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Tillage and cover effects on soil microbial properties and fluometuron degradation

Robert M. Zablotowicz · Martin A. Locke · Lewis A. Gaston

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Abstract This research concerns the influence of no tillage (NT) or conventional tillage (CT) and a ryegrass (*Lolium multiforum* Lam.) cover crop in a cotton (*Gossypium hirsutum* L.) production system on soil and ryegrass microbial counts, enzyme activities, and fluometuron degradation. Fluorescein diacetate hydrolysis, aryl acylamidase, and colony-forming units (CFUs) of total bacteria and fungi, gram-negative bacteria, and fluorescent pseudomonads were determined in soil and ryegrass samples used in the degradation study. Fluometuron (14C-labelled herbicide) degradation was evaluated in the laboratory using soil and ryegrass. The CT and NT plots with a ryegrass cover crop maintained greater microbial populations in the upper 2 cm compared to their respective no-cover soils, and CT soils with ryegrass maintained greater bacterial and fungal CFUs in the 2–10 cm depth compared to the other soils The highest enzymatic activity was found in the 0–2 cm depth of soils with ryegrass compared to their respective soils without ryegrass. Ryegrass residues under NT maintained several hundred-fold greater CFUs than the respective underlying surface soils. Fluometuron degradation in soil and ryegrass residues proceeded through sequential demethylation and incorporation of residues into nonextractable components. The most rapid degradation was observed in surface (0 to 2 cm) soil from CT and NT–ryegrass plots. However, degradation occurred more rapidly in CT compared to NT soils in the 2 to 10 cm depth. Ryegrass cover crop systems, under NT or incorporated under CT, stimulated microbiological soil properties and promoted herbicide degradation in surface soils.

Keywords Cover crop · Enzymatic activity · Herbicide metabolism · Microbial populations · No tillage · Ryegrass

R. M. Zablotowicz (✉)
USDA-ARS Southern Weed Science Research Unit,
Stoneville, MS 38776, USA
e-mail: rzablotowicz@ars.usda.gov

M. A. Locke
USDA-ARS, Water Quality & Ecology Research Unit,
Oxford, MS 38655, USA

L. A. Gaston
Department of Agronomy LSU Ag Center,
Louisiana State University,
Baton Rouge, LA 70803, USA

Introduction

The adoption of conservation management systems [no tillage (NT) and cover crops] can have beneficial effects on soil chemical, physical, and biological properties that mitigate erosion and promote sustainability (Locke and Bryson 1997; Locke et al. 2002a). Under conventional tillage (CT), plant residues are completely incorporated into the upper soil layer, whereas under NT systems, plant residues are maintained on the soil surface. 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 (Reeves 1997; Locke et al. 2006). Plant residue accumulation on the soil surface fosters higher levels of organic matter in the surface soil, promotes better fertility, improves water infiltration, and maintains lower soil temperatures. The sowing of cover crops in the fall may be integrated as part of an NT cropping system where vegetative biomass is killed by contact herbicide before planting. Under CT, the biomass may be incorporated into the soil as a green manure crop before planting. Cover crop residues remaining on the

surface can reduce evaporation, thus conserve soil moisture, lower soil surface temperatures, provide a certain degree of weed control, and minimize erosion (Liebl et al. 1992). However, crop residues remaining on the soil surface can intercept and bind herbicides, making the herbicide less available for weed control (Banks and Robinson 1986) and/or alter herbicide fate (Locke and Zablotowicz 2003; Locke et al. 2006).

Using NT and/or cover crop systems can alter enzymatic activity (Bandick and Dick 1999; Dick 1994), microbial biomass (Linn and Doran 1984; Wagner et al. 1995; Kirchner et al. 2003; Zablotowicz et al. 1998a), microbial community structure (Lupwayi et al. 1998; Feng et al. 2003), and macroflora diversity (Gaston et al. 2003; Reeleder et al. 2006). Most herbicide transformations in soil are mediated by microbial metabolism; thus modification of the soil environment and microbial populations by reduced tillage and/or cover crops can affect herbicide fate (Levanon et al. 1994; Locke and Zablotowicz 2003). In some studies, fluometuron degradation was lower under NT compared to CT conditions (Brown et al. 1994; Zablotowicz et al. 2000a). Metolachlor degraded more rapidly in soil from a vegetative filter strip compared to soil under continuous cotton (*Gossypium hirsutum* L.) production (Staddon et al. 2001), and this was attributed to higher microbial populations and activity in the filter-strip soil.

