

Effects of Incorporated Corn Residues on Glyphosate Mineralization and Sorption in Soil

CESARE ACCINELLI,^{*,†} WILLIAM C. KOSKINEN,[‡] JEFFREY D. SEEBINGER,[‡]
ALBERTO VICARI,[†] AND MICHAEL J. SADOWSKY[§]

Department of Agro-Environmental Science and Technology, University of Bologna, Viale Fanin 44,
40127 Bologna, Italy; Soil and Water Management Research Unit, Agricultural Research Service,
U.S. Department of Agriculture, St. Paul, Minnesota 55108; and Department of Soil, Water, and
Climate and BioTechnology Institute, University of Minnesota, St. Paul, Minnesota 55108

In modern agricultural systems employing conservation tillage practices, glyphosate is widely used as a preplant burndown herbicide in a wide range of crops. Conservation tillage systems are characterized by a significant presence of crop residues at the soil surface so that glyphosate is applied to a soil matrix rich in poorly decomposed crop residues. Incorporation of corn residues in the range from 0.5 to 4% caused different effects on mineralization and sorption of [¹⁴C]glyphosate in sandy and sandy loam soils. More specifically, low levels of incorporated corn residues did not affect or slightly stimulated herbicide mineralization in the sandy and sandy loam soils, respectively. In the sandy soil, incorporation of the highest level of corn residues (4%) caused a decrease in [¹⁴C]-glyphosate mineralization. [¹⁴C]Glyphosate sorption on both soil types was reduced in samples receiving high amounts of incorporated corn residues.

KEYWORDS: Glyphosate; corn residues; herbicide sorption; herbicide mineralization; genetically modified corn

INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine] is a nonselective herbicide that is widely used in several agricultural crops. The importance of glyphosate has increased in recent years due to an increased planting of glyphosate-tolerant crops (1). The major benefits of glyphosate-tolerant crops include flexibility and efficiency of weed control (2). Glyphosate-tolerant crops have encouraged the adoption of conservation tillage practices, which maintain crop residues on the soil surface (3).

Conservation tillage systems offer numerous advantages in the management of surface runoff of water, sediments, and pesticides and results in reduced sedimentation and soil erosion, improved water infiltration, decreased contamination of ground and surface waters, and conservation of nutrients and organic matter (4, 5). However, conservation tillage practices may alter the fate of pesticides in the soil ecosystem through effects on soil properties and processes and their interactions, mediated by soil organic matter, such as microbial degradation and sorption of pesticides (6). Crop residues may affect herbicide degradation and sorption depending on the degree of residue weathering and subsequent properties at the time of herbicide application (7).

Glyphosate is a nonresidual herbicide, which degrades readily in soil with an estimated half-life of 7–60 days (8). Degradation by soil microorganisms is the predominant pathway by which glyphosate is metabolized in soil (8, 9). Degradation of glyphosate is considered to be a co-metabolic process (10). Despite its high water solubility, glyphosate is strongly sorbed to soil particles and consequently has low mobility through the soil profile (11, 12). Organic matter, clay content, and iron and aluminum oxides play important roles in the sorption of glyphosate to soil (13, 14). The sorption of glyphosate relates to the amount of vacant phosphate sorption sites (10).

Glyphosate is widely used in conservation tillage as a preplant burndown herbicide. In preplant use and in glyphosate use during early growth stages of glyphosate-tolerant crops, the soil surface is only partly covered by vegetation and/or planted crop; therefore, significant amounts of glyphosate will reach the soil surface. Conservation tillage systems have significant amounts of crop residue at the soil surface, and consequently glyphosate will be applied to a soil matrix rich in poorly decomposed crop residues. No information is available on the effect of crop residues on the degradation and sorption of glyphosate in soil. The objective of this research was to determine the effects of incorporation of different ratios of corn residues to soil on mineralization and sorption of glyphosate in two different soil types.

* Corresponding author (telephone +39-051-2096670; fax +39-051-2096241; e-mail accinel@agrsci.unibo.it).

[†] University of Bologna.

[‡] U.S. Department of Agriculture.

[§] University of Minnesota.

Table 1. Properties of Ozzano (IT) and Princeton (MN) Soils

soil	textural class	particle size			pH ^a 1:2.5	organic C %
		sand %	silt %	clay %		
IT	sandy loam	63.4	22.6	14.0	8.11	0.70
MN	sand	93.5	2.7	3.8	7.20	0.94

^a Soil pH measured in 1:2.5 (w/w) soil/deionized water mixture.

Table 2. Chemical Characterization of Corn Residues Obtained from N45-A6 and N45-T5 Hybrids

corn residue	total N (%)	total C (%)	ash (%)	lignin (%)	cellulose (%)	hemi-cellulose (%)	Cry1Ab ^a toxin ($\mu\text{g g}^{-1}$ of dry residues)
N45-A6	0.733	44.30	0.45	4.37	38.03	28.11	0.35
N45-T5	0.97	44.00	0.53	4.29	38.54	27.99	

^a Quantified by enzyme-linked immunosorbent assay (QuantiPlate™ Kit for Cry1Ab/Cry1Ac, EnviroLogix, Portland, ME).

MATERIALS AND METHODS

Soils, Corn Residues, and Sample Preparation. Two soils with different physicochemical properties, representing corn production areas of the Po Valley (Italy) and of south central Minnesota, were selected for this study. Surface (0–20 cm) soil samples taken from Ozzano (Bologna, Italy; 44° 28' N, 11° 28' E) and from Princeton (Minnesota; 45° 00' N, 93° 10' E) were classified as a sandy loam (Udertic Ustochrepts, fine, mixed, mesic) and as a sandy soil (Argic Udipsamments, mixed, frigid), respectively. Soils were collected from fields that had had no glyphosate application within the previous 5 years. Collected soils were air-dried and passed through a 2-mm sieve. Physicochemical properties of the soils are given in **Table 1**.

Corn residues were collected in March 2004 from two adjacent fields that had not received glyphosate during the previous cropping seasons. Residues were from two hybrids: N45-A6, a stacked trait hybrid (Bt-protected and glufosinate-tolerant hybrid) and N45-T5 (Syngenta Seeds, Inc., NK, Golden Valley, MN), the corresponding nontransgenic isoline. Crop residues were dried at 30 °C and sieved to obtain a material with a diameter between 0.18 and 0.84 mm. Chemical properties of the corn residues are shown in **Table 2**. A sufficient amount of each corn residue type was mixed with each soil in a residue content varying from 0.0 to 8.0% (w/w). Depending on the type of conservation tillage practices, the adopted range of corn residue incorporation covers a wide range of situations in which such practices are adopted.

