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Determining Potential for Microbial Atrazine Degradation in Agricultural Drainage Ditches

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Passage of agricultural runoff through vegetated drainage ditches has been shown to reduce the amount of pesticides, such as atrazine, exiting out of agricultural watersheds. Previous studies have found that microbial communities in soil from fields treated with atrazine display enhanced rates of atrazine degradation. However, no studies have examined the potential for atrazine degradation in ditches used to drain these lands. The purpose of the current study was to determine the potential of the drainage ditch soil microbial community for atrazine degradation. Soil samples were collected from fields and adjacent drainage ditches and from nonagricultural land with no previous exposure to atrazine. Polymerase chain reaction analysis indicated widespread presence of atrazine degradation genes in fields and ditches. Potential for degradation was determined by following the decrease of atrazine in spiked soil samples over a 28-d incubation period. Greater than 95% of atrazine was degraded in field and ditch soils, whereas only 68.5 ± 1.3% was degraded in the nonagricultural control. Comparison with autoclaved soil samples indicated the primary mechanism of atrazine degradation in agricultural soils was microbially mediated, whereas its breakdown in nonagricultural soil appeared to be the byproduct of abiotic processes. Therefore, microbial communities in drainage ditch sediments have the potential to play a role in atrazine removal from agricultural runoff by breaking down atrazine deposited in sediments and limiting the amount of this herbicide carried into downstream ecosystems.

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Atrazine (6-chloro-N^2–ethyl-N^4–isopropyl-1,3,5-triazine-2,4-diamine) is one of the most widely used agricultural pesticides in the United States (USEPA, 2006). Seventy-six percent of sugarcane, 75% of corn, and 58.5% of sorghum fields are treated with this herbicide each year (USEPA, 2006). Atrazine has a high potential for leaching from soil into water (Fava et al., 2007) and, as a result of its widespread use, is frequently found in surface and ground water (Clark and Goolsby, 2000; Zablotowicz et al., 2006a; Steele et al., 2008; Iker et al., 2010). Atrazine can harm aquatic ecosystems due to its effect on aquatic life (Graymore et al., 2001; Russo and Lagadic, 2004; Fatima et al., 2007) and potential impact on amphibian development at doses as low as 0.1 μg L^{-1} (Hayes et al., 2002; Sullivan and Spence, 2003). Thus, management practices to reduce the transport of atrazine out of agricultural watersheds are of great interest.

Passage of agricultural runoff through vegetated drainage ditches has been shown to reduce levels of pesticides in agricultural runoff, thereby minimizing the amounts flushed into downstream aquatic systems (Moore et al., 2001; Cooper et al., 2004; Moore et al., 2008; Moore et al., 2011). Studies have found that microbial communities in soil previously treated with atrazine display enhanced rates of atrazine degradation compared with those with no previous atrazine exposure (Piutti et al., 2002; Zablotowicz et al., 2007; Krutz et al., 2008). However, these studies focused on soils in fields exposed to direct application of atrazine. To the authors' knowledge, there have been no studies examining the microorganisms in drainage ditch systems or the contribution they make toward the remediation of pesticides. Most agricultural fields are well drained, whereas hydrologic conditions in ditches can shift widely, ranging from complete dryness to inundation under several feet of water. It is unclear how these conditions might affect the microbial community's ability to degrade atrazine and other pesticides.

One of the initial steps in microbial atrazine mineralization is dechlorination of the s-triazine ring to yield hydroxyatrazine, which is catalyzed by chlorohydrolase enzymes encoded by the genes *atzA* and/or *trzN* (de Souza et al., 1996; Mulbry et al., 2002). After dechlorination, hydroxyatrazine is converted to cyanuric acid by two amido hydrolases encoded by *atzB* and

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Abbreviations: FDA, fluorescein diacetate; PCR, polymerase chain reaction.

atzC (Boundy-Mills et al., 1997; Sadowsky et al., 1998). These genes (*atzABC*) are highly conserved, globally distributed, and often plasmid borne (de Souza et al., 1998a, 1998b; Devers et al., 2007). Although *atzB* and *atzC* have been detected in Mississippi Delta soils displaying accelerated atrazine degradation, Zablotowicz et al. (2006b) were unable to directly amplify *atzA*. Thus far, *atzA* has only been detected in isolates from enrichment cultures of these soils (Krutz et al., 2008). However, the *trzN* chlorohydrolase gene discovered by Mulbry et al. (2002) has been detected in atrazine-adapted Mississippi soils (Krutz et al., 2008). In another study, Anderson et al. (2002) experienced difficulty detecting *atzA* without the use of southern hybridization and concluded it was not the dominant chlorohydrolase in the wetland system being studied.

