

Microbial community structure and abundance in the rhizosphere and bulk soil of a tomato cropping system that includes cover crops



Jude E. Maul^{a,*}, Jeffrey S. Buyer^a, R. Michael Lehman^b, Steve Culman^c,
Christopher B. Blackwood^d, Daniel P. Roberts^a, Inga A. Zasada^e, John R. Teasdale^a

^a USDA-ARS, Sustainable Agricultural Systems Laboratory, Beltsville, MD 44240, USA

^b USDA-ARS, North Central Agricultural Research Laboratory, Brookings, SD 44240, USA

^c Department of Crop Science, The Ohio State University, Columbus, OH 44240, USA

^d Department of Biological Sciences, Kent State University, Kent, OH 44240, USA

^e USDA-ARS, Horticultural Crops Research Laboratory, Corvallis, OR 44240, USA

ARTICLE INFO

Article history:

Received 25 April 2013

Received in revised form

21 December 2013

Accepted 6 January 2014

Available online 15 February 2014

Keywords:

Rhizosphere

Agroecology

Microbial

Crop rotation

perMANOVA

TRFLP

ABSTRACT

Understanding microbial responses to crop rotation and legacy of cropping history can assist in determining how land use management impacts microbially mediated soil processes. In the literature, one finds mixed results when attempting to determine the major environmental and biological controls on soil microbial structure and functionality. The objectives of this research were to: (1) Qualitatively and quantitatively measure seasonal and antecedent soil management effects on the soil microbial community structure in the rhizosphere of a subsequent tomato crop (*Solanum lycopersicum*) and (2) Determine phylum scale differences between the rhizosphere and bulk soil microbial community as influenced by the antecedent hairy vetch (*Vicia villosa*), cereal rye (*Secale cereale*), or black plastic mulch treatments. In this report, we use terminal restriction fragment length polymorphisms in the 16s rDNA gene to characterize changes in microbial community structure in soil samples from a field replicated tomato production system experiment at USDA-ARS Beltsville Agricultural Research Center, Beltsville, MD, USA. We found season of the year had the strongest influence on the soil microbial community structure of some of the major microbial phyla. Although we monitored just a few of the major microbial phyla (four Eubacteria and Archaea), we found that the effects of the tomato plant on the structural composition of these phyla in the rhizosphere differed dependent on the antecedent cover crop. Increased understanding of how agricultural factors influence the soil microbial community structure under field conditions is critical information for farmers and land managers to make decisions when targeting soil ecosystem services that are microbially driven.

Published by Elsevier B.V.

1. Introduction

Soil microbes play critical roles in soil biogeochemistry, soil fertility, and disease ecology (Pace, 1997). Recently, extensive efforts have been made to characterize the differential effects various plant species have on both the structure and function of the soil microbial community (Sorensen et al., 2009; Maul and Drinkwater, 2010). The influence of plants on the soil microbial community can be especially important in agricultural systems in which cash and cover crop selection can vary from short rotations (2–3 years) that might include one or two crop species to longer rotations (6–10 years) which might include six or eight species including both annual and perennial crops (Lundquist et al., 1999; Schomberg

et al., 2006; Carrera et al., 2007; Spargo et al., 2011). Understanding plant-microbe responses in the context of crop rotation and legacy of cropping history can assist in determining how land use management and changing climatic conditions impact microbially mediated soil processes.

Many interacting factors drive microbial community structure and function in soil ecosystems at different scales, making it difficult to determine the roles of individual factors, unless scale and ecosystem legacy (e.g., crop rotation, plant species legacy) are taken into consideration. For example, pH may drive soil microbial community structure at the continental scale (Fierer and Jackson, 2006), whereas differences at the regional (Green et al., 2004; Petchey and Gaston, 2009) and local scale may be driven by soil type or soil texture (Buyer et al., 1999; Wieland et al., 2001; Girvan et al., 2003). The dominant drivers of microbial community structure and function may also differ at the plot or sub-plot scale and can be driven by interactions between these larger-scale

* Corresponding author. Tel.: +1 301 504 9068; fax: +1 301 504 8370.

E-mail address: Jude.Maul@ars.usda.gov (J.E. Maul).

drivers, plant species, and land use history (Hawkes et al., 2005; Grandy et al., 2009; Maul and Drinkwater, 2010).

Agroecosystems offer excellent models for determining the underlying mechanisms controlling soil microbial community structure. In general, agroecosystems have well-defined histories, known soil types, and are managed in a manner that allows isolation of biological, edaphic, and plant species factors that have been shown to influence soil microbial community structure and function (Frey et al., 1999; Fließbach and Mader, 2000; Buckley and Schmidt, 2001; Spargo et al., 2011). Determination of factors that influence microbial community composition under field conditions has significant impact on understanding how management affects crop quality, disease ecology, and biogeochemical cycling (Buckley and Schmidt, 2001; Balsler and Mary, 2005; Zhou and Everts, 2007; Avrahami and Bohannan, 2009).

Tomatoes and other high-value crops are often grown on raised beds covered with black polyethylene mulch which offers many positive benefits, including rapid soil warming, water conservation, and weed suppression (Teasdale and Abdul-Baki, 1995; University of Maryland Extension Bulletin 236, 2012). Alternative systems that use cover crops instead of black polyethylene and minimize soil tillage and fossil fuel usage have been developed and tested at the Beltsville Agricultural Research Center (BARC), USA. Beds are formed in the fall and planted with a cover crop. In the spring, the cover crop is mowed and the crop residue left in place. The vegetable seedlings are planted directly through the cover crop residue. Minimum tillage systems modeled on this approach that use winter annual cover crops to conserve soil nitrogen and suppress spring weeds have been shown to improve soil quality while maintaining profitability (AbdulBaki et al., 1997; Abdul-Baki and Teasdale, 2007; Díaz-Pérez et al., 2008).

There are the issues regarding disease control in tomato production systems stimulated by the international phase out of the soil fumigant methyl bromide (Montreal Protocol on Substances that Deplete the Ozone Layer and the Clean Air Act, Jan 1, 2005). Cover cropping in rotation with tomatoes is emerging as a potential alternative to methyl bromide (Gilreath and Santos, 2004; Chellemi et al., 2012; Hansen and Keinath, 2013), but variability in the disease reducing efficacy among cover crop species has caused low rates of adoption among farmers. The work presented here is a step toward determining the specific effects of different cover crops on broad groups within the soil microbial community.