The phenylurea herbicide fluometuron is used for cotton production in the southeastern area of the USA and is soil-applied pre- or post-emergence control of annual grass and broadleaf weeds (Vencill 2002). Under these field conditions, half-lives of fluometuron dissipation in surface soil ranged from 9 to 38 days (Brown et al. 1996; Willian et al. 1997; Locke et al. 2005), whereas the respective half-lives under laboratory conditions ranged from 11 to 90 days (Brown et al. 1994; Willian et al. 1997; Zablotowicz et al. 2000a). Fluometuron degradation in soil typically proceeds by sequential demethylation followed by incorporation of the trifluoromethylaniline (TFMA) ring moiety into non-extractable soil components (Ross and Tweedy 1973; Zablotowicz et al. 2000a).

It is well established that degradation of fluometuron in soil can depend on enzyme activities, microbiological biomass and structure of soil and ryegrass residues. The present study was conducted to characterize counts of culturable propagules of the soil microbiological community under different tillage and ryegrass cover crop systems and evaluate the potential for fluometuron degradation under controlled conditions. Fluorescein diacetate (FDA) hydrolysis was measured because it is related to general total microbiological activity (Schnürer and Rosswall 1982), whereas aryl acylamidase activity was determined because it is involved in the cleavage of the amide bond of various acylamide and phenyl urea herbicides. Conservation management cropping systems (NT and cover crops) are becoming more readily adopted by North American growers with the widespread acceptance of herbicide resistant crops. Thus, information on the effects of NT practices and a ryegrass (*Lolium multiforum* Lam.) cover crop on microbial processes may provide complementary insight for interpreting the field persistence of fluometuron in studies (Locke et al. 2005) and in assessment of benefits of conservation management practices on soil quality and herbicide persistence.

Materials and methods

Site and field experiment

Soil used for these experiments was collected from a long-term field study conducted at the US Department of Agriculture-Agricultural Research Service Southern Weed Science Research Unit Experimental farm, Stoneville, MS. The soil was a Dundee silt loam (fine silty, mixed thermic, Aeric Ochraqualf: 18% sand, 61% silt, 21% clay, slightly acidic pH 5.7). A randomized complete block experiment of four treatments and four replicates, consisting of two tillage systems, NT and CT, each with and without a ryegrass cover crop, was used. Tillage treatments were initiated in 1990 and were maintained in the same plots throughout the study period. CT was performed each fall with three passes with an off-set disk-harrow (15 cm depth) followed by a single pass with a field cultivator (6 cm depth). In the fall of 1993, the plots were further split, with annual ryegrass planted on 12 rows of each plot. About 1 month before cotton planting, all plots received 1.1 kg ha^{-1} of glyphosate and paraquat to kill the ryegrass and spring vegetation. Two weeks after post-emergence applications, CT plots were subsoiled, disked twice, and beds formed; CT plots were cultivated another three times during the growing season. Additional management practices are presented elsewhere (Locke et al. 2005).

A composite of eight soil samples were randomly collected from the soil surface (0–2 and 2–10 cm) of each plot using a tulip bulb planter (7.5 cm diameter) 1 day before planting and herbicide application. Ryegrass residues on the surface of NT plots were collected at the same times as soil sampling. Soil samples were sieved (<2 mm) and stored moist in the dark at 5°C until analysis. Ryegrass residues were cut into 2-cm sections to facilitate enzyme assays, microbial enumeration, and degradation studies. Counts of microbial populations were assayed within 1 day of sample collection and enzyme activities within 2 to 5 days after collection. Moisture content of soils and ryegrass residues were determined after drying for 3 days at 70°C.

Microbial counts

Estimates of culturable soil microbial propagules (colony-forming units, CFUs) were determined in soil samples by serial dilutions and spiral plating (Spiral System Instruments, Bethesda, MD) on selective and semi-selective growth media, as described previously (Zablotowicz et al. 1998a; Staddon et al. 2001). Ryegrass residues (1.0 g) were homogenized in phosphate buffer (100 ml) using a sterile blender and serially diluted. Total heterotrophic bacteria, gram-negative bacteria, and fluorescent pseudomonads were enumerated on 10% tryptic soy agar containing 100 mg cycloheximide l^{-1}, 10% tryptic soy agar with crystal violet (5 mg l^{-1}), and cycloheximide (100 mg l^{-1}) and S1 media (Gould et al. 1985), respectively. Total fungal 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 days of incubation at 28°C, respectively. Microbial propagule densities were expressed as $\log_{10}$ CFU g^{-1} soil or ryegrass (oven-dry weight).

Enzyme activity

Fluorescein diacetate activity was determined by the method of Schnürer and Rosswall (1982) as modified by Zablotowicz et al. (2000b). Aryl acylamidase assays were conducted using 2-nitroacetanilide as substrate according to Zablotowicz et al. (1998b), with incubations at 28°C. Enzyme activities in ryegrass were conducted on 1.0 g of tissue, soil FDA hydrolysis on 2.0 g of soil and soil aryl acylamidase on 1.0 g of soil. All enzyme assays were triplicated using controls without substrate to correct for background. Enzyme activity is reported as nmol of product formed g^{-1} air-dried soil or ryegrass h^{-1}.

