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## Abstract

Four greenhouse studies were conducted to evaluate the effects of native prairie grasses and two pesticide-degrading bacteria to remediate atrazine and metolachlor in soils from agricultural dealerships (Alpha site soil, northwest Iowa, USA; Bravo site soil, central Iowa, USA). The Alpha soil contained a low population of atrazine-degrading microorganisms relative to the Bravo soil. Each soil freshly treated with atrazine or metolachlor was aged for a short or long period of time, respectively. An atrazine-degrading bacterium, *Agrobacterium radiobacter* strain J14a; a metolachlor-degrading bacterium, *Pseudomonas fluorescens* strain UAS-40; and a mixture of three native prairie grasses—big bluestem (*Andropogon gerardii* Vitman), yellow Indian grass (*Sorghastrum nutans* [L.] Nash), and switchgrass (*Panicum virgatum* L.)—were added to the soils after the soils were aged for long periods of time. The soils aged for short periods of time were treated with J14a, the prairie grasses, or both after aging. The J14a and the grasses significantly reduced the concentration of atrazine in Alpha soil when the soil was aged for a short period of time. However, these treatments had no statistically significant effect when the soil was aged for a long period of time or on atrazine in Bravo soil. Inoculation with UAS-40 did not enhance metolachlor dissipation in either soil, but vegetation did increase metolachlor dissipation. Our results indicate that the dissipation of atrazine by J14a is affected by the presence of indigenous atrazine-mineralizing microorganisms and probably by the bioavailability of atrazine in the soil.

## Keywords

Bacteria, Atrazine, Metolachlor, Bioavailability, Vegetation

## Disciplines

Entomology | Soil Science

## Comments

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## EVALUATION OF MICROBIAL INOCULATION AND VEGETATION TO ENHANCE THE DISSIPATION OF ATRAZINE AND METOLACHLOR IN SOIL

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**Abstract**—Four greenhouse studies were conducted to evaluate the effects of native prairie grasses and two pesticide-degrading bacteria to remediate atrazine and metolachlor in soils from agricultural dealerships (Alpha site soil, northwest Iowa, USA; Bravo site soil, central Iowa, USA). The Alpha soil contained a low population of atrazine-degrading microorganisms relative to the Bravo soil. Each soil freshly treated with atrazine or metolachlor was aged for a short or long period of time, respectively. An atrazine-degrading bacterium, *Agrobacterium radiobacter* strain J14a; a metolachlor-degrading bacterium, *Pseudomonas fluorescens* strain UA5-40; and a mixture of three native prairie grasses—big bluestem (*Andropogon gerardii* Vitman), yellow Indian grass (*Sorghastrum nutans* [L.] Nash), and switchgrass (*Panicum virgatum* L.)—were added to the soils after the soils were aged for long periods of time. The soils aged for short periods of time were treated with J14a, the prairie grasses, or both after aging. The J14a and the grasses significantly reduced the concentration of atrazine in Alpha soil when the soil was aged for a short period of time. However, these treatments had no statistically significant effect when the soil was aged for a long period of time or on atrazine in Bravo soil. Inoculation with UA5-40 did not enhance metolachlor dissipation in either soil, but vegetation did increase metolachlor dissipation. Our results indicate that the dissipation of atrazine by J14a is affected by the presence of indigenous atrazine-mineralizing microorganisms and probably by the bioavailability of atrazine in the soil.

**Keywords**—Bacteria Atrazine Metolachlor Bioavailability Vegetation

## INTRODUCTION

The widespread use of atrazine (ATR; 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) and metolachlor (MET; 2-chloro-*N*-[2-ethyl-6-methylphenyl]-*N*-[2-methoxy-1-methyl-ethyl]acetamide) as preemergent herbicides to control grass weeds and broadleaf weeds has caused the contamination of surface and groundwaters [1,2]. Agricultural dealership sites represent a potential point source of groundwater contamination [3]. Effective and low-cost remediation approaches at agricultural dealership sites are needed.

