1. Information on the study

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2. Full summary of the study according to OECD format

Glyphosate, the most commonly used herbicide in the world, can be degraded into more toxic and persistent products such as aminomethylphosphonic acid (AMPA) or non-toxic products such as sarcosine and glycine. In this study, we used liquid chromatography mass spectrometry (LC-MS) and electrospray ionization (ESI) source Q Extractive Orbitrap mass spectrometry (ESI-Orbitrap MS) to identify glyphosate degradation products and combined with sequential extraction and stable isotopes to investigate the degradation of glyphosate and transformation of phosphorous (P) product in a soil-water system. The LC-MS and ESI-Orbitrap MS results showed that glycine formed during the early stage but was rapidly utilized by soil microorganisms. AMPA started to accumulate at the late stage and was found to be 3-6 times more resistant than glyphosate against degradation; while no sarcosine was formed. The ^{18}O labeling and phosphate oxygen isotope results allowed a clear distinction of the fraction of inorganic P (P_i) derived from glyphosate, about half of which was then rapidly taken up and recycled by soil microorganisms. Our results provide the first evidence of the preferential utilization of glyphosate-derived P_i by microorganisms in the soil-water system. The rapid cycling of P_i derived from this disregarded source has important implications on nutrient management as well as water quality.

Materials and methods

Reagent and chemicals

Glyphosate (≥96 %), (Aminomethyl) phosphonic acid (≥98 %) and 9-Fluorenyl-methoxycarbonyl chloride (FMOC-Cl) (≥97 %) were obtained from Sigma-Aldrich. Isotope labeled compounds including glyphosate-2-^{13}C, ^{15}N, glycine-d_5 and sarcosine-d_3 (methyl-d_3) were purchased from Sigma-Aldrich. Other chemicals including glycine (≥99 %) and sarcosine (≥98 %) were purchased either from Acros Organics or Fisher Scientific. All the reagents were of analytical grade and stock solution were prepared with DI water.

Soil collection and incubation

A typical silt loam soil (0-15 cm depth) from the Agricultural Experiment Station research farm at the University of Delaware was used in this study. The detailed information about the soil characterization has been reported in a previous publication. After removing any plant residues and granular rock particles, the soil samples were air-dried, homogenized, passed through a 2 mm sieve, and stored until analyses.

A flowchart of the experimental and analytical approach used is shown in Figure 1. The first degradation experiment was run to identify glyphosate and its degradation products in soil as well as to determine the degradation kinetics and half-lives of major products. The soil was incubated with 1 μmol/g unlabeled glyphosate at 20ºC in the dark with 60 % water content for 175 d. A separate experiment with

dual isotope (^{13}C and ^{15}N) labeled glyphosate (1 µmol/g) spiked in soil was performed for 35 d to accurately identify degradation products. The control experiment was performed under the same condition but without glyphosate. The natural soil incubation included both biotic and abiotic degradations. Identical experiment run with autoclaved water and soil served as abiotic degradation. At selected time points, 5 g subsamples were collected into 50 mL centrifuge tubes and stored at -20ºC until further analysis. All experiments were run in duplicate under the same condition.

In order to identify P distribution and bioavailability during glyphosate degradation, the second set of experiments was performed in two ^{18}O-labeled waters ($\delta^{18}O_{H2O}$ = -6.51 and + 18.27 ‰). To collect sufficient P for isotope analyses, 5 µmol/g unlabeled glyphosate was spiked into 300 g soil, and incubated with 600 mL ^{18}O-labeled water at 20ºC in the dark for 161 d. The spiked glyphosate concentration is much higher than application dose in agriculture (about 1 kg/ha), but is required to obtain reliable phosphate isotopic analyses. The experimental containers were tightly capped to avoid any water evaporation that compromises the water oxygen isotopes. The containers were shaken every day for ~15 min to homogenize the system and then briefly ventilated to replenish ambient oxygen and to preserve the oxic condition. The control experiments were run under the same condition but in the absence of glyphosate. Subsampling and processing followed a similar procedure as described above.

Extraction and analyses of glyphosate, AMPA, glycine, and sarcosine
The extraction of glyphosate, AMPA, glycine, and sarcosine was based on the published method. Briefly, 1 g lyophilized soil samples degradation experiments were extracted with 5 mL 0.6 M KOH for 1 h by shaking at 140 rpm, then centrifuged at 2755 x g for 30 min. One mL of supernatant was removed and neutralized by HCl and then 0.12 mL of borate buffer (pH=9) and 0.12 mL FMOC-Cl (12 g/L) were added and shaken for 1 min on a vortex mixer. After an overnight reaction at room temperature, the mixture was filtered with a 0.45 µm syringe filter for LC-MS analysis.

Glyphosate, AMPA, glycine, and sarcosine standards were prepared to develop the separation method by using an Acclaim 120, C18 column (2.1 x 250 mm) under a gradient eluent program. After testing and running several programs, the optimized gradient was identified to be effective with a mixture of two mobile phases with a flow rate of 0.35 mL/min with (A) acetonitrile and (B) 5 mmol/L HAc/NH_4Ac: 0-6 min, 20-40 % A, 80-60 % B; 6-9 min, 40-75 % A, 60-25 % B; 9-10.2 min, 75-100 % A, 65-0 % B; 10.2-12 min, 100 % A, 0 % B; 12-12.1 min, 100-20 % A, 0-80 % B; 12.1-14 min, 20 % A, 80 % B.

The chromatographic separation for each sample required 14 min.

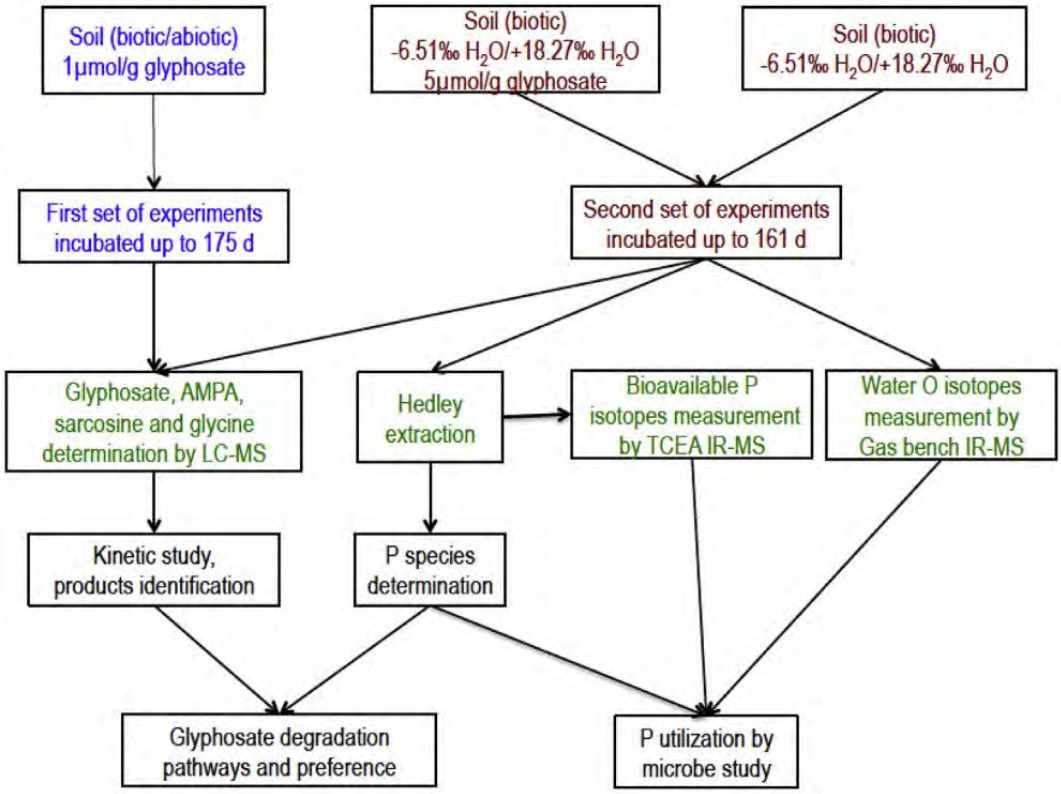

Figure 1. Flowchart outlining glyphosate degradation experiments in the water-soil system.

Glyphosate and its degradation products were identified and quantified by a Waters single quadrupole LC-MS equipped with PDA and SQ detector. The optimized MS parameters are as follows: ESI positive mode, capillary voltage 3 kV, cone voltage 40 V, desolvation temperature 200ºC, desolvation gas flow 650 L/hr, and full mass scan from 100 to 500 m/z. The unlabeled glyphosate and labeled sarcosine were quantified with labeled glyphosate and unlabeled sarcosine as internal standards. Similarly, labeled glycine was quantified by labeled glycine as an external standard to avoid any interference from glycine already present in soil. AMPA was determined by the soil spiked external standards. Labeled glyphosate degradation samples were analyzed with a high resolution mass spectrometry-Q Extractive Orbitrap Mass Spectrometry (Thermo, Germany) at the University of Delaware. Orbitrap MS data were acquired under the positive mode with scan range from 100 to 1000 m/z. Glycine formation during labeled glyphosate degradation were determined by external standard prepared by spiking labeled glycine in soil to avoid the interference of soil original glycine.

The extraction and derivatization methods for glyphosate, AMPA, glycine, and sarcosine were validated by spiking known amounts of these compounds in soil. The recovery ranged from 85 to 107 % for glyphosate, 79-93 % for AMPA, 74-88 % for glycine, and 80-97 % for sarcosine with RSD below 20 %, which is considered satisfactory. The limit of quantification (LOQ) for glyphosate and AMPA is 10 nmol/g soil and for glycine and sarcosine is 50 nmol/g in single quadrupole LC-MS, while it was largely improved by using Orbitrap (0.5 nmol/g).

Distribution of P derived from glyphosate into soil P pools

To differentiate and quantify the distribution of glyphosate-derived P in soil, samples from both control and glyphosate spiked soils (from the second set of experiments) were analyzed. A 0.3 g lyophilized soil was weighed and extracted with 30 mL DI water for 2 h using the modified Hedley et al. (1982) sequential extraction method (Tiessen et al., 1984). The supernatant was collected as H_2O extractable P_i (most labile P_i), and residual soil was extracted with 30 mL of 0.5 M $NaHCO_3$ for 16 h to collect labile and weakly adsorbed P_i. Inorganic P from those two pools represents microbially available P_i. The soil was further extracted for 16 h first with 30 mL of 0.1 M NaOH and then with 1 M HCl to obtain the NaOH extractable P_i (strongly sorbed P, fixed by Fe and Al oxides) and HCl extractable P_i (strongly

fixed Ca-P), respectively. The concentration of P_i in each pool was measured by using the phosphomolybdate blue method. The residual P in the soils after the completion of sequential extraction was quantified using ICP-MS.

Measurement of oxygen isotope ratios
Soil samples from control and glyphosate spiked (5 µmol/g) experiments with two ^{18}O-labeled waters were centrifuged first to extract waters to measure water oxygen isotopes ($\delta\,^{18}O_W$) by CO_2 equilibration method. The measurement was done in a Finnigan GasBench II coupled with an isotope ratio mass spectrometer (IRMS; Thermo, Darmstadt, Germany) in the Environmental Biogeochemistry Laboratory at the University of Delaware.

To understand the P bioavailability, the H_2O- and $NaHCO_3$- extracted P_i pools were combined and processed for the measurement of phosphate oxygen isotope ratios ($\delta\,^{18}O_P$). Five grams of lyophilized soil samples from the second set of degradation experiments were processed following the Joshi et al (2018) method to purify and finally convert P_i into silver phosphate. The O-isotope ratios were measured by a thermochemolysis/elemental analyzer (TC/EA) couples with IRMS. All isotopes from samples and standards were run at least in triplicate.

The measured $\delta\,^{18}O_P$ values of P_i were calibrated against two silver phosphate standards (YR 1aR-2 and YR 3-2, with the $\delta\,^{18}O_P$ values of -5.49 and +33.63 ‰, respectively). Similarly, the $\delta\,^{18}O_W$ values of porewater were calibrated with two USGS water standards ($\delta\,^{18}O_W$ values of -1.97 and -9.25 ‰, respectively). All isotope values are reported in per mil (‰) relative to the Vienna Standard Mean Ocean Water (VSMOW).

Results and Discussion (please refer to the section 3 of the original paper)

Degradation kinetics of glyphosate and its metabolites
The typical chromatography spectra of glyphosate, AMPA, sarcosine, and glycine are shown in Figure 2. Based on the LC-MS results, the concentrations of the compounds were calculated and are shown in Figure 3. Glyphosate gradually degraded over time and the extent of degradation reached >80 % by 35 d of incubation but traces of residual glyphosate were still detected until 175 d.

AMPA, the major metabolite of glyphosate, appeared after several days, accumulated during incubation, and reached its maximum concentration at 35 and 56 d in the experiment with 1 µmol/g and 5 µmol/g glyphosate, respectively. Afterwards, its degradation dominated over accumulation. Neither the degradation of glyphosate nor the formation of AMPA was observed in the sterilized soil incubation (abiotic only experiment), indicating microorganisms play a crucial role in degrading glyphosate in soils.

The degradation of glyphosate with time is often described according to first-order kinetics:

$$\ln(C/C_0) = -kt \qquad (1)$$

$$t_{1/2} = \ln 2/k \qquad (2)$$

where C_0 is the initial concentration, C is the concentration at time t, and k is the degradation rate constant. The maximum accumulated concentration of AMPA is used as its initial concentration since more than 80 % of glyphosate was degraded at the time. The results show that both glyphosate and AMPA degradation follow first order kinetics with a strong correlation coefficient (R^2 >0.85). The calculated half-lives of glyphosate under two sets of experiments are 28.9 and 31.5 d, respectively, consistent with the published results. A calculation based on the maximum amount of AMPA accumulated in the soil shows that the AMPA accounts for 48-68 % of the products from glyphosate degradation. It shows much longer half-lives (138.6 and 173.3 d), which highlights the high risk because of its toxicity and persistence in the environment.

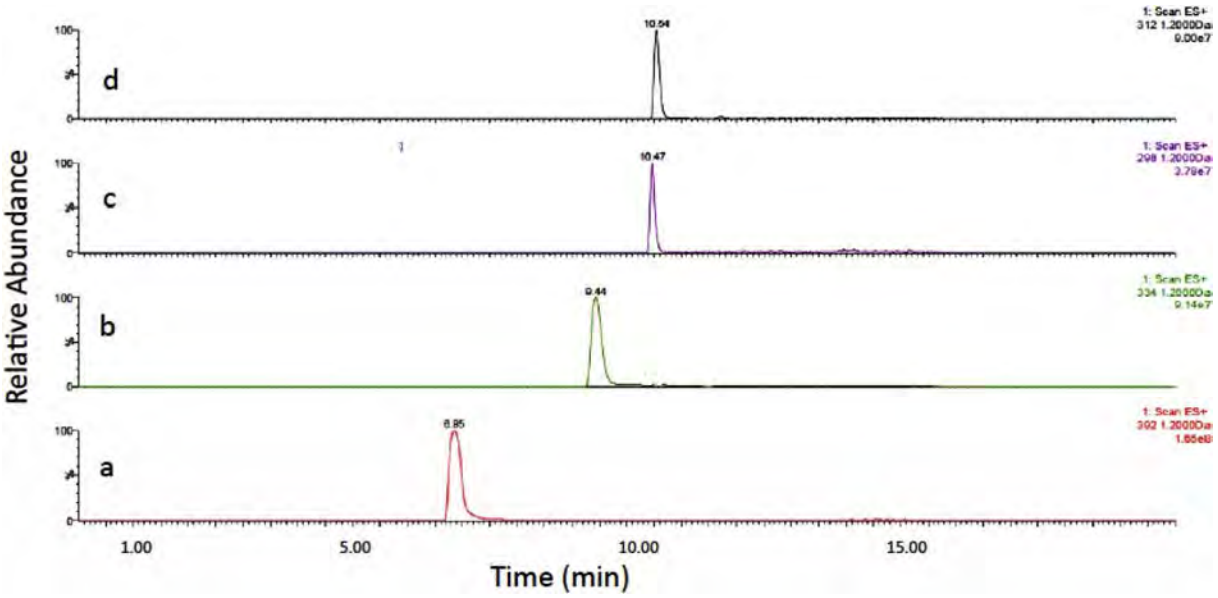

Figure 2. **Typical spectrum of glyphosate, AMPA, glycine and sarcosine analyzed by LC-MS (soil spiked with 1 µmol/g standards). a) glyphosate, b) AMPA, c) glycine, and d) sarcosine.**

Glycine is a common amino acid and commonly present in soil and other environment. The isotope labeled glyphosate provides the reliability of detection because the labeled element is present in glycine as well. Labeled glycine appeared only after few days, accumulated, and reached the highest concentration after 5 d and then decreased but was still detectable after 35 d incubation (Figure 3a).

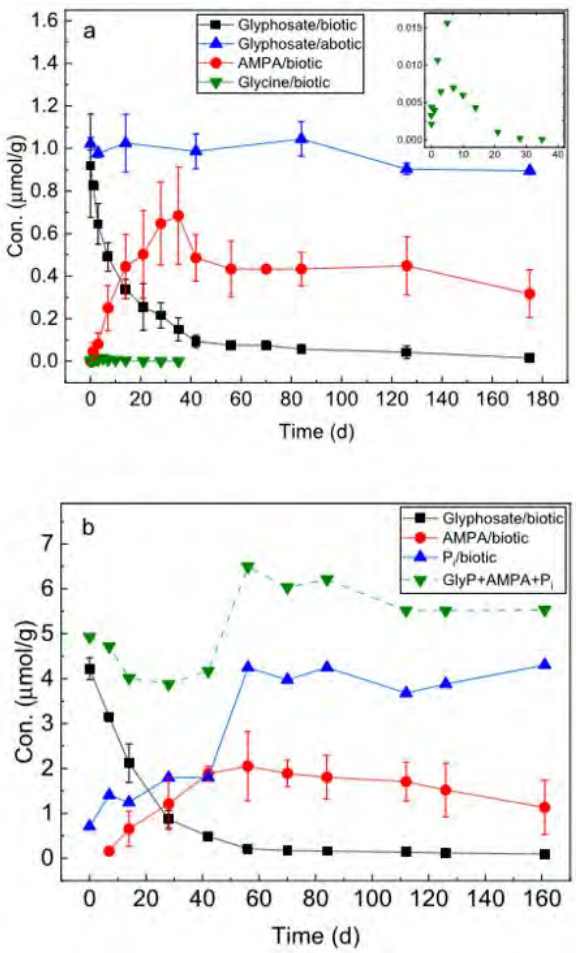

Figure 3. **Kinetics of glyphosate biotic (natural soil) and abiotic (sterilized soil) degradation and its products. a) incubated with 1 µmol/g glyphosate, and b) incubated with 5 µmol/g glyphosate. Please note that the natural soil incubation includes both biotic and abiotic components of degradation.**

The concentration of labeled glycine is low, probably due to glycine derived from glyphosate was readily incorporated into microbial biomass son after it formed. Results from a separate labeled glycine incubation experiment showed a rapid decline of soil-spiked glycine (1 µmol/g) with half-life of 0.89 d (Figure 4). Abiotic experiment showed no significant decline in glycine concentration in sterilized soil, validating methodology as well as indicating that soil microorganisms play a major role in glycine transformation. A recent study of labeled glyphosate reported the distribution of ^{13}C and ^{15}N into several amino acids including glycine, which our results corroborate. These findings, together, confirm that glyphosate derived glycine in the experiments should have rapidly utilized and metabolized by soil microorganisms.

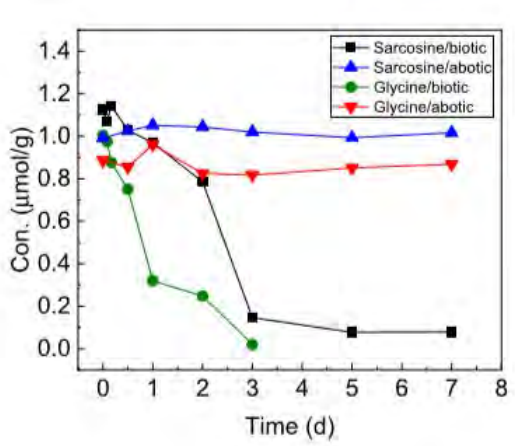

Figure 4. **Biotic (natural soil) and abiotic (sterilized soil) degradation of glycine and sarcosine in soil with spiked concentration of 1 µmol/g of each. Please note that the natural soil incubation includes both biotic and abiotic components of degradation.**

Sarcosine is a commonly recognized precursor to glycine during glyphosate degradation primarily on pure cultures that include bacteria isolated from soils, but rarely from the natural or simulated environments. In this study, sarcosine was not detected in any soil treatments including labeled glyphosate and high glyphosate (5 µmol/g) incubations. There might be three possibilities for the observed results: inefficient extraction from soil, fast oxidation of sarcosine, or presence below the detection limits of the analytical method. However, the recovery test performed by artificially spiking sarcosine in the same soil revealed that the method used could efficiently extract and accurately quantify sarcosine (yield 80-97 %). The individual incubation experiment showed that sarcosine could be degraded fast in the biotic experiment (with half-life of 0.99 d) but no significant decline in sterilized soil, which indicates degradation possible only by soil microorganisms. These lines of evidences suggest analytical method is not the reason, particularly since the high resolution Obitrap MS (detection limit of 0.5 nmol/g of sarcosine and glycine) was used. In the labeled glyphosate degradation experiments, soil samples were collected in several time points (0, 1, 2, 4 h, …until 35 d). The analytical method used successfully monitored the glycine formation and accumulation under extremely low concentration. If sarcosine was actually formed as a precursor to glycine, it should have detected by Obitrap MS since both sarcosine and glycine have similar half-lives. In a recent study, sarcosine was not detected in the abiotic degradation of glyphosate catalyzed by Mn minerals. These authors used advanced analytical methods including NMR, HPLC, and density functional theory (DFT) based electronic structure calculations and concluded that sarcosine was not a necessary intermediate product. Overall, the reliable extraction and analytical methods and intensive time point sampling verified that sarcosine was not formed during glyphosate degradation by soil microorganisms in this study.

Distribution of glyphosate-derived phosphorous in soil

Concentrations of four soil P_i pools in the control and glyphosate-spiked soils during the second set of incubations are shown in Figure 5. The experiments performed in two ^{18}O-labeled waters are considered duplicates because the difference in water oxygen isotopes does not impact the kinetics and extent of glyphosate degradation. Clearly, the control soil without glyphosate already contains high P_i and concentrations of P_i in different pools vary. It is noticeable, however, that the concentrations of P_i in these four pools remained essentially constant during the long-term incubation, with H_2O-P_i (1.01 ± 0.08 µmol/g), $NaHCO_3$-P_i (4.21 ± 0.23 µmol/g), NaOH-P_i (10.08 ± 0.91 µmol/g), and HCl-P_i (0.52 ± 0.06 µmol/g). This means that no significant transfer of P pools and organic-inorganic transformation occurred during the long-term incubation. The NaOH-P_i pool was the largest, indicating that Fe and Al minerals associated P is the major P sink in this soil, which is consistent with several other soils.

The results from the experiment in which glyphosate was spiked show that P_i derived from glyphosate

transferred into different pools, resulting in an increase of corresponding pool size. The maximum concentration of H_2O-P_i was 2.11 µmol/g at 70 d of incubation. The difference between control (soil without glyphosate) and glyphosate spiked soil shows that there was 1.06 µmol/g glyphosate-derived P_i transferred into this pool. Similarly, a significant net increase of P_i was observed in $NaHCO_3$-P_i (1.40 µmol/g), NaOH-P_i (1.93 µmol/g), and HCl-P_i (0.23 µmol/g) pools, with the highest P_i concentrations measured around 56-126 d of incubation. It is interesting that the order of P pool was the same as that in the original (control) soil: NaOH-P_i >$NaHCO_3$-P_i >H_2O-P_i >HCl-P_i. Calculated P mass balance shows that the total increase in P_i among 4 pools was 4.30 µmol/g at the end of incubation, which accounts for ~86 % of spiked glyphosate (5 µmol/g). The residual P in the control and glyphosate spiked soils were similar (7.99 ± 0.69 and 7.67 ± 0.69 µmol/g, respectively), indicating that there was no significant incorporation of glyphosate-derived P in the residual P pool. It also means that the Hedley extraction could efficiently extract almost all P and account P derived from biodegradation of glyphosate.

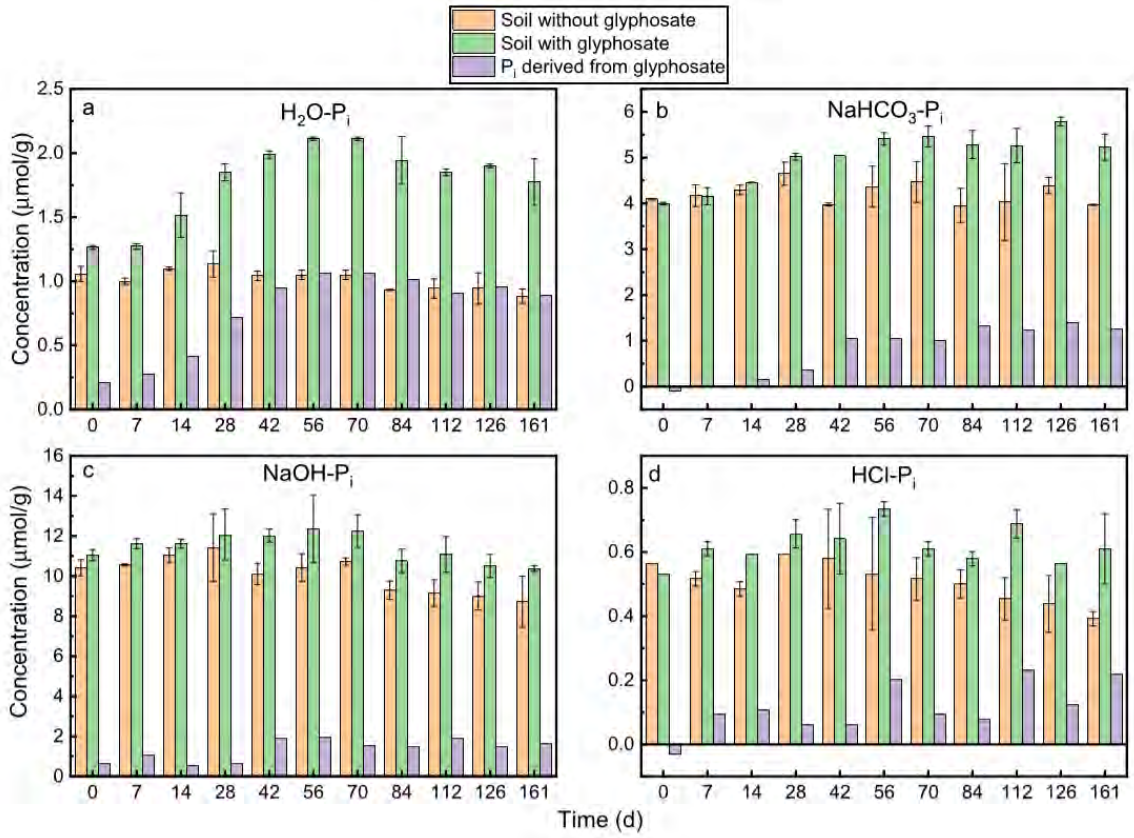

Figure 5. **Concentrations of P in different pools in original soil and glyphosate incubated soil during biotic degradation. H_2O and $NaHCO_3$ extracted P pools are considered bioavailable P in soil. Soil was spiked with 5 µmol/g glyphosate. Glyphosate derived P was calculated as the different between soil with and without glyphosate.**

In terms of distribution P_i derived from glyphosate (Figure 6), the H_2O- and $NaHCO_3$-P_i pools, which are considered readily available P_i for uptake by microorganisms and plant roots, received almost half (44 %) of it. Meanwhile, around 33-38 % of glyphosate P transformed into the NaOH-P_i pool, an unavailable or moderately bioavailable P pool depending on the soil P conditions and plant efficiency and time. This means that this conditionally unavailable P pool might be further transported into open water systems by leaching or soil erosion and could increase the risk of polluting waters. The HCl-P_i, which is not directly utilized by plants and microorganisms and normally remains as an unavailable P pool in agricultural soil, only received 3-5 % of P derived from glyphosate. These results highlight the fact that P load derived from a large amount of glyphosate application (with estimated 130 million kg used in the U.S.) cannot be ignored.

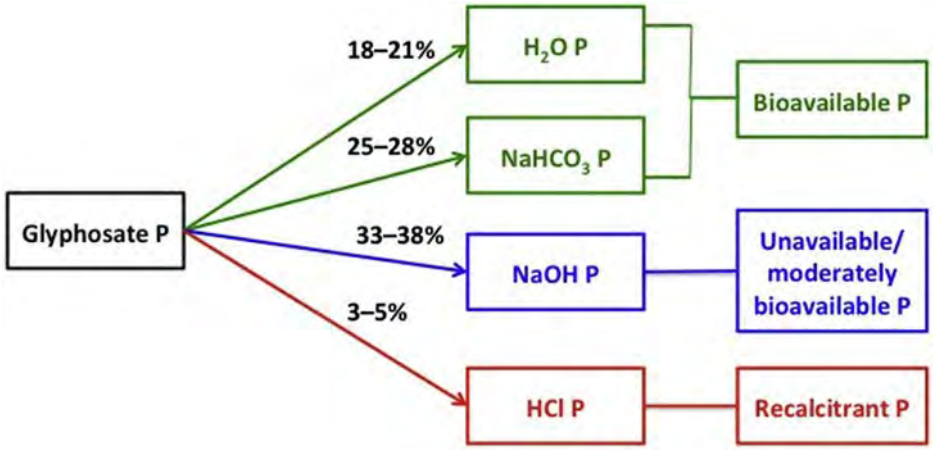

Figure 6. Distribution of glyphosate-derived P to different P pools during its biodegradation in the soil-water system. Soil incubated with 5 µmol/g glyphosate.

