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CICLO XXXII

# Environmental fate of herbicides: the application of mitigation measures and the role of conservation agriculture

**Coordinatore:** Ch.mo Prof. Sergio Casella
**Supervisore**: Prof. Roberta Masin
**Co-Supervisore**: Prof. Ester Marotta

**Dottoranda**: Laura Carretta

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# Abstract

Nowadays, agriculture is a production system predominantly controlled by human interventions, and an increasing concern is addressed to the environmental sustainability of the overall production processes. The European Union encourages the adoption of conservative agriculture as a tool to improve and sustain productivity while preserving the resources and the environment. Conservation agriculture, and in particular no-till systems, can improve soil structure and increase water infiltration, thus reducing runoff and soil erosion. However, the transition phase from conventional to conservation agriculture can pose several issues. Firstly, the lack of mechanical weed control makes weed management mainly based on herbicides. Secondly, the soil structure is still not mature and the soil tends to be prone to compaction. This could favor runoff and soil erosion, increasing the herbicides losses from the field. Among the existing mitigation measure, buffer strips are considered an effective conservation practice to reduce herbicide runoff from cultivated fields owing to the ability of vegetation to delay surface runoff, promote infiltration, and adsorb herbicides. Among the most used herbicides, both in conservation and in conventional agriculture, there is glyphosate, for weed control of the seedbed, and terbuthylazine, S-metolachlor, and mesotrione, after seeding. They are among the most frequently found pesticides in surface waters and groundwater in Italy. Therefore, it is critical to study their environmental fate once they reach the soil. Glyphosate in water and soil is primarily degraded by microorganisms, and aminomethylphosphonic acid (AMPA) is the main metabolite of glyphosate. Several studies on the environmental fate of glyphosate and AMPA have been done, but there is a lack of information about the effect of the co-formulants contained in glyphosate-based formulations on its environmental behavior. To study the environmental fate of glyphosate and AMPA it is necessary to have a sensitive and reliable analytical method. Nevertheless, the analysis of glyphosate and AMPA in water and soil is challenging due to their polar character, low volatility, and low mass, and lack of chemical groups that could facilitate their detection.

Given these premises, the overall aim of this PhD was to study the environmental fate of the above-mentioned herbicides, to evaluate the application of buffer strips as a mitigation measure, and to assess the role of conservation agriculture with regard to surface runoff and soil loss.

Chapter 1 investigates the role of conservation agriculture in runoff and soil erosion processes. This study analyzes runoff and sediment loss in a non-tilled field during the transition period from conventional to conservation agriculture in comparison to a tilled field. Chapter 2 presents an analytical method for the determination of glyphosate and AMPA in water samples by using Ultra-High Performance Liquid Chromatography coupled with mass spectrometry (UHPLC-MS/MS). The method was applied to the glyphosate and AMPA analysis in runoff water to evaluate the

efficacy of six m wide buffer strips in mitigating the runoff of these pollutants from a field in transition from conventional to conservation agriculture. Chapter 3 study the efficacy of buffer strips in reducing runoff of terbuthylazine, S-metolachlor, and mesotrione from a non-tilled field during the transition period from conventional to conservation agriculture. Four types of buffer strips with different length and composition were examined in comparison to the no-buffer situation. Chapter 4 presents an analytical method for the determination of glyphosate and AMPA in soil samples by using UHPLC-MS/MS. The method was applied to evaluate the dissipation of glyphosate and the formation/dissipation of AMPA from a field managed with conservation agriculture compared to a conventionally managed field. Chapter 5 investigates the effect of soil texture and depth on the dissipation of terbuthylazine, S-metolachlor, and mesotrione in cornfield soils. Chapter 6 examined the influence of sewage amendment on the dissipation of terbuthylazine and S-metolachlor in sandy clay soil. Chapter 7 investigate whether the presence of an alkyl polyglucoside based surfactant, Triton CG-110, has the potential to affect adsorption, leaching, and mineralization of glyphosate in two soils with different texture and washed sand.

With this thesis, insights have been gained about the environmental fate of these widely used herbicides, which can be important if we consider the widespread and recurrent use of these agrochemicals all over the world. However, the environmental fate of herbicides can vary greatly, even in the same type of soil and within the same geographical area. Therefore, further studies with different soil types and crop systems are required for a deeper understanding of the factors involved.

## Riassunto

Nei giorni nostri l’agricoltura è un sistema produttivo prevalentemente controllato dall’intervento umano, e una crescente preoccupazione è rivolta alla sostenibilità ambientale dei processi produttivi globali. L’Unione Europea incoraggia l’adozione dell’agricoltura conservativa come strumento per migliorare e sostenere la produzione, preservando al contempo le risorse e l’ambiente. L’agricoltura conservativa, e in particolare i sistemi di non-lavorazione del suolo, può migliorare la struttura del suolo e aumentare l’infiltrazione dell’acqua, riducendo così il ruscellamento superficiale e l’erosione del suolo. Tuttavia, la fase di transizione dall’agricoltura convenzionale a quella conservativa, può presentare diverse criticità. Prima di tutto, l’assenza di controllo meccanico delle malerbe rende la gestione delle infestanti prevalentemente basata sugli erbicidi. In secondo luogo, la struttura del suolo, non essendo ancora matura, tende a essere soggetta a compattamento. Questo può favorire il ruscellamento superficiale e l’erosione del suolo, andando ad incrementare le perdite di erbicida dal campo attraverso il ruscellamento. Tra le misure di mitigazione esistenti, le fasce tampone sono considerate uno strumento efficace nel ridurre il ruscellamento di erbicidi dai campi coltivati, grazie all’abilità della vegetazione di ritardare il ruscellamento, favorire l’infiltrazione e adsorbire gli erbicidi. Tra gli erbicidi maggiormente utilizzati, sia in agricoltura conservativa sia convenzionale, vi è il glifosate, per la pulizia del letto di semina, e la terbutilazina, l’S-metolaclor e il mesotrione dopo la semina. Essi sono tra i pesticidi più frequentemente trovati nelle acque superficiali e sotterranee in Italia. Per questo è di grande importanza studiare il loro destino ambientale una volta che essi raggiungono il suolo. Il glifosate nell’acqua e nel suolo è degradato prevalentemente dai microorganismi e il suo principale metabolita è l’acido aminometilfosfonico (AMPA). Sono stati condotti diversi studi sul destino ambientale di glifosate e AMPA, ma le informazioni sull’effetto dei co-formulanti contenuti nei formulati a base di glifosate sul suo destino ambientale sono per il momento molto scarse. Per studiare il destino ambientale di glifosate e AMPA è necessario poter disporre di un metodo analitico sensibile ed affidabile. Tuttavia, l’analisi di glifosate e AMPA in acqua e nel suolo è complessa per via del loro carattere polare, bassa volatilità, basso peso molecolare, e mancanza di gruppi chimici che potrebbero facilitare la rilevazione.

Date queste premesse, lo scopo complessivo di questo PhD è stato di studiare il destino ambientale degli erbicidi sopra menzionati, di studiare l’applicazione delle fasce tampone come misura di mitigazione e di valutare il ruolo dell'agricoltura conservativa per quanto riguarda il ruscellamento superficiale e l’erosione del suolo.

Il capitolo 1 studia il ruolo dell’agricoltura conservativa nei processi di ruscellamento superficiale ed erosione del suolo. Questo studio analizza il ruscellamento superficiale e la perdita di sedimento

in un campo non lavorato durante il periodo di transizione da convenzionale a conservativo in confronto con un campo lavorato. Il capitolo 2 presenta un metodo analitico per la determinazione di glifosate e AMPA in campioni acquosi usando la cromatografia liquida ad altissime prestazioni accoppiata con spettrometria di massa (UHPLC-MS/MS). Il metodo è stato applicato all'analisi di glifosate e AMPA in acqua di ruscellamento per valutare l'efficacia di una fascia tampone di sei metri nel ridurre il ruscellamenti di questi inquinanti da un campo in fase transizione tra agricoltura convenzionale e conservativa. Il capitolo 3 studia l'efficacia delle fasce tampone nel ridurre il ruscellamento di terbutilazina, S-metolaclor e mesotrione da un campo non lavorato in transizione tra agricoltura convenzionale e conservativa. Sono stati presi in considerazione quattro tipo di fasce tampone aventi diversa lunghezza e composizione e confrontati con una situazione in cui non vi era alcuna fascia tampone. Il capitolo 4 presenta un metodo analitico per la determinazione di glifosate e AMPA nel suolo usando l'UHPLC-MS/MS. Il metodo è stato applicato allo studio della dissipazione di glifosate e della formazione e dissipazione di AMPA in un campo gestito con agricoltura conservativa in confronto con un campo convenzionale. Il capitolo 5 si concentra sullo studio dell'effetto della tessitura del suolo e della profondità sulla dissipazione di terbutilazina, S-metolaclor e mesotrione in suoli coltivati a mais. Il capitolo 6 esamina l'influenza della fertilizzazione con liquame zootecnico sulla dissipazione di terbutilazina e S-metolaclor in un suolo argilloso-sabbioso. Il capitolo 7 infine indaga se la presenza di un surfattante alchilpoliglucosidico, il Triton CG-110, può influenzare l'adsorbimento, la lisciviazione e la mineralizzazione del glifosate in due suoli con differente tessitura e nella sabbia di quarzo lavata.

Con questa tesi, sono state acquisite informazioni sul destino ambientale di questi erbicidi ampiamente usati, che possono rivelarsi rilevanti se si considera l'uso diffuso e ricorrente di questi prodotti agrochimici in tutto il mondo. Tuttavia, il destino ambientale degli erbicidi può variare ampiamente, anche nello stesso tipo di suolo e all'interno della stessa area geografica. Perciò sono necessari ulteriori studi con differenti suoli e sistemi colturali per una più profonda comprensione dei fattori coinvolti.

# General introduction

The management of cropping systems is in constant evolution. Over the last decades, agriculture and crop management practices have shown deep changes to extend the cropped areas while efficiently controlling pests and diseases. Nowadays, agriculture is a production system predominantly controlled by human interventions. Soil tillage and agrochemicals application for weed and pest control are parts of these management practices. However, an increasing concern is addressed to the environmental sustainability of the overall production processes. The Common Agricultural Policy of the European Union (European Commission 2011) promoted conservation agriculture as a farming system able to deal with some major agro-environmental issues like the increasing carbon dioxide concentration in the atmosphere, the decreasing biodiversity, and the limited water availability (Basch et al. 2011; Armengot et al. 2015; Bouma and McBratney 2013). According to Food and Agriculture Organization (FAO), conservation agriculture is an approach to managing agro-ecosystems for improved and sustained productivity, increased profits and food security while preserving and enhancing the resource base and the environment (FAO 2014). Conservation agriculture aims to achieve sustainable and profitable agriculture through the application of three principles: (1) minimizing the mechanical soil disturbance (reduced tillage or no-tillage); (2) maintaining a permanent soil cover by using cover crops and crop residues; (3) diversifying crop rotations in the case of annual crops or plant association in the case of perennial crops (Hossain 2013). Conservation agriculture principles are universally applicable to all agricultural landscapes and land uses with locally formulated and adapted practices. In recent decades, there has been a great spread of conservation agriculture. In 2013, 10.9% of global croplands were under conservation agriculture, with the highest diffusion in South America (60%), but only 0.8% in Europe (Kassam et al. 2015). Reduced tillage or no-tillage is the most important component of conservation agriculture as minimal soil disturbance and permanent soil cover, the other two pillars of conservation agriculture, can only be achieved through the elimination of tillage (Shahzad, Farooq, and Hussain 2016). If applied collectively and accurately, conservation agricultural practices have a series of complementary positive outcomes. Firstly, no-tillage maintains a stable soil structure and biological activity. Secondly, the permanent organic soil cover protects the soil surface from erosion and helps to create a stable and favorable microclimate, while crop residues increase soil moisture in the soil profile, provide organic matter, reduce soil erosion, and improve soil fertility. Thirdly, crop rotations enhance the biodiversity of the system and therefore contributes to weed, pest, and diseases control (Berger, Friedrich, and Kienzle 2010). The improvement in soil structure can have also benefit on soil and water conservation as conservation agriculture can enhance the water infiltration which can, in turn, improve the water holding capacity and reduce the surface runoff and soil erosion. The reduction in runoff and soil erosion could be related to the increase in surface roughness, improved pore continuity, and surface crusting prevention provided by the permanent residue cover and the improved soil structure (Tarolli et al. 2019; Armand et al. 2009; Humberto Blanco-Canqui and Lal 2009). The mitigation of surface runoff with conservation agriculture, although still controversial, was observed in several studies (Montgomery 2007; Armand et al. 2009; Leys et al. 2010; Maetens, Poesen, and Vanmaercke 2012; Shipitalo and Edwards 1998; Wang et al. 2015).

The transition phase from conventional to conservation tillage is crucial and delicate (Knowler and Bradshaw 2007). The full benefits of conservation agriculture on the rehabilitation of the soil structure and ecosystem take tame, usually from five to seven years. During this period, the soil gradually undergoes a series of changes and adaptations to the new management. These initial transition years may pose issues which can influence farmers to leave the conservation agriculture practices. Due to the lack of mechanical weed control, weed management necessitates chemical inputs, which ideally are used in moderation as part of an integrated pest management system for safeguarding the environment and the biotic community (Knowler and Bradshaw 2007). Furthermore, the transition phase is also critical for the soil structure. The soil has low organic matter content and a poor structure, and it tends to be prone to compaction. The consequence is a potential increase in surface runoff and soil erosion that, combined with an intensified use of herbicides, dramatically increases the risk of herbicide transport to surface waters (Cessna, McConkey, and Elliott 2013). Therefore, the vulnerability of a non-tilled soil to runoff and soil erosion during the transition phase plays a critical role in determining the environmental and agricultural sustainability of this system (Kirkegaard et al. 2014; Palm et al. 2014).

As mentioned above, in conservation agriculture and particularly in the transition phase, pesticides are more than often required to achieve an efficient and economically affordable weeds and pests management, raising some concerns about environmental sustainability. In Europe, the topic of the "Sustainable Use of Pesticides" is regulated by the Directive 2009/128/EC, which objectives are to "reduce risks and impacts of pesticide use on human health and the environment and at encouraging the development and introduction of integrated pest management and of alternative approaches or techniques in order to reduce dependency on the use of pesticide" (Directive 2009/128/EC). As FAO defined, a pesticide is any substance, or a mixture of substances of chemical or biological ingredients intended for repelling, destroying or controlling any pest, or regulating plant growth (FAO and WHO 2014). Among the pesticides, herbicides account for the highest percentage of being produced and used globally (80% of all pesticide use) since they have become an integral part of modern agriculture. Herbicides play an important role in crop production because they are generally considered the most economical and efficient method for controlling weeds (Wu et al.

2015). Extensive use of herbicides has created concern as their residues are widely found in various environmental compartments and ecological niches (Silva et al. 2019; Shetty, Murugan, and Sreeja 2008). Given the extensive use of these products, there is a growing concern in identifying and understanding the mechanisms that control the environmental fate of herbicides in the soil as a source of environmental contamination. Most herbicides reach the soil through direct contact and/or after wash off from foliage (Mamy, Barriuso, and Gabrielle 2005). Once on the soil, several factors can influence their environmental fate, such as chemical and physical properties of the product, amount and frequency of use, method of application, abiotic and biotic characteristics of the environment, and meteorological conditions (Klingman, Ashton, and Noordhoff 1982). Adsorption, leaching, runoff, and dissipation of herbicides are key processes in determining their environmental fate and they are influenced by physicochemical properties of the herbicide as well as by soil and environmental conditions (Fig. 1).

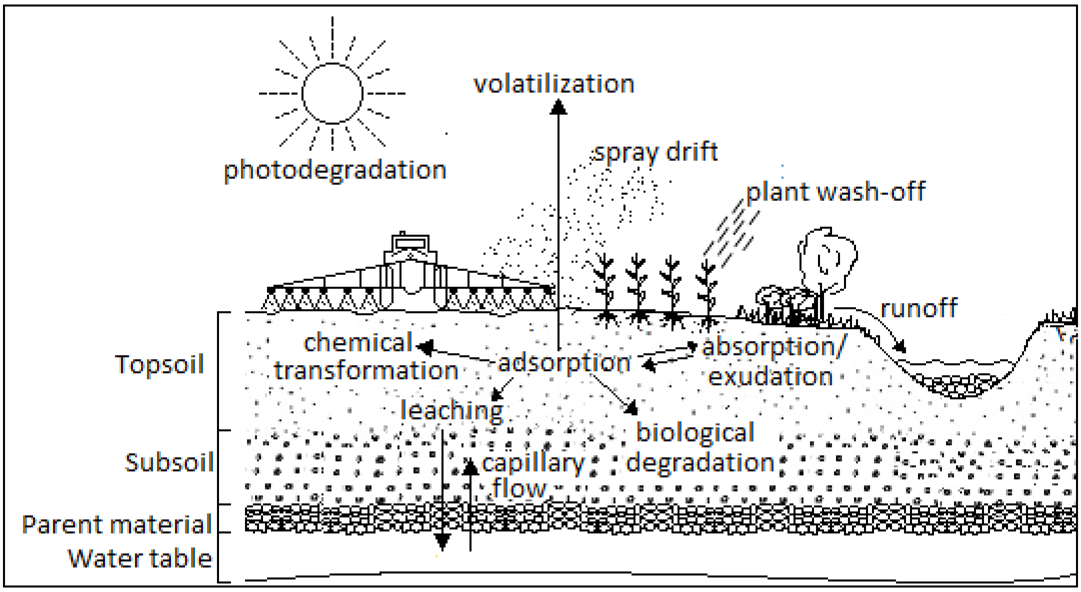

Fig. 1. Processes influencing the behaviour and fate of herbicides in the environment.

Adsorption on the soil is an important physicochemical characteristic governing the fate of herbicides in the environment. The adsorption of herbicides occurs as a result of interactions between a chemical and soil particle (Gavrilescu 2005). Herbicides adsorbs mainly to soil organic carbon, clay, and aluminum and iron (hydr)oxides since they have more sites onto which herbicides can bind, but adsorption is also dependent on soil pH, temperature, and moisture, as well as on the nature of herbicides (molecular structure, water solubility, and electrical charge). The adsorption tendency of a herbicide is expressed by its adsorption coefficient that is the ratio of pesticide concentration in the adsorbed-state and the solution phase (Gavrilescu 2005). Low adsorption coefficients (less than 500) indicate considerable potential for herbicide losses through leaching (Fushiwaki and Urano 2001). Leaching is the movement of an herbicide through the soil profile and it is an environmental concern because of the of groundwater contamination by herbicides. Leaching of an herbicide can occur via preferential flow or matrix flow. Preferential flow allows the rapid movement of the herbicide downward through cracks, root channels, wormholes, and large structural voids, reducing the likelihood for the molecule to be adsorbed or degraded along the way. Matrix flow implies a slow migration of water and dissolved herbicide through the small pores, allowing a longer contact time between soil particles and herbicide and consequently a greater possibility of retention (Cohen et al. 2007; Whitford et al. 1995). Another way in which an herbicide can be transferred from one environment component to another is surface runoff. Runoff determines the movement of water over a sloping surface that occurs when the rainfall intensity is higher than the infiltration rate in the soil. Runoff can transport herbicides from the field to surface waters, giving a significant contribution to the pollution. Herbicide runoff depends on several factors, such as slope, soil texture and moisture, rainfall amount and intensity, presence of vegetation or crop residues, and physicochemical properties of the herbicide (Gavrilescu 2005). Among the factors that influence the environmental behavior of an herbicide, dissipation is a key process because it determines the persistence of an herbicide in the soil and consequently its potential for reaching water bodies. Herbicide dissipation is of particular importance since it is the only process that effectively eliminates pesticide molecules from the environment. Dissipation is a result of the combined effect of different processes including volatilization, hydrolysis, photolysis, and chemical and microbial degradation (Lewis and Tzilivaki 2017). Microbial degradation is often the main source of pesticide degradation in soils (Waldman and Shevah 1993; Edgehill and Finn 1983). It occurs when microorganisms use herbicides as a source of carbon, energy, or other nutrients. The transformation products usually are less phytotoxic than the parent. Complete mineralization of the herbicide to $CO_2$ rarely occurs, and often a significant fraction of the herbicide forms part of a bound residue pool (Sadegh-zadeh, Wahid, and Jalili 2017). Soil organic matter content, moisture, temperature, aeration, and pH all affect microbial degradation (Gavrilescu 2005; Rice, Anderson, and Coats 2002).

Among the mitigation measures against herbicide pollution, buffer strips are considered very effective tools to mitigate the loss not only of pesticides but also of sediments and nutrients with surface runoff (Blanco-Canqui et al. 2004; Krutz et al. 2003; Lin et al. 2011; Reichenberger et al. 2007; Udawatta et al. 2002; Veum et al. 2009). Buffer strips are agricultural conservation practices that consist of non-treated areas of vegetation planted between cropland and a water body. No agrochemical application occurs on buffer strips. The runoff water has to pass through the buffer strip before entering the surface water, and during this passage, the buffer strip performs a filtering action. The vegetation of the buffer strip increases the soil infiltration capacity and surface roughness, and this decreases the speed of runoff water, promoting water infiltration and reducing runoff volume. The retention of sediments and pollutants is achieved by infiltration, sedimentation, absorption, and adsorption (Arora et al. 1996). Therefore, the dissolved phase of pesticides (carried with runoff water) and the sorbed phase (carried with sediment) are both reduced as the runoff water flows through the buffer strip. These characteristics potentially make buffer strips very promising to mitigate the negative effects in terms of pesticide runoff and soil erosion during the transition phase from conventional to conservation agriculture.

As mentioned above, herbicides are often required to manage weeds in conservation tillage and, before seeding, the most used in this agricultural system is glyphosate. Glyphosate is not common only in conservation agriculture, but it is the most used herbicide worldwide with an estimated use of 826 million kg in 2014. Glyphosate (N-phosphonomethylglycine; $C_3H_8NO_5P$) is a non-selective, systemic, broad-spectrum, post-emergent, foliar herbicide. The applications of glyphosate include weed control on transgenic glyphosate-resistant crops, pre-harvest desiccation of cereals, as well as total weed control in agricultural fields, especially in conservation agriculture, where it represents one of the most reliable options for effective weed control. Glyphosate has low acute human toxicity but its carcinogenicity is very controversial (Tarazona et al. 2017), it is also moderately toxic to birds, most aquatic organisms, earthworms and honeybees (PPDB 2019c). Glyphosate's mode of action is the inhibition of the enzyme 5-enolpyruvyl-shikimate-3- phosphate synthase (EPSPS) of the shikimate pathway, which is only found in some plants, fungi, and bacteria species. 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, Dobson, and Solomon 2000). Glyphosate is a small molecule with three polar functional groups (carboxyl, amino, and phosphonate groups), and it behaves as an amphoteric molecule (Borggaard and Gimsing 2008). When glyphosate reaches the soil, it tends to adsorb strongly to soil components such as clay, humic substances, and iron and aluminum oxides (Montgomery 2000). Glyphosate is highly polar and highly soluble in water (10.5 g $L^{-1}$ at 20°C) and it is moderately persistent in soil, with a with DT50 value (time for the dissipation of 50% of the initial concentration) ranging from 1 to 68 days (PPDB 2019c). Adsorption of glyphosate in the soil is variable, with a $k_f$ value ranging from 9.4 mL $g^{-1}$ to 700 mL $g^{-1}$, and a low GUS leaching potential index (-0.25) (PPDB 2019c). Despite its tendency of adsorb to soil component, leaching and runoff studies indicate that, under certain soil and environmental conditions, glyphosate can be transported to deeper soil layers by preferential flow through cracks and macropores, or to surface water with runoff when applied before a heavy and intense rainfall (Sasal et al. 2015). Glyphosate in water and soil is primarily degraded by microorganisms, which use glyphosate as a carbon and phosphorus source. Microbial degradation involves enzymatic reactions that break either the C-N or C-P bond, leading to aminomethylphosphonic acid (AMPA) or sarcosine, respectively, as metabolic products. AMPA is the main metabolite of glyphosate and is an aminophosphonate with a primary amine group. Ampa is chemically similar to glyphosate and has similar properties in terms of behavior and toxicity (PPDB 2019a). AMPA is polar and highly soluble in water (146 g $L^{-1}$ at 20°C), but it is more persistent in the environment than glyphosate (DT50 = 39–331 days) and it tends to accumulate in the soil (PPDB 2019a; Simonsen et al. 2008). The higher persistence of AMPA could be due to the fact there is only a reduced number of microorganisms that can mineralize AMPA. Indeed, most of the glyphosate-mineralizing microorganisms, export AMPA into the environment (Sviridov et al. 2015).

Glyphosate and AMPA contamination of water and soil is increasingly under scrutiny. A study conducted by Silva et al. (2019) revealed that, in 317 European agricultural topsoil samples analyzed for pesticides residues, glyphosate and AMPA were the most common pesticides mixtures in soils and they contributed the most to total pesticide content in soil. The contamination of surface water and groundwater by glyphosate and AMPA affects the entire Italian territory. According to the National report on pesticides in the water of 2015-2016, during 2016 a concentration of glyphosate higher than the environmental quality standards (Directive 2000/60/EC) was detected in surface waters in 24.5% of the cases, and in groundwater in 5.8% of the cases (ISPRA 2018). For AMPA the contamination of surface waters is even higher, and concentrations higher than the environmental quality standards were detected in 47.8% of monitored surface waters, whereas 4.8% of groundwater exceeded the standard. Given the widespread use of glyphosate and the extensive contamination of waters and soils, it is critical to investigate its environmental fate in the soil. Several studies concerning the environmental fate of glyphosate have been published in previous years, some conducted with the acid form of glyphosate and others with a formulated product. Formulated products also contain co-formulants, added to improve glyphosate field performance in terms of spreading, retention, and penetration through the plant (Stock and Briggs 2000). The most commonly used co-formulants are surfactants. Despite they are declared as inert, they are added to modify the behavior of the herbicide, so they may have biological and chemical activity and a potential effect on the environmental fate of the active ingredient. Several studies have been done about the ecotoxicological effects of the most used co-formulants (Defarge et al. 2016; Clair et al. 2012; Tsui and Chu 2003; Marc et al. 2005), but there is a lack of information about the effects of surfactants on the environmental fate of glyphosate.

The impact of glyphosate and AMPA in the environment is becoming increasingly under scrutiny and there is the need for sensitive and reliable methods for their determination in aqueous and soil samples. From an analytical point of view, the determination of glyphosate and AMPA at low concentration in water and soil is difficult due to their very polar character, low volatility, low mass, and lack of chemical group that could facilitate their detection (Ibáñez et al. 2005). Liquid chromatography coupled to mass spectrometry (LC-MS/MS) is the most frequently chosen method for polar analytes due to its high selectivity and sensitivity (Hanke, Singer, and Hollender 2008). However, derivatization is often required to reduce the polar character of the analyte and enable a good chromatographic separation. Among the derivatization methods, the most used for glyphosate and AMPA is the pre-column derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl) (Hanke, Singer, and Hollender 2008; Vreeken et al. 1998; Ibáñez et al. 2006; Olivo et al. 2015; Sun et al. 2017; Peruzzo, Porta, and Ronco 2008; Ibáñez et al. 2005; Catrinck et al. 2014; Oulkar et al. 2017). However, the FMOC-Cl derivatization presents some disadvantages like the interference caused by the excess of derivatizing reagent and the long derivatization reaction time. In addition, the extraction of glyphosate and AMPA from the soil is also tricky and soil components like organic matter, humic acid, and metal oxides can lead to a serious matrix effect and poor recovery rate.

Among the herbicides to be applied after seeding, in conservation agriculture as well as in conventional tillage, there are terbuthylazine, S-metolachlor, and mesotrione. Terbuthylazine (6-chloro-N-(1,1-dimethylethyl)-N'-ethyl-1,3,5-triazine-2,4-diamine) is a selective systemic herbicide that belongs to the triazines (Baćmaga et al. 2014). It is used to control grass and broad-leaved weeds in a variety of agricultural situations like maize, sorghum, apples, and citrus as well as in non-crop sites including roads, railways, and industrial sites (PPDB 2019e). It is transferred mainly through the roots of the weeds but also through their leaves, which means that it is applicable during both the pre- and post-emergence period. It acts as an inhibitor of photosynthesis at photosystem II level. It has a quite low water solubility (6.6 g $L^{-1}$ at 20°C) and it is moderately persistent in soil with a DT50 value ranging from 38 to 167 days (PPDB 2019e). A $k_f$ range from 2.1 to 10.49 mL $g^{-1}$ and a GUS leaching potential of 3.07 suggests that terbuthylazine is expected to have moderate mobility in the soil and high leaching potential. Degradation of terbuthylazine in soil produces three relevant metabolites, which are desethyl-terbuthylazine, hydroxy-terbuthylazine, and 2-hydroxy-desethyl-terbuthylazine. Desethyl-terbuthylazine is one of the main metabolites from the dealkylation degradation of terbuthylazine and it has been discussed as an environmental concern in surface and groundwater (Guzzella et al. 2003; Licia Guzzella, Pozzoni, and Giuliano 2006; Bottoni et al. 2013). Desethyl-terbuthylazine is well soluble in water (327 mg $L^{-1}$ at 20°C), poorly adsorbed to the soil ($k_f$ range 0.28-3.3 mL $g^{-1}$), and it has a high leaching potential (GUS value 3.65). Once formed, it is moderately persistent in soil with a DT50 value ranging from 41 to 94 days (PPDB 2019b).

S-metolachlor ((chloro-N-(2- ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)) is a selective systemic herbicide, produced and commercialized in an isomer mixture. It belongs to chloroacetamides and it used to control grasses and some broad-leaved weeds in a wide range of crops such as maize, soybean, and sugar beet. It is applied to crops before plants emerge from the soil, and it is adsorbed by the shoots of the seedling. Its mode of action consists of the inhibition of the cell division (Baćmaga et al. 2014). It is well soluble in water (480 mg $L^{-1}$ at 20°C), moderately mobile ($k_f$ ranging from 0.3 to 4.7 mL $g^{-1}$), and it is considered transitional about leaching potential (GUS value 1.91) (PPDB 2018). It is a low persistence compound with a DT50 ranging from 8 to 38 days.

Mesotrione (2-[4-(methylsulfonyl)- 2-nitrobenzoyl]-1,3-cyclo- hexanedione) belongs to the triketones and it is a selective, systemic, soil- and foliar-applied herbicide. It is applied pre-emergence and post-emergence to control some grass and broad-leaved weeds mainly in maize crops. It acts by inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), which affects carotenoid biosynthesis (Mitchell et al. 2001). Mesotrione is highly soluble in water (1500 mg $L^{-1}$), moderately mobile ($k_f$ ranging from 0.16 to 6.16 mL $g^{-1}$), and in transition state regarding the leaching potential (GUS value 2.69). The persistence of mesotrione is generally low with a DT50 ranging from 4 to 44 days (PPDB 2019d).

In order to optimize weed control efficacy and minimize the application costs, the application of terbuthylazine, S-metolachlor, and mesotrione in mixtures has become the rule rather than the exception in many countries (Kudsk 2017; Pannacci, Graziani, and Covarelli 2007). Given the extensive use, terbuthylazine and S-metolachlor are among the most frequently found pesticides in surface waters and groundwater in Italy (ISPRA 2018).

The overall aim of this PhD was to study the environmental fate of the above-mentioned herbicides, to evaluate the application of buffer strips as a mitigation measure, and to assess the role of conservation agriculture with regard to surface runoff and soil loss (Fig. 2).

This PhD thesis comprises 7 chapters. Chapters 2, 3, 5, and 6 are published in international peer-reviewed journals, chapters 1 and 7 have been submitted, and chapter 4 will be submitted soon. For this reason, they are all standalone chapters and they can be read independently. Consequently, some repetition occurs in parts of the introduction and materials and methods sections.

Chapter 1 investigates the role of conservation agriculture in runoff and soil erosion processes. This study analyzes runoff and sediment loss in a non-tilled field during the transition period from conventional to conservation agriculture in comparison to a tilled field.

Chapter 2 presents an analytical method for the determination of glyphosate and AMPA in water samples by using Ultra-High Performance Liquid Chromatography coupled with mass spectrometry (UHPLC-MS/MS). The method was applied to the glyphosate and AMPA analysis in runoff water to evaluate the efficacy of 6 m wide buffer strips in mitigating the runoff of these pollutants.

Chapter 3 study the efficacy of buffer strips in reducing runoff of terbuthylazine, S-metolachlor, and mesotrione from a non-tilled field during the transition period from conventional to conservation agriculture. Four types of buffer strips with different length and composition were examined in comparison to the no-buffer situation.

Chapter 4 presents an analytical method for the determination of glyphosate and AMPA in soil samples by using UHPLC-MS/MS. The method was applied to evaluate the dissipation of glyphosate and the formation/dissipation of AMPA from a field managed with conservation agriculture compared to a conventionally managed field.

Chapter 5 investigates the effect of soil texture and depth on the dissipation of terbuthylazine, S-metolachlor, and mesotrione in cornfield soils.

Chapter 6 examined the influence of sewage amendment on the dissipation of terbuthylazine and S-metolachlor in sandy clay soil.

Chapter 7 investigate whether the presence of an alkyl polyglucoside based surfactant, Triton CG-110, has the potential to affect adsorption, leaching, and mineralization of glyphosate in two soils with different texture and washed sand.

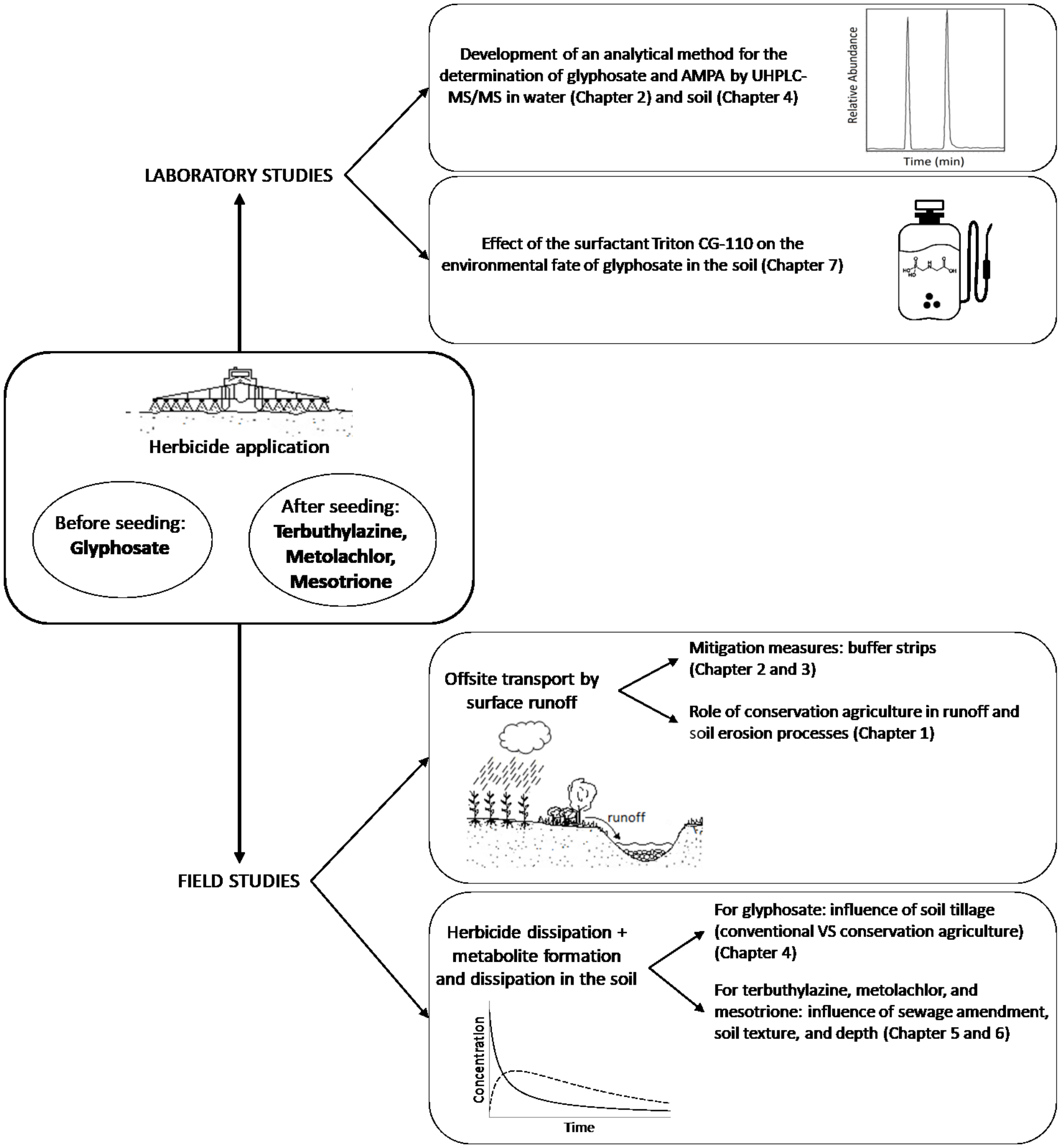

Fig. 2. Conceptual approach of the thesis, indicating the chapters and topics of the research study.

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# Chapter 1. Effects of no-till and tillage management on runoff and soil erosion: A case study in northeast Italy

## Abstract

Conservation agriculture, and in particular no-till systems, generally yields improvements in both soil characteristics (e.g. structure, and water holding capacity) and soil processes (such as runoff and hence erosion). Nevertheless, establishing the benefits of no-till takes time, and the transition period from conventional to no-till system may pose several problems. The lack of tillage makes weed management more difficult and totally based on herbicides. Especially during the transition period, the use of herbicides may be higher in no-till than in a conventional tillage system. Moreover, during the first years of no-till, the soil is prone to compaction, due to the low organic matter content and poor structure, thus favoring surface runoff and soil erosion, and increasing the risk of herbicide losses from the field. The aim of this study was to analyze runoff and soil erosion in a non-tilled field in comparison to a tilled field during the transition period. The study was conducted at the Padova University Experimental Farm, in northeast Italy. Six sub-plots 2.5 m x 5 m were set up, 3 in a tilled field (CT plot) and 3 in a non-tilled field (NT plot). Each sub-plot was equipped with a runoff water collection system. Runoff was monitored during two sampling seasons: from May to October 2017 and from May to September 2018.

At each rainfall event, runoff water volume was measured, and the amount of sediment was quantified by drying the samples taken from the collection tanks. The results showed that NT practices coincided with a reduction of more than 50% in runoff volumes and a 50% to 95% reduction in sediment losses. The reduced runoff and sediment yield in the NT plot probably translates into a lower risk of herbicide losses from the field, and this outcome can have important on-site benefits in terms of both sustainable soil management and surface water quality.

Keywords: conservation agriculture; tillage; runoff; sediment loss; rainfall.

## 1.1 Introduction

Nowadays, agriculture is a production system predominantly controlled by human interventions, such as soil tillage, agrochemicals application for weed and pest control, and synthetic fertilization. Several studies demonstrate that this system lacks sustainability, raising concerns about agroecosystems vulnerability (Foresight, 2011; MEA, 2005). Conservation agriculture was recently promoted by the Common Agricultural Policy of the European Union (CAP, Rural Development Program 2014-2020; Basch et al., 2011) to tackle some major agro-environmental issues such as the increasing carbon dioxide ($CO_2$) concentration in the atmosphere, decreasing biodiversity, and limited water availability (Armengot et al., 2015; Bouma and McBratney, 2013). Conservation agriculture is a system of agronomic practices that aims to achieve sustainable and profitable agriculture by applying three basic principles: (1) minimizing mechanical soil disturbance (reduced tillage or no-tillage), (2) maintaining permanent soil cover by using crop residues and cover crops, and (3) adopting crop rotations (Hossain, 2013). A key factor for maintaining soil structure and organic matter is to limit mechanical soil disturbance in crop management. The most important component of conservation agriculture is therefore that it reduces, or even eliminates, the use of tillage practices, thus enabling minimal soil disturbance and permanent soil cover to be achieved (Shahzad et al., 2016; Tarolli et al., 2019). Benefits on soil and water conservation with the application of minimal soil disturbance and permanent soil cover have been well documented (Jordán et al., 2010; Nyssen et al., 2008; Sun et al., 2015; Wang et al., 2015). Among the positive outcomes of conservation agriculture, it is worth mentioning the maintenance of a stable soil structure and biological activity, improving of soil fertility and soil microclimate due to the permanent organic soil cover, enhancing of water infiltration, and reduction of runoff and soil erosion (Berger et al., 2010). Higher infiltration rates can in turn reduce the losses of surface water from the field and improve water holding capacity (Thierfelder and Wall, 2009). Many field studies have been done to quantify runoff and soil erosion mitigation related to conservation tillage adoption (Armand et al., 2009; Leys et al., 2010; Maetens et al., 2012; Montgomery, 2007; Shipitalo and Edwards, 1998; Wang et al., 2015). To fully appreciate the benefits of conservation agriculture on soil and water conservation, the soil has to be “mature”, which means that it should have gone through a transition period from conventional to conservation agriculture, lasting approximately five to seven years, where the non-tilled soil gradually is subjected to a series of changes and adaptations to the new management system (Hobbs et al., 2008). The transition period presents some major difficulties. Due to the lack of mechanical weed control, weed abundance and density may be higher than in a conventional system and weed management relies mainly on chemical inputs (Armengot et al., 2015; Knowler and Bradshaw, 2007). Furthermore, due to the low organic matter content and poor structure, the soil tends to be prone to compaction, with the consequence of a potential increase in surface runoff and soil erosion during the transition period compared to conventional management. Soil compaction, combined with an intensified use of herbicides, dramatically increases the risk of herbicide losses with runoff and surface water contamination (Cessna et al., 2013). Thus, the vulnerability of a non-tilled (NT) soil to runoff and soil loss during the transition period plays a critical role in both agricultural and environmental sustainability of this system (Kirkegaard et al., 2014; Palm et al., 2014). The mechanisms underlying the effect of NT on runoff and soil erosion are contrasting. On the one hand, runoff reduction and increase in infiltration are induced by increased surface roughness (Tarolli et al., 2019), improved pore continuity, and surface crusting prevention provided by the permanent residue cover and improved soil structure (Armand et al., 2009; Blanco-Canqui and Lal, 2009). On the other hand, the absence of tillage can increase soil bulk density and decrease macroporosity, in turn decreasing infiltration rates and increasing runoff generation mechanisms (Alvarez and Steinbach, 2009; Palm et al., 2014; Sun et al., 2015). The role of NT in reducing runoff and soil erosion reported in previous research varies greatly, indicating that the effectiveness of NT may depend on practices such as residue management as well as on environmental conditions, soil type and conditions and slope characteristics (Blanco-Canqui and Lal, 2009; Leys et al., 2010; Sun et al., 2015; Truman et al., 2011).