Using quantitative PCR to screen for atrazine degrading genes, Udiković-Kolić et al. (2010) found that only 4% of the bacterial community in atrazine-contaminated soil at an agrochemical factory was capable of degrading atrazine. It is likely an even smaller portion of bacteria possesses the potential to degrade atrazine in agricultural watersheds, where atrazine concentrations are much lower. No studies have examined the degradation of atrazine in ditches used to drain agricultural fields. Thus, the purpose of the current study was to determine the potential of the drainage ditch soil microbial community for atrazine degradation.

Materials and Methods

Sample Collection

Soil samples were collected in October 2010 from two fields in a corn–soybean rotation in northeastern Mississippi: one corn field treated with atrazine in the 2010 growing season and one soybean field last treated with atrazine in the 2009 growing season. Both fields possessed adjacent drainage ditches that were also sampled. Nonagricultural soil with no previous exposure to atrazine was collected as a control from a 3 × 3 m plot at the University of Mississippi Field Station in Abbeville, Mississippi. Samples were collected from three sites in each field and each ditch, with the exception of the nonagricultural control, where only one site was sampled due to the small size of the atrazine-free plot. All equipment was rinsed with 70% ethanol between sampling sites to minimize cross-contamination. Approximately 2 kg of soil was collected from each site. Only the top 5 cm were sampled because previous research has found that rates of atrazine mineralization are highest in the top 5 cm of sediments exposed to agricultural runoff (Anderson et al., 2002). Soil samples were passed through a 2-mm sieve and stored at 4°C until analysis. Sieves were washed with soap, rinsed three times with water, rinsed with 70% ethanol, and flame sterilized between samples to minimize cross-contamination of atrazine degraders. Soil characteristics (pH, particle size, total carbon, and total nitrogen) of samples were determined by standard methods and are reported in Table 1.

Microbial Activity

Microbial hydrolytic activity in soil was determined by assaying fluorescein diacetate (FDA) hydrolysis using a protocol modified from Schnurer and Rosswall (1982). Three technical replicates were performed for each sampling site. Briefly, 1 g of soil (fresh weight) was mixed in 2 mL of 50 mmol L^{-1} potassium phosphate buffer (pH 7.6) containing 25 μg mL^{-1} FDA (Sigma) in borosilicate glass tubes. Tubes were vortexed and incubated at 28°C for 1 h with shaking. Reactions were stopped by the addition of 2 mL distilled acetone, after which tubes were vortexed and centrifuged at ~1000 × *g* for 30 min to pellet soil particles. Absorbance of supernatants was measured at 490 nm. Fluorescein concentration was calculated by comparison to a standard curve.

Atrazine Degrading Activity

Atrazine degradation potential was measured in a laboratory incubation study. Triplicate jars were prepared with 140 g of soil from each sampling site. An additional jar of soil from each sample was autoclaved (45 min at 121°C) on two consecutive days to serve as a control for abiotic processes, such as chemical hydrolysis and adsorption of atrazine to soil particles. Jars were treated with 7 mL of a 20 μg mL^{-1} aqueous solution of atrazine (Supelco, 99.9%) to yield a final concentration of 1 mg kg^{-1}. After treatment, soil moisture was adjusted to field capacity with sterile distilled water. Jars were incubated at 30°C, and 20-g samples were collected from each jar at 0, 1, 3, 7, 14, and 28 d after treatment and extracted for atrazine concentrations.

