

Soil Depth and Tillage Effects on Glyphosate Degradation

ROBERT M. ZABLOTOWICZ,^{*,†} CESARE ACCINELLI,[‡] L. JASON KRUTZ,[†] AND
 KRISHNA N. REDDY[†]

[†]Southern Weed Science Research Unit, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 350, Stoneville, Mississippi 38776, and [‡]Department of Agro-Environmental Science and Technology, University of Bologna, Viale Fanme 44, 40127 Bologna, Italy

The use of glyphosate-resistant crops facilitated the widespread adoption of no-tillage (NT) cropping systems. The experimental objectives were to determine glyphosate sorption, mineralization, and persistence at two depths [0–2 cm (A) and 2–10 cm (B)] in a silt loam managed under long-term conventional tillage (CT) or NT soybean. Relative to the other soils, organic carbon (OC) and fluorescein diacetate (FDA) hydrolytic activity were at least 1.4-fold higher in NT-A. Glyphosate K_d values ranged from 78.2 to 48.1 and were not correlated with OC. Cumulative glyphosate mineralized after 35 days was highest in NT-A soil (70%), intermediate in CT-A and CT-B (63%), and least in NT-B (51%). Mineralization was positively correlated with OC and FDA activity, but negatively correlated with K_d , indicating that sorption decreased bioavailability. Independent of tillage and depth, the half-lives for 0.01 N CaCl₂ and 0.1 N NaOH extractable residues (bioavailable residues and residues bound to iron and aluminum oxides, respectively) were ≤ 1.2 h and ≤ 14.2 days, respectively. These data indicate that glyphosate sorption and persistence are similar between the surface of NT and CT soils and that the adoption of NT will likely have minimal impact on the risk for nontarget effects of glyphosate on soil microflora or transport in surface runoff.

KEYWORDS: Biodegradation; glyphosate; mineralization; sorption; crop management

INTRODUCTION

The widespread acceptance of glyphosate-resistant cropping systems, that is, canola, corn, cotton, and soybean, facilitated the adoption of conservation management practices (1), particularly no-tillage (NT). The elimination of all tillage operations in NT systems changes soil chemical, physical, and biological properties, whereby pesticide fate is potentially altered relative to conventional tillage (CT) systems. Although the potential for glyphosate loss in runoff is lower than for the soil-applied herbicides it replaced, there is evidence of significant off-site transport under NT managements systems (2, 3). Consequently, the fate of glyphosate in NT systems must be evaluated to determine whether altered soil properties, specifically redistribution of organic matter in NT soils, will affect glyphosate persistence, sorption, and off-site transport.

Owing to the success of glyphosate-resistant cropping systems, glyphosate [*N*-(phosphonomethyl)glycine] has become the most widely used herbicide in North America (4); consequently, the herbicide's fate in soil has been extensively evaluated. In agriculturally relevant soils, glyphosate is a polyprotic acid forming monovalent and divalent anions and is sorbed primarily by aluminum and iron oxides, poorly ordered aluminum silicates, and edges of layer silicates (5). The effect of organic carbon (OC) on glyphosate sorption is poorly understood, and experimental

results often conflict with each other. Several studies imply that glyphosate sorption is positively correlated with OC, whereas others suggest that glyphosate sorption is negatively correlated with OC or that OC has no effect on sorption (3, 5). Thus, it is unknown if the accumulation of OC in NT soils will increase glyphosate sorption, thereby reducing the herbicide's off-site transport. Although sorbed by soil colloids, glyphosate is relatively bioavailable for microbial degradation, with mineralization to carbon dioxide as the primary pathway for dissipation (6–9).

The herbicide is metabolized by soil microorganisms, and two pathways have been described. First, certain bacteria, for example, *Arthrobacter* spp., *Rhizobium* spp., and *Pseudomonas* spp., possess a C–P lyase that hydrolyzes glyphosate, yielding inorganic phosphate and sarcosine (10–12). The activity of C–P lyase is regulated by phosphate, and organisms use this pathway to scavenge phosphate. Second, some organisms such as *Ochromobacter antropi* (formerly *Achromobacter* sp.) possess a glyphosate oxidoreductase that yields aminomethylphosphonic acid (AMPA) and glyoxylate (13). The metabolite, AMPA, often accumulates in glyphosate-treated soil (6, 14), and it is assumed that this pathway is common in many soil microflora, although most glyphosate-degrading organisms isolated from natural sources possess the C–P lyase, not the oxidoreductase (11).

