Biochar-Mediated [14C]Atrazine Mineralization in Atrazine-Adapted Soils from Belgium and Brazil

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ABSTRACT: Biochar addition to soil has been reported to reduce the microbial degradation of pesticides due to sorption of the active compound. This study investigated whether the addition of hardwood biochar alters the mineralization of 14C-labeled atrazine in two atrazine-adapted soils from Belgium and Brazil at different moisture regimens. Biochar addition resulted in an equally high or even in a significantly higher atrazine mineralization compared to the soils without biochar. Statistical analysis revealed that the extent of atrazine mineralization was more influenced by the specific soil than by the addition of biochar. It was concluded that biochar amendment up to 5% by weight does not negatively affect the mineralization of atrazine by an atrazine-adapted soil microflora.

KEYWORDS: biochar, pesticides, microbial adaptation

INTRODUCTION

The long-term fate of pesticides applied to agricultural soils depends on abiotic conditions such as soil properties and climatic conditions and biological degradation by the soil microflora. Pesticide sorption in soils is mainly influenced by organic matter and clay mineral content, leading to a decrease in microbial accessibility of the agrochemical. It can be expected that an increase in soil organic carbon content will generally result in a decrease of pesticide biodegradation due to increased contaminant sorption.

Investigations have demonstrated beneficial effects of biochar on plant growth, soil fertility, nutrient availability, reduction of greenhouse gas emissions, and water-holding capacity. Numerous studies have been published investigating the use of biomass-derived char (i.e., biochar) as soil amendment with respect to pesticide fate and effects on soil microbial activity.

Several studies reported the inhibiting influence of chars on pesticide degradation as a consequence of their high surface area and sorptive properties. Loganathan et al. showed that 14C atrazine mineralization using a pure culture of the specific atrazine-degrading microorganism Pseudomonas sp. strain ADP was reduced by 11% when charred material (crop-residues-derived char) was incorporated into the soil prior to atrazine application due to sorption of the herbicide. Similar observations were presented in recent studies using the 14C-labeled herbicides isoproturon and simazine and wood-derived biochars in bulk soils; the rate and extent of degradation were affected with increasing biochar concentration, showing a decreased degradation with increased biochar application amounts. Even though the simazine mineralization was influenced by the different soil properties, the mineralization decreased in the presence of biochar, probably due to reduced bioavailability as a consequence of strong and rapid sorption.

Atrazine-adapted soils, as previously described for the soils used in this study, are soils with an atrazine application history exhibiting a microbial soil community that is capable of rapidly mineralizing atrazine. The impact of biochar on atrazine degradation in adapted soils has not yet been evaluated.

In this study, we investigated the influence of biochar amendments on the biodegradation of atrazine in two atrazine-adapted field soils from Belgium and Brazil.

MATERIALS AND METHODS

The soils used were a Gleyic Luvisol (silt-loam soil) from a field in Beverst, Belgium, with an annual atrazine application history of 30 years until the day of sampling, and a Rhodic Ferralsol (heavy clay soil) collected from a field in Campinas do Sul, in southern Brazil, with a 20 years biennial atrazine application history at day of sampling; both soils were found to exhibit a high atrazine degradation capability, as reported in previous studies. Both soils were sampled from the surface layer at 0–10 cm depth (pH 5.9 for the Belgian soil, pH 5.7 for the Brazilian soil) and contained atrazine residues (extractable by means of accelerated solvent extraction and LC-MS/MS analysis) accounting for 8.3 and 4.4 μg kg−1 in the Belgian and Brazilian soil, respectively. The commercially available biochar material was prepared anaerobically from hardwood at 450–500 °C (Dynamotive USA, McLean, VA, USA) and used as received. Physical/chemical soil
Table 1. Soil Texture of the Atrazine-Adapted Soils from Belgium and Brazil; Elemental Composition and Surface Area of Soils and Biochar

