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Spatial glyphosate and AMPA redistribution on the soil surface driven by sediment transport processes – a flume experiment

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Abstract

This study investigates the influence of small-scale sediment transport on glyphosate and AMPA redistribution on the soil surface and on their off-site transport during water erosion events. Both a smooth surface (T1) and a surface with "seeding lines on the contour" (T2) were tested in a rainfall simulation experiment using soil flumes (1x0.5 m) with a 5% slope. A dose of 178 mg m^{-2} of a glyphosate-based formulation (CLINIC®) was applied on the upper 0.2 m of the flumes. Four 15-min rainfall events (RE) with 30-min interval in between and a total rainfall intensity of 30 mm h^{-1} were applied. Runoff samples were collected after each RE in a collector at the flume outlet. At the end of the four REs, soil and sediment samples were collected in the application area and in four 20 cm-segments downslope of the application area. Samples were collected according to the following visually distinguished soil surface groups: light sedimentation (LS), dark sedimentation (DS), background and aggregates.

Results showed that runoff, suspended sediment and associated glyphosate and AMPA off-site transport were significantly lower in T2 than in T1. Glyphosate and AMPA off-site deposition was higher for T2 than for T1, and their contents on the soil surface decreased with increasing distance from the application area for all soil surface groups and in both treatments. The LS and DS groups presented the highest glyphosate and AMPA contents, but the background group contributed the most to the downslope off-site deposition.

Glyphosate and AMPA off-target particle-bound transport was 9.4% (T1) and 17.8% (T2) of the applied amount, while water-dissolved transport was 2.8% (T1) and 0.5% (T2). Particle size and organic matter influenced the mobility of glyphosate and AMPA to off-target areas. These results indicate that the pollution risk of terrestrial and aquatic environments through runoff and deposition can be considerable.

Keywords: Glyphosate; Aminomethylphosphonic acid (AMPA); Runoff; Particle-facilitated transport; Sedimentation

Capsule

The downslope off-site transport and deposition of glyphosate and AMPA by water erosion onto the soil surface can be considerable, with the consequent pollution risk of off-target terrestrial environments.

1. Introduction

Glyphosate (N-phosphonomethylglycine), the active ingredient of many commercial herbicide formulations, is used worldwide for weed control (Borggaard and Gimsing, 2008; Dill et al., 2010). It is one of the most used herbicides in agriculture, particularly in combination with glyphosate-resistant crops. Due to its extensive use, concerns on its safety for both humans and the environment are growing considerably (Myers et al., 2016). Therefore, there is a pressing need to better understand all its associated risks, including its transport processes to off-target areas and consequent pollution risks of the terrestrial and aquatic environments.

Glyphosate strongly adsorbs to soil particles (Sprankle et al., 1975; Al-Rajab et al., 2008). Although it is also very soluble in water (10.2 g L^{-1}, 20 °C) (EU, 2002), it weakly desorbs from soil particles (Al-Rajab et al., 2008). Therefore, during water erosion events, glyphosate concentrations are much higher in suspended sediment than in runoff water (Yang et al., 2015a; Yang et al., 2015b). The same behaviour is observed for AMPA (aminomethylphosphonic acid), glyphosate's main metabolite (Yang et al., 2015a; Yang et al., 2015b). Due to the strong capacity of glyphosate and AMPA to adsorb to soil particles, their largest amounts and highest contents remain in the upper 0-2 cm soil layer, even after rainfall events (Yang et al., 2015a; Yang et al., 2015b). If these rainfall events cause overland flow and soil erosion by water, particle-bound glyphosate and AMPA will also be transported to off-target areas (Yang et al., 2015a; Yang et al., 2015b). Moreover, Sprankle et al. (1975) have shown that glyphosate was readily bound to clay minerals and organic matter (OM) and Bento et al. (2017) have found a positive correlation between glyphosate and AMPA contents and clay and OM contents, which are easily transported by water erosion (Palis et al., 1997; Shakesby et al., 2015).

The transport of particle-bound glyphosate and AMPA depends on the sediment movement and dynamics, which in turn are influenced by soil type, texture and land management practices. Loess soils represent about 10% of the Earth's surface, they are among the most agriculturally productive soils in the world and they are intensively used for agriculture (Haase et al., 2007; Bento et al., 2017). Large areas of loess soils are used to grow glyphosate-resistant crops, where huge amounts of glyphosate-based herbicides are applied every year (Aparicio et al., 2013; Clive, 2014; Benbrook, 2016; Bento et al., 2016). Despite the relatively good infiltration capacity of loess soils, crusting occurs often, which negatively affects the infiltration rates and, therefore, makes these soils susceptible to erosion (Le Bissonnais et al., 1998). Understanding the relationship between sediment dynamics and the transport of particle-bound glyphosate and AMPA in this type of soil is therefore of great importance.

Sediment output at the outlet of a catchment or at the end of an experimental flume gives an indication of the erosion within the catchment area. However, it doesn't give information on the sediment dynamics inside a catchment (Parsons et al., 2006; Vente et al., 2007; Baartman et al., 2013; Fryirs, 2013). Nevertheless, insight on the spatial patterns of sediment movement and (re)distribution are essential to assess particle-bound glyphosate and AMPA mobility. Connectivity of sources and sinks of sediment (re)distribution (Bracken and Croke, 2007; Bracken et al., 2015) are related to the complexity of a plot or catchment (Baartman et al., 2013). Variations in the micro-topography or obstacles to flow (e.g. soil and water conservation measures) (partly) determine this complexity: lower complexity of a plot leads to higher hydrological connectivity and therefore to higher and quicker runoff and sediment transported to the outlet (Darboux et al., 2002; Appels et al., 2011).

Using a soil-flume scale and rainfall simulations, this study aims to: 1) investigate the redistribution of glyphosate and AMPA on the soil surface driven by water erosion; and 2) quantify the transport of glyphosate and AMPA to off-target areas, including their downslope off-target deposition, driven by water erosion. The influence of different soil surface micro-topographies on glyphosate and AMPA redistribution and transport to off-target areas was also assessed.

2. Materials and Methods

2.1. Soil

We used a silty loam loess topsoil from Huldenberg, Belgium. The soil was air-dried and sieved through a 5-mm sieve to remove possible stones, while still protecting the soil macro-aggregates, and to guarantee its homogeneity for good repeatability. The soil consisted of 10% clay, 79% silt and 11% sand, with an OM content of 3.2% and a pH of 5.8. Other soil properties of the sieved soil are described in Bento et al. (2017). Prior to the experiment, the soil was tested for glyphosate and AMPA residues, according to the methodology described in section 2.3, and it was found free of glyphosate and AMPA.

2.2. Experimental design

To quantify the transport of glyphosate and AMPA to off-target areas driven by water erosion, we conducted a soil flume experiment in the rainfall simulator of the hydraulics laboratory of Wageningen University, The Netherlands. A detailed description of the rainfall simulator can be found in Lassu et al. (2015). Briefly, the rainfall simulator is 6x2.5x2.8 m (length x width x height). It is equipped with a Zehnder HMP 450 pump, which was set to 2.0 bar in this experiment, providing a constant water supply, pressure and flow. It is also equipped with two pairs of low intensity Lechler nozzles no. 460.788 (Lassu et al., 2015), sustained by two metal arms 3.35 m above the surface of the soil flume (on average; depending on the flume's slope). The spacing between the nozzles was 3.25 m. Christiansen's uniformity coefficient at the flume location was 89.5±1.1%.