We previously showed, using phospholipid fatty acid analysis (PLFA), that farming systems that employ winter annual cover crops tended to increase microbial biomass of major groups of bacteria, fungi, arbuscular mycorrhizae, and protozoans compared to black polyethylene mulch, although some groups increased more than others. The rhizosphere microbial community of the tomato plant was significantly influenced by the previous rye (*Secale cereale*) or vetch (*Vicia villosa*) cover crop species. Structural analysis of the soil microbial community also showed that the soil microbiota in the tomato's rhizosphere were responding more to the antecedent cover crop than to the indirect impact of cover crop or mulch on soil moisture and temperature (Buyer et al., 2010).

The short-term impact of a winter annual cover crop can have legacy effects on cycling of soil nutrients and microbial community structure, and if better understood could help land managers plan crop rotation schedules to target particular ecosystem services. For example, legumes add nitrogen to the soil which can be used by the following cash crop, most Poaceae (grasses) are effective at catching and recycling excess nutrients from a previous phase of the cropping rotation. Bakker et al. (2010) show that plant species diversity and the indirect effects on soil nutrient status influence the suppressive phenotype of soil streptomycetes. In addition, some leguminous cover crop species have recently been

reported to suppress plant pathogens such as *Fusarium oxysporum* (Zhou and Everts, 2007).

The objectives of this research were to: (1) Qualitatively and quantitatively measure seasonal and antecedent soil management effects on the soil microbial community structure in the rhizosphere of a subsequent tomato crop (*Solanum lycopersicum*) and (2) Determine phylum scale differences between the rhizosphere and bulk soil microbial community as influenced by the antecedent hairy vetch (*Vicia villosa*), cereal rye (*Secale cereale*), or black plastic mulch treatments.

2. Materials and methods

2.1. Field experiment

The field experimental design and sampling were described previously (Buyer et al., 2010). Briefly, replicated field plots were established in a random complete block design. Soils at the site are classified as mixed Hapludults and Endoaquults in the order Ultisols and classified as sandy loam or loamy sand, varying between 63 and 83% sand, 7–27% silt, and 2–16% clay, respectively. Raised beds were formed in the fall and seeded with rye, vetch, or left bare. In the spring, the cover crops were mowed down, while the bare plots (which were to become the black polyethylene plots) were rototilled (to kill winter annual weeds), beds reformed, and covered with black polyethylene. Soil samples were collected in September 2006 on newly formed beds; in May 2007 just before the cover crops were mowed; and July 2007, at tomato flowering. At each sampling event, four bulk soil samples per bed were taken to 15 cm with a handheld soil probe, pooled, and homogenized. As described previously (Buyer et al., 2010), rhizosphere samples were collected by uprooting two plants (one from each center bed) and collecting soil adhering to the root system. Bulk and rhizosphere samples were returned to the lab and stored at -20°C .

2.2. DNA extraction

Soils from each field replicate were homogenized and three 1.5 g sub-samples were used for three separate soil DNA extractions. DNA was extracted from soil samples using the Powersoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA). The three DNA extractions were pooled and quantified using Picogreen (Invitrogen, Grand Island, NY, USA). For all subsequent analysis, each sample was run in analytical triplicate.

2.3. Quantitative PCR

Ribosomal RNA gene copies were quantified by quantitative polymerase chain reaction (PCR; Stratagene Mx3000, Agilent Technologies, Santa Clara, CA, USA) using universal primers for bacteria (Eub338F, 518R) and fungi (5.8S, ITS1f) (Fierer et al., 2005), SYBR Green (Quantitec SYBR Green PCR Master Mix, Qiagen) double stranded detection dye, ROX as a reference dye, and bovine serum albumin (BSA; 0.4 mg ml^{-1}) as an adjuvant in $25\ \mu\text{L}$ reactions. Annealing temperature (53°C) and primer concentration (300 nM, both primers) were determined in optimization studies. Positive controls were *Escherichia coli* (ATCC#10798) and *Saccharomyces cerevisiae* (ATCC#9763). Target amplicons from the positive controls were cloned using the pGEM-T kit (Promega, Inc., Madison, WI, USA); plasmids containing inserts were quantified using Picogreen (Invitrogen, Grand Island, NY, USA) and used to create eight-point standard curves for absolute quantification of rRNA gene copy numbers. Standard curves were prepared in triplicate for each microplate analyzed. Quantitative PCR analysis was conducted with internal standards used to calculate relative cT (threshold of saturation) for each well on a 96-well plate. The cT value was then

Table 1
Phylum-specific primer sets and respective sequences[†].

Primer	Target	Sequence 3'-5'	Reverse primer
ADF681F	Alphaproteobacteria	AGTGTAGAGGTGAAATT	1392R [‡]
Beta680F	Betaproteobacteria	CRCGTGTAGCAGTGA	1392R
BLS342F	Firmicutes	CAGCAGTAGGGAATCTTC	1392R
Pln930R	Planctomycetes	Eub338F-0-III*	CTCCACCGCTTGTGTGA
Eub338	General Eubacterial	Eub338F-0-III*	1392R
A344F	Archaea	ACGGGGTGCAGCAGCGCGA	1410ArcR [¥]

[†] This table has been redrawn from Blackwood et al. (2005), AEM v. 71 no. 10.

* Eub338F-0-III, ACTCCTACGGGAGGCWGC and ACACCTACGGGTGGCWGC.

[‡] 1392R, ACGGGCGGTGTGTACA.

[¥] 1410ArcR, ACGGGCGGTGTGTGCAAGGRG.

compared to a standard curve to back calculate initial gene copy number for each rDNA target. Using the calculated copy number for each sample ANOVA was conducted and comparison of means was tested by Tukey's HSD ($\alpha=0.05$; JMP genomics 3.2, SAS Institute Inc. Cary, NC).

2.4. TRFLP microbial community analysis

DNA extracts (described above) were diluted with nuclease-free water to 2 ng μl^{-1} . DNA was amplified by PCR using an equimolar mixture of the FAM-labeled forward primers and the corresponding reverse primer. Five sets of primers were employed to target specific phylogenetic groups (Betaproteobacteria, Firmicutes, Planctomycetes, and general Eubacterial and Archaeal sets). The primers (Table 1) target the Eubacterial and Archaeal 16S rDNA genes in extracted soil DNA, resulting in the amplification of 16S products of approximately 1100 bp. Each PCR reaction contained: 1X AmpliTaq Gold[®] 360 master mix, 0.2 μM forward primer and 0.2 μM reverse primer, 10 ng of template DNA, and nuclease-free water to adjust to a total reaction volume of 50 μl . Three, replicate 50 μl reactions of each sample were amplified using a MasterCycler Gradient thermal cycler (Eppendorf, Westbury, NY) according to the following protocol: initial denaturation at 95 °C for 5 min; 27 cycles of denaturation at 95 °C for 45 s, annealing¹ for 45 s and extension at 72 °C for 1 min; and final extension at 72 °C for 10 min. The phylum specificity of the T-RFLP primers was previously demonstrated (Blackwood et al., 2005). The Archaea primer set designed specifically for this study was confirmed by performing PCR and then cloning and sequencing 96 individual clones of which 98% were positively identified as Archaeal 16s sequences (Appendix 1, submitted to NCBI, accessions numbers pending).

