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Glyphosate effects on soil rhizosphere-associated bacterial communities

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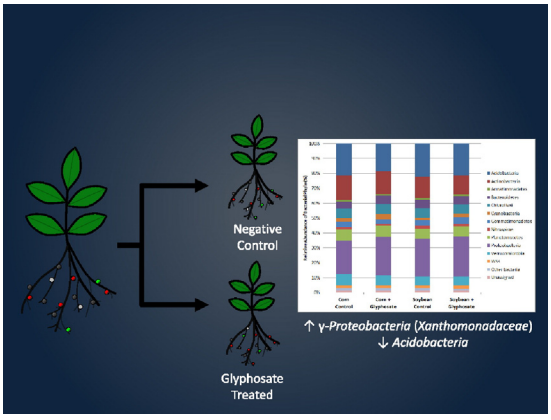
HIGHLIGHTS

- We examined the rhizosphere bacterial community composition response to glyphosate.
- Next-generation sequencing was used to examine the rhizosphere bacterial community.
- Relative abundance of *Acidobacteria* decreased in response to glyphosate exposure.
- Long-term glyphosate application could affect rhizosphere nutrient status.

GRAPHICAL ABSTRACT



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ABSTRACT

Glyphosate is one of the most widely used herbicides in agriculture with predictions that 1.35 million metric tons will be used annually by 2017. With the advent of glyphosate tolerant (GT) cropping more than 10 years ago, there is now concern for non-target effects on soil microbial communities that has potential to negatively affect soil functions, plant health, and crop productivity. Although extensive research has been done on short-term response to glyphosate, relatively little information is available on long-term effects. Therefore, the overall objective was to investigate shifts in the rhizosphere bacterial community following long-term glyphosate application on GT corn and soybean in the greenhouse. In this study, rhizosphere soil was sampled from rhizoboxes following 4 growth periods, and bacterial community composition was compared between glyphosate treated and untreated rhizospheres using next-generation barcoded sequencing. In the presence or absence of glyphosate, corn and soybean rhizospheres were dominated by members of the phyla *Proteobacteria, Acidobacteria*, and *Actinobacteria. Proteobacteria* (particularly *gammaproteobacteria*) increased in relative abundance for both crops following glyphosate exposure, and the relative abundance of *Acidobacteria* decreased in response to glyphosate exposure. Given that some members of the *Acidobacteria* are involved in biogeochemical

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processes, a decrease in their abundance could lead to significant changes in nutrient status of the rhizosphere. Our results also highlight the need for applying culture-independent approaches in studying the effects of pesticides on the soil and rhizosphere microbial community.

1. Introduction

Pesticides are substances or mixtures of substances intended for preventing, destroying, repelling or mitigating pests, and the major groups of pesticides are fungicides, herbicides, and insecticides (Grube et al., 2011). A recent comprehensive study by BCC Research of the global biopesticide and synthetic pesticide market estimated the global market of pesticides in 2014 at $61.8 billion, with a projected increase to $83.7 billion by 2019 (Lehr, 2014). Pesticides are typically used in the agricultural industry for improving crop yield and quality while also maximizing economic returns. Herbicides are the most widely used class of pesticides in agriculture (Grube et al., 2011), and of all herbicides, glyphosate has the highest use world-wide with the global market projected to reach 1.35 million metric tons by 2017 (Global Industry Analysts, 2011).

Examining the effects of pesticides, such as glyphosate, on soil and rhizosphere microbial communities is important due to the critical role of microorganisms in driving biogeochemical processes, controlling pathogens, and ultimately enabling ecosystems to function and provide services to humanity. The soil microbial community, especially the rhizosphere microbial community, impacts soil quality through its involvement in biogeochemical and nutrient cycling, long-term soil sustainability, and resistance to perturbations (Prashar et al., 2014; Topp, 2003). Within the rhizosphere, microorganisms positively affect plant health through a variety of mechanisms, including mineralization of nutrients, suppression of disease, improving plant stress tolerance, and production of phytohormones (Berendsen et al., 2012; Figueiredo et al., 2011; Gupta et al., 2000). In agricultural systems, these effects on plant health have a major impact on crop production.

