

Transfer of Atrazine Degradation Capability To Mineralize Aged ¹⁴C-Labeled Atrazine Residues in Soils

Nicolai David Jablonowski,^{*,†} Jason L. Krutz,[‡] Rosane Martinazzo,[§] Petra Zajkoska,^{||} Georg Hamacher,[⊥] Nils Borchard,[#] and Peter Burael[¶]

[†]IBG-2: Plant Sciences, Institute of Bio- and Geosciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

[‡]Delta Research and Extension Service, Mississippi State University, Stoneville, Mississippi 38776, United States

[§]Embrapa Temperate Agriculture, Brazilian Agricultural Research Corporation, BR 392, km 78, 96010-971 Pelotas, Brazil

^{||}Institute of Biotechnology and Food Science, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 81237 Bratislava 1, Slovak Republic

[⊥]Carpus+Partner AG, Forckenbeckstraße 61, 52074 Aachen, Germany

[#]IBG-3: Agrosphere, Institute of Bio- and Geosciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

[¶]Sustainable Campus, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

ABSTRACT: The degradation of environmentally long-term aged (22 years) ¹⁴C-labeled atrazine residues in soil stimulated by inoculation with atrazine-adapted soil from Belgium, the United States (U.S.), and Brazil at two different moisture regimes (50% WHC_{max}/slurried conditions) was evaluated. Inoculation of the soil containing the aged ¹⁴C-labeled atrazine residues with 5, 50, and 100% (w/w) Belgian, U.S., or Brazilian atrazine-adapted soil increased ¹⁴C-atrazine residue mineralization by a factor of 3.1–13.9, depending upon the amount of atrazine-adapted soil inocula and the moisture conditions. Aged ¹⁴C-atrazine residue mineralization varied between 2 and 8% for Belgian and between 1 and 2% for U.S. and Brazilian soil inoculum at 50% WHC_{max} but was increased under slurried conditions, accounting for 8–10% (Belgian soil), 2–7% (Brazilian soil), and 3% (American soil). The results show that an increased degradation of long-term aged ¹⁴C-labeled atrazine residues is possible by the transfer of atrazine-adapted soil microflora from different soils and regions to non-adapted soil.

KEYWORDS: atrazine, soils, adaption, transfer, mineralization

■ INTRODUCTION

Microbial degradation of pesticides in the environment represents an important dissipation process. The adaption of the soil microflora to pesticides because of repeated pesticide application results in rapid mineralization of these compounds and a reduction of the intended effects on pest organisms or weeds.^{1–4} Dissipation of the *s*-triazine herbicide atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], as one of the largest applied pesticides mainly for weed control in maize plantations worldwide, was investigated intensively. In recent years, a number of investigations were conducted describing the accelerated atrazine dissipation in field plots, which were treated with atrazine regularly, indicating a microbial adaption and enrichment of organisms capable of degrading atrazine using this pesticide as a carbon and nitrogen source.^{5–7} Soils being not adapted retain atrazine in the soil matrix for many years.⁸ This persistency of atrazine to some extent was even observed in a highly adapted soil.⁹

Little is known about the transfer of the enhanced biodegradation capability of pesticides from adapted to non-adapted soils containing pesticide residues. When using the herbicide napropamide, an induction and a transfer of biodegradation capability was documented.¹⁰ A rapid degradation was also found for the herbicide carbetamide upon repeated application onto soil, and the transfer of the degradation capability to previously non-treated soils resulting

in accelerated degradation of carbetamide through physical soil transfer was described.¹¹ Even though an accelerated mineralization of environmentally long-term aged ¹⁴C-labeled atrazine residues in soil was possible by the addition of the atrazine-degrading microorganism *Pseudomonas* species strain ADP,¹² nothing is known about the potential mineralization of these aged residues by inoculation with soil containing indigenous atrazine-adapted microorganisms. This laboratory study was conducted to evaluate the mineralization potential of aged ¹⁴C-labeled atrazine residues in soil by inoculation with atrazine-adapted, agriculturally used soils from Belgium, the United States (U.S.), and Brazil. The transfer of atrazine-degrading capabilities into soils containing long-term-aged atrazine residues provides information on the interrelation of the soil–biosphere in relation to the decontamination potential of pesticide residues in soil.

