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Consejo Superior De Investigaciones Científicas (CSIC)
Estación Experimental del Zaidín (EEZ)
Department of Environmental Protection

Interactions of soil microbiome, plant defenses and domestication in tomato.

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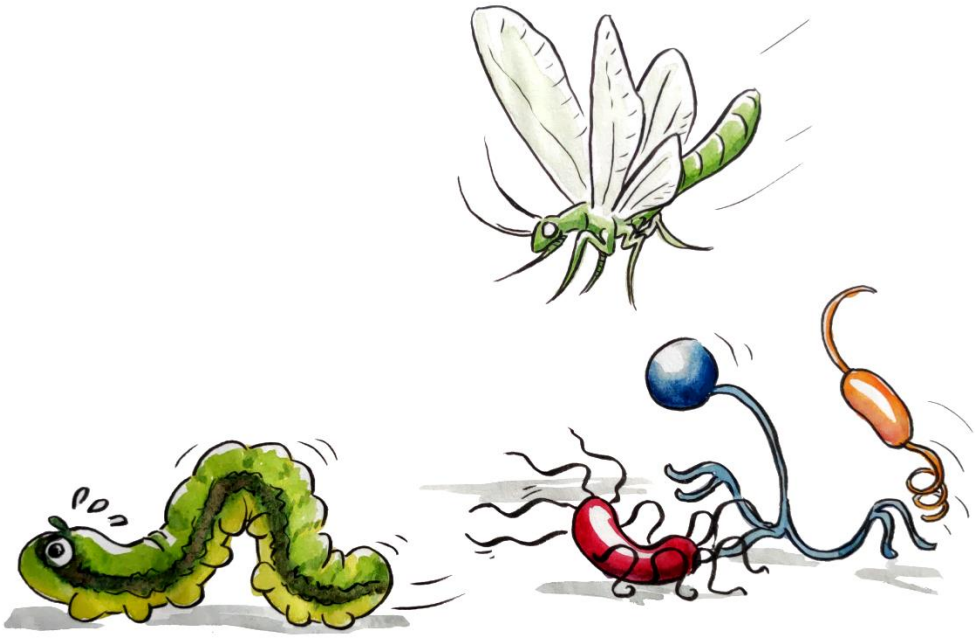

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Summary

Domestication and breeding were often focused on plant morphology, while resistance and defence traits were usually left aside. Furthermore, crop domestication could alter interactions of plants with herbivores and their natural enemies at all trophic levels. In the introduction we integrate plant-microbe-arthropod (PMA) interactions from an ecological point of view focusing on interactions with beneficial organisms both below and aboveground. Interactions with beneficial organisms are thought to be reduced during domestication and breeding but experimental evidence for this is lacking. Here we discuss how domestication and breeding can affect plant constitutive and induced defenses and if results are consistent in this direction. Thus, we show the complexity of studying domestication effects on plant and soil microbiome, resistance and defence mechanisms and propose ideas for future research to advance our understanding in this exciting field.

The objective of this thesis was to study how tomato domestication influenced belowground microbial communities, focusing on bacteria and arbuscular mycorrhizal fungi (AMF), and dive deeper into how these belowground communities affect aboveground indirect defences in tomato. This general objective was divided into two specific ones 1) To address top down effects of tomato domestication on plant microbial communities and 2) To investigate bottom up effects of microbial communities and *Spodoptera exigua* attack on volatile production and attraction of the predator *Chrysoperla carnea*.

First, we explored how tomato domestication affected belowground root-associated bacterial communities, focusing on community composition (**chapter 1**) and functionality (**chapter 2**). In **chapter 1**, two main bacterial classes were found to dominate the tomato rhizosphere of all varieties, Alphaproteobacteria and Actinobacteria. Some minority phyla, such as Acidobacteria and Gemmatimonades, were increased in modern tomato varieties compared to wild tomato varieties. Tomato fruit traits varied following the domestication degree, where wild tomatoes produced more but smaller tomatoes while modern tomatoes produced a higher plant biomass and yield. However, no effect of tomato fruit traits on the root associated bacterial community was found. On the other hand, resistance traits explained an important fraction of variation between tomatoes, especially between domesticated varieties. Lastly, evidence was found for a positive correlation between bacterial diversity and reduced resistance, suggesting that susceptible varieties harbour more diverse bacterial communities. It could be that there are other unmeasured morphological traits, such as root-associated traits, that could be linked with belowground bacterial communities.

In **chapter 2**, we show that all bacterial predicted functions were present in all tomato domestication types. The bacterial communities of wild tomato showed a higher level of aromatic degradation pathways and the Krebs cycle, indicating that modern tomato species lost degradation of recalcitrant organic compounds capacity. In line with this, reduced expression of biochemical cycles such as nitrates, sulphates and urea formation were detected in modern cultivars. Thus, it seems that the increased use of agrochemicals in modern agriculture might be connected with a reduction in metabolic pathways levels due to certain biochemical cycles. Other pathways were more highly expressed in modern tomato species, such as the synthesis of gamma-aminobutyric acid (GABA), fatty acids and jasmonic acid (JA), with plant produced JA being involved in defence against biotic stress and plant-microbe interactions, but an unclear role in the soil. Tomato landraces and wild tomato species were more connected to each other compared to modern:wild or modern:landraces pairs in terms of predicted bacterial functions in their rhizosphere.

Then, we had a look at how 1) tomato domestication and spatial location affected symbiosis with root glomeromycotan fungal communities and 2) how variation in fungal communities drives the expression of aboveground plant traits (**chapter 3**). We found similar AMF communities between varieties and no evidence for

selection of particular AMF families or genera by the different tomato genotypes. AMF communities were mainly influenced by location, especially AMF phylogenetic turnover. It therefore seems that AMF communities were driven by an unidentified environmental (soil) gradient. Despite the similarity of AMF communities, colonization levels significantly differed among tomato genotypes independently from their domestication degree, thus not supporting the hypothesis that modern tomatoes lost mycorrhizal capacity.. Aboveground plant traits also differed between varieties, with wild tomatoes generally showing increased symptom development despite lower viral incidence, higher tomato numbers and lower fruit weight. Location had a major influence on aboveground plant fruit and resistance traits. After location, AMF community composition and phylogenetic turnover explained variation in most traits, tomato variety mainly explained resistance traits and colonization while domestication explained differences in both resistance and fruit traits. Lastly, we found taxa of four different AMF genera with varying effects on aboveground plant traits, suggesting that symbiosis outcomes depend on the presence of certain AMF and can vary between taxa. Some taxa were found to positively affect plant morphology (biomass and tomato production), albeit negatively affecting resistance. We therefore believe that diverse AMF communities in the soil can be helpful in increasing plant growth and promoting tolerance to biotic stress.

Finally, in **chapter 4** we studied the effect of the natural soil microbial community and *Spodoptera exigua* attack on volatile production and attraction of the predator *Chrysoperla carnea*. As expected, the soil microbial community differed between sterile soil and natural soil treatments. However, *Rhizophagus irregularis* inoculation of natural soil did not affect soil bacterial beta diversity in the wild LA1589, while changes were observed in the modern Monita. .Feeding by *S. exigua* affected volatile production in both tomato species, but only in LA1589 an effect of soil microbiome was observed. This could be due to differences in the defense strategies between the two species, as LA1589 contains type VI trichomes, while Monita contains the *Mi* resistance gene. For both tomato species, the predator *C. carnea* preferred the sterile soil (SS) treatment, followed by inoculated natural soil (NS+Ri) and finally the non-inoculated natural soil (NS) treatment. Some volatiles were detected that could explain differences in attractiveness, with δ-elemene (LA1589) and 3-hexen-1-ol (Monita) explaining most differences in behavior. Indeed, both δ-elemene and 3-hexen-1-ol have been described as involved in insect resistance. Some *S. exigua* induced volatiles in both tomato species were described as toxic to pests, such as octanal and 2-decanone. Some volatiles were even only present after pest attack, or only induced in one of the tomato species. Other detected volatiles have been described affecting natural enemy behavior. The effect of volatiles on natural enemies may depend on the natural enemy species, as some volatiles could be attractive to one natural enemy and repellent to another. In natural soil, *Rhizophagus irregularis* inoculation enhanced the atractiveness of C. carnea. These differences in *C. carnea* behavior could be caused by differences in AMF colonization as we detected a moderate but significant increase in fungal root colonization in the inoculated natural soil compared to the non-inoculated natural soil. This indicated that *R. irregularis* has a high competitive ability. The reduced attraction of *C. carnea* to natural soil could indicate that the microbial community may promote direct defenses at the cost of indirect defenses, which requires future experiments for confirmation.

In conclusion, this thesis showed how agronomic practices and domestication affected soil bacterial communities, and the ecosystem services they provide, especially those functions related to the accumulation of organic matter in the soil. It also evidenced that soil bacterial and fungal communities of different tomato species and varieties were affected by different parameters. Bacterial communities were influenced mainly by resistance traits, which were generally non-targeted by domestication. In contrast, spatial location had profound effects on root fungal communities as well as aboveground plant traits. However, when location is taken into account, fungal communities were found to affect aboveground plant traits as well. Diving deeper into how

soil communities affect above-ground plant defence, we evidenced that pest attack affected volatile profiles in both wild and modern tomato, with soil treatment only affecting volatiles significantly in wild tomato. We identified different volatiles induced by *S. exigua* attack, some of them potentially functioning as pest or natural enemy repellents. Also, *R. irregularis* inoculation in natural soil increases *C. carnea* attraction, and could therefore be a sustainable method to enhance tomato indirect defenses.

Introduction: Plant domestication affects plant defense below and aboveground

1. History of domestication

Most of our knowledge on domestication initially came from studies of cereals [1]. Less is known about the domestication of vegetable and fruit crops such as tomato, although this area is catching on. Plant domestication and subsequent breeding by humans conducted over the last 12,000 years resulted in the modification of specific plant traits to enhance vegetative or reproductive growth, resulting in phenotypically distinct modern crop varieties [2–6]. Domesticated plants are generally classified as possessing a subset of traits which distinguish them from their wild ancestors such as increased fruit or grain size, more determinate growth and loss of dispersal mechanisms [1,4]. After domestication further selection is performed such as for grain quality, fruit shape or colour [7]. Domestication traits are generally fixed within a crop species and often absent or rare in wild populations of the crop [8]. In contrast, crop improvement traits vary among populations or cultivars of a crop [3].

In many crops, strong genetic bottlenecks occurred during domestication, as early humans used a limited number of individuals of the progenitor species after which only the seeds of the best performing plants were used to produce the next generation [7]. Such bottlenecks obviously reduced genetic diversity in various plant species, and as a result crops are often maladapted in natural environments [3,9–13]. In certain crops, the occurrence of gene flow from related species (crop-wild relatives) compensated this, increasing genetic diversity. Some domesticated crops also underwent a change in reproductive strategy such as from outcrossing to self-fertilizing which affected crop evolution and divergence between crops and their wild relatives [13]. Shifts to self-fertilization are common in fruit and seed crops [12]. Sometimes the domesticated crop has a different ploidy level, which also hampers gene flow.

Determining the history of domestication can be difficult as modern varieties only contain the genetic diversity of the lineages they are derived from [12]. Ancient lineages that were discarded, or wild populations that never participated in the process, are therefore missed. Also, some crop species originate from multiple domestication origins, such as barley, pepper and apricots [14]. Tracing domestication steps is further complicated as domesticates were moved to new environments where they sometimes crossed with local plant varieties [7,15,16].

The effects of domestication on plant pests and diseases have been extensively reviewed elsewhere [17–20], but generally with a focus on plant mechanisms involved in the interactions with a single pest or pathogen species. However, most tri-trophic plant-microbe-arthropod (PMA) interactions are more complex than the sum of individual interactions and are usually coordinated by phytohormone signalling networks [21]. Here we complement this view by discussing PMA interactions in a more ecological framework focussing on how domestication has changed the interactions of plants with beneficial organisms and their potential impact on multitrophic interactions (Figure 1).

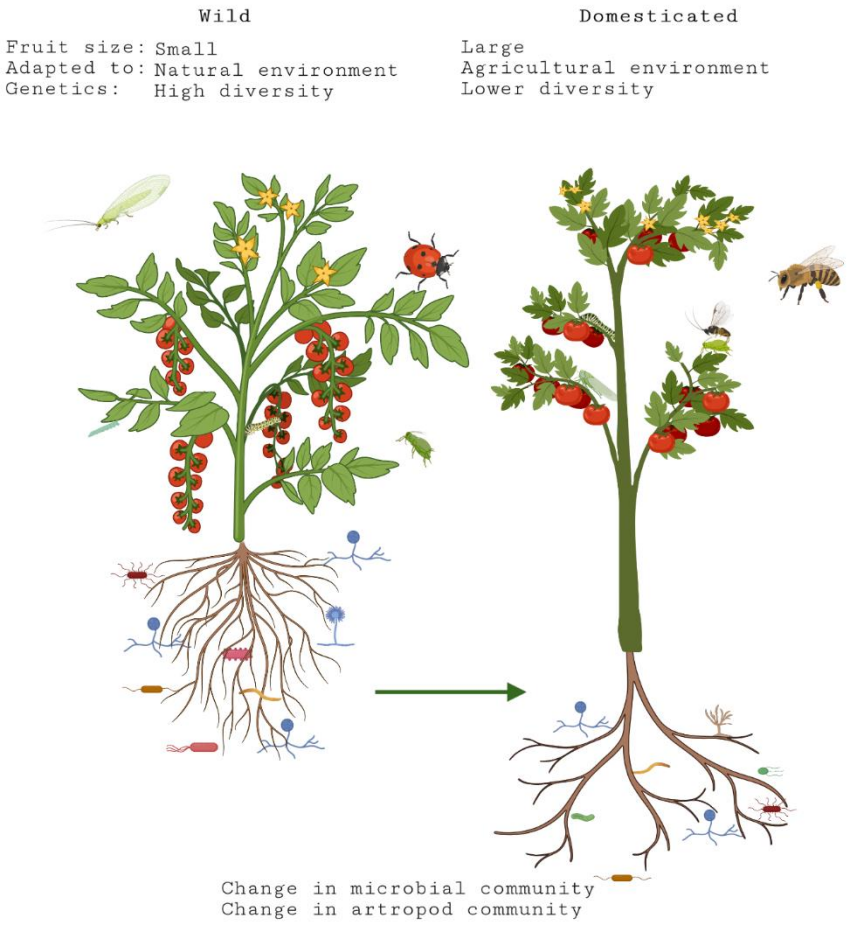

Figure 1: Overview of how domestication and breeding affected plant traits and plant-associated communities of microbes and arthropods. Figure created with Biorender.

On this topic several studies have been conducted in tomato, and therefore it may be considered as a model vegetable crop for studying such interactions. The first part of the introduction is dedicated to the domestication process of tomato. Since domestication reduced genetic diversity, it is often hypothesized that domesticated crops are less resistant to pests and pathogens and invest less in costly interactions with beneficial organisms due to increased use of pesticides and fertilizers [4,17,22,23]. However, this depends on the crop-microbe interaction studied, and examples are given. Thus, we briefly elaborate on how domestication affected plant constitutive defenses. Then, the focus will be on changes in induced direct and indirect defenses aboveground and how this may affect natural enemy attraction. Subsequently, we describe the effect of soil microbial communities (bacteria and arbuscular mycorrhizal fungi (AMF)) on plant defenses and how these interactions were altered through domestication. Combining this, the last part of the introduction discusses plant mediated tri-trophic interactions between belowground and aboveground organisms. Lastly, knowledge gaps in this exciting research field are provided.

2. Tomato domestication

Tomato (*Solanum lycopersicum L.*) originates from the Andean region [10]. The tomato genus contains 13 species, of which only *S. lycopersicum* was domesticated [9,11]. When Europeans arrived to Mexico in the 15th century, large-fruited varieties already existed [24]. Most likely, large fruit bearing mutants were

selected for by early humans from local tomato germplasm [11]. The crop experienced at least one genetic bottleneck as only small numbers of seeds (and thus accessions) moved from the Andes to Central America and from there only few were transported to Europe where it was intensely domesticated in the 18th and 19th century [10,25] . These early cultivars were selected and inherited in small communities and are therefore called heirlooms or landraces [10]. From the 20th century onwards, a large variety of morphologically distinct tomato varieties were developed through plant breeding. Most of the modern varieties are F1 hybrids. New traits generally come from genetic variation in existing cultivars, but increased attention has been placed on introgression of valuable traits from wild relatives to increase the genetic diversity of the tomato germplasm [26].

The wild species *S. pimpinellifolium* and *S. lycopersicum* var. *cerasiforme* (the latter is often referred to as a 'weedy species') are suggested to have played an important role in the early stages of domestication. The role of *S. lycopersicon* var. *cerasifome* in domestication is controversial, firstly considered a mixture of wild and cultivated tomato [27] and later a true taxonomic group with origins in South America, probably in Ecuador [25,28]. Recently, the intermediate stages of tomato domestication were studied in more detail and three distinct populations of *S. pimpinellifolium* and five populations of *S. lycopersicum* var. *cerasiforme* were detected [25]. The study suggests that *S. lycopersicon* var. *cerasifome* diverged from wild tomato over 78 thousand years ago, prior to human presence in the area and therefore likely a result of natural divergence. *S. lycopersicum* var. *cerasiforme* then spread into Peru where it mixed with local *S. pimpinellifolium* populations. Between 10-13 thousand years ago, *S. lycopersicum* var. *cerasiforme* appeared in Mexico after which it spread northwards. Cultivated tomato (*S. lycopersicum L*.) diverged about 7 thousand years ago, after which a portion of this species was taken to Europe.

Wild tomato species contain more variation at the genome level, especially within the self-incompatible (outcrossing) species [10]. Wild tomatoes are genetically variable but with little variation in fruit size and shape while cultivated varieties are phenotypically variable with little variation elsewhere in the genome. However, some phenotypic variation of cultivated tomato is also present in populations of *S. lycopersicum* var. *cerasiforme* [25]. This suggests a wild to domesticate phenotypic change in *S. lycopersicum* var. *cerasiforme* populations in south America, a reversal to wild-like phenotypes when tomato spread northwards and a second conversion to domesticated phenotypes during the transition to cultivated tomato. Fruits and seeds of domesticated tomato species are often much larger than those of their wild relatives [10,11]. For example, in *S. lycopersicon var. cerasiforme* tomatoes weight only a few grams each, while modern tomato varieties may weight up to 1 kg each [11]. Many genes, processes and proteins directly and indirectly influence fruit development, including phloem transport, floral development and male sterility. However, the variation in fruit size and shape among tomato cultivars is influenced mainly by nine major loci [11]. Some of these loci affect only fruit size or shape while extreme changes in either can be caused by the combined action of two or more loci. Many of these loci are co-localised to syntenic regions in the genome in related species, such as eggplant and pepper [29].

3. Domestication effects on plant defense against pests

3.1 Plant defense mechanisms

In contrast to constitutive defenses, which are expressed at all times, induced defenses are only expressed when plants are under attack by pathogens or herbivores. Herbivory, such as feeding and oviposition, triggers direct defenses such as toxins and feeding deterrents, and indirect defenses which are usually associated with volatile production to attract pest natural enemies [31,32]. Plants may even facilitate natural enemies by producing domatia to house arthropods or producing extrafloral nectar where they can feed on

while the pest is absent [33]. Direct defenses are often induced in response to pathogens, which are relatively immobile [30]. Induced direct defenses may be structural to strengthen physical barriers (cell wall thickening, callose deposition), activation of toxic substances such as alkaloids, programmed cell death (the hypersensitive response) to isolate and kill the threat in the case of biotrophic pathogens. In the case of herbivores, induced direct defenses often involve the production of antifeedants such as toxins and inhibitors of digestion, negatively affecting pest growth and/or survival [30]. For example, protease inhibitors are thought to act on proteases in the herbivores gut to reduce digestion of plant proteins and to protect the plant against herbivore proteases [34]. Moreover, defensins and cyclotides are small cysteine-rich proteins that play a role in herbivore defense, hampering nutrient and ion uptake by disrupting membrane integrity [30,35,36]. However, there is some overlap between constitutive and induced defenses. For example, leaf hairs, such as trichomes, may also be induced to produce defensive compounds [30,37].

One of the most immediate responses of plants to herbivory is the induction of herbivore-induced plant volatiles (HIPVs). Once the herbivore associated elicitors have been recognized, plants are able to produce and release a volatile blend that can attract predators or parasitoids [54,62,63]. The HIPV blend varies with the herbivore species, the plant species and genotype, the environmental conditions, the number of herbivore species attacking the plant and the order in which they attack [37,64,65]. HIPV release further differs between herbivore feeding guilds, with chewing and specialist insects inducing more volatiles than sap or cell content feeders or generalists [37]. Each feeding guild was shown to increase different specific volatiles, although no differences were observed in the number of compounds induced [37].

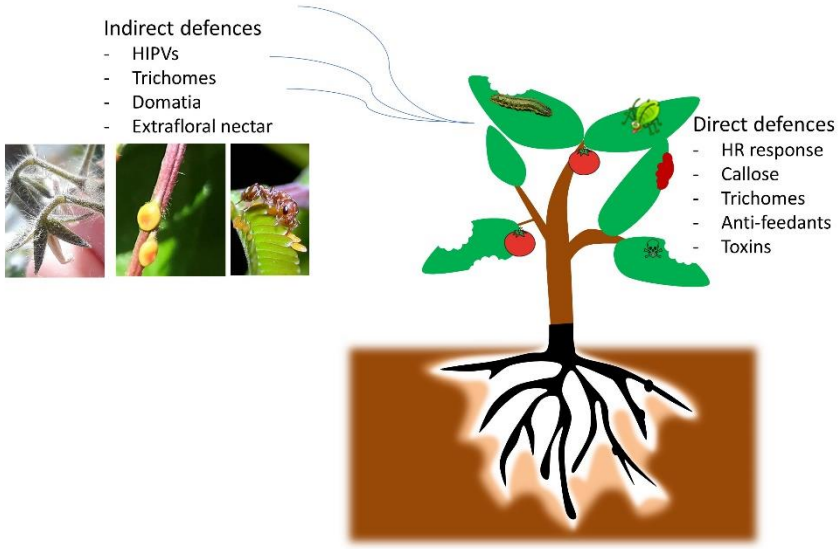

Figure 2: Some examples of direct and indirect plant defenses. Direct defenses can be structural (callose deposition, trichomes) or chemical (anti-feedants, toxins). Indirect defenses can be structural (domatia) and chemical (HIPVs produced by the plant, trichome induced volatiles, extrafloral nectar) as well.

One of the most immediate responses of plants to herbivory is the induction of herbivore-induced plant volatiles as indirect defenses (HIPVs, Figure 3). Once the herbivore associated elicitors have been recognized, plants are able to produce and release a volatile blend that can attract predators or parasitoids [38–40]. The HIPV blend varies with the herbivore species, the plant species and genotype, the environmental conditions, the number of herbivore species attacking the plant and the order in which they attack [41–43]. HIPV release

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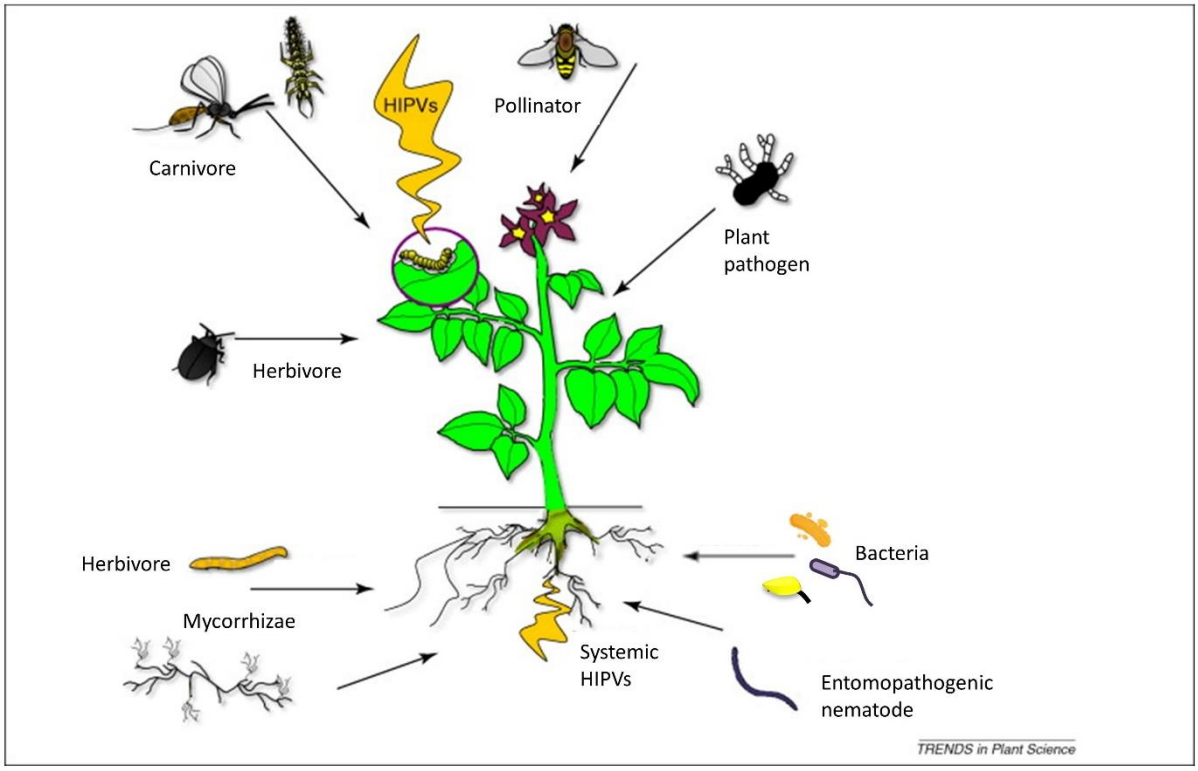

Figure 3: Production of herbivore induced plant volatiles (HIPVs) upon attack by aboveground herbivores and the interacting insects and microbes that potentially can impact the composition of these volatiles. Figure adapted from Dicke and Baldwin 2010.

3.2 Constitutive defenses

Plants evolved sophisticated defense mechanisms to deal with attack by diverse herbivore species. The expanded cultivation area of certain crops allowed additional pests to expand to new environments and generally reduced arthropod diversity [8,19,44,45]. Variation in plant traits, such as architecture, size and production of secondary compounds were shown to play major roles in shaping arthropod communities [8]. Selection for larger organs or increased productivity has been hypothesized to come with a trade-off with defense levels, often resulting in plant species that are more susceptible to pathogen and pest attack than their wild relatives [17–19,45–48]. Indeed, multiple studies related domestication to reduced physical and chemical defenses in plants, probably through the loss of defensive genes and traits as farmers selected for plants with reduced bitterness or toxicity [4,18,46,47,49]. Constraints between fast aerial growth and defense is predicted to account for differences in herbivore resistance, although this is not always the case [49].

Selection pressure for trade-offs between resistance and tolerance are expected to be higher in modern cultivars as they have usually been protected from pests using phytosanitary products. For example, breeding crops under the protection of pesticides may leave crops vulnerable as tolerance and resistance mechanisms may be lost in the process [45]. Herbivores may also convert to pests through crop cultivation. For instance, when a mixed vegetation of various wild hosts is replaced by an abundant crop host, a generalist herbivore may reach pest-level populations even when doing less well on an individual level.

Selection for one trait may impact direct and indirect chemical defenses mostly due to linkage or pleiotropic effects. Since resistance traits are potentially costly for the plant, they may have been selected against in favor of other traits for example increased palatability or increased nutrient content or reproductive growth [19,50]. Some authors even concluded that breeding for yield is often incompatible with breeding for strong resistance to pests and pathogens [19,45,46]. Moreover, modern crop varieties often provide a better food source for insects than wild varieties, making them more attractive. Therefore, arthropods often perform better on modern varieties [42].

Although correlation effects between domestication and plant defense have been found in several studies, evidence for the contrary have been found as well. For example, Turcotte et al., emphasized that the impact of domestication on plant resistance is not always consistent since allocation of resources is not limited by growth-defense trade-offs, but also to other resource sinks such as mutualistic associations or to abiotic stress tolerance [49]. Also, costs and benefits of defense are not absolute but depend on (local) ecological interactions. For example, decreased levels of defense chemicals in domesticated crops may improve development of both herbivore and parasitoid [51]. Also, the enlargement of plant structures, such as seeds, may disrupt parasitoid-herbivore interactions as herbivores could find refuge in the enlarged seed.
Although domesticated plants are generally assumed to have weakened chemical defenses, Gaillard et al. found that this can depend on the plant tissue [52]. The concentration of benzoxazinoid, the main direct chemical defense in maize, tended to be higher in leaves of wild teosinte, whereas the reverse was true for the roots. Also, the reduced insect performance on teosinte compared to cultivated maize was higher for generalist than specialist insects. In this regard, they also pointed out that weakened broad spectrum defenses in crops may have driven the development of specialist pests.
Similarly, differences in pest resistance and tolerance may depend on the plant varieties used, as intraspecific variation may be equal to or even higher than interspecific variation, which was found for maize and tomato [50,53]. In the study of Ferrero et al. in tomato, wild (*S. pimpinellifolium*, *S. habrochaites*), weedy (*S. lycopersicum* var. *cerasiforme*) and cultivated (*S. lycopersicum* var. *lycopersicum*) species were used, adding up to 23 tomato varieties [53]. The cultivated varieties included both landraces and modern cultivars. Variation was observed among the responses of closely related wild tomatoes, weedy species and landraces towards three different types of herbivores (aphids, caterpillars and nematodes). Nevertheless, the level of tolerance was generally higher in wild and early domesticated varieties. For instance, insect resistance in tomato is partly based on plant compounds present in their glandular trichomes which are widely present in wild relatives of tomato [37,54–56]. Modern tomato varieties often lack glandular trichomes, thereby reducing direct defenses against insects. Indeed, tomato varieties lacking trichomes had lower defense against aphids than varieties with trichomes [53]. Resistance or tolerance to one pest was independent of resistance or tolerance to another pest, which was confirmed in other wild-domesticated crop pairs [49]. For example, a wild tomato species showed high resistance to caterpillars but low resistance to nematodes, while a modern cultivar showed high nematode resistance but low resistance to aphids and caterpillars [53].

3.3 Inducible direct and indirect defenses

Rasmann et al. studied the trade-off between constitutive and induced resistance against herbivores finding a negative correlation between them across seven accessions of *Arabidopsis thaliana*, especially for the generalist herbivore *Spodoptera littoralis* [57]. Other examples of trade-offs between constitutive and induced resistance were found both within and across species [58,59]. In cranberry, modern varieties show reduced constitutive defenses compared to wild varieties, while induced direct resistance was not altered

[60]. However, no consistent correlation of induced resistance and defense gene induction was evidenced, indicating that herbivore defense does not depend on individual genes or molecules, but rather on a complex interactive network.

Indirect defenses are not driven by traits directly affecting the herbivore but by the ability of a plant to attract natural enemies. However, natural enemies can be affected by direct defenses both by exposure to toxins ingested by herbivores or by reduced herbivore growth [61,62]. Also, different volatiles were found to be induced in leaves and roots, suggesting that different mechanisms are responsible for volatile induction in different plant compartments. However, in the same study, some resistance mechanisms are genetically linked. Insect resistance in the leaves was positively correlated with a plants capacity to release volatile organic compounds (VOCs).

Several crops have lost secondary chemistry during domestication, either through direct selection (increased nutrient content, improved taste) or because larger organs or increased yield diluted defense levels [17,47,50]. For example, Moreira et al. found that constitutive and induced levels of glucosinolates were reduced in domesticated *Brassica oleracea* [17]. Similarly, Maag et al. found that levels of 1,4-benzoxazin-3-ones (BXs) decrease faster over three maize growth stages in cultivated lines than landraces and teosintes [50]. However, this reduction did not explain differences in defense levels. In tomato, wild varieties containing trichomes show large differences in the production of acyl sugars and flavonols [54]. Similarly, Batyrshina et al. found that whereas wild wheat varieties contain higher physical defense (trichomes), domesticated wheat had higher chemical defense (benzoxazinoids production), with chemical defense being more effective against aphids [63]. Furthermore, they showed that wild wheat contains higher levels of primary metabolites (amino acids, organic acids and sugars) than the domesticated varieties, which could explain their higher susceptibility to aphids.

Furthermore, selection for certain phenotypes may change the expression of traits we are initially not aware of, such as the interaction of plants with beneficial insects above ground. For example, tomato glandular trichomes were shown to hamper host finding by the biocontrol parasitoid of the potato tuber moth [64]. Reduced capacity to interact with insects and reduced volatile emissions as a result of domestication have been shown in maize, cranberry and lupin [19,46]. Mutyambai et al. found that commercial maize varieties do not release VOCs in response to the egg parasitoid *Chilo partellus* whereas the wild ancestor teosinte and maize landraces do [39]. Furthermore, differences between teosinte and landraces were observed regarding the quality and quantity of induced volatiles. Rasmann et al. even found a reduction of root volatiles in modern maize varieties [65]. A study on cranberry showed compromised indirect plant defense against caterpillars in modern species due to reduced induction of sesquiterpenes and jasmonic acid [60].

However, in other studies no effect of domestication on induced indirect defenses was found. For example, a study by De Lange et al. shows that a modern maize variety emits a qualitative and quantitatively distinct odor pattern after herbivory compared to two teosinte species [48]. However, when considering a larger variety of maize and teosinte species such a difference could not be verified [66]. This could be since most genetic diversity of teosinte has been maintained in modern maize varieties [50]. Furthermore, a meta-analysis comparing volatile release across several plant species did not show an effect of domestication on total volatile emission but it did show an effect on specific compounds [42]. For example, sesquiterpenes and green leaf volatiles (GLVs) were shown to often be increased in domesticated tomatoes compared to wild plants. Domestication seems to reduce blend complexity, but it is not clear whether this reduction affects its attractiveness to natural enemies, such as parasitoids.

4. Effects of domestication on soil microorganisms

4.1 The plant rhizosphere

Soil microorganisms play a major role in several ecosystem functions, i.e. regulating organic matter mineralization and biochemical nutrient cycles, contributing to humus formation, promoting plant growth, modifying plant defense and resistance to stresses and controlling
the development of various plant pathogens [67–70]. Because of their taxonomic richness, they also act as an environmental buffer retaining soil functionality even when their biological structure is perturbed, thus promoting resilience[71]. There is a high level of functional redundancy within species rich soil communities, with some 'keystone species' with unique roles in specific soil processes [71]. Keystone species can be beneficial and pathogenic. Whereas beneficial keystones increase microbiome diversity, pathogenic keystones tend to reduce microbiome diversity [72]. Soil microbes can also increase plant defense against above - and belowground herbivores (Figure 4) [22,68,73,74]. Plants even partly rely on their rhizosphere microbiome as a first line of defense [68]. Conversely, plant defense pathways can shape the rhizosphere microbiome [75]. As a result, plant growth may control rhizosphere microbial diversity directly or indirectly [76]. Moreover, many soil-borne microbes have the ability to induce plant resistance in systemic tissues, a process termed induced systemic resistance (ISR). In contrast to other types of induced resistance, ISR is induced by non-pathogenic microbes and is usually mediated by defense priming, accelerating plant defense activation upon pathogen attack [77].

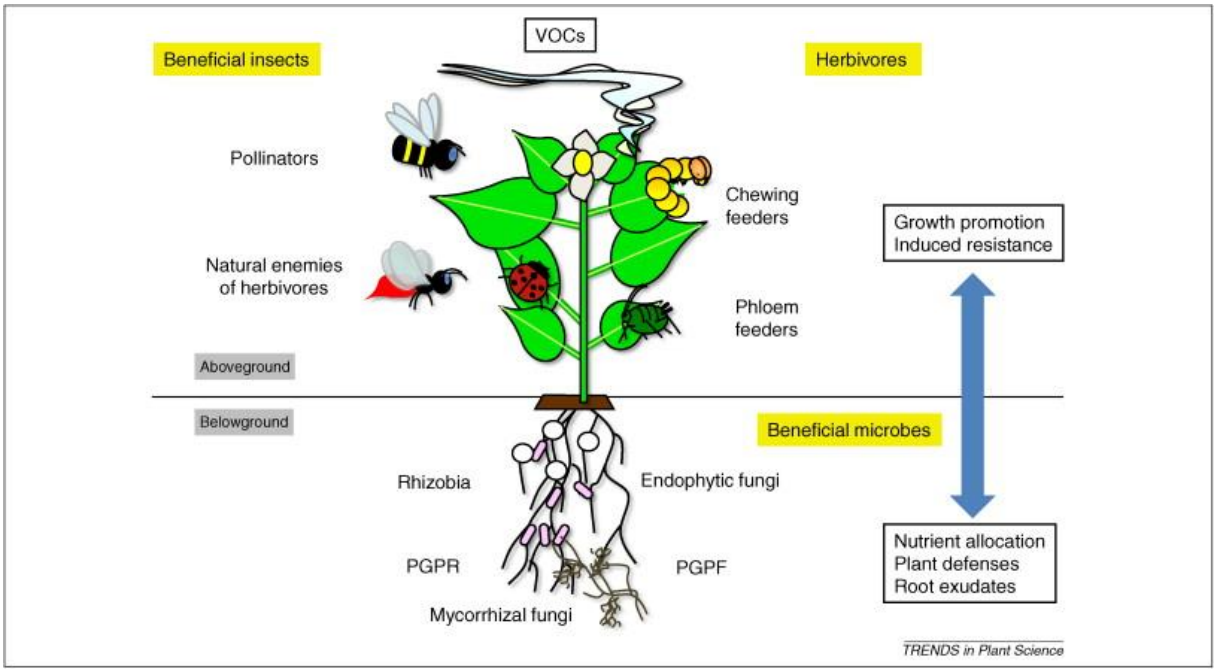

Figure 4: Overview of interactions between beneficial microbes belowground and herbivores and beneficial insects aboveground. Different soil microbes induce changes in the plant that promote plant growth and induce resistance, for example producing volatile organic compounds (VOCs) that attract natural enemies and pollinators. Aboveground herbivores affect microbial communities belowground through changes in nutrient allocations, plant defenses and root exudate composition. Figure from Pineda et al., 2010.

An important group of ISR inducing microbes are arbuscular mycorrhizal fungi (AMF). These soil fungi are obligate biotrophs that form a mutualistic symbiosis with 80% of land plant species [73,78]. The symbiosis

between plants and AMF is older than 400 million years and one of the major benefits for the plant is improved nutrient acquisition, mainly of phosphorous but also nitrogen and various micronutrients in exchange for plant carbohydrates [79–81]. Moreover, the symbiosis increase plant defense against a diversity of stresses and generally provide protection against various belowground (e.g. nematodes, root chewers) and aboveground (mainly necrotrophs and generalist leaf chewers) attackers, while biotrophic pathogens and viruses might be positively affected [82–85].

AMF may also play a role in indirect defenses such as the attraction of natural enemies [86]. Indeed, AMF symbiosis induce changes in e.g. plant root architecture, root and shoot metabolic profile and HIPV production responsible for these positive effects [87].

Plants are able to influence their microbiome by releasing a blend of chemical signals into their environment which can positively or negatively affect plants or members of the microbiome [88]. For example, root exudates are comprised of allelochemicals which allow a plant to establish a soil or rhizosphere microbiome. The nature of the interaction and the influence of the plant over its microbes could benefit the plants ability to grow or defend itself [89,90]. Apart from the plant, the environment and abiotic factors may determine microbial diversity [91]. Environmental stress such as drought or salinity, impact plant development and the ability to interact with its microbiome [68,88]. For example, abiotic stress impacts hormone pathways with consequences for root exudate composition and hence can affect microbial community diversity and function in the rhizosphere [92]. Thus, it remains difficult to determine which changes in microbiome composition are caused by the plant, the microbiome or the environment due to the complex nature of interactions between them. Likely, changes in microbiome composition are a result of a combination of factors, rather than one specifically.

4.2 Domestication affected plant-microbe interactions in soil

Next to affecting trophic interactions with herbivores and natural enemies aboveground, domestication also impacts plant-microbe interactions in the soil [22]. The plant rhizosphere is defined as the plant-root interface, which is inhabited by a unique population of microorganisms, such as bacteria and fungi. It contains a large microbial diversity originating from the surrounding bulk soil [31,68,93].

Plant have co-evolved with their rhizosphere community. In agriculture, the benefit of symbiosis is expected to be decreased since the resources that symbionts may provide are freely available through the use of fertilizers and other soil amendments [94]. Indeed, Martín-Robles et al. found that domesticated herbaceous crops often only benefit from AMF colonization under P limited conditions [95]. Crops may also reduce symbiosis if the costs of symbiosis compete with allocation to growth and reproduction. Indeed, domestication reduced dependence on *Rhizophagus* in soybean and rhizobia in legumes [76,96]. Also, domestication has decreased AMF colonization in crops such as breadfruit and flax [95,97–99]. However, it has been proposed that modern annual cultivars are generally less intensely colonized by AMF, but more responsive to their colonization [98]. A domestication independent effect has been shown in tomato, wheat, maize and barley [100–103].

Even though crops have not been directly selected for their rhizosphere microbiome, the microbiome structure might still have been altered in the process of breeding and selection [89,101]. In many crops, agricultural varieties show reduced bacterial diversity in the rhizosphere, resulting in plants with reduced mutualistic capacity [10,11,22,93,104]. Domestication effects on rhizosphere microbiomes have been found in maize, barley and soybean, although the affected bacterial groups differ between plant species [76,89,93]. Comparing differences in bacterial community structure in roots and rhizosphere of bean, barley, lettuce,

Arabidopsis and rice revealed that modern varieties tended to be enriched for Proteobacteria and Actinobacteria while Bacteroidetes were enriched in the wild relatives [105,106]. However, rice domestication had a bigger influence on fungal communities than on bacterial communities [107]. A study in bean shows that changes in root morphology during domestication altered rhizobacterial communities [108]. In maize, a core microbiome has been maintained during domestication, but abundance and activity of bacterial and fungal communities differed, with most wild varieties containing higher bacterial abundance and diversity [109]. This was confirmed by Lei et al. who found small differences in bacterial diversity in a field experiment across various plant taxa [105]. Also, relative taxa abundance in the rhizosphere of sorghum and sunflower varies little during plant development and compared to bulk soil, although some phyla and families were enriched in the rhizosphere [110]. Brisson et al. studied 10 maize accessions of three genetic groups (teosinte, inbred maize lines and modern hybrids) which showed a small amount of microbial families (bacteria and fungi) differing between groups [111]. Interestingly, a greater impact of modern hybrid development on rhizosphere communities than initial domestication was discovered, with both microbial diversity and microbial network structure showing a greater overlap between teosinte and inbred plant groups than between either and modern hybrids [111]. Furthermore, a higher bacterial diversity was discovered in the distal rhizosphere compared to the proximal rhizosphere [111]. The fungal community, however, was more diverse in the proximal rhizosphere.