Numerous studies have investigated the impacts of glyphosate on soil microbial properties using broad-scale or integrative methods such as microbial biomass, enzyme activity, and respiration. Bünemann et al. (2006) and Johnsen et al. (2001) provide exceptional reviews of this literature. Typically the results of these studies have shown no or transitory effects of glyphosate on the above mentioned microbial properties. However, the effects of glyphosate may be masked by "functional redundancy" where overall soil functions are unaffected while microbial community composition is altered and key functions mediated by specific microbial populations are affected (Imfeld and Vuilleumier, 2012). Alterations to soil microbial community composition and subsequent changes in microbial diversity could potentially have pronounced long-term effects on soil quality as well as impact plant health and therefore crop production (Bending et al., 2007; Lynch et al., 2004).

Many studies examining the effects of glyphosate on the microbial community have used culture-based methods to target specific bacterial populations of functional significance in the soil environment. For example, a study by Zobiole et al. (2011) targeted populations of *Fusarium*, fluorescent pseudomonads, Mn-transforming bacteria, and indoleacetic acid-producing bacteria in rhizosphere soils of soybean receiving glyphosate treatment and found that glyphosate treatment negatively impacted the interactions of these microbial groups, leading to increased *Fusarium* spp. abundance and reduced abundances of fluorescent pseudomonads, Mn-reducing bacteria and indole acetic acid-producing rhizobacteria. Johnsen et al. (2001) suggests, however, that by targeting specific rhizosphere bacterial populations, little information is gained regarding effects on rhizosphere bacterial community composition as a whole. Such approaches may actually cause the effects on lesser-abundant, yet still significant, taxa to be overlooked (Johnsen et al., 2001).

Mijangos et al. (2009) used DGGE in combination with Biolog Ecoplates™ and microbial biomass to assess the effects of glyphosate on rhizosphere soil microbial properties and observed a glyphosate-induced stimulation of microbial activity and functional diversity 15 days after glyphosate treatment in the culturable portion of the soil microbial community. But, this response was inconsistent when examining the microbial community 30 days after glyphosate addition. Using PLFA and bacterial 16S rRNA genotyping via T-RFLP, Widenfalk et al. (2008) showed that the herbicide glyphosate increased the abundance of branched, saturated fatty acids typical of Gram-positive bacteria in freshwater sediment. Nearly all of the research reported above on glyphosate was done under short-term conditions where a single or one season application of glyphosate was applied, and as mentioned above, often with integrative methods that might have missed subtle effects on the soil microbial community. This misses the actual field conditions in the U.S. where glyphosate tolerant (GT) cropping has now been extensively used in the major agricultural regions for 10–15 years. In addition, common agricultural practices apply commercial formulations containing glyphosate, rather than the active ingredient alone. Given that the toxicity of commercial formulations may differ from that of pure glyphosate (Sihtmäe et al., 2013; Tsui and Chu, 2003), it is important to use commercial formulations in studies investigating the effects of glyphosate-based pesticides.

Recently, Nye et al. (2014) found on the same soil type that more than 10 years of GT cropping shifted the microbial PLFA diversity compared to soil that had no history of glyphosate exposure. Although effects on overall microbial community composition and associated bacterial subgroups were noted as a result of glyphosate exposure, specific bacterial taxa affected were not identified. To fill this gap, a greenhouse study was conducted subjecting soil that had no history of glyphosate applications to GT cropping over 8 growing periods, simulating long-term field conditions. In this study, we examined the bacterial community composition from rhizosphere soil samples collected from the fourth growth period of this larger greenhouse study. And more specifically, we used next-generation barcoded sequencing, which permits detailed phylogenetic diversity analysis (Imfeld and Vuilleumier, 2012). Therefore, the objective of this particular study was to use next-generation barcoded sequencing to identify specific bacterial taxa shifts in the rhizosphere bacterial community in response to repeated glyphosate exposure on corn and soybeans.

