Bacteria of tomatoes managed with well water and pond water: Impact of agricultural water sources on carposphere microbiota

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Abstract— *We know that contamination of crops by human pathogens can occur in agricultural settings but we still do not understand precisely which environmental sources represent the highest risks. Human pathogens maybe introduced by wind, worker hygiene, plant mediated factors, insects, water sources, or any combination of these factors. To safeguard against risks to consumers from agricultural waters, FSMA regulations for U.S. crop production require the use of water with an average of less than 126 CFU per 100 ml for applications that come in direct contact with a crop. Due to availability/scarcity however, water from other sources such as agricultural ponds is commonly used. To better understand risks that may be associated with the use of "surface"(often higher microbial load) water sources, we described the bacterial microbiota associated with an agricultural pond, an agricultural well and the corresponding microbiota of tomato carpospheres managed with each water source (also described as phyllosphere). 16S rRNA gene amplicons were used for bacterial profiling of waters and tomato surfaces at four time points over a growing season. Microbial profiles differentiated surface and ground water samples throughout the season, however no significant influence on tomato fruit surfaces could be correlated to either water source. These results suggest that in certain cases, environmental pressures such as wind, dust or other airborne factors may have a more significant impact on the surface microbiology of field crops than irrigation or management water sources do.*

Keywords— Phyllosphere, carposphere, tomatoes, agricultural water sources, pond water, well water, bacterial microbiota, 16SrRNA gene.

I. INTRODUCTION

Identifying the source of food borne outbreaks is one of the most important objectives for food safety research. Contamination of fresh produce can occur at any point in the farm to fork continuum but there is evidence in certain cases – that human pathogens become associated with agricultural commodities while in the agricultural environment(7).

Contamination in the field could result from wind pressures, water sources, soils, manures, plant microbiology, insects, worker hygiene or any combination of these factors. To better understand the influence that agricultural waters may have on themicrobiology of a crop, we designed the study presented here. The Food Safety Modernization Act (FSMA) for produce safety, requires the use of water with a geometric mean of 126 or less CFU of generic *E.coli*per 100 mL of water with a statistical threshold of 410 CFU or less of generic *E.coli* in 100 mL of water for applications that come in direct contact with a crop:http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334114.htm. Given the fact that large volumes of water are needed for pesticide applications and irrigation, water demand cannot always be met with water that meets the recently mandated quality specifications. Growers routinely use water from farm ponds when other sources are no longer available. There is concern about the microbiological risks that may be introduced to a crop from the complex consortia of microbiology that is supported in waters exposed to wildlife excretions, anthropogenic pollutants, and sewage runoff – all of which have the potential to support a robust array of enteric pathogens. Groundwater sources (wells) are believed to support fewer enteric pathogens due to natural filtering mechanisms of soils and protection from wildlife pressures(11).There is of course, still ample opportunity for underground water sources to become contaminated due to depth and quality of wells, proximity to urban areas, animal husbandry, industrial pressures, seawater intrusion into aquifers and many other events. It makes sense to assume that cleaner water will result in a safer crop but few, if any studies have provided data that support this assumption. There is currently a data gap surrounding our understanding of food safety risks that may be associated with agricultural use of surface or reclaimed water sources. Studies conducted in California examined crops spray-irrigated with

two types of treated wastewater compared to well water. The treated waters showed higher fecal coliform counts than the well water but no differences could be correlated to the quality of the resulting crop(14).

Drought pressures in agriculture pose significant concerns and highlight the need for the improved understanding of risks that may be associated with all types of available water sources. Water shortages will preclude long term access to low microbial count waters for certain agricultural areas and this situation will only become more dire in the years to come, so the improved understanding of food safety risks that correlate with use of surface or reclaimed waters is valuable to sustainable agriculture and public health. To characterize the risks that may be associated with the use of a "surface" water source (agricultural pond), compared to a ground water source (water from an agricultural well), we described the bacterial microbiota of the pond and the well and the corresponding microbiota of tomato surfaces managed with each water type.