Although glyphosate sorption and dissipation in agricultural soils have been extensively evaluated (6, 8, 9, 15, 16), no study has evaluated the fate of glyphosate in NT systems. Thus, the objectives of this study were to evaluate the degradation of ¹⁴C-labeled glyphosate in soil collected from soybean plots

*Author to whom correspondence should be addressed [telephone (662) 686-5272; fax (662) 686-5422; e-mail Robert.Zablotowicz@ars.usda.gov].

maintained under continuous long-term CT or NT. As soil properties and the potential for herbicide degradation under NT can be different in the surface layer compared to lower depths (17), a component of this study was to evaluate glyphosate degradation in the upper 0–2 cm (A) and the 2–10 cm (B) soil depths. Moreover, various soil biological and chemical analyses were conducted to determine if there is a relationship between altered soil properties and glyphosate fate.

MATERIALS AND METHODS

Soils. The Dundee silt loam (fine-silty, mixed, thermic Aeric Ochraqul) evaluated in this study was collected from long-term soybean research plots maintained under NT or CT for 10 consecutive years (18). The NT plots were not tilled after the fall of 1997, whereas CT plots were tilled each year in the fall after soybean harvest and (or) in the spring before planting soybean. CT plots were tilled with a disk harrow and a field cultivator. In 2007 soil samples were collected from four replicate plots of each tillage regimen 2 months after soybean harvest at two depths (0–2 and 2–10 cm depths) using a 7.5 cm diameter probe. Ten cores from each plot were pooled, passed through a 2 mm sieve, and stored at 4 °C.

Herbicides. ¹⁴C-Glyphosate labeled at the aminomethyl carbon (2.0 GBq mmol⁻¹ specific activity, 99.5% radiological purity) was obtained from Amersham Biosciences (Pittsburgh, PA), and technical grade glyphosate was purchased from Fluka BioChemika (Sigma, St. Louis, MO).

Soil Chemical, Physical, and Biological Properties. Chemical and physical analyses were conducted on air-dried soil that was ground through a Wiley mill. Soil textural analysis was determined by using the hydrometer method (19). Electrical conductivity (EC) and pH were determined in an aqueous soil suspension (2:1 w/v) in triplicate, and total carbon and nitrogen contents were determined on duplicate samples using a Flash EA 1112 elemental analyzer (C. E. Elantech, Lakewood, NJ).

Two soil biological parameters were evaluated. Fluorescein diacetate (FDA) hydrolysis, a measure of total microbial activity, was determined using methods described by Zablotowicz et al. (20). The soil microbial community structure was assessed by using a previously described method for fatty acid methyl ester (FAME) analysis (1, 21).

Sorption. Glyphosate sorption isotherms were determined for all treatments using the batch equilibration technique. Batch solution concentrations of 7.5, 30, and 120 mg L⁻¹ were prepared in 0.01 M CaCl₂ and contained a mixture of ¹⁴C-labeled and technical grade glyphosate. A 10 mL aliquot of each solution was added to 2 g of soil in a 25 mL Corex centrifuge tube. Soil slurries were placed on a rotary shaker for 17 h at 25 °C and then centrifuged at 10000g for 10 min. Two 1-mL aliquots of the supernatant were removed and mixed with 10 mL of Ecolume, and the ¹⁴C content was determined by liquid scintillation spectroscopy (LSS; Perkin-Elmer, TriCarb4000, Meriden, CT). Sorbed glyphosate was calculated as the difference between the supernatant concentration and the amount initially added. Sorption distribution coefficients (*K_d*) were calculated using the equation

$$K_d = S_{eq}/C_{eq} \quad (1)$$

where *S_{eq}* is mg of test substance per kg of soil at equilibrium and *C_{eq}* is mg of test substance per L of supernatant at equilibrium.

Mineralization. Glyphosate mineralization potential was evaluated in biometer flasks (21). Soil, 25 g of air-dried weight equivalents, was fortified with a solution of ¹⁴C-labeled and technical grade glyphosate dissolved in distilled water. The nominal glyphosate concentration was 1 μg g⁻¹, which corresponds to 300 Bq g⁻¹ of radioactivity. Soil moisture was adjusted to 28%, and biometers were incubated in the dark at 28 °C. Glyphosate mineralization was monitored by sampling 1 N NaOH solutions. Trapped ¹⁴CO₂ in the NaOH was determined on duplicate 1 mL aliquots by LSS (Packard TriCarb 4000 series, Packard Instruments Co., Meriden, CT) using Hi-Ionic scintillation fluid (Packard Instruments), 15 mL per sample. Sampling was conducted on the basis of the rate of mineralization.

