Solanum lycopersicum (tomato) hosts robust phyllosphere and rhizosphere bacterial communities when grown in soil amended with various organic and synthetic fertilizers

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Abstract

Due to the intimate association between plants and their microbial symbionts, an examination of the influence of agricultural practices on phytobiome structure and diversity could foster a more comprehensive understanding of plant health and produce safety. Indeed, the impact of upstream crop production practices cannot be overstated in their role in assuring an abundant and safe food supply. To assess whether fertilizer type impacted rhizosphere and phyllosphere bacterial communities associating with tomato plants, the bacterial microbiome of tomato cv. 'BHN602' grown in soils amended with fresh poultry litter, commercially available sterilized poultry litter pellets, vermicompost or synthetic fertilizer was described. Culture independent DNA was extracted from bulk and rhizosphere soils, and washes of tomato blossoms and ripe fruit. PCR amplicons of hypervariable regions of the 16S rRNA gene were sequenced and profiled using the QIIME pipeline. Bulk and rhizosphere soil, and blossom and fruit surfaces all supported distinct bacterial communities according to principal coordinate analysis and ANOSIM ($R=0.87$, $p=0.001$ in year 1; $R=0.93$, $p=0.001$ in year 2). Use of microbiologically diverse organic fertilizers generally did not influence bacterial diversity, community structure or relative abundance of specific taxa on any plant organ surface. However, statistically significant differences in sand and silt contents of soil ($p<0.05$) across the field and corresponding shifts in water activity were positively ($R^2=0.52$, $p=0.005$) and negatively ($R^2=0.48$, $p=0.009$) correlated with changes in bacterial community structure in the rhizosphere, respectively. Over two harvest seasons,

45 this study demonstrated that the application of raw poultry manure, poultry litter
46 pellets and vermicompost had little effect on the tomato microbiome in the
47 rhizosphere and phyllosphere, when compared to synthetically fertilized plants.
48 Plant anatomy, and other factors related to field location, possibly associated with
49 edaphic and air characteristics, were more influential drivers of different tomato
50 organ microbiomes than were diverse soil amendment applications.

51
52 KEYWORDS: organic fertilization, fruit microbiome, flower microbiome,
53 vermicompost, poultry manure, soil characteristics

1. Introduction

Plants support diverse microbial communities above and belowground that are uniquely suited to the plant habitat and intimately connected to plant health. Microbial communities living on plant surfaces are species- and sometimes genotype-specific (Bulgarelli et al., 2012; Micallef et al., 2009b; Peiffer et al., 2013), and vary across spatial and temporal scales. In the rhizosphere, the region of soil closest to the root structure, root exudates drive composition and structure of bacterial communities, distinguishing them from those of the surrounding bulk soil (Bais et al., 2006; Micallef et al., 2009b). In the phyllosphere, dominated by leaves but also including stem, blossom, and fruit surfaces, harsh and fluctuating environmental conditions present challenges to bacterial epiphytes (Vorholt, 2012). Phyllosphere microbiota may initially be gleaned from air (Fahlgren et al., 2010; Maignien et al., 2014), nearby plants (Vorholt, 2012), or even from seed (Lopez-Velasco et al., 2013) early in life. As the plant develops, the influence of these factors may diminish, with other factors such as plant host and insect visitation becoming more influential (Alekkett et al., 2014; Ushio et al., 2015; Vorholt, 2012). In addition to differing across plant species (Knief et al., 2010; Leff and Fierer, 2013), microbial assemblages vary widely by micro-niche. Within a single tomato plant, leaf, blossom, fruit, stem, and root surfaces hosted unique bacterial and fungal communities, and leaf community diversity decreased with increasing distance from the soil (Ottesen et al., 2013), suggesting that soil may be a source for microbial communities in the phyllosphere. At an even smaller scale, bacterial communities on specific plant organs may shift in response to nutrient gradients and water availability, such as in

close proximity to stomata and leaf trichomes (Leveau and Lindow, 2001; Remus-Emsermann et al., 2012), or on particular floral structures within blossoms (Alekklett et al., 2014). Phyllosphere diversity tends to be lower than in the nutrient-rich rhizosphere, which is not as subject to extreme stresses such as UV exposure and desiccation (Bodenhausen et al., 2013; Ottesen et al., 2013). Both phyllosphere and rhizosphere bacterial community structures shift over time, showing clear successional dynamics throughout growing seasons and plant growth stages (Micallef et al., 2009a; Redford and Fierer, 2009; Shade et al., 2013; van Overbeek and van Elsas, 2008).

While plant host is known to be a strong driving factor of bacterial community composition in the rhizosphere and phyllosphere, the relative contributions of agricultural management practices are less clear. One such management practice is the incorporation of biological soil amendments of animal origin, economical, environmentally friendly, and effective sources of soil nutrients for agricultural production. These amendments, including fresh and composted manure, are often incorporated before planting and sometimes used as side dressing throughout the season. In addition to accruing organic matter and improving soil health, biological soil amendments could serve as a source of bacteria for the plant microbiome. Amendments could introduce beneficial microorganisms that directly or indirectly reduce plant pathogen and insect damage (Hadar and Papadopoulou, 2012; Liu et al., 2007; Mehta et al., 2014), or human pathogens that could undermine food safety (Islam et al., 2005; Mootian et al., 2009; Oni et al., 2015). Investigations into the

