THESIS

ELUCIDATING RHIZOBACTERIAL RESPONSE TO AUTOCLAVE DISRUPTION AND CROP INTRODUCTION WITHIN THREE DISTINCT AGRICULTURAL SOILS

Submitted by

Michael J. DiLegge

Department of Horticulture and Landscape Architecture

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2020

Master's Committee:

Advisor: Jorge M. Vivanco

Daniel K. Manter
Tiffany L. Weir
Ioannis S. Minas

ABSTRACT

ELUCIDATING RHIZOBACTERIAL RESPONSE TO AUTOCLAVE DISRUPTION AND CROP INTRODUCTION WITHIN THREE DISTINCT AGRICULTURAL SOILS

Management practices can affect the soil health properties of an agroecosystem, in turn effecting the resident soil microbial community. Insights toward how managerial practices effect soil microbial rearrangements are steadily being uncovered with next generation sequencing applications. This thesis covers research investigating how soilborne and plant-rhizospheric bacteria from three differential agricultural management systems are affected by applied disruption followed by the introduction of new plants to their sites. Two independent greenhouse experiments were conducted to evaluate plant-mediated bacterial rearrangements in soil following autoclave disruption. The first study utilized two soil types from a perennial peach orchard system experiencing negative effects of orchard replanting disease. Soils were sampled from a replanting disease (RD) site and a non-replanting disease (non-RD) block. Replanting disease soils were autoclaved; and peach, corn and tomato plants were grown in both autoclaved and unautoclaved RD soils, as well as non-RD soils. Bacterial phyla and their predicted functional genomics were assessed after autoclave disruption and plant growth.

The second experiment was an expansion of the former, utilizing autoclave disruption and the same perennial RD soil from the former study, but with the addition of conventional and organic annual agroecosystem soils. In this experiment four crops of differing plant families (corn, beet, tomato and lettuce) were introduced to examine how soil bacterial rearrangements may be influenced by distinct crop-presence after autoclave disruption. Results showed that

autoclave disruption increased plant biomass. Interestingly, the type of crop plant introduced as well as the agroecosystem soil type drove differential bacterial responses and rearrangements. These data demonstrate that both agricultural ecosystem management, paired with the family of plants grown in these ecosystems, strongly impact soil bacterial availability and rearrangement in the rhizosphere. Additionally, in agricultural sites experiencing severe long-term dysbiosis, an autoclave disruption in pair with the rotation of monocultured crops may prompt the colonization of a healthier rhizomicrobiome.

ACKNOWLEDGEMENTS

I would like to encompass my utmost appreciation and gratitude to my adviser Dr. Jorge Vivanco, as well as to the members of my graduate committee whose time, support and guidance throughout this academic exertion has been essential: Dr. Daniel Manter, Dr. Tiffany Weir and Dr. Ioannis Minas. I will remember this time for years to come.

I would also extend thanks to all who have offered advice, friendship or have lent an ear during the tough times throughout my years at Colorado State University. Major thanks to both my current and former colleagues in the Vivanco lab for great experiences, academic inspiration as well as fruitful collaboration. Additionally, I would like to thank the greater Horticulture and Landscape Architecture laboratory-cohort for guidance, providing community as well as support during the strenuous moments in both Shepardson and throughout our transition to the AZ building. Furthermore, I would like to extend thanks to current and former members of the Colorado State University Zero Waste Team; for all the positive work we've achieved (and will continue to do) in supporting the larger Fort Collins community through outlets of environmental sustainability and waste diversion. To friends and partners close to me over my years in Colorado, I want to acknowledge the patience, brace, and empathy you have shown me both during the workday and in times in our non-collegiate junctures. Thanks for all the fun.

Lastly to my parents Kimberly and Wayne DiLegge, my sister Alexis and brother-in-law Mike; thank you all for your unparalleled support, guidance and reverence throughout my life and academic journey. The pride you all have shown for me over the course of this expedition has helped motivate me to keep moving forward.

TABLE OF CONTENTS

CHAPTER 2: Soil sterilization leads to re-colonization of a healthier rhizosphere microbiome

LIST OF TABLES

LIST OF FIGURES

LIST OF SUPPLEMENTARY FILES

CHAPTER 1: Review of Recent Literature

Introduction

Until recently, the modernization of commercial agriculture has semi-disregarded the health of resident soil microbiota, and when considered; microbiota are often addressed passively in typical attempts to manufacture a profitable growing environment. Sometimes soil microbiota fall under consideration when they can be attributed to plant growth inhibitory conditions (e.g. bacterial disease, herbivorous nematodes, etc.). The actions used to alleviate these conditions usually focus on controlling the pest-pathogen in question; without consideration of how measures may affect neighboring microbiota. The application of both chemical (Schauss, et al., 2009; Chowhury et al., 2008; Schuster & Schröder, 1990) and biological pesticides (Shao & Zhang, 2017; Liao et al., 2019) have yielded adverse effects toward non-target microbes after their application in both laboratory and agroecosystem settings. As such, these off-target effects may cause detriment to native and potentially plant-benefiting microbes in both surrounding soils and in the plant rhizosphere.

The bulk of agroecosystem soils, while not penetrated by plants roots, is exposed to managerial applications and may provide a starting reservoir of plants to form associated microbial communities. These relationships are formed in the plant rhizosphere; the narrow region of soil directly influenced by root secretions and plant-associated microorganisms. Therefore, limitations or microbial imbalances in the bulk soil community stemming from management can alter the ability of rhizospheric colonization, which may result in unideal plant associated microbial communities. Recent evidence suggests that when management practices take into consideration the health of the native soil microbiota (no-till, organic matter input,

cover crop/crop rotation, etc.), these systems express high potential to provide plants with even rhizo-communities (Bonanomi et al., 2018).

Current insights revealed throughout the past decade have emphasized the ecological and economic importance of microbial evenness occurring in both bulk soil and plant rhizospheres (She et al., 2017; Hair et al., 2016; Bakker et al., 2012). Ecological roles performed by soil microbes yield promising benefits toward plant growth and development, while simultaneously increasing host defenses and the overall productivity of an agroecosystem (Lakshmanan et al., 2014). Methods tailored to combat soil microbial dysbiosis may fall under the umbrella term of "Sustainable Agriculture" (Bramhachari et al., 2017). Some of those techniques are listed as follows: crop rotation, no-tillage, organic amendments for plant nutrition, natural or biological products for plant protection from pests, and several others (Bonanomi et al., 2018). Practices such as these may allow for agroecosystems to develop high levels of microbial alpha-diversity, which in-turn provides plants with a vast array of potentially symbiotic rhizosphere taxa for recruitment, in addition to stimulating soil ecosystem cycling.

The rhizosphere also extends the plant's abilities to acquire nutrients, develop protection against outside stressors, and recruit microbial communities (Liu & Brettell, 2019). The relationship between plants and their associated microbes can be traced back over 450 million years, during initial land colonization when plants first formed relationships with newly associated microbes, allowing both to grow together in their latest terrain: the soil. More recently, a framework has been proposed by Liu & Brettell (2019) to emphasize the plants' ability to recruit beneficial microbial consortia through reallocation of their photosynthesis products, a process called rhizodeposition (Liu & Brettell, 2019). Authors further describe how these consortia may become heritable into the next plant generation (Liu & Brettell, 2019;

Lapsansky et al., 2016). Over time, outside stressors have driven the recruitment of root-associated microbial communities, allowing for the co-evolution of symbiotic plant-microbial relationships (Hassani et al., 2018). Furthering our understanding these complex plant-microbial interactions would advance the development of microbially-conscious managerial regiments for agroecosystems.

Soil microbial communities can be beneficial to plants via several functions (Manter and Steward, 2015). Recent research has focused on identifying plant-benefiting microbial keystone species, defined as low-abundance taxa that increase alpha-diversity in addition to stabilizing ecosystem function (Jones et al., 2019). In the phytobiome, these species are hypothesized to assist the plant in establishing its core microbiome (Wei et al., 2019; Harrison et al., 2018) and keystone species are likely recruited to aid microbiota in establishment when plants are introduced to soil ecosystems. Due to the long list of established plant-benefiting soil microbial functions, the introduction of agricultural management practices tailored toward benefiting resident soil microbial communities could yield economic, phytochemical/nutritional, and environmental health benefits. Although our understanding of these complex plant-microbe associations is incomplete, the work in this thesis explores mechanisms oriented toward the promotion of soil bacterial rearrangement and plant health in agroecosystems.

The effect of commercial agricultural management on soil microbial health

Intense agricultural management practices oriented toward maximizing yields and meeting worldwide food demands have over time placed extreme pressures on soil microbial communities. The global loss of soil organic carbon, increased soil compaction and increased soil erosion have also been arrtibuted to agricultural management (Edmondson et al., 2014;

Montgomery, 2007) As such, these pressures have negatively affected soil available carbon content which can cause degradation of the soil ecosystem (Franzluebbers, 2002).

Schmidt et al. (2019) demonstrates that a comparison of rhizosphere communities from organic to conventionally managed agroecosystems reveals presence of managerial-specific taxa, furthering the notion that field management influences both soil physical properties and resident microbiota (Schmit et al., 2019). It was observed that conventionally managed systems show increased levels of microbial gene functions related to plant-pathogenesis (Schmidt et al., 2019). Likely these plant pathogen-related microbes are habituated to conventional management practiced within their agroecosystem and may play a role in shaping bacterial co-occurrence networks (Schmidt et al., 2019). Other studies on organically managed agroecosystems such as Wang et al. (2012) have noted that no-till practices can lead to improvements in soil properties including increased available carbon, total nitrogen, total microbial biomass, increases in mycorrhizae populations, and increases in phospholipid fatty acid profiles related to microbial community structure, compared to annually tilled conventional systems (Wang et al., 2012).

Additional studies investigating microbial community response to organic or conventional management have shown that soil samples from organic sites showed increased nutrient availability along with increased abundance and diversity of soil microbes (Liao et al., 2018). Plants growing in plastic tunnel organic systems had higher abundance measures of taxa related to the soil organic matter turnover, and these taxa were also associated with genes linked to plant growth promotion (Liao et al., 2018). Another study by Liao et al. (2019) comparing naturally vs. conventionally managed cabbage fields showed improved soil health indicators like soil bulk density, pH, conductivity, urease and nitrate reductase activity all within their naturally

managed system (Liao et al., 2019).

Microbial responses to plant rhizodeposition

Plants grown in monocultured agroecosystems play major roles by feeding soil microbes the same rhizodeposited compounds over subsequent years. Repeated instances of the same compounds (or microbially available carbon sources) can result in conditioning native soil microbiota for repeated introduction of the same plants. Both agrochemical usage and plant rhizodeposition contribute to the implementation of a concept coined as "soil memory" (Lapsansky et al., 2016). As such, rhizodeposition is of a critical nature regarding plant-microbe interactions, and there is speculation surrounding a degree of specificity between plant genotype and responding microbes. For example, benzoxazinoid-compounds (secondary metabolites deposited by maize roots) were seen to alter root-associated microbiota of maize (Hu et al., 2018). The resulting microbial communities also benefited the next generation of maize, shown by reduced herbivore consumption of plants when grown in the same sites (Hu et al., 2018). Another study shows that when root exudates from arabidopsis were applied to soil samples previously growing arabidopsis, the exudates could maintain populations of the resident fungal community (Broeckling et al., 2008). These studies highlight the transfer of developed microbiota when the same crops (and same rhizodeposition products) are re-introduced. Other studies have been designed to demonstrate how plant rhizodeposition can alter the transcriptome of bacteria. Applying rhizodeposits collected from two distinct sugar beet varieties to *Pseudomonas aeruginosa* PA01 resulted in distinct alterations to the *P. aeruginosa* PA01 bacterial transcriptome (Mark et al., 2005). Application of different rhizodeposits induced altered metabolism, chemotaxis, type III secretion, and up or down-regulated certain homologues

showing similarities to homologues related to pathogenic and beneficial bacterial function (Mark et al., 2005).

The plants ability to properly synthesize these root-secreted compounds is strongly dependent on the overall health of the plant. When fit, plants can maintain a balanced rhizosphere community through the steady production of microbially-available carbon-based resources and are unable to do so in times of jeopardized health (Olanrewaju et al., 2019; Jalali & Suryanarayana et al., 1971). In return for these carbon-based resources, rhizomicrobiota are able to perform several symbiotic functions for plants (Olanrewaju et al., 2019; Manter & Stewart, 2015).

Plant rhizo-microbiome assemblage

Plant rhizodeposition is the primary form of communication used to interact with their biotic and abiotic surroundings (Pantigoso et al., *in press*). Certain carbon cycling processes, biochemical cycling of N and P in the rhizosphere, as well as other functions in nutrient sequestration (e.g. siderophore production) can all be traced back to plant-beneficial microbiota (Manter & Stewart, 2015). Additional studies show that a plant's physiological development may be manipulated by its associated microbiota, demonstrated by inducing earlier flowering periods in Arabidopsis via the application of a rhizospheric microbial consortium associated with early-flowering times (Panke-Buisse et al., 2015). It has been further shown that plant resource requirement is subject to change through different developmental stages, and overtime altered plant rhizodeposition products were observed to recruit differential bacteria to fit their needs (Chaparro et al., 2014). Early colonizers of the Arabidopsis rhizosphere had N-cycling related genes, later stages of plant development recruited several plant growth promoting rhizobacterial

(PGPR)-related genera, such as members of *Bacillus, Burkholderia, Cyanotheaceae* and *Bradyrhizobium* (Chaparro et al., 2014).

Importance of plant-soil feedback/plant-microbe-microbiome relationships

Plants may also upregulate their rhizodeposition in times of abiotic stress (Liu & Brettell, 2019), and this is sometimes demonstrated as increased phenolic compound production. Drought has been shown to increase plant rhizodeposition, as well as alter the metabolomic profiles of exuded carbohydrates, amino acids or other nutrients (Jones et al., 2019). Badri et al. (2013) demonstrated that bacterial and fungal community composition was shifted in pair with the supplementation of different compounds derived from Arabidopsis rhizodeposits (Badri et al., 2013). It has also been speculated that a plant's rhizomicrobiota may affect formation of rhizodeposition products (Mueller & Sachs, 2015), and that plants send signals into their rhizospheres when aboveground mechanical or insect herbivory damage occurs to recruit microbes up-regulated plant defense systems (Pineda et al., 2017).

Soil microbial dysbiosis and disease conducive soils

Over long periods of monoculture, plant pathogenic microbes can become high in abundance (Arafat et al., 2019), indirectly cultivating disease-conducive soils for the next generation of plants. Not only do disease conducive soils develop as a result of monoculturing but may also be formed when farming practices are unequipped to support the health and diversity of soil microbiota. Several of these microbially-antagonistic farming practices have led to reductions in soil drainage, structure, organic matter and nutrient availability (Lapsansky et al., 2016; Dorr de Quadros et al., 2012). Applying antimicrobial chemicals to infected systems can reduce populations of non-target and/or plant-benefiting taxa responsible for the recycling of plant detritus, soil nutrients or soil organic matter (Malik et al., 2017). Although some soil

microbes can degrade certain agrochemical- contamination (Calvayrac et al., 2012), only microbes able to withstand these intense conditions can proliferate, leading to imbalanced or dysbiotic communities. Imbalanced soil microbial communities can be indicative of diseased agroecosystem states. Studies have shown that a comparison of microbiota between diseased vs healthy sites reveals highly distinct bacterial composition and diversity, with the healthy soils possessing more-desirable microbial community conditions (Wang et al., 2017). Wang et al. (2017) also shows how non-diseased soils have higher occurrences of beneficial bacterial gene functions, as well as increased P and K nutrient availability (Wang et al., 2017).

Combating soil dysbiosis

Cover cropping and crop rotation

The use of multi-species cover crops within agroecosystems has resulted in plant-benefiting soil biological activity. It has also been shown that cover cropping can increase soil organic carbon supply, as well as increase fungal species richness (Hengen et al., 2018; Bramachari et al., 2017). Plants of different genotypes possess the ability to release differential signals into soils, and as such microbiota of different phylogenies exhibit distinct preferences for their exuded carbon. Thus, providing microbes with diverse exudation compounds (via planting diversity/crop rotation) increases microbial diversity due to responses from different plant signals. Ai et al. (2018) shows that in addition to beneficial fungal responses, providing diverse carbon sources via crop rotation also prompted variations in organic carbon content and soil aggregate distribution (Ai et al., 2018).

Disease suppressive soils

There are two types of disease suppression in soils: general suppression, which is based on competitiveness of the resident soil microbiota (Raaijmakers and Mazzola, 2016). Or the

contrary: specific suppression, which is attributed to the enhancement of microbes antagonistic to the specific causal agents of plant disease (Raaijmakers and Mazzola, 2016). Practices such as crop rotation are one of several tailored towards the development of disease suppressive soils. In support of this notion, Peralta et al. (2018) shows how increased crop rotational diversity significantly influenced bacterial community composition in polycultured agroecosystems (Peralta et al., 2018). The authors noted that their highest crop diversity plot (corn + soy + wheat + 2 cover crops) increased disease suppressive functional genomics (e.g. *prnD* gene abundance), compared to the monoculture system (Peralta et al., 2018). They additionally compared communities in fallow to those in cover crop treatments, showing that plant presence strongly increased soil disease suppressive capacity (Peralta et al., 2018). Hence, disease suppression occurring in soils originates back to the resident plant-recruited microbial populations (Expósito et al., 2017; Lapsansky et al., 2016). Recent 'omics technologies have provided new insights toward the identification of microbial consortia related to soilborne disease suppression (Exposito et al., 2017). For example, previous studies using metatranscriptomic analyses of a suppressive soil have linked members of the family Burkholderiaceae to disease-suppression (Exposito et al., 2017). It has been speculated that general disease suppression is attributed to the sulfurous volatile compound produced by *Paraburkholderia gramminis* PHS1 (Carrión et al., 2018). When soils are not conducive for disease suppression, populations of pathogenic microbes can become very high in abundance. In certain sites experiencing severe disease(s), a microbial disruption (e.g. steam or chemical sterilization) within an agroecosystem may be optimal to downgrade microbial fitness, later allowing for plants to re-colonize a more-healthy rhizosphere microbiome (Li et al., 2019).

Microbial disruption

It is known than a plant mediated selection of its rhizosphere community occurs over time and is based on microbiota available for recruitment (Mueller and Sachs, 2015). As a comparison, a human host selects for microbiota available based on the diet consumed (Zmora et al., 2018). In certain instances of systems experiencing severe microbial imbalances or dysbiosis, a drastic population reduction of microbiota may be recommended to alleviate such states. Although the off-target effects are often unintended, this population reduction is best demonstrated in human hosts by administering a course of antibiotics (Saust et al., 2016). Whereas in soil systems an application of heat or chemical sterilization may be recommended for states of severe plant disease (Li et al., 2019; Rokunuzzaman et al., 2016). This initial population reduction of microbes allows the host to preferentially recruit new microbes to fill niches left behind after the disruption (Li et al., 2019; Zmora et al., 2018). While steam-heat sterilization has variable effects on bacterial DNA in general (Yap et al., 2013), it has been shown a sterilization event reduces microbial community competition for organic matter and can provide a starting reservoir for plant 10acterial communities to be re-introduced to those sites.

The goals of this thesis

The goal of this research was to understand plant-soil microbial associations following soil disruption in order to promote soil health and agricultural ecosystem productivity. The goal was achieved by conducting two experiments in which an initial soil microbiome disruption was completed using autoclave steam sterilization, followed by the introduction of native and non-native plants to a variety of distinct agroecosystem soils. The first study used soils from a diseased peach orchard agroecosystem in attempts to alleviate disease and restore soil microbial balance. The second study applied microbiome disruption to three distinct agroecosystem soils

(conventional, organic, diseased) and subsequently introduced plants from four distinct crop families (Poaceae, Amaranthaceae, Asteraceae and Solanaceae) to examine rearrangements in rhizobacterial communities. The application of outside disruption used in both experiments provided seminal insights on the ability for a plant to recruit potential keystone species and generalist PGPRs shared by all crops after disruption.

REFERENCES

Ai, Chao, Shuiqing Zhang, Xin Zhang, Doudou Guo, Wei Zhou, and Shaomin Huang. (2018) "Distinct Responses of Soil Bacterial and Fungal Communities to Changes in Fertilization Regime and Crop Rotation." Geoderma. Elsevier

Arafat, Yasir, Muhammad Tayyab, Muhammad Umar Khan, Ting Chen, Hira Amjad, Saadia Awais, Xiangmin Lin, Wenxiong Lin, and Sheng Lin. (2019) "Long-Term Monoculture Negatively Regulates Fungal Community Composition and Abundance of Tea Orchards." Agronomy 9, no. 8. 466.

Badri, Dayakar V, Jacqueline M Chaparro, Ruifu Zhang, Qirong Shen, and Jorge M Vivanco. (2013) "Application of Natural Blends of Phytochemicals Derived from the Root Exudates of Arabidopsis to the Soil Reveal That Phenolic-Related Compounds Predominantly Modulate the Soil Microbiome." The Journal of biological chemistry. American Society for Biochemistry and Molecular Biology

Bakker, Matthew G., Daniel K. Manter, Amy M. Sheflin, Tiffany L. Weir, and Jorge M. Vivanco. (2012) "Harnessing the Rhizosphere Microbiome through Plant Breeding and Agricultural Management." Plant and Soil 360, no. 1-2 (2012): 1–13. https://doi.org/10.1007/s11104-012-1361-x.

Bonanomi, Giuliano, Marreo Lorito, Francesco Vinale, and Sheridan L Woo. (2018) "Organic Amendments, Beneficial Microbes, and Soil Microbiota: Toward a Unified Framework for Disease Suppression." Annual Reviews.

Bramhachari, P. V., Ganji Purnachandra Nagaraju, and E. Kariali. (2017) "Metagenomic Approaches in Understanding the Mechanism and Function of PGPRs: Perspectives for Sustainable Agriculture." SpringerLink. Springer, Singapore

Broeckling, Corey D, Amanda K Broz, Joy Bergelson, Daniel K Manter, and Jorge M Vivanco. (2008) "Root Exudates Regulate Soil Fungal Community Composition and Diversity." Applied and environmental microbiology. American Society for Microbiology (ASM)

Calvayrac, Christophe, Fabrice Martin-Laurent, Alexia Faveaux, Nathalie Picault, Olivier Panaud, Camille-Michel Coste, Hanene Chaabane, and Jean-François Cooper. (2012) "Isolation and Characterisation of a Bacterial Strain Degrading the Herbicide Sulcotrione from an Agricultural Soil." Pest management science. U.S. National Library of Medicine

Carrión, Víctor J., Viviane Cordovez, Olaf Tyc, Desalegn W. Etalo, Irene de Bruijn, Victor C. L. de Jager, Marnix H. Medema, Leo Eberl, and Jos M. Raaijmakers. (2018) "Involvement of Burkholderiaceae and Sulfurous Volatiles in Disease-Suppressive Soils." Nature News. Nature Publishing Group

Chaparro, Jacqueline M, Dayakar V Badri, and Jorge M Vivanco. (2014) “Rhizosphere Microbiome Assemblage Is Affected by Plant Development.” The ISME journal. Nature Publishing Group

Chowdhury, Ashim, Saswati Pradhan, Monidipta Saha, and Nilanjan Sanyal. (2008) “Impact of Pesticides on Soil Microbiological Parameters and Possible Bioremediation Strategies.” Indian journal of microbiology. Springer-Verlag,

Dorr De Quadros, Patricia, Kateryna Zhalnina, Austin Davis-Richardson, Jennie R. Fagen, Jennifer Drew, Cimelio Bayer, Flavio A.o. Camargo, and Eric W. Triplett. (2012) “The Effect of Tillage System and Crop Rotation on Soil Microbial Diversity and Composition in a Subtropical Acrisol.” Diversity 4, no. 4 (2012): 375–95. https://doi.org/10.3390/d4040375.

Edmondson, Jill L., Zoe G. Davies, Kevin J. Gaston, and Jonathan R. Leake. (2014) “Urban Cultivation in Allotments Maintains Soil Qualities Adversely Affected by Conventional Agriculture.” Besjournals. John Wiley & Sons, Ltd.

Expósito, Gómez, Ruth, de Bruijn, Irene, Postma, Joeke, and Jos M. (2017) “Current Insights into the Role of Rhizosphere Bacteria in Disease Suppressive Soils.” Frontiers. Frontiers

Franzluebbers, A.J. (2002) “Soil Organic Matter Stratification Ratio as an Indicator of Soil Quality.” Soil and Tillage Research. Elsevier.

Haig, Sarah-Jane, Christopher Quince, Robert L. Davies, Caetano C. Dorea, and Gavin Collins. (2015) “The Relationship between Microbial Community Evenness and Function in Slow Sand Filters.” mBio. American Society for Microbiology.

Harrison, Joshua G., Thomas L. Parchman, Daniel Cook, Dale R. Gardner, and Matthew L. Forister. (2018) “A Heritable Symbiont and Host-Associated Factors Shape Fungal Endophyte Communities across Spatial Scales.” Besjournals. John Wiley & Sons, Ltd.

Hassani, M.A., Durán, P. & Hacquard, S. (2018) Microbial interactions within the plant holobiont. Microbiome 6, 58 (2018). https://doi.org/10.1186/s40168-018-0445-0

Haygarth, P.M. & Ritz, K. (2009) The future of soils and land use in the UK: soil systems for the provision of land-based ecosystem services. Land Use Policy, 26S, S187–S197.

Hendgen, Maximilian, Björn Hoppe, Johanna Döring, Matthias Friedel, Randolf Kauer, Matthias Frisch, Andreas Dahl, and Harald Kellner. (2018) “Effects of Different Management Regimes on Microbial Biodiversity in Vineyard Soils.” Nature News. Nature Publishing Group.

Hu, Lingfei, Christelle A. M. Robert, Selma Cadot, Xi Zhang, Meng Ye, Beibei Li, Daniele Manzo, et al. (2018) “Root Exudate Metabolites Drive Plant-Soil Feedbacks on Growth and Defense by Shaping the Rhizosphere Microbiota.” Nature News. Nature Publishing Group.

Jalali, Bushan L., and D. Suryanarayana. (1971) “Shift in the Carbohydrate Spectrum of Root Exudates of Wheat in Relation to Its Root-Rot Disease.” Plant and Soil 34, no. 1 (1971): 261–67. https://doi.org/10.1007/bf01372783.

Jones, Piet, Benjamin J Garcia, Anna Furches, Gerald A Tuskan, and Daniel Jacobson. (2019) “Plant Host-Associated Mechanisms for Microbial Selection.” Frontiers in plant science. Frontiers Media S.A.

Lakshmanan, Venkatachalam, Gopinath Selvaraj, and P. Bais. (2014) “Functional Soil Microbiome: Belowground Solutions to an Aboveground Problem.” Plant Physiology. American Society of Plant Biologists.

Lapsansky, Erin R, Arwen M Milroy, Marie J Andales, and Jorge M Vivanco. (2016) “Soil Memory as a Potential Mechanism for Encouraging Sustainable Plant Health and Productivity.” Current Opinion in Biotechnology. Elsevier Current Trends.

Li, Kun, Michael J. DiLegge, Ioannis S. Minas, Alison Hamm, Daniel Manter, and Jorge M. Vivanco. (2019) “Soil Sterilization Leads to Re-Colonization of a Healthier Rhizosphere Microbiome.” Rhizosphere. Elsevier.

Li, Xiangzhen, Junpeng Rui, Yuejian Mao, Anthony Yannarell, and Roderick Mackie. (2013) “Dynamics of the Bacterial Community Structure in the Rhizosphere of a Maize Cultivar.” Soil Biology and Biochemistry. Pergamo.

Liao, Jianli, Liang, Yun, Huang, and Danfeng. (2018) “Organic Farming Improves Soil Microbial Abundance and Diversity under Greenhouse Condition: A Case Study in Shanghai (Eastern China).” MDPI. Multidisciplinary Digital Publishing Institute.

Liao, Jianli, Xu, Xu, Huilian, Huang, and Danfeng. (2019) “Natural Farming Improves Soil Quality and Alters Microbial Diversity in a Cabbage Field in Japan.” MDPI. Multidisciplinary Digital Publishing Institute.

Liu, Hongwei, and Laura E Brettell. (2019) “Plant Defense by VOC-Induced Microbial Priming.” Trends in plant science. U.S. National Library of Medicine.

Malik, Zaffar, Maqshoof Ahmad, Ghulam Hassan Abassi, Muhammad Dawood, Azhar Hussain, and Moazzam Jamil. (2017) “Agrochemicals and Soil Microbes: Interaction for Soil Health.” Soil Biology. Xenobiotics in the Soil Environment.

Manter, Daniel K, and Catherine Stewart. (2015) “GIBBs: A New Soil Biology Index to Quantify Beneficial Bacteria in the Soil.” Publication: USDA ARS. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America Meeting

Mark, G. Louise, J. Maxwell Dow, Patrick D. Kiely, Hazel Higgins, Jill Haynes, Christine Baysse, Abdelhamid Abbas, et al. (2005) “Transcriptome Profiling of Bacterial Responses to

Root Exudates Identifies Genes Involved in Microbe-Plant Interactions." PNAS. National Academy of Sciences.

Montgomery, David R. (2007) "Soil Erosion and Agricultural Sustainability." PNAS. National Academy of Sciences,

Mueller, U.G., and J.L. Sachs. (2015) "Engineering Microbiomes to Improve Plant and Animal Health." Trends in Microbiology. Elsevier Current Trends.

Nicomrat D, Tharajak J. and Kanthang P. (2016) "Pesticides Contaminated in Rice Paddy Soil Affecting on Cultivated Microorganism Community." Applied Mechanics and Materials. Trans Tech Publications Ltd.

Olanrewaju, Oluwaseyi Samuel, Ayansina Segun Ayangbenro, Bernard R. Glick, and Olubukola Oluranti Babalola. (2018) "Plant Health: Feedback Effect of Root Exudates-Rhizobiome Interactions." Applied Microbiology and Biotechnology 103, no. 3 (2018): 1155–66.
Panke-Buisse, Kevin, Angela C Poole, Julia K Goodrich, Ruth E Ley, and Jenny Kao-Kniffin. (2014) "Selection on Soil Microbiomes Reveals Reproducible Impacts on Plant Function." Nature News. Nature Publishing Group.

Pantigoso H.A., Yanhui H. DiLegge M.J., Vivanco J.M. (in press) Methods for Root Exudate Collection and Analysis. In The Plant Microbiome: Methods and Protocols. Springer Nature Switzerland (in press)

Peralta, Ariane L., Yanmei Sun, Marshall D. McDaniel, and Jay T. Lennon. (2018) "Crop Rotational Diversity Increases Disease Suppressive Capacity of Soil Microbiomes." The Ecological Society of America. John Wiley & Sons, Ltd.

Pineda, Ana, Ian Kaplan, and T Martijn Bezemer. (2017) "Steering Soil Microbiomes to Suppress Aboveground Insect Pests." Trends in plant science. U.S. National Library of Medicine.

Raaijmakers JM, and Mazolla M. (2016) "Soil Immune Responses." Science. American Association for the Advancement of Science.

Robinson, D.A. & Sutherland, W.J. (2002) Post-war changes in arable farming and biodiversity in Great Britain. Journal of Applied Ecology, 39, 157–176.

Rokunuzzaman, Md, Ayumi Hayakawa, Shinzo Yamane, Sota Tanaka, and Kouhei Ohnishi. (2016) "Effect of Soil Disinfection with Chemical and Biological Methods on Bacterial Communities." Egyptian Journal of Basic and Applied Sciences. No longer published by Elsevier.

Saust, Laura Trolle, Rikke Nygaard Monrad, Malene Plejdrup Hansen, Magnus Arpi, and Lars Bjerrum. (2016) "Quality Assessment of Diagnosis and Antibiotic Treatment of Infectious

Diseases in Primary Care: a Systematic Review of Quality Indicators." Scandinavian journal of primary health care. Taylor & Francis.

