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## Glyphosate effects on soil rhizosphere-associated bacterial communities



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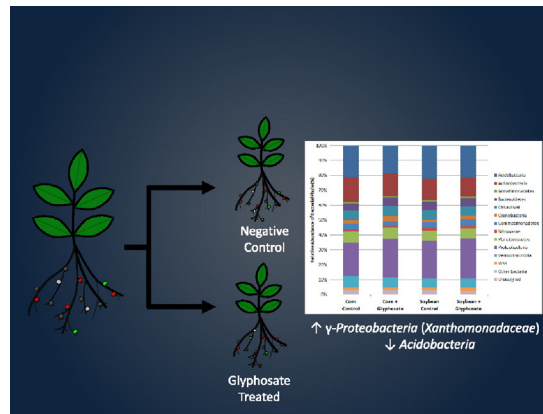
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### HIGHLIGHTS

- 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.

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## 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 Zobiolo 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

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