To our knowledge, little information exists about the effect of no-tillage on runoff and soil erosion during the transition period from conventional to conservation agriculture, when the changes in soil properties after the introduction of no-tillage are not yet fully developed. The main aim of this study was therefore to analyze runoff and soil erosion in a non-tilled field during the transition period from conventional to conservation agriculture in comparison to a tilled field (CT). The study years were the third and fourth of conservation agriculture for the NT field.

## 1.2 Materials and Methods

### *1.2.1 Site information*

The study was conducted during 2017 and 2018 at the Padova University Experimental Farm in the Po Valley, in Northeast Italy (45°12’N, 11°58’E, altitude 6 m a.s.l.). The local climate is sub-humid with a mean annual temperature of 15.6 °C. Annual rainfall in 2017 was 518 mm (the wettest month was September with 146 mm of rainfall, whereas the driest was February with 7 mm) with a total of 62 rainy days throughout this year. Annual rainfall in 2018 was 853 mm (the wettest month was October with 142 mm of rainfall, whereas the driest was December with 12 mm) with a total of 89 rainy days.

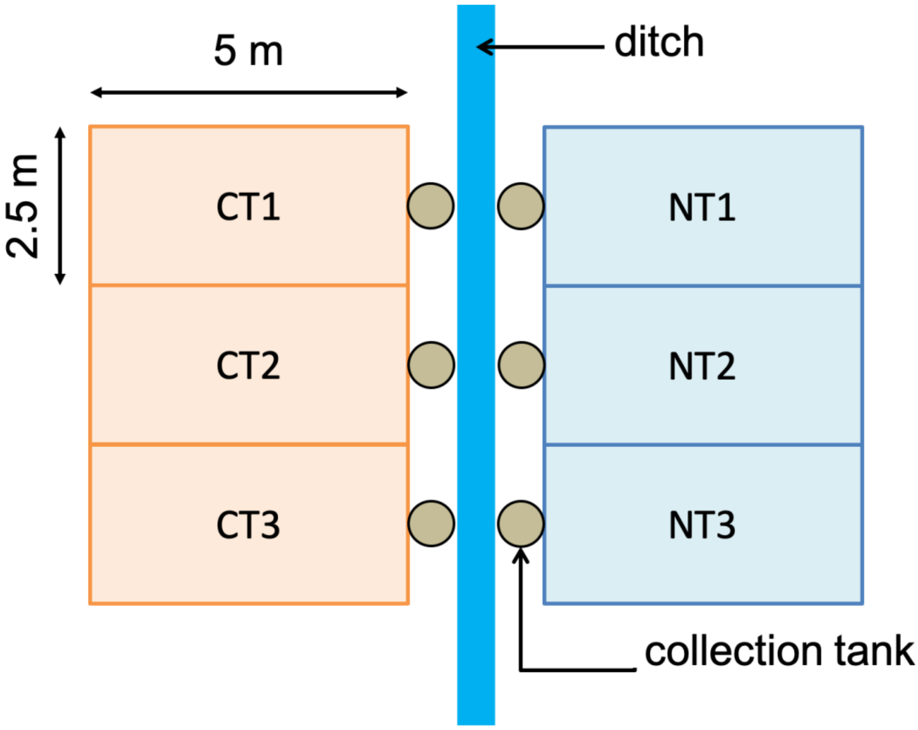

Fig. 1.1 Layout of the six experimental sub-plots installed on the CT field and NT field.

Six sub-plots (2.5 m × 5 m each) (Fig. 1.1) were set up in spring 2017, three of them in a field managed with conventional tillage (CT plot) and the other three in a field managed with conservation agriculture (NT plot) (Fig. 1.2).

Fig. 1.2 Details of NT plots. The picture was taken in summer 2017 (photo by P. Tarolli)

The NT field is a 200 m × 35 m rectangle and has been managed with conservation agriculture since 2014. The CT field is a 235 m × 40 m rectangle. The two fields face each other and are separated by a ditch. Each sub-plot is bounded on three sides by metal boards that were inserted 15 cm belowground and with 15 cm above the surface to prevent a splash effect and runoff flowing out or into adjacent sub-plots. Each sub-plot was equipped with a runoff water collection system with a 55 L tank. Because of the high volumes collected from the CT plot in some runoff events of the first sampling season, the tanks of the CT plot were replaced with 100 L tanks in July 2018.

In September 2016, a subsoiling was performed on the CT field, followed by a harrowing at the end of October. In November 2016, both fields were sown with wheat, which was harvested in June 2017. In July, the NT field was sown with soybean as a cover crop, which was harvested in October. The CT field was plowed to a 25–30 cm depth in November 2017, and the runoff water sampling was then interrupted for the winter due to the impossibility of collecting the runoff given the soil conditions. In November 2017, the NT field was sown with horseradish as a cover crop, which was terminated in April with a herbicide treatment. CT soil cultivation with a chisel was done at the end of January 2018. The runoff water sampling re-started in May 2018 when both fields were sown with maize. CT field was harrowed before maize sowing. The maize was harvested in mid-September, and the sampling of runoff water stopped at the end of October. In November 2017, before plowing the CT field, soil samples were collected from both NT and CT fields next to the runoff plots. Three disturbed soil samples for each field were collected at a 15-20 cm depth, then placed in a labelled plastic bag, sealed, and transported to the laboratory. The samples were air-dried, homogenized, sieved at particle diameter of 2 mm, and analyzed for soil texture and pH determination. Undisturbed soil samples were collected at a 15-20 cm depth to determine saturated soil hydraulic conductivity ($K_S$), saturated soil water content ($\theta_S$), and oven-dry soil bulk density (*BD*). Three samples were collected from the CT field and six from NT field, of which three in the no-track and the other three in the track position, which is the portion of soil affected by the passage of tractor wheels. The samples were collected using cylindrical samplers 7 cm tall and with an internal diameter of 7.2 cm. Disturbed and undisturbed soil samples were analyzed at the Laboratory of Soil Hydrology of the Department of Agricultural Sciences, AFBE Division, (University of Napoli Federico II, Portici, Italy).

### *1.2.2 Runoff Sampling*

The study was conducted under natural rainfall conditions. The weather station on the experimental farm, located at 30 m from the plots, with a rain gauge recording every five min was used to measure rainfall events. Runoff was monitored during two sampling seasons, from the beginning of May 2017 to the end of October 2017 (first period) and from the beginning of May 2018 to mid-September 2018 (second period). Rainfall events during the monitoring periods were analyzed to evaluate the relationships among rainfalls, the hydrological characteristics of soils, and runoff and erosion processes. For each rainfall event, the amount and duration, the maximum intensity at different time intervals (10, 30, and 60 min), and the cumulative precipitation (during the previous 7, 15, 30, and 45 days) were obtained.

At each rainfall event, the total runoff water volume collected in the tanks was measured and, for the sediment analysis, three 0.5 L water samples were collected from each sub-plot and placed in aluminum bottles, taking care to homogenize water and sediment. The samples were then transferred into plastic containers and placed in a dryer at 60 °C for 48 hours. When all the water had evaporated, the weight of the samples was measured to obtain the sediment yield for erosive events. To determine the sediment yield from each sub-plot at each runoff event, sediment concentration was multiplied by the runoff volume. In this study, the CT plot was chosen as the control group. The following two indices were selected to evaluate the effects of different tillage practices on runoff and sediment: 1) Runoff Reduction Benefit (*RRB*) in %; 2) Sediment Reduction Benefit (*SRB*) in % (Wang et al., 2017; Zhao et al., 2014). They were calculated for each runoff event and for the entire study period as follows:

$$RRB = (R_{CT} - R_{NT})/R_{CT} \times 100 \qquad (1.1)$$

$$SRB = (S_{CT} - S_{NT})/S_{CT} \times 100 \qquad (1.2)$$

where $R_{CT}$ and $R_{NT}$ are the runoff amount (mm) from CT and NT plot, respectively; $S_{CT}$ and $S_{NT}$ are the sediment loss (kg $ha^{-1}$) from CT and NT plot, respectively. These indices provide the mitigation percentage for runoff volumes and sediment losses with no-till practice compared to conventional tillage. They therefore work correctly when the runoff volumes and sediment losses are higher in CT than in NT. For the runoff events in which runoff amount or sediment losses were higher in NT than in CT, the above formulas overestimate the lack of benefit; so the indices were calculated as follows.

$$RRB = -\,[(R_{NT} - R_{CT})/R_{NT} \times 100] \qquad (1.3)$$

$$SRB = -\,[(S_{NT} - S_{CT})/S_{NT} \times 100] \qquad (1.4)$$

The rainfall conditions, runoff water samplings, and crops cultivated in NT and CT fields during the experimental periods are illustrated in Fig. 1.3.

Runoff, sediment loss, and sediment concentration data were analyzed using the TIBCO Statistica 13 software for Windows (TIBCO Software Inc.). Prior to statistical comparisons, data normality was tested using the Shapiro–Wilk test. Data normality was considered at a p-value < 0.05. For each runoff event, significant differences between CT and NT in terms of runoff volume, sediment loss, and sediment concentration were determined with Student's t-test ($\alpha = 0.05$).

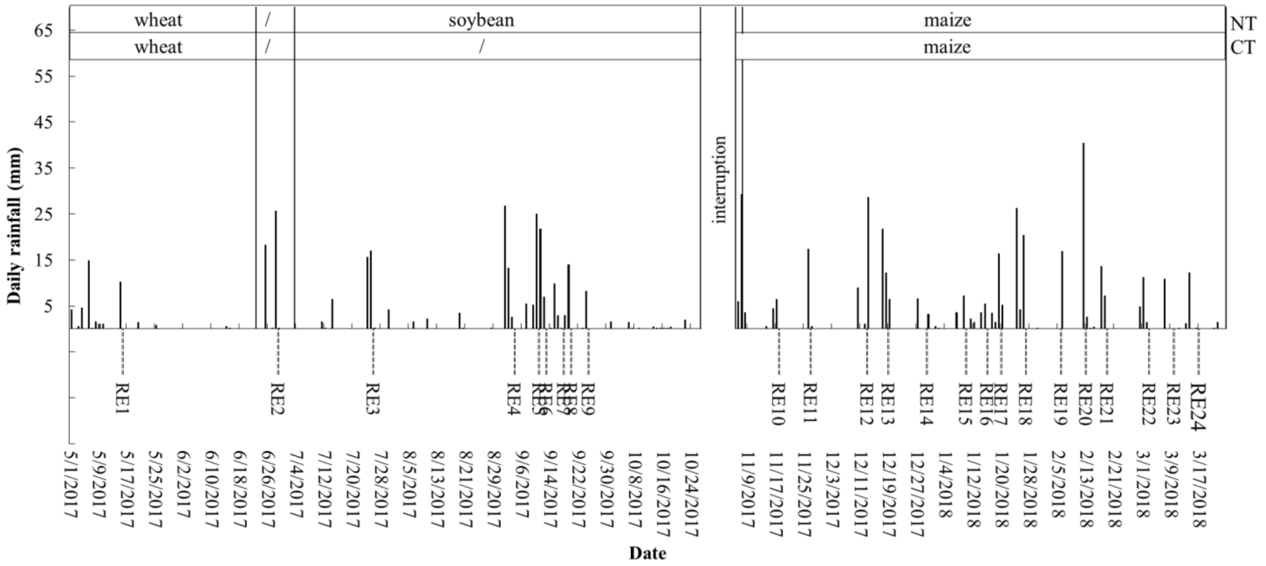

Fig. 1.3 Daily rainfall monitored from May to October 2017 and from May to September 2018. Dotted lines indicate the sampling of runoff water. The crops in NT and CT plot are indicated at the top.

## 1.3 Results and Discussion

### *1.3.1 Soil properties*

The soils in the two sites are Fulvi-Calcaric Cambisol (FAO-UNESCO, 1990), and are classified as silty-loam. The NT soil has 19.5% sand, 60.8% clay, and 19.7% silt, whereas the CT soil has 25.1% sand, 57.1% clay, and 17.8% silt. The pH is 7.85 for the NT soil and 7.38 for the CT soil.

Table 1.1 summarizes the results of the tests conducted on undisturbed soil samples. At the time of the soil sampling, the lowest value of $BD$ and the highest value of $K_s$ and $\theta_s$ were recorded in the CT soil. Tillage is supposed to decrease the degree of soil compaction and increase its porosity that yields an increase in hydraulic conductivity, which is what was observed. According to Fraser et al. (2010), tillage can also affect soil bulk density. Regarding the non-tilled soil, the track position showed $K_S$, $\theta_S$, and $BD$ values lower than those measured in the no-track position. It should be noted that the samples were collected more than one year after the last tillage of the CT field. We can therefore assume that performing the sampling soon after tillage might show more marked differences in soil hydrological properties between CT and NT soil.

Table 1.1 Mean values (± standard error) of saturated hydraulic conductivity ($K_S$), saturated soil water content ($\theta_S$), and oven-dry soil bulk density (*BD*) measured in the NT and CT plots.

| **Field** | **Sample** | **$K_S$ (cm min$^{-1}$)** | **$\theta_S$ (cm$^3$ cm$^{-3}$)** | ***BD* (g cm$^{-3}$)** |
|---|---|---|---|---|
| CT | - | $3.34\cdot10^{-2}$ ($\pm5.525\cdot10^{-3}$) | 0.483 (± 0.0245) | 1.547 (± 0.0477) |
| NT | track | $9.24\cdot10^{-4}$ ($\pm7.231\cdot10^{-4}$) | 0.419 (± 0.0078) | 1.663 (± 0.0389) |
| NT | no-track | $1.46\cdot10^{-3}$ ($\pm7.495\cdot10^{-4}$) | 0.448 (± 0.0096) | 1.737 (± 0.0397) |

### *1.3.2 Runoff volumes*

During the monitoring period, 24 runoff events (RE) occurred (Fig. 1.3). Considering both sampling periods, a total rainfall amount of 483 mm was recorded. The seasonal and inter-annual variations were very high. From May to October 2017, 263 mm of rainfall were recorded, with 146 mm concentrated in September. From May to September 2018, 377 mm of rainfall were recorded, distributed throughout the whole period. Mean rainfall intensity was mostly low, ranging from 0.13 to 25.52 mm h$^{-1}$ with an average of 2.41 mm h$^{-1}$. Rainfall characteristics of the events causing runoff (amount and duration, mean and maximum intensity at 10, 30, and 60 min, and cumulative precipitation during the previous 7, 15, 30, and 45 days) are reported in Table 1.2.

Table 1.2 Rainfall characteristics of the events generating runoff volumes.

| Runoff event | Sampling date | Rainfall amount (mm) | Rainfall duration (hours) | Maximum rainfall intensity in 5 min (mm hour$^{-1}$) | Maximum rainfall intensity in 10 min (mm hour$^{-1}$) | Maximum rainfall intensity in 30 min (mm hour$^{-1}$) | Maximum rainfall intensity in 60 min (mm hour$^{-1}$) | Mean rainfall intensity (mm hour$^{-1}$) | cumulative precipitation during the previous 7 days (mm) | cumulative precipitation during the previous 15 days (mm) | cumulative precipitation during the previous 30 days (mm) | cumulative precipitation during the previous 45 days (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RE1 | 16/5/2017 | 10.2 | 14.33 | 16.8 | 13.2 | 8 | 5.4 | 0.71 | 11.2 | 34 | 74 | 94 |
| RE2 | 29/6/2017 | 25.6 | 7.08 | 33.6 | 26.4 | 21.2 | 11.6 | 3.61 | 44.2 | 44.2 | 45 | 47.2 |
| RE3 | 26/7/2017 | 32.6 | 33.17 | 86.4 | 62.4 | 32.8 | 16.4 | 0.98 | 32.8 | 39.4 | 67 | 86 |
| RE4 | 4/9/2017 | 42.6 | 47.25 | 112.8 | 100.8 | 43.2 | 21.6 | 0.90 | 42.6 | 42.8 | 50.2 | 87.2 |
| RE5 | 11/9/2017 | 30.2 | 23.17 | 12 | 9.6 | 7.2 | 5.6 | 1.30 | 57.4 | 100.2 | 103.8 | 111.8 |
| RE6 | 13/9/2017 | 28.6 | 19.58 | 52.8 | 50.4 | 33.6 | 20.6 | 1.46 | 64.4 | 107 | 110.8 | 114.6 |
| RE7 | 18/9/2017 | 12.8 | 29.33 | 12 | 9.6 | 9.6 | 6.4 | 0.44 | 23.2 | 80.6 | 123.6 | 130.8 |
| RE8 | 20/9/2017 | 17 | 28.08 | 7.2 | 6 | 5.6 | 5 | 0.61 | 30.2 | 94.6 | 137.4 | 143.2 |
| RE9 | 25/9/2017 | 8.2 | 10.08 | 14.4 | 14.4 | 6.4 | 4.2 | 0.81 | 22.4 | 67.4 | 145.8 | 149.4 |
| RE10 | 14/5/2018 | 10.8 | 29.08 | 28.8 | 22.8 | 8 | 4.4 | 0.37 | 11.4 | 52.4 | 52.4 | 90.8 |
| RE11 | 23/5/2018 | 17.4 | 20.00 | 12 | 9.6 | 8.4 | 7 | 0.87 | 18 | 29.4 | 70.4 | 82.6 |
| RE12 | 8/6/2018 | 29.6 | 15.92 | 62.4 | 61.2 | 42.4 | 23.4 | 1.86 | 38.6 | 38.6 | 68 | 109 |
| RE13 | 14/6/2018 | 40.4 | 38.58 | 50.4 | 49.2 | 20 | 10 | 1.05 | 69 | 79 | 97 | 149.4 |
| RE14 | 25/6/2018 | 9.8 | 77.67 | 12 | 8.4 | 4 | 2.6 | 0.13 | 9.8 | 50.2 | 88.8 | 117.6 |
| RE15 | 6/7/2018 | 7.2 | 9.08 | 55.2 | 36 | 12.8 | 6.4 | 0.79 | 10.8 | 21.4 | 91.4 | 101 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RE16 | 12/7/2018 | 9 | 36.67 | 33.6 | 19.2 | 6.8 | 3.4 | 0.25 | 12.6 | 23.6 | 52.6 | 113 |
| RE17 | 16/7/2018 | 22.2 | 32.00 | 100.8 | 67.2 | 24 | 12.2 | 0.69 | 35.4 | 49.8 | 60.4 | 139.4 |
| RE18 | 23/7/2018 | 50.8 | 58.75 | 52.8 | 48 | 43.6 | 25.8 | 0.86 | 50.8 | 86.2 | 104.6 | 151.6 |
| RE19 | 2/8/2018 | 16.6 | 1.50 | 43.2 | 38.4 | 32.8 | 16.6 | 11.07 | 16.8 | 67.8 | 114 | 128.2 |
| RE20 | 9/8/2018 | 40.4 | 1.58 | 84 | 78 | 74 | 40.4 | 25.52 | 43 | 60 | 142.6 | 161.4 |
| RE21 | 15/8/2018 | 20.8 | 29.08 | 50.4 | 48 | 25.6 | 12.8 | 0.72 | 24 | 81.2 | 132.2 | 182 |
| RE22 | 27/8/2018 | 12.6 | 25.50 | 16.8 | 15.6 | 10.4 | 5.6 | 0.49 | 17.4 | 38.2 | 98.6 | 172.6 |
| RE23 | 3/9/2018 | No data[a] | | | | | | | 10.8 | 28.2 | 92.6 | 134.2 |
| RE24 | 10/9/2018 | 13.4 | 10.92 | 12 | 12 | 8.4 | 6 | 1.23 | 13.8 | 24.6 | 62.8 | 123.2 |

[a] On 3/9/2018, no precipitation data could be retrieved due to rain-gauge malfunctioning.

Fig. 1.4 reports runoff volumes measured at each runoff event in NT and CT plots. Variability in the measurements was higher in the tilled plot than in no-tillage plot. Runoff volumes measured in both NT and CT plots varied extensively over time and between events. The runoff amount in CT plot ranged from 0.08 mm (RE14) to 8.67 mm (RE18), whereas in NT plot it went from 0.07 mm (RE9) to 3.25 mm (RE20). It should be specified that for runoff events RE9 and RE20, in two out of three CT sub-plots, the runoff exceeded the maximum measurable volume of the collection tank. Therefore, the actual runoff in this event may have been higher. The values of runoff coefficient ranged from 0.84% (RE14) to 28.16% (RE1) in the CT plot and from 0.88 (RE5) to 11.19% (RE17) in the NT plot.

Higher runoff volumes were generally measured in the CT plot, although the difference was only statistically significant in 8 out of 24 runoff events (RE3, RE4, RE5, RE6, RE13, RE15, RE18, and RE20). Runoff volume was higher in NT than in CT plot Only in 4 out of 24 runoff events (RE10, RE11, RE14, and RE24),. However, the difference between runoff in NT and CT plot was only significant in RE10. This was the first runoff event of the second sampling period. It should be taken into account that, three weeks before this event, harrowing was done on the CT field. The differences in runoff volume between CT and NT observed in this event can be explained by the higher infiltration rate and surface storage of the CT soil due to increased surface roughness in the weeks after the tillage. The surface roughness that existed immediately after tillage operations in the CT field aided rainfall catchment and infiltration. A similar effect was observed by Gomez et al. (2009) and Romero et al. (2007) in olive orchards and by Myers and Wagger (1996) in conventional tillage maize. The effect of tillage on the soil morphology is time-variant and tends to decrease in time. In a study conducted on the same CT and NT fields, Tarolli et al. (2019) found that only 26 days after seedbed preparation of the tilled soil, the CT soil surface resulted smoother than NT, with consequent negative effects on water storage. Indeed, runoff coefficients in CT plot increased markedly after the first high-intensity rainfall (RE12), which may have initiated surface sealing and crusting and promoted the loss of roughness. Runoff coefficients in CT then remained high until the last event, with the exception of RE14 and RE16 when rainfall intensity was very low. As highlighted by Myers and Wagger (1996), surface sealing and crust formation appear to play an important role in the surface runoff.

Cumulated runoff in the first sampling period was lower than that in the second, for both NT plot (5.6 and 20.1 in the first and second period respectively) and CT plot (18.8 mm and 42.1 in the first and second period respectively). However, the observed differences can partly be explained by the higher total rainfall amount registered in the second period and by the occurrence of some brief and very intense rainfall events during the summer, in which a considerable amount of water fell in a short time. Considering the entire study period, the cumulated runoff was higher in CT plot than in NT (60.9 and 25.7 mm for CT and NT respectively). Overall, NT practices yielded an average reduction of about 58% in runoff volumes compared to the tilled plot. Similar results, showing a substantial reduction of runoff in conservation agriculture compared with conventional tillage, have been obtained in other geographical areas and different croplands, such as a wheat and teff in Ethiopia (Araya et al., 2011), a wheat/lupine rotation in Australia (Zhang et al., 2007), a 4-year corn/wheat/meadow/meadow rotation in Ohio, USA (Shipitalo and Edwards, 1998), and a corn planting simulation in Kentucky, USA (Seta et al., 1993). As pointed out by the latter, the lowest runoff rate from NT reflects a more rapid infiltration rate resulting from less surface sealing and more undisturbed macropores than under CT.

The generation of runoff is strongly affected by surface micro-topographical structures dictating how water exceeds storage capacity of soil surface depressions, spills out and moves through the network of depressions (Antoine et al., 2009; Appels et al., 2011; Frei and Fleckenstein, 2014). A previous study was conducted on the same NT and CT fields by Tarolli et al. (2019) to characterize surface morphology in no-tillage versus conventional tillage. As emerged from the analysis of micro-topography, obtained from a high-resolution Digital Terrain Model, NT surfaces had rougher surfaces with more pronounced concavities and convexities, allowing water to be stored in the depressions (or pockets) and making surface water flow paths more irregular. This means that, under the same wetness and rainfall conditions, NT soil has the ability to store more water in the surface concavities than CT soil.

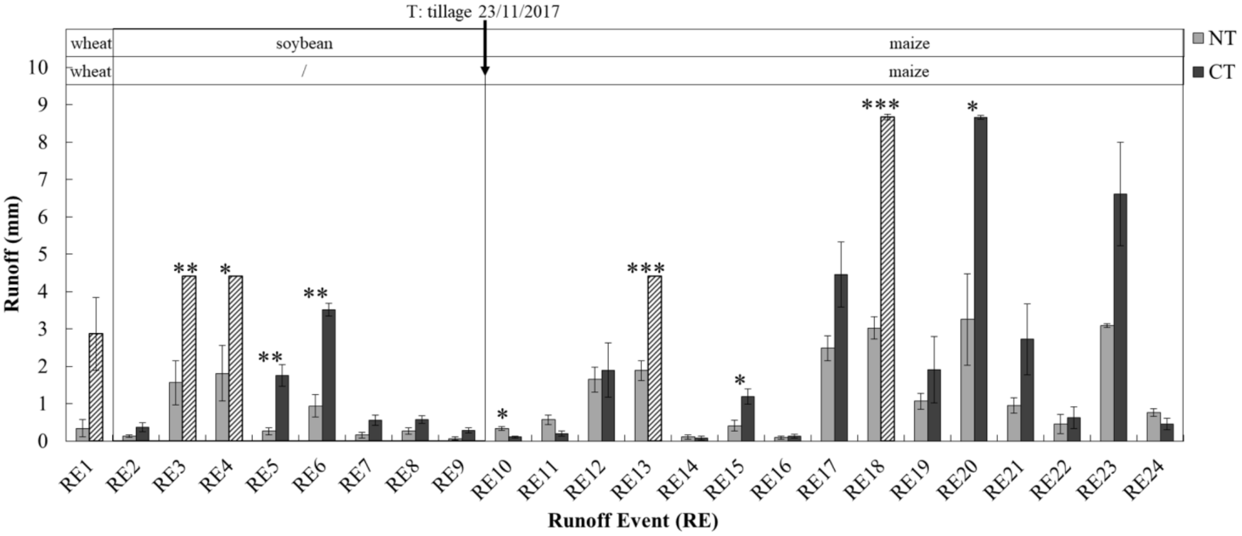

Fig. 1.4. Runoff volumes (expressed in mm) sampled from NT and CT plots at each runoff event. Vertical bars indicate standard error; the crops in CT and NT fields and the date of the tillage operation performed on CT field are indicated at the top. The dotted columns represent the events in which runoff volumes exceeded the capacity of the tanks for one or more CT sub-plots, so the runoff volume could not be precisely quantified. Asterisks indicate statistical significance levels: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$.

### *1.3.3 Sediment losses and concentrations*

Higher sediment losses were generally measured in the CT plot than in the NT plot, although the difference was statistically significant in only 10 out of 24 runoff events (RE1, RE4, RE6, RE7, RE10, RE13, RE15, RE17, RE18, RE20, and RE23). Fig. 1.5 reports sediment losses measured in NT and CT plots at each runoff event. The sediment loss in CT plot ranged from 0.63 kg ha$^{-1}$ (RE14) to 884 kg ha$^{-1}$ (RE18), whereas in NT plot they were from 0.29 kg ha$^{-1}$ (RE14) to 86 kg ha$^{-1}$ (RE3). Variability in the measurements was higher in the CT than in the NT plot., The sediment loss was higher in NT plot than CT only in runoff event RE10,but this was due to the higher runoff volume measured in NT plot at this event and not to the sediment concentration, which was lower in NT plot than in CT (sediment concentrations are reported in Fig. 1.6). The cumulative sediment loss was higher in CT (3368 kg ha$^{-1}$) than in NT (406 kg ha$^{-1}$). Higher total sediment losses were measured in the second sampling period (2782 and 247 kg ha$^{-1}$ for CT and NT respectively) compared to the first (586 and 159 kg ha$^{-1}$ for CT and NT respectively). Overall, NT practices coincided with a 50% to 95% reduction in sediment losses. In other geographical areas, Araya et al. (2011), Tiessen et al. (2010), and Schuller et al. (2007) (in Ethiopia, Canada, and Chile respectively) reported similar values of sediment loss reduction with conservation tillage, despite the fact that the NT soil in our study is not a mature conservation soil but is still going through the transition period.

Sediment concentration also appears to be affected by soil management. The concentration in runoff from NT treatment was lower than CT for the majority of runoff events, although the difference was statistically significant only for nine (RE12, RE13, RE15, RE17, RE18, RE19, RE20, RE21, and RE23) (Fig. 1.6). the sediment concentration was higher in NT than in CT for only five events in the first sampling period (RE1, RE3, RE7, RE8, and RE9), but the difference was not statistically significant for any of them. The average sediment concentration from NT was only 47% of that from CT. Indeed, the reduction in runoff rate and volume decreases the capacity of surface runoff to carry sediment (Araya et al., 2011; Myers and Wagger, 1996; Tarolli et al., 2019; Vaezi et al., 2017). What is also interesting is that the average sediment concentration from NT in the second sampling period (1.05 g $L^{-1}$) was lower than the one recorded in the first (3.51 g $L^{-1}$), whereas for CT there were no differences between the two periods. These lower concentrations may have resulted from the continued consolidation of the undisturbed soil surface over the period involved in this study leading to a lower sediment detachment during rainfall events.

Four cases of rainfall events causing runoff are shown in Fig. 1.7, two of them in 2017 and the other two in 2018. Fig. 1.7a represents a late summer rainfall, on 13/9/2017, which caused considerable runoff volumes from both CT and from NT. The event accounted for 28.6 mm of rainfall, with a maximum 5-min intensity of 52.8 mm $h^{-1}$. Measured runoff coefficients and sediment yields were 12.3% and 52.9 kg $ha^{-1}$ in the CT plot and 3.3% and 9.0 kg $ha^{-1}$ in the NT plot. Fig. 1.7b shows a rainfall event on 25/9/2017 for which light runoff was measured, that caused little soil erosion. The rainfall amount and 5-min intensity were low (8.2 mm and 14.4 mm $h^{-1}$ respectively), but this event was preceded by several others during the previous 30 days that totaled cumulative precipitation of 146 mm. This would have increased soil water content during the previous days, reaching the saturation of the soil surface during this event and generating a light saturation-excess runoff in both CT and NT. The runoff coefficients and sediment yields were 3.5% and 3.5 kg $ha^{-1}$ in the CT plot and 0.9% and 1.0 kg $ha^{-1}$ in the NT plot. Fig. 1.7c shows a rainfall occurring during summer (14/5/2018), three weeks after harrowing the CT field. The event accounted for 10.8 mm of rainfall, with a maximum 5-min intensity of 28.8 mm $h^{-1}$. Low runoff was measured in both plots but, as mentioned above, runoff in NT was higher than that measured in CT. The runoff coefficients and sediment yields were 1.0% and 3.1 kg $ha^{-1}$ in the CT plot and 3.1% and 6.3 kg $ha^{-1}$ in the NT plot. The observed values have been attributed to the effect of tillage operations on the CT soil morphology and conductivity, which is assumed to increase after tillage (Biddoccu et al., 2017). Erosion was detected in both plots, but in NT it was twice that in CT. Fig. 1.7d represents a summer storm, on 9/8/2018, which accounted for 40.4 mm of rainfall in a very short time (one hour), with a maximum 5-min intensity of 84.0 mm $h^{-1}$. This event occurred 15 weeks after the tillage of CT soil. It is likely that this short and intense rainfall increased the soil water content very rapidly in both soils. However, the CT soil having lower infiltration rate and surface storage capacity, soil saturation may have occurred earlier in CT than NT. Thus, a higher runoff caused by infiltration excess occurred in CT (8.67 mm) than in NT (3.25 mm). Sediment yield was very high in the CT plot (606.4 kg $ha^{-1}$) and low in the NT (24.4 kg $ha^{-1}$).

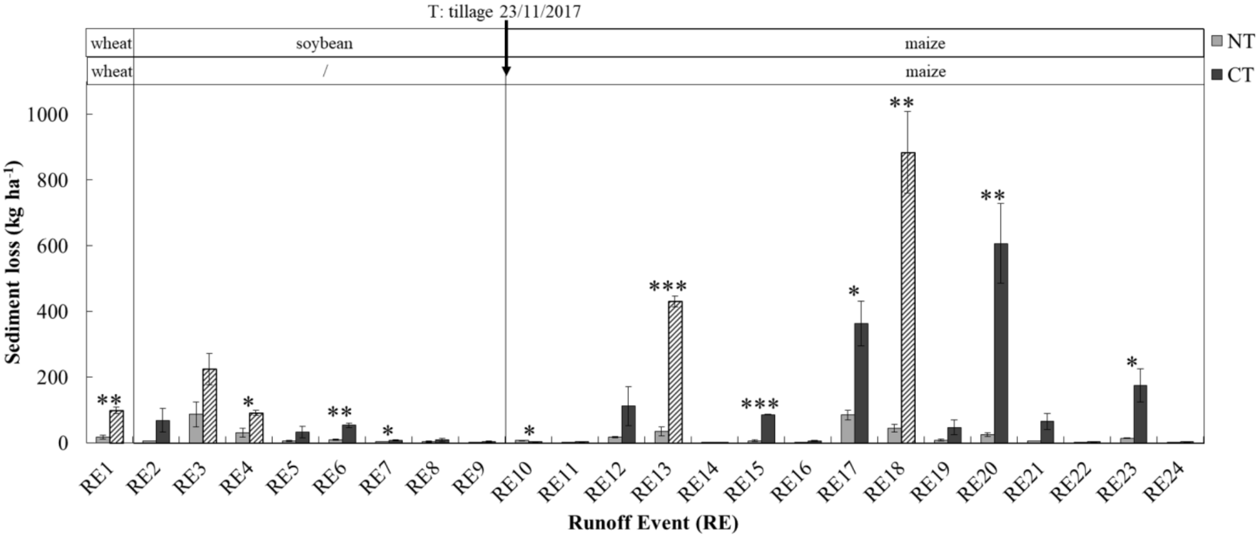

Fig. 1.5 Sediment loss from NT and CT plots at each runoff event. Vertical bars indicate standard error; the crops in CT and NT fields and the date of tillage operation performed on CT field are indicated at the top. The dotted columns represent the events in which runoff volumes exceeded the capacity of the tanks for one or more CT sub-plot, so the sediment loss could not be precisely quantified. Asterisks indicate statistical significance levels: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$.

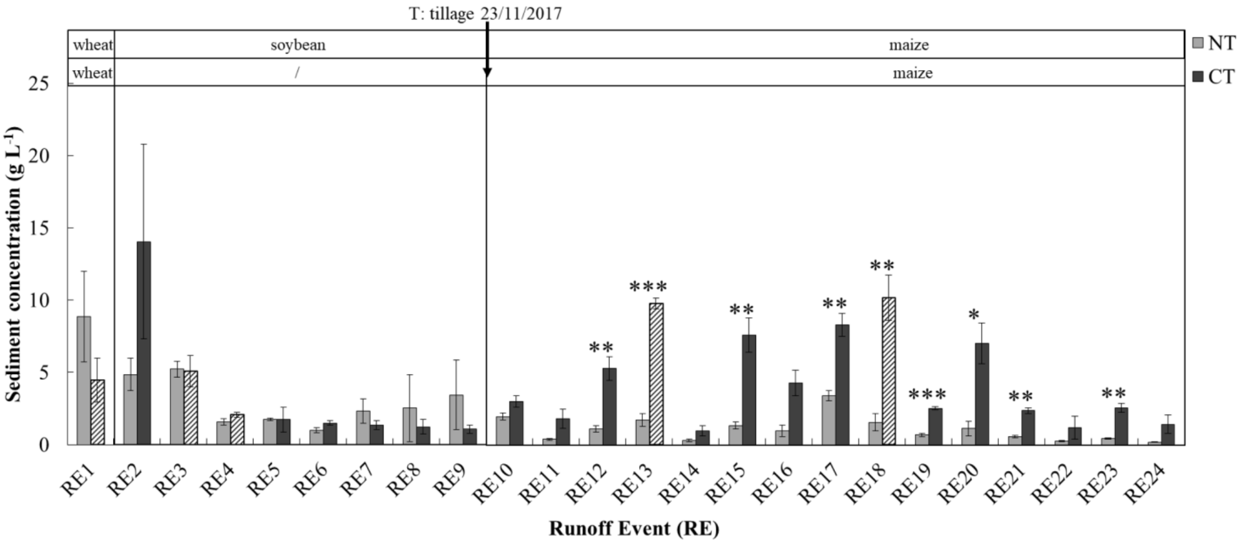

Fig. 1.6 Sediment concentration from NT and CT plots at each runoff event. Vertical bars indicate standard error; the crops in CT and NT fields and the date of tillage operation performed on CT field are indicated at the top. The dotted columns represent the events in which runoff volumes exceeded the capacity of the tanks for one or more CT sub-plot, so the sediment concentration could not be precisely quantified. Asterisks indicate statistical significance levels: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$.

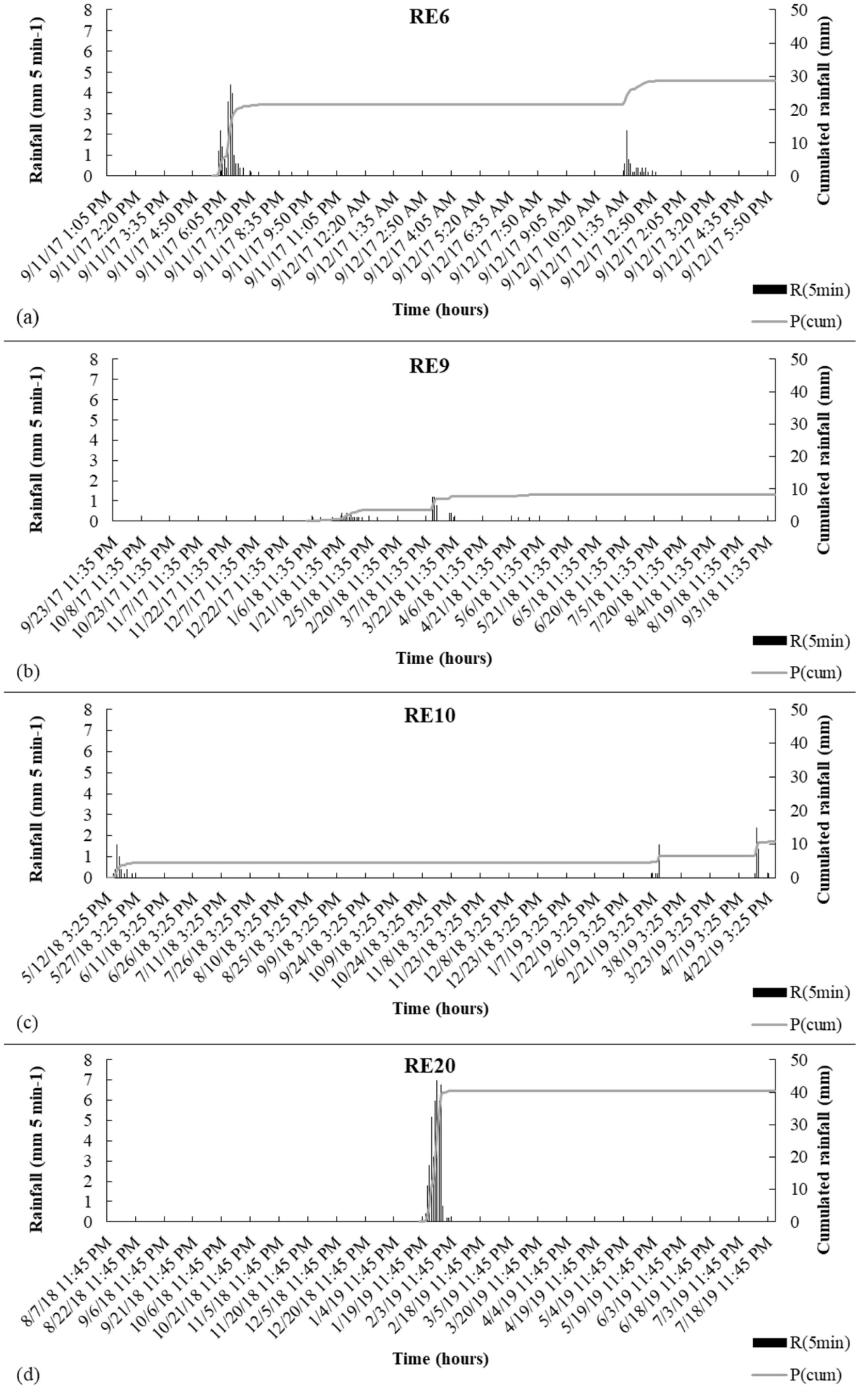

Fig. 1.7 Examples of rainfall events causing runoff with pluviometer data (5 min step). R(5min) = rain intensity at 5 min step; P(cum) = cumulative rainfall.

### *1.3.4 Evaluation of no-tillage practice on runoff and sediment loss reduction*

The values of *RRB* and *SRB* reported in Fig. 1.8 confirm a good reduction of runoff and sediment loss in NT plot compared to CT for most of the runoff events. As mentioned above, the different treatments (tillage and no-tillage) produced different surface morphology in the two soils. The NT soil, not having been subjected to tillage for four years, was not affected by the mechanical disturbance factor and could consolidate its surface morphology over time, improve surface concavities and water storage capacity, and consequently the ability to attenuate both runoff and soil erosion. Tarolli et al. (2019) found that, given the more pronounced concavities and convexities in the NT surface, the potential water depth was approximately three times that observed in CT. These high storage capacity concavities retained the rainfall water and runoff flow, delaying runoff. Consequently, the rainfall was more likely to infiltrate into the soil and generate less runoff. Conversely, on the CT soil, tillage induced a rougher surface just after seedbed preparation, but it became smoother over time and more subject to crust formation, as observed by Wang et al. (2017). As the season progressed, the CT soil decreased infiltration capacity as well as resistance to detachment, which in turn resulted in the potential for higher runoff and sediment yields. In NT, sediment can be easily intercepted and entrapped by micro-depressions, which greatly affect sediment production, and this highlights the importance of a rougher surface. These observations were also supported by Potter et al. (1995), Bewket and Sterk (2003), Barbosa et al. (2009), and Wang et al. (2017).