Schauss, Kristina, Andreas Focks, Holger Heuer, Anja Kotzerke, Heike Schmitt, Sören Thiele-Bruhn, Kornelia Smalla, et al. (2009) "Analysis, Fate and Effects of the Antibiotic Sulfadiazine in Soil Ecosystems." TrAC Trends in Analytical Chemistry. Elsevier.

Schmidt, J.E., Kent, A.D., Brisson, V.L. et al. (2019) Agricultural management and plant selection interactively affect rhizosphere microbial community structure and nitrogen cycling. Microbiome 7, 146.

Schuster, Evi, and D. Schröder. (2002) "Side-Effects of Sequentially- and Simultaneously-Applied Pesticides on Non-Target Soil Microorganisms: Laboratory Experiments." Soil Biology and Biochemistry. Pergamon.

Shao, Hainan, and Yalin Zhang. (2017) "Non-Target Effects on Soil Microbial Parameters of the Synthetic Pesticide Carbendazim with the Biopesticides Cantharidin and Norcantharidin." Nature News. Nature Publishing Group.

She, Siyuan, Jiaojiao Niu, Chao Zhang, Yunhua Xiao, Wu Chen, Linjian Dai, Xueduan Liu, and Huaqun Yin. (2017) "Significant Relationship between Soil Bacterial Community Structure and Incidence of Bacterial Wilt Disease under Continuous Cropping System." Archives of Microbiology 199, no. 2 (2017): 267–75. https://doi.org/10.1007/s00203-016-1301-x.

Wang J.J., Li X.Y., Zhu A.N., Zhang X.K., Zhang H.W., Liang W.J. (2012): Effects of tillage and residue management on soil microbial communities in North China. Plant Soil Environ., 58: 28-33.

Wang, Rui, Hongchun Zhang, Liguang Sun, Gaofu Qi, Shu Chen, and Xiuyun Zhao. (2017) "Microbial Community Composition Is Related to Soil Biological and Chemical Properties and Bacterial Wilt Outbreak." Nature News. Nature Publishing Group.

Wei, Zhong, Ville-Petri Friman Yian Gu, George A. Kowalchuk, Yangchun Xu, Qirong Shen, and Alexandre Jousset. (2019) "Initial Soil Microbiome Composition and Functioning Predetermine Future Plant Health." Science Advances. American Association for the Advancement of Science.

Yang, Dennis Tao, and Xiaodong Zhu. (2013) "Modernization of Agriculture and Long-Term Growth." Journal of Monetary Economics. North-Holland. January 26.

Yap, J.M., C.E. Goldsmith, and J.E. Moore. (2013) "Integrity of Bacterial Genomic DNA after Autoclaving: Possible Implications for Horizontal Gene Transfer and Clinical Waste Management." Journal of Hospital Infection. W.B. Saunders.

Yuan, Jun, Jacqueline M. Chaparro, Daniel K. Manter, Ruifu Zhang, Jorge M. Vivanco, and Qirong Shen. (2015) "Roots from Distinct Plant Developmental Stages Are Capable of Rapidly

Selecting Their Own Microbiome without the Influence of Environmental and Soil Edaphic Factors." Soil Biology and Biochemistry. Pergamon.

Zmora, Niv, Jotham Suez, and Eran Elinav. (2018) "You Are What You Eat: Diet, Health and the Gut Microbiota." Nature News. Nature Publishing Group, September 27, 2018. https://www.nature.com/articles/s41575-018-0061-2.

CHAPTER 2: Soil sterilization leads to re-colonization of a healthier rhizosphere microbiome[1]

Synopsis

Soil sterilization is often applied in cropping systems to remove soil-borne pathogens and nematodes. In an effort to better understand the role of sterilization on natural microbial communities and resultant plant growth, the interaction between soil sterilization and the growth of three different crops (one native crop, peach; and two non-native crops, corn and tomato) was examined in a soil known to have peach replant disease. Soil sterilization significantly increased plant growth in all crop species. The recovery of the microbial communities following sterilization was dependent upon the presence of a living plant and the final community structure differed between crop species. In all crops grown in the sterilized soil, there were common plant beneficial microbial functions that were promoted after sterilization: N fixation, P solubilization, biological control, or root growth promotion. Despite the well documented effect of soil sterilization to remove pathogens, it is also a potential means to promote rapid changes in soil microbial communities and the apparent promotion of beneficial microbes as well as a healthier soil microbiome.

[1]Li*, K., DiLegge*, M. J., Minas, I. S., Hamm, A., Manter, D.K., & Vivanco, J. (2019) Soil sterilization leads to re-colonization of a healthier rhizosphere microbiome. *Rhizosphere* https://doi.org/10.1016/j.rhisph.2019.100176. (*both authors contributed equally)

Introduction

Plants interact very closely with different kinds of microbes in the rhizosphere (Berg et al., 2005; Lynch, 1990; Raaijmakers, 2009). Some of the beneficial microorganisms that are present in the rhizosphere include nitrogen-fixing bacteria, endo-and ectomycorrhizal fungi, and plant growth-promoting rhizobacteria (PGPR) (Raaijmakers, 2009). These microbes strongly contribute to the provision of nutrient uptake by plants through nitrogen fixation (Van Rhijn and Vanderleyden, 1995; Raymond et al., 2004; Santoyo et al., 2017), P solubilization (Vassilev et al., 2006) and some can be antagonistic against plant pathogens (Berendsen et al., 2012; Hernández-León et al., 2015). Harmful microorganisms present in the rhizosphere include phytopathogenic fungi, oomycetes, bacteria, nematodes, and microbes that compete with the plant for nutrients (Van der Heijden et al., 2008).

In nature, pathogenic and beneficial microorganisms are usually kept at balance in the rhizosphere (Badri et al., 2009). However, this balance can be disrupted by biotic and abiotic stressors; causing shifts in microbial equilibrium, which can influence the growth and health of plants (Azcón-Aguilar and Barea, 1992; Linderman, 2000). Crop plants grown under intensive conditions (i.e. monocultures, high amounts of nutrient input, etc.) tend to exhibit an unbalanced microbiome which, in most cases favors microbes that are pathogenic and/or ones that compete with plants for nutrients (Van der Heijden et al., 2008; Baker et al., 2012; Yang et al., 2012; Hodge and Fitter, 2013; Shushen et al., 2016). Such an imbalance in the rhizosphere microbiome is very noticeable in re-planted tree fruit orchards and is usually referend to as orchard replanting disease (Wu et al., 2017; Zhao et al., 2016). Replanting disease is expressed symptomatically via stunted growth, low plant productivity and a decline in tree vigor leading to shortened economic life (Rutto and Mizutani, 2006). Replanting disease has become a major production problem of

the tree fruit industry – primarily due to the limited available lands suitable for tree fruit cultivation. Annual losses (due to subsequent reduction in yield and orchard longevity) are estimated to be (in severe cases) up to 20% (McKenry, 1999; Smith, 1994). There are a variety of factors that can be attributed to replanting disease, but the plant pathogenic component is the most prevalent (Proebsting and Gilmore, 1940; Israel et al., 1973; Mizutani, 1980; Gur-Coehen, 1984; Chandler, 1969; Mountain and Patrick, 1959; Chitwood, 1949). For example, fungi of the genera *Cylindrocarpon* and *Rhizoctonia*, as well as the oomycetes *Phytophthora* and *Pythium* are considered to be essential in the etiology of apple replant disease (Kelderer et al., 2012; Tewoldemedhin et al., 2011; Franke-Whittle et al., 2015). For peach replanting disease, Fusarium species (*F. equiseti, F.moniliforme, F. oxysporum,* and *F.solani*), *Alternaria tenuis*, *Myrothecium verrucaria* and *Mycelia sterilia* are the main soil-borne fungal pathogens (Wensley, 1956). For decades, soil fumigation with methyl bromide was the preferred methodology to control replanting disease, particularly prior to the re-planting of older orchards (Stirling et al., 1995; Smith, 1994). However, the use of methyl bromide and other types of chemical soil sterilizer agents were phased out by the U.S. Environmental Protection Agency in 2005. Use of replant disease-tolerant rootstocks has been a valuable alternative for apple replant disease control. However, the available replant disease-tolerant peach rootstocks promote excessive tree vigor, which is associated with delayed acclimation and reduced fruit quality (Minas et al., 2018), further increasing the impact of replant disease on peach productivity.

Soil sterilization has been shown to change the microbial properties of the rhizosphere and to promote plant growth (Eayre et al., 2000; Tian, 2009; Qin et al., 2014), presumably by reducing the inoculum of soil-borne plant diseases (Johnson, 1937; Weststeijn, 1973; Yim et al.,

2013). However, other mechanisms might be involved in the promotion of plant growth. Numerous methods of soil sterilization are available including heat (e.g., dry heat and autoclaving), chemical biocides (e.g., sodium azide, formaldehydes, propylene oxide, chloroform and methyl bromide) and r-irradiation (Kale and Raghu, 1982; Lotrario et al., 1995; Trevors, 1996; McNamara et al., 2003; Yamamoto et al., 2008; Mahmood et al., 2014). Soil sterilization is currently regarded as an unpopular methodology because it kills all soil life (Lawrence, 1956). However, the "biological desert" may be restored by re-colonization of certain microbes, to create a different microbial community in terms of cell numbers, activity and genetic structure (Wertz et al., 2007). Marshner and Rumberger (2004) found that bacterial communities rapidly re-colonize sterilized soil, and during re-colonization, the community structure changes towards higher diversity and evenness. In addition, Zhang (2016) reported that soil sterilization could change the composition and structure of soil microbial communities and increase the Shannon-Wiener diversity index of rhizosphere soil. Troelstra et al. (2001) suggested that the increased plant growth observed in sterilized soils is usually ascribed to the elimination of (often unidentified) soilborne pathogens, and that the re-colonization of the soil micro-flora following soil sterilization may produce some plant (root) growth promotion effects. Until recently, most studies about the effects of soil sterilization on rhizosphere soil microbes have been conducted using culture-dependent methods, and few studies used Denaturing Gradient Gel Electrophoresis (DGGE) and Ribosomal Intergenic Spacer Analysis (RISA) (Marschner and Rumberger, 2004; Wertz et al., 2007). Here, we evaluated the effects of orchard soil sterilization on the growth of peach saplings, as well as corn and tomato seedlings, and determined the re-arrangements of the soil bacterial community after the growth of each individual crop. The goal of this study was to determine if the effect of soil sterilization on the soil microbiome was consistent for a variety of

crop plants. This study also aided in determining which bacterial species were responsive to heat stress (from sterilization), as well as what beneficial functions may be enriched post sterilization of the soil.

Methods

Experimental soil – chemical analysis and treatment

Two different soils were used in this study: soil associated with peach replanting-disease and a soil without peach replanting-disease problems (control soil). The replanting disease soil was collected from a ten-year-old peach orchard (cv. 'Cresthaven' grafted onto 'Lovell' rootstock, Colorado State University's Experimental Orchard at Western Colorado Research Center in Orchard Mesa, CO) exhibiting replanting disease symptoms, at a depth of 20–50 cm nearby the tree trunks – the same as peach tree rhizosphere. The non-replanting disease soil was collected from a block that has grown hay (*Medicago sativa*) for the 10 years prior to soil collection and the block was located within the same experimental orchard. All soils were sifted through a No. 10 metal sieve (2-mm wide) prior to use in greenhouse studies. Standard chemical analyses of the sieved soils were conducted by the Soil, Water and Plant Testing Laboratory at Colorado State University in groups of three replicates per soil type. After sieving large debris out of it, the non-replanting disease soil received no further treatment and was referred to as control soil. The replant soil was then divided into two equal parts. The first half of the replanting disease soil was used directly and referred to as replant soil. The other half of the soil was sterilized using a STERIS brand autoclave for three 15-min liquid cycles at 121 °C. In both trials, soils exposed to the previous autoclaving conditions were referred to as sterile replant soil; although there were low, but detectable levels of bacterial DNA after autoclaving (see results for further information). Sub samples of both the sterile replant and replant soils were collected and

used for later DNA extraction to examine the effects of sterilization on the microbiome prior to implementing the outside plant-growth input.

Trial 1 – experimental setup

Soil (400 g dry weight) from each treatment was added to individual plastic pots (7 x 10 x 8.5 cm). Peach seedlings of the 'Lovell' rootstock cultivar (the same cultivar as the orchard where replant soil was collected) were obtained from Burchell Nursery Inc, in Fowler, CA. Seeds were sown in liners using potting media and kept in a greenhouse for up to 30 days. Potting media for seedling germination was called 'Burchell Canning Mix' and consisted of a peat moss and bark mixture, and substrate was not fumigated or pasteurized prior to distribution. Peach seedlings were then selected based on uniformity and were transplanted into individual plastic pots (n=10) with either: i) replant soil, ii) sterile replant soil, or iii) control soil (non-replant). For this trial (Trial 1), all pots contained a plant (i.e., no unplanted controls). All seedlings were watered daily with 60 ml of tap water. The experiment was conducted at Colorado State University's Horticultural Center Greenhouse facility, under an average temperature of 22.99 °C (29.44 °C being the maximum and 20.56 °C being the minimum temperatures). Pot location in the greenhouse were randomized via the use of the Research Randomizer program (*https://www.randomizer.org*). The peach experiment (Trial 1) was conducted over the course of an 11-week growing period. Due to sufficient available nutrient content in each of the soils used in this experiment, plants were not fertilized during growth. In the eleventh week, plants and rhizosphere soil were harvested from each of the 10 peach repetitions per treatment and analyzed as described below

Trial 2 – experimental setup

The replant disease soils were collected from the same location as previously mentioned in section 2.1, but from a later time period. Soil collection, sieving, sterilization, and chemical analyses were also the same as in section 2.1. However, prior to growing any plants, sub samples of the replant and sterile replant soil were stored at−20 °C and used for later DNA extraction to examine the effects of plant growth on the soil microbiome. Both sterile replant and replant soils (400 g dry weight) from each treatment were added to individual plastic pots (7 x 10 x 8.5 cm). Corn P0474XR, HXX/LL/RR2 (HXX contains both the Herculex I and Herculex RW genes, LL contains the Liberty Link® gene for resistance to Ignite® herbicide, RR2 contains the Roundup Ready® Corn 2 trait) and tomato (Burpee, 3750G.53) seeds were placed into Petri dishes with moistened filter paper and germinated in a growth chamber set at 26 °C. Corn and tomato plants were then transplanted into pots in an effort to maintain height uniformity across soil treatments. Heights of corn and tomato plants were measured on days 7, 14, 21, 28, 35 and 42 by the use of a ruler over the course of 6 weeks. The experiment was conducted at Colorado State University's Horticultural Center Greenhouse facility, under an average temperature of 23.74 °C (31.11 °C being the maximum and 20.56 °C being the minimum temperatures). Due to sufficient available nutrient content in each of the soils used in this experiment, plants were not fertilized during growth. The placement of the plant pots in the greenhouse were randomized via the use of the Research Randomizer program (*https://www.randomizer.org/).*

Plant biomass

Trial 1 was harvested after 11 weeks of plant growth. Trial 2 was harvested after 6 weeks of plant growth. In both trials, the above ground, below ground and total fresh mass of all plants were recorded immediately upon harvesting. The above-ground axis and belowground axis were

washed free of soil prior to fresh weight measurement. The rhizosphere soil (defined as soil attached to the roots after gentle shaking by hand) was also collected during harvest. For rhizosphere soil collection, soil that was adhered to the root was shaken off, and only the soil adhering to the roots (0–4 mm) was considered rhizosphere soil and was collected via gentle brushing of the roots (Luan et al., 2009). The rhizosphere soil samples were then transported to the laboratory and stored at −20 °C for subsequent DNA extraction. Following fresh weight measurements and rhizosphere soil collection, each plant was placed into individual paper bags,

Soil microbiome analysis

Prior to the greenhouse trials, soil DNA samples of the replant soil were collected before and after sterilization, and again after 11 or 6 weeks of greenhouse growth with either peach or corn/tomato, respectively. Of the ten plant replicates, two plant rhizosphere soil samples were combined for a total of five rhizosphere samples per treatment. Total genomic DNA (gDNA) from 0.25 ± 0.004 g of each pooled rhizosphere soil were extracted using the E.Z.N.A Soil DNA Kit (Omega Bio-Tek, Norcross, GA USA) according to the manufacturer's instructions with a final elution volume of 100 µl. The concentration of nucleic acids as well as purity of DNA samples were quantified via the use of a NanoDrop spectrophotometer and stored at −80 °C prior to microbiome analysis. The final concentration of nucleic acids ranged from 7.2 ± 2.89 ng/uL and was diluted 1:20 with molecular water to reduce PCR inhibitors.

The 16S rRNA gene was quantified and amplified for sequencing preparation based on Illumina's 16S Metagenomic Sequencing Library Preparation protocol (http://support.illumina.com). Briefly, a first round of qPCR used primers specific for the V3-4 hypervariable region with Illumina MiSeq specific attached adapter sequences for multiplexing: forward 5′ *TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGC*CTACGGGNGGCWGCAG-

3′ and reverse 5′

*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*GACTACHVGGGTATCTAATCC- 3′

with adapter sequences denoted in italics. The master mix consisted of 2 µL sample genomic DNA, 10 µL of 2X Maxima SYBR Green (Thermo Scientific, Waltham, MA, USA), and 2 µL each (10 µM) of forward and reverse primers for a total 20 µL reaction mix. The PCR thermal cycling conditions were as follows: 95 °C for 5 min, 30 cycles of 95 °C for 40 s, 55 °C for 120 s, 72 °C for 60 s, and a final annealing at 72 °C for 7 min. The resulting amplicons were purified using an in-house preparation of solid phase reversible immobilization (SPRI) magnetic beads based on the modifications of Faircloth and Glenn (2011) and original protocol of Rohland and Reich (2012). A standard curve using purified *Psuedomonas putida* KT2440 was run with the samples to quantify the starting rRNA copies g−1 soil fresh weight (FW).

A second round of PCR was performed to attach unique Illumina Nextera XT index sequences for each sample. 5 µL of first round PCR product, 25 µL of 2X Maxima SYBR Green (Thermo Scientific, Waltham, MA, USA), 10 µL water and 5 µL each of forward and reverse indices were combined for a total of 50 µL, and amplified at 95 °C for 3 min, 8 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, followed by final annealing of 72 °C for 5 min. The resulting PCR product was bead cleaned using SPRI beads, and quantified using 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 amplicon size and purity, 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 we used the Illumina MiSeq Reagent Kit v3 600-cycle for library dilution and loading onto the MiSeq (Illumina, San Diego, USA). The

library was denatured with 0.2 N NaOH, diluted to 15 pM using provided HT1 buffer, and spiked with 20% PhiX control prior to loading and running on the MiSeq.

De-multiplexed raw fastq files were processed with DADA2 (R Bioconductor package, Callahan et al., 2016) implementation included in the open source bioinformatics tool myPhyloDB version 1.2.1 (Manter et al., 2016). Briefly, all primers were removed from each sequence using the open source Python program Cutadapt (Martin et al., 2011) and sequence variants were inferred using the default pipeline in DADA2. Each sequence variant identified in DADA2 was classified to the closest reference sequence contained in the Green Genes reference database (Vers. 13_5_99) using the usearch_global option (minimum identity of 97%) contained in the open source program VSEARCH (Rognes et al., 2016). A total of 589,601 high quality sequence reads were obtained, resulting in an average of 14,740 reads per sample. Each taxonomic profile was used to determine bacterial phyla- or gene-specific abundances. Gene-specific bacterial abundances are based on a comprehensive list of functions associated with soil bacteria. The selected genes from the KEGG database (Kanehisa and Goto, 2000) incorporate processes such as nutrient cycling and availability, biocontrol of pathogens, and stress tolerance (Supplemental Table 4). The taxonomic mapping and phylogenetic reconstruction necessary to identify the population of each gene-specific abundance was performed using myPhyloDB's implementation of PICRUSt (Langille et al., 2013).

Statistical analysis

All analyses are based on plant and soil samples collected at harvest, which occurred 11 weeks after planting in the treated (replant or sterile replant) soils for trial 1 (peach) or 6 weeks after planting in the treated soils for trial 2 (corn, tomato). Significant differences in plant growth (root or shoot biomass) or bacterial abundances (total, phyla, and gene specific abundances) were

tested by one-way analysis of variance (ANOVA) and means were separated by Tukey's multiple comparison tests ($p < 0.05$) using DPS software vers. 7.05 (Tang and Zhang, 2013). In trial 2, significant differences in biomass were analyzed by two-way ANOVA with crop and soil treatment as main factors followed by Tukey's multiple comparison tests ($p < 0.05$).

Differences in the overall bacterial community composition due to crop and soil treatments were analyzed using both Principle Coordinates Analysis (PCoA, Bray-Curtis distances calculated using DADA2 sequence variants abundances) and tested for significance by perMANOVA (adonis) and treatment centroids calculated using betadisper, both functions are part of R's vegan package. A single PCoA was run combining both trials for visual comparison and centroid calculations; whereas, an independent perMANVOA was run for each crop species or the no-plant control.

Results

Soil nutrient analysis

The NO3–N, available P and available K content were measured for the control soil, replant soil, and sterile replant disease soil. In the sterile replant soil, the total NO3–N, available P and available K were 4.01 mg/kg, 15.17 mg/kg, and 258 mg/kg, respectively. In the replant soil, the total NO3–N, available P and available K were 5.57 mg/kg, 9.23 mg/kg, and 279.33 mg/kg, respectively. In the control soil (non-replant), the total NO3–N, available P and available K were 7.57 mg/kg, 13.5 mg/kg, and 215 3 mg/kg, respectively (Supplemental Table 1). There was a noticeable reduction in both the NO3–N and available K contents of the soil after sterilization, while the available P was increased in the sterilized replant soil (Supplemental Table 1).

Trial 1 - peach seedling growth

Peach biomass was significantly higher when grown in the sterile replant soils as compared to either the replant soil or the control soil treatment. Pictorial biomass differences of peach seedlings can be viewed in Figure 2-1a. Soil sterilization appeared to remove all negative impacts of replanting disease on plant growth resulting in an increase of 130% (aboveground dry weight biomass) and 249.4% (belowground dry weight biomass) as compared to the replant soil treatment (Figure 2-1, b and c). A similar, but smaller, increase in biomass was also observed when comparing the sterile replant soil to the control (non-replanting) soil. For example, plant growth was increased by 65.57% (aboveground dry weight biomass) and by 54.30% (belowground dry weight biomass) in the sterilized replant soil as compared to the control (non-replanting disease) soil treatment (Figure 2-1, b and c).

Trial 2 - corn and tomato growth

There were significant differences observed in growth between corn and tomato seedlings planted in the replant soil and sterile replant soils. Soil sterilization significantly ($p < 0.05$) increased the height of both plants at each sample date over the six-week period, and the difference in plant height increased with time (Supplemental Figs. 1a and 1b). Analysis of both fresh and dry-weight biomass at the end of the experimental course confirmed that soil sterilization significantly ($p < 0.05$) increased above- and below-ground biomass (Figure 2-2a and b). For corn, plant biomass was increased by 126.70% (aboveground) and 184.20% (belowground) when grown in sterile replant soil as compared to plants grown in replant soil. When comparing plant biomass in sterile replant soil to control soil, there was a 161.0% (aboveground) and 166.5% (belowground) increase for corn seedlings (Fig 2-2,a and b).

For tomato plants, both fresh and dry weight biomass was significantly higher in plants grown in sterile replant soil as compared with those from replant soils (Figure 2-3, a and b). For example, comparing the biomass of tomato grown in replant disease soil to sterile replant soil there was an increase of 103.7% and 104.7% for above and belowground biomass, respectively. When comparing the biomass of tomato grown in the control soil to sterile replant disease soil, there was an increase of 94.9% and 91.2% for above and below ground biomass, respectively (Figure 2-3, a and b).

Microbiome analysis

16S rRNA Abundance

In trial 1, the peach rhizosphere microbiome that developed in the replant disease soil showed significantly higher bacterial abundance compared to the sterile replant disease soil (Figure 2-4a). Similar results were observed in trial 2 for corn and tomato (Figure 2-4b). In trial 2 an additional soil control (with no plants) was added, and under this condition the microbial biomass in the sterilized counterpart of the control soil was not detectable. An increase in copies of 16s rRNA can be observed in the sterilized plant growth treatments compared to the sterilized no-plant control, suggesting a large plant contribution to the bacterial re-colonization of soil after sterilization (Figure 2-4b).

Principal coordinate analysis of rhizosphere microbiota

The effects of soil sterilization on soil microbial communities from the crops studied were determined by Illumina 16s rRNA gene sequencing analysis. A Principal Coordinates Analysis (PCoA) comprised of both trials was used to visually compare the community structural differences between treatments and displays 42.6% of the total phylogenetic variation in the data (25% x-axis, 17.6% y-axis [Figure 2-5]). After DNA extraction, all sterilized soils were

significantly different than their non-sterile counterparts based on perMANOVA analysis (peach, p=0.008; corn, p=0.006; tomato, p=0.009; no-plant control, p=0.015); however, the distances between each of these groups differed dramatically. For example, the Euclidean distance between the centroids were 0.06, 0.75, 0.79, and 0.89 for peach, tomato, corn, and no-plant controls, respectively. Thus, although still significantly different, when peach was grown in the sterilized replant soil the bacterial community recovered to its original state to the greatest degree (Figure 2-5). However, for the other two crops and the no-plant control, the sterile replant disease soil microbiota remained drastically different than their non-sterilized counterparts (Figure 2-5).

Effect of soil sterilization on relative abundance of soil bacterial phyla

Similar to the PCoA analysis, phyla-specific relative abundances were more apparent when either corn or tomato crops were grown in sterile replant soil, as opposed to the peach. At the end of the peach trial, no significant differences ($p < 0.05$) in phyla-specific abundances between the two soil treatments were observed (data not shown). However, significant differences in phyla were observed for both corn and tomato (Table 2-1). For tomato plants in sterile replant soil, the following phyla experienced significant increases in relative abundance compared to the replant disease soil: FBP, Proteobacteria, Firmicutes, and Verrucomicrobia. Additionally, the relative abundances of Acdiobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Nitrospirae and Planctomycetes were decreased in the sterilized soil. For the corn plants, the relative abundance of members of Bacteroidetes, FBP, Proteobacteria and Verrucomicrobia were significantly increased; whereas, members of Acdiobacteria, Actinobacteria, Chloroflexi, Nitrospirae and Planctomycetes experienced significant reductions

in relative abundance. For comparison, the no-plant controls showed that after sterilization the microbial community was dominated by Proteobacteria (80.7%) and Actinobacteria (13.7%).

Effect of soil sterilization on gene specific bacterial abundances

The presence of beneficial bacteria in each of the soils was performed by phylogenetic reconstruction and the proportion of the community with each of the selected genes (Table 2-2) determined. For peach, although there were observable increases in relative abundances of select PGPR-related genes, only *phzE* and *pqqC* were significantly different between the soil treatments (p=0.009) (Supplemental Table 3).

For the corn plants, the relative abundance of the following gene specific bacterial populations were significantly increased ($p < 0.05$) after corn plants were grown for 6 weeks in the sterilized soil: C-decomposition (*bglX*), N-decomposition (*ureC*), P-decomposition (*phoD*, *phoN*), S-decomposition (*aslA*), P-solubilization (*pqqC*), biological control ability (*budA*, *hcnA*) and increased root growth (*acdS*). The relative abundances of the following gene-specific bacterial populations were significantly ($p < 0.05$) reduced with corn grown in sterilized soils: C decomposition (*bglB*), P-decomposition (*E3.1.3.2*), and N-fixation (*nifDK*), biological control ability (*phzE*) and siderophore production (*entA*) (Table 2-2).

For the tomato plants, the relative abundance of the following genes were significantly increased ($p < 0.05$) after 6 weeks: N-decomposition (*ureC*), P-decomposition (*phoA*, *phoD*, *phoN*), S-decomposition (*aslA*), P-solublization (*pqqC*), biological control ability (*E3.2.1.14*, *budA*, *hcnA*), root growth stimulation (*acdS*, *ipdC*) and siderophore production (*pchB*). The relative abundance of the following gene-specific bacterial populations were significantly ($p < 0.05$) reduced after growing tomato in sterilized replanting disease soil: P-decomposition (*E3.1.3.2*), and N-fixation (*nifDK*) (Table 2-2).

When comparing the sterilized replant disease soils to non-sterilized replant soil in the absence of plants (i.e., no-plant control), the relative abundance of the following gene-specific bacterial populations increased significantly ($p < 0.05$) post-sterilization: N-decomposition (*ureC*, *amiE*), P-decomposition (*E3.1.3.2*), P-solubilization (*pqqC*), root growth (*acdS*), and S-decomposition (*aslA*) (Table 2) and the following populations significantly ($p < 0.05$) decreased due to sterilization: Cdecomposition (*E.3.2.1.21*, *bglB*, *bglx*), P-decomposition (*appA*, *phoA*, *phoD*, *phoN*), N-fixation (*nifDK*, *nifH*), biological control (*E3.2.1.14*, *hcnA*, *phzE*), root growth (*ipdC*) and siderophore production (*entA*, *mbtI*, *pchB*) (Table 2-2).

Discussion

Trial 1 – microbial causalities of replanting disease

In this study, it was clear that soil sterilization increased plant growth parameters independent of crop species. This growth promotion effect has historically been attributed to pathogen removal and resultant disease suppression (Sosnowski et al., 2009; Katan, 1981; Savory, 1966). In the peach replanting disease soil we utilized, the growth of peach seedlings was severely stunted. Predictably, the peach seedlings grown in sterile replanting soil expressed significantly increased plant growth; presumably, due to the removal of replant disease associated pathogens (Munro, 2018; Sosnowski, 2009; Katan, 1981; Savory, 1966). Peach replant disease has been linked with many different pathogens, mostly fungi and oomycetes. However, soil-based estimates of pathogens may not be the best estimate for quantification of disease development, as these estimates ignore plant susceptibility and/or climate constraints. Furthermore, based on our chosen assay (16S rRNA amplicon sequencing) and strata (soil) we cannot make any inferences about changes in fungal or oomycete pathogen presence.

Interestingly in our study, the hydrogen cyanide synthase (*hcnA*) gene was significantly increased when either corn or tomato plants were grown in the sterile replanting soil, but not when peach was reintroduced (Table 2-2). We are aware of previous studies that have identified cyanogenic bacterial members (*Pseudomonas* and *Bacillus*) in disease suppressive soils (Tsegaye et al., 2017; Zdor, 2014; Spence et al., 2014; Yang et al., 2017; Ahmad et al., 2008). Our results show that both of these bacterial genera were higher when growing corn and tomatoes in sterile replant soil (*Pseudomonas* – 0.056%, *Bacillus* – 0.019%) as opposed to the replant disease soil where both were grown (*Pseudomonas* – 0.015%, *Bacillus* – 0.008%). Notably, not all species within the genera *Pseudomonas* and *Bacillus* produce hydrogen cyanide (HCN), so we utilized a gene specific approach to estimate the population of potential cyanogenic bacteria. The *hcnA* bacterial populations in these soils were 0.056% and 0.004% for the sterile and replant disease soils, respectively. Furthermore, while cyanide may be phytotoxic at sufficient levels it has also been suggested to serve as a tool for biocontrol abilities of some *Pseudomonas* and *Bacillus* species, used as an antimicrobial (Blumer and Haas, 2000; Radhakrishnan et al., 2017).

The microbiome analysis of Trial 1 showed that there were no significant phyla-level differences between rhizospheres of peach plants growing in replant or sterile replant soils (Figure 2-5). Badri and Vivanco (2009) demonstrated that microbes residing within agroecosystem soils become conditioned to the root exudates of whichever plant is commonly cultivated there (Badri and Vivanco, 2009). The former study shows that when either the native plant or it's root exudation compounds in isolation are re-introduced to a soil, the habituated microbes are quick to respond and colonize those niches (Badri and Vivanco, 2009). Therefore, when growing a non-native plant (corn or tomato) in the sterile replant disease soil, cyanogenic

bacteria such as Pseudomonas and Bacillus spp. are assumed to be first to compete for those empty niches via the production of an unfavorable environment due to HCN synthesis.

