Soil microbes in organic vegetable production: New insights from pyrosequencing
The response of soil microbial communities to organic and conventional fertilization

A final report to The Ceres Trust

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Project Summary

Technologies for studying the composition of microbial communities are developing at a very rapid pace. A challenge for agriculture is how to use these technologies to support sustainable production. Soil microbial communities play fundamental roles in the productivity of agricultural systems. Organic methods may foster more diverse soil microbial communities beneficial for crop production that may reduce losses to pathogens and increase plant productivity.

We evaluated bacterial and fungal community responses in an established long-term experimental system comparing organic vs. conventional nutrient management for tomato production. This experiment was designed in collaboration with organic growers to represent current management practices. We used 454 pyrosequencing to simultaneously evaluate DNA from hundreds of thousands of microbes in soil samples, including species that could not be cultured using traditional techniques. We also compared the microbial communities recovered from analysis of DNA, rapidly becoming a standard technique, and from RNA, a new approach. Comparing communities recovered through DNA and RNA allowed comparison of the general pool of microbes (DNA) and the microbes that were actively metabolizing (RNA). We recovered a large number of taxa known to have important agroecological roles, and other taxa whose roles are yet to be known.

Our first hypothesis was that microbial diversity would be higher in organic compared to conventional agriculture, and the results supported this hypothesis. Our second hypothesis was that microbial diversity would be higher in DNA than in RNA samples, but higher diversity was sometimes observed in RNA samples. This may have occurred because DNA samples were dominated by a smaller number of taxa with tough dormant structures.

We are building on these analyses in a second Ceres Trust project evaluating organic management effects on microbial communities. Both these projects are the PhD research of Lorena Gomez-Montano, and the projects have also engaged two undergraduate students so far. In addition to a journal article we have published about new experiments that may contribute to understanding microbial community function (Garrett et al, 2012), the results presented in this report will be published in a peer-reviewed journal.

Introduction

Significance of the project to organic agriculture

A central idea in organic agriculture is that crop management using organic approaches helps to maintain and develop microbial communities that provide important ecosystem services. These services may include biocontrol by microbes that compete with pathogens, microbial contributions to nutrient cycling and plant nutrient uptake, or microbial contributions to soil organic matter formation. An important stumbling block to understanding the effects of organic systems on microbial communities has been the limitations of techniques available for studying these communities. New techniques, *e.g.*, 454 sequencing (pyrosequencing), make unprecedented environmental analyses affordable. Primers that are specific to a taxonomic group (Eubacteria, Fungi, Eukarya) can be used to generate lists of the relative frequencies of microbes

across multiple levels of taxonomic hierarchy (e.g., Acosta-Martinez et al., 2008). The combination of these high throughput tools with DNA-tagging (see Dowd et al., 2008; Jumpponen and Jones, 2010) allows microbial community analyses with adequate replication to address novel and important questions in detail never before available. The taxonomic identity of microbes can often be derived from public databases (GenBank) and used to infer functional roles such as beneficial mycorrhizal associates or well-known pathogens or pathogen antagonists. This new type of information allows a much more complete definition of the nature of soil microbial resources, necessary for sustainable management.

Microbial communities in soil help to maintain the productivity and health of agricultural systems (Pankhurst et al., 1996). Soil microbiota play key roles in most of the functional processes that support terrestrial ecosystems, including nutrient acquisition and recycling, degradation of agrochemicals, and nutrient cycling (Pankhurst et al., 1996; van der Heijden et al., 2008). This capacity of soil to function as a vital living system able to fulfill all these functions defines 'soil quality' (Karlen et al., 1997). Microbial diversity represents the repertoire of genetic diversity that supports the health of soil (Jain et al., 2005). However, despite the importance of soil microorganisms, very little is known about their diversity and community structure (Fierer et al., 2007). In this regard, taxonomic approaches to estimating diversity of soil microbial communities have been limited by the traditional methods and the non-culturability of the majority of the microbial species present in soil (Fierer et al., 2007; Rondon et al., 2000). Extraction of the total RNA from soil has been used to quantify the abundance of Proteobacteria, Actinobacteria, Bacteria and Eukarya under different field management regimes (fertilization, tillage, and the effect of historical cultivation on microbial community) before pyrosequencing was available for characterization (Buckley and Schmidt, 2001). Most recently, metagenomic and small subunit rRNA-based sequence analysis techniques have measured the genetic diversity of Bacteria, Archaea, Fungi and Viruses in soils collected from different ecosystems (prairie, desert and rainforest) (Fierer et al., 2007; Jumpponen, 2011). The use of 454-sequencing techniques allows analysis of millions of microorganisms, bypassing culturing (Roesch et al., 2007), and the recent development of sample-specific sequence tags (DNA tagging) for this technique allows simultaneous analysis of large numbers of individual samples, making DNA sequencing and analysis more efficient (Acosta-Martinez et al., 2008; Jumpponen and Jones, 2009; Lauber et al., 2009; Roesch et al., 2007).

Recently two studies used pyrosequencing to evaluate the effects of organic and conventional management on soil communities. Sugiyama et al. (2010) studied soil fungi and oomycetes in three organic and three conventional potato fields in Colorado, concluding that taxa classified as *Alternaria* spp. and *Ulocladium* spp. were more common in conventional farms while *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 composts or lack of pesticide use. Chaudhry et al. (2012) studied bacterial communities in a long-term organic field and a long-term conventional field in Maharashtra State, India, concluding that the organic field had higher bacterial diversity.

We studied the effects of organic and conventional soil fertilization in a long-term experiment in Olathe, Kansas. By using a designed experiment with replication we can draw more direct inference about the effects of particular treatments, to complement observational studies of the

differences among organic and conventional farms. We characterized the soil bacterial community composition in organic agriculture compared to conventional management, for two fertility levels (high and low) in a tomato crop. We also compared the microbial communities recovered when sampling DNA (which may include dormant microbes) and the microbial communities recovered when sampling RNA, later processed as cDNA (which includes those microbes that are more actively metabolizing).

Project Objectives

1. Characterize microbial communities in a long-term experiment with conventional and organic management of fertility levels
2. Evaluate the differences in bacterial and fungal communities depending on whether they are sampled through DNA or RNA
3. Provide training for a graduate student and undergraduate students through these projects

Our hypotheses included:

a. Organic management will result in higher microbial diversity than conventional management
b. Microbial communities detected through RNA (cDNA) will be less diverse than those detected through DNA

Materials and Methods

Our project built on a long-term experiment addressing organic and conventional agriculture. This USDA-IOP research project, "Effects of organic fertility management on crop health and phytochemical content of vegetables under open field and high tunnel production," is managed under the guidance of a grower advisory panel of organic farmers and grower educators. Fertility treatments under this project were planned in consultation with this group and were perceived to be relevant to common current grower practices. The research plots, which have been under continuous organic management since 2002 were USDA certified in 2003 and each year since 2006. Indiana Certified Organic is the inspector.

Study location

The field study was at the K-State Horticulture Center in Olathe, KS, in an experiment comparing organic (Indiana Organic Certification) and conventional fertilization. These field plots have been maintained in place for six years and consist of three replicate plots for each combination of management treatment (organic and conventional) and three levels of fertilization (high, medium, and low nitrogen, with levels as identified by organic growers). Including the comparison of DNA and cDNA gives a split-split plot design with whole plots (organic vs. conventional treatment) in a randomized complete block design. Each subplot to which fertilizer treatments were applied was 10.6 ft by 20 ft. Our analysis includes the three replicate plots for two levels of fertilization (high and low nitrogen), for a total of 12 experimental units. We have chosen the two extreme levels of fertilizer to compare the soil bacterial community responses, and to compare bacterial community DNA and cDNA. We sampled during the tomato part of the crop rotation in the system, following pac choi 'Mei Qing Choi'. Buckwheat was used as a cover crop between plantings in all plots.

Fertilizer treatments

High: Pre-plant fertilizer, and liquid fertilizer.

- High conventional: soluble fertilizer of KNO_3 and $Ca(NO_3)^2$ + inorganic pre-plant fertilizer
- High organic: fish hydrolysate, and compost pre-plant fertilizer

Low (or control): No added fertilizer, for either conventional or organic treatments.

Soil sampling

Soil samples were collected September 23, 2010, after harvest of the tomato plants. From each experimental unit, four 15 cm deep 5 cm dia. cores were collected in a systematic sampling scheme that avoided the edges of the plots. The subsamples from within each experimental unit were bulked, homogenized and stored on a cooler with blue -ice until 2.0g was transferred into a 15 ml bead tube that had 1.5 g beads (these bead tubes are components of the kit: RNA PowerSoil, Total RNA Isolation, MoBio, Carlsbad, CA, USA), additionally we first added 5 ml of LifeGuard solution (LifeGuard Soil Preservation solution, Mo Bio, Carlsbad, CA, USA) into each bead tube to protect the RNA integrity while the samples were transported from the field to the lab.

RNA and DNA isolation from the soil samples

RNA was isolated from the soil samples used the Total RNA Isolation kit (MoBio, Carlsbad, CA, USA), following the instructions from the manufacturer. DNA was isolated from the same soil samples with the RNA PowerSoil DNA Elution Accessory kit (Mo Bio, Carlsbad, CA, USA), following the manufacturer's instructions. The extracted RNA samples were eluted in 100 µl of solution SR7 (which is RNase/DNase free water and comes with the Total RNA Isolation kit) with 100 U of RNaseOUT (40 U/µl; Invitrogen, Carlsbad, CA, USA) to inhibit RNA degradation before reverse transcription. DNA contamination was removed from the RNA extracts using RQ1 RNase-free DNase (Promega, Madison, WI, USA) prior to first-strand complementary DNA (cDNA) synthesis. The extracted DNA samples were eluted in 100 µl of solution S5 (which is RNase/DNase free water and comes with the DNA Elution Accessory kit) To confirm the absence of contamination during the extraction a blank sample was carried through the extraction protocol and downstream sample processing. The RNA extracts were stored in -80 C until further processed. The extracted DNA samples were eluted in 100 µl of RNase/DNase free water, and stored in -80 C until further processed.