influence of soil amendment application on plant microbiota have focused primarily on the rhizosphere. Compost or manure has been reported to affect rhizosphere microbial community structure and diversity in some cases (Das and Dhar, 2012; Esperschuetz et al., 2007; Jangid et al., 2008; Lavecchia et al., 2015; Peiffer et al., 2013), but in many studies these effects are inconsistent or seasonally dependent (Gao et al., 2015; Tatti, 2012; Tian and Gao, 2014). On the other hand, the influence of fertilizer application on phytobiomes remains to be studied in depth. Diverse soil microbiomes and soil management strategies cause specific shifts in leaf metabolome composition, and in *Brassica* plants these shifts in turn influenced herbivorous insect damage and diversity of insect pests (Badri et al., 2013; Staley et al., 2010). We hypothesize therefore that amendments applied to the soil may seed the phyllosphere with new microbes, especially in lower parts of the plant close to or touching the ground, and also potentially lead to changes in plant physiology, all of which could influence the diversity of microbes on plant surfaces.

To investigate whether the use of organic fertilization on fields prior to planting can induce above and belowground changes in the harvest-time tomato microbiome, we evaluated synthetic fertilization and three organic fertilizers during tomato cultivation: fresh poultry litter, commercially available sterilized poultry litter pellets, and vermicompost. The impact of these organic fertilizers on tomato rhizobacterial communities, and blossom and fruit-associated bacterial communities was described using Illumina-based 16S rRNA gene sequencing.

2. Materials and Methods

2.1 Field design

Field studies were conducted at the University of Maryland's Wye Research and Education Centre (WyeREC) in Queenstown, MD, USA in a field classified as silt loam by the USDA Web Soil Survey (Soil Survey Staff, 2015). In both 2013 and 2014, soil amendments appropriate for both organic and conventional growing operations were applied in spring, 2 weeks prior to planting, and incorporated into the top 10 cm of field soil through raking by hand. In 2013, 4 rows (randomly chosen from a total of 8 rows) were amended with fresh poultry litter mixed from 2 anonymous Eastern Shore sources at a rate of 2,800 kg/ha. In 2014, research plots were located in the same field, but 5 new rows were prepared between 2013 row locations, so there was no additive effect of amendment application over 2 seasons. Within each row in 2014, 3 soil amendments and a synthetic fertilizer treatment were assigned randomly and applied to 1 of 4 plots within each row. The following soil amendments were chosen for their ready availability to local growers and for their potentially diverse bacterial profiles: fresh poultry litter (mixed from 2 anonymous local sources; applied at a rate of 2,800 kg/ha), sterile poultry litter pellets (microSTART60, Perdue AgriRecycle, LLC, Seaford, DE; applied at a rate of 2,800 kg/ha), and vermicompost (locally produced from domestic vegetable waste through windrow composting and subsequent digestion by red wiggler worms; applied at a rate of 6,725 kg/ha). One small bag of each soil amendment was transported back to the lab on ice and frozen at -80°C for bacterial community

analysis. In both 2013 and 2014, mineral fertilization was applied as an inorganic fertilizer control. Nutrient levels were equalized as much as possible across treatments, using supplemental mineral fertilizer as needed to reach a target nutrition profile of 140N:56P:84K kg/ha). To reduce the probability of drift, rows were spaced 4.6 m apart, with a 1.5 m buffer zone between plots within rows, where soil amendments were not applied. Field plots were mulched with black plastic and drip irrigated. Treatments had 4 (2013) or 5 (2014) independent replicate plots, each planted with 8-10 tomato plants. The tomato cultivar used was 'BHN602', a commonly used commercial, determinate variety with resistance to Verticillium wilt, Fusarium wilt, and tomato spotted wilt virus (SEEDWAY, Hall, NY). Non-sterile coated seeds were planted into 4.83x4.83x6.03 cm plug trays (T.O. Plastics, Clearwater, MN) containing LC1 potting mix (SunGro, Agawam, MA) and maintained in the greenhouse under standard conditions for approximately 6 weeks. Seedlings were transplanted into the field 2 weeks after soil amendment.

2.2 Sampling for microbial community analysis

Field samples were collected in August 2013 and September 2014, when plants had blossoms and fruit at varying levels of maturity. Three red-ripe fruit, 6-10 blossoms, and the full root ball with adhering soil were randomly sampled from one plant within each plot. Fruit and blossom samples were aseptically collected into Ziploc bags using gloved hands and 70% ethanol-sterilized pruners. After fruit and blossom sampling, each tomato plant was cut at the lower stem and removed from the plot. Soil around the roots was loosened using an ethanol-sterilized trowel, and

the roots were manually pulled from the ground with gloved hands. Loosely adhering bulk soil was firmly shaken from the root ball into a Ziploc bag (to be discarded), and the remaining root with closely adhering rhizosphere soil was moved into the final sample bag. Plant samples were transported on ice to the lab, where they were stored at 4°C and processed within 24 h. In 2013 only, bulk soil was collected from each plot for microbial community analysis. For each plot, a composite of 10 soil cores, collected 10 cm from the base of each tomato plant at a depth of 15-20 cm, was collected at planting date in early June using ethanol-sterilized soil corers. Bulk soil samples were transported on ice to the lab, where they were hand-homogenized and frozen at -80°C.