Trial 2 – introduction of non-native plants

Therefore, we also examined the effect of sterilization in this same soil on two very different crop species. Interestingly, both corn and tomato also showed significant growth increases following sterilization. As mentioned, replant disease is a complex of many different pathogens making it difficult to determine if pathogen changes in the soil are responsible for the growth increases in corn and tomato. However, the commonality of the growth response suggests either that general pathogen activity was high in the original soil or sterilization tends to promote a soil microbial community that is enriched in beneficial bacteria for a wide variety of crops.

After the 6-week growth of corn or tomato plants in sterilized replanting disease soil, each had a significant increase in the relative abundance of three bacterial phyla: Proteobacteria, Verrucomicrobia and FBP. The phylum Proteobacteria consists of mostly gram-negative members (Yang et al., 2017) as well as diazotrophs (Dommelen and Vanderleyden, 2007) and has been documented extensively as plant growth promoting rhizobacteria (PGPR). These PGPR actions include increases in plant-growth (Ali et al., 2015) and the ability to fix nitrogen and to produce aromatic polycyclic hydrocarbons for plants (Yang et al., 2017; Bruto et al., 2014). Research on Verrucomicrobia, a historically difficult-to-culture phylum (Kalam et al., 2017), is lacking although it has been observed to be ubiquitous throughout many soil types (Bergmann et al., 2011). The candidate phylum FBP, or Abditibacteriota (proposed by Tahon et al., 2018) also consists of ubiquitous members and this phylum is often observed to be widespread in extreme environments (Tahon et al., 2018). Originally recovered from an Antarctic lichen, Abditibacteriota have been found in both polar and desert ecosystems (Tahon et al., 2018). With

an exception to Proteobacteria, the relative abundance of these phyla were not increase in the control soil (without any plant input) after sterilization; suggesting that the plants favored the re-colonization of a highly desirable microbiome once the competition was removed via sterilization. Interestingly, many members of the bacterial family Firmicutes and Proteobacteria were increased in relative abundance in the sterilized soil treatments (Table 2-1). However, Firmicutes are thermotolerant spore-forming bacteria that can survive autoclaving and contain thermophilic members such as Hydrogenophilalia, and Proteobacteria have been observed to be the dominant phylum in a geothermal hot spring (Müller et al., 2013; Filippidou et al., 2016). The ability to tolerate high heat conditions likely explains the increased relative abundance of Firmicutes and Proteobacteria after autoclaving.

The identification of gene-specific beneficial bacterial populations in the soil provided important insights on increased relative abundance of bacterial genomes that have been recorded to contain specific PGPR related genes. The selected genes in this analysis perform the following six functions: N-decomposition (*ureC*), P-decomposition (*phoD*, *phoN*), S-decomposition (*aslA*), P-solubility (*pqqC*), bio control ability (*budA*, *hcnA*), and root growth (*acdS*) (Table 2-2) (Manter et al., 2016). The relative abundances of these genes were increased after growing corn and tomato in sterilized replant soil compared to those plants grown in replant disease (non-sterile) soil. The *ureC* gene belongs to the urease gene cluster and could be promoted in environments of limited nitrogen sources (Heimer et al., 2002; Cussac et al., 1992). The *phoD* and *phoN* genes involve phosphorous decomposition and are activated upon phosphorous starvation to code for phosphatase production, which increases the bioavailability of P for the plant (Rodrigues et al., 2014; Kier et al., 1979). The *aslA* gene encodes for arylsulfatases, which are enzymes that break down aromatic sulfate esters, subsequently releasing inorganic S into the soil (Schmalenberger

and Fox, 2015). The gene *pqqC* encodes for pyrroloquinoline quinone biosynthesis and is often contained within phosphorous solubilizing *Pseudomonas* spp. bacteria (Meyer et al., 2011). The root-associated PGPR and biocontrol bacterium *Bacillus amyliloquifaciens* has been observed to express the *budA* gene, coding for 2,3-butandiol compound synthesizes within the soil (Chowdhury et al., 2015; Bruto et al., 2014). This compound has been observed to induce systemic defense responses in pepper plant (Kong et al., 2018). Similarly, the biocontrol agent *Pseudomonas fluorescens* CHAO contains the *hcnA* gene and produces hydrogen cyanide synthase within the soil (Laville et al., 1998). Lastly, the *acdS* gene encodes for 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Heydarian et al., 2016). We hypothesize that the abiotic stress brought upon by soil sterilization may promote bacteria that contain the *acdS* gene; coding for ACC deaminase production in the rhizosphere. These bacteria are able to promote growth in plants experiencing abiotic stress via the removal of the amino groups from ACC, which is a precursor to stress hormone ethylene. Stromberger et al. (2017) observed that populations of ACC deaminase-producing bacteria increase in soils that lack moisture (Stromberger et al., 2017). In our experiment, we presume that dry soil autoclaving forced the bacterial populations to undergo exposure to extreme heat, subsequently promoting the relative abundance of ACC deaminase-producing bacteria.

While plant growth promotion seems to stem from microbiological input, the nutrient analyses of soils used in this study are worth mentioning (Supplemental Table 1). The results show an increase in available P post-sterilization, while simultaneously showing that both available NO3–N and K for the soil were reduced after sterilization (Supplemental Table 1). In contrast, Sinegani and Sedri (2011) show that heat sterilization reduced soil available P (Sinegani and Sedri, 2011). The literature suggests that autoclaving soils will result in an increase in the

bioavailability of nutrients like NO3–N, while the soil used in this study expressed a decrease in NO3–N after heat sterilization (Skipper and Westermann, 1973, Mahmood et al., 2014). Our findings align with those of Mahmood et al. (2014) showing that heat sterilization applied to soils reduces available K (Mahmood et al., 2014). So, it seems that the effect of heat sterilization on soil nutrients is variable and depends on the soil type.

Reshaping soil microbial communities

Autoclave sterilization displaced a myriad of soil borne microorganisms, leaving many empty niches for other microorganisms to fill. Yap et al. (2013) has demonstrated that autoclaving has variable effects on damaging gDNA from different bacteria (Yap et al., 2013). Our Figure 2-4a shows that we did have detectable measures of bacterial gDNA in soils post sterilization; however, whether this soil was isolated from thermotolerant bacterial spores or if it was intact in the soil after cell lysis is undetermined. In peach, the microorganisms habituated to the exudates (or food) provided by the peach pants were rapid to re-colonize the rhizosphere. To contrast, we changed the "food source" for these microbes by introducing non-native plants (corn and tomato), and we show significant differences in the rhizospheres of both crops. The act of heat sterilization combined with the introduction of a non-native plant successfully caused a dramatic shift in the rhizobacterial communities and allowed for increased PGPR competition as a result of microbial reduction via sterilization.

The PCoA (Figure 2-5) shows that the peach plants grown in sterilized soil were able to re-colonize their rhizosphere with a highly similar microbiome that was present prior to sterilization. This could be due to the close association between peach roots, root exudates and the soil microbial community that develops over a long period of co-adaptation (Badri and Vivanco, 2009). Microbial community variation in the rhizosphere has been previously reported

to depend on the age of plants, crop species and soil type (Badri and Vivanco, 2009). Additionally, Broeckling et al. (2007) suggested that crop species compose their soil microbiome via the mechanism of root exudation. Similar to the microbial colonization of peach plants in our study, Broeckling et al. (2007) found that *Arabidopsis* and *Medicago* maintained its own fungal community when grown in their resident soils, and interestingly observed the same fungal responses when applying isolated *Arabidopsis* or *Medicago* root exudates into the same soil. Along these lines, we suggest that the microbiota present in peach orchard replanting disease soils are habituated to the below-ground presence of peach plants and are quickly re colonized after soil sterilization and peach plant growth.

It is clear that corn and tomato were able to re-shape their rhizosphere in ways not attained by peach seedlings. Similar to our study, Qiu et al. (2013) reported increased bacterial inoculant colonization of cucumber plant rhizosphere when the inoculant bacterium was applied in combination with carbendazim, an effective soil fungicide (Qiu et al., 2013). Hence, the de-coupling of the root microbiome via steam sterilization and/or chemical fumigation may assist in colonization by beneficial microbes (Qiu et al., 2013).

In summary, here we demonstrate that soil sterilization combined with the introduction of a non-native plant successfully aids in the restoration of eubiosis in the soil microbial habitat. Our results suggest that the population reduction of microbes brought upon via heat sterilization can be rapidly recovered with the influence of a plant. Additionally, depending on the nature of co-adaptation between the plant and the soil microbiome, the new microbial community that develops after sterilization might become dramatically altered. However, we hypothesize that in any case the new community might contain beneficial microbes, potentially selected for by the plant. In agronomic ecosystems, we presume mimicking the effects observed could be achieved

via soil solarization. Thus, soil solarization/sterilization could be used as a methodology to select and isolate beneficial microbes specific to certain crops.

Figures

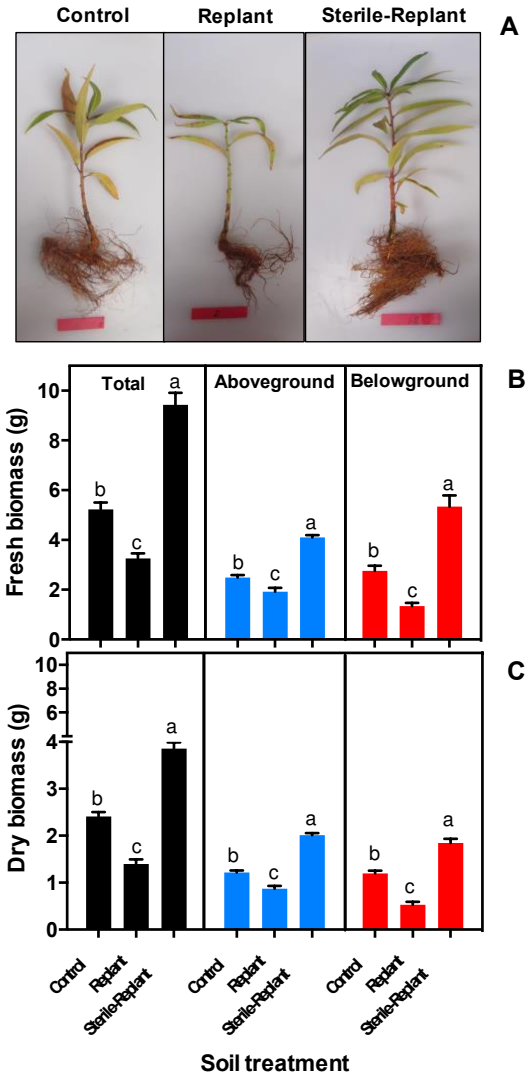


Figure 2-1: Plant growth results of peach seedlings grown in in non-replanting disease (control) soil, as well as replanting and sterile replanting disease soils (Trial 1). Growth was monitored for eleven weeks; each bar represents the mean of 10 replicates (n=10) ± standard deviation (error bars). Different letters indicate significant differences ($p < 0.05$). (A): Photographic differences in plant growth of peach seedling treatments. (B**):** Above, below and total fresh-weight biomass of peach seedlings grown in three soil treatments. (C): Above, below and gross dry-weight biomass of peach seedlings grown in three soil treatments.

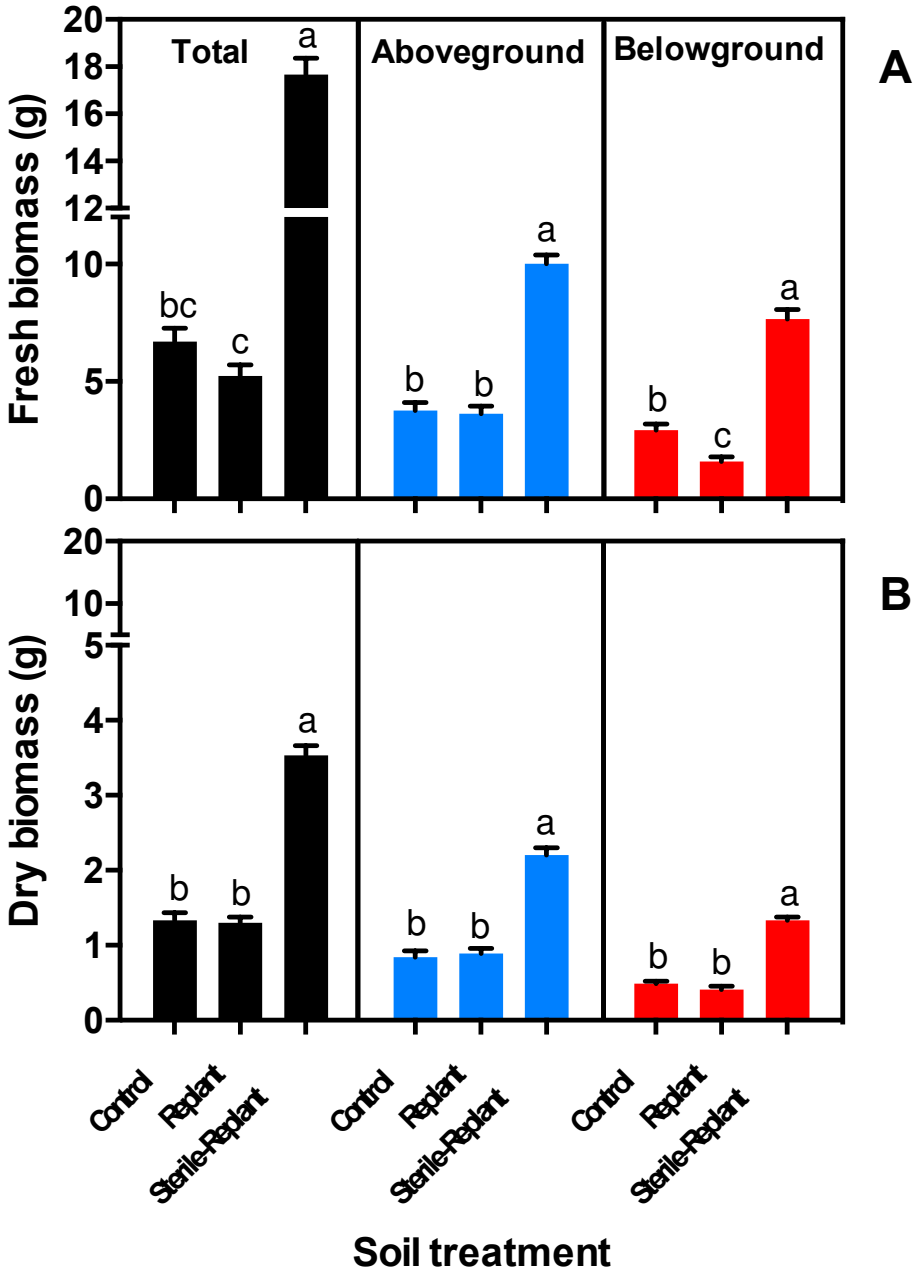


Figure 2-2: Corn plant growth results from Trial 2. Growth was monitored for six weeks; each bar represents mean for 10 replicates (n=10) ± standard deviation (error bars). Different letters indicate significant differences ($p < 0.05$). (A): Above, below, and total fresh-weight biomass of corn plants grown in three soil treatments. (B): Above, below and gross dry-weight biomass of peach seedlings grown in three soil treatments.

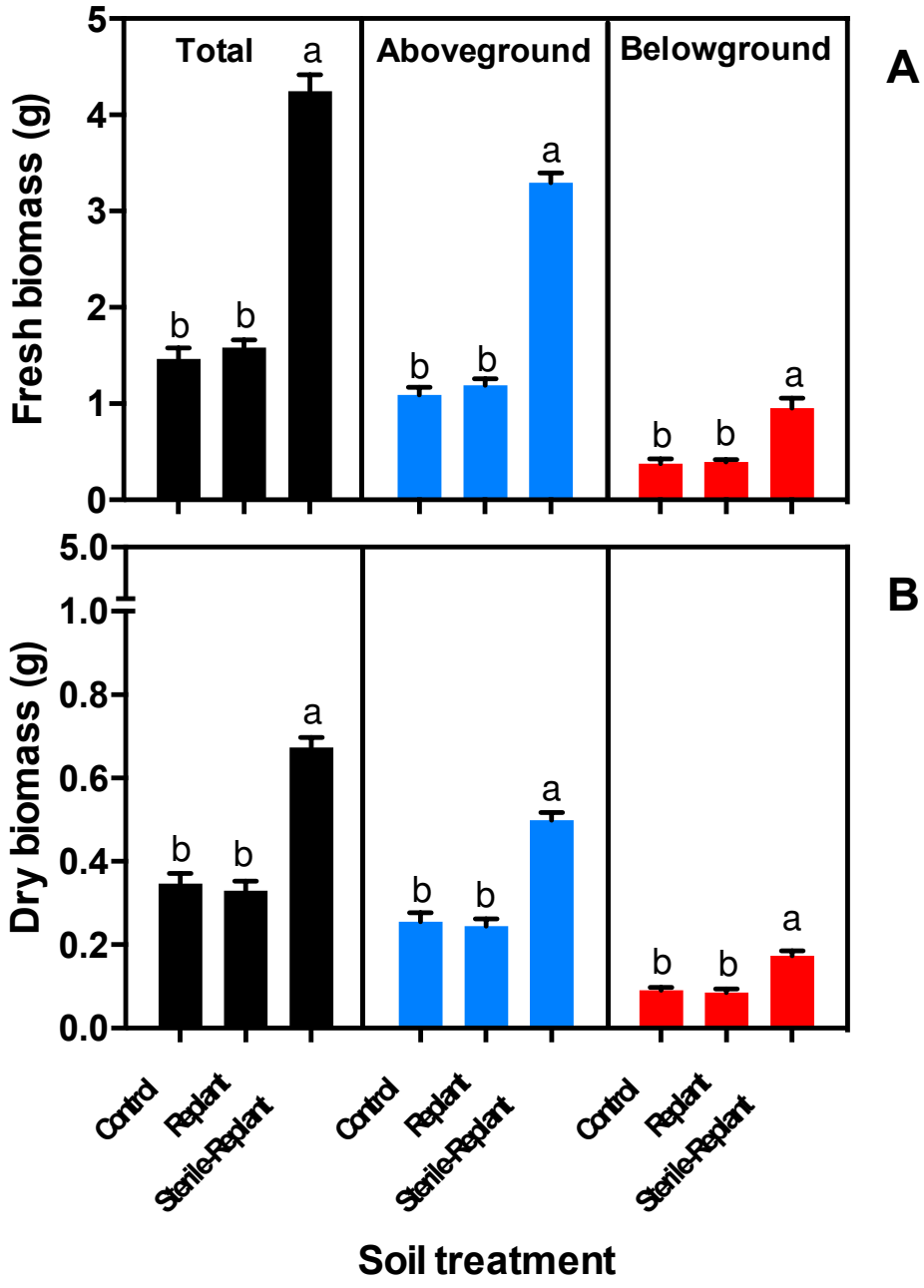


Figure 2-3: Tomato plant growth results from Trial 2. Growth was monitored for six weeks; each bar represents mean for 10 replicates (n=10) ± standard deviation (error bars). Different letters indicate significant differences ($p < 0.05$). (**A**)**:** Above, below, and total fresh-weight biomass of tomato plants grown in three soil treatments. (B): Above, below and gross dry-weight biomass of tomato plants grown in three soil treatments.

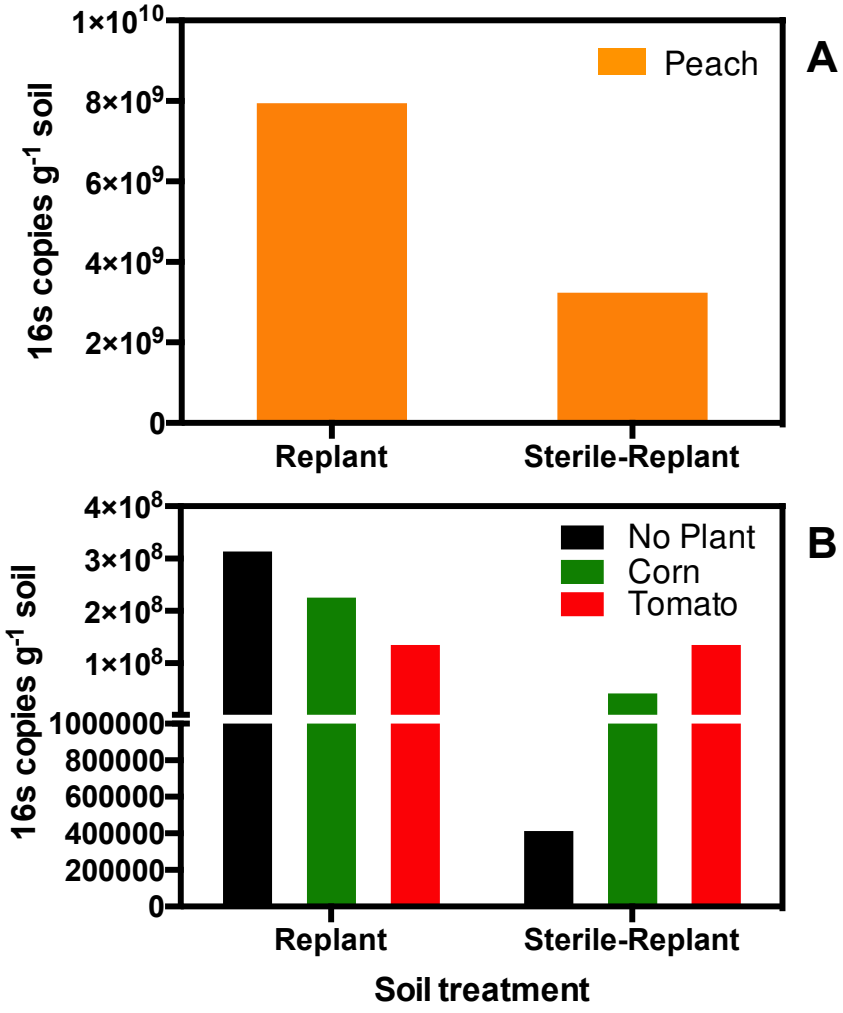


Figure 2-1: Differences in bacterial biomass (16s rRNA copies per g^{-1} soil) in sterile replanting soil compared to replanting disease soil. (A): Bacterial biomass (16s rRNA copies per g^{-1} soil l) results from Trial 1 with peach seedlings. (B): Bacterial biomass (16s rRNA copies per g^{-1} soil l) results from Trial 2 with replant disease-soil alone, as well as with corn and tomato plants grown for six weeks.

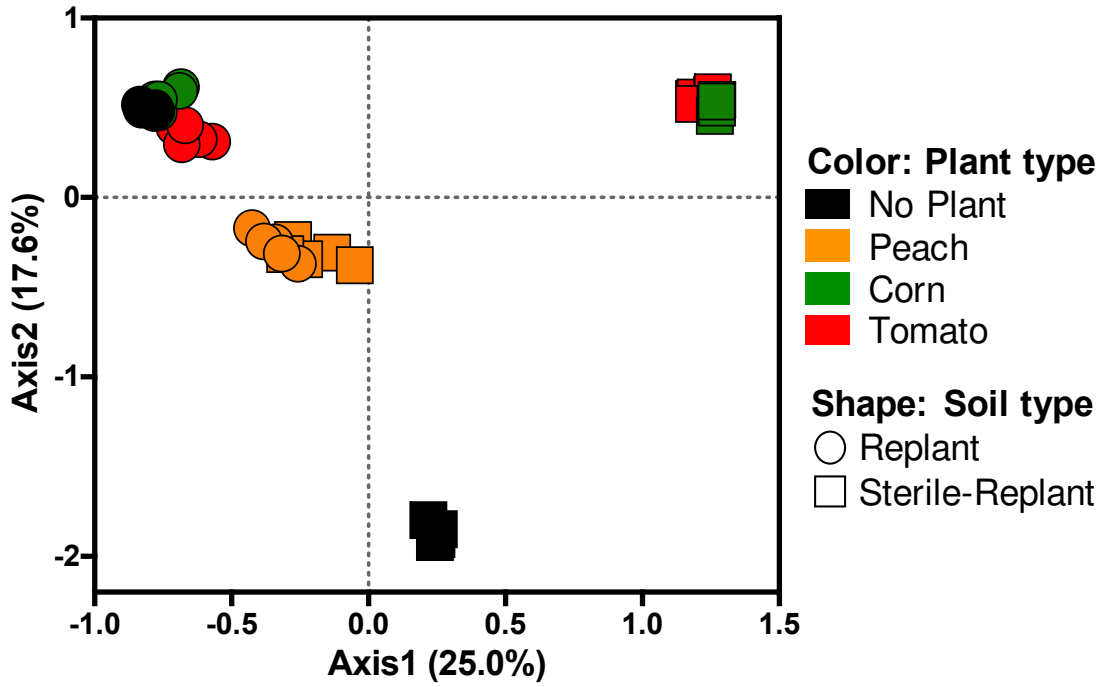


Figure 2-2:Principal Coordinate Analysis of rhizosphere microbiome of peach, corn, tomato and no-plant controls grown in sterilized and non-sterilized orchard replanting disease soil (Trials 1 and 2). Different crop treatments (or no plant samples) are indicated by different colors (black, orange, green and red for no plant, peach, corn or tomato respectively). Shapes of symbols (circle, square) indicate if the sample came plants grown in replanting disease soils or autoclaved "sterile" replanting disease soils.

Tables

Table 2-1:Effects of soil sterilization on relative abundance of 14 dominant bacterial phyla in sterilized and non-sterilized replanting disease soil alone, and with corn and tomato plants grown in the soils for 6 weeks each. Blocks highlighted in green or red represent an increase or decrease of the phylum for that row at the p=0.05 significance level, respectively.

	Corn			Tomato			No Plant-Control		
Phylum	Replant	Sterile Replant	p-value	Replant	Sterile Replant	p-value	Replant	Sterile Replant	p-value
FBP	0	0.010	<0.001	0	0.002	0.031	0	0	-
Verrucomicrobia	0.008	0.033	0.009	0.004	0.020	0.005	0.006	0	<0.001
Proteobacteria	0.289	0.604	<0.001	0.277	0.572	<0.001	0.195	0.807	<0.001
Acidobacteria	0.031	0.001	<0.001	0.051	0.002	<0.001	0.047	0.002	<0.001
Actinobacteria	0.326	0.092	<0.001	0.268	0.116	<0.001	0.372	0.137	<0.001
Chloroflexi	0.031	0	0.007	0.025	0	0.006	0.075	0	<0.001
Nitrospirae	0.011	0	<0.001	0.010	0	0.015	0.005	0	<0.001
Planctomycetes	0.020	0.004	0.073	0.015	0.008	0.032	0.012	0	<0.001
Bacteroidetes	0.011	0.058	<0.001	0.020	0.036	0.106	0.026	0.003	0.001
Cyanobacteria	0.004	0.008	0.141	0.076	0.029	0.280	0.006	0.004	0.652
Firmicutes	0.035	0.022	0.393	0.016	0.044	0.008	0.006	0.041	0.224
Gemmatimonadetes	0.015	0.011	0.177	0.018	0.007	0.043	0.019	0	<0.001
WS3	0	0	-	0.001	0	0.347	0.001	0	0.046
unclassified	0.218	0.156	0.020	0.218	0.163	0.214	0.230	0.005	<0.001

Table 2-2: Effects of soil sterilization on inferential gene relative abundance from the dominant bacterial phyla using the PICRUSt predictive algorithm. Comparisons were made between sterilized and non-sterilized replanting disease soil alone, and with corn and tomato plants grown in the soils for 6 weeks each. Blocks highlighted in green or red represent an increase or decrease for the gene representing that row at the p=0.05 significance level, respectively.

		Corn			Tomato			No Plant-Control		
Process	Gene	Replant	Sterile Replant	p-value	Replant	Sterile Replant	p-value	Replant	Sterile Replant	p-value
P Decomposition	*phoD*	0.551	0.727	<0.001	0.518	0.726	<0.001	0.637	0.314	<0.001
	phoN	0.039	0.102	<0.001	0.046	0.094	<0.001	0.049	0.017	0.039
Biocontrol	*budA*	0.005	0.012	0.010	0.004	0.011	0.049	0.004	0.075	0.114
	hcnA	0.003	0.220	<0.001	0.005	0.189	<0.001	0.005	0	0.005
N Decomposition	*ureC*	0.441	0.601	0.001	0.440	0.630	0.003	0.304	0.624	<0.001
S Decomposition	*aslA*	0.243	0.513	<0.001	0.202	0.482	<0.001	0.274	0.362	0.002
P Solubility	*pqqC*	0.143	0.359	<0.001	0.111	0.334	<0.001	0.091	0.321	<0.001
Root Growth	*acdS*	0.076	0.321	<0.001	0.068	0.265	<0.001	0.089	0.322	<0.001
N Fixation	*nifDK*	0.185	0.108	<0.001	0.213	0.084	0.035	0.128	0.005	<0.001
C decomposition	*E3.2.1.21*	0.149	0.153	0.807	0.182	0.160	0.186	0.172	0.026	<0.001
	bglB	0.555	0.365	<0.001	0.511	0.437	0.147	0.613	0.035	<0.001
	bglX	0.444	0.553	<0.001	0.504	0.529	0.578	0.552	0.331	<0.001
N Decomposition	*amiE*	0.710	0.732	0.464	0.700	0.753	0.281	0.707	0.771	0.014
P Decomposition	*E3.1.3.2*	0.168	0.091	<0.001	0.234	0.131	0.045	0.157	0.257	<0.001
	appA	0.054	0.068	0.145	0.073	0.061	0.322	0.037	0.013	0.038
	phoA	0.182	0.215	0.184	0.118	0.224	<0.001	0.195	0.054	<0.001
N Fixation	*nifH*	0.096	0.110	0.228	0.153	0.086	0.168	0.071	0.005	<0.001
Biocontrol	*E3.2.1.14*	0.413	0.418	0.895	0.388	0.465	0.048	0.502	0.011	<0.001
	phzE	0.024	0.011	0.008	0.022	0.009	0.106	0.060	0	<0.001
Root Growth	*ipdC*	0.041	0.052	0.069	0.032	0.055	0.016	0.089	0.020	<0.001
Siderophore	*entA*	0.080	0.031	<0.001	0.052	0.038	0.349	0.100	0.033	0.005
	mbtI	0.020	0.011	0.184	0.021	0.006	<0.001	0.006	0	<0.001
	pchB	0.015	0.022	0.142	0.024	0.047	0.014	0.016	0	<0.001

REFERENCES

Ahmad, F., Ahmad, I., and Khan, M. (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res.

Ali, G. S., Norman, D., and El-Sayed, A. S. (2015) Soluble and Volatile Metabolites of Plant Growth-Promoting Rhizobacteria (PGPRs). Advances in Botanical Research Plant Microbe Interactions,241-284.

Azcón-Aguilar C, Barea JM. (1992) Interactions between mycorrhizal fungi and other rhizosphere micro-organisms. In: Allen MJ, ed. Mycorrhizal functioning: an integrative plant–fungal process. New York: Chapman and Hall, 163–198.

Badri, DV, et al. (2013) Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. New Phytologist 198, 264-273

Badri, D., and Vivanco, J.M. (2009) Regulation and function of root exudates. Plant, Cell and the Environment32:666-681

Bakker MG, Manter DK, Sheflin AM, Weir TL, Vivanco JM. 2012. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. Plant and Soil 360:1-13 (Marschner Review)

Benizri, E., et al. (2005) Replant diseases: Bacterial community structure and diversity in peach rhizosphere as determined by metabolic and genetic fingerprinting. Soil Biology and Biochemistry 37:1738-1746.