Considering the entire study period, the *SRB* was greater than the *RRB*. This indicates that the no-tillage better reduced the sediment losses than runoff amount. Several studies suggest that conservation cropping systems seem to be more effective in reducing soil losses than runoff on the plot scale (Armand et al., 2009; Leys et al., 2010; Maetens et al., 2012; Montgomery, 2007; Wang et al., 2017). As mentioned above, a tilled soil is characterized by micro-depressions or furrows having the ability to store water and sediment during a rainfall event. However, with the occurrence of rainfall events, the eroded sediment accumulates in the depressions or furrows and in turn results in less space for sediment retention at a later stage. This is not the case of the NT soil, since surface morphology is assumed to be more stable and consolidates over time. Thus, with NT, the sediment reduction benefits were greater than those of runoff.

The *RRB* during the first sampling period was always greater than 50% whereas, during the second period, 4 out of 15 runoff events showed a negative value of *RRB* and overall the *RRB* was lower. This behavior was not observed for *SRB* values, which were similar in the first and second sampling period except for RE10, which was a particular case. The variability in runoff ratio and soil loss ratio between different years was described by Maetens et al. (2012) who explored the effectiveness of soil and water conservation techniques (including no-tillage) on runoff and soil erosion in Europe and the Mediterranean, analyzing 65 time-series of annual runoff and sediment loss. This paper showed that runoff ratio, calculated as the annual runoff ratio between no-tillage and conventional tillage plots, tended to increase over a six-year period following first application of the no-tillage technique, suggesting that the effectiveness of no-tillage to reduce runoff decreases over time. Since the years of the present study were the third and the fourth of no-tillage, it is likely that a slight and gradual decrease in runoff reduction effectiveness of NT is beginning, and further monitoring could confirm this hypothesis. Similarly to what we observed, Maetens et al. (2012) did not identify such a trend for sediment loss ratio, attributing this to increasing surface sealing when the soil is not tilled for several years.

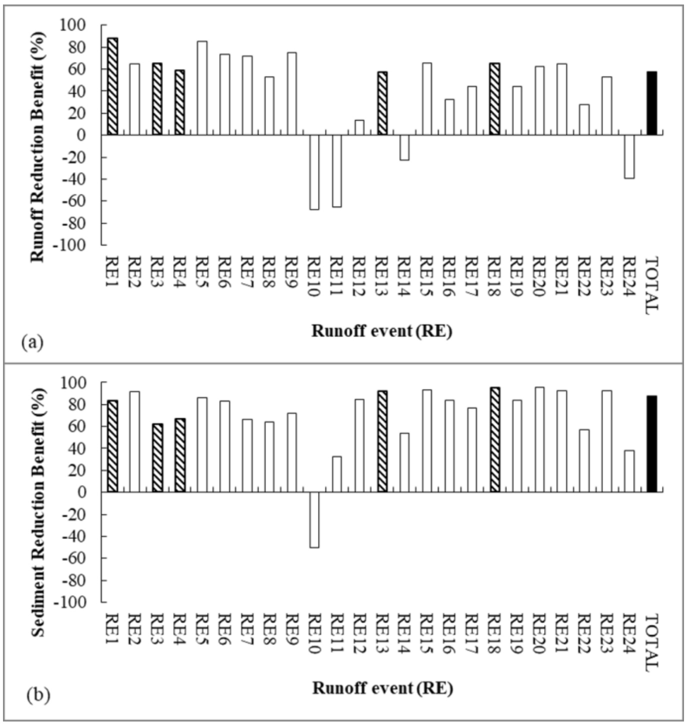

Fig. 8 Runoff Reduction Benefit (*RRB*) (a) and Sediment Reduction Benefit (*SRB*) (b) at each runoff event (white columns) and for the entire sampling period (black columns). The dotted columns represent the events in which runoff volumes exceeded the capacity of the tanks for one or more CT sub-plot.

## 1.4 Conclusion

In this study, we investigated the effect of conservation agriculture (no-tillage) on runoff and soil erosion under natural rainfall conditions in field plots. Runoff volume and soil erosion were analyzed in a no-tillage field during the transition period from conventional to conservation agriculture in comparison to a tilled field. A dataset of 24 rainfall runoff events was collected during the late spring-summer period of 2017 and 2018. This research showed that after four years of NT practices a reduction of more than 50% in runoff volumes, and a 50% to 95% reduction in sediment losses were achieved. Although the field was still in the transition period, NT was beneficial in the reduction of runoff and soil erosion, in turn promoting rainfall water and soil conservation. The reduced runoff and sediment yield in the NT plot probably translates into reduced risk of herbicide losses from the field. This has important on-site benefits, in terms of sustainable management of the soil and surface water quality. Surface morphology and soil hydrological properties in a tilled field are subjected to considerable variations over the time due to tillage operations and field management. For this reason, further and frequent monitoring of soil morphology and hydrological properties are required to provide a more solid basis to make conclusions. Since morphological parameters of a cultivated field can vary throughout the year due to soil cover, wheel traffic, and weather conditions (da Rocha Junior et al., 2016), a multi-temporal topographical survey can be particularly useful in accurately monitoring of surface changes during the seasons. This could provide useful information to model water fluxes and characterize hydrological connectivity and pesticide transport in tilled and non-tilled soil.

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# Chapter 2. A new rapid procedure for simultaneous determination of glyphosate and AMPA in water at sub µg/L level

## Abstract

Glyphosate is the most used pesticide worldwide and its impact on the environment is becoming increasingly significant. Glyphosate and its main metabolite AMPA are frequently detected in streams and rivers. In this study, an analytical method is presented that combines Ultra-High Performance Liquid Chromatography with mass spectrometry (UHPLC–ESI-MS/MS) for glyphosate and AMPA analysis in environmental water samples. The method was developed starting from an application of Waters Corporation, and involves the use of an alternative derivatizing reagent, the commercially available AccQ·Tag ™ Ultra Derivatization Kit (Waters Corporation, Milford, MA, USA). The kit contains the derivatizing reagent 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). Derivatization takes place directly in the injection vial and no sample pre-concentration is needed. The derivatization is simple, quick and robust, which fits well within the needs of a routine method for the analysis of glyphosate and AMPA. Derivatized glyphosate and AMPA were recorded in positive ion mode. The method demonstrates a good linear relationship in the concentration range from 0.2 μg/L for glyphosate and 0.05 μg/L for AMPA to 100 μg/L and an accurate recovery. The method developed has been successfully applied to the determination of glyphosate and AMPA in 23 runoff water samples collected from a field in the Po Valley (North-East Italy), an agricultural area where glyphosate is widely used.

Keywords: pesticides; LC–MS; derivatization; runoff water.

## 2.1 Introduction

Glyphosate (N-(phosphonomethyl)glycine), is an organophosphorus broad spectrum, non-selective, post-emergence herbicide. It is used in various applications for weed and vegetation control, and has become the most common pesticide worldwide with an estimated use of 1.35 million metric tons in 2017 (Global Industry Analysts Inc., 2018). Glyphosate has a low acute human toxicity but its carcinogenicity is very controversial (Tarazona et al., 2017), it is also moderately toxic to birds, most aquatic organisms, earthworms and honeybees (PPDB, 2019a). Its impact on the environment is becoming increasingly significant. Glyphosate is a small molecule with three polar functional groups (carboxyl, amino and phosphonate groups), and it behaves as an amphoteric molecule (Borggaard and Gimsing, 2008; Mazzetti, 2015). Glyphosate is highly polar and well soluble in water (11.6 g $L^{-1}$ at 20°C), and it is strongly sorbed to soil components such as clay, iron oxides and humic substances (Montgomery, 2000). These characteristics favour the transport to surface water via runoff (Beltman et al., 2001). As a consequence of its widespread use, this herbicide is classified among the frequently detected organic molecules instreams and rivers, and generally exceeds the European standard for drinking water (0.1 µg $L^{-1}$) (Botta et al., 2009). In general, glyphosate is moderately persistent in soil, with DT50 values ranging from 1 to 68days, whereas its rate of degradation in water is generally slower because there are fewer microorganisms in water than in most soils (Ghassemi, 1981; PPDB, 2019a). Glyphosate's primary decomposition route in water and soil is through microbial degradation (Franz et al., 1997). AMPA (aminomethylphosphonic acid) is the major metabolite of glyphosate and it is an aminophosphonate with primary amine group. AMPA is chemically similar to glyphosate and shows similar properties in terms of behaviour and toxicity (Jönsson et al., 2013). It is polar and well soluble in water (146.6 g $L^{-1}$ at 20°C), therefore, following a glyphosate application, AMPA is frequently detected in surface waters. Degradation of AMPA is generally slower than that of glyphosate (DT50 = 39–331days) ("Pesticide background statements," 1948; PPDB, 2019b).The determination of glyphosate and AMPA at sub µg $L^{-1}$ level is difficult due to their very polar, and in most cases ionic character, low volatility, low mass and lack of chemical groups that could facilitate their detection (Ibáñez et al., 2005).Liquid chromatography coupled to mass spectrometry(LC–MS/MS) is currently the method chosen most often for polar analytes due to its high selectivity and sensitivity (Hanke et al., 2008). Using LC–MS/MS, derivatization is often required to reduce the polar character of the analytes and enable a good chromatographic separation. Currently, the most used method involves pre-column derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) (Catrinck et al., 2014; Hanke et al., 2008; Ibáñez et al., 2006, 2005; Olivo et al., 2015; Oulkar et al., 2017; Peruzzo et al., 2008; Sun et al., 2017; Vreeken et al., 1998). However, FMOC-Cl pre-column derivatization methods have some disadvantages: (i) long derivatization reaction time, (ii)complex sample preparation, (iii) poor reproducibility in derivatization at low concentration levels, (iv) high degree of interference caused by the excess of derivatizing reagent, and (v) frequent need for pre-concentration and/or purification steps to achieve satisfactory precision, accuracy and sensitivity (quantification limits at sub-ppb level) by direct injection of the derivatized sample (Guo et al., 2016; Salazar et al., 2012). Analysis of underivatized glyphosate and AMPA has been per-formed by various approaches. In the "Quick Method for the Analysis of Highly Polar Pesticides" (QuPPe-Method) for the determination of glyphosate in foods of plant origin, the analysis of non-derivatized samples is conducted by LC–MS with a Hyper-carb column that enhances retention of polar compounds (Anastassiades et al., 2013).This method, applied to the direct injection of aqueous matrices, does not result particularly robust. It also requires special measures such as a column activation protocol and specific washings of the chromatographic system, which affect the performance of the method in terms of sensitivity and repeatability. As an alternative to liquid chromatography, and given the zwitterionic nature of glyphosate and AMPA, the analysis of glyphosate has also been conducted by both cation- and anion-exchange ion chromatography (IC) with suppressed conductivity (Zhu et al., 1999). Good retention and separation of glyphosate and AMPA were achieved with most of the IC based methods. However, this valid approach involves the need for a specific instrument. In this paper, an analytical method is presented that combines Ultra-High Performance Liquid Chromatography with mass spectrometry (UHPLC–ESI-MS/MS) for glyphosate and AMPA analysis in water samples. The method was developed starting from an application of Waters Corporation, and involves the use of an alternative derivatizing reagent to FMOC, the commercially available AccQ·Tag$^{TM}$ Ultra Derivatization Kit (Waters Corporation, Milford, MA, USA) (Waters Corporation, 2016, 2015). The kit was originally designed for amino acid analysis and it contains the derivatizing reagent 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) (Cohen and Michaud, 1993). This reagent reacts with the amino functional group present in glyphosate and in its metabolites. Derivatization takes place directly in the injection vial and no sample pre-concentration is needed. The derivatization step is simple, quick, and robust, which fits well within the needs of a routine method for the analysis of glyphosate and AMPA. The method developed has been applied to the determination of glyphosate and AMPA in runoff water samples collected from a field in the Po Valley (North-East Italy), an important agricultural area where glyphosate is widely used.

## 2.2 Materials and methods

### *2.2.1 Chemicals*

Analytical-grade standards of glyphosate and AMPA were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The compound purity was 98% and 99%, respectively. Isotope-labelled glyphosate (1,2-13C,15N) and isotope-labelled AMPA (13C,15N), used as surrogate internal standard (IS), were purchased from Toronto Research Chemicals (North York, Canada). Reagent-grade sodium tetraborate decahydrate was obtained from Honeywell Riedel-de Haën (Morris Plains, NJ, USA). UPLC-MS-grade acetonitrile was supplied by Honeywell Riedel-de Haën (Sigma-Aldrich Co. Ltd. (Steinheim, Germany)) and UPLC-MS-grade water from Scharlau (Barcelona, Spain). Ultrapure water was obtained by a Arium® basic system (Sartorius Stedim Biotech GmbH, Goettingen, Germany). Formic acid for mass spectrometry (~98%) was purchased from Honeywell Fluka (Seelze, Germany). The AccQ·Tag$^{TM}$ Ultra Derivatization Kit (AccQ·Tag$^{TM}$ Ultra Reagent Powder, AccQ·Tag$^{TM}$ Ultra Borate Buffer, and AccQ·Tag$^{TM}$ Ultra Reagent Diluent) was purchased from Waters Corporation (Milford, MA, USA).Glyphosate tends to bind to the active sites of glass (Goscinny et al., 2012). To avoid this adsorption phenomenon, borosilicate glassware was replaced by polypropylene labware. Standard stock solutions of glyphosate and AMPA were prepared dissolving 1 mg powder, accurately weighed, in 10 mL of ultrapure water obtaining a final concentration of approximately 100 mg $L^{-1}$. A 50-mg $L^{-1}$ composite standard was prepared in water by mixing and diluting the individual standard stock solutions. Glyphosate and AMPA working calibration standard and fortification solutions were prepared by serial dilution of the composite standard in water. All standard solutions were stored in polypropylene tubes. The isotope-labelled glyphosate and AMPA were purchased as1 mL of 100-µg $mL^{-1}$ stock solution in water. A 10-µg $mL^{-1}$ standard solution was prepared by dissolving 1 mL of the stock solution in 10 mL of water. Standard working solutions were prepared by diluting the intermediate standard solution in water.

### *2.2.2 Derivatization procedure*

The method involves the derivatization of glyphosate and AMPA with the AccQ·Tag$^{TM}$ Ultra Derivatization Kit. The AccQ·Tag$^{TM}$ Ultra Reagent Powder, which is the derivatizing reagent, dry 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), was reconstituted with 1 mL of AccQ·Tag$^{TM}$ Ultra Reagent Diluent (acetonitrile) in a 1.5 mL polypropylene vial. The vial was vortexed for10 s and heated on a heating block at 55°C for 15 min, until the powder was dissolved. The 5% borate buffer solution is included in the derivatization kit; however, as the kit was designed for amino acids analysis, which requires a different ratio between derivatizing agent and buffer, the quantity of buffer solution is not enough. Thus, when necessary, this was prepared by dissolving 5 g of sodium tetraborate decahydrate in 100 mL of ultrapure water. Aliquots of 200 µL of samples were transferred into 1.5 mL polypropylene vials and 225 µL of 5% borate buffer, followed by 25 µL of freshly prepared derivatizing reagent, were added. The vials were capped, vortexed for 10 s, and placed in a water bath at 55°C for 15 min, then their contents were analysed by UHPLC–ESI-MS/MS. The reaction products are stable for 5 days. Fig. 2.1 shows the reaction between AQC and the analytes.

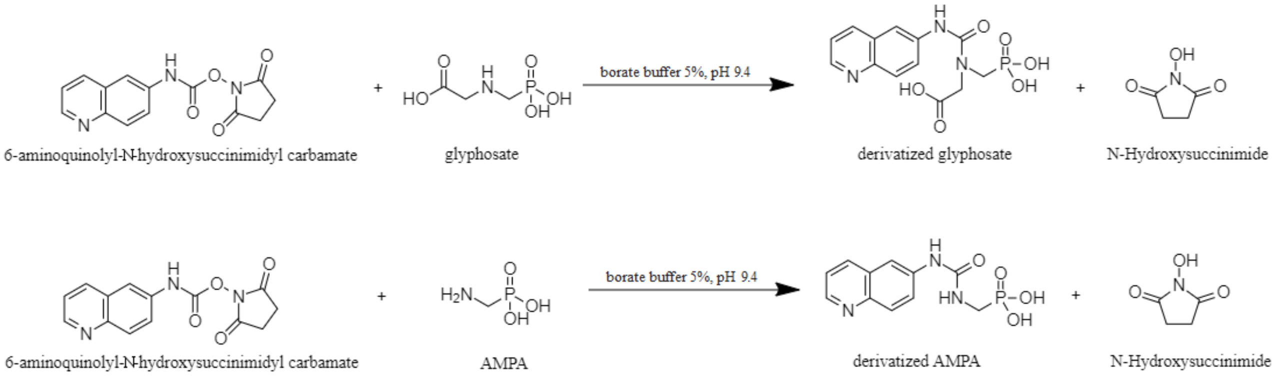

Fig. 2.1 Reactions of glyphosate and AMPA with AQC.

### *2.2.3 Instrumentation*

The analysis was conducted with a triple quadrupole mass spectrometer system (TSQ Quantiva, Thermo Scientific, Waltham, Massachusetts, USA) equipped with an ESI source and coupled to an UltiMate 3000 UHPLC System (Thermo Scientific, Waltham, Massachusetts, USA). The LC analysis was performed with a reversed-phase C18 column of 2.1 mm × 100 mm, 2.5 µm, 100 Å particle size (XSelect HSS T3 XP, Waters Corporation, Milford, MA, USA). The LC system was operated with mobile phase A (UPLC-MS-grade water) and mobile phase B (UPLC-MS-grade acetonitrile), both with 0.1% formic acid. The instrument settings for mass analysis were the following: positive ion mode, spray voltage 3500 V, vaporizer temperature 350°C, sheath gas pressure 40 (arbitrary units), auxiliary gas pressure 10 (arbitrary units), sweep gas pressure 0 (arbitrary units), ion transfer tube temperature 350°C, CID gas pressure 1.5 mTorr. Nitrogen was used as sheath gas, sweep gas, and auxiliary gas; argon was used as collision gas. The triple quadrupole analyser was operated in SRM (selected reaction monitoring) mode with 0.7 resolution for Q1 and Q3. Operating software was Xcalibur 4.0.

### *2.2.4 LC procedure*

To perform the chromatographic separation, a multi-step gradient was used for B as follows: 0% for 1 min, 0–28% in 5 min, 28–98%in 2.5 min, 98% for 1.5 min. Initial conditions were restored in 2 min and re-equilibration was achieved in 3 min. Injection volume was 20 µL. Flow rate was set at 0.4 mL min$^{-1}$. Column temperature was maintained at 40°C.

### *2.2.5 Runoff water samples*

In order to check the applicability of the proposed method to real matrices, runoff waters from a glyphosate-treated field were analysed. Runoff is an important component of herbicide mobility and in many cases is considered to be the main route for herbicide transport outside agricultural fields and surface water pollution (Dinelli et al., 1996; Wauchope, 1978). Runoff water is a complex matrix where many potential compounds may interfere. Runoff water samples were collected from a field at the Padova University Experimental Farm located in the Po Valley, North-East Italy. The experimental site is a field, managed under no-till practices, of 200 × 35 m, with a 0.8% slope down towards the ditch. The field was sown with maize and glyphosate was applied pre-sowing (1080 g ha$^{-1}$). A research programme started in 1997 in which buffer strips with different length and composition were planted. Buffer strips are non-treated areas of vegetation planted between crop-lands and a water body through which runoff must pass before entering the surface waters. Buffer strip vegetation is recognized as an effective practice to reduce herbicide runoff from cultivated fields (Krutz et al., 2005). The sampling was focused on two contrasting situations: the absence of buffer strip (NoBS) and the presence of a 6 m wide buffer strip with two rows of trees and shrubs (6BS). Each thesis (NoBS and 6BS) had two replicates. As the buffer strips are designed to reduce herbicide runoff, the majority of samples coming from the plots with buffer strip are expected to contain a lower concentration of glyphosate and AMPA compared to the plots without buffer strip. From April to November 2016, after each runoff event, runoff water was sampled using a collector system with multi-pipe divisors designed and built to measure runoff volumes (Vianello et al., 2005). Runoff water samples were placed in aluminium bottles, sealed and stored in a freezer at −18°C until analysis.

40 mL of runoff water were centrifuged at 5000 rpm for 15 min at25◦C in a Hettich® Universal 320R centrifuge with a rotor radius of 99 mm (Sigma-Aldrich Co. Ltd. Steinheim, Germany). An aliquot of the supernatant (10 mL) was filtered at room temperature (about 20°C) using regenerated cellulose membrane filter 0.20 µm pore size. 177.5 µL of runoff water samples was introduced into a 1.5-ml polypropylene vial together with 22.5 µL of isotope-labelled glyphosate and AMPA standards (0.1 µg mL$^{-1}$). Samples were derivatized according to the procedure described above. None of the samples gave peaks that interfered with the determination of glyphosate and AMPA. A test to verify that the filtration step does not entail any loss of analyte was performed by comparing the results of the analyses of two portions of the same sample of glyphosate and AMPA (50 ppb)

prepared in ultrapure water: one portion was subjected to filtration before IS addition and derivatization, while the other was treated without prior filtration. Each sample was analysed three times and the results were averaged. Based on the t-test for independent samples the results obtained by filtering the sample did not differ from those obtained without filtration.

### *2.2.6 Validation study*

Linearity of the method was evaluated analysing ten standards solutions in triplicate, in the 0.05–100 μg $L^{-1}$ range, derivatized according to the procedure described above. Precision (repeatability, expressed as relative standard deviation, in %) and recoveries were determined within a day by analysing fortified blank samples in quintuplicate. This experiment was performed at the spiking levels of 0.5, 1, and 10 μg $L^{-1}$ and, in the case of AMPA, also of 0.1 μg $L^{-1}$. The limit of detection (LOD) and the limit of quantitation (LOQ) were estimated experimentally through the injection of samples with decreasing concentrations; LOD and LOQ were determined as the analyte concentrations which give a signal-to-noise (S/N) ratio of three and ten, respectively. The specificity of the method was evaluated by analysing a blank procedure, a processed blank sample, and a blank sample spiked at the lowest fortification level assayed (LOQ). Under these conditions, the response obtained for both the blank procedure and blank samples should not exceed 30%of the response corresponding to the LOQ.

### *2.2.7 Data evaluation*

To ensure the analysis quality when processing real-world samples, blank samples fortified at the LOQ, 10 × LOQ, and 100 × LOQ concentration levels were used as quality controls distributed among the batch of samples every three-four injections. Quantification of the sample batch was considered satisfactory if the QC recoveries were in the 70–120% range. The values found in real samples were confirmed by means of the two transitions selected for each compound.

## 2.3 Results and discussion

### *2.3.1 MS optimization*

Full-scan MS spectra and product-ion MS/MS spectra of derivatized glyphosate and AMPA were recorded in positive ionization mode. Optimization of ionization parameters, collision energy and CID gas pressure, was performed by direct infusion of a 10 mg $L^{-1}$ standard solution of each compound, previously derivatized, in a flow containing 20% of acetonitrile and 0.1% of formic acid. In agreement with the application note of Waters Corporation (Waters Corporation, 2015), the transitions monitored and used for quantitation of glyphosate were 340→171 (Q) and 340→116 (q) (Fig. 2.2) and for AMPA 282→171 (Q) and 282→116 (q) (Fig. 2.3).

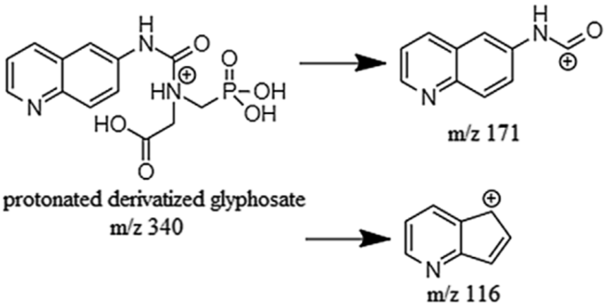

Fig. 2.2 Monitored transitions for derivatized glyphosate

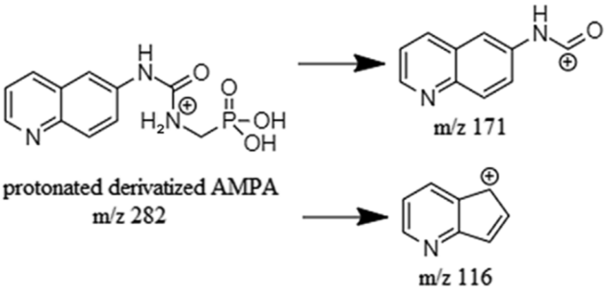

Fig. 2.3 Monitored transitions for derivatized AMPA.

These ions come from fragmentation of the derivatized molecules and are fragments of AQC. The selected reaction monitoring (SRM) transitions chosen for the determination of derivatized glyphosate and AMPA and their isotopic labelled analogues, as well as the optimized MS/MS parameters, q/Q ion ratios and retention times, are shown in Table 2.1. q/Q ion ratios and retention times are reported with their standard deviation.

Table 2.1 Analysis parameters for derivatized glyphosate, AMPA, and internal standards.

| Compound | Precursor ion (m/z) | Product ion (m/z)[a] | Collision energy (V) | q/Q ion ratios | Retention times (minutes) |
|---|---|---|---|---|---|
| Derivatized glyphosate | 340 | *Q* 171 | 18 | 0.16 ± 0.02 | 3.64 ± 0.03 |
| | | *q* 116 | 55 | | |
| Derivatized AMPA | 282 | *Q* 171 | 15 | 0.16 ± 0.02 | 3.90 ± 0.02 |
| | | *q* 116 | 49 | | |
| Derivatized isotope-labelled-glyphosate | 343 | *Q* 171 | 18 | 0.16 ± 0.01 | 3.64 ± 0.03 |
| | | *q* 116 | 55 | | |

| | | | | | |
|---|---|---|---|---|---|
| Derivatized isotope-labelled-AMPA | 284 | *Q* 171 | 15 | 0.16 ± 0.01 | 3.90 ± 0.02 |
| | | *q* 116 | 49 | | |

[a] *Q*: Transition used for quantification; *q*: transition used for confirmation.

### *2.3.2 Method validation*

To construct the calibration curves, known fixed amounts of isotopically labelled glyphosate and AMPA, used as internal standard, were spiked into a series of calibration solutions in ultrapure water and in runoff water containing known concentrations of glyphosate (0.2, 0.5, 1, 5, 10, 25, 50, 75, 100 µg L$^{-1}$) and AMPA (0.05, 0.1, 0.2, 0.5,1, 5, 10, 25, 50, 75, 100 µg L$^{-1}$) in mixture at the same concentration. The calibration solutions were derivatized in triplicate and analyzed. Standard calibration curves were generated as the ratio of the analyte peak area to the IS peak area (Aa/AIS), plotted against the ratio of the analyte amount to the IS amount (Qa/QIS). The calibre tion equation is: Aa/AIS= m·(Qa/QIS) + q. Once verified that the error on q obtained from the fitting was higher than the q value itself, the data were interpolated by the equation passing through zero, Aa/AIS= m·(Qa/QIS). Fig. 2.4 shows the calibration curves obtained for derivatized glyphosate and derivatized AMPA in ultrapure water and runoff water.

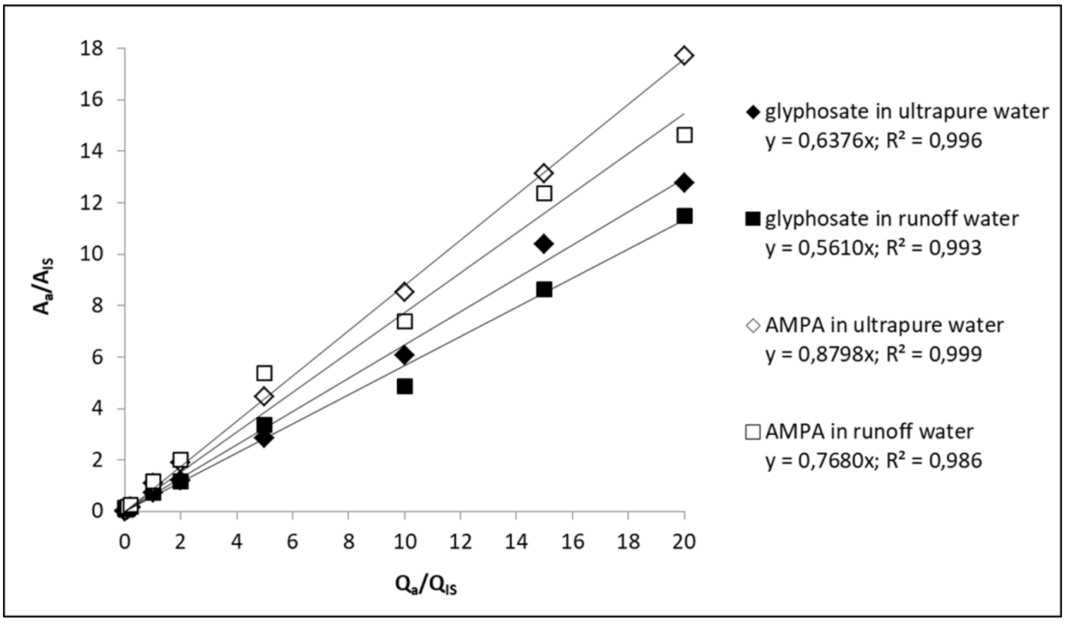

Fig. 2.4 Standard calibration curves of derivatized glyphosate and AMPA in ultrapure water and in runoff water, generated as the ratio of analyte peak area to IS peak area (Aa/AIS), plotted against the ratio of analyte amount to IS amount (Qa/QIS).

A linear response was confirmed in all cases in the entire concentration range considered, based on the squared correlation coefficients ($R^2$) obtained (Fig. 2.4), as well as randomly distributed residuals below 20%. As for the matrix effect, as can be seen in Fig. 2.4, the m value of the calibration curves constructed in runoff water differ by less than 15% with respect to the curves obtained in ultrapure water, so it was concluded that the matrix effect is negligible. The average recovery percentages in runoff water were 88% for glyphosate and 89% for AMPA. The LOD and LOQ were determined as being, respectively, 0.2 and 0.5 μg $L^{-1}$ for glyphosate and 0.05 and 0.1 μg $L^{-1}$ for AMPA. Fig. 2.5 and Fig. 2.6 compare the SRM chromatograms for derivatized glyphosate and AMPA in runoff water at LOQ level with those in the blank runoff water. The method was found to be precise and accurate, as reported in Table 2.2.

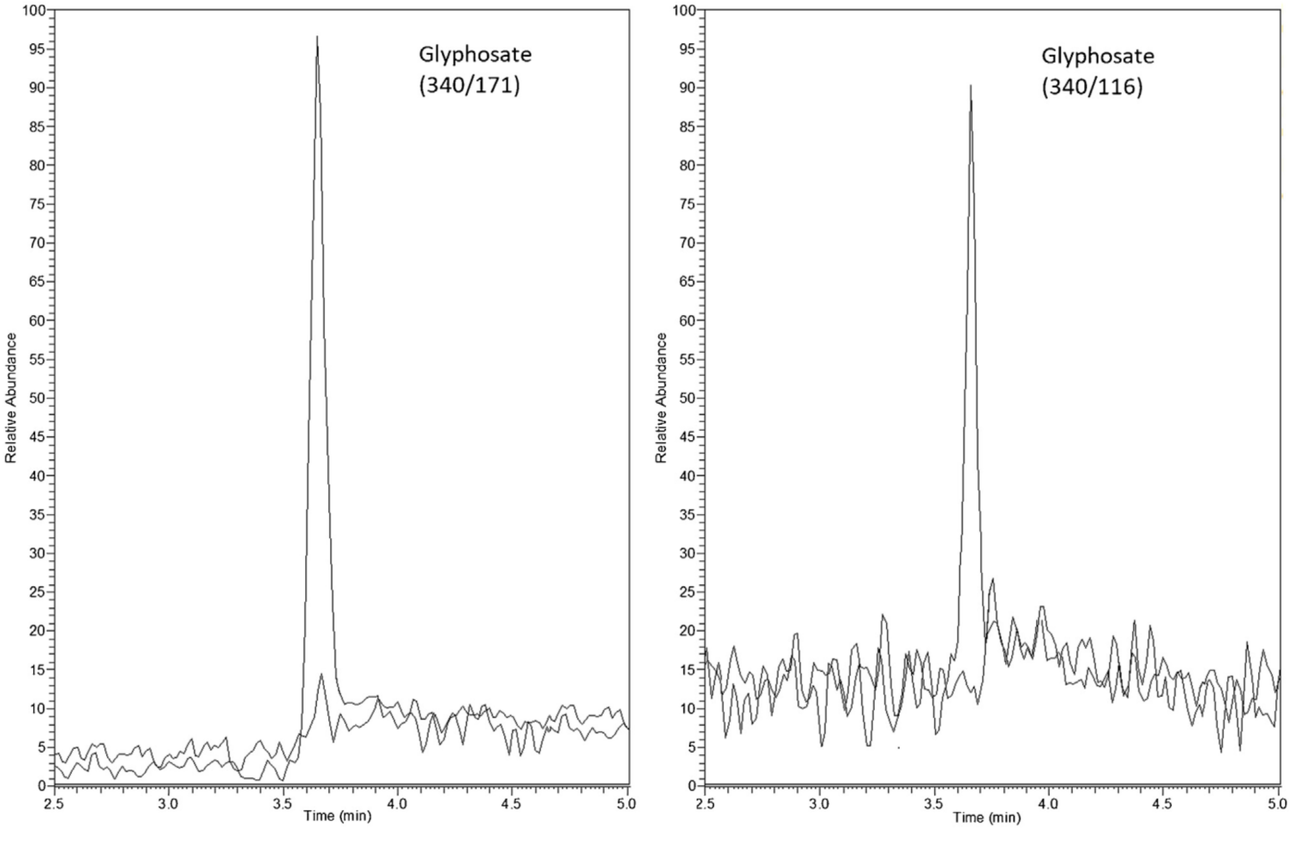

Fig. 2.5 SRM chromatograms for derivatized glyphosate in runoff water fortified with glyphosate at LOQ level (0.5 ppb) and in runoff water blank sample.

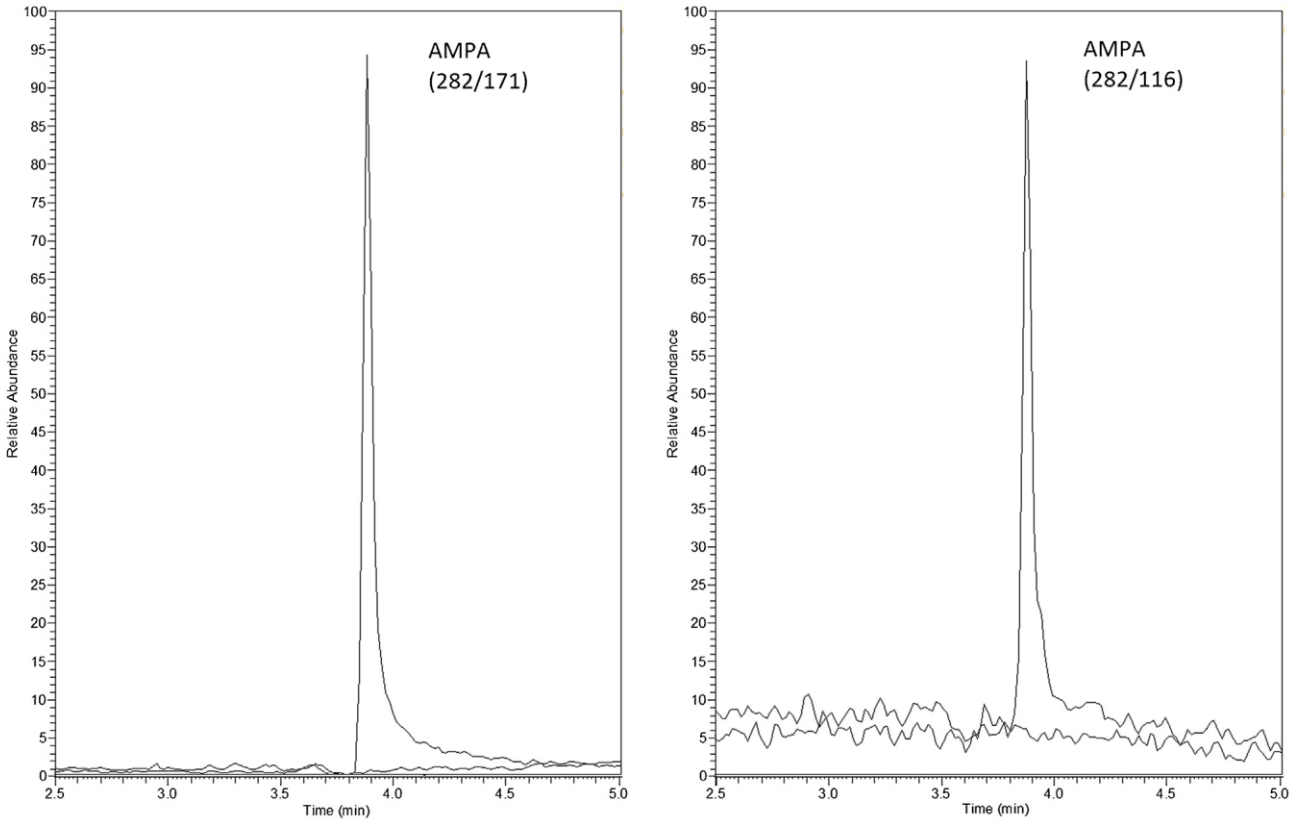

Fig. 2.6 SRM chromatograms for derivatized AMPA in runoff water fortified with AMPA at LOQ level (0.1 ppb) and in runoff water blank sample.

Table 2.2 Precision and accuracy obtained in the method validation.

| Concentration of fortified runoff water samples (ppb) | Glyphosate | | AMPA | |
|---|---|---|---|---|
| | Accuracy (%) | RDS (%) | Accuracy (%) | RDS (%) |
| 0.1 | - | - | 19 | 11 |
| 0.5 | 16 | 7 | 17 | 7 |
| 1 | 18 | 8 | 16 | 10 |
| 10 | 12 | 8 | 11 | 9 |

### *2.3.3 Analysis of runoff water samples*

The method was applied to the analysis of 23 runoff water samples collected from a field treated with glyphosate at Padova University Experimental Farm located in the Po Valley, a region with high agricultural activity. Known fixed amounts of isotopically labelled glyphosate and AMPA, used as internal standard, were spiked into each sample. Every sample was derivatized in triplicate and analysed for glyphosate and AMPA concentration. Confirmation of the compounds identity was based on, besides SRM transitions, also on retention times and on q/Q ion ratios. The requirements that retention times do not differ more than 0.1 min in samples and in the reference solutions and that the tolerance for q/Q ion ratios is within 30% were fully satisfied (Pihlström et al., 2017). Every analysis sequence included quality control samples spiked at the LOQ, 10 × LOQ and 100 × LOQ level. The quality control consisted of blank runoff water spiked with the analytes, previously analysed to confirm the absence of analytes. Satisfactory quality control recoveries were obtained for both compounds (between 70 and 120%) demonstrating the robustness of the method over the period of the analysis. In Fig. 2.7 the SRM chromatograms of Q and q for glyphosate and AMPA in a sample and in a reference runoff solution are shown. It can be seen that the q/Q ion ratios are in agreement with that reported in Table 2.1.

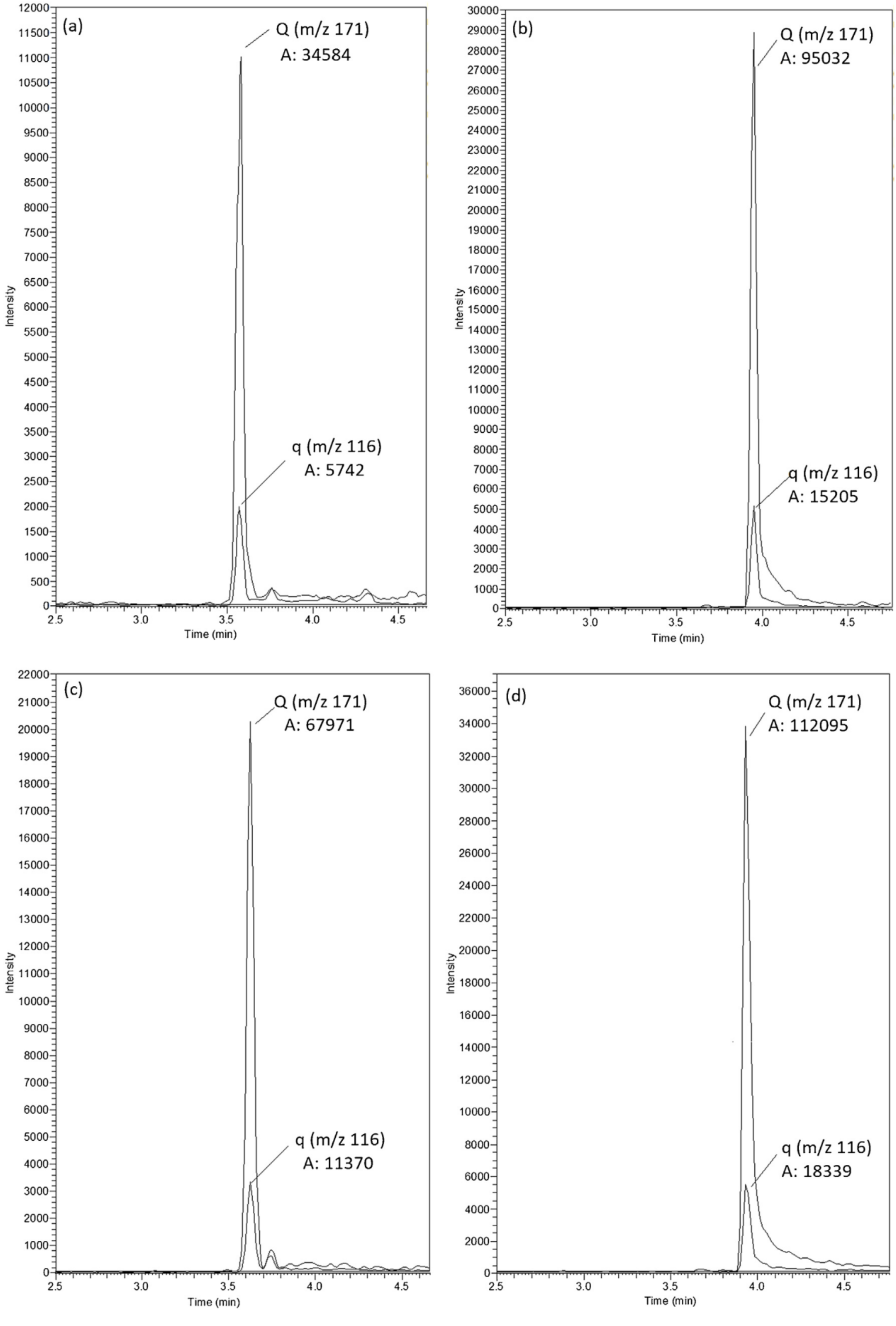

Fig. 2.7 SRM chromatograms of Q and q for glyphosate and AMPA in a runoff water sample (a, b) and in a reference runoff solution (c, d). (a) Glyphosate concentration 2.05 ppb, q/Q ion ratio 0.16;

(b) AMPA concentration 0.81 ppb, q/Q ion ratio 0.16; (c) glyphosate concentration 5 ppb, q/Q ion ratio 0.16; (d) AMPA concentration 5 ppb, q/Q ion ratio 0.16.

