The response of soil microbial communities to vegetable cropping systems analyzed for RNA- and DNA-based sampling

by

Lorena Gomez Montaño

M.S., Kansas State University, 2012

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Plant Pathology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2018

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Abstract

Soil microbial communities play fundamental and complex roles in the productivity of agriculture. However, we still have a limited understanding of the response of microbial communities to different farming systems, such as organic and conventional fertility management regimens. We applied high-throughput sequencing to develop a better understanding of how soil microbial communities (bacteria and fungi) in vegetable production respond to organic or conventional soil fertility management. Specifically, my three studies examined the following questions:

1. How do soil microbial communities from cDNA and DNA samples compare in organic and conventional fertility treatments?

2. How do soil microbial communities in a tomato cropping season respond to long-term organic vs. conventional soil fertility treatments?

3. How do soil bacterial and fungal communities respond to high tunnels, plastic mulch and organic amendments across a tomato cropping season?

The first two questions were addressed at the Kansas State University Horticulture and Extension Center in Olathe, KS, using organic and conventional field plots with three levels of fertilizer. We sampled the plots during the development of a tomato crop. The third question was addressed at a commercial farm in Lawrence, KS, during its transition to organic vegetable production, during a tomato crop. The Lawrence experiment included as treatments field plots versus high tunnels, and three organic nutrient amendments. We used 454-pyrosequencing of bacterial and fungal ribosomal markers to compare total resident (DNA) and active microbial communities (cDNA, which is DNA synthesized from a single stranded RNA template) for our first question. We used Illumina MiSeq metabarcoding of bacterial and fungal ribosomal markers for our second and third questions.

In all three studies we evaluated bacterial and fungal community responses using Simpson´s diversity index, Simpson´s evenness and richness for each experiment. For the first question, when we compared DNA and cDNA, bacterial diversity was higher in cDNA samples from organic compared to conventional management. In addition, fungal diversity from cDNA samples was higher than from DNA samples. In contrast, in the second question, bacterial and fungal diversity indices did not differ in the tomato crop under organic and conventional management systems. For our third question, high tunnels did not affect bacterial or fungal diversity. Use of plastic mulch for a tomato crop in open field plots did not affect bacterial richness, but decreased fungal richness compared to open field plots without plastic mulch. High-throughput sequencing provides a new perspective on the structure and dynamics of these communities. Information from this approach will ultimately improve our ability to manage soil for sustainable productivity by promoting beneficial microorganisms and suppressing pathogenic ones.

Table of Contents

List of Figures xii
List of Tables xv
Acknowledgements xx
Dedication xxi
Chapter 1 - Literature review and Introduction 1
References 7
Chapter 2 - The response of soil bacterial and fungal communities to organic and conventional fertilization: comparing soil DNA and RNA 15
Abstract 15
Introduction 16
Materials and Methods 22
Study location and cropping history 22
Sampling 24
RNA and DNA isolation from the soil samples 25
Reverse transcription and PCR amplification 26
Control reactions 29
Sequencing 29
Analysis of the sequence data 30
Diversity indices, most frequent taxa, and taxon responses to treatments 31
Results 32
Microbial data characterization 32
Bacteria: Diversity responses among the treatments 33
Fungi: diversity responses among the treatments 33
Dominant bacterial and fungal taxa across the treatment combinations 34
Fertility management and taxon frequencies 35
Sampling DNA compared to rRNA 37
Discussion 39
How do bacterial and fungal diversity compare between organic and conventional management? 39

Are bacterial and fungal soil communities detected through cDNA more or less diverse than those detected through DNA? ... 41
What are the most abundant bacterial and fungal taxa recovered in plots with organic and conventional fertility management? ... 42
Does the cDNA pool reveal bacterial and fungal taxa that are not present in the DNA pool, and vice-versa? ... 46
Translation of microbial community data for farm management ... 47
Conclusions ... 48
Tables and Figures ... 49
References ... 88
Chapter 3 - Soil bacterial and fungal communities in tomato production under long-term organic vs. conventional soil fertility management ... 106
Abstract ... 106
Introduction ... 107
Materials and Methods ... 110
Study site ... 110
Soil sampling and experimental design ... 112
DNA extraction, PCR amplification and sequencing ... 113
Sequence analysis ... 116
Diversity indices and statistical analysis ... 117
Bacterial and fungal community responses across time to organic and conventional soil amendments ... 118
Results ... 119
Bacterial and fungal community data characterization ... 119
Diversity responses for fungal and bacterial communities across time ... 120
Dominant bacterial and fungal taxa across sampling times ... 120
Community analyses ... 122
Discussion ... 122
Diversity responses for bacterial and fungal communities across time ... 122
Dominant bacterial and fungal taxa across sampling times ... 127
Community analyses ... 128

Conclusions ... 129
Tables and Figures ... 130
References ... 154
Chapter 4 - The effects of organic amendments and plastic mulch on soil bacterial and fungal communities over time in tomato cropping systems ... 166
Abstract ... 166
Introduction ... 167
Materials and Methods ... 171
Study location, experimental design and on-farm methodology ... 171
Soil sampling ... 173
Soil physical and chemical analyses ... 174
DNA extraction, PCR amplification and sequencing ... 174
Sequence analysis ... 178
Diversity indices ... 180
Statistical analysis ... 180
Bacterial and fungal community responses across time to soil amendments ... 181
Results ... 182
General bacterial and fungal community data characterization ... 182
Bacterial diversity ... 183
High tunnel effects ... 183
Effects of plastic mulch ... 183
Effects of nutrient amendments and sampling time ... 183
Fungal diversity ... 185
High tunnel effects ... 185
Plastic mulch effects ... 186
Effects of nutrient amendments and sampling time ... 186
Community analysis with NMDS ... 187
Dominant bacterial and fungal taxa across time ... 188
Discussion ... 189
Management effects on bacterial and fungal community diversity and composition ... 189
Dominant bacterial and fungal taxa across sampling times ... 193

Conclusions 196
Tables and Figures 197
References 233
Chapter 5 - Conclusions and future directions 248
References 251

List of Figures

Figure 2.1. Open field plots established in 2002 to compare crops grown under organic and conventional nutrient management at the K-State Horticulture Research and Extension Center in Olathe, Kansas, USA.. 81
Figure 2.2. Simpson´s diversity for the bacterial rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations.. 82
Figure 2.3. Simpson´s evenness for the bacterial rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations. 83
Figure 2.4. Richness for the bacterial rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations... 84
Figure 2.5. Simpson´s diversity for fungal rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations... 85
Figure 2.6. Richness for fungal rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations. 86
Figure 2.7. Simpson´s evenness for fungal rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations... 87
Figure 3.1. Bacterial richness in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest). 147
Figure 3.2. Fungal richness in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest). 148
Figure 3.3. Bacterial Simpson´s diversity in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest).......... 149
Figure 3.4. Fungal Simpson´s diversity in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest).......... 150

Figure 3.5. Bacterial Simpson´s evenness in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest).......... 151
Figure 3.6. Fungal Simpson´s evenness in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest).......... 152
Figure 3.7. Non-metric multidimensional scaling (NMDS) in two dimensions of *bacterial* communities from plots receiving organic (O) or conventional (C) nutrient amendments. 153
Figure 4.1. Research plots located at the Common Harvest farm in Lawrence. KS. 218
Figure 4.2. Bacterial Simpson´s diversity in a data set including observations for high tunnels and field plots without plastic mulch 219
Figure 4.3. Bacterial Simpson´s evenness in a data set including observations for high tunnels and field plots without plastic mulch 220
Figure 4.4. Bacterial richness in a data set including observations for high tunnels and field plots without plastic mulch 221
Figure 4.5. Bacterial Simpson´s diversity in a data set including observations for high tunnels and field plots without plastic mulch 222
Figure 4.6. Bacterial Simpson´s evenness in a data set including observations for field plots with and without plastic mulch 223
Figure 4.7. Bacterial richness in a data set including observations for field plots with and without plastic mulch 224
Figure 4.8. Fungal Simpson´s diversity in a data set including observations for high tunnels and field plots without plastic mulch 225
Figure 4.9. Fungal Simpson´s evenness in a data set including observations for high tunnels and field plots without plastic mulch 226
Figure 4.10. Fungal richness in a data set including observations for high tunnels and field plots without plastic mulch 227
Figure 4.11. Fungal Simpson´s diversity in a data set including observations for field plots with and without plastic mulch 228
Figure 4.12. Fungal Simpson´s evenness in a data set including observations for field plots with and without plastic mulch 229

Figure 4.13. Fungal richness in a data set including observations for field plots with and without plastic mulch 230
Figure 4.14. Non-metric multidimensional scaling (NMDS) in two dimensions of *bacterial* community composition responses to nutrient amendments 231
Figure 4.15. Non-metric multidimensional scaling (NMDS) in two dimensions of *fungal* community composition responses to nutrient amendments 232

List of Tables

Table 2.1. Maximum daily temperature (°C) and precipitation (cm) reported on Olathe, Kansas, during the summer of 2010 during the tomato cropping season (from Altamimi (2016)). .. 49

Table 2.2. Timing of field activities in the plots sampled in this study (Altamimi (2016)) at the K-State Horticulture Research and Extension Center in Olathe, Kansas. 50

Table 2.3. Overall most frequent taxa assigned to bacterial phyla recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. 51

Table 2.4. Overall most frequent taxa assigned to bacterial class recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. 52

Table 2.5. Overall most frequent taxa assigned to bacterial order recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. 53

Table 2.6. Overall most frequent taxa assigned to bacterial family recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. 54

Table 2.7. Overall most frequent taxa assigned to bacterial genera recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. 55

Table 2.8. Overall most frequent taxa assigned to fungal phyla recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. 56

Table 2.9. Overall most frequent taxa assigned to fungal class recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. 57

Table 2.10. Overall most frequent taxa assigned to fungal order recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. 58

Table 2.11. Overall most frequent taxa assigned to fungal family recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. 59

Table 2.12. Overall most frequent taxa assigned to fungal genera recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. 60

Table 2.13. Bacterial phyla that responded to the fertility treatment (high (H) vs. control (C)) or exhibited a significant management type (M= organic vs. conventional) interaction with fertility treatment (F*M), with calculated q-values. 61

Table 2.14. Bacterial genera that responded to the fertility treatment (high (H) vs. control (C)) or exhibited a significant management type (M= organic vs. conventional) interaction with fertility treatment (F*M), with calculated q-values. 62
Table 2.15. Fungal phyla that responded to fertility treatment (high (H) vs. control (C)) or exhibited a significant management type (M= organic vs. conventional) interaction with fertility treatment (F*M), with calculated q-values. 67
Table 2.16. Fungal genera that responded to fertility treatment (high (H) vs. control (C)) or exhibited a significant management type (M= organic vs. conventional) interaction with fertility treatment (F*M), with calculated q-values. 68
Table 2.17. Frequency of bacterial phyla recovered from DNA and cDNA samples from soils at Olathe, Kansas, with associated q-values. 70
Table 2.18. Frequency of fungal phyla recovered from DNA and cDNA samples from soils at Olathe, Kansas, with associated q-values. 71
Table 2.19. Frequency of bacterial genera recovered from DNA and cDNA samples from soils at Olathe, Kansas, with associated q-values. 72
Table 2.20. Frequency of fungal genera recovered from DNA and cDNA samples from soils at Olathe, Kansas, with associated q-values. 77
Table 2.21. Soil test conducted for the tomato crop in 2010 by Altamimi (2016) in the organic (ORG) and conventional (CONV) field plots located at the State Horticulture Research and Extension Center in Olathe, KS. 80
Table 3.1. Reverse bacterial primer (806R) synthesized with unique twelve base pair molecular identifier (MID) tags assigned to each sample from a long-term organic and conventional tomato management experiment. 130
Table 3.2. Reverse fungal primer (ITS4) synthesized with unique twelve base pair molecular identifier (MID) tags assigned to each sample from a long-term organic and conventional tomato management experiment. 133
Table 3.3. Type III tests of fixed effects in a GLMM analysis of bacterial richness, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. 136

Table 3.4. Type III tests of fixed effects in a GLMM analysis of fungal richness, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. 137
Table 3.5. Type III tests of fixed effects in a GLMM analysis of bacterial Simpson´s diversity, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. 138
Table 3.6. Type III tests of fixed effects in a GLMM analysis of fungal Simpson´s diversity, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. 139
Table 3.7. Type III tests of fixed effects in a GLMM analysis of bacterial Simpson´s evenness, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. 140
Table 3.8. Type III tests of fixed effects in a GLMM analysis of fungal Simpson´s evenness, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. 141
Table 3.9. The 20 overall most frequent *bacterial* phyla, orders, families, and OTUs assigned to genera recovered in MiSeq sequencing 142
Table 3.10. The 20 overall most frequent *fungal* phyla, orders, families, and OTUs assigned to genera recovered in MiSeq sequencing 144
Table 3.11. Permanova analysis for bacterial communities in organic plots for three fertility levels (control, low-and-high fertilities) across three sampling time points........ 146
Table 3.12. Permanova analysis for bacterial communities in conventional plots for three fertility levels (control, low-and-high fertilities) across three sampling time points........ 146
Table 4.1. Bacterial region primer (806R) synthesized with a unique 12-base pair molecular identifier (MID) tag assigned to each treatment in a transitional organic farm on a tomato production season........ 197
Table 4.2. Fungal region primer (ITS4) synthesized with a unique 12-base pair molecular identifier (MID) tag assigned to each treatment in a transitional organic farm on a tomato production season........ 199
Table 4.3. Soil edaphic properties at Lawrence, KS, farm evaluated (A) before tomato transplanting and (B) when tomato plants were senescent 201

Table 4.4. Physico-chemical analysis for two organic fertilizers: alfalfa meal pellets and city compost, applied in a transitional organic farm during a tomato production season. 202
Table 4.5. Type III tests of fixed effects from the ANCOVA analysis of bacterial Simpson´s diversity, in a data set including high tunnel observations and un-mulched field plot observations (“Analysis 1”). 202
Table 4.6. Type III tests of fixed effects from the ANCOVA analysis of bacterial Simpson´s evenness, in a data set including high tunnel observations and un-mulched field plot observations (“Analysis 1”). 203
Table 4.7. Type III tests of fixed effects from the ANCOVA analysis of bacterial richness, in a data set including high tunnel observations and un-mulched field plot observations (“Analysis 1”). 204
Table 4.8. Type III tests of fixed effects from the GLMM analysis of bacterial Simpson´s diversity, for a data set including mulched and un-mulched field plot observations (“Analysis 2”). 205
Table 4.9. Type III tests of fixed effects from the GLMM analysis of bacterial Simpson´s evenness, for a data set including mulched and un-mulched field plot observations (“Analysis 2”). 206
Table 4.10. Type III tests of fixed effects from the GLMM analysis of bacterial richness, for a data set including mulched and un-mulched field plot observations (“Analysis 2”). 207
Table 4.11. Type III tests of fixed effects from the ANCOVA analysis of fungal Simpson´s diversity, in a data set including high tunnel observations and un-mulched field plot observations (“Analysis 1”). 208
Table 4.12. Type III tests of fixed effects from the ANCOVA analysis of fungal Simpson´s evenness, in a data set including high tunnel observations and un-mulched field plot observations (“Analysis 1”). 209
Table 4.13. Type III tests of fixed effects from the ANCOVA analysis of fungal richness, in a data set including high tunnel observations and un-mulched field plot observations (“Analysis 1”). 210
Table 4.14. Type III tests of fixed effects from the GLMM analysis of fungal Simpson´s diversity, for a data set including mulched and un-mulched field plot observations (“Analysis 2”). 211

Table 4.15. Type III tests of fixed effects from the GLMM analysis of fungal Simpson´s evenness, for a data set including mulched and un-mulched field plot observations (“Analysis 2”). 212
Table 4.16. Type III tests of fixed effects from the GLMM analysis of fungal richness, for a data set including mulched and un-mulched field plot observations (“Analysis 2”). 213
Table 4.17. Permanova analysis for bacterial communities in field plots without plastic mulch across two sampling time points (maturity, and postharvest) on a tomato cropping season. 214
Table 4.18. Permanova analysis for bacterial communities in high tunnels across two sampling time points (maturity, and postharvest) on a tomato cropping season. 214
Table 4.19. Permanova analysis for bacterial communities in field plots with plastic mulch across two sampling time points (maturity, and postharvest) on a tomato cropping season. 215
Table 4.20. Permanova analysis for fungal communities in high tunnels across two sampling time points (maturity, and postharvest) on a tomato cropping season. 215
Table 4.21. The percentage sequences for the 20 overall most frequent bacterial phyla, orders, families and OTUs assigned to genera recovered in MiSeq sequencing of soils from tomato production at a transitional organic farm located in Lawrence, Kansas, USA. 216
Table 4.22. The percentage sequences for the 20 overall most frequent fungal phyla, orders, families and OTUs assigned to genera recovered in MiSeq sequencing of soils from tomato production at a transitional organic farm located in Lawrence, Kansas, USA. 217

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Dedication

To God who is my guide and my strength. To my beloved husband, Germán A. Vargas, and to my sweet daughter, Gabriela Vargas Gómez, for their love. To my parents, José Fernando Gómez and Adda Montaño, for their constant love and support on each step of my life; to my sisters Liliana and Luz Angela, and to my parents in-law, Honorio Vargas and Martha Orozco for their prayers and encouraging words.

Chapter 1 - Literature review and Introduction

Microbial communities are pivotal for most soil biogeochemical cycles, and soil microbial diversity and community composition have important roles in soil health (Garbeva *et al.*, 2004; Zaccardelli *et al.*, 2013). Soil health has been defined as the resilience of soil in response to stress, its ability to sustain high biological diversity and high levels of internal nutrient cycling, and its ability to maintain environmental quality and promote plant and animal health (Doran *et al.*, 1996; van Bruggen and Semenov, 2000). The first step toward developing strategies to maintain these properties is to understand the factors that may impact microbial communities in agricultural soils. For example, soil microbes respond to agricultural practices, crop selection, and environmental conditions, such as weather patterns.

In agricultural systems, soil microbial communities respond to soil management (Spedding *et al.*, 2004; Acosta-Martinez *et al.*, 2010; Yin *et al.*, 2010). The biomass and composition of soil microbial communities are affected by soil cover crops (Zelles *et al.*, 1995; Maul *et al.*, 2014), plant species (Liu *et al.*, 2016; Pii *et al.*, 2016; Sheng *et al.*, 2016), cultivation techniques (Lalande *et al.*, 2005; Ge *et al.*, 2013; Smith *et al.*, 2014), application of fertilizers and pesticides (Ibekwe *et al.*, 2004; Cycon *et al.*, 2013; Miura *et al.*, 2016), irrigation effects (Entry *et al.*, 2008; Calderon *et al.*, 2016; Luneberg *et al.*, 2018) and application of organic residues (Marschner *et al.*, 2004; Ikeda *et al.*, 2014; Vida *et al.*, 2016).

In both natural and agricultural systems, microbial interactions and activity in soils are complex and affected not only by soil management practices, but also by environmental factors such as temperature, soil moisture, and soil pH (Van Bodegom *et al.*, 2001; Fierer and Jackson, 2006; Lennon *et al.*, 2012). Previous studies have shown that environmental differences lead to

changes in edaphic properties, which can in turn alter the soil microbial communities (Lauber *et al.*, 2008; García-Orenes *et al.*, 2013). In soil, microbial communities react to environmental fluctuations, with changes in species composition and functional roles (Grayston *et al.*, 2001; Wallenstein and Hall, 2012). For example, Jumpponen (2011) followed the seasonal dynamics of fungal communities that inhabit the rhizosphere of a dominant grass species, *Andropogon gerardii* Vitman, in a tallgrass prairie ecosystem. Fungal communities in the order Helotiales predominated during spring on the roots of this dominant tallgrass prairie grass, and towards the end of the growing season were replaced by arbuscular mycorrhizal fungi. Jumpponen (2011) suggested that the presence of fungi with possible affinities to aquatic taxa may be explained by the near saturation of soil water in the beginning of the growing season, while the dominance of arbuscular mycorrhizal fungi across the later sampling time points is a common pattern in the rhizosphere environment. Ibekwe *et al*. (2017) examined the effects of salinity, temperature, and temporal variability on soil and rhizosphere microbial communities in sand tanks irrigated with prepared solutions designed to simulate saline wastewater for a spinach crop during three production seasons. They reported that temperature fluctuations affected soil bacterial community composition in soil and in the rhizosphere irrigated with saline wastewater. They concluded that microbial communities in soils impacted by saline water respond differently to irrigation water quality and season of application due to temporal effects associated with temperature.

The rapid and ongoing diversification, high adaptive capacity and physiological flexibility of microorganisms allows them to colonize a huge variety of ecosystems (Schulz *et al.*, 2013). Soils in agricultural systems often have different soil microbial communities compared to those found in unmanaged systems (Jangid *et al.*, 2008; Wu *et al.*, 2008). Agricultural soil, perturbed by human activities, has different bacterial diversity, compared to non-disturbed forest and grassland

soil (Roesch *et al.*, 2007; Acosta-Martinez *et al.*, 2008). Understanding the effects of agricultural perturbation on soil microbial communities, and how agricultural management practices affect soil communities under organic vs conventional farming systems, represents a challenge for future microbiome-based management strategies.

A major goal in sustainable agriculture is to maintain soil biological function and to promote plant health (Doran *et al.*, 1996; van Bruggen and Semenov, 2000). Organic farming aims to reduce the use of synthetic fertilizers and pesticides to improve soil quality, support beneficial microbial communities, and increase farm sustainability (Drinkwater *et al.*, 1995; Gomiero *et al.*, 2011; Henneron *et al.*, 2015). Organic farming practices are designed to try to achieve sustainability through techniques such as crop rotation, green manure, and biological pest control instead of synthetic fertilizers and pesticides (Zhengfei *et al.*, 2005). Incorporating organic amendments and managing crop residues (type and quantity) can have direct impacts on plant health and crop productivity (Bailey and Lazarovits, 2003). Furthermore, manure and byproducts of seafood and livestock industries have been used by growers to maintain productivity of agricultural soils for millennia (Lazarovits, 2001; Wolf and Snyder, 2003; Jayathilakan *et al.*, 2012).

Organic farming systems may have the potential to restore soil health and to increase agro-ecosystem resilience to stress (Azadi *et al.*, 2011). Several studies have investigated the impact of organic and conventional management practices on soil microbial communities (Esperschutz *et al.*, 2007; Sugiyama *et al.*, 2010; Orr *et al.*, 2011; Li *et al.*, 2012a; Ge *et al.*, 2013; Hartmann *et al.*, 2015; Pershina *et al.*, 2015; Lupatini *et al.*, 2017). These studies have reported that organic agriculture may increase soil microbial biomass and diversity, and may lead to distinct communities compared to those in conventional management systems. The application of organic

manure might be the main reason for differences in soil microbial communities when conventional and organic farming management are compared (Bossio *et al.*, 1998; Gunapala and Scow, 1998). Organic amendments (e.g., green manure straw and farm yard manure) and the lack of pesticides and chemical fertilizers may favor some microbial taxa, and organic amendments may introduce novel taxa. However, the effects of organic amendments on the diversity of microbial communities are complex and sometimes controversial (Hartmann *et al.*, 2015). Some studies have reported no difference or a decrease in microbial diversity and richness when organic systems were compared to conventional management (Liu *et al.*, 2007; Reilly *et al.*, 2013). Due to the diverse effects of organic and conventional farming systems on soil microbial communities, there are not always consistent patterns across systems (Hartmann *et al.*, 2015; Lupatini *et al.*, 2017).

The study of soil microbial communities has been limited by traditional methods and the non-culturability of most microbial taxa (Rondon *et al.*, 2000; Fierer *et al.*, 2007b). The complexity of microbial communities and the technical challenges to evaluating them have limited our understanding of how agricultural management practices may affect soil microbial communities. Approaches to studying soil microbial communities have evolved rapidly, including analyses of both DNA and rRNA (ribosomal RNA). DNA-based approaches have increasingly been used to study the composition of the bacterial community as a whole and to identify soil microorganisms in environmental samples (Fierer and Jackson, 2006; Swain and Ray, 2006; Acosta-Martinez *et al.*, 2008; Gomez-Montano *et al.*, 2013). These analyses are likely to reveal the “archived” past and present microbial community, so that in this sense soil DNA represents the soil history (Girvan *et al.*, 2003). rRNA, on the other hand, is more responsive to processes driving degradation, with a high turnover within cells, and can be used as an alternative for sampling community components that are active at the time of sampling (Girvan *et al.*, 2004; Mahmood *et al.*, 2005). Both DNA-

and RNA-based approaches have been used in some microbial community profiling of environmental samples. The development of high-throughput sequencing technologies has allowed the collection of many thousands of sequences from multiple samples, providing a new window into the diversity and composition of microbial communities (Acosta-Martinez *et al.*, 2010). Indexing has increased sample throughput (Hamady *et al.*, 2008; Lauber *et al.*, 2009; Brown and Jumpponen, 2015; Hamm *et al.*, 2016). High-throughput sequencing has also sparked interest in elucidating the members of the rare biosphere: microorganisms that exist at low relative abundances (Brown *et al.*, 2015). Further, the Illumina platform made possible new volumes of data (Gloor *et al.*, 2010; Caporaso *et al.*, 2011), enabling fast, affordable, reproducible, and more comprehensive assessments and comparisons of the taxonomic diversity present in complex microbial communities (Bartram *et al.*, 2011; Caporaso *et al.*, 2012). The technological advances and the development of culture-independent tools including high-throughput DNA sequencing, provide new opportunities to explore microbial diversity in soil under many different environmental conditions, thus clarifying soil microbiome responses to agricultural management (Fierer *et al.*, 2007b; Acosta-Martinez *et al.*, 2008; Lauber *et al.*, 2008; Acosta-Martinez *et al.*, 2010; Li *et al.*, 2012; Hartmann *et al.*, 2015; Brennan and Acosta-Martinez, 2017) .

The effect of anthropogenic activities on soil microbial communities, such as the effects of agriculture on bacterial communities, have been addressed in many studies but remain an important challenge (Wessén *et al.*, 2010). Further, fungi, despite their high biodiversity, and substantial ecological and economical importance, remain an understudied group of organisms (Pautasso, 2013; Rambold *et al.*, 2013). We still lack information about how bacterial and fungal diversity are affected by agriculture perturbation – including fertilizer type and the use of high tunnels and plastic mulch – in vegetable crops.

In this work, we studied both bacterial and fungal communities in soil samples taken from tomato plants grown under specific organic or conventional management strategies. We applied high-throughput sequencing to understand how soil bacterial and fungal communities in vegetable production respond to organic or conventional soil fertility management under these treatments. In our first study, we compared DNA and rRNA samples in evaluation of bacterial and fungal community responses to organic vs. conventional nutrient management. In our second study, we evaluated the soil microbial community responses to organic vs. conventional nutrient management across three sampling times in a tomato cropping season using DNA analysis. Finally, in our third study, we evaluate the effects of high tunnels and plastic mulch on soil microbes in a vegetable crop, and the effects of common organic amendments, across a tomato cropping season.

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Chapter 2 - The response of soil bacterial and fungal communities to organic and conventional fertilization: comparing soil DNA and RNA

Abstract

Soil microbes are fundamental to agricultural productivity. Organic management may foster greater soil microbial diversity, benefitting crop production and potentially reducing losses to pathogens. However, the response of microbial communities to organic and conventional farming systems has only been studied in a limited range of systems and has focused primarily on bacteria or arbuscular mycorrhizal fungi. Our first objective in this study was to evaluate bacterial and fungal community responses to organic vs. conventional nutrient management, for both a high fertility level (equivalent high levels of N from compost or synthetic fertilizers) and a control which only received nutrients from a cover crop. Our second objective was to compare *total* resident communities using extracted DNA, and the putatively *active* microbial communities using extracted rRNA (ribosomal RNA), analyzed as cDNA. We sampled soils once in a long-term field experiment with a two-year rotation of tomato and pac choi and pyrosequenced variable regions of ribosomal RNA genes (16S bacteria and 28S for fungi). The highest bacterial diversity (complement of Simpson's diversity index, 1 – D) was observed under high fertility organic management using cDNA, whereas the highest fungal diversity was observed in both conventional and organic management under control and high fertility treatments using cDNA compared to DNA. Several genera were more frequent in high fertility organic management than in

conventional management, such as the bacterial genus *Pseudomonas* and the fungal genus *Septoria*. Similarly, high fertility conventional management favored several taxa, for example the bacterial genus *Sorangium*, the subdivision *Gp4*, and the fungal genera *Cladosporium* and *Thelebolus*. Comparing the DNA and cDNA derived estimates, we found that several bacterial and fungal taxa occurred at different frequencies. For example, the bacterial genus *Pseudomonas* (phylum Proteobacteria) and the fungal genus *Septoria* (phylum Ascomycota) were more frequent in DNA derived estimates, whereas the bacterial genus *Sorangium* (phylum Proteobacteria) and the fungal genus *Glomus* (phylum Glomeromycota) were more frequent in the cDNA derived estimates. Microbial diversity was higher for the cDNA-derived estimates. The DNA and cDNA estimates provide distinct views of the community responses to management strategies and identify microbial taxa adapted to long-term organic or conventional farming systems. Ultimately, understanding these differences may contribute to management strategies to support sustainable productivity.

Introduction

Organic farming aims to reduce the use of synthetic fertilizers and pesticides to improve soil quality, support beneficial microbial communities, increase farm sustainability, and benefit human health (Drinkwater *et al.*, 1995; Gomiero *et al.*, 2011; Henneron *et al.*, 2015; Mie *et al.*, 2017). A central idea is that organic crop management supports diverse microbial communities that provide important ecosystem services, such as biocontrol by microbes that compete with pathogens, microbial contributions to nutrient cycling and plant nutrient uptake, and microbial contributions to soil organic matter formation (Kennedy and Papendick, 1995; Pankhurst *et al.*, 1996; Buckley and Schmidt, 2001; Swain and Ray, 2006; Acosta-Martinez *et al.*, 2008; van der

Heijden *et al.*, 2008; Sheik *et al.*, 2011). Microbial communities drive soil functions and help to maintain the productivity and health of agricultural systems (Pankhurst *et al.*, 1996). These communities are key to processes that support terrestrial ecosystems, including nutrient acquisition and cycling, as well as degradation of agrochemicals (Pankhurst *et al.*, 1996; van der Heijden *et al.*, 2008). The capacity of soil to function as a vital living system able to fulfill all these functions defines 'soil quality' (Karlen *et al.*, 1997). Thus, soil microbial diversity represents the repertoire of genetic potential that supports soil health (Jain *et al.*, 2005). However, despite the importance of soil microorganisms and their diversity, we are only beginning to understand the complex responses of microbial diversity to agricultural management, such as organic versus conventional systems (Hartmann *et al.*, 2015).

In organic and conventional farming systems, the use of soil amendments (organic or synthetic amendments) that are rich in nutrients – particularly N, P, and K – improves soil fertility and increases crop yield (Ahmad *et al.*, 2007; Ahmad *et al.*, 2009; Leite *et al.*, 2010) because plants directly or indirectly assimilate nutrients from the fertilizers. However, extensive use of synthetic fertilizers may negatively affect agricultural ecosystems, resulting in the loss of crop genetic diversity, a reduction in soil microbial diversity, and soil degradation (Kaur *et al.*, 2008). As a result, there is a growing interest in using alternative materials, including animal manure (Bulluck III *et al.*, 2002; Devi *et al.*, 2011; Lori *et al.*, 2017). Animal manure can deliver substantial amounts of organic matter and nutrients, especially after composting (Devi *et al.*, 2011; Galitskaya *et al.*, 2017). The addition of manure compost can also improve soil porosity, moisture, and nutrient availability, as well as biological activity in soil (Francis *et al.*, 2010; Wang *et al.*, 2011).

The study of soil microbial communities was previously limited by culture-based sampling methods, due to the non-culturability of most microbial species (Rondon *et al.*, 2000; Kirk *et al.*,

2004; Fierer *et al.*, 2007b; Thies, 2008). The complexity of microbial communities, and the technical challenges associated with evaluating them, have limited our understanding of how agricultural management practices may affect soil microbial communities. More recently, high-throughput sequencing has been used to evaluate the effects of organic and conventional management on soil communities (Sugiyama *et al.*, 2010; Li *et al.*, 2012a; Hartmann *et al.*, 2015; Pershina *et al.*, 2015). These studies have found that soil microbial communities differ between organic and conventional farming systems, and that organic farming systems may increase microbial diversity compared to conventional systems (Sugiyama *et al.*, 2010; Li *et al.*, 2012; Hartmann *et al.*, 2015). Organic amendments (green manure straw, farm yard manure, etc.) and the absence of most pesticides and chemical fertilizers may favor some microbial taxa, and organic amendments may introduce novel taxa. Sugiyama *et al.* (2010) studied soil fungi and oomycetes in three organic and three conventional potato fields in Colorado, concluding that *Alternaria* spp. and *Ulocladium* spp. were more common in conventional farms whereas *Pythium ultimum* was more common in organic farms. Sugiyama *et al.* (2010) also concluded that microbial communities in the organic farms were both more diverse and had higher evenness, potentially because of added compost or lack of pesticide use. Moreover, organic farming systems may also promote higher microbial biomass (Araujo *et al.*, 2009; Hartmann *et al.*, 2015; Lupatini *et al.*, 2017). Although an increase in diversity of biota in organic systems (such as earthworms, microarthropods, and nematodes) has been largely consistent across studies, the impact on microbiota is less clear (Hole *et al.*, 2005; Postma-Blaauw *et al.*, 2010).

Approaches to the study of soil microbial communities have evolved rapidly, including analyses of both DNA and rRNA (ribosomal RNA). Extraction of the total rRNA from soil has been used to quantify the abundance of Proteobacteria, Actinobacteria, Bacteria, and Eukarya

under a range of field management regimes before high-throughput sequencing was available for characterization (Buckley and Schmidt, 2001). Metagenomic and small subunit rRNA-based metabarcoding have been used to estimate the diversity of bacteria, archaea, fungi and viruses in soils collected from prairie, desert, and rainforest ecosystems (Fierer *et al.*, 2007b; Jumpponen, 2011). DNA-based approaches have increasingly been used to study the genetic structure of the total bacterial community and to identify soil microorganisms in environmental samples (Fierer and Jackson, 2006; Swain and Ray, 2006; Acosta-Martinez *et al.*, 2008; Gomez-Montano *et al.*, 2013). These analyses are likely to reveal the "archived" past and present microbial community, so that in this sense soil DNA represents the soil history (Girvan *et al.*, 2003). rRNA, on the other hand, is more responsive to processes driving degradation, with a high turnover rate within cells, and can be used as an alternative for sampling community components that are active at the time of sampling (Girvan *et al.*, 2004; Mahmood *et al.*, 2005).

Many studies have used DNA coding for rRNA genes or DNA-based approaches, but a limited number have used both DNA and rRNA to compare soil microbial communities in different environments (Frois D *et al.*, 1998; Griffiths *et al.*, 2000; Bastias *et al.*, 2007; Liu *et al.*, 2011; Rao *et al.*, 2012; Mikkonen *et al.*, 2014; Romanowicz *et al.*, 2016; Gill *et al.*, 2017). These studies have shown that DNA and rRNA reveal different aspects of soil microbial communities, based on the more persistent DNA and the more transient and dynamic rRNA. Most studies have found that microbial diversity in DNA is higher than in complementary DNA (cDNA, which is DNA synthesized from a single stranded RNA template) (Bastias *et al.*, 2007; Liu *et al.*, 2011; Romanowicz *et al.*, 2016). Yet some studies, for example Mikkonen *et al.* (2014), have observed that soil bacterial community richness in cDNA can be greater than in DNA. These authors concluded that this apparent increase was based not only on the slightly higher richness but also

the higher evenness of the cDNA-based community (Mikkonen *et al.*, 2014). To our knowledge, DNA and cDNA comparisons have not been used to explore the response of fungal and bacterial communities in crops under organic versus conventional management. We used both DNA and cDNA to evaluate bacterial and fungal community responses in a long-term experimental system comparing organic and conventional nutrient management in Olathe, Kansas. These farming systems differed in fertilization regimes (organic or synthetic), whereas factors such as tillage and crop rotation were kept constant. The organic system used chicken litter and fish hydrolysate composts, in contrast to the conventional system which used NPK (nitrogen-phosphorus-potassium), calcium and potassium nitrate fertilizers. Similar amounts of key nutrients from compost or synthetic fertilizers were applied in each farming system.

We addressed four questions in this experimental system. First, how do bacterial and fungal diversity compare in organic versus conventional management? We hypothesized that organic management would result in higher bacterial and fungal diversity than conventional management. The expected high diversity in organic management would support common ideas about the value of organic systems, although we do not necessarily interpret higher diversity in soil microbial communities as a system benefit. We use diversity metrics to compare systems, and to evaluate possible effects on the soil microbial community. As Shade (2016) discusses, context is necessary for interpreting the costs or benefits of diversity, where interpretation will become easier as databases relating sequence and microbial function become available.

Second, what are the most frequent bacterial and fungal taxa under these farming practices? We hypothesized that the most frequent bacterial and fungal taxa under organic management would differ from those under conventional management. Third, are bacterial and fungal soil communities detected through cDNA more or less diverse than those detected through DNA?

Based on previous studies discussed above, we would expect that DNA-based communities will have higher microbial diversity than cDNA-based communities. Extraction of DNA from soil samples allows evaluation of genetic material from dormant, dead or degraded cells (Sheik *et al.*, 2011) in addition to the active microbial population at the time of sampling. In contrast, the RNA extraction is mostly ribosomal (rRNA, analyzed as cDNA), and the number of ribosomes within a cell is indicative of protein synthesis potential (Axelrood *et al.*, 2002), often assumed to correlate with cellular activity and growth rate (Girvan *et al.*, 2003; Gilvan *et al.*, 2004; Mahmood *et al.*, 2005). Fourth, does the cDNA pool reveal some bacterial and fungal taxa that are not present in the DNA pool? We might expect that some taxa - particularly those without dormant structures - may appear in the cDNA-based communities and not in the DNA-based communities.

We used high-throughput sequencing to characterize soil bacterial and fungal communities in organic agriculture compared to conventional management, for two fertility levels (high-fertility and control) in a tomato crop. We used diversity estimates (such as species richness, Simpson´s diversity and Simpson´s evenness) to summarize bacterial and fungal community responses to these farming practices. To dissect soil microbial communities, we identified the most frequent bacterial and fungal taxa. We compared the microbial communities recovered from DNA (the general pool of microbes) and from rRNA (microbes that have the potential for active metabolism) for this set of management systems. This study provides a basic framework for evaluating soil bacterial and fungal diversity and its connection to organic and conventional farming practices.

Materials and Methods

Study location and cropping history

The field study was implemented in 2010 at the K-State Horticulture Research and Extension Center in Olathe, Kansas, USA. A long-term experiment was established in 2002 and maintained for more than eight years to compare crops grown under organic and conventional nutrient management in open field plots. These farming systems differ only in whether their source of fertilizer is synthetic or organic, with no pesticide applications included in the treatment regimen. The soil in the field plots has a silt loam texture, classified as Kennebec silt loam (Talavera-Bianchi *et al.*, 2010). A summary of weather during the 2010 tomato season from Altamimi (2016) is in Table 2.1.

The experiment followed a split plot design, with organic and conventional fertilizer treatments applied at the whole plot level, and three levels of organic or conventional fertilizer (control, low fertility and high fertility) applied at the subplot level (Figure 2.1). The current project addresses only the high-fertility treatment (described in more detail below) and the control treatment (no fertilizer added). Organic plots were managed in compliance with USDA National Organic Programs standards (https://www.ams.usda.gov/about-ams/programs-offices/national-organic-program). The field plots (9.8 m x 6.1 m) were covered with single-layer 6-mil (0.153 mm) K-50 polyethylene (Klerk's Plastic Product Manufacturing, Inc., Richburg, SC, USA) designed to control weeds and to reduce evaporation. Each whole plot, assigned to conventional or organic management, was subdivided into three 3.2 m x 6.1 m subplots, and one of the three fertilizer levels (high, low, and control) was randomly assigned to each subplot. Again, our current study addressed only the high-fertility and control treatments. Compost application rates were

based on the assumption that 50% of the nitrogen from compost would be available to plants during the growing season, while 100% would be available from conventional fertilizers (Warman and Havard, 1997). High-fertility subplots were fertilized in the beginning of the growing season, with equivalent amounts of compost or synthetic fertilizer, and received additional fertilization during the growing season as described below.

Since 2007, one half of each whole plot was planted to pac choi (*Brassica rapa* L. chinensis 'Mei Qing Choi'; Johnny's Selected Seed, Albion, ME, USA) and the other half was planted to tomato (*Lycopersicon esculentum* 'Bush Celebrity'; Totally Tomatoes, Randolph, WI, USA), with a rotation each year to change which half of the whole plot was assigned to each crop. For tomato, a single crop was grown during summer each year, where the whole plots included three rows of eighteen tomato plants, with six tomato plants included in each subplot. Pac choi was grown during spring and fall, with buckwheat (*Fagopyrum esculentum*; Albert Lea Seed, MN, USA) planted as a cover crop in between.

Conventional plots were fertilized with Jack's professional peat-lite N-P_2O_5-K_2O 20-10-20 (Allentown, PA, USA) at a rate of 98 kg nitrogen (N) /ha. Organic plots received a chicken litter source compost, MicroLeverage N-P_2O_5-K_2O 0.6-0.8-0.5 (Hughesville, MO, USA), at a rate of 197 kg/ha. Starting two weeks after planting, high-fertility plots received additional soluble fertilizer at a rate of 7.24 kg/ha of calcium nitrate and potassium nitrate for conventional plots, and an equivalent rate of fish hydrolysate for organic plots. Conventional high-fertility plots received 36.6 kg/ha of calcium nitrate ($Ca(NO_3)^2$) and 11.2 kg/ha of potassium nitrate (KNO_3), which were designed to apply calcium levels equivalent to the organic treatments. Organic high-fertility plots received 7.24 kg N/ha fish hydrolysate N-P_2O_5-K_2O 2.23-4.35-0.3 (Neptune's Harvest, Gloucester, MA, USA).

The tomato crop received one application of calcium nitrate and potassium nitrate (for conventional plots) and fish hydrolysate (for organic plots) per week during six weeks, and the spring and fall pac choi crops each received three such applications (Talavera-Bianchi *et al.*, 2010; Altamimi, 2016). Dates for seeding, fertilizer application and transplanting of tomato crop in 2010 are listed in Table 2.2, summarized from Altamimi (2016). Control plots received nutrients only from the cover crop plantings that all treatments received. Only high-fertility and no fertilizer (control) treatments were sampled in the experiment comparing DNA and rRNA sampling. Several aspects of this field experiment have been evaluated at the study site: (1) comparing the effects of the organic and conventional nutrient management systems on phenolic compounds in lettuce (Zhao *et al.*, 2007); (2) the sensory characteristics and volatiles associated with pac choi and tomatoes (Talavera-Bianchi *et al.*, 2010; Talavera-Bianchi *et al.*, 2011); (3) the response of pac choi to nitrogen fertilizer rates measured in its leaf petiole sap (Altamimi *et al.*, 2013); and (4) the effects of organic vs. conventional management and nitrogen rate on yield, soil fertility status, and plant nutrient status of tomato and pac choi grown in high tunnels and in field plots (Altamimi, 2016).

Sampling

Soil samples were collected once post-harvest on September 23, 2010, when tomato plants were approximately five months old. We sampled the three replicate subplots for the high and control levels of fertilization, for a total of 12 experimental units: 2 management treatments (organic vs. conventional) x 2 levels of fertilization (high-fertility and control) x 3 replicate subplots. This combination of treatments results in a split-split plot design, with whole plots

(organic vs. conventional fertilization) in a randomized complete block design, the first split corresponding to the fertility level, and the second split corresponding to the comparison of DNA and rRNA (Figure 2.1). We collected four soil subsamples from the middle row of each subplot, each adjacent to a different tomato plant but avoiding the root system, to represent the spatial heterogeneity within the plot and to minimize edge effects. From each experimental unit (subplot), four 15 cm deep 5 cm dia. cores were collected and placed in labeled ziploc plastic bags. Between samples, the soil corers were cleaned with water and dried with a clean paper towel to avoid soil contamination across samples. The four soil subsamples were bulked and homogenized in a clean plastic bucket to form a composite soil sample. From each composite soil sample, approximately 2.0 g was added to a 15 ml bead tube with 1.5 g beads (components of the kit: RNA PowerSoil, Total RNA Isolation, MoBio, Carlsbad, CA, USA). Into each 15 ml bead tube with 1.5 g beads, 5 ml of LifeGuard solution (LifeGuard Soil Preservation solution, MoBio, Carlsbad, CA, USA) was added before sampling to protect the RNA integrity while the samples were transported in a cooler from the field to the lab.

RNA and DNA isolation from the soil samples

Total soil RNA and DNA were isolated from the soil samples using a PowerSoil® Total RNA Isolation kit combined with DNA Elution Accessory kit (both kits from MoBio, Carlsbad, CA, USA) following the manufacturer's protocols. The extracted RNA was eluted in 100 µl of the RNA Isolation kit solution SR7 (RNase/DNase free water) with 100 U of RNaseOUT (40 U/µl; Invitrogen, Carlsbad, CA, USA) to inhibit RNA degradation before and during reverse transcription. Residual DNA was removed from the RNA extracts using RQ1 RNase-free DNase

(Promega, Madison, WI, USA) prior to first-strand complementary DNA (cDNA) synthesis. To confirm the absence of DNA in the RNA extracts, an aliquot of the RNA extract was PCR-amplified without preceding cDNA synthesis; these steps yielded no visible amplicons indicating successful removal of DNA. The DNA extracts were eluted in 100 µl of DNA Elution Accessory kit solution S5 (RNase/DNase free water). Both RNA and DNA extracts were stored at -80°C until further processing.

Reverse transcription and PCR amplification

For the reverse transcription, the extracted RNAs were quantified with an ND 1000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA), and 100 ng of each extract along with the blank extraction control were reverse-transcribed using the Thermoscript reverse transcription PCR (RT-PCR) two-step system (Invitrogen, Carlsbad, CA, USA). The bacterial 16S and fungal 28S templates were reverse transcribed using the primers 338R (Hunt *et al.*, 2011) and LR3 (Vilgalys and Hester, 1990), respectively. To denature the rRNAs before the cDNA synthesis, 100 ng of RNA for each sample was combined with 1µl of nuclease-free 10 µM reverse primer, 2 µl of 10 mM dNTPs, and the corresponding volume of nuclease free H_2O for a 12 µl final volume. Samples were incubated at 65°C for 5 min in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany). The denatured RNAs were transferred to ice and combined with 4 µl of 5 x cDNA buffer, 1 µl of 0.1 M DTT, 1 µl of RNaseOUT (Invitrogen, Carlsbad, CA, USA), 1 µl nuclease free H_2O, and 1 µl ThermoScript Reverse Transcriptase or Platinum *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA, USA) as the control for DNA contamination. The cDNAs were

synthesized in the Eppendorf Mastercycler at 50°C for 60 min, and the synthesized cDNAs were returned to ice until PCR amplification.