Berendsen RL, Pieterse CMJ, Bakker P. 2012. The rhizosphere microbiome and plant health. Trends Plant Sci 17: 478- 486.https://doi.org/10.1016/j.tplants.2012.04.001

Berg G, et al. (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol Ecol 51: 215–229

Bergmann, G. T et al. (2011) The under-recognized dominance of Verrucomicrobia in soil bacterial communities. Soil Biology and Biochemistry,43(7), 1450-1455. doi:10.1016/j.soilbio.2011.03.012

Blumer, C., and Haas, D. (2000) Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Retrieved fromhttps://www.ncbi.nlm.nih.gov/pubmed/10763748

Broeckling, C. D., et al. (2007) Root Exudates Regulate Soil Fungal Community Composition and Diversity. Applied and Environmental Microbiology,74(3), 738-744. doi:10.1128/aem.02188-07

Bruto, M., et al. (2014) Analysis of genes contributing to plant-beneficial functions in plant growth-promoting rhizobacteria and related Proteobacteria. Scientific Reports, 4(1). doi:10.1038/srep06261

Callahan, B. J. et al. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods13, 581–583

Chaparro, J. M., et al. (2013) Root Exudation of Phytochemicals in Arabidopsis Follows Specific Patterns That Are Developmentally Programmed and Correlate with Soil Microbial Functions. PLoS ONE,8(2).doi:10.1371/journal.pone.0055731

Chowdhury, S. P. (2015) Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42 – a review. Frontiers in Microbiology,6. doi:10.3389/fmicb.2015.00780
Cussac, V., Ferrero, R. L., andLabigne, A. (1992) Expression of *Helicobacter pylori* urease genes in *Escherichia coli* grown under nitrogen-limiting conditions.

Dommelen, A. V., and Vanderleyden, J. (2007) Chapter 12: Associative Nitrogen Fixation. In The Biology of the Nitrogen Cycle (pp. 179-192). Elsiver B.V. doi: 10.1016/B978-0-444-52857-5.X5000-0

Eayre CG., et al. (2000) Evaluation of methyl iodide for control of peach replant disorder. Plant Disease 84, 1177–1179.

Faircloth, B and T Glenn. (2011) Serapure_v2.2. https://ethanomics.files.wordpress.com/2012/08/serapure_v2-2.pdf

Filippidou, S., et al. (2016) A Combination of Extreme Environmental Conditions Favor the Prevalence of Endospore-Forming Firmicutes. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5094177/

Franke-Whittle IH, et al. (2015) Rhizosphere bacteria and fungi associated with plant growth in soils of three replanted apple orchards. Plant and Soil, 395:317-333

Hartmann, A. (2008) Plant-driven selection of microbes. Plant and Soil,321(1-2), 235-257. doi:10.1007/s11104-008-9814-y

Heimer, S. R., et al. (2002) Urease of enterohemorrhagic Escherichia coli: Evidence for regulation by fur and a trans-acting factor.

Hernández-León R, Rojas-Solís D, Contreras-Pérez M et al. (2015) Characterization of the antifungal and plant growth-promoting effects of diffusible and volatile organic compounds produced by Pseudomonas fluorescens strains. Biol Cont 81: 83-92.

Hodge A. and Fitter AH. (2013) Microbial mediation of plant competition and community structure. Functional Ecology, 27, 865–875

Hu, L., et al. (2018) Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nature Communications ,9(1). doi:10.1038/s41467-018-05122-7

Heydarian, Z., et al. (2016) Inoculation of Soil with Plant Growth Promoting Bacteria Producing 1-Aminocyclopropane-1-Carboxylate Deaminase or Expression of the Corresponding *acdS* Gene in Transgenic Plants Increases Salinity Tolerance in *Camelina sativa*.

Johnson, (1937) Steam sterilization of soil for tobacco and other crops. farmers' bulletin No.1692.

Kale SP and Raghu K.(1982) Efficacy of different soil sterilization methods. Chemosphere, Vol.ll, No.12,pp 1243-1247.

Kalam, S., et al. (2017) Population densities of indigenous *Acidobacteria* change in the presence of plant growth promoting rhizobacteria (PGPR) in rhizosphere. Journal of Basic Microbiology,57(5), 376-385. doi:10.1002/jobm.201600588

Kanehisa M, Goto S. (2000) KEGG: kyoto encyclopedia of genes and genomes. Nucleic acids research. 28(1):27-30.

Katan J, (1981) Solar heating (solarization) of soil for control of soil-borne pests. Annual Review of Phytopathology 19,211–36.

Kelderer M.M., Manici, L.M., Caputo, F., Thalheimer, M. (2012) Planting in the 'inter-row' to overcome replant disease in apple or-chards: a study on the effectiveness of the practice based on microbial indicators. Plant Soil 357:381–393

Kier, L. D., Weppelman, R. M., and Ames, B. N. (1979) Regulation of nonspecific acid phosphatase in Salmonella: *PhoN* and *phoP* genes.

Kong, G. H., Shin, T.S., Ryu, C.M. (2018) Stereoisomers of the Bacterial Volatile Compound 2,3-Butanediol Differently Elicit Systemic Defense Responses of Pepper against Multiple Viruses in the Field.

Langille M.G., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C. (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature biotechnology. 2013 Sep;31(9):814.

Laville, J., Blumer, C., Von Schrotter, C., Gaia, V., Défago, G., Keel, C., Haas, D.(1998) Characterization of the hcnABC Gene Cluster Encoding Hydrogen Cyanide Synthase and Anaerobic Regulation by ANR in the Strictly Aerobic Biocontrol Agent Pseudomonas fluorescens CHA0.

Lawrence, W. J. (1956) Soil Sterilization. London: Allen and Unwin.

Linderman RG. (2000) Effects of mycorrhizas on plant tolerance to diseases. Kapulnik Y, Douds Jr DD, eds. Arbuscular mycorrhizas: physiology and function. Dordrecht, The Netherlands: Kluwer Academic Publishers, 345–365.

Lotrario J.B., Stuart, B.J., Lam, T., Arands, R.R., O'Connor, O.A., Kosson, D.S. (1995) Effects of sterilization methods on the physical characteristics of soil: Implications for SorptionIsotherm Analyses. Bull. Environ. Contam. Toxicol. 54:668—67

Lynch J, (1990) The rhizosphere. Wiley, London, UK, p 458

Mahmood T., et al. (2014). Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings. 57(3):123-130.

Manter, D. K.,Korsa, M., Tebbe, C., Delgado, J.A. (2016) myPhyloDB: a local web server for the storage and analysis of metagenomic data. Database J. Biol. Databases Curation

Marschner, P., Rumberger, A. (2004) Rapid changes in the rhizosphere bacterial community structure during re-colonization of sterilized soil. Biol. Fertil. Soils 40,1–6.

Martin, M., (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal17, 10–12

McKenry, MV. (1999) The Replant Problem and it's Management. Catalina Publishing, Fresno CA.

McNamara, N.P., Black, H.I.J., Beresford, N.A., Parekh, N.R. (2003) Effects of acute gamma irradiation on chemical, physical and biological properties of soils. Appl. Soil Ecol. 24, 117–132.

Meyer, J. B., Frapolli, M., Keel, C., Maurhofer, M. (2011) Pyrroloquinoline quinone biosynthesis gene i, a novel molecular marker for studying the phylogeny and diversity of phosphate-solubilizing pseudomonads.

Minas, I. S, Tanou, G., Molassiotis, A. (2018) Environmental and orchard bases of peach fruit quality. Scientia Horticulturae 235, 307-322.

Müller, A. L., de Rezende, J.R., Hubert, C.R.J., Kjeldesn, K.U., Lagkouvardos, I., Berry, D., Jørgensen, B.B., Loy, A. (2013) Endospores of thermophilic bacteria as tracers of microbial dispersal by ocean currents. The ISME Journal,8(6), 1153-1165. doi:10.1038/ismej.2013.225

Munro, P. (2018) Linking soil biotic and abiotic factors to sweet cherry tree establishment in new and old Okanagan Valley orchards (Master's thesis, THE UNIVERSITY OF BRITISH COLUMBIA (Okanagan)). (pp. 1-206). British Columbia: University of British Columbia. doi:10.14288/1.0363959

Qin S.Q., Zhou, W., Lyu, D., Liy, L. (2014) Effects of soil sterilization and biological agent inoculation on the root respiratory metabolism and plant growth of *Cerasus sachalinensis* Kom. Scientia Horticulturae 170: 189–195

Qiu, M., Li, S., Zhou, X., Cui, X., Vivanco, J.M., Zhang, N., Shen, Q., Zhang, R. 2013. De-coupling of root–microbiome associations followed by antagonist inoculation improves rhizosphere soil suppressiveness. Biology and Fertility of Soils,50(2), 217-224. doi:10.1007/s00374-013-0835-1

RaaijmakersJ.M., Paulitz, T.C., Steinberg, C., Alabouvette, C., Moënne-Loccoz, Y. (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil. 321:341–361

Radhakrishnan, R., Hashem, A., and Abd Allah, E. F. (2017) *Bacillus*: A Biological Tool for Crop Improvement through Bio-Molecular Changes in Adverse Environments.

Raymond J, Siefert, J.L., Staples, C.R., Blankenship, R.E. (2004) The natural history of nitrogen fixation. Mol Biol Evol 21: 541-554. https://doi.org/10.1093/molbev/msh047

Rohland, N and D. Reich. (2012) Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. Genome Research 22:939-946.

Rodriguez, F., Lillington, J., Johnson, S., Timmel, C.R., Lea, S.M., Berks, B.C., (2014) Crystal structure of the Bacillus subtilis phosphodiesterase PhoD reveals an iron and calcium-containing active site.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F.(2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ4, e2584

Santoyo G, Hernández-Pacheo, C., Hernández-Salmerón, J., Hernández-León, R. (2017) The role of abiotic factors modulating the plant-microbe-soil interactions: toward sustainable agriculture. A review. Spanish Journal of Agricultural Research. 15(1), e03R01.

Savory, B. M. (1966) Specific replant diseases: Causing root necrosis and growth depression in perennial fruit and plantation crops. Farnham Royal.

Schmalenberger, A., and Fox, A. (2015) Bacterial Mobilization of Nutrients From Biochar-Amended Soils.

Shushen, Z., Vivanco, J., Manter, D.K. (2016) Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. Applied Soil Ecology. 107:324-333

Sinegani, A. A., andSedri, S. (2011) Effects of Sterilization And Temperature On The Decrease Kinetic Of Phosphorus Bioavailability In Two Different Soil Types. Journal of Soil Science and Plant Nutrition,11(2), 109-122. doi:10.4067/s0718-95162011000200010

Skipper, H. D., and Westermann, D. T. (1973) Comparative effects of propylene oxide, sodium azide, and autoclaving on selected soil properties. Soil Biology and Biochemistry,5(4), 409-414. doi:10.1016/0038-0717(73)90067-9

Sosnowski, M. R., Fletcher, J.D., Daly, A.M, Rodoni, B.C., Viljanen-Rollinson, S.L.H. (2009) Techniques for the treatment, removal and disposal of host material during programmes for plant pathogen eradication. Plant Pathology,58(4), 621-635. doi:10.1111/j.1365-3059.2009.02042.x

Stirling, GR, Dullahide, SR. and Nikulin, A. (1995) Management of lesion nematode (Pratylenchusjordanensis) on replanted apple trees. Australian Journal of Experimental Agriculture 35: 247-258.

Stromberger, M. E., Abduelafes, I., Byrne, P., Canela, M.M., Elamari, A.A., Manter, D.K., Weir, T. (2017) Genotype-Specific Enrichment of 1-Aminocyclopropane-1-Carboxylic Acid Deaminase-Positive Bacteria in Winter Wheat Rhizospheres. Soil Sci. Soc. Am. J. 81:796-805. doi:10.2136/sssaj2016.12.0437

Smith, T.J. (1994) Successful management of orchard replant disease in Washington. Acta Horticulturae 363: 161-167

Tang QY, Zhang CX. (2013) Data Processing System (DPS) software with experimental design, statistical analysis and data mining developed for use in entomological research. Insect Science. Apr;20(2):254-60.

Tahon, G., Tytgat, B., Lebbe, L., Carlier, A., Willems, A. (2018) Abditibacteriumutsteinense sp. nov., the first cultivated member of candidate phylum FBP, isolated from ice-free Antarctic soil samples. Systematic and Applied Microbiology,41(4), 279-290. doi: 10.1016/j.syapm.2018.01.009

Tewoldemedhin YT, Mazzola, M., Labuschagne, I., McLeod, A. (2011) A multi-phasic approach reveals that apple replant disease is caused by multiple biological agents, with some agents acting synergistically. Soil Biol Biochem43:1917–1927

Tian Y, (2009) Microbial properties of rhizosphere soils as affected by rotation, grafting, and soil sterilization in intensive vegetable production systems. Scientia Horticulturae. 123(2):139–47.

Trevors, J.T., (1996) Sterilization and inhibition of microbial activity in soils. J. Micro-biol. Methods 26, 53–59.

Troelstra S.R., Wagenaar, R., Smant, W., Peters, B.A.M. (2001) Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. New Phytologist 150: 697– 706.

Tsegaye, Z., Assefa, D., andBeyene, D. (2017) Properties and Application of Plant Growth Promoting Rhizobacteria. Int. J. Curr. Trend. Pharmacobiol. Med. Sci., 2(1), 30-43. doi:10.15413/ajmr.2017.0104

Van Rhijn P, Vanderleyden J. (1995) The Rhizobium-plant symbiosis. Microbiol Rev 59: 124-142.

Vassilev N, Vassileva M, Nicolaeva I. (2006) Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Appl Microbiol Biotechnol 71: 137-144. https://doi.org/10.1007/s00253-006-0380-z

Van der Heijden Marcel G.A., Bardgett R.D., Van Straalen NM. (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters, 11: 296–310 doi: 10.1111/j.1461-0248.2007.01139.x

Wertz, S, Czarnes, S, Bartoli, F, Renault, P, Commeaux, C, Guillaumaud, N, Clays-Josserand, A. (2007) Early-stage bacterial colonization between a sterilized remoulded soil clod and natural soil aggregates of the same soil. Soil Biol. Biochem. 39, 3127–3137.

Weststeijn G. (1973) Soil sterilization and glasshouse disinfection to control Fusarium oxysporum f. lycopersici in tomatoes in the Netherlands. Neth. J. P1. Path. 79 :36-40

Wu HM, Xu JJ, Wang JY, Qin XJ, Wu LK, Li ZC, Lin S, Lin WW, Zhu Q, Khan, M. U. Lin WX. (2017) Insights into the mechanism of proliferation on the special microbes mediated by phenolic acids in the *Radix pseudostellariae* rhizosphere under continuous monoculture regimes. Frontiers in Plant Science;8 (May):659.

Yamamoto, T,Ultra, VU.Jr,Tanaka, S,Sakurai, K, Iwasaki K.(2008)Effects of methyl bromide fumigation, chloropicrin fumigation and steam sterilization on soil nitrogen dynamics and microbial properties in a pot culture experiment. Soil Science and Plant Nutrition. 54, 886–894 doi: 10.1111/j.1747-0765.2008. 00319.x

Yang Jiue-in, RueggerPM, McKenry MV, Ole BJ, Borneman J. (2012) Correlations between root-associated microorganisms and peach replant disease symptoms in a California soil. PLoS ONE 7(10): e46420. https://doi.org/10.1371/journal.pone.0046420.

Yang, Y., Wang, N., Guo, X., Zhang, Y., and Ye, B. (2017) Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. Plos One,12(5). doi: 10.1371/journal.pone.0178425

Yap, J.M., Goldsmith, C.E., Moore, J.E. (2013) Integrity of bacterial genomic DNA after autoclaving: possible implications for horizontal gene transfer and clinical waste management. J Hosp Infect, 83(3):247-9, doi: 10.1016/j.jhin.2012.11.016.

Yim B, SmallaK,Winkelmann T. (2013)Evaluation of apple replant problems based on different soil disinfection treatments—links to soil microbial community structure? Plant and soil, 366: 617-631

Zdor, R. (2014) Bacterial cyanogenesis: Impact on biotic interactions. Journal of Applied Microbiology, 118(2), 267-274. doi:10.1111/jam.12697

Zhang XH, Lang DY and Zhang EH. (2016) Effects of Soil Sterilization on growth of Angelica sinensisplant and soil microbial populations in a continuous mono-cropping soill. Int. J. Agric. Biol., 18: 458–463

Zhao YP, Lin S, Chu LX, Gao JT, Azeem S, Lin WX. (2016) Insight into structure dynamics of soil microbiota mediated by the richness of replanted *Pseudostellaria heterophylla*. Scientific Reports;srep/26175

Zhou, D., Huang, X., Chaparro, J. M., Badri, D. V., Manter, D. K., Vivanco, J. M., and Guo, J. (2015) Root and bacterial secretions regulate the interaction between plants and PGPR leading to distinct plant growth promotion effects. Plant and Soil, 401 (1-2), 259-272. doi:10.1007/s11104-015-2743-7

Zhu, Q., Riley, W. J., Tang, J., and Koven, C. D. (2015) Multiple soil nutrient competition between plants, microbes, and mineral surfaces: Model development, parameterization, and example applications in several tropical forests. Biogeosciences Discussions, 12(5), 4057-4106. doi:10.5194/bgd-12-4057-2015

Zhu, S., Vivanco, J. M., and Manter, D. K. (2016) Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize.

CHAPTER 3: Amplification of crop mediated rhizomicobiome interactions in multiple soils reveals crop-specific and crop-shared bacterial community selection

Synopsis

Plants can modify their rhizosphere microbiota to variable degrees using the surrounding soil as their dominant source of microbes. Plant effects on microbial selection may be influenced by competitive interactions from the native soil microbiota. It has been demonstrated that disrupting soils can reduce populations of microbial pathogens and increase crop growth. However, very little is known about the effect of disruption on other resident members of the soil microbiome. Here, we analyzed rhizobacterial community rearrangement prompted by four crops (corn, beet, lettuce and tomato) grown in three distinct disrupted agroecosystem soils (conventional, organic, and diseased). Soil disruption exacerbated the plant effect on the soil microbiome and caused significantly altered bacterial communities between crops. Furthermore, increases in both crop-specific and crop-shared bacterial abundance were observed following crop growth across disrupted soils. For instance, corn uniquely promoted growth of *Pseudomonas* and *Sporocytophaga*, regardless of the soil. Whereas taxa such as *Bosea, Dyadobacter* and *Luteoliobacter* were shared by all crops after growth in all disrupted soils. In summary, soil disruption followed by crop growth amplified plant-mediated bacterial selection of beneficial and potentially keystones species.

Introduction

The role of the soil microbiome has become an increasingly important factor regarding agricultural crop production. Recent discoveries have revealed positive correlations between soil microbial diversity and plant health, yield, disease suppression, and soil nutrient cycling (Trivedi

et al., 2017; Maron et al., 2018). Plants actively recruit differential rhizosphere microbiota throughout growth in order to accommodate their developmental needs (He et al., 2019; Chaparro et al., 2014). Thus, cultivation of plants in microbially diverse soils may increase growth benefits (Deng et al 2019). In contrast, symptoms of soil microbial dysbiosis have been shown to occur after long term and intensive monoculture practice (Zhao et al., 2018; McDonald and Stukenbrock, 2016; Guo et al., 2018; Lin et al., 2018; Patil et al., 2017; Sande et al., 2011). Such symptoms include reduced soil bacterial diversity (Jacobsen & Hjelmsø, 2014) and/or slight rhizosphere-imbalances toward higher plant pathogen abundance (McDonald and Stukenbrock, 2016; Zhao et al 2018, Bakker et al., 2012). Additionally, studies suggest that repeated cultivation of the same plant species over time may yield some sense of microbial habituation to the rhizodeposition products of plants repeatedly grown in those same sites (Broeckling et al., 2018). However, microbial rearrangements in the rhizosphere may not solely be attributed to plant rhizodeposition, as the structure and function of soil microbial communities can also shift as a result of organic agricultural management practices (Martinez-Garcia et al., 2018). Therefore, both conventional and organic agricultural management practices can instigate variable effects on soil microorganisms, and in turn the plant's natural recruitment process of these microbes into its rhizosphere (Lin et al., 2019; Sugiyama et al., 2010).

Recent attempts to reshape imbalanced microbial communities in agroecosystem soils have shown that applying soil sterilization methods (via moist- or dry-heat, chemical fumigation, microwave or gamma-irradiation, etc.) can briefly provide a more healthy/balanced rhizosphere microbiome, which in turn can significantly promote plant growth (Li et al., 2019; Peralta et al., 2018; Wolf & Skipper, 1994). Other attempts to alleviate imbalances in rhizomicrobiota include application of known plant-growth promoting rhizobacteria (PGPRs) to field soil systems

(Trabelsi & Mhamdi, 2013). However, such studies often result in an inability of inoculated microbes to compete for resources and colonize, as resident soil microbiota are more fit to maintain colonization of the target roots/rhizosphere (Qiu et al., 2014; van Elsas et al., 2012). Interestingly, one disruption method using the fungicide carbendazim, prior to the inoculation of beneficial microbes, resulted in a much higher establishment rate compared to the same microbes applied to untreated soils (Qiu et al., 2014). Historically, chemical fumigation (e.g. with methyl bromide) has been used in commercial agriculture to disrupt established soil microbial communities. However, this method may be toxic and result in detrimental effects on surrounding ecosystems (Jacobsen & Hjelmsø, 2014; Sande et al., 2011; Noling, 1994). Moist heat or steam sterilization is a common and less harmful method used to disrupt the soil microbiome. A recent study shows that applying moist heat sterilization to soils reduced microbial load, which subsequently allowed plants to recruit distinct bacteria from the native community along with several plant growth-promoting bacterial functions (Li et al., 2019).

In this study, we explore the hypothesis that plants preferentially promote certain beneficial microorganisms during early cultivation in soils exposed to disruption allowing for either (i) low rates of microbial competition and/or (ii) reduced microbial biomass allowing the established microbiota to lessen their recalcitrance to change. To disrupt the native soil microbiome, resident microbes within three distinct agricultural ecosystem soils (organic, conventional and diseased) were exposed to moist heat sterilization (via autoclaving) and subsequently crops belonging to four different plant families (Poaceae, Amaranthaceae, Asteraceae, and Solanaceae) were grown in each of the disrupted and undisrupted soil treatments. The rhizobacterial re-arrangements induced by crops were analyzed by Illumina MiSeq sequencing of the V3-4 region of the bacterial 16s gene. After disruption of the resident

soil microbial communities, crops of different families were able to recruit differential bacterial communities, and the degree of separation was likely a direct effect of the initial bacterial composition resulting from field management of each agroecosystem. Further, certain bacterial reads were observed to be promoted by either specific single crops, or by two or more crops after growth in all disrupted soil treatments.

Methods

Soil collection, treatment, and chemical analyses

Soils used in this study were sourced from three agricultural ecosystems. Soil types, plants present during soil collection as well as geographical location of each site are as follows: (i) a USDA-certified organic cover crop field [Agricultural Research, Development and Education Center (ARDEC)-South, Specialty Crops Program, Colorado State University (CSU), Fort Collins, CO] growing mixed cover crop species (*Avena sativa* and *Vicia villosa*) and certified USDA organic since 2003, referred to as "organic soil"; (ii) a USDA ARS no-till cultivation system (ARDEC-North, USDA-ARS, Fort Collins, CO) growing corn (*Zea mays*) annually supplemented 180 lbs/acre of nitrogen and referred to as "conventional soil" and; (iii) a 10-year old peach orchard (*Prunus persica*, cv. 'Cresthaven' grafted onto 'Lovell' rootstock; Western Colorado Research Center, Orchard Mesa, Grand Junction, CO) that is symptomatic of peach replanting disease and as such is referred to as "diseased soil". Clean shovels were used to collect bulk soil from the organic site at a depth of 20-40 cm after the removal of oat and hairy vetch plants. Thus, the rhizosphere soils of oat and vetch plants were included in the soil collection. Soils from the conventional site were collected similar to the organic site, but corn plants were growing during collection and corn's rhizosphere soils were included. Soils from the

diseased site were collected nearby peach tree trunks at a depth of 20-50cm s to represent the tree rhizosphere while also being non-destructive to the orchard.

On collection day all soils were sifted through a No. 10 metal sieve (2 mm wide) prior to autoclave disruption and use in greenhouse experimentation. Sub-samples of each field soil type (organic, conventional and diseased) were collected, air dried, and stored at -20 ºC for later DNA extraction. After sieving large debris out of each soil type, soils from each field were divided into two equal parts. The first part of each soil experienced no further treatment and is referred to as undisrupted soil. The second part was exposed to steam sterilization using a STERIS brand autoclave for three 15-minute liquid cycles at 121 ºC and is referred to as the disrupted soil treatment. Standard chemical analyses of all soils were conducted by the Soil, Water and Plant Testing Laboratory at Colorado State University in groups of two replicates per treatment to determine the changes in composition brought upon by autoclaving. Parameters determined were as follows: pH, electrical conductivity, lime estimate, percent organic matter, soil texture and the following nutrient availabilities were analyzed (in ppm): NO_3-N, P, K, Zn, Fe, Mn, and Cu.

Plant growth experiment

The disrupted and undisrupted soils from each site were poured into individual plastic pots (~400 g per pot, pot size: 7 x 10 x 8.5 cm). For each soil treatment (n=6) seeds of corn, beet, lettuce, and tomato were surface sterilized with 3.0% NaClO, rinsed three times with sterile water, and imbibed in sterile water for 24 hours prior to planting. After imbibition seeds were sown into pots at a rate of 3 seeds per pot (with 10 pots per crop per soil treatment; total n = 300). After the emergence of one or more seedlings per pot, extra seedlings were removed to allow for 10 uniform replicates (at a rate of one plant per pot) within a treatment, and then the 7-week growth period began. Additionally, in all treatments, both disrupted and undisrupted soil

samples were placed into pots in the absence of plants and included in the experimental block design. These no plant controls (NPCK) provided insights to soil bacterial re-arrangements without the plant inputs. The experiment was conducted at CSU's Horticulture Center Greenhouse Facility under an average temperature of 26.26 ± 2.17 ºC (79.3 ± 3.9 ºF) and relative humidity of 38.3 ± 14.5 %. To avoid accidental transplanting of soil particles (containing microbes) from differing ecosystems via splashing during irrigation, the experimental replicates were randomized within their respective soil block treatment (i.e. crops in disrupted organic soils were randomized within the disrupted organic soil block). Plants were allowed to grow for 7 weeks and during the 7th week of growth plant growth measurements were recorded.

Plant experiment - data collection

After the 7th week of growth, plants were harvested, cut at the root-shoot axis and the above-, below- and total fresh-weight biomass measurements of each replicate were recorded. On the same day, rhizosphere soils were collected via gentle brushing of plant roots overtop Ziploc bags to remove any root-adhering soil (Inceoglu et al., 2010). Of the ten plant replicates in each treatment, two rhizosphere soil samples (or soil-core samples from the NPCKs, depth: 2-5 cm) were combined for a total of five soil or rhizosphere microbiome samples to represent each experimental treatment. Soil samples were air dried, transported to the lab and stored at -20 ºC until subsequent DNA extraction. Following fresh-weight biomass measurements and rhizosphere soil collection, plants were placed into individual paper bags, dried in an oven for 48 h at 65 ºC, and then the dry-weight biomass was recorded. The mean biomass measurements (both fresh and dry) from each treatment were compared with a two-way ANOVA model using Prism's GraphPad (Vers. 8.2.1) and pairwise comparisons were conducted using Siddak's multiple comparison tests (GraphPad Software, La Jolla California, USA).

Soil DNA Extraction and Bacterial Community Analysis

Total genomic DNA (gDNA) from 0.25 grams from three out of five soil or rhizosphere samples were extracted using the E.Z.N.A Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. Soil gDNA extractions yielded final elution volumes of 50 µl for each sample. On the same day, nucleic acid concentration and sample purity were quantified and determined via the use of a NanoDrop 2000 Spectrophotometer (Thermofischer, Waltham, MA, USA). gDNA samples were then stored at −80 ºC prior to Illumina MiSeq library preparation and downstream microbiome analyses.

Microbiome Analysis

Quantification of Bacterial Cells from gDNA

Bacterial cell counts per gram of soil were determined by performing quantitative Polymerase Chain Reaction (qPCR) of all gDNA samples against a standard curve of purified *Pseudomonas putida* KT2440 16s gDNA. For all samples, bacterial cell counts can be interpreted as *P. putida* equivalents per gram of soil. The qPCR conditions were as follows: aliquots of each quantified gDNA sample were collected to prepare template DNA at a concentration of 5 ng/ uL. The qPCR reactions were performed in 10 uL reaction volumes containing 1 uL of template DNA and 9 uL of the master mix. The master mix consisted of 5 uL SYBR Green (QuantaBio, Beverly, MA, USA), 0.5 uL of each forward and reverse primer (10 uM) and brought to a total volume of 9 uL using 3 uL of molecular grade water. The qPCR thermal cycling conditions for bacterial quantification were as follows: 95ºC for 8 minutes and 30 seconds, 30 amplification cycles (95ºC for 15 seconds, 58ºC for 30 seconds, 72ºC for 60 seconds) followed by a final annealing stage at 72 ºC for 5 minutes. The mean cell counts from

each crop treatment were compared to their respective NPCK (disrupted or undisrupted) by using a two-way ANOVA model with R Studio's aov function (Chambers et al., 1992).

Library preparation for Illumina MiSeq Sequencing

The initial soil gDNA samples were diluted 1:5 with molecular water to reduce PCR inhibitors introduced during DNA extraction. Another round of 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 second round of PCR 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, 30 amplification cycles (95ºC for 40 seconds, 55ºC for 120 seconds, 72ºC for 60 seconds) followed by a final annealing stage at 72 ºC for 7 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) (Rohland & Reich, 2012; Faircloth & Glenn, 2011).

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 Fischer 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 sequencing 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 Silva reference database (Version 132). Each taxonomic profile assigned was used to determine bacterial genus -level abundance values. Downstream analyses were conducted using R Studio's Phyloseq and vegan packages or myPhyloDB (vers. 1.2.0) (Oksanen et al., 2019; McMurdie and Holmes, 2013; Manter et al., 2013). Samples were rarified at a cutoff of 7500 reads using myPhyloDB prior to downstream analysis applications using myPhyloDB or R Studio packages. 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 model was applied to compare mean α-diversity values from each disrupted crop treatment to their respective undisrupted crop treatment. Additionally, disrupted crop treatments were compared to the disrupted NPCK treatments with R Studio's Analysis of Variance package (Chambers et al., 1992). R Studio's vegan package was used with the Bray-Curtis dissimilarity index to quantify differences in phylogeny between samples from different treatments (Oksanen et al., 2019). Distances were visualized using principal coordinates analyses (PCoA) and PCoAs were created using Prism's GraphPad (Vers 8.2.1, GraphPad Software, La Jolla California, USA). The myPhyloDB software was used to perform a complementary non-parametric multivariate statistical test, including permutational analysis of variance (perMANOVA) as well as differential abundance analyses (FDR < 0.1) to test for differences in microbial communities between treatments (Manter et al., 2013) A Venn diagram of these data was constructed using the jvenn software (Bardou et al., 2014)

Results

Soil Chemical Analysis

To determine the effect of heat treatment on soils, soil pH, electrical conductivity, lime estimate, percent organic matter, texture and parts per-million (ppm) of the following nutrients:

nitrate-nitrogen, phosphorous, potassium, zinc, iron, manganese, and copper were measured. These parameters were analyzed for all disrupted and undisrupted soil samples (Table 3-1). Across all soil sites, phosphorous and manganese were significantly increased as a result of autoclave disruption. For organic soil, P and Mn were increased by 0.299-fold (p-value 0.004) and 2.292-fold (p-value: < 0.001), respectively (Table 3-1). For conventional soil, P and Mn were increased by 0.462-fold (p-value: 0.002) and 1.792-fold (p-value: < 0.001), respectively (Table 3-1). For diseased soil, P and Mn were increased by 0.241-fold (p-value: 0.016), and 7.571-fold (p-values: < 0.001), respectively (Table 3-1). Interestingly, only the diseased agroecosystem soil experienced significant reductions in the availability of both zinc (0.137-fold, p-value: 0.002) and copper (0.318-fold, p-value: < 0.001) after autoclaving. Neither organic nor conventional soil experienced significant reductions in nutrient availability after autoclave disruption (Table 3-1).

