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Bacterial Communities on Wheat Grown Under Long-Term Conventional Tillage and No-Till in the Pacific Northwest of the United States

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ABSTRACT

Cultural practices, such as tillage, often have widespread impacts on phytobiomes. No-till has been increasingly adopted by wheat growers in the dryland cropping areas of the inland Pacific Northwest in the United States to reduce soil erosion and decrease fuel and labor inputs, yet there are limited data on how conversion to no-till impacts plant-associated bacteria in this highly productive system. To address this knowledge gap, we evaluated bacterial communities in bulk and rhizosphere soil of wheat in two locations (Idaho and Washington) for 2 years, comparing long-term no-till plots and adjacent plots under conventional tillage. In this study, members of phylum Proteobacteria were relatively more abundant in rhizosphere soil, while Acidobacteria and Gemmatimonadetes were more abundant in bulk soil than in the rhizosphere. Bacteroidetes were more frequent under conventional than conservation tillage. In general, bacterial

families were more affected by the position of the sample (rhizosphere versus bulk soil) than by tillage practices. Families generally regarded as copiotrophic (Oxalobacteriaceae, Pseudomonadaceae, and Cytophagaceae) were more abundant in rhizosphere soil than bulk in both years. On the contrary, oligotrophic families such as Gaiellaceae and those within Gemmatimonadetes were more abundant in bulk soil than in the rhizosphere. Families affected by tillage varied between the 2 years. These results suggest that bacterial communities in soil were more influenced by plant proximity (rhizosphere versus bulk soil) than by tillage practices, but that specific differences were not consistent and may vary among locations and years.

Additional keywords: agriculture, crop, management, microbiome, soil, soil ecology.

The phytobiome consists of all microbes inside and outside of the plant, the soil around the plant, and other organisms in contact with and influenced by the plant including insects, invertebrates, and nematodes. In addition, the phytobiome also includes the physical and chemical environments which provide a crucial context for biotic interactions. However, we have limited understanding of ways in which real-life management components of agriculture influence biotic interactions in the phytobiome.

Tillage, a hallmark of most agricultural systems over the last 8,000 years, is used to prepare a seed bed and manage weeds and

has vast influences on biotic and abiotic components of the phytobiome and their functioning. Conventional tillage (CT) disrupts the soils and promotes the breakdown of plant residues, increases soil erosion and degradation, accelerates soil organic matter oxidation, and disrupts soil aggregate stability (Calegari et al. 2008; Ding et al. 2002; Govaerts et al. 2006). Over the last 30 years, there has been an increased adoption of reduced tillage or no-till (NT), whereby seeds are planted directly into the residue of the previous crop (Derpsch et al. 2010). In addition to conserving soil, no-till can conserve moisture, alter soil temperatures and increase soil organic matter (Calegari et al. 2008; Lal et al. 2007). Thus, conversion to no-till is expected to result in a significant restructuring of bacterial community composition and diversity in soil and in the rhizosphere of host plants (Dorr de Quadros et al. 2012; Fierer et al. 2012).

Studies exploring tillage effects on soil microbes have reported contradictory findings. No studies have examined the interaction between tillage and rhizosphere versus bulk soil communities, as we have in our study. Numerous studies have documented a significant effect of

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tillage on soil microbial biomass, activity, composition, and diversity (Bissett et al. 2011; Ghimire et al. 2014; Gonzalez-Chavez et al. 2010; Govaerts et al. 2007, 2008; Hungria et al. 2009). However, others have reported little significant impact of tillage practices on microbial biomass or bacterial communities (Frey et al. 1999; Helgason et al. 2010; Jangid et al. 2011; Jiang et al. 2011; Wortmann et al. 2008). The inconsistency of these findings may be due to variation in microbial responses to tillage among sites, years, or differences in the methods used to characterize microbial communities. Most previous work was based on methods that have limited ability to resolve phylogenetic or taxonomic differences between microbial populations. With the development of high-throughput sequencing-based approaches, we can now acquire a more detailed snapshot of the phylogenetic composition of microbial communities. For instance, Acosta-Martinez et al. (2008) used pyrosequencing to find that Bacteroidetes were more predominant in agricultural soils (cotton–wheat–corn rotations) compared with the same soils under nondisturbed conditions. Acidobacteria subgroup GP2 were more frequent in a no-till system than in one subjected to tillage on a Kansas farm with a wheat-soybean rotation (Yin et al. 2010). Actinobacteria and Chloroflexi were more abundant in conventional farming with wheat, alfalfa, oats and flax (Li et al. 2012). The relative abundance of Actinobacteria, Betaproteobacteria, and Gammaproteobacteria was affected by tillage and correlated to the total organic carbon and clay content in soil, using pyrosequencing in a maize–wheat rotation (Navarro-Noya et al. 2013). A more recent study using pyrosequencing reported that no-till treatments had five unique phyla compared with four unique phyla in the plowed treatment, but that 99% of the operational taxonomic units (OTUs) had an abundance <1% (Sengupta and Dick 2015). Although it is widely accepted that tillage practices have the potential to change microbial community characteristics, these effects are likely complex and may vary between locations and among years. Therefore, research is needed on how agricultural management practices, such as tillage, impact microbial communities that encompass the spatial and temporal variability in soil community responses.

