THESIS

EFFECTS OF PLANT-SELECTED RHIZOBACTERIAL COMMUNITIES ON THE DROUGHT RESISTANCE OF TOMATO PLANTS

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ABSTRACT

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Drought stress has had devastating effects for vegetable growers world-wide, leading to much recent research focusing on the development of drought-resilient crops. The importance of the rhizosphere microbiome in plant performance under drought stress is under development, including the use of beneficial inoculations of PGPR and transplanting of microbial communities. However, further research is needed to fully understand plants' innate abilities in mediating rhizobacterial recruitment to benefit plant resistance to drought stress. Here, two greenhouse studies were performed to determine the efficacy of conditioned soils containing plant-selected rhizobacterial communities as a means to increase drought resilience of host plants. Soils were autoclaved to lower microbial complexity and ensure the greatest plant influence over soil rhizobacterial recruitment. Tomato plants were grown in soils, autoclaved and control, to assess microbial recruitment under a gradient of water treatments: well-watered, moderate drought and severe drought. Autoclaved soils revealed a potential amplification of plant-selective influence over microbial community assemblage for drought-specific bacteria. Inoculants derived from this study were used to observe the impacts of microbial history on a plant's ability to tolerate contemporary drought stress conditions. Microbial history was shown to have a significant effect on microbial community composition and plant performance under drought conditions. To further apply the conditioned effects of microbial communities on tomato plants under severe drought stress, a multi-generational study was performed to amplify plant-

selected microbial communities from soils previously exposed to severe drought treatment. Effects of soil conditioning and microbial history suggested the presence of bacteria, conditioned over generations of plant-selection, involved in microbially-mediated plant growth restriction of tomatoes as a drought avoidance strategy. In summary, prior exposure of plants and microbial communities to drought stress may provide beneficial traits for host plants under contemporary drought conditions.

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CHAPTER 1: LITERATURE REVIEW

Summary

Limited water resources can cause morphological, physiological and biochemical effects on plant development, ultimately resulting in reduced yield or crop loss. As drought severity and frequency increase with climate change, strategies that promote crop tolerance to drought are needed to protect global food security. Currently, researchers have utilized breeding and transgenic strategies to lessen yield losses due to drought stress. However, drought tolerance is a complex trait regulated by many genes, making this task difficult. This thesis explores strategies to promote plant resistance to drought through microbially-mediated traits. Inoculations of beneficial microbes have been shown to promote drought tolerant traits in plants. However, these approaches are still not being widely used by farmers and new adaptation strategies need to be found. Similar to suppressive soils, which develop resistance to pathogen attack over generations in field systems, plant-selected microbial communities could be a solution to greater drought tolerance in crops. Here, I reveal microbial communities recruited by tomato plants under drought stress, in hopes of amplifying plant-mediated drought strategies. The soil communities were then conditioned over generations to identify key players in these drought-resilient soil microbiomes and allow for greater host plant resilience to severe drought stress.

Global impacts of drought

Despite projected increases in water demands on a local and global scale, drought disasters are predicted to continue to increase in frequency (Leng & Hall 2019). Drought can cause a cascading effect through a country's economy, environment, and people. Decreases in precipitation and subsequent water limitations, can cause surges in forest fire frequency and

strength, erosion, loss of habitat and important ecological processes, loss of employment, and yield loss of agricultural crops (UNDRR 2019). In more developed countries, these effects can have negative, indirect impacts on citizens through economic and environmental hardships, while less developed countries can also experience direct impacts on population numbers (IPPC 2012). One of the industries most impacted by these natural disasters is agriculture. Due to their extreme and sudden nature, drought disasters have caused devastating losses in production of major agricultural crops to date (Zhanga & Huang 2011; Comas et al. 2013; Udmale et al. 2014). In addition to the limitations imposed by less precipitation, the expected increase in temperature triggered by climate change, will contribute to greater evapotranspiration and evaporation rates from plants and water tables, respectively (Overpeck & Udall 2020; IPPC 2012). In combination, the increased temperature and frequency of drought disasters will put global food security at risk.

The agricultural industry relies heavily on water in both rain-fed and more modernized irrigation systems. In recent years, many studies have looked into ways to increase the efficiency and sustainability of irrigation methods for livestock and vegetable production (De Pascale et al. 2011), including the practice of desert farming in already arid regions (Köberl et al. 2011). However, despite these advances, farmers are still unable to deal with the destructive effects of increased drought episodes and subsequent yield loss in their fields. A study in which rural farmers in India were asked about the strategies they use in dealing with the increased frequency of drought included responses such as, selling their land and other personal items, consuming less meals and borrowing money to make ends meet (Sam et al. 2020). Accordingly, global task forces have begun to push for greater funding and resources for drought risk prevention strategies. The European Environment Agency (EEA) in its 2019 Report, called for the development of adapted crops to better deal with climate change, including those adapted to

exhibit greater drought tolerance (EEA 2019). The UN has also dedicated resources to risk prevention for these inevitable drought episodes in their 2019 Global Assessment Report on Disaster Risk Reduction (GAR), including a chapter devoted to drought predictions and consequences, with focus on the agricultural sector (UNDRR 2019). The Intergovernmental Panel on Climate Change (IPCC) has discussed the disproportionate vulnerability that impending drought disasters will have on the agricultural sector, specifically those agricultural areas without the funding and advanced technology needed to replace rain-fed irrigation systems (IPCC 2012). In conclusion, drought and its impact on agricultural production is a worldwide issue and more accessible, effective means are needed to develop drought resistant crop production methods.

Plant responses to drought

Drought impacts plants on a morphological, physiological, and biochemical level (Shao et al. 2008; Hai et al. 2020). These effects can result in devastating decreases in yield for agricultural production. In the United States, drought has caused 67% of all crop yield losses over the past 50 years (Comas et al. 2013). Regardless of crop type, drought has negative impacts on plant health and performance (Ilyas et al. 2020). Drought events can vary in intensity and duration, resulting in varied drought effects to plants. Timing of drought episodes within plant development can also have a distinctive effect on the plant (Anjum et al. 2017). Here, I discuss the morphological, physiological and biochemical implications of drought stress on plant performance and development.

Morphological effects

Plants change in appearance and form in a variety of ways when responding to drought. Visually, drought causes wilt, yellowing of leaves, and suppressed development of plant parts (Ilyas et al. 2020). Drought also impedes plant growth across different crop types by reducing fresh weight, dry weight, leaf area, height, number of leaves and yield, among others (Anjum et al. 2017; Shao et al. 2008). These decreases in plant growth can be a result of size restrictive plant hormones, lack of nutrients, and impeded cell growth due to low plant turgor (Rowe et al. 2016; Anjum et al. 2011). To limit water loss, plants undergo morphological changes to reduce the rate of transpiration. Reduction in leaf size and number, and changes in stomatal density, location and quantity, limit evapotranspiration and increase survival under drought conditions (Ilyas et al. 2020). Additionally, plants can alter their root architecture by increasing root length, root density and overall root to shoot ratio to allow for greater access to limited water supplies in soils (Furlan et al. 2012). To decrease light interception surface, leaf rolling often occurs in vegetative growth under drought stress (Anjum et al. 2017). Decreased light interception allows for lower photosynthetic and transpiration rates, which helps to maintain water status. Plant morphology is affected above ground, below ground and at a microscopic level, as a response to limited water resources.

Biochemical effects

The biochemical responses of plants to drought stress have been well documented, including the production of plant hormones (Ilyas et al. 2020; Xu et al. 2010). Declines in soil moisture levels increase production and cross talk between key stress regulating phytohormones including abscisic acid (ABA), jasmonic acid (JA), auxin, ethylene, and cytokinins (Prerostova et al. 2018). ABA is the primary phytohormone involved in abiotic stress defense and regulates

stomatal closure, which determines plant growth capabilities, and a variety of signaling pathways under drought stress (de Ollas & Dodd 2016: Rowe et al. 2016). JA also controls stomatal conductance rates, root development and the scavenging of reactive oxygen species (ROS) (Prerostova et al. 2018). Ethylene levels can influence plant above- and below-ground growth, restricting plant size under stress conditions. Furthermore, ethylene has been shown to cause leaf abscission in plants to maintain water levels and is involved in multiple signaling pathways (Arraes et al. 2015). Auxin is another critical phytohormone in abiotic stress response which regulates plant growth, including root development, with the auxin most often related to drought stress being indole acetic acid (IAA) (Perostova et al. 2018). Additionally, the production of cytokinins is altered as a result of abiotic stressors. These hormones are important players in many signaling pathways and regulate plant growth and photosynthetic machinery during drought stress (Hai et al. 2020). Beyond phytohormones, drought increases the accumulation of reactive oxygen species (ROS) in plants (Nxele et al. 2017), including in a study in which ROS levels were elevated in three genotypes of maize under drought conditions (Anjum and Ashraf et al. 2017). ROS can be very harmful to plant health and ultimately cause cell death (Dortje et al. 2014). Upregulated antioxidant production, as a result of plant signaling under drought conditions, aids in drought tolerance through the scavenging of ROS (Nxele et al. 2017). Furthermore, plants upregulate the accumulation and alter the allocation of sugars and other osmolytes to decrease the water potential in plants and lessen water loss (Anjum and Ashraf 2017).

Physiological effects

Plants require water to perform many physiological functions, which can be inhibited or altered under drought stress. Initial germination of plant seeds is dependent on water level, with significantly decreased germination rates under drought conditions (Anjum et al. 2011). Net photosynthetic and transpiration rates showed a decline under drought stress across multiple crop species (Shao et al. 2008; Anjum et al. 2017; Ilyas et al. 2020). Drought induces hormone signaling pathways that regulate these rates and restrict stomatal opening or conductance rates (Ilyas et al. 2020). Decreased stomatal function results in lowered transpiration and photosynthetic rates due to a lack of CO_2 intake and gas exchange through the stomata. This allows for limited plant growth and increased survivability under drought conditions by maintaining water status. Additionally, drought results in decreased nutrient content in plant tissues (da Silva et al. 2011). This is due to a cascading effect on plant functions, beginning with a restriction in the transportation of nutrients from the soil through plant roots (Ilyas et al. 2020). This is a result of low moisture soils forming inaccessible pockets of nutrients in the soil and decreased mobility of microorganisms and plant-secreted enzymes, which help in the breakdown and acquisition of nutrients through the plant roots (Raphael et al. 2012). Furthermore, water is needed to continue transpiration and flow of nutrients via the xylem, which ultimately inhibits nutrient uptake, transportation and distribution to plant parts. Interestingly, the application of greater nutrient supply to the soil during drought times can show no increase in plant nutrient when drought is severe and sufficient amounts of nutrients already exist in the soil (Rouphael et al. 2012). Drought also impacts root exudate profiles of plants, resulting in altered plant phenotypic traits and microbial community structures in the roots and surrounding soil system (Gargallo-Garriga et al. 2018). These exudate shifts have been shown to recruit bacterial members that may impact the plant's ability to tolerate drought conditions (Xu et al. 2018).

Drought strategies of plants

Plants vary in their ability to tolerate drought stress. There are three generally accepted categories of plant adaptive strategies to deal with drought: drought escape, drought avoidance or phenotypic flexibility, and drought tolerance (Ngumbi & Kloepper 2016; Khan et al. 2018; Kooyers 2015). Drought escape is a strategy in which plants have rapid development and shortened life cycles to reach reproductive stages before harsh drought conditions result in plant death (Lakshmi et al. 2018; Kooyers 2015). Drought escape responses are triggered by soil moisture or seasonal changes such as temperature or photoperiod. For example, North American *Arabidopsis lyrata* has shown earlier flowering time under water limited conditions (Paccard et al. 2014). Farmers have begun to use this knowledge in crop planning, including the Early Soybean Planting System, in which short season cultivars of soybeans are used so that pods are set well before the potential drought season in July (Lakshmi et al. 2018). Plants exhibiting drought avoidance, or phenotypic flexibility, as a drought strategy, alter plant traits to maintain water levels. These traits can include slower plant growth, smaller or closed stomata and subsequent reduced rates of photosynthesis and transpiration (Shavrukov et al. 2017). These morphological and physiological changes result in higher water use efficiency to minimize water loss for anticipated drought conditions (Shavrukov et al. 2017). Both drought escape and drought avoidance strategies increase plant survival and fitness under extreme drought and many crops undergo both strategies to combat the onset of drought conditions (Shavrukov et al. 2017). The final means of drought adaptation in plants is drought tolerance. This strategy is the most desirable among agricultural production systems as it allows for plants to continue to grow at a normal rate and maintain yield, despite drought stressed conditions (Ngumbi & Kloepper 2016).

For example, genes of interest for cotton plants are determined to be drought tolerant based on their association with higher yield and biomass, traits often contradictory to those in plants with drought avoidance or drought escape strategies (Khan et al. 2018). Drought adaptation is a complex, polygenic trait in plants controlled by many regulatory genes and mechanisms, which make plant adaptations to drought stress a difficult trait to quantify (Khan et al. 2018; Lakshmi et al. 2018).

Current strategies for developing drought tolerant crops

Much recent research is aimed at developing strategies to increase the drought resistance of crops. Because of the complexity surrounding drought tolerance, there have been a multitude of ways in which crops, agricultural practices and soil communities have been altered in this effort (Ilyas et al. 2020). Agricultural practices are being used to better conserve water and produce greater yield, despite drought episodes, including, grafting, soil microbial alterations, applications of additional nutrients, organic matter or chemicals (Rouphael et al. 2012). Substances shown to have beneficial responses to plants internally, have also been exogenously applied including nitric oxide, nitric oxide, 24-epibrassinoide, glycine betaine, proline, silicon and other osmoprotectants to alter water intake and antioxidant accumulation in plants (Ilyas 2020). Although these applications and agricultural practices have shown some promise, further research has been performed to determine more sustainable, permanent solutions. Researchers are currently looking into the efficacy of breeding tolerant genotypes, creating transgenics with greater drought resilience, conditioning plants and microbial communities and altering rhizobacterial communities to benefit plant resistance (Ilyas 2020).

Breeding & genetically modifying crops

As previously discussed, plants have different innate strategies to deal with drought stress. Because of the existing traits within different plants, breeding has shown to be a promising strategy in developing greater drought resilient crops. Wheat and barley are important cereal crops grown around the world in a variety of climates, making them susceptible to predicted increases in drought frequency (Sallam et al. 2019). However, resilient genotypes do exist within these two cereal crop varieties. Drought resilience is a complex trait and requires breeding strategies to first determine useful criteria by which to assess genotypic tolerance to drought stress. Breeders can then breed genotypes, each with a multitude of these beneficial phenotypic traits, to create a cultivar with a combined resilience to drought (Sallam et al. 2019). For example, some plant traits identified as beneficial when breeding wheat and barley varieties include the production and accumulation of phytohormones, metabolites, enzymatic antioxidants and carotenoids, limited reductions in size and water use efficiency to maintain normal photosynthetic rates and growth, maintained nutrient uptake, and beneficial root growth and architecture, which all culminate in maintained yield results under drought stress (Sallam et al. 2019). Additionally, breeding efforts have been made across wild-type, landrace and domesticated crops. Tepary beans, a wild relative of the Common bean, has greater drought tolerance compared to its domesticated counterpart (Mwale et al. 2020). Utilizing the drought tolerant traits from the genetic pool of Tepary beans created more drought tolerant crosses with Common bean genotypes, with greater yield under drought conditions (Mwale et al. 2020).

Many studies have also looked to genetically modifying crops for greater drought resilience, despite the complex nature of the trait. Plants respond to drought stress by altering their genes, therefore, scientists point to gene regulation as an important strategy to increase

stress tolerance (Ullah et al. 2020). Drought induced genes have a wide range of morphological, physiological and biochemical impacts on plant life, including many of the aforementioned plant effects of drought. Transgenics have therefore, been created to impact a multitude of different phenotypic traits in an attempt to increase yields and drought resistance. For example, crops have been engineered to manipulate plant hormone biosynthesis and pathway signaling (Prerostova et al. 2018). More specifically, ASR proteins, found in many crop species, have been shown to be involved in plant defense responses to abiotic stresses, including drought (Gao et al 2020; Hu et al. 2013). Studies have used this knowledge to transfer ASR genes to susceptible plant species for increased drought tolerance. HaASR1, an ASR gene isolated from a desert shrub, and TaASR1, an ASR gene isolated from wheat, were transferred to *Arabidopsis thaliana* and tobacco, respectively. Both studies showed a resulting reduction in water loss and reactive oxygen species (ROS) counts, an increase in plant growth and an up-regulation of other stress-response genes (Gao et al 2020; Hu et al. 2013). Furthermore, gene expression involved in the regulation of root growth has been studied in depth as a means to confer drought tolerance (Baliey-Serres et al. 2019). Root growth alone, however, is a polygenic trait requiring the expression and reception of multiple proteins and phytohormones (Baliey-Serres et al. 2019). Uga et al. determined that the DEEPER ROOTING gene (DRO1) can regulate root growth and angle, resulting in increased rice yield under drought conditions (2013).

Although there have been many advances made in finding genes related to drought tolerant traits, breeding and genetically modifying crops can be a limited strategy (Ngumbi & Kloepper 2016). There are obvious time and labor disadvantages, however, more importantly, these methods isolate a plant as an organism independent from its surroundings. Therefore,

incorporating the relationship between soil microbes and plant genetics can help to create more practical applications for drought adaptive crops (Ngumbi & Kloepper 2016).

Bacteria mediated drought resistance

The influence of soil microbial communities on plant performance and function have been well documented. Soil microbes can impact plant health, growth, development, nutrient acquisition and defense against biotic and abiotic stresses (Ngumbi & Kloepper 2016; Jain et al. 2020; Santos-Medellin et al. 2020; He et al. 2019), making bacterial-mediated stress tolerance a hopeful strategy for drought resilience in crops. Inoculants of plant growth promoting rhizobacteria (PGPR) have shown positive impacts on plant performance under stress conditions, including drought, by modulating morphological, physiological and biochemical changes in plants (Vurukonda et al. 2016; Ngumbi & Kloepper 2016). These beneficial bacteria can directly secrete or induce plant production of osmoprotectants such as proline, choline and trehalose, which helps to maintain water status (Vurukonda et al. 2016; Ngumbi & Kloepper 2016). Inoculants of a variety of known PGPR strains have been recorded to increase proline accumulation in the leaves of agricultural crops (Ngumbi & Kloepper 2016). Additionally, rhizobacteria can secrete phytohormones and enzymes into the soil to regulate plant functions (Vurukonda et al. 2016). For example, bacteria can produce indole acetic acid (IAA), a phytohormone that can regulate cell growth and elongation in plant roots. IAA-producing bacteria can increase nutrient and water uptake under drought conditions by promoting root growth and subsequent increased root surface area (Vurukonda et al. 2016). Some PGPR can also produce ACC deaminase, an enzyme which inhibits the production of ethylene, thereby, allowing the plant to continue normal growth under drought conditions (Glick 2014). Soil

bacteria can upregulate other stress responsive genes and signaling pathways as well, including ABA, JA, and GA, to provide increased resilience against drought effects (Dodd et al. 2010). Furthermore, bacteria can secrete or induce the biosynthesis of antioxidant enzymes to increase ROS-scavenging abilities during stress (Vurukonda et al. 2016). Bacterial inoculations can also aid in drought resilience through the induction of other phenotypic traits in plants. *Arabidopsis* plants inoculated with a PGPR, *Phyllobacterium brassicacearum* strain STM196, under drought conditions, resulted in an overall increase in biomass due to the induction of late flowering time (Bresson et al. 2013).

The close association between plants and soil microbes has led to further research into the efficacy of microbial inoculants for abiotic stress resistance (Hartman & Tringe 2019). Beyond known PGPR strains, entire microbial community transfers have been studied. For instance, it is known that plants living in arid regions exhibit different phenotypic traits than those in tropical regions. However, these adaptive responses to their conditions are due to a combination of plant genetics and microbial interactions (Aguirre-von-Wobeser et al. 2018). Therefore, it's not surprising that studies have shown that drought tolerant traits can be transferrable through microbial communities from well-adapted plants (Mosqueira et al. 2019; Marasco et al. 2012; Shirinbayan et al. 2019). For example, Marasco et al. (2012) identified and isolated bacteria found in the rhizosphere of a pepper plant grown in a desert farming system. Bacteria with known drought tolerant capabilities, those exhibiting ACC-deaminase activity, were used to inoculate susceptible pepper crops, resulting in transferrable drought tolerance. Additionally, several strains of *Azotobacter* were isolated from rhizosphere soils of crops growing in arid regions and used as a bioinoculant for maize exposed to varying drought conditions (Shirinbayan et al. 2019). The bacteria from the semi-arid regions altered the response of the maize under

drought conditions, resulting in increased shoot dry weight, plant height, chlorophyll content, nitrogen, phosphorous and iron concentration (Shirinbayan et al. 2019).