Reverse transcription of soil samples

The extracted RNAs were quantified with an ND 1000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA), and 100 ng for each extracted RNA sample along with the blank extraction control were reverse –transcribed using the Thermoscript reverse transcription PCR (RT-PCR) two-step system (Invitrogen, Carlsbad, CA, USA). Universal bacterial primers 27f (Lane, 1991) and 338R were used for PCR amplification of the V1-V2 hypervariable regions of the 16S rRNA genes. For fungi, the primers LROR and LR3 (Vilgalys and Hester1994) were used for PCR amplification of the D1-D2 divergent regions of the 28S rRNA genes.

To denature the rRNAs before the cDNA synthesis, 100 ng of RNA for each one of the samples was combined with 1µl of nuclease-free 10 µM 338R primer, 2 µl of 10 mM dNTPs, and the corresponding volume (in µl) 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) which was used 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 amplification

The reverse-transcribed cDNAs were PCR-amplified with Amplitaq Gold 360 PCR Master Mix (Applied Biosystems, USA) with bacterial-specific primers (27F and 338R) that target a ca. 311 bp region of the SSU, while GoTaq Hot Start DNA polymerase (Promega, Madison, WI, USA) was used with fungal-specific primers (LROR and LR3) that target a ca. 630 bp region of the LSU.

Bacteria. The PCR reactions were conducted in a 25 µl volume with 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 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 minimized the 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 of the variable regions V1-V2 of the bacterial small subunit of the ribosome (16S rRNA) amplicons using massively parallel sequencing (MPS) (Margulies et al., 2005). For this purpose, two new primer constructs were synthesized where the 454-sequencing primer (A-primer) and the forward primer (27F) with a ten base pair (bp) DNA tag for post-sequencing sample identification in between, or the DNA capture bead anneal primer (B-primer) for the emulsion PCR (emPCR) and the reverse primer (338 R) to make the single strands on beads as required for 454 pyrosequencing (Margulies et al., 2005). The resulting sequences were as follows: 27 F-5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG NNNNNNNNNN**AGAGTTTGATCCTGGCTCAG**-3', and 338R- 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG**TGCTGCCTCCCGTAGGAGT**-3' where the underlined sequences are the 454 primers A and B, respectively, and the bold letters denote the universal 16S rRNA primers 27 F and 338 R. The 10 bp barcode within primer 27 F is denoted by 10 Ns. The second PCR reaction was carried out with initial 10 min denaturation at 95 °C followed by 5 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.

Fungi. The PCR reactions were conducted in a 25 µl volume with 2.5 µl of 10 µM forward and reverse primers, 5 µl of the cDNA template, 100 µM of each deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 1 unit GoTaq Hot Start DNA polymerase (Promega, Madison, Wisconsin), 5 µl Green Go Taq Flexi PCR buffer (Promega, Madison, Wisconsin) plus 4.8 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 53 °C, 2 min at 72°C, and a terminal elongation at 72°C for 7 min. After this first amplification, a second PCR reaction was conducted to add the A and B adapters required for direct 454 sequencing of the divergent regions D1-D2 of the fungal large subunit of the ribosome (28S rRNA) amplicons using massively parallel sequencing (MPS) (Margulies et al., 2005). For this purpose, two new primer constructs were synthesized where the 454-sequencing primer (A-primer) and the forward primer (LROR) with a ten base pair (bp)

DNA tag for post-sequencing sample identification in between, or the DNA capture bead anneal primer (B-primer) for the emulsion PCR (emPCR) and the reverse primer (LR3) to make the single strands on beads as required for 454 pyrosequencing (Margulies et al., 2005). The resulting sequences were as follows: LROR-5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGNNNNNNNNNN**CCGCTGAACTTAAGCA TATCAATA**-3', and LR3-5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG**CCGTGTTTCAAGACGGG**-3' where the underlined sequences are the 454 primers A and B, respectively, and the bold letters denote the universal 28S rRNA primers LROR and LR3. The 10 bp barcode within primer LROR is denoted by 10 Ns. The second PCR reaction was carried out with initial 10 min denaturation at 95 °C followed by 5 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.

The amplification of target-sized amplicons for bacteria and fungi was confirmed by horizontal gel electrophoresis. The PCR products were purified with Agencourt AMPure PRC purification system (AgenCourt Bioscience, Beverly, MA, USA) following the manufacturer's instructions. This clean-up system was selected because it discriminates against fragments of less than 100 bp in size, removes salts, enzymes and effectively eliminates dimers of the fusion primer constructs that exceed 40 bp in size. The clean fungal and bacterial PCR products were again quantified with the 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, a blank extraction without sample was carried through the extraction protocol. Second, to account for PCR reagent-borne contaminants, a PCR control without template DNA was included in the PCR. 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.

Analysis of the sequence data

The cDNA and DNA bacterial and fungal sequences were submitted to the computational pipeline PyroTagger v. 1.0 (Kunin and Hugenholts, 2010). This pipeline removed low quality bases and shorter sequences based on a quality filtering and length trimming. Using the algorithm 'pyroclust', the sequences were grouped in clusters at 97% similarity. The sequences were assigned to operational taxonomic units (OTUs) using RDP (Ribosomal Database Project) tools (Cole et al., 2009).

Diversity indices, common taxa, and taxon responses to treatments

From the derived OTU (Operational Taxonomic Unit) frequency data, we calculated Simpson's diversity index and Shannon's index for each plot. These diversity indices were used to test hypotheses about diversity responses among the treatments in an analysis of variance using SAS (SAS Institute Inc., Cary, NC). We also evaluated the most frequent taxa in the samples, and how individual taxa responded to the experimental treatments in generalized linear mixed models.

Results

Common taxa , and taxa responding to experimental treatments

Bacteria

The total number of bacterial sequences obtained in the field plots was 780,266. More bacterial and archaeal phyla were found to be more frequent in soil samples when DNA was extracted compared to RNA extraction (cDNA) (Figs. 1 and 2). The samples included two phyla (Crenarchaeota and Euryarchaeota) that are classified in the archaea domain. The phylum Dictyoglomi, only present in the cDNA samples, usually grows at high temperatures (Euzeby, 2011). A total of 991 genera of bacteria were obtained from the RDP classifier. Some of the genera include taxa with important ecological roles in the nitrogen cycle or in the production of antibiotics (Figs. 3 and 4). Others contain taxa that are plant pathogens that can cause important losses in crops. We recovered a number of genera of bacteria that have known roles in agroecological systems.

Some bacterial phyla and genera assignments responded to differences between organic and conventional management and to fertility treatments, and some were recovered in different frequencies from cDNA compared to DNA (Tables 1-4). Some of the observed management effects were on assignments to genera that are often important to plant health, such as *Pseudomonas*. Other genus assignments included taxa important to nitrogen fixation, such as *Rhizobium*, taxa important in nitrogen cycling, such as *Azotobacter* and *Nitrospira*, and taxa often important in plant disease, such as *Streptomyces* and *Erwinia*.

Fungi

A smaller number of fungal sequences were obtained, but estimated fungal responses to fertility treatment were large. The Ascomycota dominated the fungi, particularly in the DNA samples (Figs. 5 and 6). The assignment of sequence clusters to genera also showed greater dominance by a small number of genera for DNA samples (Figs 7 and 8). Common genus assignments included taxa often important to plant health, such as *Olpidium* (Fig. 7).

Most fungal phylum assignments and many genus assignments were recovered in different frequencies in cDNA and DNA samples (Tables 5 and 6). Significant differences in cDNA vs. DNA recovery were noted for genus assignments often important to plant health, including *Aspergillus*, *Glomus*, *Colletotrichum*, *Eutypa*, and *Alternaria*.

Diversity measures

The bacterial community exhibited higher diversity as measured by Simpson's diversity for organic management-high fertility compared to the other treatments ($p = 0.13$, Fig. 9). There was some evidence for a three-way interaction (management type * fertilizer level * DNA vs cDNA; $p = 0.13$, Fig. 9). The main difference in Simpson's diversity appears to be the higher levels of diversity for the organic high fertility treatment compared to other treatments. In an AOV, bacterial diversity in the conventional high fertility and organic high fertility treatments was

different ($p = 0.03$, Fig .9), and there was also some evidence for differences between the conventional control and organic high fertility treatments ($p = 0.06$, Fig. 9), in both cases for both DNA and cDNA. Differences in diversity between DNA and cDNA were observed only for the organic control treatment ($p = 0.03$, Fig. 9).

Also for the bacterial community there was some evidence for a three-way interaction for Shannon's index (management type * fertilizer level * DNA vs cDNA; $p = 0.06$, Fig. 10). There were statistical differences in Shannon's index between these DNA and cDNA for the organic management-high fertility treatment combination ($p = 0.01$, Fig. 10), as well as some evidence for differences between DNA vs cDNA for the conventional management-high fertility combination ($p = 0.09$, Fig. 10) and for the organic management-control combination ($p = 0.06$, Fig. 10).

For fungi, our initial estimate of the overall mean Simpson's diversity was 0.914, while the mean for DNA samples was 0.874 and the mean for cDNA samples was 0.967. Fungal diversity was lower than bacterial diversity, as anticipated. We are continuing the analysis of the effects of fertility treatments on soil fungal populations in our current Ceres Trust project.

Discussion and Conclusions

Common taxa

The analysis of soils in this experiment indicated a number of taxa known to have important roles in agroecological systems (Tables 1-6, Figs 1-8), such as *Rhizobium* (nitrogen fixing bacteria), *Glomus* (mycorrhizal fungi), and *Alternaria* (often plant pathogens). This illustrates the power of pyrosequencing to characterize a community broadly and provide insights about the whole microbial community. Interpretation of this broad community has some limitations, however, in terms of the functional roles of taxa. While some taxa have very consistent functional roles, other groups, such as *Pseudomonas* spp., include 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. When these two limitations are kept in mind, the exciting insights from these new sequencing techniques can be evaluated with appropriate caution.