Cumulative mineralization data were fitted to the two-compartment model (22)

$$P = S_{0,1}[1 - \exp(-k_1 t)] + S_{0,2}[1 - \exp(-k_2 t)] \quad (2)$$

where *P* is evolved ¹⁴C-CO₂ (%); *S_{0,1}* and *S_{0,2}* represent the initial glyphosate concentration in compartments 1 and 2, respectively (%); and *k₁* and *k₂* represent the first-order rate constants for compartments 1 and 2, respectively (days⁻¹). Compartments 1 and 2 were defined as the free (total extractable) and bound (nonextractable) glyphosate residues, respectively.

Extractable and Nonextractable Residues. Replicate flasks (250 mL polypropylene centrifuge bottles) containing 25 g of dry weight equivalent soil were treated as described previously and sampled at 0 and 14 days after treatment. Additionally, biometer flasks were destructively sampled at 3 and 35 days after treatment. The first extraction used 75 mL of 0.01 M CaCl₂ for 2 h to assess bioavailable glyphosate (6). The second extraction was overnight (20 h) with 75 mL of 0.1 N NaOH (9), which on the basis of previous studies was capable of extracting >90% of glyphosate added to soil. After each extraction, supernatants were collected by centrifugation, and extractable radioactivity was determined on duplicate aliquots using LSS with Ecolume as cocktail. Soils were air-dried, and nonextractable radioactivity was determined by oxidation of duplicate 300 mg samples (21).

Effects of Autoclaving. Soil, 25 g dry weight equivalents, collected from CT-A was placed in biometer flasks, sealed with aluminum foil, and autoclaved for 15 min at 137 °C for 3 consecutive days. Eight replicate flasks were established for autoclaved and nonautoclaved soil, with four flasks sampled at 3 and 14 days after treatment. Soil was treated with a mixture of ¹⁴C-labeled and nonlabeled glyphosate as described previously and incubated in the dark at 25 °C. Mineralization was monitored at 1, 2, 3, 5, 7, 9, 11, and 14 days after treatment. Extractable and nonextractable radioactivities were determined at days 3 and 14.

Statistical Analysis. Data were subjected to analysis of variance (ANOVA) using the general linear model procedure in SAS (26). Nonlinear regression techniques (SAS PROC NLIN) were used to estimate average *K_d* with the three herbicide concentrations. Means were separated using Fisher's protected LSD at *P* = 0.05. Pearson's correlations were determined to assess the contribution of soil characteristics to glyphosate mineralization. Principal component analysis (PCA) was used to assess FAME-based microbial community structure, with fatty acids that were very rare excluded from analysis to reduce minor experimental variation (1, 21).

RESULTS AND DISCUSSION

Soil Chemical, Physical, and Biological Properties. Soil texture was similar among soils, with average sand, silt, and clay contents of 262, 558, and 180 g kg⁻¹, respectively (data not shown). Relative to the other soils, total OC and N were at least 1.4-fold higher in NT-A (Table 1). Soil pH was higher in surface–2 cm depths than in 2–10 cm depths, regardless of tillage, whereas EC decreased in the order NT-A > NT-B = NT-C > CT-B. These trends are consistent with previous results for Dundee silt loams under NT and CT management (17).

FDA, an estimate of total microbiological activity, was higher in NT-A. Elevated FDA in NT-A was in agreement with patterns of OC accumulation and microbial populations (17). A PCA of total FAMES and subsequent ANOVA indicated a significant depth by tillage interaction (*Pr* ≥ *F* = 0.003) on the microbial community structure (Figure 1). Overall 58% of the variability was accounted for in PC1, with the greatest separation of NT-A from the other soils, and 21% of the variability in PC2.

Table 1. Soil Organic Carbon and Total Nitrogen Contents, pH, Electrical Conductivity (EC), Fluorescein Diacetate (FDA) Hydrolytic Activity, and Glyphosate Sorption (Linearized K_d) of a Dundee Silt Loam Soil As Affected by Tillage and Depth^a

tillage and depth ^b	organic C (g kg ⁻¹)	total N (g kg ⁻¹)	pH	EC ($\mu\text{S cm}^{-1}$)	FDA hydrolysis (nmol g ⁻¹ of soil h ⁻¹)	glyphosate K_d (L kg ⁻¹)
NT-A (0–2 cm)	13.31 a	1.79 a	6.02 a	155 a	140 a	48.3 b
CT-A (0–2 cm)	7.73 b	0.86 b	6.02 a	109 b	61 b	48.1 b
NT-B (2–10 cm)	9.46 b	1.04 b	5.82 b	109 b	74 b	78.2 a
CT-B (2–10 cm)	8.70 b	0.95 b	5.72 b	80 c	83 b	56.9 b

^a Mean of four replicates. Means followed by the same letter do not differ at the 95% confidence level. ^b NT, no-tillage, CT, conventional tillage.