■ MATERIALS AND METHODS

Aged ¹⁴C-Labeled Atrazine Residue Soil. The Gleyic Cambisol soil containing environmentally long-term-aged ring-¹⁴C-labeled atrazine residues originated from a long-term outdoor lysimeter

Received: March 6, 2013

Revised: June 3, 2013

Accepted: June 3, 2013

Published: June 6, 2013

study, as described and investigated in previous studies,^{8,12,13} containing 18% sand, 64.4% silt, and 17.6% clay, with an organic carbon content (C_{org}) of 1.45% and a pH of 5.9. Application of uniformly ring-¹⁴C-labeled and non-labeled atrazine was performed in 1983, 1984, and 1985 in three equal portions, totaling 133.3 mg, equivalent to a total application of 5 kg of atrazine ha^{-1} , which accounted for 56.2 MBq.⁸ The specific ¹⁴C activity of the applied atrazine was 421.6 kBq mg^{-1} . The lysimeter soil was used for continuous corn production under outdoor conditions until 2005. For the presented study, soil from the top soil layer 0–10 cm depth was used, because most residual ¹⁴C activity was found in this depth increment, corresponding to 125 Bq g^{-1} .

Soils with long atrazine application history or where atrazine impact on weeds was reported to be reduced were collected in Belgium, the U.S., and Brazil,^{7,9,14} as specified below.

Belgian Soil. The Belgian soil was a Gleyic Luvisol silt loam soil sampled from a field located in Beverst, Belgium, containing 30.1% sand, 62.1% silt, and 7.8% clay, with a C_{org} content of 1.3% and a pH of 5.9.⁹ It was used regularly for corn plantations, and atrazine was applied annually since 1973 at customary rates [0.5–2.5 kg of active ingredient (a.i.) ha^{-1}] until 2004, when atrazine application in Belgium was banned. Until 2008, manual atrazine application on a specified field plot of 324 m^2 located on the same field was conducted by scientists from the Department of Earth and Environmental Sciences, Katholieke Universiteit Leuven (KU Leuven), Leuven, Belgium, using the commercial product Gesaprim 500, at an application rate of 1 L ha^{-1} (480 g of a.i. L^{-1}).

American Soil. The U.S. soil was a Dundee silt loam (fine silty, mixed, thermic Aeric Ochraqualf, FAO: Stagnic Luvisol) with pH 6.7, 1.1% C_{org} content, and soil textural fractions of 26% sand, 55% silt, and 19% clay. The soil was collected from continuous corn plots located at the United States Department of Agriculture–Agricultural Research Service (USDA–ARS, Stoneville, MS) that had been treated with atrazine for 6 consecutive years.

Brazilian Soil. The Brazilian soil was a Rhodic Ferralsol with pH 5.7, containing 24.5% sand, 26.1% silt, and 49.4% clay, with a C_{org} content of 3.2%,¹⁴ originating from Campinas do Sul, Rio Grande do Sul, Brazil. The climate in this region is subtropical, with a mean annual temperature of 16 °C. The soil was used under no-tillage, crop-rotating mode for soybean, wheat, maize, and oat since 1995. Until sampling, the soil has been treated biennially for the past 10 years with atrazine at recommended agronomic doses (3.25 kg of a.i. ha^{-1}).

All soils were randomly selected from the upper soil layer (0–10 cm depth), air-dried, sieved (2 mm), and stored in the dark at 2 °C until further analysis.

Evaluation of Atrazine Degradation Capacity in Belgian, Brazilian, and American Soils. Prior to further experiments, the atrazine degradation capacity of the atrazine-treated soils from all sites was evaluated and further referred to as positive controls. Each soil was spiked individually in accordance with a field application dose of 1.3–2.0 mg of a.i. kg^{-1} of soil. For spiking, an ethanol stock solution containing atrazine (chemical purity of 97.4%, Sigma Aldrich, Germany) and ¹⁴C-labeled atrazine (radiochemical purity of 99% and specific ¹⁴C activity of 9.5 mCi $mmol^{-1}$, American Radiolabeled Chemicals, St. Louis, MO) was added to a homogenized subsample of 5% of each bulk soil, in accordance with previous studies.^{9,14} After ethanol evaporation, the sample was homogeneously mixed with each individual bulk soil, ¹⁴C activity was determined by oxidation of 10 subsamples of 1.0 g of dried and homogenized soil (Biological Oxidizer OX500, R.J. Harvey Instrument Corporation; Oxsolve C-400 scintillation cocktail, Zinser Analytik), and ¹⁴C radioactivity was detected by a liquid scintillation analyzer (LSC; 2500 TR, Tri-Carb, Packard, using an internal standard). Dependent upon the individual soil, the amount applied accounted for 450–500 Bq g^{-1} of dry soil. Atrazine mineralization was determined using triplicates of 10 g dry soil equivalents of each individual soil at 50% WHC_{max} and slurry conditions, placed into 250 mL Schott Duran glass bottles. For slurry conditions, 40 mL of distilled water was added to facilitate the distribution and microbial accessibility of the applied ¹⁴C-labeled atrazine under permanent shaking at 125 rpm on a horizontal shaker.

