Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil†

Mark A Weaver,* L Jason Krutz, Robert M Zablotowicz and Krishna N Reddy
USDA-ARS Southern Weed Science Research Unit, Stoneville, MS 38776, USA

Abstract: Transgenic glyphosate-resistant (GR) soybean [*Glycine max* (L.) Merr.] has enabled highly effective and economical weed control. The concomitant increased application of glyphosate could lead to shifts in the soil microbial community. The objective of these experiments was to evaluate the effects of glyphosate on soil microbial community structure, function and activity. Field assessments on soil microbial communities were conducted on a silt loam soil near Stoneville, MS, USA. Surface soil was collected at time of planting, before initial glyphosate application and 14 days after two post-emergence glyphosate applications. Microbial community fatty acid methyl esters (FAMEs) were analyzed from these soil samples and soybean rhizospheres. Principal component analysis of the total FAME profile revealed no differentiation between field treatments, although the relative abundance of several individual fatty acids differed significantly. There was no significant herbicide effect in bulk soil or rhizosphere soils. Collectively, these findings indicate that glyphosate caused no meaningful whole microbial community shifts in this time period, even when applied at greater than label rates. Laboratory experiments, including up to threefold label rates of glyphosate, resulted in up to a 19% reduction in soil hydrolytic activity and small, brief (<7 days) changes in the soil microbial community. After incubation for 42 days, 32–37% of the applied glyphosate was mineralized when applied at threefold field rates, with about 9% forming bound residues. These results indicate that glyphosate has only small and transient effects on the soil microbial community, even when applied at greater than field rates.

Keywords: glyphosate; soil microbial community; herbicide mineralization; glyphosate mineralization

1 INTRODUCTION

Few herbicides have achieved the level of acceptance and use of glyphosate. Reasons for its success include a broad weed control spectrum, its lack of undesirable effects in transgenically resistant crops and its environmental safety.[1,2] These same properties, however, have come under scrutiny. There is a growing awareness of weeds that are poorly managed in glyphosate-based systems. Some weeds, such as redvine, *Brunnichia ovata* (Walter) Shinners, are becoming limiting production factors.[3] Other weeds, including horseweed, *Conyza canadensis* (L) Cronq., and rigid ryegrass, *Lolium rigidum* (Gaud.), have developed glyphosate resistance and will require new management strategies.[4–7] Even with heightened interest in the weed management challenges of glyphosate-based systems, there is growing awareness of non-target effects of glyphosate. Glyphosate can reduce nodulation and nitrogen fixation activity in glyphosate-resistant soybean,[1,8–10] although these effects on the *Bradryhizobium japonicum*–soybean symbiosis are not consistently observed. Soybean seedlings treated with glyphosate are more susceptible to infection with *Fusarium solani* (Martius) Sacc., causing sudden death syndrome.[11] Studies by Kremer *et al*.[12] indicated that a significant amount of applied glyphosate is released into the rhizosphere, and that *Fusarium* spp. responded positively to increasing glyphosate concentrations in culture.

Questions regarding the environmental safety of glyphosate include issues of persistence and non-target effects. Improved methods for the detection of glyphosate in soil and water have led to observations of unexpected and undesirable persistence of glyphosate in soil and groundwater.[13] There is a variable degree of mineralization potential of glyphosate in soil.[14,15] Gimsing *et al*.[14] associated degradation potential with the population of *Pseudomonas* spp.

