Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil

Lars Bergström,* Elisabet Börjesson, and John Stenström

Due to the increasing concern about the appearance of glyphosate \([N-(\text{phosphonomethyl})\text{glycine}]\) and its major metabolite aminomethylphosphonic acid (AMPA) in natural waters, batch laboratory and lysimeter transport studies were performed to assess the potential for leaching of the compounds in two agricultural soils. Unlabeled and \(^{14}\text{C}\)-labeled glyphosate were added at a rate corresponding to 1.54 kg a.i. ha\(^{-1}\) on undisturbed sand and clay columns. Leachate was sampled during a period of 748 d for analyses of glyphosate, AMPA, total \(^{14}\text{C}\), and particle-bound residues. Topsoil and subsoil samples were used for determination of glyphosate adsorption, glyphosate degradation, and formation of AMPA and its degradation. The influence of adsorption on glyphosate degradation was confirmed, giving very slow degradation rate in the clay soil (half-life 110–151 d). The kinetics of AMPA suggest that although AMPA is always more persistent than glyphosate when formed from glyphosate, its degradation rate can be faster than that of glyphosate. The kinetics also suggest that apart from glyphosate being transformed to AMPA, the sarcosine pathway can be just as significant. The long persistence of glyphosate was also confirmed in the lysimeter study, where glyphosate+AMPA residues constituted 59% of the initial amount of glyphosate added to the clay soil 748 d after application. Despite large amounts of precipitation in the autumn and winter after application, however, these residues were mainly located in the topsoil, and only 0.009 and 0.019% of the initial amount of glyphosate added leached during the whole study period in the sand and clay, respectively. No leaching of AMPA occurred in the sand, whereas 0.03 g ha\(^{-1}\) leached in the clay soil.

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During the last few decades, there has been worldwide concern about the potential for contamination of surface waters and groundwater by various pesticides used on agricultural fields (Hallberg, 1989; Richards and Baker, 1993; Kreuger, 1998). Some persistent and mobile compounds can be transported long distances by water and may be found far from their site of application (Glotfelty et al., 1984). The most common way to classify the potential for different pesticides to move through soil is by short-term laboratory leaching tests, together with determination of the physico-chemical properties of the compounds (Tooby and Marsden, 1991), including soil sorption index, their half-life in soil, water solubility, and leaching indexes (Gustafson, 1989). The general opinion is that more strongly sorbed compounds are less susceptible to leaching, although this is not always the case (Bergström and Stenström, 1998). Soil is a highly complex medium involved in many interacting processes that can overshadow predictions based on intrinsic chemical properties, such as sorption. Therefore, even compounds with relatively strong sorption affinity to soil are sometimes found in natural waters (Kreuger, 1998).

Glyphosate \([N-(\text{phosphonomethyl})\text{glycine}]\) is a good example of such a compound. It was introduced by Monsanto (St. Louis, MO) in 1974 and is the most frequently used herbicide worldwide in both agricultural and nonagricultural applications. It is a nonselective herbicide that is taken up by plants after application on leaves and shoots and thereafter transported throughout the whole plant. Glyphosate is today often found in surface waters and occasionally also in groundwater, despite strong sorption to soil materials (Hance, 1976; Piccolo et al., 1994; de Jonge and de Jonge, 1999). Studies investigating the mobility of glyphosate in soil also indicate that the potential for leaching appears to be limited (Cheah et al., 1997; Fomsgaard et al., 2003), although there are studies showing that leaching may occur (Kjaer et al., 2003).

A leaching mechanism for strongly sorbed chemicals that has received increasing attention during recent years is what is referred to as colloid-facilitated transport (Grolimund et al., 1996; Hesketh et al., 2001). Through this mode of transport, in combination with preferential flow, strongly sorbed compounds can potentially migrate long distances in soil. This can be one explanation why...
glyphosate is frequently found in surface waters and sometimes even in groundwater, although there is still no strong evidence for this hypothesis.

The objective of the present study was to evaluate the potential for leaching of glyphosate and its major metabolite amino-methylphosphonic acid (AMPA) through the unsaturated zone of agricultural soils. This was done in field lysimeters containing undisturbed sand and clay profiles. In addition, adsorption, degradation, and residual amounts in soil after 2 yr were determined.

Materials and Methods

Lysimeter Experiment

Soil Characteristics, Lysimeter Collection, and Management

Three undisturbed soil columns of a sandy soil and four of a clay soil were used. The smaller number of sand columns was based on the fact that sandy soils are usually more homogeneous and therefore show less variability in flow processes (Bergström and Shirmohammadi, 1999). Some physical and chemical properties of the two soils used are listed in Table 1. The soil columns were collected using coring equipment in which a polyvinyl chloride pipe (1.18-m long and 0.295-m inner diam.) is gently pushed into the soil by a steel cylinder with cutting teeth, which rotates around the pipe as it penetrates the soil (Persson and Bergström, 1991). After collection at the two field sites, Lanna in southwest Sweden (58°21′ N, 13°08′ E) and Nåntuna close to Uppsala (59°49′ N, 17°39′ E), the columns were prepared for gravity drainage by removing about 0.07 m of soil at the base, which was replaced by gravel, two stainless steel meshes, and a fiberglass lid, giving a final length of the soil column of ~1.05 m. The lysimeters were then placed in vertical pipes permanently installed below ground at a lysimeter station located at the Swedish University of Agricultural Sciences in Uppsala, Sweden (Bergström, 1992).

