strategy for plant roots to acquire sparingly available soil P is to form associations with beneficial soil bacteria, such as those capable of phosphate decomposition and solubilization[7]. Roots form these associations by releasing chemoattractant compounds to select for certain beneficial microbes[8]. Attracting these bacteria alters the rhizosphere microbiome, a dynamic environment with diverse and populous microbial communities that is essential to plant health[8,9]. However, microbial associations with plant roots are highly influenced by the host plant as different plant species and cultivars can associate with distinct microbes[9-12]. For example, while the composition of bacterial endophytes did not change with between isogenic lines (expression and non-expression of *cry* genes), there were clear differences in root-associated microbial

communities among different maize varieties[13]. Genotypic variability in this capability has been observed in many crops including cotton[14], maize[15], wheat[16], melon[17], and lima bean[18].

Beyond inter- and intra-specific variation in microbial associations, there is also noted divergence in rhizosphere microbiome composition as a result of domestication and plant breeding events[19]. For instance, heirloom varieties of tomato harbor greater total microbial biomass in the rhizosphere when compared to modern accessions[20]. Further, the rhizosphere bacterial community of domesticated tomato varies from that of a wild progenitor, and these differences in the rhizosphere have been shown to amplify across successive planting of either domesticated or wild tomato[21]. Notably, wild tomato has also been shown to exude acid phosphatase to a greater degree[22] and more readily associate with P-solubilizing bacteria[12] than domesticated tomato. Similar patterns of divergence have been noted in other crops. Among maize accessions, it has been shown that landrace and inbred lines have greater alpha-diversity than wild teosinte[23]. These differences in the rhizosphere microbiome of wild and domesticated crops may relate to a disruption in the reliance on microbes to acquire nutrients from soil. Domesticated tomato plants, for example, are not only less responsive to beneficial soil bacterial associations[24], but they are also more likely to have negative soil-plant feedbacks than wild tomatoes[25].

Moreover, wild tomato relatives have been shown to be resilient to low-P stress[22,26]. In comparing the root-associated communities of a representative wild and domesticated tomato in P depleted conditions, it was shown that not only were there differences in alpha- and beta-diversity, but there were clear functional differences between these domestication groups. Wild tomato more intimately associated with P solubilizing bacteria than domesticated tomato, particularly in the endosphere[26]. Similarly, in conditions of low-P stress, wild tomato[26] increased

the relative abundance of P-decomposing bacteria compared to domesticated counterparts. These findings are valuable because low-P stress is a major driver of changes in the rhizosphere bacterial communities[27,28], and these microbes affect plant growth in conditions of nutrient stress[29,30]. Beneficial plant growth promoting bacteria, including strains of *Bacillus* and *Arthrobacter*, have been shown to solubilize P *in vitro*, and inoculations increase tomato biomass in conditions of P deficiency and high salt stress[31]. However, the interactions occurring among rhizosphere bacteria associated with low-P stressed crops are poorly understood. A method to elucidate microbial co-oscillations and associations affected by environmental disturbance is through generation of co-occurrence networks[32]. Thus, we aimed to utilize co-occurrence networks to explore the interactions occurring among bacterial groups in the rhizosphere of low-P stressed wild, traditional, and modern tomato. We hypothesize that network connectivity and microbial assemblages have changed across a tomato domestication gradient and are more affected by domestication than by soil nutrition.

Materials and Methods

The methods describing plant and soil selection, tomato growth conditions, DNA extraction, and PCR and amplicon sequencing presented in this manuscript are replicated from Dixon et al.[12]. Thus, all raw data used in this manuscript are culled from Dixon et al.[12]. However, all analyses and dataset presented here are new. A brief description of the replicated methodology is delineated below as well as a comprehensive description of novel *in silico* analysis.

Plant and Soil Selection

Four accessions were selected to represent each of the following tomato domestication groups: wild [*Solanum pennellii* (LA0716), *S. pimpinellifolium* (LA1580), and *S. lycopersicum* var.

cerasiforme (LA1519, LA1698)], traditional ('Rutgers' and 'Brandywine Pink', 'Matchless' and 'Marglobe'), and modern ('Bobcat', 'Quali T', Syngenta experimental line A, Syngenta experimental line B). Seeds from these selected varieties were retrieved from the University of California Davis Tomato Genetic Resources Center (TGRC) (all wild accessions), the W. Atlee Burpee Co. ('Rutgers', 'Brandywine Pink'), the Victory Seed Co. ('Matchless', 'Marglobe'), or through a donation from the Syngenta Co. (all modern accessions).

Field soil was collected from the Agricultural Research, Development, and Education Center in Fort Collins, CO. Soil was collected from the top 20 cm, avoiding topsoil. The collected soil was mixed at a 1:1 volume-to-volume ratio with sand (Quikrete Play Sand, Georgia, United States) to reach a final bioavailable P concentration, as approximated by the Olsen-P bicarbonate extraction[33], of 2.6 mg/kg.

Tomato Growth Conditions

To improve germination rates and decrease time to germination, seeds were surface sterilized following the recommendation of the TGRC[34]. Surface-sterilized seeds were placed in petri dishes on filter paper moistened with DI water. Once the seeds germinated, they were transferred to a low-nutrient potting mixture (PromixBK, Qué-bec, Canada). Seeds were kept in this potting mixture until the cotyledons fully expanded (approximately two weeks). At this point, the seedlings were transferred to pots, which contained the sand-soil mixture. Experimental units were grown in a greenhouse at Colorado State University which had the following environmental conditions: 18-21 °C daily temperature range, 17-20 °C nightly temperature range, and 16 hr photoperiod. The pots (dimensions: 11 cm width, 11 cm length) were arranged in a completely randomized design as determined by an open-source randomizing software[35], and each treatment had ten replicates (N=240). The fertilization treatment was applied one week after

transplantation. Half of the experimental units received sufficient P fertilization levels through application of triple superphosphate (TSP) (46 % P_2O_5) at rates equivalent to 163 kg/ha P_2O_5. To not conflate P deficiency symptoms with N deficiency, we fertilized all experimental units to reach N sufficiency using polymer-coated urea (44 % N) at rates equivalent to 118 kg/ha N. Plant growth was terminated eight weeks after fertilization.

Collection and DNA Extraction of Rhizosphere Soil Samples

Plants and attached soil were removed from pots and were gently shaken. Following the methodology of Pantigoso et al.[36], soil that remained adhered to the roots was defined as rhizosphere soil. Rhizosphere soil from each replicate (N = 240) was collected in sterile 15 mL centrifuge tubes and stored at -80 °C until DNA extraction. Following manufacturer's instructions, gDNA was extracted using the DNeasy Power Soil Pro kit (Qiagen, Hilden, Germany) and the automated QIAcube (Qiagen, Hilden, Germany).

PCR and minION Sequencing Conditions

All DNA-extracts from all soil samples underwent PCR and minION sequencing. DNA samples were fluorometrically quantified using a Qubit (Thermo Scientific, Illinois, United States) to determine the dilution factor of DNA extracts with sterile nuclease-free water (1-to-20 dilution). Each well contained 4 µL diluted DNA extract, 20 µL Phusion HSII master mix (Thermo Scientific, Illinois, United States), 14.4 µL nuclease-free water, and 0.8 µL of each forward (27F Bacterial Mn, 5' – TTTCTGTT-GGTGCTGATATTGC AGRGTTYGATYMTGGCTCAG – 3') and reverse (1492 Universal Mn, 5' – ACTTGCCTGTCGCTCTATCTTC TACCTTGTTACGACTT – 3') primer. The entirety of the V1-V9 region of the bacterial 16S rRNA gene was amplified. This mixture was prepared in a QIAgility (Thermo Scientific, Illinois,

United States). Amplification reactions were held in a Roche LightCycler 96 (Roche Sequencing Solutions, Indianapolis, IN) for 30 s at 98 °C followed by 25 cycles of 15 s at 98 °C, 15 s at 50 °C, and 60 s at 72 °C. There was a final extension for 5 min at 72 °C. Paramagnetic beads (AMPure XP beads, Beckman Coulter, Brea, CA) with 70 % EtOH rises were used to purify the PCR products. The PCR products were diluted with sterile, nuclease-free water to reach final DNA concentrations of 4 ng/μL. Using the PCR Barcoding Expansion kit (Oxford Na-nopore, Oxford, United Kingdom), 1 μL of sample-specific barcodes were added to 5 μL of each purified, diluted PCR product in a new PCR plate. In each well, 25 μL Phusion HSII master mix and 19 μL sterile, nuclease-free water were added. Amplification reactions for this plate included an initial cycle at 98 °C for 30 s followed by 15 cycles of 98 °C for 15, 62 °C for 15 s, and 72 °C for 60 s. The final extension for this reaction was for 5 min at 72 °C.

To prepare the library for sequencing, PCR products pooled together and purified using paramagnetic beads and 70 % EtOH. The Ligation Sequencing Kit V14 (SQK-LSK114) (Oxford Nanopore Technologies, Oxford, United Kingdom) was used, following manufacturer's instructions, to ligate samples. The ligated, pooled samples were fluorometrically quantified and diluted to reach 20 ng/μL DNA. The minION flow cell (Oxford Nanopore Technologies, Oxford, United Kingdom) was primed with a flush buffer and the 50 mM of the library was added. Signal data were collected with the MinKNOW (Oxford Nanopore Technologies, Oxford, United Kingdom) software across 48 h. Guppy (Oxford Nanopore Technologies, Oxford, United Kingdom) was used to base call and demultiplex the signal data and filter to a 70 q score. Emu[37] was used to generate taxonomic profiles. Data were filtered to remove samples with fewer than 10,000 reads.

Co-occurrence Network Generation

Data were filtered to remove any samples with less than 10,000 reads. Networks were developed in RStudio (version 4.1.2) using the igraph and microeco[38] packages. Four networks were generated: one representing all samples in unfertilized soil and three representing each tomato domestication group in unfertilized soil. Spearman's correlation was calculated using a filter threshold of 0.001, and a correlation optimization was used to identify the correlation threshold. Networks were constructed with p value threshold of 0.01 with an FDR p value adjustment. Network modules were identified using the "cluster fast greedy" method, which employs a hierarchical agglomeration algorithm to quickly detect the structures of communities within the network[39]. Generated networks were exported from RStudio using the rgexf package[40]. These cooccurrence networks were visualized using Gephi (v. 0.10.1)[41]. The Fruchterman-Reingold force-directed algorithm – which draws undirected graphs with straight edges by use of extant repulsive forces among adjacent and non-adjacent nodes[42,43] – was used for network layout. Nodes and edges were colored by the bacteria phylum. The size of nodes was proportionate to the degree number. The chorddiag package[38] was used to construct chord diagrams for the top ten most influential phyla.

Data Analysis

Keystone taxa were identified following Qiu et al.[44]; briefly, keystone taxa were those that had degree values > 6, weighted degree > 6, closeness centrality > 0.14, clustering coefficient > 0.09, and betweenness centrality < 0.05. To determine predictive functions that were present in the keystone taxa, PICRUSt2[45] was used to identify KEGG orthologs that mapped the bacterial species to genes of interest. Bacteria were classified into the following functional categories as determined by the presence of the corresponding enzymes and enzyme commission (EC) numbers listed parenthetically: stress (indolepyruvate decarboxylase, EC 4.1.1.74), siderophore

(isochorismate synthase, EC 5.4.4.2), P decomposition (alkaline phosphatase, EC 3.1.3.1), N decomposition (leucyl aminopeptidase, EC 3.4.11.1; urease, EC 3.5.1.5; amidase, EC 3.5.1.4), dissimilatory nitrate reduction (nitrite reductase, EC 1.7.2.2), C/N cycling (N-acetyl-B-glycosaminidase), C decomposition (alpha-glucosidase, EC 3.2.1.20; beta-glucosidase, EC 3.2.1.21), biocontrol (S,S-butanediol dehydrogenase, EC 1.1.1.76), and assimilatory nitrate reduction (ferredoxin-nitrite reduction, EC 1.7.7.1). A two-way ANOVA was used to determine differences in the relative abundance of keystone taxa among the three domestication groups. A Tukey's HSD test was used for pairwise comparisons. Separate ANOVA tests were run for the fertilized group and the unfertilized group to determine differences in the presence of keystone taxa for those individual fertilization groups.

Results

Soil bacterial community interactions and functionality

In unfertilized soil, bacterial communities demonstrated many strong and positive co-occurrence relationships in the tomato rhizosphere (Figure 3-1). In particular, the Pseudomonadota phylum was positively correlated with the populations of many bacterial groups, including Actinomycetota, Bacteroidota, Bacillota, Planctomycetota, Acidobacteriota, Chloroflexota, Gemmatimonadota, Candidatus Saccharibacteria, Nitrospirota, Verrucomicrobiota, and other Pseudomonadota members (Figure 3-1C, Figure 3-1D). Pseudomonadota was also the most influential phylum as illustrated in the co-occurrence network, comprising 21.6 % of the connections in the unfertilized network (Figure 3-1A) and 25.6 % in the fertilized network (Figure 3-1B). Following Pseudomonadota in unfertilized soil, Actinomycetota, Bacillota, and Bacteroidota were the most influential phyla, consisting of 20.4 %, 12.0 %, and 9.6 % of the network connections, respectively. Similarly, in fertilized soil, the most influential phyla after

Pseudomonadota were Actinomycetota, Bacteroidota, and Bacillota with 25.6 %, 11 %, and 9.8 % of the network connections. Only significant positive correlations were identified. The unfertilized network consisted of 167 nodes and 2004 edges with an average weighted degree of 18.6. The fertilized network had similar measurements: 164 nodes, 1244 edges, and an average weighted degree of 12.4. The co-occurrence network for the unfertilized soil showed strong modular structure, as indicated by a clustering coefficient of 0.728 and modularity of 0.285. For the network in the fertilized soil, there was a clustering coefficient of 0.720 and modularity of 0.420.

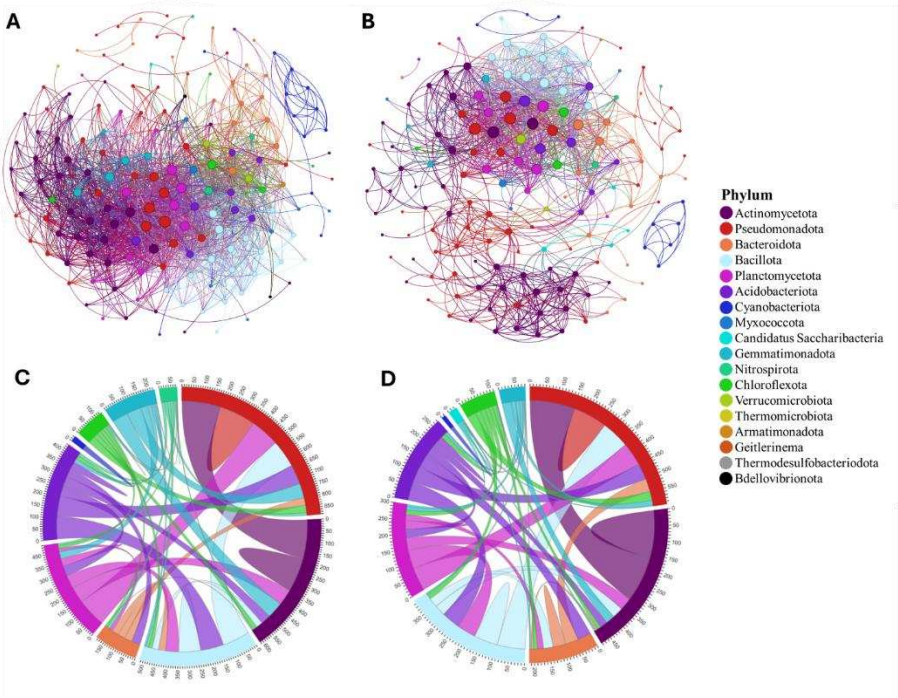

Figure 3-1. Interrelationships of bacteria in unfertilized soil of the tomato rhizosphere as illustrated through a chord and co-occurrence network diagrams. A) Co-occurrence network diagram in unfertilized soil. B). Co-occurrence network diagram in fertilized soil. C) Chord diagram in unfertilized soil. D) Chord diagram in fertilized soil. Color denotes bacterial phylum. Node size is proportional to degree (i.e., number of significant correlations). Only bacteria that were significantly correlated (optimized spearman's correlation > 0.72, $p < 0.01$) are included.

From these networks, 110 and 84 bacteria were identified as highly influential keystone taxa in unfertilized and fertilized networks, respectively (Supplemental 3-1). These keystone taxa were identified to have diverse predicted functions, including those involved in nutrient cycling,

biocontrol, and stress (Figure 3-2). In both unfertilized soil (Figure 3-1A) and P-fertilized soil (Figure 3-2B), there were no significant changes in the relative abundance of keystone taxa with different predicted functions, thus suggesting high diversity in the functions of keystone taxa. The identified keystone taxa showcased low relative abundance, with median compositions of 0.4 % in the rhizosphere of both unfertilized soil and fertilized soil (Figure 3-2).

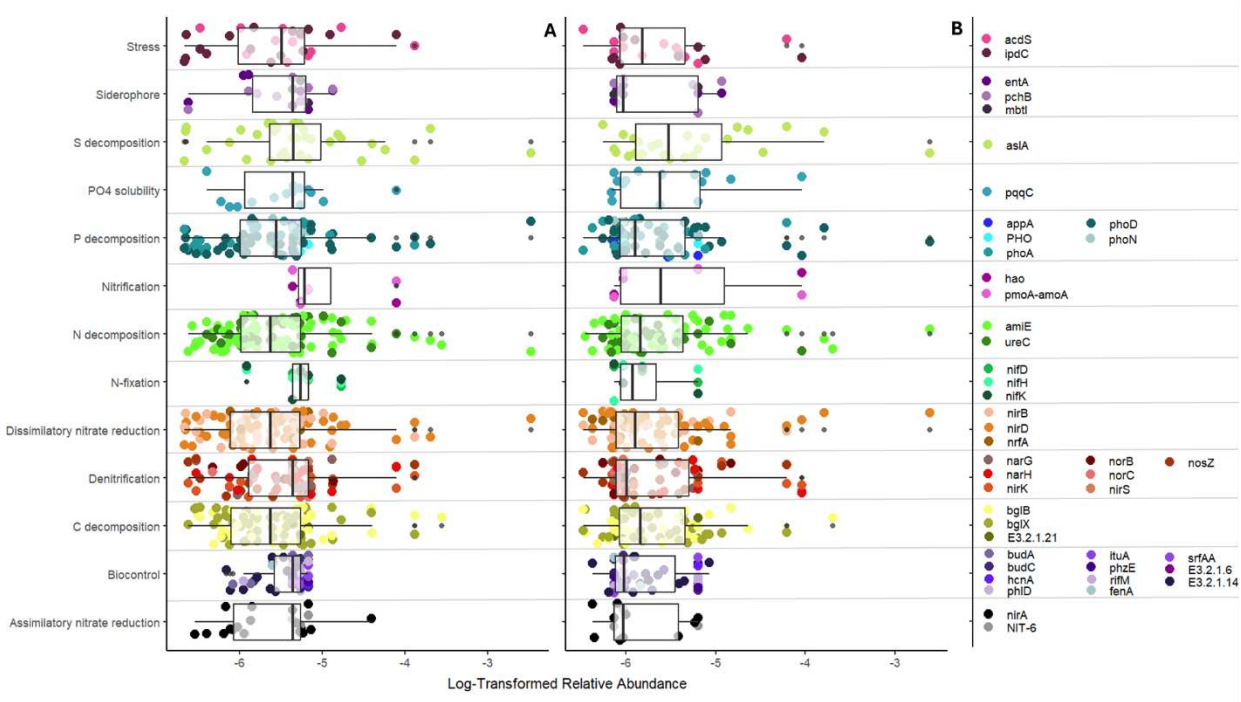

Figure 3-2. Log-transformed relative abundance with mapped predicted functions of keystone taxa. Predicted functions were identified using PICRUSt2. Each dot represents a taxon with the corresponding gene on the legend in the right panel and predictive function on the y axis. A) Key-stone taxa relative abundance in unfertilized soil. B) Keystone taxa relative abundance in fertilized soil. An ANOVA with Tukey HSD was used to determine differences in log-transformed abun-dance. There were no differences in relative abundance of the bacteria with different predicted functions.

Tomato domestication influence on soil bacterial interrelationships.

The abundance of the identified keystone taxa in fertilized and unfertilized soil varied with tomato domestication (Figure 3-3). Modern tomato accumulated a greater relative abundance of the keystone taxa compared to wild tomato in unfertilized soil (Figure 3-3 A). In unfertilized soil, the relative abundance of keystone taxa did not differ between traditional tomato and the other

domestication groups (Figure 3-3A). However, in fertilized soil, there were no differences in the relative abundance of keystone taxa (Figure 3-3B).

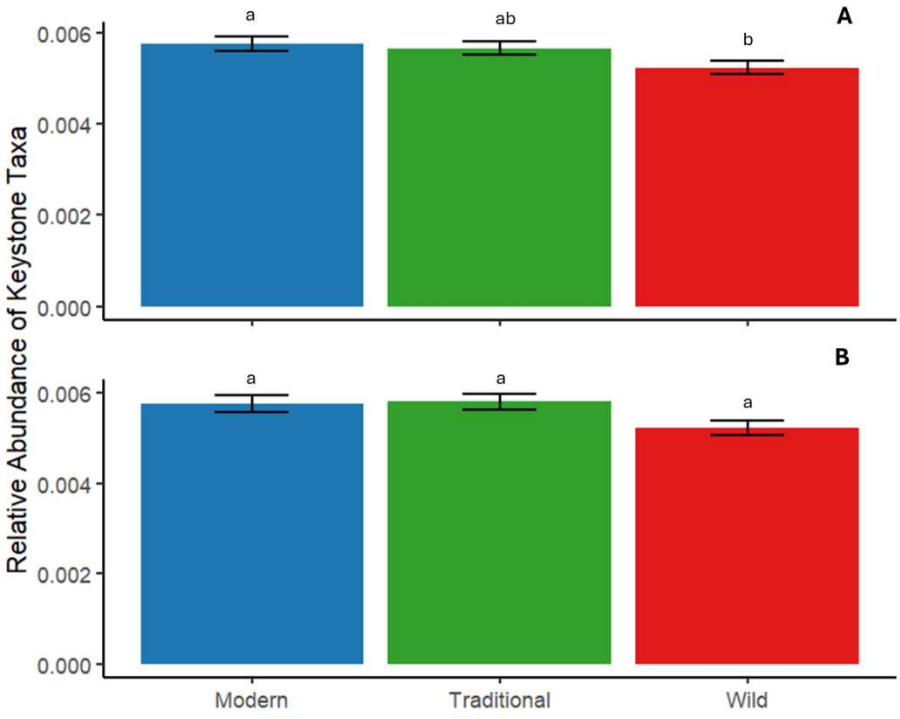

Figure 3-3. Relative abundance of the sum of keystone bacteria in the rhizosphere of wild, traditional, and modern tomato. A) Unfertilized soil and B) fertilized soil. Presented as mean ± SE. An ANVOA with Tukey HSD was run to determine differences. Letters denote significant differences (α = 0.05) in the domestication group.

To further elucidate changes occurring in the rhizosphere microbiome of wild, traditional, and modern tomato, we ran individual co-occurrence networks for each domestication group. Here, we observed varied structure of networks within each tomato domestication group (Figure 3-4). In unfertilized soil, the wild tomato rhizosphere (165 nodes, 1071 edges) had a less complex network than the other domestication groups (Figure 3-4A) with smaller node and edge numbers relative to the other traditional (174 nodes, 1708 edges) and modern (181 nodes, 2198 edges). Traditional tomato had a relatively moderate network complexity (Figure 3-4B), and modern tomato had the highest network complexity (Figure 3-4) in unfertilized soil. However, in fertilized soil, the wild tomato group increased node and edge number and became the most

complex network (193 nodes, 1613 edges) (Figure 3-4D). In fertilized soil, traditional tomato had the least complex network (175 nodes, 1173 edges) (Figure 3-4E), and modern tomato had moderate complexity (182 nodes, 1565 edges) (Figure 3-4F). However, the most influential phyla were consistent across tomato domestication groups and fertilization, with Pseudomonadota correlating to the greatest number of other phyla in the rhizosphere (Figure 3-4).

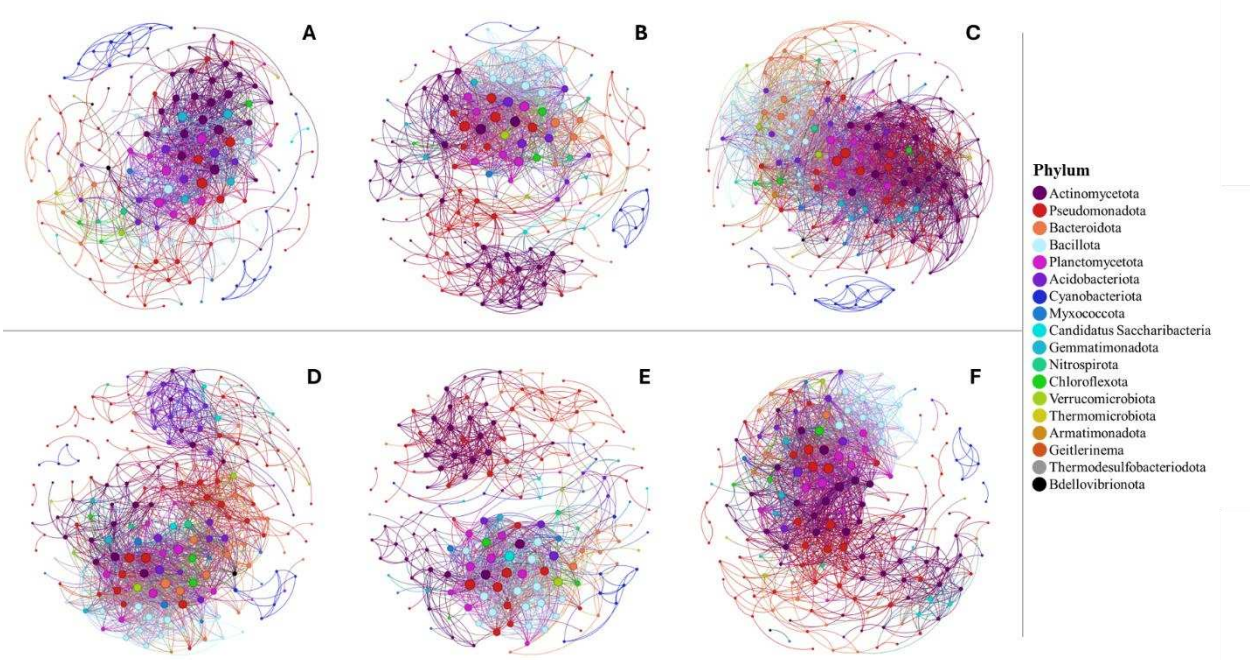

Figure 3-4. Co-occurrence network showing bacterial interrelationships in unfertilized soil of the tomato rhizosphere. Each co-occurrence network showcases correlative relationships of each tomato domestication group and fertilization treatment: unfertilized wild (A), unfertilized traditional (B), unfertilized modern (C), fertilized wild (D), fertilized traditional (E), and fertilized modern (F). Color denotes phylum. Node size is proportion to degree (i.e., number of significant correlations). Only bacteria that were significantly correlated (optimized spearman's correlation > 0.7, $p < 0.01$) are included.

Discussion

The co-occurrence network analysis of tomato domestication groups –wild, traditional, and modern – in low and high phosphorus (P) conditions revealed insightful information into varied rhizosphere microbial interactions. Here, we found that the network complexity and structure of the microbiome in unfertilized and fertilized soil was similar. Further, the most highly connected

phyla were the same in both unfertilized and P-fertilized soil. However, in examining the network structure of the different tomato domestication groups, there were clear differences. We found that with the application of P fertilizer, the wild tomato domestication group increased network complexity.