The effect of glyphosate on soil microflora and microbial processes has been an area of much research, and contrasting results have been observed by various researchers. Studies by Haney *et al*.[16,17] indicated that glyphosate application can increase soil microbial biomass, respiration and carbon and nitrogen mineralization. Studies by Busse *et al*.[18] found no effect of glyphosate when applied at 5–50 $\mu g\,g^{-1}$ soil, and only limited effects on soil microbial communities using Biolog substrate profiles. Studies on Brazilian soils[19] indicated that glyphosate is rapidly metabolized to aminophosphonic acid, and increased respiration, fluorescein diacetate (FDA) hydrolytic activity and fungal proliferation following

* Correspondence to: Mark A Weaver, USDA-ARS Southern Weed Science Research Unit, Stoneville, MS 38776, USA
E-mail: mweaver@ars.usda.gov

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glyphosate treatment. Studies conducted in the midwestern USA and under controlled conditions found limited or no effect of the glyphosate and the glyphosate-resistant soybean cropping system on soil microbial community structure, soil nematode communities, substrate-induced respiration and soil microbial biomass.[20] As a consequence of these contrasting results, the present study was designed to detect changes in soil microbial community structure and activity following glyphosate exposure in the field and the laboratory.

2 MATERIALS AND METHODS

2.1 Field studies

The experimental area was tilled with a disk harrow followed by a field cultivator in the fall of each year. Plots were treated with paraquat at 1.1 kg ha^{-1} 1–4 days prior to soybean planting to kill existing vegetation. Glyphosate-resistant soybean (AG 4702RR) was planted at a rate of 355 000 seeds ha^{-1} on 19 April 2002, 21 April 2003 and 27 April 2004 in Dundee silt loam (fine-silty, mixed, thermic Aeric Ochraqualf) on the Southern Weed Science Research farm in Stoneville, MS. Experimental units consisted of four rows of soybeans spaced 102 cm apart and 12.2 m long. Glyphosate-isopropylammonium (Roundup Ultra) was applied at 4 weeks after planting (WAP) (two- to three-trifoliate leaf stage) and 6 WAP (six- to seven-trifoliate leaf stage) to treated plots at a rate of 2.5 kg AE ha^{-1}. Non-glyphosate-treated plots were included as a control. Bulk surface soil (0–2.5 cm) was collected at time of planting, before initial glyphosate application and 14 days after each glyphosate application. Soybean roots were excavated at the last three sample dates in 2004, and rhizosphere soil was recovered from the roots. A different experimental site with similar soil conditions was used in each year.

2.2 Laboratory studies

2.2.1 Microbial community response study

The top 2.5 cm of the Ap horizon of a Dundee silt loam was collected and stored at 4 °C until use. Soil characteristics are: pH 6.3 (1:2 soil:water); cation exchange capacity 15 cmol kg^{-1}; soil organic matter content 1.1%; and soil textural fractions 26% sand, 56% silt and 18% clay. Glyphosate (98% purity; Chemserve, West Chester, PA) was added to soil at rates of 1× (47 μg g^{-1}) or 3× (140 μg g^{-1}). Rate calculations were based on a field application of glyphosate (0.84 kg ha^{-1}) and a shallow (2 mm) glyphosate–soil interaction depth.[16]

2.2.2 Glyphosate mineralization study

Mineralization of ^{14}C-glyphosate was evaluated in biometer flasks.[21] Soil (25 g dry weight equivalent) was fortified with a solution of technical-grade glyphosate (98% purity; Chem Service) and ^{14}C-labeled glyphosate (54 mCi $mmol^{-1}$ specific activity, 99% radiolabeled purity; Amersham Life Sciences) in deionized water. The initial herbicide concentration was 47 μg g^{-1} for 1× and 140 μg g^{-1} for 3×. The initial radioactivity was 190 Bq g^{-1} for 1× and 570 Bq g^{-1} for 3×. Biometers were sealed and incubated at 25 °C. Evolved ^{14}C-carbon dioxide was trapped in aqueous sodium hydroxide (0.1 M; 10 mL) and quantified by liquid scintillation spectroscopy (LSS) using Hionic-Fluor (Perkin Elmer, Boston, MA). To avoid saturation by carbon dioxide, sodium hydroxide solution was replaced on sampling days. Soils were destructively sampled at five sampling times throughout the 46 day incubation. Soils were extracted twice with aqueous sodium hydroxide (0.1 M; 60 mL), shaken at room temperature (24 h for the first extraction, 1 h for the second extraction) and centrifuged at 6000 × *g* for 10 min, and radioactivity was determined in duplicate by LSS. After extraction, air-dried soil was manually crushed into uniform particle size, and duplicate samples (0.30 g) were weighed onto Whatman 1 qualitative filter paper (Whatman Inc., Florham Park, NJ). Samples were combusted in a Packard model 306 oxidizer (Packard Instruments, Chicago, IL), and evolved ^{14}C-carbon dioxide was trapped in scintillation vials containing Carbo-Sorb + Permafluor (1 + 1 by volume, 20 mL; Perkin Elmer, Boston, MA). Radioactivity was determined by LSS. The amount of ^{14}C-carbon dioxide recovered from the combusted samples (bound residue) was added to the cumulative ^{14}C-carbon dioxide evolved, and ^{14}C was extracted to determine the mass balance of ^{14}C.