A small glass vial containing 1.5 mL of 2 M NaOH solution was placed inside the flasks to trap evolving ¹⁴CO₂, resulting from microbial mineralization of the applied ¹⁴C-labeled atrazine. The flasks were hermetically closed and stored in the dark under controlled temperature conditions at 20 ± 2 °C. Traps were replaced regularly during incubation until no further ¹⁴C-labeled atrazine mineralization was monitored (positive control in Figure 1). The NaOH solution

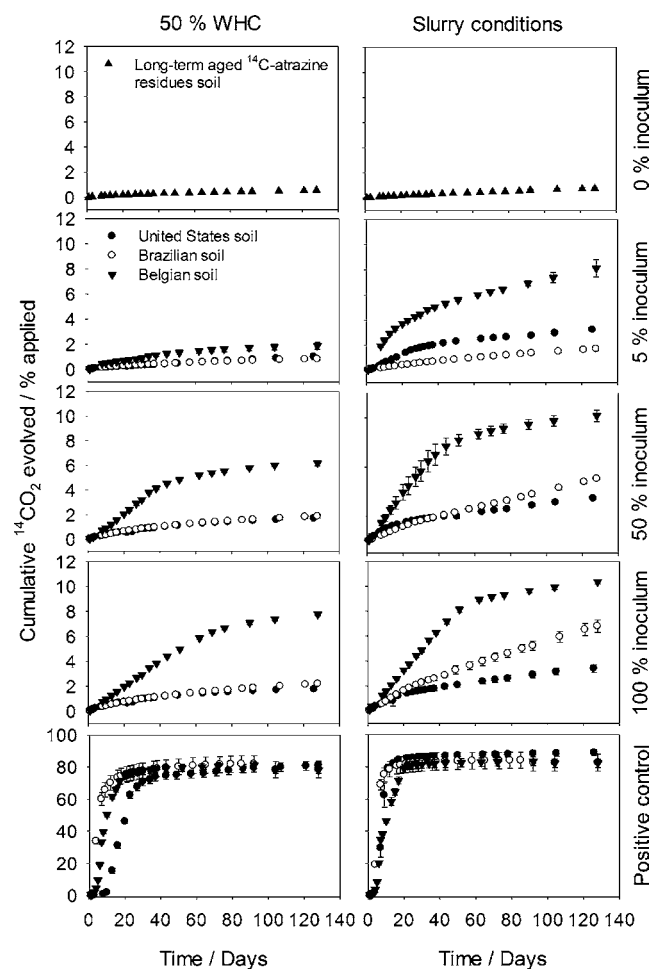


Figure 1. Cumulative ¹⁴CO₂ evolution for negative control (long-term-aged ¹⁴C-atrazine residues containing soil only), positive control (atrazine-adapted) soils from Belgium, the U.S., and Brazil, and their mixtures with long-term-aged ¹⁴C-atrazine residues containing soil at 50% WHC_{max} (left row) and slurry conditions (right row). Symbols for Belgian, American, and Brazilian soils are consistent for all figures.

containing evolved ¹⁴CO₂ was mixed with 4 mL of distilled water used for trap washing and 10 mL of scintillation cocktail (Instant Scint-Gel Plus, Perkin-Elmer), and detection of radioactivity was performed by LSC. An external standard was used for quenching correction. The presence of a bacterial community being able to rapidly degrade atrazine had to be assured as a precondition to evaluate the transfer of the degradation ability to mineralize the long-term-aged ¹⁴C-labeled atrazine residues.

Transfer of Atrazine Degradation Capacity. Samples of 10 g dry soil equivalents containing environmentally long-term-aged ¹⁴C-labeled atrazine residues at a residual ¹⁴C activity of 125 Bq g^{-1} , as described in previous studies,^{8,12,13} were placed in 250 mL Schott Duran glass bottles. The samples were thoroughly mixed with inocula of 0.5, 5, and 10 g (5, 50, and 100%, respectively) of the atrazine-adapted soils from Belgium, the U.S., and Brazil. For each inoculum, one setup was moistened to 50% WHC_{max}. A second setup was subject to slurry conditions, using 40 mL of distilled water. All setups were

conducted in triplicates and were treated in accordance with the method described in the section above. As a negative control, triplicates of aged ^{14}C -labeled atrazine residues containing soil was treated and incubated in accordance with the inocula setups. Evolving $^{14}\text{C}\text{O}_2$ resulting from microbial mineralization of the aged ^{14}C -labeled atrazine residues by the applied autochthonous microbial community present in the inocula was trapped in 1.5 mL of 2 M NaOH solution. Traps were replaced in regular intervals over a time period of 130 days of incubation and were analyzed as described in the section above.