For this experiment, a wooden soil flume 1x0.5x0.25 m (length x width x depth; Fig. 1A) was used. At the downslope end, the flume was equipped with a V-shaped metal outlet to collect the runoff water and sediment, which was covered with a plastic sheet during the rainfall simulations to avoid direct rainfall collection. The flume's bottom, which is made of an open metal frame, was left permeable by putting an Enkadrain TP filter (Enka solutions, Arnhem, Netherlands) to allow free drainage of leaching water. Because the same flume was used for all experimental runs, its sides were covered with a thin sheet of plastic in each simulation to prevent any glyphosate or AMPA from adsorbing to the wooden structure of the flume or any carryover in the subsequent simulations. The flume was set at a slope of 5%, representing loess regions of the globe where gentle slopes are predominant and which are typically used to grow glyphosate-resistant crops under non-till systems (e.g. the Pampas of Argentina) (Aparicio et al., 2013; Bento et al., 2016).

2.2.1. Treatments

To investigate the influence of different micro-topographic conditions on glyphosate and AMPA redistribution and transport during water erosion events, we employed two treatments to the soil surface (SS) of the flumes: a smooth SS (T1; see Fig. 1B), and a SS with micro-topographic disturbances that resemble seeding lines on the contour (T2; see Fig. 1C). In T2, we applied 6 "seeding lines" with a width and depth of about

1 cm each, and with 15 cm distance between each “seeding line”. The use of “seeding lines” in T2 intended to roughly mimic field conditions under non-tillage systems where glyphosate-based herbicides are intensively used to grow glyphosate-resistant crops. However, it should be noted that our very small flume scale does not allow for an exact replication of a specific crop type and field spacing between seeding lines in T2. Each treatment was done in triplicate.

2.2.2. Flume preparation

Only one flume could be tested for each rainfall simulation in order to guarantee the same rainfall intensity and rainfall characteristics. This is because the rainfall intensity distribution in the simulator has a high spatial variability (Lassu et al., 2015). The best location to place the flume at the desired rainfall intensity was selected based on pre-test results. For each run, the flume was filled with sieved, thoroughly mixed and homogenised soil to a depth of 15 cm, in three layers of 5 cm each. The bulk density of the 0-5 cm topsoil layer was 1.2±0.05 g cm^{-3}. Soil moisture (SM) was monitored at 5 and 10 cm depth using 12 EC-5 small SM sensors (Decagon, USA) connected to a CR1000 logger (Campbell Scientific, USA). Measurements were recorded every minute. On the day before each rainfall simulation, the flume was pre-wetted using a 30-min, 20 mm h^{-1} rainfall event. Runoff did not occur at this point, and the SM content increased from 6 to 20% at 5 cm depth.

2.2.3. Glyphosate application and rainfall events

Glyphosate solutions were previously prepared in 50-mL plastic tubes by diluting 49.5 µL of CLINIC® (Nufarm B.V., The Netherlands), a glyphosate-based herbicide that contains 360 g L^{-1} of glyphosate, in Millipore water to achieve a final stock solution of 356.4 mg L^{-1}. The solutions were then stored in the dark at 3 °C until use.

On the day of the experiment, 50 mL of glyphosate solution were sprayed manually in the upper 0.2 m of the flume (see red box in Fig. 1D) by a licenced staff member of Wageningen University (Netherlands). This corresponds to a flume area of 0.1 m^2 and a glyphosate application rate of 178.2 mg m^{-2} (within the recommended dose by the manufacturer against perennial weeds). During pre-tests with the manual sprayer, the referred volume of glyphosate solution was found to be enough to guarantee an homogeneous distribution of glyphosate on the soil surface. The rainfall simulation started 1 h after glyphosate application, representing a worst-case field scenario of rainfall occurring directly after glyphosate application. Each simulation consisted of four rainfall events (REs) of 15-min each (RE15, RE30, RE45 and RE60) with an intensity of 30 mm h^{-1}, and with a 30-min interval in between the events. Additionally, cumulative total rain was monitored and measured after each RE, using 6 Nortene Pluvio rain-gauges (Cellopast S.A.S., Ballée, France) installed around the flume (see Fig. 1A).

2.2.4. Sample collection

2.2.4.1 Off-site transport of glyphosate and AMPA with runoff

At the downslope end of the flume, runoff samples were collected in plastic containers after each 15-min RE, giving a total of 4 runoff samples per rainfall simulation. These samples were then subjected to centrifugation to separate the solid phase (suspended sediment) from the liquid phase (runoff water). The centrifugation process was done in two steps: 1) all collected runoff was transferred to 750-mL plastic containers and centrifuged at 3600 rpm for 20 min (D = 0.29 µm; D = particle cut-size diameter); 2) after removing most of the runoff water to a clean container, the sediments were transferred to 50-mL plastic tubes and centrifuged again at 3600 rpm for another 10 min (D = 0.39 µm). After removing the remaining water, the sediments were stored in the dark at -18 °C until glyphosate and AMPA analysis, together with an aliquot of the clean runoff water collected in a 50-mL plastic tube.

2.2.4.2 Downslope off-site deposition of glyphosate and AMPA

To investigate the transport and (re)deposition of glyphosate and AMPA on the SS, the flume was divided into five segments: the first one, upslope of the flume, corresponds to the glyphosate application area (AA; red area, Fig. 1D); the other four segments correspond to the downslope non-contaminated areas of the flume (S1-S4; green areas, Fig. 1D). Each segment was of 20x50 cm (length x width). Besides this, four SS groups were identified based on visual criteria: 1) LS – light sedimentation: deposition of material characterized by a light brown colour; 2) DS – dark sedimentation: deposition of material characterized by a dark brown colour; 3) Bg – Background: original soil where runoff passed over but no visual sediment deposition occurred; and 4) Ag – Aggregates: dark brown aggregates present on the soil surface.

At the end of the entire rainfall simulation (per replicate), 3 soil samples were collected from the AA, and 1-sample per SS group was collected in each segment (S1-S4). The sampling depth of the soil samples in the AA was 3 mm and of the SS groups in segments S1 to S4 was 2 mm. All samples were collected with plastic tea spoons into plastic sampling bags, and stored in the dark at -18 °C until glyphosate and AMPA analysis.

2.2.4.3 Sediment properties

Because the quantity of sediments (from both the runoff and the SS) was not enough to analyse for some physicochemical properties that could help explaining the glyphosate and AMPA results, we performed an extra rainfall simulation using the same methodology described in section 2.2.3, but without applying glyphosate. Samples from each SS group and from suspended sediment were collected the same way, and analysed for their OM content and particle size distribution as described by Bento et al. (2017).

2.2.5. Photographs for surface analysis

Photos of the SS area were taken at the end of each rainfall simulation to quantify the surface areas per SS group per segment for mass balance purposes. The photos were analysed using the software eCognition 5.0, and classified according to the SS groups described in section 2.2.4.2, except for the aggregates (it was not possible to accurately distinguish the background group from the aggregates). Soil surface maps were then created and exported as raster data (ASCII) to ArcGIS 10.2.1. To determine the area occupied by each SS group in each segment, a pixel:cm relation was determined by converting pixels in the images into centimetres. This was performed in ImageJ 1.51f, and a pixel:cm relation of about 37:1 was found for all images.