The remediation of ATR-contaminated soils by pure cultures of bacteria has been investigated extensively [4–7]. The effectiveness of remediation with ATR-degrading bacteria in nonsterile soils has varied. Bioaugmentation with an ATR-mineralizing bacterium, *Agrobacterium radiobacter* strain J14a, on the mineralization of ATR in a nonsterile soil has not enhanced the degradation of ATR because of the presence of indigenous ATR degraders [6]. A carbon source was needed to stimulate the mineralization of high concentrations of ATR by *Pseudomonas* sp. strain ADP [4]. Exogenous nitrogen inhibited the mineralization of ATR by M91-3, an atrazine-mineralizing bacterium [8]. Biodegradation was the primary means of MET dissipation in soil [9]. Several species of microorganisms could transform MET [10,11]. However, those organisms did not appear capable of mineralizing MET completely.

A current interest is the use of plants to remediate contaminated soils, sediments, and water. Plants remediate organic

compounds via rhizosphere degradation and via direct uptake of organics and transformation of the organics to less toxic metabolites [12]. Physicochemical properties of the compounds, plant species characteristics, and environmental conditions, such as soil properties, are the main factors that determine the rate of chemical uptake [12,13]. A large portion of the applied [<sup>14</sup>C]ATR (91%) in soil has been shown to be taken up by poplar cuttings *Populus deltoides nigra* DN 34 [14]. However, 28% and 9.9% of ATR uptake was reported in corn and *Kochia scoparia*, respectively [15,16].

The rhizosphere is the region immediately surrounding the roots of a plant. It serves as an enrichment zone for increased microbial activity via root exudation and rhizodeposition from the decay of dead root hairs and fine roots, which supply important nutritional sources for microbial growth [17]. Great density and diversity of microorganisms are present in the rhizosphere [15,17]. As a result, catabolic or cometabolic transformation occurs there. Plants also transfer oxygen to the root zone. These phenomena may enhance the transformation of organics in the root area. Studies have demonstrated the increased degradation of organics in the rhizosphere of a variety of plant species [17–19]. However, in some cases, the rhizosphere has no effect on the mineralization of organic compounds [15,20].

Bioavailability is a key factor in determining the success of various remediation strategies. As organic compounds reside in the soil over time, they become increasingly unavailable for biodegradation [21,22]. The decline in bioavailability could result from the slow diffusion of hydrophobic compounds into soil organic matter [23], sorption of some organic compounds

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Table 1. Characteristics of Alpha soil (northwest Iowa, USA) and Bravo soil (central Iowa, USA)

Characteristics <sup>a</sup>	Alpha sandy loam	Bravo loam
Sand (%)	68	32
Silt (%)	21	50
Clay (%)	11	18
OM (%)	2.5	3.9
N (%)	0.08	0.22
pH	7.8	7.5
CEC (meq/100 g)	10	14

<sup>a</sup> OM = organic matter; N = total nitrogen; CEC = cation exchange capacity.

to black carbon [24], and the diffusion of organic compounds through micropores inside of soil particles during aging [25].

The objectives of this study were to determine the influence of inoculation with herbicide-degrading bacteria on herbicide residues, to determine the effectiveness of vegetation on the dissipation of herbicides in soils, and to determine the effect of aging time in soil on the biodegradability of the herbicides. To accomplish this, four greenhouse studies were conducted to determine the degradation of ATR and MET by the bacteria and vegetation in two soils with different indigenous ATR-degrading microorganisms. After each soil was treated with ATR and MET, the soil was aged for a short period of time or a long period of time before inoculation and vegetation.

## MATERIALS AND METHODS

### Chemicals

The ATR (92.2% pure for treating the soils) and MET (97.3% pure) were obtained from Novartis Crop Protection (Greensboro, NC, USA). The ATR (98% pure analytical standard) was purchased from Chem Service (West Chester, PA, USA).

### Soils and plants

Soil samples were obtained from two agrochemical dealer sites in Iowa, USA. The two sites, denoted as the Alpha site and Bravo site, are located in northwest Iowa and in central Iowa, respectively. Surface soils (top 15 cm) were collected with the use of hand trowels. Three independent composite samples were taken from vegetated areas. Soils were stored in the dark at 4°C for less than six months, and were analyzed by A & L Midwest Laboratories (Omaha, NE, USA) to determine physical and chemical properties (Table 1).

