UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

Exploration of beneficial microorganisms associated with seeds and roots of tomatoes in native soils of Ecuador

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RESUMEN

Los microbiomas son esenciales para el desarrollo y crecimiento de las plantas. Les brinda resistencia en contra de patógenos, promueve el crecimiento vegetal y mejora la tolerancia a estrés por condiciones ambientales. Literatura previa reporta que la microbiota proveniente de suelos nativos de las plantas nativas, como ancestros de cultivos modernos, es más diverso y brinda mejores beneficios a estas. Por lo tanto, identificar qué tipo de microorganismos promueven estas funciones es relevante para la creación de agricultura sostenible en el futuro, con énfasis según la FAO en disminuir la cantidad de pesticidas y fertilizantes artificiales.

En este trabajo se realizó una revisión de literatura sobre microbiomas vegetales, direccionada al conocimiento sobre microbiomas de tomates, o de las Solanaceas en general. También, se investigó la germinación del tomate nativo *Solanum pimpinellifolium* y el tomate moderno *Solanum lycopersicum* var. Moneymaker bajo condiciones controladas del invernadero, en tratamientos con suelos agrícolas y nativos recolectados en las provincias Pichincha, El Oro y Loja en Ecuador. Las muestras de suelos fueron sometidas a análisis fisicoquímicos, para determinar el estado nutricional de cada suelo de interés. Además, la identificación y diversidad de microorganismos se dio mediante el secuenciamiento del ADN de muestras provenientes de Loja, Ecuador. A partir del secuenciamiento, múltiples herramientas bioinformáticas fueron utilizadas para poder caracterizar la diversidad taxonómica asociada con las raíces de tomates nativos en su hábitat natural.

Respecto a los microorganismos benéficos, factores edáficos y el genotipo del tomate fueron los componentes principales que influyen la comunidad microbiana. Se encontró una distinción clara entre los microbiomas del suelo testigo y la rizosfera del tomate nativo *Solanum pimpinellifolium*. En la rizosfera, *Enterobacteriaceae* formaron el núcleo del microbioma, mientras que las *Erwiniaceae* complementaron al microbioma satélite, pero únicamente en suelos nativos. Suelos agrícolas tuvieron mayor contenido de nutrientes a excepción del suelo nativo de Paltas, mientras que el suelo nativo usado para un estudio de germinación tuvo más nutrientes que el agrícola. Se concluyó que el genotipo de tomate significativamente influye en la germinación en suelos nativos; siendo *S. lycopersicum* var. Moneymaker con el mayor número de germinaciones en suelos nativos. Además, una etapa de reactivación de suelo con *S. lycopersicum* var. Moneymaker, resultó en una menor germinación para *S. pimpinellifolium* posteriormente sembrado en este, evidenciando posiblemente un efecto en la germinación por el reclutamiento inicial del microbioma por *S. lycopersicum* var. Moneymaker.

Este estudio es parte de un mejor conocimiento de la interacción de microorganismos y plantas, para determinar cómo microorganismos benéficos de suelos nativos podrían ser beneficiosos para una agricultura sostenible en el futuro.

Palabras Clave: Microbioma, tomate, Ecuador, rizosfera, suelos nativos, región andina, germinación, 16S ARNr, diversidad taxonómica, domesticación.

ABSTRACT

Microbiomes are essential for plant development and growth. They provide resistance against pathogens, promote plant growth, and improve stress tolerance towards environmental conditions. Previous literature reported that the microbiota of native soils associated with native plants, which are the ancestors of modern crops, is more diverse and confers higher benefits to the host plant. Therefore, identifying what type of microorganisms promote these beneficial functions are relevant for the creation of sustainable agriculture in the future, which according to the FAO must rely on less input of pesticides and artificial fertilizers.

In this work, a literature review on plant microbiomes was performed, directed towards the knowledge on microbiomes associated with tomato, or to the Solanaceae in general. Furthermore, the germination of the native tomato *Solanum pimpinellifolium* and the modern tomato *Solanum lycopersicum* var. Moneymaker was investigated under greenhouse conditions, cultivated in agricultural and native soils collected in the provinces of Pichincha, El Oro, and Loja (Ecuador). Soil samples were subjected to a physical-chemical analysis to determine the nutritional status of each soil. Moreover, the identification and diversity of microorganisms were determined through DNA sequencing of soil samples from Loja, Ecuador. Based on the sequencing data, a variety of bioinformatic tools were used to characterize the taxonomic diversity of bacteria associated with the roots of native tomatoes in their natural habitat.

Regarding beneficial microorganisms, prevailing soil factors and tomato genotype were found to be the main drivers of the microbiome composition. A clear distinction was found between the microbiomes of bulk soil and the rhizosphere of native tomato *Solanum pimpinellifolium*. In the rhizosphere, *Enterobacteriaceae* formed the core microbiome, while *Erwiniaceae* were only found in native soils, where they complemented the satellite microbiome.

Agricultural soils had a higher nutrient content except for the native soil of Paltas, while the native soil used for a germination study with native and modern tomatoes had more nutrients than the agricultural soil. Results showed that the tomato genotype is of significant influence on the germination of tomato seeds in native soils, as *S. lycopersicum* var. Moneymaker showed the highest germinations in native soils. Moreover, an initial soil activation phase with *S. lycopersicum* var. Moneymaker was followed by a lower germination success for *S. pimpinellifolium*, showing evidence of a possible effect on germination by the recruited microbiome of *S. lycopersicum* var. Moneymaker.

This study is part of a better understanding of the interaction of microorganisms and plants to determine how beneficial microorganisms as inhabitants of native soils could be of benefit to a more sustainable agriculture in the future.

Keywords: Microbiome, tomato, Ecuador, rhizosphere, native soils, Andes region, germination, 16S rRNA, taxonomic diversity, domestication.

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1. INTRODUCTION

Microorganisms have established associations with plants since they colonized the land. The establishment of microbial communities associated with an individual plant and its specific organs has been reported to optimize the individual's health status (Connor et al. 2018). When an alteration occurs in the normal microbiota, this commonly has negative consequences for the plant's health (Brugman et al. 2018). These health impacts emphasize the importance of understanding the interactions of plants with its microbiota. Defining the function of these interactions and underlying factors that influence microbiota or microbiome assembly, can help us understand how plants benefit from these associations with microorganisms (Compant et al. 2019). According to Cordovez et al. (2019), assembly of the microbial community is governed by the type of soil, the interaction between prevailing microbes, the plant's genotype, and plant exudates. Moreover, Sasse et al. (2018) reported that plant exudates shape the microbial communities of it, and how these vary according to the genotype of the plant and abiotic stresses it encounters. In lettuce, soil type significantly influenced the rhizosphere microbiome (Schreiter et al. 2014).

Interestingly, Kwak et al. (2018) highlighted the role of native microbiota in protecting the plant against pathogens. Tomato varieties, normally susceptible to the pathogenic fungus *Fusarium oxysporum,* once grown on native soil showed partial resistance against this pathogen (Chialva et al. 2018). Pérez et al. (2016) investigated the effects of plant domestication on the associations between microorganisms and modern cultivars. Domesticated plants have a lower self-support production system and a higher dependency on anthropogenic factors compared to their wild relatives. In general, domestication has led to the loss of various resistance traits, possibly by exposure to modern agricultural practices and as a result of modern plant breeding which focused more on production traits under perfect high-input farm conditions (Chen et al. 2015).

Plant microbiomes not only help protecting plants from pathogens. Compant et al. (2019) reported that the plant microbiome, promotes growth, immunity against pathogens, and abiotic stress tolerance, both below and aboveground Still, most of the underlying mechanisms involved in plant-microbiome interactions are not clear. Beneficial services provided by the microbiome derive from many mechanisms, for instance by improving the bioavailability of nutrients, antagonism to pathogens, and by producing plant growth promoting compounds (Hartman and Tringe, 2019). These compounds can be phytohormones (auxins, cytokinins, and others), enzymes, antibiotic, and antifungal compounds (Egamberdieva et al. 2017).

One way to understand the functionality of the microbiome is through high-throughput sequencing technologies (Cordovez et al. 2019). In this investigation, 16S rRNA sequencing was performed, providing data that allow microbial community profiling (Franzosa et al. 2015). Reads obtained by sequencing need to be quality checked, clustered, and classified before post-analysis. Typically, 16S rRNA sequencing is classified by SILVA database, quality checked by Dada2/Deblur/QIIME, and clustered into OTUs. However, clustering by ASVs improves sensitivity and specificity. Therefore, errors are corrected compared to OTUs (Fricker, Podlesny, and Fricke, 2019). Thus, sequencing results in an enormous quantity of data, which needs to be analyzed accordingly. It is important to emphasize the importance of bioinformatics pipelines to analyze these big data and to functionally understand microbial communities.

Microbiomes not only have a bright side; they also have a dark one, as microbiomes involve both beneficial and pathogenic microbes. Pathogens are known to release effectors, which are proteins that for instance modulate the release of nutrients from the plants by manipulating their metabolism in the pathogen's benefit (Fatima and Senthil-Kumar, 2015). Moreover, Snelders et al. (2018) described effectors as secreted molecules which not only affect the plant's physiology but also influences the local microbiome. Within the microbiome, microorganisms compete amongst themselves, and pathogens need to defend themselves from

antimicrobial components by degrading them or secreting toxic compounds to outcompete other microbes. Both commensal and pathogenic microbes share specific strategies to evade innate plant immunity, as plants sometimes have problems distinguishing beneficial microbes from pathogenic ones (Hacquard et al. 2017). Teixera et al. (2019) reported that both beneficial and pathogenic bacteria developed strategies to suppress the innate immune response of plants. On the one hand, the microbiome plays a vital role in conferring immunity to the host plant, but the plant's immunity influences its microbiome assembly on the other hand.