2.3. Glyphosate and AMPA analysis

Glyphosate and AMPA contents of the samples were analysed according to the methodology used by Bento et al. (2016) and Yang et al. (2015b). Briefly, glyphosate and AMPA were extracted from 2 g of soil or sediment with 10 mL of 0.6 M KOH (potassium hydroxide, p.a. 85%; Merck KGaA, Darmstadt, Germany). For sediment samples for which <2 g were available, the extraction procedure was performed by using 1 g with 5 mL of 0.6 M KOH. Isotopically-labelled glyphosate and AMPA were then added to 1-mL of the pH-adjusted supernatant and a derivatisation step was carried out using FMOC-Cl (9-fluorenylmethoxycarbonyl chloride; Sigma-Aldrich, Switzerland). For the runoff water samples, the derivatisation step was immediately performed in 1-mL of the sample. Fortified blank soil samples (0.5 mg kg^{-1}) were added as quality controls. Glyphosate and AMPA contents were then determined by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) using an XBridge™ Shield RP C18 column 100x2.1 mm (Aquity UPLC I-Class coupled to a Micromass Ultima triple-quadrupole-MS, Waters, Netherlands). The batch sample quantification was considered satisfactory when the quality-control recoveries were between 70-120%. Chemicals used, mobile phases, HPLC-MS/MS instrumentation conditions, method validation and quality control details are described in Yang et al. (2015b) and Bento et al. (2016).

2.4. Erosion quantification from the runoff samples

All suspended sediments collected with runoff for each 15-min RE had to be used for erosion quantification, including the sediments that were first extracted with KOH for glyphosate and AMPA analysis. The sediment samples were dried for 24 h at 105 °C and then weighed. In a pre-test, an increase in the weight of the dried sediment due to the use of KOH was observed and accounted for: 0.04 g for those samples using 10 mL of KOH, and 0.03 g for those samples using 5 mL of KOH.

2.5. Data analysis

Glyphosate and AMPA are presented in this study in concentration and in percentage of applied glyphosate (PAG). The data are also presented as average and standard error (SE) of the replicates. PAG for the SS data were determined considering the average bulk density of the 0-5 cm soil layer, the surface area derived from eCognition analysis (see section 2.2.5), and the sampling depth of the soil/sediment samples.

All statistical analysis used a significance level (p-value) of 0.05 and were performed in SPSS 22.

All runoff data (Fig. 2) were analysed using a mixed ANOVA, with the 15-min REs as the within-subjects factor and treatment as the between-subjects factor. A proper transformation of the data was performed whenever the normality and/or equality of variances assumptions were violated. For the glyphosate and AMPA data in suspended sediment, RE15 was excluded due to a missing value in both treatments. Whenever the sphericity assumption was violated, the Greenhouse-Geisser correction factor was used. When the interaction between the two factors was not significant, F-tests for significant differences between treatments within each RE, and one-way ANOVAS followed by Tukey or Dunnett T3 tests for significant differences between REs within each treatment, were performed. When the interaction was significant, the mixed ANOVA was followed by Bonferroni tests.

For the SS data (Fig. 3), a 3-way ANOVA was first performed to ln-transformed glyphosate contents. Because the interactions between factors (3- and 2-way) were not significant, the following tests were performed: F-tests for significant differences between treatments within each SS group and segment; and one-way ANOVAS followed by Tukey tests for significant differences between SS groups within each treatment and segment, and for significant differences between segments within each treatment and SS group. The DS group from segment S2 in T1 was excluded from analysis for differences between segments and between groups because n = 1. For data on glyphosate in PAG and on AMPA (content and PAG), non-parametric tests were applied. For differences between treatments at each level of the other 2 factors, we used Mann-Whitney U tests. For differences between segments and between SS groups at each level of the other 2 factors, we performed a Kruskal-Wallis test followed by Mann-Whitney U tests, correcting the p-value by Bonferroni. In all non-parametric tests, we used the exact probability option (1-tailed) due to the small sample size.

To test for significant differences between treatments for the SM contents and accumulated rainfall (Table 1), and for the overall glyphosate and AMPA distribution data in the AA and transported to off-target areas at the end of the rainfall simulations (Table 2), T-tests were performed. To test for significant differences between rainfall events for the SM contents (Table 1), in each treatment and for the two monitored depths, repeated measures ANOVA followed by Bonferroni tests was performed (because sphericity assumption was violated, the Greenhouse-Geisser correction factor was used instead). Ln-transformed data were used when the normality assumption was violated for a given data set.

The relationship between sediment properties and glyphosate or AMPA transport by water erosion (Table 3) was assessed by performing linear regression and Pearson correlations.

3. Results

3.1. Rainfall characteristics and SM conditions

Rainfall characteristics were similar between treatments for all 15-min REs (Table 1). No significant differences were observed for SM contents between treatments, in both SM depths and for all REs (Table 1). SM contents increased between REs for both treatments and depths. At 5 cm depth, this increase was significant between RE15 and RE30 for T2 (not significant for T1), but it increased almost steadily afterwards for both treatments. At 10 cm depth, SM was always very low for both treatments: 2.5-3.4 times lower in T1 and 3.5-5.3 times lower in T2.

3.2. Off-site runoff and soil loss in the different treatments

The results show that, overall, T1 produced higher runoff, suspended sediment and suspended load concentration per SS area than T2 (Fig. 2A-C). For T1, a significant increase in runoff water was observed between RE15 and RE30, reaching an almost steady-state afterwards (Fig. 2A). For the suspended sediment (Fig. 2B), an increase was observed between RE15 and RE30 for both treatments, although it was only significant for T2. A slight decrease was then observed between RE30 and RE45/RE60, for both treatments. This is also confirmed by the lower suspended load concentration for RE45 and RE60, when compared with the first two REs (Fig. 2C). Although an increase in runoff water and suspended sediment is observed between RE15 and RE30 for T1, a decrease is observed in the suspended load concentration for the same REs. In fact, a decrease in the suspended load concentration was observed between RE15 and RE60 for T1. A great variability in the results was also observed between replicates in this study, particularly for suspended sediment and the suspended load concentration. This is shown by the large SE bars represented in Fig. 2A-C.

3.3. Off-site transport of glyphosate and AMPA with runoff

3.3.1. Glyphosate and AMPA concentration in the runoff water

Overall, glyphosate concentrations in the runoff water varied between 0.02 and 0.08 mg L^{-1} (Fig. 2D). AMPA, on the other hand, wasn't detected in any of the runoff water samples. On average, glyphosate concentrations in the runoff water were significantly higher for T1 (0.07±0.01 mg L^{-1}) than for T2 (0.03±0.005 mg L^{-1}; Fig. 2D). They were also higher for T1 than for T2 for all REs. Between REs, both the glyphosate concentrations and the PAG transported with runoff water slightly increased from RE15 to RE30 for both treatments, reducing thereafter for T1 but remaining almost constant for T2 (Fig. 2D). The PAG transported with runoff water (Fig. 2G) was low for both treatments and for all REs (overall, <3%). Its transport was, though, significantly higher for T1 than for T2 for all REs (not significant for RE15). In total, the glyphosate transported with runoff water was 5.5 times higher for T1 than for T2.

3.3.2. Glyphosate and AMPA content in the suspended sediment

Overall, glyphosate contents in the suspended sediment varied between 3.7 and 42.9 mg kg^{-1} (Fig. 2E), while AMPA contents varied between 0.04 and 0.49 mg kg^{-1} (Fig. 2F). On average, glyphosate and AMPA contents in the suspended sediment were higher for T1 (glyphosate: 28.9±3.8 mg kg^{-1}; AMPA: 0.38±0.03 mg kg^{-1}) than for T2 (glyphosate: 11.5±2.5 mg kg^{-1}; AMPA: 0.13±0.03 mg kg^{-1}) (Fig. 2E-F). Glyphosate and AMPA contents were also higher for T1 than for T2 for all REs (Fig. 2E-F). Their contents in the suspended sediment decreased between REs for T1, although to a lesser extent for AMPA after RE45. Unlike T1, their content increased between REs for T2.