PCR reactions for bacteria included 12.5 µl of Amplitaq Gold 360 PCR Master Mix (Applied Biosystems, USA), 2.5 µl of 10 µM forward and reverse primers, and 2 µl of the cDNA or DNA template plus 5.5 µl of nuclease free water. The PCR reactions were carried out with initial 10 min denaturation at 95 °C followed by 34 cycles of 1 min at 95 °C, 1 min at 50 °C, 2 min at 72°C, and a terminal elongation at 72°C for 7 min. Longer extension steps were chosen to minimize chimeric PCR products (Jumpponen, 2007). After this first amplification, a second PCR reaction was conducted to add the A and B adapters required for direct 454 sequencing (Margulies *et al.*, 2005) to amplify the V1-V2 hypervariable regions of the 16S rRNA genes. Bacterial amplicons were generated for 454 pyrosequencing combining the A-primer (454 sequencing primer) and the 27f (forward primer; (Lane, 1991)) with a ten base pair (bp) DNA tag in between for post-sequencing sample identification (A-DNAtag-AGAGTTTGATCCTGGCTCAG); the B-primer (454 DNA capture bead anneal primer) and the 338R (reverse primer; (Hunt *et al.*, 2011)) were combined (B-TGCTGCCTCCCGTAGGAGT) to make the single strands on beads as required for 454 pyrosequencing (Margulies *et al.*, 2005).

PCR reactions for fungi included 1 unit GoTaq Hot Start DNA polymerase (Promega, Madison, Wisconsin), 2.5 µl of 10 µM forward and reverse primers, 5 µl of the cDNA or DNA template, 100 µM of each deoxynucleotide triphosphate, 2.5 mM MgCl2, 5 µl Green Go Taq Flexi PCR buffer (Promega, Madison, Wisconsin) plus 4.8 µl of nuclease free water with PCR cycle parameters with an initial 10 min denaturation at 95°C followed by 34 cycles of 1 min at 95°C, 1 min at 53°C, 2 min at 72°C, and a terminal elongation at 72°C for 7 min. For 454 sequencing, fungal amplicons on the D1-D2 variable regions of the 28S rRNA genes were generated combining

the A-primer and the LROR (forward primer; (Vilgalys and Hester, 1990)) with a ten base pair DNA tag in between (A-DNAtag-CCGCTGAACTTAAGCATATCAATA); the B-primer was combined with LR3 (reverse primer; (Vilgalys and Hester, 1990)) and the resulting amplicons (B-CCGTGTTTCAAGACGGG) were used in massively parallel sequencing (MPS) (Margulies *et al.*, 2005). Bacterial and fungal PCR reactions were conducted in a 25 µl volume. For bacteria and fungi, secondary PCR reactions were identical to those for the primary PCR except that they included primary PCR products as template and the number of PCR cycles was reduced to five. The amplification of target-sized amplicons for bacteria and fungi was confirmed by horizontal gel electrophoresis. The PCR products were purified with the Agencourt AMPure PCR purification system (AgenCourt Bioscience, Beverly, MA, USA) following the manufacturer's instructions. This clean-up system was selected because it effectively discriminates against fragments of less than 100 bp in size, removes salts and enzymes, and eliminates dimers of the fusion primer constructs that may exceed 40 bp in size. The clean bacterial and fungal PCR products were quantified with a ND1000 spectrometer.

Control reactions

To account for contaminating nucleic acids in the samples, three controls were included. First, to account for RNA/DNA contamination from the extraction system or sample handling, a blank extraction without sample was carried through the extraction protocol and used in cDNA synthesis as well as in PCR reactions. Second, to account for PCR reagent-borne contaminants, a PCR control without template DNA was included in the PCR reactions. Third, to account for DNA carryover through the RNA extraction, a control where Thermoscript reverse transcriptase was replaced with Platinum Taq polymerase was included. All these controls remained free of contaminants and yielded no visible PCR amplicons.

Sequencing

The two bacterial amplicons (one for DNA and another for cDNA) and the two fungal amplicons (one for DNA and another for cDNA) were unidirectionally sequenced using a GS-FLX Titanium technology (Roche 454 Life Sciences, Branford, CT, USA) at the Integrated Genomics Facility at Kansas State University. Each of the four libraries were sequenced in 1/4 of a plate for a sequencing reaction. The sequencing was repeated for a total of two sequencing runs, and the resulting data were merged. A total of 902,777 bacterial sequences were obtained (bacterial cDNA runs 1 and 2: 492,031 sequences; bacteria DNA runs 1 and 2: 410,746 sequences), whereas 628,791 fungal sequences (fungal cDNA runs 1 and 2: 392,677 sequences; fungal DNA runs 1 and 2: 236,114 sequences) were obtained in total. Four libraries from bacterial amplicons (two for DNA and two for cDNA) and four libraries from fungal amplicons (two for DNA and two for

cDNA) compressed as sff.files have been deposited in the data sharing repository Figshare (https://figshare.com). Both bacterial and fungal data sets are available at https://doi.org/10.6084/m9.figshare.7283093.v2

Analysis of the sequence data

The bacterial and fungal cDNA and DNA sequence data were analyzed using the software platform mothur version 1.33.3 (Schloss *et al.*, 2009). The sequence data were denoised (Quince *et al.*, 2009), trimmed, aligned, and screened for putative chimeras using the command "chimera.uchime" (Edgar *et al.*, 2011), as implemented in mothur. To minimize the effects of pyrosequencing errors (Kunin *et al.*, 2010), all reads that contained homopolymers longer than 8 bp, or that had more than one nucleotide mismatch to the barcode and two nucleotide mismatches to the PCR primer, were removed from the dataset. All sequences were truncated to 250 bp to account for declining sequence quality. Bacterial sequences were aligned against SILVA-based reference alignment (Schloss, 2009) or RDP LSU alignment (Cole *et al.*, 2009; Cole *et al.*, 2014). The curated sequences were clustered into operational taxonomic units (OTUs) using the nearest-neighbor algorithm at 97% sequence identity. Singleton OTUs were removed, and the OTUs assigned to taxon affinities using RDP 16S training set version 9 for bacteria (Schloss and Westcott, 2011) and RDP 28S training set version 7 for fungi (Liu *et al.*, 2012) with a naïve Bayesian classifier (Wang *et al.*, 2007) implemented in mothur with a minimum bootstrap support of 80% for bacteria and 60% for fungi. For each subplot, all reported OTU frequencies were determined by dividing the number of reads for any given OTU by the total number of reads obtained for that subplot. The diversity indices were estimated after subsampling the data to the

size of the lowest yielding experimental unit. On the basis of the consensus taxonomies, frequency data for OTUs at specific taxonomic ranks (genus, family, order, class and phylum) were merged and used to generate taxonomic rank-specific matrices that were the basis for the taxa-treatment analyses.

Diversity indices, most frequent taxa, and taxon responses to treatments

Using the derived OTU frequency data, we estimated the complement of Simpson's diversity index (1-D), richness (S_{Obs}) and evenness (Simpson's equitability, $E_{1/D}$) for each treatment combination using mothur (v. 1.33.3, (Schloss *et al.*, 2009)). We evaluated Simpson's diversity (1-D, or the complement of Simpson's dominance: $1 - D = 1 - \sum p_i^2$, where D = Simpson's dominance and p_i = proportion of sequences assigned to the *i*th OTU) because it is not strongly affected by the addition or loss of rare species, making it relatively stable across similar sample sizes (McCune and Grace, 2002; Magurran, 2004). Simpson´s diversity (1-D) estimates the likelihood that two randomly chosen individuals (in this case sequences) will be assigned to different species or OTUs (McCune and Grace, 2002). We also evaluated richness (S_{Obs}: number of OTUs), the observed number of OTUs in a given sample, and evenness (Simpson's equitability or $E_{1/D}$: $E_{1/D} = (1 / \sum p_i^2) / S_{Obs}$), where p_i = proportion of sequences assigned to the *i*th OTU and S_{Obs} = richness). These diversity indices were used to test hypotheses about diversity responses in generalized linear mixed models using SAS Proc GLIMMIX (SAS 9.4. Institute Inc., Cary, NC), comparing communities in control and high-fertility systems under organic and conventional management. For the diversity analyses, adjustments for multiple testing were performed using

Tukey's method. We also used generalized linear mixed models in SAS Proc GLIMMIX and p-value adjustments for multiple comparisons using Tukey's method to evaluate the most frequent taxa across the treatment combinations.

The effects of the treatment combinations (organic and conventional management; control and high-fertility levels; and DNA/rRNA (cDNA type) on the frequency of specific fungal and bacterial taxa were evaluated using generalized linear models with a binomial family (R function glm) and q-value comparisons to control the false discovery rate (R package qvalue, Storey *et al*. (2004)).

Results

Microbial data characterization

Bacteria. We obtained a total of 902,777 bacterial sequences. After trimming, alignment, and removal of chimeras, we retained 136,865 high quality bacterial sequences across the 12 subplots sampled. At 97% sequence similarity, these sequences represented 19,856 OTUs in all. The number of sequences passing our quality filtering ranged from 631 to 14,305 bacterial sequences per subplot.

Fungi. We obtained a total of 628,791 fungal sequences. After quality control (including trimming and alignment) and removal of chimeras that did not meet our minimum requirements, we retained 143,799 high quality fungal sequences across the 12 subplots sampled. At 97% sequence similarity, these sequences represented 2,147 OTUs in all. The number of sequences passing our quality filtering ranged from 563 to 13,514 fungal sequences per subplot.

Bacteria: Diversity responses among the treatments

Simpson's diversity for bacteria was higher on average in cDNA samples from the organic management-high fertility treatment than in samples from other treatment combinations. There was some evidence for a three-way interaction (management type by fertilizer level by sample extraction type (DNA vs cDNA)); $p = 0.07$, Fig.2.2), and some evidence for an effect of sample extraction type ($p = 0.10$, Fig. 2.2). The comparison of diversity estimated using DNA versus cDNA, within a management type-fertilizer level combination, indicated some evidence for differences between DNA and cDNA in the conventional management-control ($p = 0.07$, Fig. 2.2) and organic management-high fertility ($p = 0.09$, Fig. 2.2). Evenness (Simpson's equitability) responses were similar and there was some evidence for higher bacterial evenness in cDNA samples from organic management-high fertility (three-way interaction: $p = 0.07$, Fig. 2.3) compared to the other treatment combinations. Bacterial richness was higher in cDNA than in DNA samples ($p = 0.0009$, Fig.2.4), across all other treatment combinations. There was evidence for differences in richness for cDNA vs. DNA sampling within the following treatment combinations: conventional management-control ($p = 0.03$, Fig. 1.4), conventional management-high fertility ($p = 0.02$, Fig. 1.4) and organic management-high fertility ($p = 0.004$, Fig. 1.4).

Fungi: diversity responses among the treatments

For the fungal communities, there was evidence that Simpson´s diversity from cDNA samples was higher than from DNA samples, across other treatment combinations ($p = 0.002$, Fig. 2.5). Comparison of Simpson's diversity found strong evidence for differences between DNA and

cDNA estimates within treatment combinations for the conventional management-control combination ($p = 0.04$, Fig. 2.5), and the conventional management-high fertility combination ($p = 0.01$, Fig. 2.5), with some evidence for differences between DNA and cDNA samples in the organic management-high fertility combination ($p = 0.09$, Fig. 2.5).

There was also strong evidence that richness from cDNA samples was higher across all the treatment combinations compared to DNA samples ($p < 0.0001$; Fig. 1.6). Furthermore, there was some evidence ($p = 0.10$, Fig. 2.6) for a two-way interaction (sample type (DNA vs. cDNA) and fertilizer level) on fungal community richness. There was strong evidence for differences between DNA and cDNA samples ($p < 0.0001$; Fig.2.6) within each management type-fertilizer level treatment combination (conventional management-control, conventional management-high fertility, organic management-control, organic management-high fertility). In contrast, there was some evidence that fungal evenness was higher in DNA samples than in cDNA samples ($p < 0.051$, Fig. 2.7).

Dominant bacterial and fungal taxa across the treatment combinations

Bacteria. The phyla Proteobacteria and Acidobacteria were the most frequent (had greatest relative abundance) across all treatment combinations (33.9% and 19.2% respectively), with a large proportion of OTUs (12.1%) remaining unclassified at the phylum level (Table 2.3). Taxa assigned to classes Actinobacteria, Deltaproteobacteria and Alphaproteobacteria were the most frequent across the treatment combinations (15.6%, 12.3% and 11.6%, respectively), again with a large proportion (14.5%) remaining unclassified at the class level (Table 2.4). The most frequent taxa assigned to orders were Actinomycetales and Myxococcales (10.4% and 9.3% respectively),

with 20.2% unclassified to order (Table 2.5). The families Acidobacteria *incertae sedis* and Polyangiaceae were the most frequent across the treatment combinations (7.1% and 4.9% respectively), with 29.6% unclassified (Table 2.6).

The OTUs assigned to the subdivisions *Gp4* (8.1%), *Gp6* (5.6%), *Gp3* (4.7%), and to the genera *Gemmatimonas* (3.9%) and *Marmoricola* (2.8%) were the top five most frequent across the treatment combinations, with 43.7% remaining unclassified to genus (Table 2.7). **Fungi**. Ascomycota and Basidiomycota were the most frequent phyla across the treatment combinations (65.2% and 20.3% respectively), with 4.3% unclassified to phylum (Table 2.8). Sordariomycetes and Dothideomycetes were the most frequent classes across the treatment combinations (42.4% and 30.8% respectively) with 7.4% unclassified at the class level (Table 2.9). The most frequent orders were Pleosporales and Pezizales (32.5% and 21.7% respectively), with 16.2% remaining unclassified (Table 2.10). The families Trichocomaceae and Sporormiaceae were the most frequent across the treatment combinations (35.7% and 22.3% respectively), with 24.0% unclassified to family (Table 2.11). The OTUs assigned to the genera *Aspergillus* (31.5%) and *Westerdykella* (22.1%) were the most frequent, with 43.2% unclassified to genus (Table 2.12).

Fertility management and taxon frequencies

Bacteria. There was evidence that 18 bacterial phyla and 184 bacterial genera responded to the nutrient management treatments in the long-term experimental system in Olathe, Kansas (Tables 2.13, and 2.14 respectively). Management type (organic versus conventional) and fertility level (high versus control) affected the following taxa, in an analysis of both DNA and rRNA samples. Generalized linear model (GLM) analyses using q-value comparisons to control the false

discovery rate (FDR) found evidence that the bacterial phyla Actinobacteria, Firmicutes and Proteobacteria had higher frequency in the organic management-high fertility treatment combination compared to the conventional management-high fertility treatment (DNA and rRNA q-value < 0.05 for the fertility effect and its interaction with management, Table 2.13). The bacterial phyla Chloroflexi and OD1 had higher frequency in the organic management-control compared to the conventional management-control treatment (DNA and rRNA q-value < 0.05 for the fertility effect and its interaction with management, Table 2.13). Comparing high-fertility and control treatments within either organic or conventional management, we found, for instance, evidence that the bacterial phylum Proteobacteria was more frequent in the organic management-high fertility treatment compared to the organic management-control treatment (DNA and cDNA q-value < 0.006 for fertility and management interaction, Table 2.13). In contrast, there was evidence that the phylum Verrucomicrobia was more frequent in the conventional management-high fertility treatment compared to the conventional management-control treatment (DNA and cDNA q-value < 0.006 for fertility and management interaction, Table 2.13). Higher frequency of bacterial genera in the organic management-high fertility treatment compared to the conventional management-high fertility was observed for the OTUs assigned to *Bacillus*, *Ilumatobacter*, *Pseudomonas*, *Streptomyces*, and the subdivisions *Gp16* and *Gp6* (DNA and cDNA q-value < 0.05 for the fertility effect and its interaction with management, Table 2.14). There was evidence that the bacterial genera assigned to the OTUs *Blastococcus*, *Flavobacterium*, *Rhizobium*, and the subdivision *Gp1* were more frequent in the organic management-control treatment compared to the conventional management-control treatment (DNA and cDNA q-value < 0.05 for the fertility effect and its interaction with management, Table 2.14).

Fungi. Five fungal phyla and 71 fungal genera responded to the fertility management treatments in the long-term experiment (Tables 2.15, 2.16, respectively). In generalized linear model (GLM) analyses, the fungal phyla Ascomycota and Basidiomycota had higher frequency in the organic management-high fertility treatment combination compared to the conventional management-high fertility treatment (DNA and cDNA q-value < 0.05 for fertility and management interaction, Table 2.15). The fungal phylum Glomeromycota had a higher frequency in the organic management-control compared to the conventional management-control treatment (DNA and cDNA q-value < 0.05 for the fertility effect and its interaction with management, Table 2.15). The OTUs assigned to the fungal genera *Cyrenella*, *Nowakowskiella*, *Pilidiella*, *Septoria*, *Spizellomyces* and *Westerdykella* were among the most frequent in the organic management-high fertility compared to conventional management-high fertility treatment (DNA and cDNA q-value < 0.05 for the fertility effect and its interaction with management, Table 2.16). The OTUs assigned to the fungal genera *Ascobolus*, *Glomus* and *Polyschema* had a higher frequency in the organic management-control treatment compared to the conventional management-control treatment (DNA and cDNA q-value < 0.05 for the fertility effect and its interaction with management, Table 2.16).

Sampling DNA compared to rRNA

Comparing DNA and rRNA samples (processed as cDNA) revealed some general differences in the total microbial community (DNA) compared to the active microbial community (cDNA; Tables 2.17, 2.18, 2.19, 2.20). GLM analyses using q-value comparisons found that several taxa were recovered at different frequencies in DNA and cDNA, whereas others were *only*

recovered from either DNA or cDNA samples. There were broad differences at the level of bacterial phyla, where, for example, Firmicutes was approximately twice as common in DNA samples as in rRNA samples, while the phylum Verrucomicrobia was more frequent in cDNA samples (DNA and cDNA q-value < 0.05, Table 2.17). Fungal phyla also showed broad differences, where the strongly dominant Ascomycota became somewhat less common in rRNA samples, while other phyla increased in frequency (DNA and cDNA q-value < 0.05, Table 2.18). The phylum Chytridiomycota was more frequent in DNA samples, while the phyla Basidiomycota and Glomeromycota were more frequent in rRNA samples (DNA and cDNA q-value < 0.03, Table 2.18). A number of bacterial genera also differed, for example the OTUs assigned to *Gemmatimonas*, *Sorangium*, *Solirubrobacter* and the subdivision *Gp3* were most frequent in rRNA samples while the OTUs assigned to the genera *Bacillus*, *Pseudomonas*, *Nitrospira* and subdivision *Gp6* were most frequent in DNA samples (DNA and cDNA q-value < 0.01, Table 2.19). The OTUs assigned to the genus *Glomus* were three times more frequent in cDNA samples than in DNA samples, whereas the OTUs assigned to the genera *Aspergillus* and *Septoria* were at least twice as frequent in DNA samples as in cDNA samples (DNA and cDNA q-value < 0.02, Table 2.20).

Some bacterial and fungal taxa were only recovered from either the DNA or cDNA samples. The bacterial phylum Deinoccocus-Thermus was only recovered from the DNA samples (DNA and cDNA q-value < 0.01, Table 2.17). Ten bacterial genera were only present in the DNA samples, whereas twenty were present in cDNA samples (representing 8.5% and 17%, respectively, of the total bacterial genera responding to the DNA and cDNA frequencies) (Table 2.19). Many bacterial genera including *Rhizobium*, *Nocardia*, *Gemmata* and *Tetrasphaera* were only recovered from DNA samples, whereas *Geodermatophilus*, *Pseudospirillum*, *Catellatospora*

and *Rhizomicrobium* were only recovered from cDNA samples (DNA and cDNA q-value < 0.01, Table 2.19). Thirteen fungal genera were only recovered from DNA samples (including *Powellomyces*, *Blastocladiella*, *Preussia* and *Spizellomyces*), whereas seventeen were only recovered from cDNA samples (including *Thanatephorus*, *Paraglomus*, *Piriformospora* and *Waitea*; DNA and cDNA q-value < 0.02, Table 2.20).

Discussion

How do bacterial and fungal diversity compare between organic and conventional management?

We analyzed the effects of organic and conventional fertility regimens on soil bacterial and fungal diversity estimates in a tomato/pac choi cropping system. One hypothesis we evaluated was that organic management would result in higher microbial diversity than conventional management. The observations for bacterial diversity support this hypothesis, whereas those for fungal diversity do not. Bacterial diversity and evenness were higher in organic than in conventional high fertility plots (Figs. 2.2, and 2.3). The organic matter added to the high fertility organic plots, including rich substrates like fish hydrolysate, chicken litter and compost, may increase bacterial diversity. Compost contains a wealth of organic substrates, in addition to its intrinsic microbial community, so its addition to soil may change microbial communities (Perez-Piqueres *et al.*, 2006). Other studies have concluded that organic farming practices, which rely on the addition of soil organic material, promoted higher microbial gene expression, and higher abundance and diversity in the soil microbial community (Mäder *et al.*, 2002; Shannon *et al.*, 2002;

Oehl *et al.*, 2004; Hartmann *et al.*, 2015). Franciolli *et al*. (2016) studied the effects of mineral (NPK) and farmyard manure on the structure and activity of bacterial microbial communities, concluding that farmyard manure produced higher bacterial diversity. Farrell *et al*. (2010) reported that low-quality composts increased bacterial and fungal diversity and activity compared with inorganic and control treatments in the topsoil of a metal-contaminated soil. Our results are consistent with these studies in finding that organic amendments increased soil microbial diversity compared to conventional amendments. In our case, however, organic amendments only increased bacterial diversity, not fungal diversity.

Soil pH influences the diversity of bacterial communities (Ramirez *et al.*, 2010; Rousk *et al.*, 2010; Gomez-Montano *et al.*, 2013) and may drive the abundance of particular bacterial groups and the overall bacterial community composition across land-use types or across continental scales (Fierer and Jackson, 2006; Lauber *et al.*, 2008; Jenkins *et al.*, 2009; Jones *et al.*, 2009b; Rousk *et al.*, 2010). In our study site, soil pH in the organic field plots (mean pH = 7.0 across the three fertility levels) was higher than in the conventional plots (annual mean pH = 6.5 across the three fertility levels) (Table 2.21, Altamimi (2016)) - an observation consistent with other studies comparing organic and conventional systems (Drinkwater *et al.*, 1995; Clark *et al.*, 1998). Thus, the higher pH in the organic plots compared to the conventional plots may have favored higher bacterial diversity and evenness (Figs.2.2 and 2.3). Soil pH can impact the physiological constraints on soil bacteria by altering competitive outcomes or by inhibiting taxa experiencing conditions outside their preferred range. Many bacteria have intracellular pH levels close to neutral (Madigan and Martinko, 2006), and some bacteria can tolerate pH extremes better than others. Furthermore, soil pH often correlates with many other soil characteristics such as carbon availability, soil moisture, and salinity (Brady and Weil, 2007). All these factors may concurrently

contribute to driving the observed differences in bacterial community composition between plots with organic and convention fertilization.

Are bacterial and fungal soil communities detected through cDNA more or less diverse than those detected through DNA?

Our initial hypothesis was that microbial communities detected through cDNA would be less diverse than those detected through DNA, because cDNA should represent only active microorganisms at the time of sampling. In contrast, DNA should detect even dormant, dead or degraded cells, in addition to the active microbial population at the time of sampling. Contrary to our hypothesis, bacterial and fungal richness were higher in the cDNA samples compared to DNA samples (Figs. 2.4 and 2.6, respectively). Although counter to the expectation that the expressed community at a given time represents a fraction of the diversity of the total potential community (Lanzén *et al.*, 2011; Myrold *et al.*, 2014), other studies have similarly found that rRNA richness exceeds DNA richness for both bacteria (Mikkonen *et al.*, 2014) and fungi (Baldrian *et al.*, 2011). Mikkonen *et al.* (2014), similarly, observed that soil bacterial community evenness and richness were higher in cDNA than DNA in the surface communities of a field. They concluded that this apparent increase in the cDNA potentially indicated that the surface soil microbial community was already recovering from the adverse effects of contamination in polluted soil (Mikkonen *et al.*, 2014). They hypothesized that the greater cDNA evenness and richness could indicate a diversifying community relative to DNA.

In our experiment, cDNA samples exhibited higher bacterial and fungal richness (Figs. 2.4 and 2.6, respectively), perhaps because DNA samples can be dominated by taxa with very

abundant and resilient dormant structures. Another factor may be that the greater richness of the cDNA communities is observed because cDNA samples have greater relative abundance of rare taxa, where these rare taxa are not detected in samples from DNA. Rare bacterial taxa may be disproportionately active compared to common taxa (Jones and Lennon, 2010) for a number of reasons (Garbeva *et al.*, 2011; Helliwell *et al.*, 2013; Mee *et al.*, 2014; Hausmann *et al.*, 2016).

What are the most abundant bacterial and fungal taxa recovered in plots with organic and conventional fertility management?

One of our objectives was to identify the most abundant bacterial and fungal taxa under organic and conventional farming practices, averaged across fertility rate and sample type (DNA vs rRNA (cDNA)). Many bacterial and fungal taxa responded to the nutrient management treatments (Table 2.13, 2.14, 2.15 and 2.16), and to understand these responses we applied the copiotroph-oligotroph concept. This concept is an ecological classification for bacteria proposed by Winogradsky (1924), adopted by many researchers (Andrews, 1984; Andrews and Harris, 1986; Meyer, 1994; Padmanabhan *et al.*, 2003; Fierer *et al.*, 2007a). Fierer *et al.* (2007a) applied the copiotroph-oligotroph concept (similar to *r*- and *K*-strategies, respectively) to make predictions about the ecological attributes of bacterial taxa. We consider the same concept for fungi, although there are fewer studies of soil fungal communities for reference. Copiotrophic microorganisms (ecological opportunists) are more typical in soils with high nutrient availability and can exhibit high growth rates (*r*-strategies). Oligotrophic microorganisms (ecological competitors) are more typical in soils with low nutrient availability and slow growth rates (*K*-strategies). It has been proposed that the growth of copiotrophic microorganisms is stimulated by organic fertilizers (Li

et al., 2012a; Hartmann *et al.*, 2015) and that organic matter inputs increase their richness (van Diepeningen *et al.*, 2006; Hartmann *et al.*, 2015) due to high availability of organic carbon and nitrogen (van Bruggen and Semenov, 2000; Zelenev *et al.*, 2006). Greater abundance of copiotrophic microbes under organic fertilization has been observed during the short term, while in the long-run, under stable conditions, the ratio of oligotrophic to copiotrophic bacteria may be greater in organic than in conventional farming systems (Zelenev *et al.*, 2006).

In our study, the observed frequencies of bacterial phyla were consistent with results from other soil profiles (Janssen, 2006): Proteobacteria, Acidobacteria and Actinobacteria were the most frequent (Table 2.3). There were some notable differences in the frequencies for Verrucomicrobia, Planctomycetes, Gemmatimonadetes and Firmicutes. Proteobacteria, representing the most frequent phylum (34%) (Table 2.3), were more frequent under organic management compared to conventional (Table 2.13). This result was consistent with other studies where Proteobacteria tended to dominate in organic farming systems compared to conventional systems (Upchurch *et al.*, 2008; Li *et al.*, 2012a; Shange *et al.*, 2012). Proteobacteria exhibit extreme morphological, physiological and metabolic diversity, participate in global C, N and S cycling, and represent the majority of known gram-negative bacteria of medical, industrial and agricultural significance (Kersters *et al.*, 2006; Madigan and Martinko, 2006). Within this phylum, the OTUs assigned to the bacterial genera *Sorangium* and *Pseudomonas* were among the top twenty most frequent OTUs in our field plots (Table 2.7). *Sorangium*, whose frequency from rRNA (cDNA) in high-fertility conventional management was higher than in the organic treatment combinations (Table 2.14), includes saprophytic and anti-microbial members that derive nutrition from cellulose aerobically (Reichenbach and Höfle, 1993). Some *Sorangium* species exhibit slow growth and a very low apparent reproduction rate, partly due to a large investment in lipids and secondary signaling

compounds (Bolten and Muller, 2009). The OTUs assigned to the bacterial genus *Pseudomonas,* recovered from both DNA and cDNA samples in high fertility organic management were observed at almost twice the frequency as in conventional treatments (Table 2.14), an observation consistent with other studies where high frequency of *Pseudomonas* has been documented in organic farming systems (Upchurch *et al.*, 2008; Senechkin *et al.*, 2010; Chaudhry *et al.*, 2012; Li *et al.*, 2012a). Some members of these copiotrophic bacterial genera include well known plant pathogens (Madigan and Martinko, 2006), others are considered plant-growth-promoting bacteria (PGPB) and also can fix nitrogen (Park *et al.*, 2005). The higher frequency of *Pseudomonas* might help in maintaining the total N levels in organic farming soils without fertilizer supplementation (Li *et al.*, 2012a). Additionally, *Pseudomonas* species have been proposed as a primary bio-indicator of the ecological status of soil, due to their rapid responses to changes in the edaphic characteristics of the soil, their high population sizes, and their relatively easy detection using classical and modern molecular approaches (Misko and Germida, 2002; Pesaro and Widmer, 2006).

The next most abundant phyla in this study were the Acidobacteria (19.2%) and Actinobacteria (15.3%) (Table 2.3). Acidobacteria were slightly more frequent under conventional management compared to organic, while the frequency of Actinobacteria was far greater for organic than for conventional management (Table 2.13). These results corroborate previous studies where Acidobacteria tend to be more frequent under conventional farming systems (Upchurch *et al.*, 2008; Shange *et al.*, 2012). Additionally, the lower pH in our conventional field plots (Table 2.21) is consistent with the hypothesis that Acidobacteria generally prefer soil environments of low resource availability (Fierer *et al.*, 2007a) and higher acidity (Jones *et al.*, 2009b), but some acidobacterial taxa may exhibit the opposite pattern (Rousk *et al.*, 2010). Acidobacteria are widely distributed and abundant in soils but their ecological roles are poorly understood because most

members have been difficult to culture (Eichorst *et al.*, 2007; Jones *et al.*, 2009a). OTUs assigned to the uncultured bacterial subdivisions *Gp3, Gp4* and *Gp6* have been classified within the phylum Acidobacteria (Araujo *et al.*, 2012) and in our study were the three most abundant taxa (Table 2.7). The bacterial subdivisions *Gp3* and *Gp4* were more abundant in the conventional systems whereas the subdivision *Gp6* was more abundant in the organic systems (Table 2.14).

Actinobacteria, whose members are typically rod-shaped to filamentous and common inhabitants of the soil and of plant materials (Madigan and Martinko, 2006), were significantly more abundant in the organic management than in the conventional management, regardless of the nucleic acid analyzed (Table 2.13). This result was in contrast to the high abundance of Actinobacteria reported in conventionally managed croplands (Upchurch *et al.*, 2008; Li *et al.*, 2012a; Shange *et al.*, 2012). Organically farmed soils have been reported to be rich in recalcitrant carbon sources (Fließbach *et al.*, 2007) such that Actinobacteria might be expected to be more common in organically managed soils than in conventionally farmed soils. Additionally, members of this phylum play an important role in organic matter turnover and carbon cycling. They can decompose some recalcitrant carbon sources including cellulose and chitin (Jenkins *et al.*, 2009). The OTUs assigned to the genera *Streptomyces* (which includes plant pathogens and antagonists of plant pathogens) and *Conexibacter* (which includes members that may play a role in the soil nitrification process (Monciardini *et al.*, 2003; Seki *et al.*, 2012) were relatively frequent in the soils we sampled (Table 2.7). Both bacterial genera were found more frequently under organic fertility management (Table 2.14).

Among fungi, Ascomycota represent the most abundant recovered phylum (65.2%) across all treatment combinations in the soils at this study site (Table 2.8). Ascomycota were more abundant in the conventional management compared to the organic management treatment (Table

2.15) and in DNA samples compared to cDNA (Table 2.18). The largest fungal phylum, Ascomycota are distributed worldwide and represented in all land ecosystems (Kirk *et al.*, 2008). The phylum includes saprophytes and plant pathogens causing many foliar, root and canker diseases (Agrios, 2005). The top five most abundant OTUs belonged to Ascomycota and were assigned to the genera *Aspergillus* (filamentous fungi including plant pathogens, with some species producing carcinogenic aflatoxins in crops worldwide; Bennett (2010), Haqiwara *et al.* (2016)), and *Westerdykella* (species occurring worldwide on a variety of substrates including soil, mud, dung and plant material; Ebead *et al.* (2012); Table 2.12). The high frequency of *Westerdykella* in the organic field plots (Table 2.16) suggests copiotrophic traits and some *Westerdykella* species have been associated with a saprobic role in the decomposition of plant organic material in a wide variety of environmental substrates (Kruys *et al.*, 2006; Ebead *et al.*, 2012). The higher frequency of *Westerdykella* might be because the organic fertilizer introduced it to the soil and/or because the organic compounds serve as a substrate for these taxa.

Does the cDNA pool reveal bacterial and fungal taxa that are not present in the DNA pool, and vice-versa?

We hypothesized that some taxa - particularly those without dormant structures - may be detected in the cDNA-based communities and not in the DNA-based communities. Thus, some of the differences in communities recovered from DNA and cDNA may be due to the ability of some taxa to produce tough dormant (or dead) structures that can persist and be accessed through DNA sampling while they are not found in cDNA sampling or vice versa. The OTUs assigned to the genus *Aspergillus* (Ascomycota, Eurotiales, Trichocomaceae) were at least three times more

frequent in DNA samples from organic and conventional management for both fertility levels compared to cDNA samples (Tables 2.16 and 2.20). Members of this genus produce small and thick-walled spores that can persist and be accessed through DNA sampling while they are less abundant in cDNA sampling. Similarly, *Septoria* (Ascomycota, Capnodiales, Mycosphaerellaceae) is another example, where the OTUs assigned to the genus *Septoria* were at least twice as frequent in DNA samples compared to cDNA samples (Tables 2.16 and 2.20). The frequency of *Septoria* in the DNA from high fertility organic management was three times higher than the frequency in the control fertility organic management. (Altamimi, 2016) studied the same field plots sampled in our experiments, and observed in 2008 (two years before our experiment started) that *Septoria lycopersici* caused extensive defoliation of the tomato plants. Some *Septoria* species produce pycnidia, asexual fruiting bodies that can be an important mechanism of spread for foliar diseases (like leaf spots and blights) and can persist for a long period of time in the soil or plant debris (Agrios, 2005), allowing easier recovery by DNA-based molecular techniques.

Translation of microbial community data for farm management

The analysis of soils in this experiment recovered a number of bacterial and fungal taxa known to have important roles in agroecological systems (Tables 2.13 – 2.16). For example, bacterial taxa included *Nitrospira* (nitrite-oxidizing bacteria), *Streptomyces* (characterized by complex secondary metabolism), and *Pseudomonas* (sometimes plant pathogens or beneficial to plants). Examples of important fungal taxa included *Aspergillus* (common molds and pathogens), *Ascobolus* (coprophilous), and *Cladosporium* (some plant pathogens, others parasites of fungi). There is strong interest among many organic farmers in using microbial community data to guide

management decisions on farms. Interpretation of this broad community is challenging, however, because of limited information about the functional roles of taxa, and the potential for related species, or even the same species, to have very different roles. While some taxa have very consistent functional roles, other groups, such as *Pseudomonas* spp. and *Cladosporium* spp., include both important pathogens and important natural biocontrol agents. Another caveat for interpreting results is the uncertainty in categorizing taxonomic groups based on sequence databases, such that there may be errors in classification, particularly for lower taxonomic levels such as genera and species. A great deal of translation of fundamental scientific results to applied technologies will be necessary to make the use of microbial profiles a reality for day-to-day decision making on farms. Nonetheless, huge steps in microbial ecology are being made possible by new sequencing techniques to monitor soil microbial taxa at higher throughput and resolution than previously possible. This offers the potential to evaluate the success of agricultural soil management at the level of individual taxa and, potentially, taxa with individual functions.

Conclusions

The responses of microbial diversity, richness and evenness to long-term organic and conventional management were complex, and highlight current challenges to understanding soil microbial communities. We found higher bacterial diversity under the high fertility organic management using cDNA, whereas the highest fungal diversity was observed in both conventional and organic managements under control and high fertility treatments using cDNA compared to DNA. Bacterial diversity increases with organic amendments were likely due to the nutritional resources provided in the organic treatments (such as fish hydrolysate and chicken litter compost).

Further research to characterize microbes in the organic amendments, along with functional characterization of soil microbiomes produced by these treatments, would provide additional insights into how soil organic amendments may stabilize or destabilize agroecosystem processes.

Using both DNA and rRNA (cDNA) comparisons to explore the response of fungal and bacterial communities in crops under organic versus conventional management, we found higher bacterial and fungal diversity and richness in the cDNA samples compared to DNA samples. We speculate that the differences in communities recovered from DNA and rRNA (cDNA) may be due in part to the ability of some taxa to produce tough dormant (or dead) structures that can persist and be accessed through DNA sampling. Future work will be required to clarify the links between total (DNA) and active (rRNA/cDNA) community diversity, including functional gene diversity and ecological functions. These results illustrate how microbial communities respond to agricultural management, as a first step toward the development of strategies to manage microbial communities as a regular component of sustainable agricultural planning.

Tables and Figures

Table 2.1. Maximum daily temperature (°C) and precipitation (cm) reported on Olathe, Kansas, during the summer of 2010 during the tomato cropping season (from Altamimi (2016)).

Summer time defined by months	Max temperature (°C)	Precipitation (inches)
May	31	2.6
June	33	8.5
July	34	5.6
August	39	0.0
September	32	6.7

Table 2.2. Timing of field activities in the plots sampled in this study (Altamimi (2016)) at the K-State Horticulture Research and Extension Center in Olathe, Kansas.

Activities	Tomato season 2010					
Seed sown	March 30					
Pre-plant application	May 21					
Seedlings fertilized	April 13	April 22	May 9			
Seedling planted	May 25					
Soluble fertilizer added	June 7	June 15	June 22	June 30	July 6	July 13

Table 2.3. Overall most frequent taxa assigned to bacterial phyla recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. Percentage of sequences grouped in each of the top 20 OTUs assigned to bacterial phyla and p-values associated with fertilizer type (ftype: organic vs. conventional), fertilizer level (flevel: control vs. high), interaction between fertilizer type and fertilizer level (ftype*flevel), DNA/cDNA type (dtype), interaction between fertilizer type and DNA/cDNA type (ftype*dtype), interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer type-fertilizer level and DNA/cDNA type (ftype*flevel*dtype).

Phylum	Frequency (%)	p-value						
		ftype	flevel	ftype*flevel	dtype	ftype*dtype	flevel*dtype	ftype*flevel *dtype
Proteobacteria	33.907	0.953	0.436	0.953	0.000	0.000	0.000	0.000
Acidobacteria	19.187	0.676	0.669	0.717	0.001	0.000	0.000	0.000
Actinobacteria	15.303	0.891	0.557	0.943	0.000	0.000	0.000	0.000
unclassified	12.118	0.815	0.584	0.866	0.069	0.000	0.000	0.000
Chloroflexi	5.709	0.708	0.306	0.630	0.000	0.000	0.000	0.000
Bacteroidetes	3.116	0.513	0.178	0.773	0.210	0.000	0.000	0.000
Gemmatimonadetes	3.088	0.196	0.338	0.594	0.005	0.000	0.000	0.000
Firmicutes	1.966	0.368	0.157	0.845	0.000	0.000	0.000	0.000
Verrucomicrobia	1.947	0.257	0.158	0.995	0.000	0.000	0.000	0.003
Nitrospira	1.783	0.631	0.253	0.881	0.000	0.000	0.000	0.000
Armatimonadetes	0.832	0.786	0.264	0.549	0.153	0.000	0.000	0.003
Planctomycetes	0.501	0.060	0.137	0.871	0.413	0.000	0.000	0.022
OD1	0.260	0.248	0.527	0.087	0.037	0.001	0.000	0.044
Deinococcus-Thermus	0.094	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TM7	0.073	0.975	0.277	0.812	0.512	0.072	0.005	0.086
OP11	0.040	0.127	0.265	0.646	0.762	0.090	0.051	0.634
WS3	0.034	0.613	0.265	0.692	0.682	0.044	0.059	0.112
Spirochaetes	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chlorobi	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BRC1	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2.4. Overall most frequent taxa assigned to bacterial class recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. Percentage of sequences grouped in each of the top 20 OTUs assigned to bacterial class and p-values associated with fertilizer type (ftype: organic vs. conventional), fertilizer level (flevel: control vs. high), interaction between fertilizer type and fertilizer level (ftype*flevel). DNA/cDNA type (dtype), interaction between fertilizer type and DNA/cDNA type (ftype*dtype), interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer type-fertilizer level and DNA/cDNA type (ftype*flevel*dtype).

Class	Frequency (%)	p-value						
		ftype	flevel	ftype*flevel	dtype	ftype*dtype	flevel*dtype	ftype*flevel *dtype
Actinobacteria	15.602	0.891	0.557	0.943	0.000	0.000	0.000	0.000
unclassified	14.532	0.874	0.560	0.860	0.012	0.000	0.000	0.000
Deltaproteobacteria	12.279	0.830	0.478	0.871	0.000	0.000	0.000	0.000
Alphaproteobacteria	11.627	0.596	0.277	0.959	0.000	0.000	0.000	0.000
Acidobacteria_Gp4	6.485	0.199	0.422	0.386	0.775	0.000	0.000	0.000
Betaproteobacteria	5.518	0.860	0.162	0.980	0.000	0.000	0.000	0.000
Acidobacteria_Gp6	4.446	0.455	0.255	0.825	0.692	0.000	0.000	0.000
Anaerolineae	4.350	0.813	0.318	0.610	0.000	0.000	0.000	0.000
Acidobacteria_Gp3	3.799	0.215	0.312	0.368	0.000	0.000	0.000	0.000
Gemmatimonadetes	3.148	0.196	0.338	0.594	0.005	0.000	0.000	0.000
Gammaproteobacteria	2.535	0.619	0.139	0.878	0.564	0.000	0.000	0.000
Sphingobacteria	2.095	0.342	0.198	0.881	0.207	0.000	0.000	0.000
Bacilli	1.929	0.348	0.145	0.887	0.000	0.000	0.000	0.000
Nitrospira	1.817	0.631	0.253	0.881	0.000	0.000	0.000	0.000
Acidobacteria_Gp16	1.320	0.566	0.180	0.476	0.344	0.000	0.000	0.001
Subdivision3	1.300	0.250	0.176	0.869	0.000	0.000	0.000	0.007
Acidobacteria_Gp1	0.898	0.128	0.319	0.285	0.016	0.000	0.000	0.002
Acidobacteria_Gp5	0.716	0.297	0.423	0.704	0.000	0.000	0.000	0.212
Chloroflexi	0.621	0.253	0.280	0.397	0.705	0.000	0.000	0.014
Acidobacteria_Gp17	0.535	0.273	0.398	0.550	0.794	0.000	0.000	0.005

Table 2.5. Overall most frequent taxa assigned to bacterial order recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. Percentage of sequences grouped in each of the top 20 OTUs assigned to bacterial class and p-values associated with fertilizer type (ftype: organic vs. conventional), fertilizer level (flevel: control vs. high), interaction between fertilizer type and fertilizer level (ftype*flevel). DNA/cDNA type (dtype), interaction between fertilizer type and DNA/cDNA type (ftype*dtype), interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer type-fertilizer level and DNA/cDNA type (ftype*flevel*dtype).

Order	Frequency (%)	p-value						
		ftype	flevel	ftype*flevel	dtype	ftype*dtype	flevel*dtype	ftype*flevel *dtype
unclassified	20.196	0.930	0.605	0.827	0.000	0.000	0.000	0.000
Actinomycetales	10.361	0.632	0.253	0.869	0.000	0.000	0.000	0.000
Myxococcales	9.317	0.417	0.183	0.538	0.000	0.000	0.000	0.000
Acidobacteria_Gp4_ order_incertae_sedis	6.739	0.199	0.422	0.386	0.775	0.000	0.000	0.000
Rhizobiales	5.761	0.468	0.255	0.822	0.000	0.000	0.000	0.000
Acidobacteria_Gp6_ order_incertae_sedis	4.619	0.455	0.255	0.825	0.692	0.000	0.000	0.000
Anaerolineales	4.520	0.813	0.318	0.610	0.000	0.000	0.000	0.000
Acidobacteria_Gp3_ order_incertae_sedis	3.934	0.213	0.316	0.374	0.000	0.000	0.000	0.000
Sphingomonadales	3.632	0.673	0.158	0.453	0.000	0.000	0.000	0.000
Solirubrobacterales	3.434	0.521	0.299	0.676	0.000	0.000	0.000	0.000
Burkholderiales	3.299	0.854	0.168	0.851	0.000	0.000	0.000	0.000
Gemmatimonadales	3.271	0.196	0.338	0.594	0.005	0.000	0.000	0.000
Sphingobacteriales	2.177	0.342	0.198	0.881	0.207	0.000	0.000	0.000
Bacillales	2.004	0.348	0.145	0.887	0.000	0.000	0.000	0.000
Nitrospirales	1.888	0.631	0.253	0.881	0.000	0.000	0.000	0.000
Acidobacteria_Gp16_ order_incertae_sedis	1.371	0.566	0.180	0.476	0.344	0.000	0.000	0.001
Subdivision3_order_ incertae_sedis	1.351	0.250	0.176	0.869	0.000	0.000	0.000	0.007
Xanthomonadales	1.182	0.880	0.133	0.954	0.726	0.000	0.000	0.001
Acidimicrobiales	1.097	0.170	0.046	0.687	0.264	0.000	0.000	0.000
Acidobacteria_Gp1_ order_incertae_sedis	0.933	0.128	0.319	0.285	0.016	0.000	0.000	0.002

Table 2.6. Overall most frequent taxa assigned to bacterial family recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. Percentage of sequences grouped in each of the top 20 OTUs assigned to bacterial class and p-values associated with fertilizer type (ftype: organic vs. conventional), fertilizer level (flevel: control vs. high), interaction between fertilizer type and fertilizer level (ftype*flevel). DNA/cDNA type (dtype), interaction between fertilizer type and DNA/cDNA type (ftype*dtype), interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer type-fertilizer level and DNA/cDNA type (ftype*flevel*dtype).

