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Effect of tillage on microbial characteristics and herbicide degradation in a Sharkey clay soil

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Field and laboratory studies were conducted at Stoneville, MS, from 1996 to 1998 to determine the influence of subsoiling (SS) and conventional tillage (CT) of a Sharkey clay soil on microbial characteristics and herbicide degradation. Soil samples obtained from imazaquin-treated and nontreated plots from the soybean row and interrow position were analyzed. Because only the row position is actually disturbed by SS, a comparison of row and interrow position on the parameter was conducted. Imazaquin (preemergence, 140 g ai ha^{-1}) had no effect on microbial populations, microbial enzyme activity (fluorescein diacetate [FDA] hydrolysis and triphenyl-tetrazolium chloride [TTC] dehydrogenase), and organic carbon content. Estimates of microbial activity based on FDA hydrolysis and TTC dehydrogenase activity indicated greater activity under CT; however, microbial biomass and organic carbon were not affected by tillage or row position. A laboratory study assessed the degradation of carboxyl- and ring-labeled 2,4-D as influenced by tillage and row position. Soils from CT plots had an initially higher mineralization rate of ^{14}C carboxyl-labeled 2,4-D compared to soils from SS plots; however, no effect of tillage or row position was observed on the cumulative amount of $^{14}CO_2$ evolved 14 d after treatment (DAT) in 1996 and 18 DAT in 1998. In studies with ring-labeled 2,4-D, a higher $^{14}CO_2$ evolution was detected in soils obtained from SS plots, regardless of row position, whereas a greater amount of radioactivity was observed in the unextractable fraction from CT soils. Because differences in 2,4-D mineralization between tillage regimes were minimal, adoption of SS as a tillage practice for heavy clay soils in the Mississippi Delta may have a limited effect on microbial characteristics and biodegradation of soil-applied herbicides.

Nomenclature: 2,4-D; 2,4-DCP, 2,4-dichlorophenol; imazaquin; FDA, fluorescein diacetate; TTC, triphenyl-tetrazolium chloride; soybean, *Glycine max* (L.) Merr.

Key words: Enzymatic activity, microbial metabolism, subsoiling.

Tillage systems can have a major effect on the physical, chemical, and biological properties of soils. The effects of tillage systems, including conventional tillage (CT), reduced tillage, and no tillage, on soil properties have been extensively reviewed (Griffith et al. 1992; Locke and Bryson 1997). CT, mainly accomplished by a moldboard plow or disk, is characterized by a high degree of soil disturbance. Under CT, plant residues are completely incorporated into the upper soil layer, whereas under reduced-tillage and no-tillage systems, a cover of vegetation or plant residue is preserved.

Soils managed using reduced tillage generally have more surface plant residues, higher moisture content, and better structure and aggregation compared to soils managed under CT (Doran 1980; Griffith et al. 1992; Locke and Bryson 1997). Accumulation of plant residues on the soil surface fosters higher levels of organic matter in the surface soil, promoting better structure, fertility, water infiltration, and water-holding capacity (Kirchner et al. 1993; Wesley et al. 1994). Shading action of ground cover under reduced-tillage systems lowers soil surface temperatures, and fluctuations are not as dramatic compared to temperatures of soils managed under CT (Eghball et al. 1994). The increased plant residue present in soils under reduced tillage also provides a natural barrier against evaporation, promoting higher moisture levels and protecting soil surfaces from erosion.

Accelerated degradation of organic matter because of increased microbial oxidation has been observed in soils under CT, whereas soils managed under reduced tillage typically have higher amounts of organic carbon and mineralizable nitrogen (Doran 1980; Eghball et al. 1994). The presence of decaying plant material on the surfaces of soils under reduced tillage affects the quality, composition, and solubility of soil organic components and the degree of humification. Free sugars or complex polysaccharides associated with high levels of organic matter in reduced-tillage soils serve as labile substrates for microorganisms (Eghball et al. 1994). Reduced-tillage systems may create an optimal environment for microbial growth and activity and other desirable characteristics associated with soil quality (Wagner et al. 1995).

Elevated microbial and soil enzyme activity observed in soils subjected to reduced tillage can promote the biodegradation of compounds such as atrazine (Levanon et al. 1994), bentazon (Wagner et al. 1996), or fluometuron (Zablotowicz et al. 1994). Increased polymerization of organic substances also occurs under reduced tillage, which promotes formation of nonextractable humus–herbicide complexes (Foster and McKercher 1973; Greer and Shelton 1992; Locke and Bryson 1997; Stott et al. 1983). Soil microflora participate in the biotransformation of herbicides by several processes, including co-metabolism, metabolism, polymerization, and mineralization (Bollag and Liu 1990).