Mineralization data were fitted to a first-order kinetics model using SAS NLIN:

$$Y = a(1 - e^{-kt})$$

where a is the maximum mineralized (% of initial), t is time (days) and k is the first-order rate constant (day^{-1}). Sodium hydroxide extractable ^{14}C residues and bound residues at 0, 7, 14, 28 and 42 days were analyzed using a two-tailed t-test.

2.3 Microbial response parameters

2.3.1 Ester linked fatty acid methyl ester

For both the field and *in vitro* studies, microbial communities were evaluated by ester-linked fatty acid methyl ester (EL-FAME).[22–24] EL-FAMEs were separated, identified and quantified using an Agilent 6890 gas chromatograph and the MIDI EUKARY protocol, and verified using MIDI FAME standards (Microbial ID, Newark, NJ). Relatedness between samples was determined by the MIDI Sherlock version 4.5 dendrogram generating feature. This is a multivariate clustering statistical technique that expresses the overall similarity between samples. The similarity between any two samples can be determined by tracing each branch back to where they share a common branch point. Samples with high overall similarity will branch at comparatively short

distances. When treatment effects were apparent by analysis of the dendrograms of the whole-community EL-FAMEs, ANOVA was performed on individual EL-FAMES.

2.3.2 Fluorescein diacetate hydrolysis enzyme assays

Fluorescein diacetate (FDA) hydrolysis enzyme assays, modified from Schnürer and Rosswall,[25] were conducted as a general indicator of soil microbial hydrolytic activity (esterase, lipase and protease). Assays were conducted using 4 g soil (fresh weight) in 20 mL phosphate buffer (50 mM, pH 7.6) containing 0.5 mg of FDA, incubated for 1 h in a shaking incubator (28 °C, 150 rpm). Specific activity was calculated and normalized against the treatment that received no glyphosate amendments.

3 RESULTS AND DISCUSSION

3.1 Field studies

Following the second in-season glyphosate application, the microbial community was not clearly separated by glyphosate treatment. The EL-FAMEs were clustered, and soils receiving different glyphosate treatments had similar profiles; i.e. the total distance separating samples was small. Observation with pure bacterial cultures suggests that distances below 10 are generally the same species. The entire soil communities here were separated by a distance of less than 8 (total separation less than 8) (Fig. 1). In 2004, the EL-FAMEs from microorganisms in soil collected from the rhizosphere were distinct from the bulk soil (Fig. 2). The profiles from rhizosphere soil receiving different glyphosate rates, however, were similar overall (Fig. 3). A significant block effect was noted, however, for other parameters of this field study[26] associated with a texture gradient in the field. Collectively, this analysis of the microbial community based on EL-FAME agrees with conclusions[18,20,27] that glyphosate has a minimal effect on microbial community structure.