From April to November 2016 nine runoff events occurred. Glyphosate was detected in all the samples analysed from NoBS and 6BS plots. Glyphosate concentration in runoff water samples from NoBS plots ranged from 0.6 to 24.6 µg $L^{-1}$. The concentrations of glyphosate in samples from 6BS plots were lower than in NoBS plots, with values ranging from 0.5 to 12.7 µg $L^{-1}$. The highest glyphosate concentrations were recorded in the first and second runoff events (41 and 44 days after herbicide treatment) which were caused by short and intense rainfall events. AMPA was detected in all the samples analysed from NoBS plots and in only 75% of the samples from 6BS plots. AMPA concentrations were between 0.4 and 7 µg $L^{-1}$ in NoBS plots and between 0.3 and4.8 µg $L^{-1}$ in 6BS plots. Also for AMPA, highest concentrations were recorded after short and intense rainfall. As expected, in the presence of buffer strip the concentration of glyphosate and AMPA in runoff water was reduced. The buffer strips provided average con-centration reductions of 77% for glyphosate and 50% for AMPA in comparison with the plots without buffer strip.

## 2.4 Conclusions

A sensitive method based on UHPLC–ESI-MS/MS was developed for the determination of glyphosate and its major degradation product, AMPA, in waters. The method involves derivatization with the AccQ·Tag$^{TM}$ Ultra Derivatization Kit to decrease the polarity of analytes and improve their retention in the UHPLC system used. The derivatization is simple and quick, it requires small sample volumes and takes place directly in the injection vial. No sample pre-concentration was needed, significantly reducing sample preparation complexity. Thanks to the derivatization step, this method can easily be used with conventional analytical equipment available in most laboratories. Glyphosate and AMPA were recorded in positive ion mode. The method demonstrates a good linear relationship (average $R^2 \geq 0.993$) in the concentration range of 0.2–100 µg $L^{-1}$ for glyphosate and 0.05–100 µg $L^{-1}$ for AMPA. Recovery was accurate. LOD and LOQ were established to be 0.2 and 0.5 µg $L^{-1}$, respectively, for glyphosate and 0.05 and 0.1 µg $L^{-1}$ respectively for AMPA. The developed method was successfully applied to the determination of glyphosate and AMPA in 23 runoff water samples. No interfering peaks were found in any water samples at the retention windows of glyphosate and AMPA with the use of both ion transitions. These results demonstrated that simple and rapid sample preparation in combination with robustness and good sensitivity of detection were the principal advantages of this method. These characteristics make it a valid alternative to the traditional methods.

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# Chapter 3. Effect of vegetative buffer strips on herbicide runoff from a nontilled soil

## Abstract

Buffer strips can reduce herbicide runoff from cultivated fields due to the ability of vegetation to impede the flow of surface runoff, promote infiltration, and adsorb herbicides. Conservation agriculture has many environmental advantages, but the transition phase from a conventional to a conservation system is a critical period, especially for surface runoff. In 2015, a field in Italy that was transitioning from conventional to conservation agriculture was tested to analyze the efficacy of different types of buffer strips in reducing the runoff of three herbicides compared with no-buffer plots. At each runoff event, water volume was measured and terbuthylazine, metolachlor, and mesotrione concentrations were determined. Buffer strips were able to reduce the number of runoff events by 63% to 83%. The runoff volumes were significantly reduced in the presence of buffer strips, whereas no differences were found between different types of buffer strips. Among herbicides, mesotrione was not detected in runoff water samples. The highest losses of terbuthylazine and metolachlor were from plots without buffer strips during the first three runoff events. All types of buffer strips significantly reduced the total losses of terbuthylazine and metolachlor in the monitored runoff events, with a reduction of more than 99%. This study confirmed that buffer strips are a very effective mitigation system against surface water pollution by herbicides, even in agronomic situations that promote runoff.

Keywords: buffer strips; herbicides; no tillage, runoff

## 3.1 Introduction

Conservation agriculture aims to achieve sustainable and profitable agriculture through the application of three principles, which are linked to each other: (1) continuous minimum mechanical soil disturbance (reduced tillage or no tillage), (2) permanent soil cover, and (3) diversified crop rotations in the case of annual crops or plant associations in the case of perennial crops (Hossain, 2013). Reduced tillage or no tillage is the most important component of conservation agriculture as minimal soil disturbance and permanent residue cover can only be achieved through the reduction or elimination of tillage (Shahzad et al., 2016). Applied collectively, conservation agriculture practices have complementary positive outcomes: no tillage maintains stable soil structure and biological activity; a permanent organic soil cover protects the soil surface from erosion and creates a stable and favorable microclimate; cover crops provide organic matter, reduce erosion, and improve soil fertility; and crop rotation enhances the system biodiversity and therefore contributes to weed, pest, and disease control (Berger et al., 2010).

Conservation agriculture has many advantages, but some problems may arise in the transition phase from a conventional to a conservation system. A reduction in hydraulic conductivity was observed in soil measurements taken under the same crop and soil management conditions (no-till) with the increasing number of years under continuous cropping (Aparicio et al., 2002; Aparicio and Costa, 2007) also reported that bulk density at 3- to 8-cm depth was higher under no till, and the hydraulic conductivity was lower. There are field observations of a platy soil structure occurring in the first centimeters of soil in the Rolling Pampa, Argentina, with thin and flat fragments oriented parallel to the soil surface in some experiments under no till (Alvarez et al., 2009; Bonel et al., 2005; Sasal et al., 2006). Some authors have also reported continuous platy structure in silty soils under no till in other areas (Boizard et al., 2013). This platy structure alters the drainage pattern, restricts water entry into the soil, and favors surface runoff (Sasal et al., 2017).

During the transition phase, the control of pests and weeds, previously provided by conventional tillage, necessitates chemical inputs (Knowler and Bradshaw, 2007). When the crop is seeded in a nontilled seedbed, the weeds that are present before or at seeding are eliminated with herbicides. Before seeding, the most often used herbicide is glyphosate, and after seeding, herbicides most used in Italy, especially in the Po Valley, are terbuthylazine, metolachlor, and mesotrione. The contamination of water resources by terbuthylazine and metolachlor affects the entire national territory (ISPRA, 2016a), and in 2014, these pollutants were found to be the main contaminants of both surface and groundwater in the Veneto region, reaching concentrations greater than 0.1 $\mu g\ L^{-1}$ (ISPRA, 2016b). Terbuthylazine, metolachlor, and mesotrione constitute the active ingredients of the commercial product Lumax and so are frequently applied in combination.

A problem associated with the use of herbicides is that their residues and metabolites can be transported with runoff from soil to surface waters, contaminating them (Fenoll et al., 2014). Conservation agriculture, because of the permanent soil cover with cover crops and crop residues, improves soil structure and is indicated as a method to increase water infiltration (Tebrügge and Düring, 1999). Higher infiltration rates prevent losses of surface water, improving soil water status and water-holding capacity (Thierfelder and Wall, 2009). This is true for a “mature” conservation soil. However, a transition period of approximately 5 years is generally necessary before a nontilled soil improves, and benefits are seen in terms of improving soil structure (Thierfelder and Wall, 2009). During the transition phase, the typical advantages of conservation agriculture, such as higher organic matter content, are not yet evident. Furthermore, it is a critical phase for soil structure, which has low organic matter content and is prone to compaction. These conditions favor surface runoff, for which there is an increased risk compared with the prior years of conventional management. For this reason, it is extremely important to implement effective measures to limit runoff as much as possible.

Vegetative buffer strips are recognized as an effective conservation practice to mitigate the loss of sediment, nutrients, and pesticides in surface runoff (Blanco-Canqui et al., 2004; Krutz et al., 2003; Lin et al., 2011; Misra et al., 1996; Reichenberger et al., 2007; Udawatta et al., 2002; Veum et al., 2009). Buffer strips are non-treated areas of vegetation planted between croplands and a water body through which runoff must pass before entering the surface waters. The filtering capacity of riparian environments strongly depends on factors such as climate (e.g., recurrence and intensity of storm events), topography (e.g., terrain slope, size of the collector area), soil (e.g., water infiltration capacity), and vegetation (e.g., influences on soil infiltration and hydraulic roughness) (Tomer et al., 2009). These factors modify the soil filtering capacity by affecting one or more of the basic retention mechanisms, such as infiltration, sedimentation, absorption, and adsorption (Arora et al., 2010; Mayer et al., 2007). Buffer vegetation, especially grass, acts as a filter: by increasing surface roughness, it increases infiltration and decreases flow volumes and speed of runoff. Through this filtering action, pollutants are detained in the buffer zone soil, where they may undergo a number of processes, such as biotransformation, uptake by plants, or adsorption onto soil particles, before they reach surface waters (Phillips, 1989). Buffer strips also encourage sediment deposition, reducing the transport capacity of runoff (Rose et al., 2002). The plants in the vegetative buffer strips confer a higher organic matter content to the filter zone than in the adjacent cultivated field (Cardinali et al., 2014). This organic matter accumulation should increase adsorption capacity and microbial activity for herbicide degradation, thus reducing the amount of herbicide in surface runoff (Staddon et al., 2001).

The efficacy of vegetative buffer strips in mitigating herbicide runoff has been well studied. The influence of different buffer strip characteristics (length, width, slope, surface roughness, vegetation cover, presence of litter, structure, and composition) and various external conditions (rainfall regimes, season, herbicide concentrations, and time) on their effectiveness has been widely examined (Borin et al., 2005; Lerch et al., 2017; Milan et al., 2013; Misra et al., 1996; Otto et al., 2012, 2008; Phillips, 1989; Rankins et al., 2001; Vianello et al., 2005). To our knowledge, little information is available about the application of buffer strips to mitigate herbicide runoff in the context of conservation agriculture and in particular during the transition phase between conventional and conservation tillage.

Therefore, the aim of this study was to analyze the efficacy of vegetative buffer strips (1) compared with a plot without a buffer and (2) within different types of buffers in reducing the runoff of three herbicides − terbuthylazine, metolachlor, and mesotrione − from a nontilled soil. The year of the study was the first year of conservation management in a field that had been conventionally tilled in previous years.

## 3.2 Materials and Methods

### *3.2.1 Site information*

The study was conducted during 2015 at the Padova University Experimental Farm in the Po Valley, northeast Italy (45°12′N, 11°58′ E, altitude 6 m a.s.l.). The soil is classified as Fulvi-Calcaric Cambisol (FAO-UNESCO, 1990). It is silty-loam textured (11.8% clay, 44.9% silt, 43.3% sand), rich in limestone, with sub-basic pH (pH = 8.11), good organic carbon content (0.92%), and medium-low hydraulic conductivity ($4.7 \times 10^{-4}$ cm $s^{-1}$). The annual rainfall in 2015 was 533 mm (the wettest month was October with 87 mm of rain, whereas the driest was December with 0 mm) with a total of 84 rainy days. Air temperature and soil temperatures at a depth of 10 cm increased from January (minimum average, 0.2°C and 1.2° C, respectively) to July (maximum average, 30.0°C and 30.7°C, respectively). The rainfall conditions during the experimental period of May through August are illustrated in Fig. 3.1.

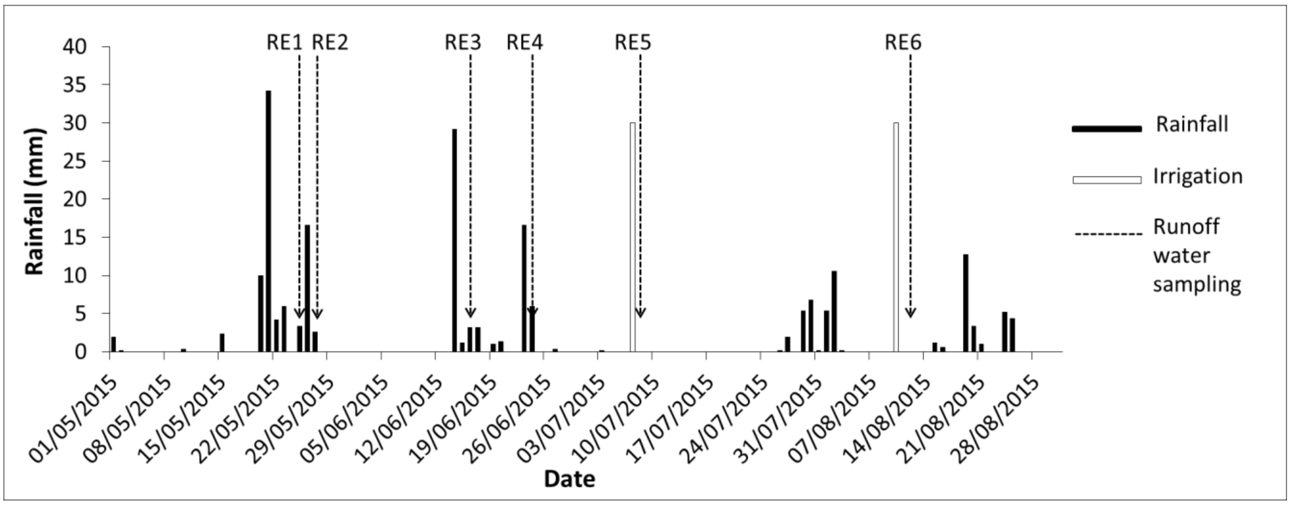

Fig. 3.1 Daily rainfall from May to August 2015. White columns indicate irrigations; the arrows indicate the sampling of runoff water.

The experimental site is a rectangular field of 200  35 m, with a 0.8% slope down toward a ditch (Fig. 3.2). Four types of buffer strips located between the cropland and the ditch are compared with plots without a buffer (WB) that were cultivated up to the edge of the ditch. The buffer strips differ in width and composition: (a) 3 m wide formed by grass cover only (3G), (b) 3 m wide with grass cover and a shrub and tree row (3G1R), (c) 6 m wide with a shrub and tree row (6G1R), and (d) 6 m wide with two rows of trees and shrubs (6G2R). The hedgerows are 1.5 and 4.5 m from the ditch. The herbaceous cover is Festuca arundinacea Schreber and the rows are of regularly alternating Viburnum opulus L. shrubs and Platanus hybrid Brot. trees. The plots are 20 × 35 m, and the five treatments have two replicates.

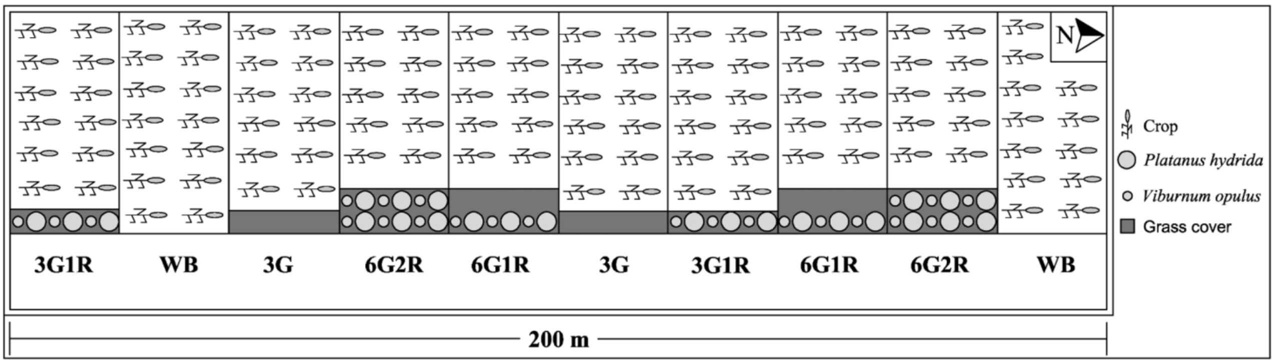

Fig. 3.2 Layout of the experimental field with the five types of vegetated buffer strips with two replicates. WB: plot without buffer strip; 3G: 3-m-wide buffer formed by grass cover only; 3G1R: 3-m-wide buffer formed by grass cover and a shrub and tree row; 6G1R: 6-m-wide buffer with a shrub and tree row; 6G2R: 6-m-wide buffer with two rows of trees and shrubs.

On May 6, 2015, the field was sod-seeded with maize, after seedbed desiccation with glyphosate, and three herbicides were applied as a formulated product (Lumax; Syngenta, Basel, Switzerland): mesotrione, S-metolachlor, and terbuthylazine. The dose of Lumax was 4 L ha$^{-1}$ with 750, 1,250, and 150 g active ingredient ha$^{-1}$ for terbuthylazine, S-metolachlor, and mesotrione, respectively.

### *3.2.2 Chemicals*

Terbuthylazine (TERB, 99% purity), S-metolachlor (METO, 98% purity), and mesotrione (MESO, 98% purity) analytical standards were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The main chemical and environmental properties were taken from the Pesticide Properties DataBase (Footprint, 2007) and are shown in Table 3.1. Stock solutions (10 mg L$^{-}$1) of each herbicide standard were prepared in methanol, protected from light and stored at −20° C. Mixtures of standard solutions with concentrations ranging from 0.02 to 2.0 μg L$^{-}$1 were injected to obtain the linearity of detector response. Methanol (HPLC grade), water (HPLC-grade), and all other chemicals (analytical-grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Table 3.1 Main chemical and environmental properties of the three tested herbicides (Footprint, 2007; MacBean 2012).

| **Properties** | **Unit** | **Mesotrione (MESO)** | **Metolachlor (METO)** | **Terbuthylazine (TERB)** |
|---|---|---|---|---|
| Molecular weight | g mol$^{-1}$ | 339.3 | 283.79 | 229.71 |
| Water solubility at 20 °C | mg L$^{-1}$ | 1500 | 480 | 6.6 |
| Groundwater Ubiquity Score (GUS) |  | 2.69 | 1.91 | 3.07 |
| $K_{OC}$ | mg L$^{-1}$ | 19–141 | 121–309 | 162–278 |
| Log $K_{OW}$ |  | 0.11 | 3.0 | 3.2 |
| $t_{1/2}$ (lab at 20 °C) | days | 19.6 | 14.5 | 75.1 |
| DT50 (field) | days | 5 | 21 | 22.4 |

### *3.2.3 Runoff Sampling*

The runoff events (RE), after which water samples were collected, occurred on the following dates: May 25, 2015; May 27, 2015; June 16, 2015; June 24, 2015; July 8, 2015; and August 12, 2015. Runoff events with daily rainfall and irrigations during the experimental period are shown in Fig. 3.1. Runoff was collected by a system with multipipe divisors designed and built to measure runoff volumes (Vianello et al., 2005); water volume was measured after each RE. Each plot had a sampler located in the ditch, and water samples were collected to determine herbicide concentrations.

Runoff samples from each plot were placed in aluminum bottles, sealed, and stored in a cooler at +4°C until analysis.

### *3.2.4 Analytical Procedure*

The procedures used were derived from previous studies (Barchanska et al., 2012; Freitas et al., 2004). To determine herbicide concentrations in water, samples (0.5 L in volume) were filtered at room temperature using 0.45-µm pore size cellulose nitrate membrane filters. After filtration, an acetate buffer was added (ca. 10%, vol/vol) to yield a pH of 4. The samples were concentrated and cleaned by solid phase extraction using an OASIS HLB sorbent cartridge (60 mg; Waters, Milford, MA) and a Baker spe-12G vacuum column processor. Cartridges were conditioned with 2 mL of MeOH followed by 2 mL of water. The samples were extracted, and the cartridges were then washed with 1 mL of a MeOH/$H_2O$ mixture (5/95, vol/vol). Excess water was removed by opening the valves to let air pass through them for approximately 20 min. The analytes were eluted with 5 mL of MeOH without the use of vacuum. The 5-mL MeOH aliquots were reduced to 50 µL with the use of a gentle stream of nitrogen gas at 45°C.

The analyses were performed by liquid chromatography-mass spectrometry using an 1100 Series Agilent Technologies system (Santa Clara, CA) equipped with binary pump, diode array detector, and MSD SL Trap mass spectrometer with ESI source. A Eurospher II (Knauer, Berlin, Germany) column C18 P with TMS endcapping, 150 × 4.6 mm i.d., 3 µm, 110 Å, was used to analyze the samples. The mobile phase consisted of water (solvent A) and methanol (solvent B), both acidified with 0.6% formic acid. The LC gradient for separation of the herbicides was as follows: from 0 to 4 min, a linear increase of solvent B from 60% to 80% and flow rate from 0.4 to 0.6 mL min$^{-}$1; from 4 to 11 min, a linear increase of solvent B from 80% to 100% at flow rate of 0.6 mL min$^{-}$1; initial conditions were re-established in 5 min, and re-equilibration time was 2 min. A 10-µL sample volume was manually injected each time. Retention times were 5.5 (±0.4), 9.0 (±0.2), and 10.1 (±0.3) min for MESO, TERB, and METO, respectively. Herbicide concentration in water samples was quantified by comparison with a calibration curve. Recovery was 79%, 85%, and 93% for MESO, TERB, and METO, respectively. The limit of detection, 3:1 signal-to-noise ratio, was 14, 20, and 70 ng kg$^{-}$1 for TERB, METO, and MESO, respectively. The limit of quantification, 10:1 signal-to-noise ratio, was 68 ng kg$^{-}$1 for TERB and METO, and MESO 20 µg kg$^{-}$1.

### *3.2.5 Statistical Analysis*

In order to assess the effects of buffer strips on runoff volumes and on the amount of total herbicide losses, a nonparametric multiple comparison with Kruskal-Wallis test was used, with significance level of differences set at P = 0.05. The test was performed with TIBCO Statistica 13.2 software for Windows (TIBCO Statistica, Palo Alto, CA). A runoff and loss reduction factor (calculated in mm for runoff volume and in mg ha$^{-1}$ for herbicide loss) was also calculated for the various buffer strips in comparison with the WB:

$$\text{Reduction (\%)} = 100 \times \left(\frac{\text{WB} - \text{buffer}}{\text{WB}}\right)$$

## 3.3 Results

### *3.3.1 Runoff Volumes*

Water volumes at RE are shown in Fig. 3.3. It can be noted that the presence of buffer strips completely intercepted the runoff waters except during a few events. The effect of the buffer strips on runoff volumes proved to be highly significant, whereas no differences were found between types of buffer strips. There were relatively few RE in the experimental period. Rainfall in 2015 (533 mm) was lower than the long-term annual mean of 854 mm. Most events were concentrated in spring and autumn. During the monitored period from herbicide treatment to full summer (99 days), 173 mm of rainfall fell, which is 32% of the annual amount. Six RE occurred with WB, resulting in 2.13 mm of runoff. Of these events, four were caused by rainfall (the total rainfall causing runoff was 108 mm), and two occurred because of irrigation (the total amount of water supplied with the irrigation was 60 mm). The buffer strips reduced the number of RE to 2 for 3G1R and 6G1R plots and to 1 for 3G and 6G2R plots, representing a reduction in number of 63% and 83%, respectively. Only in the WB plots was it possible to measure a runoff volume that in five out of six cases was approximately 0.40 mm. Regarding the plots with buffer strips, a runoff volume greater than 0.10 mm was observed only in the 6G1R during the sixth event.

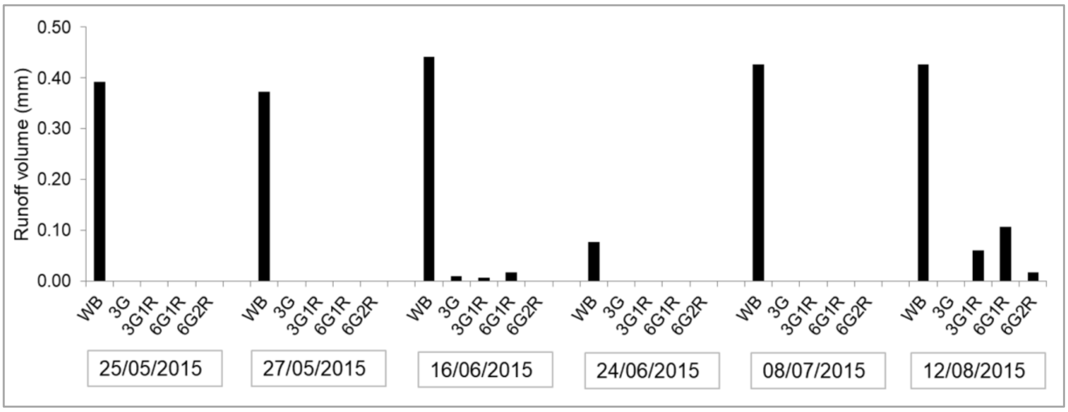

Fig. 3.3 Runoff volumes (expressed in mm) sampled at each RE.

*3.3.2 Herbicide Loss in Runoff*

Among the three herbicides investigated, MESO was not detected in runoff water samples, meaning that its concentration was always below the limit of detection. Figures 3.4 and 3.5 report the losses of TERB and METO, respectively, transported in the runoff (mg ha$^{-1}$).

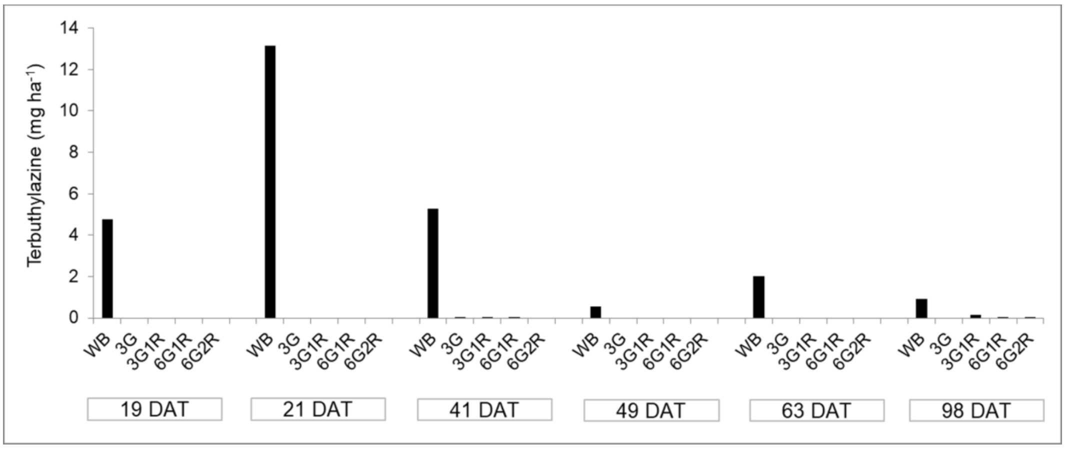

Fig. 3.4 Terbuthylazine loss (mg ha$^{-1}$) at each RE.

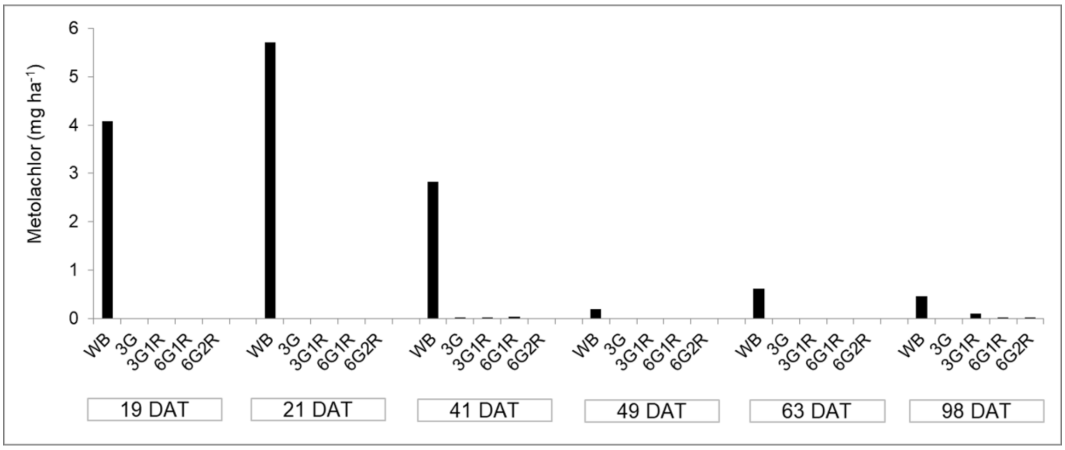

Fig. 3.5 Metolachlor loss (mg ha$^{-1}$) at each RE.

In all recorded RE, TERB and METO losses were significantly higher in WB plots than in plots with buffer strips ($P < 0.01$). The amounts of both TERB and METO lost from plots with buffer strips never reached 0.2 mg ha$^{-1}$. A herbicide loss greater than 2.5 mg ha$^{-1}$ was observed only in WB plots in the first three events. From the fourth event onward, the amount of herbicides transported with runoff water was always below 2.5 mg ha$^{-1}$. In the WB plots, TERB and METO losses were highest in the second RE, which occurred 21 days after treatment. It is interesting that, for both TERB and METO, the herbicides' mass transported in the first RE (occurred 19 days after treatment) was lower than in the second event. From the third event onward, the loss of herbicides in runoff gradually decreased, until values reached 0.91 mg ha$^{-1}$ of TERB and 0.45 mg ha$^{-1}$ of METO 98 days after treatment.

## 3.4 Discussion

### *3.4.1 Runoff Volumes*

What clearly emerged in this study is the reduction of runoff volumes in the presence of buffer strips. In accordance with other studies conducted on this site (Otto et al., 2008) and on similar buffer types (Lee et al., 2000; Schmitt et al., 1999), the buffer strips substantially reduced runoff volumes by 94.2% to 99.6%. The vegetation in the buffer strips (especially grass) has the effect of reducing flow speed and total volume of flow (Carling et al., 2001). This is a consequence of increased infiltration due to the transition created at the field-buffer interface by different soil management and vegetation cover within the buffer (Borin et al., 2005). In this study, in most cases, the presence of buffer strips was able to completely obstruct the runoff water coming from the field. Nevertheless, the scarcity of collected data does not allow the relationship between the width of the buffer strips and the runoff volumes that reach the ditch to be characterized. The same can be said regarding the plant composition (only grass cover or grass and tree row). In WB plots, higher runoff volumes were collected after brief and intense rainfall events. This is in agreement with observations at the same experimental site by Borin et al. (2005), who determined that absent buffer strips the runoff volume is positively correlated with rainfall intensity and negatively correlated with rainfall duration. Borin et al. (2005) also demonstrated that in the presence of buffer strips runoff volume is positively correlated with rainfall intensity and negatively correlated with the number of days from the previous rainfall. The buffer strip soil has high surface roughness and consequently good storage capacity. In the present case, buffer efficacy was reduced only in the third and sixth RE. In the third RE, maximum rainfall intensity was particularly F6 high, as shown in Fig. 3.6, and the three types of buffers (3G, 3G1R, and 6G1R) were unable to completely absorb the runoff water coming from the field, although the sampled runoff volumes were very low. The sixth RE occurred after the second irrigation, affecting three types of buffers (3G1R, 6G1R, and 6G2R) with larger runoff volumes than the third event, whereas the first irrigation did not generate runoff in plots with buffers. When the first irrigation was performed, 13 days had passed since the last rainfall, while at the time of the second irrigation only 6 days had passed since the last rainfall, and the higher soil water content may have allowed less water infiltration during irrigation, favoring runoff even in the presence of buffer strips.

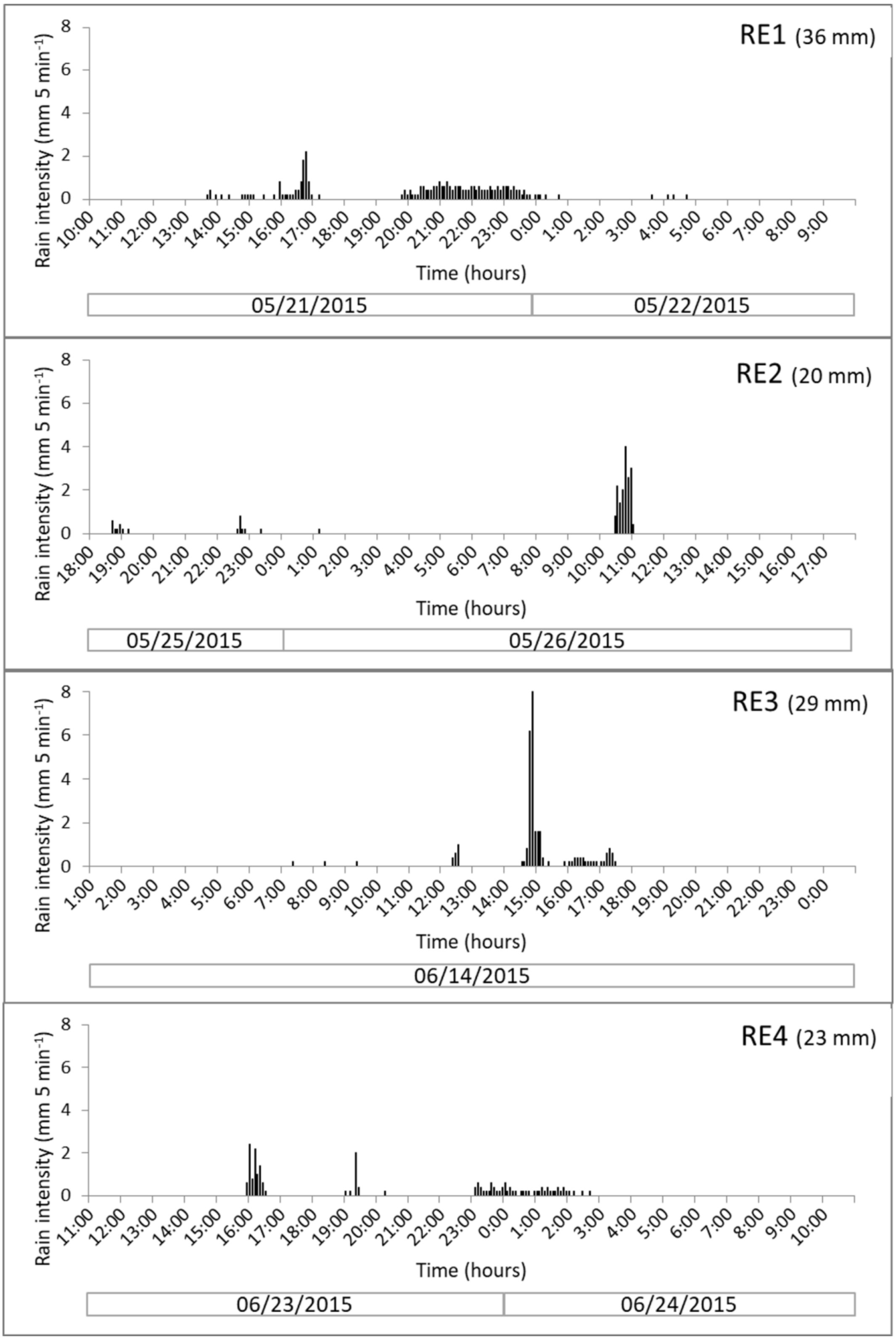

Fig. 3.6 Rainfall intensity pattern of events that caused runoff.

### *3.4.2 Herbicide Loss in Runoff*

Buffer strips were very effective in reducing the transport of herbicides to the ditch. The amount of herbicides found in the water after passing through the different types of buffers was reduced by

100% for MESO, by 99.3% to 100% for TERB, and by 99.2% to 100% for METO. This reduction is similar to that observed on the same site by Otto et al. (2012) and on similar buffer types by Arora et al. (1996) and Pätzold, Klein, and Brümmer (2007)). Krutz et al. (2005) reported that the buffer strip capacity in retaining pollutants mainly depends on plant species, and Giaccio et al. (2016) showed that pollutant retention increased significantly with sediment retention. In this sense, buffers formed by grass cover play an important role. In the present case, a 3-m-wide buffer formed by grass cover of F. arundinacea was able to retain all the runoff water and herbicides, as much as the 6-m-wide buffer with tree rows. However, the rainfall pattern during the monitored period did not promote runoff, so the data set was not large enough to validate this result.

The absence of MESO in runoff water samples may be due to its low persistence in the soil. The DT50 of MESO is 5 days (Table 3.1), so it is reasonable to assume that 19 days after treatment (first RE) this herbicide had been largely degraded in the field. The persistence of TERB and METO in soil is higher, with DT50 of 22.4 and 21 days, respectively. Therefore, when the first RE occurred, these molecules were only partially degraded in the soil. The amount of TERB transported in the runoff was higher than METO in all the events, although the dose of TERB applied with the treatment was lower than METO (750 and 1,250 g ha$^{-1}$, respectively). The reason for the higher amount of TERB in runoff water must be sought in the different physicochemical characteristics. METO is more soluble than TERB, so it immediately enters the soil solution and degrades slightly faster. Conversely, TERB has lower solubility but a higher adsorption coefficient (KOC) and greater persistence. It is therefore assumed that, when the first RE occurred, more than 50% of TERB was still present in the soil, whereas most of the applied dose of METO had already been degraded (Dousset et al., 1997).

As mentioned previously, inWB plots, herbicide losses in the first RE were less than in the second. This may be due to the lower rainfall intensity of the first event (Fig. 3.6), which resulted in lower herbicide transport compared with the second event when rainfall intensity was greater (maximum intensity of 6.6 and 16.4 mm h$^{-1}$, respectively). The third RE had the highest rainfall intensity, with a peak of 18.6mmh$^{-1}$ and a total of 29mm in 6 h. This intense rainfall also caused runoff of the herbicides retained in the buffer strips. In fact, in this event, TERB and METO were also detected in the samples from plots 3G, 3G1R, and 6G1R, although the amount of herbicide losses were much lower compared with the WB plots.

The results of this study show buffer strips are very effective in reducing runoff volumes and limiting the transport of herbicides to the ditch. As already mentioned, in the transition phase from conventional to conservation agriculture, an increase in surface runoff may occur. On the contrary, in this study, the runoff volumes were no higher than those measured on the same site in previous years when the soil was tilled (Otto et al., 2012; Vianello et al., 2005). Nevertheless, this may depend on the characteristics of rainfall events (i.e., quantity and intensity of rainfall) that differ greatly from year to year and are therefore difficult to compare.

In conclusion, this study showed that buffer strips were a very effective sytem to mitigate surface water pollution by herbicides transported by runoff, even in agronomic situations that promote runoff. Buffer strips are conceived as tools to mitigate and thus reduce problems related to agricultural pollutants, such as agrochemicals and fertilizers. However, they should not be construed as a substitute for the adoption of good agricultural practices and a careful use of herbicides. They should rather be considered as a way to reduce pollution when herbicides are used at the recommended times and doses.

In a previous work, Cardinali, Otto, and Zanin (2013)) showed that in the study area there are on average a few (1–5) ordinary RE per year, that is, not particularly intense events, whereas extreme rainfall events occur with a return period of approximately 25 to 27 years. This study, with six ordinary events, aligns with the above and confirms that buffer strips are effective against these events, with reductions greater than 90%. However, the two studies show a different herbicide loss: g ha$^{-1}$ against mg ha$^{-1}$. This may be due to the different intensity and volume of rainfall and also to the fact that the present study was conducted under conservation agriculture. Even if the results have to be confirmed, a synergistic action can be observed between the two mitigation measures, buffer strips and conservation tillage.

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# Chapter 4. A new method for the determination of glyphosate and AMPA in soil: evaluation of their dissipation in conventional and conservation agriculture

## 4.1 Introduction

Glyphosate (N-phosphonomethylglycine; $C_3H_8NO_5P$) is a non-selective, systemic, broad-spectrum, post-emergent herbicide. It is the most used herbicide worldwide with an estimated use of 826 million kg in 2014, and its impact on the environment is becoming increasingly significant (Benbrook, 2016). The applications of glyphosate include weed control on transgenic glyphosate-resistant crops, pre-harvest desiccation of cereals, as well as total weed control in agricultural fields, especially in conservation agriculture, where it is one of the most reliable options for effective weed control. Glyphosate has low acute human toxicity but its carcinogenicity is very controversial (Tarazona et al., 2017), it is also moderately toxic to birds, most aquatic organisms, earthworms and honeybees (PPDB, 2019a).

