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Rapid Development of Enhanced Atrazine Degradation in a Dundee Silt Loam Soil under Continuous Corn and in Rotation with Cotton

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Mississippi Delta cotton (*Gossypium hirsutum* L.) production in rotation with corn (*Zea mays* L.) was evaluated in field experiments from 2000 to 2005 at Stoneville, Mississippi. Plots maintained under minimum tillage were established in 2000 on a Dundee silt loam with treatments including continuous cotton or corn and alternate cotton−corn rotations. Mineralization and dissipation of ^{14}C [ring]-labeled atrazine were evaluated in the laboratory on soils collected prior to herbicide application in the first, second, third, and sixth years of the study. In soils collected in 2000, a maximum of 10% of the atrazine was mineralized after 30 days. After 1 year of herbicide application, atrazine-treated soils mineralized 52−57% of the radiolabeled atrazine in 30 days. By the sixth year of the study, greater than 59% of the atrazine was mineralized after 7 days in soils treated with atrazine, while soils from plots with no atrazine treatment mineralized less than 36%. The data also indicated rapid development of enhanced atrazine degradation in soils following 1 year of corn production with atrazine use. Atrazine mineralization was as rapid in soils under a rotation receiving biannual atrazine applications as in soils under continuous corn receiving annual applications of atrazine. Cumulative mineralization kinetics parameters derived from the Gompertz model (*k* and *ti*) were highly correlated with a history of atrazine application and total soil carbon content. Changes in the soil microbial community assessed by total fatty acid methyl ester (FAME) analysis indicated significant interactions of cropping system and sampling date, with FAME indicators for soil bacteria responsible for differences in community structure. Autoclaved soil lost all ability to mineralize atrazine, and atrazine-mineralizing bacteria were isolated from these plots, confirming the biological basis for atrazine mineralization. These results indicate that changes in degradative potential of a soil can occur rapidly and some changes in soil properties may be associated with cropping systems, which can contribute to enhanced atrazine degradation potential.

KEYWORDS: Accelerated herbicide degradation; atrazine; crop rotation

INTRODUCTION

The herbicide atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) is widely used in corn (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], and sugarcane (*Saccharum officinarum* L.) production throughout most of North America. Although atrazine was originally considered to be moderately persistent in most soils (*1*, *2*), there have been increasing reports of rapid mineralization of this herbicide during the past decade (*3*−*7*). In a recent 2 year survey of 21 soils collected from five counties in the Mississippi Delta region of the Southern United States, it was observed that rapid degradation/mineralization of atrazine was observed in soils having 1−2 years of atrazine application (*8*). Numerous species of bacteria capable of metabolizing atrazine have been isolated from diverse geographical areas, e.g., *Pseudomonas* spp. (*9*, *10*), *Pseudaminobacter* (*11*), *Alcaligenes, Ralstonia, Agrobacterium* (*12*), and *Nocardioides* (*13*). Most of the atrazine-mineralizing bacteria possess a series of atrazine-catabolizing genes with initial dechlorination by atrazine chlorohydrolase (*atzA*) and hydrolytic removal of amido-alkyl groups by two amidohydrolases (*atzB* and *atzC*) prior to cleavage of the triazine ring and subsequent mineralization (*11*, *12*). However, different pathways for atrazine

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Table 1. Soil Characteristics of Experimental Plots Collected on March 30, 2005

	g kg^{-1}					mg kg^{-1}			
cropping system	clay	sand	silt	total carbon	total nitrogen	nitrate (NO_3)	sulfate	EC[a] (μS/cm)	pH
continuous cotton	224 a[b]	339 a	437 b	12.2 b	1.33 bc	13.8 a	5.3 b	651 ab	6.75 a
continuous corn	248 a	327 a	424 b	12.8 a	1.25 c	14.1 a	5.3 b	628 ab	6.22 a
rotation 1	249 a	312 ab	438 b	13.1 a	1.58 a	18.8 a	5.6 b	525 bc	6.36 a
rotation 2	213 a	269 b	500 a	12.7 a	1.42 b	14.0 a	5.5 b	730 a	6.64 a
rotation 3	249 a	226 c	529 a	11.0 c	1.22 d	23.3 a	12.9 a	374 c	6.65 a

[a] EC, electrical conductivity. [b] Means followed by the same letter within a column do not differ significantly at the 95% confidence level.

degradation are observed in other atrazine-metabolizing bacteria such as *Nocardioides* sp. (*13*, *14*).

The Mississippi Delta region has traditionally been under intense cotton production. However, some agronomic and economic considerations have led to an examination of rotations to break the cotton monoculture system, with corn gaining grower acceptance as a profitable crop. Moderate to high levels of atrazine (5−20 μg L^{-1}) are observed in Mississippi Delta surface water (*15*, *16*); thus, potential ecotoxicological implications of atrazine contamination of surface water are a concern (*17*). Understanding the relationship between crop rotation and herbicide persistence will be important in the development of cropping systems. Some studies have implicated the development of atrazine-degrading populations with use of this herbicide (*6*, *7*). However, the actual development of atrazine-degrading potential under long-term small-plot research has not been fully explored. This study was conducted to assess changes in atrazine mineralization potential under continuous corn or corn grown in rotation with cotton and to determine if a rotation system can serve as a method to delay the development of enhanced degradation.