The rhizosphere represents a unique physical and chemical environment in direct proximity to plant roots that often harbors microbial communities distinct from those of the surrounding bulk soil community (York et al. 2016). Whereas soil physical and chemical parameters are the main determinants of bacterial communities in bulk soil, plant secretions (root exudates, mucilage, etc.) are considered primary drivers of microbial activity, composition, and diversity in the rhizosphere (York et al. 2016). Thus, even though management practices may modify bulk soil communities, the plant may compensate for these changes by recruiting a consistent suite of rhizosphere inhabitants regardless of differences in the bulk soil community. Do differences in bacterial communities between tillage practices within the bulk soil result in distinct rhizosphere communities, or does the strong organizing effect of the rhizosphere select for consistent rhizobacterial communities regardless of tillage types? Does tillage tend to homogenize communities and at what scales? Moreover, because of reduced soil disturbance under no-till, are differences between bulk and rhizosphere soil communities exacerbated in no-till systems, strengthening the effect of plant proximity in no-till systems? Understanding the interactive effects of tillage practices and plant proximity in structuring bacterial communities in field settings will be crucial for managing microbiomes in agroecosystems.

The Palouse region of eastern Washington and northern Idaho, United States, includes some of the most productive wheat cropping systems in the world, where wheat and other cereals are commonly grown in a 3-year rotation (winter wheat/spring wheat or spring barley/grain legume [pea, lentil, or chickpea]). No-till practices are especially attractive for growers in this region due to a significant reduction in soil erosion and reduction of fossil fuel inputs by reducing the number of

machinery passes on the field (Huggins and Reganold 2008; Schillinger et al. 2006), yet the effect of the adoption of no-till on soil communities has not been evaluated in Palouse agroecosystems. Here, we characterize the composition and diversity of bacterial communities from multiple locations across 2 years in side-by-side long-term conventional and no-till cereal plots. Further, we attempt to partition out the relative roles of tillage practices and plant selection in structuring soil bacterial communities by examining both rhizosphere and bulk soil communities under different tillage regimes.

MATERIALS AND METHODS

Study site, sample collection, and preparation. This study was conducted at two farm locations. The first location was at the USDA-ARS Palouse Conservation Farm (PCFS), Albion, WA. This site, about 3 miles NW of Pullman, has been in no-till management for 35 years (PCFS_NT). The crop rotation is winter wheat/spring wheat (*Triticum aestivum* L.); the soil is a Palouse silt loam, pH 5.6, with 3.36% organic matter. Next to this was a 55 × 55 m² plot that was conventionally tilled in a winter wheat/fallow rotation, as part of a disease assessment nursery (PCFS_CT). The soil was fall chiseled, spring-disked, and rod weeded three to five times during the fallow years. Two treatments were defined in this site: no-till (PCFS_NT) and conventional tillage (PCFS_CT). The second location was the Kambitsch Farm (KF), north of Genesee, ID. Replicated no-tillage and conventional tillage plots have been compared at this location since 2000, as part of the Solutions to Environmental and Economic Problems (STEEP) program and REACCH project (Regional Approaches to Climate Change for Pacific Northwest Agriculture). The crop rotation was winter wheat–spring wheat or spring barley (*Hordeum vulgare* L.)–grain legume (pea, lentil, or chickpea). The predominant soil type is also Palouse silt loam with an average pH of 5.6, 1.78% organic C, and 0.12% total N within the top 10 cm (Johnson-Maynard et al. 2007). Two treatments were defined in this location: no-till (KF_NT) and conventional tillage (KF_CT). All samples were taken from the winter wheat phase of the rotation in April to May 2008 and 2010 in both locations. There were four replicate blocks for each treatment for both locations, and soil was collected from each replicate, although only three were analyzed with pyrosequencing. For bulk soil, approximately 1 kg of soil pooled from three randomly chosen locations in the plot was taken from the top 10 cm in the root zone within the row. For rhizosphere soil, 5 to 10 plants from three randomly chosen locations in the plot were taken with intact roots. Roots with attached rhizosphere soil from wheat plants were cut from the shoot and placed in a 50-ml plastic tube containing 20 ml of sterile distilled water. The tubes were vortexed for 1 min and sonicated for 1 min, the root was removed, and the liquid was stored at –20°C for further use.