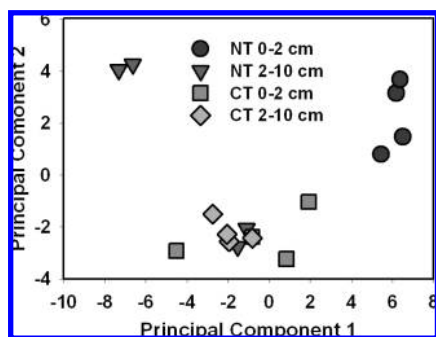


Figure 1. Principal component analysis of microbial community structure observed in two depths of no-tillage (NT) and conventional tillage (CT) Dundee silt loam soil based on total fatty acid methyl ester (FAME) technique.

Specifically, the microbial community observed in NT-A was unique compared to the other soils. Moreover, a greater number of FAMES were isolated from NT-A, and many of the unsaturated and branched fatty acids were present in greater abundance, indicating a differential stimulation of various components of the Gram-positive and -negative bacterial populations in response to accumulation of soil organic matter in the 0–2 cm surface of NT soil.

Sorption. A component of this study was to determine if altered soil chemical properties in surface NT soils, that is, higher OC contents, were positively correlated with glyphosate sorption estimates. This was of importance in that reports on the effects of OC on glyphosate sorption often conflict (1, 5). For example, several studies have reported a positive correlation between OC and glyphosate sorption, whereas others indicate that OC either has no effect on glyphosate sorption or that sorption is negatively correlated with OC. Under the conditions of this experiment, glyphosate K_d values ranged from 48.1 to 78.2 L kg⁻¹, which is consistent with previous results in the literature. However, glyphosate sorption coefficients were not correlated with OC. This was not surprising in that within the pH range of most agriculturally important soils, that is, 4–8, glyphosate is ionic, and its sorption is controlled almost exclusively by iron and aluminum oxides, poorly ordered aluminum silicates, and edges of layer silicates (5). Consequently, the accumulation of organic matter in NT soils will likely have little effect on glyphosate sorption because factors other than OC control its binding in soils.

Mineralization. In all soils there was no lag phase before the onset of glyphosate mineralization, as is consistent with other reports in the literature (7, 9, 16). The most rapid mineralization rates were observed in the initial 2 days after treatment (Figure 2). During the first 2 weeks, greater glyphosate mineralized was observed in NT-A compared to the other soils; however, by the termination of the study at 35 days, the only significant difference was that 37% greater mineralization was observed in the NT-A compared to NT-B (Table 2). The cumulative glyphosate mineralization observed in this study is more extensive than those

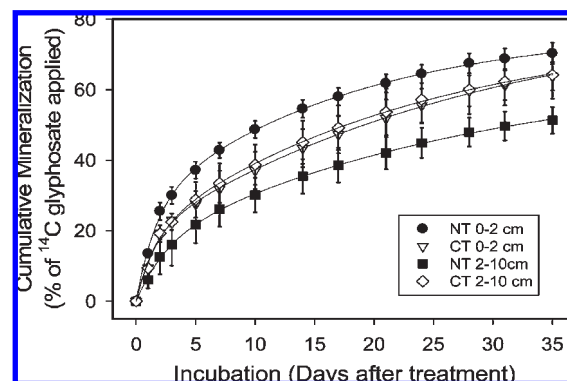


Figure 2. Cumulative mineralization of ¹⁴C-labeled glyphosate in no-tillage (NT) and conventional tillage (CT) soils collected from 0–2 and 2–10 cm depths. Means of four replicates, lines were fitted to a two-compartment four-parameter equation. Error bars represent one standard deviation.

observed in Danish (14), Norwegian (8), Italian, and Midwestern U.S. (6) soils and in a Dundee silt loam from another field in the same location (9). However, the extent of mineralization is similar to that observed in a soil managed under organic farming conditions from Germany (16). Glyphosate mineralization was positively correlated with chemical characteristics, that is, organic carbon, total nitrogen, and electrical conductivity, and microbial indices, that is, FDA hydrolytic activity, but negatively correlated with sorption (Table 3). There was no correlation of pH or soil textural components (sand, silt, and/or clay) and glyphosate mineralization, but the textural components were determined on the basis of physical size distribution. The mineralogy at these depths was not investigated, and it is possible that the amorphous iron and aluminum oxide contents may have differed in these depths or various degrees of humification existed within the clay components. However, most research indicates that glyphosate or its metabolite aminomethylphosphonic acid sorbed to iron and aluminum oxides is still bioavailable (14, 23). These data indicate that changes in chemical and biological properties associated with NT will have minimal effect on glyphosate or glyphosate residue mineralization.