Amplifying plant responses to drought stress

Recent studies have shown microbial community structure is impacted by plant selective pressures (Li et al. 2019). These pressures are regulated by root exudations, which can change as a result of different developmental and defensive demands (Chaparro et al. 2014; Xu et al. 2018). Plants can, therefore, recruit bacterial members by excreting various metabolites into the soil surrounding the rhizosphere. This idea has been further supported through studies showing differences in soil microbial communities for soils with and without plant influence, including comparisons between rhizosphere and bulk soil communities (Li et al. 2019; Pascale et al. 2020; Hartman & Tringe 2019; Naylor et al. 2017). For instance, Santos-Medellín et al. (2020), found that rice cultivars under drought conditions showed greater changes within the rhizosphere soils compared to bulk soils collected beyond the reach of plant pressures. Additionally, a study looking at the desert microbiome of palm trees showed commonalities in community membership over a range of different sites within the Sahara Desert (Mosqueira et al. 2019). The results indicated that because of the lower existing microbial complexity within desert ecosystems, plant selective pressures had a greater influence on microbial recruitment than did soil or geographic location (Mosqueira et al. 2019). Another study looked at the rhizobacteria of a desert farming pepper plant, in which differences between micro-habitats in the soils were identified (Marasco et al. 2012). These results showed significant differences in bacteria between rhizosphere soils and bulk soils, therefore, indicating plant selection of bacteria in soil communities closest to the host plant (Marasco et al. 2012). Interestingly, in a recent study,

Dagher et al. (2019) examined the efficacy of microbial bioinoculants on plants compared to crop type in shaping the rhizospheric microbial community. They found that under toxic conditions, with high levels of petroleum hydrocarbon-polluted sediments (PHCs), plant identity had a greater influence over bacterial recruitment than did the addition of *Proteobacteria* PGPR isolates. Further indicating plant selection of rhizobacteria, particularly under stressed conditions.

Microbial complexity impacts plant recruitment potential

Soil sterilization, in the form of autoclaving, is a recent tool used to reveal these plant-mediated microbial community assemblages. Similar to the lower microbial complexity in desert soils observed by Mosqueira et al. (2019), soil sterilization reduces the competitive pressures of native soil microbiota on microbial community structure (Mosqueira et al. 2019; Li et al. 2019). Alternatively, soils with high microbial complexity inhibit strong plant selection of microbial communities resulting in a greater influence of native soil communities on rhizosphere microbiomes (Liu et al. 2019). A study was performed using above- and below-ground insect herbivory, prior plant conditioning of soils and soil inoculant strengths to identify plant and microbial conditioning impacts on defense against herbivory (Wang et al. 2018). The results showed greater stress defense with lower microbial complexity, in the form of inoculants with greater filtration of microbial components (i.e. smaller mesh size used in filtration of soil inoculant resulted in greater stress response). Therefore, lessening soil microbial complexity can reveal nuanced shifts in microbial community assemblages of plants under stress, resulting in the amplification of plant stress responses (Wang et al. 2018).

Conditioning of plant-selected rhizobacterial communities

As discussed, root exudation from plant hosts can manipulate rhizobacterial community assemblage. This selective pressure is based on plant demands, pertaining to phenotypic traits which are both crop- and condition-specific (DiLegge et al. 2021; Xu et al. 2018). Therefore, conditioning these chosen microbial communities over generations can serve as a means to amplify specific microbially-mediated traits in host plants. In a recent study, Panke-Buisse et al. (2015) conditioned microbes for late and early flowering of *Arabidopsis* plants over 10 generations, which ultimately led to a shift in flowering times for 3 different genotypes. Furthermore, conditioned soils have been shown to benefit plants under biotic stresses including insect herbivory and pathogen attack (Hu et al. 2018; Schlatter et al. 2017). Soils conditioned to grass and forbs species, relayed beneficial resistance to thrips attack in a subsequent planting of chrysanthemum (Pineda et al. 2019).

Researchers have investigated suppressive soil systems for decades (Schlatter et al. 2017). These are soils conditioned by monocultured crops, which aid in pathogen resistance over generations. Soils in these types of monocropping systems have been shown to infer plant resistance to pathogens such as *Rhizoctonia* and take-all disease caused by *Gaeumannomyces graminis* var. *tritici (Schlatter et al. 2017)*. These soil communities are modulated by plant root exudation shifts under pathogen attack. Yuan et al. (2018) observed shifts in root exudates upon infection of *Pseudomonas syringae* pv *tomato* (*Pst*) in *Arabidopsis thaliana* plants including increased amino acid, nucleotide and long-chain organic acid production and simultaneous declines in sugar, alcohol and short chain organic acid exudation. These changes in the root exudation profiles of tomatoes conditioned under infection resulted in increased disease resistance over generations (Yuan et al. 2018).

Conditioning of microbiomes and plants to exhibit better stress defense is of great interest with a growing need for crops tolerant to changing climatic conditions. Therefore, researchers have begun to investigate the efficacy of conditioned soils to help crops better deal with abiotic stresses. Prior drought exposure has shown benefits to host plants under contemporary drought conditions, including multi-generational exposure and exposure within a plant's lifetime (Lau & Lennon 2012; Franks 2011; Guerrero-Zurita et al. 2020). For example, a wild-type sweet potato cultivar showed greater resilience to drought stress with repeated short-term exposures to drought stress within a single season (Guerrero-Zurita et al. 2020). Additionally, a study performed by Lau and Lennon (2012), showed the conditioning of microbes to be more effective than the conditioning of plants over 3 generations of drought treatment, further indicating the importance of microbial communities within conditioning strategies to better deal with stress. Although conditioning studies have shown promise with altering plant phenotypic traits, more studies are needed to understand how plants mediate their own rhizospheric communities under drought stress, and how those communities might be imparting drought relief to the crops.

Thesis Goals

The goal of this thesis is to better understand the role of plant-mediated microbial recruitment as means to induce greater drought resilience in tomato plants. Here, I utilized steam soil sterilization (autoclaving) to decrease microbial complexity and allow for greater plant influence of rhizobacteria. Additionally, soils were conditioned over multiple generations to amplify the effect of the crop- and stress-specific microbial community assemblage. This thesis is composed of two studies with the goal of identifying bacteria selected by the plant and beneficial to the crop under the specified water conditions.

The first study determined the effects of autoclaved soils on a tomato plant's ability to deal with drought stress. Tomato plants are drought susceptible agricultural crops which allowed for easily observed effects of beneficial microbial recruitment. Lower microbial complexity in soils allows for greater plant selective pressures on the rhizobacteria community. Therefore, I hypothesized that with autoclaved soils and subsequent lessening of microbial complexity, plants would have greater influence over their microbial symbionts and ultimately, outperform those plants grown in control soils with higher microbial complexity. The study also focused on the conditioning potential of crop- and drought-specific microbial communities. Because of the expected increase in microbial recruitment choice within autoclaved soils, microbial inoculants taken from autoclaved conditions were expected to have a greater benefit to tomato plants within the same contemporary water treatment. This effect would suggest resulting microbial communities better adapted to assist tomatoes under a given condition. The goal of the final study was to apply the conditioned microbes to subsequent generations and amplify the beneficial impacts of plant-selected, condition- and crop- specific microbial communities. These two generations were continued within autoclaved soil conditions under severe drought only, in order to determine the benefits of continued conditioning of plant-mediated microbial communities compared to soils without the conditioned bacteria.

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CHAPTER 2: LOW SOIL MICROBIAL COMPLEXITY REVEALS THE AMPLIFICATION OF TRANSFERRABLE RHIZOBACTERIAL COMMUNITIES THAT AID IN DROUGHT RELIEF FOR HOST PLANTS

Summary

Drought stress can cause shifts in rhizobacterial communities associated with crops; however, the purpose behind these microbial changes is still unclear. Here, I have furthered this research by proposing plant-selection of microbial community members as a mechanism for greater drought resilience. I exposed autoclaved soils to a drought gradient to reveal stress-specific bacterial communities and determine how these selected microbial communities impact plant performance. Soils with low initial complexity and abundance of microbial communities (i.e., autoclaved) showed an increased differentiation between microbiomes from different water treatments, compared to not autoclaved (i.e., control) soil conditions. Additionally, the resulting rhizobacterial taxa in autoclaved soils showed decreased alpha diversity with increased drought severity, indicating the development of a limited community under severe drought conditions. Autoclaved soils also resulted in increased plant biomass as compared to the control soils; with greater differences as drought severity intensified. These results suggested that the microbial communities derived from autoclaved soils had bacterial members able to better support plants under drought stress. To test this hypothesis, microbial communities from the autoclaved soils for each water treatment were transplanted to new plants undergoing contemporary drought conditions. Plants given microbial inoculants from soils previously exposed to either moderate or severe drought conditions, resulted in greater plant biomass under contemporary drought treatment, as compared to those given well-watered inoculants. The resulting rhizobacterial communities following inoculations, maintained differentiation between inoculation treatments,

regardless of contemporary conditions. These results indicate increased resilience to drought stress as a result of microbial communities selected by plants under drought conditions, within autoclaved soils. In summary, lower soil microbial complexity allowed plants increased selectivity of beneficial microbial communities under drought stressed conditions, which resulted in transferrable benefits to host plants experiencing drought stress in subsequent plantings.

Introduction

Climate change research predicts increased frequencies of drought disasters in future years, limiting water supplies for agricultural crops and causing reductions in yield around the globe (Leng & Hall 2019). Drought is one of the most damaging abiotic stresses to sustainable agriculture (Gosal et al. 2009). Studies have shown that drought tolerance is a complex, polygenic trait and alters plant health on a morphological, physiological, biochemical, and molecular level (Pandey & Shukla 2015; Yordanov et al. 2003; Huber & Bauerle 2016; Bray et al. 2000). Drought associated genes have been used in the development of transgenic crops with increased drought tolerance (Shinwari et al. 2020; Khan et al. 2019; Gao et al. 2020). In addition to genetically engineering plants, conventional and marker-assisted breeding have been performed on a wide variety of crops to increase drought tolerant traits (Sallam et al. 2019; Mwale et al. 2019; Cai et al. 2020).

Previous studies have shown that the soil microbiota, specifically the communities inhabiting the rhizosphere, can influence plant health, nutrient acquisition, and defense against biotic and abiotic stresses (Jain et al. 2020; Santos-Medellin et al. 2020; He et al. 2019). Rhizobacterial community assemblages are dependent on the profile of root-derived exudates from a given crop, in combination with native soil microbiota (Liu et al. 2019; Jain et al. 2019;

Pascale et al. 2020). Plants can differentially modulate their rhizosphere microbiomes when undergoing various biotic and abiotic stress stimuli (Pascale et al. 2020; Hartman & Tringe 2019; Naylor et al. 2017; Naylor & Coleman-Derr 2018; Preece & Penuelas 2016; Kostenko et al. 2012), throughout different developmental stages (Chaparro et al. 2014), or in response to nutrient status (He et al. 2019). Because of this close interaction between plants and microbial community assemblage within the rhizosphere, recent research has investigated the efficacy of microbes to increase drought tolerance in crops (de Vries et al. 2020). For example, a study looking at the root exudates of barley plants under drought conditions showed increased amounts of proline, potassium and phytohormones directly involved in improving root growth, osmoprotection, and stress signaling (Calvo et al. 2016). It has also been shown that certain bacteria become naturally enriched in the rhizosphere of plants undergoing drought stress, due to both root exudation changes and their ability to tolerate desiccation (Xu & Coleman-Derr 2019). Naturally occurring shifts in soil microbial communities experiencing drought have been shown to influence the drought tolerance of host plants (Zolla et al. 2013). Other studies have shown positive effects of PGPR and other drought stress-related bacteria via artificial inoculation into the rhizosphere of stressed crops (Khare et al. 2020; Rolli et al. 2014). One of those studies performed on tomatoes and peppers determined that bacteria with ACC deaminase activity, isolated from soils in arid regions, provided transferrable drought resistance (Mayak et al. 2004).

Because of the strong influence the rhizosphere microbiome has on associated plants, a soil microbial approach may be needed to further the development of drought tolerant crops. In a multi-generational study performed by Lau and Lennon (2012), the soil microbiome was shown to adapt more quickly than the plant itself under drought conditions. In addition to this study, many others have shown the effects of adapting soil microbiomes over generations. By allowing

time for the bacterial communities to adapt to the given condition or phenotypic trait over generations, the effect of the specific microbiome is amplified (Panke-Buisse et al. 2015). However, the effects of plant selection on rhizobacterial communities can sometimes be weak, particularly when compared to the surrounding effects of the native soil community (Liu et al. 2020). In order to amplify the effect of plants on rhizobacterial communities, recent studies have shown the benefits of soil perturbation, in the form of sterilization (Li et al 2019). Soil sterilization has been shown to increase plant growth, nutrient uptake and efficacy of biological inoculants for various crop types (Qin et al., 2014; Wissuwa et al. 2020). Additionally, soil sterilization can increase plant-mediated microbial recruitment by lessening the potential competing influence of resident microbiota through direct (microbe-microbe) or indirect (niche occupancy) interactions (Pineda et al. 2020; Li et al. 2019). Furthermore, soil sterilization, and the observable amplification of microbial recruitment, can aid in better understanding plants' needs under abiotic stress.

In this study, I hypothesized that (1) low soil microbial complexity will amplify the ability of plants to preferentially promote drought tolerant rhizobacterial communities due to decreased competition from resident microbiota, resulting in increased plant growth. Additionally, I hypothesized that (2) by mimicking the history of the microbiome in contemporary conditions (e.g. severe drought contemporary conditions with an inoculation of microbes historically conditioned under severe drought stress), plants will have greater microbial specialization, resulting in a more adapted crop.

Methods

Soil Collection and Sterilization

Soil was collected in March of 2020 from a USDA-certified organic cover crop field (Agricultural Research, Development and Education Center [ARDEC] South, Specialty Crops program, Fort Collins, CO), most recently having grown peppers and melons. Bulk soil was collected as well as rhizosphere soils shaken from the roots of melon and pepper plants. Soils were sifted through a No. 10 metal sieve (2 mm wide). Following sieving, half of the soil was exposed to steam sterilization using a STERIS brand autoclave for three 40-minute liquid cycles at 121 ºC and is referred to as autoclaved soils. Autoclaving of soils was used to lower initial microbial complexity and abundances of soil microbial communities. This allowed us to observe the effects of decreased microbial competition on rhizobacterial recruitment and plant performance under differing water treatments. The remaining soil was not autoclaved and is referred to as control soils. Both autoclaved and control soils were dried out in trays in the greenhouse prior to weighing and filling pots.

Sterilization Study

Tomato seeds (*Solanum lycopersicum* L.) were surface sterilized with 3.0% NaClO, rinsed three times with sterile water, and imbibed in sterile water for 24 hours prior to planting. Seeds were planted in a 72-cell seed tray in sterilized peat moss. Peat moss was sterilized the same way as mentioned above for field collected soils. Seedlings were grown in a growth chamber for 2 weeks after uniform germination. After 2 weeks, seeds were transplanted into plastic pots filled with 350g of dry autoclaved or dry control soils. For each soil treatment, plants were grown under 3 differing drought-stress conditions: well-watered (WW), moderate drought (MD), severe drought (SD) (with 9 pots per drought condition per soil treatment; total n = 54).

Microbial History Study

Tomato seeds were surface sterilized with 3.0% NaClO, rinsed three times with sterile water, and imbibed in sterile water for 24 hours prior to planting. Seeds were planted in a 72-cell seed tray in autoclaved peat moss. Peat moss was sterilized the same way as previously mentioned for soils. Seedlings were grown in growth chamber for 2 weeks after uniform germination. After 2 weeks, seeds were transplanted into plastic pots filled with 350g of dry autoclaved soil. Plants were grown under the same 3 varied drought conditions (WW, MD and SD) with 3 different inoculant types: historically well-watered (WW), historically moderate drought (MD), and historically severe drought (SD) (with 9 pots per drought condition per inoculant type: total n = 81).

Soil slurries for inoculants were created using the method from Panke-Buisse, et al. (2015). Rhizosphere soils (23.33g) from the top 3 performers in each treatment from the Sterilization Study were pooled to create a total of 70g of rhizosphere soil. Performance was determined by plant fresh weight biomass, root:shoot ratio and height. Slurries were inoculated 3 days after transplanting. This allowed us to see the effect of microbial history on assisting plants under similar or different contemporary conditions

Drought Conditions

Seedlings were watered regularly for 4 days following transplanting to allow plants to successfully establish prior to drought stress. Induction of drought took place on day 5 after transplanting. Plants were grown under specified drought conditions for 4 weeks. Drought conditions were based on the field capacity of the soil. Percent moisture was determined using the moisture tension method at Colorado State University's Soil, Water and Plant Testing

Laboratory. The field capacity percentages were calculated based on weight, using 100%, 75% and 55% for WW, MD and SD conditions, respectively. Three random pots from each treatment were weighed daily between 2:00PM and 3:00PM. The average weight was used to determine the amount of water needed to maintain the appropriate field capacities. The weight of the soil and pot were known and included in calculations to replace the water lost by transpiration and evaporation. At week 2, plants were lined up by size within each treatment and a replicate visually closest to the average size for each treatment was chosen. The chosen plants were harvested and the fresh weight above and belowground biomass was measured. This number was used in future measurements to compensate for the weight of the plant in field capacity calculations. The experiment was conducted at CSU's Horticulture Center Greenhouse Facility.

Plant Data Collection

Drought was induced for 4 weeks, after which plants were harvested. Relative water content (RWC) was measured according to Smart and Bingham (1974). Ten leaf discs from each plant were submerged in Milli-Q water for 4 hours. Relative water content (RWC) was calculated in plants as follows: (Fresh weight – Dry weight)/(Turgid weight – Dry weight) × 100 (Ortiz et al. 2015). On the same day, height was measured, plants were cut at the root-shoot axis and fresh-weight measurements were taken. Rhizosphere soils were collected for each plant by gently shaking soils off roots and storing in Ziploc bags. Above- and below-ground plant parts were placed in paper bags and dried in an oven for 72 hr at 65 ºC. Dry-weight measurements were taken following drying.

A one-way or two-way ANOVA was performed using R Studio (Version 1.2.5033) to analyze plant height, RWC, DW biomass, root length and root area in both studies.

Soil DNA Extraction

Genomic DNA (gDNA) was isolated from rhizosphere soil samples. Nine rhizosphere soil samples were homogenized in pairs, for all but one sample, with a resulting 5 total gDNA samples. Samples were extracted using Qiagen DNeasy PowerSoil Kits, according to the manufacturer's instructions. Nucleic acid concentration and sample purity were quantified and determined via the use of a NanoDrop 2000 Spectrophotometer (Thermofischer). DNA samples were then stored at −80 ºC prior to Illumina MiSeq library preparation and downstream microbiome analyses.

Library Preparation for Illumina MiSeq Sequencing

Initial soil gDNA samples were diluted 1:20 with molecular water to reduce PCR inhibitors introduced during DNA extraction. Quantitative PCR targeting the V3-V4 region of the bacterial 16S rRNA gene was performed using a modified version of primer set 341F/ 785R (341F: 5′–*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*CCTACGGGAGGCAGCAG-3′. 785R: 5′-*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*GACTACHVGGGTATCTAATCC-3′) to target bacterial 16S rRNA and to attach Illumina MiSeq adapters, denoted in italics in the above primer sequences (Klindworth et al. 2013). This qPCR reaction was performed in 20 uL reaction volumes containing 2 uL of template DNA and 18 uL of the master mix. The master mix consisted of 10 uL 2X Maxima SYBR Green (Thermo Scientific, Waltham, MA, USA), and 2 uL each (10 uM) of forward and reverse primers and brought to a total volume of 18 uL using 4 uL of molecular grade water. The PCR thermal cycling conditions were as follows: 95ºC for 5

minutes, 35 amplification cycles (94ºC for 15 seconds, 55ºC for 30 seconds, 72ºC for 60 seconds) followed by a final annealing stage at 72 ºC for 5 minutes to reduce chimeric reads. A standard curve using purified *Psuedomonas putida* KT2440 gDNA was run with the samples to quantify the starting rRNA copies per g^{-1} soil. Resulting amplicons were then purified using an in-house preparation of solid phase reversible immobilization (SPRI) magnetic beads based on a modified protocol of Faircloth and Glenn (2011) and original protocol of Rohland and Reich (2012).

