

Microbiological Characteristics of a Vegetative Buffer Strip Soil and Degradation and Sorption of Metolachlor

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ABSTRACT

Numerous studies have documented efficacy of vegetated buffer strips (VBS) in removing herbicides from surface runoff. Little is known about the fate of herbicides after deposition in buffer strip soil. Soil samples (0- to 2-cm depth) were collected from a buffer strip and an adjacent bare field (BF). Soil organic C was two-fold higher in VBS than in BF, and VBS soil maintained about 0.7 log(10) greater propagule density of total fungi and bacteria and 2 log(10) greater gram-negative bacteria and fluorescent pseudomonads. Corresponding with enhanced microbial populations, VBS exhibited higher endogenous levels of alkaline phosphatase, tetrazolium chloride dehydrogenase, aryl acylamidase, and fluorescein diacetate hydrolytic activity (1.6- to 3.8-fold greater than BF). Batch studies of metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] sorption showed greater capacity to sorb metolachlor in VBS soil than in BF (K_d 2.25 vs. 1.60 mL g⁻¹). Soil treated with ¹⁴C metolachlor (1.25 mg kg⁻¹) was incubated for 46 d in a laboratory study. Limited mineralization (<4%) was observed for both VBS and BF. Less ¹⁴C (43%) was extracted from VBS samples 46 d after treatment. Extractable fractions consisted primarily of metolachlor, although increasing amounts of polar (e.g., sulfonic acid) and nonpolar metabolites were recovered over time, especially in VBS. Metolachlor half-life was 10 and 23 d for soils from VBS and BF, respectively, attributed to higher levels of organic matter and microbial activity in VBS soils. Data suggest retention and enhanced degradation of metolachlor as it passes through VBS strips may limit further transport.

METOLACHLOR is used for pre- and postemergence control of grasses and some broadleaf weeds in a variety of crops including corn (*Zea mays* L.) and cotton (*Gossypium* spp.) (WSSA, 1994). In soils, metolachlor is metabolized by microorganisms (Miller et al., 1997) and binds to organic matter and clay minerals through hydrogen bonding and hydrophilic or lipophilic interactions (Peter and Weber, 1985; Chesters et al., 1989). Studies indicated that this degradation and sorption did not prevent movement of metolachlor and its metabolites to aquatic systems, as evidenced by the presence of parent compound and/or its metabolites in streams, ponds, and wells (Frank et al., 1990a,b; Kolpin et al., 1997; Kalkhoff et al., 1998). Kolpin et al. (1997) have associated rising levels of metolachlor in Iowa municipal wells with increased use of the herbicide. Studies suggested that metolachlor can reach water supplies through surface runoff or by leaching (Bowman et al., 1994; Funari et al., 1998). Metolachlor is not readily

degraded in aquatic environments (Liu et al., 1995), enhancing concern about its presence in these systems.

Considerable research has been devoted to decreasing the levels of herbicides and their metabolites in the environment. The use of VBS is one strategy currently used for reducing herbicide levels in surface runoff. Numerous studies have demonstrated the efficacy of VBS for herbicide removal (Klöppel et al., 1997; Lowrance et al., 1997; Patty et al., 1997) including metolachlor (Arora et al., 1996; Misra et al., 1996; Webster and Shaw, 1996; Mersie et al., 1999). However, little is known about the fate of herbicides in soil after entering VBS. While infiltration of surface runoff in grass filter strips appears to be the primary mechanism for lowering outflow concentrations of herbicides, sorption to soil and vegetative material may also be a factor (Arora et al., 1996; Misra et al., 1996).