II. MATERIALS AND METHODS

2.1 Tomato crop

Field plots were established at the University of Maryland Wye Research and Education Center in Maryland's Eastern Shore (38˚ 56', 76˚ 07'). The soil was a Nassawango silt loam. 'Juliet' grape tomato transplants were planted in the field on June 2, 2010 on black plastic mulch and trained using stakes and a four-tier string system. No specific permissions were required for collection from these research fields other than the consent of the University of Maryland agricultural research scientists and extension agents who direct the activities of this Wye Research and Education Experiment Station. The field studies did not involve endangered or protected species. The experimental design was a randomized complete block design with five blocks and three treatments. Seedlings were planted in five rows 9 m apart from each other. Within each row, each experimental unit was 9 m from the next. An experimental plot was composed of 5 grape tomato plants at an in-row spacing of 60 cm. Pesticides mixed in either ground or surface water were sprayed on July 26, August 8, August 22, August 30 and September 7. Water from a nearby pond and a well was mixed separately with standard agricultural chemicals and applied to plots with a CO_2-pressurized boom sprayer. A separate sprayer manifold consisting of nozzles, hoses and a tank was used for each treatment. These booms were used throughout the season. Standard agricultural practices for the production of shipping tomatoes in the region were used. When needed, the field plot was trickle irrigated and fertigated (application of fertilizer in water) using well water.

2.2 Sample collection and processing

Water samples were collected directly from the sprayer on July 26th, August 9th, August 22nd and September 7th 2010. After filling the appropriate 3-gallon canister to capacity with treatment water, water was run through the spray manifold before sampling. Spray catch samples were collected directly from one of five nozzles (the other four nozzles were covered), before pesticides were added to the canisters. After treatment application, the canisters and spray manifolds were rinsed out with sample water and stored with lids until the next use. Five replicates of 50 ml (surface water) and 4.75 l (ground water) were collected. Fruit samples consisting of 6 ripe tomato fruits (calyx intact) were aseptically collected a day after treatment application. Fruits were systematically harvested from different locations within the experimental unit and placed in Whirl-Pak® bags by using new gloves for each replicate and ethanol disinfection of pruning shears between samples. Samples were then transported back to the laboratory at 4˚C. Seventy milliliters of phosphate-buffered saline were added to the bags, and samples were agitated for 1 minute by hand and then sonicated for 2 minutes. The microflora wash was then transferred to polypropylene tubes and centrifuged at 30,000 x g for 30 min at 4˚C. The pellet was then transferred to a microcentrifuge tube and stored at -80˚C until DNA extraction was performed. Water samples were filtered through 0.45 µm Fisherbrand® filters (Fisher Scientific, Pittsburgh, PA). Filters were aseptically divided into four microcentrifuge tubes and stored at -80˚C. DNA extraction from filters and pellets was performed using the Zymo Research fungal/bacterial DNA extraction kit (Zymo Research, Orange, CA).

2.3 DNA sequencing

PCR amplification of the 16S rRNA bacterial gene was performed using forward primer Bact-8F (AGAGTTTGATCCTGGCTCAG) [42] and reverse primer UNI518R (ATTACCGCGGCTGCTGG) [16], designed to amplify a 527 base pair long region including variable regions V1, V2 and V3. The forward primer included the fusion primer A (CGTATCGCCTCCCTCGCGCCATCAG) in its 5' end. The reverse primer included the fusion primer B (CTATGCGCCTTGCCAGCCCGCTCAG) in its 5' end, followed by sample specific 10 bp barcodes. Standard PCRs were performed using AmpliTaq Gold LD™ (Applied Biosystems, Foster City, CA) in a 50 µl total volume (1 µl genomic DNA

as template, 1 µM each primer, 200 µM each dNTP, 2 mM MgCl2, 0.60 units AmpliTaq Gold LD, 10 x buffer provided by manufacturer). PCRs used a denaturation step at 95°C for 5 min, followed by 30 cycles of 95°C 1 min, 55°C 1 min, 72°C 1 min, with a final extension step at 72°C for 5 min. Three independent PCR amplifications were performed for each sample and subsequently pooled. After a gel based confirmation of PCR amplification, PCR products were purified using the AMPure XP beads (Agencourt, MA) following manufacturer's recommendations, and quantified using a Qubitflurometer (Life Technologies, CA). PCR products within each of the four dates were pooled and the average fragment size was assessed on a 2100 Bioanalyzer (Agilent, Santa Clara, CA) using a DNA 7500 chip. The pools of PCR products were purified of primer dimers with AMPure XP beads. Emulsion-based clonal amplification and sequencing on the 454 Genome Sequencer FLX-Titanium system were performed at the W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign according to the manufacturer's instructions (454 Life Sciences, Branford, CT). The PCR products were sequenced on five regions of a 16-region 70x75 picotiter plate. Signal processing and base calling were performed using the bundled 454 Data Analysis Software version 2.5.3.