MATERIALS AND METHODS

Study Site. This study was conducted from 2000 through 2005 in Stoneville, Mississippi, at the Southern Weed Science Research Unit experimental farm (33°26′ N, 90°55′ W). The soil at this site was a Dundee silt loam, and characteristics associated with the experimental plots at the end of the study are summarized in **Table 1**. This site was planted to glyphosate-resistant soybean the preceding year (1999) and cotton prior or soybean since 1983−1999, with no application of atrazine. Field preparation consisted of disking, subsoiling, disking, and bedding in the fall of 1999. The land was not tilled in subsequent years. The old seedbeds were raised (rebedded) with no additional tillage operations in the fall after harvest each year to maintain plots under a reduced tillage system. The raised beds also enabled furrow irrigation during the growing season. The raised beds were smoothed as-needed by removing a thin layer of soil from the top of the raised bed to plant crops in the spring. Experimental plots were fertilized by injection of urea ammonium nitrate solution (202 and 134 kg ha^{-1} for corn and cotton, respectively) into the beds at planting.

The experiment was conducted in a randomized complete block design with four replications. The rotation systems were continuous cotton, continuous corn, cotton−corn (rotation 1), and corn−cotton (rotation 2). In the sixth year (2005), glyphosate-resistant corn and cotton rotation (rotation 3), which had not received atrazine, was included for comparison. The experimental details and agronomic practices used in the study have been described elsewhere (*18*, *19*). Each treatment consisted of eight rows spaced 1.0 m apart and 45.7 m long. In 2000 and 2002, atrazine and *s*-metolachlor were applied to corn plots only at planting (1820 and 1410 g a.i. ha^{-1}, respectively), while in 2001, 2003, and 2004, atrazine and *s*-metolachlor were also applied postemergence (950 and 740 g ha^{-1}, respectively). In cotton production, fluometuron and metolachlor or pendemethalin (1120−1680 g a.i. ha^{-1} each) were applied at planting, and fluometuron plus MSMA (monosodium methanearsonate) was applied postemergent.

Soil Sampling. Each spring, soil was collected for mineralization assays from four replicates of the treatments under evaluation. Sampling points for these plots were georeferenced using various GPS equipment so that similar regions of the plots could be sampled over the course of the study. In each year, soils were collected within 2 weeks before corn planting (April 5, 2000; March 21, 2001; April 5, 2002; and March 30, 2006). Each sample consisted of a composite of 9−12 cores (5 cm diameter) collected from the surface 0−5 cm of the bed from the two middle rows of the plot. Between each sample, the probe was surface disinfected in 70% isopropanol and allowed to air dry. These soils were collected at least 4 days after paraquat application but prior to planting corn and before fertilizer or other soil-applied herbicide application. In 2005, soils were collected from all four replicate plots of the five treatments for mineralization assays, chemical analysis, and microbial community analysis. In addition, a second sample was collected for microbial community analysis 6 weeks after planting (May 15).

Analysis of Soil Chemical, Physical, and Biological Properties. Chemical and physical analyses were conducted on air-dried soil that was passed through a 2 mm sieve and milled in a Wiley mill. Soil textural analysis was determined by the hydrometer method (*20*). Electrical conductivity (EC) and pH were determined in an aqueous soil suspension (2:1) and were determined on triplicate samples. Total carbon and nitrogen contents were determined on duplicate samples using a Flash EA 1112 elemental analyzer (C.E. Elantec, Lakewood, NJ). Water extractable anions were determined using an ICS 2000 Dionex ion chromatograph (Dionex Corp., Sunnyvale, CA). Separation of anions (nitrate, sulfate, and phosphate) was performed using an IonPac AS18 hydroxide selective anion exchange column, and data were analyzed using Chromeleon software (Dionex Corp.).

Atrazine Mineralization. For each plot, 30 g of soil (air-dried weight equivalents) was added to a biometer flask (*21*), four replicate biometer flasks per treatment. Soils were processed using surface-sterilized (70% isopropanol) sieves to minimize cross-contamination. Soils were treated with a mixture of ^{14}C ring-labeled (98% radiological purity, 11.9 μCi mmol^{-1}, Syngenta Crop Protection) and unlabeled atrazine (Chem Service, Chester, PA) to attain a concentration of 1.25 μg g^{-1} and 149 Bq g^{-1} (a total of ∼252000 dpm per flask). Following treatment of soils with atrazine, soils were adjusted to 30% moisture content (w/w) by adding additional distilled water, and soils were incubated in the dark at 28 °C. To monitor atrazine mineralization, the sidearm traps of the biometer flasks were filled with 10 mL of 1 N sodium hydroxide, which was periodically removed and replaced with fresh solution. Trapped $^{14}CO_2$ in the sodium hydroxide was determined on duplicate 1 mL aliquots by liquid scintillation spectroscopy (LSS) (Packard TriCarb 4000 series, Packard Instruments Co., Meriden, CT) using Hi-Ionic scintillation fluid (Packard Instruments), 15 mL per sample. Sampling of biometer flasks was conducted based on rate of mineralization. In soil samples from 2000, biometer flasks were sampled less frequently than those in 2001, 2002, and 2006 that required daily sampling especially during the initial 9 days after treatment (DAT).