When VBS are established, continuous vegetation is maintained, and soils take on characteristics of grassland soils compared with adjacent cultivated areas. Without tillage, a litter layer from decaying above-ground vegetation and a massive network of roots alters patterns of organic matter accumulation that should substantially stimulate soil microbiological processes such as herbicide degradation. As described by Benoit et al. (1999), the highest isoproturon degradation was observed in vegetative buffer soils that also had the greatest mineralization rates of soil organic matter. Mississippi Delta soils managed under practices that enhance plant residue accumulation in surface soils (no tillage and herbicide-desiccated cover crops) provide an environment for enhanced microbial populations and soil enzyme activity (Wagner et al., 1995; Zablotowicz et al., 1998a) and more rapid degradation of the phenylurea herbicide, fluometuron (1,1-dimethyl-3-(*a,a,a*-trifluoro-*m*-tolyl)urea) (Zablotowicz et al., 1998a). The present study was conducted to assess the fate of metolachlor (degradation and sorption) and microbiological characteristics (populations and enzymatic activity) of surface soils from a 6-yr-old VBS in comparison with an adjacent cultivated cotton field. This information is needed to assess critically the fate of herbicides that are retained within VBS soils.

MATERIALS AND METHODS

The study site was on a farmer's field adjacent to Beasley Lake in Sunflower County, Mississippi (an oxbow lake formed

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Abbreviations: BF, bare field; FDA, fluorescein diacetate; LSC, liquid scintillation counting; NT, no-tillage; OC, organic carbon; TTC, tetrazolium chloride; TLC, thin-layer chromatography; VBS, vegetated buffer strip.

from a former channel of the Sunflower River), with a Dundee silt loam (fine-silty, mixed, thermic Aeric Ochraqualf) soil. A VBS and an adjacent BF were examined. The BF area was under long-term (at least 8 yr continuous) cotton production and had been exposed to metolachlor (last application in the spring of 1997). The VBS area was established as hairy vetch (*Vicia villosa* Roth) at least 6 yr prior to sampling. Predominant species in the VBS at the time of sampling included *Poa annua* L., *Capsella bursa-pastoris* (L.) Medicus, *Lamium amplexicaule* L., *Lepidium virginicum* L., *Vicia villosa* Roth, and *Stellaria media* (L.) Cyrillo. Surface soil samples (0–2 cm) were collected from six 1-m² area plots within each area (VBS or BF) on 2 April 1998. The six VBS sampling locations were spaced approximately 30 m apart and were 10 to 20 m from the edge of Beasley Lake. The corresponding BF locations were 30 to 40 m farther up the slope from the lake. One week prior to the sampling date, the BF was tilled in preparation for planting. Sample material from the VBS was shaken during collection to reduce inclusion of root material in the samples. Ten subsamples were obtained and composited for each plot. Any visible plant tissue was removed before passing samples through a 4-mm sieve. Samples were stored in a cold room at 4°C until further processing. Soil pH (1:1 w/v, 0.01 M CaCl₂), soil organic carbon (OC) (Walkley-Black, Nelson and Sommers, 1996), and particle size distribution (hydrometer method; Gee and Bauder, 1986) were determined for the soils.

Microbiological and Enzyme Assays

Total bacteria, total fungi, gram-negative bacteria, and fluorescent pseudomonads were enumerated by serial dilution on media described elsewhere (Reddy et al., 1995). Briefly, total bacteria were determined on dilute tryptic soy agar with cycloheximide and total fungi with Rose Bengal potato dextrose agar (Martin, 1950). Tryptic soy broth with crystal violet was used for gram-negative bacteria while fluorescent pseudomonads were quantified by means of S-1 media (Gould et al., 1985).

Fluorescein diacetate (FDA) was used as a substrate to estimate total microbial, heterotrophic (esterase, lipase, protease) activity (Schnürer and Rosswall, 1982). Soil (2 g) was incubated with fluorescein diacetate (0.5 mg) in 20 mL phosphate buffer (0.1 mM, pH 7.8) at 20°C for 1 h (150 rpm). The FDA hydrolysis reaction was terminated with the addition of 20 mL of acetone. The reaction mixtures were shaken for 5 min before centrifuging at 8000 × g for 10 min. Fluorescein in the supernatant was quantified spectrophotometrically at 490 nm with an extinction coefficient of 80.3 mM⁻¹ cm⁻¹. Microbial metabolic activity was estimated by triphenyl-tetrazolium chloride (TTC) dehydrogenase activity (Casida et al., 1964). Soil (2 g) was incubated statically with 4 mL of 3% (w/v) TTC for 24 h at 37°C. A second set of reactions was performed with the addition of TTC fortified with 0.1% (w/v) yeast extract (Casida, 1977). The TTC reaction was terminated with 12 mL of methanol, and the product, triphenyl-formazan, was extracted by shaking for 30 min. After centrifugation the optical density of the supernatant was measured at 485 nm and corrected for soil incubated without a substrate. Alkaline phosphatase activity was determined as described by Tabatabai (1994). Briefly, the release of *p*-nitrophenol from *p*-nitrophenyl phosphate was monitored spectrophotometrically (410 nm). Aryl acylamidase activity (Zablutowicz et al., 1998b) was estimated by monitoring hydrolysis of 2-nitroacetanilide (1 g of soil with 4 mL of 2 mM 2-NAA in phosphate buffer, 0.05 M, pH 8.0). Release of 2-nitroaniline was determined spectrophotometrically (410 nm) after 24 h of incubation at 30°C by extraction with 4 mL of methanol and centrifugation.