Plant growth experiment

Above and below ground measurements of dry-weight (DW) biomass were added together to analyze total DW biomass (Figure 1). Compared to the mean total DW of corn grown in soils with no disruption, corn DW biomass was significantly promoted after growth in all disrupted soils (p-values: < 0.0001, < 0.0001, and < 0.001 for organic, conventional and diseased, respectively). For beet, the total DW biomass was significantly promoted after growth in disrupted organic and disrupted conventional soils (p-values: 0.012 and 0.008 for organic and conventional, respectively), but not in the diseased soil. Compared to the mean DW of tomato in undisrupted soils, tomato DW was not significantly altered after growth in any of the disrupted soils. Lastly, the DW of lettuce was significantly increased after growth in disrupted

conventional and disrupted diseased soils (p-values: < 0.0001 and 0.049 for conventional and diseased, respectively), but not in the disrupted organic soil.

While some increases in plant DW were statistically insignificant, the total percent increase (and fold change) in fresh and DW biomass were calculated for all crops by comparing the crop's mean DW in each disrupted soil to the mean DW of the same crop in each undisrupted soil (Supplemental Tables 1-2). For corn, the DW biomass increased by 1.144-fold in the organic soil, by 3.019-fold in the conventional soil, and by 1.867-fold in the diseased soil after disruption. For beet, DW biomass increased by 3.206-fold in the disrupted organic soil, by 3.815-fold in the disrupted conventional soil, and by 0.514-fold in the disrupted diseased soil. Lettuce DW was promoted by 0.805-fold in the organic soils, by 2.571-fold in conventional soils, and by 1.309-fold in the diseased soils following disruption. Tomato DW increased by 1.750-fold in the disrupted organic soil, 1.155-fold in the conventional soil, and by 0.533-fold in the disrupted diseased soil (Supplemental Table 2). Tables displaying the average gain in DW biomass [as well as fresh weight bar graphs (Supplemental Figure 1)], along with percent increases observed for each treatment, can be visualized in Supplemental Tables 1 & 2.

Microbiome Analyses

qPCR bacterial cell quantification

For all soil gDNA samples, the final concentration of nucleic acids ranged from 15.2 ± 8.6 ng / uL after extraction. For qPCR analysis, bacterial cell counts from the NPCKs for each agroecosystem (disrupted or undisrupted) were compared to cell counts from crops to see changes in bacterial cells as a result of crop growth in disrupted or undisrupted soils. In organic soils, our qPCR analysis shows no significant differences observed between soils with crops compared to the NPCKs, regardless of disruption (Figure 3-2A). For conventional soils, no

significant differences in bacterial cell counts were observed between the undisrupted NPCKs and the undisrupted soil that grew crops. After disruption of the conventional soils, the lettuce rhizosphere had significantly higher bacterial cell counts, compared to the disrupted conventional NPCK (p-value: 0.011, Figure 3-2B). In diseased soil, the growth of corn in undisrupted soil allowed for a significant increase in bacterial cells, compared to cell counts from the undisrupted diseased NPCKs (p-value: 0.004, Figure 3-2C). Furthermore, after applying disruption to the diseased soil there were no significant differences in bacterial cell counts observed between the treatments and the NPCKs (Figure 3-2C).

Interestingly, the NPCKs provided insights into soil bacterial rearrangements occurring in the absence of plants following disruption. In both the organic and conventional soils, soil bacterial trends followed those observed when crops were introduced to these systems after disruption (e.g. cell counts were lessened after disruption compared to their undisrupted counterparts). Anomalously, in the diseased soil bacterial cell counts from the disrupted NPCKs were observed to increase compared to the undisrupted soils (p-value: 0.975). The associated statistical analysis for these data can be visualized in Supplemental Table 3.

Illumina MiSeq Data

After After filtering reads, removing singleton ASVs and rarifying, Illumina MiSeq paired-end sequencing generated a total of 4,264,022 reads resulting in an average of 46,348 reads per sample. Seven out of 104 total samples did not meet rarefaction criteria (min. 7500 reads) and were dropped. Bacterial diversity resulting from crop growth in disrupted soils were compared to their disrupted NPCKs within the same agroecosystem. Additional comparisons were made from the disrupted crop growth treatments to their crop-counterparts grown in the undisrupted parts of the same soil. Bacterial diversity (Shannon index) was significantly different

between communities from the disrupted and undisrupted corn grown in the diseased agroecosystem (p-value 0.041) but not for any other plant/soil combination (Table 2, Supplemental Table 4).

Principal coordinate analysis of rhizobacterial community rearrangement

In the organic soils, a permutational analysis of variance using distance matrices revealed that microbiome disruption resulted in significant differences in bacterial community phylogeny (disruption p-value: 0.001, R^2: 0.549, Axis.1: 56.14%) for the organic soils. Furthermore, our analyses revealed that each crop family was able to recruit significantly different rhizobacteria independent of disruption (crop effect p-value: 0.001, R^2: 0.1372, Axis.2: 6.06%) (Figure 3). A secondary beta-diversity analysis using betadisper further demonstrates that after the organic soils were disrupted, individual crops were able to recruit significantly different rhizobacterial communities from the same crops grown in undisrupted organic soil (p-value 0.027).

In the conventional soil, our analyses showed that disruption produced the strongest significant differences in bacterial community phylogeny (disruption p-value: 0.001, R^2: 0.693, Axis.1: 69.59%) when comparing reads from disrupted to the undisrupted conventional soil. Furthermore, crops grown in conventional soil were able to recruit significantly different rhizobacteria by plant-family, whether or not disruption occurred (crop effect p-value: 0.001, R^2: 0.060, Axis.2: 3.95%) (Figure 3-4). Our complimentary beta-diversity analysis also showed that once disrupted, crops could recruit significantly different rhizobacterial communities from their same-crop counterpart grown in undisrupted conventional soil (p-value 0.015) (Figure 3-4).

The diseased soil demonstrated the weakest effect regarding microbiome disruption. Again, soil disruption also resulted in significantly different bacterial communities when comparing reads from the disrupted to undisrupted diseased soil (disruption p-value: 0.001, R^2:

0.461, Axis.1: 47.85%). Still, in diseased soils each individual crop family recruited significantly different rhizobacteria from other crop families regardless of disruption (crop-effect p-value: 0.001, R_2: 0.148, Axis.2: 15.56%) (Figure 3-5). The beta-diversity analysis for the disrupted and undisrupted diseased agroecosystems samples revealed that crops of the same plant were unable to recruit significantly different rhizobacterial communities from their counterpart (i.e. the same crop in undisrupted diseased soil) (p-value 0.08).

Rhizobacteria recruited by crops vs NPCKs (No Plant Controls) in undisrupted and disrupted soils

In both undisrupted and disrupted treatments, bacteria showing significant alterations in abundance values were pooled across all three soil agroecosystems and grouped by crop type. The abundances of these bacteria from each crop were then compared to the respective pooled NPCK samples (undisrupted or disrupted). Differential abundance analyses revealed significantly altered bacterial genera resulting from crop presence (Tables 3-3 through 3-4). Across the pooled undisrupted treatments, four bacterial genera were significantly altered in abundance by crop presence (Table 3-3), whereas twelve bacterial genera were altered in abundance when crops were grown across the pooled disrupted treatments (Table 3-4).

When corn was grown in the undisrupted sites, no genera were increased in abundance, but one genus significantly decreased in abundance: *Azohydromonas* (p-value: < 0.001) (Table 3-3) compared to the NPCKs. After corn was grown in the disrupted sites, abundance values of the genus *Dyadobacter* were significantly promoted (p-value: < 0.001). Also, abundances of two other bacterial genera were significantly reduced after corn growth across disruption treatments: *Oscillatoria_PCC-6304* (p-value: < 0.001) and *Rhodobacter* (p-value: < 0.001) (Table 3-4).

Growing beet in the undisrupted treatments did not prompt any significant changes in bacterial abundance values when compared to the undisrupted NPCKs (Table 3-3). After growing beet in disrupted soils, both the *Dyadobacter* (p-value: < 0.001) and *Novosphingobium* (p-value: < 0.001) genera were significantly increased in abundance, while no bacterial abundances were reduced compared to the NPCKs (Table 3-4).

When lettuce was grown in undisrupted sites, the bacterial genera that increased in abundance were *Rhizorhapis* (p-value: < 0.001), *Pseudomonas* (p-value: < 0.001) and *Catellatospora* (p-value: < 0.001) (Table 3-3). After disruption, the growth of lettuce significantly promoted *Dyadobacter, Lacibacter, MM2, Neorhizobium, Niastella, Rhizorhapis, Verrucomicrobium*, *Rhodobacter* and *Qipengyuania* bacterial genera (all p-values: < 0.001, Table 3-4).

Growing tomato across the undisrupted agroecosystems did not result in significant abundance changes for any bacterial genera when compared to pooled undisrupted NPCKs (Table 3-3). However, the growth of tomato in all disrupted agroecosystems significantly increased the abundance values of the *Sphingobium* genus (p-value: < 0.001) (Table 3-4).

Difference in bacterial community recruitment by the same crops in disrupted vs undisrupted soils

After growth in the pooled disrupted soils, each crop significantly altered the abundances of several bacterial reads, compared to those recruited by their crop-counterpart samples from pooled undisrupted soils. Across all disrupted soils, growing corn resulted in significantly increased abundances of the following genera: *Roseococcus, Peredibacter, Bosea, Caenimonas, Brevundimonas, Caulobacter, Flavisolibacter, Lacibacter, Parasegetibacter, UTBCD1, Algoriphagus, Devosia, Quadrisphaera, Oligoflexus, Opitutus, Ammoniphilus, Brevibacillus,*

SH-PL14, Luteoliobacter, Solimonas, Articibacter, Pedobacter, Sphingoaurantiacus, Sphingopyxis, Dyadobacter, Larkinella, Rhabdobacter, Roseimicrobium and *Verrucomicrobium* (Table 5).

In all disrupted soils, the growth of beet plants resulted in significantly increased abundances of the following genera: *Bosea, Caenimonas, Brevundimonas, Caulobacter, Lacibacter, Sporocytophaga, Opitutus, Pseudomonas, Luteolibacter, Pedobacter, Sphingoaurantiacus, Sphingopyxis, Dyadobacter, Larkinella, Rhabdobacter* and *Stenotrophomonas* (Table 6).

Cultivation of lettuce in the disrupted soils resulted in significantly increased abundances of the following bacterial genera: *Rhodovastum, Roseomonas, Anoxybacillus, Bosea, Caenimonas, Rhodoferax, Brevundimonas, Lacibacter, Fluviicola, Algoriphagus, Cytophaga, Devosia, Flavobacterium, Knoellia, Quadrisphaera, Oligoflexus, Brevibacillus, Luteoliobacter, Articibacter, Pedobacter, Sphingoaurantiacus, Sphingopyxis, Dyadobacter, Larkinella, Rhabdobacter, Tepidsphaera, Brevifollis, Roseimicrobium,* and *Verrucomicrobium* (Table 7).

Tomato plant growth in disrupted soils significantly increased the abundances of *Anoxybacillus, Bacillus, Bosea, Chelatocossus, Caenimonas, Asticcacaulis, Brevundimonas, Lacibacter, Algoriphagus, Devosia, Flavobacterium, Yonghaparkia, Oligoflexus. Opitutus, Ammoniphilus, Aminobacter, Cereibacter, Luteoliobacter, Articibacter, Mucilaginibacter, Pedobacter, Sphingoaurantiacus, Sphingopyxis, Dyadobacter, Larkinella, Rhabdobacter, Shimazuella, Verrucomicrobium, Stenotrophomonas,* and *Thermomonas*. All bacterial reads that were significantly increased in abundance in the NPCKs after disruption (Table 9) were used to filter and remove these reads from the crop tables (Tables 5-8) to attribute abundance increases

to plant presence. The full list of bacterial taxonomic reads that were significantly increased in abundance in the NPCKs after disruption can be visualized in Table 9.

Shared bacterial abundance increases among crops following soil disruption

Across all disrupted soils, growth of corn, beet, lettuce and tomato significantly increased abundances of several unique and overlapping bacterial genera (Figure 5, Supplemental Table 5). Bacterial genera observed to increase in the disrupted NPCKs served to filter out bacteria able to proliferate in the absence of plant growth (Table 3-9). Across all disrupted agroecosystems, all four crops significantly promoted the abundance of eleven bacterial genera (and two bacterial families); *Bosea, Caenimonas, Brevundimonas, Lacibacter, Luteolibacter, Pedobacter, Sphingoaurantiacus, Aohingopyxis, Dyadobacter, Larkinella, Rhabdobacter* and the families Saccharimonadaceae and Sphingobacteriaceae (Figure 5, Supplemental Table 5). Furthermore, nine other bacterial reads were significantly increased in abundance and shared by different combinations of three out of the four crops tested. Corn, lettuce and tomato growth increased the abundance of bacterial families Cellvibrionaceae, Fibrobacteraceae, and KD3-93. The growth of beet, tomato and lettuce increased the *Algoriphagus, Devosia, Oligoflexus* and *Articibacter* bacterial genera. Beet, corn and tomato growth increased the abundance of the family Devosiaceae and the genus *Opitutus* (Figure 5, Supplemental Table 5).

Lastly, there were also several other bacterial reads observed to be significantly increased in abundance and shared in the rhizosphere by different combinations of only two out of the four crops, across all disrupted agroecosystems. Bacterial reads increased by the growth of tomato and lettuce were the genera *Anoxybacillus, Flavobacterium, PCC-7104* and *Verrucomicrobium*, and families Beijerinckiacea and Cyclobacteriaceae. Corn and lettuce growth both promoted abundances of the bacterial orders Bacillales and Candidatus_Peribacteria. The growth of corn

and tomato shared the increased abundances of the genus *Stenotrophomonas*. Beet and lettuce both promoted abundances of the genera *Quadrisphaera, Brevibacillys, SH-PL14,* and *Roseomicrobium*, in addition to the Sphingomonadaceae family as well as the order Sericytochromatia. Growing both beet and tomato promoted abundances of the genus *Ammoniphilus*, whereas the growth of both beet and corn promoted the genus *Caulobacter* and the order Microgenomatia (Supplemental Table 5).

Crop-specific bacterial abundance increases following soil disruption

Additionally, after disruption and crop growth certain bacterial reads were observed to significantly increase in abundance in the rhizospheres of specific crops. For example, the growth of beet promoted abundances of *Roseococcus, Peredibacter, Flavisolibacter, Parasegetibacter, UTBCD1* and *Solimonas* (Figure 5, Supplemental Table 5). The growth of corn significantly promoted abundances of *Sporocytophage* and *Pseudomonas* along with two bacterial families (Fibrobacteraceae and Rhodothermaceae) (Figure 5, Supplemental Table 5). Genera observed to increase in abundance in response to lettuce growth were *Rhodobastu, Roseomonas, Rhodoferax, Fluviicola, Cytophaga, Knoellia, PCC-6304, Tepidisphaera,* and *Brevifollis.* Lettuce growth also specifically promoted abundance increases of the bacterial families Burkholderiaceae, Caulobacteraceae, Opitutaceae, and Sporolactobacillaceae (Figure 5, Supplemental Table 5). The growth of tomato promoted abundance increases of fifteen bacterial reads. The genera *Bacillus, Chelatococcus, Asticcacaulis, Yongharparkia, Aminobacter, Cereibacter, Mucilaginibacter, Shimazuella,* and *Thermomonas* in addition to the bacterial families Archangiaceae, Hydrogenedensaceae, Verrucomicrobiaceae and the orders Candidatus_Peribacteria and Candidatus_Pacebacteria (Figure 5, Supplemental Table 5).

Discussion

Agroecosystem management effects disrupted rhizobacterial rearrangement

Different agroecosystem management techniques result in variable effects on the natural soil microbiota (Peralta et al., 2018; Zhao et al., 2018; McDonald & Stukenbrock, 2016). Some studies have shown that disrupting soil with autoclave heat does not provide a totally sterile environment, but that autoclave disruption is successful at reducing (without eliminating) all resident soil bacteria (Li et al., 2019; Carter et al., 2007). Therefore, an autoclave disruption was administered to each distinct agroecosystem (i.e. organic, conventional and diseased) as a means to reduce populations of native microbiota and examine the plant-influence on its bacterial community. Our findings show that a similar bacterial diversity level (Shannon index) persisted in two (conventional and diseased) of the three soil ecosystems upon collection (Table 3-2), showing a similar finding to Sugiyama et al. (2010). Notably however, after autoclave disruption and the experimental course, the organic agroecosystem soils manifested the highest value of Shannon's phylogenetic diversity compared to the conventional or diseased soils in which diversity was reduced (Table 3-2). Recent studies have shown that organic management practices often promote beneficial changes in diversity and function of the soil microbiota when compared to conventional agroecosystems (Lori et al., 2017; Bonanomi et al., 2016; Hartman et al., 2018; Lupatini et al., 2017). Additional studies show that agroecosystem management practices (i.e. annual tillage, monocropping, etc.) can lead to negative impacts on the productivity of an ecosystem (Lapsansky et al., 2016; Dorr de Quadros et al., 2012). As such, the variable effects on bacterial diversity between agroecosystems reported here are likely a result of both management and the resident microbiota within each field.

Interestingly applying soil disruption allowed plants to induce a greater number of bacterial rearrangements (12 genera) (Table 3-3) compared to when the same plants were grown in undisrupted soils (4 genera) (Table 3-4). Some of these bacteria may survive autoclave disruption due to an unknown protective characteristic, while additionally becoming habituated to a particular crop. Our findings align with the concept of soil memory; proposing that repeated presence of the same plants influence the microbial community within a soil system. These changes can promote abundances of PGPRs; in addition to the notion that these microbes can linger into the next plant generation to benefit offspring grown in the same soils (Lapsansky et al*.,* 2016). Based on this concept we propose that resident microbiota previously recruited by plants from each agroecosystem encompassed a set of functions equipped for their site and the presence of the resident crop. For this reason, applying an initial microbiome disruption was obligatory to make more apparent the influence of crop cultivation on bacterial communities from each agroecosystemm.

Agroecosystem disruption allows for crop-specific rhizobacterial recruitment

In our studies soil microbiome disruption provided both increased crop biomass as well as significant differences in bacterial communities between the disrupted and undisrupted agroecosystem treatments, across all sites [Bray Curtis distance, disruption p-values: 0.001 (for all soils), Figures 3-5]. Our findings demonstrate that crop families were able to recruit significantly different rhizomicrobiota, contingent upon its specific soil agroecosystem and whether or not disruption was administered. This crop-specific recruitment can be visualized in the organic agroecosystem as an example, where different families of crops were shown to promote abundances of distinct rhizobacterial communities; both in the undisrupted and in the disrupted treatments (crop effect p-value: 0.001, R_2: 0.1372, Axis.2: 6.06%) (Figure 3). We also

conducted a beta diversity analysis which revealed that rhizomicrobiota recruited by each crop family grown in the disrupted organic agroecosystem soils significantly differed from the same crop family grown in the undisrupted soil (p-value 0.027). These findings are likely a result of resident bacteria habituated to management factors that comprise an organic agroecosystem; such as the use of organic amendments, crop rotation, lack of chemical inputs, etc. (Hartman et al., 2018). Similar to the organic site, in the conventional agroecosystem crops were also able to recruit significantly different communities between crop-families (crop effect p-value: 0.001, R^2: 0.060, Axis.2: 3.95%) (Figure 3-4). It is known that conventional management systems often implement synthetic fertilizers and broad-spectrum pest- or herbicides, sometimes resulting in detrimental off-target effects on bacterial diversity/evenness (Chaparro et al., 2012; Krauss et al., 2011). Interestingly bacterial residents of the conventional agroecosystem site were still able to be reshaped by plants like those in the organic agroecosystem. Furthermore, our beta diversity analysis shows that crop families grown in disrupted conventional soils were able to recruit significantly different bacteria compared to the same crop families growing in undisrupted conventional soils (p-value 0.015) suggesting a significant bacterial community alteration, mediated by plants following disruption.

Crops grown in the diseased agroecosystem soils (disrupted or undisrupted) were also able to recruit different rhizobacterial communities (crop-effect p-value 0.001, R^2: 0.148, Axis.2: 15.56%). Interestingly, the disrupted diseased soil did not allow for crops of the same family to recruit different bacterial communities from their same-crop counterparts grown in undisrupted diseased soil (p-value: 0.080). We are aware that the diseased site is regularly treated with the agrochemicals glyphosate and flumioxazin, as well as other measures equipped to combat peach orchard replanting disease that may provide favorable environments for thermotolerant bacteria.

Bacillus and *Colstridium* are genera within the Firmicutes phylum and have been reported to survive heat treatments by forming thermotolerant endospores (Nicholson et al., 2000). Notably, the relative abundance (RA) values of the genus *Bacillus* in pooled undisrupted samples of organic, conventional or diseased agroecosystems were 0.036, 0.006, and 0.049, respectively. Whereas, the RA of *Clostridium* were < 0.001, < 0.001, and 0.001 in undisrupted organic, conventional and diseased soil, respectively. After disrupting each organic, conventional or diseased agroecosystem, *Bacillus* RA was increased to 0.085, 0.080, and 0.118; *Clostridium* RA was promoted to 0.001, 0.003, or remained the same at 0.001 for organic, conventional and diseased respectively (data not shown). Thus, these taxa were increased in abundance after autoclaving and crop growth, with the highest incidence occurring in the diseased agroecosystem. Likely the experimental disruption procedures promoted abundances of thermotolerant Firmicutes members *Bacillus* and *Clostridium* in all soils. Notably, both before and after applying the autoclave disruption, the highest RA of thermotolerant *Bacillus* and *Clostridium* were observed in the diseased agroecosystem soil. In support of this, our 16s qPCR assay shows that only bacterial cells in the diseased agroecosystem increased in the NPCKs after disruption, suggesting proliferation of thermotolerant bacteria after a successful competition-reduction via autoclaving (Figure 2C). Accordingly, we hypothesize that autoclave disruption provided favorable environments for thermotolerant taxa to multiply in the diseased agroecosystem (and to a lesser degree in other sites) while limiting the number of plant-available bacteria for recruitment.

Crop-shared bacterial genera recruited following agroecosystem disruption

Our differential abundance analyses revealed several bacterial abundance increases overlapping between different crops when samples were pooled after growth across all disrupted

soils (Tables 5-8, Supplemental Table 5). Eleven genera and two families (*Bosea, Caenimonas, Brevundimonas, Lacibacter, Luteolibacter, Pedobacter, Sphingoaurantiacus, Sphingopyxis, Dyadobacter, Larkinella, Rhabdobacter* and families Saccharimonadaceae and Sphingobacteriaceae, Supplemental Table 5) were observed to increase in abundance following disruption and growth of all four crops. We hypothesize that crops formed associations with some of these genera due to their plant growth promotional (or plant-symbiotic) nature. For example, members of *Bosea* and *Sphingopyxis* genera have previously been described to be plant-growth promotional taxa; and members of *Bosea* spp. can produce IAA (Yadav et al., 2015; Bjørnlund et al., 2012). When *Dyadobacter* spp. were inoculated into soil, experimental results show the genus was positively correlated with nitrogen fixation and increased nitrate reductase activity in plant leaves (Kumar et al., 2018). Additionally, *Brevundimonas, Pedobacter, Luteoliobacter, Lacibacter,* and *Caenimonas* have all been isolated from the roots of different plants (Singh et al., 2016; Raweekul et al., 2016; da Rocha et al., 2013; Jorquera et al., 2012; Kim et al., 2012). Lastly, *Larkinella*, *Rhabdobacter* and *Sphingoaurantiacus* are genera that have been previously isolated from organic amendments (*Larkinella*) or soil systems (*Rhabdobacter and Sphingoaurantiacus*) (Tan et al., 2016; Dahal et al., 2016; Anandham et al., 2015).

Abundances of other bacterial genera were increased as a result of specific recruitment by three of the four crops (but not all crops like those previously mentioned) after the disruption, and these trends were observed across pooled disrupted agroecosystems. The crop combinations that significantly increased the abundance of five bacterial genera (*Algoriphagus*, *Articibacter*, *Devosia, Oligoflexus, and Opitutus*) can be visualized in Supplemental Table 5. These genera likely possess some potential plant growth promotional abilities. For example, *Opitutus* and

Devosia are both genera associated with rice rhizosphere (Subrahmanyam et al., 2020; Moronta-Barrios et al., 2018), and *Devosia* spp. were isolated from oil palm rhizosphere in search of plant growth promoting rhizobacteria (Mohd Nor et al., 2017). Other studies show that members of the *Devosia* genus possess a myriad of plant-benefiting functions such as IAA synthesis, as well as the production of ammonia and siderophores (Rashid et al., 2011). Additionally, Rashid et al. (2011) reports that this genus is comprised of true plant-endophytes by colonizing interior tissue of tomato plants (Rashid et al., 2011). The *Algoriphagus* genus has been observed to increase their relative abundance in response plant defense inducers (salicylic acid, methyl jasmonate and abscisic acid) while decrease in relative abundance in response to ethylene (Carvalhais et al., 2014). The increased abundance response to plant-defense compounds may suggest a potential upregulation of plant-defenses carried out by members of the *Algoriphagus* genus. Literature on *Articibacter* spp. are scant, but the genus is prevalent in soybean rhizosphere during the vegetative stage (Xiao et al., 2017). In addition, *Oligoflexus tunisiensis* was isolated from the rhizosphere of both buckwheat and barley (Alkhnajari, 2019).

Crop-specific bacterial genera resulting from agroecosystem disruption

In the pooled disrupted crop treatments, there were also observations of several bacterial genera specifically increased by individual crops. The bacteria increased by beet were *Roseococus, Peredibacter, Flavisolibacter, Parasegetibacter, UTBCD1* and *Solimonas*. Some of these genera have been previously documented to be plant benefiting or associated with plant systems. The genus *Roseococcus* falls within the family Acetobacteraceae, a family associated with nitrogen fixation and plant-growth promotional abilities (Reis et al., 2015). *Peredibacter* spp. bacteria have been coined as soil-dwellers, in addition to being bacterivorous toward gram-negative soil bacteria, suggesting a potential plant biocontrol ability by *Peredibacter* (Davidov et

al., 2004). Other studies show relative abundances of *Peredibacter* also increasing after tomato was inoculated with the PGPR *Pseudmonas* sp. RU47 (Eltlbany et al., 2019). *Flavisolibacter* is a genus positively correlated with disease suppression of *Rhizoctonia solani* (Bonanomi et al., 2018). The understudied *Parasegetibacter* and *UTBCD1* genera falls within the Chitinophagaceae family, and this family has been recorded to possess plant-growth promotional genera (Madhaiyan et al., 2014; Kämpfer et al., 2011). Lastly, members of *Solimonas* have been isolated from agricultural soils growing ginseng (Kim et al., 2007).

Corn growth significantly increased abundances of the genera *Sporocytophaga* and *Pseudomonas* in addition to the families Fibrobacteraceae and Rhodothermaceae. Of promoted bacteria by corn, only *Pseudomonas* members have been extensively documented due to their plant growth promotional abilities (Rojas-Solís et al., 2018; Pham et al., 2017; Preston, 2004). However, the genus *Sporocytophaga* is widespread in soils and members such as *S. myxococcoides* are able to hydrolyze cellulose (Berg et al., 1972). Additionally, the Rhodothermaceae family bacteria also possess cellulolytic and xylanolytic activity (Liew et al., 2018). Likely both Rhodothermaceae and *Sporocytophaga* members aid in soil ecosystem cycling of plant detritus, exuding carbon sources for neighboring, potentially plant-benefiting microbes. Lastly, the family Fibrobacteraceae has been recorded to closely associate with wheat, which is in the same plant family as corn (Poaceae) (Li et al., 2017).

Lettuce growth promoted the abundance of 17 bacterial reads, and four promoted genera have been associated with plant growth or protective abilities (*Rhodoferax, Fluviicola, Cytophaga,* and *Knoellia)*. *Rhodoferax* members have been recorded to degrade chemical herbicides (Ehrig et al., 1997), and *Fluviicola* is a common member of the rice rhizosphere (Subrahmanyam et al., 2020). Bacteria within the genus *Cytophaga* are present in the barley

rhizosphere, and may also contribute to the turnover of carbon, phosphorus and nitrogen in soil ecosystems (JoHansen et al., 2002). Lastly, a species within the *Knoellia* genus was considered an endophyte of *Costus speciosus* (a type of ginger) as it was located only within leaf s of the plant (Barman et al., 2018).

Tomato growth in disrupted soils promoted nine bacterial genera (*Bacillus, Chelatococcus, Asticcacaulis, Yonghaparkia, Aminobacter, Cereibacter, Mucilaginibacter, Shimazuella,* and *Thermomonas*). *Bacillus* members have extensively been documented due to their plant growth promotional abilities (Pandey et al., 2018; Sansinenea et al., 2019; Akinrinlola et al., 2018) and *Bacillus* and *Asticcacaulis* were both considered members of the tomato endospheric bacterial community (Nieuwesteeg, 2015). Two *Muciliginibacter* spp. were recently discovered to be PGPRs by increasing root length of tomato (Madhaiyan et al., 2009). *Shimazuella* falls within the actinomycetes phylum and was isolated from *Pueraria candollei* (Kudzu) rhizosphere soil (Boonsnongcheep et al., 2017). Another genus promoted by tomato, *Yonghaparkia,* can utilize ACC (1-Aminocyclopropane-1-carboxylic acid) as a nitrogen source and isolated from the roots of *Juncus acutus* (Sharp rush) (Syranidou et al., 2017). *Aminobacter* members have been recorded to produce the plant growth hormone cytokinin (Tsavkelova et al., 2006). Lastly, *Thermomonas* members are thermotolerant (Gulmus & Gormez, 2020), explaining how these taxa were able to withstand autoclaving, although the literature on the relationship between genus and plants is lacking.

Importance of bacterial abundance increases shared by crops following disruption

Data show that some bacteria that significantly increased in abundances were associated with all crop families following disruption were indicative of PGPRs. Accordingly, we speculate that plant rhizodeposition during early growth and development plays a strong contribution to fill

"empty" niches (here "emptied" by disruption) with plant symbiotic and beneficial taxa. Since our experiment occurred during the first seven weeks of crop-growth, it is possible that some of these crop-shared genera could represent generalist-PGPRs, plant-symbiotic taxa, or bacterial keystone species. Keystone species are described as low abundance early colonizers that aid in the establishment of the plant's core microbiome (Wei et al., 2019; Harrison et al., 2018; Jones et al., 2019).

Crop-shared bacteria expressing high abundance may not represent keystone species, but instead may indicate understudied or novel PGPRs. As an example, observations of *Luteolibacter* were of particular interest as this genus was increased by all four crop families (Poaceae, Amaranthaceae, Asteraceae and Solanaceae) after growth in disrupted agroecosystems, but not increased in either NPCK (disrupted/undisrupted). The genus *Luteolibacter* falls within the Verrucomicrobia phylum, one known to be present in varying plant-soil ecosystem interactions (Aguirre-von-Wobster et al., 2018; da Rocha et al., 2013; da Rocha et al., 2011; da Rocha et al., 2010). Interestingly, this genus has been reported to occur in the rhizospheres of grass (Poaceae), leek (Amaryllildaceae) as well as potato crops (Solanaceae) (da Rocha et al., 2013). Therefore, the bacterial abundance increases shared by crops of different families may serve potential as generalist PGPRs for further experimentation.