Family	Frequency (%)	p-value						
		ftype	flevel	ftype*flevel	dtype	ftype*dtype	flevel*dtype	ftype*flevel *dtype
unclassified	29.606	0.777	0.531	0.909	0.002	0.000	0.000	0.000
Acidobacteria_Gp4_ family_incertae_sedis	7.084	0.199	0.422	0.386	0.775	0.000	0.000	0.000
Polyangiaceae	4.993	0.463	0.207	0.488	0.000	0.000	0.000	0.000
Acidobacteria_Gp6_ family_incertae_sedis	4.856	0.455	0.255	0.825	0.692	0.000	0.000	0.000
Anaerolineaceae	4.751	0.813	0.318	0.610	0.000	0.000	0.000	0.000
Acidobacteria_Gp3_ family_incertae_sedis	4.135	0.213	0.316	0.374	0.000	0.000	0.000	0.000
Sphingomonadaceae	3.732	0.624	0.153	0.440	0.000	0.000	0.000	0.000
Gemmatimonadaceae	3.438	0.196	0.338	0.594	0.005	0.000	0.000	0.000
Nocardioidaceae	3.078	0.363	0.092	0.876	0.000	0.000	0.000	0.000
Propionibacteriaceae	2.360	0.729	0.174	0.914	0.002	0.000	0.000	0.000
Nitrospiraceae	1.985	0.631	0.253	0.881	0.000	0.000	0.000	0.000
Chitinophagaceae	1.845	0.242	0.159	0.869	0.403	0.000	0.000	0.000
Bacillaceae_1	1.591	0.416	0.231	0.957	0.000	0.000	0.000	0.000
Streptomycetaceae	1.524	0.369	0.810	0.945	0.000	0.000	0.000	0.005
Acidobacteria_Gp16_ family_incertae_sedis	1.441	0.566	0.180	0.476	0.344	0.000	0.000	0.001
Subdivision3_family_ incertae_sedis	1.420	0.250	0.176	0.869	0.000	0.000	0.000	0.007
Acidobacteria_Gp1_ family_incertae_sedis	0.981	0.128	0.319	0.285	0.016	0.000	0.000	0.002
Comamonadaceae	0.974	0.630	0.163	0.803	0.000	0.000	0.000	0.001
Burkholderiales_incertae _sedis	0.964	0.487	0.379	0.885	0.000	0.000	0.000	0.014
Solirubrobacteraceae	0.879	0.502	0.475	0.733	0.000	0.000	0.000	0.006

Table 2.7. Overall most frequent taxa assigned to bacterial genera recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. Percentage of sequences grouped in each of the top 20 OTUs assigned to bacterial class and p-values associated with fertilizer type (ftype: organic vs. conventional), fertilizer level (flevel: control vs. high), interaction between fertilizer type and fertilizer level (ftype*flevel). DNA/cDNA type (dtype), interaction between fertilizer type and DNA/cDNA type (ftype*dtype), interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer type-fertilizer level and DNA/cDNA type (ftype*flevel*dtype).

Genus	**Frequency (%)**	**p-value**						
		ftype	**flevel**	**ftype*flevel**	**dtype**	**ftype*dtype**	**flevel*dtype**	**ftype*flevel *dtype**
unclassified	43.761	0.783	0.490	0.901	0.173	0.000	0.000	0.000
Gp4	8.120	0.199	0.422	0.386	0.775	0.000	0.000	0.000
Gp6	5.566	0.455	0.255	0.825	0.692	0.000	0.000	0.000
Gp3	4.740	0.213	0.316	0.374	0.000	0.000	0.000	0.000
Gemmatimonas	3.942	0.196	0.338	0.594	0.005	0.000	0.000	0.000
Marmoricola	2.879	0.219	0.101	0.978	0.000	0.000	0.000	0.001
Nitrospira	2.276	0.631	0.253	0.881	0.000	0.000	0.000	0.000
Bacillus	1.803	0.408	0.233	0.968	0.000	0.000	0.000	0.000
Gp16	1.652	0.566	0.180	0.476	0.344	0.000	0.000	0.001
3_genus_incertae _sedis	1.628	0.250	0.176	0.869	0.000	0.000	0.000	0.007
Streptomyces	1.617	0.362	0.955	1.000	0.000	0.000	0.000	0.011
Gp1	1.124	0.128	0.319	0.285	0.016	0.000	0.000	0.002
Solirubrobacter	1.008	0.502	0.475	0.733	0.000	0.000	0.000	0.006
Gp5	0.896	0.297	0.423	0.704	0.000	0.000	0.000	0.212
Steroidobacter	0.868	0.696	0.148	0.903	0.250	0.000	0.000	0.001
Conexibacter	0.791	0.261	0.497	0.731	0.000	0.000	0.000	0.148
Sorangium	0.786	0.588	0.293	0.674	0.001	0.000	0.000	0.003
Pseudomonas	0.763	0.231	0.231	0.374	0.095	0.011	0.000	0.001
Byssovorax	0.709	0.169	0.098	0.969	0.000	0.003	0.000	0.023
Gp17	0.670	0.273	0.398	0.550	0.794	0.000	0.000	0.005

Table 2.8. Overall most frequent taxa assigned to fungal phyla recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. Percentage of sequences grouped in each of the top 20 OTUs assigned to bacterial class and p-values associated with fertilizer type (ftype: organic vs. conventional), fertilizer level (flevel: control vs. high), interaction between fertilizer type and fertilizer level (ftype*flevel). DNA/cDNA type (dtype), interaction between fertilizer type and DNA/cDNA type (ftype*dtype), interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer type-fertilizer level and DNA/cDNA type (ftype*flevel*dtype).

Phylum	**Frequency (%)**	**p-value**						
		ftype	**flevel**	**ftype*flevel**	**dtype**	**ftype*dtype**	**flevel*dtype**	**ftype*flevel *dtype**
Ascomycota	65.191	0.773	0.651	0.570	0.002	0.000	0.131	0.001
Basidiomycota	20.313	0.540	0.683	0.265	0.000	0.000	0.000	0.024
unclassified	4.268	0.637	0.789	0.491	0.000	0.001	0.005	0.000
Chytridiomycota	3.892	0.745	0.525	0.919	0.000	0.000	0.001	0.000
Glomeromycota	2.975	0.293	0.627	0.991	0.000	0.007	0.002	0.012
Blastocladiomycota	1.951	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fungi_incertae _sedis	1.426	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2.9. Overall most frequent taxa assigned to fungal class recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. Percentage of sequences grouped in each of the top 20 OTUs assigned to bacterial class and p-values associated with fertilizer type (ftype: organic vs. conventional), fertilizer level (flevel: control vs. high), interaction between fertilizer type and fertilizer level (ftype*flevel). DNA/cDNA type (dtype), interaction between fertilizer type and DNA/cDNA type (ftype*dtype), interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer type-fertilizer level and DNA/cDNA type (ftype*flevel*dtype).

Class	Frequency (%)	p-value						
		ftype	flevel	ftype*flevel	dtype	ftype*dtype	flevel*dtype	ftype*flevel *dtype
Sordariomycetes	42.426	0.862	0.996	0.370	0.002	0.001	0.158	0.001
Dothideomycetes	30.817	0.664	0.348	0.386	0.000	0.000	0.267	0.000
unclassified	7.382	0.805	0.992	0.805	0.000	0.001	0.000	0.000
Eurotiomycetes	5.894	0.469	0.881	0.580	0.000	0.001	0.308	0.000
Pezizomycetes	4.353	0.751	0.709	0.159	0.000	0.000	0.001	0.037
Agaricomycetes	3.138	0.646	0.867	0.783	0.000	0.442	0.006	0.000
Chytridiomycetes	3.049	0.734	0.650	0.995	0.000	0.000	0.001	0.000
Leotiomycetes	2.213	0.750	0.010	0.052	0.169	0.226	0.007	0.007
Glomeromycetes	0.194	0.293	0.627	0.991	0.000	0.007	0.002	0.012
Tremellomycetes	0.163	0.784	0.021	0.012	0.023	0.003	0.348	0.007
Ascomycota_ incertae_sedis	0.117	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Saccharomycetes	0.36	0.621	0.279	0.864	0.648	0.645	0.461	0.368
Microbotryomycetes	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ustilaginomycetes	0.016	0.607	0.479	0.382	0.006	0.542	0.242	0.328
Blastocladiomycetes	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cystobasidiomycetes	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fungi_incertae_sedis	0,011	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Classiculomycetes	0,001	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2.10. Overall most frequent taxa assigned to fungal order recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. Percentage of sequences grouped in each of the top 20 OTUs assigned to bacterial class and p-values associated with fertilizer type (ftype: organic vs. conventional), fertilizer level (flevel: control vs. high), interaction between fertilizer type and fertilizer level (ftype*flevel). DNA/cDNA type (dtype), interaction between fertilizer type and DNA/cDNA type (ftype*dtype), interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer type-fertilizer level and DNA/cDNA type (ftype*flevel*dtype).

Order	Frequency (%)	p-value						
		ftype	flevel	ftype*flevel	dtype	ftype*dtype	flevel*dtype	ftype*flevel *dtype
Pleosporales	32.461	0.843	0.986	0.362	0.002	0.001	0.127	0.001
Pezizales	21.723	0.957	0.790	0.885	0.000	0.011	0.000	0.000
unclassified	16.252	0.751	0.709	0.159	0.000	0.000	0.001	0.037
Eurotiales	12.847	0.486	0.807	0.541	0.000	0.001	0.183	0.000
Capnodiales	11.41	0.555	0.739	0.348	0.001	0.000	0.002	0.000
Sordariales	1.546	0.615	0.119	0.949	0.002	0.005	0.004	0.945
Hypocreales	1.284	0.895	0.343	0.156	0.137	0.622	0.151	0.137
Spizellomycetales	0.577	0.547	0.711	0.780	0.001	0.000	0.011	0.000
Sebacinales	0.464	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Diaporthales	0.394	0.450	0.717	0.322	0.247	0.415	0.740	0.350
Glomerales	0.282	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tremellales	0.243	0.560	0.256	0.454	0.179	0.025	0.054	0.122
Ascomycota_ incertae_sedis	0.212	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cystofilobasidiales	0.191	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cantharellales	0.183	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Helotiales	0.162	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chaetothyriales	0.146	0.509	0.374	0.933	0.001	0.839	0.635	0.440
Rhizophydiales	0.125	0.438	0.504	0.523	0.010	0.022	0.032	0.013
Chaetosphaeriales	0.119	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Saccharomycetes_ incertae_sedis	0.12	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2.11. Overall most frequent taxa assigned to fungal family recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. Percentage of sequences grouped in each of the top 20 OTUs assigned to bacterial class and p-values associated with fertilizer type (ftype: organic vs. conventional), fertilizer level (flevel: control vs. high), interaction between fertilizer type and fertilizer level (ftype*flevel). DNA/cDNA type (dtype), interaction between fertilizer type and DNA/cDNA type (ftype*dtype), interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer type-fertilizer level and DNA/cDNA type (ftype*flevel*dtype).

Family	Frequency (%)	p-value						
		ftype	flevel	ftype*flevel	dtype	ftype*dtype	flevel*dtype	ftype*flevel *dtype
Trichocomaceae	35.692	0.832	0.973	0.359	0.002	0.001	0.141	0.001
unclassified	24.024	0.486	0.807	0.541	0.000	0.001	0.183	0.000
Sporormiaceae	22.323	0.572	0.615	0.720	0.000	0.014	0.000	0.000
Ascobolaceae	11.837	0.509	0.523	0.007	0.000	0.467	0.004	0.957
Pyronemataceae	1.938	0.943	0.841	0.374	0.000	0.010	0.002	0.028
Mycosphaerellaceae	1.422	0.413	0.253	0.260	0.010	0.411	0.257	0.000
Davidiellaceae	0.565	0.749	0.844	0.438	0.071	0.008	0.070	0.003
Lasiosphaeriaceae	0.437	0.415	0.088	0.847	0.001	0.007	0.010	0.860
Sebacinaceae	0.365	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Melanconidaceae	0.262	0.450	0.717	0.322	0.247	0.415	0.740	0.350
Glomeraceae	0.181	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Spizellomycetaceae	0.155	0.531	0.666	0.523	0.001	0.001	0.023	0.000
Tremellaceae	0.134	0.551	0.246	0.455	0.156	0.031	0.068	0.106
Ascomycota_ incertae_sedis	0.123	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Hypocreaceae	0.118	0.861	0.311	0.455	0.000	0.625	0.163	0.869
Cystofilobasidiaceae	0.120	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ceratobasidiaceae	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Herpotrichiellaceae	0.076	0.509	0.374	0.933	0.001	0.839	0.635	0.440
Helotiales_incertae_sedis	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bionectriaceae	0.062	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2.12. Overall most frequent taxa assigned to fungal genera recovered in sequencing of soils from the Experimental Research Station at Olathe, Kansas. Percentage of sequences grouped in each of the top 20 OTUs assigned to bacterial class and p-values associated with fertilizer type (ftype: organic vs. conventional), fertilizer level (flevel: control vs. high), interaction between fertilizer type and fertilizer level (ftype*flevel). DNA/cDNA type (dtype), interaction between fertilizer type and DNA/cDNA type (ftype*dtype), interaction between fertilizer level and DNA/cDNA type (flevel*dtype), and the interaction between fertilizer type-fertilizer level and DNA/cDNA type (ftype*flevel*dtype).

Genus	Frequency (%)	p-value						
		ftype	flevel	ftype*flevel	dtype	ftype*dtype	flevel*dtype	ftype*flevel *dtype
unclassified	43.215	0.816	0.969	0.358	0.001	0.001	0.163	0.001
Aspergillus	31.464	0.468	0.829	0.466	0.000	0.001	0.107	0.000
Westerdykella	22.067	0.554	0.658	0.702	0.000	0.017	0.000	0.000
Ascobolus	5.195	0.510	0.487	0.006	0.000	0.400	0.005	0.867
Septoria	1.332	0.413	0.253	0.260	0.010	0.411	0.257	0.000
Cladosporium	0.188	0.749	0.844	0.438	0.071	0.008	0.070	0.003
Geopyxis	0.119	0.685	0.995	0.635	0.001	0.109	0.000	0.000
Pilidiella	0.064	0.450	0.717	0.322	0.247	0.415	0.740	0.350
Piriformospora	0.058	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Glomus	0.057	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Polyschema	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Guehomyces	0.037	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tetracladium	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Hydropisphaera	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Saccharomycetes _incertae_sedis	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Waitea	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Paraglomus	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Emericellopsis	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Melanopsammella	0.01	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Catenomyces	0.01	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2.13. Bacterial phyla that responded to the fertility treatment (high (H) vs. control (C)) or exhibited a significant management type (M= organic vs. conventional) interaction with fertility treatment (F*M), with calculated q-values.

	Mean frequency (proportion)								q-value			
Phylum	Organic management				Conventional management							
	DNA		cDNA		DNA		cDNA		DNA		cDNA	
	H	C	H	C	H	C	H	C	F	F*M	F	F*M
Acidobacteria	0.17	0.16	0.14	0.17	0.16	0.17	0.15	0.15	0.00	0.01	0.00	0.00
Actinobacteria	0.14	0.15	0.17	0.16	0.15	0.14	0.15	0.16	0.00	0.01	0.01	0.00
Armatimonadetes	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
Bacteroidetes	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.00	0.01	0.01	0.00
Chlorobi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.00
Chloroflexi	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.01	0.00	0.00
Deinococcus-Thermus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00
Firmicutes	0.03	0.02	0.02	0.01	0.03	0.02	0.02	0.02	0.00	0.01	0.01	0.00
Gemmatimonadetes	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.00	0.01	0.01	0.00
Nitrospira	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.00
OD1	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.00
OP11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.05	0.02
Planctomycetes	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.07	0.00
Proteobacteria	0.36	0.33	0.32	0.32	0.35	0.32	0.32	0.31	0.00	0.01	0.01	0.00
Spirochaetes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00
TM7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00
unclassified	0.15	0.18	0.18	0.17	0.15	0.17	0.18	0.19	0.00	0.01	0.00	0.00
Verrucomicrobia	0.02	0.02	0.03	0.02	0.03	0.02	0.04	0.03	0.00	0.01	0.01	0.00
WS3	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.14. Bacterial genera that responded to the fertility treatment (high (H) vs. control (C)) or exhibited a significant management type (M= organic vs. conventional) interaction with fertility treatment (F*M), with calculated q-values.

Genus	Mean frequency (proportion)								q-value			
	Organic management				Conventional management							
	DNA		cDNA		DNA		cDNA		DNA		cDNA	
	H	C	H	C	H	C	H	C	F	F*M	F	F*M
3_genus_incertae_sedis	0.01	0.01	0.02	0.01	0.01	0.01	0.03	0.02	0.00	0.00	0.00	0.00
Aciditerrimonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Actinaurispora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
Actinoplanes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.01
Adhaeribacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aeromicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Agromyces	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.99	0.00
Alterococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.08	0.00
Aminobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
Amycolatopsis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.21	0.00
Anaeromyxobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Angustibacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Arenimonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Armatimonadetes_gp4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.64	0.02
Armatimonadetes_gp5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Armatimonas_Armatimonadetes_gp1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Arthrobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00
Azohydromonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
Azotobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus	0.02	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.00	0.00	0.00	0.01
Bauldia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bellilinea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00
Blastochloris	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blastococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00
Bradyrhizobium	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brevibacillus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Burkholderia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.99	0.00
Byssovorax	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00

Caenimonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
Caldilinea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Catellatospora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00
Caulobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Chelatococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Chitinophaga	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chondromyces	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00
Chthonomonas_ Armatimonadetes_gp3	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Clostridium_XI	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.05
Conexibacter	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00
Coxiella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.99	0.00
Craurococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Cryptosporangium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00
Cupriavidus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cystobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dactylosporangium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Derxia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.99	0.00
Desulfovirga	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.01
Dokdonella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Dongia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.84	0.00
Duganella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ensifer	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Euzebya	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.01	0.00	0.00
Ferruginibacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
Flavisolibacter	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
Flavitalea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.77	0.00
Flavobacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Friedmanniella	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00
Gemmata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gemmatimonas	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.00	0.00	0.00	0.00
Geobacillus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Geodermatophilus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gp1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
Gp10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gp11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gp15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Gp16	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.00	0.00	0.00	0.00
Gp17	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
Gp18	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.13	0.00	0.00	0.00

Gp2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gp22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gp25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00
Gp3	0.03	0.04	0.04	0.05	0.04	0.04	0.04	0.05	0.00	0.00	0.00	0.00
Gp4	0.03	0.02	0.02	0.02	0.04	0.02	0.02	0.02	0.00	0.00	0.00	0.00
Gp5	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
Gp6	0.04	0.03	0.03	0.04	0.03	0.04	0.03	0.03	0.00	0.00	0.00	0.00
Gp7	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Haliangium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Haliscomenobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Haloferula	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.00
Hamadaea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.99	0.00
Heliothrix	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Herbaspirillum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Hyalangium	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hydrogenophaga	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Hyphomicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00
Iamia	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ignavibacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Ilumatobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Isosphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.08	0.04
Janthinobacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kibdelosporangium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.00	0.00	0.00
Kineococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Kitasatospora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Kofleria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00
Kribbella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lechevalieria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.01
Lentzea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leptolinea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.99	0.00
Longilinea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Luedemannella	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Luteimonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
Lysobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Marmoricola	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
Massilia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Meiothermus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mesorhizobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methylibium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Methylocapsa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Methylocystis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Methylovirgula	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.20	0.00
Microbacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microlunatus	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00
Micromonospora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microvirga	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Mycobacterium	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Nannocystis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Niastella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00
Nitrosospira	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitrospira	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
Nocardia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.04
Nocardioides	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Nonomuraea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.99	0.00
Novosphingobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OD1_genus_incertae_sedis	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00
Ohtaekwangia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OP11_genus_incertae_sedis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.01	0.21	0.00
Opitutus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ottowia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.99	0.05
Paenibacillus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Paracraurococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Pasteuria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
Patulibacter	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pedobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.99	0.00
Pedomicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00
Pelomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.69	0.00
Phaselicystis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00
Phenylobacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phycicoccus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00
Phyllobacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.39	0.00
Plesiocystis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Polaromonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
Porphyrobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudaminobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.83	0.00
Pseudolabrys	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.99	0.00
Pseudomonas	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Pseudonocardia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudospirillum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.01	0.00	0.00
Pyxidicoccus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.01

Rhizobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rhizomicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rhodopila	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.01
Rhodoplanes	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Roseomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rubellimicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.08	0.00
Rubrivivax	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.99	0.00
Rubrobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00
Saccharothrix	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00
Salmonella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.45	0.00
Schlegelella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.01
Segetibacter	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Singulisphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Skermanella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
Solimonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
Solirubrobacter	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00
Solitalea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00
Sorangium	0.02	0.02	0.02	0.02	0.01	0.01	0.03	0.02	0.00	0.00	0.00	0.00
Spartobacteria_genera_ incertae_sedis	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.05
Sphingomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sphingopyxis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sphingosinicella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00
Spirilliplanes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.08	0.00
Sporichthya	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Steroidobacter	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Streptacidiphilus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.99	0.00
Streptomyces	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
Terrimonas	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Tetrasphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00
Thermoleophilum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Thermosporothrix	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
TM7_genus_incertae_sedis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Turicibacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.08	0.00
Turneriella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Umboniibacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
unclassified	0.51	0.53	0.52	0.50	0.50	0.52	0.52	0.51	0.00	0.00	0.00	0.00
WS3_genus_incertae_sedis	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zavarzinella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.08	0.00

Table 2.15. Fungal phyla that responded to fertility treatment (high (H) vs. control (C)) or exhibited a significant management type (M= organic vs. conventional) interaction with fertility treatment (F*M), with calculated q-values.

Phyla	Mean frequency (proportion)								q-value			
	Organic management				Conventional management							
	DNA		cDNA		DNA		cDNA		DNA		cDNA	
	H	C	H	C	H	C	H	C	F	F*M	F	F*M
Ascomycota	0.37	0.31	0.23	0.21	0.34	0.34	0.22	0.21	0.13	0.00	0.04	0.00
Basidiomycota	0.14	0.03	0.05	0.04	0.02	0.04	0.04	0.03	0.00	0.00	0.15	0.00
Blastocladiomycota	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.27	0.00	0.00
Chytridiomycota	0.06	0.05	0.03	0.04	0.04	0.03	0.03	0.03	0.04	0.00	0.04	0.00
Glomeromycota	0.01	0.01	0.02	0.02	0.00	0.00	0.02	0.02	0.04	0.00	0.04	0.00
unclassified	0.16	0.19	0.17	0.18	0.18	0.18	0.19	0.11	0.04	0.00	0.04	0.00

Table 2.16. Fungal genera that responded to fertility treatment (high (H) vs. control (C)) or exhibited a significant management type (M= organic vs. conventional) interaction with fertility treatment (F*M), with calculated q-values.

Genus	Mean frequency (proportion)								q-value			
	Organic management				Conventional management							
	DNA		cDNA		DNA		cDNA		DNA		cDNA	
	H	C	H	C	H	C	H	C	F	F*M	F	F*M
Acaulospora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Amplistroma	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.10
Aniptodera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Arachnomyces	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.38
Ascobolus	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00
Ascodesmis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.10	0.00	0.00
Aspergillus	0.03	0.03	0.01	0.00	0.03	0.04	0.01	0.01	0.00	0.02	0.00	0.00
Asterotremella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.01
Bionectria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Blastocladiella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.08	0.10
Boothiomyces	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.20
Camarops	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Catenomyces	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.44	0.00	0.00
Cercophora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.01	0.00
Chaetomella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.08	0.00
Chaetomidium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.01
Chytridiales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Cladosporium	0.00	0.01	0.00	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Classicula	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.01	0.00
Clavaria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.10
Corynascus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.08	0.10
Cryptococcus	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Cyphellophora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.01	0.00
Cyrenella	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.00	0.00
Dipodascopsis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Dissoconium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Doratomyces	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Emericellopsis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
Eupenicillium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.01	0.00
Geopyxis	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.02	0.00	0.38
Glomus	0.00	0.01	0.01	0.02	0.00	0.00	0.01	0.01	0.00	0.02	0.00	0.00
Guehomyces	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
Hydropisphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.00	0.00
Hypocrea	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.40
Iodophanus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.01	0.00

Kriegeria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Lobulomyces	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Lycoperdon	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00
Marcelleina	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.01	0.10
Massariosphaeria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.11	0.00	0.00
Melanopsammella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Microdiplodia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.01	0.00
Nowakowskiella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.00	0.00
Paraglomus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.00	0.00
Peziza	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Phaeodothis	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.06
Phallus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.01	0.00
Phialophora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.01	0.10
Pilidiella	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Piriformospora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.00	0.00
Polyschema	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
Powellomyces	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.01
Preussia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.10
Pyrenomyxa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Rhizophlyctis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.10
Rhizophydium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.66	0.00	0.00
Rhopalomyces	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.01	0.01
Saccharomyces	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.08	0.01
Saccobolus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Scolecobasidiella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.00	0.00
Sebacina	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.08	0.08
Septoria	0.02	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00
Spizellomyces	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.01	0.10
Tetracladium	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.03	0.05	0.00
Thanatephorus	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00	0.00
Thelebolus	0.01	0.00	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.02	0.00	0.00
Triparticalcar	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.00
unclassified	0.79	0.79	0.87	0.88	0.82	0.81	0.88	0.89	0.00	0.02	0.00	0.00
uncult_Saccharo-mycetes_incertae sedis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.36	0.08	0.00
Waitea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.44	0.00	0.00
Westerdykella	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.03	0.00	0.00

Table 2.17. Frequency of bacterial phyla recovered from DNA and cDNA samples from soils at Olathe, Kansas, with associated q-values.

	Mean frequency (proportion)		
Phylum	**DNA**	**cDNA**	**q-value**
Proteobacteria	**0.338**	0.319	**0.006**
unclassified	0.163	**0.178**	**0.000**
Actinobacteria	0.143	**0.159**	**0.006**
Acidobacteria	**0.165**	0.152	**0.000**
Bacteroidetes	**0.043**	0.042	**0.006**
Gemmatimonadetes	0.038	**0.040**	**0.006**
Chloroflexi	0.031	0.031	**0.000**
Verrucomicrobia	0.023	**0.030**	**0.006**
Firmicutes	**0.025**	0.017	**0.006**
Armatimonadetes	0.009	**0.010**	**0.000**
Nitrospira	**0.008**	0.005	**0.006**
OD1	0.005	0.005	0.006
Planctomycetes	0.004	0.004	0.068
WS3	0.003	0.003	**0.000**
TM7	0.000	**0.001**	0.006
Chlorobi	0.001	0.001	0.006
Deinococcus-Thermus	**0.001**	0.000	0.006

Table 2.18. Frequency of fungal phyla recovered from DNA and cDNA samples from soils at Olathe, Kansas, with associated q-values.

	Mean frequency (proportion)		
Phylum	**DNA**	**cDNA**	**q-value**
unclassified	0.278	**0.189**	**0.000**
Ascomycota	**0.428**	0.376	**0.000**
Basidiomycota	0.133	**0.251**	**0.000**
Chytridiomycota	**0.125**	0.096	**0.030**
Glomeromycota	0.029	**0.082**	**0.001**
Blastocladiomycota	**0.008**	0.005	**0.030**

Table 2.19. Frequency of bacterial genera recovered from DNA and cDNA samples from soils at Olathe, Kansas, with associated q-values.

	Mean frequency (proportion)		
Genus	**DNA**	**cDNA**	**q-value**
unclassified	**0.515**	0.511	**0.000**
Gp3	0.038	**0.044**	**0.000**
Gemmatimonas	0.038	**0.040**	**0.000**
Gp6	**0.038**	0.031	**0.000**
3_genus_incertae sedis	0.014	**0.021**	**0.000**
Sorangium	0.017	**0.021**	**0.000**
Solirubrobacter	0.012	**0.017**	**0.000**
Gp4	**0.027**	0.016	**0.000**
Gp16	**0.017**	0.014	**0.000**
Gp1	0.011	**0.013**	**0.000**
Streptomyces	**0.013**	0.012	**0.000**
Bacillus	**0.019**	0.010	**0.000**
Gp5	**0.009**	0.008	**0.000**
Byssovorax	0.005	**0.008**	**0.000**
Microlunatus	0.006	**0.007**	**0.000**
Conexibacter	0.004	**0.006**	**0.000**
Pseudomonas	**0.010**	0.006	**0.000**
Terrimonas	0.006	0.006	**0.000**
Rhodoplanes	**0.007**	0.005	**0.000**
Nitrospira	**0.008**	0.005	**0.000**
Marmoricola	0.005	0.005	**0.000**
Gp18	**0.007**	0.005	**0.000**

Mycobacterium	0.003	**0.005**	**0.000**
OD1_genus_incertae sedis	0.005	0.005	**0.000**
Nannocystis	0.002	**0.004**	**0.000**
Nocardioides	0.003	**0.004**	**0.000**
Spartobacteria_genera_incertae sedis	**0.006**	0.004	**0.000**
Iamia	0.003	**0.004**	**0.000**
Chthonomonas_Armatimonadetes_gp3	0.004	0.004	**0.000**
Hyalangium	**0.005**	0.004	**0.000**
Patulibacter	**0.004**	0.003	**0.000**
Roseomonas	0.001	**0.003**	**0.000**
WS3_genus_incertae sedis	0.003	0.003	**0.000**
Segetibacter	0.003	0.003	**0.000**
Haliangium	**0.004**	0.003	**0.000**
Friedmanniella	0.003	0.003	**0.000**
Kribbella	0.002	**0.003**	**0.000**
Steroidobacter	0.003	0.003	**0.000**
Caldilinea	0.002	**0.003**	**0.000**
Bradyrhizobium	0.003	0.003	**0.000**
Armatimonadetes_gp4	0.003	0.003	**0.000**
Gp7	**0.004**	0.003	**0.000**
Lysobacter	0.002	**0.003**	**0.000**
Microvirga	**0.004**	0.003	**0.000**
Phenylobacterium	0.002	**0.003**	**0.000**
Gp22	0.001	**0.002**	**0.000**
Aciditerrimonas	0.002	0.002	**0.000**
Ohtaekwangia	0.002	0.002	**0.000**
Singulisphaera	0.002	0.002	**0.000**

Blastococcus	0.002	0.002	**0.000**
Pedomicrobium	0.002	0.002	**0.000**
Caenimonas	**0.003**	0.002	**0.000**
Ilumatobacter	**0.003**	0.002	**0.000**
Opitutus	0.002	0.002	**0.000**
Brevibacillus	0.002	0.002	**0.000**
Dongia	0.002	0.002	**0.000**
Armatimonadetes_gp5	0.001	**0.002**	**0.000**
Geodermatophilus	0.000	**0.002**	**0.000**
Luedemannella	**0.003**	0.002	**0.000**
Flavobacterium	0.002	0.002	**0.000**
Caulobacter	0.001	**0.002**	**0.000**
Massilia	0.001	**0.002**	**0.000**
Aeromicrobium	0.001	**0.002**	**0.000**
Gp11	0.001	0.001	**0.000**
Pseudospirillum	0.000	**0.001**	**0.000**
Gp10	0.001	0.001	**0.000**
Herbaspirillum	0.000	**0.001**	**0.000**
Nitrosospira	0.001	0.001	**0.000**
Rubrobacter	0.001	0.001	**0.000**
Niastella	0.001	0.001	**0.000**
Anaeromyxobacter	0.001	0.001	**0.000**
Skermanella	0.001	0.001	**0.000**
Bellilinea	0.001	0.001	**0.000**
Dactylosporangium	0.000	**0.001**	**0.000**
Kitasatospora	0.000	**0.001**	**0.000**
Thermosporothrix	**0.002**	0.001	**0.000**

Cupriavidus	**0.002**	0.001	**0.000**
Paenibacillus	0.001	0.001	**0.000**
Thermoleophilum	**0.002**	0.001	**0.000**
Catellatospora	0.000	**0.001**	**0.000**
Ferruginibacter	0.001	0.001	**0.000**
Blastochloris	0.001	0.001	**0.000**
Rhizomicrobium	0.000	**0.001**	**0.000**
Novosphingobium	0.000	**0.001**	**0.000**
TM7_genus_incertae sedis	0.000	**0.001**	**0.000**
Armatimonas_Armatimonadetes_gp1	0.000	**0.001**	**0.000**
Angustibacter	0.000	**0.001**	**0.000**
Porphyrobacter	**0.002**	0.001	**0.000**
Duganella	0.001	0.001	**0.000**
Arthrobacter	0.000	**0.001**	**0.000**
Gp25	0.001	0.001	**0.000**
Azotobacter	0.001	0.001	**0.000**
Chondromyces	0.000	**0.001**	**0.002**
Chelatococcus	0.000	**0.001**	**0.002**
Hyphomicrobium	0.001	0.001	**0.000**
Solitalea	0.000	**0.001**	**0.000**
Methylocystis	0.001	0.001	**0.000**
Phaselicystis	0.001	0.001	**0.000**
Ignavibacterium	0.001	0.001	**0.000**
Gp15	0.000	**0.001**	**0.000**
Kibdelosporangium	0.000	**0.001**	**0.000**
Plesiocystis	0.001	0.001	**0.000**
Lentzea	0.001	0.001	**0.000**

Derxia	0.001	0.001	**0.005**
Longilinea	0.000	**0.001**	**0.000**
Methylocapsa	0.000	**0.001**	**0.000**
Lechevalieria	0.000	**0.001**	**0.000**
Sphingomonas	0.000	**0.001**	**0.000**
Rhizobium	**0.001**	0.000	**0.000**
Meiothermus	**0.001**	0.000	**0.000**
Ensifer	**0.001**	0.000	**0.000**
Adhaeribacter	**0.001**	0.000	**0.008**
Nocardia	**0.001**	0.000	**0.000**
Gemmata	**0.001**	0.000	**0.000**
Cystobacter	**0.001**	0.000	**0.000**
Tetrasphaera	**0.001**	0.000	**0.000**
Haloferula	**0.001**	0.000	**0.000**
Pyxidicoccus	**0.001**	0.000	**0.000**

Table 2.20. Frequency of fungal genera recovered from DNA and cDNA samples from soils at Olathe, Kansas, with associated q-values.

	Mean frequency (proportion)		
Genus	**DNA**	**cDNA**	**q-value**
unclassified	0.301	**0.379**	**0.000**
Glomus	0.094	**0.292**	**0.014**
Aspergillus	**0.231**	0.108	**0.000**
Septoria	**0.121**	0.055	**0.000**
Triparticalcar	0.103	**0.115**	**0.000**
Thelebolus	**0.008**	0.003	**0.000**
Tetracladium	0.006	0.006	**0.000**
Geopyxis	**0.011**	0.005	**0.000**
Cladosporium	**0.009**	0.005	**0.000**
Westerdykella	**0.007**	0.004	**0.000**
Ascobolus	**0.005**	0.004	**0.000**
Thanatephorus	0.000	**0.003**	**0.000**
Paraglomus	0.000	**0.003**	**0.000**
Cryptococcus	**0.008**	0.002	**0.000**
Piriformospora	0.001	**0.002**	**0.000**
Hydropisphaera	0.001	**0.002**	**0.000**
Polyschema	**0.003**	0.002	**0.000**

Massariosphaeria	0.000	**0.002**	**0.000**
Waitea	0.000	**0.002**	**0.000**
Catenomyces	**0.003**	0.002	**0.000**
Pilidiella	**0.007**	0.001	**0.000**
Rhizophydium	0.000	**0.001**	**0.000**
Hypocrea	**0.010**	0.001	**0.000**
Pyrenomyxa	0.000	**0.001**	**0.000**
Emericellopsis	**0.003**	0.001	**0.000**
Cyrenella	**0.002**	0.001	**0.000**
Camarops	0.000	**0.001**	**0.000**
Guehomyces	**0.003**	0.001	**0.006**
Acaulospora	0.000	**0.001**	**0.000**
Cyphellophora	0.000	**0.001**	**0.000**
Melanopsammella	0.000	**0.001**	**0.000**
Phaeodothis	**0.002**	0.001	**0.000**
Scolecobasidiella	0.001	0.001	0.001
Lobulomyces	0.000	**0.001**	**0.000**
Kriegeria	0.000	**0.001**	**0.000**
Chaetomidium	0.000	**0.001**	**0.000**
Eupenicillium	0.000	**0.001**	**0.000**
Microdiplodia	0.000	**0.001**	**0.000**
Chaetomella	0.000	**0.001**	**0.000**

Peziza	0.000	**0.001**	**0.000**
Lycoperdon	**0.001**	0.000	**0.000**
uncultured_Saccharomycetes incertae sedis	**0.001**	0.000	**0.000**
Nowakowskiella	**0.001**	0.000	**0.000**
Powellomyces	**0.004**	0.000	**0.000**
Boothiomyces	**0.001**	0.000	**0.000**
Blastocladiella	**0.002**	0.000	**0.000**
Asterotremella	**0.001**	0.000	**0.000**
Clavaria	**0.001**	0.000	**0.000**
Marcelleina	**0.001**	0.000	**0.000**
Phialophora	**0.001**	0.000	**0.000**
Preussia	**0.002**	0.000	**0.000**
Rhizophlyctis	**0.001**	0.000	**0.000**
Spizellomyces	**0.002**	0.000	**0.000**

Table 2.21. Soil test conducted for the tomato crop in 2010 by Altamimi (2016) in the organic (ORG) and conventional (CONV) field plots located at the State Horticulture Research and Extension Center in Olathe, KS. Mean values are reported for each fertility type (Control (C), low-fertility (L), and high-fertility (H)). Edaphic properties included pH, phosphorous (P), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), organic matter (OM), zinc (Zn), iron (Fe), cupper (Cu), manganese (Mn), and cation exchance capacity (CEC).

Mtype	Ftype	pH	P	Ca	K	Mg	Na	O.M	Zn	Fe	Cu	Mn	CEC
ORG	**Control**	7.0	80.7	3173.3	314.7	286.7	20.3	2.5	4.0	36.2	1.1	34.0	18.9
ORG	**Low**	7.0	75.3	3140.0	301.3	260.0	19.3	2.6	3.2	35.7	1.2	34.3	18.7
ORG	**High**	7.1	80.3	3343.3	315.3	283.3	20.0	2.7	3.5	37.5	1.2	35.4	19.7
Conv	**Control**	6.4	40.0	2606.7	194.0	186.7	23.0	2.2	2.0	50.1	1.3	49.5	17.7
Conv	**Low**	6.5	38.7	2540.0	202.0	190.0	23.7	2.3	2.6	47.9	1.3	46.1	18.9
Conv	**High**	6.6	37.7	2520.0	189.7	190.0	23.0	2.3	2.0	48.2	1.3	48.6	18.9

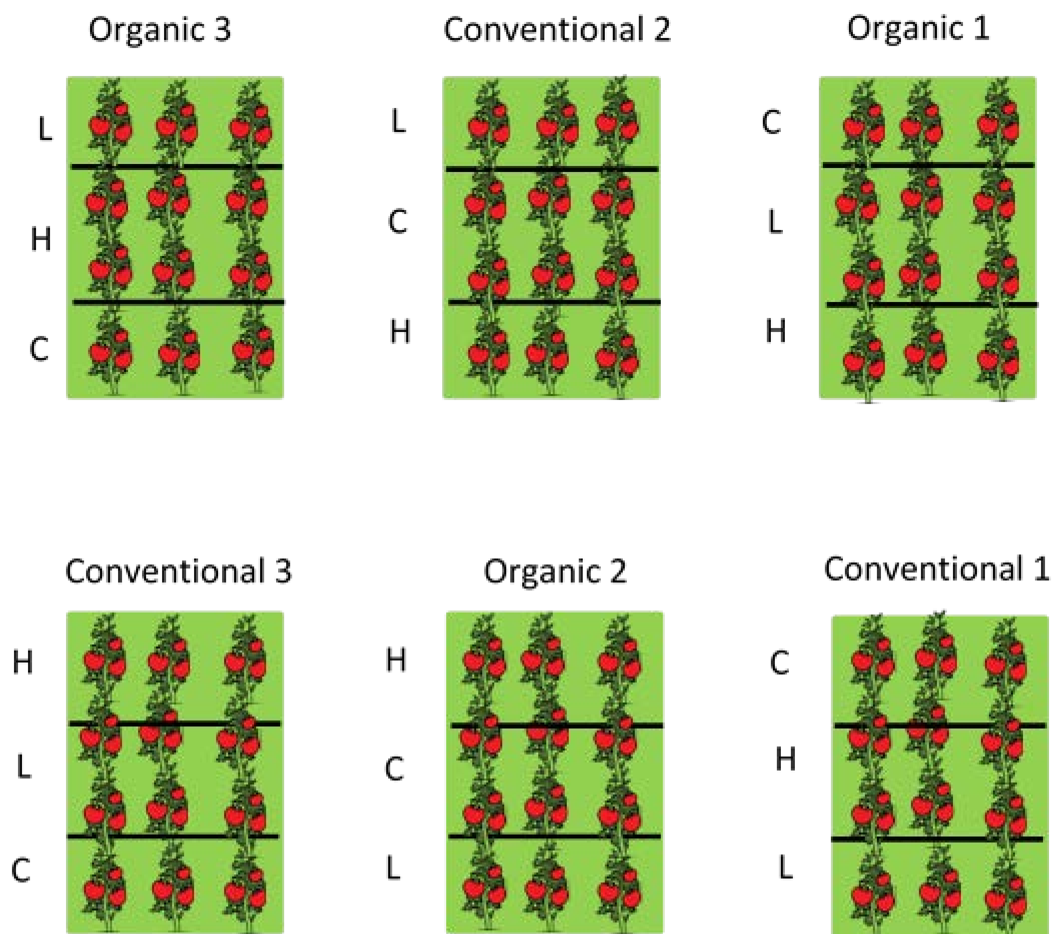

Figure 2.1. Open field plots established in 2002 to compare crops grown under organic and conventional nutrient management at the K-State Horticulture Research and Extension Center in Olathe, Kansas, USA. The experiment was arranged in a split split plot design where three organic and three conventional field plots were applied at whole plot level; three levels of fertilizers (C= control, L= low-fertility, H=high-fertility) applied at the subplot level (corresponding to the first split), and two methods of extraction (DNA or RNA) was applied to each subplot (corresponding to the second split).

Note: The pictures of the tomato plants do not correspond to the real number of tomatoes planted per subplot.

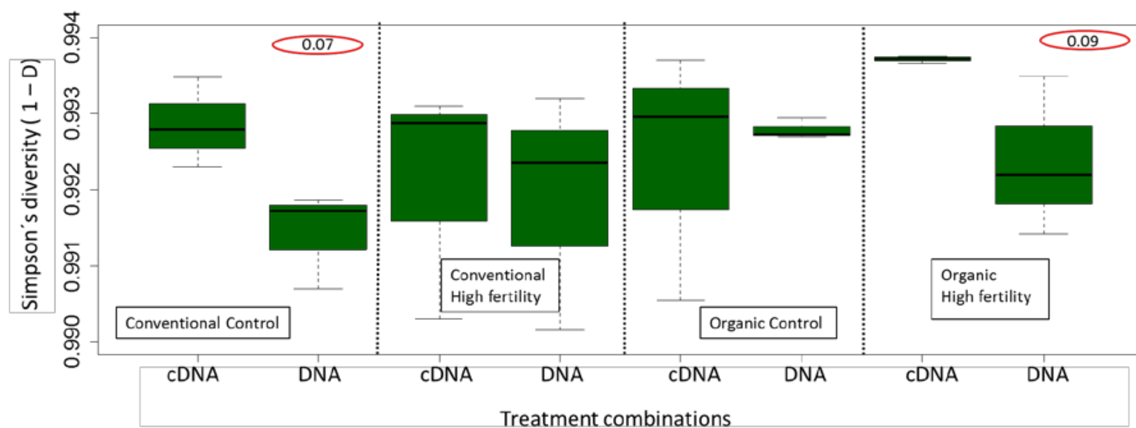

Figure 2.2. Simpson´s diversity for the bacterial rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations. The black dashed-line separates the results for soil fertility treatment combinations: conventional management control (with no added fertilizer), conventional high fertility, organic control (with no added fertilizer), and organic high fertility. In an AOV there was evidence for a three-way interaction (p = 0.07). In analyses of diversity evaluated within management type-fertilizer level combinations, there was some evidence for differences between DNA and rRNA samples in the conventional-control (p = 0.07) and organic-high fertility (p = 0.09) treatment combinations.

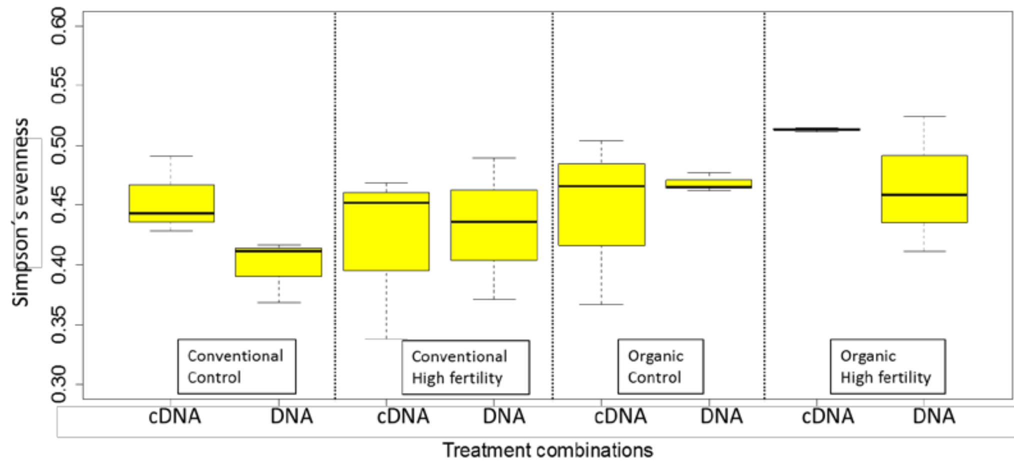

Figure 2.3. Simpson´s evenness for the bacterial rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations. The black dashed-line separates the results for soil fertility treatment combinations: conventional management control (with no added fertilizer), conventional high fertility, organic control (with no added fertilizer), and organic high fertility. In an AOV there was a higher bacterial evenness in cDNA samples from organic management-high fertility compared to the other treatment combinations (three-way interaction: management type by fertilizer level by sample extraction type, $p = 0.07$).

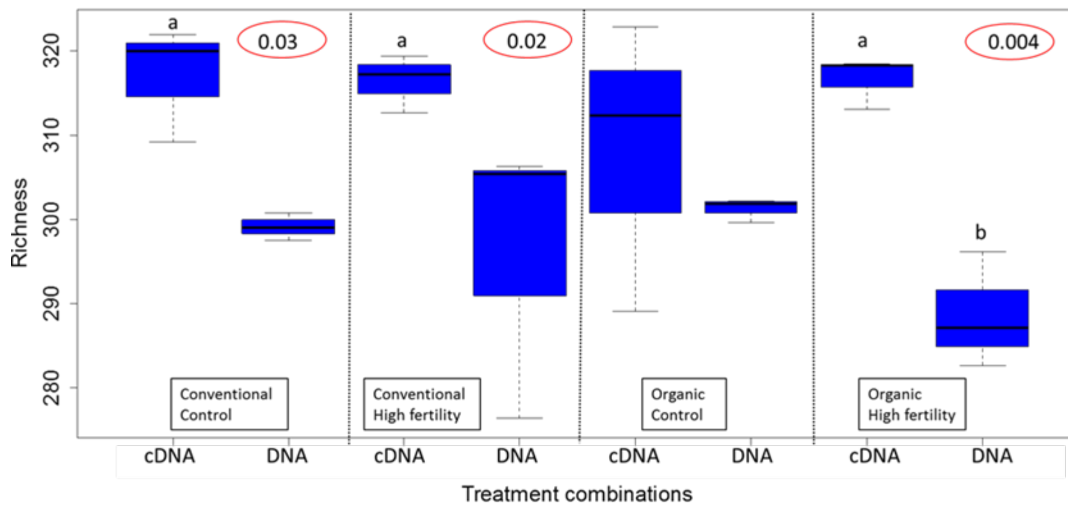

Figure 2.4. Richness for the bacterial rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations. The black dashed-line separates the results for soil fertility treatment combinations: conventional management control (with no added fertilizer), conventional high fertility, organic control (with no added fertilizer), and organic high fertility. In an AOV there was evidence for a sample type effect ($P = 0.0009$), where the observed mean richness was higher in rRNA (cDNA) samples than in the DNA samples across all other treatments. In analyses of richness for cDNA versus DNA evaluated within management type-fertilizer level combinations, there was evidence for differences in the conventional management-control ($p = 0.03$), conventional management-high fertility ($p = 0.02$) and organic management-high fertility ($p = 0.004$). Means sharing the same letters are not significantly different from each other (Tukey´s, $p < 0.05$).