Extractable and Unextractable Atrazine Residues. In addition to the biometer flasks, two 250 mL polypropylene centrifuge bottles were filled with soil and treated with atrazine as described above. These soils were assayed for extractable radioactivity at 0 and 7 DAT, in addition to soil from the biometer flasks, following the last mineralization sample (30 DAT). Methods for extraction of atrazine and metabolites are described in detail elsewhere (*8*). Soils were extracted

twice with aqueous methanol (80:20 v/v methanol and water), the methanol extracts were combined, and the total radioactivity recovered was assessed by LSS. The day 7 extracts were concentrated by rotary evaporation to remove most of the methanol, diluted in water, and passed through C_{18} solid-phase extraction (SPE) columns. The herbicide and metabolites were eluted from the SPE column in methanol and concentrated under N_2 gas and parent compound, and metabolites were determined using thin-layer chromatography (TLC) and linear imaging scanning (Bioscan Imaging System 200, Bioscan Inc., Washington, DC). Most (>95%) of the radioactivity was retained and eluted from the SPE column using this method (data not shown). Herbicides and metabolites recovered in the methanol extracts (100 μL) were spotted on silica gel plates (250 μm thick). TLC plates were developed 10 cm using toluene:ethyl acetate solvent (50:50 v/v), and R_f values for triazine standards were atrazine = 0.67, de-ethyl atrazine = 0.40, deisopropyl atrazine = 0.23, and hydroxyatrazine = 0.00[1] (*1*, *8*). The metabolites area was reported as either polar or dealkylated atrazine derivatives. Nonextractable radioactivity remaining in soils following the three extractions (bound fraction) was determined by oxidation, combusting cellulose amended soils with a biological oxidizer and collecting the radioactivity in a mixture of Carbo-Sorb E and Permafluor E cocktail (Packard Instruments Co.). Radioactivity was determined by LSS.

Biological Characterization of Soil and Isolation of Atrazine Degraders. On March 30 and May 14, 2005, soil samples were collected for microbial community analysis. Subsamples of soil from each plot were frozen at −80 °C, to preserve microbial community integrity. The soil microbial community structure was characterized by fatty acid methyl ester (FAME) using a protocol that is similar to the ester-linked method of Shutter and Dick (*22*). Methyl-ester-linked fatty acids were extracted in 0.2 N methanolic KOH at 37 °C in Teflon-capped glass tubes and neutralized with 1 M acetic acid, and the FAMEs were extracted twice into hexane. The organic phases were combined, concentrated under N_2, and dissolved in 1:1 v/v hexane:methyl-*tert* butyl ether. FAMEs were separated, identified, and quantified with a Agilent 6890 gas chromatograph using the MIDI Eukaryote protocol and MIDI FAME standards (Microbial ID, Newark, NJ). Microbial community structure was assessed using principal component (PC) analysis. To reduce minor experimental variation, only fatty acids that were present in at least half the samples and average molar percent that was at least 0.5% were considered (*23*). Following PC analysis, the contributions of treatment and sample date and interactions between treatment and sample date on PCs were analyzed using SAS PROC MIXED. Pearson's correlations were conducted to determine major fatty acids contributing to the PCs.

To isolate potential atrazine-degrading bacteria, soil was collected from continuous cotton plots that never received atrazine following harvest in August of 2005, mixed, and sieved. Five gram samples were suspended in 40 mL of water, supplemented with 20 μg mL^{-1} atrazine, and incubated on a rotary shaker at 28 °C. At various intervals following inoculation, atrazine concentrations were identified and quantified with a Waters 2695 high-performance liquid chromatography (HPLC) separations module with a Waters 996 photodiode array detector. A 2.1 mm diameter, 150 mm length Waters Symmetry C_{18} column (Waters, Milford, MA) was used for separation with an isocratic acetonitrile and water (4:6,v/v) solvent at a flow rate of 0.3 mL min^{-1}. After the atrazine levels fell below the detection threshold (0.1 μg mL^{-1}), 5 mL aliquots were transferred to flasks with 2 g of sterilized soil, 40 mL of water, and 20 μg mL^{-1} atrazine and further incubated in a rotary shaker. In cultures where atrazine levels fell below the detection threshold, 3 mL aliquots were transferred to flasks with 50 mL of mineral salts (carbon- and nitrogen-free) solution from Vogels medium (*24*) containing 20 μg mL^{-1} atrazine. Cultures that degraded atrazine were diluted and plated on atrazine-supplemented Vogel's agar. Putative atrazine-degrading colonies were individually selected after 4 days of incubation and re-examined using the above culture system.

To assess the biological basis of atrazine mineralization, soil was collected from four replicates of continuous corn plots in October 2006. Twenty-five grams of soil (oven dry weight equivalents) was placed in duplicate biometer flasks. One set of flasks was autoclaved (15 min, 137 °C) on three consecutive days, and the other set was not treated. Atrazine was added as previously described, and mineralization was monitored over 9 days of incubation.

