Glyphosate and aminomethylphosphonic acid (AMPA) behavior in loess soils and off-site transport risk assessment - Célia P. M. Bento

Glyphosate and aminomethylphosphonic acid (AMPA) behavior in loess soils and off-site transport risk assessment

Célia Patrícia Martins Bento

Propositions

1. The inappropriate use of first-order decay kinetics for glyphosate underestimates its persistence in soils.
   (this thesis)

2. Contrary to common understanding, glyphosate and AMPA are easily transported to off-target environments by both water and wind erosion.
   (this thesis)

3. The lobbying and economic interests of pesticide producing corporations strongly affect the development of herbicide-free technologies.

4. Portuguese law needs more severe punishments for arsonists in order to reduce wildfire problems.

5. The temporary house rental practices in Wageningen are environmentally unsustainable.

6. The Dutch are excellent planners and engineers, but terrible cooks.

Propositions belonging to the thesis entitled:

‘Glyphosate and Aminomethylphosphonic acid (AMPA) behavior in loess soils and off-site transport risk assessment’

Célia P. Martins Bento
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Table of contents

List of Abbreviations

AA	Application Area (chapter 4)
ADI	Acceptable Daily Intake
Al	Aluminium
Ag	Aggregates (chapter 4)
AMPA	Aminomethylphosphonic Acid
Bg	Background (chapter 4)
Bw	Body weight
C	Carbon
C_{Amax}	Maximum observed level/concentration of AMPA, i.e. concentration at which AMPA peaks
CC	Calibration Curve (chapter 2)
CO_2	Carbon dioxide
cRfD	Chronic Reference Dose
D	Absence of light (chapter 2)
DFOP	Double-First-Order-in-Parallel kinetic model
DS	Dark Sedimentation (chapter 4)
DT_{50}	Half life time or Dissipation Time of a compound, i.e. the time required for 50% of a compound to disappear from a given environmental compartment
DT_{90}	Dissipation Time of a compound, i.e. the time required for 90% of a compound to disappear from a given environmental compartment
d.w.	Dry weight
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EPSPS	5-enolpyruvyl-shikimate-3-phosphate synthase
EU	European Union
Fe	Iron
ffA	Formation fraction of AMPA
FOMC	First-Order Multi-Compartment kinetic model (also known as Gustafson and Holden model)
GM crops or GMC	Genetically Modified crops
GR crops	Glyphosate-Resistant crops
HPLC-MS/MS	High Performance Liquid Chromatography-tandem Mass Spectrometry
HS	Hockey-Stick kinetic model

HT	Herbicide Tolerance
IARC	International Agency for Research on Cancer
IR	Insect Resistance
JMPR	Joint Food and Agriculture Organization of the United Nations – World Health Organization Meeting on Pesticide Residues
L	Presence of light (chapter 2)
LOD	Limit of Detection
LOQ	Limit of Quantification
LS	Light Sedimentation (chapter 4)
MRLs	Maximum Residue Levels
N	Nitrogen
NS	Nonsterile soil (chapter 2)
OM	Organic Matter
P	Phosphorus
PAG	Percentage of Applied Glyphosate
POEA	Polyethoxylated Tallowamine Surfactant/Adjuvant
PPPs	Plant Protection Products
QC	Quality Control (chapter 2)
RE	Rainfall Event (chapter 4)
RR	Roundup-Ready
RSD	Relative Standard Deviation (chapter 2)
S	Sterile soil (chapter 2)
Sat	Saturated soil (chapter 2)
SE	Standard Error
SFO	Single First-Order kinetic model
SM	Soil Moisture
SS	Soil Surface (chapter 4)
WHC	Water Holding Capacity
w/w	weight/weight

1. General introduction

1.1 The use of glyphosate-based herbicides

Glyphosate-based herbicides are the most used worldwide for weed control in agricultural and non-agricultural systems (Benbrook, 2016; Duke and Powles, 2008) for already more than two decades (Cox, 2004; Baylis, 2000). In non-agricultural systems, glyphosate-based herbicides are commonly applied in streets and parks of urban areas, backyards, waterways, by professional gardeners, among others (Tang et al., 2015; van Stempvoort et al., 2014; Dugdale and Champion, 2012; Hanke et al., 2010; Kristoffersen et al., 2008). Under conventional agricultural systems, glyphosate-based herbicides are commonly applied before planting or between established rows in vines and tree plantations in orchards and groves (Maqueda et al., 2017; Singh et al., 2011; Duke and Powles, 2009, 2008; St. Laurent et al., 2008). Glyphosate-based herbicides are also commonly applied prior to harvesting of grain and bean crops, where they are used as desiccants (Zhang et al., 2017; Goffnett et al., 2016; Nelson et al., 2011; Cessna et al., 2002, 2000; Cessna et al., 1994). Under modern agriculture, i.e. agriculture based on the growing of glyphosate-resistant (GR) crops under non-till systems, glyphosate-based herbicides are commonly applied before planting and once or twice during the crop growing season (Benbrook, 2016; Duke and Powles, 2009, 2008; Baylis, 2000).

The largest portion of glyphosate's global use is associated to the growing of GR crops, which are biotech crops (or genetically modified crops – GM crops) that were genetically engineered to be resistant to it (Benbrook, 2016; Duke and Powles, 2008). Biotech crops, in general, are commercialized globally for >20 years (since 1996) and undergo rapid adoption worldwide (ISAAA, 2016). By 2016, >185 million ha were cultivated with biotech crops (ISAAA, 2016). The leading top-five biotech crop planting countries are the USA (72.9 million ha), Brazil (49.1 million ha), Argentina (23.8 million ha), Canada (11.6 million ha) and India (10.8 million ha), corresponding to 91% of the global biotech crop planted area in 2016 (ISAAA, 2016). Soybean (91.4 million ha; 49%), maize (60.6 million ha; 33%), cotton (22.3 million ha; 12%) and canola (8.6 million ha; 5%) are the main biotech crops grown globally, corresponding to 99% of the total cultivated area with such crops in 2016 (ISAAA, 2016).

Besides herbicide tolerance (HT), the main biotech traits grown by farmers are insect resistance (IR) and the so called "stacked traits", which combine herbicide tolerance with insect resistance (HT/IR) (ISAAA, 2016). Herbicide tolerance traits have, however, consistently dominated the market during the >20 years of biotech crops commercialization, although the "stacked traits" (HT/IR) are gaining more and more insight in the market (ISAAA, 2016). By 2016, 88% of the total area cultivated with biotech crops was using herbicide tolerant crops (HT+HT/IR) (ISAAA, 2016). All grown soybean and canola were herbicide tolerant (HT and/or HT/IR), whereas it was 90% of the grown maize and 67%

of the grown cotton (ISAAA, 2016). Herbicide tolerance traits (HT & HT/IR) mostly comprise resistance to glyphosate-based herbicides, representing >80% of all biotech crops (ISAAA, 2016; Duke and Powles, 2009, 2008).

According to Benbrook (2016), >825 000 tons of glyphosate-based herbicides were sold globally in 2014, 90.4% of them were applied in agriculture and 45.8% of them were applied in agricultural fields cultivating GR crops. Glyphosate is the top ranking active ingredient being used in agriculture in most countries, with a usage rate range in 2012 of, for example, 122.5-131.5 thousand tons in the USA (Atwood and Paisley-Jones, 2017), and 197 thousand tons in Argentina (KleffmannGroup, 2014).

1.2 Environmental & human health concerns related to the use of glyphosate-based herbicides

Glyphosate is often said to be environmentally friendly and of no or low concern to the environment and human health (Borggaard and Gimsing, 2008; Duke and Powles, 2008; Cox, 2004; Giesy et al., 2000; Williams et al., 2000). It has been regarded by regulatory authorities and scientific professionals as non- or only slightly-toxic to the environment and human health and non-carcinogenic to humans (Mink et al., 2012; Borggaard and Gimsing, 2008; Duke and Powles, 2008; Baylis, 2000; Giesy et al., 2000; Williams et al., 2000; U.S.EPA, 1993). This has mostly been based in the facts that glyphosate is non-volatile, biodegrades fast, adsorbs strongly to soil particles and that it acts in the shikimate pathway, which is absent in animals and humans (see section 1.4 for glyphosate mode of action) (Duke and Powles, 2009; Borggaard and Gimsing, 2008; Duke and Powles, 2008; Baylis, 2000; Williams et al., 2000; U.S.EPA, 1993). The same believes regard to glyphosate’s main metabolite, aminomethylphosphonic acid (AMPA), and to its commercial formulations (Williams et al., 2000).

Nevertheless, studies have shown that glyphosate, glyphosate formulations and/or AMPA can have significant adverse impacts in the environment and human health, and concerns about glyphosate’s safety are growing considerably in society (Foucart and Horel, 2018; van Bruggen et al., 2018; Cressey, 2015; Leguizamón, 2014; Arancibia, 2013; Delvenne et al., 2013; Cox, 2004). In fact, commercial glyphosate-containing products are often reported to present higher toxicity than glyphosate itself, particularly those using a polyethoxylated tallowamine (POEA) surfactant (Bonfanti et al., 2018; Duke and Powles, 2008; Peixoto, 2005; Howe et al., 2004; Giesy et al., 2000; Folmar et al., 1979).

1.2.1 Environmental health impacts associated to glyphosate, glyphosate-based herbicides and AMPA

Although several studies have reported glyphosate, glyphosate formulations and/or AMPA as non-bioaccumulative and of low toxicity to aquatic, soil and terrestrial microorganisms and invertebrates, as well as birds, mammals, amphibians and fish (Levine et al., 2015; Howe et al., 2004; Giesy et al., 2000; Williams et al., 2000), many others have proven otherwise.

Glyphosate and/or glyphosate formulations have been demonstrated to affect fish reproduction (Lopes et al., 2014), to induce neurotoxicity and cardiovascular toxicity in zebrafish (Roy et al., 2016a; Roy et al., 2016b) and to alter significantly the morphology and behavior of zebrafish larvae and adults (Bridi et al., 2017). Folmar et al. (1979) demonstrated that the glyphosate formulation Roundup and its surfactant POEA were slightly to moderately toxic to aquatic invertebrates and fish. Tate et al. (1997) demonstrated that sublethal concentrations of glyphosate significantly affected the reproduction and development of a species of freshwater snail after long-term exposure. Another study found glyphosate, the glyphosate formulation Roundup and the surfactant POEA to be acutely toxic to freshwater mussels (Bringolf et al., 2007). The glyphosate formulation Roundup was also found highly lethal to larval amphibians (96-100% mortality) in an experiment in outdoor pond mesocosms (Relyea, 2005). In another study, sublethal doses of glyphosate and glyphosate formulations led to congenital malformations in embryos of amphibians and chickens (Paganelli et al., 2010). Abnormal high malformation incidence in piglets was also associated to glyphosate exposure through contaminated feed during pregnancy (Krüger et al., 2014b). The glyphosate formulation Roundup was proven to be genotoxic to caimans (Poletta et al., 2009). Chronic exposure of earthworms to glyphosate-based herbicides has been demonstrated to affect their growth, survival, activity and reproduction success (Gaupp-Berghausen et al., 2015; Springett and Gray, 1992). Glyphosate has also been proven to affect the normal gut bacterial community of cattle and poultry by reducing beneficial bacteria, while pathogenic bacteria were resistant to it (van Bruggen et al., 2018; Krüger et al., 2013; Shehata et al., 2013).

As regards to soil health, glyphosate has been shown to have no or minor effects on soil microorganisms or to even stimulate soil microbial growth and activity by providing a source of C, N and P (FAO and ITPS, 2017; Sviridov et al., 2015; Partoazar et al., 2011; Alexa et al., 2010; Araújo et al., 2003; Accinelli et al., 2002; Dick and Quinn, 1995). However, it has also been shown to reduce soil microbial diversity and to promote shifts in the composition of soil microorganisms (van Bruggen et al., 2018; Partoazar et al., 2011; Lancaster et al., 2010; Dick and Quinn, 1995). According to van Bruggen et al. (2018), these shifts may contribute to a suppression of beneficial microorganisms and an increase of glyphosate insensitive plant/animal pathogens in soil. This may result in an increase of soil borne diseases and a reduction of plant resistance to pathogens such as, for example, infections by *Fusarium* species (van Bruggen et al., 2018; Kremer and Means, 2009; Larson et al., 2006).

Although AMPA has been reported as non-toxic to the flora and fauna (Williams et al., 2000), studies pertaining AMPA are extremely scarce and normally disregarded. In the few studies found, AMPA produced genotoxic effects and induced DNA, tissue (liver and gills) and chromosomal damages in fish (Antunes et al., 2017; Guilherme et al., 2014). In another study with earthworms, the mortality of these organisms in acute and chronic assays under field-relevant concentrations of AMPA was not affected, but the growth of earthworms resulting from parents grown in AMPA contaminated soils were adversely affected (Domínguez et al., 2016).

1.2.2 Human health impacts & concerns associated to glyphosate, glyphosate-based herbicides and AMPA

Glyphosate and AMPA have been often considered non-toxic and of no concern to human health based on evidence that their oral and dermal absorption is low to very low and that they are essentially eliminated through urine and feces unmetabolized (Williams et al., 2000). This evidence is, therefore, seen as an indication that glyphosate and AMPA don’t bioaccumulate in the body tissues (Williams et al., 2000). Besides, humans and animals don’t have the shikimate pathway in their organism (see section 1.4), emphasizing the low risk of glyphosate to animals and humans health (Mottier et al., 2013; Williams et al., 2012; Williams et al., 2000). Moreover, glyphosate and AMPA have been considered as non-carcinogenic, non-mutagenic, non-genotoxic, non-neurotoxic and non-teratogenic based on claims that there is no clear or convincing evidence proving these effects (Mink et al., 2012; Mink et al., 2011; Williams et al., 2000; U.S.EPA, 1993).

Nevertheless, the International Agency for Research on Cancer (IARC) has recently classified glyphosate as “probably carcinogenic to humans” (IARC, 2015). This has resulted in high concerns in society and in strong controversy between regulatory authorities, with the European Food Safety Authority (EFSA) disagreeing with this evaluation (EFSA, 2015). Notwithstanding, Mesnage et al. (2015), in their review study on the toxic effects of glyphosate and glyphosate formulations, concluded that glyphosate-based herbicides could be toxic below regulatory limits for chronic effects, including teratogenic, tumorigenic and hepatorenal effects. Besides, although the shikimate pathway is not shared by humans and animals, it has been reported to be present in gut bacteria, which may affect adversely the immunological system (Aitbali et al., 2018; Samsel and Seneff, 2013; Shehata et al., 2013).

In fact, several studies have demonstrated that glyphosate and glyphosate formulations could have adverse effects on human health. Studies revealed glyphosate to be linked with increasing non-Hodgkyn's lymphoma incidence (de Roos et al., 2003), to Parkinson's disease (Gui et al., 2012; Wang et al., 2011) and to increases in the number of miscarriages as well as dermatological and respiratory diseases (Camacho and Mejía, 2017). Glyphosate also induced epidermal cell death (Heu et al., 2012). Cytotoxic effects, DNA damages and/or endocrine disruption induced by glyphosate, glyphosate formulations, POEA and/or AMPA in different types of human cells were also observed (Kwiatkowska et al., 2017; Benachour and Séralini, 2009; Gasnier et al., 2009; Benachour et al., 2007), and may affect human reproduction and fetal development (Benachour et al., 2007). Thongprakaisang et al. (2013) have shown that glyphosate itself increased the cell proliferation of hormone-dependent breast cancer via estrogenic activity at low and relevant environmental concentrations, and that it may induce cancer cell growth in postmenopausal women. Jayasumana et al. (2015) associated the increase in frequency of chronic kidney disease among farmers from Sri Lanka with their exposure to glyphosate through the drinking of water from contaminated wells and by spraying glyphosate without protective equipment. Krüger et al. (2014a) stated that chronically ill humans presented significantly higher residues of glyphosate in their urine samples than healthy humans.

In studies with rats, it has been shown that acute exposure to the glyphosate formulation Roundup induced oxidative stress and (neural) cell death in immature rat hippocampus (Cattani et al., 2014) and prepubertal rat testis (de Liz Oliveira Cavalli et al., 2013). de Liz Oliveira Cavalli et al. (2013) also have shown that the acute exposure to Roundup disrupts male reproductive functions in prepubertal rats, and that Roundup concentrations 10 times lower than recommended for herbicide action are highly toxic for humans, particularly during childhood and puberty. Chronic exposure to the glyphosate formulation Glifloglex of rat offspring by ingestion of treated drinking water (Gallegos et al., 2016) and of mature male mice by inhalation (intranasal administration) (Baier et al., 2017) affected their central nervous system. Biocarb glyphosate formulation was shown to affect liver metabolism in rats exposed orally to sub-chronic treatments (Benedetti et al., 2004).

As for AMPA, few studies have been conducted so far on its effects to human health, but all existing studies have indicated it to have genotoxic effects in humans (IARC, 2015). AMPA has even been reported has more cytotoxic than glyphosate itself (Roustan et al., 2014; Benachour and Séralini, 2009). According to some studies, AMPA was found to be genotoxic to mammalian and human cells, and to induce DNA damage, chromosomal aberrations, human cell membrane damage, oxidative stress, micronuclei formation and reactive oxygen species formation (van Bruggen et al., 2018; Kwiatkowska et al., 2014; Roustan et al., 2014; Benachour and Séralini, 2009; Mañas et al., 2009a).

1.3 Loess soils, their agricultural use and relationship with glyphosate-based herbicides applications

Loess soils, or loess deposits, are soils of aeolian origin that were formed fundamentally by the accumulation of wind-blown dust and that are predominantly composed by silt-sized particles (10-50 µm) (Frechen et al., 2003; Guobin, 1999; Iriondo, 1997; Pye, 1995). The formation of this type of soils occurred during the Quaternary (mostly during glaciation periods of the Pleistocene), and they originated from the transport of great quantities of wind-blown dust from deserts and glaciated regions that then deposited over downwind wide areas (Christiansen and Hamblin, 2007; Wright, 2001; Pye, 1995; Pécsi, 1990). Loess soils represent around 10% of the Earth's land surface (Christiansen and Hamblin, 2007; Pécsi, 1990), but major deposits are observed in China, Argentina, Europe and the USA (Figure 1.1) (Pécsi, 1990). They are particularly characteristic of and widespread in semiarid regions, along the margins of great deserts, and of steppe and forest steppe areas (Figure 1.1) (Christiansen and Hamblin, 2007; Pécsi, 1990). Their thickness varies greatly, and it can range from as thin as 20 cm to as thick as >300 m (Luehmann et al., 2013; Wright, 2001; Pumpelly, 1879). This type of soils is normally described as being a loose deposit, unstratified, homogeneous, porous, permeable and easily erodible by water (Pécsi, 1990; Keyes, 1898; Pumpelly, 1879).

Loess soils are among the most fertile and agriculturally productive in the world (Catt, 2001; den Biggelaar et al., 2001; Wright, 2001; Keyes, 1898; Pumpelly, 1879). This is mostly due to their main silt composition and high porosity nature that promote a good soil aeration and penetration by plant roots, a good supply of available water for plants even during dry seasons, as well as an easy cultivation and seedbed production (Catt, 2001; Keyes, 1898; Pumpelly, 1879). Moreover, the very nutritive material naturally composing this type of soil allows crop yields in loess soils to be moderately maintained without fertilizer additions over the years, in contrast to any other soil types (Catt, 2001; Pumpelly, 1879). The best example of this is the Loess Plateau of China, which has been cultivated continuously for >4000 years without requiring the addition of manure (Pumpelly, 1879).

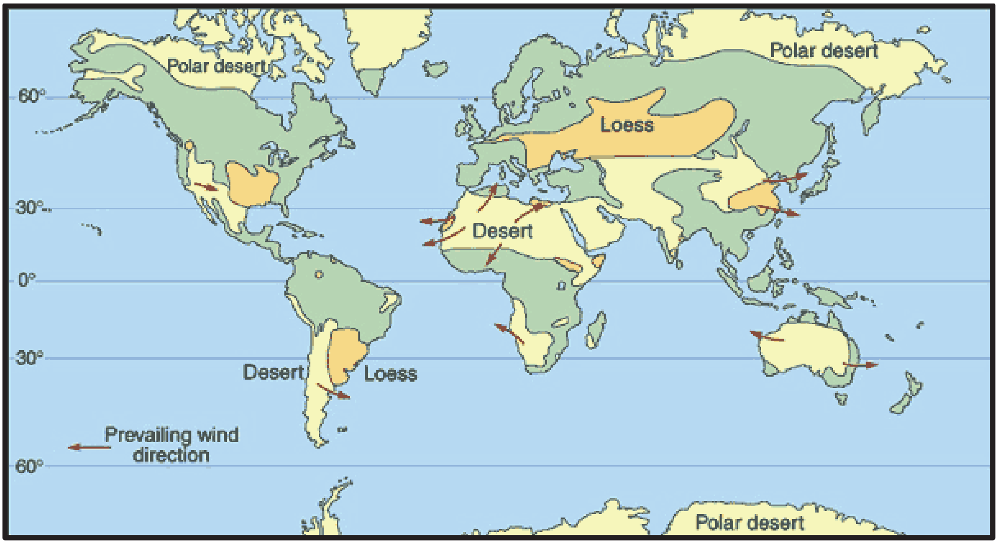


Figure 1.1 *Distribution of the major loess deposits on the globe (Source: Christiansen and Hamblin, 2007).*

Although the natural nutrient reserves of loess soils are currently not enough for maximal crop yields due to modern and intensive agricultural systems, the amount of fertilizers required are normally less than that required for other soil types (Catt, 2001; Sander et al., 1991). Consequently, loess soils are intensively used for agriculture. The Loess Plateau of China, for example, is considered the most important region for rainfed agriculture in China (Li et al., 2015). The loess Pampas of Argentina and the loess areas of the USA are another example of intensive agricultural use, where GR crops are the main and most intensively cultivated (Leguizamón, 2014; Penna and Lema, 2003; Wauchope et al., 2002; Catt, 2001). Such intensive adoption of GR crops in Argentina and the USA resulted in huge amounts of glyphosate-based herbicides being applied every year in these countries and, consequently, in their loess soil areas (Duke, 2017; Benbrook, 2016; KleffmannGroup, 2014; Leguizamón, 2014; Duke and Powles, 2009). Notwithstanding, glyphosate-based herbicides are the most used worldwide, including in loess agricultural areas of the globe where GR crop cultivations are not allowed or used, such as Europe (Silva et al., 2018; Conrad et al., 2017; Benbrook, 2016; Zhang et al., 2015).

1.4 Glyphosate mode of action

Glyphosate is the active ingredient of many herbicide commercial formulations sold around the world. It is a broad spectrum, non-selective, systemic, post-emergent, foliar applied herbicide used to control annual and perennial weeds (Benbrook, 2016; Baylis, 2000; Giesy et al., 2000; Williams et al., 2000; U.S.EPA, 1993). It was first introduced by Monsanto as Roundup herbicide in 1974 (Benbrook, 2016; Williams et al., 2012; Duke and Powles, 2008). Nevertheless, it was with the advent of GR crops in 1996 (see section 1.1) – the so called "Roundup-Ready" (RR) seeds; also introduced by Monsanto –, that its global use increased tremendously (Benbrook, 2016; Williams et al., 2012; Duke and Powles, 2008; Baylis, 2000). Glyphosate's mode of action is the inhibition of the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) of the shikimate pathway (Duke and Powles, 2008; Baylis, 2000; Giesy et al., 2000; Williams et al., 2000; Siehl, 1997). The inhibition of EPSPS by glyphosate results in the accumulation of shikimate-3-phosphate, which in turn inhibits the production of essential aromatic amino acids, affecting the synthesis of proteins (Duke and Powles, 2008; Baylis, 2000; Giesy et al., 2000; Williams et al., 2000; Siehl, 1997). This shikimate pathway is only found in plants and some fungi and bacteria, thus, glyphosate is claimed to be harmless to organisms of the animal kingdom (Dill, 2005; Giesy et al., 2000; Williams et al., 2000; Siehl, 1997).

Glyphosate's herbicidal action is only effective when directly sprayed onto the foliage of plants, from where it is assimilated by the leaves and translocated within the phloem throughout the plants (Giesy et al., 2000; Williams et al., 2000; U.S.EPA, 1993). Once glyphosate reaches the soil, it becomes inactive for its purpose and hence it is unable to kill any weeds emerging after application (Baylis, 2000; Giesy et al., 2000). Glyphosate alone has a poor absorption into plants and therefore it is normally sold as a salt – the most common ones being isopropylamine, trimesium, ammonium and sodium salts -, combined with a surfactant to aid its penetration into the plant surfaces and improve its effectiveness (Duke and Powles, 2008; Baylis, 2000; Giesy et al., 2000; Williams et al., 2000).

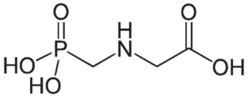
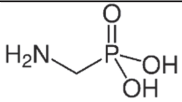
1.5 Environmental fate of glyphosate and AMPA

The environmental fate of pesticides and their metabolites can undergo different processes, which mainly depend on their properties such as volatility, chemical and microbial degradation, adsorption/desorption, and solubility in water and organic solvents. The knowledge of such properties help predicting the behavior and risk of off-target movement of pesticides in the different environmental compartments (air, soil, water), as well as their uptake and potential bioaccumulation by the biota. Nevertheless, experimental studies are always of extreme importance to confirm and judge such predictions, especially due to the complexity normally associated to such pollutants.

Glyphosate and AMPA properties

Glyphosate and AMPA have both a low vapor pressure and Henry’s law volatility constant (Table 1.1), and are, consequently, assumed as non-volatile (Williams et al., 2012; Williams et al., 2000; U.S.EPA, 1993). Both compounds are very soluble in water (Table 1.1), however they also strongly adsorb to soil particles (Table 1.1), which imprison them in the upper soil layer and turn them unlikely to leach into deeper soil layers and groundwater (Yang et al., 2015a; Yang et al., 2015b; Williams et al., 2012; Giesy et al., 2000; U.S.EPA, 1993; Ghassemi et al., 1982). Both compounds are very hydrophilic and practically insoluble in organic solvents (very low log P_{ow}; Table 1.1), and they are therefore assumed as non-bioaccumulative in living organisms (EU, 2002; Williams et al., 2000; U.S.EPA, 1993; Ghassemi et al., 1982).

Table 1.1 *Glyphosate and AMPA properties. (Sources: Lewis et al., 2016; Báez et al., 2015; EU, 2002; U.S.EPA)*

Common name	Glyphosate	AMPA
Chemical name	N-phosphonomethylglycine	Aminomethylphosphonic acid
CAS number	1071-83-6	1066-51-9
Molecular formula	$C_3H_8NO_5P$	CH_6NO_3P
Structural formula		
Exact mass	169.01 g mol^{-1}	111.01 g mol^{-1}
Vapor pressure (25°C)	1.31 x 10^{-5} Pa	8.44 x 10^{-4} Pa
Henry’s law volatility constant (25°C)	2.1 x 10^{-7} Pa m^3 mol^{-1}	2.6 x 10^{-3} Pa m^3 mol^{-1}
Solubility in water (20°C)	10.5 g L^{-1}	1467 g L^{-1}
Partition coefficient (log P_{ow}) (20°C)	-3.2	-1.6
Freundlich adsorption coefficient (k_f)	93 – 2754 μg$^{(1-1/n)}$ mL$^{1/n}$ g^{-1}	38 – 1517 μg$^{(1-1/n)}$ mL$^{1/n}$ g^{-1}
Solid/water distribution coefficient (K_d)	5.3 – 900 L Kg^{-1}	15 – 1554 L Kg^{-1}
Soil organic carbon normalized adsorption coefficient (K_{oc})	884 – 60000 L Kg^{-1}	1160 – 24800 L Kg^{-1}
*Half-life (DT_{50}) range in soil	1 – 197 days	23 – 958 days
*DT_{90} range in soil	40 – 280 days	unknown

* Time required for 50% (DT_{50}) and 90% (DT_{90}) of a compound to disappear from an environmental compartment.

1.5.1 Decay behavior in soil

Glyphosate and AMPA are considered to degrade fast in soil (Al-Rajab and Hakami, 2014; Al-Rajab and Schiavon, 2010; Giesy et al., 2000; Rueppel et al., 1977), however their persistence varies widely. Glyphosate’s half-life (DT_{50}) can range between 1 and 197 days, whereas its DT_{90} can range between 40 and 280 days (Table 1.1) (EU, 2002; Giesy et al., 2000). DT_{50} and DT_{90} is the time required for 50% and 90%, respectively, of a compound to disappear from an environmental compartment, being soil the environmental compartment referred to in the context of this thesis. AMPA is reported to be more persistent in soil than glyphosate (Yang et al., 2015a; Laitinen et al., 2006; Giesy et al., 2000; Rueppel et al., 1977), with a DT_{50} ranging between 23 and 958 days (Yang et al., 2015a; U.S.EPA, 1993). No information was found under field or laboratory conditions on the DT_{90} range of AMPA in soils. In fact, most decay studies, particularly those investigating the effects of specific factors on the decay behavior, focus mainly or even only on glyphosate, disregarding or giving little emphasis to AMPA. For both compounds, the decay behavior in soil depends mostly on the soil type and properties and on the climatic conditions (Alexa et al., 2009; Borggaard and Gimsing, 2008; Laitinen et al., 2006; Giesy et al., 2000; Ghassemi et al., 1982). Since soil properties and climatic conditions vary greatly between, as well as within, geographical areas around the globe, it is not surprising that the decay of glyphosate and AMPA also varies widely. Nevertheless, such factors are normally disregarded by farmers when applying glyphosate. This is due to the common believe that glyphosate decays fast and does not accumulate in soil, independently of the well-known heterogeneity of soil types and properties and climatic conditions. Such attitude greatly increases the risk of glyphosate and AMPA accumulation in soil and consequent off-site transport risk. An example of such risk is demonstrated by the recent study of Silva et al. (2018), who detected glyphosate and/or AMPA in 45% of European agricultural topsoils and estimated the potential spread of these compounds by water and wind erosion across Europe. This becomes particularly problematic in regions with recurrent glyphosate applications every year. In fact, a recent study has suggested glyphosate and AMPA to be “pseudo-persistent” pollutants in the loess soils of the Pampas of Argentina (Primost et al., 2017).

Glyphosate and AMPA decay in soil is reported to occur mainly by biodegradation, i.e. mediated by soil microorganisms, mostly bacteria and fungi (Sviridov et al., 2015; Giesy et al., 2000; Ghassemi et al., 1982; Rueppel et al., 1977). Abiotic degradation of glyphosate in soil, such as photodegradation, is reported to have a negligible role (Giesy et al., 2000; Ghassemi et al., 1982; Rueppel et al., 1977). Although no studies were found utilizing sterile soil to directly test the influence of abiotic degradation processes on AMPA, it is expected that these processes also have a negligible role on AMPA degradation.

Glyphosate can biodegrade by two pathways: the sarcosine pathway and the AMPA pathway (Borggaard and Gimsing, 2008; Karpouzas and Singh, 2006). The latter is normally regarded as the main degradation pathway of glyphosate in soils and the major glyphosate degradation product (Al-Rajab and Schiavon, 2010; Duke and Powles, 2008; EU, 2002; Rueppel et al., 1977). The microbial degradation of glyphosate via the sarcosine pathway is characterized by the cleavage of the C-P bond of glyphosate by C-P lyase, resulting in the release of phosphate and sarcosine (Borggaard and Gimsing, 2008; Karpouzas and Singh, 2006). Sarcosine is further transformed into glycine, which is then used for protein biosynthesis by microorganisms (Borggaard and Gimsing, 2008; Karpouzas and Singh, 2006). In the AMPA pathway, glyphosate is degraded by cleavage of the C-N bond, resulting in the release of AMPA and glyoxylate (Borggaard and Gimsing, 2008; Karpouzas and Singh, 2006). AMPA is then dephosphorylated by C-P lyases, resulting in the formation of inorganic phosphate and methylamine, and lastly mineralized into CO_2 (Borggaard and Gimsing, 2008; Karpouzas and Singh, 2006). Because AMPA is the major metabolite of glyphosate normally found in soils and the environment and it is also reported to have toxic effects in animals and humans, AMPA was the only metabolite of glyphosate being considered in this thesis.

1.5.2 Off-site transport pathways

Although glyphosate and AMPA are expected to don’t reach off-target environments, their off-site transport has been reported in several studies. Glyphosate and AMPA are frequently reported to occur in surface water (Okada et al., 2018; Primost et al., 2017; Jayasumana et al., 2015; Battaglin et al., 2014; Aparicio et al., 2013; Hanke et al., 2010; Battaglin et al., 2009; Botta et al., 2009; Struger et al., 2008; Scribner et al., 2007; Kolpin et al., 2006; Battaglin et al., 2005; Humphries et al., 2005; Scribner et al., 2003), streambed sediment (Primost et al., 2017; Battaglin et al., 2014; Aparicio et al., 2013; Peruzzo et al., 2008) and suspended matter (Okada et al., 2018; Primost et al., 2017; Aparicio et al., 2013). Despite their low potential for leaching and low volatility, they have also been reported to occur in groundwater (Okada et al., 2018; Jayasumana et al., 2015; Battaglin et al., 2014; van Stempvoort et al., 2014; Sanchís et al., 2012; Scribner et al., 2007; Brüsch, 2006), rainwater (Battaglin et al., 2014; Chang et al., 2011; Scribner et al., 2007; Humphries et al., 2005; Quaghebeur et al., 2004) and atmospheric dust (Aparicio et al., 2018; Chang et al., 2011; Humphries et al., 2005).

Considering the wide occurrence of glyphosate and AMPA in the different environmental compartments, it is of extreme importance to better understand the different transport pathways of glyphosate and AMPA to off-target areas. Off-target transport pathways of pollutants comprise drift during application, volatilization, leaching, and water and wind erosion.

The leaching potential of glyphosate and AMPA, either through direct leaching studies or through adsorption/desorption studies, has been extensively studied. The main believe is that, because glyphosate and AMPA rapidly adsorb to soil particles, which make them essentially immobile from soil, they are considered unlikely to leach (Borggaard and Gimsing, 2008; Baylis, 2000; U.S.EPA, 1993). Nevertheless, several studies have detected glyphosate and AMPA in groundwater, as shown above. Likewise, some leaching studies reported their presence in leachates (Aronsson et al., 2011; Bergstroem et al., 2011; Al-Rajab et al., 2008; de Jonge et al., 2000; Cheah et al., 1997). Although the leaching capacity and consequent risk of groundwater contamination with glyphosate and AMPA seems to be limited (Bergstroem et al., 2011; Borggaard and Gimsing, 2008; de Jonge et al., 2000; Cheah et al., 1997), several factors can affect it and increase their leaching potential through soil. Some of these factors are: preferential pathways and colloid flow through soils (e.g. macropores, fissures and cracks), soil type, soil cation composition, and soil hydrodynamic properties and biodegradation capacities (Aronsson et al., 2011; Al-Rajab et al., 2008; Borggaard and Gimsing, 2008; Dousset et al., 2007; de Jonge et al., 2000).

The atmospheric transport and deposition of glyphosate and AMPA by volatilization are considered negligible due to their very low vapor pressure (Giesy et al., 2000; U.S.EPA, 1993). Therefore, their occurrence in the atmosphere (including in rainwater) can only be associated to spray drift (glyphosate) or wind erosion (glyphosate and AMPA). In fact, Humphries et al. (2005) reported the presence of glyphosate in particles in the air rather than in air samples, suggesting that the atmospheric transport of glyphosate and AMPA occurs in association with particulate matter and not by vapor. Although the atmospheric transport of glyphosate and AMPA associated to the particle phase seems extremely likely, studies focusing on their transport with wind erosion are lacking. In fact, just very recently one study was published on the transport of glyphosate and AMPA with wind-blown material under field conditions, indicating high concentrations of these compounds in the dust of agricultural regions of Argentina (Aparicio et al., 2018).

There are some studies related to the transport of glyphosate and AMPA by water erosion, however most of them focused only on the occurrence of these compounds in the water phase (runoff water), neglecting the particle phase (Daouk et al., 2013; Birch et al., 2011; Gregoire et al., 2010; Warnemuende et al., 2007; Siimes et al., 2006). Although glyphosate and AMPA adsorb strongly to soil particles and tend to concentrate mostly in the very top surface layer, only recently studies have focused in their particle-bound transport with runoff as well (Melland et al., 2016; Napoli et al., 2016; Yang et al., 2015a; Yang et al., 2015b). Moreover, all studies on water erosion have had their main focus on the potential contamination risk of aquatic systems, disregarding their off-site transport to adjacent fields. However, runoff occurring in glyphosate-treated fields not always are close to aquatic systems and it runs through off-target downslope areas. Downslope off-site transport and deposition of particle-bound glyphosate and AMPA is therefore very likely to occur, being a source of contamination of terrestrial environments.

1.6 Objectives

This PhD thesis aims at improving and further the science on the understanding of the environmental fate of glyphosate and AMPA in the loess soil environment. In this thesis, particular attention is given to the decay behavior of glyphosate and formation and decay behavior of AMPA in loess soils, and to their off-site transport associated to the particulate matter, as a result of water and wind erosion (Figure 1.2). Loess soils were chosen as the soil environmental compartment to be investigated because: 1) they are represented in several parts of the planet, 2) they are amongst the most fertile and productive soils and therefore are intensively used for agriculture, and 3) within their extensive agricultural use, they are intensively used for the growing of GR crops in several countries, namely in the loess Pampas of Argentina – field study site of this PhD program regarding chapter 5.

