Pest Management Science *Pest Manag Sci* **64**:457–469 (2008)

Integrating soil conservation practices and glyphosate-resistant crops: impacts on soil

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Abstract

BACKGROUND: Conservation practices often associated with glyphosate-resistant crops, e.g. limited tillage and crop cover, improve soil conditions, but only limited research has evaluated their effects on soil in combination with glyphosate-resistant crops. It is assumed that conservation practices have similar benefits to soil whether or not glyphosate-resistant crops are used. This paper reviews the impact on soil of conservation practices and glyphosate-resistant crops, and presents data from a Mississippi field trial comparing glyphosate-resistant and non-glyphosate-resistant maize (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) under limited tillage management.

RESULTS: Results from the reduced-tillage study indicate differences in soil biological and chemical properties owing to glyphosate-resistant crops. Under continuous glyphosate-resistant maize, soils maintained greater soil organic carbon and nitrogen as compared with continuous non-glyphosate-resistant maize, but no differences were measured in continuous cotton or in cotton rotated with maize. Soil microbial community structure based on total fatty acid methyl ester analysis indicated a significant effect of glyphosate-resistant crop following 5 years of continuous glyphosate-resistant crop as compared with the non-glyphosate-resistant crop system. Results from this study, as well as the literature review, indicate differences attributable to the interaction of conservation practices and glyphosate-resistant crop, but many are transient and benign for the soil ecosystem.

CONCLUSIONS: Glyphosate use may result in minor effects on soil biological/chemical properties. However, enhanced organic carbon and plant residues in surface soils under conservation practices may buffer potential effects of glyphosate. Long-term field research established under various cropping systems and ecological regions is needed for critical assessment of glyphosate-resistant crop and conservation practice interactions.
Published in 2008 by John Wiley & Sons, Ltd.

Keywords: environment; no-tillage; conservation tillage; reduced tillage; cover crop; buffer; pesticide; sorption; leach; runoff; pesticide degradation; transgenic; glyphosate

1 INTRODUCTION

Conservation practices (CPs) have been used for many years to reduce sediment and chemical runoff and improve soil conditions. These practices include, among others, conservation or reduced tillage, cover crops, vegetative buffers and crop rotations. Although soil conservation measures have been practiced for a long time, their use today has been profoundly influenced by more recent glyphosate-resistant crop (GRC) technology.[1] Literature concerning long-term CP effects on the environment is growing, and some of those more recent studies include GRCs or glyphosate as a management component. Relatively few comprehensive appraisals have been published on the effects of GRCs on the environment, however, as GRC technology has only recently become available (since commercialization in the mid-1990s). In a review of the ecological risks and benefits of genetically engineered plants, Wolfenbarger and Phifer[2] noted the lack of scientifically based data to assess the environmental risks and benefits properly. They indicated that the risks and benefits of genetically engineered organisms vary spatially and temporally, rendering prediction of ecological impacts a complicated and uncertain task. Cerdeira and Duke[3] provided a comprehensive summary of environmental aspects relative to GRC. They noted several potential benefits (e.g. less herbicide and fossil fuel use) and concluded that most risks to the environment were minimal or reversible, perhaps with the exception of the potential for transgene flow to other plants. Dunfield and Germida[4] analyzed the

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(*Received 15 May 2007; revised version received 19 November 2007; accepted 19 November 2007*)
Published online 15 February 2008; DOI: 10.1002/ps.1549

impact of genetically modified crops on soil- and plant-associated microbial communities. Although the microbial communities can be altered when associated with transgenic plants, the changes are transient, variable and site specific. In another review, Motavalli *et al.*[5] concluded that no evidence was found that transgenic crops are causing significant direct effects on nutrient transformations in field environments, but that potential indirect effects have not been fully explored.

A general view in the agricultural industry is that widespread use of transgenic crop technology has enhanced environment quality. The reviews mentioned previously[1–5] all briefly address the potential environmental effects resulting from integrating CPs and genetically modified crop biotechnologies and generally support that viewpoint. The notion that positive environmental benefits ensue from adopting transgenic crop technology largely stems from the simultaneous increase in CPs since the advent of transgenic technology. Indeed, the two technologies are not only compatible, but transgenic technology has facilitated the adoption of CPs.[6] It is assumed that the same impacts of CPs on soil managed under conventional crops will occur in similarly managed soil where transgenic crops are used. The authors of this paper do not argue with the logic of these assumptions, but do note that research critically assessing the integration of soil conservation practices and transgenic technologies is lacking. Virtually no information is available that directly compares systems integrating transgenic crops and CPs with those using conventional crops and CPs. The decision by farmers to adopt transgenic technology and combine it with CPs is based on expected enhanced economic outcomes resulting from lower weed management costs and less need to till the soil rather than a goal to improve the environment.

Increased use of marginal land, less fuel consumption and improved time and labor efficiencies provide evidence that the overall impact of integrating CP and GRC technologies is positive, but what are the effects on the soil environment? Based on the reviews just cited,[1–5] some of the potential direct or indirect effects of GRC management on soil are summarized in Table 1. Are these effects additive, synergistic or negative when combined with CPs, for example, in limited-tillage soils cropped with transgenic plants as compared with those where non-transgenic crops are used? Will changes in the spectrum of herbicides used owing to GRC impact upon soil biology, and might these potential impacts be offset by CPs? Can research help to optimize benefits of both technologies? Is risk assessment needed? A 2002 CAST report[1] recommended evaluations of the environmental impacts of biotechnology-derived crops in the context of viable, currently available alternatives and practices in agriculture. A goal of this paper is to provide a comprehensive analysis of the information available, so that scientifically based conclusions can be drawn as to what those impacts might be in terms of soil. As well, areas where information gaps exist will be identified, providing the rationale for future research. Since direct comparisons of transgenic and non-transgenic technologies under the same CPs are virtually non-existent, the approach taken in this paper is to characterize components of conservation practices that may have an impact on glyphosate fate or other aspects of GR management, extrapolate the potential effects on soil and identify additional research needs. Finally, results will be reported from one of the few studies evaluating changes in CP soil under GRC or non-GRC management.