Fluometuron degradation

The potential for fluometuron degradation was studied in ryegrass residues and soils from both depths using methods described elsewhere (Zablotowicz et al. 1998a, 2000a). Ryegrass residues and soils for these studies were from samples collected at the time of planting but before fluometuron application. A preliminary mineralization study indicated that less than 3% of the fluometuron added was evolved as CO_2 during a 25-day incubation (data not shown); thus, extractions were only performed at selected intervals. Soils from all replicate plots were composited, and moisture content was determined. Soil (5.0 g oven-dried equivalent) was placed in centrifuge tubes (25-ml screw cap); fluometuron solution, containing a mixture of ^{14}C-ring labeled fluometuron (Uniformly labeled ring ^{14}C-fluometuron was a gift of Novartis, Greensboro, NC) and technical grade fluometuron (ChemService, West Chester, PA), was added to attain 840 Bq g^{-1} and 9.7 μmol kg^{-1} soil, and the soil moisture content was adjusted to 32% on a dry weight basis. Ryegrass residues (2.0 g air-dried weight) were treated to attain a similar radioactivity and fluometuron concentration, but the moisture content was adjusted to 100% (weight basis), as this enabled uniform distribution of the herbicide and provided more ideal conditions for fluometuron degradation in the laboratory microcosms (Zablotowicz et al. 1998a). After treatment, the tubes were lightly capped to promote aeration and were incubated in the dark at 28°C. Three replicate tubes of each soil treatment were sampled after 0, 4, 7, 11, 18, or 25 days. Three replicate tubes of treated ryegrass residues were sampled at 0, 4, 7, 11, or 17 days.

Fluometuron and metabolites were recovered from residues and soils with four 15-ml methanol extractions (extraction efficiency=99%). Radioactivity recovered in each methanol extract was determined using a Liquid Scintillation Counter (LSC, Model Packard TriCarb 4,000 series, Packard Instruments, Meriden, CT) with Ecolume scintillation cocktail (ICN, Costa Mesa, CA). The first two extracts were combined and reduced to 3 ml under N_2 gas. The parent herbicide and metabolites were quantified by thin layer chromatography (TLC) and linear image scanning (Bioscan Imaging System 200, Bioscan Washington, DC) using chloroform/ethanol (95:5, *v*/*v*) as solvent and 250 μm non-fluorescent silica gel TLC plates (J.T. Baker, Phillipsburg, NJ) as described elsewhere (Ross and Tweedy 1973; Zablotowicz et al. 1998a and 2000a). Rf values for standards were 0.58 fluometuron, 0.30 desmethyl fluometuron (DMF), 0.13 trifluoromethylphenylurea (TFMPU), and 0.76 trifluoromethylaniline (TFMA). Fluometuron metabolites DMF, TFMPU, and TFMA were supplied by Novartis (Greensboro, NC). Nonextractable radioactivity was determined by oxidation of soil residues after extraction. Duplicate 300-mg sub-samples of air-dried soils were mixed with 200 mg of cellulose in cellulose thimbles and combusted in a biological oxidizer (Packard TriCarb Oxidizer 306, Packard Instruments, Meridian, CT). Released $^{14}CO_2$ was trapped in a mixture of Carbo-Sorb E and Permafluor E scintillation cocktail (Packard Instrument, Meriden, CT) and was counted by LSC.

Statistical analysis

Soil chemical properties, CFUs, and enzyme activities were subjected to analysis of variance using the general linear model procedure in SAS (SAS Institute 2001). Means were separated using Fisher's protected least significant difference test at P=0.05. The fluometuron degradation data were fit to first-order kinetics using non-linear procedure (SAS, Cary, NC). The first-order degradation rate constant

(K) and the 95% confidence interval were calculated using the NLIN procedure, and the half-life ($T_{1/2}$) was calculated from K. The first-order degradation rate constant corrected for solution concentrations (K_s) based on sorption K_d was determined according to Zablotowicz et al. (2000a).

Results and discussion

Soil characteristics

Soil chemical characteristics from the pre-plant sampling (Table 1) are summarized from data presented elsewhere (Locke et al. 2005). Surface 0–2 cm soils from NT–no-ryegrass plots had 74% greater soil organic C content compared to soils from CT–no-ryegrass plots at the time of planting (Table 1). The highest organic C content was observed in surface 0–2 cm soil from NT–ryegrass plots, which was 112% greater than that from CT–ryegrass plots at the corresponding depth. In the 2–10 cm depth, soil organic C was greater in CT–ryegrass plots compared to the other treatments. Accumulation of organic C under NT in the Dundee silt loam is limited to the surface soil as has been reported elsewhere under longer duration of the NT practice (Zablotowicz et al. 2000a), and increases in soil organic C due to cover crop residues are of a magnitude similar to that reported in a cotton NT system under balansa clover (*Trifolium balansae*; Locke et al. 2002b). Both NT and use of a ryegrass cover crop decreased soil pH in both depths, with the lowest pH associated with soil from NT–ryegrass plots. K_d values of fluometuron sorption correlated with organic C content (r=0.96, Pr>0.01).