TABLE 1. Sampling dates, environmental parameters, and assays performed for microbial characterization in 1996, 1997, and 1998.[a]

					Assay[c]					
Year	Date	Sample timing	Temperature[b]	Rainfall[b]	FDA	TTC	Microbial population	Microbial biomass/ organic carbon	2,4-D mineralization	2,4-D metabolite
1996	May 15	Initial characterization	20	28	*		*			
	May 31	2 WAP	27	34	*		*		*	
	June 26	5 WAP	28	6	*		*			
1997	April 21	Initial characterization	18	0	*	*				
	June 20	2 WAP	25	79	*	*		*		
	July 13	5 WAP	30	0.3	*	*				
1998	June 17	2 WAP	29	3	*			*	*	*

[a] Abbreviations: FDA, fluorescein diacetate hydrolysis activity; TTC, triphenyl-tetrazolium chloride dehydrogenase activity; WAP, wk after soybean planting.
[b] Average temperature [(max + min)/2] and rainfall reported during the week prior to sampling.
[c] An asterisk indicates an assay was performed.

Levanon et al. (1994) suggested that the activity of microbial enzymes in polymerization and the adsorption of herbicides to organic matter are the primary mechanisms reducing the movement of herbicides under reduced tillage. The accumulation of plant residues and organic matter in the upper layer of reduced-tillage soils can create additional herbicide adsorption sites (Locke and Bryson 1997). The degree of adsorption of a specific pesticide to soil colloid can determine its bioavailability to microorganisms (Anderson 1981; Greer and Shelton 1992; Locke and Bryson 1997). Adsorbed pesticides can be protected from microbial degradation, which can increase persistence in soil.

Subsoiling (SS), or deep tillage conducted in the fall, can increase yield of nonirrigated soybean (*Glycine max*) grown on heavy clay soils of the Mississippi Delta (Wesley and Smith 1991; Wesley et al. 1994). Using a subsoiler equipped with parabolic shanks creates only a small slot in the soil surface in the area of major root development. Thus, the degree of soil disturbance is lower under SS than CT. Although the depth of cultivation is increased, SS can leave more plant residues on the soil surface. Although SS has been shown to be beneficial in several soil types, limited research has been conducted to predict the effect of SS on microbial and enzymatic activity and the effect on herbicide dissipation. Therefore, the objectives of this study were to examine the effect of tillage systems, in concert with imazaquin applied preemergence, on soil microbial characteristics and to determine the effect of SS on 2,4-D dissipation in a Mississippi Delta Sharkey clay soil.

Materials and Methods

Site and Soil Description

Field studies were conducted at the Delta Research and Extension Center, Stoneville, MS, from 1996 to 1998. The soil is a Sharkey clay (very fine, smectitic, thermic Chromic Epiaquert) with 3% sand, 32% silt, 66% clay, 3.0% organic matter, and pH 6.4. Two tillage systems, SS and CT, were evaluated. Tillage treatments were initiated 3 yr before initiation of the study and were maintained in the same plots during the 3-yr study. SS was accomplished using a subsoiler unit equipped with two parabolic shanks spaced 1 m apart. All SS plots were tilled to a depth of 40 to 45 cm each fall, with six rows (subsoiler slot positions) per plot. Although SS plots were cultivated with a spike-tooth harrow to create a smooth seedbed, the soil was less disturbed compared to the CT treatment. CT, also performed each fall, was achieved by three passes with an off-set disk harrow, adjusted to operate 15 cm deep, followed by a single pass with a field cultivator 6 cm deep.

'DPL 3589' soybean was planted 3 cm deep at a rate of 33 seeds m^{-1} of row, spaced 1 m apart with six rows per plot. In subsoiled plots, soybean rows were planted exactly over subsoiler slot positions. Immediately after planting, imazaquin was applied at 140 g ai ha^{-1} to corresponding plots. Nontreated plots receiving no imazaquin were also included for comparison. Five soil samples were randomly taken from the soil surface (0 to 5 cm) of each plot using a shovel for initial microbial characterization before soybean planting and 2 and 5 wk after planting (WAP) as summarized in Table 1. To determine the effect of row position (subsoiler shank position) on microbial populations, enzyme activity, organic carbon, microbial biomass, and biodegradation of herbicides, samples taken 2 and 5 WAP were also obtained from the soybean row and interrow (between soybean rows) positions. Soil samples were composited, passed through a 3-mm sieve, and stored moist for approximately 2 wk in the dark at 5 C until analysis.

Microbial Populations

Estimates of soil microbial propagules were determined in soil samples from all sampling dates in 1996 (Table 1) by serial dilutions and spiral plating[1] on selective and semiselective growth media, as described previously (Reddy et al. 1995; Wagner et al. 1995). Total bacteria, gram-negative bacteria, and fluorescent pseudomonads were enumerated on 10% tryptic soy agar containing cycloheximide (100 g ml^{-1}), 10% tryptic soy agar with crystal violet (5 g ml^{-1}) and cycloheximide (100 g ml^{-1}), and S1 media (Gould et al. 1985), respectively. Total fungus populations were determined on Rose Bengal potato dextrose agar (Martin 1950). Colonies of fluorescent pseudomonads, gram-negative bacteria, total fungi, and total bacteria were counted after 2, 3, 5, and 7 d of incubation at 28 C, respectively. Enumeration was conducted using four replications from imazaquin-treated and nontreated plots. Propagule counts were expressed as $\log_{10}$ colony-forming units g^{-1} soil (oven-dry wt).