Although the glyphosate content in suspended sediment was high in all samples, only a small percentage of the applied glyphosate was transported outside the flume for both treatments and for all REs (overall, <1.7%) (Fig. 2H). In fact, although the concentrations of glyphosate in runoff water were low (≤0.08 mg L^{-1}; see Fig. 2D), the total percentage of glyphosate transported with runoff water (<3%; see Fig. 2G) was almost double of that transported with suspended sediment for both treatments (<1.7%; see Fig. 2H). The amount of AMPA transported with suspended sediment was even smaller than that of glyphosate for both treatments and for all REs (overall, <0.04% of the applied glyphosate) (Fig. 2I). The transport of both compounds was, though, always higher for T1 than for T2 for all REs (Fig. 2H-I). In total, the glyphosate and AMPA transported with suspended sediment was, respectively, 7.9 and 9.5 times higher for T1 than for T2.

3.4. Downslope off-site deposition of glyphosate and AMPA

At the end of the rainfall simulations, high glyphosate contents were still detected at the surface of the AA in both treatments (T1: 36.5±3.1 mg kg^{-1}; T2: 43.4±4.4 mg kg^{-1}) (Fig. 3a-b). However, low contents were observed for AMPA (0.53±0.05 mg kg^{-1} for both treatments) (Fig. 3c-d). Downslope the AA, the highest glyphosate and AMPA contents were detected in segment S1 for all SS groups, in both treatments (Fig. 3a-d). Glyphosate contents reduced significantly thereafter by 8 to 28-times between segments S1 and S4 (Fig. 3a-b and corresponding table). Exception was for the DS group in T1, where no significant differences were observed between segments, although a 3.5-times reduction was observed until segment S3 (slightly increasing thereafter). The AMPA content in T1 also reduced until segment S3 for the DS and LS groups, but increased again in segment S4 (Fig. 3c). For the background and aggregates groups in T1 and for all SS groups in T2, the detected AMPA content in segments S2-S4 were always ≤LOQ (i.e., limit of quantification = 0.05 mg kg^{-1}) or even <LOD in a few samples (i.e., limit of detection = 0.03 mg kg^{-1}; the LOD value was assumed for statistical purposes in these cases) (Fig. 3c-d). In general, no significant differences on glyphosate and AMPA contents were observed between treatments within the same SS group and the same segment, but glyphosate contents were higher for T1 than for T2 for most of the cases (except the background group) (Fig. 3a-b). In general, glyphosate contents decreased in the order DS>LS>Ag≥Bg for both treatments and for all segments (Fig. 3a-b). Exceptions were for segment S1, where LS>DS>Ag>Bg for T1 and DS>Bg>LS>Ag for T2. AMPA contents in T1 were always highest for the DS and LS groups and lowest for the aggregates and background groups, for all segments (Fig. 3c). In T2, AMPA contents were similar in all SS groups between S2 and S4, while for S1 they decreased in the order DS>Bg>LS>Ag (Fig. 3d).

As regards the PAG, most of the recovered glyphosate and AMPA remained in the AA in both treatments (Fig. 3e-h). Nonetheless, slightly higher amounts of glyphosate and AMPA remained in the AA of T2 (glyphosate: 88.7±9.0%; AMPA: 1.7±0.2%) than in that of T1 (glyphosate: 72.5±6.1%; AMPA: 1.6±0.1%) (Fig. 3e-h; Table 2). Similar to what was observed for glyphosate and AMPA contents (Fig. 3a-d), glyphosate and AMPA amounts found downslope of the AA were highest in segment S1, and strongly reduced thereafter (Fig. 3e-h). Although the DS group presented the highest glyphosate and AMPA contents for most of the segments in both treatments (Fig. 3a-d), it contributed the least to the amounts of glyphosate and AMPA obtained outside the AA (overall for segments: $T1_{glyphosate+AMPA}$ = 0.74±0.16%; $T2_{glyphosate+AMPA}$ = 1.8±0.2%) (Fig. 3e-h). The background group contributed the most to the amounts of glyphosate and AMPA obtained outside the AA (overall for segments: $T1_{glyphosate+AMPA}$

= 5.6±0.7%; $T2_{glyphosate+AMPA}$ = 15.8±7.6%) (Fig. 3e-h). This is due to the very small areas occupied by the DS group in contrast to the very large areas occupied by the background group. For reasons explained in section 2.2.5, the PAG in the aggregates could not be determined.

3.5. Mass balance of glyphosate and AMPA distribution at the end of the experiment

According to Table 2, >85% of the applied glyphosate was recovered in total (including AMPA) in the topsoil (<0.5 cm deep) and in the runoff, in both treatments. This means that <15% of the glyphosate was lost to deeper soil layers or by its dissipation to other metabolites or its direct mineralisation. Most of the applied glyphosate remained in the surface of the AA, with a higher percentage of glyphosate and AMPA being recovered in the AA of T2 than of T1 (Table 2). The total off-target transport of glyphosate and AMPA together was <20% of the applied glyphosate, with a higher percentage being transported for T2 than for T1 (Table 2). A much higher percentage of glyphosate and AMPA was transported to the downslope SS areas than to the outlet of the flume. This is particularly true for T2, where the total glyphosate transported to the outlet of the flume was 25 times lower than that transported to the downslope SS areas. For T1, the total amount of glyphosate transported to the outlet of the flume was only 1.8 times lower than that transported to the downslope SS areas (Table 2). The particle-bound transport of glyphosate and AMPA dominated over the water-dissolved transport (Table 2). Nevertheless, this is mostly due to the glyphosate and AMPA transported to the downslope SS areas. As referred in section 3.3.2, a higher amount of glyphosate was transported dissolved in the runoff water when compared to the particle-bound transport with suspended sediment (see Fig. 2G-H). Attention needs to be paid to the high SE in the SS for T2, which shows a high variation between replicates, particularly between those of the background group. Due to the short time between glyphosate application and the rainfall simulations, the contribution of AMPA to the whole process can be considered negligible when compared to the contribution of glyphosate (Table 2).

3.6. Sediment properties and their relationship with the off-site transport of glyphosate and AMPA

The properties of the suspended sediment show that, as expected, the lighter compounds of the soil are more easily transported during a water erosion event (Table 3A). Much higher contents of OM, clay and silt were obtained in the suspended sediment than in the SS groups, whereas the sand content was much lower (Table 3A). The LS group, on the other hand, was composed by the lowest contents of clay, silt and OM and the highest sand content (Table 3A). Assuming the background group as reference for the soil properties on the surface of the flume, all groups of sediments (i.e., suspended sediment and SS groups) presented higher clay and OM contents than the background group, except the LS group. When correlating these sediment properties with the corresponding glyphosate and AMPA contents, a significantly positive correlation was found for clay and OM, whereas a significantly negative correlation was found for sand (Table 3B). The correlation with silt was positive but not significant. Except for the LS group which presented, on average, higher glyphosate and AMPA contents than the aggregates and background groups, these correlations are confirmed by our glyphosate and AMPA results (see Fig. 2E-F and Fig. 3a-d), particularly for the suspended sediment which presented the highest glyphosate and AMPA contents from all sediment groups analysed (see Fig. 2E-F).