A second PCR cycle was then conducted to attach unique Illumina Nextera XT indices to each bead cleaned sample for subsequent sample demultiplexing. Each well contained 5 uL of first round and bead-cleaned qPCR product, 25 uL of 2X Maxima SYBR Green (Thermo Scientific, Waltham, MA, USA), 5 uL each of both forward and reverse indices were combined along with 10 uL of water, bringing the total volume to 50 uL. PCR conditions were as follows: 95ºC for 3 minutes, 8 amplification cycles (95ºC for 30 seconds, 55ºC for 30 seconds and 72ºC for 30 seconds) followed by final annealing of 72ºC hold for 5 minutes. The resulting PCR product was again SPRI-bead cleaned using the same methods previously mentioned. Amplicons were then quantified using a Qubit fluorometer (Thermo Scientific, Waltham, MA, USA) prior to normalization and pooling. The final pool was run on a TapeStation system (Agilent Technologies, Santa Clara, CA, USA) to determine size and purity of amplicons, and Kapa Biosystems (Sigma-Aldrich, St Louis, MO, USA) qPCR was performed according to the manufacturers' instructions to determine concentration. The final pooled sample was diluted to 4 nM and the DNA library was denatured with 0.2 N NaOH, diluted to 10 pM using provided HT1 buffer, and spiked with 20% PhiX library standard diversity-control. Illumina's MiSeq v3 600-cycle Reagent Kit (Illumina, San Diego, USA) was used for library dilution and loading onto the

MiSeq at CSU's Next Generation Sequencing Laboratory (Fort Collins, CO).

Bacterial 16S rRNA gene sequence analysis

De-multiplexed raw fastq files were processed with the DADA2 pipeline using R Studio's Bioconductor packages (Callahan et al. 2016). Briefly, all primers were removed from each sequence using the open source Python program Cutadapt (Martin et al. 2011) and amplicon sequence variants were inferred using the default pipeline in DADA2. Each sequence variant identified in DADA2 was classified to the closest reference sequence contained within the Green Genes 13_5_99 reference database. Each taxonomic profile assigned was used to determine bacterial genus and species-level relative abundance values. Downstream analyses were conducted using R Studio's phyloseq package (McMurdie and Holmes 2013) or myPhyloDB (v 1.2.0) (Manter et al. 2013). Samples were rarified at a cutoff of 21500 reads using myPhyloDB prior to downstream analysis applications using myPhyloDB or R Studio; all samples met rarefaction criteria and no samples were removed from downstream analyses. Measurements of α-diversity assigned to treatments were determined using the Shannon diversity index, as this diversity measure accounts for both richness and evenness within each sample. A two-way ANOVA was used to compare mean α-diversity values of different drought-levels under autoclaved and control soils. Values from the Bray-Curtis dissimilarity index were calculated in myPhyloDB and used to quantify differences in microbial community structure between samples from different treatments. The myPhyloDB software was then used to visually represent distances using principal coordinates analyses (PCoA). A complementary non-parametric multivariate statistical test, a permutational analysis of variance (perMANOVA), and

differential abundance analyses (FDR < 0.1) were performed to determine differences in microbial communities between treatments (Manter et al. 2016).

Results

1. Sterilization Study

1.1.Plant performance under autoclaved and control soil conditions across a drought gradient

Plant dry weight (DW) measurements were taken for below- and above-ground biomass. The mean total DW biomass was used to analyze differences between treatment groups. Soil condition types were compared for each water treatment (Figure 1). DW of plants grown in well-watered conditions showed no significant difference between control (CK) and autoclaved (A) soil treatments (p-value = 0.381). However, as the severity of the drought increased, so did the comparative difference between autoclaved and controls soil treatments under moderate and severe drought conditions (p-value = 0.199 and p-value = 0.002, respectively). The resulting percent increase in plant biomass after autoclaving for well-watered, moderate drought and severe drought conditions were 10.08%, 23.78% and 111.31%, respectively. Additionally, a two-way ANOVA of these values revealed a significant decrease in DW plant biomass of plants grown in control soils when exposed to severe drought conditions as compared to moderate drought conditions (p-value = 1.8e-06). There was, however, no significant difference in DW biomass between plants exposed to moderate drought conditions is control soils compared to plants exposed to severe drought conditions when grown in autoclaved soils (p-value = 0.276), indicating an increase in the ability of plants in autoclaved soils to deal with severe drought treatments.

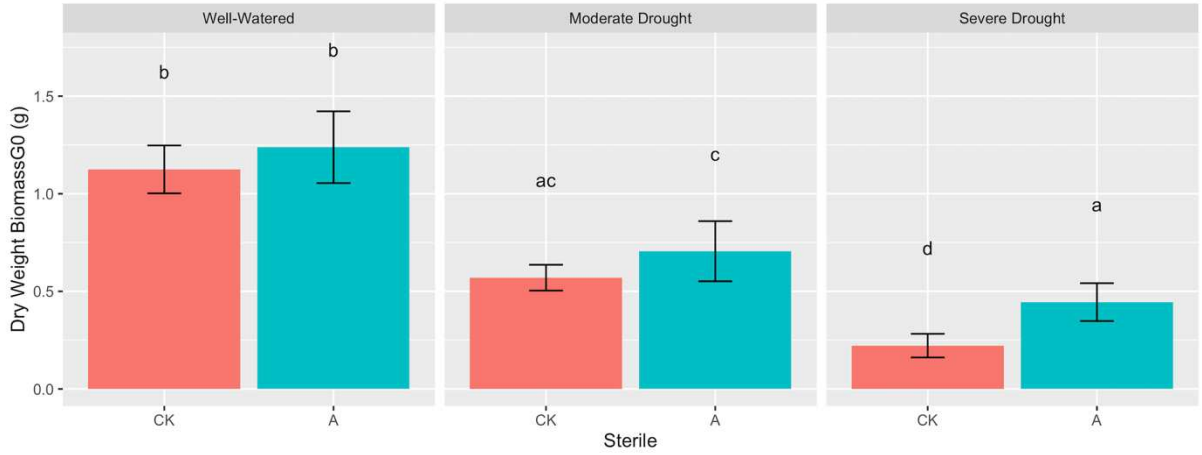

Figure 1. Mean dry weight biomass (DW) measurements for tomatoes under each water treatment. Blue bars and orange bars represent control and autoclaved soils, respectively. Different letters indicate significant differences at $p < 0.05$.

1.2 Microbial Data Analysis

1.2.1 Principal coordinate analysis of microbial community differentiation under soil and drought treatments

A principal coordinates analysis (PCoA) was performed to visually compare microbial communities from all water and soil treatment groups (Figure 2). The ordination showed clustering of microbial communities within drought treatment groups and a significant shift resulting from autoclaved soils. A permutational analysis of variance (perMANOVA), using Bray-Curtis distance matrices at the OTU level, was used to determine significant differences in rhizobacterial communities between treatment groups for autoclaved and control soils. I found significant differences between microbial communities of treatment groups as an effect of both autoclaving ($p = 0.001$) and water treatment ($p = 0.034$). Furthermore, my analysis revealed that the microbiomes of samples from each water treatment differed significantly, regardless of soil conditions (control soils: SD_MD p- value = 0.051, MD_WW p-value =0.046, SD_WW p-value = 0.014; autoclaved soils: SD_MD p-value = 0.007, MD_WW p-value = 0.009, SD_WW p-value

= 0.012) . However, the ordination shows greater separation between water treatment groups for autoclaved soils as compared to control soils. The differentiation between water treatment microbiomes was greater for autoclaved soils (drought effect p-value= 0.001) compared to control soils (drought effect p-value= 0.002). These results indicate a greater influence of water treatment on rhizobacterial recruitment when soils have lower microbial community complexity.

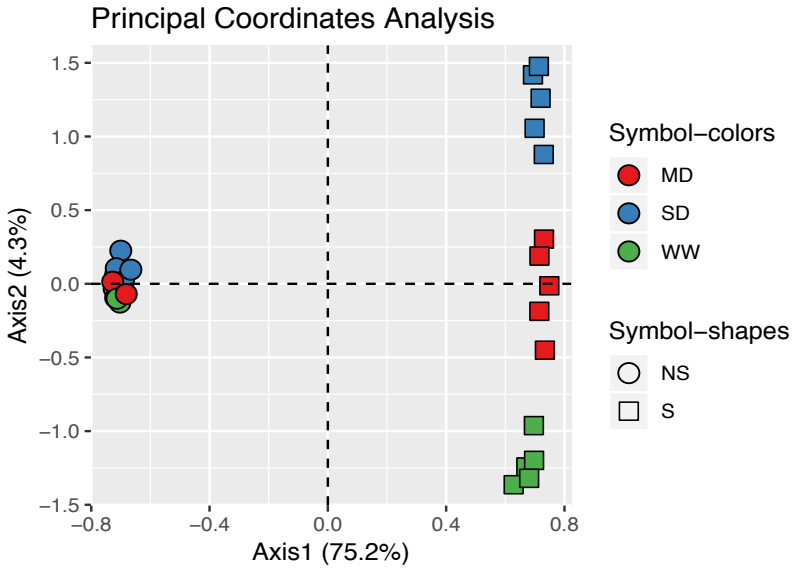

Figure 2. Principal coordinates analysis (PCoA), using Bray-Curtis distances, representing rhizobacterial communities of soil samples from each water treatment (n=5 soil samples per water treatment) and sterilization condition. Blue, red, and green represent severe drought, moderate drought, and well-watered treatments, respectively. Circles represent samples from control soils and squares represent samples from autoclaved soils.

1.2.2 Shannon diversity differences among differing water and soil conditions

Alpha diversity was calculated to the OTU level, using the Shannon Diversity index, a measure of richness and evenness of taxa in microbial communities. A two-way ANOVA of these values revealed significantly increased rhizobacterial alpha diversity under well-watered compared to severe drought conditions in autoclaved soils (Figure 3: p-value = 3.6e03). These

results show a decrease in microbial diversity within autoclaved soil treatments when plants are exposed to severe drought conditions, indicating potential drought-specific rhizobacterial recruitment under drought stress. In contrast, there is no significant difference between drought treatment rhizobacterial community diversity under control soil conditions (Figure 3). Additionally, autoclaved soils showed significantly decreased alpha diversity across all water treatments as compared to control soil treatment groups. These data suggest likely interference of competing native soil microbiota in the control soils, thereby inhibiting plant-selection of water-treatment-specific microbial communities.

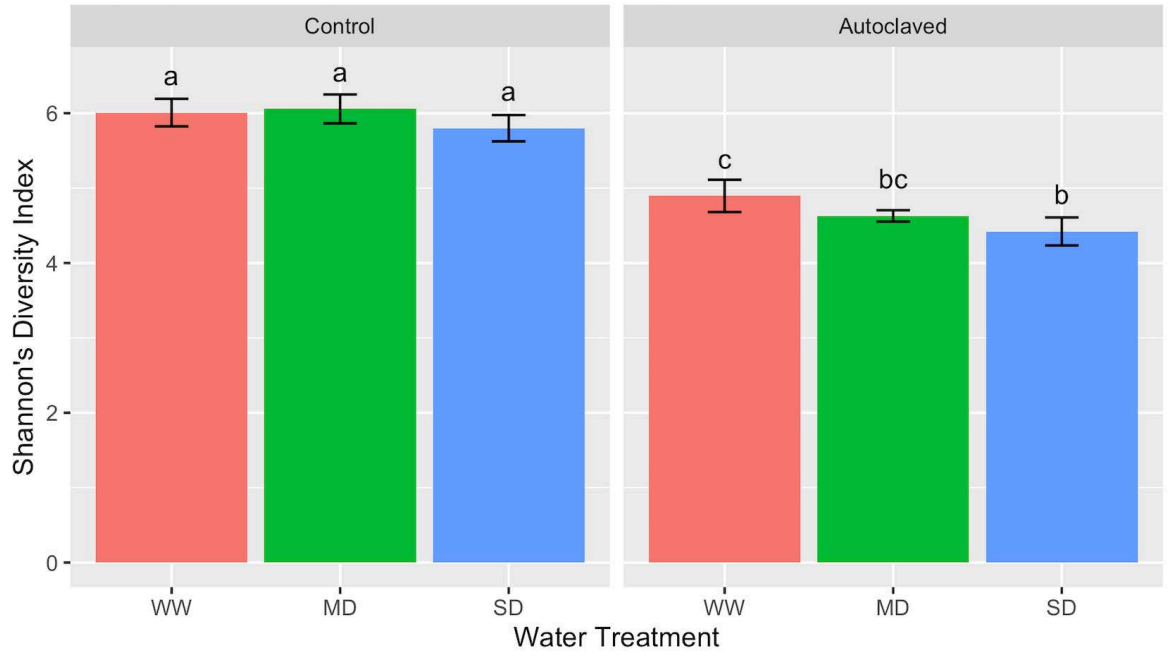

Figure 3. Alpha diversity of each water treatment group, represented using Shannon Diversity Index values, is presented here within control and autoclaved soil conditions. Different letters indicate significant differences at $P < 0.05$.

1.2.3 *Differential Abundance analysis of genus-level community member shifts under differing drought conditions*

Differential abundance analyses were performed to determine the bacterial taxonomic groups driving the differentiation of rhizobacterial communities under differing water treatments (Figure 4; Table S1; Table S2). There were no genera present in significantly different abundances in well-watered compared to moderate drought treatments or moderate drought compared to severe drought conditions in autoclaved soils. The genera present in significantly greater abundances in well-watered as compared to severe drought conditions in autoclaved soils are identified in Table S1 and Figure 4. Furthermore, the only taxon, at the genus-level, that was found to increase in abundance under severe drought conditions as compared to well-watered conditions in autoclaved soils was *(c: Betaproteobacteria, o: Burkholderiales) Oxalobacteraceae_unclassified* (Figure 4; Table S2). Figure 4 shows all significantly differing genera under severe or moderate drought conditions as compared to well-watered conditions for both autoclaved and controls soils.

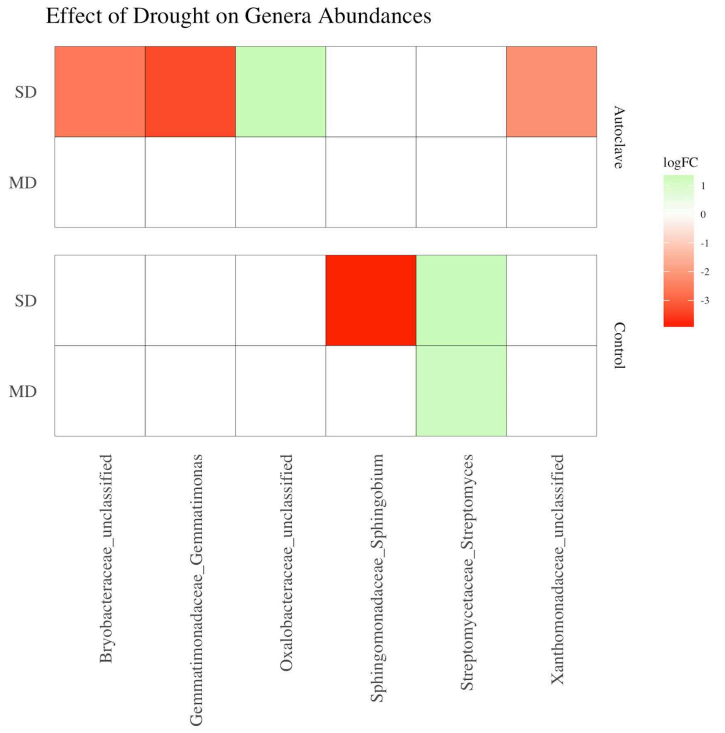

Figure 4. Genera that showed significant changes in abundance as a result of water treatment (FDR < 0.1; P < 0.05) under control and autoclaved soil conditions. The color for each cell

indicates the log fold change of that genus under severe drought (SD) or moderate drought (MD) treatments as compared to the well-watered treatment. Red represents a significant decrease in abundance; green represents a significant increase in abundance. The intensity of the color is related to the log fold change. No color signifies no significant difference in abundance between treatment groups. Taxa shown were the only genera to show significant differences in abundance within at least one treatment comparison, according to differential abundance analyses performed.

Furthermore, I performed differential abundance analyses to determine the effects of autoclaving on bacteria under differing drought treatments, to the genus-level (Figure 5). Interestingly, autoclaving showed differential effects for some genera dependent on water treatment, indicating condition-specific bacterial taxa shifts.

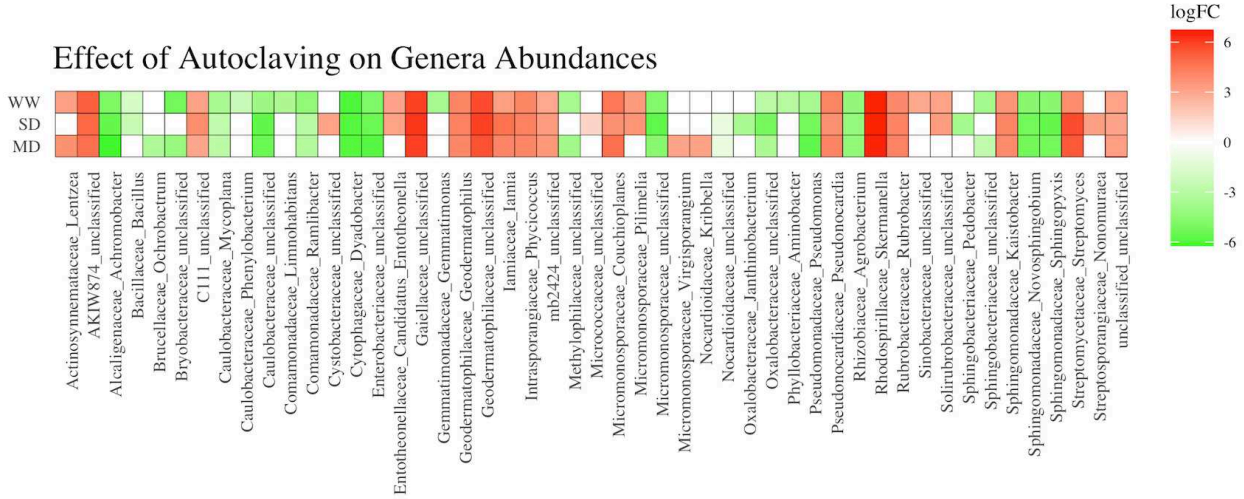

Figure 5. Genera that showed significant changes in abundance as a result of soil condition for each water treatment (FDR < 0.1; P < 0.05). The color for each cell indicates the log fold change of that genus for well-watered (WW), moderate drought (MD) or severe drought (SD) treatments under autoclaved soils as compared to control soils. Red represents a significant decrease in abundance following autoclaving; green represents a significant increase in abundance following autoclaving. The intensity of the color is related to the log fold change. No color signifies no significant difference in abundance between treatment groups. Taxa shown were the only genera to show significant differences in abundance within at least one treatment comparison, according to differential abundance analyses performed.

2. Microbial History Study

2.1 Plant performance under differing contemporary drought conditions with historically conditioned microbes

After the best performing plants from the Sterilization Study were selected and inoculants from each drought condition were created, slurries were transferred to plants in autoclaved soils undergoing the same gradient of drought stresses. This allowed us to see the effect of microbial history on assisting plants under similar or different contemporary conditions. Above and belowground biomass measurements were taken and used to determine the total DW biomass. The mean total DW biomass for each inoculation treatment was compared within each contemporary water treatment group (i.e. plants with microbial soil slurries conditioned for well-watered, moderate drought and severe drought under current well-watered conditions) (Figure 6). Plants given a microbial inoculant from previously well-watered conditions resulted in a significant decrease in plant DW biomass under contemporary moderate or severe drought conditions as compared to plants given inoculated microbial communities with prior exposure to severe drought stress. Additionally, plants grown under moderate drought conditions and given a severe drought (SD) inoculation showed no significant difference in DW biomass compared to plants grown under well-watered conditions. Furthermore, plants given the SD inoculation treatment showed no significant difference in plant DW biomass under well-watered, moderate drought or severe drought conditions as compared to plants given a well-watered (WW) inoculant under well-watered contemporary conditions. These results suggest greater plant resilience to drought with the SD inoculation treatment.

