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Changes in rhizosphere bacterial gene expression following glyphosate treatment

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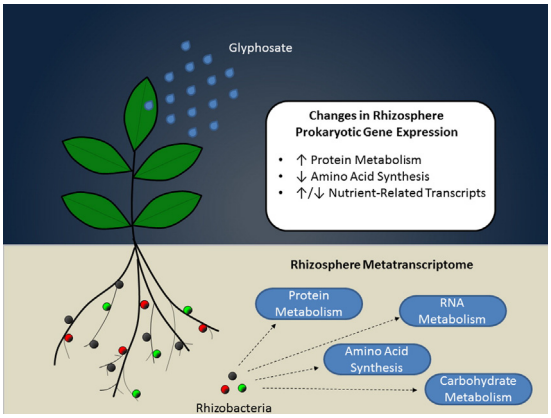
HIGHLIGHTS

- Response of rhizosphere prokaryotic metatranscriptome to glyphosate is examined.
- The metatranscriptome was dominated by RNA and carbohydrate metabolism transcripts.
- Glyphosate increased protein metabolism and decreased amino acid synthesis.
- Central carbon metabolism by bacteria was downregulated under glyphosate exposure.
- Glyphosate affects rhizosphere microbial community composition and activities.

GRAPHICAL ABSTRACT



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ABSTRACT

In commercial agriculture, populations and interactions of rhizosphere microflora are potentially affected by the use of specific agrichemicals, possibly by affecting gene expression in these organisms. To investigate this, we examined changes in bacterial gene expression within the rhizosphere of glyphosate-tolerant corn (*Zea mays*) and soybean (*Glycine max*) in response to long-term glyphosate (PowerMAX™, Monsanto Company, MO, USA) treatment. A long-term glyphosate application study was carried out using rhizoboxes under greenhouse conditions with soil previously having no history of glyphosate exposure. Rhizosphere soil was collected from the rhizoboxes after four growing periods. Soil microbial community composition was analyzed using microbial phospholipid fatty acid (PLFA) analysis. Total RNA was extracted from rhizosphere soil, and samples were analyzed using RNA-Seq analysis. A total of 20–28 million bacterial sequences were obtained for each sample. Transcript abundance was compared between control and glyphosate-treated samples using edgeR. Overall rhizosphere bacterial metatranscriptomes were dominated by transcripts related to RNA and carbohydrate metabolism. We identified 67 differentially expressed bacterial transcripts from the rhizosphere. Transcripts downregulated following glyphosate treatment involved carbohydrate and amino acid metabolism, and upregulated

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transcripts involved protein metabolism and respiration. Additionally, bacterial transcripts involving nutrients, including iron, nitrogen, phosphorus, and potassium, were also affected by long-term glyphosate application. Overall, most bacterial and all fungal PLFA biomarkers decreased after glyphosate treatment compared to the control. These results demonstrate that long-term glyphosate use can affect rhizosphere bacterial activities and potentially shift bacterial community composition favoring more glyphosate-tolerant bacteria.

1. Introduction

Crop protection chemicals, such as pesticides, are used worldwide to protect agricultural crops from suffering dramatic losses due to pests and therefore increase crop yield and quality and optimize economic returns (Imfeld and Vuilleumier, 2012). Recent forecasts predict the global market volume for pesticides will increase to 3.2 million tons by 2019, with a market value of $75.9 billion (Mordor Intelligence, May, 2014). Herbicides constitute an average of 49% of the total amount of pesticides applied globally (Grube et al., 2011).

Glyphosate is the most widely used herbicide worldwide and is projected to reach 1.35 million metric tons used by 2017 (Global Industry Analysts, 10 October, 2011). Glyphosate is a broad-spectrum, foliar-applied herbicide that has been widely used for decades in agriculture to control weeds pre-planting and post-emergence in tolerant crops (Araújo et al., 2003; Haney et al., 2002). Its mode of action is to inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) involved in the shikimate pathway (Duke and Powles, 2008). The inhibition of EPSPS leads to a buildup of shikimate and reduced synthesis of aromatic amino acids which are necessary for plant survival (Ahrens, 1994). Although, based on its physicochemical properties, glyphosate is not expected to have a drastic effect on soil microbial community function, results of studies investigating glyphosate impacts on soil microbial communities have been conflicting. Additionally, many of these studies are relatively short term and do not assess potential effects of long-term glyphosate application. Given these incongruous results, the unprecedented and widespread use of glyphosate over the past 40 years, and its predicted increase in use globally (Benbrook, 2016), studies are needed that examine the potential ecological effects of long-term glyphosate use on the soil microbial community.