Metolachlor Degradation and Sorption

Two laboratory studies were conducted simultaneously to determine the effects of VBS on metolachlor degradation in soil. Field moist soils (25 g oven-dry weight equivalent) from each of the six field plots in the VBS and BF areas were added to 250-mL Nalgene bottles (in Study I) or 250-mL biometer flasks (in Study II) and brought to 300 g kg⁻¹ soil moisture (gravimetric) with deionized water. In Study II, biometer flasks were used to assess metolachlor mineralization to ¹⁴CO₂. Otherwise, conditions for the two studies were the same. A mixture of ¹⁴C-ring labeled metolachlor (spec. act. 3.05 × 10⁵ kBq mmol⁻¹) (96% purity by TLC, Novartis, Greensboro, NC)¹ and technical grade metolachlor (99% purity, Chem Service, West Chester, PA) was applied uniformly to the soil surface (1.25 mg metolachlor kg⁻¹ soil; 3.3 kBq radioactivity per sample). Soils were incubated in the dark at 25°C. Water was added periodically to compensate for evaporation. In Study II, evolved ¹⁴CO₂ (mineralized) was trapped in the side-arm of the biometer flask by means of 10 mL 1 M NaOH and quantified by liquid scintillation counting (LSC) (Packard TriCarb 4000 Series, Packard Instruments, Meriden, CT) using Hionic-Fluor (Packard, Meriden, CT) (Bartha and Palmer, 1965; Locke and Harper, 1991). To avoid saturation by CO₂, the NaOH was replaced on sampling days. Soils from both studies were destructively sampled at nine sampling points throughout the 46-d incubation. At each sampling time, soils were extracted with methanol (50 mL per extraction). Samples were shaken at room temperature (24 h for the first extraction, 2.5 h for the second). Bottles were centrifuged at 6000 × g for 10 min and the supernatants from each extraction were pooled. Radioactivity in the extracts was quantified by LSC by Ecolume (ICN, Costa Mesa, CA). After extraction, air-dried soils were combusted to measure nonextractable ¹⁴C (Oxidizer Model 306, Packard Instruments, Downers Grove, IL). Extract volumes were reduced with a rotary evaporator. Samples were diluted in 100 mL of ultra-pure water and acidified below pH 3 prior to passing through conditioned C₁₈ solid-phase extraction columns (J.T. Baker, Phillipsburg, NJ). Metolachlor and metabolites were eluted from the column with 3 mL of methanol. Aliquots (90-μL) were spotted on thin-layer chromatography (TLC) plates (20 by 20 cm, 250-μm silica gel, Whatman, Clifton, NJ). A hexane:ethyl acetate:methylene chloride (6:3:1) solvent system (Liu et al., 1989; Locke et al., 1996) was used to develop the plates to 10 cm, and they were analyzed with a Bioscan System 200 Imaging Scanner (Bioscan, Washington, DC). Metolachlor migrated with an R_f of approximately 0.71. The presence of the sulfonic acid metabolite of metolachlor (Novartis, Greensboro, NC) was corroborated by means of two additional solvent systems (butanol:acetic acid: water 12:3:5 and butanone:acetic acid: water 10:1:1, Lamoureux and Rusness, 1989). Each study consisted of duplicate samples from each of six field plots.