2.4 Sequence processing

To generate an initial high-quality dataset, raw 16S rRNA amplicon sequences and associated quality scores were de-multiplexed and screened for length and quality using the QIIME split_libraries.py tool (3) with a minimum length of 200bp, an ambiguous base limit of 2, a mean quality score of 25 within a 50bp window, a maximum homopolymer of 8bp, and a primer mismatch limit of 2 nucleotides. Reverse primer sequences were identified and removed. Sequences passing preliminary quality screens were subsequently checked for tomato chloroplast and contaminant 18S rRNA gene sequences. Reads were searched using BLASTN (8) against a custom database containing 18S rRNA and chloroplast reference sequences and assigned to taxonomy using the RDP naïve Bayesian classifier (23). Sequences ultimately assigned to chloroplast by the RDP classifier or with a BLAST hit of ≥98.5% identity along ≥100bp were removed. Overall, 32,819 contaminant sequences were identified and removed. After initial pre-processing, 101,893 remaining sequences were organized by sample and time point for input to the CloVR-16S rRNA pipeline.Utilizing the virtual machine player VMware Fusion v4.1.2 (www.vmware.com), we executed the CloVR-16S pipeline v1.1 in CloVR image v1.0-rc3 (1) running on an Apple MacBook Pro with one available core and 2012MB of accessible RAM. The CloVR-16S pipeline was configured and submitted to the DIAG academic cloud (www.diagcomputing.org) to take advantage parallelized components within CloVR-16S. The CloVR-16S pipeline is an automated comparative analysis pipeline capable of performing robust diversity and statistical comparisons of 16S-based samples. Briefly, de-noising and quality filtering is performed in addition to chimera detection using UCHIME (6) with the SILVA rRNA GOLD database (9). Sequences passing quality screens are clustered into species-level OTUs using UCLUST (5)using a 97% identity threshold, and OTU representatives are assigned to a taxonomic lineage by the RDP naïve Bayesian classifier (23) with a 50% confidence minimum. Diversity estimators and rarefaction curves are computing using Mothur(20). Differential abundance analysis is performed on groups of interest using Metastats(16) with default parameters, and additional visualization and clustering was done using custom R scripts. We configured the pipeline to perform comparative analysis of groups by environment or date, as well as the combination of the two. For full details of the CloVR-16S protocol, see the standard operation procedure (1) available at http://precedings.nature.com/documents/6287. The CloVR pipeline screened out 1,144 additional sequences with ambiguous base calls and 16,474 sequences were detected as chimeras. A total of 84,275 sequences with an average length of 429 bp were used in the downstream analysis. The high-resolution Resphera Insight (Baltimore, MD) 16S analysis protocol was also utilized to evaluate the genus and species-level membership among environments.

2.5 Data Submission

All 16S rRNA gene fastq files have been deposited in NCBI SRA associated with Bioproject ID: PRJNA326177

III. RESULTS AND DISCUSSION

Using UCLUST with a 97% identity threshold, we identified 4,815 OTUs across all samples. In general, the microbial profiles for each water source and the tomato surfaces were very distinct from one another (Figure 1A and B). The taxonomy associated with each sample type is shown in Figure 1 A (class) and B (genus). Despite the clearly different taxonomic community structure for each water type, the bacterial profiles of the surfaces of the tomato carposphere (phyllosphere) were very similar. Rarefaction curves demonstrated that the highest bacterial diversity was associated with surface water (WS - purple) samples, compared to ground water (WG - blue) and phyllosphere samples managed with both ground and surface

water (PG - red&PS - green) (Figure1). Surface water supported approximately 400 to 500 species (OTUs at 97% similarity) per 1000 sequences sampled. Ground water (WG) supported about 100 to 150 OTUs per 1000 sequences and phyllosphere samples supported approximately 150 to 200 OTUs per 1000 sequences (Figure 2).