The main source of microorganisms for this assembly is the soil, which has been reported as the ecosystem with the highest micro-biodiversity on earth. Soil microorganisms enter through the rhizosphere, the intimate zone surrounding plant roots, and migrate to other regions of the plant to colonize their specific niche (Hunter, 2016). Toju, Okayasu, and Notaguchi (2019) recently reported that the difference in soil treatments was the main contributor to microbiome variances in the leaves of grafted tomato plants, followed by the rootstock genotype. The rhizosphere microbiome is not only important for plant health and nutrition. It may also have direct and indirect effects on plant community structure (Philippot et al. 2013). The rhizosphere microbiome composition is reported to differ between plant species mainly due to soil conditions, but it is suggested that core microbiomes might be shared by many different plant species (Fitzpatrick et al. 2018). Core microbiomes are important for plant fitness, but a microbial taxon in lower abundance (satellite) could act as a driver for essential functions as well (Compant et al. 2019). An additional concept within the microbiome field is "keystone taxa". These taxa, irrelevant of their abundance inside the community, can drive microbiome composition and functioning; individually or as a group (Banerjee, Schlaeppi, and van der Heijden, 2018).

The microbiome is not the only factor that influences a plant's health status and growth. The specific composition and characteristics of the soil too. Soil provides services to plants

such as nutrient cycling, water dynamics, and support (Hatfield et al. 2017). Without these services, plants and their associated microorganisms would not be able to grow and successfully reproduce. There are many factors that govern these services, but within the scope of this research project, the prevailing nutrient status of the soils of interest is the most important one. Natural nutrient status involves the bioavailability of nutrients and the ability of the soils to store them in excess (Hewitt, 2004). For the release and storage of soil macronutrients and micronutrients, specific processes such as dissolution-precipitation and adsorption-desorption must occur (Singh and Schulze, 2015). One way to measure this is through cation exchange capacity (CEC), for which the pH of the soil is important (Jones and Jacobsen, 2005). In general, 14 to 17 elements are listed to be essential for plant development. Some are needed in great quantities (for example nitrogen and phosphorus), while others are required in smaller con (like iron) (Grusak et al. 2016; Singh and Schulze, 2015). However, soil microorganisms influence the uptake of nutrients by plants, either directly or within symbiotic relationships which emphasizes the importance of the microbiome for plant development (Morgan and Connolly, 2013).

Within the nightshade family *Solanaceae,* its genus *Solanum* contains the section *Lycopersicon* including both the domesticated tomato, *Solanum lycopersicum*, and several wild tomatoes like *Solanum pimpinellifolium* (also commonly known in Ecuador as “tomatillo”). The habitat of these wild tomatoes is on the west side of the Andes in the desert ore semi-desert environments (Knapp and Peralta, 2016). Its habitat has a broad range, as it can also be found in dry areas on sea level, for example the endemic tomato of the Galápagos Islands, *Solanum cheesmaniae*. On the other hand, *Solanum lycopersicum* nowadays has a worldwide distribution. This process occurred due to the domestication of the tomato. According to Blanca et al. (2012), pre-domestication of *S. pimpinellifolium* occurred in the Andean region which resulted in *S. lycopersicum* var. cerasiforme. *S. lycopersicum* var. cerasiforme was then taken

to Mesoamerica where another part of the domestication process occurred, which finally resulted in *S. lycopersicum* var. lycopersicum. Because of its popularity, the Spaniards later distributed this domesticated tomato all over the world.

The current study revolves around tomatoes and their soil microbiomes. As a positive result of the domestication process, the modern tomato *S. lycopersicum* currently produces significantly larger, highly nutritious fruits compared to its native relatives. But modern tomatoes have become highly dependent on human intervention to be able to grow and to artificially protect them from plagues and diseases. Blanca et al. (2015) reported a higher overall genetic diversity within populations of native tomato *Solanum pimpinellifolium,* and landrace (*Solanum lycopersicum* var. cerasiforme), compared to modern tomatoes (*Solanum lycopersicum* var. lycopersicum). On the other hand, *S. lycopersicum* var. lycopersicum showed to have a higher frequency of six loci related specifically to fruit characteristics. This higher frequency of selected genes for fruit characteristics has a trade-off; a possible loss of traits associated with genes close to the selected gene to be maintained in the coming generations of the plant by selective sweep (Perez-Jaramillo et al. 2016). Modern tomatoes not only show a reduction in gene diversity compared to their native ancestors, but also a reduced communication of soil microbes associated with modern plants. For example, modern agricultural soils with monocultures of rice consistently led to a less diverse soil microbiome. Therefore, seedling vigor was negatively impacted, and even increased greenhouse emissions by the soil microbiome were observed (Edwards et al. 2019). One way to mitigate the consequences of this trade-off of the domestication of S. *lycopersicum* could be to generate hybrid varieties between modern tomatoes and their closest native ancestral species, S. *pimpinellifolium*, as this could potentially re-introduce important genetic resistance traits (Sharma et al. 2008).

This study focused on the effect of agricultural and native soils on the germination of tomatoes. Additionally, the microbiome of *Solanum pimpinellifolium's* rhizosphere and bulk soil grown on native soil was investigated. The objectives of this study were to characterize the bulk soil and rhizosphere, therefore, determine which aspects of it have a beneficial impact on different genotypes of tomato. These aspects involved identifying microbial taxa of the soil, and the composition of the soil. Moreover, a study of the effect of soil type on the germination of *Solanum lycopersicum* var. Moneymaker and *Solanum pimpinellifolium* was conducted. Before conducting the experiment and identification of the microbial taxa, a literature review was performed to get acquainted with the information so far on plant microbiomes. To be able to answer the research questions, we performed an experiment of soil treatments on the germination of Solanum *lycopersicum* var. Moneymaker and *Solanum pimpinellifolium,* under controlled greenhouse conditions. Soils used for the experiment came from Cayambe (Pichincha), Zapotillo (Loja), and Arenillas (El Oro). Additionally, a physical-chemical analysis of all soils used in the experiment was done, as well as a characterization of the microbial taxa (16S rRNA sequencing) of *Solanum pimpinellifolium's* rhizosphere and bulk soil grown on native and agricultural soils of Loja. Finally, we propose a back to the roots framework where we learn from tomatoes as they are grown on their native soils and apply the gained knowledge to take advantage of the beneficial associations of tomatoes with its microbes, that overall improve the health and resistance of the plant.

2. MATERIALS AND METHODS

2.1. Literature review

To narrow down the total number of articles in the initial search step, articles about "soil type and/or plant genotype", and "impact of microorganisms in plants" were found via search engine Google Scholar. Our criteria for the selection of studies related to soil type and plant genotype were the following: Studies had to relate to soil type (native and domesticated) and/or plant genotype impact on plant-microbiome interactions. Studies on plants had to be performed in a controlled environment. Our criteria for the selection of studies related to the impact of microorganisms on plants were: Microorganisms had to be inoculated onto plant tissues, plants were grown under controlled conditions, and the presence of control groups was mandatory. The collection of publications that matched our criteria served to create two literature overview tables: One table lists the publications which focused specifically on the impact of plant-microorganisms on the type of soil and host genotype. A second table listed publications with described the effects of inoculation of certain microorganisms with members of the family *Solanaceae*, primarily with different tomato species or varieties.

A search on *Dimensions* (http://dimensions.ai) was performed using combinations of search terms "plant AND microbiome", and "tomato AND microbiome", to evaluate the total number of research articles that are publicly available until 2020. Specific criteria used in Dimensions were: "Closed OR All OA OR Gold OR Green, Accepted & Submitted OR Green, Published" in the open access category, and "Article" as type of publication. With the collected information of the articles published per year which matched the specific criteria, an additional graph was created to observe the trends in the total number of articles published over the last years in the research topic of the current study.

2.2. Experiment 1: Seed germination of modern and native tomatoes in agricultural

and native soils

2.2.1. Soil sample collection.

In the south of Ecuador, in the provinces of Loja (Zapotillo) and El Oro (Arenillas), soils were collected from previously described habitats of native tomato *S. pimpinellifolium* (Morales Palacio et al. 2014). Based on personal comments of local people referring to a possible natural disease-suppressive soil, a third natural soil was collected in the province of Pichincha (Cayambe), although this location is not considered part of the native habitat of *S pimpinellifolium*. In the proximity of all natural and native soil collection sites, three additional modern agricultural soils were collected at farm sites. All soils were transported to the greenhouse facilities at Universidad San Francisco de Quito, at its campus in Cumbaya. Soils were dried for 7 days, sieved (2mm mesh size), and stored at room temperature for further processing.

2.2.2. Soil sample collection for physical-chemical analysis.

A subsample of all 6 soils was sent to the laboratory Eurofins Agro Ecuador (Cayambe) specialized at in-depth physical-chemical soil analysis, which included measurements of macronutrients and micronutrients, pH, soil conductivity, organic matter content, organic carbon content, and soil texture.

2.2.3. Experimental design – germination experiment under greenhouse conditions.

A tomato seed germination experiment was performed at the USFQ greenhouse facilities, under natural light conditions (12h light/12h darkness). The climate chamber was temperature controlled to guarantee a minimum temperature of 22ºC. For each of the soil collection sites, 16 pots were filled with 350g of the sieved native soil, and 16 pots were filled with equal amounts of the sieved agricultural soil. For each location and type of soils (agricultural or natural), seeds of either modern tomato *S. lycopersicum* var. Moneymaker

(commercial market) or native tomato *S. pimpinellifolium* (kindly provided by the National Germplasm Bank at the Universidad Nacional de Loja) were sown. The experimental design consisted of 8 pots for each type of tomato in each soil: 4 pots were a control group and the other 4 an exchange group (Figure 7). Prior to the germination experiment, a reactivation step was included to revive the microbiological activity of the dried soils. Control groups were pots seeded with the tomato genotype studied in the real experiment, while exchange groups were pots initially seeded with the opposite tomato genotype than the one studied in the real experiment. This prior reactivation step took 4-5 weeks. Plants and roots were removed from the pots. In the control pots, three seeds from the same tomato genotype were sown in the same pot, whereas three seeds from the opposite genotype were sown in the exchange pots. At the beginning of this second round of the experiment, pots were irrigated with 10% of their weight in water, and pots were randomized. Tomato seed germination were monitored and watered every 2-3 days. Seeds were considered as germinated when the cotyledon surged above the soil's surface.