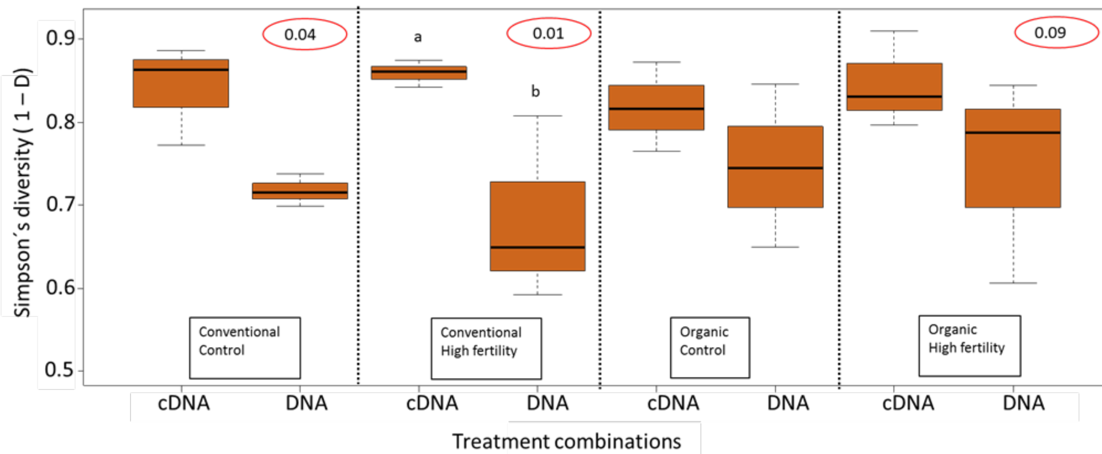

Figure 2.5. Simpson´s diversity for fungal rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations. The black dashed-line separates the results for soil fertility treatment combinations: conventional management control (with no added fertilizer), conventional high fertility, organic control (with no added fertilizer), and organic high fertility. In an AOV there was evidence for a difference between DNA and rRNA (p = 0.002). In analyses of diversity evaluated within management type-fertilizer level combinations, there was evidence for differences between DNA and rRNA samples in the following treatment combinations: conventional management-control (p=0.04), conventional management-high fertility (p = 0.01), and some evidence in for organic management-high fertility (p = 0.09). Means sharing the same letters are not significantly different from each other (Tukey´s, p < 0.05).

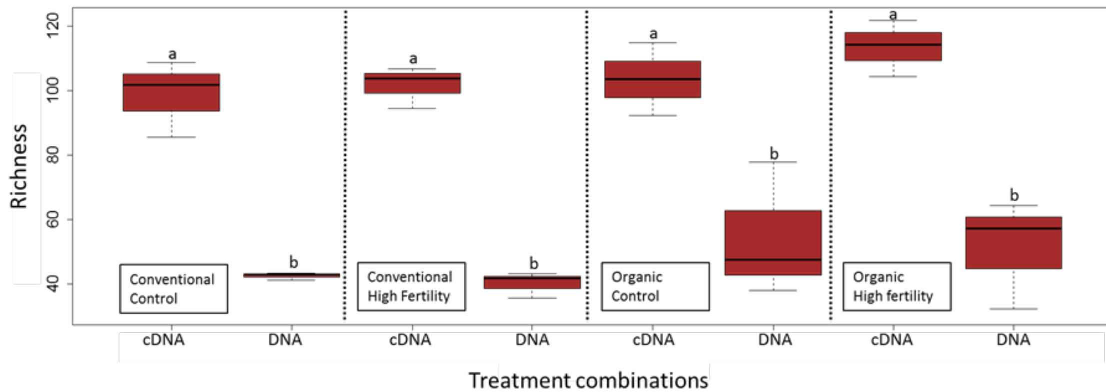

Figure 2.6. Richness for fungal rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations. The black dashed-line separates the results for soil fertility treatment combinations: conventional management control (with no added fertilizer), conventional high fertility, organic control (with no added fertilizer), and organic high fertility. In an AOV there was some evidence for a two-way interaction (fertilizer type and sample type (DNA vs. rRNA); $p = 0.10$) and strong evidence for the difference between DNA and rRNA samples ($p < 0.0001$). In analyses of richness for rRNA versus DNA evaluated within management type-fertilizer level combinations, there was evidence for differences within each combination ($p < 0.0001$). Means sharing the same letters are not significantly different from each other (Tukey´s, $p < 0.05$).

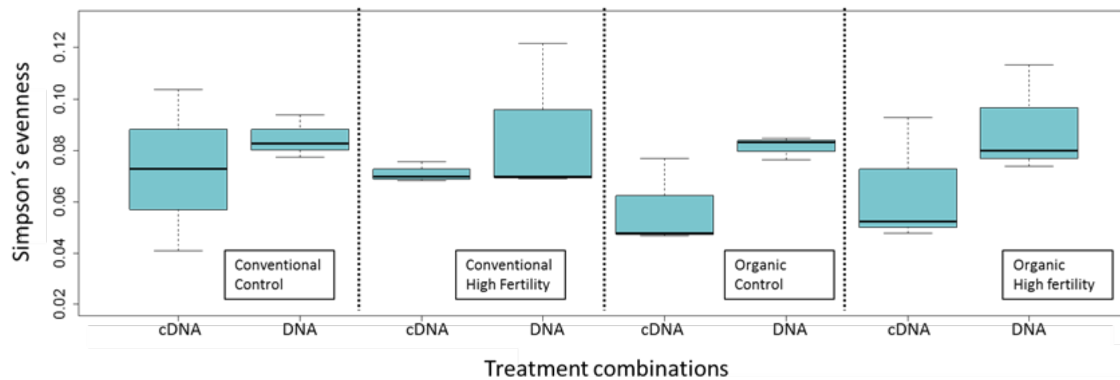

Figure 2.7. Simpson´s evenness for fungal rRNA (cDNA) or DNA communities in the field plots separated by treatment combinations. The black dashed-line separates the results for soil fertility treatment combinations: conventional management control (with no added fertilizer), conventional high fertility, organic control (with no added fertilizer), and organic high fertility. In an AOV there was evidence for a difference between evenness for rRNA and DNA ($p < 0.051$).

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Chapter 3 - Soil bacterial and fungal communities in tomato production under long-term organic vs. conventional soil fertility management

Abstract

Organic farming systems avoid the use of synthetic fertilizers, which can impact the soil microbial communities that are fundamental for agricultural productivity. Because of the importance of microbes for organic agriculture, we evaluated their response to organic and conventional fertility management. Our objective was to evaluate bacterial and fungal diversity at three stages in a tomato cropping season (pre-planting, fruit maturity, and postharvest) under three levels (high, low, and no fertilizer) of conventional and organic fertility management. Using Illumina MiSeq sequencing of the bacterial 16S and fungal ITS rRNA genes, we evaluated the responses of bacterial and fungal communities to all treatment combinations. Both bacterial and fungal richness changed across the tomato production season under conventional and organic management systems, but the detectable effects of fertility management were limited. This is in contrast to our other study in the same field experiment, in which sampling ribosomal RNA (rRNA, analyzed as complementary DNA (cDNA)) did reveal effects of fertility management on microbial diversity, while DNA samples collected at the same time did not.

Introduction

A major goal in sustainable agriculture is to maintain soil biological function and to promote plant health (van Bruggen and Semenov, 2000; Lehman *et al.*, 2015). Organic farming is designed for sustainability based on techniques such as crop rotation, green manure, and biological pest control, in place of synthetic fertilizers and with limited use of synthetic pesticides (Zhengfei *et al.*, 2005; Lupatini *et al.*, 2017). Organic farming systems may have the potential to restore soil health and to increase agro-ecosystem resilience to stress (Azadi *et al.*, 2011). Several studies have investigated the impact of organic and conventional management practices on the soil microbiome (Esperschutz *et al.*, 2007; Sugiyama *et al.*, 2010; Orr *et al.*, 2011; Li *et al.*, 2012; Ge *et al.*, 2013; Hartmann *et al.*, 2015; Pershina *et al.*, 2015; Lupatini *et al.*, 2017). These studies have reported that organic agriculture may increase soil microbial biomass and diversity, and may lead to distinct communities compared to those in conventional management systems.

Organic soil amendments, including composted or uncomposted plant residues, animal manures, and green manure, have widely different effects on the balance of soil microflora and plant diseases, depending on the nature of the residue and the preparation method (Scotti *et al.*, 2015; Zhang *et al.*, 2015). Incorporating organic amendments and managing the source and amount of crop residues can have direct impacts on plant health and crop productivity (Bailey and Lazarovits, 2003; Bonanomi *et al.*, 2018), by providing substrates for decomposing microbes (that in turn supply mineral nutrients to plants) (Rashid *et al.*, 2016) and improving soil structure and water holding capacity (Hiel *et al.*, 2018).

Soil amendments vary in their effects on soil microbial communities, in terms of effects on total microbial activity, ratios of gram-positive to gram-negative bacteria, and of bacteria to fungi (Buyer *et al.*, 2010; Bowles *et al.*, 2014). Manure and byproducts from the seafood and livestock

industries have been used by growers to maintain productivity of agricultural soils for millennia (Lazarovits, 2001). Poultry litter has been considered to be particularly valuable as organic fertilizer (Chen and Jiang, 2014) and fish protein hydrolysates are also popular as a good source of nitrogen (Chalamaiah *et al.*, 2012). Manure is often less expensive than conventional inorganic fertilizers and readily available (Staley *et al.*, 2013), and is a good source of both nutrients and organic matter (Toor *et al.*, 2006).

Soil microbiomes are key to successful agricultural systems. Several important processes in soils are mediated by soil microorganisms, including acquisition and recycling of nutrients, biological nitrogen fixation, denitrification, residue decomposition, and mineralization /immobilization of key nutrients (Fierer and Jackson, 2006; Schimel and Schaeffer, 2012; Philippot *et al.*, 2013). Microbial interactions and activity in soils are complex and affected not only by environmental factors such as temperature, soil moisture, and soil pH (Van Bodegom *et al.*, 2001; Fierer and Jackson, 2006; Lennon *et al.*, 2012), but also by soil management practices like cultivation, plowing, and mulching (Hobbs *et al.*, 2008; Power, 2010; Busari *et al.*, 2015; Guo *et al.*, 2016). Environmental differences, changes in the plant species grown, and in associated management practices, can all lead to changes in edaphic properties, which can in turn alter soil microbial communities (Lauber *et al.*, 2008; García-Orenes *et al.*, 2013).

The relationship among soil microbes, soil traits, and plant communities represents a frontier for ecology, in part due to the limitation of analytical methods. However, with technological advances and the development of culture independent tools including high-throughput DNA sequencing, there are new opportunities to explore microbial diversity in soil under many different environmental conditions, thus clarifying soil microbiome responses to agricultural management (Fierer *et al.*, 2007; Acosta-Martinez *et al.*, 2008; Lauber *et al.*, 2008;

Acosta-Martinez *et al.*, 2010; Li *et al.*, 2012; Hartmann *et al.*, 2015; Brennan and Acosta-Martinez, 2017). The effect of anthropogenic activities on soil microbial communities, such as the effects of agriculture on bacterial communities, has been addressed in many studies but remains an important challenge (Wessén *et al.*, 2010). Further, fungi, despite their high biodiversity, and ecological and economical importance, remain an understudied group of organisms (Pautasso, 2013; Rambold *et al.*, 2013). Environmental factors such as temperature, humidity, vegetation, and soil fertility may change greatly over time, with corresponding effects on soil microbiomes (Devi and Yadava, 2006; Edwards *et al.*, 2006; Frey *et al.*, 2008; Hui *et al.*, 2017).

Soil microbial communities react to environmental fluctuations, with changes in species composition and functional roles (Grayston *et al.*, 2001; Wallenstein and Hall, 2012). For example, Jumpponen (2011) found that fungal communities in the order Helotiales predominated during spring on the roots of the dominant tallgrass prairie grass *Andropogon gerardii* and towards the end of the growing season were replaced by arbuscular mycorrhizal fungi. Ibekwe *et al*. (2017) reported that temporal variability in temperature differentially affected soil bacterial community composition in soil and in the rhizosphere of spinach during three production seasons. Temporal variability in soil microbial communities, and how this variability compares across soils under organic and conventional management, represents a challenge for more complete understanding of microbial communities beyond individual snapshots.

We characterized soil bacterial and fungal communities across a season of tomato production in a long-term experiment with organic and conventional management of fertility levels. We used bacterial and fungal ribosomal markers to characterize the communities, asking the following research questions: (1) How do bacterial and fungal diversity respond to organic and conventional management across time? (2) What are the dominant bacterial and fungal taxa in

these tomato production systems? (3) How does the structure of bacterial and fungal communities change across time in response to soil amendments? Based on the literature reviewed above, we hypothesized that (1) organic management would increase bacterial and fungal diversity compared to conventional management, (2) some individual bacterial and fungal taxa would respond differently to treatment combinations, and (3) bacterial and fungal communities would change across sampling times in response to soil amendments. This work complements a previous study in which we compared DNA and RNA samples for characterizing soil bacterial and fungal community responses to organic and conventional fertilization.

Materials and Methods

Study site

We sampled during one tomato production season (2010) in a long-term experiment evaluating organic and conventional soil fertility management. The experiment was conducted at the Kansas State University Horticulture and Extension Center in Olathe, Kansas, USA, in a field with soil type Kennebec silt loam (Talavera-Bianchi *et al.*, 2010). A detailed description of the location and site management was provided in Chapter 2. These farming systems differ in fertilization (organic or synthetic), whereas factors such as tillage, cover crop and crop rotation were constant across treatments. In brief, we sampled six open field whole plots in a split plot experimental design. There were two whole plots in each of three blocks, and within each block one whole plot was randomly assigned to long-term organic management and the other whole plot to long-term conventional management. Organic plots were managed in compliance with USDA National Organic Program standards (NOP) (https://www.ams.usda.gov/about-ams/programs-

offices/national-organic-program). All plots were tilled using an earth fork followed by wheel harrowing and raking to re-form beds at 30 cm depth (Altamimi, 2016). Each whole plot was subdivided into three 3.2 m x 6.1 m subplots to which one of the three fertilizer levels was randomly assigned (high, low, and no-fertilizer control). Low- and high-fertility subplots were fertilized with equal amounts of nitrogen from compost or synthetic fertilizer at the beginning of the growing season, while high-fertility subplots received additional fertilization during the growing season. Conventional plots were fertilized with Jack's professional peat-lite N-P_2O_5-K_2O 20-10-20 (Allentown, PA, USA) at a rate of 98 kg N /ha. Organic plots received a poultry litter source compost (MicroLeverage N-P_2O_5-K_2O 0.6-0.4-4.4, Hughesville, MO, USA) at a rate of 197 kg N /ha. Starting two weeks after planting, conventional high-fertility plots received an additional 36.6 kg/ha of calcium nitrate ($Ca(NO_3)^2$) and 11.2 kg/ha of potassium nitrate (KNO_3), which were calculated to apply an amount of calcium equivalent to that present in the fish hydrolysate. Organic high-fertility plots received additional fish hydrolysate N-P_2O_5-K_2O 2.23-4.35-0.3 (Neptune's Harvest, Gloucester, MA, USA) at a rate of 7.24 kg N /ha.

Tomato (*Lycopersicon esculentum* 'Bush celebrity') (Totally Tomatoes, Randolph, WI, USA) and pac choi *Brassica rapa* L. chinensis 'Mei Qing Choi' (Johnny's Selected Seed, Albion, ME, USA) were each grown in one half of each open field plot, with a rotation between the two crops each year. In the high fertility plots, the single annual tomato crop received one fertility application per week during six weeks, and the spring and fall pac choi crops grown in rotation with tomato each received three such applications (Talavera-Bianchi *et al.*, 2010; Altamimi, 2016). All subplots (control, low fertility, and high fertility) received nutrients naturally released by the cover crop buckwheat (*Fagopyrum esculentum*; Albert Lea Seed, MN, USA), which was planted

between the spring and fall pac choi crops, and from an annual cover crop of winter rye (*Secale cereal*e).

Soil sampling and experimental design

Soil samples were collected three times during the tomato season in 2010. The first soil sample was collected before transplanting the tomato plants (April 27: pre-planting), and before the first fertility treatments were applied (May 21), but after the annual cover crop of rye (*Secale cereale*) was incorporated in control, low-and high-fertility.A second sample was collected when tomato plants were mature with flowers and fruit (July 14: maturity), after the soluble compost and synthetic fertilizers were applied for the high-fertility treatments. A final sample was collected when tomato plants were removed from field plots (November 11: postharvest). We sampled the three subplots representing three levels of fertilization (high fertility, low fertility and control) within each whole plot, for a total of 18 subplots (experimental units): 2 management types (organic vs. conventional) x 3 levels of fertilization x 3 replicates. Each experimental unit was sampled at each of the three time points for a total of 54 samples. Soil samples were systematically collected around the middle four tomato plants in the middle row in each subplot, avoiding close proximity to tomato roots, and avoiding the edges of the plots by 61 cm on each end to minimize edge effects. Soil cores were 15 cm deep and 5 cm in diameter. The four subsamples per subplot were mixed in a clean plastic bucket to form one composite sample per subplot, and placed in labeled Ziploc plastic bags on ice in a cooler during sample collection. Between subplot sampling, the soil corers were cleaned with water and dried with a clean paper towel to avoid soil contamination. From each composite sample, we collected nine subsamples of ~ 0.7 g each and

placed each in a MoBio bead solution tube for total genomic DNA extraction using the Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer´s instructions. Samples were stored at -80°C until PCR amplification.

DNA extraction, PCR amplification and sequencing

From each subplot at each time point, we extracted the soil DNA from the nine subsamples separately. We followed the Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) protocol, except that the final elution used 100 µl of buffer S5 instead of 50 µl. The DNA was quantitated with ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware) and all DNA samples were aliquoted into a 96-well plate at a concentration of 2 ng/µl. Of the nine subsamples per subplot, the three subsamples with the highest DNA content were used to produce 16S and ITS amplicons. All PCR reactions were performed in a two-step PCR process following the protocol recommended by Berry *et al*. (2011) with three technical replicates of each of the 18 experimental units per sampling time (three sampling times) including positive controls (*Escherichia coli* for bacteria and *Saccharomyces cerevisiae* for fungi) and a negative control (molecular biology grade water). The two-step approach was used to avoid barcode-specific 3'-end amplification biases (Berry *et al.*, 2011). To produce bacterial and fungal amplicons, we optimized the PCR conditions for the template concentration, $MgCl_2$ concentration, and annealing temperatures for primers described below. For doing so we used DNA from six environmental samples sampled from previous studies. All PCR reactions were performed using a MultiGene Optimax Thermal Cycler (Labnet International Inc., Edison, NJ, USA). Negative controls for DNA extractions and PCRs were included to ensure absence of contamination; no contamination was

detected on PCR products visualized via agarose gel electrophoresis. All primary PCR reactions were performed in 25 µl reaction volumes. Bacterial amplicons were generated using forward 515F (5'- GTGCCAGCMGCCGCGGTAA-3', Caporaso *et al*. (2011)) and reverse 806R (5'-GGACTACHVGGGTWTCTAAT -3', Caporaso *et al*. (2011)) primers to amplify the hypervariable V4 region of the 16S rRNA (Caporaso *et al.*, 2011). Primary PCR conditions were: 8 ng (4 µl) of DNA template, 1 µM of both forward (515F) and reverse (806R) primers, 12.5 µl of Phusion High Fidelity PCR Master Mix with HF buffer (Thermo Fisher Scientific, Grand Island, NY, USA), 3.5 µl molecular biology grade water with PCR cycle parameters with an initial denaturing step of 98 °C for 30 sec, followed by 30 cycles of 94 °C denaturing for 45 sec, 50 °C annealing for 1 min and 72 °C extension for 2 min, followed by a final extension step of 72 °C for 7 min. A total of 5µl of the reaction volume was visualized on a 1.5% agarose (*w/v*) gel to ensure presence of PCR products. The remaining volume (20 µl) of the duplicated PCR amplicons per experimental unit was pooled and cleaned using Deffinity Rapid Tips (Deffinity Genomics, West Chester, PA, USA) following the manufacturer's protocol. For the secondary PCR, 10 ml of primary PCR products in 50 µl reaction volumes were amplified using 1 µM of both forward (515F) and reverse (806R) primers, 25 µl of Phusion High Fidelity PCR Master Mix with HF buffer and 5 µl molecular biology grade water. An exception to the protocol was using only 5 cycles and the inclusion of a reverse primer joined with 12-base pair unique molecular identifier tags (MID-806R; (Caporaso *et al.*, 2011); Table 3.1). Fungal amplicons were generated using the forward ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA -3', Gardes and Bruns (1993)) and reverse ITS4 (5'-TCCTCCGCTTATTGATATGC-3', White *et al.* (1990)) primers. These primers amplify a wide range of fungal targets and the Internal Transcribed Spacer (ITS) markers are recommended as a fungal DNA barcode (Seifert, 2009; Schoch *et al.*, 2012). Primary PCR

reactions for fungi contained final concentrations or absolute amounts of reagents as follows: 20 ng (or 10µl) of DNA template, 1 µM of both forward (ITS1F) and reverse (ITS4) primers, 200 µM dNTPs, 2.5 mM $MgCl_2$, 1 unit GoTaq Hot Start DNA polymerase (Promega, Madison, Wisconsin) and 2.5 µl 5x Green GoTaq Flexi PCR buffer (Promega, Madison, Wisconsin). PCR cycling parameters included an initial denaturation at 95 °C for 10 min, then 30 cycles of denaturation at 95 °C for 1 min, annealing at 57 °C for 1 min and extension at 72 °C for 2 min, followed by a final extension step at 72 °C for 7 min. A total of 5µl of the reaction volume was visualized on a 1.5% agarose (*w/v*) gel to ensure presence of PCR products. The remaining volume (20 µl) of the duplicated PCR amplicons per experimental unit was pooled and cleaned using Deffinity Rapid Tips (Deffinity Genomics, West Chester, PA, USA) following the manufacturer's protocol. To produce fungal secondary PCR amplicons, we chose the Internal Transcribed Spacer 2 (ITS2) region of the ribosomal RNA gene repeat to target shorter reads compatible with the paired-end Illumina MiSeq and amplified with the forward primer fITS7 (5'-GTGARTCATCGAATCTTTG-3', Ihrmark *et al*. (2012)) and reverse primer ITS4 (White *et al.*, 1990). A unique sample-specific 12-base pair molecular identifier tags (MID) were incorporated in the secondary PCR using an ITS4 fusion primer (Table 3.2). Secondary PCR conditions were identical to those for the primary PCR except that they included 10 ml of primary PCR products as template, tagged reverse primers (ITS4), and the number of PCR cycles was reduced to ten.

All secondary bacterial and fungal amplicons were visualized on a 1.5% agarose (*w/v*) gel to check for amplification, pooled and cleaned with Agencourt AMPure (Beckman Coulter Inc., Pasadena, CA, USA) following the manufacturer's instructions except that we used a 1:1 ratio of AMPure bead solution to PCR volume to further discriminate against short PCR fragments. Resultant bacterial and fungal amplicons were quantified for DNA yield and pooled equimolarly

into one bacterial and one fungal amplicon library. The two libraries were AMPure® cleaned again to remove any residual short DNA contamination and finally submitted for sequencing. The bacterial and fungal libraries were sequenced at the Integrated Genomics Facility at Kansas State University (Manhattan, Kansas, USA), where Illumina-specific indices and adapters were ligated into amplicons using a NEBNext® DNA Library Prep MasterMix for the Illumina kit (Protocol E6040; New England Biolabs Inc.) and sequenced using a MiSeq Reagent kit v3 (Illumina, San Diego, CA, USA) with 600 (2 x 300) cycles. Paired .fastq files for bacteria and fungi have been deposited in the data sharing repository Figshare (https://figshare.com). The DOI for both bacterial and fungal data sets is https://doi.org/10.6084/m9.figshare.7294319.v2.

Sequence analysis

The bacterial and fungal sequence data (.fastq) were processed using mothur (version 1. 34.4; Schloss et al. (2009)). We followed most of the steps of the protocol of the standard operating procedure (SOP) for MiSeq by Kozich et al. (2013), starting by contiging the paired-end .fastq files. The paired-end read bacterial library contained 16,998,964 sequences, whereas the paired-end read fungal library contained 16,874,822 sequences. Contiged sequences were screened and culled using the following criteria: ≤ 2 mismatches to primers, ≤ 1 mismatch to barcodes (or multiple identifier (MIDs)) and homopolymeres longer than 8bp. The sequences were truncated to 250 bp, near identical sequences preclustered to reduce potential sequencing bias (Huse et al., 2008) and the remaining sequences screened for chimeras with the mothur-implemented UCHIME algorithm (Edgar et al., 2011). Bacterial sequences were aligned to SILVA-based reference alignment (Schloss, 2009) and a distance matrix calculated (dist.seqs) prior to clustering into

operational taxonomic units (OTUs). For fungal sequences, a pairwise sequence distance matrix was calculated (pairwise.seqs) prior to clustering into OTUs. After quality control and removal of chimeras, bacterial and fungal sequences were clustered into OTUs based on a 97% similarity threshold. Rare OTUs (abundance ≤ 10 across all experimental units) that might be PCR and/or sequencing artifacts were removed (Brown et al., 2015). The bacterial and fungal sequences were assigned to taxonomic affinities using a naïve Bayesian classifier (Wang et al., 2007) with a bootstrap threshold for bacterial sequences of 80% against the mothur-formatted version of the RPD training set, version 9 (Schloss and Westcott, 2011), while fungal sequences were assigned to taxonomic affinities using a bootstrap threshold of 60% against the UNITE taxonomy reference (http://unite.ut.ee/repository.php), version 6. For bacteria, sequences not assigned to Domain Bacteria (including Archaea, chloroplast, cyanobacteria, unknown and mitochondria) were omitted. For fungi, sequences not assigned to Kingdom Fungi (including Plantae, bacteria, chloroplast, and unknown) were omitted. For bacteria, the final data set with no "rare" OTUs had 4,248,145 sequences and 7,065 OTUs in total. For fungi, the final data set with no "rare" OTUs had 2,690,489 sequences and 2,814 OTUs in total. Before downstream analysis, final bacterial and fungal data sets were rarified by randomly subsampling to an equal number of sequences to calculate diversity indices estimates of the 16S and fungal ITS rRNA datasets, respectively.

Diversity indices and statistical analysis

The standardized bacterial and fungal OTU frequency data sets were used to calculate Simpson's diversity index, Simpson´s evenness, and richness for each plot using mothur (v. 1. 34.4; Schloss et al. (2009)). Simpson's diversity ($1\text{-}D = 1\text{-}\sum p_i^2$) estimates the likelihood that two

randomly chosen individuals (sequences) will be assigned to different OTUs (Simpson, 1949). It is the complement of Simpson's dominance ($D = \sum p_i^2$), where p_i is the proportion of sequences assigned to the ith OTU. We also evaluated Simpson´s evenness (also called Simpson's equitability or $E_{1/D} = (1/\sum p_i^2)/S_{obs}$, where p_i = is the proportion of sequences assigned to the ith OTU and S_{obs} = richness) and richness (S_{obs}: number of OTUs). We evaluated these diversity indices using generalized linear mixed models in SAS Proc GLIMMIX (SAS 9.4., Institute Inc., Cary, NC), comparing fungal and bacterial communities in the organic and conventional management systems. We also evaluated the most frequent taxa in the samples across three sampling times, and how individual taxa responded to the experimental treatments in generalized linear mixed models using SAS Proc GLIMMIX. We evaluated the effects of the treatments across three sampling times on dominant bacterial and fungal taxa, in an analysis using SAS Proc GLIMMIX for the following predictors: management type, fertilizer type, and sampling time. In all the analyses, p-value adjustments for multiple comparisons were performed using a Tukey-Kramer test in SAS. The package ggplot (Wickham, 2009) in the R programming environment (v.3.4.3; R Development Core Team, 2018) was used for plotting the diversity responses across the sampling times.

Bacterial and fungal community responses across time to organic and conventional soil amendments

We used non-metric multidimensional scaling (NMDS) to evaluate how microbial communities changed across time and in response to the experimental treatments. We used the

metaMDS function in the vegan package (Oksanen et al., 2018) in the R programming environment (R Development Core Team, 2018). The metaDMS function was applied with two dimensions, and a maximum number of random starts of fifty, to find the set of variables with maximum (rank) correlation using Bray-Curtis dissimilarity distances derived from the OTU table wherein OTUs were assigned at 97% sequence similarity to genera level. Community responses to fertilizer level were evaluated using a permutational multivariate analysis of variance (PERMANOVA, at 200 permutations) using the adonis function in the vegan package (Oksanen et al., 2018). Because of the split plot design of the field study, for PERMANOVA tests of the effects of soil fertility levels we divided the data into two separate data sets, one data set for each of conventional and organic management (applied at the whole plot level). Beta diversity was evaluated as species gain β_z (Koleff et al., 2003) for bacterial communities, and used as the response variable in the PERMANOVA.

Results

Bacterial and fungal community data characterization

The number of sequences passing our quality filtering ranged from 5,378 to 165,464 bacterial and from 3,884 to 104,253 fungal sequences per subplot across the three sampling times. The fifty-four samples in total for each sampling time were rarified by randomly subsampling 5,378 bacterial sequences per subplot and 3,884 fungal sequences per subplot before downstream analyses.

Diversity responses for fungal and bacterial communities across time

Generalized linear mixed model (GLMM) analyses found evidence for a sampling time effect on bacterial richness ($F = 15.26$, $p < 0.0001$, Table 3.3, Fig. 3.1) and fungal richness ($F = 12.71$, $p = 0.0001$, Table 3.4, Fig. 3.2). Bacterial and fungal richness estimates for all treatment combinations were highest pre-planting and decreased from pre-planting to maturity, then increased again post-harvest (Figs.3.1 and 3.2). There was also some evidence for the sampling time effect on bacterial Simpson´s diversity ($F = 2.61$, $p = 0.09$, Table 3.5, Fig. 3.3), but not for a sampling time effect on fungal diversity ($p = 0.24$, Table 3.6, Fig. 3.4). Trends for bacterial Simpson's diversity and bacterial richness were similar. Simpson´s evenness for bacterial and fungal communities did not differ across the three sampling times (bacterial evenness: $p = 0.31$, Table 3.7, Fig. 3.5; fungal evenness: $p = 0.23$, Table 3.8, Fig.3.6). There was not evidence that any bacterial or fungal measures of diversity responded to management type (conventional vs organic) or fertilizer level (control, low-and high-fertility; Tables 3.3 to 3.8).

Dominant bacterial and fungal taxa across sampling times

We identified dominant bacteria and fungi for all treatment combinations. We calculated the frequency of the taxa associated with a taxonomic level (phylum, order, family, and genus for both bacteria and fungi, plus species for fungi) by dividing the number of sequences of the taxa at a particular taxonomic level by the total number of sequences obtained overall, across treatment combinations and the sampling times. We found that the bacterial phyla Proteobacteria and Acidobacteria were the most frequent (25.4% and 15.1%, respectively) for all treatment

combinations and across sampling times, with 21.4% of phyla unclassified (Table 3.9). The bacterial orders assigned to Sphingobacteriales, Acidobacteriales_Gp4, and Actinomycetales were the most frequent over all treatment combinations and sampling times (6.6%, 4.7% and 4.7%, respectively), with 35.4% unclassified to order (Table 3.9). The bacterial families Chitinophagaceae, Acidobacteriaceae (for the subdivisions Gp4 and Gp6) and Spartobacteria (family *incertae sedis*) were the most frequent over all treatment combinations and sampling times (6.3%, 9.9% and 4.5%, respectively), with 44.1% unclassified (Table 3.9). Finally, the bacterial OTUs assigned to the subdivisions *Gp4* (5.4%), *Gp6* (5.3%), *Gp3* (2.4%) and to the genera *Sphingomonas* (3.1%) and *Nitrospira* (1.4%) were the top five most frequent for all treatment combinations and sampling times, with 56.1% unclassified to genus (Table 3.9).

For fungi, we found that the phyla Ascomycota, Basidiomycota, and Zygomycota were the top three most frequent phyla over all treatment combinations and sampling times (57.8%, 24.7% and 11.2%, respectively) with 4.3% of phyla unclassified (Table 3.10). The fungal orders Pleoporales and Hypocreales were the most frequent across all treatment combinations and sampling times (18.7% and 18.4%, respectively), with 9.0% of the orders unclassified (Table 3.10). Mortierellaceae and Pleosporaceae were the most frequent fungal families over all treatment combinations and sampling times (11.4% and 7.1%, respectively), with 14.3% of family unclassified (Table 3.10). The fungal OTUs assigned to the genera *Mortierella* (12.5%), *Phoma* (9.0%), *Alternaria* (4.2%), *Lysurus* (3.8%), and *Hannaella* (3.0%) were the top five most frequent fungal genera over all treatment combinations and sampling times, with 19.6% of the genera unclassified (Table 3.10). Within the fungal OTUs assigned to the genus *Mortierella*, the species *M. humilis* (3.1%), *M. exigua* (1.4%) and *M. capitata* (1.0%) were the most frequent, while *Phoma sp.* (9.5%), *Alternaria porri* (4.5%), *Lysurus cruciatus* (4.0%), and *Hannaella synensis* (3.2%)

were the most frequent species assigned to each corresponding genus. Thirty-four percent of the fungal species were unclassified (Table 3.10).

Community analyses

NMDS analyses of bacterial communities found similar composition for organic and conventional plots sampled pre-planting, with higher variability in conventional plots (Fig. 3.7). At tomato maturity, organic and conventional communities were similar to each other again, but distinct from the pre-planting communities, and organic plots were more variable (Fig. 3.7). Postharvest, communities from organic and conventional plots were distinct from each other, with organic communities more similar to pre-planting communities, and conventional communities more similar to communities at tomato maturity (Fig. 3.7). In the PERMANOVA, fertilizer levels (control, low fertility, and high fertility) for organic or conventional management did not affect community structure ($p = 0.9$; Tables 3.11 and 3.12; Fig. 3.7). For fungal communities, NMDS analysis did not converge.

Discussion

Diversity responses for bacterial and fungal communities across time

Our initial question was how bacterial and fungal diversity respond to organic and conventional management across time. In our study, neither bacterial nor fungal richness, diversity, nor evenness estimates differed with management (conventional vs organic) or fertilizer

level (control, low fertility, and high fertility; Tables 3.3 – 3.8; Figs. 3.1-3.6). These results are in contrast to previous studies in several cropping systems that compared organic and conventional farming systems (Sugiyama *et al.*, 2010; Li *et al.*, 2012; Ge *et al.*, 2013; Hartmann *et al.*, 2015; Pershina *et al.*, 2015; Lupatini *et al.*, 2017), in which organic management often increased soil microbial biomass and diversity. Organic manure was often identified as the likely reason for differences in microbial communities between conventional and organic farming management (Bossio *et al.*, 1998; Gunapala and Scow, 1998). In another study related to the current one, we sampled only once as a snapshot from the same field plots postharvest to compare total resident microbial communities by extracting DNA, versus the active microbial communities by extracting rRNA (ribosomal RNA). In that study, we likewise did not find differences in bacterial or fungal diversity when comparing conventional and organic management using the DNA approach, as in this current study. Nonetheless, we did find significant differences for bacterial and fungal diversity using rRNA-based approaches. The use of rRNA sampling may generally reveal other differences in soil microbial diversity that would not be apparent when sampling DNA. Likewise, Wang *et al.* (2012) compared microbiomes in organic and conventional soils, and concluded that different molecular approaches for assessing the diversity could lead to different results in the same study. Factors including the use of different animal and poultry manures, cover crops, extraction methods, calibration of the equipment, and the use of different PCR primers may give different outcomes (Wang *et al.*, 2012).

We evaluated the impact of nitrogen fertilizers (from organic or synthetic sources) on soil microbial communities, while other factors such as tillage, cover crop, and crop rotation were kept constant in our conventional and organic management systems. We did not find differences in bacterial or fungal diversity when comparing the different nitrogen sources applied to the

conventional and organic management treatments. Staley *et al*. (2013) using hydrolyzed fish fertilizer and poultry litter, two organic fertilizers similar to those used in our study, to evaluate yield and the content of antioxidants and phenolics of vegetable amaranth (*Amaranthus hybridus*), celosia (*Celosia argentea*), gboma (*Solanum macrocarpon*), and long bean (*Vigna unguiculata*); they reported that organic amendments did not significantly influence biomass production.

Most studies that have examined the impact of manure on soil diversity have focused primarily on cattle manure. For instance, Chaudhry *et al*. (2012) reported soil amended with composted cattle manure to be more diverse in bacteria than soil amended with inorganic fertilizers. Sun *et al*. (2004) reported bacterial communities of cattle manure-amended soil were more diverse than unamended soil. These studies addressed long-term organic and chemical amendment applications for a grain-legume cropping system and a continuous winter wheat crop, respectively. Our results contrast with these two studies, because we did not find evidence that organic-amended soil (poultry and fish hydrolysate composts) was more diverse compared to the synthetic fertilizer treatment (Jack´s peat-lite, potassium nitrate (KNO_3) and calcium nitrate ($Ca(NO_3)_2$) or compared to the control treatment. Additionally, our findings differed from previous research in which synthetic nitrogen sources decreased soil microbial diversity compared to the control treatments (Ramirez *et al.*, 2010; Chaudhry *et al.*, 2012). Nonetheless, the study conducted by Parfitt *et al*. (2005) had conclusions similar to ours. They examined seasonal pasture growth in nine adjacent hill pastures, under sheep or beef, with different long-term management, including certified organic, no fertilizer, and conventional fertilizer application. The authors reported no differences in soil microbial diversity between organic and conventional pastures using both nitrogen mineralization and microbial biomass measurements.

Most studies evaluating the effect of fertilization reported that farming systems with regular organic manure application had greater soil microbial biomass and different community structures compared to systems with synthetic fertilization (Zelles *et al.*, 1992; Marschner *et al.*, 2004; Tu *et al.*, 2006). However, these studies were based on field experiments where several factors such as crop rotation, crop varieties, soil tillage, and plant protection varied at the same time between conventional and organic soil management. Therefore, the main factor producing differences between conventional and organic farming could not be identified. Although nitrogen fertilization using either synthetic sources or organic manures is a common agricultural practice boosting world food production (Fixen and West, 2002; Canfield *et al.*, 2010), the impact of different nitrogen sources on soil microbial communities under conventional and organic farming practices is only beginning to be understood.

Bacterial and fungal richness and bacterial diversity changed across time in our study, while effects of conventional or organic fertilizers were not observed. Zhao *et al*. (2014), using 454-pyrosequencing, reported that bacterial communities in a rice-wheat cropping system varied more with sampling time during growing season than in response to combined addition of urea fertilizer and animal manure. Likewise, Orr *et al*. (2011) found that management regime effects on both the total bacterial community and the free-living diazotroph community could be secondary to other factors such as time of sampling and previous crop. Furthermore, Calbrix *et al*. (2007), in a one-year study of cover crop rotations of mustard (*Sinapis alba*) and beet (*Beta vulgaris*), reported that seasonal variation had more effect on soil microbial communities than type of organic amendment (across sewage sludge, turkey manure, and compost made of turkey and ligneous waste). Our results are similar to past research in which sampling time had a larger effect

than management on microbial diversity, richness or composition (Calbrix *et al.*, 2007; Orr *et al.*, 2011; Zhao *et al.*, 2014).

In the current study, we reported higher bacterial and fungal richness, and higher bacterial diversity, at both the pre-planting and postharvest sample times, compared to samples at maturity, suggesting changes due to weather conditions and crop growth stage. Hamm *et al.* (2016) examined the impacts of manure (solid pig manure, solid dairy manure) and granular urea nitrogen on the diversity and composition of soil bacterial communities in an annual cropping system (barley, *Hordeum vulgare*) evaluated post-planting, mid-season and post-harvest for three successive annual applications. They found that bacterial diversity and richness increased throughout the growing season. Although our results did not show a continued increase in soil microbial communities through the growing season, as was reported by Hamm et al. (2016), our results were similar in finding a response the effects of weather conditions through the season and crop growth stage.

In our field study, temperatures increased and precipitation decreased from June to August (Table 2.1). Temperature and precipitation are important environmental factors regulating microbial activity and determining the composition and diversity of soil microbial communities (Pettersson and Bååth, 2003; Bell *et al.*, 2008; Castro *et al.*, 2010; Zhao *et al.*, 2016; Stovicek *et al.*, 2017; Wang *et al.*, 2017). Interactions among temperature, moisture conditions, and plant growth stages often have important effects on soil microbial community responses (Castro *et al.*, 2010; Classen *et al.*, 2015; Tang *et al.*, 2018). For example, Wang *et al.* (2016) and Wang *et al.* (2018) reported that plant growth stage plays an important role in affecting the composition of bacterial (Wang *et al.*, 2016) and fungal communities (Wang *et al.*, 2018) on a wheat-rice (*Triticum aestivum* L., and *Oryza sativa* L.) rotation system under different fertilization regimes.

The plant growth stage, changes of climate and soil temperature, were considered important factors influencing the composition of soil microbial communities (Wang *et al.*, 2016; Wang *et al.*, 2018) .

Additionally, growing season effects on soil microbial communities may be mediated through other factors that have been reported in other studies, including expansion of the rhizosphere volume of soil with growth of an annual crop (Hamm *et al.*, 2016), crop species and varieties (Smalla *et al.*, 2001; Dunfield and Germida, 2003), and conventional and reduced-input cropping type (Lauber *et al.*, 2013).

Dominant bacterial and fungal taxa across sampling times

Our second question was about the dominant bacterial and fungal taxa in this tomato production season. The top five most frequent OTUs assigned to bacterial and fungal genera varied over all the treatment combinations. For bacteria, we found three dominant Acidobacterial subdivisions *Gp4*, *Gp6* and *Gp3,* where *Gp4* was the most frequent. Members of the phylum Acidobacteria are generally widespread and abundant across a range of ecosystems, especially soils (Kielak et al., 2016). The subdivisions *Gp4* and Gp6 have been reported as predominant in soils (Janssen, 2006; Jones et al., 2009). Other studies have reported that the highest incidence of Acidobacteria was in soils with the lowest pH (Mannisto et al., 2007; Griffiths et al., 2011), and phylogenetic clustering of acidobacterial communities became stronger as soil pH departed from neutrality (Jones et al., 2009). In our study site, soil pH in the organic field plots had a mean pH of 7.0 across the three fertility levels, while in the conventional field plots the mean pH was 6.5 (Altamimi, 2016). The difference in pH across the treatments was potentially a factor affecting the overall frequency of the members of this phylum. The bacterial genus *Sphingomonas* has been

isolated from many different land and water habitats, including from plant root systems. Members of this genus can survive low nutrient conditions, and can metabolize a wide variety of carbon sources (Feng et al., 2014). The last most abundant bacterial genus was *Nitrospira*, a ubiquitous bacterium that plays a role in the nitrogen cycle (Lucker et al., 2010) by performing nitrite oxidation in the second step of nitrification, and is able to convert urea to ammonia and CO_2 (Koch et al., 2015). Among fungal taxa, we found that *Mortierella* (phylum Zygomycota) was the most frequent genus across all treatments. Many *Mortierella* species live as saprotrophs in soil, on decaying leaves or other organic material (Webster and Weber, 2007). The next most frequent fungal genus was *Phoma* (phylum Ascomycota), which includes common inhabitants of soil (Aveskamp et al., 2008), including saprobric members (Kirk et al., 2008). The third most abundant fungal genus was *Alternaria* (phylum Ascomycota), which includes many common saprophytes (Kirk et al., 2008), several well-known plant pathogens (Woudenberg et al., 2013), and has been reported to be highly abundant in agricultural soils (Sugiyama et al., 2010).

Community analyses

For our third question, we evaluated whether bacterial and fungal communities changed across sampling times in response to soil amendments. Bacterial communities sampled for organic plots at maturity had a higher variability compared to both communities sampled for conventional plots at tomato maturity and postharvest. Houlden *et al*. (2008), evaluating the influence of plant developmental growth stage on rhizosphere microbial community structure and function in three agricultural crops – pea (*Pisum satvium*), wheat (*Triticum aestivum*), and sugar beet (*Beta vulgaris*) – found that shifts in the diversity of fungal and bacterial communities were more pronounced at

maturity for pea and sugar beets, potentially reflecting changes in both the rhizodeposition of the plant across developmental stage and changing environmental conditions.

Conclusions

We found evidence that both bacterial and fungal richness changed during the growing season under conventional and organic management systems, while we did not find evidence for effects of management type (conventional vs organic) or fertilizer level (control, low, and high fertility). Bacterial and fungal richness across treatments increased postharvest compared to maturity, suggesting changes due to weather conditions and potentially also due to crop growth stage. Bacteria assigned to the subdivision *Gp4* (phylum Acidobacteria) and to the genera *Sphingomonas* (phylum Proteobacteria) and *Nitrospira* (phylum Nitrospirae), and fungi assigned to the genera *Mortierella* (phylum Zygomycota), *Phoma* (phylum Ascomycota) and *Alternaria* (phylum Ascomycota), were the most frequent over all treatment combinations and the three sampling times. We did not detect increases in bacterial or fungal diversity with the use of organic fertilizers compared to synthetic fertilizers. Thus, our findings differed from previous studies in which organic fertilizers increased soil microbial diversity compared to synthetic nitrogen sources. One important consideration when considering the lack of evidence for management effects, however, is the relatively low statistical power of this analysis, with limited replication. However, in the same field experiment, sampling rRNA did detect fertility management effects on microbial diversity. Likewise, a related study in a nearby Kansas farm found microbial diversity responses to nutrient amendments (Chapter 4). Comparisons across such related studies could be enhanced by future research to characterize bacterial and fungal communities in manure sources, for

comparison to those in soils with and without added manure. Ultimately, understanding how these soil management choices affect the *functional* diversity of soil agroecosystems will support decision-making to strengthen desired agroecosystem processes.

Tables and Figures

Table 3.1. Reverse bacterial primer (806R) synthesized with unique twelve base pair molecular identifier (MID) tags assigned to each sample from a long-term organic and conventional tomato management experiment. The treatments include three sampling times: S1 (sample 1:pre-planting), S2 (sample 2: maturity), and S3 (sample 3: postharvest); field organic (FO) plots or field conventional (FC) plots, with replicate number indicated (FO1, FC1, FO2, FC2, FO3, FC3); and fertility level: control (C), low-fertility (L), and high-fertility (H)).