2. Materials and methods

2.1. Greenhouse study

The soil used for the study was a Blount silt loam (fine, illitic mesic Aeric Epiaqualf). Soil pH was 6.95, and soil total C was 1.47%. Soil texture was 11% sand, 48% silt, and 41% clay. Typical Blount soil clay mineralogy is characterized by illite, hydroxyl-interlayered vermiculite, kaolinite, and quartz (Dontsova and Norton, 2002). Soil was collected in 2-cm increments to a depth of 39 cm, with 37 cm from the A horizon and the remaining 2 cm from the O horizon, from soil pits at a farm undergoing organic management in Delaware County, OH. This field site was previously under rotation of alfalfa–orchard grass–corn, oats–alfalfa–orchard

grass, spelt–timothy–clover, and timothy–clover. The soil had never been exposed to glyphosate. Once collected, soil was stored in sealed plastic bags returned to the lab on ice and placed in rhizoboxes starting with the 38–39-cm increment, using ~62 g of soil per cm fill height. The soil was evenly distributed in the rhizobox and compacted to a bulk density of 1.3 g cm^{-3} and a total fresh soil weight within the rhizobox of 2500 g. A total of eight rhizoboxes were constructed as described by Bott et al. (2008). Four rhizoboxes were planted for each of two crops, corn and soybean. Two rhizoboxes per crop were treated with glyphosate (Roundup PowerMax, Monsanto Company, MO, USA; active ingredient: glyphosate, N-(phosphonomethyl) glycine, in the form of its potassium salt), and two rhizoboxes served as untreated plant controls. These eight rhizoboxes were part of a larger ongoing research project that utilized all available rhizoboxes, leading to two rhizoboxes per treatment combination in this study.

Plants were grown in eight growth periods over three years, with each growth period lasting 58 days. Plants were fertilized twice per growth period by applying 25 mL of fertilizer solution per rhizobox. Fertilizer solution was prepared by dissolving 3.745 g of Peters® 20/20/20 Professional fertilizer per liter, equaling 0.749 mg N, 0.749 mg P, and 0.749 mg K mL^{-1} of fertilizer solution. Fertilizer trace element concentrations were magnesium (0.019 mg mL^{-1}), boron (0.749 μg mL^{-1}), copper (0.002 mg mL^{-1}), iron (0.004 mg mL^{-1}), manganese (0.002 mg mL^{-1}), molybdenum (0.019 μg mL^{-1}), and zinc (0.002 mg mL^{-1}). The fertilizer was applied on days 30 and 50. The schedule for each period is outlined in Table 1.

On day 1, before planting, all rhizoboxes were sprayed with glyphosate except for the controls. Glyphosate was applied at the recommended field rate (300.79 mL ha^{-1}). Corn and soybean seedlings germinated on cotton tissue were transplanted into rhizoboxes (2 plants/box) on day 10. Roundup Ready corn (*Zea mays*; DeKalb hybrid seed brand DKC62-54 (VT3)) and soybean (*Glycine max*; OX 20-8 RR) were used. Growth stages were estimated using the shortest periods given in the Ontario Agronomy Guide (Baute et al., 2002) for corn and soybean. On days 30 and 51 (when plants reached the V–5 and V–7 growth stages, respectively), glyphosate was applied on plant leaves using a cell spreader. Soil rhizosphere samples were collected on days 31, 37, 52, and 58. This schedule was then repeated for a total of eight growth periods. The rhizosphere soil samples used in this study were collected on day 58 of the fourth growth period.

2.2. Sample collection and DNA extraction

Samples for this study were collected in the fourth growth period for corn and soybean. For the collection of rhizosphere soil samples, rhizoboxes were placed horizontally on the lab bench and clamps and the top acrylic plate were removed. Three 5-g subsamples of soil were collected using a spatula to recover soil within a 1-mm vicinity of the primary and lateral roots, avoiding the areas around the root tips and stored at −80 °C until further processing. These subsamples were processed separately, and the resulting sequence data was combined to account for variability in bacterial community composition within the rhizosphere. DNA was extracted from 500 mg of each soil rhizosphere sample using the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, CA, USA) and eluted in 50 μl. DNA extracts were quantified using a Qubit Fluorometer and the dsDNA HS Assay kit (Life Technologies, CA, USA).