While other research has not been conducted on rhizosphere co-occurrence network complexity across tomato domestication, there is a similar study conducted on rice with which our results are consistent. The finding that there is higher network complexity in wild tomato compared to lower network complexity in traditional tomato is in congruence with Chang et al.[46] who found that the microbial network complexity of wild rice was greater than its domesticated counterpart. Further, Sun et al.[47] found that wild accessions of rice have a greater abundance of bacterial chemotaxis genes than modern rice. This difference in chemotaxis between the two domesticated groups of rice is a result of changing metabolite profiles of root secretions that was affected by plant domestication[47]. Domestication was shown to reduce the abundance of sugars and alcohols in rice root exudates[47], and sugar has been shown to be a major chemoattractant for beneficial soil bacteria[48]. Sugars and other high organic C compounds that are exuded by plant roots strengthen interactions in the rhizosphere[49], and microbial interactions are approximated by measurements of cooccurrence network complexity[50]. Thus, the changing root exudate profiles may have led to greater network complexity in wild compared to domesticated crops.

While the rhizosphere network complexity was heightened for wild relatives compared to traditional and modern tomato in P-fertilized soil, it was diminished in unfertilized soil. Conversely, modern and traditional tomatoes showed little-to-no change in their network characteristics between the unfertilized and fertilized treatments. This strong response of the rhizosphere microbiome of wild tomato to P fertilizer compared to modern and traditional may

be explained by wild tomato being more responsive to P fertilization. Wild tomato has been shown to increase biomass production in response to P fertilizer to a greater degree than domesticated tomato[12]. In potato, a crop closely related to tomato, it was also shown that non-cultivated accessions were highly responsive to the addition of P fertilization compared to cultivated varieties[36]. Thus, wild tomato and its associated microbiota may be more sensitive to varied levels of exogenous P, and therefore, more subject to change with P additions than domesticated varieties.

In addition to wild tomato varying from its domesticated counterparts, modern tomato and traditional tomato also had marked differences in network complexity; the modern tomato microbiome was more complex than traditional tomato. These differences could be explained by tomato breeding patterns over time. In tomato breeding programs, there has been a heightened emphasis on introgression of traits from wild relatives since the 1970s[51], and our tested accessions of traditional tomato were developed prior to that time. It is therefore possible that the increased genetic diversity of modern tomato relative to heirloom tomato resulted in an increased complex co-occurrence network of modern tomato accessions when compared to earlier cultivars. While genetic erosion may have occurred when tomatoes were domesticated and developed for human use[52], continual introgressions of wild genetic material into modern germplasm has increased the genetic diversity of current tomato cultivars[51,53,54].

The altered complexity we observed in our tested accessions may also indicate variations in microbial diversity. Network complexity and microbial diversity have been shown to be related in a study examining tomato root-associated environments[55]. The authors found that in compartments with low alpha diversity, there was a concomitantly low measure of network complexity[55]. There has been much research into the changing bacterial diversity patterns as a

function of crop domestication[19,56-58]. Although recent research suggests that alpha diversity measurements do not differ among wild, traditional, and modern tomato[12,59], that pattern is not true for all crops. For example, while domesticated wheat harbors greater bacterial diversity, its fungal diversity is less compared to wild wheat[60]. Therefore, network complexity and diversity may be affected by domestication and major plant breeding events. For example, breeding events, such as the Green Revolution, have taken place since the development of our tested traditional tomato accessions. The Green Revolution involved the development of semi-dwarf, high-yielding crops concomitant with increased applications of fertilizers and pesticides[61]. These altered agricultural practices may have significantly influenced the diversity and composition of these microbial communities[61,62], which suggests a capability of plants to enrich microbes with functions that are advantageous under specific management regimes[63]. Such findings emphasize the influence of plant breeding and the selection on microbial dynamics within the rhizosphere. The observation that modern crops maintain microbial network complexities comparable to wild species challenges previous notions. This realization fosters a deeper under-standing of how selective trait selection by breeders can positively affect crop-microbe interactions, promoting improved resilience, plant health, and nutrient cycling of soil ecosystems[60].

Soil nutrient cycling is highly influenced by the presence of influential, keystone taxa[64]. In the current study, Pseudomonadota was associated with the greatest change in other bacteria in the tomato rhizosphere. This finding suggests that beneficial bacteria may be highly influential in rhizosphere microbial assemblage because many plant-growth-promoting bacteria are present in Pseudomonadota, and the genetics and beneficial functions of this phylum have been extensively studied[65-67]. Further, we identified numerous keystone taxa with diverse predicted functions to be present in both fertilized and unfertilized soil, thus indicating that the bacteria that are most

influential in the rhizosphere microbiome are also capable of facilitating many different soil processes. Although these keystone taxa are highly influential, they comprise a small proportion of the rhizosphere microbiome. This finding is in agreement with Shi et al.[68] who found that keystone microbes tended to have low abundances and concluded that taxa with low abundances can highly influence the functionality of the rhizosphere.

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CHAPTER 4 – WILD TOMATO RESPONDS TO A FLUSH OF EXOGENOUS NUTRITION BY INTERACTING WITH ITS RHIZOSPHERE MICROBIOME TO MAINTAIN SOLUBLE SOIL PHOSPHORUS CONCENTRATIONS

Abstract

Wild plants are periodically exposed to nutrient flushes, whereas modern cultivars are acclimated to regular nutrition from fertilizers. Here, we explored how a wild relative of a modern crop deals with flushes of phosphorus (P) fertilizer in the soil. We grew a wild and a modern tomato in P-fertilized soil, finding that the modern tomato promoted fewer P-solubilizing microbes and depleted soil P. Conversely, wild tomato had slower growth rates and P uptake along with a greater promotion of a diverse array of soil bacteria. These bacteria included P solubilizers which resulted in higher residual bioavailable soil P pools. This microbial strategy by wild tomato was likely driven by its root exudate profile, which was enriched in trehalose and glycerol compounds that we show can promote bacterial-mediated P solubilization. Subsequently, we grew modern tomato in P-fertilized soils that had either a wild or modern tomato planted previously. Modern tomato grown in wild tomato-conditioned soils showed increased biomass compared to those grown in modern tomato-conditioned soils. The increased biomass may be principally associated with residual bioavailable soil P from the wild tomato conditioning as the microbial community changes from the wild tomato were not maintained by the modern tomato variety. The microbially-dominated strategy of the wild tomato helps to maintain soluble soil P and thus may be a future strategy to be capitalized upon within modern agriculture.

Introduction

Phosphorus (P) is often a limiting nutrient in terrestrial and non-agricultural soils[1-4]. To survive in these conditions, undomesticated plants showcase great genotypic and functional diversity providing them with the ability to effectively acquire soil nutrients[1,5]. Native grasses, including *Chionochloa crassiuscula* and *Hordeum leporinum*, are examples of low-nutrient-adapted wild species that have evolved to grow in soils with low bioavailable phosphate[6]. Even though they have similar phosphate uptake capacities, native grasses have slower growth rates and less efficient biomass accumulation compared to cultivated species, enabling native grasses to reduce their annual nutrient requirement[6]. Low-nutrient-adapted plants on the whole tend to have a reduced nutrient requirement and to prioritize processes unrelated to primary growth (e.g., defense)[7]. This resource-use strategy is evident not just in plants, but also in the animal kingdom. For example, a wild relative of domesticated chicken, the red jungle fowl, tends to show greater resource allocation into survival mechanisms rather than primary production[8].

In contrast to the low nutrition common in wild species, modern relatives are adapted to conditions of high soil fertility. While wolves, for instance, are adapted to survive in periods of prolonged famine, modern dogs receive more regular feedings of diets enriched in carbohydrates[9,10]. As such, the gut microbiota of canines has shifted; modern dogs contain an abundance of taxa involved in carbohydrate metabolism[10]. Similarly, cultivated, high-yielding modern crops are bred in optimal conditions having sufficient water and nutrients[11]. The higher levels of nutrient and water inputs in agricultural systems allow for consistently healthy crop growth and sustainable yield. It is therefore possible that thousands of years of breeding, development, and events such as the Green Revolution (an agricultural event involving changing breeding practices and increased incidence of fertilization, pesticide use, and irrigation) have

promoted evolution of domesticated crops to have high-nutrient resource-acquisitive strategies[12,13]. Modern crops that were developed through conventional breeding methods tend to be adapted to stratified soil P environments and exploit their root morphology for enhanced P acquisition from the highly fertile surface horizons[14]. Thus, the ability of modern crops to display efficient P acquisition strategies by manipulating root morphology may be a result of selection of successful candidates and being domesticated in areas of high soil fertility[7,14].

At domestication, functional root traits were changed, where domesticated crops show lower specific root tip density and lower lateral root density than wild crops[15]. These traits are often associated with environments with high fertility, so early domestication events may have selected higher nutrient acquisition strategies for modern crops[16]. Crop domestication has occurred in areas of high soil fertility; post-domestication, crops were further selected and bred in conditions of even higher nutrient availability[11]. Because of changing agricultural practices from the Green Revolution, these domesticates were further acclimated to conditions of even higher nutrition through the use of synthetic fertilizers[13]. More recently, fertigation has been implemented in many crop production systems, therefore, modern crops are adjusted to growing in conditions of not just high, but frequent applications of nutrients, as fertigation systems particularly in greenhouse production can apply fertilization to high value crops in frequent pulses[17-19].

Typically, nutrient requirement studies comparing wild and domesticated crops are conducted under resource deficit conditions[20-23]. However, the mechanisms by which wild and low nutrient adapted plants respond to and survive in conditions of high nutrition remains unclear and unexplored. In natural ecosystems, wild and native plants are periodically exposed to temporal flushes of nutrients after a disturbance[7]. For example, tropical rainforests have high

concentrations of organic matter from leaves on the soil surface[24], treefalls in old growth forests create areas of high nutrition from organic matter deposition[25], and semi-arid shrub-steppe soils show marked increase in N and P concentrations as a result of vertebrate carrion decomposition[26].

Until now, the response by wild plants, like wild relatives of tomato, to these flushes of nutrients has not been addressed. To elucidate mechanisms by which wild plant species respond to a flush of exogenous nutrition, we cultivated wild tomato in conditions of excess P and compared its responses to a modern cultivar. Our overarching aim was to determine if wild tomato showcases increased reserves of soil P relative to a modern counterpart and if the wild tomato could impart benefits to both itself and to future generations of plants. Our objective was to determine if the rhizosphere microbiome of a microbially-reliant wild tomato maintains the stores of bioavailable P in soil under P-fertilized conditions compared to its domesticated counterpart, and how these wild tomato-promoted microbes may be maintaining P solubility which then promotes plant growth.

Materials and Methods

Plant growth conditions

Three trials occurred in parallel: a time course study, a root exudate study, and a soil conditioning study with successive planting cycles. For all three trials, two varieties of tomato were selected: one representative wild tomato (*Solanum pennellii,* accession no. LA0716) (“wild”) and one representative domesticated tomato (*S. lycopersicum* ‘Quali T 27’) (“modern”). Wild tomato seeds were obtained from the Tomato Genetics Resource Center at the University of California Davis, and modern tomato seeds were donated by Syngenta (Delaware, United States). Seeds

from these accessions were surface sterilized using diluted sodium hypochlorite and germinated on filter paper[22].

For the time course and soil conditioning trials, seedlings were transferred from filter paper to a low-nutrient potting mixture (PromixBK, Québec, Canada) at the stage of cotyledon emergence. Seedlings were left to establish for one week prior to transplanting them to their experimental units. An experimental unit consisted of one seedling in one pot (black plastic greenhouse pot, 11 × 11 × 13 cm) filled with growing media composed of agricultural soil and sand. Agricultural soil was collected from the Colorado State University (CSU) Agricultural Research, Development, and Education Center (ARDEC) in Fort Collins, CO. Because topsoil in agroecosystems often contains an abundance of nutrients from organic matter accumulation and fertilization[72], topsoil was avoided, and soil was collected from 5-20 cm below the surface. Soil was filtered through a 1 cm sieve to remove debris and large aggregates. To further reduce bioavailable P concentrations, sieved agricultural soil was mixed at a 1:1 volumetric ratio with sand (Quikrete Play Sand, Georgia, United States). The soil-sand mixture had a low plant-available P concentration of 4.7 mg P/kg (Olsen-P) (Table S4-1). Half of the plants were left in soils not fertilized with P and half were fertilized to P sufficiency. Pots in the fertilized treatment received triple superphosphate (TSP) (0-46-0) at rates recommended by Ward Laboratories (Kearney, Nebraska, USA) equivalent to 160 kg/ha P_2O_5 (194 mg P_2O_5 per pot). To ensure that P recovery strategies were not conflated with N recovery strategies, all pots regardless of treatment received N fertilization using a polymer-coated urea (Environmentally Smart Nitrogen) (44-0-0) at rates equivalent to 100 kg/ha N.

In the time course trial, wild and modern tomatoes were grown in unfertilized or P-fertilized soils. Every 3 weeks for 15 weeks, samples were destructively harvested to elucidate

soil P acquisition over time. In the soil conditioning trial, we sought to test the ability of wild tomato to confer beneficial P acquisition traits to modern tomato. This trial consisted of two planting cycles: the purpose of the first cycle was to alter the condition of the soil, and the second cycle was planted with only modern tomato plants to determine if the soil conditioning treatment had an effect on subsequent planting cycles. Soil was initially autoclave-sterilized to better showcase the effects from the host plant. Soil was conditioned in the first cycle by leaving the pots without a plant, planting a wild tomato, or planting a modern tomato in either unfertilized or P-fertilized soil. The plants were left to grow for five weeks, and then modern tomato was planted in all the pots after removal of the conditioning plants. The modern tomato was either fertilized or left unfertilized. These plants were also left to grow for five weeks. Both time course and soil conditioning trials were grown in a completely randomized design in a greenhouse and had seven replicates per treatment.

In the root exudate trial, seedlings were transferred to sterile test tubes filled with modified Hoagland hydroponic solution (500 µL NaH_2PO_4, 1500 µL K_2SO_4, 1000 µL $Ca(NO_3)_2$, 250 µL $MgSO_4$, 50 µL KCl, 150 µL Fe-EDTA, 25 µL H_3BO_3, 0.3 µL MoO_3, 2 µL $ZnSO_4$, 2 µL $MnSO_4$, 0.5 µL $CuSO_4$) adjusted to a pH of 7.21 and filled to cover the roots. The caps of the test tubes were covered with parafilm and left in a growth chamber, and plants were arranged in a completely randomized design. The nutrient solution was replenished twice each week, and seedlings were left to grow for three weeks.

Biomass and soil sampling

At harvest for the time course and soil conditioning trials, plants were removed from pots and were gently shaken to remove bulk soil. Bulk soil was collected for soil-P analysis. Soil remaining on the root axis was identified as rhizosphere soil, collected in sterile 15 mL conical

tubes, and stored at -80 °C until DNA extraction. Subsequently, roots were washed to remove any remaining soil-sand mixture. Plants were severed at the root-shoot interface. Root and shoot samples were placed in a drying oven at 70 ºC for 4 days, and dry biomass was recorded.

Plant and soil phosphorus analysis

For the time course and soil conditioning trials, bulk soil samples were immediately air-dried following harvest. For both trials, dry bulk soil was sieved (2 mm), and the Olsen-P bicarbonate extraction method was used to determine bioavailable soil P concentrations[73]. For the time course, water soluble P was determined through colorimetric analysis using the ascorbic acid-molybdenum blue method following Rodriguez and Soltanpour[74]. For the time course trial, approximately 10 g of dry sieved soil was mailed to Ward Laboratories (Kearney, Nebraska, USA) to determine Mehlich-3 P concentration.

Shoot samples and root samples were homogenized by grinding to a powder and then digested with nitric acid following Ippolito and Barbarick [75]. Following nitric acid digest, P concentrations were determined through inductively coupled plasma-atomic emission spectroscopy.

DNA extraction and 16S rRNA amplicon sequencing

For the time course and soil conditioning trial, rhizosphere soil samples were stored at -80 °C and were weighed to 0.25 g. Remaining rhizosphere soil was weighed to determine soil moisture content. Moisture content was calculated by measuring the fresh weight of soil samples and the oven-dried weight of samples. Total gDNA was extracted using a QIAcube instrument and DNeasy Power Soil Pro DNA isolation kit (Qiagen, Hilden, Germany) following manufacturer's

instructions. Following extraction, DNA concentration (ng/µL) was determined using Qubit Fluorometer (Thermo Scientific, Illinois, United States).

A first round of PCR was performed to isolate the full 16S gene of approximately 1500 bp using primers 27F-1492R equipped with barcode adapter sequences denoted in italics: 27F-Mn, 5′-*TTTCTGTTGGTGCTGATATTGC* AGRGTTYGATYMTGGCTCAG-3′ and 1492R-Mn, 5′-*ACTTGCCTGTCGCTCTATCTTC* TACCTTGTTACGACTT-3′. For each sample, 2-8 ng of diluted gDNA was added as well as 36 µL master mix (MM), composed of 14.4 µL nuclease-free water, Phusion HSII Polymerase (Thermo Fisher Scientific, Waltham, MA, USA), and 0.8 µL of each 10 µM forward and reverse primer. Diluted gDNA and MM solution were dispensed into each well using the QIAgility (Qiagen, Hilden, Germany). PCR conditions were 25 cycles of 98 °C for 30 s (initial denaturation), 98 °C for 15 s, 50 °C for 15 s, 72 °C for 60 s, and a final extension of 72 °C for 5 min. The PCR product was bead cleaned using lab-made paramagnetic beads[76] at a 1:1 ratio and twice cleaned with 70 % ethanol. The cleaned PCR product was eluted in 40 µL nuclease free water. DNA concentrations were measured using a Qubit fluorometer to ensure DNA retention of (appx. 4 ng/µL).

A second round of PCR was conducted for barcode ligation. A MM was prepared, consisting of 19 µL nuclease-free water and 25 µL Phusion HSII per well. This mixture was dispensed to the initial PCR product plate using the QIAgility. Subsequently, 1 µL of sample-specific barcodes (Native Barcoding Kit 96 V14, Oxford Nanopore Technologies, Oxford, United Kingdom) was added per well. PCR conditions were 25 cycles at 98 °C for 30 s (initial denaturation), 98 °C for 15 s, 62 °C for 15 s, and 72 °C for 60 s, and 1 cycle at 72 °C for 5 min (final extension). Samples were pooled equimolar at 10 µL from each sample. The pooled samples were cleaned using an equal volume of lab-made paramagnetic beads. The library was

prepared for sequencing using the Oxford Nanopore Ligation Sequencing Kit V14 (SQK-LSK114) following manufacturer instructions. The pooled final library was then loaded into a MinION flow cell R 10.4.1. Data was collected over 48 h using MinKNOW (Oxford Nanopore Technologies, Oxford, United Kingdom). Data were base-called, demultiplexed, and assigned q-scores using dorado script (Oxford Nanopore Technologies, Oxford, United Kingdom). Data were then filtered by q-score (> 70) and length (V1-V9 region, 1,000-2,000 bp) resulting in >20,000 quality filtered reads per sample. Bacterial classification of sequence reads was performed using Emu pipeline and database[77]. Resulting Emu taxonomy table was combined with sample data into a Phyloseq[78] object for further analysis. Data were run through PICRUSt2 to identify predictive functional gene abundance utilizing KEGG ontologies [79], and we selected a list of putatively beneficial genes using PICRUSt2 (Table S4-2).

Root exudate collection and metabolomics analysis

For the root exudate trial, exudates were collected after 3 weeks of growth. Following Pantigoso et al.[61], plants were gently removed from nutrient solution and triple rinsed in sterile DI water. These cleaned seedlings were placed in new sterile test tubes filled with enough sterile DI water to cover the roots. Seedlings were left in these conditions to exude for three days. Following root exudation, seedlings were removed from water and discarded. The remaining water was filtered through a 2 µm filter. To ensure adequate metabolite biomass was collected, exudates from five plants were pooled together to equate to one replicate, with three replicates per treatment. The filtrate was lyophilized, and the exudates were sent to the Environmental Molecular Science Laboratory (Richland, WA) for metabolomics analysis.

Lyophilized exudates were resuspended in 150 µl of 4:1 methanol:water solvent, vortexed and sonicated to fully dissolve. Samples were filtered through a 96-well 0.2 µm PTFE

filter prep (AcroPrep™ Advance 96-well filter plates for solvent filtration, Cytiva, Marlborough, MA) using a positive pressure system (Waters, Milford, MA) into a 96-well collection plate. Filtrates were transferred into labeled Thermo SureSTART level 3 vials (Thermo Fisher, Waltham, MA), capped, and stored at −20 °C until analysis by LC-MS.

Metabolite extracts were chromatographically resolved using reverse-phase and hydrophilic interaction liquid chromatography (HILIC) in both positive and negative ionization modes. For reverse-phase chromatography, 5 µl of metabolite extract was injected and compounds separated using a Thermo Hypersil GOLD column (2.1 × 150 mm, 3 µm particle size) with a column temperature of 40 °C and a primary flow rate of 400 µL min^{-1}. Mobile phase A (Water with 0.1 % formic acid) and B (ACN with 0.1 % formic acid) were initially 90 % and 10 %, respectively. The gradient method continued as follows: 0–2 min held at 90 % A; 2–11 min 10 % A; 11–12 min held at 10 % A; 12–12.5 min 10 % A with an increased flow rate to 500 µL min^{-1}; 12.5-13.5 min at 90 % A; 13.5-14 min held at 90 % A; 14-14.5 min at 90 % A with a decreased flow rate to 400 µL min^{-1}; 14.5-16 min held at 90 % A. For HILIC chromatography, 5 µL of metabolite extract was injected and compounds separated using an ACQUITY UPLC BEH HILIC column (2.1 × 100 mm, 1.7 µm particle size) with a column temperature of 50 °C and a primary flow rate of 300 µL min^{-1}. Mobile phase A (5 % ACN: 95 % 10 mM NH4OAc in H_2O with 0.05 % NH_4OH) and B (100 % ACN with 0.05 % NH_4OH) were initially 5 % 95 %, respectively. The gradient method continued as follows: 0–6.0 min 63 % A; 6.0-7.0 min held at 63 % A; 7.0–7.1 min 5 % A; 7.1-7.2 min 5 % A with an increased flow rate to 500 µL min^{-1}; 7.2–9.5 min held at 5 % A; 9.5-9.7 min 5 % A with a decreased flow rate to 300 µL min^{-1}; 9.7–12.0 min held at 5 % A.

Samples were analyzed by liquid chromatography-mass spectrometry (LC-MS) using a Thermo Vanquish Flex UHPLC system (Thermo Scientific, San Jose, CA) interfaced with a Thermo QExactive HF-X mass spectrometer (Thermo Scientific, San Jose, CA). The HESI probe was maintained at 350 °C and the MS inlet at 300 °C with a spray voltage of 3.7 kV for positive and 3.0 kV for negative mode. The sheath gas, aux gas, and spare gas were maintained at flow rates of 30, 8, and 1 arbitrary units, respectively. Full MS scan data were acquired at a resolving power of 120,000 FWHM at m/z 200 with a scanning range of m/z 80–800. The automatic gain control (AGC) target was set at 3E6 ions, with a maximum injection time of 20 ms. The data-dependent acquisition (dd-MS2) parameters used to obtain product ion spectra were as follows: resolving power 15,000 FWHM at m/z 200, AGC target 1E5 ions with maximum injection time of 100 ms, isolation window of 0.4 m/z, loop count 12, and an HCD (higher-energy collision dissociation) normalized collision energy (NCE) of 20, 30, and 40 eV.

For data generated, confident metabolite identifications were made using Thermo Compound Discoverer 3.3. For reverse phase and HILIC, positive and negative mode spectra are aligned using an adaptive curve with a maximum of 0.3 or 0.6 RT shift, respectively, and a 3-ppm mass tolerance. Peaks were selected based on a minimum intensity of 5e4 and a chromatographic S/N of 3. Detected features are grouped based on a mass tolerance of 3 ppm and an RT tolerance of 0.3. Compounds are assigned based on Isotopic pattern, RT, MS1, and/or MS2. All identifications and integrated peaks are manually validated and exported for statistical analysis.

After samples were acquired by LC-MS, consistent volumes of each sample (57 µL) were dried under vacuum and chemically derivatized as previously reported[80]. Briefly, the extracts were derivatized by methoxyamination and trimethylsilyation (TMS), then the samples were

analyzed by GC-MS. Samples were acquired in an Agilent GC 7890A using a HP-5MS column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies, Santa Clara, CA) coupled with a single quadrupole MSD 5975C (Agilent Technologies). One microliter of sample was injected into a splitless port at a constant temperature of 250 °C. The GC temperature gradient started at 60 °C, with a hold of temperature for 1 minute after injection, followed by an increase to 325 °C at a rate of 10 °C/minute and a 10-minute hold at this temperature. A fatty acid methyl ester standard mix (C8-28) (Sigma-Aldrich, Saint Louis, MO) was analyzed in parallel as standard for retention time calibration.

GC-MS raw data files were processed using the Metabolite Detector software[81]. Retention indices (RI) of detected metabolites were calculated based on the analysis of a FAMEs mixture, followed by their chromatographic alignment across all analyses after deconvolution. Metabolites were initially identified by matching experimental spectra to a PNNL augmented version of Agilent GC-MS metabolomics Library, containing spectra and validated retention indices for over 1200 metabolites. Then, the unknown peaks were additionally matched with the NIST20/Wiley11 GC-MS library. Metabolite identifications and quantification ions were validated and confirmed to reduce deconvolution errors during automated data-processing and to eliminate false identifications. Peak areas of metabolites detected were reported and submitted for statistical analysis.

Root exudate and bacterial application on NBRIP media

From the list of metabolites exuded by wild and modern tomato, six were selected: four highly enriched by wild tomato (trehalose, 1-stearoly-rac-glycerol, glycerol monostearate, 1-monopalmitin) and two by modern tomato (lauric acid, dodecanol). These chemicals were purchased from Thermo Fisher Scientific (Waltham, MA) (trehalose, 1-stearoly-rac-glycerol,

glycerol monostearate, lauric acid, dodecanol) and Sigma Aldrich (St. Louis, MO) (1-monopalmitin). Following Pantigoso et al.[61], a stock solution of 7 mM was made for each compound. Trehalose was dissolved in sterile Milli-Q water, but because the rest of the compounds were not water-soluble, they were dissolved in 100 % ethanol. *Azospirillum brasilense* was chosen as a representative bacterium for this experiment because it has been shown to be plant-beneficial and P-sensitive[82], it was present across samples in this study, has been shown to be capable of growth in media with ethanol[83], and would therefore be able to be grown on plates containing this solvent.

Following Islas-Valdez et al.[84], a culture of *A. brasilense* was grown to a concentration of approximately ~5.6×10^6 CFU mL^{-1} (optical density OD_{600} =0.7). This suspension was then serially diluted in a sterile diluent before 100 µL aliquots were spread onto National Botanical Research Institute's Phosphorus (NBRIP) medium. Phytin (an organic source of P) and tricalcium phosphate (TCP) (an inorganic source of P) were used as separate P sources in NBRIP formulation. The application of bacteria was concomitant with the application of 100 µL of the root exudate compound. NBRIP agar plates were incubated at 27 °C for 4 days. Subsequently, the phosphate solubilization index (PSI) was calculated by measuring the proportionate difference in the halo diameter to the colony diameter[85].