Sample	Treatment	Gene region primer (806R)	Molecular Identifier (MID) tags
1	S1-FO1-1-C	GGACTACHVGGGTWTCTAAT	ATGTCACCGCTG
2	S1-FO1-2-L	GGACTACHVGGGTWTCTAAT	TGTAACGCCGAT
3	S1-FO1-3-H	GGACTACHVGGGTWTCTAAT	AGCAGAACATCT
4	S1-FC1-1-C	GGACTACHVGGGTWTCTAAT	TGGAGTAGGTGG
5	S1-FC1-2-H	GGACTACHVGGGTWTCTAAT	TTGGCTCTATTC
6	S1-FC1-3-L	GGACTACHVGGGTWTCTAAT	GATCCCACGTAC
7	S1-FC2-1-L	GGACTACHVGGGTWTCTAAT	TCCCTTGTCTCC
8	S1-FC2-2-C	GGACTACHVGGGTWTCTAAT	ACGAGACTGATT
9	S1-FC2-3-H	GGACTACHVGGGTWTCTAAT	GCTGTACGGATT
10	S1-FO2-1-H	GGACTACHVGGGTWTCTAAT	ATCACCAGGTGT
11	S1-FO2-2-C	GGACTACHVGGGTWTCTAAT	TGGTCAACGATA
12	S1-FO2-3-L	GGACTACHVGGGTWTCTAAT	ATCGCACAGTAA

13	S1-FO3-1-L	GGACTACHVGGGTWTCTAAT	GTCGTGTAGCCT
14	S1-FO3-2-H	GGACTACHVGGGTWTCTAAT	AGCGGAGGTTAG
15	S1-FO3-3-C	GGACTACHVGGGTWTCTAAT	ATCCTTTGGTTC
16	S1-FC3-1-H	GGACTACHVGGGTWTCTAAT	TACAGCGCATAC
17	S1-FC3-2-L	GGACTACHVGGGTWTCTAAT	ACCGGTATGTAC
18	S1-FC3-3-C	GGACTACHVGGGTWTCTAAT	AATTGTGTCGGA
19	S2-FO1-1-C	GGACTACHVGGGTWTCTAAT	TGCATACACTGG
20	S2-FO1-2-L	GGACTACHVGGGTWTCTAAT	AGTCGAACGAGG
21	S2-FO1-3-H	GGACTACHVGGGTWTCTAAT	ACCAGTGACTCA
22	S2-FC1-1-C	GGACTACHVGGGTWTCTAAT	GAATACCAAGTC
23	S2-FC1-2-H	GGACTACHVGGGTWTCTAAT	GTAGATCGTGTA
24	S2-FC1-3-L	GGACTACHVGGGTWTCTAAT	TAACGTGTGTGC
25	S2-FC2-1-L	GGACTACHVGGGTWTCTAAT	CATTATGGCGTG
26	S2-FC2-2-C	GGACTACHVGGGTWTCTAAT	CCAATACGCCTG
27	S2-FC2-3-H	GGACTACHVGGGTWTCTAAT	GATCTGCGATCC
28	S2-FO2-1-H	GGACTACHVGGGTWTCTAAT	CAGCTCATCAGC
29	S2-FO2-2-C	GGACTACHVGGGTWTCTAAT	CAAACAACAGCT
30	S2-FO2-3-L	GGACTACHVGGGTWTCTAAT	GCAACACCATCC
31	S2-FO3-1-L	GGACTACHVGGGTWTCTAAT	GCGATATATCGC
32	S2-FO3-2-H	GGACTACHVGGGTWTCTAAT	CGAGCAATCCTA
33	S2-FO3-3-C	GGACTACHVGGGTWTCTAAT	AGTCGTGCACAT
34	S2-FC3-1-H	GGACTACHVGGGTWTCTAAT	GTATCTGCGCGT
35	S2-FC3-2-L	GGACTACHVGGGTWTCTAAT	CGAGGGAAAGTC
36	S2-FC3-3-C	GGACTACHVGGGTWTCTAAT	CAAATTCGGGAT
37	S3-FO1-1-C	GGACTACHVGGGTWTCTAAT	AGATTGACCAAC
38	S3-FO1-2-L	GGACTACHVGGGTWTCTAAT	AGTTACGAGCTA
39	S3-FO1-3-H	GGACTACHVGGGTWTCTAAT	GCATATGCACTG

40	S3-FC1-1-C	GGACTACHVGGGTWTCTAAT	CAACTCCCGTGA
41	S3-FC1-2-H	GGACTACHVGGGTWTCTAAT	TTGCGTTAGCAG
42	S3-FC1-3-L	GGACTACHVGGGTWTCTAAT	TACGAGCCCTAA
43	S3-FC2-1-L	GGACTACHVGGGTWTCTAAT	CACTACGCTAGA
44	S3-FC2-2-C	GGACTACHVGGGTWTCTAAT	TGCAGTCCTCGA
45	S3-FC2-3-H	GGACTACHVGGGTWTCTAAT	ACCATAGCTCCG
46	S3-FO2-1-H	GGACTACHVGGGTWTCTAAT	TCGACATCTCTT
47	S3-FO2-2-C	GGACTACHVGGGTWTCTAAT	GAACACTTTGGA
48	S3-FO2-3-L	GGACTACHVGGGTWTCTAAT	GAGCCATCTGTA
49	S3-FO3-1-L	GGACTACHVGGGTWTCTAAT	TTGGGTACACGT
50	S3-FO3-2-H	GGACTACHVGGGTWTCTAAT	AAGGCGCTCCTT
51	S3-FO3-3-C	GGACTACHVGGGTWTCTAAT	TAATACGGATCG
52	S3-FC3-1-H	GGACTACHVGGGTWTCTAAT	TCGGAATTAGAC
53	S3-FC3-2-L	GGACTACHVGGGTWTCTAAT	TGTGAATTCGGA
54	S3-FC3-3-C	GGACTACHVGGGTWTCTAAT	CATTCGTGGCGT

Table 3.2. Reverse fungal primer (ITS4) synthesized with unique twelve base pair molecular identifier (MID) tags assigned to each sample from a long-term organic and conventional tomato management experiment. The treatments include three sampling times: S1 (sample 1:pre-planting), S2 (sample 2: maturity), and S3 (sample 3: postharvest); field organic (FO) plots or field conventional (FC) plots, with replicate number indicated (FO1, FC1, FO2, FC2, FO3, FC3); and fertility level: control (C), low-fertility (L), and high-fertility (H)).

Sample	Treatment	Gene region primer (ITS4)	Molecular Identifier (MID) tags
1	S1-FO1-1-C	TCCTCCGCTTATTGATATGC	ATGTCACCGCTG
2	S1-FO1-2-L	TCCTCCGCTTATTGATATGC	TGTAACGCCGAT
3	S1-FO1-3-H	TCCTCCGCTTATTGATATGC	AGCAGAACATCT
4	S1-FC1-1-C	TCCTCCGCTTATTGATATGC	GCCAACAACCAT
5	S1-FC1-2-H	TCCTCCGCTTATTGATATGC	TTGGCTCTATTC
6	S1-FC1-3-L	TCCTCCGCTTATTGATATGC	GATCCCACGTAC
7	S1-FC2-1-L	TCCTCCGCTTATTGATATGC	TCCCTTGTCTCC
8	S1-FC2-2-C	TCCTCCGCTTATTGATATGC	ACGAGACTGATT
9	S1-FC2-3-H	TCCTCCGCTTATTGATATGC	TACCGCTTCTTC
10	S1-FO2-1-H	TCCTCCGCTTATTGATATGC	ATCACCAGGTGT
11	S1-FO2-2-C	TCCTCCGCTTATTGATATGC	TGGTCAACGATA
12	S1-FO2-3-L	TCCTCCGCTTATTGATATGC	ATCGCACAGTAA
13	S1-FO3-1-L	TCCTCCGCTTATTGATATGC	GTCGTGTAGCCT
14	S1-FO3-2-H	TCCTCCGCTTATTGATATGC	GATTATCGACGA
15	S1-FO3-3-C	TCCTCCGCTTATTGATATGC	ATCCTTTGGTTC
16	S1-FC3-1-H	TCCTCCGCTTATTGATATGC	GCCTAGCCCAAT
17	S1-FC3-2-L	TCCTCCGCTTATTGATATGC	ACCGGTATGTAC
18	S1-FC3-3-C	TCCTCCGCTTATTGATATGC	GATGTATGTGGT
19	S2-FO1-1-C	TCCTCCGCTTATTGATATGC	TGCATACACTGG

20	S2-FO1-2-L	TCCTCCGCTTATTGATATGC	AGTCGAACGAGG
21	S2-FO1-3-H	TCCTCCGCTTATTGATATGC	ACCAGTGACTCA
22	S2-FC1-1-C	TCCTCCGCTTATTGATATGC	GAATACCAAGTC
23	S2-FC1-2-H	TCCTCCGCTTATTGATATGC	GTAGATCGTGTA
24	S2-FC1-3-L	TCCTCCGCTTATTGATATGC	TAACGTGTGTGC
25	S2-FC2-1-L	TCCTCCGCTTATTGATATGC	ACTCCTTGTGTT
26	S2-FC2-2-C	TCCTCCGCTTATTGATATGC	CCAATACGCCTG
27	S2-FC2-3-H	TCCTCCGCTTATTGATATGC	ACTTGGTGTAAG
28	S2-FO2-1-H	TCCTCCGCTTATTGATATGC	TCACCTCCTTGT
29	S2-FO2-2-C	TCCTCCGCTTATTGATATGC	CAAACAACAGCT
30	S2-FO2-3-L	TCCTCCGCTTATTGATATGC	GCAACACCATCC
31	S2-FO3-1-L	TCCTCCGCTTATTGATATGC	GCACACCTGATA
32	S2-FO3-2-H	TCCTCCGCTTATTGATATGC	CGAGCAATCCTA
33	S2-FO3-3-C	TCCTCCGCTTATTGATATGC	AGTCGTGCACAT
34	S2-FC3-1-H	TCCTCCGCTTATTGATATGC	GCGACAATTACA
35	S2-FC3-2-L	TCCTCCGCTTATTGATATGC	CGAGGGAAAGTC
36	S2-FC3-3-C	TCCTCCGCTTATTGATATGC	TCATGCTCCATT
37	S3-FO1-1-C	TCCTCCGCTTATTGATATGC	AGATTGACCAAC
38	S3-FO1-2-L	TCCTCCGCTTATTGATATGC	AGTTACGAGCTA
39	S3-FO1-3-H	TCCTCCGCTTATTGATATGC	GCATATGCACTG
40	S3-FC1-1-C	TCCTCCGCTTATTGATATGC	CAACTCCCGTGA
41	S3-FC1-2-H	TCCTCCGCTTATTGATATGC	GAGAGCAACAGA
42	S3-FC1-3-L	TCCTCCGCTTATTGATATGC	TACGAGCCCTAA
43	S3-FC2-1-L	TCCTCCGCTTATTGATATGC	CACTACGCTAGA
44	S3-FC2-2-C	TCCTCCGCTTATTGATATGC	TGCAGTCCTCGA
45	S3-FC2-3-H	TCCTCCGCTTATTGATATGC	ACCATAGCTCCG
46	S3-FO2-1-H	TCCTCCGCTTATTGATATGC	TCGACATCTCTT

47	S3-FO2-2-C	TCCTCCGCTTATTGATATGC	GAACACTTTGGA
48	S3-FO2-3-L	TCCTCCGCTTATTGATATGC	GAGCCATCTGTA
49	S3-FO3-1-L	TCCTCCGCTTATTGATATGC	TTGGGTACACGT
50	S3-FO3-2-H	TCCTCCGCTTATTGATATGC	CGTGCTTAGGCT
51	S3-FO3-3-C	TCCTCCGCTTATTGATATGC	CACTCATCATTC
52	S3-FC3-1-H	TCCTCCGCTTATTGATATGC	TATCTATCCTGC
53	S3-FC3-2-L	TCCTCCGCTTATTGATATGC	TTGCCAAGAGTC
54	S3-FC3-3-C	TCCTCCGCTTATTGATATGC	CATACCGTGAGT

Table 3.3. Type III tests of fixed effects in a GLMM analysis of bacterial richness, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. The fixed effects and the two-and-three-way interactions included: management type (conventional vs organic); fertilizer level (high fertility, low fertility, and control); sampling time (pre-planting, maturity, and postharvest); and the three-way interaction management type, fertilizer level and sampling time.

Bacterial richness- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Management type	1	30	1.01	0.32
Fertilizer level	2	4	0.97	0.45
Management type* Fertilizer level	2	30	1.15	0.32
Sampling time	2	30	15.26	<0.0001
Management type* Sampling time	2	30	0.16	0.85
Fertilizer level* Sampling time	4	30	0.19	0.94
Management type* Fertilizer level* Sampling time	4	30	0.10	0.98

Table 3.4. Type III tests of fixed effects in a GLMM analysis of fungal richness, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. The fixed effects and the two-and-three-way interactions included: management type (conventional vs organic); fertilizer level (high fertility, low fertility, and control); sampling time (pre-planting, maturity, and postharvest); and the three-way interaction management type, fertilizer level and sampling time.

Fungal richness- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Management type	1	30	0.42	0.52
Fertilizer level	2	4	0.30	0.75
Management type* Fertilizer level	2	30	0.03	0.96
Sampling time	2	30	12.71	0.0001
Management type* Sampling time	2	30	1.08	0.35
Fertilizer level* Sampling time	4	30	0.73	0.57
Management type* Fertilizer level* Sampling time	4	30	0.62	0.65

Table 3.5. Type III tests of fixed effects in a GLMM analysis of bacterial Simpson´s diversity, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. The fixed effects and the two-and-three-way interactions included: management type (conventional vs organic); fertilizer level (high fertility, low fertility, and control); sampling time (pre-planting, maturity, and postharvest); and the three-way interaction management type, fertilizer level and sampling time.

Bacterial Simpson´s diversity- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Management type	1	30	0.90	0.35
Fertilizer level	2	4	1.61	0.31
Management type* Fertilizer level	2	30	1.32	0.28
Sampling time	2	30	2.61	0.09
Management type* Sampling time	2	30	0.83	0.44
Fertilizer level* Sampling time	4	30	1.42	0.24
Management type* Fertilizer level* Sampling time	4	30	0.93	0.46

Table 3.6. Type III tests of fixed effects in a GLMM analysis of fungal Simpson´s diversity, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. The fixed effects and the two-and-three-way interactions included: management type (conventional vs organic); fertilizer level (high fertility, low fertility, and control); sampling time (pre-planting, maturity, and postharvest); and the three-way interaction management type, fertilizer level and sampling time.

Fungal Simpson´s diversity- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Management type	1	30	1.46	0.23
Fertilizer level	2	4	0.63	0.57
Management type* Fertilizer level	2	30	0.52	0.60
Sampling time	2	30	1.50	0.24
Management type* Sampling time	2	30	1.57	0.22
Fertilizer level* Sampling time	4	30	0.68	0.61
Management type* Fertilizer level* Sampling time	4	30	0.28	0.88

Table 3.7. Type III tests of fixed effects in a GLMM analysis of bacterial Simpson´s evenness, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. The fixed effects and the two-and-three-way interactions included: management type (conventional vs organic); fertilizer level (high fertility, low fertility, and control); sampling time (pre-planting, maturity, and postharvest); and the three-way interaction management type, fertilizer level and sampling time.

Bacterial Simpson´s evenness- Type III test of fixed effects				
Effect	Degrees of freedom of the numerator (Num DF)	Degrees of freedom of the denominator (Den DF)	F value	Probability > F
Management type	1	30	0.13	0.71
Fertilizer level	2	4	0.72	0.53
Management type* Fertilizer level	2	30	0.86	0.43
Sampling time	2	30	1.20	0.31
Management type* Sampling time	2	30	0.87	0.43
Fertilizer level* Sampling time	4	30	0.66	0.62
Management type* Fertilizer level* Sampling time	4	30	0.19	0.94

Table 3.8. Type III tests of fixed effects in a GLMM analysis of fungal Simpson´s evenness, to evaluate conventional vs organic management systems for three fertilizer levels across three sampling times. The fixed effects and the two-and-three-way interactions included: management type (conventional vs organic); fertilizer level (high fertility, low fertility, and control); sampling time (pre-planting, maturity, and postharvest); and the three-way interaction management type, fertilizer level and sampling time.

Fungal Simpson´s evenness- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Management type	1	30	0.60	0.44
Fertilizer level	2	4	0.28	0.77
Management type* Fertilizer level	2	30	0.07	0.92
Sampling time	2	30	1.53	0.23
Management type* Sampling time	2	30	1.69	0.20
Fertilizer level* Sampling time	4	30	0.31	0.86
Management type* Fertilizer level* Sampling time	4	30	0.56	0.69

Table 3.9. The 20 overall most frequent *bacterial* phyla, orders, families, and OTUs assigned to genera recovered in MiSeq sequencing of soils from a long-term organic-conventional management experiment conducted at the Kansas State University Horticulture Extension Center in Olathe, Kansas, USA.

Phyla	Freq. (%)	Orders	Freq. (%)	Families	Freq. (%)	Genera	Freq. (%)
Proteobacteria	25.42	unclassified	35.41	unclassified	44.08	*unclassified*	56.09
unclassified	21.41	Sphingobacteria-les	6.65	Chitinopha-gaceae	6.29	*Gp4*	5.38
Acidobacteria	15.06	Acidobacteria_ Gp4_ order_incertae_sedis	4.74	Acidobacteria Gp4 family_incertae_ sedis	5.04	*Gp6*	5.27
Bacteroidetes	10.13	Actinomycetales	4.72	Acidobacteria Gp6 family_incertae sedis	4.94	*Spartobacteria_ genera_incertae_ sedis*	4.82
Actinobacteria	8.84	Acidobacteria_Gp6 order_incertae_sedis	4.65	Spartobacteria family incertae_sedis	4.53	*Sphingomonas*	3.15
Verrucomicro-bia	7.38	Spartobacteria_ order incertae_sedis	4.27	Planctomyceta-ceae	3.77	*3_genus_incertae sedis*	2.64
Planctomycetes	3.44	Rhizobiales	3.63	Sphingomonada-ceae	3.26	*Gp3*	2.45
Firmicutes	2.98	Planctomycetales	3.55	Subdivision3_ family incertae_sedis	2.48	*Nitrospira*	1.44
Chloroflexi	1.73	Sphingomonadales	3.20	Acidobacteria_ Gp3_ family_incertae_ sedis	2.30	*Gemmatimonas*	1.30
Nitrospira	1.21	Bacillales	3.06	Anaerolineaceae	1.36	*Gp16*	1.18
Gemmatimona-detes	1.09	Myxococcales	2.60	Nitrospiraceae	1.35	*Pasteuria*	0.99
Armatimona-detes	0.68	Burkholderiales	2.59	Gemmatimonadac eae	1.22	*Microlunatus*	0.85
OD1	0.18	Subdivision3_order incertae_sedis	2.33	Acidobacteria_Gp 16_ family_incertae_ sedis	1.11	*Steroidobacter*	0.78

TM7	0.18	Acidobacteria_Gp3_ order_incertae_sedis	2.17	Micromonospo-raceae	1.09	*Gp7*	0.74
Chlamydiae	0.12	Xanthomonadales	1.57	Pasteuriaceae	0.93	*Planctomyces*	0.71
Deinococcus-Thermus	0.08	Anaerolineales	1.28	Xanthomona-daceae	0.93	*Flavobacterium*	0.71
OP11	0.05	Nitrospirales	1.27	Propionibacteria-ceae	0.82	*Gp17*	0.69
Spirochaetes	0.01	Gemmatimonadales	1.15	Sinobacteraceae	0.74	*Gp1*	0.45
BRC1	0.01	Acidobacteria_Gp16 order_incertae_sedis	1.04	Nocardioidaceae	0.73	*Ohtaekwangia*	0.43
Fusobacteria	0.00	Rhodospirillales	0.92	Flavobacteriaceae	0.70	*Gp5*	0.42

Table 3.10. The 20 overall most frequent *fungal* phyla, orders, families, and OTUs assigned to genera recovered in MiSeq sequencing of soils from a long-term organic-conventional management experiment conducted at the Kansas State University Horticulture Extension Center in Olathe, Kansas, USA.

Phylum	Freq. (%)	Order	Freq. (%)	Family	Freq. (%)	Genus	Freq. (%)	Specie	Freq. (%)
Ascomycota	57.89	Pleosporales	18.71	unclassified	14.26	*unclassified*	19.57	*unclassified*	33.79
Basidiomycota	24.70	Hypocreales	18.36	Mortierellaceae	11.42	*Mortierella*	12.51	*Phoma_sp_ UASWS0872*	9.54
Zygomycota	11.24	Mortierellales	11.18	Pleosporales_ family_ *Incertae sedis*	8.68	*Phoma*	9.04	*Alternaria porri*	4.49
Unclassified	4.25	unclassified	9.03	Pleosporaceae	7.08	*Alternaria*	4.25	*Lysurus cruciatus*	4.04
Chytridiomycota	1.48	Sordariales	5.01	Nectriaceae	6.60	*Lysurus*	3.83	*Hannaella sinensis*	3.18
Glomeromycota	0.43	Phallales	4.89	Phallaceae	5.01	*Hannaella*	3.04	*Mortierella humilis*	3.06
		Tremellales	4.25	Tremellales_ family_ *Incertae sedis*	4.34	*Myrothecium*	2.79	*Stephanosporaceae sp*	1.97
		Chaetothyriales	3.07	Hypocreales_ family_ *Incertae sedis*	3.75	*Chaetomium*	2.67	*Bipolaris_ microstegii*	1.76
		Agaricales	2.82	Chaetomiaceae	3.31	*Talaromyces*	2.58	*Phallus rugulosus*	1.72
		Eurotiales	2.55	Trichocomaceae	2.60	*Cryptococcus*	2.42	*Bionectriaceae sp*	1.60
		Xylariales	2.31	Xylariales_ family_ *Incertae sedis*	2.27	*unclassified_ Stephanosporaceae*	1.86	*Exophiala equina*	1.52
		Cantharellales	2.05	Herpotrichiellaceae	2.10	*Bipolaris*	1.67	*Monographella cucumerina*	1.49
		Russulales	1.67	Stephanosporaceae	1.71	*Phallus*	1.66	*Mortierella exigua*	1.41

Filobasidiales	1.26	Bionectriaceae	1.70	*unclassified_ Bionectriaceae*	1.52	*Myrothecium inundatum*	1.32
Pezizales	1.22	Hypocreaceae	1.61	*Exophiala*	1.49	*Olpidium brassicae*	0.96
Auriculariales	1.17	Lasiosphaeriaceae	1.46	*Monographella*	1.41	*Sistotrema sp*	0.92
Corticiales	1.16	Filobasidiaceae	1.28	*Cyphellophora*	1.07	*Mortierella capitata*	0.91
Cystofilobasidiales	0.97	Ascomycota family_ *Incertae sedis*	0.99	*Olpidium*	0.91	*Xylariales sp*	0.90
Ascomycota order_ *incertae sedis*	0.97	Chaetothyriaceae	0.98	*Sistotrema*	0.87	*Rhizoctonia zeae*	0.89
Olpidiales	0.81	Cystofilobasidiaceae	0.87	*unclassified_ Xylariales*	0.85	*Minimedusa sp*	0.71

Table 3.11. Permanova analysis for bacterial communities in organic plots for three fertility levels (control, low-and-high fertilities) across three sampling time points (pre-planting, maturity, and postharvest) on a tomato cropping season.

	Degrees of freedom	Sum of squares	R^2	F value	Probability (>F)
Fertility level	2	0.043	0.052	0.659	0.940
Residual	24	0.778	0.947		
Total	26	0.821	1.000		

Table 3.12. Permanova analysis for bacterial communities in conventional plots for three fertility levels (control, low-and-high fertilities) across three sampling time points (pre-planting, maturity, and postharvest) on a tomato cropping season.

	Degrees of freedom	Sum of squares	R^2	F value	Probability (>F)
Fertility level	2	0.044	0.046	0.588	0.925
Residual	24	0.894	0.953		
Total	26	0.938	1.000		

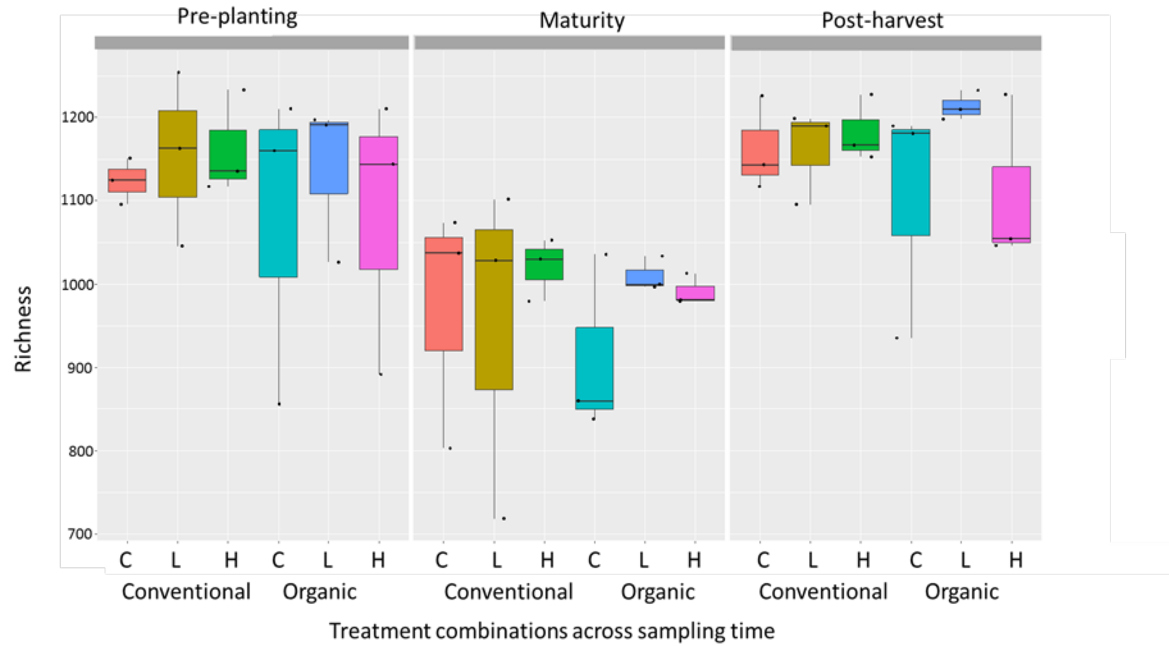

Figure 3.1. Bacterial richness in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest). GLMM analyses found strong evidence for a sampling time effect on bacterial richness (F = 15.26, p < 0.0001), but no evidence for management type (conventional vs. organic: p = 0.32) or fertility levels (p = 0.45).

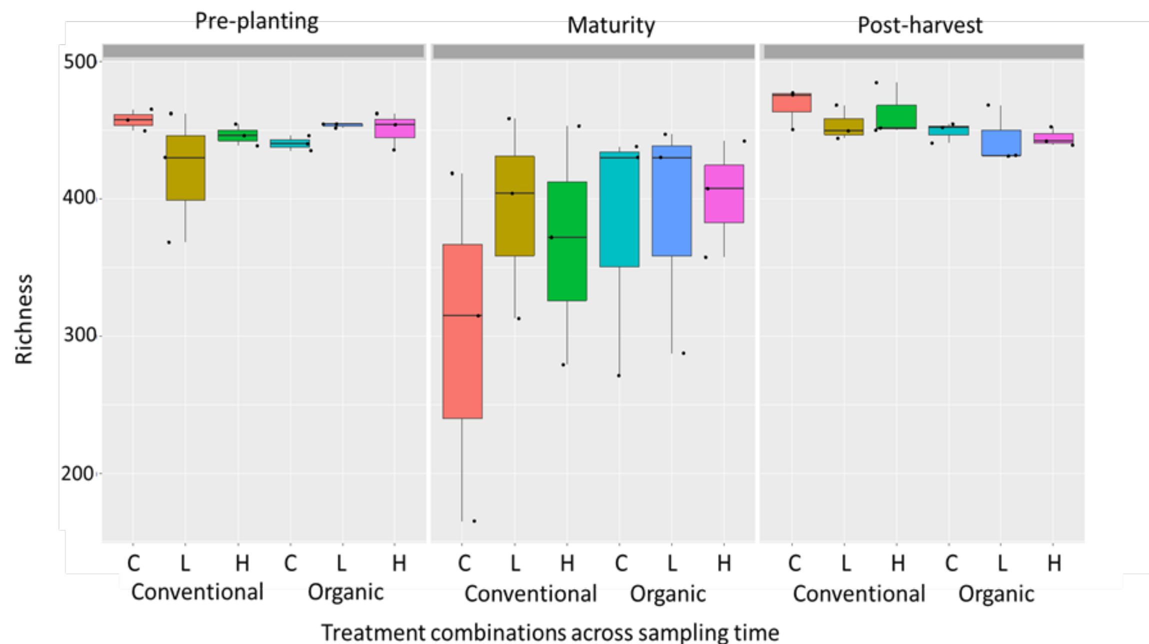

Figure 3.2. Fungal richness in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest). GLMM analyses found evidence for a sampling time effect on fungal richness ($F = 12.71$, $p = 0.0001$), but no evidence for management type (conventional vs. organic: $p = 0.52$) or fertility levels ($p = 0.75$).

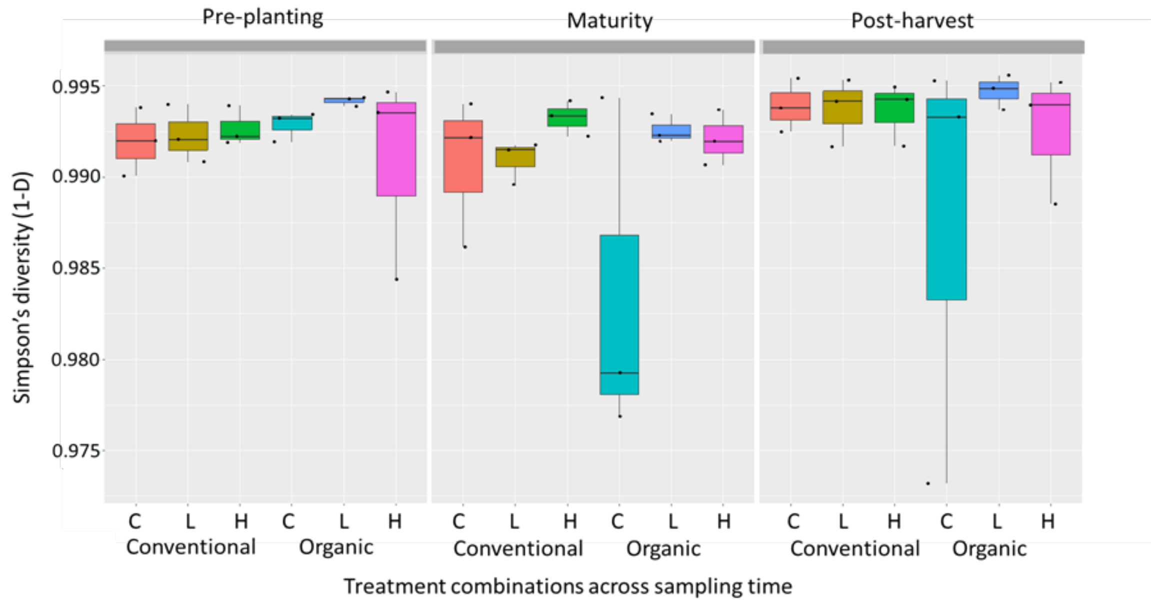

Figure 3.3. Bacterial Simpson´s diversity in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest). GLMM analyses found evidence for a sampling time effect on bacterial Simpson´s diversity (F = 2.61, p = 0.09), but no evidence for management type (conventional vs. organic: p = 0.35) or fertility levels (p = 0.31).

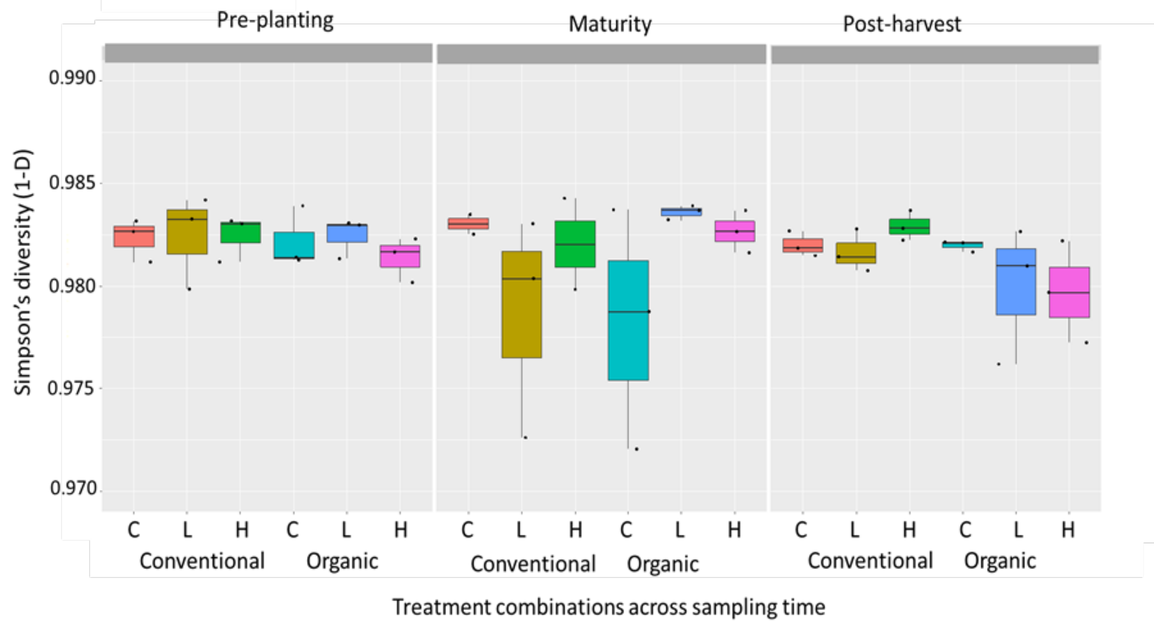

Figure 3.4. Fungal Simpson´s diversity in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest). GLMM analyses did not find evidence for any of the effects (management type, fertilizer level, sampling time) or their interactions (*p* > 0.2 for each effect and interaction).

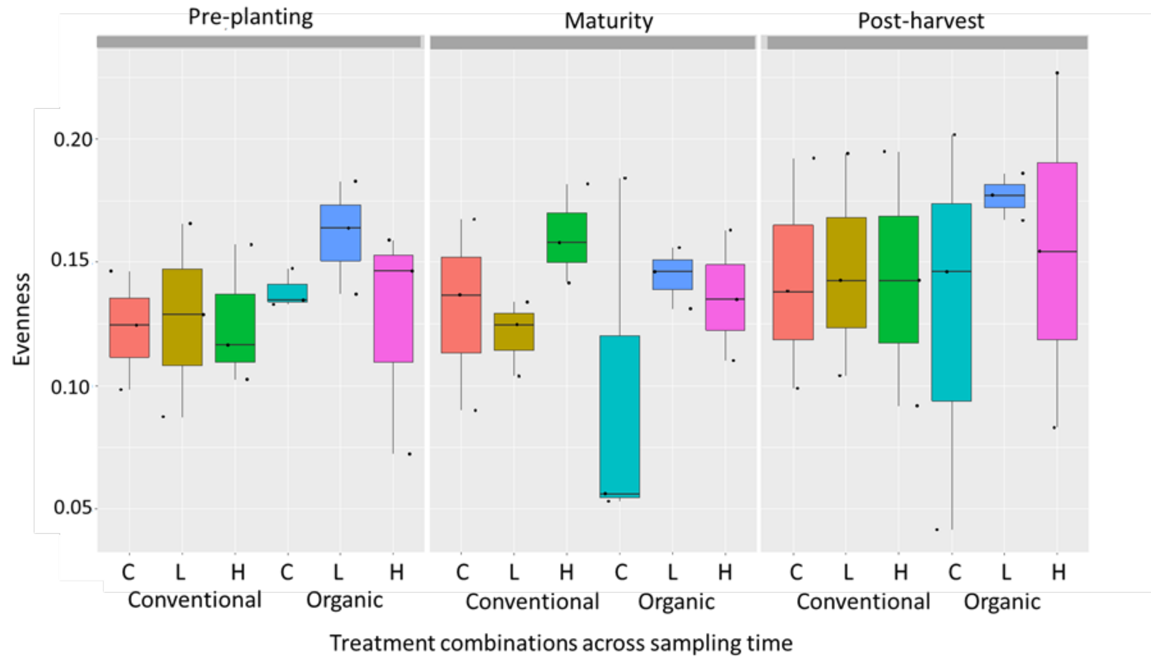

Figure 3.5. Bacterial Simpson´s evenness in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest). GLMM analyses did not find evidence for any of the effects (management type, fertilizer level, sampling time) or their interactions (*p* > 0.3 for each effect and interaction).

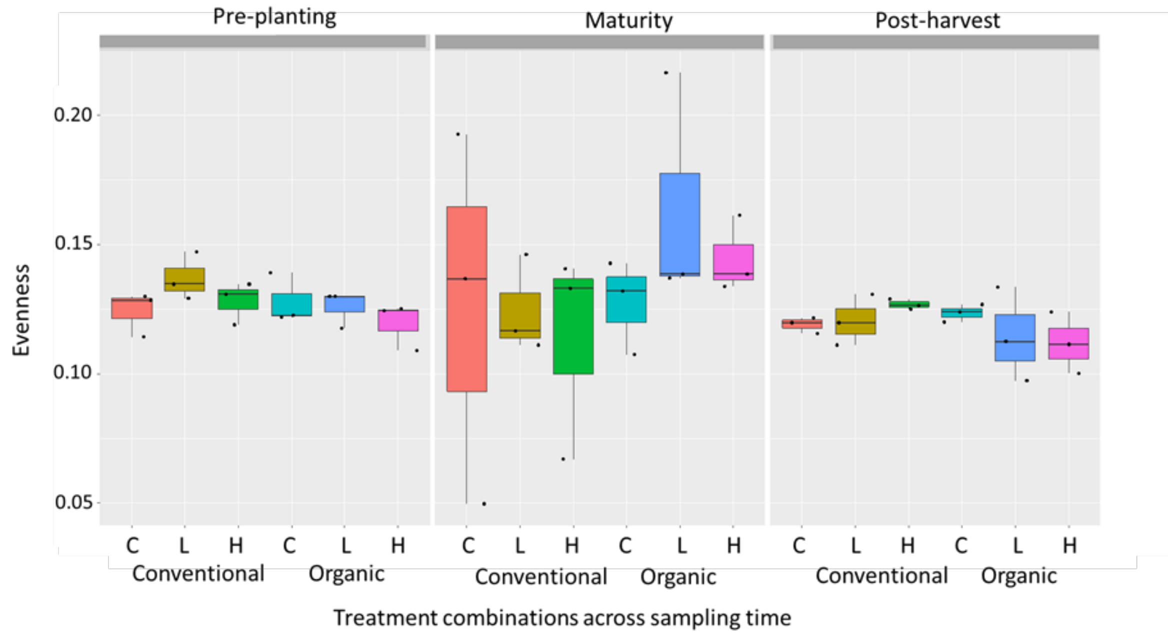

Figure 3.6. Fungal Simpson´s evenness in soil from conventional and organic tomato management systems with three fertility levels (control (C), low fertility (L), and high fertility (H)) across three sampling times (pre-planting, maturity, and postharvest). GLMM analyses did not find evidence for any of the effects (management type, fertilizer level, sampling time) or their interactions (*p* > 0.2 for each effect and interaction).

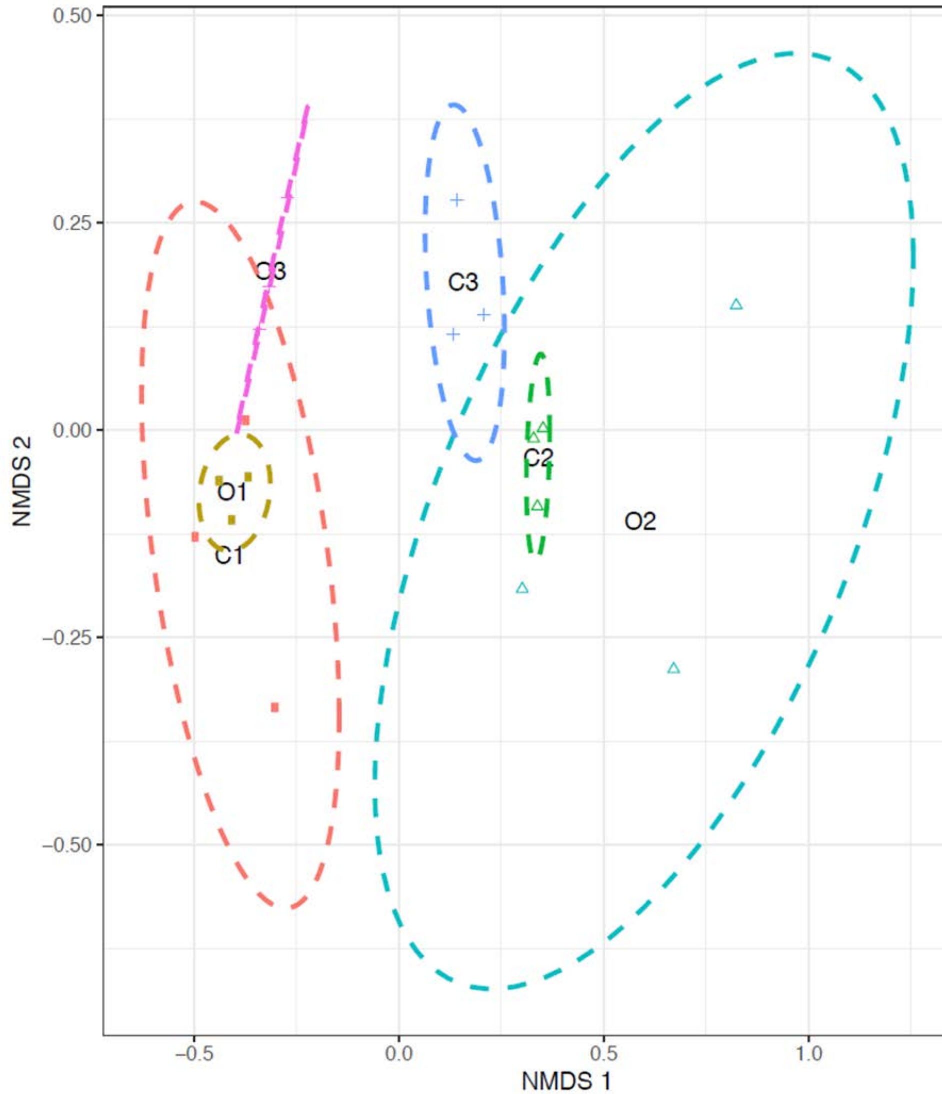

Figure 3.7. Non-metric multidimensional scaling (NMDS) in two dimensions of *bacterial* communities from plots receiving organic (O) or conventional (C) nutrient amendments. Ovals indicate 95% confidence regions for responses at each sampling time point (pre-planting (1), maturity (2), and postharvest (3)). Two convergent solutions were found after 26 trials with a final stress of 0.15 and a maximum residual of 0.0002.

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Chapter 4 - The effects of organic amendments and plastic mulch on soil bacterial and fungal communities over time in tomato cropping systems

Abstract

Soil microorganisms are central to ecosystem functioning and the sustainability of soil resources. Growing evidence suggests that organic management systems may foster more diverse soil microbial communities, which have the potential to be beneficial for crop production. As an initial step toward microbiome-informed farming systems, we evaluated the responses of soil bacterial and fungal communities to common organic farming practices. In tomato production at a transitional organic farm, we evaluated the effects of high tunnels, the use of plastic mulch, and two organic soil amendments (alfalfa meal pellets and city compost) on microbial community diversity and composition across two sampling times (maturity and postharvest). Illumina MiSeq sequencing of 16S and ITS2 rRNA gene amplicons was used to characterize communities. Organic amendments increased bacterial richness compared to the control treatment, but did not increase fungal richness, diversity or evenness. The use of high tunnels did not affect bacterial or fungal diversity. The use of plastic mulch on field plots did not affect bacterial diversity, but plastic mulch decreased fungal richness. Understanding how microbial communities respond to organic amendments, high tunnels, and plastic mulch will ultimately contribute to microbiome-informed agricultural management.

Introduction

Microbial communities are key to most soil biogeochemical cycles, and soil microbial diversity and community composition may have important roles in soil health (Garbeva *et al.*, 2004; Zaccardelli *et al.*, 2013). Soil health has been defined as the resilience of soil in response to stress, in terms of its ability to sustain high biological diversity and high levels of internal nutrient cycling, and to maintain environmental quality and promote plant and animal health (Doran *et al.*, 1996; van Bruggen and Semenov, 2000). The first step toward maintaining these properties is to understand the factors that may impact microbial communities in soils. Soil microbial communities respond to soil management (Spedding *et al.*, 2004; Acosta-Martinez *et al.*, 2010; Yin *et al.*, 2010). The biomass and composition of soil microbial communities are affected by soil cover crops (Zelles *et al.*, 1995; Maul *et al.*, 2014), plant species (Liu *et al.*, 2016; Pii *et al.*, 2016; Sheng *et al.*, 2016), cultivation techniques (Lalande *et al.*, 2005; Ge *et al.*, 2013; Smith *et al.*, 2014), application of fertilizers and pesticides (Ibekwe *et al.*, 2004; Cycon *et al.*, 2013; Miura *et al.*, 2016) and application of organic residues (Marschner *et al.*, 2004; Ikeda *et al.*, 2014; Vida *et al.*, 2016).

A central idea in organic agriculture is that organic management can better maintain the biological function of soil and promote plant health by eliminating the use of most synthetic fertilizers and pesticides, supplying regular inputs of organic matter, and using nutrient recycling strategies based on crop rotation (Stockdale *et al.*, 2001; Fließbach *et al.*, 2007; Azadi *et al.*, 2011). Organic farming has the potential to contribute to these processes through techniques that may foster more diverse soil microbial communities (Sivapalan *et al.*, 1993; Drinkwater *et al.*, 1995; Shannon *et al.*, 2002; Reeve *et al.*, 2010; Xue *et al.*, 2013), being designed to support soil health and increase agro-ecosystem resilience to stress (Mader *et al.*, 1996; Mäder *et al.*, 2002; Azadi *et al.*, 2011; Scotti *et al.*, 2013). Higher concentrations of soil microbial biomass and different

communities have been observed in systems with organic manure application, compared to systems with mineral fertilization (Zelles *et al.*, 1995; Marschner *et al.*, 2004). The application of organic manure may be a primary reason for differences in microbial communities when conventional and organic farming management are compared (Bossio *et al.*, 1998; Gunapala and Scow, 1998). In the past, technical limitations have made it difficult to evaluate the dynamics of microbial communities and to assess their role when comparing organic and conventional farming systems (Buckley and Schmidt, 2003; Nelson and Spaner, 2010). High-throughput DNA metabarcoding tools offer opportunities to dissect soil microbiota more efficiently at higher resolution, and have the potential to reveal community and taxon-level responses to agricultural management (Taberlet *et al.*, 2012).