Elemental Analysis of Soil Samples. Analysis of soil elements was performed as described previously.⁸ Briefly, homogenized and dried (105 °C) soil subsamples were used for elemental analysis (C_{org} , C_{inorg} , C_{total} , Si, Al, Ca, Fe, K, Mg, and Na) (Table 1). A 50 mg subsample was decomposed with a mixture of 0.25 g of lithium borate for 30 min at 1000 °C. The flux was dissolved in 30 mL of HCl (5%; 0.95 M, respectively) and adjusted to a total volume of 50 mL. The analysis were performed using inductively coupled plasma with optical emission spectroscopy (ICP–OES; TJA-IRIS-Intrepid spectrometer, Thermo). Carbon quantification was conducted using a Leco RC-412 multiphase carbon determinator. For nitrogen determination, a subsample of 2 mg was combusted and analyzed using a Leco TCH 600 nitrogen/oxygen/hydrogen with thermal conductivity detection.

Mineralization Kinetics and Statistical Analyses. To assess how the particular soil (Belgian, American, and Brazilian), soil moisture (50% WHC_{max} and slurry conditions), and added inoculum amounts (0, 5, 50, and 100% of atrazine-adapted soil) affected cumulative ^{14}C -atrazine mineralization at the end of the incubations, we performed a non-parametric MANOVA,¹⁵ using the Euclidian distance measures, with 5000 permutations, as described previously.¹⁶ Non-parametric MANOVA was chosen instead of a parametric MANOVA because the distribution of data was not normal and because variances between groups were not homogeneous, as tested by Shapiro-Wilks and Levene's test. Where *post hoc* pairwise comparisons were made, the Bonferroni correction was used.¹⁷

The cumulative mineralization of ^{14}C -atrazine was described by the Gompertz growth model

$$y = a \exp(-\exp(-(t - t_0)/k))$$

where a is the plateau representing the maximum percent mineralization, t is the time (day), t_0 is the abscissa of the inflection point representing the lag phase (day), and k is the Gompertz constant representing the inverse mineralization rate (day). This Gompertz equation has been used in a previous study evaluating atrazine degradation in soils.¹⁸ The data were fitted to the model using Sigma Plot 12 (Systat Software, GmbH, Erkrath, Germany).

RESULTS AND DISCUSSION

As shown in Figure 1 (positive control, bottom picture), microcosms containing the atrazine-adapted Belgian, American, and Brazilian soil at 50% WHC_{max} all showed a high mineralization capacity of 81–82%. Under slurried conditions, the mineralization was higher, accounting for 83% for Belgian soil, 88% for American soil, and 84% for Brazilian soil, respectively, all in total after 85 days, measured as evolved $^{14}\text{C}\text{O}_2$. These results evidenced a high atrazine degradation capacity in these soils. In contrast, the ^{14}C -atrazine mineralization rate of the negative control was very low, totaling 0.56% at 50% WHC_{max} and 0.75% at slurried conditions (top picture in Figure 1), but was stimulated with additions of atrazine-adapted soils (Figure 1). At slurry conditions, ^{14}C -atrazine residue mineralization was larger compared to 50% WHC_{max} conditions ($p < 0.05$; *post hoc* test; Figure 1) but of minor importance because of the low effect size (η^2) of $\approx 14\%$. As stated previously, slurried conditions were applied to favor of the diffusion of the ^{14}C -atrazine residues and to increase the mobility of the soil microorganisms to stimulate ^{14}C -atrazine residue mineralization.^{9,19}

Table 1. Elements in Soil (Weight Percent)^a

element soil	C_{org} (%)	C_{inorg} (%)	N (%)	Al (%)	Si (%)	Ca (%)	Fe (%)	K (%)	Mg (%)	Na (%)	P (%)
^{14}C -ATR residue soil	1.49 ± 0.06	0.05 ± 0.01	0.16 ± 0.01	5.93 ± 0.05	35.13 ± 0.17	0.44 ± 0.00	2.29 ± 0.01	1.46 ± 0.06	0.49 ± 0.01	0.73 ± 0.03	0.14 ± 0.01
Belgian soil	1.26 ± 0.02	0.05 ± 0.01	0.13 ± 0.00	3.19 ± 0.09	39.00 ± 0.29	0.30 ± 0.02	1.35 ± 0.21	1.53 ± 0.05	0.19 ± 0.00	0.49 ± 0.06	0.12 ± 0.01
American soil	0.96 ± 0.03	0.02 ± 0.00	0.11 ± 0.00	5.11 ± 0.02	37.30 ± 0.16	0.63 ± 0.01	1.39 ± 0.02	1.93 ± 0.02	0.37 ± 0.01	1.06 ± 0.00	0.06 ± 0.00
Brazilian soil	3.16 ± 0.04	0.03 ± 0.00	0.28 ± 0.01	11.93 ± 0.21	13.23 ± 0.12	0.15 ± 0.02	18.83 ± 0.21	0.11 ± 0.00	0.24 ± 0.01	0.04 ± 0.01	0.19 ± 0.01