2 CONSERVATION PRACTICES: THE SOIL ENVIRONMENT

Conservation management includes an array of practices and systems. In agricultural settings, these practices often are designed to conserve soil and water and improve soil quality. These practices are used in cropped areas, as well as at the field edges, and include reduced tillage, cover crops, vegetative buffer strips and waterways. Wherever they are utilized, a typical feature of these practices includes the coverage of a portion of the soil surface by vegetation or its residues. A summary of the potential effects of CPs on soil characteristics as discussed below is given in Table 2.

The surface soil crust and decomposing plant residues serve as physical barriers for evaporation, thus conserving soil moisture.[7] Increased surface coverage by plant residues also reduces exposure to erosive processes or serves as a physical impediment to water and sediment runoff, thus improving the quality of surface waters and preserving valuable soil resources. Reduction in flow velocity and increased water retention by plant residues allow water more time to infiltrate, especially by preferential flow through macropore channels under saturated conditions. Slowing runoff flow velocity also provides a greater opportunity for sediments to settle and dissolved chemicals to interact with plant residues and soil.

Table 1. Some aspects of GRC management and potential influence on soil properties

Management component	Potential influence
Less tillage, if reduced-tillage methods are adopted	Less erosion, increased plant residue accumulation and OC, increased compaction in traffic areas
Less general herbicide use	Less risk of toxic effects on soil biota
More glyphosate use	Toxicity to soil biota at high concentrations
Equipment use	Less tillage equipment if reduced tillage, potential for more spray equipment, so potentially more compaction

Table 2. Potential effects of conservation practices on soil characteristics

Parameter	Effect	Limits
OC	Increase	Primarily in the soil surface
Biological activity	Increase	Primarily in the soil surface
Macropores	Increase	Irregular pattern
Compaction	Increase	Primarily traffic areas
Aeration	Increase, decrease	May increase in non-traffic areas, decrease due to reduced tillage
Plant nutrient availability	Decrease	Primarily in the soil surface
Moisture	Increase	Below the surface crust
Temperature	Decrease	
pH	Decrease	With the use of certain acid-producing fertilizers

Limited soil disturbance and plant residue accumulation contribute to physical and biological changes in soils.[7] The undisturbed soil surface develops into a rich organic matrix of decomposing plant tissues. Under a wide variety of cropping systems, total organic carbon (OC) in surface soils often increases as degree of soil disturbance is reduced, but with only marginal changes in OC at lower soil depths.[8–10]

Associated with enhanced levels of OC in the surface of CP soils is increased biological activity.[10,11] The dynamic biological processes also impact upon plant nutrient cycling in CP soils. Accumulated plant residues with higher C:N ratios result in a slower recycling and stratification of plant nutrients within the soil profile.[9,12] The carbonaceous plant residues provide a substrate for accelerated microbial activity, resulting in immobilization of plant nutrients. Lower soil pH also has been observed in reduced-tillage soils.[7,12] These factors may lead to plant nutritional deficits that require augmentation with fertilizer.[12,13]

Undisturbed soil profiles often develop macropores and channels as a result of roots decomposing *in situ*, leaving voids, macrofauna activity (e.g. earthworms, insects) or the formation of cracks during drying in high-shrink–swell soils. Decomposing plant material contributes to the formation of larger (>0.25 mm) and more stable soil aggregates that increase porosity and the potential for water infiltration.[9,14,15] Traffic from farm equipment may contribute to soil compaction and increased bulk density,[16] and these effects may inhibit crop root development in soils under long-term no-tillage management.

3 CONSERVATION PRACTICES AND GRC IMPACTS ON SOIL

The question of interest here is what additional effects on soil might result from adding GRCs as a management component in a CP system. How would that integrated system be managed differently than conventional crops, and would those differences have any impact on soil? The approach will be to examine aspects of CP and GRC management and use information from the literature to extrapolate potential effects. Tables 1 and 2 are a starting point.

3.1 Soil physical and chemical characteristics

With some CPs, plant residues are incorporated, while in others plant residues are left on the soil surface. Incorporating plant residues exposes them to greater soil surface area and hastens their decomposition. Factors influencing glyphosate fate in soil will be discussed later, but studies indicate that increased plant residue accumulation and OC associated with CP soils may help to ameliorate potential negative effects of increased glyphosate use in GRC systems. For example, little negative effect of glyphosate on carbon mineralization, microbial biomass or soil aggregation was observed in reduced-tillage soils.[17,18] In other studies, a positive correlation between carbon mineralization and soil OC in glyphosate-treated soils suggested that increased OC offset negative glyphosate effects.[19,20] Physical interception of glyphosate by surface plant residues may reduce the concentrations reaching the soil or slow subsequent movement into the soil. The quantity, source, composition and decomposed state or age of plant residues or soil humic components influence herbicide sorption affinity, the environment for microbial activity, physical interception of herbicides and ultimately herbicide degradation.[8] More aged plant residues may have greater capacity for herbicide sorption, and this may be related to the reduction in cellulose with a proportional increase in lignin content as plant residues decompose.[21–24]

Soil compaction in traffic areas often increases with decreasing tillage, and, if strict no-tillage management is adopted, the number of post-emergence spray applications may increase, as glyphosate may be applied to desiccate existing preplant vegetation, and then multiple applications after GRC planting and emergence. The net effect would be increased compaction and less aeration in traffic areas.