<table>
<thead>
<tr>
<th>sample</th>
<th>texture (%)</th>
<th>C (%)</th>
<th>N (%)</th>
<th>H (%)</th>
<th>O (%)</th>
<th>H/C</th>
<th>O/C</th>
<th>C/N</th>
<th>SSA_{CO_2} (m² g⁻¹)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgian soil</td>
<td>sand, 30.12</td>
<td>1.26 ± 0.02</td>
<td>0.13 ± 0.00</td>
<td>na²</td>
<td>na</td>
<td>9.70</td>
<td>12.64</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>silt, 62.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>clay, 7.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazilian soil</td>
<td>sand, 24.42</td>
<td>3.16 ± 0.04</td>
<td>0.28 ± 0.01</td>
<td>na²</td>
<td>na</td>
<td>11.29</td>
<td>37.86</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>silt, 25.20</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>clay, 50.38</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>biochar</td>
<td></td>
<td>70.65 ± 0.20</td>
<td>0.20 ± 0.00</td>
<td>3.37 ± 0.02</td>
<td>21.30 ± 0.20</td>
<td>0.57</td>
<td>0.23</td>
<td>353.25</td>
<td>260.10</td>
<td>1.00</td>
</tr>
</tbody>
</table>

²: standard deviation of n = 3. Values are given in weight percent. ³: Mass ratio. ⁴: SSA_{CO_2}, specific surface area determined by CO₂ isotherms at 273 K. ⁵: R², determination coefficient of Dubinin–Radushkevich isotherm. ⁶: na, not analyzed.

and biochar properties, such as soil texture, element contents, and surface areas, are given in Table 1.

**Elemental Analyses.** The elemental composition of Belgian and Brazilian soils and biochar was determined in triplicates on LECO TCH 600, LECO CHNS-932, and TJA-IRIS-Intrepid elemental analyzers, as described elsewhere.²

**Surface Area Analyses of Soils and Biochar.** For surface area analyses, soils and biochar were outgassed at 70 °C for 16 h and at 100 °C for 2 h, respectively. The specific surface area (SSA_{CO_2} m² g⁻¹) was determined from CO₂ isotherms at 273 K. Pore and surface characteristics were performed using an Autosorb-1MP (Quintachrome) apparatus. The CO₂ isotherm was modeled using the Dubinin–Radushkevich equation.²⁴ The linear form of the Dubinin–Radushkevich equation was also used to calculate the micropore surface area based on a CO₂ molecule monolayer.

**Soil Preparation.** [²¹]Atrazine Spiking, and Biochar Soil Incorporation.** The soils were air-dried to a residual water content of 4% in the Belgian soil and 12% in the Brazilian soil, 2 mm sieved, and stored in the dark at 2 °C until further use. The atrazine-spiking solution was prepared by mixing 20 μL of ethanol [¹⁴C]atrazine solution (American Radiolabeled Chemicals, St. Louis, MO, USA) with a specific radioactivity of 6.41 kBq μL⁻¹ and 78.7 μg of atrazine [6-chloro-N-ethyl-N′-(1-methylethyl)-1,3,5-triazine-2,4-diamine] as active ingredient (ai, chemical purity = 99%) with 1980 μL of nonradioactive atrazine ethanol (EtOH, Merck Lichrosolv, ≥99.9% purity) solution containing 1290 μg of ai atrazine (chemical purity = 98%, Riedel-de Haën, Germany). The [¹⁴C]atrazine spiking solution was added to a mortared soil aliquot (5% w/w), equating a field application concentration of 3 mg atrazine kg⁻¹ of soil, and was left to air-dry until complete dryness (to avoid potential influences on microbial turnover by the EtOH as, e.g., a potential carbon source), determined by constant weight. The spiked soil was mixed with an end-over-end shaker with the remainder of the respective bulk soil portion for 1 h to achieve homogeneity. Homogeneity of the final spiked soil was tested for quantification of initial radioactivity using 10 subsamples of 0.5 g by complete combustion and [¹⁴C]-activity detection applying a Biological Oxidizer (OX500, R. J. Harvey Instrument Corp., Tappan, NY, USA) using Oxyxolve C-400 as a scintillation cocktail (Zinsser Analytic, Germany). A liquid scintillation analyzer (LSC; 2500 TR, Tri-Carb, Packard) with an internal standard was used for [¹⁴C]-activity detection. The incorporated [¹⁴C]-activity accounted for 1.05 kBq mg⁻¹ soil.