Enzyme Activity

Fluorescein diacetate (FDA) hydrolytic activity was used as an estimate of soil microbial activity during the 3 yr of the study (Table 1) using a modification of the method described by Schnürer and Rosswall (1982). Two grams of soil (fresh wt) from each sampling date was placed into 50-ml polypropylene centrifuge tubes; 15 ml 0.1 M potassium phosphate buffer (pH 7.6) and 0.5 g FDA in 250 μl acetone were added and tubes were capped and vortexed. Samples were incubated for 2 h on a reciprocal shaker at room temperature (22 to 24 C). The assay was terminated by extraction with 15 ml acetone for 30 min on a reciprocal shaker and was clarified by centrifugation (10 min at 6000 × *g*). Fluorescein (product) concentration was determined spectrophotometrically at 490 nm (extinction coefficient of 80.3 mM cm^{-1}). Experiments were conducted using two replications containing substrate and one control without substrate to correct for background.

A second assay determined triphenyl-tetrazolium chloride (TTC) dehydrogenase activity (Casida 1977) to assess soil enzyme activity in the 1997 samples (Table 1). Four grams of soil (fresh wt) was incubated in 8 ml of 3% aqueous TTC solution for 24 h at 37 C. Assays were conducted with three replicates containing TTC and one control incubated with 8 ml of deionized water. The reaction was terminated by addition of 24 ml of methanol, and soil was extracted for 30 min on a reciprocal shaker. The reaction mixture was clarified by centrifugation (10 min at 6000 × *g*). The concentration of 2,3,4-triphenyl-tetrazolium formazan (product) was determined spectrophotometrically at 485 nm after correcting for coloration due to extractable soil components.

Soil Microbial Biomass and Organic Carbon Content

Microbial biomass was determined in soil samples obtained 2 WAP in 1997 and 1998, employing a chloroform fumigation–incubation method (Jenkinson and Powlson 1976). Soil samples were sterilized by 48 h of exposure to chloroform in a dessicator, after which the dessicator was vented under vacuum several times for complete removal of residual chloroform from the soil. Twenty grams of sterilized soil (oven-dried equivalent) was transferred into biometer flasks[2] and reinoculated with 2 ml of unsterilized soil suspension (1:200 dilution). Microbial biomass was determined by comparing respiration from two replicates of sterilized, reinoculated soil and two replicates of untreated soil for each sample. CO_2 evolved from decomposition of fumigated microflora or endogenous respiration was trapped in sidearms of the flasks containing 10 ml of NaOH (1 N) during 2 wk of incubation at 28 C. Evolved CO_2 was determined by titration with 0.5 M HCl after adding 5 ml of 2 N $BaCl_2$ and phenolphthalein indicator (1 mg ml^{-1} ethanol) to a 5-ml NaOH subsample. The microbial biomass carbon (g C kg^{-1} soil, oven-dry wt) was calculated as Biomass C = F_C/0.45, where F_C is (CO_2-C evolved by fumigated soil) − (CO_2-C evolved by unfumigated soil) (Jenkinson and Ladd 1981).

The effect of tillage systems, row position, and imazaquin application on soil organic carbon (g organic C kg^{-1} soil, oven-dry wt) was determined using a modified Mebius procedure (Nelson and Sommers 1982). Triplicate samples of soils (300 mg, air dried) obtained in 1997 and 1998 2 WAP were analyzed.

2,4-D Mineralization

The effect of tillage systems and row position on herbicide degradation was evaluated in the laboratory using soil biometer flasks (Bartha and Pramer 1965) with 2,4-D as a model substrate. Radiolabeled carboxyl- and ring-labeled 2,4-D was used to determine the effect of soil management systems on various microbial transformations, including side-chain decarboxylation, ring cleavage, or oxidative coupling reactions (polymerization). Soil samples collected from herbicide nontreated plots of both tillage systems and row positions 2 WAP in 1996 and 1998 were analyzed. Studies were initiated approximately 2 wk after sampling. Twenty-five grams of soil (oven-dry wt) were placed in biometer flasks and adjusted to 30% moisture content. Soils were treated with 1.0 ml of a methanol : water (1:4, v/v) solution containing ^{14}C uniformly labeled[3] (98% radiochemical purity) and technical-grade 2,4-D[4] (99% purity) to achieve a final concentration of 10 g g^{-1} soil. Total radioactivity added per flask was 171 and 217 Bq g^{-1} soil of carboxyl-labeled material and 373 and 319 Bq g^{-1} soil of ring-labeled material in 1996 and 1998, respectively. Each tillage and row position combination was replicated with four flasks. Biometer flask side arms contained 10 ml of NaOH (1 N) to trap evolved $^{14}CO_2$ and were replaced every second day. Soils treated with carboxyl-labeled 2,4-D were incubated for 14 and 18 d, whereas soils treated with ring-labeled material were incubated for 20 and 26 d at 28 C in 1996 and 1998, respectively. Trapped $^{14}CO_2$ was determined in 1996 from 250-L aliquots of NaOH mixed with 250 L of distilled water and added to 15 ml of scintillation cocktail[5]. In 1998, evolved $^{14}CO_2$ was determined from 1-ml aliquots of NaOH added to 15 ml of scintillation cocktail.[6] Radioactivity was determined by liquid scintillation counting[7] (LSC). After completion of the 1996 study, 5 g of soil from each flask were extracted twice with methanol (15 ml and 10 ml, respectively) to determine the extractable radioactivity using a modified method described by Zablotowicz et al. (1998). In 1998, soils (25 g) were extracted twice with 50 ml of methanol. Aliquots (1 ml) were added to 15 ml of scintillation cocktail[8] and radioactivity was determined by LSC. Unextractable radioactivity was determined from air-dried methanol-extracted soils by oxidation. Duplicate 300-mg subsamples were mixed with 200 mg of cellulose into cellulose thimbles and combusted with a biological materials oxidizer[9] at 482 C for 4 min. Released $^{14}CO_2$ was trapped in a combination of carbon absorbent[10] and scintillation cocktail[11] and was counted by LSC.