Statistical analysis

All data were analyzed in R (v 4.3.3)[86] and RStudio[87] (v. 2024.12.0+467). An analysis of variance (ANOVA) using $\alpha = 0.05$ was performed on shoot and root biomass, shoot P concentration and uptake, root P concentration and uptake, Olsen-P concentration, water soluble P concentration, Mehlich-3 P concentration, P solubilizing bacterial relative abundance, P mineralizing bacterial relative abundance, and microbial biomass using Y ~ Fertilization,

Domestication, Harvest (time course trial) and Y ~ Fertilization, Conditioning (soil conditioning trial). In the time course trial, the factor "Fertilization" indicates the P-fertilization treatment (P-fertilized or unfertilized), "Domestication" indicates the type of plant used (wild or modern), and "Harvest" indicates the stage of destructive harvest (seedling, early vegetative, late vegetative, early flower, or late flower). The response variable in time course trial had to be log-transformed to satisfy the assumption of homoscedasticity. After log-transformation, ANOVA assumptions were satisfied using diagnostic plots[88]. For visualization, data were back-transformed. In the soil conditioning trial, the factor "Fertilization" indicates when plants were fertilized with TSP (P-fertilized or unfertilized) in the second planting cycle. "Conditioning" indicates the condition of the pots in the first cycle (wild plant, modern plant). A Tukey HSD was used for post-hoc comparisons using an $\alpha = 0.05$.

To compare the bacterial species that changed in abundance between treatments in the time course and soil conditioning trials, a differential abundance analysis was bacterial count data, indicating the sequence reads per sample. Count data were normalized through a relative log expression prior to testing. A Wald test was then performed on the log-2-fold change of normalized count data using the microbiomeMarker package (v 3.20)[89]. A 0.05 FDR p adjustment was used for this test. This test was used to compare the bacteria changing in abundance between each harvest point within each domestication and fertilization treatment for time course trial. This test was used in the soil conditioning trial to determine differences in count data between the soil conditioning treatments in sterile soil that was conditioned with either a wild or modern tomato in the first cycle.

To examine metabolite composition in the root exudate trial, a sparsity filter was employed using a robust Mahalanobis distance to look for potential outliers. To determine

differences, a two-tailed t test was run to assess if the normalized log2 abundance values were different between the modern and wild tomatoes. An ANOVA was run to determine differences in PSI: Y ~ Exudate, P Source, where "Exudate" indicates the type of compound used, "P Source" indicates whether phytin or TCP were used in NBRIP formulation.

Results

Divergent growth responses to phosphorus application between wild and modern tomato

Growth patterns differed for wild and modern tomato in P-fertilized soil (Figure 4-1A). Early on, both groups markedly increased shoot biomass from the first to the second stage, and during this time, modern tomato shoots were larger (Figure 4-1A). Shortly after, the modern representative slowed its rate of biomass accumulation (Figure 4-1A). Although the wild tomato also slowed growth, the biomass at the final stage of harvest was greater than the early vegetative and early flower stage. Further, by the end of the experiment, the wild representative was no different from modern (Figure 4-1A). Ultimately, after starting off with lower biomass during the first two growth stages, wild tomato caught up to modern tomato by the later harvest points. Roots of the modern tomato grew fast and accumulated their greatest biomass by the second harvest point (Figure 4-1B). After this period, newer roots were likely unable to regenerate at the same rate as the death of older roots. Unlike the growth pattern experienced by the modern tomato, wild tomato roots continued growth for the first three stages of harvest (Figure 4-1B). By the final harvest stage, wild and modern root biomass were not different (Figure 4-1B). Conversely, in unfertilized soil, modern tomato had greater shoot and root biomass than wild tomato at every harvest stage (Figure S4-1A, Figure S4-1B).

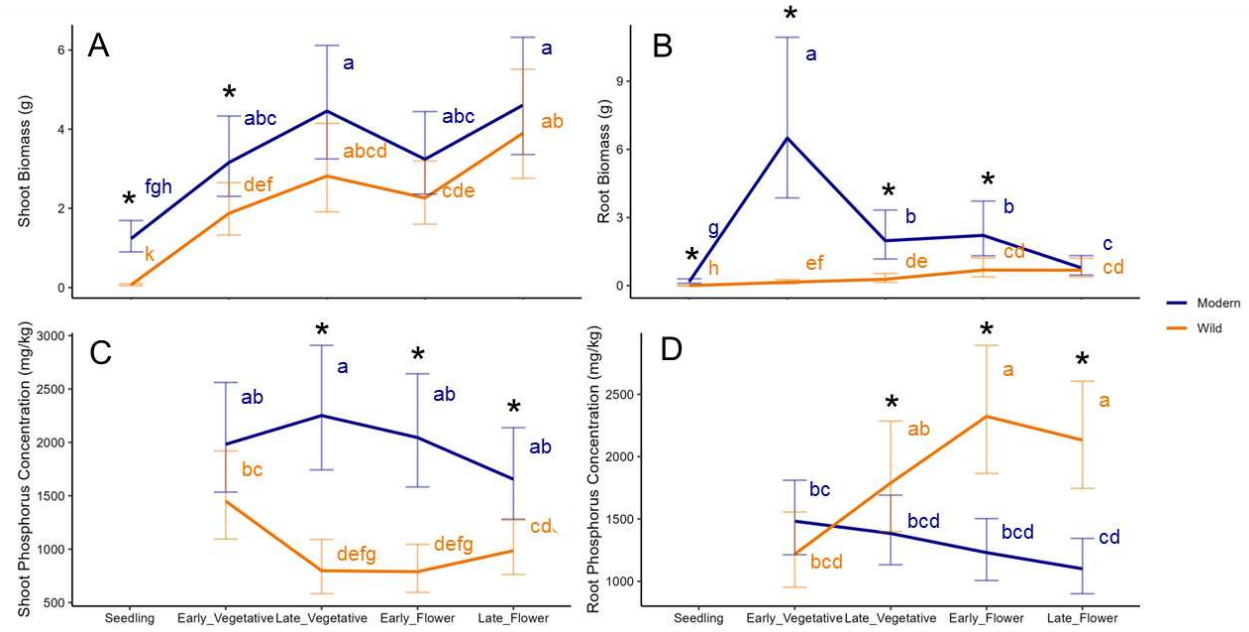

Figure 4-6. Biomass and phosphorus accumulation of a wild and a modern tomato across different developmental stages in P-fertilized soils. Dry biomass was measured from P-fertilized shoots (A) and P-fertilized roots (B). Phosphorus concentration was determined for shoots (C) and roots (D). Data were log-transformed for analysis and back-transformed for visualization. Line color denotes tomato domestication: orange (wild) and blue (modern). Bars display 95 % confidence intervals. An ANOVA with Tukey HSD was run. Different letters denote significant differences at α = 0.05. Stars denote significant difference for the pairwise contrast for wild and modern tomato at the corresponding developmental stage.

Unlike biomass accumulation, where wild caught up to modern by the final harvest point in P-fertilized soil, wild tomato maintained a lower shoot P concentration across time compared to the modern counterpart in P-fertilized (Figure 4-1C) and unfertilized (Figure S4-2A) soil. Similarly, wild tomato showed less P concentration in its shoot tissue compared to modern at every time point in P-fertilized (Figure S4-2C) and unfertilized soils (Figure S4-2D). The wild tomato concentrated more of its P in its root tissue, as its root P concentration increased with time and was larger than the modern representative for the final three harvest points (Figure 4-1D). The final two harvest points in fertilized soils also showed no differences between wild and modern tomato for root P uptake (Figure S4-2E). In unfertilized soils, these differences between the two domestication groups diminished, and the wild tomato was not different from the modern tomato at any tested harvest point for root P concentration (Figure S4-2B). For the total amount

of P in the shoot and root tissue, wild and modern were not different by the end of the experiment in high P (Figure S4-2G), and in low P, wild accumulated little P relative to the modern tomato (Figure S4-2H).

Increased soil phosphorus levels in the wild over the modern tomato

Here, we explored three pools of labile soil P that capture increasingly greater amounts of bioavailable P: water soluble P, Olsen P, and Mehlich-3 P. While all three of these tests measure orthophosphate, Mehlich-3 P can also capture *myo*-inositol hexaphosphate and its stereoisomers. The lower accumulation of shoot P and biomass by P-sufficient wild tomato could explain, in part, the corresponding levels of available P in the soil. The P-fertilized wild tomato showed greater water soluble P concentration at two of the five harvest time points (Figure 4-2A). Similarly, fertilized wild tomato maintained higher Olsen P concentrations in its soil, and for most time points, wild had greater Olsen P than modern tomato under fertilized conditions (Figure 4-2B). There was also a significant increase in Mehlich-3 for the wild tomato at the second harvest point (Figure 4-2C).

Further, there was no relative decline or increase in Olsen P (Figure 4-2B), water soluble P (Figure 4-2A), or Mehlich-3 P (Figure 4-2C) values with time for P-fertilized wild tomato; rather, wild tomato showed consistent soil P concentrations in fertilized soil throughout the entire growing period. In contrast, modern tomato depleted these soil P values relative to its initial concentration. The initial seedling stage of modern tomato showed values just as high as wild tomato for water soluble P (Figure 4-2A), Olsen P (Figure 4-2B), and Mehlich-3 P (Figure 4-2C). It subsequently utilized the reserves of bioavailable water soluble P and Olsen P resulting in significantly lower levels of P as compared to the wild (Figure 4-2) and levels that were no different than its value in soils where no P was applied (Figure S4-3). This sharp drop in

bioavailable P in modern tomato corresponds to its sharp increase in biomass. Thus, it is possible that the modern accession capitalized on the flush of exogenous nutrients (as is often applied in agricultural systems).

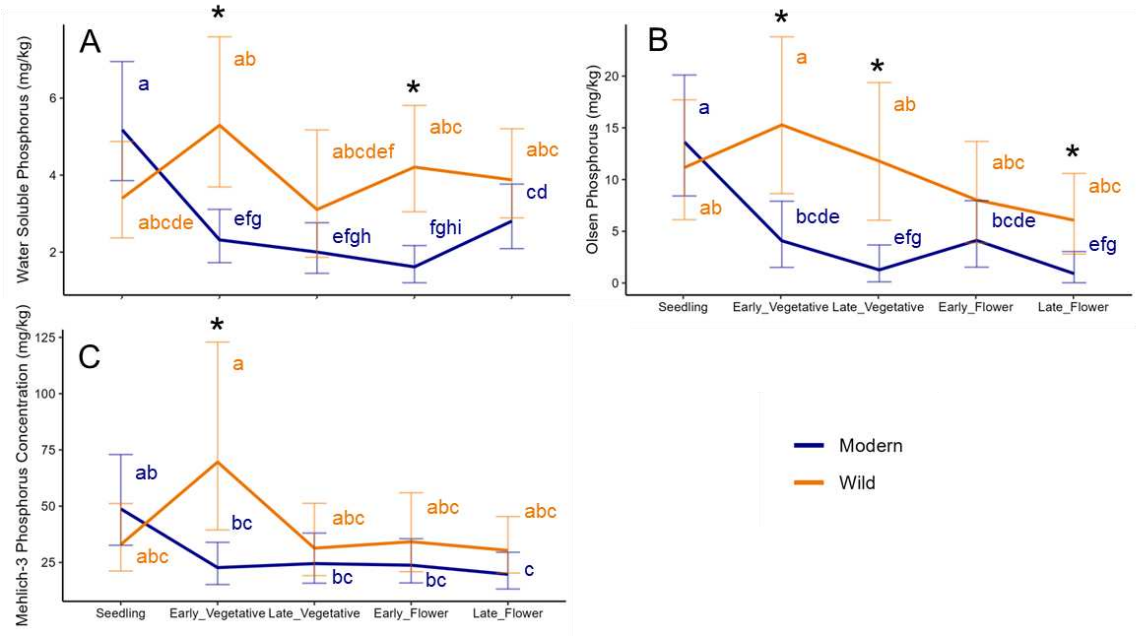

Figure 4-7. Soil phosphorus dynamics of a wild and a modern tomato across different developmental stages in P-fertilized soils. Water soluble P (A), Olsen P (B), and Mehlich-3 P (C) were measured in soils. Data were log-transformed for analysis and back-transformed for visualization. Line color denotes tomato domestication: orange (wild) and blue (modern). Bars display 95 % confidence intervals. An ANOVA with Tukey HSD was run for shoot and root biomass. Different letters denote significant differences at α = 0.05. Stars denote significant difference for the pairwise contrast for wild and modern tomato at the corresponding developmental stage.

Promotion of rhizosphere microbiota related to phosphorus by the wild tomato

Rhizosphere microbiome studies were conducted to explore possible methods by which wild tomato maintained higher extractable soil P concentrations. Notably, the pattern of development for P solubilizing bacteria in fertilized soils of wild tomato inversely paralleled that of the biomass development of tomato in fertilized soils: a rapid accumulation by wild tomato (rather than modern) early in the experiment (Figure 4-3A). This trend was not observed in unfertilized soil; at the early flower stage, modern tomato had a slight but significant increase in P solubilizer

relative abundance compared to wild, but by final harvest, there was no difference (Figure S4-4A).

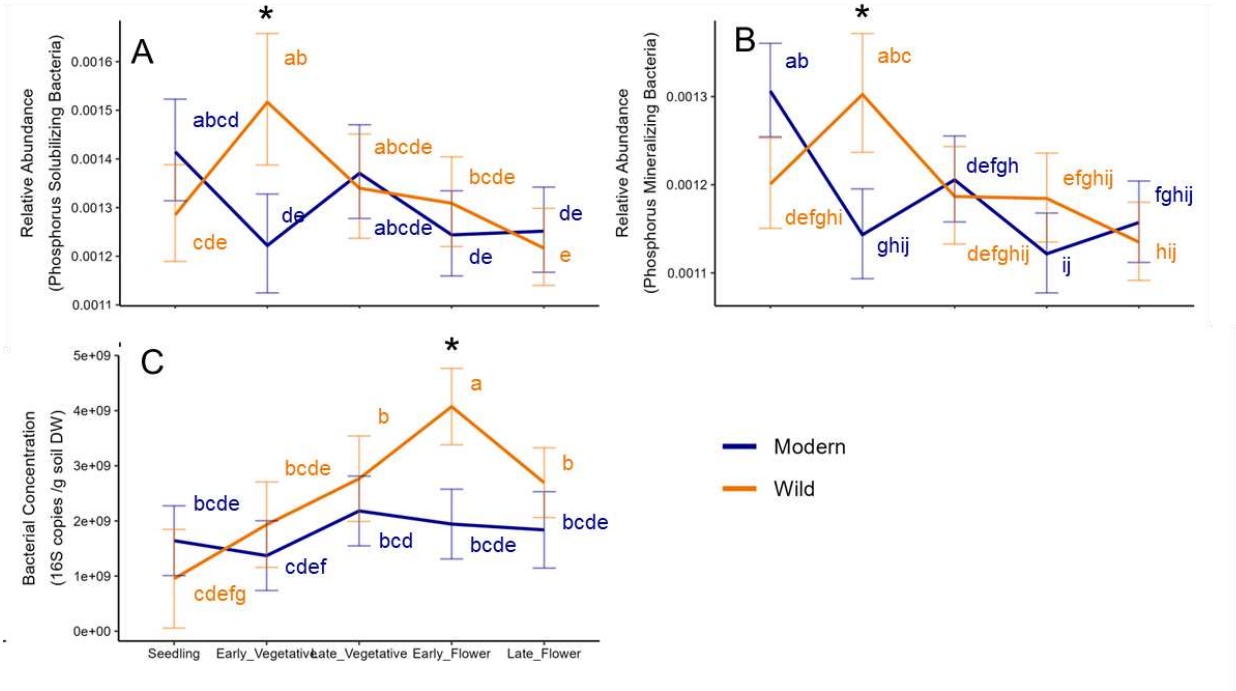

Figure 4-8. Rhizosphere bacterial dynamics of a wild and a modern tomato across different developmental stages in P-fertilized soils. The relative abundance of P solubilizing bacteria (A) and P mineralizing bacteria (B) were quantified. The bacterial biomass (16S copies per g soil dry weight) was also determined (C). Data were log-transformed for analysis and back-transformed for visualization. Line color denotes tomato domestication: orange (wild) and blue (modern). Bars display 95 % confidence intervals. An ANOVA with Tukey HSD was run for shoot and root biomass. Different letters denote significant differences at α = 0.05. Stars denote significant difference for the pairwise contrast for wild and modern tomato at the corresponding developmental stage.

The development of P mineralizing bacteria relative abundance followed the pattern of P solubilizing bacteria closely. There was again a spike in relative abundance of P mineralizers that occurred at the early vegetative stage for wild tomatoes planted in fertilized soil (Figure 4-3B). At this stage, there was greater relative abundance of P mineralizers in wild compared to modern tomato. Moreover, the P-fertilized wild tomato rhizosphere saw a decline of P mineralizing bacteria after this spike of P mineralizers (Figure 4-3B). For P-fertilized modern tomato, there was a drop in relative abundance between the first and second stages of harvest and then a

plateau (Figure 4-3B). In unfertilized soil, modern tomato had greater relative abundance compared to wild only at the last harvest stage (Figure S4-4B). These bacteria can transform recalcitrant P into bioavailable forms and are enriched early in the life history of wild tomato, thereby increasing the pool of bioavailable P. Conversely, modern tomato does not promote growth of P cycling bacteria and does not maintain stores of bioavailable P in the soil solution.

Beyond the proportionate change of certain bacterial groups, there were also notable differences in microbial biomass, which was measured as copies of the 16S gene per g of soil (Figure 4-3C). The wild tomato accession showcased a proliferation of microbial biomass at the fourth harvest stage relative to other time points and relative to the modern tomato (Figure 4-3C). Further, the wild tomato rhizosphere had an increase in microbial biomass at the stage of early flowering, whereas its modern counterpart maintained an unchanging level of microbial biomass.

Bacterial fluctuations related to changes in phosphorus application and domestication

To further elucidate changes occurring in the rhizosphere, we assessed the bacterial counts changing between each harvest stage for wild and domesticated tomato. We observed clear differences under fertilized conditions. The P-fertilized modern tomato rhizosphere showed pronounced stabilization by the later stages of development (Figure 4-4A). Conversely, wild tomato showed both greater fluctuation than modern at the same stages of development and an increase in bacterial fluctuation between the seedling and early vegetative stage for wild tomato (Figure 4-4B). These bacteria that were enriched in early vegetative stage possessed an abundance of beneficial genes. For example, there were redundant genes present for P mineralization and siderophore production (Figure 4-4B). This sharp change in bacterial dynamics at the early vegetative stage in P-fertilized soils corresponds to the rapid relative increase of P solubilizing (Figure 4-3A) and P mineralizing (Figure 4-3B) bacteria observed in

wild tomato and rapid shoot (Figure 4-1A) and root biomass (Figure 4-1B) accumulation in modern tomato, thus underscoring the importance of this time point in P recovery strategies. Although there were clear differences between wild and modern tomato when fertilizer was applied, there were minimal differences when fertilizer was omitted for both modern (Figure S4-5A) and wild tomato soils (Figure S4-5B).

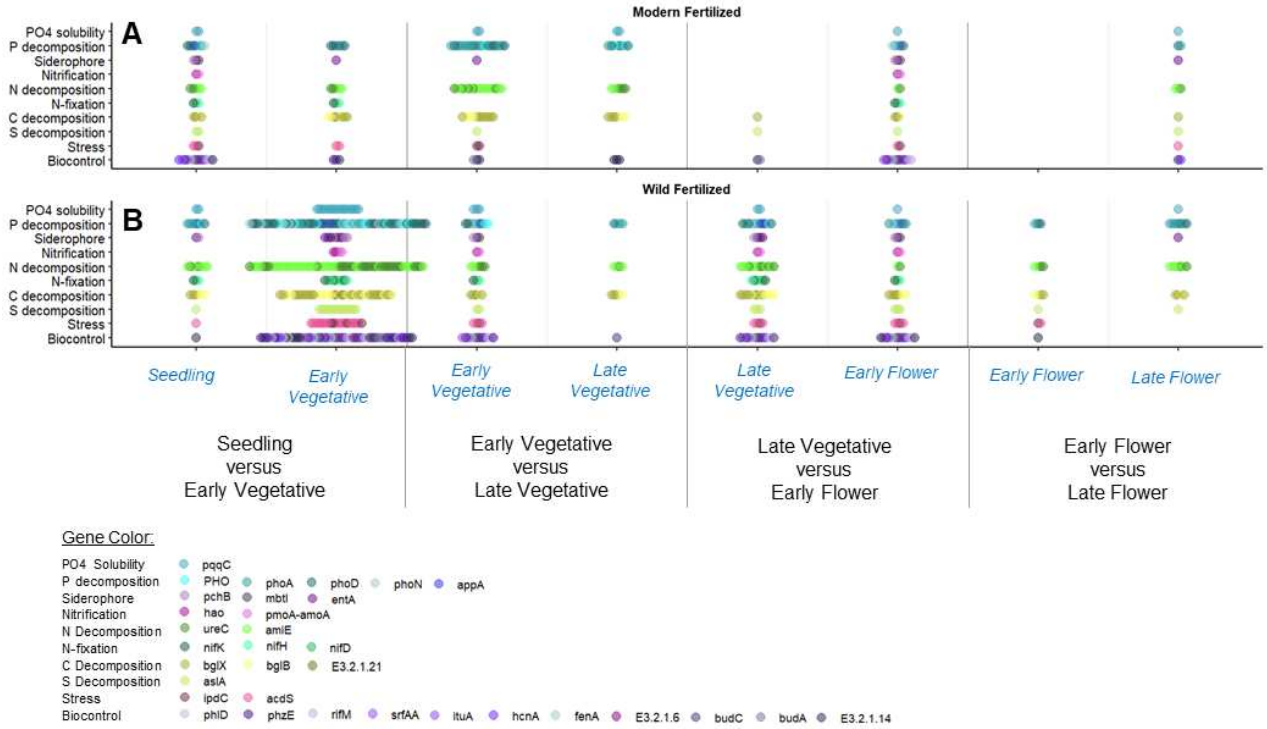

Figure 4-9. Fluctuations in the bacterial rhizosphere counts between different developmental stages in fertilized wild and modern tomatoes. A differential abundance analysis using a Wald test with an FDR p adjustment of 0.05 was conducted for each tomato domestication group: modern tomato in fertilized soil (A), wild tomato in fertilized soil (B). Bacterial count data were compared between each developmental stage, denoted in black text on the x-axis. The specific stage in which bacteria were enriched are listed on the x-axis in italicized blue text. The presence of a dot above the gray text on the x-axis indicates that the bacterium enriched in that domestication group contains a gene corresponding to a specified function on the y-axis. The different colors of dots represent different genes, and the key for these colors are listed on the bottom of the figure.

Certain bacteria were promoted by P-fertilized wild tomato. There were 7, 31, 17, 87, and 17 bacteria that differed between fertilized wild and fertilized modern tomato in the seedling (Table S4-3), early vegetative (Table S4-4), late vegetative (Table S4-5), early flower (Table S4-6), and late flower (Table S4-7) stages, respectively. Most of the species in the early flower

(Table S4-6) and late flower (Table S4-7) stages were enriched in the wild tomato rhizosphere. In exploring another comparison between fertilized wild and unfertilized wild soils, there were 85 bacteria that differed, and most were enriched in the fertilized soil (Table S4-8). Of these fluctuating bacteria among all of the above comparisons (i.e., wild vs modern at various growth stages), there were similarities in the species that were enriched by fertilized wild tomato. We identified 31 P-fertilized wild tomato-promoted bacteria (i.e., enhanced in conditions of wild high P compared to wild low P and in conditions of wild high P compared to modern high P) (Table S4-9). These bacteria contain an abundance of redundant and beneficial functions, including P mineralization, P solubilization, and siderophore production (Table S4-9).

Wild tomato-derived exudates promote bacterial phosphorus solubilizing activity

The domestication-influenced physical responses and the associations with rhizosphere microbiota because of P fertilization have been defined in the above time course trial. To illuminate the mechanisms by which the modern and wild tomato representatives interact with the soil to acquire P, we carried out a root exudate study. Briefly, the wild and modern tomato representatives were grown in full nutrient solution, and the root exudates were captured and analyzed using mass-spectrometry based metabolomics.

There were clear differences in the composition of root exudates between the tested accessions of wild and modern tomato. There were 11, 13, 3, 3, and 9 significantly different metabolites identified through LC-MS reverse phase positive mode (Figure 5A), LC-MS reverse phase negative mode (Figure 4-5B), LC-MS Hydrophilic interaction chromatography (HILIC) positive mode (Figure 4-5C), LC-MS HILIC negative mode (Figure 4-5D), and GC-MS (Figure 5E), respectively (Table S4-10). The modern tomato representative principally promoted fatty acids and organic acids (e.g., myristic acid, lauric acid, malic acid, 3-hydroxybutyric acid),

whereas wild tomato primarily exuded sugar and glycerol-related compounds (e.g., trehalose, 1-stearoylglycerol, glycerol monostearate) (Table S4-10).

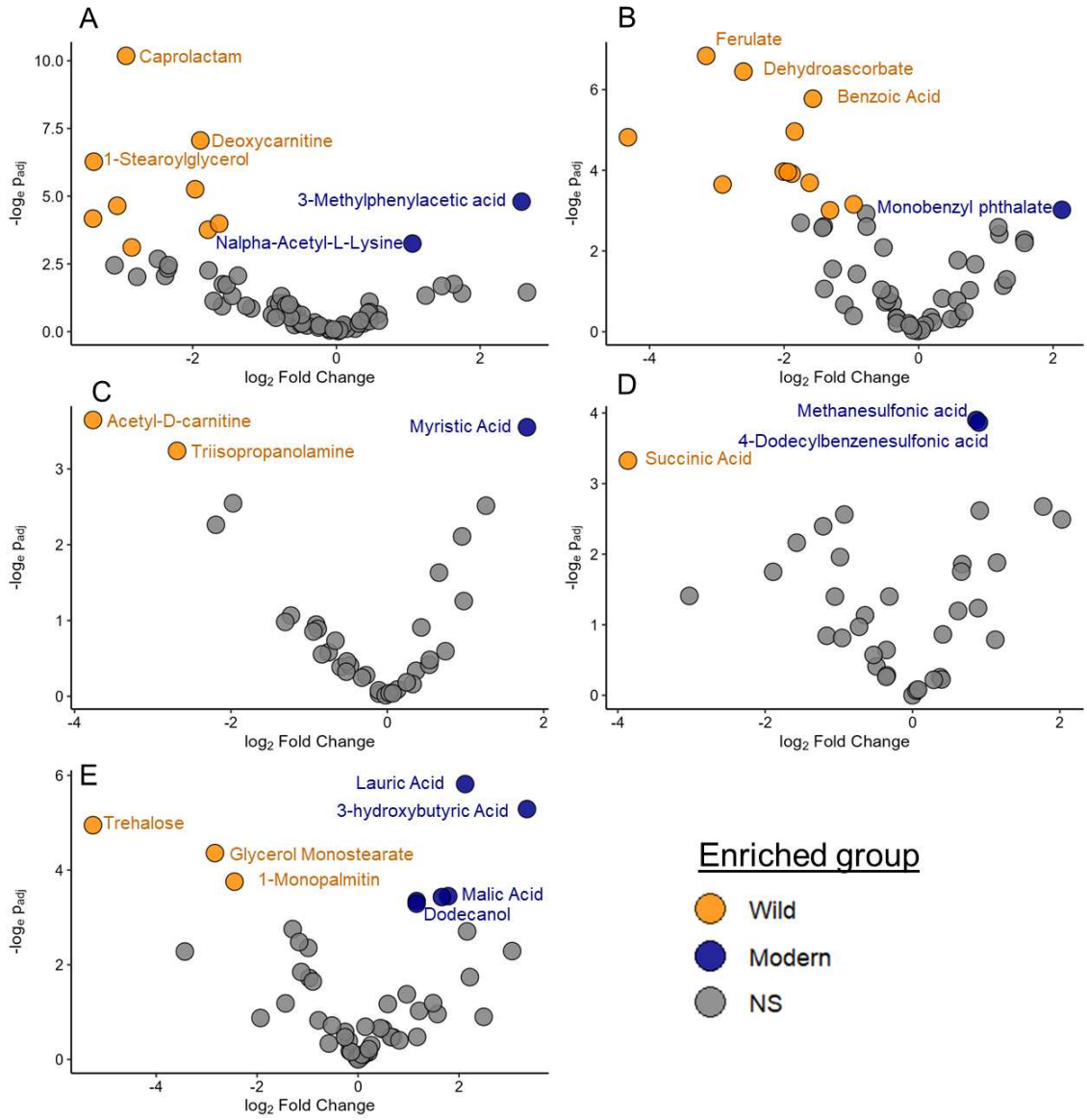

Figure 4-10 Volcano plots showing the enrichment of metabolites produced by either the wild tomato or the modern tomato. Metabolite profiles were determined through LC-MS in reverse phase positive mode (A), LC-MS in reverse phase negative mode (B), LC-MS in HILIC positive mode (C), LC-MS in HILIC negative mode (D, and GC-MS (E). Text adjacent to dots indicates the highly enriched compounds in either the wild or modern tomato exudates. Color of dots and text denote the group in which the metabolite was enriched: orange (wild tomato), blue (modern tomato), and gray (not significantly enriched).