2.2.4. Statistical analysis – seed germination data.

Soil and tomato genotype (including exchange groups) were selected factors for the germination time of either the first seed or the total number of seeds germinated as a dependent variable. A 2-way ANOVA was performed for each dependent variable. Soil type had 2 levels (agricultural and native) and plant type 4 levels (*S. lycopersicum* var. Moneymaker; *S. pimpinellifolium*; *S. lycopersicum* var. Moneymaker first then *S. pimpinellifolium*; S. *pimpinellifolium* first, then *S. lycopersicum* var. Moneymaker). These last 2 levels were exchange groups present in each type of plant for all soils. Verification for ANOVA assumptions being fulfilled was done; normality test on residuals was done by Anderson-Darling and equal variances by Levene. If normality assumption was not fulfilled, even after a Box-cox transformation, but equal variances without transformation were accomplished,

Kruskal-Wallis and Dunn tests were performed. Tukey pairwise comparison was used on models which fulfilled ANOVA assumptions to determine significant mean differences among the various treatments. α value for all tests was 0.05.

2.3. Experiment 2: Microbiome analysis of *S. pimpinellifolium* in native soils

2.3.1. Rhizosphere and bulk soil collection.

The rhizosphere from roots of native tomato *Solanum pimpinellifolium* was sampled at Calvas, Palta, and Zapotillo, in the Southern Province of Loja, Ecuador (Figure 2). Tomato plants growing in different disturbed and undisturbed habitats along farm fences, in between corn crops, or close to riverbanks or springs, were GPS referenced, photographed, and a general description of the habitat was taken. On one side of the plant, the roots were dug up and roots with soil aggregates attached were removed and placed into 50 mL Falcon tubes. Each tube received 4 mL of Life Guard Soil Preservation Solution (Qiagen, USA). Tubes were stored in a mobile cooler and brought to the laboratory the same day. In addition to the rhizosphere sample, 4 g of bulk soil in the proximity of the same tomato plant was sampled and mixed with 4 ml of Life Guard Soil Preservation Solution and stored in the cooler. In the laboratory, root samples were vortexed to retrieve the soil aggregates. Roots were removed from the tubes, and the remaining rhizosphere soil suspensions and the bulk soil suspensions were stored at -20 ºC until further processing.

2.3.2. Soil sample collection for physical-chemical analysis.

Soil samples (0.7-1 kg) from agricultural and native origin were collected in Zapotillo, Calvas, and Paltas in Loja. The agricultural soils were sampled at corn farms, whereas the native soils were sampled from natural bank rivers nearby the farm sites. All locations were selected because of the presence of individuals of *Solanum pimpinellifolium*. Collected soil samples were stored in a mobile cooler and brought to the laboratory the same day. Soil samples

were dried and sieved before sending them to a specialized laboratory for subsequent physical-chemical soil analysis as described in chapter 2.2.2. (Eurofins Agro Ecuador, Cayambe).

2.3.3. Molecular analysis – soil microbiome native tomato S. pimpinellifolium.

2.3.3.1. Soil DNA extraction.

Total DNA was extracted from the rhizosphere and bulk soil solutions, following the manufacturer's protocol from the DNeasy Power Soil Kit (QIAGEN, 2017). Extraction consisted of an initial cell lysis step by mechanical and chemical disruption of the cells. Other solutions were added to neutralize potential PCR inhibitors present in the soil samples. DNA was captured by a silica membrane through a spin column. DNA was washed and stored at -20°C.

2.3.3.2. 16S rRNA high-throughput sequencing.

DNA extractions from collected rhizosphere and soil samples were sent to Baseclear, a laboratory specialized in sequencing of microbiome DNA (Leiden, The Netherlands). Sequencing was performed through an Illumina MiSeq platform and the construction of single-end reads was done in-house by Baseclear. 16S regions v3-v4 were sequenced for bacteria (primers 341F and 805R). Reads were generated into FASTQ read sequence files and the final quality assessment processed by Baseclear before they were sent to us as final results of the sequencing runs.

2.3.3.3. 16S rRNA bioinformatic analysis.

FASTQ files were processed by Dada2 (1.12) to obtain an amplicon sequence variant (ASV) table. This ASV table was used to assign taxonomies with the Silva rRNA database (v138). ASV tables were further processed in R studio with the phyloseq R package (v. 1.30.0), to obtain a NMDS graph and a relative abundance bar plot. Relative abundance was calculated for the top 50 top ASV until a level of either bacterial family or bacterial phylum.

3. RESULTS

3.1. Literature review

3.1.1. Trends in plant and tomato microbiome research.

A literature search by *Dimensions* resulted in a total of 55,276 articles related to plant microbiomes in general, published between 1970 until April 2, 2020. A total number of 5,586 scientific articles specifically reported their results related to the tomato microbiome (since 1992). As can be observed in Figure 1, the overall interest in these topics has been growing since 2011, with 2019 as the year with most articles published on both topics. Furthermore, it is interesting that this year, already 5,485 articles on plant microbiome and 657 publications on the tomato microbiome have been published until April 2 of 2020. It is worth highlighting that the trend of articles published each year is lower prior to its next year. Thus, 2020 may exceed 2019 based on the trend of published articles on these topics.

3.1.2. Prior studies on soil/microorganism and plant interactions.

Five studies on the influence of the soil type on plants and two studies which investigated the influence of plant genotype were analyzed (Table 1). These studies showed a significant impact on the type of soil to a broad range of plants. Outcomes were a variation in microbiome community composition (comparison between native and domesticated soils), identification of edaphic factors as drivers of microbiome assemblage, and resistant native soils gave partial protection, for instance, to *Fusarium oxysporum* (Chialva et al. 2018). In one case, domesticated soil showed to have negative consequences on the overall plant performance. Regarding plant genotype: genotype, in a small but significant way seemed to contribute to the microbiome assembly. Moreover, wild ancestral plants, in comparison to their domesticated modern family members, showed to have differences in morphology and physiology, which may be contributed to shifts in their native microbiome.

A separate table (Table 2) lists seven studies of inoculated bacterial or fungal strains on tomato, and one study on potato. These studies reported an increase in dry weight/length of shoot or roots, as well as a protection against pathogens. In another study, inoculation with *Trichoderma longibrachiatum* MK1 increased the transcription of genes related to plant defense and growth. Finally, one study reported an enhancement of salinity stress tolerance thanks to the presence of the beneficial bacteria.

3.2. Experiment 1: seed germination of modern and native tomatoes in agricultural and native soils

3.2.1. Physical-chemical soil properties.

Physical-chemical analysis of soils collected in Loja, El Oro, and near Cayambe (Table 3-a) showed Loja's agricultural soil had a neutral pH, in comparison to the native soil which was slightly acidic. Organic matter percentage was higher in native soil. A trend can be seen when the increase of organic matter is correlated to total nitrogen, sulphur, calcium, potassium, sodium content, C/N, and C/S. C/N ratios were similar among soils, but considerably different for C/S ratios. Native soil resulted to have a higher content of macronutrients (except for phosphorus and magnesium) and certain micronutrients compared to the agricultural soil. It is worth highlighting that both soils were low on nutrients according to agricultural threshold values. Regarding micronutrients, the quantities of copper and cobalt were similar for both soils. Additionally, zinc and manganese content were (much) higher in native soils, while iron, boron, and molybdenum were higher in the agricultural soil. Results on the texture of both soils indicated a partially sandy loam texture, but the major texture was sandy in both soils.

As for Cayambe's soils, its native soil also had a higher content of macronutrients (except for phosphorus), but lower in micronutrients. Agricultural soil had a higher content of micronutrients, except for manganese, copper, and molybdenum. The latter two nutrients had the same content in both soil types. Manganese had a higher content in the native soil. Results

for the soils collected in El Oro showed most nutrient quantities were equal in agricultural and the native soil. Only levels of nitrogen, magnesium, sodium, iron, and cobalt were higher in the native soil. In conclusion, Cayambe was the richest in nutrients, followed by the soils from Loja. El Oro showed the lowest nutrient levels.

3.2.2. Tomato seed germination in native and agricultural soils from Loja.

Figure 3 shows the results of the germination experiment of seeds of modern tomato *S. lycopersicum* var. Moneymaker and native tomato *S. pimpinellifolium* grown in native and agricultural soils under controlled conditions in the greenhouse (Details chapter 2.2.3). Focusing on the germination time of the tomatoes in the soils of Loja, an initial ANOVA did not comply with the normality test ($p < 0.05$) but showed equal variances ($p = 0.056$). A subsequent BoX-cox transformation was realized, but the data did not follow a normal distribution either. A Kruskal-Wallis test ($p = 0.45$) revealed no significance among treatments, meaning that all treatments had similar effects on the germination time of the tomato seeds.

To test possible differences in the total amount of germinated seeds between treatment, another ANOVA was performed. This time, variances turned out to be equal ($p = 0.299$) but did not fulfill the criteria of a normal distribution ($p < 0.05$). A Kruskal-Wallis test was performed for each factor. The total amount of seeds germinated for the tomato genotype and/or exchange groups resulted to be significantly different. Consequently, a second ANOVA was realized for all tomato genotypes (including exchange groups) in each soil: ANOVA assumptions in the agricultural soil were fulfilled, and a subsequent Tukey pairwise comparison test (95% interval confidence) was performed. This test revealed that *S. pimpinellifolium* had a significantly different impact on the seed germination than activating the soil with *S. lycopersicum* var. Moneymaker. This activation step negatively affected the germination of *S. pimpinellifolium*. Equal trends were observed in the native soil, but this difference was not significant. In the

native soil of Loja, the germination of *S. lycopersicum* var. Moneymaker was significantly higher compared to the germination of *S. pimpinellifolium* (Dunn's test: p=0.017).

Figure 4 shows the results of the germination of *S. lycopersicum* var. Moneymaker and *S. pimpinellifolium* on native and agricultural soils collected in Cayambe and El Oro. In general, similar tendencies as for the soils of Loja were observed in soils of the other two regions. Moneymaker had the highest total number of seeds germinated. Interestingly, in the agricultural soil of Cayambe, it also resulted in a faster germination in comparison to other treatments. Both modern and native tomato seeds grown on different locations showed a small difference for their total seeds germinated in either soil type. As for germination per location, the soils collected in El Oro resulted in the lowest germination for *S. pimpinellifolium*, and the soils of Cayambe and Loja resulted in similar, higher germination rates (soils of Cayambe being the highest of all). Interestingly, soil type (native and agricultural) showed very similar performances for each tomato genotype of all three sample locations.