DNA extraction, purification, and sequencing. For bulk soil, DNA was extracted with an UltraClean Soil DNA Kit (MO BIO Laboratories, CA) as described previously (Yin et al. 2013). For rhizosphere soil, 2 ml of the root wash was used for DNA extraction with the alternative protocol of the UltraClean Soil DNA Kit for wet soil samples. The DNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and stored at –20°C for subsequent procedures.

The 16S rRNA genes were amplified from bulk or rhizosphere soil DNA with primers listed in Supplementary Table S1. For the 2008 samples, primers were designed to amplify the V3 hyper-variable region of bacterial 16S rRNA gene as described previously (Yin et al. 2013). For 2010 samples, primers were designed to amplify the V1 to V3 hypervariable regions of 16S rRNA gene. PCR reactions consisted of 2 ng of soil DNA, 1× reaction buffer, 0.2 mM deoxynucleotides,

0.25 μ M of each primer, 5 μ l DMSO, and 0.125 U of *Taq* DNA polymerase (Invitrogen Corp., CA) in a total volume of 25 μ l. PCR was performed using a hot start program: 80°C for 3 min; 94°C for 5 min; followed by 30 cycles of 94°C for 30 s, 55 to 60°C for 30 s, 72°C for 30 s; and a final extension at 72°C for 7 min. Each soil DNA template was amplified in five to six separate individual reactions. The presence of amplicons was confirmed by gel electrophoresis on a 1.5% w/v agarose gel and only specific amplification products were pooled. PCR products were quantified using a GelDoc quantification system (BioRad). Equimolar amounts (100 ng) of the amplicons of each sample were mixed in a single tube. PCR products were cleaned using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions for pyrosequencing.

Sequence analysis. DNA amplicons of the 2008 samples were sequenced as previously described (Yin et al. 2010). The DNA amplicons of the 2010 samples were sequenced on the genome sequencer (GS) FLX 454 pyrosequencer (Roche) at the University of Idaho. Pyrosequencing reads were trimmed to 450 flows, and denoised using the shhh.flows command in Mothur (v1.25.0) using default settings. Denoised fasta files were quality filtered to remove barcodes/primers and sequences less than 100 bp for 2008 samples and 200 bp for 2010 samples, homopolymers >6 bp, >6 ambiguous bases, and >2 primer mismatches. Open-reference OTU picking with subsampling was performed with pick_open_reference_otus.py in QIIME (v1.9.1) using a 97% similarity cutoff and the green-genes gg_13_8_otus as a reference database. OTUs were classified with the UCLUST algorithm using the gg_13_8 reference database. Chimeric OTUs were identified with ChimeraSlayer and removed, along with any nonbacterial OTU or those classified as mitochondria or chloroplasts. The OTU table was subsampled to 1,802 sequences/sample for 2008 samples and 1,200 sequences/sample for 2010 samples prior to analyses. The sequencing reads were deposited in the GenBank short-read archive under NCBI BioProject number PRJNA218469.

Statistical analysis. Because of the differences in amplification and sequencing platforms between the 2 years, data for each year was examined separately. The shifts in overall microbial community composition were tested across root proximity, tillage, and locations using permutational multivariate analysis of variance (PERMANOVA) implemented in the “Adonis” function in the vegan package in R (Anderson 2001). Community composition was represented by nonmetric MDS (NMDS) based on Bray-Curtis distances (Oksanen et al. 2015). Multiple comparisons were performed with least significant means using JMP Pro 11 (SAS Institute, Cary, NC).