For sorption studies, 5-g (oven-dried equivalent) soil samples were added to 25-mL Corex centrifuge tubes with Teflon caps. Container controls were used to correct for sorption to tubes and caps (sorption was <0.25%). ¹⁴C-metolachlor solutions (597 kBq L⁻¹) were prepared in 0.01 M CaCl₂ with metolachlor concentrations of 0.85, 7.85, and 35.55 μmol L⁻¹. Ten milliliters of solution were added to each tube, and samples were shaken for 24 h. Samples were centrifuged (8000 × g) and two 1-mL subsamples of supernatant were counted by LSC as described previously. Desorption was performed under similar conditions (5 g soil, oven-dried equivalent).

¹ Mention of a product or manufacturer is for information only and does not constitute endorsement by the USDA.

lent; 10 mL of solution). The starting concentration was 15 $\mu\text{mol L}^{-1}$ with 1510 kBq L^{-1} . Tubes were shaken for 24 h, centrifuged ($8000 \times g$), after which 5 mL were removed and replaced with 5 mL fresh 0.01 M CaCl_2 . Two 1-mL subsamples of the supernatant were removed for counting. The desorption procedure was repeated three times. The sorption-desorption experiment included five replications and was repeated once.

Degradation and Sorption Models and Statistical Analysis

Degradation of chloroacetamide herbicides such as alachlor and metolachlor occurs primarily via cometabolism, thus there is little adaptation of microorganisms to facilitate biodegradation (Stampor and Tuovinen, 1998; Zablotowicz et al., 1998c). The disappearance of total extractable ^{14}C and metolachlor was evaluated by means of the power rate equation and assumptions considered by Hamaker, 1972.

$$C = [C_0^{(1-n)} + (n-1)kt]^{1/(1-n)} \quad [1]$$

$$\frac{-dc}{dt} = kC^n \quad [2]$$

where C is the concentration of extractable ^{14}C or metolachlor at time t , C_0 is the initial concentration of metolachlor, n is the order of reaction, and k is the rate constant.

Sorption of metolachlor to soils was described by the average distribution sorption coefficient for the three concentrations (Hamaker and Thompson, 1972; Green and Karickhoff, 1990; Cleveland, 1996). Desorption was described by the average distribution desorption coefficient for two concentrations.

$$S = K_d C \quad [3]$$

where S is the amount of metolachlor sorbed to the soil ($\mu\text{mol kg}^{-1}$), K_d is the distribution sorption (or desorption) coefficient, and C is concentration of metolachlor in solution at equilibrium ($\mu\text{mol L}^{-1}$).

Nonlinear regression analysis of Eq. [1] and [2], analysis of variance, and other statistical tests were performed by SAS ver. 4.10 (SAS Institute Inc., 2000). Degradation, sorption, and desorption studies were repeated, and after statistical analysis determined no differences between data sets, the data were pooled.

RESULTS AND DISCUSSION

Soil and Microbial Parameters

The surface (0–2 cm) soils from the VBS and BF had similar texture (silt loam) (Table 1). The VBS had more than twice as much OC and half a unit lower pH than the BF (1.17% vs. 0.54%; 4.8 and 5.3, respectively). Culturable populations of all microbial taxa examined were greater in the VBS than the BF (Table 2). Differences in total bacteria were likely due to gram-negative bacteria such as fluorescent pseudomonads, as was ob-

Table 1. Physical and chemical characteristics of vegetative buffer strip (VBS) and bare field (BF) surface (0–2 cm) soils.

Soil	Sand	Silt	Clay	Organic carbon	pH
			%		
VBS	12.1	62.1	25.8	1.17	4.8
BF	11.6	60.9	27.5	0.54	5.3

Table 2. Microbial populations associated with surface (0–2 cm) soil from vegetative buffer strip (VBS) and bare field (BF) areas.

Microorganism	VBS†	BF†	LSD‡
	Log (10) colony forming units kg^{-1}		
Total fungi	7.95	7.22	0.23
Total bacteria	11.12	10.25	0.16
Gram-negative bacteria	10.00	8.34	0.39
Fluorescent pseudomonads	9.23	7.07	0.38

† Values represent the average of six replicates.