Several pure cultures isolated from these enrichments were evaluated for mineralization of ^{14}C-atrazine (data presented for six isolates MW1, MW2, MW3, MW7, MW8, and MW11). Biometer flask assemblies were filled with 20 mL of carbon and nitrogen-free mineral salts media and were autoclaved with a polyurethane foam plug between the flask and the NaOH trap. A mixture of ring-labeled atrazine and technical grade atrazine was added in 100 μL of methanol to achieve a final atrazine concentration of 20 μg mL^{-1} and 182 Bq mL^{-1}. To generate inoculum for the mineralization study, cultures were grown to midlog stage of growth ~72 h in carbon and nitrogen-free mineral salts media and atrazine (20 μg mL^{-1}). Cells were harvested by centrifugation (10 min, 8000*g*) and washed twice in sterile mineral salts media, and cell suspensions were adjusted to ~10^8 cells mL^{-1}. Biometer flasks were inoculated with 200 μL of cell suspension, and flasks were incubated on a rotary incubator at 28 °C and 60 rpm. NaOH was removed at several times between 15 and 70 h and trapped radioactivity was determined by LSC. At the termination of the study, cells were harvested by centrifugation (10 min, 8000*g*) and resuspended in mineral salts media, radioactivity was recovered in the cell suspension, and culture supernatant was determined.

Statistical Analysis. Soil chemical and physical properties, methanol recovery of radioactivity, mineralization, and nonextractable ^{14}C were subjected to analysis of variance (ANOVA) using the general linear model procedure in SAS (*25*). Means were separated using Fisher's protected LSD test at $P = 0.05$. Atrazine mineralization was fitted to second-order degradation kinetics using the three-parameter Gompertz growth model [$y = ae^{-e[-k(t-ti)]} + ct$] (Sigma Plot version 7.0). This model has been used extensively in several recent studies evaluating atrazine degradation (*8*, *18*, *26*−*28*), and a growth model describes important parameters of microbial growth-mediated herbicide degradation. Parameters determined include *a*, the plateau representing maximum percent mineralization; *ti*, the abscissa of the inflection point; and *k*, the mineralization rate constant. In soils sampled in 2000 before atrazine was applied to the study site, the lowest and nearly linear rate of mineralization was observed; parameter *a* was constrained to cumulative mineralization observed at day 30. No other constraints were used for describing mineralization in the other 3 years.

RESULTS AND DISCUSSION

Soil Properties under Various Cropping Systems. Soils from these plots were all silt loams with a similar amount of clay; however, soils from the rotation 2 and rotation 3 plots had less sand and more silt than the soil from continuous cotton or corn plots (**Table 1**). There were significant differences in total carbon and nitrogen contents among treatments, with the highest content in soils from rotation 1 and the lowest from rotation 3. Soils cropped under continuous corn had a lower total nitrogen content as compared to soils under a corn−cotton rotation or continuous cotton. A cropping system with continuous corn or a corn−cotton rotation would more likely accumulate carbon as compared to continuous cotton because of crop residues remaining after harvest in a corn vs a cotton system. Moreover, the high demand for nitrogen use in high-yielding corn would also cause a greater depletion of total soil nitrogen. There was no significant difference in most water-soluble anions (nitrate, nitrite, and phosphate) among the five treatments, although sulfate concentration was highest in soil from the rotation 3 plots. Typical extractable nitrate present in soils at the time of sampling was between 10 and 20 μg NO_3 g^{-1} soil. The lowest EC was observed in soil collected from rotation 3 plots. Further discussion of changes in soil organic matter under these cropping systems is presented elsewhere (*19*).

Atrazine Mineralization. Before initiation of this 6 year field study, a low level of atrazine mineralization (i.e., 10.5% over a 30 day incubation) was observed in soils collected from these

Table 2. Mineralization Characteristics of a Dundee Silt Loam Soil as Affected by Cropping Systems in 2000–2005

year/cropping system	cumulative mineralization		Gompertz parameters[a]		
	day 7	day 30	*a*	*k*	*ti*
2000	1.5[b]	10.5	10.5	0.11	17.9
2001					
continuous cotton	3.3 b[c]	25.9 b	23.9 b	0.150 b	12.7 a
continuous corn	39.9 a	51.6 a	48.4 a	0.471 a	3.1 b
rotation 1 (cotton[d])	2.5 b	27.8 b	27.9 b	0.171 b	14.3 b
rotation 2 (corn)	37.4 a	50.9 a	48.4 a	0.451 a	3.4 b
2002					
continuous cotton	12.5 b	41.1 b	41.0 b	0.244 b	8.8 a
continuous corn	47.6 a	57.7 a	57.7 a	0.673 a	2.0 b
rotation 1 (corn)	45.8 a	56.3 a	56.3 a	0.736 a	2.1 b
rotation 2 (cotton)	41.3 a	53.8 a	53.8 a	0.733 a	2.1 b
2005					
continuous cotton	36.8 b	67.6 a	66.8 a	0.301 c	5.6 a
continuous corn	63.8 a	73.1 a	69.5 a	0.665 a	2.2 b
rotation 1 (cotton)	59.6 a	74.0 a	72.1 a	0.445 b	3.3 b
rotation 2 (corn)	64.2 a	73.9 a	70.6 a	0.568 a	2.4 b
rotation 3, no atrazine	24.7 b	58.2 b	58.4 b	0.233 c	6.7 a

