Review

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Agronomic and environmental implications of enhanced *s*-triazine degradation

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Abstract

Novel catabolic pathways enabling rapid detoxification of *s*-triazine herbicides have been elucidated and detected at a growing number of locations. The genes responsible for *s*-triazine mineralization, i.e. *atzABCDEF* and *trzNDF*, occur in at least four bacterial phyla and are implicated in the development of enhanced degradation in agricultural soils from all continents except Antarctica. Enhanced degradation occurs in at least nine crops and six crop rotation systems that rely on *s*-triazine herbicides for weed control, and, with the exception of acidic soil conditions and *s*-triazine application frequency, adaptation of the microbial population is independent of soil physiochemical properties and cultural management practices. From an agronomic perspective, residual weed control could be reduced tenfold in *s*-triazine-adapted relative to non-adapted soils. From an environmental standpoint, the off-site loss of total *s*-triazine residues could be overestimated 13-fold in adapted soils if altered persistence estimates and metabolic pathways are not reflected in fate and transport models. Empirical models requiring soil pH and *s*-triazine use history as input parameters predict atrazine persistence more accurately than historical estimates, thereby allowing practitioners to adjust weed control strategies and model input values when warranted.

Keywords: enhanced biodegradation; modeling; leaching; weed control; pesticide

1 INTRODUCTION

It is arguable that no highly substituted *s*-triazine compound had been released into the biosphere until the commercial launch of simazine in 1958; thus, it was deemed unlikely that microbial populations would possess the enzymatic ability rapidly to degrade these xenobiotic compounds.[1,2] *s*-Triazine persistence data collected over subsequent decades supported this assumption, and consequently these compounds have been considered recalcitrant. Current weed control programs and pesticide transport models function under this historic paradigm, that is, *s*-triazine herbicides are persistent, provide season-long residual weed control and are susceptible to off-site transport. However, recent data indicate that bacterial adaptations have occurred, thereby enabling rapid mineralization of highly substituted *s*-triazine herbicides. Reviews focusing on the evolutionary significance of these metabolic pathways exist,[3,4] but the scope of this manuscript is to expound on the agronomic and environmental implications of these bacterial adaptations. Specifically, the intention is to:

- characterize the pathways enabling *s*-triazine mineralization;
- describe the agronomic and environmental significance of these pathways;
- delineate the physiochemical properties of soils harboring microbial populations able rapidly to degrade *s*-triazine herbicides;
- project where adaptation may occur based on global soil data;
- determine if cultural practices, that is, herbicide use history, cropping history, residue management practices, and N fertility, affect adaptation;
- identify management practices for improving weed control with *s*-triazine herbicides in affected areas;
- propose input values for modeling atrazine fate, transport and risk assessment;
- describe a multiple linear regression model that predicts atrazine persistence in soil at the global scale.

2 MATERIALS AND METHODS

2.1 Bacterial phylogeny and taxonomy

Bacterial species reported to contain atrazine-metabolizing genes, i.e. *atzABCDEF* and *trzNDF*, or unidentified genes that function like

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atzA or *trzN*, were used to query 16S ribosomal sequences from the Ribosomal Database Project (RDP).[5] When available, a type species was used to represent a reported species; otherwise, the most similar species was used. In cases where the reported species has been renamed or reassigned, the synonyms listed with the National Center for Biotechnology Information GenBank database were used to identify currently accepted taxonomy. In cases where an atrazine-degrading bacterium was not identified at the species level, a type species for that genus was used in its place. These 16S sequences were collected, and a dendogram with bootstrap values was constructed using RDP 10.10 Tree Builder.

2.2 Harmonized world soil database

The harmonized world soil database (HWSD) v.1.1 was used to identify surface soils with sand, silt, clay, organic carbon and pH between the tenth and ninetieth percentile and maximum and minimum values of known *s*-triazine-adapted soils.[6] The HWSD-viewer was used to visualize where these soils occur, and an ARC-GIS shape file containing the latitude and longitude of known adapted soils was overlaid on the HWSD-viewer.

2.3 LEACHM simulations

Two one-dimensional leaching estimation and chemistry models (LEACHMs) were constructed to compare the potential for atrazine and its metabolites to leach through *s*-triazine-adapted and non-adapted soils.[7,8] For the non-adapted simulation, the half-lives for atrazine (60 days), desethylatrazine (DEA) (52 days), deisopropylatrazine (DIA) (36 days) and hydroxyatrazine (HA) (60 days) were assumed to be constant for 0–30 cm depth.[9,10] Persistence estimates increased linearly from 30 to 100 cm depth, with maximum half-life values at the bottom of the root zone threefold higher than that in the upper horizon: 180 days for atrazine, 156 days for DEA, 108 days for DIA and 180 days for HA.[10–12] The percentage of atrazine converted to DEA, DIA and HA was set to reflect historic trends, that is, 72% for DEA, 18% for DIA and 10% for HA.[12] For adapted soils, the half-life for atrazine (6 days), DEA (10 days), DIA (8 days) and HA (6 days) was reduced relative to non-adapted soils and assumed to be constant for 0–30 cm depth.[9–11] Again, persistence estimates increased linearly from 30 to 100 cm depth, but the maximum half-life value at the bottom of the root zone was 11-fold higher than that in the upper horizon: 66 days for atrazine, 110 days for DEA, 88 days for DIA and 66 days for HA.[9–10,13] The proportion of atrazine converted to daughter products was set to reflect alterations in the historic metabolic pathway, that is, 22% for DEA, 7% for DIA and 71% for HA.[9,14]

Ten-year simulations (1995–2004) for the top meter of unsaturated silty loams beneath a field in the Morgan Creek, Maryland, watershed subjected to annual rotations of corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] were constructed for *s*-triazine-adapted and non-adapted soils. The simulation includes five biennial applications of 2.08 kg ha^{-1} atrazine in the spring, preceding the planting of corn.[15] Physical and chemical properties of atrazine and its metabolites are those described previously.[16]

2.4 Multiple linear regression analysis

2.4.1 *Initial data survey from the literature*

Atrazine dissipation was assumed to follow first-order kinetics. Reported and derived atrazine half-life values were normalized to 20 °C using a Q_{10} of 2.2. Soils were identified as adapted by one of four methods: (1) *s*-triazine half-life value <30 days; (2) mineralization assay;[12] (3) dissipation assay;[17] or (4) detection of *atzABCDEF* or *trzNDF* genes. The physiochemical range of *s*-triazine-adapted soils was subsequently identified by constructing box plots for the percentage sand, silt, clay and organic carbon and pH using Sigma plot 10.0. Soils identified as potential outliers via the box-plotting technique, that is, values outside the 10 and 90 percentiles, were omitted from the dataset. Using these criteria, 98 soils with known atrazine use histories and persistence estimates were selected for model development.

2.4.2 *Model development*

Multiple linear regression was used to determine the importance of soil physiochemical parameters and herbicide use history in predicting atrazine persistence in soil (SAS Proc ReG, stepwise option). Parameters evaluated included percentage sand, silt, clay and organic carbon, soil pH (1 : 1 aqueous soil solution paste), consecutive years of atrazine applications, ranging from 0 (no applications) to 5 (five consecutive applications), and atrazine use history in the last five years, where soils receiving an atrazine application = 1 and soils not receiving an atrazine application = 2.

3 RESULTS

3.1 Historic *s*-triazine dissipation pathways

s-Triazine dissipation in soil has been the focus of many studies over the last 50 years because of its widespread use and frequent detection in surface and groundwater.[4,18] The existing paradigm on the rate and path of *s*-triazine dissipation is based almost exclusively on work conducted prior to 1993. Thus, many weed scientists assume that *s*-triazine herbicides dissipate in the soil via either non-biological, chemical hydrolysis or biologically mediated *n*-dealklyation reactions, and that these compounds are more persistent as soil pH increases (Fig. 1).

3.1.1 *s-Triazine dissipation prior to 1993*

The basic assumption prior to the 1990s was that the halogen, methylthioether and *N*-alkyl substitutions on the *s*-triazine ring impede the ability of soil microorganisms to metabolize most herbicidal *s*-triazines.[18] Hence, atrazine and other *s*-triazines were considered to be poorly biodegradable in the soil, as was supported by early research. For example, Cook[1] was able to isolate soil bacteria that could grow on cyanuric acid and related *s*-triazines, but these microbes were not able to grow on *s*-triazine herbicides.

The proposed pathway of *s*-triazine degradation prior to the discovery of microbes that could rapidly degrade these herbicides is reported in Fig. 1. There were two branches in this pathway: (1) a chemical hydrolysis pathway that resulted in the formation of hydroxylated products, and (2) a biological system that *N*-dealkylated the side chains. The biological pathway does not detoxify the herbicides because the metabolites are still phytotoxic, so it was proposed that the primary method for detoxifying *s*-triazines in the soil was through chemical hydrolysis.[19,20]

3.1.2 *Chemical hydrolysis of s-triazines in the soil*

The hydroxyl analogs of atrazine, simazine and propazine can be produced in strongly acid or basic solutions via chemical hydrolysis.[21] Early researchers studying the route of *s*-triazine herbicide dissipation in soil found the hydroxylated metabolites of atrazine, propazine and simazine.[22–25] The formation of these metabolites occurred more rapidly in the presence of soil than

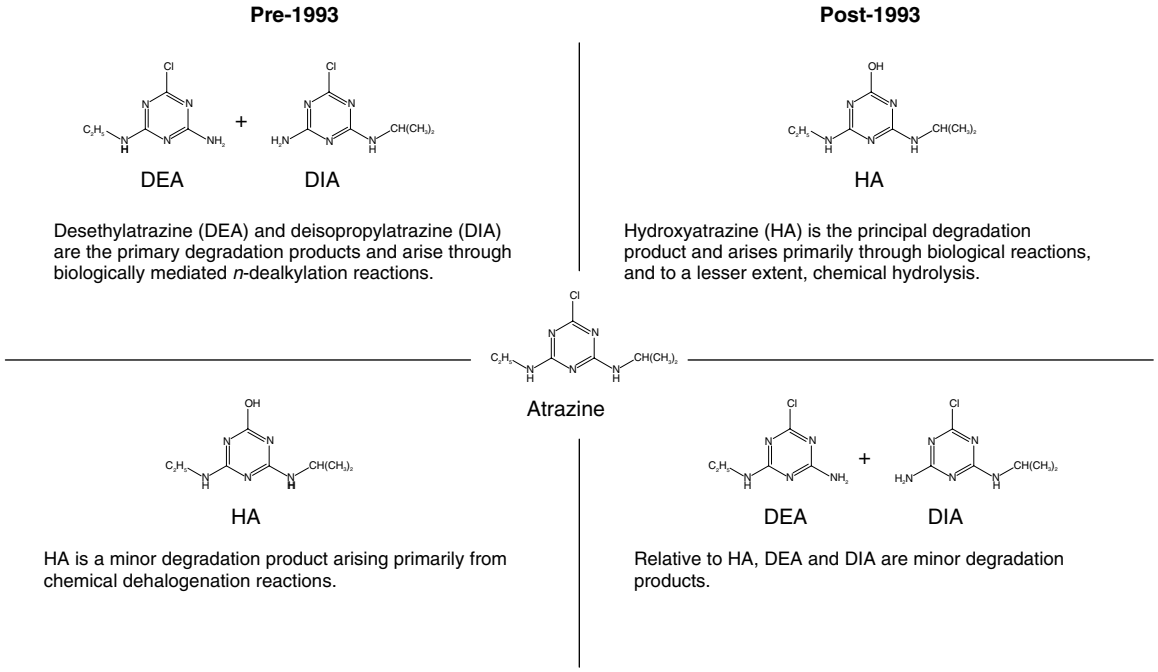

Figure 1. Degradation pathway for atrazine in soil, pre- and post-1993. Adapted from Wackett *et al*[4].

in an aqueous solution.[22] Treating soil with either 200 ppm of sodium azide or heat (95 °C) did not decrease the formation of the hydroxylated metabolites of atrazine, simazine and propazine, thus supporting the assumption that this process was not biologically mediated.[23]

Russell *et al*.[26] demonstrated how montmorillonite clay could catalyze the formation of hydroxytriazines from atrazine and propazine. The hydrolysis occurs in three steps. First, the chloro-*s*-triazine is sorbed to a soil surface through hydrophobic interactions of the alkyl side chain. Then a proton is transferred to one of the ring N atoms. This protonation facilitates nucleophilic attack on the C atom in the 2-position, resulting in the formation of the hydroxylated metabolite.[27]

The rate of abiotic hydrolysis is dependent on pH, with greater hydrolysis as pH decreases. Research prior to the 1990s showed that the persistence of the triazines was the shortest in soils with pH less than 6 and greatest in soils with pH greater than 7. The conclusion from these results was that the primary method of detoxification of the *s*-triazines was chemical hydrolysis, as the hydroxylated metabolites are tightly bound to soil and are non-phytotoxic.[24,28]

3.1.3 *Biological degradation of s-triazines*

Initial work on the metabolism of ^{14}C ring-labeled atrazine in the soil showed minimal or no release of $^{14}CO_2$ from the soil (Fig. 2).[24,29] The rate of $^{14}CO_2$ release was related to the level of organic matter in the soil and general microbial activity.[30] Researchers at the time concluded that ring cleavage of the *s*-triazine played little or no role in the dissipation of the herbicide.

Sirons *et al*.[20] found that atrazine was microbially converted into de-ethylated atrazine (DEA) as a major metabolite and into a de-isopropylated metabolite (DIA) as a minor metabolite. Krutz *et al*.[11] also found that there was more DEA formed compared with DIA in non-adapted soils from Colorado and Mississippi. DEA and DIA bind loosely to soil, and DEA has been frequently found in soil water at greater depths and at higher concentrations in groundwater than atrazine or DIA.[31] Early studies showed that more $^{14}CO_2$ was released when the radiolabel was on the aminoethyl substituent than when it was in the ring or on the aminoisopropyl substituent.[21] However, *N*-dealkylation was not considered to be a detoxification step, as DEA and DIA are still phytotoxic. It was proposed that part of the long-term activity of atrazine was due to the formation of these metabolites.[20] Field

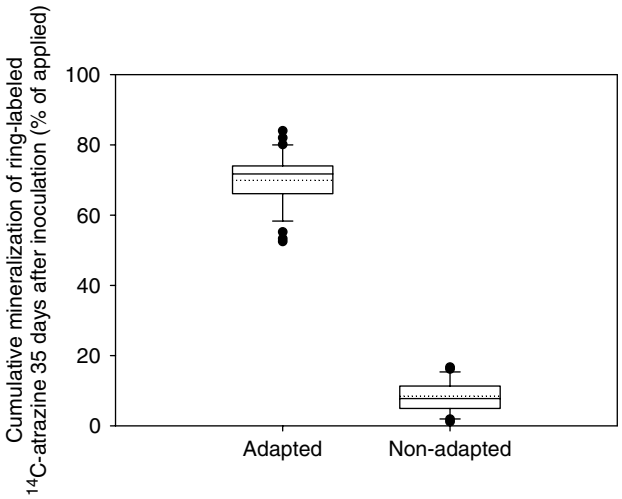

Figure 2. Box plot comparisons of published data for cumulative $^{14}CO_2$ evolution of ^{14}C-ring-labeled atrazine 35 days after inoculation in *s*-triazine-adapted and non-adapted soil. Aadapted from Krutz *et al*.[12] Boundary of box closest to zero indicates the 25th percentile, a solid line within the box marks the median, a dashed line within the box delineates the mean and the boundary of the box furthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentile, and solid dots indicate outliers. The number of independent observations is 22 for non-adapted soils and 54 for *s*-triazine-adapted soils.[75,85,107–110,121,122,125,154–157].