Although glyphosate is not intentionally applied directly to the soil, a significant portion may reach the soil surface during pre-seeding or early-season applications. Glyphosate tends to adsorb strongly to soil components, particularly iron and aluminum oxides. On one hand, the high solubility (10.5 g/L at 20 °C) may increase the risk of being transported in the aqueous phase (PPDB, 2019a). On the other, the extensive adsorption can lower the potential to contaminate surface waters or groundwater, but it contributes to the accumulation of glyphosate in soil (Gimsing et al., 2004a; Sidoli et al., 2016; Vereecken, 2005). Herbicide residues and their metabolites in soil are an environmental concern since they can be transported from the fields to surface water and groundwater, contaminating them (Fenoll et al., 2014). The contamination of surface and groundwater by glyphosate affects the entire Italian territory. According to the National report on pesticides in the water of 2015-2016, during 2016 a concentration higher than the environmental quality standards (Directive 2000/60/EC, 2000) was detected in surface waters in 24.5% of cases, and in groundwater in 5.8% of cases (ISPRA, 2018).

Glyphosate is moderately persistent in soil, with a DT50 value (time for dissipation of 50% of the initial concentration) ranging from 1 to 68 days (PPDB, 2019a). Glyphosate is primarily degraded by biological activity, although evidence of an abiotic pathway via metal interaction has been reported (Ascolani Yael et al., 2014). Two pathways of microbial degradation have been described. The first involves the oxidative cleavage of the C-N bond to yield aminomethylphosphonic acid (AMPA). The second implicates the breaking of the C-P bond by a C-P lyase to generate sarcosine (Eddy et al., 2008; Franz et al., 1997). AMPA is the main degradation product of glyphosate. This compound is an aminophosphonate with a primary amine group. Ampa is chemically similar to glyphosate and has similar properties in terms of behavior and toxicity (PPDB, 2019b). AMPA is polar and well soluble in water (146 g/L at 20 °C), but it is more persistent in the environment than glyphosate (DT50 = 39–331 days) and tends to accumulate in the soil (Simonsen et al., 2008).

### *4.1.1 Analysis of glyphosate and AMPA*

Analysis of glyphosate and AMPA is challenging due to their physico-chemical properties, including amphoteric character, water solubility, and high polarity (Ma et al., 2008). Their chemical structure lacks the necessary functional groups that might be helpful for direct detection (Ibáñez et al., 2005). Most methods developed until now require a derivatization procedure to enable analysis by gas chromatography or high-performance liquid chromatography. Currently, the most used method involves pre-column derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl) (Catrinck et al., 2014; Hanke et al., 2008; Ibáñez et al., 2006, 2005; Olivo et al., 2015; Oulkar et al., 2017; Peruzzo et al., 2008; Sun et al., 2017; Vreeken et al., 1998). However, the FMOC-Cl pre-column derivatization presents some disadvantages like the interference caused by the excess of derivatizing reagent and the long derivatization reaction time. In addition, the extraction of glyphosate and AMPA from the soil is tricky. Most soil samples are characterized by the presence of organic matter, humic acid, and metal oxides, which can lead to a serious matrix effect and poor recovery rate. Several studies have been published in this research area. In these, methods based on LC associated with different extraction and cleanup sample treatments are most frequently used (Botero-Coy et al., 2013; Druart et al., 2011; Ghanem et al., 2007; Hanke et al., 2008; Hidalgo et al., 2004; Ibáñez et al., 2006, 2005; Nedelkoska and Low, 2004) but severe matrix effects, low recoveries, and poor reproducibility are often found when complex samples such as natural waters and soils are analyzed (Druart et al., 2011; Ghanem et al., 2007; Ibáñez et al., 2006, 2005).

### *4.1.2 Environmental fate of glyphosate and conservation agriculture*

Given the widespread contamination by glyphosate and AMPA, there is a growing need to identify and understand the mechanisms that control the fate of glyphosate in the soil as a source of environmental contamination. Once a herbicide reaches the soil, several processes can influence its environmental fate, such as volatilization, leaching, adsorption and degradation (Long et al., 2014). Some factors are related to the intrinsic properties of the herbicide (e.g. adsorption, solubility and persistence), and others depend on the physicochemical and biological characteristics of the soil (e.g. organic content, pH, moisture, microbial biomass, pore connectivity). The soil properties are also affected by the climate, as well as crops and soil management (Holland, 2004; Okada et al., 2016a). Among the factors influencing the environmental behavior of a herbicide, degradation is a key process since it determines its persistence in soil and consequently the potential for reaching water bodies. The dissipation rate and degradation mechanism of a herbicide in the soil are influenced by physical and chemical characteristics of the herbicide itself, as well as by soil properties weather conditions and applied dose (Otto et al., 1997; Rice et al., 2002; Wu et al., 2015). As mentioned above, glyphosate is a very effective option for weed control in conservation agriculture. Conservation agriculture is defined by the FAO as the agriculture that improves livelihoods of farmers through the application of three main principles: minimal soil disturbance (reduced tillage or no-tillage), permanent soil cover and crop rotations (Corsi, 2019). The elimination of tillage operations in no-till (NT) systems lead to a change in soil physical, chemical, and biological properties. In some cases, the annual input of crop residues on the soil surface increases the organic matter content (Levanon et al., 1994). Furthermore, the lack of physical soil mixing results in the stratification of soil organic matter content, with the top few centimeters of soil usually having the highest contents (Karlen et al., 1989; Lal et al., 1994; Reicoskj et al., 1995). The increased organic matter content promotes the retention of certain pesticides and increases the numbers and activity of the microbial population (Levanon et al., 1994; Novak et al., 1996). Microbial populations can affect the dissipation and mobility of herbicides in the soil. Consequently, herbicide fate, including dissipation, is potentially altered relatively to conventional tillage (CT) systems. The dissipation of glyphosate and the formation/dissipation of AMPA in NT system must therefore be evaluated to determine whether and to what extent the altered soil properties will affect their persistence. Although glyphosate dissipation in agricultural soils has been extensively evaluated (Accinelli et al., 2005; Gimsing et al., 2004b; La Cecilia et al., 2018; Simonsen et al., 2008; Stenrød et al., 2006; Weaver et al., 2007; Zablotowicz et al., 2009) few studies, all performed in laboratory conditions, have evaluated the fate of glyphosate and AMPA in NT systems.

### *4.1.3 Aim of the study*

A method is presented for the determination of glyphosate and AMPA in soil samples by using Ultra-High Performance Liquid Chromatography coupled with mass spectrometry (UHPLC–ESI-MS/MS). The method is based on a previously published procedure developed for the determination of these molecules in water samples (Carretta et al., 2019), and involves the use of the commercially available AccQ·Tag$^{TM}$ Ultra Derivatization Kit (Waters Corporation, Milford, MA, USA). A detailed investigation was conducted to improve extraction, purification and detection of glyphosate and AMPA. The optimized method was validated based on three representative soil samples from Italy and was applied to evaluate the dissipation of glyphosate and the formation/dissipation of AMPA from a field managed with conservation agriculture compared to a conventionally managed field. For this purpose, a field experiment was performed in North-East

Italy. The year of the study was the fifth year of conservation tillage for the NT field. As soil properties and the potential herbicide dissipation under NT can be different in the surface layer compared to lower depths (Zablotowicz et al., 2007), a component of this study was to evaluate glyphosate dissipation in the upper (0−5 cm) and lower (5−20 cm) soil depths.

## 4.2 Materials and Methods

### *4.2.1 Chemicals*

Analytical-grade standards of glyphosate and AMPA were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The compound purity was 98% and 99%, respectively. Isotope-labelled glyphosate (1,2-$^{13}$C, $^{15}$N) and isotope-labelled AMPA ($^{13}$C, $^{15}$N), used as surrogate internal standard (IS), were purchased from Toronto Research Chemicals (North York, Canada). Reagent-grade sodium tetraborate decahydrate was obtained from Honeywell Riedel-de Haën (Morris Plains, NJ, USA). Potassium hydroxide (86.6% purity) was purchased from VWR International (Leuven, Belgium). Hydrochloric acid (37%) was obtained from (Sigma-Aldrich Co. Ltd. (Steinheim, Germany)). UPLC-MS-grade acetonitrile was supplied by Honeywell Riedel-de Haën (Sigma-Aldrich Co. Ltd. (Steinheim, Germany)) and UPLC-MS-grade water from Scharlau (Barcelona, Spain). Ultrapure water was obtained by an Arium® basic system (Sartorius Stedim Biotech GmbH, Goettingen, Germany). Formic acid for mass spectrometry (~98%) was purchased from Honeywell Fluka (Seelze, Germany). The AccQ•Tag$^{TM}$ Ultra Derivatization Kit (AccQ•Tag$^{TM}$ Ultra Reagent Powder, AccQ•Tag$^{TM}$ Ultra Borate Buffer, and AccQ•Tag$^{TM}$ Ultra Reagent Diluent) was purchased from Waters Corporation (Milford, MA, USA). Glyphosate tends to bind to the active sites of glass (Goscinny et al., 2012); to avoid this adsorption phenomenon, borosilicate glassware was replaced by polypropylene labware.

Standard stock solutions of glyphosate and AMPA were prepared by dissolving 1 mg powder, accurately weighed, in 10 mL of ultrapure water obtaining a final concentration of approximately 100 mg/L. A 20-mg/L composite standard solution was prepared in ultrapure water by mixing and diluting the individual standard stock solutions. Glyphosate and AMPA working calibration standard and fortification solutions were prepared by serial dilution of the composite standard in ultrapure water. All standard solutions were stored in polypropylene tubes at 4 °C.

The isotope-labelled glyphosate and AMPA were purchased as 1 mL of 100-µg/mL stock solution in water. A composite 10-µg/mL standard solution was prepared by mixing 1 mL of each stock solution in 10 mL of ultrapure water. Standard working solutions were prepared by diluting the intermediate standard solution in water to reach a concentration equal to 1 µg/mL of both glyphosate and AMPA.

### *4.2.2 Soil sampling and characterization for the method validation*

In the method development and validation, three soils with different physical-chemical-mineralogical characteristics (Table 4.1) were used: a non-tilled soil (NT), a conventionally tilled soil (CT), and a vineyard soil (VN). When it comes to real agricultural soils, which are treated more or less frequently with glyphosate, it is not easy to find completely blank soil samples, since traces of glyphosate and especially of AMPA can be detected even several months after the treatment. To select these soils, a preliminary screening was performed among a broader range of soil samples collected at the Padova University Experimental Farm (North-East Italy) and in another agricultural area of North-East Italy (Valdobbiadene), in order to identify the soils with the lowest content of glyphosate and AMPA. Soil samples NT and CT were collected from the 5-20 cm horizon of two fields located at the Padova University Experimental Farm (North-East Italy). Soil NT has been managed under no-tillage since 2015 whereas soil CT is managed under conventional tillage. The last treatment on the field NT was performed six months before the sampling with the product Taifun MK at a dose of 1.8 kg/ha of active ingredient, while the field CT was treated 14 months before the sampling with the product Taifun MK at a dose of 1.6 kg/ha of active ingredient. Soil sample VN comes from a vineyard located in Valdobbiadene (North-East Italy), and it was collected from the 15-25 cm horizon. The texture is mainly controlled by the loamy fraction. It has a low OC content and high pH. The field VN was treated 3 years before the sampling.

Table 4.1 Main physical and chemical characteristics of the three soils. (n.a. = not analyzed).

| Soil sample | NT |  | CT |  | VN |
|---|---|---|---|---|---|
|  | Soil depth |  | Soil depth |  | Soil depth |
|  | 0-5 cm | 5-20 cm | 0-5 cm | 5-20 cm | 15-25 cm |
| % Sand | 45.2 | 41.2 | 51.2 | 51.2 | 43.5 |
| % Silt | 31.4 | 31.4 | 29.4 | 29.4 | 40.3 |
| % Clay | 23.4 | 27.4 | 19.4 | 19.4 | 16.2 |
| OC (% dm) | 1.48 | 1.07 | 0.92 | 0.98 | 0.57 |
| $N_{tot}$ (% dm) | 0.16 | 0.12 | 0.10 | 0.11 | 0.07 |
| pH | 8.21 | 8.23 | 8.15 | 8.19 | 8.51 |
| EC (dS/m) | 0.167 | 0.141 | 0.135 | 0.158 | 0.151 |
| P Olsen (mg/kg dm) | 90.7 | 68.3 | 87.2 | 94.3 | 8.3 |
| CEC (meq/100g) | 18.4 | 18.8 | 13.2 | 14.6 | 9.4 |
| Fe (mg/kg dm) | 656 | 671 | 588 | 593 | n.a. |
| Al (mg/kg dm) | 319 | 331 | 119 | 125 | n.a. |

### *4.2.3 Sample preparation*

All soil samples were air-dried, homogenized, and sieved at Ø 2 mm. Aliquots of 2 g of soil were transferred to polypropylene centrifuge tubes (50 mL). 122 µL of isotope-labelled glyphosate and AMPA standards (1 µg/mL) were added before extraction of the target analytes, in order to account for all uncontrolled losses of analytes. Samples were extracted by shaking with 0.6 M KOH (10 mL) on a mechanical shaker for 1 hour at 280 rpm (IKA yellow line RS 10 control orbital shaker), and then centrifuged at 6000 rpm for 10 min at 6 °C. An aliquot of the supernatant (4 mL) was filtered at room temperature (about 20 °C) using a regenerated cellulose membrane filter 0.20 µm pore size. A clean-up step was performed on the filtered samples by passing them through Oasis® HLB Plus light cartridges (30 mg sorbent per cartridge, 30 µm particle size) in order to extract amino compounds, which could compete with the analytes in the derivatization. Afterwards, 1 mL of the sample was transferred to a 2 mL plastic tube, and 85 µL of HCl 6 M were added to adjust the pH to approximately 9 before derivatization. An aliquot of 200 µL of the sample was introduced into a 1.5-ml polypropylene vial. The sample was then derivatized according to our previously reported procedure (Carretta et al., 2019), using the AccQ•Tag$^{TM}$ Ultra Derivatization Kit. The AccQ•Tag$^{TM}$ Ultra Reagent Powder, which is constituted of 6-aminoquinolyl-n-hydroxysuccinimidyl carbamate (AQC), was reconstituted with 1 mL of AccQ•Tag$^{TM}$ Ultra Reagent Diluent in a 1.5 mL glass vial. The vial was vortexed for 10 seconds and heated on a heating block at 55 °C for 15 minutes until the powder was dissolved. The 5% borate buffer solution is included in the derivatization kit; however, as the kit was designed for amino acids analysis, which requires a different ratio between derivatizing agent and buffer, the quantity of buffer solution is not enough. Thus, when necessary, this was prepared by dissolving 5 g of sodium tetraborate decahydrate in 100 mL of ultrapure water. 225 µL of 5% borate buffer solution, followed by 25 µL of freshly prepared derivatizing reagent, were added. The vials were capped, vortexed for 10 seconds, and placed in a water bath at 55 °C for 15 minutes, then glyphosate and AMPA concentration was analyzed by UHPLC-ESI-MS/MS. The reaction products are stable for 5 days.

### *4.2.4 Instrumentation*

The analysis was conducted with a triple quadrupole mass spectrometer system (TSQ Quantiva, Thermo Scientific, Waltham, MA, USA) equipped with an ESI source and coupled to an UltiMate 3000 UHPLC System (Thermo Scientific, Waltham, MA, USA). The LC analysis was performed with a reversed-phase C18 column of 2.1 mm × 100 mm, 2.5 µm, 100 Å particle size (XSelect HSS T3 XP, Waters Corporation, Milford, MA, USA). The LC system was operated with mobile phase

A (UPLC-MS-grade water) and mobile phase B (UPLC-MS-grade acetonitrile), both with 0.1% formic acid. Mobile phases, LC gradient, and instrumentation conditions of the UHPLC-MS/MS (i.e. column temperature, flow rate, MS method optimization, source parameters, and transitions) were as described by Carretta et al. (2019).

### *4.2.5 Validation study*

The linearity of the method was evaluated in NT, CT and VN soils analyzing ten standards solutions in triplicate, in the 50-1500 μg/kg soil dry weight (d.w.) range, corresponding to a range of 9.2-276 μg/L in soil extracts. Precision (repeatability, expressed as relative standard deviation, in %) and recoveries were determined within a day for NT, CT, and VN soils by analyzing fortified blank samples in quintuplicate. This experiment was performed at the spiking levels of 50, 200, and 500 μg/kg soil d.w. The limit of detection (LOD) and limit of quantitation (LOQ) were estimated experimentally through the injection of samples with decreasing concentrations; LOD and LOQ were determined as the analyte concentrations that give a signal-to-noise (S/N) ratio of three and ten, respectively. The specificity of the method was evaluated by analyzing a blank procedure, a processed blank sample, and a blank sample spiked at the lowest fortification level assayed (LOQ). Under these conditions, the response obtained for both the blank procedure and blank soil samples should not exceed 30% of the response corresponding to the LOQ.

### *4.2.6 Data evaluation*

To ensure the analysis quality when processing real-world samples, blank samples fortified at 50, 200, and 500 μg/kg soil d.w. levels were used as Quality Controls (QC) distributed among the batch of samples every three-four injections. Quantification of the sample batch was considered satisfactory if the QC recoveries were in the 70–120% range. The values found in real samples were confirmed using the two transitions selected for each compound.

### *4.2.7 Application to real samples*

The developed UHPLC–ESI-MS/MS method was applied to the analysis of 52 soil samples as part of a field experiment aiming to study the dissipation of glyphosate and the formation/dissipation of AMPA in two soils under different tillage managements, conservation agriculture (field NT) and conventional tillage (field CT).

The study was conducted from October 2018 to April 2019 at the Padova University Experimental Farm in the Po Valley (Veneto Region, NE Italy 45°21’N; 11°58’E; 6 m a.s.l.). On NT field residues of the previous crop (maize) were left on the surface after maize harvesting (conducted on

17/09/2018). On 18/10/2018 a pre-sowing treatment was applied on both NT and CT field with glyphosate as a formulated product (Roundup Power 2.0, Monsanto, St. Louis, MO, USA). The applied dose was 4 L/ha, which corresponds to 1.44 kg/ha of active ingredient. The dissipation of glyphosate and the formation/dissipation of AMPA in the treated fields were followed for 182 days after their application. The soils were sampled before the herbicide treatment to be used as a control. Soon after treatment, soil samples were taken to assess the initial concentration of glyphosate (soil concentration at 0 Days After Treatment, DAT), then at increasing times from spraying to evaluate the field dissipation kinetics (1, 4, 7, 13, 19, 29, 50, 90, 126, 161, and 182 DAT). Soil samples were collected with a soil auger (3.5 cm i.d. × 25 cm length) at 0-5 and 5-20 cm depth. On each sampling date, five sub-samples per field were collected and placed in a labelled plastic bag, sealed, and transported to the laboratory where they were air-dried, homogenized and sieved at Ø 2 mm. Daily rainfall in the experimental period of October 2018 through April 2019 is illustrated in Fig. 4.1.

Dissipation of glyphosate and formation/dissipation of AMPA were studied by modeling the experimental data according to the FOCUS work group guidance document on degradation kinetics (FOCUS, 2006). Differential equations were used. The kinetic model for glyphosate was the first-order multicompartment, also known as Gustafson and Holden (FOMC; Eq. (4.1)):

$$\frac{dC}{dt} = -\frac{\alpha}{\beta} C \left(\frac{t}{\beta} + 1\right)^{-1} \qquad (4.1)$$

where $C$ is the glyphosate concentration at time = $t$, $\alpha$ and $\beta$ are respectively the shape and location parameter for the variation of the degradation rate over time.

The model for the metabolite AMPA was composed of a FMOC degradation model for glyphosate and a SFO degradation model for AMPA. The rate equation for the AMPA was Eq. (4.2):

$$\frac{dM}{dt} = -\frac{dC}{dt} \times ff \times k_2 M \qquad (4.2)$$

where $M$ is the metabolite concentration at time $t$, $ff$ is the formation fraction of AMPA, and $k_2$ is the constant degradation rate for the metabolite.

Differential equations were integrated numerically by using the Runge-Kutta method with a time-step equal to 0.1 days. Integrated models were fitted to the observed data by using maximum likelihood, based on the BFSG algorithm and log-normal likelihood. Standard errors were derived by the Hessian of the objective function at maximum likelihood. Whenever necessary, the parameter $ff$ was constrained to 1, to avoid unrealistic values ($ff$ > 1). The goodness of fit was assessed by using graphical analyses of residuals.

The estimated trend of concentrations over time for both glyphosate and AMPA were used to derive the DT50 for glyphosate (time required for the concentration to drop to half the initial estimated value), the maximum concentration value for AMPA, and the DT50 for AMPA (i.e. the time required for the concentration to drop to half the maximum estimated value). Standard errors for these estimates were obtained by using the delta method. DT50 values were compared across the treatments by using heteroscedastic t-tests (Motulsky and Christopoulos, 2003).

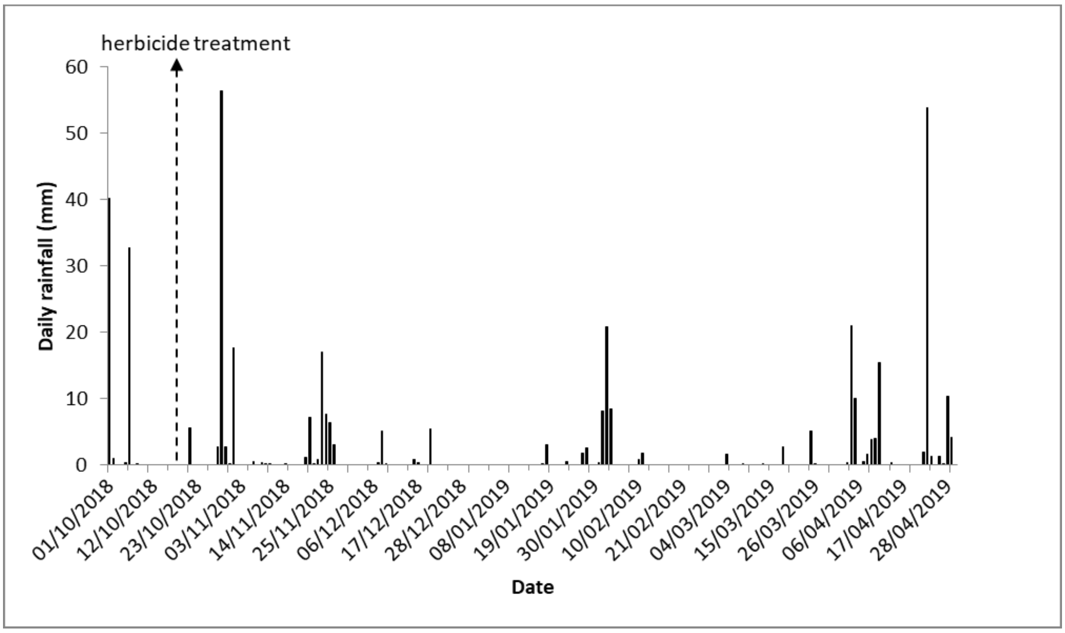

Fig. 4.1 Daily rainfall from October 2018 to April 2019 at the Padova University Experimental Farm.

## 4.3 Results and Discussion

### *4.3.1 Method validation*

In agreement with the application note of Waters Corporation (Waters Corporation, 2015) and the previously published method for water samples analysis (Carretta et al., 2019), the transitions monitored and used for quantification of glyphosate were 340→171 (Q) and 340→116 (q), and for AMPA 282→171 (Q) and 282→116 (q). The q/Q ion ratios were 0.16±0.02 for both glyphosate and AMPA. The retention times were 3.64±0.03 min for glyphosate and 3.90±0.02 min for AMPA.

To construct the calibration curves, NT, CT and VN soil samples were spiked with known fixed amounts of isotopically labelled glyphosate and AMPA, used as internal standard, and with known concentrations of glyphosate (15, 20, 50, 100, 200, 500, 1000, and 1500 μg/kg) and AMPA (6, 15, 20, 50, 100, 200, 500, 1000, and 1500 μg/kg) in mixture at the same concentration. The calibration solutions were derivatized in triplicate and analyzed. Standard calibration curves were generated as the ratio of the analyte peak area to the IS peak area (Aa/AIS), plotted against the ratio of the analyte amount to the IS amount (Qa/QIS). The calibration equation is: $Aa/AIS = m\cdot(Qa/QIS) + q$.

Once verified that the error on q obtained from the fitting was higher than the q value itself, the data were interpolated by the equation passing through zero, Aa/AIS = m·(Qa/QIS). The calibration curves obtained for derivatized glyphosate and derivatized AMPA (Fig. 4.2) in ultrapure water, and NT, CT and VN soils are shown.

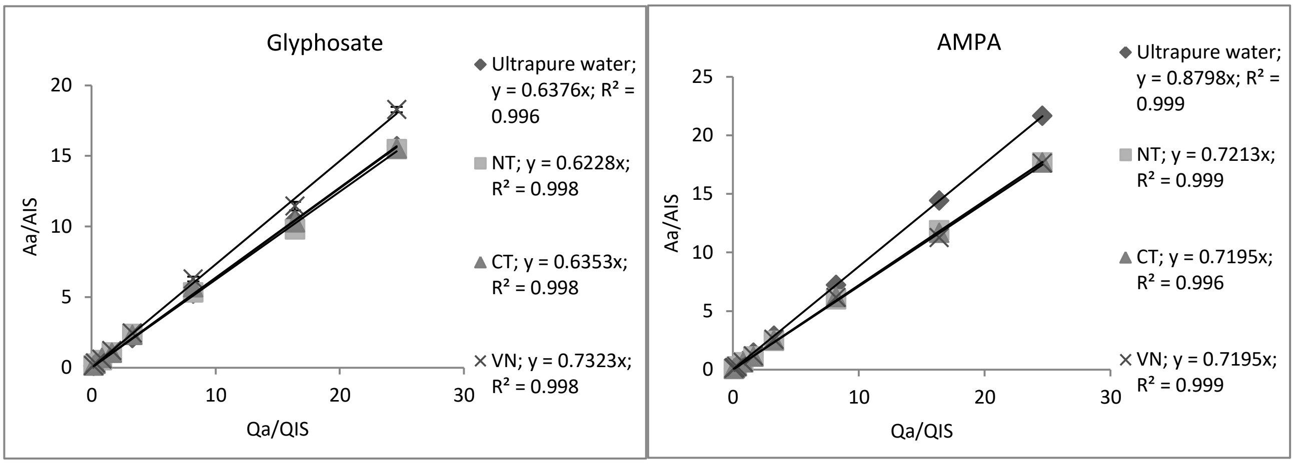

Fig. 4.2 Standard calibration curves of derivatized glyphosate and AMPA in ultrapure water, and NT, CT and VN soils, generated as the ratio of analyte peak area to IS peak area (Aa/AIS), plotted against the ratio of analyte amount to IS amount (Qa/QIS). Vertical bars represent the standard deviation.

A linear response was confirmed in all cases in the entire concentration range considered, based on the squared correlation coefficients ($R^2$) obtained, as well as randomly distributed residuals below 20%. The average recovery percentages for glyphosate were 98%, 100%, and 115% for soil NT, CT and VN, respectively, whereas for AMPA recoveries were 81% for VN soil and 82% for NT and CT soils. The LOD and LOQ were determined as 15 and 50 µg/kg for glyphosate and 6 and 20 µg/kg L for AMPA, respectively. Fig. 4.3 and Fig. 4.4 compare the SRM chromatograms for derivatized glyphosate and AMPA in CT soil spiked at LOQ level with those in the non-contaminated (blank) CT soil. The response obtained for blank soil samples did not exceed 30% of the response corresponding to the LOQ.

Matrix effects were evaluated based on the slopes of regression lines plotted from results obtained in matrices versus standard solutions. The matrix effect was below 15% and 20% for glyphosate and AMPA, respectively, so the method was considered acceptable for quantifying the analytes in the evaluated matrices. The method was found to be precise and accurate, as reported in Table 4.2.

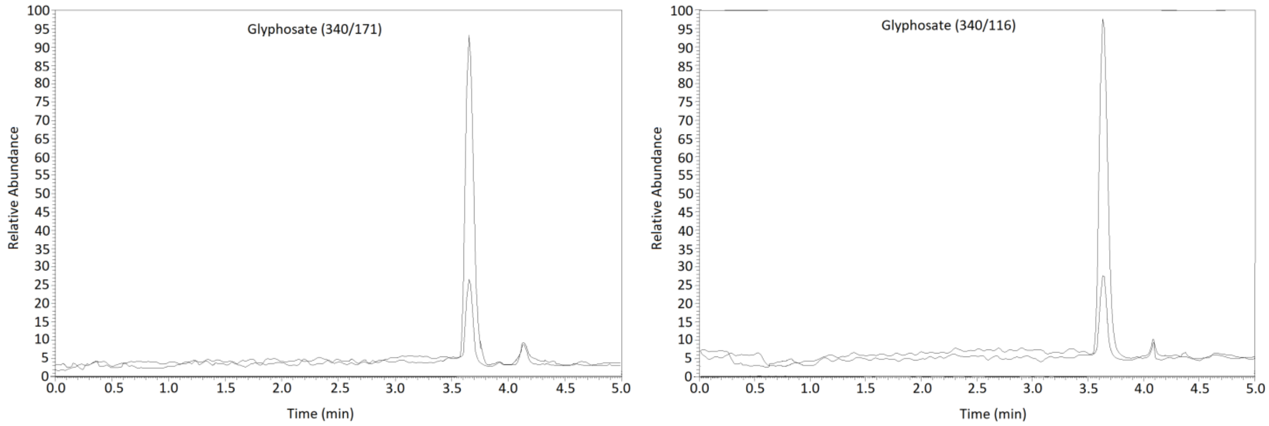

Fig. 4.3 SRM chromatograms for derivatized glyphosate in CT soil sample fortified with glyphosate at LOQ level (50 µg/kg) and in CT soil blank sample.

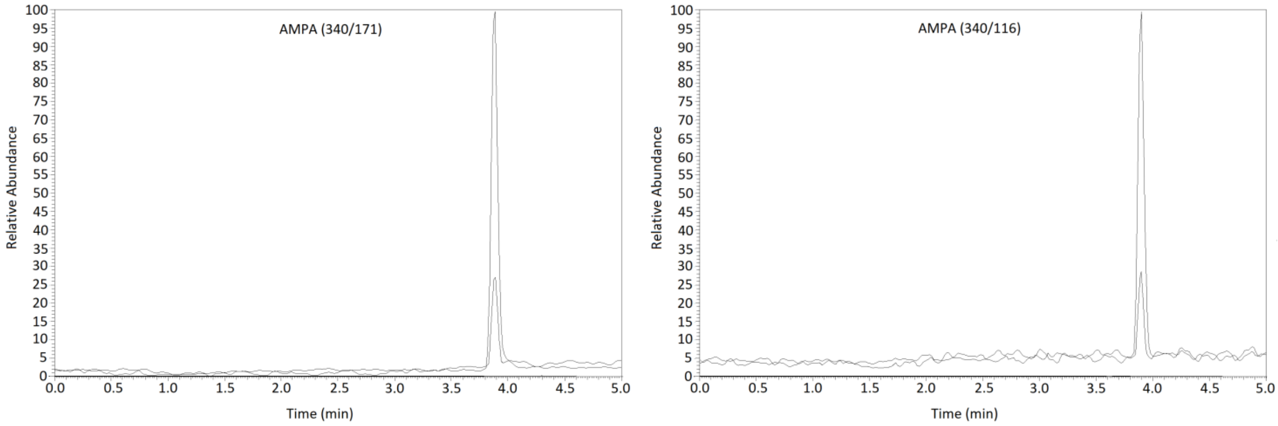

Fig. 4.4 SRM chromatograms for derivatized AMPA in CT soil sample fortified with AMPA at LOQ level (20 µg/kg) and in CT soil blank sample.

Table 4.2 Precision and accuracy obtained in the method validation.

| Soil | Concentration of fortified soil samples (µg/kg) | Glyphosate | | AMPA | |
|---|---|---|---|---|---|
| | | Accuracy (%) | RDS (%) | Accuracy (%) | RDS (%) |
| NT | 50 | 12 | 7 | 11 | 8 |
| | 200 | 15 | 8 | 11 | 11 |
| | 500 | 14 | 6 | 14 | 9 |
| CT | 50 | 17 | 10 | 16 | 7 |
| | 200 | 11 | 9 | 17 | 8 |
| | 500 | 12 | 5 | 13 | 7 |
| VN | 50 | 16 | 11 | 15 | 10 |
| | 200 | 15 | 8 | 18 | 9 |
| | 500 | 15 | 7 | 11 | 9 |

### *4.3.2 Glyphosate dissipation and AMPA formation/dissipation in tilled and non-tilled soil*

The method was applied to the analysis of 52 soil samples collected from two fields treated with glyphosate at the Padova University Experimental Farm located in the Po Valley. Known fixed amounts of isotopically labelled glyphosate and AMPA, used as internal standard, were spiked into each sample. Every sample was extracted and derivatized in triplicate and analyzed for glyphosate and AMPA concentration. Confirmation of the identity of the compounds was based, besides SRM transitions, also on retention times and q/Q ion ratios. The requirements that retention times do not differ more than 0.1 min in samples and the reference solutions and that the tolerance for q/Q ion ratios is within 30% were fully satisfied (Pihlström et al., 2017). Every analysis sequence included quality control samples spiked at the 50, 200, and 500 µg/kg levels. The quality control consisted of "blank" soil samples (specifically the CT and NT soil samples used for the method validation, alternating them in the sequence) spiked with the analytes. Satisfactory quality control recoveries were obtained for both compounds (between 70 and 120%), demonstrating the robustness of the method throughout the analysis. In Fig. 4.5 the SRM chromatograms of Q and q for glyphosate and AMPA in a soil sample are shown. It can be seen that the q/Q ion ratios are in agreement with that reported in section 3.1.

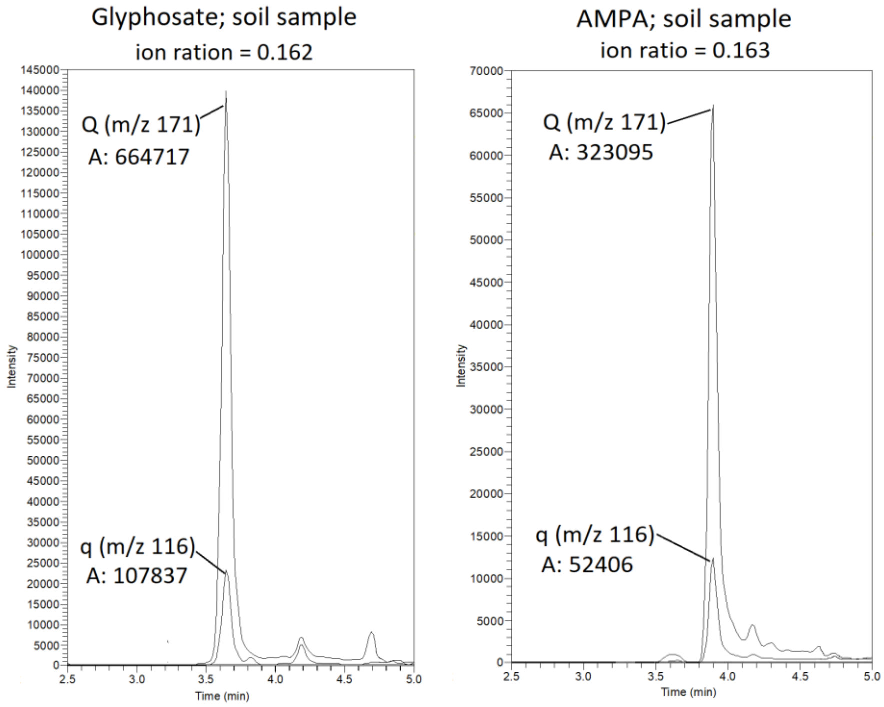

Fig. 4.5 SRM chromatograms of Q and q for glyphosate and AMPA in a soil sample. Glyphosate concentration: 1404 µg/kg soil d.w.; AMPA concentration: 148 µg/kg soil d.w.

One day before the glyphosate treatment, glyphosate and AMPA concentrations were measured in the soil samples. In NT soil samples at 0-5 cm depth, the concentration was 45 µg/kg for glyphosate and 94 µg/kg for AMPA, whereas at 5-20 cm glyphosate was below the limit of detection and only AMPA could be detected, at a concentration of 15 µg/kg. The AMPA concentration in NT soil is notable if we consider that the maximum AMPA concentration detected during the experiment was 424 and 52 µg/kg for 0-5 and 5-20 cm layer, respectively. In CT soil samples, the glyphosate and AMPA concentrations measured one day before the treatment were below the limit of detection for both sampling depths. It has to be noted that CT soil was treated with glyphosate 14 months before the soil sampling, whereas the NT soil was treated only 6 months before the sampling, therefore a lower AMPA concentration can be expected in CT soil than in NT.

The measured concentration of glyphosate 0 DAT at 0-5 cm depth was four times higher in CT soil than in NT soil (1414 µg/kg and 353 µg/kg, respectively), whereas in the 5-20 cm layer the concentrations were similar in both soils (86 µg/kg and 84 µg/kg, respectively) and markedly lower than those in the surface layer. Maize crop residues on the NT soil surface could have played a role in determining the lower concentration of glyphosate detected in the NT soil soon after treatment. Several studies have observed that crop residues distributed on the soil surface, as is done in conservation agriculture, can intercept a considerable amount of the applied herbicide, thus reducing the amount of chemical reaching the weeds and soil (Chauhan et al., 2006; Gaston et al., 2001; Ghadiri et al., 1984; Khalil, 2018). Crop residues physically separate herbicides from the soil surface, and Chauhan et al. (2006) showed that they can intercept from 15 to 80% of the applied herbicide, affecting herbicide efficacy, persistence and fate. The potential interception of herbicides by crop residues may depend on the biomass of the residues as well as on their external surface area. Once intercepted by the residues, herbicides can be adsorbed on them and subsequently washed off by rainfall (Gaston et al., 2001; Unger and Kaspar, 1994). None of the previously mentioned studies were focused specifically on glyphosate. The observed effect of crop residues on its interception is of particular importance since glyphosate is the most used herbicide in conservation agriculture where the retention of crop residues is one of the main pillars.

The lower concentration detected in the deeper soil layer can be motivated by the fact, observed by Ratcliff et al. (2006) and Stratton (1990), that glyphosate movement in soil is limited by chemical adsorption to the surface centimeters, although actual penetration differs somewhat depending on the soil type (Sprankle et al., 1975).

Estimated parameters for the fitting curves of glyphosate and the metabolic product AMPA in NT and CT soils at 0-5 and 5-20 cm depth are reported in Table 4.3.

Table 4.3 Glyphosate and AMPA dissipation kinetics parameters in tilled and non-tilled soil at 0-5 and 5-20 cm depth, by fitting Gustafson and Holden (FOMC) or single first-order (SFO) kinetic models. See Section 2.7 for treatments abbreviations.

| | Glyphosate following FOMC | | | AMPA following SFO* | |
|---|---|---|---|---|---|
| Treatment | $C_0$ ± SE (µg/kg soil d.w.) | α ± SE | β ± SE | $C_0$ ± SE (µg/kg soil d.w.) | $k$ ± SE (day$^{-1}$) |
| NT 0-5 cm | 378 ± 15.7 | 0.33 ± 0.019 | 1.25 ± 0.311 | 169 ± 9.0 | 0.001 ± 0.0003 |
| NT 5-20 cm | 79 ± 2.1 | 0.27 ± 0.014 | 1.43 ± 0.312 | 30 ± 1.2 | 0.007 ± 0.0002 |
| CT 0-5 cm | 1302 ± 24.8 | 0.88 ± 0.099 | 8.09 ± 1.630 | 70 ± 16.8 | 0.010 ± 0.0005 |
| CT 5-20 cm | 84 ± 4.8 | 0.83 ± 0.129 | 6.37 ± 2.129 | 11 ± 1.5 | 0.005 ± 0.0007 |

*The parameter *ff* was constrained to 1 to avoid unrealistic values (*ff* > 1).

In both tillage systems and depths, an immediate dissipation response was observed to the glyphosate applied without any lag phase (Fig. 4.6). A rapid mineralization start without a lag phase was also observed by Gimsing et al. (2004b), Mamy et al. (2005) and Zablotowicz et al. (2009). In our soils the initial dissipation was fast, and 30 to 38% of the applied glyphosate was degraded within the first four days. The fast initial decrease in glyphosate concentrations was followed by a slower decline which was particularly noticeable in NT soil at both depths. Several studies observed that the readily available form of glyphosate (either soluble or desorbed into the soluble soil phase) allows for its fast initial dissipation, but that it decreases at a later stage, resulting in a slower dissipation rate of the remaining glyphosate from the adsorbed phase (Bento et al., 2016; Eberbach, 1998; Gimsing et al., 2004b; Okada et al., 2017; Simonsen et al., 2008). A possible explanation for the observed bi-phasic pattern of glyphosate degradation in both the tested soils could be the fact that only the fraction of the pesticide in soil solution is available for degradation (Linn and Scow, 1993). Non-linear sorption with Freundlich exponent below 1, which often occurs for glyphosate (Cederlund et al., 2016; Garba et al., 2018; Glass, 1987; Okada et al., 2016b), results in a decreasing availability of the herbicide in soil solution with decreasing concentration. This may have decreased its degradation rate at later stages of the experiment. Moreover, in field studies, seasonal changes in temperature and/or soil moisture can affect the degradation rate and cause deviations from first-order kinetics (e.g. degradation rate may decrease in winter due to lower temperatures).

The DT50 of glyphosate and AMPA in tilled and non-tilled soil at 0-5 and 5-20 cm depth and the results of the heteroscedastic t-tests are summarized in Table 4.6.