[a] Gompertz parameters: *a*, the plateau representing maximum percent mineralization; *ti*, the abscissa of the inflection point; and *k*, the mineralization rate constant. [b] Samples collected in 2000 are presented as the mean of eight replicates, four from plots from continuous corn and four from plots for continuous cotton. [c] Means of four replicates; means followed by the same letter within a column and a year do not differ significantly at the 95% confidence level. [d] The crop indicated in parentheses was grown in the previous year.

plots (**Table 2**). Following the first application of atrazine to corn, soils collected in the spring of 2001 mineralized 50.9−51.6% of the atrazine during a 30 day incubation. A highly variable but low to moderate rate of mineralization (27% cumulative after 30 day) was observed in plots that were previously cropped to cotton and did not receive atrazine (continuous cotton and rotation 1). In 2002, a similar level of atrazine mineralization was observed in soils from plots under continuous corn or rotations 1 or 2, while significantly lower mineralization was found in soil from continuous cotton soils. By the sixth year of the study, a similar level of cumulative mineralization (i.e., 68−74% of the atrazine applied) was observed for all of the originally sampled plots, including continuous cotton, during a 30 day incubation. However, soil from rotation 3 plots exhibited less mineralization. Studies conducted in Ohio (*6*) demonstrated that a 3 year corn rotation with reduced atrazine use was sufficient in reducing accelerated atrazine mineralization as compared to a continuous corn cropping system. Data from our studies indicate that there is a rapid dispersal and development of an atrazine-degrading potential under an alternate yearly rotation of atrazine use as under yearly atrazine application. Data for atrazine mineralization modeled from the Gompertz growth model in soils collected from continuous corn and continuous cotton plots are presented in **Figure 1a**,**b**, respectively. Examining the cumulative mineralization at 7 days after incubation, an extended lag in atrazine mineralization was observed in soils with no history of atrazine, even in the sixth year of the study. For all treatments and years, the atrazine mineralization kinetics was fit well by the Gompertz growth equation; however, for soils from 2000 and 2001, a constraint of a = cumulative mineralization at day 30 needed to be used. The length of the lag phase (*ti*) progressively decreased for the continuous corn plots over time. However, following 1 year of corn cultivation and atrazine application (2002), there were only limited differences in *ti* among continuous corn or rotations 1 or 2 treatments. Similarly, the

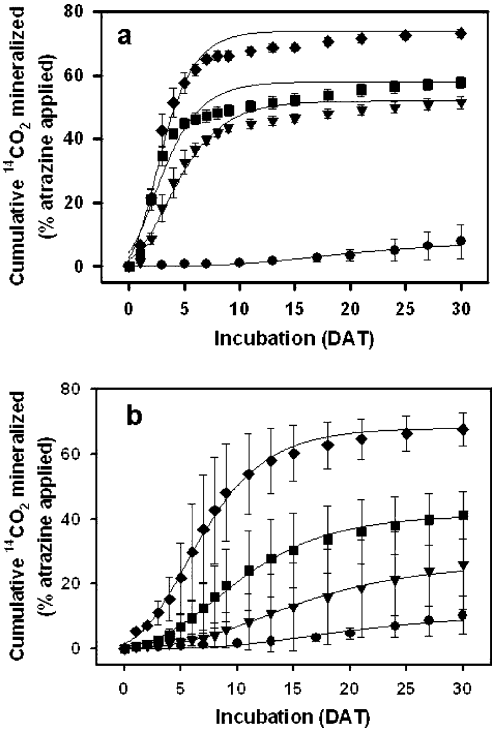

Figure 1. Mineralization of atrazine in soils collected from continuous corn (**a**) or continuous cotton plots (**b**) sampled in 2000 (●), 2001 (▼), 2002 (■), and 2005 (◆). Each symbol is represented by the mean and standard deviation; the modeled curve was generated from the Gompertz growth equation.

mineralization constant, *k*, increased rapidly following the first application of atrazine. The *k* values observed for atrazine-treated soils are in a similar range to those reported by others for soils able to rapidly mineralize atrazine (*5*, *8*, *26*, *28*).

The development of enhanced atrazine mineralization in soil from plots that did not receive atrazine occurred rapidly. Although these soils were maintained under minimum tillage, transport of degraders may have occurred from implements used for bed formation, tractor tires, harvesting equipment, and irrigation or runoff. Because of randomization of treatments, all continuous cotton plots were not aligned in front or in back of plots receiving atrazine, and the development of accelerated degradation was randomly distributed in this experiment. This apparent movement of atrazine-degrading microorganisms is similar to the level of contamination previously observed by researchers conducting assessments of rhizobia that nodulate various legumes (*29*) and makes conducting long-term atrazine fate studies under various cropping systems challenging.