Residual (background) concentrations of ATR and MET from Alpha site were low, with amounts  $\leq 0.3$   $\mu\text{g/g}$  soil [16]. A trace amount of trifluralin (0.1  $\mu\text{g/g}$  soil) was also detected in the Alpha soil. For the Bravo soil, ATR, MET, pendimethalin, and trifluralin were detected, with amounts  $\leq 0.9$   $\mu\text{g/g}$  dry soil [16]. The soils have different numbers of indigenous ATR-mineralizing microorganisms [16]. The two soils were chosen for the current research to test the effect of indigenous ATR-mineralizing microorganisms on the degrading ability of the inoculated ATR-degrading bacteria. Because the average background concentrations of contaminants were low, soils were spiked with a herbicide mixture of ATR and MET.

The plants used in this study were the mixture of three species of native prairie grasses: Big bluestem (*Andropogon gerardii* Vitman), yellow Indian grass (*Sorghastrum nutans* [L.] Nash), and switchgrass (*Panicum virgatum* L.).

### Microorganisms

The two herbicide-degrading bacteria used in the studies included *A. radiobacter* strain J14a, which degrades ATR [6], and *Pseudomonas fluorescens* strain UA5-40, which degrades alachlor [26] and MET [18]. The UA5-40 strain was supplied by R. Zablotowicz, Agricultural Research Service, U.S. Department of Agriculture (Stoneville, MS). The J14a was grown in a basal minimal salts medium supplemented with vitamins, trace elements, and ATR [6]. The ATR was the sole N source. The UA5-40 was grown in a half-strength tryptic soy broth containing 50 mg/L of MET. When the bacteria were grown to late log phase on a rotary shaker, they were centrifuged and resuspended in sterile phosphate buffer [6]. Cell densities were determined by plate count.

### Soil study

The current research is made up of four different studies. In each study, the general procedures are as follows. The soils were weighed into 900-ml treatment jars. An acetone solution containing a mixture of ATR and MET was applied uniformly onto the soils. The soils were mixed well on brown wrapping paper to evaporate acetone and homogenize the treated soils. The soils were placed in Ray Leach Cone-Tainers<sup>TM</sup> (Stuewe & Sons, Corvallis, OR, USA) and were covered with aluminum foil. The soils were aged in the greenhouse at a temperature of  $27 \pm 2^\circ\text{C}$ , and a light cycle of 12:12 h light:dark. The soils were watered with approximately 5 ml of tap water per cone on a weekly basis during the aging period. After the soils were treated with phosphate buffer containing the bacteria,  $\text{NH}_4\text{NO}_3$ , vegetation, or a combination of treatments, the soils were placed in the cones and kept in the greenhouse. Water was added to the soils on a daily basis to maintain adequate moisture until the end of the study. For the vegetation treatment, the mixture of the three native grasses was planted in a small tray in uncontaminated soil in the greenhouse until the height of the grasses ranged from 10 to 20 cm. Then the soil was washed from the grasses with tap water, and the grasses were transplanted into the treated soils in the cones. Each cone contained 6 to 12 grass plants (a mixture of the three species of native prairie grasses).

*Alpha soil short-term study.* This experiment was designed to examine the influence of a mixture of the three native prairie grasses and J14a on the dissipation of ATR and MET after the Alpha soil treated with ATR and MET was aged for a short period of time. Alpha soil was treated uniformly with a mixture of ATR and MET solutions to obtain a soil concentration of 100  $\mu\text{g/g}$  soil (dry wt) for ATR and 25  $\mu\text{g/g}$  soil (dry wt) for MET. The chemicals in the soil were aged for 13 d before adding J14a ( $10^7$  cells/g of soil), vegetation, both J14a ( $10^7$  cells/g of soil) and vegetation, and phosphate buffer. Each treatment was replicated four times, and each replication contained 80 g of soil (dry wt). The study ended 71 d postchemical application. Concentrations of ATR and MET were determined at 13 and 71 d postchemical application. The ATR or MET remaining then is reported as a percentage of the concentrations at day 13.