Certain root exudates that were highly enriched in either the wild tomato (glycerol monostearate, 1-stearoylglycerol, 1-monopalmitin, trehalose) or the modern tomato (lauric acid, dodecanol) (Figure 4-5, Table S4-10) were selected for downstream analysis to test if wild and modern tomato differentially affected microbiota capable of increasing P availability to the plant. Metabolites were added to media with *Azospirillum brasilense*[27] to visually determine the P solubilizing index (calculated as the diameter of the halo divided by the diameter of the colony). Wild tomato-derived exudate compounds promoted the P-solubilizing activity of *A. brasilense*, particularly glycerol monostearate and 1-stearoylglycerol (Figure 4-6A, Figure 4-6B). Trehalose increased P solubilization by *A. brasilense* in tricalcium phosphate (TCP) media (Figure 4-6B). The tested modern tomato exudates resulted in neither solubilization nor colony growth (Figure 4-6A, Figure 4-6B). Regardless of exudate treatment, *A. brasilense* showed a greater P solubilization index when grown in phytin media (Figure 4-6A) compared to tricalcium phosphate (TCP) (Figure 4-6B). These exudates were also tested alone without the supplementation of *A. brasilense.* However, no compound produced solubilization directly (data not shown).

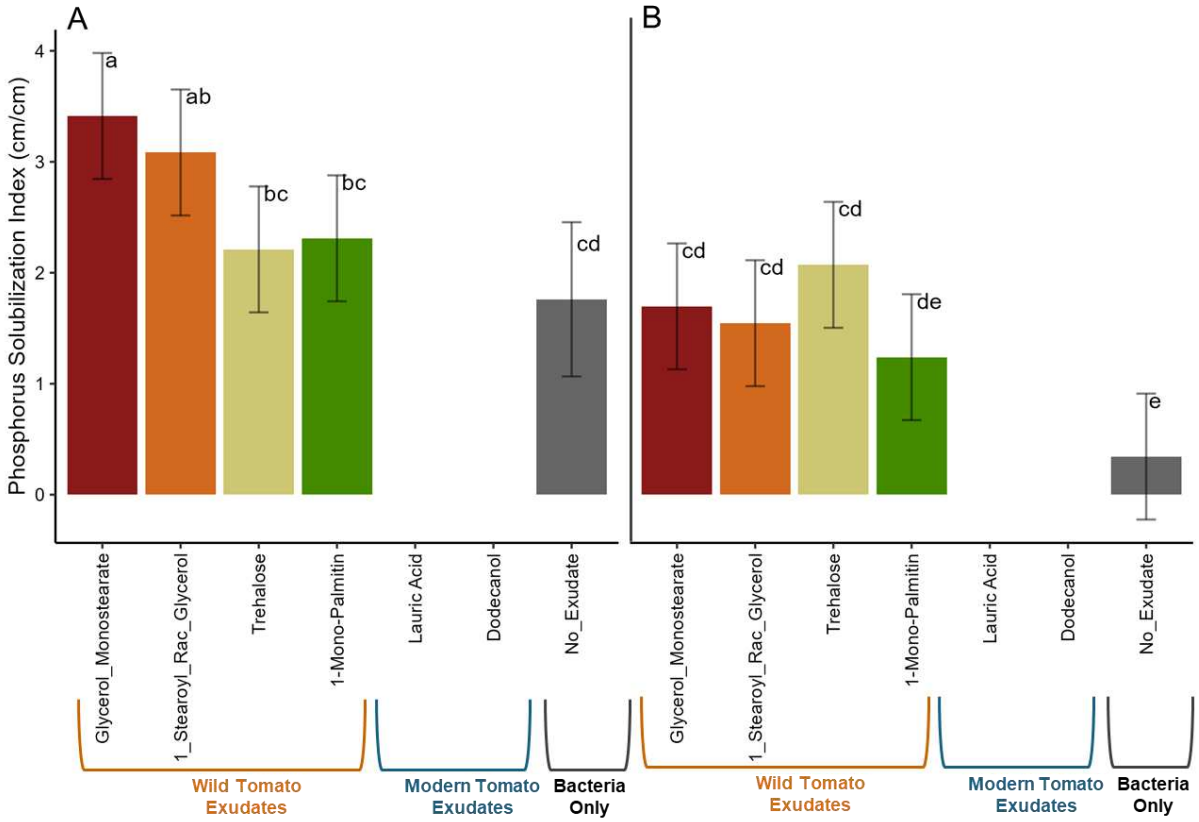

Figure 4-11. Phosphorus solubilization index (cm/cm) of Azospirillum brasilense plated with metabolites derived from wild tomato (glycerol monostearate, 1-stearoylglycerol, 1-monopalmitin, trehalose) and modern tomato (lauric acid, dodecanol) exudates. "No_Exudate" indicates the treatment of Azospirillum brasilense plated alone without any exudate addition. A. brasilense and exudates were plated on NBRIP media using either phytin (A) or tricalcium phosphate (B) as the phosphorus source. An ANOVA with Tukey HSD test were run at α = 0.05. Bars represent the 95 % confidence interval. Different letters denote significant differences at α = 0.05. Labels on the x axis without any corresponding bar indicates that the exudate did not show any growth of bacterial colonies.

Conditioning soils with fertilized wild tomato slightly alters modern tomato growth

In the previous results, we observed that in P-fertilized soils, wild tomato promoted the growth of beneficial P-cycling bacteria (Figure 4-3) along with several other potentially beneficial bacteria in the rhizosphere (Figure 4-4), particularly between the seedling and vegetative stages of development. Further, higher concentrations of bioavailable P were observed that allowed for the greater growth of wild tomato in later harvest stages for tomato (Figure 4-2). To determine how these changes may benefit subsequent cycles of plant growth, we grew a wild tomato, a

modern tomato, or left the pots empty as a no plant control in soils that were either P-fertilized or unfertilized. Henceforth, these soil conditioning treatments are referred to as “wild-conditioned”, “modern-conditioned”, and “no plant-conditioned” soils, respectively. These soil conditioning treatments continued for five weeks (i.e., equivalent to between the seedling and early vegetative stage defined in the previous time course trial), and then a modern tomato was planted in all pots. The modern tomatoes planted in the second cycle were either P-fertilized or left unfertilized.

We found that although conditioning sterile soils with P-fertilized wild tomato produced little-to-no differences in biomass compared to the no plant control, it did produce plants that were larger than those grown in soils where a P-fertilized modern tomato was initially planted (Figure 4-7A, Figure 4-7B). Regardless of whether the second cycle received fertilization, plants in soils with an initial modern tomato had smaller shoots than those in the wild-conditioned soils (Figure 4-7A). Root biomass accumulation showed a similar pattern. Fertilized wild tomato-conditioned soils increased root biomass of modern tomato compared to fertilized modern-tomato conditioned soils, but it did not change the biomass compared to the no plant-conditioned soils (Figure 4-7B). Fertilized modern-conditioned soils showed the greatest root P concentration of the soil conditioning treatments (Figure 4-7C). However, when accounting for biomass, modern-conditioned soils had a reduced P uptake (Figure 4-7D). Unlike the differences observed in soils that were initially fertilized, in soils that were unfertilized in the first cycle, there were few differences among the soil conditioning treatments for shoot biomass (Figure S4-6A) and root biomass (Figure S4-6B).

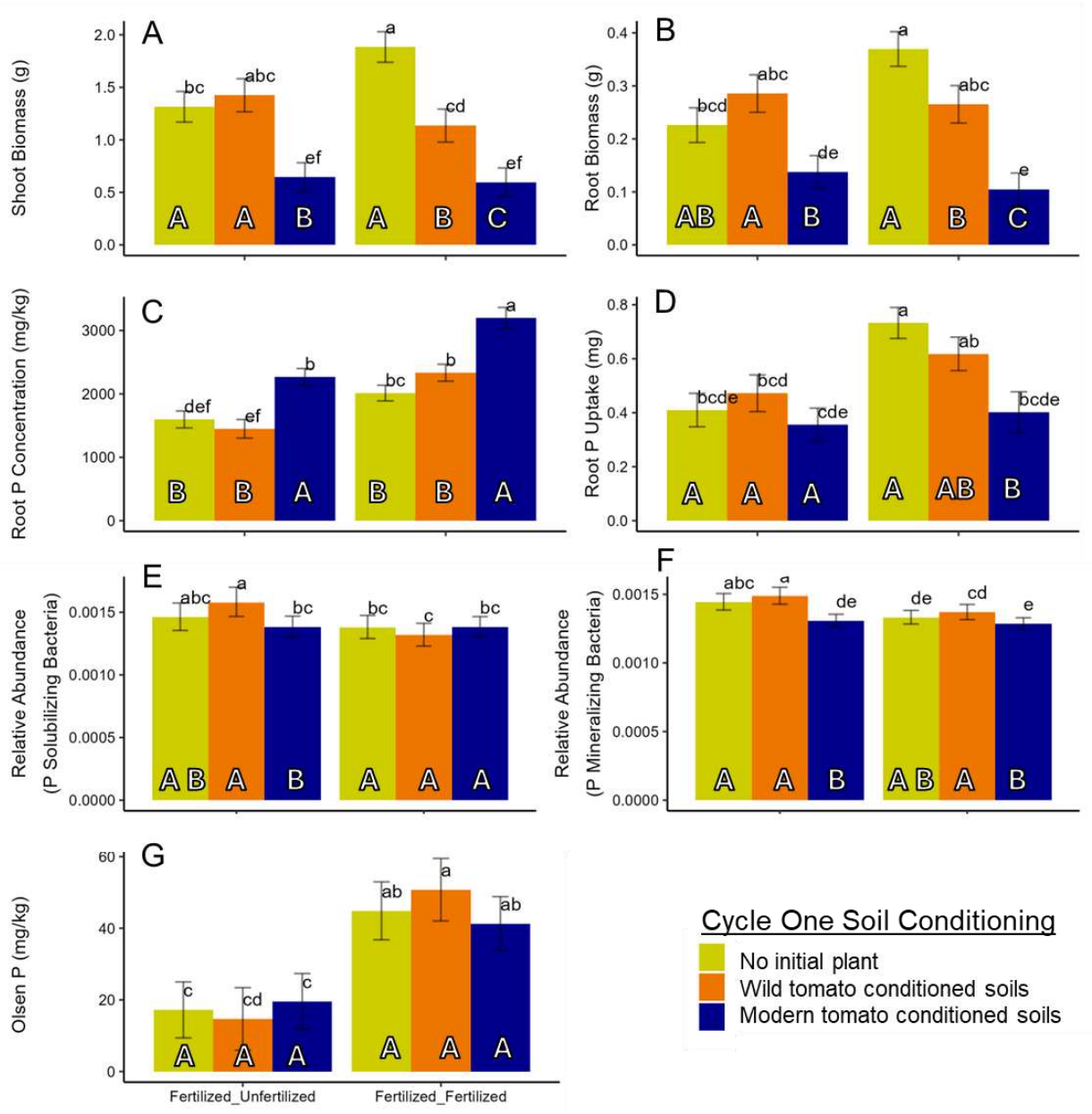

Figure 4-12. Above- and belowground impacts of conditioning soils with fertilizer and wild tomato on modern tomato growth. Modern tomato shoot biomass (A), root biomass (B), root P concentration (C), root P uptake (D), rhizosphere phosphorus solubilizer relative abundance (E), rhizosphere phosphorus mineralizer relative abundance (F), and soil Olsen phosphorus (G) were determined and analyzed using an ANOVA with Tukey HSD test. Results are displayed as mean ± standard error. Different letters denote significant difference in either biomass or relative abundance at α = 0.05. The x-axis represents the fertilization patterns in the soil conditioning cycle and in the second cycle. Bar color represents how soils were conditioned in cycle 1: yellow (no plant-conditioned), orange (wild-conditioned), and blue (modern-conditioned). Different lowercase letters denote differences in the interactive effect. Different uppercase letters denote differences of the soil conditioning treatment within a specific fertilization pattern.

Modern tomato has a rhizosphere microbiome resilient to change

The relative abundance of P-solubilizing bacteria was slightly but significantly influenced by the soil conditioning treatment. The only significant difference in P-fertilized soils was between the modern-conditioned soils compared to the wild-conditioned soils under the fertilization pattern of P-fertilized then unfertilized (Figure 4-7E). This trend was similar for P mineralizing bacteria, but regardless of fertilization pattern, P mineralizers were enriched in the wild-conditioned soils compared to the modern-conditioned soils (Figure 4-7F). It is important to note that although wild-conditioned soils resulted in greater P mineralizer relative abundance compared to modern-conditioned soils, neither of these treatments were different from the no plant control. Therefore, these changes observed between our wild and modern conditioning treatments are significant, but minor. Under low fertility (i.e., when soils were not fertilized in the first planting cycle), the wild tomato relative could not promote P solubilizing or P mineralizing bacterial growth. The relative abundance of P solubilizing (Figure S4-7E) and P mineralizing (Figure S4-7F) bacteria in wild-conditioned and modern-conditioned soils were not significantly different when soils followed the fertilization pattern of unfertilized then fertilized. Further, the changes caused by the soil conditioning treatments did not persist in the soil P pools, and Olsen P was not affected by the soil conditioning treatment (Figure 4-7G).

We examined the bacterial community composition between the wild-conditioned soils to the communities from the time course trial (P-fertilized wild tomato and P-fertilized modern tomato). A perMANOVA revealed that these three conditions produced distinct microbial communities. However, a PCoA ordination illustrated greater overlap between the fertilized modern soils (initial time course trial) and the wild-conditioned soils (this soil conditioning trial) compared to the overlap between fertilized wild soils (time course trial) and the wild-conditioned

soils (soil conditioning trial) (Figure S4-7). Therefore, the bacteriome dissimilarity may be greater between P-fertilized wild tomatoes and modern tomatoes conditioned with a wild tomato.

Although the modern tomato was not able to maintain the microbiome cultivated by the wild tomato, certain bacterial species persisted. We examined highly abundant bacteria in modern tomato soils conditioned with a P-fertilized wild tomato (Figure S4-8) and compared those species to those promoted by fertilized wild tomato in the above time course trial (Table S4-3, Table S4-4, Table S4-5, Table S4-6, Table S4-7). There was some compositional consistency in the highly abundant species in P-fertilized wild-conditioned soils (soil conditioning trial) and the P-fertilized wild tomato (time course trial). For example, *Trichocoleus desertorum* and *Geitlerinema* sp. *PCC 7407* were enriched in both modern tomato planted in wild-conditioned soils (Figure S4-8) and in P-fertilized wild tomato soils during the time course trial in the early vegetative and the late vegetative stages. These species are plant beneficial; *T. desertorum* and *G.* sp. *PCC 7407* both contain genes associated with C decomposition and N decomposition. The plants in the initial soil conditioning trial were grown through the early vegetative stage, the stage in which these bacteria were enriched in wild tomato soils in the initial time course study. Thus, the wild tomato is imparting some change on the soil composition, that not only appears during its own growing period, but also persists through a second planting cycle with a different plant.

Discussion

Wild plants – more so than their domesticated counterparts – rely on microbial associations to grow in a phosphorus (P) deficit[22,29,30]. To grow in conditions of limited nutrition, wild crop relatives tend to demonstrate insensitivity to low P[21,31] and allocate resources for survival, not production[7]. These strategies are essential for adapting to natural soils where P concentrations

limit plant growth[32,33]. However, natural soils also experience sporadic occurrences of concentrated sections of P through events such as cadaver decomposition[34], faunal fecal deposits[35], and treefall occurrences[25]. In our current study, we observed how wild tomato responded to a similar flush of exogenous nutrition: by intimately interacting with its associated soil microbiota via its root exudates to promote soil P availability, resulting in enhanced plant acquisition and development.

To increase levels of bioavailable P, wild tomato showed slower P uptake and promoted beneficial bacterial associations at the early vegetative stage. This stage showed great fluctuations in the rhizosphere for P-fertilized wild tomato. Many bacteria were differentially abundant between this stage and the previous seedling stage. This microbially-informed strategy differs from that which was employed by modern tomato. Modern tomato – at the early vegetative stage – rapidly accumulated root tissue biomass, thereby exploiting the fertilizer-P reserves. The concentrations of water-soluble, Olsen, and Mehlich-3 P decreased with time for P-fertilized modern tomato, particularly between the first and second harvest stages. These changing concentrations illustrate a depletion in varied soil pools by modern tomato. While water soluble P measures only dissolved reactive phosphate[36], Olsen P tends to primarily capture orthophosphate, and Mehlich-3 may extract other forms of P (e.g., orthophosphate, orthophosphates mono-/di-esters)[37,38]. Thus, the modern tomato utilizes and depletes P across a range of labile soil pools to levels that were no different from the unfertilized control.

The divergent P acquisition strategies by the representative wild and modern tomato (i.e., microbially facilitated for wild and root scavenging for modern) are most apparent at the early vegetative harvest stage. It has been shown in tomato that there are dynamic changes occurring during the first four weeks of development (equivalent to between the seedling and early

vegetative stage in this current study), but after four weeks, the rhizosphere stabilizes[39]. Thus, the wild and modern tomato representatives may have been imposing strong host selection power on their microbial communities near the early vegetative stage, resulting in distinct communities and a separation in P recovery strategies. In both wild tomato and domesticated tomato, growth to this approximate stage (four weeks) was sufficient to produce differences in the rhizosphere bacteriome[40]. Thus, by this period, host plants imparted change on their rhizosphere communities. Here, wild tomato promoted plant beneficial P solubilizing and P mineralizing bacteria. This result is consistent with the findings from other studies that found that wild tomato harbors P -solubilizing and -mineralizing bacteria to a greater extent than modern tomato[21,22]. In our current study, P-fertilized modern tomato showed a relatively stable rhizosphere bacteriome assemblage, with little fluctuation occurring. This result is consistent with Yu et al.[21], who suggested that wild tomato forms stronger and more intimate relationships with the surrounding soil microbial communities than modern tomato.

The presence of these P solubilizing bacteria likely allows for the phosphates from P-fertilizer to stay in soil solution as opposed to being precipitated in the soil as insoluble mineral forms. Bioavailable phosphates in soil solution precipitate to secondary P minerals and must be dissolved to become plant available again[41]. Through their organic acid activity, P solubilizing bacteria increase the available P status of soils[12,42,43]. At the point when P -solubilizing and -mineralizing bacteria increased in the P-fertilized wild tomato soils, we observed a concomitant increase in water-soluble, Olsen, and Mehlich-3 P in the P-fertilized wild compared to the P-fertilized modern tomato soils. We thus posit that the increased bioavailable inorganic P in the wild tomato soils is because of the P solubilizing bacteria the plant root exudates effectively culture. This conclusion is consistent with current research[44,45]. Across a variety of soil types,

application of P-solubilizing bacteria increased the concentration of Olsen P[44,45]. Thus, wild plants capable of enhancing growth of P-solubilizing organisms in the soil may result in increased reserves of plant-available soil solution phosphate. The concentration of water-soluble, Olsen, and Mehlich-3 P in P-fertilized wild tomato soils maintained at higher levels than the modern representative and at levels that were unchanging with time. The available P is preserved by wild tomato for uptake and biomass accumulation in the later plant growth stages. Most likely, the unavailable P that is targeted by wild tomato is loosely bound, which makes it easier to recover in the current generation of growth. Conversely, the modern tomato was unable to proliferate P solubilizing and mineralizing bacteria, so it was unable to maintain stores of bioavailable P.

These changes in bioavailable P may also have been driven by microbial abundance. Soil microbes immobilize P by incorporating it into their biomass[46]. Microbial biomass has therefore been shown to be a valuable predictor of P immobilization, as greater microbial biomass indicates greater immobilization[47]. The microbial biomass in the rhizosphere of our tested accession of wild tomato not only was greater than the modern accession, but it also increased with time. Thus, microbial proliferation may have led to an increase in the extractable forms of P as immobilization and mineralization occur in soils.

The wild tomato accession likely is regulating its composition and biomass of microbiota through root exudate activity. Chemicals secreted by plant roots mediate interactions occurring in the rhizosphere[48]. The wild tomato accession in this study promoted the exudation of trehalose, a sugar compound. Sugars are a carbon source and have been hypothesized to cause a proliferation of soil microbial activity when produced as an exudate[39,49]. Our tested wild tomato increased microbial biomass with time and relative to the modern tomato, and this change may be caused

by the increased sugar exudation. Sugars also tend to be exuded earlier in plant development[50]. This trend supports our result in which P-fertilized wild tomato showcased great fluctuations in the rhizosphere microbiome at the early vegetative stage. It is possible that the exuded sugar compounds increased microbial communities resulting in great change in the rhizosphere. Trehalose, preferentially exuded by wild tomato, is also an important root exudate that promotes plant growth. In a study with maize, for example, it was found that exogenous application of trehalose increased growth and stress tolerance to low P[51]. Trehalose being a carbon source might explain its promotion of the growth of our tested soil bacterium. By the time we calculated the P solubilization index after 4 days of incubation, the P dissolved to the point at which we could not calculate the P solubilization index. The wild tomato in our study also exuded an abundance of glycerol compounds. We observed this growth in our current study wherein glycerol compounds (glycerol monostearate and 1-stearoyl glycerol) increased the P solubilizing activity of *Azosprillum brasilense,* a P solubilizing bacterium commonly used as an inoculant for its plant growth promoting properties[27]. The *Azosprillum* genus tends to be slower growing than other rhizosphere genera, like *Pseudomonas*[52]. The promotion of P solubilization by glycerol and *A. brasilense* therefore suggests a strong indirect solubilizing capacity of the selected glycerol compounds. Glycerol has been shown to be a carbon source for microbes[53]. Therefore, the production of glycerol and sugar compounds by wild tomato are likely driving the proliferation of plant-beneficial rhizobacteria that ultimately allowed for wild tomato to consistently grow and develop across time.

Rather than being enriched in sugars and glycerol compounds, the modern tomato accession promoted exudation of organic acids. There is strong evidence in current literature that organic acids promote direct P solubilization[54-57]. The tested metabolites from modern tomato

(lauric acid and dodecanol) did not increase P solubilization of *A. brasilense* and inhibited its growth. Lauric acid and dodecanol have been shown to have antimicrobial properties[58,59]. Root exudates with antimicrobial properties can indicate pathogen suppressive strategies[60]. Thus, the difference in the bacterial suppression and bacterial promotion between the modern and wild tomato accessions suggests that they showcase different strategies for growth and development. Wild tomato enhances intimate associations in the rhizosphere. Modern tomato, as illustrated through its strong production of antimicrobial compounds and organic acids, does not intimately associate with its rhizosphere microbiota. Instead of concerted plant-microbe recovery of soil P, modern tomato is likely directly acquiring P. Although the tested modern tomato exudates were unable to directly solubilize P, other metabolites that were highly promoted by modern tomato (but not selected for testing in plates) have been shown to directly solubilize P. For example, in recent studies by Pantigoso et al.[55,61], malic acid[61] has been shown to directly solubilize P from sparingly available tricalcium phosphate media. Organic acids have been widely documented in the literature to solubilize sparingly available forms of soil P[54,61-63]. The primary mechanism by which organic acids promote plant P uptake is by their strong dissolution capacity[64]. Therefore, because our tested modern tomato accession increased exudation of organic acids relative to the wild accession, it is likely engaging in a direct physical strategy to recover soil P.

The properties of the modern tomato metabolites observed in the root exudates trial may also explain some of the results observed in the soil conditioning trial. The antimicrobial activity by modern tomato exudates suggests that the modern tomato is unable to continue the growth of the wild tomato-promoted bacteria. It is possible that any changes produced by the wild tomato on rhizosphere microbiota related to soil P cycling would not have been able to continue when the modern plant was subsequently grown. In our soil conditioning trial, we showed that the

changes imposed by the wild tomato can slightly but significantly increase the growth and P solubilizer relative abundance of a subsequent generation of modern tomato. However, this increase did not translate into increased Olsen P by the end of the second planting cycle. This finding is consistent with Chen et al.[65] who found that even though genotypes of cotton varied in shoot biomass, root biomass, and shoot P concentration, the Olsen P concentrations were unchanged.

Likely as a result of its root exudate activity, the modern tomato was unable to maintain the populations of the wild-promoted bacteria. However, there was some consistency in the bacteria promoted by P-fertilized wild tomato and the bacteria that were highly abundant in modern tomato that previously had a wild tomato planted. Thus, certain bacteria (i.e., *Trichocoleus desertorum* and *Geitlerinema* sp. *PCC 7407*) are promoted by wild tomato and maintained by modern tomato. These bacteria are also plant-beneficial, possessing genes associated with C and N decomposition. *T. desertorum* plays a role in the weathering of calcareous stone material[66], a process that may be promoting soil P cycling in our tested soils which are calcareous. *Geitlerinema* sp. *PCC7407* has also been found to be enriched in the rhizosphere of wild tomato compared to heirloom or modern tomato[22]. Moreover, both *T. desertorum* and *G.* sp. *PCC 7407* are members of Cyanobacteria. In a recent study comparing the effects of Cyanobacteria application to different forms of organic fertilizers, researchers found that Cyanobacteria inoculation impacted microbial community composition and increased microbial biomass[67]. Cyanobacteria are known for their plant-growth-promoting abilities and are often used as a biofertilizer[68]. Thus, in addition to carrying over to the next cycle of planting with a different species, the wild tomato microbiome also promoted plant-beneficial soil nutrient cycling. This microbial legacy effect, however, has been shown to diminish with time[69]. We grew

our modern tomato for approximately five weeks, so it is possible these effects observed by the wild tomato may attenuate if tomato were grown for a longer period.

There were also notable species enriched specifically by P-fertilized wild tomato. *Rhizobacter gummiphilus,* for instance, is a species promoted during the early vegetative stage by P-fertilized wild tomato. Past research has shown that this bacterium has been enriched in this tested accession (LA0716) relative to other tomatoes[22]. This bacterium is a P solubilizer and P mineralizer, so it can transform P into bioavailable forms from organic and inorganic pools. Therefore, in conditions of high legacy P, *R. gummiphilus* may be contributing to the heightened levels of soil P in wild tomato. In low P soil, the wild tomato was small, showed little change in the rhizosphere between harvest stages, and did not accumulate soil P. Thus, wild tomato was responsive to the high fertility from fertilization. Similar results have been observed in wild wheat[70] and potato[20] where the wild relative exhibited less growth than the domesticated in conditions of a P deficit.