Table 1
Schedule of events per growth period.

Day	Event
1	Glyphosate burn down spray
10	Corn and soybean planted
30	Glyphosate spray[a]
31	Collection of rhizosphere soil samples
37	Collection of rhizosphere soil samples
51	Glyphosate spray[b]
52	Collection of rhizosphere soil samples
58	Collection of rhizosphere soil samples

[a] Exact application at V3–V5 growth stages.
[b] Exact application at V6–V7 growth stages.

2.3. Sequencing library construction

PCR primers (515F/806R) designed by Caporaso et al. (Caporaso et al., 2012) were used to amplify the bacterial V4 hypervariable region of the 16S rRNA gene. Each primer contained the sequence adapter regions used by Caporaso et al. (Caporaso et al., 2012), and the reverse PCR primers contain a 12-base Golay barcode. Three sequencing primers were designed based on those of Caporaso et al. (Caporaso et al., 2012) to yield the 5′ read, the 3′ read, and the index read. See Table 2 for a description of the primers used.

PCR reagent mixes contained 12.5 μl KAPA HiFi HotStart Ready Mix (2×), 0.75 μl each of the forward and reverse primers (10 μM final concentration), 10 ng genomic DNA, and PCR water for a total reaction volume of 25 μl. The following touchdown PCR conditions were used: initial denaturation at 95 °C for 2 min followed by 32 cycles of denaturation at 98 °C for 20 s, annealing beginning at 61 °C and ending at 50 °C for 30 s, and extension at 72 °C for 30 s. The annealing temperature was lowered 1 °C every cycle until reaching 50 °C, which was used for the remaining cycles. Following this, a final extension of 72 °C for 10 min was used. PCR products were purified by ethanol precipitation and verified on a 1% agarose gel. Positive amplicons were quantified using a Qubit Fluorometer and the dsDNA HS Assay kit (Life Technologies, CA, USA).

Amplicons were pooled at equimolar concentrations, and the resulting pooled library was size-selected to remove smaller primer dimers. Since the 16S rRNA gene amplicon was approximately 420 bp, the E.Z.N.A. Size Select-IT Kit (Omega Bio-Tek, GA, USA) was used on the pooled bacterial 16S rRNA gene library, targeting 150–500 bp fragments. The library was quantified using a Qubit Fluorometer and dsDNA HS Assay kit (Life Technologies, CA, USA). The library was denatured with 0.2 N NaOH and diluted with pre-chilled HT1 buffer (Illumina, CA, USA) to a final concentration of 8 pM. The denatured and diluted library was spiked with 40% denatured PhiX and sequenced separately on an Illumina MiSeq (Illumina, CA, USA) using the sequencing primers mentioned above and a 300-cycle (2 × 150) MiSeq Reagent Kit v2 (Illumina, CA, USA).

2.4. Data analysis

Paired-end reads were assembled using PANDAseq (Bartram et al., 2011; Masella et al., 2012), and all downstream processing of sequences was completed using the QIIME pipeline v1.5.0 (Caporaso et al., 2010b). Assembled sequences were quality filtered using USEARCH v7 (Edgar, 2010), retaining only sequences >75 bases in length with expected

Table 2
Primers used for amplification of bacterial 16S rRNA gene V4 hypervariable region.

Primers	Sequence (5′ to 3′)[a]
Forward (515F)	aatgatacggcgaccaccgagatctacacTATGGTAATT**gt**GTGCCAGCMGCCGCGGTAA
Reverse (806R)	caagcagaagacggcatacgagat**NNNNNNNNNNNN**[b]AGTCAGTCAG**cc**GGAC TACHVGGGTWTCTAAT
Sequencing	
Read 1	TATGGTAATT**gt**GTGCCAGCMGCCGCGGTAA
Read 2	AGTCAGTCAG**cc**GGACTACHVGGGTWTCTAAT
Index	ATTAGAWACCCBDGTAGTCC**gg**CTGACTGACT