*Alpha soil long-term study.* This experiment was designed to examine the influence of a mixture of the three native prairie grasses and the bacterial strains J14a and UA5-40 on the dissipation of ATR and MET after the Alpha soil treated with ATR and MET was aged for a long period of time. Alpha soil was treated uniformly with a solution that provided 194  $\mu\text{g/g}$  soil of ATR and 170  $\mu\text{g/g}$  soil of MET. The purpose of

adding high initial concentrations of ATR and MET in the Alpha soil long-term study was to have measurable amounts of the compounds after a long period of aging. The soils were aged for 213 d before adding J14a ( $10^7$  cells/g of soil), UA5-40 ( $10^7$  cells/g of soil), both J14a and UA5-40 (each at  $10^7$  cells/g of soil), phosphate buffer, both J14a ( $10^7$  cells/g of soil) and vegetation, both UA5-40 ( $10^7$  cells/g of soil) and vegetation, J14a and UA5-40 (each at  $10^7$  cells/g of soil) and vegetation, and both phosphate buffer and vegetation. Each treatment was replicated four times, and each replication contained 115 g of soil (dry wt). The study ended 269 d postchemical application. Concentrations of ATR and MET were determined at 213 and 269 d postchemical application. The ATR or MET remaining then is reported as a percentage of the concentrations at day 213.

**Bravo soil short-term study.** This experiment was designed to examine the influence of a mixture of the three native prairie grasses and nitrogen fertilizer on the dissipation of ATR and MET residues after the Bravo soil treated with ATR and MET was aged for a short period of time. Bravo soil was treated uniformly with a mixture of ATR and MET, providing 119  $\mu\text{g/g}$  soil of ATR and 34  $\mu\text{g/g}$  soil of MET. The chemicals in the soil were aged 14 d before adding  $\text{NH}_4\text{NO}_3$  (equivalent to 89.7 kg/ha), vegetation, both  $\text{NH}_4\text{NO}_3$  (equivalent to 89.7 kg/ha) and vegetation, and phosphate buffer. Each treatment was replicated four times, and each replication contained 80 g of soil (dry wt). The study was ended at 71 d postchemical application. The concentrations of ATR and MET were determined at 14 and 71 d postchemical application. The ATR or MET remaining then is reported as a percentage of the concentrations at day 14.

**Bravo soil long-term study.** This experiment was designed to examine the influence of a mixture of the three native prairie grasses, a mixture of J14a and UA5-40, and nitrogen fertilizer on the dissipation of ATR and MET, after the Bravo soil treated with ATR and MET was aged for a long period of time. Bravo soil was treated uniformly with a mixture of ATR and MET solutions to obtain 104  $\mu\text{g/g}$  soil of ATR and 25  $\mu\text{g/g}$  soil of MET. Because of the mineralization of ATR by indigenous ATR-mineralizing microorganisms in the Bravo soil [16], the chemicals in the soil were aged for 56 d so that a measurable amount of ATR remained after aging. Then, the soils were treated with inoculation only, addition of  $\text{NH}_4\text{NO}_3$  only, inoculation plus the addition of  $\text{NH}_4\text{NO}_3$ , addition of phosphate buffer only (control), inoculation plus vegetation, addition of  $\text{NH}_4\text{NO}_3$  plus vegetation, inoculation plus the addition of  $\text{NH}_4\text{NO}_3$  and vegetation, and addition of phosphate buffer plus vegetation. Inoculation included both J14a and UA5-40, each at  $10^7$  cells/g of soil. The amount of  $\text{NH}_4\text{NO}_3$  added was equivalent to 89.7 kg/ha. Each treatment was replicated four times, and each replication contained 115 g of soil (dry wt). Each replication contained more soil than that in the short-term studies because of the need for enumeration of ATR-mineralizing microorganisms at the end of the study. The study ended 171 d postchemical application. Concentrations of ATR and MET were determined at 56 and 171 d postchemical application. The ATR or MET remaining then is reported as a percentage of the concentrations at day 56. A  $^{14}\text{C}$  most probable number experiment that used [ $^{14}\text{C}$ -U-ring]ATR as a nitrogen source [16] was done to determine the number of ATR-mineralizing microorganisms (both native and inoculated J14a) present in Bravo soil at the end of the study. An aliquot of 5 g (wet wt) was taken from each replication of each treatment. The soil

aliquots from four replications of each treatment were combined and mixed. A 10-g subsample was taken from the combined soil of each treatment and was added to 90 ml of sterile phosphate buffer. Tenfold serial dilutions were made by sequential transfer of 1-ml subsamples into 9-ml sterile phosphate buffer. Aliquots of 100  $\mu\text{l}$  of each soil dilution and 500  $\mu\text{l}$  of [ $^{14}\text{C}$ ]ATR treating solution containing a mineral salts broth and trace elements were pipetted to sterile shell vials. The shell vials were stoppered with sterile foam plugs and were placed in scintillation vials containing 1 ml of 1 N NaOH. The scintillation vials were incubated at 20 to 23°C for 50 d. The radioactivity in the scintillation vials was used to determine the positive most probable number tubes.