2.3 Plant surface washing, DNA isolation and amplicon sequencing

Aseptically collected samples were washed with sterile deionized water and sonicated for 6 min to dislodge microbial cells from the plant surface. Rhizosphere washes were pelletized at 1773 *g* for 30 min at 4°C using a Sorvall centrifuge with an SA-600 rotor. Blossom and fruit washes were filtered through sterile 0.22 µm nitro-cellulose filters (Nalgene Nunc International Corporation, Rochester, NY). Pellets and filters were frozen at -80°C until further processing. Total community DNA was extracted from frozen rhizosphere pellets and bulk soil samples using the Powerlyzer Powersoil kit (MoBio Laboratories, Carlsbad, CA) and from plant surface-wash filters using the Powerwater kit (MoBio Laboratories). Bacteria represent the most abundant inhabitants of the phyllosphere, therefore community profiling targeted the 16S region of the prokaryotic 30S small ribosomal subunit,

which contains both highly conserved and highly variable regions. In 2013, the V4 region of the 16S rRNA gene was amplified using 515F-806R primers as recommended by the Earth Microbiome Project (Caporaso et al., 2012), and libraries were sequenced on an Illumina MiSeq (v2) using 251 bp paired-end sequencing. In 2014, the V1-V3 region of the 16S rRNA gene was chosen with the aim of obtaining a higher resolution within the Enterobacteriaceae, a group with high significance for food safety and plant pathology. This region was amplified using 8F-533R primers (Ottesen et al., 2013), and sequencing was carried out using 300 bp paired-end sequencing on the Illumina MiSeq (v3), following Illumina's protocol for 16S Metagenomic Sequencing Library Preparation (Illumina part # 15044223 rev. B). PhiX (50% in 2013; 25% in 2014) was spiked into each run to provide diversity necessary for cluster generation.

2.4 Sequencing data analysis

Quality filtering and sequence analysis were carried out using QIIME v. 1.8 (Caporaso et al., 2010b) and Mothur v. 1.34 (Schloss et al., 2009). Alignment was performed using PyNAST (Caporaso et al., 2010a) and the Greengenes Core reference alignment (DeSantis et al., 2006; McDonald et al., 2012), and taxonomy assignment utilized the RDP Classifier 2.2 (Wang et al., 2007). Reads that failed to match the reference database were clustered *de novo* using uclust (Edgar, 2010). Prior to alignment, sequences went through several quality filtering steps to remove chimeras (Edgar et al., 2011), non-target sequences (chloroplast and mitochondria),

and sequences less than 100 bp in length. A final operational taxonomic unit (OTU) table was created excluding unassigned sequences and singletons.

To ensure comparability between samples, within each comparison all samples were rarefied to a common sequencing depth as recommended by Weiss et al. (Weiss et al., 2015). After analyzing the data at several rarefaction depths, numbers were chosen that allowed the inclusion of as many replicates as possible without loss of statistical signal. Plant organ types were analyzed separately to assess the influence of soil amendment treatment and bulk soil properties on microbial diversity for each of these diverse niches. Comparisons across all sample types from 2014 were performed at a depth of 2,450 sequences per sample. When analyzing plant parts separately, the rarefaction level was adjusted: 1,670 for fruit, 1,980 for blossoms, and 5,950 for the rhizosphere. Rhizosphere communities tended to have higher alpha diversity compared to phyllosphere communities, and as such higher thresholds were chosen for rhizosphere samples when possible. In 2013, rarefaction was employed as follows: 5,570 for soil, 4,545 for rhizosphere, 6,330 for blossom, 3,875 for fruit samples. Comparisons including the full set of samples were carried out with 3,606 sequences per sample..

UniFrac was utilized in QIIME to calculate beta diversity metrics weighted by phylogenetic distance (Chang et al., 2011; Lozupone and Knight, 2005). Principal coordinate analysis (PCoA) plots were created to visualize beta diversity across treatments utilizing a weighted UniFrac distance matrix. After filtering to include

only OTUs present in at least 75% of samples, as recommended by QIIME documentation, significant differences in relative abundance among OTUs were assessed through a Kruskal-Wallis test utilizing an FDR correction (group_significance.py in QIIME). ANOSIM, an analysis of similarity test, was implemented using R's Vegan package (Oksanen et al., 2013) to assess significance of treatment influence on microbial community structure (999 permutations per test). Pairwise comparisons within treatments were carried out using a 2-sided 2-sample *t*-test of distance through QIIME's make_distance_boxplots.py script. Vectors representing the relationship between soil characteristics and bacterial community profiles at 97% identity were generated through 999 permutations of Vegan's envfit function for all 2014 sample types. A tree displaying the differences between plant organs and soil amendments was created using FastTree (Price et al., 2009) and visualized using FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). Sequencing data were deposited in the NCBI Sequence Read Archive (SRA) database under accession number SRP074759.

2.5 Soil properties

In 2014, bulk soil was collected from the top 10 cm of each plot using ethanol-sterilized scoops. Soil samples were sent to Waters Agricultural Lab, Inc. (Camilla, GA) for analysis. The following soil properties were measured for soil from each plot: available P, exchangeable K, Mg, Ca, and H, soil pH, cation exchange capacity (CEC), percent base saturation of cation elements, organic matter, and soil texture (% sand, silt, and clay). Water activity (A_w) was assessed using a Pa_wkit water

activity meter (Aqualab, Pullman, WA). Significant differences between treatments were assessed using ANOVA and pairwise comparisons utilized Tukey's HSD test (JMP Pro v.11).