Atrazine Extraction and Detection

The initial concentration of atrazine in soil samples was determined from 10 g of dried soil using ethyl acetate as described previously (Bennett et al., 2000), with a detection limit of 0.01 μg g^{-1}. For degradation studies, samples were analyzed with a water miscible solvent (methanol) so that soil could be extracted immediately after collection at each time point without having to oven dry or wait for it to air dry. Soil samples (20 g dry weight equivalent) were weighed into 50-mL glass centrifuge tubes. Atrazine was recovered by extracting with 20 mL 80% methanol and agitating overnight on an orbital shaker (Model 6000, Eberbach). Extracts were centrifuged (10 min at 150 × *g*), and the supernatant was poured off. Soil was extracted a second time with 10 mL 80% methanol and shaken for 2 h. Supernatants from both

Table 1. Soil characteristics.†

Sample area	Particle size distribution			Total C	Total N	pH
	Sand	Silt	Clay			
	%					
Corn ditch	49.3 ± 10.6	49.6 ± 10.2	1.1 ± 0.4	0.56 ± 0.11	0.043 ± 0.008	4.98 ± 0.12
Corn field	14.4 ± 2.5	83.9 ± 2.6	1.7 ± 0.1	0.99 ± 0.03	0.088 ± 0.004	5.70 ± 0.09
Soybean ditch	82.8 ± 3.1	16.8 ± 2.9	0.4 ± 0.3	0.23 ± 0.02	0.019 ± 0.003	5.92 ± 0.49
Soybean field	33.9 ± 10	64.8 ± 9.8	1.4 ± 0.2	1.58 ± 0.12	0.146 ± 0.013	5.10 ± 0.28
Nonagricultural control	89.6 ± 3.5	10 ± 3.2	0.4 ± 0.2	0.38 ± 0.13	0.026 ± 0.009	4.88 ± 0.045

† All values reported as mean ± SE.

extractions were combined and concentrated down to 1 mL on an N-EVAP112 nitrogen evaporator (Organomation Associates Inc.) to remove all methanol. Concentrated supernatants were brought up to 20 mL with deionized water and cleaned by loading onto 3-mL C_{18}–solid phase extraction columns. Atrazine was eluted from the C_{18} with 6 mL methanol. The methanol eluate was concentrated to 1.5 mL. Atrazine was detected and quantified on an Agilent 1100 series high-performance liquid chromatograph equipped with a G1313A autoinjector, a quaternary pump, diode-array and fluorescence detectors, an Eclipse XDB C18 column (4.6 × 250 mm, 5 μm), a thermostated column compartment, and an Agilent ChemStation. Analysis was done at flow rate of 1.0 mL min^{-1}, temperature of 25°C, injection volume of 20 μL, and mobile phase 55% acetonitrile:45% deionized water with diode array detector. The detection limit for moist soils extracted with methanol was 0.005 μg g^{-1}.

Screening for Atrazine Mineralization Genes

Before sieving, approximately 0.3 g (fresh weight) of soil was set aside from each sample and stored at −20°C until DNA extraction. DNA was extracted using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc.) according to the manufacturer's instructions. To check for PCR inhibitors, amplification of the 16S ribosomal RNA gene using the universal bacterial primers Bac8 and Univ1492 was attempted on each DNA extract following previously described procedures (Jackson et al., 2001). A lack of amplified products after this reaction in most samples suggested the presence of PCR inhibitors, so a nested PCR approach was used to screen samples for the presence of the *atzA*, *atzB*, *atzC*, and *trzN* genes using primers listed in Table 2. *Arthrobacter aurescens* TC1 containing *trzN*, *atzB*, and *atzC* (Sajjaphan et al., 2004); the *atzA*-carrying plasmid pMD4 (de Souza et al., 1995); and nonatrazine metabolizing *Escherichia coli* DH5α were used as positive and negative controls, respectively. Polymerase chain reactions contained 50 mmol L^{-1} KCl, 10 mmol L^{-1} Tris-HCl (pH 8.3 at 25°C), 1.5 mmol L^{-1} Mg^{2+}, 0.4 μmol L^{-1} each primer, 200 μmol L^{-1} each dNTP, 1.25 U Taq polymerase, and 2 μL DNA extract in a final volume of 50 μL. Amplification reactions were performed using the following cycle: 95°C for 2 min, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 7 min. Nested PCR reactions were performed by taking 2 μL of product from the first reaction and reamplifying it with another set of primer pairs (Table 2) under the same reaction and cycle conditions except with a 30-s extension time. Polymerase chain reaction products were resolved on a 1% agarose gel and examined for bands in the expected size range. For samples yielding negative results, the original and nested PCRs were repeated using DNA extracts diluted 10- or 100-fold to further overcome PCR inhibition.