[¹⁴C]Glyphosate Mineralization. Twenty grams of both Ozzano (IT) and Princeton (MN) soils (air-dried basis) containing different amounts of the two corn residue types (0.0, 0.5, 2.0, and 4.0% w/w) were weighed into 250-mL glass flasks with three replicates. A solution of unlabeled (chemical purity > 98%) and [¹⁴C]-labeled glyphosate (*N*-phosphonomethyl-2-¹⁴C-glycine; radiopurity > 99%, specific activity = 1.18 10⁶ MBq g⁻¹) in distilled water at a final concentration of 5.71 μg of active ingredient (ai) mL⁻¹ (1.48×10^{-3} MBq mL⁻¹) was prepared, and 3.5 mL was added to each soil sample to obtain a final glyphosate concentration of 1 $\mu\text{g g}^{-1}$ of air-dried soil. Unlabeled glyphosate and radiolabeled glyphosate were provided by Sigma Chemical Co. (St. Louis, MO). The soil moisture of the treated soil samples was adjusted to the gravimetric water content of -33 kPa using distilled water, and the samples were then incubated in the dark at 25 °C. The moisture level of incubated samples was checked at 7-day intervals and adjusted to the initial -33 kPa, if needed. During the 28-day incubation period, glyphosate mineralization was monitored by trapping evolved ¹⁴CO₂ in vials containing 5 mL of a 1 M NaOH solution. NaOH solution was replaced every 2 days, and the flasks were aerated. Trapped ¹⁴CO₂ was determined by mixing a 1-mL aliquot of NaOH solution with 5 mL of EcoLite scintillation cocktail (ICN Pharmaceuticals Inc., Costa Mesa, CA), and the amount of radioactivity was determined by liquid scintillation counting (LSC) for 10 min using a 1500 Tri-Carb Packard (Meriden, CT) liquid scintillation analyzer.

Prior to analysis, samples were kept in the dark for 12 h. No chemiluminescence was observed.

Soil Extraction. At 3, 14, and 28 days after treatment (DAT), the distribution between solution phase and weakly sorbed ¹⁴C residues in incubated soil samples was determined using a two-step procedure. At each sampling time, triplicate samples receiving no corn residues (control) and samples with 4% corn residues were removed, and all of the soil in each flask (20 g) was transferred to Teflon centrifuge tubes by washing with 200 mL of 0.01 M CaCl₂. The samples were shaken for 14 h in a horizontal shaker at 20 °C. The suspension was centrifuged at 2500 rpm for 30 min and the supernatant transferred to preweighed glass vials. A 50-mL aliquot was removed and filtered through a 0.2- μm filter. Radioactivity of the filtered supernatant was determined by LSC after 1-mL aliquots were mixed with 5 mL of scintillation cocktail. The remaining filtered extract was saved for later determination of the amount of glyphosate in the extracted ¹⁴C residues, as described below. Soil was then extracted with 200 mL of 0.1 M K₂HPO₄ (pH 8) following the same procedure as for the CaCl₂ extraction.

The aqueous extracts were analyzed by high-performance liquid chromatography (HPLC) on a 1090 model (Hewlett-Packard Co., Palo Alto, CA) equipped with a 0.46 \times 25 cm Luna NH₂ column (Phenomenex Inc., Torrance, CA). HPLC fractions were collected on the basis of the retention times of glyphosate determined previously (~9 min) (9). Fractions were mixed with liquid scintillation cocktail and quantified by LSC. Soil-bound ¹⁴C residues after extraction with the two procedures were quantified by combusting subsamples (0.3 g on air-dried basis) of the extracted soils with a Packard sample oxidizer (Packard Instrument Co., Meriden, CT). Radioactivity was quantified by LSC.

[¹⁴C]Glyphosate Sorption/Desorption Isotherms. Isotherms for the sorption of glyphosate on IT and MN soil samples receiving different ratios of corn residues (0.0, 0.5, 2.0, 4.0, and 8.0% w/w) were determined using the batch equilibrium method. Two grams (air-dried basis) of each soil–corn residue combination was weighed into 50-mL glass centrifuge tubes, and 10 mL of [¹⁴C]glyphosate solution prepared in 0.01 M CaCl₂ was added. Sorption isotherms were determined using triplicate samples at six initial glyphosate concentrations, ranging from 0.2 to 120 $\mu\text{g mL}^{-1}$. Radiolabeled glyphosate was added to unlabeled solutions to give an initial radioactivity of ~50 Bq mL⁻¹. Tubes were sealed with Teflon-lined caps and shaken at 20 °C for 14 h. Samples were centrifuged at 2500 rpm for 10 min. Five-milliliter aliquots of supernatant were removed and filtered through a 0.2- μm filter, and a 1-mL fraction was added to 5 mL of scintillation cocktail. The same procedure adopted for the estimation of glyphosate sorption to soil–corn residue mixture was repeated using samples prepared with the two corn residues alone, except that 0.5 g (air-dried basis) of corn residues was used. Preliminary investigations showed that equilibrium was attained in <14 h and that there was no significant glyphosate sorption to glass centrifuge tubes or to the filters.

The amount of sorbed glyphosate was calculated from the concentration differences between the supernatant of the equilibrated solutions and those of the corresponding initial solutions. Sorption data were fitted to the log form of the Freundlich equation: $\log C_s = \log K_f + (1/n) \log C_e$, where C_s is the concentration of glyphosate sorbed ($\mu\text{g g}^{-1}$ of soil), C_e is the equilibrium concentration ($\mu\text{g mL}^{-1}$ solution), and K_f and $1/n$ are empirical Freundlich constants. Values of K_f and $1/n$ were estimated by linear regression after a log–log transformation.

Desorption isotherms for glyphosate on the two soils receiving 0.0 and 8.0% corn residue incorporation rates were conducted using samples from the initial concentrations of 60 and 120 $\mu\text{g mL}^{-1}$. Desorption of glyphosate was determined by replacing a 4-mL. After solution addition, tubes were shaken for 14 h and centrifuged as described above. The desorption cycle was repeated four times.