Following PCR, amplified products from the two identical 50 μl reactions for each sample were pooled. Pooled products were verified by electrophoresis on a 1.5% agarose gel. A 30- μl restriction enzyme digest of each sample was prepared as follows: 5 U *Sau96I*, 1X reaction buffer 4, 0.1 μg μl^{-1} BSA, and nuclease-free water were combined to a volume of 15 μl , then added to 15 μl of pooled amplified DNA for each sample. Restriction digestion was carried out in a MasterCycler Gradient thermal cycler (Eppendorf, Westbury, NY) at 37 °C for 4.5 h, with a final step of 70 °C for 15 min to stop the reaction.

Digested DNA was purified using a Performa[®] DTR Edge Plate (Edge BioSystems, Gaithersburg, MD) and vacuum concentrated to near dryness on a Savant Speed Vac (Thermo Fisher Scientific, Waltham, MA, USA). Samples were resuspended in 20 μl of HiDi formamide containing 0.75% GeneScan[™] 500 LIZ[™] size standard (Applied Biosystems Inc., Foster City, CA, USA). Terminal

¹ Annealing temperatures followed protocols were described in Blackwood et al. (2005).

fragment-size analysis was performed using a 3730 ABI Prism Genetic Analyzer (Applied Biosystems Inc.) in conjunction with GeneMapper[™] (Applied Biosystems Inc.), and SeqMan Pro software (DNA Star Lasergene v 8, GATC Biotech, Constance, Germany).

2.5. Statistical analysis and ordination of factors

Datasheets directly from the 3730 ABI Prism Genetic Analyzer containing soil microbial community fingerprints determined by terminal restriction fragment length polymorphisms (TRFLP) and a sample label sheet were uploaded to the online TRFLP analysis software package T-REX (<http://trex.biohpc.org/>; Culman et al., 2009). A merged labeled datasheet was created, screened for spurious data points, and evaluated for acceptable peak intensity within T-REX. The labeled, transposed dataset was then Hellinger-transformed (square root of the proportional data). All subsequent analyses (permutational multivariate analysis of variance (perMANOVA) and redundancy analysis; RDA) were conducted using this Hellinger-transformed dataset.

perMANOVA (Anderson, 2001) was conducted within T-REX in a series of runs designed to partition explained variance into combinations of factors measured during the field experiment. Analysis was conducted separately on each phylum to discriminate the individual treatment effects on each phylum. perMANOVA was run individually for each phylum (*Eubacteria* (*gen.*), *Firmicutes*, *Betaproteobacteria*, *Planctomycetes*, and *Archaea*) and the percent variance explained was calculated by dividing the treatment sum of squares by the experimental sum of squares. The full model (including all main effects and interactions) was run with the perMANOVA function within TREX.

Canoco 4.5 (MicroPowercomputing, Ithaca, NY) was used to conduct RDA with the same dataset previously used for perMANOVA. The RDA is a linear constrained ordination of the soil microbial community structure as determined by the TRFLP fingerprint pattern. The TRFLP band counts and band size data were used as the "species" dataset and was run individually for each phylum (*Eubacteria* (*gen.*), *Firmicutes*, *Betaproteobacteria*, *Planctomycetes*, and *Archaea*). Environmental variables were used in the RDA to determine the qualitative impact of each factor on the structure of the microbial community. Environmental variable included season (fall, spring, or summer), location (bulk or rhizosphere), and cover (rye, vetch, or black plastic).

3. Results

In a previous study, both rye and vetch cover crops produced abundant above-ground biomass. Tomato marketable yields were lower for rye cover crop compared to the other two treatments, however these differences were not significantly different (Buyer et al., 2010).

Table 2

Quantitative PCR of fungal (28S) or bacterial (16S) rDNA gene copy number in spring soils after cover crop termination or black poly row cover installation.

Cover	rDNA gene copy number		
	Black poly	Rye	Vetch
Fungi ^a	1.9E + 08 ^b	3.99E + 08 ^{a,b}	7.12E + 08 ^a
Bacteria	9E + 08 ^b	1.26E + 09 ^{a,b}	2.21E + 09 ^a
F:B [∫]	0.21 ^b	0.32 ^{ab}	0.35 ^a

^aSuperscript letters that differ within row indicate significant differences among cover types. ($\alpha = 0.05$).

[∫] Indicates fungal to bacterial ratio.

3.1. Quantitative PCR

Tomato production systems that utilized a hairy vetch winter annual cover crop resulted in a higher abundance of fungal and bacterial rDNA in soils compared to black poly, as determined by 28S and 16S rDNA specific quantitative PCR (Table 2). Ribosomal DNA abundance is a surrogate indicator of cellular biomass and activity (Klappenbach et al., 2000). The soil in the vetch cover crop treatment was significantly greater than the black polyethylene treatment. The rye cover crop had intermediate levels of fungal and bacterial abundances but was not significantly different from the black polyethylene or the vetch treatments. The fungal:bacterial ratio was also significantly different among the soil surface management treatments with vetch having the highest ratio of fungi to bacteria (Table 2).

3.2. perMANOVA

Using perMANOVA, we partitioned the variance explained by our TRFLP database for each phyla into the effects of *season* (fall, spring, or summer); *soil cover* (vetch, rye, or black polyethylene); and *location* relative to the tomato root (bulk or rhizosphere; Table 3). When comparing all treatments, season explained more of the variation in the microbial community structure than soil cover or location. When season was factored as the only main effect the overall percentage of the variation explained was significant for all phyla (29.12%, $p = 0.014$). Because the tomato plants were present in the soil only during the summer months, the factor *location* (bulk or tomato rhizosphere) excluded spring or fall. In the summer months, the proximity to the tomato plant root (bulk or rhizosphere soil) had a significant effect on the structure of the microbial community regardless of phylum (13.81%, $p = 0.064$) and the Firmicutes showed the only significant response to tomato rhizosphere (3.38%, $p < 0.1$) (Table 3). Antecedent cover crop management had a non-significant effect on the structure of the community structure. The cumulative effect of all of the factors was determined and the community structure of individual phyla was 20–27% driven by the combined effects of season, location, and cover (Table 3). The strongest effects were detected in the Eubacteria (approx. 27%) followed by the Betaproteobacteria (approx. 24%). In general, season had the strongest effect on the structure

of the soil microbial community, followed by rhizosphere and bulk soil differences, and then soil cover or antecedent cover crop.