3.2 Soil biology

Biological processes in soil are influenced by a number of factors, including moisture, nutrient availability, pH, organic substrate, temperature, habitat and the concentration of toxic substances. From discussion in the previous section it is apparent that all of these factors can be impacted upon by CPs (Table 2), but the effects of GRCs on soil biology are less clear.

Differences in composition of transgenic plants may influence soil biology as the residues decompose and release constituents. One implicit difference between the composition of GRC and non-GRC plants is the introduced genetic material, and there is concern that the genetic material may be transferred to soil organisms. Also, other transgenic traits (e.g. *Bt* gene for insect resistance) often are included with GRCs, and plants with those traits sometimes differ in starch, protein, lignin, toxins and nitrogen composition when compared with non-GRC plants.[25–27] Ecological ramifications, however, are not certain, as there are no consistent trends, and effects may be transitory. Further research is needed to evaluate how differences in plant composition between GRC and non-GRC might be influenced by CPs. Depending on the CP adopted, incorporation of plant residues may vary from none to minimal, resulting in slower decomposition, so differences in composition of transgenic plant residues might impact upon carbon and nutrient cycling.

Soil microbial communities are diverse, and responses to glyphosate are equally varied, although there is concern that increased glyphosate use may alter soil microbial community structure, tipping the balance in favor of less sensitive organisms. For example, Araújo *et al.*[28] showed that, in Brazilian soils, culturable bacterial populations were not affected by glyphosate, while fungi and actinomycete populations increased in response to glyphosate. However, several researchers have shown that amending glyphosate to soil did not significantly affect microbial populations or activities,[19,20,29–33] except where soil was treated with excessively high glyphosate concentrations.[30] Others have observed that glyphosate applied at low concentrations stimulates microbial populations and activity parameters such as enzymes, respiration and nitrogen mineralization.[19–20,28,29,34–36] In any case, minor stimulation of microbial populations owing to an increase in glyphosate use under GRC would only add to already enhanced populations and activities in surface CP soils, but those increases may not be measurable, and may be transitory.[30,33] If glyphosate concentrations are excessive, the increased plant residue accumulation and OC in CP soils may provide some buffer against potential deleterious effects.

Stimulating beneficial populations should cause little or no negative ecological impact, but the incidence of disease in some GRCs has generated concern that soil-borne plant pathogens might be favored or that pathogen antagonists are detrimentally impacted upon. Results have been mixed. In some studies, pathogens or disease incidences due to glyphosate application were enhanced,[37,38] while in others there was little or no effect.[38–42] Some of the variability in response to glyphosate application might be due to environmental conditions such as soil moisture.[43] Concerns also have been raised about increased plant disease risk in CP systems because the accumulation of plant residues coupled with a cooler and moist soil environment may provide an improved habitat for plant pathogens.[44] Rotating crops may help to reduce the incidence of disease in any of these systems.[44] However, combining CP and GRC might actually compound problems if the use of GRCs promotes monocrop systems and a less diverse array of herbicides for weed control, leading to the development of soil-borne diseases and increased incidence of glyphosate-resistant weeds and weed shifts. Additional long-term research is needed to address these complex interactions.

A common practice in CPs with or without GRCs is to desiccate existing weeds or cover crops before planting a crop. Early-season diseases can occur when planting into cool, moist soils, and this problem might be compounded in the presence of freshly decomposing plant residues. Increasing the interval between desiccation of existing vegetation and crop planting may reduce early-season diseases.[45]

Glyphosate intercepted by soil has relatively no herbicidal activity, but may be taken up by plants through the roots and translocated within the plant. This is only likely to occur, however, at high concentrations. As glyphosate is not normally applied directly to the soil, high concentrations in soil would be rare and should not affect any but the most sensitive plant species.[46] Plant residues covering CP soils may reduce the quantity of glyphosate entering the soil, and increased OC may sorb glyphosate and limit further mobility (see discussion below).

In an early review, Eijsackers[47] indicated that effects of glyphosate on soil fauna were small or absent, but that further research was needed. More recent work supports that analysis.[48,49] Glyphosate did not influence soil nematode populations under either no-tillage or conventional tillage.[50] Indirect effects of glyphosate on fauna may involve degradation or alteration of habitat, e.g. reduced vegetative cover.[51] However, the habitat degradation did not have long-term (more than one season) negative impacts. Therefore, habitat degradation is not likely to be a significant issue in CP soils where plant residues often decompose slowly.

4 CONSERVATION PRACTICES AND GLYPHOSATE FATE IN SOIL

Depending on site-specific needs, many combinations of herbicides are included with glyphosate in weed management strategies for GRCs. Several studies have evaluated the effects of herbicide dissipation in systems where CPs are used, but relatively little research has assessed glyphosate fate in these systems. Thus, the focus of this section will be on glyphosate fate in the soil environment in the context of CPs. Only a brief discussion of general glyphosate interactions in soil will be presented, as another paper in this issue addresses this topic in greater detail.[52]

The primary ways that glyphosate contacts soil are (a) direct application (non-target) and (b) removal from plant tissue in washoff. Although the target for glyphosate is weed foliage, a significant percentage is also intercepted by the GRCs, as application can take place at prescribed stages of crop growth. As glyphosate is relatively soluble, its susceptibility to washoff depends on the rainfastness of the formulation used, the interval between application and rainfall, the kinetics of glyphosate uptake and metabolism by plants and the affinity of glyphosate for sorption to foliage of intercepted plants. In systems with CPs, glyphosate often is used to desiccate weed or cover crop foliage before planting a crop. Once glyphosate is absorbed by plants, its release back into the environment depends on the rate of plant decomposition, as glyphosate will primarily remain sequestered in plant tissue until the plant dies and decomposes. Some glyphosate also may exude from plant roots after translocation.[37] Once in the soil matrix, glyphosate is subject to sorption, *in situ* degradation or removal in surface runoff or by leaching.