#### *Extraction and gas chromatographic analysis*

Extraction and gas chromatographic analysis methods have been described by Anhalt et al. [27]. Briefly, the soils were extracted three times with ethyl acetate by mechanical shaking. The extracts were concentrated by rotary evaporation. The concentrated extracts were analyzed with a gas chromatograph with a flame thermionic detector. The extraction efficiency for ATR and MET was 107% ( $\pm 9\%$ ) and 98% ( $\pm 0.1\%$ ), respectively, on the basis of spike recovery tests. Quantitation limit = (the concentration [ $\mu\text{g/ml}$ ] of the standards required to give a signal-to-noise ratio of 2:1)  $\cdot$  (10 ml of the soil extract)/25 g soil. For ATR and MET this limit was evaluated as 0.078 and 0.313  $\mu\text{g/g}$ , respectively.

#### *Statistical analysis*

All studies were evaluated with analysis of variance and a factorial design with vegetation, bacterial inoculation, or nitrogen fertilizer as the main factors. Confidence intervals for the most probable number procedure were determined by Cochran's methods [28].

## RESULTS

### *Alpha soil short-term study*

The average concentrations of ATR and MET before vegetation and inoculation were  $93.3 \pm 4.5$  and  $24.2 \pm 2.0$   $\mu\text{g/g}$ , respectively. The mixture of prairie grasses had a significant effect on the dissipation of both ATR and MET ( $p = 0.0020$  and  $0.0092$  for ATR and MET, respectively; Table 2). The J14a significantly decreased the percentage of ATR remaining ( $p = 0.0025$ ; Table 2); however, J14a had no significant effect on the dissipation of MET (Table 2).

### *Alpha soil long-term study*

The concentrations of ATR and MET in the Alpha soil long-term study after 213 d of aging were  $4.3 \pm 1.4$  and  $71.0 \pm 18.2$   $\mu\text{g/g}$ , respectively. No statistical difference was seen in the ATR remaining between the vegetated soil and the un-vegetated soil at 269 d postchemical treatment, which was 90.5% for the vegetated soil and 94.6% for the un-vegetated soil. However, the percentage of MET remaining in the vegetated soil at day 269 was significantly less than that in the un-vegetated soil ( $p = 0.0001$ ; Table 3). Inoculation of J14a or UA5-40 did not significantly enhance the degradation of ATR and MET. The ATR remaining in the soil inoculated with J14a and the MET remaining in the soil receiving the inoculation of UA5-40 at the end of the study was 93.3% and 72.6%, respectively, whereas in the uninoculated soil, the ATR and MET remaining were 91.6% and 75.5%, respectively. No second-order or third-order interactions were significant.

Table 2. Dissipation of atrazine and metolachlor in Alpha soil (northwest Iowa, USA) 58 d after vegetation and inoculation with *Agrobacterium radiobacter* J14a. Atrazine and metolachlor were aged for 13 d before vegetation and inoculation. Data are reported as percent herbicide remaining at 71 d postchemical application

Herbicide	% Herbicide remaining			<i>p</i>	% Herbicide remaining			<i>p</i>
	J14a	No J14a	SEM <sup>a</sup>		Vegetation	No vegetation	SEM	
Atrazine	2.5	13.4	0.02	0.0025	2.3	13.6	0.02	0.0020
Metolachlor	67.7	53.7	6.1	0.1284	47.4	74.0	6.1	0.0092

<sup>a</sup> SEM = standard error of the mean.