^a ^{14}C -Atrazine (ATR) residue soil = experimental soil containing the environmentally long-term-aged ^{14}C -labeled atrazine residues. Mean ± standard deviation of $n = 3$. Mean variation for Al, Ca, Fe, K, Mg, Na, and P: for concentration >1%, ±3%; for concentration <1 and >0.1%, ±10%; and for concentration <0.1%, ±20%.

The extent of mineralization of long-term-aged ^{14}C -labeled atrazine residues was dependent upon the amount of atrazine-adapted soil inocula and the moisture conditions. Aged ^{14}C -atrazine residue mineralization varied between 2 and 8% for Belgian soil and between 1 and 2% for American and Brazilian soils at 50% WHC_{max} but was increased under slurried conditions, accounting for 8–10% (Belgian soil), 3% (American soil), and 2–7% (Brazilian soil) (Figure 1). In other words, in comparison to the small ^{14}C -atrazine residue mineralization in the negative control, the largest additions of American and Brazilian soil inocula tripled and quadruplicated (by 3.1 and 3.9 for American and Brazilian soils, respectively), while Belgium soil multiplied (by 13.9) aged ^{14}C -atrazine residue mineralization at 50% WHC_{max} . However, at slurry conditions, mineralization of aged ^{14}C -atrazine residues even increased (by 4.5 for American soil and 9.1 for Brazilian soil and an equally high increase by 13.8 for Belgian soil compared to the mineralization rate of the ^{14}C -atrazine residue negative control soil without inoculum; Figure 1).

Cumulative mineralization of ^{14}C -atrazine was significantly affected, as indicated by MANOVA's for soil, moisture, inoculum amount, and their interaction terms (Table 2).

Table 2. Results from MANOVA To Assess Differences in Mineralization Rates of ^{14}C -Atrazine Residues, Using Three Factors: (1) Soil (Belgian, American, and Brazilian Soils), (2) Soil Moisture (50% WHC_{max} and Slurry), and (3) Added Amounts of Atrazine-Adapted Soil [0, 5, 50, and 100% (w/w)]^a

source	df	SS	F	η^2
soil (S)	2	197.8	1671	28.6 ^b
moisture (M)	1	96.5	1631	14.0 ^b
amount (A)	3	244.2	1375	35.3 ^b
SM	2	9.3	79	1.3 ^b
SA	6	89.7	253	13.0 ^b
MA	3	27.3	154	3.9 ^b
SMA	6	24.2	68	3.5 ^b
residual	48	2.8		
total	71	691.9		

^adf, degrees of freedom; SS, sum of squares; F, F value; and η^2 , values indicates how important (in percentage terms) each independent variable is in explaining variations in the data sets.²⁵ ^b $p < 0.001$.

However, the effect size (η^2) indicated that the interactions were of minor importance, except the soil \times inoculum amount interaction term. The inoculum amount and soil were by far the most important sources of variation, explaining ≈ 35 and $\approx 29\%$, respectively, of the data variance (Table 2). However, there were no significant differences between all mixtures of atrazine-adapted soils and non-adapted ^{14}C -atrazine residue soil, while in comparison to negative controls, each inocula addition irrespective of size increased cumulative ^{14}C -atrazine residue mineralization significantly ($p < 0.05$; *post hoc* test). Interestingly, the added inocula amount of atrazine-adapted soil from Belgium affected ^{14}C -atrazine residue mineralization differently from those from Brazil and the U.S. (Figure 1). The Belgium soil induced a significantly ($p < 0.05$; *post hoc* test) larger cumulative ^{14}C -atrazine mineralization at inoculation rates of 50 and 100% compared to mixtures containing inocula from American and Brazilian soils. Hence, despite stronger effects of the added inocula amounts on the ^{14}C -atrazine residue mineralization, interaction between the soils and the

respective biocenosis as a consequence of, e.g., pedogenesis and land cover has to be considered.