In times of high fertility, wild tomato maintains bioavailable soil P through its activity in the rhizosphere microbiome. In contrast, modern crop relatives, like the modern tomato in this study, express resource acquisitive strategies and take up nutrients rapidly. They are adapted to grow in highly fertile conditions[4] and rely on quick uptake of nutrients when they are available. This finding of divergent strategies in wild and modern tomato is valuable because P from fertilizers is easily lost from soil solution to retention reactions[71].

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CHAPTER 5 – SUGGESTIONS FOR FUTURE RESEARCH

One of the findings of this dissertation is that the microbiome of the wild tomato was unable to persist through to the subsequent cycles of plant growth. This trial comprised of a crop rotation analog, wherein a modern tomato was planted following planting of an initial wild tomato. Although there is evidence provided by the chapters in this dissertation that *Solanum pennellii* harbors a markedly distinct microbiome with high microbial biomass and enhanced phosphorus (P) solubilizer relative abundance, there was little benefit observed when it preceded modern tomato planting. Therefore, future research aims may focus on developing methods to harness the unique and putatively plant beneficial properties of the wild tomato rhizosphere and effectively transfer them to modern cultivars. Two possible avenues for these aims include (1) co-planting to transfer the whole tomato rhizosphere microbiome and (2) applying signaling metabolites directly to soils with modern plants.

Rather than conducting a crop rotation study, a co-planting (i.e., intercropping) study may provide stronger results. By co-planting *S. pennellii* with a modern cultivar, the whole root exudates will be able to be continuously produced in the root zone of the modern crop. The rhizosphere of *S. pennellii* has been shown in this dissertation to promote microbial biomass and P solubilizing bacteria, so by providing the modern tomato with these whole root exudates, beneficial soil bacteria in the rhizosphere may be able to proliferate and increase plant growth relative to not planting *S. pennellii* as an intercropped species.

Although the proposed co-planting study may provide benefit to the modern tomato representative, this study would not be able to elucidate which metabolites are promoting plant growth. To address this objective, we can apply root exudates enriched by wild tomato into the

soils with modern tomato planted. By composing treatments of separate blends and individual metabolites, we can link which metabolite is correlated with the increased growth by modern tomato. Like the proposed co-planting study, this trial also provides the modern tomato with signaling metabolites that may be able to promote the P solubilizing capacity of native microbiota.

These proposed next steps involve elucidating the ability of wild tomato root exudates to increase soil P bioavailability by enhancing microbial P solubilization. Overall, this work lays the foundation for development of approaches that promote soil P cycling while reducing reliance on external P inputs.

APPENDICES

Supplemental Table 2-1. KEGG orthologs used for predictive function identification. References used to support the function mentioned in the main text are provided in the "Reference" column.

Enzyme	Gene	Function	KEGG	Reference
Acetolactate decarboxylase	*budA*	Biocontrol	K01575	Chlebek, D.*, et al.* Genetic Determinants of Antagonistic Interactions and the Response of New Endophytic Strain Serratia quinivorans KP32 to Fungal Phytopathogens. *International Journal of Molecular Sciences* 2022;23(24):15561.
(S,S)-butanediol dehydrogenase	*budC*	Biocontrol	K18009	Dudeja, S.S.*, et al.* Bacterial endophytes: molecular interactions with their hosts. *Journal of Basic Microbiology* 2021;61(6):475-505.
Chitinase	*E3.2.1.14*	Biocontrol	K01183	Gomaa, E.Z. Chitinase production by Bacillus thuringiensis and Bacillus licheniformis: their potential in antifungal biocontrol. *The journal of Microbiology* 2012;50:103-111.
Isochorismate synthase	*ISC*	Antifungal	EC.5.4.4.2_1	Perez, E.*, et al.* The importance of chorismate mutase in the biocontrol potential of Trichoderma parareesei. *Frontiers in Microbiology* 2015;6:1181.
Surfactin family lipopeptide synthetase A	*srfAA*	Antibacterial	K15654	Mora, I., Cabrefiga, J. and Montesinos, E. Cyclic lipopeptide biosynthetic genes and products, and inhibitory activity of plant-associated Bacillus against phytopathogenic bacteria. *PLoS One* 2015;10(5):e0127738.
1-aminocyclopropane-1-carboxylate deaminase	*acdS*	Root Growth	K01505	Manter, D.K., Hamm, A.K. and Deel, H.L. Community structure and abundance of ACC deaminase containing bacteria in soils with 16S-PICRUSt2 inference or direct acdS gene sequencing. *Journal of Microbiological Methods* 2023:106740.
Isochorismate lyase	*PCH*	Chelation	EC.4.2.99.21_1	Cunrath, O.*, et al.* A cell biological view of the siderophore pyochelin iron uptake pathway in p seudomonas aeruginosa. *Environmental microbiology* 2015;17(1):171-185.
Beta-glucosidase	*E3.2.1.21*	Carbon Decomposition	E3.2.1.21	Adetunji, A.T.*, et al.* The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of soil science and plant nutrition* 2017;17(3):794-807.
Indolepyruvate decarboxylase	*ipdC*	Stress	K04103	Malhotra, M. and Srivastava, S. Organization of the ipdC region regulates IAA levels in different Azospirillum brasilense strains: molecular and functional analysis of ipdC in strain SM. *Environmental Microbiology* 2008;10(5):1365-1373.
2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase	*entA*	Siderophore	K00216	Penwell, W.F., Arivett, B.A. and Actis, L.A. The Acinetobacter baumannii entA gene located outside the acinetobactin cluster is critical for siderophore production, iron acquisition and virulence. *PloS one* 2012;7(5):e36493.
Nitrous-oxide reductase	*nosZ*	Denitrification	K00376	Dandie, C.*, et al.* Analysis of denitrification genes and comparison of nosZ, cnorB and 16S rDNA from culturable denitrifying bacteria in potato cropping systems. *Systematic and applied microbiology* 2007;30(2):128-138.
Isochorismate pyruvate lysase	*pchB*	Siderophore	K02364	Olucha, J.: University of Kansas; 2012. Structural, Functional and Computational Characterization of Pseudomonas aeruginosa Siderophore Biosynthetic Pathway Accessory Proteins PchB and PvdA.
Beta-glucosidase	*bglX*	Carbon Decomposition	K05349	Adetunji, A.T.*, et al.* The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of soil science and plant nutrition* 2017;17(3):794-807.
Beta-glucosidase	*bglB*	Carbon Decomposition	K05350	Adetunji, A.T.*, et al.* The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of soil science and plant nutrition* 2017;17(3):794-807.

Amidase	*amiE*	Nitrogen Decomposition	K01426	Ochiai, S.*, et al.* AmiE, a novel N-acylhomoserine lactone acylase belonging to the amidase family, from the activated-sludge isolate Acinetobacter sp. strain Ooi24. *Applied and Environmental Microbiology* 2014;80(22):6919-6925.
Alkaline phosphatase	*phoA*	Phosphorus Decomposition	K01077	Orchard, E., Webb, E. and Dyhrman, S. Characterization of phosphorus-regulated genes in Trichodesmium spp. *The Biological Bulletin* 2003;205(2):230-231.
Alkaline phosphatase	*phoD*	Phosphorus Decomposition	K01113	Zhu, X.*, et al.* Distribution characteristics of phoD-harbouring bacterial community structure and its roles in phosphorus transformation in steppe soils in Northern China. *Journal of Soil Science and Plant Nutrition* 2021;21:1531-1541.
Pyrroloquinoline-quinone synthase	*pqqC*	Phosphorus Solubilization	K06137	Misra, H., Rajpurohit, Y. and Khairnar, N. Pyrroloquinoline-quinone and its versatile roles in biological processes. *Journal of biosciences* 2012;37:313-325.
Acid phosphatase	*PHO*	Phosphorus Decomposition	K01078	Hammond, J.P., Broadley, M.R. and White, P.J. Genetic responses to phosphorus deficiency. *Annals of botany* 2004;94(3):323-332.
Acid phosphatase	*appA*	Phosphorus Decomposition	K01093	Touati, E. and Danchin, A. Cloning and characterization of the pH 2.5 acid phosphatase gene, appA: cyclic AMP mediated negative regulation. *Molecular and General Genetics MGG* 1987;208:499-505.
Pyrroloquinoline-quinone synthase	*pqs*	P Cycling	EC.3.4.11.1_1	Misra, H., Rajpurohit, Y. and Khairnar, N. Pyrroloquinoline-quinone and its versatile roles in biological processes. *Journal of biosciences* 2012;37:313-325.
Alkaline phosphatase	*alp*	P Cycling	EC.3.1.3.1_1	Sharma, U., Pal, D. and Prasad, R. Alkaline phosphatase: an overview. *Indian journal of clinical biochemistry* 2014;29:269-278.
Acid phosphatase	*AcP*	P Cycling	EC.3.1.3.2_1	Fuchs, K.R., Shekels, L.L. and Bernlohr, D.A. Analysis of the ACP1 gene product: classification as an FMN phosphatase. *Biochemical and biophysical research communications* 1992;189(3):1598-1605.
3-phytase	*3PH*	P Cycling	EC.3.1.3.8_1	Sajidan, A.*, et al.* Molecular and physiological characterisation of a 3-phytase from soil bacterium Klebsiella sp. ASR1. *Applied microbiology and biotechnology* 2004;65:110-118.
4-phytase	*4PH*	P Cycling	EC.3.1.3.26_1	Ranjan, K. and Sahay, S. Identification of phytase producing yeast and optimization and characterization of extracellular phytase from Candida parapsilosis. *Int J Sci Nat* 2013;4(4):583-590.
Arylsulfatase	*aslA*	Sulfur Decomposition	K01130	Cregut, M. and Rondags, E. New insights in agar biorefinery with arylsulphatase activities. *Process Biochemistry* 2013;48(12):1861-1871.
Methane/ammonia monooxygenase	*pmoA-amoA*	Nitrification	K10944	Van Kessel, M.A.*, et al.* Complete nitrification by a single microorganism. *Nature* 2015;528(7583):555-559.
Nitrogenase Fe protein	*nifH*	Nitrogen Fixation	K02588	Gaby, J.C. and Buckley, D.H. A comprehensive evaluation of PCR primers to amplify the nifH gene of nitrogenase. 2012.
Nitrogenase Mo-Fe protein	*nifK*	Nitrogen Fixation	K02586	Fani, R., Gallo, R. and Liò, P. Molecular evolution of nitrogen fixation: the evolutionary history of the nifD, nifK, nifE, and nifN genes. *Journal of molecular evolution* 2000;51:1-11.
Nitrogenase Mo-Fe protein	*nifD*	Nitrogen Fixation	K02591	Fani, R., Gallo, R. and Liò, P. Molecular evolution of nitrogen fixation: the evolutionary history of the nifD, nifK, nifE, and nifN genes. *Journal of molecular evolution* 2000;51:1-11.
Hydroxylamine dehydrogenase	*hao*	Nitrification	K10535	Bergmann, D.J., Hooper, A.B. and Klotz, M.G. Structure and sequence conservation of hao cluster genes of autotrophic ammonia-oxidizing bacteria: evidence for their evolutionary history. *Applied and environmental microbiology* 2005;71(9):5371-5382.
Nitrite reductase (NADH)	*nirD*	Dissimilatory Nitrate Reduction	K00363	Hu, R.*, et al.* Evidence for assimilatory nitrate reduction as a previously overlooked pathway of reactive nitrogen transformation in estuarine suspended particulate matter. *Environmental Science & Technology* 2022;56(20):14852-14866.
Nitrite reductase (cytochrome c-552)	*nrfA*	Dissimilatory Nitrate Reduction	K03385	Hu, R.*, et al.* Evidence for assimilatory nitrate reduction as a previously overlooked pathway of reactive nitrogen transformation in estuarine suspended particulate matter. *Environmental Science & Technology* 2022;56(20):14852-14866.

Ferrodoxin-nitrite reductase	*nirA*	Assimilatory Nitrate Reduction	K00366	Frías, J.E. and Flores, E. Induction of the nitrate assimilation nirA operon and protein-protein interactions in the maturation of nitrate and nitrite reductases in the cyanobacterium Anabaena sp. strain PCC 7120. *Journal of Bacteriology* 2015;197(14):2442-2452.
Nitrate reductase	*narG*	Denitrification	K00370	Chèneby, D.*, et al.* Genetic characterization of the nitrate reducing community based on narG nucleotide sequence analysis. *Microbial ecology* 2003;46(1):113-121.
Nitrate reductase	*narH*	Denitrification	K00371	Petri, R. and Imhoff, J.F. The relationship of nitrate reducing bacteria on the basis of narH gene sequences and comparison of narH and 16S rDNA based phylogeny. *Systematic and Applied Microbiology* 2000;23(1):47-57.
Nitrite reductase (NO forming)	*nirK*	Denitrification	K00368	Dandie, C.*, et al.* Analysis of denitrification genes and comparison of nosZ, cnorB and 16S rDNA from culturable denitrifying bacteria in potato cropping systems. *Systematic and applied microbiology* 2007;30(2):128-138.
Nitric oxide reductase	*norB*	Denitrification	K04561	Schmidt, I., van Spanning, R.J. and Jetten, M.S. Denitrification and ammonia oxidation by Nitrosomonas europaea wild-type, and NirK-and NorB-deficient mutants. *Microbiology* 2004;150(12):4107-4114.
Glycine dehydrogenase	*hcnA*	Chelation	K10814	Rijavec, T. and Lapanje, A. Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. *Frontiers in microbiology* 2016;7:1785.
Phloroglucinol synthase	*phlD*	Antifungal	K15431	Yang, F. and Cao, Y. Biosynthesis of phloroglucinol compounds in microorganisms. *Applied Microbiology and Biotechnology* 2012;93:487-495.
Iturin family lipopeptide synthetase A	*ituA*	Antifungal	K15661	Dang, Y.*, et al.* Enhanced production of antifungal lipopeptide iturin A by Bacillus amyloliquefaciens LL3 through metabolic engineering and culture conditions optimization. *Microbial cell factories* 2019;18:1-14.
Fengycin family lipopeptide synthetase D	*fenA*	Antifungal	K15667	Moyne, A.-L., Cleveland, T.E. and Tuzun, S. Molecular characterization and analysis of the operon encoding the antifungal lipopeptide bacillomycin D. *FEMS Microbiology Letters* 2004;234(1):43-49.
AHBA synthesis associated protein	*rifM*	Antibiotic	K16017	Chen, S.*, et al.* Biosynthesis of ansatrienin (mycotrienin) and naphthomycin: identification and analysis of two separate biosynthetic gene clusters in Streptomyces collinus Tü 1892. *European journal of biochemistry* 1999;261(1):98-107.
2-amino-4-deoxychorismate synthase	*phzE*	Antibiotic	K13063	McDonald, M.*, et al.* Phenazine biosynthesis in Pseudomonas f luorescens: Branchpoint from the primary shikimate biosynthetic pathway and role of phenazine-1, 6-dicarboxylic Acid. *Journal of the American Chemical Society* 2001;123(38):9459-9460.
Salicylate synthetase	*mbtI*	Chelation	K04781	Harrison, A.J.*, et al.* The structure of MbtI from Mycobacterium tuberculosis, the first enzyme in the biosynthesis of the siderophore mycobactin, reveals it to be a salicylate synthase. *Journal of bacteriology* 2006;188(17):6081-6091.
Isochorismatase	*entB*	Chelation	EC.3.3.2.1_1	Chlebek, D.*, et al.* Genetic Determinants of Antagonistic Interactions and the Response of New Endophytic Strain Serratia quinivorans KP32 to Fungal Phytopathogens. *International Journal of Molecular Sciences* 2022;23(24):15561.
Urease alpha subunit	*ureC*	Nitrogen Decomposition	K01428	Adetunji, A.T.*, et al.* The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of soil science and plant nutrition* 2017;17(3):794-807.
Acid phosphatase	*phoN*	Phosphorus Decomposition	K09474	Adetunji, A.T.*, et al.* The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of soil science and plant nutrition* 2017;17(3):794-807.
Nitrite reductase (NAD(P)H)	*NIT-6*	Assimilatory Nitrate Reduction	K00366	Besson, S., Almeida, M.G. and Silveira, C.M. Nitrite reduction in bacteria: A comprehensive view of nitrite reductases. *Coordination Chemistry Reviews* 2022;464:214560.

Nitrite reductase (NO forming)	*nirS*	Denitrification	K15864	Braker, G. and Tiedje, J.M. Nitric oxide reductase (norB) genes from pure cultures and environmental samples. *Applied and environmental microbiology* 2003;69(6):3476-3483.
Nitric oxide reductase	*norC*	Denitrification	K02305	Braker, G. and Tiedje, J.M. Nitric oxide reductase (norB) genes from pure cultures and environmental samples. *Applied and environmental microbiology* 2003;69(6):3476-3483.

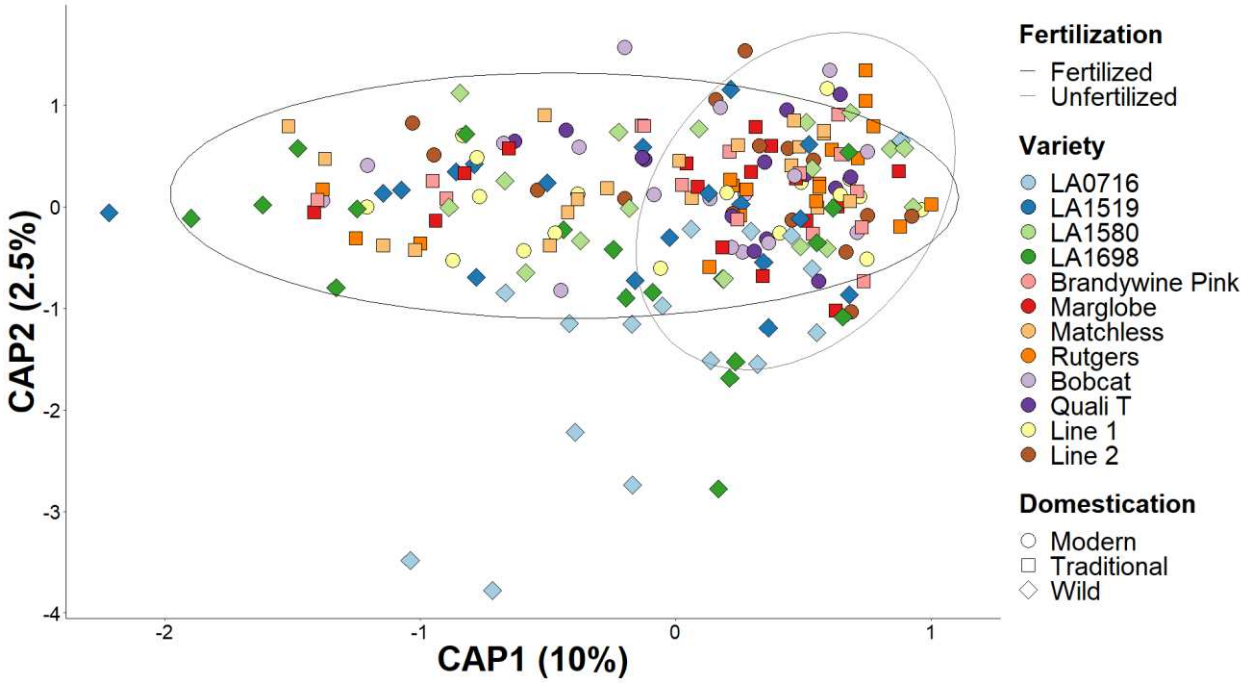
Supplemental Table 2-2. Domestication categorization and approximate year of development for the tested tomato accessions.

Tomato Species	Accession Name	Approximate Year Developed	Domestication Group
Solanum lycopersicum	Quali T 27	2010s	Modern
Solanum lycopersicum	Bobcat	2010s	Modern
Solanum lycopersicum	Line 1	2020s	Modern
Solanum lycopersicum	Line 2	2020s	Modern
Solanum lycopersicum	Brandywine Pink	1890s	Traditional
Solanum lycopersicum	Rutgers	1925	Traditional
Solanum lycopersicum	Marglobe	1930s	Traditional
Solanum lycopersicum	Matchless	1889	Traditional
Solanum lycopersicum var. *cerasiforme*	LA1580	Unknown	Wild
Solanum lycopersicum var. *cerasiforme*	LA1698	Unknown	Wild
Solanum pimpinellifolium	LA1519	Unknown	Wild
Solanum pennellii	LA0716	Unknown	Wild

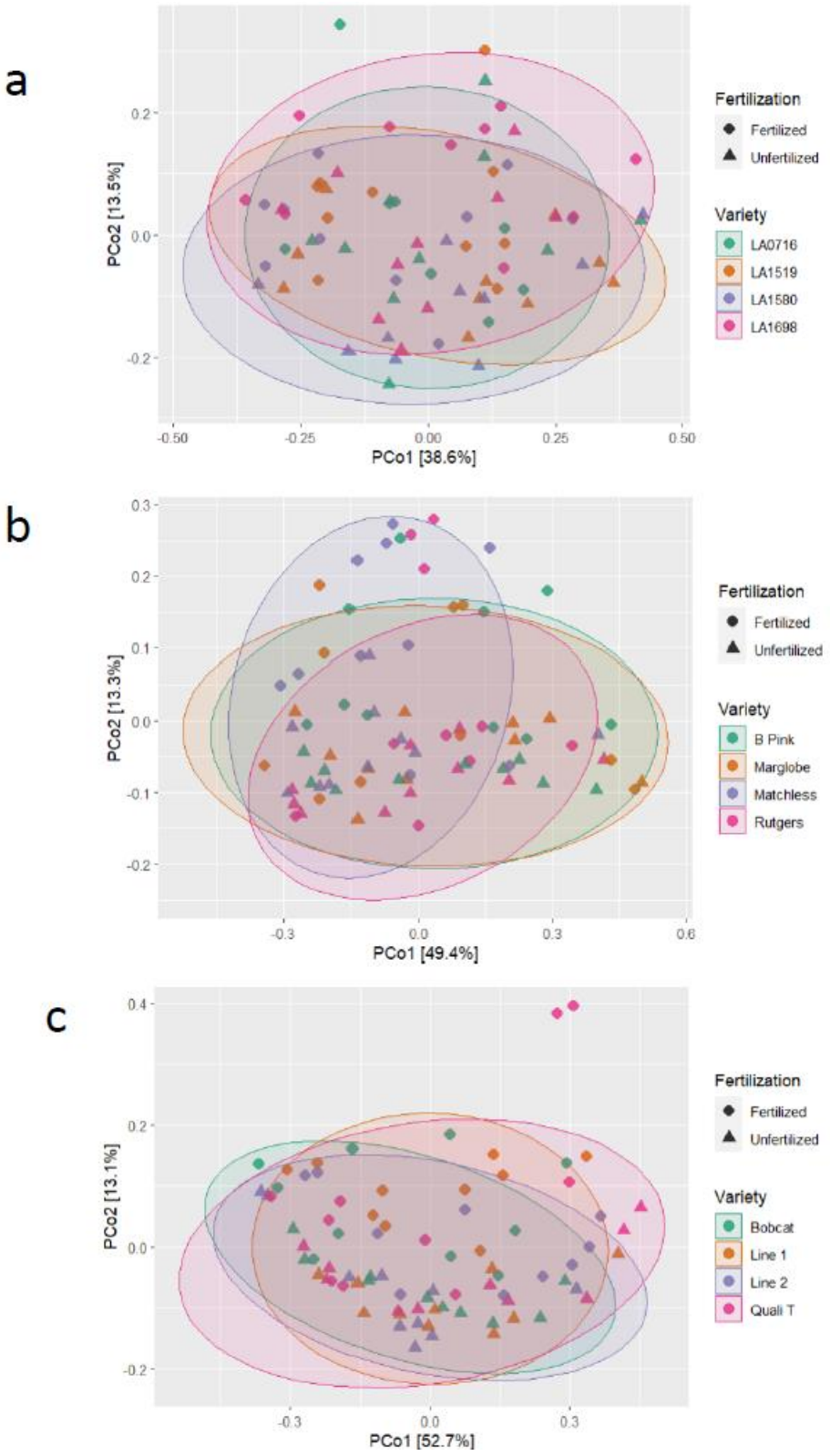
Supplemental Table 2-3. Non-varied relative abundance of bacteria with predictive functions among tomatoes across a domestication gradient in unfertilized soil. Bacterial relative abundance (presented as mean ± SEM) in unfertilized soil is shown for each tomato domestication group (Modern, Traditional, Wild). An ANOVA (F and FDR-adjusted p values presented) with Tukey HSD at α = 0.05 was used to determine differences in the relative abundance of rhizosphere bacteria as a function of tomato domestication group. Different letters within each row denote significant differences in the relative abundance of the bacteria with corresponding tested gene. For data that did not have normally distributed residuals, a log-transformation was run (denoted as "L" in the F-value column). "Function" represents the predictive function for the bacteria with the corresponding gene listed in the "Gene" column. "KEGG" represents the KEGG entry used to for each corresponding gene. This table illustrates the genes are not significantly different (Table 1-2 shows the values for the genes that are significantly different).