#### Bravo soil short-term study

The average concentrations of ATR and MET before vegetation and addition of N fertilizer were  $68.6 \pm 22.7$  and  $30.6 \pm 2.4$   $\mu\text{g/g}$ , respectively. The MET was less persistent in the vegetated soil than in the unvegetated soil ( $p = 0.0008$ ; Table 3). However, the dissipation of ATR was not influenced by the presence of the prairie grasses. The amount of ATR remaining was 13.6% in the vegetated soil compared with 12.6% in the unvegetated soil. No significant differences were seen in the percentage of ATR or MET remaining between N-amended soil and soil without N fertilizer. In the N-amended soil, the ATR and MET remaining was 12.9 and 58.0%, respectively, whereas 13.3% of ATR and 59.3% of MET remained in the nonamended soil. The second-order interaction (N fertilizer  $\times$  vegetation) was not significant for the degradation of ATR or MET.

#### Bravo soil long-term study

The concentrations of ATR and MET in the Bravo soil long-term study before treatment with vegetation, J14a and UA5-40, and nitrogen fertilizer were  $45.4 \pm 10.8$  and  $27.5 \pm 2.7$   $\mu\text{g/g}$ , respectively. Dissipation of MET was significantly greater in the vegetated soil than in the unvegetated soil ( $p = 0.0229$ ; Table 3). However, no enhanced dissipation of ATR was seen in the vegetated soil compared with that in the unvegetated soil. At the end of the study, the amount of ATR remaining was 9.9 and 10.3% in the vegetated and unvegetated soil, respectively. The N fertilizer did not have any significant influence on the degradation of ATR or MET, with 9.0% ATR remaining and 62.4% MET remaining in the N-amended soil compared with 11.3% ATR remaining and 61.9% MET remaining in the nonamended soil. No significant difference was seen in the amount of ATR remaining between the soil inoculated with J14a and UA5-40 and the uninoculated soil (11.3 and 9.0%, respectively). The inoculation of J14a and UA5-40 did not significantly decrease the remaining MET, with 64.9% remaining in the inoculated soil and 59.2% in the uninoculated soil. Third-order interactions (inoculation  $\times$  N fertilizer  $\times$  vegetation) and all second-order interactions (inoculation  $\times$  N

fertilizer, inoculation  $\times$  vegetation, and N fertilizer  $\times$  vegetation) were not significant.

The most probable number showed that significantly more ATR-mineralizing microorganisms were found in the inoculated and vegetated soil than in the inoculated and unvegetated soil (Table 4). However, in the uninoculated soil, the vegetation and no vegetation treatments were not significantly different (Table 4). Also, ATR-mineralizing microorganisms were significantly more numerous in the N-amended soil than in the soil without the amendment of N. In the soil without vegetation and N amendment, ATR-mineralizing microorganisms were significantly more numerous in the uninoculated soil than in the inoculated soil, probably because of the indigenous ATR-mineralizing microorganisms.

## DISCUSSION

#### Dissipation of ATR by bacteria

Strain J14a augmentation was successful in enhancing biodegradation of ATR only in the Alpha soil short-term study. Previous reports showed that 94 to 98% of [<sup>14</sup>C-U-ring]ATR was mineralized by J14a in medium [6,29]. Although mineralization was not monitored in this study, the rapid dissipation of ATR is consistent with those findings. Glutathione *S*-transferase activity on ATR was not observed in UA5-40 [26]. Therefore, it was not a surprise that UA5-40 did not have an effect on the dissipation of ATR. Inoculation of J14a in the Alpha soil long-term study failed to enhance the dissipation of ATR. One possible reason might be the limited bioavailability of ATR. Although bioavailability of ATR was not measured in this study, the comparison of ATR remaining at the beginning of inoculation in the Alpha short-term study and long-term studies (93.3 and 4.3  $\mu\text{g/g}$ , respectively) indicates that the bioavailable amount of ATR in the Alpha long-term study had decreased markedly. Another study conducted in this laboratory showed that bioavailability of ATR, as measured by the amounts of <sup>14</sup>CO<sub>2</sub> evolved, was not significantly different between the Alpha soil aged for 6 d and the Alpha soil aged for 68 d [30]; however, the soil was aged for 213 d in the Alpha long-term study. Chung and Alexander [21]

Table 3. The concentration of metolachlor before vegetation, the duration of vegetation in soils, and the dissipation of metolachlor in Alpha soil (northwest Iowa, USA) long-term study, Bravo soil (central Iowa, USA) short-term study, and Bravo soil long-term study. Data are reported as percent metolachlor remaining at the end of each study

Study	Concentration ( $\mu\text{g/g}$ )	Duration of vegetation (d)	With vegetation (%)	Without vegetation (%)	SEM <sup>a</sup>	<i>p</i>
Alpha soil long-term	71.0	56	55.4	94.0	2.3	0.0001
Bravo soil short-term	30.6	57	50.0	67.3	0.03	0.0008
Bravo soil long-term	27.5	115	57.1	67.5	2.7	0.0229

<sup>a</sup> SEM = standard error of the mean.