Furthermore, whereas the rhizosphere fungi of wild soybean contained a diversity of potential functions, the functions of rhizosphere fungi in cultivated soybean were mainly related to nutrient uptake [76]. Chang et al. also showed that pathogenic soil fungi have a higher impact on cultivated than on wild soybean, revealing reduced resistance during domestication [76].

The effect of soil type in shaping microbial communities was also shown in maize [5]. Similarly as for bacteria, soil type is a major factor driving the community of AMF and pathogenic fungi in soybean [76]. Field conditions were found to affect mycorrhizal responsiveness with modern cultivars showing reduced growth in the absence of mycorrhizal fungi [98]. Overall, agricultural practices and soil type are thought to be main drivers of rhizosphere microbial communities in agricultural settings, while abiotic factors and plant species are more important in natural systems [110].

5. Tri-trophic interactions

Changes in a plant phenotype or in its metabolic profile during domestication and breeding may have unexpected effects on tri-trophic interactions between plants, herbivores and natural enemies [115]. Organisms occurring below and above ground may affect each other directly or indirectly by using a resource required by the other organism or by activating plant defense mechanisms [30]. Furthermore, interactions with other trophic levels may also shift when multiple attackers are involved [30]. For example, the simultaneous attack of below and aboveground herbivores was shown to alter the produced HIPV blend, when compared to plants being attacked by only a single herbivore species [41]. Apart from attackers, beneficial microbes may also impact the third trophic level by improving the attraction, attack rate and performance of parasitoids [86,116]. In fact, inoculation of specific organisms, such as the fungal endophyte *Fusarium solani* in tomato, may increase attraction of predators [117]. Moreover, other non-pathogenic soil microbes such as mycorrhizal fungi and certain rhizobacteria were shown to increase attraction of predatory mites to bean plants and parasitoid wasps to *Arabidopsis* [118,119]. However, non-pathogenic rhizobacteria can also have negative effects on tri-trophic interactions by reducing parasitoid attraction [120]. Likely, these differences may be related to the biotic and abiotic context in which the plant and interacting organisms are growing [74,121].

Additional complexity is derived from the presence of microorganisms on the plant surface and inside herbivores, or the potential interaction with beneficial insects (i.e. predators, parasitoids and pollinators). Phyllosphere microorganisms (mainly bacteria, but also fungi, viruses, archaea and algae) are present both on the surface of plant organs such as leaves, stems, buds and flowers or inside plant tissues [122]. Some of the benefits from these interactions include pathogen or pest suppression. For example, *Sphingomonas* bacteria were found to suppress disease symptoms and pathogen growth of *Pseudomonas syringae* and *Xanthomonas campestris* in *Arabidopsis* [123]. Furthermore, fungal or bacterial endophytes living inside asymptomatic plant tissues may change plant volatile production which, in turn, increases natural enemy attraction or hyperparasitoid behaviour. This effect was shown in grasses where the endophyte *Epichloë* provide both direct defense (alkaloid production) and indirect defense by attracting more aphid predators [124]. Endophytic fungi may also benefit the plant by translocating nitrogen from killed herbivores to the plant through their hyphae [125]. Microbes were also shown to be present inside floral nectar, extrafloral nectar and honeydew, food sources for many adult parasitoids [122]. These microbes may alter the composition of the nectar thereby contributing to its flavor and scent which could affect plant-insect interactions [122,124]. For example, pollinators may be negatively or positively affected, and changes in parasitoid attraction and survival have been reported [122]. Similarly, the honeydew bacteria Staphylococcus sciuri was shown to produce chemicals that increase attraction and oviposition of hoverflies to pea aphid infested plants [126].

It should be noted that most studies regarding tri-trophic interactions have been performed using single organisms instead of whole microbiomes. However, to be able to translate results to field situations, it is important to consider whole microbiomes in the study of tri-trophic interactions between microbes, plant and insects [127]. For example, *Nerium oleander* plants growing in soils amended with a vermicompost-borne microbiome showed a different HIPV blend after aphid attack compared to those growing in sterilized soil [128]. Furthermore, the vermicompost treated plants were more attractive to the parasitoid *Chrysoperla carnea*. Considering the many factors potentially influencing these multitrophic interactions, it is relevant that in the last years some studies are addressing tri-trophic interactions under field conditions [129–131].

6. Concluding remarks and future perspectives

In summary, crop domestication alters interactions of plants with herbivores and their natural enemies at all trophic levels by 1) changing the nutritional value and defensive properties of the plant, thus affecting herbivore host selection and influencing herbivore fitness and abundance; 2) altering arthropod communities, 3) influencing the performance of parasitoids, predators and pollinators and 4) altering the microbial communities associated to the plant, as these changes may modulate plant interactions with the herbivores and enemies [8,18,132]. Although some trends are described, for example that changes in volatile production associated to domestication often mean that modern varieties are less well protected, changes are not always consistent in that direction. When comparing a small number of wild and domesticated species, a domestication effect might be found, whereas studies comparing a wider range of accessions often point to high level of intraspecific variation.

So, taken together, the relation between crop domestication and the plants interactions with beneficial (including the plant and soil microbiota, and pest natural enemies) and deleterious organisms (such as insect herbivores) is very complex, and we have highlighted some research gaps in this field of research. First of all, most studies were performed on staple crops such as weed and maize. Secondly, studies are often comparing few varieties of a certain plant species. Thirdly, domestication effects on plant defense are often studied

using single or few herbivore or pathogen species. Lastly, experimental conditions may influence the observed natural enemy attraction, so that the context dependency of the outcome is generally not properly addressed.

7. Objectives

The general objective of this thesis was to study how tomato domestication influenced belowground microbial communities and further explore the effect of these belowground communities on aboveground indirect defenses in tomato. To investigate this, the main objective was divided into two, the first objective being elaborated in chapter 1-3 and objective 2 in chapter 4.

Objective 1: Top down effects of tomato domestication on plant microbial communities

Chapter 1: Resistance and not plant fruit traits determine root-associated bacterial community composition along a domestication gradient in tomato.

Chapter 2: Tomato domestication affects potential functional molecular pathways of root-associated soil bacteria.

Chapter 3: Arbuscular mycorrhizal fungal community drive fruit development and pathogen incidence in field cultivated tomatoes

Objective 2: Bottom up effects of soil microbial community on tomato defenses

Chapter 4: Trophic interactions between soil microbiome, volatiles induced by Spodoptera exigua and behavior of the predator Chrysoperla carnea after inoculation with Rhizophagus irregularis in tomato plants.

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Objective 1: Top down effects of tomato domestication on plant microbial communities

1. General methods

1.1 Field experiment

Seeds of 27 *Solanum lycopersicum* Mill., *S. habrochaites* and *S. pimpinellifolium* accessions were obtained from the Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC) germplasm bank, based on their degree of domestication (Table 1). On 19 April 2018, 10 one-month-old seedlings per variety, 270 in total, were randomly distributed in an experimental field in the IHSM La Mayora (Málaga, Spain, 36.77° N, 4.04° W). The field soil is classified as a eutric regosol soil (1). Plants were exposed to the natural community of insects and became naturally infected by two common viral diseases transmitted by whiteflies: tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV). Plants were grown until 16 July 2018, when they were in the fruiting stage. The aboveground biomass was weighted, the number of tomatoes produced counted and the total tomato fruit weight measured.

On 28 June 2018, plant symptoms were scored according to the symptom severity scale described by Kone et al. (2), which runs from 0 = no disease symptoms to 10 = severe leaf distortion/necrosis/narrowed or shoes-string leaf. The frequency of virus infection per tomato variety was performed using tissue blot hybridisation methodologies described by Navas-Castillo et al. and Fortes et al. (3,4) for TYLCV and ToCV, respectively.

Table 1: Domestication degree per tomato variety. Those varieties that were classified differently in chapter 2 are depicted after the / sign.

Tomato variety	Species	Domestication degree
H. de Toro	*Solanum lycopersicum*	modern
BC5	*Solanum lycopersicum*	modern
Cazorla	*Solanum lycopersicum*	modern
Com 1	*Solanum lycopersicum*	modern
Com 2	*Solanum lycopersicum*	modern
Com 3	*Solanum lycopersicum*	modern
Com 4	*Solanum lycopersicum*	modern
Edkawy	*Solanum lycopersicum*	modern / landrace
Flor Baladre	*Solanum lycopersicum*	modern / landrace
Kalohi	*Solanum lycopersicum*	modern / landrace
LA1589	*Solanum pimpinellifolium*	wild
LA1777	*Solanum habrochaites*	wild
Marmande	*Solanum lycopersicum*	modern / landrace
Mellilero	*Solanum lycopersicum*	modern / landrace
MEX 3	*Solanum lycopersicum*	modern / landrace
MEX 33	*Solanum lycopersicum*	modern / landrace

MEX 89	*Solanum lycopersicum cerasiforme*	early domesticated / landrace
MM	*Solanum lycopersicum*	modern
Monita	*Solanum lycopersicum*	modern
Moruno	*Solanum lycopersicum*	modern / landrace
PE55	*Solanum lycopersicum*	early domesticated / wild
Penjar	*Solanum lycopersicum*	modern / landrace
Pera	*Solanum lycopersicum*	early domesticated / landrace
Periana	*Solanum lycopersicum cerasiforme*	modern
PI134418	*Solanum habrochaites*	wild
SanMarzano	*Solanum lycopersicum*	modern
T0 937	*Solanum pimpinellifolium*	wild

1.2 Soil processing

1.2.1 Soil collection

The soil attached to the main and secondary roots was taken by shaking. The root-associated soil from each plant was placed in separate plastic bags and kept at 4 °C until laboratory analyses (60 days). Then, samples from each variety were pooled and ground together using a mortar and pestle and sieved twice (2-mm mesh) and immediately stored at −20 °C until molecular analyses were performed. Freezing is often considered the best option to store soil samples for microbiome analysis. However, research shows that storage at 4 °C for up to 30 days has a minimal effect on microbiome composition (5–7).

1.2.2 Soil chemical characterization

For each variety, two replicates of air-dried field soil samples were used to determine chemical properties at the Scientific Instrumentation Service, EEZ-CSIC, Granada, Spain. Total N and soil organic C were determined with the aid of the Leco-TruSpec CN elemental analyser (LECO Corp., St Joseph, MI, USA). Total mineral content was determined by the digestion method with HNO3 65%:HCl 35% (1:3; v-v) followed by analysis using inductively coupled plasma optical emission spectrometry (ICP-OES) (ICP 720-ES, Agilent, Santa Clara, CA, USA).

1.2.3 Molecular analyses of soil bacteria

DNA was extracted separately from eight 1 g soil subsamples using the bead-beating method with the PowerSoil® DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA) according to the manufacturer's instructions. Extracts of four subsamples were pooled and further concentrated at 35 °C to a final volume of 20 µL using a Savant Speedvac® concentrator, resulting in two replicates per variety. Bacterial communities were analysed using Illumina MiSeq, and to determine the bacterial communities, we amplified the V3-V4 hypervariable regions of the 16S rRNA gene using the ProV3V4 primers with the following sequences: 5' CCTACGGGNBGCASCAG 3' and 5' GACTACNVGGGTATCTAATCC 3' (8,9). The amplified region was approximately 464 bp. The products were sequenced on the Illumina MiSeq platform using a 2 × 250 nucleotide paired-end protocol (genomic facilities of the López-Neyra Institute of Parasitology and Biomedicine, IPBLN-CSIC, Granada, Spain). To minimise amplification of mitochondria and chloroplasts, blockers were used (9). Between the two PCR steps, amplicons were purified and after the second PCR step, amplicons were pooled in an equimolar manner.

SEED2 was used for the initial steps of processing the resulting sequences (10). First, we merged forward and reversed sequences. Then, sequences containing ambiguous bases (N) and with a quality score below

30 were removed. Primer sequences were removed and sequences trimmed to 400 bp. Afterwards, the sequences were clustered into operational taxonomic units (OTUs) using the UPARSE method by setting the OTU radius to 3%, so selecting sequences at 97% similarity. OTUs with just one read were removed together with chimeric sequences. Finally, a consensus sample x OTU matrix was prepared and the most abundant sequence per OTU was selected as representative. Taxonomy was assigned to each OTU using the classify.seqs algorithm in mothur software together with the SILVA database version 132 (11,12). At this stage, no archaea were detected in the samples.

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Chapter 1: Resistance and not plant fruit traits determine root-associated bacterial community composition along a domestication gradient in tomato

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Abstract: Soil bacterial communities are involved in multiple ecosystem services, key in determining plant productivity. Crop domestication and intensive agricultural practices often disrupt species interactions with unknown consequences for rhizosphere microbiomes. This study evaluates whether variation in plant traits along a domestication gradient determines the composition of root-associated bacterial communities; and whether these changes are related to targeted plant traits (e.g., fruit traits) or are side effects of less-often-targeted traits (e.g., resistance) during crop breeding. For this purpose, 18 tomato varieties (wild and modern species) differing in fruit and resistance traits were grown in a field experiment, and their root-associated bacterial communities were characterised. Root-associated bacterial community composition was influenced by plant resistance traits and genotype relatedness. When only considering domesticated tomatoes, the effect of resistance on bacterial OTU composition increases, while the effect due to phylogenetic relatedness decreases. Furthermore, bacterial diversity positively correlated with plant resistance traits. These results suggest that resistance traits not selected during domestication are related to the capacity of tomato varieties to associate with different bacterial groups. Taken together, these results evidence the relationship between plant traits and bacterial communities, pointing out the potential of breeding to affect plant microbiomes.

Keywords: breeding; microbiomes; rhizosphere bacterial communities; tomato domestications; traits

1. Introduction

Over time, agricultural techniques have changed to meet the growing demand for food and agricultural products of the world population. Agricultural productivity has increased over the last century through, amongst others, improved varieties and increased use of agrochemicals, leading to environmental issues, weakened cropping systems and increased demands for sustainable agriculture [1].

The plant rhizosphere contains a large diversity of microorganisms that are involved in multiple ecosystem services, including an increase in plant nutrition and disease suppression [2]. The composition of the plant-associated microbiome is determined by the interplay of the host plant characteristics and the surrounding soil conditions [2–4]. Plants structure their microbiome through the release of a specific blend of exudates, the composition of which depends on the plant species or variety, being phylogenetically conserved, even at the genotype level [5,6]. Conversely, soil microorganisms affect a variety of host plant traits, including nutritional content and morphology, as well as activating defense pathways and the emission of plant volatile organic compounds [2,5,6], thereby altering the interactions with insect herbivores or plague enemies aboveground [7].

Through domestication, modern plant varieties have been developed with different fruit shapes, colours and sizes adapted to consumer preferences [8,9]. Indeed, in many crops, breeding has focused on phenotype, such as fruit size and yield [9,10], and other traits favouring resistance and defense have been left aside, yielding crops more susceptible to pests and diseases [11–13]. As an example, between the studied traits that could impact plant resistance, glandular trichomes of tomato cultivars have been seen to provide refuges for pests that hamper host finding by parasitoids [14]. Moreover, reduced volatile emissions due to domestication have been shown in maize, cranberry and lupin [15–17], with potential effects on pest repulsion/attraction. In a previous field study evaluating the response of 23 tomato accessions to important agricultural pests, higher tolerance levels were evidenced in wild and early domesticated accessions than in modern ones, although the differences could not be linked to the phylogenetic distance between accessions [11]. The effects of domestication on plant traits are, thus, complex, and a diversity of genes and gene sets have been associated with domestication (reviewed in [18]). The reduced genetic and associated trait

diversity in domesticated varieties could indirectly impact ecosystem services, such as pest control by natural enemies [14,19,20]. Indeed, genetically diverse fields harbour diverse insect populations, including natural enemies, thereby improving biological pest control [19,20].

Despite this knowledge on domestication effects, the potential role of plant-associated microbiomes in plant resistance or tolerance to diseases has been overlooked. It is known that in many crops such as tomato, domestication and breeding together with increased use of pesticides and fertilisers have resulted in varieties with reduced investment in costly beneficial plant–microbe interactions below and aboveground [1,6,7,9,14,21]. Genetic variation between varieties affects morphological traits such as root growth, architecture and exudate composition, which may impact microbiome assembly [21]. It is then assumed that, despite the lack of studies on plant-associated microbiomes along the domestication process, humans have potentially altered microbiome compositions by, e.g., altering plant metabolic activity [12,23,24].

There are potential links between plant fruit traits (selected during breeding) and the degree of domestication with associated soil microbiomes. Studies in pear and pepper have shown that varieties with large fruits or high fruit sets divert more photosynthates to growth than smaller fruited varieties [22,23], potentially altering the release of exudates through roots. Modern cultivars also differ in root architecture compared to their wild relatives, and the consequent changes in root exudation profiles may also impact rhizosphere community composition [6]. In general, modern cultivars have shallower roots due to readily available macronutrients and water in agricultural fields and higher exudation of simple sugars [24]. A study comparing wild and modern bean varieties showed that differences in root length partly explains the divergence in rhizobacterial communities [25]. Since resistance traits are not very specific and often involve changes in metabolite profiles at the root or systemic level, we expect that soil-associated bacterial communities are affected to a wider extent by these traits than those related to fruit. Furthermore, resistant plants are expected to recruit microbes to alleviate stress [21].

In this study, we used 18 tomato varieties with the aim to: (i) determine how root associated bacterial community composition is influenced by domestication, (ii) reveal trends in tomato traits during domestication by differentiating between fruit and resistance traits, and (iii) explore to what extent these traits are associated with root-associated bacterial community composition. We expect that crop domestication caused differences in resistance and fruit traits across tomato varieties, with resistance traits having a higher impact on bacterial community composition. Knowledge on the extent to which root-associated bacterial microbiomes covary with targeted (fruit) and untargeted (resistance) domestication traits will provide insights into the potential consequences of breeding for plant microbiome composition.

2. Results

2.1. Plant domestication influence soil bacterial communities

Figure 1 shows the relative bacterial abundance of the tomato root-associated soils based on the 16S rRNA gene in all 27 tomato varieties. Two main bacterial classes, Alphaproteobacteria and Actinobacteria, dominated the total bacterial community with no differences observed between plant groups. Minority phyla such as Acidobacteria ($F = 7.152$, $p = 0.002$) and Gemmatimonadetes ($F = 4.720$, $p = 0.013$) were significantly less represented in the rhizosphere of wild tomato species than in tomato landraces and modern commercial cultivars. At the family level, the relative abundance of the *Gemmatimonadaceae* ($F = 4.133$, $p = 0.022$), *Microbacteriaceae* ($F = 5.419$, $p = 0.007$), and *Streptomycetaceae* ($F = 4.752$, $p = 0.022$) families decreased, while *Sphingomonadaceae* ($F = 7.887$, $p = 0.001$) increased in wild tomato relatives. Again, no differences between tomato landraces and modern commercial cultivars were detected.

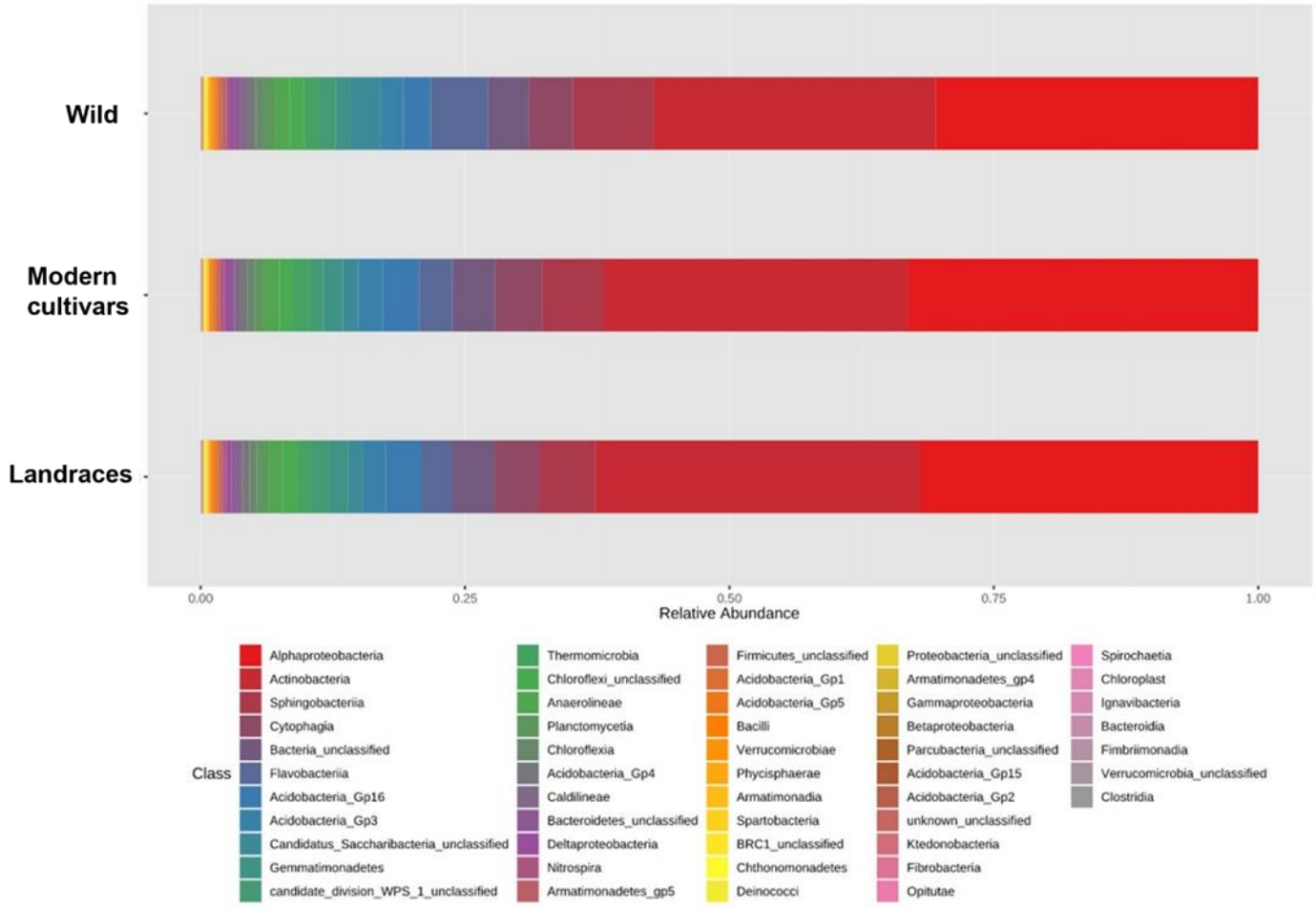

Figure 1. Relative abundance of bacteria of tomato rhizosphere soils. Wild: wild tomato related species; Landraces: tomato landraces; Modern: modern commercial cultivars.

The relative abundances of Acidobacteria_Gp16_unclassified (F = 3.701, p = 0.031), *Hyphomicrobiaceae* (F = 6.736, p = 0.002), and *Nocardioidaceae* (F = 4.179, p = 0.021) were different between wild and commercial cultivars, while landraces had intermediate values, generally not differing from the other two groups.
Linear discriminant analysis (LDA) at the genus level showed *Pedobacter* (*Sphingobacteriaceae*), *Rodococcus*, *Skermanella* and the proteobacterium *Microvirga* to be mainly responsible for the differences between the three tomato clusters (Figure *2*). In addition, minor changes in bacterial diversity were observed at the OTU level (Table 1), as indicated by a significant decrease in the evenness of crop wild relatives (F = 6.623, p = 0.003).

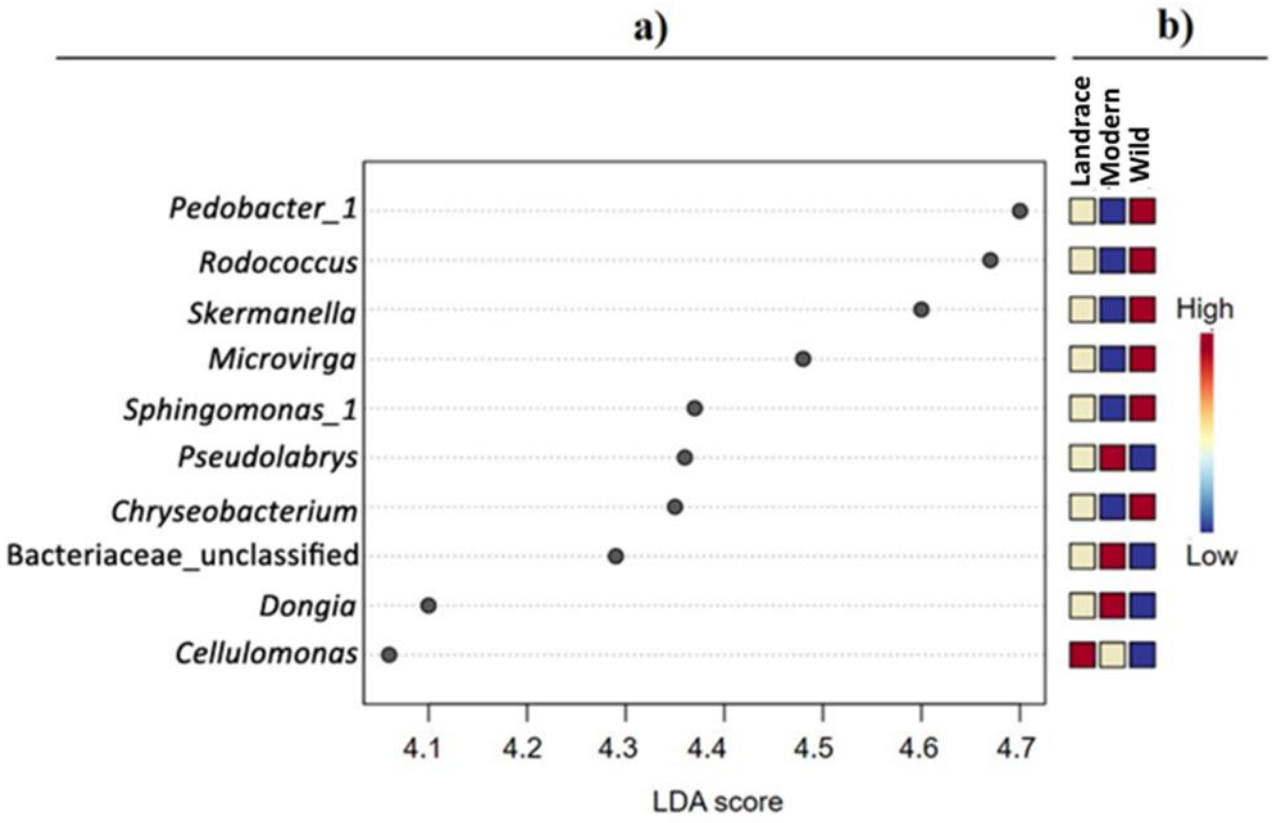

Figure 2. (a) Linear discriminant analysis (LDA) scores and, (b) heatmap from blue (low) via white to red (high) of genus relative abundances in root-associated soil of wild tomato related species (wild), tomato landraces (landrace), and modern commercial cultivars (modern)

Table 1. Richness estimates and diversity indices (means ± SE) for 16S rRNA libraries of tomato rhizosphere soils. Different letters indicate a significant difference among tomato varieties ($p < 0.05$, ANOVA, Dunn's post hoc-Bonferroni corrected p values) when exist. Wild: wild tomato related species; Lanrance: tomato landraces; Modern: modern commercial cultivars

	Wild	**Landrace**	**Modern**
Shannon–Wiener Diversity Index	5.79 ± 0.26	6.23 ± 0.06	6.18 ± 0.09
Shannon Entropy	8.35 ± 0.37	8.98 ± 0.08	8.92 ± 0.12
Species Richness	3260 ± 346	3727 ± 155	3471 ± 231
Total Abundance	52,838 ± 3338	59,144 ± 1702	57,100 ± 2964
Simpson Diversity Index	0.031 ± 0.013	0.010 ± 0.001	0.011 ± 0.002
Evenness	0.719 ± 0.021 b	0.759 ± 0.004 a	0.764 ± 0.005 a
Chao-1	4453 ± 450	4983 ± 241	4619 ± 329

2.2. Plant traits affected by domestication

For the rest of the results, we used the 18 tomato varieties which were used in the greenhouse experiment as well. The PCA ordination showed a clear trend from modern to wild tomato varieties from left-up to right-down positions of the ordination (Figure 3); wild tomato varieties produced more and smaller tomatoes than early domesticated and modern varieties, whereas modern varieties produced the highest plant biomass (both with and without pests) (see Table S1 for details on traits).

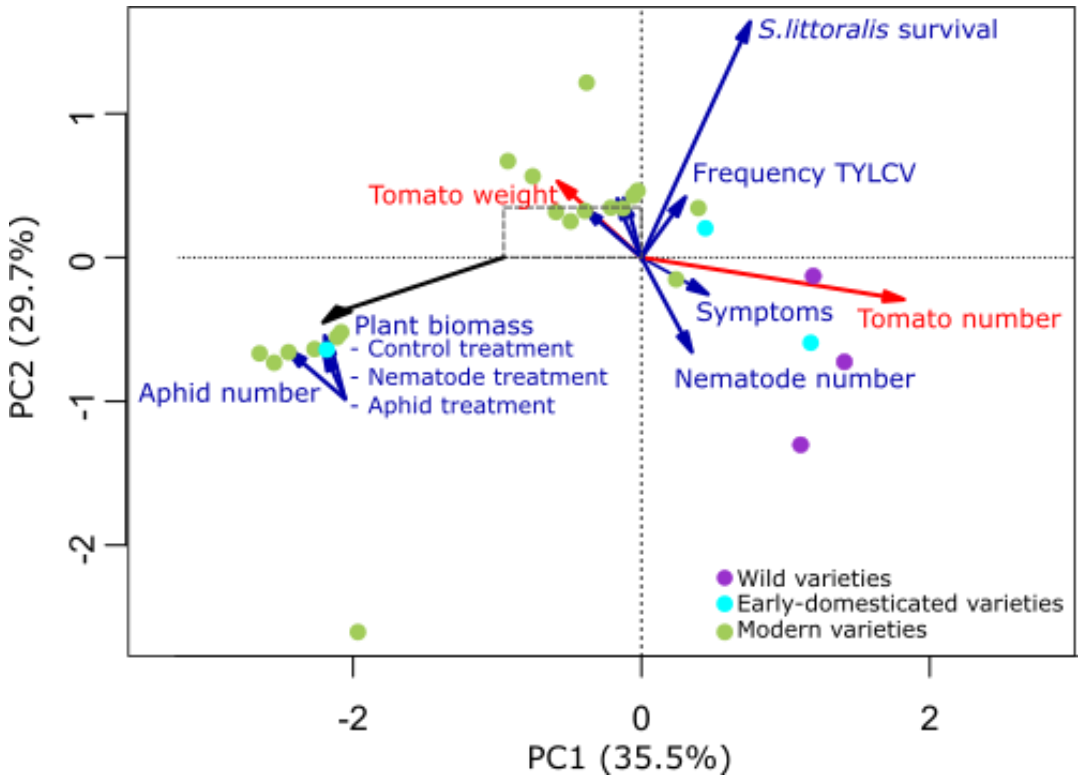

Figure 3. Principal components analysis (PCA) of plant traits (fruit in red and resistance in blue) of 18 varieties of tomato (*Solanum lycopersicum* Mill., *S. habrochaites* and *S. pimpinellifolium*). Tomato varieties were classified into wild (purple), early domesticated (light blue) and modern (green): TYLCV, tomato yellow leaf curl virus frequency of infection; *S. littoralis*, *Spodoptera littoralis*; Symptoms, plant symptom development according to symptom severity scale. A detail of shorter axes is provided (dashed square) to improve readability. For clarification, very short axes, i.e., those of minor importance, were not shown in the ordination.

2.3. Plant traits and soil characteristics influence soil bacterial communities

Bacterial OTU richness and Simpson and Shannon diversity indices significantly increased with *S. littoralis* survival and occurrence of tomato yellow leaf curl virus (TYLCV), mainly due to resistance traits showed by the variety Periana, and decreased with soil Si and Sr (Table S2). A set of plant traits and soil characteristics was found to drive the OTU composition of bacterial communities; in other words, they represented the minimum number of variables that explained a major proportion of OTU variation across samples [26]. For resistance, TYLCV was the most significant factor explaining the variation in soil bacterial OTU communities (Table S3). CN ratio, Si and Ni in soil were similarly selected. No significant effects were identified for fruit traits. These traits, together with the tomato phylogeny (four PCOA axes), were used to partition the variation of OTU bacterial community composition (Figure 4a,b). Total explained variation reached 25% (Table S4). Soil (9.4%) explained most variation (alone or combined with tomato phylogeny), whereas tomato phylogeny (5.4%) and resistance (4.3%) explained a smaller part of the variation.

The variation partitioning was repeated, excluding wild varieties, as a way to avoid bias caused by the fact that cultivated tomato belonged to the same tomato species (*S. lycopersicum*). Conversely, wild tomatoes belonged to different plant species. In this analysis, TYLCV was again selected together with soil CN ratio, C and As. In this case, the total explained variation reached 23% (Figure 4c,d), with resistance explaining most variation (9.7%), followed by soil (7.4%) and phylogeny (1%).

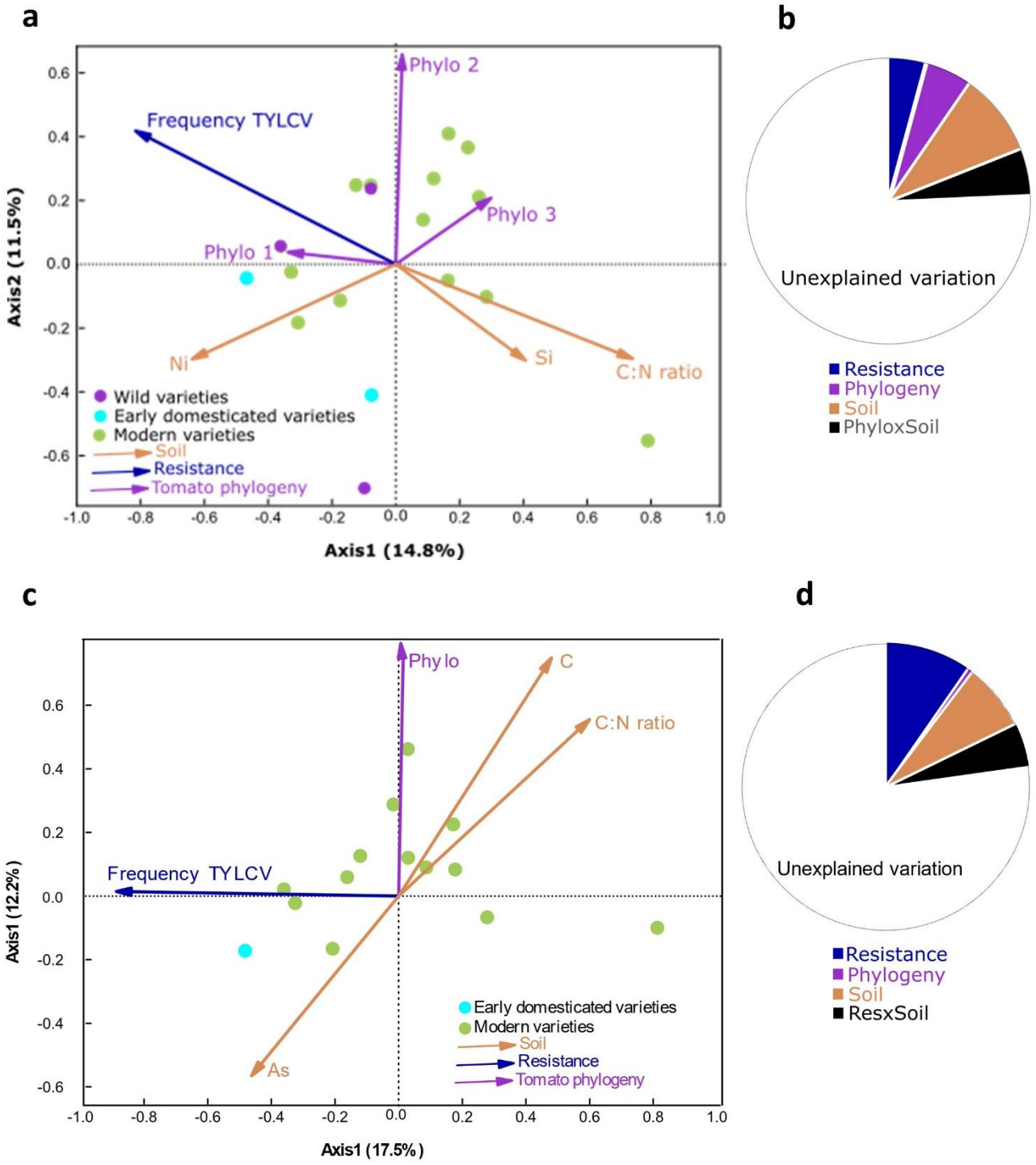

Figure 4. Redundancy analysis of bacterial OTU composition of tomato driven by plant (resistance and fruit morphology), soil (nutrients) and tomato phylogeny: (**a**) redundancy analysis; (**b**) varpart, including all three groups of tomato varieties; (**c**) redundancy analysis; (**d**) varpart, excluding wild varieties.

3. Discussion

Root traits selected during domestication were previously suggested to have a significant influence on the composition of the rhizosphere microbiome [27,28]. We found similar core bacterial microbiome members in tomato landraces and modern commercial cultivars, but detected small, though significant, differences in bacterial communities associated with both their rhizospheres and those of wild tomato relatives (Figure 1).

At family level, *Gemmatimonadaceae* (phylum Actinobacteria), *Microbacteriaceae* and *Streptomycetaceae* (Gemmatimonadetes) were represented less in the rhizosphere of wild tomato related species. At genera level, domestication gradually reduces the presence of the ubiquitous soil bacterium *Pedobacter*, the aromatic substrate metabolizer *Rhodococcus* and the alphaproteobacteria *Skermanella* and *Microvirga*, the latter considered a symbiotic nitrogen-fixing bacterium.

Previous studies highlighted the effect of plant species on the microbial composition and OTU abundance of the rhizosphere microbiome [29,30]. Domesticated crops often have shallow roots and shifts in traits such as leaf size and root architecture. Changes in these morphological traits results in increased litter quality, lower C:N ratio and root exudate composition, which could influence microbial community composition [6,21,31,32]. In this study, bacterial diversity at the OTU level was found to remain virtually unchanged along the domestication gradient, although evenness levels were significantly lower in the rhizosphere of tomato wild relatives. Evenness refers to the similarity of OTU frequencies in bacterial populations. Even though species evenness and richness are complementary, no differences were observed in the latter; the number of soil bacterial phyla recruited by wild type crops was similar to other tomatoes. Nevertheless, evenness does not necessarily translate into optimal diversity; ecosystem functions at the bacterial community level are more important than the bacterial species. As several species in an ecosystem may fulfil a similar function (redundancy), their even distribution is not essential as long as the function itself remains active. However, a more even species distribution within a bacterial community is assumed to make the ecosystem more resilient, as the risk of losing an essential component of the functional network would be much lower.

In this study, the effect of tomato domestication on root-associated bacterial community composition was observed from a trait perspective, allowing for a mechanistic interpretation of the identified patterns. Fruit traits (i.e., tomato number and weight) varied according to tomato domestication. However, resistance traits, non-related to tomato domestication, drove most of the explained variation in bacterial OTU composition.

The spectra of studied tomato varieties showed that modern varieties contained heavier and fewer fruits than wild or early domesticated varieties, consistent with modern tomatoes being large and diverse in shape, whereas wild tomatoes are generally small and round [33]. Even though tomatoes have not been selected for their root-associated microbiome, their structure might be altered through changes in root exudates, usually tied to root morphological characteristics, which feed and filter root microbiota [6,22,32,33].

Furthermore, Leff et al. found that faster-growing sunflower varieties had lower bacterial diversity in the rhizosphere [34]. Moreover, some authors linked bacterial-associated diversities to increased plant growth [35]. However, we did not find an effect of aboveground plant morphology on root-associated bacterial community structure. This could be due to the lack of links between the morphological traits used in this study and the more impacting belowground traits on root-associated microbiomes. For example, Legay et al. showed that belowground (root-associated) traits, such as root C:N ratio and root diameter, explained more variation in microbial properties than aboveground (leaf) traits [36]. Furthermore, the main driver of bacterial communities close to the roots is recruitment from the bulk soil. However, even though some authors found differences in microbial communities between bulk and rhizosphere soil [37], others found a difference in both compartments with the phyllosphere [38]. In addition, diversity indices were found to be lower or higher in the rhizosphere compared with the bulk soil [39,40]. The effect of soil nutrients suggests that environmental variables such as soil type impact microbial communities [2,41]. For example, Peiffer et al. showed in different maize varieties that plant genotype affects OTU richness within a field, and this genotypic effect varies between field environments [42]. Thus, it was suggested that environmental factors such as pH and geographic patterns interact to shape maize rhizosphere microbiota. Furthermore, microbes

themselves influence community structure by producing secondary metabolites such as antibiotics and toxins to compete with other microbes and successfully establish in the rhizosphere [2,5].

Despite this evidence, explaining the change in bacterial microbiomes through domestication seems difficult due to the contradictory results often found. For example, Leff et al. observed little effect of the domestication of sunflower on overall rhizosphere bacterial communities [34]. By contrast, Shenton et al. found that rhizosphere bacterial communities of wild rice differ in species richness and composition compared with cultivated rice, which was not correlated to the genetic distance of the plants [27]. However, in our study, resistance traits that were not aligned with the domestication degree (see [11]) were responsible for an important fraction of the variation on root-associated bacterial communities, especially between domesticated varieties, aligning with the evidence that plants shape microbial communities as an additional layer of defense. For example, plants under attack may recruit microorganisms that alleviate biotic stress or actively repress pathogen proliferation [21,43]. As far as we know, only one study has observed a difference between microbial communities associated with wild and domesticated plants, in this case, rice, in their response to a biotic challenge [44]. Plant resistance may also impact the functional profile of associated bacterial communities. For example, common bean genotypes resistant to *Fusarium oxysporum* contained bacterial communities enriched in genes encoding antifungal compounds [45]. Plant genetic factors related to immunity were shown to play a role in structuring the microbial community [46]. For example, Lebeis et al. showed how plant defense hormones, especially salicylic acid, shape root bacterial communities [47]. We also found a positive correlation between bacterial diversity and reduced plant resistance traits (*S. littoralis* survival and TYLCV infection). In this sense, it has been proposed that higher bacterial diversities should be associated with increased plant resistance due to the observed recruitment of beneficial microbes [9,48,49]. However, our results clearly aligned with Doornbos et al., who postulated that more susceptible plants harbour more diverse bacterial communities than resistant plants [43], but this is not always the case [34,48].

The limitations of sequencing technology were described in a recent review [49]. Most studies focus on bacteria, while other organisms such as fungi, viruses and archaea may be important as well [24,38,39]. Furthermore, often studies are conducted on a small scale, which limits detection of low-abundance taxa that could have a leading role in microbial community structure and function. DNA-based sequencing does not allow to determine whether the bacterial OTUS are functional. Furthermore, the diversity profile found could depend on the primer set used [42]. The V3–V4 region, as used in this study, was found to detect the highest phylum diversity compared with other 16S primers.

