Potential microbial community impacts from increased use and application dose of glyphosate

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Background

Since its introduction in the 1970's, glyphosate (N-(phosphonomethyl) glycine) has become an active ingredient of agricultural herbicides in over 130 countries and is used on 150 food and non-food products (Battaglin et al., 2014). Glyphosate's success may be attributed to the introduction of genetically modified glyphosate-resistant (GR) soybeans in 1996, followed by maize and cotton. Prior to the introduction of GR crops, growers relied on several herbicides with different modes of action. The introduction of glyphosate and GR crops allowed growers to manage weeds post-emergent through foliar application. In addition, GR crops grown without tillage, known as conservation tillage, can reduce soil erosion and increase water adsorption (Dill, 2005; Nail et al., 2007).

In 2004, GR crops accounted for 85% soybeans, 60% cotton, and 18% maize planted in the United States (Dill, 2005). As of 2008, 96% of soybeans planted in the United States and 64% planted internationally were GR crops (Dill et al., 2008). Since the introduction of glyphosate in 1974, glyphosate-based herbicide volume has increased 100-fold globally and accounts for 56% of total global herbicide used for both GR and conventional crops (Benbrook, 2016; Coupe et al., 2016; Duke et al., 2012; Myers et al., 2016).

Glyphosate mode of action

Glyphosate is a phosphonate of organophosphorus compounds (Duke et al., 2012) and inhibits an enzyme known as 5-enolpyruvyl shikimate 3-phosphate synthase (EPSPS). EPSPS produces essential aromatic amino acids through a metabolic route known as the shikimate pathway. This pathway is found only in plants, some bacteria, algae, and fungi (Borggaard & Gimsing, 2008). Animals do not have a shikimate pathway and therefore do not contain the enzyme that is inhibited by glyphosate. However, emerging research suggests glyphosate may also impact other enzymes found in mammals and therefore further research is required to fully assess potential risks to people and animals (Gill et al., 2018; Krüger et al., 2014).

Weed Resistance

Increased glyphosate use has been attributed to the emergence of glyphosate-resistant weeds (Powles et al., 2008). By reducing the abundance of species susceptible to glyphosate, spaces for naturally resistant weeds were opened, such as tropical spiderwort (*Commelina benghalensis*) and Asiatic dayflower (*Commelina communis*). In addition, weeds have acquired glyphosate resistance, including ragweed (*Ambrosia artemisiifolia*), hairy fleabane (*Conyza bonariensis*), and horseweed (*Conyza canadensis*) (Nandula et al., 2005). To effectively eradicate glyphosate-resistant weeds, herbicides with a different mode of action are suggested.

Glyphosate in soil

Within 24 hours after foliar application, glyphosate is adsorbed by the plant and released by plant roots with little to no change in chemical structure (Kepler et al., 2018; Kremer et al., 2005). Glyphosate may also enter the soil when plant tissue degrads, or from direct applications to the soil (Duke et al., 2012). Once in the soil, glyphosate will bind to soil particles and is no longer bioavailable. However, glyphosate does not always adsorb directly to soil and instead can be metabolized by microorganisms (Al-Rajab & Schiavon, 2010; Borggaard & Gimsing, 2008; de Jonge et al., 2001; Prata et al., 2003).

Soil characteristics can influence glyphosate adsorption, especially soil organic matter and minerals containing iron, magnesium, aluminum, and iron oxides (Borggaard & Gimsing, 2008). In addition, if soil phosphorus concentrations are greater than approximately 0.06 pounds per acre feet^3 of soil, glyphosate will compete for sorption sites due to the phosphate component in glyphosate (de Jonge et al., 2001; Prata et al., 2003). If phosphorus binds to the available sites, then glyphosate will remain bioavailable in soil. Glyphosate may also become bioavailable depending on soil pH and may be more readily released from soil particles if the soil is a basic (pH 7.9) clay loam as opposed to an acidic (pH 5.7) sandy loam (Al-Rajab & Schiavon, 2010). The mineralization rates of glyphosate in clay loam soils can be nearly 8% higher than in sandy loam soils. Shallow aquifers below sandy and loamy soil with a low cation exchange capacity had lower concentrations (>0.1 micrograms per liter) of glyphosate, whereas higher concentrations between 0.1 and 0.7 milligrams per liter were found after rain events in Argentina soil (Peruzzo et al., 2008). This indicates that soils with low cation exchange capacity are less able to adsorb glyphosate in the soil and will instead leach through the soil profile or become available for microorganisms to metabolize (Candela et al., 2007). While many studies of glyphosate adsorption have been conducted in Europe, soil adsorption dynamics in Florida agricultural areas requires more research due to differences in climate, parent material, and other soil forming factors that contribute to different soil characteristics. Studies of glyphosate in Florida were primarily conducted between the 1980’s to early 1990’s and focused on exotic species eradication and glyphosate in water (Baird et al., 1983; Miles et al., 1997; Tanner et al., 1992). Further research in Florida would allow insight into glyphosate degradation by microorganisms that may impact agricultural areas.

Glyphosate degradation

Degradation of glyphosate rarely occurs in sterile soils and therefore the process is primarily attributed to microbial degradation (Borggaard & Gimsing, 2008). When glyphosate is degraded by microorganisms, aminomethyl phosphonic acid (AMPA) is released (Sviridov et al., 2015). AMPA can break down to inorganic phosphate and methylamine, and this inorganic phosphate may be utilized as an alternative phosphorus source by microorganisms (Borggaard & Gimsing, 2008; Forlani et al., 1999; Sviridov et al., 2015). However, it is unclear if the inorganic phosphorus accumulated from glyphosate degradation in soil is used by plants and microorganisms.