In this PhD thesis, the following research questions are addressed:

i. What is the decay kinetics behavior of glyphosate and the formation and decay kinetics behavior of AMPA in loess soil under laboratory and field conditions, and which factors affect this process the most? (Chapters 2 & 5)
ii. What is the role of wind erosion on the (long-range) atmospheric transport of glyphosate and AMPA, and to what extent does wind erosion contribute to glyphosate and AMPA human exposure? (Chapter 3)
iii. What is the off-site transport risk of dissolved and particle-bound glyphosate and AMPA from loess soil driven by water erosion, and how do different soil surface micro-topographies affect their redistribution, transport and deposition into off-target terrestrial and aquatic environments? (Chapters 4 & 5)
iv. What is the dynamics of glyphosate and AMPA in the loess soil surface layer of fields cultivated with GR crops and with long-term repeated applications of glyphosate-based herbicides? (Chapter 5)

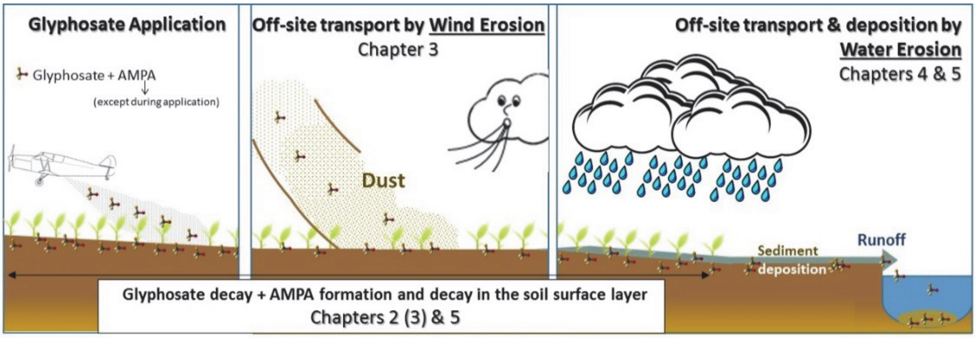


Figure 1.2 *Outline of this PhD thesis, indicating the chapters and topics of the research study.*

1.7 Outline of this thesis

This PhD thesis comprises 6 chapters. The research questions of this thesis are addressed in chapters 2 to 5 and all of them have been published (chapters 2 to 4) or are under review (chapter 5) in highly ranked international peer reviewed journals. For this reason, they are all standalone publications and they can be read independently. Consequently, some repetition occurs in parts of the introduction and methods sections. Figure 1.2 summarizes the outline of this thesis as well as the main topics addressed in each chapter.

Chapter 1 comprises the general introduction of this PhD thesis with focus on the use of glyphosate-based herbicides worldwide and in loess soils, the environmental and human health impacts and social concerns related to their use, and the glyphosate and AMPA environmental fate in the soil compartment.

Chapters 2 to 4 comprise laboratory experiments whereas chapter 5 comprises a field experiment in agribusiness fields of the loess Pampas of Argentina.

Chapter 2 addresses the decay kinetics behavior of glyphosate and the formation and decay kinetics behavior of AMPA as affected by temperature, soil moisture and light/darkness, under biotic and abiotic conditions. The decay kinetics of both compounds are investigated using the “ModelMaker 4” decay kinetics model.

Chapter 3 investigates the role of wind erosion on the atmospheric transport of glyphosate and AMPA and discusses the potential contribution of this transport pathway to human exposure. This study was performed in a wind-tunnel and glyphosate and AMPA were analyzed in different size fractions of the wind-blown sediment derived from loess soil.

Chapter 4 assesses the influence of small-scale sediment transport and of different soil surface micro-topographies on the off-site transport of glyphosate and AMPA with water erosion to terrestrial and aquatic environments, as well as their redistribution, transport and deposition on the soil surface. This study was performed in loess soil using rainfall simulations and flumes.

Chapter 5 investigates the dynamics of glyphosate and AMPA in the soil surface layer of fields cultivating GR crops in the loess Pampas of Córdoba province, in Argentina. The cultivation areas studied have a long-term history of repeated applications of glyphosate-based herbicides. Focus is given to the role of decay kinetic processes and to the off-site transport risk with water erosion. The decay kinetics of glyphosate and the formation and decay kinetics of AMPA under field conditions are here investigated, using “ModelMaker 4”.

Moreover, glyphosate and AMPA concentrations in runoff water and water-eroded sediment are quantified.

To conclude, chapter 6 summarizes the major conclusions of this research and discusses the implications of these findings. Furthermore, this chapter addresses recommendations for a more sustainable use of glyphosate-based herbicides and suggests promising herbicide-free alternatives to (agribusiness) farmers. This chapter concludes with directions for future research work.

1.8 Study Area (related to Chapter 5)

The study area is located in the loess Pampas of Córdoba province, in Argentina, an area highly affected by intensive agriculture of GR crops. The province has a total surface of 165 321 km^2 and around 3 million inhabitants. The city of Córdoba is the second most populated in the country, with around 1.3 million inhabitants (census 2010). According to the köppen climate classification, the city of Córdoba and most of the province are characterized by a humid subtropical climate, with hot and humid summers and cold and dry winters. The mean annual precipitation in the province increases from west (600 mm) to east (950 mm), but that of the city of Córdoba and of the study area varies between 750 and 850 mm (Figure 1.3). Around 80% of the total annual rainfall, though, occurs during spring and summer months (October to March). The mean annual temperature in the city of Córdoba and in the study area is 16.7°C, but temperatures as low as -8°C and as high as 42°C can occur. The soil type of the study area is Mollisols, sub-order Ustolls, on loess (Figure 1.3).

In the province of Córdoba, the agricultural area has doubled between 1988 and 2008, from 7.2 to 14.4 million ha (INDEC, 2009, 1992). Agriculture focuses mostly on the growing of soybeans, maize, wheat, sorghum, chickpeas and also peanuts in the sandy Pampa, south of the province (Figure 1.3) (Togni, 2013). However, soybean and maize production clearly dominate the agricultural system, with the most prevalent crop rotation plan being a biennial and triennial soybean-maize rotation (Togni, 2013). Since these are summer crops, the result is the existence of a fallow period during winter. This fallow period is known in Córdoba province and in the country as chemical fallow (“barbecho químico”), meaning that herbicides, namely glyphosate-based ones, are applied during winter. Nevertheless, some farmers apply a crop rotation plan that, besides soybean or maize in the summer, a winter crop is also grown, mostly wheat (Togni, 2013).

In the province of Córdoba, as well as in Argentina and in the study area, the advent of GR crops resulted in a shift of the agricultural management practices from tillage to non-tillage systems. The adoption of non-tillage systems resulted in a great reduction of soil erosion problems by water and wind, but it also highly increased the use of herbicides, particularly glyphosate-based herbicides (Togni, 2013). Glyphosate is the most used herbicide in the province, followed by 2,4-D and atrazine (Lantieri et al., 2009).

In Argentina, GR crops are one of the main reasons for the immense increase of agricultural production since the mid-90s (Trigo and Cap, 2004). GR soybean was the first transgenic crop introduced in the country and its cultivated area (as % of the total soybean planted area) increased from <1% in 1996/97 to 100% in 2003/04 (Trigo, 2016, 2011). Currently, Argentina is the third largest soybean producer worldwide, with a production of 58.8 million tons and a harvested area of 19.5 million ha in 2016 (FAO, 2018). As a consequence, the use of glyphosate-based herbicides also increased dramatically since the adoption of GR soybeans in 1996, being the most used pesticide in the country (Aparicio et al., 2013; Trigo, 2011). By 2012, 197 000 tons of glyphosate-based herbicides were applied in the country, 98% of which in chemical fallows, and soybean and maize cultivations (KleffmannGroup, 2014).

Despite the great usage of glyphosate-based herbicides in Argentina in the last decades, only recently more attention is being paid to its impacts and fate. In fact, these are mostly a consequence of the societal pressure on the Argentine authorities due to public concerns on its effects to human health (Leguizamón, 2014; Arancibia, 2013; Delvenne et al., 2013). Besides, the loess region of Córdoba province is prone to wind and water erosion (Apezteguía et al., 2009). However, except for the very recent study of Aparicio et al. (2018) on the transport of glyphosate and AMPA with wind-blown sediment, studies on these transport pathways in Argentina are missing.

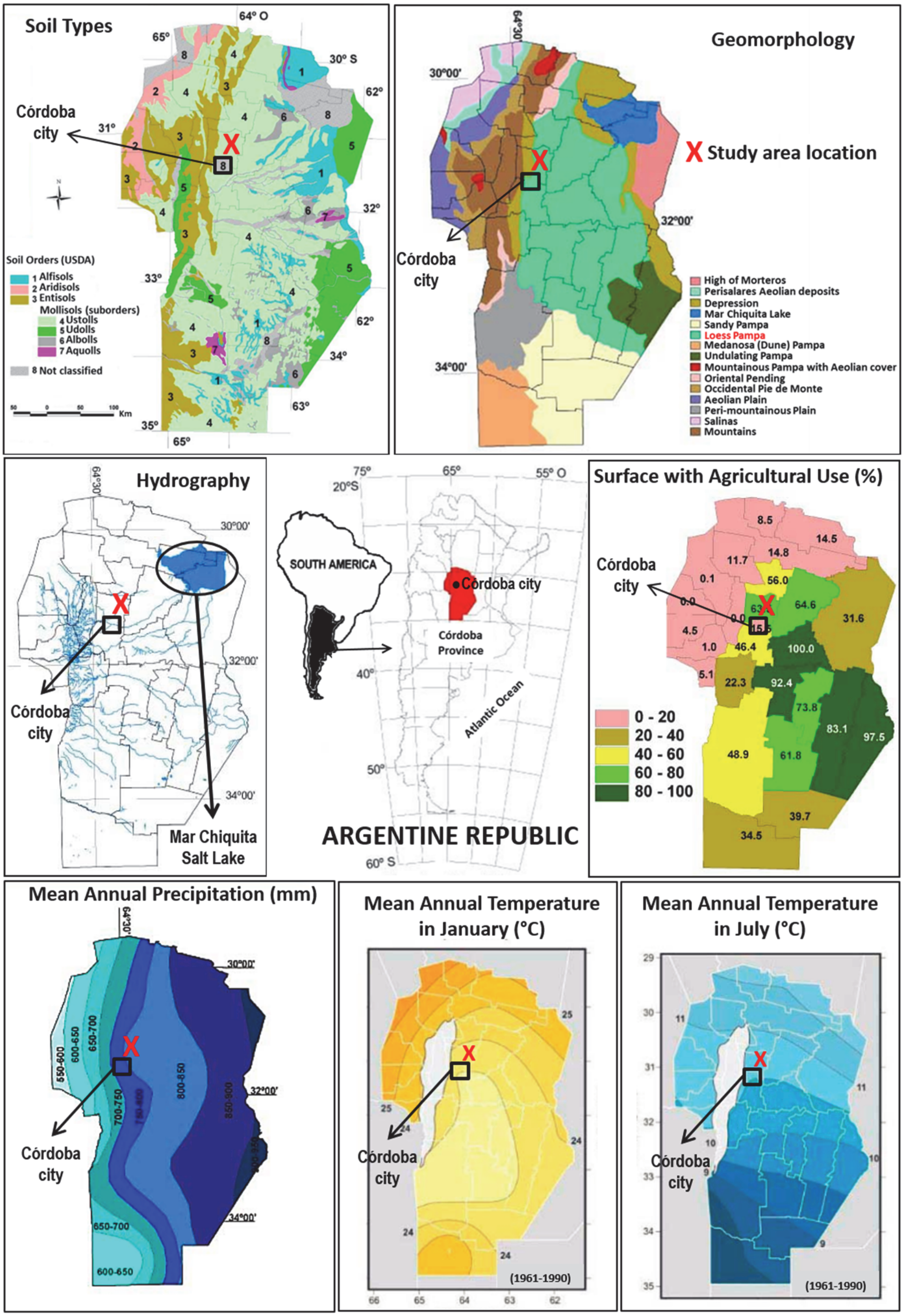


Figure 1.3 *Location of Córdoba province, Córdoba city and study area, and characterization of the province and study area by soil type, geomorphology, hydrography, agricultural use, mean annual precipitation and mean annual temperature in the summer (January) and winter (July) (adapted from Cruzate et al., 2008)*

2. Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness

The decay kinetics of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) were studied in loess soil, under biotic and abiotic conditions, as affected by temperature, soil moisture (SM) and light/darkness. Nonsterile and sterile soil samples were spiked with 16 mg kg^{-1} *of glyphosate, subjected to three SM contents (20% WHC, 60% WHC, saturation), and incubated for 30 days at 5°C and 30°C, under dark and light regimes. Glyphosate and AMPA decay kinetics were fit to single-first-order (SFO) or first-order-multicompartment (FOMC) models, per treatment combination. AMPA kinetic model included both the formation and decline phases. Glyphosate decay kinetics followed SFO at 5°C, but FOMC at 30°C. AMPA followed SFO decay kinetics for all treatments. Glyphosate and AMPA decay occurred mostly by microbial activity. Abiotic processes played a negligible role for both compounds. Under biotic conditions, glyphosate decay and AMPA formation/decay were primarily affected by temperature, but also by SM. Light regimes didn't play a significant role. Glyphosate* DT_{50} *varied between 1.5–53.5 days, while its* DT_{90} *varied between 8.0–280 days, depending on the treatment. AMPA persisted longer in soil than glyphosate, with its* DT_{50} *at 30°C ranging between 26.4–44.5 days, and its* DT_{90} *between 87.8–148 days. The shortest* DT_{50}/DT_{90} *values for both compounds occurred at 30°C and under optimal/saturated moisture conditions, while the longest occurred at 5°C and reaching drought stress conditions. Based on these results, we conclude that glyphosate and AMPA dissipate rapidly under warm and rainy climate conditions. However, repeated glyphosate applications in fallows or winter crops in countries where cold and dry winters normally occur could lead to on-site soil pollution, with consequent potential risks to the environment and human health.*

To our knowledge, this study is the first evaluating the combined effect of temperature, soil moisture and light/darkness conditions on AMPA formation/decay kinetics and behavior.

Based on:

Bento, C. P. M., Yang, X., Gort, G., Xue, S., van Dam, R., Zomer, P., Mol, H. G. J., Ritsema, C. J. & Geissen, V. 2016. Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. Science of The Total Environment. 572, 301-311.

2.1. Introduction

Loess soils are present in several parts of the world, but major deposits are observed in the USA, Argentina, China and some European countries. Such soils are normally very fertile and are among the most agriculturally productive in the world. In general, these soils are intensively used for agriculture, a great part of them to grow genetically modified crops (GMC), where huge amounts of pesticides are applied every year, especially glyphosate-based herbicides. The USA, for example, which is the world leader in growing GMC, applied ca. 94.3 thousand tons of glyphosate-based herbicides in 2007 (39% of the total herbicides applied in the country) (Grube et al., 2011). Argentina, currently the third largest GMC producer, applied ca. 197 thousand tons of glyphosate-based herbicides in 2012 (80% of the total herbicides used in the country) (Lupi et al., 2015; KleffmannGroup, 2014). Despite the huge amounts of glyphosate-based herbicides used, monitoring of their residues in these soils and the related risks to the environment and human health is still rarely practiced. For realistic risk assessment studies, persistence studies in this type of soil under different scenarios are still required.

Glyphosate (N-phosphonomethylglycine; $C_3H_8NO_5P$) is a non-selective, systemic, broad spectrum, post-emergent herbicide extensively used worldwide for weed control, especially under intensive agriculture where glyphosate-resistant (GR) crops are commonly used (Bergstroem et al., 2011; Al-Rajab and Schiavon, 2010; Bonny, 2008; Eberbach, 1999). Glyphosate can biodegrade by two pathways in soil, but its primary and main metabolic pathway is its degradation to AMPA (aminomethylphosphonic acid; CH_6NO_3P) (Al-Rajab and Hakami, 2014; Borggaard and Gimsing, 2008; Barrett and McBride, 2005; Eberbach, 1999). It is considered to be easily degraded in soil, but its persistence varies widely, with its half-life (DT_{50}) ranging between 1-197 days and its DT_{90} between 40-280 days (Yang et al., 2015a; Bergstroem et al., 2011; Laitinen et al., 2006; EU, 2002; Giesy et al., 2000). AMPA is found to be more persistent in soil than glyphosate, with its half-life ranging between 23-958 days (Yang et al., 2015a; Laitinen et al., 2006; U.S.EPA, 1993). Glyphosate volatilization from soil is assumed to be negligible due to its low vapor pressure (1.31×10^{-5} Pa, 25°C) (EU, 2002; Giesy et al., 2000; U.S.EPA, 1993). It strongly adsorbs to soil particles (Cáceres-Jensen et al., 2009; Al-Rajab et al., 2008), but it is also very soluble in water (10.5 g L^{-1}, 20°C) (EU, 2002).

Glyphosate is often applied all year round, including winter periods (for weed control in fallows or winter crops), especially in agriculture regions that are intensively cultivated. According to Benbrook (2012), glyphosate is usually applied 1.5 times in GR soybean at doses between 0.67-0.90 kg a.i. ha^{-1} in the USA, but its application is increasing every year due to weed resistance. Glyphosate is also often applied as harvest aid on wheat and barley, or to control weeds mixed with these winter crops (Benbrook, 2012). In Argentina, glyphosate is normally applied throughout the year, often before harvest of winter crops, but also during late autumn and winter, prior to sowing wheat/barley or during fallow (personal communication). According to a survey among farmers (unpublished data) from the Pampa region of Córdoba province, in Argentina, glyphosate applications can vary from 2 to 7 times per year, at doses that can vary between 0.36-1.44 kg a.i. ha^{-1}. However, application rates as high as 2.2 kg a.i. ha^{-1} may be used for the control of perennial weeds. It can be applied 1 to 4 times during the growing season of GR soybean or GR maize, and 1 to 3 times during fallow (winter period).

The degradation of pesticides consists of biotic (microbial degradation) and abiotic (e.g. photodegradation and chemical degradation) processes; which of these a given pesticide undergoes depends on its structural affinity to specific types of transformation and on the environmental conditions (Fenner et al., 2013). Microbial degradation is considered the predominant pathway affecting pesticides fate in soil. Nevertheless, recent research has proven that naturally-occurring chemicals in soil, namely naturally abundant levels of reduced sulphur compounds and dissolved organic matter, can promote pesticides degradation (Fenner et al., 2013; Zeng et al., 2012; Zeng et al., 2011). Glyphosate decay in soil is reported to occur mainly by microbial degradation whereas soil photodegradation and abiotic degradation are considered to play a minor role (Sviridov et al., 2015; Alexa et al., 2009; Ghassemi et al., 1982; Rueppel et al., 1977). Nevertheless, Barrett and McBride (2005) and Ascolani Yael et al. (2014) have shown that abiotic degradation can occur in the presence of manganese oxide or metals, respectively. Temperature and soil moisture are two of the most important factors regulating microbial activity in soils, thus affecting glyphosate decay and AMPA formation/decay. Studies that tested separately the effect of temperature or soil moisture on glyphosate decay have shown that glyphosate mineralization rates increase with increasing temperature (Stenrød et al., 2005; Strange-Hansen et al., 2004; Eberbach, 1999; Eberbach, 1998; Heinonen-Tanski, 1989) and increasing soil moisture (Grundmann et al., 2008; Schroll et al., 2006). However, laboratory studies often focus on experiments at room temperature (20-25°C) and/or 10°C (Al-Rajab and Hakami, 2014; Al-Rajab and Schiavon, 2010; Alexa et al., 2010; Alexa et al., 2009; Eberbach, 1998), while glyphosate is also regularly applied before sowing winter crops or in fallows during winter, when lower temperatures are common. Furthermore, there is a lack of laboratory studies combining altogether the effect of temperature, soil moisture and light/darkness on glyphosate decay and AMPA formation/decay under biotic and abiotic conditions. Moreover, the majority of studies focus only on the parent compound glyphosate, not taking into account its main degradation product AMPA. Thus, understanding more in detail the role of these factors, combined, on glyphosate decay and AMPA formation/decay, especially under extreme conditions, can give a better insight of glyphosate and AMPA behavior in soil and to the potential risks to the environment. Such data can also be of great importance for use in environmental risk assessments and environmental fate models.

Glyphosate is often assumed to follow single first-order (SFO) decay kinetics. This is the typically used type of kinetics for glyphosate because the equation is simple and the DT_{50}/DT_{90} values are easy to calculate (Borggaard and Gimsing, 2008; Simonsen et al., 2008). However, the application of such simple kinetic model to glyphosate decay does not always result in a good fit (Yang et al., 2015a; Bergstroem et al., 2011; Gimsing et al., 2004b; Eberbach, 1998), especially for a proper estimation of DT_{90} which can easily be underestimated by such model. AMPA is often given little attention in decay kinetic studies under laboratory or field conditions. Nonetheless, all studies found determined its decay kinetics from the maximum observed amount onwards (Yang et al., 2015a; Simonsen et al., 2008), except Bergstroem et al. (2011). This is considered a conservative approach that underestimates the decay of metabolites. Therefore, fitting of decay kinetics to metabolites should take into account its simultaneous formation and decay, whenever possible (FOCUS, 2006).

The aims of this study are: 1) to evaluate the best fit decay kinetics of glyphosate and AMPA under different conditions, taking into account the simultaneous formation and decay of AMPA in the models; 2) to evaluate the combined effect of temperature, soil moisture and light/darkness on glyphosate decay and AMPA formation/decay in loess soil, under biotic and abiotic conditions; and 3) to understand which factors have a major role on glyphosate decay and AMPA formation/decay. These results can be used as a basis to assess the potential risks to the environment and human health.

2.2 Materials and Methods

2.2.1 Soil

The soil used in this study was a silty loam loess soil from Nagelbeek, in the region of Limburg, in The Netherlands. The soil was collected from the surface (0-10cm) in a harvested wheat field, after removing the litter layer. Undisturbed soil samples, in triplicate, were collected at the surface with metal rings, to determine bulk density and the water retention curve.

The soil was then sieved with a 2-mm sieve at field soil moisture, and immediately stored at 4°C until use (no longer than three months as recommended by the OECD guideline 307 (OECD, 2002)). Since disturbed and sieved soil was used in this experiment, bulk density and the water retention curve were also determined in disturbed, slightly compacted, sieved soil. Prior to the experiment, a subsample of the soil was collected and oven-dried (105°C) for 24h to determine the initial soil moisture content. Background information on the history of glyphosate applications in this soil was not available. To check for any background residues of glyphosate and AMPA, the soil was analyzed prior to the experiment. Soil properties and glyphosate and AMPA background residues are provided in Table 2.1.

*T**able 2.1** Soil Properties from the Dutch loess soil used in this study.*

Parameters	**Loess Soil, Nagelbeek, Limburg region**	
Soil depth (cm)	0-10	
Particle Size Distribution:		
<0.002mm (clay) (%)	26.5	
0.002-0.05mm (silt) (%)	59.2	
>0.05mm (sand) (%)	14.3	
pH water	7.1	
pH $CaCl_2$	6.7	
Organic matter (OM) (g kg^{-1})	33.1	
Organic carbon (OC) (g kg^{-1})	19.2	
Inorganic carbon (g kg^{-1})	0.80	
N total (g kg^{-1})	0.96	
P total (g kg^{-1})	0.77	
P available (mg P kg^{-1})	1.90	
K available (mg K kg^{-1})	116	
Mg available (mg Mg kg^{-1})	143	
Na available (mg Na kg^{-1})	8.0	
CEC (mmol+ kg^{-1})	100	
C/N ratio	9.0	
Carbonates (%)	<0.2	
	Undisturbed Soil	**Disturbed Soil**
Bulk density (g cm^{-3})	1.53 ± 0.05	0.84 ± 0.01
Soil Saturation (pF0) (% $_{w/w}$)	26.6 ± 1.9	47.7 ± 0.9
Soil Water Holding Capacity (WHC) (pF1) (% $_{w/w}$)	25.7 ± 1.7	39.1 ± 0.5
Soil Field Capacity (pF2) (% $_{w/w}$)	24.8 ± 0.9	34.2 ± 0.8
Soil Wilting Point (pF4.2) (% $_{w/w}$)	9.4 ± 0.6	5.2 ± 0.1
Glyphosate background (mg kg^{-1})	0.05 ± 0.03 (= LOQ)	
AMPA background (mg kg^{-1})	0.18 ± 0.07	

2.2.2 Facilities

This study was performed in a climate chamber (12 m^2) of the greenhouse facilities of Wageningen University, The Netherlands. Climate factors such as temperature, relative air humidity and light were controlled through automatic control panels. It was equipped with 2 long tables (each table was 0.85x3.80 m^2) divided by a central corridor. Above and along each table were 12 TL-D Xtra Polar 36W/840 fluorescent lamps (Philips, The Netherlands), configured in 3 series, each series with 4 lamps in parallel. The light intensity produced by the array of lamps was dependent on temperature and distance from the source area. Luminosity above each table could be controlled separately.

2.2.3 Glyphosate decay and AMPA formation/decay

2.2.3.1 Preparation of glyphosate

Glyphosate (98%) was dissolved in Millipore water to achieve a final stock solution with a concentration of 1040 mg L^{-1}. A concentration of glyphosate in soil of 16 mg kg^{-1} was used for all experiments. This concentration corresponded to a maximum application rate of 2.2 kg a.i. ha^{-1} (recommended for chemical fallow against perennial weeds), assuming a soil depth of 1 cm.

2.2.3.2 Experimental Design

This experiment employed a factorial design with 2 microbiological soil conditions x 2 temperatures x 3 soil moisture contents x 2 light regimes. The microbiological soil conditions used were nonsterile and sterile soil to test the effect of glyphosate decay and AMPA formation/decay under biotic and abiotic conditions, respectively. For temperature, we chose to use 5°C and 30°C, to represent extreme climate conditions. The sterile soil treatment level was only tested at 30°C, because the results obtained allowed us to assume that a similar behavior would occur for both temperature levels. For soil moisture, three levels were chosen: 20% of water holding capacity (WHC) (shortage of water), 60% of WHC (optimal conditions) and saturation (excess of water, i.e. a soil moisture >100% WHC – corresponds to “Soil Saturation (pF0)” in Table 2.1). The two light regimes were absence and presence of light to test the role of photolysis under different temperatures and soil moisture contents on glyphosate decay and AMPA formation/decay. In order to represent natural conditions, light was applied for 12 h day^{-1} for those samples subject to light regimes. Real light intensity, determined as Photosynthetically Active Radiation (PAR: λ = 400-700 nm), was measured with a LI-190 quantum sensor (LI-COR, USA) at the table height (0.97 m), at both temperatures, giving an average PAR of 42±7 µmol m^{-2} s^{-1} at 5°C, and of 75±9 µmol m^{-2} s^{-1} at 30°C. For dark conditions, a black and white poly panda film was installed around one of the tables in order to prevent any light from entering. The experiment totalized 18 treatments. Each treatment was done in triplicate, totalizing 21 samples per treatment (3 samples per treatment per sampling day; 7 sampling days in total). The treatment abbreviations used in this study were: 1) for microbiological soil conditions: NS – nonsterile soil, S – sterile soil; 2) for temperature: 5 – 5°C, 30 – 30°C; 3) for soil moisture: Sat – saturated soil, 60 – 60% WHC, 20 – 20% WHC; 4) for light regimes: L – presence of light, D – absence of light.

In this experiment, the relative air humidity inside the climate chamber was set to 70±5% at both temperatures. In the climate chamber, the samples were separated by dark and light conditions, but set randomly in each table. Before applying glyphosate, a pre-incubation period of 3 days was performed for the nonsterile soils at the corresponding testing temperatures (5±1°C and 30±1°C). This was done to allow germination and removal of seeds and to re-establish microbial metabolism at the desired temperature (OECD, 2002). No pre-incubation was performed for the sterile soils because the purpose it is used for is not expected to occur in sterile soils. After applying glyphosate, the samples were incubated for a maximum period of 30 days.

Nonsterile Soil

This experiment was carried out by weighing 65 g (dry weight, d.w.) of nonsterile soil into plastic pots of 280 mL. The soil was slightly compacted using a small manual soil compactor. In order to guarantee as much as possible the same compaction in all samples, the soil compactor was set to fall 10 times by gravity at the pot height. Soils were then adjusted for the desired soil moisture contents (see section 2.2.3.2), by weighing the pots and adding Millipore water as needed, and pre-incubated. After the pre-incubation period, 1 mL of glyphosate solution was added to the soil (see section 2.2.3.1). Soil moisture content was controlled at this stage and in a daily basis, and Millipore water was added when necessary. The pots were left open during the entire (pre-)incubation period at both temperatures. Control samples (without glyphosate) were used and prepared the same way.

Sterile Soil

For the sterile soil decay experiment, 15 kg of the stored soil were transferred to a heat-resistant plastic bag and sealed. The soil was autoclaved for 1h at 121°C and 18 psi, and then left at room temperature for 24h. This procedure was repeated for 3 consecutive days. Simultaneously, several glass bottles containing Millipore water were sealed with lids equipped with septa, and autoclaved at the same conditions as soil. The sterilized water was used in the sterile soil decay experiment to prepare the glyphosate solution and to adjust the soil moisture content of the soil samples.

The sterile soil was then prepared as described for the nonsterile soil above. Some additional steps were, however, performed. After weighing the soil, 1 mL of 1 g kg^{-1} NaN_3 solution (Sodium Azide, 99%) was added to prevent microbial activity during the incubation period, due to possible contamination of the soil with microorganisms or to poor sterilization. The soil was then thoroughly mixed to homogenize the NaN_3. After applying the glyphosate and adjusting the soil moisture, the pots were sealed with 2 layers of cling film and rubber elastics to prevent soil contamination with microorganisms. Cling film was used to allow the contact of soil with light for those samples under light regimes. Soil moisture for these samples was controlled weekly.

The soils in each plastic pot (nonsterile and sterile) were collected at 0, 1, 2, 3, 5, 14 and 30 days after glyphosate application. The soil in each plastic pot was then thoroughly mixed, transferred to plastic bags and frozen at -18°C until analysis.

2.2.4 Glyphosate and AMPA analysis

2.2.4.1 Chemicals

Glyphosate (98%), AMPA (98.5%), sodium tetraborate decahydrate (≥99.5%, ACS grade), and ammonium acetate (NH_4Ac, 98%) were purchased from Sigma-Aldrich Co. (USA). Isotopically-labelled glyphosate (1,2-^{13}C, ^{15}N) and AMPA (^{13}C, ^{15}N) were used as internal standards, and purchased from Dr. Ehrenstorfer (Augsburg, Germany). FMOC-Cl (9-fluorenylmethoxycarbonyl chloride, ≥99.0%) was purchased from Sigma-Aldrich (Switzerland). Potassium hydroxide (KOH, p.a. 85%), hydrochloric acid (HCl, 37% ACS, ISO, Reag. Ph Eur grade), and ammonia solution (NH_3, 25%) were purchased from Merck KGaA (Darmstadt, Germany). Methanol (MeOH, 99.98%) and acetonitrile (ACN, 99.95%, HPLC grade) were purchased from Actu-All Chemicals (The Netherlands). Formic acid (CH_2O_2, p.a., ≥98%) was purchased from Merck (The Netherlands). Mixed glyphosate and AMPA isotope-labelled standards (mix IS GLY/AMPA) of 5 µg mL^{-1} were prepared in Millipore water. Standard stock solutions of glyphosate and AMPA at concentrations of 2000 µg mL^{-1} were prepared in Millipore water. Mixed glyphosate and AMPA (mix GLY/AMPA) standard solutions of 100 µg mL^{-1} were prepared from glyphosate and AMPA stock solutions. Mixed GLY/AMPA standard solutions of 10 µg mL^{-1} and 1 µg ml^{-1} were prepared from 100 µg mL^{-1} mix GLY/AMPA standard solution. KOH 0.6 M, HCl 6 M and borate buffer 5% were prepared in Millipore water, and 6.5 mM FMOC-Cl was prepared in ACN. All stock solutions and dilutions were stored at 4°C until use.

2.2.4.2 Soil extraction

All samples were thawed and homogenized manually before extraction. Soil subsamples of 2 g were transferred to 50 mL centrifuge plastic tubes, and extracted with 10 mL of KOH 0.6 M, by shaking mechanically in an end-over-end shaker for 1h. The samples were then centrifuged at 3500 rpm for 15 min. Afterwards, 1 mL of the soil extract was transferred to a 10 mL plastic tube, and 80 µL of HCl 6 M were added to adjust the pH to approximately 9 before derivatization.

2.2.4.3 Derivatization

For the derivatization step, 20 µL of 5 µg mL^{-1} mix IS GLY/AMPA were first added to the pH-adjusted soil extract. Then, 0.5 mL of borate buffer 5% and 0.5 mL of FMOC-Cl 6.5 mM were added. The tubes were shaken for a few seconds in a vortex mixer and incubated for 30 min. at room temperature, after which the reaction was stopped by adding 50 µL formic acid. The tubes were shaken again for a few seconds in a vortex mixer, and 0.5 mL of the mixture were transferred to plastic LC vials integrated with 0.45 µm PTFE filters. Solvent standards of 0, 0.01, 0.02, 0.04, 0.1, 0.2, 0.4, 1, 2 and 4 µg mL^{-1} were prepared from mix GLY/AMPA standard solutions of 100 µg mL^{-1}, 10 µg mL^{-1} and 1 µg ml^{-1}, in Millipore water. For each batch of samples, the solvent standards were derivatized the same way as the samples.

2.2.4.4 HPLC-MS/MS

Glyphosate and AMPA concentrations were determined by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), using an XBridge^{TM} Shield RP C18 column, 3.5µm particle size, 100x2.1 mm i.d. (Aquity UPLC I-Class coupled to a Micromass Ultima triple-quadrupole MS, Waters, The Netherlands). Mobile phases and instrumentation conditions of the HPLC-MS/MS (i.e. column temperature, flow rate, MS method optimization, source parameters and transitions) were as described by Yang et al. (2015b). The LC gradient times for the separation described by Yang et al. (2015b) were, however, slightly adjusted at the end of the run: the final isocratic stage was shortened by 2 min., reducing the run time to 13 min.

2.2.4.5 Validation and quality control

Glyphosate and AMPA responses were normalized to their corresponding isotope-labelled internal standards, thereby correcting for any ion suppression or enhancement effects during the HPLC-MS/MS measurements. Solvent standards were always run at the beginning, middle and end of each batch of samples. Two standard calibration curves (CCs) were determined for each compound, based on the average normalized responses of the solvent standards, for each batch of samples. The CCs were determined considering the following range of standard concentrations: 1st CC – from 0 to 0.2 µg mL^{-1}, for both compounds; 2nd CC – from 0.1 to 4 µg mL^{-1} for glyphosate, and from 0.1 to 2 µg mL^{-1} for AMPA (AMPA often showed poor linearity above 2 µg mL^{-1}, therefore the higher standard concentrations were excluded from CC calculations for this compound). Linearity was satisfactorily achieved for all CCs, with correlation coefficients >0.99 and residuals within ±20%. An initial method validation was performed using two Argentinean loess soils from the pampas of Córdoba province. This involved analysis of duplicate blanks and six replicates of each soil spiked at 0.05, 0.2 and 0.5 mg kg^{-1}. The average recovery and RSD obtained for glyphosate were 87% and 10%, respectively. The average recovery and RSD obtained for AMPA were 85% and 12%, respectively. With each batch of samples analyzed, spiked samples were included for quality control purposes. For this, besides the Argentinean loess soils, also a Dutch loess soil from the region of Limburg, and a Chinese loess soil from the Loess Plateau were used. These samples were spiked at 0.05, 0.1, 0.2, 0.5 and 1 mg kg^{-1}. Recoveries obtained for the different soils and at the different levels were consistent with those obtained during the initial validation. The average recovery and RSD obtained for glyphosate for all quality control samples (n=73) were 86% and 13%, respectively. For AMPA, the average recovery was 92% and the RSD was 16%. The limits of detection (LOD) for glyphosate and AMPA were defined as the lowest concentration at which the analytical process can reliably differentiate from background levels. LODs were determined considering a signal in the chromatogram at the lowest analyte concentration assayed that was 3 times the average of the background noise of blank soil samples (S:N=3). The LODs for glyphosate and AMPA (n=15) were 0.02 and 0.03 mg kg^{-1}, respectively. The limits of quantification (LOQ) were defined as the lowest concentration assayed and validated, which gave a satisfactory average recovery (70-120%) and precision (≤20% RSD). The LOQ was 0.05 mg kg^{-1} for both compounds (n=28).

The analytical results for the soil were determined using the two corresponding CCs of each compound. In case the response of the sample was below the average response of the 0.2 µg mL^{-1} solvent standard, the CC with the lower concentration range was used for quantification. If not, the CC with the higher concentration range was used. With each batch of samples, two blank soil samples were fortified at 1 mg kg^{-1} and added as quality control samples to the batch. The final extract of these samples was analyzed twice, at the beginning and at the end of each batch of samples, thereby ensuring the quality of the analysis when processing real samples. The quantification of the sample batch was considered satisfactory when the QC recoveries were between 70-120%. The analytes were considered identified when the retention time was the same as the isotopically labelled internal standards and the ion ratio of the 2 transitions measured for each compound deviated less than ±30% of the reference ion ratio as determined from the calibration standards.

2.2.5 Data Analysis

All glyphosate and AMPA concentrations were converted to percentage of initially applied glyphosate prior to any data analysis, and are presented as such in this study.

2.2.5.1 Select kinetic model

To determine and select the kinetic models that best describe glyphosate decay and AMPA formation/decay for the different treatments applied, the FOCUS work group guidance document on degradation kinetics (FOCUS, 2006) was followed. The kinetic models parameters, for both glyphosate and AMPA, were estimated using ModelMaker 4 (A.P.Benson). To select the model that best describes glyphosate decay and AMPA formation/decay, the indicators recommended by FOCUS (2006) to assess the goodness of fit and to compare the models were used (visual assessment of curve fits and residual plots, chi-square (χ^2) model errors, t-test).

In a first step, two kinetic models were used to fit glyphosate decay kinetics alone: single first-order (SFO; Eq. 2.1) and first-order multicompartment, also known as Gustafson and Holden (FOMC; Eq. 2.2).

SFO: $$C_t = C_0 e^{-kt} \qquad (2.1)$$

FOMC: $$C_t = \frac{C_0}{\left(\frac{t}{\beta}+1\right)^{\alpha}} \qquad (2.2)$$

where C_t is the glyphosate concentration at time t, C_0 is the initial glyphosate concentration (t=0), k is the degradation rate constant (SFO), β is a location parameter (FOMC) and α is a shape parameter determined by the coefficient of variation of k values (FOMC).