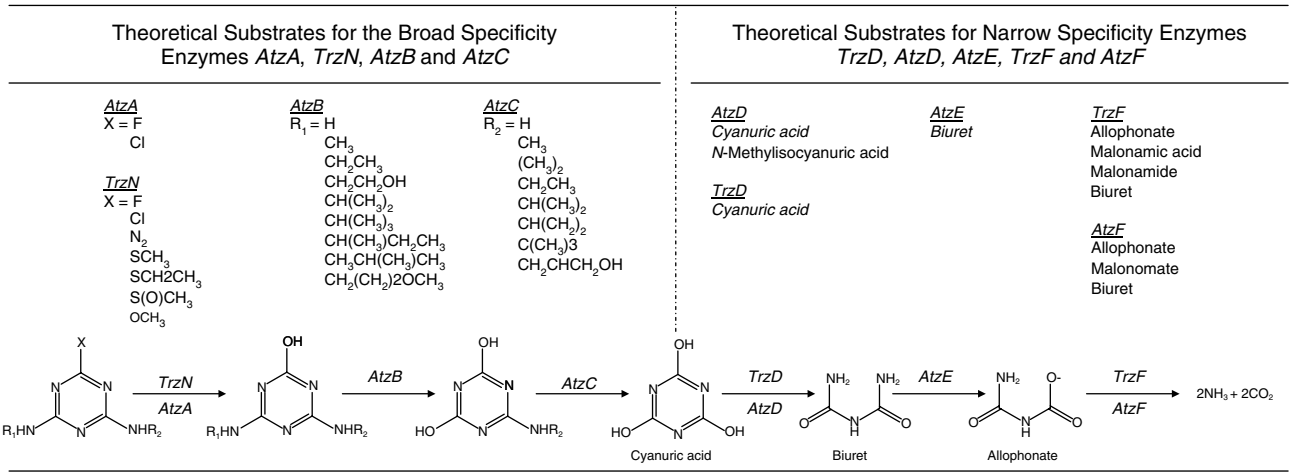

Figure 3. *s*-Triazine metabolism by soilborne bacteria with *atzABCEEF* and *trzNDF* gene homologs. Adapted from Shapir *et al*[3].

work from the 1970s to the 1990s supported this interpretation, as atrazine and other *s*-triazines were shown to have very long residual activity, with half-lives ranging from 28 to 178 days, depending on soil and rate applied.[32–35] Hiltbold and Buchanan[36] estimated that atrazine persistence increased from 9 to 29 days with each unit increase in pH, depending on soil. They also reported that atrazine degraded more rapidly in acid soils compared with basic soils, but that microbes played a greater role in degradation as pH increased.[34,36] Based on these observations, the average atrazine half-life was estimated to be 60 days.[13]

The authors submit that the dissipation pathway in Fig. 1 is accurate for soils that do not have an *s*-triazine application history or where *s*-triazine herbicides are rarely applied. For example, Krutz *et al*.[11] compared atrazine metabolism in non-adapted and adapted soils from Colorado and Mississippi. They found that in soils with no history of atrazine use the half-life was between 32 and 128 days, depending on temperature and moisture. Conversely, in soils with an *s*-triazine application history the atrazine half-life values varied from 1 to 12 days. Moreover, the pattern of metabolite formation in non-adapted soils was similar to that reported by earlier researchers, with an accumulation of DEA, DIA and HA. Thus, in non-adapted soils, the widely accepted paradigm on *s*-triazine dissipation in soil is probably correct. However, in soils harboring microbial populations able rapidly to mineralize *s*-triazine herbicides, the historic pathway is likely overshadowed by a new, biologically mediated dehalogenation pathway.

3.2 *atzABCDEF* and *trzNDF* homologs

3.2.1 *Novel s-triazine catabolic pathways*

The discovery of two bacterial isolates, *Pseudomonas* sp. ADP and *Nocardioides* sp. *C190*, changed current understanding of atrazine's fate in the environment. *Pseudomonas* sp. ADP was isolated from an atrazine-contaminated soil collected from an agricultural chemical dealership in Little Falls, Minnesota, USA,[37–39] and *Nocardioides* sp. *C190* was isolated from a Canadian agricultural soil with a prior atrazine use history.[40,41] The isolation of *Pseudomonas* sp. ADP was unprecedented in that the bacterium mineralized atrazine, a feat deemed unlikely by microbiologists from 1958 until the bacterium's isolation in 1994. Conversely, *Nocardioides* sp. *C190* could not mineralize atrazine but degraded a broader range of *s*-triazine herbicides than did *Pseudomonas* sp. ADP.[40,41] In the succeeding years, the underlying principles regulating *s*-triazine catabolism by these bacteria were elucidated. Herein, a review is given of the bacterial genes *atzABCDEF* and *trzNDF* and the corresponding enzymes *AtzABCDEF* and *TrzNDF* responsible for rapid *s*-triazine mineralization in soil.

The *atzABCDEF* and *trzNDF* genes code for unique enzymes that catabolize diverse *s*-triazine herbicides (Fig. 3). In this pathway, atrazine degradation is initiated by either *AtzA* or *TrzN*, Fe(II) and Zn(II) metalloenzymes respectively, which dehalogenate atrazine, yielding hydroxyatrazine.[41–46] *AtzA* and *TrzN* share less than 27% sequence identity and have different substrate specificities.[42,43,45] *AtzA*'s substrate range includes six chlorotriazine herbicides, i.e. atrazine, simazine, propazine, terbuthylazine, mesoprazine and sebuthylazine, and their mono-*n*-dealkylated metabolites. Conversely, *TrzN*'s substrate range encompasses the six chlorotriazines above, in conjunction with six methoxytriazines, i.e. atraton, methometon, prometon, secbumeton, simeton and terbumeton, four methylthiotriazines, i.e. ametryn, desmetryn, methoprotyne and terbutryn, the mono-*N*-dealkylated metabolites of the preceding chloro, methoxy and methylthiotriazines herbicides and a few pyrimidine compounds. The succeeding enzyme in the pathway, hydroxyatrazine *N*-ethylaminohydrolase (*AtzB*), is a Zn(II) metalloenzyme that hydrolytically converts hydroxyatrazine to *N*-isopropylammelide.[47] *AtzB*'s substrate range includes the preceding hydroxylated chloro, methoxy and methylthiotriazine herbicides and their corresponding hydroxy-mono-*N*-dealkylated metabolites.[48] The third enzyme in the pathway, *N*-isopropylaminohydrolase (*AtzC*), is a Zn(II) metalloenzyme that converts *N*-isopropylammelide and related *s*-triazine metabolites to cyanuric acid.[49,50] One of two cyanuric acid hydrolases, *AtzD* or *TrzD*, then cleaves the *s*-triazine ring, yielding biuret.[51–53] Biuret is converted to allophanate via *AtzE*, at which point one of two enzymes, *AtzF* or *TrzF*, hydrolyze allophanate, yielding 2 mol of ammonia and carbon dioxide respectively.[54–57] Relative to the upstream genes in this pathway, *AtzDEF* and *TrzDF*'s substrate range is comparatively restricted.[44,52–55,57]

3.2.2 *Biological and spatial distribution of the s-triazine catabolism genes*

The *atzABCDEF* and *trzNDF* genes occur in diverse phyla and are highly conserved and globally dispersed (Fig. 4; Table 1). Specifically, bacterial isolates from four phyla originating from six

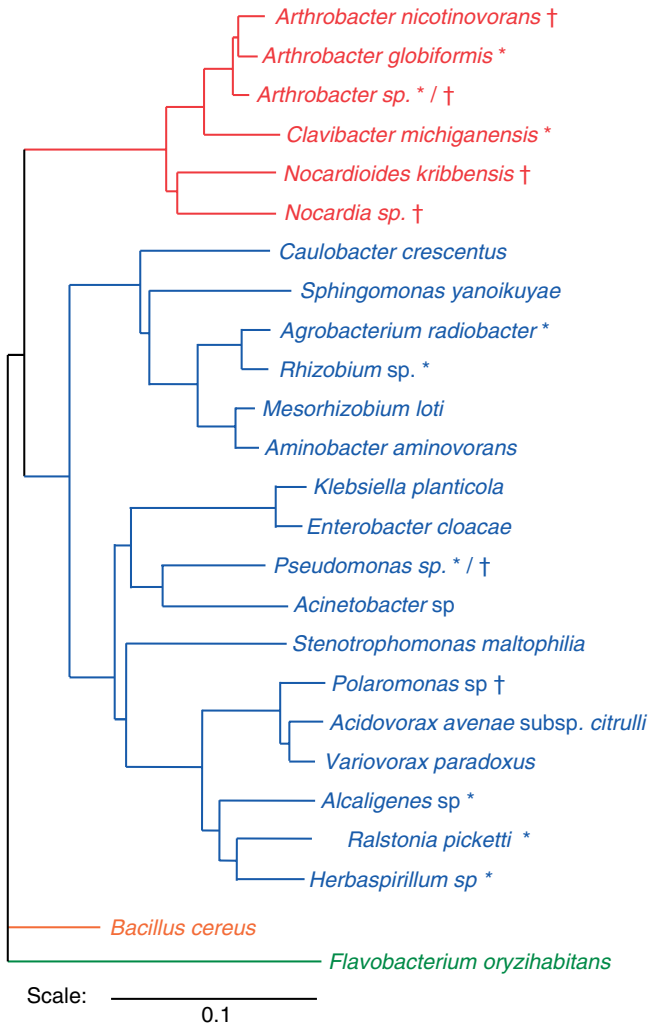

Figure 4. Phylogenetic relationship of bacterial species reported to contain atrazine-metabolizing genes, i.e. *atzABCDEF* or *trzNDF*. When available, a type species was used to represent a reported species; otherwise, the most similar species was used. In cases where the reported species has been renamed or reassigned, the synonyms listed with the National Center for Biotechnology Information GenBank database were used to identify currently accepted taxonomy. In cases where an atrazine-degrading bacterium was not identified at the species level, a type species for that genus was used in its place. Red, blue, orange and green denotes Actinobacteria, Proteobacteria, Firmicutes and Bacteroideles phyla respectively. The symbols * and † denote detection of *atzA* and *trzN* gene respectively.

continents bear homologs of *s*-triazine catabolism genes with high sequence identity to those of *Pseudomonas* sp. ADP or *Arthrobacter aurescens* TC1. With noted exceptions,[58–61] most isolates contain some or all of the *atzABCDEF* and *trzNDF* homologs on one or more self-transmittable plasmids.[55,59,60,62] The prevailing explanation for the detection of *atzABCDEF* and *trzNDF* homologs in diverse phyla, therefore, is horizontal gene transfer arising primarily from plasmid conjugation[40,55,59–61,63–67] and, to a lesser extent, transduction.[67,68] The highly conserved sequence identity among *atzABC* and *trzN* homologs is consistent with a recent, single origin with subsequent global dispersal.[59,69,70] Intercontinental transport of *atzABCDEF* and *trzNDF* genes is undocumented, but airborne microbial communities are colonized by soil bacteria which remain viable after transatlantic atmospheric deposition.[71,72]

3.3 Agronomic implications of enhanced degradation

3.3.1 *Reduced residual weed control*

Detecting *atzABCDEF* and *trzNDF* gene homologs in bacterial isolates spanning diverse phyla and continents indicates potential for widespread enhanced *s*-triazine degradation. Enhanced degradation is the phenomenon whereby a soil-applied pesticide is rapidly degraded by a population of microorganisms that have developed the ability to use the compound as a carbon, energy and (or) nutrient source because of previous exposure to it or an analog. Agronomic implications of enhanced degradation, namely reduced residual weed control, are compounded when structurally similar pesticides also degrade rapidly in adapted soils, a phenomenon referred to as cross-adaptation. As the substrate range of this catabolic pathway encompasses most commercially available *s*-triazine herbicides, cross-adaptation among compounds in this herbicide class is also likely (Fig. 3). In the following section, the agronomic implications of enhanced s-triazine degradation are discussed, the geographic distribution of adapted soils is described, basic soil physiochemical properties associated with enhanced degradation are delineated, the impact of agronomic practices on enhanced degradation is determined and, finally, strategies to curtail adverse agronomic effects attributed to this phenomenon are proposed.

Reduced residual weed control is the primary agronomic concern for soils exhibiting enhanced degradation. Residual weed control depends on the herbicide concentration required for efficacy and the herbicide's persistence in soil, which are pest and soil specific.[73] Atrazine half-life values in *s*-triazine-adapted soil are approximately tenfold lower than historic estimates, 60 days (Fig. 5); thus, for a given soil–pest complex, residual weed control may be tenfold lower in *s*-triazine-adapted soil than in non-adapted soil. For example, if an atrazine concentration of 0.4 mg kg^{-1} is required to achieve $\geq$85% control of pitted morningglory in a Dundee silt loam, then acceptable residual weed control would last 80 days in a non-adapted soil but only 8 days in an *s*-triazine-adapted soil (Fig. 6).

Enhanced degradation can reduce atrazine's residual control of sensitive weed species.[12,74–77] Under laboratory and greenhouse conditions, *Nasturtium officinale* R.Br. and *Solanum nigrum* L. died 15 days after sowing in soil containing atrazine incorporated at 4 mg kg^{-1}.[77] Inoculating this same soil with atrazine-degrading bacteria prior to incorporating atrazine at 4 mg kg^{-1} resulted in normal plant growth.[77] Under greenhouse conditions, the application of atrazine at 1.82 kg ha^{-1} to an *s*-triazine-adapted Dundee silt loam from Mississippi, USA, harboring native bacteria possessing *atzABC* and *trzN* gene homologs did not control *Ipomoea lacunosa* L.[74] Moreover, under typical Mississippi Delta, USA, corn production systems, neither atrazine nor simazine applied at 1.2 kg ha^{-1} controlled *Sida spinosa* L. in an *s*-triazine-adapted field site.[12,75] It was postulated, therefore, that instances of reduced residual weed control with atrazine in soils with a prior use history in the USA, including Colorado, Hawaii, Louisiana, Mississippi, Tennessee and Texas, were associated with enhanced degradation and not *s*-triazine-resistant weed biotypes, improper application techniques or lack of activation.[12] Consequently, there is a need to identify where soils exhibiting enhanced *s*-triazine degradation presently occur.