4. Materials and methods

4.1. Data field experiment

The setup of the field experiment is explained in general methods (1.1 Field experiment). Table S1 shows the average values obtained for each tomato variety. Aboveground biomass, the number of produced tomatoes and the total tomato fruit weight were used as fruit traits, while virus frequency and plant symptoms were used as resistance traits (Table S1B). Soil chemical characterization is shown in Table S1C. Soil processing is described in general methods (1.2 Soil processing).

For the bacterial community composition (section 2.1), the 27 tomato varieties were grouped into: (1) wild tomato species (accessions NR0407 (PE55), NR1021 (LA1589), NR0136 (PI134418), NR0699 (LA1777), NR0937 (T0937)), (2) tomato landraces (accessions NR0025 (Mellilero), NR0006 (Kalohi), NR0044 (Flor Baladre), NR0213 (Mex3), NR0275 (Mex 89), NR0237 (Mex 33), NR0469 (Pera), NR0166 (Moruno), NR0063

(Marmande), NR0705 (Edkawi), NR0612 (De Penjar)), and (3) modern commercial cultivars (accessions ABL104 (BC5), NR0561 (ANL101), NR0071 (San Marzano), NR0816 (Monita), NR0080 (Moneymaker), NR1080 (Periana), NR0504 (Cazorla). COM1, COM2, COM3 and COM4 cultivars, which are protected under plant variety rights, have no accession number).

For section 2.2 and 2.3, we used data from 18 tomato accessions that were selected because there were greenhouse data on resistance traits available (see section 4.2). In this case, tomato varieties were grouped into wild (*Solanum habrochaites* and *S. pimpinellifolium*) and early domesticated and modern (including *S. lycopersicum var. cerasiforme* and *S. lycopersicum lycopersicum*) (Table S1A). This classification was established by Ferrero et al. [11] and here verified using a k-means clustering analysis based on the measured traits [50].

4.2. Resistance to pests data

In a previous glasshouse experiment performed by Ferrero et al. (see details in [11]) in steam-sterilised sand–peat mixture, the response of the same 18 tomato varieties to root-knot nematodes, aphids and *Spodoptera littoralis* were determined after 6 weeks of growth. Plant biomass (dry weight) was measured under control (no pest) and pest treatment conditions.

For aphids, the total number of individuals were counted; for nematodes, the number of root knots/mg root (nematode number); and the mean increase in *S. littoralis* larvae weight per day (survival) was determined. These were included in the list of plant resistance traits (Table S1D). The averages across the tomato variety replicates were used as the trait values characteristic of each variety.

The plant phylogenetic tree developed by Ferrero et al. [11] was used to extract a phylogenetic distance matrix between tomato varieties. The variation in this matrix was decomposed by principal coordinates analysis, and the four generated axes were fed into subsequent analyses (capscale function in R package Vegan).

4.3. Study of bacterial community composition

Molecular analysis of soil bacteria is described in general methods (1.2.3 Molecular Analyses of Soil Bacteria). OTU abundance information was normalized to the abundance value of the sample with the least number of sequences. Alpha diversity indices generated by SEED2 were used to compare bacterial richness and diversity in tomato accessions. Statistically significant differences in alpha diversity, the bacterial composition of the group of tomato varieties and predictive metagenomics profiling data were evaluated using generalized lineal model (GLM) with degree of domestication as fixed factor. We checked fixed factors for significance with Wald test from car package [51] and multiple comparisons between levels of the fixed factor were tested using Tukey's test with the package lsmeans and emmeans [52]. For each model, residuals were examined for model validation. Beta diversity, or species complexity differences between groups of tomato varieties, was determined by linear discriminant analysis (LDA) effect size (LEfSe) using the MicrobiomeAnalyst web server. Taxa with an LDA score > 4 were considered important biomarkers of each group given that a p value < 0.05 indicates significant differences between groups. Data were analyzed using R version 3.6.3 [53] and R Studio version 1.1.456 [54].

The rarefaction curves of the selected 18 tomato varieties were visualised in Microbiome Analyst to confirm that all samples reached the plateau (55,56) (Figure S1). The two replicates per variety were summed for the statistical analysis.

All Illumina sequence raw data were deposited in the Sequence Read Archive (SRA) service of the European Bioinformatics Institute (EBI) database (BioProject ID: PRJNA693664).

4.4. Statistical analyses

Before the statistical analyses, plant traits were averaged per variety and log transformed. The OTU abundance table was Hellinger transformed. The relationship between fruit and resistance traits and the domestication degree of the tomato varieties was visualised via principal components analysis.

Bacterial OTU richness and Simpson and Shannon diversity indices were calculated using the abundance OTU x sample matrix after the Hellinger transformation of data. Their relationship with plant traits and soil variables were tested via Spearman correlation.

The relative influence of tomato fruit traits, resistance traits, phylogeny of tomato and soil chemical composition on bacterial OTU composition was tested by variation partitioning approach based on redundancy analysis (RDA, 29). The minimum number of variables inside each of these explaining factor classes explaining a major part of OTU variation was selected via forward selection using ordistep.

These analyses were carried out in R software using the R packages Vegan [55], picante [56], FD [57], dplyr [58] and lme4 [59].

5. Conclusions

In our study, we found that fruit traits (tomato number and weight) varied according to tomato domestication, while resistance traits drove most of the explained variation in bacterial OTU composition. These mechanisms highlight a direct effect of plant defense mechanisms on root-associated bacterial community composition and are consistent with the found dependence of bacterial communities on resistance traits. In summary, this study reveals how non-targeted traits during domestication shape the bacterial community of tomato, but further research is required to confirm the mechanisms behind the relationship between bacterial communities and plant resistance.

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7. Supplementary material

Table S1. Experimental variables included in the multivariate analysis. A) Domestication degree per tomato variety. B) Data from the field experiment: plant traits. C) Data from field experiment: soil characterization. D) Data from Ferrero et al. 2019: plant traits.

A) Domestication degree

Tomato variety	Species	Domestication degree
H. de Toro	*Solanum lycopersicum*	Modern
BC5	*Solanum lycopersicum*	Modern
Edkawi	*Solanum lycopersicum*	Modern
Flor Baladre	*Solanum lycopersicum*	Modern
Kalohi	*Solanum lycopersicum*	Modern
LA1589	*Solanum pimpinellifolium*	Wild
Marmande	*Solanum lycopersicum*	Modern
Melillero	*Solanum lycopersicum*	Modern
Mex 89	*Solanum lycopersicum* var. *cerasiforme*	Early-domesticated
Moneymaker	*Solanum lycopersicum*	Modern
Monita	*Solanum lycopersicum*	Modern
Moruno	*Solanum lycopersicum*	Modern
PE55	*Solanum lycopersicum*	Early-domesticated
De Penjar	*Solanum lycopersicum*	Modern
Periana	*Solanum lycopersicum var. cerasiforme*	Modern
PI134418	*Solanum habrochaites*	Wild
San Marzano	*Solanum lycopersicum*	Modern
T0 93715	*Solanum pimpinellifolium*	Wild

B) Plant traits field experiment. Plant state: Plant symptom characterization; Frequency ToCV: Percentage of tomato replicates with chlorosis virus detected with tissue-blot hybridization; Frequency TYLCV: Percentage of tomato replicates with yellow leaf curl virus detected with tissue-blot hybridization.

Tomato variety	Total plant biomass (Kg)	Tomato fruit weight (Kg)	Tomato fruit number	State	Frequency ToCV	Frequency TYLCV
H. de Toro	0.41	99.2	0.5	3.00	1.00	0.8
BC5	0.57	207.91	5.7	4.3	0.20	0.3
Edkawi	0.325	107.05	0.875	2.75	1.00	0.7
Flor Baladre	0.33	225.04	0.33	2.22	1.00	0.89
Kalohi	0.39	165.33	1.1	2.2	1.00	0.6
LA1589	0.526	51.06	61.3	6.6	0.80	0.8
Marmande	0.33	412.95	3.5	2.33	1.00	0.5
Melillero	0.52	201.94	1.8	2.7	0.9	0.4
Mex 89	0.76	85.19	65.78	5.78	0.9	0.8
MM	0.363	403.82	4.2	3.00	1.00	0.7
Monita	0.29	98.24	2.09	2.45	1.00	0.7
Moruno	0.24	137.82	4.00	2.29	1.00	0.25

PE55	0.28	46.00	5.17	3.00	1.00	0.625
De Penjar	0.6725	282.56	12.5	3.9	1.00	0.4
Periana	0.5	69.34	0.3	2.7	0.9	0.1
PI134418	0.39	21.12	17.89	7.44	0.67	0.5
San Marzano	0.63	110.17	2.11	2.44	1.00	0.5
T0 93715	0.63	7.88	15.2	7.4	0.4	0.6

Tomato variety	Nitrogen (%)	Carbon (%)	C:N ratio	Al	As	Ca	Cd	Co
H. de Toro	0.29	5.48	19.07	32272.45	30.64	9512.67	2.29	21.63
BC5	0.30	5.77	19.25	29903.35	26.45	10998.40	2.23	19.42
Edkawi	0.31	6.03	19.47	30849.13	29.29	9355.71	2.23	21.20
Flor Baladre	0.25	4.00	16.03	40469.74	30.25	8871.26	2.40	22.48
Kalohi	0.31	5.25	17.18	34563.67	27.25	11063.83	2.07	19.38
LA1589	0.30	5.79	19.14	44769.84	32.79	7804.21	2.22	21.64
Marmande	0.36	9.51	26.35	47279.17	33.44	10332.83	2.09	22.12
Melillero	0.27	5.05	19.05	48191.47	32.23	8737.64	2.27	21.26
Mex 89	0.24	3.57	14.78	36127.94	35.02	7748.17	2.40	22.77
Moneymaker	0.32	6.79	21.11	39290.18	31.92	8788.23	2.22	20.71
Monita	0.35	7.83	22.27	36863.42	27.85	9099.69	2.15	20.85
Moruno	0.31	6.69	21.30	33901.28	28.67	10937.73	2.13	20.73
PE55	0.21	3.42	16.68	44845.01	37.77	8121.07	2.40	23.68
De Penjar	0.26	4.19	15.89	43683.51	31.67	8550.81	2.29	22.27
Periana	0.32	6.75	21.14	46608.99	23.73	11773.04	2.03	20.42
PI134418	0.29	5.16	18.05	52079.30	35.37	8814.62	2.42	24.27
San Marzano	0.29	5.32	18.33	48274.30	25.99	7567.39	2.14	22.05
T0 93715	0.33	5.44	16.44	34162.53	33.24	7739.21	2.26	21.82

C) Field experiment Chemical composition of soil (ppm (mg/Kg))

Tomato variety	Cr	Cu	Fe	K	Li	Mg	Mn	Na
H. de Toro	47.01	39.16	32775.74	7891.33	29.24	7987.08	846.29	0.04
BC5	44.87	40.29	31155.15	7694.72	26.80	8344.68	771.62	0.03
Edkawi	45.56	39.34	33169.75	7519.68	28.77	7550.65	875.68	0.03
Flor Baladre	54.91	40.06	35588.47	9655.52	33.43	8372.00	863.50	0.04
Kalohi	48.22	38.63	31031.98	8877.17	27.95	8465.32	859.65	0.04
LA1589	57.71	40.92	33620.68	11887.42	31.11	7584.11	888.58	0.05
Marmande	59.18	38.17	31873.98	12308.45	28.36	8032.74	925.72	0.06
Melillero	61.57	38.24	34490.29	12655.27	31.47	8438.64	807.50	0.05
Mex 89	50.77	40.46	35408.39	8917.46	30.71	7884.02	980.78	0.03
Moneymaker	52.42	41.88	33572.50	9825.97	28.13	8131.44	727.50	0.04
Monita	50.65	39.99	32321.24	9701.17	27.78	7800.04	793.15	0.04
Moruno	47.21	38.34	31601.04	8521.75	26.27	8424.78	882.49	0.04

PE55	57.48	39.02	35385.81	11384.10	30.59	7764.41	1022.06	0.05
De Penjar	56.89	38.22	34266.37	11362.56	30.16	8187.77	874.02	0.05
Periana	58.46	38.14	30811.28	12562.43	28.25	8992.66	729.31	0.06
PI134418	64.50	40.21	35617.88	13259.06	32.10	8677.31	968.29	0.06
San Marzano	59.35	34.75	33049.06	12385.03	28.82	7979.41	1177.24	0.06
T0 93715	48.03	38.65	33498.50	8794.56	27.40	7232.60	955.40	0.03

Tomato variety	Ni	P	Pb	S	Si	Sr	Ti	V	Zn
H. de Toro	45.93	707.46	22.47	411.73	2877.92	47.73	1148.59	59.06	125.51
BC5	40.74	715.53	21.64	489.64	2728.97	57.07	1128.88	56.16	131.67
Edkawi	43.74	818.07	22.09	470.31	2652.96	58.89	1077.34	56.82	106.75
Flor Baladre	48.04	767.09	23.16	373.89	3398.72	52.20	1318.87	69.95	109.48
Kalohi	41.15	832.00	21.53	481.18	3204.44	54.61	1212.39	60.98	99.80
LA1589	45.09	968.39	23.32	409.23	4196.16	50.89	1375.55	73.44	84.85
Marmande	43.99	992.85	23.92	487.64	4799.41	61.69	1490.84	77.27	84.34
Melillero	45.56	887.12	23.33	388.67	4237.66	51.75	1534.15	79.85	83.94
Mex 89	48.31	1020.34	24.51	341.58	3054.83	42.80	1191.39	64.45	97.92
Moneymaker	43.16	822.85	23.24	406.64	3350.30	54.84	1232.17	66.60	91.76
Monita	43.02	1072.14	22.11	471.51	3359.55	53.64	1332.99	63.90	85.51
Moruno	42.95	933.49	23.01	422.84	3230.94	56.79	1149.81	59.14	96.92
PE55	48.33	1236.94	25.20	314.81	3843.06	47.52	1304.76	73.95	100.50
De Penjar	45.86	1064.76	24.39	390.81	3542.49	50.39	1380.06	73.00	103.77
Periana	40.29	937.59	22.04	547.08	4490.26	65.13	1555.12	76.24	96.26
PI134418	49.26	1174.52	24.65	414.23	3914.28	47.82	1479.69	84.41	93.77
San Marzano	49.03	992.56	21.64	347.10	4147.62	49.36	1422.21	79.08	84.83
T0 93715	45.16	1129.40	24.36	404.48	4089.26	47.52	1175.80	60.73	107.31

D) Ferrero et al. (2019) resistance traits. Averages per tomato variety. Total plant biomass (g): Total plant biomass (dry weight) of control plants (no pest attack); Spodoptera exigua survival: Mean increase in weight per day; Plant biomass (aphid treatment) (g): Plant biomass (dry weight) under aphid pressure; Aphid number: Number of aphids in the plant at the end of experiment; Plant biomass (nematode treatment)(g): Plant biomass (dry weight) under nematode pressure; Nematode number: Number of root knots/ mg root.

Tomato variety	Total plant biomass (g)	Spodoptera exigua survival	Plant biomass (aphid treatment) (g)	Aphid number	Plant biomass (nematode treatment) (g)	Nematode number
H. de Toro	3.997	0.47	3.66	70.64	4.96	13.63
BC5	3.708	0.33	3.13	32.86	3.42	47.14
Edkawi	4.746	0.60	2.56	66.83	4.01	79.41
Flor Baladre	3.818	0.60	3.09	67.60	4.10	27.21
Kalohi	3.901	0.33	3.30	81.80	4.21	21.23
LA1589	2.815	0.27	1.51	77.93	2.25	126.30
Marmande	4.065	0.40	2.91	62.60	4.76	22.55

Melillero	4.624	0.27	3.26	83.77	3.65	9.53
Mex 89	2.328	0.13	2.13	28.40	2.62	9.92
Moneymaker	4.077	0.47	3.24	74.79	3.69	25.36
Monita	4.286	0.87	2.42	86.53	3.94	0.47
Moruno	4.471	0.67	4.29	95.47	4.76	24.00
PE55	3.981	0.73	3.14	64.57	4.66	47.89
De Penjar	3.718	0.47	2.96	52.69	4.03	7.83
Periana	2.12	0.00	1.90	79.80	2.11	33.01
PI134418	1.437	0.13	0.93	5.71	1.71	42.84
San Marzano	4.141	0.60	3.27	75.93	4.96	31.24
T0 93715	4.589	0.80	3.95	68.60	4.43	47.94

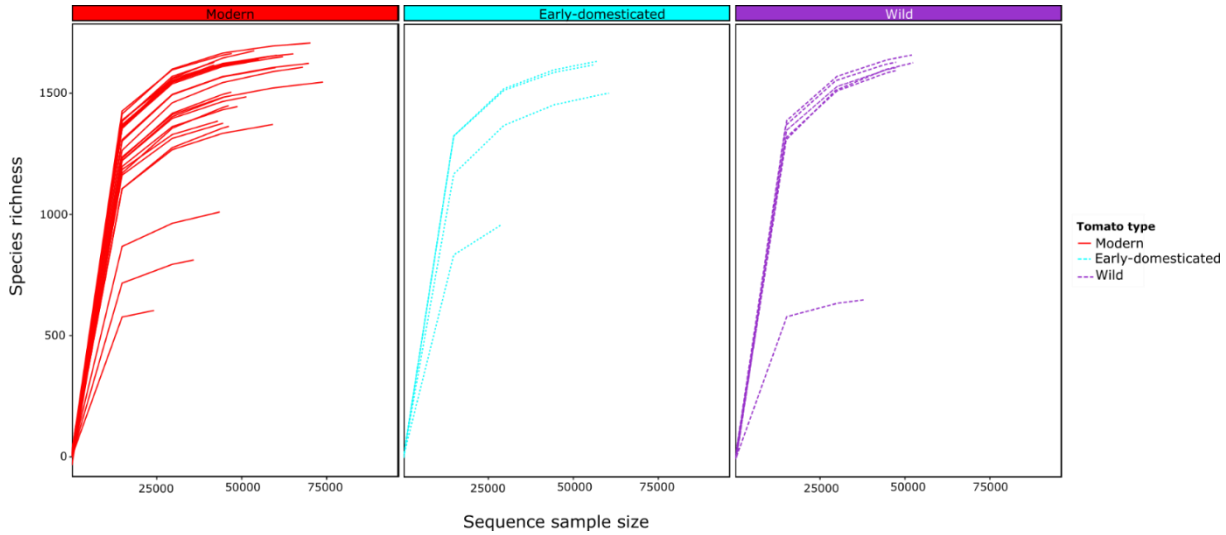

Figure S1: Rarefaction curves of 18 varieties of tomato (*Solanum lycopersicum* Mill.. *S. habrochaites* and *S. pimpinellifolium*). Tomato varieties were classified into wild (purple). early-domesticated (light blue) and modern (red).

Table S2. Pearson correlation test of bacterial diversity indexes and plant traits and soil variables. R **coefficients** are shown. Asterisks indicate significance: * $p < 0.05$; ** $p < 0.01$. For details in plant traits see Table S1.

Variable type	Variable	S	Simpson	Shannon
Plant traits	Biomass (Field exp)	0.06	-0.017	0.007
	Tomato Fruit Weight	0.196	0.281	0.191
	Tomato Fruit Number	0.164	0.212	0.234
	Plant State	0.095	0.068	0.114
	Frequency ToCV	0.034	0.024	0.023
	Frequency TYLCV	**0.637 ****	**0.62 ****	**0.685 ****
	Biomass (Ferrero et al (2019))	0.02	0.099	0.083
	Spodoptera survival	**0.524 ***	**0.604 ****	**0.631 ****
	Aphid Biomass	0.013	0.01	0.032
	Aphid number	-0.211	-0.185	-0.21
	Nematode_biomass	0.107	0.129	0.153
	Nematode number	-0.047	-0.128	-0.067
Soil variables	Nitrogen	-0.226	-0.006	-0.188
	Carbon	-0.335	-0.111	-0.291
	C:N ratio	-0.397	-0.199	-0.353
	Al	-0.274	-0.31	-0.287
	As	0.169	0.119	0.231
	Ca	-0.214	-0.175	-0.261
	Cd	0.399	0.276	0.409
	Co	0.078	-0.01	0.099
	Cr	-0.229	-0.275	-0.246
	Cu	0.225	0.26	0.248
	Fe	0.286	0.187	0.304

K	-0.344	-0.365	-0.354
Li	0.336	0.185	0.291
Mg	-0.062	-0.112	-0.148
Mn	0.205	0.127	0.231
Na	-0.351	-0.37	-0.373
Ni	0.351	0.238	0.361
P	-0.379	-0.365	-0.315
Pb	-0.068	-0.125	-0.021
S	-0.307	-0.178	-0.328
Si	**-0.496 ***	**-0.513 ***	**-0.50 ***
Sr	**-0.479 ***	-0.343	**-0.486 ***
Ti	-0.358	-0.378	-0.389
V	-0.243	-0.29	-0.262
Zn	0.252	0.14	0.208

Table S3. Stepwise model selection of redundancy analyses for plant traits (fruit and resistance) and soil nutrient variables. Asterisks indicate significance: .p < 0.1; * p < 0.05; ** p < 0.01. For details in plant traits see Table S1.

Dataset	Variable type	Variable	Df	AIC	F	p
Whole dataset	Resistance traits	Frequency TYLCV	1	-37.574	22.245	**0.005****
	Soil variables	Si	1	-37.616	17.284	**0.010****
		Ni	1	-37.488	18.409	**0.005****
		CN ratio	1	-37.167	21.262	**0.005****
Excluding wild var.	Resistance traits	Frequency TYLCV	1	-31.06	2.269	**0.005****
	Soil variables	As	1	-31.510	18.147	**0.020***
		Carbon	1	-30.544	26.009	**0.005****
		CN ratio	1	-30.498	26.396	**0.005****
		S	1	-31.905	15.075	0.080 .

Table S4. Variation partitioning of bacterial OTU community composition in plant traits (fruit and resistance). tomato phylogeny and soil variables. Either considering the whole dataset or only domesticated tomato varieties. Asterisk indicates significant p values: . p < 0.1; * p < 0.05. For details in plant traits see Table S1.

Dataset	Partition	Df	R^2
Whole dataset	Resistance traits	1	0.043 .
	Phylogeny	4	0.054
	Soil	2	**0.094 ***
	Resistance × Phylogeny	0	0
	Resistance × Soil	0	0
	Phylogeny × Soil	0	0.053
	Resist. × Phylo × Soil	0	0
	All	0	0
	Residuals	0	0.757
Excluding wild var.	Resistance traits	1	**0.097 ***
	Phylogeny	1	0.007
	Soil	3	**0.074 ***
	Resistance × Phylogeny	0	0
	Resistance × Soil	0	0.050
	Phylogeny × Soil	0	0
	Resist. × Phylo × Soil	0	0
	All	0	0
	Residuals	0	0.772

Chapter 2: Tomato domestication affects potential functional molecular pathways of root-associated soil bacteria

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Abstract: While it has been well evidenced that plant domestication affects the structure of the root-associated microbiome, there is a poor understanding of how domestication-mediated dif-ferences between rhizosphere microorganisms functionally affect microbial ecosystem services. In this study, we ex-plore how domestication influenced functional assembly patterns of bacterial communities in the root-associated soil of 27 tomato accessions through a transect of evolution, from plant ancestors to landraces to modern cultivars. Based on molecular analysis, functional profiles were predicted and co-occurrence networks were constructed based on the identification of co-presences of functional units in the tomato root-associated microbiome. The results re-vealed differences in eight metabolic pathway categories and highlighted the influence of the host genotype on the potential functions of soil bacterial communities. In general, wild tomatoes dif-fered from modern cultivars and tomato landraces which showed similar values, although all an-cestral functional characteristics have been conserved across time. We also found that certain functional groups tended to be more evolution-arily conserved in bacterial communities associ-ated with tomato landraces than those of modern varieties. We hypothesize that the capacity of soil bacteria to provide ecosystem services is affected by agronomic practices linked to the do-mestication process, particularly those related to the preservation of soil organic matter.

Keywords: bacterial functions; co-presence networks; metagenomics; microbial ecology; plant domestication

1. Introduction

The coevolutionary framework for analyzing interactions between plants and soil microorganisms has mainly been used for organisms involved in rhizosphere processes. Given that rhizosphere microbiomes are part of complex food webs affecting large numbers of nutrients released by the plant, it has been suggested that plants attract and select beneficial microbiomes by first releasing signals and then filtering species [1,2]. Rhizopshere microbiota are well known to play a critical role in both the adapta-tion of plants to the environment, but also contribute to a wide range of essential eco-system services, such as carbon and nutrient cycling, plant growth promotion, soil structure stability, food web interactions and soil atmosphere gas exchange, which ul-timately affect soil productivity and sustainability [3].

In addition to plant genetics and developmental stage [4,5], other factors includ-ing soil management, agronomic practices, pathogen presence, soil pH, nutrient con-tent, and moisture, have been suggested to affect root microbial community structure [6–8]. However, the question of how the host and its environment regulate microbiome assembly and co-occurrence in plant species has not been addressed yet. This is of par-ticular interest for crops in the context of plant–soil feedback, where plants can change soil biology and chemistry in ways that could affect subsequent plant growth [9].

Crop genetic diversity is usually reduced during plant domestication, which is associated with the selection of certain morphological traits such as root architecture and exudate composition, leading to striking differences between crops and their wild relatives [10,11]. Therefore, domestication is expected to have a direct impact on the type and diversity of below-ground microorganisms [9]. Indeed, domestication and genetic selection have progressively differentiated the microbiota of modern crops from those of their wild progenitors. It has also been postulated that crops are more likely to display negative feedbacks as compared to wild relatives, as domestication potentially disrupted beneficial rhizosphere associations [12]. Previous studies of cul-tivated plants evidenced differences between bacteria associated with differing plant genotypes such as wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.) and

tomato (*Solanum lycopersicum* Mill.) [13–15], suggesting that traits se-lected during domestication could have a significant influence on rhizosphere microbiota composition.

Although the structure of root-associated microbial communities is widely ac-cepted to depend, to a greater or lesser degree, on the plant genotype, little is known about whether domestication-mediated differences between rhizosphere microorgan-isms functionally affect microbial ecosystem services. In this scenario, an evaluation of functional soil microbial genes could help to determine the effect of domestication on functional redundancy or co-occurrence of basic metabolic capacity in the rhizospheres of crop varieties and their wild ancestors [16]. This is essential to identify agricultural practices that resulted in reduced trade-offs between agricultural productivity and the provision of ecosystem services.

This study aims to explore how plant domestication influences the assembly pat-terns of soil microbial communities by metagenomic analysis of bacterial communities and predicted functions in the rhizosphere of different tomato varieties along a domestication gradient.

2. Results

2.1. Bacterial community functional analysis

We used metagenomics analysis to predict the functional potential of the bacterial community and to explore associated metabolic pathway networks using Kyoto Encyclopedia of Gene and Genome (KEGG) clusters.

At the level of functional units of gene sets, all tomato varieties shared all the 181 predicted functions related to soil bacteria. However, 68 of them differed among tomato domestication types (Table 1). In general, wild tomatoes differed from modern cultivars and tomato landraces that usually showed similar values, but generally tomato landraces had intermediate values between modern cultivars and wild relatives. For example, the levels of the aromatic degradation metabolic pathway category, except for module M00541 (benzoyl-CoA degradation), tended to be significantly higher in bacteria growing in wild tomato accessions, indicating that tomato landraces drive bacterial communities with similar levels of predicted functions as modern commercial cultivars. Similarly, while the values for the metabolic categories of nitrogen, sulfur, cofactor/vitamin and purine were decreased in modern cultivars with respect to wild varieties, no differences between wild and landrace cultivars were detected. By contrast, lipo-polysaccharide and lipid metabolic pathway levels were clearly higher in both landrace and modern cultivars with respect to their wild relatives.

Table 1. Functional units of gene sets in metabolic pathways (KEGG modules) of tomato rhizosphere soils differentially represented among tomato varieties. Different letters indicate a significant difference among tomato varieties (ANOVA, Dunn's post hoc-Bonferroni corrected *p* values). WTRS: wild tomato related species; TL: tomato landraces; MCC: modern commercial cultivars.

Pathway Modules		**WTRS**	**TL**	**MCC**	p-**value**
Amino acid metabolism; Arginine and proline metabolism	M00015_Proline biosynthesis, glutamate =>proline	1656a	1609b	1626b	0.021
	M00023_Tryptophan biosynthesis, chorismate => tryptophan	3173b	3234a	3205a	0.009
	M00037_Melatonin biosynthesis, tryptophan => serotonin => melatonin	69b	77a	74ab	0.042

	M00040_Tyrosine biosynthesis, chorismate => arogenate => tyrosine	508a	464b	467b	5.579×10^{-6}
	M00042_Catecholamine biosynthesis, tyrosine => dopamine => noradrenaline => adrenaline	95b	106a	104ab	0.023
	M00533_Homoprotocatechuate degradation, homoprotocatechuate => 2-oxohept-3-enedioate	491a	474b	473ab	0.047
Amino acid metabolism; Aromatic amino acid metabolism	M00545_Trans-cinnamate degradation, trans-cinnamate => acetyl-CoA	1048a	1004b	1014b	0.002
Amino acid metabolism; Branched-chain amino acid metabolism	M00036_Leucine degradation, leucine => acetoacetate + acetyl-CoA	7057a	6938b	6874b	6.597×10^{-5}
Amino acid metabolism; Cysteine and methionine metabolism	M00017_Methionine biosynthesis, apartate => homoserine => methionine	5393b	5464a	5422a	0.007
	M00035_Methionine degradation	2031b	2101a	2065b	6.836×10^{-5}
	M00338_Cysteine biosynthesis, homocysteine + serine => cysteine	233c	276a	257b	9.38×10^{-11}
Amino acid metabolism; Lysine metabolism	M00031_Lysine biosynthesis, mediated by LysW, 2-aminoadipate => lysine	81b	106a	98b	1.069×10^{-5}
Amino acid metabolism; Other amino acid metabolism	M00118_Glutathione biosynthesis, glutamate => glutathione	944a	876b	880b	7.781×10^{-8}
	M00027_GABA (gamma-Aminobutyrate) shunt	2018a	1942b	1921b	1.037×10^{-5}
Amino acid metabolism; Polyamine biosynthesis	M00133_Polyamine biosynthesis, arginine => agmatine => putrescine => spermidine	865b	895a	879ab	0.016
	M00134_Polyamine biosynthesis, arginine => ornithine => putrescine	872a	838b	841b	0.008
	M00136_GABA biosynthesis, prokaryotes, putrescine => GABA	677a	620b	636b	6.265×10^{-9}
Amino acid metabolism; Serine and threonine metabolism	M00555_Betaine biosynthesis, choline => betaine	1473a	1377b	1383b	4.206×10^{-11}
Carbohydrate metabolism; Central carbohydrate metabolism	M00006_Pentose phosphate pathway, oxidative phase, glucose 6P => ribulose 5P	1535ab	1523b	1546a	0.050
	M00077_Chondroitin sulfate degradation	105b	118a	123a	6.408×10^{-5}

	M00008_Entner–Doudoroff pathway, glucose-6P => glyceraldehyde-3P + pyruvate	1993a	1905c	1928b	2.838×10^{-6}
	M00009_Citrate cycle (TCA cycle, Krebs cycle)	12,529a	12,667b	12,563a	0.000
	M00011_Citrate cycle, second carbon oxidation, 2-oxoglutarate => oxaloacetate	9185b	9286a	9207b	0.001
	M00003_Gluconeogenesis, oxaloacetate => fructose-6P	5474b	5544a	5498b	0.008
	M00633_Semi-phosphorylative Entner–Doudoroff pathway, gluconate/galactonate => glycerate-3P	85b	91ab	95a	0.038
Carbohydrate metabolism; Other carbohydrate metabolism	M00061_D-Glucuronate degradation, D-glucuronate => pyruvate + D-glyceraldehyde 3P	1694a	1654b	1680ab	0.015
	M00081_Pectin degradation	113b	129a	132a	6.318×10^{-5}
	M00114_Ascorbate biosynthesis, plants, glucose-6P => ascorbate	2958ab	2995a	2949b	0.027
	M00131_Inositol phosphate metabolism, Ins(1,3,4,5)P4 => Ins(1,3,4)P3 => myo-inositol	1003a	969b	968ab	0.027
	M00550_Ascorbate degradation, ascorbate => D-xylulose-5P	27a	19b	18b	1.195×10^{-5}
	M00554_Nucleotide sugar biosynthesis, galactose => UDP-galactose	199b	207ab	217a	0.005
	M00565_Trehalose biosynthesis, D-glucose 1P => trehalose	3380b	3603a	3666a	2.2×10^{-16}
Energy metabolism; Carbon fixation	M00170_C4-dicarboxylic acid cycle, phosphoenolpyruvate carboxykinase type	1302c	1357a	1321bc	1.883×10^{-5}
	M00172_C4-dicarboxylic acid cycle, NADP—malic enzyme type	3505a	3444b	3445b	0.030
	M00173_Reductive citrate cycle (Arnon-Buchanan cycle)	10,778b	10,891a	10,850ab	0.004
	M00374_Dicarboxylate-hydroxybutyrate cycle	7259b	7345a	7333a	0.012
	M00620_Incomplete reductive citrate cycle, acetyl-CoA => oxoglutarate	2168b	2224a	2231a	0.001

Energy metabolism; Methane metabolism	M00344_Formaldehyde assimilation, xylulose monophosphate pathway	913b	944a	942ab	0.032
	M00345_Formaldehyde assimilation, ribulose monophosphate pathway	749b	808a	800a	6.137×10^{-7}
	M00346_Formaldehyde assimilation, serine pathway	3166b	3234a	3226ab	0.007
	M00356_Methanogenesis, methanol => methane	22b	26ab	27a	0.039
	M00358_Coenzyme M biosynthesis	177b	190a	198a	0.000
	M00378_F420 biosynthesis	82b	93a	89ab	0.055
	M00563_Methanogenesis, methylamine/dimethylamine/trimethylamine => methane	465a	434b	464a	3.724×10^{-6}
Energy metabolism; Nitrogen metabolism	M00530_Dissimilatory nitrate reduction, nitrate => ammonia	1864a	1823b	1848a	0.018
Energy metabolism; Sulfur metabolism	M00176_Assimilatory sulfate reduction, sulfate => H2S	2814a	2741b	2766ab	0.006
Glycan metabolism; Glycosaminoglycan metabolis	M00076_Dermatan sulfate degradation	115b	129a	135a	2.073×10^{-5}
	M00077_Chondroitin sulfate degradation	105b	118a	123a	6.408×10^{-5}
	M00078_Heparan sulfate degradation	191b	215a	224a	2.272×10^{-7}
	M00079_Keratan sulfate degradation	475b	526a	547a	1.002×10^{-12}
Glycan metabolism; Lipopolysaccharide metabolism	M00060_KDO2-lipid A biosynthesis, Raetz pathway, LpxL-LpxM type	3058b	3132a	3124a	0.002
	M00064_ADP-L-glycero-D-manno-heptose biosynthesis	692b	743a	771a	3.151×10^{-7}
Lipid metabolism; Fatty acid metabolism	M00082_Fatty acid biosynthesis, initiation	3785b	3861a	3842ab	0.015
	M00083_Fatty acid biosynthesis, elongation	9121b	9218a	9214a	0.017
	M00086_beta-Oxidation, acyl-CoA synthesis	1699b	1743a	1746ab	0.016
Lipid metabolism; Lipid metabolism	M00113_Jasmonic acid biosynthesis	428b	454a	438b	0.002
Metabolism of cofactors and vitamins;	M00116_Menaquinone biosynthesis, chorismate => menaquinol	943b	1026a	977b	1.104×10^{-10}

Cofactor and vitamin metabolism	M00117_Ubiquinone biosynthesis, prokaryotes, chorismate => ubiquinone	2772a	2703b	2707ab	0.010
	M00122_Cobalamin biosynthesis, cobinamide => cobalamin	2143a	2105b	2153a	0.002
	M00128_Ubiquinone biosynthesis, eukaryotes, 4-hydroxybenzoate => ubiquinone	74a	64b	67ab	0.011
Nucleotide metabolism; Purine metabolism	M00546_Purine degradation, xanthine => urea	2126a	2089b	2125a	0.011
Xenobiotics biodegradation; Aromatics degradation	M00537_Xylene degradation, xylene => methylbenzoate	215a	199b	200ab	0.015
	M00541_Benzoyl-CoA degradation, benzoyl-CoA => 3-hydroxypimeloyl-CoA	59b	67a	67ab	0.024
	M00548_Benzene degradation, benzene => catechol	27a	20b	21b	0.000
	M00551_Benzoate degradation, benzoate => catechol/methylbenzoate => methylcatechol	124a	108b	110b	0.004
	M00568_Catechol ortho-cleavage, catechol => 3-oxoadipate	445a	421b	433ab	0.012
	M00569_Catechol meta-cleavage, catechol => acetyl-CoA/4-methylcatechol => propanoyl-CoA	466a	441b	430b	0.002
	M00637_Anthranilate degradation, anthranilate => catechol	90a	73b	82b	1.726×10^{-5}

Amino acid metabolism pathways exhibited no clear tendency, although in modern commercial culti-vars, cysteine and methionine pathway levels were higher and those of other amino acid pathways were lower. A similar variable pattern was observed with respect to both central carbohydrate and other carbohydrate metabolic pathways in the category of carbohydrate metabolism.

Finally, a marked increase in the carbon fixation and methane metabolic subfunctions and in the met-abolic pathway categories glycan metabolism and lipid metabolism, respectively, was observed in the modern commercial cultivars.

2.2. Functional networks of KEGG orthologous groups

Figure 2 and Table 2 show the co-presence networks and the topological properties of functional net-works, respectively, for the modern:wild, landraces:wild and modern:landraces pairs. An increase in the average number of neighbors and a decrease in the characteristic path length were found in landraces:wild pairs (Table 2). Additionally, an increase in the network radius and diameter were detected in the pair modern:landraces. Finally, the pair modern:wild showed the largest number of KEGG-module nodes and the

largest number of edges or internode connections in the network. The clustering coefficient, which reflects the tendency of organisms to form relatively high-density clusters, was zero. Co-occurrence networks are generated by connecting pairs of terms using a set of criteria defining co-occurrence. These networks connect across, rather than between, nodes. Every node, in which none of whose neighbors connect to each other, has a clustering coefficient of zero.

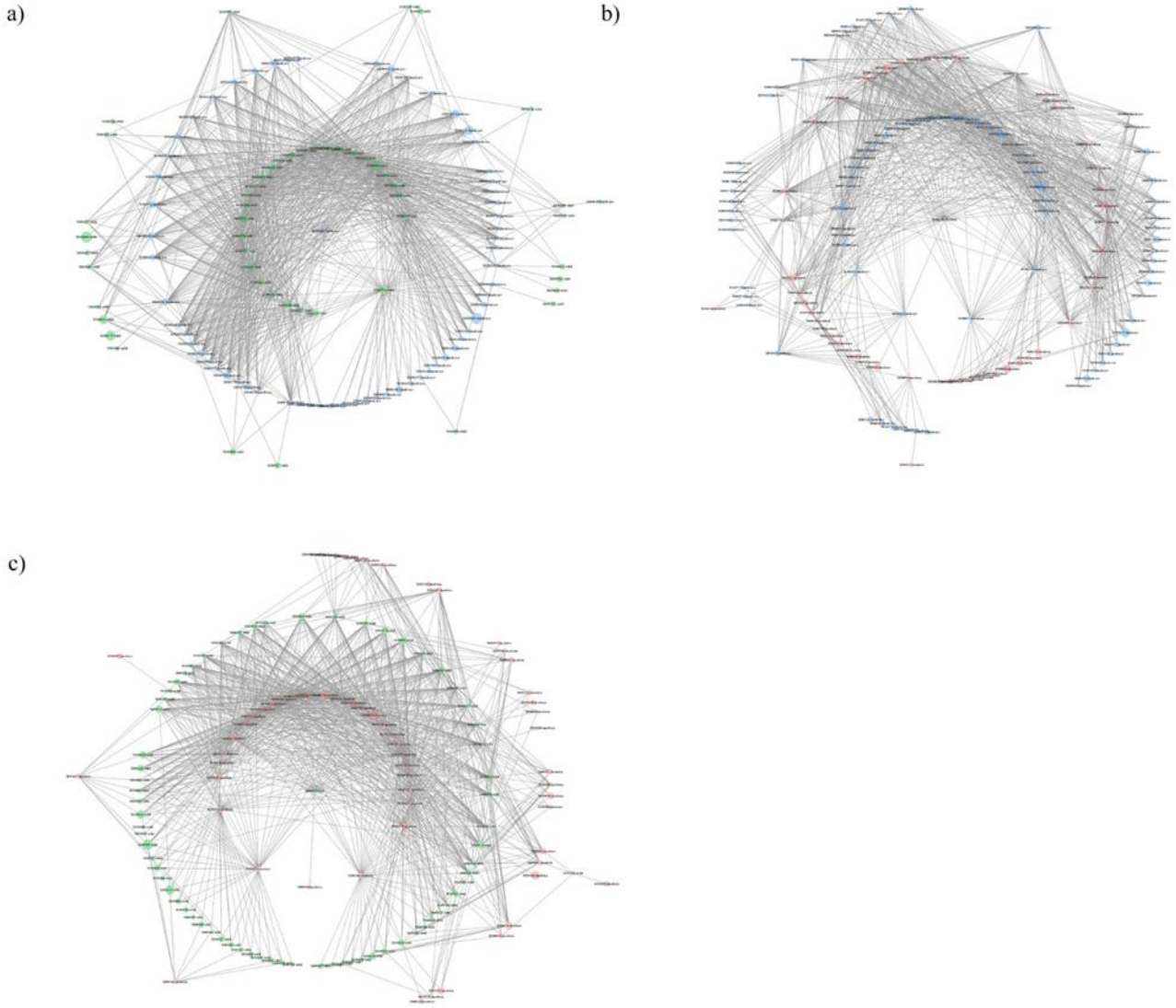

Figure 2. Co-presence network for the couples: (**a**) tomato landraces:wild tomato related species, (**b**) modern commercial cultivars:tomato landraces, (**c**) modern commercial cultivars:wild tomato related species. Node sizes reflect average relative abundance of each KEGG module. The line thickness is proportional to the edge weight. Node colors: green for wild tomato related species, blue for tomato landraces and red for modern commercial cultivars.

Table 2. Topological properties of pairwise functional networks. WTRS: wild tomato related species; TL: tomato landraces; MCC: modern commercial cultivars.