Table 4. Effect of vegetation, inoculation of *Agrobacterium radiobacter* J14a and *Pseudomonas fluorescens* UA5-40, and  $\text{NH}_4\text{NO}_3$  on the population of atrazine-mineralizing microorganisms in Bravo soil (central Iowa, USA) at 171 d postchemical application (corresponding 95% confidence intervals are given in parentheses)

Treatment	Microorganisms (cells/g soil $\times 10^4$ )	
	With vegetation	Without vegetation
Inoculation, N <sup>a</sup>	21.7 (6.6–72)	1.6 (0.5–5.3)
Inoculation, no N	0.7 (0.2–2.4)	0.1 (0.02–0.2)
No inoculation, N	31.5 (9.5–104)	4.6 (1.4–15)
No inoculation, no N	2.7 (0.8–8.8)	3.1 (0.9–10)

<sup>a</sup> N = ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ).

showed that only 11.1 to 79.5% of the initial concentrations were extracted after ATR was aged for 200 d in 16 soils. Sequestration was complete after 200 d of aging in most of the soils [21]. Therefore, the remarkable loss of ATR in the Alpha soil long-term study is probably related to sequestration of the compound in the soil. The lower concentration of ATR at the beginning of inoculation in the Alpha long-term study is probably caused by the longer aging time of the treated soil compared with that in the Alpha short-term study.

The absence of a response to J14a inoculation in the Bravo soil appears to be related to the rapid degradation of ATR by indigenous ATR degraders and might be related to lack of competitiveness of J14a in the soil with the indigenous ATR degraders. The number of indigenous ATR-mineralizing microorganisms was low in the Alpha soil [6,16]. However, a much higher number of ATR-mineralizing microorganisms was noted in the Bravo soil. The number of indigenous ATR-mineralizing microorganisms in the Bravo control soil was either not significantly different or was greater than those in the soils with various treatments (Table 4). The number of indigenous ATR-mineralizing microorganisms in Bravo soil is comparable to those found by others [6,16]. The large indigenous population of ATR-mineralizing microorganisms in Bravo soil was effective in mineralizing ATR, and addition of J14a did not generally increase total ATR mineralization [6]. Anhalt et al. [27] reported that the concentration of ATR decreased from 50 to  $<3 \mu\text{g/g}$  after 160 d of incubation in Bravo soil. Another study conducted in this laboratory showed that 62 and 49% of the applied [ $^{14}\text{C}$ ]ATR was evolved as  $^{14}\text{CO}_2$  after 36 d of incubation in *K. scoparia* rhizosphere soil and nonrhizosphere soil from the Bravo site, respectively [19]. A different study from this laboratory showed that 2 and 7.3% of the applied [ $^{14}\text{C}$ ]ATR were extractable from rhizosphere Bravo soils and unvegetated Bravo soils treated at  $50 \mu\text{g/g}$  after 36 d of incubation, respectively [16]. The rapid dissipation of ATR in Bravo soil indicates that remediation of ATR contamination in Bravo soil might not be necessary.

#### Dissipation of MET by bacteria

Strain UA5-40 augmentation was not successful in increasing biodegradation of MET in either the Alpha or the Bravo soil. Liu et al. [31] reported that a strain of *Streptomyces* sp., which can transform MET in growth media, failed to transform MET in a nonsterile soil. In this study, MET is more persistent than ATR in both soils because the average concentrations of MET and ATR at the beginning of the Alpha soil long-term study were 71 and  $4.3 \mu\text{g/g}$ , respectively. Arthur et al. [16] and Perkovich et al. [19] showed that  $<10\%$  of the added MET was mineralized by indigenous bacteria in both Alpha and

Bravo soils. This indicates that indigenous MET degraders were not active in mineralization of MET in both soils. Others also noted that the majority of MET applied was recovered in the solvent extracts after 160 d of incubation in soils [27]. This indicates that the sequestration of MET into the soil micropores is not rapid. The UA5-40 was capable of metabolic transformation of alachlor via glutathione-S-transferase-mediated dechlorination [26] and did transform MET in media [18]. Sufficient nutrients and a significant increase of UA5-40 in soils are probably needed to drive significant metabolic activity of UA5-40 in the current studies.