2,4-D Metabolite Determination

Formation of metabolites from ring-labeled 2,4-D in CT and SS plots in 1998 soil samples was determined using thin layer chromatography (TLC) as described by Zablotowicz et al. (1998). Twenty-five grams of soil (interrow position, oven-dry wt) were placed in 250-ml polypropylene bottles, treated with ^{14}C uniformly ring–labeled[5] and technical grade 2,4-D[6] in a methanol : water solution (1:4, v/v) to attain a concentration of 10 g g^{-1} soil with 319 Bq g^{-1} soil. Moisture content was adjusted to 30%. Samples were

TABLE 2. Soil microbial populations in a Sharkey clay soil at 2 and 5 wk after soybean planting (WAP) as influenced by tillage systems and row position in 1996.

		Microbial population							
		2 WAP				5 WAP			
Tillage system	Row position	Total fungi	Total bacteria	Gram-negative bacteria	Fluorescent pseudomonads	Total fungi	Total bacteria	Gram-negative bacteria	Fluorescent pseudomonads
		$\log_{10}$ colony-forming units g^{-1} soil							
Conventional	Row	5.31	7.78	6.17	4.44	4.99	7.34	5.30	2.27
Conventional	Interrow	5.56	8.03	5.96	4.62	4.83	7.28	5.15	2.26
Subsoiling	Row	4.94	7.87	6.19	4.56	4.94	7.28	5.78	3.84
Subsoiling	Interrow	5.19	7.83	6.03	4.28	4.66	7.31	5.44	2.64
LSD (0.05)		0.37	0.23	NS	NS	0.13	NS	0.41	1.54

incubated in the dark at 28 C for 4 or 8 d and were extracted twice with methanol as described previously (Zablotowicz et al. 1994). Methanol extracts were reduced in a rotary evaporator to approximately 10 ml. The methanol concentrate was diluted with 100 ml of deionized water, acidified to pH 3.0 with HCl (1 N), and passed through a C-18 solid-phase extraction column.[12] Extracted 2,4-D and metabolites were eluted from the column with 3 ml methanol. Aliquots (40 μl) were spotted on 250-μm silica TLC plates[13] and developed 10 cm with benzene : acetic acid : acetone (25:25:1, v/v/v) solvent. The distribution of radioactivity in the developed TLC plates was analyzed with an imaging scanner.[14] The R_f values for 2,4-D and 2,4-dichlorophenol (2,4-DCP) were 0.16 and 0.40, respectively.

Statistical Analysis

The field experiment was conducted using a split-split plot design with four replications. Data obtained from microbial enumeration, FDA, TTC, and organic carbon were analyzed with tillage treatment as the main plot unit, herbicide treatment (imazaquin or no herbicide) as the subplot, and row position the sub-subplot. Data obtained from soil microbial biomass determination and 2,4-D mineralization were analyzed as a split plot design (only nontreated soils were assessed). All data were subjected to analysis of variance, and means were separated using Fisher's Protected LSD test. All statistical determinations were made at the 0.05 level of significance, unless otherwise noted. Because of year interactions, data from each year of the study were analyzed separately.

Results and Discussion

Effect of Imazaquin

Preemergent application of imazaquin at 140 g ai ha^{-1} did not affect microbial characteristics of a Sharkey clay soil. Although deleterious effects of certain herbicides on soil microbial populations have been reported in the literature (Biederbeck et al. 1987; Levanon et al. 1994; Moorman and Dowler 1991; Narain Rai 1992), no effect of imazaquin on microbial populations, soil enzyme activity, microbial carbon, or organic carbon was observed in this study (data not shown). Subsequently, data were averaged over imazaquin treatment and analyzed as a split plot design. Because of an interaction, the combination of tillage system and row position are discussed separately.