4. Discussion

4.1. Off-site transport of glyphosate and AMPA by runoff water and suspended sediment

Insight on the mobility, redistribution and transport of glyphosate and AMPA over the soil surface during water erosion events as a consequence of sediment dynamics can help assessing the off-target transport risk of these compounds to adjacent fields. This study shows that differences in the soil surface micro-topography/morphology influence the soil hydrological and sediment connectivity, and consequently, the redistribution of water, sediment and associated glyphosate and AMPA. In the smooth SS (T1), preferential runoff pathways were observed already in the first RE, indicating a high hydrological and sediment connectivity. The SS with "seeding lines on the contour" (T2; hereinafter referred as "seeding lines"), on the other hand, showed a low connectivity in the first RE due to obstacles promoted by the seeding lines, which consequently resulted in a slow formation of preferential flow paths for water and sediment through time. These results are in agreement with the hypothesis of Baartman et al. (2013), which says that "connectivity decreases with increasing morphological complexity": surfaces with low morphological complexity (smooth SS) result in a more effective hydrological and sediment connectivity and erosion response than more complex morphological surfaces (seeding lines). As shown by our study, these differences on the SS micro-topography/morphology have direct consequences on the off-site transport of glyphosate and AMPA by runoff and suspended sediment. In soil surfaces with low complexity (smooth SS), runoff and associated suspended sediment transported high contents of glyphosate and AMPA off-site already in the first RE, which decreased in the subsequent REs. A decrease in glyphosate and AMPA contents over time in the runoff water and suspended sediment was also observed by

Yang et al. (2015b) for smooth SS. In soil surfaces with higher complexity, and consequently lower connectivity (seeding lines), lower glyphosate and AMPA contents were transported off-site in the first RE, but they increased in the subsequent REs. The opposite trends of the REs through time between T1 and T2 observed for glyphosate and AMPA in the suspended sediment and, to a lesser extent, in the runoff water also indicate that the seeding lines in T2 delay both sediment and water movement by obstructing the flow. In the smooth SS, sediment and water could be transported directly from the AA to the outlet and thus the sediment reached the outlet faster and in higher quantities. The trend in T2 suggests that for longer lasting rainfall events, the sinks promoted by the seeding lines are filled, and transported runoff, sediment and adsorbed glyphosate and AMPA increase over time. The seeding lines also seem to promote a delaying and buffering effect on the off-site transport of glyphosate and AMPA to the outlet, since much higher amounts of glyphosate and AMPA were recovered on the soil surface of T2. Nevertheless, this effect also promoted a higher off-site transport of these compounds to the areas downslope the AA. In fact, the background group contributed the most to the total off-site transport of glyphosate and AMPA observed in T2. This suggests that the buffering/delaying effect of the seeding lines promoted a longer presence of contaminated runoff water on the surface of the soil, which in turn promoted a longer time for the re-adsorption of glyphosate and AMPA to the soil particles.

The results from the mass balance indicate that the total amount of glyphosate and AMPA transported with runoff (T1: 4.5%; T2: 0.7%) was limited compared to the amounts recovered in the AA (T1: 74.0%; T2: 90.4%). Yang et al. (2015b), in a flume experiment with a smooth surface and bare loess soil, also reported much higher amounts of glyphosate being recovered from the SS than in the runoff. Besides, they reported a total glyphosate and AMPA transport with runoff of 14%, which is quite higher than what we obtained for a smooth surface. They used, however, steeper slopes (18% and 36%) and a higher rainfall intensity (60 mm h^{-1}).

Although glyphosate and AMPA adsorb strongly to soil particles, research on their transport by water erosion during rainfall events has focused mostly on their content in runoff water (Siimes et al., 2006; Warnemuende et al., 2007; Gregoire et al., 2010; Birch et al., 2011; Coupe et al., 2012; Daouk et al., 2013). Only recently, more attention has been paid to their particle-bound transport with runoff (Yang et al., 2015a; Yang et al., 2015b; Melland et al., 2016; Napoli et al., 2016). Our study indicates that the contribution of total particle-bound transport was much higher than the total water-dissolved transport, particularly for T2. Nevertheless, if we only consider the particle-bound contribution from runoff (excluding the contribution from the SS), higher amounts of glyphosate were transported dissolved in water (T1: 2.8%; T2: 0.5%) than bound to soil particles (T1: 1.7%; T2: 0.2%). This contradicts the findings by Yang et al. (2015b), who reported that much higher amounts of glyphosate and AMPA were transported bound to soil particles (10%) than dissolved in runoff water (4%). In a field experiment with loess soil, Yang et al. (2015a) also reported a much higher percentage of particle-bound transport of glyphosate than of water-dissolved transport. Besides the trapping effects of the seeding lines in T2, the low slope (5%) used in our study most probably explains these results. This study also indicates that OM and clay minerals are rapidly and easily transported with runoff, and that these are significantly and positively correlated with glyphosate and AMPA contents in sediment. Therefore, OM and clay particles seem to facilitate the rapid particle-bound transport of glyphosate and AMPA. The rapid transport of OM and fine particles with water-eroded sediment has also been reported before (Palis et al., 1997; Polyakov and Lal, 2004; Shakesby et al., 2015). Likewise, the high glyphosate and AMPA contents linked with OM and clay minerals have also been suggested and/or proven by other studies (Sprankle et al., 1975; Bento et al., 2017).

4.2. Off-site deposition of glyphosate and AMPA

Although various studies have assessed the off-target transport of glyphosate and AMPA during rainfall events (Peruzzo et al., 2008; Daouk et al., 2013; Sasal et al., 2015; Melland et al., 2016), their transport to adjacent fields is normally disregarded. However, as shown in this study, the transport of sediment to adjacent areas can be a source of pollution. In our study, the transport and deposition of glyphosate and AMPA downslope the AA was much higher than that transported to the outlet, particularly in the SS with seeding lines (T2). This off-site deposition in the downslope areas decreased rapidly over distance though, particularly in T2. These results show once again the influence of SS micro-topography and morphological complexity on hydrological and sediment connectivity during water-erosion events. Higher morphological complexity, in this study promoted by the seeding lines, result in lower hydrological and sediment connectivity, which in turn promote a higher soil surface deposition and a lower erosion response. This affects, in the same order of magnitude, the off-site deposition of glyphosate and AMPA on the soil surface and the off-site transport of these compounds to the outlet. Although glyphosate contents in the DS and LS groups kept high over distance, particularly in the smooth SS, they also decreased. Nevertheless, the suspended sediment in runoff presented much higher glyphosate and AMPA contents than the SS groups, suggesting that the former travelled directly from the AA to the outlet. The background group corresponds to the area on the soil surface where visible transport processes did not occur. While the DS and LS groups are both active sediment transport groups, the glyphosate and AMPA recovered from the background group has most likely been transported there by runoff water containing desorbed glyphosate and AMPA. When rainfall occurs, some glyphosate and AMPA desorb from the soil to the water phase, and they are transported with runoff water, as shown by our results on glyphosate concentrations in runoff water. Nevertheless, during overland water flow and/or (re)infiltration of the runoff water, some of the desorbed glyphosate may re-adsorb into soil particles. The rate to which glyphosate adsorbs to and desorbs from soil particles depends mostly on soil properties, and its desorption can vary from values as low as 0.6% to as high as 80% of the adsorbed glyphosate (Piccolo et al., 1994; Sørensen et al., 2006). Piccolo et al. (1994) also suggested that "glyphosate adsorption on soils is far from being permanent".