Statistics

All analyses were conducted in JMP version 8.0.1 (SAS Institute Inc.). Atrazine degradation data were fitted to the first-order decay kinetics equation in JMP:

$$A = A_0 e^{-kt} \quad [1]$$

where A_0 is the concentration of atrazine at time zero (mg kg^{-1}), k is the first-order rate constant (d^{-1}), and t is time (days). Half-life ($T_{1/2}$) of atrazine for each sample site was calculated from the equation:

$$T_{1/2} = \ln\frac{2}{k} \quad [2]$$

The Pearson correlation coefficient was calculated to determine the potential correlation between FDA activity and atrazine half-life. Two-way ANOVA was conducted to determine the effects of landscape (field or ditch) and crop (corn or soybean) on atrazine half-life. For all other comparisons, one-way ANOVA followed by Tukey's honestly significant difference test was used to determine potential differences in pH, total C, total N, FDA activity, percent atrazine decrease, and atrazine half-life between sample types (field, ditch, or nonagricultural) and percent atrazine decrease between soil samples and autoclaved controls. All analyses were conducted using an α of 0.05.

Results and Discussion

Characterization of select physical and chemical properties of soil samples are reported in Table 1. Field soils differed from ditches in some aspects. These differences were expected owing to the different functions that fields and ditches serve and, consequently, the different conditions and management practices they are subjected to. Ditches tended to be more sandy, whereas fields had a higher silt content. Similarly, fields also had higher total C ($p \le 0.0412$) and total N ($p \le 0.0210$) than ditches, although C and N in soils were not higher than 2 and 0.2%, respectively. Characteristics of nonagricultural control soil more closely matched those in ditches. Both were sandy and had similar total C ($p \ge 0.6693$) and N ($p \ge 0.6778$) content. There was no significant difference in pH between fields, ditches, and the nonagricultural control ($p = 0.1203$). However, all soils were

Table 2. Primers used in this study for the detection of atrazine-degrading genes and overall bacterial DNA.

Primer	Sequence	Reference
Bac8f	AGAGTTTGATCCTGGCTCAG	Jackson et al. (2001)
Univ1492r	GGTTACCTTGTTACGACTT	Jackson et al. (2001)
atzAF	CCATGTGAACCAGATCCT	de Souza et al. (1998a)
atzAR	TGAAGCGTCCACATTACC	de Souza et al. (1998a)
atzBF	CATGGCGGCGGCAGGTGTG	de Souza et al. (1998a)
atzBR	CCCGGGCCAGCTCCATTTCA	de Souza et al. (1998a)
atzCF	AGTCAGCGAAGGGCGTAGGTATCA	de Souza et al. (1998a)
atzCR	GACAAATCCGGGAGACACAAGGTT	de Souza et al. (1998a)
trzN10	CACCAGCACCTGTACGAAGG	Mulbry et al. (2002)
trzN11	GATTCGAACCATTCCAAACG	Mulbry et al. (2002)
atzAFn	ACGGGCGTCAATTCTATGAC	this study
atzARn	ATCTGACAGGGCTGTGATCC	this study
atzBFn	CAACGACAGCTCGAACCTC	this study
atzBRn	ACACCACCTTGCCGTTGAT	this study
atzCFn	GTTGAAAACGGAGGCCATAA	this study
atzCRn	CCCTTTGCATCAATTTCGTC	this study
trzN10n	AAGGCGTCCTGACCAGAAG	this study
trzN11n	TCGATGAGACCCAGACAGTG	this study

acidic, which is known to slow down biotic atrazine degradation (Mueller et al., 2010).

