EFFECTS OF SELECTED SURFACTANTS ON NUTRIENT UPTAKE AND SOIL MICROBIAL COMMUNITY

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APPROVAL PAGE

The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

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presented by Mona-Lisa L. Banks,

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and hereby certify that, in their opinion, it is worthy of acceptance.

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DEDICATION

I dedicate this thesis to my father, Mr. Stanley Banks and in memory of my mother Mrs. Claudette Banks.

Mom and dad you both stressed the importance of education in us your children from an early age. You toiled to provide the financial and moral support we needed, in order to give us a decent foundation to our academic career. With this foundation, I stepped out into the world earning and striving to make you proud parents. This thesis reflects that dedication, and I say thank you for all your hard work and the confidence you have always had in me.

Rest in Peace Mother

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TABLE OF CONTENTS

LIST OF TABLES

Tables **Page**

LIST OF FIGURES

Figures **Page**

ABSTRACT

Surfactants with solvent and wetting abilities are used in the formulation of herbicides to enhance spraying capabilities. These chemicals eventually enter into the soil and may disrupt different chemical, physical and biological processes. The aim of this study was to examine the effects on nutrient uptake in corn and soil microbial community due to application of surfactants at different rates, herbicides, and surfactant-herbicide combinations in silt loam and silty clay loam soils. Surfactants used were Activator 90, Agri-Dex and Thrust. Herbicides used were glyphosate, atrazine, and bentazon. Corn was planted in fertilized soils and moisture levels maintained. After seven weeks, plant foliage were ground and stored for elemental analyses with Inductively Coupled Plasma Emission Spectrophotometer (ICP). Soil samples were analyzed with Polymerase Chain Reaction (PCR-DGGE) and Phospholipid Fatty Acids analyses (PLFA) to assess microbial diversity.

The treatments did not greatly affect nutrient uptake by corn. Visual differences between both soils and among treatments were observed with DNA band expression, although few significant differences due to treatments were found. PLFA biomarkers were variably affected by soils and different treatments relative to control. In general treatments did not significantly affect plant nutrient uptake or microbial community, perhaps as a result of the onetime application of small quantities of chemicals. Differences between both soils reflected the

overall contribution of soil texture, chemical and physical characteristics. Formulation additives should be considered when evaluating effects of pesticide applications on plant quality and soil biology.

CHAPTER 1

INTRODUCTION

Over the years, use of pesticides in crop production systems has increased about fouty fold (Ridgeway et al., 1978). These pesticides include insecticides, fungicides and herbicides. However, the use of these pesticides have raised concern due to potential negative impacts on the environment and human health. Herbicides are used to eradicate unwanted plants from crop fields and other locations. In the application of herbicide, it is often desirable to include chemicals such as adjuvants, which have solvent and wetting abilities to enhance spraying capabilities of the herbicide coverage over large areas. The Weed Science Society of America defines an adjuvant as any substance in a herbicide formulation that is added to the spray tank to modify the activity and application characteristics of the herbicide (Hazen, 2000). Two of the main types of adjuvants used with herbicides are surfactants and crop oil concentrates (COC). Crop oil concentrates are a mixture of a surfactant and non-phytotoxic oil that also reduces surface tension. This oil may be vegetable- or petroleum-based, and keeps the leaf surface moist longer than water, allowing more time for penetration of the herbicide into the targeted plant. This type of adjuvant is most widely used with post-emergent herbicides.

The word surfactant is derived from the term “surface active agent.” Surfactants are organic molecules, sometimes referred to as amphiphiles as they have both a hydrophilic head and a hydrophobic tail. Surfactants function at the interface between compounds with different solubility by lowering the surface tension of a liquid. In doing so, the herbicide mixture is altered to an oil-like mixture allowing for easier emulsifying, dispersing, spreading, and wetting. There are four basic groups of surfactants: anionic, cationic, nonionic and amphoteric. Anionic surfactants produce a negative charge and are the most widely used accounting for 50% of the world’s production (Salager, 2002). They are used in detergents, soaps, and wetting agents. Cationic surfactant produces a positive charge and is commonly used as a bactericide. Although it strongly attaches to plant surfaces it is highly phytotoxic when not mixed with other surfactants. Nonionic surfactants do not form an electrical charge and increase pesticide penetration through plant cuticles. Amphoteric surfactants can have either a positive or a negative charge depending on the pH of the spray solution. These surfactants are seldom used in agriculture (Czarnota & Thomas, 2006) but are widely used in pharmaceuticals and cosmetics (Nielloud & Marti-Mestres, 2000).

Surfactants have been widely studied over the years to examine their different effects on the environment. They have proven to be positive in helping to improve plant quality by reducing pest and weeds. However, negative effects are associated with high toxicity of surfactants to organisms in aquatic systems (Mann & Bidwell, 2001). The soil and its components carry out important roles

in the production of the food supply. The introduction of surfactants into the soil environment may cause some chemical reactions, which may interfere with the transport of nutrients and reduce quality of soil for microorganisms. Therefore, it is vital that research be carried out to examine the effects of the surfactants on plant nutrient uptake and on microbial populations and activities.

OBJECTIVES OF STUDY

1. Determine the effect of different surfactants on plant nutrient uptake. The constituents of plant samples analyzed with Inductively Coupled Plasma Emission Spectrophotometer (ICP) will determine the differences in macronutrient, micronutrient and trace element concentrations in corn plants as a result of different rates of applied surfactants. We hypothesize that treatments with different chemicals used will cause variations in nutrient uptake indirectly through direct effects on soil.

2. Examine the changes in quantity and diversity of soil microbial community using polymerase chain reaction combined with denaturing gradient gel electrophoresis (PCR-DGGE) in association with application of surfactants, herbicides and soil texture. We hypothesize that the treatments with different chemicals used will cause differences in soil microbial community.

3. Examine the effect of different surfactants, herbicides, and soil texture on soil microbial community composition using Phospholipid Fatty Acid Analysis (PLFA) technology. We hypothesize that the application of different surfactants at different rates in combination with different herbicides results in changes in soil microbial PLFA profiles using two different soils.

EXPERIMENTAL APPROACH

SOIL AND EXPERIMENTAL LOCATION

Two different soils, with silt loam and silty clay loam soil textural classes, were chosen and used in this study to examine the interaction with surfactant on soil microbial diversity. Silt loam soil was collected from the Lincoln University Carver Farm near the Moreau River in Jefferson City, Missouri. It is classified as a Wrengart silt loam (fine-silty, mixed, superactive, mesic Fragic Oxyaquic Hapludalfs), located on the terrace of the floodplain, with a slope of 5 to 9%, very deep and moderately well drained. Soil samples were collected from the soil surface to a depth of 30 cm. Particle size analysis for the silt loam was % sand: 15, % silt: 65, and % clay: 20. The clay soil was collected at the University of Missouri-Columbia Bradford Farm from a plot that previously had the topsoil removed to expose the claypan layer or argillic horizon. The soil at this location is classified as a Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualfs) with slope of 1 to 4% eroded and poorly drained. Collection was achieved within 35 to 85 cm of the original soil surface yielding samples with clay content of 30 to 60%. Subsequent soil textural analyses classified the collected soil textural class as a silty clay loam. Particle size analysis for the silty clay loam was % sand: 20, % silt: 42, and % clay: 37.5.

Representative soil samples were taken from the collection sites using hand-held push probe. Soils were analyzed for concentrations of total organic carbon, total nitrogen, soil test phosphorus, exchangeable potassium,

exchangeable calcium and exchangeable magnesium. Other properties such as organic matter content, pH, and cation exchange capacity were determined for both soils (Table 1.1). Soils were dried, passed through sieve to remove large particles and stored at 4°C prior to the greenhouse study.

The greenhouse study was conducted at the Lincoln University Dickenson Research Greenhouse. The greenhouse offered a controlled environment for the study with temperature and lighting settings at suitable conditions for growing field corn (*Zea mays* L. type 'Indenta'). Field corn was chosen as it is widely grown in Missouri. Pots were randomized on greenhouse benches (Table 1.3 and Figure 1.1). A controlled environment was used to isolate the treatment effects. Using the greenhouse versus planting in the field eliminated interference of factors that may have limited growth and nutrient uptake, such as pests, diseases, drought conditions, severe heat and cold temperatures.

SURFACTANTS AND HERBICIDES USED

Three extensively used surfactants were used in the study: alkylphenol ethoxylate plus alcohol ethoxylate (Activator 90; non-ionic; Loveland Industries, Inc., Greeley CO), polyethoxylate (Agri-Dex; non-ionic; Helena Chemical Company, Collierville TN) and a blend of ammonium sulfate, drift reduction/deposition polymers and anti-foam agent (Thrust; anti-foam agent; Loveland Industries, Inc., Greeley CO). These surfactants/adjuvants are classified differently based on their chemical structure. Activator 90 is a non-

ionic surfactant, Agri-Dex is non-ionic crop oil concentrate (COC) and Thrust is a blend of milled ammonium sulfate drift reduction/deposition and anti-foam agent. We examined the effects of surfactants alone as well as surfactants mixed and applied with herbicides. The herbicides used were glyphosate (Gly-4 Plus; Universal Crop Protection Alliance LLC, Eagan MN), atrazine (AAtrex; Universal Crop Protection Alliance LLC, Eagan MN) and bentazon (Basagran; Micro Flow Company LLC, Memphis TN). The surfactants and herbicides are paired together as seen in Table 1.2 as this is how they are commonly used in agriculture application. Glyphosate's mode of action is inhibition of the 5-enolpyruvylshikimimate-3-phosphate synthase (EPSPS) in the shikimic acid pathway. Glyphosate is adsorbed to soil particles, readily degraded by soil microbial organisms, and typically has a half-life of 50 days (Franz et al., 1997). Atrazine and bentazon function as photosynthesis inhibitors with half-lives of 60 and 20 days, respectively. Atrazine and bentazon are both degraded by microbial action and atrazine also by hydrolysis (WSSA, 2007).

Atrazine and bentazon were applied after planting. Glyphosate is a preemergent, burndown herbicide and was applied accordingly. Application of chemicals was carried out in accordance with the manufacturer's recommended rates except for treatments of surfactant only, where surfactants were applied at different rates (Table 1.2). Addition of these chemicals will help us to examine and understand their interaction with different nutrients, and soil microbial community diversity in different soils.

Table 1.1. Soil properties for silt loam and silty clay loam

Textural class	pH ($CaCl_2$)	OM %	Tot. org. C %	CEC $cmol_c\ kg^{-1}$	Tot. N $mg\ kg^{-1}$	Bray 1 P $mg\ kg^{-1}$	Exc. K $mg\ kg^{-1}$	Exc. Ca $mg\ kg^{-1}$	Exc. Mg $mg\ kg^{-1}$
Silt loam	5.61	2.18	1.27	12.4	0.122	47.08	107	1214	233
Silty clay loam	4.52	1.89	1.10	22.2	0.096	1.34	57	1524	252

*Abbreviation: Exc., exchangeable; org., organic; tot., total.

Table 1.2. Chemicals used and treatment rates applied to pots in greenhouse experiment (L ha^{-1})

	Treatment rates			
Treatments	**Surfactant**	**Surfactant x 2**	**Herbicide**	**Surfactant + Herbicide**
Activator-90	0.02 ml	0.04 ml		
Glyphosate			0.0065 ml	
Activator-90 + Glyphosate				0.02 ml + 0.0065 ml
Agridex	0.02 ml	0.04 ml		
Atrazine			0.0167 ml	
Agri-Dex + Atrazine				0.02 ml + 0.0167 ml
Thrust	0.01 g	0.02 g		
Bentazon			0.0083 ml	
Thrust + Bentazon				0.01 g + 0.0083 ml
Control	0	0	0	0

METHODS OF ANALYSES

Plant and soil nutrient analyses were done using the aqua regia method. The aqua regia method is adequate for breaking down plant material (Novozamsky et al, 1996) for analyses of nutrient concentration. Plants samples were digested using an Ethos EZ Microwave Digestion Lab-Station (Milestone Inc., Shelton, CT 06484 USA). Digested samples were diluted and appropriately stored for analyses. Total element analyses of the samples were determined using Inductively Coupled Plasma Emission Spectrophotometer (ICP). Total organic carbon and total nitrogen were determined by the combustion method using a LECO TrueSpec carbon/nitrogen analyzer (LECO Corporation, St. Joseph, MI, USA).

One of the significant roles played by soil microorganisms is metabolizing various chemical compounds introduced to the soil such as pesticides, fertilizers and toxic organic and inorganic substances (Susarla et al, 2002). It is important that we understand how different surfactants affect different microbial communities in the soil. Denaturing Gradient Gel Electrophoresis (DGGE) of Polymerase Chain Reaction (PCR), a fingerprinting technique was used to examine how microbial groups, based on specific gene sequences, change due to environmental and experimental factors. Microbial cell composition markers were characterized with the use of cell structure procedure based on detection of microbial Phospholipid Fatty Acids (PLFA). Both methods have proven to be of valid support to traditional methods (Ercolini, 2003).

GREENHOUSE EXPERIMENTAL LAYOUT

Table 1.3. Treatments applied and pot numbers used in randomizing the greenhouse experiment.

Treatment	Pot Number	Treatment	Pot Number	Treatment	Pot Number
Control	1 – 3				
Activator-90 alone	4 – 6	Agri-Dex alone	16 – 18	Thrust	28 – 30
Activator-90 X 2	7 – 9	Agri-Dex x 2	19 – 21	Thrust x 2	31 – 33
Gly-4	10 – 12	Atrazine	22 – 24	Bentazon	34 – 36
Activator-90 +Gly-4	13 – 15	Agri-Dex + Atrazine	25 – 27	Thrust + Bentazon	37 – 39

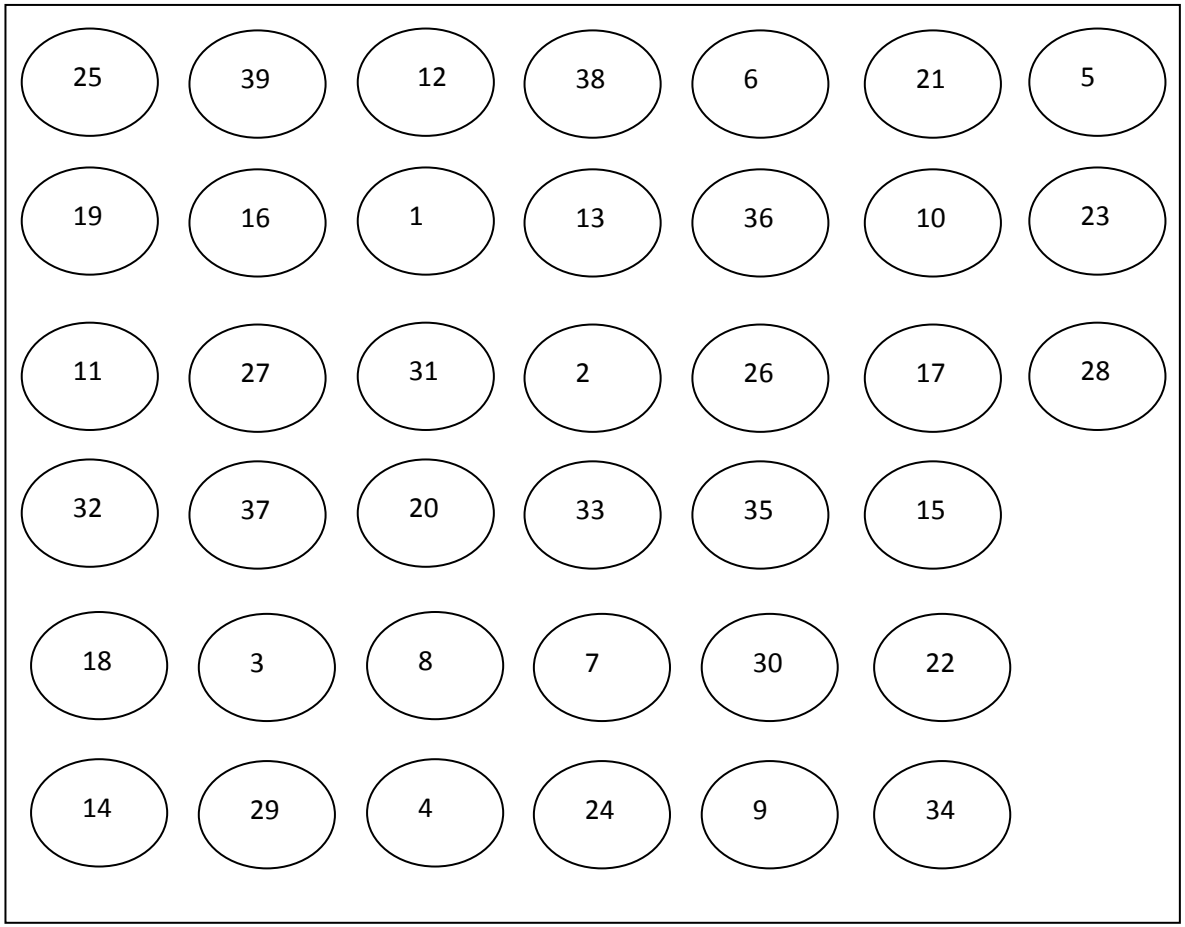

Figure 1.4. Diagram showing the randomization of pots used in the experiment in the greenhouse. This layout was used for both silt loam and silty clay loam soils.

Table 1.5. Table of abbreviations		
Abbreviation	**Meaning**	**Page**
ANOVA	Analyses of Variance	44
CLPP	Community Level Physiological Profiling	20
COC	Crop Oil Concentrate	1
DGGE	Denaturing Gradient Gel Electrophoresis	4
HLPC	High Pressure Liquid Chromotography	105
ICP	Inductively Coupled Plasma Emission Spectrophotometer	4
LSD	Least Significant Difference	108
PCA	Principal Component Analyses	108
PCR	Polymerase Chain Reaction	4
PLFA	Phospholipid Fatty Acids Analyses	4

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CHAPTER 2

LITERATURE REVIEW

SURFACTANTS AND SOIL

Surfactants are used in large amounts in different industries such as agriculture, detergents and cosmetics to modify spreading characteristics. When surfactants are used with agricultural chemicals they eventually enter into soil and water systems. Soil and water contamination with hazardous compounds has been a global problem. Surfactants entering the environment can possibly disrupt interactions of different chemical, physical and biological processes, which may affect aquatic and terrestrial life. Therefore, it is critical to understand the fate, behavior and transport of surfactants upon entering the ecosystem.

There have been several scientific studies on surfactants over the years. Because surfactants are organic molecules and subject to potential biodegradation, most compounds have half lives of about 3 weeks or less (Valoras et al., 1976). Valoras et al. (1976) examined the effects of soil moisture on the degradation of nonionic surfactants. They reported that the rate of surfactant degradation decreased when soil water content level was not suitable for microbial growth. He also found that it was unlikely for surfactants to

accumulate in high concentrations in the soil and that ground water pollution is decreased by microbial degradation of surfactants.

Surfactants have been useful in increasing the efficient and rapid uptake of herbicides into the cuticle of plant leaves (Reddy & Singh, 1992; Riechers et al., 1994). Surfactant interaction with various minerals have been studied to understand their different behaviors upon entering the soil. Adsorption of surfactants to clay particles has been reported in different studies (Hower, 1970; Sánchez-Martín et al., 2000). Adsorption of surfactants to clay surfaces depends on the chemistry of the surfactant and minerology of the clay. Ray et al. (1995) demonstrated that nonionic surfactants had a greater affinity and held more strongly to montmorillonite than kaolinite clays. Podoll (1987) showed that sorption of poly ethylene glycose to sediments was related to the fraction of clay and not the fraction of organic carbon.

Surfactants have also been widely studied as a source for reducing oil contamination in the environment. In a study by Dwarakanath et al. (1999) using laboratory soil column experiments, anionic surfactants served as a means of remediation for nonaqueous phase liquids (NAPLs) trapped in aquifers. Laboratory studies have been conducted on the remediation of various NAPLS such as gasoline, transmission fluid and other chemicals with the use of surfactants resulting in recoveries of ≤ 85%. (Ellis et al., 1986; Ang & Abdul, 1991).

Although there are studies demonstrating the benefits of using surfactants, there are some concerns on the negative effects of these products. Studies conducted by Peters et al. (1992) and Bourbonais et al. (1995) highlighted difficulties with surfactant precipitation and removing surfactant residues from soil. An important property of the soil is hydraulic conductivity or the ease in which water moves through the soil. The use of surfactants has been studied and has been recognized as a cause in decreased soil hydraulic conductivity (Renshaw et al., 1997) since surfactants are adsorbed into clays and organic materials. Adsorption and precipitation of surfactants to clay surfaces are caused by formed crystals, gels, and macroelmulsion rather than microemulsion that are better for transport and have a lower viscosity (Pope & Wade, 1995). Non-ionic surfactants may form admicelles on clay surfaces or micelles on voids of clays (Sánchez-Martín et al., 2000). This only occurs with uncharged surfactants as it is independent on specific charges. Brownawell et al. (1990) and Cano & Dorn (1996) found that the main factor involved in nonionic surfactant adsorption was by clay swelling as opposed to organic carbon content in the soil. This makes the compositon of the soil important to the adsorption of nonionic surfactants. Other problems detected included pore plugging and impermeability (Ziegenfuss, 1987) while attempting to use surfactant as a remediation agent**.** There are indications that abnormal ear growth and cob length in corn could possibly be caused by different surfactants (Nielsen et al., 2008). Growth abnormalitities in corn are caused by several factors such as temperature stress, nutrient difficiency, drought conditions, flooding, genetics of the plant, and poor

pollination. Most of these factors directly affect the soil and root system of plants.