3.3. Experiment 2: microbiome analysis of native tomato *S. pimpinellifolium* in soils of Loja

3.3.1. Physical-chemical soil properties of native and agricultural soils in Loja.

Physical-chemical analysis of soils collected at six locations in the Southern province of Loja (Table 3-b), showed Calvas' agricultural soil had the highest pH value (7.5), while the agricultural soil from Zapotillo had the lowest pH (5.7). As a trend, pH values of native soils were higher than agricultural soils, except for the soils from Calvas. Moreover, all soils, except the agricultural soil of Zapotillo (acid), were close to a neutral pH value. Agricultural soils resulted to possess a higher percentage of organic matter and organic carbon (except Paltas' native soil, which had the highest % of all). A trend can be seen in native soils when the increase of organic matter is correlated to total nitrogen, calcium, boron content, whereas in agricultural soils it only correlated to silicon and calcium content. In general, agricultural soils had a

considerably higher content of macronutrients compared to native soils, except for the native soil collected near Paltas. As for micronutrients, native soils had a higher content of zinc and iron in comparison to agricultural soils, with again the exception for Paltas, as its agricultural soil had a higher content in comparison to its native soil. Copper and molybdenum content in all soil samples were lower than the detection limit as established by the laboratory. The remaining micronutrients showed high variances for all soil sampling sites. Thus, no general pattern was established for the type of soil. It is worth highlighting that most soils exceeded the minimum threshold values as established by Eurofins Agro Ecuador to perform agriculture. As for ratios, C/N ratio of all soils was similar, except the native soil of Zapotillo. Only the native soil of Paltas was similar to the agricultural soil of Zapotillo. Contrastingly, C/S ratios considerably varied among soils, only the ratios of agricultural soils from Zapotillo and Paltas were similar. As for soil texture, the major texture in all soils, except Calvas (silt), was sandy. Most soils had a sandy clay loam texture component as well (except Calvas).

3.3.2. 16S rRNA analysis of native and agricultural soils in Loja.

Characterization of the rhizosphere and bulk soil microbiome of native tomato *S. pimpinellifolium* in its native habitat of the province of Loja was performed. Figure 5 shows the results of a Non-Metric Multidimensional Calculation (NMDS), which revealed a clear difference between the microbiomes in bulk soils and the rhizosphere of *S. pimpinellifolium* at all sampling locations. Regarding soil type (agricultural vs. native soils), no difference was observed. However, when each soil was analyzed individually, differences in local microbiomes were revealed within each site. Rhizosphere samples collected in native soils at Calvas and Zapotillo were the most similar among all soils of interest.

As shown in Figure 6, there is a clear difference between bacterial communities living close to the roots of native tomatoes, compared to microbial communities in the nearby soils (away from tomato roots). Figure 6b clearly shows that Proteobacteria dominated in the

rhizosphere communities, while Firmicutes were the most abundant phylum in the microbiome of the bulk soil samples. In general, the native tomato rhizosphere samples showed a higher relative abundance compared to the bulk soil (except Paltas soil), which in other words indicates enrichment of the microbial communities in the rhizosphere, compared to the local bulk soil. Moreover, within the bulk soil samples, the ones collected in native soils resulted in a higher relative microbial abundance compared to the bulk soils collected at the farm sites, with Calvas as the only exception. The native soil collected in Calvas had a low abundance of bacteria in its bulk soil but was enriched in the rhizosphere. In contrast, within the rhizosphere samples, agricultural soils resulted to possess a higher relative abundance than native soils (again Paltas soil proved to be the exception). On the family level, *Enterobacteriaceae* dominated in the tomato rhizosphere, while *Bacillaceae* dominated in the bulk soil samples of Loja (Figure 6c and 6d).

As for the rhizosphere microbiomes, *Enterobacteriaceae* seemed to construct the core microbiome in all sample locations. Families which were identified to be part of the satellite microbiomes were *Erwiniaceae*, *Sphingomonadaceae, Yersiniaceae*, and *Bacillacea*. Specifically, *Yersiniaceae* was only present in Zapotillo's native soil, which was the only soil that was dominated by this family. *Sphingomonadaceae* were present only in low abundance for Paltas' agricultural and native soils. Paltas' native soil was the only one to harbor *Bacillaceae* (although in low abundance). *Erwiniaceae* was present in low abundance only in the rhizosphere of tomatoes sampled in all native soils. It is worth noting that the core microbiome abundance was highly reduced in both soils collected in Palta.

Regarding Bulk soil, the core microbiome among all locations was dominated by *Bacillaceae,* whereas *Planococcaceae* and *Rubrobacteriaceae* were identified to form part of the satellite microbiome. *Planococcaceae* were present in Calvas' agricultural soil and Paltas' native soil, however in low abundance. *Rubrobacteriaceae* (in low abundance as well) was

present in agricultural soils of Calvas and Paltas. In general, a variation of taxon abundance was found among soil sites. What is clearly visible in all the sample locations, is the different microbiomes between the rhizosphere and bulk soil.

4. DISCUSSION

In general, local soil characteristics are an important factor to influence the rhizosphere microbiome. To determine the edaphic factors of the agricultural and native soils collected, subsamples of all soils were shipped to a specialized laboratory for a physical-chemical soil analysis. The pH values and general soil textures of all samples were within the range desirable for the growth of native tomato cultivation *S. pimpinellifolium* (Plants For A Future, 2020). The overall acidic pH levels of the native soils of Zapotillo and Calvas, and the agricultural soil collected in Paltas, could explain their relatively low content of nitrogen, phosphorus, boron, and molybdenum (United States Department of Agriculture, 2011). Although the agricultural soil of Zapotillo resulted in the lowest pH of all samples, the macronutrient content was higher than its native soil. Overall, the agricultural soils of Calvas and Zapotillo showed higher nutrients contents than their native soils, whereas the opposite results were observed in Paltas, where the native soil was richer in nutrients. A possible reason why agricultural soils of Loja tend towards being more nutritious, might be the application of fertilizers and other agricultural practices to make the soil more arable. The most plausible cause was soil amendment as samples were collected after corn harvesting. For example, the high difference in calcium soil stocks between agricultural and native soils (Table 3) could be due to liming. Liming generally improves Ca, Mg, and P content (Fageria and Moreira, 2011). Although agricultural soil' parameters are generally higher as reported by other studies, most soil parameters of the agricultural and native soils of interest in Loja resulted to be within the desired range for agricultural practices. The Organization of American States in collaboration with the government of Ecuador stated that although soils in Loja, in general, were low in phosphorus, with a medium level of organic matter and nitrogen, and of variable texture. These soils could be used for agriculture (1994).

A reason why the native soil in Paltas resulted to have a higher content of nutrients compared to the other two native soils of Zapotillo and Calvas, could be due to the difference in organic matter content. Forest soils, like the native soil collection site in Paltas, contain more organic carbon than any other type of soil (Boyle and Powers, 2013). Soil organic carbon is the major constituent of soil organic matter (European Soil Data Centre, 2020). Moreover, soil organic matter is correlated with a higher nutrient storage capacity (Brady, 2016). Furthermore, the native soil sampling site near was located at the foot of a shrubland slope. In general, the foot of a slope is considered to be a site where nutrients accumulate, due to erosion and run-off (Bo-Jie et al. 2004).

Levels of total organic matter content in native soils correlate with the quantities of nitrogen, boron, and the C/S ratios. Other influences of local nutrient concentrations in the soils are land use, weathering of rocks and soils, precipitation, application of synthetic fertilizers, or animal manure (Shand, 2007; Efretuei, 2016; Han et al. 2017). The different geographic locations in Loja, the altitude differences or the variance in land use (Table 4), could explain the smaller differences we observed in the macro and micronutrient concentrations in soil sample sites (Thompson et al. 2005).

Results of the physical-chemical analysis from the native and agricultural soils collected for the germination study showed that native soils had a higher content of nutrients, except for Cayambe's native soil. A possible reason is soil erosion by agricultural practices, leaving the soil exposed to rain and water: washing away soil nutrients (Parikh and James, 2012). As for Cayambe's agricultural soil, agricultural practices to make the soil more arable could have been preceded the moment of soil collection.

Regarding soil textures, the major soil texture of all soils resulted to be sandy with clay, except for the soils of Calvas, where the textures were mostly silty. Allard et al. (2016) associated sand and silt content as factors that influenced shifts in the rhizobacterial community of

tomatoes. If variation of the rhizosphere bacterial community is based solely on sand and silt content, a higher content of silt and lower levels of sand could explain the increase in *Enterobacteriaceae* abundance (seen also in their study, though not significant).

In general, water activity, low content of nitrogen and phosphorus have proven to be factors that influence soil microbial communities' composition, and the root microbiome of plants (Hartman and Tringe, 2019). These factors and their interactions could explain the different microbial compositions as observed in the results of the 16S rRNA analysis. In this study, the rhizosphere and bulk soil samples of the agricultural soils of Calvas, Zapotillo, and the native soil of Paltas revealed a higher content of nitrogen and phosphorus than their counterparts. These similar trends could also be observed for the bacterial abundance, as they were also higher than their counterparts, which might demonstrate how differences in edaphic factors of agricultural and native soils drive microbiome composition. The exception to this was Zapotillo's agricultural bulk soil bacterial abundance, its native soil had a higher abundance and lower nutrients contents. In this context, it is important to mention iron. Iron was deficient only in the soils collected in Paltas. Interestingly, these soils had a much lower abundance of *Enterobacteriaceae*. As iron is one of the essential metal ions for many cellular processes (Porcheron et al. 2013), it is possible that the low content of iron in these soils may have impacted more *Enterobacteriaceae*as. This family is known for having a variety of mechanisms to specifically sequester iron (Carpenter and Payne, 2014). Moreover, *Enterobacteriaceae* are interesting as on the one hand they are known in biocontrol applications as plant growth bacteria, as they can fix nitrogen and solubilize phosphorus, but on the other hand, some species are known to act as plant pathogens (Jha et al. 2011). It is therefore important to analyze the sequences generated in this study on the species level as well so that the microbiome community members in these native soils can be identified on the species level. This might reveal functional information on the species that were encountered.