3.3.2 *Distribution of adapted soils*

For this section, soils were designated as adapted by one of four methods, that is, whole soil extracts or bacterial isolates

Table 1. Bacteria reported to contain the *atzABCDEF* and (or) *trzNDF* genes

Bacterium	Genes	Accession number	Reference
Acidovorax avenae subsp. *Citrulli*	*trzD*	AF086815.2	51
Acidovorax sp. JLS4	*trzD*		52
Acinetobacter sp. C-1	*atzB*	AM901597.2	159
Agrobacterium J14a	*atzABC*		160,161
Agrobacterium radiobacter	*atzD*		52
Agrobacterium radiobacter J14a	*atzA* like		143,162
Agrobacterium sp.	*atzABCDEF*		59
Agrobacterium sp.	*atzA*		99
Agrobacterium tumefaciens	*atzB*		96,99,
Alcaligenes sp. SG1	*atzABC, trzD*		52,143,161
Aminobacter aminovorans	*atzC, trzD*		163
Arthrobacter sp.	*trzN*		59
Arthrobacter aurescens TC1	*trzN, atzBC*	CP000475.1	46,64,164
		AY456696.1	
Arthrobacter crystallopoietes strain Cit2	*atzBC*	AF364904.1	163
Arthrobacter globiformis	*trzN, atzC*		97
Arthrobacter nicotinovorans	*atzB*	AY650036.1	79
Arthrobacter nicotinovorans strain HIM	*atzABC*	AY650035.1	79
Arthrobacter sp.	*trzN*		59
Arthrobacter sp.	*trzN, atzBC*		165
Arthrobacter sp.	*trzN, atzBCD*		61
Arthrobacter sp.	*trzN, atzBC*		166
Arthrobacter sp.	*atzB*		96
Arthrobacter sp.	*trzN, atzBCD*		61
Arthrobacter sp. AD1	*atzA*		58
Arthrobacter sp. AD1	*atzA*	AF543694.1	58
Arthrobacter sp. AD25	*trzN*	DQ989289.1	Li and Cai, unpublished
	atzD	DQ989288.1	
Arthrobacter sp. AD26	*trzN*	EU091479.1	Li *et al.*, unpublished
	atzB	EU621846.1	Zhu *et al.*, unpublished
	atzC	EU621847.1	Zheng *et al.*, unpublished
Arthrobacter sp. AD26-2	*trzN*	EU400620.1	Wang *et al.*, unpublished
Arthrobacter sp. AD30	*trzN, atzBC*	FJ161691.1	Zheng *et al.*, unpublished
		FJ161693.1	
		FJ161695.1	
Arthrobacter sp. AG1	*trzN, atzBC*		165
Arthrobacter sp. MCMB-436	*trzN, atzBCD*	AY589015.1	61
		AY589016.1	
		AY589013.1	
		AY594331.1	
Beta proteobactria	*atzA*	AB194097.1	167
Beta proteobactria	*atzB*	AB194098.1	167
Caulobacter crescentus	*atzB*		96
Chelatobacter heintzii = *Aminobacter aminovorans*	*trzD, atzABC*		168
Chelatobacter heintzii strain Cit1	*atzABCD*	AF364900.1	163
		AF364901.1	
		AF364902.1	
		AF364903.1	
Clavibacter			161
Clavibacter michiganensis ATZ1	*atzABC*		127,143,161
Enterobacter cloacae	*trzD*	AF342826.1	51,169
Flavobacterium oryzihabitans	*atzB*		96
Flavobacterium sp.	*atzBC*		96
Herbaspirillum sp. B601	*atzABC*	DQ089655.2	Bazhanov unpublished
		AY965854.2	
		AY965855.2	

Table 1. (*Continued*)

Bacterium	Genes	Accession number	Reference
Isolate 38/38	*atzABC*		161
Klebsiella planticola 99	*trzD*		52
Klebsiella sp.	*atzA*		99
Mesorhizobium loti	*atzF*	BA000012.4	170
Nocardia sp.	*trzN, atzB*		96
Nocardioides kribbensis	*trzN, atzBC*		97
Nocardioides panacihumi (three isolates)	*trzN, atzC*		97
Nocardioides sp.	*trzN*		59
Nocardioides sp.	*trzN, atzBC*		59
Nocardioides sp.	*trzN, atzBC*		171
Nocardioides sp. C190	*trzN*		40,41
Nocardioides sp. SP12	*trzN; atzBC*	AF537327 AF537330 AF537329	171
Nocardioides sp. AN3	*trzN*	AB427184.1	172
Nocardioides sp. C190	*trzN*	AF416746.1	41
Nocardioides sp. CMU5	*trzN, atzB*	EF088652.1 EF088653.1	97
Nocardioides sp. MTD22	*trzN*	AB427183.1	172
Nocardioides sp. SP12	*trzN, atzBC*	AF537328.1 AF537330.1 AF537329.1	119,171
Polaromonas sp.	*trzN, atzBC*		59
Pseudaminobacter sp.	*AtzABC*		40
Pseudaminobacter sp. C147	*AtzABC*		39,40, 173
Pseudaminobacter sp. C195	*AtzABC*		39,40,173
Pseudomonas putida	*AtzB*		96
Pseudomonas sp. CN1	*TrzD*		52
Pseudomonas sp. NRRLB-12227	*TrzD*		53
Pseudomonas sp. NRRLB-12228	*TrzD*		52
Pseudomonas sp.	*atzABCDEF*		175
Pseudomonas sp. AD39	*AtzC*	FJ161696.1	Zheng *et al.*, unpublished
Pseudomonas sp. AD39	*AtzB*	FJ161694.1	Zheng *et al.*, unpublished
Pseudomonas sp. AD39	*AtzC*	FJ161696.1	Zheng *et al.*, unpublished
Pseudomonas sp. AD39	*TrzN*	FJ161692.1	Zheng *et al.*, unpublished
Pseudomonas sp. ADP	*atzABCDEF*		39
Pseudomonas sp. ADP	atzABCDEF	U66917.2	47
Pseudomonas sp. C-15	*AtzB*	AM901596.2	159
Pseudomonas sp. CN1	*AtzAC*		87
Pseudomonas sp. YAYA6	*atzA* like		174
Ralstonia M91-3	*AtzABC*		161,176,177
Ralstonia picketti D	*atzA* like		143
Ralstonia picketti D	*AtzD*		52
Ralstonia sp. M91-3	*TrzD*		52
Rhizobium leguminosarum	*AtzBC*		96
Rhizobium sp.	*atzA*		178
Rhizobium sp.	*AtzC*		96
Rhizobium sp. PATR	*AtzA*		143,178
Sinorhizobium sp.	*trzN, atzBC*		59
Sphingomonas yanoikuyae	*AtzB*		96
Stenotrophomonas maltophilia	*AtzA*		163
Stenotrophomonas sp.	*AtzAB*		179
Variovorax paradoxus	*AtzB*		96
ß-proteobacterium	*atzABCDEF*		167

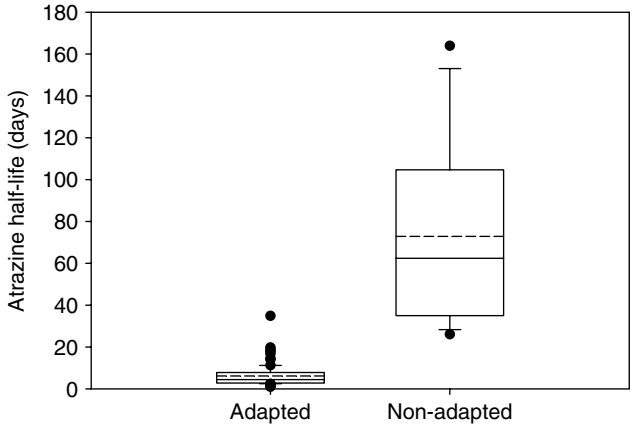

Figure 5. Box plot comparisons of published data for atrazine dissipation in *s*-triazine-adapted and non-adapted soil. Boundary of box closest to zero indicates the 25th percentile, a solid line within the box marks the median, a dashed line within the box delineates the mean and the boundary of the box furthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentile, and solid dots indicate outliers. The number of independent observations is 14 for non-adapted soils and 84 for *s*-triazine-adapted soils.[11,82,84,85,91,104,105,107,108,125,158]

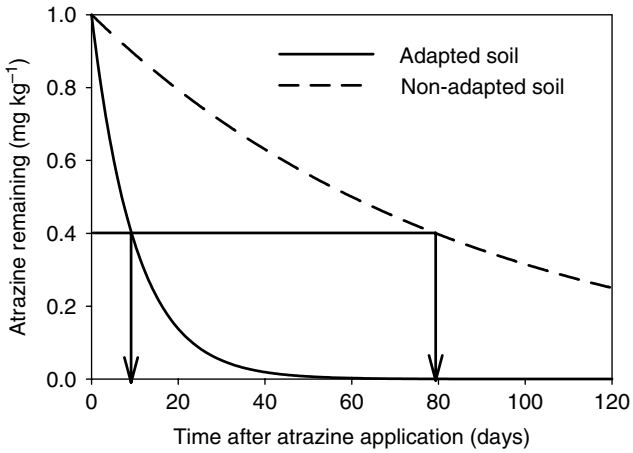

Figure 6. Theoretical example of residual weed control with atrazine in adapted and non-adapted soils. In this example, 0.4 mg kg^{-1} atrazine is required for 85% weed control. In a non-adapted soil, residual atrazine does not decline to 0.4 mg kg^{-1} until 80 days after atrazine application, while the atrazine concentration will decline to that same level in an adapted soil in only 10 days.

were positive for *atzABCDEF* or *trzNDF* gene homologs, dissipation assays,[17] mineralization assays[12] or they were declared as such by the author. Within this context, *s*-triazine-adapted soils are reported for the Hawaiian island (Shaner, private communication), the New Zealand islands[78–81] and all continents except Antarctica (Table 2; Fig. 7). Enhanced *s*-triazine degradation is not, therefore, a geographically localized problem, and, in order to determine where adaptation may occur in the future, the physiochemical range of known adapted soils was delineated.

3.3.3 *Physiochemical ranges*

Soil data from the aforementioned studies were evaluated to delineate the physiochemical range of *s*-triazine-adapted soils. Enhanced degradation occurs in soils ranging in texture from 3 to 90% sand, 4 to 60% silt and 3 to 56% clay, which covers all possible soil texture classes (Fig. 8). However, 90% of soils exhibiting enhanced degradation have textures ranging from 5 to 60% sand, 19 to 74% silt and 15 to 41% clay. Transposing these data onto the textural triangle reveals that 90% of known *s*-triazine-adapted soils group into the silty clay, clay loam, silty clay loam, sandy clay loam, loam, silt loam or sandy loam classes (Fig. 9). Furthermore, adaptation has been documented in soils with organic carbon contents ranging from 0.5 to 46%, with 90% of these soils having total organic carbon levels between 1.0 and 2.8% (Fig. 8). Soil pH levels ranging from 5.1 to 8.6 are reported for *s*-triazine-adapted soils, with 90% of the soils having a pH between 5.8 and 8.1 (Fig. 8).

Table 2. Intercontinental reports of enhanced *s*-triazine degradation

Continent	Country/region	Reference
Asia	China	58,165,166,180,181
	Croatia	59,98,182
	India	61,183
	Iran	184
	Israel	185
	Japan	94,167,172,186–188
Australia	New South Wales	91,189
Africa	Kenya	190
Europe	Belgium	82,88,191,192
	France	40,59,62,83,90,95,103,110, 117–119,125,126;163, 171,178,193
	Hungary	88,194
	Ireland	88
	Italy	159
	Spain	93,195–197
	Switzerland	174,198,199
North America	California	17,96,127,143,161
	Colorado	9,11,17,104,112
	Delaware	89
	Florida	200
	Illinois	17,201
	Indiana	161,202
	Iowa	153
	Louisiana	161
	Maryland	16
	Mexico	128
	Minnesota	37–39,161,203
	Mississippi	9,11,12,17,75,85,105,114
	Nebraska	97,160,161,204
	North Carolina	205
	North Dakota	99
	Ohio	68,111,123,161,176,177,206,207
	Ontario	40,41,83
	Pennsylvania	208
	Quebec	40,46,209,210
	South Dakota	211,212
	Tennessee	68,84
	Texas	109,113
South America	Argentina	107,108,121,122,213
	Chile	175,179,214,215
	Colombia	216

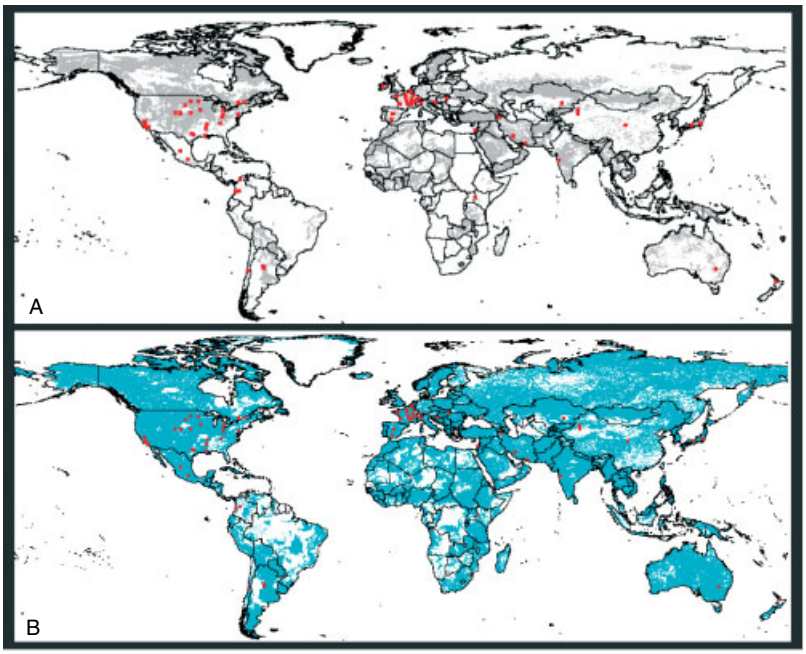

Figure 7. Global distribution of surface soils with physiochemical properties (percentage sand, silt, clay and organic carbon, and pH) consistent with those known to be exhibiting enhanced *s*-triazine degradation. Locations known to be exhibiting enhanced *s*-triazine degradation are noted with red dots. Panel A has highlighted in grey those soils with physiochemical properties within the 10–90th percentile range of known adapted soils. Panel B has highlighted in blue those soils with physiochemical properties within the minimum–maximum range of known adapted soils.

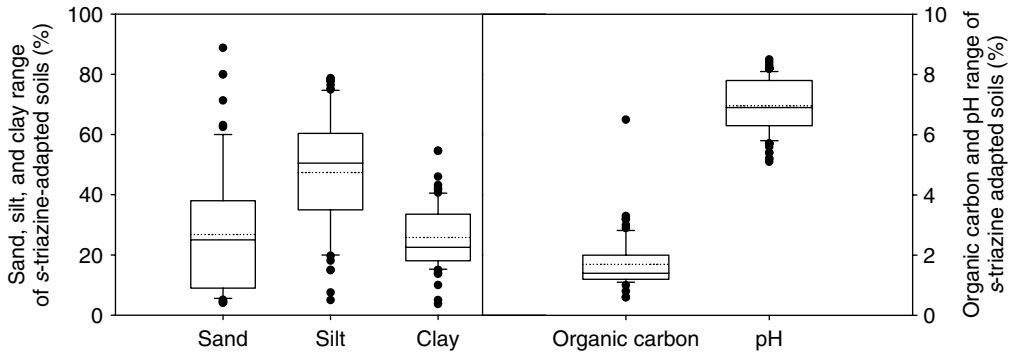

Figure 8. Physicochemical range of known *s*-triazine-adapted soils.

3.3.4 Soil pH as the principal physiochemical driver

Of the evaluated soil physiochemical properties, only acidic soil conditions restrict adaptation, particularly in Europe.[82,83] In two European screening studies spanning 83 soils varying widely in texture and pH, enhanced degradation was not observed in soils with pH <6.1.[82,83] In North America, enhanced degradation occurs in soils with pH ≥ 5.1, but the dissipation rate is reduced at pH <5.5.[84,85] For instance, the average atrazine half-life for a Tennessee, USA, *s*-triazine-adapted soil with a pH ranging from 5.7 to 7.5 was 2.8 days ($n = 20$; SD $= 0.27$).[84] When the soil pH ranged from 5.1 to 5.4, however, the average atrazine half-life was 11.4 days ($n = 4$; SD $= 1.29$). Thus, atrazine half-life increased fourfold at pH ≤ 5.4, yet was still fivefold lower than the historic persistence estimate, 60 days.

The pH effects on microbial ecology likely contribute to increased *s*-triazine persistence in adapted soils. For example, *s*-triazine catabolism in agricultural soils is mediated primarily by bacterial consortia.[68,86–99] Soil bacterial diversity and richness are maximal at neutral pH but decline as pH decreases, a trend consistent from field to continent scale.[100,101] Moreover, as soil pH declines, the microbial population shifts from bacterial to fungal dominated.[102] Thus, increased *s*-triazine persistence in acidic soils and (or) the inability of acidic soils to exhibit enhanced degradation are likely a function of decreased diversity and richness among the atrazine-degrading bacterial community.