Chemical Analysis of the Liquid Phase. Triplicate samples of the IT and MN soils receiving two different ratios (0.0 and 4.0%) of corn residue incorporation were prepared and analyzed following the same procedure adopted for the estimation of glyphosate sorption on soil. The sole difference was that no glyphosate was contained in the added 0.01 M CaCl₂ solution. After 14 h of shaking, samples were centrifuged at 2500 rpm for 10 min and supernatant was saved for pH measurement and chemical analysis. Elemental analysis of filtered (0.2 μm) supernatant

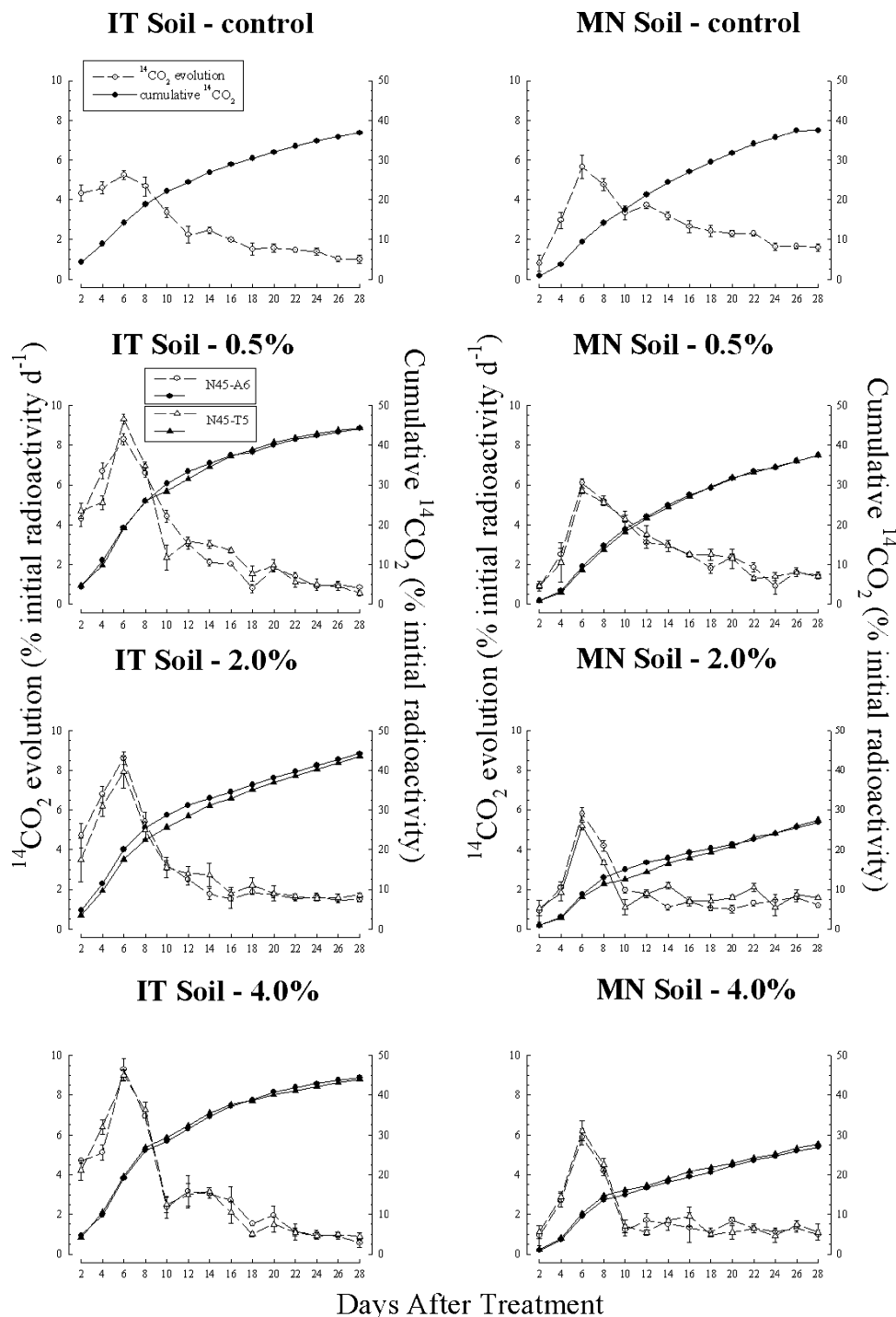


Figure 1. Effect of incorporation of different ratios (0.0–4.0%) of corn residues on $^{14}\text{CO}_2$ evolution from Ozzano (IT) and Princeton (MN) soils. The incorporated corn residues were obtained from a Bt-protected and glufosinate-tolerant hybrid (N45-A6) and the correspondent isolate (N45-T5). Bars represent standard errors of the mean.

samples was performed using a Perkin-Elmer Optima 300 DV ICP spectrometer (Boston, MA).

Enumeration of Culturable Bacteria. At the end of the 28-day incubation period, total indigenous bacteria of incubated soil samples receiving 4% corn residue addition were enumerated by dilution plate count. Ten grams (air-dried soil) of each incubated soil sample was mixed with 95 mL of 0.1% sodium pyrophosphate buffer (PBS, pH 7) and shaken on a horizontal shaker for 20 min at 200 rpm. Samples were decimally diluted in sterile 0.1% PBS, and 100- μL aliquots were spread-plated onto 10% tryptone soy agar supplemented with cycloheximide (50 $\mu\text{g mL}^{-1}$). Plates were incubated at 27 $^{\circ}\text{C}$, and colonies were enumerated after 4, 6, and 10 days. Measurements were done in triplicate, and nonincubated soil samples were included.

RESULTS AND DISCUSSION

^{14}C Glyphosate Mineralization. Significant amounts of glyphosate were mineralized in the two soils. At the end of the 28-day incubation, total accumulated $^{14}\text{CO}_2$ levels in the IT and MN control soils were not different ($P > 0.05$) and accounted for 36.8 and 37.4% of the total applied ^{14}C as glyphosate, respectively (Figure 1). These findings confirmed the short persistence of glyphosate in soil (15, 16).