Extractable and Nonextractable Atrazine Residues. In the laboratory incubation studies, the recovery of extractable ^{14}C residues and nonextractable radioactivity for the 4 years is presented in **Table 3**. Generally, a mass balance of greater than 95% was observed at day 0, and 85−97% was observed at 30 DAT (data not shown). Extractable ^{14}C is inversely related to the amount of atrazine mineralized, e.g., the greater mineralized less extractable. Because of a rapid loss of atrazine from certain treatments, the recovery of atrazine and metabolites was only assessed at the extractions conducted at 7 DAT. In 2000 and 2001 soils with no atrazine exposure, greater than 91% of the extractable radioactivity was recovered as atrazine at 7 DAT. However, with greater exposure to atrazine, about 50% or less of the extractable radioactivity was recovered as atrazine at 7 DAT. In 2000, most of the metabolites extractable in methanol at 7 DAT were dealkylated derivatives, while in 2002 and 2006

Table 3. Recovery of ^{14}C-Atrazine at 7 and 30 DAT in Soils Collected from Various Cropping Systems from 2000 to 2006

year/cropping system	total extractable radioactivity		extractable atrazine	polar metabolites	dealkylated atrazine	nonextractable	
	7 DAT	30 DAT	7 DAT	7 DAT	7 DAT	7 DAT	30 DAT
2000	84.1[a]	60.9	79.2	1.8	5.1	15.2	19.4
2001							
continuous cotton	77.9 a[b]	42.4 a	73.1 a	3.5 b	1.4 c	16.2 a	15.4 a
continuous corn	36.3 b	22.8 b	19.7 b	13.1 a	3.6 a	15.0 a	7.7 b
rotation 1 (cotton[c])	77.5 a	37.7 a	70.8 a	5.4 b	1.1 bc	14.7 a	13.5 ab
rotation 2 (corn)	38.2 b	22.1 b	22.5 b	12.6 a	3.1 ab	8.6 b	11.6 ab
2002							
continuous cotton	63.3 a	26.7 a	51.5 a	10.8 a	2.3 b	17.6 a	16.1 a
continuous corn	33.1 b	17.4 c	17.9 b	10.8 a	5.9 a	15.3 a	9.9 bc
rotation 1 (corn)	32.5 b	18.2 c	17.4 b	10.9 a	2.7	14.2 ab	8.7 c
rotation 2 (cotton)	35.7 b	21.3 b	23.7 b	10.0 a	3.7 ab	11.3 b	13.6 ab
2005							
continuous cotton	43.7 a	15.3 b	20.1 a	16.5 a	7.1 a	8.0 a	15.3 a
continuous corn	13.5 b	11.6 bc	6.5 b	5.2 b	1.8 b	8.4 a	5.7 b
rotation 1 (cotton)	16.0 b	11.7 bc	8.1 b	6.2 b	1.7 b	7.6 a	4.7 b
rotation 2 (corn)	14.0 b	10.2 c	7.2 b	5.5 b	1.3 b	6.8 ab	4.9 b
rotation 3 (no atrazine)	56.7 a	22.3 a	28.7 a	19.1 a	8.9 a	6.3 b	21.7 a

[a] Samples collected in 2000 are the means of eight replicates. [b] The crop indicated in parentheses was grown in the previous year. [c] Means of four replicates; means followed by the same letter within a column and a year do not differ significantly at the 95% confidence level.

Table 4. Pearson Correlations between Five Atrazine Mineralization Parameters and History of Atrazine Exposure or Soil Chemical and Physical Properties in Soil Sampled in 2005[a]

soil property	Gompertz mineralization parameters[b]			cumulative mineralization	
	a	*k*	*ti*	day 7	day 30
atrazine xposure	0.4899*	0.8769***	–0.8038***	0.7782***	0.5978**
total carbon	0.6815***	0.6258***	–0.6860***	0.7117***	0.7640***
total nitrogen	0.4067	0.1284	–0.2499	0.4051	0.4447*
pH	–0.4060	–0.3953	0.5192*	–0.3953	–0.3785
EC	0.3575	–0.4015	–0.4324	0.4330	0.4590*
nitrate	0.153	–0.2540	0.1393	–0.0238	0.0538
sulfate	–0.7918***	–0.5744**	0.6545**	–0.6245**	–0.7883***
sand	0.3418	0.2932	–0.2544	0.2168	0.3268
silt	–0.3531	–0.3317	0.4043	–0.2504	–0.3250
clay	0.0096	0.0309	–0.1415	0.0018	–0.0074

[a] Significant correlations at the $P < 0.05$ level are indicated by *; $P < 0.01$ by **; and $P < 0.001$ by ***. [b] Gompertz parameters: *a*, the plateau representing maximum percent mineralization; *ti*, the abscissa of the inflection point; and *k*, the mineralization rate constant.

more than 70% of the metabolites recovered at 7 DAT were polar hydroxylated atrazine compounds that remained at the origin in the TLC systems used for metabolite characterization, regardless of cropping system. An increase in the accumulation of polar metabolites suggests increased microbial populations possessing an atrazine chlorohydrolase. Overall, most of a small fraction of the ^{14}C label was recovered in the nonextractable components, and the relative recovery of ^{14}C in the nonextractable fraction decreased with exposure to atrazine. In 2006, the two treatments that never received atrazine (continuous cotton and the glyphosate-resistant corn−cotton rotation) had significantly greater accumulation of polar metabolites and nonextractable ^{14}C residues as compared to the three cropping systems that were exposed to atrazine for 2−5 years.