After selecting the model that best describes glyphosate decay, AMPA data were added and the SFO model was used to fit AMPA formation/decay kinetics. This kinetic model included both the formation and decline phases of AMPA in order to describe its complete behavior. By using this approach, an additional parameter had to be estimated – the formation fraction of AMPA (*ffA*). The stepwise approach to fitting was followed, i.e. first glyphosate parameters initially determined were kept fixed and the model parameters for AMPA (C_0, *ffA* and k) were optimized and fitted; then, the fitted glyphosate and AMPA parameters were used as initial values, and were optimized and fitted altogether.

2.2.5.2 Statistical analysis

To determine the DT_{50} and DT_{90} values and to evaluate the effects of the different treatments on glyphosate decay and AMPA formation/decay, the statistical software SAS 9.3 was used.

Treatments effects: DT_{50}/D_{T90} values and glyphosate decay under biotic conditions

DT_{50} and DT_{90} values for glyphosate and AMPA were calculated using the FOMC equation for glyphosate and SFO for AMPA, by rewriting the equations to determine DT_{50} and DT_{90} as parameter estimates. FOMC was chosen in these calculations for glyphosate because its decay curves for the treatments at 5°C did not show any differences between SFO and FOMC kinetic models, and the treatments at 30°C followed FOMC. The formation of AMPA was taken into account in the equation to determine AMPA DT_{50} and DT_{90}. C_0 of glyphosate and AMPA, *α* and *ffA* parameters were fixed. Comparing the decay kinetics of glyphosate for the different treatments under biotic conditions was carried out by comparing the DT_{50} values. An ANOVA (analysis of variance) table was created to evaluate the main effects of factors temperature, light, and soil moisture, and their interactions on glyphosate decay under biotic conditions, followed by pairwise comparisons using F-tests ($p<0.05$, corrected by Bonferroni). The sums of squares for the ANOVA table were obtained as differences in residual sums of squares of two nested non-linear models, with and without the factor of interest. Multiple pairwise comparisons to test for differences between all DT_{50} values and between all DT_{90} values of glyphosate and AMPA were also performed using F-tests ($p<0.05$, corrected by Bonferroni).

Treatments effects: biotic vs. abiotic and AMPA formation/decay under biotic conditions
Linear regression analysis followed by F-tests ($p<0.05$, corrected by Bonferroni when applicable) was applied to evaluate: 1) the effects of biotic (nonsterile) and abiotic (sterile) conditions on glyphosate decay and AMPA formation/decay for each of the treatments, 2) the treatment effects on glyphosate decay and AMPA formation/decay within abiotic conditions, and 3) the treatment effects on AMPA formation/decay under biotic conditions. Because glyphosate decay and AMPA formation/decay follow nonlinear models, data were log-transformed before applying linear regression analysis. For glyphosate, a linear regression model with treatment dependent intercepts and linear and quadratic effects of time was applied. For AMPA, a linear model with treatment dependent intercepts and linear and quadratic effects of log(time) was applied. The linear and quadratic coefficients were used to evaluate the main effects of temperature, light, and soil moisture, and their interactions, showing results in an ANOVA table.

2.3 Results

The decay behavior of glyphosate and AMPA is presented in Figure 2.1. The decay behavior of both compounds differed significantly between abiotic (sterile) and biotic (nonsterile) conditions (glyphosate: $F=373.2$; $p<0.0001$; AMPA: $F=53.5$; $p<0.0001$), independently of the treatment combination. The experimental data (Figure 2.1) show a visible negligible decay of glyphosate and formation of AMPA under abiotic conditions (6.9±5.2% of the initially applied glyphosate dissipated after 30 days; 0.7±0.4% of AMPA was formed after 30 days). AMPA residues detected during the incubation period under abiotic conditions are, therefore, expected to correspond mostly to background residues already existing in the soil prior to the experiment (1.8±0.3% of AMPA existing at day 0; see Figure 2.1 and Table 2.1). Moreover, no significant effects of soil moisture and light/darkness were observed within abiotic conditions for glyphosate ($p=0.99$). Nonetheless, as opposed to glyphosate, the statistical tests revealed that 4 out of 6 treatments under abiotic conditions formed some AMPA through time, although at an extremely slow pace (0.9±0.3% after 30 days, for the 4 treatments). The 4 treatments comprised the 3 treatments subject to light regimes and 30-SD-60. Under biotic conditions, glyphosate disappeared almost completely at 30°C since only 3% of the initially applied glyphosate remained in the soil. Exception was for treatment 30-NS-L-20 where only 77±5% was dissipated. At 5°C, the lowest decay values were recorded (52±12%) for glyphosate.

2.3.1 Glyphosate and AMPA decay kinetic models

Under abiotic conditions, it was not possible to determine the kinetic parameters and DT_{50}/DT_{90} values for glyphosate and AMPA due to their negligible decay and formation, respectively, during the incubation period. Under biotic conditions, the best fitting model for glyphosate was dependent on temperature (Table 2.2; Figure 2.1). At 5°C, the SFO model was the best fit, although the FOMC model also fit the data well, as the curves coincided for most of the treatments (graphs not shown). At 30°C, the FOMC model was the best fit for glyphosate. Although the χ^2 error values were <15% for the SFO fits, their residual plots indicated systematic deviations for the later sampling dates up to measured DT_{90} (graphs not shown). This underestimates the DT_{90} values, i.e., it predicts a faster degradation of glyphosate than observed. For AMPA, the SFO model was used to fit the data (Table 2.2) since its formation/decay was reasonably well described by this model (see visual fit in Figure 2.1). However, its decay rates and DT_{50}/DT_{90} values could not be reliably determined for all treatments at 5°C and one treatment at 30°C (30-NS-L-20), because a plateau and decline phases were not reached during the incubation period (Tables 2.2 and 2.3, Figure 2.1).

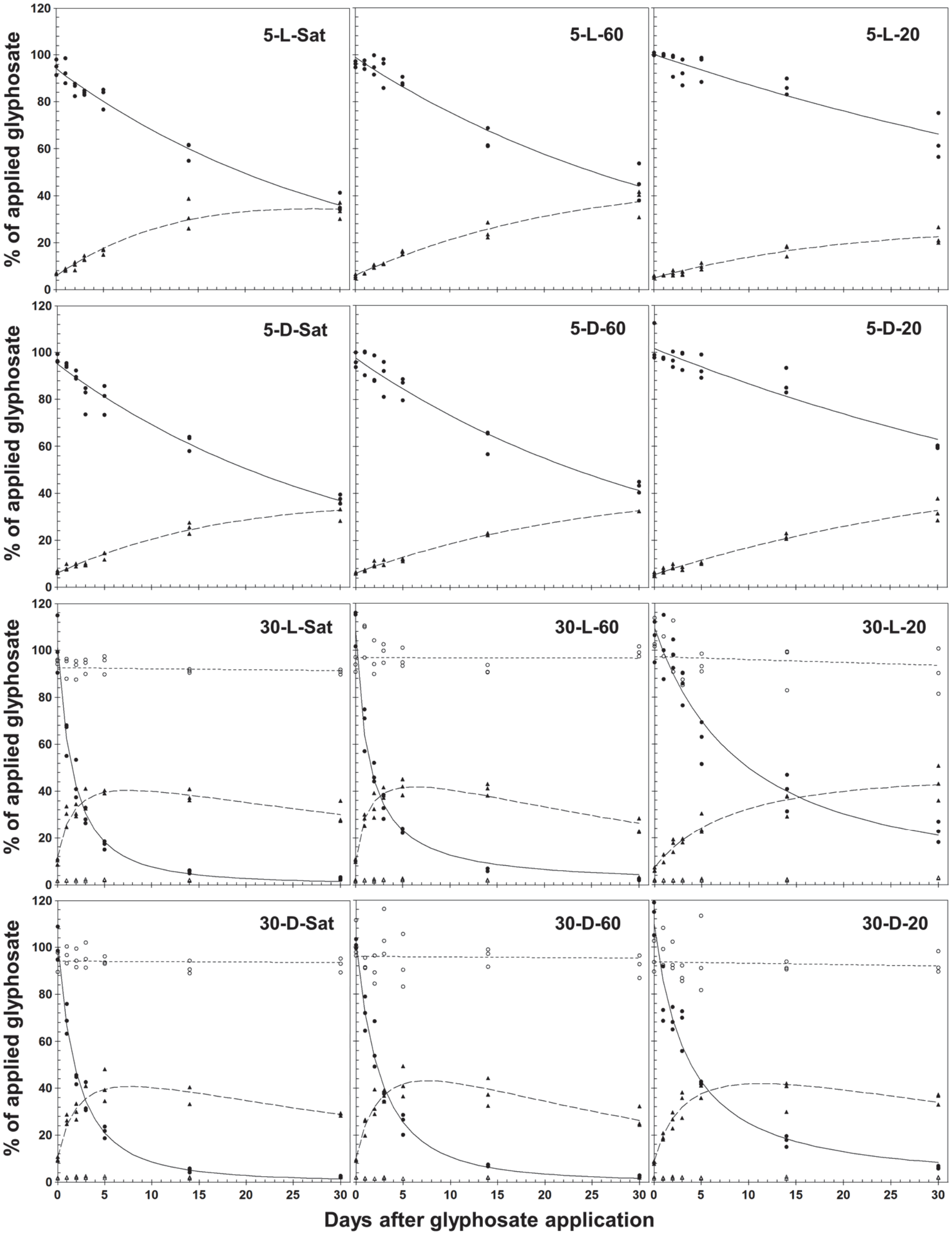


Figure 2.1 *Decay kinetics of glyphosate (●) and AMPA (▲) in nonsterile (solid symbols) and sterile (open symbols) soil at 5°C and 30°C, under different soil moistures (Sat, 60, 20) and with light or in darkness (L, D). Symbols represent the experimental data and lines the theoretical kinetic models*

Table 2.2 *Glyphosate and AMPA decay kinetics parameters in nonsterile soil for the different treatments applied, by fitting Single First-Order (SFO) or Gustafson and Holden (FOMC) kinetic models. See section 2.2.3.2 for treatments abbreviations.*

	Glyphosate following SFO or FOMC					AMPA following SFO			
Treatments	C_0 ± SE (% of applied)	k ± SE (day^{-1}) (SFO)	α ± SE (FOMC)	β ± SE (FOMC)	χ^2 error (%)	C_0 ± SE (% of applied)[a]	ffA ± SE	k ± SE (day^{-1})	χ^2 error (%)
5-NS-L-Sat	94.1 ± 1.0	0.03 ± 0.002*	-	-	1.6	5.8 ± 1.3	1.00 ± 0.17*	[b]	-
5-NS-L-60	98.8 ± 1.2	0.03 ± 0.002*	-	-	2.3	5.9 ± 1.4	0.71 ± 0.19*	[b]	-
5-NS-L-20	100 ± 1.1	0.01 ± 0.001*	-	-	2.0	4.9 ± 1.4	0.88 ± 0.42*	[b]	-
5-NS-D- Sat	95.1 ± 1.0	0.03 ± 0.002*	-	-	2.7	5.9 ± 1.2	0.64 ± 0.15*	[b]	-
5- NS-D-60	97.5 ± 1.1	0.03 ± 0.002*	-	-	1.5	6.0 ± 1.3	0.54 ± 0.17*	[b]	-
5- NS-D-20	102 ± 1.1	0.02 ± 0.001*	-	-	2.5	5.3 ± 1.4	0.82 ± 0.30*	[b]	-
30- NS-L- Sat	102 ± 2.7	-	1.9 ± 0.6*	3.3 ± 1.4*	2.8	11.5 ± 2.5	0.37 ± 0.05*	0.02 ± 0.004*	4.7
30- NS-L-60	109 ± 2.5	-	1.1 ± 0.2*	1.5 ± 0.4*	4.7	9.5 ± 2.4	0.43 ± 0.04*	0.03 ± 0.004*	4.3
30- NS-L-20	110 ± 3.4	-	1.4 ± 0.6*	12.8 ± 7.4*	6.0	7.3 ± 3.0	0.43 ± 0.13*	[b]	-
30- NS-D- Sat	101 ± 2.3	-	2.1 ± 0.7*	4.6 ± 1.9*	3.4	10.5 ± 2.1	0.40 ± 0.04*	0.02 ± 0.004*	3.7
30- NS-D-60	102 ± 3.1	-	2.4 ± 0.8*	6.3 ± 2.7*	4.3	10.1 ± 2.3	0.48 ± 0.05*	0.03 ± 0.005*	2.6
30- NS-D-20	110 ± 3.0	-	1.3 ± 0.3*	4.6 ± 1.7*	6.9	8.8 ± 3.0	0.47 ± 0.08*	0.02 ± 0.007*	6.1

a. AMPA results were converted to equivalent mass of glyphosate, so C_0 for AMPA represents the initial concentration of AMPA in % of applied equivalent glyphosate.

b. Plateau and decline phases for AMPA were not reached during the experimental period, thus it was not possible to reliably determine AMPA parameters, especially the decay rate (k).

*Estimated parameter is significantly different from zero (t-test; $p<0.05$).

Table 2.3 *Glyphosate and AMPA DT_{50} and DT_{90} values in nonsterile soil, and incubation time (t_{Amax}) at which AMPA concentration peaks (C_{Amax}), for the different treatments. See section 2.2.3.2 for treatments abbreviations.*

Treatment	Glyphosate		AMPA			
	$^{\#}DT_{50}$ ± SE (days)	$^{\#}DT_{90}$ ± SE (days)	$^{\$}DT_{50}$ ± SE (days)	$^{\$}DT_{90}$ ± SE (days)	t_{Amax} (days)	C_{Amax} (% of applied)$^{b)}$
5-NS-L-Sat	17.8 ± 1.4 b	93.2 ± 10.1 b	a)	a)	>30	n.a
5-NS-L-60	24.9 ± 2.1 b	130 ± 7.1 b	a)	a)	>30	n.a
5-NS-L-20	53.5 ± 6.2 a	280 ± 40.4 a	a)	a)	>30	n.a
5-NS-D- Sat	18.5 ± 1.4 b	96.5 ± 10.5 b	a)	a)	>30	n.a
5- NS-D-60	22.2 ± 1.8 b	116 ± 13.1 b	a)	a)	>30	n.a
5- NS-D-20	48.8 ± 5.3 a	255 ± 35.6 a	a)	a)	>30	n.a
30- NS-L- Sat	1.5 ± 0.1 f	8.0 ± 0.7 f	34.6 ± 5.5 a	115 ± 18.1 a	8	40.4
30- NS-L-60	1.8 ± 0.1 e,f	9.4 ± 0.9 e,f	26.4 ± 3.4 a	87.8 ± 11.4 a	6	41.8
30- NS-L-20	10.6 ± 0.8 c	55.2 ± 5.6 c	a)	a)	>30	n.a
30- NS-D- Sat	1.8 ± 0.1 e,f	9.2 ± 0.8 e,f	33.7 ± 5.2 a	112 ± 17.4 a	8	40.7
30- NS-D-60	2.2 ± 0.1 e	11.3 ± 1.0 e	31.6 ± 4.7 a	105 ± 15.5 a	8	43.1
30- NS-D-20	4.1 ± 0.3 d	21.5 ± 2.1 d	44.5 ± 8.1 a	148 ± 26.8 a	11	41.9

n.a – not available

[#] Glyphosate DT_{50} and DT_{90} values were determined using the FOMC model for all treatments, and by fixing C_0 and α, in order to allow for statistical comparisons.

[\$] AMPA DT_{50} and DT_{90} values were determined by taking into account the FOMC model for glyphosate (for the formation phase of AMPA), and by fixing C_0 of AMPA, α and *ffA*.

a) Plateau and decline phases for AMPA were not reached during the experimental period, thus it was not possible to reliably determine AMPA DT_{50} and DT_{90}.

b) AMPA results were converted to equivalent mass of glyphosate, so C_{Amax} represents the peak concentration of AMPA in % of applied equivalent glyphosate.

Different lowercase letters within the same column mean significant differences between DT_{50}s and between DT_{90}s (F-test; $p<0.05$ - corrected by Bonferroni).

2.3.2 Treatments effects under biotic conditions

Under biotic conditions, glyphosate decay and AMPA formation/decay were primarily affected by temperature and secondly by soil moisture (Table 2.4A). Light regimes showed no significant effects on glyphosate decay and AMPA formation/decay (Table 2.4A). Exception was for AMPA for treatments 30-NS-L-20 vs. 30-NS-D-20, where AMPA formation/decay was significantly faster under dark conditions.

The greater influence of temperature on glyphosate decay and AMPA formation/decay has been demonstrated by several ways: the much higher F-values for temperature (Table 2.4A); the significant differences between both temperatures for all treatments (Table 2.4B); the significant differences between both temperatures for all DT_{50} and DT_{90} values for glyphosate (Tables 2.3 and 2.4-B1); and the immediate degradation of glyphosate to AMPA (C_0 of AMPA; see Table 2.2), which increased with increasing temperature (3.1 and 5.3 times higher than the background values of AMPA at 5°C and 30°C, respectively). On the other hand, a much higher fraction of glyphosate (*ffA*) was degraded to AMPA at very low temperatures ($ffA_{5°C}$=76.5±16.8% vs. $ffA_{30°C}$=43.0±4.1%), although with higher variation as well (Table 2.2). Nevertheless, the *ffA* results at 5°C need to be interpreted with care, since the plateau and decline phases were not reached at this temperature, which can result in an erroneous estimation and consequent misinterpretation of this parameter.

The effect of soil moisture was more evident on glyphosate decay than on AMPA formation/decay (for SM, $F_{glyphosate}$=104.58 vs. F_{AMPA}=15.66 (Table 2.4A)). For glyphosate, our results show significant differences between all soil moisture contents tested, for both temperatures (Table 2.4-B1). For AMPA, only at 30°C a significant slower formation and decay of AMPA occurred for soils reaching drought stress conditions (20% WHC) (Table 2.4-B2). The absence of significant differences between the DT_{50}/DT_{90} values also demonstrate the lower influence of soil moisture on AMPA formation/decay (Table 2.3).

2.3.3 DT_{50}/DT_{90} values

The DT_{50} and DT_{90} values of glyphosate under biotic conditions varied widely in this loess soil and they depended on the treatment combination (Table 2.3). On average, glyphosate decayed 8.4 times faster at 30°C than at 5°C. The variations in the range of DT_{50} and DT_{90} values of glyphosate for each temperature were mostly related to the influence of soil moisture (see SMxT in Table 2.4-A1). The wetter the soil the faster it was for glyphosate to decay from soil. Glyphosate DT_{50} was, on average, 1.25 times shorter for saturated soils than for those at 60% WHC (Table 2.4-B1). This difference was, however, not so strong when comparing these high moisture soils with those reaching drought stress conditions (20% WHC). On average, glyphosate DT_{50} was 2.9 and 3.6 times shorter for soils at 60% WHC and saturated, respectively, than for those at 20% WHC (Table 2.4-B1). This statement is reinforced by the pairwise comparisons between all DT_{50}/DT_{90} values (Table 2.3). These were not significant between saturated soils and soils at optimal moisture conditions (except 30-NS-L-Sat vs. 30-NS-D-60), but were significant between these high moisture soils and those at 20% WHC, at both temperatures. According to our results, glyphosate decay is, on average, 11 times faster at high temperatures for soils under saturated and optimal (60% WHC) moisture conditions, but only 7 times faster when soil moisture approaches drought stress (≤20% WHC) (Table 2.4-B1). Based on the combined effect of temperature with soil moisture (Table 2.4-B1), glyphosate persists longer in this loess soil in the following order (from low to higher persistence): warm+moist < warm+dry < cold+moist < cold+dry.

DT_{50} and DT_{90} values were always larger for AMPA than those of glyphosate at the same treatment conditions (Table 2.3). However, this higher persistence of AMPA decreased with decreasing soil moisture (at 30°C): AMPA persisted 21 times longer than glyphosate in saturated soils, 15 times longer in soils at 60% WHC and 11 times longer in soils at 20% WHC.

Table 2.4 *Effect of temperature, light and soil moisture on glyphosate decay (DT_{50}) and AMPA formation/decay under biotic conditions.*

A. ANOVA

	A1. Glyphosate (based on DT_{50})			A2. AMPA (based on linear regression)		
Source	df	Type III SS	F-value	df	Type III SS	F-value
Temperature (T)	1	23540.60	* 638.71	2	2.23	* 345.02
Light (L)	1	78.00	2.12	2	0.004	0.64
Soil Moist. (SM)	2	7708.90	* 104.58	4	0.20	* 15.66
L x T	1	0.30	0.01	2	0.03	* 5.31
SM x T	2	3133.50	* 42.51	4	0.24	* 18.32
L x SM	2	98.80	1.34	4	0.04	* 3.07
L x SM x T	2	40.90	0.55	4	0.09	* 7.12
Error	238	8771.90		216	0.70	

B. Pairwise comparisons between treatments with regard to SM x T (averaged over light levels) [#]

	B1. Glyphosate (based on DT_{50}±SE; days)			B2. AMPA (based on linear regression)		
Temperature	Soil Moisture			Soil Moisture		
	Saturated	60% WHC	20% WHC	Saturated	60% WHC	20% WHC
5°C	18.2±1.0 a, A	23.6±1.4 b, A	51.2±4.2 c, A	a, A	a, A	a, A
30°C	1.7±0.1 a, B	2.0±0.1 b, B	7.3±0.4 c, B	a, B	a, B	b, B

* Significant differences between treatment levels ($p<0.05$).

[#] p-value was corrected by Bonferroni for multiple comparisons.

Different lowercase letters within the same row mean significant differences between soil moisture levels; different capital letters within the same column mean significant differences between temperature levels (F-test, $p < 0.05$).

Note: Although the ANOVA table for AMPA (A2) shows significant differences with regard to LxT and LxSMxT, only the treatments 30-NS-L-20 vs. 30-NS-D-20 were significantly different from each other.

2.4 Discussion

2.4.1 Glyphosate decay

Our study indicates that the type of kinetic model for glyphosate decay in this loess soil depends on temperature. SFO kinetics was the best fit at 5°C, while FOMC bi-phasic kinetics, i.e. a decay kinetics that is described by a fast initial decline followed by a slower decline, was the best fit at 30°C. Eberbach (1998), in a study with four types of soil (loamy sand, clay, silty clay loam and loam) tested at two temperatures (25°C and 10°C), suggested bi-phasic decay kinetics of glyphosate at 25°C for all soils, in accordance to our results at 30°C. At 10°C, this author suggested SFO decay kinetics for the clay and silty clay loam soils, but bi-phasic for the loamy sand and loam soils. Based on our own visual assessment of his figure on glyphosate decay at 10°C, we believe, however, that the loam soil followed SFO instead.

Thus, the decay kinetics followed by these soils at 10°C are also in accordance with our findings, except for the loamy sand soil. The latter presented a very low clay and organic carbon content when compared to the other 3 soils studied by Eberbach (1998) and to our silty loam soil, which might explains its bi-phasic behavior at this low temperature. Glyphosate adsorption tends to be strongly related to the clay and organic matter content of the soil (although the latter varies considerably between humic substances) (Vereecken, 2005). Thus, weaker bonds and a higher readily available form (either soluble or desorbed into the soluble soil phase) are expected to occur in the loamy sand soil of Eberbach (1998) even at low temperatures. Some studies suggest that glyphosate has this readily available form that allows for its fast initial decay, but that it declines in a later stage, resulting in a much slower glyphosate decay rate that only derives from the adsorbed phase (Simonsen et al., 2008; Gimsing et al., 2004b; Eberbach, 1999; Eberbach, 1998). Our study suggests that, under our conditions, this adsorbed phase may have been reached at concentrations below 20-25% of the initially applied glyphosate, which explains the underestimation of DT_{90} when using SFO for cases with high decay rates. This slower decay of the adsorbed glyphosate poses a potential off-site risk through erosion related particulate transport. It is actually not considered in pesticide transport models, which only use SFO decay kinetics (Yang et al., 2016; Yang et al., 2015a).

According to our study, glyphosate decays mostly by microbial activity. Abiotic processes were proven to have a negligible role on glyphosate decay, and this is in accordance to what has been reported elsewhere (Ghassemi et al., 1982; Rueppel et al., 1977; Sprankle et al., 1975). Under biotic conditions, temperature and soil moisture were the factors that affected glyphosate decay the most. The presence of light, on the other hand, played a minor role. Nevertheless, this minor influence seems to depend on soil moisture. A positive influence (faster glyphosate decay) was observed for soils with high moisture contents (saturated and at 60% WHC), but it was negative (slower glyphosate decay) for soils reaching drought stress conditions. Since photodegradation didn’t play a role on glyphosate decay under abiotic conditions, the observed effect suggests that light affected the microbial activity, but this seems unrelated with photodegradation. Rueppel et al. (1977) also concluded that photodegradation of glyphosate in soil, if occurring, has a very minor role under natural conditions.

Glyphosate persists much longer in soil when temperatures are very low, and the drier the soil the longer it persists. Studies where the effect of temperature on glyphosate decay has been tested also have shown that its decay rate increases with increasing temperature (Stenrød et al., 2005; Strange-Hansen et al., 2004; Eberbach, 1999; Eberbach, 1998; Heinonen-Tanski, 1989). The influence of soil moisture (SM) on glyphosate decay has also been reported and confirms our results, i.e. increasing SM increases glyphosate decay

(Grundmann et al., 2008; Schroll et al., 2006). Schroll et al. (2006), in a study at 20°C and where they tested the effect of increased water content on pesticide mineralization using glyphosate (12 SM contents ranging from 5% to 100% of WHC), report that: 1) soils under extreme drought stress conditions (≤-20MPa, equivalent to ≤≈13% WHC) result in minimal pesticide mineralization, 2) SM contents between -20MPa and -0.015MPa (≈40% WHC) show a linear correlation (an increase in SM results in an increase of pesticide mineralization), and 3) at SM contents approximating WHC, pesticide mineralization is "considerably reduced". Our study shows, however, that glyphosate decay increases with increasing soil moisture (from 20% WHC to saturation), not confirming the reduction referred by Schroll et al. (2006) for SM contents approximating WHC. Nevertheless, as shown in our study, the difference between glyphosate decay in saturated soils and soils at 60% WHC is smaller than that between soils at 20% and 60% WHC. No studies were found testing the combined effect of soil moisture and temperature.

The experimental data also revealed a clear influence of temperature and soil moisture in the DT_{50} and DT_{90} values (Table 2.3). These were much shorter at high temperatures combined with high moisture soils than those at low temperatures combined with soils reaching drought stress conditions. Rueppel et al. (1977), in a greenhouse decay study with a silty loam soil at temperatures between 26-32°C and 11% SM (w/w; which should correspond to ≈30% WHC for this type of soil), reported a DT_{50} of 3 days and DT_{90} of 14 days. These values are comparable to our results at 30°C, since they are larger than those we obtained at 60% WHC but shorter than those we obtained at 20% WHC. Rampoldi et al. (2014), in a laboratory experiment with 2 silty loam loess soils (as classified by the texture triangle) at 28°C and 85% WHC, report an average DT_{50} of 4.0 and 4.6 days. These half-lives are larger than the range we report for soils under optimal/saturated conditions at 30°C. Since temperature and SM were similar for both studies, this is probably due to the higher and neutral pH and much lower P available observed in our soil (Table 2.1). Forlani et al. (1999) report maximal microbial activity and degradation rates under conditions of neutral pH in soil. Pipke and Amrhein (1988) and Dick and Quinn (1995) report that some bacteria strains are able of using glyphosate as a source of P when the latter is a limiting factor in soil. No laboratory studies reporting DT_{50} nor DT_{90} at 5°C were found. Under field conditions, Yang et al. (2015a), in a 2-year field study in Chinese loess soil, reported a DT_{50} of 3.5 days for glyphosate, with no significant differences between both years. The reported field half-life was comparable to our results at 30°C, despite the fluctuations in temperature (between 14°C and 30°C) and soil moisture (between 5% and 25% (gravimetric water content)). To our knowledge, DT_{90} estimations under field conditions are rarely reported and the inappropriate use of SFO kinetics in some studies would underestimate it. Nevertheless, Mamy et al. (2008), in a field study during the growing season of a winter crop in a clay-loam soil in France, reported that <10% of applied glyphosate was detected 34 days after application. This suggests a $DT_{90}<34$ days during a winter period, which is much shorter than the shorter DT_{90} we report at 5°C (93.2 days). This might be explained by some lixiviation and transport to deeper soil layers, but mostly by the temperature fluctuation during the field experiment. Temperatures during this field experiment ranged, in average, between 0°C and 17°C and, from the observation of precipitation data, the soil was normally under optimal/wet conditions.

Our study indicates that glyphosate applied in cold conditions leads to a risk of slow decay and, therefore, longer presence in soils than often estimated in models for risk assessment.

2.4.2 AMPA formation and decay

Our study indicates that AMPA follows first-order (SFO) decay kinetics regardless of temperature, soil moisture or light regimes. Although AMPA is often given little attention in decay kinetic studies under laboratory or field conditions, the studies found also reported SFO for this compound (Yang et al., 2015a; Bergstroem et al., 2011; Simonsen et al., 2008). Nonetheless, all the studies determined its decay kinetics by fitting the SFO model from the maximum observed amount of AMPA onwards, not taking into account its formation from glyphosate (Yang et al., 2015a; Simonsen et al., 2008). Exception was our current study and the one by Bergstroem et al. (2011). According to FOCUS (2006), this is a conservative approach that underestimates the decay and, consequently, overestimates the DT_{50}/DT_{90} values of metabolites.

Similar to glyphosate, AMPA forms/decays mostly by microbial activity whereas abiotic processes play a minor role. Under biotic conditions, temperature and soil moisture were also the factors that affected AMPA formation/decay the most, but the presence of light played a negligible role. To our knowledge, our study is the first discussing the combined effect of temperature, soil moisture and light/darkness on AMPA formation/decay kinetics. So far, focus has always been given mostly (or even only) to glyphosate, especially when soils are subject to different conditions.

AMPA clearly persists longer in soil when temperatures are very low. Besides the inexistence of a decline phase for all treatments at 5°C, the strong influence of temperature has also been demonstrated by the increasing initial concentration of AMPA (at day 0) with increasing temperature. This, though, also shows that glyphosate immediately degrades to AMPA within the first 2h after applying glyphosate (average time between sampling and freezing the samples). Paudel et al. (2015) also reported that the majority of degradation products of glyphosate are formed within 1 min of reaction. Although the results on the formation fraction of AMPA (*ffA*) need to be treated with care for reasons already described in section 2.3.2, the percentage of glyphosate being degraded to AMPA also seems to be strongly influenced by temperature. Much more AMPA has been formed at 5°C than at 30°C. This suggests that glyphosate degradation to other metabolites was reduced at low temperatures, while AMPA formation was beneficiated. Even though no identification of soil microorganisms has been performed in the present study, our *ffA* results suggest that the soil microorganisms degrading glyphosate to AMPA in our soil were more resistant and active at very low temperatures than those promoting the formation of other metabolites.

Although soil moisture has been proven in the present study to influence AMPA formation/decay, its effect is less evident than for glyphosate. The stronger effect of soil moisture on glyphosate decay, when compared to AMPA, suggests that other decay processes of glyphosate, such as its degradation to other metabolites (e.g. sarcosine), are being more strongly affected by soil moisture than its formation to AMPA.

According to our results, AMPA decays slower in soil than glyphosate under the same conditions. This is in accordance to what has been reported elsewhere (Yang et al., 2015a; Bergstroem et al., 2011; Mamy et al., 2008). This higher persistence of AMPA is expected though, because it is being formed from the degradation of glyphosate. Thus, AMPA has to be as or more persistent than glyphosate. In fact, AMPA, at high temperatures, dissipates at rates similar to that of glyphosate at very low temperatures (Table 2.2). A similar decay rate for AMPA has been reported under field conditions by Yang et al. (2015a). Our results also suggest that a lag-phase might be occurring for AMPA during the first days of its formation or at least its decay occurs very slowly. This is based on the fact that the average *ffA* and C_{Amax} are very similar at 30°C (see Tables 2.2 and 2.3). According to FOCUS (2006), the maximum observed level of AMPA (C_{Amax}) should normally be lower than the actual formation fraction (*ffA*), as a result of the simultaneous formation and degradation of the metabolite. Laboratory decay kinetic studies where AMPA is directly applied to soil and treated as parent compound are, however, lacking in literature. Therefore, further research is needed for a better comprehension of AMPA decay and behavior in soil.

The results on DT_{50} and DT_{90} values at 30°C also confirm the higher persistence of AMPA in this loess soil, when compared to those of glyphosate. Yang et al. (2015a), in a 2-year field study in Chinese loess soil, reported a half-life for AMPA of 23 days in the first year and 35 days in the second year. The reported half-life for the second year is within the DT_{50} range obtained in our study, despite being a field study with fluctuations in temperature and SM. However, for the first year, it is shorter than the lower range of 26.4 days that we achieved. Their field decay rates in the first year were slightly higher than those in their second year and than our laboratory decay rates, thus explaining their shorter DT_{50} for the first year.

Simonsen et al. (2008), in a laboratory study with a loam soil at 14.3°C, reported an AMPA DT_{50} of 32 days and DT_{90} of 106 days. Although their incubation temperature has been less than half of our higher incubation temperature, both DT_{50} and DT_{90} values are within the range we report in our study.

2.5 Conclusions

From the combined effect of temperature with soil moisture we conclude that glyphosate decays fast under warm and moist conditions, but it persists 30 times longer under cold and dry conditions. AMPA persists longer in soil than glyphosate, even under warm and moist conditions. The type of decay kinetic model followed by glyphosate in loess soil depends on temperature, thus its DT_{90} values should be estimated using the appropriate kinetic model to avoid its underestimation. From a practical point of view, repeated glyphosate applications in fallows or winter crops in countries where cold and dry winters normally occur might increase the risk of on-site soil pollution due to accumulation of these chemicals.

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3. Glyphosate and AMPA distribution in wind-eroded sediment derived from loess soil

Glyphosate is one of the most used herbicides in agricultural lands worldwide. Wind-eroded sediment and dust, as an environmental transport pathway of glyphosate and of its main metabolite aminomethylphosphonic acid (AMPA), can result in environmental- and human exposure far beyond the agricultural areas where it has been applied. Therefore, special attention is required to the airborne transport of glyphosate and AMPA. In this study, we investigated the behavior of glyphosate and AMPA in wind-eroded sediment by measuring their content in different size fractions (median diameters between 715 and 8 µm) of a loess soil, during a period of 28 days after glyphosate application. Granulometrical extraction was done using a wind tunnel and a Soil Fine Particle Extractor. Extractions were conducted on days 0, 3, 7, 14, 21 and 28 after glyphosate application. Results indicated that glyphosate and AMPA contents were significantly higher in the finest particle fractions (median diameters between 8 and 18 µm), and lowered significantly with the increase in particle size. However, their content remained constant when aggregates were present in the sample. Glyphosate and AMPA contents correlated positively with clay, organic matter, and silt content. The decay of glyphosate over time was very low, which was most probably due to the low soil moisture content of the sediment. Consequently, the formation of AMPA was also very low. The low decay of glyphosate in our study indicates that the risk of glyphosate transport in dry sediment to off-target areas by wind can be very high. The highest glyphosate and AMPA contents were found in the smallest soil fractions (PM_{10} and less), which are easily inhaled and, therefore, contribute to human exposure.

Based on:

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3.1 Introduction

Glyphosate (N-phosphonomethylglycine, $C_3H_8NO_5P$) is the active ingredient of many commercial formulations of herbicides extensively used worldwide for weed control. Its annual global production has been estimated at approximately 720 000 tons in 2012 (IARC, 2015). It is intensively used in agriculture, particularly in combination with genetically modified crops (GMC) that are resistant to it.

Glyphosate is a broad-spectrum, systemic, post-emergent herbicide that decays in soil mostly by microbial activity (Bento et al., 2016; Schnurer et al., 2006; Giesy et al., 2000). Its persistence in soil varies widely, with a half-life ranging between 1-197 days, depending on temperature, soil moisture, soil type, soil binding extent, microbial breakdown and phosphate levels (Bento et al., 2016; EU, 2002; Giesy et al., 2000; Nomura and Hilton, 1977). The main metabolic pathway of glyphosate is its degradation to aminomethylphosphonic acid (AMPA), which is more persistent in soil than glyphosate itself (half-life ranging between 23-958 days) (Bento et al., 2016; Yang et al., 2015a; U.S.EPA, 1993).

Glyphosate and AMPA are considered non-volatile and, therefore, their loss to the atmosphere via volatilization is considered negligible (EU, 2002; Giesy et al., 2000; U.S.EPA, 1993). However, offsite movement can occur through spray drift (Marrs et al., 1993; Payne, 1992; Payne et al., 1990) or by airborne particulate transport. Glyphosate and AMPA strongly adsorb to soil particles (Beltran et al., 1998; Cheah et al., 1997). However, their adsorption coefficients can vary greatly, with reported Freundlich adsorption coefficients (K_f) ranging between 93-2754 $\mu g^{(1-1/n)}\ mL^{1/n}\ g^{-1}$ for glyphosate and 38-1517 $\mu g^{(1-1/n)}\ mL^{1/n}\ g^{-1}$ for AMPA (Báez et al., 2015). The degree to which glyphosate and AMPA adsorb to soil depends mostly on soil properties such as clay content, soil phosphate content, soil aluminum and iron oxides/hydroxides, pH, and organic matter (Borggaard and Gimsing, 2008; Gimsing et al., 2004a; de Jonge and de Jonge, 1999; Sprankle et al., 1975). Glyphosate and AMPA transport to off-target areas by wind erosion is, therefore, very likely. In fact, the occurrence of glyphosate and/or AMPA in the atmosphere (air and/or rain) has already been reported in some studies (Messing et al., 2011; Humphries et al., 2005; Quaghebeur et al., 2004). Since glyphosate is non-volatile and AMPA is a degradation product of glyphosate, their occurrence in the atmosphere can only be associated to spray drift (glyphosate) or transport in wind-eroded sediment (glyphosate and AMPA). Nevertheless, work on their transport by wind erosion hasn't been published so far, even though glyphosate is the most widely used herbicide worldwide.