SURFACTANTS AND MICROBIAL ACTIVITY

A large number of microbes exist in the soil environment; millions per gram of soil. There are different types of microbes in the soil, such as algae, protozoa, bacteria and fungi. The functions they perform are very complex and critical for nutrient and carbon cycling and other metabolic activities including plant growth promotion and disease suppression. Soil microbes obtain energy by decomposing organic residues in the soil (Paul & Clark, 1989). During metabolism, soil microorganisms convert nutrients from organic to inorganic forms for plant uptake. Microbes contribute to natural systems by sequestration of carbon dioxide, soil respiration, enzymatic activities, soil aggregation, organic matter decomposition, and nutrient mineralization (Smith, 1994). The process of nitrogen fixation is carried out exclusively by prokaryotic microorganisms. When conditions are compatible for plant and microbes, a symbiotic relationship for N fixation may develop. Soil microbial populations are influenced by the quality and quantity of substrate in the soil and by soil physical and chemical properties and climatic conditions. They are most active in warm, moist soils with temperatures ranging from 25°C to 35°C. Soil biological components are very responsive to changes in the environment making microbial diversity and their functions suitable measures for soil quality (Releeder et al., 2006). The production or diversity of microbial communities is affected by several

environmental and growth factors such as moisture, temperature, nutrient availability, and management practices (Petersen et al., 2002). Some studies have reported that soil texture was the main factor that governed soil microbial diversity (Girvan et al., 2003; Fang et al., 2005).

Surfactants are used in different quantities for various products such as agricultural pesticides, detergents and cosmetics to modify spreading characteristics (Schramm, 2001; Krogh et al., 2003). Surfactants are commonly used as a formulation component with herbicides and other pesticides to modify the effects or surface tension of other liquids. With application, these chemicals eventually enter soil and water systems (Buhler, et al., 1993). Surfactants entering the environment may possibly disrupt interactions of various chemical, physical and biological processes. Chemical, biological, and physical properties can be affected by changes in management practices, which can alter microbial populations and activities (Ekenler & Tabatabai, 2003; Griffiths et al., 2004). The addition of surfactants to the soil environment may alter microbial activity by impacting sorption sites in the soil, competing with nutrients, increasing solubility of pesticides, increasing toxicity or by serving as carbon sources for some components of the microbial community. This interaction depends on the chemical properties of the surfactant and the soil since different surfactants may affect different microbial communities (Oros-Sichler et al., 2007). It is likely that surfactant toxicity contributes to the composition of microbial community structure (Lozada et al. 2007). In this particular study, the community shaping was speculated to be attributed to the surfactants being degraded by certain

populations or toxicity toward other components. The effects of some nonionic surfactants vary in different soils as the half-life may be extended. This may affect microbial processes depending on the surfactant half-life and toxicity (Charnay et al., 2000) which could possibly affect plant growth and soil productivity.

Microbes are known to degrade natural and synthetic chemicals in the soil environment (Atlas, 1986). Some chemicals are more complex than others and may remain in the soil resulting in negative effects on various microbial populations (Zhang et al., 2010). Through the process of biodegradation, microorganisms reduce the effects of various contaminants and toxins on the environment (Ying, 2006). Due to microbes sensitivity to environmental changes, the application of chemicals may affect or alter the metabolic efficiency of microbes (Tardioli et al., 1997; Mechri et al., 2008).

It is important that we understand the variability of composition found within the soil ecosystem. In the past, there have been different labor intensive and culture dependent methods used to measure microbial diversity. These methods relied on determination of bacterial diversity based on phenotypic characteristics through processes of isolation, culture, and identification (Atlas, 1984). Some of these methods underestimated the quantity of microbes present due to partial detection or recovery of genetic information (Gafan et al., 2005). Plate counts and community level physiological profiling (CLPP) are often commonly used (Jenkinson and Powlson, 1976b; Turco et al., 1994). With

advances in technology, new procedures were developed to provide information on a wider range of soil microbial diversity and to examine these as biological indicators. Some of these procedures include phospholipid fatty acid (PLFA) and polymerase chain reaction - denaturing gradient gel electrophoresis (PCR-DGGE).

Phospholipid fatty acid (PLFA) differences in carbon chain length and phospholipids are useful to recognize various microbial species (Leckie, 2005). The variations detected based on PLFA profiles have been linked to changes in microbial community compositions (Baah et al., 1992; Cavigelli et al., 1995; Petersen and Klug, 1994). Management practices such as tillage, cropping systems and addition of various chemicals have altered microbial community structure based on PLFA profiles (Acosta-Martinez et al., 2010; Dick et al., 2010; Ratcliff et al., 2006; Ibekwe and Kennedy, 1998). Cationic surfactants were found to be more toxic to PLFA groups, with gram-negative affected more than gram-positive bacteria, and that the level of toxicity was related to sorption in the soil (Sarkar et al., 2009 and Nye et al., 1994).

Denaturing gradient gel electrophoresis (DGGE) is a fingerprinting technique used to observe and quantify uncultured bacteria based on distinctive gene sequences present within DNA extracted from soil. This technique has been utilized in assessment of various natural habitats since its introduction in the early 1990's (Muyzer et al., 2003; Vallaeys et al., 1997; Jensen et al., 1998; Siqueira et al., 2005; Sadet et al., 2007). Microbial populations have been

successfully characterized using this technique (McGaig et al., 2001). It separates different DNA sequences in the DNA mixture when the soil extract is passed through a chemical denaturant after the amplification of 16S rDNA by PCR.

Microorganisms contribute to overall ecosystem functioning through mediation of various metabolic processes, and therefore it is important that we understand the microbial response to the introduction of various synthetic chemicals in to the environment. Microbial communities or sub-populations of the community can be affected by these chemicals (Bittman et al., 2005; Ratcliff et al., 2006; Dick et al., 2010). Application of PLFA and DGGE-PCR analyses are ways to quantify the changes in microbial communities due to impacts of surfactants in the environment in a more extensive manner compared with traditional culture-based methods. Understanding the effects of surfactants on the diversity of microbial communities using these new technologies is therefore an area that is worth investigating.

SURFACTANTS AND HERBICIDES

The use of herbicides has been practiced as part of modern crop production systems since their introduction in the 1940's (Aldrich and Kremer, 1997). It has benefited crop production by eliminating unwanted weeds and minimizing competition for nutrients, space and light. In recent years, herbicide-

tolerant crops have been developed, which have some economic advantages and have sparked an increase in the use of herbicides (Burnside, 1992). The increased use of herbicides has become a major concern to human, animal and environmental health (Pimentel et al., 1992).

For weed management, herbicides and surfactants are utilized to improve weed control efficacy. Herbicides are used with formulation additives or surfactants as it modifies spreading and absorption characteristics of the herbicide (Schramm, 2001; Grogh et al., 2003). They are paired together based on chemical characteristics that enhance foliar uptake of post-emergence herbicides (Liu, 2004). This serves as one of the routes for synthetic surfactants to enter the ecosystem (Haney, et al., 2000). A substantial amount of different herbicides and surfactants are used commercially and all react differently in the soil system based on their chemical composition. This may result in different chemical, biological and physical processes in the soil that may altered when these chemicals interact with each other (Smith and Hayden, 1982; Ray et al.; 1995).

Degradation of herbicides in the soil takes place by both microbial and chemical processes (Fuesler & Hanafey, 1990). Microbes may use herbicide compounds as a source of carbon with the aid of enzymes during metabolism to convert them to forms that no longer exhibit herbicide characteristics. Li et al. (2008) reported biological degradation was key in the degradation of bentazon and atrazine. It was concluded that combined application of the herbicides and

formulation of individual herbicide with a nonionic surfactant reduced the degradation time of the herbicides. Hydrolysis is the chemical process that causes herbicides to sometimes change to a deactivated form in the soil when reacting with water. Many herbicides are absorbed and translocated in plants as a part of their mode of action. The more vigorous the plant is growing the more herbicide it will absorb. Herbicides have different modes of actions where inhibition of certain metabolic pathways takes place. Likewise, they differ in the half-lives and in the way they are degraded. Similar to surfactants, many herbicides are also adsorbed to soil particles. It is crucial to assess herbicide fate in the environment relative to toxicity, availability to soil biological processes including degradation, and persistence in soil. Adsorption of herbicides to soil particles greatly depend on herbicide chemistry, soil organic matter content, clay content, and other soil physicochemical properties (Liu et al., 2008).

Several factors affect the availability of herbicides to soil microbes involved in degradation such as soil nutrient composition and content, pH, temperature and moisture levels (Weber et al. 1993). Another concern is herbicide mobility in the soil, which is affected by the physicochemical and biological properties of the herbicides and soils (Weber et al., 2007). Sadeghi et al. (2000) found greater leaching of atrazine in silt loam compared to a sandy loam soil was a result of macropore flow mechanisms regardless of tillage practice. Other research showed that certain surfactants were useful in reducing the mobility of the specific herbicides which can result in increased degradation and decreased risk to the environment (Hua et al., 2009).

Herbicides may reduce enzyme activity and populations of various organisms in soil (Toyota et al., 1999; Sannino and Gianfreda, 2001). Some herbicides, such as glyphosate, are easily adsorbed to clay and can be degraded in different environments (Ahrens, 1994; Barja and dos Santos Afonso, 2005; Pessagno, et al.; 2008). Other herbicides, such as atrazine, tend to accumulate in the soil requiring consortia comprised of different microbial groups to sequentially degrade the herbicide (Satsuma, 2009; Zablotowicz et al., 2002). Haney et al. (2000) stated that glyphosate was directly and rapidly degraded by microbes, even at high rates of application.

However, many concerns have been raised about the effects of herbicides on microbial activity over the past several years (Upchurch et al., 1966; Charnay et al., 2000; Haney et al., 2000). Harris et al. (1995) found that herbicides did not affect soil microbial counts to the extent of different management practices, such as tillage, no-tillage, and burning of crop residue. Lupwayi et al. (2010) reported that continuous application of herbicides reflected more significant changes in soil microbial process. Changes have been detected in bacterial and fungal communities of soil due to application of various herbicides (Ratcliff et al., 2006). Charnay et al. (2000) found that the degradation of herbicides was decreased when formulated with different surfactants. Crouzet et al (2010) concluded in a study with pure herbicide and formulated herbicide that microbial activity was affected, but only when the chemicals were used at rates much greater than recommended. Haney et al. (2000) saw a direct correlation with enhancement of microbial activity and the amount of carbon and nitrogen added

through glyphosate treatments. Based on the literature, it would be useful to study the impact of herbicide and surfactant formulations on the soil microbial community structure, and nutrient uptake in soils with different texture and chemical properties.

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CHAPTER 3

EFFECTS OF SELECTED SURFACTANTS ON NUTRIENT UPTAKE IN CORN (*ZEA MAYS* L.)

INTRODUCTION

Surfactants (surface active agent) are synthetic chemicals used in various industries for their modification properties in formulation liquids allowing for easier emulsifying, dispersing, spreading, and wetting. These industries include agriculture, detergency, pharmacy, and cosmetics. North America is one of the leading consumers of surfactants and the world has seen a rapid increase in its usage (Underwood, 2000). In the agricultural industry, surfactants are used in the formulation of different pesticide and this has also increased in previous years (Ridgeway et al., 1987). They are common ingredients used for herbicide formulations to aid in the eradication of weeds. There are four different classifications of surfactants: anionic, cationic, nonionic, and amphoteric. They have proven to be positive in helping to improve crop quality by reducing pests and weeds (Haller and Stocker, 2003). Surfactants used with agricultural chemicals eventually enter into soil and water systems after application. These

chemicals have a half-life of three weeks or less (Valoras et al., 1976). Biodegradation of some of these chemicals by microorganisms may reduce adverse effects on the environment (Ying, 2006). Soil moisture influences the degradation of surfactants, with the rate of degradation decreasing with decreased water content as it was not suitable for microbial growth (Valoras et al., 1976). Degradation is determined by the chemical nature of the surfactant and the soil chemical environment relative to nutrients and oxygen (Ying, 2006; Ang & Abdul 1992). Surfactants entering the environment can possibly disrupt different chemical, physical and biological interactions. Therefore, it is critical that we understand the fate, behavior and transport of surfactants upon entering the ecosystem.

There have been studies over the years to examine effects on the environment as it pertains to plant, soil and water contamination. Positive aspects linked to the utilization of surfactants include their ability to increase the efficiency and rapid uptake of herbicides into the cuticle of plant leaves (Reddy & Singh, 1992; Riechers et al., 1994). Studies have also been conducted on their interaction with soil moisture as a means to improve water use efficiency and reducing nutrient loss in to the environment (Poulter, 2009). Surfactants have also been useful in the transport or mobility of the herbicide bentazon in soil. Hua et al. (2009) concluded that surfactants (non-ionic) were valuable in reducing the mobility of the specific herbicides which can result in increased degradation and decreased risk to the environment.

Although considerable efforts have examined how surfactants affect aquatic and other organisms, very little has focused on surfactant effects on plant nutrient uptake/translocation and plant growth. A study conducted on the use of nonionic surfactants showed that these compounds increased root growth (depth and volume) in corn (Brumbaugh and Pertersen, 2001). Pertaining to plant nutrition, a study by Walworth & Kopec (2004) with turfgrass and bermuda grass did not show either positive or negative effects on macronutrient and micronutrient uptake due to surfactant application.

Likewise, some negative effects have been associated with the use of surfactants especially in aquatic environments where nonionic surfactants caused an increase in toxicity (Mann & Bidwell, 2001). Composition of the soil is critical to the adsorption of surfactants as clay swelling is a main contributor to this process (Brownawell et al.,1990; Cano and Dorn 1996). Nonionic surfactants have shown a greater affinity and are held more strongly to montmorillonite than kaolinite clays (Ray et al. 1995). Its also been recorded that surfactants decrease hydraulic conductivity (Renshaw et al., 1997) as it adsorbed onto clays and organic materials. Surfactants used as remediating agents may cause pore plugging and impermeability (Ziegenfuss, 1987). Abnormalities in crops are generally caused by several factors such as temperature stress, nutrient deficiency, drought conditions, flooding, genetics of the plant and poor pollination. However, some reports suggest possible involvement of different surfactants in abnormalities in corn ear growth and cob length (Nielsen et al., 2008). Recently the surfactant alkylphenol ethoxylate, a

component of a pyraclostrobin fungicides applied to corn to control fungal diseases, was implicated as a causal agent of “arrested ear development” in corn that results in improper or incomplete development of the ear (Schmitz et al., 2011). Most reports of surfactant effects on plants deal with above-ground vegetative or reproductive development, while few have pertained to below-ground or root development and uptake.

Organic and synthetic surfactants as soil contaminants are influenced by humic substances (Buffle, 1988; Koopal et al., 2004). Humic substances are organic amphiphilic component of humus with functional groups, which play critical roles in plant nutrition and soil fertility. Humic substances interact with other molecules through different forces. The various chemical components cause humic substances to vary in solubility due to pH, molecular mass, surface activity, and aggregation (Piccolo and Mbagwu, 1989; Terashima et al., 2004; Gamboa and Olea, 2006). Cationic surfactants bind to humic substances at very low concentrations. The mobility of surfactant and humic substance complexes in soil or aquatic environment is affected by variable precipitation under different conditions (Ishiguro, 2007).

As in the case of surfactant use, the herbicides has increased over the last few decades due to their effectiveness in weed management in nearly all crop production systems. The main function of herbicides is to disrupt essential metabolic pathways that are vital for plant survival, specifically targeted at weeds. There are different categories of herbicides now available including selective, non-selective, pre-plant and post-plant herbicide classes. One advancement by

the herbicide industry was the combination of active ingredients with surfactants in herbicide formulations to ensure better mixing, uptake, spreading and retention on plant surfaces. Several environmental factors, such as moisture, soil type, nutrient content, pH, and temperature, influence the effectivenes of herbicides (Smith and Hayden, 1982). Herbicide degradation or longevity in soils is greatly influenced by microbial composition, moisture, and temperature (Laabs et al., 2002). Under aerobic conditions surfactants are easily degraded but tend to persist in anaerobic conditions (Fernández Cirelli, 2010). Such reactions may influence nutrient cycling in soil.

Studies have been conducted to evaluate the fate and transport of herbicides relative to physical, chemical and biological changes in the environment. Some studies have shown that microbial numbers are not affected by herbicides with different management practices (Harris et al.,1995). Haney et al. (2000) later reported that addition of glyphosate strongly correlated with carbon and nitrogen mineralization and did not negatively affect soil microbial activity. Glyphosate is strongly adsorbed to soil particles with variable sorption (as reflected by the sorption coefficient, Kd) across soil horizons (Farenhorst et al., 2009). Atrazine, one of the most extensively used herbicides, is widely considered persistent and may be detected in water bodies affecting aquatic species both directly and indirectly (Graymore et al., 2001). Soil texture and management have been shown to influence the sorption and movement of herbicides (Sadeghi et al., 2000; Rae et al., 1998).

The soil has an integral role in the production of the food supply. The introduction of surfactants into the soil environment may cause some chemical reactions or physical changes that may interfere with the transport and availability of nutrients to plants. Therefore the research objective was to examine the effects of surfactants on plant nutrient uptake by determining differences in micronutrient and macronutrient concentrations in corn plants treated with different application rates of surfactants and surfactant-herbicide combinations.

METHODS AND PROCEDURE

Soils and chemicals used in study

Soil samples collected were silt loam (20 % clay) from Lincoln University Carver Farm (38° 31’ 36.1” N, 92° 8’ 22.9” W) and silty clay loam (37.5 % clay) from University of Missouri Bradford Farm (38° 53’ 48” N, 92° 12’ 23.5” W). Soils were classified as Wrengart silt loam (fine-silty, mixed, active, mesic Fragic Oxyaquic Hapludalfs) and Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualfs), respectively. The silt loam soil 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 five years. The 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 plus 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 using standard procedures of the University of Missouri Soil and Plant Testing Laboratory (Buchholz et al., 1983). Soils were processed, air dried and analyzed for chemical and physical characteristics (Table 3.1). Surfactants used in this study were alkylphenol ethoxylate plus alcohol ethoxylate (Activator 90; non-ionic; Loveland Industries, Inc., Greeley CO), polyethoxylate (Agri-Dex; non-ionic; Helena Chemical Company, Collierville TN) and a blend of ammonium sulfate, drift reduction/deposition polymers and anti-foam agent (Thrust; anti-foam agent; Loveland Industries, Inc., Greeley CO). Herbicides used were glyphosate (Gly-

4 Plus; Universal Crop Protection Alliance LLC, Eagan MN), atrazine (AAtrex; Universal Crop Protection Alliance LLC, Eagan MN), and bentazon (Basagran; Micro Flow Company LLC, Memphis TN). Surfactants and herbicides were applied alone and in combination to both soils. Non-treated soils were controls (Table 3.2). Application followed label rates provided for the surfactants and herbicides, and calculated to per pot of 4000 g soil. Treatment mixtures of deionized water and chemical(s) were applied directly to potted soils. Some treatments were applied prior to planting or after planting depending on the herbicide associated with it; preemergent or postemergent.

Two-gallon pots (20.3 cm dia. by 20.3 cm in height) lined with polyethylene plastic bags, were filled with 4000 g of air-dried soil, fertilized and limed based on recommendations determined from soil test results. Fertilizers used in the study to meet soil test recommendations were ammonium nitrate (NH_4NO_3), ammonium phosphate ($(NH_4)_2HPO_4$), potassium chloride (KCl) and ammonium sulfate ($(NH_4)_2SO_4$) used for additional nitrogen half way through the growth period. Field capacity was calculated for each soil, were brought to field capacity and then watered daily to maintain field capacity levels of 21 and 24% for the silt loam and the silty clay loam, respectively. Untreated hybrid field corn (*Zea mays* L. type 'Indenta') was used as the experimental crop. Treatments were replicated three times and arranged in a randomized complete complete block design on greenhouse benches. In each pot, six field corn seeds were planted and thinned to two plants per pot after two weeks of growth. Temperature in the greenhouse varied from 18 to 27°C throughout the day.

Additional lighting was also provided to increase day light period. After seven weeks of growth from seeding (8-leaf stage - V8) the corn foliage was harvested by cutting at the soil surface. Fresh foliage weights were recorded and foliage allowed to dry at 70 °C for 72 hours after which the dry weights were recorded. Dried plants were ground and stored for digestion in sample bags. Soil samples were collected, air dried and passed through a 2 mm mesh sieve (number 10) and stored in soil sample plastic bags for digestion.

Digestion and analyses of samples

Digestion of foliage and soil samples was done using a microwave aqua regia acid digestion method with Nitric Acid-Perchloric Acid mixture (HNO_3-$HClO_4$) and Hydrochloric Acid- Nitric Acid-Hydrofluoric Acid (HCl-HNO_3-HF) mixture, respectively (Novozamsky et al, 1996; Papp and Fischer, 1987). Plant and soil samples of 0.25 g were weighed into vessels and acid mixture added. The teflon vessel was properly sealed and allowed to digest and cool for 30 minutes at 180 °C using an Ethos EZ Microwave Digestion Lab-Station (Milestone Inc., Shelton, CT 06484 USA). Each vessel was carefully opened after cooling and digested material filtered into a 25 ml flask. The volume of the digested material was diluted to 25 ml with deionized water. Diluted digested samples were stored at 4 °C until elemental analysis with an Inductively Coupled Plasma Emission Spectrophotometer (ICP). Samples were diluted three times prior to analyses. Along with samples, blanks, Standard Reference Materials (SRM) and automatic recalibration of the ICP were used as quality controls.