3.3.5 Herbicide use history as the principal cultural management practice

A prerequisite for enhanced degradation is the selection of soilborne bacteria with an adaptation that enables rapid pesticide degradation.[9] To date, atrazine-degrading bacteria or the *atzABCDEF* and *trzNDF* gene homologs have been detected in agricultural soils only when these have been previously exposed to *s*-triazine herbicides.[17,83,85,103,104] This likely signifies that *s*-triazine herbicides are the primary agents responsible for the selection of *s*-triazine-degrading bacteria with *atzABCDEF* and *trzNDF* gene homologs. The development of enhanced atrazine degradation in

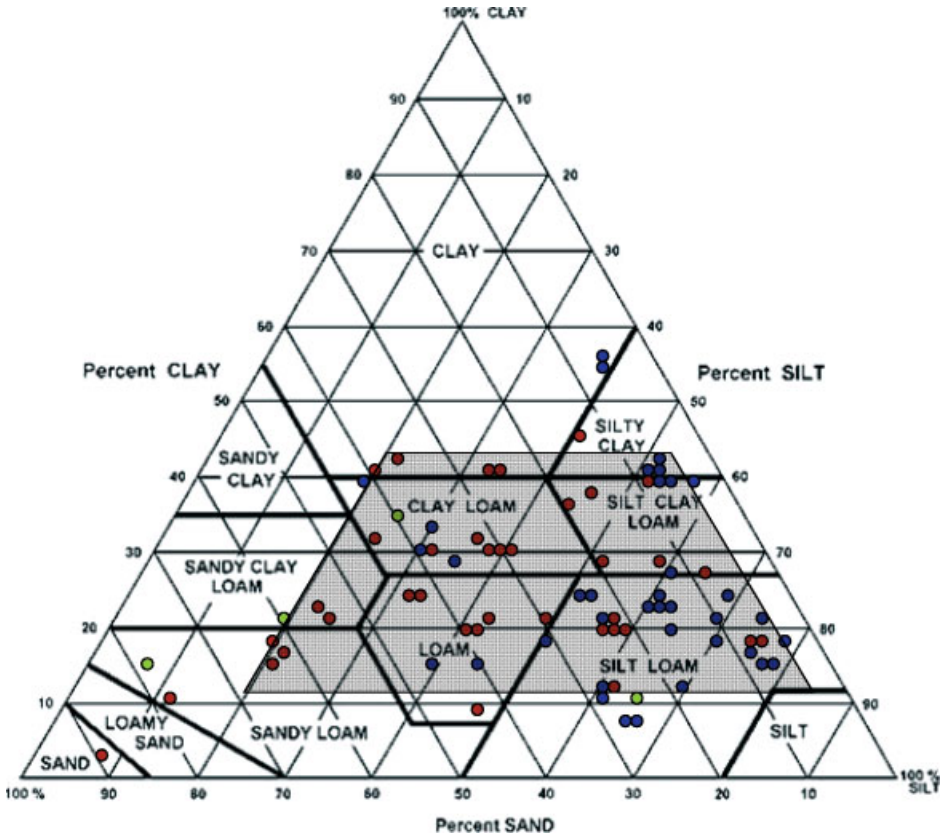

Figure 9. Textural triangle revealing 10–90th percentile of known *s*-triazine-adapted soils.

agricultural soils will depend primarily on herbicide use history, particularly *s*-triazine application frequency.

Not only do recurrent *s*-triazine applications select for bacteria able rapidly to degrade the herbicide, but frequent application increases the degrader population and (or) their activity. The relationship between application frequency and atrazine-degrader number/activity is inferred from European and North American screening studies,[17,82,83,85,104] but is clearly illustrated with data from a 6 year replicated field study conducted in Mississippi, USA, on a Dundee silt loam.[105] Prior to receiving an *s*-triazine application, the Dundee silt loam mineralized 9.2% of ring-labeled ^{14}C-atrazine 30 days after application, which is typical for non-adapted soil (Figs 3 and 10). One year after the first atrazine application, the soil mineralized 55% of ring-labeled ^{14}C-atrazine by 30 days after application, a value representative of *s*-triazine-adapted soils. Atrazine mineralization increased incrementally with successive applications until year 5, at which time it plateaued at approximately 80%. Results from this replicated field study indicate that one atrazine application, even at a typical field rate for corn, can induce enhanced degradation, and that successive applications increase either the atrazine-degrader population and (or) their activity.

Decreasing the *s*-triazine application frequency may slow the development and (or) minimize the adverse agronomic effects associated with enhanced degradation. One *s*-triazine application every 2 years, however, does not retard adaptation,[74,82,83,105–110] or increase residual weed control.[74] Atrazine dissipation in soil with an application frequency of once every 36 months is reduced relative to soils receiving annual applications,[111,112] but weed control data are lacking for this application interval. The authors suspect that an application frequency of once every 48 months may suppress adaptation, but field data are required for verification.

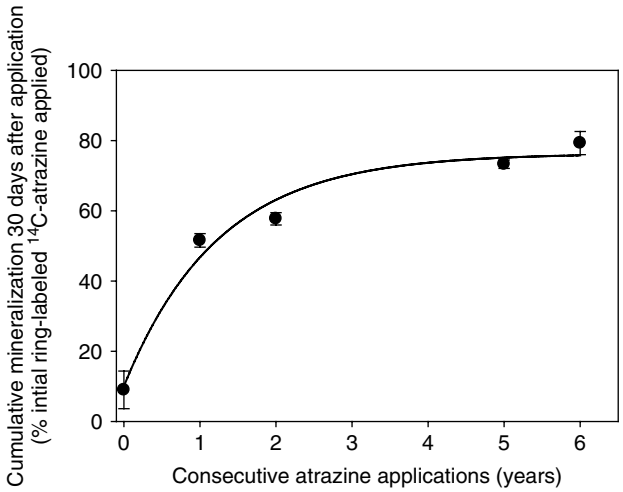

Figure 10. Mineralization of ring-labeled ^{14}C-atrazine as a function of *s*-triazine exposure history. One exposure increased cumulative atrazine mineralization from 10 to 50%. After five exposures, cumulative atrazine mineralization approached 80%. Adapted from Zablotowicz *et al*[114].

The coapplication of glyphosate or glufosinate with atrazine increases the herbicide's persistence in *s*-triazine-adapted soils. For instance, at 8 and 12 days after the application of atrazine (3.86 mg kg^{-1}) and glyphosate (0, 43, 86, 129, 172 or 215 mg kg^{-1}) to a Weswood silt loam, extractable atrazine was positively correlated with glyphosate rate; yet, regardless of glyphosate rate,

Table 3. Crop and crop rotations exhibiting enhanced *s*-triazine degradation

Crop	Reference
	Monocultures
Avocado	215
Citrus	Hanson, unpublished
Corn	9,11,12,17,62,68,74,75,82–86,88–90, 95,97,103–105,109–112,114,117–119, 121–123,125–127,163,171,178,181,184, 191,192,200,207,209–211
Grape	17
Olive	93
Pineapple	Shaner, unpublished
Sorghum	113
Sugar cane	158,190
Turf	88,167,205
	Rotations
Corn–barley	82,110
Corn–cotton	17,74,85,105
Corn–sorghum	17,109
Corn–soybean	17,82,104,107,108,121,122,153,207
Corn–sunflower	104
Corn–wheat	82,89,103,110
Corn–wheat–soybean	111,123,207
Corn–wheat–fallow	112
Corn–wheat–sunflower	112

atrazine half-life was $\leq$8 days.[113] Similarly, 5 days after the application of atrazine (2.5 mg kg^{-1}) and glufosinate (0, 10, 20 or 40 mg kg^{-1}) to a Dundee silt loam, extractable atrazine was positively correlated with glufosinate rate, but, regardless of treatment, atrazine's half-life was $\leq$5 days.[114] These data demonstrate that the co-application of glyphosate or glufosinate with atrazine transiently increases the herbicide's persistence in *s*-triazine-adapted soils. It is unlikely, however, that field application rates of glyphosate or glufosinate increase the residual activity of *s*-triazine herbicides under field conditions.

3.3.6 *Cropping history*

Enhanced degradation is reported for nine crops and six crop rotation systems that rely on *s*-triazine herbicides for residual weed control (Table 3). The authors submit, therefore, that enhanced degradation can occur in any cropping system where this class of herbicides remains a component of the weed control program if soil pH is greater than approximately 5.4 and the application frequency is greater than once every 4 years. Other crops potentially affected by enhanced *s*-triazine degradation include those treated with ametryn, i.e. bananas and plantains, with atrazine, i.e. macadamia nuts, conifers and guava, with prometon, i.e. industrial sites and railroad rights of way, with prometryn, i.e. cotton, celery and pigeon peas, and with simazine, i.e. strawberries, almonds, nectarines, apples, blueberries, caneberries, cranberries, established Christmas trees, filberts, lemons, pears, pecans, shelterbelts, sour cherries, walnuts, peaches, plums, sweet cherries, lemons, oranges and grapefruit.[115]

Although no production system impedes *s*-triazine adaptation, there is evidence that corn facilitates adaptation through a rhizosphere effect. The rhizosphere is a zone of soil under the direct influence of plant roots; thus, it is carbon enriched and maintains a larger and more diverse microbial population than non-rhizosphere soil.[116] Accordingly, atrazine-degrader numbers, diversity, activity and survival are often greater in the rhizosphere of corn than in bulk soil.[86,95,117–119] It is unknown whether the rhizosphere of other crops exhibit a similar stimulatory effect on the *s*-triazine-degrader population, as these studies are lacking.

3.3.7 *Residue management systems*

Residue management practices, that is, production systems that leave 30% or more of the crop residues on the soil surface after planting, can alter pesticide persistence relative to conventional tillage systems.[120] Residue management systems evaluated to date do not appear to retard the development of enhanced *s*-triazine degradation. *s*-Triazine-adapted soils occur in conventional tillage,[85,107–109] reduced tillage,[9,11–12,74–75,105,121] no-tillage[84,105,107,108,121,122] and cover crop production systems.[121,123] Studies comparing enhanced atrazine degradation among residue management systems are limited, but, when evaluated, atrazine dissipation is similar among NT, CT and cover crop systems.[121] Thus, residue management systems do not appear to impede adaptation when *s*-triazine herbicides are a component of the weed control program.

3.3.8 *Exogenous N*

Isolation techniques and ^{15}N-labeling studies reveal that catabolizing bacteria can utilize *s*-triazine herbicides as an N source.[124] It was postulated that this relationship could be exploited to alter *s*-triazine persistence in adapted soils. For example, under laboratory conditions, the addition of exogenous N to pure culture and native *s*-triazine-adapted soils transiently suppressed *s*-triazine degradation by adapted microbial populations, with the suppression level depending primarily on the bacterial population responsible for degradation and the N source applied.[82,89,114,125–130] Additionally, atrazine-catabolizing genes, particularly *atzDEF*, are subject to a complex regulatory circuit that is modulated by N availability and the presence of degradation products, e.g. cyanuric acid.[129,130] Thus, coinciding N fertilization with *s*-triazine applications could increase herbicide persistence. Field data from Colorado, Tennessee and Mississippi, USA, indicate that *s*-triazine half-life values are $\leq$9 days under typical corn production systems, even when N is applied at recommended field rates, approximately 150 kg ha^{-1}.[12,75,84,104] It is unlikely, therefore, that synchronizing *s*-triazine applications with N fertilization will appreciably extend the residual activity of *s*-triazine herbicides in adapted soils.

3.3.9 *Minimizing effects of enhanced s-triazine degradation*

There are three historical approaches to dealing with enhanced pesticide degradation:[131]

- increase pesticide application rate and frequency;
- switch to pesticides from alternative chemical families;
- integrated pest management (IPM), that is, utilize a combination of pest control strategies including mechanical, physical, genetic, biological, cultural and chemical.

Applying the approaches described above to the problem of enhanced *s*-triazine degradation reveals one feasible solution, IPM. For example, increasing application rate and frequency is not a viable option in that regulatory limits may be violated. Switching to another herbicide is not a sensible option because of cross-adaptation among *s*-triazine herbicides, regional and

global registration restrictions on alternative herbicide families and limited economically available alternative herbicides, particularly for corn. Integrated pest management, therefore, is the most feasible option for controlling enhanced atrazine degradation.

In the context of IPM, two strategies can be employed:[131,132]

- prevent the development of enhanced degradation;
- circumvent the consequence of enhanced degradation in adapted soils.

In the case of enhanced *s*-triazine degradation, the present analysis indicates that the phenomenon can occur in any cropping system if (1) the soil pH is above approximately 5.4 and (2) *s*-triazines are applied more than once every 3–4 years. Consequently, if the aim is to prevent enhanced degradation, then *s*-triazine herbicides should not be applied more than once every 4 years. This will necessitate the use of alternative herbicides, which, as stated above, may not be feasible owing to regional registration restrictions and limited economically viable alternative chemistries. Conversely, if the objective is to maintain acceptable weed control in the context of existing enhanced degradation, then a number of interconnected strategies could be employed:

- supplement *s*-triazine residual weed control with additional pre-emergent chemistries from alternative herbicide families;
- plant herbicide-resistant varieties and rely on post-emergence applications and (or) cultivation for weed control;
- apply *s*-triazine herbicides post-direct, thereby taking advantage of foliar uptake.

3.4 Environmental implications of enhanced degradation

3.4.1 Fate, transport and environmental risk assessment predictions

Pesticide persistence is an integral component of fate, transport and risk assessment models. Sensitivity analysis, that is, investigations into how model performance varies along with changes in the key assumptions on which the model projections are based, indicates that inaccurate persistence estimates adversely affect model predictions.[80,133–141] The primary environmental concern associated with enhanced *s*-triazine degradation, therefore, is that historic *s*-triazine persistence estimates, if used as default input parameters, will result in erroneous fate, transport and environmental risk predictions for adapted soils. Herein, the differences in atrazine persistence between adapted and non-adapted soils are described, how these differences will affect environmental modeling and risk assessment is discussed and alternative default input parameters for *s*-triazine-adapted soils are recommended.

3.4.2 Historic atrazine persistence estimates

The USEPA indicates that atrazine's average half-life in soil under aerobic laboratory conditions is 3–4 months,[142] which is consistent with Wauchope's review of the literature[13] and the present authors' analysis of published data for non-adapted soils (Fig. 5). Conversely, the average atrazine half-life under aerobic laboratory conditions in *s*-triazine-adapted soils is 6 days (Fig. 5). The differences in atrazine persistence between non-adapted and *s*-triazine-adapted soils are significant in that a theoretical twofold decrease in herbicide persistence can reduce the predicted off-site loss tenfold.[134]

3.4.3 Metabolite formation and persistence in adapted and non-adapted soils

The concentration of atrazine's mono-*N*-dealkylated metabolites is typically lower in *s*-triazine-adapted soil than in non-adapted soil, which has been attributed to two competing processes.[9,11] First, atrazine-degrading consortia containing *atzABCDEF* and *trzNDF* gene homologs circumvent biologically mediated *N*-dealkylation reactions, thereby focusing atrazine dissipation primarily through the novel *s*-triazine pathway (Fig. 3).[11] This hypothesis is supported by modeling data, which indicate that, in *s*-triazine-adapted soils, 71, 23 and 6% of the parent compound is converted to HA, DEA and DIA respectively.[14] Conversely, in non-adapted soils, the projected conversion of atrazine to daughter products is 10% for HA, 72% for DEA and 18% for DIA.[9] Second, the mono-*N*-dealkylated metabolites of atrazine are substrates for *atzA* and *trzN*, and thus, in *s*-triazine-adapted soils, DEA and DIA could be quickly converted to hydroxy-*s*-triazine intermediates.[44,143] For example, the mean half-life value for DEA and DIA in non-adapted soil is 52 and 36 days respectively.[144–146] In *s*-triazine-adapted soil, however, the mean half-life is 10 days for DEA and 8 days for DIA.[9] The differences in metabolite formation and dissipation between *s*-triazine-adapted soils are significant in that the *n*-dealklyated metabolites are included in atrazine's risk assessment.[142]