	MCC:WTRS	TL:WTRS	MCC:TL
Number of nodes	133	116	132
Number of edges	1005	1003	1001
Average number of neighbors	15,113	17,293	15,167
Network diameter	6	6	7
Network radius	3	3	4
Characteristic path length	2.542	2.371	2.577
Clustering coefficient	0.000	0.000	0.000
Network density	0.114	0.150	0.116
Network heterogeneity	0.850	0.780	0.869
Network centralization	0.230	0.325	0.309
Connected components	1	1	1

Highly connected clusters were retrieved for every network, four for the pair modern:wild and three for the other two pairs (Figures 3, 4 and 5). On a closer analysis, we detected some links in highly connected clusters. Thus, bacterial functional units M00026 (histidine biosynthesis, PRPP ≥ histidine), M00032 (lysine degradation, lysine ≥ saccharopine ≥ acetoacetyl-CoA), M00141 (C1-unit interconversion) and M00376 (3-hydroxypropionate bi-cycle) were highly connected in the soil of tomato landraces and wild relatives (Figure 3a), whereas modern varieties and wild relatives were con-nected by modules M00141, M00376, M00021 (cysteine biosynthesis, serine ≥ cysteine) and M00089 (triacylglycerol biosynthesis)(Figure 4a–c). Finally, modules M00026 and M00141 were highly represented in modern varieties and tomato landraces (Figure 5a,b).

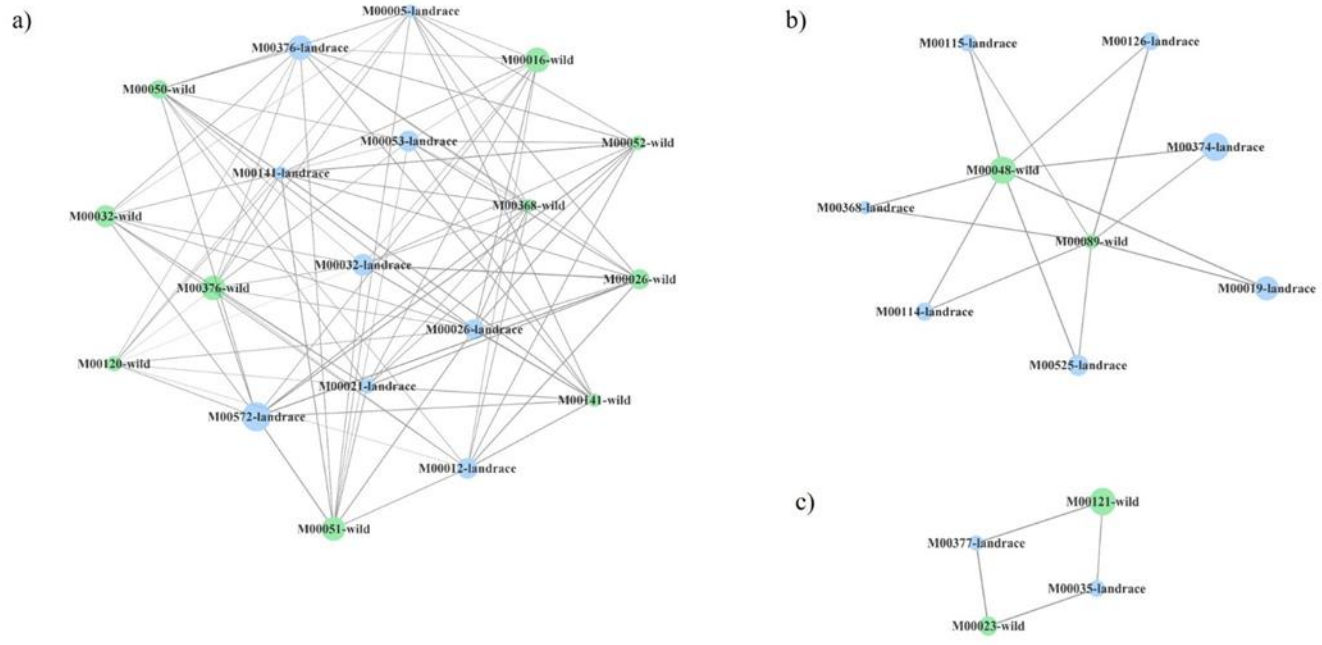

Figure 3. Three (a–c) most connected clusters in co-presence networks for the couple tomato landraces (blue):wild tomato related species (green). Node sizes reflect average relative abundance of each KEGG module. The line thickness is proportional to the edge weight.

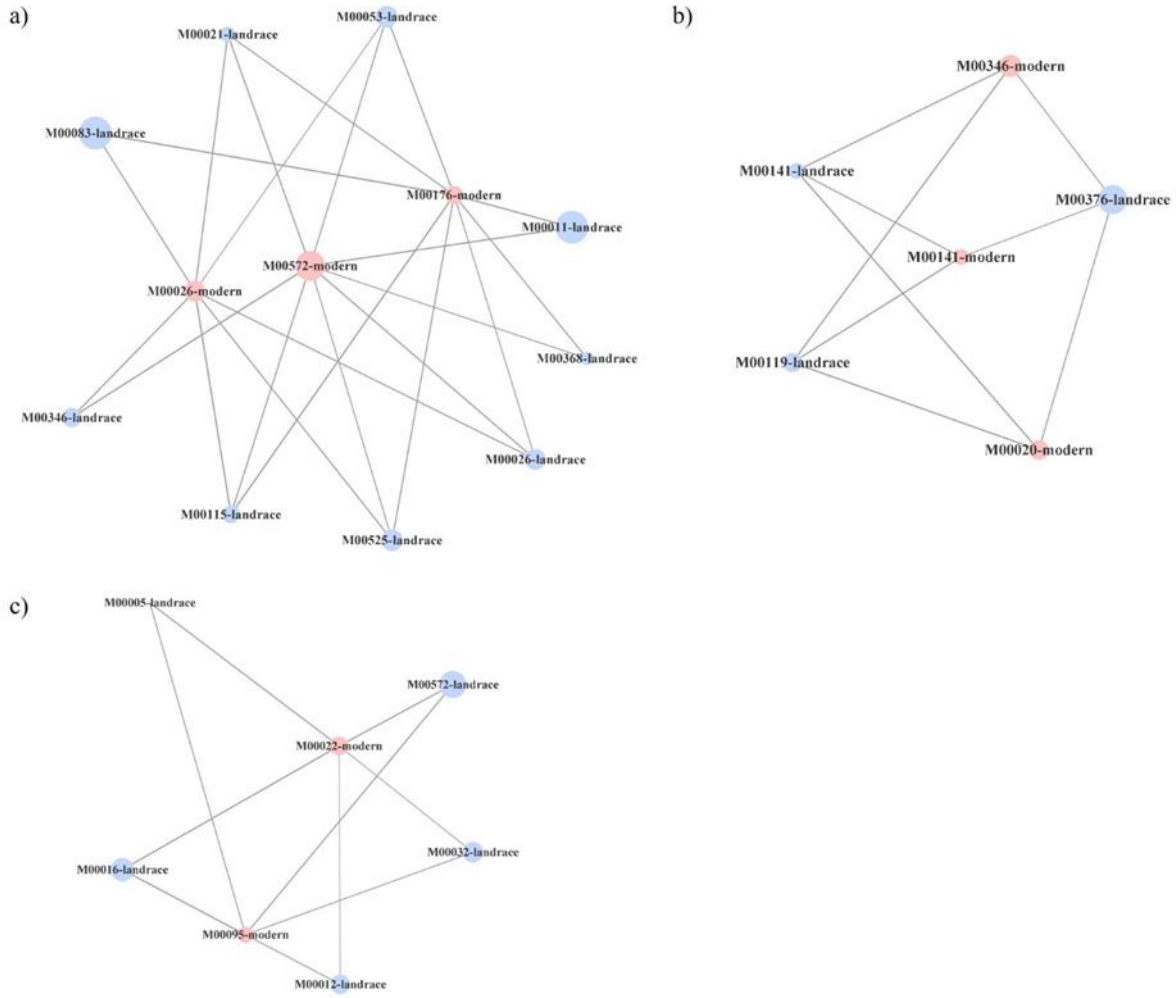

Figure 4. Three (a–c) most connected clusters in co-presence networks for the couple modern commercial cultivars (red):tomato landraces (blue). Node sizes reflect average relative abundance of each KEGG module. The line thickness is proportional to the edge weight.

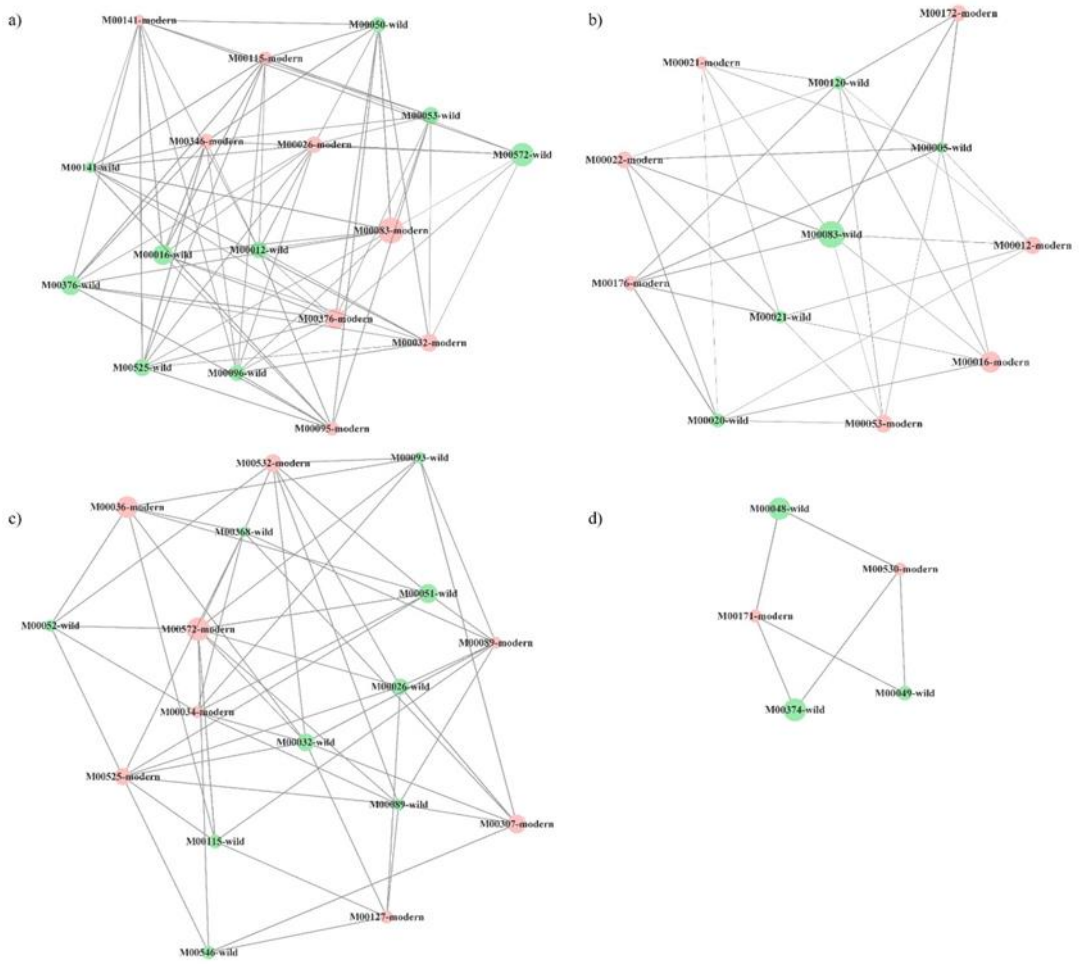

Figure 5. Four (a–d) most connected clusters in co-presence networks for the couple modern commercial cultivars (red):wild tomato relates species (green). Node sizes reflect average relative abundance of each KEGG module. The line thickness is proportional to the edge weight.

3. Discussion

Using metagenomic analysis, the functional potential of the bacterial community was predicted and the associated metabolic pathway network explored (Table 1). The levels of the global metabolic pathway for aromatic degradation were significantly higher in bacteria associated to accessions of tomato wild relatives. The modules belonging to this pathway catalyze reactions involving various polyphenols such as catechol. Humification is known to involve biotic and abiotic transformations of soil litter layer materials into mature humic substances, where catechol and o-quinones derived from biotic activity in humic substance synthesis play a fundamental role [17]. In addition, the increase in catechol promotes the formation of humic substances through abiotic reactions in the catechol–Maillard system [18]. Thus, the observed decrease in the degradation of aromatic compounds to catechol indicates a loss of degradation capacity due to cultivation. Organic matter and humic substances play an important role in improving soil fertility and structure, water retention capacity and C sequestration in the environment [19], which diminishes along the domestication gradient. Another possible hypothesis is that plants affect microbial populations, and changes in environmental conditions, soils and cultivation techniques—with the gradual abandonment of organic materials in favor of agrochemicals—could reduce the degradation capacity of recalcitrant organic compounds associated with domestication and breeding. On the other hand, with respect to the carbon cycle, organic C taken up by microorganisms is partitioned into growth, metabolite excretion, and respiration

[20]. We detected an increase in the Krebs cycle of wild tomato related species belowground. After incorporation into the bacterial biomass, C is usually converted into stable organic matter or decomposed and respired as CO_2 depending on the chemical recalcitrance and degree of protection of the organic matter [21].

In this context, it has been suggested that crop wild relatives establish beneficial interactions with microbes more frequently than domesticated cultivars [22]. Given the abandonment of some agricultural practices related to exogenous organic matter inputs and the preservation of endogenous C, a concomitant loss of bacterial functions dealing with recalcitrant organic matter has been occurring for many years. It has also been evidenced that agronomic practices, such as tillage, irrigation and the use of other inputs such as pesticides and fertilizers influence the belowground diversity and functions of soil microbes [23]. We therefore postulate that a loss in bacterial functions related to soil organic matter preservation occurs during tomato domestication.

A similar trend was detected in metabolic pathways related to biochemical cycles, such as the reduction in nitrates and sulphates and the formation of urea from purine metabolism. The decrease in these pathways that play a key role in plant growth could be attributed to the domestication process, or more precisely, to the emergence of modern commercial cultivars. Similar to the observations in the C-cycle, the increasing use of agrochemicals in modern agriculture may, in some way, be connected to the reduction on metabolic pathway levels caused by certain biochemical cycles.

Carbon fixation was more common in bacteria associated with modern commercial cultivars. This important process in soil carbon cycling is carried out by CO_2-fixing and CO-oxidizing bacteria and can reduce atmospheric CO_2 concentrations, thus indirectly mitigating global warming [20,24,25]. However, as no differences in the synthesis of ribulose 5 phosphate, an intermediary in the carbon fixation Calvin cycle, can be attributed to domestication, it is not possible to draw a clear picture of the effects of domestication on this ecosystem service.

On the other hand, pathways such as fatty acid and jasmonic acid biosynthesis were more commonly found in the rhizosphere of modern and landrace varieties. Fatty acids are involved in multiple functions, ranging from cell membrane constituents to cell signaling. Fatty acids have been used as indices of soil quality and even to describe food web connections [26], thus, positive feedback compared with their wild ancestors could be attributed to tomato crops. Jasmonic acid (JA) and its derivatives (collectively known as jasmonates) play an important role in regulating plant defenses against biotic stresses, and facilitating beneficial interactions between plants and microbes in the root zone [27,28]. JA signaling has been suggested to have evolved during land colonization by plants exposed to new biotic and abiotic stresses [29], and symbiotic relationships with microbes, including plant growth promoting bacteria and mycorrhizal fungi. Moreover, microbe induced systemic resistance to pathogens and pests involve JA signaling [30,31]. However, although JA production by bacteria and fungi in soil has been reported [32], its impact on plant–microbiome interactions remains unclear. Finally, regarding signalling, we detected a significant increase in the biosynthesis of gamma-aminobutyric acid (GABA) in wild tomato species compared to the groups that included cultivars. GABA is involved in inter-bacterial communication and interactions between bacteria and their host [33]. Furthermore, GABA production has been associated with bacterial overcoming of environmental stress [34].

Overall, these findings highlight the influence of tomato domestication on some molecular pathways of the associated soil bacteria, although all ancestral functional characteristics of bacteria have been conserved across time. However, we wonder whether there is a pattern of bacterial functional abundance associated with the tomato soil related to the domestication degree. To shed some light on this point, we calculated interactions between functional units of gene sets in metabolic pathways, which may help to address the question of how microbial genes work together to support specific microbiome functions [35]. In this study, we assessed pairwise relationships between bacterial functional units based on metagenomic sequencing of bacteria growing on tomato plants along a domestication gradient The highest connectance levels in

bacterial communities were found in landraces:wild pairs due to an increase in network density as measured by the higher average number of connections estab-lished expressed by the average number of neighbors (Table 2). In addition, the increased connectance in the landraces:wild pairing with respect to the other two pairs was related to the decrease in the characteristic path length, defined as the average number of steps along the shortest paths for all possible pairs of network nodes. These changes suggest an intensification of microbial connectance relative to the pairs modern:landraces and modern:wild. Finally, an increase in the pair modern:landraces regarding the network radius and diameter measuring the longest of all the shortest calculated paths in the network, suggests a decrease in module-pathway connectance.

Highly connected clusters, or sets of nodes most of which are connected with one another, were then explored (Figures 3, 4 and 5). Again, the highest connectance was detected for the pair landraces:wild varieties and nodes representing the same module in the two different types of tomato were recovered in a single cluster. For the rest of the pairs, even if they shared the same number of common modules, they were recovered in two or three different clusters. Overall, the above results suggest that certain functional groups such as the synthesis of certain amino acids or carbohydrate metabolism tend to be more evolutionarily conserved in bacterial communities associated with tomato landraces than those of modern varieties. However, we also found that most of the metabolic routes of bacteria associated to either landraces or modern cultivars with those associated to their ancestors were different. In this scenario, a possible process of divergent evolution in tomato lines, that is, the process by which groups of the same common ancestor evolve and accumulate differences in response to changes in both environmental conditions and biotic factors, could be debated. Nevertheless, further investigation is needed to clarify how tomato domestication has driven specific bacterial functions in root-associated soil.

4. Materials and methods

4.1. Field experiment

The setup of the field experiment is explained in general methods (1.1 Field experiment). In this study, cluster assays of the 27 tomato accessions, based on their degree of domestication, were carried out: (1) wild tomato species (accessions NR0407, NR1021, NR0136, NR0699, NR0937), (2) tomato landraces (accessions NR0025, NR0006, NR0044, NR0213, NR0275, NR0237, NR0469, NR0166, NR0063, NR0705, NR0612), and (3) modern commercial cultivars (accessions ABL104, ANL101, NR0071, NR0816, NR0080, NR1080, NR0504. COM1, COM2, COM3 and COM4 cultivars, which are protected under plant variety rights, have no accession number). Detailed information on soil characteristics is given in Supplementary Material Table S1.

4.2. Predictive metagenomics profiling

Soil processing and is molecular analysis of soil bacteria is described in general methods section 1.2 (Soil processing). To determine the potential functional metabolic capabilities of soil bacterial communities, we used Tax4fun, an open-source R package, which predicts the functional capabilities of these communities based on 16S datasets. Tax4Fun is applicable to output obtained from the SILVAngs web server [46]. Tax4fun was implemented in Shotgun Data Profiling (SDP) module of MicrobiomeAnalyst to predict functional pathways based on Kyoto Encyclopedia of Gene and Genome (KEGG) annotations (https://www.kegg.jp/) [47,48]. KEGG functional annotations were based on modules, i.e., functional units of gene sets in the KEGG metabolic pathways database that can be linked to specific metabolic capacities and other phenotypic features [44].

4.3. Functional networks

Similarities on functional profiles across tomato types were studied by looking for correlations in the abundance of modules. CoNet plug-in method [49] in Cytoscape software v.3.8.2 [50] was used to visualize

these relationships by building co-occurrence networks. Thus, two nodes representing the same module in different tomato types should be connected in the case that both tomatoes have a similar pattern of abundance for that module. Thus, building networks by tomato type pairs gives an idea of the conservation of modules across domestication (i.e., the number of links between the same module in different tomato types). Co-occurrence networks were constructed based on the identification of significant positive associations, that is, co-presences of functional units in the tomato root-associated microbiome. Due to the different number of samples/tomato varieties in each domestication type, for arranging the construction of network, the number of samples in each domestication type was adjusted to the tomato type with the least number. The selection and order of samples was arranged randomly. This analysis was repeated 5 times by shuffling the input sample order to avoid spurious results. To run the analysis, KEGG modules having less than 20 reads were discarded from the analyses. KEGG module abundance was normalized by sample. A total of 2000 permutations were set up by keeping edge number constant. The significance of co-presences were evaluated by a combination of Spearman and Pearson correlations and Bray–Curtis dissimilarity (see e.g., [48,51], corrected for multiple testing using Bonferroni). Finally, the MCODE Cytoscape plugin [52] with default settings was then used to detect highly connected network modules. Only modules with an MCODE score greater than 2.0 were retained for analysis [35].

5. Conclusions

In our study we found that core bacterial microbiome is similar between tomato landraces and modern commercial cultivars with small differences with wild tomato. These findings highlight the influence of the host genotype on the potential functions of soil bacterial communities. Furthermore, we found that differences in eight biological metabolic pathways between wild tomatoes compared with tomato landraces and modern commercial. Thus, we conclude that all ancestral functional characteristics of bacteria have been conserved across time. In the light of these results, it becomes apparent that the capacity of soil bacteria to provide ecosystem services is affected by agronomic practices linked to the domestication process, particularly those related to the preservation of soil organic matter. We also assayed the relationships between functional units of bacteria growing on tomato plants along a domestication gradient, finding the highest levels of connection between bacterial communities driven by tomato landraces and their wild ancestors.

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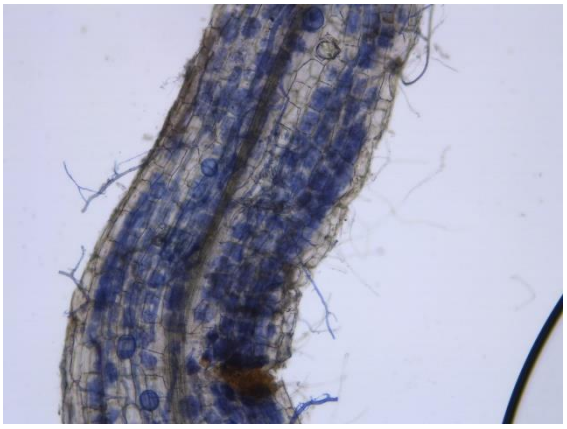
7. Supplementary material

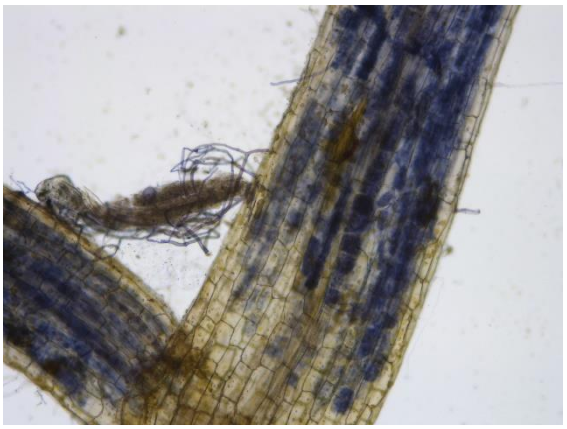
Table S1: TN: Total Nitrogen (%), TOC: Total Organic Carbon (%), and elemental analysis of nutrients (ppm) in soil

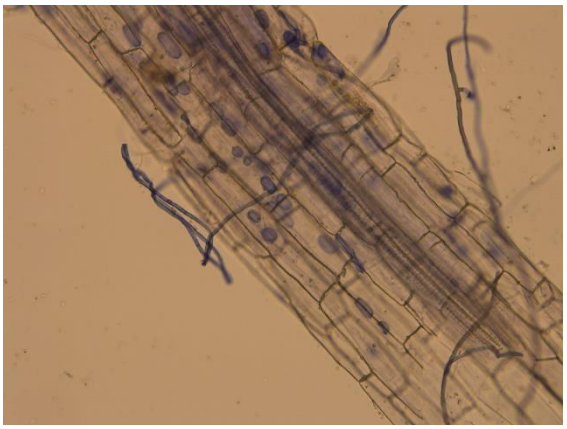
TN	**TOC**	**Al**	**As**	**Ca**	**Cd**
0.303±0.008	1.760±073	40938±1244	31±0.613	9226±230	2.22±0.022
Co	**Cr**	**Cu**	**Fe**	**K**	**Li**
22±0.250	54±1.10	39±0.264	33233±298	10525±342	29±0.403
Mg	**Mn**	**Na**	**Ni**	**P**	**Pb**
8086±78	875±20	0.046±0.002	46±0.884	995±41	24±0.585
S	**Si**	**Sr**	**Ti**	**V**	**Zn**
432±11	3718±110	53±0.919	1326±27	69±1.62	102±3.37



Chapter 3: Arbuscular mycorrhizal fungal communities drive fruit development and pathogen incidence in field cultivated tomatoes

Authors: Lisanne Smulders, Emilio Benítez, Maria Jose Pozo, Álvaro López-García





Abstract: Increasing demands for sustainable agriculture has raised interest in the use of rhizosphere microbial communities to enhance plant growth and defence. Arbuscular mycorrhizal fungi (AMF) form symbiosis with plant roots and provide the plant with nutrients and protection in exchange for carbohydrates and lipids. Changes in species distribution of associated AMF communities may result in different expression of aboveground plant traits. We studied whether the spatial distribution of AMF in field soils affected the functioning of tomato plants, i.e. the development of their fruits and the incidence of pathogenic viruses. We found that AMF communities did not differ across the 26 studied tomato accessions representing a range of domestication levels, i.e. from wild to modern, and were mainly driven by spatial location, especially in terms of their phylogenetic composition. Once spatial distribution was considered, the AMF taxonomic and phylogenetic composition significantly explained the expression of most aboveground plant traits, except tomato chlorosis virus (ToCV) incidence and plant biomass. Furthermore, we found that AMF composition explained variation in root colonization. Besides, we could identify a set of known AMF species whose presence determined decreased pathogenic incidence or increased plant fruit development.

1. Introduction

In agricultural environments the increased use of fertilizers and pesticides led to crops that are often more attractive to insect pests and diseases [1]. Therefore, there are increased demands to switch to organic practices and the engineering of plant associated microbiomes, e.g. rhizosphere microbial communities. This group of microbes is involved in multiple ecosystem services, such as increasing plant nutrition and disease suppression [2], making them a primary target for developing next generation agricultural systems [3].

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs requiring a host plant to survive. Symbiosis between plants and AMF have existed for over 400 million years and occur in 80% of land plant species [4,5]. In exchange for carbohydrates and lipids, AMF provide the plant with nutrients (mainly phosphorous) and increased resistance to biotic and abiotic stresses such as drought and salinity [2,6,7]. Indeed, AMF trigger changes in the plant root architecture and their metabolic profile, and accumulation of defense compounds [8,9]. Besides protection against root pathogens, mycorrhizal colonization can also increase the plant resistance to aboveground attackers by priming plant defenses. This mycorrhiza induced resistance (MIR) is generally effective against necrotrophic pathogens and leaf chewers [10–13]. In contrast, its protective role against viruses and biotrophic pathogens is more controversial, with varying results [14]. For example, in tomato AMF were found both to prime tolerance against one virus [15] but increase susceptibility against another [16]. Furthermore, AMF may positively affect other beneficial plant interactions, e.g. by attracting parasitoids [17].

However, experimental evidences revealed that there is high functional diversity among AMF species: e.g. in general some are more prone to provide nutrients and others stress resistance [18,19]. Moreover, AMF functionality has been seen to be phylogenetically conserved across AMF families, allowing the use of their phylogeny as a proxy of their function [20]. Indeed, analyzing AMF phylogenetic turnover can be used to quantify the existence of shifts in their functioning [19,21,22]. Hence, the taxonomic and phylogenetic composition of the AMF community with which a plant is associated will be related to the functioning of the symbiosis and, consequently, the plant performance. On the other hand, the effect of AM symbiosis on plants may also depend on plant genotype and their degree of domestication [23,24]. For example, domesticated breadfruit varieties showed reduced but more diverse AMF communities, which potentially indicated lower responsiveness to the symbiosis [25]. Moreover, domestication reduced the responsiveness of tomato to *Trichoderma* colonization [26]. In addition, high levels of intraspecific variation in maize and tomato has also been evidenced regarding the level of defense induction by AMF [27–29].

Despite specific effects of different AMF taxa on plant functioning and performance, AMF community composition varies intrinsically across plant varieties and is shaped by soil conditions [30]. Studies at large-scale (comparing different habitats) have evidenced that AM fungal communities are structured more by environmental characteristics than by host plant identity [21,22,31]. In general AMF species distribution follows soil gradients such as pH or C/N ratio [31]. Furthermore, interactions across AMF species, such as competition for limiting resources, also potentially structure community composition [22]. Lastly, processes dependent on probabilistic events, such as dispersal limitation, play a role in spatially structuring their communities [21,31]. Indeed, at small spatial scales (plot level), spatial distance and phylogenetic patterns are important drivers of their communities [32]. Thus, the effects of AMF on plant functioning under field conditions is influenced by different processes and can be confounded with several environmental gradients. The effects of mycorrhiza on plant functioning have often been observed under controlled greenhouse conditions [33–35] which seem more likely to show significant effects [36]. Considering the biotic spatial heterogeneity usually found in soil [37] and the dependence on the interplay between AMF identity and plant variety when assessing the plant functioning, we aimed to analyze how the spatial variation in the AMF community composition affected the response of tomato plants in terms of fruit development and pathogen infection. The study included the characterization of AMF communities in a cultivated area where tomato varieties ranging from wild to modern cultivars were grown. Particularly, we aimed: 1) to explore the taxonomic and phylogenetic variation of AMF communities in the studied field in terms of spatial location and tomato type (modern, early domesticated and wild varieties); and 2) with this information, to study whether the found variability in root AMF communities drive the expression of plant traits related to fruit development and pathogen infection. We hypothesized that spatial location, as far as it could represent environmental abiotic gradients, would be more important that tomato variety to drive AMF community composition. In turn, the found spatial variability in AMF community composition would be linked to the expression of plant traits, especially those involved in pathogen resistance.

2. Results

2.1 Source of AMF community composition

Most VTs detected were from the family *Glomeraceae* (21). Other VTs belonged to the *Claroideoglomeraceae* (3), *Diversisporaceae* (3), *Archaeosporaceae* (1) and *Paraglomeraceae* (2).

Only spatial location was found to significantly drive the VT compositional turnover of AMF communities (explained variation 6.07%, $p<0.001$, Table S2), meanwhile tomato type and variety did not explain it ($p=0.674$). Thus, AMF communities were similar between the different tomato varieties used in this experiment.

2.2 Source of AMF phylogenetic turnover

Variation in AMF phylogenetic turnover was explained by spatial location in a wider extent (17.68%, $p<0.001$, Table S2). Tomato type and variety did not explain it ($p=0.911$). Thus, tomatoes belonging to different varieties and domestication degrees did not select for different phylogenetic groups of AMF (e.g. particular genera or families).

2.3 Source of variation of tomato traits

By including both AMF community and spatial variation into one model, explanation due to either can be studied independently of the other. The fitted models showed that spatial position explained most variation in plant traits (Table 1). As found for the AMF communities, this variation can be understood as possible

environmental gradients affecting the expression of tomato traits. Except for colonization, spatial position explained between 19.0 and 51.4% of variance in tomato traits (see R2 calculation for each variable type in Table 1). Tomato variety (nested in tomato type) mainly explained resistance traits and colonization, ranging from 17.3 to 30.0%. Tomato type, i.e. domestication degree, per se significantly explained variation in virus incidence (TYLCV, 17.3%), plant symptoms (29%) and fruit traits (ranging from 9.4 to 16.4%). Plant symptoms and fruit traits varied between varieties, with wild varieties generally showing more symptoms, and higher tomato number with lower weight (Figure 1). Similarly, wild varieties seem better protected to TYLCV, while varying little between most modern and early domesticated varieties. Thus, domestication appeared to lead to less but heavier tomatoes and decreased plant symptoms, and reduced TYLCV resistance.

Table 1. Linear modeling of plant resistance traits, fruit traits and AMF colonization. Spatial location (space, MEM axes), tomato type and variety (nested in tomato type), and (A) PCOA axes of taxonomic AMF community composition or (B) PCOA axes of AMF phylogenetic turnover, were tested as predictors. Values indicate combined R2 for each model predictor type. Significant drivers are shown with asterisks: *p<0.05, **p<0.01, ***p<0,001.

A

		Space	**Tom.Type**	**Tom.Type: Variety**	**PCOAs$_{AMF\text{-}tax}$**
Resistance	TYLCV	0.329***	0.041**	0.173***	0.190***
	TOCV	0.190*	0.151**	0.029	0.112
	Symptoms	0.254***	0.039***	0.290***	0.258***
Morphology	Plant biomass	0.264**	0.096*	0.048*	0.146
	Fruit number	0.514***	0.164***	0.057***	0.184***
	Fruit weight	0.394***	0.111***	0.112***	0.244***
AMF Colonization		0.062**	0.015	0.300***	0.333***

B

		Space	**Tom.Type**	**Tom.Type: Variety**	**PCOAs$_{AMF\text{-}phy}$**
Resistance	TYLCV	0.329***	0.017	0.190***	0.217***
	TOCV	0.190*	0.051*	0.052	0.155
	Symptoms	0.254***	0.030***	0.290***	0.261***
Morphology	Plant biomass	0.264*	0.058**	0.053*	0.139
	Fruit number	0.514***	0.005	0.130***	0.232***
	Fruit weight	0.394***	0.022*	0.150***	0.291**
AMF Colonization		0.062**	0.000	0.311***	0.288***

Both AMF community variables (composition and phylogeny) were found to significantly drive all tomato traits except TOCV and plant biomass. The explained variance ranged between 18.4 and 33.3% (Table 1). Significant differences in AMF colonization were detected between tomato varieties (Figure 1E). Only a slight trend following the domestication degree was detected (0 to 1.5% explained variance), even though domestication significantly affected resistance and morphological traits (Table 1). Furthermore, both AMF community composition as AMF phylogeny explained ca. 30% of variation in AMF colonization.

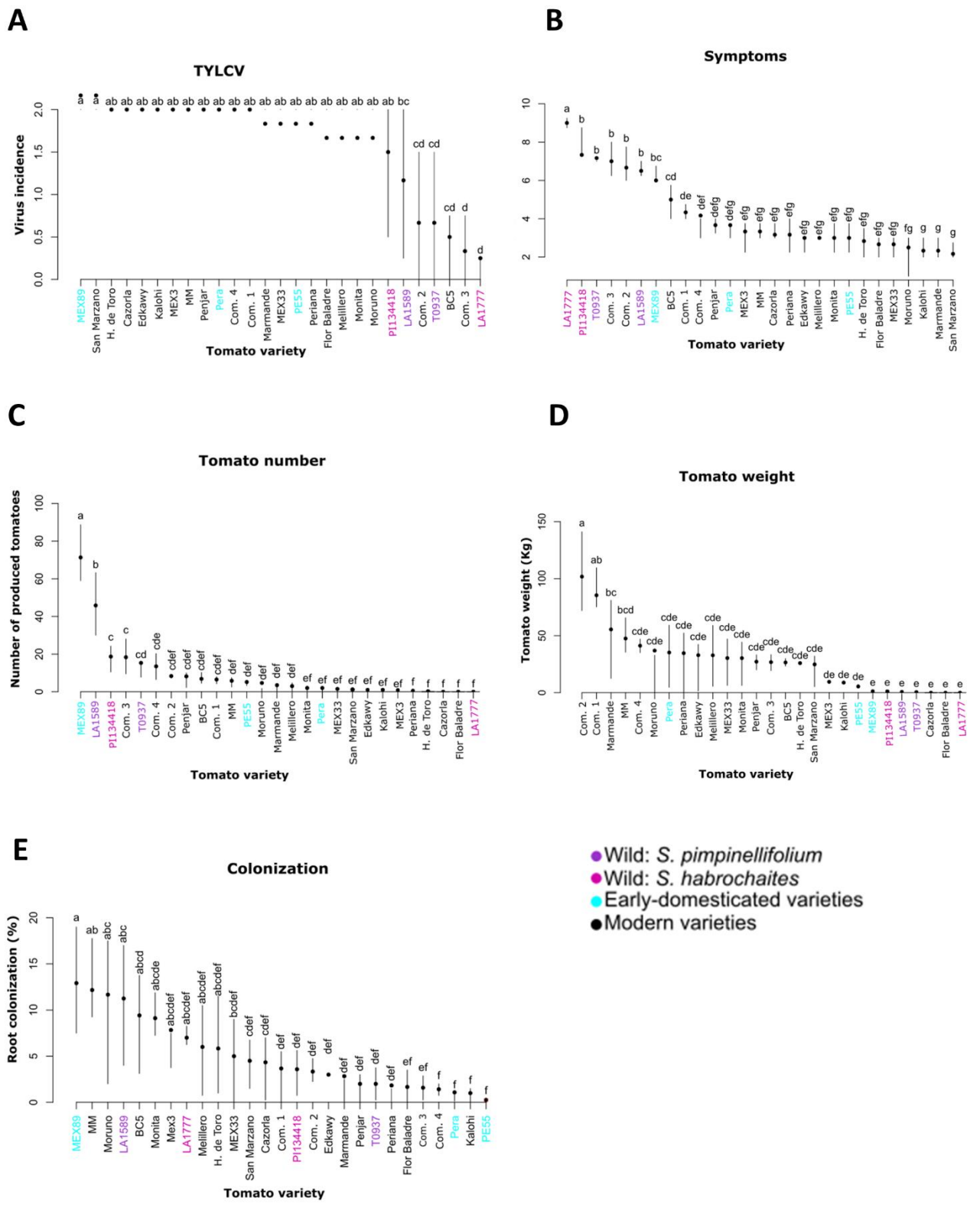


Figure 1. Average TYLCV infection level (A), plant symptoms (B), tomato number (C), tomato weight (Kg, D) and root colonization level (%, E) of 27 tomato varieties (*Solanum lycopersicum* Mill., *S. pimpinellifolium* and *S. habrochaites*). Tomato varieties were divided into wild (purple for *S. pimpinellifolium* and pink for *S. habrochaites*), early-domesticated (light blue) and modern. The mean is shown (dot) with the interquartile range (IQR). Averages of bars not sharing letters significantly differ according to duncan post hoc test.

2.4 Correlations between VTs and plant traits

A total of 19 VTs were correlated with PCOAsAMF-tax that significantly drove tomato traits (Table 2, Table S3), of which six were known species. Most VTs belonged to the *Glomus* genus (11). Other genera included *Diversispora* (3), *Claroideoglomus* (2), *Paraglomus* (2) and *Dominikia* (1). The response of plant traits to the abundance of these VTs was variable.

In general terms, VTX054 and VTX419 had negative effects on tomato resistance (TOCV, symptoms), but positive effects on morphology (Table 2). On the other hand, VTX113 and VTX114 (*Rhizoglomus*) reduce symptoms and had negative effects on biomass, while VTX155 (*Dominikia iranica*) showed the opposite pattern, increasing symptoms and biomass.

We found six VTs with positive effects on plant resistance, with three VTs (VTX065, VTX113, VTX114) reducing plant symptoms and three VTs (VTX065, VTX130, VTX419) reducing viral incidence. On the other hand, five VTs (NewVTX1, VTX062, VTX155, VTX418 and VTX419) increased virus incidence. However, except NewVTX1 and VTX062, despite this negative effect on resistance, positive effects on biomass (all three) and tomato number (only VTX419) were observed.

Considering morphology, six VTs (VTX143, VTX153, VTX155, VTX199, VTX418, VTX419) had positive effects, mostly on biomass and tomato number. Of these VTX153 and VTX199 showed an inverse relation between tomato weight and tomato number. In total, ten VTs had negative effects on morphology.

Correlations with significant PCOAsAMF-phy were studied at the family and genus level (Table S4). Plant symptoms were negatively correlated to PCOAsAMF-phy indicating a higher abundance of *Diversisporaceae* at the family and genus level when lower symptom development. Biomass was positively correlated to *Diversisporaceae* and negatively to *Glomus* and *Paraglomus* genera.

Table 2. VT species that correlative either positively (+) or negatively (-) with different plant traits (resistance and morphology) or AMF colonization.

VTX	Species	Positive	Negative
VTX056	*Claroideoglomus* sp.		Symptoms, biomass, tomato weight
VTX193	*Claroideoglomus* sp.	Tomato weight	
VTX054	*Diversispora celata*	TOCV, symptoms, biomass, tomato number	
VTX060	*Diversispora celata*		Tomato number
VTX062	*Diversispora* sp.	TOCV	
VTX155	*Dominikia iranica*	Symptoms, biomass	
VTX065	*Funneliformis* sp.		TYLCV, symptoms
VTX130	*Glomus* sp.		TOCV
VTX143	*Glomus* sp.	Tomato number	
VTX153	*Glomus* sp.	Tomato weight	Tomato number
NewVTX1	*Glomus* sp.	TYLCV, symptoms	
VTX418	*Glomus* sp.	Symptoms, biomass	Tomato number
VTX419	*Glomus* sp.	TOCV, symptoms, biomass, tomato number	TYLCV
VTX199	*Glomus macrocarpum*	Tomato number	Tomato weight
VTX108	*Rhizoglomus* sp.		Tomato number

VTX113	*Rhizoglomus intraradices*		Symptoms, biomass
VTX114	*Rhizoglomus irregularis*		Symptoms, biomass
VTX001	*Paraglomus* sp.	Tomato weight	Tomato number
VTX281	*Paraglomus* sp.	Symptoms, biomass	Tomato number

3. Discussion

3.1 Sources of taxonomic and phylogenetic variation of AMF communities in the studied field

As a first step to study the effect of AMF communities on tomato traits, we explored if these communities varied across space and domestication degree of tomatoes. AMF community composition was similar across tomato varieties and domestication degrees, in agreement with studies comparing AMF communities in different maize and chickpea varieties [38,39]. Location was found to be a major component influencing both AMF community composition but especially AMF phylogenetic turnover. The latter evidence points out towards an environmental (soil) gradient driving their communities, as far as phylogeny could be understood as a proxy for niche requirements [40,41]. Similarly, Horn et al showed that in a grassland (small scale) spatial distance and phylogenetic turnover mainly predicted AMF community composition [32]. This is further supported by another study comparing sand and clay soils and two studies of Dumbrell et al. showing that AMF community composition differs between soil types and environments such as over a pH gradient [21,22,31]. In accordance, the clear effect of space on the phylogenetic turnover points out towards the effect of non-measured environmental variables [40]. However, changes in phylogenetic turnover can also be caused by stochastic processes such as dispersal (degree of movement between communities), drift (changing population sizes due to chance) and speciation causing spatial imprints on AMF communities [22,31,42]. Nevertheless, it has been seen that environment is often more important than space to explain the distribution of AMF communities [31,43].

Thus, in the present study we interpret the AMF community spatial signature as a consequence of an unidentified soil gradient. As far as this gradient could be affecting plant functioning as well, space was also included in the subsequent analyses of plant traits together with AMF community information to try to disentangle the pure, non-confounded effect, of AMF communities on plant functioning.