3.3. Redundancy analysis

Redundancy analysis and accompanying biplots allow visualization of the direction and magnitude of environmental factors on microbial community structure and were used to explore the same TRFLP dataset used for the perMANOVA analysis. These results corresponded well with the variance partitioning conducted in the perMANOVA analysis (Table 3). Consistent with results from the perMANOVA, decomposition of variance of RDA results (data not shown) indicated that season played the largest role in driving the structure of the soil microbial populations among all phyla measured (mean 6.4%), and the effect of season on structuring any one of the phyla was the greatest for the general Eubacterial primer set (8.5%). When the effects of season were isolated and only the summer sampling period was considered again, there was a similar trend in the decomposition of variance to what was observed in the perMANOVA analysis in that location (rhizosphere or bulk) tended to have a stronger effect than cover during the summer months in 3 out of 5 phyla.

Results from RDA are presented graphically in Figs. 1 and 2. The plots are a visual display of how the variance explained in the experiment is distributed among all of all the variables measured. The area of each plot represents on average 63% of the variation in microbial community structure accounted for by all of the variables, although the cumulative variation explained by all measured variables on soil microbial community structure was only 10–30% of the total variance (which corresponds to the sum of all explained variance determined by perMANOVA). The cumulative variation explained by the effects of the treatment on the microbial community structure points out that 72–79% of the variation in microbial community structure could not be explained by the controlled factors in this experiment.

Vector length in RDA biplots indicate the strength and direction the microbial community shift relative to the influence of the indicated environmental variables (season, cover crop, rhizosphere, or bulk soil). If the composition of the microbial community shifts in reproducible ways in response to one or a combination of environmental variables, we would see a strong correlation between the vector and the data points in the biplot representing microbial community similarity based on RDA.

Tomato rhizosphere soils were collected only in the summer, which was the only time-point where tomato plants were present. We therefore split the RDA into two. First, we analyzed bulk soil from all the seasons and all treatments. Second, we analyzed bulk and rhizosphere soil from all treatments but only in the summer. Each analysis was a complete factorial, which balanced the statistical design and simplified interpretation of the results.

3.4. Archaea

For Archaea in bulk soil (Fig. 1A), community structure in fall and spring was similar to each other and less similar to the

Table 3

Percent variation in microbial community structure explained by each factor using perMANOVA.

Phyla/group	Season (Spring, Summer or Fall)	Location (rhizosphere or bulk soil)	Mulch (cover crop; rye or vetch) or black polyethelene	Location:mulch interaction	Season:mulch interaction	Full model (factor sums)	Unexplained variation
Archaea	5.33	2.71	4.46	2.25	5.74	20.50	79.50
Firmicutes	6.85*	3.38*	2.25	3.57	5.90	21.96	78.04
Betaproteobacteria	6.47	2.62	2.40	4.10	9.06	24.65	75.35
Eubacteria	8.60*	3.24	3.85	4.29	7.38	27.38	72.62
Planctomycetes	5.62	2.82	2.63	2.98	6.30	20.36	79.64

Significance level; * $\alpha = 0.1$

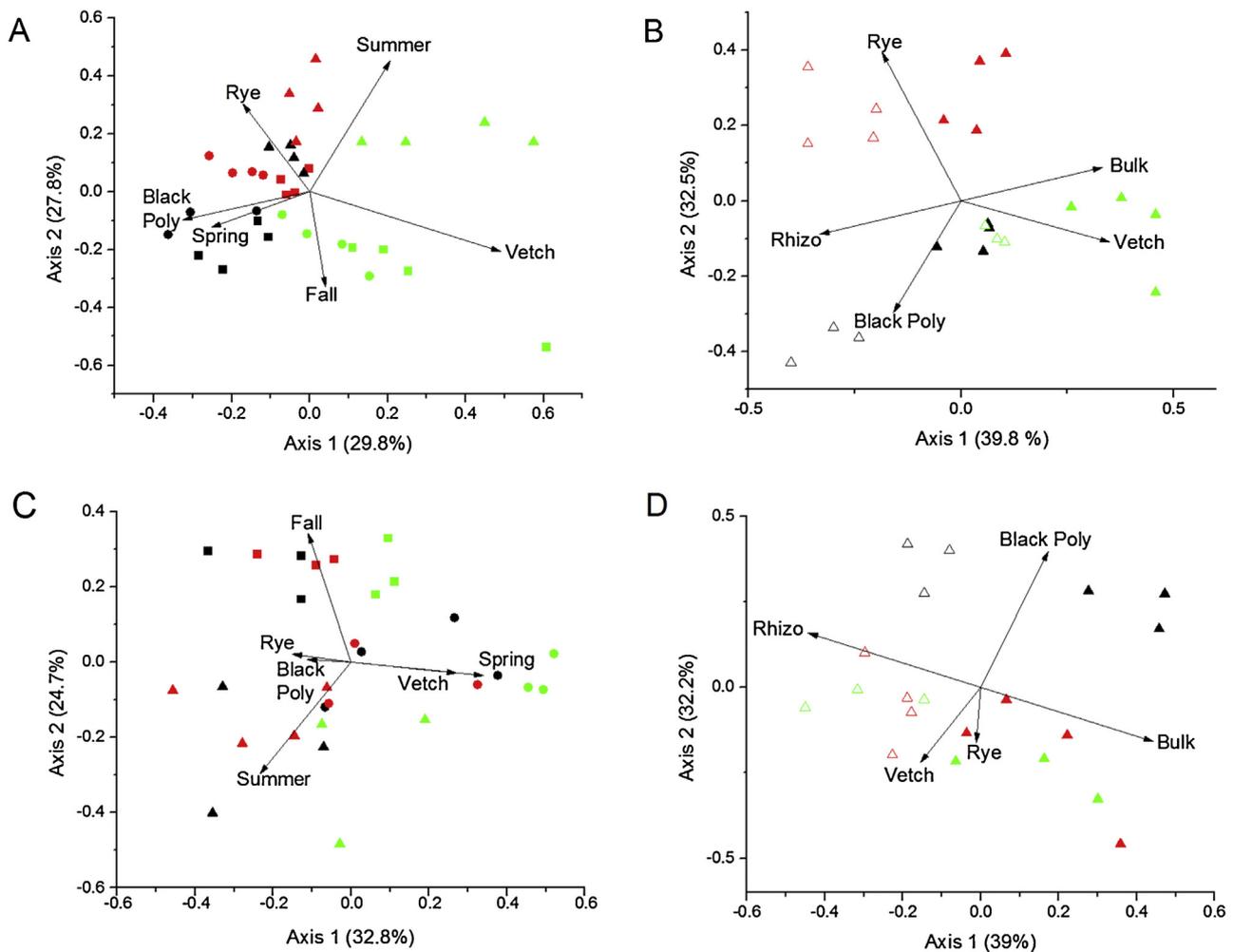


Fig. 1. Redundancy analysis of soil microbial community structure as determined by phylum-specific TRFLP. A: Archaea bulk soil, all seasons. B: Archaea, summer only. C: Eubacteria bulk soil, all seasons. D: Eubacteria, summer only.

community structure in summer. Black poly and rye treatments were more similar to each other than they were to plots under vetch. Focusing solely on the summer treatment (Fig. 1B), the community structure in plots under black poly and vetch was more similar to each other and less similar to those under rye. Bulk and rhizosphere soil community structure was clearly different among all three cover treatments.