Given that the P_i derived from glyphosate is steady means that it was gradually released as the degradation continues and distributed more into the bioavailable pool, and it may be a better P source for plants. Phosphorus fertilizer is the major P supply for plants with estimated 4 billion kg used in the U.S in 2014 with 50-70 % use efficiency. However, its fast P release kinetics do not match the dynamic needs of different crop growth stages well and this offset causes nutrient loss from soil to aquatic systems. Given the slow but steady P release from glyphosate degradation, it might be slightly more synchronous than commercial fertilizers, but still too fast than plant needs. Furthermore, multiple sprays of glyphosate during the crop lifetime (average of 1.6 times per crop year) support the possibility of fractionating more into bioavailable P that plants can readily take up. This demands reconsidering glyphosate not only use as a herbicide but a bonus P source to crops and should be included in estimations of crop P needs to improve the P efficiency of plant uptake as well reducing the P loss from agricultural soils.

Bioavailability of glyphosate-derived phosphorus

Once inside the cell, P_i is involved in several metabolic reactions catalyzed by enzymes including incorporation into cell biomass and ATP-ADP conversion. One of the unique enzymes is pyrophosphatase (PPase), which is highly conserved across all three domains of life, catalyzes the hydrolysis of pyrophosphate into P_i. This is a reversible reaction and leads to exchange of all four O atoms in P_i with O in ambient water and thus achieves O-isotopic equilibrium between phosphate and water. The equilibrium isotope depends on the temperature and water oxygen isotope value.

To further test the bioavailability and rate of microbial utilization of glyphosate-derived Pi, phosphate oxygen isotopes ($\delta^{18}O_P$) of P_i in the soil incubated with and without glyphosate were measured and compared with the equilibrium isotope values calculated from the temperature and oxygen isotopes of water ($\delta^{18}O_w$) in the experiments. The $\delta^{18}O_w$ values remained constant at -6.51 ± 0.30 ‰ and +18.27 ± 0.12 ‰ for two ^{18}O-labeled water experiments in the long-term incubation except at the end of the experiment (161 d), when an inadvertent evaporation resulted in slight enrichment of isotopes (-4.90 ‰ and +20.71 ‰, respectively). The expected isotopic equilibrium value ($\delta^{18}O_{P\text{-}eq}$) was calculated based on the (Chang and Blake, 2015) equation as:

$$\delta^{18}O_{p-eq} = e^{\left(\frac{14.43}{T} - 0.0265\right)} \times (\delta^{18}O_w + 1000) - 1000 \qquad (3)$$

The $\delta^{18}O_{P\text{-}eq}$ values in the experiments incubated with -6.51 ‰ and +18.27 ‰ water are +15.83 ± 0.31 ‰ and +41.16 ± 0.12 ‰ (Figure 7), respectively, and remained constant during the incubation period (except at 161 d, in which water mass was not conserved). The starting isotope values of extracted P_i were consistent in all treatments: 20.77 ± 0.26 ‰, 21.02 ± 0.10 ‰, 21.38 ± 0.42 ‰ and 21.21 ± 0.16 ‰ in two controls (soil without glyphosate) and two glyphosate spiked experiments with -6.51 ‰ and +18.27 ‰ ^{18}O-labeled waters, respectively. It means that there are no different O sources or contaminants that might have impacted isotope values during the incubation period, besides the degradation of glyphosate.

The measured $\delta^{18}O_P$ values in the bioavailable P in ^{18}O spiked (+18.27 ‰) water became gradually heavier (Figure 7a), shifting towards the equilibrium values (+41.16 ‰) and reached 32.04 ‰ at the end of incubation. This result reveals the rapid uptake of the available P by soil microorganisms and the release of cycled P back to the soil. At the early stage, $\delta^{18}O_P$ values of P_i in the soil spiked with glyphosate were consistent with those in original control experiments. However, they became lighter after 14 d and remained 1.2-2.5 ‰ lighter for a long period. This is due to the contribution from a much lighter isotope value of P_i (~4-9 ‰) derived from glyphosate. The newly derived P_i from glyphosate degradation mixed with soil P_i pool and turned them into isotopic lighter and away from the equilibrium value (around +41.2 ‰). This result is consistent with P_i distribution that P_i was heavily released from glyphosate from 14 d to 84 d (Figure 5) and preserving isotope record of the lighter glyphosate derived P_i in the system (see Figure 5). However, the difference in isotope values between those two treatments gradually narrowed and eventually erased at 161 d, indicating that the soil microorganisms were efficient to uptake and cycle almost all of bioavailable P in the soil both from originally present soil and from glyphosate derived P_i.

The isotope trend in the experiments performed in -6.51 ‰ water (Figure 7b) is comparable to heavy water, but with a minor difference. For example, the $\delta^{18}O_P$ values in glyphosate spiked soil became much lighter and reached the equilibrium value sooner than those from control soil (without glyphosate). The reason is that the P_i derived from glyphosate carries much lighter $\delta^{18}O_P$ values (as explained above), which brings the isotope values close to equilibrium (which is lighter: +15.83 ± 0.31 ‰, due to the lighter water oxygen isotopes). The gap between the two treatments was 0.8 ‰, and then increased to 2.3 ‰ due to the large contribution of lighter isotopes of glyphosate derived Pi, but with the enhancement of microbe turnover, it decreased again but still 1.6 ‰ off at the end of the incubation.

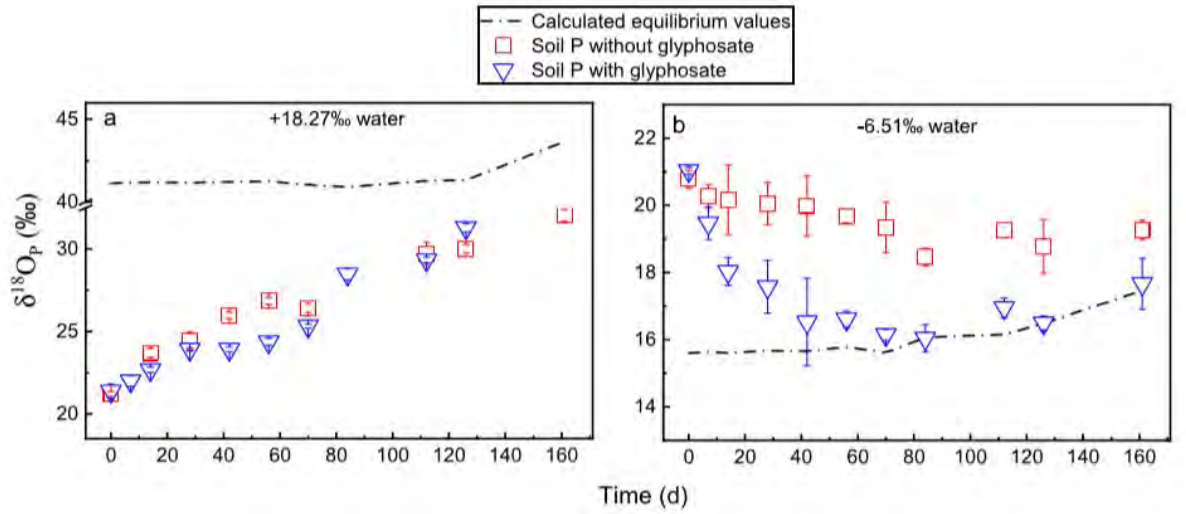

Figure 7. Changes in phosphate oxygen isotopes during glyphosate biodegradation in the water-soil system. The calculated equilibrium values assumes all P is completely recycled by microorganisms. The closer the isotope values toward the equilibrium values, the higher the extent of P cycling.

The observed results explained above provide several new insights on degradation of glyphosate

and its metabolites and recycling of glyphosate derived-P and together have several implications on the fate and impact of glyphosate in soils.

First, it proves that the isotope signature of glyphosate degradation can be detected in the experiments mimicking environmental systems. Second, it indicates that the degradation of glyphosate is faster than the microbial uptake and turn-over of P, so that the unique signature could be measured at the early to middle stage of the reaction.

Third, if the $\delta^{18}O_P$ values of the P derived from organic compounds are farther/closer to the equilibrium range compared to those present in-situ, they could easily shift/overprint bulk isotope value (due to mixing), leading to the inaccurate estimation of the biological activities.

Microbial turnover of P in the soil-water system

To evaluate the extent of P taken up and recycled by soil microorganisms, the P turnover was calculated from the starting $\delta^{18}O_P$ values ($\delta^{18}O_{P\text{-}t0}$) at 0 h, measured values at time t ($\delta^{18}O_{P\text{-}t}$) and the equilibrium values ($\delta^{18}O_{P\text{-}eq}$):

$$\%P\ \ turnover = \frac{(\delta^{18}O_{p-t} - \delta^{18}O_{p-to})}{(\delta^{18}O_{p-eq} - \delta^{18}O_{p-t})} \times 100 \qquad (4)$$

As the equation shows, the closer the values of $\delta^{18}O_{P\text{-}t0}$ to $\delta^{18}O_{P\text{-}eq}$, the higher the microbial turnover efficiency. The results show that P_i in the control experiment was rapidly exchanged by soil microorganisms and driven closer to the equilibrium values, with the turnover efficiency of 22-28 % at 56 d and 45-48 ‰ at 161 d in two ^{18}O-labeled waters (Figure 8). As expected, the efficiency of P turnover was similar irrespective of the starting isotopic values of ^{18}O-labeled water (-6.51 ‰ or +18.27 ‰).

In the glyphosate spiked experiments, the $\delta^{18}O_P$ value at time t ($\delta^{18}O_{P\text{-}t/spike}$) is the sum of glyphosate derived P_i ($\delta^{18}O_{P\text{-}t/gly}$) and the original P_i from control soil ($\delta^{18}O_{P\text{-}t/con}$), which can be calculated from a simple mass balance equation as follows:

$$\delta^{18}O_{p-t/spike} = x\delta^{18}O_{p-t/gly} + (1-X)\delta^{18}O_{p-t/con} \qquad (5)$$

where *x* is the fraction of P_i derived from glyphosate in the spiked samples. We calculated the starting isotope values of glyphosate derived P_i in two ^{18}O-labeled water systems at 0 h using previous results, which are +6.92 ‰ and ±12.14 ‰ in -6.51 ‰ and +18.27 ‰ waters, respectively. Based on the starting values of glyphosate-derived P_i, its microbial turnover was calculated using equation (4). As shown in Figure 8, the trend of P turnover in the soils receiving glyphosate-derived P_i was similar to that of control soil (without glyphosate), but the recycling efficiency was higher (67-75 ‰). Overall, phosphate oxygen isotopes allowed discrimination of sources and variable recycling efficiency of soil P vs glyphosate-derived P_i.

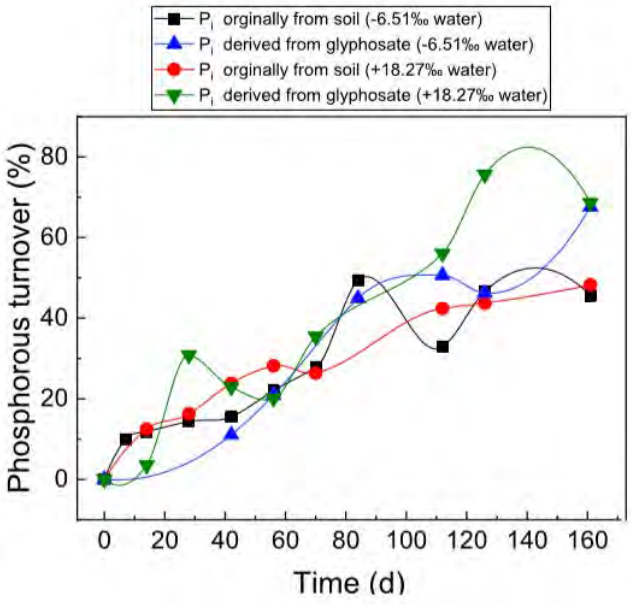

Figure 8. Microbial turnover efficiency of soil P and glyphosate-derived P.

Glyphosate degradation pathways in soil

To understand the degradation pathways and specific preferences in the soil system studied, the released P_i extractable from four pools were combined together. The total P mass from glyphosate source was also calculated by adding glyphosate, AMPA, and released P_i and are shown in Figure 3b. The released P_i steadily increased and reached the peak concentration around 56 d. AMPA remained at the accumulation stage and started to degraded at that time only when more than 80 % of glyphosate was already degraded. There was slight decrease in total P (from original concentration of 4.92 μmol/g) at the early stage of degradation, and then remained almost constant during the incubation period. Consider the efficient extraction of the glyphosate-derived P_i, it implies that there might be some other non-detected P speciation during the early stage of glyphosate degradation besides glyphosate, AMPA, and inorganic P. A potential P compound could be methylphosphonic acid, which can be generated synchronously if glycine forms directly from glyphosate. Based on the data generated in this study and foregoing assumptions and published results, revised pathways and temporal preference of glyphosate degradation in the soil-water system is proposed and shown in Figure 9. Under the action of soil microorganisms, at the early stage of degradation, glyphosate is cleaved at C(3)-N position to form glycine and methyl-phosphonic acid, the latter one is further degraded to form P_i, which accumulates in the system. Another bond cleavage occurs at C(2)-N position and form AMPA and glyoxylic acid. AMPA accumulates at the late stage of degradation. No sarcosine was generated in the soil-water system in this study, so it is not the required intermediate metabolite to form glycine.

Conclusion

In this study, we studied degradation glyphosate and its metabolites and successfully utilized phosphate oxygen isotopes to confirm the biological availability of glyphosate-derived P in the simulated soil-water system. The broader conclusions derived from this study and the implications thereof are as follows:

1) A satisfactory method of extraction and separation of glyphosate and its major metabolites in soil was developed, which could be used to identify the fate of glyphosate in a variety of environments. The absence of degradation in sterilized soil showed the soil microorganisms play the essential role on the degradation of glyphosate. Temporal presence of glycine and AMPA varied as well as their microbial uptake and degradation. AMPA was found to be 3-6 times resistant than glyphosate against degradation, which brings a higher concern to the safety of environment.

2) The distribution of glyphosate-derived P_i in a soil was investigated. About half of the glyphosate-derived P_i transferred into the readily bioavailable P pool. A slow but steady release of P_i from the degradation of glyphosate could mean that its supply could be slightly more synchronous with plant P demand during plant growth especially because it is applied more than one time during a crop cycle. This means that a higher proportion of glyphosate-derived P, than P from commercial fertilizers which release P all at once, could be taken up by plants.

3) Glyphosate-derived P_i has a distinct isotopic signature and can aid in identification of its source. The natural environment, however, is complex and could pose additional challenges, most likely due to the low content of glyphosate and inappropriate sampling time could miss to detect significant offset of isotope values. This is because isotope signature could be erased or overprinted due to biological cycling of glyphosate-derived P.

4) ^{18}O-labeling in water and application of phosphate oxygen isotope method allowed explicit understanding of microbial uptake and extent of biological turnover of glyphosate derived-P. The microbial turnover of original P in soil and glyphosate-derived P was comparable, but it was found that the microorganisms were more efficient to utilize and recycle glyphosate-derived P. The research tool developed could be further used to investigate the extent of microbial activities in soils and other natural environments.

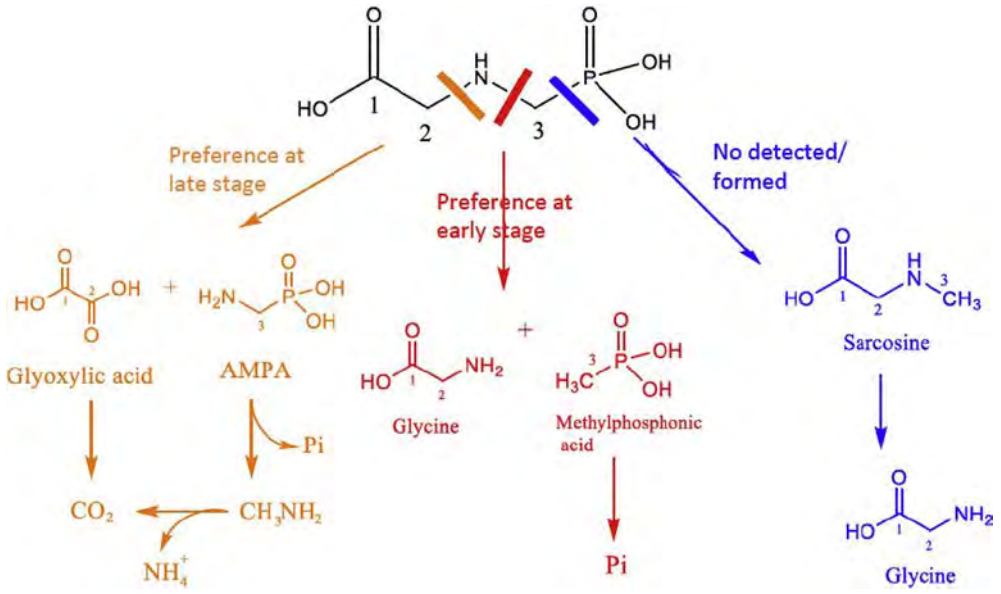

Figure 9. (Bio)degradation pathways of glyphosate and preference of degradation in the water-soil system used in this study.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes a soil degradation experiment with glyphosate, where also AMPA was identified as metabolite. The main focus of the article is the transformation of glyphosate-derived phosphorous. In general, the methods and results are well described but the relevant experiment (incubation with 1 µmol/g non-labelled glyphosate for 175 d) shows some deviations from the OECD 307 study guideline: soil properties are not reported, exact soil water content during incubation is unclear, the mass of soil incubated is not clearly stated (1 g soil was used for extraction). Further, concentrations of glyphosate and AMPA are only presented in figures, not as tabulated values. DT_{50} values according to SFO were calculated for glyphosate and AMPA (based on max. concentration) but details on fits and statistics are not provided.

The article is therefore considered as reliable with restrictions (Category 2).

1. Information on the study

Data point:	KCA 7.1.3.1.1
Report author	Tévez, H., et al.
Report year	2015
Report title	pH dependence of Glyphosate adsorption on soil horizons
Document No	Boletín de la Sociedad Geológica Mexicana Vol 67, Num. 3 , 2015, P. 509-516
Guidelines followed in study	None
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (the soil used for the experiment does not reflect European conditions (climate and soil characterization))

2. Full summary of the study according to OECD format

Pesticides bring many problems to the environment and to human health. The first rationale for their use is increased food production. Glyphosate N-(phosphonomethyl)glycine (PMG) is a non-selective, post emergent, and broad spectrum herbicide, very well known for its extensive application in agriculture worldwide. PMG adsorption experiments were carried out in three horizons of a Typic Haplustoll soil from the Province of Santiago del Estero, Argentina. Adsorption isotherms were fitted using Freundlich and Langmuir models. The affinity constants (K_F and K_L), the adsorption intensity (l/n) and the maximum surface coverage (Γ_{max}) were obtained. The results show the dependence of the parameters K_L and Γ_{max} with pH and also with the different horizons and particle size.

Materials and Methods

Chemicals
All chemicals utilized were of analytical reagent grade and were used without further purification. All solutions and soil dispersions were prepared using Milli-Q water. All PMG solution concentrations ranged from 0.05 to 10 mM prepared daily.

Study area
Climate is semiarid mesothermal, with an average annual temperature of 19.6 °C and rainfall of between 600 and 750 mm per year concentrated in the spring-summer period. Samples were taken up to 130 cm of depth from three very well differenced horizons classified as Ap (0 - 18 cm), AB (18 - 50 cm) and BC (105 - 130 cm).

Characterizations
The fresh soil samples were air-dried and ground. pH was measured in 0.0 1 M $CaCl_2$ solution. Organic matter (OM) content and soils chemical analysis were determined by the dichromate oxidation method. The available phosphorus (P) is the inorganic P, that is extractable at pH 8.5 and was determined following the experimental procedure described in Olsen et al., 1954 and Page et al., 1982. The total surface area (Sw) was measured by H_20 adsorption (Torres-Sanchez and Falasca, 1997). The total iron oxides (Fe_{tot}) and amorphous iron oxides (Fe_{amorph}) were established by dithionite (Holmgren, 1967) and oxalate method (McKeague, 1967), respectively. Soils samples were mixed with Lithium Metaborate/Lithium Tetraborate ($LiBO_2$, /$Li_2B_4O_7$) and fused in a furnace. The molten melt was completely dissolved in acidic media of 5 % nitric acid. This solution was analyzed for major and selected trace elements by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). The sample composition are reported as oxide percentage. The mineralogical composition and quantitative analysis of the soils were determined by X-ray Diffraction (XRD) and using the Rietveld method (Rietveld, 1969). Point of zero net proton charge (PZNPC) or point of zero salt effect (PZSE) is the pH where the net adsorption of protons and hydroxyl ions on the surfaces is independent of electrolyte concentration. Titration curves, when surface charge is plotted against pH, frequently showed a common intersect ion point that match with PZNPC.

Table 1. Characteristics of agriculture soils profile from Santiago del Estero/Argentina.

Horizon	pH ($CaCl_2$ 1:2.5)	OM ($g\ C.Kg^{-1}$)	P ($\mu g.g^{-1}$)	Sw ($m^2.g^{-1}$)	Fe_{amorph} ($mg.g^{-1}$)	Fe_{tot} ($mg.g^{-1}$)	PZNPC (pH)
Ap	5.90	23.30	43.34	188	0.239	1.66	7.1
AB	5.75	17.10	6.67	259	0.158	1.91	7.4
BC	6.02	12.10	1.19	242	0.095	0.99	8.1

Adsorption experiment

The adsorption of herbicide by the soils was studied using batch experiments. Solutions of different concentration of glyphosate was added to soil samples dispersions. Dispersions were kept in constant agitation overnight at constant pH, ionic strength and room temperature to reach equilibrium. The sample was filtered and adsorbed glyphosate was calculated from the difference between the total added ligand and the supernatant concentration (Ce). PMG was evaluated by ion chromatography. Two plastic anion columns were coupled in series to serve both as pre-column and analytical chromatographic column. The typical experimental error is lower than 5 % for all results.

Table 2. Chemical Analysis of agriculture soils profile from Santiago del Estero, Argentina.

Horizon	Chemical Analysis (%)						
	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O
Ap	64.6	12.25	3.51	1.47	1.14	1.70	2.43
AB	63.0	13.65	4.10	1.34	1.40	1.50	2.53
BC	63.0	14.05	4.42	1.44	1.62	1.56	2.66

pH effect

The pH dependence of the glyphosate uptake by soil horizons was investigated using batch isotherm experiments in a pH range from 2 to 8 with a soil concentration of 9.1 g/L and different initial concentrations of PMG at a constant ionic strength of 0. 1 M of KNO_3. The pH was measured using a Metrohm 644 pH-meter with a combined glass microelectrode. Adsorption experiments were conducted in triplicate following the procedure described above. There were no significant differences within each replicate ($p < 0.0\ 1$). The expressed values represent the average of the obtained results.

Isotherms Modeling

The relationship between the ligand uptake and the sorbate equilibrium concentration as constant temperature is known as the adsorption isotherm. The adsorbent capacity of a certain material is related to the material balance adsorption: the sorbate that disappears from solution must be in the adsorbent. Freundlich and Langmuir models were chosen and applied for describing the equilibrium data.

Table 3. Mineralogical Composition of agriculture soils profile from Santiago del Estero, Argentina. Values in parenthesis represent estimated standard deviations.

Horizon	Mineralogical Composition (%)				
	Quartz	Sanidine Feldspar	Andesine Feldspar	Illite	Magnetite
Ap	45.2 (0.4)	9.6 (0.9)	24.7 (0.8)	18.6 (1.4)	1.3 (0.2)
AB	39.8 (0.5)	9.6 (0.8)	23.5 (0.7)	25.9 (1.5)	1.2 (0.3)
BC	46.2 (0.4)	9.3 (0.9)	19.9 (0.9)	24.7 (1.3)	1.2 (0.3)

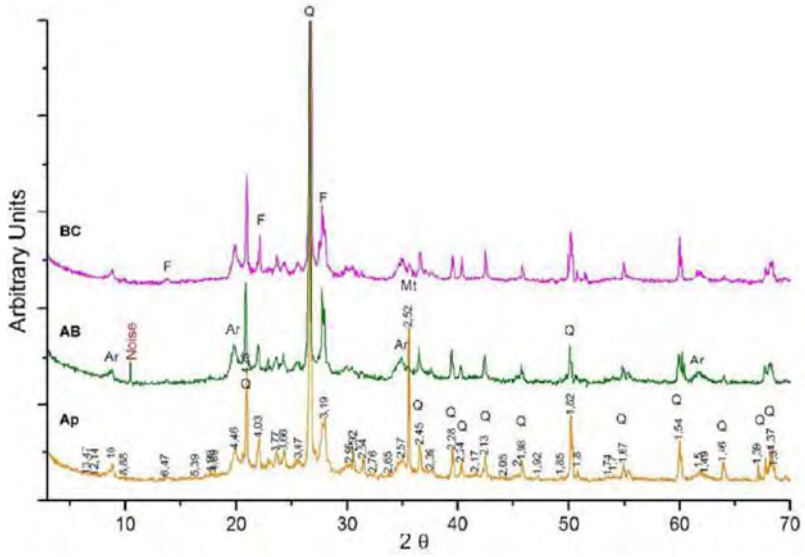

Fig. 1. XRD or the three soil horizons. Q: Quartz, Ar: Clay, F: Feldspar, Mt: Magnetite.

Results and Discussion

Soil characteristics, chemical analysis, mineralogical composition and quantitative analysis are presented in Table 1, 2 and 3 respectively. XRD of the three soil horizons are shown in Figure 1. The experimental curves of PZNPC recorded for the BC horizon are illustrated in Figure 2. Similar behavior was found for all the horizons that showed PZNPC values in the range of 7.1 - 8.1 (Table 1) following the sequence: Ap<AB<BC. PZNPC value can be explained by the absence of clay minerals with a negative permanent charge, while the presence of 2: 1 clays shift the PZNPC to lower pH values (Table 3). The higher PZNPC value for the horizons corresponds to horizon BC that contains similar amount of quartz, lower amount of feldspars (andesine) and high amount of illite. PZNPC increase with andesine feldspar content and OM decrease. The determination coefficients of a linear fit were $R^2_{andesine} = 0.9971$ and $R^2_{OM} = 0.9189$. The analysis of the three parameters variations in a 3D plot presented a determination coefficient of $R^2 = 1.0000$ and a constant variance test of $p < 0.0001$. The PMG adsorption isotherms of soils dispersions equilibrated at different pH values are shown in Figure 3. The Freundlich model parameters values (K_Γ , and l/n) were calculated and are given in Table 4. The 1/n values vary between 0.1 and 1, which indicates that this model could be used for interpreting the data. The correlation between experimental and calculated curves had a p-level between 0.137 and 0.0035; the determination coefficients were between 0.7578 and 0.9953 for different pHs and horizons.

The Langmuir model was also applied to make an interpretation of PMG adsorption isotherms on soil dispersions equilibrated at different pH values. This is shown in Figure 3, where solid lines are calculated using this model and Γ_{max} and K_L are given.

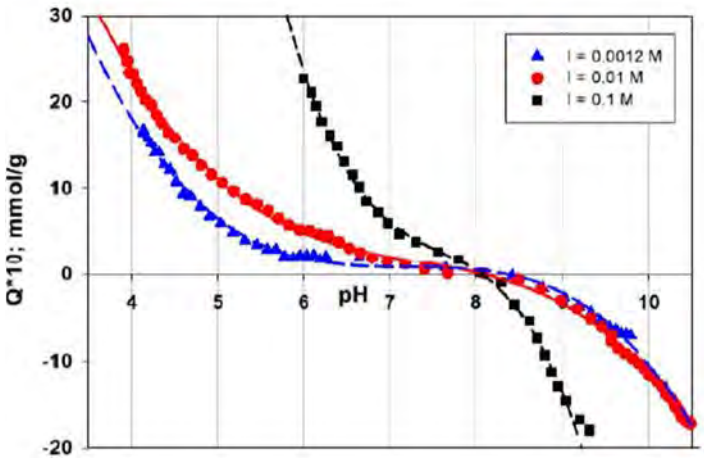

Fig. 2. Potentiometric titration curves or the dispersions or the BC horizon at three ionic strengths ($I = \frac{1}{2}\Sigma_i C_i Z_i$).

The isotherm model parameters were obtained by a non-linear optimization using the Solver-Excel tool. The parameters values were obtained from the plot of the inverse of the surface coverage as a function of the inverse of the equilibrium concentration. Results of the adsorption and surface coverage calculations were normalized with Sw data and the various horizons were contrasted. The correlation between experimental and calculated curves had a p-level between 0.050 and 0.001; the determination coefficients (R^2) obtained were between 0.9300 and 0.9999; and were higher than those obtained using the Freundlich model. Thus, the Langmuir model would better represent the adsorption process of PMG on the Santiago del Estero Province soil.