**Incubation Experiments.** The influence of biochar amendments on the mineralization of [¹⁴C]atrazine applied to soil was determined as follows. For both soils, triplicates of 10 g of dry soil equivalents were weighed into 250 mL Duran glass bottles (biomicrocosms). Quantities of 0.01, 0.1, and 0.5 g of biochar (equal to 0.1, 1, and 5%) by weight of dry soil, in accordance with low and high agronomic addition rates, as previously described²⁵ were added to the biomicrocosms. The amount of biochar added was chosen as a multiple of an estimated percentage of 0.02% (w/w) biochar deposited on agricultural soils after the crop burning of one harvest.²⁵ All experiments were conducted under 60% water-holding capacity (WHC_{max}) using distilled water and under slurred conditions using 0.01 M CaCl₂ solution (1 + 4, w/v, soil + solution). Controls with soil only were set up in triplicates for both soils and moisture conditions. During the incubation period of 88 days, the evolution of ¹⁴CO₂ resulting from [¹⁴C]atrazine mineralization was analyzed as described elsewhere.²¹

**Mineralization Kinetics.** The cumulative mineralization of [¹⁴C]atrazine was described by the Gompertz growth model

\[ y = a \exp(-\exp(-(t - t_o)/k)) \]

where \( a \) is the plateau representing the maximum percent mineralization, \( t \) is the time (days), \( t_o \) is the abscissa of the inflection point representing the lag phase (days), and \( k \) is the Gompertz constant representing the inverse mineralization rate (days). This model has been used in several studies evaluating atrazine degradation in soils.²⁵,²⁶ The data were fitted to the model using Sigma Plot 12 (Systat Software, GmbH, Erkrath, Germany).

**Statistical Analyses.** To assess how soil (Belgian vs Brazilian soil), soil moisture (WHC at 60% and slurry), and added biochar amounts [0.0, 0.1, 1.0, and 5.0% (w/w)] affected [¹⁴C]-atrazine mineralization during the incubation period of 88 days, we performed a nonparametric MANOVA,²⁷ using the Euclidian distance measures and 4999 permutations. Nonparametric MANOVA was chosen instead of a parametric MANOVA because the distribution of data was not normal and due to inhomogenous variances between groups, as tested by Shapiro–Wilk and Levine tests. When post hoc pairwise comparisons were made, the Bonferroni correction was used.²⁸

**RESULTS AND DISCUSSION.** A higher soil organic carbon content of 3.16% was determined in the Brazilian soil compared to the Belgian soil (1.26%; Table 1). However, the C/N ratio was in a similar range for both soils, being 9.70 and 11.29 for the Belgian and Brazilian soils, respectively. The biochar showed high carbon and oxygen contents of 71 and 21%, respectively, and the elemental composition was comparable to that of other hardwood biochars reported in the literature.²⁹ The specific surface areas for the biochar and the Belgian and Brazilian soils were 260, 13, and 38 m² g⁻¹, respectively (Table 1). A high specific surface area of biochars was found to increase atrazine sorption.¹¹,³⁰ This may elude microbial access, hence suppressing the mineralization of [¹⁴C]atrazine as applied in our study.¹¹

However, compared to control treatments the cumulative [¹⁴C]atrazine mineralization rates [day⁻¹] were larger for all treatments with a biochar application rate of 5% (w/w) (Table 2). These mineralization rates determined for atrazine-treated
soils and their biochar-amended mixtures were in a range reported for other atrazine-treated soils.\textsuperscript{25,26,32} Moreover, for the Brazilian soil amended with 5\% biochar (w/w) and at 60\% of the maximum WHC\textsubscript{max}, a shorter lag phase ($t_0$) was observed (Table 2). Both an increased mineralization rate and a shorter lag in treatments with 5\% biochar (w/w) may indicate an improved bioavailability and/or biodegradation of $[^{14}$C]atrazine.

The Belgian soil without biochar addition showed a high $[^{14}$C]atrazine mineralization of 78\% at 60\% WHC\textsubscript{max} after 88 days of incubation. This mineralization accounted for 84\% under slurry conditions. Mineralization remained comparably high in all treatments containing biochar, accounting for 78\% (+0.1\% biochar), 77\% (+1.0\% biochar), and 71\% (+5.0\% biochar) at 60\% WHC\textsubscript{max} conditions (Figure 1a) and 85\% (0.1\% biochar), 83\% (1\% biochar), and 76\% (5\% biochar) at slurry conditions (Figure 1b) after 88 days.

