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Soil microbial community response to surfactants and herbicides in two soils

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ABSTRACT

The environmental impacts of herbicides on desirable plants and the soil biota are of public concern. The surfactants that are often used with herbicides are also under scrutiny as potentially harmful to soil biological systems. To address these concerns, we used two soils, a silt loam and a silty, clay loam from south central Missouri, to investigate the impacts of herbicides and surfactants on soil microbial communities using phospholipid fatty acid (PLFA) analysis. The surfactants used in this study were alkylphenol ethoxylate plus alcohol ethoxylate (Activator 90), polyethoxylate (Agri-Dex), and a blend of ammonium sulfate, drift reduction/deposition polymers and anti-foam agent (Thrust). The herbicides were glyphosate, atrazine and bentazon. Surfactants and herbicides were applied to soils at label rate, either alone or combined, to 4000 g soil per pot. The two soils differed in history, texture, some chemical characteristics and several microbial community characteristics. A few of the chemicals altered some of the components of the microbial community after only one application of the chemical at field-rate. The Cole County, MO silt loam showed larger changes in the microbial community with application of treatments. For the Boone County, MO silty clay loam, Activator 90, Agri-Dex and bentazon treatments increased microbial biomass determined by PLFA; Thrust decreased PLFA markers, bacteria to fungi ratio; and Agri-Dex at both rates decreased monounsaturated fatty acids. Changes in the microbial community due to herbicides or surfactants were minimal in this study of a single application of these chemicals, but could be indicators of potential long-term effects. Long-term studies are needed to determine the changes in the microbial community after several years of annual applications of herbicides and surfactants on a wide array of soil types and management practices.

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1. Introduction

Herbicides are routinely applied to more than 90% of most U.S. crops at rates varying from g to kg ha^{-1} (Gianessi and Reigner, 2007; Center for Food Safety, 2008; Singh and Ghoshal, 2010). In 2007, 84 and 35 million kg of glyphosate and atrazine applied in the U.S. (U.S.E.P.A., 2011), respectively, raise concerns regarding potential impacts on soil microbial activity and community structure. In addition, surfactants are often used with herbicides as additives to enhance foliar uptake of post-emergence herbicides (Liu, 2004). While some of these chemicals may not be applied directly to soils, a substantial amount may contact soil during application or rainfall events (Haney et al., 2000). Some herbicides, such as glyphosate, move little in soil and may be easily adsorbed to clay and organic matter and slowly degrade in water and soil (Ahrens, 1994; Pessagno et al., 2008; Barja and dos Santos Afonso, 2005). Others persist for many years in soil or enter groundwater (Kolpin, 1996; Boyd, 2000). Herbicide degradation is affected by soil factors including nutrient composition and content, pH, temperature and moisture (Weber et al., 1993). Like glyphosate, bentazon and atrazine are degraded by biological processes (Li et al., 2008). Atrazine-degrading microorganisms may accumulate in soil receiving frequent atrazine applications and coexist with the indigenous soil microbial community while metabolizing the herbicide (Satsuma, 2009; Zablotowicz et al., 2002). The addition of nonionic surfactants to soil with herbicides reduced herbicide degradation compared with the herbicides applied alone (Li et al., 2008). Herbicides and surfactants differ in chemical composition and react differently when incorporated into the soil system due to

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differences in chemical properties and interactions with soil components and environmental factors (Smith and Hayden, 1982; Ray et al., 1995).

Glyphosate and atrazine may reduce enzyme activity and populations of various organisms in soil (Toyota et al., 1999; Sannino and Gianfreda, 2001). Ratcliff et al. (2006) found that bacterial and fungal populations were altered with application of various herbicides. When pure herbicide (mesotrione as active ingredient) and formulated herbicide were added to soils, the microbial activity was affected by application of pure herbicide (mesotrione) and formulated herbicide only at rates 10 times and 100 times greater than the recommended rate (Crouzet et al., 2010). These chemicals can directly or indirectly affect microbial communities or sub-populations of the community and these changes can be expressed either as short-term or long-term effects (Bittman et al., 2005; Ratcliff et al., 2006; Dick et al., 2010). Soil management history can also affect microbial composition and responses (Girvan et al., 2003).

Microbial community structure, often used as an indicator in monitoring soil quality, is affected by various environmental and growth factors, such as moisture, temperature, nutrient availability, and management practices (Petersen et al., 2002). Assessment of microbial community composition within the soil ecosystem is helpful in determining if management practices and environmental conditions are aggrading or degrading to soils. Phospholipid fatty acid (PLFA) profiles, characteristic of soil microbial communities, differ with management practices including tillage, cropping system, and addition of various chemicals (Acosta-Martínez et al., 2010; Dick et al., 2010; Ratcliff et al., 2006; Ibekwe and Kennedy, 1998). The objective of this study was to examine the short-term effect of surfactants and herbicides on soil microbial community composition using PLFA analysis. We hypothesized that surfactants and herbicides added to different soils may alter soil microbial community structure as indicated by PLFA profiles and these profiles could provide an indication of potential long-term effects on the soil biota.