Numerous studies have reported that prior application can lead to rapid atrazine degradation in soil from agricultural fields (Barriuso and Houot, 1996; Ostrofsky et al., 1997; Yassir et al., 1999; Zablotowicz et al., 2006b, 2007; Shaner and Henry, 2007;Krutz et al., 2008). The field and ditch soils from corn and soybean fields examined in the current study exhibited enhanced degradation, with >95% of atrazine spiked into soils degraded after 28 d (Fig. 1). These observations are consistent with those of Zablotowicz et al. (2007), who found that atrazine mineralization rates in fields receiving biannual atrazine treatment were similar to those treated annually. In comparison, significantly (p < 0.0001) less of the spiked atrazine (68.5%) was degraded in control nonagricultural soil, which is in agreement with the results of Barriuso and Houot (1996), who found that atrazine mineralization was related to land history, such that the highest mineralization rates were observed in soil from continuous maize production treated with atrazine annually, whereas rates were lowest in land never treated with atrazine.

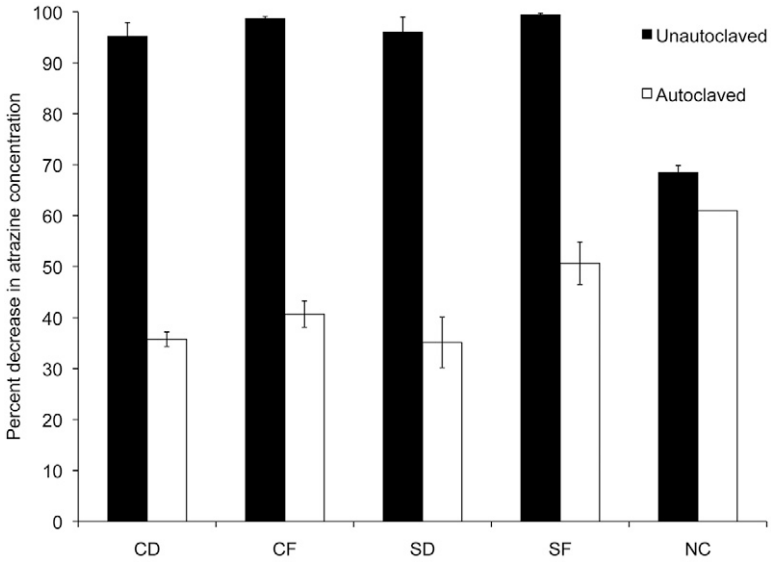

Fig. 1. Total percent decrease in methanol-extractable atrazine after 28-d incubation at 30°C in autoclaved and unautoclaved soil spiked with atrazine to yield a target concentration of 1 mg kg^{-1}. Soil samples were collected from a corn field (CF) and adjacent ditch (CD), a soybean field (SF) and adjacent ditch (SD), and a nonagricultural plot with no history of atrazine application as a control (NC). Values are mean ± SE of replicates per sample.

A two-way ANOVA of atrazine half-life found no interaction between landscape (field vs. ditch) and crop (corn vs. soybean) (p = 0.87). Although rates of degradation were slightly less in ditches compared with both field soils (Fig. 2), the differences in half-life were not statistically significant (p = 0.12); this may be the result of the small sample size. Atrazine appeared to dissipate more rapidly in ditches than in nonagricultural soil, where the half-life of atrazine was 17.9 ± 1 d, 3 to 12 times longer than the half-lives observed in ditches and fields (Table 3). However, owing to the presence of an outlier in the soybean ditch and the small sample size, this difference was only significant between control and corn ditch soils (p = 0.04) (Table 3). Overall, degradation was substantially slower in soil with no history of prior atrazine treatment in this and several other studies (Ostrofsky et al., 1997; Yassir et al., 1999; Anderson et al., 2002; Zablotowicz et al., 2006b; Shaner and Henry, 2007; Krutz et al., 2008).

The FDA activity within fields and ditches was highly variable (Table 3). Therefore, there were no significant differences between any of the sampling areas (p = 0.94). In addition, there was no strong correlation between FDA activity or the half-life of atrazine in soil (r = 0.2032; p = 0.15) (Fig. 3). Fluorescein diacetate hydrolysis is a measure of microbial activity in soil (Schnurer and Rosswall, 1982). Thus, the lack of correlation between FDA activity and atrazine half-life indicates that differences in atrazine degradation potential between sites were the result of specific adaptation of the microbial community to metabolize atrazine rather than overall microbial activity.