3.2 Sources of tomato trait variation

As a way to infer possible effects of tomato domestication on symbiotic performance, we analyzed AMF root colonization. It significantly differed across tomato varieties but it was independent of tomato type, as shown for other crops such as wheat [44] and maize [29]. Thus, even though AMF communities were similar at the compositional level, tomato varieties developed different colonization patterns, suggesting some control of the plant variety on this symbiotic trait. Although the ability to establish mycorrhizal colonization has been described to be under genetic control, our results do not support various studies evidencing reduced mycorrhizal capacity due to domestication [23–25]. In contrast, the found variation in the studied plot of both AMF taxonomy and phylogeny allowed that the same plant variety could have faced different AMF communities in the experimental plot whose inherent characteristic would have modified the final symbiotic output, i.e. the colonization extent. Moreover, as seen in the models including the AMF phylogeny, the shift in AMF families, not necessarily a shift in species/virtual taxa, can cause a change in the observed colonization pattern. Indeed, the colonization strategies of AMF have been seen to be conserved in their phylogeny [45].

Domestication seemed to have reduced resistance to TYLCV while plant symptom development was reduced, which could mean increased tolerance to TYLCV infection. Similarly, in maize, tolerance to Western corn rootworm increased with domestication but decreased with breeding [46]. However, maize tolerance to corn leafhopper was not affected by domestication [47].

Next, we modeled whether the observed differences in AMF communities influence aboveground plant traits. Since AMF are known to be functionally diverse, it can be expected that plants hosting different AMF communities differ in the expression of resistance traits or production [4,48]. We observed an effect of AMF community composition and phylogenetic turnover on aboveground plant traits. As explained, this effect was independent on possible underlying spatially structured soil variables. When looking behind these patterns, we observed that most VTs (63.3%) were correlated to any of the measured plant traits. We detected six VTs with positive effects on resistance, from the genera *Claroideoglomus*, *Funneliformis*, *Glomus* and *Rhizoglomus*. Powell et al. who found phylogenetic conservation of AMF functional traits and host benefits, with variation mostly associated to early divergences between e.g. Glomerales and Diversisporales [20]. Indeed, five VTs had negative effects on resistance, from the genera *Dominikia*, *Diversispora* and *Glomus*. Nevertheless, their study included only four AMF families. Interestingly, most of the *Glomus* taxa and the single *Domininikia* taxa showed a positive effect on plant morphology. Indeed, AMF are not expected to protect against viruses, although different results have been shown [15,16]. The results could be also influenced by a differential attraction of the virus vector whitefly *Bemisia tabaci* according to the plant health state [14,49]

We found six VTs of the genera *Dominikia*, *Funneliformis* and *Glomus* with positive effects on morphology. Moreover, we found two VTs with an inverse relation between tomato weight and tomato number. Furthermore, we found ten VTs with negative effects on morphology, from the genera *Claroideoglomus*, *Diversispora*, *Glomus*, *Paraglomus* and *Rhizoglomus*. Different studies found that members of the *Claroideoglomeraceae*, *Diversisporaceae* and *Paraglomeraceae* had positive effects on Chrysanthemum biomass [50] and protect against plant-feeding nematodes [19], in contrast to our found negative effect of *Claroideoglomus* on plant biomass.

Some AMF species are well studied as they are used regularly as model species, such as *Funneliformis mosseae* which is known to protect against infection by oomycetes [51], chewing caterpillars [52,53] and nematodes [54,55]. To this we can add a positive effect of *Funneliformis* on TYLCV resistance by reducing the virus incidence and plant symptoms. *Rhizoglomus irregularis* was found to provide enhanced resistance to *Botrytis cinerea* [11] and chewing caterpillars [53]. Indeed, the detected *Rhizoglomus* taxa provided reduced plant symptoms. Also, *Rhizoglomus* provided most benefits to leek growth, followed by species of *Funneliformis*¸ *Claroideoglomus* and *Diversispora* [56]. Indeed, we found a positive correlation of *Diversispora* with biomass.

These results together show how the outcome of interactions between AMF fungi and plants depends on the fungal species that are present in the soil and can vary between taxa of the same genus. Even though increased AMF diversity generally results in increased ecosystem functioning [57] results between and within studies are often inconsistent [58–60].

4. Materials and methods

4.1 Field experiment

The setup of the field experiment is explained in general methods (1.1 Field experiment). Table S1 shows the values obtained for each plant. Virus frequency and plant symptom development were used as resistance traits (Table S1A), while aboveground biomass, the number of produced tomatoes and the total tomato fruit weight were used as morphological traits (Table S1B). Soil processing is described in general methods (1.2 Soil processing) and the results are shown in Table S1C.

4.2 Determination of mycorrhizal colonization

Mycorrhizal colonization was determined using the ink staining method of Vierheilig et al. [61]. Roots were incubated in 10% KOH for 20 minutes at 90°C. Afterwards, roots were washed and acidified in 2% acetic acid for 5 minutes. Then the staining solution containing 5% ink (Lamy T51) in 2% acetic acid was added and roots were incubated at 60°C in a WNB water bath with shaking device (Memmert GmbH + Co.KG, Germany) for about 20 minutes. Roots were then washed to remove the ink solution and stop the staining reaction. The degree of mycorrhizal colonization (expressed as the percentage of total root length colonized by AMF) was calculated with the gridline intersection method using a Nikon SMZ800 stereomicroscope and bright field conditions [62].

4.3 Molecular analysis of root fungi

Diversity of AMF in the roots was determined by Illumina Miseq sequencing [9] on four to six plant replicates per tomato variety. DNA was extracted from 100mg fresh roots from each sample. Roots were dried (50ºC 24h) and grinded with glass beads in a Retsch MM301 mixer mill (Retsch Gmbh, Haan, Germany) for two minutes at 30Hz. DNA was extracted from the resulting powder using the DNA plant kit (Bioline, Memphis, Tennessee, USA) following the manufacturer's instructions. PCR reaction targeted the 18S rRNA gene using the Glomeromycota-specific primers NS31 [63] and AML2 [64]. The products were sequenced on the Illumina MiSeq platform using a 300 × 2 nucleotide paired-end protocol (genomic facilities of the López-Neyra Institute of Parasitology and Biomedicine, IPBLN-CSIC, Granada, Spain) further details in [65]. Raw data was demultiplexed and barcodes removed. Samples were returned as individual per-sample fastq files from the sequencing facility.

The original 9,617,755 reads were processed with the amplicon sequence variant (ASV) analysis pipeline known as Divisive Amplicon Denoising Algorithm (DADA2 v. 1.16 [66]): Sequences were trimmed to 295bp (forward) and 290bp (reverse), primers removed and expected errors set up to a maximum of 2. Sequences were dereplicated and the error rate model inferred and used to implement the sample inference algorithm to remove Illumina sequencing errors. Forward and reverse reads were merged, and the amplicon sequence variant (ASV) abundance table generated. After chimera removal, LULU curation algorithm was used to further reduce sequencing errors [67]. Then, taxonomy was assigned using SILVA database v132 and the RDP algorithm [68]. To improve the taxonomic assignment, the SILVA database was supplemented with Glomeromycotan sequences from MaarjAM database [69]. After removing non-glomeromycotan sequences, ASVs were clustered by Virtual Taxa (VT) at the 97% similarity level against the MaarjAM database (accessed on 18TH October 2021). One ASV (6 reads) showing a low overlap in MaarjAM database was discarded from further analyses. ASVs non-fitting at a minimum of 97% were aligned together with the rest of ASVs using MAFFT [70] and clustered at 97% using VSEARCH [71]. Those ASVs clustering with VT-named ASVs were added to the existing cluster and those clustering alone were considered as new VTs. This resulted in the

identification of 30 VTs, from which two were considered new VTs. After inspecting the rarefaction curves, samples with less than 150 reads (44 out of 270 samples) were excluded from further analyses since they did not reach the plateau (Figure S1).

4.4 Statistical analysis

For all statistics, R version 4.0.4 (2021-02-15) was used. The number of reads per VT was used as a proxy of relative abundance of each VT in a sample. This abundance matrix was Hellinger-transformed prior to subsequent analyses.

4.4.1 Spatial modelling

In order to model the spatial distribution of both AMF communities and tomato traits, the spatial position of samples was decomposed by Moran Eigenvector Mapping (MEM) to factor the spatial autocorrelation at multiple scales [72]. This method allows testing different types of spatial structures. We followed the pipeline published by Horn et al. [32], available at [73]. The best spatial model was selected using the Akaike Information Criterion AIC [74] independently for each response variable (see subsections below). Subsequently, the best linear combination of eigenvectors (five MEM axes per selected model) was chosen on the basis of the highest correlation with the data and the lowest AIC (Figure S2). The selected five MEM axes were fed as the spatial component in further analysis.

4.4.2 Source of AMF community composition turnover (Hypothesis 1)

To explore the relative influence of location (MEM axes) and tomato type (wild, early-domesticated or modern) on fungal AMF community composition a variation partitioning approach based on redundancy analysis (RDA) was used [75]. The explained variance by each group of factors and their covariation was obtained by means of varpart function (vegan R package [75,76]). The significance of location and tomato type were estimated via partial RDA, by controlling for the other variables meanwhile estimating the effect of the former in each case.

4.4.3 Source of AMF phylogenetic turnover (Hypothesis 1)

To determine factors behind possible phylogenetic turnover across AMF communities and hence, changes in the functional profile of AMF communities, a phylogenetic tree was constructed using IQ-Tree algorithm [77]. For each VT, the sequence of the most abundant ASV was used as the representative sequence and all of them were aligned using MAFFT online tool [70]. Phylogenetic turnover of AMF communities was obtained by using the comdist function (picante R package [78]). This function uses the VT abundance matrix and a VT x VT phylogenetic distance matrix (obtained from the phylogenetic tree) to calculate mean phylogenetic distances across samples. This analysis results in a sample x sample distance matrix whose source of variation was analyzed via distance-based RDA (dbRDA). A similar variation partitioning analysis (previous subsection) was performed, hence elucidating the effects of spatial position and tomato variety on AMF phylogenetic turnover.

4.4.4 Source of variation of tomato plant functioning (Hypothesis 2)

The source of variation of tomato traits was analyzed by linear modeling (lm function, nlme R package [79]) including the composition of AMF communities, spatial location (MEM axes), tomato type and tomato variety (nested in tomato type) as predictors. Complementarily, the response of tomato traits was also modelled by using the AMF community phylogenetic turnover.

Modeling the effect of AMF in this way allowed to truly test the variation on tomato traits independently on possible environmental gradients that together affect the AMF communities and tomato traits. Once both types of variables are included in models, the explanation due to one of them is independent of the other ones.

For including the AMF community composition in these models, the sample x VT abundance matrix was decomposed into new variables via Principal Coordinates Analyses (PCoA). The resulting new variables (axes) were fed into the linear models. To avoid including every new calculated axis, a prior selection was performed by creating linear models of combinations of up to four axes [79] (hereafter PCoAsAMF-tax). The best combination of axes was selected according to the lowest AIC and used in a final linear model for each tomato trait. Similarly, the AMF phylogenetic turnover was decomposed into PCoA axes (hereafter PCoAsAMF-phy) and AIC selected to be included in tomato trait models. All five MEMs of spatial variables for each trait were also included in the tomato trait models. For tomato fruit average weight and tomato fruit number models, those non-fruiting tomato varieties were excluded.

If any PCoA axes were found to drive tomato traits, they were correlated (via Pearson correlation) with the abundance of individual VTs. In this way, we could relate the significant PCoA axis with particular VTs. In case that the abundance of a VT correlated with more than one PCoA axes, we only retained the one showing the highest correlation (higher correlation coefficient). The significant phylogenetic PCoA axes were similarly correlated with specific taxa but in this case with the abundance of AMF genera and families.

5. Conclusions

In conclusion, we show high levels of interspecific variation in AMF colonization between tomato varieties and species with high and low colonization levels in all tomato types. However, AMF community composition was not distinguishable between varieties. Spatial location and not tomato domestication influences AMF community composition and phylogenetic turnover in tomato. Furthermore, spatial location also influences aboveground plant morphology and resistance traits, but taking this variation into account, AMF communities independently influenced aboveground plant traits as well. Since we did not detect differences in soil nutrient composition between varieties, it could be an effect of unmeasured environmental variables such as pH or an imprint of the dispersal of *B. tabaci*. We identified one *Dominikia* and two *Glomus* taxa with positive effects on morphology, although they scored low on resistance. Furthermore, we identified one *Funneliformis* taxa with positive effects on TYLCV resistance and reducing plant symptoms and one *Dominikia* taxa with positive effects on plant biomass, although increasing plant symptoms. Within the genera *Claroidemoglomus*, *Diversispora*, *Glomus* and *Paraglomus*, we found varying effects of different taxa on resistance and morphology. Since we found different fungal genera and different taxa within genera to correlate with plant traits in different ways, we think that it is important to take into account the whole fungal community when studying their effect on plant biotic and abiotic stress.

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7. Supplementary material

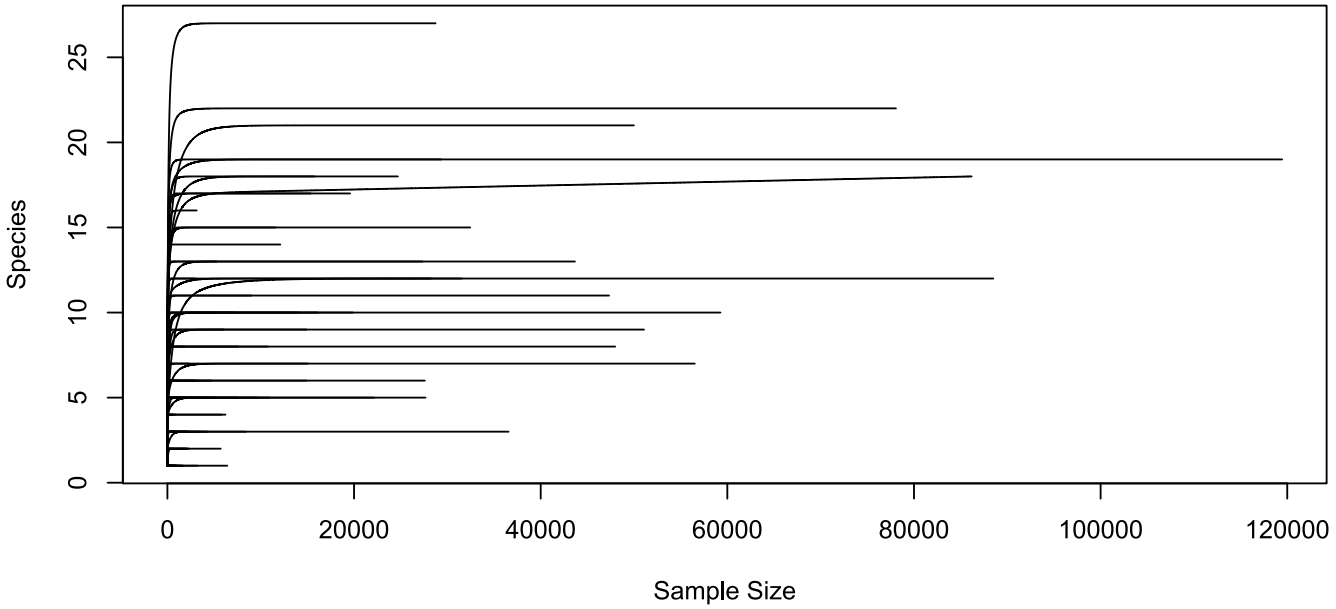


Figure S1. Rarefaction curves of roots of 27 varieties of tomato (Solanum lycopersicum Mill.. S. habrochaites and S. pimpinellifolium).

Table S1. Experimental variables included in the multivariate analysis. Results are shown for individual plants (A-B) or two soil replicates (C). A) Plant traits: Resistance B) Plant traits: Morphology. C); Soil chemical characteristics.

A) Plant traits: resistance. Frequency ToCV: Percentage of tomato replicates with tomato chlorosis virus detected with tissue-blot hybridization; Frequency TYLCV: Percentage of tomato replicates with tomato yellow leaf curl virus detected with tissue-blot hybridization; State: Plant symptoms characterization; Colonization: Percentage (%) colonization of roots.

Variety	Frequency TOCV	Frequency TYLCV	State	Colonization (%)
H. de Toro	3	2	5	7
H. de Toro	3	2	2	13
H. de Toro	2	2	2	13
H. de Toro	2	2	2	0
H. de Toro	1	2	2	1
H. de Toro	1	2	4	1
BC5	2	0	5	2.5

BC5	0	1	4	5
BC5	1	0	7	22
BC5	1	2	4	13
BC5	0	0	4	14
BC5	1	0	6	0
Cazorla	0	2	4	1
Cazorla	0	2	3	0
Cazorla	0	2	2	7
Cazorla	2	2	3	11
Cazorla	0	2	4	7
Cazorla	0	2	3	0
Com. 1	1	0	7	4
Com. 1	2	0	7	4
Com. 1	1	0	7	0
Com. 1	2	2	3	0
Com. 1	1	2	6	14
Com. 1	0	2	4	0
Com. 2	2	0	8	0
Com. 2	0	0	7	3
Com. 2	2	0	8	3
Com. 2	1	0	7	4
Com. 2	2	0	5	2
Com. 2	1	2	6	0
Com. 3	3	2	2	0
Com. 3	0	2	3	6
Com. 3	1	2	2	0
Com. 3	3	1	6	5
Com. 3	2	0	9	0
Com. 3	1	1	4	2.5
Com. 4	0	2	9	4.5
Com. 4	2	0	8	8
Com. 4	0	2	7	0
Com. 4	3	2	4	1.5
Com. 4	2	2	3	2
Com. 4	2	2	4	0
Edkawy	1	2	3	5
Edkawy	1	2	3	8
Edkawy	3	2	3	5
Edkawy	1	2	2	0
Edkawy	1	2	2	0
Edkawy	2	2	5	0
FlorBaladre	2	2	2	0

FlorBaladre	1	2	2	2
FlorBaladre	2	2	2	4
FlorBaladre	2	2	3	4
FlorBaladre	3	2	3	0
FlorBaladre	0	0	4	0
Kalohi	1	2	2	0
Kalohi	1	2	3	0
Kalohi	2	2	3	0
Kalohi	0	2	1	2
Kalohi	2	2	2	4
Kalohi	1	2	3	0
LA1589	1	2	7	14
LA1589	3	1	6	23
LA1589	1	2	7	2
LA1589	3	0	5	18
LA1589	3	2	7	1
LA1589	3	0	7	10
LA1777	0	0	10	9
LA1777	0	0	9	8
LA1777	0	0	9	7
Marmande	0	1	8	4
Marmande	0	2	3	3
Marmande	2	2	2	0
Marmande	2	2	2	0
Marmande	3	2	1	13
Marmande	1	1	3	0
Mellilero	1	2	3	1
Mellilero	0	0	3	13
Mellilero	0	2	3	0
Mellilero	2	2	3	3
Mellilero	2	2	2	17
Mellilero	2	2	4	0
MEX 3	1	2	3	3
MEX 3	0	2	6	8
MEX 3	2	3	3	3
MEX 3	2	2	2	2
MEX 3	1	1	4	22
MEX 33	1	2	3	6
MEX 33	2	2	2	6
MEX 33	2	2	4	0
MEX 33	2	2	3	9
MEX 33	0	2	3	0

MEX 89	2	2	2	0
MEX 89	1	2	2	12
MEX 89	1	1	2	9
MEX 89	3	2	6	15
MEX 89	2	2	7	21
MEX 89	0	2	6	16
MM	1	2	4	20
MM	2	2	7	5
MM	2	3	6	0.5
MM	2	2	3	10
MM	0	2	3	18
MM	1	2	4	17
Monita	0	2	3	0
Monita	2	2	3	9
Monita	2	2	4	19
Monita	2	2	4	9
Monita	0	2	3	7
Monita	2	2	2	8
Moruno	1	2	3	13
Moruno	2	0	4	18
Moruno	2	2	2	0
Moruno	1	2	1	18
Moruno	1	2	1	28
Moruno	2	2	6	16
PE55	0	2	3	0
PE55	0	0	1	8
PE55	0	2	3	0
PE55	3	2	4	0
PE55	1	2	3	1
PE55	1	2	1	0
Penjar	0	2	3	0
Penjar	3	2	5	0.5
Penjar	2	1	2	0
Penjar	2	2	5	0
Penjar	0	2	4	0
Penjar	1	2	4	0
Pera	2	2	3	0
Pera	2	2	2	8
Pera	0	2	4	4
Pera	2	2	3	0.5
Pera	0	2	3	1
Pera	0	2	3	0

Periana	1	2	4	0
Periana	2	2	3	1
Periana	0	2	6	4
Periana	0	2	1	9
Periana	0	1	5	2
Periana	0	2	4	0
PI134418	0	2	3	0
PI134418	1	2	2	0
PI134418	0	2	4	0
PI134418	2	2	3	6
PI134418	3	0	9	3
PI134418	0	3	8	0
SanMarzano	2	2	8	2.5
SanMarzano	1	2	3	2
SanMarzano	2	2	3	1
SanMarzano	2	3	3	7
SanMarzano	2	2	2	6
SanMarzano	2	2	1	8
T0 937	2	2	8	3
T0 937	2	0	6	6
T0 937	1	0	8	5
T0 937	2	2	8	3
T0 937	3	0	7	1
T0 937	2	2	7	0

B) Plant traits: Morphology. Biomass (kg): Total plant biomass; Tomato Number: Number of tomatoes; MeanWeight (kg): Mean weight of tomatoes; x and y: field coordinates.

Variety	Biomass (kg)	Tomato Number	MeanWeight (kg)	x	y
H. de Toro	0.55	0	0.00	28.8	6.4
H. de Toro	0.2	0	0.00	44.4	1.6
H. de Toro	0.4	0	0.00	40.8	4.8
H. de Toro	0.15	0	0.00	44.4	3.2
H. de Toro	0.1	0	0.00	37.2	0.8
H. de Toro	0.7	1	155.68	2.4	8.8
BC5	0.6	12	56.01	10.8	8.8
BC5	0.9	6	23.67	3.6	8.8
BC5	0.95	8	31.63	42	4
BC5	0.25	0	0.00	28.8	3.2
BC5	0.3	4	23.20	31.2	4
BC5	1.1	11	24.64	42	4.8
Cazorla	0.45	0	0.00	6	8

Cazorla	0.55	0	0.00	30	0.8
Cazorla	0.35	0	0.00	12	8.8
Cazorla	0.8	0	0.00	36	5.6
Cazorla	0.9	0	0.00	9.6	8
Cazorla	0.75	0	0.00	16.8	8
Com. 1	0.4	0	0.00	20.4	0.8
Com. 1	1.05	10	0.53	33.6	5.6
Com. 1	0.2	11	0.43	27.6	4.8
Com. 1	0.3	5	116.12	43.2	2.4
Com. 1	1.1	8	90.10	38.4	4
Com. 1	0.25	0	0.00	21.6	4
Com. 2	1.8	28	38.08	28.8	8.8
Com. 2	0.55	14	33.85	30	2.4
Com. 2	0.85	28	23.49	24	4
Com. 2	1.2	9	161.06	12	8
Com. 2	0.6	5	56.11	7.2	7.2
Com. 2	0.95	7	76.48	16.8	7.2
Com. 3	0.35	1	32.35	22.8	7.2
Com. 3	0.75	0	0.00	22.8	4
Com. 3	0.95	0	0.00	21.6	7.2
Com. 3	0.6	8	17.91	26.4	3.2
Com. 3	1.9	30	31.96	32.4	4.8
Com. 3	0.45	2	15.44	26.4	0.8
Com. 4	0.6	26	0.91	40.8	1.6
Com. 4	0.2	9	0.32	28.8	8
Com. 4	0.35	19	0.65	38.4	1.6
Com. 4	0.35	15	32.69	34.8	4
Com. 4	0.35	6	42.35	40.8	2.4
Com. 4	0.8	8	46.55	31.2	5.6
Edkawy	0.3	1	18.60	22.8	1.6
Edkawy	0.45	2	50.26	24	8.8
Edkawy	0.4	2	122.74	31.2	4.8
Edkawy	0.25	1	5.80	34.8	0
Edkawy	0.15	0	0.00	38.4	0.8
Edkawy	0.3	0	0.00	28.8	5.6
FlorBaladre	0.25	0	0.00	42	3.2
FlorBaladre	0.4	0	0.00	18	8
FlorBaladre	0.35	0	0.00	8.4	8.8
FlorBaladre	0.55	0	0.00	25.2	8.8
FlorBaladre	0.35	0	0.00	22.8	8.8
FlorBaladre	0.35	0	0.00	22.8	6.4
Kalohi	0.4	1	53.18	40.8	5.6

Kalohi	0.6	5	0.00	4.8	8.8
Kalohi	0.55	0	0.00	1.2	7.2
Kalohi	0.25	0	0.00	22.8	0.8
Kalohi	0.15	0	0.00	8.4	7.2
Kalohi	0.6	0	0.00	24	0.8
LA1589	0.6	27	0.63	50.4	2.4
LA1589	0.3	36	0.36	31.2	2.4
LA1589	0.6	28	0.63	49.2	2.4
LA1589	0.05	71	0.75	25.2	4.8
LA1589	0.7	43	1.08	39.6	5.6
LA1589	0.8	70	0.90	27.6	6.4
LA1777	1.2	0	0.00	21.6	0.8
LA1777	0.35	0	0.00	33.6	0
LA1777	0.3	0	0.00	20.4	0
Marmande	0.25	0	0	2.4	28.8
Marmande	0.65	4	127.22	43.2	0
Marmande	0.2	0	0.00	37.2	0
Marmande	0.3	1	84.18	36	0
Marmande	0.03	0	0.00	46.8	1.6
Marmande	0.25	4	50.05	39.6	0.8
Mellilero	0.55	12	71.55	27.6	0
Mellilero	0.95	5	71.05	25.2	5.6
Mellilero	1.05	1	66.30	27.6	8.8
Mellilero	0.35	4	22.34	45.6	1.6
Mellilero	0.1	0	0.00	33.6	2.4
Mellilero	1.05	8	37.10	34.8	4.8
MEX 3	0.45	0	0.00	14.4	8
MEX 3	0.55	0	0.00	20.4	1.6
MEX 3	0.3	0	0.00	7.2	8
MEX 3	0.3	0	0.00	20.4	2.4
MEX 3	0.35	5	57.178	3.2	37.2
MEX 33	0.225	0	0.00	36	1.6
MEX 33	0.5	0	0.00	33.6	6.4
MEX 33	0.55	0	0.00	38.4	7.2
MEX 33	0.35	2	49.92	31.2	0
MEX 33	0.2	2	25.37	21.6	8.8
MEX 89	0.25	0	0.00	38.4	5.6
MEX 89	0.2	4	68.23	40.8	0
MEX 89	0.35	1	38.64	0	21.6
MEX 89	1.25	59	1.63	39.6	2.4
MEX 89	1	59	1.16	26.4	5.6
MEX 89	0.75	107	1.09	22.8	0

MM	0.4	28	1.38	43.2	0.8
MM	0.95	85	1.43	34.8	1.6
MM	1.2	90	0.94	26.4	7.2
MM	0.3	4	29.05	31.2	0.8
MM	0.45	0	0.00	1.2	8
MM	0.5	8	67.49	31.2	1.6
Monita	0.5	2	73.33	14.4	7.2
Monita	0.5	6	60.42	33.6	0.8
Monita	0.75	15	54.85	12	7.2
Monita	0.4	1	77.79	44.4	5.6
Monita	0.5	3	49.31	15.6	8
Monita	0.25	0	0.00	38.4	0
Moruno	0.25	3	29.57	30	3.2
Moruno	0.5	5	25.21	27.6	3.2
Moruno	0.2	0	0.00	27.6	7.2
Moruno	0.3	0	0.00	37.2	4.8
Moruno	0.35	0	0.00	31.2	7.2
Moruno	0.4	2	177.40	48	2.4
PE55	0.45	1	43.61	22.8	8
PE55	0.03	0	0.00	6	8.8
PE55	0.1	25	0.60	32.4	0.8
PE55	0.4	15	8.99	27.6	0.8
PE55	0.25	4	7.84	44.4	0.8
PE55	0.1	0	0.00	25.2	0
Penjar	0.45	5	5.50	28.8	0
Penjar	0.2	4	5.43	26.4	4
Penjar	0.3	3	4.87	20.4	3.2
Penjar	1	11	20.51	14.4	8.8
Penjar	1.2	25	26.59	22.8	5.6
Penjar	0.7	3	35.39	2.4	7.2
Pera	0.325	1	57.10	22.8	4.8
Pera	0.45	7	19.92	45.6	2.4
Pera	1	2	3.75	26.4	0
Pera	0.55	2	60.78	24	4.8
Pera	0.3	0	0.00	27.6	2.4
Pera	0.55	1	77.36	25.2	4
Periana	0.85	7	18.83	1.2	8.8
Periana	0.6	0	0.00	21.6	8
Periana	0.85	2	54.47	21.6	1.6
Periana	0.35	0	0.00	39.6	4.8
Periana	0.5	1	22.50	3.6	7.2
Periana	0.55	0	0.00	6	7.2

PI134418	0.6	1	123.25	20.4	8
PI134418	0.95	0	0.00	38.4	4.8
PI134418	0.75	1	62.27	15.6	7.2
PI134418	0.35	1	1.67	37.2	2.4
PI134418	0.65	42	1.28	28.8	7.2
PI134418	0.45	15	1.47	39.6	3.2
SanMarzano	0.6	22	56.00	33.6	1.6
SanMarzano	0.45	6	22.56	33.6	4
SanMarzano	0.55	24	47.13	34.8	6.4
SanMarzano	0.55	3	64.21	25.2	1.6
SanMarzano	0.3	1	21.18	36	3.2
SanMarzano	0.35	2	31.20	36	4.8
T0 937	0.85	8	81.56	30	0
T0 937	0.7	12	165.16	38.4	3.2
T0 937	0.95	9	70.47	13.2	8
T0 937	0.85	47	0.39	24	2.4
T0 937	0.35	7	0.45	43.2	4
T0 937	0.6	17	0.45	24	1.6

C) Chemical composition of soil (ppm (mg/Kg)).

	Nitrogen (%)	**Carbon (%)**	**Al**	**As**	**Ca**	**Cd**	**Co**	**Cr**
H. de Toro	0.28	5.31	31392.19	31.06	9865.29	2.31	22.55	46.10
H. de Toro	0.30	5.64	33152.72	30.22	9160.04	2.27	20.72	47.91
BC5	0.29	5.67	29131.57	27.42	10935.73	2.25	19.76	44.19
BC5	0.30	5.86	30675.13	25.48	11061.06	2.22	19.08	45.56
Cazorla	0.31	6.32	40615.66	27.12	10485.12	2.13	19.86	54.38
Cazorla	0.32	6.87	39956.16	27.98	11198.14	2.15	19.70	53.52
Com. 1	0.31	5.92	47631.27	31.50	8035.45	2.22	22.60	59.47
Com. 1	0.30	6.13	44677.52	32.50	7609.60	2.15	22.09	56.66
Com. 2	0.25	4.00	39698.88	31.16	9326.35	2.18	20.90	53.03
Com. 2	0.25	4.00	35107.24	29.49	9656.82	2.12	20.09	48.65
Com. 3	0.30	5.11	42240.64	29.43	9997.15	2.23	21.67	56.08
Com. 3	0.32	5.38	35457.85	33.37	9972.01	2.24	21.04	49.69
Com. 4	0.30	5.58	32885.62	29.51	9805.13	2.10	20.78	46.55
Com. 4	0.32	5.44	39757.99	30.63	10265.03	2.10	21.22	52.31
Edkawy	0.30	5.95	30699.05	30.31	9330.06	2.24	21.94	45.69
Edkawy	0.30	5.62	30999.21	28.26	9381.36	2.22	20.47	45.42
Flor Baladre	0.37	9.19	36361.37	27.71	8742.37	2.31	21.04	50.42
Flor Baladre	0.35	9.83	44578.12	32.80	9000.14	2.49	23.92	59.40
Kalohi	0.26	4.70	35408.87	27.69	11226.76	2.12	20.12	49.19
Kalohi	0.27	5.40	33718.47	26.80	10900.91	2.02	18.65	47.25
LA1589	0.24	3.90	43807.07	31.93	7516.21	2.16	21.16	56.19

LA1589	0.26	4.08	45732.62	33.65	8092.22	2.29	22.13	59.22
LA1777	0.37	5.75	50272.00	35.35	8121.66	2.32	22.95	62.22
LA1777	0.34	5.99	45711.52	29.26	7821.81	2.25	21.72	58.65
Marmande	0.23	3.54	44967.65	33.87	10099.07	2.08	21.80	57.26
Marmande	0.26	3.60	49590.69	33.01	10566.59	2.10	22.44	61.09
Mellilero	0.33	6.87	46385.08	30.04	8718.77	2.26	20.91	59.97
Mellilero	0.32	6.71	49997.86	34.42	8756.51	2.28	21.60	63.18
MEX3	0.34	7.43	56030.90	36.13	8535.59	2.41	24.81	68.15
MEX3	0.36	8.23	50440.12	33.97	8303.46	2.38	24.15	62.91
MEX33	0.32	6.63	47233.73	31.62	8560.69	2.29	22.40	60.08
MEX33	0.31	6.75	32389.95	31.63	9603.18	2.21	21.66	45.82
MEX89	0.34	7.20	37035.45	35.19	7724.91	2.38	22.74	51.63
MEX89	0.30	6.30	35220.43	34.84	7771.43	2.42	22.80	49.92
MM	0.19	3.41	35323.15	35.72	9122.46	2.30	21.60	48.79
MM	0.22	3.43	43257.21	28.12	8454.01	2.15	19.83	56.06
Monita	0.26	4.13	31940.49	28.06	9246.73	2.13	20.74	46.11
Monita	0.26	4.25	41786.34	27.63	8952.64	2.18	20.96	55.19
Moruno	0.35	8.13	31963.20	28.11	11083.93	2.10	19.67	45.24
Moruno	0.37	8.57	35839.36	29.23	10791.54	2.16	21.78	49.18
PE55	0.29	5.07	44168.48	37.30	8475.16	2.38	24.29	56.66
PE55	0.28	5.25	45521.53	38.25	7766.99	2.41	23.06	58.30
Penjar	0.32	6.54	41827.30	32.44	8512.55	2.33	22.99	55.31
Penjar	0.34	6.80	45539.73	30.91	8589.08	2.25	21.55	58.47
Pera	0.28	5.04	41154.41	31.66	9668.35	2.12	20.95	54.23
Pera	0.30	5.59	41115.49	27.76	9596.23	1.99	19.92	53.33
Periana	0.30	4.54	43938.50	23.78	11952.10	2.02	20.07	55.93
Periana	0.30	4.70	49279.48	23.69	11593.97	2.03	20.76	60.99
PI134418	0.31	5.30	52236.71	38.19	9401.07	2.55	25.43	65.86
PI134418	0.30	5.22	51921.89	32.56	8228.17	2.29	23.12	63.13
SanMarzano	0.33	5.43	48067.16	25.18	7565.04	2.09	22.47	58.59
SanMarzano	0.33	5.45	48481.45	26.81	7569.74	2.19	21.63	60.12
T0 937	0.36	6.57	35247.47	32.31	7619.86	2.28	22.40	49.20
T0 937	0.35	6.75	33077.59	34.16	7858.56	2.24	21.24	46.86

	Cu	**Fe**	**K**	**Li**	**Mg**	**Mn**	**Na**	**Ni**
H. de Toro	38.91	32884.60	7715.46	29.87	8129.90	859.45	340.17	46.81
H. de Toro	39.41	32666.89	8067.20	28.61	7844.27	833.13	358.55	45.04
BC5	39.82	31537.22	7574.54	27.13	8318.36	796.13	312.83	41.42
BC5	40.76	30773.08	7814.89	26.47	8371.00	747.10	319.92	40.07
Cazorla	38.92	31782.97	9933.59	28.56	8474.42	745.17	480.71	40.52
Cazorla	38.65	31634.32	9692.08	28.95	8627.71	765.60	454.28	41.69
Com. 1	36.67	33753.09	12555.15	29.94	7859.90	847.06	565.07	44.61

Com. 1	38.36	33214.65	11826.61	29.96	7544.80	855.30	511.31	47.34
Com. 2	43.82	33010.07	10410.38	27.21	8068.36	793.49	428.00	44.48
Com. 2	37.41	32242.99	9092.00	26.92	8037.04	821.07	369.44	42.67
Com. 3	39.04	33438.98	11558.29	28.41	8198.91	845.45	462.52	44.41
Com. 3	39.18	33015.22	9805.96	27.31	8165.70	850.19	356.06	42.97
Com. 4	38.69	31529.00	8508.47	26.64	7567.12	809.98	344.69	43.89
Com. 4	38.37	31745.99	10410.05	26.86	7793.94	779.58	442.83	42.52
Edkawy	39.77	33229.64	7516.14	28.91	7555.72	901.50	318.93	43.96
Edkawy	38.92	33109.86	7523.21	28.62	7545.59	849.86	341.42	43.52
Flor Baladre	38.94	34226.53	8579.76	31.37	8042.48	783.41	380.06	44.96
Flor Baladre	41.17	36950.40	10731.29	35.50	8701.52	943.59	495.11	51.11
Kalohi	40.10	31773.77	9089.21	28.69	8551.69	847.60	404.29	42.42
Kalohi	37.15	30290.19	8665.13	27.20	8378.96	871.71	385.47	39.88
LA1589	40.14	32570.64	11860.36	31.09	7382.72	863.94	499.59	44.32
LA1589	41.70	34670.71	11914.49	31.14	7785.51	913.23	499.39	45.87
LA1777	40.47	34582.15	13024.54	33.38	7938.23	866.55	625.28	48.25
LA1777	38.12	34026.72	11568.04	31.95	7697.12	935.99	501.42	46.16
Marmande	38.23	31745.07	11671.55	27.78	7810.13	926.73	559.35	43.41
Marmande	38.11	32002.88	12945.34	28.94	8255.36	924.71	662.70	44.57
Mellilero	38.24	34215.23	12156.68	30.90	8335.72	801.76	509.73	44.49
Mellilero	38.23	34765.36	13153.86	32.05	8541.56	813.24	578.95	46.63
MEX3	43.04	36565.06	14358.61	34.98	8891.20	1053.77	655.29	50.53
MEX3	38.83	35666.63	12815.07	31.98	8431.28	995.74	552.16	47.48
MEX33	39.78	34378.78	12238.69	31.82	8080.95	956.27	547.74	47.23
MEX33	38.77	32755.69	8292.66	28.46	7675.72	925.75	337.95	44.92
MEX89	40.07	35214.03	9120.53	30.90	7823.48	1000.45	363.46	48.14
MEX89	40.85	35602.76	8714.39	30.52	7944.56	961.12	323.75	48.48
MM	48.10	34663.10	8516.76	29.16	8486.97	732.66	358.69	45.00
MM	35.65	32481.91	11135.17	27.11	7775.91	722.34	505.94	41.32
Monita	40.69	31956.20	8348.95	26.83	7640.57	805.10	342.63	42.81
Monita	39.29	32686.28	11053.40	28.73	7959.50	781.20	462.59	43.22
Moruno	38.05	31159.02	7972.43	25.40	8351.63	841.30	336.64	41.65
Moruno	38.62	32043.06	9071.07	27.14	8497.93	923.68	373.79	44.25
PE55	39.09	35490.55	11253.80	31.14	7758.05	1056.35	490.20	49.10
PE55	38.96	35281.08	11514.40	30.05	7770.78	987.78	496.02	47.56
Penjar	38.49	34347.55	10794.30	30.32	8208.32	898.17	438.16	46.41
Penjar	37.95	34185.19	11930.81	30.01	8167.22	849.87	497.98	45.32
Pera	40.33	32235.05	10921.52	27.33	7928.88	792.37	487.25	88.61
Pera	37.58	30554.31	10945.81	26.42	7960.42	702.82	475.98	40.35

Periana	39.86	30766.02	11727.84	27.65	8851.45	702.41	536.66	39.74
Periana	36.42	30856.54	13397.02	28.84	9133.88	756.21	667.94	40.84
PI134418	42.69	37459.94	13165.85	33.20	9093.31	1026.69	561.72	50.89
PI134418	37.72	33775.82	13352.27	30.99	8261.32	909.89	608.38	47.63
SanMarzano	35.11	32409.61	12339.04	28.58	7864.82	792.80	597.91	52.92
SanMarzano	34.39	33688.51	12431.03	29.06	8094.00	1561.69	587.94	45.13
T0 937	39.26	33665.19	9079.23	27.69	7266.96	958.42	354.45	45.64
T0 937	38.04	33331.82	8509.88	27.11	7198.24	952.38	332.67	44.68

	P	**Pb**	**S**	**Si**	**Sr**	**Ti**	**V**	**Zn**
H. de Toro	719.98	22.45	412.22	2852.25	48.11	1114.43	57.71	113.61
H. de Toro	694.95	22.50	411.23	2903.60	47.35	1182.76	60.41	137.42
BC5	692.91	22.06	487.96	2634.74	56.72	1113.13	55.37	136.93
BC5	738.14	21.21	491.31	2823.20	57.42	1144.62	56.94	126.41
Cazorla	653.60	21.43	470.67	3350.59	56.32	1338.62	69.53	108.83
Cazorla	709.61	21.30	479.13	3167.53	56.84	1349.38	68.72	177.35
Com. 1	1079.11	24.21	444.79	4266.28	53.03	1419.18	77.60	84.71
Com. 1	1074.94	23.08	420.60	4460.31	50.54	1397.81	72.99	83.79
Com. 2	1066.83	23.21	480.99	3721.03	51.66	1310.94	67.63	162.44
Com. 2	1144.38	24.18	485.13	3251.61	52.09	1215.96	61.65	109.90
Com. 3	1731.19	24.72	451.25	3766.92	52.84	1432.48	71.40	108.76
Com. 3	1706.17	51.07	432.51	3787.77	50.26	1233.58	62.61	97.50
Com. 4	1294.46	23.16	499.80	3657.23	54.32	1187.36	58.33	87.69
Com. 4	1246.36	24.31	516.82	4079.30	57.29	1285.34	66.70	137.63
Edkawy	807.69	22.48	457.59	2544.95	58.91	1071.27	56.87	104.03
Edkawy	828.44	21.71	483.03	2760.96	58.87	1083.41	56.76	109.48
Flor Baladre	755.93	21.81	355.64	2951.11	49.64	1237.32	63.87	104.72
Flor Baladre	778.25	24.51	392.14	3846.32	54.77	1400.42	76.02	114.23
Kalohi	838.35	22.50	485.75	3235.95	55.96	1248.29	62.63	102.87
Kalohi	827.65	20.56	476.61	3172.94	53.26	1176.50	59.32	96.72
LA1589	936.10	22.85	389.51	4239.70	49.55	1323.76	71.22	81.23
LA1589	1000.68	23.79	428.94	4152.63	52.23	1427.34	75.65	88.47
LA1777	1098.41	25.77	433.16	4048.86	59.02	1519.15	82.11	92.03
LA1777	1042.98	20.88	438.52	4324.54	56.19	1444.49	75.96	83.34
Marmande	984.76	23.33	474.05	4564.74	59.37	1474.06	73.76	83.11
Marmande	1000.95	24.51	501.23	5034.07	64.02	1507.63	80.77	85.57
Mellilero	887.29	23.31	380.50	4314.14	51.13	1515.69	77.23	81.63
Mellilero	886.95	23.34	396.83	4161.18	52.37	1552.61	82.48	86.25
MEX3	856.02	23.79	338.87	4569.25	55.51	1640.70	90.47	87.79
MEX3	826.83	25.93	329.73	4487.35	53.49	1524.52	82.56	90.56
MEX33	976.11	27.76	470.06	4075.78	52.51	1429.37	77.08	96.56

MEX33	1037.66	23.38	455.40	3252.99	50.38	1095.94	57.31	87.85
MEX89	1050.31	24.84	367.33	3086.21	43.49	1195.80	65.26	104.61
MEX89	990.37	24.19	315.84	3023.44	42.11	1186.99	63.63	91.22
MM	823.11	25.10	410.72	2969.72	55.30	1172.99	61.74	98.08
MM	822.59	21.38	402.56	3730.89	54.38	1291.35	71.47	85.44
Monita	1070.93	22.57	487.14	3204.50	54.10	1217.58	57.68	90.46
Monita	1073.35	21.65	455.88	3514.60	53.17	1448.41	70.12	80.56
Moruno	960.33	21.97	429.54	3078.54	56.86	1112.79	56.49	99.09
Moruno	906.64	24.06	416.14	3383.34	56.72	1186.82	61.78	94.75
PE55	1283.17	25.86	318.47	4176.98	47.76	1308.49	72.73	102.92
PE55	1190.71	24.53	311.16	3509.14	47.28	1301.03	75.16	98.08
Penjar	1072.19	25.28	403.33	3209.85	50.44	1348.15	70.66	101.99
Penjar	1057.33	23.50	378.28	3875.12	50.34	1411.98	75.34	105.56
Pera	1022.23	23.89	520.10	3996.35	55.26	1389.16	69.49	182.41
Pera	1008.09	22.29	494.50	4247.12	53.68	1371.23	68.36	82.66
Periana	926.56	21.58	555.95	4123.09	64.93	1480.62	72.19	93.42
Periana	948.61	22.49	538.22	4857.43	65.32	1629.63	80.30	99.10
PI134418	1269.37	26.67	423.26	3805.20	49.35	1511.51	85.31	95.71
PI134418	1079.67	22.62	405.21	4023.37	46.30	1447.88	83.52	91.83
SanMarzano	1006.17	21.94	344.53	3724.66	49.52	1410.75	77.86	83.24
SanMarzano	978.95	21.34	349.66	4570.59	49.21	1433.66	80.31	86.42
T0 937	1134.20	24.49	393.96	4183.82	47.43	1202.30	62.21	110.40
T0 937	1124.61	24.23	414.99	3994.70	47.61	1149.30	59.26	104.21

Table S2. Variation partitioning of fungal VT community composition in tomato type and spatial location. Either considering the VT community composition or the VT phylogenetic turnover between species. Asterisk indicates significant p values: *** $p < 0.001$.