2. Materials and methods

2.1. Soils and chemicals

We conducted the study with soils from the A horizons of a Wrengart silt loam (20% clay; fine-silty, mixed, active, mesic Fragic Oxyaquic Hapludalfs) collected from the Lincoln University Carver Farm, Cole County, Jefferson City, MO (38°31′36.1″ N, 92°8′22.9″ W), and a Mexico silty clay loam (37.5% clay; fine, smectitic, mesic Vertic Epiaqualfs) collected from University of Missouri Bradford Farm, Boone County, Columbia, MO (38°53′48″ N, 92°12′23.5″ W). The soils were located 56 km (26 miles) from each other. The Cole County, MO silt loam prior to collection was under continuous tall fescue (*Festuca arundinacea* L.) with annual fertilizer applications of N-P-K (60-30-30) for no less than 5 years. The Boone County, MO silty clay loam was under permanent broomsedge grass (*Andropogon virginicus* L.) due to its low pH, and had not been fertilized recently. No known herbicide or surfactant applications had been made to the two sites prior to soil sampling. Bulk soils were air dried, sieved to pass a 2 mm screen and analyzed for chemical and physical characteristics (Buchholz et al., 1983; Table 1). The two soils differed in that Cole County, MO silt loam was slightly acidic with low CEC and high P and K, while the Boone County, MO silty clay loam was very acidic with a high CEC and low P and K.

Herbicides were applied at a dose simulating recommended field application rates (atrazine, 2.24 kg a.i. ha^{-1}; bentazon, 1.12 kg a.i. ha^{-1}; glyphosate, 0.84 kg a.i. ha^{-1}). Surfactants were applied at the recommended dose and at two times field application rates (alkylphenol ethoxylate plus alcohol ethoxylate [Activator 90] 2.3 L ha^{-1}; polyethoxylate [Agri-Dex] 2.3 L ha^{-1}; ammonium sulfate + drift reduction/deposition polymers + anti-foam agent blend [Thrust] 2.8 L ha^{-1}). Surfactant–herbicide combinations used in commercial formulations were applied at field application rates and included Activator 90 + glyphosate, Agri-Dex + atrazine and bentazon + Thrust. Non-treated soils served as controls.

2.2. Herbicide and surfactant treatments

To determine effects of surfactants, herbicides and surfactant–herbicide combinations on the soil microbial community, a greenhouse experiment was conducted twice. Two-gallon pots (20.3 cm dia. by 20.3 cm in height) were filled with 4000 g of air-dried soil, fertilized and limed in accordance with fertility recommendations for field corn (*Zea mays* L. cultivar 'Indenta') based on soil test analyses (Lory et al., 1998). Soils were brought to field capacity and watered daily to maintain field capacity. Surfactant and herbicide treatments were prepared at designated rates using deionized water and were applied directly to pot surface. Surfactants used in this study were Activator-90 (Actv), Agri-Dex (Agrd), and Thrust (Thrst). Herbicides used were glyphosate (Glyp), atrazine (Atrz), and bentazon (Bent). Application followed label rates for the surfactants and herbicides, and amounts per pot were for 4000 g soil per pot (Table 2). Six seeds of field corn were planted in each pot and later thinned to two plants per pot. Treatments were replicated three times and arranged in a randomized complete block design on greenhouse benches. The study was replicated twice with similar results each time and the second study is reported here.

Temperature in the greenhouse varied from 18 to 27 °C throughout the day. Supplemental lighting was used to increase the daylight period to 14 h light and 10 h dark. The corn grew equally well in each soil. Seven weeks after seeding, when small roots were found throughout the pot, the corn foliage was harvested by cutting at the soil surface and the roots were carefully removed from the soil. Samples were collected from root-free soil, stored in plastic bags at 4 °C and processed for PLFA.

2.3. Phospholipid fatty acid analysis

Phospholipid fatty acid analysis (PLFA) of the soil samples followed the methods of Bligh and Dyer (1959), modified by Petersen and Klug (1994). Reagents used in the procedure were high pressure liquid chromatography (HPLC) grade supplied by Sigma (St. Louis, MO) unless otherwise stated. Two-gram soil samples were added to Teflon-lined screw cap culture tubes and extracted. The total lipid extract was fractionated into glyco-, neutral, and polar lipids (Ibekwe and Kennedy, 1998). The polar lipid fraction was transesterified with mild alkali to recover the PLFA as methyl esters in hexane. Solid phase extraction was used to separate the samples for phospholipid analysis with 100-mg silica columns (Varian, Palo Alto, CA) as described in Pritchett et al. (2011). A gas chromatograph (Agilent Technologies GC 6890, Palo Alto, CA) equipped with a fused silica column, flame ionizer detector, and integrator was used to analyze fatty acid methyl esters. Integration and analysis of samples were operated with ChemStation software (Agilent Technologies GC 6890, Palo Alto, CA). Microbial Identification Systems, Inc. (Newark, DE) software provided parameters that were used for peak identification and integration of areas.

Peak chromatographic responses were converted to mole responses by using internal standards and recalculation of responses was done as needed. Various peaks were used as markers for bacteria as described in Pritchett et al. (2011). Briefly, peaks that correspond to carbon chain lengths of 12–20 carbons are generally associated with microorganisms and were handled