All management practices performed on the lysimeters were intended to reproduce field conditions as closely as possible. Just before sowing in each year, the soil in each lysimeter was hand-tilled to simulate light harrowing. Spring barley (Hordeum distichum L.) was sown at a rate of 2 g per lysimeter on 21 May 2006, 26 May 2007, and 30 May 2008. On each occasion, mineral fertilizers were applied at rates of 100 kg N ha⁻¹, 22 kg P ha⁻¹, and 56 kg K ha⁻¹. The barley was harvested on 1 Sept. 2006, 28 Sept. 2007, and 16 Sept. 2008 by cutting the aboveground plant parts at ground level.

In addition to natural precipitation, all lysimeters received supplemental irrigation on two occasions during the 2-yr experimental period (in total, 22 mm). On each occasion, water was added with spray bottles over a few hours at rates typical of heavy rain storms, but not exceeding the infiltration capacity of the soil.

Chemical Application

Glyphosate was applied to two lysimeters of the sand soil and to three lysimeters of the clay soil on 18 Sept. 2006 at a rate corresponding to 1.54 kg a.i. ha⁻¹, which represents a normal dose in Swedish cereal production systems. Radiolabeled [¹⁴C] glyphosate (ARC 1313 glyphosate-[phosphonomethyl-¹⁴C]), 50 mCi mmol⁻¹, American Radiolabeled Chemicals, Inc., St. Louis, MO) was used to obtain fast screening of the leachate samples using scintillation counting analysis. The radiolabeled portion (5.32 MBq) was mixed with formulated (Roundup BIO, contains 486 g glyphosate L⁻¹ as isopropylamin salt, Monsanto Crop Sciences), unlabeled glyphosate (in total 10.5 mg lysimeter⁻¹), which was dissolved in 11 mL (0.16 mm) of water. This solution was applied to the lysimeters by dripping it on the soil surface using a syringe. After the solution had been applied, 5 mL (0.07 mm) of water was drawn up into the syringe and also applied to each lysimeter. In addition to glyphosate, KBr at a rate of 0.268 g Br⁻ per lysimeter (~40 kg Br⁻ ha⁻¹) was applied to provide information on the movement of water through the soil columns. The KBr was dissolved in water (0.4 g KBr in 5 mL), which was applied separately to the lysimeters, also using a syringe.

Soil and Water Sampling

On 17 Oct. 2007, samples of the topsoil (0–30 cm) and subsoil (30–80 cm) of each soil were collected for determination of adsorption and degradation characteristics. These samples were taken from the lysimeter of each soil used as control (i.e., no glyphosate added). Three soil cores from each lysimeter were collected with a tube drill. The individual samples were then mixed by layers into a topsoil and a subsoil sample for each lysimeter. On 5 Oct. 2008, after leaching measurements were terminated, soil samples were collected from the lysimeters to which glyphosate had been applied to determine the residual amounts of glyphosate and AMPA about 2 yr after application. Three cores

<table>
<thead>
<tr>
<th>Table 1. Selected soil characteristics of the Lanna clay and the Nåntuna sand. Standard laboratory methods were used throughout (Bergström et al., 1994).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Clay</td>
</tr>
<tr>
<td>0–30</td>
</tr>
<tr>
<td>30–60</td>
</tr>
<tr>
<td>60–90</td>
</tr>
<tr>
<td>Sand</td>
</tr>
<tr>
<td>0–30</td>
</tr>
<tr>
<td>30–60</td>
</tr>
<tr>
<td>60–90</td>
</tr>
</tbody>
</table>

† Gr = gravel, >2 mm; Sa = sand, 0.06–2 mm; Si = silt, 0.002–0.06 mm; Cl = clay, <0.002 mm.
‡ Determined in water.
§ Equivalent to porosity.
Nonlinearity. To determine if negligible amount of AMPA had been formed. In this branched pathway, \( k \) for glyphosate degradation in Eq. [2] equals the sum of \( k \) for AMPA formation and \( k \) for sarcosine formation. Then \( k = k - k \) and the fractions of glyphosate transformed into AMPA and sarcosine are \( k/k \) and \( k/k \), respectively. Since no more than 100% of the glyphosate can be transformed into AMPA, the upper limit for \( k \) is \( k \), which case \( k = 0 \). The maximum concentration of AMPA, \( c_{A_{\text{max}}} \), occurs at time \( t_{\text{max}} \) when \( dc_A/dt = 0 \). Inserting this value into Eq. [3], replacing \( c_G \) and \( c_A \) in Eq. [3] by their expressions in Eq. [2] and [4], respectively, and rearranging gives the following:

\[
t_{\text{A_{max}}} = \frac{\ln(k) - \ln(k)}{k - k}
\]

Nonlinear Regression

Least squares fits of data on adsorption and on residual values of glyphosate and AMPA were fitted to their respective equations by nonlinear regression. Residual values of AMPA were fitted using the values of \( c_{G0} \) and \( k \) for glyphosate degradation as obtained from Eq. [2]. The calculations were performed on a PC with the application SigmaPlot for Windows version 10.0 (Systat Software, Inc., San Jose, CA); the nonlinear regression method is based on the Levenberg and Marquardt method.

\[
c_G(t) = c_{G0} e^{-kt}
\]

where \( c_G \) (mg kg\(^{-1}\)) is the residual concentration of glyphosate at time \( t \) days after application, \( c_{G0} \) (mg kg\(^{-1}\)) is the initial concentration of glyphosate, and \( k \) (d\(^{-1}\)) is the first-order rate coefficient for degradation.