Dataset	**Partition**	**Df**	**Explained variance**
VT community	Spatial location	5	**0.061*** **
	Tomato type	0	0.001
	Location x type	2	0.002
	Residuals		0.936
VT phylogenetic turnover	Spatial location	5	**0.177*** **
	Tomato type	0	0.000
	Location x type	1	0.000
	Residuals		0.830

Table S3: Pearson correlation test of significant fungal MDS axes (AMF VT) and plant traits. R coefficients are shown. Asterisk indicates significant p values: .*** p <0.001, ** p <0.01, * p <0.05.

Virtual taxa	**Genus**						
		IL	**TOC**		**Symptoms**		
		MDS15	MDS13	MDS30	MDS1	MDS10	MDS15
	Model estimate	0.467	-1.756	2.642	-2.366	-0.231	0.821
VTX338	*Archaeospora*	0.120	-0.019	0.048	-0.042	0.006	0.120
VTX055	*Claroideoglomus*	-0.088	-0.004	0.052	-0.050	0.110	-0.088
VTX056	*Claroideoglomus*	-0.051	-0.042	-0.004	-0.042	**0.571*** **	-0.051
VTX193	*Claroideoglomus*	-0.059	-0.065	-0.041	-0.010	0.164	-0.059
VTX054	*Diversispora*	-0.111	**-0.263** **	0.021	-0.006	**-0.244** **	-0.111
VTX060	*Diversispora*	-0.053	-0.028	-0.019	-0.001	-0.089	-0.053
VTX062	*Diversispora*	-0.080	-0.041	**-0.916*** **	-0.127	-0.018	-0.080
VTX063	*Glomus*	-0.148	0.002	0.058	-0.167	0.024	-0.148
VTX064	*Septoglomus*	0.014	-0.045	0.044	-0.006	-0.027	0.014
VTX065	*Glomus*	**-0.196***	-0.146	-0.002	-0.042	-0.007	**-0.196***
VTX067	*Glomus*	0.049	-0.053	0.052	-0.019	0.023	0.049
VTX098	*Glomus*	-0.105	-0.040	-0.002	0.128	-0.039	-0.105
NewVTX2	*Glomus*	-0.072	-0.038	0.004	-0.017	0.024	-0.072
VTX105	*Glomus*	-0.058	-0.042	0.010	0.030	0.059	-0.058
VTX108	*Glomus*	0.134	0.062	0.004	0.099	-0.070	0.134
VTX113	*Glomus*	-0.057	-0.030	-0.011	**0.503*** **	-0.001	-0.057
VTX114	*Glomus*	-0.049	-0.027	-0.020	**0.469*** **	0.001	-0.049
VTX130	*Glomus*	-0.145	**0.600*** **	0.002	-0.041	-0.177	-0.145
VTX143	*Glomus*	-0.107	0.008	0.036	-0.123	0.034	-0.107
VTX153	*Glomus*	0.034	0.017	-0.089	-0.008	-0.086	0.034
VTX155	*Glomus*	-0.040	0.010	-0.105	**-0.401*** **	-0.010	-0.040
VTX199	*Glomus*	0.020	-0.007	-0.015	-0.082	0.009	0.020
VTX214	*Glomus*	0.059	0.004	-0.004	-0.015	-0.026	0.059
VTX342	*Glomus*	-0.156	0.022	0.057	-0.072	-0.006	-0.156
VTX409	*Glomus*	-0.110	-0.006	0.053	-0.158	0.032	-0.110
VTX418	*Glomus*	0.099	-0.179	-0.005	-0.037	**-0.260** **	0.099
VTX419	*Glomus*	**-0.251** **	**-0.343*** **	-0.002	-0.046	**-0.468*** **	**-0.251** **
VTX001	*Paraglomus*	-0.094	0.002	-0.114	-0.146	-0.078	-0.094
VTX281	*Paraglomus*	-0.090	-0.042	-0.001	-0.026	**0.209***	-0.090
NewVTX1	*NA*	0.209*	-0.022	0.061	-0.004	-0.012	**0.209***

Virtual taxa	**Species**					
		Biomass		**Tomato number**		

		MDS1	MDS10	MDS16	MDS23	MDS25
	Model estimate	-0.042	0.006	-0.066	-0.006	-0.104
VTX338	*Archaeospora*	-0.050	0.110	-0.025	-0.049	0.068
VTX055	*Claroideoglomus*	-0.042	**0.571*** **	0.016	-0.016	-0.163
VTX056	*Claroideoglomus*	-0.010	0.164	0.033	0.009	0.004
VTX193	*Claroideoglomus*	-0.006	**-0.244** **	**-0.198***	-0.061	-0.134
VTX054	*Diversispora*	-0.001	-0.089	**0.256** **	-0.117	0.002
VTX060	*Diversispora*	-0.127	-0.018	-0.003	-0.024	-0.085
VTX062	*Diversispora*	-0.167	0.024	0.000	-0.041	-0.027
VTX063	*Glomus*	-0.006	-0.027	0.006	-0.022	0.036
VTX064	*Septoglomus*	-0.042	-0.007	0.094	0.044	-0.026
VTX065	*Glomus*	-0.019	0.023	0.006	-0.097	-0.032
VTX067	*Glomus*	0.128	-0.039	0.049	-0.119	-0.029
VTX098	*Glomus*	-0.017	0.024	0.007	-0.097	-0.068
NewVTX2	*Glomus*	0.030	0.059	0.045	-0.036	0.049
VTX105	*Glomus*	0.099	-0.070	**0.305*** **	0.040	-0.001
VTX108	*Glomus*	**0.503*** **	-0.001	0.028	-0.013	0.006
VTX113	*Glomus*	**0.469*** **	0.001	0.041	-0.004	0.009
VTX114	*Glomus*	-0.041	-0.177	0.026	0.010	0.033
VTX130	*Glomus*	-0.123	0.034	0.067	**0.230***	-0.005
VTX143	*Glomus*	-0.008	-0.086	-0.035	-0.028	**-0.227***
VTX153	*Glomus*	**-0.401*** **	-0.010	-0.030	-0.036	-0.062
VTX155	*Glomus*	-0.082	0.009	-0.055	-0.098	**0.801*** **
VTX199	*Glomus*	-0.015	-0.026	-0.015	-0.030	0.039
VTX214	*Glomus*	-0.072	-0.006	0.017	0.024	-0.027
VTX342	*Glomus*	-0.158	0.032	0.011	-0.045	0.121
VTX409	*Glomus*	-0.037	**-0.260** **	**0.727*** **	-0.010	-0.043
VTX418	*Glomus*	-0.046	**-0.468*** **	**-0.207***	-0.006	0.009
VTX419	*Glomus*	-0.146	-0.078	0.019	-0.022	**-0.194***
VTX001	*Paraglomus*	-0.026	**0.209***	0.034	**-0.312*** **	0.168
VTX281	*Paraglomus*	-0.004	-0.012	-0.043	-0.066	-0.013
NewVTX1	*NA*	-0.042	0.006	-0.066	-0.006	-0.104

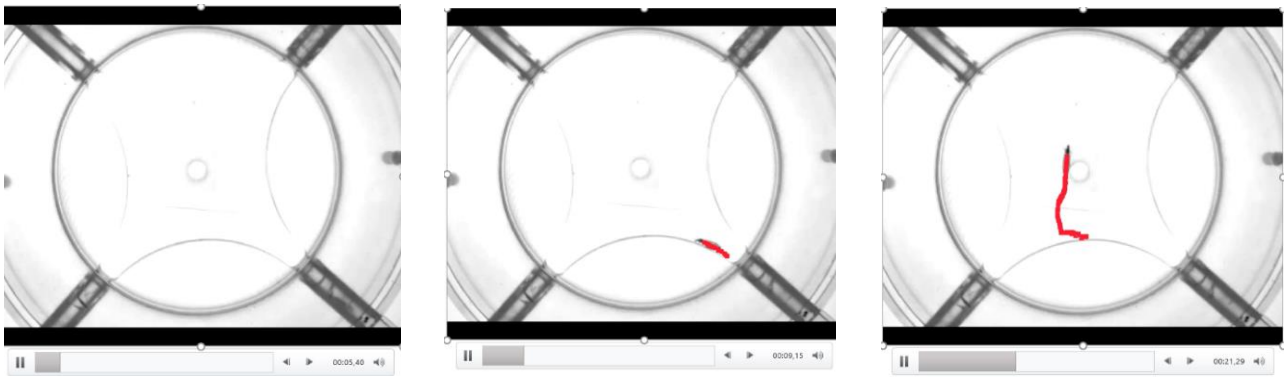
Virtual taxa	**Genus**		
		Tomato weight	
		MDS9	MDS25
	Model estimate	-32.316	-18.944
VTX338	Archaeospora	-0.027	-0.104
VTX055	Claroideoglomus	0.094	0.068
VTX056	Claroideoglomus	**0.203***	-0.163
VTX193	Claroideoglomus	**-0.705*** **	0.004
VTX054	Diversispora	0.015	-0.134

VTX060	Diversispora	0.046	0.002
VTX062	Diversispora	-0.096	-0.085
VTX063	Glomus	-0.013	-0.027
VTX064	Septoglomus	0.007	0.036
VTX065	Glomus	0.021	-0.026
VTX067	Glomus	-0.032	-0.032
VTX098	Glomus	0.038	-0.029
NewVTX2	Glomus	-0.049	-0.068
VTX105	Glomus	-0.039	0.049
VTX108	Glomus	0.025	-0.001
VTX113	Glomus	0.040	0.006
VTX114	Glomus	0.062	0.009
VTX130	Glomus	-0.001	0.033
VTX143	Glomus	-0.007	-0.005
VTX153	Glomus	-0.069	**-0.227***
VTX155	Glomus	0.029	-0.062
VTX199	Glomus	-0.032	**0.801*****
VTX214	Glomus	-0.063	0.039
VTX342	Glomus	-0.040	-0.027
VTX409	Glomus	-0.082	0.121
VTX418	Glomus	0.083	-0.043
VTX419	Glomus	0.049	0.009
VTX001	Paraglomus	-0.118	**-0.194***
VTX281	Paraglomus	0.116	0.168
NewVTX1	NA	-0.023	-0.013

Table S4. Pearson correlation test of significant fungal phylogenetic MDS axes (AMF VT) and plant traits. R coefficients are shown. Asterisk indicates significant p values: .*** p <0.001, ** p <0.01, * p <0.05.

AMF family	**Model estimates**				
	TYLCV	**TYLCV**	**TYLCV**	**Biomass**	**Symptoms**
	MDS1	MDS3	MDS6	MDS19	MDS74
	1.062	1.246	-0.381	0.455	0.645
Archaeosporaceae	0.007	0.028	**0.209***	-0.048	-0.002
Claroideoglomeraceae	**0.336*****	**-0.519*****	-0.054	-0.040	0.005
Diversisporaceae	-0.035	-0.042	0.078	**0.199***	0.006
Glomeraceae	-0.126	0.108	0.086	**-0.349*****	**-0.322*****
Paraglomeraceae	**0.511*****	**0.347*****	0.137	**-0.298****	0.004
AMF genus					
Archaeospora	0.007	0.028	**0.209***	-0.048	-0.002
Claroideoglomus	**0.336*****	**-0.519*****	-0.054	-0.040	0.005
Diversispora	-0.035	-0.042	0.078	**0.199***	0.006

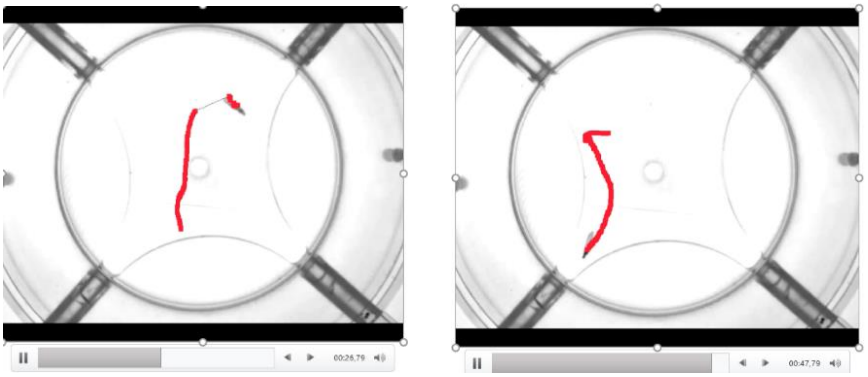
Glomus	-0.125	0.107	0.082	**-0.350*** **	**-0.325*** **
Paraglomus	**0.511*** **	**0.347*** **	0.137	**-0.298** **	0.004
Septoglomus	-0.064	0.034	0.018	0.027	-0.116



Objective 2: Bottom up effects of soil microbial community on tomato defenses

Chapter 4: Role of plant volatiles in mediating the effect of the soil microbiome on the behavior of the predator *Chrysoperla carnea* in tomato.

Authors: Lisanne Smulders, Emilio Benítez, Maria Jose Pozo, Rafa Alcala Herrera, Álvaro López-García



A**bstract:** Soil microbial communities are known to affect plant growth and plant defenses, including indirect defences affecting the attraction of natural enemies. Although domestication is expected to result in crops that are more attractive to pests and less attractive to pest natural enemies, studies in maize and tomato evidenced high levels of intraspecific variation. Microbial inoculants, including those based on arbuscular mycorrhizal fungi (AMF), potentially enhance plant defenses, but their effect under natural conditions are not well characterized. In this study, we used a natural soil with and without inoculation with the AMF *Rhizophagus irregularis* to study the effect of inoculation on the natural soil microbial community. A sterile soil with reduced bacterial diversity and no AMF inoculation was also tested to clarify the role of soil-borne bacteria. We studied interactions between the soil microbiome and tomato in their response to attack by the pest *Spodoptera exigua* (Hübner) and attraction of the predator *Chrysoperla carnea* (Steph.) in two tomato species, one wild (*Solanum pimpinellifolium*, var. LA1589) and one modern (*Solanum lycopersicum*, var. Monita). Volatile profiles were affected by pest attack in both tomatoes, while soil microbiome only affected volatiles in the wild LA1589. The generalist predator *C. carnea* preferred tomatoes grown in sterile soil in both wild and modern tomatoes and was least attracted to the uninoculated natural soil. We identified some volatiles with potential pest or natural enemy repellent activity. Furthermore, *R. irregularis* inoculation increased *C. carnea* attraction comparing natural soils in both tomato species, indicating that AMF inoculation may enhance indirect defense in tomato.

1. Introduction

The anthropogenic selection of plant varieties, known as plant domestication, has impacted the relationship between plants and their associated soil microbiomes, even though they were not directly selected for [1,2]. Plants affect rhizosphere microbial communities through root exudate composition and modulation of plant defense pathways [3]. Apart from the plant, the environment and abiotic factors may impact microbial diversity [4,5]. For example, drought or salinity impact plant development and its ability to interact with its microbiome [5]. Soil microbes influence the soil community structure by producing secondary metabolites to compete with other microbes and successfully establish in the rhizosphere [6,7].

The rhizosphere is inhabited by a unique population of microorganisms, such as bacteria, fungi and archaea, which are involved in multiple ecosystem services such as nutrient cycling, disease suppression and pest control [6,7]. For example, arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that provide the plant with nutrients and increased resistance to biotic and abiotic stresses such as drought and salinity [6,8]. The benefits of these symbioses are thought to decrease in modern crops, as fertilisers and other soil amendments have made the resources usually provided by microbial symbionts freely available [9,10]. Indeed, agricultural varieties often show reduced bacterial diversity in the rhizosphere, thereby reducing potential mutualistic capacity [11–15]. However, in this thesis we did not find an effect of domestication on the community composition of bacteria in the soil (chapter 2) or AMF in the roots (chapter 4).

Apart from interactions with belowground organisms, domesticated crops are often more attractive to insect pests and pathogens due to the selection of morphological traits such as larger fruits or increased yields [16–19]. Multiple studies have found reduced physical and chemical defenses in modern plants, probably through the loss of defensive genes and traits in favour of traits such as reduced bitterness or toxicity [17,20–22]. During domestication, secondary chemistry decreased in several crops, due to direct selection or dilution of defense levels [23,24]. Furthermore, reduced volatile emissions and interactions with insects as a result of domestication were shown in maize, cranberry and lupin [16,25]. However, other studies showed that intraspecific variation in defense levels was equal to or higher than interspecific variation in maize and

tomato [24,26]. This variability could be explained by plants allocating their resources to different sinks such as growth, defense, mutualistic associations or abiotic stress tolerance [22].

Although effects of domestication on symbioses with microbial partners, microbial communities and plant defense have been well studied, the effect of natural soil communities on plant defense is less well known [9,27–30]. Soil microbial communities have been shown to mediate the production of herbivore induced plant volatiles (HIPVs) and the attraction of pest natural enemies [31,32]. Beneficial microbes may alter HIPV production through changes in chemical and physical plant traits [32]. For example, soil microbial communities affected HIPV emission after aphid attack, influencing the preference of the predator *Chrysoperla carnea* [33]. Similarly, tomatoes colonized with the fungal endophyte *Fusarium solani* attracted more predators than non-colonized plants [34]. Beneficial microbes, including AMF, were even found to enhance the attack rate, performance and attraction of parasitoids [31,35].

The effects of microbial inoculants such as AMF are usually studied in sterile soils, thus not taking into account interactions between specific AMF and the natural soil microbiome. Therefore, in this study, we used a natural soil inoculated or not with the AMF *Rhizophagus irregularis* to study how AMF inoculation influences the plant response. We studied the interaction between the soil microbiome and two tomato species in their response to attack by the beet armyworm *Spodoptera exigua*, and their interaction with the generalist predator *C. carnea*. As insect resistance in tomato is partly based on plant compounds present in their glandular trichomes, we selected a wild tomato species with type VI trichomes and a modern tomato species without these trichomes [36–39] .

We hypothesize that the soil microbiome impacts HIPVs production and attraction of *C. carnea*, and that the modern tomato species would be less responsive to the microbiome composition and/or inoculation than wild tomato species in terms of volatile production and attraction of *C. carnea*. Specifically, we aimed to: 1) Observe changes in volatile production after *S. exigua* attack; 2) Determine the effect of soil microbial community on volatile production before and after *S. exigua* attack; 3) Determine the effect of soil microbial community on *C. carnea* behavior; and 4) Find correlations between volatile production and *C. carnea* behavior.

2. Results

2.1 Plant growth and mycorrhizal colonization

Plant growth was not affected by soil treatment in Monita (Table S3A). In contrast, for LA1589, shoot weight was significantly higher in the NS treatment compared to the SS (p=0.003**) and NS+Ri treatment (p=0.018*). Root weight was significantly higher only in the SS treatment compared to the NS treatment (p=0.037*).

AMF colonization was addressed both at the histochemical and molecular level. Histochemical staining of fungal structures in the roots showed no significant differences in the colonization level in plants growing in natural soil regardless of the fungal inoculation in either tomato species (Table S3). For wild LA1589, colonization levels were 6±1.91% in NS and 7.1±9.7% in NS+Ri plants. For Monita, colonization levels were 8.1±1.9% in NS and 6.1±1.04% in NS+Ri. Remarkably, colonization levels were very similar among both tomato species. Absence of colonization was confirmed in the SS treatment. A detailed analysis of the different fungal structures showed also no significant differences regarding the root colonization intensity, arbuscules, vesicle and appressoria content between NS and NS+Ri treatments in either tomato species (Table S3B and S3C). However, molecular quantification of fungal DNA within the root tissues revealed differences among treatments. Generic primers for glomeromycota, amplifying most AMF species, and specific primers amplifying only *R. irregularis* were used. Inoculation with *R. irregularis* resulted in higher total AMF levels in the roots, although differences were significant only in LA1589 ($p<0.05$, Figure 1A).

However, *R. irregularis* colonization levels increased significantly in both tomato species ($p<0.05$, Figure 1). Thus, colonization level and intensity were similar in both tomato species, but *R. irregularis* presence in the roots significantly increased in the NS+Ri treatment compared to the non-inoculated NS treatment.

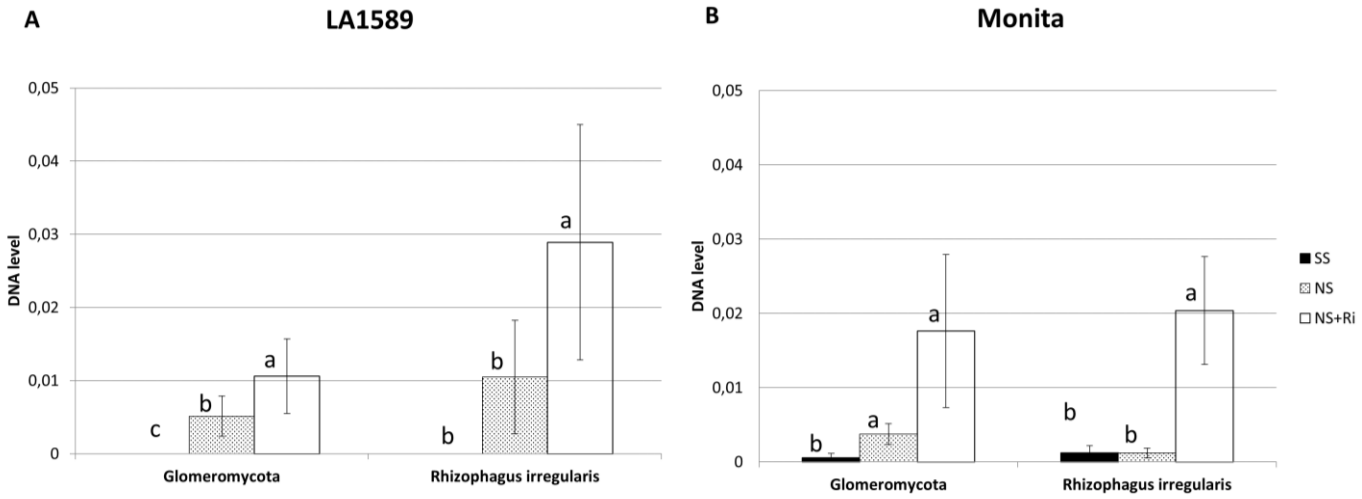


Figure 1: Molecular quantification of mycorrhizal fungi within the roots. Fungal DNA content in the root was quantified and normalized to the plant DNA. Glomeromycota DNA levels (using generic primers for Glomeromycota), and *Rhizophagus irregularis* DNA levels of this species in roots of wild tomato species LA1589 (A) and modern tomato species Monita (B). Results shown are the average level (means ± SE) of five plants per treatment. Statistically significant differences ($p<0.05$) are depicted with different letters according to Kruskall Wallis.

2.2 Bacterial community composition affected by *S. exigua* attack.

We studied the bacterial community composition in the soil to check whether these could explain differences between soil treatments. No differences were found in bacterial composition, total and water-soluble C, or nutrient content between the soils used for both varieties. However, both species drove bacterial community composition differently during the growing period (LA1589 PERMANOVA $F=5.620$, $p=0.023$*; Monita PERMANOVA $F=10.98$, $p=0.009$**, Figure 2A). Although the effect of steam sterilization on the bacterial community was clear and maintained different to the other treatments throughout the growth period of both tomato varieties; NS and NS+Ri treatments showed similar bacterial beta diversity (the degree of community differentiation between samples) after growing the wild LA1589, while the effect of inoculation was clear in the modern Monita (Figure 2A).

For both tomato species, a clear effect of soil treatment (LA1589 PERMANOVA $F=9.014$, $p=0.003$**; MONITA PERMANOVA $F=7.785$, $p=0.003$**, Figure 2B) but not of pest attack (LA1589 PERMANOVA $F=0.849$, $p=0.539$; MONITA PERMANOVA $F=0.591$, $p=0.634$, Figure 2C) on the structure of the bacterial population were evidenced. However, the beta diversity analyses showed some differences between species, suggesting that bacterial communities of wild tomato were more sensitive to pest attack.

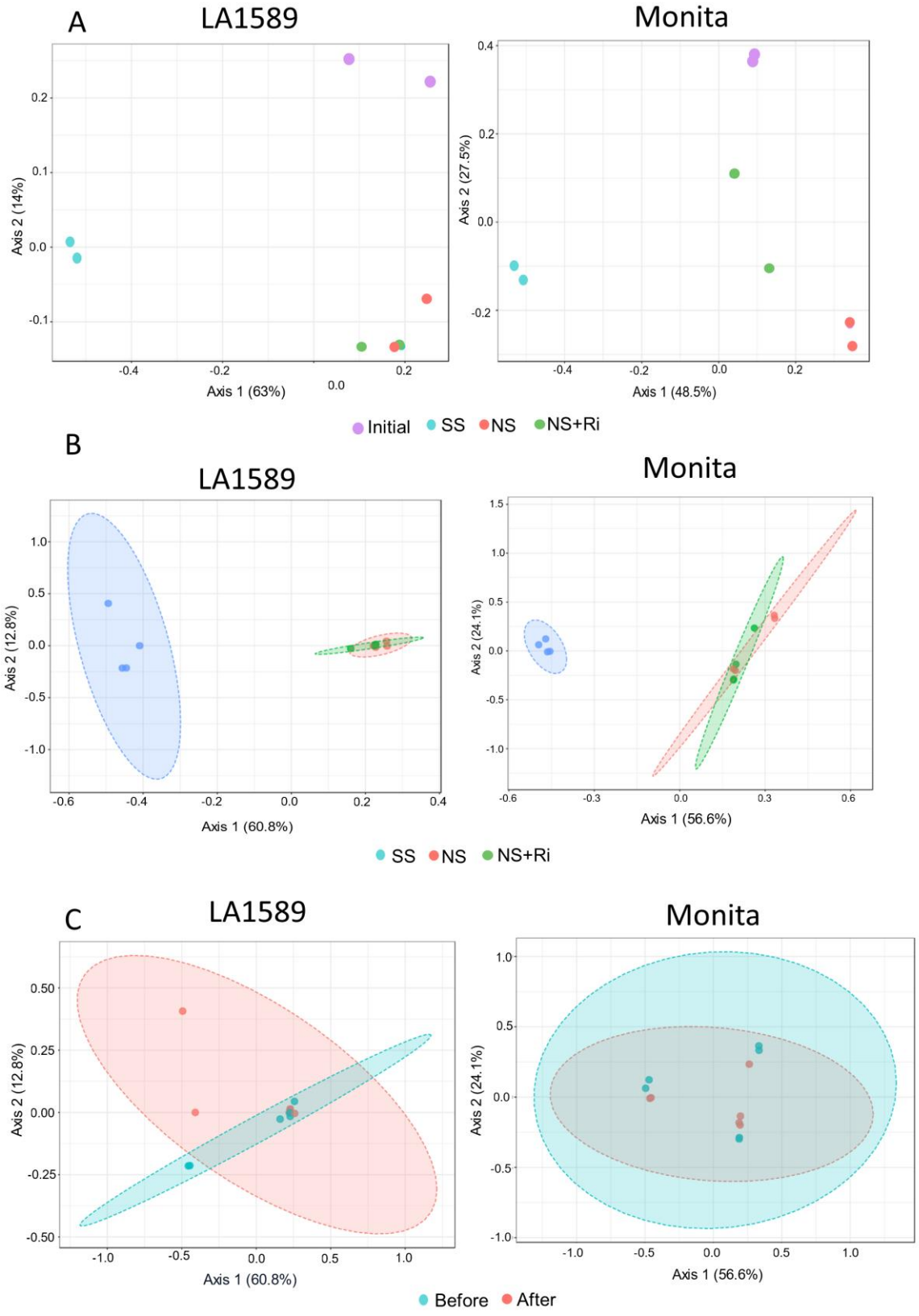


Figure 2: Beta diversity analysis of soil microbial communities of wild tomato species LA1589 and modern tomato species Monita in the different soil treatments sterile soil (SS), natural soil (NS) and inoculated natural soil (NS+Ri). (A) beta diversity of the bacterial communities in the bulk soil collected at the beginning (initial) and end of the experiment in both varieties. (B) beta

diversity in the different soil treatments for LA1589 and Monita. (C) beta diversity of LA1589 and Monita soil communities as affected by pest attack. For each soil treatment there were four samples, two before pest attack and two after pest attack.

2.3 Soil treatment and herbivory impact volatile production

Volatile profiles were analysed for the different soil treatments before and after S. exigua attack by GC-MS. Volatile profiles were significantly different depending on both soil treatment (treatment) and pest (before and after attack) (Overall PERMANOVA, Table 1). Remarkably, soil treatment was only significantly affecting the volatile profiles in LA1589, as shown when running separate PERMANOVAs and evidenced by the significant soil treatment × variety interaction.

Table 1: PERMANOVA results on the effects of soil treatment, pest attack and variety on volatile profiles. Models are shown considering both tomato species together (both), or separately for wild species LA1589 and modern species Monita. Significant differences are highlighted in bold. Asterisks indicate significance: * p < 0.05; ** p < 0.01; ***p<0.001.

		F	R^2	p
Both	Treatment	5.599	0.086	**0.003****
	Pest	33.370	0.171	**0.001*****
	Variety	34.093	0.523	**0.001*****
	Treatment:Pest	0.501	0.005	0.747
	Treatment:Variety	5.483	0.056	**0.003****
	Pest:Variety	2.490	0.013	0.092
	Treatment:Pest:Variety	0.366	0.004	0.852
LA1589	Treatment	75.310	0.329	**0.001*****
	Pest	134.697	0.295	**0.001*****
	Treatment:Pest	-0.399	-0.017	1.000
Monita	Treatment	0.5668	0.036	0.650
	Pest	147.157	0.463	**0.002****
	Treatment:Pest	14.738	0.093	0.258

The PCA ordination of volatiles in wild species LA1589 clearly separates the NS treatment from both the SS and the NS+Ri treatments (Figure 3A). Furthermore, volatile profiles differed before and after pest attack, irrespective of soil treatment. This ordination confirms the results revealed by PERMANOVA, with both treatment and pest being significant (Table 1).

The PCA ordination of volatiles in modern species Monita clearly separates volatile profiles before and after attack, while they are similar between soil treatments (Figure 3B). These results are confirmed by PERMANOVA, with only pest being significant (Table 1). Thus, wild tomato variety LA1589 seems more responsive to soil treatment than modern tomato variety Monita.

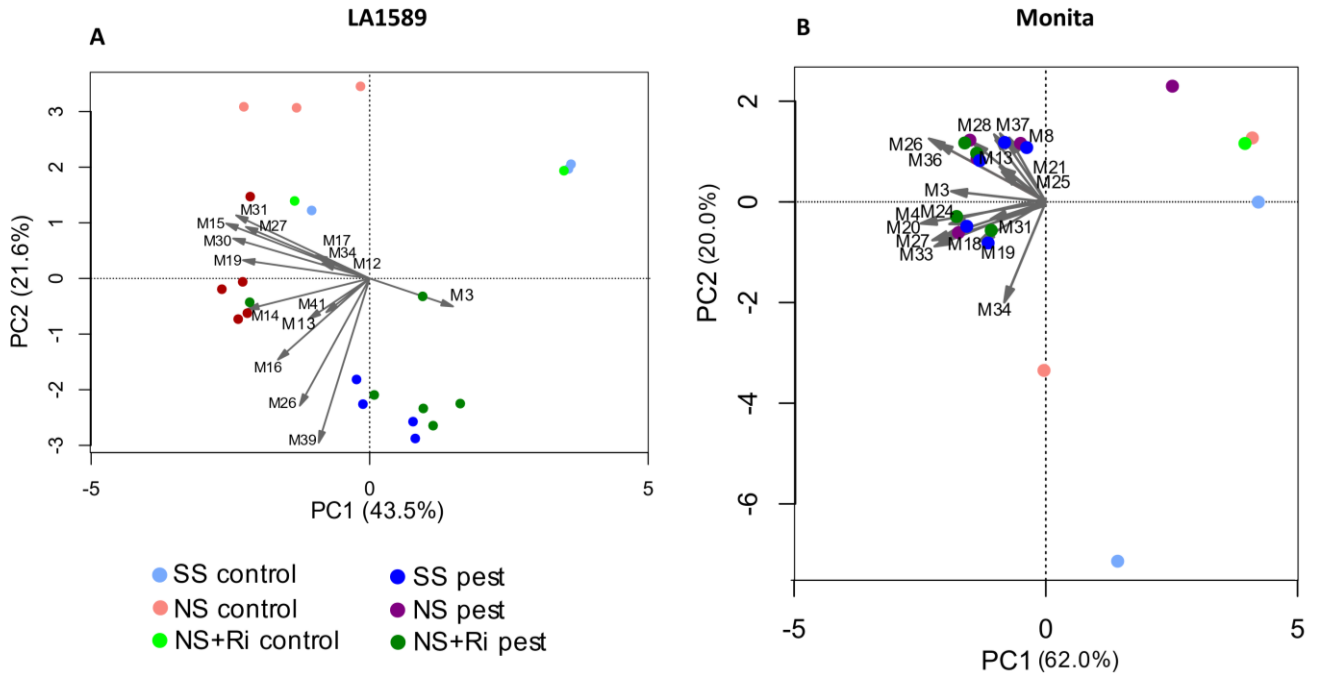


Figure 3: Principal Components Analysis (PCA) of volatile profiles in wild tomato species LA1589 (A) and modern tomato species Monita (B). Treatments are sterile soil (SS, blue), natural soil (NS, red) and inoculated natural soil (NS+Ri, green). Volatile profiles before attack (control) are depicted in light shades, while volatile profiles after attack (pest) are depicted in dark shades. The volatiles corresponding to each number can be found in Table S1.

Differential accumulation of volatiles was observed among treatments (Table 2). Some volatiles were herbivore inducible in both species, such as octanal (M13) and 2-nonanone (M21, Figure S1A). Some were even only at detectable levels under herbivory, such as decanal (M39) in LA1589 and bornyl acetate (M26) and linalool oxide (M36) in Monita.

Table 2: Results of ANOVA and Kruskall Wallis tests for significant differences in volatile production between soil treatments or pest attack for both tomato varieties separately. Results are depicted as F-value$^{df,\ residuals}$. Significant differences are highlighted in bold. Asterisks indicate significance: * p < 0.05; ** p < 0.01; ***p<0.001.

Volatile	LA1589			Monita		
	Pest	Treatment	Pest* Treat-ment	Pest	Treatment	Pest* Treatment
M1: toluene	$0.644^{1,18}$	$0.452^{1,18}$	$0.001^{1,18}$	$3.601^{1,10}$	$0.413^{1,10}$	$0.080^{1,10}$
M2: hexanal			2.022^{5}	$0.238^{1,10}$	$0.522^{1,10}$	$0.531^{1,10}$
M3: 2-hexenal	$1.503^{1,18}$	**$5.786^{1,18}$***	$1.305^{1,18}$	$0.041^{1,10}$	$0.851^{1,10}$	$0.666^{1,10}$
M4: 3-hexen-1-ol				$1.516^{1,10}$	$0.000^{1,10}$	$0.181^{1,10}$

M5: o-xylene	4.406[1,18]	0.323[1,18]	0.004[1,18]	**7.763[1,10]***	0.757[1,10]	0.302[1,10]
M6: p-Xylene	3.933[1,18]	0.414[1,18]	0.021[1,18]	**7.445[1,10]***	0.164[1,10]	0.035[1,10]
M7: α-pinene			9.315[5]	0.036[1,10]	0.061[1,10]	0.038[1,10]
M8: 6-methyl-2-hep-tanone				1.009[1,10]	**5.018[1,10]***	3.406[1,10]
M9: 1,3,5-cyclohepta-triene ,3,7,7,-trimethyl-			**17.388[5]****	0.018[1,10]	0.001[1,10]	0.010[1,10]
M10: 6-methyl-5-hep-ten-2-one				**7.651[1,10]***	0.625[1,10]	0.522[1,10]
M11: mircene				0.094[1,10]	0.001[1,10]	0.000[1,10]
M12: 2-carene	0.520[1,18]	**9.158[1,18]****	0.342[1,18]	0.067[1,10]	0.006[1,10]	0.058[1,10]
M13: octanal			**17.117[5]****	43.436[1,10]	**157.376[1,10] ***	**114.794[1,10] ***
M14: α-phellandrene			**13.876[5]***			0.417[3]
M15: α-terpinene			**15.086[5]***			0.223[3]
M16: m-cymene			**16.953[5]****	0.610[1,10]	0.016[1,10]	0.030[1,10]
M17: limonene B phellandrene	1.030[1,18]	**9.065[1,18]****	0.319[1,18]			0.189[3]
M18: β-(E)-ocimene	0.546[1,18]	2.451[1,18]	0.760[1,18]			0.077[3]
M19: α-terpinolene	0.039[1,18]	2.253[1,18]	0.141[1,18]			0.136[3]
M20: p-cymenene				1.021[1,10]	0.000[1,10]	0.122[1,10]
M21: 2-nonanone	**5.073[1,18]***	1.303[1,18]	1.232[1,18]	**7.285[1,10]***	3.767[1,10]	2.794[1,10]
M22:	**16.071[1,18]**	0.112[1,18]	0.436[1,18]	2.220[1,10]	0.017[1,10]	0.087[1,10]

nonanal	***					
M23: ethyl phenol				**11.746^{1,10}****	3.814^{1,10}	2.960^{1,10}
M24: methyl salicylate						1.704^{3}
M25: 2-decanone	3.812^{1,18}	2.702^{1,18}	2.989^{1,18}	**7.082^{1,10}***	4.678^{1,10}	3.702^{1,10}
M26: bornyl acetate			**19.291^{5}****			**8.186^{3}***
M27: δ-elemene			**14.69^{5}***	0.861^{1,10}	0.004^{1,10}	0.064^{1,10}
M28: $C_{13}H_{18}$						**8.28^{3}***
M29: tetradecane	**8.385^{1,18}****	0.135^{1,18}	2.536^{1,18}	5.539^{1,10}*	0.011^{1,10}	0.223^{1,10}
M30: β-caryophyllene	0.001^{1,18}	2.000^{1,18}	0.101^{1,18}	1.437^{1,10}	0.071^{1,10}	0.016^{1,10}
M31: humulene			**16.223^{5}****	1.378^{1,10}	0.078^{1,10}	0.024^{1,10}
M32: hexadecane				1.201^{1,10}	0.000^{1,10}	0.053^{1,10}
M33: γ-terpinen			3.614^{5}	0.460^{1,10}	0.000^{1,10}	0.120^{1,10}
M34: $C_{10}H_{16}$			2.947^{5}			1.475^{3}
M35: decane	**7.235^{1,18}***	0.260^{1,18}	**0.403^{1,18}***	**5.932^{1,10}***	2.006^{1,10}	1.251^{1,10}
M36: linanool oxide				**238.634^{1,10} *****	0.000^{1,10}	0.001^{1,10}
M37: nonanol				2.155^{1,10}	**5.232^{1,10}***	4.032^{1,10}
M38: dodecane	**5.890^{1,18}***	0.192^{1,18}	2.157^{1,18}	**11.921^{1,10}****	1.364^{1,10}	1.420^{1,10}
M39: decanal			**14.154^{5}***	**9.249^{1,10}***	0.270^{1,10}	0.691^{1,10}
M40: tridecane	**7.139^{1,18}***	0.477^{1,18}	2.877^{1,18}			**8.52^{3}***
M41: undecane			**17.213^{5}****			

Other volatiles were only herbivore induced by one tomato species (Figure S1B). In LA1589 m-cymene and (M16) and nonanal (M22) were herbivore induced in all treatments, in Monita ethyl phenol (M23), linalool oxide and dodecane (M38) were herbivore induced in all treatments. Interestingly, in Monita most other volatiles (9 out of 15) were only herbivore induced in the SS treatment.

2.4 *C. carnea* behavior affected by soil treatment in both tomato species.