4.3. From small flume scales to field scales

This study quantified the transport and (re)distribution of glyphosate and AMPA with sediment after erosive rainfall events. However, the relations shown in this small-scale laboratory study cannot be directly translated to the field or catchment scale. Upscaling erosion and sediment dynamics from flume – plot to field – catchment scales is known to be difficult (e.g. Delmas et al., 2012). Particularly, the connectivity and the spatial source-sink distribution are different in field situations as compared to laboratory experiments, and largely influence runoff and sediment redistribution. This particularly concerns treatment T2 in which we mimicked the effect of micro-topographic disturbances caused by seeding lines. Furthermore, the very small scale of the flumes does not allow to exactly replicate a specific crop and field spacing between seeding lines. Larger scale flume experiments as well as field experiments to quantify glyphosate and AMPA transport with sediment and their redistribution and deposition in adjacent fields are therefore recommended. Investigations into the effect of different soil cover percentages promoted by the litter remaining on the soil surface from previous crops are also recommended. Moreover, given the high variation in our results, the number of replicates (n = 3) performed per treatment has proven to be a limitation for this type of experiment. Guaranteeing the exact same conditions in a flume with disturbed/sieved soil is difficult and most probably explains the variation obtained. Thus, in future research, a higher number of replicates should be used in this type of experiments.

5. Conclusions

Desorbed glyphosate and AMPA into the runoff water, as well as particle-bound transport, may result in considerable amounts of glyphosate and AMPA being transported, re-adsorbed and deposited onto adjacent off-target areas (8-18% of the applied glyphosate after four rain events). Moreover, the particle-bound transport of these compounds is mostly associated with OM and clay minerals, which are easily and rapidly transported with runoff. There is, thus, a risk of pollution of the terrestrial (adjacent fields) and aquatic (surface waters) environments. Differences in the micro-topography of the two soil surfaces tested in this study affected runoff and sediment dynamics and, consequently, glyphosate and AMPA mobility. The presence of (micro)topographic disturbances can work as buffer zones, helping to reduce the runoff and erosion rates and, consequently, the amount of glyphosate and AMPA transported with runoff and suspended sediment. Nevertheless, if they are not created/present in the application area and on their downslope edges, they may increase the off-target transport of glyphosate and AMPA to adjacent fields. Field studies on the extent to which glyphosate and AMPA can be transported and deposited onto adjacent off-target fields need further attention.

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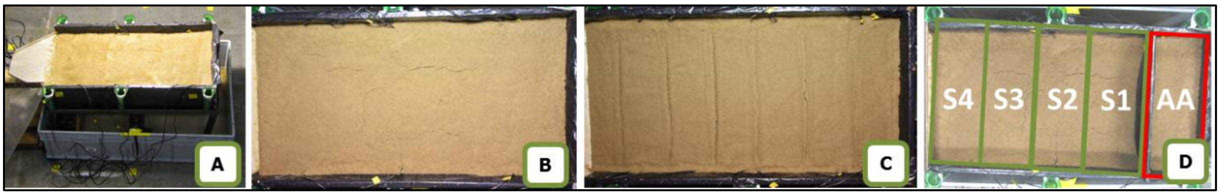


Figure 1: A. Soil flume; B. Treatment T1 – Smooth surface; C. Treatment T2 – "Seeding lines"; D. Glyphosate application area (AA; red) and sampling segments (S1-S4; green).

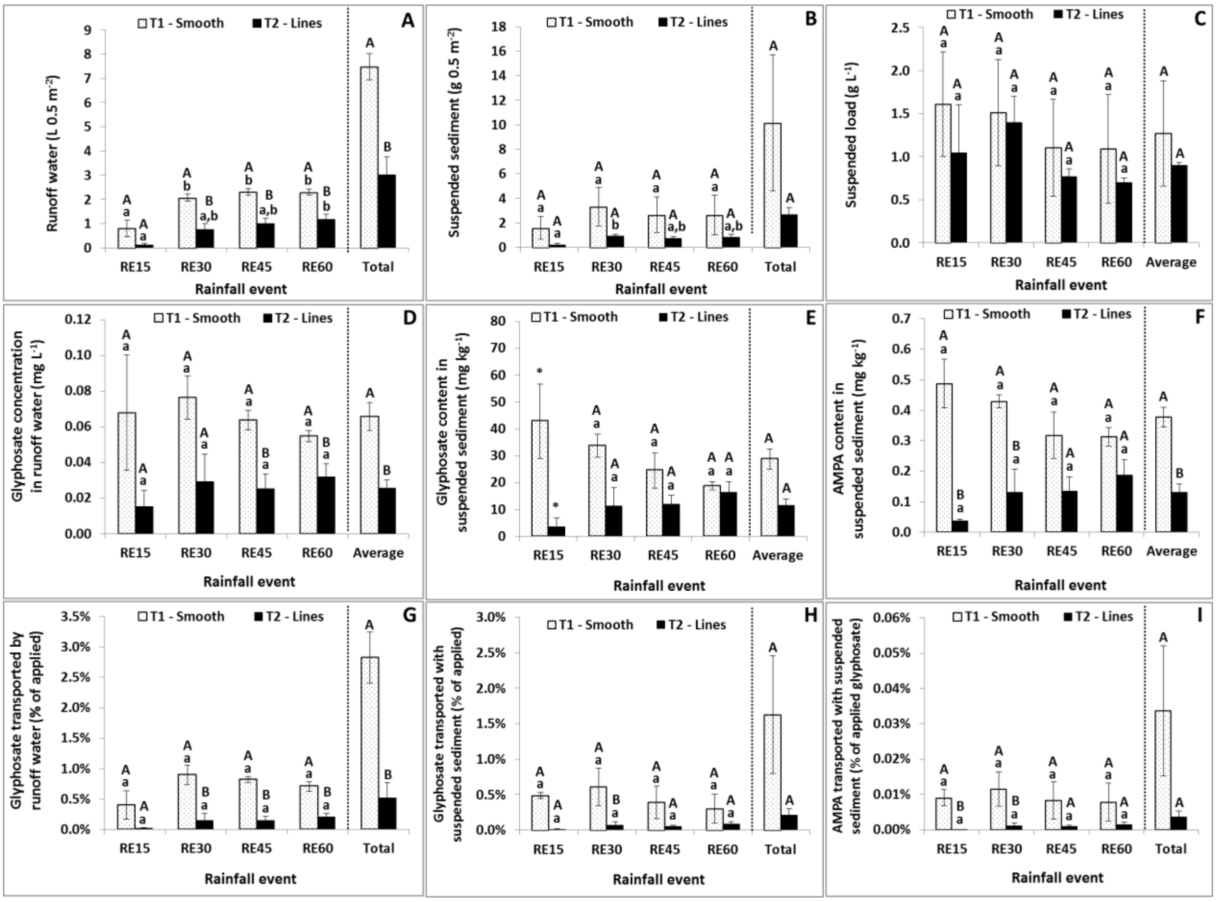
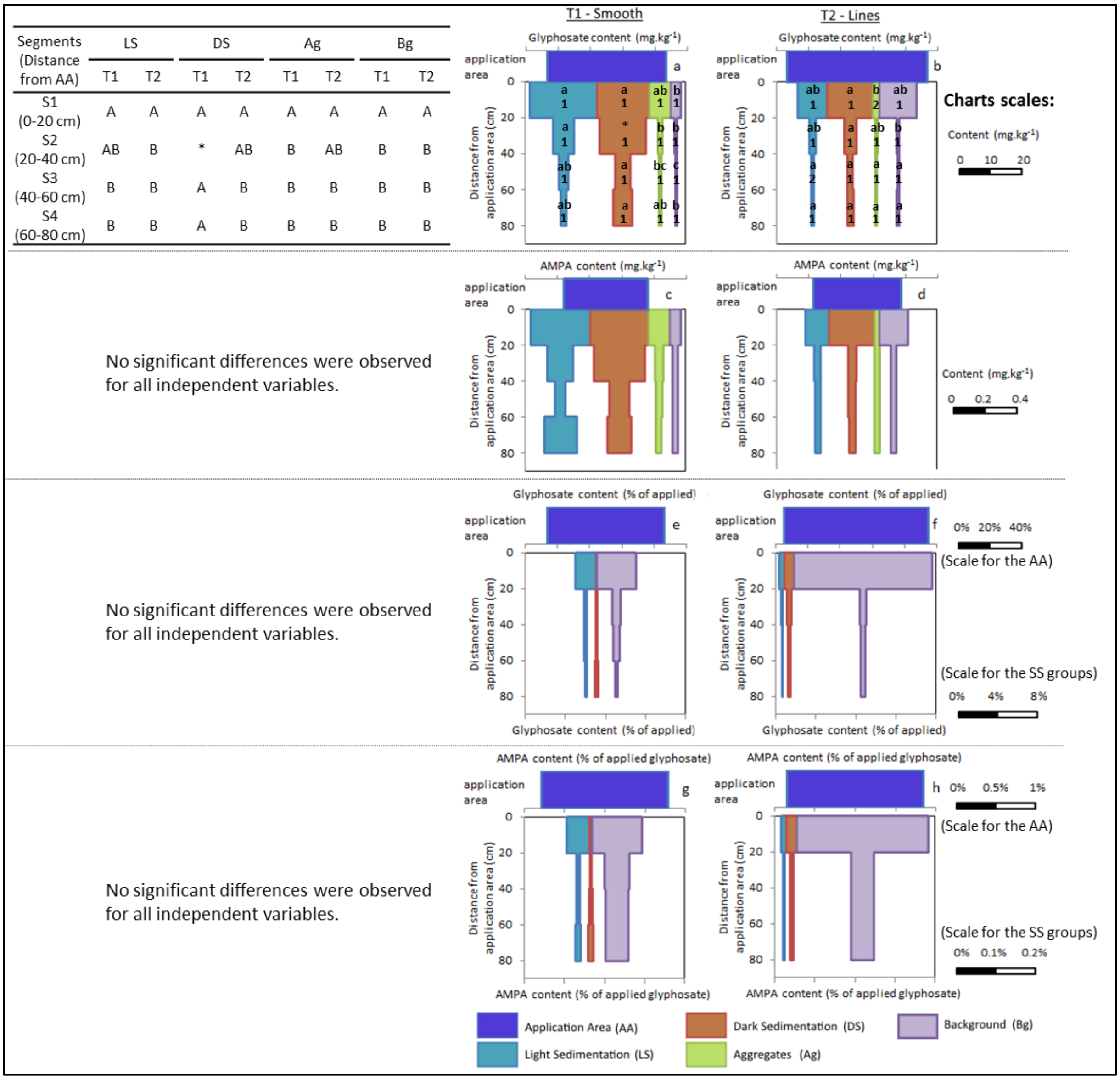