3.4.4 Temperature and moisture effects on atrazine degradation in adapted soils

Prominent transport models contain submodules that predict pesticide degradation as a function of temperature and moisture:[11,147]

$$C_t = C_0\, e^{-k(T,\,\theta)} \quad (1)$$

$$T_{1/2} = 0.693/k \quad (2)$$

$$k_{(T,\,\theta)} = k_{ref} Q_{10}[(T - T_{ref})/10](\theta/\theta_{ref})\beta \quad (3)$$

$$Q_{10} = T_{1/2\ 20\,^\circ C}/T_{1/2\ 10\,^\circ C} \quad (4)$$

$$\beta = -[\log(T_{1/2\ 70\%FC}) - \log(T_{1/2\ 40\%FC})]/\log(70/40) \quad (5)$$

where C_t is the pesticide concentration in soil at time t (mg kg^{-1}), C_0 is the initial pesticide concentration (mg kg^{-1}), $k_{(T,\theta)}$ is the rate constant at actual temperature T (°C) and soil moisture θ, k is the first-order rate constant in reciprocal days (day^{-1}) at reference temperature and moisture, Q_{10} is the factor by which degradation increases when T increases by 10 °C, T is the actual temperature (°C), T_{ref} is the reference temperature, θ is the actual soil moisture [% field capacity (FC)], θ_{ref} is the reference soil moisture (40% FC) and β is the moisture exponent. FOCUS, which reviewed 148 Q_{10} estimates for various pesticides, concluded that Q_{10} does not vary significantly among pesticides, and recommended a default Q_{10} of 2.20, which is standard for most fate and transport models.[148] On review of subsequent data, however, the Panel on Plant Protection Products and their Residues (PPR-Panel) reversed their decision:

- There are group-specific and compound-specific differences in Q_{10}.
- One median Q_{10} value for all pesticides should not be assumed.
- Compound-specific Q_{10} values should be used rather than default values.
- Until such data are available, a default Q_{10} value of 2.58 is recommended.[149]

To date, one study has evaluated temperature and moisture effects on atrazine degradation in *s*-triazine-adapted soils, and separate Q_{10} values for adapted (3.00) and non-adapted soils (2.20) were recommended.[11] Until these findings are substantiated,

Table 4. Leaching estimation and chemistry model (LEACHM) simulations for the Morgan Creek, Maryland, watershed. Projections are based on 10 year simulations (1995–2004) for the top metre of s-triazine-adapted or non-adapted unsaturated silty loams when subjected to annual rotations of corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.]. Five biennial applications of 2.08 kg ha^{-1} of atrazine were applied in the spring preceding the planting of corn

Soil	Compound	MF ratio[a]	$T_{1/2}$[b] (days)	V_{mhf}[c]	Applied/produced (g ha^{-1})	Leached (g ha^{-1})	Residual (g ha^{-1})	Leached (% applied)
Adapted	Atrazine		6	11	10 400	23	0	0.22
	Desethylatrazine	0.22	10	11	2350	25	0	0.24
	Deisopropylatrazine	0.07	8	11	600	4	0	0.04
	Hydroxyatrazine	0.71	6	11	7420	9	0	0.09
	Total residues					61	0	0.59
Non-adapted	Atrazine		60	3	10 400	284	35	2.73
	Desethylatrazine	0.72	52	3	7250	464	69	4.46
	Deisopropylatrazine	0.18	36	3	1810	67	8	0.64
	Hydroxyatrazine	0.10	60	3	1010	22	14	0.21
	Total residues					837	126	8.04

[a] MF ratio is the ratio of atrazine converted to desethylatrazine, deisopropylatrazine and hydroxyatrazine.[9,12,14]
[b] $T_{1/2}$ is the near-surface half-life for atrazine, desethylatrazine, deisopropylatrazine and hydroxyatrazine.[9–11]
[c] V_{mhf} is the maximum multiple of the near-surface half-life that the half-life reaches at 100 cm.[10]

it is prudent to adjust default Q_{10} values from 2.20 to 2.58, regardless of herbicide use history. Conversely, moisture effects on atrazine degradation indicate that differential β values are not required for modeling atrazine fate and transport in *s*-triazine-adapted and non-adapted soils.[11] This finding corroborates that of FOCUS, which recommended a default β of +0.8 for pesticide fate and transport modeling, as variations in measurements of β for individual pesticides are as great as variations between pesticides.[148]

3.4.5 Soil depth effects on atrazine degradation in adapted soils

Atrazine persistence, regardless of herbicide use history, generally increases with depth in the soil profile.[9,78,81,108,145,146,150–153] Models like RZWQM and LEACHM, therefore, allow the user to vary the maximum half-life factor, that is, the multiple of the near-surface half-life that the half-life reaches at 100 cm.[10] Data from Colorado and Mississippi, USA, indicate that the maximum half-life factor will be at least 3–4-fold higher in *s*-triazine-adapted soil than in non-adapted soil.[9] For example, if the maximum half-life factor for non-adapted soil is 3, then a maximum half-life factor ranging from 9 to 12 would be appropriate for the same soil exhibiting enhanced degradation.

3.4.6 LEACHM predictions for adapted and non-adapted soils

LEACHM predictions were notably different between *s*-triazine-adapted and non-adapted soils (Table 4). By accounting for the altered metabolic pathway and reduced persistence estimates for the parent and daughter products in *s*-triazine-adapted soil, leaching potential relative to non-adapted soil was reduced 12.3-fold for atrazine, 18.5-fold for DEA, 16.8-fold for DIA, 2.4-fold for HA and 13.7-fold for total *s*-triazine residues. LEACHM simulations demonstrate that, if historic dissipation pathways and rate constants are assumed for *s*-triazine-adapted soils, then herbicide fate, transport and risk assessment errors will be considerable. Given the number of soils known to exhibit enhanced degradation, coupled with the projections on where this phenomenon can occur (Fig. 7), there is a need to develop a reliable method to predict atrazine persistence in agricultural soils.

3.5 Predicting atrazine persistence across the agricultural landscape

A multiple linear regression model able to predict atrazine half-life values in soil was constructed using the stepwise procedure in SAS (model 1):

$$\log_{10}(T_{1/2}) = 0.18080 - [0.62740 \times \log_{10}(\text{pH})] - [0.32927 \times \log_{10}(C_{yr})] + 0.87216h \quad \text{(model 1)}$$

where $T_{1/2}$ is the atrazine half-life value standardized to 20 °C via the Arrhenius equation (days), pH is minus the decimal logarithm of the hydrogen ion activity in a 1 : 1 aqueous soil solution paste, C_{yr} is the consecutive years of atrazine applications, ranging from 0 (no applications) to 5 (five consecutive applications), and h is the atrazine use history in the last 5 years, where soils receiving an atrazine application = 1 and soils not receiving an atrazine application = 2. The model and all parameters contained in it were highly significant: $P_{0.05}$ for full model <0.0001, $P_{0.05}$ for pH <0.0001, $P_{0.05}$ for C_{yr} = 0.0206 and $P_{0.05}$ for h < 0.0001. Conversely, tillage, soil texture and organic carbon did not significantly contribute to model 1 (data not reported).

Model 1 grouped 90 soils from five continents, including Africa, Australia, Europe, North America and South America, into one of three categories:

- adapted, i.e. atrazine half-life ≤15 days;
- intermediate, i.e. atrazine half-life ranging from 15 to 30 days;
- non-adapted, i.e. atrazine half-life >30 days (Table 5).

The model accurately grouped 11 of 12 non-adapted soils and 73 of 77 adapted soils. Model deficiencies were primarily associated with discriminating between adapted and intermediately adapted soils, particularly in Europe. Moreover, model 1 was a better predictor of atrazine half-life values than the historic estimate (Table 5). It is likely, therefore, that this model, or an updated version of it, could be used to generate initial atrazine persistence estimates for environmental and risk assessment modeling.

Table 5. Observed and predicted atrazine half-life ($T_{1/2}$) values in soils from five continents that have or have not been exposed to atrazine

Continent	Country/region	$T_{1/2}$ predicted (days)[a]	$T_{1/2}$ observed (days)	Error reduction (%)[b]	Reference
		Soils with an atrazine use history			
Africa	Africa	5.3	18.7	149.3	158
South America	Argentina	4.5	11.3	370.7	107
Australia	Australia	3.2	7.0	703.0	91
Europe	Belgium	4.5	2.3	2400.7	82
Europe	Belgium	4.7	2.8	2012.2	82
Europe	Belgium	4.5	3.1	1800.6	82
Europe	Belgium	3.6	3.2	1768.5	82
Europe	Belgium	2.7	4.2	1299.2	82
Europe	Belgium	4.0	4.3	1290.8	82
Europe	Belgium	3.0	4.3	1269.0	82
Europe	Belgium	4.8	4.5	1224.0	82
Europe	Belgium	4.5	4.6	1197.0	82
Europe	Belgium	2.8	4.6	1160.0	82
Europe	Belgium	4.4	5.0	1101.6	82
Europe	Belgium	4.2	6.3	823.5	82
Europe	Belgium	4.7	6.7	764.4	82
Europe	Belgium	5.1	7.9	621.9	82
Europe	Belgium	5.6	8.5	575.0	82
Europe	Belgium	5.5	9.5	492.2	82
Europe	Belgium	5.8	9.5	495.5	82
Europe	Belgium	5.6	10.2	441.6	82
Europe	Belgium	5.4	10.5	426.2	82
Europe	Belgium	4.1	10.9	388.2	82
Europe	Belgium	3.9	14.5	240.3	82
Europe	Belgium	5.6	16.8	190.0	82
Europe	Belgium	6.4	19.8	135.6	82
Europe	Belgium	5.6	34.9	−11.8	82
North America	Colorado	6.1	1.5	3595.3	104
North America	Colorado	3.5	2.0	2824.0	104
North America	Colorado	2.8	2.0	2862.2	104
North America	Colorado	4.1	2.2	2543.0	104
North America	Colorado	3.0	2.5	2281.6	104
North America	Colorado	3.0	2.6	2194.1	104
North America	Colorado	3.2	4.0	1380.1	11
North America	Colorado	5.8	4.2	1290.6	104
North America	Colorado	10.6	10.4	474.6	104
Europe	France	2.7	8.9	504.1	125
North America	Mississippi	3.8	1.0	5618.1	11
North America	Mississippi	3.5	3.1	1821.8	105
North America	Mississippi	6.4	3.2	1676.3	105
North America	Mississippi	6.4	4.1	1306.2	85
North America	Mississippi	5.3	4.4	1242.5	85
North America	Mississippi	3.8	4.6	1187.8	85
North America	Mississippi	5.3	4.8	1140.4	85
North America	Mississippi	4.8	4.8	1149.5	85
North America	Mississippi	6.7	4.9	1087.8	85
North America	Mississippi	4.4	5.0	1088.6	105
North America	Mississippi	5.1	5.3	1027.6	105
North America	Mississippi	7.0	5.5	964.1	85
North America	Mississippi	6.4	5.5	973.7	85
North America	Mississippi	5.1	5.7	941.4	105
North America	Mississippi	7.0	5.9	898.8	85
North America	Mississippi	6.4	5.9	909.2	105
North America	Mississippi	5.3	6.2	859.4	85
North America	Mississippi	6.4	7.7	662.0	85
North America	Mississippi	6.7	10.0	467.0	85

Table 5. (*Continued*)

Continent	Country/region	$T_{1/2}$ predicted (days)[a]	$T_{1/2}$ observed (days)	Error reduction (%)[b]	Reference
North America	Mississippi	8.8	10.7	442.6	85
North America	Mississippi	9.6	14.1	293.5	85
North America	Mississippi	6.4	18.0	168.7	85
North America	Tennessee	3.2	2.4	2366.4	84
North America	Tennessee	4.0	2.5	2238.6	84
North America	Tennessee	4.8	2.6	2123.2	84
North America	Tennessee	3.8	2.6	2161.8	84
North America	Tennessee	3.4	2.6	2176.6	84
North America	Tennessee	4.9	2.7	2039.4	84
North America	Tennessee	4.6	2.7	2051.5	84
North America	Tennessee	4.0	2.7	2072.8	84
North America	Tennessee	4.1	2.7	2069.2	84
North America	Tennessee	3.3	2.7	2098.6	84
North America	Tennessee	4.7	2.8	1974.5	84
North America	Tennessee	3.6	2.8	2014.6	84
North America	Tennessee	4.2	3.0	1858.5	84
North America	Tennessee	4.2	3.0	1860.8	84
North America	Tennessee	4.4	3.1	1794.7	84
North America	Tennessee	5.6	3.2	1698.6	84
North America	Tennessee	7.3	3.3	1597.6	84
North America	Tennessee	4.8	3.5	1578.1	84
North America	Tennessee	5.9	3.7	1463.3	84
North America	Tennessee	4.2	3.7	1506.9	84
North America	Tennessee	6.3	10.6	425.1	84
		Soils with no known atrazine use history			
Africa	Africa	51.3	164.0	−5.3	190
Africa	Africa	130.0	88.0	−15.9	190
South America	Argentina	56.3	121.8	−3.1	107
South America	Argentina	48.6	142.2	−8.0	108
Europe	Belgium	71.4	53.0	−21.5	82
North America	Colorado	44.1	26.1	60.9	104
North America	Colorado	40.6	36.0	54.0	11
Europe	France	37.0	99.0	−23.2	125
North America	Mississippi	52.8	30.5	23.5	85
North America	Mississippi	51.3	32.0	27.1	11
North America	Mississippi	47.4	37.0	34.2	105
North America	Mississippi	48.6	38.2	29.9	85
North America	Mississippi	51.3	71.8	−12.1	85
North America	Mississippi	47.4	81.0	−15.6	85

[a] Predicted values are based on model 1: $\log_{10}(T_{1/2}) = 0.18080 - [0.62740 \times \log_{10}(\text{pH})] - [0.32927 \times \log_{10}(C_{yr})] + 0.87216h$, where $T_{1/2}$ is the atrazine half-life value standardized to 20 °C via the Arrhenius equation (days), pH is minus the decimal logarithm of the hydrogen ion activity in a 1 : 1 aqueous soil solution paste, C_{yr} is the consecutive years of atrazine applications, ranging from 0 (no applications) to 5 (five consecutive applications), and h is the atrazine use history in the last 5 years, where soils receiving an atrazine application = 1 and soils not receiving an atrazine application = 2. The model and all parameters contained in it were highly significant: $P_{0.05}$ for full model <0.0001, $P_{0.05}$ for pH <0.0001, $P_{0.05}$ for $C_{yr} = 0.0206$ and $P_{0.05}$ for $h < 0.0001$.

[b] Reduction in error afforded by model 1 relative to historic estimate, 60 days, that is, error reduction = [(60 days − observed)/observed] ×100 − [(model 1 projection − observed)/observed] ×100. Negative values indicate less error associated with historic estimate relative to model 1.

4 CONCLUSIONS AND FUTURE RESEARCH NEEDS

Basic and applied research indicates a recent bacterial adaptation that enables rapid detoxification of *s*-triazine herbicides. The genes that allow bacteria to use *s*-triazine herbicides as a sole C and (or) N source are primarily plasmid mediated, are susceptible to horizontal gene transfer, occur in at least four bacterial phyla and have been identified on six continents. The substrate range of these metabolic pathways encompasses most commercially available *s*-triazine herbicides and has been linked to enhanced degradation in agricultural soils from around the globe.

Linking these microbial adaptations with enhanced degradation has significant agronomic implications. Decreased residual weed control with *s*-triazine herbicides has been confirmed under laboratory, greenhouse and field conditions. With the exception of *s*-triazine application frequency and soil pH, cultural management practices and soil physiochemical properties do not retard adaptation. Thus, it appears that enhanced degradation can occur

in any agricultural soil with a pH greater than approximately 5.4, given sufficient exposure to *s*-triazine herbicides.

From an environmental perspective, the development and subsequent spread of enhanced *s*-triazine degradation will require the historic default input values to be changed if accurate fate, transport and risk assessment modeling is desired. For example, when the altered metabolic pathway and reduced persistence estimates for the parent and daughter products in *s*-triazine-adapted soil were accounted for, atrazine's leaching potential was reduced 13.7-fold relative to LEACHM simulations that relied on historic input values.

At completion of this manuscript, several questions remain:

- How widespread is enhanced *s*-triazine degradation?
- Does atrazine provide an economic return on investment when applied to soils exhibiting enhanced degradation?
- What are typical half-life values for atrazine, DEA, DIA and HA as a function of their geographic position, depth in the soil profile and herbicide use history?
- What are regulatory agencies using as estimates for the conversion of atrazine to daughter products, regardless of *s*-triazine use history?
- Will the suggested modeling parameters better predict atrazine fate and transport under actual field conditions?

Given the potential impact of these new metabolic pathways on *s*-triazine efficacy and off-site transport, the authors submit that a large-scale screening study is required to address these concerns.