Key factors influencing herbicide dissipation that can be manipulated by CPs include quantity and character of soil OC, soil structure, quantity, age and composition of accumulated plant residues, soil chemistry, aeration, soil microflora, soil moisture and chemistry and structure of the herbicide. The potential effects of some of these factors on glyphosate sorption, degradation and mobility in CP soil are summarized in Table 3 and discussed below.

4.1 Glyphosate sorption in soil

Glyphosate sorption in soil is a key factor contributing to its vulnerability to both degradation and transport. Glyphosate is an anion and is water soluble ($11\,600\,\mu g\,L^{-1}$ at 25 °C), is stable in water over a range of pH and has a low log K_{ow} (-3.3), but sorption patterns among soils and experiments vary considerably, sometimes leading to conflicting conclusions regarding its ultimate fate in the environment. Sorption mechanisms such as anion exchange and hydrogen bonding because of the zwitterionic nature of glyphosate (a net positive charge at $pH < 2$ and a negative charge at $pH > 2.6$) may account for the wide range in sorption coefficients (e.g. K_f 0.6–215) observed for glyphosate.[53–58]

Several studies have shown that soil organic matter has a positive effect on glyphosate sorption,[21,58–63] but mechanisms are not completely understood, and there are conflicting observations.[53] Piccolo *et al.*[64] proposed that glyphosate sorption to various humic substances was by hydrogen bonding among various acidic and oxygen-containing groups on both glyphosate and the humic materials, and the lower pH sometimes observed in reduced-tillage soils may facilitate this potential mechanism. Glyphosate sorption to organic matter also may be indirect through bridging mechanisms. Glyphosate ligand groups (amine-N, carboxylate-O and phosphonate-O) combine with metal ions and clay minerals,[55,65–66] and these complexes serve as bridges for sorption to negative reactive soil surfaces, including organic C.[21,59–61,67,68]

4.2 Glyphosate degradation in soil

Glyphosate degradation in soil generally is regarded as an initially rapid process followed by a slower continuous phase.[21,62,69–71] Degradation patterns and kinetics for glyphosate degradation differ with soil type and conditions, and, although glyphosate is considered to be relatively less persistent than many other herbicides, half-lives in soil vary considerably from 6 days to more than 60 days.[71–73] Mineralization to carbon dioxide is a major endpoint for glyphosate dissipation, while volatilization potential is limited.[63,70,74–78] Glyphosate degradation appears to be mediated primarily by microbial processes,[21,70,79,80] and two metabolites are documented for glyphosate: sarcosine[81–83] and aminomethylphosphonic acid (AMPA), with AMPA relatively more persistent in the soil environment.[84,85] Both glyphosate degradation processes offer only limited energy gained by the transformations, and therefore the greater availability of carbonaceous substrates under CP may provide stimulus to cometabolism of glyphosate initiated by either C–P lyase or glyphosate-oxidoreductase.

Few trends universally characterize the effects of CPs on herbicide degradation because of a wide range of herbicide chemistries, cropping practices, climate, topography and other factors. In studies with several herbicides, CPs have had a varied influence on patterns of herbicide metabolite accumulation, degradation,

Table 3. Potential effects of conservation practices on glyphosate fate

	Effect on glyphosate sorption	Degradation	Mobility – leaching	Mobility – runoff
OC increase	+, no effect	+, −	−	−
Biological activity increase		+		
Macropores increase			+	−
Compaction increase			−	+
Aeration decrease		+		
Moisture increase		+	+	+
pH decrease	+, −	−	−	−

mineralization and incorporation as non-extractable residues,[8] and this may extend to glyphosate as well. Although the potential impacts of CPs or GRCs on the dynamics of glyphosate and metabolite degradation in soil are not well studied, some observations can be made on the basis of what is known about soil characteristics, including soil moisture, biological activity, OC, pH and immobilization of nitrogen and phosphorus.

Increased sorption resulting from higher OC in CP soils may reduce bioavailability,[21,63,86] although some research showed that sorbed glyphosate may be susceptible to degradation.[21,80] Increased sorption of glyphosate has been observed in soil amended with maize residues,[22] but incorporation of >4% dry matter of maize residues was required to increase glyphosate sorption significantly. As plant residues decompose, they form humic components in soil that are associated with polymerization and binding of herbicides or metabolites into non-extractable fractions. In studies where radiolabeled glyphosate was used, herbicide residues not removed from soil by various extractants were defined as the non-extractable fraction.[63,74–75] Von Wirén-Lehr *et al.*[75] evaluated the mineralization of glyphosate applied directly to soil or sequestered within plant residues that were added to soil. Initial mineralization of glyphosate in plant residues occurred as rapidly as the mineralization of glyphosate directly applied to soil. This was attributed to their association with easily biodegradable plant constituents, such as starch, protein and pectin fractions. However, a greater percentage of glyphosate in plant residues was non-extractable, indicating that this component was sequestered in plant components, such as lignin, which are more resistant to decomposition.