Elements analyzed were phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), aluminum (Al), chromium (Cr), sodium (Na), nickel (Ni), and lead (Pb). Total organic carbon and total nitrogen was determined by the combustion method using a LECO TrueSpec carbon/nitrogen analyzer (LECO Corporation, St. Joseph, MI, USA).

Statistical analyses were used to interpret the results for the plant and soil nutrient concentrations. The research was conducted as a factorial experiment (three surfactants at two different rates with control, and three herbicides) in a randomized blocked design. Each treatment was replicated three times. Analysis of variance (ANOVA) was calculated with the PROC GLM (SAS Institute, 2003) to examine the possible effects that surfactants might have on nutrient uptake. The Student Newman Kuels Test was used to compare treatment means at $P \leq 0.05$.

RESULTS AND DISCUSSION

There were differences in chemical and physical characteristics of the two soils used in this. The silt loam soil had a higher pH (0.01 M $CaCl_2$) of 5.61 compared to the silty clay loam soil pH of 4.52 (Table 5.1a), both not suitable for corn production. Also, the silt loam soil was higher in total organic C, N, P, and K compared to the silty clay loam. Cation exchange capacity values of the silty clay loam were double the CEC of the silt loam soil and Mg in the silty clay loam was higher than the silt loam.

Fresh weight and dry weight

In our analyses we examined fresh weight and dry weight for plants, and elemental concentrations in both plants and soils. Foliage fresh and dry weights were statistically similar among treatments (Figure 3.1, A and B). During the greenhouse study, it was generally noted that plants in the silty clay loam were smaller than plants grown in the silt loam, apparently reflecting differences in soil conditions.

Textural differences between the two soils likely contribute to differences in porosity, pore size distribution and moisture retention affecting air permeability with the silty clay loam soil being more subject to changes in these properties than the silt loam (Riley et al., 2005). Increased tortuosity and more tightly bound nutrients to soil particles are conditions that can affect plant growth. Soil with coarser particles may produce greater root growth and foliage growth than soils

with finer soil particles as root interception, mobility of nutrients, and air movement is not restricted (Lapen et al., 2001).

Greater variation in fresh weight accumulation by plants grown was apparent in the silty clay loam compared with silt loam where fresh weight biomass was more uniform across treatments. This implies that differences physiochemical properties of the soils may cause some variation in response to treatments in the silty clay loam (Kumar et al., 2004). This is expected as plants grown in soil with high clay contents may have some restrictions to conditions suitable for plant growth and nutrient availability. There were not visual signs of crop injury due to the treatments applied. In the early stages of growth (V4), the silty clay loam plants exhibited limited symptoms of phosphorus deficiency by displaying a slight purple color to leaves. After a few days symptoms were no longer evident suggesting that at early stages (V4), when growth is vigorous, the plant was lacking available phosphorus from the soil for translocation to established vegetative biomass due to limited root development. This can partly be attributed to the soil physiochemical state where nutrients are not readily released to soil solution for plant uptake (Bagyaraj et al., 2000; Richardson, 1994).

Plant Nutrient Concentrations

Proper plant growth greatly depends on efficient plant nutrient uptake from the soil solution. We examined various nutrients to determine specific treatment effects. Plant tissue N and (C:N) values were not significantly different between

either soils or for the treatments applied to the soils. However, in the silt loam (Figure 3.2A) N increased slightly for all treatments and C:N decreased when compared to the control. The increase in N concentration for plants grown in silt loam amended with the treatments may result from the use of added chemicals as food sources for soil microorganisms thereby contributing to increased mineralization of N (Jonasson et al., 2006). Similar values were seen in the silty clay loam (Figure 3.2B) but the same trend was not observed. Although there was a difference in plant fresh and dry weights, with highest biomass accumulated when grown in silt loam, the silty clay loam plants had slightly higher N concentrations. Silty clay loam properties including smaller pore space and greater water holding capacity may lead to greater retention of soil solution allowing the finer roots more time for direct access to the soil solution while, in the silt loam, the soil solution more readily percolates through the soil volume and likely leached to the bottom of the pot.

For the elemental concentrations determined by ICP, nutrients were categorized as macronutrients, micronutrients and trace elements. Plant macronutrient uptake in silt loam (Table 3.3) showed no significant difference between treatments but nutrient concentration was higher when compared to the control. For the surfactants, Activator-90 increased S (P=0.0001) in the silty clay loam (Table 3.4). No difference was observed for macronutrients with Agri-Dex application at the recommended rate in the silty clay loam for corn tissue. Thrust significantly decreased macronutrient uptake when applied at the recommended rates to the silty clay loam for Ca (P=0.0003), Mg (P=0.0076), and S (P=0.0001).

Silty clay loam receiving herbicide alone showed decreased plant macronutrient uptake with glyphosate for P (*P*=0.0003) and S, and in atrazine for S. Decreased macronutrient uptake has also been detected in previous studies (Ellis et al., 1983; Wilson and Stewart, 1973) in which herbicides also reduced nutrient uptake and resulted in plant growth being reduced.

As observed for macronutrients, micronutrient concentrations for corn grown in silt loam (Table 3.5) showed no significant differences, but most treatments increased nutrient uptake when compared to the control. An increase in Cu concentration was noted for all treatments when compared to the control in the silt loam soil. In the silty clay loam (Table 3.6), significance was only seen in Cu (*P*=0.0081) and Zn (*P*=0.0313) among the different treatments. Activator-90 at the recommended rate increased micronutrient uptake, while the combination of the Activator-90 and Gly-4 Plus decreased nutrient uptake. Glyphosate has been reported to immobilize cationic nutrients in soil and reduce uptake into several plants (Cakmak et al., 2009). It is interesting that apparent glyphosate nutrient immobilization was greatest in silty clay loam with initially low nutrient content compared with silt loam having higher nutrient contents. Thrust added to silt loam was also related to decreases in plant uptake of Mo and Zn.

Trace element (Table 3.7 and Table 3.8) concentrations were only significantly different between treatments for Na (*P*=0.0049) when plants were grown in silt loam soil. Treatments had variably effects on trace element uptake except for Ni where all treatments increased uptake when compared to the

control. In the silty clay loam, no significance was observed in the trace elements and concentrations of all trace elements varied among treatments.

The surfactant, Activator-90, seemed to have a generally positive effect on nutrient uptake as it increased the concentrations of micronutrients and macronutrients in plant tissue in both the silt loam and silty clay loam. The application of the Activator-90 at the double rate further increased macronutrient concentration in the silt loam but not in the silty clay loam. This was not the case for other surfactants used in the study. Agri-Dex alone and at the double rate increased plant tissue concentration of macronutrients in the silt loam but decreased them in the silty clay loam. Thrust decreased macronutrient concentration in the silty clay loam but was variable for the micronutrients and had variable effects on nutrient uptake the silt loam soil. Application of thrust at the double rate increased nutrient uptake with silt loam, but decreased for silty clay loam for both macronutrients and micronutrients. Overall, the effect on plant nutrient concentration due to application of surfactants at the double rate was not significantly different from that of the recommended rate.

Nutrient uptake (Tables 7.1 – 7.4) was variably affected by the different application rates of surfactants, herbicides, and combination treatments. However these effects were extremely small and not significantly different for most elements. This may be due to the low concentrations of chemicals applied under actual field rates that were also used in the study. The effects pose no significant positive or negative effect to nutrient uptake or plant growth in the two soils and one corn hybrid used in our study.

CONCLUSION

This study detected differences between soil types reflected in accumulation of foliage fresh and dry weight of corn grown for 37 days. Plants grown in the silt loam had higher fresh and dry weights than plants grown in the silty clay loam. The carbon to nitrogen ratio of plant tissue was slightly higher for the silt loam than the silty clay loam and also varied among the different treatments within each soil. Nutrient uptake values for plants grown in both soils did not differ greatly. Translocation of most of the nutrients was not significantly affected by the different treatments applied partly due to the small amounts of chemicals applied to soils in the study. However, we did observe that the silt loam soil had a general increase in plant nutrient concentration while a general decrease was observed for the silty clay loam soil. Surfactants applied at double rates did not have significant effects relative to the recommended rates. No consistent effect was observed for nutrient uptake in corn across the different surfactants, herbicides, and combination treatments.

Soil textural class coupled with the chemical nature of the surfactants and herbicides contributed to variations in nutrient uptake when compared to the controls. When assessing effects of herbicides on plant nutrient uptake, one should consider both active ingredient and formulation additives, including surfactants, used during field application.

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TABLES AND FIGURES

Table 3.1. Soil properties for silt loam and silty clay loam

Textural class	pH ($CaCl_2$)	OM %	Tot. org. C %	CEC $cmol_c\ kg^{-1}$	Tot. N $mg\ kg^{-1}$	Bray 1 P $mg\ kg^{-1}$	Exc. K $mg\ kg^{-1}$	Exc. Ca $mg\ kg^{-1}$	Exc. Mg $mg\ kg^{-1}$
Silt loam	5.61	2.18	1.27	12.4	0.122	47.08	107	1214	233
Silty clay loam	4.52	1.89	1.10	22.2	0.096	1.34	57	1524	252

*Abbreviation: Exc., exchangeable; org., organic; tot., total.

Table 3.2. Chemicals used and treatment rates applied to pots in greenhouse experiment (L ha^{-1})

	Treatment rates			
Treatments	**Surfactant**	**Surfactant x 2**	**Herbicide**	**Surfactant + Herbicide**
Activator-90	0.02 ml	0.04 ml		
Glyphosate			0.0065 ml	
Activator-90 + Glyphosate				0.02 ml + 0.0065 ml
Agridex	0.02 ml	0.04 ml		
Atrazine			0.0167 ml	
Agri-Dex + Atrazine				0.02 ml + 0.0167 ml
Thrust	0.01 g	0.02 g		
Bentazon			0.0083 ml	
Thrust + Bentazon				0.01 g + 0.0083 ml
Control	0	0	0	0

Table 3.3. Plant macronutrient concentration (%) for corn (V8 growth stage) grown in silt loam

Treatment	P	K	Ca	Mg	S
Control	0.19	1.90	0.48	0.39	0.16
Act	0.22	2.05	0.55	0.43	0.16
Act x 2	0.21	2.15	0.60	0.44	0.17
Gly-4	0.22	2.14	0.54	0.44	0.18
Comb	0.22	2.10	0.53	0.40	0.19
Control	0.19	1.90	0.48	0.39	0.16
Agri	0.22	2.23	0.54	0.42	0.16
Agri x 2	0.19	2.02	0.58	0.42	0.18
Atraz	0.20	2.19	0.55	0.40	0.16
Comb	0.22	1.88	0.54	0.39	0.19
Control	0.19	1.90	0.48	0.39	0.16
Thrust	0.22	1.77	0.51	0.45	0.16
Thrust x 2	0.22	1.90	0.55	0.45	0.16
Bent	0.22	1.96	0.55	0.38	0.18
Comb	0.21	1.76	0.50	0.35	0.16

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine. No significant differences (P=0.05) among treatments were detected based on Student Newman Kuels test.

Table 3.4. Plant macronutrient concentration (%) for corn (V8 growth stage) grown in silty clay loam

Treatment	P		K	Ca		Mg		S	
Control	0.20	abc	2.42	0.60	ab	0.43	a	0.16	b
Act	0.22	a	2.51	0.67	a	0.44	a	0.18	a
Act x 2	0.22	ab	2.37	0.54	bc	0.43	ab	0.16	bc
Gly-4	0.15	d	2.02	0.55	abc	0.38	ab	0.12	d
Comb	0.16	cd	1.95	0.47	bc	0.40	ab	0.13	d
Control	0.20	abc	2.42	0.60	ab	0.43	a	0.16	b
Agri	0.18	abcd	1.92	0.51	bc	0.44	a	0.15	bcd
Agri x 2	0.18	abcd	1.97	0.48	bc	0.35	ab	0.14	bcd
Atraz	0.17	cd	1.87	0.48	bc	0.37	ab	0.13	cd
Comb	0.18	bcd	2.03	0.49	bc	0.35	ab	0.14	bcd
Control	0.20	abc	2.42	0.60	ab	0.43	a	0.16	b
Thrust	0.17	cd	1.76	0.41	c	0.31	b	0.13	cd
Thrust x 2	0.16	cd	1.92	0.50	bc	0.37	ab	0.13	cd
Bent	0.17	cd	1.94	0.53	bc	0.40	ab	0.14	bcd
Comb	0.18	abcd	2.51	0.58	ab	0.46	a	0.13	cd

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine. Values within columns followed by same letters are not significantly different (P=0.05). Means were compared using Student Newman Kuel test.

Table 3.5. Plant micronutrient concentration (mg kg^{-1}) for corn (V8 growth stage) grown in silt loam

Treatment	Cu	Fe	Mn	Mo	Zn
Control	5.86	71.38	38.24	0.08	32.48
Act	6.70	75.81	45.85	0.53	38.26
Act x 2	6.72	74.42	49.19	0.27	38.74
Gly-4	6.54	73.16	39.87	0.40	34.82
Comb	6.18	70.78	42.32	0.58	35.12
Control	5.86	71.38	38.24	0.08	32.48
Agri	6.95	97.13	46.40	0.00	36.39
Agri x 2	5.89	152.43	44.04	0.43	28.19
Atraz	7.17	71.08	47.78	0.41	34.76
Comb	6.90	82.21	45.35	0.36	36.80
Control	5.86	71.38	38.24	0.08	32.48
Thrust	6.63	82.93	36.69	0.66	36.58
Thrust x 2	7.03	78.56	42.21	0.36	38.54
Bent	6.58	64.93	41.18	0.54	35.75
Comb	5.90	79.05	43.62	0.54	33.04

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine. No significant differences (P=0.05) among treatments were detected based on Student Newman Kuels test.

Table 3.6. Plant micronutrient concentration (mg kg^{-1}) for corn (V8 growth stage) grown in silty clay loam

Treatment	Cu	Fe	Mn	Mo	Zn
Control	7.29 ab	71.12	21.31	0.56	23.79 ab
Act	8.56 a	121.83	26.20	0.57	36.09 a
Act x 2	7.60 ab	79.88	22.48	0.24	23.98 ab
Gly-4	6.30 b	88.65	23.04	0.48	17.38 b
Comb	6.72 ab	57.23	19.90	0.52	19.51 b
Control	7.29 ab	71.12	21.31	0.56	23.79 ab
Agri	7.48 ab	70.70	22.46	0.58	19.94 b
Agri x 2	7.44 ab	73.18	22.23	0.53	28.66 ab
Atraz	6.53 b	187.78	19.07	0.6	18.38 b
Comb	7.15 ab	56.79	22.39	0.57	21.65 ab
Control	7.29 ab	71.12	21.31	0.56	23.79 ab
Thrust	7.40 ab	64.64	21.47	0.25	17.31 b
Thrust x 2	7.04 ab	60.65	26.48	0.46	18.21 b
Bent	7.17 ab	72.23	20.17	0.27	18.37 b
Comb	8.60 a	68.34	23.69	0.22	22.84 ab

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine. Values within columns followed by same letters are not significantly different (P=0.05). Means were compared using Student Newman Kuel test.

Table 3.7. Plant trace element concentration (mg kg^{-1}) for corn (V8 growth stage) grown in silt loam

Treatment	Al	Cr	Na		Ni	Pb
Control	69.70	0.60	72.14	ab	1.20	0.47
Act	92.45	0.60	83.52	ab	1.39	0.46
Act x 2	100.54	0.65	106.52	ab	1.50	0.58
Gly-4	86.00	0.62	76.82	ab	1.26	0.96
Comb	74.48	0.56	82.12	ab	1.41	0.51
Control	69.70	0.60	72.14	ab	1.20	0.47
Agri	91.12	0.73	133.58	a	1.77	0.81
Agri x 2	197.80	1.47	74.31	ab	4.79	0.38
Atraz	86.76	0.55	66.22	b	1.39	0.57
Comb	55.09	0.50	75.87	ab	1.38	0.21
Control	69.70	0.60	72.14	ab	1.20	0.47
Thrust	89.30	0.64	93.12	ab	1.40	0.43
Thrust x 2	85.42	0.67	82.65	ab	1.53	2.10
Bent	81.64	0.52	78.25	ab	1.35	0.89
Comb	60.12	0.63	70.81	ab	1.53	0.24

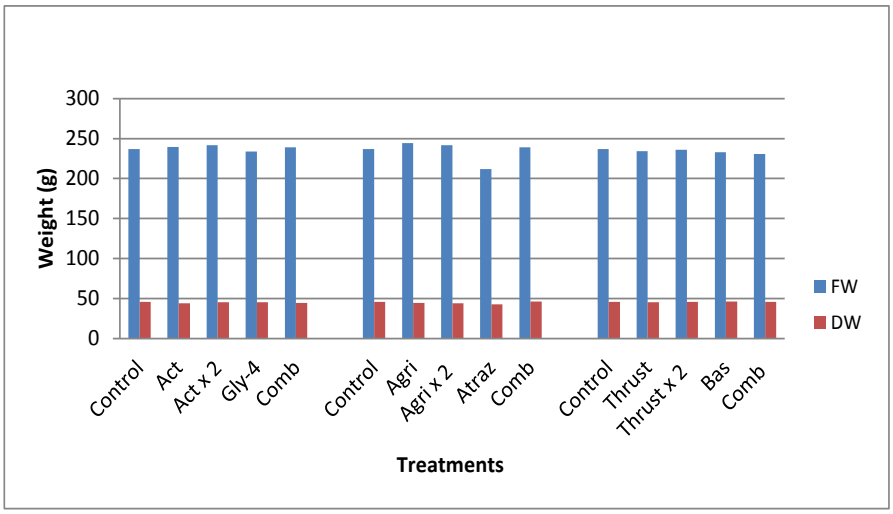
Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine. Values within columns followed by same letters are not significantly different (P=0.05). Means were compared using Student Newman Kuel test.

Table 3.8. Plant trace element concentration (mg kg^{-1}) for corn (V8 growth stage) grown in silty clay loam

Treatment	Al	Cr	Na	Ni	Pb
Control	35.20	0.86	117.17	2.81	1.04
Act	122.60	1.35	153.84	4.44	1.10
Act x 2	41.80	1.29	165.63	5.40	1.30
Gly-4	76.30	0.98	98.28	3.13	0.75
Comb	25.20	0.63	87.10	1.75	0.56
Control	35.20	0.86	117.17	2.81	1.04
Agri	29.90	0.92	183.29	3.23	0.87
Agri x 2	32.30	0.65	93.31	2.49	1.05
Atraz	309.70	1.50	145.13	6.16	0.70
Comb	25.90	0.66	134.46	2.58	0.69
Control	35.20	0.86	117.17	2.81	1.04
Thrust	17.80	0.67	74.10	2.61	0.16
Thrust x 2	30.20	0.56	131.00	1.45	0.62
Bent	27.90	1.05	116.71	4.18	1.19
Comb	55.00	0.00	108.02	1.88	3.68

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine. No significant differences (P=0.05) among treatments were detected.

A



B

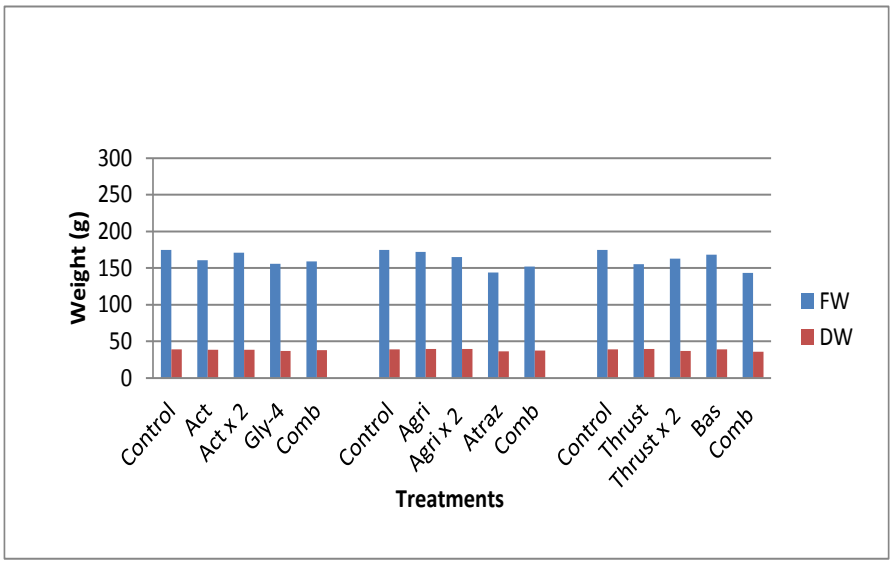
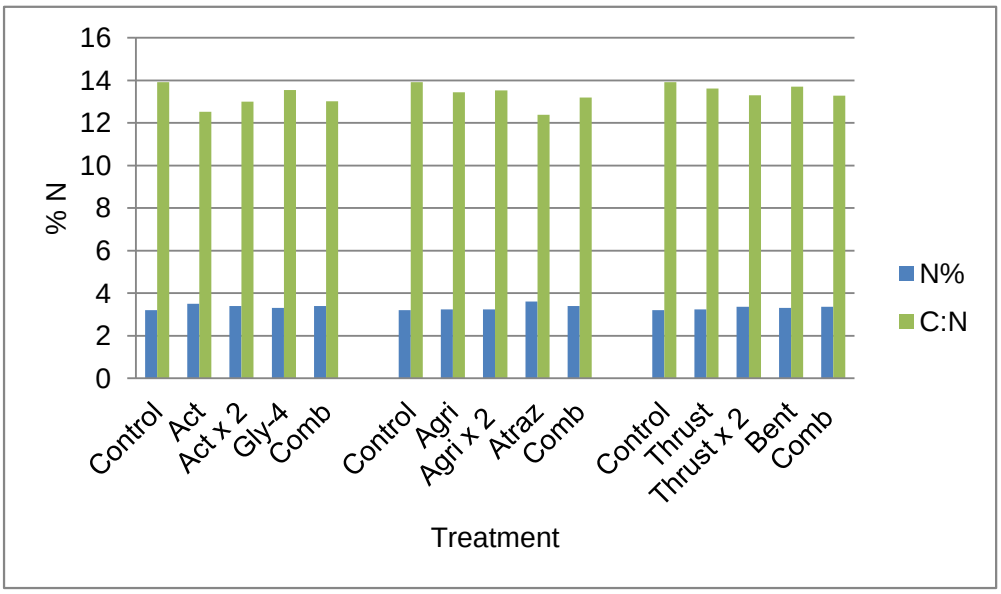


Figure 3.1. Foliage fresh weight (FW) and dry weight (DW) of corn grown in silt loam (A) and silty clay loam (B) soil. Corn plants were harvested at V8 growth stage (37 days after emergence). Act=Activator; Gly-4=glyphosate; Agri=Agri-Dex; Atraz=atrazine; Bent=Bentazon. No significant differences (P=0.05) between treatments were detected.