Fertility management and taxa frequency

Many taxa responded to the fertility management treatments in the long-term experiment (Tables 1 and 3). For example, taxa classified as *Pseudomonas* exhibited a significant management (organic vs. conventional) by fertility level interaction. The frequency of *Pseudomonas* recovered from DNA in high fertility organic management was twice the frequency in the control, while it was lower in conventional treatments and did not shift as much with conventional fertility treatments. Evaluating cDNA captured this difference even more strikingly, where the frequency in the high fertility organic management was six times that in the control treatment. Sampling RNA (to yield cDNA) should give information about the most active taxa, and it appears that for taxa like *Pseudomonas* the effect of the fertility treatment was most detectable in this sample.

Sampling DNA compared to RNA

Comparing evaluation of DNA samples to RNA samples (processed as cDNA) revealed some general differences in the total microbe community (DNA) compared to the active microbe community (RNA, cDNA). Several taxa were recovered in different frequencies from DNA compared to cDNA. There were broad differences at the level of bacterial phyla, where for example Gemmatimonadetes were approximately twice as common in DNA samples than in cDNA samples and several other phyla exhibited significant differences as well (Table 4). Fungal phyla also showed striking differences, where the strongly dominant Ascomycota became somewhat less common in cDNA samples, allowing other phyla to increase in frequency (Table 6). A number of genera also differed, where for example *Glomus*, *Olpidium*, *Alternaria*, and *Eutypa* were more frequent in cDNA samples while *Aspergillus* and Colletotrichum were more frequent in DNA samples (Tables 2 and 5). Some of the differences between 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. Several taxa that have proven difficult to recover from soil in the past were recovered here in cDNA samples, such as the Glomeromycota, an extremely important group for the proper functioning of soil and plant health. Recovery of the Blastocladiomycota, many of which are pathogens, also suggests that this approach may be useful for evaluating biocontaminants. The recovery of Neocallimastigomycota is interesting, because these are often symbiotic associates of ruminants. If this annotation is correct, it suggests that there may be a novel group of soil fungi that deserves further study.

Microbial diversity

The observations for microbial diversity support our initial hypotheses. Bacterial diversity as measured by Simpon's diversity was higher in organic than in conventional high fertility plots (Fig. 9). Interestingly, the diversity in the organic control plots was also relatively high in DNA samples. This may have occurred because of interplot interference between the organic subplots in the split plot experiment, perhaps if tillage between seasons moved soil among the subplots within a management treatment whole plot. For Shannon's index, cDNA samples from the high fertility organic treatments had the highest diversity. Fungi were less diverse than bacteria, which is a common result for soil. For fungi, greater evenness of taxonomic representation is apparent in the cDNA compared to DNA samples. Our initial hypothesis was that microbial communities detected through RNA (cDNA) would be less diverse than those detected through DNA. In contrast, it appears that in this experiment cDNA samples may exhibit more diversity and evenness, perhaps because DNA samples can become loaded with those taxa with tougher dormant structures.

Translation of microbial community data for farm management

There is strong interest among many organic farmers in using microbial community data to guide management decisions on farms. 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. The huge steps in microbial ecology made possible by new sequencing techniques will need to be matched by new experimental techniques for understanding the functional roles of microbes, as we discuss in a synthesis paper (Garrett et al., 2012). The experiment and results supported by Ceres Trust benefit from and contribute to the great leap forward in characterizing microbial communities and their responses to management.

The first step is to understand what taxa are present, and how they respond to management, as new taxa will continue to be discovered in soil for some time to come. Adding information about the agroecological function of little-known taxa will be another important step.

Linking with our current Ceres Trust project
Our current 'Phase II' project supported by Ceres Trust also addresses organic management effects on soil microbial communities. We are continuing the analysis of bacteria and fungi in these communities, building on the initial results reported here. A synthesis of both projects will be presented at the end of the Phase II project.

Outreach

In addition to discussions at farmer field days, Lorena Gomez-Montano and other members of our team have presented information about this project and the role of soil microbes in plant productivity at the following meetings and seminars.

1. Midwest Organic & Sustainable Education Service (MOSES) 23rd annual meeting.
February 23-25, 2012, La Crosse, Wisconsin
Oral presentation: Soil Microbes in Organic Vegetable Production

2. American Phytopathological Society (APS).
August 4-8, 2012, Providence, Rhode Island
Poster presentation: Soil fungal and bacterial communities in organic vs. conventional vegetable production: Capturing the active players through soil RNA analysis
This poster is included as Figure 11.

3. K-State Research and the State: Graduate Student Poster Session.
November 6, 2012, K-State Student Union, KS Ballroom, Manhattan, KS
Title of the presentation: Soil fungal and bacterial communities in organic vs. conventional vegetable production: Capturing the active players through soil RNA analysis

4. KSU Department of Plant Pathology student seminar series, November 7, 2012

Additional outreach activities for this project are planned in conjunction with our current associated Ceres Trust project.

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Addenda

The following pages include tables, figures, and photos summarizing the project.

Table 1. Bacterial genera that responded to the fertility treatment (high vs. control) or exhibited a significant management type (organic vs. conventional) interaction with fertility treatment. The q-values < 0.05 are in bold font, and those < 0.1 are in green font.

Genus	Mean frequency (proportion)								q-value			
	Organic management				Conventional management							
	DNA		cDNA		DNA		cDNA		DNA		cDNA	
	High fertility	Control fertility	High fertility	Control fertility	High fertility	Control fertility	High fertility	Control fertility	Fertility effect	Fertility * management interaction	Fertility effect	Fertility * management interaction
Acidisphaera	0.000	0.001	0.001	0.001	0.000	0.000	0.001	0.001	0.605	**0.044**	1.000	1.000
Amycolatopsis	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.253	1.000	**0.001**	0.917
Anaeromyxobacter	0.028	0.031	0.025	0.028	0.029	0.027	0.023	0.027	0.161	**0.004**	0.860	1.000
Arthrobacter	0.004	0.002	0.008	0.004	0.002	0.001	0.002	0.002	1.000	0.547	1.000	**0.000**
Bacillus	0.022	0.024	0.018	0.012	0.024	0.023	0.017	0.016	0.713	1.000	1.000	**0.000**
Balneimonas	0.008	0.004	0.005	0.003	0.002	0.003	0.001	0.002	0.956	**0.000**	0.234	**0.000**
Blastococcus	0.003	0.002	0.004	0.003	0.004	0.003	0.003	0.004	0.713	1.000	0.398	**0.001**
Brevibacillus	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.436	0.057	0.234	1.000
Byssovorax	0.007	0.006	0.019	0.016	0.007	0.006	0.021	0.018	0.491	1.000	0.068	1.000
Chitinophaga	0.001	0.000	0.001	0.001	0.000	0.001	0.001	0.001	0.436	**0.004**	1.000	0.892
Chloroflexus	0.004	0.005	0.005	0.007	0.005	0.004	0.006	0.005	0.268	**0.037**	0.234	**0.002**
Cobetia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.065	1.000	1.000
Conexibacter	0.013	0.015	0.016	0.016	0.015	0.014	0.020	0.021	1.000	0.067	0.601	1.000
Croceicoccus	0.002	0.002	0.001	0.001	0.002	0.002	0.001	0.002	1.000	1.000	0.062	0.190
Cupriavidus	0.001	0.001	0.002	0.001	0.000	0.001	0.001	0.001	**0.038**	**0.032**	1.000	**0.000**
Desulfovirga	0.001	0.002	0.002	0.003	0.002	0.002	0.002	0.002	1.000	1.000	1.000	0.097
Flammeovirga	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.144	0.093
Flectobacillus	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.683	**0.037**	1.000	1.000
Geopsychrobacter	0.022	0.023	0.021	0.030	0.020	0.022	0.021	0.024	1.000	1.000	1.000	**0.000**
Geotoga	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.062	**0.011**	1.000	1.000