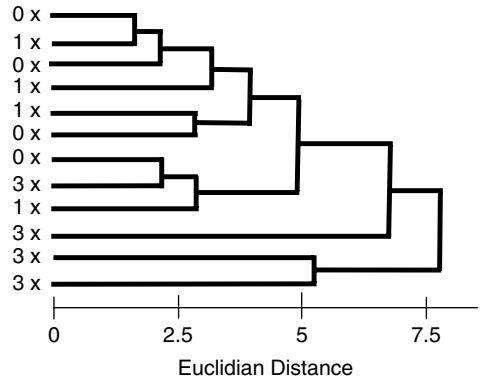

Figure 1. Separation of EL-FAME profiles from microbial communities in bulk soil following the second in-season glyphosate application.

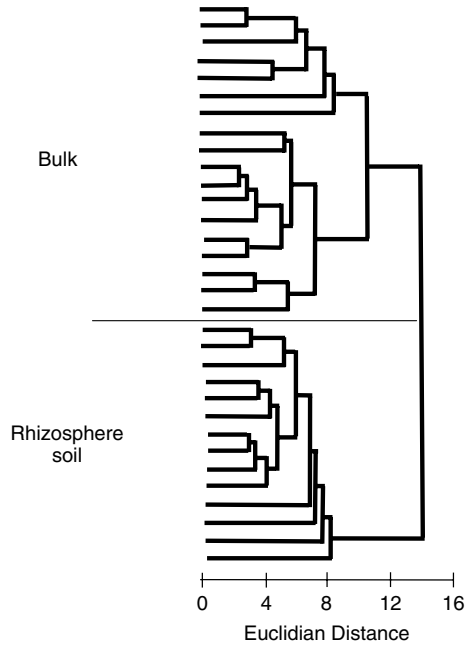

Figure 2. Separation of EL-FAME profiles from microbial communities in bulk soil and the soybean rhizosphere following the second in-season glyphosate application.

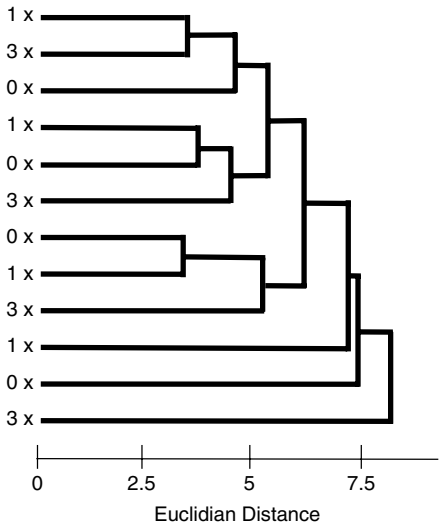

Figure 3. Separation of EL-FAME profiles of microbial communities in soybean rhizosphere soil following the second in-season glyphosate application.

3.2 *In vitro* microbial community response to glyphosate

Analysis of the EL-FAMEs after 3 days incubation revealed slightly less overall variation (overall Euclidian distance <6) and a separation of the profiles corresponding to the level of glyphosate treatment (Fig. 4). By the seventh day after incubation, however, the community structure had reconverged (Fig. 5). Individual EL-FAMEs that differed in relative abundance after 3 days incubation with glyphosate were 12:0 ($P = 0.048$, increased in response to 1× or 3× glyphosate rates), 16:1 ω 7c ($P = 0.01$, increased in response to 1× glyphosate, associated with gram-negative bacteria[28]), 20:0 ($P = 0.05$, increased in

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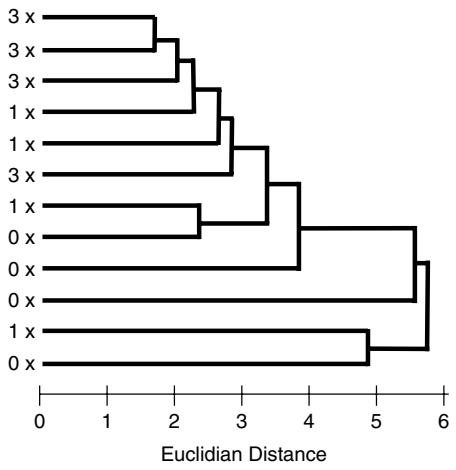

Figure 4. Separation of EL-FAME profiles from microbial communities in soil incubated *in vitro* alone or with 1× or 3× labeled rates of glyphosate for 3 days.