Five atrazine mineralization characteristics (Gompertz kinetic parameters [*a*, *k*, *ti*] and cumulative mineralization at 7 and 30 DAT) were correlated with with various soil properties and history of atrazine exposure (**Table 4**). The strongest correlation observed was with atrazine history (0−5 years) and *k*, *ti*, and cumulative mineralization at 7 DAT. Cumulative mineralization was also highly correlated with two soil chemical parameters: total soil carbon and extractable sulfate. The total organic carbon content varied between 10.2 and 13.3 g kg^{-1} of soil in individual plots and may have accounted for some of the internal variability in mineralization among the various treatments. The positive correlation with organic carbon may indicate that available organic substrates support the maintenance and development of atrazine-metabolizing organisms. Studies by Abdelhafid et al. (*30*) and Rhine et al. (*23*) indicate that there are contrasting effects of organic carbon and nitrogen on atrazine degradation in adapted and unadapted soils. Adapted soils exhibited little effect of organic amendments; however, supplemental mineral nitrogen depressed atrazine ring mineralization (*29*). Moderate to weak correlations were observed with soil pH, EC, and total nitrogen content. Only one parameter, *ti*, was positively correlated with soil pH. Other studies conducted on Canadian and French soils indicated a strong relationship between increased atrazine ring mineralization and increased soil pH (*4*). There was no relationship between soil texture and atrazine mineralization, as observed in other studies (*8*), indicating that atrazine sorption to soil colloids may have had only limited effects reduced availability of atrazine for mineralization in soils evaluated in these studies.

Biological Characterization of Soil and Isolation of Atrazine Degraders. Cropping system and time of sampling had a significant effect on soil microbial community structure as evident by PC analysis of total FAMEs (**Figure 2** and **Table 5**). The factor with the greatest contribution to PC1 was date

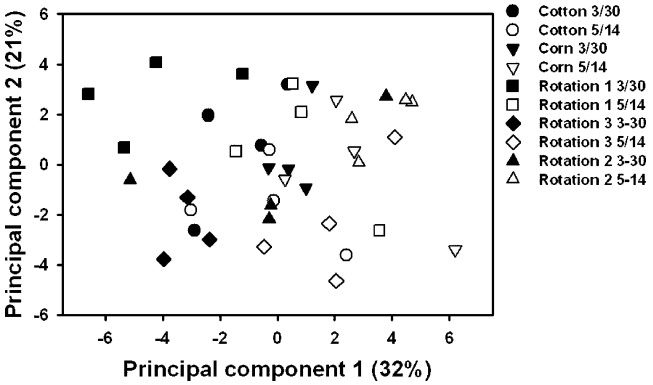

Figure 2. Soil microbial community analysis based upon PC analysis of total FAME analysis of soil sampled from six cropping systems, before corn planting and atrazine application, and six weeks following application in 2005.

Table 5. Contributions of Cropping System and Sampling Date to Soil Total FAMEs and Correlation of Dominant Fatty Acids Contributing to Soil Community Structure of Plots of Various Cropping Systems as Analyzed by Principal Component (PC) Analysis

PC1	PC2
Pr > F	Pr > F
cropping system, 0.0018	cropping system, 0.0005
date, <0.0001	date, 0.3048
cropping system × date, 0.1198	cropping system × date, 0.1619
fatty acids contributing to PC1	fatty acids contributing to PC2
+,[a] 17:1 G iso (<0.0001)	+, 17:0 (<0.0001)
+, 15:0 iso (<0.0001)	+, 15:0 (<0.0001)
+, 16:0 iso (<0.0001)	–, 18:0 (<0.0001)
+, 19:0 cyclo c1l-12 (<0.0001)	+, 20:4 (<0.0001)
–, 18:2 Ω6c (<0.0001)	+, 18:1Ω9t alcohol (<0.0001)
+, 17:1 iso (<0.0001)	–, 16:0 (<0.0001)
+, 17:0 anti (<0.0001)	+, 20:0 (0.0002)
+, 15:0 anti (<0.0001)	

[a] Indicates if the fatty acid is positively or negatively correlated with the PC.

of sampling while cropping system had a highly significant contribution to principle components 1 and 2. For PC1, ANOVA indicated that plots maintained under continuous cotton were significantly different than continuous corn (0.01% level) and that continuous corn was significantly different than rotations 2 and 3 (0.01% level). Pearson correlations conducted on dominant fatty acids contributing to the two PCs indicate that branched fatty acids representing Gram-positive bacteria were positively contributed to PC1, while the fungal FAME 18:2 Ω 8c was negatively correlated with PC1 (**Table 5**). For PC2, ANOVA indicated a significant effect of cropping system with no effect of sampling date or interaction of cropping system and sample date. In PC2, rotations 1 and 2 were significantly different than rotation 3 and continuous cotton was significantly different than rotations 2 and 3 (0.05% level). The major contributors to PC2 were various unsaturated fatty acids that are common components of most microorganisms and not associated with any particular group of microorganisms. In addition, 18:1Ω9t alcohol (associated with Gram-negative bacteria) positively contributed to PC2 (**Table 5**). The studies by Rhine et al. (*23*) indicated that both atrazine exposure and artificial carbon and nitrogen inputs significantly altered microbial community structure using total FAME analysis. In this current study, various cropping systems associated with atrazine application altered microbial communities under field conditions.