‡ Across a row, differences are significant at Fisher LSD, $\alpha = 0.05$.

served in some cover crop soils (Wagner et al., 1995; Zablotowicz et al., 1998a).

Four endogenous enzyme activities were 1.9 to 3.8 fold higher in the VBS than the BF (Table 3). Overall, these enzyme assays are indicative of greater microbial activity in the VBS. The exception was exogenous dehydrogenase activity when the soil was supplemented with 0.1% (w/v) yeast extract (Table 3). The higher dehydrogenase activity for BF measured when the soil was supplemented with a carbon source suggests that the microbial populations in the BF were limited by carbon and/or nitrogen. Indeed, even though VBS contained more OC than BF, the BF would be expected to contain a lower proportion of decomposable C and a higher proportion of humified C than the VBS soils (Benoit et al., 1999). Similar dehydrogenase activity in VBS soils with and without yeast extract indicates that maximum activity for the indigenous microbial population occurs without enrichment, or perhaps by catabolite repression occurs from additional nutrients. Higher aryl acylamidase activity in the VBS is significant as this may indicate a greater potential for degradation of herbicides such as metolachlor, propanil [*N*-(3,4-dichlorophenyl)propanamide], and linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] (Zablotowicz et al., 1998b). FDA activity is a generic indicator of esterase activity and suggests a greater potential for herbicide ester bond cleavage in the VBS (Zablotowicz et al., 2000). Although alkaline phosphatase activity is considered to be less than acid phosphatase in acid soils (Tabatabai, 1994), alkaline phosphatase activity in the more acidic VBS soil was about twice that of the field soil. Alkaline phosphatase is produced solely by microorganisms,

Table 3. Enzyme activities in surface (0–2 cm) soils collected from vegetative buffer strip (VBS) and bare field (BF) areas.

Enzyme	VBS†	BF†	LSD‡
	Product formation $\mu\text{mol kg}^{-1} \text{ soil h}^{-1}$		
FDA§	653.4	172.6	157.6
TTC-dehydrogenase (no carbon source)¶	4.3	1.6	1.1
TTC-dehydrogenase (0.1% yeast extract)¶	5.6	18	5.7
Alkaline phosphatase	271	141	50
2-NAA-aryl acylamidase	25.5	8.6	3

† Values represent the average of six replicates.

‡ Across a row, differences are significant at Fisher LSD, $\alpha = 0.05$.

§ FDA = fluorescein diacetate hydrolysis.

¶ TTC = triphenyl-tetrazolium chloride dehydrogenase activity.

Table 4. Sorption partition coefficients K_d and K_{oc} derived for metolachlor sorption and desorption in soils collected from vegetative buffer strip (VBS) and bare field (BF) areas.

	Sorption $K_d^\dagger$ (s.e.)	K_{oc} (s.e.)	Desorption $K_d^\dagger$	% desorbed ‡
VBS	2.25*** (0.04)	192 (3.8)	3.09*** (0.17)	35.4
BF	1.60 (0.04)	295 (6.7)	2.59 (0.18)	38.0

† Parameter values within a column are significantly different ($P < 0.001$; F -test Hinds and Milliken, 1987).

‡ Expressed as a percentage of metolachlor sorbed.

while acid phosphatase is of both microbial and plant origin (Tabatabai, 1994).

Soil enzyme activity measured in these assays represents enzymes associated with living organisms, especially dehydrogenase activity. Other hydrolytic enzyme activity may also be associated with dormant or dead organisms or enzymes immobilized in clay-humus colloids (Burns, 1982). Nevertheless, increased levels of enzymatic activity observed in VBS soils agree with elevated microbial populations, suggesting the potential for enhanced herbicide degradation. Similar results were obtained by Benoit et al. (1999), who found greater CO_2 evolution in a grass buffer strip than in an adjacent field.