3.5. Eubacteria and specific Eubacterial phyla

Eubacterial community structure in bulk soil (Fig. 1C) was clearly different in fall, spring, and summer treatments, while rye and black poly treatments were more similar to each other than to those taken from plots under vetch. During the summer (Fig. 1D), the black poly soil communities were most distinct from vetch and rye, while the rhizosphere and bulk communities were clearly different from each other.

Firmicutes in bulk soil (Fig. 2A) were affected by season, but cover crop treatment had very little effect. A cover crop effect on Firmicute community structure was found in summer months (Fig. 2B), with vetch soils maintaining a more unique soil microbial community when compared to rye and black poly soils.

In bulk soil, the Betaproteobacteria (Fig. 2C) were affected by both season and treatment. Rye and black poly treatments were more similar to each other than to vetch. However, during the summer (Fig. 2D), vetch and black poly soil communities were more

similar to each other and rye soil communities were most dissimilar. The microbial communities that inhabit the rhizosphere and bulk soils in all of our treatments were clearly different from each other.

The Planctomycetes were affected by both season and treatment in bulk soil (Fig. 2E), with vetch and rye more similar to each other than to black poly. The same treatment effects were observed in the summer (Fig. 2F), when the rhizosphere and bulk samples were also clearly distinguishable.

4. Discussion

A goal of this research was to determine how season, antecedent cover crop, and soil surface management such as black plastic mulch affect phylum-level structure of the soil microbial community in the bulk and rhizosphere soils of a subsequent tomato cash crop. Although the black plastic mulch treatment was roto-tilled, whereas the cover crop treatments were not, we do not believe that this single tillage event drives microbial community structure in these field sites where tillage is historically common.

Phylum-specific primer pairs for TRFLP analysis of the soil microbial community gave insight into how the soil microbial community changed in response to agronomic techniques such as using leguminous and grass cover crops in rotation with tomato. The data showed that season, as expected, had the greatest effect on the structure of the soil microbial community, followed by

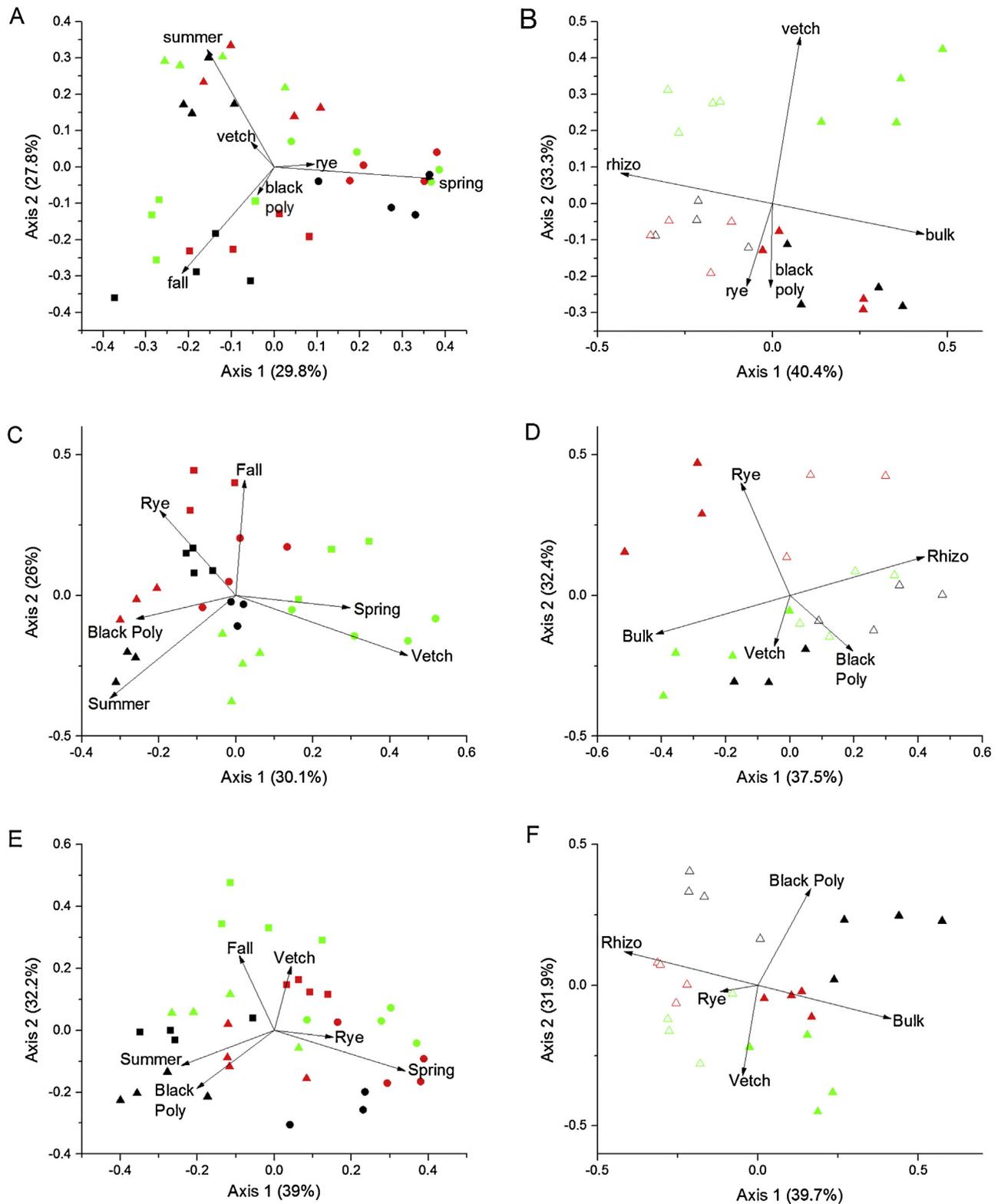


Fig. 2. Redundancy analysis of soil microbial community structure as determined by phylum-specific TRFLP. A: Firmicutes bulk soil, all seasons. B: Firmicutes, summer only. C: Betaproteobacteria bulk soil, all seasons. D: Betaproteobacteria, summer only. E: Planctomycetes bulk soil, all seasons. F: Planctomycetes, summer only.

location (bulk or rhizosphere). Although in this study we did not find a significant effect of antecedent cover crop or other soil surface management, some studies have found that antecedent cover crops can affect the structure of the microbial community (Buyer et al., 2010; Chellemi et al., 2012; Kuffner et al., 2012; Lekberg et al., 2012).