Glyphosate selection of microorganisms

Microorganisms that do not benefit plant growth or soilborne plant pathogens may increase if glyphosate is used continuously after 2 weeks (Johal & Huber, 2009; Kremer et al., 2009; Zhan et al., 2018; Ziobole et al., 2011). Bacterial and fungal taxa must acquire an enzyme to breakdown glyphosate due to an inhibition of the EPSPS enzyme in the shikimate pathway needed to produce amino acids. (Sviridov et al., 2015). The ability to breakdown glyphosate provides microorganisms with phosphorus in an otherwise phosphorus-limited environment. Microorganisms have been found to utilize glyphosate two weeks after glyphosate exposure (Kremer et al., 2009; Moore et al., 1983). Carbon dioxide concentrations, microbial activity, and AMPA residuals of glyphosate can indicate glyphosate degradation. Carbon dioxide production was 10-15% higher and AMPA 1.5 times higher in soils exposed to glyphosate for 6 and 10 years than soils that had never been exposed (Araujo et al., 2003). Microbial activity, measured in micrograms of fluorescein diacetate (FDA) hydrolysis gram^{-1} of soil, increased exponentially from 2.16 in soils that had never been exposed to 41.56 to 78.05 micrograms gram^{-1} of soil for soils with applications of glyphosate of 6 and 10 years. These results suggest microorganisms were stimulated by glyphosate application.

Soil bacteria that may increase in abundance after glyphosate exposure include *Bacillus cereus, Enterobacter sp., Flavobacterium sp., pseudomonas species, Arthrobacter sp., Comamonas odontotermitis,* and *Geobacillus caldoxylosilyticus* (Kremer et al., 2009; Zhan et al., 2018). Selected fungal communities include *Aspergillus niger, Mucor IIIR, Penicillium IIR, Penicillium chrysogenum, Scopulariopsis sp, Trichoderma harzianum, and Fusarium sp.* (Kremer et al., 2009; Zhan et al., 2018). These microorganisms are capable of utilizing glyphosate as an energy source and therefore have been found to be selected after glyphosate application (Zobiole et al., 2010). In addition, gram-negative bacteria have been speculated to increase in soil exposed to glyphosate. Resistance may be due to the cell wall structure, but requires further research (Liu et al., 2018)

Glyphosate and plant susceptibility to disease

Glyphosate may impact the abundances of bacteria and fungi with the shikimate pathway after high dose application rates and/or long-term use. For example, the biomass ratio of fungi to bacteria decreased by 38% after 100 days of glyphosate use at both recommended application dose rates (50 mg of glyphosate /kg soil) and high application dose rates (500 mg of glyphosate /kg soil) in Chinese agricultural soils (Liu et al., 2018). This suggests fungi in those soils had a higher sensitivity to glyphosate than bacteria, which could lead to a shift in fungal species present. For example, long-term applications of glyphosate (10 years) on GR soybean crops had higher root colonization by *Fusarium spp.* (Kremer et al., 2009; Zhan et al., 2018; Zobiole et al., 2011). *Fusarium sp.* are capable of utilizing glyphosate as a substrate (Martinez et al., 2018), therefore glyphosate application may potentially increase fusarium disease risk (Johal & Huber, 2009; Martinez et al., 2018).

Glyphosate-sensitive bacteria include rhizosphere-inhabiting manganese (Mn)-reducing fluorescent pseudomonads and Acidobacteria (Blagodatskaya et al., 2013; Ziobole et al., 2011). Both bacteria are common in agricultural soils and have been reported to decrease in abundance in response to single glyphosate applications on Mexican silt loam soil (Ziobole et al., 2011). This may be problematic for growers because a reduction of Mn-reducers suggests Mn, a trace mineral critical for plant disease defenses, is not available for plant uptake (Kremer et al., 2009; Johal & Huber, 2009). Acidobacteria can enhance plant growth, possibly due to iron acquisition and production of plant hormones (Kalam et al., 2017; Kielak et al., 2016; Lugtenberg and Kamilova, 2009). Reduction of both microorganisms in the presence of glyphosate have been correlated to increased disease incidences including Corynespora root rot of soybean, take-all of cereal crops, diseases caused by Xylella fastidiosa, and fusarium diseases (Johal & Huber, 2009).

Conclusion

Glyphosate-based products account for more than half of globally used herbicides (Benbrook, 2016; Coupe et al., 2016; Duke et al., 2012; Myers et al., 2016). However, glyphosate does not always adsorb directly to soil or remain on plant foliage and may instead be degraded by microorganisms (Al-Rajab & Schiavon, 2010; Borggaard & Gimsing, 2008; de Jonge et al., 2001; Prata et al., 2003). Emerging research suggests glyphosate in soil may reduce the abundance of some beneficial microorganisms that stimulate plant growth while increasing the abundance of microorganisms potentially associated with crop disease (Johal & Huber, 2009). Due to variabilities in glyphosate fate based on unique soil characteristics, the interactions of glyphosate and Florida agricultural soils require further research to allow insight in glyphosate degradation by microorganisms.

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