Microbial Populations

At initial characterization (before soybean planting), microbial populations did not differ between tillage management systems (data not shown). Soil samples collected from CT plots 2 WAP in 1996 contained higher counts of total fungi than that found in soils from SS plots for their respective row positions (Table 2). Total bacteria were greatest in soil from CT plots in the interrow position; however, all other microbial populations evaluated were not affected by tillage or row position. In contrast, higher numbers of gram-negative bacteria and fluorescent pseudomonads 5 WAP were found in soil from SS plots in the row position compared to both positions in soil from CT plots. The number of fluorescent pseudomonads was low 5 WAP, which may have been related to high temperatures and drier soil.

Microbial populations and their enzyme activities in soils are subject to seasonal fluctuations (Kaiser and Heinemeyer 1993; Kirchner et al. 1993). Warmer temperatures and adequate moisture typically found in the spring promote the growth of crops and other vegetation, providing additional substrate for proliferation of soil microbes. Water availability is a key environmental parameter influencing the quantity and diversity of microflora in soil (Anderson 1981, 1984). Increased rainfall and temperature and the presence of soybean roots may have promoted microbial growth at later sampling dates (Table 2). Although microbial populations increased from the initial sampling time to 2 WAP, numbers declined by 5 WAP (Table 2). In 1996, dry soil conditions in combination with higher average temperatures were present 5 WAP (Table 1). Surface soil temperatures increased during the season, resulting in periods of water deficit that typically reduce microbial populations. Thus, effects of tillage on microbial populations in a Sharkey clay soil are difficult to assess because of temporal variations.

Although significant differences in microbial populations were observed, the effects of tillage system or row position were not consistent. The effect of SS on microbial propagules observed in this study was much lower than effects reported in previous studies that compared no-tillage and CT systems in other Mississippi Delta soils (Reddy et al. 1995; Wagner et al. 1996; Zablotowicz et al. 1994). Soils used in these studies had lighter texture (e.g., Dundee silt loam [fine-silty, mixed, thermic Aeric Ochraqualf]), which may have been more responsive to tillage than Sharkey clay soils. Subtle changes in the soil microbial community ob-

TABLE 3. Fluorescein diacetate (FDA) hydrolysis and triphenyl-tetrazolium chloride (TTC) dehydrogenase activity in a Sharkey clay soil as influenced by tillage system and row position.[a]

		FDA hydrolysis activity							TTC dehydrogenase activity		
		1996			1997			1998	1997		
Tillage	Position	Initial	2 WAP	5 WAP	Initial	2 WAP	5 WAP	2 WAP	Initial	2 WAP	5 WAP
		nmol g^{-1} soil h^{-1}									
Conventional	Row	264	261	265	155	193	126	299	2.5	1.1	1.6
Conventional	Interrow	—	276	271	—	217	108	301	—	1.2	1.0
Subsoiling	Row	198	155	249	103	131	111	166	1.2	0.6	1.3
Subsoiling	Interrow	—	178	229	—	163	76	215	—	0.8	1.0
LSD (0.05)		NS	46	NS	NS	28	34	71	1.1	0.4	NS
LSD (0.10)		48	—	—	41	—	—	—	—	—	—

[a] Abbreviation: WAP, weeks after soybean planting.

served in this study would be expected to have minimal effect on the potential for microbial herbicide metabolism.

Microbial Enzyme Activity

At initial sampling dates in 1996 and 1997, increased FDA activity (33 to 50%) was associated with soils from CT compared to SS plots; however, these differences were only statistically different at the 0.10 level of significance (Table 3). During all 3 yr of the study, CT soils (from both row and interrow positions) had higher FDA hydrolytic activity compared to soil from SS plots 2 WAP. In 1996 5 WAP, no differences in FDA hydrolysis attributable to tillage or row position were observed, although in 1997, soils collected from SS plots in the interrow position had lower FDA activity than other soil treatments. FDA hydrolysis is a simple, nonspecific, but sensitive technique to determine microbial activity in soils (Dick 1984; Reddy et al. 1995; Wagner et al. 1995). The substrate, FDA, is nonspecific for a wide spectrum of enzymes, including proteases, lipases, and esterases, and its hydrolysis has been highly correlated with the de-esterification of fenoxaprop-ethyl (Zablotowicz et al. 2000). In addition, a good correlation was found between FDA activity and soil respiration. However, FDA hydrolytic activity may represent enzymatic activity from living and dead cells as well as extracellular enzymes associated with clay minerals, organic matter, or fungal biomass (Schnürer and Rosswall 1982). FDA hydrolysis has also been suggested as a useful parameter to assess soil quality (Dick 1994).

TTC dehydrogenase activity is based on the ability of a soil to use TTC as an alternative electron acceptor and is used as an indicator for total microbial activity estimated only in living cells (Casida 1977). At initial characterization in 1997, TTC dehydrogenase activity was 108% greater in CT compared to SS soils (Table 3). Higher dehydrogenase activity was also observed in CT soils compared to SS soils collected 2 WAP when comparing respective row positions. However, no differences were observed 5 WAP. Results from the TTC assays confirmed results from the FDA hydrolysis assays, indicating greater biological activity in soils from CT compared to SS plots, regardless of row position. The magnitude of enzyme activity is dependent on several factors, such as microbial populations and composition, environmental conditions (temperature, moisture, pH), and carbon substrate availability (Dick 1984, 1994; Sinsabaugh 1994). Under CT, incorporation of plant residues into the surface soil provided more labile carbon substrates to support microbial activity and may be the major factor responsible for higher enzyme activities in CT soils compared to SS soils.