Not only are the soil edaphic factors influencing the rhizosphere microbiome, plants themselves too. Cordovez et al. (2019) highlighted several studies on how the plant's genotype, plant exudates, and specific interactions between microorganisms select for the microbial community. This could explain why proteobacteria dominated the rhizosphere soil sample, and firmicutes dominated the bulk soil samples. This result is recently confirmed by Cheng et al. (2020), who have reported proteobacteria as the major constituent phylum in 11 varieties of *S. lycopersicum* grown on natural fields.

The interaction by certain microorganisms to others, independent of their abundances in the microbiome, could also be a factor in the variances observed in the microbiome composition across all samples. Banerjee, Schlaeppi, and van der Heijden (2020) reported that keystone taxa influence microbiome structure, independent of their abundance. Keystone taxa could explain why Zapotillo's agricultural bulk soil (higher concentration of nutrients) had a lower bacterial abundance than its native soil (lower concentration of nutrients).

Interestingly, the rhizosphere *of S. pimpinellifolium* showed higher bacterial abundances compared to the bulk soil samples. According to Berlanas et al. (2019), the rhizosphere is more nutritious than bulk soil given that plants release rhizodeposits (nutrients and others), while bulk soil is mostly oligotrophic. Thus, the rhizosphere could have been more abundant in bacteria in response to the release of nutrients and exudates from the tomatoes. The core microbiome for the rhizosphere samples was formed by *Enterobacteriaceae*. Lee et al. (2019) have also reported *Enterobacteriaceae* as part of the tomato core microbiome (relative abundance higher than 60%) which were cultivated at different geographical locations. It is possible Enterobacteriaceae enrichment and selection in the rhizosphere of *S. pimpinellifolium* may be due to improving its fitness. Compant et al. (2019) have reported that core microbiomes are selected by plants due to improving their fitness. On the other side, *Erwiniaceae* was a satellite microbiome of the rhizosphere found only in all native soils of

Loja. The same argument could be applied to this observation. Jousset et al. (2017) have reported the local abundance of taxons present in certain habitats are important for key ecological processes and provide protection against pathogens in plants, as they stimulate the plant immune system. Therefore, *Erwiniaceae* could be providing beneficial functions to *S. pimpinellifolium.* Within this family, genera like *Erwinia* and *Pantoea* have species that are known plant pathogens, but other species have been reported to be associated with plant and boost plant growth (Palmer et al. 2018).

Sphingomonadaceae were found only in soils collected near Paltas, although in low abundances. Certain species of this family are known for being plant pathogens, plant growth bacteria, and antagonist to pathogens (Glaeser and Kämpfer, 2014). It could be that *Sphingomonadaceae* had a higher abundance in the soils with lower abundances of Enterobacteriaceae due to antagonistic effects. Roy et al. (2019) reported almost a non-existent abundance of *Sphingomonadaceae* (0.66%) in *S. lycopersicum* rhizosphere and reported *Enterobacteriaceae* as the highest abundant (94.6%). Another result of the current study was that *Yersiniaceae* were found only in the rhizosphere from Zapotillo's agricultural soil. Previously published information on the *Serratia clade* indicated its beneficial influence plants. Multiple species of *Serratia* are known as plant growth bacteria (Caneschi et al. 2019).

Regarding bulk soil, *Bacillaceae* formed the core microbiome. *Bacillaceae* are widely distributed across nature. They can be found in different habitats and is therefore considered asrobust bacteria. This family acts as a biofertilizer, participates in the nitrogen/carbon cycle, and promotes plant's health (Mandic-Mulec, Stefanic, and van Elsas, 2018). Satellite microbiomes contained *Rubrobacteriaceae* and *Planococcaceae*, which are also found in different locations and soil types. Most genera from *Planococcaceae* have optimal growth when pH values are higher than 7 (Shivaji, Srinivas, and Reddy, 2014). Soils with a pH higher than 7 were the only ones to show an abundance of *Planococcaceae*. No literature was found

to describe a relevant function to the soil or plants by neither *Planococcaceae*, nor *Rubrobacteriaceae*. Most species of *Rubrobacteriaceae* can be found in high temperature environments, others in church walls, and even in a marine sponge (Norman, King, and Friesen, 2017).

Results of the NMDS analysis revealed no difference between the microbiomes of the agricultural and native soils between bulk soil and rhizosphere (Figure 5). However, if considered individually, differences between soils can be observed. The most plausible reason for not observing a clear difference could be due to location sampling of agricultural soils. Samples were collected at the outside border of farm fields, not in the center of an agricultural field. The only soil sampling site in the center of an agricultural field was at Calvas.

The results of Experiment 1 related to the germination of modern and native tomatoes in native and agricultural soils, showed no significant differences among soil types, but seeds of modern tomato *S. lycopersicum* var. Moneymaker had a significantly higher number of total seeds germinated in comparison to the native tomato *S. pimpinellifolium*. A possible explanation is the seed vigor of *S. lycopersicum* var. Moneymaker, as its seeds are bigger and had a more uniform size. Khan et al. (2012) reported a correlation of seed size and seedling establishment, but in germination characteristics, it was not consistent for tomato (*Solanum lycopersicum* cv. Moneymaker x *Solanum pimpinellifolium*). They also found that most of the alleles which had a positive effect on seedling traits were present in *S. lycopersicum* var. Moneymaker. Furthermore, Peñaloza, and Durán (2015) reported that this correlation and further developed composition, genetics, and metabolism may be contributing to this effect. Thus, it is possible that *S. lycopersicum* var. Moneymaker may have better alleles for seedling traits due to the plant breeding selection process that led to this variety. On the other hand, larger seeds have a higher germination rate in other species due to higher nutrient storage in these seeds (Chacon, Bustamante, and Henriquez, 1998; van Mölken et al. 2005). Another

possible reason is storage conditions. Seeds of *S. lycopersicum* var. Moneymaker were obtained from a commercial distributor. Commercial seeds must comply with quality standards in order to be sold. On the other hand, *S. pimpinellifolium* was obtained through the national germplasm bank at the UNL in Loja, whose storage conditions of these seeds are unknown.

As seeds can recruit microorganisms and confer horizontal transmission of these (Nelson et al. 2018), exchange groups were performed to investigate the recruitment of microorganisms by initial seed affected germination of seeds planted in the second rounds of Experiment 1. Agricultural soil initially seeded with *S. lycopersicum* var. Moneymaker and then with *S. pimpinellifolium* gave a significantly lower total number of germinated seeds of *S. pimpinellifolium*. Other exchange groups did not show significant differences in germination. Based on the low macronutrient content of the agricultural soils, seed germination could have been more dependent on a possible symbiosis with microorganisms. Repas et al. (2017) reported this dependency by a symbiotic fungus to increase the germination speed and rate of tomato on nutrient poor oily soils in comparison to control treatments. It is possible that the recruited microorganisms for *S. lycopersicum* var. Moneymaker in the activation phase of the soil created a negative soil feedback for subsequent germination of *S. pimpinellifolium* in the second round of the experiment. As species are more distantly related, negative soil feedback becomes stronger (Bukowski, Schittko, and Petermann, 2018). However, negative soil feedback can occur even in plants in monocultures of the same species (Xue, Bezemer, and Berendse, 2018). Thus, negative soil feedback could have even occurred in these closely related species of tomato. However, plant soil feedbacks are complex to study and are still unpredictable (De Long et al. 2019). This could explain why a significant negative or positive plant-soil feedback could not be observed in other exchange groups.

5. CONCLUSIONS

This work is one of the first of its kind to use a multidisciplinary approach to explore the rhizosphere microbiome of native tomato *Solanum pimpinellifolium* in its native soils in the south of Ecuador. *S. pimpinellifolium's* core rhizosphere microbiome was formed by *Enterobacteriaceae*. This family has been known to be beneficial for the plant, although some species are pathogenic. As for satellite microbiomes, *Erwiniaceae* was present only in the tomato's rhizosphere in native soils. It is possible this family may have been selected by the tomato for improving its fitness on undisturbed soil. No clear taxonomic distinction of agricultural and native soil microbiomes could be observed, possibly due to location sampling of agricultural soils. However, a clear difference was observed between bulk soil and tomato's rhizosphere microbiome, where the rhizosphere had a bigger bacterial abundance. This might demonstrate how plants and keystone taxa can select certain taxons and enrich them. A variation in microbiome among soils of distinct locations and land use history shows how important edaphic factors are. Furthermore, it reinforces the idea that they are drivers of microbial community assembly. Soils whose nutrients content were high, specifically in nitrogen and phosphorus, correlated with the highest bacterial abundance. It is worth highlighting how important the microbiome of a plant is for its germination, especially in soils with nutrient deficiencies. The microbiome is not the only key player during the germination process, also the plant's genotype, and soil edaphic factors as well. Modern tomato *Solanum lycopersicum* var. Moneymaker proved to have a higher germination in total number of seeds, compared to the native tomato *Solanum pimpinellifolium.* This might be a result of the plant breeding selection process that led to the variety Moneymaker, which now possesses alleles with positive effects on seed vigor. But more research is required in this field to understand the roles and the function of the microbial community in plants on agricultural and in native soil.