A branched reaction scheme was applied to describe the degradation of glyphosate to AMPA and sarcosine (Karpouzas and Singh, 2006; Borggaard and Gimsing, 2008) and the degradation of AMPA (Fig. 1). According to this scheme and assuming first-order kinetics, the rate of AMPA formation and degradation is then

\[
dc_A/dt = 0.66k_e c_G - k_2 c_A
\]

where \( c_A \) (mg kg\(^{-1}\)) is the concentration of AMPA at the time \( t \). Because the concentrations of glyphosate and AMPA were expressed in units mg kg\(^{-1}\), the value of \( c_{G0} \) obtained from Eq. [2] was multiplied by the stoichiometric factor 0.66 (i.e., the ratio of the molecular weights of the dominant species of AMPA and glyphosate at pH 7) in these calculations. The equation describing the concentration of AMPA was obtained by combining Eq. [2] and [3], and integrating:

\[
c_A = \frac{0.66k_1 c_{G0}(e^{-kt} - e^{-k_2 t})}{k_2 - k}
\]

Dehydration Study

Glyphosate dissolved in water (1.4 mg mL\(^{-1}\)) was applied drop-wise (1.0 mL) to 15 g of fresh soil. The soil was dried and mixed, after which an additional amount of fresh soil (to give 140 g DW in total) was thoroughly mixed into the spiked soil to give an initial concentration of 10 µg glyphosate per g DW of soil. Portions corresponding to 10 g of dry soil were transferred to 50-mL plastic tubes. The water content was adjusted to 60% of the water-holding capacity. The tubes were sealed with plastic caps that allow gas exchange and incubated at 20°C in the dark. After 2, 4, 8, 16, 32, and 64 d, two tubes were put in the freezer (−20°C) until analysis for residual concentrations of glyphosate and the metabolite AMPA. The weight of the tubes was measured once a week during the incubation, and when necessary, the moisture content was adjusted to 60% of the water-holding capacity.

Residual values of glyphosate were used for a least squares fitting procedure to determine values of the parameters of the function for first order exponential decay:

\[
c_G(t) = c_{G0} e^{-kt}
\]
Glyphosate and AMPA Analyses

Reagents

Analytical standards used for calibration were (trivial names in italics): \(N\)-(phosphonomethyl)glycine, glyphosate, (Riedel-de-Haén, Sigma-Aldrich, Sweden AB) and (aminomethyl)-phosphonic acid, AMPA, (Dr. Ehrenstorfer GmbH, Augsburg, Germany). Internal standards were \(^1\)C, \(^1\)N; \(^2\)D-labeled AMPA and \(^1\)C; and \(^1\)N-labeled glyphosate (LCG Standards AB, Borás, Sweden). Concentrated HCl, ethyl acetate, and NaOH (analytical reagent grade from VWR, Stockholm, Sweden), were used for extraction and solvation. The AG1-X8, 100–200, formate form cal reagent grade from VWR, Stockholm, Sweden), were used for ion exchange and clean-up. Trifluoroacetic anhydride (TFAA) and trifluoroethanol (TFE), both analytical reagent grade from Sigma Aldrich Sweden AB (Stockholm, Sweden), were used for the derivatization. The 0.22-μm glass fiber filters \# GSWP04700 were from Millipore VWR (Stockholm, Sweden).

Calibration

Stock solutions of glyphosate and AMPA were diluted in water to concentrations of 100 μg mL\(^{-1}\) and stored at +4°C. A solution containing 1 μg mL\(^{-1}\) of glyphosate and AMPA was prepared daily as a working standard. The labeled glyphosate and AMPA were diluted in deionized water to a concentration of 1 μg mL\(^{-1}\) and stored at −20°C in 2-mL portions.

Clean-Up and Derivatization

Water Samples

A 50-mL volume of a water sample and 0.1 μg each of glyphosate and AMPA internal standard were adjusted to pH 2 with 6 M HCl in a plastic tube. The sample was left to precipitate for 1 h and centrifuged at 5000 rpm for 10 min. The upper, clear phase was adjusted to pH 7 to 8. Ag1-X8 (2.3 g) was weighed into an empty 6 mL-plastic column equipped with a piece of cotton at the top and was adjusted to pH 7 to 8. Ag1-X8 (2.3 g) was weighed into an empty 6 mL-plastic column equipped with a piece of cotton at the top, and the column was wetted with deionized water. A 3-mL (200 mg) C18 SPE column was activated with 3 mL of methanol and 3 mL of water and connected on top of the AG1-X8 column. An empty 75-mL plastic column was connected on top of the C18 and Ag1-X8 columns, and the sample was applied at a rate of 2 mL min\(^{-1}\). The two upper columns were removed and the analytes were eluted with 3×4 mL of 0.6 M HCl at a rate of 1 mL min\(^{-1}\) and collected in a 100-mL pear-shaped flask. The sample was evaporated to approximately 2 mL under vacuum, quantitatively transferred to an 8-mL glass tube and evaporated to dryness under an air stream at 50°C. The derivatization was performed by adding 1 mL of trifluoroethanol and 2 mL of trifluoroacetic anhydride, and the sample was held at 100°C for 1 h. After being cooled to room temperature, the sample was evaporated under nitrogen and redissolved in 1.00 mL of ethyl acetate before analysis.