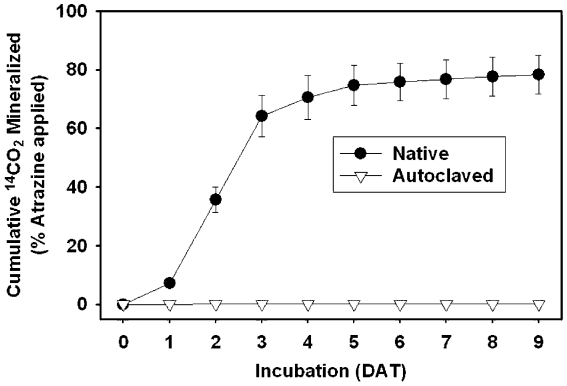

Figure 3. Effects of autoclaving on atrazine mineralization in an adapted Dundee silt loam. Means and standard deviations of four replicates for native and autoclaved soil.

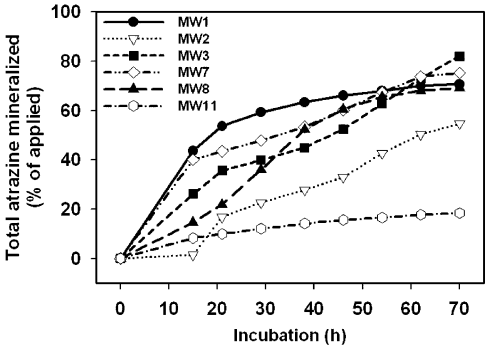

Figure 4. Mineralization of atrazine by six pure cultures of bacteria (MW1, MW2, MW3, MW7, MW8, and MW11) isolated from rotation plots grown on N-free Vogel's media with atrazine as the sole nitrogen source.

Both fungal and bacterial components of the soil microbial community contribute to various processes in atrazine degradation (*31*); bacteria typically are responsible for cleavage of the triazine ring and total mineralization, while fungi metabolize atrazine mainly by *N*-dealkylation. In adapted soils, the populations of atrazine degraders are rather small (100−10000 g soil^{-1}) (*8*, *23*) and a small component of the total bacterial community. The FAME community studies indicate that cropping systems can favor enhanced bacterial dominance that may be associated with establishment of bacteria capable of enhanced atrazine degradation. Bacterial diversity as measured by amplification of 16s DNA and DGGE was affected up to 45 days following application of atrazine when formulated material was applied at greater than 10 mg kg^{-1} (*32*).

The effect of autoclaving soil that has received 6 years of continuous atrazine application is presented in **Figure 3**. Native soil exhibits a rapid rate of atrazine mineralization, while soil that has been autoclaved demonstrated a 100% loss in atrazine mineralization potential. The effect of soil sterilization provides indirect evidence that atrazine degradation occurring in this soil is biologically based. In addition, pure cultures of bacteria capable of mineralizing ring-labeled atrazine have been isolated from this study (**Figure 4**) and the results have been confirmed in a repeat incubation. A total of six bacterial strains, having unique fatty acid profiles and each a distinct species of Gram-negative bacteria (*Klebsiella pneumoniae*, *Moraxella nonliquefaciens*, *Pseudomonas fluorescens*, *Pseudomonas* sp., *Serratia odorifer*, and *Stenotrophomonas maltophila*), have been confirmed as capable of mineralizing atrazine as a sole carbon and nitrogen source. Isolates MW1, MW3, and MW7 had almost no lag before rapid mineralization, while MW2 and MW8 had a delay before initiation of mineralization. Although there was

a consistent loss of atrazine in HPLC assays, isolate MW11 exhibited only minimal mineralization (<20%). A mass balance of greater than 95% was observed in the pure culture studies, with less than 5% of the radioactivity associated with washed cells and most of the residual radioactivity present in the cell-free supernatant. The magnitude and rates of atrazine ring mineralization are similar to those reported by others for pure bacterial cultures (*9*, *11*, *33*). As all six atrazine-degrading bacteria were unique based on fatty acid content, this suggests a mobile genetic element, e.g., plasmid similar to that maintained by atrazine-degrading *Pseudomonas* spp. ADP may be responsible for dispersal of the atrazine-degrading phenotype within diverse genera. Further metabolic and genetic characterization of these and other isolates is in progress and will be reported elsewhere. The isolation of pure cultures of atrazine-mineralizing bacteria confirms the biological nature of atrazine mineralization in these plots.

In conclusion, these studies have confirmed results of previous studies (*8*) that under Mississippi Delta conditions enhanced atrazine degradation develops within a year of atrazine exposure. The isolation of atrazine-mineralizing bacteria confirms the biological nature of enhanced atrazine mineralization. The implication of accelerated atrazine degradation on the potential for control of broadleaf weeds can be a problem as atrazine is typically an economic option for control of weeds such as morning glory (*Ipomoea* spp.) in this geographical area (*18*). Important information gained from this study indicates that a rotation of alternate corn and cotton is not sufficient to reduce enhanced rates of atrazine dissipation. From a research design perspective, these studies demonstrate that even though large-scale plots were used, control plots that never received atrazine also rapidly developed the potential for atrazine mineralization, albeit slower than soil from plots receiving atrazine.

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