Comparing the small ^{14}C -atrazine residue mineralization rates of the negative control (i.e., only soil containing the long-term-aged ^{14}C -atrazine residues) to the increased ^{14}C -atrazine mineralization rates of its mixtures with atrazine-adapted soils confirms that long-term-aged ^{14}C -atrazine residues are bioaccessible and biodegradable by an indigenous, atrazine-adapted soil microflora from different soils and locations (Figure 1). The mineralization rates of the freshly applied ^{14}C -atrazine in the atrazine-adapted soils were in a range reported for other atrazine-treated soils.^{16,18,20,21} The extent of ^{14}C -atrazine residue biomineralization by the inoculated heterogeneous soil microflora is in the same range or even higher compared to the degradation rate of a specialized atrazine-degrading microorganism, as demonstrated in a previous study using the atrazine-degrading strain *Pseudomonas* species ADP.¹² Evidently, this is because the consortia of microorganisms present in the adapted soils have higher complementary atrazine degradation capabilities than a single, even specialized strain. However, in comparison to the mineralization in mixtures with American and Brazilian atrazine-adapted soil inocula, additions of atrazine-adapted soil from Belgium were most effective to stimulate ^{14}C -atrazine mineralization, despite the added inoculum amounts (Figure 1). In fact, the ^{14}C -atrazine mineralization capacities were in line with markedly changed mineralization rates (Table 3).

We assume that the increased degradation capacity of the Belgian soil is related to similar soil properties and abiotic conditions also present in the soil containing the ^{14}C -atrazine residues. The similar soil (e.g., pH and C_{org} content) and humidity conditions in the Belgian and aged atrazine residues containing soil may have promoted the establishment of a comparable soil micro environment, facilitating the degradation of the ^{14}C -atrazine residues when the Belgian atrazine-adapted soil was used as inoculum. This would be in-line with another study, suggesting that the differing atrazine-degrading capabilities is due to the effect of site characteristics on the degradative mechanisms of the indigenous microbial consortium.²² These are related to local factors, such as land cover and soil type.²³ Here, the used soils were collected from arable land, but the soil types differed. Thus, in comparison to the negative control soil (Germany, silty loam Gleyic Cambisol) the soil properties of the atrazine-adapted Belgian soil (silty loam Gleyic Luvisol) were similar, whereas soils from the U.S. (silty loam Stagnic Luvisol) and Brazil (clay Rhodic Ferrasol) differed from the negative control in either their soil texture or water regime. Consequently, this indicates that degradation capacities of aged atrazine residues can be improved by mixing with each atrazine-adapted soil type but most effectively by additions of atrazine-adapted soil of similar type and properties. Further, it can be hypothesized that the most competent degrading consortium of microorganisms in the Belgian soil is due to the longest atrazine application history, resulting in the establishment of a strong atrazine-degrading bacterial community being able to degrade even long-term-aged ^{14}C -atrazine residues effectively (Figure 1). An increased release of soil-bound ^{14}C -atrazine residues by soil-born *Pseudomonas* species was found to result in a concomitant degradation of these ^{14}C -atrazine residues and the soil organic matter,²⁴ which may support our findings. However, these hypotheses need further investigation using more different atrazine-adapted soil types

Table 3. Gompertz Mineralization Characteristics of the ¹⁴C-Atrazine Applied to the Atrazine-Adapted Soils from Belgium, the U.S., and Brazil and Their Mixtures with the Non-atrazine-Adapted Soil Containing the Long-Term-Aged ¹⁴C-Atrazine (ATR) Residues^a