Particle-Bound Glyphosate and AMPA in Leachate

Leachate samples from three lysimeters of each soil were analyzed for particle-bound glyphosate and AMPA. These samples comprised two samples from the untreated lysimeters and four samples from the glyphosate-treated lysimeters on sampling occasions when the highest concentrations of glyphosate and AMPA were detected in the leachate. A 300-mL portion of each sample was filtered through a 0.22-μm glass fiber filter. The filter was weighed before and after filtration, dried at 105°C, and the dry weight of the particles was calculated. The dry filter and the particles were analyzed for glyphosate and AMPA by extraction with 7 mL of 0.1 M NaOH following the same procedure as for soil samples (see below).

Soil Samples

Ten grams of soil were extracted with 40 mL (for the degradation study) or 75 mL (for the lysimeter soil residue analysis) of 0.1 M NaOH by shaking for 30 min at 200 rpm, sonicated for 10 min and centrifuged for 10 min at 5000 rpm. The internal standards (0.1 μg each of glyphosate and AMPA) were added to a portion (40 μL and 4 mL for the degradation and the lysimeter studies, respectively) of the clear upper part of the sample, which was then analyzed according to the procedures described for the water samples. The portion from the degradation study was evaporated and derivatized directly after precipitation of the extract, since no column clean-up was needed due to the high residual concentrations in these samples.

Instrumentation

The gas chromatography–mass spectrometry (GC–MS) analyses were performed with a Hewlett-Packard 6890 GC (Agilent Technologies Sweden AB), equipped with a 30 m by 0.25 mm i.d. (0.25-μm film thickness) fused silica capillary column (HP-5 for GC–MS), a mass spectrometer 5973, a split/splitless injector, and the software Chemstation, all from Agilent Technologies (Kista, Sweden). One microliter of the samples was injected (in the splitless mode at 270°C, oven temperature 70°C). After 2 min, the oven temperature was raised to 170°C at 30°C min\(^{-1}\) and then from 170 to 250°C at 120°C min\(^{-1}\). Helium (N47 grade, 99.997%) was used as the carrier gas and the flow rate was 1.2 mL min\(^{-1}\). The mass spectrometer was operated in the electron impact (EI) mode; the transfer line and manifold temperatures were 270 and 230°C, respectively. Fragment ions were detected by selected ion monitoring (SIM) and used for identification of the AMPA and glyphosate derivates as shown in Table 2.

Verification of compound identification was based on comparison of the areas of the selected ions in the samples with

Table 2. Molecular weights, retention times (RT) and specific selected ions for compound derivatives.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Molecular weight</th>
<th>RT (min)</th>
<th>Quantification ion (m/z, % relative abundance)</th>
<th>Qualification ion (m/z, % relative abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPA†</td>
<td>371</td>
<td>4.49</td>
<td>126 (100)</td>
<td>302 (23)</td>
</tr>
<tr>
<td>AMPA‡</td>
<td>375</td>
<td>4.49</td>
<td>130 (100)</td>
<td>306 (22)</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>511</td>
<td>4.49</td>
<td>411 (100)</td>
<td>384 (50)</td>
</tr>
<tr>
<td>Glyphosate‡</td>
<td>513</td>
<td>5.35</td>
<td>413 (100)</td>
<td>386 (48)</td>
</tr>
</tbody>
</table>

† AMPA = aminomethylphosphonic acid.
‡ Internal standard.
those of the standards. For quantification, the response areas for AMPA and glyphosate target ions were calculated in relation to those of the internal standards. The response was found to be linear in the practical concentration range (2.5–100 pg) of individual components injected.

The quantification levels for glyphosate and AMPA were 0.1 μg L⁻¹ in water and 0.01 μg g⁻¹ in soil. In some samples, however, the quantification level was higher due to the specific background.

Bromide Analyses
Bromide concentrations were measured using a Dionex ICS-90 ion chromatograph (Dionex, Sunnyvale, CA). Before analysis, an aliquot (2 mL) of each leachate sample was filtered through a membrane filter and, if needed, diluted into a 2-mL vial. The detection limit was 0.1 mg L⁻¹.

Results and Discussion
Adsorption of Glyphosate
The high correlation coefficients ($R^2 \geq 0.997$; Table 3) obtained when sorption data for both soils and soil layers were fitted to the Freundlich adsorption isotherm show that they could be accurately described by this model. The values of the $K_f$ parameter obtained were considerably higher in the clay soil than in the sand and are similar to values previously reported for glyphosate sorption to soils of similar textures (Vereecken, 2005). In the sand, $K_f$ was higher in the topsoil than in the subsoil, whereas the opposite was true for the clay. The correlation between $K_f$ and the amount of clay in the different soils was 0.987. Although based on only four soils (topsoil and subsoil in the respective soils), this result supports the generally held view that glyphosate is primarily sorbed to clay particles and their associated iron oxides (Vereecken, 2005). Normalization of the distribution coefficients for glyphosate should therefore also account for the amount of clay and oxides present in soil and not organic carbon only, which is used to calculate $K_{oc}$.

The $1/n$ parameter, which expresses the degree of linear relationship between $c_{soil}$ and $c_{aq}$, was close to 1 for both layers of the clay soil and the sand topsoil, showing an almost constant distribution coefficient between sorbed and dissolved glyphosate in these soil layers in the range of concentrations studied. In the subsoil of the sand, the parameter $1/n$ was 0.82, indicating that the availability of sites for sorption in this layer becomes limiting at high glyphosate concentrations.