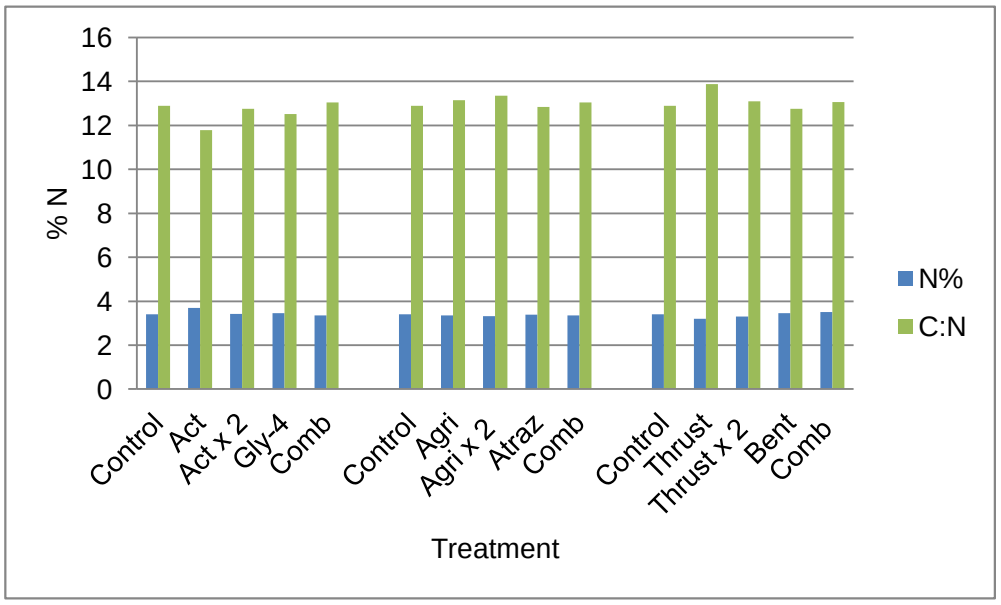


Figure 3.2. Percent nitrogen (N) and carbon:nitrogen (C:N) for silt loam (A) and silty clay loam soil (B). Corn plants were harvested at V8 growth stage (37 days after emergence). Act=Activator; Gly-4=glyphosate; Agri=Agri-Dex; Atraz=atrazine; Bent=Bentazon. No significant differences (P=0.05) between treatments were detected.

CHAPTER 4

EFFECTS OF SELECTED SURFACTANTS, HERBICIDES, AND SOIL TEXTURE ON DENATURING GRADIENT GEL ELECTROPHORESIS PROFILES (DGGE) OF THE SOIL MICROBIAL COMMUNITY

INTRODUCTION

Soil microorganisms play an important role in soil chemical, physical and biological processes. Soil biological parameters are often used as indicators to assess soil productivity and quality as affected by various management practices (Reeleeder et al., 2006). Soil microorganisms are critical components of the ecosystem and contribute to a wide array of ecosystem functions (Hackle et al., 2004; Wolters and Schaefer, 1996). Microorganisms have the natural capability to degrade chemicals (Atlas, 1986). Biodegradation of chemicals, such as surfactants and herbicides, by microorganisms is critical in reducing the efficiency and negative impacts of these chemicals on the environment. Degradation can be heavily influenced by environmental conditions and the chemical nature of the substances entering soil (Ying, 2006). However, the application of certain chemicals may result in alteration of metabolic efficiency

due to the sensitivity of soil microorganisms to environmental changes (Tardioli et al., 1997 and Mechri et al., 2008). Surfactants and herbicides are widely used in conventional cropping systems and these chemicals, due to their unique structures and properties, can react differently when contacting the soil system (Smith, 1982; Ray et al.; 1995).

Additions of chemicals, such as surfactants, may alter microbial activity by impacting sorption sites in the soil, increasing solubility of pesticides, increasing toxicity or serving as carbon or nutrient sources for some components of the microbial community. Surfactants are used in different industries, with widest use in detergents and agricultural pesticides. The main function of surfactants is modification of effects or surface tension of other chemicals with which they are combined. In agriculture, surfactants are used as formulation additives to different pesticides due to the capability to modify spreading characteristics (Schramm, 2001; Grogh et al., 2003). This serves as one entry point for surfactants appearing in the soil system. Once in the soil system, the chemical nature of the surfactants may change due to the chemistry of the soil and vice versa. There are different surfactants used in the industry and as a result different chemical, biological and physical process in the soil may be affected or altered as a result of the different surfactants used. Although soil microorganisms have the capability to naturally degrade some of the chemicals, due to the complex structures of some chemicals they may tend to linger in the soil system compared to naturally-occurring organic substances and possibly impose adverse effects on certain microbial groups (Zhang et al., 2010).

Degradation of certain surfactant compounds, such as the alkylphenol ethoxylate group, is mediated by microorganisms in soils and sediments after a period of metabolic acclimation (Ying, 2006).

In crop production, surfactant usage is often associated with use of herbicides. The surfactant and herbicides are combined together based on chemical factors that promote surface contact with plant surfaces and increase foliar uptake of post-emergence herbicides (Liu, 2004). Neither post-emergence herbicides nor surfactants are routinely applied directly to soils, but a substantial amount may contact soil during application or rainfall events (Haney, et al., 2000). Some herbicides such as glyphosate are easily adsorbed to clay and can be biodegraded in water and soil (Ahrens, 1994; Pessagno et al., 2008, Barja and dos Santos Afonso, 2005). Several factors, such as nutrient composition and content, pH, temperature and moisture levels, affect the availability of herbicides to soil microorganisms involved in degradation (Weber et al. 1993). Pesticides may reduce enzyme activity and populations of various organisms in the soil (Toyota et al., 1999; Sannino and Gianfreda, 2001). In a study done by Ratcliff et al. (2006), bacterial and fungal composition was altered due to the application of different herbicides.

Molecular biological techniques have made analyses of microorganisms less labor intensive while providing a more accurate or thorough description of microbial diversity in the environment. Cultural approaches once used to determine bacterial diversity examined phenotypic characteristics through a

process of isolation, culture, and identification (Atlas, 1984). In this approach, only a small portion of the total microbial population possessing incomplete genetic information was uncovered, thereby underestimating the actual quantity of microorganisms present (Gafan et al., 2005). A more advanced method now frequently used to reveal more detailed molecular/genetic information is polymerase chain reaction (PCR) combined with denaturing gradient gel electrophoresis (DGGE) or PCR-DGGE. The method was introduced to the field of microbial ecology in the early 1990's (Muyzer et al., 2003) and has been useful in a wide range of natural habitats (Vallaeys et al., 1997; Jensen et al., 1998; Siqueira et al., 2005; Sadet et al., 2007). Denaturing gradient gel electrophoresis (DGGE) is a fingerprinting technique used to observe uncultured bacteria by quantifying the amount of distinctive DNA present. It separates different DNA sequences in a mixture when passed through a chemical denaturant after the amplification of the conserved gene sequence in bacteria, 16S rDNA, by PCR. DNA sequences differ in base formation/composition giving them various thermal properties due to the bonding between complementary or neighboring base pairs. A staining process is used to visualize the different bands produced during electrophoretic separation. Soil microbial populations have been successfully characterized using this technique (McGaig et al., 2001).

Microorganisms contribute to overall ecosystem functioning through mediation of various metabolic processes, and therefore it is important that we understand the microbial response to the introduction of various synthetic chemicals in the environment. Our understanding of the effects of surfactants on

diversity of microbial communities is very limited, therefore this is an area that is worth investigating. The purpose of the study is to understand the association of surfactants, herbicides and soil texture on the quantity and diversity of soil microbial community using PCR-DGGE. We hypothesize that the treatments with different chemicals used will cause differences in microbial composition and activity.

MATERIALS AND METHODS

Soils and chemicals used in study

Bulk soil samples used in this study were a silt loam soil (20 % clay) from the Lincoln University Carver Farm (38° 31' 36.1" N, 92° 8' 22.9" W) and a silty clay loam soil(37.5 % clay) from the University of Missouri Bradford Farm (38° 53' 48" N, 92° 12' 23.5" W). Soils are classified as Wrengart silt loam (fine-silty, mixed, active, mesic Fragic Oxyaquic Hapludalfs) and Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualfs), respectively. No known herbicide plus 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 based on standard soil test procedures of the University of Missouri Soil and Plant Testing Laboratory (Buchholz et al., 1983) (Table 4.1). Surfactants used in this study were alkylphenol ethoxylate plus alcohol ethoxylate (Activator 90; non-ionic; Loveland Industries, Inc., Greeley CO), polyethoxylate (Agri-Dex; non-ionic; Helena Chemical Company, Collierville TN) and a blend of ammonium sulfate, drift reduction/deposition polymers and anti-foam agent (Thrust; anti-foam agent; Loveland Industries, Inc., Greeley CO). The herbicides used were glyphosate (Gly-4 Plus; Universal Crop Protection Alliance LLC, Eagan MN), atrazine (AAtrex; Universal Crop Protection Alliance LLC, Eagan MN) and bentazon (Basagran; Micro Flow Company LLC, Memphis TN). Surfactants and herbicides were applied alone, and in combination to both soils: non-treated soils served as controls (Table 4.2).

Application followed label rates provided for the surfactants and herbicides, and calculated to per pot of 4000 g soil. Treatment mixtures of deionized water and chemical(s) were applied directly to soils dispensed into the pots.

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 soil recommendations based from soil test results. Field capacity was calculated for each soil and soils in pots were brought to field capacity then watered daily to maintain field capacity levels. Field corn (*Zea mays* L. type 'Indenta') was used as the experimental crop. 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. Temperature in the greenhouse varied from 18 to 27 °C throughout the day with supplemental light to increase day light period.. After seven weeks of growth (from seeding) the corn foliage was harvested by cutting shoots at the soil surface, followed by careful removal of roots from the soil. At this point very small roots were present throughout the soil. Soil samples were collected from the root area by shaking roots into a bag. Field moist soil was stored in soil sample plastic bags at 4 °C and processed for analyses.

Microbial Analyses

Soils were air dried and passed through a 2-mm mesh sieve (number 10). Total organic carbon and total nitrogen was determined with combustion using a LECO TrueSpec carbon/nitrogen analyzer (LECO Corporation, St. Joseph, MI,

USA). Soil DNA was extracted from samples (0.25 g) using the Power Soil DNA® Isolation Kit (MO BIO Laboratories, Inc). Briefly, soil was added to a micro-bead suspension and vortexed for 10 min followed by centrifugation at 10,000 X g for 30 s; the supernatant was re-centrifuged after protein precipitation and then purified by passing through a spin column by centrifuging at 10,000 X g for 30 s. Soil DNA concentration (ng μl^{-1}) was determined using Gene Quant Pro UV spectrophotometer at 260 nm. An end volume of 50 µl was then stored for PCR reaction at -60°C.

Bacterial primers used to amplify extracted soil 16s rDNA fragments were F984GC-R1378 (Heuer et al., 1997). A PCR mixture of 50 µl consisting of each primer, DNA template and REDTaq ReadyMix was used for the PCR reaction. An Eppendorf Mastercycler Thermal Cycler (Perkin-Elmer) was used to carry out the PCR reaction. The reaction program used was 94°C for 4 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min and a final extension at 72°C for 10 min and then held at 4°C. PCR products of the same length but different internal sequences were obtained, which were then ready to be separated using DGGE. A 1% agarose solution in 1x TBE (Tris-borate, boric acid, and Na_2-EDTA) buffer with ethidium bromide was used to create a gel in an UVT tray. The gel was loaded into the electrophoresis tank and PCR standards and products carefully added to the gel. TBE buffer was added to the tank to cover the gel to a depth of 1mm. The tank was closed and connected to the power supply to allow DNA to move towards the red lead (positive anode) for 2 hours at 120 V.

A BioRad system with 8% (wt/vol) polyacrylamide gel with linear denaturing gradient urea formamide ranging from 37 – 57% was used to do the DGGE. The gel was placed in an Upper Buffer Chamber and preheated to 60°C before loading samples. DNA samples with 35 µl of the PCR products were carefully loaded into wells of the gel. Four known bacterial markers were used (*Bacillus cereus, Bacillus pumilus, Flavobacterium balustinum and Pseudomonas fluorescens*) and added to each side of the gel. The gel was run in 1x TAE buffer at 60°C at 130 V for 6.5 hours using BioRad Power Pac 300; removed and stained with SYBR green I (1:10,000 dilution) in TAE (Tris acetate, sodium acetate, and Na_2-EDTA) buffer before visualization in a GeneGenius Gel Document System under UV light. Genetool Software (Syngene) was used to digitize the band positions and intensity for each soil treatment and estimate diversity of microorganisms.

Statistical analyses were used to interpret the results for DNA, carbon, nitrogen, peak height and raw volume between the silt loam and silty clay loam soils. Analysis of variance (ANOVA) was calculated with the PROC GLM (SAS Institute, 2003) to examine the possible effects of different treatments on DNA, carbon, nitrogen, peak height and raw volume. ANOVA was used to determine significant effects and, where F-values were significant (P=0.05), mean separations were conducted using the Student Newman Kuels Test.

RESULTS AND DISCUSSION

There were differences in chemical and physical characteristics of the two soils used in this study which affects the biological properties and microbial community composition. The silt loam soil had a higher pH (0.01 M $CaCl_2$) of 5.61 compared to the silty clay loam soil pH of 4.52 (Table 5.1a), both not suitable for corn production. Also, the silt loam soil was higher in total organic C, N, P, and K compared to the silty clay loam. Cation exchange capacity values of the silty clay loam were double the CEC of the silt loam soil and Mg in the silty clay loam was higher than the silt loam.

DNA was extracted from soil and concentrations were quantified with a Gene Quant Pro UV spectrophotometer. Extracted soil DNA contents differed between the silt loam and silty clay loam soils with *P*-values of 0.0001 and 0.0487, respectively (Figure 4.1 and Table 4.3). Previous studies have shown that DNA extracted directly from soil may correlate with soil microbial biomass (Marstorp et al., 2000), therefore, the soil DNA contents detected in our study may reflect differences in microbial biomass between the silt loam and silty clay loam (Table 4.4). This is supported by differences found in microbial biomass for both soils determined by PLFA analysis (Chapter 5), data of which are presented for comparison in Table 4.4. Soil DNA measurements are apparently useful in differentiating effects of management practices on soil quality and productivity (Reeleeder et al., 2006; Udawatta et al., 2008). Introduction of herbicide formulations into soil as part of weed management practices may have similar

effects on microbial biomass measured as extracted DNA. Activator-90 applied at both rates, glyphosate alone, Activator-90 plus glyphosate, atrazine, Agri-Dex plus atrazine, and bentazon and bentazon plus Thrust resulted in significantly lower soil DNA contents than the control. Soil DNA in Agri-Dex alone treated soils was significantly higher than the control; Agri-Dex at 2X rate and both rates of Thrust also increased soil DNA, although not significantly. In the silty clay loam, mean separation showed no significant differences; atrazine treatment was associated with the highest concentration of soil DNA.

Increased soil DNA resulting from atrazine treatment is in agreement with a prior study where atrazine-degrading bacteria populations increased resulting in rapid degradation of atrazine (Martin-Laurent et al., 2003; Zablotowicz et al., 2006). Rapid degradation of atrazine may reduce weed control while minimizing movement of the chemical in the environment. Herbicide-alone treatments of glyphosate, atrazine and bentazon in the silt loam decreased DNA concentration when compared to the non-treated control. In the silty clay loam, glyphosate decreased DNA concentration. Herbicides may possibly adsorb to soil mineral and organic components. This process of sorption has been reported to limit microbial degradation due to decreased accessibility to microorganisms (Selim et al., 1999; Koskinen et al., 2001). Herbicides can affect soil DNA, as observed in other studies with gylphosate (Barriuso et al., 2010; Kremer and Means, 2009). These studies showed that glyphosate affected microbial communities and microbial processes associated with corn growth. All three surfactants applied at label and 2x rates in both soils showed similar effects on soil DNA concentration.

This suggests that addition of surfactants may affect soil biological community structure (Zhu et al., 2010). Overall, results imply that treatments differentially affected the microbial community, particularly with the silt loam.

Carbon (C) and nitrogen (N) were analyzed for both soils for all treatments (Figure 4.2 and Table 4.3). Carbon showed no significance in the silt loam or silty clay loam soils. Nitrogen showed significance in both silt loam and silty clay loam with $P = 0.0005$ and $P = 0.0009$, respectively. In the silt loam soil, the treatments of herbicides alone and combinations of herbicide and surfactant decreased N content when compared to the control. This indicated that these treatments affected the mineralization of N in the silt loam soil. Higher N contents were seen in some silty clay loam treatments receiving the same treatments. This could have been due to soil physical and chemical properties. Nitrogen mineralization is known to vary with soil type, physical and chemical soil properties and different environmental parameters (Kiese et al., 2002; Singh and Kashyap, 2007; Owen et al., 2010). Atrazine-degrading soil bacteria reportedly mineralize N from atrazine (Zablotowicz et al., 2006), however, the amount of N released from atrazine applied at label rate may not significantly contribute to overall soil N and the mineralized N may be readily scavenged by the soil bacterial community. Several studies report decreases in soil N content or in processes that contribute to N concentration in leguminous plants (Kremer and Means, 2009; Drew et al., 2007; Anderson et al., 2004).

The DGGE profiles prepared for both soils show representative lanes or tracks resolved in the gels for each treatment (Figures 4.3 and 4.4). Each track showed the separation of the amplified sequences of the DNA primer that differ according to individual bacterial sequence resulting in different bands representing the diverse bacterial components in the soil sample. Each band represents a different microbial species based on band position. Some bands are more prominent than others and are proportional with DNA intensity calculated as raw volume for each band (Figures 4.5 and 4.6). In Figures 4.5 and 4.6, representing quantified band patterns resulting from DGGE of extracted DNA from Thrust plus bentazon treated silt loam and silty clay loam, respectively, the numbers on the x axis are migration distances for each band; the y axis represents peak height for each band, determined on the basis of band density using the densitometer program in the Genetool software. The area under each peak is then determined and, with peak height, is used to calculate raw volume as a quantitative expression for each band. At times it is hard to interpret visually the differences between bands, and plots are a good way to quantify these differences. Visual differences of the DGGE profiles indicated likely differences in the bacterial communities between soils and between treated and non-treated soils. In each figure, there is a visual difference between the control track (group 1) and the treated tracks (group 2, 3, and 4). Icoz et al. (2008), in his study with different varieties of corn with DGGE analyses, reported that there were no differences between varieties for microbial diversities but reported seasonal differences. Ying (2006) suggested that upon exposure to surfactants of the

alkylphenol ethoxylate class (Activator 90), environmental microbial communities undergo a period of acclimation before the onset of degradation. This suggests a possible shift in microbial community composition to include a component capable of degrading the surfactant compound, which may appear as an additional DNA band on the DGGE pattern thereby altering community structure for groups 2, 3, and 4.

Within the different groups, there are differences within each track with the peak height and raw volume of the prominent bands. Cumulative peak height and raw volume of bands for each track were analyzed. Both silt loam and silty clay loam soil showed significant differences among the treatments applied to each soil (Table 4.5). In the silt loam, the control DNA band measurements were significantly less than for herbicide and surfactant treatments (P = 0.0001). There were no significant differences between treatments but variations due to treatments were apparent. In the silty clay loam, significant treatment effects were detected for band height and raw volume (P = 0.0001). Agri-Dex and Thrust at both rates significantly affected height and raw volume. Atrazine alone and atrazine plus Agri-Dex showed significance in height. Microbial response to combinations of atrazine plus Agri-Dex also significantly differed relative to raw volume measurements. Examination of the bands or data showed variations between the chemicals and different rates applied compared to control. Comparing the herbicide alone treatments to the formulated treatments, there was an increase with formulated treatments for peak height and raw volume. This suggests that formulated herbicides may stimulate bacterial growth. Ros et

al. (2006) reported that formulated atrazine applications at different rates increased bacteria numbers and resulted in different banding patterns. Furthermore, Zablotowicz et al. (2006) suggested that atrazine-degrading bacteria in soil increased >1000 times on exposure to atrazine at field rates, which could likely affect microbial community composition detectable by soil DNA analysis. Also, glyphosate released in rhizospheres of corn and soybean (*Glycine max* (Merr.) L.) enhanced populations of selected *Pseudomonas* and *Agrobacterium* species (Kremer and Means, 2009), which might also influence soil DNA profiles.