First, we determined whether *C. carnea* behavior was affected by species and soil treatment. We found an effect of species (PERMANOVA, F=41.212, p=0.039*) and treatment (F=213.614, p=0.001***) but there was no Treatment*variety interaction (F=0.009, p=0.811). Thus, analyses were performed for each species separately. Indeed, soil treatment affected the behavior of *C. carnea* in both tomato species, (Glm modelling p<0.001, Table 3). Neither tomato species showed significant differences in behavior between male and female individuals (p=1), therefore in further analysis the results are not separated by sex.

Table 3: Glm models of the effect of soil treatment and sex of *C. carnea* on the preference (choice of treatment) of *C. carnea*. Significant differences are indicated in bold.

Model	Statistical test (distribution)	Fixed factor/s	X^2	d.f.	Significance
Olfactometer LA1589	GLM (binomial)	Treatment	32.63	2	**<0.001**
		Sex	0	1	1
Olfactometer Monita	GLM (binomial)	Treatment	23.69	2	**<0.001**
		Sex	0	1	1

Next, it was determined which of the measured behavioral parameters were affected by soil treatment (Table 4). Strikingly, for both LA1589 and Monita, the SS treatment was most attractive, followed by the NS+Ri treatment, and the NS treatment was the least preferred. *C. carnea* speed was not significantly affected by the chosen treatment. In contrast, for LA1589 the distance moved was lower in those individuals choosing the NS+Ri treatment, while the time to choose did not significantly differ among treatments. In Monita, the distance moved and the time to choose were higher in those individuals that chose the NS treatment. Overall the results evidence a preference for the SS vs NS, and *R. irregularis* inoculation in NS increased *C. carnea* attraction to intermediate levels.

Table 4: Average values for the parameters measured with the Ethovision program for modern tomato species Monita and wild tomato species LA1589: choice of treatment (Treatment choice), number of individuals choosing treatment (N), speed (cm/s), distance moved before making a choice (distance moved, cm) and time to make a choice (time to choose, s). Significant differences of each tomato species separately are indicated by different letters for each parameter (Kruskall-Wallis tests, p<0.05).

Species	Treatment choice	N	speed	distancemoved	timetochoose
LA1589 (wild)	SS	45**a**	1.09±0.08	13.84±2.08**a**	15.26±1.98
	NS+Ri	28**b**	1.11±0.11	9.88±0.66**b**	14.08±2.49
	NS	11**c**	1.20±0.17	13.03±0.29**a**	15.08±3.39
Monita (modern)	SS	69**a**	1.64±0.09	10.67±0.82**b**	10.29±1.50**b**
	NS+Ri	48**b**	1.66±0.11	9.86±0.73**b**	13.02±4.46**b**

	NS	30**c**	1.60±0.15	13.58±0.68**a**	20.56±5.91**a**

2.5 Correlation between volatiles and *C. carnea* behavior in both tomato species.

Since the soil treatment affects the volatile pattern as well as the behavior of *C. carnea,* we asked whether there would be a correlation between volatile emission and *C. carnea* behavior. For both tomato species, *C. carnea* behavior showed a separation of the natural soil treatment (NS) from the other treatments (PCA ordination, Figure 4). In LA1589, volatile M27 (δ-elemene) was selected by ordistep to explain most differences in its behavior, which seems to explain the differences between NS plants with the others (Figure 4A). For Monita volatile M4 (3-hexen-1-ol) was selected by ordistep to explain most differences in behavior between plants and seems to be responsible for this difference (Figure 4B).

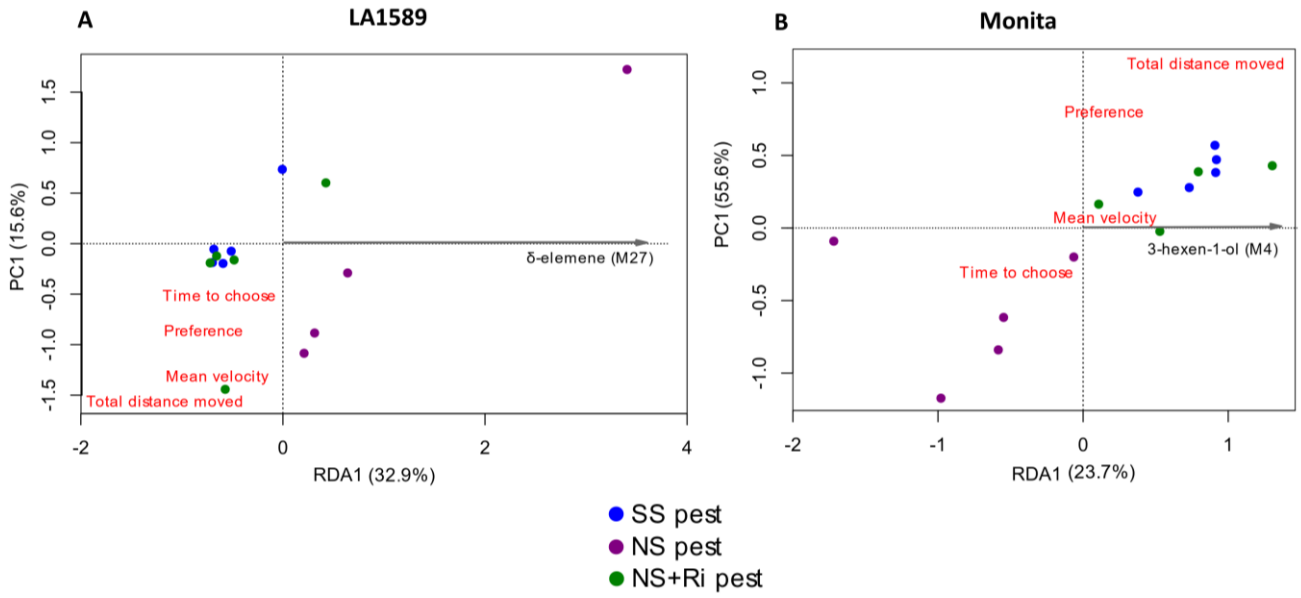


Figure 5: Principal Components Analysis (PCA) of *C. carnea* behavior in wild tomato species LA1589 and modern tomato species Monita. Treatments are natural soil (NS, purple), sterile soil (SS, green) and inoculated natural soil (NS+Ri, blue). Volatiles, as selected with ordistep, that affect differences in behavior are depicted with grey arrows.

δ-elemene was shown to drive distance moved ($p<0.01$), time to choose ($p<0.05$) and preference ($p<0.05$) in LA1589 (Table 5). Furthermore, δ-elemene was significant in an adonis model containing all parameters ($p<0.01$). 3-hexen-1-ol was shown to drive time to choose (ANOVA $p<0.05$, Table 5) in Monita.

Table 5: Results of selected volatiles in Monita and LA1589 driving all behavioral parameters (All, PERMANOVA) or each behavioral parameter separately (ANOVA). Results indicate F values^{df}. Asterisks indicate significance: * p < 0.05; ** p < 0.01; ***p<0.001.

	Name	**All**	**Speed**	**Distancemoved**	**Timetochooce**	**Preference**
LA1589	δ-elemene (M27)	**54.483^{1} ***	3.251^{1}	**58.535^{1} ****	**5.006^{1} ***	**56.249^{1} ***
Monita	3-hexen-1-ol (M4)	37.591^{1}	0.367^{1}	49.615^{1}	**6.258^{1} ***	0.083^{1}

Lastly, we tested whether δ-elemene and 3-hexen-1-ol were correlated to high or low levels of other volatiles to identify volatiles with a positive or negative correlation with *C. carnea* behavior and therefore could explain observed differences in attraction. Correlation analysis shows that δ-elemene (selected in LA1589) negatively correlates to α-pinene (M7, $p<0.05$) and positively to some other sesquiterpenes such as humulene (M31) and terpenes such as limonene + β-phellandrene (M17) and β-caryophyllene (M30, Table S5). In total δ-elemene correlated to 9 other volatiles. Some terpenes were expressed at a higher level in the NS treatment, the least attractive treatment, compared to both the NS and NS+Ri treatments (Figure S1C). 3-hexen-1-ol (selected in Monita) positively correlates to 34 other volatiles, out of 39 volatiles detected, indicating that 3-hexen-1-ol is showing the general pattern of volatile emission.

3. Discussion

In this study the effect of manipulation of soil microbiota by steam sterilization or AMF inoculation on volatile production and behavior of *C. carnea* upon induction by the generalist caterpillar *S. exigua* was studied in two tomato species with a different domestication degree (*S. pimpinellifolium* var. LA1589 and *S. lycopersicum* var. Monita), which differ in defense traits [26]. For instance, the wild tomato LA1589 contains type VI trichomes which have been associated with high levels of insect resistance against different pests including e.g. aphids, the leafminer *Liriomyza trifolii* and the caterpillar *S. exigua* [40]. In contrast, the modern tomato Monita contained the *Mi* gene, which has been isolated from *Solanum habrochaites* and confers resistance to phloem feeders such as the aphid *Macrosiphum euphorbiae*, the whitefly *Bemisia tabaci*, the tomato psyllid *Bactericerca cockerelli* and three species of nematodes [41,42]. However, no report on the effect of the *Mi* gene on chewing caterpillars were found.

Other factors, such as plant age, may have had an impact. Indeed, Monita plants were about half the age of the LA1589 plants (8 weeks versus 14 weeks). Since volatiles are known to change with growing conditions and during plant development [43–45], age may have affected experimental results. However, it should be noted that plants of both species were of similar developmental stage. Thus, it is likely that most differences observed in the volatiles profiles are related to the different resistance strategies of these two plant species. As expected, natural volatile emissions were affected by pest attack in both species. However, soil treatment only had a significant impact in LA1589, but not in Monita. Pest attack is known to affect volatile profiles and HIPVs are described in many plant systems, of which some may be toxic to the pest. For example, type VI trichomes, which were only present in LA1589, contain sesquiterpenes toxic to *S. exigua* such as δ-elemene, α-curcumene, and α-humulene [40]. Others may be involved in natural enemy attraction e.g. *Tetranychus urticae* induced volatiles attractive to the predator *Phytoseiulus persimilis* in tomato [40].

For both wild LA1589 and modern Monita, the predator *C. carnea* preferred the SS treatment followed by the NS+Ri and finally the NS treatment (Table 3). We identified seven volatiles which were induced by *S. exigua* in both tomato species. For example, bornyl acetate was induced in SS and NS treatments and has been described as toxic to the aphid *Myzus persicae* and the spidermite *Tetranychus urticae* [46,47]. Furthermore, there were four (LA1589) and eight (Monita) volatiles specifically induced in either tomato species. Since 1,3,5-cycloheptatriene,3,7,7,-trimethyl-, limonene + β-phellandrene, α-phellandrene, α-terpinene, 2-carene, δ-elemene and humulene (LA1589) were highest in the NS treatment, the least attractive treatment, and lower in the SS and NS+Ri treatments, we hypothesise they may function as pest repellents. This could be a direct defense to prevent pest attack, as it was shown for α-terpinene being toxic to the nematode *Ditylenchus destructor* [48]. Similarly, limonene and humulene were described as toxic to

different pest insects [49,50]. Interestingly, tetradecane was induced in LA1589, but was reduced in response to *Tuta absoluta* [51].

Remarkably, δ-elemene and α-humulene were especially present in the least attractive LA1589-NS treatment and less present in the other two treatments, with δ-elemene even absent from the most attractive SS treatment. This could be due to reduced suitability of *S. exigua* as a food source and potentially indicates a trade-off between direct and indirect defenses in LA1589. Thus, the absence of δ-elemene in the SS treatment would improve *S. exigua* performance, reducing direct defenses, and increase attraction of *C. carnea*, thus increasing indirect defenses.

Other HIPVs could be involved in natural enemy attraction. For example, decanal and octanal were induced in SS (both species) and LA1589-NS+Ri treatments, the most attractive to *C. carnea*. They were described as a HIPV in cotton with decanal being involved in parasitoid foraging and octanal attracting the predatory bug *Deraeocoris punctulatus* and the syrphid fly *Paragus quadrifasciatus* [52]. The attractive properties of HIPVs to natural enemies is not universal and depends on the organism exposed to them. For example, nonanal, induced in LA1589, has been shown to attract natural enemies such as the predatory bug *Orius similis*, but not the green lacewing *C. cinica* [52,53]. Moreover, in Monita linalool was induced in SS and NS treatments, and was described as repellent to the parasitoid *Aphidius ervi* [54] but attractant to the predator lady beetle [55]. Other volatiles such as 2-carene, α-phellandrene, humulene, α-phellandrene and α-terpinene were described as potentially involved in the attraction of different predators and parasitoids [55–58], although we detected them at higher levels in the least attractive NS treatment.

Thus, we were able to detect some potential volatiles explaining differences in *C. carnea* behavior, but there could be more. Most volatiles released by plants are present in low levels, with a few highly abundant ones [59]. Highly abundant compounds are sometimes unattractive or even repellent to natural enemies when presented alone or their absence does not reduce natural enemy attraction [60]. In fact, minor compounds could play major roles in natural enemy attraction as natural enemies are known to perceive subtle changes in volatile emissions [61]. For example, *Cotesia marginiventris* was attracted to polar volatiles which are often produced in very low levels, even below the GC-MS threshold level [62]. Thus, it is possible that that we could have missed volatiles explaining differences in *C. carnea* attraction.

On the other hand, blend complexity could also explain differences in natural enemy attraction. For example, reduced blend complexity may include critical compounds or blends that are important for natural enemy attraction [63]. For example, the egg parasitoid *Chrysonotomyia ruforum* was found to respond to the ¬sesquiterpene (E)-β-farnesene, a volatile induced by *Diprion pini* oviposition, only when contrasted with background odor from pines without eggs [60]. However, a too low dose did not induce a response while a too high dose worked as a repellant.

The different attractiveness of both natural soil treatments could be explained by differences in AMF colonization. Despite similar extension of mycorrhizal colonization as quantified by the binocular, precise molecular quantification revealed a significant increase of *R. irregularis* within the roots of inoculated plants of both tomato species. However, inoculation only significantly increased total AMF colonization levels in LA1589 (Figure 1A and 1B). Moreover, *R. irregularis* inoculation induced *C. carnea* attraction comparing natural soil treatments. These results indicate a high competitive ability of R. irregularis so it may outcompete other native AMF in the soil.

The reduced attraction in NS compared to SS could indicate that the soil microbiome has a negative effect on *C. carnea* attraction, which was partially reversed by AMF. Indeed, Pineda et al. found that aphid infested *Arabidopsis thaliana* was less attractive to the parasitoid *Diaeretiella rapae* in the presence of rhizobacteria, due to increased volatile production [64].

R. irregularis inoculation of natural soil induced *C. carnea* attraction as compared with the uninoculated natural soil. The different attractiveness of the two natural soil treatments could be explained by differences in AMF colonization. Mycorrhizal symbiosis was established in plants growing in natural soil without inoculation. The extension of mycorrhizal colonization in the roots of both treatments was similar as determined by histochemical staining, but precise molecular quantification revealed a significant increase of *R. irregularis* within the roots of inoculated plants of both tomato species. These results indicate a high competitive ability of *R. irregularis* so it may outcompete other native AMF in the soil, and the results support the role of *R. irregularis* in positively impacting *C. carnea* atraction.

The positive effect of the SS on the attraction of the predator could also be an artifact due to the sterilization procedure. For example, steam-sterilization may not kill all microbes and those remaining dominated the soil community resulting in a differential impact on plant defense. For example, tomatoes growing in natural soils elicited the expression of defense genes as compared with a sterile substrate [65]. This could be caused by differences in the soil microbial community, which differed between the SS and natural soil treatments in both tomato varieties. So the natural microbial community could promote direct defenses, at the cost of indirect defenses [66], although such an effect was not detected in milkweed [67].

Taken together, it seems that wild tomato LA1589 was more responsive to the soil microbiome than modern tomato Monita, coinciding with the assumption that modern crops have reduced mutualistic capacity [12,13]. Despite the differences observed, we cannot generalise an effect of domestication, as high intraspecific variation has been observed in maize and tomato [24,26, and in chapter 2 and 4 of this thesis]. In addition, to have a more complete overview of the microbial effects on plant indirect defenses, the microbial composition in other compartments, such as the endosphere, should be explored.

4. Materials and methods

Experiments were arranged to characterize a wild (*Solanum pimpinellifolium* var. LA1589) and a modern (*Solanum lycopersicum* var. Monita) tomato species. These experiments were carried out in subsequent years, in 2019 for Monita and 2020 for LA1589. We used an internal control (*Solanum lycopersicum* L. cv. Moneymaker) detecting no significant differences in the GC-MS volatile peak area between years.

4.1 Experimental set-up

Soil and seeds used in the experiment were provided by the Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC) germplasm bank. The field soil used in the experiment is classified as an Eutric Regosol soil [68]. Soil was collected from IHSM-UMA-CSIC, sieved with a 1cm2 sieve, mixed, air-dried and stored in bags until the start of the experiment (natural soil treatment, NS). For the sterile soil treatment (SS), soil was steam sterilized for three consecutive days at 95°C for 45 minutes. Afterwards, soil was air-dried and stored. For all treatments tomato seeds were disinfected for three hours in a fuming hood containing 3% HCl in bleach solution. Seeds (n=30) were sown in commercial soil substrate vermiculite (2:1) and seedlings were transplanted after 2-3 weeks. For transplantation, pots (12cm diameter, 13 cm height) were prepared containing a mix of 1.5kg soil and 2/3 of volume vermiculite and 200mL of water. For each treatment, 10 plants were transplanted. After transplanting, the plants of the NS+Ri treatment were inoculated with 1mL *Rhizophagus irregularis* spores (Koppert Biological Systems, Berken en Rodenrijs, Netherlands), corresponding to 1000 spores. Afterwards, plants were provided with another 50mL of water and grown in the greenhouse (16:8 L:D, 24:16°C, 60-80% RH). Plant were watered and provided with Long Ashton nutrient solution [69] containing 25% of the standard phosphorus (P) concentration as required.

Deviations from the above setup for the different varieties were the following. Soil for both tomato species was collected in the same year, in winter for LA1589 and in spring for Monita. Plant growth started in summer for LA1589 and in autumn for Monita. Behavioral analysis was done in autumn for both species. Since wild species LA1589 grew slower and produced smaller leaves than modern species Monita, LA1589 plants were older (14 weeks) during volatile and behavioral analysis than Monita plants (8 weeks). We used plants in the same developmental stage in both years.

4.2 Insect rearing

4.2.1 *Spodoptera exigua*

Spodoptera exigua H. (Lepidoptera: Noctuidae) eggs were provided by Andermatt Biocontrol AG (Grossdietwil, Switzerland) and reared in the laboratory of Dr. Herrero for more than 50 generations. After hatching, larvae were reared on artificial diet [70] at 25±3 ºC with 70±5% relative humidity and a 16 h/8 h diurnal photoperiod. For wild species LA1589 five *S. exigua* larvae of stages L2/L3 were used, for modern species Monita three *S. exigua* larvae of stages L4/L5 were used. Larvae were left to feed for 24 hours in a tent to prevent escape. Before starting the predator behavioral assay, larvae were removed from the plants.

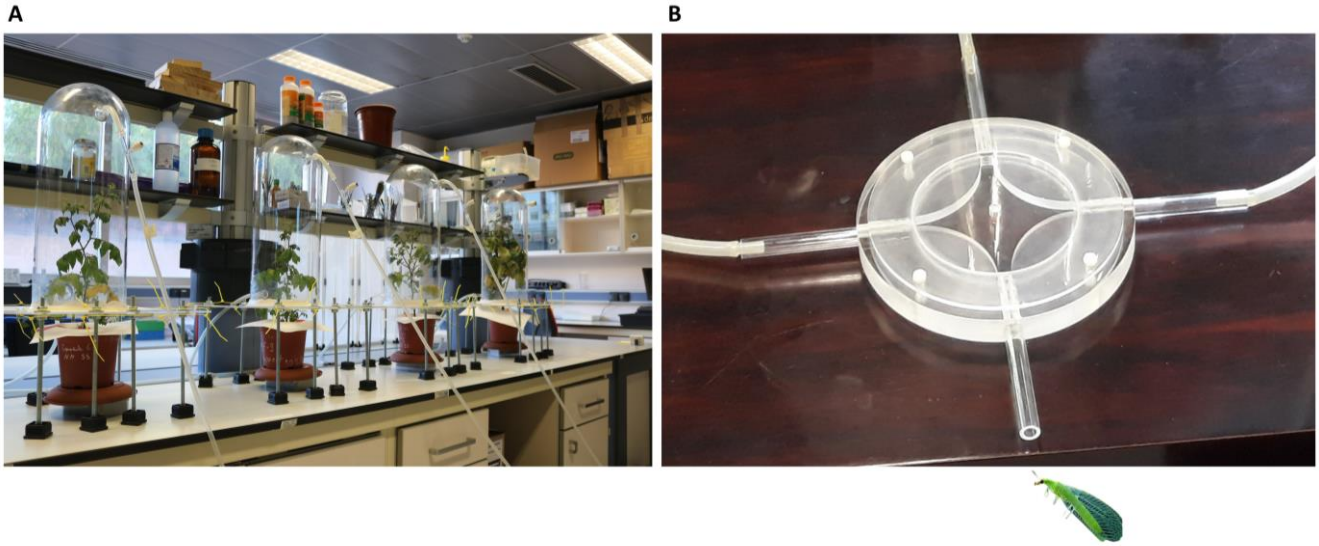
4.2.1 *Chrysoperla carnea*

The *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae) larvae were supplied by Koppert Biological Systems (La Mojonera, Almería, Spain). Larvae were individually reared in Petri dishes and fed on *Ephestia kuehniella* Zell. (Lepidoptera: Pyralidae) eggs. Upon emergence, *C. carnea* adults were collected daily and kept in boxes (28 cm diameter, 15 cm high) with an ovipositing surface; they were fed on honey:pollen (1:1, v:v) and mineral water, and maintained in a controlled environment cabinet at 25°C, 50-60% RH and 16:8 (L:D) h. Adult *C. carnea* were sexed by examining the ventral surface of the abdominal tip. Both males and females were used for bioassays.

4.3 Volatile collection

Closed glass chambers (40x40x140cm), sufficiently large to accommodate the plants and completely separated from the pots containing the soil were connected from the top to the glass tubing of the olfactometer through transparent polytetrafluoroethylene tubes (Figure 6A). A solid-phase microextraction (SPME) fibre was inserted into each arm of the olfactometer for volatile collection (Table S1). Using air pressure, a synthetic pure air at an airflow rate of 1.2 l min-1 [71] per channel was drawn into the bottom of the chambers. Volatiles were taken before introducing the pest *S. exigua* (volatiles before attack) from selected plants (n=3). Moreover, volatiles were collected from empty chambers (background volatiles). Volatiles were taken (volatiles after attack) with SPME fibres during the behavioral assay (n=5). Volatiles (before and during attack) were taken during 2 hours.

Figure 6: Overview of experimental set-up during behavioral assays. Set-up of plants in closed glass chambers with tubes (A) being connected to a closed-system multi-tube olfactometer in a separate room to avoid physical attraction to plants (B). The fourth arm was used as an entry point for the predator lacewing *C. carnea*.



4.4 Behavioral assay with *C. carnea*

A closed-system multi-tube olfactometer was used to assess the choice and behavior of the predator *C. carnea* (Figure 6B). Natural enemy attraction was monitored in a four arms-olfactometer connected to EthoVision XT integrated video tracking system (Noldus Information Technology, Wageningen, The Netherlands). The EthoVision software automatically determines the location of the individual insect in the area and calculates several movement parameters derived from changes in position. The parameters chosen were (1) total distance moved (cm), (2) mean velocity (mm/s) and (3) time to choose (Table S2). Volatiles were taken for the duration of the assay, which was approximately 2 hours.

Behavioral tests of adult predator *C. carnea* were carried out under artificial light between 09:00 and 18:00 at 28±2°C. A white circular paperboard arena was placed around the olfactometer to prevent visual perturbations. *C. carnea* adults were inserted into a single branch of the olfactometer and were left to choose between the three branches of the device (each connected to a different soil treatment, Figure 6B), with a maximum observation period of 5 min. If the insects, which were used only once and then released, did not enter the arena, they were excluded from the data analysis. At the end of the tests used plants were harvested. Behavioral tests were conducted on five executive days for each treatment to account for diurnal variation (one plant per treatment per day).

4.5 Harvesting and mycorrhizal colonization

At harvest, shoot and root fresh weight were measured. Roots were separated from the soil, washed and dried to remove attached soil before determining their weight. An aliquot of the roots was cut into 1cm pieces and kept in 50mL Eppendorf tubes to determine mycorrhizal colonization. Another part of the roots was wrapped in aluminium foil, flash frozen in liquid nitrogen and stored at -80°C for DNA extraction. Soil samples from each pot were placed in separate polyethylene plastic bags and immediately stored at -80°C until molecular analyses were performed.

Mycorrhizal colonization was determined using the ink staining method of Vierheilig et al. [72]. Roots were incubated in 10% KOH at 60°C overnight. Afterwards, roots were washed and acidified in 2% acetic acid for

5 minutes. Then the staining solution containing 5% ink (Lamy T51) in 2% acetic acid was added and roots were incubated at 60°C in a water bath WNB with shaking device (Memmert GmbH + Co.KG, Germany) for about 20 minutes. Roots were then washed to remove the ink solution and stop the staining. The degree of mycorrhizal colonization (expressed as the percentage of total root length colonized by AMF) was calculated with the gridline intersection method using a Nikon SMZ800 stereomicroscope with bright field conditions [73].

Afterwards, mycorrhizal fragments were mounted on slides (at least 15 per plant) to determine the colonization intensity in terms of arbuscules, vesicles and appressoria by the Trouvelot method using a Leica DM1000 LED microscope (Leica Microsystems CMS GmbH) for examination [74]. For molecular quantification of mycorrhizal fungi within the roots, frozen roots were grinded in liquid nitrogen with mortar and pestle to a fine powder. DNA was extracted from 100mg grinded roots of 5 individual plants using the DNA plant kit (Bioline, Memphis, Tennessee, USA) following the manufacturer's instructions. Real-time qPCR was performed using the iCycler iQ5 system (Bio-Rad) to determine the presence of glomeromycotan fungi using the HgEF primers encoding for the glomeromycotan elongation factor, and the presence of *R. irregularis* using the Ri28S primers encoding the 28S nuclear large ribosomal subunit [75]. Primer sequences are HgEF 5' TTGCTTTCGTCCCAATATCC '3 and 5' AGTGGAAGACGAAGGGGTTT '3; Ri28S 5' TTCGGGTAATCAGCCTTTCG '3 and 5' TCAGAGATCAGACAGGTAGCC 3'. Expression values were normalized using the housekeeping gene SlEF-1α [76], which encodes for the tomato elongation factor-1α (primer sequences are SlEF-1α 5' GATTGGTGGTATTGGAACTGTC '3 and 5' AGCTTCGTGGTGCATCTC 3'). Relative quantification of normalised AMF or *R. irregularis* values was performed using the comparative 2-Δ(ΔCt) method [77]. A negative qPCR result after 35 cycles indicated absence of fungus. Two technical replicates were used for each plant, so 10 values per treatment.

4.6 Molecular analysis of soil bacteria

For molecular analysis of soil bacteria, eight 1g aliquots from each soil sample were DNA extracted using the bead-beating method with the aid of a PowerSoil® DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA) according to the manufacturer's instructions. Extractions of every treatment were pooled into two groups and concentrated at 35 °C to a final volume of 20 µl using a Savant Speedvac® concentrator. The V3-V4 hypervariable regions (primers 5' CCTACGGGNBGCASCAG 3' and 5′ GACTACNVGGGTATCTAATCC 3′ [78,79] of the 16S rRNA gene were used to characterize the bacterial communities of the two replicates per sample using the Illumina MiSeq platform at the genomic facilities of the López-Neyra Institute of Parasitology and Biomedicine (IPBLN-CSIC). Raw sequences were pre-processed using the SEED2 platform [80] by first merging forward and reverse sequences. Quality filtering excluded sequences containing ambiguous bases (N) and those with a quality score of less than 30. Primers were removed and sequences trimmed to 400bp length. The sequences were then clustered using the UPARSE method: Operational Taxonomic Unit (OTU) radius set of 3% and sequence similarity of 97%. Singletons and chimeric sequences were removed. Taxonomic assignment of OTUs was performed using the classify.seqs algorithm in mothur software against the SILVA v132 database [81,82]. An abundance sample x OTU matrix were generated using OTU reads as a proxy of abundance using the Marker Data Profiling module in the MicrobiomeAnalyst tool [83,84]. The most abundant sequence per OTU was selected as representative. Rarefaction curves were visualized using MicrobiomeAnalyst to confirm that all samples reached a plateau. Beta diversity analysis were performed using the phyloseq package [85] to test species complexity between groups using the MicrobiomeAnalyst web-based platform.

4.7 Statistical analysis

4.7.1 Effect of soil treatment and pest attack on volatile production

For volatile analysis the volatile data was log transformed. To check for the effect of treatments: soil treatment, pest attack and tomato variety we used a permutational ANOVA (PERMANOVA, function Adonis, vegan R package [86]) including every interaction between explanatory factors (euclidean distance was used as measure of dissimilarity and 1000 permutations). Since in the initial model we observed a significant interaction between soil treatment and tomato variety, we run separate models for each variety. Then, the volatile patterns of each plant were shown using redundancy analysis (RDA, [87].

To test which volatiles significantly differed between soil treatment, *S. exigua* presence and their interaction, linear models (lm) were run on each volatile and tomato variety, separately. Since the NS+Ri treatment of Monita only had one replicate, this treatment was not taken into account before attack. The goodness of fit was checked by plotting the residuals (function simulateResiduals, package DHARMa [88]) and an ANOVA test was used to determine whether each volatile was affected by soil, pest treatment or their interaction (type 3 ANOVA function, car R package [89]). When significant, lsmeans (package emmeans [90]) was used as a post hoc test to perform pairwise comparisons. If data did not meet normality and/or homocedasticity, the non-parametric test Kruskall Wallis (package agricolae, [91]) was used. When significant, pairwise comparisons were performed with dunn test (package rstatix, [92]). The same process was repeated for Monita using only the data after attack to include the NS+Ri treatment and check for significant differences between soil treatments, but none were detected.

For beta diversity, Bray–Curtis distance and Permutational ANOVA (PERMANOVA) were used to evaluate the distance between samples and the statistical significance of the clustering pattern, respectively.

4.7.2 Effect of soil treatment on *C. carnea* behavior

To check for the effect of treatment and tomato variety we used a permutational ANOVA (PERMANOVA, function Adonis, vegan R package [86]) including every interaction between explanatory factors (euclidean distance was used as measure of dissimilarity and 1000 permutations). Glm models using a binomial distribution were run to test whether Crisopa behavior was affected by soil treatment (SS, Ni or Ri) or sex (female or male) of the Crisopa insect. Since there were no significant differences between male and female Crisopa, sex was not taken into account in further analysis. Tukey test was performed to see which treatments were significantly different. Then the non-parametric test Kruskall Wallis (package agricolae, [91]) was used to test whether speed, distance moved and time to choose differed between soil treatments. Models were run separately for tomato varieties.

4.7.3 Correlation between volatile production and *C. carnea* behavior

An RDA was run using the Crisopa response as the response variables (log-transformed) and volatiles as explanatory variables. For this analysis, only the volatiles taken after pest attack were considered. The main volatiles driving differences in Crisopa behavior were selected via forward selection using ordistep, after which only the selected volatiles were used in the RDA.

4.7.4 Effect of AMF inoculation on colonization level and intensity

Data for fungal colonization intensity were log-transformed. For the molecular data, we calculated fold changes between the NS and the NS+Ri treatment. To determine whether fungal colonization, as determined by the microscope and by molecular methods, differed between treatments, we used PERMANOVA when

data met normality (function Adonis, vegan R package [86]) and the non-parametric test Kruskall Wallis if data did not meet normality (package agricolae, [91]).

5. Conclusions

In summary, we conclude that pest attack affected volatile blends in both wild and modern tomato, with soil microbiota being only significant in wild LA1589. We found volatiles that were induced by *S. exigua* in both tomato species or specifically in one of them, which could be interesting targets for *S. exigua* performance assays to determine their function as pest or natural enemy repellants. Furthermore, some of these volatiles e.g. decanal, nonanal and linalool oxide were described to function in natural enemy attraction or repellence, so could be useful to test their effects on the attraction of different predators and parasitoids. The behavior of the predator *C. carnea* was affected by soil treatment in both LA1589 and Monita. According to the data in both tomato species, we conclude that *R. irregularis* inoculation increases *C. carnea* attraction in natural soil. Since the increased *R. irregularis* colonization levels coincided with increased attraction of a generalist predator, inoculation could be a potentially sustainable way to enhance plant indirect defenses for environmental friendly crop protection.

6. References

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7. Supplementary material

Table S1: Peak area of volatiles detected in each sample of modern tomato Monita and wild tomato LA1589

		toluene	hexanal	2-hexenal	3-hexen-1-ol	o-xylene	p-Xylene
Species	**Treatment**	M1	M2	M3	M4	M5	M6
LA1589	SS_control	230502.542	187995.338	103597.222	0	36775.956	128218.519
LA1589	SS_Pest	333051.852	97697.902	41179.167	0	83860.656	293088.889
LA1589	NS_Control	407090.051	118780.886	0	0	64672.131	189314.815
LA1589	NS_Pest	532699.346	126816.783	5164.583	0	126578.689	440284.444
LA1589	NS+Ri_Control	297262.164	97925.408	22180.556	0	47830.601	153066.667
LA1589	NS+Ri_Pest	362405.664	128099.301	22845.833	0	109714.754	335528.889
Monita	SS_control	259160.131	46863.636	0	11524.561	47364.754	34755.556
Monita	SS_Pest	1806782.135	164475.524	31816.667	246520.702	619431.148	326691.111
Monita	NS_Control	504892.157	232167.832	17140.625	12568.421	87229.508	47677.778
Monita	NS_Pest	3503520.697	205348.252	18404.167	253460.351	1004450.820	518108.889
Monita	NS+Ri_Control	318065.359	14916.084	0	0	115942.623	47566.667
Monita	NS+Ri_Pest	2476797.930	228236.014	48557.292	316550.877	947245.902	510975.000

		α-pinene	6-methyl-2-heptanone	1.3.5-cycloheptatriene.3.7.7.-trimethyl-
Species	**Treatment**	M7	M8	M9
LA1589	SS_control	3377424.679	0	0
LA1589	SS_Pest	980113.462	0	0
LA1589	NS_Control	458552.885	0	324844.025
LA1589	NS_Pest	370556.731	0	185864.151
LA1589	NS+Ri_Control	1722568.910	0	0
LA1589	NS+Ri_Pest	850225.000	0	40846.038
Monita	SS_control	569293.269	17417.127	1670188.500
Monita	SS_Pest	547345.192	414180.110	704659.400

Monita	NS_Control	884500.000	49621.547	1798222.698
Monita	NS_Pest	674576.923	594592.265	836141.125
Monita	NS+Ri_Control	74033.654	62055.249	69049.000
Monita	NS+Ri_Pest	732246.394	658776.243	747845.250

		6-methyl-5-hepten-2-one	mircene	2-carene	octanal	α-phellandrene
Species	**Treatment**	M10	M11	M12	M13	M14
LA1589	SS_control	0	0	233761.384	86977.778	43116.120
LA1589	SS_Pest	0	0	76453.552	341213.333	48300.000
LA1589	NS_Control	0	0	5873003.643	146822.222	942054.645
LA1589	NS_Pest	0	0	3512079.781	461026.667	721101.639
LA1589	NS+Ri_Control	0	0	117040.073	97044.444	57393.443
LA1589	NS+Ri_Pest	0	0	848902.732	280190.000	169263.934
Monita	SS_control	27197.368	312657.051	8247193.989	0	1973793.033
Monita	SS_Pest	242150.000	169488.462	4236190.164	692090.000	982899.180
Monita	NS_Control	52789.474	238028.846	9466551.913	185583.333	2394428.279
Monita	NS_Pest	285978.947	212015.385	4032987.978	911660.000	1002538.525
Monita	NS+Ri_Control	28355.263	16237.179	537327.869	69950.000	117774.590
Monita	NS+Ri_Pest	376575.658	265623.397	5002476.776	1258033.333	1180913.934

		α-terpinene	m-cymene	limonene + β-phellandrene	β-(E)-ocimene
Species	**Treatment**	M15	M16	M17	M18
LA1589	SS_control	4692.884	11673.669	3608093.250	57833.333
LA1589	SS_Pest	3284.270	110538.655	2614212.944	44228.750
LA1589	NS_Control	271129.213	22379.552	70329937.370	11120.833
LA1589	NS_Pest	275869.663	101556.303	51766004.175	19356.250
LA1589	NS+Ri_Control	7413.858	11264.706	3487738.344	46772.917
LA1589	NS+Ri_Pest	52605.618	83147.899	13305578.288	22067.500
Monita	SS_control	858359.551	697787.815	152419050.104	159984.375
Monita	SS_Pest	473478.652	1133478.151	75971803.758	103885.000
Monita	NS_Control	854634.831	592613.445	158784425.887	123215.625
Monita	NS_Pest	394464.045	926612.605	78489340.292	116746.250
Monita	NS+Ri_Control	44016.854	81861.345	11826993.737	0
Monita	NS+Ri_Pest	551724.719	1510529.412	100673528.184	155309.375

		α-terpinolene	p-cymenene	2-nonanone	nonanal	ethyl phenol
Species	**Treatment**	M19	M20	M21	M22	M23
LA1589	SS_control	11056.225	0	39386.612	239599.388	0

LA1589	SS_Pest	18002.410	0	198314.754	1985211.009	0
LA1589	NS_Control	51586.345	0	82146.175	344776.758	0
LA1589	NS_Pest	63353.012	0	231327.049	1920677.064	0
LA1589	NS+Ri_Control	12329.317	0	46433.060	294938.838	0
LA1589	NS+Ri_Pest	16595.181	0	113131.967	1447634.862	0
Monita	SS_control	171852.410	28584.906	18032.787	635568.807	17200.272
Monita	SS_Pest	122727.711	117643.396	787475.410	2076693.578	852913.351
M*onita*	*NS_Control*	*157867.470*	*26570.755*	*83446.721*	991348.624	84284.741
Monita	NS_Pest	118024.096	129549.057	1159608.197	2259214.679	1201945.504
Monita	NS+Ri_Control	17487.952	0	80*663.934*	203944.954	76806.540
Monita	NS+Ri_Pest	162971.386	205879.717	1486816.598	3163215.596	1598072.207

		methyl salicylate	2-decanone	bornyl acetat*e*	δ-elemene	$C_{13}H_{18}$
Species	**Treatment**	M24	M25	M26	M27	M28
LA1589	SS_control	0	41089.431	10462.121	4788.423	0
LA1589	SS_Pest	0	76453.552	252260.606	0	0
LA1589	NS_Control	0	125551.220	0	45443.114	0
LA1589	NS_Pest	0	3512079.781	177403.030	46073.054	0
LA1589	NS+Ri_Control	0	56817.886	13606.061	6656.687	0
LA1589	NS+Ri_Pest	0	848902.732	150360.606	2805.988	0
Monita	SS_control	18630.653	58163.415	0	37464.072	25262.542
Monita	SS_Pest	57797.990	2930830.244	236478.788	104137.725	1405662.876
Monita	NS_Control	59741.206	258651.220	0	20425.150	82489.967
Monita	NS_Pest	49283.417	3906897.561	230546.970	96681.437	1720349.164
Monita	NS+Ri_Control	0	285931.707	0	0	121200.669
Monita	NS+Ri_Pest	83211.055	5419739.024	329130.682	143721.557	2428392.140

		tetradecane	β-caryophyllene	humulene	hexadecane	γ-terpinen
Species	**Treatment**	M29	M30	M31	M32	M33
LA1589	SS_control	71764.423	72840.278	13376.682	0	55849.162
LA1589	SS_Pest	754228.846	53500.000	3369.507	0	51455.866
LA1589	NS_Control	103825.321	309572.917	60294.469	0	32026.071
LA1589	NS_Pest	651626.923	540543.750	98760.538	0	50267.039
LA1589	NS+Ri_Control	89192.308	53881.944	11417.040	0	34828.678
LA1589	NS+Ri_Pest	288754.808	33072.917	4881.614	0	30982.123
Monita	SS_control	158098.558	428416.667	73327.354	151105.381	106634.078
Monita	SS_Pest	936306.731	1323318.750	223700.448	277782.960	73384.358
Monita	NS_Control	174564.904	252666.667	41865.471	160701.794	114731.844
Monita	NS_Pest	881856.731	1041270.833	172614.350	285706.726	72706.145
Monita	NS+Ri_Control	67961.538	28739.583	0	37278.027	0

Monita	NS+Ri_Pest	1340609.375	1266552.083	214003.363	411595.291	98843.575

		terpene $C_{10}H_{16}$	decane	linanool oxide	nonanol	dodecane
Species	**Treatment**	M34	M35	M36	M37	M38
LA1589	SS_control	0	162010.499	0	0	123769.671
LA1589	SS_Pest	0	653248.031	0	0	915845.494
LA1589	NS_Control	21452.539	273030.184	0	0	206759.657
LA1589	NS_Pest	7886.093	864695.276	0	0	935775.966
LA1589	NS+Ri_Control	0	197140.420	0	0	155879.828
LA1589	NS+Ri_Pest	0	537731.496	0	0	408790.558
Monita	SS_control	52390.728	52895.669	0	13659.091	62221.030
Monita	SS_Pest	19133.775	956504.724	87478.400	1331985.455	1325506.438
Monita	NS_Control	55612.583	186021.654	0	49313.636	150618.026
Monita	NS_Pest	11829.139	1412809.449	114051.200	1853536.364	1524071.245
Monita	NS+Ri_Control	0	118019.685	0	nonanol	dodecane
Monita	NS+Ri_Pest	17192.053	1672615.157	143362.000	M37	M38

		decanal	tridecane	undecane
Species	**Treatment**	M39	M40	M41
LA1589	SS_control	0	43726.241	120476.596
LA1589	SS_Pest	666526.761	423402.553	936657.021
LA1589	NS_Control	0	78967.376	*264526*.241
LA1589	NS_Pest	424138.028	386161.702	1043424.681
LA1589	NS+Ri_Control	0	61114.894	191995.745
LA1589	NS+Ri_Pest	450436.620	*167354.894*	*54*9627.234
Monita	SS_control	78774.648	49612.766	0
Monita	SS_Pest	1010385.915	810806.809	0
Monita	NS_Control	121274.648	83361.702	0
Monita	NS_Pest	959997.183	856775.319	0
Monita	NS+Ri_Control	74881.818	134356.223	65492.958
Monita	NS+Ri_Pest	2677290.909	2218024.678	1532595.070

Table S2: Average values for the parameters measured with the Ethovision program for each sample for modern tomato species Monita and wild tomato species LA1589: Mean velocity (cm/s), Total distance moved before making a choice (Totaldistance-moved, cm), time to make a choice (timetochoice, s), and percentage of individuals choosing the sample (Preference, %).