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7. TABLES

Table 1. Outcomes of studies on type of soil and host genotype

Plant	Outcome	Author(s)
Soybean	Firmicutes enriched in rhizosphere grown on agricultural soil while Bacteroidetes on native soil. Soybean genotype slightly tunes soybean rhizosphere microbiome assembly	Liu et al. (2019)
Rice	Domestication through monoculturing creates changes in microbiome composition. Uncultivated soils and domesticated soils rhizosphere were significantly different. Soil domestication had negative consequences for plant performance	Edwards et al. (2019)
Annual crops	In broad terms, domestication has made majority of the annual crops less resistant to herbivores, due to changes in morphological traits and loss of metabolites toxic for herbivores	Chen, Gols, and Benrey, (2015)
Tomato	Tomatoes susceptible to *Fusarium oxysporum* when grown in resistant/conducive native soils in comparison to control substrate elicited stress and defense responses. Resistant native soils partially protected the tomato in comparison to the conducive soil	Chialva et al. (2018)
Modern cultivars	Higher abundance of Bacteroidetes on/in the roots of wild common bean. Changes in root architecture, root exudation, and plant physiology comparing domesticated plants and their wild parts. These changes could explain the shift of microbiomes of wild and domesticated plants	Pérez-Jaramillo, Mendes, and Raaijmakers, (2018)
Tomato	Edaphic factors drove rhizobacterial assemblage, not fertilizer or organic/synthetic soil amendments. Factors such as soil texture (silt and sand content) and water activity	Allard et al. (2016)
Barley	Host genotype drives in a small, but significant way the stratification of root and rhizosphere microbiota. Genotypes tested were wild, landrace, and domesticated barley	Bulgarelli et al. (2015)

Studies on the impact of native and agricultural soils or plant genotype on different plant species.

Table 2. Outcomes of inoculated strains on tomatoes and others

Plant	Strain	Outcome	Author(s)
Wild Tomato	*Sphingomonas* sp. LK11	Wild type tomatoes had a significant increase in root and shoot length under non saline conditions. GOT-3 tomatoes only had in root length. Under salinity stress LK11 significantly improved growth on both tomatoes type, possibly by expression of glutathione-related genes	Abdul et al. (2017)
Tomato and mung bean	*Bacillus cereus* and *Klebsiella variicola*	*B. cereus* inoculated on tomato significantly increased shoot length. *K. variicola/B. cereus* inoculated on mug bean significantly increased dry weight and shoot length. Inoculations had significantly increased mineral uptake in both plants	Saqib et al. (2020)
Tomato and lulo	diazotrophic/N-scavenging bacteria	Inoculated tomatoes and lulus with strains belonging to *Rhizobium* sp., *Cupriavidus* sp., and *Pseudomonas* sp. had a significant correlation with root/shoot dry weight, biomass accumulation increasement	Zuluaga et al. (2020)
Tomato	*Trichoderma longibrachiatum* MK1	In vitro grown tomato plantlets inoculated with MK1 increased transcription of genes associated to cell wall reinforcement, ROS scavenging, defense, protein synthesis and localization. Authors suggest these mechanisms activate induced systematic resistance to pathogens and plant growth	De Palma et al. (2016)
Tomato	Endophytic bacteria of desert cactus	"*B. megaterium* RR10, *B. amyloliquefaciens* CBa_RA37, *E. cloacae* CEc_LGR7, and *K. pneumoniae* CKp-RR19 significantly enhanced the germination and the subsequent root and shoot elongation of tomato in greenhouse conditions"	Eke et al. (2019)
Tomato	*Pseudomonas syringae* pathovar *tomato* (Pst) and field grown tomato leaves phyllosphere	Inoculated tomatos' leaves with phyllosphere provided protection against Pst. A significant lower density of Pst was found on treated leaves. Protection was dose dependent	Berg and Koskella (2018)

Tomato	*Pseudomonas* sp. consortia and *Ralstonia solanacearum*	Inoculated *Pseudomonas* sp. consortia in roots reduced pathogen abundance and disease incidence. Effects were dependent in *Pseudomonas* richness	Hu et al. (2016)
Potato	9 *Pseudomonas* strains from roots and rhizosphere of field grown potato consortia and *Phytophthora infestans*	Inoculated potato leaves with Pseudomonas strains inhibited mycelial growth and zoospore release of *Phytophthora infestans.* Some strains individually and others in combination gave protective effects	De Vrieze et al. (2018)

Studies on different species or varieties of tomato and potato inoculated with a consortium of microorganisms or induvial strains.

Table 3. Chemical and physical analysis of different locations within Loja

			(b) Experiment 2: Microbiome Analysis						(a) Experiment 1: Germination	
Parameter	**Target Value**	**Unit**	**Calvas agrícola**	**Calvas nativo**	**Zapotillo agrícola**	**Zapotillo nativo**	**Paltas agricola**	**Paltas nativo**	**Loja agricola**	**Loja nativo**
Total N stock	2850-4000	kgh N/ha	7250	3230	4600	1920	2970	8390	2410	3670
C/N ratio	13-17	-	11	13	12	8	10	12	13	15
N-suppling capacity	95-145	kgh N/ha	130	50	75	40	55	140	35	50
S-plant available	20-30	kgh S/ha	18	8	21	15	11	18	175	23
Total S stock	570-915	kgh S/ha	1265	540	1150	665	670	1350	450	500
C/S ratio	50-75	-	66	75	48	24	45	76	70	113
S-supplying capacity	20-30	kgh S/ha	19	7	21	15	13	18	7	3
P-plant available	3.7-6.6	kgh P/ha	1.1	<0.9	6.1	10.8	<0.9	2.4	2.1	2
P-soil stock	385-535	kgh P/ha	405	125	490	420	90	285	330	135
K-plant available	200-310	kgh K/ha	165	165	100	80	75	200	130	240
K-soil stock	335-470	kgh K/ha	470	320	425	280	910	115	225	245
Ca-plant available	205-480	kgh Ca/ha	315	25	25	25	280	20	170	25
Ca-soil stock	5330-7995	kgh Ca/ha	17955	12360	14320	8485	14515	14430	4585	4620
Mg-plant available	375-470	kgh Mg/ha	530	850	900	1300	1660	560	910	1140
Mg-soil stock	230-495	kgh Mg/ha	1470	190	955	1245	2305	795	800	790
Fe-plant available	7140-12840	g Fe/ha	7470	9310	10200	15670	6200	<5380	<6140	<5810
Zn-plant available	1430-2140	g Zn/ha	<280	610	420	490	440	<270	<300	430

Mn-plant available	9130-14270	g Mn/ha	<730	1570	3480	3250	1220	1020	3550	12980
Cu-plant available	115-185	g Cu/ha	<60	<60	<65	<65	<60	<55	<65	70
B-plant available	285-430	g B/ha	1245	315	540	520	335	1275	940	890
Mo-plant available	290-14270	g Mo/ha	<10	<10	<10	<10	<10	<10	10	<10
Acidity (pH)	-	-	7.5	6.6	5.7	6.6	6.6	7.2	7.1	6.3
C-organic	-	%	3	1.4	1.8	0.5	1	3.8	1.1	2
Organic matter	-	%	5.9	4.6	3.2	2.5	4.6	7.5	3.4	5.4
Soil texture	-	-	Silty Clay	Loam	Sandy Clay Loam	Sandy Loam	Sandy Clay Loam	Sandy Clay Loam	Sandy loam	Sandy loam

Nutrient quantities and other edaphic factors of each soil used in this study. Target value is the range established by Eurofins Agro Ecuador (Cayambe) for agricultural use.

Table 4. Site-soil type by sample code

Sample_code	Site	Soil Type	Location	Altitude	Land use
BZ1	Bulk soil	Agricultural	Zapotillo	172 masl	Maize
BC3	Bulk soil	Agricultural	Calvas	1169 masl	Maize and Yucca
RZ1	Rhizosphere	Agricultural	Zapotillo	172 masl	Maize
RC3	Rhizosphere	Agricultural	Calvas	1169 masl	Maize and Yucca
BZ6	Bulk soil	Native	Zapotillo	231 masl	Riverbank
RZ6	Rhizosphere	Native	Zapotillo	231 masl	Riverbank
BP7	Bulk soil	Agricultural	Paltas	966 masl	Maize and Peanut
RP7	Rhizosphere	Agricultural	Paltas	966 masl	Maize and Peanut
BC8	Bulk soil	Native	Calvas	1196 masl	Natural Vegetation
RC8	Rhizosphere	Native	Calvas	1196 masl	Natural Vegetation
BP11	Bulk soil	Native	Paltas	999 masl	Dry Forest
RP11	Rhizosphere	Native	Paltas	999 masl	Dry Forest

Altitude and land use of each type of soil per location.

8. FIGURES

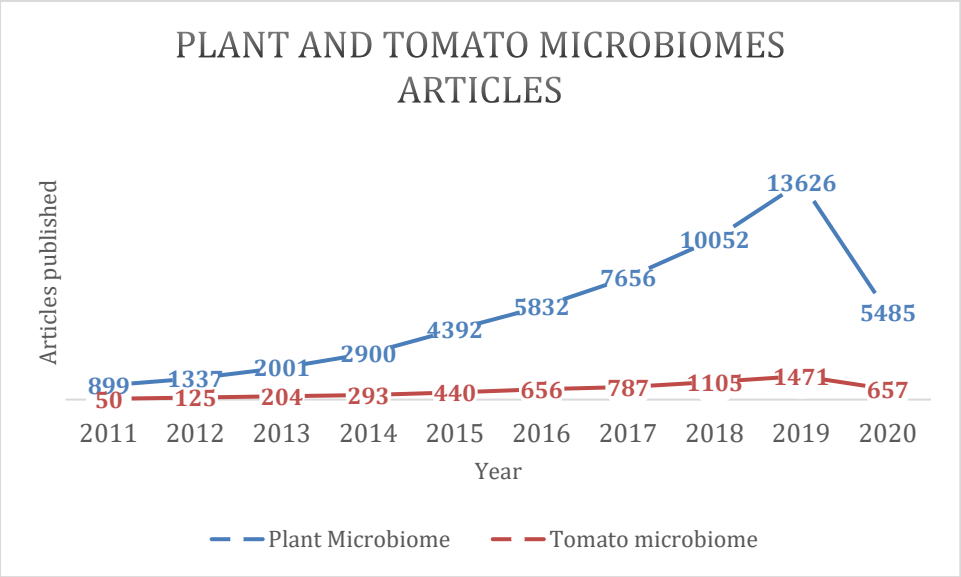


Figure 1. Trends on plant and tomato microbiome. Number of articles published per year about plant and tomato microbiome.