In field and ditch soils, >95% of atrazine spiked into soils had degraded after 28 d, whereas <51% was degraded in the autoclaved soils (Fig. 1). In contrast to field and ditch soils, there was no significant difference in the percent atrazine decrease observed in autoclaved and unautoclaved nonagricultural soil (p = 0.87). These results indicate that a large part of atrazine dissipation in fields and ditches was microbially mediated, whereas its loss in nonagricultural soil was mainly the product of abiotic processes, such as adsorption to soil particles and chemical hydrolysis.

The detection of atrazine degradation genes is usually limited to soil displaying enhanced degradation (Anderson et al., 2002; Piutti et al., 2002; Krutz et al., 2008). For most samples collected in the current study, atrazine genes were not detected in original DNA soil extracts without the use of template dilution and nested PCR, which is in agreement with Shapir et al. (2000), who found nested PCR to be the most effective

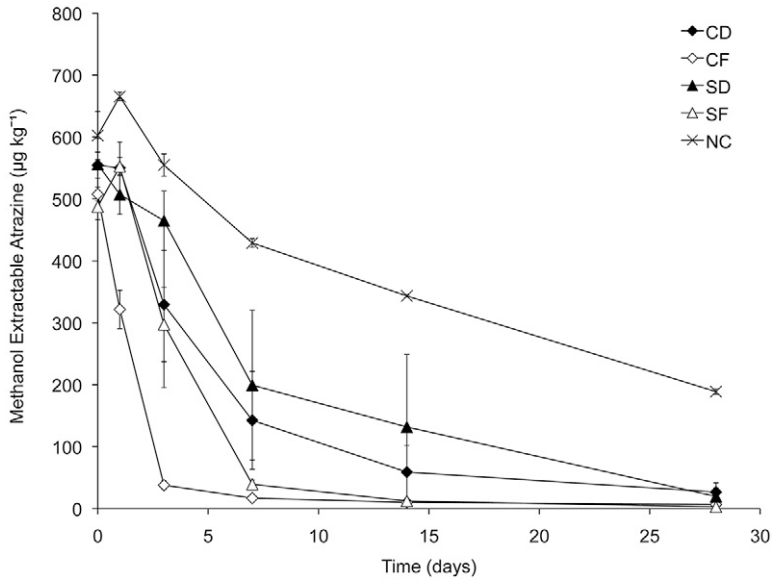

Fig. 2. Loss of atrazine over time in soils spiked to yield a target concentration of 1 mg kg^{-1} atrazine. Soil samples were collected from a corn field (CF) and adjacent ditch (CD), a soybean field (SF) and adjacent ditch (SD), and a nonagricultural plot with no history of atrazine application as a control (NC). Values are mean ± SE of replicates per sample.

way to predict atrazine mineralization in soil. Polymerase chain reaction screening indicated the widespread presence of atrazine degradation genes in all samples from agricultural sites (Table 4). Prior research on agricultural soils in Mississippi failed to detect the *atzA* gene by direct PCR amplification (Zablotowicz et al., 2006b). Therefore, its detection in all but one agricultural sample was unexpected. The difference in *atzA* detection between these two studies was attributed to the use of nested PCR and to differences in soil type and cropping history between northwest Mississippi, where Zablotowicz et al. (2006b) sampled, and northeast Mississippi, where the current study was conducted. The nested PCR approach may also aid in detecting *atzA* in other locations that previously reported difficulty with this gene. Five sixths of drainage ditch sampling sites possessed a full complement of the atrazine degradation genes screened (*atzA*, *atzB*, *atzC*, and *trzN*). Three of the agricultural soil samples (CD3, SF2, and SF3) were not positive for all degradation genes. However, it is possible that these genes were present but at low copy numbers that could not be detected by PCR and gel visualization. In addition, Martin-Laurent et al. (2006) reported the isolation of some atrazine-degrading bacterial communities that only possessed genes representing truncated atrazine degradation pathways. None of the atrazine degradation genes was detected in nonagricultural soil. Of the genes screened in the current study, *trzN* proved to be the best predictor of enhanced atrazine degradation activity, being the only gene detected in all of the soils that displayed rapid atrazine breakdown.