soil	50% WHC _{max}				slurry conditions			
	a (%)	k (day)	t ₀ (day)	R ²	a (%)	k (day)	t ₀ (day)	R ²
	Negative Control (No Inoculum)							
¹⁴ C-ATR residue soil	0.56 ± 0.03	37.5 ± 3.9	26.9 ± 2.3	0.98 ^b	0.87 ± 0.05	46.9 ± 4.3	42.3 ± 3.3	0.99 ^b
	Experiments with 5% Adapted Soil Inoculum							
Belgian soil	1.85 ± 0.06	25.9 ± 2.2	20.3 ± 1.3	0.98 ^b	7.16 ± 0.30	20.6 ± 3.0	14.6 ± 1.7	0.95 ^b
American soil	1.11 ± 0.06	43.9 ± 3.6	43.0 ± 3.1	0.99 ^b	2.83 ± 0.07	16.3 ± 1.3	18.5 ± 0.9	0.98 ^b
Brazilian soil	0.83 ± 0.03	34.3 ± 2.9	27.7 ± 1.7	0.99 ^b	1.82 ± 0.08	40.2 ± 3.1	38.8 ± 2.3	0.99 ^b
	Experiments with 50% Adapted Soil Inoculum							
Belgian soil	5.93 ± 0.07	17.5 ± 0.7	21.2 ± 0.4	1.00 ^b	9.64 ± 0.12	17.0 ± 0.7	18.9 ± 0.5	1.00 ^b
American soil	1.63 ± 0.04	25.9 ± 1.8	23.0 ± 1.1	0.99 ^b	3.08 ± 0.16	26.4 ± 3.8	18.2 ± 2.2	0.95 ^b
Brazilian soil	1.83 ± 0.06	30.1 ± 2.5	24.9 ± 1.5	0.98 ^b	5.81 ± 0.41	48.2 ± 5.2	46.9 ± 4.4	0.98 ^b
	Experiments with 100% Adapted Soil Inoculum							
Belgian soil	7.82 ± 0.10	25.6 ± 0.8	29.3 ± 0.5	1.00 ^b	10.13 ± 0.10	19.7 ± 0.6	23.0 ± 0.4	1.00 ^b
American soil	1.69 ± 0.04	23.1 ± 1.7	18.6 ± 1.1	0.98 ^b	3.15 ± 0.13	26.5 ± 2.8	21.4 ± 1.7	0.97 ^b
Brazilian soil	2.17 ± 0.07	32.4 ± 2.8	24.9 ± 1.6	0.98 ^b	7.50 ± 0.42	43.7 ± 4.1	42.3 ± 3.2	0.99 ^b
	Positive Control, Atrazine-Adapted Soil							
Belgian soil	78.1 ± 0.7	3.6 ± 0.2	7.1 ± 0.1	0.99 ^b	82.3 ± 1.0	4.3 ± 0.3	7.6 ± 0.2	0.99 ^b
American soil	77.5 ± 0.6	5.6 ± 0.3	15.8 ± 0.3	1.00 ^b	86.6 ± 0.3	1.7 ± 0.1	7.1 ± 0.1	1.00 ^b
Brazilian soil	78.0 ± 0.8	2.7 ± 0.3	3.8 ± 0.2	0.98 ^b	82.6 ± 0.4	1.6 ± 0.1	4.6 ± 0.1	1.00 ^b

^aThe soils and their mixtures with the ¹⁴C-atrazine residue soil were incubated for 130 days. Data are shown as the mean and standard errors. *a*, maximum percent mineralization; *k*, Gompertz mineralization constant; *t*₀, lag phase; and *R*², coefficient of determination. ^bSignificant at a level of *p* < 0.001.

with various application histories to be transferred to similar, non-adapted soils containing aged atrazine residues.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +49-2461-61-8682. Fax: +49-2461-61-2492. E-mail: n.d.jablonowski@fz-juelich.de and/or n.d.jablonowski@daad-alumni.de.

Funding

The financial support for Rosane Martinazzo [National Council for Scientific and Technological Development (CNPq)] and Petra Zajkoska [IAESTE Program, German Academic Exchange Service (DAAD)] is gratefully acknowledged. The entire experiment was conducted at IBG-3: Agrosphere.

Notes

The authors declare no competing financial interest.

REFERENCES

- (1) Walker, A.; Parekh, N.; Roberts, S.; Welch, S. Evidence for the enhanced biodegradation of napropamide in soil. *Pestic. Sci.* **1993**, *39*, 55–60.
- (2) Sanyal, D.; Kulshrestha, G. Effects of repeated metolachlor applications on its persistence in field soil and degradation kinetics in mixed microbial cultures. *Biol. Fertil. Soils* **1999**, *30*, 124–131.
- (3) Sorensen, S.; Aamand, J. Rapid mineralisation of the herbicide isoproturon in soil from a previously treated Danish agricultural field. *Pest Manage. Sci.* **2003**, *59*, 1118–1124.
- (4) Krutz, L. J.; Zablotowicz, R. M.; Reddy, K. N.; Koger, C. H., III; Weaver, M. A. Enhanced degradation of atrazine under field conditions correlates with a loss of weed control in the glasshouse. *Pest Manage. Sci.* **2007**, *63*, 23–31.
- (5) Pussemier, L.; Goux, S.; Vanderheyden, V.; Debongnie, P.; Tresinie, I.; Foucart, G. Rapid dissipation of atrazine in soils taken from various maize fields. *Weed Res.* **1997**, *37*, 171–179.

- (6) Shaner, D. L.; Henry, W. B. Field history and dissipation of atrazine and metolachlor in Colorado. *J. Environ. Qual.* **2007**, *36*, 128–134.

- (7) Krutz, L. J.; Shaner, D. L.; Accinelli, C.; Zablotowicz, R. M.; Henry, W. B. Atrazine dissipation in s-triazine-adapted and nonadapted soil from Colorado and Mississippi: Implications of enhanced degradation on atrazine fate and transport parameters. *J. Environ. Qual.* **2008**, *37*, 848–857.