Loess soils represent ≈10% of the global land surface and are among the most agriculturally productive soils in the world. They are present in many parts of the world such as the USA, Argentina, China and Europe. A great part of them are intensively used for agriculture and to grow GMC (den Biggelaar et al., 2001). Consequently, huge amounts of pesticides, particularly glyphosate-based herbicides, are applied every year in these soils.

Agricultural soils account for a significant source of airborne particulate matter because of wind erosion and tillage activities (Gill et al., 2006). Therefore, off-site airborne transport of glyphosate and AMPA from farmland in loess soils is extremely likely. This off-site transport is mostly associated to the finest particles (dust), which can travel over large distances. Small particles can travel from ≈500 km (particle sizes between 10 and 20 μm) to thousands of kilometers (particle sizes <10 μm) during moderate wind storms (Pye, 1987). Moreover, in a sediment sample, the largest amounts of pesticides are normally adsorbed to the fine particles (O'Hara et al., 2000; Agassi et al., 1995). Their contents in these particles can also be much higher than those in the parent topsoil (Clymo et al., 2005). The inhalation of polluted particles is strongly linked to human diseases such as asthma, heart and lung diseases, and cancer (Moorthy et al., 2015; Prüss-Ustün et al., 2011; Ichinose et al., 2005; Järup, 2003; Besancenot et al., 1997). As for glyphosate, the International Agency for Research on Cancer (IARC) classified it, in 2015, as "probably carcinogenic to humans" (IARC, 2015). Although the European Food Safety Authority (EFSA) disagreed with this evaluation, it proposed for the first time for glyphosate an acute reference dose (ARfD) of 0.5 mg kg^{-1} of body weight (bw) (EFSA, 2015). EFSA has set this same dose as an acceptable daily intake (ADI) for consumers. An acceptable operator exposure level (AOEL) of 0.1 mg kg^{-1} of bw day^{-1} has also been proposed (EFSA, 2015). It is, therefore, very important to study the transport of glyphosate and AMPA in wind-eroded sediment, particularly for fine particles (dust), which may contribute to human exposure. Risk assessment studies on human health could also benefit and improve from such information.

This study investigates the occurrence of glyphosate and AMPA in wind-eroded sediment derived from loess soil produced during wind erosion in a wind tunnel. It aims to 1) investigate the distribution of glyphosate and AMPA in different size fractions of wind-eroded sediment; 2) study the effect of several physicochemical parameters (clay, silt, organic matter) on glyphosate and AMPA transport during wind erosion; and 3) estimate the potential of glyphosate and AMPA to become transported over large distances from the pollution source.

3.2 Materials and Methods

3.2.1 Soil

We used the topsoil of a silty loam loess soil from Huldenberg, Belgium. The soil was air-dried and then sieved through a 1-mm sieve. It was tested for glyphosate and AMPA residues and found free of glyphosate and AMPA.

The main soil properties of the sieved soil are shown in Table 3.1 . Fig. 3.1 shows the grain size distribution of the soil after disintegration of all aggregates.

Table 3.1 *Soil properties of the loess soil used in this study.*

Parameters	Value
Particle Size Distribution:	
<2 µm (clay) (%)	10
2-50 µm (silt) (%)	79
>50 µm (sand) (%)	11
pH $CaCl_2$	5.8
Organic matter (OM) (%)	3.2
Particle density (g cm^{-3})	2.5
N total (g kg^{-1})	1.7
P available (mg kg^{-1})	0.4
K available (mg kg^{-1})	209
Mg available (mg kg^{-1})	121
Na available (mg kg^{-1})	10
C/N ratio	9

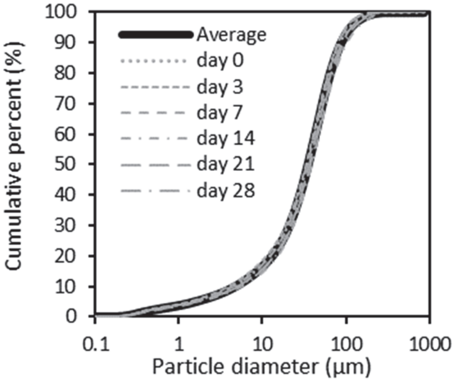


Figure 3.1 *Particle size distribution of the start soil after disintegration of all aggregates.*

3.2.2 Glyphosate preparation and application in the soil

3.2.2.1 Preparation of glyphosate solution

Glyphosate solution was prepared by diluting 980 µL of CLINIC®, a glyphosate-based herbicide that contains 360 g L^{-1} of glyphosate, in Millipore water to achieve a final stock solution of 0.42 g L^{-1}. A concentration of glyphosate in soil of 8.4 mg kg^{-1} was used in this study, which corresponds to an application rate of 1.26 kg a.i. ha^{-1} (typically applied in agricultural fields), assuming a soil depth of 1 cm and a bulk density of 1.5 g cm^{-3}.

3.2.2.2 Application in soil

A plastic sheet was put on the ground and an approximately 5-cm thin layer of the air-dried and sieved soil (42 kg) was spread on it. The soil was then sprayed with the prepared glyphosate solution (see section3.2.2.1). During the application, the soil was thoroughly mixed with a rake. The soil was then stored in a plastic bag at room temperature (22°C) and dark conditions.

A small portion of the soil was collected after glyphosate application and oven-dried (105°C) for 24h to determine the initial soil moisture content, which was found to be 5.4% (w/w).

3.2.3 Facilities and Instrumentation

The experiment was carried out in the facilities of the Geography and Tourism Research Group of the Katholieke Universiteit Leuven, Belgium. A closed-return wind tunnel was used. The tunnel has two test sections, both of which were used in this study. The dimensions of the large test section are 760 cm (length) x 120 cm (width) x 60 cm (height), and those of the small test section are 150 cm (length) x 35 cm (width) x 30 cm (height). A detailed description of the wind tunnel can be found in the technical report by Goossens and Offer (1988); a scheme of the tunnel is shown in Figure 3.2a.

Apart from the wind tunnel, we also used a modified version of the Soil Fine Particle Extractor developed in a previous study by Goossens (2012). The setup is shown in Figure 3.2b. This instrument draws up the sediment, previously spread on a table, with a plastic hose attached to a BASE 440 three-engine vacuum cleaner connected to a cyclone dust separator (RIBO, Villanova, Italy). The hose is 300 cm long and 4 cm in diameter; the separator is 70 cm high and 40 cm in diameter. Coarse particles settle in the separator and are thus removed from the sample. Separation is accomplished by the circular motion of the particles and enhanced by selective gravitational settling. Some of the smallest particles remain suspended in the separator. After initial separation in the separator, the dust enters a tube 139 cm long and 16 cm in diameter, which operates as an elutriator. Dust is then accelerated through a small pipe 36 cm long and 7.6 cm in diameter and hits an impactor (diameter: 8.7 cm) installed near the bottom of a settling chamber. Only the finest particles will suspend in the chamber. These particles then enter a 200-cm long plastic tube. Further granulometrical separation is performed in this tube, which operates as a second elutriator. Particles then enter the vacuum cleaner and settle in a 50-L deposition chamber, where they can be collected. Three 1200-W engines that generate a suction rate up to 510 $m^3 h^{-1}$ and create an under pressure of 2200-mm H_2O power the instrument. For this study, only one engine (170 $m^3 h^{-1}$) was used.

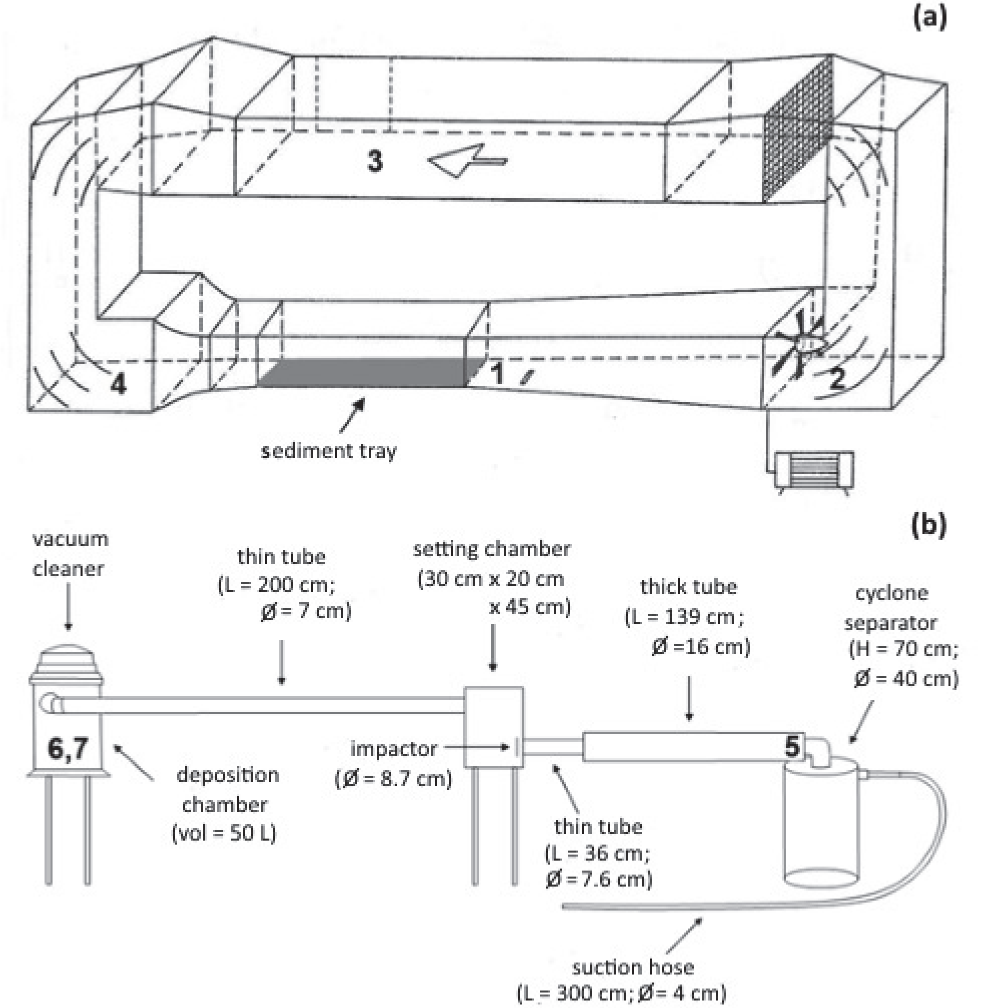


Figure 3.2 *Experimental setup for the sediment extractions. (a) Leuven wind tunnel, with locations of the sampling sites 1 to 4; (b) Soil Fine Particle Extractor, with locations of the sampling sites 5 to 7.*

3.2.4 Experimental design

To perform each experimental run, a total of 8 kg of pre-treated soil (enough to fill the sediment tray in the wind tunnel) was taken one day before each experimental run. The soil was then oven-dried at 37.5°C for 24h to ensure a soil moisture ≤2% (the highest soil moisture allowed to guarantee wind erosion; see Nourzadeh et al. (2013). Soil samples (in duplicate) were always taken before and after the drying process to control for any effect on glyphosate decay and AMPA formation/decay.

The oven-dried soil was then subjected to wind erosion, which was carried out in the wind tunnel. In the small test section, a tray 150 cm long x 35 cm wide x 2 cm deep was installed. The upwind 75 cm were filled with a piece of wood; the downwind 75 cm were covered with a thin sheet of plastic (to avoid direct contact between the glyphosate-treated soil and the metal of the tray). The oven-dried soil was then put into the tray. Its surface was carefully flattened using a slat. The wind tunnel was then closed and turned on to allow the soil sediment to erode until the entire tray was empty. We used a free-stream wind speed of 10.0 m s^{-1}, which was well above the deflation threshold of the sediment used (6.5 m s^{-1} according to visual observations made before the test). It took approximately 1h until the tray was empty.

After each run, sediment samples (in triplicate) were collected (≥2 g for most of the samples; and always ≥1 g) at 4 different places in the wind tunnel using a clean brush (Figure 3.2a). The distances from the trailing edge of the tray were as follows: sample 1: 10 cm; sample 2: 480 cm, sample 3: 1290 cm, and sample 4: 1865 cm. Due to aeolian selection, the samples become finer as they are taken further from the source.

Because of the restricted length of the wind tunnel, sample 4 was the finest sample that could be obtained with the wind tunnel technique. To collect even finer samples we used the Soil Fine Particle Extractor and 3 more samples were collected (Figure 3.2b). After each wind tunnel run, the tunnel was first thoroughly cleaned with the vacuum cleaner. A sample (sample 7) was then taken from the deposition chamber of the vacuum cleaner, which at this stage was directly connected to the cyclone separator. The sediment in the separator was then mixed with the remaining dust in the deposition chamber and put on a clean table. After assembling the entire Soil Fine Particle Extractor, the sediment on the table was sucked up and samples 5 and 6 were collected just downwind from the cyclone separator (sample 5) and in the deposition chamber of the vacuum cleaner (sample 6) (see Figure 3.2b).

All experimental runs (wind tunnel + Soil Fine Particle Extractor) and collection of samples were conducted on days 0, 3, 7, 14, 21 and 28 after glyphosate application. All samples were stored in plastic tubes and frozen at -18°C until glyphosate and AMPA analysis.

3.2.5 Sample analysis

3.2.5.1 Particle size distribution and organic matter content

To analyze the particle size distribution of samples 2 to 7 we used a Malvern Mastersizer S laser particle size analyzer (Malvern Ltd, Malvern, UK). Sample 1, which exclusively consisted of large aggregates, was analyzed optically with a microscope. For the latter sample, we collected a subsample from the main sample and measured the nominal diameter of all aggregates. Using these data, the aggregate size distribution of the sample could be determined. To get an idea of the internal particle size distribution of the large aggregates themselves, we also collected several of these aggregates, carefully crushed and dispersed them, and then analyzed them with the Mastersizer instrument.

The organic matter (OM) content was estimated by oxidation at 600°C and detected by close infra-red using an SC-144DR equipment (LECO Corporation, St Joseph, MI, USA). When there was insufficient sample for analysis, the triplicates were mixed together.

3.2.5.2 Glyphosate and AMPA content

Glyphosate and AMPA contents in the samples were analyzed as described by Bento et al. (2016). Briefly, glyphosate and AMPA were extracted from 1 g of soil or wind-eroded sediment with 5 mL of 0.6 M KOH (potassium hydroxide, p.a. 85%). After shaking and centrifuging the samples, 1 mL of the supernatant was transferred to a 10-mL plastic tube. Isotopically labelled glyphosate and AMPA were added at this point and then a derivatization step was carried out with FMOC-Cl to improve retention and MS/MS detection as described by Bento et al. (2016). Solvent standards with isotopically labelled internal standards were prepared together with all the samples for each batch of samples, and derivatized the same way. Glyphosate and AMPA contents were then determined by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) using an XBridge™ Shield RP C18 column 100 x 2.1 mm i.d. (Aquity UPLC I-Class coupled to a Micromass Ultima triple-quadrupole MS, Waters, The Netherlands). Chemicals used, mobile phases and instrumentation conditions of the HPLC-MS/MS were as described by Yang et al. (2015b) and Bento et al. (2016).

With each batch of samples, two blank soil samples of the loess soil used in this study were fortified at 0.5 mg kg^{-1} and added as quality control (QC) samples. To ensure the quality of the analysis when processing real samples, the fortified samples were analyzed twice, at the beginning and at the end of each batch. The quantification of the sample batch was considered satisfactory when the QC recoveries were between 70 and 120%. A detailed description of the method validation and quality control can be found in Bento et al. (2016).

3.2.6 Statistical analysis

All statistical analyses were performed in SPSS 22, and the graphs in Figure 3.5 were produced in SigmaPlot 10.0. A one-way ANOVA to ln-transformed data followed by Dunett T3 post-hoc tests was performed to test for significant ($p<0.05$) differences in clay, silt or

OM content between extracted size fractions of the wind-eroded sediment. Besides, a power function was applied to the non-aggregated samples (sample 3-7) to test the correlation between the clay or OM content and the particle size of the samples. To test for significant differences of glyphosate or AMPA residues between extracted size fractions of the wind-eroded sediment, an analysis of covariance (ANCOVA) to ln-transformed data followed by Bonferroni tests was performed ($p<0.05$). The assumption of homogeneity of regression slopes was not violated. Moreover, a categorical principal components analysis (non-linear PCA) was performed to determine the relationship between sediment properties (clay, silt, OM) and glyphosate or AMPA content in the wind-eroded sediment. The loading of a given variable was considered meaningful if its absolute value was ≥0.40 for a given component. Besides, a Pearson correlation was computed to assess the relationship between glyphosate or AMPA contents and clay, silt or OM.

A reconstruction of the distribution of glyphosate in the original soil in the sediment tray before the start of each wind tunnel experiment was also performed. This was done by considering the glyphosate content for a large number of narrow grain size classes, which could be estimated by applying an exponential regression analysis to the data (only the samples without aggregates, i.e., samples 3-7).

3.3 Results and discussion

3.3.1 Physicochemical composition of the wind-eroded sediment

3.3.1.1 Particle size distribution

The particle size distribution of the different extracted fractions of the wind-eroded sediment is shown in Figure 3.3a. Sample 1 was composed of large, macroscopic aggregates only. Sample 2 consisted of individual grains and micro-aggregates, mixed with a few macroscopic aggregates. Samples 3-7 only contained individual grains with some small micro-aggregates (as verified under the microscope) and were mostly composed of particles ≤100 µm in diameter. More than 96% of the particles of samples 5-7 were ≤50 µm in diameter. The median diameters of the samples were: 715±69 µm (sample 1), 58±2 µm (sample 2), 33±1 µm (sample 3), 29±1 µm (sample 4), 18±1 µm (sample 5), 8±1 µm (sample 6) and 11±3 µm (sample 7). These median diameters are further used as reference codes in the data analysis presented here.

Crushing of the macroscopic aggregates (sample 1) and analyzing their grain size distribution showed that the aggregates are perfect compositions of the original tray sediment (Figure 3.3b), with a median particle diameter of 36±2 µm for both the aggregates and the original tray soil.

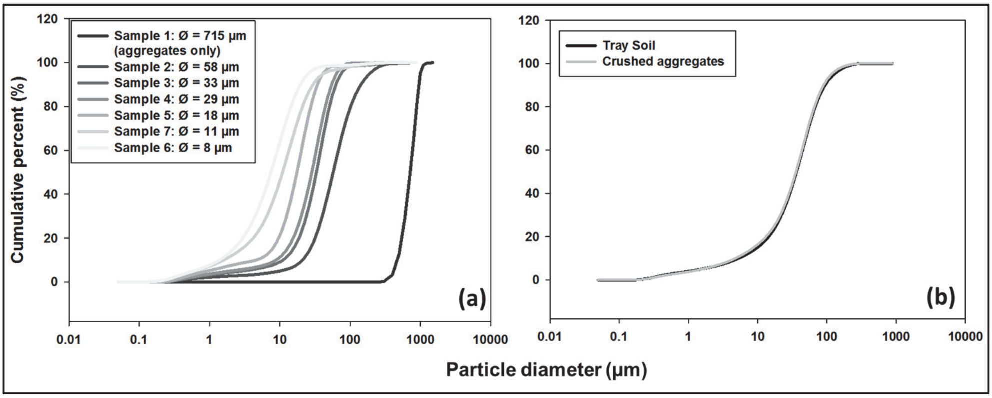


__Figure 3.3__ Particle size distribution of (a) the different extracted fractions of wind-eroded sediment; (b) the crushed aggregates and the original sediment in the sample tray. Ø = median diameter.

3.3.1.2 Clay, silt and OM content

The clay (<2 µm), silt (2-50 µm) and OM content of the different extracted fractions of the wind-eroded sediment are shown in Figure 3.4. The clay content was significantly higher for the finest extracted size fraction (median diameter of 8 µm) and lowered significantly with increasing particle size (Figure 3.4), except for the samples with a 715-µm median diameter which consisted exclusively of macroscopic aggregates. A strong negative correlation was also observed between the clay content and the particle size of the non-aggregated samples (median diameters between 8 and 33 µm; Clay (%) = 67.7 MDES$^{-0.78}$, R^2 = 0.99; MDES = median diameter of the extracted sample). Likewise, the OM content was highest for the finest extracted fractions (samples with median diameter of 8 and 11 µm) and lowered significantly with increasing particle size (Figure 3.4). Nevertheless, this decrease in OM was no longer significant after a particle size ≥33 µm. A strong negative correlation was also observed between the OM content and the particle size of the non-aggregated samples (OM (%) = 13.1 MDES$^{-0.61}$, R^2 = 0.90).

All samples were mostly composed of silt (Figure 3.4). The silt content decreased as the samples became coarser, but to a lower extent compared to clay and OM. In the aggregated samples (median diameters of 58 and 715 µm), the silt content was significantly lower than in the non-aggregated samples.

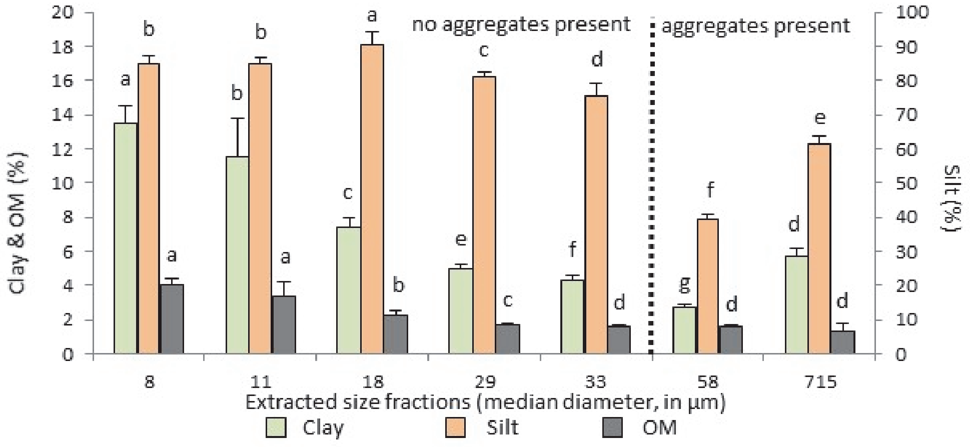


Figure 3.4 *Clay, silt and organic matter (OM) content of the extracted size fractions. The 715-µm samples consist exclusively of large aggregates. Different lowercase letters within the same type of bars mean significant differences in silt or clay or OM between extracted size fractions ($p<0.05$).*

3.3.2 Glyphosate and AMPA content in the wind-eroded sediment

3.3.2.1 Relationship between glyphosate or AMPA and particle size

Glyphosate content (Figure 3.5a) varied between 5.5 and 16 µg g^{-1}, with a significantly higher content in the finest extracted fractions (median diameters from 8 to 18 µm). AMPA content (Figure 3.5b), on the other hand, was rather low, varying between 0.07 and 0.7 µg g^{-1}. Here too, AMPA content was significantly higher in the finest extracted fractions. In Figure 3.6, the relationship between glyphosate (or AMPA) content and particle size of the wind-eroded sediment is better shown. Here, it is clearly visible that glyphosate and AMPA contents were highest in the finest samples (median diameter: 8 µm) and became lower with increasing particle size until ≈33 µm (Figure 3.6a-b). Note that this does not necessarily mean that the highest amounts of glyphosate and AMPA in a sample occur in the finest fractions of that sample: the mass of coarse grains is much higher than that of fine grains, so even when the concentration is higher in the fine fractions it is possible that the coarse fractions contain more glyphosate and AMPA in weight. A larger spread was observed for AMPA (Figure 3.6b) than for glyphosate (Figure 3.6a). However, this is not meaningful since it just reflects the increase of AMPA content in the course of time (see Figure 3.5b). For the individual days, the lower AMPA content with increasing particle size became better visible. It also became stronger over time. The effect of the presence of macroscopic aggregates in a sample was also very prominent (Figure 3.6). Once macroscopic aggregates were present (samples with median diameters of 58 and 715 µm), glyphosate and AMPA contents remained constant regardless of how numerous or how large the aggregates were. This seems to be related with the fact that the aggregates are perfect compositions of the original soil in the sediment tray (Figure 3.3b), regardless of their size. Because, in an aggregate, the largest mass is represented by the coarsest grains, glyphosate and AMPA contents will be rather low, approaching the concentration in the coarsest individual grains, albeit a little higher because of the presence of a higher percentage of fine particles in the aggregates.

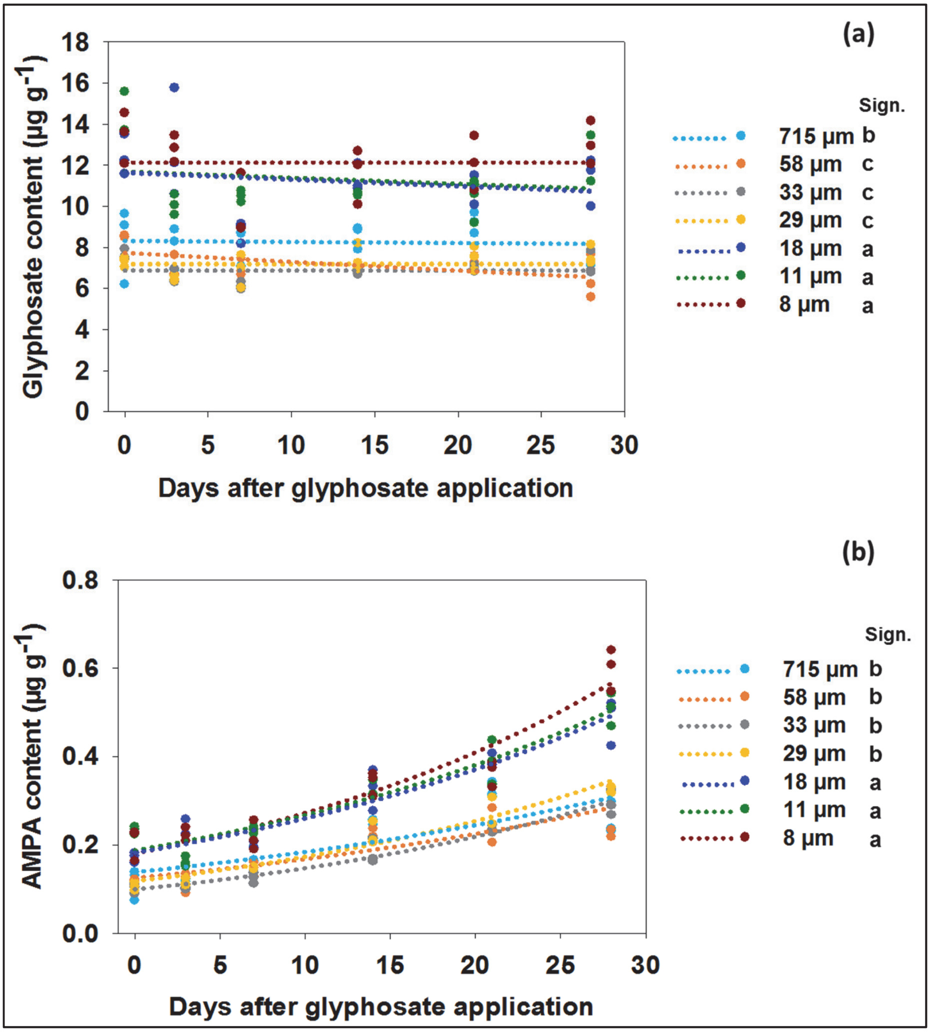


Figure 3.5 *Glyphosate (a) and AMPA (b) contents in the different extracted size fractions of the wind-eroded sediment during the 28 days after glyphosate application, and respective trendlines. Note the different vertical scales between (a) and (b). To the right of the legends, different lowercase letters mean significant differences in glyphosate (a) and AMPA (b) content between extracted size fractions, using an ANCOVA followed by Bonferroni tests ($p<0.05$).*

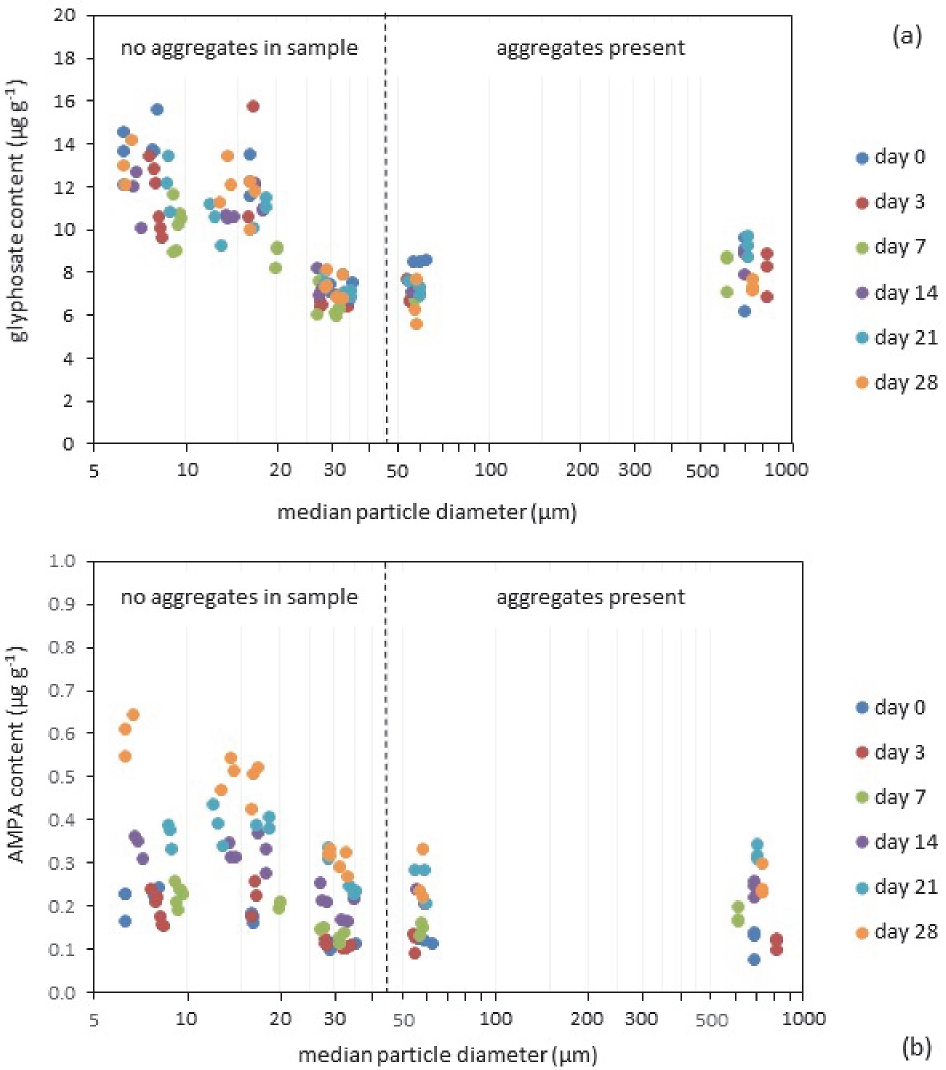


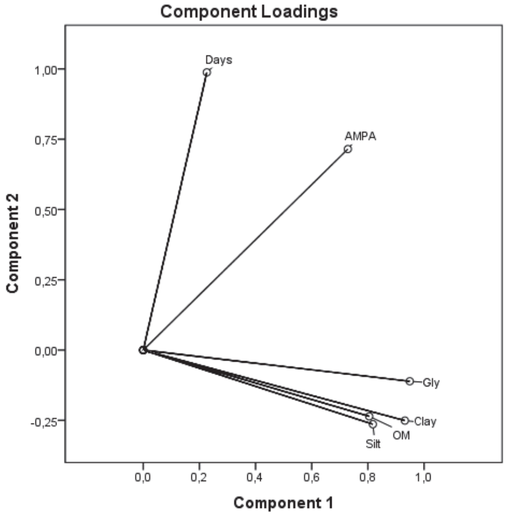
Figure 3.6 *Relationship between (a) glyphosate content and particle size, (b) AMPA content and particle size.*

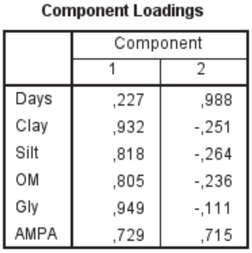
When comparing the glyphosate content in the different sediment fractions with its content in the parent soil, it was, on average, 1.4 times higher in the finest fractions of the wind-eroded sediment (median diameters between 8 and 18 µm) than in the parent soil. In contrast, the coarsest fractions (median diameters between 29 and 58 µm) had glyphosate contents that were, on average, 1.2 times lower than that in the parent soil. Only the samples entirely composed of macroscopic aggregates (median diameter of 715 µm) matched the glyphosate content of the parent soil, confirming once again that the large aggregates are perfect compositions of the original soil in the sediment tray. Clymo et al.

(2005) also reported a much higher concentration of the herbicide pendimethalin in the $PM_{2.5}$ fraction when compared to their field soil, but not for the herbicide metolachlor. According to these authors, pendimethalin is less volatile than metolachlor and therefore, the former has a higher affinity to the particle phase while the latter has a higher affinity to the gas phase. Glyphosate is also non-volatile and tends to strongly adsorb to soil particles; therefore its preference to the particle phase is also expected.

3.3.2.2 Relationship between glyphosate or AMPA and clay, silt and OM

Figure 3.7 shows the results of the categorical principal components analysis performed to determine the relationship between the studied sediment properties (clay, silt and OM) and glyphosate and AMPA content. The proportion of variance-accounted-for by the first component is 61.1%, whereas the second component accounts for 28.1%. Thus, the two components together account for a considerable proportion (89.2%) of the variance. All sediment properties analyzed in this study loaded in the first component together with glyphosate and AMPA, whereas only the duration of the experiment (days) loaded in the second component together with AMPA (Figure 3.7). The studied sediment properties do, therefore, play a major role in adsorbing glyphosate and AMPA. The duration of the experiment, on the other hand, was only meaningful for AMPA.





Component Loadings

	Component 1	Component 2
Days	,227	,988
Clay	,932	-,251
Silt	,818	-,264
OM	,805	-,236
Gly	,949	-,111
AMPA	,729	,715

Figure 3.7 *Categorical Principal Components Analysis (non-linear PCA). Gly – glyphosate; OM – organic matter.*

The order to which glyphosate and AMPA contents in the wind-eroded sediment are influenced by the studied sediment properties is as follows: clay > OM > silt (Figure 3.7). Glyphosate content correlates significantly and positively to the clay content (R^2=0.63,

$p<0.01$). For coarser soil fractions, such as silt, the relationship with glyphosate content is considerably less expressed ($R^2=0.27$) but still significant ($p<0.01$). Significantly positive correlations were also observed between AMPA content and clay ($R^2=0.16$, $p<0.01$), and AMPA content and silt ($R^2=0.10$, $p<0.01$) (Figure 3.7).

Organic matter also appears as a strong factor influencing glyphosate adsorption to wind-eroded sediment (Figure 3.7): glyphosate content correlates significantly and positively to the OM content ($R^2=0.49$, $p<0.01$). However, one should realize that a positive correlation between glyphosate content and OM would be observed anyway because both are a function of particle size (both are higher for smaller particles, see Figure 3.4). Therefore, the effect of OM on glyphosate adsorption cannot be confirmed with certainty.

In summary, these results show that the highest concentrations of glyphosate and AMPA in the finest fractions are related to the higher clay and OM content in these same fractions, although the role of silt cannot be ignored. Sprankle et al. (1975) also reported that glyphosate was readily adsorbed to clay and OM, and that less glyphosate was adsorbed by a sandy loam soil than by a clayey loam soil.

3.3.2.3 Glyphosate and AMPA content through time and consequences for their airborne off-site transport with dust

The fact that glyphosate and AMPA contents are highest in the fine fractions of the soil has important consequences for the airborne off-site transport of these compounds, because particles <20 µm have the capacity of being transported in long-term suspension. This can easily be shown by calculating the aeolian threshold for long-term suspension, which, according to the model of Pye and Tsoar (1990), is $u_\infty/u_* < 0.1$, where u_∞ is the terminal fall velocity and u_* the friction velocity. Using this criterion, 20-µm particles are already transported in long-term suspension when $u_* < 0.3$ m s^{-1}. Assuming a roughness length z_0 of 3-10 cm (typical value for agricultural areas, depending on the type of crop, see Ramli et al. (2009)), this corresponds to a 10-m height wind speed of 3.5-4.4 m s^{-1}, which are very typical values for many inland agricultural areas. For 10-µm particles, the critical wind speed is much lower: only 1.2-1.4 m s^{-1} (at 10 m height). At these wind speeds, particles are able to travel tens to even several hundreds of km before they settle back to the Earth's surface.

During the 4-week experiment, nearly no glyphosate decay took place (Figure 3.5a). Consequently, the formation of AMPA (Figure 3.5b) was very slow and remained low during the experimental period. Glyphosate and AMPA decay mostly by microbial activity (Bento et al., 2016; Gimsing et al., 2004b; Nomura and Hilton, 1977), and for the latter a minimum soil moisture is required (Bento et al., 2016; Schroll et al., 2006). In our study, the soil moisture content during storage, after applying glyphosate but before the 24-h drying process prior to each wind tunnel test, was 5.4%. This soil moisture content revealed to be very low to allow for soil microbial activity and consequent glyphosate decay. According to Bento et al. (2016), the decay of glyphosate and AMPA in a silty loam loess soil is already significantly slower at a soil moisture content of 8% (w/w; 20% of water holding capacity). Schroll et al. (2006) also showed that pesticide mineralization (using glyphosate) was nearly inexistent for soil moisture contents ≤2.6% (w/w; equivalent to the ≤-20 MPa reported by these authors). Very important in this context is that wind erosion of fine, dusty particles only occurs when the topsoil (and, therefore, also the particles themselves) is sufficiently dry. Nourzadeh et al. (2013) tested several types of loamy soils using a field wind tunnel and found that the maximum moisture content to allow wind erosion of these soils was only 2%, well below the limit for a substantial decay of glyphosate. Besides wind erosion, for many silty soils, tillage erosion is a second (and in many cases even more important) mechanism for emission of fine particulates. For tillage-emitted particles, the probability for off-site transport is also highest when the particles are dry. Since the decay of glyphosate in our study occurred already extremely slowly for a soil moisture content of 5.4%, its decay would be nearly inexistent for such dry wind-eroded sediment. Therefore, if glyphosate is applied during a dry period and emission of fine particles happens thereafter (either by wind erosion if the soil cover is still small, or by tillage activities if there is already some cover), then the potential for airborne glyphosate transport to off-site areas is considerable. In fact, Farenhorst et al. (2015), in a 2-year study where bulk deposition samples were collected and analyzed for several pesticides and some metabolites, showed that glyphosate and AMPA were the compounds detected at the highest concentrations. These authors also reported that glyphosate “accounted for 65% of the total pesticide deposition over the 2 years” and that its deposition was >5 times larger in the dryer year. Other studies also reported the occurrence of glyphosate and/or AMPA in the atmosphere (Messing et al., 2011; Humphries et al., 2005; Quaghebeur et al., 2004).