[a] Lowercase letters denote adapter sequences, underlined letters are pad regions, lowercase bold letters are linker regions, and uppercase letters are primer sequences specific to the V4/ITS1 regions.
[b] N's represent location of 12-base Golay barcode; see Caporaso et al. (2012) for a listing of the barcodes used.

errors >1.0. Chimeric sequences were identified and removed using USEARCH v6.1 (Edgar, 2010). Sequences were assigned to operational taxonomic units (OTUs) using open reference OTU picking in USEARCH v6.1 at a 97% similarity threshold. Reads were first clustered against the Greengenes 16S rRNA gene database (Aug 2013 release) (DeSantis et al., 2006), and then all remaining reads that did not cluster were clustered *de novo*. A representative sequence was chosen for each OTU based on which sequence was the most abundant for that given OTU. Taxonomy was assigned to the representative sequence of each OTU using uclust with the Greengenes 16S rRNA gene database (Aug 2013 release) (DeSantis et al., 2006). In addition, sequences identified as chloroplast following taxonomic assignment were removed. Reads were aligned using PyNAST (Caporaso et al., 2010a) against a reference alignment of the Greengenes core set (McDonald et al., 2011).

The generated OTU table was rarified to an even sampling depth (29,205 sequences per sample) using the *single_rarefaction.py* script in the QIIME pipeline, and this rarified OTU table was used to calculate alpha diversity metrics, including OTU abundance, Chao1 (Chao, 1984), Faith's phylogenetic diversity (Faith, 1992), and Shannon's index (Shannon, 1948). Alpha diversity metrics were compared between control and glyphosate-treated samples for each crop using the *compare_alpha_diversity.py* script in the QIIME pipeline which implements a nonparametric two-sample t-test with 999 Monte Carlo permutations. Beta diversity metrics were also estimated using the rarified OTU table, including unweighted and weighted UniFrac distances (Lozupone and Knight, 2005). Weighted UniFrac distances were compared using a multiple response permutation procedure (MRPP) with 999 permutations. Principal coordinates analysis was performed using the *beta_diversity_through_plots.*py script in the QIIME pipeline which uses the weighted UniFrac distances to generate plots and aid in visualization of the relationships among the various samples and treatments. All sequences obtained in this study were submitted to the NCBI Sequence Read Archive (SRA) and are available under the study accession number PRJNA284763.

3. Results

3.1. Sequencing summary

Following assembly and quality filtering, a total of 505,391 bacterial 16S rRNA gene sequences were obtained with a range of 29,205 to 66,702 sequences per rhizobox and a mean of 49,249 sequences per rhizobox. All rarefaction curves tended to approach a plateau, indicating that the number of sequences obtained was sufficient to describe the bacterial diversity within these samples (Supplemental Fig. S1).

3.2. Rhizosphere bacterial community diversity

Alpha diversity estimates were similar between corn and soybean rhizospheres as well as among the control and glyphosate-treated samples (Table 3). Mean OTU abundance within corn and soybean control rhizospheres was 3814 and 3849, respectively. This increased slightly following glyphosate treatment to 4001 OTUs in corn and 3893 in soybean. Chao1 richness estimates increased in corn following glyphosate treatment (e.g. 5872 to 6154) but decreased in soybean (e.g. 5946 to 5754). Phylogenetic diversity observed within the corn rhizosphere was 182.29 in controls and 189.66 in glyphosate-treated samples, and there was no increase in phylogenetic diversity for soybean rhizosphere samples following glyphosate treatment. Shannon's diversity estimates were similar between control and glyphosate-treated rhizosphere samples with a mean Shannon's diversity estimate of 10.2. Weighted UniFrac distances showed that rhizosphere beta diversity varied by plant species ($p = 0.029$; $\alpha = 0.10$) but was fairly similar overall between control and glyphosate-treated samples ($p = 0.78$; $\alpha = 0.10$). Fig. 1 contains a PCoA plot of these results.