Examination of the data comparatively for both soils, differences between both soils for raw volume are readily apparent. The silt loam exceeded silty clay loam in DNA band contents expressed as raw volume regardless of treatment (Table 4.5). This indicates that soil texture is a main factor affecting diversity of the soil bacterial community, as demonstrated in previous studies (Fang et al., 2005; Girvan et al., 2003; Marschner et al., 2001).

CONCLUSION

Soil DNA contents significantly differed due to the various treatments added in the silt loam. In the silty clay loam soil, atrazine treatment was associated with the highest DNA concentration. This suggests that the atrazine degrading bacteria population was increased due to atrazine mineralization resulting in an overall higher soil DNA concentration. In both soils, herbicide-alone treatments other than atrazine and bentazon in silty clay loam decreased DNA concentrations. The addition of all three surfactants caused positive, negative and neutral effects on soil DNA. Varied results were also seen for C and N in silt loam and silty clay loam when compared to the control. Decreased total N suggesting reduced mineralization was observed with glyphosate, atrazine and bentazon treatments in the silt loam compared to control. The opposite was observed in the silty clay loam suggesting that soil properties affected N mineralization.

Visual differences were seen in the DGGE profiles between the silt loam and silty clay loam soil. Differences with the different treatments were observed based on the intensity of the different DNA bands. Non-treated control differed in DNA patterns from treatments, expressed as height and raw volume of DNA extracted from silt loam. In the silty clay loam, there were significances with the control and treatments. Also in the silt loam, formulated treatments seemed to stimulate bacterial growth compared to herbicide alone treatments. Difference between both soils for DNA expressed as raw volume reflected the contribution

of soil type to diversity of microbial community. Differences among treatments were mostly nonsignificant, however, trends for differential effects were noted. The lack of definitive treatment effects may be due to the application to soil of small quantities of chemicals, which represented actual field rates. The application of PCR-DGGE techniques was useful to examine differences between both soils and the different treatments applied. Based on this study in a controlled greenhouse environment, these results should be considered in further more rigorous field studies.

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TABLES AND FIGURES

Table 4.1. Soil properties for silt loam and silty clay loam

Textural class	pH ($CaCl_2$)	OM %	Tot. org. C %	CEC $cmol_c\ kg^{-1}$	Tot. N $mg\ kg^{-1}$	Bray 1 P $mg\ kg^{-1}$	Exc. K $mg\ kg^{-1}$	Exc. Ca $mg\ kg^{-1}$	Exc. Mg $mg\ kg^{-1}$
Silt loam	5.61	2.18	1.27	12.4	0.122	47.08	107	1214	233
Silty clay loam	4.52	1.89	1.10	22.2	0.096	1.34	57	1524	252

*Abbreviation: Exc., exchangeable; org., organic; tot., total.

Table 4.2. Chemicals used and treatment rates applied to pots in greenhouse experiment (L ha^{-1}**)**

	Treatments rates			
Treatments	**Surfactant**	**Surfactant x 2**	**Herbicide**	**Surfactant + Herbicide**
Activator-90	0.02 ml	0.04 ml		
Glyphosate			0.0065 ml	
Activator-90 + Glyphosate				0.02 ml + 0.0065 ml
Agridex	0.02 ml	0.04 ml		
Atrazine			0.0167 ml	
Agri-Dex + Atrazine				0.02 ml + 0.0167 ml
Thrust	0.01 g	0.02 g		
Bentazon			0.0083 ml	
Thrust + Bentazon				0.01 g + 0.0083 ml
Control	0	0	0	0

Table 4.3. The effect of silt loam, silty clay loam and herbicide/surfactant treatments on DNA, total organic carbon, and total nitrogen contents. Different letters after values within each column indicate statistical difference at P = 0.05 based on Student Newman Kuels Test.

		Silt loam			Silty clay loam		
	Treatments	DNA (ng/µl)	Total Carbon %	Total Nitrogen %	DNA (ng/µl)	Total Carbon %	Total Nitrogen %
Group 1	Control	10.90 bc	1.19 a	0.14 ab	6.17 ab	1.24 a	0.12 bc
Group 2	Act	3.93 ef	1.19 a	0.14 ab	5.80 ab	1.18 a	0.10 c
	Act x 2	5.27 ef	1.19 a	0.14 ab	7.67 ab	1.26 a	0.14 ab
	Gly-4	9.37 cd	1.19 a	0.13 b	5.73 ab	1.08 a	0.13 abc
	Comb	6.60 def	1.18 a	0.13 b	7.83 ab	1.07 a	0.11 bc
Group 3	Agri	14.90 a	1.21 a	0.15 ab	8.17 ab	1.13 a	0.13 ab
	Agri x 2	14.23 ab	1.19 a	0.14 ab	6.70 ab	1.18 a	0.15 a
	Atraz	7.63 de	1.19 a	0.13 b	9.23 a	1.11 a	0.13 ab
	Comb	7.23 def	1.17 a	0.13 b	7.97 ab	1.15 a	0.12 abc
Group 4	Thrust	13.57 ab	1.18 a	0.14 b	7.30 ab	1.12 a	0.12 abc
	Thrust x 2	12.57 ab	1.17 a	0.14 b	7.30 ab	1.09 a	0.12 abc
	Bent	6.63 def	1.18 a	0.13 b	7.03 ab	1.13 a	0.14 ab
	Comb	5.73 def	1.17 a	0.13 b	6.40 ab	1.09 a	0.11 bc

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate.
Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine.
Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine.

Table 4.4. Comparison of soil microbial biomass (MB) based on PFLA analysis (Chapter 5) and soil DNA contents. Different letters after values within columns indicate statistical difference at P = 0.05 based on Student Newman Kuels Test

	Silt loam				Silt clay loam			
	DNA (ng/µl)		MB (mol%)		DNA (ng/µl)		MB (mol%)	
Control	10.9	bc	119.8	b	6.17	ab	75	c
Act	3.93	ef	108.5	b	5.8	ab	122.3	bc
Act x 2	5.27	ef	377.2	a	7.67	ab	118.1	bc
Gly-4	9.37	cd	144.3	b	5.73	ab	92.4	c
Comb	6.6	def	153.1	b	7.83	ab	111.5	bc
Agri	14.9	a	151.5	b	8.17	ab	255.6	ab
Agri x 2	14.23	ab	119.3	b	6.7	ab	221.1	abc
Atraz	7.63	de	164	b	9.23	a	99.5	bc
Comb	7.23	def	88.7	b	7.97	ab	146.3	bc
Thrust	13.57	ab	141.8	b	7.3	ab	104.8	bc
Thrust x 2	12.57	ab	109.4	b	7.3	ab	125	bc
Bent	6.63	def	219.5	ab	7.03	ab	305.4	a
Comb	5.73	def	77.3	b	6.4	ab	156.6	abc

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine.

Table 4.5. The effect of silt loam, silty clay loam and herbicide/surfactant treatments on height and raw volume DNA separated by PCR-DGGE analyses. Different letters after values within columns indicate statistical difference at P = 0.05 based on Student Newman Kuels Test.

		Silt loam					Silty clay loam				
	Treatments	No. of bands	Height		Raw Vol.		No. of bands	Height		Raw Vol.	
Group 1	Control	15	92.6	b	5375.8	b	14	92.6	cd	5172.7	cde
Group 2	Act	21	120.2	a	7064.8	a	15	80.8	d	4402.8	e
	Act x 2	24	136.7	a	8000.4	a	17	106.8	bcd	5867.9	bcd
	Gly-4	25	131.9	a	7718.9	a	16	110.9	bc	5903.3	bcd
	Comb	23	121.3	a	7048.4	a	20	116.6	abc	6335.1	abc
Group 3	Agri	23	128.7	a	7408.4	a	21	129.6	ab	6874.4	ab
	Agri x 2	22	129.7	a	7457.7	a	23	142.6	a	7516.9	ab
	Atraz	25	144.2	a	8309.0	a	23	122.2	ab	6457.2	abc
	Comb	24	128.4	a	7496.2	a	26	125.8	ab	6756.6	ab
Group 4	Thrust	23	140.5	a	8208.0	a	21	127.5	ab	6974.3	ab
	Thrust x 2	25	133.7	a	7814.9	a	24	128.8	ab	7104.1	ab
	Bent	22	125.4	a	7371.1	a	22	92.9	cd	5157.5	cde
	Comb	23	127.2	a	7435.8	a	22	107.3	bcd	5904.8	bcd

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate.
Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine.
Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine.

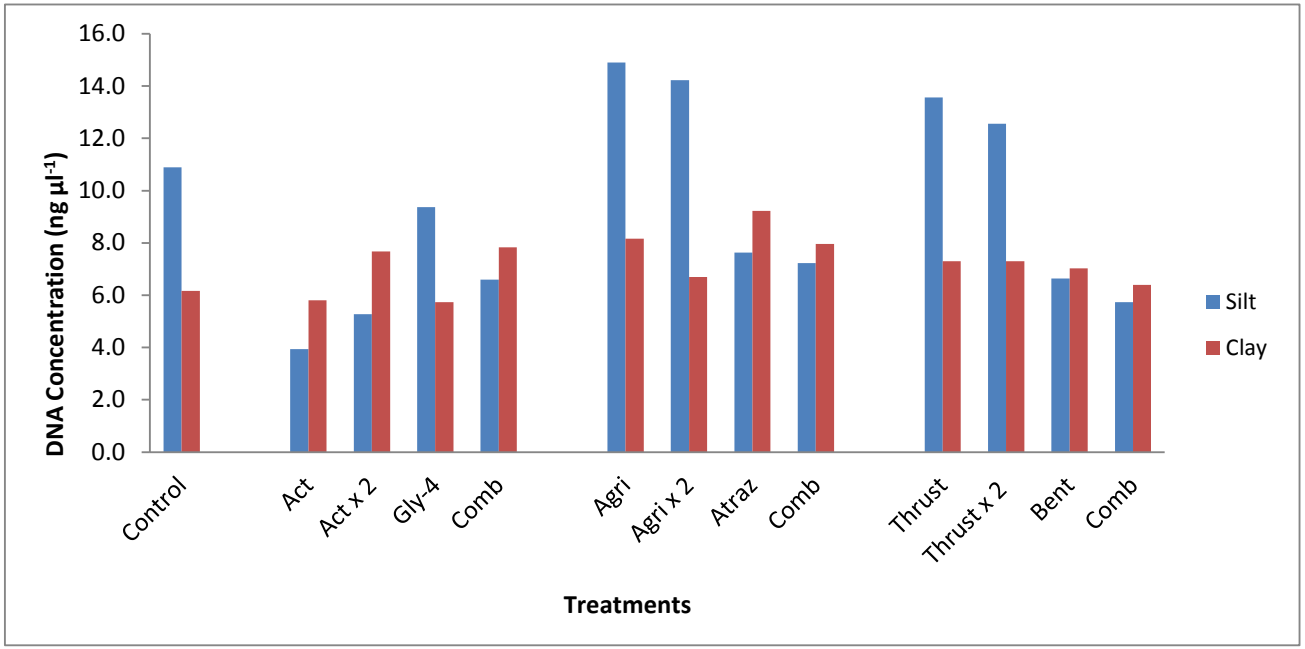

Figure 4.1. Graph of DNA concentration for silt loam and silty clay loam soil. Extracted soil DNA showed significance in both silt loam and silty clay loam with *P* values of 0.0001 and 0.0487, respectively. Statistical difference based on Student Newman Kuels Test of P = 0.05.

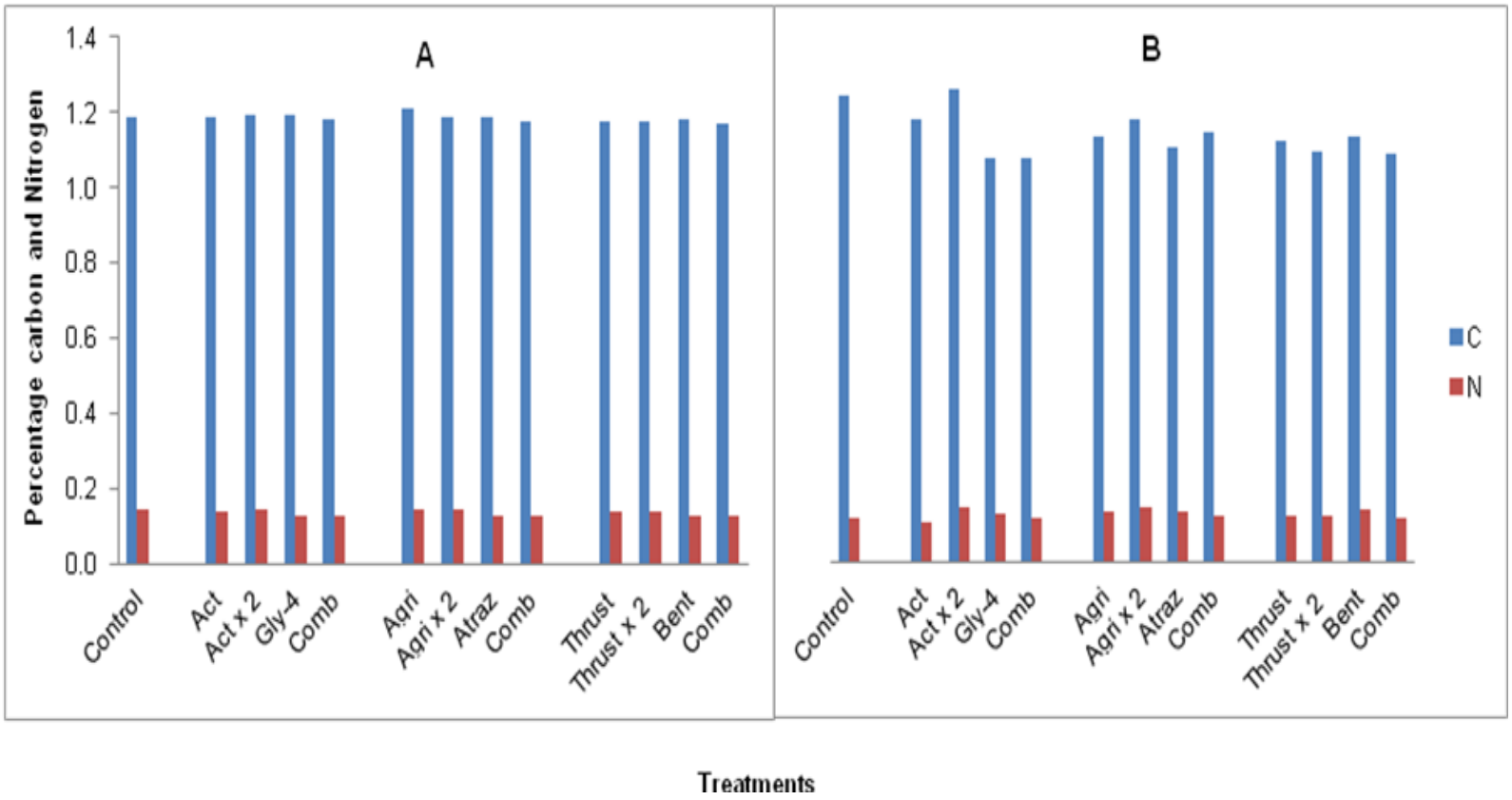

Figure 4.2. Graph of total carbon (C) and nitrogen (N) percentage for silt loam (A) and silty clay loam (B) soil. Carbon was not significant for both soils. Nitrogen showed significance in both silt loam and silty clay loam with *P* = 0.0005 and *P* = 0.0009, respectively. Statistical difference based on Student Newman Kuels Test of P = 0.05.

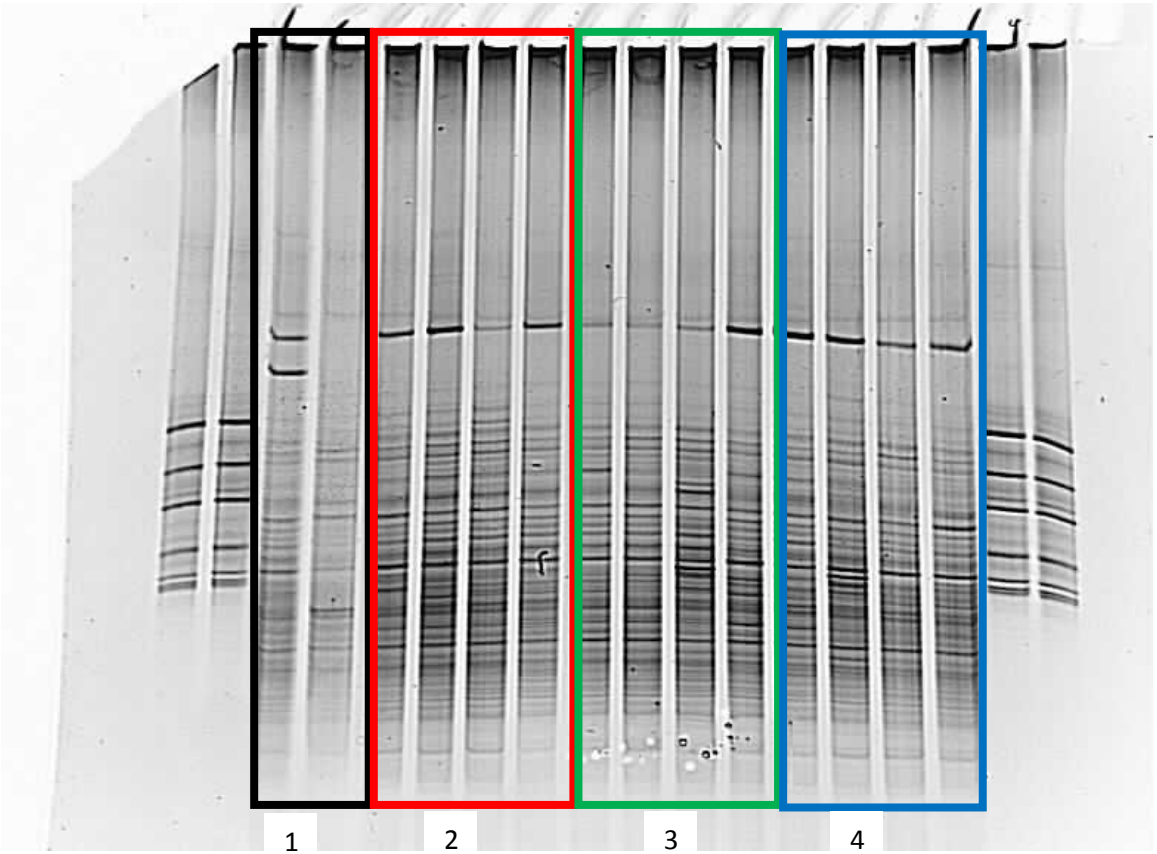

Figure 4.3. DGGE profiles for silt loam soil. Profiles are divided into 4 different groups. Group 1 contains the controls, one with plant and one without plant, group 2: various treatments of Activator-90 and glyphosate, group 3: various treatments of Agri-Dex and atrazine, and group 4: various treatments of Thrust and bentazon.

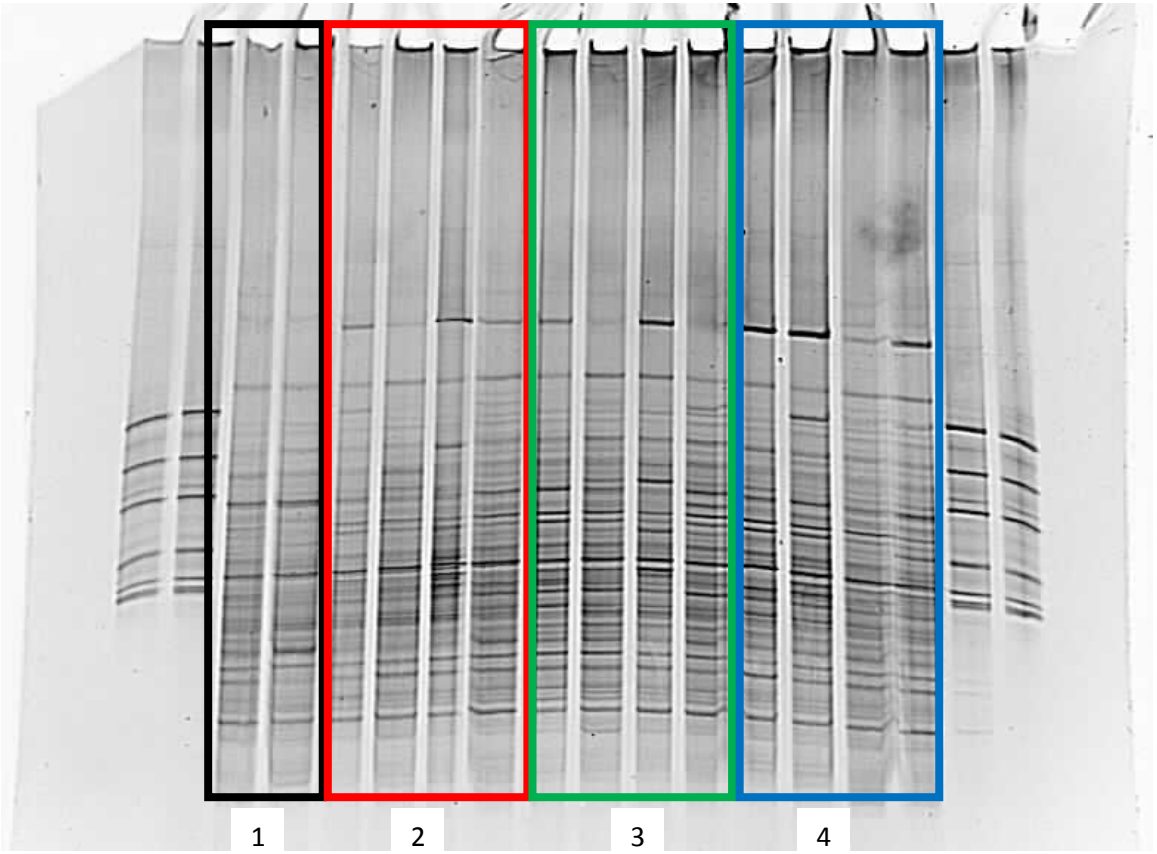

Figure 4.4. DGGE profiles for silty clay loam soil. Profiles are divided into 4 different groups. Groups 1 contains the controls, one with plant and one without plant, group 2: various treatments of Activator-90 and glyphosate, group 3: various treatments of Agri-Dex and atrazine, and group 4: various treatments of Thrust and bentazon.