Sorption and Desorption

A higher sorption K_d value for the VBS soil indicates a greater capacity to sorb metolachlor (Table 4). The desorption K_d value for the VBS was moderately higher than the BF, indicating a greater equilibrium shift in favor of sorption for the VBS soil (Table 4). Again, this results from the larger sorption capacity in the VBS soil. Higher organic carbon in the VBS is one of the most significant factors in relation to greater sorption in these soils (Obrigawitch et al., 1981; Braverman et al., 1986; Wood et al., 1987; Locke, 1992; Miller et al., 1997). These results are consistent with the observations of Mersie et al. (1999) and support the hypothesis that enhanced metolachlor retention in VBS may reduce further mobility.

The K_{oc} values were higher for the BF soil than the VBS soil (Table 4). Calculating the K_{oc} can sometimes be used to adjust for the effect of organic carbon content on herbicide sorption. However, factors other than organic carbon content influence herbicide sorption, and may result in slightly inflated K_{oc} values. Differences in characteristics of the organic matter between the soils may influence the K_{oc} value (Benoit et al., 1999). Although total organic carbon content was higher in the VBS soil resulting in higher total sorption of metolachlor, a greater proportion of the organic carbon in the BF was likely more humified than that in the VBS soil. This may have caused greater affinity for metolachlor in BF even though capacity to sorb was not as large as VBS. Although OC is likely the primary sorbent (Miller et al., 1997), metolachlor sorption has also been correlated with clay content (Peter and Weber, 1985). Clay has less impact on sorption when the clay:OC ratio is less than 30 (Green and Karickhoff, 1990) as was the case with the VBS soils (clay:OC = 22:1). Therefore,

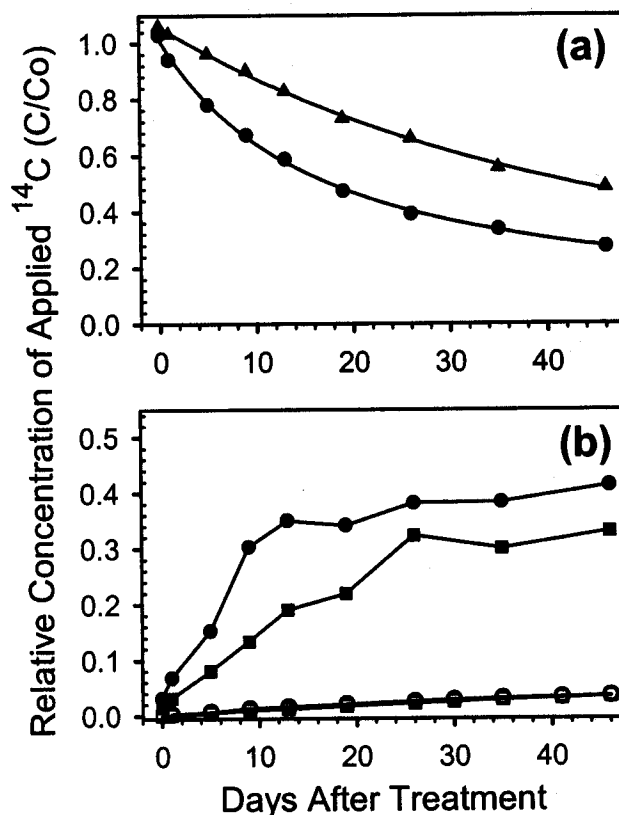


Fig. 1. Fate of ^{14}C during the course of the experiment. Results for extractable ^{14}C are shown in (a) with curves representing nonlinear regression fits of the first-order equation. Symbols for (a) are ● VBS extractable and ▲ BF extractable. The nonextractable and mineralized components are illustrated in (b). Symbols for (b) are ○ VBS Mineralized, □ BF Mineralized, ● VBS nonextractable, and ■ BF nonextractable.

the slightly higher clay to organic carbon ratio (52:1) of the BF may raise the apparent K_{oc} value.