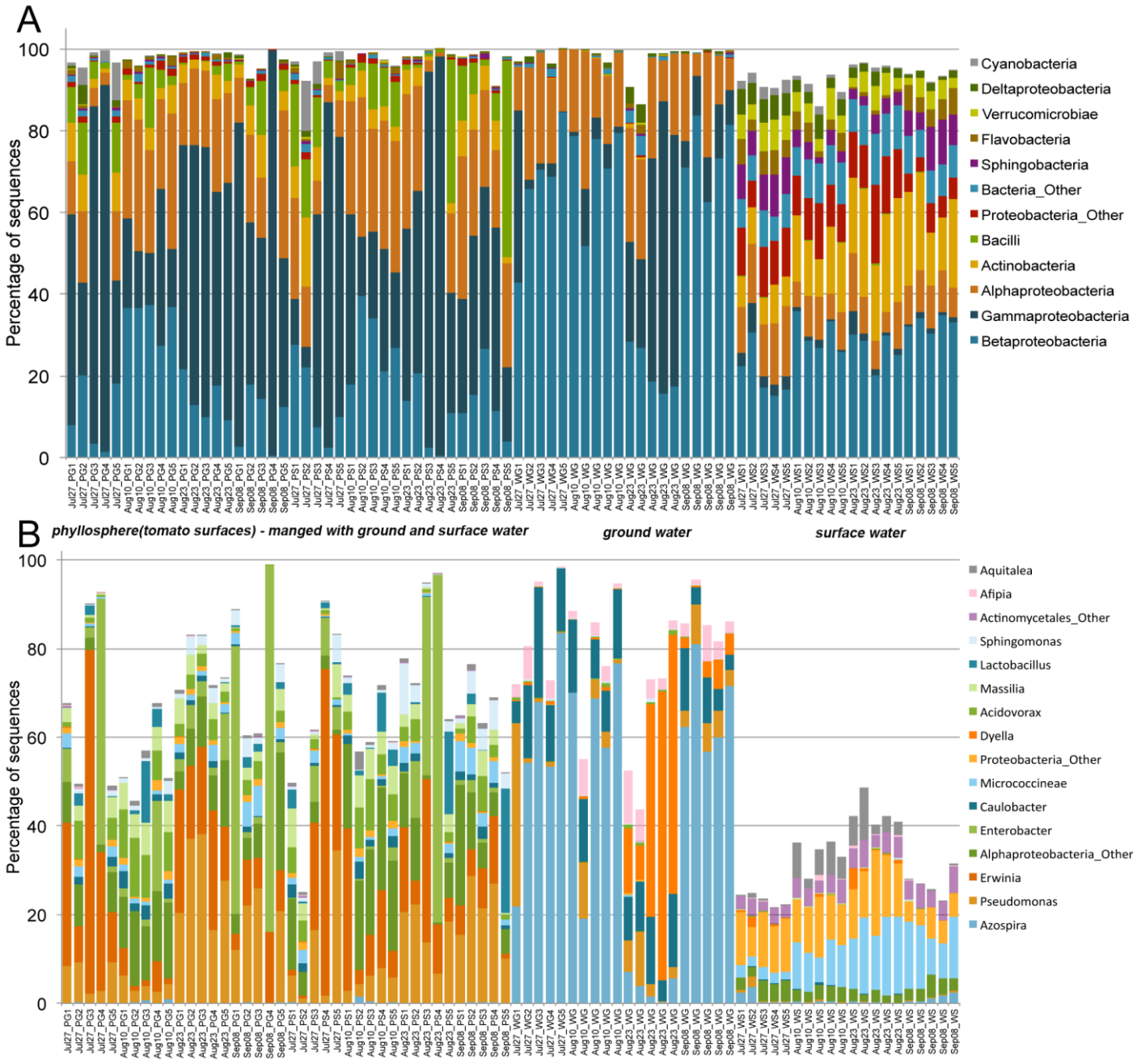

FIGURE 1. DISTRIBUTION OF PREVALENT TAXONOMIC MEMBERS ACROSS SAMPLES.

For (A) class-level assignments and (B) genus-level assignments. The percentage of sequences assigned to each taxon is shown along the y-axis. Phyllosphere and ground water samples (PG & PS) were dominated by Proteobacteria, while surface water (WS) communities were much more diverse, and contained higher levels of Actinobactera, Sphingobacteria, and Verrucomicrobiae. *Pseudomonas* and *Erwinia* were well represented in phyllosphere samples (PG & PS), and *Azospira* and *Afipia* were prominent in ground water samples (WG). Taxonomic structure is distinct by environment.