We previously showed by PLFA analysis that total microbial biomass and fungal biomass were highest under vetch, intermediate under rye, and lowest under black polyethylene (Buyer et al., 2010). PLFA analysis further showed that season significantly affected the abundance of Gram-positive bacteria, Gram-negative bacteria, and fungi. In the current study, quantitative PCR indicated

the same pattern of treatment effects on Eubacterial and fungal DNA abundance (Table 2). Although we do not have quantitative PCR data across all seasons, the changes observed in the ordination of influencing factors (Figs. 1 and 2) can be used to better understand some of the underlying changes in microbial community structure while the tomato crop is actively growing during the season.

We discuss the community structure analysis focusing on two broad spatially and temporally defined groups. We focus first on the complete dataset that includes bulk soil and all of the sampling dates. The second group is limited to analysis of both bulk and tomato rhizosphere soil in the summer months. We do this for three major reasons, the first of which is, the strong effects of season dominate the explainable variance when all sampling dates are considered in the analysis (except for Archaea, Fig. 1a). Secondly, splitting the data into two groups, each of which is a complete factorial, simplifies interpretation of the data and allows for balanced statistical analysis of bulk vs. rhizosphere comparisons. Finally, focusing on the choice of cover crop species or ground cover is a manageable variable in applied agroecosystem decision making, therefore, conducting analysis focusing on the specific effects of cover crop choice on the rhizosphere of cash crop makes sense from the agricultural management perspective. The interactions between cover crop choice and cash crop rhizosphere microbial community can have significant effects on crop disease ecology (Zhou and Everts, 2007) and performance (Fatima et al., 2012). The strong effect of season may mask the effects of cover crop species or mulch treatment on the structure of the rhizosphere microbial community where critical plant/microbe interactions are occurring.

4.1. All sampling dates, bulk soil only

Comparing cover crop vector lengths among RDA PCA plots (Fig. 2A–E), the community structure of Firmicutes appears to be less responsive to cover cropping than other phyla studied. This is qualitatively consistent with Table 3 and with our earlier conclusion that Gram-positive bacteria were less active than other bacteria in processing cover crop derived carbon and other rhizodeposits (Buyer et al., 2010). This correspondence is intriguing that most Firmicutes are Gram-positive (Wilkins, 2009), but without quantitative PCR using the phylum-specific primers we cannot say that the observed response of Gram-positive bacteria is driven solely by the Firmicutes.

The Betaproteobacteria appear to be weakly responsive to season and cover crop when compared to other phyla tested. This is inconsistent with the changes observed in the Gram-negative biomass marker using PLFA in previous studies (Buyer et al., 2010), and suggest that these bacteria genetically classified in the phylum Betaproteobacteria may have sub-populations that are functionally responding differently to cover crop treatments and proximity to roots (bulk and rhizosphere; Zablutowicz et al., 2007). It is intriguing that the Gram-negative Firmicutes are strongly influenced by cover crops, whereas the Gram-positive Firmicutes are not strongly influenced by cover crops but relatively strongly influenced by seasonal effects. This is supportive of the hypothesis that under our field conditions the soil dwelling Gram-negative bacteria may tend to be copiotrophic as a community whereas the Gram-positive bacteria may tend to be oligotrophic (Buyer et al., 2010). There is precedent for this finding in laboratory incubations in which labile carbon sources were used to induce microbial activity and then qPCR was used to quantify the abundance of Gram-negative Betaproteobacteria and the Gram-positive Firmicutes (Fierer et al., 2007).

Whereas the structure of the Eubacterial communities that studied showed significant response to season, the structure of the

Archaeal community was equally influenced by season and the cover crop or plastic mulch treatments across all sampling. It is well known that Archaea associate with plant roots and in some cases colonize roots at unexpectedly high levels relative to total soil microbial diversity (Simon et al., 2000). A possible explanation for this observation in our data is that the presence of different plant species in a system has a disproportionately greater influence on Archaeal community structure compared to Eubacteria. Archaea commonly make up only 2–10% of the total 16S rDNA gene copies present in terrestrial soils (Bates et al., 2011) and the total number of markers of soil community richness (TRFs) is lower in the Archaeal sample in this experiment (data not shown). The low relative abundance of Archaea may result in amplification of the effects of plants because changes in the relative abundance of just a few Archaea species will result in significant changes in vector length and direction in PCA biplots.

4.2. Summer bulk and rhizosphere soil

In all cases, there was an evidence of a rhizosphere effect, with the domain- or phylum-level community structure. Firmicutes show a clear difference between bulk soil and tomato rhizosphere (Table 3). However, treatment effects were different between phyla. The antecedent effects of cover crop, and the effects of black poly mulch, are different for the different domains, phyla, and cover crops studied in this experiment.

In the summer months, the community structure of the Firmicutes was strongly impacted by the differences between the bulk soil and the rhizosphere soil. In addition, vetch and rye cover crops had contrasting effects on the structure of the Firmicute community composition. The microbial community in the black poly treatment was intermediate in structure to either of the covers. It may be that some individual members of the Firmicutes can colonize the tomato rhizosphere, thus altering Firmicutes community structure in the rhizosphere compared to bulk soil, even though the phylum Firmicutes as a whole tend to be less abundant in the rhizosphere than other phyla.

In general, the mulch treatment effects (vetch, rye, and black poly) on the soil microbial community structure were contrasting to each other and intermediate to strong effect of location (bulk, rhizosphere) which indicates the strong effects of micro-habitat during the summer growing months and the direct influence of the growing crop on rhizosphere microbial community structure. We previously concluded that vetch cover cropping affected the tomato rhizosphere, either by providing labile nutrients for soil microbial growth or synergistic effect between nutrients released by the cover crop and the tomato rhizosphere (Buyer et al., 2010).