Gene	Function	KEGG	F	p	Relative Abundance		
					Modern	Traditional	Wild
budA	Biocontrol	K01575	2.94^{L}	0.112	0.085 ± 0.001 a	0.085 ± 0.002 a	0.095 ± 0.004 a
budC	Biocontrol	K18009	3.62^{L}	0.072	0.008 ± 0.006 a	0.079 ± 0.002 a	0.091 ± 0.005 a
E3.2.1.14	Biocontrol	K01183	1.05	0.482	0.106 ± 0.003 a	0.166 ± 0.003 a	0.168 ± 0.005 a
ISC	Antifungal	EC.5.4.4.2_1	3.91^{L}	0.061	0.059 ± 0.001 b	0.059 ± 0.002 b	0.068 ± 0.004 a
srfAA	Antibacterial	K15654	4.34^{L}	0.057	0.058 ± 0.001 b	0.059 ± 0.002 b	0.068 ± 0.004 a
acdS	Root Growth	K01505	3.94	0.061	0.154 ± 0.002 b	0.158 ± 0.004 ab	0.170 ± 0.006 a
PCH	Chelation	EC.4.2.99.21_1	4.23	0.059	0.075 ± 0.002 b	0.074 ± 0.003 b	0.087 ± 0.005 a
E3.2.1.21	Carbon Decomposition	E3.2.1.21	3.81^{L}	0.063	0.055 ± 0.001 ab	0.055 ± 0.002 b	0.065 ± 0.004 a
ipdC	Stress	K04103	1.19	0.430	0.122 ± 0.002 a	0.121 ± 0.002 a	0.127 ± 0.004 a
entA	Siderophore	K00216	2.46^{L}	0.140	0.087 ± 0.001 a	0.092 ± 0.002 a	0.096 ± 0.004 a
nosZ	Denitrification	K00376	0.83	0.536	0.159 ± 0.002 a	0.162 ± 0.005 a	0.166 ± 0.005 a
pchB	Siderophore	K02364	2.82^{L}	0.113	0.082 ± 0.001 a	0.084 ± 0.002 a	0.092 ± 0.004 a
bglX	Carbon Decomposition	K05349	2.69	0.123	0.405 ± 0.005 a	0.417 ± 0.006 a	0.432 ± 0.012 a
bglB	Carbon Decomposition	K05350	1.44	0.356	0.358 ± 0.005 a	0.370 ± 0.005 a	0.368 ± 0.007 a
amiE	Nitrogen Decomposition	K01426	0.04	0.977	0.722 ± 0.005 a	0.723 ± 0.005 a	0.725 ± 0.007 a
phoA	Phosphorus Decomposition	K01077	0.52	0.671	0.248 ± 0.004 a	0.253 ± 0.005 a	0.255 ± 0.006 a
phoD	Phosphorus Decomposition	K01113	0.24	0.837	0.507 ± 0.004 a	0.506 ± 0.004 a	0.511 ± 0.007 a
pqqC	Phosphorus Solubilization	K06137	4.08	0.059	0.197 ± 0.003 b	0.199 ± 0.003 b	0.213 ± 0.006 a
PHO	Phosphorus Decomposition	K01078	4.14	0.059	0.107 ± 0.003 ab	0.104 ± 0.004 b	0.123 ± 0.007 a
appA	Phosphorus Decomposition	K01093	3.91^{L}	0.061	0.063 ± 0.001 b	0.064 ± 0.002 b	0.073 ± 0.004 a
pqs	P Cycling	EC.3.4.11.1_1	0.02^{L}	0.980	0.129 ± 0.002 a	0.129 ± 0.002 a	0.131 ± 0.004 a
alp	P Cycling	EC.3.1.3.1_1	0.66	0.610	0.425 ± 0.003 a	0.433 ± 0.005 a	0.425 ± 0.006 a
AcP	P Cycling	EC.3.1.3.2_1	0.43^{L}	0.711	0.071 ± 0.003 a	0.067 ± 0.002 a	0.071 ± 0.005 a
3PH	P Cycling	EC.3.1.3.8_1	0.59^{L}	0.643	0.076 ± 0.003 a	0.076 ± 0.003 a	0.083 ± 0.005 a
4PH	P Cycling	EC.3.1.3.26_1	0.42^{L}	0.711	0.010 ± 0.001 a	0.010 ± 0.000 a	0.009 ± 0.006 a

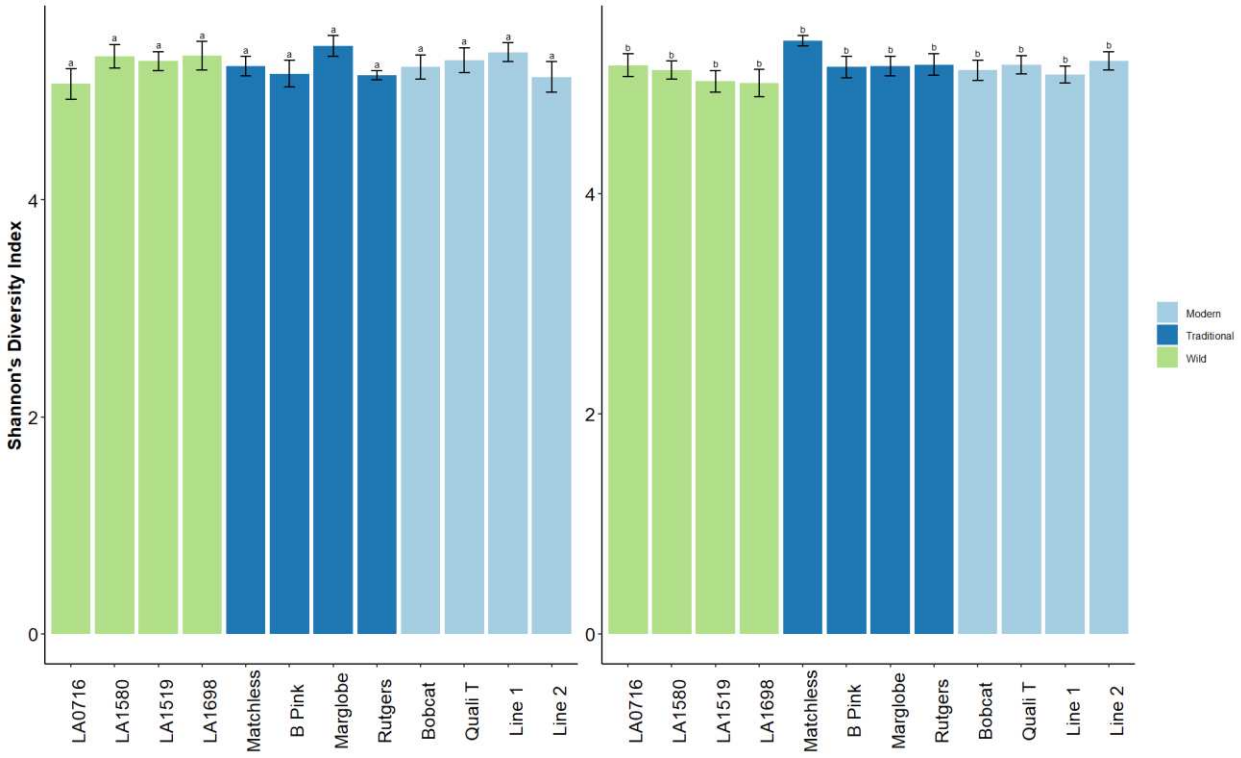
aslA	Sulfur Decomposition	K01130	1.37	0.372	0.413 ± 0.006 a	0.408 ± 0.005 a	0.398 ± 0.008 a
pmoA-amoA	Nitrification	K10944	2.49	0.140	0.082 ± 0.002 a	0.080 ± 0.002 a	0.089 ± 0.004 a
nifH	Nitrogen Fixation	K02588	3.22^{L}	0.095	0.095 ± 0.002 b	0.099 ± 0.003 ab	0.108 ± 0.005 a
nifK	Nitrogen Fixation	K02586	3.11	0.098	0.394 ± 0.003 b	0.402 ± 0.006 ab	0.417 ± 0.009 a
nifD	Nitrogen Fixation	K02591	3.18^{L}	0.095	0.094 ± 0.002 b	0.099 ± 0.003 ab	0.108 ± 0.005 a
hao	Nitrification	K10535	2.63	0.128	0.080 ± 0.002 a	0.079 ± 0.002 a	0.087 ± 0.004 a
nirD	Dissimilatory Nitrate Reduction	K00363	0.82	0.536	0.573 ± 0.005 a	0.568 ± 0.006 a	0.562 ± 0.008 a
nrfA	Dissimilatory Nitrate Reduction	K03385	2.19^{L}	0.176	0.076 ± 0.001 a	0.078 ± 0.002 a	0.084 ± 0.004 a
nirA	Assimilatory Nitrate Reduction	K00366	2.90^{L}	0.112	0.168 ± 0.005 a	0.166 ± 0.005 a	0.194 ± 0.011 a
narG	Denitrification	K00370	0.95	0.505	0.165 ± 0.002 a	0.169 ± 0.003 a	0.173 ± 0.005 a
narH	Denitrification	K00371	0.88	0.529	0.169 ± 0.002 a	0.173 ± 0.003 a	0.176 ± 0.005 a
nirK	Denitrification	K00368	0.21^{L}	0.840	0.179 ± 0.002 a	0.182 ± 0.004 a	0.184 ± 0.005 a
norB	Denitrification	K04561	2.82^{L}	0.113	0.142 ± 0.002 a	0.148 ± 0.004 a	0.161 ± 0.007 a



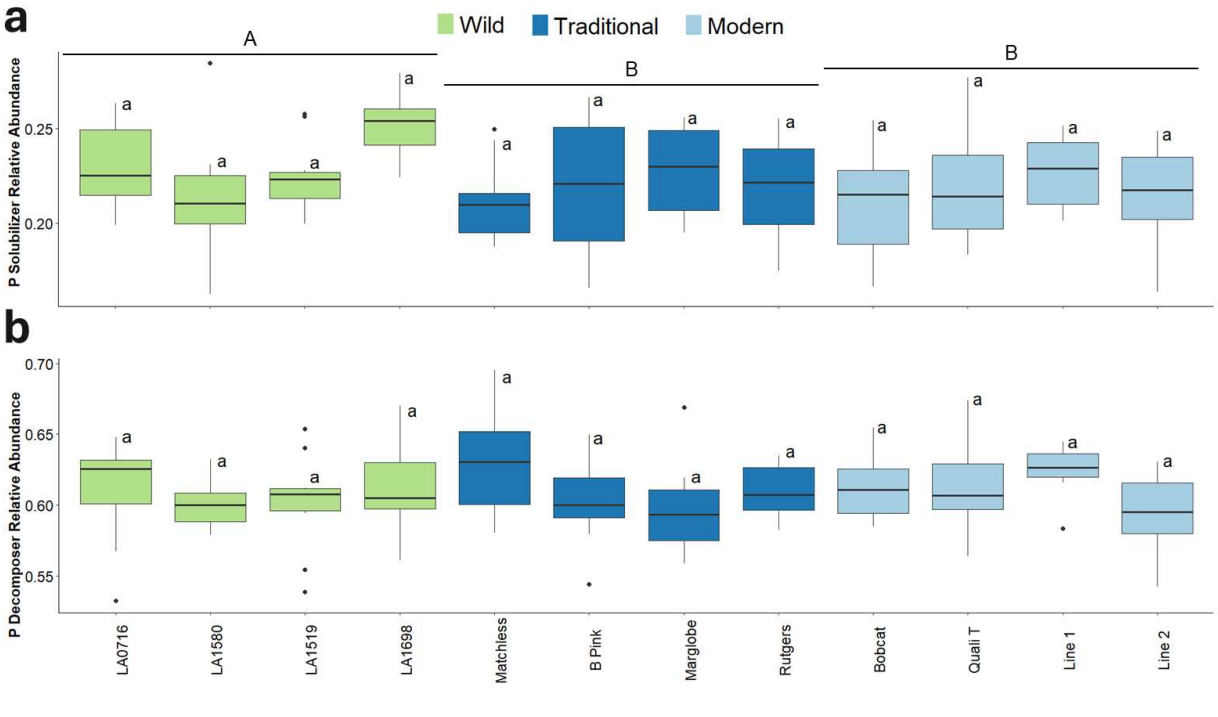
Supplemental Figure 2-1. Distance-based redundancy analysis (db-RDA) showing the clustering based on Bray-Curtis dissimilarity of the bacterial community structure in the tomato rhizosphere. The ellipses represent the fertilization treatment: fertilized (black) and unfertilized (gray). The color represents the variety: LA0716 (light blue), LA1519 (dark blue), LA1580 (light green), LA1698 (dark green), 'Brandywine Pink' (pink), 'Marglobe' (red), 'Matchless' (light orange), 'Rutgers' (dark orange), 'Bobcat' (light purple), 'Quali T' (dark purple), Line 1 (yellow), Line 2 (brown). The shape represents the level of domestication: modern (circle), traditional (square), and wild (diamond).



Supplemental Figure 2-1. Principal coordinate analysis (PCoA) clustering of the bacterial community structure in the tomato rhizosphere. The ellipses and colors indicate the different tomato varieties: LA0716, 'Brandywine Pink', 'Bobcat' (yellow); LA1519, 'Marglobe', 'Quali T' (light blue); LA1580, 'Matchless', Line 1 (dark blue); LA1698, 'Rutgers', V8053 (red). The shapes represent the fertilization treatment: fertilized (circle) and unfertilized (triangle).



Supplemental Figure 2-2. Shannon's diversity index of tomatoes across a domestication gradient. Different colored bars indicate the domestication level: wild (green), traditional (dark blue), and modern (light blue). An ANOVA was run with a Tukey HSD test for post-hoc comparison. Different lowercase letters denote significant differences ($p < 0.05$) for the fertilization level: left panel indicates fertilized tomatoes and right panel indicates unfertilized tomatoes. There were no significant differences among tomato variety or domestication group ($p > 0.05$).



Supplemental Figure 2-3. Phosphorus (P) solubilizing and P decomposing bacteria relative abundance in fertilized soil. ***a*** *P solubilizing bacteria in fertilized soil. An ANVOA showed differences in shoot P uptake for Domestication main effect.* ***b*** *P decomposing bacteria in unfertilized soil. An ANVOA showed no differences in shoot P uptake for any tested effect. Different colored bars indicate the domestication level: wild (green), traditional (dark blue), and modern (light blue). An ANOVA was run with a Tukey HSD test for post-hoc comparison. Different lowercase letters denote significant differences ($p < 0.05$) for the different varieties. Different uppercase letters denote significant differences ($p < 0.05$) for the different domestication groups. Treatments sharing a common letter are not significantly different.*

Supplemental Table 3-1. Identified Keystone Taxa in Fertilized and Unfertilized soil

Taxa	Fertilization Group
Vicinamibacter silvestris	Unfertilized
Stenotrophobacter roseus	Unfertilized
Stenotrophobacter namibiensis	Unfertilized
Stenotrophobacter terrae	Unfertilized
Luteitalea pratensis	Unfertilized
Nitrospira japonica	Unfertilized
Nitrospira moscoviensis	Unfertilized
Povalibacter uvarum	Unfertilized
Steroidobacter denitrificans	Unfertilized
Woeseia oceani	Unfertilized
Pirellula staleyi	Unfertilized
Planctomycetes bacterium Pla175	Unfertilized
Chryseolinea serpens	Unfertilized
Chryseolinea soli	Unfertilized
Ohtaekwangia koreensis	Unfertilized
Pseudarthrobacter sulfonivorans	Unfertilized
Pseudarthrobacter sp. NIBRBAC000502772	Unfertilized
Arthrobacter sp. *QXT-31*	Unfertilized
Arthrobacter sp. *KBS0702*	Unfertilized
Flavisolibacter ginsenosidimutans	Unfertilized
Flavisolibacter tropicus	Unfertilized
Bacillus oryzisoli	Unfertilized
Metabacillus litoralis	Unfertilized
Skermanella rosea	Unfertilized
Skermanella mucosa	Unfertilized
Sphingomonas daechungensis	Unfertilized
Sphingomonas sp. *AE3*	Unfertilized
Fimbriimonas ginsengisoli	Unfertilized
Microvirga subterranea	Unfertilized
Paenibacillus sp. *JDR-2*	Unfertilized
Paenibacillus castaneae	Unfertilized
Paenibacillus prosopidis	Unfertilized
Chthoniobacter flavus	Unfertilized
Nordella oligomobilis	Unfertilized
Peribacillus muralis	Unfertilized
[Brevibacterium] frigoritolerans	Unfertilized
Peribacillus simplex	Unfertilized
Bacillus megaterium	Unfertilized
Aciditerrimonas ferrireducens	Unfertilized

Aquihabitans daechungensis	Unfertilized
Ilumatobacter fluminis	Unfertilized
Tepidiforma bonchosmolovskayae	Unfertilized
Anabaena cylindrica	Unfertilized
Crinalium epipsammum	Unfertilized
Planctomycetes bacterium Pla85_3_4	Unfertilized
Thermostilla marina	Unfertilized
Planctomycetales bacterium	Unfertilized
Microlunatus phosphovorus	Unfertilized
Microlunatus panaciterrae	Unfertilized
Microlunatus ginsengisoli	Unfertilized
Friedmanniella endophytica	Unfertilized
Microlunatus aurantiacus	Unfertilized
Gaiella occulta	Unfertilized
Brevitalea deliciosa	Unfertilized
Brevitalea aridisoli	Unfertilized
Solirubrobacter soli	Unfertilized
Terrimonas soli	Unfertilized
Acidibacter ferrireducens	Unfertilized
Candidatus Solibacter usitatus	Unfertilized
Ammoniphilus resinae	Unfertilized
Bacillus circulans	Unfertilized
Rubrobacter spartanus	Unfertilized
Rubrobacter radiotolerans	Unfertilized
Rubrobacter xylanophilus	Unfertilized
Daejeonella oryzae	Unfertilized
Limisphaera ngatamarikiensis	Unfertilized
Planctomycetes bacterium ETA_A8	Unfertilized
Methyloceanibacter caenitepidi	Unfertilized
Gemmatirosa kalamazoonesis	Unfertilized
Roseisolibacter agri	Unfertilized
Gemmatimonas aurantiaca	Unfertilized
Microvirga zambiensis	Unfertilized
Microvirga ossetica	Unfertilized
Cohnella abietis	Unfertilized
Gemmatimonas phototrophica	Unfertilized
Microlunatus sp. *KUDC0627*	Unfertilized
Steroidobacter agariperforans	Unfertilized
Peribacillus asahii	Unfertilized
Solirubrobacter ginsenosidimutans	Unfertilized
Baekduia soli	Unfertilized

Thioalkalivibrio sulfidiphilus	Unfertilized
Neobacillus niacini	Unfertilized
Bacillus sp. *S3*	Unfertilized
Neobacillus mesonae	Unfertilized
Burkholderiales bacterium GJ-E10	Unfertilized
Microvirga brassicacearum	Unfertilized
Arenimicrobium luteum	Unfertilized
Adhaeribacter terreus	Unfertilized
Fimbriiglobus ruber	Unfertilized
Longimicrobium terrae	Unfertilized
Acidimicrobium ferrooxidans	Unfertilized
Sandaracinus amylolyticus	Unfertilized
Stigmatella aurantiaca	Unfertilized
Rhodoplanes sp. *Z2-YC6860*	Unfertilized
Egibacter rhizosphaerae	Unfertilized
Amaricoccus macauensis	Unfertilized
Blastococcus saxobsidens	Unfertilized
Georgfuchsia toluolica	Unfertilized
Caldilinea aerophila	Unfertilized
Bacillus sp. *1NLA3E*	Unfertilized
Zavarzinella formosa	Unfertilized
Brevibacillus thermoruber	Unfertilized
Acidothermus cellulolyticus	Unfertilized
Haliangium tepidum	Unfertilized
Planctomycetes bacterium Pan265	Unfertilized
Planctomycetes bacterium Pan216	Unfertilized
Planctomycetes bacterium Mal4	Unfertilized
Labilithrix luteola	Unfertilized
Loriellopsis cavernicola	Unfertilized
Conexibacter woesei	Unfertilized
Steroidobacter denitrificans	Fertilized
Ilumatobacter fluminis	Fertilized
Pseudarthrobacter sp. *NIBRBAC000502772*	Fertilized
Aciditerrimonas ferrireducens	Fertilized
Stenotrophobacter roseus	Fertilized
Flavisolibacter tropicus	Fertilized
Gemmatirosa kalamazoonesis	Fertilized
Microlunatus ginsengisoli	Fertilized
Arthrobacter sp. *PGP41*	Fertilized
Nitrospira japonica	Fertilized
Chryseolinea soli	Fertilized

Chthoniobacter flavus	Fertilized
Arthrobacter sp. *YN*	Fertilized
Arthrobacter pascens	Fertilized
Gaiella occulta	Fertilized
Stenotrophobacter namibiensis	Fertilized
Chryseolinea serpens	Fertilized
Arthrobacter sp. *FB24*	Fertilized
Candidatus Saccharibacteria bacterium oral taxon 955	Fertilized
Arthrobacter alpinus	Fertilized
Povalibacter uvarum	Fertilized
Skermanella rosea	Fertilized
Rubrobacter xylanophilus	Fertilized
Nordella oligomobilis	Fertilized
Vicinamibacter silvestris	Fertilized
Planctomycetes bacterium Pla175	Fertilized
Limisphaera ngatamarikiensis	Fertilized
Luteitalea pratensis	Fertilized
Sphingomonas sediminicola	Fertilized
Microvirga subterranea	Fertilized
Woeseia oceani	Fertilized
Sphingomonas daechungensis	Fertilized
Thermostilla marina	Fertilized
Candidatus Solibacter usitatus	Fertilized
Rubrobacter radiotolerans	Fertilized
Bacillus megaterium	Fertilized
Ohtaekwangia koreensis	Fertilized
Brevitalea deliciosa	Fertilized
Bacillus oryzisoli	Fertilized
Microlunatus panaciterrae	Fertilized
Steroidobacter agariperforans	Fertilized
Pseudarthrobacter sp. *NIBRBAC000502771*	Fertilized
Planctomycetales bacterium	Fertilized
Stenotrophobacter terrae	Fertilized
Arthrobacter crystallopoietes	Fertilized
Arthrobacter sp. *UKPF54-2*	Fertilized
Planctomycetes bacterium Mal4	Fertilized
Caldilinea aerophila	Fertilized
Brevitalea aridisoli	Fertilized
Methyloceanibacter caenitepidi	Fertilized
Litorilinea aerophila	Fertilized
Paenibacillus sp. *JDR-2*	Fertilized

Planctomycetes bacterium Pla85_3_4	Fertilized
Haliangium tepidum	Fertilized
Rubrobacter spartanus	Fertilized
Planctomycetes bacterium ETA_A8	Fertilized
Skermanella aerolata	Fertilized
Paenibacillus castaneae	Fertilized
Metabacillus litoralis	Fertilized
Arthrobacter sp. Rue61a	Fertilized
Bacillus circulans	Fertilized
Bacillus sp. *S3*	Fertilized
Arthrobacter sp. *24S4-2*	Fertilized
[Brevibacterium] frigoritolerans	Fertilized
Gemmatimonas phototrophica	Fertilized
Microlunatus sp. KUDC0627	Fertilized
Paraflavitalea soli	Fertilized
Neobacillus niacini	Fertilized
Pseudarthrobacter phenanthrenivorans	Fertilized
Cohnella abietis	Fertilized
Acidibacter ferrireducens	Fertilized
Tepidiforma bonchosmolovskayae	Fertilized
Microvirga zambiensis	Fertilized
Brevibacillus thermoruber	Fertilized
Nocardioides alpinus	Fertilized
Adhaeribacter terreus	Fertilized
Paenibacillus prosopidis	Fertilized
Daejeonella oryzae	Fertilized
Burkholderiales bacterium GJ-E10	Fertilized
Devosia ginsengisoli	Fertilized
Skermanella mucosa	Fertilized
Peribacillus simplex	Fertilized
Bacillus sp. *1NLA3E*	Fertilized
Nitrospira moscoviensis	Fertilized

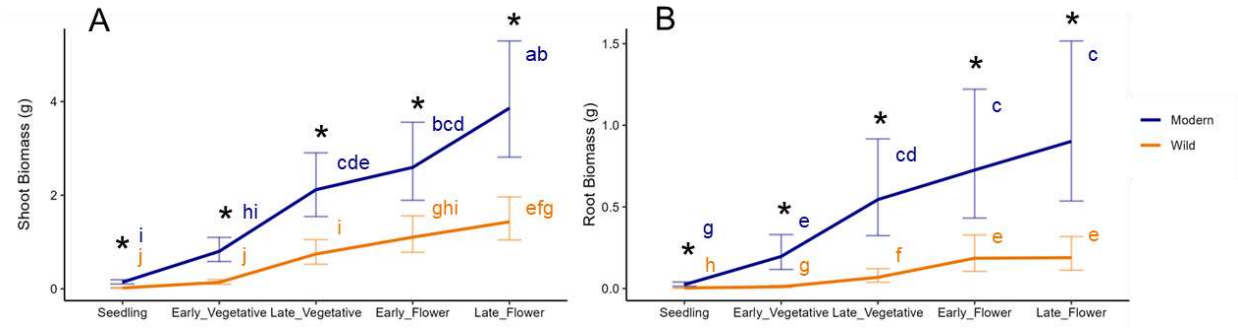
Supplemental Table 4-1. Chemical properties of soil-sand mixture. The Olsen phosphorus test was used to determine phosphorus concentration.

Characteristic	Value
pH	8.7
Soluble Salts 1:1 (mmho/cm)	0.30
Organic Matter (LOI-%)	1.2
Phosphorus (mg/kg)	4.7
KCl Nitrate-N (mg/kg)	13.2
Potassium (mg/kg)	176
Calcium (mg/kg)	3911
Magnesium (mg/kg)	416
Sodium (mg/kg)	58
M-3 Sulfate (mg/kg)	71.5
Zinc (mg/kg)	1.25
Iron (mg/kg)	6.7
Manganese (mg/kg)	5.5
Copper (mg/kg)	0.48
Sum of cations (mg/100g)	23.7
Base Saturation – Potassium (%)	2
Base Saturation – Calcium (%)	82
Base Saturation – Magnesium (%)	15
Base Saturation – Sodium (%)	1

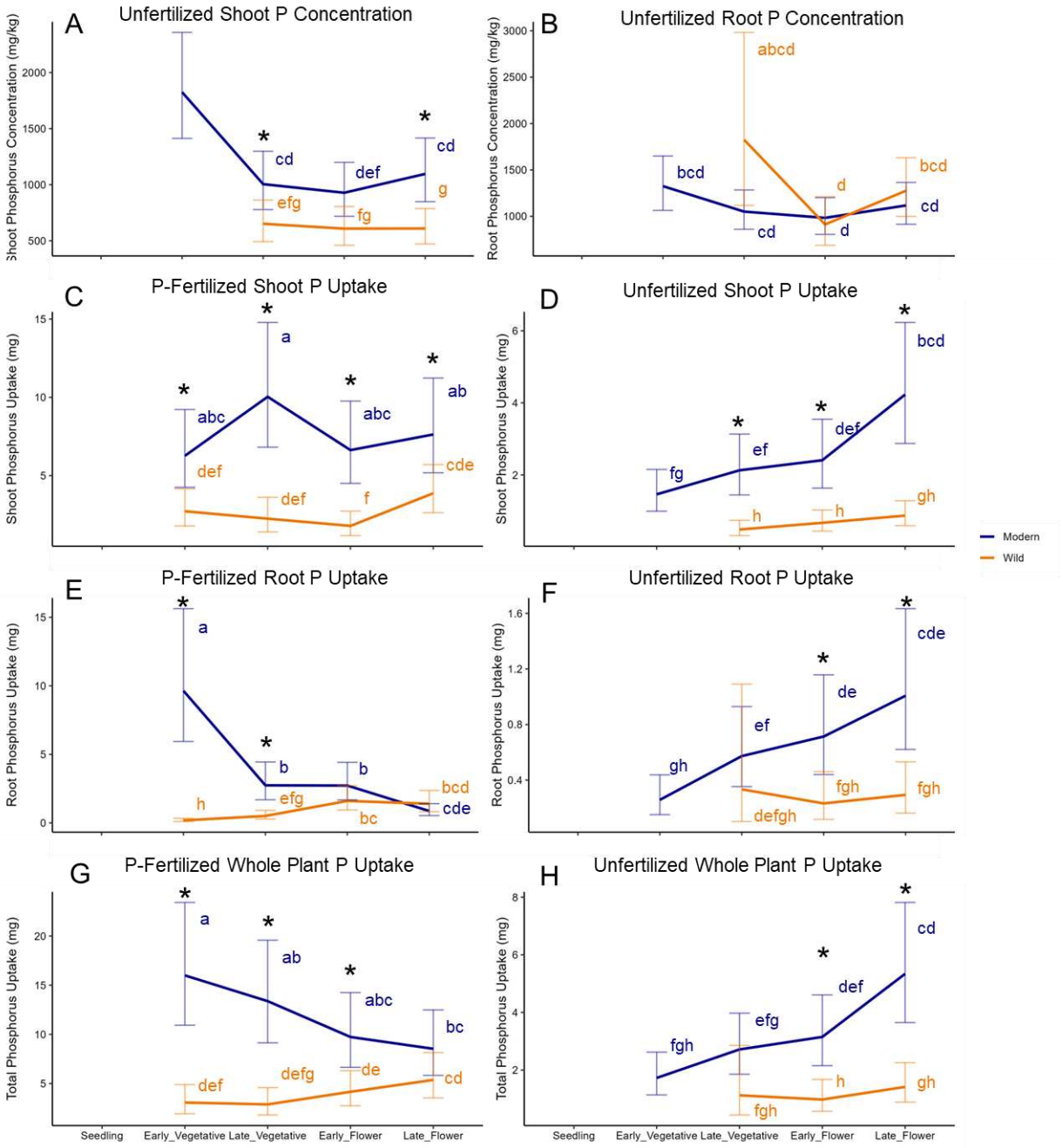
Supplemental Table 4-2. Predictive genes identified using PICRUSt2 and their beneficial functions. The "Gene" column denotes the genes selected for analysis, and "Enzyme" represents the corresponding enzyme the gene encodes. "KEGG Ontology" is the identifier in the KEGG database, and "Function" is the predictive function the gene carries out.