Degradation of Glyphosate and AMPA
Best fits of glyphosate and AMPA residue data to Eq. [2] and [4], respectively, are shown in Fig. 2 and the parameter values obtained in Tables 4 and 5, respectively. Initial extraction efficiencies of glyphosate were 112 to 123% as shown by comparing the initial concentrations obtained (Table 4) with the

<table>
<thead>
<tr>
<th>Soil</th>
<th>$c_{G0}$†</th>
<th>$k$</th>
<th>$R^2$</th>
<th>$t_{1/2}$‡</th>
<th>DT₉₀§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand topsoil</td>
<td>11.21 ± 0.33</td>
<td>0.041 ± 0.003</td>
<td>0.978</td>
<td>16.9</td>
<td>56.2</td>
</tr>
<tr>
<td>Sand subsoil</td>
<td>12.27 ± 0.19</td>
<td>0.019 ± 0.001</td>
<td>0.985</td>
<td>36.5</td>
<td>121</td>
</tr>
<tr>
<td>Clay topsoil</td>
<td>11.99 ± 0.15</td>
<td>0.0063 ± 0.0005</td>
<td>0.948</td>
<td>110</td>
<td>365</td>
</tr>
<tr>
<td>Clay subsoil</td>
<td>11.61 ± 0.41</td>
<td>0.0046 ± 0.0014</td>
<td>0.562</td>
<td>151</td>
<td>501</td>
</tr>
</tbody>
</table>

† $c_{G0}$ = initial concentration of glyphosate; $k$ = first-order rate coefficient for degradation; $t_{1/2}$ = half-life; DT₉₀ = time for 90% degradation.
‡ Calculated as ln(2)/$k$.
§ Calculated as ln(10)/$k$. 

Fig. 2. Best fits (A) to Eq. [2] of data on glyphosate and (B) to Eq. [4] of data on aminomethylphosphonic acid (AMPA) concentrations for sand topsoil (†), sand subsoil (○), clay topsoil (▷), and clay subsoil (△) (mean ± SE, $n = 2$). dw = dry weight.
Table 5. Coefficients ($\pm$ SE, $n = 12$) obtained by nonlinear regression for formation and degradation of aminomethylphosphonic acid (AMPA) according to Eq. [4].

<table>
<thead>
<tr>
<th>Soil</th>
<th>$k_1$†</th>
<th>$k_3$</th>
<th>$t_{1/2}$‡</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand topsoil</td>
<td>0.0216 ± 0.0011</td>
<td>0.0115 ± 0.0019</td>
<td>60.4</td>
<td>0.965</td>
</tr>
<tr>
<td>Sand subsoil</td>
<td>0.0092 ± 0.0005</td>
<td>0.0076 ± 0.0018</td>
<td>91.3</td>
<td>0.988</td>
</tr>
<tr>
<td>Clay topsoil</td>
<td>0.0063 ± 0.0005</td>
<td>0.0199 ± 0.0013</td>
<td>34.9</td>
<td>0.973</td>
</tr>
<tr>
<td>Clay subsoil</td>
<td>0.0028 ± 0.0006</td>
<td>0.0071 ± 0.0087</td>
<td>97.6</td>
<td>0.901</td>
</tr>
</tbody>
</table>

† $k_1$ = first-order rate coefficient for degradation of glyphosate to AMPA; $k_3$ = first-order rate coefficient for degradation of AMPA; $t_{1/2}$ = half-life.
‡ Calculated as ln(2)/$k_1$.
§ In the clay topsoil, $k_1$ became 0.0073, i.e., larger than $k_3$ (first-order rate coefficient for degradation; Table 4), leading to a formation fraction > 1, and was therefore set equal to $k_3$.

Fig. 3. Relationship between half-life ($t_{1/2}$) for glyphosate and Freundlich adsorption coefficient ($K_f$), and between $t_{1/2}$ for aminomethylphosphonic acid (AMPA) and % organic matter.

nominal value of 10 μg g$^{-1}$ DW. All parameter values were significantly different from zero ($p < 0.05$, $n = 12$). The models gave good fits of the data for all soils ($R^2 \geq 0.90$), except for glyphosate in the clay subsoil ($R^2 = 0.56$). This poor fit could be due to difficulties in getting glyphosate homogeneously distributed in this clay-rich (56.1%) subsoil with no organic matter (0%). Another explanation could be that the $R^2$ values obtained by nonlinear and linear regression are not comparable. In nonlinear regression, $R^2$ refers to the fraction of the variance explained and is the model efficiency (EF). A disadvantage of EF is its dependency on the slope of the curve, as it is always relatively small for relatively flat decline patterns, or can even be negative for curves describing for instance formation and degradation of metabolites, irrespective of the scatter of measured data around the calculated curve (FOCUS, 2005). Therefore, from visual inspection of the fits to the data (Fig. 2) and from the generally small standard errors in the parameters determined (Tables 4 and 5), we concluded that the equations provide relevant quantitative information.

Glyphosate degraded relatively rapidly in the sand, with a half-life of 16.9 and 36.5 d in the topsoil and subsoil, respectively (Table 4). In the clay, very long half-life values of 110 and 151 d were obtained, and remarkable values of 365 and 500 d for 90% degradation (DT$_{90}$). These half-life values are within the range previously reported for glyphosate degradation in agricultural soils (Giesy et al., 2000). There was a high correlation between half-life and $K_f$ (Fig. 3), suggesting that adsorption is important for the amount of glyphosate available in the soil water for degradation.

The concentration of AMPA steadily increased during the incubation period of 64 d in all soils except the sand topsoil (Fig. 2B), where it peaked after 43.4 d at 2.4 mg of AMPA kg$^{-1}$, representing 32.4% of the initial amount of glyphosate added (Table 6). The degradation rate of AMPA, as quantified by $k_3$, gave a half-life of 35 to 98 d, with slower rates in the subsoil (Table 5). The correlation between these half-life values and the amount of organic matter was $-0.973$ (Fig. 3), suggesting that increasing amounts of organic matter, or perhaps AMPA-degrading microorganisms dwelling there, increase degradation rates.