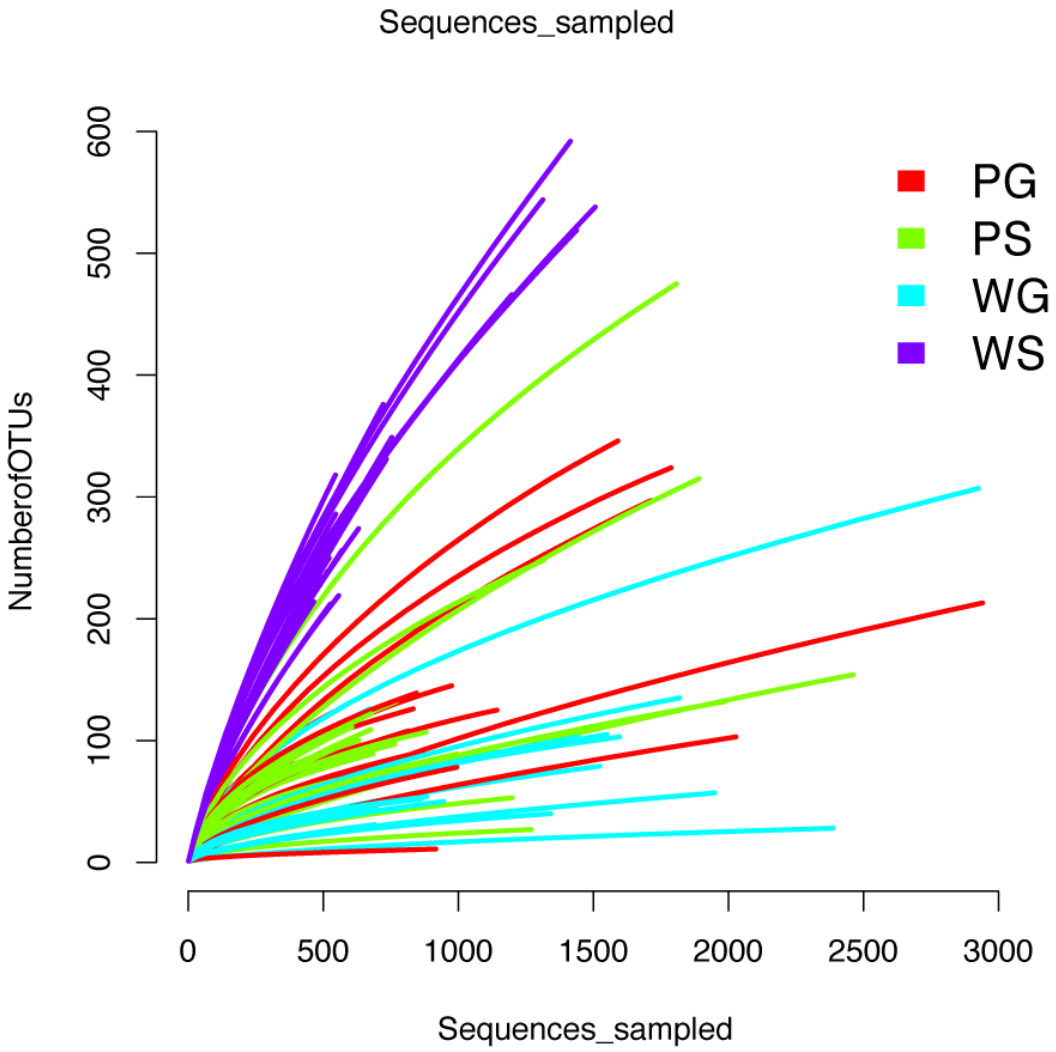

FIGURE 2. RAREFACTION ANALYSIS OF PHYLLOSPHERE AND WATER SAMPLES.

(A) Using species-level OTUs clustered at 97% identity, the diversity for each sample type is shown by number of OTUs per sequences sampled. Surface water (WS) samples tended to be the most diverse. There was considerable overlap in the number of OTUs observed in the other three environments (PS, PG, and WG).

The observation that microbial communities from both water sources (surface and ground) were distinct but that those differences did not persist in carposphere (phyllosphere) samples is also clearly evident in the ordinations created from unweighted unifrac clustering, and Bray Curtis dissimilarity. All methods of plotting the data supported the observation that surface water and ground water form distinct clusters, but phyllosphere (tomato surface) samples – despite being managed with different water sources cannot be separated by this treatment (Figure 3A and B).

The most abundant taxa observed in surface water samples (WS) were members of Micrococcineae, Proteobacteria and Actinomycetales. For ground water (WG) *Azospira* and *Caulobacter*were the most dominant genera. None of these communities appears to have been directly transferred to phyllosphere samples (PS & PG) whichwere primarily dominated by commonly observed genera in phyllosphere datasets such as; *Erwinia*, *Pseudomonas*, *Enterobacter* and an Alphaproteobacteria taxon (Figure 1 B) (2, 10).

No OTUs were universally present in all samples and only five otus were present in at least one sample from each time point and each environment (water sources and tomato phyllospheres). Those were classified as: *Cupriavidus*, (Burkholderiaceae); *Caulobacter*, (Caulobacteraceae); *Pseudomonas*, (Pseudomonadaceae); *Pelomonas*,(Comamonadaceae); and *Ralstonia*, (Ralstoniaceae).