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REFERENCES

1 Cook AM, Biodegradation of *s*-triazine xenobiotics. *FEMS Microbiol Rev* **46**:93–116 (1987).
2 Timmons FL, A history of weed control in the United States and Canada. *Weed Sci* **53**:748–761 (2005).
3 Shapir N, Mongodin EF, Sadowsky MJ, Daugherty SC, Nelson KE and Wackett LP, Evolution of catabolic pathways: genomic insights into microbial *s*-triazine metabolism. *J Bacteriol* **189**:674–682 (2007).
4 Wackett LP, Sadowsky MJ, Martinez B and Shapir N, Biodegradation of atrazine and related *s*-triazine compounds: from enzymes to field studies. *Appl Microbiol Biotechnol* **58**:39–45 (2002).
5 Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, *et al*, The Ribosomal Database Project: improved alignments and new tools for rRNA analysis (2009).
6 Batjes NH, Harmonized soil profile data for applications at global and continental scales: updates to the WISE database. *Soil Use Manag* **25**:124–127 (2009).
7 Hutson JL and Wagenet RJ, LEACHM: *Leaching Estimation and Chemistry Model: A Process-Based Model of Water and Solute Movement, Transformations, Plant Uptake and Chemical Reactions in the Unsaturated Zone Continuum, Vol. 2, Version 3*. Water Resources Institute, Cornell University, Ithaca, NY (1992).
8 Hutson JL, LEACHM: *Leaching Estimation and Chemistry Model: A Process-Based Model of Water and Solute Movement, Transformations, Plant Uptake and Chemical Reactions in the Unsaturated Zone: Model Description and User's Guide, Version 4.1*. Water Resources Institute, Cornell University, Ithaca, NY (2005).
9 Krutz LJ, Shaner DL and Zablotowicz RM, Comparative fate of atrazine in three depths of *s*-triazine-adapted and non-adapted soils from Colorado and Mississippi. *J Environ Qual* **in review** (2010).
10 Wauchope RD, Rojas KW, Ahuja LR, Ma Q, Malone RW and MA L, Documenting the pesticide processes module of the ARS RZWQM agroecosystem model. *Pest Manag Sci* **60**:222–239 (2004).
11 Krutz LJ, Shaner DL, Accinelli C, Zablotowicz RM and Henry WB, Atrazine dissipation in *s*-triazine-adapted and non-adapted soil from Colorado and Mississippi: implications of enhanced degradation on atrazine fate and transport parameters. *J Environ Quality* **37**:848–857 (2008).
12 Krutz LJ, Burke IC, Reddy KN, Zablotowicz RM and Price AJ, Enhanced atrazine degradation: evidence for reduced residual weed control and a method for identifying adapted soils and predicting herbicide persistence. *Weed Sci* **57**:427–434 (2009).
13 Wauchope RD, Butler TM, Hornsby AG, Augustine-Beckers PM and Burt PP, The SCS/ARS/CES pesticide properties database for environmental decision making. *Rev Environ Contam Toxicol* **123**:1–155 (1992).
14 Webb RMT, Sandstrom MW, Krutz LJ and Shaner DL, Simulations of branched serial first order decay of pesticides. *J Environ Qual* **in review** (2010).
15 Hancock TC, Sandstrom MW, Vogel JR, Jr, Webb RMT, Bayless ER and Barbash JE, Pesticide fate and transport throughout unsaturated zones in five agricultural settings, USA. *J Environ Qual* **37**:1086–1100 (2008).
16 Webb RMT, Wieczorek ME, Nolan BT, Hancock TC, Sandstrom MW, Barbash JE, *et al*, Variations in pesticide leaching related to land use, pesticide properties, and unsaturated zone thickness. *J Environ Qual* **37**:1145–1157 (2008).
17 Shaner DL, Henry WB, Krutz LJ and Hanson B, Rapid assay for detecting enhanced atrazine degradation in soil. *Weed Sci* **55**:528–535 (2007).
18 Mandelbaum RT, Sadowsky MJ and Wackett LP, Microbial degradation of *s*-triazine herbicides, in *The Triazine Herbicides*, ed. by LeBaron HM, McFarland JF and Burnside OC. Elsevier Science Publishers, San Diego, CA, pp. 301–328 (2008).
19 Khan SU and Marriage PB, Residues of atrazine and its metabolites in an orchard soil and their uptake by oat plants. *J Agric Food Chem* **25**:1408–1413 (1977).
20 Sirons GJ, Frank R and Sawyer T, Residues of atrazine, cyanazine and their phytotoxic metabolites in a clay loam soil. *J Agric Food Chem* **21**:1016–1020 (1973).
21 Esser HO, Dupuis G, Ebert E, Marco G, Vogel C, Marco G, *et al*, *s*-Triazines, in *Herbicides: Chemistry, Degradation, and Mode of Action*, 2nd edition, ed. by Kearney PC and Kaufman DD. Marcel Dekker, New York, NY, pp. 129–208 (1975).
22 Armstrong DE, Chesters G and Harris RF, Atrazine hydrolysis in soil. *Soil Sci Soc Amer Proc* **31**:61–66 (1967).
23 Harris CI, Fate of chloro-*s*-triazines herbicides in soil. *J Agric Food Chem* **15**:157–162 (1967).
24 Skipper HD, Gilmour CM and Furtick WR, Microbial versus chemical degradation of atrazine in soils. *Soil Sci Soc Amer Proc* **31**:653–656 (1967).
25 Best JA and Weber JB, Disappearance of *s*-triazines as affected by soil pH using a balance-sheet approach. *Weed Sci* **22**:364–373 (1974).
26 Russell JD, Cruz M, White JL, Bailey GW, Payne WR, Jr, Pope JD, Jr, *et al*, Mode of chemical degradation of *s*-triazines by montmorillonite. *Science* **160**:1340–1342 (1968).
27 Laird DA and Koskinen WC, Triazine-soil interactions, in *The Triazine Herbicides*, ed. by LeBaron HM, McFarland JF and Burnside OC. Elsevier, San Diego, CA, pp. 275–300 (2008).
28 Clay SA and Koskinen WC, Adsorption and desorption of atrazine, hydroxyatrazine and *S*-glutathione atrazine in two soils. *Weed Sci* **38**:262–266 (1990).
29 Dao TH, Lavy TL and Sorensen RC, Atrazine degradation and residue distribution in soil. *Soil Sci Soc Am J* **43**:1129–1134 (1979).
30 McCormick LL and Hiltbord AE, Microbiological decomposition of atrazine and diuron in soil. *Weeds* **14**:77–82 (1966).
31 Koskinen WC and Banks PA, Soil movement and persistence of triazine herbicides, in *The Triazine Herbicides*, ed. by Le Baron HM, McFarland JF and Burnside OC. Elsevier, San Diego, CA, pp. 355–386 (2008).
32 Buchanan GA and Hiltbold AE, Performance and persistence of atrazine. *Weed Sci* **21**:413–415 (1973).
33 Frank R and Sirons GJ, Dissipation of atrazine residues in soils. *Bull Environ Contam Toxicol* **34**:541–548 (1985).
34 Kells JJ, Rieck CE, Blevins RL and Muir WM, Atrazine dissipation as affected by surface pH and tillage. *Weed Sci* **28**:101–104 (1980).

35 Roeth FW, Lavy TL and Burnside OC, Atrazine degradation in two soil profiles. *Weed Sci* **17**:202–205 (1969).
36 Hiltbold AE and Buchanan GA, Influence of soil pH on persistence of atrazine in the field. *Weed Sci* **25**:515–520 (1977).
37 Mandelbaum RT, Wackett LP and Allen DL, Mineralization of the *s*-triazine ring of atrazine by stable bacterial mixed cultures. *Appl Environ Microbiol* **59**:1695–1701 (1993).
38 Mandelbaum RT, Wackett LP and Allen DL, Rapid hydrolysis of atrazine to hydroxyatrazine by soil bacteria. *Environ Sci Technol* **27**:1943–1946 (1993).
39 Mandelbaum RT, Allan DL and Wackett LP, Isolation and characterization of a *Pseudomonas* sp. that mineralizes the *s*-triazine herbicide atrazine. *Appl Environ Microbiol* **61**:1451–1457 (1995).
40 Topp E, Zhu H, Nour SM, Houot S, Lewis M and Duppels D, Characterization of an atrazine-degrading *Pseudaminobacter* sp. isolated from Canadian and French agricultural soils. *Appl Environ Microbiol* **66**:2773–2782 (2000).
41 Mulbry WW, Zhu H, Nour SM and Topp E, The triazine hydrolase gene *trzN* from *Nocardioides* sp. strain C190: cloning and construction of gene-specific primers. *FEMS Microbiol Lett* **206**:75–79 (2002).
42 de Souza ML, Seffernick J, Martinez B, Sadowsky MJ and Wackett LP, Atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP: gene sequence, enzyme purification, and protein characterization. *J Bacteriol* **178**:4894–4900 (1996).
43 Seffernick JL, McTavish H, Osborne JP, de Souza ML, Sadowsky MJ and Wackett LP, Atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP is a metalloenzyme. *Biochemistry* **41**:14430–14437 (2002).
44 Shapir N, Rosendahl C, Johnson G, Andreina M, Sadowsky MJ and Wackett LP, Substrate specificity and colorimetric assay for recombinant *TrzN* derived from *Arthrobacter aurescens* TC1. *Appl Environ Microbiol* **71**:2214–2220 (2005).
45 Shapir N, Pedersen C, Gill O, Strong L, Seffernick J, Sadowsky MJ, *et al*, *TrzN* from *Arthrobacter aurescens* TC1 is a zinc amidohydrolase. *J Bacteriol* **188**:5859–5864 (2006).
46 Strong LC, Rosendahl C, Johnson G, Sadowsky MJ and Wackett LP, *Arthrobacter aurescens* TC1 metabolizes diverse *s*-triazine ring compounds. *Appl Environ Microbiol* **68**:5973–5980 (2002).
47 Boundy-Mills KL, de Souza ML, Mandelbaum RT, Wackett LP and Sadowsky MJ, The *atzB* gene of *Pseudomonas* sp. strain ADP encodes the second enzyme of a novel atrazine degradation pathway. *Appl Environ Microbiol* **63**:916–923 (1997).
48 Seffernick JL, Aleem A, Osborne JP, Johnson G, Sadowsky MJ and Wackett LP, Hydroxyatrazine *N*-ethylaminohydrolase (*AtzB*): an amidohydrolase superfamily enzyme catalyzing deaminatin and dechlorination. *J Bacteriol* **189**:6989–6997 (2007).
49 Sadowsky MJ, Tong Z, de Souza M and Wackett LP, *AtzC* is a new member of the amidohydrolase protein superfamily and is homologous to other atrazine-metabolizing enzymes. *J Bacteriol* **180**:152–158 (1998).
50 Shapir N, Osborne JP, Johnson G, Sadowsky MJ and Wackett LP, Purification, substrate range, and metal center of *AtzC*: the *N*-isopropylammelide aminohydrolase involved in bacterial atrazine metabolism. *J Bacteriol* **184**:5376–5384 (2002).
51 Eaton RW and Karns JS, Cloning and comparison of the DNA encoding ammelide aminohydrolase and cyanuric acid amidohydrolase from 3 *s*-triazine degrading bacterial strains. *J Bacteriology* **173**:1363–1366 (1991).
52 Fruchey I, Shapir N, Sadowsky MJ and Wackett LP, On the origins of cyanuric acid hydrolase: purification, substrates, and prevalence of *atzD* from *Pseudomonas* sp. strain ADP. *Appl Environ Microbiol* **69**:3653–3657 (2003).
53 Karns JS, Gene sequence and properties of an *s*-triazine ring-cleavage enzyme from *Pseudomonas* sp. strain NRRLB-12227. *Appl Environ Microbiol* **65**:3512–3517 (1999).
54 Cheng G, Shapir N, Sadowsky MJ and Wackett LP, Allophanate hydrolase, not urease, functions in bacterial cyanuric acid metabolism. *Appl Environ Microbiol* **71**:4437–4445 (2005).
55 Martinez B, Tomkins J, Wackett LP, Wing R and Sadowsky MJ, Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. *J Bacteriol* **183**:5684–5697 (2001).
56 Shapir N, Sadowsky MJ and Wackett LP, Purification and characterization of allophanate hydrolase (AtzF) from *Pseudomonas* sp. strain ADP. *J Bacteriol* **187**:3731–3738 (2005).
57 Shapir N, Cheng G, Sadowsky MJ and Wackett LP, Purification and characterization of *TrzF*: biuret hydrolysis by allophanate hydrolase supports growth. *Appl Environ Microbiol* **72**:2491–2495 (2006).
58 Cai B, Han Y, Liu B, Ren Y and Jiang S, Isolation and characterization of an atrazine-degrading bacterium from industrial wastewater in China. *Lett Appl Microbiol* **36**:272–276 (2003).
59 Devers M, Azhari NE, Kolic N and Martin-Laurent F, Detection and organization of atrazine-degrading genetic potential of seventeen bacterial isolates belonging to divergent taxa indicate a recent common origin of their catabolic functions. *FEMS Microbiol Lett* **273**:78–86 (2007).
60 Devers M, Rouard N and Martin-Laurent F, Genetic rearrangement of the *atzAB* atrazine-degrading gene cassette from pADP1::Tn5 to the chromosome of *Variovorax* sp. MD1 and MD2. *Gene* **392**:1–6 (2007).
61 Vaishampayan PA, Kanekar PP and Dhakephalkar PK, Isolation and characterization of *Arthrobacter* sp. strain MCM B-436, an atrazine-degrading bacterium, from rhizospheric soil. *Internat Biodet Biodeg* **60**:273–278 (2007).
62 Rousseaux S, Soulas G and Hartmann A, Plasmid localization of atrazine-degrading genes in newly described *Chelatobacter* and *Arthrobacter* strains. *FEMS Microbiol Ecol* **41**:69–75 (2002).
63 de Souza ML, Wackett LP and Sadowsky MJ, The *atzABC* genes encoding atrazine catabolism are located on a self-transmissible plasmid in *Pseudomonas* sp. strain ADP. *Appl Environ Microbiol* **64**:2323–2326 (1998).
64 Sajjaphan K, Shapir N, Wackett LP, Palmer M, Blackmon B, Tomkins J, *et al*, *Arthrobacter aurescens* TC1 atrazine catabolism genes *trzN, atzB*, and *atzC* are linked on a 160-kilobase region and are functional in *Escherichia coli*. *Appl Environ Microbiol* **70**:4402–4407 (2004).
65 Hirkala DLM and Germida JJ, Field and soil microcosm studies on the survival and conjugation of a *Pseudomonas putida* strain bearing a recombinant plasmid, pADPTel. *Can J Microbiol* **50**:595–604 (2004).
66 Devers M, Henry S, Hartmann A and Martin-Laurent F, Horizontal gene transfer of atrazine-degrading genes (*atz*) from *Agrobacterium tumefaciens* st96-4 pADP1::TN5 to bacteria of maize cultivated soil. *Pest Manag Sci* **61**:870–880 (2005).
67 Sorensen SJ, Bailey M, Hansen LH, Kroer N and Wuertz S, Studying plasmid horizontal transfer *in situ*: a critical review. *Nature* **3**:700–710 (2005).
68 Ghosh D, Roy K, Williamson KE, White DC, Wommack KE, Sublette KL, *et al*, Prevalence of lysogeny among soil bacteria and presence of 16s rRNA and *trzN* genes in viral community DNA. *Appl Environ Microbiol* **74**:495–502 (2008).
69 de Souza ML, Wackett LP, Boundy-Mills KL, Mandelbaum RT and Sadowsky MJ, Cloning, characterization, and expression of a gene region from *Pseudomonas* sp. strain ADP involved in the dechlorination of atrazine. *Appl Environ Microbiol* **61**:3373–3378 (1995).
70 Scott C, Jackson CJ, Coppin CW, Mourant RG, Hiltron ME, Sutherland TD, *et al*, Catalytic improvement and evolution of atrazine chlorohydrolase. *Appl Environ Microbiol* **75**:2184–2191 (2009).
71 Maron PA, Lejon DPH, Carbalho E, Bizet K, Lemanceau P, Ranjard L, *et al*, Assessing genetic structure and diversity of airborne bacterial communities by DNA fingerprinting and 16s rDNA clone library. *Atmos Environ* **39**:3687–3695 (2005).
72 Prospero JM, Blades E, Mathison G and Naidu R, Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil dust. *Aerobiologia* **21**:1–9 (2005).
73 Walker SR, Robinson GR and Hargreaves PA, Weed control with atrazine and chlorsulfuron is determined by herbicide availability and persistence in soils. *Aust J Agric Res* **48**:1003–1009 (1997).
74 Krutz LJ, Zablotowicz RM, Reddy KN, Koger III CH and Weaver MA, Enhanced degradation of atrazine under field conditions correlates with a loss of weed control in the glasshouse. *Pest Manag Sci* **63**:23–31 (2007).
75 Krutz LJ, Burke IC, Reddy KN and Zablotowicz RM, Evidence for cross-adaptation between *s*-triazine herbicides resulting in reduced efficacy under field conditions. *Pest Manag Sci* **64**:1024–1030 (2008).
76 Vaishampaya PA and Kanekar PP, Use of atrazine sensitive leguminous plants as biological indicators to evaluate the atrazine