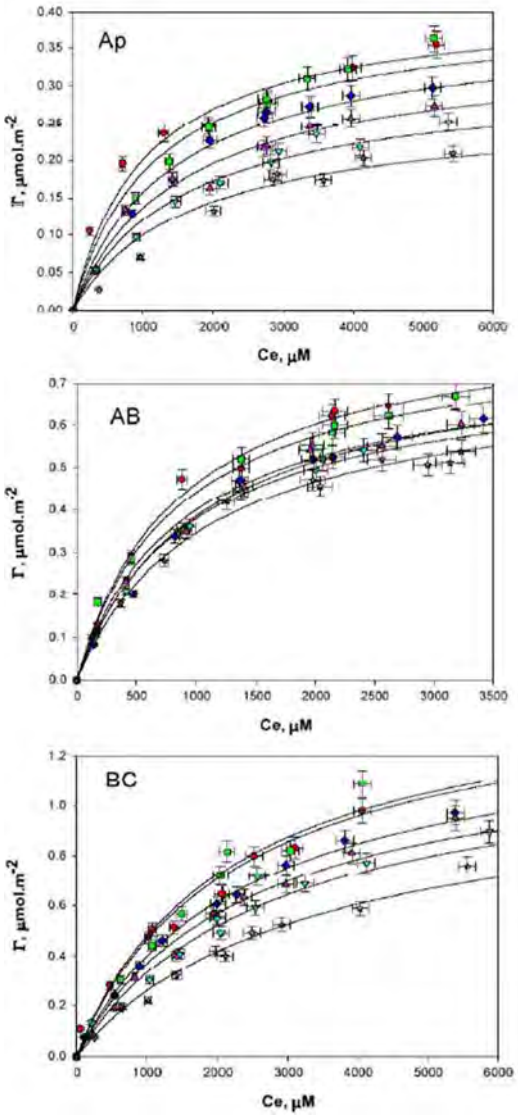

Fig. 3. Adsorption isotherm or PMG on horizon Ap, AB and BC. Solid lines are calculated using Langmuir model with constants and maximum surface coverage detaled in Table 5 . •: pH 2, •:pH 3, +: pH 4, : pH 5, pH 6 and *: pH 8.

The dependence of the surface coverage with PMG concentration in the various horizons at constant pH = 5 is shown in Figure 4. Horizon Γ_{max} sequence is Ap<AB<BC. This behavior is similar to those found for PZNPC. The dependence of the surface coverage with pH in the various horizons is also shown in Figure 3. The adsorption capacity increases from pH 8 to 2. This pH effect was normally observed during the adsorption of anionic species. Consequently, PMG interaction with the surface occurs throughout the anionic chemical groups (carboxylate or phosphonate) and not through the amine gr up (pKa = 10.14) that is positively charged at the studied pH range (Figure 5). The surface coverage decrease, $\Delta\Gamma_{max}$ for horizon Ap is around 41 % for this pHs range (Table 5). This difference is lower for

horizons BC, 27 % , and AB, 12 %. The highest adsorption capacity is obtained by horizon BC followed by horizon AB, and the lowest for horizon Ap. A similar sequence was obtained for PZNPC (Table 1), indicating that the horizon with higher positive surface charge presents higher PMG surface coverage. The ratio of the Γ_{max} of the horizons ($R_{H1/H2}$) was calculated where H1 and H2 denote two different horizons, Γ_{maxH1} and Γ_{maxH2} indicate the maximum coverage of H1 and H2 horizons, respectively. This ratio between the horizons BC and AB was $R_{BC/AB}$ = 46 %, between horizons BC and Ap was $R_{BC/AP}$ = 72 % and between horizon AB and Ap was $R_{AB/Ap}$ = 50 %. These percentages are opposed to the phosphate content that follows the order Ap > AB > BC. The highest adsorption constants correspond to horizon AB (Table 5). The changes in the adsorption affinity between horizon BC and AB reach ΔK_L = 46 % while horizon BC decreases 73 % in respect to horizon Ap.

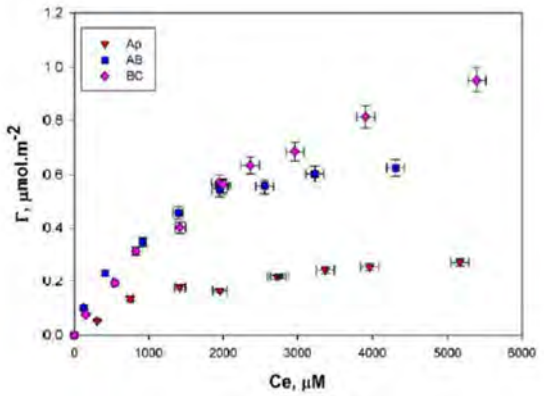

Fig. 4. Adsorption isotherm of PMG on horizon Ap, AB and BC at pH 5.

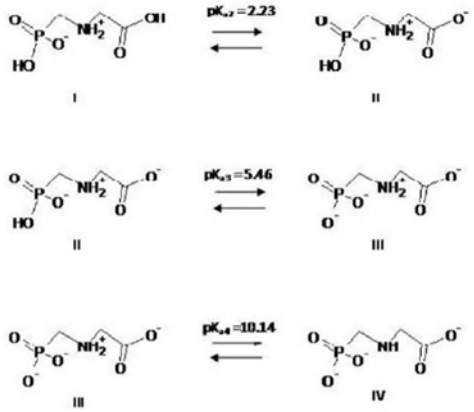

Fig. 5. PMG acid-base equilibrium.

The greater slope of the adsorption curves in the AB horizon indicate that PMG binds more strongly to the active sites of this horizon. Thus, the active site of PMG adsorption on the AB horizon could be the surface iron atoms and the higher adsorption in this horizon is directly related to higher iron content.
The adsorption on horizon BC does not reach maximum coverage in experimental conditions. The adsorption isotherms with a low initial slope describe an adsorption process with characteristic adsorption constants of low energy interaction (Figure 3). The constant and the equilibrium reactions of acid-base dissociation of glyphosate (Barja and dos-Santos-Afonso, 1998) are shown in Figure 5, where I, II and III are the main species presents in the studied pH range.

Table 4 . Freundlich parameters (in $\mu mol^{1-1/n}.m^{-2}$) for glyphosate adsorption on Santiago del Estero Province soils.

Horizon		Ap			AB			BC	
pH	K_F*10^3	1/n	R^2	K_F*10^3	1/n	R^2	K_F*10^3	1/n	R^2
2	7.3	0.46	0.9449	19.7	0.44	0.9743	5.3	0.64	0.9842
3	6.7	0.47	0.9953	18.4	0.45	0.9894	4.9	0.65	0.9759
4	5.9	0.47	0.9646	16.2	0.46	0.9654	4.7	0.63	0.9852
5	5.6	0.46	0.9729	16.2	0.45	0.9652	4.6	0.63	0.9892
6	5.0	0.48	0.7578	15.1	0.46	0.9862	4.3	0.62	0.9812
8	3.8	0.47	0.9453	14.3	0.45	0.9749	3.0	0.64	0.9893

Conclusions

The major factor in PMG adsorption on soil samples is given by the pH, which could be due to the influence of this parameter on the PMG molecule and on the surface charge of the soil particles. PMG adsorption increase with acidity, and this increase correspond to the adsorption of a ligand with a negative net charge. Sorption of glyphosate in soils is similar to the adsorption of the organic molecule on the soil components such as clay minerals, iron oxides and OM. For these soils with a low organic matter contents and/or similar amounts of clay in the various horizons, the adsorption would be determined by the content of phosphorous, iron oxide and the specific surface. Regarding the relative adsorption capacity of the soil, the adsorption process has a different behavior profile, where the deeper horizon (BC) has a higher capacity retention for this herbicide.

Table 5. Langmuir parameters for PMG adsorption on soils or Santiago del Estero Province, Argentina

Horizon		Ap			AB			BC	
pH	$\Gamma_{max.}$ $\mu mol.m^{-2}$	$K_{L.}$ $L.mmol^{-1}$	R^2	$\Gamma_{max.}$ $\mu mol.m^{-2}$	$K_{L.}$ $L.mmol^{-1}$	R^2	$\Gamma_{max.}$ $\mu mol.m^{-2}$	$K_{L.}$ $L.mmol^{-1}$	R^2
2	0.41	0.91	0.9572	0.81	0.93	0.9936	1.50	0.40	0.9331
3	0.40	0.87	0.9723	0.82	0.93	0.9833	1.49	0.42	0.9664
4	0.38	0.73	0.9874	0.78	0.92	0.9925	1.36	0.41	0.9955
5	0.34	0.67	0.9769	0.76	0.92	0.9932	1.27	0.40	0.9854
6	0.31	0.63	0.9633	0.74	0.91	0.9974	1.23	0.39	0.9858
8	0.24	0.61	0.9424	0.72	0.90	0.9926	1.10	0.28	0.9830

The lower adsorption in the AB and Ap horizons could be influenced by the higher content of phosphorus. However, the strength of the interaction, as given by the Langmuir Model Constant K_L is larger on horizon A B and would be linked to the illite and iron oxide content that have a better distribution in AB. It should be noted that the Langmuir adsorption model is the best fit to the adsorption experimental results in these soils, although the Freundlich model has a good fit for some pHs. Given the adsorption extent found in this study, it is expected that pesticides will be retained in these soils. This strong interaction could prevent the pesticides movement into the ground water. On the other hand, this retention rate could result in the release of the herbicide on the environment due to displacement by runoff.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes the adsorption of non-labelled glyphosate on an agricultural soil from Argentina. The pH-dependency was evaluated in addition. The soil and climate conditions do not reflect European conditions. However, the soil characterization and the results for adsorption, Freundlich isotherm and pH dependency are well described.

The study is therefore classified as relevant with restrictions (Category 2).

1. Information on the study

Data point:	KCA 7.1.2.2.1
Report author	Todorovic, G., et al.
Report year	2014
Report title	Influence of soil tillage and erosion on the dispersion of glyphosate and aminomethylphosphonic acid in agricultural soils
Document No	International Agrophysics, 2014, 28, 93-100
Guidelines followed in study	None
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (study design not sufficiently described to relate results to real field conditions, details on analytical method and statistics are missing)

2. Full summary of the study according to OECD format

Erosion processes can strongly influence the dissipation of glyphosate and aminomethylphosphonic acid applied with Roundup Max® in agricultural soils; in addition, the soil structure state shortly before erosive precipitations fall can be a key parameter for the distribution of glyphosate and its metabolite. Field rain simulation experiments showed that severe erosion processes immediately after application of Roundup Max® can lead to serious unexpected glyphosate loss even in soils with a high presumed adsorption like the Cambisols, if their structure is unfavourable. In one of the no-tillage-plot of the Cambisol, up to 47% of the applied glyphosate amount was dissipated with surface run-off. Moreover, at the Chernozem site with high erosion risk and lower adsorption potential, glyphosate could be found in collected percolation water transported far outside the 2x2 m experimental plots. Traces of glyphosate were found also outside the treated agricultural fields.

Materials and Methods

The experiments were carried out where following soil tillage systems were compared in 3 field replications:
– conventional tillage (CT) with plough with and without cover crop during winter period;
– no-tillage (NT) with cover crop during winter period.
The investigated soils were a Chernozem from loess at Pixendorf and a sandy stagnic Cambisol from tertiary carbonate free sediments at Kirchberg, Austria. In order to investigate the influence of erosion and tillage on glyphosate and AMPA, two rain simulation experiments were conducted in 3 field replications (1, 2, 3) within the CT and NT plots. For this, Roundup Max® was applied onto rain simulation soil plots according to the common agricultural practice (2% herbicide solution. In both sites, the vegetation cover degree was typically higher in the NT-plots (80-100% of weed cover) than in the CT-plots (only few yield residues of maize) and the application was carried out in sunny and not windy weather shortly before starting the rain simulation experiment (worst case scenario). The average slope in both sites was 12-15% at the Cambisol and 10% at the Chernozem. Both sites are known as rather erodible. The soil surface of the Chernozem immediately before the rain simulation was crumby; in turn, the cambisol had a crusted, dry, and cracky surface. The rain simulator was designed as a potable equipment, the spray pattern was generated by full jet nozzles, the rain fall intensity was controlled with intermittent spraying.

During 60 min of rain simulation with 30 mm, run-off fractions were collected at different time intervals at the Chernozem and averagely at the Cambisol and cooled in boxes. In the laboratory, the run-off samples were immediately centrifuged to separate the liquid from the solid phase. Immediately after the rain simulation, soil samples were collected within the simulation soil plots of 2x2 m at different depths

(0-2, 2-5, 5-10, 10-15, and 15-20 cm at the Chernozem and at 0-2 and 2-5 cm at the Cambisol). Glyphosate and AMPA were analyzed according to Rampazzo *et al.*, 2013. All physical and chemical analyses on soil samples were carried out according to the standard methods.

Table 1. Fe-oxide distribution in the investigated soils

Site	Soil type (WRB)	Depth (cm)	Fe_o (mg kg^{-1})	Fe_d (mg kg^{-1})	Fe_o/Fe_d
Pixendorf	Chernozem	0-5	983	7 970	0.12
		5-20	1 040	8 378	0.12
Kirchberg	Cambisol	0-5	3 422	14 843	0.23
		5-20	3 726	15 032	0.25

Fe_o – amorphous (weakly crystallized) Fe-oxides, oxalate-soluble; Fe_d – well crystallized Fe-oxides, dithionite-soluble.

Results and Discussion

The Chernozem shows the development from loess with typical silty texture (topsoil 0-20 cm, 12% clay, 65% silt, 23% sand, pH 7.3, 15% $CaCO_3$ and 3% OM), whereas the Cambisol is a loamy sandy soil (topsoil 0-20 cm, 14% clay, 33% silt, 53% sand, pH 5.7, no $CaCO_3$ and 3% OM). The Chernozem exhibited a low content and the Cambisol a high content of Fe oxides and therefore the expected sorption capacity for glyphosate and AMPA was theoretically higher at the Cambisol (Table 1).
Figure 1 shows the amount of total (liquid and solid) run-off after the rain simulation experiments on the Chernozem. Before glyphosate and AMPA were analyzed, a separation of the solid and liquid run-off phase in the laboratory was carried out. The CT-plots produced the highest run-off amounts because of their lower protecting weed cover, causing a splash of the surface by the erosive precipitation with consequent loss of infiltrability. On the other hand, the amount of runoff at the Chernozem was 10 times lower than the Cambisol because of its crumby structure with a better infiltration rate during the rainfall simulation, whereas the soil surface of the Cambisol was compacted and crusty. The different amounts of run-off between the 3 field replications of the Chernozem (Fig. 1) were due to the inhomogeneity of the field conditions. Consequently, the total amount of glyphosate washed out of the plots by liquid run-off at the Chernozem was much higher in the CT-plots than in the NT-plots (Fig. 2).

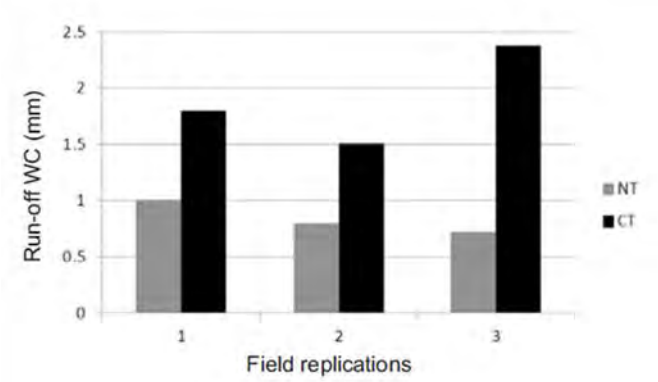

Fig. 1. Chernozem: total run-off of the conventional tillage (CT) and no-tillage (NT) plots in the 3 field replications, WC – water column.

A fractionation of the time-dependent glyphosate contents in run-off-fractions of the Chernozem at time intervals of 15 min is shown in Fig. 3a. As it was expected, the first fraction showed the highest contents in both variables CT and NT and then decreasing with time. The CT-plots showed again higher glyphosate contents than the NT-plots, which instead showed higher glyphosate concentration (less dilution) at the same time (Fig. 3b).

According to Gjettermann *et al.,* 2011, desorption kinetics are important for evaluating the significance of dissolved and particle-facilitated transport of glyphosate. Consequently, the separation from water and solid phases should be done within a short time of minutes. We managed to do this within 30 min from field sampling. The contents of glyphosate and AMPA in the solid phase of run-off in the Chernozem are shown in Figs 3c,d. The glyphosate contents retained by the run-off sediment is an analogue to that in the total and fractionated runoff (Figs 2, 3a), where the first collected fraction of run-off sediment contains the highest amounts of glyphosate which then generally decreases in the following fractions and the CT-plots shows higher amounts than the NT-plots. Analogous is the distribution of AMPA in the sediment (Fig. 3d). Since the loss of glyphosate by run-off was higher in the CT-plots (Fig. 3a), the amount of glyphosate and AMPA adsorbed by the Chernozem immediately after the rain simulation experiments was consequently higher in the NT-plots (Fig. 4). Moreover, there is a clear depth function of the adsorption of glyphosate and AMPA through the soil immediately after Roundup Max® application and rainfall simulation at the Chernozem. The glyphosate and AMPA contents clearly decreased with soil depth.

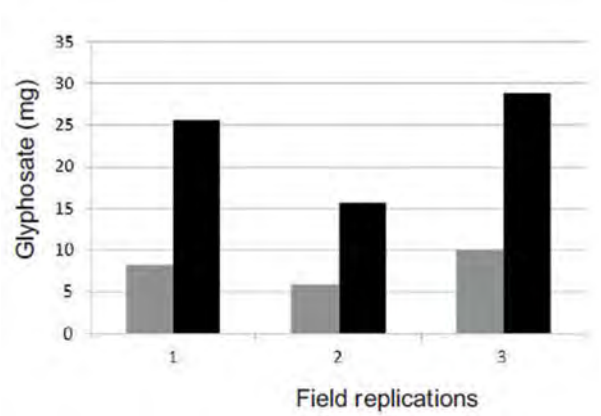

Fig. 2. Chernozem: total amounts of glyphosate in liquid run-off at the 3 field replication plots. Legend as in Fig. 1.

The Chernozem had a favourable crumby structure in the NT-plots, with no cracks, no preferential flow, and optimal conditions for water retention in the upper soil layers at the moment of the rainfall simulation experiment, so that more than 50% of the adsorbed glyphosate was retained in the first 5 cm of the soil. The fact that AMPA could already be detected 1 h after the Roundup Max® application underlines the quick glyphosate degradation in soil, as reported by Mamy *et al.*(2005) as well. The total (liquid and solid) amount of surface run-off in the Cambisol is shown in Fig. 5. The Cambisol had a dry, crusty, and very deeply cracky soil surface of the CT-plots before starting the rainfall simulation and therefore the first amount of the precipitation quickly infiltrated in the cracks, but very soon a splash process and loss of infiltration took place due to the fine sandy texture and low surface protection by weeds. This led to a higher surface run-off of the CT-plots than the NT-plots. Consequently, Figs 6 and 7a show that the contents and concentrations of glyphosate in the liquid run-off of the NT-plots of the Cambisol were much higher than in the CT-plots. In the dry and cracky soil surface of the CT-plots, it took some time before run-off started and glyphosate could easily enter deeper into the soil; on the other hand, the NT-plots had a nearly 100% weed cover, as reported also by Locke and Bryson (1997); consequently, this might buffer potential effects of glyphosate in the soil (Locke *et al.,* 2008).
In this study, most of the applied glyphosate adhered to the photosynthetically active plant organs (stem and leaves) immediately after application; consequently, glyphosate was literally washed out of the 2x2 m simulation plots with runoff and had less time to infiltrate the soil surface (Fig. 8a). Based on the high content of pedogenical Fe-oxides (15 000 mg Fe_d/kg, Table 1), high soil adsorption of glyphosate was expected for the Cambisol. The surprisingly high loss of glyphosate by surface run-off (in one of the 3 field replications about 47% of the applied glyphosate) measured in this study confirmed the crucial effect of soil structure and preferential flow on the dissipation of glyphosate after heavy erosive precipitations, which were also be observed by other scientists. The contents of glyphosate and AMPA in the solid phase of run-off at the Cambisol are shown in Figs 7b, c, respectively. The concentrations of glyphosate and AMPA in the solid phase of run-off at the Cambisol are similarly distributed to the

corresponding aqueous fractions of run-off; they are mostly higher in the NT-plots than in the CT-plots. Figure 8 shows the content of glyphosate and AMPA adsorbed by the soil immediately after the rain simulation experiments at the Cambisol. Immediately after the rain simulation experiment, a very clear distribution in the soil appears: glyphosate and AMPA are first adsorbed in the upper 0-2 cm of the soil and only a small amount reaches the next soil depth of 2-5 cm. In general, the NT-plots show a clearly lower content of glyphosate and AMPA as compared to the CT-plots. This is explained by the respectively higher glyphosate contents in run-off of NT-plots (Fig. 6). The soil losses of the Chernozem and Cambisol through erosion processes are shown in Fig. 9.

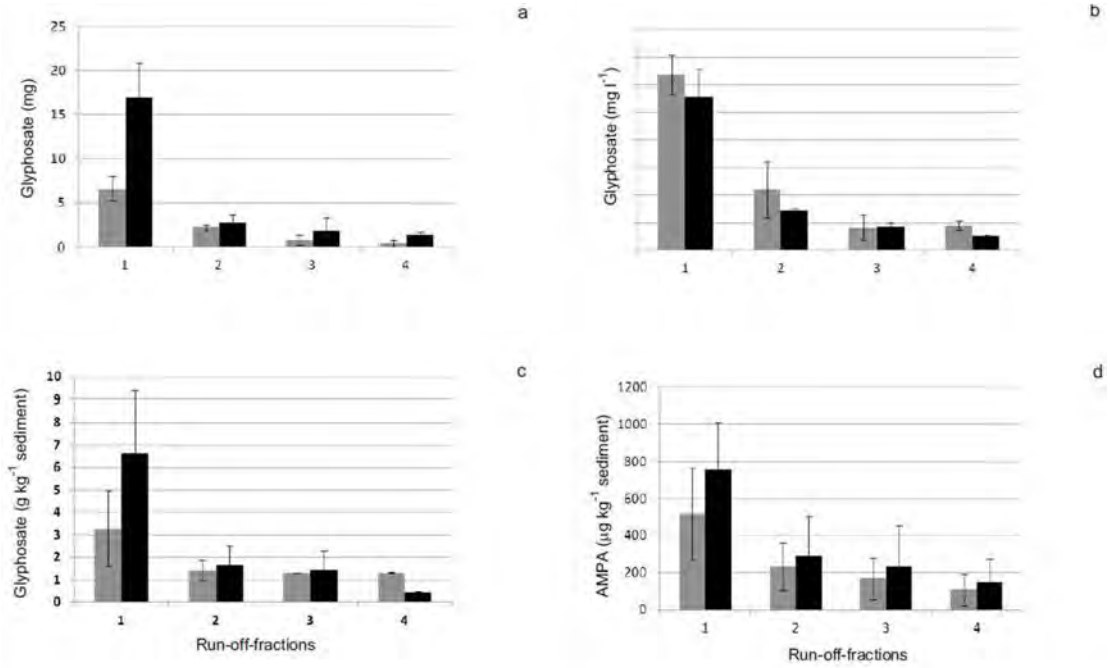

Fig. 3. Chernozem: a – glyphosate amount, b – glyphosate concentrations in liquid, and c – glyphosate contents, d –AMPA contents in the solid phase of run-off-fractions at 15-min intervals (average of 3 field replications). Legend as in Fig. 1.

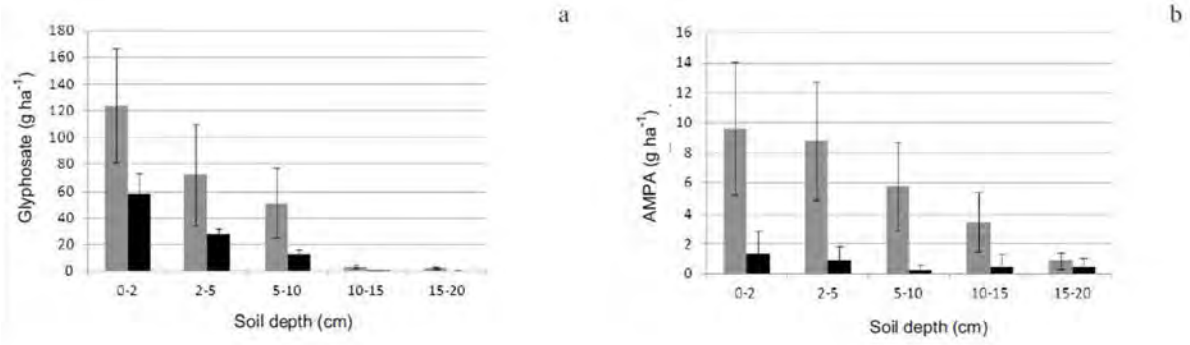

Fig. 4. Chernozem: a – glyphosate contents, and b – AMPA contents in the soil within the rain simulation plots (average value from the 3 field replications). Legend as in Fig. 1.

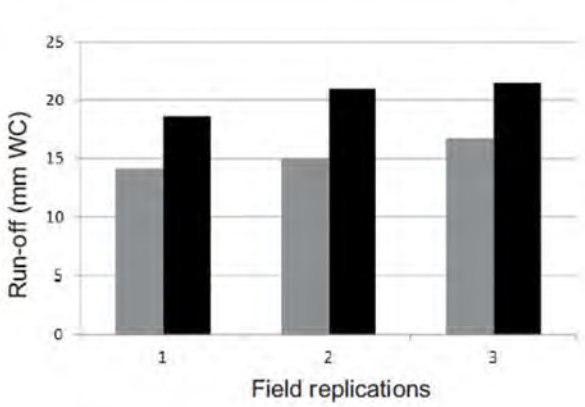

Fig. 5. Cambisol: total run-off of the CT- and NT-plots in the 3 field replications, mm WC – mm water column. Legend as in Fig. 1.

At both sites, the soil loss from the CT-plots, measured as sediment in the surface run-off, was higher than from the NT-plots because of the much lower vegetation cover before the simulation experiment, splash, and reduction of infiltration. The loss of the Cambisol soil was 10 times higher than that of the Chernozem. The reason for this is that the two experimented soils had a completely different soil structure and surface conditions before starting the rain simulation. The Chernozem had a very friable, crumby, permeable structure after the wheat yield. The Cambisol stood right after the corn yield, the soil surface was crusty and less permeable, except for shrinking cracks which swelled during the experiment.

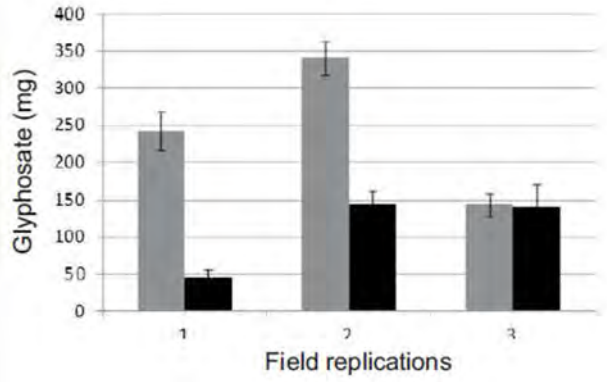

Fig. 6. Cambisol: total amounts of glyphosate in liquid run-off at the 3 field replication plots. Legend as in Fig. 1.

The Chernozem at Pixendorf and surroundings is generally known as a location with high erosion risk because of the high silt amount (> 60 mass %) and especially with corn crop, where deep gully erosion forms. The erosion rills discharge downslope to an artificial run-off retention basin at the footslope of the experimental field. This basin can run over and flow downwards on different paths and is collected through further toeslope retention basins. Water samples from both retention basins were analyzed and traces of glyphosate, which were surely not connected with rain simulation experiments, were found (Fig. 10). Moreover, soil percolation water samples at the footslope of the experimental field were collected at two depths from previously installed stations and analyzed for glyphosate and AMPA. Since the rainfall simulation experiment was conducted at the topslope whereas the percolation water samples were collected at the footslope (100 m distance) at the same time, it seems unlikely that the measured amounts of glyphosate and AMPA were influenced by the rain simulation, but are probably residual amounts from previous field application, confirming the possibility of dissipation through natural processes.

The results show that small amounts of glyphosate and its metabolite can dissipate through soil percolation, mainly depending on the physico-chemical adsorption and structural properties of soils, and

were found in collected soil solutions at two different depths far out of the 2x2 m precipitation plots (Fig. 11).

Conclusions

1. The rain simulation experiments clearly showed that even in a potentially high glyphosate adsorbing soil like the Cambisol, erosion and surface run-off can lead to severe glyphosate loss if the soil structure state *eg* compaction degree, crusting, infiltrability, pore size distribution, in the case of erosive precipitations shortly after Roundup Max® application, is unfavourable. In this study, in one of the NT-plot repetitions, up to 47% of the applied glyphosate amount were dispersed with run-off.
2. Traces of glyphosate in collected percolation soil water at Pixendorf, probably from previous conventional field application of Roundup Max®, confirmed the general low glyphosate adsorption capacity of Chernozems from Loess and the risk of transport towards groundwater.
3. Analysis of water from run-off retention basins in the landscape in the surroundings of the investigated Chernozem confirmed that through high erosion processes, especially in maize crop, glyphosate is partly transported outside the treated agricultural fields.