Table 4.6 Values of DT50 of glyphosate and AMPA in NT and CT soil at 0-5 and 5-20 cm depth.

| Compound | Soil | Depth | DT50 ± SE (days) | Difference[a] | p-value[b] |
|---|---|---|---|---|---|

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| Glyphosate | NT | 0-5 cm | 9.1 ± 1.43 | NT 0-5 cm − NT 5-20 cm | -8.9 | 0.0011 |
|  | NT | 5-20 cm | 18.0 ± 2.21 | CT 5-20 cm − NT 5-20 cm | -9.8 | 0.0003 |
|  | CT | 0-5 cm | 9.7 ± 0.65 | NT 0-5 cm − CT 0-5 cm | -0.6 | 0.3592 |
|  | CT | 5-20 cm | 8.2 ± 1.15 | CT 5-20 cm − CT 0-5 cm | -1.4 | 0.1474 |
| AMPA | NT | 0-5 cm | 981 ± 321.7 | NT 5-20 cm − NT 0-5 cm | -822.3 | 0.0084 |
|  | NT | 5-20 cm | 158 ± 5.2 | NT 5-20 cm − CT 5-20 cm | -74.0 | 0.0137 |
|  | CT | 0-5 cm | 131 ± 5.2 | CT 0-5 cm − NT 0-5 cm | -849.4 | 0.0069 |
|  | CT | 5-20 cm | 232 ± 31.2 | CT 0-5 cm − CT 5-20 cm | -101.1 | 0.0018 |

[a] Difference between DT50 values

[b] p-values for the null hypothesis of no significant difference (heteroscedastic t-test)

The DT50 of glyphosate in NT soil at 0-5 cm was not significantly different from that in CT soil at the same depth, whereas a significantly higher DT50 was observed in NT than in CT soil at 5-20 cm (18.0±2.21 and 8.2±1.15 days, respectively). In CT soil no significant difference between the two depths was observed in terms of DT50, whereas in NT soil glyphosate dissipated faster at 0-5 than at 5-20 cm (DT50: 9.1±1.4 and 18.0±2.21 days, respectively).

Although the DT50 values alone do not allow us to describe a clear effect of conservation agriculture on glyphosate kinetics, these results suggest that soil tillage could to some extent affect glyphosate dissipation. Contrasting results were obtained by Okada et al. (2017) and Zablotowicz et al. (2009), by measuring the dissipation of glyphosate in tilled and non-tilled soil in laboratory studies under controlled conditions. They did not observe any significant difference between the two soil management systems despite NT's effect on soil chemical, physical and biological properties. The higher DT50 that we observed in the deeper layer of NT soil could be only partially explained by the differences in soil properties between tilled and non-tilled soil which, however, does not explain why no difference in the DT50 was observed between the surface layer of NT and CT soils. However, when it comes to field studies, several factors, which are not completely under control, act together influencing the dissipation of a pesticide in the soil. As previously mentioned, the amount of glyphosate detected at 0-5 cm soon after treatment was largely higher in CT than NT soil, leading to a different initial concentration of glyphosate. As observed by Nourouzi et al. (2012), the initial concentration of glyphosate applied to the soil has an effect on the dissipation of the herbicide. Increasing in the initial glyphosate concentration decreases its degradation, probably because microbial growth and activity are inhibited by the toxicity exerted by the high concentrations of glyphosate. Therefore, as the initial glyphosate concentration increases, the time of biodegradation of glyphosate also increases, making it scarcely helpful to compare the DT50 values in the 0-5 cm layer of CT and NT soils where the initial concentration was so different.

Instead, a more reliable comparison is possible between the 5-20 cm soil layers. In this case, we hypothesize that adsorption processes may have played a role in determining the observed difference. After five years of conservation management, chemical properties of the NT soil have been altered in comparison to CT soil. The NT soil showed higher organic carbon content (OC) and cation exchange capacity (CEC) (see Table 1). Several studies have reported a positive correlation between OC content and glyphosate adsorption (Okada et al., 2016b; Prata et al., 2007; Schuette, 1998; Zablotowicz et al., 2009). Glyphosate adsorption can be also associated with CEC since glyphosate can be complexed with cations released from the clays via cation exchange reactions (Glass, 1987). Although glyphosate adsorption was not investigated in this study, we can hypothesize higher adsorption in NT soil, and consequently a lower bioavailability for degradation. Soil OC is not the only soil component that can adsorb glyphosate. It is known that soil minerals can play an important role in this process. The pH of studied soils is only slightly above the pH range of the most agriculturally important soil, which is 4-8. In this pH range, glyphosate is ionic, and its sorption is mainly controlled by iron and aluminum oxides, poorly ordered aluminum silicates, and edge layer silicates (Borggaard and Gimsing, 2008). The mineralogy in these soils was not investigated, but soil elemental analysis indicates a higher Al and Fe content in NT soil than in CT soil (see Table 1). It is therefore possible that the amorphous iron and aluminum oxide contents differed in these soils and layers, thus influencing glyphosate adsorption. However, Gimsing et al. (2004) and Zablotowicz et al. (2009) observed that iron and aluminum oxide-bound glyphosate could also be desorbed in response to the removal of the bio-degradable pool of glyphosate during degradation, to replenish the bio-degradable pool. However, this depends on soil type and the forms and degrees of inclusion of oxide-bound glyphosate, which in turn influence the desorption kinetics that, together with the microbial mineralization activity, regulate the overall glyphosate degradation rate.

Besides the DT50, observing the experimental data it becomes clear that the dissipation behavior of glyphosate is notably different in NT soil than in CT. Despite a higher initial concentration in CT soil compared to NT, at the end of the study the concentration detected at 0-5 cm in NT soil was almost twice that in CT soil, whereas at 5-20 cm this difference was even more pronounced with a ratio of about 3:1 between NT and CT.

The DT50 values found in this study, although being within the typical values from 1 to 68 days (PPDB, 2019a), were generally low. One of the possible explanations can be found in the soil pH, which is slightly above 8. Indeed, as soil pH increases, glyphosate becomes more anionic, increasing its repulsion by the negatively charged particles of the soil and therefore becoming more bio-available and more easily degradable (Zhao et al., 2009). Overall, the DT50 values found are in agreement with those reported by Zhang et al. (2015) and Stenrød et al. (2006) for loam soils, and by Al-Rajab and Hakami (2014), Eberbach (1998) and Simonsen et al. (2008) for different soil types, but lower than the values found in other studies (Mamy et al., 2008; Peres et al., 2003).

The formation and dissipation of the metabolic product AMPA in NT and CT soils at 0-5 and 5-20 cm depth is represented in Fig. 4.6. AMPA began to form in the early stages after treatment in all the soils and depths. The fact that AMPA could already be detected a few hours after glyphosate application underlines the quick glyphosate degradation in soil, as reported by Mamy et al. (2005) and Todorovic et al. (2013). The fast decrease of glyphosate concentration at the beginning of the dissipation study was reflected by an increase in AMPA concentration in all cases. The fact that AMPA is formed when glyphosate is degraded clearly means that the persistence of AMPA has to be longer than that of glyphosate. Similarly to what has been observed for glyphosate, AMPA concentrations were lower in the deeper soil layer. Although the vertical mobility of AMPA is higher than that of glyphosate, AMPA is strongly adsorbed by the soil compounds, and most of this molecule tends to remain in the first centimeters of soil (Mamy et al., 2005).

The maximum concentration of AMPA was estimated to be 344±7.3 and 53±1.2 µg/kg soil d.w. in NT soil at 0-5 and 5-20 cm, respectively and 578±9.6 and 49±2.1 µg/kg soil d.w. in CT soil at 0-5 and 5-20 cm, respectively. The time of maximum AMPA concentration was estimated to be 70 and 18 days in NT soil at 0-5 and 5-20 cm, respectively and 28 and 36 days in CT soil at 0-5 and 5-20 cm, respectively. In the surface layer, AMPA DT50 was higher in NT than in CT soil (981±321.7 and 131±5.2 days for NT and CT, respectively), whereas the contrary was observed in the deeper layer, where a lower DT50 was observed in NT (158±5.2 and 232±31.2 days for NT and CT respectively). Although differences between the DT50 of AMPA in all soils and depths were statistically significant, it was not possible to delineate a clear effect of soil tillage on AMPA kinetics. The observed differences could rather be attributed to the variability of experimental data, which in some cases (namely NT soil at 0-5 cm and CT soil at 5-20 cm) complicated the curve fitting. In the case of CT soil at 5-20 cm, the concentration of AMPA increases too much and for too long, compared to the glyphosate dissipation rate. To obtain a good fit, the formation fraction (*ff*) should not be constrained to 1. However, in this case, *ff* values much higher than 1 would be obtained, and this is not realistic (FOCUS, 2006). There were large standard errors associated with the DT50 of AMPA in NT soil at 0-5 cm and in CT soil at 5-20 cm. Moreover, in NT soil at 0-5 cm, the slope of the decreasing part of the curve was negligible. Modeling of the data was therefore difficult, and the reported DT50 values of AMPA should be interpreted cautiously. The DT50 values of AMPA in NT soil at 5-20 cm and in CT soil at 5-20 are in agreement with the DT50 range reported on the Pesticide Properties Database (PPDB, 2019b).

AMPA was still detected in the soil on the last sampling date, 182 DAT, in all soils and depths. This is in agreement with what was observed by Mamy et al. (2005) in a field study with a lower glyphosate application rate. Although the initial parent molecule concentration and amount of AMPA formed were higher in CT than in NT soil at 0-5 cm depth, at the end of the experiment the measured concentration of AMPA in NT soil was twice that in CT soil (284 µg/kg and 136 µg/kg for NT and CT, respectively). In the deeper soil layer, the amount of AMPA formed and its concentration measured at the end of the experiment were comparable.

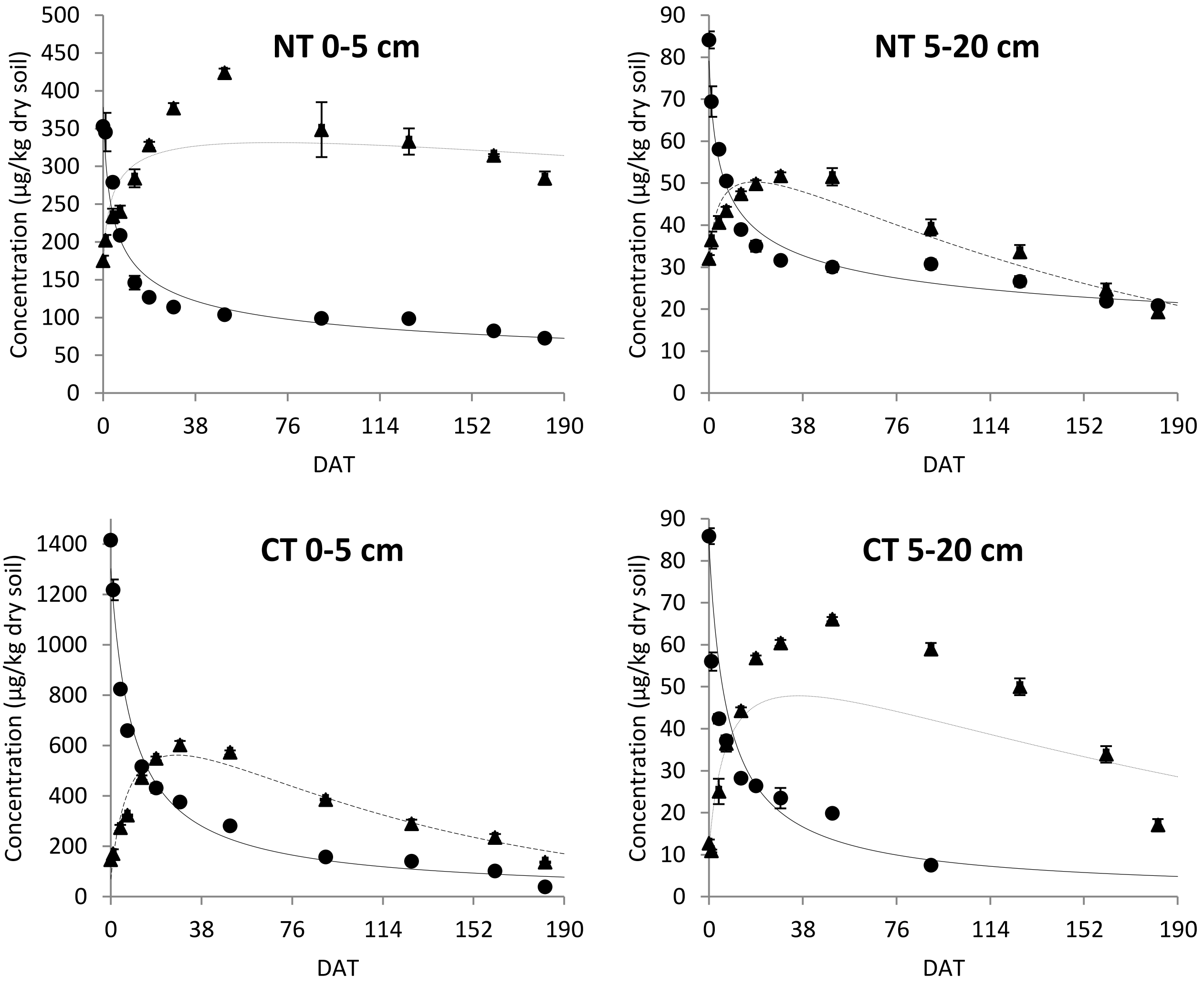

Fig. 4.6 Dissipation kinetics of glyphosate (●) and AMPA (▲) in CT and NT soils at 0-5 and 5-20 cm depth. Symbols represent the experimental data (expressed in µg/kg soil d.w.) and lines (solid lines for glyphosate and dashed lines for AMPA) the theoretical kinetic models. Error bars represent the standard deviation.

The grey dotted lines represent the cases in which the theoretical kinetic of AMPA could not be considered fairly descriptive of the observed results (NT 0-5 cm and CT 5-20 cm).

## 4.4 Conclusions

A method is presented based on UHPLC-ESI-MS/MS for the determination of glyphosate and AMPA in the soil. This method allows the rapid, accurate and selective determination of glyphosate and AMPA in soil samples. Satisfactory results were obtained for both glyphosate and AMPA in the validation experiment using isotope-labelled glyphosate and AMPA as internal standards. The method demonstrates a good linear relationship in the concentration range of 15-1500 µg/kg for glyphosate and 6-1500 µg/kg for AMPA. Recovery was accurate. LOD and LOQ were established to be 15 and 50 µg/kg, respectively for glyphosate and 6 and 20 µg/kg, respectively for AMPA.

The developed method was successfully applied to the determination of glyphosate and AMPA in 52 soil samples to study glyphosate dissipation and AMPA formation and dissipation in tilled and non-tilled soil at two different depths. In all the studied soils and depths the initial dissipation of glyphosate was fast, followed by a decrease in the rate. This study showed that conservation agriculture could slow down the dissipation of glyphosate in comparison with conventional agriculture, but no clear effect was observed for AMPA. After six months, residues of glyphosate and AMPA were still present in the soils except for glyphosate in the deeper layer of the tilled soil. Crop residues, distributed on the surface of the NT field as is done in conservation agriculture, intercepted a significant amount of glyphosate at the time of application, thus reducing the amount of herbicide that reached the soil surface in NT compared to CT soil. The crop residues that were on the NT field during the period of the study derived from a maize crop. It could be interesting to deepen this aspect of the research by studying how residues from different crops and cover crops intercept and adsorb the applied glyphosate, and how much could be washed off during more or less intense rainfall events.

Conservation agriculture has several positive outcomes that make it a valid soil management system, especially when the primary aim is to improve the soil structure and properties of highly exploited soils. To get the maximum benefits from this soil management system and to limit the risk of glyphosate and AMPA pollution as much as possible it is recommended to always adopt good agricultural practices and use this herbicide carefully at the recommended times and doses.

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# Chapter 5. Dissipation of terbuthylazine, metolachlor, and mesotrione in soils with contrasting texture

## Abstract

This study evaluates the dissipation of terbuthylazine, metolachlor, and mesotrione at different depths in soils with contrasting texture. The field trial was conducted at the Padua University Experimental Farm, north-east Italy. The persistence of three herbicides was studied in three different soil textures (clay soil, sandy soil, and loamy soil) at two depths (0–5 and 5–15 cm). Soil organic carbon content was highest in the clay (1.10%) followed by loam (0.67%) and sandy soil (0.24%); the pH of soils was sub-alkaline. Terbuthylazine, metolachlor, and mesotrione were applied on maize as a formulated product (Lumax®) at a dose of 3.5 L $ha^{-1}$. Their dissipation in the treated plots was followed for 2 months after application. The concentrations of herbicides were analyzed by liquid chromatography-mass spectrometry. The dissipation of terbuthylazine, metolachlor, and mesotrione could be described by a pseudo first-order kinetics. Terbuthylazine showed the highest DT50, followed by metolachlor and mesotrione. Considering the tested soil, the highest DT50 value was found in clay soil for terbuthylazine and metolachlor, whereas for mesotrione there was no difference among soils. Significant differences were found between the two soil depths for terbuthylazine and metolachlor, whereas none were found for mesotrione. These results suggest that soil texture and depth have a strong influence on the dissipation of terbuthylazine and metolachlor, whereas no influence was observed on mesotrione because of its chemical and physical properties.

Keywords: herbicide; DT50; sand; clay; loam; depth.

## 5.1 Introduction

Herbicides play an important role in crop production because they are generally considered the most economical and efficient method for controlling weeds (Wu et al., 2015). Their use is also common in non-crop environments. Studying the environmental fate of herbicides is crucial because they are one of the major sources of environmental pollution. The widespread use of herbicides can lead to the presence of their residues and metabolites in the soil (Fenoll et al., 2014). Increasing persistence of herbicide residues in the soil will increase the probability of transport of herbicides from fields to surface and ground water resources (Long et al., 2014).

The environmental fate of herbicides is influenced by several processes, including volatilization, leaching, adsorption, and degradation (Long et al., 2014). Among these, degradation is a key process because it determines persistence of the herbicides in soil and consequently their potential for reaching water bodies. For this reason, determination of the herbicide dissipation rate in agricultural soil is important for monitoring their environmental impact and potential effect on water quality (Dyson et al., 2002). Dissipation in soil is governed by chemical and physical properties of the herbicide and soil as well as by climatic conditions (Rice et al., 2002). Several studies have demonstrated that soil pH and organic carbon influence herbicides degradation. Degradation is also correlated with soil texture, especially the clay content (Dyson et al., 2002; Lehmann et al., 1992; Walker et al., 1985; Walker and Allen, 1984; Walker and Thompson, 1977). Biodegradation has been shown to be the primary mechanism of herbicide degradation in soil (Long et al., 2014; Ma et al., 2001; O'Connell et al., 1998; Rice et al., 2002; Shelton and Parkin, 1991; Taylor-Lovell et al., 2002; Wu et al., 2011).

Terbuthylazine, metolachlor, and mesotrione are three widely used herbicides that constitute the active ingredients of the commercial product known as Lumax®. Lumax® is a selective pre-emergence herbicide quite effective in controlling annual grass and broadleaf weeds in maize crops, and it contains terbuthylazine, metolachlor and mesotrione in weight percentages of 28, 17, and 3, respectively (Pinna et al., 2014). Terbuthylazine, metolachlor, and mesotrione are widely used in Italy, especially in the Po Valley, where maize is a widespread crop. Contamination of water resources by terbuthylazine and metolachlor affects the entire national territory, but the values found in Northern Italy, especially in Veneto, are higher than the national average, because of their intense use in this region (ISPRA, 2016a). In 2014, terbuthylazine and metolachlor were found to be the main contaminants of both surface and groundwater in Veneto, even reaching concentrations greater than 0.1 mg $L^{-1}$ (ISPRA, 2016b).

Terbuthylazine (6-chloro-N-(1,1-dimethyl-ethyl)-N0-ethyl-1,3,5-triazine-2,4-diamine) is a selective systemic herbicide that belongs to the triazines (Baćmaga et al., 2014). It is mainly absorbed through the roots, but also through foliage. Terbuthylazine acts by inhibition of photosynthesis at the photosystem II level. The Herbicide Resistance Action Committee (HRAC) classified terbuthylazine as C1 (GIRE, 2016). Metolachlor ((chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) is a substance that belongs to the chloroacetamides and is used to control mainly grass weeds, being absorbed by the shoots of seedlings (Baćmaga et al., 2014). The metolachlor mode of action consists in the inhibition of cell division, and HRAC classifies it as K3 (GIRE, 2016). Mesotrione (2-[4-(methylsulfonyl)- 2-nitrobenzoyl]-1,3-cyclohexanedione) belongs to the triketones and is used to eradicate mono- and dicotyledonous weeds (Baćmaga et al., 2014). It acts by inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), which affects carotenoid biosynthesis, and HRAC classifies it as F2. (GIRE, 2016; Mitchell et al., 2001).

The objective of this study was to investigate the dissipation of terbuthylazine, metolachlor, and mesotrione at different depths in cornfield soils with contrasting textures. The selected soils are very common and widespread in Northern Italy. Herbicide residues in soils were monitored over time, to determine their persistence under different conditions. The study was conducted to gain a better understanding of the factors that influence the dissipation of terbuthylazine, metolachlor, and mesotrione in soils, and to evaluate the environmental fate implications of these relationships in this agronomic and environmental context.

## 5.2 Materials and methods

### *5.2.1 Site information*

The study was conducted in 2012 at the Padua University Experimental Farm in the Po Valley (Veneto Region, NE Italy 45°21′N; 11°58′E; 6 m a.s.l.). Annual rainfall was 631 mm (the wettest month was October with 137 mm of rain, while the driest month was July with 0.4 mm) with a total of 107 rainy days. Air temperature and soil temperatures at -10 cm increase from January (minimum average: -2.2°C and 2.1° C, respectively) to August (maximum average: 33.4°C and 27.7° C, respectively). Weather conditions during the experimental period of May through July are illustrated in Fig. 5.1.

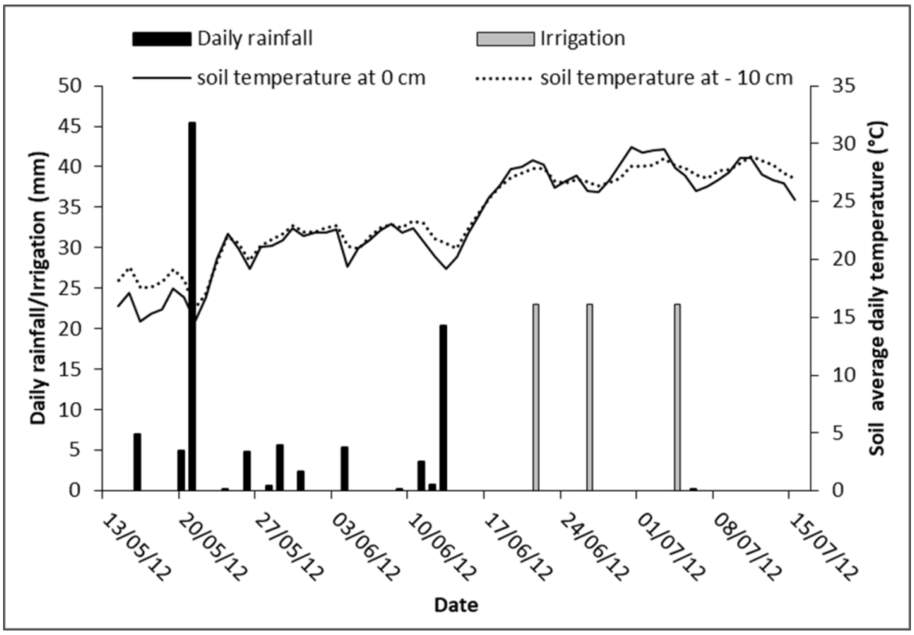

Fig. 5.1 Weather conditions at the Padua University Experimental Farm in the Po Valley (Italy) during the experimental period.

This experiment is settled in areas of soil confined by concrete boxes with an open bottom placed into the ground, 4 m$^2$ in area and 80 cm deep. Experimental treatments derive from a randomized complete block design of three types of soil, hereinafter called clay ("clay"), sandy clay loam ("loam"), and sandy ("sand") in relation to their dominant property.

The soils were brought from three locations in the Veneto region: clay soil from the southwestern plain, sandy soil from the central coastal area and loam soil from the southern plain. In each lysimeter, samples for soil analysis were taken in April 2012 with an auger at 15 cm depth, then air-dried and passed through a 2 mm sieve. Physical and chemical characteristics of the soil (Table 5.1) were analyzed (n = 3): textural classification (Klute et al., 1986); pH (Sørensen S., 1909); organic carbon (Walkley and Black, 1934) and total nitrogen (Kjeldahl, 1883).

Management and mineral fertilization treatments (200 kg ha$^{-1}$ y$^{-1}$ N – 100 $P_2O_5$ – 240 $K_2O$) were the same in all plots.

Table 5.1 Main physical and chemical characteristics of the 0-5 cm layer and 5-15 cm layer of the three soils.

| Parameters | Unit | Depth | Clay | Loam | Sand |
|---|---|---|---|---|---|
| Sand (2 mm – 50 μm) | % | 0-15 cm | 30 | 58 | 90.5 |
| Silt (50 – 2 μm) | % | 0-15 cm | 19 | 21 | 5.5 |
| Clay (< 2 μm) | % | 0-15 cm | 51 | 21 | 4 |

| | | | | | |
|---|---|---|---|---|---|
| OC* | % | 0-5 cm | 1.84 | 1.36 | 0.42 |
| | | 5-15 cm | 2.24 | 1.27 | 0.63 |
| N | % | 0-5 cm | 0.17 | 0.10 | 0.03 |
| | | 5-15 cm | 0.17 | 0.11 | 0.03 |
| pH ($H_2O$) | | 0-5 cm | 8.16 | 8.03 | 8.23 |
| | | 5-15 cm | 8.26 | 8.02 | 8.27 |

* OC = Organic carbon

### *5.2.2 Chemicals*

Terbuthylazine (99% purity), metolachlor (98% purity), and mesotrione (98% purity) analytical standards were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The main chemical and environmental properties were taken from the Pesticide Properties DataBase (PPDB) (Footprint, 2007), and are shown in Table 5.2.

Table 5.2 Main chemical and environmental properties of the three tested herbicides (from Footprint 2007, http://www.eu-footprint.org/ppdb.html).

| Properties | Unit | Mesotrione (MESO) | Metolachlor (METO) | Terbuthylazine (TERB) |
|---|---|---|---|---|
| Molecular weight | | 339.3 | 283.79 | 229.71 |
| Water solubility at 20 °C | mg $L^{-1}$ | 1500 | 480 | 6.6 |
| Melting point | °C | 165.3 | -61.1 | 176 |
| $K_{Foc}$ | mL $g^{-1}$ | 19-170** | 189** | 224** |
| GUS* | | 2.69 | 1.91 | 3.07 |
| $t_{1/2}$ (lab at 20 °C) | days | 19.6 | 14.5 | 75.1 |
| DT50 (field) | days | 5 | 21 | 22.4 |

* GUS = Groundwater Ubiquity Score

** $K_{Foc}$ values are taken from The Pesticide Manual (British Crop Protection Council, 2012).

Stock solutions (100 mg $mL^{-1}$) of each herbicide standard were prepared in methanol, protected from light and stored at -20°C. Mixtures of standard solutions, with concentrations ranged from 0.1–2.5 mg $mL^{-1}$, were injected to obtain the linearity of detector response and detection limits (LOD) of the herbicides. Methanol (HPLC-grade), water (HPLC-grade), and all other chemicals (analytical-grade) were purchased from Sigma-Aldrich (St. Louis, MO).

### *5.2.3 Field experiment*

On 14th May a pre-emergence treatment was applied with terbuthylazine, metolachlor, and mesotrione, hereinafter called TERB, METO, and MESO, as a formulated product (Lumax®, Syngenta). The herbicides were applied using a 9 m wide boom portable field sprayer equipped with 19 flat-fan nozzles (110) and a spray pressure of 200 kPa. Adequate space between plots (4 m) was used as a buffer zone to avoid any effects between the different treatments applied (Vasileiadis et al., 2017). The applied dose was 3.5 L ha$^{-1}$ with 131, 656, and 1,094 g active ingredient ha$^{-1}$ for MESO, TERB, and METO, respectively. The dissipation of TERB, METO, and MESO in the treated plots was followed for 2 months after their application.

The soil was sampled before herbicides treatment to be used as a control. Soon after treatment, soil samples were taken to assess initial concentration in the cultivated soil (soil concentration at 0 d after treatment), then at increasing times from spraying to evaluate field dissipation kinetics (1, 2, 3, 4, 9, 11, 16, 23, 38, and 60 days after treatment). Soil samples were collected with a soil auger (3 cm i.d. × 10 cm length) at 0–5 and 5–15 cm depth. On each sampling date, five sub-samples per plot were collected and placed in a labeled plastic bag, sealed, transported to the laboratory in a cooler and stored at -20°C until analysis. Soil water content was measured after soil drying at 105°C to express results on a dry weight basis.

### *5.2.4 Determination of soil dissipation half-lives (DT50) of the herbicides*

The dissipation of TERB, METO, and MESO could be described by a pseudo first-order kinetics:

$C_t = C_0 \times e^{-kt}$

where $C_0$ and $k$ denote y-intercept values and the slope of dissipation lines, respectively, $C_t$ is the concentration of herbicide in soil, and $t$ is the post-application time in days. Hence, their half-lives (DT50) were calculated from the equation (DT50) = ln 2/k.

The procedures used for sample preparation derived from previous studies (Barchanska et al., 2012). For analyzing herbicide concentration, soil samples (20 g, on dry weight basis) were placed in dark bottles, and 50 mL of methanol–0.1 M HCl (9–1, v/v) was added. The bottles were shaken for an hour at 200 rpm at room temperature (about 18°C). Mixtures were then centrifuged for 15 min at 3,200 rpm (Heraeus Christ Labofuge GL, Germany) and the supernatant was filtered at 0.45 mm pore size. The filtrate was then evaporated at 38°C using a IKA® RV 8 rotary evaporator (IKA®, Werke GmbH & Co.KG, Staufen, Germany) and the residue was dissolved in 50 mL of 0.1 M HCl. Subsequently, the extraction was conducted using a TELOS neo™ PRP polymeric SPE column (60 mg, Kinesis) and a J.T. Baker SPE-12G glass column processor at a flow rate of 15 mL min$^{-1}$. After extraction, the analytes were eluted with 5 mL of methanol without the vacuum, and methanol aliquots were reduced to approximately 50 mL with the use of a nitrogen gas stream at 45°C.

The soil (without analytes) was spiked with herbicides at a concentration of 0.4, 1, and 2.2 µg ml$^{-1}$ for defining the analyte recoveries. Extraction was then the same as reported above. The recoveries in soil were: 75, 76%, and 81% for MESO, TERB, and METO, respectively.

The analyses were performed by LC-MS using an 1100 Series Agilent Technologies system (CA, US), equipped with binary pump, diode array detector, and MSD SL Trap mass spectrometer with ESI source. A Eurospher II (Knauer, Berlin, Germany) column C18 P with TMS endcapping, 150 × 4.6 mm i.d., 3 µm, 110 Å was used to analyze the samples; the mobile phase consisted of 0.6% formic acid in water (solvent A) and methanol (solvent B).

Gradient elution program: from 0 to 4 min a linear increase of solvent B from 60% to 80% and flow rate from 0.4 to 0.6 mL min$^{-1}$, from 4 to 11 min a linear increase of solvent B from 80% to 100% at flow rate of 0.6 mL min$^{-1}$; initial conditions were re-established in 5 min and re-equilibration time was 2 min.

A 10 mL sample volume was manually injected each time. Retention times were 5.5 (±0.4), 9.0 (±0.2), and 10.1 (±0.3) min for MESO, TERB, and METO, respectively.

The LOD, 3:1 signal-to-noise ratio, was 14, 20, and 90 ng kg$^{-1}$ for TERB, METO, and MESO, respectively.

### *5.2.5 Statistical analysis*

Statistical analyses were conducted using the Statistica 13.2 software for Windows (Statistica, Oklahoma, USA). A factorial analysis of variance (ANOVA) was performed to reveal the effects of sample depth and soil texture on the observed DT50 values of TERB, METO, and MESO. Fisher's least significant differences (LSD), at a confidence level of 95%, were determined for comparing treatment group means.

## 5.3 Results and discussion

Results of dissipation studies for TERB, METO, and MESO at various sampling times are plotted in Fig. 5.2 for the 0–5 cm layer and in Fig. 5.3 for 5–15 cm layer. The data are presented as percent of each compound dissipated from the initial application on soil.

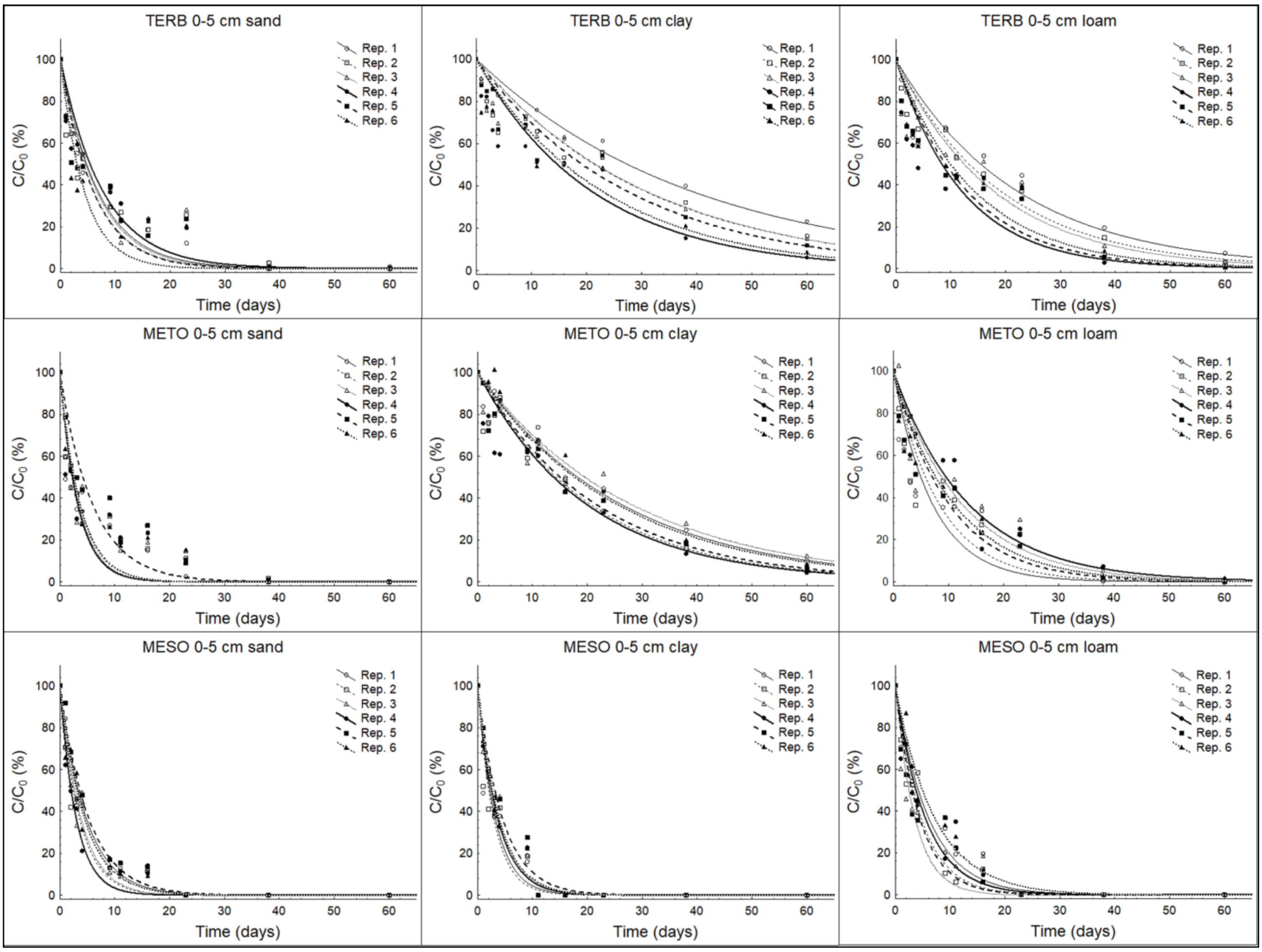

Fig. 5.2 Plots of dissipation of TERB, METO, and MESO in 0-5 cm soil layer.

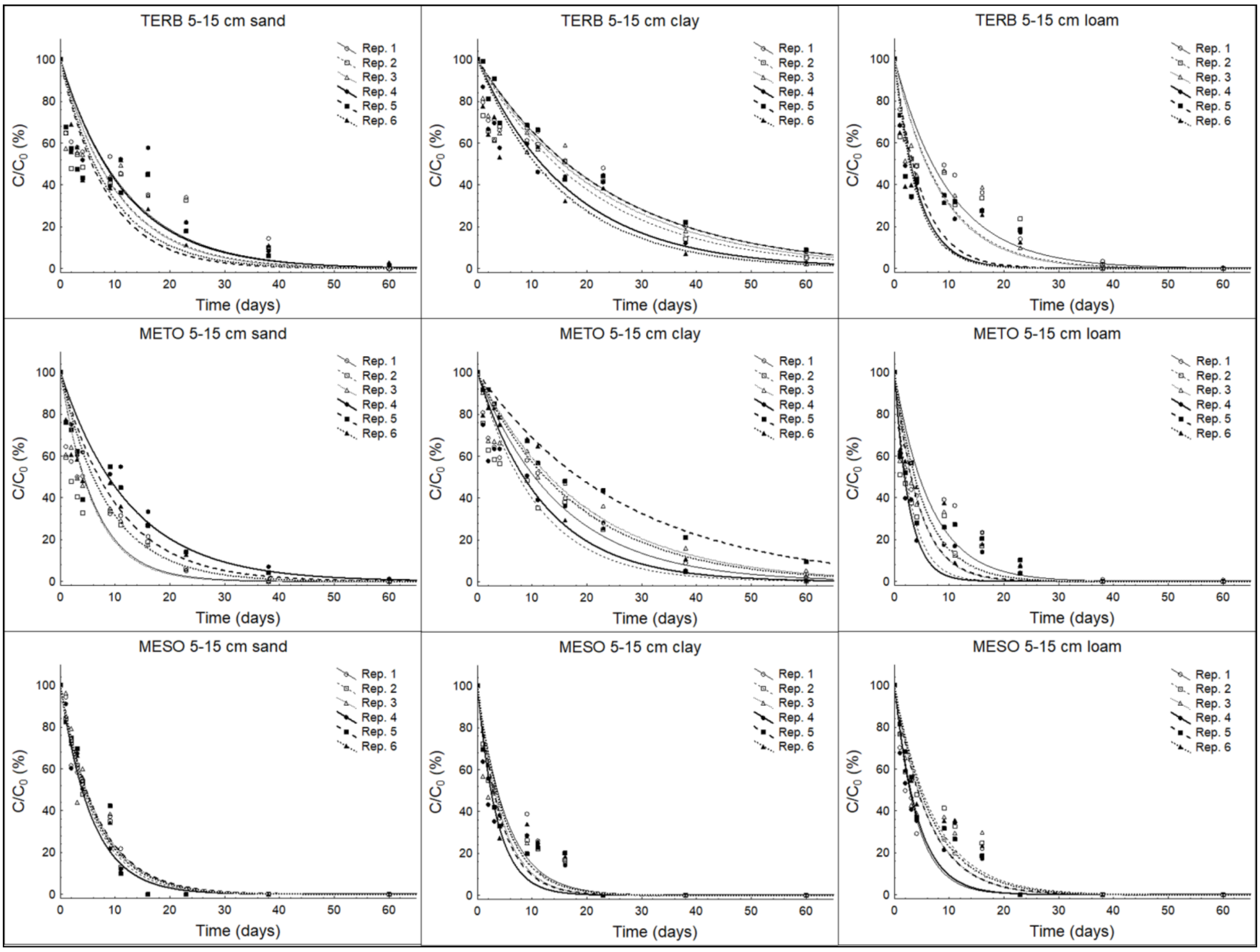

Fig. 5.3 Plots of dissipation of TERB, METO, and MESO in 5-15 cm soil layer.

The dissipation of TERB, METO, and MESO followed a pseudo first-order kinetics, with an $R^2$ value ranging from 0.70 for TERB in sandy soil at 5–15 cm to 0.99 for MESO in sandy soil at 0–5 cm. The degradation rate constants ranged from 0.025 to 0.247 day$^{-1}$ for TERB, from 0.036 to 0.378 day$^{-1}$ for METO, and from 0.138 to 0.340 day$^{-1}$ for MESO. The corresponding half-lives ranged from 2.8 to 27.5 days for TERB, from 1.8 to 19.5 days for METO, and from 2.0 to 5.0 days for MESO. ANOVA showed that all the effects of the principal factors (herbicide, soil texture, and depth) and their interaction effects were statistically significant, as reported in Table 5.3.

Table 5.3 Results of factorial ANOVA.

| Source | d.f.[a] | Sum of square | Mean of square | F-value | *P* |
|---|---|---|---|---|---|
| Soil | 2 | 978.82 | 489.41 | 100.00 | < 0.0001 |
| Depth | 1 | 61.77 | 61.77 | 12.62 | 0.0006 |
| Herbicide | 2 | 869.80 | 434.90 | 88.86 | < 0.0001 |
| Soil × Depth | 2 | 202.56 | 101.28 | 20.69 | < 0.0001 |
| Soil × Herbicide | 4 | 635.10 | 158.78 | 32.44 | < 0.0001 |

| | | | | | |
|---|---|---|---|---|---|
| Depth × Herbicide | 2 | 83.00 | 41.50 | 8.48 | 0.0004 |
| Soil × Depth × Herbicide | 4 | 71.74 | 17.93 | 3.66 | 0.0082 |
| Error | 90 | 440.49 | 4.89 | | |

[a] Degree of freedom

Soil texture and depth had a strong influence on the dissipation of TERB and METO, whereas none was observed for MESO (Fig. 5.4).