Interestingly, the effect of inoculation type on plant DW biomass under contemporary drought conditions increased as the severity of drought increased. Under severe drought conditions, plants with microbial inoculants conditioned with prior exposure to either degree of

drought stress significantly outperformed plants given microbial inoculants conditioned for well-watered treatment. In contrast, there was no significant difference in plant DW biomass between inoculation treatments under well-watered conditions, indicating that microbial history has a greater influence on plant growth when under stress.

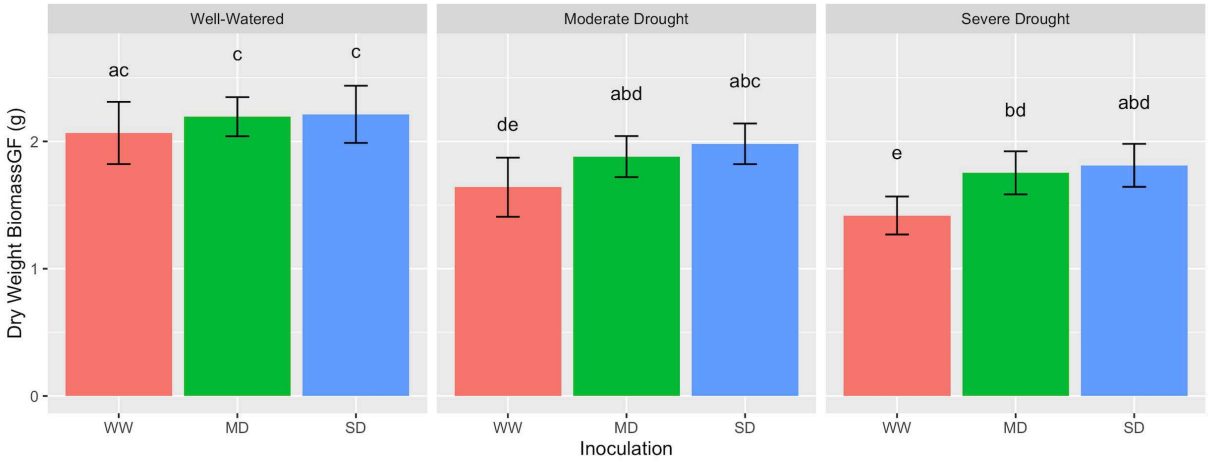

Figure 6. Mean dry weight biomass (DW) measurements for tomatoes with different inoculation types and contemporary water conditions. Red bars, green bars and blue bars represent well-watered inoculation (WW), moderate drought inoculation (MD) and severe drought inoculation (SD) treatments, respectively. Each panel from left to right represents inoculation treatments within well-watered, moderate drought and severe drought contemporary conditions. Different letters indicate significant differences at $p < 0.05$.

2.2 Microbial Data Analysis

2.2.1 Principal coordinate analysis of microbial communities with historically conditioned microbes under differing contemporary drought conditions

A PCoA was performed to analyze microbial differences between inoculation types under severe drought and well-watered contemporary drought conditions (Figure S1). These results revealed rhizobacterial communities of each inoculation treatment, derived from water treatment-specific microbiomes, maintained differentiation under varying contemporary drought conditions. Thereby, indicating a relatively strong influence of microbial history on

rhizobacterial recruitment, regardless of contemporary conditions (inoculation effect: p-value = 0.001). Despite the continued differentiation between treatment groups, there was a significant shift in all 3 inoculation treatments when comparing severe drought and well-watered contemporary conditions (drought effect: p-value = 0.001).

2.2.2 Differential Abundance analysis of genus-level community member shifts under differing drought conditions

Differential abundance analyses were performed to determine the bacterial taxonomic groups driving the differentiation of rhizobacterial communities. In order to identify water treatment-specific taxa, differential abundance analyses were performed to compare bacterial microbial communities across different contemporary water conditions with each inoculation type. There were no taxa present, to the genus level, that showed significantly different abundances as a result of a well-watered inoculation when comparing MD and SD contemporary conditions or MD and WW contemporary conditions. There were also no significantly different genera shown between MD and WW contemporary conditions with a moderate drought inoculation. There were no genera present in significantly different abundances between SD and MD contemporary drought conditions with a severe drought inoculation. Bacterial taxa found to be significantly different between treatments, however, are indicated in supplementary data (Tables S3-S7).

From these significantly different taxa, I identified two genera that showed consistent trends in abundance dependent on water treatment. *Nocardioidaceae_unclassified* was found to be present in significantly greater abundances under SD compared to WW contemporary conditions for both well-watered and severe drought inoculation treatments. This taxon also

showed increased abundances under MD compared to WW contemporary conditions with a severe drought inoculation treatment, indicating that *Nocardioidaceae_unclassified* may be preferentially selected under increasing drought conditions. In contrast, *Comamonadaceae_unclassified* showed significantly increased abundance levels under WW compared to SD contemporary conditions and MD compared to SD contemporary conditions for well-watered and moderate drought inoculation treatments, respectively. These trends suggest that *Comamonadaceae_unclassified* is susceptible to water-limited conditions.

Discussion

Autoclaved soil allows for greater microbial differentiation between water treatments

My data showed greater differentiation in microbial community composition for water treatments with plants grown in autoclaved soils, compared to those grown in control soils (sterilization effect: p-value= 0.001 and 0.002, respectively). These findings are consistent with previous studies having shown the efficacy of soil sterilization as a means to reveal crop- and condition-specific rhizobacteria (Pineda et al. 2020; Li et al. 2019). Additionally, plants grown under autoclaved conditions showed a pattern of decreasing microbial alpha diversity in rhizosphere soils as drought severity increased. This trend was not observed for control soils, in which there were no significant differences in alpha diversity between water treatments. The specificity of the resulting water treatment microbiomes suggests a potential increase in plant selectivity on rhizobacterial communities following autoclaving. This increased influence of plant-selection on the soil microbiome has previously been attributed to the decrease in competition from native soil microbiota (Li et al. 2019). In addition, plants secrete different root exudates dependent on environmental and developmental demands (Pascale et al. 2020;

Chaparro et al 2014), including drought specific metabolites (Xu et al. 2018). These findings, in combination with my own, point to the sterilization of soils as a means to increase plant influence on microbial recruitment.

Effects of lower microbial community complexity was amplified with increasing drought severity

The effects of soil sterilization extend beyond microbial community composition, impacting aspects of plant performance. Soil sterilization has resulted in changes to nutrient uptake, stress defenses and increased plant biomass (Wang et al. 2018; Hu et al. 2018). Although recent studies have shown that plants undergoing abiotic stress can benefit from soil sterilization (Fu et al. 2020; Torres-Martinez et al. 2020), more research is needed to determine the importance of the resulting recruited microbiomes. Here I have analyzed both plant performance and microbial community composition to better understand the impact of soil sterilization, in the form of autoclaved soils. My data showed increased plant DW biomass under autoclaved soil conditions (Figure 1). Interestingly, I observed an amplification of this autoclave effect under increasing drought stress levels. A potential explanation for this phenomenon could be due to the increased importance of plant-selected rhizobacterial communities when plants are undergoing abiotic stress. There was no significant difference in plant biomass under well-watered conditions, when microbial communities selected by the plants were not necessary for plant survival. Contrastingly, plants grown under severe drought stress, when microbial communities were needed for plant adaptation, showed the greatest difference in plant DW biomass between autoclaved and control soil conditions.

Xu et al. (2018), among other studies (e.g., Xu & Coleman-Derr 2019), have observed an increase in the relative abundance of monoderm, or gram-positive, bacterial species under

drought conditions. Although the mechanisms behind these shifts are still being studied, they have found evidence that this shift in microbial community composition is beneficial to plant performance (Xu et al. 2018). Additionally, a study looking into the microbiome of plants conditioned within a desert farming ecosystem showed increased abundances in bacteria exhibiting PGP traits that aid in greater drought tolerance, including the genera *Acinetobacter*, *Citrobacter*, *Achromobacter* and *Klebsiella* (Marasco et al. 2012). These studies indicate the possibility of plant-selection of rhizobacterial communities as a means to help mediate drought stress. The significant increase I observed in plant biomass following autoclaving, further supports a potential recruitment of taxa with the ability to benefit plant performance under drought conditions. These effects overtime could become evident in not autoclaved soils; however, due to the reduced microbial influence of native soil microbiota, I was not able to see these effects within a single generation. Furthermore, the greater differentiation between the autoclaved and control soils as the drought severity intensified, can be explained by the increased importance of these beneficial, plant-selected bacteria.

Host plant strategy utilized in altering functional capabilities of rhizobacterial communities

Root exudates released into the soil surrounding the plant roots have the ability to modulate community composition and function through the promotion or inhibition of different microbial community members (Chaparro et al. 2012; Veach et al. 2020). For example, Lorenz et al. (2006) drew correlations between the enzymatic activity with the resulting nutrient cycling functions of soils, and the microbial community membership shifts under heavy metal contaminated soils. Furthermore, plants can impact microbial community function directly as a result of horizontal gene transfer or specific signaling metabolites from host plants to soil

microbes (Guan et al. 2016; Li et al. 2016). It is unclear which is a more effective or efficient means of providing beneficial traits to plants (i.e. microbiome membership modification vs. microbiome functional adaptation), particularly under stressful conditions. As there often exists great functional redundancy in highly diverse soil communities (Griffiths et al. 2013), it can be hypothesized that altering the functional outcomes of soil microbes may be difficult regardless of the approach taken by the plant. Here, our findings show that microbial community membership is significantly different as an effect of water treatment, to a greater extent within autoclaved soils. These results indicate that host plants chose to modify microbial community membership, and with lower microbial complexity soils, were able to significantly impact plant performance under drought stress as a result. Therefore, our data show the potential for plants modifying microbial community composition as an indirect, but effective, way to change whole-community function for host plant benefits. Further research into the functional impacts of drought conditioned soil systems on the gene expression and enzymatic activities of soil microbes is needed to better understand the comparative effects of microbial modification strategies of plants.

Microbial History Study combines plant selection and microbial conditioning

Studies have shown the benefits of microbial transplants to increase a plant's ability to exhibit specific traits, including better stress defense strategies (Panke-Buisse 2015; Mayak et al. 2004). Microbial transplants are studies in which microbial communities, through soil slurries or soil transfers, are passed from one plant to another. Mayak et al. (2004) showed the efficacy of microbial transplants in transferring drought tolerance traits to otherwise drought susceptible crops. In addition to environmental conditions, rhizobacterial communities are dependent on a

number of factors, including crop type (Li et al. 2019). As previously mentioned, plants are able to mediate microbial community composition through shifts in root exudates, depending on different demands and environmental stimuli (Pascale et al. 2020). As a result, the increase in DW plant biomass that I observed when plants were given microbial inoculations previously conditioned for drought treatments, is an example of a microbial transplant conditioned for both a specific water treatment and crop type. Additionally, utilizing lower initial microbial complexity is a means to reveal crop- and stress-specific bacterial communities and therefore, my method of using soil slurries from the autoclaved treatment groups in the Sterilization Study allowed for the transplant of more highly plant-selected microbiomes, specific to the demands and root exudations of tomato plants under the 3 different water treatments.

Mimicking microbes favored plant performance under contemporary water conditions

I hypothesized that within the Microbial History Study, plants given microbial inoculations which mimicked their current, or contemporary, conditions would outperform plants given microbial inoculations different from their contemporary conditions. My results revealed that well-watered microbes showed a decreased ability to aid plants under contemporary drought conditions. Surprisingly, similar to the results from the Sterilization Study, the effects of the inoculation treatments were amplified as drought severity intensified. There were no differences in plant DW biomass for inoculation treatments under well-watered conditions (Figure 6). However, there was a significant difference between well-watered inoculation treatment groups compared to severe drought inoculations, for both contemporary drought conditions (Figure 6). This could be explained by a greater demand for microbial contributions to plant survival under drought stress.

Furthermore, previous studies indicate that plants and soils with prior exposure to drought conditions are better able to tolerate contemporary drought stress (Guerrero Zurita et al. 2020). In a study looking at evolution over generations, the soil microbiome was shown to adapt more quickly than the plant itself when conditioned for drought (Lau & Lennon 2012). Here, I observed the ability of the rhizosphere microbiome to adapt in a single generation in autoclaved soils, to develop a microbial community able to better assist tomato plants under drought conditions in a subsequent generation.

Bacterial taxa fluctuated in abundance dependent on water treatment

I identified specific bacterial taxa that fluctuated with water treatments. Differential Abundance Analyses were performed to identify taxa showing significant increases or decreases in abundance as the field capacity percentages lowered (Tables S1-S7). The aim here was to better understand the plant selection of these taxa when under differing water treatments.

In the Sterilization Study, *Oxalobacteraceae_unclassified* increased under severe drought conditions compared to well-watered, in the autoclaved soils (p-value = 6.4e-04). The *Oxalobacteraceae* family has been shown to increase in abundance in previous drought studies under increasing temperatures and drought severity (Xiong et al. 2014). Members of the *Oxalobacteraceae* family are known to provide a variety of functions to soil communities including oxalic acid metabolism, nitrogen fixation, phosphorus uptake and plant growth promotion (Carper et al. 2018; Baldani et al. 2014). Additionally, members of this family are known to have genes encoding for ACC deaminase production, which can lower stress ethylene levels and help plants to continue to grow under drought stressed conditions (Baldani et al. 2014). A study performed in Milpa ecosystems, in which maize is rain-fed without irrigation,

showed higher abundances in the rhizosphere soils of maize compared to bulk soils or soil alone, indicating plant selection of these bacteria under water limiting conditions (Aguirre-von-Wobeser et al. 2018). *Herbaspirillum seropedicae*, a member of the *Oxalobacteraceae* family, was successfully used as an inoculation to better assist common bean under drought conditions (Da Piedade Melo et al. 2017). Furthermore, members of the *Oxalobacteriaceae* family have been identified in suppressive soils, conditioned to aid plants in greater resistance to pathogenic attack, specifically to *Rhizoctonia* infection (Schillinger & Paulitz 2014; Yin et al. 2013). My findings are in congruence with these studies, suggesting that the presence of *Oxalobacteraceae_unclassified* may be beneficial to plant performance under severe drought stress.

Additionally, I found a trend of increasing abundances of *Nocardioidaceae_unclassified* under contemporary drought treatments for well-watered and severe drought inoculation types (Tables S4, S6 & S7). These results suggest plant-selection of this taxon under drought conditions. In a study performed by Conn et al. (2008), a member of the *Nocardioidaceae* family was identified as having a priming effect on plant stress defenses in *Arabidopsis thaliana,* resulting in greater defense response with inoculation. Furthermore, the order *Actinomycetales*, of which *Nocardioidaceae_unclassified* belongs, was an influential bacterial member isolated from the rhizosphere of drought-tolerant transgenic sugar cane (Zhao et al. 2020). At the phyla level, *Actinobacteria* have been identified in numerous studies as having beneficial impacts on drought tolerance and plant growth (Palaniyandi et al. 2013). The existing literature in combination with my findings suggest that *Nocardioidaceae_unclassified* may be a potential plant selected bacteria to aid in drought tolerance.

In the Sterilization Study, *Gemmatimonadaceae_Gemmatimonas* was found to increase in abundance under increasing soil moisture content, showing significantly increased abundances under well-watered conditions compared to severe drought conditions in autoclaved soils (Table S1). Furthermore, Differential Abundance Analyses for soil microbial communities from the Microbial History Study revealed a significant increase in abundance for (c: *Gemm-1*, o: *unclassified*) *unclassified_unclassified* under well-watered compared to severe drought contemporary conditions with the well-watered inoculation treatment (Table S3). These taxa both belong to the phylum *Gemmatimonadetes,* one of the most abundant phyla found in agricultural soils (DeBruyn et al. 2011). Upon further investigation, this phylum showed increased abundances following soil disturbance in the form of steam sterilization, similar to the process I used to sterilize my soils (Kim et al. 2013). Here I saw a similar increase following autoclaving under well-watered conditions (Figure 5). In the same study of Milpa ecosystems as mentioned above, Aguirre-von-Wobeser et al. found a decrease in the abundance of *Gemmatimonadetes* near plant roots, indicating the suppression of this taxa by the presence of plants in a drought conditioned environment (Aguirre-von-Wobeser et al. 2018). Furthermore, this taxon is known to decrease in soils with unfavorable conditions, including in the rhizosphere of tomatoes exhibiting tomato blight (Zhang et al. 2020). These findings are congruent with the initial colonization of this bacteria observed within my rhizosphere soil samples following sterilization. However, due to its inability to proliferate when faced with increasing drought severity and plant selectivity, the abundance of this taxa was significantly decreased in severe drought treatments.

Conclusions

My studies suggest increased plant-selection of rhizobacterial communities following soil autoclaving. This is seen in the increased sensitivity of microbial community composition to varying drought conditions. These shifts, in combination with increased plant biomass, indicate the presence of drought specific bacterial recruitment to assist tomato plants under stressful conditions. To further reveal the impact of these microbial communities, my second study showed greater plant performance with previously conditioned microbes. Therefore, bacterial communities cultivated under autoclaved soils may have been selected based on the plants' needs under different water conditions. Here I have shown some insight into the benefits of plant-mediated microbial recruitment. Further research into the resulting bacterial communities, over generations of drought-specific selection, need to be performed to better understand the capabilities of plants in selecting for defensive microbial communities under drought.

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CHAPTER 3: CONDITIONED SOILS REVEAL PLANT-SELECTED MICROBIAL COMMUNITIES THAT IMPACT PLANT DROUGHT RESPONSE

Summary

Rhizobacterial communities can contribute to plant trait expression and performance, including plant tolerance against abiotic stresses such as drought. The conditioning of microbial communities related to disease resistance over generations has been shown to develop suppressive soils which aid in plant defense responses. Here, I applied this concept for the development of drought resistant soils. I hypothesized that soils conditioned under severe drought stress and tomato cultivation over generations, will allow for plant selection of rhizobacterial communities that provide plants with improved drought resistant traits. Autoclaved soils were used as a tool to lower microbial community complexity to determine plant-selection of rhizobacterial members. A slurry was used to condition soils for tomato growth under severe drought stress over two generations. The initial soil slurry used in the first generation, was obtained in a previous study, derived from autoclaved soils under identical conditions. Surprisingly, the plants with the conditioned microbial inoculant showed significantly decreased plant biomass in both generations. Additionally, the microbial communities within these generations were significantly different in community composition when comparing soils from inoculation treatment groups across generations (i.e., conditioning effect), as well as when comparing inoculated and control soils within each generation (i.e., microbial history effect). These findings indicate a significant effect of conditioning and microbial history on the resulting microbiome of tomato plants undergoing drought stress. A few bacterial taxa which showed increased abundances under inoculation treatments, and

corresponding decreases in plant biomass, were members of the *Phyllobacteriaceae* and *Oxalobacteraceae* families. Additionally, the *Phyllobacteriaceae*, *Nocardioidaceae* and *Oxalobacteraceae* families showed significant increases as a result of drought conditioning over generations. These taxa have been previously reported as important rhizobacterial community members aiding in drought tolerance of different plant species. Our results, in combination with these other studies, indicate a potential drought avoidance strategy in which recruited microbial communities restrict the size of a plant in order to better deal with limited water resources.