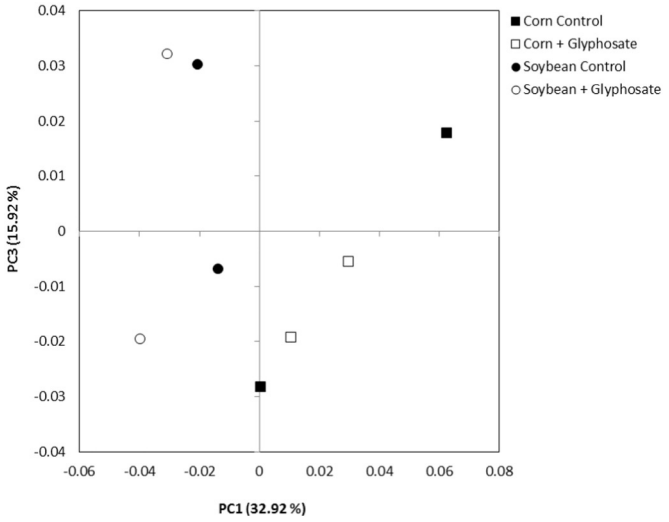

Fig. 1. PCoA plot based on weighted Unifrac distances generated for control (solid symbols) and glyphosate-treated (hollow symbols) rhizosphere bacterial communities of corn (squares) and soybean (circles) following four growth periods.

3.3. Rhizosphere bacterial community composition

Control samples and those receiving long-term glyphosate applications showed similarities in bacterial community composition at the phylum level. Control and treatment rhizosphere samples for both corn and soybean were dominated by members of the phyla *Proteobacteria, Acidobacteria*, and *Actinobacteria* (Fig. 2). The abundance of *Proteobacteria*-affiliated sequences increased in response to glyphosate treatment ($p = 0.096$). Corn rhizosphere samples showed an increase from an average of 22.9 ± 1.5% *Proteobacteria* sequences to 25.9 ± 0.9%. Soybean rhizosphere sample *Proteobacteria* sequences increased from an average of 25.4 ± 1.2% to 27.2 ± 0.2%. Within the sequences identified as belonging to the phylum *Proteobacteria*, no one bacterial class dominated for either corn or soybean. The *alphaproteobacteria, betaproteobacteria*, and *gammaproteobacteria* classes were present in controls samples for corn and soybean (ranging from approximately 5.2–8.3% relative

Table 3
Alpha diversity metrics for rhizosphere samples collected from control and glyphosate-treated rhizospheres of corn and soybean. Values represent mean ± 1SE.

	Observed OTUs	Chao1 richness estimate	Faith's phylogenetic diversity	Shannon's index
Corn				
Control	3814 ± 60	5872 ± 233	182.3 ± 3.1	10.2 ± 0.03
Glyphosate	4001 ± 86	6154 ± 111	189.7 ± 4.3	10.3 ± 0.05
Soybean				
Control	3849 ± 2	5946 ± 49	184.2 ± 0.2	10.2 ± 0.04
Glyphosate	3893 ± 101	5754 ± 210	184.2 ± 4.0	10.2 ± 0.08
Crop Effect[a]	0.656	0.428	0.604	0.91
Treatment Effect[a]	0.201	0.928	0.318	0.241

[a] Values represent p-values calculated using a nonparametric two-sample t-test with 999 Monte Carlo permutations.

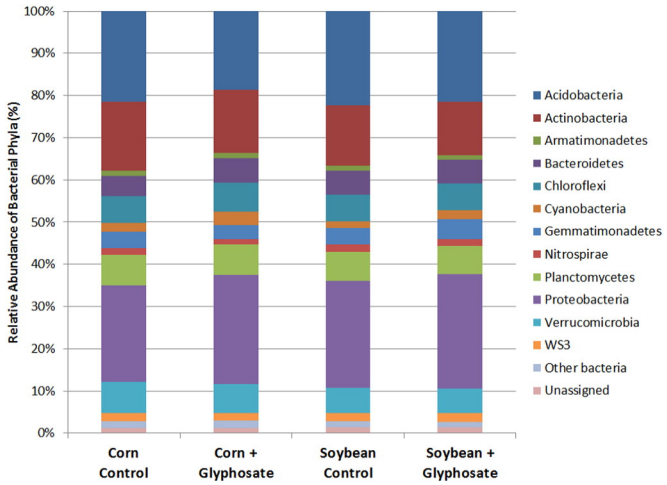

Fig. 2. Relative abundance of bacterial phyla present in control and glyphosate-treated rhizosphere bacterial communities of corn and soybean following four growth periods.

abundance for each), as well as to a lesser extent the *deltaproteobacteria* (3.2% corn, 4.1% soybean).