3. Results

3.1 Sequencing run metrics

Sequencing of samples collected over two years was performed separately. Sequencing of 2014 samples resulted in 9.3 million total sequences for each of the forward and reverse reads (for the 60 samples taken through the full analysis). Reads 1 and 2 were merged at an average efficiency of 58%. For all unmerged read pairs, read 1 was included for further analysis. After removing low quality or non-target sequences (less than 100 bp long, sequences identified as phiX, chimeric, chloroplast, or mitochondrial), 3.4 million reads remained for OTU picking. For 2013 samples, sequencing on the MiSeq v2 platform resulted in 1.4 million total sequences for the 56 samples included in this study. Merging efficiency was 84%, and after quality filtering 1.3 million sequences remained for further analysis.

3.2 Plant organ as a driver of bacterial communities

Principal Coordinates Analysis (PCoA) performed through QIIME and analysis by R-Vegan function ANOSIM showed that rhizosphere, blossoms, and fruit supported distinct bacterial communities, with the greatest distance observed between rhizosphere soil and the two phyllosphere groups (Figure 1). Plant organ drove

variation in bacterial community structure more than any other factor in 2013 ($R=0.87$, $p=0.001$, $n=56$) and 2014 ($R=0.93$, $p=0.001$, $n=60$). When 2013 and 2014 data were analyzed together, sample type (soil, rhizosphere, blossom, fruit) consistently explained the majority of variation among samples ($R=0.69$, $p=0.001$, $n=116$). Year also had a significant, albeit weaker, effect on bacterial community composition ($R=0.37$, $p=0.001$, $n=116$).

At the phylum level, the largest difference between above- and below-ground bacterial communities was observed in the Proteobacteria, which were much more dominant on fruit and blossoms compared to bulk soil and rhizosphere in both 2013 and 2014. In 2013, blossom and fruit surfaces were dominated by Pseudomonadaceae (50% on blossom and 40% on fruit) and Enterobacteriaceae (39% and 26%, respectively). Dominant taxa in rhizobacterial communities in 2013 belonged to the Bacillaceae (13.6%) and Pseudomonadaceae (12.5%), both of which were highly enriched compared to the surrounding bulk soil. In 2014, Pseudomonadaceae were elevated in blossoms compared to roots, however they were much less prevalent than in 2013, at 9% relative abundance. Instead, Xanthamonadaceae dominated on blossoms (32.6%), while fruit supported a high relative abundance of Rhizobiaceae (14.3%), mostly explained by the genus *Agrobacterium*, at 13.6%. Both the Pseudomonadaceae and Xanthamonadaceae families contain pathogens that can infect tomato; it is possible that these pathogens occurred in the field, however resolution is not high enough to differentiate between pathogenic and non-pathogenic members of this taxa.

Members of the Paenibacillaceae, a group known to include several bacteria with biocontrol activity against plant and human pathogens, were detected in all sample types over both years, with highest prevalence in the rhizosphere (~1% relative abundance both years). The rhizosphere samples harbored the highest phylogenetic diversity, with an average of 1,764 unique OTUs (97% identity) identified at a rarefaction level of 2,450 sequences per sample in 2014. Alpha (within sample) diversity in blossoms and fruit was significantly lower, with 357 and 693 OTUs identified at the same rarefaction level, respectively ($p=0.003$) in 2014.

3.3 Influence of soil amendment on tomato-associated bacterial communities

The data from 2013 indicated a potentially weak influence of poultry litter amendment on tomato blossom ($R=0.34$, $p=0.076$, $n=8$) and rhizosphere ($R=0.16$, $p=0.051$, $n=16$), but not fruit ($R=0.04$, $p=0.319$, $n=16$), bacterial communities (Figure 2). Bacterial communities profiled from bulk soil did not respond to soil amendment ($R=0.02$, $p=0.293$, $n=16$), however row location in the field appeared to influence bulk soil bacterial community structure ($R=0.58$, $p=0.002$, $n=16$).

In 2014, to better control for the confounding influence of soil parameters and strengthen the study design, the field was blocked by row (with all treatments incorporated into plots within each row). We were also able to introduce greater replication and two more soil amendment treatments. Despite these changes and

the use of a longer 16S rRNA gene fragment for sequencing, no effect of soil amendments on the tomato microbiome was observed in 2014. Soil amendment was not a significant factor for bacterial community structure in the rhizosphere (Figure 2), and no significant differences were observed at any taxonomic level. Likewise, in 2014, blossom and fruit surfaces hosted convergent bacterial communities across soil amendment treatments (Figure 2). When analyzed within field row in a nested perMANOVA utilizing a Bray-Curtis distance measure, soil amendment had no influence on 2014 bacterial community structure on any tomato plant surface studied ($p>0.05$). To verify that the 2013 detection of a weak soil amendment treatment effect on blossom-associated bacterial community structure was not attributable to a higher sampling depth in 2013, 2013 blossom data was reanalyzed at the 2014 rarefaction level of 1,980 sequences per sample. The weak effect of poultry litter application on blossom microbiome remained ($R=0.34$, $p=0.095$, $n=8$).