In organic farming systems, which are often low-input agro-ecosystems, plant productivity and ecosystem functionality are supported by cover crops and/or livestock manure (Lammerts van Bueren *et al.*, 2002). Organic manure application generally increases soil microbial diversity and richness (Zhen *et al.*, 2014; Hartmann *et al.*, 2015; Francioli *et al.*, 2016), and increases soil nutrient levels and organic matter content (Mäder *et al.*, 2002; Liang *et al.*, 2012).

The effects of organic amendments on soil microbial community composition may change over time. Soil microbial biomass and community composition can respond rapidly to changing environmental conditions (Schloter *et al.*, 2003), so microbial characteristics are potentially valuable indicators for assessing the effect of agricultural management practices (Gil-Sotres *et al.*, 2005). Seasonal changes in soil communities may be hastened or slowed by agricultural systems that affect soil temperature and moisture, such as the use of high tunnels or plastic mulch. Organic management in greenhouses may increase microbial biomass carbon, and bacterial and fungal

phospholipid fatty acid (PLFA) concentrations, compared to conventional management in greenhouses and both conventional and organic management in open fields (Ge *et al.*, 2013).

High tunnels, or hoop houses, are open greenhouse-like structures that provide a protected environment for crop production throughout the year. In contrast to traditional greenhouse production where plants are planted on trays and pots in benches, in high tunnel production plants are planted directly in the ground. Because natural rain is excluded, the soil in high tunnels is very dry outside the irrigation zone. The primary function of high tunnels is to modify the crop environment by elevating temperatures to allow earlier planting in the spring, earlier ripening, and extended fall harvest (Lamont, 2005; Kadir *et al.*, 2006) . Other benefits include protection from wind and rain damage, reduction of some diseases and insects compared to the open field, and typically greater crop yield and quality relative to open fields (Wells and Loy, 1993; Lamont, 2005; Zhao and Carey, 2009). Only a few studies have addressed the effects of high tunnels on microbial communities (Ibekwe and Kennedy, 1998; Yao *et al.*, 2006; Shi *et al.*, 2009; Ge *et al.*, 2013). Ge *et al.* (2013) used phospholipid fatty acid (PLFA) analyses to evaluate high tunnel effects, concluding that they produced greater microbial activity and biomass compared to open fields. In contrast, other studies, using PLFA and/or community level physiological profiles (CLPPs), found a more diverse microbial communities in open field plots (Ibekwe and Kennedy, 1998; Yao *et al.*, 2006; Shi *et al.*, 2009). Ge *et al.* (2013) concluded that high tunnels could enhance the effects of organic management by creating more favorable environmental conditions that increase soil microbial activity and diversity. On the other hand, the high tunnel microclimate, and the rapid accumulation of nutrients and salinity, could impact soil microbial functional diversity. There is thus a risk of soil degradation, (Yao *et al.*, 2006; Shi *et al.*, 2009).

Plastic mulch, the application of plastic on the soil surface with regularly spaced holes for plants to grow through, is used to limit soil water evaporation, maintain good soil structure, and protect crops from soil contamination (Kasirajan and Ngouajio, 2012). Plastic mulch has been used extensively in vegetable production since the 1960s (Lamont, 2005). Benefits from mulching include weed control, reduced erosion and leaching of nutrients, maintenance of soil moisture (Carter and Johnson, 1988; Tindall *et al.*, 1991), and enhanced microbial activity (Hankin *et al.*, 1982). Plastic mulch is often reported to have effects such as decreasing the amount of water loss caused by evaporation (Kasirajan and Ngouajio, 2012), increasing soil temperatures, and effects on soil nutrient availability (Wang *et al.*, 2015). Plastic mulch in combination with manure application may improve nutrient availability (Lamont, 2005; Li *et al.*, 2009; Cuello *et al.*, 2015). Most studies of plastic mulch have addressed soil moisture, structure, and nutrition, and crop yield (Li *et al.*, 1999; Li *et al.*, 2009; Gao *et al.*, 2014; Steinmetz *et al.*, 2016), while plastic mulch effects on the soil micro-ecological environment have been neglected (Steenwerth *et al.*, 2002). Changes in soil microbial communities under plastic mulch have been reported (Li *et al.*, 2009; Chen *et al.*, 2014; Dong *et al.*, 2017; Farmer *et al.*, 2017). Plastic mulch combined with organic amendments may increase microbial diversity (Hou *et al.*, 2007; Dong *et al.*, 2017; Farmer *et al.*, 2017). In the long term, new technologies for monitoring individual microbial taxa may provide strategies to make organic farming systems more productive and profitable, by providing information about the effects of management decisions such as plastic mulching on microbial communities, and of microbial communities on production.

The objectives of this study were to determine the responses of soil microbial communities across the tomato production season to (a) the use of high tunnels, (b) the use of plastic mulch, and (c) organic nutrient amendments. We used Illumina MiSeq metabarcoding of bacterial and fungal

ribosomal markers to examine the response of soil microbial diversity in a farm transitioning to organic vegetable production. Based on the literature discussed above, we first hypothesized that the use of high tunnels and plastic mulch would affect microbial communities, and that organic nutrient amendments would increase bacterial and fungal diversity compared to the control (no amendment). Similar temporal dynamics of the microbial communities might be observed earlier in high tunnels and later in the open field, if sampling frequency is high enough to detect the lag. Second, we characterized soil microbial taxa that were most frequent over all treatment combinations and sampling times during a tomato growing season. Third, we used non-metric multidimensional scaling (NMDS) to evaluate changes in community structures across time in response to soil amendments.

Materials and Methods

Study location, experimental design and on-farm methodology

The research site was at Common Harvest farm in Lawrence, Kansas, at 38°96´N and 95°2´W. The farm's cropping history goes back to 2009, when the farm was cropped with soybean and maize. In 2010, it was used as a horse pasture. Later in 2010, the farm was in conversion from conventional agriculture to organic certification and in 2014 was certified organic by the Oklahoma Department of Agriculture, Food and Forestry. The soil type is Eudora kimo complex, predominantly a sandy loam. The soil is approximately 6.5% sand, 78% silt and 15% clay, based on a 2011 test at the Kansas State University Soil testing lab. Annual mean temperature in the area is ~12 °C and mean annual rainfall is 1 m (http://www.usclimatedata.com). The research plots

were put in place in early 2011 and included two high tunnels (29.3 m by 6 m each, separated by a 3 m sidewalk) and eight adjacent open field plots (14 m by 6 m each, separated by 3 m fescue grass (*Festuca* sp.) buffers) (Fig. 4.1). The experiment implemented a split-plot design, in which field plots with plastic mulch, field plots without plastic mulch, or high tunnels were assigned at the whole plot level. (Note that for practical reasons the high tunnels were not interspersed with the open field plots, but the nearest open field plot was only 9 m from the high tunnels so they were in a similar environment). Four of the eight open field whole plots were randomly assigned the plastic mulch treatment and were covered with a single layer of 1.0 mil embossed black plastic mulch (Grower´s solution, Cookeville, TN, USA), while the other four field plots were uncovered. The endwalls from the high tunnels were open throughout the summer.

Within each whole plot, there were three 4.6 m by 6 m subplots, with one of three nutrient management treatments randomly assigned to each subplot. Within each subplot, three rows of six tomato plants each (*Lycopersicum esculentum* cv. "Mountain Fresh") were planted. All subplots were tilled using a rototiller powered by a farm tractor and were raked to 30 cm depth. The three nutrient management treatments were alfalfa meal pellets, city compost, and no nutrient addition (the control). There was a total of 30 subplots ((2 high tunnels + 8 field plots) x 3 nutrient management treatments). The ten subplots receiving alfalfa meal pellets (N-P_2O_5 –K_2O 1-3-5, Bradfield Organics® Luscious Lawn and Garden Fertilizer, RitePack Inc., St. Joseph, MO, USA) were fertilized before transplanting (using 9 kg per 92 m^2). The ten subplots receiving city compost were also fertilized before transplanting using ca. 0.63 cm of compost to every 2.54 cm depth of tilled soil. The city compost from Lawrence was made from the weekly curbside collection of residential yard trimmings and small woody debris which included grass, leaves, garden pruning and small woody waste collected from commercial lawn care providers

(https://lawrenceks.org/swm/lawncompostsale/). The remaining ten subplots assigned to the control were not fertilized. The analysis of high tunnel effects was based on a comparison of results in two high tunnel whole plots and in four field whole plots without plastic mulch. The analysis of plastic mulch effects was based on a comparison of results in the four field whole plots with plastic mulch and in the four field whole plots without the plastic mulch treatment.

Soil sampling

Soil associated with the tomato crop was sampled twice in 2011, to evaluate how soil communities changed between maturity and tomato senescence. Sampling took place July 1st, at mid-season (mature tomato plants: some plants with flowers and green fruits), and Oct 7th, at the end of the season (post-harvest: tomato plants senescent but still in place in the plots). In each of the 30 subplots (experimental units), six soil cores (15 cm deep, 5 cm diam.) were sampled systematically near the base of the middle four tomato plants in the middle row, avoiding the subplot edges by 61 cm on each end. The soil corers were cleaned with water and dried with a clean paper towel after each subplot was sampled, to avoid cross-contamination between subplots. The six soil cores from each subplot were bulked and homogenized in a clean plastic bucket to form one composite soil sample per subplot. Samples were stored in labeled plastic bags on ice in a cooler during sample collection and transport. From each composite sample, we collected ~400 g soil to assess physical and chemical soil properties and another ca. 0.7 g for DNA extraction, which was stored in MoBio bead solution tubes (Ultra Clean Soil DNA Isolation Kit; MoBio Laboratories, Carlsbad, CA, USA) to protect the DNA integrity. Nine bead solution tubes with ~0.7 g of soil per tube were used for each composite sample. Upon arrival at the laboratory, soils

for DNA extraction samples were frozen at -80 °C until PCR amplification, and soil for edaphic analyses was oven-dried at 50 °C for 96 h.

Soil physical and chemical analyses

Soil physical and chemical properties were analyzed twice during the tomato season by the Kansas State Research and Extension Soil Testing Laboratory from the Department of Agronomy at Kansas State University, Manhattan, Kansas, USA. The first soil testing was conducted before tomato plants were transplanted on the subplots (pre-planting); the second was conducted at the end of the season (postharvest). Soil particle size (texture) was estimated using a modification of the Bouyoucos hydrometer method (Bouyoucos, 1962). Soil pH was measured in water using the slurry method (1:1 soil: water), electrical conductivity (EC) was estimated by the saturated paste method, and the exchangeable cation concentration (i.e. calcium, magnesium, sodium, and potassium) using ammonium acetate (NH_4OAc) extraction (Warncke and Brown, 1998). The Cation Exchange Capacity (CEC) was calculated by adding the concentrations of the total exchangeable bases (K, Na, Ca, Mg) with exchangeable acidity (concentration of H^+ and Al^{+3}). The percentage soil organic matter (SOM) (Walkley-Black method), total N (Kjeldahl method), and P (Bray P1 method) were also measured.

DNA extraction, PCR amplification and sequencing

For each experimental unit, we extracted the soil DNA from the nine subsamples separately. We followed the Ultra Clean Soil DNA Isolation Kit protocol (MoBio Laboratories,

Carlsbad, CA, USA), except that the final elution used 100 µl of buffer S5 instead of 50 µl. All DNA samples were quantified with an ND 1000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA). Of the nine subsamples per experimental unit (subplot), the three with the highest DNA content with their duplicates were used to produce first PCR amplicons for both fungi and bacteria in 25 µl reaction volumes. All PCR reactions were performed in a two-step process following protocol recommended by Berry *et al.* (2011), including positive controls (*Escherichia coli* for bacteria and *Saccharomyces cerevisiae* for fungi) and a negative control (molecular biology grade water). The two-step approach was used to avoid a 3'-end amplification bias generated with DNA tags and to prevent potential barcode-specific PCR biases (Berry *et al.*, 2011). For producing first PCR bacterial and fungal amplicons, we optimized the PCR conditions for template, $MgCl_2$ concentrations as well as the annealing temperatures for all primers described below including DNA from six environmental samples. All PCRs were performed on MultiGene Optimax Thermal Cycler (Labnet International Inc., Edison, NJ, USA). Negative controls for DNA extractions and PCRs were included to ensure absence of contamination; no contamination was detected on PCR products visualized via an agarose gel electrophoresis.

Bacteria. In the primary PCR, bacterial amplicons were generated using a forward 515F (5'-GTGCCAGCMGCCGCGGTAA-3', Caporaso *et al.* (2011)) primer and 806R (5'-GGACTACHVGGGTWTCTAAT -3', Caporaso *et al.* (2011)) reverse primer to amplify the V4 region of the 16S rRNA (Caporaso *et al.*, 2011). The primary PCR conditions were: 8 ng (4 µl) of DNA template, 1 µM of both forward (515F) and reverse (806R) primers, 12.5 µl of Phusion High Fidelity PCR Master Mix with HF buffer (Thermo Fisher Scientific, Grand Island, NY, USA), 3.5 µl molecular biology grade water with PCR cycle parameters with an initial denaturing step of 98 °C for 30 sec, followed by 30 cycles of 94 °C denaturing for 45 sec, 50 °C annealing for 1 min and

72°C extension for 2 min, followed by a final extension step of 72°C for 7 min. Each separate PCR was visualized on a 1.5% agarose (*w/v*) gel to ensure presence of PCR products. The remaining volume (20 µl) of the duplicated PCR amplicons per experimental unit was pooled and cleaned using Deffinity Rapid Tips (Deffinity Genomics, West Chester, PA, USA) following the manufacturer's protocol. For the secondary PCR, 10 µl of primary PCR products in a 50 µl reaction volume were amplified using 1 µM of both forward (515F) and reverse (806R) primers, 25 µl of Phusion High Fidelity PCR Master Mix with HF buffer and 5 µl molecular biology grade water, following the manufacturer's protocol with the exception of using only 5 cycles and the inclusion of a reverse primer joined with 12-bp unique molar identifier tags (MID-806R; Table 4.1). All technical replicates and their duplicates for secondary PCR also were visualized on a 1.5% agarose (*w/v*) gel to check for amplification. After secondary PCR visualization, 25 µl remaining PCR volume was pooled per experimental unit and cleaned with Agencourt AMPure (Beckman Coulter Inc., Pasadena, CA, USA) as per manufacturer's instructions except that we used a 1:1 ratio of AMPure bead solution to PCR volume to further discriminate against short PCR fragments. Resultant amplicons were quantified for DNA yield and pooled equimolarly and cleaned again with Agencourt AMPure® and finally submitted for sequencing.

Fungi. The fungal Internal Transcribed Spacer region (ITS) was PCR-amplified for producing the first amplicons. First fungal amplicons were amplified with the forward primer ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA -3', Gardes and Bruns (1993)) and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3', White *et al.* (1990)). The ITS1F and ITS4 primer pair was used to amplify the intervening 5.8S rDNA and the adjacent highly variable ITS1 and ITS2 regions (White *et al.*, 1990). These primers amplify a wide range of fungal targets and the ITS markers are recommended as the fungal DNA barcode (Seifert, 2009; Schoch *et al.*, 2012).

The primary PCRs contained final concentrations or absolute amounts of reagents as follows: 20 ng (or 10µl) of DNA template, 200 mM dNTPs, 10 mM of both forward (ITS1F) and reverse (ITS4) primers, 2.5 µM $MgCl_2$, 1 unit GoTaq Hot Start DNA polymerase (Promega, Madison, Wisconsin) and 2.5 µl 5x Green GoTaq Flexi PCR buffer (Promega, Madison, Wisconsin). PCR cycling parameters included an initial denaturation at 95°C for 10 min, then 30 cycles of denaturation at 95 °C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 2 min, followed by a final extension step at 72°C for 7 min. Each separate PCR (3 technical replicates and their duplicates) was visualized on a 1.5% agarose (*w/v*) gel to ensure presence of PCR products. The remaining volume (20 µl) of the duplicated PCR amplicons per experimental unit was pooled and cleaned using Deffinity Rapid Tips (Deffinity Genomics, West Chester, PA, USA) following the manufacturer's protocol. For producing fungal secondary PCRs amplicons, we chose the Internal Transcribed Spacer 2 (ITS2) region of the ribosomal RNA gene repeat. ITS2 was chosen to target shorter reads available with the paired-end Illumina MiSeq and amplified with the forward primer fITS7 (5'-GTGARTCATCGAATCTTTG-3', Ihrmark *et al.* (2012)) and reverse primer ITS4 (White *et al.*, 1990). A unique sample-specific 12-base pair sequence barcode was incorporated in the secondary PCR using an ITS4 fusion primer synthesized with sample specific DNA-tags (MID-ITS4; Table 4.2). Secondary PCR conditions were identical to those for the primary PCR except that they included 10 µl of primary PCR products as template, tagged reverse primers (ITS4), and the number of PCR cycles was reduced to ten. All technical replicates and their duplicates for secondary PCR also were visualized on a 1.5% agarose (*w/v*) gel to check for amplification. The secondary PCR amplicons were cleaned using Agencourt AmPure XP magnetic 96-well SPRIplate system (Beckman Coulter, Indianapolis, Indiana) following the manufacturer's protocol with 1:1 ratio of bead solution to reaction volume to discriminate against nontarget small

DNA fragments. The three technical replicates per experimental unit (30 experimental units in total) and their duplicates were combined and the experimental units equimolarly pooled into one amplicon library. The libraries were AmPure cleaned again to remove any residual short DNA contamination and submitted for sequencing.

The sequencing from both bacterial and fungal libraries was performed by the Integrated Genomics Facility at Kansas State University (Manhattan, KS, USA), where Illumina-specific primers and adapters for fungi and bacteria were ligated into amplicons using a NEBNext® DNA Library Prep MasterMix for Illumina kit (Protocol E6040; New England Biolabs Inc.) and sequenced using a MiSeq Reagent kit v3 (Illumina, San Diego, CA, USA) with 600 (2 x 300) cycles. Paired .fastq files for bacteria and fungi have been deposited in the data sharing repository Figshare (https://figshare.com). Both bacterial and fungal data sets are available at https://doi.org/10.6084/m9.figshare.7283498.v2.

Sequence analysis

The obtained bacterial and fungal sequence data (.fastq) were processed using mothur (version 1. 34.4; Schloss *et al.* (2009)). We followed most of the steps at the protocol of the standard operating procedure (SOP) for MiSeq by Kozich *et al.* (2013), including starting by contiging the paired-end .fastq files. Pair-end fastq files were overlapped to form contiguous reads in a single fasta file. The paired-end read bacterial library contained 19,032,266 sequences, whereas the paired-end read fungal library contained 17,064,332 sequences. Contiged sequences from all the samples were screened and culled using the following criteria: 2 mismatches to the primers, 1 mismatch to the MID (multiple identifier) tags and maximum 8 homopolymeric regions.

The sequences were truncated to 250 bp, and pre-clustering was done to reduce noise and potential sequencing bias as recommended (Preusse *et al.*, 2007; Huse *et al.*, 2008) allowing for two differences between bacterial sequences and for three differences between fungal sequences. Bacterial sequences were aligned against SILVA-based reference alignment (Schloss, 2009) and a distance matrix calculated prior clustering, while a pairwise sequence distance matrix was calculated for fungal sequences prior to clustering into operational taxonomic units (OTUs). Chimeras were detected and removed with the mothur-implemented UCHIME algorithm (Edgar *et al.*, 2011). The clustering of the non-chimeric sequences to OTUs was done by clustering at 97% similarity threshold using a nearest neighbor-joining method. Then, the bacterial sequences were assigned to taxonomic affinities using the naïve Bayesian classifier (Wang *et al.*, 2007) with a bootstrap threshold of 80% against the RPD training set, version 9 (Schloss and Westcott, 2011); while fungal sequences were assigned to taxonomic affinities using a bootstrap threshold of 60% against the UNITE taxonomy reference (Abarenkov *et al.*, 2010), version 6. For bacteria, sequences not assigned to Domain Bacteria (including Archaea, chloroplast, cyanobacteria, unknown and mitochondria) were omitted. For fungi, sequences not assigned to Kingdom Fungi (including Plantae, bacteria, chloroplast, and unknown) were omitted. Rare OTUs (abundance ≤ 10 across all experimental units) that may have been PCR and/or sequencing artifacts were removed (Brown *et al.*, 2015). For bacteria, the final data set with no "rare" OTUs had 4,683,092 sequences and 7,970 OTUs in total. For fungi, the final data set with no "rare" OTUs had 2,343,690 sequences and 4,832 OTUs in total. Before downstream analysis, final bacterial and fungal data sets were rarified by randomly subsampling to an equal number of sequences to calculate diversity indices estimates of the 16S and fungal ITS rRNA datasets, respectively.

Diversity indices

For the standardized bacterial and fungal OTU frequency data, we calculated Simpson's diversity index, Simpson´s evenness, and richness for each plot using mothur (v. 1. 34.4; Schloss *et al.* (2009)). Simpson's diversity ($1\text{-}D = 1-\sum p_i^2$) estimates the likelihood that two randomly chosen individuals (sequences) will be assigned to different OTUs (Simpson, 1949). It is the complement of Simpson's dominance ($D = \sum p_i^2$), where p_i is the proportion of sequences assigned to the *i*th OTU. We also evaluated Simpson´s evenness (also called Simpson's equitability or $E_{1/D} = (1/\sum p_i^2)/S_{obs}$, where p_i = is the proportion of sequences assigned to the *i*th OTU and S_{obs} = richness) and richness (S_{obs}: number of OTUs) that correspond to the observed number of OTUs in a given community.

Statistical analysis

Simpson's diversity, Simpson´s evenness, and richness were evaluated to test hypotheses about how fungal and bacterial diversity respond to the use of high tunnels, plastic mulch and organic amendments across two sampling times on a tomato season. Two type of analyses were performed. In the first analysis (Analysis 1), we tested the high tunnel effect on soil microbial diversity in an analysis of covariance (ANCOVA) with three soil amendment treatments (control, alfalfa meal pellets, and city compost), two plot types (high tunnel and field plots without plastic mulch), and with two sampling times (maturity and post-harvest) as repeated measures. Note that the test of high tunnel effects is an approximate test, and true randomization of high tunnel locations in the experiment was not a practical option. In the second analysis (Analysis 2), we

tested the plastic mulch effect on soil microbial diversity with the presence or absence of plastic mulch applied at the whole plot level, the soil amendment treatments applied at the subplot level, and repeated measures across two sampling times. Generalized linear mixed models in SAS Proc GLIMMIX (SAS 9.4., Institute Inc., Cary, NC) were used for the analysis of plastic mulch and soil amendments in this data subset. Tukey´s method was used for adjusting p-values for multiple comparison tests in both analyses. The effects of organic amendment and sampling time were evaluated in both Analysis 1 and Analysis 2. Plots of diversity responses to treatments were generated using the ggplot2 package (Wickham, 2016) in R (R Development Core Team, 2018).

We also used generalized linear mixed models in SAS Proc GLIMMIX and p-value adjustments for multiple comparisons using Tukey's method to evaluate the response of the most frequent taxa to the treatment combinations.

Bacterial and fungal community responses across time to soil amendments

We used non-metric multidimensional scaling (NMDS) in a two dimensional visualization to evaluate how microbial communities changed across time and in response to the experimental treatments, using the vegan package (Oksanen *et al.*, 2018) in R (R Development Core Team, 2018). We used the metaMDS function (Oksanen *et al.*, 2018) in vegan, with 50 as the maximum number of random starts and using Bray-Curtis dissimilarity distances for taxa assigned to genera. Bacterial and fungal communities were compared for the two sampling dates for each treatment combination using the ordiellipse function (Oksanen *et al.*, 2018) to illustrate 95% confidence regions. In a permutational multivariate analysis of variance (PERMANOVA) test for nutrient amendment effects, we divided the data into three separate data sets: (1) from high tunnels, (2)

from field plots with plastic mulch, and (3) from field plots without plastic mulch. To evaluate variation between community structures from different treatments, beta diversity was measured using species gain β_z (Koleff *et al*., 2003) for bacterial and fungal communities by treatment. The data were partitioned in a PERMANOVA with 200 permutations using the adonis function in the vegan package (Oksanen *et al.*, 2018) in R (R Development Core Team, 2018).

Results

General bacterial and fungal community data characterization

We acquired a total of 19,032,266 bacterial and 17,064,332 fungal sequences from the high tunnels and field plots. After trimming, alignment for the bacterial sequences, and removal of chimeras, we retained 4,683,092 high quality bacterial sequences and 2,343,690 high quality fungal sequences across the two high tunnels and eight field plots. At 97% sequence similarity, the bacterial sequences represented 7,970 OTUs and the fungal sequences represented 4,832 OTUs. The number of sequences passing our quality filtering ranged from 1,354 to 107,874 bacterial sequences and 3,226 to 53,434 fungal sequences per subplot across the three seasons and ninety samples in total. The data were rarified by randomly subsampling 1,354 bacterial sequences per subplot and 3,226 fungal sequences per subplot before downstream analyses.

Bacterial diversity

High tunnel effects

To evaluate the high tunnel effect on bacterial diversity, we evaluated three diversity measures (Simpson's diversity, Simpson's evenness,and richness) in high tunnels in comparison to the responses in open field plots (field plots without plastic mulch). We found no evidence for a high tunnel effect on bacterial diversity Simpson´s diversity: p = 0.52, and Simpson´s evenness: p = 0.54, and richness: p = 0.66; see plot type effect in Tables 4.5 to 4.7).

Effects of plastic mulch

To evaluate the plastic mulch effect, we compared open field plots with and without plastic mulch for three diversity measures: Simpson´s diversity, Simpson´s evenness, and richness). We did not find support for any effects of plastic mulch on bacterial diversity measurements (Simpson´s diversity: p = 0.54, Simpson´s evenness: p = 0.42, and richness: p = 0.32; see mulch type effect in Tables 4.8 to 4.10).

Effects of nutrient amendments and sampling time

These effects were evaluated both in "Analysis 1", an ANCOVA of data including the high tunnel observations and the un-mulched field observations, and in "Analysis 2", GLMM analyses of data including the mulched and un-mulched field observations. Following is a summary of results across these two analyses. In Analysis 1 (Tables 4.5 to 4.7), there was evidence for an effect of nutrient amendement on bacterial Simpson´s diversity (type III test: p = 0.05, Table 4.5,

and Fig. 4.2), but less evidence for a sampling time effect ($p = 0.11$, Table 4.5). There was some evidence for an effect of nutrient amendments on bacterial evenness (Simpson´s equitability) (type III test: $p = 0.05$, Table 4.6, and Fig. 4.3), and for a sampling time effect (type III test: $p = 0.09$, Table 4.6, and Fig. 4.3). Bacterial diversity and evenness were greater postharvest for the control soils in both high tunnels and open field plots, compared to soils treated with alfalfa or city compost (Fig. 4.2 and Fig. 4.3).

Bacterial richness strongly responded to the nutrient amendment treatment (type III test: $p = 0.008$, Table 4.7, and Fig. 4.4), and also some evidence was found for the interaction between plot type (high tunnel vs. un-mulched field plots) and nutrient amendment (type III test: $p = 0.07$, Table. 4.7, and Fig. 4.4). No evidence was found for a sampling time effect on bacterial richness ($p = 0.78$). Bacterial richness was greater at maturity for city compost soils in field plots across the two sampling times (Fig. 4.4). The differences of the least squares means (LSM) showed higher bacterial richness for soils treated with city compost in field plots compared to both alfalfa in field plots ($p = 0.05$, after Tukey-Kramer adjustment, Fig. 4.4) and alfalfa in high tunnels ($p = 0.03$, after Tukey-Kramer adjustment, Fig. 4.4) at maturity and post-harvest. There was also some evidence for higher bacterial richness in control soils for high tunnels compared to both soils treated with alfalfa in field plots ($p = 0.07$, after Tukey-Kramer adjustment, Fig. 4.4) and alfalfa in high tunnels ($p = 0.04$, after Tukey-Kramer adjustment, Fig. 4.4) at maturity and post-harvest.

In Analysis 2 (Tables 4.8 to 4.10), there was strong evidence for a nutrient amendment effect on both bacterial diversity and evenness (type III test: $p = 0.03$, and $p = 0.008$, respectively, Table 4.8, and Table 4.9), and some evidence for a two-way interaction between mulch type (presence or absence of plastic mulch) and sampling time (type III test: $p = 0.06$, and $p = 0.03$, Simpson´s diversity and Simpson´s evenness, respectively, Tables 4.8 and 4.9). Similar results

were observed for Simpson´s diversity and Simpson´s evenness (Fig. 4.5 and Fig. 4.6), where evidence was found for higher bacterial diversity and evenness in control soils compared to soils treated with alfalfa in un-mulched open field plots (p = 0.04, and p = 0.03, after Tukey-Kramer adjustment, respectively, Fig. 4.5 and Fig. 4.6), and also some evidence was found for higher bacterial diversity and evenness in control soils for mulched field plots compared to alfalfa soils in un-mulched open field plots (p = 0.07, and p = 0.05, after Tukey-Kramer adjustment, respectively, Fig. 4.5 and Fig. 4.6) at maturity and post-harvest.

Bacterial richness also responded to the nutrient amendment treatment (type III test: p = 0.005, Table 4.10 and Fig. 4.7). There was evidence for higher bacterial richness in soils treated with city compost for un-mulched field plots compared to alfalfa soils for both un-mulched field plots (p = 0.06, after Tukey-Kramer adjustment, Fig. 4.7) and for mulched field plots (p = 0.04, after Tukey-Kramer adjustment, Fig. 4.7) at maturity and post-harvest.

Fungal diversity

High tunnel effects

We used an ANCOVA to test whether there were differences in three fungal diversity measurements (Simpson´s diversity, Simpson´s evenness, and richness) between high tunnels and open field plots (field plots without plastic mulch). We did not find evidence for a high tunnel effect on fungal diversity (Simpson´s diversity: p = 0.25; Simpson´s evenness: p = 0.27; and richness: p = 0.37; see plot type on Tables 4.11 to 4.13).

Plastic mulch effects

We evaluated the effects of plastic mulch on fungal communities in open fields (outside high tunnels) using three diversity measurements: Simpson´s diversity, Simpson´s evenness, and richness). We found some evidence for higher fungal richness in soils without plastic mulch for all nutrient amendments across sampling times (type III test for mulch type effect ($p = 0.06$; Table 4.16 and Fig. 4.13), but a plastic mulch effect was not observed for fungal diversity ($p = 0.41$, Table 4.14) or for fungal evenness ($p = 0.94$, Table 4.15).

Effects of nutrient amendments and sampling time

As for bacteria, these effects were evaluated both in "Analysis 1", an ANCOVA of data including the high tunnel observations and the un-mulched field observations, and in "Analysis 2", GLMM analyses of data including the mulched and un-mulched field observations. Following is a summary of results across these two analyses. In Analysis 1, there was not evidence for a sampling time effect for fungal diversity (Simpson´s diversity: $p = 0.18$, Table 4.11, and Fig. 4.8), but there was some evidence for fungal evenness ($p = 0.10$, Table 4.12, and Fig. 4.9), and some evidence for a sampling time effect for fungal richness (type III test: $p = 0.07$, Table 4.13, and Fig. 4.10). There was also some evidence for a nutrient amendment effect for fungal evenness (type III test: $p = 0.07$, Table 4.12, and Fig. 4.9), but not for fungal richness ($p = 0.9$, Table 4.13) or fungal diversity ($p = 0.37$, Table 4.11). Fungal evenness increased from maturity to postharvest for the three nutrient amendments (control, alfalfa, and city compost) for the un-mulched field plots compared to the similar responses observed for the three nutrient amendments at maturity and post-harvest on high tunnels (Fig. 4.9). A similar pattern was observed for fungal richness (Fig.

4.10), where the three nutrient amendments for the un-mulched field plots increased from maturity to post-havest, but not for the three nutrient amendments on the high tunnels (Fig. 4.10).

In Analysis 2, there was some evidence for a sampling time effect on fungal diversity (type III test: $p = 0.06$, Table 4.14, and Fig. 4.11), while strong evidence was found for a sampling time effect for both fungal fungal evenness (type III test: $p = 0.008$, Table 4.15, and Fig. 4.12) and fungal richness (type III test: $p < 0.0001$, Table 4.16, and Fig. 4.13). There was not evidence for a nutrient amendment effect on any of the diversity measurements (Simpson´s diversity: $p = 0.83$; Simpson´s evenness: $p = 0.39$; and richness: $p = 0.9$; Tables 4.14, 4.15, and 4.16, respectively). In general, fungal diversity, evenness, and richness increased from maturity to postharvest across all treatment combinations in Analysis 2 (Figs. 4.11 to 4.13).

Community analysis with NMDS

For bacterial communities, there was some overlap of confidence regions in the NMDS of communities sampled from high tunnels and plots with plastic mulch at maturity and postharvest, while communities from field plots without plastic mulch were more distinct across sampling time points (Fig. 4.14). In the PERMANOVA, split into three analyses by whole plot treatment, there was evidence for an effect of nutrient amendments on bacterial communities in field plots without plastic mulch ($p = 0.02$, Table 4.17), but not evidence for nutrient amendments in high tunnels ($p = 0.18$, Table 4.18) or in field plots with plastic mulch ($p = 0.72$, Table 4.19). For fungal communities, the confidence regions in the NMDS for high tunnel and mulched-field plot communities overlapped, but were distinct from the non-mulched field communities (Fig. 4.15). In high tunnels, there were distinct communities at maturity and postharvest, but these differences

were less clear outside the high tunnels (Fig 4.15). In the PERMANOVA – split into three separate analyses for high tunnels, mulched field plots, and unmulched field plots – there was little evidence for an effect of amendments on fungal communities, with the most evidence for an amendment effect in the high tunnels (p = 0.11, Table 4.20).

Dominant bacterial and fungal taxa across time

We characterized which soil microbial taxa were most frequent across the treatment combinations. The bacterial phyla Proteobacteria and Acidobacteria were the most frequent across the two sampling times (27.3% and 17.3%, respectively), with a small proportion of OTUs (7.1%) remaining unclassified at the phylum level (Table 4.21). The most frequent orders to which taxa were assigned were Actinomycetales and Acidobacteriales (order *incertae sedis*) (22.4% and 19.5% respectively), with 10.1% unclassified to order (Table 4.21). The families Acidobacteria (family *incertae sedis*) and Nevskiales were the most frequent across the treatment combinations 11. 7% and 10.4%, respectively), with 25.4% unclassified (Table 4.21). The OTUs assigned to the subdivisions *Gp4* (9.9%), *Gp6* (9.6%), and the genera *Spartobacteria* (8.1%), *Flavobacterium* (7.1%), and *Steroidobacter* (5.6%) were the top five most frequent across the treatment combinations, with 40.2% unclassified to genus (Table 4.21).

The most frequent fungal taxa assigned to phylum, order, family and OTUs assigned to genus and species were identified across the treatment combinations and the two time points. Ascomycota was the most frequent fungal phylum across the treatment combinations (55.2%), with 5.8% unclassified to phylum (Table 4.22). The most frequent orders were Hypocreales and Mortierellales (22.3% and 14.3% respectively), with 10.5% unclassified (Table 4.22). The fungal

families Mortierellaceae and Nectriaceae were the most frequent across the treatment combinations (13.4% and 10.9%, respectively), with 25.4% unclassified to family (Table 4.22). The OTUs assigned to the genera *Mortierella* (11.8%) and *Hannaella* (8.7%) were the most frequent, with 41.3% unclassified to genus (Table 4.22). For *Mortierella*, we found that the OTUs assigned to the species *M. humilis* (5.6%), *M. exigua* (2%) and *M. capitata* (1.9%) were among the twenty most frequent species across the treatment combinations. The OTUs assigned to the species *Hannaella sinensis* (8.1%), *Phoma* sp. (7.5%) and *Haematonectria haematococca* (6.4%) were among the top five most frequent species across the treatment combinations, with 51.1% unclassified to species (Table 4.22).

Discussion

Management effects on bacterial and fungal community diversity and composition

Our first objective was to determine the response of soil microbial communities to organic nutrient amendments across the tomato season. We evaluated the hypothesis that organic nutrient amendments would increase bacterial and fungal richness and diversity compared to the unamended control, across sampling times. We found that the application of organic amendments (alfalfa meal pellets and city compost) increased bacterial richness compared to the control treatment (Figs. 4.4, and 4.7), but did not increase fungal richness. For bacteria, the application of city compost significantly increased richness compared to soils treated with alfalfa (Figs. 4.4, and 4.7); while for fungi, neither alfalfa meal pellets nor city compost increased fungal diversity, evenness or richness (Figs.4.8 to 4.10). Our results for bacteria are consistent with previous studies where the use of organic fertilizers and soil amendments generally increased the richness and

diversity of soil microbial communities (Quilty and Cattle, 2011; Zhen *et al.*, 2014; Hartmann *et al.*, 2015; Francioli *et al.*, 2016) For instance, organic fertilizers tend to increase total microbial activity and rates of nutrient cycling, and can increase microbial diversity, and richness (Ngosong *et al.*, 2010; Li *et al.*, 2012a; Hartmann *et al.*, 2015; Lori *et al.*, 2017), in some cases by introducing novel taxa (Perez-Piqueres *et al.*, 2006) or by stimulating microbial groups that are known to prefer nutrient-rich environments (Francioli *et al.*, 2016) due to the nutritional resources provided in the organic amendments (Hamm *et al.*, 2016). Furthermore, the addition of organic fertilizers may increase global microbial biomass and enhance soil enzyme activity (Debosz *et al.*, 2002). Effects on bacterial richness may vary with the type of amendment applied, the specific nutritional resources it provides, and changes in the soil organic matter (Bonanomi *et al.*, 2018). Hamm *et al.* (2016) studied the impacts of manure (including solid pig manure and solid dairy manure) and granular urea N additions on the diversity and composition of soil bacterial communities in a barley (*Hordeum vulgare* L.) cropping system. They found that bacterial richness increased with manure treatment as a result of the nutritional resources provided in the organic amendments.

The type and quantity of organic fertilizer can affect the physico-chemical properties of the soil, which in the long-term have a significant influence on productive capacity (Tejada *et al.*, 2006; Melero *et al.*, 2007), and can also strongly influence the soil microflora (Crecchio *et al.*, 2001; Tejada *et al.*, 2006; Kowaljow and Mazzarino, 2007). Based on our physical chemical tests, alfalfa meal pellets were acidic (pH = 5.7), in contrast to city compost (pH = 7.6, Table 4.4). The alfalfa meal pellets also were higher in total N, total C, ammonium (NH_4), nitrate (NO_3), P, K, Mg, and Na compared to city compost (Table 4.4). Soil pH is one of the most important factors explaining differences in both bacterial and fungal communities in soil. For instance, Lauber *et al.* (2009) found that overall bacterial community composition correlated with differences across the

range of soil pH at the continental scale. Zhang *et al.* (2016) found that fungal community composition may be directly affected by soil pH due to species-specific physiological constraints on fungal survival and growth. The soil pH in our subplots receiving city compost, in both high tunnels and field plots, increased from the first soil test (average pre-planting: pH = 6.5, Table 4.3A) to the second test (average during post-harvest: pH = 7.1, Table 4.3B). Previous studies have found greater bacterial richness and diversity near a neutral pH (Fierer and Jackson, 2006; Lauber *et al.*, 2009; Ramirez *et al.*, 2010). Soil pH is often strongly associated with the composition of particular bacterial groups and community composition across geographic scales (Lauber *et al.*, 2009; Shen *et al.*, 2013). Typically there is higher bacterial diversity in neutral soils and lower in acidic and alkaline soils (Fierer and Jackson, 2006). Fungal species often have a wider pH range, often covering 5-9 pH units without significant inhibition of growth (Nevarez *et al.*, 2009). The pH consistently decreased in subplots treated with alfalfa meal pellets from pre-planting (mean pH = 6.6, Table 4.3A) to post-harvest (mean pH = 6.1, Table 4.3B). Previous studies have found that fungi generally do well in acidic conditions (Matthies *et al.*, 1997; Rousk *et al.*, 2010), but some – like *Mortierella* – also perform well in neutral to slightly alkaline conditions (Mueller *et al.*, 2004). The fungal genus *Mortierella* was the most frequent taxon across the treatment combinations in our study (Table 4.22). Besides pH, fungal communities also vary with soil nutrients including total N, P, organic matter and C: N ratios (Lauber *et al.*, 2008; Zhang *et al.*, 2016). Alfalfa had a higher percentage of micro- and macronutrients compared to city compost in our study (Table 4.3), which might contribute to the diversity of fungal communities.

We also evaluated the effects of high tunnels and plastic mulch, which generally support faster crop development and might similarly "speed up" shifts in microbial communities across the season. We hypothesized that the use of high tunnels and plastic mulch would affect richness

and diversity of bacterial and fungal communities across sampling times. We did not observe high tunnel or plastic mulch effects on bacterial or fungal communities for any of the diversity measurements. Our results are different from other studies. For example, Ge *et al.* (2013) found higher fungal biomass and phospholipid fatty acid (PLFA) levels in organic-greenhouse management compared to organic open-field plots management. Thus, they concluded that the combined effects of organic and greenhouse cultivation improved soils more than expected given the contribution of each factor alone. Additionally, the authors found a relatively low bacterial-to-fungal PLFA ratio in organically managed soils. They concluded that the increase in fungal biomass in the organic systems may be attributed to both an increase in nutrient inputs from manure application and an increase in bacterial biomass in the conventional system (Ge *et al.*, 2013). They also hypothesized that the more diverse microbial community in the organic plots benefitted from the warm, moist, and stable conditions in the greenhouses (Ge *et al.*, 2013). We found that organic amendments (in our case alfalfa meal pellets or city compost) did not increase fungal diversity, evenness orrichness beyond the use of high tunnels alone. The fungal genera *Mortierella*, *Hannaella* and *Phoma* were among the most frequent in field plots for all treatments and across the three sampling times in the tomato crop (Table 4.22). The fungal genera *Mortierella, Hannaella and Phoma* included species reported to be thermophilic and/or thermotolerant (Mouchacca, 1999; Mueller *et al.*, 2004), and particularly some *Phoma* species have often been found in greenhouses (Boerema *et al.*, 2004).

Fungal richness in field plots for all treatment combinations was higher without plastic mulch compared to the mulched field plots, across sampling times (Fig. 4.13). Dong *et al.* (2017) studied the effect of plastic film mulching (spring mulching, autumn mulching, and no mulching) on soil bacterial and fungal communities during maize cultivation in a rain-fed region. They

reported that soil bacterial and fungal richness and diversity increased under the spring and autumn mulching treatments compared to the no-mulch control. Farmer *et al.* (2017) investigated the effect of long-term fertilization (inorganic N fertilizer, organic pig compost, and combined manure and inorganic N fertilizer) and film mulching (presence/absence) on soil properties and bacterial community structure in a maize cropping system. They found that film mulching and manure fertilization significantly increased bacterial diversity and richness during long-term fertilization. Our results are in contrast to those of Dong *et al.* (2017) and Farmer *et al.* (2017) in that the use of plastic mulch or the combined effect of plastic mulch and organic fertilization enhanced both bacterial and fungal community richness and diversity. The incongruence between our results and those of Dong *et al.* (2017) and Farmer *et al.* (2017) might be due to differences in the type of organic fertilizer, the type of plastic mulch, and the crop type. Cesarano *et al.* (2017) also reported that the amount and frequency of the application of organic amendments impacts the soil microbiome.

Dominant bacterial and fungal taxa across sampling times

We characterized soil microbial taxa that were dominant (higher relative abundance) for particular organic amendments across the two sampling times for a tomato crop. The most dominant bacteria in this experiment were Proteobacteria (27.3%, Table 4.21), in agreement with other studies assessing the composition of soil bacterial communities using sequencing approaches (Janssen, 2006; Roesch *et al.*, 2007; Spain *et al.*, 2009; Acosta-Martinez *et al.*, 2010; Li *et al.*, 2012b). Proteobacteria represented the most abundant phylum, approximately 50% of the clone library of 16S rRNA genes from a wide range of ecosystems across North America (Fierer *et al.*,

2007). In addition, the phylum Proteobacteria has been reported as dominating in organic farming systems compared to conventional systems (Upchurch *et al.*, 2008; Li *et al.*, 2012b; Shange *et al.*, 2012). In this phylum, OTUs assigned to the bacterial genus *Steroidobacter* were in the top ten most frequent genera on this transitional farm (Table 1.5). This genus includes motile, non-endospore-forming bacteria that can grow between 28-32 °C and at a pH of 7.0-7.5 (Fahrbach *et al.*, 2008; Sakai *et al.*, 2014; Gong *et al.*, 2016). *Steroidobacter* spp. have been isolated from soil in a vegetable field (Sakai *et al.*, 2014) and from a forest soil (Gong *et al.*, 2016).

The phyla Acidobacteria and Bacteroidetes were also very frequent in our study (17.3 %, and 14.1%, Table 4.21), and are commonly observed at a high frequency in agricultural systems (Roesch *et al.*, 2007; Acosta-Martinez *et al.*, 2008; Acosta-Martinez *et al.*, 2010; Pershina *et al.*, 2015; Brennan and Acosta-Martinez, 2017; Lupatini *et al.*, 2017). The phylum Acidobacteria has been detected in agricultural systems managed with organic fertilizers amendments (Nunes da Rocha *et al.*, 2013; Pershina *et al.*, 2015; Lupatini *et al.*, 2017), while several studies have reported that the relative abundance of the phylum Bacteroidetes increased with the intensity of cropping (Acosta-Martinez *et al.*, 2008; Acosta-Martinez *et al.*, 2010; Brennan and Acosta-Martinez, 2017) and with high C availability (Fierer *et al.*, 2007). Acosta-Martinez *et al.* (2008) found that the phylum Bacteroidetes was the most predominant in soil in agricultural production (Cotton-Winter wheat-corn and cotton-cotton) compared to the same soil under non-disturbed grass systems (pasture monoculture and a diverse mixture of grasses) in a study of farms in the Texas High Plains, USA. It has been proposed that the dominance of Bacteroidetes in agricultural systems could be due to their ability to rapidly exploit bioavailable organic matter and colonize aggregates (Weiss *et al.*, 1996; Abell and Bowman, 2005). The OTUs assigned to the Acidobacterial subdivisions *Gp4* and *Gp6* were the first two most frequent taxa across all treatments and across

sampling times (9.9% and 9.6%, respectively, Table 4.21), while the genus *Flavobacterium* (Bacteroidetes) was the fourth most frequent in this experiment (7.1%, Table 4.21). Additionally, *Flavobacterium* is one of the most frequent genera of soil bacteria in libraries of 16S rRNA and 16S rRNA genes (38% clones; Janssen, 2006).