Figure 2: Runoff water (A), suspended sediment (B), suspended load concentration (C), glyphosate content and amount transported with runoff water (D, G), and glyphosate and AMPA contents and amounts transported with suspended sediment (E-F, H-I), for each 15-min rain event (RE) and for the total rain, in the different treatments (average ± SE). Different lowercase letters mean significant differences between REs for the same treatment (p<0.05). Different capital letters mean significant differences between treatments for the same RE (p<0.05). *Excluded from mixed ANOVA analysis due to missing values. Note the different vertical scales between all graphs.



Segments (Distance from AA)	LS		DS		Ag		Bg	
	T1	T2	T1	T2	T1	T2	T1	T2
S1 (0-20 cm)	A	A	A	A	A	A	A	A
S2 (20-40 cm)	AB	B	*	AB	B	AB	B	B
S3 (40-60 cm)	B	B	A	B	B	B	B	B
S4 (60-80 cm)	B	B	A	B	B	B	B	B

No significant differences were observed for all independent variables.

No significant differences were observed for all independent variables.

No significant differences were observed for all independent variables.

Figure 3: Glyphosate (a-b, e-f) and AMPA (c-d, g-h) redistribution on the SS of the flumes at the end of the entire rainfall simulations (average) for both treatments. Note the different charts scales. Different capital letters mean significant differences between segments within the same treatment and SS group ($p<0.05$). Different lowercase letters mean significant differences between SS groups within the same treatment and segment ($p<0.05$). Different numbers (1, 2) mean significant differences between treatments within the same SS group and segment ($p<0.05$). *Excluded from statistical analysis due to missing values (n = 1). For a better reading of the values (average ± SE) presented in this figure, refer to Table S1 in the supplementary material.

Table 1: Cumulative rainfall characteristics and soil moisture (SM) contents per treatment, for each 15-min rainfall event (RE). SM corresponds to measurements right before the starting of each RE.

	RE 15	RE 30	RE 45	RE 60
Cumulative rain (mm) – T1	7.2 ± 0.9 A	15.1 ± 1.9 A	22.9 ± 3.0 A	30.6 ± 3.8 A
Cumulative rain (mm) – T2	7.4 ± 0.8 A	14.8 ± 1.5 A	22.3 ± 2.3 A	29.4 ± 3.1 A
SM-T1 at 5 cm depth (%)	17.7 ± 3.5 A; a	21.0 ± 4.9 A; a, b	22.9 ± 4.1 A; b	23.9 ± 2.9 A; b
SM-T2 at 5 cm depth (%)	17.9 ± 0.7 A; a	23.5 ± 0.1 A; b	25.4 ± 1.0 A; b	26.7 ± 1.3 A; b
SM-T1 at 10 cm depth (%)	5.5 ± 0.9 A; a	6.1 ± 1.3 A; a, b	7.4 ± 2.2 A; a, b	9.4 ± 3.4 A; b
SM-T2 at 10 cm depth (%)	4.2 ± 0.7 A; a	4.4 ± 0.7 A; a, b	5.0 ± 0.2 A; a, b	7.6 ± 0.4 A; b

Different capital letters mean significant differences ($p<0.05$) between treatments for the same parameter and same RE.

Different lowercase letters within the same row mean significant differences ($p<0.05$) between REs.

Table 2: Glyphosate and AMPA distribution in the AA and transported to off-target areas at the end of the rainfall simulations, for both treatments (average±SE).