4.3 Glyphosate mobility in soil

Glyphosate and its primary metabolite, AMPA, are generally regarded as relatively immobile in soil because of sorption,[21,69,87] but exceptions have been noted.[88] Several studies indicate that preferential flow is likely the most important mechanism for glyphosate leaching,[86,89–92] and the promotion of macropore channels under CP might enhance the potential for glyphosate movement. Some of the glyphosate leaching through macropores may be colloid-facilitated transport,[89,93] including that of humic macromolecules, which would be prevalent in CP soils. Increased OC in CP soils and corresponding glyphosate retention or degradation could offset some of the risk of glyphosate leaching via preferential flow.[93,94]

Glyphosate and its most persistent metabolite, AMPA, have been detected in surface water bodies.[95,96] Only a few studies have evaluated glyphosate runoff loss in tilled[56,70] and reduced-tillage systems.[97–99] In the limited number of studies where effects of tillage on glyphosate were directly compared, however, minimal differences due to tillage were observed.[97]

5 A CASE STUDY COMPARING GRC AND NON-GRC EFFECTS ON SOIL CHARACTERISTICS UNDER CONSERVATION TILLAGE

5.1 Background

Continuous and intensive cotton production has dominated the Mississippi Delta region for decades, but, as alternative crops such as maize gain popularity, research is needed to address management issues relevant to those cropping systems. Widespread adoption of transgenic technology in cropping systems has generated concerns as to long-term implications on the environment, but, as indicated previously, only limited research has addressed how the combination of GRC and CP may influence soil and water quality. A study was conducted to evaluate agronomic and economic aspects of conservation tillage maize and cotton rotations under GRC and non-GRC systems, and results were reported by Reddy *et al.*[100] This paper reports on soil-related environmental aspects from that study.

5.2 Materials and methods

5.2.1 Study site

A 6 year conservation tillage field study was conducted from 2000 to 2005 at the USDA-ARS Southern Weed Science Research Unit farm, Stoneville, MS (33°26′N, 90°55′W). The soil was a Dundee silt loam (fine-silty, mixed, active, thermic Typic Endoqualf) with initial soil conditions of pH 6.7, 1.1% organic matter, a CEC of 15 cmol kg^{-1} and soil particle size fractions of 26% sand, 55% silt and 19% clay. The experimental area had a history of GR soybean [*Glycine max* (L.) Merr.] production the preceding year (1999) and conventional cotton prior to 1999. Initial field preparation consisted of disking, subsoiling, disking and bedding in the fall of 1999. During the subsequent years, reduced-tillage management was implemented for the entire area as no further tillage was done with the exception of refurbishing raised seedbeds each fall after harvest.

The study was conducted in a randomized complete block design with four replications. There were four rotation systems for each GR and non-GR cultivar. Rotation systems included continuous cotton, continuous maize, maize–cotton (rotation 1) and cotton–maize (rotation 2). The reverse rotation sequences were added to make comparisons between rotation and monoculture systems each year. Each treatment consisted of eight rows spaced 102 cm apart and 45.7 m long.

The experimental area was treated with paraquat at 1.1 kg ha^{-1} 1–4 days prior to crop planting to kill existing vegetation. Weed management consisted of a glyphosate-based program for the GR cultivars and a non-glyphosate herbicide-based program for the non-GR cultivars, as summarized elsewhere.[100] Fertilizer application and insect control programs were standard for cotton and maize production.

5.2.2 Soil sampling and analysis of soil chemical and physical properties

Soil samples from the surface (0–5 cm depth) were collected from all plots prior to planting or fertilizer application in 2003–2005. Soil samples consisted of a composite of nine subsamples collected randomly from the middle four rows of the plot. Bulk soil samples were homogenized by passing the soil through a 2 mm sieve.

Chemical and physical analysis was conducted on air-dried soil that was milled and passed through a 2 mm sieve. Electrical conductivity (EC) and pH were determined in an aqueous soil suspension (2:1). Total carbon and nitrogen content was determined on duplicate samples using a Flash EA 1112 elemental analyzer (CE Elantec, Lakewood, NJ). Water-extractable anions were determined using a ICS 2000 Dionex ion chromatograph (Dionex Corp., Sunnyvale, CA). Anion separation (nitrate, sulfate and phosphorus) was performed using an IonPac AS18 hydroxide selective anion-exchange column, and data were analyzed using Chromeleon software (Dionex Corp., Sunnyvale, CA). Further details of the management and results of soil evaluations from this project can be found in reports by Reddy *et al.*[100] and Zablotowicz *et al.*[101] Soil carbon and nitrogen were subjected to analysis of variance using the general linear model procedure in SAS (Version 8.1; SAS Institute, Cary, NC). Means were separated using Fisher's LSD.

5.2.3 Soil sampling and biological characterization

For microbial community analysis, two soil samplings were made in 2005 using surface disinfected (70% isopropanol) probes 7.5 cm in diameter. The first sampling was prior to fertilization and crop planting (30 March), and the second sampling was after crop (maize or cotton) establishment (14 May). Soil subsamples from each plot were frozen at −80 °C to preserve microbial community integrity. Soil microbial community structure was characterized by fatty acid methyl ester (FAME) extraction and analysis using a protocol modified from Schutter and Dick,[102] as described elsewhere.[101] FAMEs were identified and quantified using gas chromatography (Agilent 6890 gas chromatograph, flame ionization detector) equipped for MIDI Eukaryote protocol and calibrated using MIDI FAME standards (Microbial ID, Newark, NJ). Microbial community structure was subjected to principal component analysis using SAS software (SAS, Cary, NC). Only FAMEs that were present in at least half the samples and with an average molar percentage of at least 0.5 were included in the analysis. The contributions of cropping system and herbicide regime and interactions between cropping system and herbicide regime on principal components were analyzed using SAS Proc Mixed. Each sample date was analyzed separately. Pearson's correlations were conducted to determine FAMEs that contributed to the principal components.