3.4 Microbiome analysis of organic soil amendments

The soil amendments themselves supported phylogenetically diverse bacterial assemblages ($p<0.001$, UniFrac Monte Carlo significance test). Vermicompost harbored a highly diverse bacterial community most similar to that of the rhizosphere, while the poultry litter and poultry pellets were characterized by a less diverse community dominated by several shared families (Figure 3). Dominant taxa in vermicompost were Hyphomicrobiaceae, Acidimicrobiales and Bacillaceae. On the other hand, the most predominant groups in poultry manure and poultry litter pellets were the Staphylococcaceae, Dermabacteraceae, Lactobacillaceae and

Aerococcaceae (Figure 3). While bacterial assemblages in poultry pellets were most similar to those in fresh poultry litter, a large proportion of the DNA isolated from the former samples could likely have persisted from non-viable organisms killed during the sterilization process.

Despite differences in bacterial communities among these diverse soil amendments, a significant differential shift in bacterial community structure or diversity in the mature plant rhizosphere or phyllosphere at harvest was not observed. Additionally, application of diverse soil amendments did not lead to changes in most physico-chemical soil characteristics, the only measurable difference being an elevated Cation Exchange Capacity (CEC) in vermicompost-amended plots (Table 1).

3.5 Impact of edaphic factors on tomato-associated bacterial communities

While soil amendment application did not exert a drastic influence on tomato-associated bacterial communities, soil physico-chemical characteristics may have played a role. Rhizosphere samples taken from field rows 4 and 5 supported phylogenetically similar bacterial communities, as did those from rows 2 and 3, and both pairs differed from row 1, regardless of soil amendment treatment applied (Figure 4B).

Analysis of bulk soil collected from all plots showed a clear gradient in soil texture ($p<0.001$) and Mg concentration ($p=0.016$) through the field, coupled with a weakly significant gradient in water activity ($p=0.080$) (Table 1, Figure 4A). At the 97%

identity level, shifts in rhizosphere beta diversity were correlated with continuous silt ($R^2=0.61$, $p=0.015$) and water activity ($R^2=0.61$, $p=0.009$) gradients in the field. Vector fitting revealed that levels of nutrients Mg ($R^2=0.50$, $p=0.044$) and K ($R^2=0.50$, $p=0.044$) were also correlated with rhizosphere beta diversity, with Mg (kg/ha) increasing toward row 5 and K+ (% nutrient saturation) decreasing toward row 5 (Figure 4B, Supplemental Table 1).

While row and its associated soil characteristics tended to influence beta diversity both in the rhizosphere and on ripe fruit surfaces (Figure 4B), statistically significant differences were observed at the family level only in the rhizosphere (Figure 5). Rhizosphere soil collected from rows 4 and 5 hosted lower percentages of Bacillaceae and Mycobacteriaceae and higher percentages of Oxalobacteriaceae and Pseudomonadaceae compared to the other rows. Shifts in fruit-associated beta diversity differed by row as well, with samples from rows 1 and 5 clustering together and rows 3 and 4 forming another cluster, with both clusters diverging from row 2. While row did not significantly drive blossom-associated bacterial beta diversity, rows 3 and 4 tended to cluster more closely together, as did rows 1 and 2.

A closer look at the relative abundance of taxa across rows in each sample type revealed that common trends in beta diversity across plant organs could not be attributed to shifts in the abundance of the same taxa (Figure 5). Although levels of Xanthomonadaceae were consistent across all rhizosphere samples, relative abundance appeared to be slightly higher on blossoms, increasing by at least 20% in

rows 4 and 5 compared to the rest of the field (although this variation was not significant). On fruit, Xanthomonadaceae was highest in rows 3 and 4 compared to the rest of the field, with row 5 being lowest. While row was used as a blocking factor throughout sample processing, no other factor (placement on PCR plate, DNA extraction date/lot of kit, indexing primers used) had a significant influence on beta diversity. Location in the field based on the perpendicular gradient (column) also had no significant effect on any sample type, and only one soil characteristic, K, differed significantly between columns in the field (Table 1, Supplementary Table 1).

4. Discussion

Using a phylogenetic approach, this study found that the application of three locally available organic soil amendments—fresh poultry litter, sterile poultry litter pellets, and vermicompost—did not exert a remarkable differential influence from synthetic fertilizer on tomato rhizosphere, blossom or fruit-associated bacterial communities when applied before planting. At harvest time, tomato plants supported bacterial communities that were plant organ-specific but generally independent of soil amendment. This finding indicates that tomato plants are robust hosts to epiphytic bacteria with the ability to maintain a consistent selective pressure on plant-associated microbiota, despite changing agricultural inputs. Although we observed no effect attributable to soil amendment, our study was limited to one time point, and differences in plant-associated microbiomes in response to soil amendment may have existed earlier during plant development. Studies of the cucumber and bean rhizosphere found that microbial community response to compost

amendments was strong during early plant establishment but decreased throughout the season as plants matured (Copeland et al., 2015; Gao et al., 2015). This suggests that over time plant-mediated pressures override the influence exerted by the soil amendments, however, additional research is needed to determine the contribution of such agricultural inputs to the crop microbiome early in plant development.