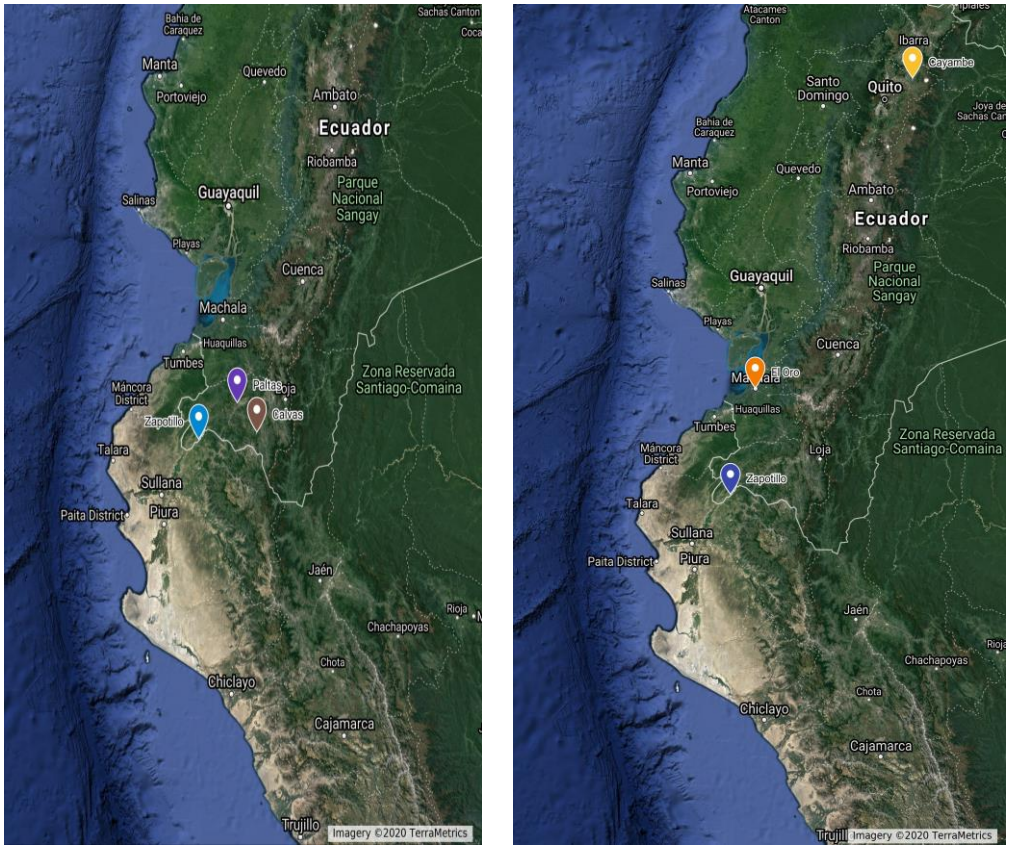


Figure 2. Map of soil samples collected. Geographical locations of soils sampled for microbiome analysis of *S. pimpinellifolium* (left). Geographical locations of soils sampled for germination experiment (right). Each location had a sample of native and agricultural soil.

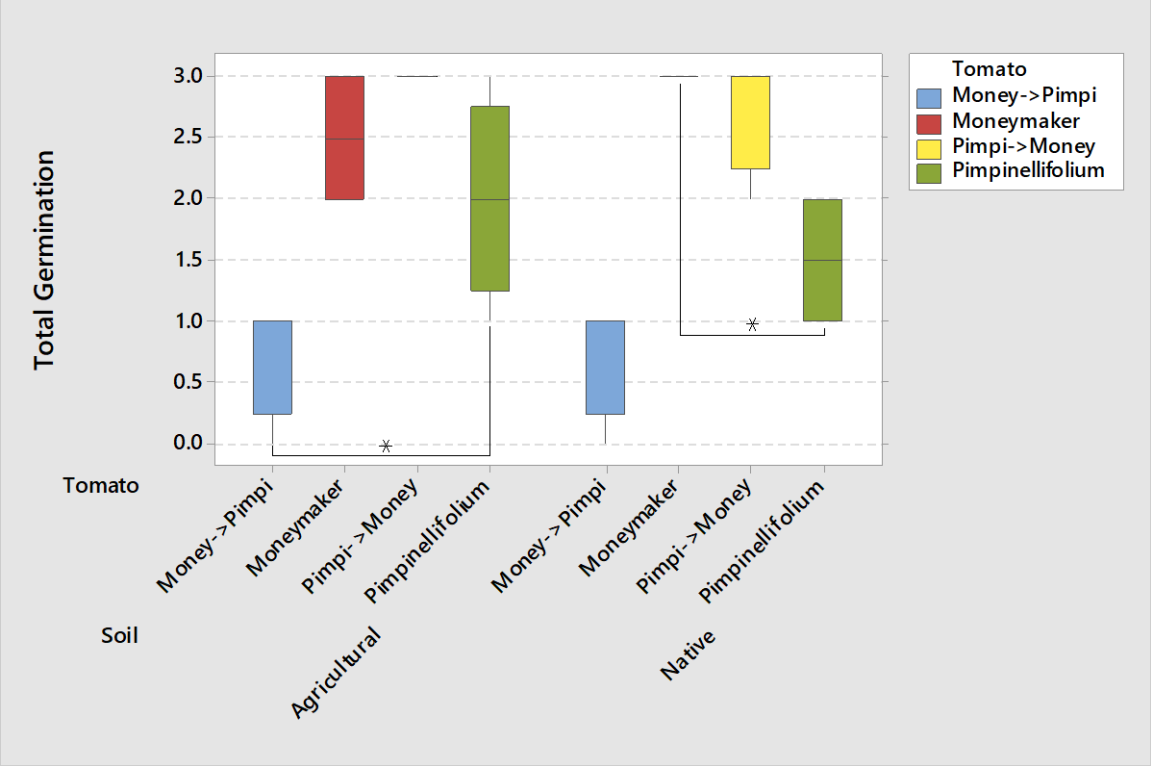
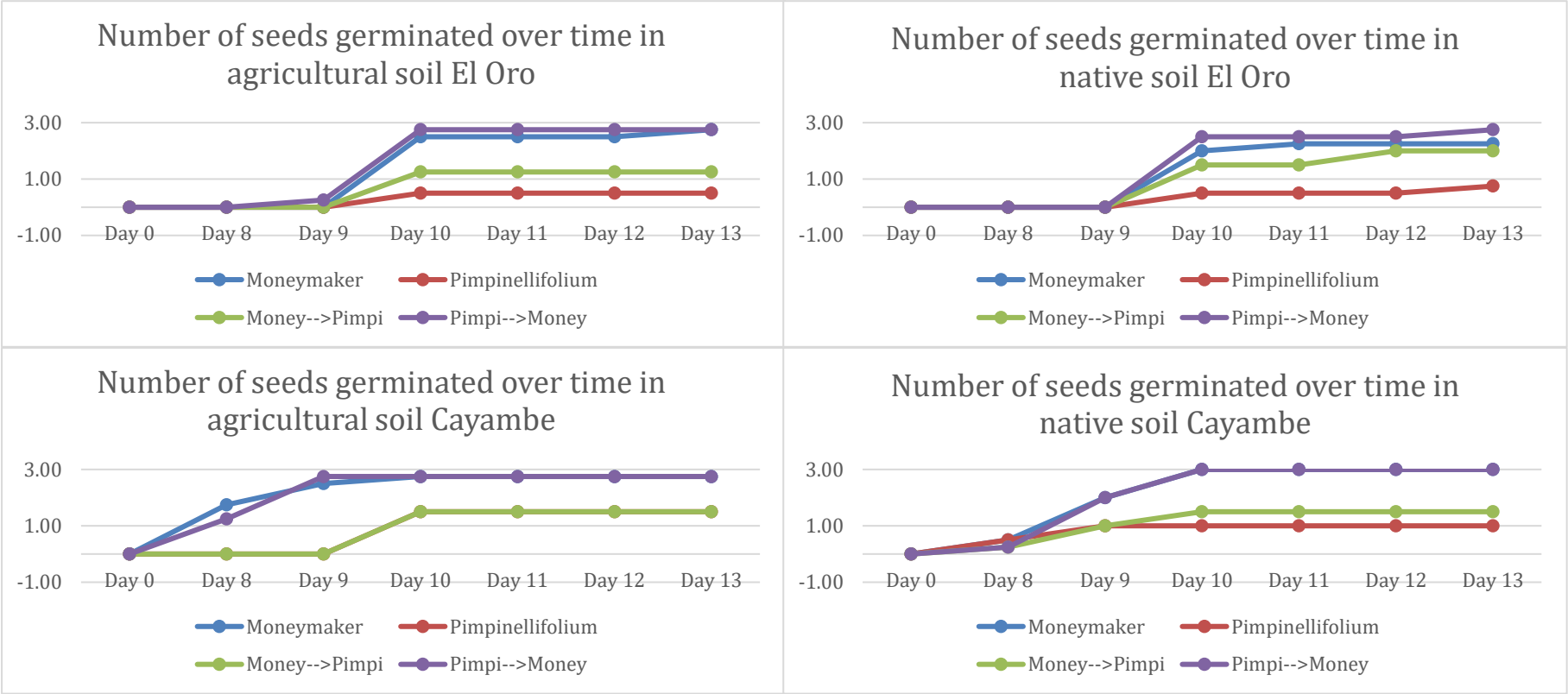


Figure 3. Boxplot of total seed germination in all soil treatments. Visualization of number of seeds germinated in soil treatments.