The most dominant fungal phylum was Ascomycota (55.2%, Table 4.22). This phylum has a central role in most land-based ecosystems (Schoch *et al.*, 2009). It includes important decomposers, breaking down both organic materials (such as dead leaves and animals) and large molecules (such as cellulose or lignin), and thus has important roles in nutrient cycling (Ma *et al.*, 2013). The most frequent genus in our study was the OTUs assigned to *Mortierella* (11.8%, Table 4.22). *Mortierella* (Zygomycota – Mucorales – Mortierellaceae, Hibbett *et al.* (2007)) includes members that are generally opportunistic, not pathogenic for plants or animals, although some species can be pathogenic to humans (Dix and Webster, 1995). This widespread genus includes members that live as saprotrophs in soil, decaying leaves and other organic material, while other species lives on fecal pellets or on shed exoskeletons of microarthropods (Li *et al.*, 2018). Some *Mortierella* spp. produce arachidonic acid (ARA) which is considered the most important fatty acid used in medicine, pharmacology, cosmetics, the food industry (Dyal and Narine, 2005) and agriculture (Eroshin and Dedyukhina, 2002). *M. humilis* have been found to produce ARA (Eroshin *et al.*, 1996) and it was one of the most abundant species across the treatment combinations (5.6%, Table 4.22). Fungi generally grow well in acidic conditions (Matthies *et al.*, 1997), but some fungi, including *Mortierella* and *Peziza,* grow well in neutral to slightly alkaline conditions (Yamanaka, 2003), such as in our experiment plots (pH of 7 to 7.3, Table 4.3A and B), especially in those amended with city compost or under high tunnel conditions (Table 4.3A and B). The second most frequent genus was the OTUs assigned to *Hannaella sinensis* (Basidiomycota

–Tremellales, Wang and Bai (2008) (Wang and Bai, 2008)). This species has been reported on plant leaf surfaces and along with the other six species associated with the genus *Hannaella*, these yeast-like taxa are often important phyllosphere inhabitants (Wang and Bai, 2008; Boekhout *et al.*, 2011) and associated with soils (Landell *et al.*, 2014).

Conclusions

In this study, the application of nutrient amendments such as city compost increased bacterial richness. Using high-throughput sequencing to evaluate the effect of high tunnels and plastic mulch on soil microbes in a tomato crop, the use of high tunnels did not have significant effects on fungal or bacterial diversity. The use of plastic mulch on field plots decreased fungal richness. Our results contrast with others reporting that the use of plastic mulch or the combination of plastic mulch and organic fertilization increased both bacterial and fungal community richness and diversity (Dong *et al.*, 2017; Farmer *et al.*, 2017). We evaluated the plastic mulch effect in the short term (within a tomato season), whereas future studies of mulching over a longer period will provide more insights about long-term effects on soil microbial communities on farms. Long-term benefits for farming will result from developing a broader database of information about microbiome responses to farm management, in combination with more information about functional roles of microbes.

Tables and Figures

Table 4.1. Bacterial region primer (806R) synthesized with a unique 12-base pair molecular identifier (MID) tag assigned to each treatment in a transitional organic farm on a tomato production season. The treatments include two sampling times: S1 (sample time 1: maturity), and S2 (sample time 2: post-harvest). Soil samples were collected from high tunnels (HT) or open field plots (F) with three fertility treatments: control (C), alfalfa meal pellets (A), and city compost (CC). Open field plots were treated with the presence or absence of plastic mulch: NM (no mulch) or PM (with plastic mulch).

Sample	Treatment	Gene region primer (806 R)	Molecular Identifier (MID) tags
1	S2-HT1-1-A	GGACTACHVGGGTWTCTAAT	AGATTGACCAAC
2	S2-HT1-2-CC	GGACTACHVGGGTWTCTAAT	AGTTACGAGCTA
3	S2-HT1-3-C	GGACTACHVGGGTWTCTAAT	GCATATGCACTG
4	S2-HT2-1-C	GGACTACHVGGGTWTCTAAT	CAACTCCCGTGA
5	S2-HT2-2-A	GGACTACHVGGGTWTCTAAT	TTGCGTTAGCAG
6	S2-HT2-3-CC	GGACTACHVGGGTWTCTAAT	TACGAGCCCTAA
7	S2-F1-1-NM-C	GGACTACHVGGGTWTCTAAT	CACTACGCTAGA
8	S2-F1-2-NM-A	GGACTACHVGGGTWTCTAAT	TGCAGTCCTCGA
9	S2-F1-3-NM-CC	GGACTACHVGGGTWTCTAAT	ACCATAGCTCCG
10	S2-F2-1-PM-CC	GGACTACHVGGGTWTCTAAT	TCGACATCTCTT
11	S2-F2-2-PM-C	GGACTACHVGGGTWTCTAAT	GAACACTTTGGA
12	S2-F2-3-PM-A	GGACTACHVGGGTWTCTAAT	GAGCCATCTGTA
13	S2-F3-1-PM-CC	GGACTACHVGGGTWTCTAAT	TTGGGTACACGT
14	S2-F3-2-PM-A	GGACTACHVGGGTWTCTAAT	AAGGCGCTCCTT
15	S2-F3-3-PM-C	GGACTACHVGGGTWTCTAAT	TAATACGGATCG
16	S2-F4-1-NM-A	GGACTACHVGGGTWTCTAAT	TCGGAATTAGAC
17	S2-F4-2-NM-C	GGACTACHVGGGTWTCTAAT	TGTGAATTCGGA
18	S2-F4-3-NM-CC	GGACTACHVGGGTWTCTAAT	CATTCGTGGCGT
19	S2-F5-1-NM-A	GGACTACHVGGGTWTCTAAT	TACTACGTGGCC
20	S2-F5-2-NM-C	GGACTACHVGGGTWTCTAAT	GGCCAGTTCCTA
21	S2-F5-3-NM-CC	GGACTACHVGGGTWTCTAAT	GATGTTCGCTAG
22	S2-F6-1-PM-C	GGACTACHVGGGTWTCTAAT	CTATCTCCTGTC
23	S2-F6-2-PM-CC	GGACTACHVGGGTWTCTAAT	ACTCACAGGAAT
24	S2-F6-3-PM-A	GGACTACHVGGGTWTCTAAT	ATGATGAGCCTC
25	S2-F7-1-PM-C	GGACTACHVGGGTWTCTAAT	GTCGACAGAGGA
26	S2-F7-2-PM-CC	GGACTACHVGGGTWTCTAAT	TGTCGCAAATAG
27	S2-F7-3-PM-A	GGACTACHVGGGTWTCTAAT	CATCCCTCTACT
28	S2-F8-1-NM-CC	GGACTACHVGGGTWTCTAAT	TATACCGCTGCG
29	S2-F8-2-NM-A	GGACTACHVGGGTWTCTAAT	AGTTGAGGCATT
30	S2-F8-3-NM-C	GGACTACHVGGGTWTCTAAT	ACAATAGACACC
31	S3-HT1-1-A	GGACTACHVGGGTWTCTAAT	CGGTCAATTGAC

32	S3-HT1-2-CC	GGACTACHVGGGTWTCTAAT	GTGGAGTCTCAT
33	S3-HT1-3-C	GGACTACHVGGGTWTCTAAT	GCTCGAAGATTC
34	S3-HT2-1-C	GGACTACHVGGGTWTCTAAT	AGGCTTACGTGT
35	S3-HT2-2-A	GGACTACHVGGGTWTCTAAT	TCTCTACCACTC
36	S3-HT2-3-CC	GGACTACHVGGGTWTCTAAT	ACTTCCAACTTC
37	S3-F1-1-NM-C	GGACTACHVGGGTWTCTAAT	CTCACCTAGGAA
38	S3-F1-2-NM-A	GGACTACHVGGGTWTCTAAT	GTGTTGTCGTGC
39	S3-F1-3-NM-CC	GGACTACHVGGGTWTCTAAT	CCACAGATCGAT
40	S3-F2-1-PM-CC	GGACTACHVGGGTWTCTAAT	TATCGACACAAG
41	S3-F2-2-PM-C	GGACTACHVGGGTWTCTAAT	GATTCCGGCTCA
42	S3-F2-3-PM-A	GGACTACHVGGGTWTCTAAT	CGTAATTGCCGC
43	S3-F3-1-PM-CC	GGACTACHVGGGTWTCTAAT	GGTGACTAGTTC
44	S3-F3-2-PM-A	GGACTACHVGGGTWTCTAAT	ATGGGTTCCGTC
45	S3-F3-3-PM-C	GGACTACHVGGGTWTCTAAT	TAGGCATGCTTG
46	S3-F4-1-NM-A	GGACTACHVGGGTWTCTAAT	AACTAGTTCAGG
47	S3-F4-2-NM-C	GGACTACHVGGGTWTCTAAT	ATTCTGCCGAAG
48	S3-F4-3-NM-CC	GGACTACHVGGGTWTCTAAT	AGCATGTCCCGT
49	S3-F5-1-NM-A	GGACTACHVGGGTWTCTAAT	GTACGATATGAC
50	S3-F5-2-NM-C	GGACTACHVGGGTWTCTAAT	GTGGTGGTTTCC
51	S3-F5-3-NM-CC	GGACTACHVGGGTWTCTAAT	TAGTATGCGCAA
52	S3-F6-1-PM-C	GGACTACHVGGGTWTCTAAT	TGCGCTGAATGT
53	S3-F6-2-PM-CC	GGACTACHVGGGTWTCTAAT	ATGGCTGTCAGT
54	S3-F6-3-PM-A	GGACTACHVGGGTWTCTAAT	GTTCTCTTCTCG
55	S3-F7-1-PM-C	GGACTACHVGGGTWTCTAAT	CGTAAGATGCCT
56	S3-F7-2-PM-CC	GGACTACHVGGGTWTCTAAT	GCGTTCTAGCTG
57	S3-F7-3-PM-A	GGACTACHVGGGTWTCTAAT	GTTGTTCTGGGA
58	S3-F8-1-NM-CC	GGACTACHVGGGTWTCTAAT	GGACTTCCAGCT
59	S3-F8-2-NM-A	GGACTACHVGGGTWTCTAAT	CTCACAACCGTG
60	S3-F8-3-NM-C	GGACTACHVGGGTWTCTAAT	CTGCTATTCCTC

Table 4.2. Fungal region primer (ITS4) synthesized with a unique 12-base pair molecular identifier (MID) tag assigned to each treatment in a transitional organic farm on a tomato production season. The treatments include two sampling times: S1 (sampling 1: maturity), and S2 (sampling 2: post-harvest). Soil samples were taken from high tunnels (HT) or open field plots (F) with three fertility treatments: control (C), alfalfa meal pellets (A), and city compost (CC). Open field plots had the presence or absence of plastic mulch: NM (no mulch) or PM (with plastic mulch).

Sample	Treatment	Gene Region primer (ITS4)	Molecular Identifier (MID) tags
1	S2-HT1-1-A	TCCTCCGCTTATTGATATGC	AGATTGACCAAC
2	S2-HT1-2-CC	TCCTCCGCTTATTGATATGC	AGTTACGAGCTA
3	S2-HT1-3-C	TCCTCCGCTTATTGATATGC	GCATATGCACTG
4	S2-HT2-1-C	TCCTCCGCTTATTGATATGC	CAACTCCCGTGA
5	S2-HT2-2-A	TCCTCCGCTTATTGATATGC	GAGAGCAACAGA
6	S2-HT2-3-CC	TCCTCCGCTTATTGATATGC	TACGAGCCCTAA
7	S2-F1-1-NM-C	TCCTCCGCTTATTGATATGC	CACTACGCTAGA
8	S2-F1-2-NM-A	TCCTCCGCTTATTGATATGC	TGCAGTCCTCGA
9	S2-F1-3-NM-CC	TCCTCCGCTTATTGATATGC	ACCATAGCTCCG
10	S2-F2-1-PM-CC	TCCTCCGCTTATTGATATGC	TCGACATCTCTT
11	S2-F2-2-PM-C	TCCTCCGCTTATTGATATGC	GAACACTTTGGA
12	S2-F2-3-PM-A	TCCTCCGCTTATTGATATGC	GAGCCATCTGTA
13	S2-F3-1-PM-CC	TCCTCCGCTTATTGATATGC	TTGGGTACACGT
14	S2-F3-2-PM-A	TCCTCCGCTTATTGATATGC	CGTGCTTAGGCT
15	S2-F3-3-PM-C	TCCTCCGCTTATTGATATGC	CACTCATCATTC
16	S2-F4-1-NM-A	TCCTCCGCTTATTGATATGC	TATCTATCCTGC
17	S2-F4-2-NM-C	TCCTCCGCTTATTGATATGC	TTGCCAAGAGTC
18	S2-F4-3-NM-CC	TCCTCCGCTTATTGATATGC	CATACCGTGAGT
19	S2-F5-1-NM-A	TCCTCCGCTTATTGATATGC	TACTACGTGGCC
20	S2-F5-2-NM-C	TCCTCCGCTTATTGATATGC	GGCCAGTTCCTA
21	S2-F5-3-NM-CC	TCCTCCGCTTATTGATATGC	GATGTTCGCTAG
22	S2-F6-1-PM-C	TCCTCCGCTTATTGATATGC	CTATCTCCTGTC
23	S2-F6-2-PM-CC	TCCTCCGCTTATTGATATGC	ACTCACAGGAAT
24	S2-F6-3-PM-A	TCCTCCGCTTATTGATATGC	ATGATGAGCCTC
25	S2-F7-1-PM-C	TCCTCCGCTTATTGATATGC	GTCGACAGAGGA
26	S2-F7-2-PM-CC	TCCTCCGCTTATTGATATGC	TGTCGCAAATAG
27	S2-F7-3-PM-A	TCCTCCGCTTATTGATATGC	CATCCCTCTACT
28	S2-F8-1-NM-CC	TCCTCCGCTTATTGATATGC	ATGTGTGTAGAC
29	S2-F8-2-NM-A	TCCTCCGCTTATTGATATGC	TTCTCTCGACAT
30	S2-F8-3-NM-C	TCCTCCGCTTATTGATATGC	ACAATAGACACC
31	S3-HT1-1-A	TCCTCCGCTTATTGATATGC	CGGTCAATTGAC
32	S3-HT1-2-CC	TCCTCCGCTTATTGATATGC	GCTCTCCGTAGA
33	S3-HT1-3-C	TCCTCCGCTTATTGATATGC	GCTCGAAGATTC

34	S3-HT2-1-C	TCCTCCGCTTATTGATATGC	AGGCTTACGTGT
35	S3-HT2-2-A	TCCTCCGCTTATTGATATGC	TCTCTACCACTC
36	S3-HT2-3-CC	TCCTCCGCTTATTGATATGC	ACTTCCAACTTC
37	S3-F1-1-NM-C	TCCTCCGCTTATTGATATGC	CTCACCTAGGAA
38	S3-F1-2-NM-A	TCCTCCGCTTATTGATATGC	GTGTTGTCGTGC
39	S3-F1-3-NM-CC	TCCTCCGCTTATTGATATGC	CCACAGATCGAT
40	S3-F2-1-PM-CC	TCCTCCGCTTATTGATATGC	TATCGACACAAG
41	S3-F2-2-PM-C	TCCTCCGCTTATTGATATGC	GATTCCGGCTCA
42	S3-F2-3-PM-A	TCCTCCGCTTATTGATATGC	CGTAATTGCCGC
43	S3-F3-1-PM-CC	TCCTCCGCTTATTGATATGC	GGTGACTAGTTC
44	S3-F3-2-PM-A	TCCTCCGCTTATTGATATGC	ATGGGTTCCGTC
45	S3-F3-3-PM-C	TCCTCCGCTTATTGATATGC	TAGGCATGCTTG
46	S3-F4-1-NM-A	TCCTCCGCTTATTGATATGC	AACTAGTTCAGG
47	S3-F4-2-NM-C	TCCTCCGCTTATTGATATGC	ATTCTGCCGAAG
48	S3-F4-3-NM-CC	TCCTCCGCTTATTGATATGC	AGCATGTCCCGT
49	S3-F5-1-NM-A	TCCTCCGCTTATTGATATGC	GTACGATATGAC
50	S3-F5-2-NM-C	TCCTCCGCTTATTGATATGC	GTGGTGGTTTCC
51	S3-F5-3-NM-CC	TCCTCCGCTTATTGATATGC	ATGCCATGCCGT
52	S3-F6-1-PM-C	TCCTCCGCTTATTGATATGC	GACATTGTCACG
53	S3-F6-2-PM-CC	TCCTCCGCTTATTGATATGC	ATGGCTGTCAGT
54	S3-F6-3-PM-A	TCCTCCGCTTATTGATATGC	GTTCTCTTCTCG
55	S3-F7-1-PM-C	TCCTCCGCTTATTGATATGC	CGTAAGATGCCT
56	S3-F7-2-PM-CC	TCCTCCGCTTATTGATATGC	GCGTTCTAGCTG
57	S3-F7-3-PM-A	TCCTCCGCTTATTGATATGC	GTTGTTCTGGGA
58	S3-F8-1-NM-CC	TCCTCCGCTTATTGATATGC	GGACTTCCAGCT
59	S3-F8-2-NM-A	TCCTCCGCTTATTGATATGC	CTCACAACCGTG
60	S3-F8-3-NM-C	TCCTCCGCTTATTGATATGC	CTGCTATTCCTC

Table 4.3. Soil edaphic properties at Lawrence, KS, farm evaluated (A) before tomato transplanting and (B) when tomato plants were senescent, including soil organic matter (SOM). For field plots (FP), the mean (±SD) from four subplots is presented. For high tunnels (HT), the mean (±SD) from two subplots is presented. Other abbreviations: NM = no plastic mulch; PM = with plastic mulch; C = no amendment applied (control); A = alfalfa meal pellets; CC = city compost. Mean and standard deviation is provided for each plot.

A)

Soil test conducted pre-planting							
Plot	**pH**	**SOM (%)**	**Total N (%)**	**P (ppm)**	**K (ppm)**	**Ca (ppm)**	**Mg (ppm)**
FP-NM-C	6.2 ± 0.1	2.3 ± 0.2	0.1 ± 0.0	63.7 ± 16.5	187.6 ± 13.9	2163.5 ± 91.7	135.6 ± 14.8
FP-PM-C	6.3 ± 0.1	2.4 ± 0.1	0.1 ± 0.0	70.3 ± 19.8	176. 8 ± 16.8	2172.1 ± 47.5	134.2 ± 8.6
FP-NM-A	6.2 ± 0.1	1.9 ± 0.1	0.1 ± 0.0	66.1 ± 18.6	178. 6 ± 25.2	2160.21 ± 95.3	133.9 ± 9.8
FP-PM-A	6.4 ± 0.3	2.0 ± 0.3	0.1 ± 0.0	54.8 ± 13.9	172.9 ± 13.7	2208.9 ± 146.0	128.5 ± 8.5
FP-NM-CC	6.3 ± 0.1	2.2 ± 0.3	0.1 ± 0.0	63.5 ± 27.7	171.9 ± 12.9	2220.7 ± 82.4	130.8 ± 10.1
FP-PM-CC	6.3 ± 0.1	2.3 ± 0.3	0.1 ± 0.0	64.4 ± 9.1	177.1 ± 5.9	2127.3 ± 109.9	131.9 ± 10.5
HT-C	7.0 ± 0.6	2.6 ± 0.0	0.2 ± 0.0	342.5 ± 225.6	417.9 ± 170.9	2522.9 ± 385.8	134.3 ± 0.3
HT-A	7.1 ± 0.5	2.5 ± 0.0	0.2 ± 0.0	344.5 ± 300.5	387.3 ± 184.4	2715.2 ± 464.1	152.1 ± 0.6
HT-CC	7.1 ± 0.5	2.6 ± 0.5	0.2 ± 0.0	318.6 ± 331.6	379.9 ± 244.4	2641.5 ± 410.1	150.8 ± 1.1

B)

Soil test conducted post-harvest							
Plot	**pH**	**SOM (%)**	**Total N (%)**	**P (ppm)**	**K (ppm)**	**Ca (ppm)**	**Mg (ppm)**
FP-NM-C	6.2 ± 0.3	2.3 ± 0.1	0.2 ± 0.0	57.4 ± 11.5	205. 7 ± 4.1	1997.5 ± 117.3	170.6 ± 7.1
FP-PM-C	6.0 ± 0.3	2.3 ± 0.4	0.2 ± 0.0	58.5 ± 16.3	191.2 ± 20.9	1954.2 ± 44.6	167.1 ± 9.0
FP-NM-A	6.1 ± 0.3	2.1 ± 0.2	0.1 ± 0.0	69.5 ± 15.2	241.1 ± 44.7	1913.7 ± 125.2	170.3 ± 13.1
FP-PM-A	5.6 ± 0.3	2.5 ± 0.3	0.2 ± 0.0	74.6 ± 9.3	238.3 ± 25.5	1966.5 ± 57.8	184.6 ± 14.9
FP-NM-CC	7.1 ± 0.2	5.4 ± 1.0	0.3 ± 0.0	116.7 ± 11.4	564.8 ± 44.9	2743.2 ± 53.0	259.5 ± 14.6
FP-PM-CC	6.9 ± 0.2	4.1 ± 0.9	0.3 ± 0.0	106.3 ± 15.8	453.0 ± 87.0	2541.9 ± 188.9	240.4 ± 24.7
HT-C	7.1 ± 0.5	2.3 ± 0.2	0.2 ± 0.0	301.0 ± 246.0	434.7 ± 184.7	2492.2 ± 264.0	183.4 ± 8.3
HT-A	6.6 ± 0.9	2.6 ± 0.3	0.2 ± 0.0	311.5 ± 255.3	419.2 ± 224.1	2463.4 ± 691.5	202.7 ± 3.3
HT-CC	7.3 ± 0.1	5.7 ± 0.1	0.3 ± 0.0	318.5 ± 275.1	913.9 ± 464.9	3078.6 ± 119.5	304.0 ± 32.8

Table 4.4. Physico-chemical analysis for two organic fertilizers: alfalfa meal pellets and city compost, applied in a transitional organic farm during a tomato production season. Soil tests included evaluation of soil organic matter (SOM), ammoniacal nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N).

Physical chemical analysis											
Fertilizer	pH	SOM (%)	Total N (%)	Total C (%)	NH_4-N (ppm)	$N0_3$-N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)
Alfalfa	5.7	14	3	39	236.	105	1480	27510	4604	1867	993
City Compost	7.6	14	2	29	26	42	811	4408	5537	1051	411

Table 4.5. Type III tests of fixed effects from the ANCOVA analysis of bacterial Simpson´s diversity, in a data set including high tunnel observations and un-mulched field plot observations ("Analysis 1"). The fixed effects and the two-way interaction included: plot type (high tunnels vs. un-mulched field plots); nutrient amendment (alfalfa, city compost, and control); interaction between plot type and nutrient amendment; and sampling time (maturity and postharvest).

Bacterial Simpson´s diversity –HT effect- Type III test of fixed effects				
Effect	Degrees of freedom of the numerator (Num DF)	Degrees of freedom of the denominator (Den DF)	F value	Probability > F
Plot type	1	5	0.47	0.52
Nutrient amendment	2	5	6.05	0.05
Plot type* Nutrient amendment	2	5	1.18	0.37
Sampling time	1	5	3.75	0.11

Table 4.6. Type III tests of fixed effects from the ANCOVA analysis of bacterial Simpson´s evenness, in a data set including high tunnel observations and un-mulched field plot observations (“Analysis 1”). The fixed effects and the two-way interaction included: plot type (high tunnels vs. un-mulched field plots); nutrient amendment (alfalfa, city compost, and control); interaction between plot type and nutrient amendment; and sampling time (maturity and postharvest).

Bacterial Simpson´s evenness –HT effect- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Plot type	1	5	0.43	0.54
Nutrient amendment	2	5	5.47	0.05
Plot type* Nutrient amendment	2	5	0.56	0.60
Sampling time	1	5	4.31	0.09

Table 4.7. Type III tests of fixed effects from the ANCOVA analysis of bacterial richness, in a data set including high tunnel observations and un-mulched field plot observations ("Analysis 1"). The fixed effects and the two-way interaction included: plot type (high tunnels vs. un-mulched field plots); nutrient amendment (alfalfa, city compost, and control); interaction between plot type and nutrient amendment; and sampling time (maturity and postharvest).

Bacterial richness –HT effect- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Plot type	1	5	0.21	0.66
Nutrient amendment	2	5	14.52	0.008
Plot type* Nutrient amendment	2	5	4.41	0.07
Sampling time	1	5	0.08	0.78

Table 4.8. Type III tests of fixed effects from the GLMM analysis of bacterial Simpson´s diversity, for a data set including mulched and un-mulched field plot observations ("Analysis 2"). The fixed effects and the two-and-three-way interactions included: nutrient amendment (alfalfa, city compost, and control); mulch type (mulched and un-mulched field plots); interaction between nutrient amendment and mulch type; sampling time (maturity and postharvest), interaction between nutrient amendment and sampling time; interaction between mulch type and sampling time; and the three-way interaction nutrient amendment, mulch type and sampling time.

Bacterial Simpson´s diversity-PM effect -Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Nutrient amendment	2	30	4.01	0.03
Mulch type	1	3	0.46	0.54
Nutrient amendment* Mulch type	2	30	1.96	0.15
Sampling time	1	30	0.01	0.94
Nutrient amendment* Sampling time	2	30	0.36	0.70
Mulch type* Sampling time	1	30	3.64	0.06
Nutrient amendment* Mulch type* Sampling time	2	30	0.41	0.66

Table 4.9. Type III tests of fixed effects from the GLMM analysis of bacterial Simpson´s evenness, for a data set including mulched and un-mulched field plot observations ("Analysis 2"). The fixed effects and the two-and-three-way interactions included: nutrient amendment (alfalfa, city compost, and control); mulch type (mulched and un-mulched field plots); interaction between nutrient amendment and mulch type; sampling time (maturity and postharvest), interaction between nutrient amendment and sampling time; interaction between mulch type and sampling time; and the three-way interaction nutrient amendment, mulch type and sampling time.

Bacterial Simpson´s evenness-PM effect -Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Nutrient amendment	2	30	5.73	0.008
Mulch type	1	3	0.88	0.42
Nutrient amendment* Mulch type	2	30	1.63	0.21
Sampling time	1	30	0.25	0.62
Nutrient amendment* Sampling time	2	30	0.16	0.85
Mulch type* Sampling time	1	30	5.07	0.03
Nutrient amendment* Mulch type* Sampling time	2	30	0.52	0.59

Table 4.10. Type III tests of fixed effects from the GLMM analysis of bacterial richness, for a data set including mulched and un-mulched field plot observations ("Analysis 2"). The fixed effects and the two-and-three-way interactions included: nutrient amendment (alfalfa, city compost, and control); mulch type (mulched and un-mulched field plots); interaction between nutrient amendment and mulch type; sampling time (maturity and postharvest), interaction between nutrient amendment and sampling time; interaction between mulch type and sampling time; and the three-way interaction nutrient amendment, mulch type and sampling time.

Bacterial richness-PM effect- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Nutrient amendment	2	30	6.37	0.005
Mulch type	1	3	1.38	0.32
Nutrient amendment* Mulch type	2	30	0.14	0.86
Sampling time	1	30	0.17	0.68
Nutrient amendment* Sampling time	2	30	1.56	0.23
Mulch type* Sampling time	1	30	0.30	0.58
Nutrient amendment* Mulch type* Sampling time	2	30	0.06	0.93

Table 4.11. Type III tests of fixed effects from the ANCOVA analysis of fungal Simpson´s diversity, in a data set including high tunnel observations and un-mulched field plot observations ("Analysis 1"). The fixed effects and the two-way interaction included: plot type (high tunnels vs. un-mulched field plots); nutrient amendment (alfalfa, city compost, and control); interaction between plot type and nutrient amendment; and sampling time (maturity and postharvest).

Fungal Simpson´s diversity –HT effect- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Plot type	1	5	1.67	0.25
Nutrient amendment	2	5	1.21	0.37
Plot type* Nutrient amendment	2	5	0.77	0.51
Sampling time	1	5	2.34	0.18

Table 4.12. Type III tests of fixed effects from the ANCOVA analysis of fungal Simpson´s evenness, in a data set including high tunnel observations and un-mulched field plot observations (“Analysis 1”). The fixed effects and the two-way interaction included: plot type (high tunnels vs. un-mulched field plots); nutrient amendment (alfalfa, city compost, and control); interaction between plot type and nutrient amendment; and sampling time (maturity and postharvest).

Fungal Simpson´s evenness –HT effect- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Plot type	1	5	1.53	0.27
Nutrient amendment	2	5	4.62	0.07
Plot type* Nutrient amendment	2	5	0.35	0.71
Sampling time	1	5	3.88	0.10

Table 4.13. Type III tests of fixed effects from the ANCOVA analysis of fungal richness, in a data set including high tunnel observations and un-mulched field plot observations ("Analysis 1"). The fixed effects and the two-way interaction included: plot type (high tunnels vs. un-mulched field plots); nutrient amendment (alfalfa, city compost, and control); interaction between plot type and nutrient amendment; and sampling time (maturity and postharvest).

Fungal richness –HT effect- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Plot type	1	5	0.94	0.37
Nutrient amendments	2	5	0.11	0.90
Plot type* Nutrient amendments	2	5	1.32	0.34
Sampling time	1	5	5.02	0.07

Table 4.14. Type III tests of fixed effects from the GLMM analysis of fungal Simpson´s diversity, for a data set including mulched and un-mulched field plot observations ("Analysis 2"). The fixed effects and the two-and-three-way interactions included: nutrient amendment (alfalfa, city compost, and control); mulch type (mulched and un-mulched field plots); interaction between nutrient amendment and mulch type; sampling time (maturity and postharvest), interaction between nutrient amendment and sampling time; interaction between mulch type and sampling time; and the three-way interaction nutrient amendment, mulch type and sampling time.

Fungal Simpson´s diversity-PM effect -Type III test of fixed effects				
Effect	Degrees of freedom of the numerator (Num DF)	Degrees of freedom of the denominator (Den DF)	F value	Probability > F
Nutrient amendment	2	30	0.18	0.83
Mulch type	1	3	0.87	0.41
Nutrient amendment* Mulch type	2	30	1.48	0.21
Sampling time	1	30	3.56	0.06
Nutrient amendment* Sampling time	2	30	0.16	0.85
Mulch type* Sampling time	1	30	0.95	0.33
Nutrient amendment* Mulch type* Sampling time	2	30	0.84	0.44

Table 4.15. Type III tests of fixed effects from the GLMM analysis of fungal Simpson´s evenness, for a data set including mulched and un-mulched field plot observations ("Analysis 2"). The fixed effects and the two-and-three-way interactions included: nutrient amendment (alfalfa, city compost, and control); mulch type (mulched and un-mulched field plots); interaction between nutrient amendment and mulch type; sampling time (maturity and postharvest), interaction between nutrient amendment and sampling time; interaction between mulch type and sampling time; and the three-way interaction nutrient amendment, mulch type and sampling time.

Fungal Simpson´s evenness-PM effect -Type III test of fixed effects				
Effect	Degrees of freedom of the numerator (Num DF)	Degrees of freedom of the denominator (Den DF)	F value	Probability > F
Nutrient amendment	2	30	0.96	0.39
Mulch type	1	3	0.00	0.94
Nutrient amendment* Mulch type	2	30	0.02	0.97
Sampling time	1	30	7.97	0.008
Nutrient amendment* Sampling time	2	30	0.82	0.45
Mulch type* Sampling time	1	30	0.18	0.67
Nutrient amendment* Mulch type* Sampling time	2	30	0.22	0.80

Table 4.16. Type III tests of fixed effects from the GLMM analysis of fungal richness, for a data set including mulched and un-mulched field plot observations ("Analysis 2"). The fixed effects and the two-and-three-way interactions included: nutrient amendment (alfalfa, city compost, and control); mulch type (mulched and un-mulched field plots); interaction between nutrient amendment and mulch type; sampling time (maturity and postharvest), interaction between nutrient amendment and sampling time; interaction between mulch type and sampling time; and the three-way interaction nutrient amendment, mulch type and sampling time.

Fungal richness-PM effect- Type III test of fixed effects				
Effect	**Degrees of freedom of the numerator (Num DF)**	**Degrees of freedom of the denominator (Den DF)**	**F value**	**Probability > F**
Nutrient amendment	2	30	0.10	0.90
Mulch type	1	3	8.13	0.06
Nutrient amendment* Mulch type	2	30	1.12	0.34
Sampling time	1	30	23.77	<.0001
Nutrient amendment* Sampling time	2	30	0.27	0.76
Mulch type* Sampling time	1	30	0.04	0.84
Nutrient amendment* Mulch type* Sampling time	2	30	0.53	0.59

Table 4.17. Permanova analysis for bacterial communities in field plots without plastic mulch across two sampling time points (maturity, and postharvest) on a tomato cropping season.

Bacteria_Field plots without plastic mulch ~ Fertilizer type					
	Degrees of freedom	Sum of squares	R^2	F value	Probability (>F)
Fertility level	2	0.068	0.140	1.710	0.025
Residual	21	0.421	0.859		
Total	23	0.489	1.000		

Table 4.18. Permanova analysis for bacterial communities in high tunnels across two sampling time points (maturity, and postharvest) on a tomato cropping season.

Bacteria_High tunnel plots ~ Fertilizer type					
	Degrees of freedom	Sum of squares	R^2	F value	Probability (>F)
Fertility level	2	0.043	0.216	1.242	0.189
Residual	9	0.458	0.783		
Total	11	0.201	1.000		

Table 4.19. Permanova analysis for bacterial communities in field plots with plastic mulch across two sampling time points (maturity, and postharvest) on a tomato cropping season.

Bacteria_Field plots with plastic mulch ~ Fertilizer type					
	Degrees of freedom	Sum of squares	R^2	F value	Probability (>F)
Fertility level	2	0.058	0.065	0.731	0.726
Residual	21	0.837	0.934		
Total	23	0.895	1.000		

Table 4.20. Permanova analysis for fungal communities in high tunnels across two sampling time points (maturity, and postharvest) on a tomato cropping season.

Fungi_High tunnel plots ~ Fertilizer type					
	Degrees of freedom	Sum of squares	R^2	F value	Probability (>F)
Fertility level	2	0.131	0.166	0.895	0.104
Residual	9	0.657	0.833		
Total	11	0.788	1.000		

Table 4.21. The percentage sequences for the 20 overall most frequent bacterial phyla, orders, families and OTUs assigned to genera recovered in MiSeq sequencing of soils from tomato production at a transitional organic farm located in Lawrence, Kansas, USA.

Phylum	Freq. (%)	Order	Freq. (%)	Family	Freq. (%)	Genus	Freq. (%)
Proteobacteria	27.26	Actinomycetales	22.45	unclassified	25.43	*unclassified*	40.18
Acidobacteria	17.33	Acidobacteria_Gp4_ order_incertae_sedis	19.53	Acidobacteria_Gp4_ family_incertae_sedis	11.69	*Gp4*	9.97
Bacteroidetes	14.13	unclassified	10.12	Acidobacteria_Gp4_ family_incertae_sedis	11.27	*Gp6*	9.63
Actinobacteria	12.51	Rhizobiales	7.86	Nevskiales	10.37	*Spartobacteria_ genera_incertae_ sedis*	8.06
Verrucomicrobia	7.45	Sphingobacteriales	4.87	Spartobacteria_ family_incertae_sedis	9.78	*Flavobacterium*	7.13
unclassified	7.08	Bacillales	3.92	Chitinophagaceae	6.34	*Steroidobacter*	5.60
Firmicutes	4.09	Planctomycetales	3.69	Flavobacteriaceae	4.14	*Planctomyces*	5.07
Planctomycetes	3.96	Burkholderiales	3.54	Acidobacteria_Gp3_ family_incertae_sedis	4.07	*Nitrospira*	4.09
Chloroflexi	2.43	Acidobacteria_Gp6_ order_incertae_sedis	3.28	Subdivision3_family _incertae_sedis	3.27	*Gp1*	2.51
Nitrospira	1.09	Spartobacteria_ order_incertae_sedis	3.18	Sinobacteraceae	2.21	*Pseudomonas*	1.63
Gemmatimonadetes	0.68	Xanthomonadales	2.84	Micromonosporaceae	2.02	*Gemmatimonas*	1.59
Chlamydiae	0.43	Myxococcales	2.52	Planctomycetaceae	1.96	*Pasteuria*	1.33
TM7	0.40	Subdivision3_ order_incertae_sedis	2.36	Xanthomonadaceae	1.93	*Gp16*	0.94
Armatimonadetes	0.49	Acidobacteria_Gp3_ order_incertae_sedis	2.21	Nocardioidaceae	1.07	*Bradyrhizobium*	0.81
OD1	0.16	Flavobacteriales	2.17	Nitrospiraceae	0.99	*Gp3*	0.73
Chlorobi	0.04	Rhodospirillales	1.81	Bacteroidetes_ family_incertae_sedis	0.85	*Ohtaekwangia*	0.23
OP11	0.05	Nitrospirales	1.02	Sphingobacteriaceae	0.76	*3_genus_ incertae_sedis*	0.19
Deinococcus-Thermus	0.02	Acidobacteria_Gp1_ order_incertae_sedis	0.94	Gemmatimonadaceae	0.69	*Tumebacillus*	0.13
Spirochaetes	0.02	Bacteroidetes_ order_incertae_sedis	0.86	Sphingomonadaceae	0.57	*TM7_genus_ incertae_sedis*	0.10
BRC1	0.01	Sphingomonadales	0.75	Pasteuriaceae	0.52	*Opitutus*	0.04

Table 4.22. The percentage sequences for the 20 overall most frequent fungal phyla, orders, families and OTUs assigned to genera recovered in MiSeq sequencing of soils from tomato production at a transitional organic farm located in Lawrence, Kansas, USA.

Phylum	Freq. (%)	Order	Freq. (%)	Family	Freq. (%)	Genus	Freq. (%)	Species	Freq. (%)
Ascomycota	55.21	Hypocreales	22.27	unclassified	25.41	unclassified	41.26	unclassified	51.13
Basidiomycota	22.48	Mortierellales	14.32	Mortierellaceae	13.46	*Mortierella*	11.83	*Hannaella sinensis*	8.13
Zygomycota	13.27	Pleosporales	13.76	Nectriaceae	10.90	*Hannaella*	8.77	*Phoma_sp_ UASWS0872*	7.55
unclassified	5.89	unclassified	10.52	Pleosporales_ family_incertae sedis	6.81	*Phoma*	7.42	*Haematonectria haematococca*	6.41
Chytridiomycota	1.83	Tremellales	6.25	Tremellales_ family_ incertae_sedis	6.56	*Haemato-nectria*	6.51	*Mortierella humilis*	5.62
Glomeromycota	1.04	Sordariales	5.24	Pleosporaceae	5.62	*Myrothecium*	4.14	*Alternaria porri*	3.27
		Russulales	3.78	Hypocreales_ family_ incertae_sedis	4.73	*Alternaria*	3.08	*Lysurus cruciatus*	2.12
		Phallales	3.44	Stephanos-poraceae	3.82	*Gibberella*	3.01	*Mortierella exigua*	1.99
		Chaetothyriales	3.26	Phallaceae	2.64	*Lysurus*	2.77	*Mortierella capitata*	1.95
		Eurotiales	2.91	Trichoco-maceae	2.37	*unclassified Stephanos-poraceae*	1.86	*Gibberella intricans*	1.90
		Agaricales	2.83	Chaetomiaceae	2.24	*unclassified Bionec-triaceae*	1.54	*Stephanos-poraceae_sp*	1.61
		Xylariales	1.99	Hypocreaceae	2.18	*Fusarium*	1.48	*Exophiala equina*	1.48
		Ascomycota_ order_incertae_ sedis	1.47	Herpotrichie-llaceae	2.12	*Cryptococcus*	1.22	*Bionectriaceae sp*	1.37
		Cantharellales	1.32	Lasios-phaeriaceae	2.09	*Talaromyces*	1.07	*Monographella cucumerina*	0.94
		Corticiales	1.28	Xylariales_ family_ incertae sedis	1.75	*Exophiala*	0.86	*Bipolaris microstegii*	0.88
		Capnodiales	1.07	Ascomycota_ family_ incertae_ sedis	1.49	*Phallus*	0.77	*Rhizoctonia zeae*	0.83
		Filobasidiales	1.01	Bionec-triaceae	1.46	*Monographella*	0.64	*Staphylotrichum sp*	0.79
		Pezizales	0.87	Corticiaceae	1.39	*Bipolaris*	0.52	*Phallus rugulosus*	0.71
		Cystofilo-basidiales	0.76	Davidiella-ceae	1.16	*Waitea*	0.41	*Myrothecium sp*	0.68
		Rhizophlyc-tidales	0.41	Filobasidia-ceae	1.05	*Trichoderma*	0.38	*Remersonia sp*	0.62

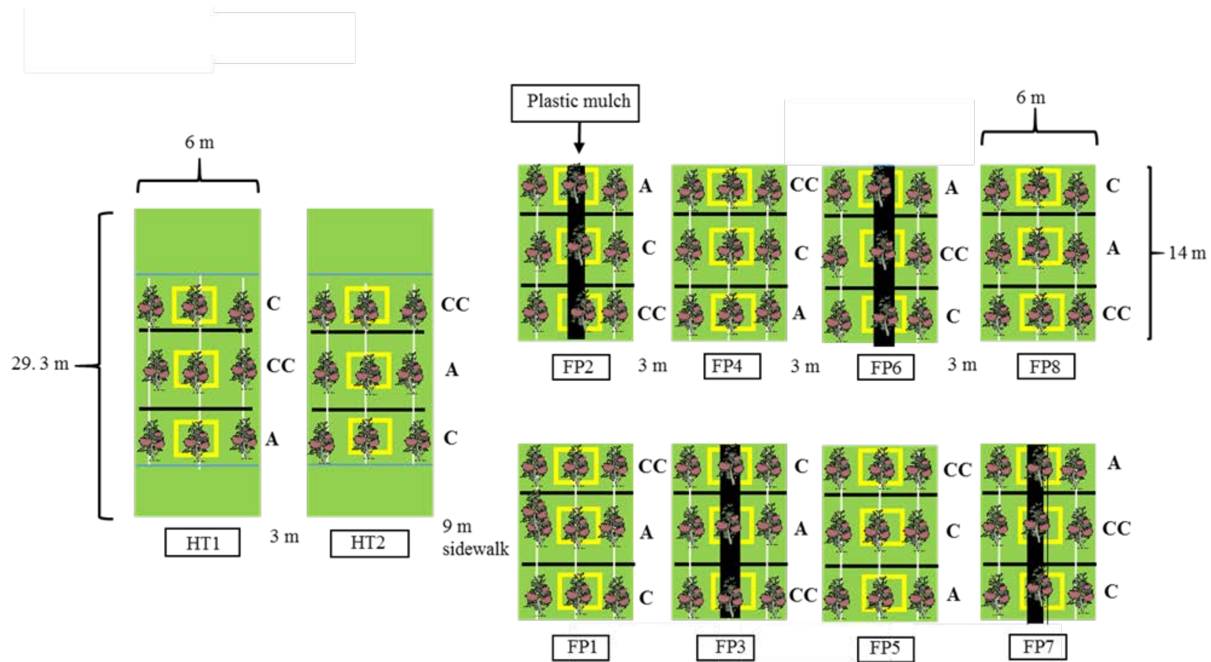

Figure 4.1. Research plots located at the Common Harvest farm in Lawrence. KS. Two high-tunnels (HT1, and HT2; 29.3 m by 6 m each. separated by a 3 m sidewalk) and eight adjacent open field plots (FP1 to FP8; 14 m by 6 m each. separated by a 3 m sidewalk) were divided into subplots for random assignment to three nutrient management treatments: control (C), alfalfa meal pellets (A), and city compost (CC). A 9 m wide sidewalk separated the high tunnels (HT) and field plots (FP). Tomato (*Lycopersicum esculentum* "Mountain Fresh") was planted in all experimental plots. In the open field plots, four open field plots (FP2, FP3, FP6, and FP7) were covered with a single layer of embossed black plastic mulch (designated as black lines in the figure), while the other four field plots (FP1, FP4, FP5, and FP8) were uncovered. Soil samples were taken systematically near the base of the middle four tomato plants in the middle row from each plot. In the figure, the four tomato plants are represented by only one tomato plant per subplot, and the middle rows are designated by a yellow square in each subplot.

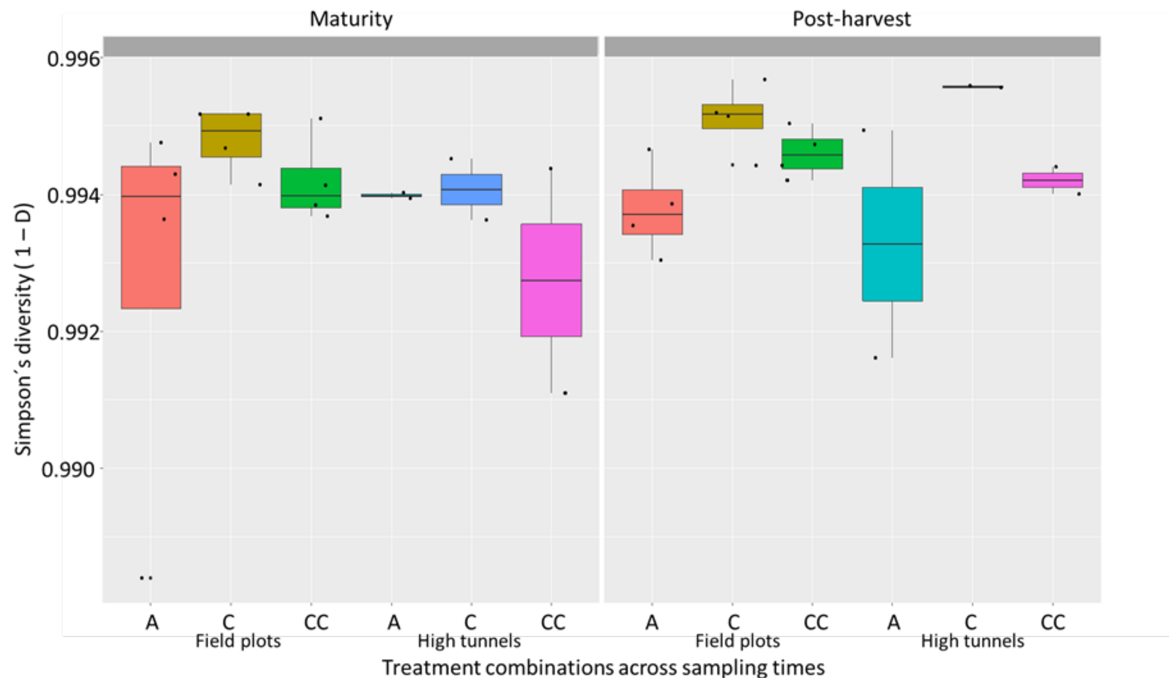

Figure 4.2. Bacterial Simpson´s diversity in a data set including observations for high tunnels and field plots without plastic mulch, where nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)), across two sampling times: maturity and postharvest. The ANCOVA analyses found evidence for the effect of nutrient amendment (F = 6.05, p = 0.05).