Introduction

Plant-mediated rhizobacterial selection in soils is well documented (Rolfe et al. 2019; Chaparro et al. 2012; Pineda et al. 2019; DiLegge et al. 2020). This selective pressure is regulated by root exudates of plants which can alter the microbial community composition within rhizosphere soils (Chaparro et al. 2012). Root exudation profiles and subsequent microbial community assemblage are regulated by genotype, among other factors. DiLegge et al. (2020), showed several crop-specific bacterial taxa when analyzing the rhizosphere soil communities of 4 different crop species under identical growing conditions. Additionally, these exudates can shift as a result of plant developmental stages, exposure to various stress conditions, and nutrient demands (Chaparro et al. 2014). For example, in a study performed by Santos-Medellin et al. (2020), microbial community shifts were observed under drought stressed conditions in cultivars of rice. The soil samples analyzed were from bulk soil, endophytic and rhizospheric communities. The rhizosphere bacterial communities showed the greatest change in microbial membership under drought conditions, indicating stronger plant influence on soils closest to the plant (Santos-Medellin et al. 2020). Although plants can modulate the microbial

communities colonizing the rhizosphere, the microbial community structure is generally a combined influence of plant and native soil microbiota (Liu et al. 2019). Therefore, decreasing microbial community complexity of soils has been shown to amplify the effects of plant selection on rhizobacterial communities. A study examined the microbial community composition of date palm roots, grown in different areas of the Sahara Desert (Mosqueira et al. 2019). Despite the heterogeneity of the plant sites, the results showed similar trends in microbial communities of date palm roots across experimental plots. These results indicate greater plant influence on rhizobacterial selection, due to the decreased microbial complexity of the desert soils (Mosqueira et al. 2019). Other studies have looked into the artificial removal of native soil microbiota, which resulted in increased crop- or condition-specific microbial community recruitment (DiLegge et al. 2020, Pineda et al. 2019). Additionally, Li et al. (2019) examined the effects of autoclaving on the soil microbiome, identifying significant shifts in community composition with the presence of crops compared to soil alone conditions, indicating plant-mediated microbial recruitment.

Utilizing this knowledge of plant-selected rhizobacteria, researchers have investigated the ability of plant-mediated soil microbial communities to impact plant health, performance and phenotypic traits (Pascale et al. 2020). Conditioning soil communities is a way to amplify the existing microbial community for a specific plant response. For example, Panke-Buisse et al. (2015) conditioned microbial inoculants for early and late flowering in *Arabidopsis thaliana* over 10 generations. Following the tenth generation, the inoculant was used on 4 different genotypes, all of which showed significant shifts in flowering time as a result of the inoculation treatment (Panke-Buisse et al. 2015). Furthermore, plants can condition their own soils in response to particular stress exposures. Plant modulation of rhizobacterial communities as an adaptive

strategy to deal with stress conditions can be referred to as the "cry for help" hypothesis (Rolfe et al. 2019), in which plants recruit the bacterial communities needed to benefit the plant under a particular stress. This phenomenon has been observed for biotic and abiotic stresses, including soil communities aiding in pathogen and herbivory resistance (Rolfe et al. 2019; Wang et al. 2018).

Suppressive soils are a well-known example of the effects of conditioned microbial communities on plant health and performance under biotic stressors (Schlatter et al. 2017). These adaptive soils have two generally accepted types of suppression: specific and general (Schlatter et al. 2017; Gomez et al. 2017; Rolfe et al. 2019). Within both types of suppression, a microbially-mediated plant defense against soil pathogens is induced by the recruitment of particular beneficial microbes. General suppression is a whole community response, in which, native rhizobacteria outcompete the pathogen for available resources creating low levels of protection against a variety of different pathogens (Gomez et al. 2017). This type of suppression is often correlated to increased microbial biomass and can be strengthened by amending soils with additional organic matter (Mousa & Raizada et al. 2016). Although it is not transferrable, it can still be conditioned overtime to benefit crops in an infected field (Schlatter et al. 2017; Mousa & Raizada et al. 2016). Specific suppression, however, is due to key players in the microbial community that are increased by root exudates from infected plants (Gomez et al. 2017; Schlatter et al. 2017; Mousa & Raizada et al. 2016). These specific taxa protect the plant from infection through directly damaging the pathogen or indirectly inducing plant defense responses. A well-known example of specific suppression happens in soils of wheat and barley monocultures. A phenomenon called Take-all decline (TAD), is a conditioned response of soil communities to continued exposure to Take-all disease, caused by *Gaeumannomyces*

graminis var. *tritici*. Plants infected with *Gaeumannomyces graminis* var. *tritici* have shown a consistent trend of plant infection for several years followed by sudden plant resistance after continued monocropping of wheat and barley (Gomez et al. 2017; Schlatter et al. 2017; Mousa & Raizada et al. 2016). Recently, it was identified that the bacteria responsible for this suppr*ession was* 2,4-diacetylphloroglucinol (2,4-DAPG)-producing *Pseudomonas fluorescens* (Kwak & Weller 2013; Schlatter et al. 2017). Interestingly, in TAD suppressive soil systems, plant susceptibility returns when a crop is planted during the conditioning generations that is not susceptible to the take-all pathogen, including oats and alfalfa (Raaijmakers & Weller 1998). These findings show plants' abilities to select for beneficial microbial communities as a successful stress defense strategy.

Suppressive soils have led to many other recent studies looking into the conditioning of soil microbial communities as an adaptive strategy for crop resilience to other biotic and abiotic stresses (Wang et al. 2018; Lau & Lennon 2012; Pineda et al. 2020). Here, I used the combined understanding of suppressive soils, transferrable microbial inoculants, and artificially lowering the complexity of soils to propose the conditioning of plant-mediated, drought-resistant soils. I reasoned that through generational conditioning and amplified plant influence of drought-specific microbial communities I could reveal plant-chosen microbial taxa which benefit plant health and performance under severe drought conditions.

Methods

Soil Collection

Soil was collected in June of 2020, from a USDA-certified organic cover crop field (Agricultural Research, Development and Education Center [ARDEC] South, Specialty Crops

program, Fort Collins, CO), most recently having grown peppers and melons. Bulk soil was collected as well as rhizosphere soils shaken from the roots of melon and pepper plants. Soils were sifted through a No. 10 metal sieve (2 mm wide). Following sieving, half of the soil was exposed to steam sterilization using a STERIS brand autoclave for three 40-minute liquid cycles at 121 ºC and is referred to as autoclaved soils. Autoclaving of soils was used to lower initial microbial complexity and abundances of soil microbial communities. This allowed us to observe the effects of decreased microbial competition on rhizobacterial recruitment and plant performance under severe drought stress. The remaining soil that was not autoclaved and is referred to as not autoclaved soils. Both autoclaved and not autoclaved soils were dried out in the greenhouse prior to weighing and filling pots.

Experimental Design

Tomato seeds (*Solanum lycopersicum* L.) were surface sterilized with 3.0% NaClO, rinsed three times with sterile water, and imbibed in sterile water for 24 hours prior to planting. Seeds were planted in sterile full-strength MS media in petri dishes. Seeds were placed in a growth chamber for 11 days, allowing for germination and root and shoot emergence. After 11 days, seeds were transplanted into plastic pots filled with 350g of dry autoclaved or not autoclaved soils. For each soil treatment, plants were grown under severe drought (SD) conditions at 55% Field Capacity for 3 weeks. This was a two-generation study in order to determine the potential impacts of a conditioned drought stress-specific microbiome.

Each generation had 4 treatments groups, including an inoculated and control treatment for both autoclaved and not autoclaved soil (with 7 pots per treatment: total n = 28). Soil slurries for inoculants were created using the method from Panke-Buisse, et al. (2015). Rhizosphere soils

(23.33g) from the top 3 performers in the severe drought inoculation treatment under contemporary severe drought conditions from the Microbial History Study (Chapter 2) were pooled to create a total of 70g of rhizosphere soil. Performance was determined by plant fresh weight biomass, root:shoot ratio and height. The second generation had inoculants from generation 1. Rhizosphere soils were created from the top 3 performers of each inoculation treatment and the same soil slurry process was used as previously mentioned. Slurries were inoculated 3 days after transplanting for both generations.

Inoculation treatments were used to determine the impact of microbial history on plant performance and resulting microbial communities. Analyzing the results over generations allowed us to determine trends in plant performance and microbiome composition related to conditioning effects.

Drought Conditions

Seedlings were watered regularly for 4 days following transplanting to allow plants to successfully establish prior to drought stress. Induction of drought was 5 days after transplanting. Plants were grown under severe drought conditions based on the field capacity of the soil. Percent moisture was determined using the moisture tension method at Colorado State University's Soil, Water and Plant Testing Laboratory. The field capacity percentages were calculated based on weight, using 55% for severe drought (SD) conditions. Three random pots from each treatment were weighed daily between 2:00PM and 3:00PM. The average weight was used to determine the amount of water needed to maintain the 55% field capacity. The weight of the soil and pot were known and included in calculations to replace the water lost by transpiration and evaporation. At week 2, plants were lined up by size within each treatment and

a replicate visually closest to the average size for each treatment was chosen. The chosen plants were harvested and the fresh weight above and belowground biomass was measured. This number was used in future measurements to compensate for the weight of the plant in field capacity calculations. The experiment was conducted at CSU's Horticulture Center Greenhouse Facility.

Plant Data Collection

Drought was induced for 3 weeks for each generation, after which plants were harvested. Relative Water Content (RWC) was measured according to Yuan et al. (2010). Three randomly selected leaves from each plant were submerged in Milli-Q water for 24 hours. Relative water content (RWC) was calculated in plants as follows: (Fresh weight – Dry weight)/(Turgid weight – Dry weight) × 100 (Ortiz et al. 2015). On the same day, height was measured, plants were cut at the root-shoot axis and fresh-weight measurements were taken. Rhizosphere soils were collected for each plant by gently shaking soils off roots and storing in Ziploc bags. Above- and below-ground plant parts were placed in paper bags and dried in an oven for 72 hrs at 65 ºC. Dry-weight measurements were taken following drying.

A one-way and two-way ANOVA were performed using R Studio (Version 1.2.5033) to analyze plant height, RWC, DW biomass, root length and root area in both studies.

Soil DNA Extraction

Genomic DNA (gDNA) was isolated from rhizosphere soil samples. Five rhizosphere soils were used for 5 total gDNA samples per treatment. Samples were extracted using Qiagen DNeasy PowerSoil Kits, according to the manufacturer's instructions. Nucleic acid concentration

and sample purity were quantified and determined via the use of a NanoDrop 2000 Spectrophotometer (Thermofischer). DNA samples were then stored at –80 ºC prior to Illumina MiSeq library preparation and downstream microbiome analyses.

Library Preparation for Illumina MiSeq Sequencing

Initial soil gDNA samples were diluted 1:20 with molecular water to reduce PCR inhibitors introduced during DNA extraction. Quantitative PCR targeting the V3-V4 region of the bacterial 16S rRNA gene was performed using a modified version of primer set 341F/ 785R (341F: 5′–*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*CCTACGGGAGGCAGCAG-3′. 785R: 5′-*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*GACTACHVGGGTATCTAATCC-3′) to target bacterial 16S rRNA and to attach Illumina MiSeq adapters, denoted in italics in the above primer sequences (Klindworth et al. 2013). This qPCR reaction was performed in 20 uL reaction volumes containing 2 uL of template DNA and 18 uL of the master mix. The master mix consisted of 10 uL 2X Maxima SYBR Green (Thermo Scientific, Waltham, MA, USA), and 2 uL each (10 uM) of forward and reverse primers and brought to a total volume of 18 uL using 4 uL of molecular grade water. The PCR thermal cycling conditions were as follows: 95ºC for 5 minutes, 35 amplification cycles (94ºC for 15 seconds, 55ºC for 30 seconds, 72ºC for 60 seconds) followed by a final annealing stage at 72 ºC for 5 minutes to reduce chimeric reads. A standard curve using purified *Psuedomonas putida* KT2440 gDNA was run with the samples to quantify the starting rRNA copies per g^{-1} soil. Resulting amplicons were then purified using an in-house preparation of solid phase reversible immobilization (SPRI) magnetic beads based on a

modified protocol of Faircloth and Glenn (2011) and original protocol of Rohland and Reich (2012).

A second PCR cycle was then conducted to attach unique Illumina Nextera XT indices to each bead cleaned sample for subsequent sample demultiplexing. Each well contained 5 uL of first round and bead-cleaned qPCR product, 25 uL of 2X Maxima SYBR Green (Thermo Scientific, Waltham, MA, USA), 5 uL each of both forward and reverse indices were combined along with 10 uL of water, bringing the total volume to 50 uL. PCR conditions were as follows: 95ºC for 3 minutes, 8 amplification cycles (95ºC for 30 seconds, 55ºC for 30 seconds and 72ºC for 30 seconds) followed by final annealing of 72ºC hold for 5 minutes. The resulting PCR product was again SPRI-bead cleaned using the same methods previously mentioned. Amplicons were then quantified using a Qubit fluorometer (Thermo Scientific, Waltham, MA, USA) prior to normalization and pooling. The final pool was run on a TapeStation system (Agilent Technologies, Santa Clara, CA, USA) to determine size and purity of amplicons, and Kapa Biosystems (Sigma-Aldrich, St Louis, MO, USA) qPCR was performed according to the manufacturers' instructions to determine concentration. The final pooled sample was diluted to 4 nM and the DNA library was denatured with 0.2 N NaOH, diluted to 10 pM using provided HT1 buffer, and spiked with 20% PhiX library standard diversity-control. Illumina's MiSeq v3 600-cycle Reagent Kit (Illumina, San Diego, USA) was used for library dilution and loading onto the MiSeq at CSU's Next Generation Sequencing Laboratory (Fort Collins, CO).

Bacterial 16S rRNA gene sequence analysis

De-multiplexed raw fastq files were processed with the DADA2 pipeline using R Studio's Bioconductor packages (Callahan et al. 2016). Briefly, all primers were removed from

each sequence using the open source Python program Cutadapt (Martin et al. 2011) and amplicon sequence variants were inferred using the default pipeline in DADA2. Each sequence variant identified in DADA2 was classified to the closest reference sequence contained within the Green Genes 13_5_99 reference database. Each taxonomic profile assigned was used to determine bacterial genus and species-level relative abundance values. Downstream analyses were conducted using R Studio's phyloseq package (McMurdie and Holmes 2013) or myPhyloDB (v 1.2.0) (Manter et al. 2013). Samples were rarified at a cutoff of 5000 reads using myphyloDB prior to downstream analysis applications using myphyloDB or R Studio. All samples met rarefaction criteria and no samples were removed from downstream analyses. Measurements of α-diversity assigned to treatments were determined using the Shannon diversity index and observed richness. A two-way ANOVA was used to compare mean α-diversity values of inoculated and control treatment groups in autoclaved soils. Values from the Bray-Curtis dissimilarity index were calculated in myPhyloDB and used to quantify differences in microbial community structure between samples from different treatments. The myPhyloDB software was then used to visually represent distances using principal coordinates analyses (PCoA). A complementary non-parametric multivariate statistical test, a permutational analysis of variance (perMANOVA), and differential abundance analyses (FDR < 0.1) were performed to determine differences in microbial communities between treatments (Manter et al., 2016).

Results

1. Autoclaved soil studies

1.1. Plant DW biomass differences as a result of inoculation with historically conditioned microbes

Plant dry weight measurements (DW) were taken for below and above ground biomass. I performed a two-way ANOVA to analyze differences in mean total DW between treatment groups. Inoculation treatments were compared under autoclaved soil conditions within each generation to determine the impacts of microbial history. Inoculations were created from soil slurries containing microbial communities conditioned for severe drought stress, thereby, mimicking the plants' contemporary conditions. In both generations 1 and 2, plants grown in autoclaved soils with an inoculation showed a significant decrease in plant DW biomass compared to those grown without inoculation (Figure 7). The percent change in biomass from the control to the inoculated treatment groups for generation 1 and 2 were -33.68% and -30.41%, respectively. The results showed no significant difference in DW biomass as a result of conditioning effects, as seen by comparing the inoculated treatment group from both generations (Figure 7).

A two-way ANOVA was performed to analyze differences in above- and belowground DW biomass between treatments within each generation. Belowground DW biomass showed no significant difference between inoculated and control treatments for either generation. Interestingly, aboveground DW biomass significantly decreased with inoculation for both generation 1 (p-value = 0.0397) and generation 2 (p-value = 0.0319).

It is important to note that beyond size, there were no visual differences between plants given inoculations as compared to those in the control treatments for each generation.

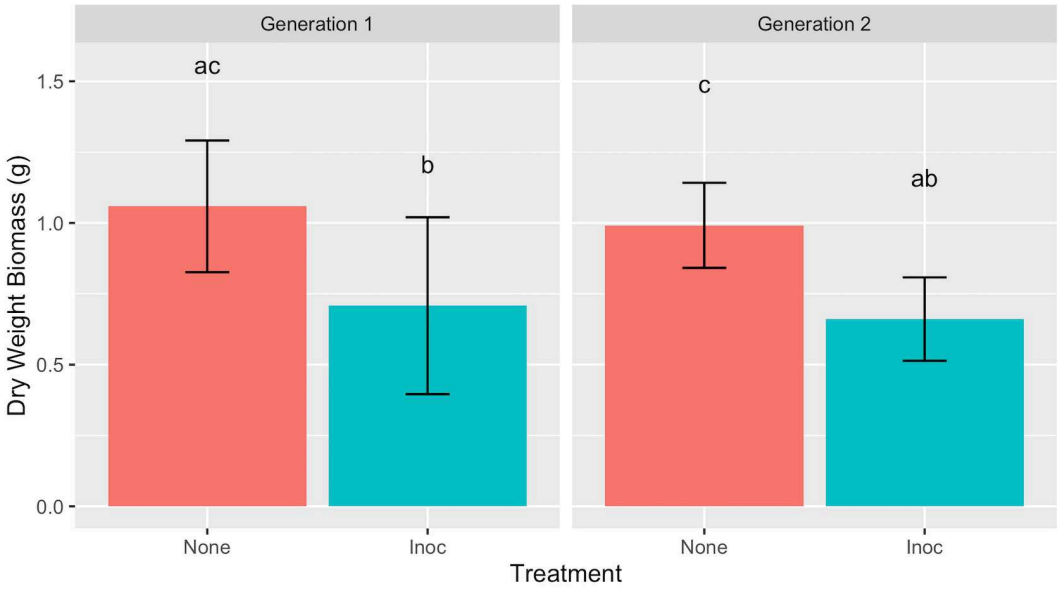


Figure 7. Mean plant DW biomass of tomatoes under inoculation and control treatments for each generation. Red bars represent control treatments with no inoculation. Blue bars represent inoculated treatments. Different letters indicate significant differences at $P < 0.05$.

1.2. Effects of inoculation treatment on alpha diversity of microbial communities

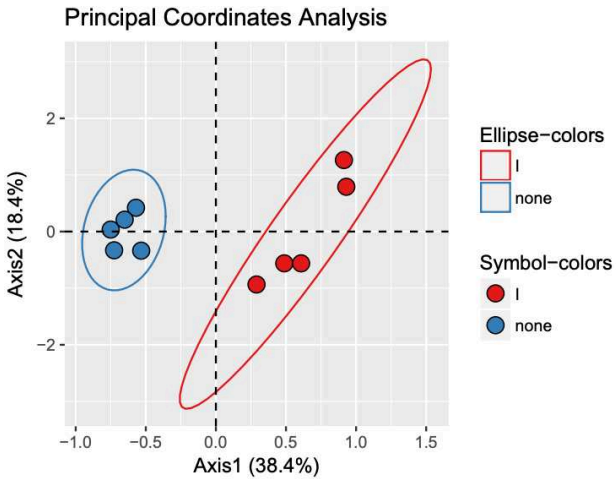
Alpha diversity was calculated, to the OTU level, using the Shannon Diversity index and the observed richness values. Here I utilized both units of measurement to better analyze changes in the microbial communities, as a result of inoculation. A two-way ANOVA was performed using the values calculated from both indexes. The results showed no significant difference in alpha diversity between inoculated and control treatments, for either measure of alpha diversity (Figure S2; Figure S3). These results indicate that, despite shifts at the whole community and taxa level for microbial community structure as a result of inoculation, the alpha diversity values were not affected.