Fig. 1. Relative abundances of bacterial phyla in soil derived from the 2 years (2008 and 2010). Sequences in 3% operational taxonomic units were classified and grouped at the phylum level. The frequency of group sequences <1% were not included.

Significance was accepted at an α level of 0.05. Heatmaps were generated using an R script. Microbial diversity was evaluated using Shannon diversity, richness, and Simpson's diversity in R.

RESULTS

Bacterial community composition. Pyrosequencing yielded a total of 75,623 sequences in the 2008 samples, providing 1,802 to 4,939 sequences (138 ± 22 bp in length) for each sample, which were binned into 4,445 operational taxonomic units (OTUs) belonging to 279 bacterial families. A total of 70,801 sequences were in the 2010 samples, providing 1,200 to 3,727 sequences (265 ± 9 bp in length) for each sample, which were binned into 4,338 OTUs belonging to 261 bacterial families. The soil in 2008 was dominated by phyla Proteobacteria (41.9%), Actinobacteria (22.0%), Gemmatimonadetes (13.1%), Bacteroidetes (8.7%), and Acidobacteria (8.4%) (Fig. 1). In 2010, the most abundant taxonomic group was Actinobacteria (39.4%), followed by Proteobacteria (30.9%), Bacteroidetes (9.6%), and Acidobacteria (6.4%) (Fig. 1). Phyla that were rare but found in all samples included Chloroflexi (2.5%), TM7 (2.02%), and Verrucomicrobia (1.4%) in 2008. Infrequent phyla in 2010 included Chloroflexi (3.6%), TM7 (2.4%), Verrucomicrobia (1.4%), and Planctomycetes (1.6%) (Fig. 1).

Bacterial community composition varies with root proximity, tillage treatment, and location. Root proximity, tillage and location all significantly impacted bacterial community structures at the CTU level in both years (Table 1, Fig. 2). The effects of treatments on bacterial community structure in 2008 were much more apparent than in 2010 (Fig. 2A and B). In particular, in 2008 there was a clear differentiation in communities by location and between tillage practices. However, the effect of tillage on bacterial community structure depended on the sampling location, and was much stronger at PCFS than at KF. Further, within each location $\times$ tillage treatment in 2008, rhizosphere communities clustered separately from bulk soil communities. Intriguingly, at KF, communities in the plant rhizosphere appeared to be less impacted by tillage than those at PCFS. Although location, tillage, and root proximity were significant factors, patterns in bacterial community structure in 2010 were less apparent. Moreover, the relative impacts of tillage and root proximity in 2010 varied among locations. For example, at KF, there was little clear clustering of bacterial communities by either tillage type or root proximity, suggesting relatively less variability in community composition at this location. In contrast, at PCFS, communities from bulk soil were distinct

TABLE 1
Permutational multivariate analysis of variance results testing bacteria community responses for treatments (root proximity, tillage, and location)^z

Factor	2008		2010	
	r^2	P	r^2	P
Tillage	0.103	0.001	0.066	0.001
Location	0.130	0.001	0.087	0.001
Root proximity	0.109	0.001	0.077	0.001
Tillage $\times$ location	0.076	0.002	0.053	0.040
Tillage $\times$ root proximity	0.043	0.070	0.046	0.145
Location $\times$ root proximity	0.047	0.030	0.071	0.001
Tillage $\times$ location $\times$ root proximity	0.044	0.063	0.046	0.139

^z Root proximity: bulk soil versus rhizosphere soil; tillage: no-till versus conventional tilling; and location: Palouse Conservation Farm versus Kambsch Farm. Bold numbers indicate significance ($P < 0.05$). P values are based on 999 permutations.