3.3.3 Potential contribution of glyphosate and/or AMPA contaminated airborne dust to human exposure

Figure 3.8 shows the reconstruction of the distribution of glyphosate in the original non-aggregated soil in the sediment tray before the start of each wind tunnel experiment. As expected, the glyphosate distribution was nearly identical for the six experimental runs, and it was predominantly concentrated in the finest fractions. On average for the six experimental runs, 13% of the glyphosate in the original soil was concentrated in the $PM_{2.5}$ fraction (particles <2.5 µm), 15% in the PM_4 fraction, and 28% in the PM_{10} fraction. It is currently unknown whether the distribution of glyphosate in Figure 3.8 also applies to the macroscopic aggregates, but because the aggregates are almost perfect compositions of the original soil in the sediment tray (see Figure 3.3b) the distribution of glyphosate within the aggregates is probably not far off from that shown in Figure 3.8. For AMPA, 14% was concentrated in the $PM_{2.5}$ fraction, 15% in the PM_4 fraction, and 29% in the PM_{10} fraction.

These results reconfirm that glyphosate and AMPA are considerably susceptible to be transported with airborne dust. After having accomplished their airborne transport trajectory, the glyphosate and/or AMPA containing soil particles will settle to the ground, thereby contaminating the deposition area. When the deposition is induced by rainfall and the particles and the soil become wet, glyphosate and/or AMPA will most probably further decay. When dry deposition occurs and the conditions remain dry for a while, glyphosate may remain in the deposited sediment until the soil becomes wet and the soil microorganisms active. But during their airborne transport, the particles may also become inhaled, especially the finest fractions (PM_{10} and smaller), which can penetrate deeply into the human respiratory system (Kim et al., 2015). Some of the smallest particles ($PM_{2.5}$) may even remain fixed in the lungs (Brauer et al., 2001).

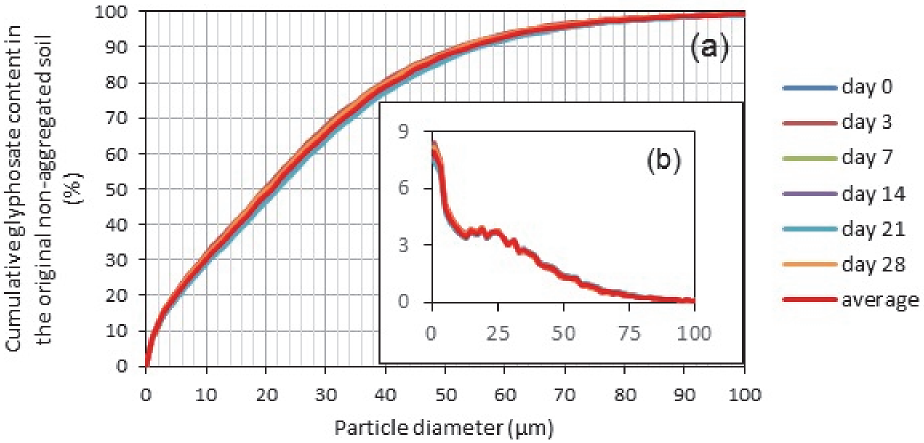


Figure 3.8 *Calculated cumulative (a) and non-cumulative (b) distribution of glyphosate in the original soil (after destruction of the aggregates) for the six experimental days.*

Inhalation of such fine particulates has already been associated to various health problems and around 2.1 million people worldwide are estimated to die every year from lung and respiratory system diseases caused by fine particulate matter (Kim et al., 2015; Shah et al., 2013). The risks to human health are expected to increase even more when these fine particulates are polluted with adsorbing chemicals. Bartoš et al. (2009), who assessed the airborne exposure health risks associated to several toxic compounds, reported that 83-94% of the cumulative cancer risk was due to particle-bound chemicals. To our knowledge, a human health risk assessment focusing on exposure to glyphosate and AMPA contaminated airborne particulates has not been done so far. As shown by our study, airborne particulates can be contaminated with very high concentrations of glyphosate and AMPA. According to Williams et al. (2000), the oral and dermal absorption of glyphosate and AMPA is low to very low, and they don't bioaccumulate. Any ingested glyphosate or AMPA is, therefore, expected to be eliminated unmetabolized through the urine or feces (Williams et al., 2000; Brewster et al., 1991). Nevertheless, in the review article of Williams et al. (2000), values of absorbed glyphosate in the body system of rats between 15% and 36% were reported, and absorbed percentages were dependent on the applied dose levels and on single or repetitive oral exposures. Brewster et al. (1991), who studied the tissue distribution of glyphosate in rats after a single oral dose, reported that significant (>1%) doses of administered glyphosate were detected in the small intestine, bone, colon and kidney. These authors also reported that >94% of the body burden was unmetabolized glyphosate and that only 1.2% of the administered dose of glyphosate was still remaining in some tissues after one week, mostly in the bone.

Glyphosate is reported as being harmless to humans or mammals mostly due to the absence of the shikimate pathway in animals (Helander et al., 2012; Brewster et al., 1991). However, studies have shown that glyphosate induced DNA damage (Mañas et al., 2009b), inhibited the activity of human serum enzymes (El-Demerdash et al., 2001), and that glyphosate exposure was related to Parkinson's disease (Gui et al., 2012; Wang et al., 2011; Barbosa et al., 2001). AMPA, on the other hand, has been shown to induce DNA damage and to produce chromosomal aberrations in human lymphocites (Mañas et al., 2009a). According to the IARC (2015), there are few studies on the effects of AMPA, but the existing ones all gave positive results as regards its genotoxicity. The IARC (2015) also reported that there is strong evidence that glyphosate and AMPA can induce oxidative stress. Inhalation of glyphosate is normally disregarded and considered a minor route of exposure in humans due to its low vapor pressure (IARC, 2015). Nevertheless, as shown in our study, very high concentrations of glyphosate may occur in airborne particulate matter that can be inhaled by humans and, thus, exposure through this route might have been underestimated in assessments done so far.

The considerations above show that there is sufficient reason to take off-site airborne transport of glyphosate and/or AMPA contaminated soil fractions seriously, especially during sufficiently long periods of drought.

3.4 Conclusions

Our study indicates that glyphosate and AMPA contents are highest in sediment particles <10 µm (PM_{10}), and that their content diminishes with increasing particle size. The risk of off-site airborne transport of glyphosate and AMPA with dust is, therefore, very high. Because glyphosate and AMPA hardly decay under dry conditions of the soil, this risk is intensified if glyphosate is applied in arid and semi-arid areas or during long periods of drought. If glyphosate and AMPA contaminated PM_{10} fractions of soil are emitted to the atmosphere, they may be inhaled by humans and animals. This contributes to the risk of human and animal exposure and, therefore, more attention should be paid to this route of exposure in environmental and human health risk assessment studies. Moreover, glyphosate applications during dry periods in regions susceptible to wind erosion should be avoided.

Acknowledgements

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

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.

Based on:

Bento, C. P. M., Commelin, M. C., Baartman, J. E., Yang, X., Peters, P., Mol, H. G., Ritsema, C. J. & Geissen, V. 2018. Spatial glyphosate and AMPA redistribution on the soil surface driven by sediment transport processes – A flume experiment. Environmental Pollution. 234, 1011-1020.

4.1 Introduction

Glyphosate (N-phosphonomethylglycine), the active ingredient of many commercial herbicide formulations, is used worldwide for weed control (Dill et al., 2010; Borggaard and Gimsing, 2008). It is one of the most used herbicides in agriculture, particularly in combination with glyphosate-resistant (GR) 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 (Al-Rajab et al., 2008; Sprankle et al., 1975). Although it is also very soluble in water (10.5 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 behavior 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 (Shakesby et al., 2015; Palis et al., 1997).

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 (Bento et al., 2017; Haase et al., 2007). Large areas of loess soils are used to grow GR crops, where huge amounts of glyphosate-based herbicides are applied every year (Benbrook, 2016; Bento et al., 2016; Clive, 2014; Aparicio et al., 2013). 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 (Baartman et al., 2013; Fryirs, 2013; de Vente et al., 2007; Parsons et al., 2006). 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 et al., 2015; Bracken and Croke, 2007) 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 (Appels et al., 2011; Darboux et al., 2002).

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.

4.2 Materials and Methods

4.2.1 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 4.2.3, and it was found free of glyphosate and AMPA.

4.2.2 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; Figure 4.1A) 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 GR crops under non-till systems (e.g. the Pampas of Argentina) (Bento et al., 2016; Aparicio et al., 2013).

4.2.2.1 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 Figure 4.1B), and a SS with micro-topographic disturbances that resemble seeding lines on the contour (T2; see Figure 4.1C). 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 GR 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.

4.2.2.2 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 homogenized 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.

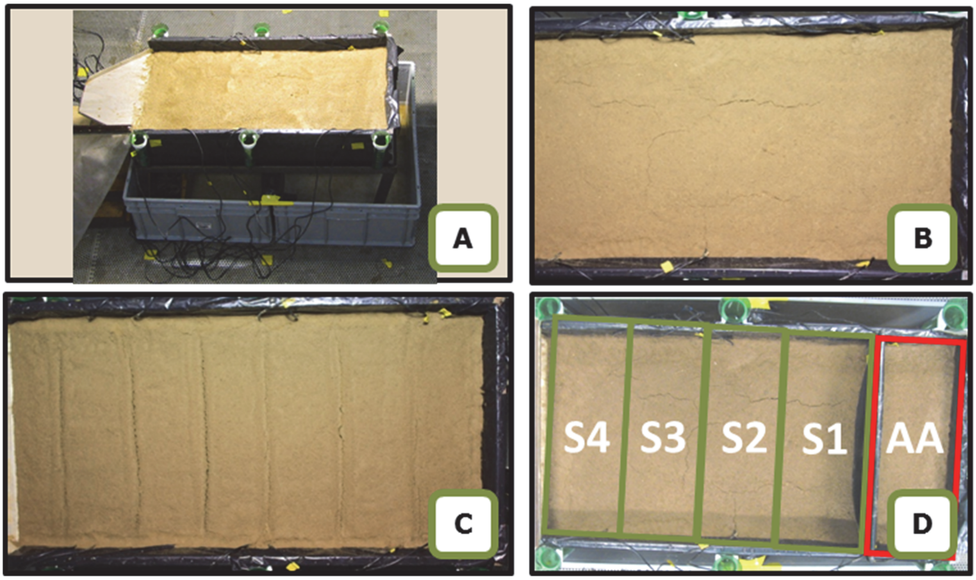


Figure 4.1 *A. Soil flume; B. Treatment T1 – Smooth surface; C. Treatment T2 – "Seeding lines"; D. Glyphosate application area (AA; red) and sampling segments (S1-S4; green).*

4.2.2.3 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 Figure 4.1D) by a licensed 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 1h 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 Figure 4.1A).

4.2.2.4 Sample collection

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.

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, Figure 4.1D); the other four segments correspond to the downslope non-contaminated areas of the flume (S1-S4; green areas, Figure 4.1D). Each segment was 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 color; 2) DS – dark sedimentation: deposition of material characterized by a dark brown color; 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.

Sediment properties

Because the quantity of sediments (from both the runoff and the SS) was not enough to analyze 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 4.2.2.3, but without applying glyphosate. Samples from each SS group and from suspended sediment were collected the same way, and analyzed for their OM content and particle size distribution as described by Bento et al. (2017).

4.2.2.5 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 analyzed using the software eCognition 5.0, and classified according to the SS groups described in section 4.2.2.4 (downslope off-site deposition), 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 centimeters. This was performed in ImageJ 1.51f, and a pixel:cm relation of about 37:1 was found for all images.

4.2.3 Glyphosate and AMPA analysis

Glyphosate and AMPA contents of the samples were analyzed 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 derivatization step was carried out using FMOC-Cl (9-fluorenylmethoxycarbonyl chloride; Sigma-Aldrich, Switzerland). For the runoff water samples, the derivatization 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).

4.2.4 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 24h 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.

4.2.5 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 4.2.2.5), 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 (Figure 4.2) were analyzed 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 (Figure 4.3), 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 4.1), 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 4.2), T-tests were performed. To test for significant differences between rainfall events for the SM contents (Table 4.1), 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 4.3) was assessed by performing linear regression and Pearson correlations.

4.3 Results

4.3.1 Rainfall characteristics and SM conditions

Rainfall characteristics were similar between treatments for all 15-min REs (Table 4.1). No significant differences were observed for SM contents between treatments, in both SM depths and for all REs (Table 4.1). 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.

Table 4.1 *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.

4.3.2 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 (Figure 4.2A-C). For T1, a significant increase in runoff water was observed between RE15 and RE30, reaching an almost steady-state afterwards (Figure 4.2A). For the suspended sediment (Figure 4.2B), 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 (Figure 4.2C). 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 Figure 4.2A-C.

4.3.3 Off-site transport of glyphosate and AMPA with runoff

4.3.3.1 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} (Figure 4.2D). 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}; Figure 4.2D). 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 (Figure 4.2D). The PAG transported with runoff water (Figure 4.2G) 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.

4.3.3.2 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} (Figure 4.2E), while AMPA contents varied between 0.04 and 0.49 mg kg^{-1} (Figure 4.2F). 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}) (Figure 4.2-F). Glyphosate and AMPA contents were also higher for T1 than for T2 for all REs (Figure 4.2E-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%) (Figure 4.2H). In fact, although the concentrations of glyphosate in runoff water were low (≤0.08 mg L^{-1}; see Figure 4.2D), the total percentage of glyphosate transported with runoff water (<3%; see Figure 4.2G) was almost double of that transported with suspended sediment for both treatments (<1.7%; see Figure 4.2H). 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) (Figure 4.2I). The transport of both compounds was, though, always higher for T1 than for T2 for all REs (Figure 4.2H-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.

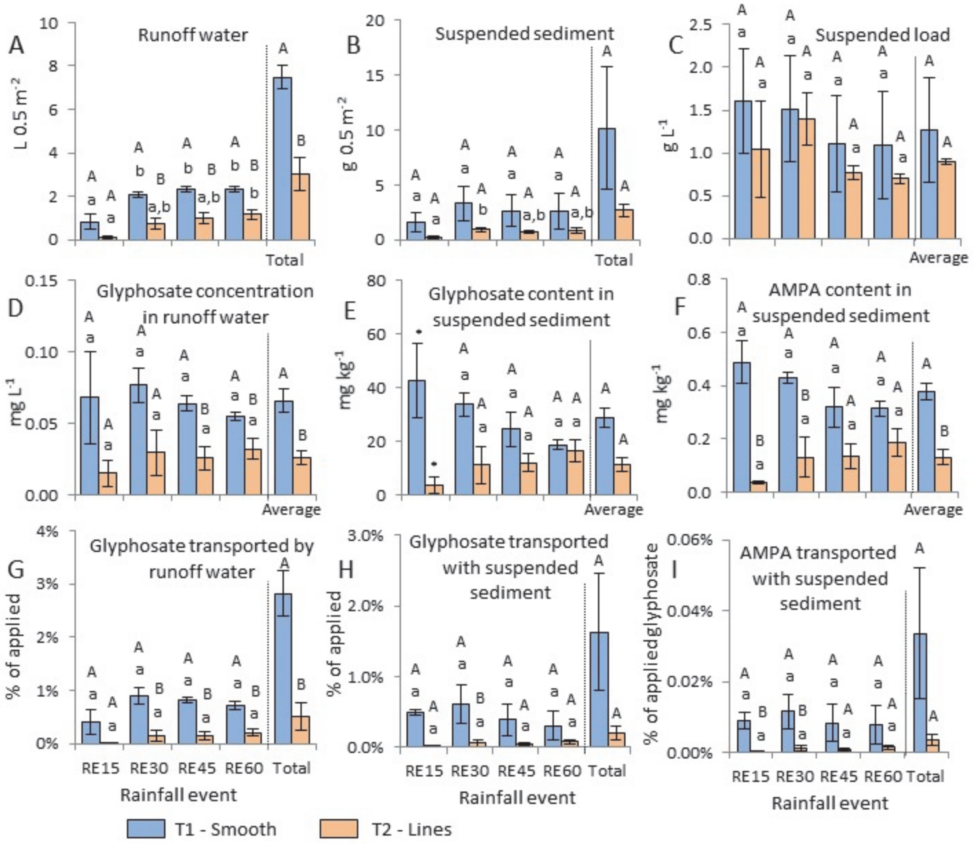


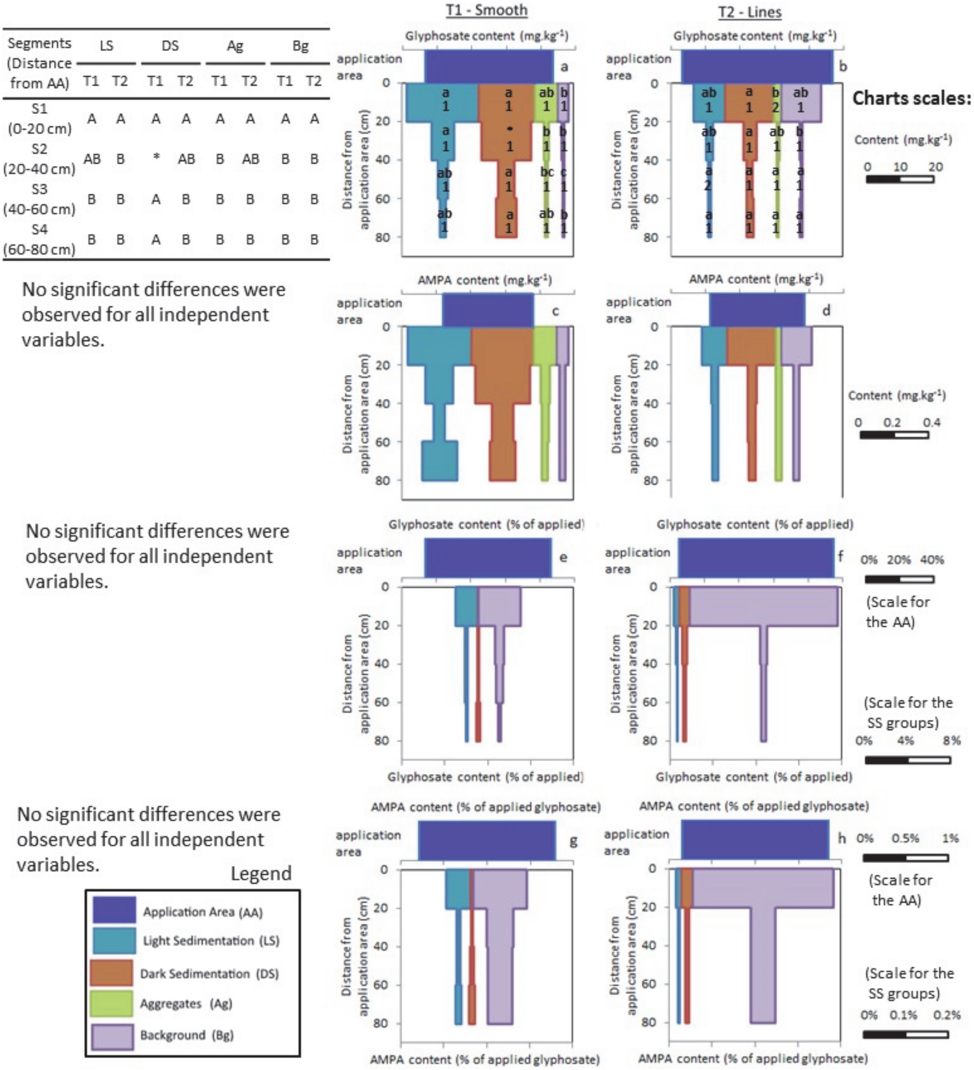
Figure 4.2 *Runoff water (A), suspended sediment (B), suspended load concentration (C), glyphosate content and amount transported with runoff water (D, G), and glyphosate and AMPA contents and amounts transported with suspended sediment (E-F, H-I), for each 15-min rain event (RE) and for the total rain, 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.*

4.3.4 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}) (Figure 4.3a-b). However, low contents were observed for AMPA (0.53±0.05 mg kg^{-1} for both treatments) (Figure 4.3c-d). Downslope the AA, the highest glyphosate and AMPA contents were detected in segment S1 for all SS groups, in both treatments (Figure 4.3a-d). Glyphosate contents reduced significantly thereafter by 8 to 28 times between segments

S1 and S4 (Figure 4.3a-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 (Figure 4.3c). 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) (Figure 4.3c-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) (Figure 4.3a-b). In general, glyphosate contents decreased in the order DS>LS>Ag≥Bg for both treatments and for all segments (Figure 4.3a-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 (Figure 4.3c). 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 (Figure 4.3d).

As regards the PAG, most of the recovered glyphosate and AMPA remained in the AA in both treatments (Figure 4.3e-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%) (Figure 4.3e-h; Table 4.2). Similar to what was observed for glyphosate and AMPA contents (Figure 4.3a-d), glyphosate and AMPA amounts found downslope of the AA were highest in segment S1, and strongly reduced thereafter (Figure 4.3e-h). Although the DS group presented the highest glyphosate and AMPA contents for most of the segments in both treatments (Figure 4.3a-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%) (Figure 4.3e-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%) (Figure 4.3e-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 4.2.2.5, the PAG in the aggregates could not be determined.



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 4.3 *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 S4.1 in the supplementary material.*

4.3.5 Mass balance of glyphosate and AMPA distribution at the end of the experiment

According to Table 4.2, >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 decay to other metabolites. 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 4.2). 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 4.2. 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 4.2). The particle-bound transport of glyphosate and AMPA dominated over the water-dissolved transport (Table 4.2). Nevertheless, this is mostly due to the glyphosate and AMPA transported to the downslope SS areas. As referred in section 4.3.3.2, a higher amount of glyphosate was transported dissolved in the runoff water than in the particle-bound transport with suspended sediment (see Figure 4.2G-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 4.2).

Table 4.2 *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}

4.3.6 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 4.3A). 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 4.3A). The LS group, on the other hand, was composed by the lowest contents of clay, silt and OM and the highest sand content (Table 4.3A). 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 4.3B). 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 Figure 4.2E-F and Figure 4.3a-d), particularly for the suspended sediment which presented the highest glyphosate and AMPA contents from all sediment groups analyzed (see Figure 4.2E-F).

Table 4.3 *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 ($p<0.05$).

4.4 Discussion

4.4.1 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 (Daouk et al., 2013; Coupe et al., 2012; Birch et al., 2011; Gregoire et al., 2010; Warnemuende et al., 2007; Siimes et al., 2006). Only recently, more attention has been paid to their particle-bound transport with runoff (Melland et al., 2016; Napoli et al., 2016; Yang et al., 2015a; Yang et al., 2015b). 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 (Shakesby et al., 2015; Polyakov and Lal, 2004; Palis et al., 1997). Likewise, the high glyphosate and AMPA contents linked with OM and clay minerals have also been suggested and/or proven by other studies (Bento et al., 2017; Sprankle et al., 1975).

4.4.2 Off-site deposition of glyphosate and AMPA

Although various studies have assessed the off-target transport of glyphosate and AMPA during rainfall events (Melland et al., 2016; Sasal et al., 2015; Daouk et al., 2013; Peruzzo et al., 2008), 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 (Sørensen et al., 2006; Piccolo et al., 1994). Piccolo et al. (1994) also suggested that “glyphosate adsorption on soils is far from being permanent”.

4.4.3 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.

4.5 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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Supplementary Material

Table S4.1 *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).

5. Dynamics of glyphosate and AMPA in the soil surface layer of glyphosate-resistant crop cultivations in the loess Pampas of Argentina

This study investigates the dynamics of glyphosate and AMPA in the soil surface layer of two fields growing glyphosate-resistant (GR) crops in the loess Pampas of Córdoba Province, Argentina. Glyphosate decay and AMPA formation/decay were studied after a single application, using decay kinetic models. Furthermore, glyphosate and AMPA concentrations were investigated in runoff to evaluate their off-site risk. During a 2.5-month study, cultivations of GR soybean and maize received an application of 1.0 and 0.81 kg a.e. ha^{-1}, respectively, of Roundup UltraMax©. Topsoil samples (0-1, 1-2 cm) were collected weekly (including before application) and analyzed for glyphosate, AMPA and soil moisture (SM) contents. Runoff was collected from runoff plots (3 m^2) and weirs after 2 erosive rainfall events, and analyzed for glyphosate and AMPA contents (water, eroded-sediment). Under both cultivations, background residues in soil before application were 0.27-0.42 mg kg^{-1} for glyphosate and 1.3-1.7 mg kg^{-1} for AMPA. In the soybean area, the single-first-order (SFO) model performed best for glyphosate decay. In the maize area, the bi-phasic Hockey-Stick (HS) model performed best for glyphosate decay, due to an abrupt change in SM regimes after high rainfall. Glyphosate half-life and DT$_{90}$ were 6.0 and 19.8 days, respectively, in the soybean area, and 11.1 and 15.4 days, respectively, in the maize area. In the soybean area, 24% of the glyphosate was degraded to AMPA. In the maize area, it was only 5%. AMPA half-life and DT$_{90}$ were 54.7 and 182 days, respectively, in the soybean area, and 71.0 and 236 days, respectively, in the maize area. Glyphosate and AMPA contents were 1.1-17.5 times higher in water-eroded sediment than in soil. We conclude that AMPA persists and may accumulate in soil, whereas both glyphosate and AMPA are prone to off-site transport with water erosion, representing a contamination risk for surface waters and adjacent fields.

Based on:

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5.1 Introduction

The use of glyphosate-based herbicides in Argentina has increased dramatically since the introduction of glyphosate-resistant (GR) crops in 1996, being the most used pesticide in the country (Aparicio et al., 2013; Trigo, 2011). By 2012, 197 000 tons of glyphosate-based herbicides were applied, approximately 10 times more than in 1996, mostly in chemical fallows (47%), soybean cultivations (42%), and maize cultivations (9%) (KleffmannGroup, 2014; Trigo, 2011). Nevertheless, only recently more attention is being paid to the impacts and fate of glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) in the country.

Glyphosate is considered to decay fast in soil, with some reported half-lives <5 days and DT_{90}<10 days (Bento et al., 2016; Yang et al., 2015a; Al-Rajab and Schiavon, 2010). However, glyphosate was also found to persist in soil for longer periods of time, namely when adverse soil/climate conditions are present (e.g. dry soil, low temperatures) (Bento et al., 2016; Schroll et al., 2006; Heinonen-Tanski, 1989), or in soils with strong adsorption capacity (Al-Rajab and Schiavon, 2010). Some studies reported a bi-phasic decay behavior for glyphosate, i.e. a fast initial decay rate – associated to its mobile phase available in the soil solution –, followed by a slower decay rate – associated to its strongly bound phase, which is not readily available for microorganisms (Bento et al., 2016; Al-Rajab and Schiavon, 2010; Eberbach, 1998). AMPA displays similar behavior in terms of sorption and degradation (Bento et al., 2016; Simonsen et al., 2008; Feng and Thompson, 1990). However, AMPA is found to have longer half-lives than its parent (Bento et al., 2016; Yang et al., 2015a; Simonsen et al., 2008). Considering that glyphosate and AMPA may persist in soil for longer periods than normally reported and the high glyphosate application frequency and rates in agricultural fields (Benbrook, 2016), there is a risk of continuously detecting considerable amounts of these compounds in the topsoil, or even of their accumulation over the years.

Several authors stated that glyphosate and AMPA are unlikely to reach areas outside the targeted agricultural fields, mostly due to their strong capacity to adsorb to soil particles (Al-Rajab et al., 2008; Duke and Powles, 2008; Kjaer et al., 2003; Feng and Thompson, 1990). However, glyphosate and AMPA contents remaining in the topsoil are prone to be transported to off-target areas during water erosion events (Yang et al., 2015a; Yang et al., 2015b; Todorovic et al., 2014). Shipitalo et al. (2008), for example, reported glyphosate and AMPA concentrations in runoff water up to 182 and 31 $\mu g\ L^{-1}$, respectively, in Appalachian watersheds (USA). Two studies in different parts of Buenos Aires province, in Argentina, reported the occurrence of glyphosate and AMPA in surface water, suspended sediment and sediment from stream beddings (Aparicio et al., 2013; Peruzzo et al., 2008). Considering that the loess Pampas of Argentina are prone to water erosion (Apezteguía et al., 2009), the off-site transport risk of glyphosate and AMPA with eroded soil is high. However, studies on their transport with water erosion in the country are still missing.

This study aims at investigating the dynamics of glyphosate and AMPA in the soil surface layer of fields cultivated with GR soybeans and GR maize, both under long-term application of glyphosate-based herbicides, in Córdoba province, Argentina. Focus is given to the role of decay processes of glyphosate and to the formation/decay of AMPA, taking into account the background contents as a consequence of long-term applications. Furthermore, the concentration of these compounds in runoff water and water-eroded sediment is studied to evaluate their off-site risks.

5.2 Materials and methods

5.2.1 Study site

5.2.1.1 General characteristics

This study was performed in a farmland of Córdoba province, Argentina, located in a Piedmont area where the Pampas begin to slope upwards to meet the Sierras Chicas. It belongs to the climate region of the dry Pampas, with a predominant temperate climate, characterized by hot and humid summers and cold and dry winters. Mean annual precipitation is 846 mm, with 80% of the rainfall occurring between October and March. The mean annual temperature is 16.7°C, with a mean annual minimum of 11.2°C (absolute minimum: -7.6°C) and mean annual maximum of 24.3°C (absolute maximum: 42.4°C). The soil type is Mollisols on loess. The agricultural areas under study are dedicated to the cultivation of GR soybean and maize for >15 years according to a soybean-maize rotation plan under non-tillage systems. Glyphosate-based herbicides are also applied for >15 years, since GR crops were adopted. For at least the last 5 years, glyphosate-based herbicides are applied 3-5 times a year with a ground boom sprayer, at application rates varying between 0.8 and 1.5 kg a.e. ha^{-1}.

5.2.1.2 Specific characteristics

This study was performed between 28-11-2014 and 10-2-2015 in two agricultural areas cultivated with GR soybean (*Glycine max* (L.) Merr.) and GR maize (*Zea mays* L.), respectively. Soybeans were cultivated in 220 ha with an average slope of 1.8%, while the maize was cultivated in 93 ha with an average slope of 1.7%. The soil was partially covered with stubble from the last harvested crop. The soybean was sown on 11/12-10-2014 and harvested in March 2015. The maize was sown between 28-11 and 1-12-2014 and harvested in July 2015. During sowing, nitrogen fertilizer was applied in the maize field. Soil varied from loam (soybean) to clayey loam (maize). Soil properties from both cultivation areas, and temperature and rainfall data during the study period are presented in supplementary material (Table S5.1; Figure S5.1).

Due to serious water erosion problems in the past, both cultivation areas have soil bunds to reduce soil erosion risks. The soil bunds (60-70 m apart) were built along the contour lines and are discharging into grass channels running through the lowest points in each cultivation area. These channels divide the cultivation areas into small catchments, whereas the soil bunds divide these catchments into sub-catchments. This study was performed in one of the small catchments per cultivation area. The soybean catchment is 35 ha, whereas the maize catchment is 50 ha.

5.2.1.3 Glyphosate application

Before the study period began, glyphosate was last applied on 12-10-2014 in the soybean area and on 30-10-2014 in the maize area. In both cases, the applied rate was 0.97 kg a.e. ha^{-1} of Roundup full® II (Monsanto, Argentina), mixed with other pesticides.

During the study period, glyphosate was applied once, using the granular commercial formulation Roundup UltraMax© (Monsanto, Argentina). The application rates were 1.0 and 0.81 kg a.e. ha^{-1} in the soybean (on 2-12-2014) and maize (on 26-12-2014) cultivations, respectively. The granular formulation was prepared in water with a commercial formulation of SpeedWet® (SpeedAgro, Argentina), an ethoxylated nonylphenol-based coadjuvant, and applied as a solution mixture with other pesticides.

5.2.2 Soil sampling for glyphosate decay and AMPA formation/decay

To determine the glyphosate and AMPA contents in the soil surface layer and to investigate their decay behavior, 6 composite soil samples (5:1) per cultivation area were collected weekly (before and after glyphosate application) in the defined catchments (see section 5.2.1.2). These composite samples were collected in 3 sub-catchments (delineated by the soil bunds): 3 at a depth of 0-1 cm and 3 at a depth of 1-2 cm. The latter were only collected from beginning of January 2015. Each composite sample per depth was collected from each sub-catchment. The samples were collected randomly from bare patches of the soil using a metal ring, put into plastic bags and stored at -20°C until analysis.

Soil moisture was also monitored weekly at 0-5 cm depth in each cultivation area to investigate its role on the decay rate of glyphosate and AMPA. For this, samples (in triplicate) were collected on the same days as those for glyphosate and AMPA, using a 100cc metal ring. It was determined by weight difference before and after drying at 105°C.

5.2.3 Off-site glyphosate and AMPA transport with runoff

To investigate the transport of glyphosate and AMPA with runoff during water erosion events, 3 plots and 3 weirs (sub-catchment scale) were installed in each cultivation area. The plots (Figure S5.2A; supplementary material) were 3x1 m with the outlet at the lowest point, connected to a 200-L barrel. They were delineated by metal sheets driven 10 cm deep into the soil and retaining a height of 20 cm above the soil surface. Each plot was installed in the middle of a sub-catchment (see section 5.2.1.2), halfway between the soil bunds. The weirs were located at the outlet of 3 of the sub-catchments, where the surface runoff discharges into the grass channel. Each weir (Figure S5.2B; supplementary material) consisted of a metal sheet of 140x10 cm, hammered 9 cm deep into the ground, with a crest of 1 cm above the soil surface. The spillway had a width of 120 cm and was enclosed by a 30 cm high metal sheet at either side, driven 10 cm into the soil. Runoff spilling over the crest was sampled by using two 20-cm wide collectors. Each collector was connected to a 200-L barrel by a PVC hose (diameter: 51 mm). Installation of the plots and weirs was finished on 28-11-2014 in the soybean area and on 9-12-2014 in the maize area.

Three erosion-inducing rainfall events occurred during the study period: on 6-01-2015 (69.7 mm), 12-01-2015 (54.7 mm) and 9-02-2015 (56.0 mm). However, due to miscommunication with the farmer, runoff samples of the events of January were collected together. Due to problems with the barrels during the rainfall events (some have floated, while others have overflowed), it was not possible to quantify the erosion rates and related glyphosate and AMPA transport. The runoff in each barrel was stirred until a homogenous mix was observed. Then, two 1-L bottles of sample were collected (one for OM content and another for glyphosate and AMPA analysis). At the laboratory, the eroded sediment were separated from the runoff water by filtration, using paper filters of 8-µm pore size. For OM, the eroded sediment were analyzed using the loss-by-ignition method. For glyphosate and AMPA analysis, the runoff water and eroded sediment samples were stored at -20°C until analysis.

5.2.4 Glyphosate and AMPA analysis

Glyphosate and AMPA were analyzed by HPLC-MS/MS, using an XBridge™ Shield RP C18 column 100x2.1 mm, 3.5 µm particle size and an Aquity UPLC I-Class coupled to a Micromass Ultima triple-quadrupole MS (Waters, The Netherlands). The extraction (with potassium hydroxide (KOH), at a 1:5 ratio) and derivatization (with FMOC-Cl (9-fluorenylmethoxycarbonyl chloride)) methods, chemicals used, as well as the method validation and quality control were as described by Yang et al. (2015b) and Bento et al. (2016). A sample batch was accepted when recoveries of the quality control were between 70-120%. The limits of quantification (LOQ) of the method for both glyphosate and AMPA in soil and in water are 0.05 mg kg^{-1} and 10 µg L^{-1}, respectively. The limits of detection (LOD) of the method for glyphosate and AMPA in soil are 0.02 and 0.03 mg kg^{-1}, respectively. The LODs of the method for glyphosate and AMPA in water are 5.8 and 8.4 µg L^{-1}, respectively.

5.2.5 Data analysis

To study the field decay kinetics of glyphosate and AMPA in soil, we used ModelMaker 4.0 (A.P. Benson). For this purpose, the data were converted to percentage of applied glyphosate. The guidance document of FOCUS (2006) was followed to determine and select the models that best describe glyphosate decay and AMPA formation/decay in the upper soil layer (0-1 cm) of each cultivation area. The day of glyphosate application was considered as time zero. No sampling was possible to be performed on the application dates. Therefore, the theoretical glyphosate content in the soil immediately after application was calculated by:

$$C_0 = C_p + \left(\frac{a \cdot 100}{d \cdot \rho_b}\right) \tag{5.1}$$

where C_0 is the initial glyphosate content (mg kg^{-1}); C_p is the glyphosate content measured prior to the application (mg kg^{-1}) assuming that no decay occurred between the last sampling date and the application date, a is the application rate of glyphosate (kg a.e. ha^{-1}), d is the soil sampling depth (m) and ρ_b is the soil bulk density (kg m^{-3}).

Four models were tested to describe the glyphosate decay kinetics: a single first-order model (SFO, eq. 5.2); and three bi-phasic models – the First-Order-Multi-Compartment model (FOMC, eq. 5.3), the Hockey-Stick model (HS, eqs. 5.4a and 5.4b) and the Double-First-Order-in-Parallel model (DFOP, eq. 5.5).