1.3. Effects of microbial history on rhizobacterial communities

1.3.1. Microbial community differentiation as an effect of microbial history

Two Principal Coordinate Analyses (PCoA) were performed to visually represent differences between inoculation and control treatments groups within each generation. Analyzing differences between treatment groups within a given generation revealed the effects of microbial history on the resulting microbial communities. I observed a significant shift in microbial community structure with inoculation as compared to control treatments for both generation 1 and generation 2 (Figure 8). A perMANOVA, using Bray-Curtis distance matrices at the OTU level, was used to determine significance of microbial shifts. In generation 1, there was a significant difference between the rhizobacterial community resulting from the inoculated treatment as compared to the control (p-value = 0.005). Furthermore, there was a significant difference between microbial communities when comparing the inoculated and control groups within generation 2 (p-value = 0.005). These results indicate a significant impact of microbial history on resulting rhizobacterial communities.

(A) Generation 1



(B) Generation 2

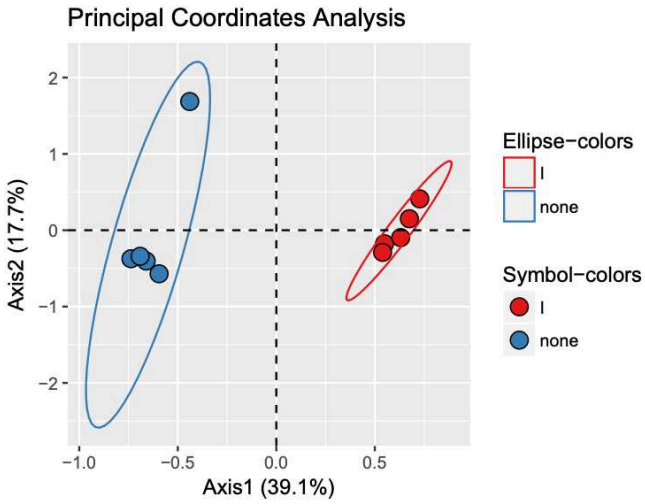


Figure 8. Principal coordinates analysis (PCoA), using Bray-Curtis distances, representing rhizobacterial communities of soil samples from inoculated and control treatments (n=5 soil samples per water treatment) within generation 1 (A) and generation 2 (B). Red and blue represent circles inoculated (I) and control (none) soils, respectively.

1.3.2. Mimicking microbial history of soils affects genus-level microbial community abundances

Differential Abundance Analyses were performed to determine taxa, to the genus-level, that significantly differed as a result of microbial inoculation. Genera that showed significantly different abundances in inoculated compared to control soils were identified for generation 1 (Table S8) and generation 2 (Table S9). The fluctuations in abundance of these taxa can be attributed to changes in the microbial history of the rhizosphere soils. Among these differing taxa, *Xanthomonadaceae_unclassified* showed a significant increase in inoculation treatment within both generations, indicating potential plant selection for this bacterium under severe drought conditions. Alternatively, *Chthoniobacteraceae_unclassified* showed a significant decrease in inoculated soils as compared to control soils within each generation. These results show that this taxon is consistently restricted in abundance when given an inoculant with microbes historically conditioned for severe drought stress, suggesting that this bacterium is not selected by plants under drought stress.

1.4. Effects of conditioning on rhizobacterial communities

1.4.1. Microbial community differentiation of microbiomes conditioned under severe drought over generations

A Principal Coordinate Analysis (PCoA) was performed to visually compare microbial communities from each generation of severe drought conditioning, including the microbial composition from the initial soil slurry community (i.e., generational foundation [GF]) (Figure 9). This analysis identified the impact of conditioning soil communities over generations under a given condition and specific crop-type. The PCoA revealed significantly different microbial communities from soil samples resulting from each generation. A permutational analysis of variance (perMANOVA), using Bray-Curtis distance matrices at the OTU level, was used to verify significant differences visually observed. These data showed significant community shifts of rhizobacterial communities as an effect of generation (p-value= 0.001). These findings suggest an impact of conditioning soils on microbial community composition with each additional planting.

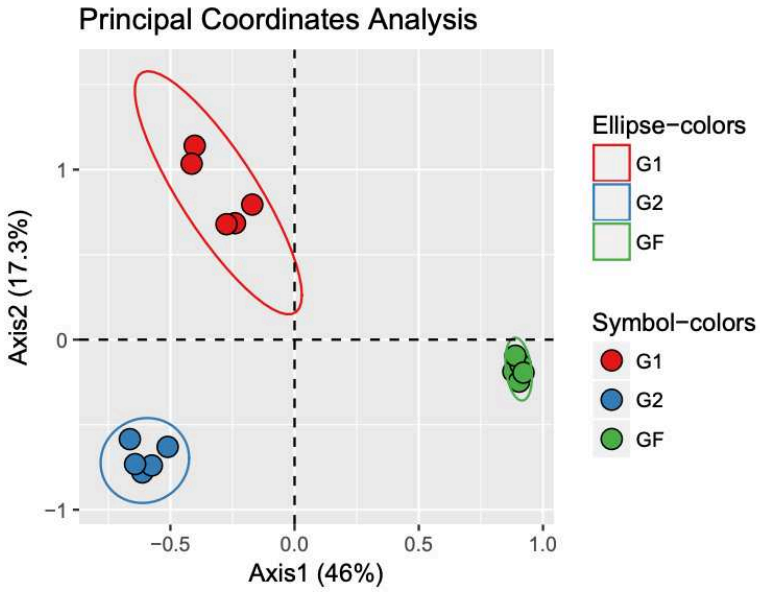


Figure 9. Principal coordinates analysis (PCoA), using Bray-Curtis distances, representing rhizobacterial communities of soil samples from inoculated treatments (n=5 soil samples per water treatment) from generation 1 (G1), generation 2 (G2) and the initial soil slurry (GF). Green, red and blue circles represent GF, G1 and G2, respectively.

1.4.2. Effects of severe drought conditioning on genus-level microbial community abundances

Differential Abundance Analyses were performed to determine taxa, to the genus-level, that significantly differed in abundance between treatment groups. To best identify key players in the conditioning of soil microbial communities under severe drought stress over generations, comparisons were made between inoculation treatments from the initial soil slurry, generation 1 and generation 2. In this way, I was able to identify genera that were increased or decreased in abundance with continued exposure to severe drought conditions. Genera which significantly differed in abundance between inoculated soils from generation 1 and generation 2 were identified (Table S10). Additionally, taxa observed to significantly change in abundance from the initial soil slurry as compared to the inoculated soils from generation 1 or generation 2 were recorded (Table S11; Table S12). Genera which showed increasing trends with generational

conditioning of soils were identified. These taxa showed significant increases in abundances for at least two of the previously mentioned comparisons between inoculation groups from differing generations (Table 1). Additionally, all of these taxa were observed to show significant increases in abundance in generation 2 inoculated treatment as compared to the initial soil slurry microbiome. The shifts in abundance of these taxa suggest that these genera are selected for by tomato plants under severe drought conditions over continued exposure.

Table 1. Bacterial taxa, to the genus level, which showed differing abundances when comparing inoculated treatment groups. GF signifies microbial community membership from the initial soil slurry used to inoculate G1. Green boxes and red boxes represent significant increases and decreases, respectively. Gray boxes represent no significant differences in abundance between the two treatment groups. The two generational treatments used in a comparison are identified at the top of each column.

Family_Genus	GF to G1	G1 to G2	GF to G2
Nocardioidaceae_Aeromicrobium			
Nocardioidaceae_Pimelobacter			
Phyllobacteriaceae_Chelativorans			
Phyllobacteriaceae_Aminobacter			
Oxalobacteraceae_Janthinobacterium			
Oxalobacteraceae_unclassified			

2. Not autoclaved soil studies

2.1. Comparative effects of inoculation on plant performance in autoclaved and not autoclaved soils

Plant DW biomass measurements were taken and mean total DW biomass was compared between treatment groups within autoclaved and not autoclaved soil conditions. I compared the differences in DW biomass for plants with inoculation under autoclaved and not-autoclaved conditions within each generation (Table 2). These comparisons provided insight into the inoculation's ability to impact plant performance under varying degrees of contemporary microbial complexity. As previously mentioned, inoculation treatments for autoclaved soils in both generations showed a significant decrease in plant biomass (Figure 6). Interestingly, in not autoclaved soils, the inoculation treatment showed no significant difference in plant biomass compared to the control group for either generation (Table 2). Although neither result was significant there was a noted difference in the percent change between the inoculated group and the control group for the two generations. Generation 1 showed a slight increase from the control to the inoculated treatment group with a percent change of +11.11% (p-value= 0.893). Generation 2 showed a slight decrease from the control to the inoculated treatment group with a percent change of -5.75% (p-value= 0.903). These changes could indicate a potential lag in the effect of the inoculation when microbial complexity is high.

Table 2. Mean DW biomass for tomato plants under different treatment groups within each generation. Control treatments were plants without inoculation. Percent change is the difference in mean DW biomass between control and inoculated treatments within each generation and soil type.

	Autoclaved Soils			Not Autoclaved Soils		
	Control	Inoculated	Percent Change	Control	Inoculated	Percent Change
Generation 1	1.06	0.71	-33.02%	0.36	0.4	+11.11%
Generation 2	0.99	0.66	-33.33%	0.87	0.82	-5.75%

2.2. Conditioning effects of microbial community composition within not autoclaved soils

Soils that were not autoclaved showed no significant shifts in microbial community composition as a result of conditioning. A Principal Coordinate Analysis (PCoA) was performed to visually compare microbial communities from inoculated and control soil treatments within each generation. This allowed us to observe differences in resulting rhizobacterial communities as a result of inoculation of previously conditioned microbes, within a high complexity soil environment. A permutational analysis of variance (perMANOVA), using Bray-Curtis distance matrices at the OTU level, showed no significant differences between inoculated and not inoculated treatments for either generation. However, there was a visual increase in microbiome differentiation between generation 2 treatment groups as compared to the overlapping microbial communities from generation 1 treatment groups (Figure S4).

2.3. Effects of conditioning and microbial history on genus-level microbial community abundances

I analyzed the bacterial abundances of two taxa of interest within not autoclaved soil treatments, *Phyllobacteriaceae_Aminobacter* and *Phyllobacteriaceae_Chelativorans*, to determine differences in rhizobacterial recruitment as a result of higher soil microbial complexity. I performed differential abundance analyses to identify abundance changes as an effect of microbial history and soil conditioning. In contrast to autoclaved soil conditions, there were no significant differences in abundance observed for either bacteria within not autoclaved soils. However, there were slight differences in abundance when comparing different treatment groups (Table S13).

Discussion

Plant selected microbial communities aid in drought avoidance strategy

I found that tomato plants, given microbial inoculants conditioned for monocultured tomato plants under severe drought stress, showed significantly decreased DW plant biomass for both generations (Figure 7). Furthermore, when broken down by above- and belowground biomass measurements, the significant decrease was seen in aboveground biomass only, with no significant differences shown for belowground biomass. These results suggest an adaptation of the microbial community inoculated with conditioned soils to restrict plant vegetative growth. Our findings suggest that decreased plant biomass may be an alternative adaptive strategy for plants to better deal with drought stress.

There are three known strategies plants use in dealing with drought stress: drought tolerance, drought escape and drought avoidance (Ngumbi & Kloepper 2016; Khan et al. 2018; Kooyers 2015). Drought tolerance is manifested by the plant's normal growth and function, despite drought exposure (Ngumbi & Kloepper 2016). Drought escape is a tactic used by plants in which drought stress triggers rapid development to reach reproductive stages before the onset of harsher conditions (Lakshmi et al. 2018; Kooyers 2015). Drought avoidance, sometimes called phenotypic flexibility, is a strategy in which plants change their morphology or physiology in order to maintain water status in certain organs. This strategy can result in a wide range of altered plant traits including changes to stomatal rates or abundance, slowed plant growth or decreased leaf area and size (Shavrukov et al. 2017). The decreased plant biomass I observed, as a result of microbial inoculation, suggests that the microbial community may be employing a drought avoidance strategy to better help the host plant deal with the severe drought stress.

In a study performed by Bresson et al. (2013), a PGPR strain, *Phyllobacterium brassicacearum* STM196, was inoculated into soil communities of *Arabidopsis* plants. Plants

exposed to water deficit conditions and given this inoculation, were shown to have delayed growth rates compared to plants under water deficit without the inoculation. Interestingly, the plants with the PGPR inoculant showed greater plant biomass at the time of bolting, despite reduced vegetative growth leading up to bolting stage. These findings suggest that *Phyllobacterium brassicacearum* STM196 restricted the plant's rate of development and subsequently, prolonged vegetative growth, allowing the plant to accumulate greater biomass over a longer period of time (Bresson et al. 2013). Similar to these findings, I observed decreased plant growth as a result of microbial inoculation under severe drought stress. Additionally, our microbial analysis identified *Phyllobacteriaceae_Aminobacter* and *Phyllobacteriaceae_Chelativorans*, two other members of the *Phyllobacteriaceae* family, as increasing with soil conditioning and microbial inoculation. These two taxa showed significantly increased abundances under inoculated treatment as compared to control soils in generation 2 (Table S9). Abundances also increased with conditioning from generation 1 to generation 2 as well as overall conditioning effect from the initial soil slurry to generation 2 (Table 2). Further research is needed to understand the effects of these specific taxa on tomato plant morphology and the final biomass measurements at flowering or yield, however, our results indicate that these taxa may be key drivers in the reduced plant biomass accumulation as a result of inoculation.

Soil conditioning affects plant performance and rhizobacterial community composition

Conditioning soils for tomato planting under severe drought treatment led to significant effects on microbial community assemblage. I observed significant microbial community shifts following each generation of conditioning, beginning with the initial soil slurry inoculant (Figure

4). These effects were seen on the whole community level and in specific bacterial abundances. These shifts were expected, as soils exposed to consistent plant types and environmental conditions over generations have been shown to alter microbial communities of plants (Schlatter et al. 2017). Furthermore, I observed changes in plant DW biomass as a result of conditioned soils (Figure 7). As mentioned above, I suggest that the observed decrease in biomass may be indicative of increased drought resilience by reducing growth rates of host plants. The changes in microbial community membership and plant phenotype are reminiscent of studies observing suppressive soils. In these studies, monocultured crops under biotic stress are able to alter microbial community composition in a way that eventually results in resistance to pathogenic attack (Schlatter et al. 2017; Gomez et al. 2017; Rolfe et al. 2019). Researchers have begun to investigate similar conditioned responses of soils for drought resistance, including a study performed by Lau and Lennon in which it was observed phenotypic changes in plants grown in soils conditioned under drought stress (Wang et al. 2018; Lau & Lennon 2012). This study, in combination with more recent findings indicating prior drought exposure as a benefit for plant resistance to contemporary drought conditions, suggests that conditioned soils have the potential to develop resistance against drought (Guerrero-Zurita et al. 2020). The effects of conditioning observed in our study further these findings by identifying specific taxa related to these microbial and phenotypic changes.

Soil conditioning may alter plant drought strategy

As previously discussed, the microbial communities from the initial soil slurry were significantly different in composition as compared to those from the following two generations, indicating a significant effect of conditioning. In addition to microbiome composition, the

resulting impacts on plant performance also showed differences when comparing plants grown in the earlier generations of the soil slurry development (Chapter 2) as compared to inoculated plants grown in generations 1 and 2 of this study. Initially, there was a significant increase in plant DW biomass as a result of autoclaved soils under severe drought stress (Sterilization Study, Chapter 2). There was a similar trend with significantly greater plant DW biomass under contemporary severe drought conditions when plants were given a microbial inoculant conditioned for severe drought stress, as compared to plants given a microbial inoculant conditioned for well-watered treatments (Microbial History Study, Chapter 2). These findings suggested that the microbial communities revealed through autoclaving and previous exposure to drought conditions, provided drought tolerant traits for plants to continue to grow at a higher rate under drought stressed conditions. Conversely, in this study, our findings suggest that the microbial communities conditioned over generations have contributed to a drought avoidance trait for the plant by slowing the rate of growth for host plants. Despite both studies utilizing the same severe drought conditions, measured at 50% field capacity, the function of the microbial communities may have been altered with continued conditioning time.

Our findings indicate a potential shift in functional traits of the microbial communities within the inoculation treatment, as a result of increased exposure to severe drought stress. Although inoculated plants showed a decrease in biomass when microbes were conditioned over generations, this may be the result of greater adaptation for the host plants. Similarly, a meta-analysis performed by Li et al. (2021), showed that in contrast to popular breeding strategies for drought tolerant traits in wheat cultivars, wild cultivars with greater drought avoidance strategies, actually showed greater yields and resulting aboveground biomass under severe drought conditions. However, the domesticated cultivars bred for drought tolerant traits showed greater

success under less severe drought stressed conditions (Li et al. 2021). This study, in combination with our findings, suggest drought strategies may benefit plants differently dependent on the intensity of drought stress. Additionally, our study provides support for the functional adaptation of microbial communities over extended conditioning periods for more successful plant performance under severe drought stress. Further research is needed to investigate these microbial changes as a result of different conditioning lengths and different degrees of drought stress to better understand how microbial communities may adapt over generational growing systems.

Genus level impacts of conditioning and microbial history lead to drought-specific microbiome

I observed significant differences in bacterial abundances as a result of conditioning and microbial history effects. Conditioning effects are seen when comparing microbial communities over generations of soils exposed to severe drought and tomato plants; microbial history effects are those differences observed within a given generation as a result of soil inoculant. Notable taxa, to the genus level, that were significantly impacted by microbial history were *(O: Gemmatimonadales) unclassified*, *Bryobacteraceae_unclassified*, *Chthoniobacteraceae_unclassified* and *Xanthomonadaceae_unclassified*. *(O: Gemmatimonadales) unclassified and Chthoniobacteraceae_unclassified* both showed a trend towards decreased bacterial abundance with inoculation of historically severe drought conditioned microbes (Table S8; Table S9). *Chthoniobacteraceae_unclassified* showed significant declines in abundance when comparing inoculated and control soils for both generations. I observed a decrease in abundance of *(O: Gemmatimonadales)* with inoculation for generation 1 only. Interestingly, I observed similar trends for decreasing abundances with

drought exposure for two other members of the phylum *Gemmatimonadetes* in a previous study (Table S1; Table S3). These findings suggest that these taxa may be susceptible to drought conditions and not needed for plant survival under drought stress. In contrast, *Bryobacteraceae_unclassified* and *Xanthomonadaceae_unclassified* showed significant increases as a result of inoculant with historically conditioned microbial communities. Surprisingly, these taxa were observed to decrease in abundance under severe drought conditions as compared to well-watered conditions in an earlier study (Table S1; Table S3). These data indicate plant selection of these taxa under more favorable conditions, which may be the reason for our observed increase in abundance as a result of inoculation treatment.

Conditioning effects influenced abundance levels of a few select taxonomic groups, including *Nocardioidaceae_Aeromicrobium, Nocardioidaceae_Aeromicrobium, Phyllobacteriaceae_Chelativorans Phyllobacteriaceae_Aminobacter, Oxalobacteraceae_Janthinobacterium* and *Oxalobacteraceae_unclassified*. These genera all showed increased abundance levels as a result of conditioning soil communities under tomato cultivation and severe drought conditions. This was quantified using differential abundance analyses comparing genus-level abundances in inoculated treatment groups from generation 1, generation 2 and the initial soil slurry microbial community (Table 2). All of these bacteria showed an increase in at least 2 generational comparisons including an overall increase from conditioning when comparing abundances in the initial soil slurry to generation 2. The families *Oxalobacteraceae* and *Nocardioidaceae* were both observed to increase in abundance under severe drought conditions in a previous study (Table S2; Tables S4, S6 & S7). *Oxalobacteraceae,* the only family to show an increase in abundance under severe drought as compared to well-watered conditions within autoclaved soils, has shown similar trends in other

studies (Xiong et al. 2014). Members of this family are known to have drought tolerant traits, including ACC deaminase production (Baldani et al. 2014). Furthermore, members of this family have been found in soil rhizobacterial communities native to arid regions and have been shown to be successful inoculants to induce drought tolerance in susceptible crops (Aguirre-von-Wobeser et al. 2018; Da Piedade Melo et al. 2017). *Nocardioidaceae* is another bacterial family that showed similar trends in this study as it did in our prior study. A member of this family, *Nocardioidaceae_unclassified,* showed increased abundance levels under drought as compared to well-watered conditions with microbial inoculants conditioned for well-watered or severe drought treatment. Upon further investigation, relatives of this taxa have been shown to be important microbial community members in drought-related soil studies. *Nocardioides albus EN46*, a member of the *Nocardioidaceae* family, was identified as benefitting Arabidopsis plants under biotic stress by promoting defensive priming of stress response pathways (Conn et al. 2008). Furthermore, the order, *Actinomycetales*, and phyla, *Actinobacteria,* have been identified as important rhizobacterial members influencing plant performance and drought tolerance in other studies (Zhao et al. 2020; Palaniyandi et al. 2013). The genera *Phyllobacteriaceae_Chelativorans* and *Phyllobacteriaceae_Aminobacter* were observed to increase in abundance as a result of both conditioning over generations and microbial history within generation 2. This taxon is discussed previously and may be a key driver of the plant size restriction I observed as a result of inoculation.