There were no significant differences in ^{14}C glyphosate mineralization between the two corn residue types regardless of the level of the residues (Figure 1). The concentration of

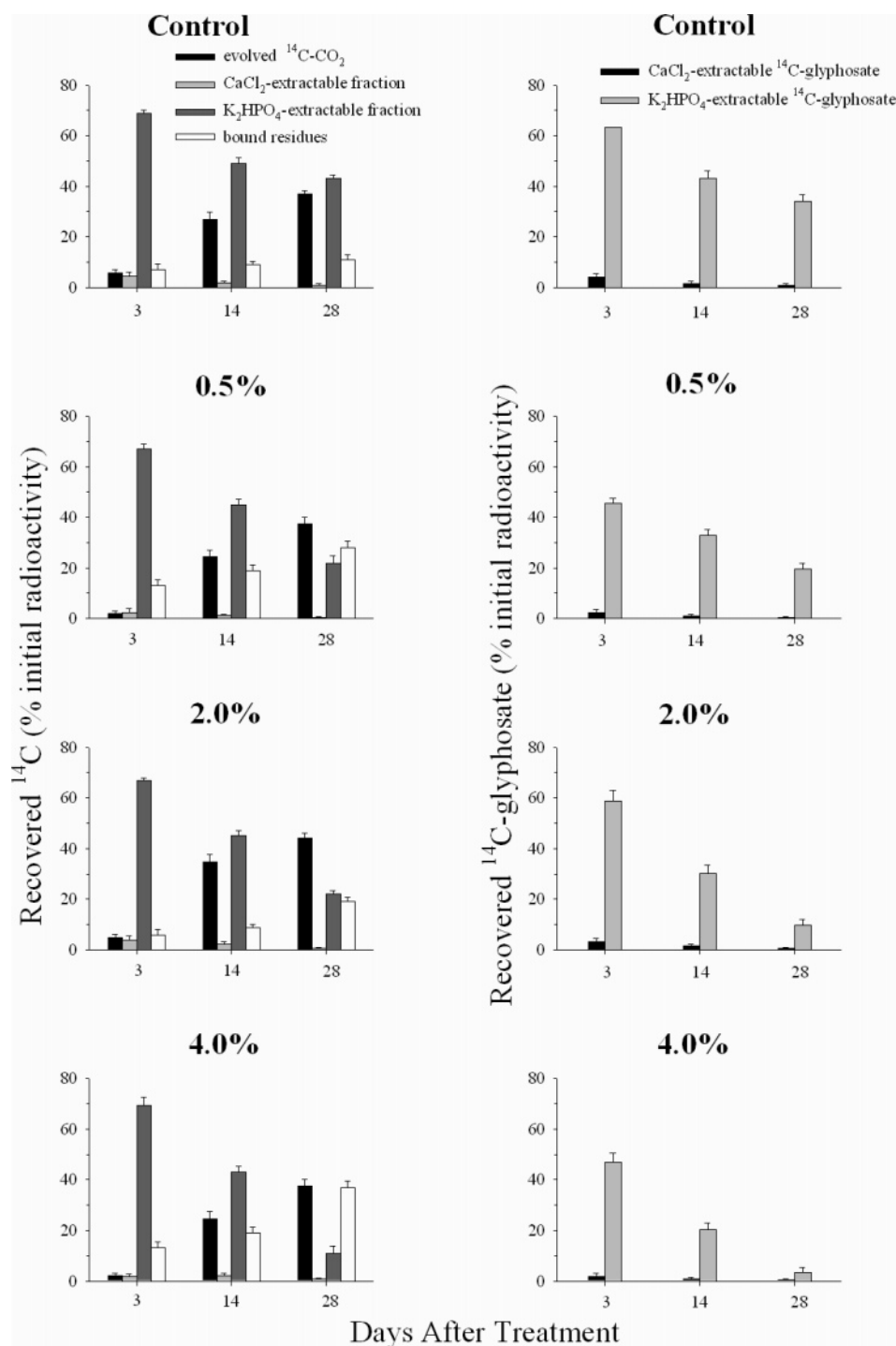


Figure 2. Repartition of applied [^{14}C]glyphosate into evolved $^{14}\text{CO}_2$, CaCl_2 , and K_2HPO_4 -extractable fractions and bound residues in Ozzano (IT) soil receiving different ratios of corn residues from the transgenic corn.

the insecticidal Cry1Ab toxin in the added corn residue was $0.35 \mu\text{g g}^{-1}$ of dry residues (**Table 2**). Addition of 0.5% corn residue in the IT soil caused a significant increase in the total recovered $^{14}\text{CO}_2$. In the MN soil the mineralization of glyphosate was significantly reduced by corn residue at 2 and 4%, but not at 0.5%.

Glyphosate is relatively resistant to chemical hydrolysis, thermal decomposition, and photolysis (17). Microbial degradation is the predominant mechanism of glyphosate transformation in soil (9, 10, 18). Some microbial strains have been isolated that are able to grow in artificial media using glyphosate as sole C, N, or P (10). However, degradation of glyphosate in the soil is a nonspecific, co-metabolic process (10). The absence

of a lag phase and the patterns of $^{14}\text{CO}_2$ evolution in this study support this conclusion. An initial rapid mineralization rate followed by a slower, constant mineralization rate is the typical pattern of co-metabolic degradation processes of xenobiotics (10, 19). The average cumulative $^{14}\text{CO}_2$ evolution rates in the IT and MN soil, among the different corn residue incorporation levels, were 43.0 and 31.6% of the total ^{14}C applied, respectively. These findings confirmed the ability of soil microorganisms to mineralize glyphosate. In general, the intensity of the co-metabolic processes is related to the composition and activity of the soil microbial community (20). The incorporation of corn residues resulted in the addition of readily metabolizable substrate, which was mainly composed of organic C (**Table 2**).