Above-ground, the use of diverse organic soil amendments did not lead to consistent changes in microbial community structure or relative abundance of bacterial taxa. In 2013, the application of poultry manure may have shifted blossom- and rhizosphere-associated bacterial community structure but not relative abundance of specific taxa. This effect was not detected in 2014. Weather patterns and field management practices were similar between 2013 and 2014 sampling periods, but the field setup in the first year of sample collection did not include replication within rows. It is possible that the difference in effect could be attributed to field gradients, or other factors that we did not measure. In any case, our findings do suggest that interactions with one or more unidentified factors may occur that could augment the influence of the organic fertilizers used in this study.

Although fertilizer type appeared to be a less significant factor than expected, by contrast, location in the field exerted a measurable effect on crop-associated bacterial assemblages. Statistically significant increases in silt and sand content across the field from rows 1 to 5 were associated with gradual changes in soil water activity, which in turn were paralleled with shifts in rhizobacterial community

447 structure. Relative abundance measures of some taxa were significantly different
448 across these soil characteristic gradients. Previous work has reported the effect of
449 edaphic factors on the rhizosphere. Both water availability (Fierer et al., 2003;
450 Reichel et al., 2014) and soil texture (Schreiter et al., 2014) have been previously
451 identified as drivers of rhizosphere community structure, and both of these factors
452 likely modulate the availability of nutrients such as Mg and K. Soil organic matter
453 has been identified as a factor for cucumber rhizobacterial community structure,
454 whereas compost use exerted no long-term impact (Tian and Gao, 2014). On the
455 other hand, a study of the grape microbiome showed that the use of compost over
456 several years, leading to changes in physico-chemical soil characteristics, did not
457 induce changes in the rhizosphere microbiome (Tatti, 2012). Shifts in phyllosphere
458 bacterial communities from different rows were less dramatic compared to
459 rhizosphere samples, with significant differences in beta diversity but not relative
460 abundances of bacterial taxa observed. Soil conditions, such as C:N ratio and pH
461 have previously been shown to influence phyllosphere microbial community
462 structure (Zarraonaindia et al., 2015). Our results support this trend, although we
463 observed a weaker response in the phyllosphere compared to the rhizosphere. Fruit
464 surface bacterial community groupings somewhat followed the gradient of
465 increasing water activity and changing soil texture from rows 1 to 5, but additional
466 associations were noted, showing that at least one additional factor (again
467 associated with field location) was a strong driver of the tomato microbiome above-
468 ground. Microbial communities collected from fruit surfaces from rows 1 and 5
469 were statistically similar, sharing a high prevalence of certain families such as

Sphingomonadaceae and Microbacteriaceae. While border rows were employed in the study design to attenuate any edge effect, rows 1 and 5 were still closest to the edge of the field, which was flanked on each side by roads used by farm vehicles. Dust from passing vehicles may have influenced microbial diversity on fruit surfaces on the outer rows, causing them to host similar bacterial communities despite different soil conditions.

In this study, soil amendments were applied to supplement existing bulk soil nutrition as an alternative to synthetic nutrition alone. Manure- or compost-based amendments may be chosen for nutrient management in organic or conventional growing operations due to their widespread availability, affordable cost, and effectiveness in releasing nutrients slowly throughout the season. Many organic growers use animal-derived fertilizer (fresh or composted manure) as a primary source of plant nutrition, and it has been posited that organically grown produce could therefore have a higher risk of contamination with enteric human pathogens. Many consumers on the other hand assume that organically grown produce is “safer” than its conventional counterpart (Berlin et al., 2009; Williams and Hammitt, 2001). In actual fact, studies do not tend to support this – many studies comparing the microbiological safety of conventional versus organic produce tend to show no differences in microbiological safety risk (Bourn and Prescott, 2002; Diez-Gonzalez and Mukherjee, 2009; Magkos et al., 2006; Marine et al., 2015; Pagadala et al., 2015). Many of these studies have used bacterial indicators of fecal contamination, such as generic *E. coli* and fecal coliforms, to assess risk, however these indicators have been

shown to have little to no correlation with the presence of pathogens (Pachepsky et al., 2014; Wu et al., 2011). Better and more comprehensive methods are needed to assess the relative risk of agricultural management practices, including use of manure, on produce safety.

By understanding the ecological influence of biological soil amendment use on plant-associated microbial communities, we will come closer to understanding how certain nutrient management practices influence food safety risk in agriculture. Samples of soil amendments and rhizosphere soil, blossoms and fruit were analyzed for the foodborne pathogens *Salmonella enterica* and *Listeria monocytogenes* (data not shown). No foodborne pathogens were detected from the soil amendments used in this study, so the potential for transmission to the field and survival throughout the season could not be assessed. Instead, we investigated the potential for soil amendment application to directly or indirectly influence the makeup of bacterial assemblages in the tomato rhizosphere, and on blossom and fruit surfaces, finding that location of the plant in the field and plant organ were much more influential.

5. Conclusions

Investigating the impact of soil edaphic characteristics on the tomato microbiome was not the aim of this study, but the effect of row (and its associated soil texture and water activity gradients) was notable, especially in contrast to the apparent lack of influence of soil amendments. While the plant host itself, and the organ-specific

niches it provides, regulated bacterial community structure to a large extent, this study showed that field location and associated soil characteristics had a stronger influence than poultry litter fertilizer or vermicompost. The effect was more marked belowground, but certain shifts were also observed in phyllosphere communities. This study suggests therefore that in the short term, poultry litter-based manure and vermicompost amendments applied to soil before transplanting of seedlings are not important determinants of the tomato microbiome at the time of harvest. On the other hand, location in the field, which may be subject to variable environmental conditions such as changes in soil characteristics or air quality, may be important factors to evaluate. This segues to possible effects of long-term organic fertilization, which tends to build organic matter over time and alters physical characteristics, which would be expected to exert important influences. Long-term studies are needed to test this hypothesis, determine whether such changes are also specific to plant developmental stage, and how these complex factors contribute to crop health and safety.