The rhizosphere is the narrow zone of soil surrounding plant roots that is specifically influenced by plant root activities and/or is in association with root hairs and plant-produced materials (Walker et al., 2003). Rhizosphere soil contains a diverse microbial community, with bacteria being the most dominant members (Saharan and Nehra, 2011). Phospholipid fatty acid (PLFA) biomarkers have been used to assess the effects of pesticides on microbial community composition (Nye et al., 2014; Rosenbaum et al., 2014; Widenfalk et al., 2008). Although these studies provide insight into the effects of glyphosate on microbial community composition, they provide no information on its effects on microbial activity. In order to examine the effects of pesticides, such as glyphosate, on microbial activities both the structural and functional responses of the microbial community need to be assessed (Widenfalk et al., 2008; Zabaloy et al., 2008).

Functionally, bacteria within the rhizosphere can influence plant health in many ways. Many soilborne, bacterial plant pathogens can reside within the rhizosphere and have deleterious effects on plant growth and health. These effects are often a result of the production of metabolites or by nutrient competition and inhibition of beneficial rhizobacteria (Sturz and Christie, 2003). Conversely, rhizobacteria, such as plant growth-promoting rhizobacteria (PGPR), have been shown to exert a variety of beneficial effects on plants, including improved seed germination, seedling vigor, plant growth and development, biocontrol, and productivity (Mendes et al., 2013). Rhizobacteria are also involved in biogeochemical processes within the soil such as carbon, nitrogen, and phosphorus cycling. Given the impact of these microbial functions on overall plant health and subsequent crop yield, it is important to determine whether or not these functions are impaired as a result of the addition of pesticides such as glyphosate.

Several studies have noted variable effects when reviewing glyphosate impacts on soil microbial community function (Bünemann et al. (2006) and Duke et al. (2012)). Araújo et al. (2003) collected soil of two different types with and without a prior history of glyphosate exposure, applied 2.16 mg kg^{-1} of technical glyphosate, and measured microbial activity via respiration and FDA hydrolysis at 2, 4, 8, 16, 24, and 32 days after treatment. The soils with a history of glyphosate exposure (6 and 11 years) had a 10–15% higher increase in respiration when treated with glyphosate than soils with no glyphosate history. Microbial activity measured by FDA hydrolysis increased significantly (9–19%) with time and glyphosate addition. Zabaloy et al. (2008) applied glyphosate at a rate of 150 mg kg^{-1} to agricultural soil with a history of glyphosate exposure. Soil microcosms were sampled 3, 7, 14, and 21 days after treatment to measure respiration, FDA hydrolysis, and dehydrogenase activity. Although the response varied by soil type, a 42% and 28% increase in respiration was observed initially for the glyphosate-treated soils but then dissipated over time. Glyphosate had no discernable effect on FDA hydrolysis in one soil, but caused reduced FDA hydrolysis within the first two weeks after treatment in the other soil. And, dehydrogenase activity showed no consistent changes following glyphosate treatment.

Haney et al. (2000) found C and N mineralization increased in agricultural soils with increasing glyphosate application rates of 47, 94, 140, and 234 μg g^{-1} (Haney et al., 2000). Zobiole et al. (2011) observed only transient effects of glyphosate on dehydrogenase, β-glucosaminidase, β-glucosidase, and respiration following a single application of glyphosate at the rates of 800, 1200, and 2400 g a.e. ha^{-1}. Sannino and Gianfreda (2001) studied the effects of multiple pesticides at a mean application dose ranging from 40 to 200 mg kg^{-1} on invertase, urease, and phosphatase activity using several different soil types. In this study, glyphosate stimulated invertase and urease activity but showed up to a 98% reduction in phosphatase activity. These results indicate a variable response to glyphosate by the microbial community and highlight the impact of soil type, time, glyphosate history, and dosage rate on the microbial community's response to glyphosate.

All of the functions mentioned above are conventional, broad-scale measures of microbial activity. Although these measures provide useful information about glyphosate's effects on major indicators of microbial activity (e.g. respiration, nutrient cycling), these measures result in limited knowledge of bacterial functions possibly affected by glyphosate. Hence, there is a wide range of possible functional responses among rhizobacteria to glyphosate treatment that may be missed using these conventional methods (Imfeld and Vuilleumier, 2012; Jacobsen and Hjelmsø, 2014; Zabaloy et al., 2012).

Metatranscriptomic analyses, such as RNA-Seq, measure the expression of many genes and functions at once by detecting mRNA transcripts rather than focusing on a single functional response to a given treatment. Metatranscriptomics has been used in many instances to investigate microbial community functional diversity in various habitats, as well as to examine the microbial community response to a given environmental alteration (Damon et al., 2012; Goffredi et al., 2015; Mason et al., 2012; Qi et al., 2011; Shrestha et al., 2009; Urich et al., 2008; Zakrzewski et al., 2012). For instance, Urich et al. (2008) used a metatranscriptomic approach to simultaneously study the structural and functional diversity of a soil microbial community, and Shrestha