Extractable and Nonextractable Residues. Estimated $t_{1/2}$ values for 0.01 N CaCl₂ extractable residues, interpreted as the bioavailable fraction, were ≤ 1.2 h for all soils (Table 2). The rapid decline in bioavailable glyphosate was attributed primarily to a sorption mechanism. Within the pH range evaluated, glyphosate forms mono- and (or) divalent anions that readily sorb to iron and aluminum oxides (5, 15, 25, 26). For example, at 4 h after application, $\geq 70\%$ of glyphosate residues were recovered in the 0.1 N NaOH extract; that is, a fraction sorbed to iron and aluminum oxides (15). Similarly, Gimsing et al. (15) reported that 1 day after the application of glyphosate to six Danish surface soils, $< 2\%$ of the residues were bioavailable, whereas $> 50\%$ were sorbed to iron and aluminum oxides. Collectively, the glyphosate bioavailable fraction behaves similarly among agricultural soils given that the labile fraction is governed principally by the affinity of the mono- and divalent glyphosate

Table 2. Recovery of Radioactivity in Two Depths of Soil from No-Tillage (NT) and Conventional Tillage (CT) Soils Treated with ^{14}C -Glyphosate^a

incubation time/soil ^b	% of initial ^{14}C -glyphosate applied recovered				
	CaCl ₂ extractable	NaOH extractable	nonextractable	mineralized	total recovery
4 h					
NT-A (0–2 cm)	7.7 b	70.3 b	15.9 a		93.9 b
CT-A (0–2 cm)	11.5 a	75.6 a	12.4 a		98.9 a
NT-B (2–10 cm)	8.5 b	76.7 a	11.5 a		96.3 a
CT-B (2–10 cm)	9.4 b	76.3 a	13.4 a		98.2 a
day 3					
NT-A (0–2 cm)	1.0 a	61.5 b	10.0 a	26.3 a	98.5 ab
CT-A (0–2 cm)	0.5 b	62.5 b	12.2 a	20.1 b	95.2 b
NT-B (2–10 cm)	0.8 ab	62.4 b	8.9 a	19.5 b	103.2 a
CT-B (2–10 cm)	0.5 b	76.6 a	12.4 a	17.1 c	95.2 b
day 14					
NT-A (0–2 cm)	0.2 a	36.8 a	8.0 a	54.6 a	99.7 a
CT-A (0–2 cm)	0.1 a	41.0 a	8.4 a	43.9 b	92.9 ab
NT-B (2–10 cm)	0.1 a	44.5 a	7.8 a	35.4 c	87.9 b
CT-B (2–10 cm)	0.1 a	41.5 a	9.6 a	45.4 b	96.4 a
day 35					
NT-A (0–2 cm)	0.1 a	15.3 c	2.9 a	70.3 a	88.6 a
CT-A (0–2 cm)	0.3 a	19.3 bc	4.2 a	63.8 a	87.6 a
NT-B (2–10 cm)	0.1 a	28.3 a	4.8 a	51.3 b	84.5 a
CT-B (2–10 cm)	0.1 a	20.0 b	5.3 a	62.9 a	88.3 a

^a Mean of four replicates. Means followed by the same letter do not differ at the 95% confidence level. ^b NT, no-tillage, CT, conventional tillage.

Table 3. Pearson Correlation Coefficients of Glyphosate Mineralization Measured at 7 and 35 Days with Various Soil Properties

soil property	mineralization at 7 days	mineralization at 35 days
FDA hydrolysis	0.651/0.006 ^a	0.545/0.029
soil carbon	0.710/0.002	0.472/0.065
total soil nitrogen	0.700/0.003	0.448/0.081
electrical conductivity	0.602/0.013	0.412/0.113
pH	0.074/0.784	0.141/0.603
sand content	−0.074/0.785	−0.181/0.503
silt content	−0.321/0.226	−0.202/0.452
clay content	0.191/0.479	0.305/0.253
K _d	−0.538/0.032	−0.730/0.001

^a Coefficient/Pr > F.

and (or) aminomethylphosphonic acid anions for iron and aluminum oxides, regardless of tillage or depth in the soil profile.