Atrazine degradation in drainage ditch soils was highly variable, which may be indicative of patchy exposure and deposition of atrazine transported in runoff. In addition, the presence of vegetation is known to stimulate atrazine mineralization in soil (Marchand et al., 2002; Piutti et al., 2002; Singh et al., 2004). Because vegetation at the drainage ditch sites sampled in the current study was sporadic, this could also explain the high variability in atrazine half-life observed in drainage ditch samples.

A number of studies have found that the maize rhizosphere influences atrazine mineralizing members of the soil microbial community. Piutti et al. (2002, 2003) found that *atzC* and *trzN* levels were higher in the maize rhizosphere than in bulk soil, and Martin-Laurent et al. (2006) found that, although *trzN* was prevalent in the rhizosphere and bulk soil, *atzA* was found more frequently in communities from the rhizosphere. The maize rhizosphere may also enhance atrazine breakdown through chemicals in root exudates. One such chemical, DIMBOA (3,4-dihydro-2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3-one), is a hydroxamic acid that promotes rapid chemical hydrolysis of atrazine (Wenger et al., 2005). However, DIMBOA and its analogs break down rapidly in soil with half-lives <62 h, whereas its more persistent breakdown product, MBOA, does not stimulate atrazine hydroxylation (Raveton et al., 1997; Macías et al., 2004). Given that the current study sampled bulk soil after corn was harvested, it is unlikely this compound was present in concentrations high enough to influence the atrazine dissipation observed, although the effects of other root exudates (either from corn, soybean, or ditch vegetation) remaining in soil samples cannot be discounted. Because the collection of rhizosphere samples was not the focus of the current study, future research looking specifically at the rhizosphere and the impact of drainage ditch vegetation on atrazine degradation may provide a better understanding of this system.

Table 3. Fluorescein diacetate activity and atrazine degradation parameters in soil collected from fields, drainage ditches, and nonagricultural control.†

Sample area	FDA‡ activity	Degradation constant	Half-life
	nmol gdw^{-1} h^{-1}		d
Corn ditch	62.9 ± 14.5	0.264 ± 0.130	4.5 ± 2.1
Corn field	65.5 ± 5.6	0.456 ± 0.026	1.5 ± 0.1
Soybean ditch	57.6 ± 22.2	0.172 ± 0.062	6.1 ± 3.1
Soybean field	48.9 ± 17	0.285 ± 0.043	2.6 ± 0.4
Nonagricultural soil	50 ± 18.6	0.039 ± 0.002	17.9 ± 1.0

† All values reported as mean ± SE.

‡ Fluorescein diacetate activity.

Dissipation of atrazine in sediments was observed in the current study. However, several intermediates in the breakdown of atrazine are known to accumulate in the soil and water, and several of these products still possess some toxicity. Since 1993, the major route of atrazine degradation observed in previously exposed soils is via *atzABC/trzN* mediated dehalogenation leading to production of hydroxyatrazine and later cyanuric acid intermediates (Wackett et al., 2002; Shapir et al., 2007; Krutz et al., 2010). Because the *atzABC/trzN* genes were detected in the agricultural soils analyzed in the current study, it is likely this is the primary route of biotic atrazine degradation occurring in these systems, although reverse transcriptase PCR would be necessary to confirm these genes are actively expressed during degradation. Before 1993, biotic dealkylation of atrazine resulting in desethylatrazine and deisopropylatrazine intermediate products was the dominant atrazine degradation pathway observed in soils and is still seen in atrazine-exposed soils today, though to