TABLE 4. Influence of tillage system and row position on radioactivity recovered from a Sharkey clay soil treated with 10 μg g^{-1} 2,4-D (^{14}C carboxyl-labeled) in 1996 and 1998.[a]

			Cumulative $^{14}CO_2$ recovery											
			Incubation time (DAT)											
Year	Tillage	Position	2	4	6	8	10	12	14	16	18	ME	UE	Recovery
			% of applied											
1996	Conventional	Row	22.5	41.6	57.7	69.5	74.4	76.4	77.7	—	—	1.0	15.0	93.7
	Conventional	Interrow	21.6	41.5	59.5	70.8	75.5	77.0	77.9	—	—	1.0	15.5	94.4
	Subsoiling	Row	19.0	38.1	51.6	66.9	73.6	75.9	77.1	—	—	1.0	14.9	92.9
	Subsoiling	Interrow	19.5	38.7	55.3	68.3	73.3	75.2	76.5	—	—	0.9	15.0	92.6
	LSD (0.05)		1.1	2.6	3.2	2.0	2.0	NS	NS	—	—	NS	NS	NS
1998	Conventional	Row	18.9	42.3	59.8	70.6	76.0	79.2	81.0	82.1	82.9	1.0	12.2	96.1
	Conventional	Interrow	18.4	36.0	51.8	64.0	70.1	74.6	77.3	79.0	80.3	1.6	13.3	95.1
	Subsoiling	Row	13.2	33.2	51.8	63.9	70.0	74.0	76.7	78.5	80.0	1.7	11.8	93.5
	Subsoiling	Interrow	15.7	38.1	56.6	68.0	72.8	76.3	78.5	79.9	81.0	1.3	11.9	94.1
	LSD (0.05)		1.7	8.2	NS	6.5	NS	NS	NS	NS	NS	0.5	NS	NS

[a] Abbreviations: DAT, days after treatment; ME, methanol-extractable fraction; UE, unextractable fraction.

Soil Microbial Biomass and Organic Carbon

Tillage system and row position had no effect on either soil microbial biomass carbon or total soil organic carbon (data not shown). Only small differences in microbial populations attributed to tillage systems have been observed in this study; thus, alterations in soil microbial biomass would not have been expected. Crop rotation sequences and climate have been found to have greater influence on soil microbial biomass than tillage (Biederbeck et al. 1987; Eghball et al. 1994; Moorman and Dowler 1991). Cropping systems that produce more plant residues and do not greatly disturb the soil surface tend to have greater amounts of soil organic matter (Eghball et al. 1994). Subsoiling techniques cause minimal disturbance to the soil surface; therefore, an increase in soil organic matter due to reduced oxidation would only be expected over several years.

2,4-D Mineralization and Metabolite Determination

Although 2,4-D was chosen as a model substrate, differences in degradation of 2,4-D due to tillage system may be used as an indicator for biodegradation characteristics of other herbicides in a Sharkey clay soil. In 1996, a more rapid initial mineralization ($^{14}CO_2$ evolution) of carboxyl-labeled 2,4-D was detected in CT compared to SS (Table 4). Specifically, in the initial 8 d after treatment (DAT), cumulative $^{14}CO_2$ evolution was higher in CT compared to SS soils for their respective row positions. In soils collected in 1998, initially higher mineralization was observed 2 DAT when comparing CT and SS soils, regardless of row position. Mineralization detected in the row position 4 DAT was significantly higher in CT (Table 4). This initial, more rapid mineralization of carboxyl-labeled 2,4-D in CT compared to SS soils may reflect levels of increased microbial activity, as observed in the FDA hydrolytic and TTC dehydrogenase enzyme assays.

Although the highest initial rate of mineralization of carboxyl-labeled 2,4-D occurred in CT soils, the level of $^{14}CO_2$ evolution in soils from SS plots increased over time to a level equivalent to that of CT soils, indicating that microbial populations in SS soils adapted to degrade 2,4-D. Mineralization of the carboxyl-labeled 2,4-D side chain occurred without a lag period, indicating the presence of metabolizing microorganisms and enzyme systems to facilitate this transformation (Kunc and Rybarova 1983). In both years, about 78 and 83% of the applied carboxyl-labeled 2,4-D was mineralized to $^{14}CO_2$ after 14 and 18 d of incubation, respectively (Tables 4). Minimal radioactivity (about 1%) was recovered by methanol extraction at termination of studies, indicating almost complete metabolism of the acetic acid moiety of 2,4-D. Between 12 and 15% of initially applied radioactivity was associated with the unextractable fraction. A satisfactory level of mass balance (total recovery) of 93 to 96% was observed.