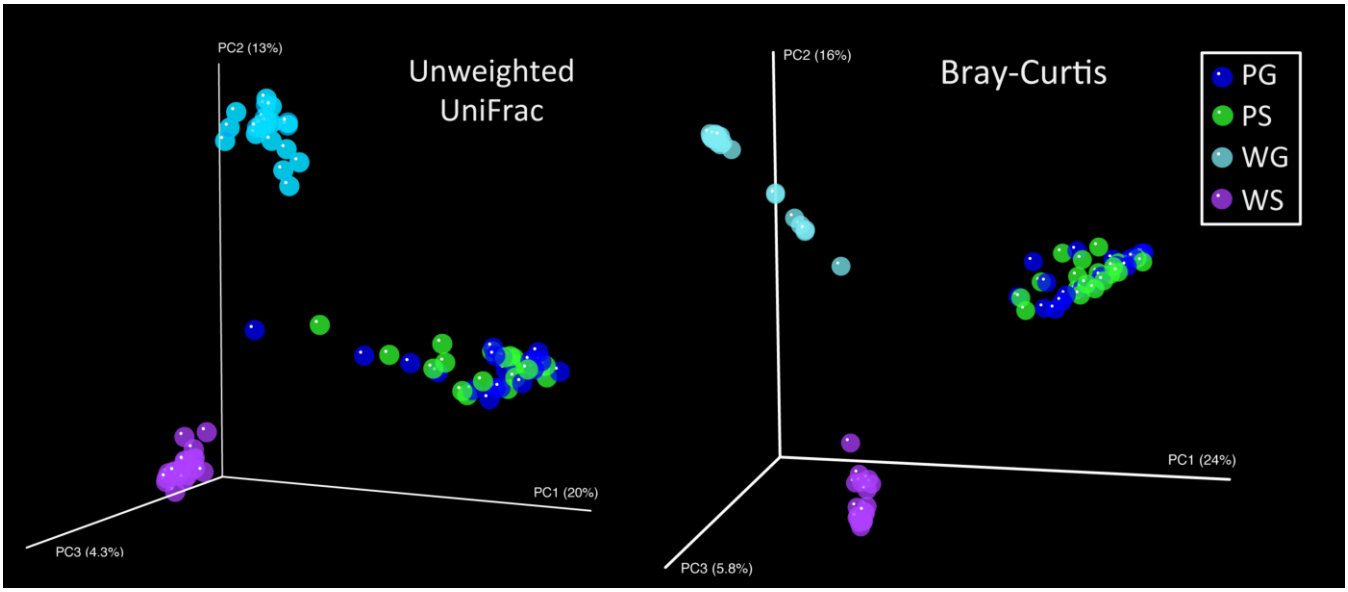

FIGURE 3. BRAY CURTIS AND UNWEIGHTED UNIFRAC PCOA

Using the UniFracunweighted distance metric, and Bray Curtis dissimilarity metric, ground water WG (light blue), surface water WS (purple), and tomato phyllosphere samples cluster distinctly. Phyllosphere samples treated with different water sources PG (blue) and PS (green) are not easily differentiated.