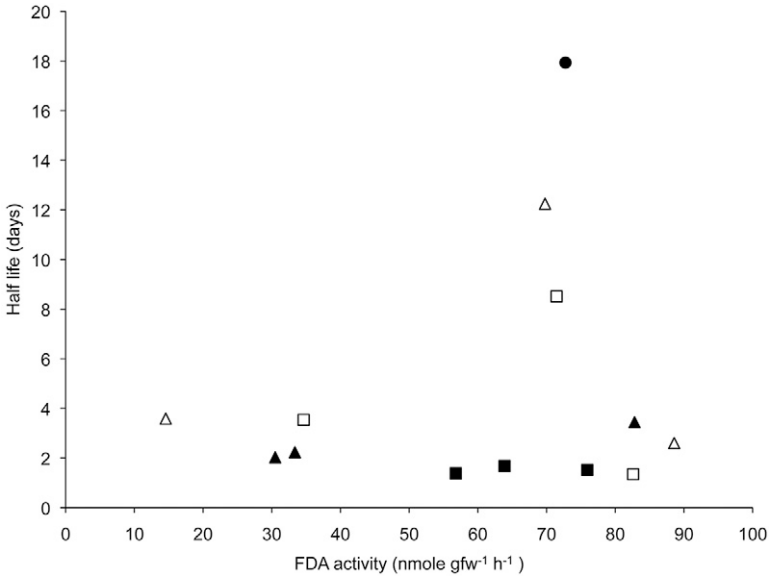

Fig. 3. Scatter plot of atrazine half-life (days) plotted against microbial activity as determined from fluorescein diacetate (FDA) activity (nmol gdw^{-1} h^{-1}) in corn field (closed squares) and adjacent ditch (open squares), soybean field (closed triangles) and adjacent ditch (open triangles), and nonagricultural control (closed circle).

Table 4. Presence of atrazine degradation genes in corn ditch and field, soybean ditch and field, and nonagricultural control samples.

Sample†	*trzN*	*atzA*	*atzB*	*atzC*
CD1	+	+	+	+
CD2	+	+	+	+
CD3	+	+	–	–
CF1	+	+	+	+
CF2	+	+	+	+
CF3	+	+	+	+
SD1	+	+	+	+
SD2	+	+	+	+
SD3	+	+	+	+
SF1	+	+	+	+
SF2	+	+	–	+
SF3	+	–	–	+
NC	–	–	–	–
pMD4‡	–	+	–	–
TC1§	+	–	+	+
DH5α¶	–	–	–	–

† CD, corn ditch; CF, corn field; NC, nonagricultural control; SD, soybean ditch; SF, soybean field.

‡ Plasmid pMD4 containing *atzA*.

§ *Arthrobacter aurescens* TC1 containing *trzN*, *atzB*, and *atzC*.

¶ *Escherichia coli* DH5α negative control.

a lesser extent (Wackett et al., 2002; Krutz et al., 2008; Krutz et al., 2010). Desethylatrazine and deisopropylatrazine still possess phytotoxic effects (Sirons et al., 1973). In addition, the cyanuric acid intermediate of the *atzABC/trzN* pathway is known to have deleterious effects on kidney function when combined with melamine (Pang et al., 2011; Ding et al., 2012). Thus, although the current study was unable to analyze samples for atrazine degradation intermediates, toxic byproducts of this herbicide may still be present in drainage ditches even after original concentrations have dissipated.

Moore et al. (2001) found higher concentrations of atrazine in drainage ditch sediments than water over the course of a 28-d study. In addition, the half-life of atrazine in drainage ditch waters was more than 3 times lesser than in sediments. However, the rapid decrease of atrazine in the water column may have been the result of settling and uptake in sediments and ditch vegetation in addition to degradation. In spite of its relatively short half-life in drainage ditch water, atrazine and its degradation products are still detected in rivers and groundwater aquifers (Clark and Goolsby, 2000; Steele et al., 2008; Iker et al., 2010). In addition, Kröger et al. (2012) detected atrazine in water and sediments from agricultural drainage ditches in concentrations of 13.5 μg L^{-1} and 271 μg kg^{-1}, respectively, at several sites in the Lower Mississippi Alluvial Valley. Given that atrazine is capable of leaching from soil into water (Fava et al., 2007), any atrazine deposited into drainage ditch sediments can potentially be released back into the water column. As a result, microbial communities with the ability to degrade atrazine in these ditch sediments could contribute to lowering concentrations of this herbicide in the water and decrease the amounts transported into downstream ecosystems.

Conclusion

Runoff from agricultural land is capable of leaching agrochemical pollutants from soil in fields and transporting it through drainage ditches into downstream receiving systems. Results from the current study demonstrate that microbial communities present in the sediments of these ditches are capable of breaking down atrazine at an accelerated rate. Thus, these communities have the potential to remove atrazine deposited in drainage ditches, thereby limiting the amounts of this herbicide carried into downstream ecosystems.

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