from rhizosphere communities and clustered by tillage. However, PCFS rhizosphere communities in 2010 were less variable than bulk soil communities and did not cluster distinctly by tillage type. Together, these findings suggest that the relative importance of tillage and root proximity in structuring bacterial communities can vary with location and with year. Although the impacts of tillage and root proximity on bacterial community structure varied with location and year, there were no significant interactions between tillage treatment and root proximity (Table 1), indicating that bacterial communities did not differ consistently with root proximity in different tillage treatments. For example, although rhizosphere communities differed between tillage treatments at PCFS in 2008, there were no clear patterns at KF in 2008 or in either location in 2010 (Fig. 2). Thus, in some location–year combinations, differences in bulk soil communities between tillage treatments are also reflected by differences in rhizosphere communities, though in other locations or years, the plant rhizosphere effect may overcome tillage-induced variation in community composition (e.g., KF in 2008 and PCFS in 2010), or other uncharacterized variables may overwhelm both tillage and rhizosphere effects on bacterial communities (e.g., KF in 2010). These results highlighted the need for future work examining management impacts on bacterial communities to evaluate greater spatial and temporal ranges, rather than site- or time-specific snapshots. Trends apparent in one location at one time may not be indicative of

trends across time or large areas, especially when there is a wide range of climatic, precipitation, soil types, and cropping systems across biogeographical zones.

Bacterial family distribution as affected by root proximity, tillage treatment, and location. Multiple comparisons were performed to determine which bacterial families were differentially abundant across root proximity, tillage management, and location. The most abundant families across all soil samples in 2008 were Sphingomonadaceae, Sphingobacteriaceae, and Chitinophagaceae (Fig. 3; Supplementary Table S2). Some bacterial families were significantly influenced by plant proximity (rhizosphere versus bulk soil). For example, Sphingobacteriaceae were more frequent in rhizosphere soil than in bulk. The relative abundance of Chitinophagaceae was lower in rhizosphere soil compared with bulk soil, but was particularly higher in conventional tillage soil. Similar to Sphingobacteriaceae, Xanthomonadaceae were more frequent in rhizosphere soil compared with bulk soil. The most abundant families in the 2010 samples were Gaiellaceae, Sphingomonadaceae, Geodermatophilaceae, Oxalobacteraceae, and Xanthomonadaceae (Fig. 4; Supplementary Table S3). Gaiellaceae and Sphingomonadaceae were more abundant in bulk soil compared with rhizosphere soil. Conversely, Oxalobacteraceae were more abundant in rhizosphere soil. Families that were more abundant in bulk soil compared with rhizosphere in both years included Gaiellaceae, Gemmatimonadetes-N1423WL-unclassified, Gemmatimonadetes-Ellin5290-unclassified, Acidobacteria-iii1-15-unclassified, and Betaproteobacteria_SC-I-84_unclassified, while some families were more abundant in bulk soil in one year only (Figs. 3 and 4). In contrast, families Oxalobacteraceae, Pseudomonadaceae, and Cytophagaceae were more abundant in rhizosphere soil in both years. Similarly, some families were more abundant in rhizosphere soil in one year only (Figs. 3 and 4).

The effect of tillage on the family level was shown in Figures 3 and 4. Families affected by tillage varied between the 2 years. For instance, in 2008, Hyphomicrobiaceae, Koribacteraceae, and Acidobacteriaceae were relatively more abundant in the no-till soil; while Micrococcaceae, Gaiellaceae, Nocardiodaceae, and Streptomycetaceae were relatively more abundant in the CT soil (Fig. 3). Compared with 2008, in 2010 families such as Conexibacteraceae and Ellin6075 were relatively more abundant in the no-till soil and Xanthomonadaceae and Gemmatimonadetes-unclassified was more abundant in the conventional tillage soil (Fig. 4).

Location also affected the relative abundance of bacterial families. Family Ellin6075 was relatively more abundant in PCFS soil in both years, while Geodermatophilaceae was more frequent in KF soil compared with PCFS soil (Figs. 3 and 4). In addition, some families were relatively more abundant in PCFS in 2008 including Chitinophagaceae, Microbacteriaceae, Sinobacteraceae, and Cytophagaceae; but families Sphingomonadaceae, Micrococcaceae, Gaiellaceae, and Bradyrhizobiaceae were more abundant in KF soil. Similarly, families Acidobacteriaceae and Nakamurellaceae were more abundant in PCFS soil in 2010 only. In contrast, Propionibacteriaceae, Pseudonocardaceae, and Kineosporiaceae were more abundant in KF soil.

Bacterial phyla distribution as affected by root proximity, tillage treatment, and location. The relative abundance of bacterial phyla varied among root proximity, tillage management and farm location treatments and were not consistent. The differences were analyzed by performing multiple comparisons in JMP. The results showed that plant proximity and location affected bacterial phyla distribution (Fig. 5). Phyla Acidobacteria and Gemmatimonadetes were significantly more abundant in bulk soil than in rhizosphere in both years (Fig. 5A and B). Acidobacteria were much more abundant at PCFS compared with KF in 2008 soil, while Gemmatimonadetes were higher at KF than PCFS (Fig. 5A).