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Figure Captions

Figure 1. Principal Coordinates Analysis (PCoA) illustrating differences in bacterial community structure on the surfaces of tomato blossoms, fruit, rhizosphere, and soil in 2013 (A) and 2014 (B). A distance matrix weighted by abundance and utilizing UniFrac distances was used to calculate principle coordinates. Percent variation explained by each principle coordinate is marked on each axis. For each year, the strength of the sample type grouping is denoted by p values for the ANOSIM R statistic, representing the strongest correlation as it approaches 1.

Figure 2. Influence of soil amendment application on tomato plant surface-associated bacterial communities at time of tomato harvest in 2013 (top) and 2014 (bottom). Principle Coordinates Analysis utilizing a distance matrix weighted by OTU abundance and UniFrac distance between related taxa was performed to compare the beta diversity between tomato rhizosphere, fruit, and blossom bacterial communities from plots amended with poultry litter (L), poultry pellets (P), vermicompost (V), or mineral nutrition (C). To compare similarity between groups, R and p values were generated using ANOSIM. As R approaches 1, samples collected from plots treated with the same soil amendments are more similar to each other than to those collected from differently amended plots.

Figure 3. Phylogenetic relationship of tomato plant organ surfaces and starting soil amendments. The top 20 most abundant families within each sample type are

shown, scaled up to 100%, at the tips of the tree. The tree was created in FastTree and R utilizing UniFrac distances.

Figure 4. Soil texture (bars) and water activity (line) by tomato row in the field (A) and within plant organ beta diversity by row, represented by PCoA plots (B). Letters in bars representing sand and silt fractions in panel (A) denote significant differences in those soil components ($p < 0.05$). R and p values shown in panel (B) were calculated through ANOSIM using a Bray-Curtis distance metric. As R approaches 1, samples within a row are more similar to each other than to samples from other rows. Soil characteristics exhibiting significant ($p < 0.05$) correlation with ordination configuration are shown as vectors in panel (B): Potassium percent base saturation is represented as $K^+(\%)$, Magnesium level as $Mg\text{ (kg/ha)}$, relative silt as $Silt\text{ (\%)}$, and water activity as A_w . Vector direction shows increasing gradients of soil characteristic values, and the length of each arrow is proportional to the strength of the correlation between the variable and the ordination.

Figure 5. Mean relative abundance of families identified from tomato rhizosphere, fruit, and blossom surfaces from rows 1-5 in 2014. Families with a relative abundance greater than 0.01 for at least one row within each tomato organ are shown (after filtering to OTUs present in $>25\%$ of each sample type). FDR-corrected p values from a Kruskal-Wallis test are given for differences within a row. Relative abundances are coded by color, with the highest abundances marked in dark green and the lowest abundances marked in light yellow.

Table 1

Table 1. Differences in soil characteristics among 2014 tomato plots considering application of different soil amendments and field location by row and column, as determined by ANOVA (*p* values are shown).

Soil Factor	Soil Amendment ^a	Row ^b	Column ^b	Trends
Sand (%)	0.399	0.002*	0.752	Decreasing from row 1 to 5
Clay (%)	0.678	0.108	0.642	
Silt (%)	0.572	0.0003*	0.574	Increasing from row 1 to 5
pH	0.414	0.408	0.35	
Organic Matter (%)	0.178	0.435	0.456	
CEC (meq/100g)	0.031*	0.713	0.491	Highest in vermicompost plots
Water Activity (A _w)	0.794	0.084	0.297	Highest in rows 4 and 5
P (kg/ha)	0.343	0.488	0.315	
K (kg/ha)	0.991	0.401	0.021*	Increasing from column 1 to 4
Ca (kg/ha)	0.207	0.193	0.976	
ENR (kg/ha)	0.224	0.382	0.469	
Mg (kg/ha)	0.061	0.018*	0.983	Higher in row 5 than 2 and 3; Higher in amended plots compared to controls