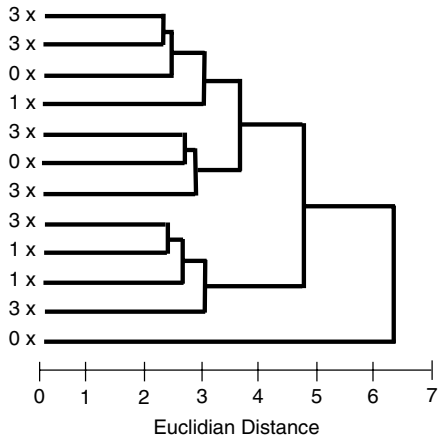

Figure 5. Separation of EL-FAME profiles from microbial communities in soil incubated *in vitro* alone or with 1× or 3× labeled rates glyphosate for 7 days.

response to glyphosate) and 18:1 ω 9t alcohol ($P = 0.05$, increased in response to glyphosate). Concurrently, normalized FDA enzyme activity revealed a small but significant reduction in hydrolytic activity in soils treated with threefold label rates of glyphosate at 3 and 14 days after treatment (Fig. 6). Other time points and glyphosate concentrations were not significantly different from the non-glyphosate-treated soil.

The repressed FDA activity in response to glyphosate treatment, while small, is in contrast to other reports of microbial stimulation subsequent to glyphosate application[12,19] Others have reported that glyphosate increased microbial biomass.[16,19] Glyphosate has stimulated soil fungi populations in other studies,[12,19] but no significant effect of glyphosate on the abundance of fungi-specific biomarkers (16:1 ω 5c; 18:2 ω 6c) was observed in the present study.

The measured *in vitro* responses were small and transient. The transient nature of these effects suggests that monitoring for microbial community shifts 14 days after treatment in field studies could miss

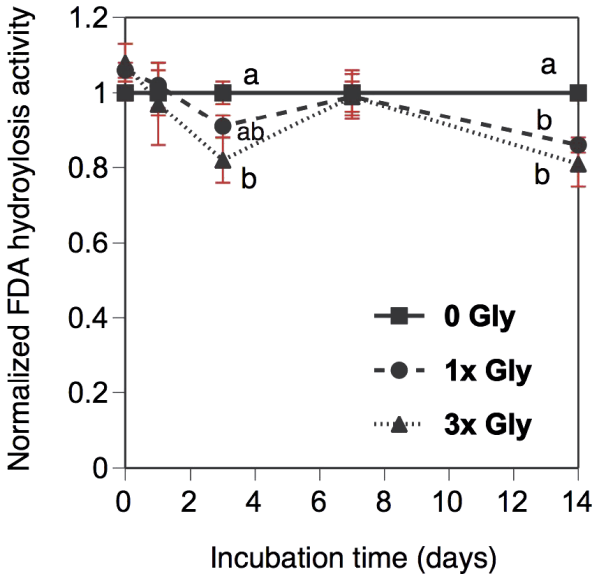

Figure 6. Normalized fluorescein diacetate (FDA) hydrolytic activity in soils incubated *in vitro* with glyphosate.

these short-lived effects. The use of homogenized soil in the laboratory study appeared to reduce plot-to-plot variability that occurred in the field, enabling detection of subtle changes in the soil microbial community.