Number of seeds germinated over time in agricultural soil El Oro
3.00
1.00
-1.00
Day 0
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Moneymaker
Pimpinellifolium
Money-->Pimpi
Pimpi-->Money
Number of seeds germinated over time in native soil El Oro
3.00
1.00
-1.00
Day 0
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Moneymaker
Pimpinellifolium
Money-->Pimpi
Pimpi-->Money
Number of seeds germinated over time in agricultural soil Cayambe
3.00
1.00
-1.00
Day 0
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Moneymaker
Pimpinellifolium
Money-->Pimpi
Pimpi-->Money
Number of seeds germinated over time in native soil Cayambe
3.00
1.00
-1.00
Day 0
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Moneymaker
Pimpinellifolium
Money-->Pimpi
Pimpi-->Money

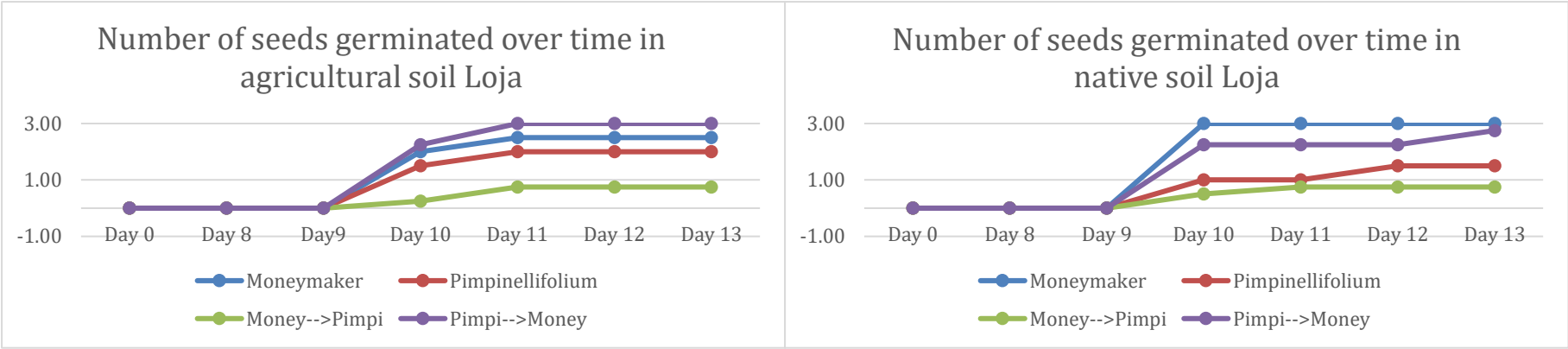


Figure 4. Germination over time of tomato on different soils. Seeds germinated of *Solanum pimpinellifolium* and *Solanum lycopersicum* var. Moneymaker with their exchange groups per day in agricultural and native soils of different locations of Ecuador.

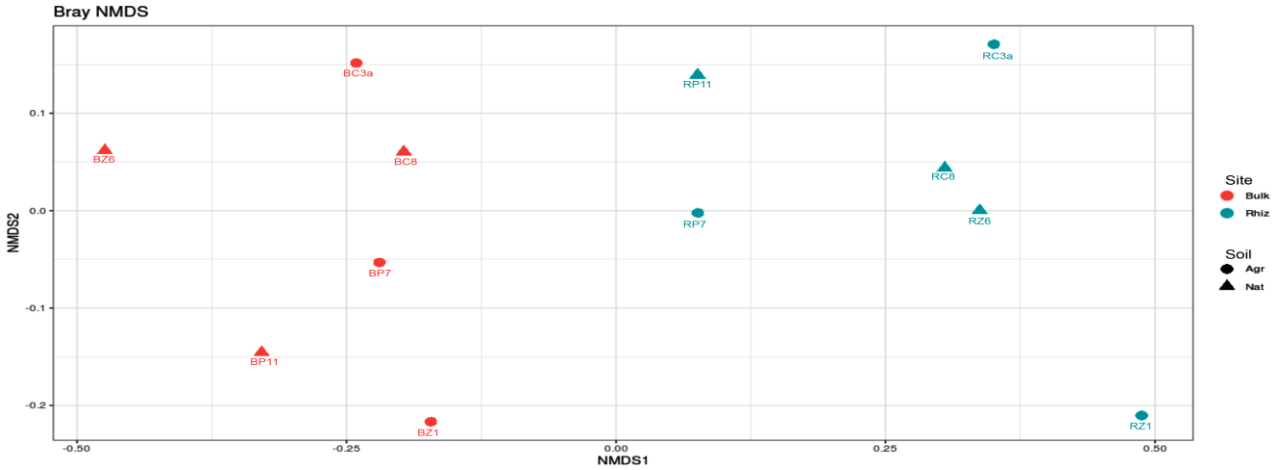


Figure 5. Multivariate analysis of soil type-site. Visualization of data by soil type and site grouped. Type of soil with site attributed to the codes can be found on Table 4.

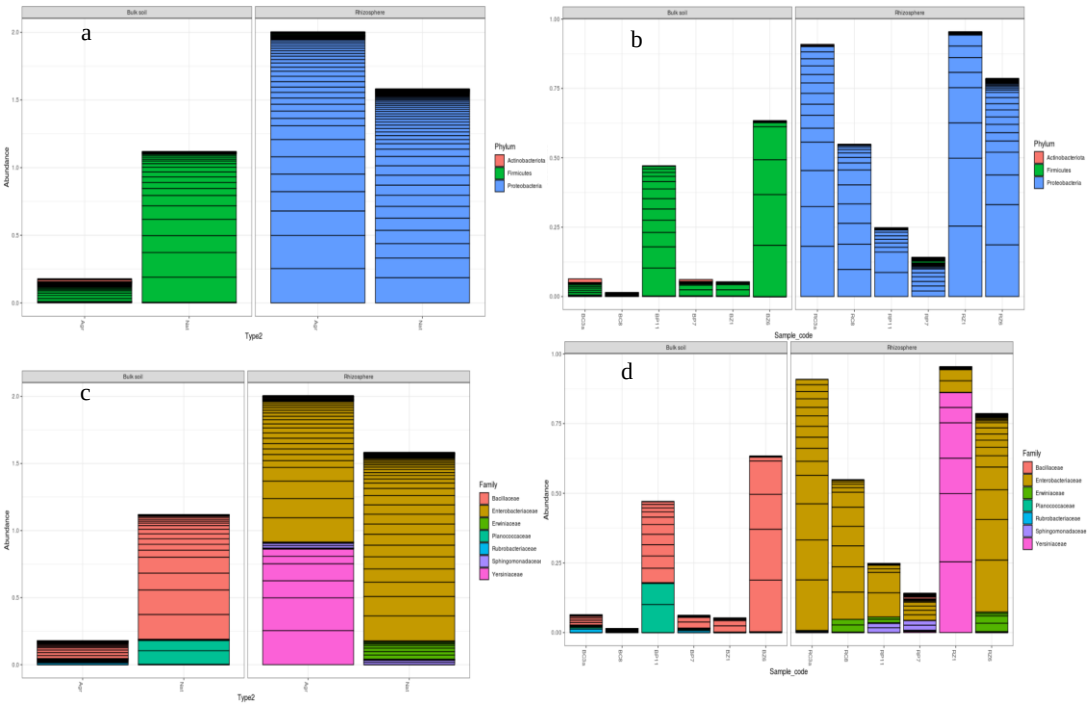


Figure 6. Relative abundance graphs of microbiome by soil type and location. Colors represent a given taxon in the graph. Type of soil with site attributed to the codes on X axis can be found on Table 4. a) Phylum relative abundance by agricultural and native soil in rhizosphere and bulk soil. b) Phylum relative abundance by site in rhizosphere and bulk soil. c) Family relative abundance by agricultural and native soil in rhizosphere and bulk soil. d) Family relative abundance by site in rhizosphere and bulk soil.

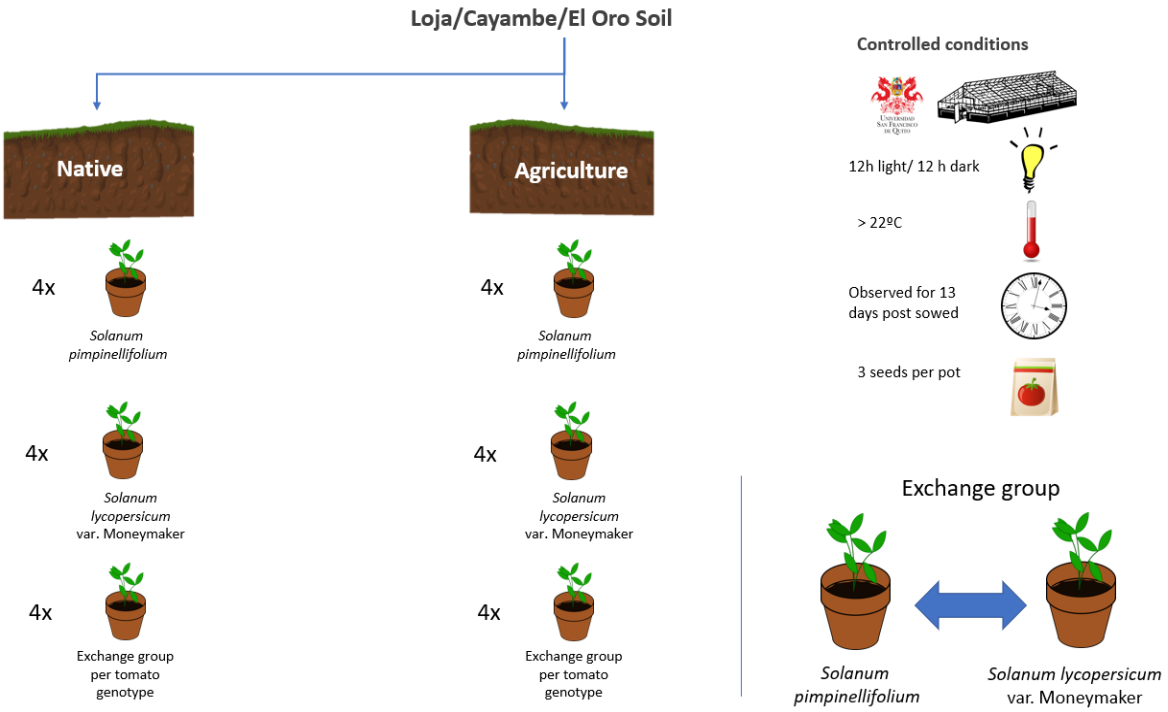


Figure 7. Seed germination experiment design. Layout of conditions and soil treatments done to *Solanum pimpinellifolium* and *Solanum lycopersicum* var. Moneymaker.