Higher microbial community complexity weakens conditioning effects

Suppressive soils often take several years in field conditions to create resilient responses in plants, therefore, by lessening microbial complexity I hoped to accelerate this conditioning

effect in a greenhouse experiment. Our results revealed that inoculation had a significant effect on plant DW biomass in autoclaved soils, however, under not autoclaved soil conditions I found no significant difference in biomass for either generation (Table 1). Native microbial complexity is known to have an influence on rhizosphere communities (Liu et al. 2019). Furthermore, it has been documented that lower soil microbial complexity allows for greater plant influence as compared to soils with complex communities of native soil microbiota (Mosqueira et al. 2019). For this reason, I expected to see a weakened impact of both plant selection and microbial history on contemporary plant performance and microbial community composition. This furthers the importance of utilizing lower microbial complexity when working in a controlled, greenhouse setting to determine plant influence on microbial recruitment. Interestingly, although there was no significant decrease in plant biomass or change in microbial community composition, both of these factors showed a slight shift after two generations of conditioning within not autoclaved soils. Microbial community composition in generation 1 compared to generation 2 showed greater differentiation between inoculated and control treatments (Figure S4). Additionally, in generation 2, there was a slight decrease in plant biomass when comparing inoculated to control soil conditions (Table 1). This suggests that there may be a delay in the effects of plant selection and microbial inoculation under conditioning settings.

Similar weakened effects were seen at the genus-level. In not autoclaved soils, *Phyllobacteriaceae_Chelativorans* and *Phyllobacteriaceae_Aminobacter* showed no significant differences in abundances when analyzing the effects of conditioning or microbial history. However, *Phyllobacteriaceae_Aminobacter* showed a similar pattern to the differences in abundance seen in autoclaved soils, with a slight increase in generation 2 inoculated treatment as compared to control and a slight increase in abundance in generation 2 as compared to

generation 1 inoculated treatments. *Phyllobacteriaceae_Chelativorans* showed no differences in abundance when analyzing microbial history or conditioning effects. Furthermore, these taxa showed slight decreases when comparing the microbiome of the initial soil slurry to that of the inoculated treatment in generation 2. These results indicate that the higher microbial complexity of not autoclaved soils interfered with the selection of these taxa under severe drought conditions. This is a similar pattern seen in suppressive soils when a different crop is planted in a monocultured system and disrupts the conditioned soil microbiome (Raaijmakers & Weller 1998). In these soils, the pathogen resistance is negatively impacted by the interference of a new crop and set of root exudates, similar to the disruption I saw in our soils with the addition of greater amounts of native soil microbiota.

Conclusions

Our findings suggest significant impacts of soil conditioning and microbial history on plant performance and microbial community composition for tomato cultivation under severe drought stress. Microbial inoculation of soils previously conditioned for similar stresses and crop type have been shown to increase plant resistance to abiotic and biotic stressors. Our results suggest a similar trend through the microbially-mediated restriction of plant vegetative growth under drought stress. This strategy can be a way for plants to better regulate water loss under severe drought conditions. Furthermore, I identified a lack of significant impact of soil conditioning and microbial history on soils with higher microbial complexity. Thereby, indicating the importance of amplifying plant influence on soil microbial communities to accelerate the effects of soil conditioning for greenhouse experiments. Further research is needed to understand impacts of conditioned soil communities on resulting biomass and yield outcomes.

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APPENDICES

Table S1. Bacterial taxa, to the genus level, observed to significantly decrease in abundance under severe drought compared to well-watered conditions in autocalved soils from the Sterilization Study (FDR<0.01; p<0.01).

Family_Genus	baseMeanWW	baseMeanSD	logFC	p-value	FDR
Xanthomonadaceae_unclassified	5.252	1.038	-2.191	0.0001	0.019522
Bryobacteraceae_unclassified	4.534	0.646	-2.578	0.000058	0.019522
Gemmatimonadaceae_Gemmatimonas	2.262	0.104	-3.368	0.000535	0.062581

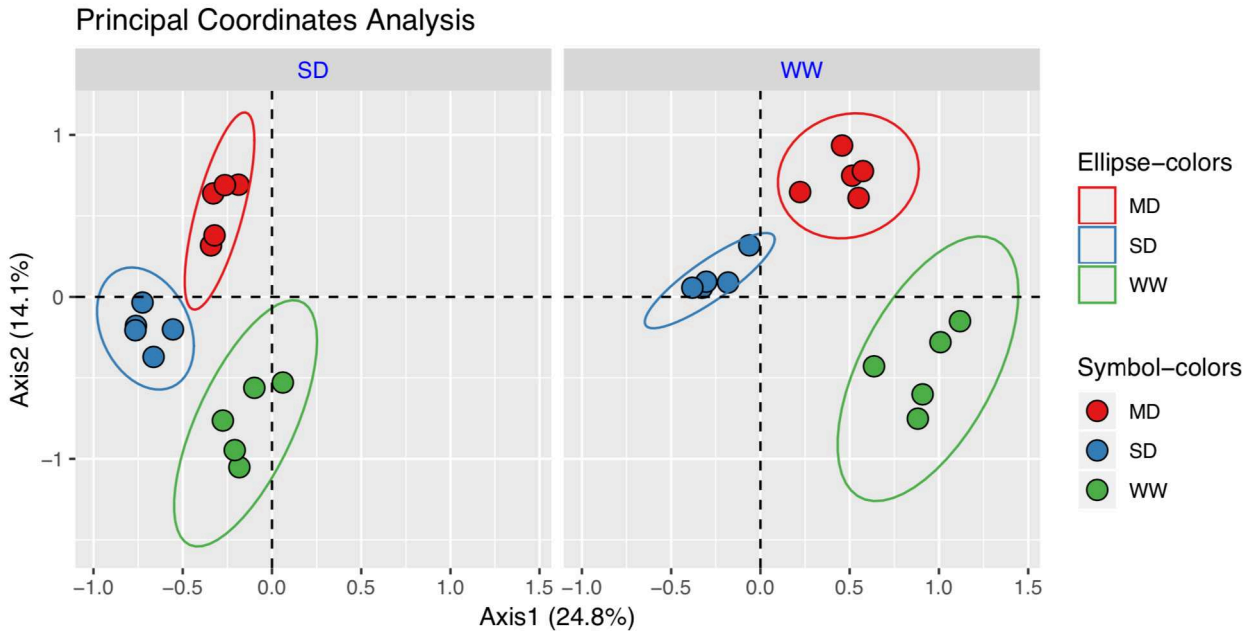


Figure S1. Principal Coordinates Analysis (PCoA), representing rhizobacterial communities of soil samples from each inoculation treatment (n=5 soil samples per inoculation treatment) and water condition. Blue, red and green represent severe drought, moderate drought and well-watered inoculations, respectively. The left pane represents inoculation treatments under contemporary severe drought conditions. The right pane represents inoculation treatments under contemporary well-watered conditions. Confidence ellipsoids are used to show significant clustering of each inoculation group.

Table S2. Bacterial taxa, to the genus level, observed to significantly increase in abundance under severe drought compared to well-watered conditions in autoclaved soils from the Sterilization Study (FDR<0.01; p<0.01).

Family_Genus	baseMeanWW	baseMeanSD	logFC	p-value	FDR
Oxalobacteraceae_unclassified	5.07	12.924	1.349	0.00064	0.062581

Table S3. Bacterial taxa, to the genus level, observed to significantly decrease in abundance under severe drought compared to well-watered contemporary conditions with the well-watered inoculation treatment (FDR<0.01; p<0.01). Unclassified genera are listed with their highest classification level (c: class, o: order).

Family_Genus	baseMeanWW	baseMeanSD	logFC	p-value	FDR
Comamonadaceae_unclassified (c: Gemm-1, o: unclassified)	13.254	0.686	-4.043	8.71E-17	3.41E-14
unclassified_unclassified	7.9	0.864	-3.02	1.18E-08	1.54E-06
Xanthomonadaceae_Dokdonella	4.2	0.888	-2.093	6.25E-04	4.88E-02

Table S4. Bacterial taxa, to the genus level, observed to significantly increase in abundance under severe drought compared to well-watered contemporary conditions with the well-watered inoculation (FDR<0.01; p<0.01).

Family_Genus	baseMeanWW	baseMeanSD	logFC	p-value	FDR
Nocardioidaceae_unclassified	4.278	16.95	1.956	1.97E-10	3.85E-08
Pseudonocardiaceae_Amycolatopsis	1.804	6.778	1.84	8.85E-05	8.65E-03

Table S5. Bacterial taxa, to the genus level, observed to significantly decrease in abundance under severe drought compared to moderate drought contemporary conditions with the moderate drought inoculation treatment (FDR<0.01; p<0.01).

Family_Genus	baseMeanMD	baseMeanSD	logFC	p-value	FDR
Comamonadaceae_unclassified	10.882	1.734	-2.563	0.00014	0.05444

Table S6. Bacterial taxa, to the genus level, observed to significantly increase in abundance under severe drought compared to well-watered conditions with the severe drought inoculation treatment (FDR<0.01; p<0.01).

Family_Genus	baseMeanWW	baseMeanSD	logFC	p-value	FDR
Nocardioidaceae_unclassified	4.83	13.764	1.487	2E-06	0.00087

Table S7. Bacterial taxa, to the genus level, observed to significantly increase in abundance under moderate drought compared to well-watered contemporary conditions with the severe drought inoculation treatment (FDR<0.01; p<0.01).

Family_Genus	baseMeanWW	baseMeanMD	logFC	p-value	FDR
Nocardioidaceae_unclassified	4.83	12.038	1.3	6.3E-05	0.02445

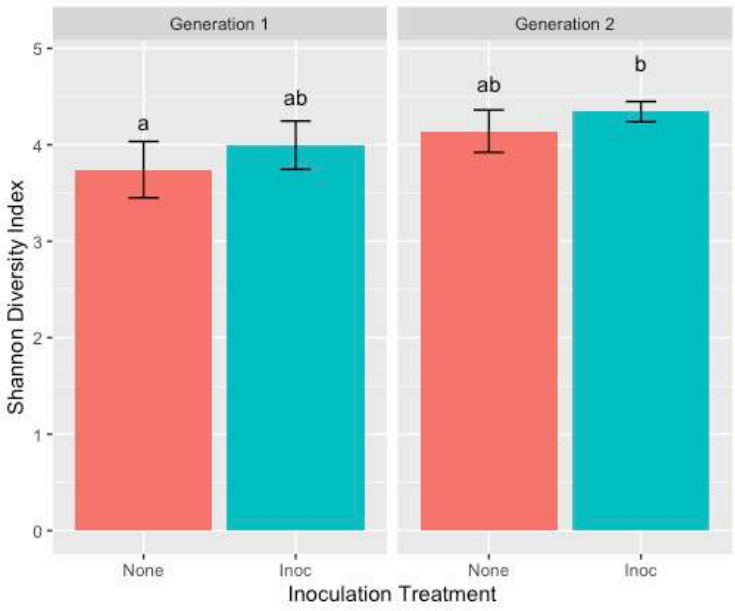


Figure S2. Mean alpha diversity values of each treatment group represented using Shannon Diversity Index values. Values for inoculated and control treatments are presented here within generation 1 and generation 2. Different letters indicate significant differences at $p < 0.05$.

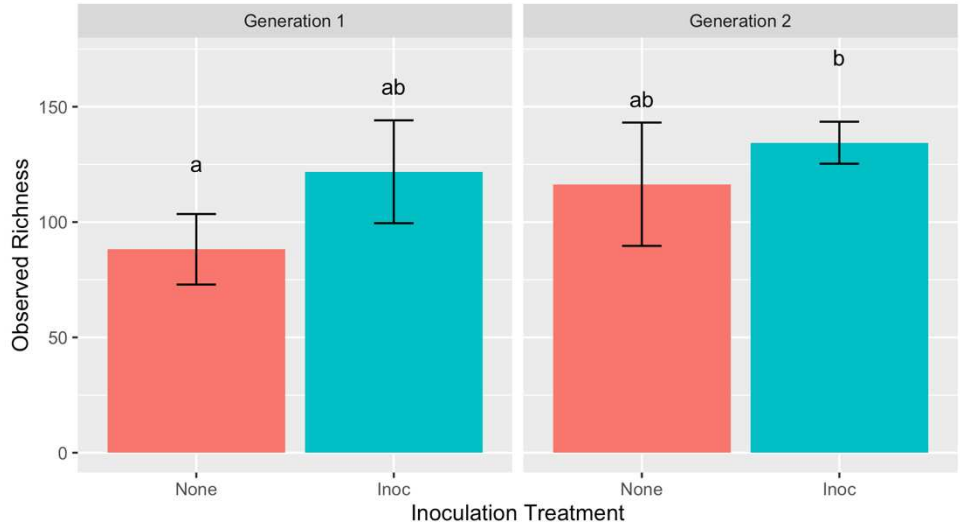


Figure S3. Mean alpha diversity values of each treatment group represented using observed richness values. Values for inoculated and control treatments are presented here within generation 1 and generation 2. Different letters indicate significant differences at $p < 0.05$.

Table S8. Bacterial taxa, to the genus level, observed to significantly differ in abundance in the inoculated treatment (I) as compared to the control (C) in generation 1 (FDR<0.01; p<0.01).

Family_Genus	baseMeanI	baseMeanC	logFC	p-value	FDR
Xanthomonadaceae_unclassified	167.04	0	-10.385	8.51E-15	1.8552E-12
Micromonosporaceae_Couchioplanes	99.106	0	-9.633	1.0161E-08	1.1076E-06
Comamonadaceae_Hydrogenophaga	45.792	0	-8.521	2.7084E-07	1.9681E-05
Pirellulaceae_unclassified	17.244	0	-7.119	2.1227E-06	0.00011569
(o: Ellin6067) unclassified_unclassified	14.924	0	-6.912	6.0419E-06	0.00022852
Bryobacteraceae_unclassified	14.59	0	-6.88	6.2896E-06	0.00022852
(o: Gemmatimonadales) unclassified_unclassified	0	31.716	7.992	0.00024082	0.00749997
RB40_unclassified	13.542	0	-6.774	0.00029473	0.00803149
Nocardioidaceae_Aeromicrobium	0	27.51	7.793	0.00039061	0.00946153
Planctomycetaceae_Planctomyces	9.946	0	-6.332	0.00068627	0.01496074
(o: Pedosphaerales) unclassified_unclassified	0	8.19	6.057	0.00099051	0.01963013
Chthoniobacteraceae_unclassified	0	6.87	5.805	0.00213901	0.03885859
Cytophagaceae_unclassified	12.76	0.738	-3.901	0.0030617	0.05134229

Table S9. Bacterial taxa, to the genus level, observed to significantly differ in abundance in the inoculated treatment (I) as compared to the control (C) in generation 2 (FDR<0.01; p<0.01).

Family_Genus	baseMeanI	baseMeanC	logFC	p-value	FDR
Xanthomonadaceae_unclassified	38.838	0	-8.284	6.3187E-12	1.3775E-09
Rhizobiaceae_Sinorhizobium	0	39.192	8.297	6.7073E-10	7.3109E-08
Rhodospirillaceae_unclassified	26.644	0	-7.742	2.296E-08	1.6384E-06
Methylobacteriaceae_Methylobacterium	0	27.088	7.766	3.0062E-08	1.6384E-06
RB40_unclassified	26.946	0.284	-6.048	3.7389E-07	1.6301E-05
Phyllobacteriaceae_Chelativorans	17.462	0	-7.136	9.5151E-07	3.4571E-05
Pirellulaceae_unclassified	13.768	0	-6.796	3.5576E-06	8.6174E-05
(o: Ellin6067)unclassified_unclassified	13.482	0	-6.766	3.5248E-06	8.6174E-05
Planctomycetaceae_Planctomyces	13.986	0	-6.818	2.9406E-06	8.6174E-05
Chitinophagaceae_unclassified	21.1	0.472	-5.152	5.676E-05	0.00123736
Sphingomonadaceae_Sphingomonas	0	19.238	7.275	9.9778E-05	0.00197742
Sporichthyaceae_unclassified	44.904	2.108	-4.334	0.00021064	0.00382667
Chitinophagaceae_Flavihumibacter	6.384	0	-5.702	0.00023115	0.00387628
Cytophagaceae_unclassified	7.058	0	-5.845	0.00029843	0.00464705
mb2424_unclassified	9.336	0	-6.241	0.00035658	0.00518234
Chthoniobacteraceae_unclassified	0	11.678	6.56	0.00043213	0.00588781
Chitinophagaceae_Flavisolibacter	0.44	15.78	4.816	0.00061873	0.00793433
Cytophagaceae_Larkinella	0	8.496	6.11	0.000835	0.0101128
Bryobacteraceae_unclassified	4.524	0	-5.217	0.00267812	0.03072795
Nocardioidaceae_unclassified	31.312	208.838	2.734	0.00369072	0.03831314
Phyllobacteriaceae_Aminobacter	74.032	5.394	-3.745	0.00354015	0.03831314

Caulobacteraceae_Mycoplana (o: Myxococcales)	0	6.874	5.808	0.00418256	0.04144537
unclassified_unclassified	21.134	1.27	-3.929	0.00608603	0.05768496
Hyphomicrobiaceae_Hyphomicrobium	5.444	0	-5.479	0.00657123	0.05968867
Oxalobacteraceae_Janthinobacterium	9.25	0	-6.231	0.00859442	0.07494332

Table S10. Bacterial taxa, to the genus level, observed to significantly differ in abundance in inoculated soils from generation 1 (G1) compared to inoculated soils from generation 2 (G2) (FDR<0.01; p<0.01).

Family_Genus	**baseMeanG2**	**baseMeanG1**	**logFC**	**p-value**	**FDR**
Micromonosporaceae_Couchioplanes	0	99.106	9.634	4.3348E-10	9.4498E-08
Nocardioidaceae_Aeromicrobium	19.91	0	-7.324	1.3315E-07	1.0553E-05
Phyllobacteriaceae_Chelativorans	17.462	0	-7.135	1.4522E-07	1.0553E-05
Phyllobacteriaceae_Aminobacter	74.032	11.22	-2.706	1.6929E-06	9.2263E-05
Bradyrhizobiaceae_Balneimonas	1.5	43.894	4.764	1.992E-05	0.0008685
Xanthomonadaceae_unclassified	38.838	167.04	2.102	0.00051865	0.01884435
Oxalobacteraceae_unclassified	27.08	219.738	3.017	0.00105321	0.03280004
Nocardioidaceae_unclassified	31.312	222.544	2.827	0.0015197	0.04141192

Table S11. Bacterial taxa, to the genus level, observed to significantly differ in abundance in inoculated soils from the generational foundation soil slurry (GF) compared to inoculated soils from generation 1 (G1) (FDR<0.01; p<0.01).