Microbial counts

The effects of tillage and a ryegrass cover crop on counts of culturable soil microorganisms are summarized in Table 2. Although plate counts may estimate less than 10% of the total propagule density of various soil microflora, differences in CFU among tillage and cover crop treatments, especially in surface soil, reflect a stimulation of soil microflora in response to these management practices. The ryegrass cover crop residues had a greater effect on CFU of various microflora than did tillage practice. The highest estimates of CFU total heterotrophic bacteria, gram-negative bacteria, fluorescent pseudomonads, and total fungi were generally in the surface 0–2 cm of NT and CT soils under herbicide-desiccated ryegrass residues (Table 2). In the lower 2–10 cm depth, increases in microbial counts were observed to a greater extent under CT–ryegrass compared to soil from other treatments.

The greatest enhancement of soil bacterial counts due to ryegrass cover crop was with gram-negative bacteria, e.g., fluorescent pseudomonads. Increases in total bacteria numbers attributable to ryegrass were in the order of $\log_{10}$ 0.36 to 0.52 CFU g^{-1} soil for NT and CT respectively, while increases in gram-negative bacteria were $\log_{10}$ 1.07 and 0.87 CFU g^{-1} comparing CT–ryegrass plots to those without ryegrass in the 0–2 and 2–10 cm depths, respectively. Maintaining cover crop residues on the surface (NT) or incorporation (CT) provides a stimulating substrate for microbial growth. The microbial counts associated with the surface 0–2 cm of soils from NT–ryegrass plots were similar to those reported in previous studies assessing the effects of ryegrass, hairy vetch (*Vicia villosa*), and rye (*Secale cereale* L.) cover crops on microbial populations in a similar Dundee soil (Wagner et al. 1995; Zablotowicz et al. 1998a). In an Alabama cotton production system, higher total phospholipids (PFLA) were recovered from long-term NT soils compared to CT soils (Feng et al. 2003). In addition, altered PFLA profiles were observed in NT soils indicating changes in gram-positive-to-gram-negative bacteria and fungal-to-bacteria ratios, demonstrating that NT practices can increase the microbial biomass and change community

Table 1 Organic C content, pH, and fluometuron sorption (linearized K_d) of a Dundee silt loam soil, as affected by tillage and ryegrass cover crop

Soil	Total organic C (g C kg^{-1})	pH	Fluometuron K_d (μmol kg^{-1})
0–2 cm			
NT–ryegrass	21.4 a[b]	5.15 d	5.04 (0.04)[c]
CT–ryegrass	10.1 c	5.73 c	2.07 (0.02)
NT–none	14.9 b	6.03 b	2.39 (0.04)
CT–none	9.4 c	6.25 a	1.65 (0.03)
2–10 cm			
NT–ryegrass	5.9 b	5.33 c	1.44 (0.03)
CT–ryegrass	6.8 a	5.45 c	2.01 (0.03)
NT–none	5.7 b	5.78 b	1.61 (0.04)
CT–none	6.0 b	6.06 a	1.81 (0.03)

[a] From Locke et al. (2005)
[b] Means followed by the same letter within a column for a given depth do not differ significantly at the 95% confidence level.
[c] Standard deviation

Table 2 Colony-forming units (CFU) enumerated from ryegrass and a Dundee silt loam soil as affected by tillage and ryegrass cover crop in 1995

Soil	Colony forming units $\log_{10}$ g^{-1} soil			
	Total heterotrophic bacteria	Gram-negative bacteria	Fluorescent pseudomonads	Total fungi
Ryegrass	10.77[a]	10.03	7.96	7.53
Soil 0–2 cm				
NT–ryegrass	8.38 a[b]	7.04 a	5.92 a	5.22 a
CT–ryegrass	8.28 a	7.07 a	5.90 a	5.17 a
NT–none	8.02 b	6.62 b	5.40 b	4.99 a
CT–none	7.76 c	6.00c	5.06 b	4.59 b
Soil 2–10 cm				
NT–ryegrass	7.61 bc	6.43 b	5.46 b	4.48 b
CT–ryegrass	8.27 a	6.99 a	6.17 a	5.21 a
NT–none	7.56 c	6.20 c	5.10 b	4.27 b
CT–none	7.74 b	6.12 c	5.31 b	4.58 b