Following glyphosate treatment, all classes of *Proteobacteria* increased in relative abundance. *Gammaproteobacteria* sequences increased the most for both crops with an increase of 1.5% in corn and 0.7% in soybean. The majority of *gammaproteobacteria* sequences in control samples (2.6% corn, 3.2% soybean) were identified as belonging to the order *Xanthomonadales*, mainly from the families *Sinobacteraceae* and *Xanthomonadaceae*. Within the families *Sinobacteraceae* and *Xanthomonadaceae*, the majority of sequences matched reference sequences from unidentified genera. Other lower abundance genera present from these families included *Steroidobacter* within the family *Sinobacteraceae* and *Arenimonas, Dokdonella, Luteibacter, Lysobacter, Pseudoxanthomonas*, and *Thermomonas* within the family *Xanthomonadaceae*. The relative abundance of *Xanthomonadaceae* sequences increased for both crops following glyphosate treatment ($p = 0.081$). The response of *Sinobacteraceae* to glyphosate treatment varied with crop ($p = 0.003$). In corn, *Sinobacteraceae* relative abundance decreased (2.2% to 1.5%) following glyphosate treatment, but in soybean relative abundance of *Sinobacteraceae* increased (1.5% to 2.1%).

In contrast, the relative abundance of members of the phylum *Acidobacteria* showed a decrease in response to glyphosate treatment ($p = 0.083$). In corn, the average relative abundance of *Acidobacteria* sequences decreased from 21.5 ± 1.1% in the control samples to 18.7 ± 0.8% in glyphosate-treated samples. For soybean there was also a decrease in the average relative abundance of *Acidobacteria* sequences from 22.3 ± 0.6% in control samples to 21.5 ± 0.3% in glyphosate-treated samples. The *Acidobacteria* subgroup 6, a dominant *Acidobacteria* subgroup in soils with few cultured representatives, made up the majority of *Acidobacteria* sequences for both corn (45.9%) and soybean (49.1%) and decreased in abundance following glyphosate treatment for corn, and to a lesser extent soybean. The average relative abundance of *Actinobacteria* also decreased following glyphosate treatment from 16.45% to 14.95% in corn and 14.35% to 12.6% in soybean ($p = 0.445$).

4. Discussion

Analysis of the corn and soybean rhizosphere microbiota indicated dominance by the members of the bacterial phyla *Proteobacteria, Acidobacteria*, and *Actinobacteria*. All of these phyla contain taxa commonly found within soil rhizospheres that are capable of having various effects on plant health including beneficial and pathogenic interactions (Berendsen et al., 2012; Lee et al., 2008; Philippot et al., 2013). For example, many Gram-negative fluorescent *Pseudomonas* species fall within the phylum *Proteobacteria* and have been reported to benefit plants by stimulating plant growth and exhibiting traits involved in biological control of plant diseases (Lugtenberg and Kamilova, 2009).

The results of this study showed subtle alterations to rhizosphere bacterial community composition following the application of the herbicide glyphosate. The largest shifts in relative abundance were observed for *Proteobacteria* (specifically *gammaproteobacteria*) and *Acidobacteria*. The increase in γ-*Proteobacteria* relative abundance for both corn and soybean rhizosphere samples was driven by increases in bacteria from the family *Xanthomonadaceae* following glyphosate treatment, suggesting that *Xanthomonadaceae* are adapted to and/or enriched by environments containing glyphosate. Previous studies also noted an increased abundance of bacteria from the family *Xanthomonadaceae* in response to long-term fertilization and have cited a potential importance of *Xanthomonadales* in the bacterial population dynamics of altered soils (Campbell et al., 2010). Interestingly, members of the novel family *Sinobacteraceae* (Order *Xanthamonadales*) have been isolated from a soil that was adjacent to and contaminated by a chemical factory that produced herbicides, including glyphosate (personal communication Zhou et al., 2008), suggesting that the members of this family are increased in their abundance and metabolic activity in response to herbicide contamination of soils.