In 1996, a higher initial mineralization rate of ring-labeled 2,4-D occurred in soils from CT compared to SS plots in the interrow position (Table 5). However, more 2,4-D was mineralized to $^{14}CO_2$ in soils from SS compared to CT, regardless of row position at the termination of the study (18 DAT). In 1998, a higher mineralization rate was detected 2 DAT in soils obtained from CT compared to SS,

TABLE 5. Influence of tillage system and row position on radioactivity recovered from a Sharkey clay soil treated with 10 μg g^{-1} 2,4-D (^{14}C ring-labeled) in 1996 and 1998.[a]

Year	Tillage	Position	Cumulative $^{14}CO_2$ recovery												ME	UE	Recovery
			Incubation time (DAT)														
			2	4	6	8	10	12	14	16	18	20	22	24	26			
			% of applied														
1996	Conventional	Row	4.4	15.0	25.1	34.9	39.4	41.4	42.8	44.3	45.3	—	—	—	—	3.5	35.9	84.8
	Conventional	Interrow	5.0	16.5	29.6	38.4	41.7	43.1	44.2	45.4	46.3	—	—	—	—	3.2	34.0	82.5
	Subsoiling	Row	4.5	15.4	23.9	35.3	40.5	42.8	44.4	45.9	48.0	—	—	—	—	3.9	30.6	81.9
	Subsoiling	Interrow	4.3	14.9	25.0	36.3	41.6	43.7	45.2	46.7	47.7	—	—	—	—	3.5	30.1	81.3
	LSD (0.05)		0.5	NS	2.0	1.8	1.5	1.5	1.5	1.4	1.2	—	—	—	—	0.5	3.7	NS
1998	Conventional	Row	3.2	15.1	28.7	38.7	44.0	47.6	49.9	51.4	52.6	53.6	54.4	54.9	55.4	3.0	28.8	87.2
	Conventional	Interrow	3.0	12.6	25.3	35.6	41.7	45.9	48.8	50.6	52.1	53.3	54.3	54.9	55.4	3.1	29.0	87.5
	Subsoiling	Row	2.4	13.4	27.3	38.3	44.2	48.5	51.4	53.3	55.0	56.4	57.5	58.3	59.0	3.3	25.2	87.5
	Subsoiling	Interrow	2.6	13.8	27.8	38.7	44.4	48.3	51.0	52.9	54.5	55.7	56.7	57.4	58.0	3.1	25.6	86.7
	LSD (0.05)		0.2	1.8	2.6	2.4	2.0	1.8	1.6	1.4	1.2	1.1	1.0	0.8	0.8	0.2	2.0	NS

[a] Abbreviations: DAT, days after treatment; ME, methanol-extractable fraction; UE, extractable fraction.

TABLE 6. Influence of tillage systems on metabolite formation in a Sharkey clay soil treated with 10 μg g^{-1} 2,4-D (^{14}C ring-labeled) in 1998.[a]

	Incubation time (DAT)							
	4				8			
Tillage system	ME	2,4-D	2,4-DCP	UE	ME	2,4-D	2,4-DCP	UE
	% of applied							
Conventional	34.7	21.1	12.9	37.7	12.3	6.9	5.2	34.1
Subsoiling	35.2	26.7	7.7	39.8	16.6	10.0	6.5	32.0
LSD (0.05)	NS	3.3	3.8	NS	NS	NS	NS	NS

[a] Abbreviations: DAT, days after treatment; 2,4-DCP, 2,4-dichlorophenol; ME, methanol-extractable; UE, unextractable.

regardless of row position (Table 5). However, at later sampling dates, cumulative $^{14}CO_2$ evolution increased in SS compared to CT soils regardless of row position.

Total cumulative mineralization of ring-labeled 2,4-D was approximately 45 to 59% of the applied radioactivity in 1996 and 1998, respectively (Table 5). Only approximately 4% of the initial radioactivity was recovered by methanol extraction, indicating nearly complete degradation of applied 2,4-D. A higher percentage of ^{14}C was recovered in the unextractable fraction in CT compared to SS soils, regardless of row position in both years of the study. The trend of greater mineralization of the 2,4-D ring in SS soils and greater incorporation of ring-labeled 2,4-D in the unextractable fraction in CT soils suggests altered pathways of 2,4-D metabolism due to tillage. Perhaps oxidative coupling of the 2,4-D ring moiety into soil humus was facilitated under CT, whereas more complete oxidation occurred in SS soils. Mass balance using ring-labeled 2,4-D ranged from 81 to 87%, with a higher efficiency in 1998 (Table 5). Although mineralization studies were conducted in soil biometer flasks to minimize loss of radioactivity, some loss of volatile compounds can be expected in this controlled environment (Bartha and. Pramer 1965). Possible loss of ^{14}C could have occurred via volatilization of 2,4-D itself and the metabolites, 2,4-DCP and 2,4-dichloroanisole (Smith 1985), and could have occurred during sampling of NaOH solutions or during soil extraction. However, volatility losses of 2,4-DCP and 2,4-dichloroanisole would only be detectable for the ring-labeled 2,4-D.