The Brazilian soil without biochar showed similar atrazine mineralization at both moisture regimens, accounting for 39 and 37\% at 60\% WHC\textsubscript{max} and slurry conditions, respectively (Figure 1c,d). Biochar amendment had a highly stimulating effect on the $[^{14}$C]atrazine mineralization in the Brazilian soil, accounting for 50\% (0.1\% biochar), 48\% (1.0\% biochar), and 46\% (5.0\% biochar; Figure 1c). With increasing amounts of added biochar, the $[^{14}$C]atrazine mineralization was even more pronounced at slurry conditions, accounting for 49\% (0.1\% biochar), 51\% (1.0\% biochar), and 62\% (5.0\% biochar; Figure 1d) after 88 days.

Organic amendments have been used as soil additives to increase soil sorption capacity, resulting in a decreased pesticide concentration in the soil solution.\textsuperscript{35,34} Our results showed

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Table 2. Gompertz Mineralization Characteristics of $[^{14}$C]atrazine Applied to the Atrazine-Adapted Soils from Brazil and Belgium, Which Were Amended with Different Amounts of Biochar [0.0, 0.1, 1.0, and 5.0\% (w/w)]\textsuperscript{a}

<table>
<thead>
<tr>
<th>Soil</th>
<th>Control</th>
<th>0.1% Biochar</th>
<th>1.0% Biochar</th>
<th>5.0% Biochar</th>
<th>Control</th>
<th>0.1% Biochar</th>
<th>1.0% Biochar</th>
<th>5.0% Biochar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgian soil</td>
<td>72.4 ± 2.5</td>
<td>74.0 ± 1.7</td>
<td>72.4 ± 2.5</td>
<td>68.0 ± 0.6</td>
<td>75.5 ± 5.1</td>
<td>78.7 ± 4.3</td>
<td>78.6 ± 3.3</td>
<td>74.1 ± 0.5</td>
</tr>
<tr>
<td>Brazilian soil</td>
<td>42.6 ± 1.5</td>
<td>53.0 ± 0.7</td>
<td>49.8 ± 0.6</td>
<td>44.4 ± 0.4</td>
<td>35.6 ± 1.7</td>
<td>52.4 ± 2.8</td>
<td>53.7 ± 2.7</td>
<td>61.9 ± 1.0</td>
</tr>
</tbody>
</table>

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\(a\) Data are shown as the mean and standard error. \(k\), Gompertz mineralization constant, 1/\(k\) representing inverse mineralization rate; \(t_0\) lag phase; \(R^2\), coefficient of determination.

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Figure 1. Mineralization of $[^{14}$C]atrazine in (a, b) Belgian and (c, d) Brazilian soil at 60\% WHC\textsubscript{max} and slurry conditions with biochar amendment. Lines with solid lines and dots represent the positive control indicating the atrazine mineralization potential in Belgian and Brazilian soil without any biochar amendment. Different letters indicate significant differences (\(p < 0.05\)); ns, not significant differences between mineralization. Lines are visual aids only. Data points represent mean values of \(n = 3\). Error bars indicate standard deviation of \(n = 3\) and appear when bigger than data point.
elevated Gompertz mineralization rates of \([^{14}C]\text{atrazine}\) in both soils at the highest biochar concentration and a significantly increased cumulative mineralization in the Brazilian soil. This outcome contradicts the assumption that biochar soil amendments reduce atrazine biodegradation by strong sorption of this chemical. We assume that the high atrazine mineralization in the presence of the used biochar may be attributed to cometabolic degradation due to easily degradable compounds associated with the biochar.\(^{35,36}\) Even though a reduced microbial atrazine mineralization was reported in the presence of crop-residue-derived char using an atrazine-degrading organism,\(^{23}\) our study highlights the noninhibiting influence of highly carbonaceous biochar amendments on atrazine degradation by native soil bacteria. It is likely that a community of atrazine-degrading microorganisms in our soil samples exhibits stronger atrazine mineralization capabilities compared to single atrazine-degrading strains. With regard to the above-mentioned publications, our results further demonstrate that biochar effects on pesticides in soils cannot be generalized due to the highly diverse nature of biochars and the complexity of soil systems.