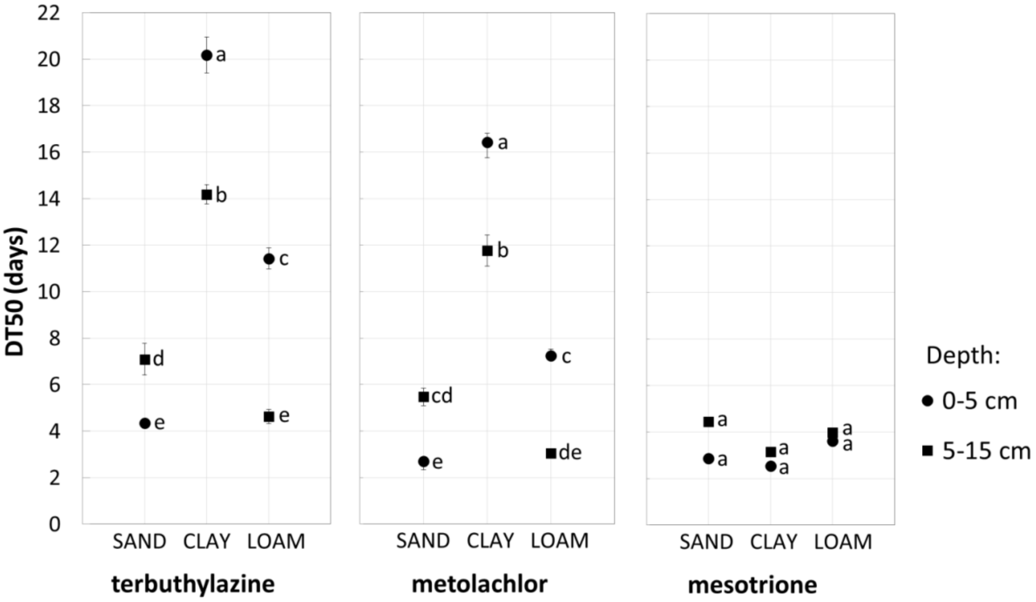

Fig. 5.4 DT50 values of TERB, METO, and MESO in sandy, clay and loam soil at 0-5 cm and 5-15 cm. Error bars represent the standard error of the mean (n=6). Different letters next to the indicators denote significant differences ($p < 0.05$) between soil textures and depths for each herbicide, within the individual boxes.

At a depth of 0–5 cm, the highest DT50 value of TERB was found in clay soil (20.2 ± 0.8 days), followed by loam (11.4 ± 0.5 days) and sandy soil (4.4 ± 0.1 days). In the 5–15 cm layer the highest DT50 value for TERB was found in clay soil (14.2 ± 0.4 days), but contrariwise, the dissipation was faster in loam than in sandy soil (4.6 ± 0.3 days and 7.1 ± 0.7 days, respectively). METO showed similar behavior, as the highest DT50 was found in clay soil both at 0–5 cm and at 5–15 cm (16.4 ± 0.4 days and 11.8 ± 0.7 days, respectively). Also for METO, at 0–5 cm the dissipation was faster in sandy than in loam soil (2.7 ± 0.1 days and 7.2 ± 0.3 days, respectively), whereas at 5–15 cm METO degraded faster in loam than in sandy soil, although the difference between the DT50 in loam and sandy soil at 5–15 cm was not statistically significant.

Overall, the DT50 values of TERB and METO measured in this study were shorter than typical values reported in the literature in field studies (Barra Caracciolo et al., 2005; Long et al., 2014; Stipičević et al., 2015; Wu et al., 2011). Herbicide degradation rates rise as temperature and soil moisture increase because both chemical and microbial decomposition rates speed up with higher temperatures and moisture levels (Furmidge and Osgerby, 1967; Sahid and Teoh, 1994). Garrett et al. (2015) showed that the key period for significant leaching of these herbicides to water bodies is within approximately the first month after spray application when the soil content of the applied herbicides is high. For this reason, heavy rainfall events close to spray application have been noted to promote herbicides leaching down the soil profile, Meteorological data collected over the period of the present trial shows that there was a heavy rainfall event seven days after application of the herbicides, with 45 mm of rain in one day (Fig. 5.1). It is possible to hypothesize that this event, combined with the low OC of the soils, and consequently the low adsorption of herbicides, would have led to herbicide leaching into the deeper layers, especially for TERB, which is a highly leachable herbicide, as shown in Table 5.2. This phenomenon may have occurred mainly in the sandy soil, because of the lower OC compared to loam and clay soil. Measurements at 0–5 and 5–15 cm do not evaluate leaching, and then the amount of each herbicide potentially moved by leaching cannot be determined. Furthermore, the degradation rate rises with increasing soil moisture content. Hence, it is possible that, in the days immediately after the rainfall, the higher soil moisture may have increased degradation of TERB and METO.

Regarding the influence of soil texture, these results highlight that TERB and METO degraded slowly in clay soil than in loam and sand. It has been proven that degradation of TERB and METO in soils is dependent on clay content (Stipičević et al., 2015; Wu et al., 2011). In fact, soils high in clay have the greatest potential to bind herbicides to soil particles, with a corresponding decrease in leaching and loss through volatilization. In addition, among the tested soils, clay soil has the highest OC content, which also contributes to herbicides adsorption, affecting in turn their bioavailability and biodegradation (Shea, 1989; Six et al., 2002). Because of the high adsorption of herbicides to clay and organic matter, a slower degradation rate can be expected from a soil with higher clay and OC content, leaving the herbicides less available for microorganism decomposition (Choi et al., 1988; Furmidge and Osgerby, 1967). These hypotheses are supported by previous studies that demonstrated an inverse relationship between microbial decomposition and sorption of TERB (Cabrera et al., 2007; Dousset et al., 1997; López-Piñeiro et al., 2012) and METO (Wu et al., 2011) in the soil.

The DT50 of TERB and METO was also significantly affected by depth, but in a different way depending on the type of soil. In clay and loam soil, the DT50 of TERB and METO was higher at a

0–5 cm depth than at 5–15 cm, whereas only in sandy soil the DT50 was higher at 5–15 cm than at 0–5 cm. The OC in sand is lower than in clay and loam. As mentioned above, adsorption of herbicides on soil is highly dependent on OC, so it is assumed that there was less adsorption in the sandy soil compared to clay and loam. For this reason, it is probable that herbicide leaching in the sandy soil was much more pronounced than in the other soils. Consequently, in sandy soil, a greater transport of herbicides into the lower layers would be expected, and this can contribute to explaining the faster dissipation of TERB and METO at 0–5 cm than at 5–15 cm.

In clay and loam soil, depth affected the DT50 of TERB and METO in a way different from what happened in the sandy soil. An important factor that affects the dissipation of TERB and METO is soil moisture. Sahid and Teoh (1994) and Rice et al. (2002) showed that, at higher soil moisture levels, an increased degradation of TERB and METO is expected since higher moisture levels hinder herbicide adsorption onto soil particles, making the molecules more readily available for degradation by soil microbes. Moreover, relatively higher soil moisture is good for stimulating the activity of microorganisms (Su et al., 2017). Soil moisture measured in the soil samples at 0–5 cm was on average significantly lower than that in the samples at 5–15 cm, with a more pronounced difference in clay and loam soil, as shown in Fig. 5.5. This probably contributes to explaining the lower DT50 values found in the 5–15 cm layer compared to the 0–5 cm layer, in clay and loam soil.

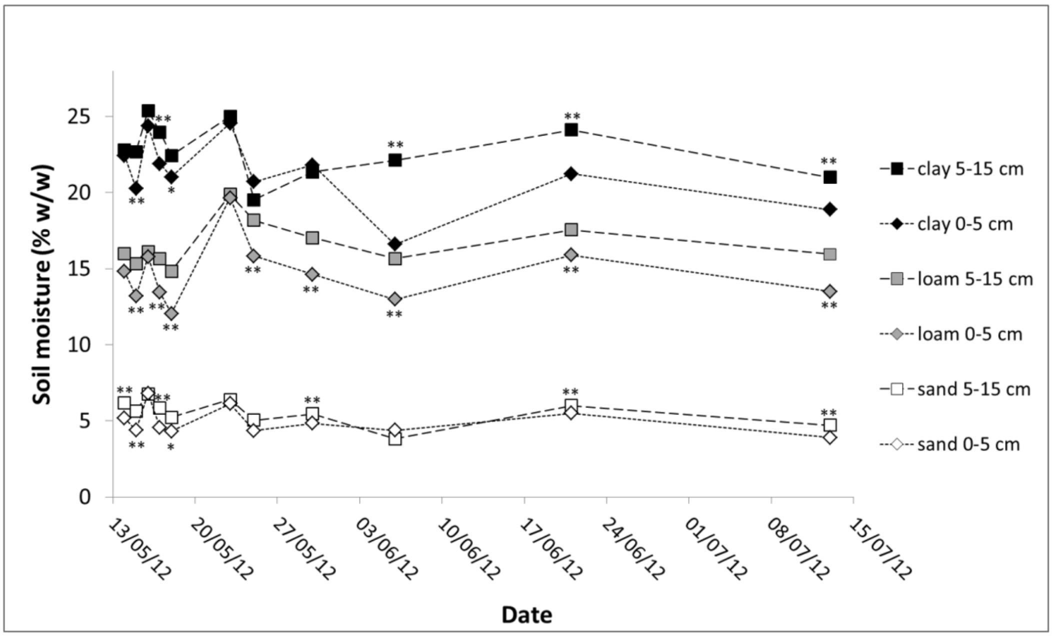

Fig. 5.5 Soils moisture values at each sampling date ($p < 0.05$ *; $p < 0.01$ **).

Contrary to what was observed for TERB and METO, the dissipation of MESO appears to be unaffected by soil texture and depth, and the DT50 values found are rather lower than those reported by some authors. The dissipation rate of each compound under field conditions is the result of a variety of complex dynamic physical, chemical, and biological processes. Su et al. (2017) and Dumas et al. (2016) showed that the solubility of MESO increases at basic pH, with ionization and there is an inverse correlation between the DT50 of MESO and soil pH. Dyson et al. (2002) demonstrated that mesotrione degradation is correlated more strongly with the pH than with other variables, and that the DT50 decreases with increasing pH, with a regression line of: log10 (DT50) = - 0.192 pH + 2.18. The pH of the tested soils is sub-alkaline, ranging between 7.64 and 7.79, and the low DT50 values found for MESO in all the tested soils and depths are in agreement with those described by the regression model reported above.

## 5.4 Conclusions

Soil texture and depth had a strong influence on the dissipation of terbuthylazine and metolachlor, whereas none was observed on mesotrione. The variations of DT50 values of terbuthylazine and metolachlor between soils and depths may have been due to the influence that soil texture, organic matter, and soil moisture have

on the persistence of these herbicides. Because of its chemical and physical properties, the persistence of mesotrione was less affected by soil texture and depth, whereas the soil pH influenced its persistence, leading to low DT50 values in all studied soils.

Terbuthylazine and metolachlor showed medium-low persistence, less than 20 days. Nevertheless, the analyses of deep and surface water indicate a widespread presence of these herbicides in the Po Valley (ISPRA, 2016b). This is due to the widespread use of these molecules on maize and the high rainfall during maize sowing period in spring. In addition, the trial has highlighted that intense rainfall events occurring near distribution, cause the transfer of herbicides to both surface and ground waters. For this reason, it seems very important to improve precision herbicide application technologies to reduce herbicide use, such as band spraying (banding herbicide on the crop row in conjunction with inter-row mechanical hoeing) (Davis and Pradolin, 2016) or other emerging solutions proposed by the agricultural robotics (Emmi et al., 2014; Pérez-Ruìz et al., 2014). These innovative practices seem to be very promising and their application will result in a reduction of herbicide pollution and limitation of the contamination of both surface water through runoff and ground water through leaching.

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# Chapter 6. Effect of sewage amendment on the dissipation of terbuthylazine, its degradation compound desethyl-terbuthylazine, and S-metolachlor in a field study

## Abstract

This study evaluates the effect of sewage amendment (SA) on the dissipation of terbuthylazine, its degradation compound desethyl-terbuthylazine, and S-metolachlor in the soil. The experiment was conducted at Padua Experimental Farm (Italy). Herbicides dissipation was evaluated in soils differently fertilized for three years: with inorganic fertilizer, with sewage sludge, and with a combination of them. Terbuthylazine and S-metolachlor were applied on sorghum as a formulated product at a dose of 2.8 L ha$^{-1}$, and their dissipation was followed for 2.5 months. The concentrations of herbicides and one metabolite in soil were analyzed by liquid chromatography-mass spectrometry. The dissipation of terbuthylazine and S-metolachlor followed a pseudo first order kinetics; they dissipated faster in soil amended only with inorganic fertilizer than in soils amended with sewage or sewage + inorganic fertilizer. The reduction in mineralization of the herbicides after sewage addition can be attributed to the reduced herbicide availability to microorganisms. The degradation of terbuthylazine led to the formation of desethyl-terbuthylazine. SA slowed down the formation and the degradation of desethyl-terbuthylazine, leading to a higher amount measured at the end of the incubation. These findings have practical implications for the assessment of the environmental fate of terbuthylazine and S-metolachlor in agricultural areas.

Keywords: herbicides; persistence; triazines; chloroacetamide; sewage.

## 6.1 Introduction

Terbuthylazine (TER), a chloro-s-triazine herbicide and S-metolachlor (S-MET), a choloroacetanilide herbicide, are among the pesticides most used in Italy, especially in Northern regions. TER and S-MET are selective herbicides, widely used for the control of grasses and some broadleaf weeds in maize and sorghum.

A notable problem associated with the widespread use of these herbicides concerns the presence of their residues and metabolites in the soil, which can be transported from fields to surface water and groundwater (Fenoll et al., 2014a). Contamination of surface water resources by TER and S-MET affects the entire national territory, but the detection frequency in Northern Italy, especially in Veneto (ranging from 31 to 35%), is higher than the national average (ranging from 9.5 to 20%), because of their intense use in this region (ISPRA, 2018a). In 2014, terbuthylazine and S-metolachlor were found to be the main contaminants of both surface and groundwater in Veneto, reaching concentrations even greater than 0.1 mg $L^{-1}$, the limit established in the Directive for Drinking Water (ISPRA, 2018b). Desethyl-terbuthylazine, one of the main metabolites of TER, is also frequently detected at levels above 0.1 mg $L^{-1}$ in groundwater (Funari et al., 1995; Otto et al., 2007).

Dissipation rate and degradation mechanism of a herbicide in the soil are influenced by herbicide physical and chemical characteristics as well as by applied dose, distribution times, and soil characteristics such as moisture, temperature, organic matter content, texture, microbial activity, plus weather conditions that occur after the application (Otto et al., 1997; Rice et al., 2002; Wu et al., 2015). In particular, pesticide behavior in soils may be modified after organic amendment, depending on the organic materials used and the pesticide properties themselves (Alvey and Crowley, 1995). Sewage amendments (SAs) are frequently added to soils as an economic means to fertilize them. Nutrients in SA are used to replace or supplement commercial chemical fertilizers, while its organic matter can improve soil structure, reduce its erosion, and improve crop yields (Metzger and Yaron, 1987; Tester, 1990). However, the addition of SA can affect the rate and pathways of herbicides degradation in soils, depending on the nature and reactivity of the SA and their effect on microbial activity, as well as on the herbicide properties (Alvey and Crowley, 1995). Indeed, the organic matter present in the soil, or supplied with fertilizers, plays an important role in limiting the action of herbicides because it can absorb them more or less strongly and release them gradually, thus affecting the degradation of the active ingredient. The herbicides can also be immobilized by organic matter, so that they can be absorbed by the roots (Tejada et al., 2013; Wu et al., 2011).

Under controlled laboratory conditions, an increase in sorption and a decrease in leaching of TER and S-MET were observed in soils with organic amendment (Cabrera et al., 2009, 2007; Dolaptsoglou et al., 2007; Fenoll et al., 2014b; Singh, 2003; Wang et al., 2010). Cabrera et al. (2008) and Peña et al. (2013) also found that biodegradation of TER and S-MET is reduced in soils enriched with organic amendment, which has been attributed in part to microbial preference to use the exogenous organic matter rather than herbicide. These results suggest that in some cases herbicide persistence can be increased by organic amendment, and the risk of soil and water resources contamination consequently increased.

Given the significant contamination of water in Italy by TER and S-MET, and the relevance of this ecological problem, we conducted a study to investigate the influence of SA on the dissipation of TER and S-MET in soil. For this purpose, a field experiment was performed in North-East Italy. Their dissipation was evaluated in soils that had received different fertilization for three years: soil without amendment (inorganic fertilizer, IF), soil with SA applied and soil amended with a combination of IF and SA (IF+SA). The findings of this study could provide information regarding the consequences that the use of such amendments may have on the environmental fate of these herbicides.

## 6.2 Materials and methods

### *6.2.1 Site information*

The study was conducted at the Padua University Experimental Farm in the Po Valley, North-East Italy in 2014. Annual rainfall was 1311mm (the wettest month was July with 189mm while the driest was October with 40 mm) with a total of 115 rainy days. Air temperature and soil temperature at -10 cm depth increased from January (minimum average: -1.2 °C and 4.5 °C, respectively) to August (maximum average: 34.0 °C and 26.6 °C, respectively). The weather conditions during the experimental period of May through August are illustrated in Fig. 6.1.

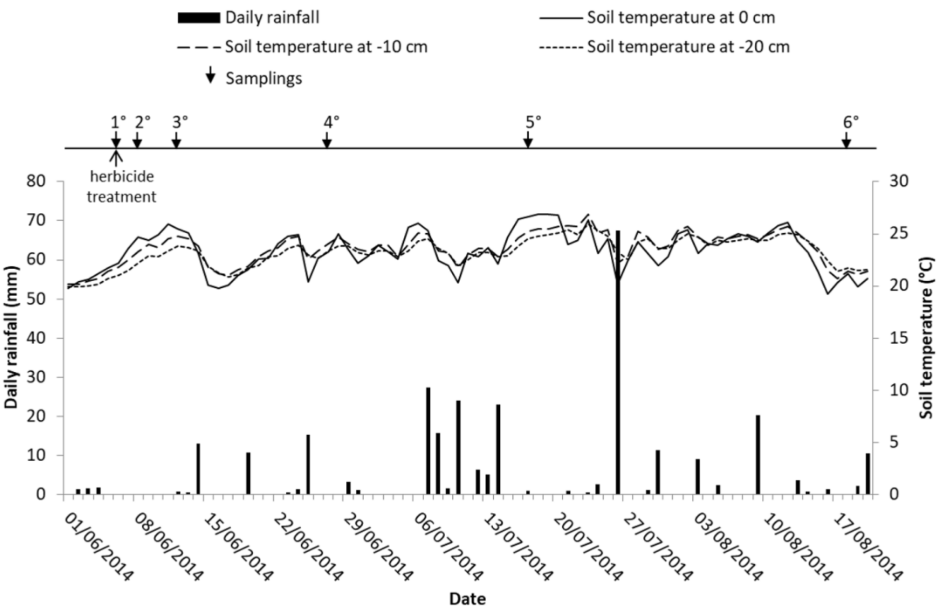

Fig. 6.1 Daily rainfall and average soil temperature from May to August 2014 at the Padua University Experimental Farm.

### *6.2.2 Soil, organic amendments and inorganic fertilizer*

The soil is a sandy clay according to ISSS classification: clay (%), 32 ± 2.4; silt (%), 31 ± 2.2; sand (%), 37 ± 1.1 (n = 3, ±standard deviation, SD).

Inorganic fertilizers used were: triple superphosphate (TSP, Helm AG) 46%, $P_2O_5$; potassium sulfate 50%, $K_2O$ (KALISOP® fine max. 1.0% Cl, $K_2O$ KALI GmbH); and urea (YaraVera EURA 46, Yara Italia SpA). Chemical characteristics of the sewage and the amended soils and their macronutrient, micronutrient, and metal contents are listed in Table 6.1. Organic carbon and nitrogen analyses were performed with a Vario Macro instrument (Elementar Analysensysteme GmbH, Hanau, Germany). Metal content in sewage and soil samples was analyzed by a Spectro Arcos ICP EOS analyzer (Spectro Analytical Instruments GmbH, Kleve, Germany).

Table 6.1 Chemical properties of sewage amendment and soils amended with IF (inorganic fertilizer), SA (sewage amendment), and IF+SA (inorganic fertilizer + sewage amendment).

| Properties | Unit | Sewage amendment | IF | SA | IF+SA |
|---|---|---|---|---|---|
| OC | g $kg^{-1}$ | 23.43 ± 0.006 | 6.42 ± 0.128 | 7.60 ± 0.080 | 6.46 ± 0.077 |
| N | % | 1.71 ± 0.007 | 0.13 ± 0.012 | 0.16 ± 0.004 | 0.12 ± 0.001 |
| pH | | 7.30 | 8.07 ± 0.017 | 8.05 ± 0.009 | 8.06 ± 0.009 |
| $P_2O_5$ | % | 0.14 ± 0.03 | | | |

| | | | | | |
|---|---|---|---|---|---|
| $K_2O$ | % | 0.26 ± 0.03 | | | |
| P | mg kg$^{-1}$ d.w. | 6366 | 900 | 880 | 1034 |
| K | mg kg$^{-1}$ d.w. | 61397 | 12234 | 9495 | 12287 |
| Ca | mg kg$^{-1}$ d.w. | 19451 | 93449 | 96147 | 92129 |
| Mg | mg kg$^{-1}$ d.w. | 9739 | 39106 | 37724 | 36455 |
| Na | mg kg$^{-1}$ d.w. | 5791 | 790 | 586 | 709 |
| Cd | mg kg$^{-1}$ d.w. | 0.493 | 0.415 | 0.362 | 0.321 |
| Cr | mg kg$^{-1}$ d.w. | 3 | 42 | 36 | 30 |
| Cu | mg kg$^{-1}$ d.w. | 36 | 41 | 37 | 30 |
| Fe | mg kg$^{-1}$ d.w. | 1214 | 25453 | 22364 | 19157 |
| Mn | mg kg$^{-1}$ d.w. | 144 | 595 | 548 | 480 |
| Ni | mg kg$^{-1}$ d.w. | 6 | 27 | 21 | 18 |
| Pb | mg kg$^{-1}$ d.w. | nd | 30 | 25 | 22 |
| Zn | mg kg$^{-1}$ d.w. | 228 | 97.3 | 82.9 | 73.9 |

### *6.2.3 Chemicals*

Terbuthylazine (TER, 99% purity), desethyl-terbuthylazine (TER-D, 99.5% purity), 2-hydroxy-terbuthylazine (TER-2H, 97.5% purity), S-metolachlor (S-MET, 98% purity), and metolachlor oxanilic acid (OA, 98% purity) analytical standards were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). TER (6-chloro-N-(1,1-dimethylethyl)-N0- ethyl-1,3,5- triazine-2,4-diamine) is a selective systemic herbicide that belongs to the triazines (Baćmaga et al., 2014). It is mainly absorbed not only through the roots, but also through foliage. TER acts by inhibition of photosynthesis at the photosystem II level. The Herbicide Resistance Action Committee (HRAC) classified TER as C1 (GIRE, 2016). S-MET ((chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)) is a substance that belongs to the chloroacetamides and is used to control mainly grass weeds, being absorbed by the shoots of seedlings (Baćmaga et al., 2014). The S-MET mode of action consists in the inhibition of cell division, and HRAC classifies it as K3 (GIRE, 2016). The main chemical and environmental properties were taken from The Pesticide Properties DataBase (Footprint, 2007) and are shown in Table 6.2.

Table 6.2 Selected chemical properties and environmental parameters of the herbicides and their principal metabolites (from FOOTPRINT, 2007).

| Properties | Unit | S-Metolachlor (S-MET) | Oxanilic acid (OA) | Terbuthylazine (TER) | 2-hydroxy-terbuthylazine (TER-2H) | Desethyl-terbuthylazine (TER-D) |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| Molecular weight | g $mol^{-1}$ | 283.8 | 279.3 | 229.7 | 211.3 | 201.7 |
| Water solubility at 20 °C | mg $L^{-1}$ | 480.0 | 238 | 6.6 | 7.2 | 327.1 |
| Melting point | °C | -61.1 | – | 176.0 | – | – |
| Freundlich constant (*Kf*) | | 1.88 | 0.094 | 5.1 | 3.9 | 1.4 |
| $DT_{50}$ (field studies) | days | 11-31 | 128 | 10-36 | 453 | 16-223 |
| GUS leaching potential index | | 1.91 | 6.61 | 3.07 | 4.59 | 3.65 |

FOOTPRINT (2007) The FOOTPRINT pesticide properties database. University of Hertfordshire as part of the EU-funded FOOTPRINT project (FP6-SSP-022704). Available at: http://www.eu-footprint.org/ppdb.html). Accessed 28 March 2018.

Stock solutions (100 µg $mL^{-1}$) of each herbicide standard were prepared in methanol, protected from light and stored at -20 °C. Mixtures of standard solutions, with concentrations ranging from 0.1 to 2.5 µg $mL^{-1}$, were injected to obtain the linearity of detector response and the detection limits (LOD) of the herbicides studied. Methanol (HPLC-grade), water (HPLC-grade) and all other chemicals (analytical-grade) were purchased from Sigma-Aldrich (St. Louis, MO).

### *6.2.4 Field experiment*

Three subplots (15×15 m) were randomly selected from a 5 ha field with three different fertilizers: IF, SA, and IF+SA (Fig. 6.2). For three years (2012, 2013, and 2014), the experimental area was plowed to a 25-cm depth.

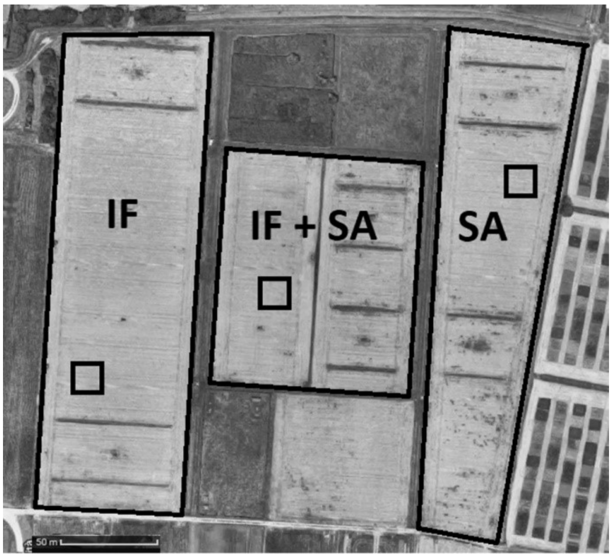

Fig. 6.2 Layout of the experimental field with three different fertilizers: inorganic fertilizer (IF), SA and a combination of IF and SA (IF+SA).

Table 6.3 reports the fertilizations performed in IF, SA, and IF+SA plots in the years 2012, 2013, and 2014. The amounts of SA incorporated in the plots before seeding corresponded to about 300 kg ha$^{-1}$ of nitrogen, 136 kg ha$^{-1}$ of $P_2O_5$, and 195 kg ha$^{-1}$ of $K_2O$. Standard agronomic practices were followed to equal the amount of SA in IF plots: 136 kg ha$^{-1}$ of $P_2O_5$ and 195 kg ha$^{-1}$ of $K_2O$ before seeding and 150 kg ha$^{-1}$ of N twice in mid-June during the inter-row cultivation. Before seeding, IF+SA plots received half of the total amount with SA (150 kg ha$^{-1}$ of N, 68 kg ha$^{-1}$ of $P_2O_5$, and 98 kg ha$^{-1}$ of $K_2O$) and half of $P_2O_5$ and $K_2O$ with inorganic fertilizer, with the remaining 150 kg ha$^{-1}$ of N with urea during the inter-row cultivation.

Table 6.3 Fertilization and sewage amendments during the years 2012, 2013, and 2014 in the inorganic fertilized (IF), sewage amendment (SA), and their combination (IF+SA) plots.

| | Date | Amendment | N (kg ha$^{-1}$) | $P_2O_5$ (kg ha$^{-1}$) | $K_2O$ (kg ha$^{-1}$) |
|---|---|---|---|---|---|
| Year 2012 | | | | | |
| IF | 09/05/2012 | Inorganic (TSP 46% and KALISOP® 1.0% Cl) | | 136 | 195 |
| | 07/06/2012 | Inorganic (YaraVera EURA 46%) | 150 | | |
| | 18/06/2012 | Inorganic (YaraVera EURA 46%) | 150 | | |
| SA | 06/05/2012 | Sewage (0.37% N; 0.17% $P_2O_5$; 0.24% $K_2O$) | 300 | 136 | 195 |
| IF+SA | 06/05/2012 | Sewage (0.37% N; 0.17% $P_2O_5$; 0.24% $K_2O$) | 150 | 65 | 93 |
| | 09/05/2012 | Inorganic (TSP 46%; KALISOP® 1.0% Cl) | | 65 | 93 |
| | 07/06/2012 | Inorganic (YaraVera EURA 46%) | 150 | | |
| Year 2013 | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| IF | 13/06/2013 | Inorganic (TSP 46%; KALISOP® 1.0% Cl) | | 153 | 370 |
| | 19/06/2013 | Inorganic (YaraVera EURA 46%) | 150 | | |
| | 25/06/2013 | Inorganic (YaraVera EURA 46%) | 150 | | |
| SA | 10/06/2013 | Sewage (0.24% N; 0.12% $P_2O_5$; 0.29% $K_2O$) | 300 | 153 | 370 |
| IF+SA | 10/06/2013 | Sewage (0.24% N; 0.12% $P_2O_5$; 0.29% $K_2O$) | 150 | 76 | 185 |
| | 13/06/2013 | Inorganic (TSP 46%; KALISOP® 1.0% Cl) | | 76 | 185 |
| | 19/06/2013 | Inorganic (YaraVera EURA 46%) | 150 | | |
| Year 2014 | | | | | |
| IF | 20/05/2014 | Inorganic (TSP 46%; KALISOP® 1.0% Cl) | | 153 | 370 |
| | 20/06/2014 | Inorganic (YaraVera EURA 46%) | 150 | | |
| | 30/06/2014 | Inorganic (YaraVera EURA 46%) | 150 | | |
| SA | 17/05/2014 | Sewage (0.24% N; 0.12% $P_2O_5$; 0.29% $K_2O$) | 300 | 153 | 370 |
| IF+SA | 17/05/2014 | Sewage (0.24% N; 0.12% $P_2O_5$; 0.29% $K_2O$) | 150 | 76 | 185 |
| | 20/05/2014 | Inorganic (TSP 46%; KALISOP® 1.0% Cl) | | 76 | 185 |
| | 20/06/2014 | Inorganic (YaraVera EURA 46%) | 150 | | |

Table 6.4 reports the crops and herbicide treatments during the years 2012, 2013, and 2014 in every plot. In detail, in 2012 and 2013, the field was shown with maize; in 2012 4 L ha$^{-1}$ of the formulated product (Lumax®, Syngenta) was applied at the dose of 750 and 1250 g active ingredient ha$^{-1}$ for terbuthylazine and S-metolachlor, respectively. In 2013 3.6 L ha$^{-1}$ of the formulated product (Lumax®, Syngenta) was applied at the dose of 675 and 1125 g active ingredient ha$^{-1}$ for terbuthylazine and S-metolachlor, respectively. In May 2014, the plots were seeded with sorghum. TER and S-MET were applied on June 6, 2014 as a formulated product (Primagram® Gold, Syngenta) using a conventional rear-mounted boom sprayer at 200 kPa pressure using 2.8 L ha$^{-1}$ at the dose of 524 and 875 g active ingredient ha$^{-1}$ for terbuthylazine and S-metolachlor, respectively. The soil was sampled before herbicide treatments to analyze its physico-chemical characteristics. The soil was also sampled before treatment as a control sample, and soon after treatment soil samples were taken to assess initial herbicide concentration in the cultivated soil (soil concentration at 0 d after treatment), then at increasing times from spraying to evaluate field dissipation kinetics (3, 6, 21, 41, and 73 days after treatment). Topsoil samples were collected with a soil auger (5 cm i.d. × 20 cm length) at 0–20 cm depth. At each sampling date, five different subsamples were collected along the diagonals of each subplot. A sample for each plot was obtained by mixing the five subsamples and placed in a labeled plastic bag, sealed, transported to the laboratory in a cooler

and stored at -20 °C prior to analysis. Soil water content was measured after soil drying at 105 °C to express results on a dry weight basis.

Table 6.4 Crops and herbicide treatments during the years 2012, 2013, and 2014 in every plot.

| Year | Crop | Sowing date | Formulated product | Dose (L ha$^{-1}$) | Treatment date |
|---|---|---|---|---|---|
| 2012 | Maize | 11/05/2012 | Lumax (S-Metolachlor + terbuthylazine + mesotrione) | 3.5 | 11/05/2012 |
| 2013 | Maize | 15/06/2013 | Lumax (S-Metolachlor + terbuthylazine + mesotrione) | 3.6 | 15/06/2013 |
| 2014 | Sorghum | 22/05/2014 | Primagram gold (S-Metolachlor + terbuthylazine) | 2.8 | 06/06/2014 |

### *6.2.5 Determination of Herbicides Concentration*

The procedures used derived from previous studies (Barchanska et al., 2012). To ascertain herbicide concentration, soil samples (20 g, on dry weight basis) were placed in dark bottles (n = 3) and 50 mL of methanol-0.1 M HCl (9-1, v/v) was added. The bottles were shaken for an hour at 200 rpm at room temperature (about 18 °C). Mixtures were then centrifuged for 15 min at 3,200 rpm (Heraeus Christ Labofuge GL, Germany) and upernatant filtered at 0.45 mm pore size. The filtrate was then evaporated at 38 °C using an IKA® RV 8 rotary evaporator (IKA® , Werke GmbH & Co.KG, Staufen, Germany) and the residue was dissolved in 50mL of 0.1 M HCl. Subsequently, the extraction was conducted using a TELOS *neo*™ PRP polymeric SPE column (60 mg, Kinesis) and a J.T. Baker SPE-12G glass column processor at a flow rate of 15mL min$^{-1}$. After extraction, the analytes were eluted with 5ml of methanol without the vacuum and methanol aliquots were reduced to 50 ml with the use of a nitrogen gas stream at 45 °C.

To define the analyte recoveries, soil without analytes (blank) was spiked with herbicides at a concentration of 0.4, 1 and 2.2 µg mL$^{-1}$. Then, the extraction was the same as reported above. The recoveries (n = 6) were 73 ± 1.9% for TER-D, 75 ± 2.3% for OA, 94 ± 2.7% for TER-2H, 98 ± 1.3% for TER, and 99 ± 0.8% for S-MET.

The analyses were performed by LC-MS using a 1100 Series Agilent Technologies system (CA, US), equipped with binary pump, diode array detector, and MSD SL Trap mass spectrometer with ESI source. A Eurospher II (Knauer, Berlin, Germany) column C18 P with TMS endcapping, 150 × 4.6mm i.d., 3 µm, 110 Å was used to analyze the samples, the mobile phase consisted of 0.6% formic acid in water (solvent A) and methanol (solvent B). The program of gradient elution started with 40% of solvent B, constant for 3min, followed by a linear increase of solvent B from 40% to 80% from 3 to 10 minutes, constant for 3min, followed by a linear increase of solvent B from 80% to 100% from 13 to 18 min. Initial conditions were re-established in 5 min and re-equilibration time was 2 min. The flow rate was 0.6 mL min$^{-1}$. A 10 mL sample volume was manually injected each time. Retention times were 2.7 (±0.05), 11.0 (±0.14), 13.7 (±0.09), 14.5 (±0.11), and 18.4 (±0.18), min for TER-2H, TER-D, TER, S-MET, and OA, respectively. The limit of detection (LOD), 3:1 signal-to-noise ratio, was 14 and 20 ng kg$^{-1}$ for TER (and its metabolites) and S-MET (and its metabolites), respectively. The limit of quantification (LOQ), 10:1 signal-to-noise ratio, was 68 ng kg$^{-1}$ for TER and S-MET (and their metabolites).

### *6.2.6 Statistical analysis*

Herbicide dissipation was evaluated by interpolating the field data of soil concentration (mg kg$^{-1}$) with a model of first order dissipation defined by a single parameter (k):

$$C_t = C_0 \times e^{-kt} \qquad (6.1)$$

where $C_0$ and $k$ denote $y$-intercept values and the slope of the dissipation lines, respectively, $C_t$ is herbicide concentration in the soil, and $t$ is the post-application time in days. Hence, their half-lives (DT50) were calculated from the equation:

$$DT50 = ln\ 2/k \qquad (6.2)$$

The metabolites are gradually formed by the herbicide, they reach a peak and then undergo a decrease. The occurrence of a metabolite ($M$) in time ($t$), when $M$ is being formed by the decay of the parental molecule ($P$) at reaction rate k and is itself decaying at reaction rate $k_0$, can be described by the two exponential model (Otto et al., 1997):

$$M = (P_0 - P_0 \times e^{-t \times k}) \times (e^{-t \times k'}) \qquad (6.3)$$

where $P_0$ is the concentration of parent molecule (TER) transformable into metabolite (TER-D). $P_0$ would coincide with the initial parent molecule concentration in the soil only if the herbicide disappearance was attributable solely to its transformation into that metabolite.

The time of maximum occurrence of TER-D, $t(M_{max})$, was calculated with (Otto et al., 1997):

$$t(M_{max}) = 1/k \times [ln(1/k + 1/k') - ln(1/k)] \qquad (4)$$

Model fit was performed by using non-linear estimation module of TIBCO Statistica (TIBCO Software Inc.), DT50 values were compared across treatments by using heteroscedastic t-tests (Motulsky and Christopoulos, 2003).

## 6.3 Results and discussion

### *6.3.1 Herbicide degradation*

The specific purposes of this study were to point out the role of SA in the dissipation of TER and S-MET. The results of dissipation studies in IF, SA, and IF+SA plots are plotted in Fig. 6.3 for TER and TER-D and in Fig. 6.4 for S-MET. The data are presented as percentage of each compound dissipated from the initial concentration. Estimated parameters for the fitting curves of TER, S-

MET and the metabolic product TER-D in tested soils with different fertilization are reported in Table 6.5.

Table 6.5 Estimated parameters for the fitting curves of TER, S-MET and the metabolic product TER-D in tested soils with different fertilization.

| Herbicides | Amended soil | $R^2$ | k |
|---|---|---|---|
| TER | IF | 0.98 | 0.102±0.0192 |
|  | SA | 0.99 | 0.048±0.0051 |
|  | IF+SA | 0.99 | 0.053±0.0071 |
| S-MET | IF | 0.96 | 0.083±0.0207 |
|  | SA | 0.99 | 0.066±0.0035 |
|  | IF+SA | 0.97 | 0.051±0.0098 |
| **Herbicides** | **Amended soil** | $R^2$ | **k'** |
| TER-D | IF | 0.50 | 0.248±0.0622 |
|  | SA | 0.93 | 0.095±0.0103 |
|  | IF+SA | 0.97 | 0.128±0.0098 |

TER and S-MET dissipation followed a pseudo first-order kinetics, with an R2 value ranging from 0.96 for S-MET in IF-amended soil to 0.99 for S-MET in SA-amended soil. The DT50 of TER and S-MET in IF, SA, and IF+SA plots are summarized in Table 6.6. The DT50 of TER in soil amended with IF (7 days) was significantly lower than the DT50 in soil amended with IF+SA and SA (13 and 14 days, respectively). Similarly, for S-MET, the lowest DT50 was observed in soil amended with IF (8 days) but, unlike TER, its degradation was slightly faster in the soil amended only with SA (11 days) than in the soil amended with IF+SA (14 days).

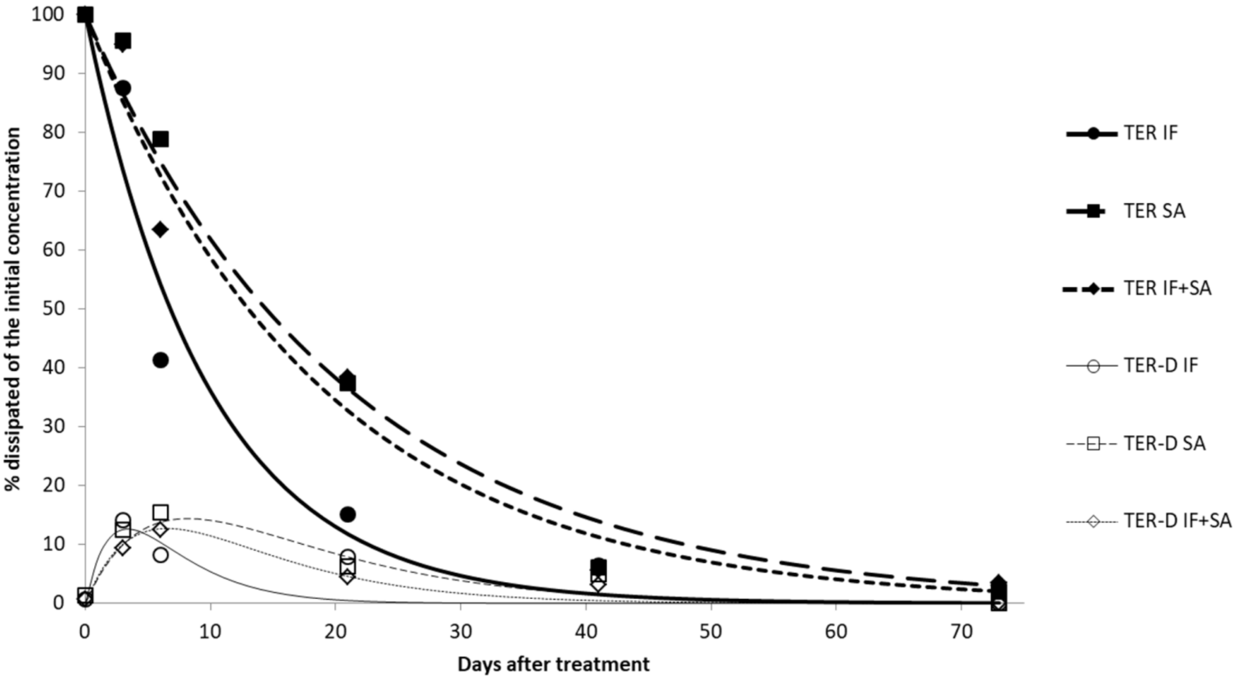

Fig. 6.3 Degradation of terbuthylazine (TER) and its metabolic product (TER-D) in soils with different fertilization expressed as percentage of each compound dissipated from the initial concentration ($C/C_0\%$). Lines represent the fitting curves and symbols represent the observed values.