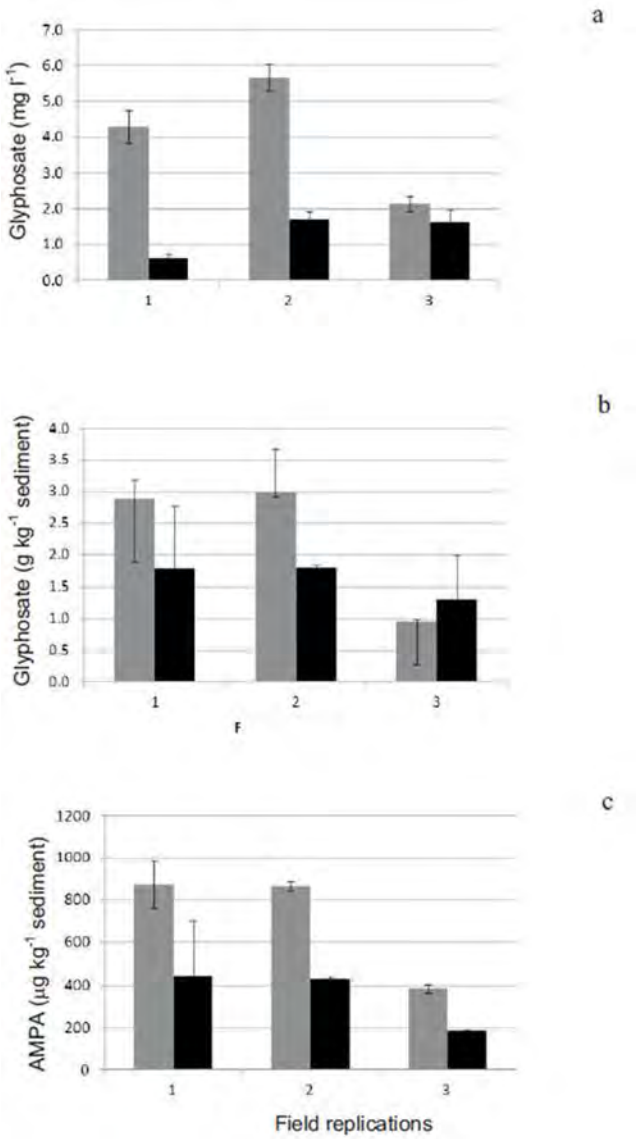

Fig. 7. Cambisol: a – glyphosate concentrations in liquid, b – glyphosate, and c –AMPA contents in the solid phase of run-off at the 3 field replications (average of the 60 min rain simulation). Legend as in Fig. 1.

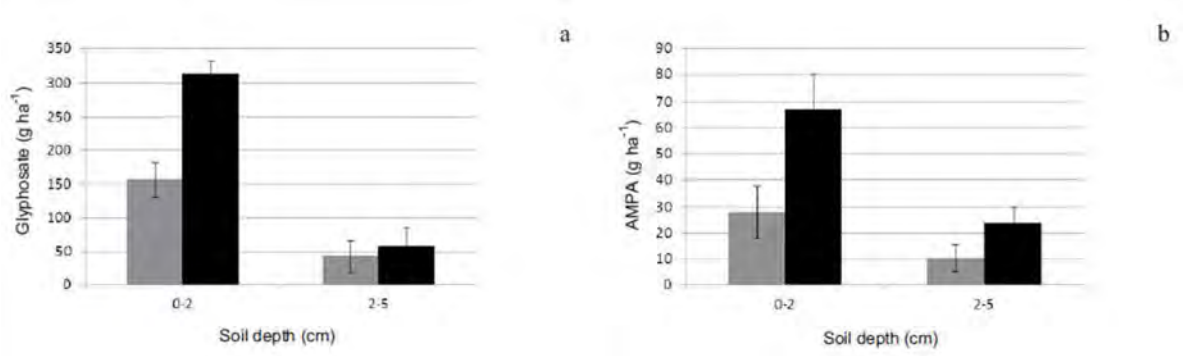

Fig. 8. Cambisol: a – glyphosate and b – AMPA contents in the soil within the rain simulation plots (average value from the 3 field replications). Legend as in Fig. 1.

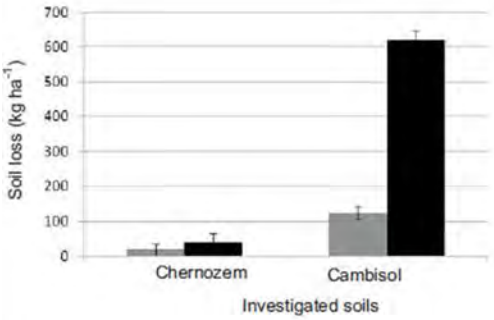

Fig. 9. Total soil loss of the investigated soils after the rain simulation experiments (averages of 3 field replications). Legend as in Fig. 1.

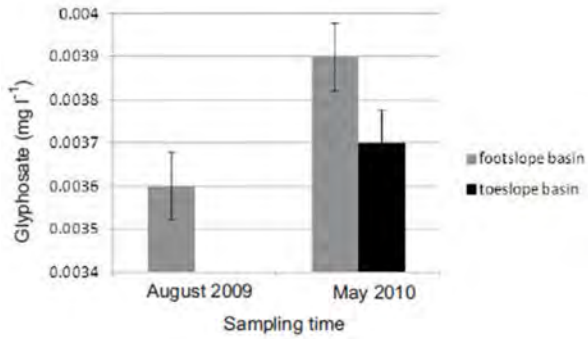

Fig. 10. Glyphosate concentrations in natural run-off retention basins outside the experimental fields.

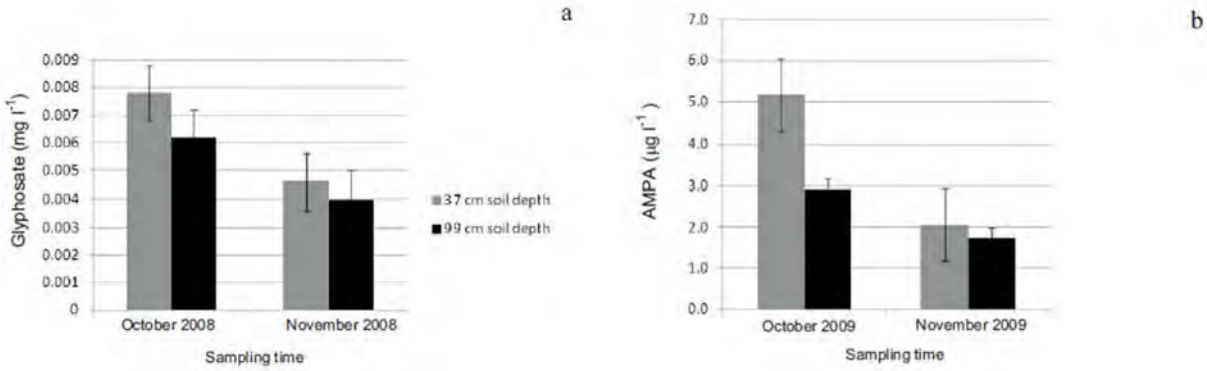

Fig. 11. Concentrations of: a – glyphosate, and b – AMPA in percolation water at 2 different times and soil depths.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes the runoff behavior of glyphosate and AMPA in two field experiments in two different European agricultural soils with artificial rainfall. No details on the description of the analytical method and of statistical analysis are provided.
The study is therefore classified as relevant to the data requirement but only as supplementary information (Category 2).

1. Information on the study

Data point:	KCA 7.1.4.3
Report author	Ulen, B., et al.
Report year	2014
Report title	Spatial variation in herbicide leaching from a marine clay soil via subsurface drains
Document No	Pest Management Science (2014)
Guidelines followed in study	None
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (essential parameters to derive endpoint missing)

2. Full summary of the study according to OECD format

BACKGROUND: Subsurface transport via tile drains can significantly contribute to pesticide contamination of surface waters. The spatial variation in subsurface leaching of normally applied herbicides was examined together with phosphorus losses in 24 experimental plots with water sampled flow-proportionally. The study site was a flat, tile-drained area with 60% marine clay in the topsoil in southeast Sweden. The objectives were to quantify the leaching of frequently used herbicides from a tile drained cracking clay soil and to evaluate the variation in leaching within the experimental area and relate this to topsoil management practices (tillage method and structure liming).
RESULTS: In summer 2009, 0.14, 0.22 and 1.62%, respectively, of simultaneously applied amounts of MCPA, fluroxypyr and clopyralid were leached by heavy rain five days after spraying. In summer 2011, on average 0.70% of applied bentazone was leached by short bursts of intensive rain 12 days after application. Peak flow concentrations for 50% of the treated area for MCPA and 33% for bentazone exceeded the Swedish no-effect guideline values for aquatic ecosystems. Approximately 0.08% of the glyphosate applied was leached in dissolved form in the winters of 2008/2009 and 2010/2011. Based on measurements of glyphosate in particulate form, total glyphosate losses were twice as high (0.16%) in the second winter. The spatial inter-plot variation was large (72–115%) for all five herbicides studied, despite small variations (25%) in water discharge.
CONCLUSIONS: The study shows the importance of local scale soil transport properties for herbicide leaching in cracking clay soils.

Materials and Methods

The field site is located in a flat valley with a clay soil of marine origin in eastern Sweden. The experimental field was tile-drained in 2006 to 0.9 m depth. Twenty-four of these plots were used in the present experiment. The plots are situated in two rows of 14 plots at varying distance from an open ditch that acts as the recipient of drainage water from the surrounding valley. Three management practices were randomly assigned to the plots: Conventional autumn ploughing, shallow autumn tillage and structure-liming (i.e. liming carried out to reduce phosphorus leaching and to improve crop yield by improving soil structure). Soil pH and total organic carbon (OC) content are given in Table 1. There were no significant differences ($P > 0.05$) in soil pH and OC between treatments.

Table 1

Mean values and standard deviations (SD) of soil pH and concentrations (%) of organic carbon (OC) at the start of the project in the autumn of 2007 and five years later in the spring of 2012 after repeated different tillage treatments and after structure liming first year

			Shallow tillage		Structure-limed		Conventional ploughing	
Sampling time	Property	Depth (cm)	Mean	SD	Mean	SD	Mean	SD
2007	pH	0–2	6.4	0.2	6.3	0.1	6.4	0.1
2012	pH	0–2	6.1	0.2	6.5	0.5	6.1	0.1
2007	OC (%)	0–23	2.4	0.5	2.5	0.5	2.5	0.5
2007	OC (%)	23–60	1.4	0.3	1.5	0.4	1.5	0.3
2007	OC (%)	60–90	0.6	0.1	0.6	0.1	0.6	0.1
2012	OC (%)	0–2	2.7	0.2	2.7	0.3	2.6	0.3

Pesticide leaching

We studied the leaching of seven different pesticides with contrasting properties (Table 2). It should be noted that both pesticide half-lives and adsorption partitioning coefficients are dependent on soil properties and the values presented in Table 2 may, therefore, not be representative for the clay soil at this site. Pesticide leaching was studied in two different crop rotations (Table 3), both with oats and peas during the last two years (2010–2011). In crop rotation I (20 plots), conventional autumn ploughing was compared with shallow autumn tillage and the effects of previous structure-liming in autumn 2007 were examined. Glyphosate was applied before sowing in spring 2008 to control couchgrass in eight shallow-tilled plots in crop rotation I (Table 4). In early summer the same year, the low-dose substances thifensulfuron-methyl and tribenuron-methyl were applied in both rotations. In autumn 2008 glyphosate was applied after harvest (four plots) in crop rotation II in order to control couchgrass and volunteer cereals. The three pesticides clopyralid, fluroxypyr and MCPA (all ingredients in the same commercial product) were sprayed for weed control on 9 June 2009 in crop rotation I (20 plots) and on 23 June 2010 in both rotations (24 plots). Glyphosate was applied after harvest in September 2010 and bentazone was applied on 11 June 2011 in both rotations (24 plots). Most applications were made in the evening, with no wind and always in the recommended dose. The total loads of herbicides applied (Table 4) were similar to those reported from agricultural catchments within the Swedish National Pesticide Monitoring Programme. Precipitation was measured at the site with unheated tilting bucket equipment and collected in a data logger.

Table 2

Herbicide properties and leaching potential data taken from the Pesticide Properties Database (PPDB, 2010)[11] Substance

DT_{50}lab[a](days)	DT_{50}field[b](days)	K_{oc}[c] ($cm^3\,g^{-1}$)	GUS[d]	pK_a[e]
Bentazone	13	14	55.3	2.30	3.28
MCPA	24	25	74[f]	2.94	3.73
Fluroxypyr	1	51	195[f]	0	2.94
Clopyralid	34	11	5.0	5.06	2.01
Glyphosate	12	12	1435	−0.49	2.34
Thifensulfuron-methyl	4	4	28.3	1.53	4.4
Tribenuron-methyl	14	14	35	2.88	4.7

[a] Degradation half-life for aerobic conditions measured in the laboratory.
[b] Degradation half-life for aerobic conditions measured in the field.
[c] Adsorption distribution coefficient to organic carbon.
[d] Groundwater ubiquity score.
[e] Acid dissociation constant.
[f] Freundlich adsorption coefficient to organic carbon.

Table 3

Year, crop, date and commercial brand name of herbicides applied in 2008–2011 in crop rotations I and II (number of conventionally ploughed plots/total number of treated plots)

Year	Rotation I Crop	Date	Herbicide (16/20 plots)	Rotation II Crop	Date	Herbicide (4/4 plots)
2008	Spring barley	24/4	Glypro Bio[a, b]	Winter wheat	26/6	Harmony 50T Plus[c]
	Spring barley	26/6	Harmony 50T Plus[c]	After W wheat	16/8	Glypro Bio[b]
2009	Spring barley	9/6	Ariane S[d]	Winter wheat	6/5	Harmony 50T Plus[c]
2010	Oats	23/6	Ariane S[d]	Oats	23/6	Ariane S[d]
	Oats	22/9	Glypro Bio[b]	After oats	22/9	Glypro Bio[b]
2011	Pea	11/6	Basagran[e]	Pea	11/6	Basagran[e]

[a] Only in eight shallow-tilled plots.
[b] Active ingredient glyphosate (49%).
[c] Active ingredients thifensulfuron-methyl (37%) and tribenuron-methyl (17%).
[d] Active ingredients MCPA (20%), fluroxypyr (4%) and clopyralid (2%).
[e] Active ingredient bentazone (87%).

Table 4

Year, date of application, substance analysed in drainage water, crop and applied dose of detected substance, together with the general dose (in g ha^{-1}) applied in Swedish monitored small catchments in 2008–2011

Year	Date	Substance	Crop	Dose (g ha^{-1})	General dose (g ha^{-1})
2008	24/4	Glyphosate	Before barley	707	748
2008	26/4	Thifensulfuron-methyl	Spring barley	4	6
		Tribenuron-methyl	Spring barley	2	3
2008	16/8	Glyphosate	After winter wheat	1060	1116
2009	26/5	Thifensulfuron-methyl	Winter wheat	6	6
		Tribenuron-methyl	Winter wheat	3	3
2009	9/6	Clopyralid	Spring barley	52	48
		Fluroxypyr	Spring barley	104	81
		MCPA	Spring barley	520	590
2010	23/6	Fluroxypyr	Oats	104	75
		MCPA	Oats	520	510
		Glyphosate	After oats	1060	1110
2011	11/6	Bentazone	Pea	475	500

Water sampling and analysis

Water discharge from each plot was measured with tilting vessels in an underground basement where sampling of drainage water also took place. The water was sampled flow-proportionally, with every subsample representing 0.003 mm discharge in summer and 0.04 mm discharge in the rest of the year. The bulk samples were collected weekly (or for the first flow events following application more frequently). The concentration of thifensulfuron-methyl and tribenuronmethyl (in 2008) was determined with solid-phase extraction followed by liquid chromatography and mass spectrometry (LCMS) and the concentration of clopyralid, fluroxypyr and MCPA (in 2009) by the same solid-phase extraction and by derivatisation and gas chromatography/mass spectrometry (GC/MS). Fluroxypyr and MCPA (in 2010) and bentazone (in 2011) were analysed by mass spectrometric determination (LC-MS/MS). Dissolved glyphosate (DissGly) and its main metabolite AMPA were analysed in winter 2008/2009 and 2010/2011, which involved ion exchange and derivatisation, followed by final identification and quantification by GC/MS. In winter 2010/2011, glyphosate analysis included particulate glyphosate (PartGly), which was trapped using a cellulose acetate filter with pore size 0.45 μm.

Table 5

Monthly precipitation (Prec) and total snow accumulation (Snow acc) in winter periods (October–April current year and January–April following year), water discharge (Flow) and ratio Flow/Prec for the experimental years and long-term (1988–2011) average

Year		May	June	July	August	September	October–April	Snow acc (cm)
2008	Prec (mm)	30	5	44	42	8	385	14
	Flow (mm)	10	1	0	4	5	395	
	Flow/Prec	0.33	0.20	0	0.03	0.63	0.99	
2009	Prec (mm)	45	95[a]	94	54	35	384	50
	Flow /mm)	3	43	3	1	0	370	
	Flow/Prec	0.07	0.45	0.03	0.02	0	0.85	
2010	Prec (mm)	53	39	155[b]	95	51	290	75
	Flow (mm)	16	3	8	87	42	310	
	Flow/Prec	0.30	0.08	0.05	0.92	0.82	0.85	
2011	Prec (mm)	40	70	26	138	72	358	3
	Flow (mm)	3	14	0	0	4	350	
	Flow/Prec	0.08	0.20	0	0	0.06	0.96	
1988–2011	Prec (mm)	40	67	82	69	63	338	32
	Flow (mm)	7	12	2	18	10	360	
	Flow/Prec	0.15	0.18	0.02	0.21	0.16	0.97	

[a] Maximum intensity 46 mm day^{-1} in the middle of the month.
[b] Maximum intensity 79 mm day^{-1} at the end of the month.

Results and Discussion

Concentrations of pesticides in drain water

The sulphonylureas (thifensulfuron-methyl and tribenuronmethyl) were not detected above LOD in 2008. Because of the fast dissipation of these substances, they were not analysed for in subsequent years. Unlike these low-dose substances, detectable levels of all other herbicides were found every year in drain flow in the first 1–2 months after early summer application. Detectable concentrations of fluroxypyr and MCPA were also observed 31 days after application (Table 6) in the samples taken after flooding of the measuring station. Detection of pesticides in the first few rainfall/drainage events after application is consistent with the flow was the simultaneous arrival of clopyralid and fluroxypyr on 14–16 June 2009, despite large differences in K_{oc} values (Table 2). However, since only five days had passed between application and rainfall, the substances might not have been in equilibrium with the soil solid material due to slow kinetics. Dissolved glyphosate was detected in consecutive events in autumn 2008. Both particle-bound glyphosate and dissolved glyphosate were detected in the discharge from all fast-flow events in autumn 2010. Levels above the $C_{\text{no effect}}$ concentrations were observed in 50% of the plots for MCPA and in 33% for bentazone (Table 6). Levels of glyphosate, AMPA, clopyralid and fluroxypyr were on all occasions below their $C_{\text{no effect}}$ concentrations. The coefficient of variation in the most important leaching event for the substances studied varied between 72 and 115% between all different plots (including different treatments) and increased in the order bentazone < clopyralid < fluroxypyr < PartGly < MCPA < DissGly. These highly variable pesticide concentrations were not significantly correlated to the basic soil factors pH value, clay content and organic matter content in the topsoil, which only showed minor variance (2, 17 and 10%, respectively).

Table 6

Year, date of application of substance (including glyphosate metabolite AMPA) and glyphosate in dissolved (diss) form and total glyphosate, numbers of plots (Plots), number of days (No. days) until major rain event, Swedish guideline values for no effect ($C_{no\ effect}$), maximum (Max) and mean concentration in the main drainage event, ratio of number of plots with concentration exceeding $C_{no\ effect}$ to total number of plots treated (Ratio $C_{no\ effect}$) and total period (days) after application when values exceeding $C_{no\ effect}$ were detected

Year	Date	Substance	No. days	$C_{no\ effect}$ (µg L^{-1})	Maximum (µg L^{-1})	Mean (µg L^{-1})	Ratio $C_{no\ effect}$	Period (days)
2008	16/8	Glyphosate diss.	47	100	1.2	0.48	0/4	—
		AMPA	47	500	0.3	—	—	—
2009	9/6	Clopyralid	5	50	5.5	2.2	0/20	—
		Fluroxypyr	5	100	1.7	0.67	0/20	—
		MCPA	5	1	5.5	2.0	10/20	5–14
2010	23/6	Fluroxypyr[a]	31	100	0.3	0.081	0/24	—
		MCPA[b]	31	1	0.04	0.007	0/24	—
2010	22/9	Glyphosate diss.	33	100	3.9	0.58	0/24	—
		Glyphosate total	33	100	9.4	2.2	0/24	—
		AMPA	33	500	0.7	—	—	—
2011	11/6	Bentazone	12	30	63	23.9	8/24	12–16

[a] Generally only analysed in dissolved form.
[b] Late collection of sample, as the measuring station was flooded.

Leaching losses of pesticides

The amount of pesticide leached in summer periods from conventionally ploughed plots sprayed simultaneously with the same herbicide in 2009–2011 varied between 0.2 and 3.3 g/ha (0.1–1.6% of amount applied) (Table 7). Leaching losses above 1% are generally associated with large rainfall amounts shortly after application. However, for our case the hydrological conditions did not represent 'worst-case' leaching conditions and hence the large leaching losses demonstrate the great potential for preferential transport in this soil. Losses exceeding 0.1% took place from 22 to 24 plots (92–100% of the experimental area) for clopyralid and bentazone, while the relative losses of MCPA exceeding 0.1% represented 42% of the area. The relative leaching losses of the substances studied here are presented in Table 7. Surprisingly, autumn application of glyphosate in 2008 and 2010 resulted in quite similar losses in dissolved form in the following winters (0.9 g/ha corresponding to 0.08% of applied amounts; Table 7), irrespective of whether the main discharge took place after autumn rain followed by a mild winter (2008) or in connection with snowmelt after a cold winter with continuous snow cover (2011). Due to slow degradation during the winter of 2010/2011 owing to long-lasting snow cover, glyphosate was available for leaching during the main snowmelt event, which was fast and probably resulted in preferential transport.

Table 7

Year, date, applied substance, including the sum of the three components in the commercial product Ariane S, mean losses from all ploughed plots with standard deviation (SD), mean losses relative to applied amount, range of the relative losses and area with relative losses exceeding 0.1 g ha^{-1}. Glyphosate was analysed in both dissolved (diss.) and particulate (part.) form in 2010

Year	Date	Substance	Mean (g ha^{-1})	SD	Relative losses (%)	Range of relative losses (%)	Area (%)
2008	16/8	Glyphosate diss.	0.89	0.64	0.084	0.02–0.17	25
2009	9/6	Clopyralid	0.84	0.70	1.62[c]	0.09–4.55	92
		Fluroxypyr	0.24	0.36	0.22	0.002–0.96	60
		MCPA	0.71	0.89	0.14	0.003–0.49	42
		Sum	1.81	3.03	0.34	0.02–1.36	60
2010	23/6	Fluroxypyr[a]	> 0.03	0.02	> 0.03	—	—
2010	22/9	Glyphosate diss.[b]	0.90	0.32	0.085	0.05–0.12	—
		Glyphosate part.	0.82	0.25	0.064	0.08–0.10	—
		Glyphosate total	1.72	1.47	0.15	0.12–0.23	100
2011	11/6	Bentazone	3.31	2.92	0.70[b]	0.42–2.16	100

[a] From late sample after flooding of the measuring station.
[b] Period 22 September–15 April and calculated from the same four plots as treated in 2008.
[c] Relative losses of clopyralid were significantly greater ($P < 0.01$) than losses of fluroxypyr applied simultaneously.

Herbicide correlation with particulate phosphorus and plot position

In spite of the small variation in the amounts of water discharge between plots, there was a large variation in herbicide losses for all substances resulting from the highly varying concentrations in drainage water. Similar relationships have previously been reported between total glyphosate and PP for the same field. Due to their strong sorption, both glyphosate and PP are considered to leach mainly through preferential transport in macropores. Our results suggest that preferential transport dominates leaching also for the weakly sorbed substances at this site. In addition, since the pesticides which were applied at the soil surface were leaching with a similar pattern as PP, this suggests that the topsoil was the major source of leached PP. We did not observe any surface runoff in the direction of the Recipient ditch during the experimental period. Lateral flows below the soil surface and e.g. on a plough pan were also unlikely to occur, since there was no distinct plough pan at the site. There was no correlation between the topsoil (0–23cm) pH and the plot position. However, topsoil OC clearly increased with decreasing distance between plot mid-point and the recipient ditch (R^2 = 0.70%, P < 0.001) and pH in the deeper subsoil (60–90 cm) decreased (R^2 = 78%, P < 0.001). The concentration of all pesticides tended to increase with decreasing distance between plot position and the recipient ditch. The relationship was significant for bentazone, and was also significant from a total ranking of all pesticides detected (Fig. 1).

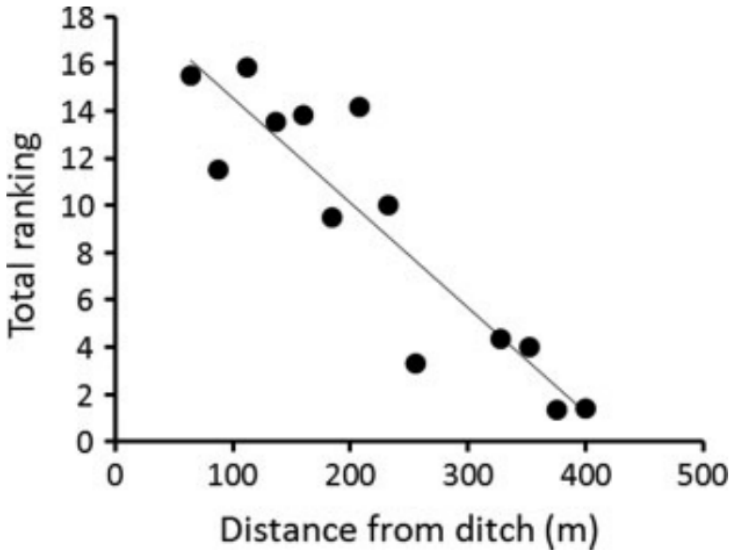

Fig. 1. Total ranking of mean concentration of clopyralid, fluroxypyr, MCPA, bentazone, dissolved glyphosate and particulate glyphosate related to the distance between the ditch and the centre of the respective plot. The estimates were made for the observed concentrations in the major event for every substance. The slope of the regression line is significantly different from zero (P < 0.001).

Effects of soil management, soil structure, pH and organic matter

There was a general tendency for larger losses of all substances from shallow-tilled plots than from ploughed plots, with or without previous structure liming (Table 8). The apparent differences, which were not significant for any single substance, increased in the order clopyralid < MCPA < bentazone < fluroxypyr < total glyphosate. However, estimated for all five substances lumped together (paired *t*-test), the difference between shallow-tilled and ploughed structure-limed plots was significant (P < 0.05), both before and after adjustment to the effect of plot position in relation to the ditch. From soils where preferential flow and transport are important, ploughing is generally considered to reduce pesticide leaching by interrupting continuous macropores. For our case the larger losses from the shallow-tilled plots may also have been an effect of shallow and uneven accumulation of crop residues in these plots which resulted in uneven infiltration and preferential herbicide transport along straw residues. At the study site, it has been demonstrated that structure liming (quicklime) significantly improves soil aggregate stability measured as a decrease in readily dispersed clay. Improved aggregate stability should influence the transport of glyphosate which adsorbs strongly to clay particles. However, the improved aggregate stability did not result in any significantly smaller losses of glyphosate from structure limed plots compared to conventionally tilled plots.

Table 8

Year of application, mean and standard deviation (SD) of transported masses of the applied substances (in g ha^{-1}) from tilled, structure-limed (+ ploughed) and conventionally ploughed plots

		Shallow tillage Mean			Structure-limed Mean			Conventional ploughing Mean		
Year	Substance	Un-adjusted	Adjusted	SD	Un-adjusted	Adjusted	SD	Un-adjusted	Adjusted	SD
2009	Clopyralid	1.16	1.14	0.77	1.07	1.13	0.95	0.73	0.71	0.59
2009	Fluroxypyr	0.36	0.35	0.29	0.25	0.26	0.28	0.22	0.21	0.25
2009	MCPA	1.11	1.09	1.03	0.86	0.88	1.25	0.63	0.61	0.82
2011	Bentazone	4.78	4.73	2.84	3.52	3.75	3.81	3.40	3.32	2.53
2010	Glyphosate[a]	3.81	3.77	2.58	0.85	0.90	1.13	1.59	1.56	1.56

Note: Mean transported losses are given both as unadjusted values and values adjusted for the distance to the ditch.
[a] Total glyphosate in both particulate and dissolved form in the period 22 September 2010–15 April 2011.

For ionisable pesticides, leaching is also affected by soil pH, with weaker sorption at higher pH. Based on the p*K*a values of the substances studied here and the small differences in pH between treatments (Table 8), any pH effects on leaching were probably minor. The topsoil OC content is often higher under long term shallow tillage than under conventional tillage, which has consequences for pesticide sorption and degradation. However, in our case the OC content was not significantly different between treatments and there were no significant differences in subsoil OC between plots with different management regimes. The coefficient of variation in relative leaching losses between all substances for the shallow-tilled plots varied between 40 and 92%. The coefficient of variation in the relative leaching losses from all plots and for all substances combined (92–156%) varied even more. In conclusion, the variation in relative leaching losses between plots within the same treatment was larger than that between different substances. This finding also demonstrates that the differences in transport pathways through the soil between plots have a larger effect on pesticide concentrations than the differences in pesticide properties.