^aBlocked by row
^bBlocked by soil amendment
**p*<0.05

Figure1

Figure2

Figure3

Figure4

Figure5

	Family	FDR_P	Row				
			1	2	3	4	5
Rhizosphere	Bacillaceae	0.035*	0.156	0.102	0.110	0.060	0.050
	Hyphomicrobiaceae	0.110	0.072	0.077	0.075	0.072	0.061
	Sphingomonadaceae	0.643	0.076	0.065	0.059	0.062	0.060
	Micrococcaceae	0.054	0.060	0.059	0.015	0.022	0.020
	Bradyrhizobiaceae	0.091	0.031	0.024	0.024	0.042	0.039
	Streptomyces	0.041*	0.018	0.038	0.048	0.033	0.018
	Rhodospirillaceae	0.057	0.016	0.023	0.029	0.024	0.030
	Gaiellaceae	0.376	0.018	0.029	0.026	0.021	0.022
	Microbacteriaceae	0.501	0.026	0.027	0.017	0.022	0.024
	Comamonadaceae	0.047*	0.037	0.014	0.011	0.024	0.028
	Oxalobacteraceae	0.036*	0.018	0.010	0.011	0.030	0.031
	Mycobacteriaceae	0.040*	0.023	0.025	0.025	0.013	0.010
	Xanthomonadaceae	0.412	0.015	0.014	0.017	0.024	0.016
	Paenibacillaceae	0.081	0.019	0.015	0.027	0.014	0.012
	Acidobacteria-6p_11-15f_	0.141	0.013	0.014	0.017	0.020	0.021
	Rhizobiales.f_	0.095	0.022	0.013	0.013	0.018	0.018
	Rhizobiales.Other	0.062	0.013	0.019	0.021	0.016	0.012
	Chitinophagaceae	0.354	0.017	0.013	0.015	0.019	0.015
	Burkholderiaceae	0.069	0.027	0.010	0.004	0.017	0.013
	RB41.f_	0.053	0.017	0.003	0.005	0.019	0.023
	Nocardoidaceae	0.058	0.003	0.013	0.012	0.013	0.010
	Alphaproteobacteria.Other	0.037*	0.014	0.012	0.011	0.006	0.006
	Phyllobacteriaceae	0.091	0.008	0.006	0.007	0.015	0.014
	Solibacteraceae	0.291	0.010	0.008	0.009	0.010	0.013
	Solirubrobacteriales.f_	0.102	0.009	0.012	0.012	0.008	0.007
	Solibacteriales.f_	0.060	0.007	0.008	0.009	0.011	0.011
	Caulobacteraceae	0.206	0.006	0.011	0.010	0.008	0.009
	Fireflyaceae	0.145	0.005	0.007	0.008	0.010	0.010
	Burkholderiales.Other	0.037*	0.004	0.005	0.004	0.010	0.017
	Actinomycetales.Other	0.280	0.010	0.008	0.007	0.008	0.005
	Pseudomonadaceae	0.039*	0.005	0.002	0.001	0.010	0.019
	Betaproteobacteria.o_f_	0.042*	0.004	0.005	0.007	0.008	0.011
	Alphaproteobacteria.o_f_	0.037*	0.004	0.002	0.004	0.013	0.011
	Enterobacteriaceae	0.265	0.001	0.002	0.000	0.003	0.003
Blossom	Xanthomonadaceae	0.268	0.247	0.339	0.293	0.457	0.661
	Pseudomonadaceae	0.598	0.070	0.150	0.033	0.113	0.182
	Microbacteriaceae	0.396	0.248	0.087	0.127	0.016	0.016
	Enterobacteriaceae	0.332	0.020	0.020	0.228	0.171	0.025
	Sphingomonadaceae	0.396	0.123	0.067	0.119	0.070	0.025
	Rhizobiaceae	0.407	0.118	0.059	0.087	0.010	0.006
	Shewanellaceae	0.396	0.025	0.021	0.082	0.073	0.035
	Gamma proteobacteria,Other	0.191	0.066	0.103	0.007	0.014	0.009
	Pseudomonadales,Other	0.618	0.037	0.034	0.045	0.026	0.014
	Comamonadaceae	0.534	0.008	0.018	0.001	0.034	0.017
	Methylobacteriaceae	0.268	0.016	0.020	0.001	0.003	0.007
	Phyllobacteriaceae	0.326	0.003	0.015	0.000	0.001	0.000
	Burkholderiales,Other	0.191	0.003	0.015	0.000	0.000	0.000
	Bradyrhizobiaceae	0.276	0.003	0.011	0.000	0.000	0.000
Fruit	Rhizobiaceae	0.423	0.105	0.206	0.116	0.238	0.307
	Sphingomonadaceae	0.222	0.243	0.155	0.121	0.131	0.208
	Microbacteriaceae	0.105	0.194	0.084	0.232	0.068	0.248
	Pseudomonadaceae	0.772	0.126	0.131	0.060	0.080	0.094
	Xanthomonadaceae	0.137	0.039	0.037	0.132	0.158	0.036
	Enterobacteriaceae	0.230	0.061	0.077	0.135	0.034	0.019
	Shewanellaceae	0.100	0.007	0.038	0.074	0.083	0.003
	Pseudomonadales,Other	0.121	0.033	0.071	0.048	0.016	0.020
	Methylobacteriaceae	0.137	0.021	0.056	0.012	0.064	0.024
	Oxalobacteriaceae	0.243	0.030	0.049	0.005	0.009	0.026
	Gamma proteobacteria,Other	0.222	0.006	0.030	0.013	0.014	0.006
	Comamonadaceae	0.158	0.017	0.006	0.003	0.015	0.021
	Sphingobacteriaceae	0.222	0.018	0.013	0.002	0.000	0.011
	Bradyrhizobiaceae	0.521	0.002	0.001	0.000	0.034	0.002
	Alcaligenaceae	0.222	0.002	0.012	0.009	0.014	0.000
	Micrococcaceae	0.103	0.015	0.001	0.001	0.001	0.017
	Rhodobacteraceae	0.121	0.025	0.000	0.000	0.004	0.003
	Rhizobiales,Other	0.250	0.007	0.002	0.004	0.016	0.005
	[Weekseiliaceae]	0.262	0.004	0.012	0.002	0.000	0.005
	Actinomycetales,Other	0.105	0.001	0.000	0.004	0.013	0.002