Gp1	0.004	0.006	0.003	0.003	0.019	0.018	0.012	0.011	1.000	**0.020**	1.000	1.000
Gp17	0.007	0.008	0.004	0.004	0.004	0.007	0.004	0.004	**0.007**	0.991	1.000	0.430
Gp2	0.001	0.001	0.000	0.001	0.002	0.001	0.002	0.001	**0.038**	**0.027**	1.000	0.703
Gp3	0.010	0.011	0.015	0.021	0.019	0.017	0.038	0.036	0.220	0.217	1.000	**0.000**
Gp4	0.042	0.056	0.040	0.053	0.068	0.067	0.057	0.057	1.000	**0.000**	1.000	**0.000**
Gp5	0.004	0.006	0.010	0.012	0.006	0.006	0.014	0.012	1.000	0.119	0.084	0.011
Gp7	0.007	0.009	0.001	0.002	0.012	0.009	0.003	0.003	0.253	**0.000**	1.000	0.182
Herpetosiphon	0.004	0.005	0.005	0.007	0.006	0.006	0.007	0.006	1.000	0.753	1.000	**0.026**
Hydrocarboniphaga	0.001	0.000	0.000	0.001	0.000	0.001	0.000	0.000	0.994	0.067	0.458	1.000
Kaistella	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.001	**0.038**	0.184	1.000	1.000
Kofleria	0.007	0.008	0.019	0.021	0.010	0.008	0.019	0.021	0.113	0.058	1.000	0.726
Lysobacter	0.002	0.001	0.001	0.000	0.001	0.001	0.001	0.000	1.000	1.000	1.000	**0.032**
Marinithermus	0.002	0.002	0.001	0.001	0.004	0.003	0.001	0.001	**0.014**	0.412	1.000	1.000
Marmoricola	0.005	0.004	0.018	0.014	0.008	0.006	0.019	0.018	0.831	1.000	0.820	**0.002**
Massilia	0.001	0.000	0.001	0.000	0.001	0.000	0.001	0.001	0.436	1.000	1.000	**0.071**
Meiothermus	0.001	0.004	0.000	0.003	0.000	0.002	0.000	0.001	**0.000**	**0.021**	**0.000**	0.856
Meiothermus	0.001	0.004	0.000	0.003	0.000	0.002	0.000	0.001	**0.000**	**0.021**	**0.000**	0.856
Methylibium	0.004	0.003	0.013	0.011	0.004	0.004	0.010	0.010	1.000	1.000	1.000	**0.018**
Methylovirgula	0.009	0.008	0.011	0.012	0.010	0.010	0.014	0.013	1.000	1.000	1.000	**0.001**
Microlunatus	0.009	0.008	0.008	0.008	0.004	0.006	0.005	0.005	0.292	**0.043**	0.630	1.000
Microvirga	0.002	0.002	0.002	0.002	0.001	0.001	0.001	0.001	1.000	1.000	0.820	0.083
Modestobacter	0.004	0.004	0.006	0.006	0.003	0.003	0.005	0.005	1.000	1.000	0.675	0.078
Niastella	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	1.000	1.000	1.000	**0.018**
Nitrosospira	0.001	0.002	0.002	0.001	0.003	0.002	0.003	0.001	**0.014**	**0.006**	0.000	0.260
Nocardioides	0.005	0.003	0.009	0.007	0.003	0.002	0.004	0.004	1.000	0.262	1.000	**0.000**
Nonomuraea	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	1.000	1.000	**0.018**	**0.033**
Novosphingobium	0.005	0.004	0.002	0.003	0.004	0.005	0.002	0.002	0.378	**0.007**	0.943	0.215
OP11_genera_incertae_sedis	0.001	0.001	0.000	0.000	0.001	0.002	0.000	0.000	**0.007**	**0.044**	1.000	1.000
Oxobacter	0.002	0.002	0.001	0.001	0.002	0.004	0.002	0.001	**0.000**	1.000	1.000	1.000
Persicirhabdus	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	**0.014**	0.828	1.000	1.000

Phaselicystis	0.004	0.005	0.019	0.021	0.004	0.005	0.020	0.018	1.000	1.000	**0.001**	**0.001**
Phenylobacterium	0.001	0.001	0.002	0.002	0.001	0.002	0.003	0.003	**0.045**	1.000	0.597	0.537
Pseudomonas	0.005	0.002	0.013	0.002	0.001	0.001	0.001	0.001	1.000	**0.022**	0.518	**0.000**
Rudaea	0.003	0.002	0.001	0.001	0.002	0.001	0.000	0.001	0.268	1.000	0.062	0.071
Solirubrobacter	0.017	0.017	0.024	0.023	0.017	0.019	0.027	0.027	**0.045**	0.161	1.000	0.508
Sphingobium	0.001	0.000	0.001	0.000	0.001	0.000	0.001	0.001	0.527	1.000	1.000	**0.002**
Sphingomonas	0.013	0.009	0.006	0.003	0.006	0.007	0.002	0.003	1.000	**0.020**	**0.011**	**0.000**
Sphingopyxis	0.005	0.006	0.002	0.002	0.008	0.011	0.003	0.003	**0.001**	0.612	0.665	1.000
Sporichthya	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	1.000	1.000	0.068	0.188
Subdivision3_genera_incertae_sedis	0.004	0.005	0.018	0.025	0.005	0.006	0.023	0.024	1.000	1.000	1.000	**0.000**
Swaminathania	0.004	0.003	0.003	0.005	0.003	0.003	0.004	0.003	1.000	1.000	0.366	**0.000**
Variovorax	0.001	0.000	0.001	0.000	0.000	0.000	0.001	0.001	0.527	0.058	1.000	0.190
Zavarzinella	0.003	0.003	0.005	0.007	0.003	0.004	0.007	0.006	0.956	1.000	1.000	0.088

Table 2. Bacterial genera that had different cDNA and DNA frequencies.

	Mean frequency (proportion)		
Genus	**cDNA**	**DNA**	**q-value**
Gemmatimonas	0.022	**0.042**	**0.000**
Chondromyces	**0.008**	0.002	**0.000**
Kofleria	**0.020**	0.008	**0.001**
Conexibacter	**0.018**	0.014	**0.001**
Oceanibaculum	**0.002**	0.002	**0.004**
Haliangium	**0.005**	0.001	**0.004**
Byssovorax	**0.019**	0.007	**0.026**
Coraliomargarita	0.001	**0.002**	**0.038**
Gp6	0.046	**0.062**	**0.039**
Sphingopyxis	0.003	**0.007**	**0.039**
Ottowia	0.008	**0.015**	**0.043**
Meiothermus	0.001	**0.002**	0.060
Mycobacterium	0.003	**0.005**	0.060
Saccharococcus	0.001	**0.002**	0.060
Pseudolabrys	0.002	**0.010**	0.063
Gp3	**0.028**	0.014	0.064
Subdivision3_genera_incertae_sedis	**0.023**	0.005	0.077
Oxobacter	0.001	**0.002**	0.078
Marmoricola	**0.017**	0.006	0.087
Massilia	**0.001**	0.001	0.087
Acidisphaera	**0.001**	0.000	0.087

Table 3. Bacterial phyla that responded to the fertility treatment (high vs. control) or exhibited a significant management type (organic vs. conventional) interaction with fertility treatment. The q-values < 0.05 are in bold font, and those < 0.1 are in green font.

Phylum	Mean frequency (proportion)								q-value			
	Organic management				Conventional management							
	DNA		cDNA		DNA		cDNA		DNA		cDNA	
	High fertility	Control fertility	High fertility	Control fertility	High fertility	Control fertility	High fertility	Control fertility	Fertility effect	Fertility * management interaction	Fertility effect	Fertility * management interaction
Acidobacteria	0.171	0.189	0.144	0.168	0.215	0.206	0.186	0.182	0.115	**0.000**	0.452	**0.000**
Actinobacteria	0.188	0.174	0.216	0.192	0.173	0.173	0.199	0.201	0.385	**0.000**	0.306	**0.000**
Bacteroidetes	0.062	0.052	0.050	0.047	0.043	0.044	0.038	0.038	1.000	**0.000**	0.452	0.075
Chlorobi	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	**0.039**	0.268	0.452	1.000
Chloroflexi	0.052	0.058	0.056	0.063	0.045	0.048	0.058	0.055	1.000	0.268	**0.031**	**0.000**
Cyanobacteria	0.001	0.001	0.001	0.000	0.001	0.001	0.000	0.000	1.000	1.000	0.306	**0.006**
Deinococcus-Thermus	0.003	0.006	0.001	0.004	0.004	0.005	0.001	0.002	0.543	0.067	**0.004**	**0.000**
Firmicutes	0.089	0.093	0.068	0.066	0.090	0.093	0.070	0.068	**0.005**	0.401	0.432	0.960
Gemmatimonadetes	0.034	0.040	0.020	0.023	0.046	0.047	0.024	0.023	0.119	0.268	0.445	0.052
Nitrospira	0.016	0.017	0.011	0.008	0.018	0.017	0.008	0.009	**0.026**	0.072	0.432	0.090
OP10	0.004	0.005	0.002	0.002	0.005	0.004	0.003	0.004	0.543	0.268	**0.031**	0.960
OP11	0.001	0.001	0.000	0.000	0.001	0.002	0.000	0.000	**0.001**	**0.006**	0.766	1.000
Planctomycetes	0.009	0.010	0.016	0.018	0.010	0.012	0.020	0.018	0.245	1.000	0.432	**0.006**
Proteobacteria	0.339	0.319	0.380	0.366	0.310	0.308	0.342	0.348	0.162	0.072	0.445	0.090
Verrucomicrobia	0.015	0.019	0.027	0.034	0.023	0.024	0.033	0.035	1.000	**0.006**	0.432	**0.000**

Table 4. Bacterial phyla that had different cDNA and DNA frequencies.

	Mean frequency (proportion)		
Phylum	**cDNA**	**DNA**	**q-value**
Gemmatimonadetes	0.022	**0.042**	**0.000**
Actinobacteria	**0.202**	0.177	**0.000**
Firmicutes	0.068	**0.091**	**0.001**
Acidobacteria	0.170	**0.195**	**0.031**
Deinococcus-Thermus	0.002	**0.005**	**0.041**
Proteobacteria	**0.359**	0.319	0.056
Bacteroidetes	0.043	**0.050**	0.086
Spirochaetes	0.0002	**0.0003**	0.093

Table 5. Fungal genera that had different cDNA and DNA frequencies.