Conclusions

Concentrations of the herbicides bentazone, clopyralid, fluroxypyr, MCPA and glyphosate were measured in subsurface drain discharge from a clay field during a four-year study. Despite hydrological conditions not representing a worst case scenario for leaching, the relative leaching losses of all herbicides studied were large compared to values reported in the literature. Measured concentrations of bentazone and MCPA exceeded Swedish guideline values based on predicted no effect on aquatic ecosystems for 50 and 33% of the plots for MCPA and bentazone, respectively. All substances studied (except sulphonyl ureas which were not detected), irrespective of sorption strength, showed similar leaching patterns. These observations clearly demonstrate that preferential transport in macropores is the dominant transport process at this site. The variation in relative leaching losses between plots within the same treatment was greater than that between different substances. Crack stabilisation by gyttja, especially in the deeper subsoil, was suggested as an important explanatory factor for this large spatial variation in pesticide leaching, although it was not possible to investigate differences in gyttja content between plots. Continuous macropores connecting the soil surface to the subsoil may be a factor contributing to the generally large pesticide losses observed after shallow tillage. However, careful studies of soil macropore systems, including topsoil and subsoil properties, are needed to explain the unpredictability in leaching at this site.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes a leaching experiment from a Swedish marine clay soil with agricultural land use. Glyphosate among other herbicides was considered in analysis. The study provides supportive information but not all parameters to derive endpoints are reported.
The study is therefore classified as reliable with restrictions (Category 2).

1. Information on the study

Data point:	KCA 7.1.4.3
Report author	Ulen, B., et al.
Report year	2012
Report title	Particulate-facilitated leaching of glyphosate and phosphorus from a marine clay soil via tile drains
Document No	Acta Agriculturae Scandinavica Section B - Soil and Plant Science, 2012; 62: Supplement 2, 241-251
Guidelines followed in study	None
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (Not enough information provided to check validity)

2. Full summary of the study according to OECD format

Losses of commonly used chemical pesticides from agricultural land may cause serious problems in recipient waters in a similar way to phosphorus (P). Due to analytical challenges concerning determination of glyphosate (Gly), transport behaviour of this widely used herbicide is still not well known. The objective of the present study was to quantify and evaluate leaching of Gly in parallel with P. Leaching losses of autumn-applied Gly (1.06 kg/ha) via drainage water were examined by flow-proportional sampling of discharge from 20 drained plots in a field experiment in eastern Sweden. Samples were analysed for Gly in particulate-bound (PGly) and dissolved (DGly) form. The first 10 mm water discharge contained no detectable Gly, but the following 70 mm had total Gly (TotGly) concentrations of up to 6 µg/L, with 62% occurring as PGly. On average, 0.7 g TotGly/ha was leached from conventionally ploughed plots, compared with 1.7 g TotGly/ha from shallow-tilled plots (cultivator to 12 cm working depth). Higher Gly losses occurred in snowmelt periods in spring, but then with the majority (60%) as DGly. All autumn concentrations of PGly in drainage water were significantly correlated ($p < 0.001$) to the concentrations of particulate-bound phosphorus (PP) lost from the different plots (Pearson correlation coefficient 0.84), while PP concentrations were in turn significantly correlated to water turbidity (Pearson correlation coefficient 0.81). Leaching losses of TotGly were significantly lower (by 1.3 g/ha; $p < 0.01$) from plots that had been structure-limed three years previously and ploughed thereafter than from shallow-tilled plots. Turbidity and PP concentration also tended to be lowest in discharge from structure-limed plots and highest from shallow-tilled plots. This difference in TotGly leaching between soil management regimes could not be explained by differences in measured pH in drainage water or amount of discharge. However, previously structure-limed plots had significantly better aggregate stability, measured as readily dispersed clay (RDC), than unlimited plots. The effects of building up good soil structure, with strong soil aggregates and an appropriate pore system in the topsoil, on mitigating Gly and P losses in particulate and dissolved form should be further investigated.

Materials and Methods

Experimental plots and soil characteristics

The experiment was done on 20 drained plots in an experimental field with a sub-surface drainage water collection system constructed on a flat plain close to the Lake Bornsjön reservoir. Drainage water flows to a sampling and measuring station and is recorded with tilting vessels and data logger. The data logger controls the flow proportional sampling by means of small tube pumps in the basement of the station. After a certain volume of water has passed, the suction tube is first cleaned by reverse pumping and thereafter a small volume is sampled. The flow-proportional (composite) sampling took place in dark

glass vessels at relatively cold temperature and in darkness for a maximum of one week prior to freezing the water samples and transport to the laboratory before analysis.
Clay content (60%), is high throughout the profile (Table 1), with small spatial variation in both topsoil and sub-soil (variance less than 0.5%). pH and soil concentration of P are uniformly distributed in the experimental area (variance less than 15%). In the soil profile, the pH (dry soil samples) varies between 5.2 and 6.9, with the lowest values occurring in the 70-100 cm layer, which includes the tile drains at approximately 90 cm depth. Under wet conditions the pH in the upper sub-soil is higher than that under dry conditions (6.9 compared with 6.6). Overall, the soil profile generally demonstrates a high ability to sorb P to the soil matrix.
The soil horizon has a strongly aggregated structure, especially in the deeper part, with approximately 10 cm wide and 10-20 cm prismatic aggregates in the layer 43-100 cm. Water retention is very high. In an adjoining field with an old drainage system, the deeper soil horizon is very wet, the aggregates similarly very prismatic and the structure is easily destroyed by digging.

Table 1. Selected physical and chemical properties of the soil at the study site.

	Soil depth (cm)					
Properties	0–10	10–30	30–50	50–70	70–100	Reference for the method
Particle size distribution						
<0.002 mm (clay) (%)	60	60	59	61	54	Eriksson et al. (1998)
0.002–0.02 mm (%)	31	30	30	31	34	Eriksson et al, (1998)
0.02–0.2 mm (%)	9	10	11	8	12	Eriksson et al. (1998)
Organic matter (%)	3.9	1.9	0.1	0.0	0.0	Eriksson et al. (1998)
pH_{H_2O}[a]	6.0	6.2	6.6	6.5	5.2	ISO (2005)
P_{Olsen} (mmol kg^{-1})[a]	0.59	0.53	0.13	0.17	0.68	Olsen and Sommers (1982)
P_{AL} (mmol kg^{-1})[a]	1.4	1.0	0.3	0.4	1.0	Egnér et al. (1960)
Al_{ox} (mmol kg^{-1})[a]	116	106	71	77	88	Schwertmann (1964)
Fe_{ox} (mmol kg^{-1})[a]	165	169	158	181	118	Schwertmann (1964)
Al_{AL} (mmol kg^{-1})[a]	10.3	9.9	9.8	9.6	16.1	Ulén (2006)
Fe_{AL} (mmol kg^{-1})[a]	9.4	10.1	8.8	9.4	12.5	Ulén (2006)
PSI_2 (mmol kg^{-1}).[a]	7.3	7.8	7.3	7.2	10.5	Börling et al. (2004)
P_{Olsen}/PSI_2[a] (%)	8.1	6.8	1.8	2.4	6.4	Börling et al. (2004)
DPS_{AL}[a] (%)	8.7	6.2	2.0	2.5	4.3	Ulén (2006)

[a]Data from Andersson et al. (2012).

Glyphosate application and cultivation practices.
No Gly had been applied to the actual experimental plots for the previous three years. Quicklime (CaO) had been applied in dry conditions on the stubble in four plots in 2007 Phosphorus fertilization was 11 kg/(ha year), always applied in mineral form in spring. This is a moderate load, since the area has special restrictions. When starting the experiment the aim was to avoid P limitation of the crop and therefore 20 kg/(ha year) were applied in 2007-2011 for all plots except four. Glyphosate was applied on 22 September 2010 as the commercial product Glypro Bio, at a rate equal to 1.06 kg/ha active substance. Twelve days later, the conventional and structure-limed plots were stubble-harrowed (Table 2) and eight plots were shallow-tilled (12 cm) twice and reconsolidated with a rib-roller. After a further 10 days, the conventionally ploughed plots (8) and the structure-limed plots (4) were mould-board-ploughed and the soil was inverted to a depth of 23 cm.

Table 2. Management regime in the different treatments (A-E) in 2010, where A+B (eight plots) represent regular conventional autumn ploughing, C (four plots) represents previous structure liming and D+E (eight plots) represent regular shallow tillage in autumn.

Treatment	Management	Date
A+B, C, D+E	Harrowing (0–5 cm)	16 May
A+B, C, D	Fertilization, seed drilling[a]	17 May
E	Fertilization (broadcasting)	17 May
A+B, C, D+E	Sowing (oats)	17 May
A+B, C, D+E	Harvesting	27 August
A+B, C, D+E	Glyphosate application ($1.06\ kg\ ha^{-1}$)	22 September
A+B, C	Stubble harrowing	4 October
D+E	Cultivation (8 cm) twice	4 October
A, B, C	Conventional ploughing (23 cm)	14 October

[a]No P fertilization to B plots.

Weather, discharge and water sampling procedure

Autumn 2010 was short, with permanent snow from the end of November (Fig. 1). Owing to the thickness of the snow cover, soil freezing was limited despite low air temperatures. The main snowmelt took place in late March and the first two weeks in April. The glass vessels with flow-proportional samples in the station basement were observed regularly (at least weekly) and when at least 300 mL turbid water had been collected from most plots, sub-samples were taken from every plot for Gly analysis. When there was a moderate amount of water or less turbid water in the glass vessel, sampling was performed only for analysis of P and turbidity for reasons of economy. Such sampling occurred in total on five sampling occasions. On 28 March, 186 days after glyphosate application in autumn, turbidity was observed once again in the flow-proportionally sampled water and additional water was collected for Gly analysis, which was performed on the 14 most turbid samples.

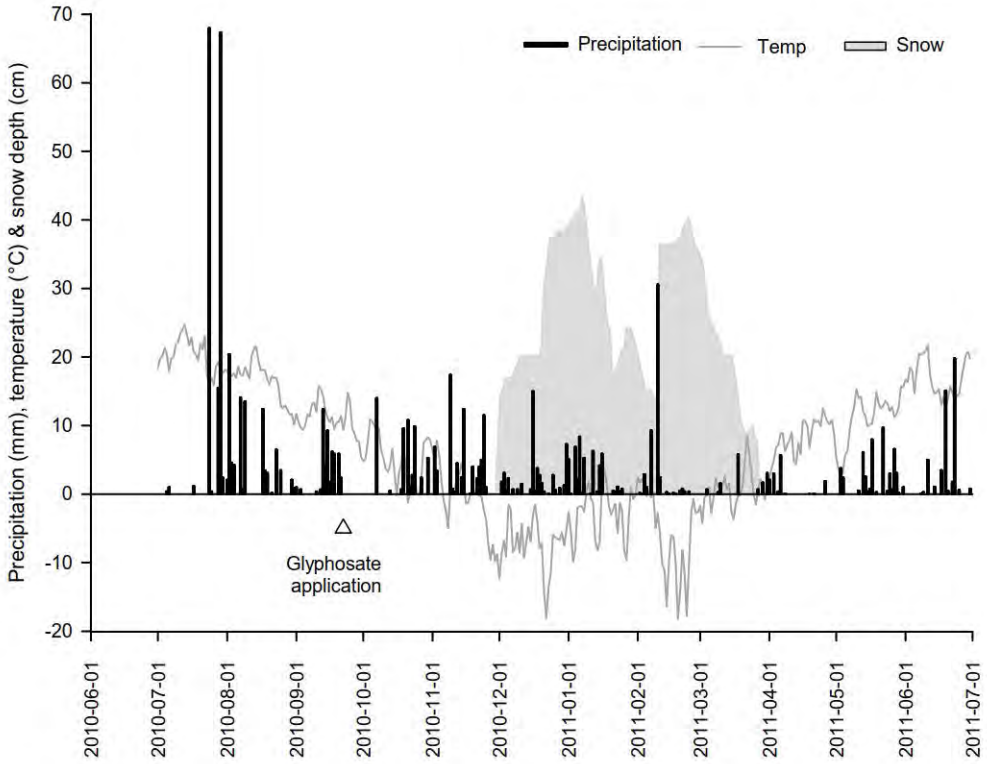

Fig. 1. Temperature (°C), precipitation (mm) and snow cover (mm) on the experimental field in 2010-2011.

Water analysis
Total P was analysed as soluble molybdate-reactive P after acid oxidation with $K_2S_2O_8$ (ECS, 1996). DRP was analysed after pre-filtration using filters with pore diameter 0.45. Particulate P (PP) is the absolute dominant P fraction, while non-mineral forms of dissolved P are very small, and accordingly the difference between TotP and DRP was taken as PP. The concentration of particles was analyzed from thawed samples as turbidity on a HACH 2100 turbidometer. Before analysing Gly, each thawed sample was thoroughly shaken by hand, centrifuged and filtered. The filtered water was used for analysis of DGly, including AMPA, after pH adjustment (pH 7-8) with either diluted HCl or NaOH. After a few more rounds of extraction, centrifugation and filtration, the pH of the samples was adjusted to 2 in order to precipitate any humic acids and to harmonize with the method used for stream and lake sediment. After dilution, the pH was readjusted to 7-8.
The same analytical procedure was used for both PGly and DGly and involved ion-exchange and derivatization, using a modified version of Mogadati et al. (1996), followed by final identification and quantification by gas chromatography-mass spectrometry (GC-MS).

Soil aggregate stability
Soil samples from plots with structure liming, conventional ploughing and reduced tillage were analysed in the laboratory for aggregate stability, expressed as readily dispersed clay (RDC). Slightly moist samples were collected from the topsoil (0-20 cm) on 27 August 2010, before post-harvest stubble cultivation, and gently transported to the laboratory. Four sub-samples representing 12 aggregates (8-10 mm) were prepared for each plot and gently wet-sieved (0.6 mm mesh opening) with a slow oscillating movement. After 4 hours sedimentation (to allow all particles larger than clay to settle; Sheldrick & Wang, 1993), the content of dispersed clay still in solution was determined by turbidometer.

Data calculations and statistical analyses
The mean and standard deviation were calculated for the experimental parameters determined in all flow-proportional samples (four or eight parallel samples) from replicate plots for the different treatments. If no residue of Gly or AMPA was detected in a given sample, the value 0 was used for calculating the mean. Pearson correlation and regression linear relationships were determined between the parameters total glyphosate (TotGly_PGly_DGly), TotP, PGly, PP and turbidity for the autumn period (27 September - 15 November) and between TotP and turbidity for the spring period (21 March - 11April). Any differences in glyphosate concentrations between the different soil treatments were analysed using Bonferroni post test assuming equal variance and a significance level of $p < 0.05$. Leaching losses from the different plots in the autumn period were calculated by multiplying discharge by measured flow-proportional concentrations in the periods between sample collections. In the spring period, transport of TotGly was estimated from measured values from 14 plots on 28 March.

Results and Discussion

Glyphosate and phosphorus concentrations in water
One week after Gly application in autumn, when 10 mm discharge had passed through the tile drainage system, no Gly or AMPA was present in detectable quantities in the discharge (Table 3). In the following 7-8 weeks, representing 70 mm water discharge, relatively high and quantifiable concentrations of both DGly and PGly were detected in practically all water samples and, in addition, dissolved AMPA was frequently observed. The concentrations varied greatly from plot to plot and TotGly concentrations of up to 5-6 µg/L were recorded for some plots. High PGly concentrations were generally associated with high DGly concentrations and the two forms of Gly were significantly correlated to each other (Pearson correlation 0.35; $p < 0.002$). Hence, more DGly seemed to leach with mobilized soil particles with high Gly content. Mean DGly concentration in discharge in the autumn (22/9-15/11) was 1.03 µg/L for plots with shallow tillage; 0.43 µg/L for plots with conventional ploughing and 0.36 µg/L for plots with structure liming (differences not statistically significant).

Table 3. Discharge, pH (in stored composite samples) and flow-proportional concentrations of dissolved glyphosate (DGly), AMPA, particulate glyphosate (PGly), dissolved reactive phosphorus (DRP), particulate P (PP) and turbidity (Turb) in five periods 2010 - 2011 (n.d. = not detected)

Period	22/9–27/9	28/9–25/10	26/10–8/11	8/11–15/11	21/3–28/3
Conventional ploughing					
Discharge (mm)	8.2±3.0	9.2±4.3	25.5±11.1	33.5±13.1	72.9±30.2
pH	6.5	7.2	7.0	6.7	6.6
DGly (μg L^{-1})	n.d.	0.43±0.32	0.43±0.34	0.39±0.21	0.31±0.34
AMPA (μg L^{-1})	n.d.	0.05	0.04	0.03	n.d.
PGly (μg L^{-1})	n.d.	0.67±0.63	0.61±0.67	0.60±0.34	0.22±0.43
DRP (mg L^{-1})	0.021±0.011	0.021±0.011	0.018±0.007	0.020±0.007	0.048±0.019
PP (mg L^{-1})	0.132±0.068	0.122±0.010	0.161±0.166	0.168±0.144	0.124±0.039
Turb (NTU)	64±26	36±7	62±24	60±20	30±16
Structure liming					
Discharge (mm)	10.4±4.1	13.5±5.4	29.1±11.4	30.1±5.1	74.4±25.5
pH	6.9	7.3	7.2	6.9	6.6
DGly (μg L^{-1})	n.d.	0.24±0.20	0.30±0.21	0.24±0.27	0.23±0.25
AMPA (μg L^{-1})	n.d.	0.03	e.d.	0.05	0.08
PGly (μg L^{-1})	n.d.	0.40±0.48	0.41±0.53	0.33±0.58	0.16±0.35
DRP (mg L^{-1})	0.018±0.007	0.017±0.008	0.015±0.005	0.020±0.006	0.047±0.027
PP (mg L^{-1})	0.075±0.066	0.066±0.074	0.093±0.131	0.100±0.106	0.078±0.032
Turb (NTU)	46±30	34±11	64±26	46±31	28±6
Shallow tillage					
Discharge (mm)	10.8±5.3	15.6±6.6	25.9±10.2	29.7±6.4	76.4±23.5
pH	6.8	7.2	7.1	6.8	6.6
DGly (μg L^{-1})	n.d.	1.15±0.89	1.28±1.42	0.99±0.64	0.82±0.93
AMPA (μg L^{-1})	n.d.	0.05	0.23	1.3	0.02
PGly (μg L^{-1})	n.d.	1.99±1.64	1.42±1.44	1.89±1.48	0.57±0.84
DRP (mg L^{-1})	0.024±0.007	0.024±0.007	0.023±0.008	0.027±0.007	0.047±0.021
PP (mg L^{-1})	0.142±0.078	0.236±0.181	0.411±0.355	0.275±0.151	0.136±0.029
Turb (NTU)	88±44	50±17	99±45	81±31	52±43

Similar to TotGly, the majority of TotP was lost in particulate form. The proportion of PP was higher (90%) than the proportion of PGly (60%). The present study site Gly was tilled down (10 or 23cm depth) in autumn after spraying which would facilitate the dispersion of Gly. A clear and positive correlation between TotGly and TotP concentrations and between PGly and PP concentrations was recorded (Fig. 2). In turn, PP concentrations could be quite well predicted from turbidity (Fig. 2). In contrast, DRP concentrations were generally low (0.018-0.027 mg/L) and DGly concentrations were more weakly correlated to DRP concentrations ($r = 0.65$; $p < 0.001$). Glyphosate is commonly suggested to compete with phosphate ions for adsorption sites, but at the present site, with high sorption capacity of the soil particles, this seemed not to be the case, since the correlation was positive. Mean PGly concentrations in the autumn were 1.73 µg/L in discharge from shallow-tilled plots; 0.62 µg/L for conventional ploughed plots; and 0.36 µg/L for structure-limed plots, all differences being statistically significantly different ($p < 0.001$). This implies that colloid P, colloid glyphosate and dissolved pesticides, although mobilized with different mechanisms (de Jonge et al., 2009), may be transported via macropore flow.

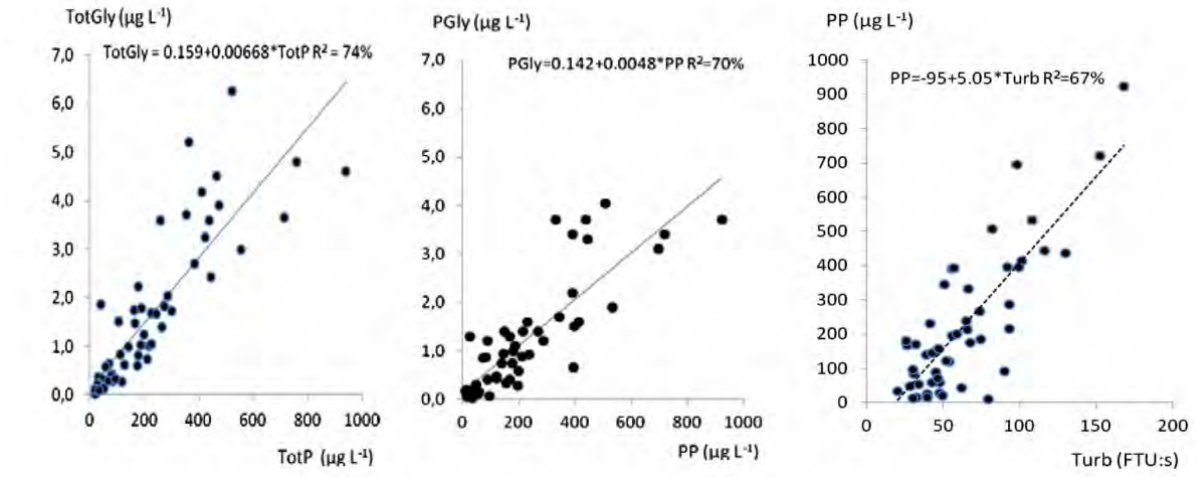

Fig. 2. Regression equation for the relationship between concentrations of: (a) total glyphosate (TotGly) and total phosphorus (TotP); (b) particulate glyphosate (PGly) and particulate P (PP); and (c) PP and turbidity (NTUs) in the period 27 September - 15 November 2010. Corresponding Pearson correlations (0.86, 0.84 and 0.82, respectively) were all significant ($p < 0.001$).

Glyphosate and phosphorus concentrations and losses in spring versus autumn period

As with Gly and P, pH was measured in the cumulative flow-proportionally sampled water and may have changed in the glass vessel. However, measured pH generally did not differ between the three treatments and pH in discharge from the previously structure-limed plots was similar to that in discharge from the unlimited plots (Table 3). The pH tended to be lower (6.6) in the snowmelt period (Table 3). The measured drop in (logarithmic-based) pH value is equal to 75% less H^+ ions, which may have influenced both the electrical charge of Gly and the hydrogen bonds of the minerals, and which may explain the high concentrations of DGly in snowmelt. The snowmelt water had low electric conductivity and DRP concentrations that were twice as high as those in the autumn discharge water. The PGly concentrations found in snowmelt in the present study were generally lower than the DGly concentrations and remained at nearly the same level as in autumn. Consequently, the relative proportions of DGly and PGly were reversed from autumn to spring (snowmelt) (Table 4). However, the latter case is based on a more limited number of analyses ($n = 14$).

Table 4. Number of samples analysed (n), relative proportions of dissolved glyphosate (DGly) and particulate glyphosate (PGly) in total glyphosate (TotGly) and relative proportions of dissolved reactive P (DRP) and particulate P (PP) in total phosphorus (TotP) in autumn (28/9 - 15/11 2010) and in a snowmelt period in spring (21 - 28/3 2011), based on flow-proportional concentrations.

Glyphosate			Phosphorus		
Fraction	Autumn	Spring	Fraction	Autumn	Spring
n	80	14	No	80	20
DGly/TotGly (%)	40	60	DRP/TotP (%)	10	32
PGly/ TotGly (%)	60	40	PP/TotP (%)	90	68

In practice, half-life degradation rate may be several months. However, as indicated here; ratio PGly/turbidity was only 20-40% lower in March than in November. Simultaneously, PGly/PP ratio decreased by 30% on average (from 0.54% in autumn to 0.15% in spring). Correspondingly the topsoil colloids may be more depleted of P in spring than in autumn, since the ratio PP to turbidity was lower and had a lower slope in snowmelt than in autumn.

Therefore, there may be similarities between Gly and P transport behavior in spite of the fact that P exists in a large P pool in topsoil and that yearly net P load to the soil in recent years has been six-fold higher than the glyphosate load.

Since the major water discharge took place during the snowmelt period, glyphosate losses tended to be higher in spring than in autumn (Tables 5 and 6). In relation to applied amount, losses were approximately 0.1% in spring and 0.05% in autumn for the conventionally ploughed plots. The main reason for the high spring discharge was the intensive snowmelt taking place after a winter with much snow accumulation. These results indicate the importance of such a snowmelt period for Gly losses, confirming findings by Laitinen et al. (2009). Snow accumulation also had great consequences for P losses.

Table 5. Discharge and transport of dissolved glyphosate (DGly), particulate glyphosate (PGly), total glyphosate (TotGly), dissolved reactive phosphorus (DRP), particulate P (PP) and total P (TotP) from conventionally ploughed, structure-limed (and ploughed) and shallow-tilled plots in the period 28/9 - 15/11 2010.

Period 28/9–15/11	Conventional	Structure-limed	Shallow-tilled
Discharge (mm)	69 ± 28	74 ± 23	72 ± 22
DGly (g ha^{-1})	0.25 ± 0.13	0.12 ± 0.10	0.65 ± 0.54
PGly (g ha^{-1})	0.45 ± 0.53	0.19 ± 0.19	1.01 ± 0.75
TotGly (g ha^{-1})	0.70 ± 0.60	0.31 ± 0.31**	1.65 ± 0.96
DRP (kg ha^{-1})	0.012 ± 0.004	0.012 ± 0.003	0.018 ± 0.007
PP (kg ha^{-1})	0.104 ± 0.082	0.048 ± 0.044	0.192 ± 0.111
TotP (kg ha^{-1})	0.117 ± 0.084	0.060 ± 0.044	0.209 ± 0.114

**Significantly lower ($p < 0.05$) than in shallow-tilled plots.

Table 6. Discharge (mm) and leaching losses of dissolved glyphosate (DGly), particulate glyphosate (PGly) and total glyphosate (TotGly) as a percentage of original amount applied from conventionally ploughed, structure-limed (and ploughed) and shallow-tilled plots based on measurements in autumn (28/9 - 15/11 2010) and more rough estimates in the most intensive spring snowmelt period (31/3 - 11/4).

	Conventional		Structure-limed		Shallow-tilled	
	Autumn	Snowmelt	Autumn	Snowmelt	Autumn	Snowmelt
Discharge (mm)	69	170	74	169	72	160
DGly (%)	0.024	–	0.011	–	0.061	–
PGly (%)	0.041	–	0.018	–	0.095	–
TotGly (%)	0.066	0.09	0.029	0.05	0.156	0.19

Glyphosate and phosphorus losses under different soil management regimes

In the autumn period, TotGly leaching losses were on average 0.70 g/ha from the conventionally ploughed plots (Table 6). TotGly losses from structure-limed plots were significantly lower ($p < 0.05$) than from shallow-tilled plots, expressed in absolute terms (Table 5), and also as a percentage of applied amount of Gly (Table 6). Fewer particles with attached Gly and P are expected to mobilize from soil aggregates that are less prone to dispersion. The structure-limed plots had significantly ($p < 0.05$) better aggregate stability (lower RDC values) in autumn than the conventionally ploughed and shallow-tilled plots (Fig. 3), which may explain the clear tendency for lower losses of both PGly and PP from this treatment (Table 5).

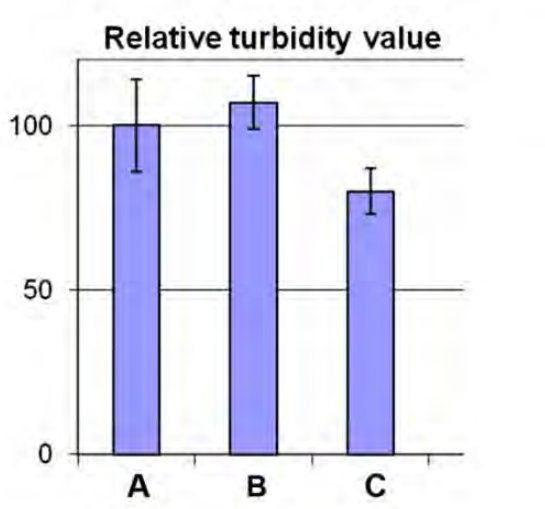

Fig. 3. Readily dispersed clay (RDC) in the topsoil from (A) conventionally ploughed, (C) structure-limed and (B) shallowtilled plots. The soil was sampled in September 2010, three years after structure liming

Leaching losses of both PGly and PP tended to be highest from the regular shallow-tilled plots (Table 5). Any enhanced amounts of stubble residues in the topsoil, combined with higher potential biological activity and organic matter content, did not seem to have improved aggregate stability from plots (Fig. 3). However, significantly higher organic matter content was not expected, since such major changes may take at least 10 years. Sorption of Gly is generally not increased in the presence of more straw residues as a consequence of reduced tillage. Therefore, the straw may have facilitated water transport rather than providing new sorption sites after the mixing and reconsolidation of the soil surface. In addition, shallow and uneven accumulation of crop residues on the shallow-tilled plots possibly resulted in uneven infiltration and rapid lateral water movement compared with annually ploughed plots. This is a factor that should be further investigated.