5.3 Results and discussion

5.3.1 Soil chemical evaluations

In the earlier report by Reddy *et al.*,[100] impacts of rotation or crop on soil properties from 2000 to 2005 were evaluated only in the context of non-GRC management, while in the present study all combinations of cropping system (continuous cotton or maize, cotton–maize and maize–cotton rotations) and herbicide management (GRC vs non-GRC) were considered for the years 2003 to 2005. For the non-GRC soils there was a gradual increase in OC over time (2000 to 2005), presumably resulting from the introduction of reduced-tillage management in that area.[100] Since soil in GRC plots was not sampled until 2003, the authors can only speculate that there was a similar increase in OC in the GRC system owing to reduced tillage.

The cropping system and herbicide regime used had a significant effect on several soil parameters, but the most consistent trend from 2003 to 2005 was that soil associated with continuous GR maize always had the highest soil carbon and nitrogen (Table 4). There were

Table 4. Soil carbon and nitrogen content in soil sampled from continuous (cont.) maize or cotton or rotations of maize and cotton under either GRC or non-GRC variety in a reduced-tillage system

	2003		2004		2005	
	GR	Non-GR	GR	Non-GR	GR	Non-GR
			Soil carbon (g kg^{-1})[a]			
Cont. maize	13.8 a	11.6 bc	14.7 a	12.3 bc	15.4 a	12.8 bc
Cont. cotton	9.7 d	10.1 d	11.1 d	11.7 cd	11.9 c	12.2 c
Rotation 1[b]	11.0 cd	12.5 b	11.9 c	12.9 b	12.2 c	13.1 b
Rotation 2[b]	11.8 bc	11.7 bc	12.3 bc	12.4 bc	12.5 bc	12.7 bc
			Soil nitrogen (g kg^{-1})[a]			
Cont. maize	1.60 a	1.39 bc	1.64 a	1.20 b	1.69 a	1.25 c
Cont. cotton	1.18 d	1.21 d	1.28 b	1.29 b	1.32 bc	1.34 bc
Rotation 1	1.22 c	1.49 ab	1.36 b	1.56 a	1.41 b	1.58 a
Rotation 2	1.23 c	1.34 b	1.25 b	1.39 b	1.29 c	1.43 b

[a] For soil carbon or nitrogen, means within a column or row followed by the same letter are not significantly different at $P < 0.05$, with the exception of soil carbon in 2005 which was significantly different at $P < 0.06$.

[b] Rotations for 2003 to 2005, respectively, were cotton–maize–cotton (rotation 1) and maize–cotton–maize (rotation 2).

a few differences in EC and pH among the different rotation and herbicide management systems, but these effects varied from year to year with no apparent trends (data not shown). In GRC plots, OC was always lower in continuous cotton than in continuous maize, and this same trend often was observed for the non-GRC soil (Table 4). Continuous cotton consistently had the lowest soil carbon and nitrogen, and no difference in those parameters was observed between GRC and non-GRC cotton. No discernible trends were observed for the rotation systems, leading to speculation that potential effects contributed by either crop were disrupted by the annual rotation. As the effects of rotation were difficult to discern using analysis of variance, comparisons of soil carbon and nitrogen were made between treatments that had a maize crop the previous year and those where the previous year's crop was cotton. This evaluation included the continuous maize and cotton treatments as well. For GRC, the correlations of soil carbon and nitrogen where maize was grown the previous year were 0.53^{**} and 0.51^{**} for carbon and nitrogen respectively. There was a weaker correlation of previous-year maize and soil carbon for non-GR (0.31^{*}), but no significant correlation for soil nitrogen.

These data present compelling evidence that components of GR maize management influenced soil carbon and nitrogen in a different way to that of non-GR maize. Maize has higher biomass, and it would be expected to contribute to soil carbon. Indeed, this was the case for both continuous GR and non-GR maize. On the other hand, cotton would not be expected to leave much residual biomass. These anticipated trends were observed in the present study. Although it was not as obvious, rotations where GR maize was the previous year's crop contributed to higher soil carbon and nitrogen. Why would either soil carbon or nitrogen be greater in GR maize than in non-GR maize? The same fertilizer rate was used, and the same maize cultivars with the exception of the transgene were used. The only other difference in management was in the way weeds were controlled. Only glyphosate was used with no pre-emergent herbicide in the GR maize, whereas in non-GRC maize both pre-emergent and post-emergent herbicides were used. Weed control efficacy for these two systems was reported previously,[100] and no major weed problems resulting from either herbicide regime were identified. However, these weed evaluations were made during mid-season.

Although no evaluations were made of winter weed populations, the authors speculate that weed biomass in areas with GR maize was higher than in those with non-GRC maize during fall and winter months where residual herbicide activity may have reduced weed density and biomass. Winter weed growth may have contributed to the higher soil carbon and nitrogen observed in GR maize. Petersen and Röver[103] evaluated three mulch systems (straw, non-winter-hardy and winter-hardy cover crop) and tillage (rotary band tillage vs plowing) in GR sugar beet. They determined that integrating winter-hardy cover crops reduced the risk of nitrogen loss by leaching because the nitrogen was immobilized in the cover crop biomass during the winter months. This is similar to what was observed in the present study, with the exception that nitrogen may have been immobilized in winter weed biomass instead of a cover crop. To extrapolate this further, one strategy for reducing the risk of nutrient loss is to reduce fertilizer rates. In conservation systems where nutrients are sequestered in weed or crop residue biomass, leaching losses might be reduced. This underscores the need for nutrient management in CP systems to be integrated with weed management, with weed and soil evaluations conducted during fallow as well as during growing seasons.