Fig. 2. Two-dimensional nonmetric multidimensional scaling (NMDS) ordination plots of bacterial community structure based on Bray-Curtis distances. **A**, NMDS derived from 2008 samples; and **B**, NMDS derived from 2010 samples.

Proteobacteria, the most abundant phylum, were significantly more abundant in rhizosphere soil than in bulk in 2008 and significantly more abundant at PCFS compared with KF in 2010 (Fig. 5A and B). Bacteroidetes were affected by all treatments in 2008 and much more abundant in rhizosphere soil and PCFS (Fig. 5A). Among relatively low abundant phyla, Chloroflexi and TM7 were higher in bulk soil than in rhizosphere in 2008, while Firmicutes showed the opposite pattern and were higher in rhizosphere soil (Fig. 5A). The other two low abundance phyla were affected by location in 2010 samples. Verrucomicrobia were significantly more frequent at KF site, while Firmicutes were more frequent at PCFS (Fig. 5B). On the contrary, tillage treatment exerted relatively small influences on bacterial phyla distribution and varied over time in this study. Acidobacteria were much more abundant in no-till soil compared with conventional tillage in 2008 samples, while Gemmatimonadetes were much more abundant in conventional tillage soil compared with no-till in 2010 (Fig. 5A and B).

Effect of root proximity, tillage treatment, and location on microbial community diversity. The diversity of bacterial communities was calculated with the Shannon diversity index, richness index, and Simpson's diversity index to determine how microbial diversity changed with root proximity, tillage treatment and location (Table 2). The Simpson's index is more weighted toward community

evenness, while the Shannon index is weighted toward community richness. Tillage had no significant effects on any of the indices of bacterial diversity. The effect of bulk/rhizosphere on Shannon diversity was not significant in both years. The richness in the 2008 samples was greater than that of 2010. There was a greater richness in rhizosphere soil than bulk soil at KF in the 2008 samples, but not in 2010. Higher Simpson diversity was observed in the 2010 samples. There was a significant difference in the Shannon diversity between the PCFS and KF sites in 2008, and the Shannon diversity was higher at KF compared with PCFS, but it was not different in 2010. The Simpson diversity was higher at KF compared with PCFS in the 2008 samples, while the Simpson diversity in the 2010 samples showed an opposite pattern of being lower at KF compared with PCFS (Table 2). In summary, the results were not consistent, but varied among year and location.

DISCUSSION

In describing the phytobiome in agricultural ecosystems, it is important to consider how management and cultural practices influence microbial communities and their relationships with plants. Crops are highly manipulated, in terms of genotypes planted, crop rotation, residue management, fungicide and herbicide inputs, and

Fig. 3. Heatmap illustrating the relative abundance of bacterial families from conventionally tilled and no-till systems at the Palouse Conservation Farm (PCFS) and the Kambitsch Farm (KF) in 2008. *, higher abundance in bulk soil; **, higher abundance in rhizosphere soil; ▲, higher abundance in conventional tillage (CT) soil; ▲▲, higher abundance in no-till (NT) soil; ●, higher abundance at KF soil; and ●●, higher abundance at PCFS soil. Differences in abundance of bacterial family were considered significant with $P < 0.05$ or false discovery rate < 0.1 (least significant means using JMP Pro 11).

Fig. 4. Heatmap illustrating the relative abundance of bacterial families from conventionally tilled and no-till systems at the Palouse Conservation Farm (PCFS) and the Kambitsch Farm (KF) in 2010. *, higher abundance in bulk soil; **, higher abundance in rhizosphere soil; ▲, higher abundance in conventional tillage (CT) soil; ▲▲, higher abundance in no-till (NT) soil; ●, higher abundance at KF soil; and ●●, higher abundance at PCFS soil. Differences in abundance of bacterial family were considered significant with $P < 0.05$ or false discovery rate < 0.1 (least significant means using JMP Pro 11).

cultural practices (e.g., tillage) with profound effects on plant–microbe interactions, and especially the role they play in promoting plant health and interactions with pathogens. This is one of the first studies to examine how tillage affects bacterial communities of the phytobiome in both the bulk and rhizosphere soil in long-term wheat cropping system plots. As expected, plant proximity significantly influenced the soil bacterial community composition and the communities of the bulk and rhizosphere soil varied greatly between locations and among sampling years, but tillage effects were stronger with bulk than rhizosphere communities in many instances. This work also demonstrated that although both tillage and plant proximity impact bacterial communities, the relative role of these factors may differ among years and locations.