There was no major difference in amount of discharge between the different treatments (Table 6). Topsoil structure should be further explored in connection with topsoil susceptibility to preferential flow and transport under different agricultural management regimes. In addition, there was a great variation in concentrations between different plots. Both ‘gyttja’ (cohesive matter of organic origin settled in marine or lake sediment) and oxidized iron (rust) have been frequently observed in soils at the present site. Such material might strengthen the crack walls and make them into permanent pathways, which could explain the general fast transport of particulate-bound glyphosate and P at the present site.

The source of the Gly leaching in this study was the tilled topsoil (0-12 or 0-23 cm), which was possibly the main source of P leaching too. Besides the total amount applied, risk assessment of leaching is often based on the sorption/desorption properties of the actual substance. However, according to the results of the present study, factors such as soil structure, macropore topology and macropore flow may be of great importance.

Conclusion

This study has demonstrated that a significant proportion of glyphosate (Gly) leaching losses may occur in particulate form from clay soils with high amounts of sorption sites available. Crack stabilization by gyttja, especially in the deeper sub-soil, might be an important explanatory factor for fast vertical transport of Gly and phosphorus (P) at the study site. The crack might also be an important explanatory factor for the great spatial variability in Gly and P, in both particulate and dissolved form, at the study site. Structure liming of the topsoil was demonstrated to reduce total Gly leaching losses compared with unlimited soils, while shallow tillage may not be a suitable way to mitigate particle-facilitated transport of Gly and P via tile drains from this type of clay soil. Proper agricultural management and improved topsoil structure can counteract fast macropore flow in this type of clay soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a field leaching experiment with glyphosate in Sweden on an agriculturally used soil. The article provides no information to check the validity of the study against current standards.
The study is therefore classified as reliable with restrictions (Category 2).

1. Information on the study

Data point:	KCA 7.2.2.3
Report author	Wang, S. et al.
Report year	2016
Report title	(Bio)degradation of glyphosate in water-sediment microcosms - A stable isotope co-labeling approach
Document No	Water Research 99 (2016) 91-100
Guidelines followed in study	OECD guideline 308
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (water/sediment systems may have received inputs of glyphosate or AMPA within the previous 4 years; extremely high application rate)

2. Full summary of the study according to OECD format

Glyphosate and its metabolite aminomethylphosphonic acid (AMPA) are frequently detected in water and sediments. Up to date, there are no comprehensive studies on the fate of glyphosate in water sediment microcosms according to OECD 308 guideline. Stable isotope co-labeled $^{13}C_3{}^{15}N$-glyphosate was used to determine the turnover mass balance, formation of metabolites, and formation of residues over a period of 80 days. In the water-sediment system, 56 % of the initial $^{13}C_3$-glyphosate equivalents was ultimately mineralized, whereas the mineralization in the water system (without sediment) was low, reaching only 2 % of ^{13}C-glyphosate equivalents. This finding demonstrates the key role of sediments in its degradation. Glyphosate was detected below detection limit in the water compartment on day 40, but could still be detected in the sediments, ultimately reaching 5 % of $^{13}C_3{}^{15}N$-glyphosate equivalents. A rapid increase in $^{13}C_3{}^{15}N$-AMPA was noted after 10 days, and these transformation products ultimately constituted 26 % of the $^{13}C_3$-glyphosate equivalents and 79 % of the ^{15}N-glyphosate equivalents. In total, 10 % of the ^{13}C label and 12 % of the ^{15}N label were incorporated into amino acids, indicating no risk bearing biogenic residue formation from $^{13}C_3{}^{15}N$-glyphosate. Initially, glyphosate was biodegraded via the sarcosine pathway related to microbial growth, as shown by co-labeled $^{13}C_3{}^{15}N$-glycine and biogenic residue formation. Later, degradation via AMPA dominated under starvation conditions, as shown by the contents of ^{13}C-glycine. The presented data provide the first evidence of the speciation of the non-extractable residues as well as the utilization of glyphosate as a carbon and nitrogen source in the water-sediment system. This study also highlights the contribution of both the sarcosine and the AMPA degradation pathways under these conditions.

Materials and Methods

Chemicals

All the chemicals used were analytical or reagent grade and were obtained from the Carl Roth Company (Karlsruhe, Germany) if not specified otherwise. Resin for amino acid purification (Dowex 50W-X8, 50-100 mesh) was purchased from VWR/Merck (Darmstadt, Germany). Methanol and ammonium acetate for ultraperformance liquid chromatography-mass spectrometry (UPLC/MS) measurements were provided by Biosolve (Valkenswaard, Netherlands). Labeled $^{13}C_3{}^{15}N$-glyphosate was purchased from IsoSciences Company (Trevose, PA, USA). The isotopic enrichment of the labeled glyphosate was 99 at % for ^{13}C and 98 at % for ^{15}N; the chemical purity was 98 %.

Sediments and water

The sediments and associated water were collected from the Getel creek, Harz Mountains in Saxony-Anhalt, Germany. The catchment of this creek comprises agricultural lowlands with continuous

crop rotation and pesticide application. It is thus a high risk area for exposure to pesticides. The sediments contained 38 % (±0.7 %) sand (>0.05 mm), 62 % (±0.7 %) silt + clay (<0.05 mm), 85 mg/g (±2 mg/g) total organic carbon and 15 mg/g (±1 mg/g) total nitrogen. The pH of the sediments and creek water was 7.1 and 8.8, respectively. The content of total organic carbon of the suspended matter in the creek water was 8 mg/L (±1 mg/L), and the content of total nitrogen was 3 mg/L (±0.6 mg/L). Neither glyphosate nor AMPA were detected in the sediments or creek water. Sediments and associated water were taken from the upper layer (up to 5 cm) of the Getel creek sediment. The sediments were separated from the water by filtration, wet sieved and gently homogenized.

Incubation experiment

Degradation experiments were conducted according to the OECD guideline 308 in biometer flasks to address the transformation in aquatic sediment systems. Six incubations were performed: 1) water-sediment without glyphosate (non-amended control), 2) water-sediment with unlabeled glyphosate (unlabeled control), 3) water-sediment with labeled glyphosate (biotic system), 4) water with unlabeled glyphosate (unlabeled control), 5) water with labeled glyphosate and 6) sterilized water sediment with labeled glyphosate (abiotic system). The two controls without glyphosate and unlabeled glyphosate were used to correct for the natural abundances of ^{13}C (~1.1 at %) and ^{15}N (~0.37 at %) in the sediment, and water systems without sediment were prepared to test the effect of sediment on the microbial degradation of glyphosate. Abiotic controls were incubated to distinguish between abiotic and biotic degradation of $^{13}C_3{}^{15}N$-glyphosate. In these controls, sediment and water were sterilized by autoclaving three times at 120°C for 20 min prior to incubation.

50 g (dw) of sediment and 90 mL of creek water containing either unlabeled or labeled glyphosate were added to glass bottles. The initial concentration of glyphosate was 50 mg/L in water and water-sediment systems, except in the blanks containing no glyphosate. This concentration is well above environmentally relevant levels, but it was required to obtain reliable isotopic enrichment results in the water-sediment systems given the limited sensitivity of $^{13}C/^{15}N$ isotope analytical methods and the high background due to natural abundance of the heavy isotopes in the controls. To assess the overall fate and turnover at lower concentrations that were closer to environmentally relevant concentrations, additional water-sediment experiments at 3 mg/L (minimum ^{13}C and ^{15}N label detection limit) were prepared. Incubation experiments were conducted in the dark and at constant temperature (20°C) for 80 days. The bottles were sampled after 0, 5, 10, 20, 40 and 80 days (abiotic, blank, water and 3 mg/L systems only after 80 days). At each sampling time, the respective systems were destructively sampled, and the water and sediments were separated by filtration and subjected to further analyses. The CO_2 evolved from the mineralized glyphosate was trapped in 2 M NaOH; the NaOH solution was exchanged at regular intervals. Because the pH of the water was >7.0, a certain amount of CO_2 originating from the mineralization of glyphosate may partition into the water phase, which therefore has been analyzed in addition to the NaOH traps. Mineralization in biotic and abiotic systems includes $^{13}CO_2$ in both the sodium hydroxide and water phases.

Chemical analyses

A general mass balance of the ^{13}C and ^{15}N labels in the systems was set up based on the contents and isotopic compositions of CO_2, the extractable glyphosate and its metabolites and either ^{13}C or ^{15}N in the total NER. Proteins were hydrolyzed, and the amino acids (AA) were extracted and analyzed for their concentration and isotopic composition to estimate the extent that C and N from $^{13}C_3{}^{15}N$-glyphosate were incorporated into microbial biomass and ultimately into biogenic residues. Proteins are the main constituents of microbial biomass (50 % of cells); therefore, the quantification of biogenic residues formation was based on a factor of 2 for both ^{13}C and ^{15}N-amino acids (AA).

CO_2 measurements

The ^{13}C labeled CO_2 was quantified by measuring the total inorganic C in a 2 M NaOH solution on a total organic carbon analyzer. The isotopic composition of CO_2 (at % ^{13}C) was measured by

GC-combustion-isotope ratio mass spectrometry (GC-C-irMS; Finnigan MAT252 Thermo Electron coupled to a Hewlett Packard 6890 GC) with a Porabond Q-HT Plot FS column (50 m - 0.32 m - 5 µm).

Extractable glyphosate and AMPA

Glyphosate and AMPA were extracted with borate buffer (40 mM, pH 9.2) from sediments and derivatized with 0.5 mL of fluorenylmethyloxycarbonyl (Fmoc). The water samples were directly derivatized with Fmoc in borate buffer. The concentrations of glyphosate and AMPA were determined by UPLC-MS i-Class system (Waters, Manchester, UK) with an Acquity UPLC HSS T3 column (1.7 µm, 2.1 x 100 mm; Waters, Milford, MA, USA). The temperatures of the column and the autosampler were set at 60°C and 4°C, respectively. The injection volume was 10 µL. The eluents were 5 mM NH_4 acetate (pH 8) in water (eluent A) and methanol (eluent B). The flow rate was set to 0.6 mL/min. The gradient program was as follows: 0-3 min 5 % B, 7-8 min 95 % B, 8.1-10 min 5 % B. The MS analysis was performed using a Xevo TQ-S mass spectrometer (Waters, Manchester, UK) equipped with an ESI source in negative ion mode working in multiple reaction monitoring (MRM) mode. A capillary voltage of 2 kV and a desolvation temperature of 600°C were used. The flow of the desolvation gas was set at 1000 L/h. Unlabeled glyphosate and AMPA were used for calibration and as internal standards for correction of possible matrix effects which may occur during the measurement of glyphosate and AMPA concentrations. Transitions, cone voltages, and collision energies were automatically tuned for the compounds: $^{13}C_3{}^{15}N$-glyphosate (m/z 172 / m/z 154, cone: 58 V, collision energy: 10 V; m/z 172 / m/z 63, cone: 58 V, collision energy: 16 V) and $^{13}C_3{}^{15}N$-AMPA (m/z 112 / m/z 63, cone: 58 V, collision energy: 16 V; m/z 112 / m/z 79, cone: 58 V, collision energy: 10 V). The detection limit (LOD) of glyphosate was determined at 20 µg/L, and the LOD for AMPA was 30 µg/L based on the signal-to-noise method (signal >3 S/N). For the entire procedure, including the extraction of the sediment samples, the detection limits were 0.608 mg/kg (glyphosate) and 0.912 mg/kg (AMPA). The recovery of glyphosate and AMPA was >98 %. The values of the coefficient of determination (R^2) for all calibration curves were greater than 0.99. The relative error of UPLC-MS measurements was <10 %.

Non-extractable residues (NER)

After the extraction of glyphosate and AMPA, the sediment sample containing unextracted ^{13}C and ^{15}N label as NER was airdried. An aliquot of 4-5 mg was weighed and combusted using an elemental analyzer-combustion-isotope ratio mass spectrometer combination (EA-C-irMS; Euro EA 3000, Eurovector, Milano, Italy + Finnigan MAT 253, Thermo Electron, Bremen, Germany). Glyphosate-derived C and N were calculated as the excess ^{13}C and ^{15}N over the controls. The values of the coefficient of determination (R^2) for all calibration curves were greater than 0.99.

Amino acids (AA)

Amino acids were analyzed in the living microbial biomass AA fraction of sediment (bioAA) and in the total AA pool of the sediment fraction (tAA). Microbial biomass was extracted from the sediment with ion exchanger and sodium deoxycholate/polyethylenglycol solution. The sediment and microbial biomass pellets containing accordingly tAA and bioAA were hydrolyzed using 6 M HCl. Thereafter, the hydrolysate was purified over a cation exchange resin. The detailed extraction, purification and derivatization methods for bioAA and tAA were described previously. The identity and quantity of AA were measured using GC-MS, HP 6890 with a BPX-5 column. The isotopic composition of the respective AA (at % ^{13}C and at % ^{15}N) was determined by GC-C-irMS, Finnigan MAT 253 coupled to a Trace GC, with a BPX-5 column. The details on the analytical conditions for AA separation by GC-MS and GC-C-irMS are reported in Nowak et al. (2013). For quantification and identification of respective AA in samples, an external standard containing all detectable AA in the samples (alanine, glycine, threonine, valine, leucine, isoleucine, proline, aspartate, glutamate, phenylalanine and lysine) was used. The internal standard L-norleucine was added to each sample before hydrolysis to estimate the losses in soil AA analyses. The recovery of all measured AA was >90 %, except from threonine (>80 %). The measured isotopic compositions were corrected for shifts due to derivatization.

Data analyses and mass balance

All incubation experiments and chemical analyses were conducted in triplicate, and the data are presented as averages of three replicates. Mineralization, extractable and non-extractable $^{13}C_3$ ^{15}N-glyphosate residues were quantified for each sampling date in order to set up the full carbon and nitrogen mass balance, and to determine the compound degradation kinetics. The contents of the $^{13}CO_2$, ^{13}C and ^{15}N-NER, ^{13}C and ^{15}N-AA (bioAA + tAA) were based on quantitation of the total concentration of the respective carbon or nitrogen pool and on analyzing the excess of ^{13}C (^{15}N) over the controls (non-amended without glyphosate and unlabeled containing unlabeled glyphosate) as described by Lerch et al. (2009). The results were expressed as a percentage of ^{13}C or ^{15}N label relative to the initial $^{13}C_3$-glyphosate equivalents or ^{15}N-glyphosate equivalents. The total uncertainty of the carbon pool in CO_2 and of the carbon and nitrogen pools in NER was <10 %, whereas the total uncertainty of the determination of at % ^{13}C and at % ^{13}N isotope signatures was <0.5 % for unlabeled samples, but <3 % for the labeled ones. The relative average error of the label excess (based on Gaussian error propagation) was <10 % for CO_2 and NER.

The total uncertainty of carbon and nitrogen pool in tAA and bioAA was <15 %. The total uncertainty on the determination of at % ^{13}C and at % ^{13}N isotope analysis was <0.5 % for unlabeled samples, but <1 % for labeled ones. The relative average error of the label excess (based on Gaussian error propagation) was <10 % for tAA and bioAA.

The recovery of the ^{13}C and ^{15}N labels expressed as a percentage of the initially applied isotope label equivalents ranged from 93 to 110 % for C and from 86 to 110 % for N. Incorporation of the ^{13}C and ^{15}N labels into the microbial biomass and thus the total content of biogenic residues formed during degradation of $^{13}C_3{}^{15}N$-glyphosate in the water-sediment system were estimated from ^{13}C -tAA and ^{15}N-tAA, considering that AA constituted approximately 50 % of the total C and total N in the biomass. The recovery of microbial biomass extraction is estimated at 40 %. The bioAA results are presented both as the original data and the recalculated values based on 40 % extraction efficiency, but interpretation of bioAA was based on the original data.

Results and Discussion

Mineralization of $^{13}C_3$-glyphosate

Mineralization of $^{13}C_3$-glyphosate in the biotic water-sediment system consisted of three periods (Figure 1): an initial short lag-phase from day 0 to day 10 characterized by low mineralization rates (0.3 %/day), day 10-40 characterized by the highest mineralization rate (1.2 %/day), and day 40-80 characterized by decreasing mineralization rates to 0.4 %/day. At the end of incubation, a total of 56 % of the $^{13}C_3$-glyphosate had been mineralized. Abiotic processes played a minor role in the mineralization of $^{13}C_3$-glyphosate (<20 %). The mineralization rates of $^{13}C_3$-glyphosate in the water system (without sediment) were very low and increased slowly during the first ten days (~0.1 %/day). Thereafter, the mineralization rate decreased and only 2 % of $^{13}C_3$-glyphosate equivalents were mineralized at the end, demonstrating the key role of sediments in the mineralization of $^{13}C_3$-glyphosate. Mineralization of $^{13}C_3$-glyphosate at 3 mg/L was slightly higher (65 % of $^{13}C_3$-glyphosate equivalents) than at 50 mg/L. The acclimation period at 50 mg/L was longer (10 days vs. 5 days for 3 mg/L). The mineralization rate in the initial phase (0 – 10 days) was two-fold higher at 3 mg/L (0.6 %/day) than at 50 mg/L (0.3 %/day) and 1.3-fold higher in the second phase (10 – 40 days; 1.6 %/day compared to 1.2 %/day, respectively). In the third phase (40 – 80 days), the mineralization rate was 2-fold lower at 3 mg/L (0.2 %/day) than at 50 mg/L (0.4 %/day).To date, there are no reports on the mineralization of $^{13}C_3$-glyphosate in water-sediment systems. Although glyphosate was below the detection limit in the sediment and associated water used in the present experiments, prior exposure to this herbicide is very likely due to input from the agricultural area in the catchment, with major biodegradation occurring in the sediment phase. In contrast to the high $^{13}CO_2$ evolution from $^{13}C_3$-glyphosate equivalents, no or minimal mineralization of ^{15}N-glyphosate was found in the present study because the total recovery of the ^{15}N label ranged from 86 to 110 %.

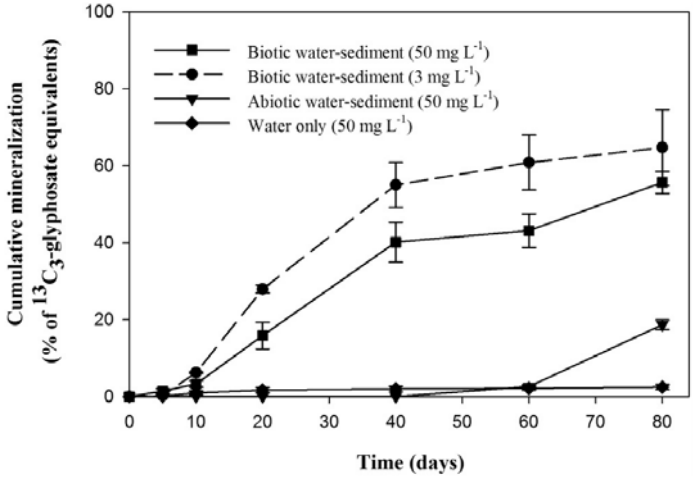

Figure 1. **Cumulative mineralization of $^{13}C_3$-glyphosate in water-sediment and water only systems over 80 days given as percentages of applied $^{13}C_3$-glyphosate equivalents.**

Turnover of $^{13}C_3{}^{15}N$-glyphosate

The content of $^{13}C_3{}^{15}N$-glyphosate in the biotic 50 mg/L water sediment system decreased rapidly until day 40 (Fig. 2A), indicating its low persistence reflected in its half-life (DT_{50}) of 15 days. In the water compartment, glyphosate dissipated rapidly during the first five days. Thereafter, elimination of this herbicide continued slowly until its ultimate removal by day 40. From day 40 onwards, $^{13}C_3{}^{15}N$-glyphosate was detected only in the sediment compartment although it was initially spiked in the water phase. This indicates that elimination from the water compartment was a combined process of sorption onto sediments and microbial transformation. A quick partitioning of glyphosate from the water compartment to the sediments had already been observed on day 0. At the initial sampling, which was performed 3 h after the addition of the glyphosate-spiked water to allow for particle sedimentation, 16 % of the initially added $^{13}C_3{}^{15}N$-glyphosate was already detected in the sediment phase. The high abundance of the silt + clay fraction (62 %), which is typically rich in oxides, of the sediments might explain the rapid elimination of glyphosate from the water by adsorption to the sediments. The turnover kinetics of $^{13}C_3{}^{15}N$-glyphosate in the sediment was much slower than in the water. A maximum amount of $^{13}C_3{}^{15}N$-glyphosate (51 % of the initially added $^{13}C_3{}^{15}N$ label) was detected in the sediments on day 5. Therefore, a potential risk by residual glyphosate in the sediment is given. Thereafter, elimination of $^{13}C_3{}^{15}N$-glyphosate from sediments was rapid (days 5-40), followed by a slower disappearance towards the end to ultimately result in 5 % of the initially added $^{13}C_3{}^{15}N$ label.

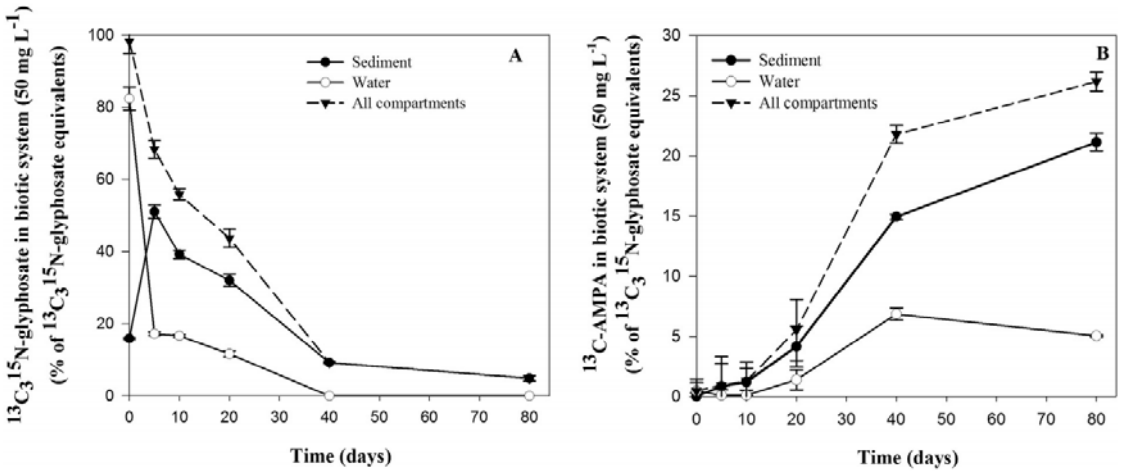

Figure 2. **Distribution of the extracted $^{13}C_3{}^{15}N$-glyphosate (A) and $^{13}C1$-AMPA (B) in biotic water-sediment systems (50 mg/L) over 80 days expressed as the percentage of applied $^{13}C_3{}^{15}N$-glyphosate. (Please note: ^{13}C-AMPA only contains one labeled carbon atom; the second metabolite glyoxylate contains the other two).**

The decrease in $^{13}C_3{}^{15}N$-glyphosate in both the water and sediment compartments during days 5-40 parallels the increasing mineralization of glyphosate. At the same time, a large amount of the recovered $^{13}C_3{}^{15}N$-glyphosate from the water-sediment system was associated with the sediments (~74 %), whereas only ~25 % was dissolved in the water. Finally, when $^{13}C_3{}^{15}N$-glyphosate was detected only in the sediment compartment (40 – 80 days), mineralization kinetics were slower indicating a limited bioavailability of glyphosate adsorbed onto sediment particles (Katagi, 2013). In the first ten days of the present experiments, only low contents of ^{13}C in AMPA (1 % of $^{13}C_3{}^{15}N$-glyphosate equivalents; Figure 2B) and ^{15}N in AMPA (4 % of $^{13}C_3{}^{15}N$-glyphosate equivalents) were observed. Thereafter, (10-40 days), a rapid increase in the $^{13}C_3{}^{15}N$-AMPA contents was noted and was accompanied by the rapid degradation of $^{13}C_3{}^{15}N$-glyphosate in the water-sediment system with concomitant $^{13}CO_2$ formation. From day 40 onwards, when glyphosate partitioned into the sediment and its mineralization rate decreased, the increase in the $^{13}C_3{}^{15}N$-AMPA contents slowed down. At the end of the experiment, ^{13}C in AMPA accounted for 26 % of the $^{13}C_3$-glyphosate equivalents. As only one of the three labeled ^{13}C atoms from the glyphosate, but all of the ^{15}N (one atom) is retained in AMPA (Figure 4) and the percentages are referred to the initial amount of labeled atoms (not molecules), the percentage of ^{15}N-AMPA was generally 3-fold higher than that of ^{13}C-AMPA and thus amounted to 79 % of the initially added ^{15}N-glyphosate. Similar to $^{13}C_3{}^{15}N$-glyphosate, the recovered $^{13}C_3{}^{15}N$-AMPA from the system was mostly associated to the sediment (70-90 %), whereas the residual (10-30 %) was dissolved in the water phase. In contrast to $^{13}C_3{}^{15}N$-glyphosate, $^{13}C_3{}^{15}N$-AMPA was more persistent; this was indicated by its continuous increase until the end of the experiment, indicating that $^{13}C_3{}^{15}N$-AMPAwas degraded more slowly than it was produced from glyphosate, as reported earlier (Mamy et al.,2005). Unfortunately, our data do not allow quantification of microbial AMPA degradation due to the simultaneous formation and degradation. Due to the continuing production of AMPA at a higher rate than degradation, a potential risk may be given by this metabolite. Compared to the biotic systems, the abiotic controls, water without sediment and biotic systems at 3 mg/L showed much lower formation of $^{13}C_3{}^{15}N$-AMPA from glyphosate.

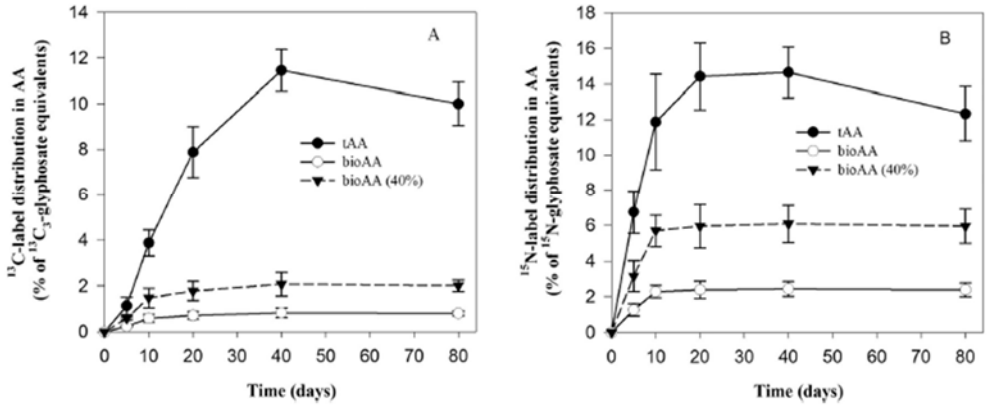

Figure 3. **Time dependent ^{13}C- (A) and ^{15}N-label (B) incorporation into tAA, bioAA and recalculated bioAA (40 % extraction efficiency) during microbial degradation of $^{13}C_3{}^{15}N$-glyphosate in biotic water-sediment system (50 mg/L) expressed as the percentage of applied $^{13}C_3{}^{15}N$-glyphosate equivalents.**

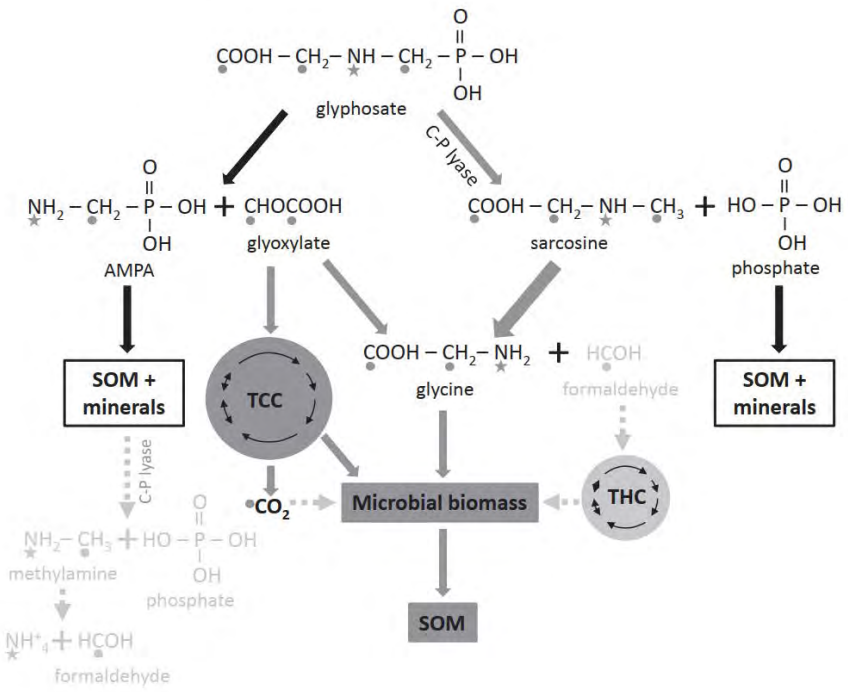

Figure 4. **Pathways of microbial degradation of glyphosate through sarcosine and AMPA in biotic water-sediment system (50 mg/L). Dark grey arrows: biogenic residue formation; black arrows: xenobiotic NER formation; TCC: tricarboxylic acid cycle; THC: tetrahydrofolate cycle; grey circles = ^{13}C label; grey stars = ^{15}N label; Light grey = presumed further degradation.**

Incorporation of the ^{13}C and ^{15}N labels into AA and biogenic residues

The total AA pool of sediment (tAA) includes the AA in the living biomass and in the dead and decaying necromass. Neither ^{13}C nor ^{15}N enrichment in AA was detected in the suspended particles in either the water compartment of the abiotic sediment-water systems or the water systems. In the biotic water-sediment systems, ^{13}C label incorporation into the bioAA fraction was observed in the first sampling, and the contents of ^{13}C-bioAA increased rapidly to a maximum on day 40 (0.83 % of $^{13}C^{3}$-glyphosate equivalents; Figure 3A). A continuous flux of ^{13}C--labeled AA from the living biomass to the non-living fraction OM was noted from day 5 onwards. ^{13}C-tAA initially increased sharply whereas from day 20 onwards, the ^{13}C-bioAA remained nearly constant, and approximately 92 % of the ^{13}C label in the tAA could be attributed to the non-living OM. In contrast to the ^{13}C bioAA, the ^{13}C-tAA contents slightly decreased after 40 days. At the end of the experiment, the contents of ^{13}C in tAA reached 10 % of the initially added $^{13}C_3$-glyphosate. Considering a protein content of 50 % in bacterial cells (Nowak et al., 2013), we arrive at a total of 20 % ^{13}C-biogenic residues at the end of the experiment. Similar to ^{13}CAA, incorporation of the ^{15}N label into bioAA and tAA was also observed starting from day 5 (Figure 3B). In contrast to ^{13}C-bioAA, ^{15}N-bioAA contents plateaued on day 10 (2.38 % of ^{15}N-glyphosate equivalents). The incorporation of ^{15}N-bioAA into the non-living OM fraction was also similar to that of ^{13}C-bioAA, starting rapidly (on day 5), and approximately 81 % of the ^{15}N-tAA was stabilized in the non-living OM at the end. The rapid initial increase in ^{15}N-tAA continued until day 20 and then remained stable until day 40. Analogous to ^{13}C-tAA, ^{15}N-tAA decreased slightly towards the end.