5.3.2 Soil microbial evaluations

Cropping system, herbicide system and time of sampling had a significant effect on soil microbial community structure, as discerned by principal component analysis of total FAMEs extracted from soil (Figs 1 and 2; Tables 5 and 6). Prior to planting maize in March, prior herbicide regime was the major contributor to principal component 1 (PC1) ($Pr > 0.037$), with branched-chain FAMEs (15:a, 15:0 iso, 16:0 iso, 17:1 g iso) and the mycorrhizal FAME (16:1 ώ5c) positively correlating with PC1, and saturated FAMEs (20:0, 21:0 and 22:0) negatively correlating with PC1. Cropping system was the dominant contributor to PC2 and PC3 ($Pr > 0.002$). The saturated FAMES (18:0, 24:0 and 14:0) and the gram-positive branched FAME 17:0 iso and the hydroxylated FAMEs positively correlated with PC2. The unsaturated gram-negative bacterial FAMEs (18:1 ώ9c and 20:4 ώ6c) and the cyclic FAME of 17:0 positively correlated with PC3, and the unsaturated FAME (17:1 ώ8c), the fungal FAME 18:2 ώ6c and the saturated FAME 16:0 negatively correlated with PC3.

In soil collected on 14 May after cotton planting and the first glyphosate application, significant

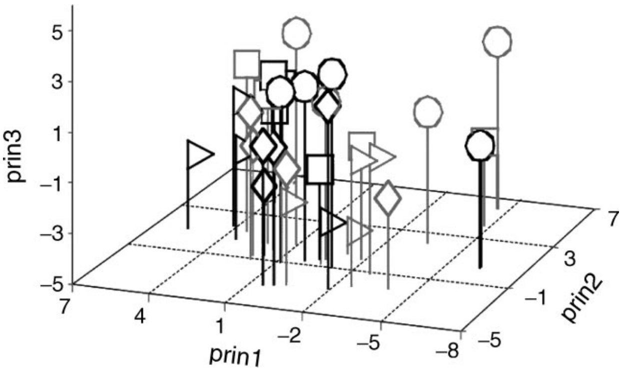

Figure 1. Soil microbial community structure based upon principal component analysis of total FAME profiles of soils sampled from plots planted to glyphosate-resistant crop management (grey) and conventional herbicide management (black). Soil was sampled on 30 March 2005. Continuous cotton (circles), continuous maize (squares), rotation 1 (flags), rotation 2 (diamonds).

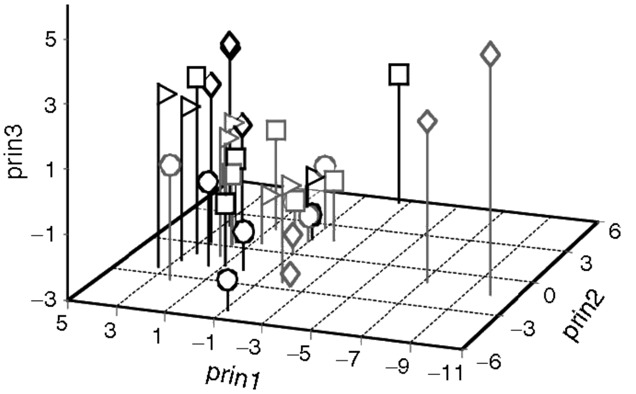

Figure 2. Soil microbial community structure based upon principal component analysis of total FAME profiles of soils sampled from plots planted to glyphosate-resistant crop management (grey) and conventional herbicide management (black). Soil was sampled on 30 May 2005. Continuous cotton (circles), continuous maize (squares), rotation 1 (flags), rotation 2 (diamonds).

Table 5. Analysis of variance of principal component analysis of FAME characterization of soils from maize cotton cropping systems on 30 March 2005

Source	PC1 (32.1%)	PC2 (18.2%)	PC3 (13.1%)
	Pr > *F*		
System	0.949	0.0015	0.0018
GR	0.0373	0.4133	0.8166
Sys × GR	0.1900	0.0948	0.9543
	FAMEs correlating with principal components (*Pr* > 0.02)		
	15:0 anti (+)	Unknown (−)	16:0 (−)
	16:iso (+)	17:0 iso (+)	18:1 ω9c (−)
	15:0 iso (+)	18:0 (+)	17:0 cycl (+)
	17:1 g iso (+)	24:0 (+)	20:4 ω6c (+)
	16:1 ω5c (+)	14:0 (+)	17:0 anti
	20:0 (−)	19:1 OH (+)	17:1 ω8c (+)
	21:0 (−)	17:0 anti (−)	18:2 ω6c (−)
	22:0 (−)		

Table 6. Analysis of variance of principal component analysis of FAME characterization of soils from maize cotton cropping systems on 14 May 2005