The plant root proximity exhibited a stronger effect than tillage on the community of families. Families such as Oxalobacteraceae and Pseudomonadaceae of the Proteobacteria, Sphingobacteriaceae of the Bacteroidetes were more frequent in the rhizosphere. These are considered copiotrophic r-strategists (Fierer et al. 2007). This agrees with the results of Donn et al. (2014) who showed that the predominant rhizosphere families on wheat were Oxalobacteraceae and Flavobacteriaceae. Oxalobacteraceae were also shown to be in higher frequency in the rhizosphere of diseased wheat roots attacked by *Rhizoctonia solani* growing in stunted patches (Yin et al. 2013). Phenazine-producing *Pseudomonas* spp. have been shown to be numerous in the rhizosphere of wheat grown in a lower-rainfall zone of central Washington State (Parejko et al. 2013). The study by Marques et al. (2014) also supported a stronger rhizosphere effect on some bacterial taxa in field-grown sweet potato including *Sphingobium* (family Sphingomonadaceae), *Pseudomonas*, and *Chryseobacterium* (family Oxalobacteraceae).

Tillage may specifically impact some bacterial families and these impacts appeared to vary from 2008 to 2010. Considering both years, only 24 families were affected by tillage, compared with 43 that were affected by root proximity (Figs. 3 and 4). In general, most families such as Oxalobacteraceae, Pseudomonadaceae, Gaiellaceae, whether examined in bulk or rhizosphere soil, were not affected by tillage, and the results were inconsistent from year to

Fig. 5. The relative abundance of bacterial phyla significantly affected by root proximity, tillage management, and location. **A**, Phyla in 2008 soil; and **B**, phyla in 2010 soil. PCFS: USDA-ARS Palouse Conservation Farm; KF: Kambitsch Farm; Rhizo: rhizosphere soil; CT: conventional tillage; and NT: no-till. All phyla listed in the figure were significantly affected by treatments ($P \leq 0.05$, least significant means using JMP Pro 11). Bars represent standard deviation.

year. But those families that did show a tillage effect were also affected by root proximity. In 2008, there were 11 bulk soil families affected by tillage, compared with only six rhizosphere families. For example, Solirubrobacterales-unclassified was more common in no-till in 2008. *Solirubrobacter* was the most common genus in Solirubrobacteriaceae. Li et al. (2012) found this genus more abundant in a conventional than an organic wheat production system. Rhodospirillaceae was also more common in no-till in 2008. Within Rhodospirillaceae, *Azospirillum* was identified with the most confidence. *Azospirillum* is a diazotrophic free-living bacterium that can fix nitrogen and has been extensively studied as a plant growth promoting bacterium. In 2008, Chitinophagaceae was more dominant in conventional tillage soil, but a similar trend was seen in 2010 although it was not significant at the 0.05 level. Taken together, these findings suggested that tillage might affect some bacterial taxa in certain years, but not the entire bacterial community.

Alternatively, differences in our ability to detect treatment differences between years may be related to our use of different primers or sequencing technology in 2008 versus 2010, although

TABLE 2
Effect of root proximity (bulk versus rhizosphere), tillage treatment, and location on ecological indices of bacterial communities^z

Samples		Shannon		Richness		Simpson	
		2008	2010	2008	2010	2008	2010
Location	PCFS	5.80 ± 0.13 B	6.10 ± 0.03	698 ± 33 B	523 ± 10	112.01 ± 37.64 B	349.84 ± 18.14 A
	KF	6.09 ± 0.25 A	6.06 ± 0.05	792 ± 83 A	515 ± 16	200.12 ± 71.00 A	320.20 ± 38.31 B
Tillage	CT	5.95 ± 0.21	6.09 ± 0.04	738 ± 61	521 ± 12	159.91 ± 62.19	337.62 ± 36.21
	NT	5.94 ± 0.28	6.08 ± 0.05	749 ± 92	518 ± 15	152.22 ± 81.00	331.68 ± 29.80
Bulk/Rhizo	Bulk	5.87 ± 0.18	6.09 ± 0.04	708 ± 55 B	525 ± 11	143.63 ± 47.90	337.73 ± 32.29
	Rhizo	6.02 ± 0.27	6.07 ± 0.05	775 ± 83 A	514 ± 14	168.50 ± 86.63	335.00 ± 33.39