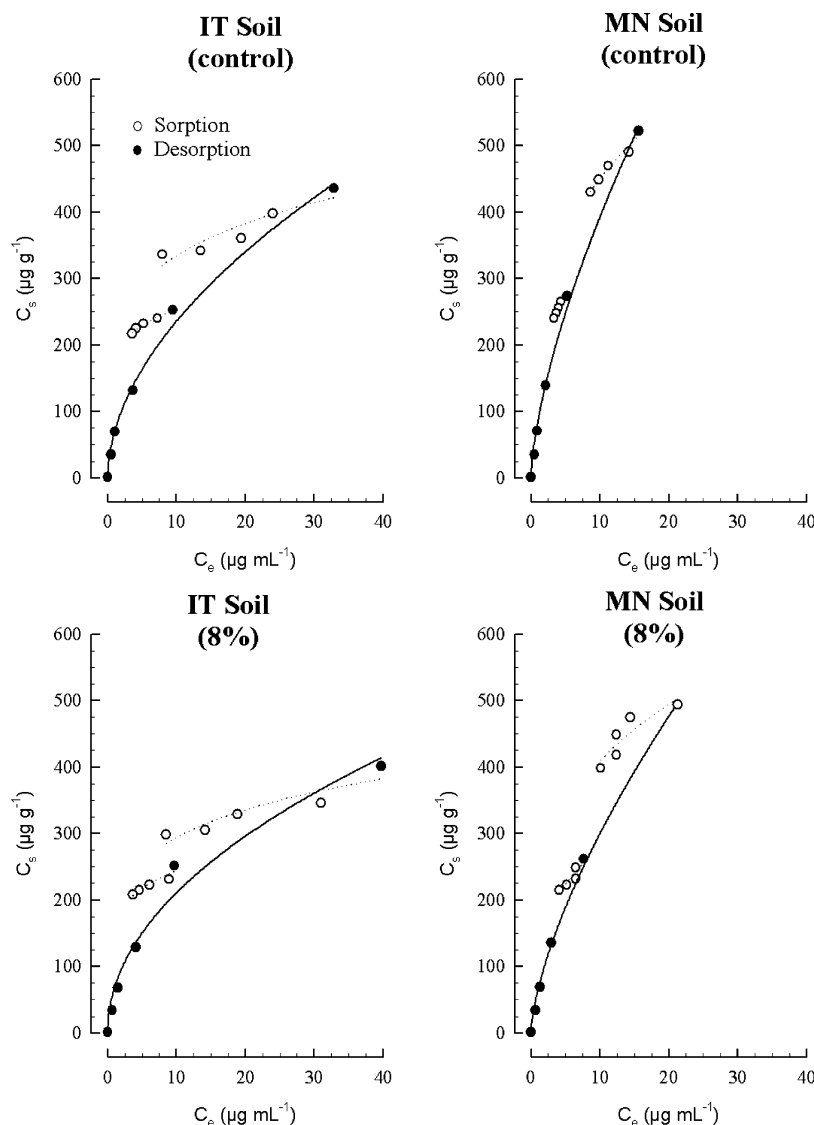


Figure 3. Effect of the incorporation of the highest corn residue level (8%) from the N45-A6 hybrid on sorption/desorption isotherms of $[^{14}\text{C}]$ glyphosate on Ozzano (IT) and Princeton (MN) soils.

In the IT and MN soils, incorporation of the lowest (0.5%) corn residue level resulted in 32 and 23% increases of the total soil carbon, respectively. The addition of readily available substrate stimulates the soil microbial community and increases the co-metabolic processes (21). This phenomenon can partly explain the increase of glyphosate mineralization observed in the IT soil following the addition of 0.5% of corn residues. A higher corn residue addition did not further stimulate the soil microbial community, presumably by causing an excessive alteration of soil C/N ratio. The MN soil had higher initial organic C than the IT soil, and the addition of corn residue resulted in an inhibitory effect of $[^{14}\text{C}]$ glyphosate mineralization due to a change in the C/N ratio.

Soil Extraction. In both the IT and MN control soils, the amount of extractable ^{14}C residues and $[^{14}\text{C}]$ glyphosate decreased throughout the 28-day incubation. The decrease was greater for samples receiving the highest corn residue level (4%). The decrease of labeled extractable residues and parent compound was due to the degradation and formation of bound, unextractable residues. No significant differences ($P > 0.05$) between the corn isoline residues obtained from the N45-A6 and N45-T hybrids were observed. The partition of applied $[^{14}\text{C}]$ glyphosate into CaCl_2 - and K_2HPO_4 -extractable and bound residues in the IT soil receiving different ratios of corn residues

from the transgenic corn is shown in **Figure 2**. These results show that pesticide bioavailability decreases with increasing pesticide–soil contact time (22, 23). As in other similar studies, the CaCl_2 -extractable fraction represented the “readily available pesticide” and the K_2HPO_4 -extractable fraction gave the sorbed concentration (24).

Most of the $^{14}\text{CO}_2$ evolution occurred within the first 2 weeks after treatment (**Figure 2**). The phenomenon was greater in the IT than in the MN soil. Because corn residue addition stimulated $[^{14}\text{C}]$ glyphosate mineralization in the IT soil, the observed decrease of pesticide bioavailability throughout the incubation period played a minor role in the microbial degradation of the pesticide. In addition, because similar pesticide bioavailability was observed in the two soils, the inhibitory effect of corn residue incorporation on the herbicide mineralization in the MN soil suggests the hypothesis that other factors should be considered for a correct explanation of this phenomena. These findings are comparable with data obtained by von Wirén-Lehr et al. (15).

$[^{14}\text{C}]$ Glyphosate Sorption/Desorption Isotherms. Glyphosate sorption to the IT and MN soils, over the initial solution concentration range from 0.5 to $120 \mu\text{g mL}^{-1}$, was adequately described by the linearized form of the Freundlich equation (**Figure 3**). In both soils, isotherm slopes were significantly less

Table 3. Glyphosate Sorption Coefficients Determined in the Ozzano (IT) and Princeton (MN) Soil as a Function of Different Ratios of Incorporated Corn Residues

corn residue	incorporated amount (%)	IT soil			MN soil		
		K_f^a ($\mu\text{g}^{1-1/n} \text{g}^{-1} \text{mL}^{1/n}$)	$1/n^b$	r^2	K_f ($\mu\text{g}^{1-1/n} \text{g}^{-1} \text{mL}^{1/n}$)	$1/n$	r^2
N45-A6 N45-T5	0.5	43.01 (43.74–47.61)	0.79 (0.06)	0.99	62.16 (63.38–67.37)	0.88 (0.04)	0.99
		42.52 (42.42–43.11)	0.76 (0.06)	0.98	63.28 (63.12–64.04)	0.88 (0.04)	0.99
N45-A6 N45-T5	2.0	43.70 (43.60–44.30)	0.75 (0.05)	0.98	62.89 (62.73–63.65)	0.88 (0.04)	0.98
		42.90 (42.81–43.51)	0.78 (0.06)	0.98	63.31 (63.14–64.06)	0.88 (0.04)	0.99
N45-A6 N45-T5	4.0	42.75 (42.65–43.34)	0.76 (0.05)	0.98	62.52 (62.34–63.26)	0.89 (0.05)	0.99
		38.26 (39.08–39.72)	0.79 (0.06)	0.98	61.63 (61.46–62.37)	0.88 (0.04)	0.99
N45-A6 N45-T5	8.0	38.06 (37.98–38.61)	0.78 (0.56)	0.98	62.78 (62.60–63.52)	0.88 (0.04)	0.99
		35.22 (35.75–39.50)	0.81 (0.07)	0.99	44.58 (45.46–49.15)	0.88 (0.04)	0.99
N45-A6 N45-T5		35.02 (34.95–35.53)	0.81 (0.07)	0.99	43.81 (43.69–44.40)	0.88 (0.04)	0.99