	Mean frequency (proportion)		
Genus	**cDNA**	**DNA**	**q-value**
Chromocleista	0.001	**0.275**	**0.000**
Phaeoisaria	0.058	**0.127**	**0.000**
Catabotrys	**0.051**	0.006	**0.000**
Spizellomycete	0.005	**0.064**	**0.000**
Plectosphaera	**0.037**	0.011	**0.000**
Spizellomyces	**0.032**	0.006	**0.000**
Catenomyces	**0.028**	0.003	**0.000**
Spiromastix	**0.028**	0.008	**0.000**
Ascobolus	**0.014**	0.001	**0.000**
Cyllamyces	**0.023**	0.003	**0.000**
Neocallimastix	**0.021**	0.002	**0.000**
Karlingiomyces	**0.020**	0.005	**0.000**
Olpidium	**0.021**	0.002	**0.000**
Entrophospora	**0.030**	0.000	**0.000**
Zeloasperisporium	0.003	**0.011**	**0.000**
Kappamyces	**0.011**	0.001	**0.000**
Oedogoniomyces	**0.012**	0.002	**0.000**
Glomus	**0.015**	0.000	**0.000**
Corynascus	**0.009**	0.002	**0.000**
Catenophlyctis	**0.011**	0.005	**0.000**
Lasiobertia	**0.007**	0.001	**0.000**
Chaetomidium	**0.119**	0.099	**0.000**
Botryozyma	**0.007**	0.001	**0.000**
Triangularia	0.002	**0.008**	**0.000**
Sphaeriothyrium	**0.014**	0.007	**0.000**

Eichleriella	**0.004**	0.001	**0.000**
Coelomomyces	**0.007**	0.002	**0.000**
Poria	0.000	**0.012**	**0.000**
Coniochaeta	0.025	**0.037**	**0.000**
Piriformospora	**0.004**	0.000	**0.000**
Chaunopycnis	0.002	**0.008**	**0.000**
Powellomyces	**0.004**	0.001	**0.000**
Schizosaccharomyces	**0.005**	0.001	**0.000**
Cheilymenia	**0.004**	0.001	**0.000**
Saccharata	**0.006**	0.000	**0.000**
Paraglomus	**0.006**	0.001	**0.000**
Cercophora	**0.026**	0.023	**0.000**
Aspergillus	0.008	**0.012**	**0.000**
Scortechiniella	0.008	**0.013**	**0.000**
Troposporella	**0.004**	0.000	**0.000**
Fusculina	**0.008**	0.000	**0.000**
Batrachochytrium	**0.003**	0.000	**0.000**
Protodontia	**0.003**	0.000	**0.001**
Anungitopsis	**0.002**	0.000	**0.001**
Athelia	**0.002**	0.000	**0.001**
Doassansiopsis	0.000	**0.003**	**0.001**
Falcocladium	**0.003**	0.000	**0.001**
Rhizophydium	**0.003**	0.001	**0.002**
Albertiniella	**0.001**	0.000	**0.002**
Conoplea	**0.002**	0.000	**0.002**
Spinulosphaeria	**0.006**	0.002	**0.002**
Arthrocladiella	0.000	**0.002**	**0.003**
Chalastospora	**0.005**	0.003	**0.004**
Phlyctochytrium	0.077	**0.085**	**0.005**
Boothiomyces	0.002	**0.003**	**0.005**

Cymatoderma	**0.001**	0.000	**0.006**
Peziza	**0.001**	0.001	**0.011**
Eutypa	**0.003**	0.003	**0.012**
Thelebolus	0.003	**0.006**	**0.012**
Stomiopeltis	**0.002**	0.002	**0.013**
Alternaria	**0.003**	0.001	**0.017**
Jugulospora	0.000	**0.001**	**0.018**
Rhizocladosporium	0.000	**0.002**	**0.043**
Lachnocladium	**0.001**	0.000	**0.045**
Dimargaris	**0.001**	0.000	**0.047**
Clitopilopsis	**0.002**	0.001	**0.049**
Colletotrichum	0.013	**0.015**	0.053
Pilidiella	0.002	**0.005**	0.053
Podospora	**0.010**	0.008	0.054
Chytridium	**0.001**	0.000	0.057
Phragmidium	**0.010**	0.005	0.077
Geosiphon	**0.001**	0.000	0.097

Table 6. Fungal phyla that had different cDNA and DNA frequencies.

	Mean frequency (proportion)		
Phylum	**cDNA**	**DNA**	**p-value**
Ascomycota	0.582	**0.763**	**0.000**
Blastocladiomycota	**0.049**	0.014	**0.000**
Glomeromycota	**0.062**	0.002	**0.000**
Neocallimastigomycota	**0.044**	0.004	**0.000**
Chytridiomycota	**0.201**	0.173	**0.000**
Basidiomycota	**0.052**	0.035	**0.000**
Zygomycota	**0.001**	0.000	**0.009**

Figure 1. Bacterial phyla recovered through **cDNA**.

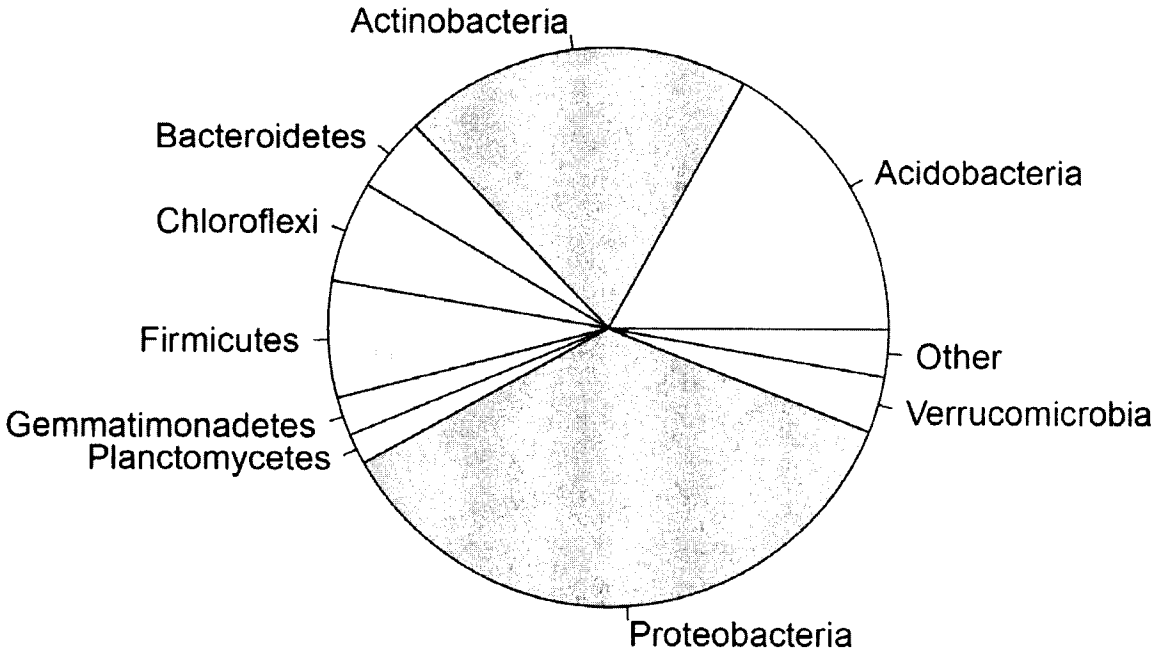

Figure 2. Bacterial phyla recovered through **DNA**.

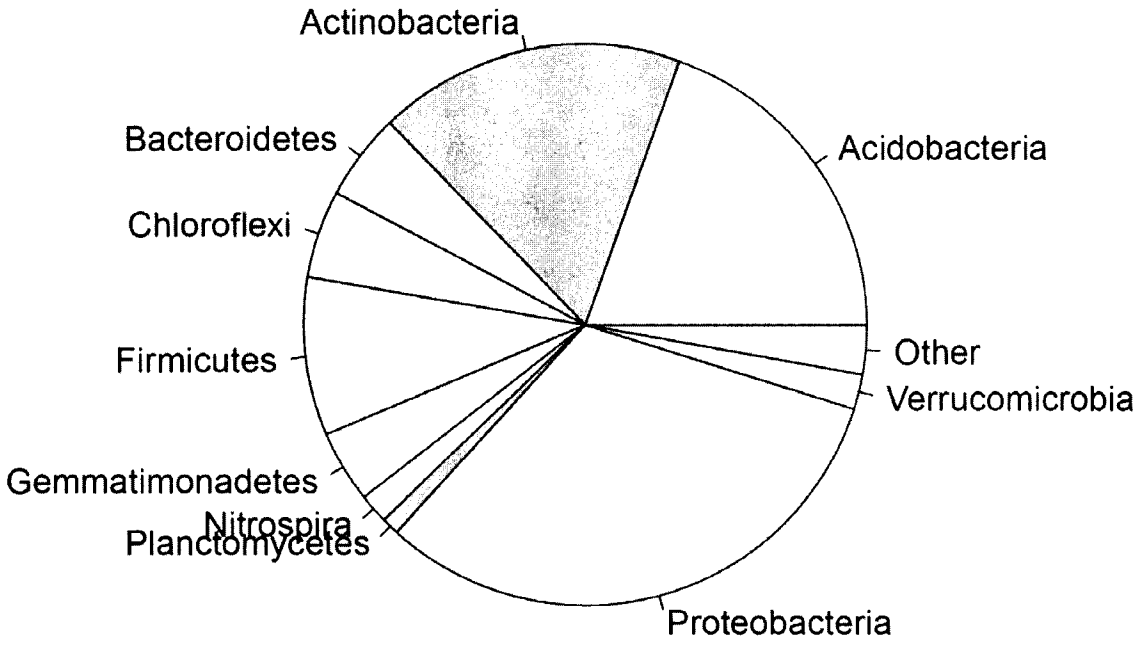

Figure 3. Bacterial genera recovered through **cDNA** (where genera present with frequency at least 0.01 are designated).