Source	PC1 (32.1%)	PC2 (18.2%)	PC3(13.1%)
	Pr > *F*		
System	0.214	0.124	0.081
GR	0.001	0.895	0.391
Sys × GR	0.002	0.0597	0.685
	FAMEs correlating with principal components (*P* > 0.02)		
	18:0 (−)	15:0 iso (+)	15:0 (+)
	16:1 ω7c (−)	17:1 iso (+)	17:0 (+)
	16:0 (−)	18:2 ω6c (−)	18:1 ω9t OH (−)
	20:0 (+)	19:1 ω8 OH (+)	22:0 (−)
	14:0 (+)	16:0 iso (+)	24:0 (−)
	18:1 ω9t (+)	17:0 iso (+)	17:0 anti (−)
	17:0 anti (+)	17:0 cyclo (+)	21:0 (−)
	24:0 (+)	17:0 anti (+)	17:0 iso (−)
	12:0 (+)	16:1 ω7c (+)	
	20:4 ω6c (+)	15:0 anti (+)	

contributions of herbicide regime or interaction between cropping system and herbicide were observed in the first two components, with different FAMEs correlating with the principal components. Various saturated FAMEs were major positive and negative contributors to PC1 (12:0, 14:0, 20:0 and 24:0 positive, and 16:0 and 18:0 negative), and likewise with unsaturated FAMEs (16:1 ω7c negative, and 18:1 ω9t and 20:4 ω6c positive). In PC2 the interaction between cropping system and herbicide contributed to the microbial community structure, with the gram-positive FAMEs (15:0 iso, 16:0 iso, 17:0 iso and 17:0 anti) and gram-negative FAMES (19:1 ω8 OH and 16:1 ω7c) being positively correlated with PC1, and the fungal FAME 18:2 ω8c being negatively correlated with PC2.

Soil microbial community structure was dynamic in response to agronomic inputs, and changes in community structure also may have been impacted upon by soil chemical properties and environmental conditions before soil sampling. Glyphosate is relatively short lived in a Dundee silt loam soil (30–50% mineralized within 30 days),[33] and alterations of the microbial community may be due to indirect effects of continuous glyphosate use, e.g. soil carbon and nitrogen modifications as previously discussed. As glyphosate inhibits aromatic amino acid synthesis in many soil bacteria and fungi as well as in plants, it may exhibit short- to long-term effects on soil biological processes. Glyphosate adds labile carbon and phosphorus, and can directly stimulate biological properties such as soil microbial biomass, specific components of the microflora and respiration activity. In a long-term study evaluating the effects of a conventional or glyphosate-based weed control program under various maize–cotton cropping systems, the authors observed that continuous GR maize maintained greater soil organic carbon and nitrogen compared with conventional maize. Soil microbial community structure based on total fatty acid methyl ester analysis indicated that branched FAMEs representing gram-positive bacteria and a mycorrhizal FAME were positively correlated with a conventional herbicide system, and saturated fatty acids were negatively correlated. Subtle changes in microbial community structure may be due to many factors, including pesticide use. Previous reports[29,33] on short-term effects of glyphosate application noted subtle or no changes in FAME-based community structure in soils or soybean rhizospheres following glyphosate application. The changes observed here are also minor, but do support the argument that long-term research on GRC systems is needed to evaluate potential alterations of the soil microbial ecosystem. A similar contribution of GRC was observed in PC1 of both sampling dates, indicating some level of consistency, but unfortunately these were the only dates this analysis was conducted. Some of the differences in microbial community structure may be related to non-target effects of the glyphosate cropping system, as differences in soil carbon and nitrogen in plots maintained under various cropping and herbicide regimes.

6 SUMMARY AND CONCLUSIONS

In varying degrees, and often dependent on site-specific factors, less disturbance of soil and increased plant residue accumulation under CP management results in a variety of conditions that might influence glyphosate fate in soil: higher soil OC, less distribution of applied nutrients (particularly those that are immobile, such as phophorus) in soil, nutrient immobilization, lower pH, higher and more diverse microbial populations and enhancement of preferential flow pathways. Higher OC and lower pH might enhance soil sorption of glyphosate, rendering it less bioavailable and more immobile. Higher microbial populations may contribute to more rapid glyphosate degradation. Overall, characteristics of CP-managed soils relative to glyphosate should contribute to the environment in a beneficial way, and, if GRC helps to promote these conditions, the net contribution of combined CP and GRC on the environment will be positive. It should be noted, however, that most of the potential effects appear to be minor and transient.

The case study reported in this paper corroborates some of the literature reviewed previously. Results from the case study indicated that higher plant biomass during the fallow months contributed to increased carbon and nitrogen sequestration in GR maize under reduced-tillage management. Evaluation of the microbial community structure indicated that, following 5 years of continuous GRC, there was a significant effect of GRC altering soil community structure, but that differences in microbial populations between GRC and non-GRC were subtle and dynamic. Increased sequestration of carbon and nutrients and diverse soil microbial populations are desirable attributes and benefit the environment.

It is logical to extrapolate that the potential for pollution and soil quality degradation will be reduced under GRC systems, as glyphosate alone (more frequently) or in combination with other herbicides (less frequently) is used to manage weeds. Adopting CPs may reduce this potential even further. From another perspective, increased glyphosate use with a concomitant decrease in the use of other herbicides has increased the risk of weed species evolving glyphosate resistance.[104] This trend might reduce glyphosate input in the future and increase use of less environmentally benign herbicides. So, in the end, have we really reduced input of herbicides that may be detrimental to the environment?

Although it is possible to extrapolate and speculate on the positive or negative benefits of either CP or GRC, virtually no research has addressed the joint impacts of CPs and GRCs on the environmental quality by making side-by-side comparisons of GRC and non-GRC systems under either conservation or conventional conditions. CP and GRC technologies appear to be compatible, but are there additional or new risks associated with combining GRC and CP than exist with either alone, i.e. is there need for risk assessment? Anecdotally, if you extend the logic of what is known, it appears there is no significant added risk of combining the two technologies, but the corroboration research is lacking. Does the science need to be done? Additional research would be useful to confirm whether there are no benefits or if benefits such as decreased runoff and improved soil quality are synergistic or additive. Modeling could help in this respect, but more ground truth data would make the modeling efforts more credible.

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