SFO: $C = C_0 \cdot e^{-k \cdot t}$ (5.2)

FOMC: $C = C_0 / (\frac{t}{\beta} + 1)^{\alpha}$ (5.3)

HS: $C = C_0 \cdot e^{-k_1 \cdot t}$ for $t \leq t_b$ (5.4a)

$C = C_0 \cdot e^{-k_1 \cdot t_b} \cdot e^{-k_2 \cdot (t - t_b)}$ for $t > t_b$ (5.4b)

DFOP: $C = C_0 \cdot (g \cdot e^{-k_1 \cdot t} + (1 - g) \cdot e^{-k_2 \cdot t})$ (5.5)

where C is the glyphosate content (%) at time t (days), C_0 is the glyphosate content (%) at t=0, k, k_1 and k_2 are decay rate constants (day^{-1}), α is a shape parameter determined by the coefficient of variation of rate constant values (-), β is a location parameter (-), t_b is the time (days) at which the rate constant changes from k_1 to k_2, and g (-) is the fraction of C_0 in the compartment with rate constant k_1.

To determine the formation/decay kinetics of AMPA, the SFO model was used. The AMPA formation kinetics was described by the fraction of glyphosate that decayed to AMPA (*ffA*). For each model, the glyphosate decay parameters were optimized first; C_0 of glyphosate was fixed to 100% in all models. Next, the AMPA formation and decay parameters were optimized. C_0 of AMPA was fixed to its content (in %) observed in each field at the last sampling date before glyphosate application; it was assumed that no decay occurred during this period. Then, both glyphosate and AMPA parameters were optimized together. For both fields, all four models were executed for glyphosate and the model with the best fit was selected based on the indicators recommended by FOCUS (2006) (visual assessment of curve fits and residual plots, χ^2 model errors, t-test). The half-life (DT_{50}) and DT_{90} of both compounds were calculated based on the selected best fit model, according to the specific equations presented by FOCUS (2006). Only the results obtained for the selected best model of glyphosate are presented in this study.

To test for significant differences ($p<0.05$) of glyphosate and AMPA contents in the soil between sampling dates per cultivation area and per soil depth, a one-way ANOVA followed by Tukey or Dunnett's T3 post-hoc tests was performed. For significant differences ($p<0.05$) of glyphosate and AMPA contents in the soil between soil depths per cultivation area and date, an independent-samples T-test was performed. For significant differences ($p<0.05$) of AMPA contents in the soil between cultivation areas per soil depth, an independent-samples T-test was performed. The datasets were verified for their normality and equality of variances, and an ln-transformation was performed whenever these assumptions were violated. Statistics were performed using SPSS 22. Results of the statistical tests can be consulted in supplementary material (Table S5.2), together with the glyphosate and AMPA contents (in mg kg^{-1}) in soil at both sampling depths per sampling date and per cultivation area.

5.3 Results

5.3.1 Temperature, rainfall and SM content

During the study period, the average temperature was 22.5±2.9°C (Figure S5.1). Total precipitation was 358.2 mm, with 81% of the rainfall occurring in January (54%) and the first 10 days of February (27%) (Figures 5.1-5.2, Figure S5.1). The SM content from both cultivation areas at 0-5 cm depth remained below the soil wilting point (soybean: 21% w/w; maize: 19% w/w) until 6-01-2015. The lowest SM content was measured on 6-01-2015, right before the first erosive rainfall event (Figures 5.1-5.2 Table S5.2). Thereafter, SM in the maize cultivation area increased 1.3 times, on average, being above soil wilting point and quite stable (Figure 5.2; Table S5.2). In the soybean field, the SM content showed alternating high and low values (Figures 5.1, Table S5.2).

5.3.2 Glyphosate decay and AMPA formation/decay

Glyphosate and AMPA were detected in all soil samples from both cultivation areas, before and after glyphosate application (Figures 5.1-5.2; Table S5.2). Although, in both cultivation areas, the last glyphosate application was about 2 months before the one applied during the study period, glyphosate and AMPA were still detected in the 0-1 cm soil layer in a range of 0.27-0.42 mg kg^{-1} for glyphosate and 1.3-1.7 mg kg^{-1} for AMPA (Figures 5.1-5.2; Table S5.2). No significant differences of glyphosate contents were obtained between the sampling dates before application (soybean: 28-11-2014; maize: 9 to 22-12-2014) and the last sampling date (9-2-2015) (Figures 5.1-5.2; Table S5.2). This indicates that, for a similar time period after glyphosate application, the glyphosate content decreased to comparable levels in both cultivation areas. Glyphosate and AMPA contents were always significantly higher at a soil depth of 0-1 cm compared to those at 1-2 cm soil depth (Figures 5.1-5.2; Table S5.2). AMPA contents in the soybean area were significantly higher than those in the maize area, for both soil depths.

Based on the glyphosate application rates, the expected average glyphosate content in the soil immediately after application (day 0) at a soil depth of 0-1 cm was 10.9 and 7.2 mg kg^{-1} in the soybean and maize cultivation areas, respectively. Comparing these with the glyphosate contents obtained on the first sampling date after application, a decrease of 45.0% (7 days after) in the soybean area, and of 48.6% (11 days after) in the maize area was observed (Figures 5.1-5.2; Table S5.2). Nevertheless, a high variation on glyphosate content in the first sampling date after application was observed in the soybean area (Figure 5.1). This is likely a consequence of the variability on litter cover and crop growth observed in this cultivation area, which certainly affected the glyphosate reaching the soil during its application.

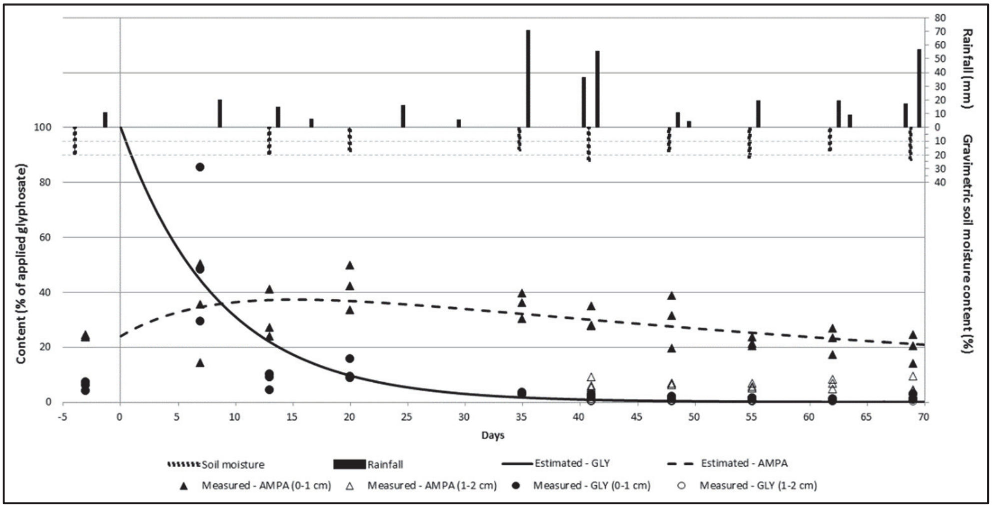

Figure 5.1 *Glyphosate and AMPA residues at 0-1 and 1-2 cm soil depth, their decay behavior in the top 0-1 cm soil, and rainfall and soil moisture contents under GR soybean. Day of glyphosate application (t = 0) was 2-12-2014. AMPA results were converted to equivalent mass of glyphosate.*

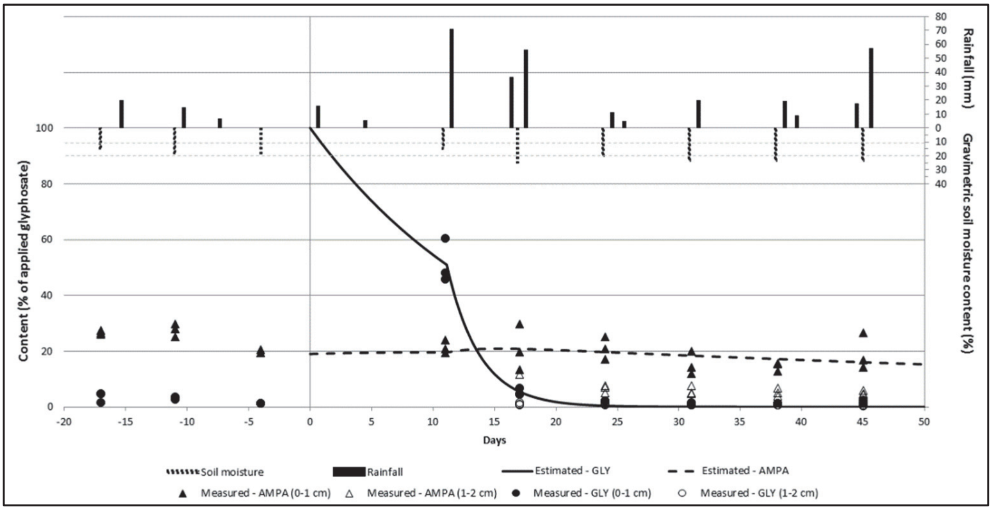

Figure 5.2 *Glyphosate and AMPA residues at 0-1 and 1-2 cm soil depth, their decay behaviour in the top 0-1 cm soil, and rainfall and soil moisture contents under GR maize. Day of glyphosate application (t = 0) was 26-12-2014. AMPA results were converted to equivalent mass of glyphosate.*

Soybean cultivation area

The SFO model resulted in the best fit for the decay of glyphosate in the soybean area (Figure 5.1). However, although this model presented the lowest χ^2 error value describing the glyphosate decay, it was still very high (47.2%; Table 5.1). Therefore, the simulated glyphosate behavior only represents a tendency. The high χ^2 error is the result of the great variation of measured glyphosate contents between replicates on the first sampling date, and the underestimation by the model of the latter sampling values. The glyphosate DT_{50} and DT_{90} estimated by this model were 6.0 and 19.8 days, respectively.

AMPA initial content on the day of application was fixed at 24% of the applied glyphosate, which corresponds to its last measured content in soil prior to glyphosate application. The SFO model fit for AMPA was well described, with a χ^2 error below the 15% threshold (Table 5.1). According to the model estimations, only 24% of the applied glyphosate degraded to AMPA (Table 5.1), and its decay occurred quite slowly during the study period (Figure 5.1, Table 5.1). As a result, AMPA DT_{50} and DT_{90} estimated by the model were 54.7 and 182 days, respectively.

Table 5.1 *Decay parameters of glyphosate using the SFO and HS model for the soybean and maize field, respectively, and the formation and decay parameters of AMPA using the SFO model.*

	Glyphosate following SFO or HS				AMPA following SFO[a]		
Field	k ± SE (SFO) (day^{-1})	k_1 ± SE (HS)	k_2 ± SE (HS)	χ^2 error (%)	ffA ± SE -	k ± SE (day^{-1})	χ^2 error (%)
Soybean	0.12 ± 0.02*	-	-	47.2[b]	0.24 ± 0.09*	0.013 ± 0.006*	9.0
Maize	-	0.06 ± 0.004*	0.37 ± 0.07*	9.7	0.05 ± 0.04#	0.010 ± 0.006#	12.4

k, k_1 and k_2 - decay rates; *ffA* – formation fraction of AMPA.

[a] AMPA results were converted to equivalent mass of glyphosate.

[b] The χ^2 error is above the acceptable threshold of 15%.

* Estimated parameter is significantly different from zero (*t*-test; p<0.05).

\# Estimated parameter is significantly different from zero (*t*-test; p<0.1).

Maize cultivation area

In the maize area, the decay of glyphosate showed a particular behavior. The HS model, with a decay rate change (t_b) occurring 11 days after glyphosate application, provided the best fit (Figure 5.2). In the 11 days between the application day and the first sampling, the glyphosate content decreased slowly (k_1 = 0.06 day^{-1}), compared to the rapid decay observed in the subsequent period (k_2 = 0.37 $days^{-1}$) (Table 5.1, Figure 5.2). This change coincided with the start of a period of heavy rainfall, beginning in the evening of 6-01-2015, which in turn marked a shift in the SM regime observed in this cultivation area (Figure 5.2). The results from the t-test for the HS parameters and the χ^2 error were satisfactory (Table 5.1). The glyphosate DT_{50} and DT_{90} estimated by this model were 11.1 and 15.4 days, respectively.

The initial AMPA content was fixed at 19% of the applied glyphosate, corresponding to its last measured concentration in soil prior to glyphosate application. The SFO model performed reasonably well for AMPA, with a χ^2 error below the 15% threshold and a good visual fit (Table 5.1, Figure 5.2). The lack of perceived formation and decay of AMPA is noticeable, with a model estimation of only 5% of the applied glyphosate degrading into AMPA. The AMPA DT_{50} and DT_{90} estimated by the model were 71.0 and 236 days, respectively. Notwithstanding, these values need to be interpreted with care since the t-test of the estimated parameters were only significant at $p<0.1$.

Comparison between both cultivation areas

When comparing both cultivation areas, we see that glyphosate DT_{50} is nearly twice as long in the maize area than in the soybean area. Indeed, the decay rate described by k_1 (HS; maize) is half of that described by k (SFO; soybean) (Table 5.1). However, the second phase decay rate described by k_2 (HS model; Table 5.1) in the maize area is 3 times higher than that of the soybean area. Although a longer DT_{50} for glyphosate was observed in the maize area, its persistence was lower than in the soybean area (i.e., a shorter DT_{90} in the maize area). This lower persistence in the maize area was due to the faster glyphosate decay induced by heavy rainfall and higher SM content 11 days after glyphosate application. According to the models results, the degradation of glyphosate to AMPA was nearly 5 times higher in the soybean area than in the maize area (Table 5.1). The decay of AMPA, however, was similar in both cultivation areas (Table 5.1), although AMPA persisted longer in the maize area.

5.3.3 Glyphosate and AMPA contents in eroded sediment and runoff water

Although not all plots and weirs yielded runoff during the water erosion events, glyphosate and AMPA were detected in all collected sediment samples (Table 5.2). The highest glyphosate and AMPA contents were detected in the maize cultivation area during the water-erosive events of 6/12-01-2015 (11-17 days after glyphosate application), with ranges between 4.2 and 10.9 mg kg^{-1} (Table 5.2). These contents were 1.1 to 8.3 times higher than those obtained in the parent soil at the same time (Table 5.2). In fact, glyphosate and AMPA contents were always higher in the eroded sediment than in the parent soil, except for AMPA in the plots of the soybean area (Table 5.2). The highest sediment/soil ratios were obtained for glyphosate (maize: 16.4; soybean: 17.5) at the sub-catchment scale (weirs) in the erosive rainfall event of 9-02-2015, which occurred 45 (maize) and 69 (soybean) days after glyphosate application (Table 5.2). This occurred despite the already relatively low glyphosate content observed in the soil surface layer (0-1 cm) when this rainfall event took place (Table 5.2). In nearly all cases, the sediment/soil ratios were higher at the sub-catchment scale than at the plot scale (Table 5.2). The transported OM with eroded sediment was unrelated with the glyphosate and AMPA contents (linear regression: $R^2_{glyphosate} = 0.08$; $R^2_{AMPA} = 0.15$).

Table 5.2 *Glyphosate and AMPA contents (average±SD) in eroded sediment and runoff water collected from the soybean and maize cultivation areas after water-erosive events, and corresponding sediment:soil ratios. Soil values refer to the last sampling date before each water-erosive event (see Table S5.2.).*

Code	**OM**	**Glyphosate**				**AMPA**			
	(sed) (%)	Soil ($\mathrm{mg\ kg^{-1}}$)	Sed	Sed/soil ratio -	Runoff water ($\mathrm{\mu g\ L^{-1}}$)	Soil ($\mathrm{mg\ kg^{-1}}$)	Sed	Sed/soil ratio -	Runoff water ($\mathrm{\mu g\ L^{-1}}$)
***SP35-41 (n=3)**	2.0 ± 0.7	0.36 ± 0.05	1.4 ± 0.7	3.9	< LOD	2.5 ± 0.3	2.2 ± 0.1	0.88	< LOD
***SW35-41 (n=0)**	-		-	-	-		-	-	-
***MP11-17 (n=2)**	2.3 ± 0.2	3.7 ± 0.6	4.2 ± 0.3	1.1	< LOD	1.0 ± 0.1	8.3 ± 2.7	8.3	< LOD
***MW11-17 (n=3)**	2.6 ± 1.8		10.9 ± 6.7	2.9	35.4 ± 27.5		4.7 ± 1.0	4.7	11.3 ± 4.4
#SP69 (n=2)	7.2 ± 6.9	0.21 ± 0.11	1.3 ± 1.5	6.1	22.8 ± 22.8	1.4 ± 0.4	1.1 ± 0.8	0.77	< LOD
#SW69 (n=1)	4.2 ± -		3.7 ± -	17.5	23.4 ± -		2.0 ± -	1.4	< LOD
#MP45 (n=1)	3.4 ± -	0.13 ± 0.05	0.54 ± -	4.2	< LOD	0.91 ± 0.31	2.0 ± -	2.2	< LOD
#MW45 (n=2)	2.5 ± 1.6		2.1 ± 1.0	16.4	< LOD		2.6 ± 0.6	2.8	< LOD

S (soybean field); M (maize field); P (plot); W (weir – sub-catchment scale); dd (days after application); Sed (eroded sediment); OM (Organic matter content of the eroded sediment); * rainfall events of 06-01 & 12-01-2015; # rainfall event of 09-02-2015

Our results show that glyphosate was only detected in 3 runoff water samples and AMPA in only 1 (Table 5.2). This indicates the higher affinity of these compounds to be transported adsorbed to eroded sediment. The maximum glyphosate and AMPA concentrations detected were 35.4 and 11.3 µg L^{-1}, respectively. In the maize area, glyphosate and AMPA were only detected in the runoff water at the sub-catchment scale and for the erosive rainfall events of January 2015 (Table 5.2). In the soybean area, glyphosate was detected at the plot and sub-catchment scales, but only in the runoff water from the erosive rainfall event of 9-02-2015 (Table 5.2). AMPA was not detected in runoff water for all erosive rainfall events occurring in the soybean area (Table 5.2).

5.4 Discussion

Studies pertaining the environmental fate of glyphosate and AMPA in Argentina have focused mostly on their occurrence in soil and surface waters (Pérez et al., 2017; Primost et al., 2017; Ronco et al., 2016; Lupi et al., 2015; Aparicio et al., 2013; Peruzzo et al., 2008). No field studies under real-world agricultural management activities were found on the dynamics of glyphosate and AMPA in the soil surface layer, in which the role of their decay processes and loss by runoff were both investigated. In fact, there were no studies looking into these processes individually in Argentina.

5.4.1 Glyphosate dynamics in the soil surface layer of agribusiness fields

Despite the great geographical proximity (approx. 1 km apart) between the two cultivation areas studied here, differences on glyphosate decay and persistence were observed. Since glyphosate and AMPA decay mostly by microbial activity (Bento et al., 2016), factors such as temperature, SM and soil properties strongly influence the microbial breakdown (Bento et al., 2016; Schroll et al., 2006; Eberbach, 1998). Consequently, the persistence of glyphosate and AMPA varies greatly between, as well as within, geographical areas (Silva et al., 2018; EU, 2002).

As shown in our study, glyphosate followed a first-order decay kinetic model (SFO) in the soybean area, whereas it followed a bi-phasic decay kinetic model (HS) in the maize area. However, as referred in section 5.3.2 for the soybean area, the SFO fit shows only a tendency due to the uncertainty resulting from the very high χ^2 error. The consistent model underestimation observed for the soybean area in the later stages of the decay process suggests that a bi-phasic model should have been applied instead. This has also been suggested elsewhere (Bento et al., 2016; Eberbach, 1998). However, higher χ^2 errors for the bi-phasic models were obtained. Moreover, the estimated parameters for the bi-phasic models were not significantly different from zero by the t-test ($p<0.05$), as it is required. Therefore, the SFO model was still the best fit for the soybean area.

In the maize area, the decay rate was affected by an abrupt change in the SM regime after heavy rainfall. Although the same amount of rain occurred in both cultivation areas, this shift from dry to wet conditions in a steady basis was only observed in the maize area, as opposed to the alternating low and high SM contents observed in the soybean area. We could not find a plausible explanation for this behavioral difference. This shift from a dry to a wet phase in the maize area resulted in an increase of the glyphosate decay rate by a factor of six. Only the HS model performed satisfactorily under these conditions. This model has also performed best in a study on glyphosate decay in water (Maqueda et al., 2017), as well as in another study on the decay of the herbicide isoproturon in soil (Díez and Barrado, 2010). In these studies, however, the HS model was applied similarly to other bi-phasic models, i.e. to represent an initial rapid decay followed by a lower decay rate. The reverse, as observed in our study, was not found in any other study.

The DT_{50} of 6.0 days in the loam loess soil of the soybean area was comparable to that of 6-9 days obtained under laboratory conditions in other agricultural soils (Simonsen et al., 2008; Eberbach, 1998). However, it was longer than the 3.5 days found in a Chinese loess soil under field conditions (Yang et al., 2015a). In the maize area, which was a clayey loam loess soil, the DT_{50} was 11.1 days. As referred previously, the HS model is bi-phasic, using two decay rates (k_1, k_2). If we consider each phase separately, we could calculate the theoretical half-life values to be 11.5 days if dry conditions would remain, which is only slightly longer than the actual estimated DT_{50}, and 1.9 days if wet conditions would have been present from the start. Schroll et al. (2006) studied the effect of SM on the decay of glyphosate, and found a linear (positive) correlation between SM and glyphosate decay. A similar correlation was found by Bento et al. (2016). Therefore, the distinct change in the decay rates in the maize area can largely be attributed to the increase in SM after the heavy rainfall event of 6-01-2015. Additionally, the occurrence of erosive rainfall events also contributed to the loss of glyphosate and AMPA from the soil surface layer through surface transport with runoff. Leaching is believed to have had a minor contribution on glyphosate and AMPA losses from the soil surface layer, since their contents were significantly lower at 1-2 cm soil depth compared to those at 0-1 cm soil depth. Moreover, glyphosate contents were relatively low (close to LOQ for most of the sampling periods) at 1-2 cm soil depth and quite stable over time. The high tendency for glyphosate to remain adsorbed to soil particles at the very topsoil surface layer and its low propensity to leach to deeper soil layers has also been proven elsewhere (Yang et al., 2015a; Yang et al., 2015b; Al-Rajab et al., 2008; Kjaer et al., 2003).

5.4.2 AMPA dynamics in the soil surface layer of agribusiness fields

Similar to glyphosate, differences on AMPA persistence were observed between the cultivation areas studied. This confirms that site-specific external factors (e.g. SM, soil properties, microbial diversity) can vary greatly, affecting the decay behavior and persistence of these compounds.

High AMPA background residues were found in the soil surface layer (0-1 cm) of both cultivation areas before glyphosate application (1.3-1.7 mg kg^{-1}). These high background residues suggest that AMPA is accumulating in soil. Aparicio et al. (2013) also found AMPA residues ranging between 0.30-2.3 mg kg^{-1} in cultivation areas of the province of Buenos Aires, in Argentina. Primost et al. (2017) reported much higher AMPA residues when compared to those in our study: an average AMPA content of 4.2 mg kg^{-1}, with a record world maximum reported of 38.9 mg kg^{-1}, in soils “under real-world agricultural management practices” (comparable to the management practices of our study site) of the Pampas of Entre Ríos province, Argentina. These authors also stated that, “under current practices, (glyphosate) application rates are higher than dissipation rates” and that glyphosate and AMPA are to be considered “pseudo-persistent” compounds (Primost et al., 2017). Our maximum AMPA background residues (1.7 mg kg^{-1}) are, however, slightly lower than the 1.9 mg kg^{-1} reported by Silva et al. (2018) for agricultural topsoils from the European Union.

The decay rate of AMPA is considerably lower than that of glyphosate, resulting in longer DT_{50} and DT_{90} in both cultivation areas. The longer persistence of AMPA compared to glyphosate has also been reported elsewhere (Bento et al., 2016; Yang et al., 2015a; Bergstroem et al., 2011). Notwithstanding, The DT_{50} of 54.7 and 71.0 days obtained in our study were larger than the 23-35 days found under field conditions in a Chinese loess soil (Yang et al., 2015a). A low decay rate suggests that AMPA may accumulate in soil and may persist in higher quantities than glyphosate. In our study, glyphosate peaked in the period shortly after application, decreasing significantly thereafter. AMPA, on the other hand, remained almost constant during the study period and at higher concentrations than glyphosate, suggesting that AMPA is accumulating in these cultivation areas. Nevertheless, the formation rate of AMPA was also low. According to the models results, only 24% of the decayed glyphosate appeared as AMPA in the soybean area, while in the maize area its formation was negligible. Bento et al. (2016) found higher formation rates of AMPA (37-48%) in a laboratory experiment with loess soil at 30°C. Besides degrading to AMPA, glyphosate has a second known degradation pathway to sarcosine (Borggaard and Gimsing, 2008). It is expected that, in this study, glyphosate degraded primarily to sarcosine, particularly in the maize area. If so, the high AMPA content in the soil of the maize area is most probably a residue of earlier glyphosate applications that is extremely slowly decaying. There is no clear explanation to why glyphosate degraded to AMPA before, but not so distinctly after this application in both cultivation areas (although more clearly in the maize area). One possible reason may be the formulation of glyphosate that was applied. The granular properties of Roundup UltraMax® may have caused a preference for the sarcosine degradation pathway. On the other hand, the microorganisms responsible to degrade glyphosate to AMPA may have been affected by some external factor, which would explain this shift in degradation pathways. For example, Dual Gold®, a herbicide applied in the mix for the maize area, is known to impact microbial communities adversely (Joly et al., 2012). However, no studies were found relating this effect to glyphosate degrading microorganisms.

5.4.3 Glyphosate and AMPA contents in eroded soil and runoff water

Our results show higher glyphosate and AMPA contents in eroded sediment than in runoff water. This is in accordance with findings from elsewhere (Bento et al., 2018; Yang et al., 2015a; Yang et al., 2015b). In fact, glyphosate was only detected in 3 runoff water samples and AMPA in 1. For those runoff water samples >LOD, the maximum glyphosate and AMPA concentrations detected were 35.4 and 11.3 µg L^{-1}, respectively, which are well above the 0.1 µg L^{-1} EU limit for pesticides in drinking water. In the eroded sediment, the maximum glyphosate and AMPA contents obtained were 10.9 and 8.3 mg kg^{-1}, respectively. These values are higher than the maximum of 0.56 mg kg^{-1} of glyphosate and 0.21 mg kg^{-1} of AMPA in suspended sediment found in various streams in Buenos Aires Province (Aparicio et al., 2013) or the 0.58 mg kg^{-1} of glyphosate and 0.48 mg kg^{-1} of AMPA in suspended sediment in waterways of Entre Ríos Province (Primost et al., 2017), both studies in the Pampas of Argentina. It is important to refer though that our results in eroded sediment are not affected by a dilution factor, as they were in the above mentioned studies. Our measurements were performed to suspended sediment collected from runoff samples originating directly from the studied cultivation areas.

Glyphosate and AMPA contents were higher in the eroded sediment than their content in the parent soil for all cases, except for the plots in the soybean area. This is in accordance with the findings of Yang et al. (2015a). This result can be explained by the fact that surface transport occurred mostly in the form of sheet erosion. In this type of erosion, only the upper few millimeters of soil are transported (Whiting et al., 2001), which contain the highest glyphosate and AMPA contents (Yang et al., 2015a; Yang et al., 2015b; Rampazzo et al., 2013).

Glyphosate and AMPA contents were higher in the eroded sediment obtained at the sub-catchment scale (weirs) than those at the plot scale. Among these results were some particular high ratios, namely the glyphosate ratio found at the sub-catchment scale in both cultivation areas in the erosive event of 9-02-2015. This is likely a product of glyphosate-rich sediment accumulation in front of the weirs during previous rainfall events. Glyphosate and AMPA strongly adsorb to clay and fine silt particles (Bento et al., 2018; Bento et al., 2017). Glyphosate-rich suspended sediment is the last type of sediment to be deposited at the end of a water erosion event, leading to an accumulation of such sediment at the lowest point of a catchment (Bento et al., 2018). The soil samples were collected in the middle of the cultivation areas and are unlikely to take this effect into account. Some authors found that glyphosate transport is particularly high during erosion events that occur shortly after application (Todorovic et al., 2014; Screpanti et al., 2005). From our sediment/soil ratios obtained at the catchment scale, this was not particularly obvious.

5.5 Conclusions

From this study we conclude that the decay is the dominant process describing the glyphosate dynamics, and SM content is a very important factor influencing the decay rate. AMPA dynamics, on the other hand, didn’t show a clear pattern in our study. The high background residues of AMPA before glyphosate application suggest that AMPA may accumulate in soil, particularly in cultivation areas with repeated glyphosate applications. Nevertheless, long-term (>1 year) decay studies under current-use agricultural management activities are recommended to confirm this. The great variability in the decay behavior and persistence of glyphosate and AMPA observed in our study indicates the need to perform site-specific decay studies.

From the high glyphosate and AMPA contents obtained in eroded sediment we also conclude that water erosion represents a great risk of glyphosate and AMPA contamination of surface water bodies and adjacent fields. This is particularly true when erosive rainfall events occur in the form of sheet erosion.

Acknowledgements

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Supplementary Material

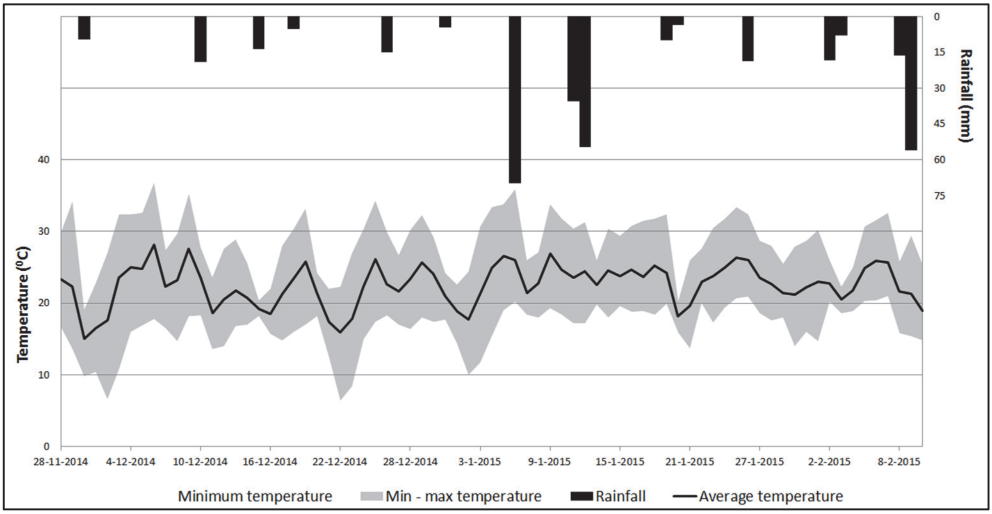


Figure S5.1 *Rainfall and average, minimum and maximum temperatures during the study period. Temperature data were provided by the national meteorological services of Argentina. Rainfall data were collected from on-farm rainfall gauges monitored at 7:00 each morning.*

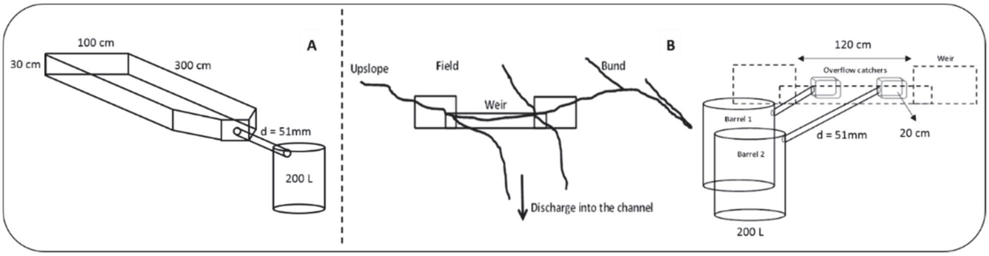


Figure S5.2 *Schematic representation of the plots (A) and weirs (B).*

Table S5.1 *Soil properties of the soybean and maize cultivation areas.*

Parameter	Soybean field	Maize field
Sample depth (cm)	0-10	0-10
Particle size distribution		
<0.002 mm (clay) (%)	26.3	27.4
0.002-0.05 mm (silt) (%)	44.2	40.0
>0.05 mm (sand) (%)	29.5	32.6
pH in $CaCl_2$	5.8	5.9
Organic matter (OM) (%)	5.1	4.6
Bulk density (g cm^{-3})[a]	1.0	1.1

[a] Bulk density was measured at 0-5cm depth and it's the average of 3 locations in each cultivation area.

Table S5.2 *Glyphosate and AMPA contents (average±SD) on the top 0-1cm and 1-2 cm soil, and soil moisture (SM) contents (average±SD) on the top 0-5cm soil, in the soybean and maize cultivation areas.*

		Soybean$^{\#}$			Maize$^{\#}$		
Sampling date	Soil depth (cm)	Glyphosate (mg kg^{-1})	AMPA (mg kg^{-1})	SM (%)	Glyphosate (mg kg^{-1})	AMPA (mg kg^{-1})	SM (%)
28-11-2014	0-1	0.42 ± 0.12* bc	1.7 ± 0.03* a	19.6 ± 3.4	-	-	-
9-12-2014	0-1	6.0 ± 3.1 e	2.4 ± 1.3 a	n.d.	0.27 ± 0.13* bc	1.3 ± 0.04* a	15.6 ± 2.4
15-12-2014	0-1	0.88 ± 0.33 cd	2.2 ± 0.7 a	19.5 ± 1.6	0.23 ± 0.03* bc	1.3 ± 0.1* a	18.9 ± 0.1
22-12-2014	0-1	1.2 ± 0.4 d	3.0 ± 0.6 a	16.6 ± 1.9	0.09 ± 0.01* a	0.93 ± 0.03* a	19.5 ± 1.3
6-01-2015	0-1	0.36 ± 0.05 bc	2.5 ± 0.3 a	16.6 ± 2.3	3.7 ± 0.6 d	1.0 ± 0.1 a	14.9 ± 1.2
12-01-2015	0-1	0.28 ± 0.06 ab; A	2.2 ± 0.3 a; A	24.0 ± 2.1	0.38 ± 0.09 c; A	0.99 ± 0.39 a; A	24.6 ± 2.2
	1-2	0.10 ± 0.05 a; B	0.71 ± 0.21 a; B		0.08 ± 0.03 b; B	0.57 ± 0.23 a; A	
19-01-2015	0-1	0.19 ± 0.05 ab; A	2.2 ± 0.7 a; A	18.0 ± 0.5	0.16 ± 0.001 ab; A	0.99 ± 0.19 a; A	19.9 ± 0.7
	1-2	0.07 ± 0.02 a; B	0.69 ± 0.03 a; B		0.07 ± 0.02 ab; B	0.47 ± 0.10 a; B	
26-01-2015	0-1	0.17 ± 0.02 ab; A	1.6 ± 0.1 a; A	22.7 ± 0.9	0.09 ± 0.02 a; A	0.73 ± 0.19 a; A	23.8 ± 1.8
	1-2	0.06 ± 0.02 a; B	0.61 ± 0.10 a; B		0.05 ± 0.004 ab; B	0.41 ± 0.11 a; A	
2-02-2015	0-1	0.13 ± 0.01 a; A	1.6 ± 0.3 a; A	17.7 ± 2.0	0.10 ± 0.01 a; A	0.69 ± 0.07 a; A	23.8 ± 2.4
	1-2	0.07 ± 0.02 a; B	0.69 ± 0.18 a; B		0.05 ± 0.01 ab; B	0.37 ± 0.11 a; B	
9-02-2015	0-1	0.21 ± 0.11 ab; A	1.4 ± 0.4 a; A	23.0 ± 0.9	0.13 ± 0.05 ab; A	0.91 ± 0.31 a; A	23.4 ± 0.9
	1-2	0.10 ± 0.05 a; B	0.62 ± 0.33 a; B		0.03 ± 0.01 a; B	0.36 ± 0.06 a; B	

n.d.: no data

Different lowercase letters within the same column indicate significant differences between sampling dates for the same soil depth ($p<0.05$).

Different capital letters within the same column indicate significant differences between soil depths for the same sampling date ($p<0.05$).

[#] Glyphosate formulation was applied on 2-12-2014 (soybean) and 26-12-2014 (maize)

* Background contents

6. Synthesis

6.1 General conclusions

This PhD thesis addresses four key questions that aim to investigate the environmental fate of glyphosate and AMPA in the loess soil environment. Special focus was given to the decay of glyphosate and to the formation/decay of AMPA in loess soils, under laboratory and field conditions, and to their off-site transport by wind (wind tunnel experiment) and water (flume experiment and field scale conditions) erosion.

The insights gained through this PhD research improve and further the science on understanding the environmental fate of glyphosate and AMPA in loess soils, particularly regarding AMPA, which until now has received little attention. Particular insights were attained regarding the off-site transport of these compounds with wind erosion, a transport pathway that has been largely disregarded so far. The findings of this PhD thesis can be applied not only to the loess Pampas of Argentina, where social concern about the effects of repeated applications of glyphosate are strongly increasing, but also to other loess regions of the world. Moreover, the insights of this PhD research are a valuable contribution to and basis for environmental fate modelling studies, and can help in the redesign and improvement of the principles behind exposure and human health risk assessments.