Family_Genus	**baseMeanG1**	**baseMeanGF**	**logFC**	**p-value**	**FDR**
Paenibacillaceae_Ammoniphilus	10.118	0	-6.359	4.2167E-06	6.7994E-05
Planococcaceae_Planomicrobium	5.93	0	-5.602	0.00798295	0.03739065
Micrococcaceae_Arthrobacter	5.832	0	-5.579	0.00887632	0.04017705
Rhizobiaceae_Sinorhizobium	5.15	0	-5.403	0.01155735	0.04828981
Oxalobacteraceae_Janthinobacterium	5.064	0	-5.377	0.00376108	0.01993541
unclassified_unclassified	50.598	1.18	-5.284	6.7833E-14	8.7505E-12
Rhodobacteraceae_Rhodobacter	4.426	0	-5.188	0.00499053	0.02504958
Oxalobacteraceae_unclassified	218.99	7.746	-4.804	5.4049E-08	1.3945E-06
Bacillaceae_unclassified	4.928	0.066	-4.727	0.00912833	0.04060531
unclassified_unclassified	2.004	0	-4.093	0.0034506	0.01894159
Brucellaceae_Ochrobactrum	1.922	0	-4.037	0.02279772	0.08044487
Bradyrhizobiaceae_Balneimonas	44.14	2.604	-4.025	3.1595E-07	6.1966E-06
unclassified_unclassified	1.582	0	-3.774	0.02295371	0.08044487
Planococcaceae_unclassified	1.37	0	-3.583	0.02780158	0.09471523
Alteromonadaceae_Cellvibrio	8.506	0.686	-3.416	0.00489434	0.02504958
Cytophagaceae_unclassified	12.818	1.3	-3.188	3.9033E-05	0.00050352
Cytophagaceae_Adhaeribacter	4.252	0.42	-3.01	0.0081158	0.03739065
Pseudomonadaceae_Pseudomonas	16.546	2.254	-2.812	0.00327871	0.0183893
Bacillaceae_Bacillus	139.366	21.884	-2.668	3.3625E-07	6.1966E-06

Bradyrhizobiaceae_unclassified	9.62	1.872	-2.29	0.01160453	0.04828981
Micrococcaceae_unclassified	198.59	43.602	-2.188	5.6076E-05	0.00068893
Nocardioidaceae_Pimelobacter	45.61	11.312	-2.004	0.012386	0.05072361
Pirellulaceae_unclassified	18.06	5.284	-1.753	0.00285038	0.01691943
Sphingomonadaceae_unclassified	6.036	21.112	1.781	0.01766111	0.06603718
Comamonadaceae_Ramlibacter	6.146	23.124	1.886	0.00378618	0.01993541
Comamonadaceae_Hydrogenophaga	45.164	182.798	2.01	0.01365797	0.05505869
Sphingomonadaceae_Sphingobium	8.65	38.228	2.124	1.6493E-05	0.0002364
Erythrobacteraceae_unclassified	2.936	16.278	2.418	0.00642379	0.03127054
Sphingomonadaceae_Sphingopyxis	8.574	47.674	2.454	0.00010366	0.00102862
Verrucomicrobiaceae_unclassified	1.526	10.182	2.639	0.01714454	0.065108
Flavobacteriaceae_Flavobacterium	0	0.762	2.825	0.02790061	0.09471523
Sphingomonadaceae_Novosphingobium	0	0.822	2.92	0.02307334	0.08044487
Ellin517_unclassified	0	0.854	2.968	0.02090783	0.07597494
Geodermatophilaceae_unclassified	0	0.868	2.988	0.02003558	0.07384541
Beijerinckiaceae_unclassified	0	0.92	3.062	0.01716025	0.065108
Rhizobiaceae_Kaistia	0	0.936	3.084	0.01637532	0.0640126
Rhizobiaceae_Agrobacterium	2.524	22.58	3.096	7.1199E-05	0.00083497
unclassified_unclassified	6.146	54.322	3.112	0.00667348	0.03188441
Caulobacteraceae_Arthrospira	0	0.994	3.161	0.0138753	0.05507428
Xanthobacteraceae_Ancylobacter	0	1.012	3.183	0.01078843	0.04639026
Pseudonocardiaceae_Pseudonocardia	0	1.042	3.221	0.01070389	0.04639026
C111_unclassified	0	1.366	3.574	0.00315497	0.01808847
Sinobacteraceae_unclassified	0	1.41	3.616	0.00288548	0.01691943
unclassified_unclassified	1.24	16.688	3.618	3.0394E-05	0.00041272
Microbacteriaceae_Agrococcus	0	1.494	3.693	0.00504875	0.02504958
Thermoactinomycetaceae_unclassified	0	1.69	3.858	0.00121966	0.00806853
unclassified_unclassified	0	1.718	3.88	0.00108576	0.00737171
Cellulomonadaceae_Actinotalea	0	1.728	3.888	0.00177654	0.01091303
unclassified_unclassified	0	1.844	3.976	0.00079821	0.00588396
Verrucomicrobiaceae_Luteolibacter	0	1.882	4.003	0.00077314	0.00586678
Hyphomicrobiaceae_Rhodoplanes	0	1.98	4.072	0.00137587	0.00865789
Comamonadaceae_Methylibium	0	2.318	4.287	0.00033635	0.00271182
Bradyrhizobiaceae_Bosea	0	2.322	4.289	0.00044528	0.00348128
unclassified_unclassified	0	2.336	4.297	0.00127273	0.00820911
Microbacteriaceae_Microbacterium	0	2.558	4.422	0.00082456	0.00590938
unclassified_unclassified	0	2.774	4.534	0.00018618	0.00165636
Verrucomicrobiaceae_Prosthecobacter	0	2.924	4.606	0.00106072	0.00737171
Rhizobiaceae_Rhizobium	0	3.006	4.645	0.00024112	0.00207359
Cytophagaceae_Dyadobacter	1.038	31.538	4.763	1.4175E-08	4.5715E-07
Phyllobacteriaceae_Chelativorans	0	3.294	4.772	8.5425E-05	0.00088158
Comamonadaceae_Variovorax	0	3.454	4.838	0.0002938	0.00244515
Methylophilaceae_Methylotenera	0	3.484	4.85	8.2896E-05	0.00088158
Rhizobiaceae_Shinella	0	3.726	4.943	8.2392E-05	0.00088158
Nocardioidaceae_Aeromicrobium	0	4.336	5.156	0.00017539	0.00161613
Rhodobacteraceae_Rubellimicrobium	0	5.266	5.429	9.4998E-06	0.00014417
Streptomycetaceae_Streptomyces	0	5.498	5.489	0.00012636	0.0012074

Comamonadaceae_Delftia	0	5.722	5.546	3.0901E-06	5.3149E-05
Sphingobacteriaceae_unclassified	0	7.822	5.989	9.5248E-08	2.0478E-06
unclassified_unclassified	0	8.036	6.027	6.4036E-08	1.5019E-06
Caulobacteraceae_Mycoplana	0	11.266	6.508	1.7315E-08	4.9637E-07
Comamonadaceae_Pelomonas	0	12.126	6.613	7.6203E-09	2.8086E-07
Sphingomonadaceae_Sphingomonas	0	16.644	7.066	2.9947E-12	1.5453E-10
Caulobacteraceae_unclassified	0	21.32	7.421	1.9239E-14	4.9638E-12
Nocardioidaceae_Nocardioides	0	23.12	7.537	4.572E-09	1.966E-07
unclassified_unclassified	0	24.022	7.592	1.0712E-12	6.9091E-11
Pseudonocardiaceae_Amycolatopsis	0	27.154	7.768	1.153E-13	9.9156E-12

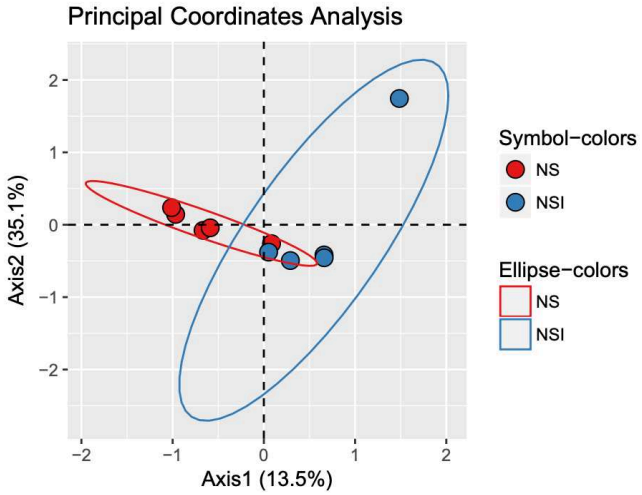
Table S12. Bacterial taxa, to the genus level, observed to significantly differ in abundance in inoculated soils from the generational foundation soil slurry (GF) compared to inoculated soils from generation 2 (G2) (FDR<0.01; p<0.01).

Family_Genus	**baseMeanG1**	**baseMeanGF**	**logFC**	**p-value**	**FDR**
AK1AB1_02E_unclassified	0.636	0	-2.605	0.03601489	0.09679
Alteromonadaceae_Cellvibrio	0.686	12.31	3.94	4.815E-05	0.00035493
Bacillaceae_Bacillus	21.884	240.288	3.451	4.9658E-27	6.4059E-25
Bacillaceae_unclassified	0.066	16.08	6.408	4.3104E-11	7.489E-10
Beijerinckiaceae_unclassified	0.92	0	-3.063	0.01198924	0.03818795
Bradyrhizobiaceae_Bosea	2.322	0	-4.29	0.00020808	0.00116705
Bradyrhizobiaceae_unclassified	1.872	10.324	2.389	0.02662663	0.0730816
Bryobacteraceae_unclassified	25.526	4.604	-2.438	6.2277E-06	6.427E-05
Caulobacteraceae_Arthrospira	0.994	0	-3.161	0.00912347	0.03017762
Caulobacteraceae_Mycoplana	11.266	0	-6.509	2.1006E-09	3.0109E-08
Caulobacteraceae_Phenylobacterium	14.686	2.844	-2.317	0.00545352	0.01876009
Caulobacteraceae_unclassified	21.32	0	-7.422	1.5395E-15	5.6743E-14
Cellulomonadaceae_Actinotalea	1.728	0	-3.889	0.00121097	0.00529544
Chitinophagaceae_Flavisolibacter	3.098	0.414	-2.578	0.00497629	0.01758744
Chitinophagaceae_unclassified	3.442	21.188	2.58	6.1202E-06	6.427E-05
Comamonadaceae_Azohydromonas	1.612	0	-3.796	0.00126196	0.00542642
Comamonadaceae_Delftia	5.722	0	-5.547	1.3004E-06	1.4587E-05
Comamonadaceae_Hydrogenophaga	182.798	84.964	-1.102	0.00024058	0.00129309
Comamonadaceae_Methylibium	2.318	0	-4.288	0.00017014	0.00099766
Comamonadaceae_Pelomonas	12.126	0	-6.614	9.1924E-10	1.4823E-08
Comamonadaceae_Variovorax	3.454	0	-4.839	0.0001147	0.00070461
Cytophagaceae_Adhaeribacter	0.42	3.232	2.623	0.02513073	0.0697175
Cytophagaceae_Dyadobacter	31.538	0.23	-6.477	4.5773E-19	2.9524E-17
Cytophagaceae_unclassified	1.3	6.906	2.305	0.00472882	0.01694494
Flavobacteriaceae_Flavobacterium	0.762	0	-2.826	0.02188918	0.06274899
Gemmatimonadaceae_Gemmatimonas	15.204	36.184	1.246	0.00051547	0.00247089
Geodermatophilaceae_unclassified	0.868	0	-2.989	0.0145757	0.04372711
Haliangiaceae_unclassified	4.846	0	-5.313	8.6114E-06	7.9348E-05
Hyphomicrobiaceae_Rhodoplanes	1.98	0	-4.073	0.00050772	0.00247089
Hyphomonadaceae_unclassified	1.632	10.422	2.588	7.7205E-05	0.00051074
mb2424_unclassified	1.52	9.534	2.555	0.00185665	0.00760342
Methylobacteriaceae_unclassified	3.944	0	-5.024	2.4408E-05	0.00019679

Methylophilaceae_Methylotenera	3.484	0	-4.851	2.3013E-05	0.00019152
Microbacteriaceae_Agrococcus	1.494	0	-3.694	0.00270934	0.01059107
Microbacteriaceae_Microbacterium	2.558	0	-4.423	0.00026218	0.00138047
Microbacteriaceae_unclassified	0	6.748	5.783	0.00140776	0.00595414
Micrococcaceae_Arthrobacter	0	2.002	4.09	0.01355703	0.04165031
Micrococcaceae_unclassified	43.602	186.794	2.097	6.9996E-05	0.00048808
Micromonosporaceae_Couchioplanes	65.412	0	-9.033	8.0941E-32	2.0883E-29
Micromonosporaceae_unclassified	572.368	197.006	-1.536	4.8223E-09	6.5482E-08
Nocardioidaceae_Aeromicrobium	4.336	20.374	2.202	0.00052674	0.00247089
Nocardioidaceae_Nocardioides	23.12	0	-7.538	1.8973E-09	2.8795E-08
Nocardioidaceae_Pimelobacter	11.312	37.204	1.708	0.00019152	0.00109805
Nocardioidaceae_unclassified	130.06	30.962	-2.065	0.00301883	0.01162475
Opitutaceae_Opitutus	9.902	27.936	1.486	0.00049379	0.00247089
Oxalobacteraceae_Janthinobacterium	0	8.956	6.186	0.00091614	0.00414675
Oxalobacteraceae_unclassified	7.746	27.594	1.819	0.00436972	0.01587869
Paenibacillaceae_Ammoniphilus	0	29.85	7.906	2.0237E-19	1.7404E-17
Paenibacillaceae_Aneurinibacillus	0	3.438	4.833	0.00525311	0.01831489
Paenibacillaceae_Cohnella	2.91	15.06	2.323	0.02229619	0.06321336
Phyllobacteriaceae_Aminobacter	10.326	75.094	2.849	1.257E-12	2.7026E-11
Phyllobacteriaceae_Chelativorans	3.294	17.53	2.37	1.2099E-05	0.00010764
Phyllobacteriaceae_Mesorhizobium	4.618	0	-5.245	8.0234E-06	7.6668E-05
Phyllobacteriaceae_unclassified	1.268	0	-3.477	0.00375923	0.01405624
Pirellulaceae_unclassified	5.284	13.888	1.375	0.00931054	0.03040656
Planctomycetaceae_Planctomyces	4.388	14.2	1.668	0.0040647	0.01498132
Planococcaceae_unclassified	0	6.612	5.752	0.00021984	0.00120679
Pseudomonadaceae_Pseudomonas	2.254	23.584	3.32	0.00016972	0.00099766
Pseudonocardiaceae_Amycolatopsis	27.154	0	-7.769	1.8338E-15	5.9139E-14
Pseudonocardiaceae_Pseudonocardia	1.042	0	-3.222	0.00815265	0.02767611
RB40_unclassified	5.482	26.708	2.261	1.8216E-07	2.3498E-06
Rhizobiaceae_Agrobacterium	22.58	0	-7.504	5.6198E-17	2.4165E-15
Rhizobiaceae_Kaistia	0.936	0	-3.085	0.0112961	0.03642992
Rhizobiaceae_Rhizobium	3.006	0	-4.646	8.8435E-05	0.00057041
Rhizobiaceae_Shinella	3.726	0	-4.944	2.1151E-05	0.0001819
Rhodobacteraceae_Rhodobacter	0	19.46	7.293	1.169E-13	3.016E-12
Rhodobacteraceae_Rubellimicrobium	5.266	0	-5.43	6.6461E-06	6.595E-05
Sinobacteraceae_unclassified	1.41	0	-3.617	0.00233144	0.00939862
Sphingobacteriaceae_Pedobacter	1.558	0	-3.75	0.00171051	0.00711793
Sphingomonadaceae_Novosphingobium	0.822	0	-2.921	0.01736482	0.05091049
Sphingomonadaceae_Sphingobium	38.228	19.688	-0.951	0.01356057	0.04165031
Sphingomonadaceae_Sphingomonas	16.644	0	-7.067	3.0398E-13	7.1298E-12
Sphingomonadaceae_Sphingopyxis	47.674	3.924	-3.559	4.354E-11	7.489E-10
Sphingomonadaceae_unclassified	21.112	6.87	-1.6	0.01592416	0.04722338
Sporichthyaceae_unclassified	23.95	45.032	0.909	0.03014229	0.08186012
Staphylococcaceae_Staphylococcus	2.296	32.736	3.764	7.0781E-12	1.4047E-10
Streptomycetaceae_Streptomyces	5.498	0	-5.491	0.00010882	0.00068476
Thermoactinomycetaceae_unclassified	1.69	0	-3.859	0.0010567	0.00470047
unclassified_unclassified	1.18	39.214	4.916	6.114E-18	3.1548E-16
unclassified_unclassified	24.022	0	-7.593	1.7616E-14	5.0499E-13
unclassified_unclassified	54.322	4.188	-3.657	2.4553E-07	3.0166E-06

unclassified_unclassified	49.7	103.926	1.064	3.1818E-05	0.00024876
unclassified_unclassified	2.774	0	-4.535	4.7517E-05	0.00035493
unclassified_unclassified	0	4.762	5.29	7.6005E-05	0.00051074
unclassified_unclassified	1.844	0	-3.977	0.00062217	0.00286642
unclassified_unclassified	347.132	577.328	0.736	0.0025711	0.01020528
unclassified_unclassified	16.688	5.446	-1.592	0.0032777	0.01243599
unclassified_unclassified	0	1.828	3.967	0.01320041	0.041533
Verrucomicrobiaceae_Luteolibacter	1.882	0	-4.004	0.00052025	0.00247089
Verrucomicrobiaceae_Prosthecobacter	2.924	0	-4.607	0.00035023	0.00180719
Verrucomicrobiaceae_unclassified	10.182	1.578	-2.595	0.01374791	0.04172896
Xanthobacteraceae_Ancylobacter	1.012	0	-3.184	0.00905233	0.03017762
Xanthomonadaceae_Dokdonella	0.194	3.858	3.645	0.02116284	0.06134847
Xanthomonadaceae_Lysobacter	24.184	7.4	-1.689	0.02494179	0.0697175
Xanthomonadaceae_Pseudoxanthomonas	25.962	0.728	-4.933	1.0901E-06	1.2784E-05
Xanthomonadaceae_unclassified	93.162	39.568	-1.231	5.1424E-05	0.00036854

(A) Generation 1



(B) Generation 2

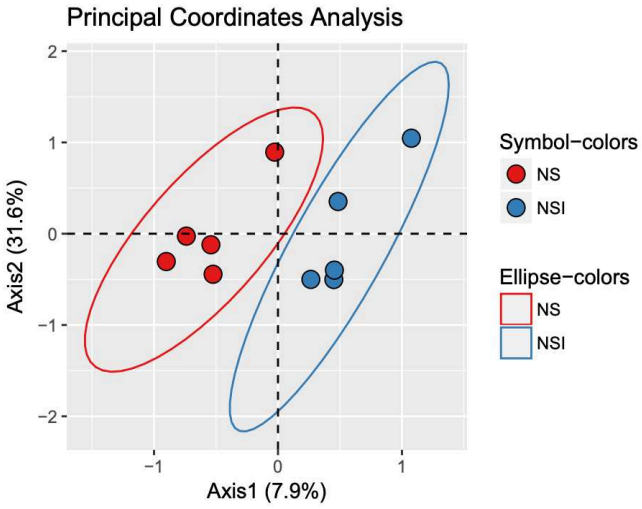


Figure S4. Principal Coordinates Analysis (PCoA), using Bray-Curtis distances, representing rhizobacterial communities of soil samples from inoculated and control treatments (n=5 soil samples per water treatment) in not autoclaved soils within generation 1 (A) and generation 2 (B). Blue and red circles represent inoculated (NSI) and control (NS) soils, respectively.

Table S13. Differences in abundance for *Phyllobacteriaceae_Chelativorans* and *Phyllobacteriaceae_Aminobacter* in inoculated treatment groups from each generation in not autoclaved soils: initial soil slurry (GF), generation 1 (NA_G1) and generation 2 (NA_G2) (FDR<0.01; p<0.01).

Family_Genus	**baseMeanGF**	**baseMeanNA_G2**	**logFC**	**p-value**	**FDR**
Phyllobacteriaceae_Chelativorans	0.364	0	-1.968	1.12E-01	5.22E-01
Phyllobacteriaceae_Aminobacter	1.086	0.482	-0.997	2.80E-01	1.00E+00
Family_Genus	**baseMeanNA_G2**	**baseMeanNA_G1**	**logFC**	**p-value**	**FDR**
Phyllobacteriaceae_Chelativorans	0	0	0	1	1
Phyllobacteriaceae_Aminobacter	0.482	0	-2.279	0.124986	1
Family_Genus	**baseMeanGF**	**baseMeanNA_G1**	**logFC**	**p-value**	**FDR**
Phyllobacteriaceae_Chelativorans	0.364	0	-1.967	1.14E-01	6.67E-01
Phyllobacteriaceae_Aminobacter	1.086	0	-3.275	6.12E-03	8.26E-02