	Glyphosate (% of applied)		AMPA (% of applied glyphosate)		TOTAL (glyphosate + AMPA) (% of applied glyphosate)	
Treatment	T1: Smooth	T2: Lines	T1: Smooth	T2: Lines	T1: Smooth	T2: Lines
Application Area (AA)	72.5 ± 6.1 a	88.7 ± 9.0 a	1.6 ± 0.1 a	1.7 ± 0.2 a	74.0 ± 6.2 a	90.4 ± 9.2 a
Off-target transport:						
- Soil surface (SS) (∑SS groups)	7.8 ± 1.2 a	17.6 ± 7.4 a	0.38 ± 0.02 a	0.56 ± 0.17 a	8.2 ± 1.2 a	18.2 ± 7.6 a
- Total transport w/ runoff (water + susp. sed.)	4.5 ± 1.2 b	0.72 ± 0.36 a	0.03 ± 0.02 a	0.004 ± 0.002 a	4.5 ± 1.2 b	0.72 ± 0.36 a
- Water-dissolved transport (runoff water)	2.8 ± 0.4 b	0.51 ± 0.26 a	< LOD	< LOD	2.8 ± 0.4 b	0.51 ± 0.26 a
- Particle-bound transport (susp. sed. + SS)	9.4 ± 1.8 a	17.8 ± 7.3 a	0.42 ± 0.04 a	0.56 ± 0.16 a	9.9 ± 1.8 a	18.4 ± 7.5 a
- Total transported (TT) (runoff + SS)	12.3 ± 2.2 a	18.4 ± 7.1 a	0.42 ± 0.04 a	0.56 ± 0.16 a	12.7 ± 2.2 a	18.9 ± 7.3 a
TOTAL recovered (AA + TT)	84.7 ± 10.3 a	109 ± 6 a	2.0 ± 0.2 a	2.3 ± 0.1 a	86.7 ± 10.5 a	111 ± 6 a

Different lowercase letters mean significant differences between treatments ($p<0.05$); susp. sed.: suspended sediment; LOD: Limit of Detection of AMPA = 0.008 mg L^{-1}

Table 3: Sediment properties (A) and their relationship with the transported glyphosate and AMPA by water erosion (B).

A. Sediment properties

SS Group / Time step	Clay (%) (0-2 µm)	Silt (%) (2-50 µm)	Sand (%) (>50 µm)	OM (%)
Light sedimentation (LS)	7.1	69.2	23.7	0.89
Dark sedimentation (DS)	9.9	75.8	14.3	2.1
Aggregates	9.6	77.6	12.8	2.4
Background	9.4	77.7	12.9	1.3
Suspended sediment T15	15.1	80.0	4.9	---
Suspended sediment T30	13.2	83.1	3.7	3.6
Suspended sediment T45	13.5	82.6	3.8	3.8
Suspended sediment T60	10.9	83.8	5.3	3.0

B. Relationship between sediment properties and glyphosate or AMPA contents

	Clay (C)	Silt (St)	Sand (S)	OM
Glyphosate (G)	G = 3.0 C – 21.5; $R^2 = 0.74$*	G = 1.3 St – 93.1; $R^2 = 0.48$	G = –1.1 S + 22.7; $R^2 = 0.64$*	G = 7.3 OM – 7.0; $R^2 = 0.74$*
AMPA (A)	A = 0.03 C – 0.16; $R^2 = 0.66$*	A = 0.01 St – 0.79; $R^2 = 0.37$	A = – 0.01 S + 0.27; $R^2 = 0.52$*	A = 0.07 OM – 0.02; $R^2 = 0.65$*

* Significant correlation between the sediment property and the compound by Pearson ($p<0.05$).

Table S1: Glyphosate and AMPA (average±SE) redistribution on the soil surface of the flumes at the end of the rainfall simulations for both treatments.

Treatment	Distance from AA (cm)	Glyphosate content [mg kg^{-1}; (% of applied)]				AMPA content [mg kg^{-1}; (% of applied glyphosate)]			
		Light Sedimentation	Dark Sedimentation	$Aggregates	Background	Light Sedimentation	Dark Sedimentation	$Aggregates	Background
T1 (Smooth Soil Surface)	0-20	*20.8 ± 6.5 (2.0 ± 0.4)	*16.3 ± 3.8 (0.18 ± 0.10)	6.9 ± 1.9	3.1 ± 0.4 (3.8 ± 0.3)	*0.37 ± 0.03 (0.06 ± 0.001)	*0.36 ± 0.05 (0.01 ± 0.004)	0.13 ± 0.04	0.07 ± 0.003 (0.13 ± 0.002)
	20-40	6.3 ± 2.1 (0.21 ± 0.08)	#14.3 ± … (0.10 ± …)	1.0 ± 0.3	0.5 ± 0.1 (0.70 ± 0.13)	0.16 ± 0.06 (0.01 ± 0.003)	#0.31 ± … (0.003 ± …)	0.05 ± 0.01	0.03 ± 0.00 (0.06 ± 0.001)
	40-60	2.5 ± 0.7 (0.20 ± 0.10)	*4.6 ± 0.4 (0.16 ± 0.12)	0.7 ± 0.2	0.5 ± 0.1 (0.59 ± 0.19)	0.06 ± 0.01 (0.01 ± 0.003)	*0.12 ± 0.01 (0.01 ± 0.004)	0.04 ± 0.01	0.03 ± 0.001 (0.06 ± 0.003)
	60-80	*1.6 ± 0.4 (0.06 ± 0.03)	5.8 ± 3.1 (0.33 ± 0.18)	0.5 ± 0.2	0.2 ± 0.1 (0.25 ± 0.11)	*0.20 ± 0.16 (0.01 ± 0.01)	0.15 ± 0.06 (0.01 ± 0.01)	0.03 ± 0.0004	0.03 ± 0.002 (0.05 ± 0.003)
T2 (Surface with “seeding lines”)	0-20	9.3 ± 1.3 (0.48 ± 0.11)	14.1 ± 1.5 (0.90 ± 0.27)	2.3 ± 0.2	11.3 ± 6.3 (13.8 ± 7.5)	0.15 ± 0.02 (0.01 ± 0.003)	0.28 ± 0.03 (0.03 ± 0.01)	0.03 ± 0.002	0.18 ± 0.09 (0.33 ± 0.17)
	20-40	1.7 ± 1.0 (0.11 ± 0.08)	3.6 ± 0.7 (0.37 ± 0.16)	0.5 ± 0.1	0.5 ± 0.2 (0.63 ± 0.20)	0.04 ± 0.01 (0.003 ± 0.001)	0.05 ± 0.01 (0.01 ± 0.003)	0.03 ± 0.00	0.03 ± 0.00 (0.06 ±0.002)
	40-60	0.5 ± 0.1 (0.02 ± 0.01)	1.7 ± 0.8 (0.22 ± 0.12)	0.4 ± 0.2	0.3 ± 0.1 (0.39 ± 0.11)	0.03 ± 0.00 (0.002 ± 0.0005)	0.04 ± 0.01 (0.01 ± 0.002)	0.03 ± 0.00	0.03 ± 0.00 (0.06 ± 0.0002)
	60-80	0.7 ± 0.1 (0.02 ± 0.01)	1.7 ± 0.8 (0.24 ± 0.16)	0.3 ± 0.1	0.4 ± 0.1 (0.45 ± 0.14)	0.03 ± 0.00 (0.001 ± 0.001)	0.04 ± 0.01 (0.01 ± 0.003)	0.03 ± 0.00	0.03 ± 0.001 (0.06 ± 0.001)

* n=2; # n=1; for samples with the symbols * and #, it means that the SS (soil surface) group was not observed in 1 or 2 of the replicates, respectively, at the corresponding distance from the application area (AA).

$ Mass balance of glyphosate and AMPA in the aggregates in percentage of applied glyphosate could not be determined because it was not possible to accurately distinguish the background group from the aggregates in the software used to determine the areas occupied by the different SS groups.

Values of AMPA highlighted in red mean that all samples from triplicates were <LOD and that the LOD value was assumed for statistical purposes.

Values of AMPA highlighted in orange mean that the average value of the triplicates fell between LOD and LOQ (with at least 1 triplicate detected <LOD).