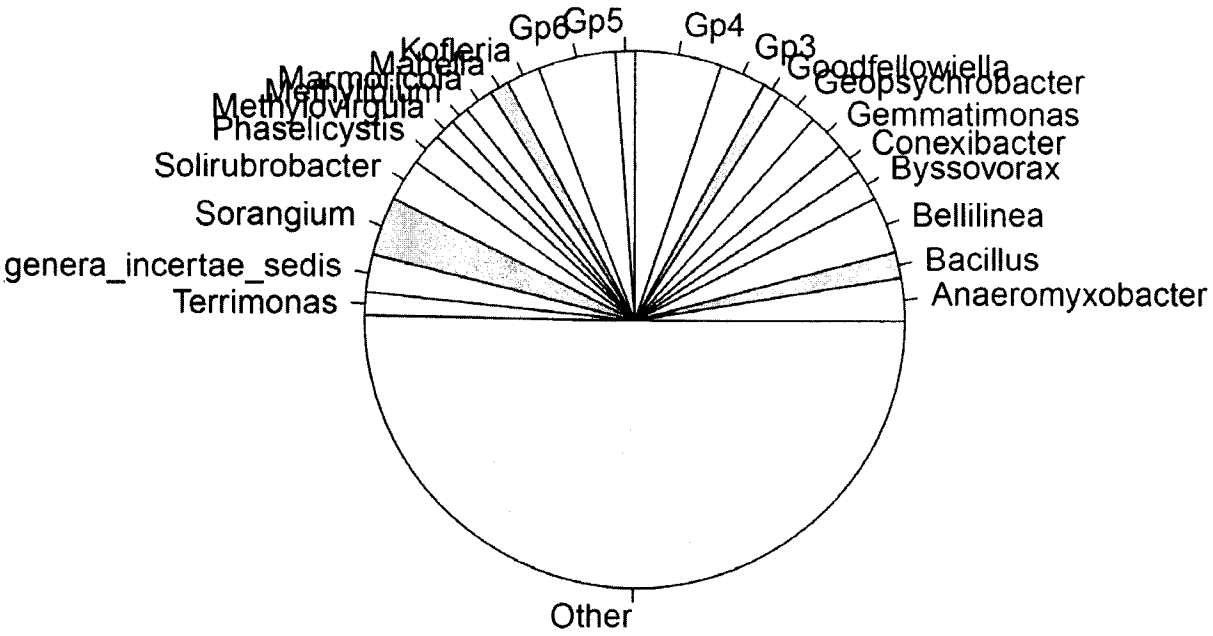

Figure 4. Bacterial genera recovered through **DNA** (where genera present with frequency at least 0.01 are designated).