Gene	Enzyme	KEGG Ontology	Function
hcnA	glycine dehydrogenase	K10814	Biocontrol
budA	acetolactate decarboxylase	K01575	Biocontrol
budC	(S,S)-butanediol dehydrogenase	K18009	Biocontrol
E3.2.1.6	endo-1,3(4)-beta-gulcanase	K01180	Biocontrol
E3.2.1.14	chitinase	K01183	Biocontrol
phlD	phloroglucinol synthase	K15431	Biocontrol
ituA	iturin family lipopeptide synthetase A	K15661	Biocontrol
fenA	fengycin family lipopeptide synthetase D	K15667	Biocontrol
srfAA	surfactin family lipopeptide synthetase A	K15654	Biocontrol
rifM	AHBA synthesis associated protein	K16017	Biocontrol
phzE	2-amino-4-deoxychorismate synthase	K13063	Biocontrol
ipdC	indolepyruvate decarboxylase	K04103	Stress
acdS	1-aminocyclopropane-1-carboxylate deaminase	K01505	Stress
mbtI	salicylate synthetase	K04781	Siderophore
entA	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase	K00216	Siderophore
pchB	isochorismate pyruvate lysase	K02364	Siderophore
bglX	beta-glucosidase	K05349	C decomposition
bglB	beta-glucosidase	K05350	C decomposition
E3.2.1.21	beta-glucosidase	K01188	C decomposition
amiE	amidase	K01426	N decomposition
ureC	urease alpha subunit	K01428	N decomposition
pqqC	pyrroloquinoline-quinone synthase	K06137	PO_4 solubility
phoA	alkaline phosphatase	K01077	P decomposition
phoD	alkaline phosphatase	K01113	P decomposition
PHO	acid phosphatase	K01078	P decomposition

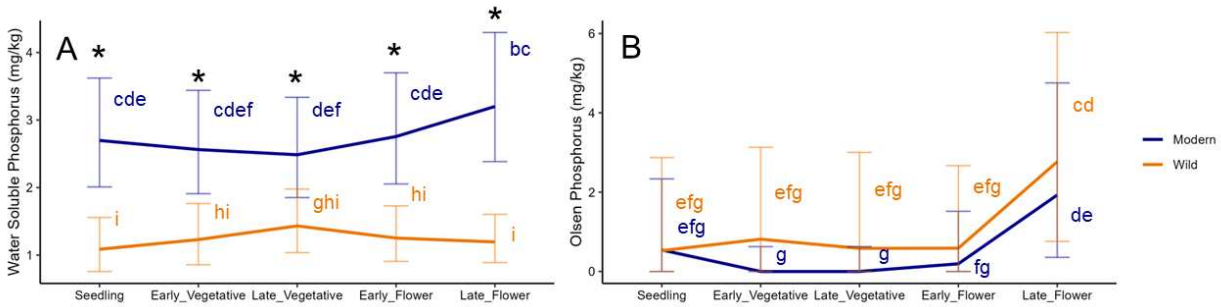
appA	acid phosphatase	K01093	P decomposition
phoN	acid phosphatase	K09474	P decomposition
aslA	Arylsulfatase	K01130	S decomposition
nifH	nitrogenase Fe protein	K02588	N-fixation
nifD	nitrogenase Mo-Fe protein	K02591	N-fixation
nifK	nitrogenase Mo-Fe protein	K02586	N-fixation
pmoA-amoA	methane/ammonia monooxygenase	K10944	Nitrification
hao	hydroxylamine dehydrogenase	K10535	Nitrification



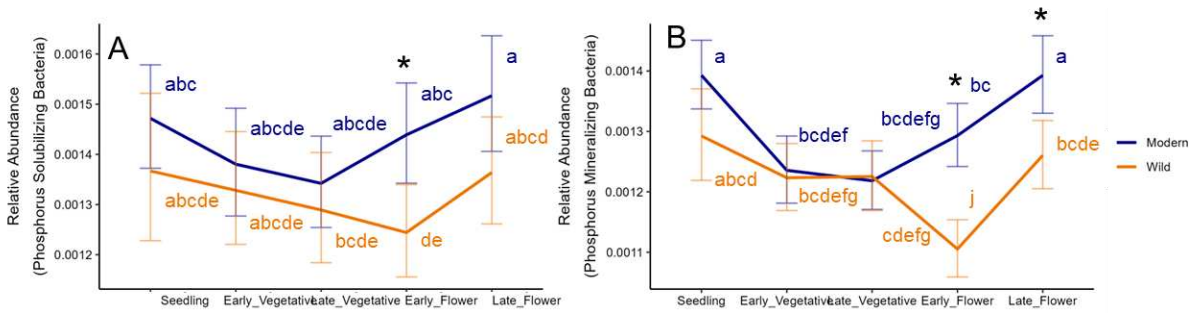
Supplemental Figure 4-1. Biomass accumulation of a wild and a modern tomato across different developmental stages in unfertilized soils. Shoot biomass (A) and root biomass (B) of a wild and a modern tomato accession grown in unfertilized soil were determined. Data were log-transformed for analysis and back-transformed for visualization. Line color denotes tomato domestication: orange (wild) and blue (modern). Bars display 95 % confidence intervals. An ANOVA with Tukey HSD was run. Different letters denote significant differences at α = 0.05. Stars denote significant difference for the pairwise contrast for wild and modern tomato at the corresponding developmental stage.



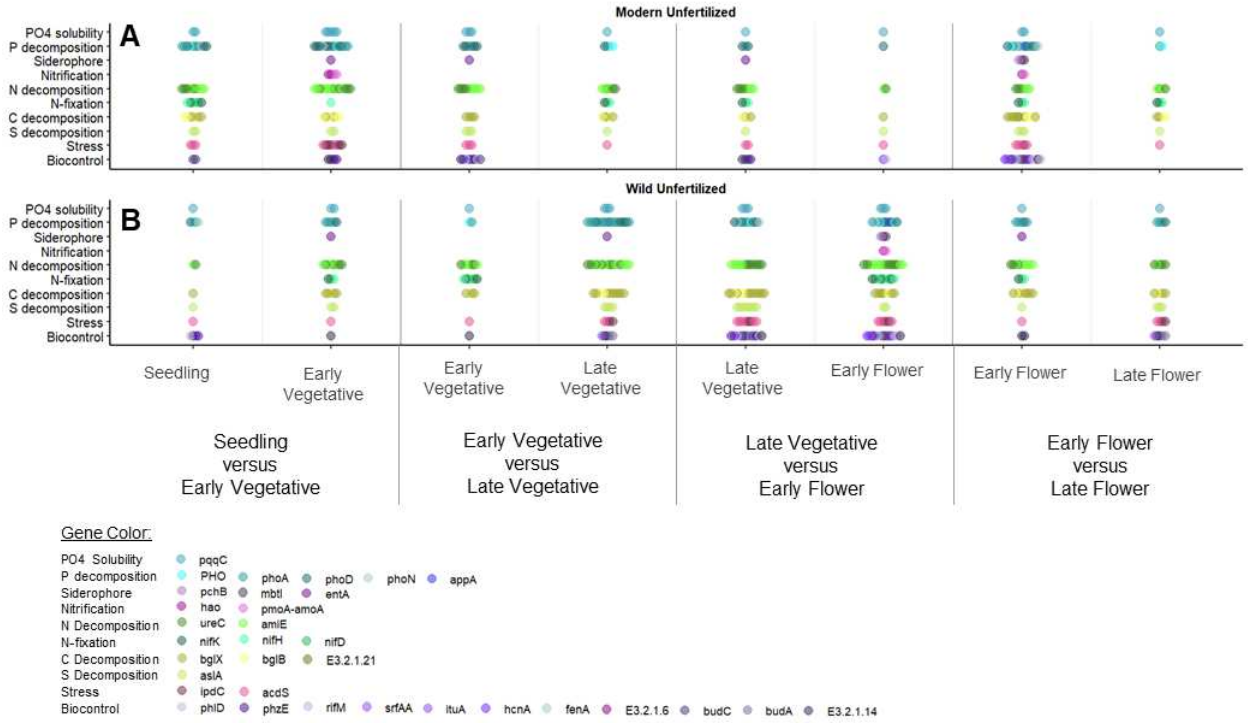
Supplemental Figure 4-2. Soil phosphorus dynamics of a wild and a modern tomato across different developmental stages in unfertilized soils and P-fertilized soils. Shoot P (A) and root P (B) concentration in unfertilized soils were determined. Shoot P uptake in P-fertilized (C), shoot P uptake in unfertilized (D), root P uptake in fertilized (E), and root P uptake in unfertilized (F) soils were measured. Finally, total P in fertilized (G) and unfertilized soils (H) were calculated. Data were log-transformed for analysis and back-transformed for visualization. Line color denotes tomato domestication: orange (wild) and blue (modern). Bars display 95 % confidence intervals. An ANOVA with Tukey HSD was run. Different letters denote significant differences at α = 0.05. Stars denote significant difference for the pairwise contrast for wild and modern tomato at the corresponding developmental stage.



Supplemental Figure 4-3. Soil P dynamics of a wild and a modern tomato across different developmental stages in unfertilized soils. Water soluble P (A) and Olsen P (B) were measured. Data were log-transformed for analysis and back-transformed for visualization. Line color denotes tomato domestication: orange (wild) and blue (modern). Bars display 95 % confidence intervals. An ANOVA with Tukey HSD was run. Different letters denote significant differences at α = 0.05. Stars denote significant difference for the pairwise contrast for wild and modern tomato at the corresponding developmental stage.



Supplemental Figure 4-4. Phosphorus solubilizing and mineralizing bacterial relative abundance of a wild and a modern tomato across different developmental stages in unfertilized soil. Phosphorus solubilizers (A) and mineralizers (B) were measured for wild and modern tomato in low P soils. Data were log-transformed for analysis and back-transformed for visualization. Line color denotes tomato domestication: orange (wild) and blue (modern). Bars display 95 % confidence intervals. An ANOVA with Tukey HSD was run. Different letters denote significant differences at α = 0.05. Stars denote significant difference for the pairwise contrast for wild and modern tomato at the corresponding developmental stage



Supplemental Figure 4-5. Fluctuations in the bacterial rhizosphere counts between different developmental stages in unfertilized wild and modern tomatoes. A differential abundance analysis using a Wald test with an FDR p adjustment of 0.05 was conducted for each tomato domestication group: modern tomato soil (A), wild tomato (B). Bacterial count data were compared between each developmental stage, denoted in black text on the x-axis. Bacteria that were enriched in a certain developmental stage are listed on the x-axis in italicized blue text. The presence of a dot above the gray text on the x-axis indicates that there a bacterium enriched in that domestication group contains a gene corresponding to a specified function on the y-axis. The different colors of dots represent different genes, and the key for these colors are listed on the bottom of the figure.

Supplemental Table 4-3. Differentially abundant bacteria in phosphorus (P) fertilized soils between wild and modern tomato at the seedling stage. A Wald test with an FDR p adjustment of 0.05 was conducted to determine species enrichment. The bacteria in the "Taxa" column denote the species that were differentially abundant. The "Enrich Group" column indicates the domestication group in which the corresponding bacteria were enriched in the fertilized wild tomato soils. The "Function" column shows an abbreviation of the predictive function of the bacteria as determined through PICRUSt2. The functions are abbreviated as the following: Biocontrol ("Bioc"), nitrogen decomposition ("N Dec"), phosphorus decomposition ("P Dec"), phosphate solubilization ("P Sol"), sulfur decomposition ("S Dec"), stress control ("Stress").

Enrich Group	Species	Function
Wild	*Crinalium epipsammum*	C Dec, N Dec, P Dec
Wild	*Cyanothece* sp. *PCC 7425*	C Dec, N Dec, P Dec
Modern	*Arenimonas daechungensis*	N Dec, P Dec
Modern	*Chiayiivirga flava*	N Dec, P Dec
Modern	*Pseudomonas chlororaphis*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Modern	*Pseudomonas fluorescens*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Modern	*Sphingobium* sp. *MI1205*	Bioc, C Dec

Supplemental Table 4-4. Differentially abundant bacteria in phosphorus (P) fertilized soils between wild and modern tomato at the early vegetative stage. A Wald test with an FDR p adjustment of 0.05 was conducted to determine species enrichment. The bacteria in the "Taxa" column denote the species that were differentially abundant. The "Enrich Group" column indicates the domestication group in which the corresponding bacteria were enriched in the fertilized wild tomato soils. The "Function" column shows an abbreviation of the predictive function of the bacteria as determined through PICRUSt2. The functions are abbreviated as the following: Biocontrol ("Bioc"), nitrogen decomposition ("N Dec"), phosphorus decomposition ("P Dec"), phosphate solubilization ("P Sol"), sulfur decomposition ("S Dec"), stress control ("Stress").

Enrich Groupc	Species	Function
Wild	*Geitlerinema* sp. *PCC 7407*	C Dec, N Dec
Wild	*Methylibium petroleiphilum*	C Dec, N Dec, P Dec, P Sol, Stress
Wild	*Niastella gongjuensis*	Bioc, C Dec, S Dec
Wild	*Pseudomonas corrugata*	Bioc, C Dec, N Dec, P Dec, P Sol, Stress
Wild	*Pseudomonas fluorescens*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Wild	*Pseudomonas kilonensis*	Bioc, C Dec, N Dec, P Dec, P Sol, Stress
Wild	*Pseudomonas putida*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress, Side
Wild	*Pseudomonas* sp. *R5-89-07*	C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Wild	*Pseudomonas synxantha*	Bioc, C Dec, N Dec, P Dec, P Sol, Stress
Wild	*Rhizobacter gummiphilus*	C Dec, N Dec, P Dec, P Sol
Wild	*Trichocoleus desertorum*	C Dec, N Dec
Modern	*Arenimonas daechungensis*	N Dec, P Dec
Modern	*Asanoa endophytica*	Bioc, C Dec, N Dec, P Dec, Stress
Modern	*Asticcacaulis biprosthecium*	C Dec, N Dec, P Dec
Modern	*Bacillus mesophilus*	C Dec, N Dec
Modern	*Bacillus methanolicus*	N Dec, P Dec
Modern	*Bacillus pumilus*	Bioc, C Dec, N Dec, P Dec, Side
Modern	*Bacillus* sp. *S3*	C Dec, N Dec, P Dec, P Sol
Modern	*Bacillus* sp. *X1(2014)*	N Dec
Modern	*Cytobacillus gottheilii*	N Dec, P Dec
Modern	*Devosia* sp. *1566*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress, Side, NFix, Nitr
Modern	*Edaphobaculum flavum*	C Dec

Modern	*Lysinibacillus* sp. *SGAir0095*	N Dec, P Dec
Modern	*Mesobacillus foraminis*	N Dec, P Dec
Modern	*Mesobacillus subterraneus*	C Dec, N Dec, P Dec
Modern	*Metabacillus indicus*	N Dec, P Dec
Modern	*Neobacillus Cucumis*	C Dec, N Dec, P Dec, P Sol, S Dec
Modern	*Neobacillus mesonae*	C Dec, N Dec, P Dec
Modern	*Neobacillus niacin*	C Dec, N Dec, P Dec, S Dec
Modern	*Paenisporosarcina indica*	N Dec, P Dec
Modern	*Solibacillus silvestris*	N Dec, P Dec, Side

Supplemental Table 4-5. Differentially abundant bacteria in phosphorus (P) fertilized soils between wild and modern tomato at the late vegetative stage. A Wald test with an FDR p adjustment of 0.05 was conducted to determine species enrichment. The bacteria in the "Taxa" column denote the species that were differentially abundant. The "Enrich Group" column indicates the domestication group in which the corresponding bacteria were enriched in the fertilized wild tomato soils. The "Function" column shows an abbreviation of the predictive function of the bacteria as determined through PICRUSt2. The functions are abbreviated as the following: Biocontrol ("Bioc"), nitrogen decomposition ("N Dec"), phosphorus decomposition ("P Dec"), phosphate solubilization ("P Sol"), sulfur decomposition ("S Dec"), stress control ("Stress").

Enrich Group	Species	Function
Wild	*Geitlerinema* sp. *PCC 7407*	C Dec, N Dec
Wild	*Leptolyngbya* sp. *O-77*	Bioc, C Dec, N Dec, NFix, Nitr, P Dec, P Sol, S Dec, Side, Stress
Wild	*Micavibrio aeruginosavorus*	C Dec
Wild	*Microbacterium chocolatum*	Bioc, C Dec, N Dec, Stress
Wild	*Rhizobacter dauci*	C Dec, N Dec, P Dec, P Sol
Wild	*Rhizobacter fulvus*	C Dec, N Dec, P Dec, P Sol
Wild	*Rhizobacter gummiphilus*	C Dec, N Dec, P Dec, P Sol
Wild	*Rhizorhapis suberifaciens*	C Dec, N Dec, P Dec, S Dec
Wild	*Rhodoferax ferrireducens*	C Dec, N Dec, P Dec, Stress
Wild	*Sphingobium amiense*	Bioc, C Dec, P Dec
Wild	*Sphingobium boeckii*	Bioc, C Dec, N Dec, P Dec
Wild	*Sphingobium chungbukense*	C Dec, S Dec
Wild	*Sphingobium* sp. *YG1*	C Dec, N Dec, P Dec, S Dec
Modern	*Chryseobacterium wanjuense*	C Dec
Modern	*Ilumatobacter nonamiensis*	N Dec, S Dec
Modern	*Lewinella cohaerens*	C Dec, N Dec, P Dec, Stress
Modern	*Nocardioides* sp. *JS614*	Bioc, C Dec, N Dec, S Dec

Supplemental Table 4-6. Differentially abundant bacteria in phosphorus (P) fertilized soils between wild and modern tomato at the early flower stage. A Wald test with an FDR p adjustment of 0.05 was conducted to determine species enrichment. The bacteria in the "Taxa" column denote the species that were differentially abundant. The "Enrich Group" column indicates the domestication group in which the corresponding bacteria were enriched in the fertilized wild tomato soils. The "Function" column shows an abbreviation of the predictive function of the bacteria as determined through PICRUSt2. The functions are abbreviated as the following: Biocontrol ("Bioc"), nitrogen decomposition ("N Dec"), phosphorus decomposition ("P Dec"), phosphate solubilization ("P Sol"), sulfur decomposition ("S Dec"), stress control ("Stress").

Enrich Group	Species	Function
Wild	*Acidovorax defluvii*	N Dec, P Dec, Stress
Wild	*Acidovorax facilis*	N Dec, P Dec, Stress
Wild	*Acidovorax* sp. *1608163*	N Dec, P Dec, Stress
Wild	*Acidovorax* sp. *KKS102*	N Dec, P Dec, Stress
Wild	*Acidovorax* sp. *RAC01*	Bioc, C Dec, N Dec, NFix, Nitr, P Dec, P Sol, S Dec, Side, Stress
Wild	*Acidovorax* sp. *T1*	N Dec, P Dec, Stress
Wild	*Acidovorax valerianellae*	N Dec, P Dec
Wild	*Acidovorax wautersii*	Bioc, N Dec, P Dec, Stress
Wild	*Agrobacterium* sp. *RAC06*	Bioc, C Dec, N Dec, NFix, Nitr, P Dec, P Sol, S Dec, Side, Stress
Wild	*Agrobacterium tumefaciens*	C Dec, N Dec, NFix, P Dec, Side, Stress
Wild	*Bosea* sp. *Tri-49*	Bioc, C Dec, N Dec, NFix, Nitr, P Dec, P Sol, S Dec, Side, Stress
Wild	*Cellvibrio* sp. *KY-GH-1*	Bioc, C Dec, N Dec, NFix, Nitr, P Dec, P Sol, S Dec, Side, Stress
Wild	*Ciceribacter thiooxidans*	C Dec, N Dec, P Dec
Wild	*Devosia riboflavina*	Bioc, C Dec, N Dec, P Dec
Wild	*Dyadobacter fermentans*	Bioc, C Dec, N Dec, P Dec, S Dec
Wild	*Dyadobacter ginsengisoli*	Bioc, C Dec, N Dec, P Dec, S Dec
Wild	*Dyadobacter jiangsuensis*	Bioc, C Dec, N Dec, P Dec, S Dec
Wild	*Ensifer adhaerens*	C Dec, N Dec, P Dec, S Dec, Stress
Wild	*Flavobacterium hauense*	Bioc, C Dec, N Dec, P Dec, Stress
Wild	*Legionella cherrii*	Bioc, N Dec, P Dec, Stress
Wild	*Legionella fallonii*	N Dec, P Dec, Stress

Wild	*Luteolibacter gellanilyticus*	C Dec, N Dec, P Dec, S Dec
Wild	*Lysobacter soli*	Bioc, C Dec, N Dec, P Dec, P Sol, Side
Wild	*Methylibium petroleiphilum*	C Dec, N Dec, P Dec, P Sol, Stress
Wild	*Methylibium* sp. *Pch-M*	C Dec, N Dec, P Dec, P Sol
Wild	*Micavibrio aeruginosavorus*	C Dec
Wild	*Microbacterium chocolatum*	Bioc, C Dec, N Dec, Stress
Wild	*Microbacterium hominis*	Bioc, C Dec, N Dec, Stress
Wild	*Microbacterium thalassium*	Bioc, C Dec, N Dec, Stress
Wild	*Microbacterium yannicii*	Bioc, C Dec, N Dec, S Dec
Wild	*Mitsuaria chitosanitabida*	Bioc, C Dec, N Dec, P Dec
Wild	*Neorhizobium galegae*	Bioc, C Dec, N Dec, NFix, P Dec
Wild	*Nocardia globerula*	C Dec, N Dec, P Dec, S Dec, Side
Wild	*Novosphingobium* sp. *P6W*	Bioc, C Dec, N Dec, P Dec, S Dec
Wild	*Pseudomonas brassicacearum*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Wild	*Pseudomonas chlororaphis*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Wild	*Pseudomonas fluorescens*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Wild	*Pseudomonas frederiksbergensis*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Wild	*Pseudomonas orientalis*	C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Wild	*Pseudomonas putida*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Side, Stress
Wild	*Pseudomonas synxantha*	Bioc, C Dec, N Dec, P Dec, P Sol, Stress
Wild	*Pseudomonas syringae*	Bioc, C Dec, N Dec, P Dec, P Sol, Stress
Wild	*Pseudorhodoferax soli*	C Dec, N Dec, P Dec, P Sol, S Dec, Side, Stress
Wild	*Pseudoxanthomonas japonensis*	C Dec, N Dec, P Dec
Wild	*Pseudoxanthomonas mexicana*	C Dec, N Dec, P Dec
Wild	*Rhizobacter dauci*	C Dec, N Dec, P Dec, P Sol
Wild	*Rhizobacter fulvus*	C Dec, N Dec, P Dec, P Sol
Wild	*Rhizobacter gummiphilus*	C Dec, N Dec, P Dec, P Sol
Wild	*Rhizobium deserti*	Bioc, C Dec, N Dec, NFix, P Dec
Wild	*Rhizobium rhizoryzae*	C Dec, N Dec, P Dec

Wild	*Rhizobium sp. WL3*	Bioc, C Dec, N Dec, NFix, Nitr, P Dec, P Sol, S Dec, Side, Stress
Wild	*Rhizorhapis suberifaciens*	C Dec, N Dec, P Dec, S Dec
Wild	*Rhodococcus* sp. *S2-17*	C Dec, N Dec, P Dec, S Dec
Wild	*Rhodoferax ferrireducens*	C Dec, N Dec, P Dec, Stress
Wild	*Shinella yambaruensis*	C Dec, N Dec, P Dec, S Dec
Wild	*Sphingobium amiense*	Bioc, C Dec, P Dec
Wild	*Sphingobium boeckii*	Bioc, C Dec, N Dec, P Dec
Wild	*Sphingobium chlorophenolicum*	Bioc, C Dec, N Dec, P Dec, S Dec
Wild	*Sphingobium chungbukense*	C Dec, S Dec
Wild	*Sphingobium cloacae*	C Dec, P Dec, S Dec
Wild	*Sphingobium indicum*	C Dec, N Dec, P Dec, S Dec
Wild	*Sphingobium* sp. *TKS*	C Dec, S Dec
Wild	*Sphingobium* sp. *YG1*	C Dec, N Dec, P Dec, S Dec
Wild	*Sphingobium yanoikuyae*	Bioc, C Dec, P Dec, S Dec
Wild	*Sphingomonas colocasiae*	C Dec, N Dec, P Dec, S Dec
Wild	*Variovorax* sp. *PMC12*	C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Wild	*Xanthomonas campestris*	C Dec, N Dec, P Dec, P Sol, Side
Wild	*Xanthomonas citri*	C Dec, N Dec, P Dec, P Sol, Side
Wild	*Xanthomonas oryzae*	C Dec, N Dec, P Dec, P Sol, Side
Wild	*Xanthomonas sacchari*	Bioc, C Dec, N Dec, P Dec, P Sol
Modern	*Aggregicoccus edonensis*	Bioc, C Dec, N Dec, P Dec, Side
Modern	*Bacillus weihaiensis*	C Dec, N Dec, P Dec
Modern	*Blastopirellula retiformator*	P Dec, S Dec, Stress
Modern	*Cytobacillus gottheilii*	N Dec, P Dec
Modern	*Desulfuromonas soudanensis*	Bioc, C Dec, N Dec, NFix, Nitr, P Dec, P Sol, S Dec, Side, Stress
Modern	*Domibacillus robiginosus*	N Dec, P Dec
Modern	*Ectothiorhodospira mobilis*	NFix, P Dec
Modern	*Egibacter rhizosphaerae*	C Dec, N Dec

Modern	*Massilia oculi*	Bioc, C Dec, N Dec, P Dec
Modern	*Nocardioides islandensis*	Bioc, C Dec, N Dec, P Dec, S Dec
Modern	*Nocardioides* sp. *JS614*	Bioc, C Dec, N Dec, S Dec
Modern	*Nocardioides terrigena*	Bioc, C Dec, N Dec, P Dec, S Dec
Modern	*Paraburkholderia sp. SOS3*	Bioc, C Dec, N Dec, NFix, Nitr, P Dec, P Sol, S Dec, Side, Stress
Modern	*Pontibacter chitinilyticus*	Bioc, C Dec, P Dec
Modern	*Streptomyces* sp. *S501*	Bioc, C Dec, N Dec, P Dec, S Dec, Stress
Modern	*Thermobaculum terrenum*	C Dec
Modern	*Vulgatibacter incomptus*	N Dec

Supplemental Table 4-7. Differentially abundant bacteria in phosphorus (P) fertilized soils between wild and modern tomato at the late flower stage. A Wald test with an FDR p adjustment of 0.05 was conducted to determine species enrichment. The bacteria in the "Taxa" column denote the species that were differentially abundant. The "Enrich Group" column indicates the domestication group in which the corresponding bacteria were enriched in the fertilized wild tomato soils. The "Function" column shows an abbreviation of the predictive function of the bacteria as determined through PICRUSt2. The functions are abbreviated as the following: Biocontrol ("Bioc"), nitrogen decomposition ("N Dec"), phosphorus decomposition ("P Dec"), phosphate solubilization ("P Sol"), sulfur decomposition ("S Dec"), stress control ("Stress").