As indicated by MANOVA statistical analysis, mineralization of \([^{14}C]\text{atrazine}\) was significantly affected for soil and soil \(\times\) biochar interaction (Table 3). The soils were by far the most

Table 3. Results from MANOVA To Assess Differences in Mineralization Rates of \([^{14}C]\text{atrazine}\) Using Three Factors: Soil (Belgian and Brazilian Soils), Soil Moisture (WHC\(_{\text{max}}\) at 60% and Slurry), and Added Biochar Amounts [0.0, 0.1, 1.0, and 5.0% (w/w)].\(^{16}\)

<table>
<thead>
<tr>
<th>source</th>
<th>df</th>
<th>SS</th>
<th>(F)</th>
<th>(\eta^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>soil (S)</td>
<td>1</td>
<td>0.9514</td>
<td>76.26**</td>
<td>54.73</td>
</tr>
<tr>
<td>moisture (M)</td>
<td>1</td>
<td>0.0038</td>
<td>0.31</td>
<td>0.22</td>
</tr>
<tr>
<td>added biochar (B)</td>
<td>3</td>
<td>0.0621</td>
<td>1.65</td>
<td>3.57</td>
</tr>
<tr>
<td>S (\times) M</td>
<td>1</td>
<td>0.0070</td>
<td>0.56</td>
<td>0.40</td>
</tr>
<tr>
<td>S (\times) B</td>
<td>3</td>
<td>0.2045</td>
<td>5.46*</td>
<td>11.77</td>
</tr>
<tr>
<td>M (\times) B</td>
<td>3</td>
<td>0.0065</td>
<td>0.17</td>
<td>0.37</td>
</tr>
<tr>
<td>S (\times) M (\times) B</td>
<td>3</td>
<td>0.1036</td>
<td>2.77</td>
<td>5.96</td>
</tr>
<tr>
<td>residual</td>
<td>21</td>
<td>0.3992</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>47</td>
<td>1.7382</td>
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</tr>
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</table>

| df, degrees of freedom; SS, sum of squares; \(F\), \(F\) value; \(\eta^2\), \(\eta^2\) values indicate how important (in percentage terms) each independent variable is in explaining variations in the data sets.\(^{36}\); \(p =\) permutation \(p\) value, probability levels: *, \(p < 0.01\); **, \(p < 0.001\). |

...important source of variation, explaining \(\approx 55\%\) of the variance in the data, despite the improved mineralization kinetics at higher biochar application rates (5% w/w). Because biochar did not generally affect the extent of mineralized \([^{14}C]\text{atrazine}\), interactions of \([^{14}C]\text{atrazine}\) with the different soils were the main factor affecting its mineralization (Table 3). Thus, \([^{14}C]\text{atrazine}\) mineralization was significantly increased (\(p < 0.05\)) in Brazilian soil, but not in Belgian soil (Figure 1). This finding emphasizes the importance of physical/chemical soil properties supporting sorption and mineralization processes and the presence of a microbial community being able to rapidly mineralize atrazine. However, biochar amendments may prime pesticide mineralization, as shown in our study.

The high microbial atrazine degradation capability in the used atrazine-adapted soils resulted in a rapid mineralization of the freshly applied \([^{14}C]\text{atrazine}\) even in the presence of biochar. However, it remains unknown if atrazine residues present in the used soils from former field applications as mentioned above were also subject to mineralization. As reported earlier, aged atrazine residues remained accessible to the specific atrazine-degrading microorganism \(\text{Pseudomonas sp. strain ADP}\).\(^{23}\) However, atrazine is not entirely degraded even in the atrazine-adapted Belgian soil, as described in a previous study.\(^{21}\) We assume that the microbial atrazine mineralization occurs more rapidly than strong sorption on biochar, which would, in turn, result in an exclusion from bioavailability.\(^{19}\)

The presence of the biochar used may not reduce the efficiency of pesticides in soils because the molecules remain bioaccessible to the greatest extent. However, more research is needed to investigate the herbicidal efficiency in biochar-amended soils and the effects of biochar on pesticide metabolite formation and leaching. Because each particular biochar has different properties as a result of different biomasses and operational parameters used in production,\(^{37}\) its suitability for environmental application cannot be generalized. Further investigations under real environmental conditions are required.

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**Notes**

The authors declare no competing financial interest.

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