[a] Mean of four replicates not statistically analyzed with soil data
[b] Means followed by the same letter within a column for a given sample do not differ significantly at the 95% confidence level.

structure. Microbial counts of ryegrass residues from the surface of NT plots were several hundred-fold greater than those from the surface 0–2 cm of soil from NT–ryegrass plots (Table 3). Similar values were reported in other studies evaluating the effects of rye or hairy vetch in a soybean production system (Zablotowicz et al. 1998a; Wagner et al. 1995). Most heterotrophic bacteria enumerated in this study utilize the readily available substrates such as simple sugars and amino acids, while many fungi can utilize more complex plant constituents, e.g., cellulose, hemicellulose, and lignin. Fungal colonization is important in crop residue degradation, and many of these fungi, i.e., *Rhizopus japonicum* and *Cunninghamella echinulata*, may also produce cytochrome P-450 eznymes that demethylate phenylurea herbicides (Wallnofer et al. 1973; Tillimans et al. 1976).

Soil enzymatic activity

The highest soil enzymatic activities were found in surface soils sampled from NT–ryegrass plots (Table 3); CT–ryegrass and NT–no-ryegrass plots had higher activity than surface CT with no cover. In the lower 2–10 cm depth, the highest FDA activity was observed in CT–ryegrass plots. Both enzyme activities were over two- to eleven-fold higher in ryegrass residues than that measured in any surface soil. FDA hydrolytic activity and respiration in a NT Gigger silt loam surface soil (0 to 3 cm) were 84 and 38% greater than that in CT, respectively, (Gaston et al. 2003), but neither wheat nor hairy vetch cover crops had much effect on FDA hydrolytic activity. These soil assays assess activities of enzymes associated with living cells and those present in microbial debris or humic acid-clay complexes (Burns 1982). Aryl acylamidases are hydrolytic enzymes that can cleave the amide bond of various acylamide carbamate and phenyl urea herbicides (Hoagland and Zablotowicz 2000). Thus, both FDA-hydrolysis and 2-nitroacetanilide aryl acylamidase activity can be related to hydrolytic reactions involved in the degradation of certain herbicides (Zablotowicz et al. 1998a,b, 2000b).

Fluometuron degradation

A more rapid rate of fluometuron degradation was observed in the surface 0–2 cm NT and CT soils beneath ryegrass and

Table 3 Hydrolytic enzyme activities of a Dundee silt loam as affected by soil depth, tillage, and ryegrass

Tillage cover crop	Fluorescein diacetate hydrolysis (fluorescein formed nmol $g^{-1}h^{-1}$)		Aryl Acylamidase (2-nitroaniline formed nmol g^{-1} h^{-1})	
	0 to 2 cm	2 to 10 cm	0 to 2 cm	2 to 10 cm
Ryegrass	3400[a]		105[a]	
NT–ryegrass	1509 a[b]	402 b	41.4 a	19.4 ab
CT–ryegrass	974 b	895 a	19.0 c	24.1 a
NT–none	809 b	305 b	26.6 b	14.2 b
CT–none	404 c	455 b	9.4 d	12.3 b

[a] Mean of four replicates not analyzed statistically with soil data
[b] Means followed by the same letter within a column for a given sample do not differ significantly at the 95% confidence level.

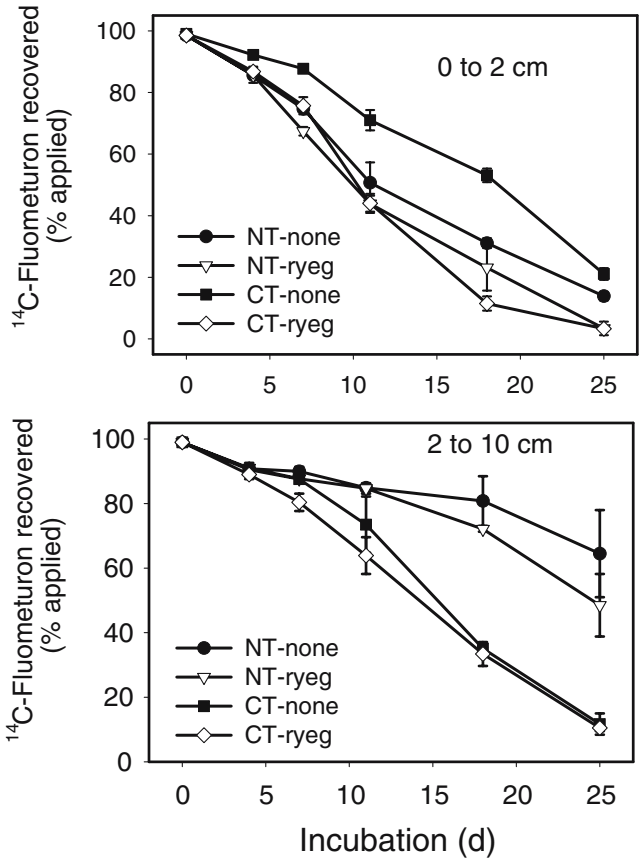

Fig. 1 Effect of tillage, ryegrass cover crop, and soil depth on the recovery of methanol-extractable ^{14}C-labeled fluometuron during a 25-day laboratory incubation study. Values are mean and standard deviation of three replicates