		Crisopa behavior			
Species	**Treatment**	**Meanvelocity**	**Totaldistancemoved**	**Timetochoice**	**Preference**
LA1589	SS_Pest	148.278	538.500	11.252	50.674
LA1589	NS_Pest	141.508	145.450	19.290	21.786

LA1589	NS+Ri_Pest	137.908	553.228	13.643	34.428
Monita	SS_Pest	53.368	710.158	16.470	54.960
Monita	NS_Pest	56.620	73.562	12.274	11.392
Monita	NS+Ri_Pest	70.276	677.796	14.678	33.650

Table S3: Average values for plant growth and colonization data. A) Plant growth and histochemical colonization level. B) Colonization intensity in terms of vesicles and arbuscules. C) Colonization intensity in terms of appressoria.

A) Plant growth (shoot and root weight), histochemical colonization level by gridline intersection method (Mycorrhization %). For the Trouvelot method: F% percentage of mycorrhizal (containing arbuscules, vesicles, hyphae or appressoria) root fragments; M% abundance of mycorrhizal colonization in root system and m% intensity of mycorrhizal colonization in root fragments. For details on the Trouvelot categories, see Gianinazzi-pearson et al. (1986).

Species	Treatment	Shoot weight(g)	Root weight (g)	Mycorrization (%)	F%	M%	m%
LA1589	SS	13.06	18.71	0			
LA1589	NS	18.45	11.23	5.95	86.58	18.75	20.87
LA1589	NS+Ri	14.31	14.13	7.10	75.42	11.44	14.50
Monita	SS	17.05	4.78	0			
Monita	NS	16.69	5.79	7.00	85.48	11.85	15.88
Monita	NS+Ri	17.05	5.82	5.70	72.41	12.61	17.80

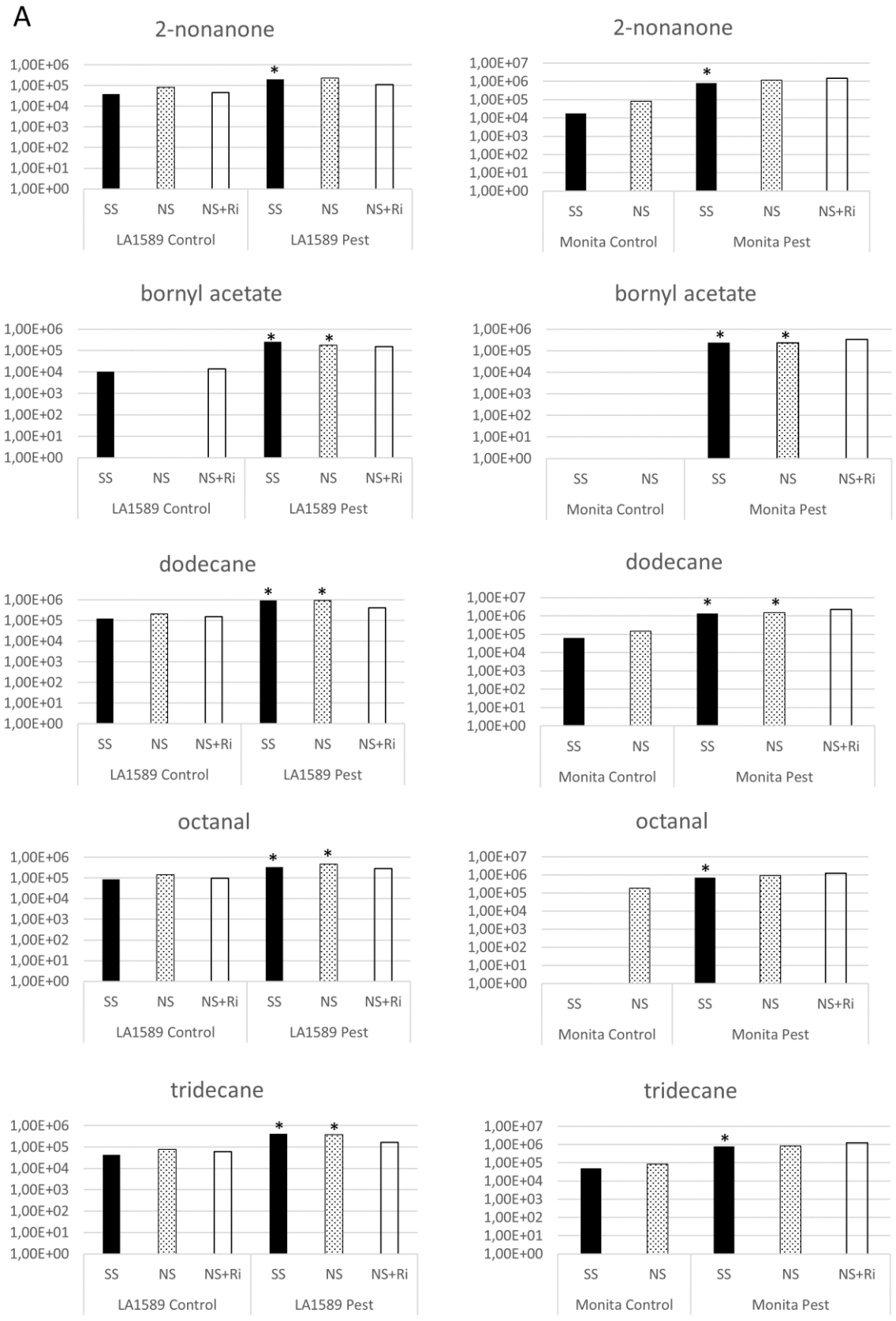
B) Colonization intensity of arbuscules and vesicles. Arbuscule (a) and vesicle (v) abundance in mycorrhized root fragments, Arbuscule (A) and vesicle (V) abundance in the root system. mA1, mA2 etc are the percentage of mycorrized root fragments rated A1, rated A2 etc. For details on the Trouvelot categories, see Gianinazzi-pearson et al. (1986).

		Arbuscules					Vesicles				
Species	Treatment	a%	A%	mA3	mA2	mA1	v%	V%	mV3	mV2	mV1
LA1589	SS										
LA1589	NS	81.71	15.64	66.01	30.76	3.24	20.86	4.62	3.34	28.77	31.29
LA1589	NS+Ri	71.31	13.19	55.85	27.62	16.53	21.37	3.43	2.75	34.16	15.42
Monita	SS										
Monita	NS	72.34	8.78	45.67	53.10	1.23	16.05	2.19	0	25.72	31.88
Monita	NS+Ri	85.34	10.48	74.32	21.14	4.42	34.41	3.57	27.15	8.37	30.77

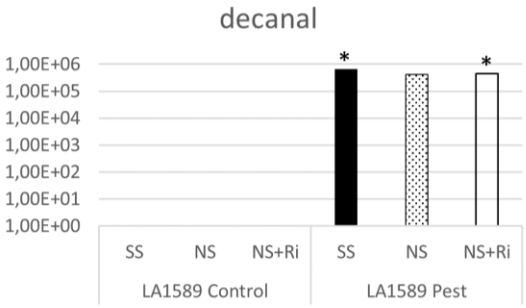
C) Colonization intensity (Trouvelot) in terms of appressoria found in each fragment. mA9, mA8 etc are the percentage of mycorrized root fragments with 9 appressoria, with 8 appressorias etc. %Ap: average number of appressoria per treatment. For details on the Trouvelot categories, see Gianinazzi-pearson et al. (1986).

Species	Treatment	mC9	mC8	mC7	mC6	mC5	mC4	mC3	mC2	mC1	%Ap
LA1589	SS										
LA1589	NS	0	4.54	0	3.34	5.75	8.20	11.49	25.77	9.75	1.46
LA1589	NS+Ri	2.61	1.93	1.93	4.80	5.63	2.86	34.48	6.12	15.64	1.14
Monita	SS										

Monita	NS	0	0	0	0	0	21.15	20.03	29.43	9.97	1.28
Monita	NS+Ri	0	0	0	0	0.44	7.03	14.23	23.14	20.82	0.70



(A) Volatiles induced by S. exigua in both LA1589 and Monita.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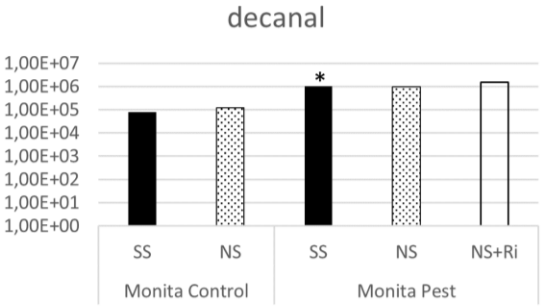
LA1589 Control
LA1589 Pest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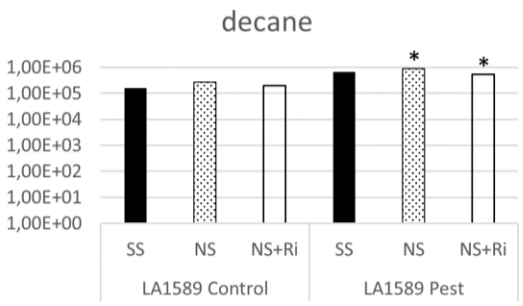
Monita Control
Monita Pest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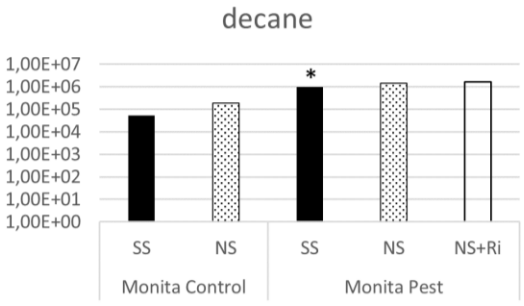
LA1589 Control
LA1589 Pest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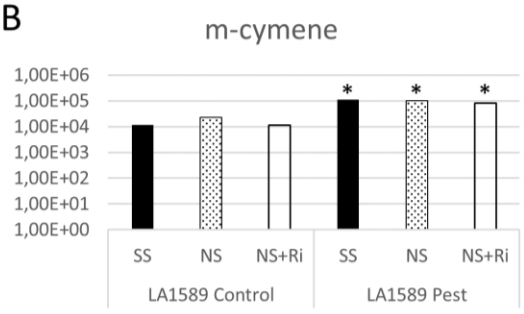
Monita Control
Monita Pest

B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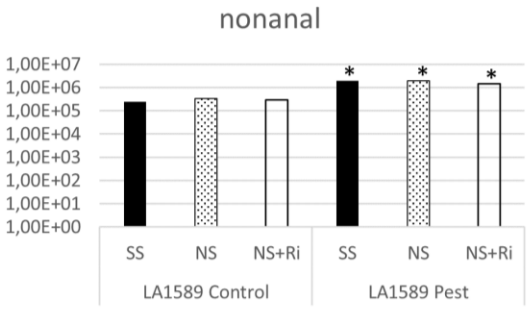
LA1589 Control
LA1589 Pest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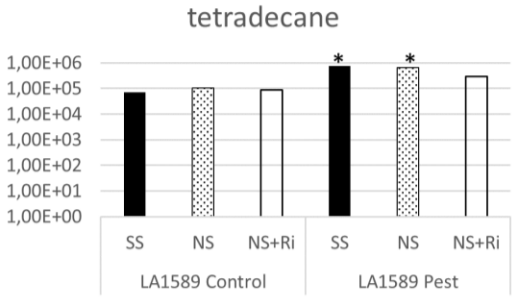
SS NS NS+Ri SS NS NS+Ri
LA1589 Control
LA1589 Pest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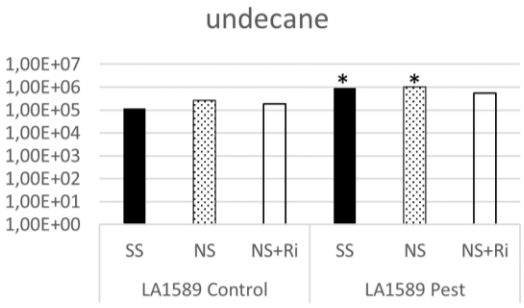
LA1589 Control
LA1589 Pest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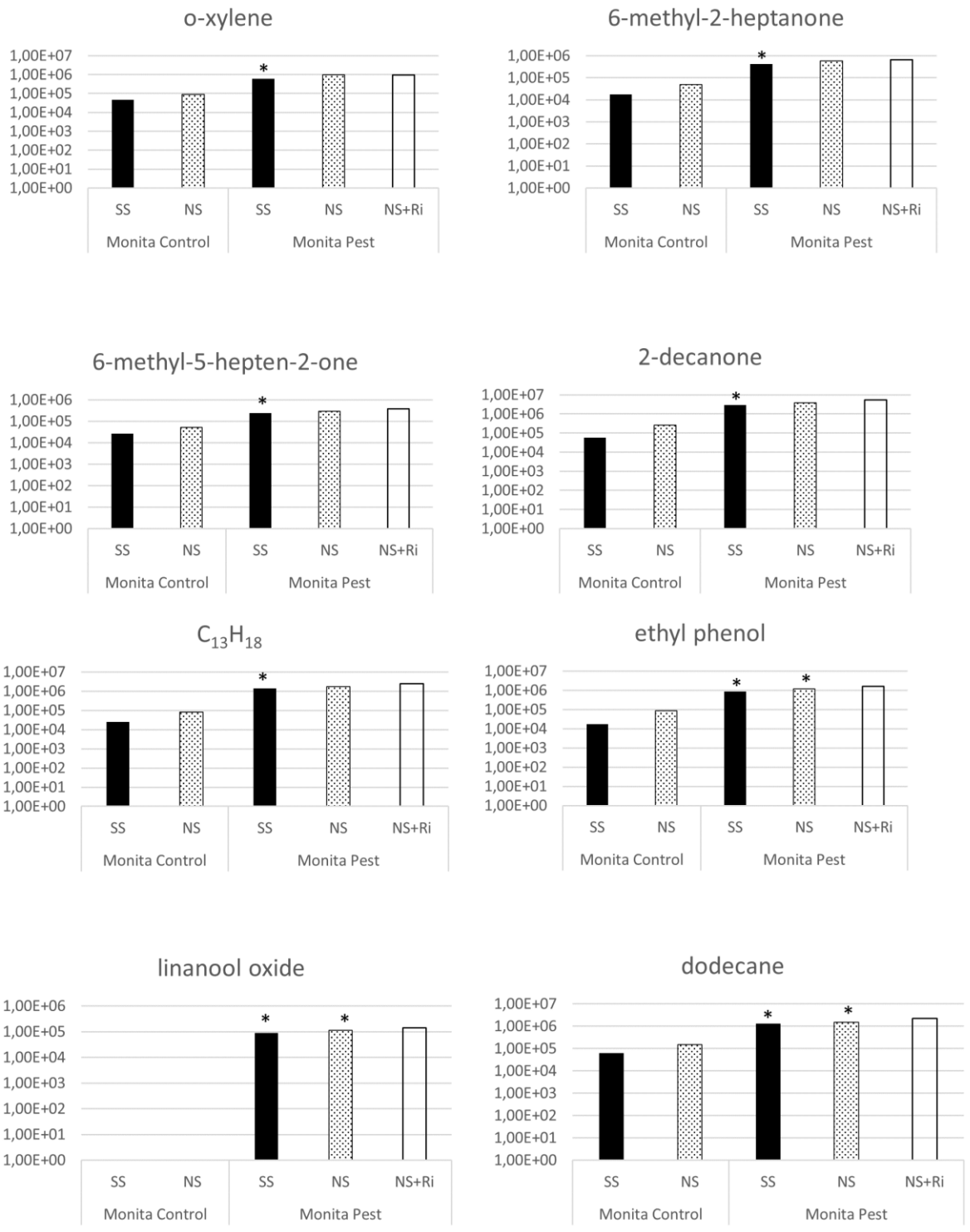
SS NS NS+Ri SS NS NS+Ri
LA1589 Control
LA1589 Pest



(B) Volatiles induced by S. exigua in either LA1589 or Monita.

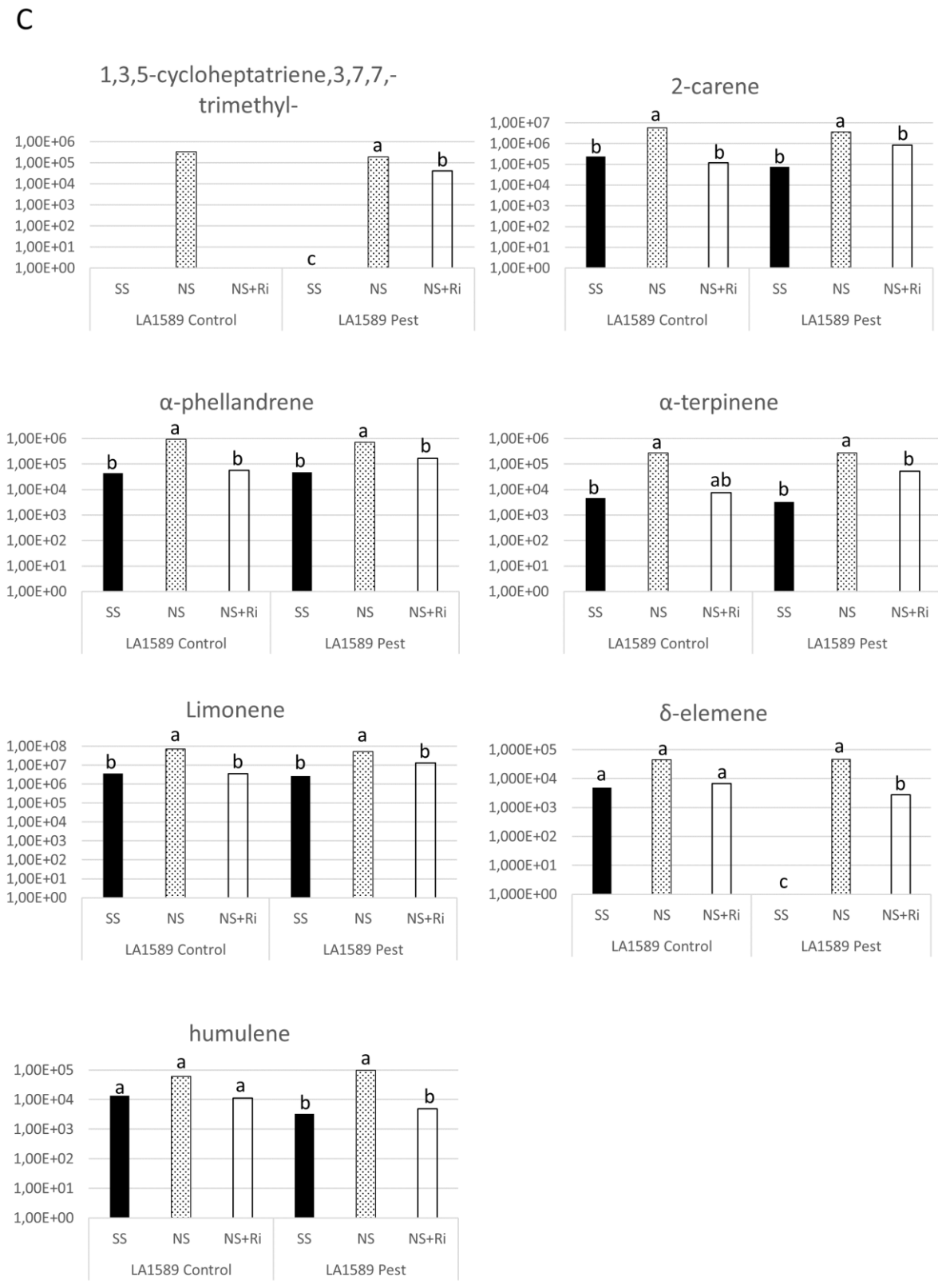


Figure S1: Volatiles significantly different for each tomato variety. (A) Volatiles induced by S. exigua in both LA1589 and Monita. (B) Volatiles induced by S. exigua in either LA1589 or Monita. (C) Volatiles with higher expression in the NS treatment in wild species LA1589. Stars indicate significant differences between control and pest conditions of the same treatments, while letters indicate significant differences within control or pest conditions.

Table S5: Pearson correlation test between selected volatiles in LA1589 and Monita and other volatiles. R coefficients are shown. Positive correlation means that when the level of the selected volatile is high, so is the other volatile. A negative correlation means that a high level of the selected volatile coincides with a low level of the other. Asterisks indicate significance: * $p < 0.05$; ** $p < 0.01$; ***$p<0.001$. Volatiles only detected in Monita are highlighted in yellow.

Volatile name	**LA1589 δ-elemene**	**Monita 3-hexen-1-ol**
M7: α-pinene	-0.522*	0.714**
M9: 1,3,5-cycloheptatriene,3,7,7,-trimethyl-	0.997***	0.567*
M12: 2-carene	0.960***	0.584*
M14: α-phellandrene	0.934***	0.625*
M15: α-terpinene	0.840***	0.659*
M17: limonene + β-phellandrene	0.957***	0.657*
M19: α-terpinolene	0.619*	0.982***
M30: β-caryophyllene	0.680**	
M31: humulene	0.902***	
M2: hexanal		0.917***
M5: o-xylene		0.818***
M6: p-Xylene		0.833***
M8: 6-methyl-2-heptanone		0.590*
M10: 6-methyl-5-hepten-2-one		0.861***
M13: octanal		0.897***
M16: m-cymene		0.814***
M18: β-(E)-ocimene		0.627*
M21: 2-nonanone		0.868***
M22: nonanal		0.893***
M25: 2-decanone		0.855***
M26: bornyl acetate		0.923***
M27: δ-elemene		0.931***
M29: tetradecane		0.869***
M33: γ-terpinen		0.984***
M35: decane		0.829***

M38: dodecane		0.877***
M39: decanal		0.820***
M40: tridecane		0.859***
M11: mircene		0.703**
M20: p-cymenene		0.986***
M23: ethyl phenol		0.795***
M24: methyl salicylate		0.766**
M28: $C_{13}H_{18}$		0.853***
M32: hexadecane		0.850***
M36: linanool oxide		0.696**
M37: nonanol		0.897***

General discussion

Current agriculture is met with the challenge of producing enough food to minimise malnutrition and support population expansion while at the same time limiting collateral damage to the environment (1). A key element in the transition to sustainable agriculture is preserving biodiversity and ecosystem services provided by the soil microbiome. Soil microbiota contribute to nutrient absorption, resistance to biotic and abiotic stresses, plant growth and development (2). Intensive management practices put selection pressure on microorganisms through modification of their habitats by tillage, high nutrient content and low plant diversity (3). Domestication has clearly changed plant phenotype, e.g. bigger fruits and higher yields (4–6). These changes are generally thought to have reduced plant defenses and interactions with beneficial microorganisms below and aboveground (7–12). Negative effects of domestication are hypothesised to be derived from reduced genetic diversity and increased use of agrochemicals such as fertilisers and pesticides (13–15). The use of fertilisers resulted in an excess of nutrients, mitigating the need of establishing mutualistic associations that require exchanging carbohydrates for nutrients provided by a beneficial microbe. However, recent studies evidenced similar levels of interspecific and intraspecific variation between wild and domesticated species and varieties (16–19). Therefore, the effect of soil microbiomes on domesticated varieties may be improved, decreased or have remained unchanged compared to their ancestors. Even though current plant breeding efforts take into account plant defenses, the effects on belowground microbial communities have often been overlooked. It is clear that microbial communities differ between plant species and varieties and even between plant developmental stages, but the effect of those changes on plant defense mechanisms is not well known (2,20,21). Furthermore, apart from the plant, soil microbial communities are influenced by soil management and characteristics such as soil type and pH (22,23). In this thesis we studied how tomato domestication influenced belowground communities and further explored the effect of microbial communities on aboveground indirect defenses.

We first studied the top down effects of tomato domestication on plant microbial communities, focusing on bacteria (**chapter 1 and 2**) and arbuscular mycorrhizal fungi (AMF, **chapter 3**). We compared plants along a domestication degree, including two wild species (*Solanum habrochaites* and *S. pimpinellifolium*) to modern species, including intermediate varieties (*S. lycopersicum* var. *cerasiforme* and *S. lycopersicum* var. *lycopersicum*) growing under field conditions. **Chapter 1** focused on how domestication and plant traits affected soil bacterial community diversity. Plants structure their microbiome by releasing a specific blend of exudates which differs between plant species and varieties (15,24). Domestication and subsequent breeding in many crops has been focused on phenotype and traits for resistance and defense were often left aside leaving crops vulnerable to pests and diseases (10,13,25). However, the role of plant associated microbiomes in plant resistance and defense are not well known. Since genetic variation between varieties affect traits such as root growth or exudate composition and under the hypothesis that domesticated crops reduced investment in plant-microbe interactions, we explored if domestication has potentially altered microbiome composition (26–28).

Two main bacterial classes, Alphaproteobacteria and Actinobacteria, were found to dominate the rhizosphere of all tomatoes regardless of their plant group. However, some minority phyla such as Acidobacteria and Gemmatimonades were more abundant in modern tomatoes compared to wild tomatoes. At the family level modern tomatoes showed higher levels of *Gemmatimonadaceae*, *Microbacteriaceae*, and *Streptomycetaceae* and a lower level of *Sphingomonadaceae*. At the genus level the effect of domestication was also evident, reducing the level of e.g. the aromatic substance metabolizer *Rhodococcus* and the Alphaproteobacteria *Skermanella* and *Microvirga*, the latter considered a nitrogen fixing bacterium.

As expected, tomato fruit traits varied according to the domestication degree, with wild tomatoes producing more but smaller tomatoes, whereas modern tomatoes produced the highest plant biomass and yield. Several studies linked plant traits such as leaf size, root architecture or fast growth to changes in soil community composition. For example, increased litter quality, lower soil C:N ratio and changes in root exudate composition were identified to influence microbial community composition (15,27,29,30). However, we did not find an effect of aboveground plant morphology on root associated bacterial community structure, in line with results from Leff et al. (30,31). It could be that the environmental impacts on microbial communities masked the impact of morphology, or that some other morphological traits should have been considered.

We found that resistance traits explained an important fraction of variation between bacterial communities especially between domesticated varieties. This is in line with plants shaping microbial communities to increase plant defenses (27). Furthermore, we evidenced a positive correlation between bacterial diversity and reduced resistance, suggesting that susceptible plants generally harbour more diverse bacterial communities (28,32). Alternatively, the activation of defense signalling in more resistant plants may provoke a stronger filter for microbes, resulting in a less diverse community (33).

In **chapter 2**, we studied the influence of tomato domestication on the ecosystem services e.g. nutrient cycling and plant growth promotion provided by the bacterial community (34). Traits selected during domestication could have influenced the composition of rhizosphere microbiota, as evidenced in wheat (*Triticum aestivum*), rice (*Oryza sativa*), barley (*Hordeum vulgare*) and tomato (*Solanum lycopersicum*) (28,35,36). As previously mentioned, soil environment also influenced soil microbial communities (37–39).

All predicted functions were present in all plant groups. However, the soil bacterial communities of certain tomato groups showed different proportions of specific functions. The bacterial communities of wild tomatoes showed a higher level of genes related to aromatic degradation, indicating a loss of degradation capacity due to cultivation (40,41). Furthermore, an increase in the Krebs cycle in the soil bacterial population of wild tomato species was observed. After being taken up by bacteria, carbon is usually converted into stable organic matter or decomposed and released as CO_2 (42). Similarly, the bacterial communities of modern cultivars showed a reduction in biochemical cycles such as for nitrates, sulphates and urea formation. We therefore propose that the increased use of agrochemicals may be connected to reduced metabolic pathway levels due to certain biochemical cycles. Moreover, a reduction in the biosynthesis of gamma-aminobutyric acid (GABA) was observed in bacteria associated to wild tomato compared to modern cultivars. GABA is involved in communication between bacteria and bacteria-host communication (43). On the other hand, pathways such as fatty acid and jasmonic acid (JA) synthesis were expressed more in the microbiome of modern cultivars. Fatty acids are involved in functions such as cell membrane constituents and cell signalling and have been used to indicate soil quality (44). JA and its derivatives (collectively named jasmonates) play an important role in plant defenses against biotic stress and interactions between plants and root microbes (45,46). However, the role of JA production in the soil remains unclear, and its role as a potential driver of microbial communities deserves further investigation.

Interactions between functional units of gene sets could help answer how microbial genes work together to support specific functions (47). Bacterial communities were more connected comparing tomato landraces with wild tomato species due to increased network density. Also, the increased connectance of landraces:wild pairs was due to a decrease in the characteristic path length, suggesting that the connection between microbes is more intense in landraces:wild pairs as compared to modern:landraces and modern:wild pairs.

In **chapter 3**, we focused on fungal communities. AMF are obligate biotrophs forming a symbiosis with 80% of land plants (48,49). AMF provide the plant with nutrients (mainly phosphorus) and increased resistance to biotic and abiotic stresses in exchange for plant produced carbohydrates and lipids (38,50–52). As AMF play a role in the distribution of soil nutrients they may indirectly influence the soil community structure (53). In breadfruit domestication decreased AMF colonization but in other crops such as maize and tomato modern varieties show both high and low colonization levels (54–58). AMF communities on the large scale are structured mainly by environmental gradients such as pH or C/N ratio, competition for limiting resources and neutral processes e.g. dispersal limitation (59–61). At smaller scales, spatial distance and phylogenetic patterns tend to be more important than environment (62). Therefore, in **chapter 3** we had a look at i) how spatial location and tomato type affects fungal communities and ii) how the observed variation in fungal communities drive the expression of aboveground plant traits.

We found similar AMF communities between tomato genotypes in line with studies comparing different varieties of chickpea and maize (63,64). Furthermore, the different tomato genotypes did not select particular AMF families or genera. Instead, location was important for AMF community composition, but especially for AMF phylogenetic turnover. AMF phylogenetic turnover refers to the relatedness between AMF taxa within a community, e.g. plants selecting a community of closely related AMF taxa (clustering) or a community with more distantly related AMF taxa (overdispersion).

A phylogenetically diverse AMF community is considered important for the uptake of phosphorus and reduced root colonization by pathogens (65). It seems that the AMF communities were driven by an unidentified environmental (soil) gradient. Indeed, several studies on grasslands evidenced that AMF composition can be explained by spatial location and by environmental gradients such as pH (60,62). However, changes in AMF communities can also change due to stochastic processes such as dispersal, drift and speciation (66). Nevertheless, environment is found as more important than location in explaining AMF communities (59,67).

Next, we sought to infer the influence of AMF communities on aboveground plant fruit and resistance traits independently from location. Since AMF affect plant growth and defense mechanisms, different AMF communities may differ in their effect on plant morphology and resistance (48,53). Although AMF communities were very similar among the tomato varieties, root colonization significantly differed among varieties independent of tomato type. Our results thus do not support reduced mycorrhizal capacity due to domestication found in several studies (15,54,56). This could be a result of the influence of location with tomato varieties being exposed to different AMF communities within the experimental plot resulting in modification of the symbiotic output (60). Other plant traits, such as TYLCV incidence, symptom development and tomato fruit traits, differ between varieties. Generally, wild tomatoes showed increased symptom development, a higher tomato number with and a lower fruit weight. Location was again a major influencer of variation in aboveground plant traits. Besides location, both AMF community composition and phylogeny explained variation in most traits, except TOCV incidence and plant biomass. Furthermore, tomato variety mainly explained resistance and colonization, while the domestication degree explained differences in both resistance and fruit traits.

Lastly, we correlated whether changes in plant traits were driven by the presence of specific fungal families, genera or taxa. Each fungal family may contribute to different functional traits to the plant (48,53). Phylogenetic conservation of functional traits and plant benefits were associated with early divergences (68,69). We found taxa within the genera *Claroidemoglomus*, *Diversispora*, *Glomus* and *Paraglomus* with varying effects on tomato resistance and morphology, suggesting that the outcome of symbiotic AMF-plant interactions depends on the present AMF and can vary between taxa of the same genus. It has been

evidenced that variation in AMF functional traits is mostly associated with early divergences between e.g Glomerales and Diversisporales (70). We found taxa from *Dominikia* and *Glomus* with positive effects on morphology, albeit negative effects on resistance. AMF are not considered to protect against viruses (71), although increased resistance to viruses has been observed in some cases (72). Furthermore, it could be an effect of the attraction of the vector whitefly *Bemisia tabaci* which may prefer healthier looking plants (73,74).

In line with the observed domestication trend from many small tomatoes to few heavy tomatoes, there were two AMF taxa with inverse correlations between tomato number and tomato weight. Moreover, we identified one *Funneliformis* taxa with positive effects on resistance and one *Dominikia* taxa with positive effects on plant biomass. Therefore, whether the outcome of plant-AMF interactions is positive or negative depends on which AMF are present in the soil and could also depend on vector distribution patterns in the field. We think that diverse AMF communities in the soil can be helpful in increasing plant growth and reducing biotic stress in field grown tomato due to complementarity or increased chances of a beneficial taxa being present (75). Fungal diversity has been found to contribute to variation in fitness outcomes (76,77), although results between and within studies are often inconsistent (78).

The second objective of this thesis was to address the effects of soil microbial communities on volatile production and attraction of the predator *Chrysoperla carnea* (Steph.) upon *Spodoptera exigua* (Hübner) attack (**chapter 4**). In the previous chapters the focus has been on how soil microbiomes differ between tomato species and varieties and how different microbial communities affect plant traits, but indirect defences and the third trophic level were not addressed, and this is the focus of the last chapter.

As stated above, the benefits of symbioses are expected to decrease in modern crops, and reduced bacterial diversity and consequent mutualistic capacity were indeed found in agricultural varieties (15,79,80). Soil microbial communities may alter volatile production through changes in chemical and physical plant traits and thereby influence the attraction of natural enemies (81). Several crops have lost secondary chemistry during domestication due to direct selection or diluted defense levels (7,82). For example, reduced volatile emissions were shown in domesticated cranberries and Brassicaceae (8,83,84). However, in maize and tomato intraspecific variation was shown to be equal or even higher than interspecific variation, indicating that other parameters besides domestication play a role as well (16,19). Therefore we compared two species of tomato, one wild species (*Solanum pimpinellifolium* L.) and one modern species (*Solanum lycopersicum* L.), to study how the interactions between the natural soil microbiome, and tomato impacts plant indirect defences to herbivory.

Even though pest attack affected volatile profiles in both wild (LA1589) and modern (Monita) tomato species, soil microbiome only affected volatiles in LA1589. This could be the result of intrinsic differences between tomato species such as in defense traits, with the wild LA1589 containing type VI trichomes (85) and the modern Monita containing the *Mi* gene (86,87). Plants were of the same developmental stage, but of different ages (8 weeks of Monita and 14 weeks in LA1589). Volatile profiles may differ depending on light conditions (88) or fruit developmental stage (89).

Surprisingly, the generalist predator *C. carnea* preferred the sterile soil (SS) treatment followed by the NS+Ri and lastly the NS treatments in both tomato species. We detected some volatiles that could explain differences in *C. carnea* attraction, with δ-elemene in wild tomato and 3-hexen-1-ol in modern tomato explaining most variation in *C. carnea* behavior as selected by ordistep. δ-elemene has been described as a pheromone correlated to other terpenes involved in insect resistance such as β-phellandrene, α-phellandrene and β-caryophyllene (90,91). However, δ-elemene was produced more in the NS treatment

and less in the SS treatment. 3-hexen-1-ol, which was absent from LA1589, was found to be induced in intact tomato plants after *T. absoluta* oviposition (90,92).

We detected volatiles induced by *S. exigua* in both tomato species which were described as toxic to pests, such as octanal and 2-decanone. Some of these volatiles, decanal in LA1589 and bornyl acetate in Monita were even only present after pest attack. Bornyl acetate has been described as a toxin to aphids and spidermites (93,94). Furthermore, other volatiles were only pest induced in one tomato species, such as nonanal and tetradecane in LA1589 and ethyl phenol and dodecane in Monita. In LA1589 some volatiles were present in higher levels in the NS treatment compared to the other treatments and could be potentially involved in direct defense by functioning as pest repellents. For example, limonene and humulene have been described as toxic to different pest insects (95,96).

Other volatiles could be involved in natural enemy attraction, such as decanal and octanal which were described as HIPVs involved in natural enemy behavior in other studies (97). These volatiles were induced in the SS treatment (both species) and LA1589-NS+Ri treatment. However, the attractiveness of natural enemies to plants may depend on the natural enemy species. For example, 2-carene, α-phellandrene and α-terpinene were described as attractants for other predators and parasitoids (98–101), but were present in higher levels in the least attractive NS treatment.

There could be more volatiles that explain differences in *C. carnea* behavior. Minor compounds, even present in levels undetectable by GC-MS, could be responsible for differences in natural enemy attraction (102). Also, natural enemies could react to differences in blend complexity, rather than changes in individual volatiles (103). Furthermore, it could be due to differences in AMF colonization. A moderate although significant increase in total AMF (LA1589 only) and *Rhizophagus irregularis* colonization (both species) were evidenced in the inoculated natural soil (NS+Ri) compared to the natural soil (NS) treatment, indicating a high competitive ability of *R. irregularis*.

The reduced attraction to natural soil compared to sterile soil indicated a negative effect of soil microbiome on *C. carnea* attraction, but requires further experiments for confirmation. Indeed, a study in *Arabidopsis* found decreased attraction of a parasitoid in the presence of rhizobacteria due to increased volatile production (104). It could even be an artifact of the sterilization procedure which may not have killed all microbes with those remaining having a differential impact on plant defenses. The soil microbial community differed between the sterile soil and natural soil treatments in both tomato species. In natural soil, AMF inoculation did not change bacterial communities in the wild LA1589, while a change was observed in the modern Monita. Therefore, it could be that the natural microbial community promotes direct defenses through priming (51,52), at the cost of indirect defenses (105). However, no such trade-off has been detected in milkweed (106).

We therefore conclude that pest attack affected volatile blends in wild and modern tomato, with the soil microbiome only being significant in wild tomato. We identified volatiles induced by *S. exigua* in both tomato species, some common and some species specific. Behavior of the predator *C. carnea* was affected by soil treatment in both species. Some volatiles could potentially function as pest or natural enemy attractants or repellents and could be tested individually or in mixes in pest performance or natural enemy attraction assays to confirm their function. We also conclude that *R. irregularis* inoculation increases *C. carnea* attraction in natural soil despite the presence of AMF already in the natural soil. Regarding the comparison between wild LA1589 and modern Monita, a wider variety of wild and modern tomato cultivars should be used to be able to draw conclusions on whether this concerns an effect of domestication. Furthermore, different varieties should be used at the same time to synchronize experimental conditions.

Taken together, the results in this thesis indicate that all tomato species and varieties tested shared a similar core microbiome with only small differences between wild tomato and both tomato landraces and modern commercial cultivars. Soil bacterial communities were shaped by resistance traits, non-targeted by domestication. We also found that agronomic practices linked to domestication affected ecosystem services provided by soil bacterial communities, especially those related to organic matter preservation. In contrast, fungal community composition was mainly affected by spatial location, and not by plant traits. Spatial location and tomato domestication also affected aboveground plant traits. Taking this variation into account, an effect of fungal community composition on plant traits could be detected. Moreover, specific fungal taxa were correlated to the expression of aboveground plant traits, but we could not identify AMF families or genera that generally enhance or reduce tomato resistance or morphology. Lastly, we conclude that pest attack affected volatile profiles in both wild and modern tomato, with soil treatment only affecting volatiles in wild tomato. We found different volatiles induced by *S. exigua* attack, of which seven were common in both tomato species, four specific to the wild LA1589 and eight specific to the modern Monita. We also identified volatiles that potentially function as pest or natural enemy repellents. Lastly, we conclude that *R. irregularis* inoculation increases *C. carnea* attraction in natural soil, which could be a potential sustainable way to enhance tomato indirect defenses for environmental friendly crop protection. The use of microorganisms such as AMF not only improves plant mineral nutrition thereby reducing fertiliser use, but also improves water supply and other ecological functions.

Some outstanding questions still remain. Regarding the first objective, can we confirm the potential functions of root associated bacterial and fungal communities using RNA sequencing? How do resistance traits shape bacterial communities, which plant mechanisms are responsible for this effect? Moreover, can we identify other plant traits that might be involved in plant-microbe-insect interactions? We did not find effects of morphology, but it could be that belowground root traits, such as root length and diameter, might be more important than aboveground traits in explaining variation between bacterial communities (107).

Regarding the second objective, why was sterile soil more attractive to C. carnea than the natural soil? Could it be that the native soil microbiome promotes direct defenses at the cost of indirect defenses? So, *S. exigua* would perform worse on the natural soil treatments resulting in a lower HIPV production compared to the sterile soil. However, since *S. exigua* performance was not measured, we cannot support this conclusion. We already showed that initial soil bacterial communities differed between sterile soil and natural soil, and developed differently during cultivation with the different plant species. Our next goal is the analysis of the root endophytic microbial communities of all treatments at the DNA (compositional) and RNA (functional) level. To confirm that the observed effect is indeed associated to the microbial communities present in the natural soil, different experiments should be carried out: the sterile soil could be amended with a microbial wash of the natural soil (SS+wash), or the sterile soil can be supplemented with 10% of natural soil as inoculum (SS+NS). If the amended soil reduces *C. carnea* attraction as compared to the unamended one, then a negative microbiome effect on indirect defenses can be confirmed. Evaluation of the caterpillar performance feeding on those plants and analyzing plant defense compounds/ genes in plant leaves will help to clarify whether such a reduction on indirect defenses may be related to a trade off with direct defenses or on the contrary, deleterious microorganisms present in the natural communities are responsible for the observed effect.

Conclusions

1. Tomato landraces and modern tomato cultivars share a core microbiome but small differences can be observed with wild tomato.

2. Fruit traits varied according to the domestication gradient, with wild tomatoes producing more but smaller tomatoes and modern tomato producing the highest plant biomass.

3. Soil bacterial communities were shaped mainly by resistance traits, non-targeted by domestication. Furthermore, we found a positive correlation between bacterial diversity and reduced resistance

4. Domestication affected ecosystem services provided by soil bacterial communities, especially those related to organic matter preservation. All ancestral functions were conserved over time, with some metabolic pathways differing between wild tomato and the other tomato groups (landraces and modern). Furthermore, a higher level of connection between those bacterial communities driven by tomato landraces and their wild ancestors was found.

5. Spatial location has profound effects on root fungal communities and aboveground plant traits. When this variation is taken into account, fungal community composition also affected plant fruit and resistance traits.

6. Mycorrhizal colonisation varies between tomato varieties, independently of their domestication degree.

7. Spodoptera exigua attack impacts volatile profiles in both wild (Solanum pimpinellifolium var. LA1589) and modern tomato (Solanum lycopersicum var. Monita). However, soil treatment only had a significant effect on the volatile profile of wild tomato LA1589.

8. The behavior of the generalist predator Chrysoperla carnea was affected by soil treatment in both wild and modern tomato, with volatiles δ-elemene (Solanum pimpinellifolium var. LA1589) and 3-hexen-1-ol (Solanum lycopersicum var. Monita) being correlated to these differences.

9. Inoculation of natural soil with Rhizophagus irregularis enhanced the attraction of the predator Chrysoperla carnea to challenged plants in both LA1589 and Monita.

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