Conclusions

Our results indicate that bacterial responses to crop growth are amplified after soil disruption. Additionally, after disruption of the organic or conventional soils, recruited taxa significantly differed from bacteria recruited by the same plant families grown in the undisrupted organic or conventional soil, and this effect did not occur in the diseased soil. Accordingly, the implementation of managerial practices to promote soil microbiome re-arrangement should be

considered in agriculture. Lastly, across all disrupted agroecosystems crops were shown to develop both crop-specific and crop-shared relationships alongside several bacterial genera. Crop-specific associations between these genera and their hosts may aid in future determination of core microbiota for the plant species tested; whereas crop-shared taxa (such as *Luteolibacter* and other potential PGPRs) may be of interest in future determination of novel and generalist plant growth promoting rhizobacteria.

Figures

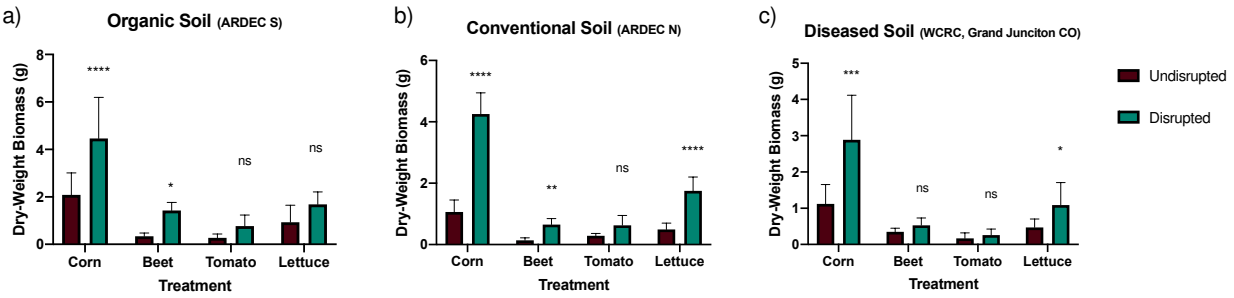


Figure 3-1: Mean dry-weight (DW) biomass measurements for each crop in each soil treatment (n=10 per crop per soil treatment, ± standard deviation). (a) DW biomass results from crops grown in organic soil (b) DW biomass of crops grown in conventional soil (c) DW biomass of all crops grown in diseased soil. Red or blue bars represent the mean DW biomass for each crop in undisrupted or disrupted soil respectively. Significant differences between mean DW biomass of crops grown in disrupted or undisrupted soils are denoted by "*", "**", "***" or "****".

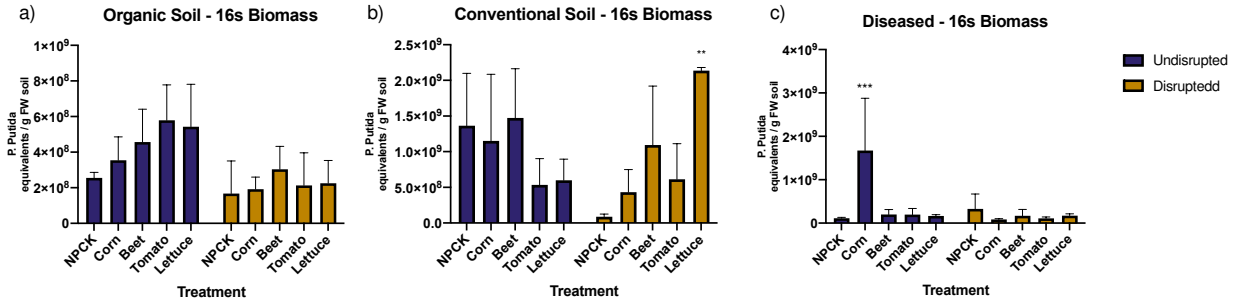


Figure 3-2: Mean bacterial cell counts per gram of fresh weight of each soil (n=3 per crop per soil treatment, ± standard deviation). (a) bacterial cell counts resulting after crop growth in organic soil (b) bacterial cell counts resulting after crop growth in conventional soil (c) bacterial cell counts resulting after crop growth in diseased soil. Purple or yellow bars represent the mean number of bacterial cells for each crop grown in undisrupted or disrupted soils, respectively Bacterial cell counts can be interpreted as *P putida* equivalents per gram of fresh-weight soil Significant differences in cell counts between the NPCKs and crop treatments are denoted by "*".

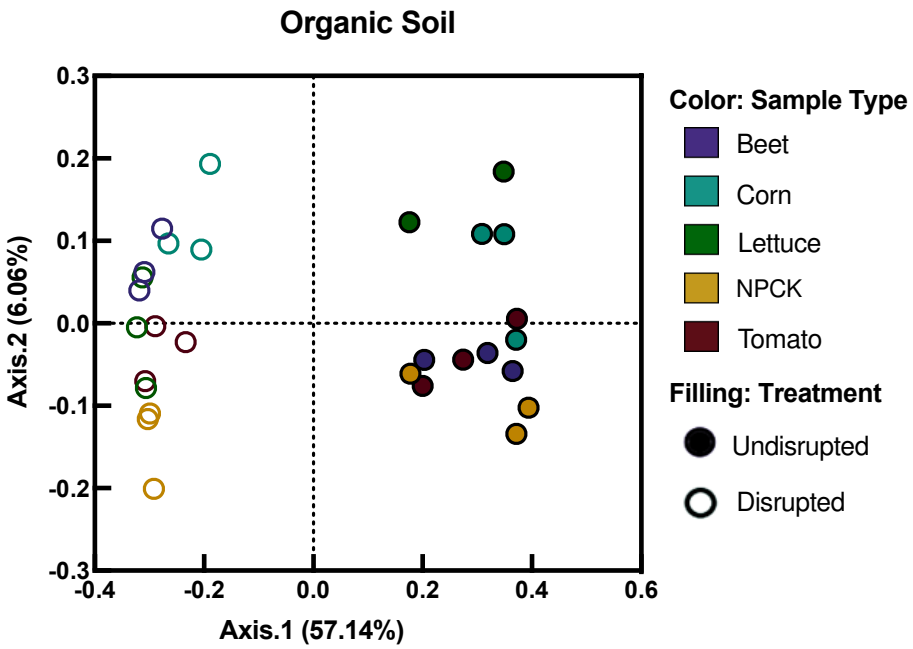


Figure 3-3: Principal Coordinate Analyses (PCoA) of rhizobacterial communities after crop growth in undisrupted and disrupted organic agroecosystem soils. Colored circles indicate samples treated with disruption, whereas hollow circles indicate undisrupted samples, respectively. Purple, teal, green, yellow or red color represents rhizospheric samples from beet, corn, lettuce, NPCKs or tomato samples, respectively.

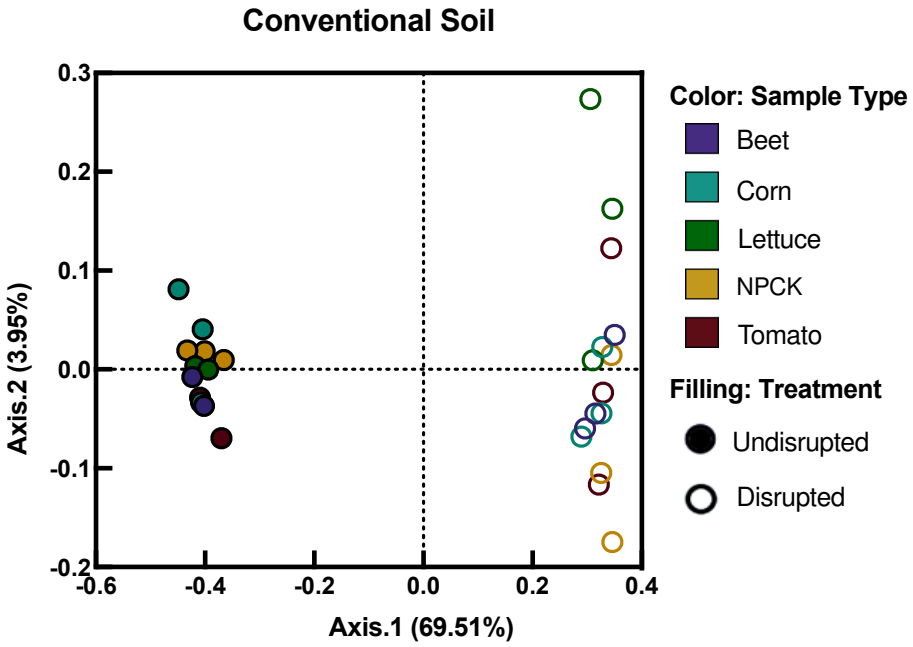


Figure 3-4**:** Principal Coordinate Analyses (PCoA) of rhizobacterial communities after crop growth in undisrupted and disrupted conventional agroecosystem soils. Colored circles indicate samples treated with disruption, whereas hollow circles indicate undisrupted soils, respectively. Purple, teal, green, yellow or red color represents rhizospheric samples from beet, corn, lettuce, NPCKs or tomato samples, respectively.

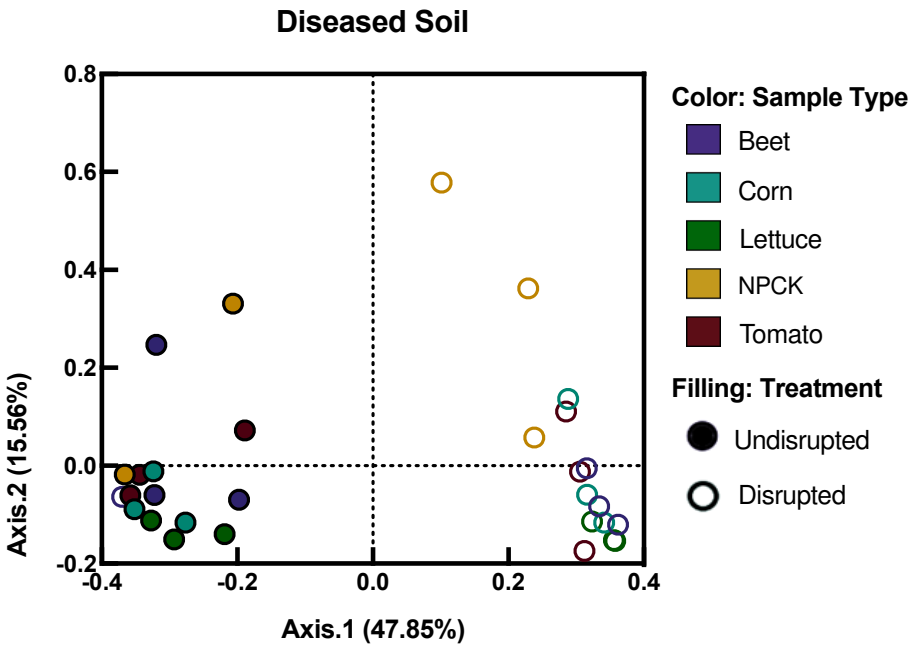


Figure 3-5: Principal Coordinate Analyses (PCoA) of rhizobacterial communities after crop growth in undisrupted and disrupted diseased agroecosystem soils. Colored circles indicate samples treated with disruption, whereas hollow circles indicate undisrupted soils, respectively. Purple, teal, green, yellow or red color represents rhizospheric samples from beet, corn, lettuce, NPCKs or tomato samples, respectively.

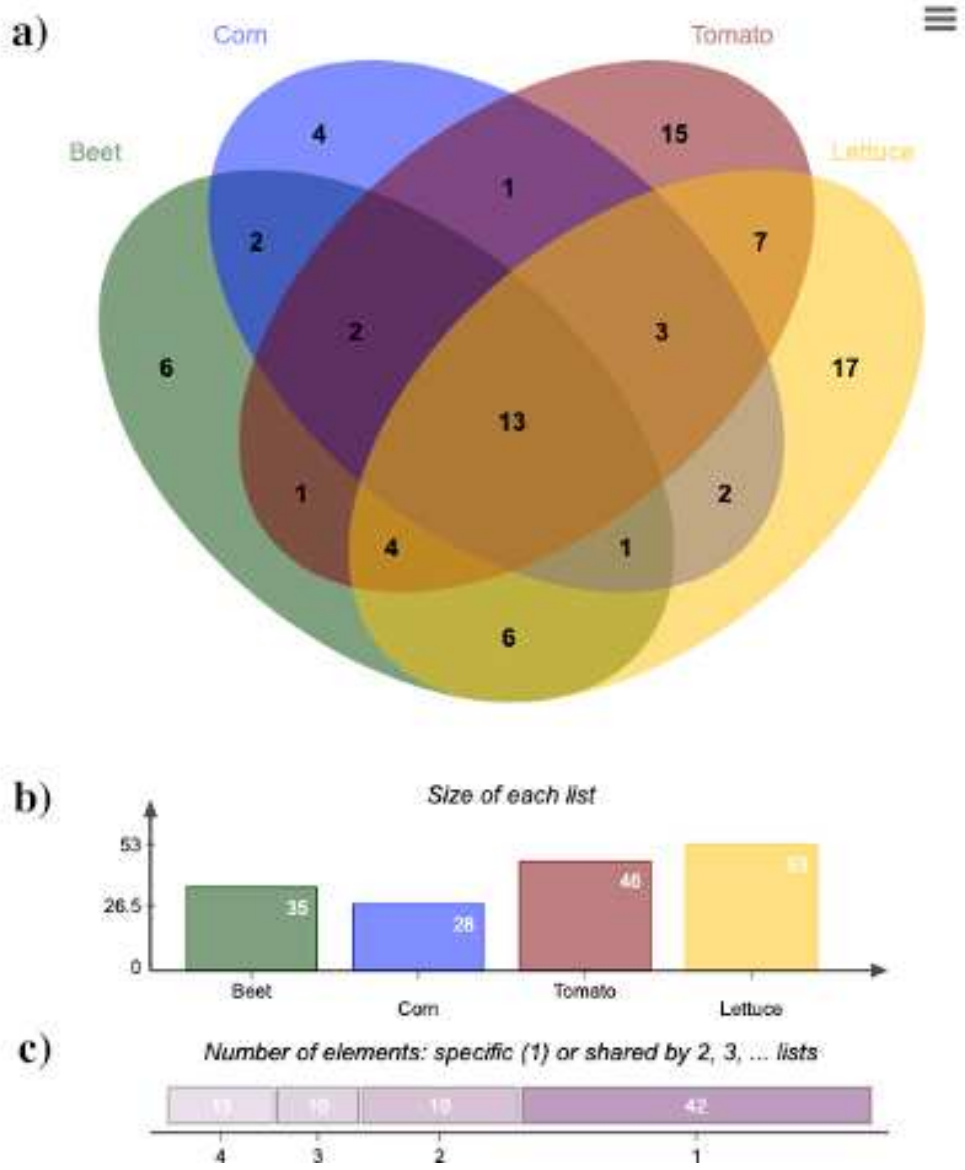


Figure 3-6: Venn diagram displaying the shared and unique bacterial reads from pooled crop rhizospheres after disruption. Bacterial reads comprising the figure were calculated from Tables 3-6. The list of full crop-shared or crop specific bacterial reads can be visualized in Supplemental Table 5. (A) Venn diagrams noting the number of shared or specific genera for each crop (green = beet, blue = corn, red = tomato, yellow = lettuce). (B) Bar graph showing the size of bacterial increased in abundance by each crop after disruption. (C) Display showing the number of bacterial reads unique to one (42) or shared by two, three or four crops (19, 10, 13, respectively).

Tables

Table 3-1: Chemical analyses of soils (n=2 per agroecosystem) tested. Green- or red-highlighted cells in the p-value column (within each larger agroecosystem column) indicate significant increases or decreases in the parameter tested as a result of applying disruption to the soils.

(n=2)	Organic Soil			Conventional Soil			Diseased Soil		
	Undisrupted	Disrupted	p-value	Undisrupted	Disrupted	p-value	Undisrupted	Disrupted	p-value
pH	7.62	7.745	0.341	7.985	8.175	0.094	7.82	7.93	0.448
EC (mmhos/cm)	2.45	2.58	0.909	0.6	0.7	0.966	0.615	0.71	0.973
Lime Estimate	high	med/high	-	high	high	-	high	high	-
% Organic Matter	2.85	2.25	0.480	2.75	2.85	0.999	2.5	2.3	0.984
NO3-N ppm	24.15	23.8	0.991	7.35	6.45	0.709	0.65	0.8	1.000
P ppm	17.75	22.4	0.004	11.25	16.45	0.002	14.35	17.8	0.016
K ppm	566.5	525	0.072	465	437.5	0.282	254.5	240.5	0.811
Zn ppm	1.4	1.2	0.178	1.15	0.9	0.081	4	3.45	0.002
Fe ppm	7.6	7.55	1.000	5.55	7.35	0.302	13.3	10.45	0.066
Mn ppm	12	39.5	0.000	1.85	35	0.000	4.2	36	0.000
Cu ppm	3.7	3.35	0.233	2.3	2.1	0.689	7.7	5.25	0.000
Texture	Silty Clay / Clay	Silty Clay	-	Silty clay / clay	Silty Clay	-	Sandy clay / SC loam	Sandy Clay	-

Table 3-2: Shannon's diversity index. Shannon's diversity index values were recorded for all samples and determined using the R's vegan package (Vers. 25-6). (NPCK= No plant controls).

Soil Type	Treatment	Crops				
		Corn	Beet	Tomato	Lettuce	NPCK
Organic	Field Collected					2.40
	Undisrupted	4.31	4.64	4.50	3.88	4.48
	Disrupted	4.44	4.41	4.63	4.66	4.65
Conventional	Field Collected					4.63
	Undisrupted	3.98	4.40	4.44	4.38	4.36
	Disrupted	4.44	4.14	4.16	4.22	3.82
Diseased	Field Collected					4.46
	Undisrupted	5.00	4.41	4.99	4.63	4.40
	Disrupted	4.15	4.19	4.28	4.16	3.63

Table 3-3: Shared and unique significant abundance increases of genus-level bacteria, prompted by each crop in all undisrupted agroecosystems (FDR: False discovery rate. Gray highlighting indicates the genus was not significantly altered within the crop rhizosphere).

Genus	No-Plant basemean	Beet basemean	p-value	FDR	No-Plant basemean	Corn basemean	p-value	FDR	No-Plant basemean	Lettuce basemean	p-value	FDR	No-Plant basemean	Tomato basemean	p-value	FDR
Azohydromonas					101.401	50.701	< 0.001	0.000								
Catellatospora									0.000	11.543	< 0.001	0.071				
Pseudomonas									9.969	177.193	< 0.001	0.071				
Rhizorhapis									0.000	31.760	< 0.001	0.000				

Table 3-4: Shared and unique significant abundance increases of genus-level bacteria, prompted by each crop in all disrupted agroecosystems (FDR: False discovery rate. Gray highlighting indicates the genus was not significantly altered within the crop rhizosphere)

Genus	No-Plant basemean	Beet basemean	p-value	FDR	No-Plant basemean	Corn basemean	p-value	FDR	No-Plant basemean	Lettuce basemean	p-value	FDR	No-Plant basemean	Tomato basemean	p-value	FDR
Dyadobacter	0.000	9.726	< 0.001	0.100	0.000	16.783	< 0.001	0.041	0.000	54.051	< 0.001	0.000				
Lacibacter									0.000	7.470	< 0.001	0.046				
MM2									0.000	8.532	< 0.001	0.046				
Neorhizobium									0.000	9.853	< 0.001	0.010				
Niastella									2.757	50.557	< 0.001	0.046				
Novosphingobium	15.540	313.827	< 0.001	0.030												
Oscillatoria_PCC-6304					57.927	0.300	< 0.001	0.041								
Qipengyuania									150.482	12.853	< 0.001	0.046				
Rhizorhapis									0.000	32.106	< 0.001	0.000				
Rhodobacter					43.719	0.000	< 0.001	0.041	43.719	0.000	< 0.001	0.036				
Sphingobium													2.074	113.283	< 0.001	0.005
Verrucomicrobium									0.000	23.053	< 0.001	0.046				

Table 3-5: Bacterial genera observed to significantly increase in abundance after corn growth across all disrupted soils, compared to abundances of the same genera across undisrupted soils growing corn. If any abundance increases were observed from taxa listed in the NPCKs (Table 3-9), they were removed from this table so included taxa may be attributed to corn presence. Unclassified genera increased in abundance after disruption are listed with their highest classification level (k: kingdom, p: phylum, etc.) (FDR: False discovery rate).

Family_*Genus*	Basemean Disrupted	Basemean Undisrupted	p-value	FDR
(c: Microgenomatia o: unclassified) unclassified_*unclassified*	2.64	0.00	0.013	0.073
(c: Parcubacteria o: unclassified) unclassified_*unclassified*	19.93	2.56	0.019	0.098
(c: Sericytochromatia o: unclassified) unclassified_*unclassified*	25.33	2.44	0.000	0.004
(o: Candidatus_Nomurabacteria) unclassified_*unclassified*	12.40	0.40	0.000	0.006
(p: BRC1 c: unclassified o: unclassified) unclassified_*unclassified*	3.57	0.00	0.010	0.064
Acetobacteraceae_*Roseococcus*	7.36	0.11	0.005	0.036
Bacillaceae_*Fictibacillus*	181.87	6.99	0.000	0.000
Bacteriovoracaceae_*Peredibacter*	5.27	0.23	0.004	0.032
Beijerinckiaceae_*Bosea*	57.21	10.20	0.001	0.012
Burkholderiaceae_*Caenimonas*	20.82	3.43	0.017	0.091
Burkholderiaceae_*Massilia*	356.84	11.81	0.000	0.000
Burkholderiaceae_*Noviherbaspirillum*	119.20	13.11	0.000	0.004
Caulobacteraceae_*Brevundimonas*	182.33	4.60	0.000	0.000
Caulobacteraceae_*Caulobacter*	30.50	7.04	0.011	0.067
Caulobacteraceae_*Phenylobacterium*	104.01	18.95	0.000	0.000
Cellvibrionaceae_*Cellvibrio*	46.83	5.19	0.001	0.008
Chitinophagaceae_*Cnuella*	15.05	0.15	0.001	0.009
Chitinophagaceae_*Flaviaesturariibacter*	15.83	0.66	0.001	0.006
Chitinophagaceae_*Flavisolibacter*	33.54	9.96	0.010	0.064
Chitinophagaceae_*Lacibacter*	32.61	0.86	0.000	0.002
Chitinophagaceae_*Parasegetibacter*	2.33	0.00	0.014	0.078
Chitinophagaceae_*UTBCD1*	8.02	0.43	0.015	0.082
Chthoniobacteraceae_*Chthoniobacter*	227.75	24.58	0.000	0.000
Cyclobacteriaceae_*Algoriphagus*	14.72	0.00	0.001	0.012
Devosiaceae_*Devosia*	210.08	44.28	0.000	0.002
Devosiaceae_*unclassified*	30.39	8.36	0.017	0.091
Family_XVIII_*unclassified*	5.13	0.10	0.011	0.067
Gemmatimonadaceae_*Gemmatirosa*	11.04	0.73	0.010	0.064
Hymenobacteraceae_*Adhaeribacter*	192.42	14.84	0.000	0.000
Hymenobacteraceae_*Pontibacter*	68.36	3.40	0.000	0.000
Kineosporiaceae_*Quadrisphaera*	28.27	5.71	0.017	0.091
Micrococcaceae_*Paenarthrobacter*	193.92	10.95	0.000	0.000
Micromonosporaceae_*Micromonospora*	427.62	21.32	0.000	0.005
Micropepsaceae_*unclassified*	29.45	3.82	0.000	0.003
Oligoflexaceae_*Oligoflexus*	18.92	1.72	0.010	0.064
Opitutaceae_*Opitutus*	69.90	17.40	0.004	0.033
Paenibacillaceae_*Ammoniphilus*	10.23	0.46	0.004	0.035

Family_*Genus*	Basemean Disrupted	Basemean Undisrupted	p-value	FDR
Paenibacillaceae_*Brevibacillus*	26.57	0.00	0.000	0.001
Paenibacillaceae_*Cohnella*	11.82	0.00	0.000	0.000
Pedosphaeraceae_*unclassified*	174.40	17.73	0.000	0.000
Planococcaceae_*Paenisporosarcina*	32.70	7.29	0.005	0.036
Rhizobiaceae_*unclassified*	274.41	7.47	0.000	0.000
Rubinisphaeraceae_*SH-PL14*	17.04	3.52	0.011	0.067
Rubritaleaceae_*Luteolibacter*	108.16	22.46	0.000	0.001
Saccharimonadaceae_*unclassified*	28.73	5.24	0.013	0.074
Solibacteraceae__*Paludibaculum*	13.51	0.42	0.000	0.002
Solimonadaceae_*Solimonas*	1.45	0.00	0.018	0.093
Sphingobacteriaceae_*Arcticibacter*	3.56	0.00	0.001	0.012
Sphingobacteriaceae_*Pedobacter*	100.58	4.36	0.000	0.000
Sphingobacteriaceae_*unclassified*	23.29	0.34	0.000	0.002
Sphingomonadaceae_*Porphyrobacter*	33.33	7.11	0.008	0.056
Sphingomonadaceae_*Sphingoaurantiacus*	33.34	0.91	0.000	0.000
Sphingomonadaceae_*Sphingopyxis*	49.53	3.53	0.000	0.000
Sphingomonadaceae_*unclassified*	43.52	13.26	0.003	0.028
Spirosomaceae_*Dyadobacter*	17.19	0.70	0.004	0.032
Spirosomaceae_*Larkinella*	2.05	0.00	0.014	0.081
Spirosomaceae_*Rhabdobacter*	3.50	0.00	0.005	0.036
Streptomycetaceae_*Allostreptomyces*	15.99	0.06	0.002	0.023
Thermoactinomycetaceae_*unclassified*	9.69	0.13	0.001	0.007
Verrucomicrobiaceae_*Roseimicrobium*	28.47	3.09	0.001	0.011
Verrucomicrobiaceae_*unclassified*	26.60	4.86	0.015	0.083
Verrucomicrobiaceae_*Verrucomicrobium*	10.21	0.34	0.010	0.064

Table 3-6: Bacterial genera observed to significantly increase in abundance after beet growth across all disrupted soils, compared to abundances of the same genera across undisrupted soils growing beet. If any abundance increases were observed from taxa listed in the NPCKs (Table 3-9), they were removed from this table so included taxa may be attributed to corn presence. Unclassified genera increased in abundance after disruption are listed with their highest classification level (k: kingdom, p: phylum, etc.) (FDR: False discovery rate).

Family_*Genus*	Basemean Disrupted	Basemean Undisrupted	p-value	FDR
(c: Microgenomatia o: unclassified) unclassified_*unclassified*	3.272	0.000	0.016	0.096
(o: Bacillales) unclassified_*unclassified*	3.212	0.000	0.007	0.050
(o: Candidatus_Nomurabacteria) unclassified_*unclassified*	14.864	0.655	0.006	0.047
(o: Candidatus_Peribacteria) unclassified_*unclassified*	8.944	0.313	0.002	0.020
Bacillaceae_*Fictibacillus*	150.049	14.446	0.001	0.009
Beijerinckiaceae_*Bosea*	46.467	8.114	0.000	0.006
Burkholderiaceae_*Caenimonas*	31.039	2.475	0.001	0.008
Burkholderiaceae_*Massilia*	384.284	32.979	0.000	0.003
Burkholderiaceae_*Noviherbaspirillum*	117.858	10.135	0.000	0.004
Caulobacteraceae_*Brevundimonas*	79.498	16.118	0.006	0.047
Caulobacteraceae_*Caulobacter*	47.218	3.675	0.001	0.008
Caulobacteraceae_*Phenylobacterium*	115.163	25.510	0.003	0.028
Cellvibrionaceae_*unclassified*	22.956	0.256	0.001	0.010
Chitinophagaceae_*Cnuella*	11.328	0.000	0.001	0.007
Chitinophagaceae_*Flaviaesturariibacter*	7.139	0.194	0.003	0.023
Chitinophagaceae_*Lacibacter*	51.383	4.399	0.000	0.005
Chthoniobacteraceae_*Chthoniobacter*	248.652	17.794	0.000	0.000
Cytophagaceae_*Sporocytophaga*	8.361	0.194	0.000	0.001
Devosiaceae_*unclassified*	39.638	9.033	0.003	0.028
Family_XVIII_*unclassified*	8.143	0.000	0.000	0.002
Fibrobacteraceae_*possible_genus_04*	14.154	0.000	0.000	0.002
Fibrobacteraceae_*unclassified*	2.182	0.000	0.010	0.064
Gemmatimonadaceae_*Gemmatirosa*	7.441	0.000	0.000	0.005
Hymenobacteraceae_*Adhaeribacter*	276.732	18.739	0.000	0.000
Hymenobacteraceae_*Pontibacter*	55.630	6.030	0.005	0.038
KD3-93_*unclassified*	4.543	0.000	0.002	0.022
Micrococcaceae_*Paenarthrobacter*	146.627	3.266	0.000	0.000
Micromonosporaceae_*Micromonospora*	321.553	24.236	0.000	0.005
Micropepsaceae_*unclassified*	26.648	5.524	0.008	0.057
Opitutaceae_*IMCC26134*	8.010	0.000	0.000	0.001
Opitutaceae_*Opitutus*	125.033	9.715	0.000	0.000
Paenibacillaceae_*Paenibacillus*	17.409	0.733	0.010	0.066
Pedosphaeraceae_*unclassified*	208.781	11.805	0.000	0.000
Planococcaceae_*Lysinibacillus*	4.951	0.079	0.010	0.066
Planococcaceae_*Paenisporosarcina*	31.080	4.104	0.000	0.005
Pseudohongiellaceae_*BIyi10*	5.754	0.000	0.001	0.010
Pseudomonadaceae_*Pseudomonas*	133.050	16.445	0.012	0.075
Rhizobiaceae_*unclassified*	336.043	5.524	0.000	0.000

Family_*Genus*	Basemean Disrupted	Basemean Undisrupted	p-value	FDR
Rhodothermaceae_*unclassified*	3.311	0.055	0.007	0.052
Rubritaleaceae_*Luteolibacter*	54.161	4.633	0.000	0.004
Saccharimonadaceae_*unclassified*	24.852	1.439	0.013	0.080
Solibacteraceae__*Paludibaculum*	8.306	0.000	0.000	0.001
Sphingobacteriaceae_*Pedobacter*	160.149	3.950	0.000	0.000
Sphingobacteriaceae_*unclassified*	22.756	0.228	0.000	0.000
Sphingomonadaceae_*Sphingoaurantiacus*	22.806	1.783	0.002	0.019
Sphingomonadaceae_*Sphingopyxis*	54.493	2.409	0.000	0.002
Spirosomaceae_*Dyadobacter*	9.642	0.134	0.001	0.014
Spirosomaceae_*Larkinella*	3.476	0.000	0.006	0.045
Spirosomaceae_*Rhabdobacter*	12.881	0.106	0.000	0.000
Streptomycetaceae_*Allostreptomyces*	23.138	0.015	0.000	0.002
Thermoactinomycetaceae_*Baia*	5.542	0.000	0.007	0.052

Table 3-7: Bacterial genera observed to significantly increase in abundance after lettuce growth across all disrupted soils, compared to abundances of the same genera across undisrupted soils growing lettuce. If any abundance increases were observed from taxa listed in the NPCKs (Table 3-9), they were removed from this table so included taxa may be attributed to lettuce presence. Unclassified genera increased in abundance after disruption are listed with their highest classification level (k: kingdom, p: phylum, etc.) (FDR: False discovery rate).