for NT soil without ryegrass compared to CT soils without ryegrass (Fig. 1). Fluometuron degradation was adequately modeled using a first-order equation (Table 4). Calculated half-lives were similar in surface 0–2 cm NT and CT–ryegrass soil (9.4 and 9.2 days, respectively), intermediate in NT–no-ryegrass soil (11.4 days), and greatest in CT–no-ryegrass soil (14.5 days; Table 4). In another study assessing the interaction of tillage and a hairy vetch cover crop on fluometuron degradation in a Lexington silt loam, Brown et al. (1994) indicated that the half-lives in soils sampled from the 0–4 cm depth of CT and NT soils with a hairy vetch cover crop were 71 and 78 days, respectively, while the half lives in CT and NT soils from the same depth without a cover crop were 49 and 57 days, respectively. The greater persistence of fluometuron in that study was attributed to decreased fluometuron bioavailability at lower soil pH. In the current study, soil pH was lowest under a NT–ryegrass system, but fluometuron degradation rates were not decreased. In the 0–2 cm layer from the companion field evaluations (Locke et al. 1995), the relative order of fluometuron dissipation was NT+ryegrass> NT+none>CT+ryegrass>CT+none (the NT+ryegrass did not follow first-order kinetics, as there was interception and release from ryegrass residues). In another NT system with a greater accumulation of organic matter and a greater fluometuron K_d (Zablotowicz et al. 2000a), it was proposed that limited herbicide bioavailability due to sorption decreased fluometuron degradation in surface soil, i.e., the portion of fluometuron sorbed was less subject to degrada-

Table 4 Fluometuron degradation rate constants (K), calculated half-lives ($T_{1/2}$), and degradation rate constants corrected for fluometuron in solution and calculated half-life ($T_{corr\ 1/2}$) in ryegrass residues and soil as affected by tillage, ryegrass cover crop, and soil depth

Tillage–cover crop	Depth (cm)	K[a] (day^{-1})	C.L.[b] (day^{-1})	$T_{1/2}$ (days)	K (corrected for solution phase fluometuron)	$T_{1/2}$ (corrected for solution phase fluometuron)
Ryegrass	Surface	0.0817	0.0717, 0.0917	8.5	0.6563	1.06
NT–ryegrass	0–2	0.0736	0.0630, 0.0841	9.4	1.309	0.52
CT–ryegrass	0–2	0.0752	0.0591, 0.0913	9.2	0.6746	1.02
NT–none	0–2	0.0605	0.0532, 0.0677	11.5	0.5422	1.28
CT–none	0–2	0.0478	0.0390, 0.0566	14.5	0.3105	2.23
NT–ryegrass	2–10	0.0227	0.0193, 0.0262	30.5	0.132	5.25
CT–ryegrass	2–10	0.0525	0.0431, 0.0613	13.2	0.4042	1.71
NT–none	2–10	0.0137	0.0100, 0.0173	50.6	0.0869	7.97
CT–none	2–10	0.0533	0.0356, 0.0580	13.0	0.2980	2.32

[a] First-order rate constant
[b] Lower and upper limits of 95% confidence limits