^a Numbers in parentheses are 95% confidence interval. ^b Numbers are mean $1/n \pm$ standard deviation. ^c Correlation coefficients of linear regression of linearized Freundlich isotherms.

Table 4. Glyphosate Sorption Coefficients for Corn Residues Obtained from the N45-A6 and N45-T5 Hybrids

corn residue	K_f^a ($\mu\text{g}^{1-1/n} \text{g}^{-1} \text{mL}^{1/n}$)	$1/n^b$	r^2
N45-A6	4.28 (3.91–4.16)	0.84 (0.04)	0.98
N45-T5	4.12 (3.99–4.18)	0.85 (0.04)	0.99

^a Numbers in parentheses are 95% confidence interval. ^b Numbers are mean $1/n \pm$ standard deviation. ^c Correlation coefficients of linear regression of linearized Freundlich isotherms.

than 1, thus indicating that glyphosate sorption was concentration dependent. Differences in $1/n$ values did not permit a direct comparison of the sorption capacities (K_f coefficients) of the two soils. However, considering that the K_f coefficient estimated in the MN soil was ~ 1.5 times greater than that in the IT soil (Table 3), a significantly higher sorptive capacity of the former with respect to the latter soil can be assumed.

Addition of corn residues did not change the relative differences in the sorptive capacities between the two soils, regardless of the incorporated amount (Table 3). There were no differences between corn residues from the two isolines. Incorporation of corn residues from 0.5 to 2% and from 0.5 to 4% did not affect sorption of [^{14}C]glyphosate to the IT and the MN soil, respectively. In contrast, incorporation of the highest corn residue amount (8%) reduced the sorption capacities of both soils for glyphosate. A smaller reduction was observed in the IT soil with 4% of incorporated corn residues. Although the incorporation of such high amounts ($\geq 4\%$) exceeds crop residues left at the soil surface with conservation tillage practices, these findings support the concept that, depending on the sorption mechanisms involved and the type and weathering of the crop residues, a decrease in sorption capacity of soil following crop residue incorporation cannot be excluded (7).

Sprinkle et al. (19) showed that the addition of mature organic matter to soil samples seeded with wheat severely inactivated glyphosate. In contrast, the addition of 5–10% cellulose did not inactivate glyphosate. Added corn residues were mainly cellulose and hemicellulose (Table 2), and these materials had low affinity for glyphosate (Table 4). Consequently, the observed decrease of glyphosate sorption on soil receiving corn residue was not likely to be due to a reduction of the sorptive capacity of the soil per se. The observed decrease of herbicide sorption on soil mixed with the highest level of corn residue (8%) is possibly due to coverage of the soil sorptive sites by the corn residues. However, considering the decrease of labeled degradates and glyphosate throughout the 28-day incubation period, especially in soils receiving corn residue addition

(Figure 2), it can be assumed that the binding of glyphosate and its degradation products to corn residue increased over the incubation time. According to Kögel-Knabner (25), as decomposition of corn residues incorporated into the soil proceeds, the concentration of cellulose rapidly decreases. In contrast, the concentration of lignin tends to increase over time. Dao (7) observed that the capacity of wheat residues incorporated into the soil to retain metribuzin changed as a function of incubation time. The increased sorption capacity of decaying wheat residues was associated with a decline in cellulose concentration or, conversely, the lignin enrichment of the residues. More detailed information on the effect of weathering on the sorptive properties of corn residues to glyphosate is needed.

Glyphosate desorption was hysteretic (Figure 3 and Table 5). In contrast to sorption, the incorporation of 8% corn residue in the two soils had similar desorption isotherms. The lowest hysteresis coefficients were in the soil with the lowest sorptive capacity (26). In the IT soil hysteresis was more pronounced at the lowest initial glyphosate concentration, regardless of corn residue addition (Table 5). This phenomenon was not observed in the MN soil. For each soil type, comparable hysteresis coefficients in control and soil–corn residue mixtures suggested that similar sorption mechanisms were involved in the two different systems.

Chemical Composition of the Liquid Phase. The pH, inorganic phosphate concentration, and ionic strength of the liquid phase can modify glyphosate sorption on soil (27, 28). Sorption increased with decreased solution pH and the increased presence of Al and Fe ions at the soil sorptive surface. In addition, glyphosate competes with inorganic phosphate for sorption sites (10).

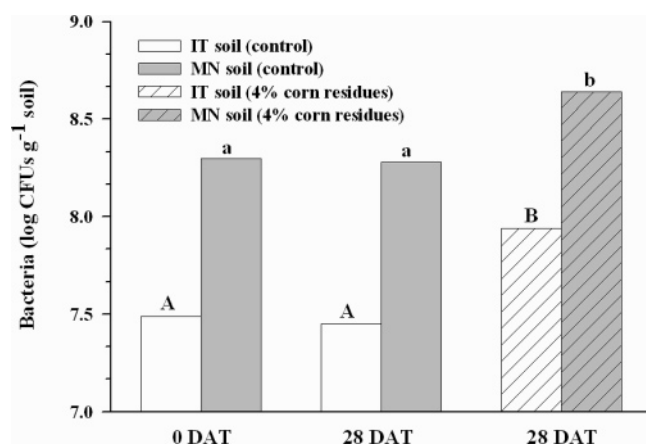
Because the liquid phase was prepared in 0.01 M CaCl_2 , calcium and chloride were the two dominant chemical species in the liquid phase. Table 6 provides concentrations of representative chemical species in the supernatant of the three studied materials (soil, soil–corn residue mixture, and corn residues alone). Similar to the glyphosate sorption experiment, the liquid phase was prepared in 0.01 M CaCl_2 ; calcium and chloride were consequently the two dominant chemical species (data not shown). Excluding iron, the concentrations of the other chemicals were different ($P < 0.05$) in the liquid phase of the two control soils. Incorporation of the two corn residues reduced these differences. The concentration of phosphorus remained higher ($P < 0.05$) in the MN soil than in the IT soil. Incorporation of corn residues increased the concentration of total organic carbon in the supernatant. However, the increase was lower than expected on the basis of an additive effect. This