The main findings of this PhD research are:

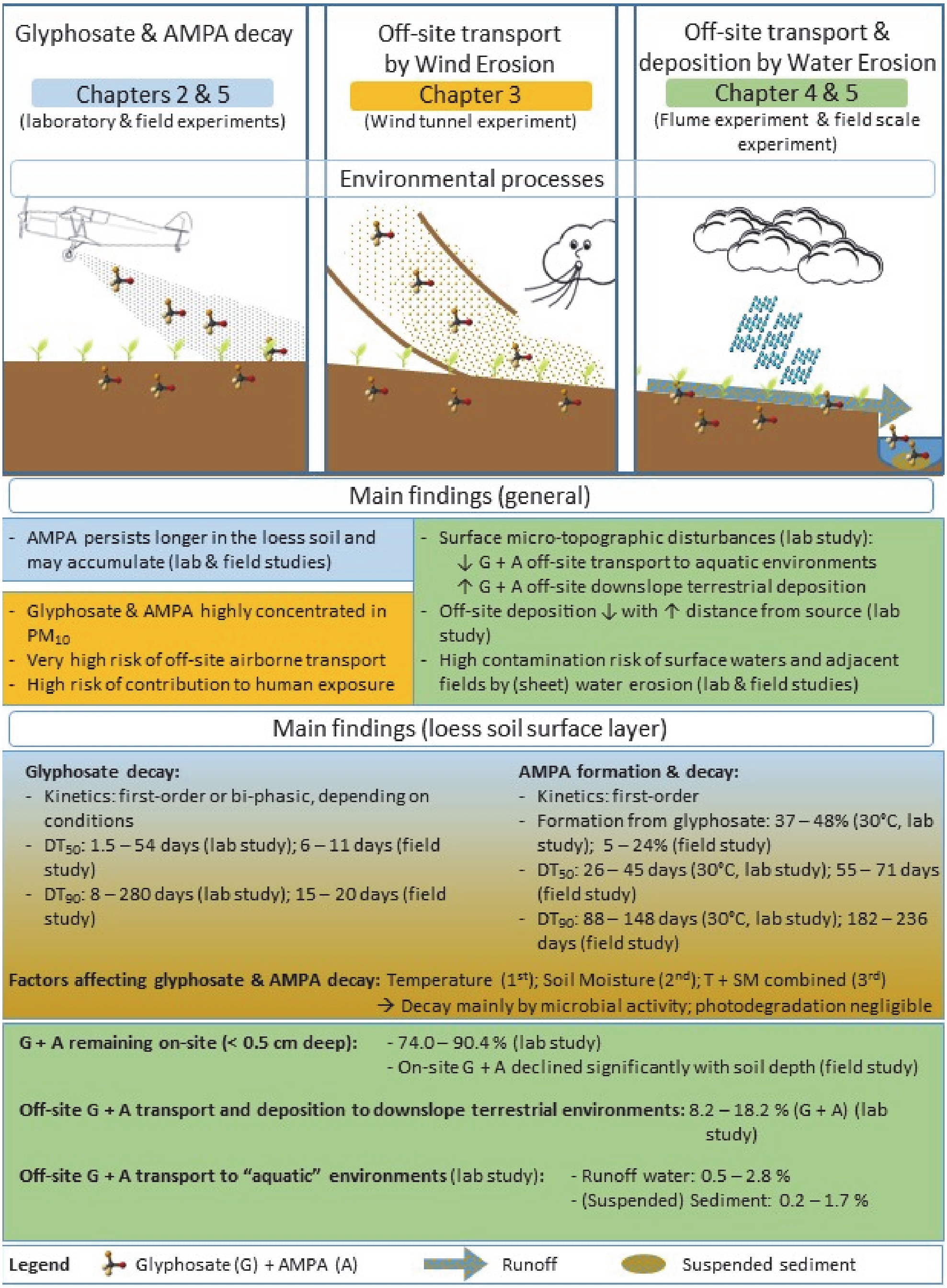
- The decay of glyphosate and AMPA in loess soils is mostly a microbiological process and is fastest under warm and moist soil conditions and slowest under cold and dry soil conditions. AMPA persists longer in loess soil than glyphosate, and tends to accumulate. Glyphosate and AMPA DT_{50} and DT_{90} and, consequently, their persistence, vary widely in the same type of soil, due to variable climate and soil moisture conditions. Repeated glyphosate applications, particularly under dry soil conditions, increase the risk of accumulation of these compounds in loess soils and, consequently, of on-site soil pollution and off-site transport with wind and water erosion.
- The type of decay kinetics followed by glyphosate in loess soils is mostly temperature dependent, but abrupt soil moisture changes from dry to moist also play a role. When high decay rates occur, glyphosate tends to follow bi-phasic rather than first-order decay kinetic models. The inappropriate use of first-order decay kinetic models in these cases underestimates glyphosate's DT_{90} and, consequently, gives a wrong prediction of its persistence in loess soils. Efforts should be made to apply the correct decay kinetics in environmental fate models.
- AMPA decay is reasonably well described by first-order kinetic models, but the short duration of the studies did not allow a complete overview of its decay behavior in loess soils. AMPA formation was taken into account, which provides a better estimation of its decay behavior. Glyphosate degradation into AMPA was extremely variable (5-100%)

amongst different temperature conditions and between laboratory and field conditions. Longer experimental periods under different conditions are needed for more in-depth investigation of the formation and decay behavior of AMPA.

- Glyphosate and AMPA contents are highest in eroded soil particles <10 µm (PM_{10}), and their content decreases with increasing eroded soil particle size. Therefore, glyphosate and AMPA long-range off-site transport risk with wind erosion (dust) is very high. Consequently, the risk of inhalation of contaminated dust by animals and humans can be considerable, contributing to the risk of human and animal exposure to glyphosate and AMPA. This route of exposure needs more attention in risk assessment studies.
- During water erosion events, the particle-bound transport of glyphosate and AMPA is as or even more important than the water-dissolved transport. Erosive rainfall events occurring in the form of sheet erosion may represent a higher particle-bound off-site transport risk of glyphosate and AMPA. This is because glyphosate and AMPA concentrate in the very top soil surface layer (0-1 cm), adsorbed to clay minerals and OM, which are easily and rapidly transported by this type of water erosion. The pollution risk of aquatic systems is therefore high.
- The risk of deposition of glyphosate and AMPA into off-target downslope fields during water erosion events can be considerable, particularly in soil surfaces with (micro-)topographic disturbances. Nevertheless, their off-site deposition onto adjacent fields decreases with increasing distance from the glyphosate treated area. Notwithstanding, the pollution risk of off-target terrestrial areas is high.
- The dynamics of glyphosate under field conditions in the loess soils is mostly dominated by its microbial degradation, with soil moisture playing an important role on its decay rate. The processes dominating AMPA dynamics under field conditions didn’t show a clear pattern. AMPA decay under field conditions was limited and may be accumulating in the loess soils. Due to the tendency of glyphosate and AMPA to remain in the soil surface layer, water and wind erosion play an important role in glyphosate and AMPA dynamics in loess soils.

6.2 General discussion

In light of the four research questions presented in chapter 1, the overall findings of this PhD thesis are summarized in Figure 6.1 and synthesized in the discussion below.



- AMPA persists longer in the loess soil and may accumulate (lab & field studies)

- Glyphosate & AMPA highly concentrated in PM_{10}
- Very high risk of off-site airborne transport
- High risk of contribution to human exposure

- Surface micro-topographic disturbances (lab study):
  ↓ G + A off-site transport to aquatic environments
  ↑ G + A off-site downslope terrestrial deposition
- Off-site deposition ↓ with ↑ distance from source (lab study)
- High contamination risk of surface waters and adjacent fields by (sheet) water erosion (lab & field studies)

Glyphosate decay:
- Kinetics: first-order or bi-phasic, depending on conditions
- DT_{50}: 1.5 – 54 days (lab study); 6 – 11 days (field study)
- DT_{90}: 8 – 280 days (lab study); 15 – 20 days (field study)

AMPA formation & decay:
- Kinetics: first-order
- Formation from glyphosate: 37 – 48% (30°C, lab study); 5 – 24% (field study)
- DT_{50}: 26 – 45 days (30°C, lab study); 55 – 71 days (field study)
- DT_{90}: 88 – 148 days (30°C, lab study); 182 – 236 days (field study)

Factors affecting glyphosate & AMPA decay: Temperature (1st); Soil Moisture (2nd); T + SM combined (3rd)
→ Decay mainly by microbial activity; photodegradation negligible

G + A remaining on-site (< 0.5 cm deep): - 74.0 – 90.4 % (lab study)
- On-site G + A declined significantly with soil depth (field study)

Off-site G + A transport and deposition to downslope terrestrial environments: 8.2 – 18.2 % (G + A) (lab study)

Off-site G + A transport to "aquatic" environments (lab study): - Runoff water: 0.5 – 2.8 %
- (Suspended) Sediment: 0.2 – 1.7 %

***Figure 6.1** Synopsis of the overall findings of this PhD thesis on the fate of glyphosate (G) and AMPA (A) in loess soils: glyphosate decay and AMPA formation and decay (Chapters 2 & 5, blue); off-site transport of G & A by wind (chapter 3, orange) and water (chapters 4 & 5, green) erosion.*

6.2.1 Decay kinetics of glyphosate, formation and decay kinetics of AMPA and factors affecting these processes in loess soils

Glyphosate

This research thesis focused mostly on the effect of temperature, soil moisture and light/darkness on glyphosate decay rates and kinetics. Temperature (chapter 2) and soil moisture (alone and combined) (chapters 2, 3 and 5) were proven to have a major effect on glyphosate decay, whereas the effect of light (photodegradation) was negligible (chapter 2). These findings are in accordance with those reported by other studies (EFSA, 2015; Grundmann et al., 2008; Schroll et al., 2006; Eberbach, 1998; Rueppel et al., 1977). According to the findings of chapter 2, glyphosate tends to decay quickly under warm and humid climates, whereas it will decay more slowly under cold and dry climates. Considering that the climate of the study area in Argentina is characterized by hot and humid summers and cold and dry winters, repeated glyphosate applications during the year, especially autumn/winter applications in chemical fallows, are likely to increase glyphosate persistence. Moreover, as shown in chapter 5, dry soil conditions during summer also occur, which also slows the decay of glyphosate, as proven in chapter 2, and increases the risk of glyphosate persistence and accumulation in the loess soil.

This PhD research demonstrated that the decay kinetics of glyphosate in loess soils can follow both first-order (SFO) and bi-phasic (FOMC, HS) models, depending on temperature (chapter 2) and soil moisture (chapter 5). EFSA (2015), in its most recent peer review risk assessment on glyphosate, also reports bi-phasic decay kinetic models (FOMC or DFOP) for most soils analyzed under laboratory conditions at temperatures >20°C, and under field conditions. No information was available on the type of decay kinetics for glyphosate at (very-)low temperatures, except for one type of soil (loamy sand) at 10°C, which followed a bi-phasic (DFOP) decay kinetic model (EFSA, 2015). In this PhD research, the SFO decay kinetic model was mostly associated with very low temperatures and consequent low decay rates, whereas bi-phasic models (FOMC) were associated with high temperatures and consequently higher decay rates (chapter 2).

Changes in the type of decay kinetics for glyphosate depending on temperature have also been observed by Eberbach (1998). However, changes due to soil moisture, as observed in the field study (chapter 5), have not been previously reported. In fact, as demonstrated in some studies (Grundmann et al., 2008; Schroll et al., 2006) and in the laboratory study of chapter 2, while soil moisture normally affects the decay rates, it has not been associated with the decay kinetics. Laboratory studies normally investigate the effect of soil moisture based on the fact that the same sample is subject to a constant soil moisture content throughout the entire experimental period (as in chapter 2). Besides, except for the laboratory study in chapter 2, studies only observed the effect of soil moisture on glyphosate decay rates, but not on decay kinetics (Grundmann et al., 2008; Schroll et al., 2006).

Glyphosate decay is mostly a process promoted by microbial activity, as demonstrated in our laboratory study (chapter 2) and elsewhere (Sviridov et al., 2015; Ghassemi et al., 1982; Moshier and Penner, 1978; Rueppel et al., 1977). As also demonstrated in the laboratory study (chapter 2), microbial activity is negatively affected by dry soil conditions, which in turn reduces glyphosate decay rates. However, when sufficient rainfall occurs and soil moisture conditions increase, microbial activity is enhanced and consequently the glyphosate decay rate also increases, as demonstrated in chapter 5. Such a shift in the decay rates, if significant – as it was in chapter 5 in the maize cultivation area, changing from 0.06 days^{-1} (dry soil conditions) to 0.37 days^{-1} (moist soil conditions) – results in a different type of decay kinetics for glyphosate. In the case of the maize cultivation area (chapter 5), this shift led glyphosate to follow the bi-phasic HS decay kinetic model, instead of an SFO or FOMC decay kinetic model if the soil moisture conditions would remain unchanged. This type of scenario certainly occurs quite often under field conditions, particularly in semi-arid and arid regions, and therefore needs further attention for a proper estimation of the glyphosate decay kinetic models and consequent decay rates and endpoints (DT_{50} and DT_{90}).

Despite the factors investigated in chapter 2, other factors have also been proven to affect glyphosate’s decay rate and kinetics, mainly the type and properties of soil (Borggaard and Gimsing, 2008; Gimsing et al., 2004b; Eberbach, 1999; Eberbach, 1998). Glyphosate decay tends to behave differently between different types of soils, and most studies associated this to the different adsorption/desorption capacities of the different types of soils (Borggaard and Gimsing, 2008; Gimsing et al., 2004b; Eberbach, 1999; Eberbach, 1998). Glyphosate tends to adsorb strongly to clay minerals, Fe and Al oxides and, although controversial between studies, to OM (Vereecken, 2005; Gimsing et al., 2004b; Eberbach, 1998). On the other hand, because glyphosate decay is mostly a microbial process, the strongly adsorbed glyphosate is believed to be unavailable to microorganisms. The bi-phasic decay kinetic behavior of glyphosate is believed, also in this research thesis, to be mostly related to this strongly adsorbed phase (chapter 2).

Considering all these factors, this project proves that the decay behavior of glyphosate is very variable, resulting in a wide range of DT_{50} and DT_{90}. Consequently, the persistence of glyphosate in soil also varies greatly. The results from the laboratory study alone (chapter 2), where only one type of soil (loess soil) was investigated, confirm the wide variability in glyphosate DT_{50} and DT_{90} based simply on different climatic conditions and soil moisture contents. Moreover, the field study presented in chapter 5 also proves that the decay behavior of glyphosate varies even within areas with great geographical proximity and subject to the same climatic conditions.

The recent glyphosate peer review assessment of EFSA (2015) also reported that “glyphosate exhibits low to very high persistence in soil”. Such great variability in the decay behavior of glyphosate between, as well as within, geographical areas, and due to different climatic conditions, soil moisture and different soil types and properties, indicates the need to perform site-specific decay studies. Considering the recurrent applications of glyphosate in the same fields every year and that they are normally applied mixed with other pesticides, such decay studies should be performed with some frequency to monitor possible changes over the medium-to-long-term. This would allow better insight into the possible effects of other pesticides and of climate change. Such monitoring studies would also allow prediction of potential increased risk of glyphosate and AMPA accumulation in soil and trigger application of mitigation measures to avoid serious soil contamination and consequent off-site risk.

AMPA

Despite it being the major metabolite formed from the degradation of glyphosate and having a proven toxicity to organisms and humans (see section 1.2), decay studies of AMPA in soil are scarce. The glyphosate peer review assessment of EFSA (2015) mentions that decay studies of AMPA in soil, particularly in acidic soils, are missing and need further attention, yet few studies have been initiated. The few studies found that investigated the decay kinetics of AMPA in soil, looked at only the maximum observed amount onwards (Yang et al., 2015a; Simonsen et al., 2008), except Bergstroem et al. (2011) and EFSA (2015) (in their recalculation of AMPA endpoints, based in several studies, under field conditions), which is a conservative approach that underestimates the decay of metabolites (FOCUS, 2006).

In this PhD research, the simultaneous formation and decay of AMPA was taken into account in the decay kinetic studies performed in chapters 2 and 5. Such an approach not only provides a better estimation of the decay behavior of AMPA (FOCUS, 2006), but also allows estimation of the percentage of glyphosate that was degraded to AMPA (AMPA formation) and more accurate determination of the time after glyphosate application at which AMPA content peaked in soil.

In both studies performed on the formation and decay kinetics of AMPA (chapters 2 and 5), a single first-order (SFO) model fitted the data satisfactorily, regardless of climatic conditions and soil moisture. This is in accordance with the other studies that investigated the decay kinetics of AMPA (EFSA, 2015; Yang et al., 2015a; Bergstroem et al., 2011;

Simonsen et al., 2008). The estimated decay rates of AMPA were low under both laboratory and field conditions, varying between 0.01 (field; chapter 5) and 0.03 (lab; chapter 2) days^{-1}. Similar decay rates of AMPA were obtained under field conditions in a loess soil by Yang et al. (2015a) and under laboratory conditions in other soil types (Bergstroem et al., 2011; Simonsen et al., 2008).

Although soil moisture did not significantly affect the decay rates of AMPA (chapter 2), temperature played an important role. Similar to what has been seen with glyphosate, photolysis (or photodegradation) was negligible. Unfortunately, our laboratory study (chapter 2) with sterile soil did not include the direct application of AMPA to ascertain with a high degree of confidence the influence of abiotic processes on the decay of AMPA. Nevertheless, the results obtained from the decay of glyphosate under abiotic conditions suggest that, as for glyphosate, the degradation of AMPA is mostly a microbiological process. However, studies applying AMPA directly into (sterile) soil are needed to confirm this. The few laboratory studies found that investigated the decay kinetics of AMPA did not take into account the influence of temperature, soil moisture, light/darkness or abiotic degradation in the process.

The estimated formation of AMPA from glyphosate in loess soil was much lower under field conditions (5-24%; chapter 5) than under laboratory conditions (37-48%; chapter 2) for similar temperatures (30°C under laboratory conditions). Although the estimated results on AMPA formation at 5°C need to be taken into account with care because the plateau and decline phases were not reached during the experimental period (see chapter 2, section 2.3.2), these were even higher (54-100%). Bergstroem et al. (2011), in their laboratory study at 20°C with different types of soil, reported similar results (48-100%) to those we obtained at 5°C. Nevertheless, as happened in our experiment at 5°C, they did not reach plateau and decline phases during their 64 days of experiment. EFSA (2015), in their recalculated values from studies under field conditions and in different types of soils, reported a formation of AMPA ranging between 33.2% and 50.8% (n=3), which is above our values on AMPA formation under field conditions (chapter 5). Such variability on the percentage of AMPA that has been formed from glyphosate, under both laboratory and field conditions, is most probably related to the activity and composition of the soil microbial communities under different conditions. Unfortunately, no identification of soil microorganisms was possible during this PhD research to corroborate these suspicions and to better understand the factors involving and affecting the different patterns on the degradation of glyphosate to AMPA. It is important to note that AMPA formation has rarely been studied. Apart from this thesis, only the study by Bergstroem et al. (2011) and the report of EFSA (2015) have contemplated this. Therefore this is a topic needing further attention.

The studies performed in this PhD research on AMPA decay kinetics confirmed a stronger persistence of AMPA in soil compared to glyphosate, for all conditions tested and under laboratory and field conditions. This is in accordance with other studies (EFSA, 2015; Yang et al., 2015a; Bergstroem et al., 2011; Simonsen et al., 2008; Mamy et al., 2005; Grunewald et al., 2001; Rueppel et al., 1977). Our laboratory and field studies also confirmed that the DT_{50} (26-71 days) and DT_{90} (88-236 days) values of AMPA can vary widely (Figure 6.1). In fact, several studies report that AMPA can be persistent in the soil environment and that it tends to accumulate in soil (Okada et al., 2018; Grandcoin et al., 2017; Primost et al., 2017; EFSA, 2015; Aparicio et al., 2013; Coupe et al., 2012; Al-Rajab and Schiavon, 2010; Simonsen et al., 2008; Mamy et al., 2005; Gimsing et al., 2004b). The field study from this PhD thesis (chapter 5) also confirms the great persistence of AMPA in both cultivation areas studied, and also suggests that it is accumulating in soil: background AMPA contents in soil (before glyphosate application) were quite high (1.3-1.7 mg kg^{-1}), and remained almost constant during the 2.5 month study period (after glyphosate application).

Considering the risk of AMPA accumulation in soil, the recurrent glyphosate applications every year in agricultural fields, and the proven toxicity of this compound by some studies, it is of extreme importance to better understand the environmental fate of AMPA and the environmental and human health risks associated with it. However, the duration of the decay studies contemplating AMPA, including those from this PhD thesis, were too short to have a complete overview of its behavior. More studies on the formation and decay of AMPA contemplating longer experimental periods, under laboratory and field conditions, are needed.

6.2.2 Role of wind erosion on the atmospheric transport of glyphosate and AMPA and its potential contribution to human exposure

Wind erosion is often neglected as a transport pathway of pesticides into the environment (Aparicio et al., 2018; Huber et al., 2000; Larney et al., 1999). Proving this is the great number of studies existing on the transport of these pollutants by water erosion in contrast to those found on their transport by wind erosion – a "dirty" search on Scopus database gave 845 studies related to their transport by water erosion [((TITLE-ABS-KEY (pesticides) OR TITLE-ABS-KEY (herbicides))) AND (TITLE-ABS-KEY (water AND erosion))], but 116 related to their transport by wind erosion [((TITLE-ABS-KEY (pesticides) OR TITLE-ABS-KEY (herbicides))) AND (TITLE-ABS-KEY (wind AND erosion))]. Moreover, this transport pathway is often considered irrelevant, even by authorities (Huber et al., 2000; U.S.EPA, 1993). Nevertheless, studies have proven that wind erosion plays a role on the off-site transport of pesticides into the environment, particularly those with high affinity to the particle phase (Aparicio et al., 2018; Balayiannis et al., 2009; Cessna et al., 2006; Clay et al., 2001; Larney et al., 1999).

Regarding glyphosate and AMPA, there were no studies on their transport by wind erosion until very recently, despite their known strong adsorption capacity to soil particles and their proven occurrence in the atmosphere (see chapter 1). The study presented in chapter 3 of this PhD thesis and, afterwards, the study of Aparicio et al. (2018) were, to the best of my knowledge, the first ones investigating this transport pathway for glyphosate and AMPA.

The wind-tunnel experiment (chapter 3) demonstrated that glyphosate and AMPA are prone to be transported by wind erosion. The significantly highest concentrations of glyphosate and AMPA in the finest particles (<18 µm) confirm that the long-range atmospheric transport of these compounds is extremely likely. In the field experiment carried out by Aparicio et al. (2018) in semi-arid regions of Argentina, glyphosate and AMPA concentrations were highest in the wind-blown material at a height of 150 cm (which comprises the smallest particle size they measured), and their concentrations decreased with decreasing height (i.e. increasing particle size), confirming the wind-tunnel results in chapter 3. The authors reported concentrations of 0.25 and 0.22 mg kg^{-1} of glyphosate and AMPA, respectively, in the wind-blown sediment at a height of 150 cm, and this material was 60 (glyphosate) and 3 (AMPA) times more enriched than the parent soil (Aparicio et al., 2018). The wind-tunnel results (chapter 3) and the results of Aparicio et al. (2018) confirm that high concentrations of glyphosate and AMPA can be easily detected in PM_{10}, which are easily transported over large distances (thousands of kilometers), and which are easily inhaled. Therefore, glyphosate and AMPA transported by this pathway represents a risk to the environment and human health and needs further attention.

The wind-tunnel experiment (chapter 3) has also shown that glyphosate decayed very slowly over time due to the very low soil moisture content of the sediment. The negative influence of low soil moisture contents on glyphosate decay has also been proven in chapters 2 and 5 and by Schroll et al. (2006). Moreover, glyphosate and AMPA tend to remain in the very thin surface layer of soils (0-1 cm soil depth or less), as demonstrated in chapters 4 and 5 and elsewhere (Yang et al., 2015a; Yang et al., 2015b), which is highly exposed to wind erosion. This soil surface layer is also more directly exposed to sunlight and heat and, consequently, soil water evaporation and dryness will here occur faster. This reduces soil microbial activity at this surface layer and, consequently, increases the persistence of glyphosate and AMPA, as demonstrated in the field decay study in the maize cultivation area (chapter 5). As a result, the airborne off-site transport risk of glyphosate and AMPA with dust is increased. Furthermore, the field results in the maize cultivation area confirm the conclusion of chapter 3 that if glyphosate is applied during a drought period and if it persists for some time (as happened in the maize area for the first 11 days after glyphosate application), the airborne off-site transport risk of glyphosate and AMPA with dust is intensified.

As referred in the discussion section of chapter 3, inhalation of fine particles ($<PM_{10}$) has already been associated to various health problems and even death, and these are expected to increase when pollutants, such as glyphosate and AMPA, are adsorbed to these fine particles. Moreover, as referred above, the highest concentrations of glyphosate and AMPA are found in these fine particles due to their great affinity to clay and OM (as proven in chapters 3 & 4). However, human health risk assessments based on this route of exposure have been disregarded so far for these compounds, but it certainly needs more attention and should be integrated in risk assessment studies.

6.2.3 Off-site transport risk of glyphosate and AMPA driven by water erosion in loess soils

As referred in chapter 1, most studies investigating the off-target transport of glyphosate and AMPA with water erosion focused only on the concentration of these compounds in the water phase (Daouk et al., 2013; Birch et al., 2011; Gregoire et al., 2010; Warnemuende et al., 2007; Siimes et al., 2006), while just a few recent studies contemplated the particle-bound phase as well (Melland et al., 2016; Napoli et al., 2016; Yang et al., 2015a; Yang et al., 2015b).

In the water erosion studies (chapters 4 and 5), high glyphosate and AMPA contents were observed in the eroded sediment, ranging between 0.50 and 42.9 mg kg^{-1} for glyphosate and between non-detectable (<LOD) and 8.3 mg kg^{-1} for AMPA. In runoff water, glyphosate and AMPA concentrations were always lower than in sediment in both studies, although, when detected, they were always above the 0.1 µg L^{-1} EU limit for drinking water – between non-detectable and 76.5 µg L^{-1} for glyphosate and non-detectable and 11.3 µg L^{-1} for AMPA. Therefore, the particle-bound phase is a source of pollution as (or even more) important as the water-dissolved phase, which needs more attention. Moreover, the main focus of the studies investigating the off-site transport of glyphosate and AMPA has been to the contamination of aquatic systems. However, as demonstrated in the flume experiment (chapter 4), the contamination of terrestrial environments downslope treated areas is very likely and needs further attention.

Consistent with previous studies (Yang et al., 2015a; Yang et al., 2015b), chapters 4 and 5 demonstrate that the particle-bound off-target transport of glyphosate and AMPA can be considerable. According to the results of chapter 4, the glyphosate and AMPA off-site transport with the particle-bound phase ranged between 9.4-17.8% (including the downslope off-site deposition), whereas the off-site transport with the water-dissolved phase ranged between 0.5-2.8%. This is in accordance with the results of Yang et al. (2015a) and Yang et al. (2015b) in a Chinese loess soil. Nevertheless, when only considering the glyphosate and AMPA off-site transport to the outlet of the flume, i.e. excluding the terrestrial off-site deposition in the downslope areas, higher amounts of glyphosate and AMPA were transported with the water-dissolved phase (0.5-2.8%) than with the particle-bound phase (0.2-1.7%). This contradicts the findings of Yang et al. (2015a) and Yang et al. (2015b), but it is most probably explained by the much lower slope used in our flume experiment, as referred in chapter 4. In the flume experiment, the used slope intended to represent the loess regions of the Pampas of Argentina, which are characterized by gentle slopes and are intensively used for the growing of GR crops under non-tillage systems.

One should be aware that the flume experiment (chapter 4) represents a worst-case scenario of rainfall occurring 1h after glyphosate application, whereas chapter 5 represents field conditions under normal agribusiness management activities. Erosive rainfall events in the field study (chapter 5) occurred several days to >2 months after glyphosate was applied. Moreover, high glyphosate and AMPA contents, as well as detectable glyphosate concentrations in runoff water, were observed in the rainfall event occurring >2 months after glyphosate application in the soybean cultivation. Considering this and that glyphosate seems to be applied in GR crop fields every 2-3 months (on average) in Argentina, it can be stated that the major risk of glyphosate and AMPA off-site transport by water erosion is not limited to erosive rainfall events occurring shortly after application, as stated by some studies (Yang et al., 2015b; Todorovic et al., 2014; Borggaard and Gimsing, 2008; Screpanti et al., 2005). In fact, the results from chapter 5 indicate that, under current glyphosate application practices in Argentina, there is a continuous risk of glyphosate and AMPA to be transported in high concentrations to off-target areas. Consequently, there is a high risk of continuously detecting these compounds in surface waters (water, suspended matter and riverbed sediment) and in off-target terrestrial environments.

In chapter 4, it was also demonstrated that micro-topographies on the soil surface help reducing the runoff and erosion rates and, consequently, the off-target transport of glyphosate and AMPA to aquatic environments. The results also have shown that the deposition of glyphosate and AMPA into off-target terrestrial environments was enhanced in soil surfaces with micro-topographic disturbances. Moreover, in both types of soil surface studied, the transport and deposition of glyphosate and AMPA downslope the glyphosate treated area reduced with increasing distance from the source. No studies were found in the literature contemplating the transport and deposition of glyphosate and AMPA to adjacent fields or the role of different soil (micro-)topographies on their transport and deposition into downslope off-target fields. Nevertheless, Aparicio et al. (2018) reported the presence of 2.4 and 6.3 µg kg^{-1} of glyphosate and AMPA, respectively, in the soil of an Argentinean field that hasn't been treated with glyphosate, and refers that these low values could only be detected in that field as a result of soil particle movement from surrounding fields where glyphosate has been applied.

The results from chapter 4 relate to glyphosate and AMPA, but certainly such results also apply to other pesticides and pollutants, particularly those with similar properties to glyphosate and AMPA. These results also demonstrate that the transport and deposition of glyphosate and AMPA, as well as other pollutants, to off-target adjacent fields and surface waters can be minimized by the creation of proper physical and/or biological barriers within, as well as at the downslope edges, of treated fields.

6.2.4 Dynamics of glyphosate and AMPA in the loess soil surface of fields cultivated with GR crops and under long-term repeated glyphosate applications

The cultivation areas of Argentina investigated in chapter 5 are fields under long-term history of repeated applications of glyphosate. The glyphosate and AMPA contents found in the top 0-1 cm soil surface layer prior to glyphosate application indicate that, under current agricultural management practices, these compounds are omnipresent in these fields. The high background contents of AMPA indicate that this compound might be even accumulating in these fields. A field survey conducted by an MSc. student to farmers of the loess Pampas of Córdoba province, in Argentina, indicates that the pesticide application and agricultural management practices are similar in the entire region, with glyphosate being the most applied pesticide (data not published). Moreover, several studies in Argentina confirm the occurrence of high glyphosate and AMPA contents in agricultural soils of the country (Aparicio et al., 2018; Okada et al., 2018; Primost et al., 2017; Lupi et al., 2015; Aparicio et al., 2013; Peruzzo et al., 2008). Some also suggest that both glyphosate and AMPA are accumulating in soil, particularly AMPA (Okada et al., 2018; Primost et al., 2017). Considering these facts, it seems reasonable to conclude that the widespread use of glyphosate-based herbicides, as well as the current agricultural management practices in the province and in the country, are resulting in the omnipresence of these compounds in the Argentinean environment and in their accumulation in soil.

According to the results of chapter 5, glyphosate and AMPA remain mostly in the very top surface layer of the loess soils studied (0-1 cm), as indicated by the significantly lower glyphosate and AMPA contents in the subsequent soil layer (1-2 cm). The flume experiment (chapter 4) also confirms these findings, as well as other studies performed in loess soils (Lupi et al., 2015; Yang et al., 2015a; Yang et al., 2015b). Leaching of glyphosate and AMPA to deeper soil layers and groundwater seems, therefore, to be limited in this type of soils, as also suggested by other studies (Yang et al., 2015a; Yang et al., 2015b; Williams et al., 2012; U.S.EPA, 1993). Nevertheless, the fact that glyphosate and AMPA remain and are omnipresent in the very top surface layer of these soils allow me to state with some degree of confidence that there is a recurrent high risk of glyphosate and AMPA to be transported off-site with water and wind erosion. Although it was not possible to determine the amount of glyphosate and AMPA transported with water erosion in the field study (chapter 5), the high contents found in the eroded sediment, even >2 months after glyphosate application, indicate the recurrent high off-target transport risk. Even though the off-target transport of these compounds with wind erosion was not assessed in this field study, field observations during the study period suggest that the risk of their transport with wind erosion is also high. This is particularly true when glyphosate is applied before sowing and after sowing in an early stage of the growing crop, in fields with low litter cover from the previous crop. Such situations were observed during the field study. Field observations during the study period have also shown that soybean stubbles from the previous year give little soil cover, leaving the soil almost bare and easily subject to wind erosion. This transport pathway will, however, be more limited when the crops are sufficiently grown and cover most of the field area. By then, water erosion and decay processes will play a major role.

6.3 Implications and recommendations

6.3.1 Implications for the environment and human health

Loess soils are reported to be easily erodible by water, but also by wind (Catt, 2001; Pécsi, 1990), and to be more erodible than many other soil types (Catt, 2001). In fact, the Midwestern loess soils of the USA suffered from heavy and increased dust storms in the 1930s due to extreme drought and bad agriculture practices (Catt, 2001). The change in agricultural management practices in the Argentinean loess areas from tillage to non-tillage systems in the late 1990s due to the advent of GR crops clearly reduced the water and wind erosion problems faced by farmers (Trigo, 2016; Togni, 2013). Nevertheless, this agricultural management change has also resulted in excessive use of glyphosate-based herbicides. The link between glyphosate-based herbicides use and non-tillage systems is very strong (FAO and ITPS, 2017), and it is clearly evident in Argentina (Trigo, 2016, 2011). This excessive use of glyphosate-based herbicides, not only in Argentina, but also worldwide, calls for immediate and concrete measures to reduce glyphosate use and minimize its potential adverse impacts to the environment and human health. Some recommendations and alternatives will be addressed in section 6.3.3.

The insights gained in this PhD research about the environmental fate of glyphosate and AMPA in the loess soil environment are important not only to the Argentinean society and policy makers, but also worldwide, due to the widespread and recurrent use of glyphosate-based herbicides. Such widespread and recurrent use is resulting in high social concerns about glyphosate safety. Consequently, policy makers, particularly from Argentina and Europe, are under great pressure. Proof of this is, for example, the recent approval of glyphosate for only another 5 years in Europe, instead of the normal 10 years.

Environmental Implications

The main findings of this PhD research confirm that the persistence of glyphosate and AMPA in soil can vary greatly, even in the same type of soils and within the same geographical areas, and that these compounds, particularly AMPA, might be accumulating in soil. Adding to this, is the high periodicity at which glyphosate is being applied in agricultural fields. The results of the field study (chapter 5) suggest that the frequency of glyphosate applications in Argentina are greater than the soil microorganisms capacity to degrade glyphosate and AMPA. As a result, a glyphosate and AMPA concentration baseline seems to be omnipresent in the soil surface that, under current agricultural management practices, may lead to their accumulation in soil over time. As shown in several studies, this is already resulting in a continuous presence of glyphosate and AMPA in the different environmental compartments (Aparicio et al., 2018; Okada et al., 2018; Silva et al., 2018; Battaglin et al., 2014; Aparicio et al., 2013). In the short/medium term, if nothing is done, this may lead to serious soil and environmental pollution problems with glyphosate and AMPA that need to get more attention and action from global and local authorities.

Since glyphosate and AMPA decay rates are strongly affected by climatic and soil moisture conditions, climate change may certainly play a role in the persistence of these compounds in soil, as well as on their off-site transport. According to climate change predictions, precipitation patterns are shifting, temperatures are rising and an increase in the frequency and severity of extreme events is being observed, namely strong and lasting droughts and devastating flood events (WWF, 2018; Barros et al., 2015). In fact, during my stay in the region of the study area, heavy rainfall events were observed, with a great rise in the river flow crossing the city of Córdoba, Argentina, carrying high amounts of suspended sediment (see Figure 6.2). There are also personal communications referring to an increase in dust storm events in the city and in the region in the last years. This is of concern considering that the loess soils from Argentina have been reported to have the highest wind erosion rates and the highest PM_{10} emissions to the atmosphere compared to other soil types in the country (Aimar, 2016).

Briefly considering different climate change scenarios, the rise in temperature, if associated with wetter conditions, will positively affect the decay rates of glyphosate and AMPA in soil, reducing their persistence over time. However, if or when this rise comes associated with high evapotranspiration rates and drought, glyphosate and AMPA decay rates will be negatively affected and their persistence in soil will increase. This persistence will certainly be even more pronounced if current excessive glyphosate applications remain unchanged. Such scenarios also have consequences on the off-site transport of glyphosate and AMPA with water and wind erosion, particularly because glyphosate and AMPA tend to concentrate mostly in the top soil surface layer, which is more prone to water and wind erosion.

Figure 6.2 *Canal "La Cañada" in Córdoba city under normal flow (left) and right after a heavy rainfall event (right) in October 2014.*

The frequency of heavy rains and the severity of flood events are increasing in Argentina. When such events occur after glyphosate application and/or after a relatively long dry period, large amounts of glyphosate and AMPA are extremely likely to be transported to off-target environments. In fact, the probability of heavy rainfall and flood events in the loess Pampas of Argentina is highest during the spring/summer months, which coincides with the growing of the most important GR cash crops (soybean, maize) and, consequently, with the highest frequency of glyphosate applications. According to Gaitan et al. (2017), the average soil loss with water erosion in the province of Córdoba, Argentina, is estimated in 4.2 t ha^{-1} $year^{-1}$, but in the loess Pampas of the province it can range between 0.5 and 30 t ha^{-1} $year^{-1}$. Taking into account the average glyphosate and AMPA concentrations in the water-eroded sediment from chapter 5, it can be estimated that there is an average potential loss of glyphosate of 20 g ha^{-1} $year^{-1}$ (loess Pampas: 2.5-147 g ha^{-1} $year^{-1}$) and of AMPA of 15 g ha^{-1} $year^{-1}$ (loess Pampas: 1.8-108 g ha^{-1} $year^{-1}$) by water erosion in the province of Córdoba alone. According to Silva et al. (2018), potential glyphosate and AMPA losses into the environment with water erosion from European agricultural soils can range between 9.8 and 47.7 g ha^{-1} $year^{-1}$. These estimations indicate that great amounts of glyphosate and AMPA are reaching off-target terrestrial and aquatic environments every year, with consequent potential impacts to the environment and human health. Long-term field studies on glyphosate and AMPA losses with water erosion should be performed and then incorporated in environmental fate models and risk assessment studies for a more accurate prediction of their impacts to the environment and human health.