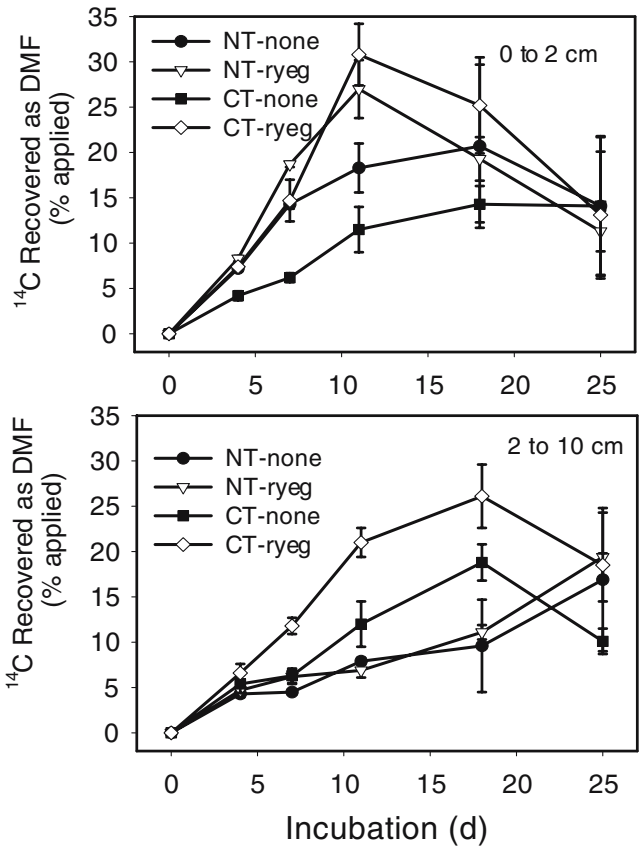

Fig. 2 Effect of tillage, ryegrass cover crop, and soil depth on the percentage of fluometuron present as methanol-extractable ^{14}C-desmethyl fluometuron (DMF) during a 25-day laboratory incubation study. Values are mean and standard deviation of three replicates

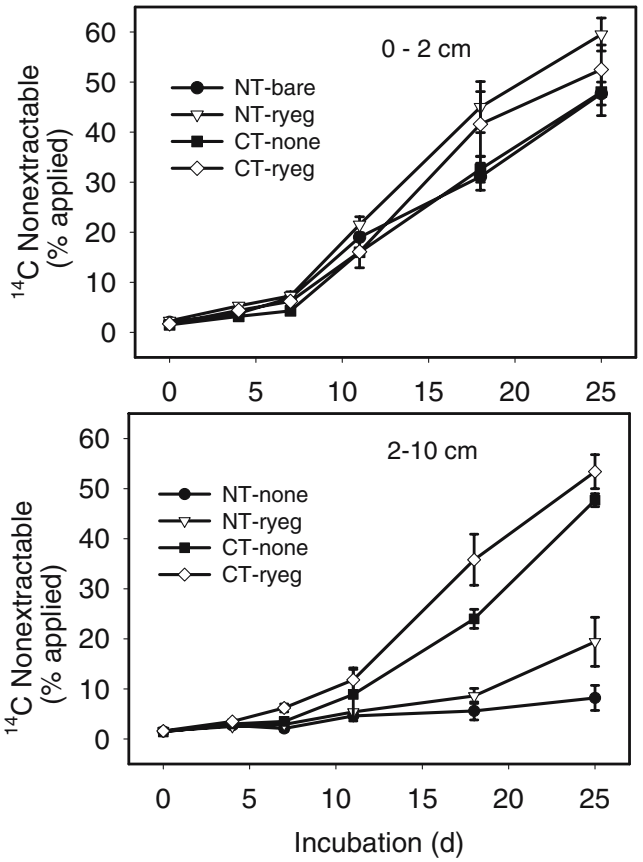

Fig. 3 Effect of tillage, ryegrass cover crop, and soil depth on the percentage of fluometuron present as nonextractable ^{14}C residues as determined by oxidation. Values are mean and standard deviation of three replicates

tion than that in solution. For the present study, the highest fluometuron K_d (6.04) was in the surface NT + ryegrass soil, where fluometuron degradation proceeded. When the degradation constants were calculated based on amount of fluometuron in solution, the relative degradation rates are NT–ryegrass>CT–ryegrass=ryegrass=NT–none>CT–none in the surface 0–2 cm soil. This was similar to the order obtained without adjusting for sorption, although degradation rates were dramatically enhanced (Table 4), as solution fluometuron was the basis for calculations. Although higher enzymatic activity was found in ryegrass residues and NT–ryegrass 0–2 cm soils compared to CT–ryegrass 0–2 cm soils, a similar fluometuron half-life was observed in these three systems. However, when degradation rate is corrected for sorption, differences in enzyme activity and degradation agree better for soil. In the 2–10 cm depth, fluometuron degraded more rapidly in CT soils compared to NT soils, and only a minimal effect of ryegrass was observed. Half-lives were similar in CT–ryegrass and CT–no-ryegrass soils (13.2 and 13.0 days) but lower than NT–ryegrass soil (44 days) and NT–no-ryegrass soil (73 days) in the 2–10 cm depth. The slower rate of fluometuron degradation observed in the lower 2–10 cm depths of NT soils compared to CT soils in the current study, even when based on fluometuron in solution, agreed with other studies (Zablotowicz et al. 2000a,b). This may be attributed to lower microbiological activity and availability of organic substrates