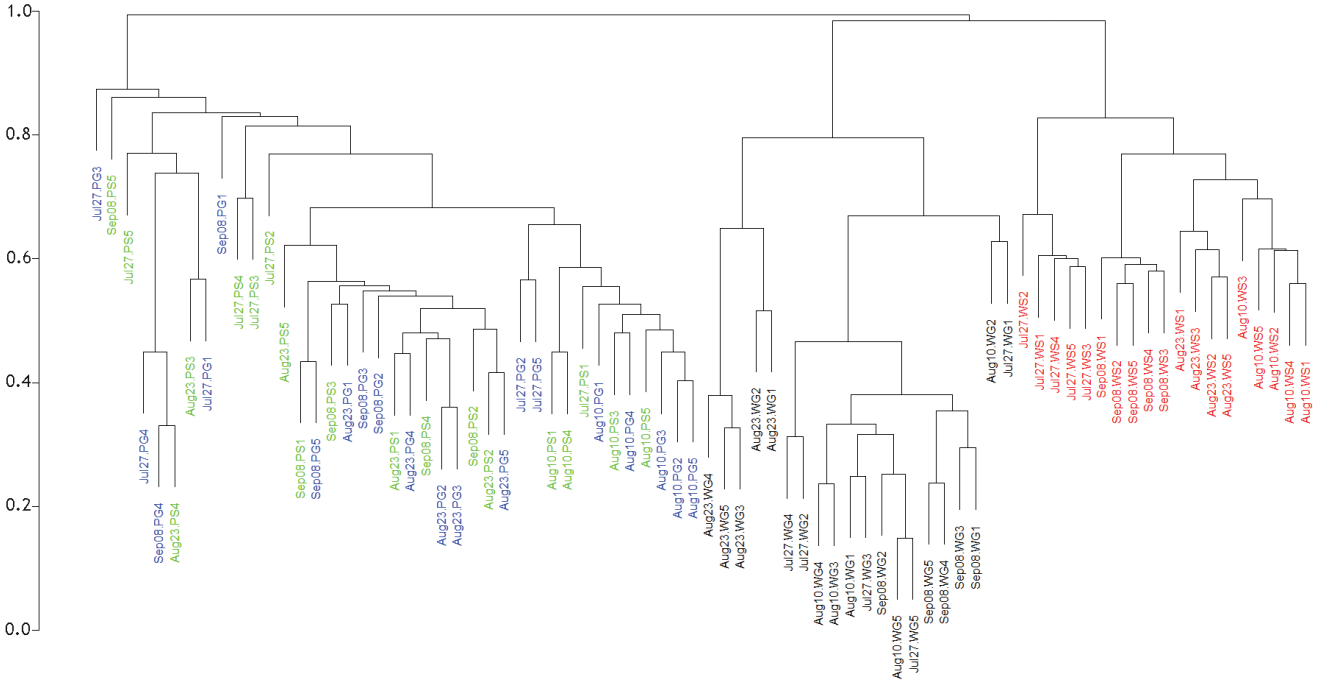

FIGURE 4. DENDROGRAM OF COMMUNITY RELATEDNESS FOR PG, PS, WS AND WG SAMPLES.

As with all other methods of describing the taxonomic diversity and community structure of the phyllosphere communities (tomato surfaces) managed with surface and ground water (PS green &PG blue) and the water sources themselves (WG black and WS red) –there is distinct clustering of each environment -without the water treatments impacting the phyllosphere environment.

To further examine the possibility of bacterial species from water environments being introduced to the crop environment, we used the Resphera Insight pipeline(Baltimore, MD), which has been optimized to assign taxonomy to species such as *S. enterica*. No sequences in the dataset from any sample type were assigned to *S. enterica* but a few species with emerging or opportunistic health implications were identified, such as *Brevundimonas vancanneytii* in ground water (WG), and *Massilia timonae* and *Cupriavidus gilardii* from phyllosphere samples (PS, PG).

In most cases, incidence of specific taxonomic groups, as previously observed in taxonomic histograms (Figure 1A and 1B), appeared to be driven by the environment. The phyllosphere environment could not be separated by the treatments of surface and ground water. In general, species observed in water environments, were not present in phyllosphere samples. It was difficult to document bacterial species that co-occurred in multiple environments. At the genus level – for example; *Pseudomonas* and *Cupriavidus*, representation was spread across multiple environments: phyllosphere , surface water, and ground water. However when species-specific identity could be assigned as in the case of *Pseudomonas psychrotolerans* and *Cupriavidus gilardii*, then incidence was no longer shared across multiple environments, but restricted to a single environment.

Using a similar experimental design to the study presented here, we previously established that bacterial communities on tomato surfaces treated with surface and ground water could not be differentiated by a variety of statistical methods – despite the fact that each water source (surface and ground) had its own distinct microbial profile(21). Those results were based on one single time-point and thus there were limits to the conclusions that could be drawn. The work presented here, demonstrates the same phenomenon at four distinct time-points spanning a complete growing season. These findings provide additional support for the observation that water sources used to manage field crops do not appear to represent the most significant drivers of plant surface microbiology and thus may not represent the most significant risks for introduction of bacterial pathogens to crops. There is evidence in the literature that weather events and geography as opposed to farming practices are the most influential drivers of phyllosphere microbial ecology(4, 13, 15, 18, 19, 22). The most important risk determinants for the contamination of a lettuce crop in the Mid-Atlantic were shown to be seasonality and weather events(12). Indeed, this trend has been well established for human disease, so it is not at all surprising that the same phenomenon might be true for agriculture(17). We certainly do not wish to suggest that water quality is not important for agriculture. Water clearly has the potential to vector both human and plant pathogens. It is possible that our data did not provide significant resolution to observe human pathogens if they were present and perhaps our sequencing depth may not have been sufficient to describe important low abundance taxa.

IV. CONCLUSION

Despite the aforementioned caveats, we demonstrated with confidence that the primary drivers of the bacterial microbiology observed in the tomato carposphere (phyllosphere) was not significantly influenced by water sources as diverse as well water and agricultural pond water. As we continue to evolve guidance recommendations and regulations for crop safety, it makes sense to continue monitoring microbial loads in agricultural waters but also perhaps to include additional surveillance of the microbial loads associated with air pressures that come in contact with crops, as these may represent the primary drivers of carposphere surface microbiota.

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REFERENCES

[1] Angiuoli, S., M. Matalka, A. Gussman, K. Galens, M. Vangala, D. Riley, C. Arze, J. White, O. White, and W. F. Fricke. 2011. CloVR: A virtual machine for automated and portable sequence analysis from the desktop using cloud computing. BMC bioinformatics 12:356.