^z Treatments within each effect followed by different letters are significantly different at $P \leq 0.05$ or false discovery rate ≤ 0.1 . Abbreviations: PCFS, USDA-ARS Palouse Conservation Farm; KF, Kambitsch Farm; rhizo, rhizosphere soil; CT, conventional tillage; and NT, no-till.

the reason for this is unknown. In general, patterns in microbial communities evaluated with different sequencing technologies or primer sets are consistent, though primer biases may result in different relative abundance estimates for some taxa (Claesson et al. 2010; Luo et al. 2012; Tremblay et al. 2015). Further, although reads from the 2008 sampling were short (~135 bp), this does not prevent us from classifying most sequences to the family level (~50% of OTUs representing ~72% of sequences were classified to family in 2008), and still offers useful insight into community composition and diversity. Thus, it is unlikely that differences in sequencing technologies or primers would result in the large differences in beta-diversity patterns observed between sampling years. Future studies incorporating different sequencing technologies, protocols, or multiple sequencing runs at the same or different sequencing centers may benefit by sequencing replicate samples within and across to assess intraexperiment bias and calibrate data accordingly (Z. Song, D. Schlatter, D. Gohl, and L. Kinkel, *unpublished data*). Because sequencing technologies and protocols continue to develop at a rapid pace, it will be especially important to develop appropriate controls for longitudinal studies, which will be critical for understanding the long-term temporal patterns in microbial community responses to management practices, such as tillage. By archiving DNA, these new technologies could be used to verify these trends. It may also be important to “ground truth” whatever major “keystone” community members are identified. This could be done by designing qPCR primers from the OTUs and sampling the same archived DNA to see if the trends were similar.

The strength of the responses of bacterial communities to tillage, root proximity, and locations differed between years. This high degree of variation, and specifically communities less clearly differentiated by treatments in 2010 versus 2008, suggests that microbial community responses to tillage or root proximity are contingent upon on factors that vary across short time scales, such as weather patterns or plant phenology. Notably, 2010 was much wetter and warmer than 2008. In Pullman in 2010, the average temperature in January, February, March, and April were 5.5, 2.2, 2.3, and 2.3°C warmer than 2008. Precipitation was 177, 104, and 77% more in 2010 than in 2008, in April, May, and June, respectively. The trends at the Kambitsch site, 55 km from Pullman, would be similar. Because soil moisture is limiting in dryland ecosystems and a critical driver of microbial community structure (Bainard et al. 2016), the unusually high soil moisture may have limited our ability to detect differences in community structure between treatments that are often associated with a moisture gradient, such as root proximity or no-till versus conventional tillage.

This work demonstrates how agricultural practices, specifically tillage, impacts bacterial communities in soil and in wheat rhizospheres.

Unexpectedly, our results suggest that tillage may exert a smaller influence on bacterial communities of wheat than proximity to the root, though the strength of these impacts appeared to depend on the location and sampling year. Soil depth may have a stronger effect on microbial communities, as indicated by Stroobants et al. (2014), who demonstrated that bacterial communities in a winter wheat field were different between the top soil and subsoil. In our study, we sampled the top layer of soil where differences would be expected to be greatest in terms of organic matter and residue levels. Our results agree with those of Donn et al. (2014), who found that tightly bound rhizosphere communities were less affected by crop rotation (wheat–wheat versus wheat–fallow) than the loosely bound rhizosphere communities. Overall, crop rotation and variety exerted relatively small influences on rhizosphere communities compared with the developmental stage of the plant (Donn et al. 2014). Our results suggest that rhizosphere communities are similarly buffered or protected against changes exerted by tillage, although this depended on the location and sampling year. The lack of consistent trends from year to year or location to location suggests the highly dynamic nature of bacterial communities, and a resilience to many cultural factors such as tillage. On the other hand, fungal communities may be more sensitive to tillage, because of the effects of soil disturbance and the difference in plant residue that fungi would encounter in the two tillage systems.

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