^{15}N-tAA amounted to 12 % of the initially added ^{15}N-glyphosate at the end (similar to ^{13}C-tAA), and 24 %was observed for ^{15}N-biogenic residues based on a conversion factor of two for biomass in general. The dominant incorporation of both the ^{13}C and ^{15}N labels into the glycine was observed throughout the experiment and was most pronounced in the initial incubation period (see Tables 1 and 2A, B). Various AA were progressively enriched in both isotopes over time. In general, incorporation of ^{15}N proceeded faster than that of ^{13}C (2A, 2B). In contrast to ^{13}C, the ^{15}N label disappeared from $^{13}C^{15}$N-glycine quickly and was distributed within different AA more rapidly than the ^{13}C label.

The results based on the ^{13}C- and ^{15}N-colabeling technique allowed comprehensive insight into the C and N fluxes from the colabeled $^{13}C_3{}^{15}N$ -glyphosate via microbial biomass to the non-living OM. Microorganisms assimilated the carbon and nitrogen from glyphosate to synthesize biomass compounds, as shown by the ^{13}C and ^{15}N-labeled bioAA. After death and cell lysis, their biomass constituents were progressively incorporated into the non-living OM fraction where they were stabilized and ultimately formed non-toxic biogenic residues.

Table 1. ^{13}C label distribution in diverse ^{13}C-bioAA (A) and ^{13}C-AA in the non-living SOM (B) during biodegradation of $^{13}C_3$-glyphosate in biotic water-sediment system (50 mg/L).

(A)

Incubation time (days)	^{13}C-bioAA (% of $^{13}C_3$-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.06 (±0.01)	0.07 (±0.00)	0.07 (±0.02)	0.07 (±0.00)	0.06 (±0.01)
Glycine	n.d.	**0.17** (±0.02)	**0.18** (±0.01)	0.14 (±0.01)	0.15 (±0.03)	**0.11**↓ (±0.01)
Threonine	n.d.	n.d.	n.d.	0.07 (±0.00)	0.06 (±0.01)	0.06 (±0.02)
Valine	n.d.	n.d.	0.10 (±0.02)	0.10 (±0.01)	0.09 (±0.02)	0.09 (±0.00)
Leucine	n.d.	n.d.	n.d.	n.d.	0.14 (±0.00)	0.12 (±0.01)
Isoleucine	n.d.	n.d.	0.11 (±0.00)	0.14 (±0.00)	0.05 (±0.01)	0.04 (±0.02)
Proline	n.d.	n.d.	n.d.	0.05 (±0.03)	0.04 (±0.01)	0.09 (±0.00)
Aspartate[a]	n.d.	n.d.	0.02 (±0.00)	0.03 (±0.00)	0.04 (±0.02)	0.08 (±0.01)
Glutamate[b]	n.d.	n.d.	0.06 (±0.03)	0.07 (±0.03)	0.05 (±0.02)	0.05 (±0.00)
Phenylalanine	n.d.	n.d.	n.d.	n.d.	0.10 (±0.03)	0.07 (±0.01)
Lysine	n.d.	n.d.	0.04 (±0.01)	0.04 (±0.03)	0.04 (±0.06)	0.03 (±0.01)
Total	**n.d.**	**0.23 (± 0.03)**	**0.58 (± 0.07)**	**0.71 (± 0.13)**	**0.83 (± 0.21)**	**0.80 (± 0.10)**

(B)

Incubation time (days)	^{13}C-non-living AA (% of $^{13}C_3$-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.4 (±0.1)	0.6 (±0.1)	1.3 (±0.3)	1.4 (±0.1)	0.7 (±0.1)
Glycine	n.d.	**0.5** (±0.2)	**1.0** (±0.1)	1.0 (±0.1)	0.5 (±0.1)	0.6 (±0.2)
Threonine	n.d.	n.d.	n.d.	0.4 (±0.1)	0.1 (±0.1)	0.5 (±0.1)
Valine	n.d.	n.d.	n.d.	1.0 (±0.6)	1.1 (±0.1)	1.3 (±0.3)
Leucine	n.d.	n.d.	n.d.	n.d.	1.6 (±0.3)	1.2 (±0.1)
Isoleucine	n.d.	n.d.	n.d.	0.7 (±0.1)	1.1 (±0.1)	0.9 (±0.3)
Proline	n.d.	n.d.	0.5 (±0.1)	0.5 (±0.1)	0.5 (±0.1)	0.3 (±0.1)
Aspartate[a]	n.d.	n.d.	0.5 (±0.1)	0.5 (±0.1)	1.1 (±0.2)	0.8 (±0.1)
Glutamate[b]	n.d.	n.d.	0.7 (±0.1)	0.5 (±0.1)	1.1 (±0.1)	1.6 (±0.2)
Phenylalanine	n.d.	n.d.	n.d.	n.d.	1.0 (±0.3)	0.6 (±0.1)
Lysine	n.d.	n.d.	n.d.	1.3 (±0.1)	1.3 (±0.4)	0.9 (±0.3)
Total	**n.d.**	**0.9 (± 0.3)**	**3.3 (± 0.5)**	**7.2 (± 1.6)**	**10.8 (± 2.0)**	**9.4 (± 1.9)**

[a] Incl. Asparagine.
[b] Incl. Glutamine; n.d. - not detectable; values are presented as averages ± standard deviation; values printed in bold show characteristic values; arrows illustrates increases or decreases of the respective AA compared to the preceding sampling event.

Table 2. ^{15}N label distribution in diverse ^{15}N-bioAA (A) and ^{15}N-AA in the non-living SOM (B) during biodegradation of ^{15}N-glyphosate in biotic water-sediment system (50 mg/L).

(A)

Incubation time (days)	^{15}N-bioAA (% of ^{15}N-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.01 (±0.08)	0.09 (±0.1)	0.03 (±0.02)	0.15 (±0.02)	0.23 (±0.07)
Glycine	n.d.	**0.70** (±0.07)	**1.20**↑(±0.01)	**1.20** (±0.09)	**0.80**↓(±0.10)	**0.43**↓(±0.07)
Threonine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valine	n.d.	0.38 (±0.07)	0.28 (±0.03)	0.28 (±0.02)	0.30 (±0.05)	0.28 (±0.02)
Leucine	n.d.	0.17 (±0.02)	0.17 (±0.04)	0.14 (±0.07)	0.40 (±0.09)	0.38 (±0.03)
Isoleucine	n.d.	n.d.	0.30 (±0.01)	0.28 (±0.01)	0.09 (±0.07)	0.13 (±0.06)
Proline	n.d.	n.d.	0.04 (±0.04)	0.04 (±0.13)	0.05 (±0.04)	0.22 (±0.05)
Aspartate[a]	n.d.	n.d.	n.d.	0.13 (±0.06)	0.10 (±0.09)	0.22 (±0.10)
Glutamate[b]	n.d.	n.d.	0.10 (±0.03)	0.19 (±0.07)	0.19 (±0.08)	0.20 (±0.02)
Phenylalanine	n.d.	n.d.	0.06 (±0.03)	0.08 (±0.02)	0.21 (±0.04)	0.19 (±0.02)
Lysine	n.d.	n.d.	0.04 (±0.03)	0.01 (±0.01)	0.14 (±0.05)	0.10 (±0.08)
Total	**n.d.**	**1.26 (±0.24)**	**2.28 (±0.32)**	**2.38 (±0.5)**	**2.43 (±0.63)**	**2.38 (±0.52)**

(B)

Incubation time (days)	^{15}N-non-living AA (% of ^{15}N-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.2 (±0.1)	0.6 (±0.1)	0.4 (±0.1)	0.8 (±0.2)	0.2 (±0.1)
Glycine	n.d.	**1.1** (±0.1)	1.2 (±0.2)	1.2 (±0.1)	**0.8**↓(±0.1)	**0.7**↓ (±0.1)
Threonine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valine	n.d.	0.8 (±0.1)	1.4 (±0.2)	1.5 (±0.5)	1.3 (±0.2)	1.0 (±0.2)
Leucine	n.d.	0.7 (±0.1)	1.2 (±0.8)	2.8 (±0.4)	1.8 (±0.1)	1.5 (±0.1)
Isoleucine	n.d.	n.d.	1.5 (±0.1)	1.2 (±0.2)	1.6 (±0.1)	1.4 (±0.3)
Proline	n.d.	n.d.	0.6 (±0.1)	0.8 (±0.1)	0.8 (±0.1)	0.4 (±0.1)
Aspartate[a]	n.d.	n.d.	0.1 (±0.2)	0.5 (±0.1)	1.0 (±0.1)	0.6 (±0.2)
Glutamate[b]	n.d.	**1.2** (±0.1)	1.0 (±0.1)	0.7 (±0.1)	1.4 (±0.1)	1.8 (±0.1)
Phenylalanine	n.d.	n.d.	0.7 (±0.1)	1.4 (±0.1)	1.4 (±0.1)	1.2 (±0.1)
Lysine	n.d.	n.d.	0.1 (±0.1)	1.6 (±0.1)	1.7 (±0.1)	0.9 (±0.1)
Total	**n.d.**	**4.0 (±0.5)**	**8.4 (±2.0)**	**12.1 (±1.8)**	**12.6 (±1.2)**	**9.7 (±1.4)**

[a] Incl. Asparagine.
[b] Incl. Glutamine; n.d. - not detectable; values are presented as averages ± standard deviation; values printed in bold show characteristic values; arrows illustrates increases or decreases of the respective AA compared to the preceding sampling event.

Indication of different (bio)degradation pathways of glyphosate in water-sediment systems

Based on the detailed glyphosate turnover mass balance and the patterns of ^{13}C and ^{15}N labeled AA over time, particularly of the dominant glycine, we could distinguish between two degradation pathways of this herbicide in water-sediment system. The dominance of co-labeled ^{13}C^{15}N-glycine especially in the first sampling event indicates its formation via the sarcosine pathway (Borggaard and Gimsing, 2008; Singh and Walker, 2006; see also Figure 4). However, the occurrence of the sarcosine pathway in soil or sediment has not yet been proven (Borggaard and Gimsing, 2008; Singh andWalker, 2006). We could not detect sarcosine in our experiment, but this compound is rapidly oxidized to glycine and thus does not accumulate. The formed glycine is directly incorporated into microbial biomass, resulting in the observed occurrence of co-labeled ^{13}C^{15}N-glycine in the living biomass AA. The negligible mineralization (3 % of ^{13}C equivalents initially applied) with high simultaneous removal of $^{13}C_3{}^{15}$N -glyphosate and the maximum contents of ^{13}C and ^{15}N glycine on day 10 also support the hypothesis that glyphosate is initially degraded via the sarcosine pathway. Hence, the sarcosine pathway was actually contributing at the beginning of glyphosate degradation, whereas the AMPA pathway dominated in the later degradation phase. A later decrease of co-labeled $^{13}C_3{}^{15}$N-glycine (10-20 days) was accompanied by a rapid increase in AMPA over time.

The risk potential of glyphosate residues in water-sediment systems

To date, there is no detailed information on the metabolic fate of glyphosate residues and their distribution in the water-sediment system. The present results provide detailed insight into the biodegradation processes of $^{13}C_3{}^{15}$N-glyphosate in the water-sediment system and into the transformation of this herbicide into AMPA, microbial biomass and NER. Since glyphosate is biodegraded and the NER are dominantly biogenic residues, the highest potential risk is provided by the significant concentrations of AMPA.

Non-extractable $^{13}C_3$-glyphosate residues were formed immediately (6 % of the initially added ^{13}C label, see Table 3). The NER contents increased until day 10 and then remained on a high level. From day 20 onwards, their contents decreased and ultimately reached 23 % of the $^{13}C_3$-glyphosate equivalents. The chemical composition of the NER formed during degradation of glyphosate is not yet known, and their

analyses are limited to quantification. In the present study, glyphosate was initially a source of xenobiotic ^{13}CNER formation that was dominant until day 10 (Figure 5A). However, immobilized glyphosate in the NER was microbially degradable, as shown by the continuous decrease of xenobiotic NER over time, specifically of ^{13}C-xenobiotic NER. Microorganisms used the carbon and nitrogen from $^{13}C_3{}^{15}$N-glyphosate to synthesize their biomass compounds, as shown by the ^{13}C and ^{15}N incorporation into microbial AA, leading to biogenic residues in OM after cell death and lysis. Based on the ^{13}C-tAA content, 20 % of the ^{13}C-biogenic residues were formed and constituted the major fraction of ^{13}C-NER (87 %, Figure 5A). These results agree with previous studies on biogenic residue formation during biodegradation of ^{13}C-labeled pesticides or pharmaceuticals.

Table. 3. **Mass balance of $^{13}C_3{}^{15}$N-glyphosate degradation in biotic and abiotic water-sediment systems and in water over 80 days (% of initially applied ^{13}C- and ^{15}N-label equivalents).**

		% of initial ^{13}C				
	Time (days)	**Mineralization**	**Glyphosate**	**AMPA**	**NER**	**Recovery**
	0	n.d.	98 (± 1)	0.2 (±0.1)	6 (± 1)	104 (± 2)
Biotic	**5**	1 (± 1)	68 (± 2)	1 (± 1)	28 (± 1)	98 (± 4)
	10	3 (± 1)	56 (± 2)	1 (± 1)	33 (± 5)	93 (± 7)
	20	16 (± 0)	44 (± 2)	6 (± 1)	35 (± 1)	101 (± 5)
	40	40 (± 1)	9 (± 1)	22 (± 1)	31 (± 1)	102 (± 4)
	80	**56 (± 3)**	**5 (± 1)**	**26 (± 1)**	**23 (± 2)**	**110 (± 6)**
Abiotic	**80**	19 (± 1)	41 (± 2)	11 (± 1)	26 (± 0)	97 (± 3)
Only water	**80**	2 (± 1)	90 (± 2)	2 (± 0)	n.d.	94 (± 3)

		% of initial ^{15}N			
	Time (days)	**Glyphosate**	**AMPA**	**NER**	**Recovery**
	0	98 (± 1)	1 (± 1)	5 (± 0)	104 (± 2)
Biotic	**5**	68 (± 2)	3 (± 1)	20 (± 2)	91 (± 6)
	10	56 (± 2)	4 (± 1)	26 (± 3)	86 (± 6)
	20	44 (± 2)	17 (± 3)	30 (± 1)	91 (± 6)
	40	9 (± 1)	65 (± 4)	31 (± 0)	105 (± 5)
	80	**5 (± 1)**	**79 (± 5)**	**26 (± 0)**	**110 (± 6)**
Abiotic	**80**	41 (± 2)	33 (± 1)	26 (± 3)	100 (± 6)
Only water	**80**	90 (± 2)	6 (± 1)	n.d.	96 (± 2)

n.d. - not detectable; values are shown as averages ± standard deviation

The kinetics of ^{15}N-NER formation showed a similar pattern to that of ^{13}C-NER and reached 26 % of the initially added ^{15}N-glyphosate (Table. S2). Analogous to the ^{13}C-biogenic residues, the ^{15}N-NER were primarily biogenic (Figure 5B); at the end of the experiment, the ^{15}N-biogenic residues amounted to 24 % of ^{15}N-glyphosate equivalents and constituted 92 % of the ^{15}N-NER. In contrast to the ^{13}C-biogenic residues, the ^{15}N-biogenic residues were formed rapidly, which is in line with the metabolization and mineralization of $^{13}C_3{}^{15}$N-glyphosate via the sarcosine pathway without N mineralization in the initial degradation phase. The contents of extractable $^{13}C_3{}^{15}$N-glyphosate residues (31 % of the $^{13}C_3$-glyphosate equivalents and 84 % of the ^{15}N-glyphosate equivalents) comprised a large proportion of the ^{13}C and ^{15}N-isotope mass balance at the end, with AMPA accounting for almost all of these residues (Table. S2). The percentage of ^{15}N-AMPA was 3-fold higher than that of ^{13}C-AMPA because only one out of three ^{13}C atoms, but all ^{15}N atoms from the co-labeled glyphosate are transferred to AMPA during metabolization (see Figure 4). In the sediment-water systems nearly all of the NER could be explained by biogenic residues bearing no potential risk. However, high contents of extracted AMPA were detected, which typically biodegrades slower than glyphosate. The detailed fate of AMPA needs to be investigated to assess the potential risks related to this degradation product of glyphosate. In contrast to previous studies in which biogenic residues remained constant, ^{13}C and ^{15}N biogenic

residues from glyphosate slightly decreased towards the end of the experiment. Total hydrolysable ^{13}C- and ^{15}N-labeled AA decreased progressively after 69 days in sediments incubated with ^{13}C-glucose and ^{15}N-labeled ammonium, which is in agreement with the present study.

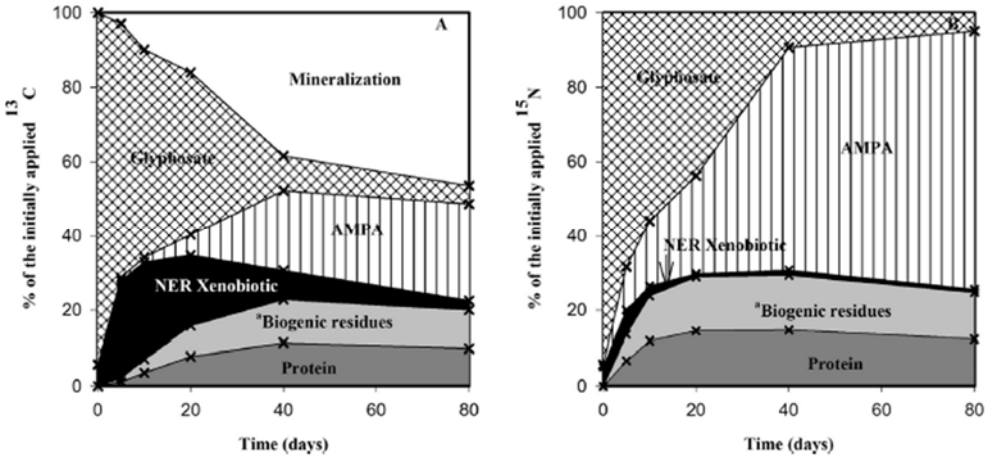

Figure 5. **Detailed mass balance including biogenic residue formation (A) of $^{13}C_3$-glyphosate and (B) of ^{15}N-glyphosate in biotic water-sediment system (50 mg/L). a: Biogenic residues were calculated based on a conversion factor of 2 for proteins.**

Conclusions

- This is the first detailed glyphosate turnover mass balance including NER speciation in water-sediment systems using stable isotope co-labeled tracers (^{13}C and ^{15}N).

- Sediment plays a key role in the microbial degradation of glyphosate via both the sarcosine and AMPA pathway.

- At the end, nearly all of the NER can be assigned to non-toxic biogenic residues after degradation of the parent compound.

- Accumulation of main metabolite of glyphosate, AMPA, may because for environmental concern; therefore, an additional investigation of the fate of AMPA in water-sediment systems is needed.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article reports the results from a water-sediment dissipation experiment with ^{13}C-^{15}N-labelled-glyphosate, conducted according to OECD guideline 308. The methods and results are generally well described and conclusive. However, the water and associated sediment were taken from a German small water body located in agricultural lowlands with continuous crop rotation and pesticide application, which is considered a high risk area for exposure to pesticides. Thus, it cannot be excluded that the water/sediment system received inputs of glyphosate or AMPA within the previous 4 years, as is required in OECD 308 guideline. Further, for the main experiment, the application rate was extremely high (50 mg/L, equivalent to an application rate of 150 kg/ha when assuming overspray of a 30 cm deep water body) while in OECD 308 guideline it is recommended that the test concentration should be close to application rate or environmental concentrations.

The article is therefore classified as reliable with restrictions (Category 2).

E-Fate: Reliability criteria for the detailed assessment of full-text documents

Data requirements (indicated by the corresponding EU data point)	Criteria for "Reliable" articles	Criteria met? Yes / No / Uncertain
General criteria for reliability considered for all data requirements indicated by the corresponding EU data points as specified in EC Regulation (EU) No 283/2013	1. For guideline-compliant studies (GLP studies): OECD, OPPTS, ISO, and others. The validity/quality criteria listed in the corresponding guidelines met.	No
	2. Previous exposure to other chemicals is documented (where relevant).	No
	3. The test substance is dissolved in water or non-toxic solvent	Yes
	4. Glyphosate, when the test substance, is sufficiently documented - identity of the test material reported (i.e. purity, source, content, storage conditions)	Yes
	5. Only glyphosate is the tested substance (excluding mixture), and information on application of glyphosate is described	Yes
	6. The endpoint measured can be considered a consequence of glyphosate (or a glyphosate metabolite)	Yes
	7. Study design / test system is well described, including when relevant: concentration in exposure media (dose rates, volume applied, etc.), dilution/mixture of test item (solvent, vehicle) where relevant.	Yes
	8. Analytical verifications performed in test media (concentration)/ collected samples, stability of glyphosate in test media documented	Yes
	9. An endpoint can be derived. Findings do deliver a regulatory endpoint, and/or is useful as supporting information	Yes
	10. Assessment of the statistical power of the assay is possible with reported data.	Yes
	11. If statistical methodology was applied for findings reported, then the data analysis applied is clearly reported (e.g., checking the plots and confidence intervals)	Yes
	12. Field locations relevant/comparable to European conditions. Soils not completely matching the OECD criteria but from Europe or to some extent representative for the European Agriculture.	Yes
	13. Characterization of soil: texture (sandy loam, silty loam, loam, loamy sand), pH (5.5-8.0), cation exchange capacity, organic carbon (0.5-2-5 %), bulk density, water retention, microbial biomass (~1 % of organic carbon)	No
	14. Other soils where information on characterization by the parameters: pH, texture, CEC, organic carbon, bulk density, water holding capacity, microbial biomass	No
	15. For tests including agricultural soils, they should not have been treated with test substance or similar substances for a minimum of 1 year	No
	16. For soil samples, sampling from A-horizon, top 20 cm layers; soils freshly from field preferred (storage max 3 months at 4 +/- 2°C).	No
	17. Data on precipitation is recorded	No
	18. The temperature was in the range between 20-25°C and the moisture was reported	Yes
	19. The presence of glyphosate identified in samples collected from groundwater, soil, surface waters, sediments or air from European areas	Yes
	20. Analytical results present residues measurements which can be correlated with the existing residues definition of glyphosate	Yes

E-Fate: Reliability criteria for the detailed assessment of full-text documents

Data requirements (indicated by the corresponding EU data point)	Criteria for "Reliable" articles	Criteria met? Yes / No / Uncertain
	21. Analytical methods clearly described and adequate Statement of specificity and sensitivity of the analytical methods is included	Yes
	22. Radiolabel characterization: purity, specific activity, location of label	Yes
	23. If degradation kinetics are included: expect to see data tables provided, model description. Statistical parameters for kinetic fit.	Yes
	24. Glyphosate monitoring data: description of matrix analysed, and analytical methods fully described as above.	No
	25. For environmental fate studies: clear description of application rate and relevance to approved uses.	Yes

1. Information on the study

Data point:	KCA 7.1.3.1.1
Report author	Zhelezova, A., et al.
Report year	2017
Report title	Effect of Biochar Amendment and Ageing on Adsorption and Degradation of Two Herbicides
Document No	Water Air Soil Pollut (2017) 228: 216
Guidelines followed in study	OECD 106 (2000)
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (not all validity criteria reported for the sorption experiment, no guideline followed, no endpoint can be derived according to current guidance for the degradation experiment)

2. Full summary of the study according to OECD format

Biochar amendment can alter soil properties, for instance, the ability to adsorb and degrade different chemicals. However, ageing of the biochar, due to processes occurring in the soil over time, can influence such biochar-mediated effects. This study examined how biochar affected adsorption and degradation of two herbicides, glyphosate (N-(phosphonomethyl)-glycine) and diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) in soil and how these effects were modulated by ageing of the biochar. One sandy and one clayey soil that had been freshly amended with a wood-based biochar (0, 1, 10, 20 and 30% w/w) were studied. An ageing experiment, in which the soil-biochar mixtures were aged for 3.5 months in the laboratory, was also performed. Adsorption and degradation were studied in these soil and soil-biochar mixtures, and compared to results from a soil historically enriched with charcoal. Biochar amendment increased the pH in both soils and increased the water-holding capacity of the sandy soil. Adsorption of diuron was enhanced by biochar amendment in both soils, while glyphosate adsorption was decreased in the sandy soil. Ageing of soil-biochar mixtures decreased adsorption of both herbicides in comparison with freshly biochar-amended soil. Herbicide degradation rates were not consistently affected by biochar amendment or ageing in any of the soils. However, glyphosate half-lives correlated with the Freundlich Kf values in the clayey soil, indicating that degradation was limited by availability there.

Materials & Methods

Soil Sampling and Processing

The soil samples were collected in September 2015 from arable fields at two locations: Länna (L) (59° 52′ N, 17° 58′ E) and Ulleråker (U) (59° 49′ N, 17° 39′ E). Soil sampling at L was performed in two parts of the arable field: an untreated part (L) and a historically charcoal-enriched part (LB). Because of the long-term charcoal amendment, the latter soil was characterised by lower bulk density and higher loss on ignition and water-holding capacity (WHC) than the unamended soil from the same field, which leads to higher yields in dry years. In each soil, about 10 samples were taken from the upper layer (5–15 cm below surface) and pooled. After sieving, the Ø < 2 mm fraction was homogenised and stored at −20 °C in plastic bags until the start of the experiment. Moisture content and WHC were measured for all soil samples. Moisture content was determined by drying at 110 °C for 10 h, while WHC was defined as the moisture content after saturation of 30 g soil with distilled water for 10 h followed by 4 h of free drainage. Chemical and physical properties of the three soils studied (L, LB, U) were determined by a commercial laboratory and are presented in Tables 1 and 2.

Preparation and Ageing of Soil-Biochar Mixtures

The biochar used was the commercial product *Skogens kol*, which is produced from a mixture of about 80% hardwood, mainly birchwood (*Betula* sp.) and 20% wood from Norway spruce (*Picea abies*), by slow pyrolysis with a maximum process temperature of 380– 430 °C (Cederlund et al. 2016). Soil-biochar mixtures were prepared by mixing soil (L and U) with sieved biochar (Ø < 2 mm) at a rate of 1, 10, 20 and 30% biochar per unit soil dry weight (designated L1, L10, L20 and L30 and U1, U10, U20 and U30). WHC was determined as described above and pH for all mixtures was measured in a 1:2 slurry of soil and distilled water (*w*/*v*) after shaking and stabilisation for 10 h. Biochar ageing was performed with soil-biochar mixtures made from U soil. These mixtures were incubated in darkness at 20 °C for 3.5 months. The moisture content was adjusted to 55% of WHC and monitored and adjusted weekly by addition of deionised water.

Chemicals Used in Herbicide Adsorption and Degradation Experiments

Glyphosate (N-(phosphonomethyl)-glycine, CAS [1071-83-6], 98%) and diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), CAS [330-54-1], 99.0%) were provided by Dr. Ehrenstorfer GmbH, Augsburg, Germany. ^{14}C-labelled diuron ([ring-U-^{14}C], 96.4%, 5.71 MBq/mg) and glyphosate ([P- methylene-^{14}C], 4.87 MBq/mg) were provided by the Institute of Isotopes Co. Ltd., Budapest, Hungary.