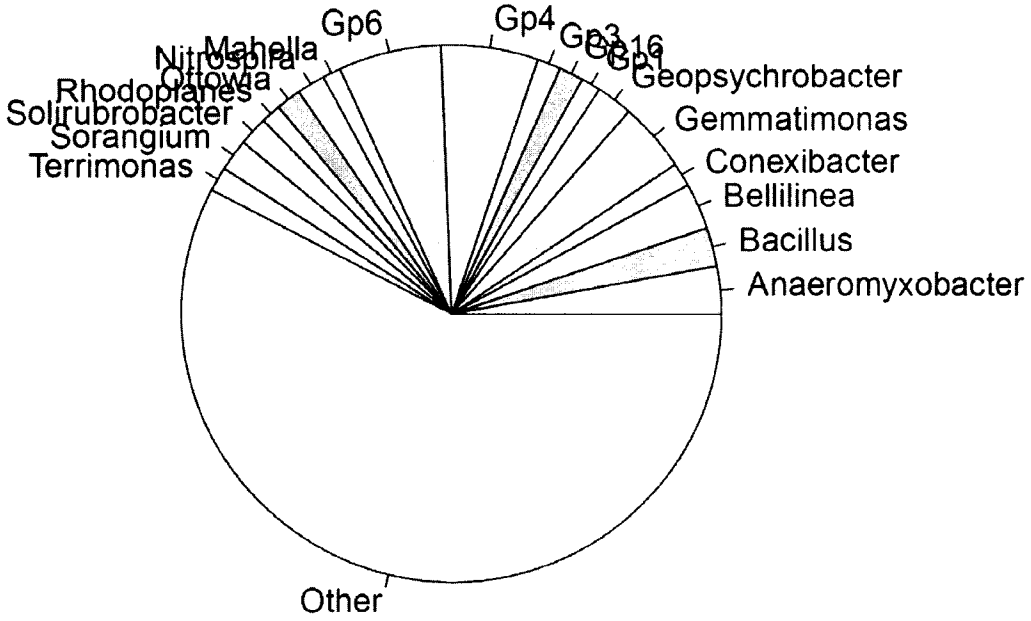

Figure 5. Fungal phyla recovered through **cDNA.**

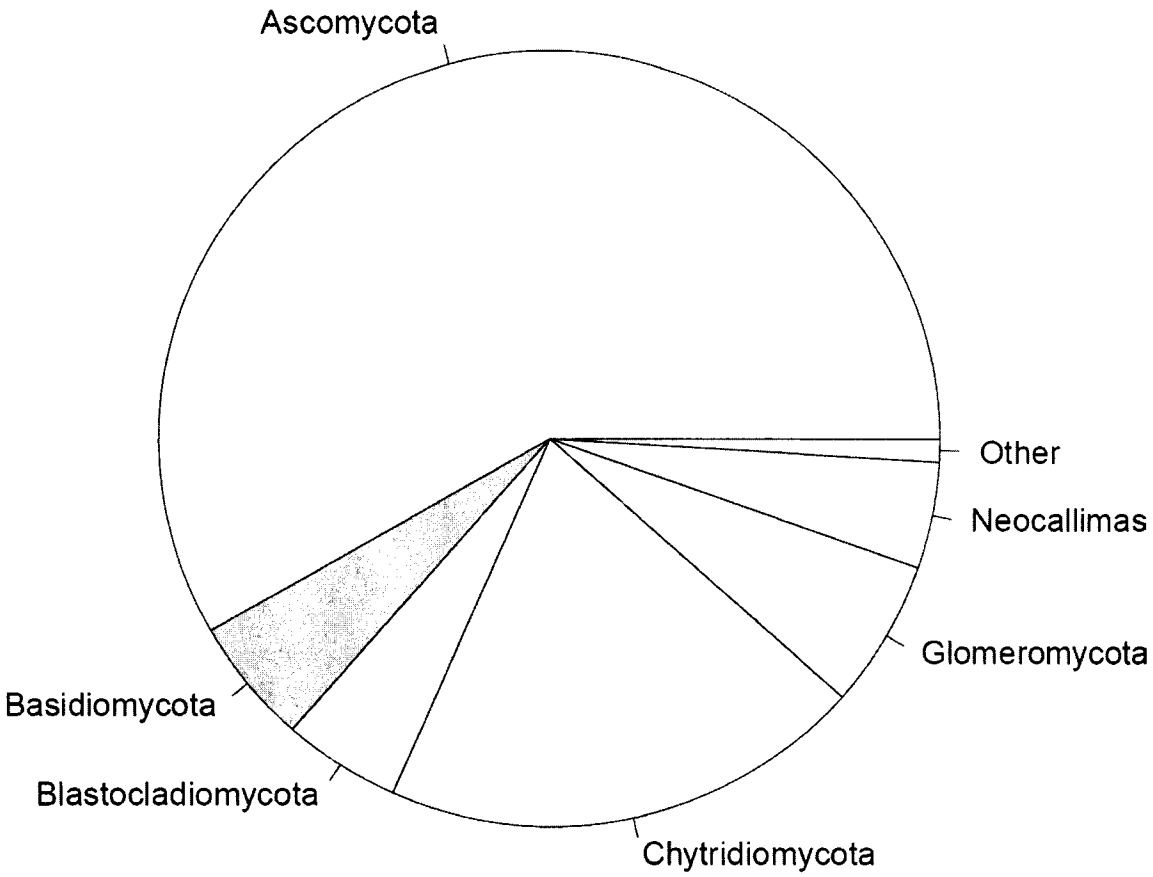

Figure 6. Fungal phyla recovered through **DNA.**

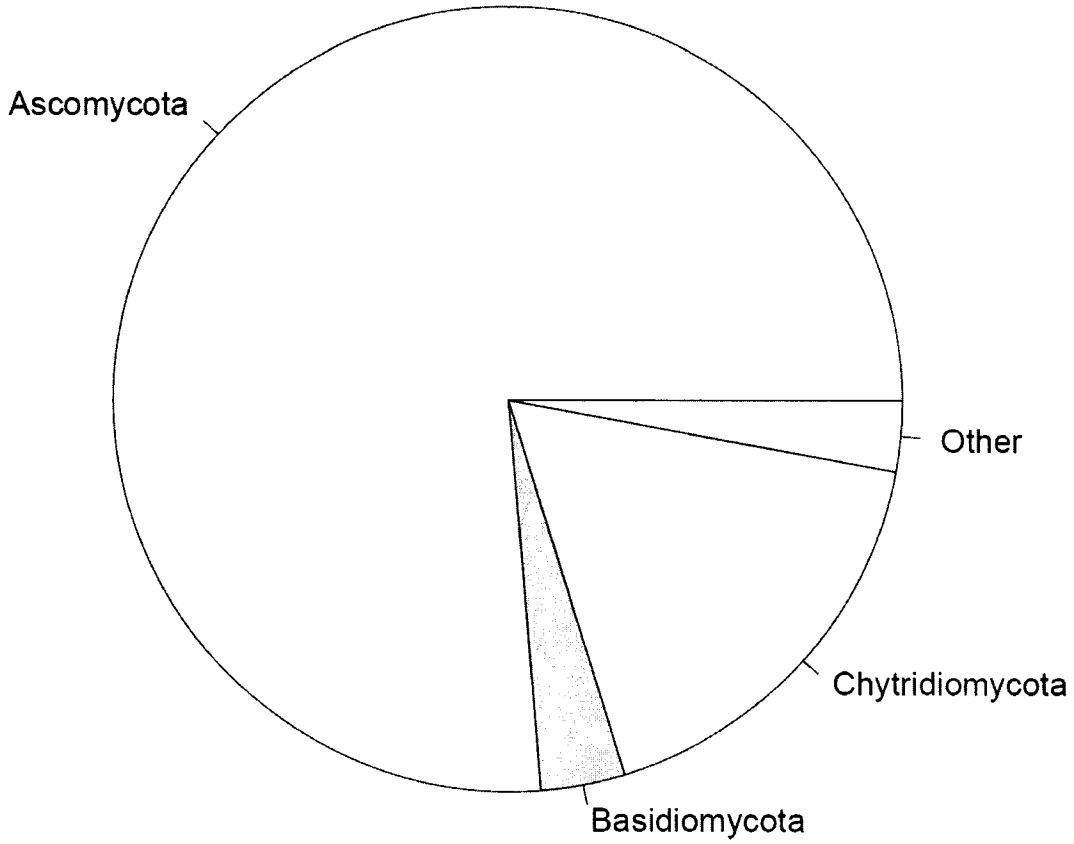

Figure 7. Fungal genera recovered through **cDNA.**

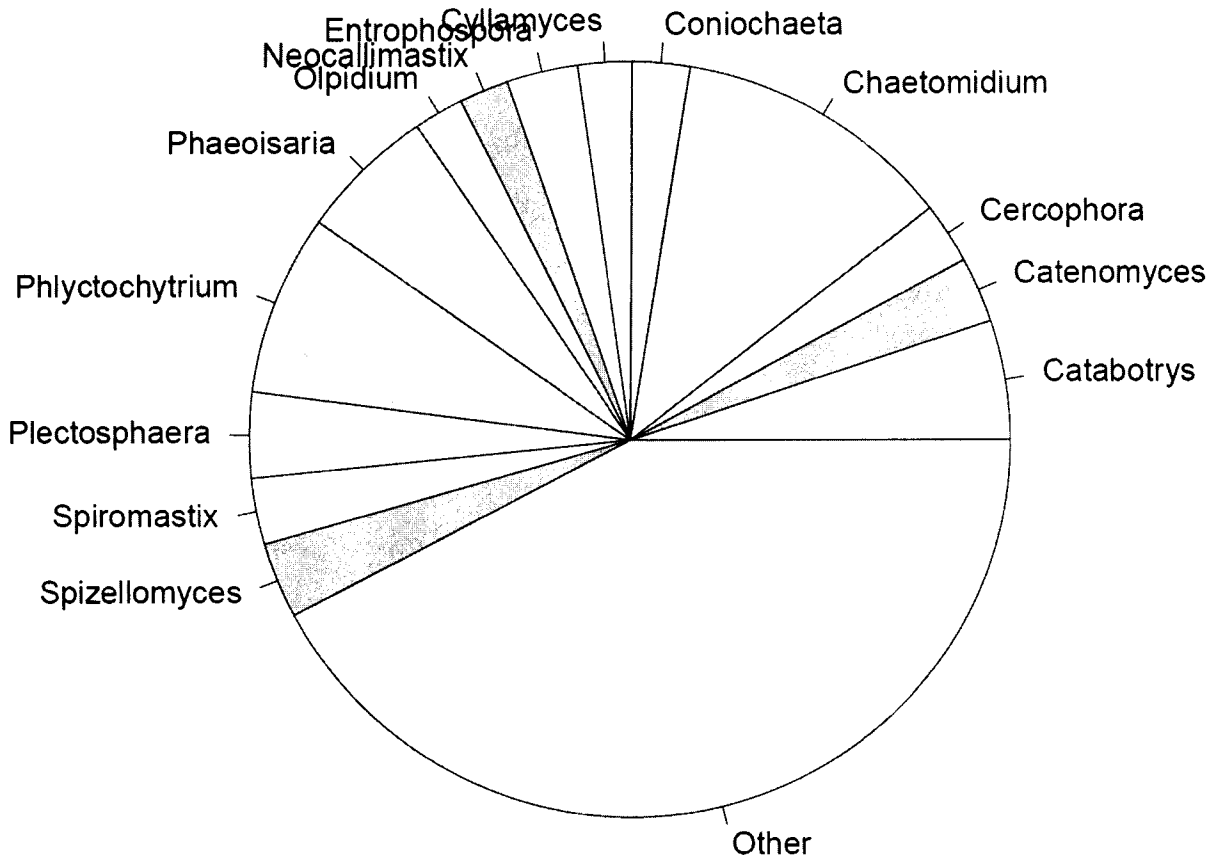

Figure 8. Fungal genera recovered through **DNA**.

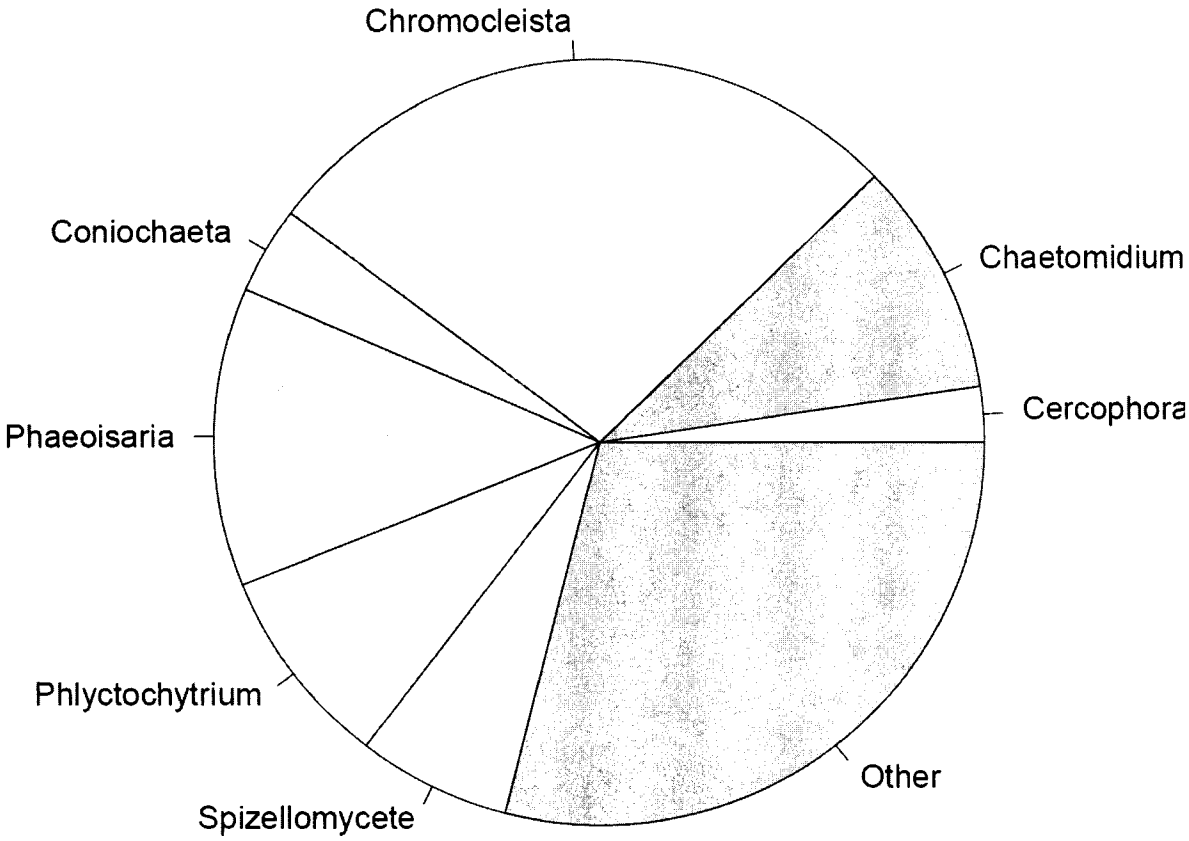

Fig 9. Box plots showing Simpson's diversity for the bacterial cDNA/DNA communities in the field plots under different treatment combinations. The orange dashed-line separates the results for each management-fertility treatment combination. An AOV gives some evidence for a three-way interaction (P = 0.13).

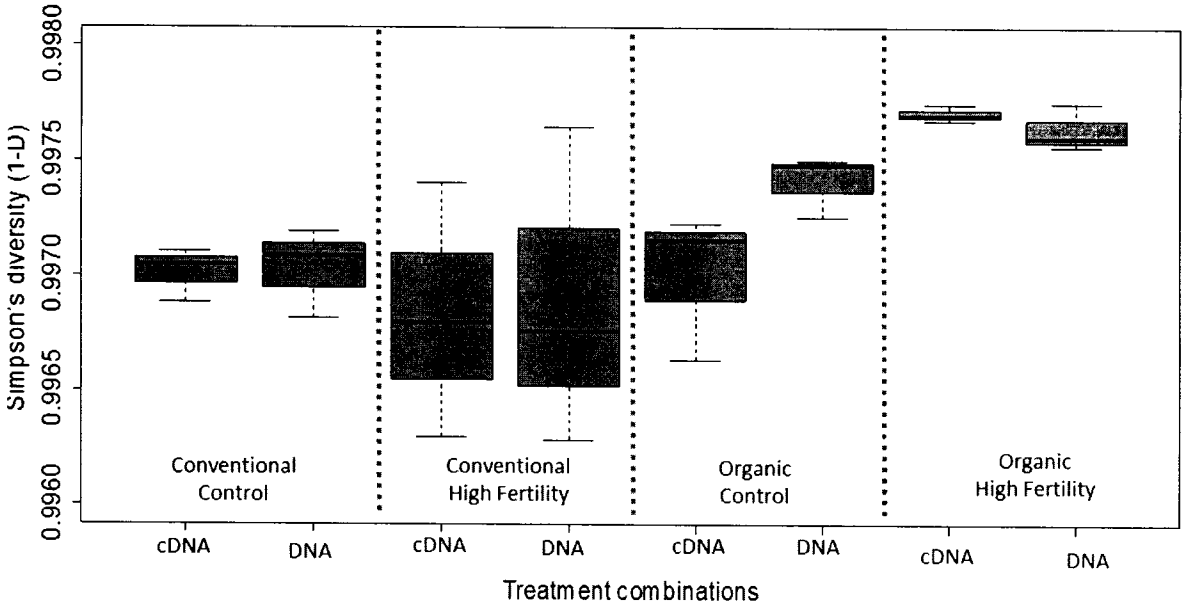

Figure 10. Box plots showing Shannon's index for the bacterial cDNA/DNA community in the field plots under different treatment combinations. The green dashed-line separates the results for each management-fertility treatment combination. An AOV gives some evidence for a three-way interaction (P = 0.06).