Degradation and Metabolite Formation

Extractable ^{14}C decreased over the course of the experiment for both soils (Fig. 1a) and was described by a first-order model (Table 5). Half-lives for the extractable ^{14}C fraction were 18 and 38 d for the VBS and BF, respectively (Table 5). After 46 d of incubation, 28% of the initial ^{14}C could be extracted from the VBS soils

Table 5. Parameter values (C_0 = initial concentration; k = rate constant; n = order of reaction) describing the disappearance of methanol-extractable ^{14}C and metolachlor in soils collected from vegetative buffer strip (VBS) and bare field (BF) areas. Values were calculated with a first-order equation (Hamaker, 1972).

Parameter	Extractable ^{14}C		Metolachlor	
	VBS †	BF	VBS	BF
C_0	100 (2.16)	100 (0.51)	100 (0.88)	100 (0.80)
k	0.0217 (0.011)	0.0161 (0.008)	0.0269 (0.0046)	0.0220 (0.006)
N	1.14 (0.11)	1.03 (0.12)	1.23 (0.043)	1.07 (0.065)
Half-life (days)	18	38	10	23

† Values in parentheses are the asymptotic standard error for the parameter value.

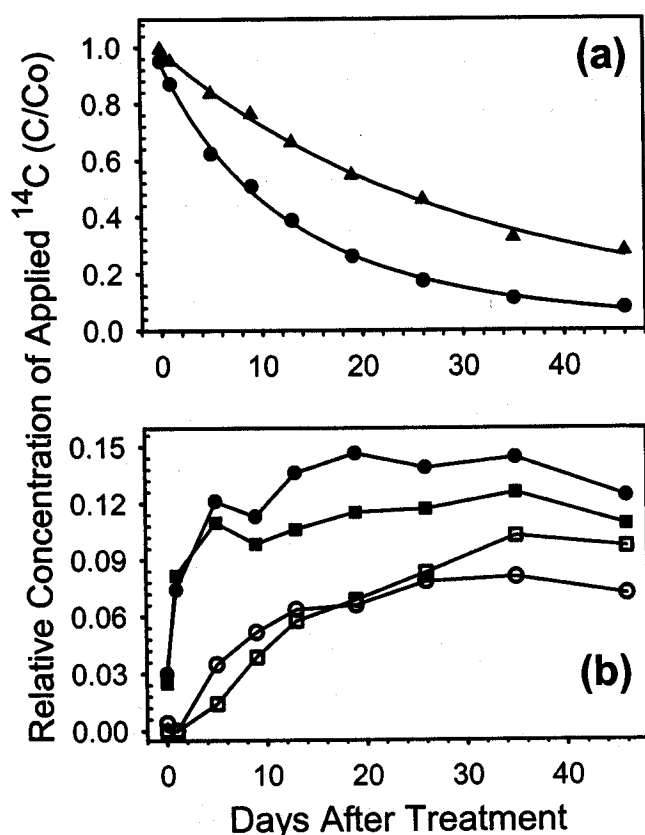


Fig. 2. TLC analysis of methanol extractable ^{14}C . Disappearance of metolachlor over time (a) with curves representing nonlinear regression fits of the first-order equation. Symbols for (a) are ● VBS metolachlor and ▲ BF metolachlor. Appearance of non-polar and polar metabolites are shown in (b). Symbols for (b) are ● VBS non-polar, ■ BF non-polar, ○ VBS polar, and □ BF polar.

while 48% was recovered from the BF soils. Conversely, nonextractable ^{14}C increased with time and was significantly higher for the VBS ($\text{LSD } \alpha = 0.05$) after Day 1 with the exception of Day 26 (Fig. 1b). The extractable-bound results are consistent with the sorption experiment and likely reflect the higher levels of organic matter and microbial activity in the VBS soils. Total CO_2 evolution did not differ between VBS and BF, and did not account for more than 4% of the total ^{14}C for either soil (Fig. 1b). Limited mineralization of metolachlor has been observed by others (Levanon et al., 1994; Miller et al., 1997; Stampor and Tuovinen, 1998) including in rhizosphere soils (Anderson and Coats, 1995). Locke et al. (1996) also found low rates of mineralization for another chloroacetamide, alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide]. In contrast to our mineralization results, Benoit et al. (1999) found that ^{14}C - CO_2 release from isoproturon was dramatically higher in grass buffer soils (15% in upper 2 cm soil) than those from an adjacent field (2%) suggesting more complete mineralization in the former. Metolachlor has double *ortho* substitution in the aniline ring and *N*-alkyl substitution that hinders mineralization of the aniline ring (Villareal et al., 1994).