- (8) Jablonowski, N. D.; Koeppchen, S.; Hofmann, D.; Schaeffer, A.; Burauel, P. Persistence of ¹⁴C-labeled atrazine and its residues in a field lysimeter soil after 22 years. *Environ. Pollut.* **2009**, *157*, 2126–2131.

- (9) Jablonowski, N. D.; Hamacher, G.; Martinazzo, R.; Langen, U.; Köppchen, S.; Hofmann, D.; Burauel, P. Metabolism and persistence of atrazine in several field soils with different atrazine application histories. *J. Agric. Food Chem.* **2010**, *58*, 12869–12877.

- (10) Walker, A.; Welch, S.; Roberts, S. Induction and transfer of enhanced biodegradation of the herbicide napropamide in soils. *Pestic. Sci.* **1996**, *47*, 131–135.

- (11) Hole, S.; McClure, N.; Powles, S. Rapid degradation of carbetamide upon repeated application to Australian soils. *Soil Biol. Biochem.* **2001**, *33*, 739–745.

- (12) Jablonowski, N. D.; Modler, J.; Schaeffer, A.; Burauel, P. Bioaccessibility of environmentally aged ¹⁴C-atrazine residues in an agriculturally used soil and its particle-size aggregates. *Environ. Sci. Technol.* **2008**, *42*, 5904–5910.

- (13) Jablonowski, N. D.; Koeppchen, S.; Hofmann, D.; Schaeffer, A.; Burauel, P. Spatial distribution and characterization of long-term aged ¹⁴C-labeled atrazine residues in soil. *J. Agric. Food Chem.* **2008**, *56*, 9548–9554.

- (14) Martinazzo, R.; Jablonowski, N. D.; Hamacher, G.; Dick, D. P.; Burauel, P. Accelerated degradation of ¹⁴C-atrazine in Brazilian soils from different regions. *J. Agric. Food Chem.* **2010**, *58*, 7864–7870.

- (15) Anderson, M. J. A new method for non-parametric multivariate analysis of variance. *Aust. Ecol.* **2001**, *26*, 32–46.

- (16) Jablonowski, N. D.; Borchard, N.; Zajkoska, P.; Fernández-Bayo, J. D.; Martinazzo, R.; Berns, A. E.; Burauel, P. Biochar-mediated ¹⁴C-atrazine mineralization in atrazine-adapted soils from Belgium and Brazil. *J. Agric. Food Chem.* **2013**, *61*, 512–516.

(17) Quinn, G.; Keough, M. *Experimental Design and Data Analysis for Biologists*; Cambridge University Press: Cambridge, U.K., 2002.

(18) Krutz, L. J.; Burke, I. C.; Reddy, K. N.; Zablotowicz, R. M.; Price, A. J. Enhanced atrazine degradation: Evidence for reduced residual weed control and a method for identifying adapted soils and predicting herbicide persistence. *Weed Sci.* **2009**, *57*, 427–434.

(19) Wenk, M.; Baumgartner, T.; Dobovsek, J.; Fuchs, T.; Kucsera, J.; Zopfi, J.; Stucki, G. Rapid atrazine mineralisation in soil slurry and moist soil by inoculation of an atrazine degrading *Pseudomonas* sp. strain. *Appl. Microbiol. Biotechnol.* **1998**, *49*, 624–630.

(20) Zablotowicz, R.; Weaver, M.; Locke, M. Microbial adaptation for accelerated atrazine mineralization/degradation in Mississippi Delta soils. *Weed Sci.* **2006**, *54*, 538–547.

(21) Zablotowicz, R. M.; Krutz, L. J.; Reddy, K. N.; Weaver, M. A.; Koger, C. H.; Locke, M. A. Rapid development of enhanced atrazine degradation in a dundee silt loam soil under continuous corn and in rotation with cotton. *J. Agric. Food Chem.* **2007**, *55*, 852–859.

(22) Chirnside, A. E. M.; Ritter, W. F.; 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 Biol. Biochem.* **2007**, *39*, 3056–3065.

(23) *European Atlas of Soil Biodiversity*; Jeffery, S., Gardi, C., Jones, A., Montanarella, L., Marmo, L., Miko, L., Ritz, K., Peres, G., Römbke, J., van der Putten, W. H., Eds.; European Commission, Publications Office of the European Union, Luxembourg, 2010.

(24) Khan, S. U.; Behki, R. M. Effects of *Pseudomonas* species on the release of bound ¹⁴C residues from soil treated with [¹⁴C]atrazine. *J. Agric. Food Chem.* **1990**, *38*, 2090–2093.

(25) Plaistow, S. J.; Lapsley, C. T.; Benton, T. G. Context-dependent intergenerational effects: The interaction between past and present environments and its effect on population dynamics RID C-6493-2009. *Am. Nat.* **2006**, *167*, 206–215.