Family_*genus*	Basemean Disrupted	Basemean Undisrupted	p-value	FDR
(c: Sericytochromatia o: unclassified) unclassified_*unclassified*	41.45	1.49	0.000	0.002
(class: Berkelbacteria o: unclassified) unclassified_*unclassified*	6.79	0.12	0.014	0.079
(k: Archea c: Woesearchaeia) unclassified_*unclassified*	3.29	0.00	0.019	0.097
(k: unclassified p: unclassified c: unclassified o: unclassified) unclassified_*unclassified*	5.64	0.04	0.007	0.055
(o: Bacillales) unclassified_*unclassified*	5.66	0.20	0.002	0.018
(o: Candidatus_Nomurabacteria) unclassified_*unclassified*	8.99	0.09	0.016	0.088
(o: Candidatus_Peribacteria) unclassified_*unclassified*	18.30	0.20	0.001	0.016
(o: Micavibrionales) unclassified_*unclassified*	14.48	1.53	0.016	0.088
(o: Rhodospirillales) unclassified_*unclassified*	5.88	0.41	0.002	0.019
Acetobacteraceae_*Rhodovastum*	2.98	0.10	0.015	0.084
Acetobacteraceae_*Roseomonas*	23.15	5.67	0.016	0.088
Bacillaceae_*Anoxybacillus*	9.02	0.13	0.008	0.059
Bacillaceae_*Fictibacillus*	189.32	28.51	0.002	0.025
Beijerinckiaceae_*alphaI_cluster*	7.94	0.76	0.019	0.097
Beijerinckiaceae_*Bosea*	46.16	4.30	0.000	0.001
Burkholderiaceae_*Caenimonas*	30.83	3.68	0.010	0.068
Burkholderiaceae_*Noviherbaspirillum*	152.96	23.94	0.004	0.031
Burkholderiaceae_*Rhodoferax*	10.11	0.00	0.011	0.070
Burkholderiaceae_*unclassified*	54.92	13.73	0.021	0.099
Caedibacteraceae_*Candidatus_Nucleicultrix*	4.19	0.00	0.000	0.005
Caulobacteraceae_*Brevundimonas*	52.82	8.65	0.002	0.022
Caulobacteraceae_*Phenylobacterium*	104.94	22.76	0.000	0.005
Caulobacteraceae_*unclassified*	6.35	0.47	0.006	0.049
Cellvibrionaceae_*Cellvibrio*	112.67	5.44	0.000	0.004
Cellvibrionaceae_*unclassified*	40.80	0.00	0.000	0.000
Chitinophagaceae_*Cnuella*	10.65	0.00	0.000	0.006
Chitinophagaceae_Lacibacter	50.95	0.33	0.000	0.000
Chthoniobacteraceae_*Chthoniobacter*	337.52	22.61	0.000	0.000
Crocinitomicaceae_*Fluviicola*	3.62	0.30	0.016	0.088
Cyclobacteriaceae_*Algoriphagus*	6.35	0.00	0.000	0.000
Cyclobacteriaceae_*unclassified*	4.73	0.00	0.009	0.062
Cytophagaceae_*Cytophaga*	1.31	0.00	0.020	0.098
Devosiaceae_*Devosia*	158.77	53.89	0.012	0.071
env.OPS_17_*unclassified*	10.29	0.25	0.004	0.035

Family_*Genus*	Basemean Disrupted	Basemean Undisrupted	p-value	FDR
Family_XVIII_*unclassified*	21.78	0.06	0.000	0.000
Fibrobacteraceae_*unclassified*	1.88	0.00	0.013	0.077
Flavobacteriaceae_*Flavobacterium*	21.75	1.90	0.001	0.010
Gemmatimonadaceae_*Gemmatirosa*	12.58	0.13	0.006	0.048
Hymenobacteraceae_*Adhaeribacter*	369.78	39.37	0.000	0.000
Intrasporangiaceae_*Knoellia*	7.30	0.00	0.016	0.088
KD3-93_*unclassified*	5.66	0.12	0.017	0.091
Kineosporiaceae_*Quadrisphaera*	31.07	2.97	0.003	0.027
Micrococcaceae_*Paenarthrobacter*	160.32	25.93	0.004	0.031
Micromonosporaceae_*Micromonospora*	308.70	12.90	0.000	0.001
Micropepsaceae_*unclassified*	25.37	2.51	0.000	0.007
Nodosilineaceae_*Nodosilinea_PCC-7104*	12.94	0.51	0.019	0.097
Oligoflexaceae_*Oligoflexus*	26.99	2.67	0.011	0.069
Opitutaceae_*IMCC26134*	8.79	0.00	0.000	0.000
Opitutaceae_*unclassified*	5.06	0.00	0.012	0.070
Oscillatoriaceae_*Oscillatoria_PCC-6304*	2.14	0.00	0.012	0.073
Paenibacillaceae_*Brevibacillus*	19.66	0.17	0.004	0.034
Paenibacillaceae_Cohnella	13.29	0.00	0.000	0.005
Pedosphaeraceae_*unclassified*	290.74	19.28	0.000	0.000
Planococcaceae_*Paenisporosarcina*	33.26	6.98	0.007	0.049
Pseudohongiellaceae_*BIyi10*	6.30	0.08	0.001	0.012
Rhizobiaceae_*unclassified*	335.95	3.27	0.000	0.000
Rubinisphaeraceae_*SH-PL14*	19.03	3.52	0.020	0.099
Rubritaleaceae_Luteolibacter	54.46	4.99	0.000	0.001
Saccharimonadaceae_*unclassified*	11.82	0.80	0.016	0.088
Solibacteraceae__*Paludibaculum*	14.41	1.06	0.011	0.070
Sphingobacteriaceae_*Arcticibacter*	8.78	0.11	0.007	0.053
Sphingobacteriaceae_*Pedobacter*	125.37	15.39	0.000	0.000
Sphingobacteriaceae_*unclassified*	14.16	1.23	0.001	0.010
Sphingomonadaceae_*Porphyrobacter*	19.78	1.42	0.013	0.075
Sphingomonadaceae_*Sphingoaurantiacus*	17.54	0.79	0.001	0.010
Sphingomonadaceae_*Sphingopyxis*	26.68	1.24	0.000	0.007
Sphingomonadaceae_*unclassified*	28.21	7.87	0.020	0.099
Spirosomaceae_Dyadobacter	53.58	0.17	0.000	0.000
Spirosomaceae_*Larkinella*	9.54	0.00	0.002	0.023
Spirosomaceae_*Rhabdobacter*	16.81	0.00	0.000	0.001
Sporolactobacillaceae_*unclassified*	6.03	0.00	0.005	0.039
Streptomycetaceae_*Allostreptomyces*	13.79	0.01	0.001	0.008
Tepidisphaeraceae_*Tepidisphaera*	10.08	1.31	0.004	0.034
Thermoactinomycetaceae_*unclassified*	8.63	0.07	0.002	0.019
Verrucomicrobiaceae_*Brevifollis*	5.12	0.00	0.014	0.078
Verrucomicrobiaceae_*Roseimicrobium*	47.54	3.59	0.002	0.018
Verrucomicrobiaceae_*Verrucomicrobium*	23.12	0.00	0.000	0.007

Table 3-8: Bacterial genera observed to significantly increase in abundance after tomato growth across all disrupted soils, compared to abundances of the same genera across undisrupted soils growing tomato. If any abundance increases were observed from taxa listed in the NPCKs (Table 3-9), they were removed from this table so included taxa may be attributed to tomato presence. Unclassified genera increased in abundance after disruption are listed with their highest classification level (k: kingdom, p: phylum, etc.) (FDR: False discovery rate).

Family_*genus*	**Basemean Disrupted**	**Basemean Undisrupted**	**p-value**	**FDR**
(c: Berkelbacteria f: unclassified) unclassified_*unclassified*	3.60	0.10	0.012	0.069
(c: Microgenomatia o: unclassified) unclassified_*unclassified*	2.14	0.00	0.020	0.099
(c: OPB56) unclassified_*unclassified*	12.90	2.66	0.003	0.024
(f: Candidatus_Peribacteria) unclassified_*unclassified*	15.03	0.97	0.007	0.044
(f: Candidatus_Pacebacteria) unclassified_*unclassified*	2.52	0.00	0.019	0.097
(p: BRC1 c: unclassified o: unclassified unclassified) unclassified_*unclassified*	6.34	0.06	0.002	0.016
Archangiaceae_*unclassified*	11.27	0.21	0.008	0.049
Bacillaceae_*Anoxybacillus*	4.80	0.00	0.010	0.058
Bacillaceae_*Bacillus*	307.27	126.93	0.013	0.072
Bacillaceae_*Fictibacillus*	184.91	15.52	0.000	0.001
Beijerinckiaceae_*alphaI_cluster*	6.03	0.38	0.009	0.053
Beijerinckiaceae_*Bosea*	37.79	4.46	0.000	0.001
Beijerinckiaceae_*Chelatococcus*	12.27	0.00	0.005	0.037
Burkholderiaceae_*Caenimonas*	27.97	3.11	0.003	0.024
Burkholderiaceae_*Massilia*	379.32	62.75	0.003	0.028
Burkholderiaceae_*Noviherbaspirillum*	159.81	11.16	0.000	0.000
Caulobacteraceae_*Asticcacaulis*	6.32	0.00	0.000	0.001
Caulobacteraceae_*Brevundimonas*	108.10	18.16	0.002	0.016
Caulobacteraceae_*Phenylobacterium*	99.44	21.84	0.002	0.016
Cellvibrionaceae_*Cellvibrio*	51.12	5.95	0.017	0.091
Cellvibrionaceae_*unclassified*	15.53	0.42	0.005	0.035
Chitinophagaceae_*Cnuella*	8.40	0.22	0.002	0.016
Chitinophagaceae_*Lacibacter*	27.98	0.06	0.000	0.000
Chthoniobacteraceae_*Chthoniobacter*	240.34	27.47	0.000	0.000
Cyclobacteriaceae_*Algoriphagus*	5.35	0.00	0.003	0.027
Cyclobacteriaceae_*unclassified*	8.48	0.21	0.006	0.039
Devosiaceae_*Devosia*	207.26	54.32	0.003	0.025
Devosiaceae_*unclassified*	32.62	8.22	0.007	0.048
env.OPS_17_*unclassified*	10.46	0.68	0.011	0.065
Family_XVIII_*unclassified*	8.16	0.18	0.000	0.000
Fibrobacteraceae_*unclassified*	5.46	0.00	0.018	0.094

Family_*Genus*	Basemean Disrupted	Basemean Undisrupted	p-value	FDR
Flavobacteriaceae_*Flavobacterium*	21.01	3.39	0.014	0.078
Hydrogenedensaceae_*unclassified*	5.76	0.09	0.012	0.069
Hymenobacteraceae_*Adhaeribacter*	198.26	25.52	0.000	0.001
KD3-93_*unclassified*	1.91	0.00	0.010	0.058
Microbacteriaceae_*Yonghaparkia*	13.51	0.72	0.013	0.073
Micrococcaceae_*Paenarthrobacter*	135.00	3.58	0.000	0.000
Micromonosporaceae_*Micromonospora*	444.21	23.90	0.000	0.005
Micropepsaceae_*unclassified*	24.03	4.19	0.008	0.052
Nodosilineaceae_*Nodosilinea_PCC-7104*	112.69	8.37	0.009	0.053
Oligoflexaceae_*Oligoflexus*	20.86	2.79	0.008	0.052
Opitutaceae_*Opitutus*	65.38	21.95	0.014	0.078
Paenibacillaceae_*Ammoniphilus*	25.47	4.24	0.006	0.042
Paenibacillaceae_*Cohnella*	12.12	0.00	0.000	0.000
Paenibacillaceae_*Paenibacillus*	28.58	1.89	0.003	0.024
Pedosphaeraceae_*unclassified*	161.92	15.91	0.000	0.000
Planococcaceae_*Lysinibacillus*	8.74	0.75	0.015	0.084
Planococcaceae_*Paenisporosarcina*	27.90	3.73	0.001	0.012
Rhizobiaceae_*Aminobacter*	8.15	0.26	0.008	0.052
Rhizobiaceae_*unclassified*	272.96	5.69	0.000	0.000
Rhodobacteraceae_*Cereibacter*	2.88	0.00	0.015	0.083
Rubritaleaceae_*Luteolibacter*	41.91	4.04	0.000	0.000
Saccharimonadaceae_*unclassified*	22.73	0.41	0.003	0.022
Solibacteraceae__*Paludibaculum*	4.78	0.26	0.018	0.096
Sphingobacteriaceae_*Arcticibacter*	3.39	0.00	0.005	0.038
Sphingobacteriaceae_*Mucilaginibacter*	5.08	0.00	0.001	0.010
Sphingobacteriaceae_*Pedobacter*	78.09	12.97	0.000	0.002
Sphingobacteriaceae_*unclassified*	14.62	0.17	0.000	0.000
Sphingomonadaceae_*Sphingoaurantiacus*	33.83	4.94	0.003	0.025
Sphingomonadaceae_*Sphingopyxis*	27.74	0.81	0.000	0.000
Spirosomaceae_*Dyadobacter*	7.01	0.20	0.017	0.091
Spirosomaceae_*Larkinella*	2.19	0.00	0.003	0.024
Spirosomaceae_*Rhabdobacter*	11.81	0.00	0.000	0.000
Streptomycetaceae_*Allostreptomyces*	23.62	0.00	0.000	0.002
Thermoactinomycetaceae_*Baia*	12.72	0.00	0.002	0.015
Thermoactinomycetaceae_*Shimazuella*	4.08	0.00	0.011	0.066
Thermoactinomycetaceae_*unclassified*	8.88	0.26	0.002	0.016
Verrucomicrobiaceae_*unclassified*	28.33	3.13	0.003	0.024

Family_*Genus*	Basemean Disrupted	Basemean Undisrupted	p-value	FDR
Verrucomicrobiaceae_*Verrucomicrobium*	2.76	0.00	0.020	0.099
Xanthomonadaceae_*Stenotrophomonas*	8.17	0.08	0.005	0.037
Xanthomonadaceae_*Thermomonas*	5.33	0.00	0.008	0.049

Table 3-9: Bacterial genera observed to significantly increase in abundance in the disrupted NPCKs, compared to abundance of the same genera across undisrupted NPCKs. Unclassified genera increased in abundance after disruption are listed with their highest classification level (k: kingdom, p: phylum, etc.). Taxa listed in Table 3-9 served as a control to filter out bacterial reads able to proliferate after disruption in the absence of plant growth.

Family _ *Genus*	Basemean Disrupted	Basemean Undisrupted	P-value	FDR
(c: Parcubacteria o: unclassified) unclassified_*unclassified*	32.76	2.02	0.004	0.042
(o: Rhodospirillales) unclassified_*unclassified*	12.94	1.42	0.005	0.052
(p: BRC1 c: unclassified o: unclassified) unclassified_*unclassified*	4.74	0.00	0.009	0.078
Archangiaceae_*Anaeromyxobacter*	4.11	0.00	0.005	0.053
Bacillaceae_*Fictibacillus*	168.66	10.44	0.001	0.015
Burkholderiaceae_*Massilia*	321.81	38.00	0.007	0.062
Burkholderiaceae_*Noviherbaspirillum*	78.58	10.57	0.006	0.059
Burkholderiaceae_*Rhizobacter*	19.52	0.19	0.004	0.043
Caedibacteraceae_*Candidatus_Nucleicultrix*	5.19	0.00	0.001	0.015
Caulobacteraceae_*Phenylobacterium*	83.01	19.15	0.007	0.064
Cellvibrionaceae_*Cellvibrio*	112.84	4.46	0.000	0.001
Chitinophagaceae_*Cnuella*	13.46	0.00	0.000	0.000
Chitinophagaceae_*Flaviaesturariibacter*	18.52	2.23	0.010	0.085
Chthoniobacteraceae_*Chthoniobacter*	220.57	46.65	0.001	0.019
Cyanobacteriaceae_*Annamia_HOs24*	27.06	0.00	0.005	0.051
Desulfarculaceae_*unclassified*	5.52	0.00	0.001	0.015
Family_XVIII_*unclassified*	12.28	0.00	0.000	0.009
Gemmatimonadaceae_*Gemmatirosa*	13.09	0.00	0.000	0.003
Hymenobacteraceae_*Adhaeribacter*	151.18	20.55	0.000	0.003
Hymenobacteraceae_*Pontibacter*	127.99	8.95	0.000	0.002
Micrococcaceae_*Paenarthrobacter*	59.90	0.39	0.000	0.000
Micromonosporaceae_*Micromonospora*	459.05	10.73	0.000	0.001
Micropepsaceae_*unclassified*	29.95	4.06	0.005	0.054
Nitrosomonadaceae_*Nitrosomonas*	3.62	0.00	0.002	0.027
Opitutaceae_*IMCC26134*	6.06	0.00	0.002	0.029
Paenibacillaceae_*Cohnella*	19.35	0.00	0.000	0.002
Paenibacillaceae_*Paenibacillus*	29.09	0.21	0.002	0.022
Pedosphaeraceae_*unclassified*	173.34	16.43	0.000	0.000
Planococcaceae_Lysinibacillus	16.43	0.39	0.009	0.078
Planococcaceae_Paenisporosarcina	36.03	3.66	0.003	0.035
Pseudohongiellaceae_*BIyi10*	7.89	0.99	0.009	0.078
Rhizobiaceae_*unclassified*	280.32	2.35	0.000	0.000
Solibacteraceae__*Paludibaculum*	7.77	0.00	0.000	0.002
Sphingomonadaceae_Porphyrobacter	31.88	2.08	0.009	0.078
Streptomycetaceae_*Allostreptomyces*	16.13	0.00	0.002	0.028
Thermoactinomycetaceae_*Baia*	7.64	0.00	0.003	0.039
Thermoactinomycetaceae_*unclassified*	6.70	0.19	0.009	0.078
Verrucomicrobiaceae_*Prosthecobacter*	5.38	0.14	0.002	0.030

REFERENCES

Aguirre-von-Wobeser E, Rocha-Estrada J, Shapiro LR, de la Torre M (2018) Enrichment of Verrucomicrobia, Actinobacteria and Burkholderiales drives selection of bacterial community from soil by maize roots in a traditional milpa agroecosystem. PLoS ONE 13(12): e0208852.

Akinrinlola, Rufus J., Yuen, Gary Y., and Anthony O. "Evaluation of Bacillus Strains for Plant Growth Promotion and Predictability of Efficacy by In Vitro Physiological Traits." International Journal of Microbiology. Hindawi, October 3, 2018.

Alkhnajari, A. (2019) Microbial diversity of buckwheat rhizosphere in wirework-infested and non-infested soils using metagenomics [Island Scholar Master's thesis]. University of Prince Edward Islands

Anandham, R., Premalatha, N., Jee, H. J., Weon, H. Y., Kwon, S. W., Krishnamoorthy, R., … Gopal, N. O. (2015). Cultivable bacterial diversity and early plant growth promotion by the traditional organic formulations prepared using organic waste materials. International Journal of Recycling of Organic Waste in Agriculture, 4(4), 279–289. doi: 10.1007/s40093-015-0107-1

Patil, S.M., Hiremath, B.T., Nadagouda and M.P., Potdar. 2017. Impact of Fertigation and Target Yield Levels on Soil Microbial Biomass and Cane Yield of Ratoon Sugarcane.Int.J.Curr.Microbiol.App.Sci. 6(4): 1718-1722. doi:

Bakker, M.G., Manter, D.K., Sheflin, A.M. et al. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. Plant Soil 360, 1–13 (2012).

Bardou, P., Mariette J., Escudlié, F., Djemiel, C., Klopp, C., (2014) jvenn: an interactive Venn diagram viewer. BMC Bioinformatics 2014, 15:293 doi:10.1186/1471-2105-15-293 -

Barman, D., & Dkhar, M. S. (2018). Plant Growth-Promoting Potential of Endophytic Bacteria Isolated from Costus speciosus in Tropical Deciduous Forest of Eastern Himalaya. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. doi:10.1007/s40011-018-0998-5

Berg, B., Hofsten, B. V., & Pettersson, G. (1972). Electron microscopic Observations on the Degradation of Cellulose Fibres by Cellvibrio fulvus and Sporocytophaga myxococcoides. Journal of Applied Bacteriology, 35(2), 215–219. doi: 10.1111/j.1365-2672.1972.tb03692.x

Bjørnlund, L., Liu, M., Rønn, R., Christensen, S., & Ekelund, F. (2012). Nematodes and protozoa affect plants differently, depending on soil nutrient status. European Journal of Soil Biology, 50, 28–31. doi: 10.1016/j.ejsobi.2011.11.012

Bonanomi, G., Cesarano, G., Antignani, V., Maio, C. D., Filippis, F. D., & Scala, F. (2018). Conventional farming impairs Rhizoctonia solani disease suppression by disrupting soil food web. Journal of Phytopathology, 166(9), 663–673. doi: 10.1111/jph.12729

Bonanomi, Giuliano, Francesca De Filippis, Gaspare Cesarano, Antonietta La Storia, Danilo Ercolini, and Felice Scala. "Organic Farming Induces Changes in Soil Microbiota That Affect Agro-Ecosystem Functions." Soil Biology and Biochemistry. Pergamon, September 23, 2016.

Boonsnongcheep, P., Nakashima T., Takahashi, Y., Prathanturarug, S. (2016) Diversity of Endophytic Actinomycetes Isolated from Roots and Root Nodules of Pueraria candollei Grah. ex Benth. and the Analyses of Their Secondary Metabolites. Chiang Mai J. Sci. 2017; 44(1) : 1-14

Broeckling, Corey D., Amanda K. Broz, Joy Bergelson, Daniel K. Manter, and Jorge M. Vivanco. "Root Exudates Regulate Soil Fungal Community Composition and Diversity." Applied and Environmental Microbiology. American Society for Microbiology, February 1, 2008.

Callahan et al., 2016: Callahan, B. J. et al. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583

Carter, David O., David Yellowlees, and Mark Tibbett. "Autoclaving Kills Soil Microbes Yet Soil Enzymes Remain Active." Pedobiologia. Urban & Fischer, May 24, 2007.

Carvalhais, L. C., Dennis, P. G., & Schenk, P. M. (2014). Plant defence inducers rapidly influence the diversity of bacterial communities in a potting mix. Applied Soil Ecology, 84, 1–5. doi: 10.1016/j.apsoil.2014.06.011

Chambers, J. M., Freeny, A and Heiberger, R. M. (1992) Analysis of variance; designed experiments. Chapter 5 of Statistical Models in S eds J. M. Chambers and T. J. Hastie, Wadsworth & Brooks/Cole.

Chaparro, J.M., Sheflin, A.M., Manter, D.K. et al. Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48, 489–499 (2012).

Chaparro, Jacqueline M, Dayakar V Badri, and Jorge M Vivanco. "Rhizosphere Microbiome Assemblage Is Affected by Plant Development." The ISME journal. Nature Publishing Group, April 2014.

da Rocha UN, Plugge CM, George I, van Elsas JD, van Overbeek LS (2013) The Rhizosphere

da Rocha, UN, Jan Dirk van Elsas, Leonard Simon van Overbeek, (2011) Verrucomicrobia subdivision 1 strains display a difference in the colonization of the leek (Allium porrum) rhizosphere, FEMS Microbiology Ecology, Volume 78, Issue 2, November 2011, Pages 297–305

da Rocha, Ulisses Nunes da, Jan Dirk van Elsas, and Leonard Simon van Overbeek. (2010) "Real-Time PCR Detection of Holophagae (Acidobacteria) and Verrucomicrobia Subdivision 1

Groups in Bulk and Leek (Allium Porrum) Rhizosphere Soils." Journal of Microbiological Methods. Elsevier, August 27, 2010.

Dahal, R. H., & Kim, J. (2016). Rhabdobacter roseus gen. nov., sp. nov., isolated from soil. International Journal of Systematic and Evolutionary Microbiology, 66(1), 308–314. doi: 10.1099/ijsem.0.000714

Davidov, Y., & Jurkevitch, E. (2004). Diversity and evolution of Bdellovibrio-and-like organisms (BALOs), reclassification of Bacteriovorax starrii as Peredibacter starrii gen. nov., comb. nov., and description of the Bacteriovorax–Peredibacter clade as Bacteriovoracaceae fam. nov. International Journal of Systematic and Evolutionary Microbiology, 54(5), 1439–1452. doi: 10.1099/ijs.0.02978-0

Deng, Siwen, Heidi M.-L. Wipf, Grady Pierroz, Ted K. Raab, Rajnish Khanna, and Devin Coleman-Derr. "A Plant Growth-Promoting Microbial Soil Amendment Dynamically Alters the Strawberry Root Bacterial Microbiome." Nature News. Nature Publishing Group, November 27, 2019.

Dorr de Quadros, P.; Zhalnina, K.; Davis-Richardson, A.; Fagen, J.R.; Drew, J.; Bayer, C.; Camargo, F.A.; Triplett, E.W. The Effect of Tillage System and Crop Rotation on Soil Microbial Diversity and Composition in a Subtropical Acrisol. Diversity 2012, 4, 375-395.

Ehrig, A., Müller, R. H., & Babel, W. (1997). Isolation of phenoxy herbicide-degradingRhodoferaxspecies from contaminated building material. Acta Biotechnologica, 17(4), 351–356. doi: 10.1002/abio.370170411

Eltlbany, N., Baklawa, M., Ding, G.-C., Nassal, D., Weber, N., Kandeler, E., … Smalla, K. (2019). Enhanced tomato plant growth in soil under reduced P supply through microbial inoculants and microbiome shifts. FEMS Microbiology Ecology, 95(9). doi: 10.1093/femsec/fiz124

Faircloth, B and T Glenn. (2011) Serapure_v2.2. https://ethanomics.files.wordpress.com/2012/08/serapure_v2-2.pdf

Guo, Zhen, Jichang Han, Juan Li, Yan Xu, and Xiaoli Wang. "Effects of Long-Term Fertilization on Soil Organic Carbon Mineralization and Microbial Community Structure." PLOS ONE. Public Library of Science. Accessed March 14, 2020.

Hartman, K., van der Heijden, M.G.A., Wittwer, R.A. et al. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6, 14 (2018).

He, Y, et al. "Co-Inoculation of Bacillus Sp. and Pseudomonas Putida at Different Development Stages Acts as a Biostimulant to Promote Growth, Yield and Nutrient Uptake of Tomato." Journal of Applied Microbiology, U.S. National Library of Medicine, July 2019,

Inceoglu, O, Salles, Joana Falcão, Leo van Overbeek, and Jan Dirk van Elsas. “Effects of Plant Genotype and Growth Stage on the Betaproteobacterial Communities Associated with Different Potato Cultivars in Two Fields.” Applied and Environmental Microbiology. American Society for Microbiology, June 1, 2010.

Jacobsen, Carsten Suhr, and Mathis Hjort Hjelmsø. “Agricultural Soils, Pesticides and Microbial Diversity.” Current Opinion in Biotechnology. Elsevier Current Trends, September 30, 2013.

Jari Oksanen, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, Eduard Szoecs and Helene Wagner (2019). vegan: Community Ecology Package. R package version 2.5-6.

Johansen, J., & Binnerup, S. (2002). Contribution of Cytophaga-like Bacteria to the Potential of Turnover of Carbon, Nitrogen, and Phosphorus by Bacteria in the Rhizosphere of Barley (Hordeum vulgare L.). Microbial Ecology, 43(3), 298–306. doi: 10.1007/s00248-002-2006-z

Jorquera, M. A., Shaharoona, B., Nadeem, S. M., Mora, M. D. L. L., & Crowley, D. E. (2012). Plant Growth-Promoting Rhizobacteria Associated with Ancient Clones of Creosote Bush (Larrea tridentata). Microbial Ecology, 64(4), 1008–1017. doi: 10.1007/s00248-012-0071-5

Kämpfer, P., Lodders, N., & Falsen, E. (2011). Hydrotalea flava gen. nov., sp. nov., a new member of the phylum Bacteroidetes and allocation of the genera Chitinophaga, Sediminibacterium, Lacibacter, Flavihumibacter, Flavisolibacter, Niabella, Niastella, Segetibacter, Parasegetibacter, Terrimonas, Ferruginibacter, Filimonas and Hydrotalea to the family Chitinophagaceae fam. nov. International Journal of Systematic and Evolutionary Microbiology, 61(3), 518–523. doi: 10.1099/ijs.0.023002-0

Kim, M. K., Kim, Y.-J., Cho, D.-H., Yi, T.-H., Soung, N.-K., & Yang, D.-C. (2007). Solimonas soli gen. nov., sp. nov., isolated from soil of a ginseng field. International Journal of Systematic and Evolutionary Microbiology, 57(11), 2591–2594. doi: 10.1099/ijs.0.64938-0

Kim, S.-J., Weon, H.-Y., Kim, Y.-S., Moon, J. Y., Seok, S. J., Hong, S.-B., & Kwon, S.-W. (2012). Caenimonas terrae sp. nov., isolated from a soil sample in Korea, and emended description of the genus Caenimonas Ryu et al. 2008. Journal of Microbiology, 50(5), 864–868. doi: 10.1007/s12275-012-1587-6

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O. (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013 Jan 7;41(1):e1. doi: 10.1093/nar/gks808. Epub. PMID: 22933715; PMCID: PMC3592464. (2012).

Krauss J, Gallenberger I, Steffan-Dewenter I (2011) Decreased functional diversity and biological pest control in conventional compared to organic crop fields. PLoS One 6:e19502

Kumar, S., Suyal, D. C., Bhoriyal, M., & Goel, R. (2018). Plant growth promoting potential of

Lapsansky, Erin R, Arwen M Milroy, Marie J Andales, and Jorge M Vivanco. “Soil Memory as a Potential Mechanism for Encouraging Sustainable Plant Health and Productivity.” Current opinion in biotechnology. U.S. National Library of Medicine, April 2016.

Li, Kun, Michael J. DiLegge, Ioannis S. Minas, Alison Hamm, Daniel Manter, and Jorge M. Vivanco. “Soil Sterilization Leads to Re-Colonization of a Healthier Rhizosphere Microbiome.” Rhizosphere. Elsevier, November 11, 2019.

Li, S., Peng, M., Liu, Z., & Shah, S. S. (2017). The Role of Soil Microbes in Promoting Plant Growth. Molecular Microbiology Research. doi: 10.5376/mmr.2017.07.0004

Liew, K.J., Teo, S.C., Shamsir, M.S. et al. Complete genome sequence of Rhodothermaceae bacterium RA with cellulolytic and xylanolytic activities. 3 Biotech 8, 376 (2018).

Lin, Weiwei, Manhong Lin, Hongyan Zhou, Hongmiao Wu, Zhaowei Li, and Wenxiong Lin. “The Effects of Chemical and Organic Fertilizer Usage on Rhizosphere Soil in Tea Orchards.” PloS one. Public Library of Science, May 28, 2019.

Lori, M., Symnaczik, S., Mader, P., De Deyn, G., Gattinger, A., 2017. Organic farming enhances soil microbial abundance and activity–a meta-analysis and meta-regression. PLOS ONE, 12(7): e0180442. DOI: 10.1371/journal.pone.0180442.

Lupatini M, Korthals GW, de Hollander M, Janssens TKS and Kuramae EE (2017) Soil Microbiome Is More Heterogeneous in Organic Than in Conventional Farming System. Front. Microbiol. 7:2064. doi: 10.3389/fmicb.2016.02064

Madhaiyan, M., Poonguzhali, S., Lee, J.-S., Senthilkumar, M., Lee, K. C., & Sundaram, S. (2009). Mucilaginibacter gossypii sp. nov. and Mucilaginibacter gossypiicola sp. nov., plant-growth-promoting bacteria isolated from cotton rhizosphere soils. International Journal Of Systematic And Evolutionary Microbiology, 60(10), 2451–2457. doi: 10.1099/ijs.0.018713-0

Madhaiyan, M., Poonguzhali, S., Senthilkumar, M., Pragatheswari, D., Lee, J.-S., & Lee, K.-C. (2014). Arachidicoccus rhizosphaerae gen. nov., sp. nov., a plant-growth-promoting bacterium in the family Chitinophagaceae isolated from rhizosphere soil. International Journal Of Systematic And Evolutionary Microbiology, 65(Pt 2), 578–586. doi: 10.1099/ijs.0.069377-0

Manter, D. K., Korsa, M., Tebbe, C., Delgado, J.A. (2016) myPhyloDB: a local web server for the storage and analysis of metagenomic data. Database J. Biol. Databases Curation

Maron, P.-A., Sarr, A., Kaisermann, A., Lévêque, J., Mathieu, O., Guigue, J., … Ranjard, L. (2018, May 1). High Microbial Diversity Promotes Soil Ecosystem Functioning. Retrieved from

Martin et al., 2011 (cutadept): Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10–12

Martínez-García, Laura B., Gerard Korthals, Lijbert Brussaard, Helene Bracht Jørgensen, and Gerlinde B. De Deyn. “Organic Management and Cover Crop Species Steer Soil Microbial Community Structure and Functionality along with Soil Organic Matter Properties.” Agriculture, Ecosystems & Environment. Elsevier, May 3, 2018.