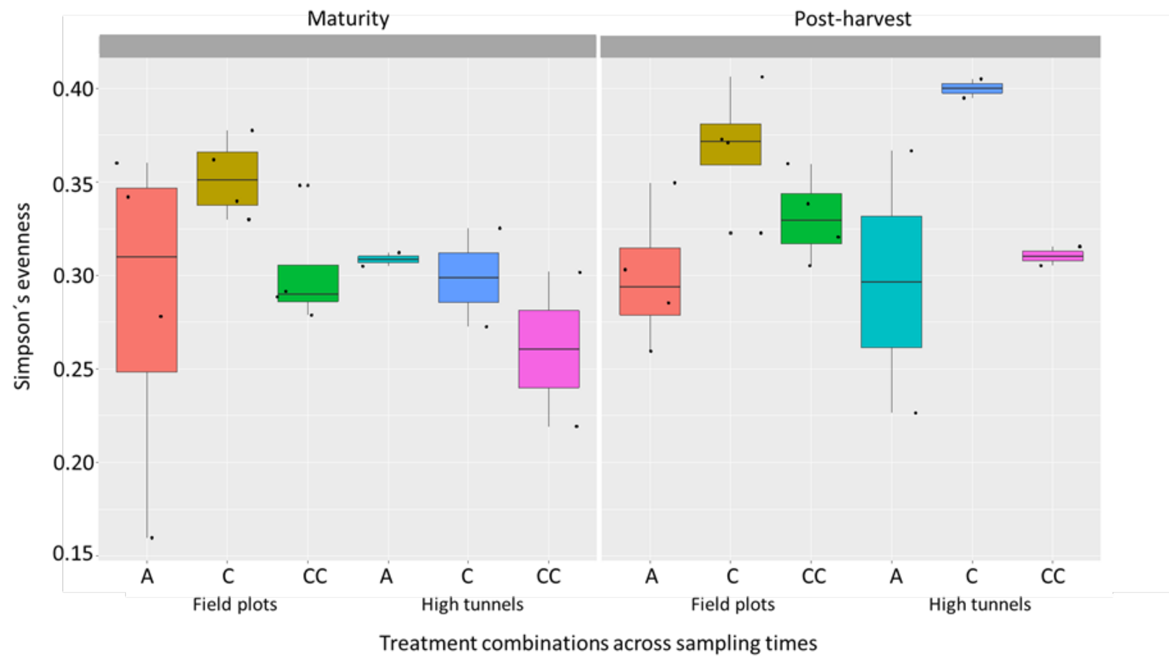

Figure 4.3. Bacterial Simpson´s evenness in a data set including observations for high tunnels and field plots without plastic mulch, where nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)), across two sampling times: maturity and postharvest. The ANCOVA analyses found some evidence for a nutrient amendment effect (F = 5.47, p = 0.05), and a borderline evidence for a sampling time effect (F = 4.31, p = 0.09).

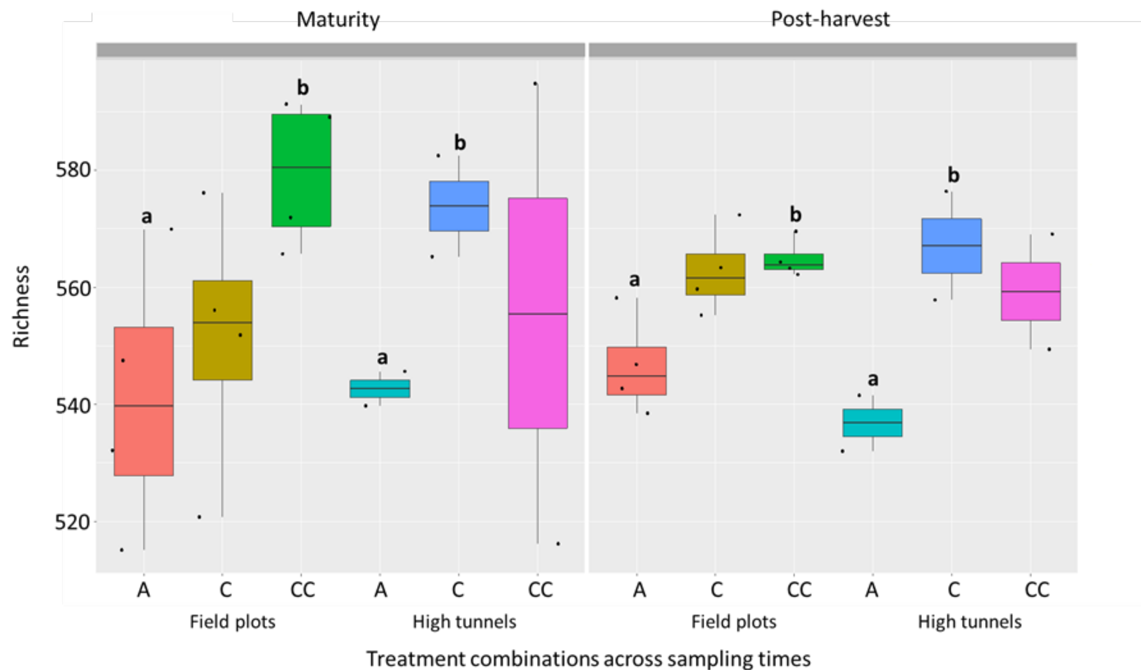

Figure 4.4. Bacterial richness in a data set including observations for high tunnels and field plots without plastic mulch, where nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)), across two sampling times: maturity and postharvest. The ANCOVA analyses found strong evidence for a nutrient amendment effect (F = 14.52, p = 0.008) and some evidence for the interaction between plot type (high tunnels vs. un-mulched field plots) and nutrient amendment (F = 4.41, p = 0.07). Comparisons of least square means (LSM) found evidence for differences between: alfalfa vs. city compost in field plots (t = -4.16, p = 0.05, after Tukey-Kramer adjustment); alfalfa in field plots vs. control in high tunnels(t = -3.91, p = 0.07, after Tukey-Kramer adjustment); city compost in field plots vs. alfalfa in high tunnels (t = 4.76, p = 0.03, after Tukey-Kramer adjustment); and alfalfa vs. control in high tunnels (t = -4.52, p = 0.04, after Tukey-Kramer adjustment).

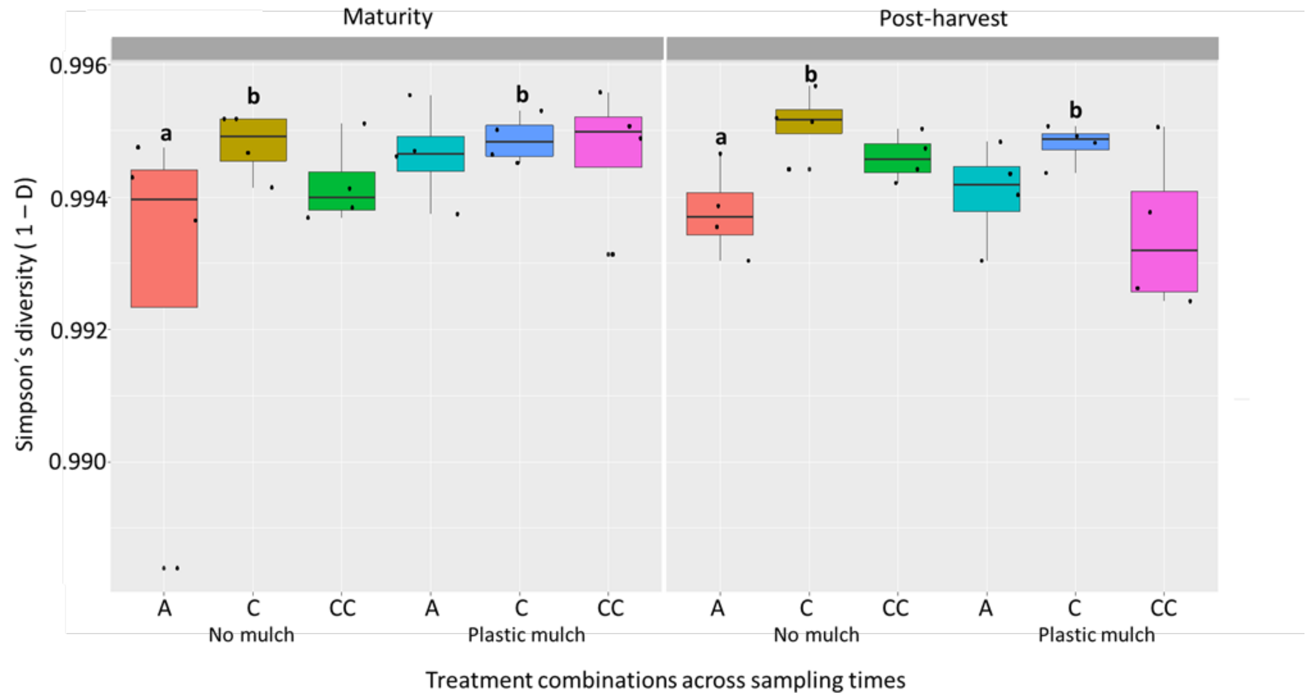

Figure 4.5. Bacterial Simpson´s diversity in a data set including observations for high tunnels and field plots without plastic mulch, where nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)), across two sampling times: maturity and postharvest. A GLMM analyses found evidence for a nutrient amendment effect (type III test: $F = 4.01$, $p = 0.03$), and the two-way interaction: mulch type (presence/absence) and sampling time ($F = 3.64$, $p = 0.06$). Comparisons of least square means (LSM) found evidence for differences between: alfalfa vs. control in field plots without plastic mulch ($t = -3.09$, $p = 0.04$, after Tukey-Kramer adjustment), and alfalfa in un-mulched field plots vs. control in mulched field plots ($t = -2.88$, $p = 0.07$, after Tukey-Kramer adjustment).

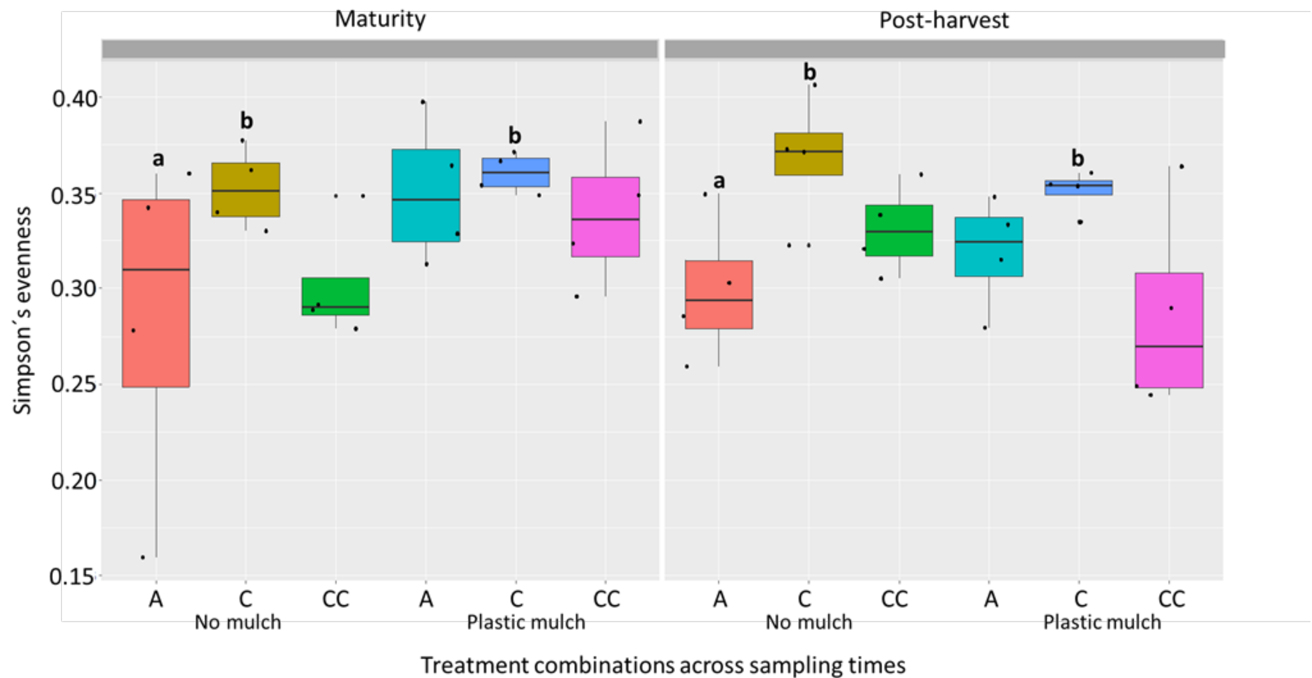

Figure 4.6. Bacterial Simpson´s evenness in a data set including observations for field plots with and without plastic mulch, in which nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)) across two sampling times: maturity and post-harvest. A GLMM analysis found evidence for a nutrient amendment effect (type III test: F = 5.73, p = 0.008), and the two-way interaction: mulch type (presence/absence) and sampling time (F = 5.07, p = 0.03). Comparisons of least square means (LSM) found evidence for differences between: alfalfa vs. control in un-mulched field plots (t = -3.22, p = 0.03, after Tukey-Kramer adjustment), and alfalfa in un-mulched field plots vs. control in mulched field plots (t = -3.00, p = 0.05, after Tukey-Kramer adjustment).

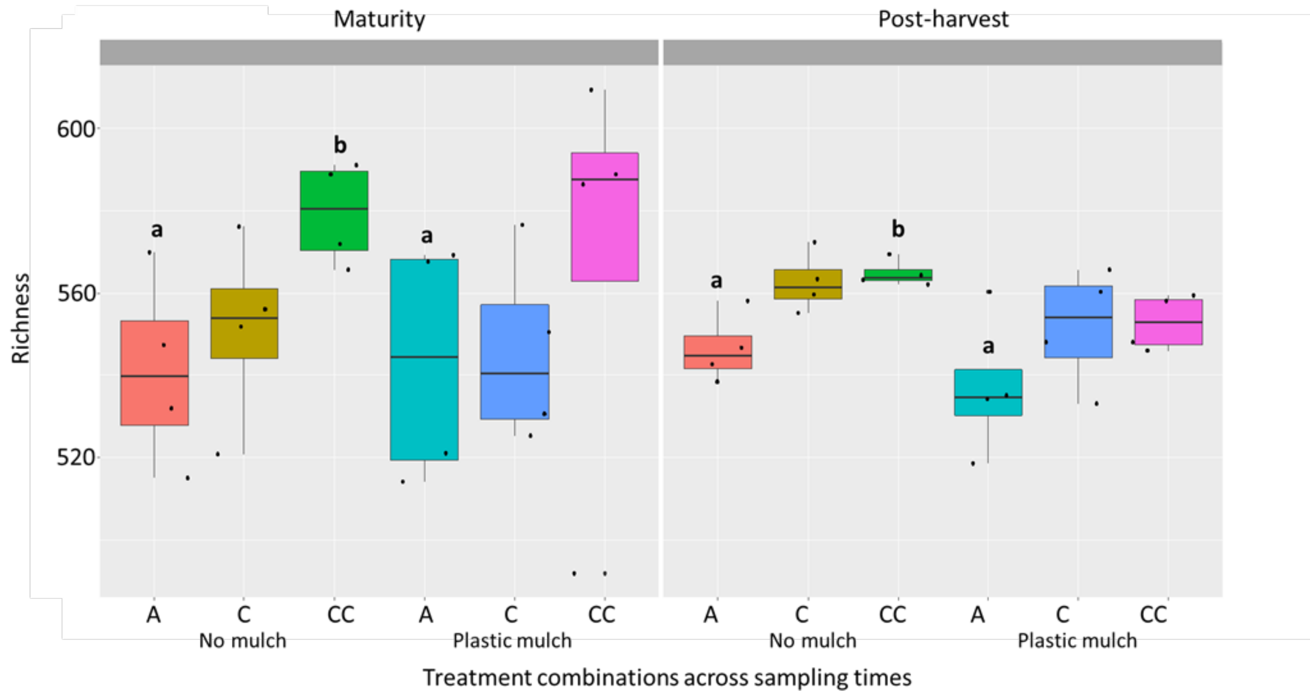

Figure 4.7. Bacterial richness in a data set including observations for field plots with and without plastic mulch, where nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)) across two sampling times: maturity and post-harvest. A GLMM analyses found evidence for the effect of nutrient amendment (F = 6.37, p = 0.005). Comparisons of least square means (LSM) found evidence for differences between: alfalfa vs. city compost for un-mulched field plots (t = -2.89, p = 0.06, after Tukey-Kramer adjustment), and alfalfa for mulched field plots vs. city compost for un-mulched field plots (t = -3.11, p = 0.04, after Tukey-Kramer adjustment).

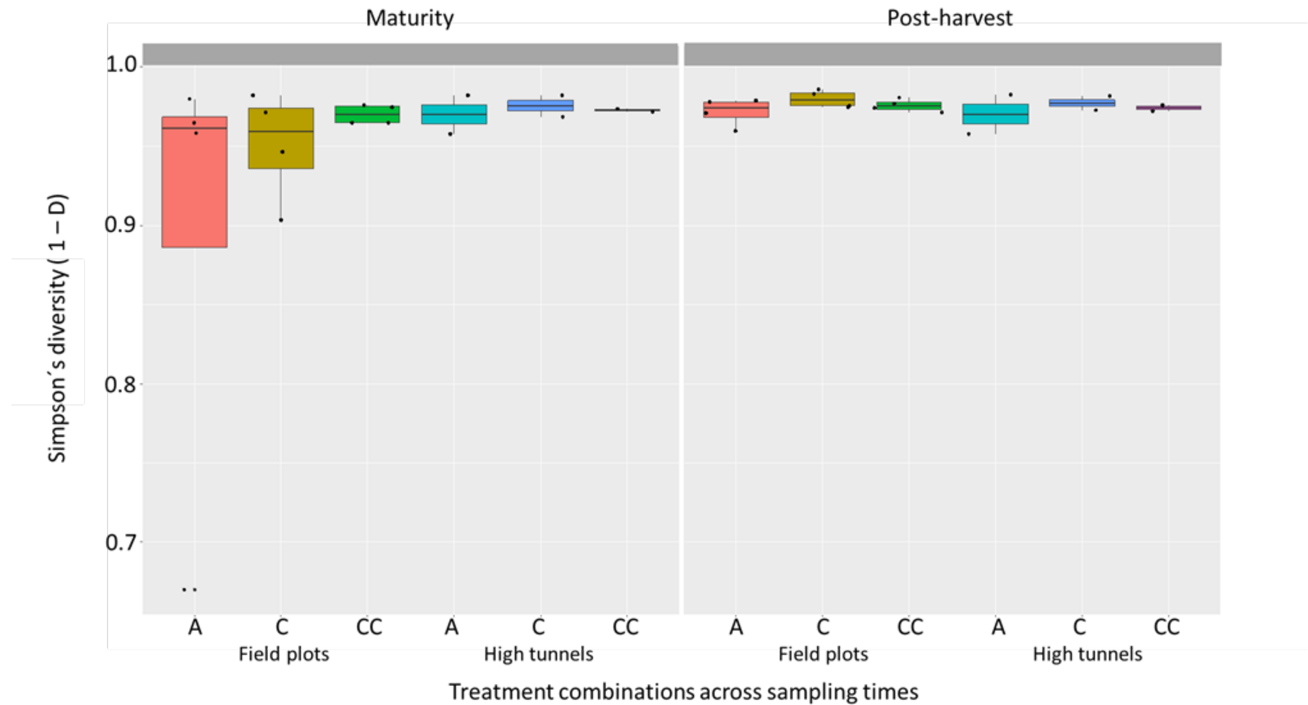

Figure 4.8. Fungal Simpson´s diversity in a data set including observations for high tunnels and field plots without plastic mulch, where nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)), across two sampling times: maturity and post-harvest. The ANCOVA analyses did not find evidence for any of the effects (nutrient amendment, high tunnel, and sampling time) or their interactions (*$p > 0.2$*).

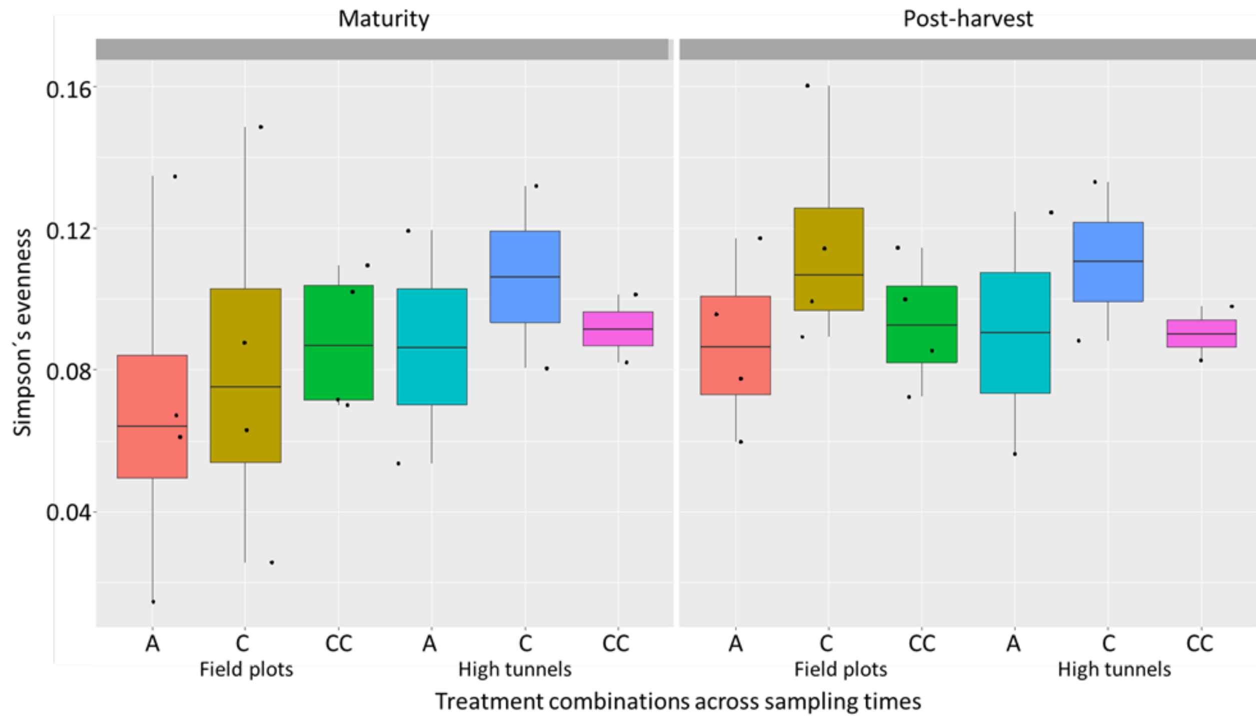

Figure 4.9. Fungal Simpson´s evenness in a data set including observations for high tunnels and field plots without plastic mulch, where nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)), across two sampling times: maturity and post-harvest. The ANCOVA analyses found some evidence for a nutrient amendment effect ($F = 4.62$, $p = 0.07$), and a borderline evidence for a sampling time effect ($F = 3.88$, $p = 0.10$).

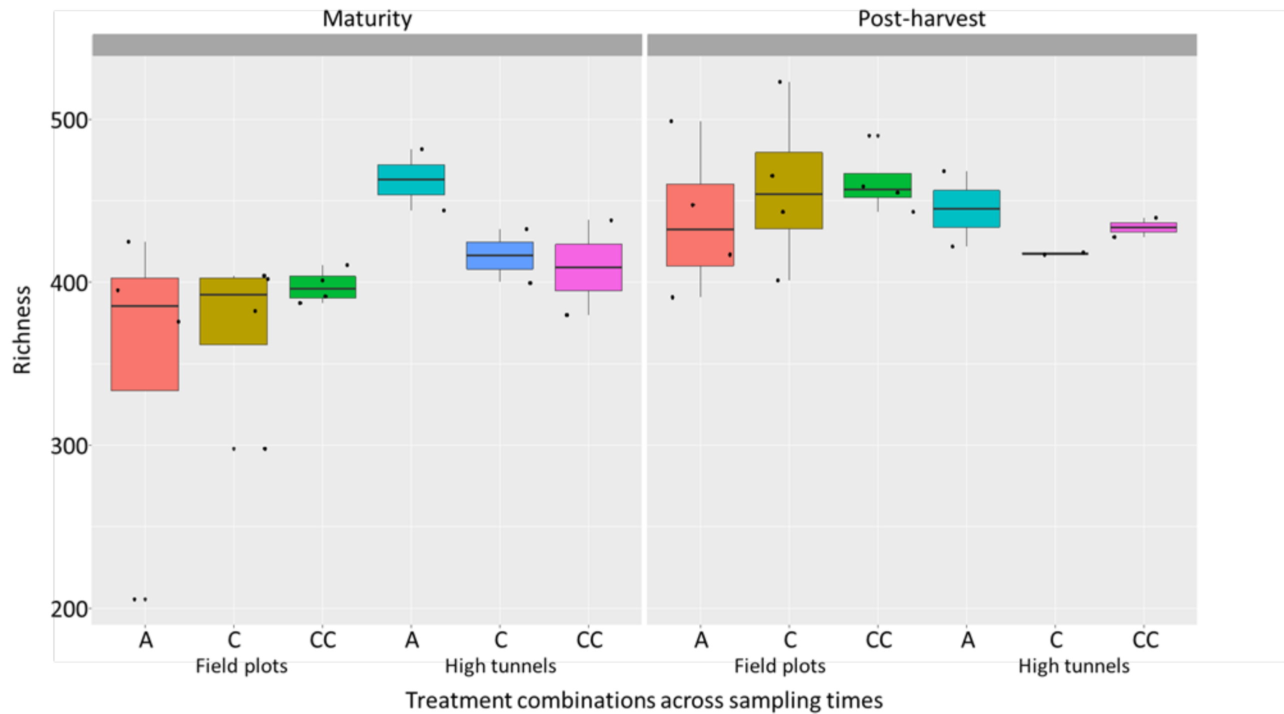

Figure 4.10. Fungal richness in a data set including observations for high tunnels and field plots without plastic mulch, where nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)), across two sampling times: maturity and post-harvest. The ANCOVA analyses found some evidence for a sampling time effect (F = 5.02, p = 0.07).

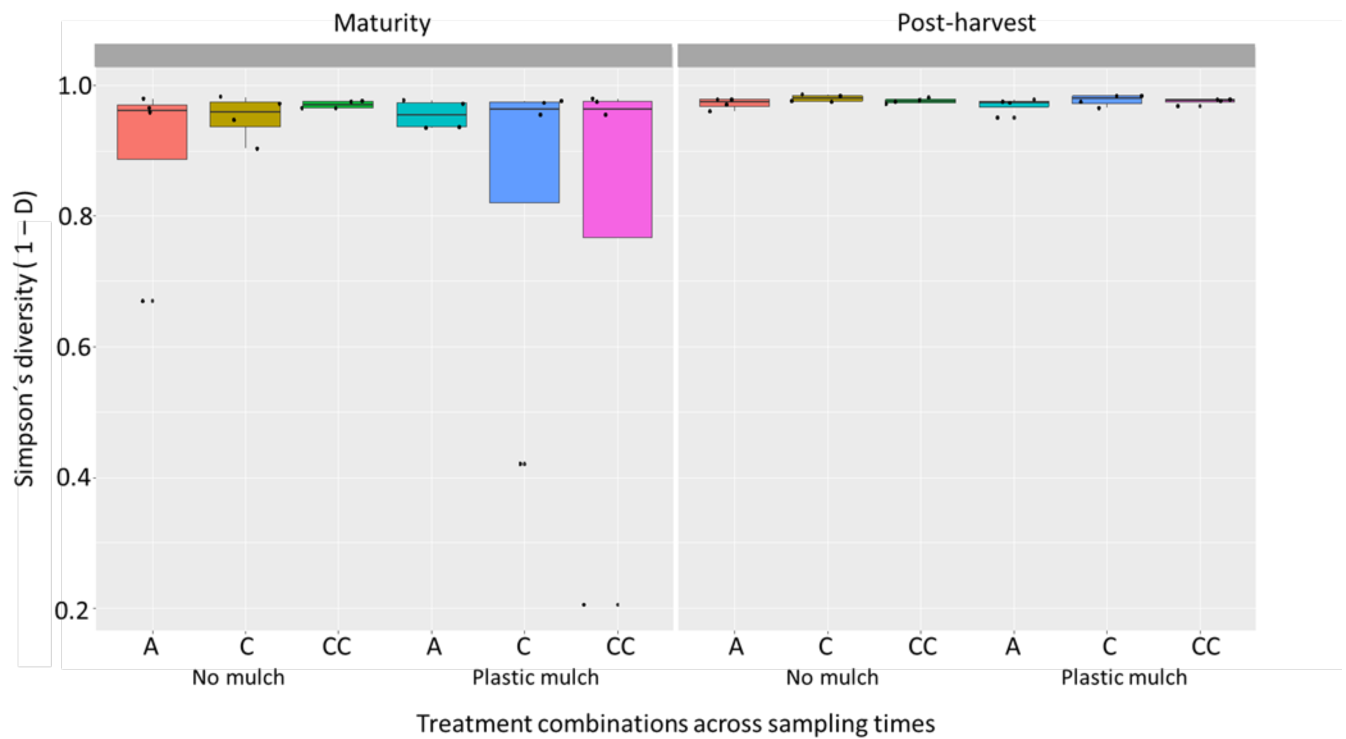

Figure 4.11. Fungal Simpson´s diversity in a data set including observations for field plots with and without plastic mulch, where nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)) across two sampling times: maturity and post-harvest. A GLMM analyses found some evidence for a sampling time effect (type III test: $F = 3.56$, $p = 0.06$).

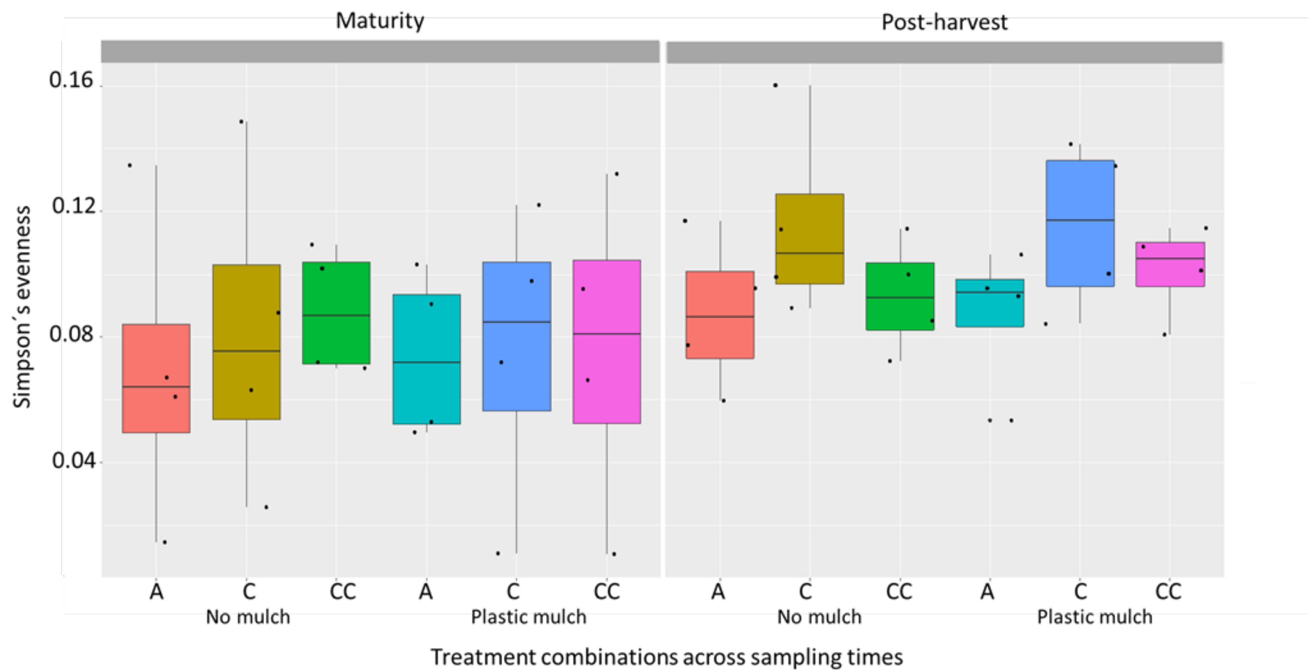

Figure 4.12. Fungal Simpson´s evenness in a data set including observations for field plots with and without plastic mulch, in which nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)) across two sampling times: maturity and post-harvest. A GLMM analysis found strong evidence for a sampling time effect (F = 7.97, p = 0.008).

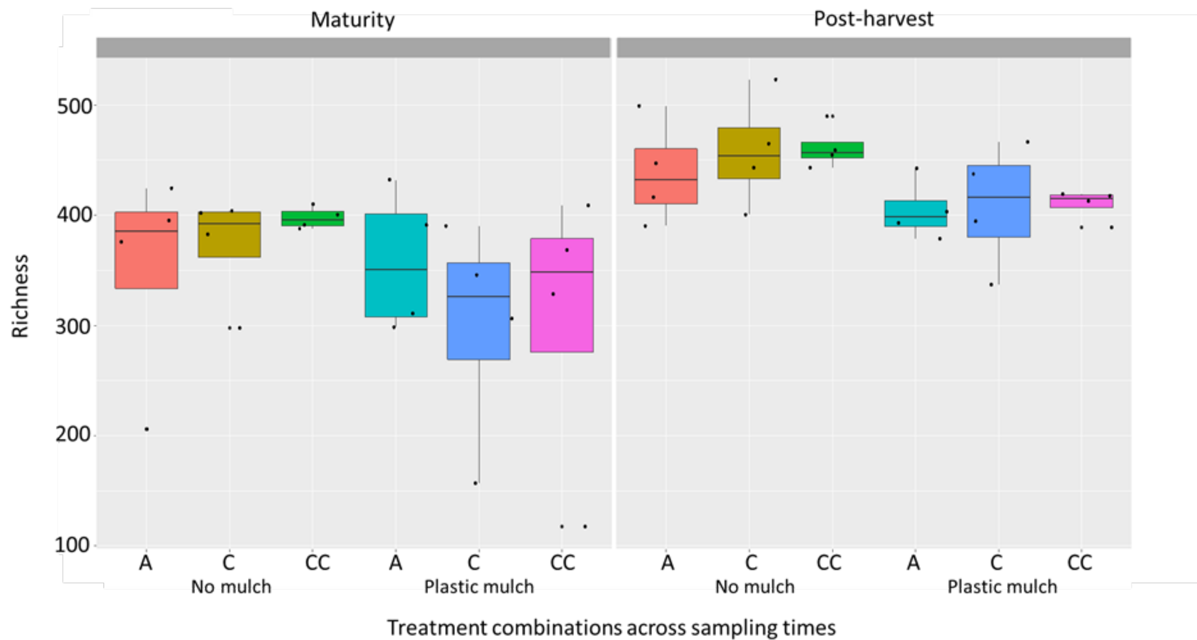

Figure 4.13. Fungal richness in a data set including observations for field plots with and without plastic mulch, where nutrient amendments were alfalfa meal pellets (A), control (C, no additional amendments added) and city compost (CC)) across two sampling times: maturity and post-harvest. A GLMM analyses found some evidence for a mulch type effect (F = 8.13, p = 0.06), and a strong evidence for a sampling time effect (F = 23.77, p < 0.0001).

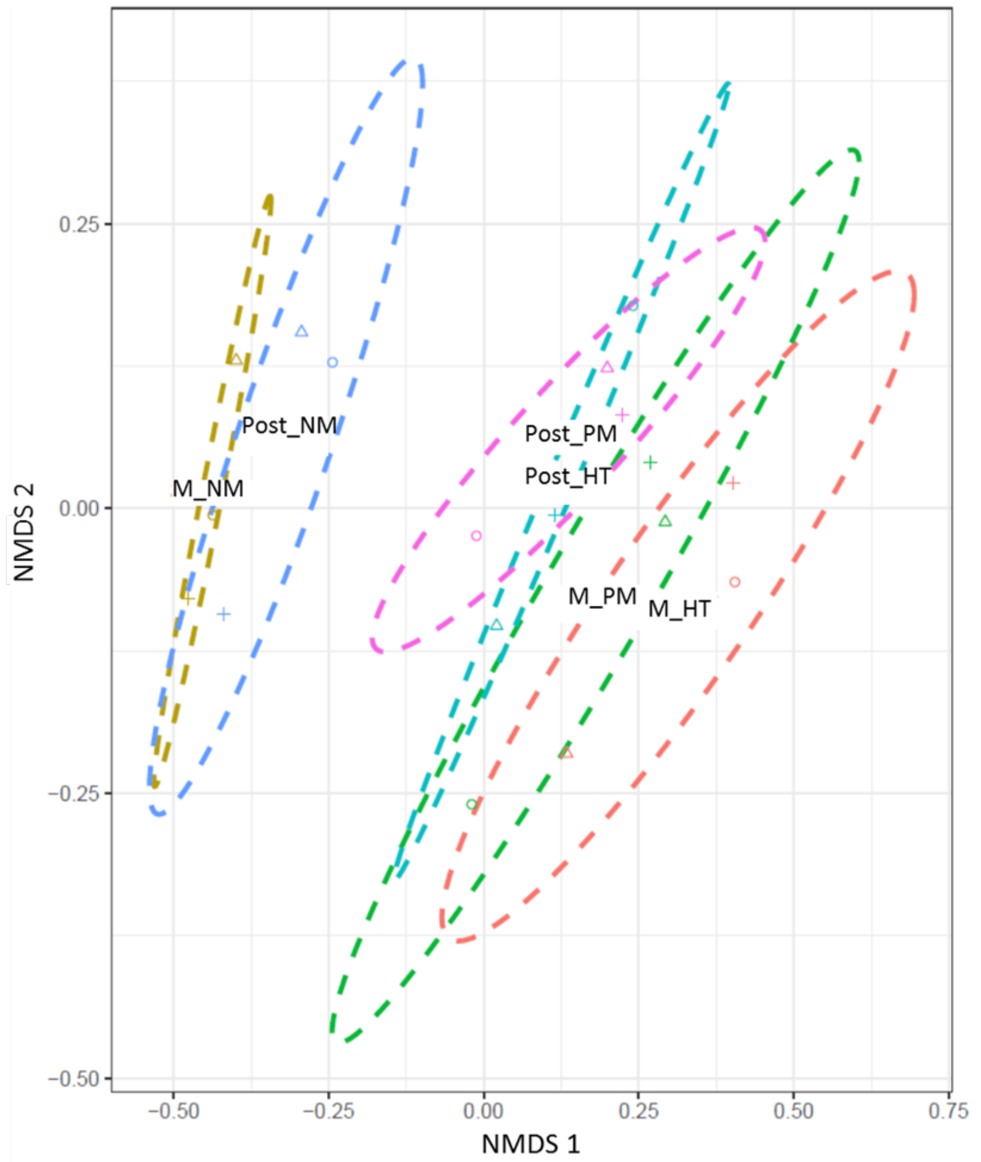

Figure 4.14. Non-metric multidimensional scaling (NMDS) in two dimensions of *bacterial* community composition responses to nutrient amendments (alfalfa meal pellets (circles), control (triangles), or city compost (plus signs). Circles indicate 95% confidence regions for responses in each treatment (high tunnel (HT), field plot with plastic mulch (PM), or field plot without plastic mulch (NM)) across two sampling time points (maturity (M), and postharvest (Post). Two convergent solutions found after twenty trials with a final stress of 0.12 and a maximum residual of 0.0006.

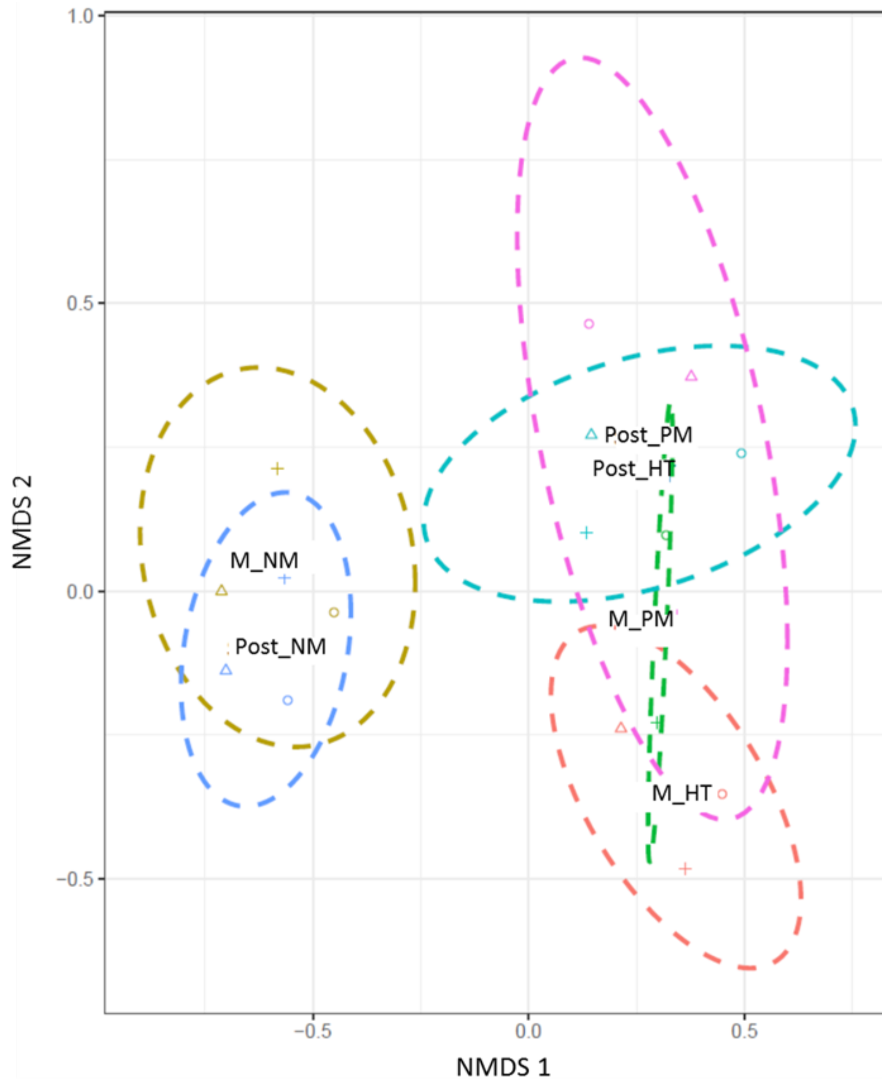

Figure 4.15. Non-metric multidimensional scaling (NMDS) in two dimensions of *fungal* community composition responses to nutrient amendments (alfalfa meal pellets (circles), control (triangles), or city compost (plus signs). Circles indicate 95% confidence regions for responses in each treatment (high tunnel (HT), field plot with plastic mulch (PM), or field plot without plastic mulch (NM)) across two sampling time points (maturity (M), and postharvest (Post). Two convergent solutions found after twenty trials with a final stress of 0.14 and a maximum residual of 0.052.

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Chapter 5 - Conclusions and future directions

We studied bacterial and fungal communities in soil samples taken from tomato production plots under specific organic or conventional management strategies. We applied high-throughput sequencing to better understand how soil bacterial and fungal communities respond to organic or conventional soil fertility management under these treatments. In our first study titled "*The response of soil bacterial and fungal communities to organic and conventional fertilization: comparing soil DNA and RNA,"* we used both DNA and ribosomal RNA (rRNA, analyzed as complementary DNA (cDNA)) to evaluate bacterial and fungal community responses in a long-term experimental system. The two methods sometimes reached different conclusions. For example, in the high fertility organic management treatment, bacterial diversity was higher using cDNA compared to DNA. In addition, the highest fungal diversity occured using cDNA compared to DNA under control and high fertility treatments for both organic and conventional systems. The frequencies of several bacterial and fungal taxa differed between in the two methods. For example, *Pseudomonas* and *Aspergillus* were more frequent in DNA derived estimates, for bacterial and fungi, respectively. In contrast, using the cDNA analysis, *Sorangium* (bacteria) and *Glomus* (fungi) were more frequent. We hypothesized that the biological differences in the long-term survival structures produced by various taxa may have led to differences in communities recovered from DNA, which includes dead/dormant cells, and cDNA, which would be focused on living/active cells. Future research avenues could include studies to examine functional gene diversity and ecological functions.

In our second study titled "*Soil bacterial and fungal communities in tomato production under long-term organic vs. conventional soil fertility management*", we characterized soil

bacterial and fungal communities across a growing season in tomato production in a long-term experiment comparing organic and conventional management strategies using differing fertilizer input levels. Our study showed that sampling time significantly affected both bacterial and fungal richness under conventional and organic management systems, while management types (conventional vs organic) and fertilizer levels (control, low-and-high-fertility) had only minimal effects. Bacterial and fungal richness across treatments increased postharvest. The characterization of soil microbial communities found that the bacteria assigned to the subdivisions *Gp4* (phylum Acidobacteria) and to the genera *Sphingomonas* (phylum Proteobacteria) and *Nitrospira* (phylum Nitrospirae), and fungi assigned to the genera *Mortierella* (phylum Zygomycota), *Phoma* (phylum Ascomycota) and *Alternaria* (phylum Ascomycota), were the top three dominant taxa across all treatment combinations and the three sampling times. Neither bacterial nor fungal diversity nor richness was affected by whether organic fertilizers versus synthetic fertilizers were applied. Thus, our findings are not consistent with previous studies in which organic fertilizers have been reported to increase soil microbial diversity compared to synthetic nitrogen sources (Zelles *et al*., 1992; Marschner *et al*., 2004; Tu *et al*., 2006). Characterization of functional diversity of the soil agroecosystem in response to these treatments would provide a greater understanding of how soil amendments may stabilize agroecosystem processes.

Finally, in our third study titled "*The effects of organic amendments and plastic mulch on soil bacterial and fungal communities over time in tomato cropping systems*", we evaluated the effect of high tunnels and plastic mulch on soil microbes in a vegetable crop under two organic amendments. We found that the application of the city compost amendment, but not alfalfa meal pellets, increased bacterial richness without affecting the increase of fungal richness, diversity or evenness. The use of high tunnels did not have a significant effect on fungal or bacterial diversity.

The use of plastic mulch on field plots decreased fungal richness, but did not affect bacterial richness or diversity. Our results differ from prior work in which plastic mulch enhanced community richness and diversity for bacteria and fungi (Dong et al., 2017; Farmer et al., 2017). Our experiment explored plastic mulch effects in only one season. Multi-season experiments would provide more insights into longer-term effects on soil microbial communities.

There is strong interest among many organic farmers to exploit microbial community data to guide farm management decisions. However, interpretation of such broad community data has some limitations, because information about the functional roles of taxa is limited, and closely related species or even conspecific strains may have very different roles. While some taxa have very consistent functional roles, other groups, such as the bacterial *Pseudomonas* spp. and fungal *Cladosporium* spp., include both important pathogens and important natural biocontrol agents. Our methods have limited resolution to the species level, let alone specific strains, pathotypes, or other sub-specific groupings. Another caveat for interpreting these community data is the uncertainty in categorizing taxonomic groups based on sequence databases: there may be errors in classification, particularly at taxonomic levels such as genera and species in those databases. A great deal of translation of of this type of fundamental scientific results to applied technologies will be necessary to make the use of microbial community profiles a reality for day-to-day decision making on farms. Nonetheless, huge steps in microbial ecology enabled by new sequencing techniques have permitted monitoring soil microbial taxa at a greater volume and resolution than previously possible. This offers the potential to evaluate the success of agricultural soil management at the level of individual taxa and, ultimately, taxa with desired, beneficial functions.

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