Concomitantly, upon treatment with glyphosate, in both corn and soybean there were decreases in the relative abundance of *Acidobacteria*, particularly the *Acidobacteria* subgroup 6. *Acidobacteria* have been found to be dominant members of rhizosphere soil and are believed to be highly involved in biogeochemical processes within the rhizosphere particularly for cellulose degradation (Eichorst et al., 2011; Lee et al., 2008; Štursová et al., 2012). Long-term decreases in the abundance of these bacteria could impair the ability of soil to perform certain biogeochemical reactions performed by these organisms. The decrease of *Acidobacteria* was more dramatic in corn, suggesting that any subsequent effects on biogeochemical processes due to reduced *Acidobacteria* taxa abundance and/or activity would be more pronounced in corn. This has implications for growing corn with GT cropping which may exacerbate the reduction of *Acidobacteria* taxa over a corn–soybean rotation. The abundance of this same subgroup of *Acidobacteria* was previously shown to decrease in subsurface sediments contaminated with uranium (Barns et al., 2007), indicating that these taxa are responsive and sensitive to environmental change and may serve as useful bioindicators of environmental alteration.

Although effects of glyphosate on specific bacterial taxonomic groups were observed, there was no overall effect of glyphosate on bacterial community diversity. This highlights the need to examine the microbial diversity response to herbicide application at a finer level both taxonomically as well as functionally rather than solely looking at net diversity responses. In addition, bacteria within the phylum *Acidobacteria* are somewhat recalcitrant to cultivation, especially given their high abundance in soil (George et al., 2011; Janssen, 2006). The high relative abundance of *Acidobacteria* ribotypes observed within the rhizosphere of this study and their reduced abundance in glyphosate-treated rhizosphere soil would not have been observed using culture-based methods but was made possible by employing next-generation sequencing instrumentation.

The results of this study are specific to the soil used and alternative results could have occurred on other soil types. This is because soil texture, mineralogy, pH, and organic matter have a major impact on the microbial community structure and secondly, on the fate, decomposition, and sorption of glyphosate and its metabolites, most notably aminomethylphosphonic acid (AMPA) as the major decomposition intermediate (Ascolani Yael et al., 2014; Franz et al., 1997). Thus, each soil type would have a variable rate of decomposition and degree of sorption and toxicity of glyphosate or AMPA for susceptible populations. Less is known about AMPA sorption, but for glyphosate it is primarily

sorbed on surfaces of variable-charge clay and inorganic precipitates. Sorption increases as surface area of the minerals increases and pH decreases (Duke et al., 2012). Thus the amount of glyphosate (and presumably AMPA) (Rampazzo et al., 2013) sorption capacity would be relatively high for our study because this soil has a high clay content at 41% (and therefore high surface area), but from a pH perspective this soil at 6.9 is less favorable for glyphosate sorption. However, relatively little is known about rates of microbial toxicity for glyphosate or AMPA in soil solution or in any sorbed fraction. In part this is due to inability to accurately determine what is biologically available to microorganisms (Duke et al., 2012). Furthermore in our case, we did not have several soil types with variable levels of chemical and physical properties in order to draw inferences and indirectly determine toxicity effects on microbial community members.

Given the wide-spread and increasing use of glyphosate, the results of our study are important because they show that shifts in subpopulations (particularly *Xanthamonadales, Acidobacteria*) are possible under long-term GT cropping. However, this does not provide any information on whether this shift affects functional capability of the soil under GT cropping. Thus, our research provides justification for more detailed, culture-independent investigations on function and delivery of ecosystem services or negative impacts such as crop pathogens.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.11.008.

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