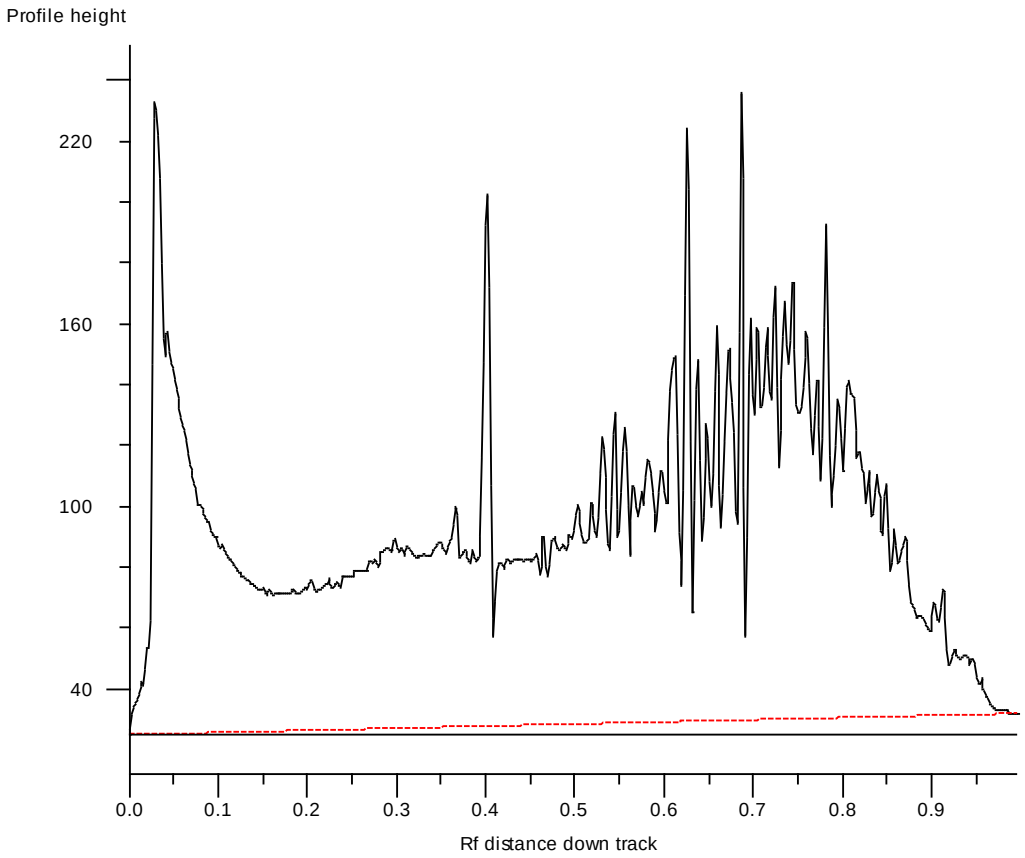

Figure 4.5. Standard graph generated using Genetool software showing a representative lane (Thrust and bentazon combination treatment) from the DGGE image using DNA extracted from silt loam soil. Each peak represents an individual DNA band, the height of which is relative to band density as detected by densitometry accommodated by the GeneGenius imaging system.

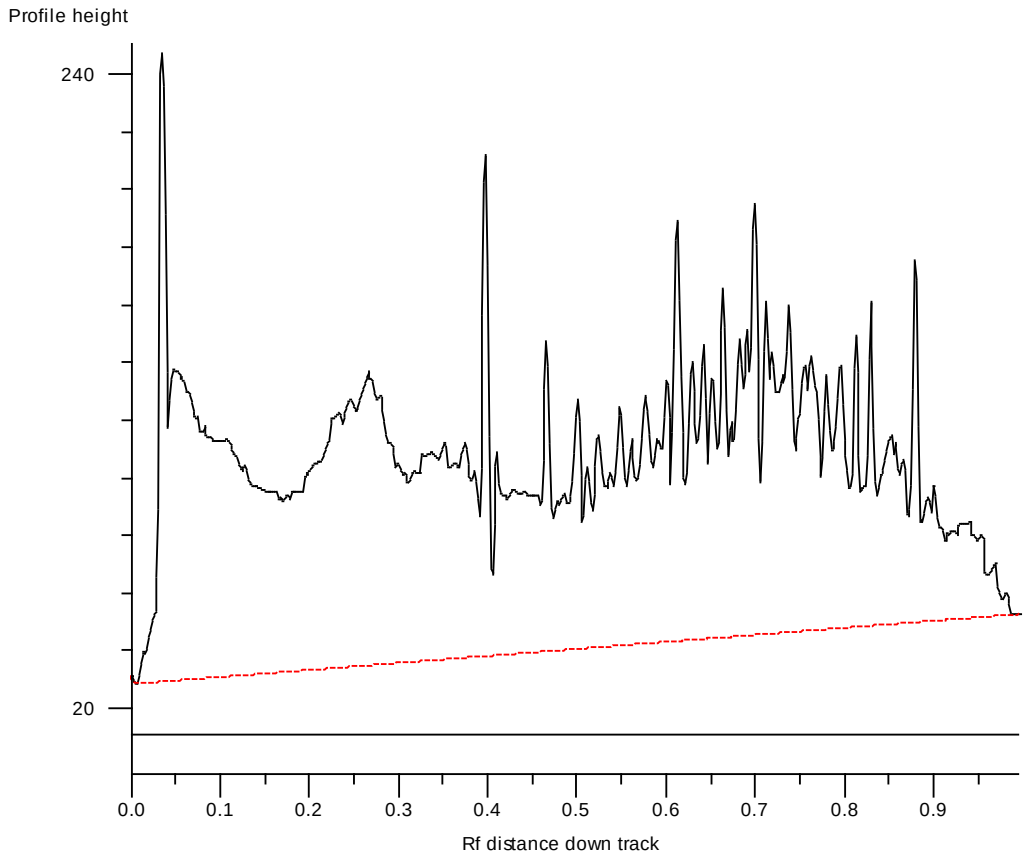

Figure 4.6. Standard graph generated using Genetool software showing a representative lane (Thrust and bentazon combination treatment) from the DGGE image using DNA extracted from silty clay loam soil. Each peak represents an individual DNA band, the height of which is relative to band density as detected by densitometry accommodated by the GeneGenius imaging system.

CHAPTER 5

RESPONSE OF THE SOIL MICROBIAL COMMUNITY TO SELECTED SURFACTANTS, HERBICIDES, AND SOIL TEXTURE

INTRODUCTION

Microbial community structure and function measurements are often used as indicators for monitoring soil quality, because the biological constituent of soil is sensitive to changes to the environment. Many soil processes are microbially mediated, such as carbon and nitrogen cycling, mineralization of other nutrients, enzymatic activities, soil aggregation, and decomposition of organic matter (Smith, 1994). Microbial community structure is affected by various environmental and growth factors, such as moisture, temperature, nutrient availability, and management practices (Petersen et al., 2002). Assessment of microbial communities and

community composition diversity within the soil ecosystem are helpful in determining if management practices and environmental conditions are aggrading or degrading to soils. Several labor-intensive and culture-dependent methods are used to measure microbial biomarkers and include plate counts and community level physiological profiling (CLPP) (Jenkinson and Powlson, 1976b; Turco et al., 1994). Culture-independent methods including phospholipid fatty acid analysis (PLFA) examine more of the total soil microbial community and provide information on diversity, which can be used as an indicator of biological activity in soil. The application of PLFA analysis is based on carbon chain lengths of phospholipids in microbial cells, which vary among species and are useful in identification of microbial species (Leckie, 2005). Cell membranes are composed of phospholipids and the major microbial groups can be separated based on the long-chain fatty acid composition unique to each group (Vestal and White, 1989). Different PLFA patterns are associated with different microbial communities that can be distinctive under different soil properties and thereby can be used to identify changes in composition of microbial communities due to soil or crop management practices (Baah et al., 1992; Cavigelli et al., 1995; Petersen and Klug, 1994).

Phospholipid fatty acid profile differences can be attributed to management practices including tillage, cropping system, and addition of various chemicals (Acosta-Martinez et al., 2010; Dick et al., 2010; Ratcliff et al., 2006; Ibekwe and Kennedy, 1998). Herbicides and surfactants differ in chemical composition and react differently when incorporated into the soil system due to soil properties and environmental factors (Smith and Hayden, 1982; Ray et al., 1995). Bacterial and

fungal composition of soil may be altered due to the application of different herbicides (Ratcliff et al., 2006). Surfactants are used in varying amounts in different chemical products, such as agricultural pesticides, detergents and cosmetics to modify spreading characteristics (Schramm, 2001; Krogh et al., 2003). Surfactants are used with agricultural chemicals including herbicides and insecticides to modify the effects of surface tension of liquid carriers in the pesticide formulation. These chemicals eventually enter soil and water systems (Buhler, et al., 1993). Through biodegradation pathways, microorganisms can modify the structure and activity of some of these chemicals to reduce their adverse effect on the environment (Ying, 2006). Surfactants entering the environment can possibly disrupt interactions of various chemical, physical and biological processes. Use of nonionic surfactants has resulted in preferential sorption based on clay content of the soil (Podoll, 1987). Ray et al. (1995) reported that nonionic surfactants had a greater affinity for and held more strongly to montmorillonite clay than kaolinite. Cationic surfactants were more toxic to gram-negative than to gram-positive bacteria and the level of toxicity was attributed to the extent of sorption in the soil (Sarkar et al., 2009; Nye et al., 1994).

Surfactants are often used with herbicides as an additive and enhance foliar uptake of post-emergence herbicides (Liu, 2004). These chemicals are not applied directly to soils, but a substantial amount may contact soil during application or rainfall events (Haney et al., 2000). Some herbicides, such as glyphosate, are easily adsorbed to clay and can be biodegraded in water and soil (Ahrens, 1994; Pessagno et al., 2008, Barja and dos Santos Afonso, 2005). Atrazine-degrading microorganisms may accumulate in soil receiving frequent atrazine applications, and

coexist with the indigenous soil microbial community, which may metabolize the herbicide (Satsuma, 2009; Zablotowicz et al., 2002). Herbicide degradation is affected by soil factors including nutrient composition and content, pH, temperature and moisture (Weber et al. 1993). In a study conducted with bentazon and atrazine, it was concluded that biological degradation was key for both herbicides. It was also stated that the herbicides added with a nonionic surfactant reduced the degradation time of the herbicides, compared to the herbicides applied alone and when herbicides were applied simultaneously, degradation time increased (Li et al., 2008). Pesticides 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 due to the application of different herbicides. In a study conducted with pure herbicide (mesotrione as active ingredient) and formulated herbicide, it was concluded that microbial activity was affected but only when the chemicals were used at rates much 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 were expressed either as a short-term or long-term effect (Ratcliff et al., 2006; Dick et al., 2010; Bittman et al., 2005). It is also likely that soil history was a determining factor in microbial composition and responses (Girvan et al., 2004). Application of PLFA analysis is one way to quantify the changes to the soil microbial community due to potential impacts of pesticides and surfactants in the environment.

The objective of this study was to examine the effect of various surfactants, herbicides, and soil texture on soil microbial community composition using PLFA

analysis. We hypothesize that surfactants and herbicides added to different soils will alter soil microbial PLFA profiles.

MATERIALS AND METHODS

Soils and chemicals

Two soil types, a silt loam and a silty clay loam were used in this study. A Wrengart silt loam (20% clay; fine-silty, mixed, active, mesic Fragic Oxyaquic Hapludalfs) was collected from the Lincoln University Carver Farm, Jefferson City, MO (38° 31' 36.1" N, 92° 8' 22.9" W). A Mexico silty clay loam (37.5% clay; fine, smectitic, mesic Vertic Epiaqualfs) was collected from University of Missouri Bradford Farm, Columbia, MO (38° 53' 48" N, 92° 12' 23.5" W). Bulk soils were air dried, sieved to pass a 2 mm screen and analyzed for chemical and physical characteristics (Buchholz et al., 1983; Table 5.1a). No known herbicide plus surfactant applications had been made to the two sites prior to soil sampling.

Surfactants used in this study were alkylphenol ethoxylate plus alcohol ethoxylate (Activator 90; non-ionic; Loveland Industries, Inc., Greeley CO), polyethoxylate (Agri-Dex; non-ionic; Helena Chemical Company, Collierville TN) and a blend of ammonium sulfate, drift reduction/deposition polymers and anti-foam agent (Thrust; anti-foam agent; Loveland Industries, Inc., Greeley CO). The herbicides were glyphosate (Gly-4 Plus; Universal Crop Protection Alliance LLC, Eagan MN), atrazine (AAtrex; Universal Crop Protection Alliance LLC, Eagan MN), and bentazon (Basagran; Micro Flow Company LLC, Memphis TN). Surfactants and herbicides were applied to soils at label rate, either alone or in combination; calculated to per pot of 4000 g soil (Table 5.1b). Non-treated soils served as

controls. Treatment mixtures of deionized water and chemical(s) were applied directly to potted soils.

To determine effects of surfactants and herbicide-surfactant combinations on the soil microbial community, a greenhouse experiment was conducted. Two gallon pots (20.3 cm dia. by 20.3 cm in height) were filled with 4000g of air-dried soil, fertilized and limed in accordance with fertility recommendations for field corn (*Zea mays*) based on soil test results (Lory et al. 1998). Soils were brought to field capacity and watered daily to maintain field capacity levels. Surfactant and herbicide treatments were prepared at designated rates using deionized water and were applied directly to pots. Six seeds of field corn (*Zea mays* L. type 'Indenta') 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. Temperature in the greenhouse varied from 18 to 27^{o}C throughout the day. Additional lighting was also provided to increase day light period. 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. Soil samples were collected, stored in soil sample plastic bags at 4^{o}C and processed for PLFA.

Phospholipid Fatty Acid Analysis (PLFA)

Investigation of soil phospholipid fatty acid (PLFA) was based on the methods of Bligh and Dyer (1959), and modified by Petersen and Klug (1994). Reagents used in the procedure were high pressure liquid chromatography (HLPC) grade supplied by Sigma (St. Louis, MO) unless otherwise stated. Two-gram soil samples

were added to Teflon-lined screw cap culture tubes (16 mm – 100 mm) and subjected to the following series of steps; saponification at 100^{o}C, acid methylation at 80^{o}C, and an alkaline wash. Long-chain fatty acids and other lipid compounds making up methyl esters were extracted with hexane. Nonadecanoic acid methyl ester was incorporated to allow for quantification of lipids identified on a molar basis. Solid phase extraction was used to separate the samples for phospholipid analysis with 100-mg silica columns (Varian, Palo Alto, CA). This was followed by conditioning of the columns with 3 mL hexane, 1.5 mL hexane/chloroform (1:1) and 100 mL chloroform plus a slight vacuum (25.4-50.8 mm of Hg) applied to the column after each solvent. Rinsing of the columns under vacuum was carried out with the sequential addition of 1.5 mL chloroform/2-propanol (1:1) and 1.5 mL 2% acetic acid in diethyl ether. Methanol (2 mL) was used to elute the phospholipids from the columns. In preparation for PLFA extraction, the sample was evaporated in the presence of nitrogen. The organic phase was evaporated to a dry state under nitrogen then re-dissolved with 75 mL hexane:methyl tertiary butyl ether (1:1). 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 the Eukary methods parameters that were used for peak identification and integration of areas. Peak chromatographic responses were converted to mol responses by using internal standards and recalculation of responses were done as needed. Carbon chains with

lengths of 12 to 20 carbons are correlated with microorganisms. A relationship determined by Bailey et al. (2002) was used to calculate biomass.

Bacteria to fungal ratio were calculated for samples. Fatty acids were designated by the number of carbon atoms, followed by a colon, the number of double bonds, and then by the position of the first double bond from the methyl (ω) end of the molecules. The following relationships of fatty acid profiles with various microorganisms are also presented in tabular form (Table 5.1c). Branched fatty acids are indicated by 'i' and 'a' for iso and anteiso branching, respectively. The prefix 'cyc' designates cyclopropane fatty acids. Different peaks were used as markers for bacteria: 12:0 3OH, i14:0, 15:0, a15:0, i15:0, i15:0 g, cyc15:1, i16:0, 16:1ϖ7, cis16:1 ϖ7, trans16:1v7, a17:0, cyc17:0, i17:0, 17:1 ϖ 6, i17:1 ϖ 7, 18:1 ϖ 7, cis18:1 ϖ 7, cis18:1 ϖ9, cyc19:0, cyc19:0, C11-12, cyc19:0, cis19:1 ϖ 9 (Vestal and White, 1989). Markers used for fungi; 16:1 ϖ 5, cis16:1 ϖ 5, 18:1 ϖ 9, 18:2 ϖ 6, cis18:2 ϖ 6, 18:2 ϖ 9, 18:3 ϖ 3, 18:3 ϖ 6, cis18:3 ϖ6 (Federle, 1986; Wander et al., 1995; Zelles et al., 1995; Frostegard et al., 1993; Sundh et al., 1997). Gram-positive bacteria markers were; i14:0, i15:0, a15:0, i15:0 g, i16:0, i17:0, cis18:1 ϖ 9 (O'Leary and Wilkinson, 1988; Wander et al., 1995; Zelles et al., 1995; Sundh et al., 1997). Gram-negative bacteria markers were; 15:1 ϖ 6c, *cis*16:1v7t, cy17:0, *cis*18:1v7; cy19:0, cyc19:0, *cis*19:1v9 (Ratledge and Wilkinson, 1988; Wander et al., 1995; Zelles et al., 1995; Sundh et al., 1997). Mycorrhizal markers were 16:1 ϖ 5, *cis*16:1 ϖ 5, 18:2 ϖ 6, *cis*18:2 ϖ 6, 18:2 ϖ 9 (Balser et al., 2005; Belen Hinojosa et al., 2005). Ratios of the cyclopropyl fatty acids to monoenoic precursors and the

total saturated to total monounsaturated fatty acids we used to calculate stress indicators (Kieft et al., 1997; Bossio and Scow, 1998; Fierer et al., 2003). Peaks used to calculate cyclopropyl fatty acids to monoenoic precursor ratios were cyc17:0 to cis16:1 ϖ 7 and cyc19:0 to *cis*18:1 ϖ 7. The ratio of total saturated to total monounsaturated fatty acids used the ratio of the sum of 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0 to sum of *cis*16:1 ϖ 11, *cis*16:1 ϖ 9, *cis*16:1 ϖ 7, *cis*16:1 ϖ 5, *cis*17:1 ϖ 9, *cis*17:1v8, *cis*17:1v7, and *cis*17:1 ϖ 5. Monounsaturated fatty acids from 14:0 to 19:0 were also evaluated (Bossio and Scow, 1998) (Table 5.1c).

Statistical Analyses

The data for the different parameters was analyzed for different soil types and treatments using SAS PROC GLM (2002). ANOVA was used to determine significant effects and, where F-values were significant (P=0.05), mean separations were conducted using Fisher's Protected Least Significant Difference (LSD) test. Fatty acid percentages spanned a wide range and were log transformed for principal component analyses (PCA) in SAS. PCA was used and a covariance analysis between factors was performed in order to reduce data dimensionality and to observe unknown trends with microbial populations (Tabachnick and Fidell, 2001). PCA was used to show differences and similarities between the two soils and also with the various treatments used in the study. Further comparison was done using canonical analysis. Multi-dimensional plots were used to present data for better understanding of the relationship. We adopted Kaiser's rule (Joliffe, 1986) and only variables with an eigenvalue greater than one were used for further analysis when the correlation matrix is used.

RESULTS AND DISCUSSION

There were differences in chemical and physical characteristics of the two soils used in this study which affects the biological properties and microbial community composition. The silt loam soil had a higher pH (0.01 M $CaCl_2$) of 5.61 compared to the silty clay loam soil pH of 4.52 (Table 5.1a), both not suitable for corn production. Also, the silt loam soil was higher in total organic C, N, P, and K compared to the silty clay loam. Cation exchange capacity values of the silty clay loam were double the CEC of the silt loam soil and Mg in the silty clay loam was higher than the silt loam.