3.3 Mineralization of glyphosate *in vitro*

At both treatment concentrations, mineralization of glyphosate followed first-order kinetics (Fig. 7), and estimates for parameters a and k were significantly different between treatments. Specifically, a and k were greater for 1× than 3×. These data indicate that cumulative mineralization and the rate of mineralization, on a percentage basis, were reduced at the 3× rate of glyphosate compared with the 1× glyphosate rate. The absolute mass of glyphosate mineralized was greater in the 3× treatments than in the 1× treatments for any given time point. Similarly, sodium hydroxide extractable ^{14}C residues were greater for the 3× glyphosate rate than for the 1× rate at all time periods excluding 0 days (Table 1). In

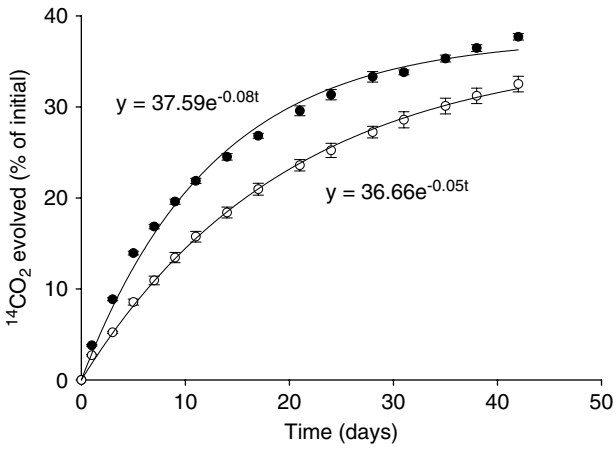

Figure 7. Mineralization of glyphosate *in vitro*: ●, treatments with 1× rate of glyphosate; ○, treatments with 3× rate of glyphosate.

Table 1. Bound and sodium hydroxide extractable ^{14}C residues in glyphosate-treated soil

	0 days		7 days		14 days		28 days		42 days	
TRT	Bound (%)	Extract (%)	Bound (%)	Extract (%)	Bound (%)	Extract (%)	Bound (%)	Extract (%)	Bound (%)	Extract (%)
1×	4.7 (1.3)	92.2 (1.4)	6.0 (1.1)	75.8 (1.3)	6.4 (1.2)	64.1 (1.7)	6.3 (0.3)	58.4 (1.0)	5.7 (0.8)	53.9 (1.0)
3×	3.0 (0.5)	93.2 (1.7)	8.2 (1.2)	78.5 (1.3)	8.7 (0.5)	70.7 (2.4)	8.5 (0.4)	63.9 (0.7)	6.6 (1.4)	59.1 (2.4)
P	0.125	0.565	0.107	0.027	0.001	0.014	0.001	0.001	0.250	0.046

The values in parentheses are standard deviations.
The P value for each column is the probability that the 2 means could be equal.

contrast, bound residue formation was greater at day 14 and 28 in the 3× glyphosate rate compared with the 1× rate.

The rates of mineralization observed in this study are similar to those reported elsewhere.[14,15] The studies by De Andrea *et al*.[29] found that, after repeated application of glyphosate, mineralization of glyphosate decreased. This is similar to the present studies with lower rates of degradation at higher concentrations. Although FDA activity was reduced by glyphosate in concurrent assays, a similar level of reduced activity was observed in both treatments. It is possible that higher rates of application may impede glyphosate degradation potential, but it is also possible that physiochemical processes lower activity. Several studies have shown increased microbial biomass in response to glyphosate,[16,19] and it might be expected that a greater microbial biomass would enhance the potential for glyphosate degradation.

Measurements of soil quality often include parameters for soil microbial activity and microbial diversity.[30,31] Management practices, including chemical inputs, may lower soil quality directly or indirectly through perturbations to the soil microbial community.[32] The impact of glyphosate on soil microbes and microbial processes in this study was small and short lived. This microbial resilience, coupled with the lack of soil persistence, indicates that soil quality will not be reduced by glyphosate application in similar agroecosystems.

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