McDonald, Bruce A., Stukenbrock, Eva H. (2016) “Rapid Emergence of Pathogens in Agro-Ecosystems: Global Threats to Agricultural Sustainability and Food Security.” Philosophical Transactions of the Royal Society B: Biological Sciences, December 5, 2016.

McMurdie and Holmes (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE. 8(4):e61217

Moronta-Barrios F, Gionechetti F, Pallavicini A, Marys E, Venturi V (2018) Bacterial microbiota of rice roots: 16S-based taxonomic profiling of endophytic and rhizospheric diversity, endophytes isolation and simplified endophytic community. Microorgani 6(1):14

Nicholson, W L, N Munakata, G Horneck, H J Melosh, and P Setlow. “Resistance of Bacillus Endospores to Extreme Terrestrial and Extraterrestrial Environments.” Microbiology and molecular biology reviews : MMBR. American Society for Microbiology, September 2000.

Nieuwesteeg, N. (2015) Biological control of fungal plant pathogens by tomato endosphere bacteria. [Unpublished Master’s Thesis] Wageningen University and Research Center

Noling, J. W., & Becker, J. O. (1994). The Challenge of Research and Extension to Define and Implement Alternatives to Methyl Bromide . The Journal of Nematology, 573-586.

Nor, M. N. M., Sabaratnam, V., & Tan, G. Y. A. (2017). Devosia elaeis sp. nov., isolated from oil palm rhizospheric soil. International Journal of Systematic and Evolutionary Microbiology, 67(4), 851–855. doi: 10.1099/ijsem.0.001683

Oztas Gulmus, E., & Gormez, A. (2019). Characterization and biotechnological application of protease from thermophilic Thermomonas haemolytica. Archives of Microbiology. doi:10.1007/s00203-019-01728-7

Pandey, Chitra, Vivek K. Bajpai, Yogesh Kumar Negi, Irfan A. Rather, and D.K. Maheshwari. “Effect of Plant Growth Promoting Bacillus Spp. on Nutritional Properties of Amaranthus Hypochondriacus Grains.” Saudi Journal of Biological Sciences. Elsevier, March 6, 2018.

Peralta, Ariane L., Yanmei Sun, Marshall D. McDaniel, and Jay T. Lennon. “Crop Rotational Diversity Increases Disease Suppressive Capacity of Soil Microbiomes.” The Ecological Society of America. John Wiley & Sons, Ltd, May 15, 2018.

Pham, V.T.K., Rediers, H., Ghequire, M.G.K. et al. The plant growth-promoting effect of the nitrogen-fixing endophyte Pseudomonas stutzeri A15. Arch Microbiol 199, 513–517 (2017).
Preston, G.M. (2004) Plant perceptions of plant growth-promoting Pseudomonas Phil. Trans. R. Soc. Lond. B359907–918

psychrotolerantDyadobactersp. for pulses and finger millet and impact of inoculation on soil chemical properties and diazotrophic abundance. Journal of Plant Nutrition, 41(8), 1035–1046. doi: 10.1080/01904167.2018.1433211

Qiu et al., 2014: Qiu M, Li S, Zhou X, Cui X, Vivanco JM, Zhan N, Shen Q, Zhang R (2014) De-coupling of root- microbiome associations followed by antagonist inoculation improves rhizosphere soil suppressiveness. Biology and Fertility of Soils 50:217-224

Rashid, Shimaila, Trevor C. Charles, and Bernard R. Glick. "Isolation and Characterization of New Plant Growth-Promoting Bacterial Endophytes." Applied Soil Ecology. Elsevier, October 24, 2011. https://www.sciencedirect.com/science/article/abs/pii/S0929139311002198?via=ihub.

Raweekul, W., Wuttitummapor, S, Sodchuen, W., Kittiwongwattana, C. (2016). Plant growth promotion by endophytic bacteria isolated from rice (Oryza sativa). Thammasat Int Jol of Sci and Tech. 21(1)

Reis, V. M., & Teixeira, K. R. D. S. (2015). Nitrogen fixing bacteria in the familyAcetobacteraceaeand their role in agriculture. Journal of Basic Microbiology, 55(8), 931–949. doi: 10.1002/jobm.201400898

Rohland, N and D. Reich. (2012) Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. Genome Research 22:939-946

Rojas-Solís, D., Zetter-Salmón, E., Contreras-Pérez, M., Rocha-Granados, M. D. C., Macías-Rodríguez, L., & Santoyo, G. (2018). Pseudomonas stutzeri E25 and Stenotrophomonas maltophilia CR71 endophytes produce antifungal volatile organic compounds and exhibit additive plant growth-promoting effects. Biocatalysis and Agricultural Biotechnology, 13, 46–52. doi: 10.1016/j.bcab.2017.11.007

Sande, Doris, Jeffrey Mullen, Michael Wetzstein, and Jack Houston. "Environmental Impacts from Pesticide Use: a Case Study of Soil Fumigation in Florida Tomato Production." International journal of environmental research and public health. MDPI, December 2011.

Sansinenea, Estibaliz. "Bacillus Spp.: As Plant Growth-Promoting Bacteria." SpringerLink. Springer, Singapore, January 1, 1970.
Selects for Particular Groups of Acidobacteria and Verrucomicrobia. PLoS ONE 8(12): e82443. doi: 10.1371/journal.pone.0082443

Singh, N., Marwa, N., Mishra, S. K., Mishra, J., Verma, P. C., Rathaur, S., & Singh, N. (2016). Brevundimonas diminuta mediated alleviation of arsenic toxicity and plant growth promotion in Oryza sativa L. Ecotoxicology and Environmental Safety, 125, 25–34. doi: 10.1016/j.ecoenv.2015.11.020

Subrahmanyam G., Kumar A., Sandilya S.P., Chutia M., Yadav A.N. (2020) Diversity, Plant Growth Promoting Attributes, and Agricultural Applications of Rhizospheric Microbes. In:

Yadav A., Singh J., Rastegari A., Yadav N. (eds) Plant Microbiomes for Sustainable Agriculture. Sustainable Development and Biodiversity, vol 25. Springer, Cham

Sugiyama A, Vivanco JM, Jayanty SS, Manter DK (2010). Pyrosequencing assessment of soil microbial communities in organic and conventional potato farms. Plant Disease 94:1329-1335

Syranidou, E., Christofilopoulos, S., Politi, M., Weyens, N., Venieri, D., Vangronsveld, J., & Kalogerakis, N. (2017). Bisphenol-A removal by the halophyte Juncus acutus in a phytoremediation pilot: Characterization and potential role of the endophytic community. Journal of Hazardous Materials, 323, 350–358.

Tan, Y., Wang, Y., Wang, D., Wang, G., & Zheng, S. (2016). Sphingoaurantiacus capsulatus sp. nov., isolated from mountain soil, and emended description of the genus Sphingoaurantiacus. International Journal of Systematic and Evolutionary Microbiology, 66(12), 4930–4935. doi: 10.1099/ijsem.0.001447

Trabelsi, Darine, and Ridha Mhamdi. “Microbial Inoculants and Their Impact on Soil Microbial Communities: a Review.” BioMed research international. Hindawi Publishing Corporation, 2013.

Trivedi, P., Schenk, P. M., Wallenstein, M. D., & Singh, B. K. (2017, September). Tiny Microbes, Big Yields: enhancing food crop production with biological solutions.

van Elsas, Jan Dirk, Mario Chiurazzi, Cyrus A Mallon, Dana Elhottova, Václav Kristufek, and Joana Falcão Salles. “Microbial Diversity Determines the Invasion of Soil by a Bacterial Pathogen.” Proceedings of the National Academy of Sciences of the United States of America. National Academy of Sciences, January 24, 2012.

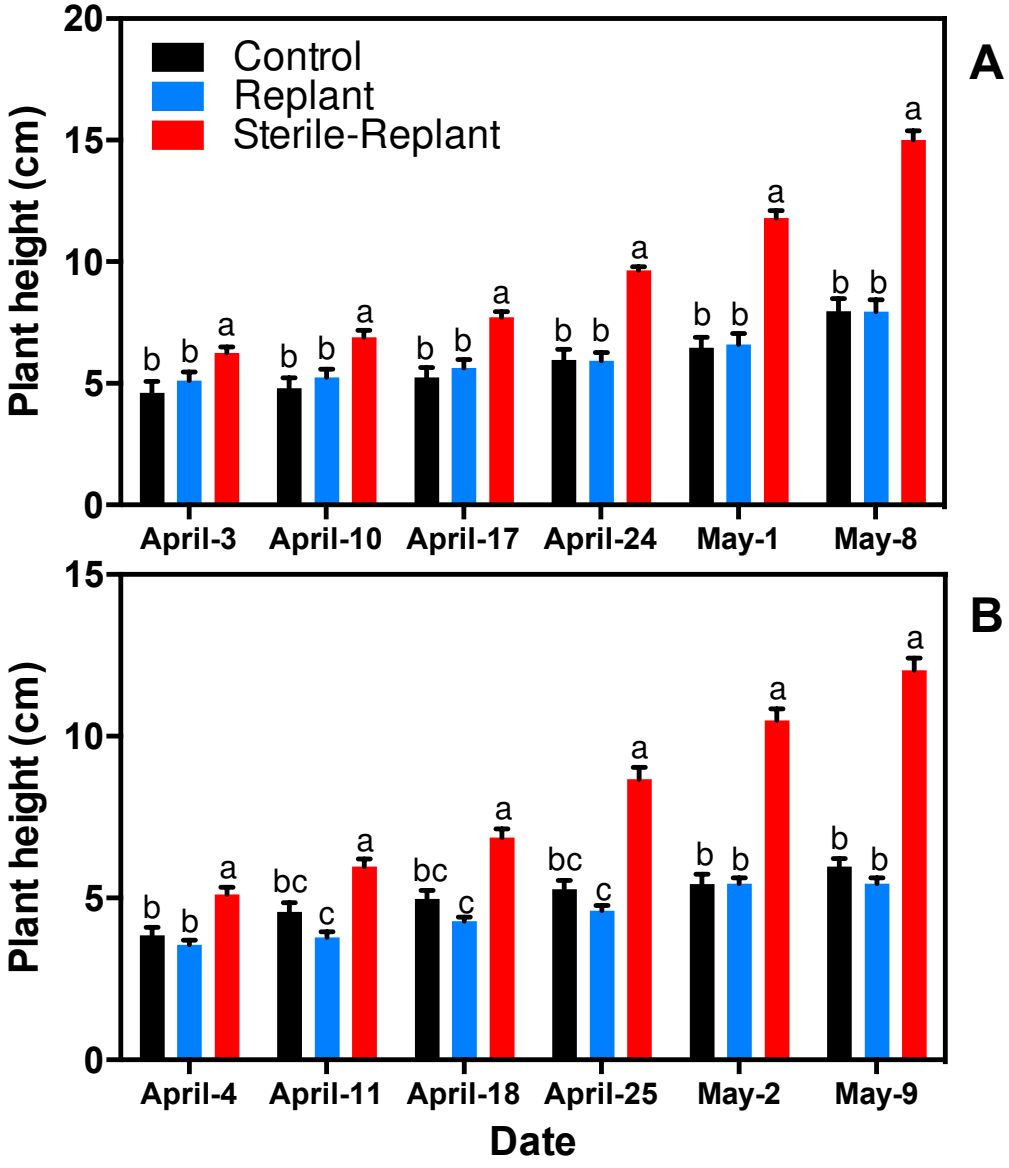
Wolf, Duane C., and Horace D. “Soil Sterilization.” Methods of Soil Analysis: Part 2-Microbiological and Biochemical Properties. Accessed March 14, 2020.

Xiao, X., Fan, M., Wang, E. et al. Interactions of plant growth-promoting rhizobacteria and soil factors in two leguminous plants. Appl Microbiol Biotechnol 101, 8485–8497 (2017). https://doi.org/10.1007/s00253-017-8550-8

Yadav A., Singh J., Rastegari A., Yadav N. (eds) Plant Microbiomes for Sustainable Agriculture. Sustainable Development and Biodiversity, vol 25. Springer, Cham

Yadav, A.N., Sachan, S.G., Verma, P., Saxena, A.K. (2015) Prospecting cold deserts of north western Himalayas for microbial diversity and plant growth promoting attributes. Journal of Bioscience and Bioengineering. 119(6), 683-693

Zhao, Qingyun, Wu Xiong, Yizhang Xing, Yan Sun, Xingjun Lin, and Yunping Dong. “Long-Term Coffee Monoculture Alters Soil Chemical Properties and Microbial Communities.” Nature News. Nature Publishing Group, April 17, 2018.



Supplemental Figure 2-1: Weekly height measurements (cm) of corn and tomato plants in trial 2. Different letters denote significant differences ($P < 0.05$) and standard deviation from the mean is represented in the error bars.

Supplemental Table 2-1**:** The available nutrient contents of different soil treatments in this experiment (mg/kg).

Soil Treatment	**NO3-N** (mg/kg)	**Available P** (mg/kg)	**Available K** (mg/kg)
Replant soil	5.57	9.23	279.33
Sterile replant soil	4.10	15.7	258.00
Control soil (non-replant)	7.57	13.5	153.00

Supplemental Table 2-2: Percent Increase in Total Dry-Weight Production (g)

Crop	Replant soil DW (g)	Sterile replant soil DW (g)	% Increase
Corn	1.25	3.5	180
Tomato	0.34	0.66	94.118
Peach	1.5	3.8	153.33

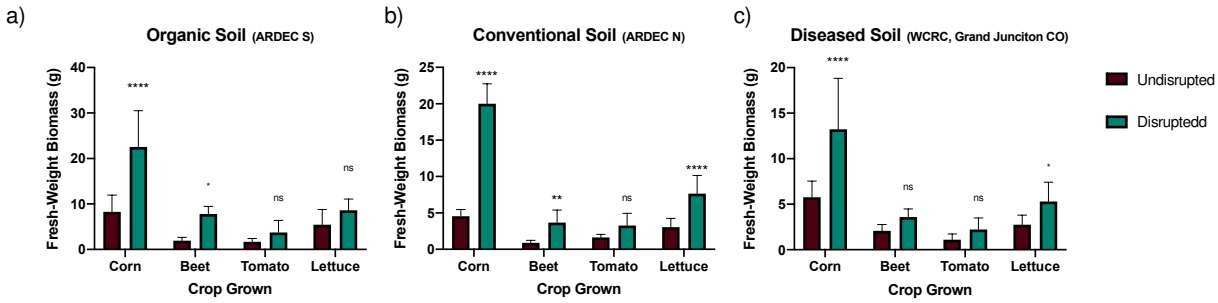
Supplemental Table 2-3: Effects of soil sterilization on inferential gene relative abundances from the dominant bacterial phyla in replant and sterile replant disease soil with peach plants grown in the soils for 11 weeks. Blocks highlighted in green or red represent an increase or decrease at the p=0.05 significance level, respectively.

		Peach		
Process	**Gene**	**Replant**	**Sterile replant**	**p-value**
C Decomposition	E3.2.1.21	0.000	0.004	0.337
	bglB	0.710	0.393	0.766
	bglX	0.278	0.084	0.678
N Decomposition	*amiE*	0.182	0.182	0.873
	ureC	0.377	0.450	0.231
P Decomposition	E3.1.3.2	0.442	0.241	0.822
	appA	0.233	0.205	0.335
	phoA	0.275	0.100	0.557
	phoD	0.490	0.381	0.145
	phoN	0.026	0.060	0.150
S Decomposition	*aslA*	0.292	0.098	0.227
N Fixation	*nifDK*	0.284	0.109	0.604
	nifH	0.259	0.084	0.444
P Solubility	*pqqC*	0.406	0.081	0.040

Biocontrol	E3.2.1.14	0.657	0.314	0.161
	budA	0.246	0.232	0.147
	hcnA	0.004	0.004	0.956
	phzE	0.000	0.012	0.003
Root growth	*acdS*	0.138	0.097	0.286
	ipdC	0.019	0.015	0.971
Siderophore	*entA*	0.011	0.005	0.626
	mbtI	0.000	0.004	0.095
	pchB	0.002	0.002	0.990

Supplemental Table 2-4: KEGG orthologues selected for PICRUSt predictive analysis.

	KEGG		
Process	Gene	Entry	Definition
C Decomposition	E3.2.1.21	K01188	beta-glucosidase
	bglB	K05350	beta-glucosidase
	bglX	K05349	beta-glucosidase
N Decomposition	*amiE*	K01426	amidase
	ureC	K01428	urease alpha subunit
P Decomposition	E3.1.3.2	K01078	acid phosphatase
	appA	K01093	acid phosphatase
	phoA	K01077	alkaline phosphatase
	phoD	K01113	alkaline phosphatase
	phoN	K09474	acid phosphatase
S Decomposition	*aslA*	K01130	arylsulfatase
N Fixation	*nifDK*	K02591, K02586	nitrogenase Mo-Fe protein
	nifH	K02588	nitrogenase Fe protein
P Solubility	*pqqC*	K06137	pyrroloquinoline-quinone synthase
Biocontrol	E3.2.1.14	K01183	chitinase
	budA	K01575	acetolactate decarboxylase
	hcnA	K10814	glycine dehyrdrogenase (cyanide-forming)
	phzE	K13063	2-amino-4-deoxychorismate synthase
Root growth	*acdS*	K01505	1-aminocyclopropane-1-carboxylate deaminase
	ipdC	K04103	indolepyruvate decarboxylase
Siderophore	*entA*	K00216	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase
	mbtI	K04781	salicylate synthetase
	pchB	K02364	Isochorismate pyruvate lysase



Supplemental Figure 3-1: Mean crop fresh-weight (FW) biomass (a) grown in organic soil (b) conventional soil and (c) diseased soil (n=10 / treatment, ± standard deviation). Significant increases in biomass of each crop are denoted by "*", "**" or "***" above each. Siddak's multiple comparison test using GraphPad (Vers 8.2.1).

Supplemental Table 3-1: Average Plant Fresh Weight (FW) Biomass and Percent Increases

Average Fresh Weight (g)		Crops Tested			
Soil	Treatment	Corn	Beet	Tomato	Lettuce
Organic	Unautoclaved	8.280	1.945	1.691	5.460
	Autoclaved	22.540	7.820	3.740	8.610
	% Increase	172.222	302.057	121.171	57.692
Conventional	Unautoclaved	4.550	0.913	1.630	3.050
	Autoclaved	20.000	3.760	3.270	7.650
	% Increase	339.560	311.829	100.613	150.820
Diseased	Unautoclaved	5.760	2.080	1.100	2.740
	Autoclaved	14.689	3.590	2.220	5.290
	% Increase	155.015	72.596	101.818	93.066

Supplemental Table 3-2: Average Plant Dry Weight (DW) Biomass and Percent Increases

Average Dry Weight (g)		Crops Tested			
Soil	Treatment	Corn	Beet	Tomato	Lettuce
Organic	Unautoclaved	2.080	0.340	0.280	0.931
	Autoclaved	4.460	1.430	0.770	1.680
	% Increase	114.423	320.588	175.000	80.451
Conventional	Unautoclaved	1.060	0.135	0.290	0.490
	Autoclaved	4.260	0.650	0.625	1.750
	% Increase	301.887	381.481	115.517	257.143
Diseased	Unautoclaved	1.120	0.350	0.169	0.472
	Autoclaved	3.211	0.530	0.259	1.090
	% Increase	186.706	51.429	53.254	130.932

Supplemental Table 3-3: Pairwise comparison of qPCR results for values of 16s rRNA copies per g FW rhizosphere or bulk soil (NS= Non "sterile" and soils were not autoclaved before experimentation S=Sterile and soils were autoclaved before experimentation)

16s rRNA copies / g FW Soil - Comparison of Disrupted Vs. Undisrupted Soils

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Organic Soil				
NoPlant:Unautoclaved-Beet:Unautoclaved	-202242348	-662224516	ns	0.8530598
NoPlant:Unautoclaved-Corn:Unautoclaved	-99531654	-559513822	ns	0.9983772
NoPlant:Unautoclaved-Lettuce:Unautoclaved	-288537662	-748519831	ns	0.4746212
Tomato:Unautoclaved-NoPlant:Unautoclaved	323777128	-136205040	ns	0.3284444
NoPlant:Autoclaved-Beet:Autoclaved	-55721412	-515703580	ns	0.9999857
NoPlant:Autoclaved-Corn:Autoclaved	-136426493	-596408661	ns	0.9844926
NoPlant:Autoclaved-Lettuce:Autoclaved	22368828	-437613341	ns	1
Tomato:Autoclaved-NoPlant:Autoclaved	-34844228	-494826396	ns	0.9999998
Conventional Soil				
NoPlant:Unautoclaved-Beet:Unautoclaved	-111707062	-1810238653	ns	0.9999999
NoPlant:Unautoclaved-Corn:Unautoclaved	210997912	-1487533680	ns	0.9999823
NoPlant:Unautoclaved-Lettuce:Unautoclaved	764427629	-934103963	ns	0.8363816
Tomato:Unautoclaved-NoPlant:Unautoclaved	-827567533	-2526099125	ns	0.7698208
NoPlant:Autoclaved-Beet:Autoclaved	-1003978478	-2702510070	ns	0.5514078
NoPlant:Autoclaved-Corn:Autoclaved	-773297144	-2471828736	ns	0.8276529
NoPlant:Autoclaved-Lettuce:Autoclaved	-2046485876	-3745017467	**	0.0108405
Tomato:Autoclaved-NoPlant:Autoclaved	524863771	-1173667821	ns	0.9797232
Diseased Soil				
NoPlant:Unautoclaved-Beet:Unautoclaved	-86778106	-1254318809	ns	0.9999998

NoPlant:Unautoclaved-Corn:Unautoclaved	-1560104315	-2727645018	***	0.0039314
NoPlant:Unautoclaved-Lettuce:Unautoclaved	-50719652	-1218260356	ns	1
Tomato:Unautoclaved-NoPlant:Unautoclaved	81312294	-1086228409	ns	0.9999999
NoPlant:Autoclaved-Beet:Autoclaved	162085172	-1005455532	ns	0.9999546
NoPlant:Autoclaved-Corn:Autoclaved	160028973	-1007511731	ns	0.9999592
NoPlant:Autoclaved-Lettuce:Autoclaved	157904694	-1009636010	ns	0.9999636
Tomato:Autoclaved-NoPlant:Autoclaved	-220716905	-1388257608	ns	0.9994323

Supplemental Table 3-4: Pairwise comparison between values of Shannon's alpha diversity assigned to each treatment. (Above) Comparison of Shannon's α-diversity values between disrupted and undisrupted treatments. (Below) Comparison of Shannon's α-diversity values between communities detected in disrupted no plant treatments and disrupted plant treatments (e.g. Crop: Autoclaved= disrupted, Crop: Unautoclaved=undisrupted).

Shannon's Alpha Diversity Value Comparison of Disrupted Crop Vs. Undisrupted Crop Treatments

Organic Soil

Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
Beet:Unautoclaved-Beet:Autoclaved	0.230	-0.403	0.863	0.943	ns
Corn:Unautoclaved-Corn:Autoclaved	-0.122	-0.755	0.511	0.999	ns
Lettuce:Unautoclaved-Lettuce:Autoclaved	-0.785	-1.493	-0.077	0.023	ns
Tomato:Unautoclaved-Tomato:Autoclaved	-0.128	-0.761	0.505	0.999	ns
SoilOnly:Unautoclaved-SoilOnly:Autoclaved	-0.176	-0.809	0.457	0.989	ns

Conventional Soil

Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
Beet:Unautoclaved-Beet:Autoclaved	0.261	-0.617	1.138	0.981	ns
Corn:Unautoclaved-Corn:Autoclaved	-0.320	-1.104	0.465	0.886	ns
Lettuce:Unautoclaved-Lettuce:Autoclaved	0.167	-0.710	1.044	0.999	ns
Tomato:Unautoclaved-Tomato:Autoclaved	0.283	-0.594	1.160	0.969	ns

SoilOnly:Unautoclaved-SoilOnly:Autoclaved	0.534	-0.251	1.318	0.354	ns

Diseased Soil

Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
Beet:Unautoclaved-Beet:Autoclaved	0.225	-0.602	1.052	0.991	ns
Corn:Unautoclaved-Corn:Autoclaved	0.849	0.022	1.676	0.041	*
Lettuce:Unautoclaved-Lettuce:Autoclaved	0.467	-0.360	1.294	0.608	ns
Tomato:Unautoclaved-Tomato:Autoclaved	0.717	-0.110	1.544	0.125	ns
SoilOnly:Unautoclaved-SoilOnly:Autoclaved	0.774	-0.053	1.601	0.079	ns

Shannon's Alpha Diversity Value Comparison of Disrupted Soil Alone Vs. Disrupted Crop Treatments

Organic Soil

Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
SoilOnly:Autoclaved-Beet:Autoclaved	0.241	-0.392	0.874	0.926	ns
SoilOnly:Autoclaved-Corn:Autoclaved	0.219	-0.414	0.852	0.957	ns
SoilOnly:Autoclaved-Lettuce:Autoclaved	-0.010	-0.643	0.623	1.000	ns
Tomato:Autoclaved-SoilOnly:Autoclaved	-0.021	-0.654	0.612	1.000	ns

Conventional Soil

Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
SoilOnly:Autoclaved-Beet:Autoclaved	-0.317	-1.102	0.467	0.890	ns
SoilOnly:Autoclaved-Corn:Autoclaved	-0.482	-1.266	0.303	0.481	ns
SoilOnly:Autoclaved-Lettuce:Autoclaved	-0.393	-1.178	0.391	0.721	ns
Tomato:Autoclaved-SoilOnly:Autoclaved	0.336	-0.449	1.120	0.856	ns

Diseased Soil

Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
SoilOnly:Autoclaved-Beet:Autoclaved	-0.561	-1.388	0.266	0.373	ns
SoilOnly:Autoclaved-Corn:Autoclaved	-0.523	-1.350	0.304	0.464	ns
SoilOnly:Autoclaved-Lettuce:Autoclaved	-0.534	-1.361	0.293	0.438	ns
Tomato:Autoclaved-SoilOnly:Autoclaved	0.649	-0.178	1.475	0.210	ns

Supplemental Table 3-5: Crop-specific and crop-shared bacterial abundance increases resulting from growth in disrupted agroecosystems.

Crop specific taxa	
Crop	**Taxa**
Beet	Acetobacteraceae_*Roseococcus*
	Bacteriovoracaceae_*Peredibacter*
	Chitinophagaceae_*Flavisolibacter*
	Chitinophagaceae_*Parasegetibacter*
	Chitinophagaceae_*UTBCD1*
	Solimonadaceae_*Solimonas*
Corn	Cytophagaceae_*Sporocytophaga*
	Fibrobacteraceae_*possible_genus_04*
	Pseudomonadaceae_*Pseudomonas*
	Rhodothermaceae_*unclassified*
Lettuce	(class: Berkelbacteria o: unclassified) unclassified_*unclassified*
	(k: Archea c: Woesearchaeia) unclassified_*unclassified*
	(k: unclassified p: unclassified c: unclassified o: unclassified) unclassified_*unclassified*
	(o: Micavibrionales) unclassified_*unclassified*
	Acetobacteraceae_*Rhodovastum*
	Acetobacteraceae_*Roseomonas*
	Burkholderiaceae_*Rhodoferax*
	Burkholderiaceae_*unclassified*
	Caulobacteraceae_*unclassified*
	Crocinitomicaceae_*Fluviicola*
	Cytophagaceae_*Cytophaga*
	Intrasporangiaceae_*Knoellia*
	Opitutaceae_*unclassified*
	Oscillatoriaceae_*Oscillatoria_PCC-6304*
	Sporolactobacillaceae_*unclassified*
	Tepidisphaeraceae_*Tepidisphaera*
	Verrucomicrobiaceae_*Brevifollis*
Tomato	(o: Candidatus_Peribacteria) unclassified_*unclassified*
	(o:Candidatus_Pacebacteria) unclassified_*unclassified*
	(p: BRC1) unclassified_*unclassified*
	Archangiaceae_*unclassified*
	Bacillaceae_*Bacillus*
	Beijerinckiaceae_*Chelatococcus*
	Caulobacteraceae_*Asticcacaulis*
	Hydrogenedensaceae_*unclassified*
	Microbacteriaceae_*Yonghaparkia*
	Rhizobiaceae_*Aminobacter*
	Rhodobacteraceae_*Cereibacter*
	Sphingobacteriaceae_*Mucilaginibacter*
	Thermoactinomycetaceae_*Shimazuella*
	Verrucomicrobiaceae_*unclassified*

	Xanthomonadaceae_*Thermomonas*
Shared by two or more crops	
Crop	**Taxa**
Beet + Corn	(c: Microgenomatia o: unclassified) unclassified_*unclassified*
	Caulobacteraceae_*Caulobacter*
Beet + Tomato	Paenibacillaceae_*Ammoniphilus*
Beet + Lettuce	(c: Sericytochromatia o: unclassified) unclassified_*unclassified*
	Kineosporiaceae_*Quadrisphaera*
	Paenibacillaceae_*Brevibacillus*
	Rubinisphaeraceae_*SH-PL14*
	Sphingomonadaceae_*unclassified*
	Verrucomicrobiaceae_*Roseimicrobium*
Corn + Tomato	Xanthomonadaceae_*Stenotrophomonas*
Corn + Lettuce	(o: Bacillales) unclassified_*unclassified*
	(o: Candidatus_Peribacteria) unclassified_*unclassified*
Tomato + Lettuce	Bacillaceae_*Anoxybacillus*
	Beijerinckiaceae_alphaI_*cluster*
	Cyclobacteriaceae_*unclassified*
	env.OPS_17_*unclassified*
	Flavobacteriaceae_*Flavobacterium*
	Nodosilineaceae_*Nodosilinea_PCC-7104*
	Verrucomicrobiaceae_*Verrucomicrobium*
Shared by Three or more crops	
Crop	**Taxa**
Beet + Corn + Tomato	Devosiaceae_*unclassified*
	Opitutaceae_*Opitutus*
Beet + Corn + Lettuce	(o: Candidatus_Nomurabacteria) unclassified_*unclassified*
Beet + Tomato + Lettuce	Cyclobacteriaceae_*Algoriphagus*
	Devosiaceae_*Devosia*
	Oligoflexaceae_*Oligoflexus*
	Sphingobacteriaceae_*Arcticibacter*
Corn + Lettuce + Tomato	Cellvibrionaceae_*unclassified*
	Fibrobacteraceae_*unclassified*
	KD3-93_*unclassified*
Shared by all four crops	
Crops	**Taxa**
Beet + Corn + Lettuce + Tomato	Beijerinckiaceae_*Bosea*
	Burkholderiaceae_*Caenimonas*
	Caulobacteraceae_*Brevundimonas*
	Chitinophagaceae_*Lacibacter*
	Rubritaleaceae_*Luteolibacter*
	Saccharimonadaceae_*unclassified*
	Sphingobacteriaceae_*Pedobacter*
	Sphingobacteriaceae_*unclassified*

Sphingomonadaceae_*Sphingoaurantiacus*
Sphingomonadaceae_*Sphingopyxis*
Spirosomaceae_*Dyadobacter*
Spirosomaceae_*Larkinella*
Spirosomaceae_*Rhabdobacter*