Table 6. Derived parameter values on fraction of glyphosate degraded to aminomethylphosphonic acid (AMPA) ($k/k$), rate constant for formation of sarcosine ($k_3$), incubation time ($t_{\text{amino max}}$) at which the AMPA-concentration peaks ($c_{\text{amino max}}$), and $c_{\text{amino max}}$ as fraction of initially added glyphosate.

<table>
<thead>
<tr>
<th>Soil</th>
<th>$k_3$†</th>
<th>$k_3$‡</th>
<th>$t_{\text{amino max}}$§</th>
<th>$c_{\text{amino max}}$¶</th>
<th>$c_{\text{amino max}}$ fraction#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand topsoil</td>
<td>0.0190</td>
<td>0.0098</td>
<td>43.4</td>
<td>2.40</td>
<td>32.4</td>
</tr>
<tr>
<td>Sand subsoil</td>
<td>0.0098</td>
<td>0.0098</td>
<td>80.4</td>
<td>2.40</td>
<td>32.4</td>
</tr>
<tr>
<td>Clay topsoil</td>
<td>0.0018</td>
<td>0.0018</td>
<td>84.7</td>
<td>1.47</td>
<td>18.6</td>
</tr>
<tr>
<td>Clay subsoil</td>
<td>0.0215</td>
<td>0.0215</td>
<td>174</td>
<td>1.34</td>
<td>17.5</td>
</tr>
</tbody>
</table>

† Values of $k$ (first-order rate coefficient for glyphosate degradation) from Eq. [2] (Table 4) and of $k_3$ (first-order rate coefficient for degradation of glyphosate to AMPA) from Eq. [4] (Table 5).
‡ $k_3 = k - k_1$.
§ Calculated according to Eq. [5].
¶ Obtained by inserting derived values of $k$, $k_3$, $c_{\text{amino}}$, and $t_{\text{amino max}}$ into Eq. [4].
# Calculated as ($c_{\text{amino max}} \times 1.52 \times 100$)/$c_{\text{amino}}$, where 1.52 is the stoichiometric factor for conversion of AMPA to glyphosate concentration.
The degradation of AMPA is reported to be slower than that of glyphosate (Giesy et al., 2000). In the Footprint database, AMPA is classified as persistent, with a typical half-life of 151 d, compared with 12 d for glyphosate (Footprint, 2009). The fact that AMPA is formed when glyphosate is degraded clearly means that the persistence of AMPA has to be equal to or longer than that of glyphosate. However, we did not find any previous study in which the degradation of AMPA was studied and compared with that of glyphosate in the same soil. In a study where glyphosate degraded with a half-life of 9 d, Simonsen et al. (2008) estimated a half-life of 32 d for AMPA from the descending part of data on AMPA residues. However, this is a worst-case scenario as these data represent the sum of AMPA formation from degradation of the glyphosate still present and AMPA degradation. This does not reveal how fast the AMPA molecule per se is degraded. Our data suggest that the AMPA degradation rate can be faster than that for glyphosate, for instance in soils with high clay content, which slows down glyphosate degradation, and high organic matter content, which stimulates AMPA degradation (Fig. 3).

Microbial degradation is the main process controlling the disappearance of glyphosate in soil, and there are two well-described biological pathways for such degradation that give AMPA and sarcosine as the respective metabolites (Karpouzas and Singh, 2006; Borggaard and Gimsing, 2008). It has recently been shown that ligninolytic enzymes can also transform glyphosate into AMPA (Pizzul et al., 2009). Because AMPA is the only significant soil metabolite found in soil degradation studies, it is frequently suggested that metabolism of glyphosate in soil usually proceeds via the AMPA pathway (Giesy et al., 2000; Karpouzas and Singh, 2006). However, the fractions of AMPA formed in our study (48–100%, Table 6) suggest that both pathways can be active in soil, with up to 52% not following the AMPA pathway. Reasons for the sarcosine pathway not being considered significant in soil could be that soil residues of sarcosine are not determined in most studies and that sarcosine rapidly degrades to glycine (Karpouzas and Singh, 2006) in biologically active soil.

Precipitation and Drainage Conditions

Daily precipitation and average air temperatures at the lysimeter station are shown in Fig. 4. Over the 2-yr study period (15 Sept. 2006–15 Sept. 2008), cumulative precipitation was 1192 mm, which, in combination with supplemental irrigation, resulted in a total water input to the lysimeters of 1214 mm. This total water input is slightly higher (10%) than the long-term average precipitation for the Uppsala region (554 mm yr−1). Average air temperature during the experimental period (7.8°C) was also higher than the long-term average at Uppsala (5.3°C).

A few weeks after glyphosate was applied, from 30 September onward, rain events were quite frequent (Fig. 4), and precipitation totaled 232 mm by the end of 2006. This clearly created worst-case conditions for leaching of the herbicide, and the average amounts of leachate were 169 and 156 mm from the sand and clay soil, respectively, during this period. Peak weekly amounts of leachate, reaching 42 (sand) and 33 (clay) mm, occurred 8 wk after herbicide application (Fig. 5). During 2007, precipitation was close to the normal for the area, although quite unevenly distributed. During periods with low evaporation (November, December, and January), monthly precipitation was about 60 mm, which was clearly above the average and increased the risk of leaching. In 2008, precipitation was again above normal, causing large amounts of leachate (Fig. 5).