degradation efficiency of a bacterial inoculum. *World J Microbiol Biotechnol* **23**:447–449 (2007).
77 Wenk M, Bourgeois M, Allen J and Stucki G, Effects of atrazine-mineralizing microorganisms on weed growth in atrazine-treated soils. *J Agric Food Chem* **45**:4474–4480 (1997).
78 Aislabie J, Hunter D, Ryburn J, Fraser R, Northcott GL and Di HJ, Atrazine mineralisation rates in New Zealand soils are affected by time since atrazine exposure. *Aust J Soil Res* **42**:783–792 (2004).
79 Aislabie J, Bej AK, Ryburn J, Lloyd N and Wilkins A, Characterization of *Arthrobacter nicotinovorans* HIM, an atrazine-degrading bacterium from agricultural soil New Zealand. *FEMS Microbiol Ecol* **52**:279–286 (2005).
80 Di JH, Sparling GP, Lee R and Magesan GN, The effect of mineralization rates of atrazine in surface and subsurface soils on its groundwater contamination potential. *Aust J Soil Res* **39**:175–183 (2001).
81 Sparling G, Dragten R, Aislabie J and Fraser R, Atrazine mineralisation in New Zealand topsoils and subsoils: Influence of edaphic factors and numbers of atrazine-degrading microbes. *Aust J Soil Res* **36**:557–570 (1998).
82 Pussemier L, Goux S, Vanderheyden V, Debongnie P, Tresinie I and Foucart G, Rapid dissipation of atrazine in soils taken from various maize fields. *Weed Res* **37**:171–179 (1997).
83 Houot S, Topp E, Yassir A and Soulas G, Dependence of accelerated degradation of atrazine on soil pH in French and Canadian soils. *Soil Biol Biochem* **32**:615–625 (2000).
84 Mueller TC, Steckel LE and Radosevich M, Effect of soil pH and previous atrazine use history on atrazine degradation in a Tennessee field soil. *Weed Sci* **in press** (2010).
85 Zablotowicz RM, Weaver MA and Locke MA, Microbial adaptation for accelerated atrazine mineralization/degradation in Mississippi Delta soils. *Weed Sci* **54**:538–547 (2006).
86 Alvey S and Crowley DE, Survival and activity of an atrazine-mineralizing bacterial consortium in rhizosphere soil. *Environ Sci Technol* **30**:1596–1603 (1996).
87 de Souza ML, Newcombe D, Alvey S, Crowley DE, Hay A, Sadowsky MJ, *et al*, Molecular basis of a bacterial consortium: interspecies catabolism of atrazine. *Appl Environ Microbiol* **64**:178–184 (1998).
88 Goux S, Agathos SN and Pussemier L, Metabolic characterization of fifteen atrazine-degrading microbial communities. *J Ind Microbiol Biotechnol* **21**:254–259 (1998).
89 Rhine ED, Fuhrmann JJ and Radosevich M, Microbial community responses to atrazine exposure and nutrient availability: linking degradation capacity to community structure. *Microbiol Ecol* **46**:145–160 (2003).
90 Martin-Laurent F, Cornet L, Ranjard L, Lopez-Gutierrez L, Philippot L, Schwartz C, *et al*, Estimation of atrazine-degrading genetic potential and activity in three French agricultural soils. *FEMS Microbiol Ecol* **48**:425–435 (2004).
91 Popov VH, Cornish PS, Sultana K and Morris EC, Atrazine degradation in soils: the role of microbial communities, atrazine application history, and soil carbon. *Aust J Soil Res* **43**:861–871 (2005).
92 Smith D, Alvey S and Crowley DE, Cooperative catabolic pathways within an atrazine-degrading enrichment culture isolated from soil. *FEMS Microbiol Ecol* **53**:265–273 (2005).
93 Santiago-Mora R, Martin-Laurent F, De Prado R and Franco AR, Degradation of simazine by microorganisms isolated from soils of Spanish olive fields. *Pest Manag Sci* **61**:917–921 (2005).
94 Satsuma K, Kameshiro M, Hayashi O, Sato K and Kato Y, Characterization of a *Nocardioides*-based, atrazine-mineralizing microbial colony isolated from Japanese riverbed sediment. *J Pestic Sci* **31**:420–423 (2006).
95 Martin-Laurent F, Barres B, Wagschal I, Piutti S, Devers M, Soulas G, *et al*, Impact of the maize rhizosphere on the genetic structure, the diversity and the atrazine-degrading gene composition of cultivable atrazine-degrading communities. *Plant Soil* **282**:99–115 (2006).
96 Smith D and Crowley DE, Contribution of ethylamine degrading bacteria to atrazine degradation in soils. *FEMS Microbiol Ecol* **58**:271–277 (2006).
97 Vibber LL, Pressler MJ and Colores GM, Isolation and characterization of novel atrazine-degrading microorganisms from an agricultural soil. *Appl Microbiol Biotechnol* **75**:921–928 (2007).
98 Kolic NU, Hrsak D, Begonja AK, Petric I, Stripicevic S, Soulas G, *et al*, Combined metabolic activity within an atrazine mineralizing community enriched from agrichemical factory soil. *Internat Biodeter Biodeg* **60**:299–307 (2007).
99 Siripattanakul S, Wirojanagud W, McEvoy TM, Limpiyakorn T and Khan E, Atrazine degradation by stable mixed culture enriched from agricultural soil and their characteriazation. *J Appl Microbiol* **106**:986–997 (2009).
100 Fierer N and Jackson JB, The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* **103**:626–631 (2006).
101 Wakelin SA, Macdonald LM, Rogers SL, Gregg AL, Golber TP and Baldock JA, Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. *Soil Biol Biochem* **40**:803–813 (2008).
102 Paul EJ and Clark FE, Soil as a habitat for organisms and their reactions, in *Soil Microbiology and Biochemistry*, 2nd edition, ed. by Paul EJ and Clark FE. Academic Press, San Diago, CA, pp 12–32 (1996).
103 Barriuso E and Houot S, Rapid mineralization of the *s*-triazine ring of atrazine in soils in relation to soil management. *Soil Biol Biochem* **28**:1341–1348 (1996).
104 Shaner DL and Henry WB, Field history and dissipation of atrazine and metolachlor in Colorado. *J Environ Qual* **36**:128–134 (2007).
105 Zablotowicz RM, Krutz LJ, Reddy KN, Weaver MA, Koger CH and Locke MA, Rapid development of enhanced atrazine degradation in a Dundee silt loam under continuous corn and in rotation with cotton. *J Agric Food Chem* **55**:852–859 (2007).
106 Goux S, Shapir N, Fantroussi SE, Lelong S, Agathos SN and Pussemier L, Long-term maintenance of rapid atrazine degradation in soils inoculated with atrazine degraders. *Water Air Soil Poll Focus* **3**:131–142 (2003).
107 Hang S, Barriuso E and Houot S, Behavior of ^{14}C-atrazine in Argentinean topsoils under different cropping managements. *J Environ Qual* **32**:2216–2222 (2003).
108 Hang S, Barriuso E and Houot S, Atrazine behaviour in the different pedological horizons of two Argentinean non-till soil profiles. *Weed Res* **45**:130–139 (2005).
109 Krutz LJ, Gentry TJ, Sensema SA, Pepper IL and Tierney DP, Mineralisation of atrazine, metolachlor and their respective metabolites in vegetated filter strip and cultivated soil. *Pest Manag Sci* **62**:505–514 (2006).
110 Yassir A, Lagacherie B, Houot S and Soulas G, Microbial aspects of atrazine biodegradation in relation to history of soil treatment. *Pestic Sci* **55**:799–809 (1999).
111 Ostrofsky EB, Robinson JB, Traina SJ and Tuovinen OH, Effect of cyanuric acid amendment on atrazine mineralization in surface soils and detection of the *s*-triazine ring-cleavage gene *trzD*. *Soil Biol Biochem* **33**:1539–1545 (2001).
112 Shaner DL, Wiles L and Hansen N, Behavior of atrazine in limited irrigation cropping systems in colorado: Prior use is important. *J Environ Qual* **38**:1861–1869 (2009).
113 Krutz LJ, Senseman SA and Haney RL, Effect of Roundup Ultra on atrazine degradation in soil. *Biol Fertil Soils* **38**:115–118 (2003).
114 Zablotowicz RM, Krutz LJ, Weaver MA, Accinelli C and Reddy KN, Glufosinate and ammonium sulfate inhibit atrazine degradation in adapted soils. *Biol Fertil Soils* **45**:19–26 (2008).
115 Senseman SA, *Herbicide Handbook*, 9th edition. Weed Science Society of America, Lawrence, KS (2009).
116 Curl EA and Truelove B, *The Rhizosphere*, Springer, New York, NY (1986).
117 Marchand AL, Piutti S, Lagacherie B and Soulas G, Atrazine mineralization in bulk and maize rhizosphere. *Biol Fertil Soils* **35**:288–292 (2002).
118 Lopez-Gutierrez JC, Philippot L and Martin-Laurent F, Impact of maize mucilage on atrazine mineralization and *atzC* abundance. *Pest Manag Sci* **61**:838–844 (2005).
119 Piutti S, Hallet S, Rousseaux S, Philippot L, Soulas G and Martin-Laurent F, Accelerated mineralisation of atrazine in maize rhizosphere soil. *Biol Fertil Soils* **36**:434–441 (2002).
120 Locke MA and Bryson CT, Herbicide–soil interactions in reduced tillage and plant residue management systems. *Weed Sci* **45**:307–320 (1987).
121 Hang S, Houot S and Barriuso E, Mineralization of ^{14}C-atrazine in an entic Haplustoll as affected by selected winter weed control strategies. *Soil Tillage Res* **96**:234–242 (2007).
122 Hang S, Houot S and Barriuso E, Vertical variation of atrazine mineralization capacity in soils. *Agriscientia* **2**:87–95 (2007).