Table 5. Glyphosate Desorption Coefficients at Two Initial Solution Concentrations (C_i) Determined in Untreated (Control) Soils and Soil Samples Receiving the Incorporation of 8% Corn Residues of the N45-A6 Hybrid

corn residue	incorporated amount (%)	IT soil				MN soil			
		$C_i = 60 \mu\text{g mL}^{-1}$		$C_i = 120 \mu\text{g mL}^{-1}$		$C_i = 60 \mu\text{g mL}^{-1}$		$C_i = 120 \mu\text{g mL}^{-1}$	
		$1/n_{\text{f-des}}^a$	r^2	$1/n_{\text{f-des}}$	r^2	$1/n_{\text{f-des}}$	r^2	$1/n_{\text{f-des}}$	r^2
N45-A6	8.0	0.15 (0.01)	0.95	0.18 (0.05)	0.84	0.29 (0.04)	0.95	0.30 (0.03)	0.96
		0.16 (0.03)	0.88	0.18 (0.04)	0.86	0.31 (0.07)	0.85	0.28 (0.08)	0.80

^a Numbers are mean $1/n_{\text{f}} \pm$ standard deviation.**Table 6.** Mean Concentration of Representative Chemicals in the Liquid Phase Obtained by Applying the Batch Equilibrium Method to Untreated (Control) Soil Samples, Soil Samples Receiving the Incorporation of 4% Corn Residues, and Sole Corn Residues

sample type	$\mu\text{g mL}^{-1}$							
	Fe	K	Mg	Mn	Na	P	Zn	TOC
IT soil (control)	0.12 ^a	62.52	31.61	0.14	15.15	1.33	0.004	16.2
MN soil (control)	0.12	6.65	59.07	1.49	4.76	2.10	0.171	33.1
IT soil + 4% corn residues	0.13	11.90	27.92	1.19	5.46	0.44	0.061	37.5
MN soil + 4% corn residues	0.17	12.09	66.40	1.70	5.01	2.05	0.090	60.5
corn residues alone	0.03	15.03	11.35	0.15	1.80	0.36	0.013	68.0

^a Data are the average of the two corn residue isolines.**Figure 4.** Effect of incorporation of 4% corn residues from the N45-A6 hybrid on viable bacteria population in the Ozzano (IT) and Princeton (MN) soils. CFUs, colony-forming units. Columns with the same letters do not differ ($P > 0.05$).

phenomenon was observed with most of the analyzed chemicals. In some cases (i.e., potassium in the IT soil), incorporation of corn residues caused a significant concentration decrease ($P < 0.05$), suggesting that chemical composition of the supernatant resulting from corn residue incorporation would not be correctly predicted by a simplistic additive approach.

Culturable Soil Bacteria. By the end of the 28-day incubation period, the total number of bacteria in the two soils was enhanced ($P < 0.05$) by the 4% level of corn residues over that in nontreated (control) soils (**Figure 4**). The highest increase of colony-forming units (CFUs) was observed in the IT soil. These findings support the stimulatory effect of corn residue incorporation on glyphosate mineralization observed in the IT soil. Differences in the total bacteria populations of the two control soils did not reflect the capability of the soil to degrade glyphosate. This confirmed that the total culturable bacteria level represents only a small percentage of the soil bacteria population. Other bacterial groups, not estimated or underestimated by the

adopted methodology, may be involved in glyphosate mineralization processes. Residues of these two isolines did not affect the number of CFUs ($P > 0.05$) (data not shown), thus confirming the absence of detrimental effect of the Cry1Ab toxin on soil bacteria (29).

Conclusions. Two soils of different physicochemical characteristics were used to determine the effect of corn residue addition on the mineralization and sorption of [^{14}C]glyphosate on soil. The results suggest the following:

(i) Incorporation of a moderate level (0.5%) of corn residues stimulated or did not affect glyphosate mineralization in the IT sandy loam, but did not affect it in the MN sandy soil. Increasing ratios of corn residue decreased [^{14}C]glyphosate mineralization in MN soil.

(ii) The acid form of glyphosate showed low affinity to soil particles. This phenomenon reduced glyphosate sorption in soil with a high level (8%) of corn residues.

(iii) Corn residues from the transgenic corn and the correspondent nontransgenic isolate exhibited no differences in their effects on glyphosate sorption and mineralization.

(iv) The presence of a significant amount of the Cry1Ab toxin in the transgenic corn residues did not affect total culturable numbers of soil bacteria.

(v) Even in the cases of significant reduction of glyphosate mineralization and sorption, the results confirmed the short persistence and high retention of glyphosate in soil. Levels of added corn residue (8%) that reduced mineralization and sorption of glyphosate greatly exceeded the amounts of corn residue incorporated or left on the soil surface of fields managed following conservation practices.

ABBREVIATIONS USED

C_e , herbicide equilibrium concentration; C_s , sorbed herbicide concentration; DAT, days after treatment; HPLC, high-performance liquid chromatography; IT, Ozzano soil; K_f and $1/n$, empirical Freundlich constants; LSC, liquid scintillation counting; MN, Princeton soil; PBS, sodium pyrophosphate buffer.

LITERATURE CITED

- (1) Shaner, D. L. The impact of glyphosate-tolerant crops on the use of other herbicides and on resistance management. *Pest Manag. Sci.* **2000**, *56*, 320–326.
- (2) Dill, G. M. Glyphosate-resistant crops: history, status and future. *Pest Manag. Sci.* **2005**, *61*, 219–224.
- (3) Blevins, R. L.; Frye, W. W. Conservation tillage: an ecological approach to soil management. *Adv. Agron.* **1993**, *51*, 33–78.
- (4) Franzluebbers, A. J.; Hons, F. M.; Zuberer, D. A. Tillage and crop effects on seasonal dynamics of soil CO_2 evolution, water content, temperature, and bulk density. *Appl. Soil Ecol.* **1995**, *2*, 95–109.
- (5) Gaston, L. A.; Boquet, D. J.; Bosch, M. A. Fluometuron sorption and degradation in cores of silt loam soil from different tillage and cover crop system. *Soil Sci. Soc. Am. J.* **2003**, *67*, 747–755.