The cumulative amounts of leachate from the lysimeters each year are shown in Table 7. In total over the 2-yr period, the amount of leachate was 572 (± 17) kg ha−1 for the sand and 461 (± 15) from the clay soil. In relation to water input, these amounts constituted 47 and 38% of precipitation plus irrigation, which is considerably higher than in other similar leaching studies performed in Sweden (Bergström and Jokela, 2001).

Leaching of Bromide

The total amount of bromide that leached during the 2-yr period was 30.2 (± 1.2) kg ha−1 for the sand and 20.1 (± 2.6) kg ha−1 for the clay, which was equivalent to 75.4 (± 2.9) and 50.3 (± 6.4) % of that applied. It is reasonable to assume that most of the missing bromide was taken up by plants and removed from the soils during the growing season 2007 (Schnabel et al., 1995; Kohler et al., 2005). If this was the case (cf. Fig 6), and as the bromide was applied in a pulse-like manner, it is possible to carry out a quantitative analysis of the bromide breakthrough.
curve (BTC), allowing us to obtain an estimate of the effective pore volume (i.e., the volume of water in soil that participates in solute transport) (Butters et al., 1989). This is equivalent to the first normalized temporal moment of the BTC (e.g., Valocchi, 1985), which was obtained by numerical integration of the BTC utilizing the trapezoidal method (Atkinson, 1989).

The effective pore volumes for bromide were 165 and 194 mm in the sand and clay, respectively.

The concentrations of bromide in leachate are shown in Fig. 6. In the sand, the peak concentration reached on average 28.5 mg L$^{-1}$ on 24 Nov. 2006, after 147 mm of accumulated leachate (i.e., an effective pore volume of 0.9), followed by a steady decrease to levels below 0.2 mg L$^{-1}$ from the beginning of March 2008 onward. In the clay soil, two bromide peaks occurred, reaching about 10 mg L$^{-1}$, one in the beginning of January 2007 and the other in the beginning of April 3 mo later, after 180 and 277 mm of cumulative drainage, respectively (i.e., effective pore volumes of 0.9 and 1.4). However, during the first 6 wk after application bromide concentrations reached 4 mg L$^{-1}$ after only about 40 mm of accumulated drainage (equivalent to an effective pore volume of 0.2), which indicates that some preferential flow occurred in the clay (cf. Fig. 6C).

**Leaching of Glyphosate and AMPA**

Average concentrations of glyphosate and AMPA in leachate are shown in Fig. 7. In the sand, the average peak concentration of glyphosate reached 0.36 μg L$^{-1}$ in the beginning of March 2007, when temperatures were consistently above freezing (Fig. 4), about 25 wk after pesticide application. During this period, the amount of leachate was about 250 mm (i.e., equivalent to 1.5 effective pore volumes). Thereafter the glyphosate concentration decreased and the average concentration was below 0.1 μg L$^{-1}$ from 16 March 2007 onward. This leaching pattern indicates limited preferential transport of the herbicide through the sand profile, although some preferential transport must have occurred considering the strong sorption of glyphosate (Table 3) and thereby expected large retardation. This is a flow behavior reported in several other leaching studies in sandy soils (e.g., Bergström and Shirmohammadi, 1999).

The fact that the glyphosate peak occurred about 15 wk later than the corresponding bromide peak (Fig. 6) is a reflection of bromide being a nonreactive tracer. In the clay soil, the initial glyphosate peak occurred in the beginning of December and reached 0.23 μg L$^{-1}$ after about 150 mm of water (i.e., equivalent to 0.8 effective pore volumes) had leached out of the soil columns. This considerably smaller amount of leachate suggests that glyphosate was partly transported through preferential flow paths in the clay profile, as was the case for bromide.

This flow pattern has been documented earlier in this clay soil for reactive solutes (Djodjic et al., 1999; Bergström, 1995) and for nonreactive tracers (Bergström and Jarvis, 1993; Bergström and Shirmohammadi, 1999). However, the highest glyphosate peak (0.44 μg L$^{-1}$) in leachate from the clay soil coincided with that in the sand, i.e., in the beginning of March 2007. This glyphosate peak was washed out of the columns slightly earlier than the corresponding bromide peak (Fig. 6), which was
rather unexpected. Apart from preferential flow, another explanation could be that the highly water-soluble bromide diffused into micropores in the clay soil relatively soon after application and once in these pores it was largely protected from percolating water (Bergström and Stenström, 1998). From July 2007 onward, the average glyphosate concentration in clay soil leachate was <0.1 μg L⁻¹, although single samples had concentrations slightly exceeding the detection limit (0.1 μg L⁻¹). Average concentrations of AMPA in leachate were at or below 0.1 μg L⁻¹ in both soils (Fig. 7), with the highest concentration (0.30 μg L⁻¹) in a sample from one of the clay lysimeters.