#### Plant effects on dissipation of ATR and MET

Vegetation significantly enhanced the dissipation of ATR only in the Alpha soil short-term study. Another study conducted in this laboratory also showed that the concentrations of ATR were significantly reduced 28 d after the prairie grasses were planted in the Alpha soil, which was aged for 50 d before vegetation [32]. Arthur et al. [16] reported that *K. scoparia* significantly decreased the extractable ATR in Alpha soil. Alvey and Crowley [15] noted that corn enhanced the formation of hydroxyatrazine. In the Alpha soil long-term study, vegetation did not result in significant enhancement of ATR dissipation. Uptake from roots by plants is one of the ways to remediate the contaminants, and the uptake depends on the concentrations of the chemicals in soil water [12,14]. The average concentrations of ATR before vegetation in the Alpha soil short-term study and in the Alpha soil long-term study were 93.3 and  $4.3 \mu\text{g/g}$ , respectively. The difference in the ATR concentrations before vegetation in the Alpha soil short- and long-term studies is probably the reason for the different effect of vegetation on the dissipation of ATR in the two studies.

The conditions of the Alpha and Bravo soil short-term studies are very similar; however, the effect of vegetation is different. Vegetation had no effect on increasing dissipation of ATR in Bravo soil, and this failure appears to be related to the effective mineralization of ATR by indigenous ATR degraders in that soil. Addition of the prairie grasses resulted in enhanced dissipation of MET in both studies. Others have noted that corn and aquatic plants, such as coontail (*Ceratophyllum demersum*), American elodea (*Elodea canadensis*), and common duckweed (*Lemna minor*), were effective in enhancing the degradation of MET in soil [18] or water [33], respectively.

The enhanced dissipation of ATR and MET by the plants might be caused by the increased uptake of ATR and MET by roots and then transformation by the plants [12,14] or by the transport of the compounds to the root zone by evapotranspiration of the plants and subsequent transformation by rhizosphere microflora [17]. Another study conducted in this laboratory showed that ATR and MET, as well as their biotransformation products, were present in big bluestem, yellow Indian grass, and switchgrass after they were grown in the treated soil [34]. The ATR and MET can also be metabolized by other plants [35–37].

#### The effect of nitrogen fertilizer on the dissipation of ATR

Atrazine can be used by ATR-degrading bacteria as a nitrogen source [8]. The purpose of adding nitrogen fertilizer in the treatments was to test whether exogenous N can inhibit ATR-degrading bacteria to use ATR as a N source. Our data showed that nitrogen fertilizer had no effect on the dissipation

of ATR by J14a and the indigenous ATR degraders. Our result is consistent with the findings of Struthers et al. [6] and Bichat et al. [8], who reported that degradation of ATR by J14a was not affected by the presence of exogenous N in medium.

### CONCLUSION

The effects of J14a on the degradation of ATR depend on the bioavailability of ATR and the presence of indigenous ATR degraders in soil. When the bioavailability of ATR was not limited (93.3  $\mu\text{g/g}$  before inoculation), J14a significantly decreased the ATR residues in Alpha soil, which contained low numbers of indigenous ATR-mineralizing microorganisms. On the other hand, when the bioavailability of ATR was low (4.3  $\mu\text{g/g}$  before inoculation), no accelerated dissipation was seen with inoculation in the same soil. In soil with a high population of indigenous ATR-mineralizing microorganisms, inoculation of J14a or addition of the prairie grasses did not influence the dissipation of ATR. Nitrogen fertilizer did not affect the reduction of ATR concentration by J14a and indigenous ATR degraders. Native prairie grasses significantly decreased the MET residues in both soils. Augmentation with J14a and phytoremediation with native prairie grasses could provide an inexpensive, effective, and aesthetically pleasing way to remediate ATR- and MET-contaminated soils.

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