Enrich Group	Species	Function
Wild	*Acidovorax carolinensis*	N Dec, P Dec, Stress
Wild	*Cellvibrio* sp. *KY-GH-1*	Bioc, C Dec, N Dec, NFix, Nitr, P Dec, P Sol, S Dec, Side, Stress
Wild	*Luteolibacter pohnpeiensis*	C Dec, N Dec, P Dec, S Dec
Wild	*Lysobacter soli*	Bioc, C Dec, N Dec, P Dec, P Sol, Side
Wild	*Microbacterium chocolatum*	Bioc, C Dec, N Dec, Stress
Wild	*Microbacterium hominis*	Bioc, C Dec, N Dec, Stress
Wild	*Pseudomonas synxantha*	Bioc, C Dec, N Dec, P Dec, P Sol, Stress
Wild	*Pseudorhodoferax soli*	C Dec, N Dec, P Dec, P Sol, S Dec, Side, Stress
Wild	*Pseudoxanthomonas mexicana*	C Dec, N Dec, P Dec
Wild	*Rhizobacter dauci*	C Dec, N Dec, P Dec, P Sol
Wild	*Rhizorhapis suberifaciens*	C Dec, N Dec, P Dec, S Dec
Wild	*Sphingobium boeckii*	Bioc, C Dec, N Dec, P Dec
Wild	*Sphingobium chlorophenolicum*	Bioc, C Dec, N Dec, P Dec, S Dec
Wild	*Sphingobium chungbukense*	C Dec, S Dec
Wild	*Sphingobium indicum*	C Dec, N Dec, P Dec, S Dec
Modern	*Adhaeribacter swui*	C Dec, N Dec, P Dec
Modern	*Sphingobium* sp. *MI1205*	Bioc, C Dec

Supplemental Table 4-8. Differentially abundant bacteria in between fertilized wild and unfertilized wild tomato. A Wald test with an FDR p adjustment of 0.05 was conducted to determine species enrichment. The bacteria in the "Taxa" column denote the species that were differentially abundant. The "Enrich Group" column indicates the fertilization treatment in which the corresponding bacteria were enriched. The "Stage" column denotes the harvest stage at which the corresponding bacterium was enriched in wild tomato. The "Function" column shows an abbreviation of the predictive function of the bacteria as determined through PICRUSt2. The functions are abbreviated as the following: Biocontrol ("Bioc"), nitrogen decomposition ("N Dec"), phosphorus decomposition ("P Dec"), phosphate solubilization ("P Sol"), sulfur decomposition ("S Dec"), stress control ("Stress").

Species	Enrich Group	Stage	Function
Acidovorax sp. KKS102	Fertilized	Seedling	N Dec,P Dec,Stress
Acidovorax carolinensis	Fertilized	Early_Vegetative	N Dec,P Dec,Stress
Acidovorax sp. RAC01	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,N Fix,Nitr,P Dec,P Sol,S Dec,Side,Stress
Arthrobacter tumbae	Unfertilized	Early_Vegetative	C Dec,N Dec
Bacillus licheniformis	Unfertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,Side
Bacillus sp. X1(2014)	Unfertilized	Early_Vegetative	N Dec
Bhargavaea ginseng	Unfertilized	Early_Vegetative	N Dec,P Dec,S Dec
Chelatococcus daeguensis	Unfertilized	Early_Vegetative	N Dec,Stress
Cytobacillus gottheilii	Unfertilized	Early_Vegetative	N Dec,P Dec,N Dec,P Dec
Massilia albidiflava	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,P Sol
Massilia umbonate	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,P Sol
Pelomonas saccharophila	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec
Pseudoduganella violaceinigra	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,Side
Pseudomonas chlororaphis	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,P Sol,S Dec,Stress
Pseudomonas corrugata	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,P Sol,Stress
Pseudomonas frederiksbergensis	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,P Sol,S Dec,Stress
Pseudomonas kilonensis	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,P Sol,Stress,
Pseudomonas putida	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,P Sol,S Dec,SideStress
Pseudomonas sp. R5-89-07	Fertilized	Early_Vegetative	C Dec,N Dec,P Dec,P Sol,S Dec,Stress,

Pseudomonas synxantha	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,P Sol,Stress,
Pseudomonas syringae	Fertilized	Early_Vegetative	Bioc,C Dec,N Dec,P Dec,P Sol,Stress,
Rhizobacter dauci	Fertilized	Early_Vegetative	C Dec,N Dec,P Dec,P Sol
Rhizobacter fulvus	Fertilized	Early_Vegetative	C Dec,N Dec,P Dec,P Sol
Rhizobacter gummiphilus	Fertilized	Early_Vegetative	C Dec,N Dec,P Dec,P Sol
Rhodoferax ferrireducens	Fertilized	Early_Vegetative	C Dec,N Dec,P Dec,Stress
Rhodoferax sediminis	Fertilized	Early_Vegetative	N Dec,P Dec
Sphingobium chungbukense	Fertilized	Early_Vegetative	C Dec,S Dec
Sphingobium indicum	Fertilized	Early_Vegetative	C Dec,N Dec,P Dec,S Dec
Bacillus cereus	Unfertilized	Late_Vegetative	Bioc,C Dec,N Dec,P Dec,Side,Stress
Bacillus sp. S3	Unfertilized	Late_Vegetative	C Dec,N Dec,P Dec,P Sol
Bacillus sp. Y1	Unfertilized	Late_Vegetative	C Dec,N Dec,P Dec
Metabacillus indicus	Unfertilized	Late_Vegetative	N Dec,P Dec
Neobacillus mesonae	Unfertilized	Late_Vegetative	C Dec,N Dec,P Dec
Neobacillus niacin	Unfertilized	Late_Vegetative	C Dec,N Dec,P Dec,S Dec
Oxalophagus oxalicus	Unfertilized	Late_Vegetative	N Dec,P Dec,Stress
Paenibacillus mucilaginosus	Unfertilized	Late_Vegetative	Bioc,C Dec,N Dec,P Dec,S Dec,Stress
Rhizobium leguminosarum	Fertilized	Late_Vegetative	C Dec,N Dec,N Fix,P Dec,S Dec,Stress
Rhodoferax saidenbachensis	Fertilized	Late_Vegetative	N Dec,P Dec
Solibacillus silvestris	Unfertilized	Late_Vegetative	N Dec,P Dec,Side
Aliterella antarctica	Unfertilized	Early_Flower	C Dec,N Dec
Devosia honganensis	Unfertilized	Early_Flower	C Dec,N Dec
Dyadobacter jiangsuensis	Fertilized	Early_Flower	Bioc,C Dec,N Dec,P Dec,S Dec
Dyadobacter psychrophilus	Fertilized	Early_Flower	Bioc,C Dec,N Dec,P Dec,S Dec
Ectothiorhodospira mobilis	Unfertilized	Early_Flower	N Fix,P Dec
Herbaspirillum hiltneri	Unfertilized	Early_Flower	Bioc,N Dec,P Dec,S Dec,Stress
Herpetosiphon aurantiacus	Unfertilized	Early_Flower	C Dec

Legionella fallonii	Fertilized	Early_Flower	N Dec,P Dec,Stress
Lysobacter soli	Fertilized	Early_Flower	Bioc,C Dec,N Dec,P Dec,P Sol,Side
Mesorhizobium tianshanense	Fertilized	Early_Flower	N Dec,N Fix,P Dec,P Sol,Stress
Methylotenera versatilis	Fertilized	Early_Flower	N Dec,P Dec
Microbacterium chocolatum	Fertilized	Early_Flower	Bioc,C Dec,N Dec,Stress
Microbacterium hominis	Fertilized	Early_Flower	Bioc,C Dec,N Dec,Stress
Microbacterium thalassium	Fertilized	Early_Flower	Bioc,C Dec,N Dec,Stress
Microbacterium yannicii	Fertilized	Early_Flower	Bioc,C Dec,N Dec,S Dec
Mucibacter soli	Fertilized	Early_Flower	C Dec
Nocardia globerula	Fertilized	Early_Flower	C Dec,N Dec,P Dec,S Dec,Side
Novosphingobium sp. P6W	Fertilized	Early_Flower	Bioc,C Dec,N Dec,P Dec,S Dec
Paenibacillus agaridevorans	Unfertilized	Early_Flower	Bioc,C Dec,N Dec,P Dec
Pontibacter brevis	Unfertilized	Early_Flower	C Dec,N Dec,P Dec,C Dec,N Dec,P Dec
Pseudomonas brassicacearum	Fertilized	Early_Flower	Bioc,C Dec,N Dec,P Dec,P Sol,S Dec,Stress
Pseudomonas fluorescens	Fertilized	Early_Flower	Bioc,C Dec,N Dec,P Dec,P Sol,S Dec,Stress
Pseudomonas marincola	Unfertilized	Early_Flower	C Dec,N Dec,P Dec,P Sol,S Dec,Stress,
Pseudomonas orientalis	Fertilized	Early_Flower	C Dec,N Dec,P Dec,P Sol,S Dec,Stress,
Pseudorhodoferax soli	Fertilized	Early_Flower	C Dec,N Dec,P Dec,S Dec,Side,Stress,
Pseudoxanthomonas japonensis	Fertilized	Early_Flower	C Dec,N Dec,P Dec
Pseudoxanthomonas Mexicana	Fertilized	Early_Flower	C Dec,N Dec,P Dec
Rhizobium rhizoryzae	Fertilized	Early_Flower	C Dec,N Dec,P Dec,
Rhizorhapis suberifaciens	Fertilized	Early_Flower	C Dec,N Dec,P Dec,S Dec
Rhodococcus sp. S2-17	Fertilized	Early_Flower	C Dec,N Dec,P Dec,S Dec
Singulisphaera acidiphila	Fertilized	Early_Flower	Bioc,C Dec,N Dec,P Dec,S Dec,Stress
Sphingobium yanoikuyae	Fertilized	Early_Flower	Bioc,C Dec,P Dec,S Dec

Sphingomonas colocasiae	Fertilized	Early_Flower	C Dec,N Dec,P Dec,S Dec
Vulgatibacter incomptus	Unfertilized	Early_Flower	N Dec
Xanthomonas campestris	Fertilized	Early_Flower	C Dec,N Dec,P Dec,P Sol,Side
Xanthomonas citri	Fertilized	Early_Flower	C Dec,N Dec,P Dec,P Sol,Side
Xanthomonas oryzae	Fertilized	Early_Flower	C Dec,N Dec,P Dec,P Sol,Side
Xanthomonas sacchari	Fertilized	Early_Flower	Bioc,C Dec,N Dec,P Dec,P Sol
Cellvibrio sp. KY-GH-1	Fertilized	Late_Flower	Bioc,C Dec,N Dec,N Fix,Nitr,P Dec,P Sol,S Dec,Side,Stress
Lysinibacillus sp. SGAir0095	Fertilized	Late_Flower	N Dec,P Dec,N Dec,P Dec
Mucibacter soli	Fertilized	Late_Flower	C Dec
Paenibacillus protaetiae	Unfertilized	Late_Flower	Bioc,C Dec,N Dec,P Dec
Reyranella massiliensis	Fertilized	Late_Flower	Bioc,C Dec,N Dec,P Dec,P Sol,S Dec
Sphingobium boeckii	Fertilized	Late_Flower	Bioc,C Dec,N Dec,P Dec
Trichocoleus desertorum	Fertilized	Late_Flower	C Dec,N Dec

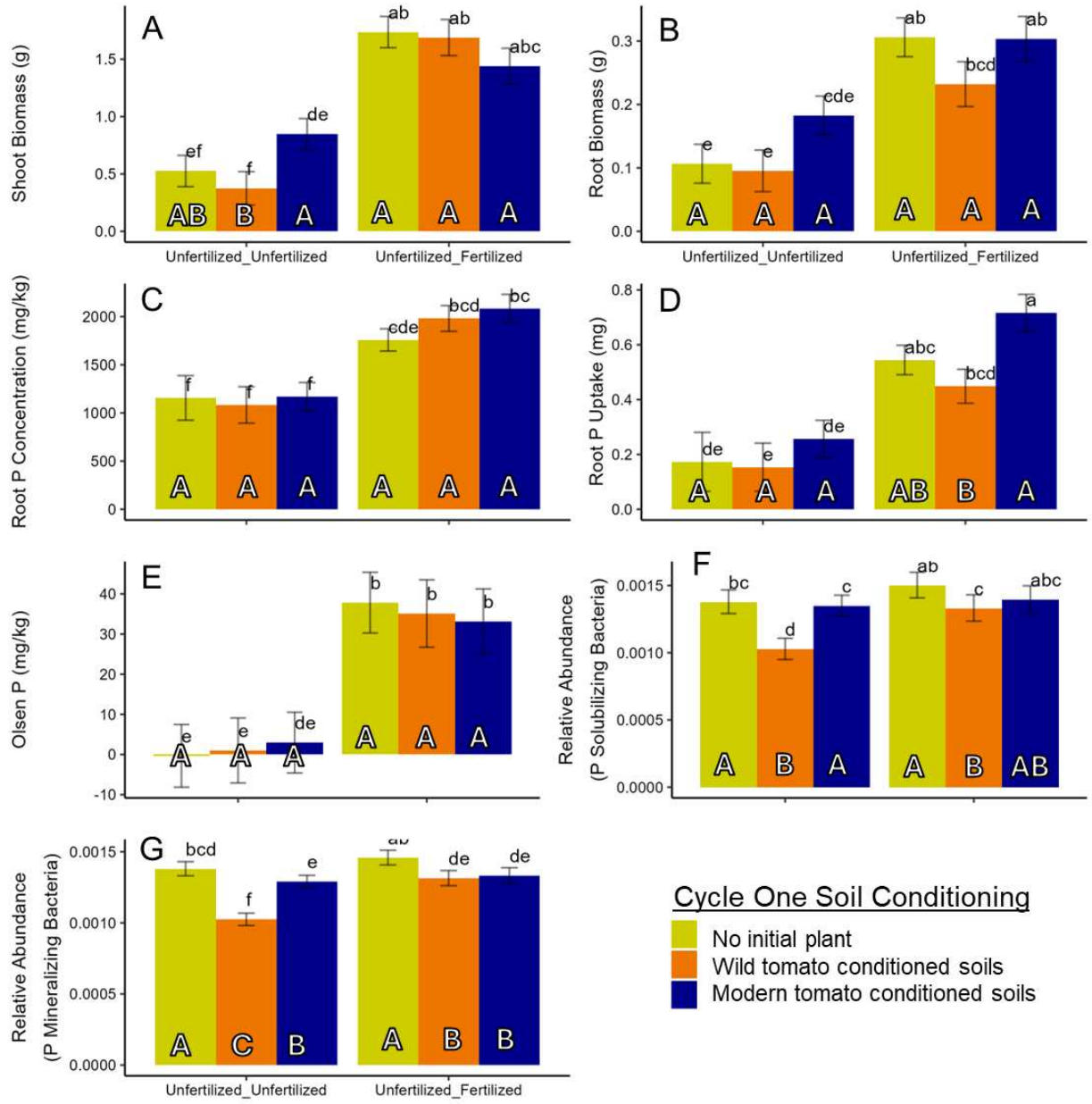
Supplemental Table 4-9. Bacteria promoted by wild tomato in phosphorus (P) fertilized conditions. The bacteria in the "Taxa" column denote the species that were enriched in fertilized wild tomato compared to fertilized modern tomato and were enriched in fertilized wild compared to unfertilized wild. A Wald test with an FDR p adjustment of 0.05 was conducted to determine species enrichment. The "Stage" column indicates the stage in which the corresponding bacteria were enriched in the fertilized wild tomato soils. The "Function" column shows the predictive function of the bacteria as determined through PICRUSt2. The functions are abbreviated as the following: Biocontrol ("Bioc"), nitrogen decomposition ("N Dec"), nitrogen fixation ("N Fix"), nitrification ("Nitr"), phosphorus decomposition ("P Dec"), phosphate solubilization ("P Sol"), sulfur decomposition ("S Dec"), stress control ("Stress").

Stage	Taxa	Function
Early Vegetative	*Pseudomonas corrugata*	Bioc, C Dec, N Dec, P Dec, P Sol, Stress
Early Vegetative	*Pseudomonas putida*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Side, Stress
Early Vegetative	*Pseudomonas* sp. R5-89-07	C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Early Vegetative	*Pseudomonas synxantha*	Bioc, C Dec, N Dec, P Dec, P Sol, Stress
Early Vegetative	*Rhizobacter gummiphilus*	C Dec, N Dec, P Dec, P Sol
Early Vegetative, Early Flower	*Pseudomonas fluorescens*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Early Flower	*Dyadobacter jiangsuensis*	Bioc, C Dec, N Dec, P Dec, S Dec
Early Flower	*Legionella fallonii*	N Dec, P Dec, Stress
Early Flower	*Microbacterium hominis*	Bioc, C Dec, N Dec, Stress
Early Flower	*Microbacterium thalassium*	Bioc, C Dec, N Dec, Stress
Early Flower	*Microbacterium yannicii*	Bioc, C Dec, N Dec, S Dec
Early Flower	*Nocardia globerula*	C Dec, N Dec, P Dec, S Dec, Side
Early Flower	*Novosphingobium* sp. P6W	Bioc, C Dec, N Dec, P Dec, S Dec
Early Flower	*Pseudomonas brassicacearum*	Bioc, C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Early Flower	*Pseudomonas orientalis*	C Dec, N Dec, P Dec, P Sol, S Dec, Stress
Early Flower	*Pseudorhodoferax soli*	C Dec, N Dec, P Dec, S Dec, Side, Stress
Early Flower	*Pseudoxanthomonas japonensis*	C Dec, N Dec, P Dec
Early Flower	*Pseudoxanthomonas mexicana*	C Dec, N Dec, P Dec
Early Flower	*Rhizobium rhizoryzae*	C Dec, N Dec, P Dec
Early Flower	*Rhizorhapis suberifaciens*	C Dec, N Dec, P Dec, S Dec
Early Flower	*Rhodococcus* sp. S2-17	C Dec, N Dec, P Dec, S Dec
Early Flower	*Sphingobium yanoikuyae*	Bioc, C Dec, P Dec, S Dec

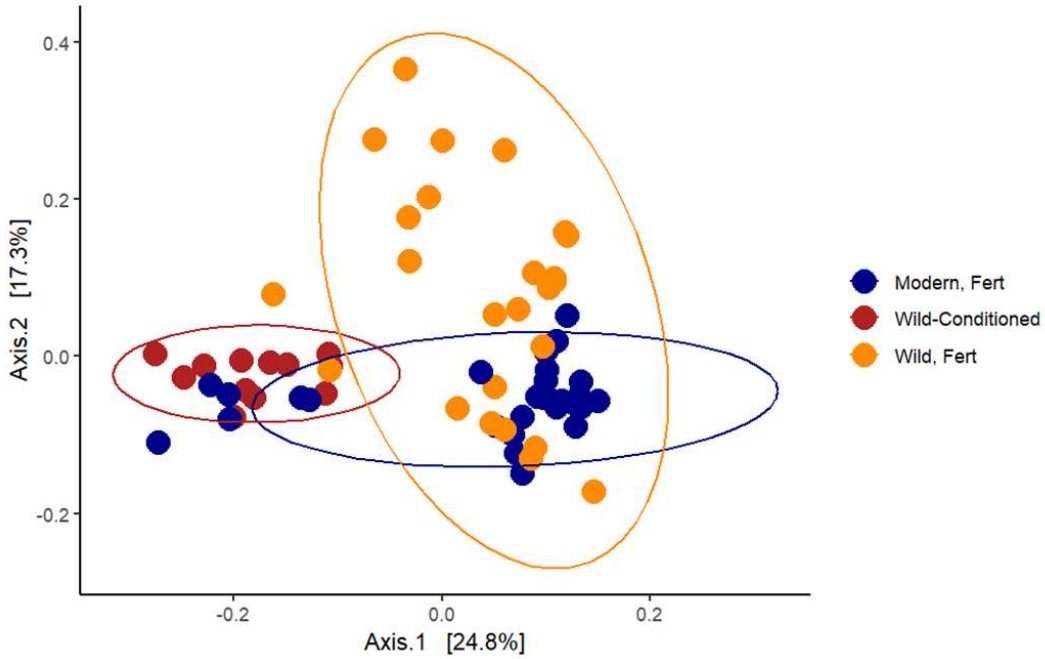
Early Flower	*Sphingomonas colocasiae*	C Dec, N Dec, P Dec, S Dec
Early Flower	*Xanthomonas campestris*	C Dec, N Dec, P Dec, P Sol, Side
Early Flower	*Xanthomonas citri*	C Dec, N Dec, P Dec, P Sol, Side
Early Flower	*Xanthomonas oryzae*	C Dec, N Dec, P Dec, P Sol, Side
Early Flower	*Xanthomonas sacchari*	Bioc, C Dec, N Dec, P Dec, P Sol
Early Flower, Late Flower	*Lysobacter soli*	Bioc, C Dec, N Dec, P Dec, P Sol, Side
Early Flower, Late Flower	*Microbacterium chocolatum*	Bioc, C Dec, N Dec, Stress
Late Flower	*Cellvibrio* sp. KY-GH-1	Bioc, C Dec, N Dec, N Fix, Nitr, P Dec, P Sol, S Dec, Side, Stress
Late Flower	*Sphingobium boeckii*	Bioc, C Dec, N Dec, P Dec

Supplemental Table 4-10. Metabolites enriched by wild and modern tomato. "Metabolite" indicates the metabolite that was enriched in the corresponding "Enrich Group" column. The method of metabolite analysis is listed in the "Method" column.

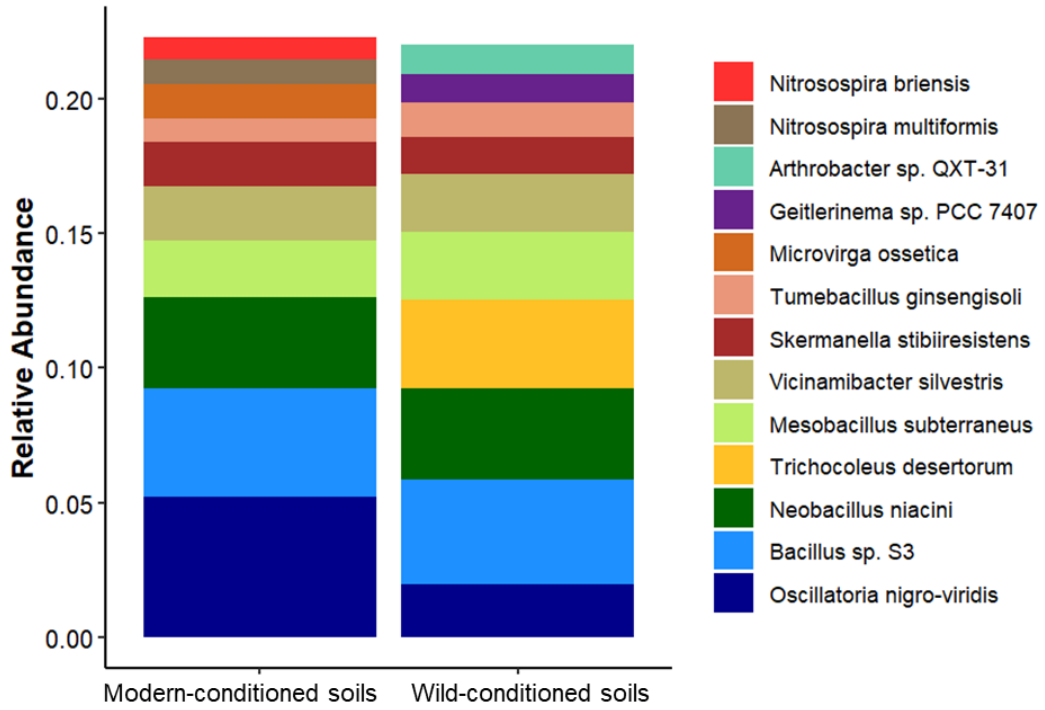
Metabolite	Fold change	p value	Enrich Group	Method
1-monopalmitin	-2.44691	0.023384	Wild	GCMS
glycerol monostearate	-2.83482	0.012791	Wild	GCMS
Trehalose	-5.251	0.007097	Wild	GCMS
1-dodecanol	1.1603	0.035255	Modern	GCMS
3,4-dihydroxybutanoic acid	1.15899	0.037256	Modern	GCMS
3-hydroxybutyric acid	3.345143	0.00505	Modern	GCMS
lauric acid	2.121505	0.00298	Modern	GCMS
malic acid	1.786035	0.031773	Modern	GCMS
succinic acid	1.660416	0.032411	Modern	GCMS
Succinic acid	-3.86284	0.035942	Wild	HILIC_Neg
Methanesulfonic acid	0.866746	0.020203	Modern	HILIC_Neg
4-Dodecylbenzenesulfonic acid	0.901228	0.020964	Modern	HILIC_Neg
Acetyl-D-carnitine	-3.76523	0.026097	Wild	HILIC_Pos
Triisopropanolamine	-2.68913	0.039242	Wild	HILIC_Pos
Myristic acid	1.786313	0.028722	Modern	HILIC_Pos
Glycerate	-1.84274	0.007002	Wild	RP_Neg
Aspartate	-1.88687	0.019897	Wild	RP_Neg
Dehydroascorbate.[Peak.1]	-2.60455	0.001585	Wild	RP_Neg
Citrate.[Peak.1]	-1.31787	0.049386	Wild	RP_Neg
Phosphoric acid	-4.32161	0.008077	Wild	RP_Neg
Benzoic acid.[Peak.2]	-0.96624	0.04258	Wild	RP_Neg
Benzoic acid.[Peak.1]	-1.57222	0.003105	Wild	RP_Neg
5-Oxo-L-Proline.[Peak.2]	-2.00517	0.018902	Wild	RP_Neg
5-Oxo-L-Proline.[Peak.1]	-1.94424	0.019045	Wild	RP_Neg
Uridine	-2.91475	0.026027	Wild	RP_Neg
(S)-Lactate.[Peak.1]	-1.61932	0.024958	Wild	RP_Neg
Ferulate	-3.15919	0.001073	Wild	RP_Neg
Monobenzyl phthalate	2.129514	0.048795	Modern	RP_Neg
Caprolactam	-2.92707	3.78E-05	Wild	RP_Pos
Triisopropanolamine	-3.05074	0.009593	Wild	RP_Pos
Decylamine, N,N-dimethyl-, N-oxide	-1.78717	0.023232	Wild	RP_Pos
1-Stearoylglycerol Peak 2	-3.38853	0.015389	Wild	RP_Pos
1-Stearoylglycerol Peak 1	-3.37724	0.001886	Wild	RP_Pos
5-Oxo-L-Proline.[Peak.1]	-1.63184	0.018528	Wild	RP_Pos
Aspartate	-1.96556	0.005212	Wild	RP_Pos
Deoxycarnitine	-1.8938	0.00086	Wild	RP_Pos
L-Glutamine	-2.84895	0.044657	Wild	RP_Pos
3-Methylphenylacetic acid	2.577738	0.008222	Modern	RP_Pos
Nalpha-Acetyl-L-Lysine	1.058319	0.038693	Modern	RP_Pos



Supplemental Figure 4-6. Above- and belowground impacts of conditioning soils with fertilizer and wild tomato on modern tomato growth. Modern tomato shoot biomass (A), root biomass (B), root P concentration (C), root P uptake (D), Olsen P (E), as well as rhizosphere phosphorus solubilizer (F) and phosphorus decomposer (G) relative abundance were determined were that were initially unfertilized and analyzed using an ANOVA with Tukey HSD test. Results are displayed as mean ± standard error. Different letters denote significant difference in either biomass or relative abundance at α = 0.05. The x-axis represents the fertilization patterns in the soil conditioning cycle and in the second cycle. Bar color represents how soils were conditioned in cycle 1: yellow (no plant-conditioned), orange (wild-conditioned), and blue (modern-conditioned). Different lowercase letters denote differences in the interactive effect. Different uppercase letters denote differences of the soil conditioning treatment within a specific fertilization pattern.



Supplemental Figure 4- 7. Principal coordinate analysis (PCoA) ordination of the bacterial community structure in the wild and modern tomato rhizosphere. The ellipses and colors indicate the different tomato treatments across two different trials: dark blue (fertilized modern tomato, time course trial), orange (fertilized wild tomato, time course trial), red (modern tomato conditioned in soils that had an initially fertilized wild tomato).



Supplemental Figure 4-8. Abundant taxa in modern tomato soils. The ten most abundant bacterial species were plotted for the rhizosphere of modern tomato that was planted in soils that were initially fertilized. The x axis denotes the soil conditioning treatment: modern or wild conditioned soils.