Estimated $t_{1/2}$ values for total extractable ^{14}C -glyphosate residues, that is, 0.01 N CaCl₂ extracts + 0.1 N NaOH extracts (glyphosate and aminomethylphosphonic acid), were ≤ 14.2 days for all soils (Supporting Information Table 1). These estimates are consistent with other reports for glyphosate $t_{1/2}$ values and offer insight into the behavior of glyphosate in agricultural soils (6, 27). From an applied perspective, these data indicate that converting from CT to NT will not significantly alter glyphosate persistence, despite NT's effect on soil chemical, physical, and biological properties (Table 1). From a theoretical perspective, it is interesting to note that $\geq 70\%$ of glyphosate residues were sorbed to iron and aluminum oxides by 4 h after application; yet, glyphosate $t_{1/2}$ values were < 14.2 days for all soils. This implies that glyphosate sorbed to iron and aluminum oxides is readily desorbed into soil solution and (or) is available for microbial degradation while sorbed to the oxides. The current paradigm is that sorbed glyphosate is not biologically available and that desorption is the rate-limiting step in microbial degradation. This theory prevails because of the negative relationship between glyphosate K_d and $t_{1/2}$ values. However, Schnurer et al. (23) noted that glyphosate sorbed to the iron oxide goethite underwent microbial degradation. Thus, one can deduce that both processes, that is,

Table 4. Recovery of ^{14}C -Glyphosate from Autoclaved and Nonautoclaved Dundee Silt Loam

		CaCl ₂	NaOH	total	
		mineralized	extractable	extractable	nonextractable
day 3					
autoclaved	0.4 ^a	1.2	83.0	9.7	94.5
nonautoclaved	23.6	0.3	59.6	9.5	93.2
LSD 0.05 ^b	1.3	0.5	6.0	3.5	2.8
day 14					
autoclaved	1.7	0.8	86.0	11.1	99.6
nonautoclaved	51.0	0.2	33.0	10.4	94.6
LSD 0.05	1.8	0.3	4.8	2.4	6.4

^a Mean of four replicates ^b Fisher least significant difference.

desorption followed by degradation in soil solution and degradation of sorbed glyphosate, likely occur simultaneously.

Nonextractable glyphosate residues were independent of tillage or depth throughout the study (Table 2). At 4 h after treatment, between 11.5 and 15.9% of the ^{14}C -glyphosate applied was recovered by oxidation. Conversely, by 35 days after treatment, nonextractable ^{14}C residues decreased to a low of 2.9–5.3%. This temporal trend suggests that these nonextractable glyphosate residues were initially bioavailable and subject to mineralization. The relatively low repartitioning of the nonextractable ^{14}C -glyphosate into nonextractable residues is consistent with results elsewhere (6, 9). The relatively low incorporation of ^{14}C into nonextractable residues (2.9–5.2%) also indicates that the increased levels of OC in the surface of NT soils will not affect long-term binding of glyphosate or metabolites.

Effects of Autoclaving Soil on Glyphosate Degradation. $^{14}\text{CO}_2$ evolution was negligible over 14 days of incubation in autoclaved CT soil, whereas mineralization of glyphosate in native biologically active soil (51% in 14 days) was similar to that observed in a previous study (Table 4). There was no effect of autoclaving on CaCl₂ extractable glyphosate residues as a similarly low level was readily extractable in autoclaved and native soils in a neutral aqueous system, indicating the sorption was mediated via chemical, not biochemical, processes such as oxidative coupling to

organic matter. The lower amount of NaOH extractable residues recovered in native soil corresponds with the ~50% mineralized in the 14 day study.

In conclusion, adoption of conservation management systems such as NT should not prolong the residence of glyphosate in soil, which when sequestered in the upper soil surface glyphosate is readily decomposed by soil microflora. There have been some reports on nontarget effects of glyphosate on certain soil and rhizosphere microbial populations or activity (28–30). The current studies indicate that in soils where glyphosate is the principal herbicide used in glyphosate-resistant cropping systems, there will be a limited persistence of glyphosate in the soil environment and thus pose only minimal potential for nontarget effects on the soil community (9, 31).

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Supporting Information Available: Tabular presentation of kinetics of glyphosate dissipation from the CaCl₂ and NaOH extracts. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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