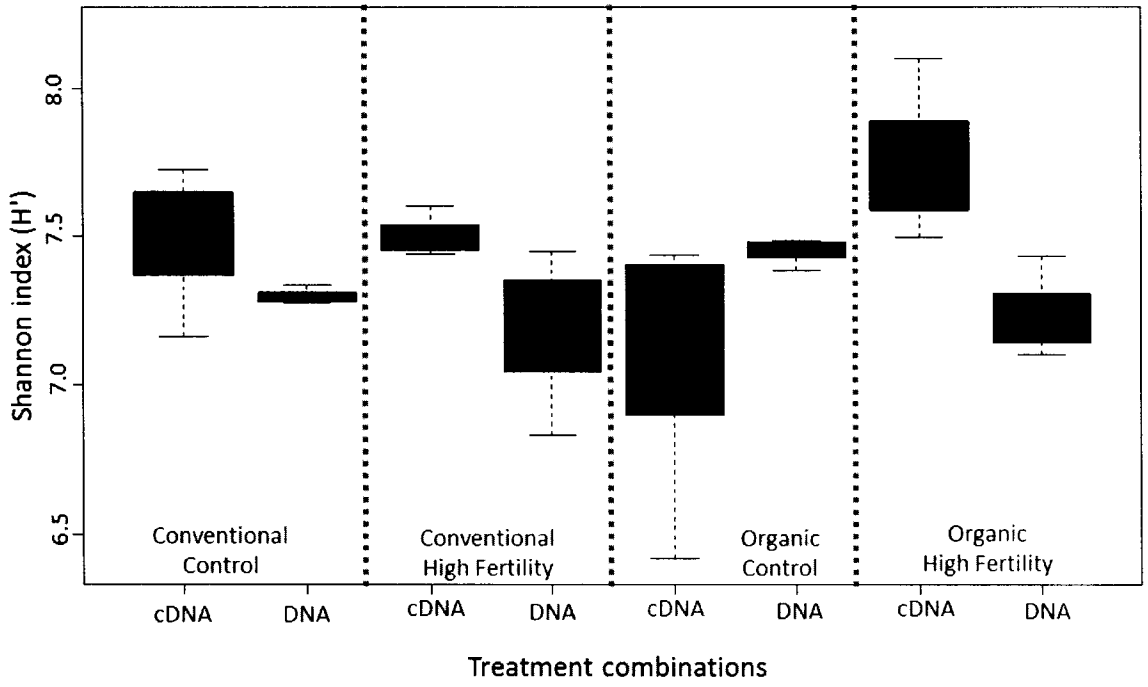

Fig 11. Gomez-Montano et al. 2012 poster from the national meeting of the American Phytopathological Society

Soil fungal and bacterial communities in organic vs. conventional vegetable production: Capturing the active players through soil RNA analysis

Lorena Gomez-Montano (logomez@ksu.edu)[1], Ari Jumpponen[2], Megan Kennelly[1] and Karen A. Garrett[1]
[1]Department of Plant Pathology, [2]Division of Biology, Kansas State University

Introduction

Soil microbes are fundamental to the productivity of agricultural systems. Organic management may foster more diverse soil microbial communities beneficial for crop production, with the potential to reduce losses to pathogens. We evaluated active microbial community responses in a six-year field experiment with two-year rotation of tomato and pac choi. We compared microbial communities in organic vs. conventional nutrient management with low and high fertility levels. We also wanted to understand the active microbial community, as opposed to the community sampled by DNA extraction, which may include dormant and moribund taxa. A new frontier for microbial ecology is the study of soil community RNA. We used 454-pyrosequencing and DNA-tagging to compare *total* resident fungal, archaeal and bacterial communities using extracted DNA and the *actively metabolizing* microbial communities using extracted RNA.

In this first phase of the project, we have analyzed soil archaeal and bacterial diversities, as reported here. In the next phase of the project we will analyze fungal, archaeal and bacterial communities in more detail in organic vs. conventional management.

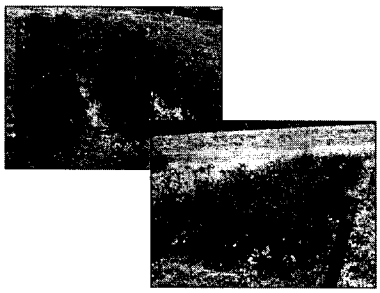

Figure 1. Photos illustrating field plots located at the K-State Horticulture Center in Olathe, KS with four months old tomato plants under organic and conventional fertilization (FO1: Field organic plot 1; FC2: field conventional plot 2).

Objectives

1. Characterize the soil bacterial community composition in organic agriculture compared to conventional management, for two fertility levels in a tomato crop.
2. Compare the active (cDNA pool) and the resident (DNA pool) soil microbial communities in organic vs. conventional nutrient managements.

Hypotheses

1. The diversity of the soil bacterial community will be higher under organic management compared to conventional.
2. The DNA pool will have a more diverse bacterial community compared to the cDNA pool.
3. The cDNA pool will reveal some bacterial taxa that are not present in the DNA pool.

Methods

- A total of twelve experimental units were sampled after harvest of the tomato plants.
- RNA and DNA were isolated from the soil samples using Total RNA Isolation kits and RNA PowerSoil DNA elution kits, respectively.
- The extracted rRNAs were reverse transcribed and cDNAs were synthesized.
- A list of cDNA and DNA sequences was obtained using 454 pyrosequencing.
- Diversity estimators such as Inverse Simpson's dominance were used to estimate taxon diversity based on 97% similarity.
- The diversity indices were used to test hypotheses about diversity responses among the treatments in an ANOVA using SAS.

Results

➢ Diversity responses among the treatments

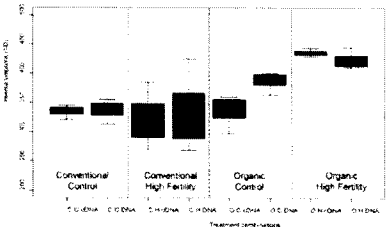

Figure 2. Box plots showing Inverse Simpson's index for the bacterial cDNA/DNA community in the field plots under different treatment combinations. The green dashed-line separates the results for conventional (C) and organic (O) management. The second treatment was the fertilizer level: control (C) or high (H). The third treatment was the type of DNA: DNA or cDNA (RNA). Each entry on the x-axis is located as follows: management type, fertilizer level, and DNA type. An ANOVA gives some evidence for the three-way interaction ($P<0.10$) and a management-fertility interaction ($P<0.06$).

Results cont.

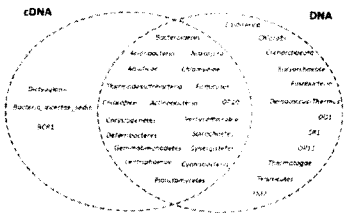

Figure 3. Venn diagram showing the most abundant bacterial and archaeal phyla in the cDNA and DNA samples, where the phyla in only the cDNA or DNA sets were those that were at least 2-fold more frequent in one type of sample than the other. A higher proportion of phyla were more frequent in DNA samples than in cDNA samples. The identity of phyla was inferred using RDP (Ribosomal Database Project) tools.

Conclusions

- In the first phase of our research we found that the bacterial community showed higher diversity as measured by Inverse Simpson's index for organic (O) management compared to conventional (C) management (Figure 2).
- There were higher levels of bacterial diversity for the organic high fertility compared to the other treatments (Figure 2).
- A higher proportion of bacterial and archaeal phyla were more common in DNA samples than in cDNA samples (Figure 3).
- This pyrosequencing approach allowed us to identify taxa with important ecological roles in the nitrogen cycle or in the production of antibiotics. We recovered a number of genera of bactena that have roles in agroecological systems. These included *Nitrospira* and *Azotobacter*, both with important roles in the nitrogen cycle, and *Pseudomonas* and *Erwinia*, often plant pathogens or antagonists of plant pathogens.

Future Perspectives

- In the next phase of our research we will evaluate fungal, archaeal and bacterial communities in these agricultural managements in more detail.

Acknowledgments

This work was supported by The Ceres Trust and the Kansas Agricultural Experiment Station.

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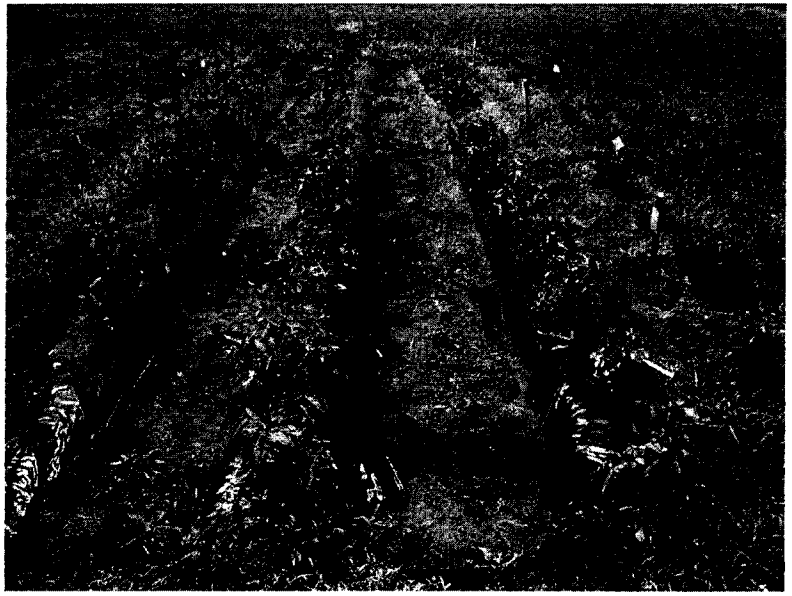
Images of the experiment, sampling, team, and outreach

Plots early in the season



Experimental plots as tomatoes are developing

Anita Stanescu and Lorena Gomez-Montano collecting soil cores for analysis of microbial communities. Lorena Gomez-Montano is developing this project as her PhD thesis.

Anita Stanescu, Kalen Menke, and Lorena Gomez-Montano at the research site

A field day at Jill Elmer's farm, where we discussed this project and the follow-up project at Jill Baron and Tom Buller's farm.