In 1998, an additional short-term incubation study assessed ^{14}C ring-labeled 2,4-D dissipation and metabolite formation in soil from CT and SS plots. Approximately 35 and 15% of the initial radioactivity was recovered by methanol extraction after 4 and 8 DAT, respectively (Table 6). Likewise, about 38 and 33% of the initial 2,4-D was unextractable 4 and 8 DAT, respectively. TLC analysis of methanol extracts indicated a rapid dissipation of 2,4-D with 21 and 27% of the initial 2,4-D recovered 4 DAT from CT and SS, respectively. Results also indicate that 2,4-DCP was the major metabolite formed and accumulated in a Sharkey clay (Table 6). A higher percentage of 2,4-DCP was initially accumulated in CT (Table 6), supporting results of a more rapid mineralization of the acetate side chain of 2,4-D in CT compared to SS soils. Up to 43% of the extractable radioactivity 8 DAT was recovered as 2,4-DCP.

Mineralization of carboxyl-labeled 2,4-D occurred more rapidly and to a greater extent compared to ring-labeled 2,4-D. Microbial metabolism of 2,4-D proceeds by initial cleavage of the ether-linked acetic acid side chain forming glycolic acid, which can be utilized as an energy source and readily metabolized to CO_2 (Foster and McKercher 1973; Kunc and Rybarova 1983; Stott et al. 1983). Mineralization of the ring structure of 2,4-D molecules requires ring cleavage and dechlorination. Transformation of the ring moiety of 2,4-D is mediated by a limited group of microorganisms possessing specific enzyme systems. The mineralization of ring-labeled 2,4-D exhibited a lag period up to 4 d in both soils, indicating growth or an adaptation period of 2,4-D–metabolizing bacteria. Typically, the acetic acid side chain is used for general metabolism by soil microorganisms. Thus, greater $^{14}CO_2$ evolution was observed in soils treated with carboxyl-labeled 2,4-D compared to ring-labeled 2,4-D. In contrast, 2,4-D and its metabolites can be subjected to enzyme-mediated oxidative coupling reactions and incorporated into relatively resistant humic acid polymers (Sarkar et al. 1988; Wilson and Cheng 1978; Zablotowicz et al. 1994). In general, incorporation of phenolic compounds into humic acid polymers or diffusion into the interior of humic acid particles reduces the concentration of free 2,4-D molecules in soil solutions, thereby stabilizing them against biodegradation. Incorporation of 2,4-D and its metabolites into soil as chemically bound compounds can resist solvent extractions (Sarkar et al. 1988; Stott et al. 1983; Wilson and Cheng 1978, Zablotowicz et al. 1994). Continuous incorporation of plant residues in CT may stimulate microorganisms that produce enzymes responsible for oxidative coupling of phenolic compounds and may explain the higher unextractable portion of ring-labeled herbicide in soil from CT plots.

Tillage systems evaluated in this study resulted in small and transient changes in microbial populations. Microbial activity measured by two distinct assays was consistently higher in CT compared to SS soils, which most likely enabled the initially more rapid mineralization rate of carboxyl-labeled 2,4-D in CT systems. CT operations completely incorporated crop residues into the upper soil layer (plow layer), thereby providing intimate contact between microorganisms and substrate. Although the soil microbial biomass and organic matter content were not affected by tillage systems, crop residues incorporated in CT soils may have provided readily available carbon and nitrogen sources to support increased microbial activity. Because SS had only minor effects on biodegradation of 2,4-D, minimal effects on microbial decomposition of other soil-applied herbicides would be expected. Previous work also indicated that SS does not affect the environmental fate of a common soil-

applied herbicide used in soybean (Seifert 1999). Therefore, adoption of SS techniques in the Mississippi Delta would most likely have minimal effects on soil microbial properties and the biodegradation of soil-applied herbicides.

Sources of Materials

[1] Spiral System Instruments, 7830 Old Georgetown Road, Bethesda, MD 20814.

[2] Bellco Glass Inc., 340 Edrudo Road, Vineland, NJ 08360.

[3] 2,4-D (ring- and carboxyl-labeled), Sigma Chemical Co., 3050 Spruce Street, St. Louis, MO 63103.

[4] 2,4-D (technical grade), ChemService, P.O. Box 3108, West Chester, PA 19381.

[5] ScintiSafe Plus 50% scintillation cocktail, Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA 15219.

[6] Hi-Ionic-Fluor scintillation cocktail, Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA 15219.

[7] Model Packard TriCarb 4000 series, Packard Instruments Co., 800 Research Parkway, Meriden, CT 06450.

[8] EcoLume scintillation cocktail, ICN, 3300 Hyland Avenue, Costa Mesa, CA 92626.

[9] Packard TriCarb Oxidizer 306, Packard Instruments Co., 800 Research Parkway, Meridian, CT 06450.

[10] Carbo-Sorb E, Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450.

[11] Permafluor E$^+$, Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450.

[12] J. T. Baker, 222 Red School Lane, Phillipsburg, NJ 08865.

[13] Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA 15219.

[14] Bioscan Imaging System 200, Bioscan, Inc., 4590 Macarthur Boulevard, Washington, DC 20007.

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