4.3. Implications and future directions

Eubacterial and fungal biomass increased with vetch cover cropping compared to black polyethylene mulch. We did not have the capacity to conduct the qPCR in a phylum-specific manner so we have limited ability to determine whether surface mulches preferentially influence specific groups of bacteria and fungi. The presence of vetch in a crop rotation increases soil nutrient and labile organic matter concentration (Bayer et al., 2001; Parr et al., 2011). Increases in soil nutrients and available labile organic matter may account for the increase in bacterial and fungal biomass under the vetch cover crop. Another consideration is that the soil temperature under the black polyethylene surface mulch was 3.4–5.7°C warmer than the soils under the vetch cover and ranged between 18 and 28°C (Teasdale and Abdul-Baki, 1995). The higher temperature under the black polyethylene may have shifted the soil temperature beyond optimal for some of the native soil microbes resulting in relatively higher biomass in the vetch treatments.

The seasonal effects observed in this experiment can overwhelm the subtle, locally driven legacy effects of crop rotation on cash crop rhizosphere dynamics (Table 3). Herein lies a major hurdle in managing soil microbial ecology to target-specific ecosystem functions because even though there are large seasonal shifts in the community structure, it is well documented that the intimate plant-microbe interactions taking place in the rhizosphere influence important plant/environment interactions such as disease ecology (McSpadden Gardener and Weller, 2001; Zhou and Everts, 2007), nutrient cycling (Cheng, 2009), and biogeochemical transformations (Richardson, 2001). Seasonal sampling (through the different phases of a crop rotation) allowed us to capture broad community changes that occurred as the seasons changed and established the context for the finer scale sampling (bulk and rhizosphere) during the summer months. These results suggest that the development of production systems which utilize cover crops as a biocontrol for plant pathogens during summer vegetable production (Chellemi et al., 2012; Hansen and Keinath, 2013) should consider scouting for soil pathogens throughout the year to capture the legacy effects of antecedent cover crop species.

It is clear that agricultural management decisions such as cover crop species selection for a rotation can have both a short-term (Hu et al., 1997; Maul and Drinkwater, 2010) and a legacy effect on both the subsequent cash crop (Fatima et al., 2007) and the soil microbial community (van Bruggen and Semenov, 2000). Within the agricultural community, we are beginning to see crop selection as a tool for disease control via indirect management of the resident soil microbial ecosystem (Zhou and Everts, 2007). Moving forward, in order to make science-based recommendations to land managers and farmers we must have a better understanding of the linkages between the large-scale effectors of change in the microbial community (season, soil type, pH, soil disturbance, moisture content) and the fine-scale effectors of change (rhizosphere, cover crop species). In this experiment by monitoring (season) or holding (soil type, pH, soil disturbance) many of the constant large-scale effectors we were able to focus on the effects of the antecedent cover crop species on the microbial community structure in the rhizosphere of a tomato crop. Ongoing research on the direct and indirect effects of antecedent cover crops on cash crops is designed to ask the questions; do the plant decomposition products alter gene expression in an actively growing plant and does the cover crop/cash crop rotation foster increased plant fitness through the legacy effect of the decomposing cover crop on the soil microbial community structure and function, with the eventual goal of recommending crop rotation choices that selectively influence the soil microbial community.

5. Conclusions

Eubacterial and fungal biomass increased with vetch cover cropping compared to black polyethylene mulch. The community structure of Archaea, Eubacteria, Firmicutes, Betaproteobacteria, and Planctomycetes were most affected by season. Cover crop species and mulch also affected the community structure of each domain and phylum, but the effects were different for each domain and phylum. There were clear rhizosphere effects on the community structure of every domain and phylum as well. These results are consistent with those previously found using PLFA analysis but provide a more detailed analysis of community structure at the phylum level.

References

Abdul-Baki, A.A., Teasdale J.R., 2007. Sustainable production of fresh-market tomatoes and other vegetables with cover crop mulches. Farmers' bulletin; FB-2280, U.S. Dept. of Agriculture, Agricultural Research Service, Washington, D.C.

- AbdulBaki, A.A., Morse, R.D., Teasdale, J.R., Devine, T.E., 1997. Nitrogen requirements of broccoli in cover crop mulches and clean cultivation. *J. Vegetable Crop Prod. [USA]* 3, 85–100.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46.
- Avrahami, S., Bohannon, B.J.M., 2009. N₂O emission rates in a California meadow soil are influenced by fertilizer level, soil moisture and the community structure of ammonia-oxidizing bacteria. *Glob. Change Biol.* 15, 643–655.
- Bakker, M.G., Glover, J.D., Mai, J.G., Kinkel, L.L., 2010. Plant community effects on the diversity and pathogen suppressive activity of soil streptomycetes. *Appl. Soil Ecol.* 46, 35–42.
- Balsler, T.C.F., Mary, K., 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry* 73, 395–415.
- Bates, S.T., Berg-lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. Examining the global distribution of dominant archaeal populations in soil. *ISME J.* 5, 908–917.
- Bayer, C., Martin-Neto, L., Mielniczuk, J., Pillon, C.-N., Sangoi, L., 2001. Changes in soil organic matter fractions under subtropical no-till cropping systems. *Soil Sci. Soc. Am. J.* 65, 1473–1478.
- Blackwood, C.B., Oaks, A., Buyer, J.S., 2005. Phylum- and class-specific PCR primers for general microbial community analysis. *Appl. Environ. Microbiol.* 71, 6193–6198.
- Buckley, D.H., Schmidt, T.M., 2001. The structure of microbial communities in soil and the lasting impact of cultivation. *Microb. Ecol.* 42, 11–21.
- Buyer, J.S., Roberts, D.P., Russek, C.E., 1999. Microbial community structure and function in the spermosphere as affected by soil and seed type. *Can. J. Microb.* 45, 138–144.
- Buyer, J.S., Teasdale, J.R., Roberts, D.P., Zasada, I.A., Maul, J.E., 2010. Factors affecting soil microbial community structure in tomato cropping systems. *Soil Biol. Biochem.* 42, 831–841.
- Carrera, L.M., Buyer, J.S., Vinyard, B., Abdul-Baki, A.A., Sikora, L.J., Teasdale, J.R., 2007. Effects of cover crops, compost, and manure amendments on soil microbial community structure in tomato production systems. *Appl. Soil Ecol.* 247–255.
- Chellemi, D.O., Wu, T., Graham, J.H., Church, G., 2012. Biological impact of divergent land management practices on tomato crop health. *Phytopathology* 102, 597–608.
- Cheng, W., 2009. Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C–N budgets. *Soil Biol. Biochem.* 41, 1795–1801.
- Culman, S., Bukowski, R., Gauch, H., Cadillo-Quiroz, H., Buckley, D., 2009. T-REX: software for the processing and analysis of T-RFLP data. *BMC Bioinformatics* 10, 171.
- Díaz-Pérez, J.C., Phatak, S.C., Ruberson, J., Silvoy, J., Morse, R., 2008. Utilization of mulches increase yield and improve weed control in no-till organic broccoli. In: Endale, D.M. (Ed.), Proc. 30th Southern Conserv. Agric. Syst. Conf. Tifton, Georgia.
- Fatima, T., Handa, A.K., Mattoo, A.K., 2007. Polyamines cross-talk with phospholipase A2 to regulate gene expression in tomato fruit and other plant models. *FASEB J.* 21, A1044-b-.
- Fatima, T., Teasdale, J.R., Bunce, J., Mattoo, A.K., 2012. Tomato response to legume cover crop and nitrogen: Differing enhancement patterns of fruit yield, photosynthesis and gene expression. *Func. Plant Biol.* 39, 246–254.
- Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364.
- Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl. Environ. Microb.* 71, 4117–4120.
- Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. *PNAS* 103, 626–631.
- Fliessbach, A., Mader, P., 2000. Microbial biomass and size-density fractions differ between soils of organic and conventional agricultural systems. *Soil Biol. Biochem.* 32, 757–768.
- Frey, S.D., Elliott, E.T., Paustian, K., 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biol. Biochem.* 31, 573–585.
- Gilreath, J.P., Santos, B.M., 2004. Methyl bromide alternatives for weed and soilborne disease management in tomato (*Lycopersicon esculentum*). *Crop Protection* 23, 1193–1198.
- Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Appl. Environ. Microb.* 69, 1800–1809.
- Grandy, A.S., Strickland, M.S., Lauber, C.L., Bradford, M.A., Fierer, N., 2009. The influence of microbial communities, management, and soil texture on soil organic matter chemistry. *Geoderma* 150, 278–286.
- Green, J.L., Holmes, A.J., Westoby, M., Oliver, I., Briscoe, D., Dangerfield, M., Gillings, M., Beattie, A.J., 2004. Spatial scaling of microbial eukaryote diversity. *Nature* 432, 747–750.
- Hansen, Z.R., Keinath, A.P., 2013. Increased pepper yields following incorporation of biofumigation cover crops and the effects on soilborne pathogen populations and pepper diseases. *Appl. Soil Ecol.* 63, 67–77.
- Hawkes, C.V., Wren, I.F., Herman, D.J., Firestone, M.K., 2005. Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecol. Lett.* 8, 976–985.
- Hu, S., Grunwald, N.J., van Bruggen, A.H.C., Gamble, G.R., Drinkwater, L.E., Shennan, C., Demment, M.W., 1997. Short-term effects of cover crop incorporation on soil carbon pools and nitrogen availability. *Soil Sci. Soc. Am. J.* 61, 901–911.