Higher concentrations of BtoF, GN, and Ana were observed in the silt loam compared with the silty clay loam soil (Table 5.2). Furthermore, comparison of the treatments within the different soils showed variations among the treatments. Analysis of variance showed differences in the response of the various biomarkers to treatments (Table 5.2). In the silt loam, treatments affected Bac (P=0.003), Fun (P=0.0307), GN (P=0.0001), GP (P=0.0001), Aer (P=0.003), and Ana (P=0.0001) biomarkers. The microbial populations in the silty clay loam only showed differences due to treatment for BtoF (P=0.0342) and Mono (P=0.0367). More microbial groups in the silt loam were affected by treatment when compared to the silty clay loam.

Biomarkers for the fatty acids were analyzed with SAS and examined for significance (see Tables 5.3a, 5.3b, 5.4a, and 5.4b). For the silt loam soil, the treatments affected the microbial groups differently (Tables 5.3a and 5.3b). For silt loam, Activator-90 surfactant at the recommended rate decreased Bac, GN and Aer.

Activator-90 at 2X rate increased BM and Fun and decreased Bac, BtoF ratio, GN, GP, Aer and Ana and Mono fatty acids. Glyphosate alone and combined with Activator-90 decreased Bac, BtoF, GN, Aer, Ana, and increased GP. Agri-Dex at the recommended rate decreased GN and Ana, and at 2X rate showed decreased Bac, GN Aer, Ana, and increased StoM, a stress indicator. Atrazine showed a decrease in Bac, GN, Aer, Ana, and an increase in GP. Combinations of Agri-Dex with atrazine and Thrust with bentazon decreased Bac, GN, Aer and Ana. Thrust at the recommended and 2X rates decreased Bac, GN, GP, Aer and Ana. Bentazon decreased Bac, BtoF, GN, Aer, Ana and increased GP. Over all, all treatments except Agri-Dex decreased bacterial markers relative to control. Fungi increased with Activator-90 at 2X rate in silt loam. Bacteria to fungi ratio values decreased relative to control for Activator-90 2X, glyphosate, Activator-90 and glyphosate combination, and bentazon. Gram-negative bacteria biomarkers were less with all treatments compared to control. Gram-positive bacteria biomarkers were higher than the control for glyphosate, Activator-90 and glyphosate combination, atrazine and bentazon and lower for Agri-Dex and atrazine combination and Thrust and bentazon. Aerobic and anaerobic bacteria biomarkers were significantly affected by all chemical treatments except Agri-Dex and Activator-90, respectively, applied individually at recommended rate.

In the silty clay loam, only a few differences were observed (Tables 5.4a and 5.4b). Agri-Dex applied at the recommended rate and bentazon significantly increased MB. All other treatments showed an increase in MB when compared to control. The application of glyphosate and atrazine in combination with surfactants in

the silty clay showed an increase in MB when compared to the control. Suggested that the presence of the surfactants may have enhanced degradation of the herbicides resulting in higher BM (Li et al., 2008). Both rates of Agri-Dex decreased Mono, a stress indicator. Heipieper et al. (1996), reported that a shift in monounsaturated fatty acids from a lower number to a higher number was evidence of stress. Bentazon and Thrust decreased Ana bacteria. In contrast, decreases in biomarkers of the bacterial community occurred with nearly all treatments in the silt loam. This indicates that the bacterial community was sensitive to subtle changes in the soil environment (Pennanen et al., 1996; Wilkenson et al., 2002). A general increase in MB developed in both soils with Activator-90 at 2X rate in the silt loam and Agri-Dex and bentazon in the silty clay loam showing significance. Haney et al. (2000) showed that glyphosate applied to soil did not significantly affect MB. Glyphosate may either stimulate or inhibit soil microbial activity based on the concentration used and on soil properties (Carlisle and Trevors, 1986). The increases in MB suggest that the different chemicals added to soil may have been used as carbon sources by soil microorganisms (Wardle and Ghani, 1995; Fierer et al., 2003). Gram-positive bacteria were increased with addition of herbicides in the silt loam, but decreased in the silty clay loam. In previous studies, the GP component exhibited growth fluctuations during the degradation of soil-applied herbicides (Pipke et al., 1987; Strong et al., 2002; Schmalenberger and Tebbe, 2002; Seeger et al., 2010). The application of herbicide alone to both soils had some affect on the microbial community. Based on these reports, it is possible that

species composition and perhaps herbicide metabolic ability of the GP component varied considerably between the silt loam and the silty clay loam.

Microbial stress indicators are used to detect shifts in microbial community due to unfavorable conditions such as temperature, availability of substrate, water availability, and toxicity caused by various substances including heavy metals. Cyclopropane is produced under limited carbon source (Bossio and Scow, 1998). Monoenic acids are associated with high substrate availability. Ratio of cyclopropyl fatty acids to monoenoic fatty acids might be a good indicator for monitoring stress on the microbial community. The ratio of total saturated to total monounsaturated fatty acids used the ratio of the sum of 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0 to sum of cis16:1v11, cis16:1v9, cis16:1v7, cis16:1v5, cis17:1v9, cis17:1v8, cis17:1v7, and cis17:1v5. The ratio of cyclopropyl fatty acids to monoenoic fatty acids increased with the Activator-90 and glyphosate combination treatment in the silt loam. These biomarkers were not significantly different for Activator-90 and glyphosate treatment in the silty clay loam although it decreased with both application rates of Agri-Dex (Table 5.4b).

Ordinate analysis was used to observe differences or similarities among the treatments in the silt loam and silty clay loam soil. It was also used to graphically display the clustering of both soils. Soil variation was observed between the silt loam and silty clay loam. Plots of the different components showed a distinctive separation of the microbial community of silt loam and silty clay loam soils (Figure 5.1, a-d). For the silt loam the x axis explained 81% of the variability and the y axis

accounted for 6% of the variability. The silty clay loam had 80% of its variability explained in the x axis and 14% for the y axis. The differences between the two soils based on the separation reflect different fatty acid combinations, which indicate diverse microbial composition present in each soil. This difference may be attributed to differences in soil physical and chemical properties, and management practices (Table 5.1a). These findings support previous studies where the original soil chemistry and texture were main contributors to differences detected in the diversity of microbial community (Fang et al., 2005; Girvan et al., 2003; Marschner et al., 2001). Fang et al. (2005), using carbon substrate utilization, also detected differences between sandy loam and silty clay soils used to grow corn. The separation of treatments within each soil is variable with a combination of clusters and distinct separations observed. Another likely contributor to variations in the microbial community is the addition of plant–based carbon substrates as root exudates as the plant matures. Exudates may differ between soils and even within treatments due to a combination of chemical and physical factors affecting the plant thereby altering the microbial community (Brimecombe et al., 2001).

Similar to using ordination to view differences and similarities between the two soils, the same approach was used to compare the different treatment effects on PLFA patterns within each soil. Further analyses were done using eigenvalues to determine the variance. Both positive and negative eigenvalues were observed for both soils for all four ordinates (O1, O2, O3, and O4) for various treatments in the data distribution pattern. Greater effects were observed in the silt loam compared to the silty clay loam (Tables 5.5a and 5.5b) with mean separation.

Silt loam was significant in O3 (P = 0.0507), and O4 (P = 0.0045). Ordinate 2 was not significant (P = 0.0786), but showed difference in the mean separation. The combination treatments, Activator-90 plus glyphosate, Agri-Dex plus atrazine; atrazine alone; and Thrust at 2X application rate were significantly higher than the control for O2. Ordinate 3 showed significantly higher eigenvalues for all treatments except for Agri-Dex plus atrazine and Thrust alone. For O4, glyphosate alone treatment was significantly different than the control. This suggests that glyphosate may have been adsorbed to soil particles making it unavailable to microbial degradation (Pessagno et al., 2008). Other studies demonstrated that adjuvants or surfactants aid in the sorption of herbicide to soil by modifying the solubility limiting bioavailability (Beighel et al., 1999; Krogh et al., 2003). In the silty clay loam no significance between the four ordinates was detected, but different effects were observed. Based on the mean separation, Agri-Dex at both application rates O1 (P = 0.1048) and bentazon alone for O2 (P = 0.0856) had different effects on PLFA compared to the control. The addition of surfactants at both application rates did not significantly affect PLFA profiles for the silt loam or silty clay loam.

Ordination graphs were used to plot the eigenvalues of the two soils with the different treatments (Figures 5.2 and 5.3). From the graphs plotted with O3 and O2, the two soils showed a difference in the separation of the treatments for PLFA. The distance between the different treatments in the ordination graph signifies similarities; the shorter the distance the greater the similarity. In the silt loam there is a distribution of the treatments along the y-axis (O3) with the control with negative values and majority of treatments with greater concentrations. The clay is distributed

along the x-axis (O2) with the control and some treatments having similarities. This agrees with the biomarker data reflecting more activity in the silt loam than the silt clay loam. The microbial community structure in silt loam and silty clay loam was altered to some extent by herbicide and surfactants applied to soil.

The ordination data for both soils in the present study did not reflect any great significance based on the different one-time application of treatments. This may suggest that soil properties and continuous chemical application together are primary factors as related to microbial composition and response versus one-time applications of surfactants and herbicides used in our study (Girvan et al., 2003; Lupwayi et al., 2010 ; Seghers, et al., 2003; El Frantroussie., 1999).

CONCLUSION

The PLFA microbial analyses indicated differences in the microbial community between the silt loam and silty clay loam soils. Total Bac mol percent decreased in most treatments relative to the control in silt loam soil. There were few differences among treatments in the silty clay loam. Significant differences were seen in MB with bentazon application and in Mono for Agri-Dex at both application rates based on mean separation. Each soil contained different combinations of the various microbial groups. Although there was a general decrease in the total Bac values, MB increased when most treatments were applied to both soils. Also, the response of GP bacteria were different between both soils with the herbicide alone treatments of glyphosate, atrazine and bentazon. There was a significant increase in GP observed in silt loam and a general decrease observed in silty clay loam compared to control, implying different types of GP present in each soil. In general, no significant increases were observed for the stress indicators used in the analyses, although a common increase was observed when compared to the control. The application of surfactants at two different rates, herbicides and combination treatments showed varying changes relative to non-treated controls among the different biomarkers analyzed in the study.

Although most changes were not significantly affected by different treatments applied, the application of relatively small quantities of chemicals, which represented actual field rates, to soils affected the expression of treatment effects observed in the study. The use of PLFA profiles indicated that the microbial community responded to changes due to surfactants and herbicide treatments applied and

these differences due to treatment were greater for the microbial groups in the silt loam. The treatments had much less of an impact with the silty clay. These results show some changes in the microbial populations with additions of herbicides and surfactants, but these changes varied with soil texture and were only for a short-term incubation study and one application of the compounds. Further long-term studies are needed that investigate the effect of multiple applications of herbicides and surfactants and multiple years on the microbial community.

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TABLES AND FIGURES

Table 5.1a. Soil properties for silt loam and silty clay loam

Textural class	pH ($CaCl_2$)	OM %	Tot.org. C %	CEC $cmol_c\ kg^{-1}$	Tot. N $mg\ kg^{-1}$	Bray 1 P $mg\ kg^{-1}$	Exc. K $mg\ kg^{-1}$	Exc. Ca $mg\ kg^{-1}$	Exc. Mg $mg\ kg^{-1}$
Silt loam	5.61	2.18	1.27	12.4	0.122	47.08	107	1214	233
Silty clay loam	4.52	1.89	1.10	22.2	0.096	1.34	57	1524	252

*Abbreviation: Exc., exchangeable; org., organic; tot., total.

Table 5.1b. Chemicals used and treatment rates applied to pots in greenhouse experiment (L ha^{-1})

	Treatment rates			
Treatments	**Surfactant**	**Surfactant x 2**	**Herbicide**	**Surfactant + Herbicide**
Activator-90	0.02 ml	0.04 ml		
Glyphosate			0.0065 ml	
Activator-90 + Glyphosate				0.02 ml + 0.0065 ml
Agridex	0.02 ml	0.04 ml		
Atrazine			0.0167 ml	
Agri-Dex + Atrazine				0.02 ml + 0.0167 ml
Thrust	0.01 g	0.02 g		
Bentazon			0.0083 ml	
Thrust + Bentazon				0.01 g + 0.0083 ml
Control	0	0	0	0

Table 5.1c. Phospholipid fatty acids used as biomarkers for microorganisms.		
Indicates	**Reference**	**PLFA biomarker**
Bacteria	Vestal and White, 1989	12:0 3OH, i14:0, 15:0, a15:0, i15:0, i15:0 g, cyc15:1, i16:0, 16:1ϖ7, cis16:1 ϖ7, trans16:1v7, a17:0, cyc17:0, i17:0, 17:1 ϖ 6, i17:1 ϖ 7, 18:1 ϖ 7, cis18:1 ϖ 7, cis18:1 ϖ9, cyc19:0, cyc19:0, C11-12, cyc19:0, cis19:1 ϖ 9
Fungi	Federle, 1986 Wander et al., 1995 Zelles et al., 1995 Frostegard et al., 1993 Sundh et al., 1997	16:1 ϖ 5, cis16:1 ϖ 5, 18:1 ϖ 9, 18:2 ϖ 6, cis18:2 ϖ 6, 18:2 ϖ 9, 18:3 ϖ 3, 18:3 ϖ 6, cis18:3 ϖ6
Gram-positive	O'Leary and Wilkinson, 1988 Wander et al., 1995 Zelles et al., 1995 Sundh et al., 1997	i14:0, i15:0, a15:0, i15:0 g, i16:0, i17:0, cis18:1 ϖ 9
Gram-negative	Ratledge and Wilkinson, 1988 Wander et al., 1995 Zelles et al., 1995 Sundh et al., 1997	15:1 ϖ 6c, *cis*16:1v7t, cy17:0, *cis*18:1v7; cy19:0, cyc19:0, *cis*19:1v9
Ratio of Cyclopropyl and monoenoic fatty acid (stress indicator)	Kieft et al., 1997 Bossio and Scow, 1998 Fierer et al., 2003	cyc17:0 to cis16:1 ϖ 7 and cyc19:0 to *cis*18:1 ϖ 7
Total saturated to total monounsaturated fatty acid	Bossio and Scow, 1998	*cis*16:1 ϖ 11, *cis*16:1 ϖ 9, *cis*16:1 ϖ 7, *cis*16:1 ϖ 5, *cis*17:1 ϖ 9, *cis*17:1v8, *cis*17:1v7, and *cis*17:1 ϖ 5,14:0, 15:0, 16:0, 17:0, 18:0, 20:0

Table 5.2. Probability values from analysis of variance for microbial biomarker groupings of phospholipids fatty acid profiles based on silt loam and silty clay loam. Samples taken from Cole and Boone County Missouri respectively. Microbial biomarker groupings were microbial biomass (MB), bacterial (Bac), fungal (Fun), bacteria to fungi ratio (BtoF), gram-negative bacteria (GN), gram-positive bacteria (GP), aerobic bacteria (Aer), anaerobic bacteria (Ana), ratio saturated to monounsaturated (StoM), and monounsaturated (Mono).

	Soil type	
Biomarkers	Silt Loam	Silty Clay Loam
	P values	
MB	0.4054	0.1442
Bac	*0.0030	0.8149
Fun	*0.0307	0.5124
BtoF	0.1350	*0.0342
GN	*0.0001	0.3731
GP	*0.0001	0.7822
Aer	*0.0030	0.8222
Ana	*0.0001	0.1300
StoM	0.1690	0.4960
Mono	0.1521	*0.0367

*Statistically different at $P \leq 0.05$

Table 5.3a. The effect of silt loam texture and treatments on microbial biomarker groupings of phospholipids fatty acid profiles. Samples taken from Cole County. Microbial biomarker groupings were microbial biomass (MB), bacterial (Bac), fungal (Fun), bacteria to fungi ratio (BtoF), gram-negative bacteria (GN), gram-positive bacteria (GP).

	MB		Bac		Fun		BtoF		GN		GP	
Treatments	Mol %											
Control	119.8	b	0.2193	a	0.0302	bcde	7.61	ab	0.1655	a	0.0538	def
Act	108.5	b	0.1855	b	0.0308	bcde	6.25	abcd	0.1383	b	0.0472	fg
Act x 2	377.2	a	0.1446	d	0.0525	a	3.43	d	0.1017	de	0.0429	gh
Gly-4	144.3	b	0.1790	bc	0.0463	ba	3.88	cd	0.1073	cde	0.0717	a
ActGly	153.1	b	0.1593	bcd	0.0356	bcde	4.47	cd	0.0937	e	0.0656	abc
Agri	151.5	b	0.1884	ab	0.0383	abcd	4.98	bcd	0.1307	bc	0.0578	cde
Agri x 2	119.3	b	0.1854	b	0.0411	abc	4.61	bcd	0.1249	bcd	0.0605	bcd
Atraz	164.0	b	0.1612	bcd	0.0350	bcde	4.63	bcd	0.0925	e	0.0687	ab
AgriAtraz	88.7	b	0.1423	d	0.0195	e	5.11	abcd	0.1128	bcde	0.0295	i
Thrust	141.8	b	0.1577	bcd	0.0289	cde	5.80	abcd	0.1157	bcde	0.0420	gh
Thrust x 2	109.4	b	0.1519	cd	0.0312	bcde	5.37	abcd	0.1139	bcde	0.0381	ghi
Bent	219.5	ab	0.1615	bcd	0.0368	abcd	4.43	cd	0.0927	e	0.0688	ab
ThrustBent	77.3	b	0.1696	bcd	0.0254	cde	6.80	abc	0.1340	cb	0.0356	hi

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; ActGly=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; agriAtraz=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; ThrustBent=Thrust plus atrazine. Different letters after numbers within same column indicates statistical difference at P =0.05 with Fisher's LSD.

Table 5.3b. The effect of silt loam texture and treatments on microbial biomarker groupings of phospholipids fatty acid profiles. Samples taken from Cole County. Microbial biomarker groupings were aerobic bacteria (Aer), anaerobic bacteria (Ana), ratio saturated to monounsaturated (StoM), and monounsaturated (Mono).

	Aer		Ana		StoM		Mono	
Treatments	Mol %							
Control	0.2193	a	0.1655	a	0.00	b	0.5095	abcd
Act	0.1855	b	0.1383	ab	0.00	b	0.5722	abc
Act x 2	0.1446	d	0.1017	de	1.98	ab	0.3718	d
Gly-4	0.1790	bc	0.1073	cde	34.00	ab	0.4976	abcd
ActGly	0.1593	bcd	0.0937	e	36.06	a	0.4687	bcd
Agri	0.1884	ab	0.1307	bc	21.52	ab	0.5248	abcd
Agri x 2	0.1854	b	0.1249	bcd	19.69	ab	0.5532	abc
Atraz	0.1612	bcd	0.0925	e	32.47	ab	0.4735	bcd
AgriAtraz	0.1423	d	0.1128	bcde	0.00	b	0.6034	abc
Thrust	0.1577	bcd	0.1157	bcde	25.89	ab	0.5587	abc
Thrust x 2	0.1519	cd	0.1139	bcde	21.14	ab	0.6185	ab
Bent	0.1615	bcd	0.0912	e	30.90	ab	0.4240	cd
ThrustBent	0.1696	bcd	0.1340	bc	0.00	b	0.6578	a

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; ActGly=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; agriAtraz=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; ThrustBent=Thrust plus atrazine. Different letters after numbers within same column indicates statistical difference at P =0.05 with Fisher's LSD.

Table 5.4a. The effect of silty clay loam texture and treatments on microbial biomarker groupings of phospholipids fatty acid profiles. Samples taken from Boone County. Microbial biomarker groupings were microbial biomass (MB), bacterial (Bac), fungal (Fun), bacteria to fungi ratio (BtoF), gram-negative bacteria (GN), gram-positive bacteria (GP).

	MB		Bac		Fun		BtoF		GN		GP	
Treatments	Mol %											
Control	75.0	c	0.1230	a	0.0548	a	2.27	bcd	0.0681	ab	0.0549	ab
Act	122.3	bc	0.1254	a	0.0487	ab	2.57	bcd	0.0701	ab	0.0553	ab
Act x 2	118.1	bc	0.1364	a	0.0463	ab	3.00	abc	0.0797	a	0.0567	ab
Gly-4	92.4	c	0.1192	a	0.0401	ab	3.09	abc	0.0663	ab	0.0529	ab
ActGly	111.5	bc	0.0991	a	0.0501	ab	2.15	cd	0.0562	b	0.0429	b
Agri	255.6	ab	0.1156	a	0.0512	ab	2.33	bcd	0.0585	b	0.0560	ab
Agri x 2	221.1	abc	0.1316	a	0.0560	a	2.39	bcd	0.0582	b	0.0734	a
Atraz	99.5	bc	0.1191	a	0.0486	ab	2.44	bcd	0.0712	ab	0.0479	ab
AgriAtraz	146.3	bc	0.1279	a	0.0411	ab	3.22	ab	0.0689	ab	0.0590	ab
Thrust	104.8	bc	0.0935	a	0.0560	a	1.73	d	0.0543	b	0.0392	b
Thrust x 2	125.0	bc	0.1111	a	0.0432	ab	2.58	bcd	0.0548	b	0.0563	ab
Bent	305.4	a	0.1052	a	0.0589	a	2.06	cd	0.0547	b	0.0504	ab
ThrustBent	156.6	abc	0.1148	a	0.0478	ab	2.40	bcd	0.0637	ab	0.0511	ab

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; ActGly=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; agriAtraz=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; ThrustBent=Thrust plus atrazine. Different letters after numbers within same column indicates statistical difference at P =0.05 with Fisher's LSD.