On the other hand, drought conditions will certainly increase the transport risk of glyphosate and AMPA with wind erosion. Aimar (2016) estimated soil losses by wind erosion in loess regions of Argentina ranging between 1 and 27 t ha^{-1} $year^{-1}$, and 40-45% of this is material in suspension, i.e. emitted to the atmosphere. If we consider the average glyphosate and AMPA concentrations in wind-blown material (at 150 cm height) as reported in semi-arid regions of Argentina by Aparicio et al. (2018), it can be estimated that there is an average potential loss of glyphosate of 0.1-3 g ha^{-1} $year^{-1}$ and of AMPA of 0.1-2.7 g ha^{-1} $year^{-1}$ with dust. According to Silva et al. (2018), potential glyphosate and AMPA losses to the environment with wind erosion from European agricultural soils can range between 1.9 and 3 g ha^{-1} $year^{-1}$. These estimations indicate that glyphosate and AMPA are transported in relatively high amounts every year to the atmosphere and over long-range distances. Therefore, the environmental and human health risks associated to the occurrence of glyphosate and AMPA in the atmosphere due to contaminated dust, disregarded so far, need to be re-evaluated and integrated in risk assessment studies.

According to the estimations of glyphosate and AMPA transport by water and wind erosion above, the study region in Argentina alone is prone to transport as much or more glyphosate and AMPA to off-target environments by these pathways every year than all of Europe together. Although much higher amounts of particle-bound glyphosate and AMPA can be transported with water erosion, their transport with wind erosion is more likely to promote a direct exposure to humans by inhalation.

Human Health Implications

The recurrent glyphosate applications and consequent continuous occurrence of glyphosate and AMPA in the different environmental compartments of Argentina in particular, but also worldwide, represents a major threat to human health. Although glyphosate is already for many years the most heavily applied pesticide worldwide, its usage continues to rise (Benbrook, 2016; Myers et al., 2016). This means that the occurrence and persistence of glyphosate and AMPA in the environment also continues to rise, and the risk of their accumulation in the environment is equally rising. With it the animal and human exposure risk rises as well. Such constant presence of these compounds in the environment and their tendency to increase in concentration in the coming years leads me to state that animal and human exposure to glyphosate and AMPA have become a long-term chronic exposure problem rather than just an acute exposure problem. This position is supported by the recent publication by van Bruggen et al. (2018) who state that there is increasing evidence on animal data suggesting the occurrence of health effects associated to chronic and ultra-low doses due to the accumulation of glyphosate and AMPA in the environment.

When looking into Argentina in particular, increasing cases of cancer, miscarriages and birth defects have been reported in inhabitants living in rural areas or at the borders of cities surrounded by agricultural fields regularly sprayed with glyphosate and other pesticides, including the province of Córdoba (Leguizamón, 2014; López et al., 2012; Lantieri et al., 2009). Attempts to link these health effects to glyphosate through epidemiological studies have been made (Leguizamón, 2014; López et al., 2012). However, the use of many other pesticides, including other herbicides such as 2,4-D and dicamba, mixed with glyphosate makes it difficult to prove such link (Leguizamón, 2014; López et al., 2012). Besides the ubiquity of these compounds in the different environmental compartments, they are also being regularly detected in food, feed and water (Myers et al., 2016; Mesnage et al., 2015). This means that glyphosate and AMPA are being regularly ingested, but also inhaled through contaminated dust, as proven by the wind tunnel experiment (chapter 3) and by Aparicio et al. (2018).

Despite the increasing exposure to glyphosate-based herbicides, glyphosate continues to be considered safe for humans by some parties (Mink et al., 2012; Williams et al., 2012; Mink et al., 2011). Except for the IARC (IARC, 2015), this same belief is shared by most competent authorities such as EFSA (EFSA, 2015), the European Chemicals Agency (ECHA), the Joint Food and Agriculture Organization of the United Nations – World Health Organization Meeting on Pesticide Residues (JMPR) and several national authorities outside the EU such as Canada, Japan, Australia and New Zealand (EU, 1995-2018). This has resulted in the most recent approval of glyphosate for another 5 years in the EU by the European Commission and member states (EU, 2017a, 1995-2018). Notwithstanding, because there is evidence that the adjuvant POEA is the one posing a risk to human health instead, the EU banned this co-formulant from glyphosate-based herbicides in 2016 (EU, 2017a, 2016b, 1995-2018).

Glyphosate is amongst the herbicides with the highest maximum residue levels (MRLs) in most pulses, cereals, oilseeds, oil fruits and wild fungi, ranging between 2 and 50 ppm, whereas it is normally ≤0.1 ppm for most of the other herbicides (EU, 2016a). In the USA, the MRLs can go even higher, up to 400 ppm in animal feed (USEPA, 2002). Glyphosate is also amongst the pesticides with the highest acceptable exposure limits, normally referred to as chronic reference dose (cRfD) in the USA or as acceptable daily intake (ADI) in the EU (Myers et al., 2016). The cRfD in the USA for glyphosate is 1.75 ppm bw day^{-1} (Myers et al., 2016; USEPA, 2002). In the EU, the ADI was 0.3 ppm bw day^{-1} since 2002, but it was increased to 0.5 ppm bw day^{-1} in 2017, with the recent approval of glyphosate (EU, 2017c). Nevertheless, an acute reference dose (ARfD) equivalent to the ADI was established as well for the first time in the EU (it was nonexistent before this approval) (EU, 2017c, 2002). Because all these limits are normally based in toxicity studies, this means that glyphosate is indeed less harmful to humans than the other herbicides and pesticides in general. However, there can also be adverse side effects from long term exposure.

According to Mesnage et al. (2015), there is a “coherent body of evidence indicating that glyphosate-based herbicides could be toxic below the regulatory lowest observed adverse effect level for chronic toxic effects”. Nevertheless, with the advent of GR crops and the consequent need to accommodate the glyphosate use patterns associated with them, regulators have allowed an increase in the MRLs in several crops (e.g. maize, soybeans, canola, alfalfa) and related livestock feeds, and consequently on the acceptable exposure limits (Myers et al., 2016). Unfortunately, these exposure limits and MRLs were set based on old, outdated studies, performed before the substantial increase on glyphosate use currently observed, particularly in the USA (Myers et al., 2016).

All things considered, it can be inferred that the current excessive use of glyphosate-based herbicides worldwide, the consequent increase of animal and human exposure through ingestion of contaminated food and water as well as the risk of inhalation associated to dust, and the results from animal and epidemiological studies (many revised in chapter 1), point to the need for policy makers to revisit glyphosate toxicity and the tolerable exposure/daily intake limits, and to consider long-term chronic exposures more carefully in their evaluations. Other studies have also reported this same concern and need (van Bruggen et al., 2018; Benbrook, 2016; Myers et al., 2016; Mesnage et al., 2015).

6.3.2 Implications and recommendations for pesticide regulatory and supervision authorities

Public concerns regarding the environmental and human health safety of glyphosate-based herbicides is immense. Pesticide regulators and policy makers in general are, therefore, under intense pressure to take proper action. However, the lobbying and economic interests behind pesticide corporations strongly contrast with the public opinion. It is certainly not an easy task for pesticide regulators and policy makers.

According to the EU rules, there is a distinction between active substances, which are approved at the EU level, and the authorization of plant protection products (PPPs or commercial formulations), which are the responsibility of national authorities in each EU Member State (EU, 2017b, 1995-2018). This means that at a regulatory level, glyphosate is tested alone in toxicity studies for its approval, even though it is never used in this form but rather as a mixture with adjuvants in commercial formulations (Mesnage et al., 2015). Nevertheless, many studies have demonstrated that glyphosate formulations and/or the surfactant POEA are more toxic than glyphosate itself (Bonfanti et al., 2018; Mesnage et al., 2013; Duke and Powles, 2008; Bringolf et al., 2007; Peixoto, 2005; Howe et al., 2004; Folmar et al., 1979). This challenges the concept and principle behind the approval of pesticides. Policy makers need to rethink the way pesticides are approved and need to consider an approach in which all ingredients and/or the whole mixture of a pesticide is taken into account. Some progress in this regard was made at the EU level when the European Commission banned the surfactant POEA in 2016 (EU, 2017a, 2016b). Such example should be followed by Argentinean authorities and other countries worldwide.

Moreover, pesticide regulators and policy makers need to reconsider the toxicity of glyphosate and its metabolites, particularly associated with long-term chronic low-dose exposures, and consequent ADI limits. AMPA is the major metabolite of glyphosate and it has been proven to be as (or even more) toxic as glyphosate (Roustan et al., 2014; Benachour and Séralini, 2009). Nevertheless, it is not clear if AMPA and other toxic metabolites are regulated, although EFSA (2015) has noted that AMPA presents similar toxicity to that of glyphosate and that glyphosate's reference values apply to AMPA. The pesticide database platform from the European Commission only presents MRLs for active ingredients, no reference is made to AMPA or metabolites in general (EU, 2016a). In the glyphosate renewal review report, presented ADI limits only refer to glyphosate, no mention is made to AMPA (EU, 2017c). The pesticide database from the European Commission should be extended to metabolites.

Another point that must be considered by pesticide regulators and policy makers is the need to legislate MRL values of pesticides, including glyphosate and AMPA, in soils. Regulations exist for MRLs in drinking water, food and feed, but they are nonexistent for soils. Such nonexistence of regulatory MRLs for soils represents a very high risk of on-site soil pollution with pesticides and, consequently, of off-site transport and animal and human exposure. The regulation of MRLs for pesticides in soils would certainly prevent soil pollution, but most importantly would reduce their presence in the soil environment, with a consequent reduction of the off-site transport and exposure risks of pesticides.

Although the use of pesticides may have been seen as the only viable solution to increase food production many decades ago, there are nowadays an increasing number of alternatives that could be as efficient. Some of these alternatives are discussed in section 6.3.3. Policy makers and pesticide regulators need to promote and ultimately require a shift from a herbicide-based agriculture to a more environmentally sustainable and even herbicide-free agriculture. Technological methods already exist and new developments are increasingly of interest, especially in a society with an increasing demand for organic/biological food. Therefore, policy makers have the obligation and public responsibility to increase their efforts towards a more sustainable and environmentally friendly agriculture, by promoting and applying concrete measures to (agribusiness) farmers towards a significant reduction or even complete elimination of the use of herbicides.

An example of concrete measures would be devoting more investment and research funding to Private Public Partnership projects focused on the development of cost-effective non-herbicide weed management alternatives, as well as to the improvement of already existing solutions. In Argentina, for example, local authorities could make use of their experimental stations, belonging to INTA (Instituto Nacional de Tecnología Agropecuaria – National Institute of Agricultural Technology) and spread all over the country, to invest in research and field tests on alternatives to herbicides (either elimination or significant reduction). Thereafter, Argentinean authorities should require the start of awareness programs based on demonstrations to agribusiness farmers. To guarantee their effectiveness, policy makers and local governments could make these demonstration programs mandatory. Similar approaches should be performed and become mandatory in other countries and in Europe, using existing and available facilities.

Supervision authorities also need to more regularly inspect the application of the regulations and use of recommended doses, and to monitor and make publicly available data on the types and amounts of pesticides applied. Information on treated crops, amount of pesticide used per application and per year, frequency of application, and residues in soil, surface and groundwater, and the atmosphere is needed. Although this type of information, or part of it, is available in some countries, it does not exist or is extremely difficult to obtain in most countries, including Europe. The existence of such information as a mandatory regulation would promote transparency and increase public trust. It would also allow for a better evaluation of the need for new measures towards a more sustainable and healthy environment.

In Argentina, some regulatory developments have been achieved, but they were rather provincial regulations. In the province of Córdoba, a law (law no. 9164) was approved in 2004 restricting the aerial and terrestrial application of pesticides within a specific radius from the limits of cities and rural agglomerations, according to toxicological classes. Similar laws were applied in some other provinces. The restrictions were: for aerial applications – prohibition to apply pesticides from classes I and II in a radius <1500 m, and prohibition to apply pesticides from classes III and IV in a radius <500 m; for terrestrial applications – prohibition to apply pesticides from classes I and II in a radius <500 m, but pesticides from classes III and IV can be applied to the limits of the cities or rural areas. Glyphosate belongs to class III (WHO, 2010), therefore it can be applied by terrestrial means up to the limits of the cities and rural areas. Such a law aims to reduce human exposure due to spray drift, however, it does not protect from contaminated dust. To address this, Argentinean local authorities should install physical biological barriers around the limits of cities and rural agglomerations, such as evergreen trees or shrub fences. This would at least reduce the risk of inhalation of contaminated air/dust by inhabitants living in the vicinities of the agricultural fields.

6.3.3 Recommendations for a more sustainable agricultural management

Alternatives that are environmentally and economically appealing are starting to arise for a herbicide-free agriculture. But certainly, in order to convince (agribusiness) farmers, the alternatives need to be reliably effective, cost-effective, economically viable for farmers (i.e. the same or less costly as current herbicide-based practices), less or as time consuming as the current herbicide-based practices and not complicated (Shaner and Beckie, 2014).

As a short-to-medium-term solution, it is recommended that farmers, particularly agribusiness farmers, make a greater effort to apply herbicides based on precision agriculture technology. The adoption of this type of technology avoids the uniform spraying of herbicides in entire field areas, while also minimizing herbicide costs and preventing unnecessary environmental risks. Machines already exist and are in use by agribusiness farmers that are equipped with global positioning systems (GPS) for the electronic mapping of yields within an agricultural field. Such maps are further used in the sowing tractors to apply site-specific variable-rate fertilizers according to the field needs. Such GPS mapping-based technology or similar approaches could be used for the application of herbicides.

Some of this technology already exists for weed control (Nordmeyer, 2015; Shaner and Beckie, 2014; Lopez-Granados, 2011; Slaughter et al., 2008). Examples of variable-rate site-specific herbicide applications include technologies based on sensors, such as vision machines, spectral analysis and remote sensing (Shaner and Beckie, 2014; Lopez-Granados, 2011; Slaughter et al., 2008; Lee et al., 1999). Such technologies are, however, still facing some technical challenges (Shaner and Beckie, 2014; Lopez-Granados, 2011). Therefore, incentives need to be created to improve the quality of this type of technology and further convince agribusiness farmers, who are financially capable, to invest in it.

As medium-to-long-term solutions, greater effort is recommended on the development of reliable herbicide-free alternatives that (also economically) can convince agribusiness farmers to move away from herbicide-based agriculture, or at least significantly reduce the amount of herbicides applied every year. Several herbicide-free solutions have already been developed but need to be improved and to gain visibility among (agribusiness) farmers. In a society with increasing interest in technology, the development of robotics for weed management or the use of nanotechnology is already a reality, and the use of drones is also triggering interest (Shaner and Beckie, 2014; Lopez-Granados, 2011).

In the field of robotics, a number of unmanned and autonomous vehicles for inter- and intra-row weeding have already been developed and sound a promising technology (Shaner and Beckie, 2014; Slaughter et al., 2008). Robotic systems can comprise mechanical, thermal, chemical and electrical weed control solutions (Slaughter et al., 2008). A few examples of mechanical robotic weeders are knife weeders (Hussain et al., 2018; Pérez-Ruiz et al., 2012; Slaughter et al., 2008), rotating hoe weeders (Hussain et al., 2018; Slaughter et al., 2008; Åstrand and Baerveldt, 2002), torsion-weeders (Ascard and Fogelberg, 2002), among others. Camera equipped drones have not yet been adapted for weed control management, but are also a promising emerging technological solution that could offer low-cost sensing and highly flexible spatial resolution for site-specific herbicide applications (Shaner and Beckie, 2014; Lopez-Granados, 2011) or for a better management plan on where to send autonomous vehicles for mechanical weeding.

Other alternatives also exist, including the use of better cover and green manure crops that create unfavorable conditions for weed germination and growth, strategic and precision tillage in order to not jeopardize erosion control measures promoted by non-tillage systems, crop rotation, and the use of biological/natural products for weed control (Shaner and Beckie, 2014). For the latter, very few bio-herbicides have been created and commercialized so far, and advancements on this type of weed control are still very limited (Shaner and Beckie, 2014). Moreover, most of these type of measures are clearly not appealing to farmers in general, particularly agribusiness farmers, otherwise they would have been already extensively adopted.

A total ban of glyphosate-based herbicides is being strongly requested by society, particularly in Europe and Argentina. I do agree that the use of glyphosate-based herbicides needs to be strongly reduced. However, under current agricultural management practices, I am of the opinion that a total ban of glyphosate may result in a greater environmental and human health problem rather than a solution. This is because, under current agricultural management practices that strongly rely on herbicides, and in the absence of readily available and sufficiently tested non-chemical alternatives, a total ban of glyphosate will likely result in its substitution by other, more toxic herbicides, posing a greater risk to the environment and human health. In fact, companies have already created new GMCs to be resistant to 2,4-D and dicamba herbicides, for example, which are more toxic than glyphosate. Therefore, before a probable ban of glyphosate, it is necessary that society demands from governments and policy makers a shift in agricultural management practices from a herbicide-based system to a herbicide-free system. Society needs to demand that greater governmental and industrial funds are applied to the development and improvement of herbicide-free alternatives.

Special focus is required on herbicide-free solutions for the growing of soybeans, maize and cotton under non-tillage systems, as these are amongst the main crops produced by agribusiness farmers in Latin America and the USA, and on which the greatest proportion of glyphosate-based herbicides are applied. Society also needs to demand from governments and policy makers that, in the medium/long term, regulations are created to make these herbicide-free solutions mandatory and to progressively ban or strongly restrict the use of herbicides. Only in this way, will a ban of glyphosate have beneficial impacts to the environment and human health.

6.4 Research challenges and future research directions

This PhD thesis has focused on the decay behavior and wind and water erosion related transport pathways of glyphosate and AMPA in a loess soil environment, with special focus on loess agricultural areas with extensive and intensive production of GR crops under non-tillage systems. While this research clearly contributes to a better understanding of the processes involving glyphosate and AMPA fate in the soil environment, a number of topics remain to be investigated and assessed, namely:

- A deeper understanding of the environmental fate of glyphosate and AMPA in other soil types, in different crop systems and under different agricultural management practices, such as tillage;
- A deeper understanding of the formation and decay behavior of AMPA using longer experimental periods under laboratory and field conditions;
- Controlled studies on how, for example, crop type, glyphosate formulation and pesticide mixtures affect glyphosate and AMPA adsorption, decay, glyphosate degradation pathways (AMPA vs. sarcosine), AMPA formation and decay, and glyphosate and AMPA off-site transport;
- Field monitoring studies of the off-site transport of glyphosate and AMPA with wind erosion;
- Long-term (>1 year) field monitoring studies of the off-site transport of glyphosate and AMPA with water erosion and in agricultural fields receiving repeated glyphosate applications;
- Field studies of the off-site terrestrial deposition of glyphosate and AMPA;
- Comparative field studies of the environmental fate of glyphosate and AMPA (decay, off-site transport) between large-scale farming systems (agribusiness) and small-scale farming systems (family based, subsistence farming and/or small commerce in local markets).

Another topic that needs further attention from the scientific community is the surfactants used in pesticide formulations. This PhD thesis focused exclusively on glyphosate and AMPA, but the surfactant POEA may be as or more important than glyphosate itself, since it has been shown by several studies to be more toxic than glyphosate alone. Therefore, studies focusing on the so called “inert diluents”, that are these surfactants, need special attention. More studies on their environmental fate and occurrence, particularly those with already proven toxicity such as POEA, are required.

Lastly, further attention is needed from the scientific community for investigation of the impacts and effects of pesticide mixtures (including glyphosate) on the environment and human health. Further attention from the scientific and industrial communities is also needed for the development of new, and improvement of existing, economically and environmentally sustainable herbicide-free solutions for agriculture that promote sufficient and at least equal yields as current herbicide-based agricultural practices.

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English summary

For many decades now, the use of pesticides is considered the most viable solution in conventional agriculture to increase food production. As a result, great amounts of pesticides are applied every year in agricultural fields. Amongst all pesticides applied, glyphosate-based herbicides lead the market worldwide for more than two decades, with more than 825 000 tons sold globally in 2014, mostly as a result of the introduction of glyphosate-resistant (GR) crops in 1996 by Monsanto company. The huge and still increasing use of glyphosate-based herbicides is leading to its frequent occurrence in the different environmental compartments, as well as in feed and food. Despite being sold by pesticide companies as an environmentally friendly product and of no concern to the environment and human health, its intensive use worldwide is resulting in high social concerns about its safety, especially after being classified as possible carcinogenic to humans in 2015 by the International Agency for Research on Cancer (IARC).

Loess soils are amongst the most productive and fertile soils and, consequently, are intensively used for agriculture. They represent around 10% of the Earth's land surface, with major deposits occurring in China, USA, Argentina and Europe. Of particular interest are the loess deposits from Argentina and USA, which are intensively used for the production of GR crops and, consequently, are heavily subject to the application of glyphosate-based herbicides every year. Because loess soils are easily erodible by water and wind, the advent of GR crops promoted a change in agricultural management practices from tillage to non-tillage systems. Consequently, weeds are controlled chemically rather than mechanically before and after sowing. Such an agricultural management change helped reducing water and wind erosion problems faced by farmers, but also resulted in the excessive use of glyphosate-based herbicides.

Despite being the most used pesticide worldwide, the environmental fate of glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) is still not well understood. The decay behavior and persistence of these compounds is very variable across different soils and climatic regions, and AMPA is poorly studied so far. Although these compounds strongly adsorb to soil particles, studies on their off-site transport with wind erosion were nonexistent until very recently (2018), and most studies on their transport with water erosion disregarded the particle-bound phase.

In this context, this PhD thesis aims at better understanding the environmental fate of glyphosate and AMPA in the loess soil environment. Special focus is given to: 1) the decay

kinetics of glyphosate and the formation and decay kinetics of AMPA; 2) the off-site transport of glyphosate and AMPA associated to the particle-bound phase, as a result of wind and water erosion. These processes were studied under laboratory (chapters 2 to 4) and field (chapter 5) conditions. The field study was performed in agribusiness fields of the loess Pampas of Argentina.

Chapter 2 investigates the decay kinetics of glyphosate and the formation and decay kinetics of AMPA in loess soil under laboratory conditions. The combined effect of temperature (5 and 30°C), soil moisture (20% WHC, 60% WHC, saturation) and light/darkness under biotic and abiotic conditions was addressed. Glyphosate and AMPA decay was mostly a microbiological process; abiotic (photodegradation) processes were negligible. Under biotic conditions, glyphosate followed first-order (SFO) kinetics at 5°C, but bi-phasic (FOMC) at 30°C. Efforts are needed to apply the correct decay kinetics for glyphosate in environmental fate models to avoid the underestimation of glyphosate's DT_{90}. AMPA followed always SFO kinetics. A higher formation of AMPA was observed at 5°C (54-100%) than at 30°C (37-48%). Persistence of glyphosate (DT_{50}: 1.5-54 days; DT_{90}: 8-280 days) and AMPA (at 30°C - DT_{50}: 26-45 days; DT_{90}: 88-148 days; endpoints not achieved at 5°C) was very variable, depending on temperature and soil moisture conditions. From the DT_{50} and DT_{90} values, it can be concluded that AMPA persisted longer than glyphosate for all conditions. Glyphosate and AMPA decayed fastest under warm and moist soil conditions and slowest under cold and dry soil conditions. Therefore, repeated glyphosate applications under dry and cold conditions, as they happen in fallows or before sowing winter crops, may lead to glyphosate and AMPA accumulation in soil and to on-site soil pollution.

Chapter 3 investigates the role of wind erosion on the atmospheric transport of glyphosate and AMPA. This experiment was performed with loess soil in a wind-tunnel. The distribution of glyphosate and AMPA in different size fractions of the wind-eroded sediment and the relationship with clay, silt and organic matter (OM) were investigated. An estimation for the potential off-site transport of these compounds over large distances from the pollution source was also performed. The results indicated that glyphosate and AMPA contents are highest in the finest particle fractions of the wind-eroded sediment (<18 µm), decreasing significantly with increasing particle size. The results also showed that glyphosate and AMPA correlate positively with the clay, OM and silt contents of the wind-eroded sediment (clay > OM > silt). The low soil moisture content of the sediment resulted in a very low decay of glyphosate and formation/decay of AMPA over time. We concluded that the risk of glyphosate and AMPA to be transported to the atmosphere over large distances is very high, due to the low decay of glyphosate in dry sediment and the highest glyphosate and AMPA contents in PM_{10}. Consequently, because PM_{10} or smaller particles are easily inhaled, the risk of human exposure to contaminated dust is also high. Therefore, more attention needs to be paid to this route of exposure in risk assessment studies.

Chapter 4 investigates the influence of small-scale sediment transport and of different soil surface micro-topographies on the off-site transport of glyphosate and AMPA with water erosion. The redistribution, transport and deposition onto the loess soil surface was also assessed. Smooth soil surfaces and soil surfaces resembling seeding lines on the contour were investigated, using soil flumes (1 x 0.5 m) with a 5% slope. Glyphosate (178 mg m^{-2}) was sprayed on the upper 0.2 m of the flumes. Four 15-min rainfall events with 30-min interval in between and a total rainfall intensity of 30 mm h^{-1} were applied. When taking into account the off-site deposition to downslope soil surface areas, glyphosate and AMPA particle-bound off-site transport was higher (9.4-17.8%) than the water-dissolved one (0.5-2.8%). When only considering the off-site transport to the outlet of the flume (i.e. excluding the downslope terrestrial deposition), glyphosate and AMPA water-dissolved off-site transport was higher (0.5-2.8%) than the particle-bound one (0.2-1.7%). The results have shown that the presence of micro-topographic disturbances (“seeding lines”) reduces the runoff and erosion rates into the outlet of the flumes and, consequently, the off-site transport of glyphosate and AMPA to aquatic environments. However, their off-site deposition to downslope terrestrial environments is enhanced. The results also indicated that the off-site downslope transport of these compounds reduced with increasing distance from the source, for both types of soil surfaces. We concluded that the particle-bound transport of glyphosate and AMPA is as or more important than their water-dissolved transport. The pollution risk of off-target terrestrial and aquatic environments can be considerable. Buffer zones, promoted by soil (micro-)topographic barriers, within the treated areas as well as at the downslope edges of treated fields are recommended to reduce the off-target transport of these compounds into terrestrial and aquatic environments.

Chapter 5 investigates the dynamics of glyphosate and AMPA in the soil surface of fields cultivated with GR crops (soybean and maize) in the loess Pampas of Córdoba province, in Argentina. The cultivation areas studied have a long-term history of repeated applications of glyphosate-based herbicides. Focus was given to the role of decay kinetic processes and to the off-site transport risk. Glyphosate decay kinetics and AMPA formation/decay kinetics were investigated after a single application with the glyphosate commercial formulation Roundup UltraMax© (soybean area: 1.0 kg a.e. ha^{-1}; maize area: 0.81 kg a.e. ha^{-1}). Moreover, glyphosate and AMPA concentrations were quantified in runoff water and eroded sediment after 2 erosive rainfall events. Under both cultivation areas, background residues in soil before application were 0.27-0.42 mg kg^{-1} for glyphosate and 1.3-1.7 mg kg^{-1} for AMPA. Glyphosate and AMPA contents were higher in the top 0-1 cm soil surface layer and were significantly lower in the subsequent soil surface layer (1-2 cm). Therefore, leaching of these compounds into deeper soil layers and groundwater seems to be limited. Glyphosate followed SFO decay kinetics in the soybean cultivation area, but bi-phasic (Hockey-Stick – HS) in the maize cultivation area. The latter was due to an abrupt change in the soil moisture conditions from dry to moist after a significant rainfall event, which resulted in an increase of the glyphosate decay rate from 0.06 to 0.37 days^{-1}. AMPA followed SFO decay kinetics, but its formation (5-24%) and decay rates (0.01 days^{-1}) were low. Glyphosate DT_{50} in soil varied between 6 (soybean) and 11 (maize) days, whereas its DT_{90} varied between 15 (maize) and 20 (soybean) days. AMPA persisted much longer in soil than glyphosate, with its DT_{50} varying between 55 (soybean) and 71 (maize) days and its DT_{90} between 182 (soybean) and 236 (maize) days. The low decay rates of AMPA in both cultivation areas indicate that it persists for long periods in soil and suggest that it may be accumulating in soil. During erosive rainfall events, higher glyphosate and AMPA contents were observed in eroded sediment than in runoff water. Glyphosate and AMPA contents were also 1-18 (glyphosate) and 0.8-8 (AMPA) times higher in water-eroded sediment than in soil. The results indicated that the risk of glyphosate and AMPA off-site transport with water erosion can be considerable, even 2 months after glyphosate application, particularly associated to sheet erosion. The tendency of glyphosate and AMPA to remain in the top soil surface layer also suggests that wind erosion may play an important role on the off-site transport of these compounds, particularly when soil cover with stubbles is low, before sowing and after sowing until the crop covers sufficiently most of the soil surface.

Overall, this PhD thesis improved the knowledge and furthered the science of the environmental fate of glyphosate and AMPA in the loess soil environment. This is particularly true for AMPA, which until now has received little attention. This PhD thesis is a major contribution to the understanding of glyphosate decay kinetics and particularly of AMPA formation and decay kinetics. Glyphosate decay kinetic studies presented in this PhD thesis indicate the need to incorporate bi-phasic kinetic models in environmental fate modelling studies in order to more reliably determine glyphosate persistence in soil. More and longer studies are still required under laboratory and field conditions to better understand the formation and decay behavior of AMPA. Another major contribution of this PhD research regards the off-site transport of glyphosate and AMPA with wind erosion, a transport pathway that has been disregarded so far. Field studies focusing on the off-site and long-range transport of glyphosate and AMPA with wind erosion are needed in different types of soil and in fields under different tillage systems. Glyphosate and AMPA risk assessments need to consider the exposure to contaminated dust. This PhD thesis also contributed for a better understanding of glyphosate and AMPA off-site transport with water erosion, not only to aquatic environments but also to terrestrial environments. Chapter 6 concludes with recommendations to pesticide regulatory and supervision authorities and farmers for a more sustainable agricultural management, suggesting several alternatives to significantly reduce the use of glyphosate-based herbicides and to promote a shift for a herbicide-free agricultural system.

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Célia Bento

About the author

Célia Bento was born on 27 January 1982 in Coimbra, Portugal. In 2006, she obtained her BSc degree (3+2 year major – pre-Bologna) in Environmental Engineering from the Polytechnic Institute of Coimbra / Agriculture Higher School of Coimbra (IPC/ESAC), Portugal. During her BSc, she did 2 theses and a voluntary extra-curricular training. The first BSc thesis focused on assessing the global and per stage removal efficiency of the Wastewater Treatment Plant of Olhalvas, in Leiria, Portugal. Thereafter, she did a 1-year voluntary extra-curricular training in the EMAS@SCHOOL Project (LIFE03 ENV/P/00501) focused on the quantitative and physical-chemical characterization of various livestock and green waste materials to assess the construction of a composting unit on the ESAC campus. In 2006, she completed her second BSc thesis on the assessment of soil fertility under different land uses and soil types in Maroon communities of the state of Mato Grosso, Brazil. This was part of the EU-project Guyagrofor (EU FP6 funding, https://cordis.europa.eu/project/rcn/73705_en.html).

From 2007 to 2012, Célia worked as a research assistant at IPC/ESAC in EU FP6 funded projects. She worked with the EU-project Guyagrofor until it ended in 2010. During this time she returned several times to the Mato Grosso area of Brazil and also completed a post-Bologna MSc programme in Environmental Engineering at the University of Aveiro, Portugal (2009) based on her work as a research assistant in the Guyagrofor EU-project. Her MSc thesis focused on sustainable management techniques in Agroforestry systems to improve soil fertility and soil water conservation, again in Maroon communities of Mato Grosso. Between 2010 and 2012, she worked with the EU-project DESIRE (http://www.desire-project.eu), in close collaboration with the project team of Swansea University, UK assessing post-fire soil erosion in burned forest areas of Portugal.

In 2011, Célia applied for a PhD grant from the Portuguese Foundation for Science and Technology (Fundação para a Ciência e a Tecnologia - FCT), which was approved. As a consequence, in May 2012, she started her PhD study at the Soil Physics and Land Management (SLM) group of Wageningen University, in The Netherlands. Her PhD project, as presented in this dissertation, has concerned the environmental fate of glyphosate and AMPA in the loess soil environment, with special focus on the decay behavior of these compounds and their off-site transport by water and wind erosion. The main study site for this project was in Argentina. During her PhD, Célia supervised several MSc students and presented her research results at several international and national conferences.

Currently, Célia works as a post-doc researcher in the Environmental Chemistry department of the Institute for Coastal Research, based at the Helmholtz-Zentrum Geesthacht (HZG), in Germany.

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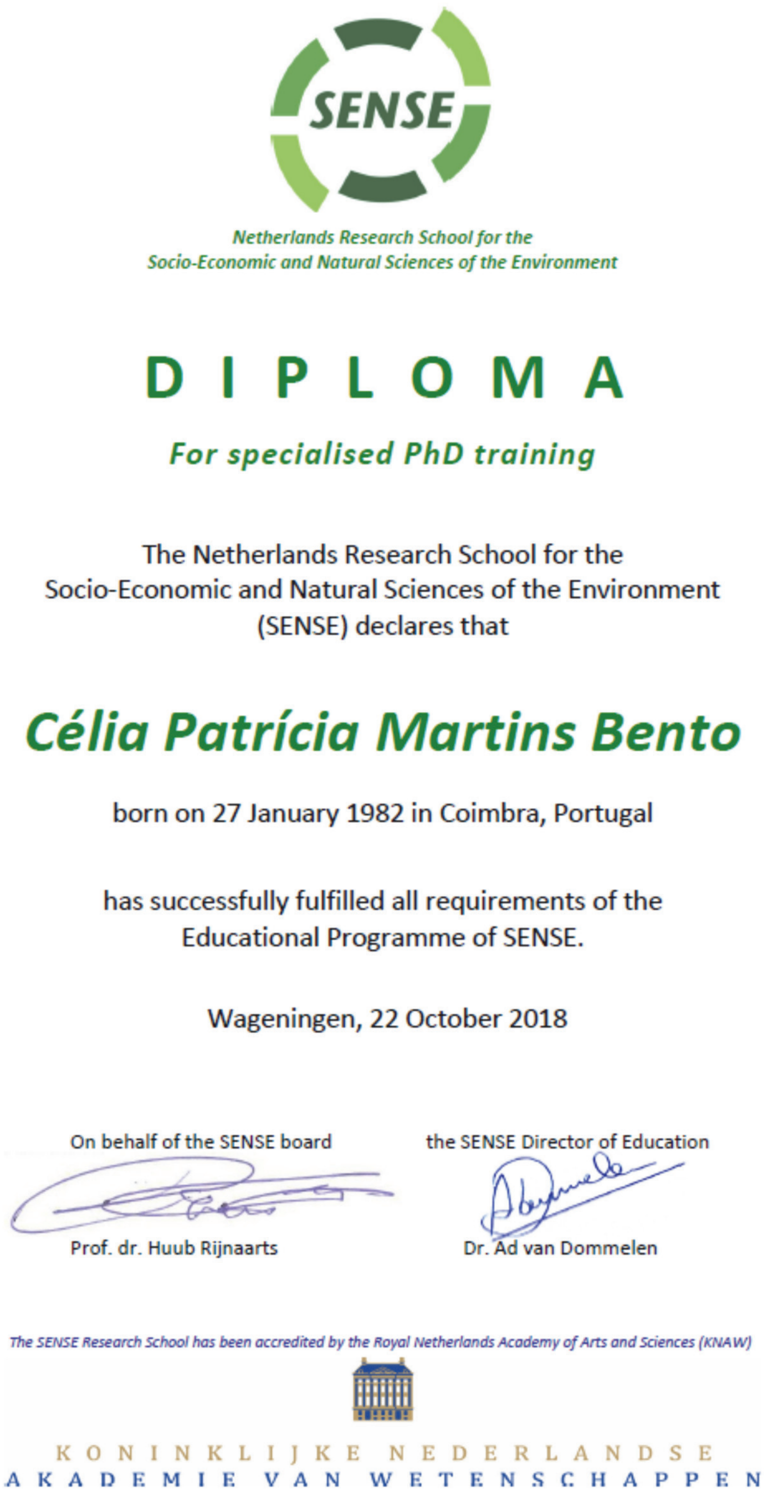

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- Environmental research in context (2012)
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- Research in context activity: 'Co-organizing SENSE-WIMEK Symposium on Hazard, Risk and Sustainability in the Soil Environment, Wageningen, 14 October 2015'

Selection of other PhD and Advanced MSc Courses

- Techniques for Writing and Presenting a Scientific Paper (2013)
- Environmental Analytical Techniques, Wageningen University (2013)
- Career Assessment (2016)

Site specific training and activities

- High-Performance Liquid Chromatography tandem Mass Spectrometry (HPLC-MS/MS), RIKILT, Wageningen (2013)
- Revision of the "Government review of the IPCC AR5 SYR Report, Ministry of Infrastructure and the Environment, The Netherlands (2014)

Management and Didactic Skills Training

- Supervising four MSc student with thesis (2014-2017)
- Supervising BSc student with internship entitled 'Exploratory study on human health by exposure to pesticides in the province of Córdoba, Argentina' (2014)
- Teaching in the MSc course 'Genetically Modified Soybeans and the use of glyphosate (herbicide) in Argentina' (2014)
- Teaching in the MSc course 'GMOs and glyphosate in Argentina – Off-target transport and related consequences to the environment and human health' (2015)

Oral Presentations

- *Wind erosion processes and related glyphosate transport in the loess Pampean region of Córdoba province, Argentina.* Conference on Desertification and Land Degradation, 17-18 June 2013, Ghent, Belgium
- *Wind erosion as an environmental transport pathway of glyphosate and AMPA.* EGU General Assembly Conference, 26-27 April 2017, Vienna, Austria

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