- Klappenbach, J.A., Dunbar, J.M., Schmidt, T.M., 2000. rRNA operon copy number reflects ecological strategies of bacteria. *Appl. Environ. Microb.* 66, 1328–1333.
- Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schlöter, M., Zechmeister-Boltenstern, S., Jandl, R., Schindlbacher, A., Sessitsch, A., 2012. Effects of season and experimental warming on the bacterial community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing. *FEMS Microb. Ecol.* 82, 551–562.
- Lekberg, Y., Rosendahl, S., Michelsen, A., Olsson, P.A., 2012. Seasonal carbon allocation to arbuscular mycorrhizal fungi assessed by microscopic examination, stable isotope probing and fatty acid analysis. *Plant Soil*, 1–9.
- Lundquist, E.J., Scow, K.M., Jackson, L.E., Uesugi, S.L., Johnson, C.R., 1999. Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. *Soil Biol. Biochem.* 31, 1661–1675.
- Maul, J., Drinkwater, L., 2010. Short-term plant species impact on microbial community structure in soils with long-term agricultural history. *Plant Soil* 330, 369–382.
- McSpadden Gardener, B.B., Weller, D.M., 2001. Changes in populations of rhizosphere bacteria associated with take-all disease of wheat. *Appl. Environ. Microb.* 67, 4414–4425.
- Pace, N.R., 1997. A molecular view of microbial diversity and the biosphere. *Science* 276, 734–740.
- Parr, M., Grossman, J.M., Reberg-Horton, S.C., Brinton, C., Crozier, C., 2011. Nitrogen delivery from legume cover crops in no-till organic corn production. *Agron. J.* 103, 1578–1590.
- Petchey, O., Gaston, K., 2009. Effects on ecosystem resilience of biodiversity, extinctions, and the structure of regional species pools. *Theor. Ecol.* 2, 177–187.
- Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Func. Plant Biol.* 28, 897–906.
- Schomberg, H., Endale, D., Calegari, A., Peixoto, R., Miyazawa, M., Cabrera, M., 2006. Influence of cover crops on potential nitrogen availability to succeeding crops in a Southern Piedmont soil. *Biol. Fert. Soil.* 42, 299–307.
- Simon, H.M., Dodsworth, J.A., Goodman, R.M., 2000. Crenarchaeota colonize terrestrial plant roots. *Environ. Microb.* 2, 495–505.
- Sorensen, J., Nicolaisen, M.H., Ron, E., Simonet, P., 2009. Molecular tools in rhizosphere microbiology—from single-cell to whole-community analysis. *Plant Soil* 321, 483–512.
- Spargo, J.T.C., M. A. Mirsky, S. B. Maul, J. E. Meisinger, J.J., 2011. Mineralizable soil nitrogen and labile soil organic matter in diverse long-term cropping systems. *Nutrient Cycling in Agroecosystems* 90, 253–266.
- Teasdale, J.R., Abdul-Baki, A.A., 1995. Soil temperature and tomato growth associated with black polyethylene and hairy vetch mulches. *J. Am. Soc. Horticultural Sci.* 120, 848–853.
- University of Maryland Extension Bulletin 236, 2012. Maryland Commercial Vegetable Production Recommendations for 2012. In: Extension, U.o.M. (Ed.). University of Maryland Extension.
- van Bruggen, A.H.C., Semenov, A.M., 2000. In search of biological indicators for soil health and disease suppression. *Appl. Soil Ecol.* 15, 13–24.
- Wieland, G., Neumann, R., Backhaus, H., 2001. Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. *Appl. Environ. Microb.* 67, 5849–5854.
- Wilkins, W., 2009. *Bergey's Manual of Systematic Bacteriology*. Williams and Wilkins, Baltimore, MD.
- Zablotowicz, R.M., Locke, M.A., Gaston, L.A., 2007. Tillage and cover effects on soil microbial properties and fluometuron degradation. *Biol. Fert. Soil.* 44, 27–35.
- Zhou, X.G., Everts, K.L., 2007. Effects of host resistance and inoculum density on the suppression of Fusarium wilt of watermelon induced by hairy vetch. *Plant Dis.* 91, 92–96.