A mass balance of ^{14}C recovered ranged from 82 to 100% (average s.d. 1.88) in the BF samples and from

71 to 100% (average s.d. 1.45) in VBS. Although greater than 93% was recovered during the first 13 d of incubation, lower ^{14}C recovery was obtained for both treatments as the incubation progressed. Volatilization can be an important dissipation mechanism for metolachlor, ranging from 6 to 22% in one field study, with greater losses in plant-residue managed soils (Prueger et al., 1999). Metolachlor volatilization was not measured in the present study, but may account for some of the discrepancy in ^{14}C recovery, especially in VBS.

TLC analysis indicated that most of the extracted ^{14}C was metolachlor during the first 17 d of incubation (Fig. 2a). More metolachlor ($\text{LSD } \alpha = 0.05$) was extracted from the BF soils throughout the course of the experiment. First-order kinetics described metolachlor degradation for both soils, and the half-lives were 10 and 23 d, respectively, for the VBS and BF (Table 5). At the end of the experiment, 7% of initial metolachlor remained extractable in the VBS while 26% could still be recovered from the BF. More metolachlor was extracted from the BF soils on and after Day 5. For BF, metolachlor was the primary constituent of the extracts throughout the study period (Fig. 2). In the VBS, metolachlor was the dominant compound in the extract until Day 26 when the amount of metabolites was equivalent to or greater than metolachlor (Fig. 2). Significantly more nonpolar metabolites (those which migrated between 0.2 to 0.6 in the hexane:ethyl acetate:methylene chloride solvent) were isolated from the VBS soils on Days 13, 19, and 26. Polar compounds (those which remained at the origin of the TLC plate) were similar for both soils during most of the incubation period, but more polar compounds were recovered from the BF soils during the last 2 wk (Fig. 2b). This may be due to slower degradation rates in the BF soils, resulting in an accumulation of non-polar compounds. Combining polar and nonpolar values revealed significantly higher metabolite accumulation early in the incubation (Days 5, 9, 13, and 19) from VBS soil. The presence of the sulfonic acid metabolite was confirmed using the two polar solvent systems in several samples from Day 19. Sulfonic acid comprised between 26 and 60% of the total polar metabolites in these later samples. Studies by Locke et al. (1996) indicated that sulfonic acid was the major metabolite of the chloroacetamide alachlor during degradation in soil. Polar metabolites of the chloroacetamides may also result from glutathione conjugation mediated by gram-negative bacteria (Zablotowicz et al., 1994). Overall, our results are similar to those of Anderson et al. (1994) who found enhanced degradation of metolachlor in rhizosphere soils. Further, Mersie et al. (1999) found little metolachlor in leachate from a tilted-bed runoff experiment, suggesting that degradation, sorption, or both had taken place.

Together, the results indicate a greater potential for retention (binding) and microbial degradation of metolachlor in the vegetative buffer soil due to increased sorption capacity and higher microbial activity in the VBS soils. Enhanced metabolite formation may increase retention of metabolites by facilitating oxidative reduc-

tive binding mechanisms to the soil components (Hsu and Bartha, 1974; Bollag et al., 1986; Miller et al., 1997). Comparable results were observed by Locke et al. (1996) when contrasting the fate of alachlor in no-tillage (NT) and conventionally tilled soils. Enhanced alachlor sorption was noted in the NT soils and thought to be due to higher levels of organic residues. This study only examined soils from the vegetated area and did not take into consideration potential effects of vegetative material in retaining and degrading herbicides (Zablotowicz et al., 1998b). Sorption of metolachlor to the plant material should further reduce availability (Arora et al., 1996; Misra et al., 1996). Overall, the greater microbial activity and sorption in the VBS should reduce the amount of metolachlor in surface runoff or leaching (Levanon et al., 1994). Additional leaching studies of VBS, such as those by Mersie et al. (1999), should be performed to test these observations further.

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