Table 5.4b. The effect of silty clay loam texture and treatments on microbial biomarker groupings of phospholipids fatty acid profiles. Samples taken from Boone County. Microbial biomarker groupings were aerobic bacteria (Aer), anaerobic bacteria (Ana), ratio saturated to monounsaturated (StoM), and monounsaturated (Mono).

	Aer		Anaer		StoM		Mono	
Treatments	Mol %							
Control	0.1230	a	0.0681	abc	23.88	ab	0.6804	ab
Act	0.1254	a	0.0701	abc	39.54	a	0.5045	abcd
Act x 2	0.1364	a	0.0797	a	26.42	ab	0.5392	abc
Gly-4	0.1192	a	0.0663	abc	30.36	ab	0.6758	ab
ActGly	0.0991	a	0.0561	bc	27.22	ab	0.6535	ab
Agri	0.1146	a	0.0512	bc	17.14	ab	0.2967	d
Agri x 2	0.1316	a	0.0505	c	24.16	ab	0.3639	cd
Atraz	0.1191	a	0.0712	ab	29.85	ab	0.6875	a
AgriAtraz	0.1279	a	0.0689	abc	47.42	a	0.5449	abc
Thrust	0.0935	a	0.0543	bc	22.61	ab	0.5892	abc
Thrust x 2	0.1111	a	0.0548	bc	38.05	a	0.4791	abcd
Bent	0.1052	a	0.0547	bc	33.85	ab	0.4484	bcd
ThrustBent	0.1148	a	0.0607	abc	41.33	a	0.5475	abc

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; ActGly=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; agriAtraz=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; ThrustBent=Thrust plus atrazine. Different letters after numbers within same column indicates statistical difference at P =0.05 with Fisher's LSD.

Table 5.5a. Results of analysis of variance of eigenvalues from phospholipid fatty acid profiles from silt loam and treatments. Samples taken from Cole County Missouri.

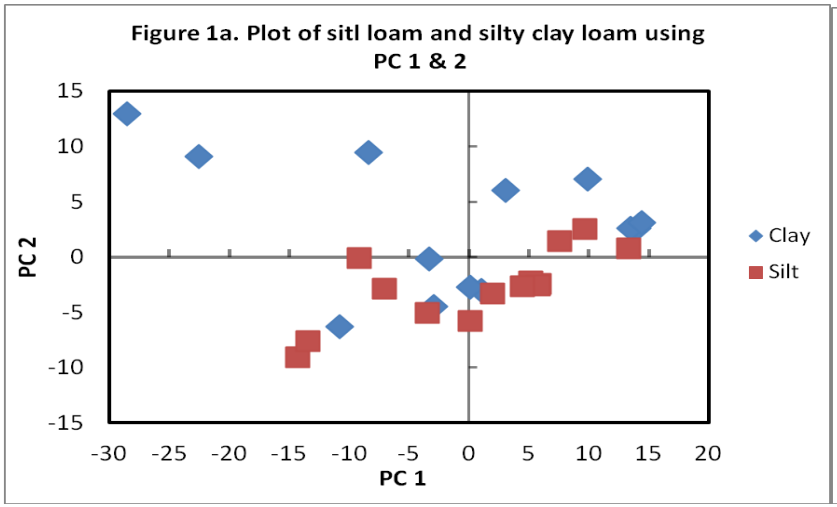
	O1		O2		O3		O4	
Treatments	*P* = 0.123		*P* = 0.0786		*P* = 0.0507		*P* = 0.0045	
Control	0.31	abc	-4.44	d	-5.15	c	0.52	abcd
Act	6.15	ab	-1.52	bcd	-0.63	b	1.73	abc
Act x 2	-15.33	c	-4.02	cd	-0.14	b	3.65	a
Gly-4	-4.21	abc	-1.06	bcd	2.03	ab	-4.14	f
ActGly	-8.74	bc	4.86	a	0.61	ab	-2.49	def
Agri	2.00	abc	-1.34	bcd	-0.45	b	-0.57	bcdef
Agri x 2	5.21	abc	-0.59	abcd	0.34	ab	-1.12	cdef
Atraz	-7.32	bc	1.81	abc	1.18	ab	-3.39	ef
AgriAtraz	8.10	ab	3.50	ab	-0.93	bc	2.66	ab
Thrust	4.43	abc	-1.15	bcd	-1.63	bc	-0.30	bcde
Thrust x 2	10.49	ab	3.31	ab	-0.10	b	1.00	abcd
Bent	-14.98	c	-0.11	abcd	4.63	a	0.96	abcd
ThrustBent	13.89	a	0.73	abcd	0.23	b	1.49	abc

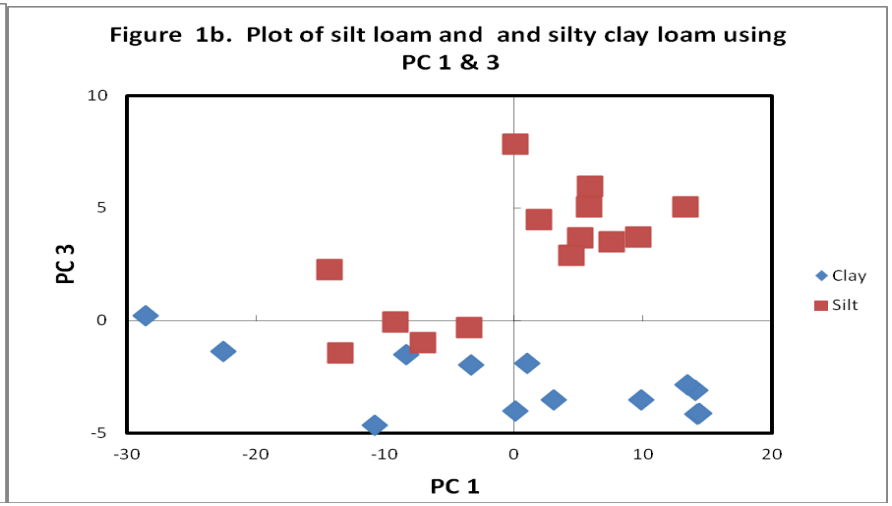
Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; ActGly=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; agriAtraz=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; ThrustBent=Thrust plus atrazine. Different letters after numbers within same column indicates statistical difference at P =0.05 with Fisher's LSD.

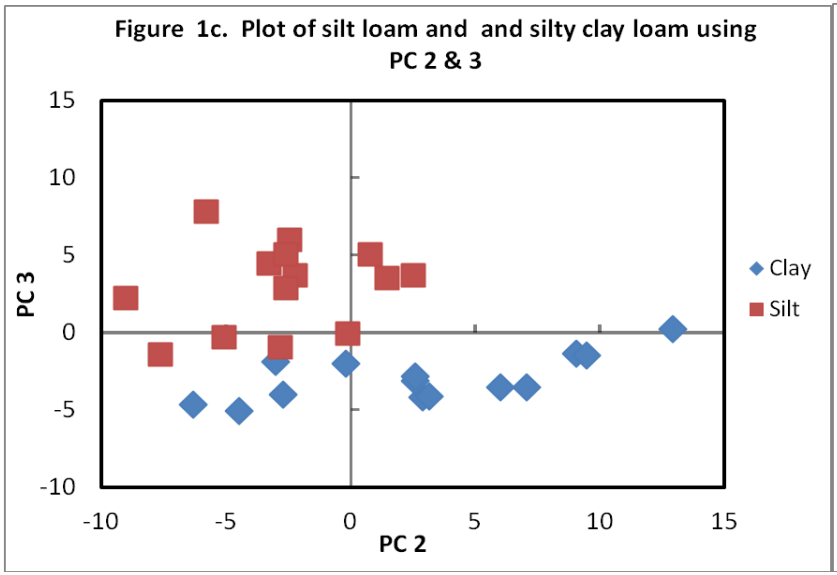
Table 5.5b. Results of analysis of variance of eigenvalues from phospholipid fatty acid profiles from silty clay loam and treatments. Samples taken from Boone County Missouri.

	O1		O2		O3		O4	
Treatments	*P* = 0.1048		*P* = 0.0856		*P* = 0.7286		*P* = 0.7128	
Control	-15.41	c	2.11	ab	0.84	ab	-0.03	a
Act	1.44	abc	-3.09	abc	-1.10	ab	1.07	a
Act x 2	-3.19	bc	-5.23	abc	-0.85	ab	-0.65	a
Gly-4	-4.18	bc	3.63	ab	-1.60	ab	-0.34	a
ActGly	-10.74	c	5.79	a	0.78	ab	-1.59	a
Agri	28.32	a	6.43	a	-1.06	ab	1.84	a
Agri x 2	21.79	ab	3.20	ab	-0.35	ab	2.47	a
Atraz	-15.62	c	2.26	ab	0.51	ab	0.30	a
AgriAtraz	0.28	abc	-7.84	bc	-3.45	b	-0.48	a
Thrust	7.79	abc	5.87	a	1.45	ab	-1.12	a
Thrust x 2	-2.45	bc	-5.32	abc	0.07	ab	-1.26	a
Bent	7.74	abc	-10.34	c	4.02	a	-0.91	a
ThrustBent	-1.34	bc	-5.48	abc	0.05	ab	-1.31	a

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; ActGly=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; agriAtraz=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; ThrustBent=Thrust plus atrazine. Different letters after numbers within same column indicates statistical difference at P =0.05 with Fisher's LSD.







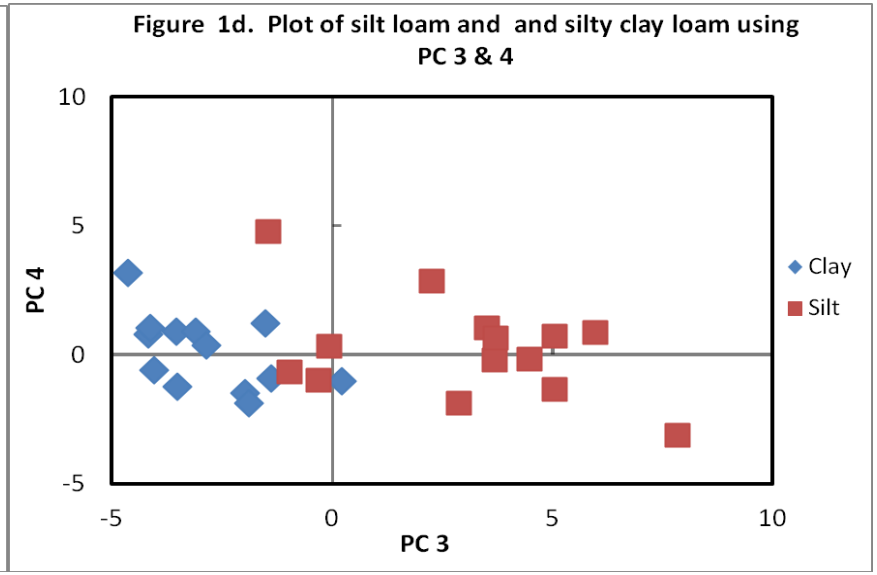


Figure 5.1. Plots of ordinate values showing the difference between biomarkers in silt loam and silty clay loam.

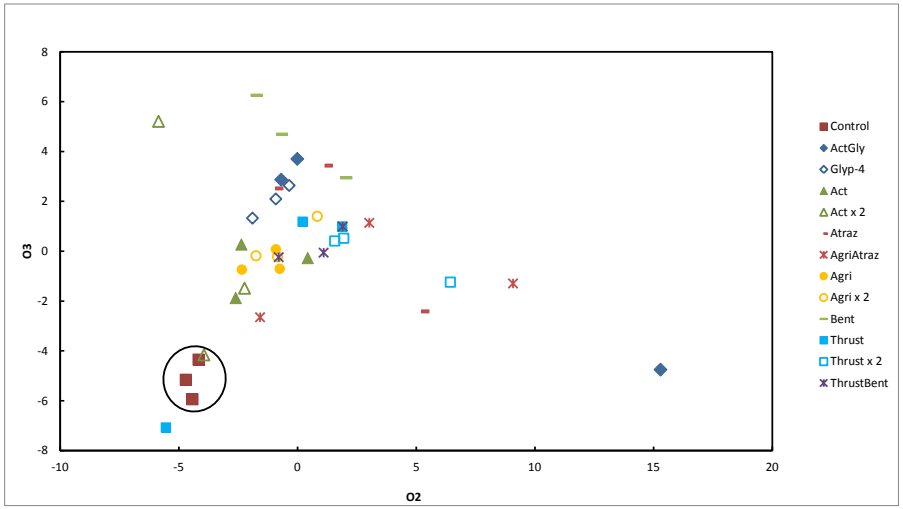


Figure 5.2. Ordination graph plot from the eigenvalues from phospholipid fatty acid profiles from silt loam and treatments. Circled portion contains the control.

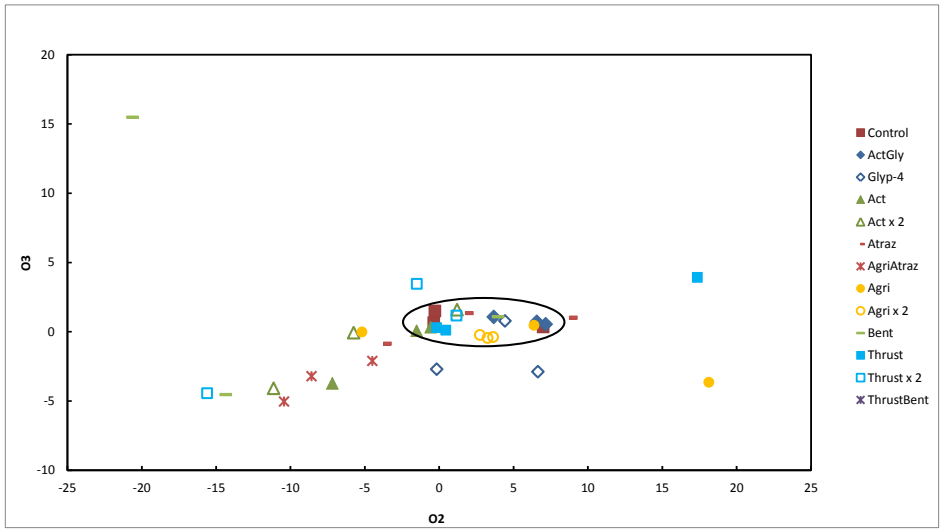


Figure 5.3. Ordination graph plot from the eigenvalues from phospholipid fatty acid profiles from silty clay loam and treatments. Circled portion contains the control.

CHAPTER 6

OVERALL SUMMARY

The aim of this study was to examine the effects of herbicides and surfactants rates on nutrient concentration in corn and on the diversity of the soil microbial community. Based on results presented in Chapters 3, 4, and 5, few significant effects associated with the treatments were detected. However, some notable effects with either negative, neutral or positive treatment responses compared with non-treated, control soils were detected.

In Chapter 3, considering plant nutrient uptake, fresh and dry weight of corn biomass differed between both soils. Soil carbon and nitrogen ratio (C:N) was higher in the silt loam compared with silty clay loam. Nutrient concentrations in corn did not differ between either soil. Surfactants applied at twice the recommended rate did not significantly affect nutrient uptake for corn planted in either soil. The various surfactant and herbicide treatments did not significantly affect nutrient uptake by corn, suggesting that corn growth and development under adequate nutrient availability are not negatively affected by the chemicals used in this study applied to silt loam or silty clay loam at label rates or twice the label rate for surfactants.

Chapter 4 presented results for DNA and PCR-DGGE analyses for characterizing the soil microbial community and the potential effects of the applied chemicals. Visual differences between both soils and among treatments were observed in the expression of DNA band intensity, although when DNA profiles were quantified, few significant differences due to treatment were found. Soil DNA concentrations were significantly affected by the various treatments in silt loam but these differences were more variable in silt clay loam soil. Differences between both soils likely reflected the overall contribution of soil texture, chemical and physical properties on characteristics of the microbial community.

Phospholipid fatty acid (PLFA) analytical results presented in Chapter 5 indicated differences in the microbial communities between the silt loam and the silty clay loam soil. Microbial biomass increased for most treatments in both soils. Based on these results, different types of bacteria appeared to be present at different levels in both soils. The application of surfactant treatments at two rates, and the herbicide and combination treatments variably affected different PLFA biomarkers relative to control soils.

When herbicides are applied in the field, formulation additives should also be considered when evaluating effects of chemical additions on the soil microbial community. Most treatments in this study did not cause significant responses from plant nutrient uptake or microbial community structure standpoints, perhaps as a result of the one-time application of small quantities of chemicals applied at recommended rates. The results from this study, conducted under greenhouse

conditions to simulate the field environment, suggest that future studies under actual field conditions with multiple applications and different rates are necessary to confirm that these responses occur under field condition or determine if other specific factors are involved in expression of responses.

APPENDIX

Table 7.1. Plant macronutrient uptake (μg^{-1}) for corn (V8 growth stage) grown in silt loam

Treatment	P	K		Ca	Mg	S
Control	87818	871553	ab	220907	177020	74380
Act	98197	905356	ab	242655	189750	71257
Act x 2	97068	972517	ab	268557	197257	76706
Gly-4	98066	970110	ab	246609	201398	82231
Comb	97983	933261	ab	234615	180204	82453
Control	87818	871553	ab	220907	177020	74380
Agri	96955	995844	a	241212	186712	73226
Agri x 2	82681	891211	ab	251689	183945	80464
Atraz	85886	932216	ab	234546	172711	69610
Comb	101787	867955	ab	249104	181395	86346
Control	87818	871553	ab	220907	177020	74380
Thrust	101430	799192	b	230742	203357	73089
Thrust x 2	100182	871595	ab	251603	206402	74219
Bas	100065	902278	ab	252325	172711	81844
Comb	96991	800939	b	227347	158189	73445

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine. Significant differences (P=0.05) among treatments were detected based on Student Newman Kuels test.

Table 7.2. Plant macronutrient uptake (μg^{-1}) for corn (V8 growth stage) grown in silty clay loam

Treatment	P		K		Ca		Mg	S	
Control	79823	ab	948614	ab	234256	ab	170584	62702	ab
Act	85438	a	955938	b	257327	a	170434	67975	a
Act x 2	83606	a	910629	abc	208483	abc	166329	60375	abc
Gly-4	56155	c	738604	abc	205141	abc	141242	46033	e
Comb	60828	c	733148	abc	175755	bc	151368	47660	de
Control	79823	ab	948614	ab	234256	ab	170584	62702	ab
Agri	72824	abc	764183	abc	201506	abc	173392	58663	bcd
Agri x 2	70946	abc	776633	abc	188605	bc	138816	54254	bcde
Atraz	61248	c	677142	c	173840	bc	135742	48010	de
Comb	65517	bc	753677	abc	181691	bc	131370	51531	cde
Control	79823	ab	948614	ab	234256	ab	170584	62702	ab
Thrust	65461	bc	692985	c	160926	c	122677	52320	bcde
Thrust x 2	60122	c	711595	bc	186092	bc	138512	49448	de
Bas	64827	bc	751500	abc	204007	abc	154251	52953	bcde
Comb	65477	bc	899738	abc	208801	abc	165987	47653	de

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine. Significant differences (P=0.05) among treatments were detected based on Student Newman Kuels test.

Table 7.3. Plant micronutrient concentration (μg^{-1}) for corn (V8 growth stage) grown in silt loam

Treatment	Cu	Fe	Mn	Mo	Zn
Control	269	3275	1756	4	1488
Act	296	3347	2019	23	1689
Act x 2	305	3372	2229	12	1749
Gly-4	297	3320	1808	18	1580
Comb	275	3138	1884	25	1568
Control	269	3275	1756	4	1488
Agri	310	4324	2075	0	1626
Agri x 2	259	6452	1924	19	1247
Atraz	310	3045	2051	19	1492
Comb	320	3821	2100	17	1702
Control	269	3275	1756	4	1488
Thrust	300	3760	1660	30	1654
Thrust x 2	322	3602	1931	16	1766
Bas	303	2994	1895	24	1649
Comb	270	3601	1986	24	1506

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine. No significant differences (P=0.05) among treatments were detected based on Student Newman Kuels test.

Table 7.4. Plant micronutrient concentration (μg^{-1}) for corn (V8 growth stage) grown in silty clay loam

Treatment	Cu		Fe	Mn	Mo	Zn	
Control	286	abc	2779	834	22	933	ab
Act	331	a	4726	1003	23	1396	a
Act x 2	293	abc	3058	864	10	921	ab
Gly-4	234	c	3298	851	17	648	b
Comb	255	bc	2172	749	20	738	b
Control	286	abc	2779	834	22	933	ab
Agri	297	abc	2817	891	23	790	ab
Agri x 2	295	abc	2886	880	21	1140	ab
Atraz	237	bc	6853	692	22	667	b
Comb	267	abc	2113	835	22	801	ab
Control	286	abc	2779	834	22	933	ab
Thrust	290	abc	2523	836	10	684	b
Thrust x 2	260	abc	2246	978	18	677	b
Bas	277	abc	2788	778	11	709	b
Comb	309	ab	2450	852	8	820	ab

Act=Activator-90; Act x 2=Activator-90 doubled; Gly-4=glyphosate; Comb=Activator-90 plus glyphosate. Agri=Agri-Dex; Agri x 2=Agri-Dex doubled; Atraz=atrazine; Comb=Agri-Dex plus atrazine. Thrust; Thrust x 2=Thrust doubled; Bent=bentazon; Comb=Thrust plus atrazine. Significant differences (P=0.05) among treatments were detected based on Student Newman Kuels test.