[2] Balint-Kurti, P., S. J. Simmons, J. E. Blum, C. L. Ballare, and A. E. Stapleton. 2010. Maize leaf epiphytic bacteria diversity patterns are genetically correlated with resistance to fungal pathogen infection. Mol Plant Microbe Interact 23:473-84.

[3] Caporaso, J. G., K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen, and R. Knight. 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26:266-7.

[4] Copeland, J. K., L. Yuan, M. Layeghifard, P. W. Wang, and D. S. Guttman. 2015. Seasonal Community Susscession of the Phyllosphere Microbiome. Molecular Plant Microbe Interactions 28:274-285.

[5] Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460-1.

[6] Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics.

[7] Greene, S. K., E. R. Daly, E. A. Talbot, L. J. Demma, S. Holzbauer, N. J. Patel, T. A. Hill, M. O. Walderhaug, R. M. Hoekstra, M. F. Lynch, and J. A. Painter. 2008. Recurrent multistate outbreak of Salmonella Newport associated with tomatoes from contaminated fields, 2005. Epidemiology and Infection 136:157-165.

[8] Group, N. A. T. T. W. 2008, posting date. Commodity specific food safety guidelines for the fresh tomato supply chain 2nd. [Online.]

[9] Gu, L., Z. Bai, B. Jin, Q. Hu, H. Wang, G. Zhuang, and H. Zhang. 2010. Assessing the impact of fungicide enostroburin application on bacterial community in wheat phyllosphere. J Environ Sci (China) 22:134-41.

[10] Kinkel, L. L. 1997. Microbial population dynamics on leaves. Annu Rev Phytopathol 35:327-47.

[11] Lopez-Velasco, G., G. E. Welbaum, R. R. Boyer, S. P. Mane, and M. A. Ponder. 2011. Changes in spinach phylloepiphytic bacteria communities following minimal processing and refrigerated storage described using pyrosequencing of 16S rRNA amplicons. J Appl Microbiol 110:1203-14.

[12] Marine, S. C., S. Pagadala, F. Wang, D. M. Pahl, M. V. Melendez, W. L. Kline, R. A. Oni, C. S. Walsh, K. L. Everts, and R. L. Buchanan. 2015. The growing season, but not the farming system, is a food safety risk determinant for leafy greens in the mid-Atlantic region of the United States. Applied and environmental microbiology 81:2395-2407.

[13] Moulas, C., C. Petsoulas, K. Rousidou, C. Perruchon, P. Karas, and D. G. Karpouzas. 2013. Effects of systemic pesticides imidacloprid and metalaxyl on the phyllosphere of pepper plants. BioMed research international 2013.

[14] Nisiotou, A. A., K. Rantsiou, V. Iliopoulos, L. Cocolin, and G. J. Nychas. 2011. Bacterial species associated with sound and Botrytis-infected grapes from a Greek vineyard. Int J Food Microbiol 145:432-6.

[15] Osono, T., and A. Mori. 2005. Seasonal and leaf age-dependent changes in occurence of phyllosphere fungi of giant dogwood. Mycoscience.

[16] Ottesen, A. R., J. R. White, D. N. Skaltsas, M. J. Newell, and C. S. Walsh. 2009. Impact of Organic and Conventional Management on the Phyllosphere Microbial Ecology of an Apple Crop. Journal of Food Protection 72:2321-2325.

[17] Patz, J. A., D. Campbell-Lendrum, T. Holloway, and J. A. Foley. 2005. Impact of regional climate change on human health. Nature 438:310-317.

[18] Perazzolli, M., L. Antonielli, M. Storari, G. Puopolo, M. Pancher, O. Giovannini, M. Pindo, and I. Pertot. 2014. Resilience of the Natural Phyllosphere Microbiota of the Grapevine to Chemical and Biological Pesticides. Applied and Environmental Microbiology 80:3585-3596.

[19] Polymenakou, P. N. 2012. Atmosphere: A Source of Pathogenic or Beneficial Microbes? Atmosphere 3:87-102.

[20] Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn, and C. F. Weber. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537-41.

[21] Telias, A., J. White, D. Pahl, A. Ottesen, and C. Walsh. 2011. Bacterial community diversity and variation in spray water sources and the tomato fruit surface. BMC microbiology 11:81.

[22] Thompson, I. P. B. M. J. F., J.S.; Fermor, T.R.; Lilley, A.K.; Lynch, J.M.; McCormack, P.J.; McQuilken, M.P.; Purdy, K.J.; Rainey, P.B.; Whipps, J.M. 1993. Quantitative and qualitative seasonal changes in the microbial community from the phyllosphere of sugar beet (Beta vulgaris). Plant and Soil:177-191.

[23] Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73:5261.