The average total amount of glyphosate that leached from the sand was 0.13 (± 0.03) g ha⁻¹ and from the clay soil 0.28 (± 0.08) g ha⁻¹. These amounts correspond to 0.009 and 0.019% of the amount of glyphosate applied to the soils. No leaching of AMPA occurred in the sand, whereas 0.03 g ha⁻¹ leached in the clay soil. Total leaching of the ¹⁴C applied in September 2006 was on average 0.31% from the sand soil and 0.25% from the clay. This shows that constituents other than glyphosate and AMPA that were not positively identified formed the major proportion of the total radioactivity in leachate. The leaching rates determined in this study are quite small compared with those in many other studies. For example, in a study performed by Al-Rajab et al. (2008), which included microlysimeters of three soils (clay loam, silty clay loam, and sandy loam), the amounts of glyphosate leached during 11 mo ranged between 0.11 and 0.28% of the amount applied. However, there are also studies showing similar results to those obtained in the present experiment. In a study in France performed using lysimeters filled with calcareous soil (Landry et al., 2005), leaching of glyphosate was between 0.02 and 0.06% of that applied after 680 mm of rainfall. Similarly, Cheah et al. (1997) recovered 0.04 to 0.07% of applied glyphosate in lysimeter leachate after 200 mm of simulated rainfall. However, the conditions in all the above-mentioned studies were quite different from those in this study; the lysimeters were only 9.8 to 25 cm long, the experimental periods were considerably shorter (a few days to 1 yr), and the amounts of rainfall were much smaller (200 to 869 mm). These differences certainly have to be taken into account in a comparison of results.

No glyphosate or AMPA was determined to be particle-bound, even though large quantities of particles were present in leachate from the clay soil. It is noteworthy that the particles were operationally defined as those being retained on a 0.22-μm glass-fiber filter. Some studies have shown that colloid-facilitated transport of glyphosate can occur. For example, de Jonge et al. (2000) showed in a study on lysimeters filled with undisturbed topsoil of a sandy loam that 1 to 27% of leached glyphosate was particle-bound. Considering the overall low total concentrations of glyphosate in the present study (Fig. 7), the particle-bound proportion would be below the detection limit (0.1 μg L⁻¹) if it constituted less than 25% of what was leached. It is also important to bear in mind that topsoil lysimeters only include about 30% of the profiles used in this study and may in fact, as indicated above, generate results that are quite atypical of results obtained in full-length lysimeters, such as those used here. The underlying subsoil can act as a sink or source for particles leaching through the soil profile.

The trend for glyphosate to leach in larger amounts from clay soils than from sandy soils is relatively well documented.

Fig. 6. Average concentrations of bromide in the leachate (mean ± SD, n = 2 for sand and n = 3 for clay), (A) over time and (B) in response to cumulative leachate, and (C) the normalized cumulative tracer mass as a function of effective pore volume for both soils. Open symbols (□) refer to the clay soil and filled symbols (■) to the sand.

In a Danish study, this was attributed to periods of high intensity rainfall shortly after application, when glyphosate was located on the soil surface and thereby exposed to rapid water transport in clay macropores extending up to the surface (Kjaer et al., 2003).
Residues of Glyphosate and AMPA in Soil

Residues of glyphosate and AMPA in the 0- to 30-, 30- to 60-, and 60- to 90-cm soil layers 748 d after application are shown in Fig. 8. Residues were found in the 0- to 30-cm layer in all lysimeters and also in the 30- to 60-cm layer in one of the lysimeters with clay soil, possibly due to preferential flow in clay macropores and translocation in plant roots (Laitinen et al., 2007). No residues were found in the 60- to 90-cm layer in any of the lysimeters. Considering the worst-case conditions prevailing for leaching after application of glyphosate in the autumn of 2006, these results confirm the generally low mobility found for these compounds (Giesy et al., 2000; Vereecken, 2005).

No glyphosate was detected in one of the sand lysimeters, and 0.019 mg kg⁻¹ remained at 0 to 30 cm in the other one. Low concentrations could be expected from the fast degradation in the sand topsoil (laboratory half-life 16.9 d). The concentrations of AMPA (0.026 and 0.090 mg kg⁻¹) remaining can be due to a combination of slow degradation (laboratory half-life 60.4 d) and continuous supply from degradation of remaining glyphosate. Related to the initial amount of glyphosate added, the remaining glyphosate residues represented 2.7% and total residues of glyphosate + AMPA, calculated as glyphosate equivalents, represented 27%.

In the clay soil, glyphosate and AMPA were found in all three lysimeters, probably due to very slow degradation of glyphosate in the topsoil and subsoil (Table 4), and thereby a long-term supply of AMPA, slow degradation of AMPA in the clay subsoil (Table 5), and 100% formation of AMPA from glyphosate degradation in the topsoil (Table 6). Glyphosate residues represented 5.1% and total residues 59% of the initial amount of glyphosate added. Similar field persistence of glyphosate and AMPA residues was found in a sandy soil in Finland, where total residues in the 0- to 60-cm layer accounted for 72% of the amount applied 20 mo after application (Laitinen et al., 2009).

Conclusions

The influence of adsorption on glyphosate degradation was confirmed, giving very slow degradation in the clay soil. The kinetics of AMPA residues suggest that although AMPA is always more persistent than glyphosate when formed from glyphosate, its degradation can be faster, for instance in soils with a high clay content, which slows down glyphosate degradation, and a high organic matter content, which stimulates AMPA degradation. The kinetics also suggest that apart from glyphosate being transformed to AMPA, the sarcosine pathway can be just as significant. The long persistence of glyphosate was also confirmed in the lysimeter study, where glyphosate+AMPA residues constituted 59% of the initial amount of glyphosate added to
the clay soil 748 d after application. However, despite quite frequent rain events and large amounts of precipitation in the autumn and winter after application, these residues were mainly located in the topsoil, confirming the generally low mobility reported for these compounds. This conclusion is also supported by the small amounts of glyphosate and AMPA leached during the whole study period. Possible residues of glyphosate and AMPA due to transport on particles > 0.22 μm were below the limit of detection (0.1 μg L⁻¹), and this does not appear to be an important transport mechanism in the soils included in this study.

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References