Measurement of Herbicide Adsorption in Soils and Soil-Biochar Mixtures

Adsorption was determined in a batch-equilibrium system according to OECD guideline 106 (OECD 2000). A pre-study was performed to estimate the time when the equilibrium between adsorbed herbicide and herbicide in solution was reached (8, 24 and 32 h). In all cases, equilibrium was reached within 24 h. For high-percentage soil-biochar mixtures with U soil, an additional pre-study was performed to estimate an appropriate soil to solution ratio as defined in the OECD guideline. Soil and soil-biochar mixtures, corresponding to 1 g of soil or mixture dry weight, were weighed into tubes (15-mL glass tubes for diuron and 50-mL polypropylene tubes for glyphosate) and adjusted with 0.01 M $CaCl_2$ to reach the appropriate soil-solution ratio. This was 1:40 for all samples with glyphosate and for U20, U30, U20a and U30a with diuron and 1:4 for all other samples with diuron. The samples were shaken for 24 h (20 °C, 200 revolutions/min). After that, herbicides were added to reach concentrations of 1, 5, 10, 50 and 100 μg/g dry weight (dw) soil for glyphosate and 0.1, 0.5, 1, 5 and 10 μg/g dw soil for diuron, due to its lower water solubility. In addition, a fixed amount (10 μL for glyphosate and 20 μL for diuron) of ^{14}C- labelled herbicide was added to each tube to reach an activity of 2000 DPM (3.333×10^{-5} MBq) per sample. There were two replicate tubes of each concentration. After 24 h, the tubes were centrifuged (3000 revolutions/min for 30 min), samples of supernatant were transferred to scintillation vials (4 mL for diuron and 10 mL for glyphosate samples) and Quicksafe A (Scintvaruhuset, LAB-service, Uppsala, Sweden) was added directly before measurement of scintillation. ^{14}C activity was measured on a Beckman LS 6000TA liquid scintillation counter (Beckman Counter Inc., Fullerton, CA). Controls without herbicides were measured for all samples to exclude the level of background radioactivity. The data obtained were fitted using the linear form of the Freundlich isotherm.

Herbicide Degradation Experiment

The herbicides were dissolved in water (glyphosate) or methanol (diuron) and added dropwise to a fraction (10%) of the soils and soil-biochar mixtures. Water and methanol were allowed to evaporate from the samples for 10 h. The herbicide-treated part was then mixed with the rest of each sample to give an initial nominal concentration of 10 mg/kg soil dry weight. Portions of soil corresponding to 5 g of dry weight were weighed into 50-mL plastic tubes and the water content was adjusted to 60% of WHC and kept at this level for the duration of the experiment. The tubes were sealed with caps and were incubated at 20 °C in the dark. After 1, 2, 5, 8, 16, 23 (only for U samples) and 31 days of incubation, two replicate tubes from each treatment were placed in the freezer (−20 °C) for future extraction and analysis.

Table 1. Chemical properties of the soils studied

Soil	Code	HCl extracted K (mg 100 g^{-1})	HCl extracted P (mg 100 g^{-1})	Al-K[a] (mg 100 g^{-1})	Al-P[a] (mg 100 g^{-1})	Total C (%)	Total N (%)	pH
Charcoal-amended soil from Länna	LB	68.35	85.02	3.82	16.48	17.57	0.37	5.57
Untreated soil from Länna	L	229.36	78.77	37.28	16.67	4.86	0.34	5.27
Soil from Ulleråker	U	287.59	68.16	34.95	4.87	1.36	0.1	6.41

[a] Al–K/Al-P = ammonium lactate-extractable K and P—Swedish standard method for estimation of plant available K and P fractions (Otabbong et al. 2009)

Table 2. Physical properties of the soils studied

Soil code	Clay Ø < 0.002 mm	Fine silt 0.002–0.006 mm	Medium silt 0.006–0.02 mm	Coarse silt 0.02–0.06 mm	Fine sand 0.06–0.2 mm	Medium sand 0.2–0.6 mm	Coarse sand 0.6–2 mm	Loss on ignition %
LB	n.d.	n.d.	n.d.	n.d.	5.9	3.3	4.1	39.4
L	66.5	14.8	9.1	6.5	2.1	0.7	0.3	13.7
U	7.5	3.2	2.4	3.2	12.1	63.8	7.8	3.3

n.d. not determined

Data from the degradation experiment after recovery correction were used to estimate herbicide half-life. Recovery was calculated as:

$$\text{Recovery} = \left(\frac{C_0}{C_{\text{nominal}}}\right) \times 100$$

where *C0* is the herbicide concentration determined at day 0. Natural logarithms of remaining concentrations for days 0–31 were plotted against time, giving the first- order rate constant *k* as the slope of the linear regression line. Half-life (*T*½) was calculated as:

$$T^{1}/_{2} = \frac{\ln 2}{k}$$

Analysis of Diuron

For diuron extraction from soil and soil-biochar mixtures, the following protocol was used: 10 mL methanol were added using a Vogel pipette to the tubes with sample. The tubes were shaken at 200 revolutions/min for 60 min, centrifuged at 4000 revolutions/min for 10 min and the supernatant was filtered (OOH Whatman; 11 cm). Portions (1 mL) of filtrate were transferred to sample vials and HPLC analysis was performed according to the protocol in Cederlund et al. (2007). Standard solutions with concentration range 0.05–50 µg/mL were analysed with extracts from samples. The HPLC was equipped with a G1314A UV detector, a G1311A pump, a G1329A auto injector (Agilent Technologies AB; 1100 Series; Sweden) and a Zorbax SB-C18 column (12.5 × 4.6 mm, 5 mm; ChromTech AB, Sundbyberg, Sweden).

Analysis of Glyphosate

Extraction of glyphosate, derivatisation and measurement on GC-MS were performed using the same reagents for analytical standards, glyphosate extraction and internal standards as previously described (Bergström, Börjesson, and Stenström, 2011).

Results & Discussion

Effect of Biochar on Soil Water-Holding Capacity and pH

The studied soils had different physical texture: the dominant particle fractions in the L soil were clay and fine silt, while the U soil was dominated by medium and fine sand. The texture of the LB soil could not be fully determined due to its high organic matter content, as traces of organic C remained in the sample after digestion (oxidation by H_2O_2). Coming from the same field as L, it is likely that the LB soil was also dominated by clay. However, the proportion of sand was higher (Table 2). This agrees with Kihlberg et al. (unpublished), who also reported a coarser particle size distribution in LB compared with L soil, but also did not subdivide particles with Ø < 0.06 mm. The WHC of the clayey L soil (53%) was higher than in the sandy U soil, where it was only 27%, and was not affected by biochar addition. However, the LB soil, which was historically amended by charcoal, had a higher WHC (57%) than the L soil with or without fresh biochar amendment. In the sandy soil, the WHC increased from 27 to 42% with biochar addition and was correlated positively (r = 0.98) with the biochar percentage (Fig. 1). Biochar addition increased the pH from 5.27 to 6.07 in the L soil and from 6.41 to 7.69 in the U soil (Fig. 2). Ageing of the biochar led to a further pH increase in most of the soil-biochar mixtures (U10a-U30a). In the LB soil, the pH was higher (5.77) than in the L soil. The pH of soil-biochar mixtures was correlated with the percentage of biochar added in all cases (r = 0.99 for L soil-biochar mixtures; r = 0.99 for fresh U soil-biochar mixtures; r = 0.98 for aged U soil- biochar mixtures).

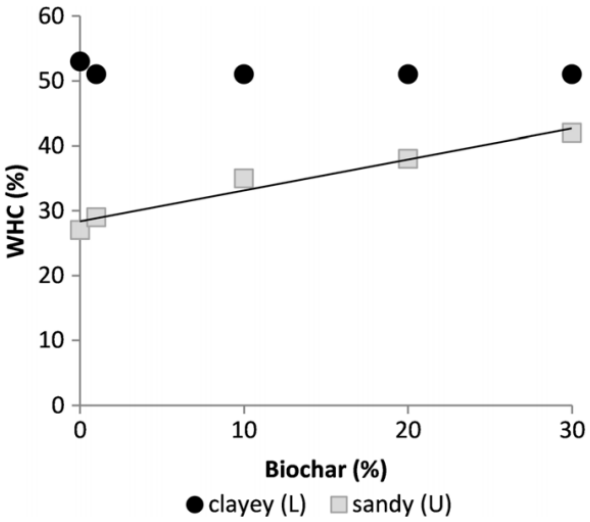

Fig. 1. Water-holding capacity (WHC) of the soil samples ± standard deviations plotted against biochar percentage added

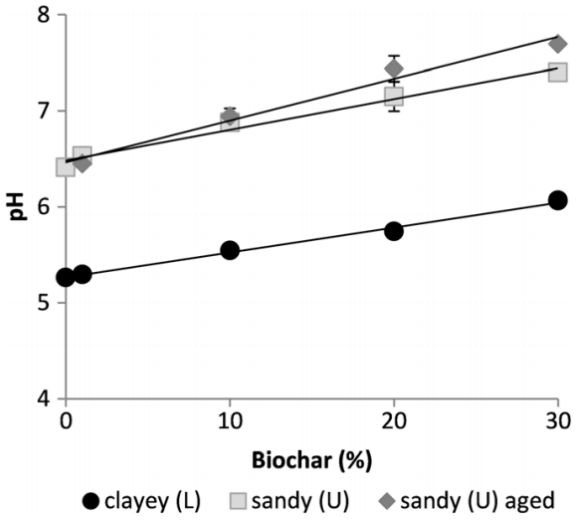

Fig. 2. pH of the soil samples (N = 2) ± standard deviations plotted against biochar percentage added

Adsorption of Diuron

Biochar amendment increased diuron adsorption in both the L and U soils (Fig. 3). In the LB soil, K_F was 364 $\mu g^{1-1/n}(mL)^{1/n} g^{-1}$, which is quite close to the K_F value of the L20 soil-biochar mixture. K_F values in the aged soil-biochar mixtures were lower than in mixtures with fresh biochar addition. There were positive correlations between the diuron K_F values and biochar percentage for L, U and aged U soils (r = 0.96, r = 0.95, and r = 0.95, respectively).

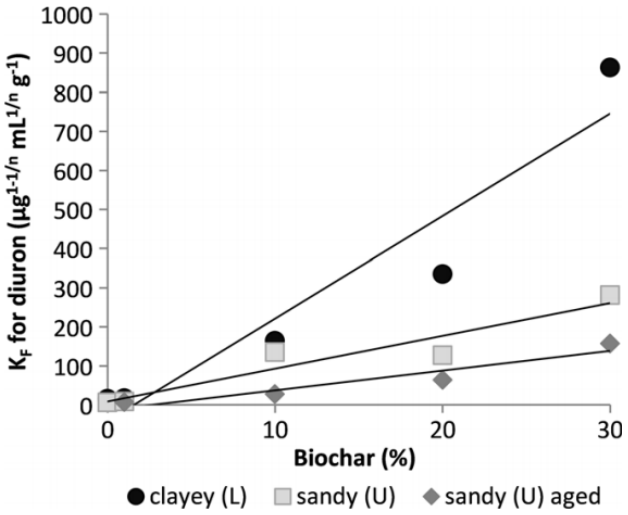

Fig. 3. Freundlich K_F values for diuron plotted against biochar percentage added in samples from Länna (L) and Ulleråker (U)

Adsorption of Glyphosate

Glyphosate was more strongly adsorbed in the L soil (K_F = 1218 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}) than in the U soil (K_F = 146 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}). No consistent effect of biochar amendment on glyphosate adsorption in L soil was observed (Fig. 4). A very high K_F value was observed for the sample with 1% biochar addition (K_F = 1892 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}), while the K_F values for the unamended L soil and the other soil-biochar mixtures varied between 1099 and 1294 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}. The LB soil had a much lower *KF* value (539 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}) than the L soil and soil-biochar mixtures. However, in the U soil, glyphosate adsorption was correlated negatively (r = −0.99) with the biochar percent- age (Fig. 4). Ageing of the biochar decreased adsorption further.

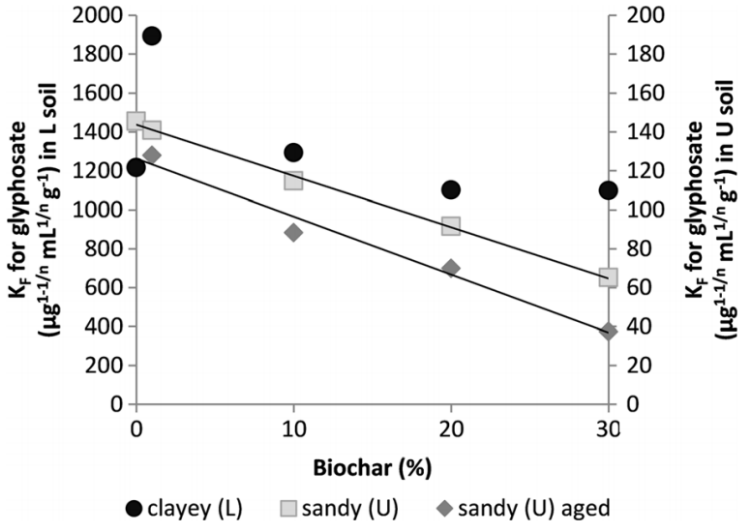

Fig. 4. Freundlich K_F values for glyphosate plotted against biochar percentage added in samples from Länna (L) and Ulleråker (U)

Table 3. Freundlich parameters (K_F, 1/n and R^2 value) for adsorption and half-life of diuron and glyphosate

Sampling site		Adsorption						Degradation			
		Diuron			Glyphosate			Diuron		Glyphosate	
		K_F[a]	1/*n*	R^2	K_F[a]	1/*n*	R^2	$T½$[b]	R^2	$T½$[b]	R^2
Länna	LB	364	0.859	0.99	539	0.890	0.99	36	0.965	17	0.97
	L	15.21	0.863	0.99	1218	0.842	0.99	40	0.963	87	0.606
	L1	17.10	0.807	0.99	1892	0.872	0.99	47	0.708	187	0.333
	L10	164	0.859	0.99	1294	0.806	0.99	42	0.853	151	0.385
	L20	335	0.822	0.99	1102	0.796	0.99	56	0.918	131	0.402
	L30	863	0.978	0.96	1099	0.780	0.98	45	0.86	51	0.945
Ulleråker	U	5.73	0.798	0.99	145.5	0.783	0.99	112	0.663	182	0.482
	U1	8.60	0.586	0.95	140.9	0.765	0.99	58	0.718	83	0.767
	U10	135	0.789	0.99	114.8	0.754	0.99	33	0.866	66	0.674
	U20	127	0.727	0.85	91.6	0.780	0.99	35	0.868	78	0.621
	U30	281	0.753	0.97	65.2	0.750	0.99	40	0.888	53	0.861
	U1a	6.44	0.760	0.99	127.9	0.761	0.99	37	0.71	51	0.716
	U10a	27.4	0.824	0.99	88.3	0.776	0.99	27	0.785	81	0.683
	U20a	64	0.547	0.94	70.0	0.788	0.99	29	0.849	49	0.917
	U30a	157	0.686	0.97	37.4	0.751	0.99	35	0.871	68	0.885

[a] The unit of K_F is $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}

[b] The unit of $T½$ is days

Degradation of Diuron

In the L and U soils and soil-biochar mixtures, from 20 to 50% of the added diuron was degraded during the experimental period. Diuron half-life varied between 40 to 56 days in the L soil, was 36 days in the LB soil and varied between 26 to 112 days in the U soil (Fig. 5). No correlation was seen between the biochar percentage and diuron half-life in any of the soils. However, in the U soil, the half-life was shorter in all samples with biochar addition compared with the unamended soil. Here, it should be noted that the half-life of 112 days found for the U soil without biochar may be a less accurate estimation, since the dynamics of diuron degradation did not fit well with a first-order kinetic model in this sample. The degradation kinetics of all other samples followed first-order kinetics reasonably well, with R^2 values of 0.7–0.96. Ageing of the biochar consistently decreased diuron half-life in the U soil.

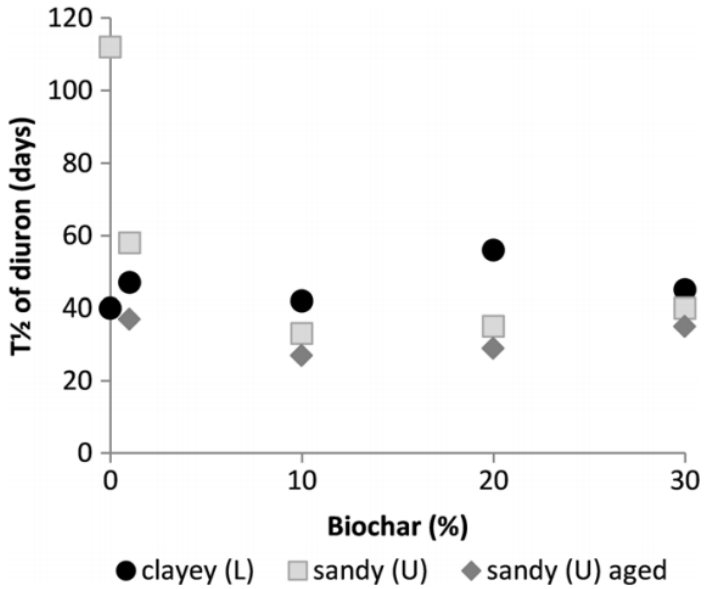

Fig. 5. Diuron half-life in the Länna (L) and Ulleråker (U) soils and soil-biochar mixtures

Degradation of Glyphosate

In the L and U soils and soil-biochar mixtures, 10–70% of the added glyphosate was degraded during the experimental period. Glyphosate half-life in the L soil varied between 51 and 187 days. However, in the L, L1, L10 and L20 samples, the data fitted poorly to the first-order kinetic model (R^2 = 0.33–0.61), mostly due to great variation in glyphosate concentrations during the first week of degradation. This fact can explain the some-what inconsistent pattern of half-life variation for the soil-biochar mixes. However, degradation in the LB and L30 samples followed first-order kinetics well (R^2 = 0.97 and 0.94). The shortest glyphosate half-life (19 days) was observed in the LB soil. Degradation of glyphosate was relatively slow in the unamended U soil, but was faster in all samples with biochar amendment. In the unamended U soil, the half- life of glyphosate was 182 days, while in the U soil- biochar mixtures, it varied between 49 and 83 days. However, as in the case of diuron, data from the un-amended U soil were a poor fit to the first-order model (R^2 = 0.48) and the degradation rate in the biochar-amended samples did not appear to be related to the biochar percentage added. The fastest degradation was observed in the U1a and U20a soil-biochar mixtures, but ageing of the biochar did not consistently affect degradation rates (Fig. 6). No correlations between glyphosate half-life and amount of added biochar were found for any of the L and U soils (Fig. 6). However, the half-life was correlated with the K_F value for glyphosate (r = 0.88) in samples of the L soil when the LB sample was included (Fig. 7). In the U soil and soil-biochar mixtures, the adsorption coefficient of glyphosate was generally lower and its half-life was not correlated with the K_F value (Fig. 7).

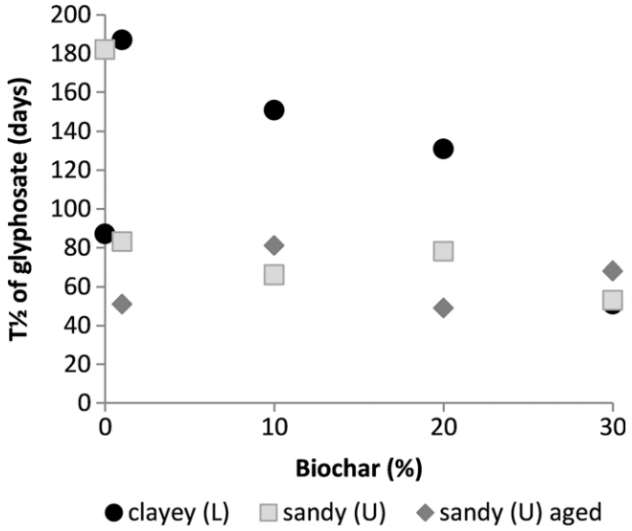

Fig. 6. Glyphosate half-life in the Länna (L) and Ullerråker (U) soils and soil-biochar mixtures

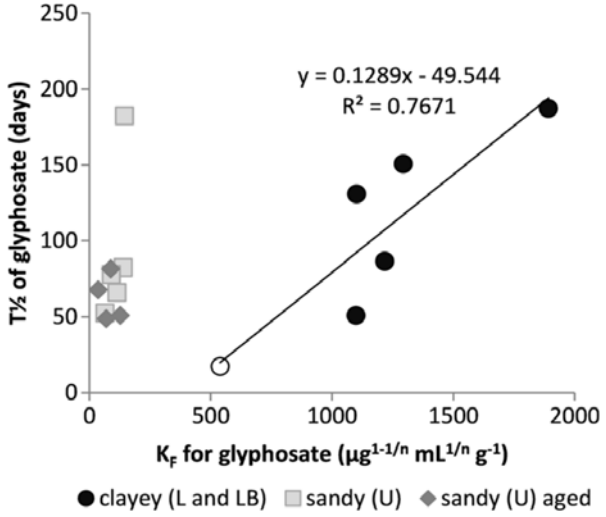

Fig. 7. Correlation between glyphosate half-life and adsorption coefficient (KF). The open circle is for the LB soil

Effects of Biochar on Herbicide Adsorption

Diuron adsorption increased after biochar amendment in both the L and U soils. This effect of biochar addition has been observed in previous studies for silty loam and sandy soil. Biochar contains many adsorption sites that can bind non-polar herbicides, so diuron adsorption increased with amount of biochar added, and the risk of it leaching is lower. The increased pH obtained with biochar addition is not likely to have contributed to the increased sorption since diuron is uncharged at relevant soil pH-levels. In a previous study, we also found that pH has no effect on diuron adsorption when studying this particular biochar without soil (Cederlund et al. 2016).

Biochar addition decreased glyphosate adsorption in the sandy U soil, but not in the clayey L soil. The difference in effects of biochar on glyphosate adsorption between the L and U soils may be explained by the different soil texture and physical properties of these soils. The decreased glyphosate adsorption in the U soil is likely to be related to the induced pH changes. According to several studies, soil pH is negatively correlated with glyphosate adsorption (Gimsing et al. 2004b; Mamy and Barriuso 2005; Vereecken 2005). Increased soil pH can increase the negative charge of both soil surfaces and glyphosate itself, which leads to enhanced repulsion. Glyphosate has a pH-dependent OH^- group with a pKa value of 5.7, so its charge is likely to have been affected in the pH range studied here. The same relationship with pH has been observed for glyphosate adsorption on pure biochar: Herath et al. (2016) studied the effect of pH on adsorption of glyphosate on a rice husk biochar and found that the adsorption percentage varied from 75 to 85% at pH 3–5, decreased to 75–65% at pH 6–8 and then significantly dropped to 55% at pH 9. However, in a previous study, we showed that glyphosate adsorption by the studied biochar was low at both low and high pH (Cederlund et al. 2016). In the L soil, there was no linear relationship between glyphosate adsorption coefficient and biochar amendment. The overall strong adsorption in this soil possibly contributed to masking the relatively minor effects of the biochar. It is known that inorganic components of soil, such as Al- and Fe-oxides, adsorb glyphosate effectively (Gimsing et al. 2004a) and that this herbicide is less available in soils with a high clay content. The induced pH changes in this soil also occurred over a different pH interval, which may have contributed to the less clear outcome.

Effects of Biochar Ageing on Adsorption

Short-term ageing of the biochar mixtures in the laboratory decreased adsorption of both herbicides. This suggests that processes that have the potential to reduce sorption, such as organo-mineral interactions with the biochar surface (Pignatello et al. 2006; Singh and Kookana 2009; Lin et al. 2012), were the dominant forces affecting the biochar during our ageing experiment. For diuron, our results are consistent with findings in a field study on biochar amendment of Australian ferrosols, in which diuron and atrazine adsorption to soils amended by poultry litter and paper mill biochar was significantly reduced after 32 months of ageing. For glyphosate, it is possible that the further increase in pH during the 3 months of ageing contributed to the additional decrease observed in sorption. Although we cannot know the original properties of the charcoal applied to the historically charcoal-enriched LB soil, it may be informative to compare the adsorption results from this soil. In LB, the K_F value for diuron was comparable to that determined in the 20% soil-biochar mixture (L20) and, considering that the total carbon content of the LB soil is about 18% (Table 1), this suggests limited effects of ageing. However, for glyphosate, the K_F value of the LB soil was only 539 $\mu g^{1-1/n}\ mL^{1/n}\ g^{-1}$, which is only about half the K_F value found for any of the fresh biochar mixtures or the unamended L soil (Table 3). Since the adsorption of glyphosate on the biochar itself is very weak, this low adsorption is difficult to explain in terms of reduced adsorptive affinity of the charcoal. It is more likely to reflect a reduced affinity for glyphosate of the soil itself. Kihlberg et al. (unpublished) suggest that the heat from the charcoal kilns in LB may have contributed to sintering the clay particles in the soil, causing a shift towards a coarser particle size distribution. Heating clay soils to 500 °C has been shown to change soil physical texture and increase the amount of silt and sand particles. Such a reduction in the proportion of clay would consequently reduce the amount of surfaces available for glyphosate adsorption. Heating may also cause other mineralogical changes in soil that affect adsorption, for instance de Santana et al. (2006) reported reduced interaction between glyphosate and Al_2O_3 and Fe_2O_3 in soil after burning. Our results for glyphosate differ somewhat from those of Kumari et al. (2016), who found that glyphosate sorption was

increased in a silty loam soil amended with the same wood-based biochar that we used (*Skogens kol*) after 7–10 months of ageing under field conditions. The application rates used in their study varied from 10 to 100 Mg biochar/ha added to the topsoil layer (0– 10 cm), which corresponds to about 0.8–8% of biochar per gramme dry weight assuming a bulk density of the soil of 1.3 g/cm³. Increases in glyphosate sorption occurred in plots amended with 10, 20 and 40 Mg/ha of biochar (i.e. corresponding to 0.8, 1.6 and 3.2% *w*/*w*), while the plot amended with 100 Mg/ha, where the glyphosate adsorption was the same as in the unamended plots, was considered to be an outlier (Kumari et al. 2016). In the present study, the clayey L soil with the lowest application rate was the outlier: the adsorption coefficient in the L1 soil-biochar mixture was much higher than in L soil without amendment, while the adsorption coefficient in the L10, L20 and L30 soil-biochar mixtures was the same or lower than in the unamended clayey L soil. However, we cannot offer an explanation for this pattern. In the sandy U soil, the adsorption of glyphosate was reduced after the ageing process, which can be explained by a further pH increase and low affinity to sorb glyphosate in both sandy soil and biochar itself.

Herbicide Degradation before and after Biochar Amendment

Microbial degradation of chemicals in soil has often been reported to be limited by strong sorption (Bergström et al. 2011; Gimsing et al. 2004a; Wu et al. 2011). Moreover, pesticide degradation is often inhibited after fresh biochar addition (Kookana 2010), which can be explained by a decrease in their bioavailability. In the present case, it seems that despite the fact that adsorption of diuron increased in both soils and that adsorption of glyphosate decreased in the sandy soil, biochar amendment had no clear effect on either diuron or glyphosate degradation. However, even though neither the K_F value nor the half-life of glyphosate was clearly correlated with the added biochar percentage in the clayey L soil, the half-life was correlated with the K_F value (Fig. 7). This indicates that in the case of glyphosate in the clayey L soil, which had K_F values >1000 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}, availability of glyphosate may have been a rate-limiting factor for its degradation, while in the other cases adsorption was too weak to have an effect.

Conclusion

As hypothesised, fresh biochar addition increased diuron adsorption in both clayey (L) and sandy (U) soils. However, glyphosate adsorption decreased only in the sandy U soil. These effects are most likely due to adsorption of diuron on the biochar itself, while in the case of glyphosate the decreased sorption may be explained by an increase in soil pH after biochar addition. No consistent effect of biochar amendment on herbicide degradation was observed in the studied soils, which contradicts our initial hypothesis. However, there was a positive relationship between adsorption and glyphosate half-life in the clayey soil-biochar mixtures, indicating that availability may be the rate-limiting step, but only where adsorption is strong. The consequences of biochar ageing under laboratory conditions were further increases in soil pH and a reduction in adsorption of both herbicides. Changes in biochar adsorptive properties during ageing in soil should be taken into consideration when planning its use in agriculture and for soil remediation purposes.

Assessment and conclusion by applicant:

The study describes the adsorption and degradation behavior of two pesticides in two different agricultural soils from Northern Europe after amendment of a biochar at different portions. Data on adsorption and degradation were evaluated for Glyphosate as well for the original soils without biochar amendment. The study design is well described and the adsorption parameters are sufficiently reported. The adsorption experiments was conducted according to the OECD guideline 106. However, not all validity criteria could be cross-checked as they are not reported. For the evaluation of the degradation behavior of Glyphosate, no information on the use of a specific guideline was reported. The results were only evaluated against Single First Order kinetics with partly poor fits. No information of the Glyphosate findings for the samples at different time points are reported, i.e. no additional kinetic evaluation is possible with the presented data. The study is therefore classified as reliable with restrictions (Category 2).