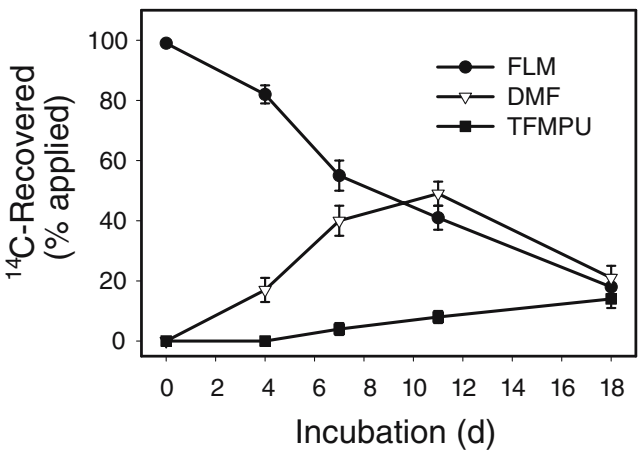

Fig. 4 Recovery of ^{14}C-fluometuron and metabolites by methanol extraction from ryegrass residues during an 18-day laboratory incubation study. Values are mean and standard deviation of three replicates

to support co-metabolic processes of fluometuron degradation (e.g., *N*-dealkylation).

In the 0–2 cm soils, greater DMF accumulation was observed in NT soils and CT with ryegrass compared to CT soils with the highest accumulation at day 11, declining thereafter (Fig. 2a). Patterns observed in the present study are similar to those in the companion field study (Locke et al. 2005), where soils associated with cover crop residues tended to enhance occurrence of DMF. However, appearance of DMF in the current laboratory study occurred slightly earlier than in the field studies. In soils from the 2–10 cm depth, the greatest and most rapid DMF accumulation was observed in CT–ryegrass soils (Fig. 2b). A low amount of DMF accumulation was observed in NT soils corresponding with a slow rate of fluometuron dissipation.

Accumulation of the ^{14}C-ring into nonextractable soil components was greatest in NT and CT soils from ryegrass in the 0–2 cm soil depth (Fig. 3a), while in the lower 2–10 cm depth, significantly greater incorporation of the ^{14}C-ring into nonextractable soil components was observed in CT soils. The greatest level of TFMPU was observed in surface CT and NT soils from ryegrass plots (4 to 6% of applied) with less than 2% recovered in NT and CT soils without ryegrass (data not shown). A mass balance of recovery of ^{14}C-applied of 76 to 90% was observed.

Fluometuron degradation in ryegrass residues followed a linear kinetics (Fig. 4), with extractable fluometuron decreasing more rapidly than in soil, consistent with results from the companion field study (Locke et al. 2005). Concurrent with a decrease in methanol-extractable fluometuron was an accumulation of both *N*-dealkylated metabolites (DMF and TFMPU). A maximum accumulation of total metabolites was found at 11 days, with subsequent decrease in methanol-extractable DMF. There was a linear accumulation of TFMPU after 4 days after a significant formation of DMF. Nonextractable ^{14}C accumulation in ryegrass residues is not presented, as this property was highly variable with significant loss of plant material during the laboratory incubation and drying of samples; thus a mass balance of recovery is not presented. Dissipation of fluometuron in ryegrass residues occurred as rapidly in this laboratory incubation (half-life of 8.5 days, Table 4) as it did under field conditions (half-life of 3 to 9 days, Locke et al. 2005) where leaching and/or runoff may have also been responsible for some of the dissipation. As the ryegrass incubations were conducted under greater moisture content than soils, there was less fluometuron partitioned on the ryegrass, and the calculated degradation rates were similar with and without consideration of sorption K_d.

These laboratory assessments of fluometuron degradation under controlled conditions indicated that tillage and herbicide-desiccated ryegrass cover crop influenced the potential for fluometuron degradation. The results also support observations made in the companion field evaluations (Locke et al. 2005). In the 0–2 cm soil depth, where the most rapid degradation of fluometuron was observed, metabolite accumulation and incorporation of the trifluoromethylphenyl ring label into nonextractable components was observed in CT and NT soils underneath ryegrass residues. More rapid accumulation of fluometuron into non-extractable components agreed with greater microbial counts and soil enzyme activities in NT and CT surface soils from ryegrass plots compared to respective soils with no cover crop. Adding organic amendments to a Dundee silt loam increased the degradation rate of fluometuron and cyanazine, with ryegrass residues being more stimulatory than either cornmeal or poultry litter (Wagner and Zablotowicz 1997).

Conclusions

These studies demonstrate that employing a herbicide-desiccated ryegrass cover crop in either a NT or CT cotton production can have stimulatory effects on microbial populations and microbial activity and improve overall soil quality as well as the potential for degradation of fluometuron. Under field conditions, it is often difficult to demonstrate management effects on herbicide degradation, as factors such as interception of the herbicide by crop residues (Locke et al. 2005) and leaching through the soil profile make analysis of herbicide degradation more difficult to interpret.

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