- (6) Zablotowicz, R. M.; Locke, M. A.; Gaston, L. A.; Bryson, C. T. Interactions of tillage and soil depth on fluometuron degradation in a Dundee silt loam soil. *Soil Tillage Res.* **2000**, *57*, 61–68.
- (7) Dao, T. H. Field decay of wheat straw and its effects on metribuzin and S-ethyl metribuzin sorption and elution from crop residues. *J. Environ. Qual.* **1991**, *20*, 203–208.
- (8) Giesy, J. P.; Dobson, S.; Solomon, K. R. Ecotoxicological risk assessment for Roundup™ herbicide. *Rev. Environ. Contam. Toxicol.* **2000**, *167*, 35–120.
- (9) Accinelli, C.; Screpanti, C.; Vicari, A.; Catizone, P. Influence of insecticidal toxins from *Bacillus thuringiensis* subsp. *kurstaki* on the degradation of glyphosate and glufosinate-ammonium in soil samples. *Agric. Ecosyst. Environ.* **2004**, *103*, 497–507.
- (10) Torstensson, L. Behaviour of glyphosate in soils and its degradation. In *The Herbicide Glyphosate*; Grossbard, E., Atkinson, D., Eds.; Butterworth: London, U.K., 1985; pp 137–149.
- (11) Smith, A. E. Persistence and transformation of the herbicide [¹⁴C]-glufosinate-ammonium in prairie soils under laboratory conditions. *J. Agric. Food Chem.* **1988**, *36*, 393–397.
- (12) Zaranyika, M. F.; Nyandoro, M. G. Degradation of glyphosate in the aquatic environment: an enzymatic kinetic model that takes into account microbial degradation of both free and colloidal (or sediment) particle adsorbed glyphosate. *J. Agric. Food Chem.* **1993**, *41*, 838–842.
- (13) Glass, R. L. Adsorption of glyphosate by soils and clay minerals. *J. Agric. Food Chem.* **1987**, *35*, 497–500.
- (14) Gerritse, R. G.; Beltran, J.; Hernandez, F. Adsorption of atrazine, simazine, and glyphosate in soils of the Gngangara Mound, Western Australia. *Aust. J. Soil Res.* **1996**, *34*, 599–607.
- (15) von Wirén-Lehr, S.; Komossa, D.; Gläsgen, W. E.; Sandermann, H., Jr.; Scheunert, I., Jr. Mineralization of [¹⁴C]glyphosate and its plant-associated residues in arable soils originating from different farming systems. *Pestic. Sci.* **1997**, *51*, 436–442.
- (16) Eberbach, P. L. Influence of incubation temperature on the behavior of triethylamine-extractable glyphosate (N-phosphonomethylglycine) in four soils. *J. Agric. Food Chem.* **1999**, *47*, 2459–2467.
- (17) Moore, J. K.; Braymen, H. D.; Larson, A. D. Isolation of a *Pseudomonas* sp. which utilizes the phosphonate herbicide glyphosate. *Appl. Environ. Microbiol.* **1983**, *46*, 316–320.
- (18) Tebbe, C. C.; Reber, H. H. Utilization of the herbicide phosphinothricin as a nitrogen source by soil bacteria. *Appl. Microbiol. Biotechnol.* **1988**, *29*, 103–105.
- (19) Sprankle, P.; Meggitt, W. F.; Penner, D. Adsorption, mobility, and microbial degradation of glyphosate in the soil. *Weed Sci.* **1975**, *23*, 229–234.
- (20) Felsot, A. S.; Shelton, D. R. Enhanced biodegradation of soil pesticides: interactions between physicochemical processes and microbial ecology. In *Sorption and Degradation of Pesticides and Organic Chemicals in Soil*; SSSA Book Series 22; Soil Science Society of America: Madison, WI, 1993; pp 227–251.
- (21) Dzantor, E. K.; Felsot, A. S. Microbial responses to large concentrations of herbicides in soil. *Environ. Toxicol. Chem.* **1991**, *10*, 649–655.
- (22) Koskinen, W. C.; Rice, P. J.; Anhalt, J. A.; Sakaliene, O.; Moorman, T. B.; Arthur, E. L. Sorption–desorption of “aged” sulfonylaminocarbonyltriazolinone herbicide in soil. *J. Agric. Food Chem.* **2002**, *50*, 5368–5372.
- (23) Koskinen, W. C.; Anhalt, J. A.; Sakaliene, O.; Rice, P. J.; Moorman, T. B.; Arthur, E. L. Sorption–desorption of two “aged” sulfonylaminocarbonyltriazolinone herbicide metabolites in soil. *J. Agric. Food Chem.* **2003**, *51*, 3604–3608.
- (24) Cox, L.; Koskinen, W. C.; Yen, P. Y. Changes in sorption of imidacloprid with incubation time. *Soil Sci. Soc. Am. J.* **1998**, *62*, 342–347.
- (25) Kögel-Knabner, I. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol. Biochem.* **2002**, *34*, 139–162.
- (26) Cox, L.; Koskinen, W. C.; Yen, P. Y. Sorption–desorption of imidacloprid and its metabolites in soils. *J. Agric. Food Chem.* **1997**, *45*, 1468–1472.
- (27) Miles, C. J.; Wallace, L. R.; Moye, H. A. Determination of glyphosate herbicide and (amminomethyl)phosphonic acid in natural waters by liquid chromatography using pre-column fluorogenic labeling with 9-fluorenylmethyl chloroformate. *J. Assoc. Off. Anal. Chem.* **1996**, *69*, 458–461.
- (28) de Jonge, H.; de Jonge, L. W. Influence of pH and solution composition on the sorption of glyphosate and prochloraz to a sandy loam soil. *Chemosphere* **1999**, *39*, 753–763.
- (29) Saxena, D.; Stotzky, G. *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworm, nematodes, protozoa, bacteria, and fungi in soil. *Soil Biol. Biochem.* **2001**, *33*, 1225–1230.

Received for review January 26, 2005. Revised manuscript received March 29, 2005. Accepted March 29, 2005.

JF050186R