123 Ostrofsky EB, Traina SJ and Tuovinen OH, Variation in atrazine mineralization rates in relation to agricultural management practice. *J Environ Qual* **26**:647–657 (1997).
124 Bichat F, Sims GK and Mulvaney RL, Microbial utilization of heterocyclic nitrogen from atrazine. *Soil Sci Soc Am J* **63**:100–110 (1999).
125 Abdelhafid R, Houot S and Barriuso E, Dependence of atrazine degradation on C and N availability in adapted and non-adapted soils. *Soil Biol Biochem* **32**:389–401 (2000).
126 Abdelhafid R, Houot S and Barriuso E, How increasing availabilities of carbon and nitrogen affect atrazine behaviour. *Biol Fertil Soils* **30**:333–340 (2000).
127 Alvey S and Crowley DE, Influence of organic amendments on biodegradation of atrazine as a nitrogen source. *J Environ Qual* **24**:1156–1162 (1995).
128 Garces RAG, Hansen AM and Van Afferden M, Mineralization of atrazine in agricultural soil: inhibition by nitrogen. *Environ Toxicol Chem* **26**:844–850 (2007).
129 Garcia-Gonzalez V, Govantes F, Shaw LJ, Burns RG and Santero E, Nitrogen control of atrazine utilization in *Pseudomonas* sp. strain ADP. *Appl Environ Microbiol* **69**:6987–6993 (2003).
130 Garcia-Gonzalez V, Govantes F, Porrua O and Santero E, Regulation of the *Pseudomonas* sp. strain ADP cyanuric acid degradation operon. *J Bacteriol* **187**:155–167 (2005).
131 Arbeli Z and Fuentes CL, Accelerated biodegradation of pesticides: an overview of the phenomenon, its basis and possible solutions: and a discussion on the tropical dimension. *Crop Prot* **26**:1733–1746 (2007).
132 Felsot AS and Tollefson JJ, Evaluation of some methods for coping with enhanced biodegradation of soil insecticides, in *Enhanced Biodegradation of Pesticides in the Environment*, ed. by Racke KD and Coats JR. American Chemical Society, Washington, DC, pp. 192–213 (1990).
133 Bakhsh A, Ma L, Ahuja LR, Hatfield JL and Kanwar RS, Using RZQWM to predict herbicide leaching losses in subsurface drainage water. *Trans Am Soc Agric Eng* **47**:1415–1426 (2004).
134 Boesten JJTI and Van der Linden AMA, Modeling the influence of sorption and transformation on pesticide leaching and persistence. *J Environ Qual* **20**:425–435 (1991).
135 Chinkuyu A, Meixner T, Gish T and Daughtry C, Predictions of pesticide losses in surface runoff from agricultural fields using GLEAMS and RZWQM. *Trans Am Soc Agric Eng* **48**:585–599 (2005).
136 Cryer SA and Havens PL, Regional sensitivity analysis using a fractional factorial method for the USDA model GLEAMS. *Environ Modeling Software* **14**:613–624 (1999).
137 Dann RL, Close ME, Lee R and Pang L, Impact of data quality and model complexity on prediction of pesticide leaching. *J Environ Qual* **35**:628–640 (2006).
138 Donigian AS, Jr, and Carsel RF, Modeling the impact of conservation tillage practices on pesticide concentrations in ground and surface waters. *Environ Toxicol Chem* **6**:241–250 (1987).
139 Leterme B, Vanclooster M, Van Der Lindent T, Tiktak A and Rounsevell DA, Including spatial variability in monte carlo simulations of pesticide leaching. *Environ Sci Technol* **41**:7444–7450 (2007).
140 Malone RM, Ahuja LR, Ma L, Wauchope RD, Ma Q and Rojas KW, Application of root zone water quality model (RZWQM) to pesticide fate and transport: an overview. *Pest Manag Sci* **60**:205–221 (2004).
141 Persicani D, Pesticide leaching into field soils: sensitivity analysis of mathematical models. *Ecol Model* **84**:265–280 (1996).
142 *Decision Documents for Atrazine*. [Online]. United States Environmental Protection Agency (USEPA) (2006). Available: http://www.epa.gov/oppsrrd1/REDs/atrazine_combined_docs.pdf [25 August 2009].
143 Seffernick JL, Johnson G, Sadowsky MJ and Wackett LP, Substrate specificity of atrazine chlorohydrolase and atrazine-catabolizing bacteria. *Appl Environ Microbiol* **66**:4247–4252 (2000).
144 Bottoni P, Keizer J and Funari E, Leaching indices of some major triazine metabolites. *Chemosphere* **32**:1401–1411 (1996).
145 Kruger EL, Somasundaram L, Kanwa RS and Coats JR, Persistence and degradation of [^{14}C] atrazine and [^{14}C] deisopropylatrazine as affected by soil depth and moisture conditions. *Environ Toxicol Chem* **12**:1959–1967 (1993).
146 Kruger EL, Rice PJ, Anhalt JC, Anderson TA and Coats JR, Comparative fates of atrazine and deethylatrazine in sterile and nonsterile soils. *J Environ Qual* **26**:95–101 (1997).
147 Beulke S, Beinu WV, Brown CD, Mitchell M and Walker A, Evaluation of simplifying assumptions on pesticide degradation in soil. *J Environ Qual* **34**:1933–1943 (2005).
148 *Soil Persistence Models and EU Registration*. [Online]. FOCUS. Available: http://ec.europa.eu/food/plant/protection/evaluation/guidance/soil_en.pdf [28 August 2009].
149 Culleres DB, Boesten J, Bolognesi C, Boobis A, Buchert A, Capri E, *et al*, Opinion on a request from EFSA related to the default Q_{10} value used to describe the temperature effect on transformation rates of pesticides in soil. *EFSA J* **622**:1–32 (2007).
150 Accinelli C, Dinelli G, Vicaria A and Catizone P, Atrazine and metolachlor degradation in subsoils. *Biol Fertil Soils* **33**:495–500 (2001).
151 Blume E, Bischoff M, Moorman TB and Turco RF, Degradation and binding of atrazine in surface and subsurface soils. *J Agric Food Chem* **52**:7382–7388 (2004).
152 Miller JL, Wollum III AG and Weber JB, Degradation of Carbon-14-atrazine and Carbon-14-metolachlor in soil from four depths. *J Environ Qual* **26**:633–638 (1997).
153 Reungsang A, Moorman TB and Kanwar RS, Transport and fate of atrazine in Midwestern riparian buffer strips. *J Am Water Resources Assoc* **37**:1681–1692 (2001).
154 Hayar S, Munier-Lamy C, Chone T and Schiavon M, Physico-chemical versus microbial release of 14C-atrazine bound residues from a loamy clay soil incubated in laboratory microcosms. *Chemosphere* **34**:2683–2697 (1997).
155 Langenbach T, Schroll R and Paim S, Fate and distribution of ^{14}C-atrazine in a tropical oxisol. *Chemosphere* **40**:449–455 (2000).
156 Mersie W, Seybold C and Tsegaye T, Movement, adsorption and mineralization of atrazine in two soils with and without switchgrass (*Panicum virgatum*) roots. *Eur J Soil Sci* **50**:343–349 (1999).
157 Mordaunt CJ, Gevao B, Jones KC and Semple KT, Formation of non-extractable pesticide residues: observations on compound differences, measurement and regulatory issues. *Environ Pollut* **133**:25–34 (2005).
158 Getenga ZM, Doerfler U and Schroll R, Study of atrazine degradation in soil from Kenyan sugarcane cultivated fields in controlled laboratory conditions. *Toxicol Environ Chem* **91**:195–207 (2009).
159 Martin M, Gibello A, Lobo C, Nande M, Garbi C, Fajardo C, *et al*, Application of fluorescence *in situ* hybridization technique to detect simazine-degrading bacteria in soil samples. *Chemosphere* **71**:703–710 (2008).
160 Moscinski JK, Jayachandran K and Moorman TB, Mineralization of the herbicide atrazine by *Agrobacterium radiobacter*, in *Abstracts of the 96th General Meeting of the American Society for Microbiology*, American Society for Microbiology, Washington, DC, p. 458 (1996).
161 de Souza ML, Seffernic J, Martinez B, Sadowsky MJ and Wackett LP, The atrazine catabolism genes *atzABC* are widespread and highly conserved. *J Bacteriol* **180**:1951–1954 (1998).
162 Struthers JK, Jayachandran K and Moorman TB, Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil. *Appl Environ Microbiol* **64**:3368–3375 (1998).
163 Rousseaux S, Hartmann A and Soulas G, Isolation and characterization of new gram-negative and gram-positive atrazine degrading bacteria from different French soils. *FEMS Microbiol Ecol* **36**:211–222 (2001).
164 Mongodin EF, Shapir N, Daugherty SC, DeBoy RT, Emerson JB, Shvartzbeyn A, *et al*, Secrets of soil survival revealed by the genome sequence of *Arthrobacter aurescens* TC1. *Plos Genetics* **2**:2094–2106 (2006).
165 Dai XZ, Jiang JD, Gu LF, Pan RQ and Li SP, Study on the atrazine-degrading genes in *Arthrobacter* sp. AG1. *Chin J Biotechnol* **23**:783–793 (2007).
166 Li Q, Li Y, Zhu X and Cai B, Isolation and characterization of atrazine-degrading *Arthrobacter* sp. AD26 and use of this strain in bioremediation of contaminated soil. *J Environ Sci* **20**:1226–1230 (2008).
167 Iwasaki A, Takagi K, Yoshioka Y, Fujii K, Kojima Y and Harada N, Isolation and characterization of a novel simazine-degrading β-proteobacterium and detection of genes encoding *s*-triazine-degrading enzymes. *Pest Manag Sci* **63**:261–268 (2007).
168 Devers M, Soulas G and Martin-Laurent F, Real-time reverse transcription PCR analysis of expression of atrazine catabolism genes in two bacterial strains isolated from soil. *J Microbiol Meth* **56**:3–15 (2004).

169 Karns JS and Eaton RW, Genes encoding *s*-triazine degradation are plasmid-borne in *Klebsiella pneumonia* strain 99. *J Agric Food Chem* **45**:1017–1022 (1997).
170 Kaneko T, Nakamura Y, Sato S, Asamizu E, Kato T, Sasamoto S, *et al*, Complete genome structure of the nitrogen fixing symbiotic bacterium *Mesorhizobium loti*. *DNA Res* **7**:331–338 (2000).
171 Piutti S, Semon E, Landry D, Hartmann A, Dousset S, Lichtfouse E, *et al*, Isolation and characterization of *Nocardioides* sp. SP12, an atrazine-degrading bacterial strain possessing the gene *trzN* from bulk- and maize rhizosphere soil. *FEMS Microbiol Lett* **221**:111–117 (2003).
172 Yamazaki K, Fujii A, Iwasaki A, Takagi K, Satsuma K, Harada N, *et al*, Different substrate specificities of two triazine hydrolases (*TrzN*s) from *Nocardioides* species. *FEMS Microbiol Lett* **286**:171–177 (2008).
173 Topp E, A comparison of three atrazine-degrading bacteria for soil bioremediation. *Biol Fertil Soils* **33**:529–534 (2001).
174 Yanze-Kontchou C and Gschwind N, Mineralization of the herbicide atrazine as a carbon source by a Pseudomonas strain. *Appl Environ Microbiol* **60**:4297–4302 (1994).
175 Hernandez M, Villalobos P, Morgante V, Gonzalez M, Reiff C, Moore E, *et al*, Isolation and characterization of a novel simazine-degrading bacterium from agricultural soil of central Chile, *Pseudomonas* sp. MHP41. *FEMS Microbiol Lett* **286**:184–190 (2008).
176 Radosevich M, Traina SJ, Hao Y and Tuovinen OH, Degradation and mineralization of atrazine by a soil bacterial isolate. *Appl Environ Microbiol* **61**:297–302 (1995).
177 Stamper DM, Hallberg KB, Radosevich M, Traina SJ and Tuovinen OH, Phylogenetic and biochemical characterization of an atrazine-mineralizing bacterial isolate, in *Abstracts of the 97th General Meeting of the American Society for Microbiology*, American Society for Microbiology, Washington, DC, pp. 525 (1997).
178 Bouquard C, Ouazzani J, Prome J, Michel-Briand Y and Plesiat P, Dechlorination of atrazine by a *Rhizobium* sp. isolate. *Appl Environ Microbiol* **63**:862–866 (1997).
179 Hernandez GM, Morgante V, Perez AM, Biaggini PB, Noew PM, Vergara MG, *et al*, Novel *s*-triazine-degrading bacteria isolate from agricultural soils of central Chile for herbicide bioremediation. *Electronic J Biotechnol* **11**:5 (2008).
180 Hu J, Dai XZ and Li SP, The isolation and identification of a gram positive atrazine-degradation bacterium BTAHI. *China Environ Sci* **24**:738–742 (2004).
181 Jiang Z, Ma Y, Wang R and Zhang Y, Isolation and identification of two high effective atrazine degrading microbe. *2nd Internat Conf Bioinformatics and Biomedical Engineering, ICBBE*, **4535438**: 4233–4235 (2008).
182 Kolic NU, Martin-Laurent F, Devers M, Petric I, Kolar AB and Hrsak D, Genetic potential, diversity and activity of an atrazine-degrading community enriched from a herbicide factory effluent. *J Appl Microbiol* **105**:1334–1343 (2008).
183 Singh P, Suri CR and Cameotra SS, Isolation of a member of *Acinetobacter* species involved in atrazine degradation. *Biochem Biophys Res Comm* **317**:697–702 (2004).
184 Dehghani M, Nasseri S, Amin S, Naddafee K, Taghavi M, Yunesian M, *et al*, Isolation and identification of atrazine-degrading bacteria from corn field soil in Fars Province of Iran. *Pakistan J Biol Sci* **10**:84–89 (2007).
185 Shapir N, Mandelbaum RT and Fine P, Atrazine mineralization by indigenous and introduced *Pseudomonas* sp. strain ADP in sand irrigated with municipal wastewater and amended with composted sludge. *Soil BiolBiochem* **32**:887–897 (2000).
186 Harada N, Takagi K, Fujii K and Iwasaki A, Transformation of methylthio-*s*-triazines via sulfur oxidation by strain JUN7, a *Bacillus cereus* species. *Soil Biol Biochem* **38**:2952–2957 (2006).
187 Satsuma K, Characterisation of new strains of atrazine-degrading *Nocardioides* sp. isolated from Japanese riverbed sediment using naturally derived river ecosystem. *Pest Manag Sci* **62**:340–349 (2006).
188 Satsuma K, Complete biodegradation of atrazine by a microbial community isolated from a naturally derived river ecosystem (microcosm). *Chemosphere* **77**:590–596 (2009).
189 Zwieten LV and Kennedy IR, Rapid degradation of atrazine by *Rhodococcus* sp. NI86/21 and by an atrazine-perfused soil. *J Agric Food Chem* **43**:1377–1382 (1995).
190 Getenga ZM, Dorfler U and Schroll R, Laboratory degradation studies of ^{14}C-atrazine and -isoproturon from sugarcane cultivated fields under Kenyan tropical conditions. *Bull Environ Toxicol* **82**:678–682 (2009).
191 Shapir N, Goux S, Mandelbaum RT and Pussemier L, The potential of soil microorganisms to mineralize atrazine as predicted by MCH-PCR followed by nested PCR. *Can J Microbiol Rev Can Microbiol* **46**:425–432 (2000).
192 Vanderheyden V, Debongnie P and Pussemier L, Accelerated degradation and mineralization of atrazine in surface and subsurface soil materials. *Pestic Sci* **49**:237–242 (1997).
193 Martin-Laurent F, Piutti S, Hallet S, Wagscha I, Philippot L, Catroux G, *et al*, Monitoring of atrazine treatment on soil bacterial, fungal and atrazine-degrading communities by quantitative competitive PCR. *Pest Manag Sci* **59**:259–268 (2003).
194 Vargha M, Takats Z and Marialgeti K, Degradation of atrazine in a laboratory scale model system with Danube river sediment. *Water Res* **39**:1560–1568 (2005).
195 Mahia J and Diaz-Ravina M, Atrazine degradation and residue distribution in two acid soils from temperature humid zone. *J Environ Qual* **36**:826–831 (2007).
196 Mahia J, Martina A and Diaz-Ravina M, Extractable atrazine and its metabolites in agricultural soils from the temperate humid zone. *Environ Geochem Health* **30**:147–152 (2008).
197 Sanchez M, Garbi C, Martinez-Alvarez R, Ortiz LT, Allende JL and Martin M, *Klebsiella planticola* strain DSZ mineralizes simazine: physiological adaptations involved in the process. *Appl Microbiol Biotechnol* **66**:589–596 (2005).
198 Gschwind N, Rapid mineralization of the herbicide atrazine by a mixed microbial community, in *Proc Internat Symp Environmental Aspects of Pesticide Microbiology*, Sigtuna, Sweden. Department of Microbiology, Swedish University of Agricultural Scienses, Uppsala, Sweden, pp. 204–206 (1992).
199 Gschwind N, Biologischer Abbau des Herbizids Atrazin in einem Modellabwasser. *Gwf-Wasser/Abwasser* **134**:65–69 (1993).
200 Potter TL and Bosch DD, Summer cover crops reduce atrazine leaching to shallow groundwater in Southern Florida. *J Environ Qual* **36**:1301–1309 (2007).
201 Ames RA and Hoyle BL, Biodegradation and mineralization of atrazine in shallow subsurface sediments from Illinois. *J Environ Qual* **28**:1674–1681 (1999).
202 Assaf NA and Turco RF, Accelerated biodegradation of atrazine by a microbial consortium is possible in culture and soil. *Biodegradation* **5**:29–35 (1994).
203 Gan J, Becker RL, Koskinen WC and Buhler DD, Degradation of atrazine in two soils as a function of concentration. *J Environ Qual* **25**:1064–1072 (1996).
204 Jenks BM, Roeth FW, Martin AR and McCallister DL, Influence of surface and subsurface soil properties on atrazine sorption and degradation. *Weed Sci* **46**:132–138 (1998).
205 Hixson AC, Shi W, Weber JB, Yelverton FH and Rufty TW, Soil organic matter changes in turfgrass systems affect binding and biodegradation of simazine. *Crop Sci* **49**:1481–1488 (2009).
206 Anderson KC, Wheeler KA, Robinson JB and Tuovinen OH, Atrazine mineralization potential in two wetlands. *Water Res* **36**:4785–4784 (2002).
207 Ostrofsky EB, Robinson JB, Traina SJ and Tuovinen OH, Analysis of atrazine-degrading microbial communities in soils using most-probable-number enumeration, DNA hybridization, and inhibitors. *Soil Biol Biochem* **34**:1449–1459 (2002).
208 Chirnside AEM, Ritter WF and Radosevich M, Isolation of a selected microbial consortium from a pesticide-contaminated mix-load site soil capable of degrading the herbicides atrazine and alachlor. *Soil Biology Biochem* **39**:3056–3065 (2007).
209 Bigwaneza PC, Fortin J, Antoun H, Ndayegamiye A and Cote D, Effect of long-term liquid pig manure application on atrazine mineralization in a soil cultivated with maize. *Biol Fertil Soils* **38**:191–199 (2003).
210 Topp E, Tessier L and Gregoric EG, Dairy manure incorporation stimulates rapid atrazine mineralization in an agricultural soil. *Can J Soil Sci* **3**:403–409 (1996).
211 Liu Z, Clay SA and Clay DE, Spatial variability of atrazine and alachlor efficacy and mineralization in an eastern South Dakota field. *Weed Sci* **50**:662–671 (2002).
212 Strong LC, McTavish H, Sadowsky MJ and Wackett LP, Field-scale remediation of atrazine-contaminated soil using recombinant *Escherichia coli* expressing atrazine chlorohydrolase. *Environ Microbiol* **2**:91–98 (2000).

213 Hang S, Nassetta M, Canas AI, Rampoldi EA, Fernandez-Danigia MV and Diaz-Zorita M, Changes in the atrazine extractable residues in no-tilled mollisols. *Soil Tillage Res* **96**:243–249 (2007).
214 Dinamarca MA, Cereceda-Balic F, Fadic X and Seeger M, Analysis of *s*-triazine-degrading microbial communities in soils using most-probable-number enumeration and tetrazolium-salt detection. *Internat Microbiol* **10**:209–215 (2007).
215 Moran AC, Muller A, Manzano M and Gonzalez B, Simazine treatment history determines a significant herbicide degradation potential in soils that is not improved by bioaugmentation with *Pseudomonas* sp. ADP. *J Appl Microbiol* **101**:26–35 (2006).
216 Arbeli Z and Fuentes CL, Dominance of atrazine chlorohydrolase gene *trzN* over its analogous *atzA* in 13 agricultural soils from three departments in Colombia. *Internat Biodeter Biodeg* **62**:14 (2008).