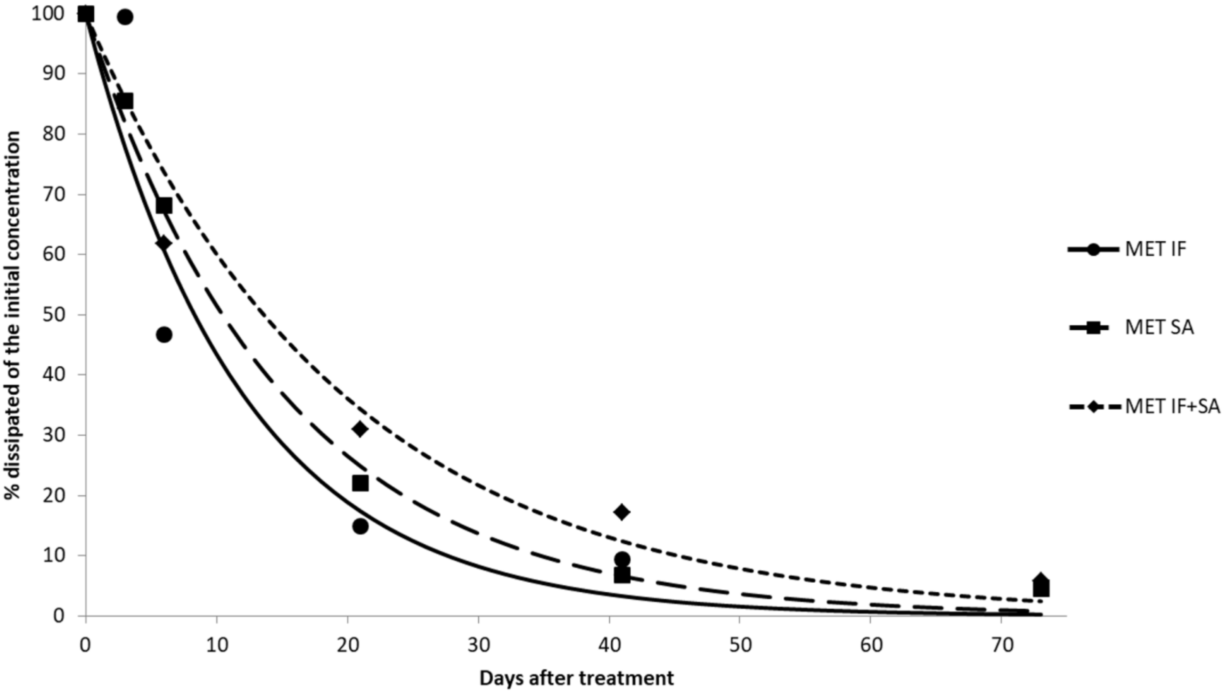

Fig. 6.4 Degradation of S-metolachlor (S-MET) in soils with different fertilization expressed as percentage of each compound dissipated from the initial concentration ($C/C_0\%$). Lines represent the fitting curves and symbols represent the observed values.

In this study, the OC content in the soil amended with SA is significantly higher than in the IF-amended soil (7.6 and 6.4, respectively). Soil organic matter is a key factor that controls the fate of organic substances in soil through adsorption, solubility and moisture interactions (Graber et al., 2001; Sánchez-Camazano et al., 1997; Worrall et al., 2001). Some authors (Cox et al., 1997; Felsot and Shelton., 1993; Gan et al., 1998; Guo et al., 1993; Topp et al., 1996) stated that the addition of SA amendment can promote biodegradation by enhancing microbial populations and activities. On the other hand, we observed a reduction in herbicide mineralization after organic amendment to soil as has also been reported by other studies (Albarrán et al., 2004; Delgado-Moreno and Peña, 2009; Fenoll et al., 2014b; López-Piñeiro et al., 2011). These authors attributed the slower degradation to reduced herbicide availability to microorganisms because of the sorption to the organic amendment or to a toxic effect of the organic amendment on the soil microbial population. Singh et al. (2001) found that sorption of TER and S-MET is predominantly controlled by the soil organic matter content. Furthermore, Wang et al. (2010) found that the addition of organic amendments to a soil with low native organic matter, like the soil tested in this study, may enhance soil sorption of TER, thus reducing its availability in soil solution for microbial decomposition. Furthermore, although organic amendments have generally been shown to increase the amount and activity of soil microbial biomass, soil microorganisms in SA- and IF+SA-amended soil may have preferentially used the organic matter of SA as a carbon and/or nitrogen source instead of TER and S-MET. The negative effects of soil organic matter on the degradation of other nonpolar pesticides have been documented by a few studies (Dolaptsoglou et al., 2007; Walker, 1991). In general, the DT50 values of TER and S-MET found in our study were shorter than typical values reported in the literature for field studies (Barra Caracciolo et al., 2005; Long et al., 2014; Stipičević et al., 2015; Wu et al., 2011). It should be noted that the soil tested had been treated with TER and S-MET repeatedly over the past two decades, with consecutive treatments in the previous four years. It is hence reasonable to expect some adaptation of the indigenous soil microorganisms for TER and S-MET degradation. After SA addition, however, the exogenous microorganisms added through SA, and not yet adapted for such a degradation process, could have prevailed over the indigenous soil microflora limiting the mineralization of the herbicides. It is known that one of the factors that seem to play an important role in the rapid dissipation of s-triazine herbicides is the previous herbicide use history. (Krutz et al., 2010) found that the DT50 of atrazine in soil with s-triazine application history is lower than in soil with no history of s-triazine use due to an adaptation of bacteria that enables rapid pesticide degradation (Krutz et al., 2008). Likewise, repeated S-MET applications may result in its enhanced degradation because of microbial acclimation, as found by Sanyal and Kulshrestha (1999).

Table 6.6 Values of DT50 of TER and S-MET in tested soils.

| Herbicides | Amended soil | DT50 | 95% Confidence interval | | Difference[1] | | p-value[2] |
|---|---|---|---|---|---|---|---|
| TER | IF | 6.80 | 3.51 | 10.08 | IF − SA | -7.63 | 0.002 |
| | SA | 14.43 | 10.51 | 18.35 | SA − IF+SA | 1.41 | 0.278 |
| | IF+SA | 13.02 | 8.54 | 17.50 | IF − IF+SA | -6.22 | 0.008 |
| S-MET | IF | 8.32 | 3.00 | 13.64 | IF − SA | -2.14 | 0.171 |
| | SA | 10.46 | 9.04 | 11.88 | SA − IF+SA | -3.15 | 0.133 |
| | IF+SA | 13.61 | 6.88 | 20.35 | IF − IF+SA | -5.29 | 0.072 |

### *6.3.2 Metabolites*

The formation and dissipation of the metabolic product (TER-D) of TER in soils amended with IF, SA, and IF+SA is represented in Fig 6.3. The data are expressed as percentage of the initial TER concentration. TER-D was the only metabolite detected for which it was possible to study the kinetics. The other metabolites were found only on a few sampling dates and their occurrence kinetics were not clear (data not shown). Because of their chemical structure, triazine herbicides and their N-heterocyclic derivates like TER may be metabolized in complex pathways and used as sources of carbon and nitrogen (Barra Caracciolo et al., 2005; Gebendinger and Radosevich, 1999; Kontchou and Gschwind, 1999; Radosevich et al., 1995; Topp et al., 2000). In soil, one of the primary mechanisms of its transformation has been found to be a biotic oxidative N-deethylation with the formation of TER-D (Funari et al., 1998; Klotz et al., 1997; Ostrofsky et al., 2002). TER-D dechlorination may also then occur with the formation of TER-2H (Di Corcia et al., 1999; Guzzella et al., 2003; Klotz et al., 1997).

Referring to Eq. (3), the estimated P0, expressed as percentage of the initial TER concentration in the soil, were 30% for IF- and SA-amended soil and 31% for IF+SA-amended soil. The value of estimated P0 confirms that TERD is an important product of TER degradation in soil since, according to the model adopted, at least 30% of the TER applied gives rise directly to TER-D.

A small amount (0.50 mg $kg^{-1}$) of TER-D was already present in the samples taken 0 d after treatment for all the treatments. The residual presence of TER-D in the soil samples can be attributed to the above-mentioned herbicide treatments with TER performed in the tested field in previous years. The increase in TER-D concentration in the differently amended soil, and thus the transformation of the applied TER into TER-D, began 3 days after treatment. The decrease in TER concentration in our study was initially accompanied by an increase in TER-D concentration up to the time of maximum occurrence, and then the decay phase of TER-D occurred. The time of maximum occurrence of TER-D, $t(M_{max})$, calculated with Eq. (4), was lower in IF-amended soil (3.7 days) than in IF+SA- and SA-amended soil (6.6 and 6.9 days, respectively). The estimated maximum abundance of TER-D, expressed as percentage of initial TER concentration, was 12.5, 14.4, and 12.7% for IF-, SA-, and IF+SA-amended soil, respectively. The percentage of metabolite detected after 73 days was lower than 0.1% of the parent compound for all the treatments. At the end of the study (73 days), TER-D concentration was slightly higher in the SA-amended soil. The presence of SA had a limited impact on the formation and subsequently the degradation of the metabolic product TER-D. The addition of SA to the soil led to a slightly slower formation of TER-D from TER and to a negligibly higher amount of metabolite measured at the end of the incubation in SA-amended soil.

## 6.4 Conclusion

Addition of organic amendments to the soil is a common and effective practice in agriculture to maintain or improve soil fertility. However, the persistence of pesticides applied in the amended soils might be affected by modifications to soil chemical and microbial conditions after the addition of organic amendments. Our data show a slight increase in TER and S-MET persistence in soil with a history of herbicide use with the addition of SA. We also highlighted that this agronomic practice slightly influences the formation and dissipation of the main metabolite of TER.

Despite soils in the Po Valley treated with TER and SMET for decades showing a low persistence of these herbicides, the risk of water contamination by these compounds is still high. A higher persistence of these compounds in the soil may extend the risk of water contamination instead of reducing it. In areas already affected by a high level of contamination by these herbicides or metabolites, any agronomic practice that could increase the persistence and thus an extended release of these molecules through the soil profile towards waters should be done with particular caution. For example, a good practice in the case of addition of the organic amendment to soil could be to perform the herbicide treatment only on the row, in order to reduce the total amount of herbicide applied to the soil and limit the risks for surface waters and groundwater.

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# Chapter 7. Decyl glucoside surfactant Triton CG-110 does not significantly affect the environmental fate of glyphosate in soil at environmentally relevant concentrations

**Abstract**

Glyphosate is the most used herbicide worldwide, and its impact on the environment is increasingly under scrutiny. Glyphosate is applied as formulated products that contain co-formulants, among which are the surfactants. The aim of this study was to investigate whether the presence of an alkyl polyglucoside-based surfactant, Triton CG-110 can affect adsorption, leaching, and mineralization of glyphosate in soil. The adsorption and mineralization experiments were conducted in two soils with different textures (sandy and clay) and in washed sand, with and without Triton CG-110. Glyphosate and surfactant mixtures were applied at realistic field rates and proportions. Because of ponding and very scarce leaching from the real soil, the leaching experiments were conducted only with washed sand. The results showed a moderate reduction in glyphosate adsorption only in washed sand and in sandy soil, whereas adsorption was unaffected in clay soil. The presence of Triton CG-110 did not significantly affect glyphosate leaching in washed sand or glyphosate mineralization in any of the tested soils. The results show that Triton CG-110 is unlikely to affect the environmental fate of glyphosate in the soil at environmentally relevant concentrations.

Keywords: herbicide; alkyl polyglucosides; adsorption; leaching; mineralization.

## 7.1 Introduction

Glyphosate (N-(phosphonomethyl)glycine) is the most common herbicide worldwide with an estimated use of 826 million kg in 2014, and is likely to remain so for the foreseeable future (Benbrook, 2016). Glyphosate is a foliar herbicide and is used for selective weed control on transgenic glyphosate-resistant crops, for pre-harvest desiccation of cereals, as well as for total weed control in agricultural fields and non-crop areas. Glyphosate is a small molecule with three polar functional groups (carboxyl, amino and phosphonate groups), and behaves as an amphoteric molecule (Borggaard and Gimsing, 2008; Mazzetti, 2015). Glyphosate is highly polar and well soluble in water (10.5 g $L^{-1}$ at 20 °C), and is strongly sorbed to soil components, particularly iron- and aluminium oxides (Gimsing et al., 2004a; Sidoli et al., 2016). Although glyphosate is not intentionally applied directly to the soil, a significant portion of the herbicide may reach the soil surface during pre-seeding or early-season applications. Adsorption, degradation and leaching of glyphosate exhibit considerable variation depending on soil composition and properties as well as on climatic conditions, and consequently, its persistence in the environment and its potential for reaching water bodies vary extensively (de Jonge et al., 2001; Gimsing et al., 2007, 2004a, 2004b; Laitinen et al., 2006; Sørensen et al., 2006). Given the widespread use of glyphosate, it is critical to investigate its environmental fate, including sorption, degradation and leachability. Several studies concerning the environmental fate of glyphosate have been published in previous years, some conducted with the acid form of glyphosate and others with formulated products.

Glyphosate and other pesticides are not introduced into the environment as pure active ingredients, but as formulated products that also contain co-formulant chemicals, also called adjuvants, as well as various other additives. The co-formulants are included in the formulated products to improve glyphosate performance in different ways, modifying spray droplet formation, droplet impaction and spray retention on the leaf surface, spreading, coverage and deposit formation (Stock and Briggs, 2000). The most commonly used co-formulants are the surfactants, which play an important role in the retention and penetration of glyphosate through the plant cuticle, enhancing its uptake, translocation and field performance (Travlos et al., 2017), and it is not inconceivable that surfactants may also alter the environmental fate of glyphosate. The identities of the co-formulants (declared as inert) are generally confidential and protected according to art. 63 of Regulation (EC) No. 1107/2009 ("Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC," 2009). Furthermore, the co-formulants added to glyphosate in formulations differ between countries and manufacturers and can vary over time.

Consequently, scientists often do not have access to compositional information and description of the structural characteristics of the co-formulants in products.

This lack of information is problematic, since despite the common meaning of the word “inert”, inert ingredients are added in order to modify the behaviour of the herbicide and may have biological and chemical activity of their own. In a recent review, Mesnage et al. (2019) presented an overview of the most common surfactants contained as co-formulants in glyphosate-based herbicides, obtainable from material safety data sheets (MSDS) or determined from their previous experimental investigations of pesticide composition (Mesnage et al., 2019). Among them, the most common in glyphosate-based herbicide formulations over the last 40 years have been ethoxylated amines, a class of surfactants with the acronym POEA (PolyOxyEthylene Amine) (European Food Safety Authority (EFSA), 2015). The major surfactant from the POEA family is polyethoxylated tallow amine. It is well-known that the glyphosate-based formulations containing polyethoxylated tallow amine are more toxic than glyphosate alone, through both in vitro (Clair et al., 2012; Defarge et al., 2016) and in vivo studies (Marc et al., 2005; Tsui and Chu, 2003). Overall, the European Food Safety Authority stated that “compared to glyphosate, a higher toxicity of the POE-tallow amine was observed on all endpoints investigated” (European Food Safety Authority EFSA, 2015). Consequently, glyphosate-based formulations containing polyethoxylated tallow amine have been banned in Europe since July 2016 and have been replaced by other, presumably less toxic, but also less studied compounds.

The effects of these replacement co-formulants on the environmental fate of glyphosate in the soil are mostly unknown. Some glyphosate-based herbicides recently introduced in the European market contain a blend of alkyl polyglucoside (CAS number 68515-73-1) and nitroryl (CAS number 226563-63-9) (Mesnage et al., 2019). Alkyl polyglucosides (APG) are a class of non-ionic surfactants made up of a hydrophilic sugar moiety coupled to a hydrophobic alkyl chain. They are produced from renewable resources such as fatty alcohols and glucose derived from starch. A complex mixture of alkyl mono-, di-, tri-, and oligoglucosides are produced in industrial processes (von Rybinski and Hill, 1998). APG are used in cosmetic products, for industrial cleaners and in agricultural applications. They are generally considered safe and environmentally-friendly because of their low toxicity and high biodegradability (Iglauer et al., 2009; Zhang et al., 2012). APG appear to increase the water solubility and bioavailability of hydrophobic organic contaminants in the soil. For this reason, the use of APG in environmental applications has raised great interest. For example, they have been found to enhance the phytoremediation of soil contaminated by polycyclic aromatic hydrocarbons (PAHs) (Liu et al., 2013a, 2013b), improve the washing of weathered crude oil contaminated soils (Han et al., 2009), increase the biodegradation of diesel oil (Kaczorek et al., 2015), and stimulate the organic matter degradation and composting process of dairy manure (Zhang et al., 2011). Recently, Cederlund and Börjesson (2016) investigated the influence of an APG surfactant, applied as a foaming agent during hot water weed control, on the mobility of some organic contaminants in soil. The results of that study indicated that both adsorption and leaching of glyphosate in washed sand were affected at higher surfactant concentration (Cederlund and Börjesson, 2016). This suggests that APG may affect the environmental fate of glyphosate to some extent and that it could be interesting to study these potential effects also in natural soil where interactions of surfactant and contaminant with organic matter and other soil components can occur, and using concentrations that would be present in soil after applying a glyphosate-formulation containing APG-surfactants.

The aim of this study was thus to investigate whether the presence of an APG-based surfactant, Triton CG-110, has the potential to affect adsorption, leaching and mineralization of glyphosate in the soil. The working hypothesis was that the effects of Triton CG-110, although potentially significant at higher concentrations, would be slight or undetectable when applied at realistic field rates. We tested this hypothesis by studying the adsorption, leaching and mineralization behaviour of glyphosate in two soils with different textures and in washed sand with and without the addition of Triton CG-110.

## 7.2 Materials and Methods

### *7.2.1 Chemicals*

Certified glyphosate (N-(phosphonomethyl)glycine, (CAS number 1071-83-6), 98.6%), was obtained from Dr. Ehrenstorfer, Augsburg, Germany. Radiolabelled glyphosate [P-methylene-$^{14}$C], > 95%, 11.103 MBq mg$^{-1}$ was provided by the Institute of Isotopes Co. Ltd., Budapest, Hungary. Ultima Gold™ (Perkin Elmer Inc., Waltham, MA, USA) was used for the scintillation counting analysis. Calcium chloride (≥ 95%), hydrochloric acid (≥ 37%), and Triton CG-110 (CAS number 68515-73-1) were purchased from Sigma-Aldrich Sweden AB (Stockholm, Sweden). Triton CG-110 is a non-ionic surfactant and it is a mixture of 58.0-62.0% D-glucopyranose, oligomeric, decyl octyl glycoside and 38.0-42.0% water. Sodium hydroxide (99%) was obtained from VWR Chemicals (Leaven, Belgium) and barium chloride dihydrate (99%) was from Merck KGaA (Darmstadt, Germany).

### *7.2.2 Soil sampling and processing*

The soil samples were collected in October 2018 from a field located just north of Ultuna (Uppsala, Sweden; 59° 49’ N, 17° 39’ E). The texture of the soil is predominantly clayey but parts of the field have been covered by sand washed out from the nearby Uppsala esker. The field has been managed as grassland since 2009. The grass is regularly mowed and used as a substrate for biogas production. No pesticides have been applied since 2009, but 2000 kg ha$^{-1}$ of Vinasse (a by-product of the sugar or ethanol industry used as fertilizer) were applied in 2018. For both soil types (sandy soil, SS; clay soil, CS), a bucket was sampled from the upper layer (5-15 cm below surface). After sieving, the Ø < 4 mm fraction was homogenised and stored at +2 °C in plastic bags until the start of the experiment, except for a portion of one kilogram of each soil that was air dried, sieved at Ø < 1 mm, and stored at room temperature for the leaching experiments. In a natural soil, both surfactants and contaminants can interact with soil organic matter, which could potentially influence their adsorption, mineralization and leaching behaviour (Cederlund and Börjesson, 2016). To disentangle the effect of organic matter on the behaviour of glyphosate, washed sand (WS) (Ø = 0.5 ± 0.1 mm; Rådasand AB (Lidköping, Sweden)) was also used in the experiments.

Moisture content was determined for SS and CS by drying the samples at 105 °C for 10 hours; subsequently, loss of ignition was determined by heating the soil at 550 °C for 5 hours. The water holding capacity (WHC) was determined for WS, SS, and CS as the moisture content after saturation of 30 g of sample with distilled water for 10 hours followed by 5 hours of free drainage. The pH was measured both in distilled water and in 0.01 M $CaCl_2$ solution, in a 1:2 slurry of soil and liquid (w/v) after shaking and stabilisation for 1 h. Soil texture of SS and CS, and chemical and physical properties of the three soils (Table 7.1), were determined at the Soil and Plant and Soil Physics laboratories of the Department of Soil and Environment of the Swedish University of Agricultural Sciences (SLU, Uppsala, Sweden). The natural soils (SS and CS) had different physical texture: the dominant particle fractions in SS soil were medium and fine sand, while the CS soil was dominated by clay.

Table 7.1 Chemical and physical properties of SS, CS, and WS soil.

| | SS | CS | WS |
|---|---|---|---|
| Clay (%) (Ø < 0.002 mm) | 14.8 | 47.6 | n.d. |
| Silt (%) (0.002 – 0.06 mm) | 14.1 | 22.3 | n.d. |
| Sand (%) (0.06 – 2 mm) | 70.5 | 28.4 | n.d. |
| Gravel (%) (2 – 20 mm) | 0.6 | 1.8 | n.d. |
| Water content (%) | 14.01 | 17.20 | n.d. |
| Loss on ignition (%) | 3.9 | 4.9 | n.d. |
| WHC (%) | 29.94 | 42.05 | 18.89 |
| pH in water | 6.43 | 8.14 | 6.99 |

| | | | |
|---|---|---|---|
| pH in $CaCl_2$ 0.01 M | 5.75 | 7.53 | 6.81 |
| HCl-P (mg kg$^{-1}$) | 580.4 | 600.6 | 146.0 |
| AL-P (mg kg$^{-1}$) | 114.2 | 130.9 | 17.4 |
| HCl-K (mg kg$^{-1}$) | 1630.4 | 6479.9 | 426.2 |
| AL-K (mg kg$^{-1}$) | 64.5 | 341.6 | 7.5 |
| Tot. C (%) | 1.63 | 1.92 | <0.02 |
| Tot. N (%) | 0.13 | 0.18 | <0.02 |

n.d. not determined

### *7.2.3 Surface tension*

Surface tension values of 0–1% Triton CG-110 solution were measured on a Krüss K6 manual tensiometer using the ring tear-off method. The measured values of interface tension were corrected according to Harkins and Jordan (Harkins and Jordan, 1930). The critical micelle concentration (CMC) was determined as the inflection point in a plot of the surface tension against the log Triton CG-110 concentration, i.e. the point where lowest surface tension is reached (Fig. 7.1).

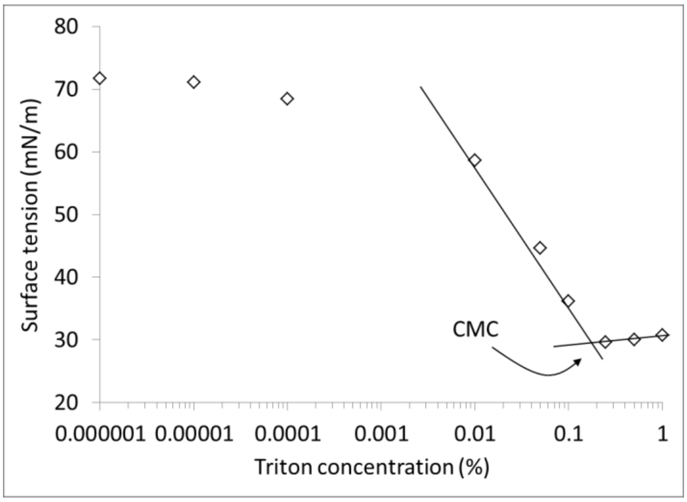

Fig. 7.1 Effect of alkyl polyglucoside concentration (log scale) on surface tension (n = 3) ± standard deviation. The arrow indicates the critical micelle concentration (CMC).

### *7.2.4 Adsorption of glyphosate in the soil*

#### *7.2.4.1 Screening test*

A screening test was performed with fixed glyphosate content and ten different concentrations of Triton CG-110 covering a wide range in order to investigate if there is an effect on the adsorption of glyphosate to WS, SS and CS, and from which concentration of Triton CG-110 this effect begins to be relevant. The glyphosate content was set to 5 µg g$^{-1}$ of soil dry weight. This value was chosen to be representative of how the herbicide would be used in the field, assuming an application of 1.3 kg a.i. ha$^{-1}$ being mixed into the top 2 cm of soil and a bulk density of 1.3 g cm$^{-3}$. The concentrations of Triton CG-110 were 0, 0.000001, 0.00001, 0.0001, 0.01, 0.05, 0.1, 0.25, 0.5 and 1%, ranging from $5.7 \cdot 10^{-06}$-fold lower to 5.7-fold higher than the CMC, and were selected to simulate two different situations (as outlined below), plus a range of intermediate levels.

The first case takes into consideration the proportion between glyphosate and surfactant in the commercial formulation; the second case considers the concentration of the surfactant in the spraying solution to be applied to the field. According to the available MSDS, the glyphosate-based formulations that include APG as an adjuvant can have variable content of this surfactant, usually 20% or less. The formulation Roundup Platinum, contains 480 g L$^{-1}$ of glyphosate and 20% (w/v) surfactant, and has a density of 1342.6 g L$^{-1}$. If Roundup Platinum is applied at a glyphosate dose equal to 5 µg g$^{-1}$ of soil dry weight, the amount of surfactant applied to the soil will be 2.80 µg g$^{-1}$ of soil dry weight, which corresponds to 0.000007% in the final volume of $CaCl_2$ in the adsorption experiment. This first situation is represented by the lower concentrations of Triton CG-110 used in our experiments.

However, in adsorption experiments, the soil is diluted to a slurry, and consequently, the concentration of surfactant is diluted (along with the glyphosate concentration) to levels that are below what would be expected in the field directly after application of a glyphosate herbicide. Therefore, considering the importance of surfactant concentration (rather than amount) for its behaviour, we also wanted to simulate this second case.

According to directions for use of the most common glyphosate-based formulations, a product containing 480 g L$^{-1}$ of glyphosate and 20% of Triton CG-110 is normally diluted with water to a final spray volume of 100 to 400 L ha$^{-1}$ when applied in the field. Assuming an application rate of 1.3 kg a.i ha$^{-1}$, as in the previous example, the resulting concentration of the surfactant in the spraying solution varies from 1.8 to 7.3 g L$^{-1}$ with spray volumes of 100-400 L ha$^{-1}$, which would correspond to between 0.34 and 1.34% in the final volume of $CaCl_2$ in the adsorption experiment. This second situation is represented by the higher concentrations of Triton CG-110.

The adsorption of glyphosate was measured at 20 °C in a batch equilibrium system. The screening tests were performed for WS, SS and CS soils. An aliquot of soil, corresponding to 1 g of soil dry weight, was weighed into 50-mL polypropylene tubes and adjusted with 0.01 M $CaCl_2$ to reach the appropriate soil-solution ratio. This was 1:40 for all samples. The samples were shaken for 24 h (20 °C, 200 revolutions min$^{-1}$). After that, the slurry was spiked with a mixture of $^{14}C$-labelled and unlabelled glyphosate in 0.01 M $CaCl_2$ to give a final concentration of 5 µg g$^{-1}$ of soil dry weight and an activity of 2000 DPM ($3.333 \times 10^{-5}$ MBq) per sample, together with Triton CG-110 in 0.01

M $CaCl_2$ to reach the concentrations indicated above. There were three replicate tubes of each concentration. The samples were shaken for 24 h (20 °C, 200 revolutions $min^{-1}$) and the pH of the samples with 0 and 1% of Triton CG-110 was measured. The pH after equilibrium was reached was 6.64, 5.77 and 7.29 in 0% Triton CG-110 samples and 5.01, 5.46 and 6.34 in 1% Triton CG-110 samples for WS, SS and CS, respectively. After that, the tubes were centrifuged (3000 revolutions $min^{-1}$ for 20 min). Aliquots of supernatant (1 mL) were transferred to scintillation vials and 4 mL of scintillation cocktail Ultima Gold™ (PerkinElmer, Waltham, MA, USA) were added directly before the measurement of scintillation. The $^{14}C$ activity was measured on a Beckman LS 6000TA liquid scintillation counter (Beckman Counter Inc., Fullerton, CA). Controls without herbicide were measured for all samples to exclude the level of background radioactivity.

#### *7.2.4.2 Adsorption Isotherms*

To determine adsorption isotherms, adsorption of glyphosate to the soil was measured for five different concentrations (1, 5, 10, 50 and 100 µg $g^{-1}$ of soil dry weight) in three replicate samples. Two approaches were adopted for the determination of the adsorption isotherms. In the first, three fixed Triton CG-110 concentrations (0, 0.00001 and 0.5% in the final volume of $CaCl_2$) were selected based on the above considerations and the results of the screening test. In the second one, the amounts of Triton CG-110 applied to the soil were proportional to the glyphosate amounts based on three hypothetical herbicide formulations containing 0, 15 and 30% Triton CG-110, respectively (w/v). In relation to the glyphosate concentration reported above, in this second case the concentrations of the surfactant in solution were 0.000001, 0.000005, 0.00001, 0.00005 and 0.0001% for the samples with 15% Triton CG-110 in the formulation, and 0.000002, 0.00001, 0.00002, 0.0001 and 0.0002% for the samples with 30% Triton CG-110. The procedure used to measure the adsorption of glyphosate was the same as that described in section 2.4.1.

Adsorption data were fitted by nonlinear regression to the Freundlich adsorption isotherm using Statistica 13 (TIBCO Software Inc.):

$$Q_e = K_f C_e^{(1/n)} \qquad (7.1)$$

where $Q_e$ (µg $g^{-1}$) is the adsorbed amount, $C_e$ (µg $mL^{-1}$) is the concentration in the aqueous phase, $K_f$ [$\mu g^{1-1/n}$ $(mL)^{1/n}$ $g^{-1}$] is the Freundlich adsorption coefficient, and *1/n* (–) the measure of nonlinearity.

#### *7.2.5 Leaching*

A miniature column system was constructed using plastic syringes (inner diameter = 2.55 cm; 10 cm height) with round filter paper in both ends. These smaller columns were chosen in order to reduce the amount of soil in the columns since glyphosate tends to adsorb strongly on the soil and its mobility is generally low. Previous studies conducted with larger columns (inner diameter = 4 cm; 37 cm height) packed with washed sand showed low leaching of glyphosate (less than 2.5% of applied) (Cederlund et al., 2017; Cederlund and Börjesson, 2016). Dry soil was put into the columns in small portions and the columns were packed by gently knocking them on a soft rubber plate. The columns were connected to a peristaltic pump, IPC 12 (Labinett AB, Gothenburg, Sweden) and before the start of each leaching experiment 0.01 M $CaCl_2$ was pumped from the bottom to the top to saturate the system.

To test the feasibility of the leaching experiment with real soil a preliminary test was initially performed. WS, SS and CS soils filled columns were contaminated with labelled and unlabelled glyphosate applied evenly on the surface of the columns. The columns were leached with 0.01 M $CaCl_2$ at a flow rate of about 0.2 mL min$^{-1}$ (corresponding to a rainfall intensity of 23.5 mm h$^{-1}$). The CS-columns ponded soon after the start of the experiment, which points to ineffective macropores transport so that no data could be retrieved with CS soil. No glyphosate was detected in the leachate from the SS-columns within the experimental duration of three pore volumes (PV). Therefore, the leaching experiment was only conducted with the WS.

For the leaching experiment with WS, labelled (3700 Bq) mixed with unlabelled glyphosate and Triton CG-110 were applied evenly over the surface of the columns. The total amount of glyphosate applied per column was 0.092 mg, which corresponds to an application rate of 1.8 kg ha$^{-1}$. The amounts of Triton CG-110 applied to the soil were proportional to the glyphosate amount based on three hypothetical glyphosate-based formulations containing 0, 15 and 30% Triton CG-110, respectively (w/v). Three replicate columns were leached for every Triton CG-110 level. The columns were leached with 0.01 M $CaCl_2$ at 0.2 mL min$^{-1}$ flow rate for 8 hours. Portions of leachate were collected every 40 min in scintillation vials, the vials were weighed, and 4 mL of scintillation cocktail (Ultima Gold™) were added to 1 mL of sample. The $^{14}C$ activity was measured as described above for the adsorption experiments.

The cumulative leached fraction of glyphosate (leached/total amount added) was plotted against the total volume eluted. These cumulative leaching curves were fitted to the Gompertz equation using Statistica 13 (TIBCO Software Inc.):

$$y = ae^{-e^{-\left(\frac{x-x_0}{b}\right)}} \qquad (7.2)$$

where $y$ is the cumulative amount leached (% of added) after $x$ mm of leachate has leached through the column, $a$ is the upper asymptote of the curve, i.e. the cumulatively leached fraction at $x_{max}$, $x_0$ is the inflection point of the curve (where rate of leaching is the highest), and $b$ is a parameter that describes the slope of the curve. Using the derived parameters, the cumulative leaching after 75 mm of leachate was calculated for all the columns and used for comparisons between treatments and compounds.

### *7.2.6 Mineralization*

For mineralization experiment, SS and CS samples were sieved (Ø < 4 mm) and stored at +2 °C until used.

Triplicate soil samples corresponding to 20 g of soil dry weight (20 g for WS, 23.6 g for SS and 24.2 g for CS) were weighed into small plastic jars. Unlabelled glyphosate, $^{14}$C-labelled glyphosate and Triton CG-110 dissolved in deionized water, was mixed into each jar for 30 s with a spatula to obtain final radioactivity and total glyphosate content of about 1600 Bq and 5 µg $g^{-1}$ dry weight. The amounts of Triton CG-110 applied to the soil were proportional to the glyphosate amount based on three hypothetical glyphosate-based formulations containing 0, 15 and 30% Triton CG-110, respectively (w/v). Distilled water was added to adjust the water content to 60% of the WHC of the soils. The water content was kept at this level for the entire duration of the experiment. The plastic jars were installed in airtight glass jars together with two scintillation vials containing NaOH (0.2 M; 4 ml) to trap carbon dioxide. The glass jars were incubated in the dark at 20 °C and the base traps were changed regularly; one was used for determination of the release of radioactivity from the soil through the mineralization of glyphosate ($^{14}$C-$CO_2$) and the other for assessment of the basal respiration (total-$CO_2$). The $^{14}$C activity was measured by liquid scintillation after 1 mL of NaOH had been mixed with 4 mL of Ultima Gold™. The $^{14}$C liberated was corrected for background radiation in controls without herbicide. The total amount of $CO_2$ that had been released from the soil was determined by titration. Aliquots of NaOH (2 ml) were mixed with $BaCl_2$ (0.1 M; 4 ml) and titrated with HCl (0.1 M) using a TIM 850 Titration Manager (Radiometer Analytical, Copenhagen, Denmark). The basal respiration was calculated by linear regression of accumulated $CO_2$ data versus time.

$^{14}$C-$CO_2$ evolution in SS and CS soils was fitted to the first order kinetics equation using Statistica 13 (TIBCO Software Inc.):

$$Y = a(1 - e^{-kx}) \qquad (7.3)$$

where $a$ is the maximum $^{14}$C mineralized (expressed in % of applied herbicide), $Y$ is the amount mineralized at time $x$, $k$ is the first-order rate constant ($day^{-1}$), and $x$ is time (days), using non-linear regression.

The mineralization of glyphosate in WS was described by a logistic model instead of the first-order model as above using Statistica 13 (TIBCO Software Inc.):

$$Y = \frac{a}{(1+(x/x_0)^b)} \qquad (7.4)$$

where $a$ is the maximum $^{14}C$ mineralized (expressed in % of applied herbicide), $Y$ is the amount mineralized at time $x$, $x_0$ (days) is the time at which the cumulated glyphosate mineralization reaches half the final cumulated glyphosate mineralization, $b$ is a shape coefficient, and $x$ is time (days), using non-linear regression.

### *7.2.7 Statistical analysis*

The data were analyzed using Statistica 13 (TIBCO Software Inc.).

For the adsorption experiment, the best-fit $K_f$ values were compared across the treatments by using heteroscedastic t-test with a significance level of $p < 0.05$ (Motulsky and Christopoulos, 2003). This test can be used to compare one parameter of the same model applied to different data sets, when the nonlinear regression program cannot fit models with shared parameters. The t ratio is calculated as:

$$t = \frac{\left| logK_{f_{treatment\ A}} - logK_{f_{treatment\ B}} \right|}{\sqrt{SE^2{}_{treatment\ A} + SE^2{}_{treatment\ B}}} \qquad (7.5)$$

For the leaching experiment, differences between treatments were tested for statistical significance using a one-way ANOVA with a significance level of $p < 0.05$.

For the mineralization experiment, each soil type was treated separately, and the differences between treatments were tested for statistical significance using a one-way ANOVA with a significance level of $p < 0.05$.

## 7.3 Results and discussion

### *7.3.1 CMC of the Triton CG-110 extract*

Pesticide-surfactant interactions in the soil are very complex and depend on several parameters, including the adsorption characteristics of the surfactant and pesticide, their solubility, type of soil, and concentration of surfactant in the soil-water system compared with the CMC. The CMC-value of the surfactant is usually the most important parameter that affects the ability of a surfactant to mobilise organic contaminants in soil. Surfactants are surface-active molecules with a polar head group and a non-polar chain. When dissolved in water at low concentration, surfactant molecules exist as monomers that accumulate mainly at the water surface, having little or no effect on solubilisation of hydrophobic organic contaminants. Above the CMC, micelles are formed that are responsible for the capacity of surfactants to solubilise hydrophobic organic compounds (Cao et al., 2008; Cederlund and Börjesson, 2016; Haigh, 1996). For the Triton CG-110 extract examined in this study, the CMC was determined to be about 0.17% (w/v) (Fig. 7.1). However, due to its polar nature, glyphosate is very soluble in water (10.5 g $L^{-1}$, 20 °C), thus it is unlikely that it would be incorporated into the hydrophobic portions of the micelles (Wyrill et al., 1977). No significant effect on the solubility of glyphosate in the presence of an APG-based surfactant at concentrations from 0 to 0.3% was reported in a previous study (Cederlund and Börjesson, 2016).

### *7.3.2 Effects on glyphosate adsorption*

The results of the screening test showed that glyphosate adsorption to WS decreased with increasing surfactant concentration, going from 2.30 µg $g^{-1}$ of glyphosate adsorbed in the soil without the surfactant to 0.26 µg $g^{-1}$ with 1% Triton CG-110 in solution (Fig. 7.2). A similar effect on glyphosate adsorption on washed sand with different concentrations of an APG surfactant was observed in a previous study (Cederlund and Börjesson, 2016). A modest effect on glyphosate adsorption was observed in SS soil, where the amount of glyphosate adsorbed with 1% Triton CG-110 (3.82 µg $g^{-1}$) decreased by 23% compared to the amount without surfactant (5.02 µg $g^{-1}$). In CS, the glyphosate adsorption to soil appeared to be only slightly affected by the Triton CG-110 concentration in the solution (Fig. 7.2). Overall, weaker adsorption of glyphosate was observed on WS compared to SS and CS soils. This is in agreement with the evidence, pointed out in several studies, that glyphosate adsorption is related to the clay content and cation-exchange capacity of the soil (Glass, 1987; Sprankle et al., 1975; Vereecken, 2005).

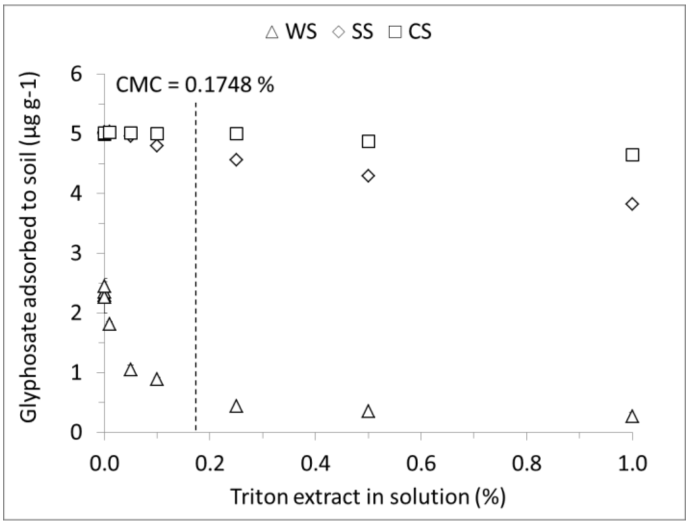

Fig. 7.2 Effect of Triton CG-110 concentration on glyphosate adsorption to WS, SS and CS soil. The error bars represent standard deviation (n = 3).

Consequently, the Freundlich $K_f$-values were considerably higher in CS and SS than in WS (Fig. 7.3, Table 7.2). With the three fixed concentrations of Triton CG-110 (0, 0.00001 and 0.5%), the glyphosate adsorption to WS and SS soil was affected by the surfactant concentration, whereas no significant effect was observed for CS (Fig. 7.3a, 7.3c, 7.3e). Fits were generally good ($R^2 \geq 0.95$), indicating that data for all the soils could be described well by the Freundlich equation. Adsorption was non-linear, as indicated by the 1/n values being consistently below one. The results of the heteroscedastic t-tests on the $K_f$ values are reported in Supplementary Table 7.S1. In WS and SS, the highest $K_f$ values were observed in the absence of Triton CG-110 (13.5 and 165.4 $\mu g^{1-1/n}$ $(ml)^{1/n}$ $g^{-1}$, respectively). Increasing the concentration of surfactant in solution from 0 to 0.00001 and 0.5% led to a significant decrease in the $K_f$ values of glyphosate in WS (10.3 and 4.0 $\mu g^{1-1/n}$ $(ml)^{1/n}$ $g^{-1}$, respectively), whereas in SS a significant reduction was observed only with the highest surfactant concentration ($K_f$ = 90.8 $\mu g^{1-1/n}$ $(ml)^{1/n}$ $g^{-1}$). Larger decreases are seen for higher Triton CG-110 concentration, especially for SS soil. However, it should be taken into account that although the highest surfactant concentration used in this first experiment (0.5%) simulated the concentration in the spraying solution, the proportion between the amount of surfactant and that of glyphosate was not representative of what would be the case if a real glyphosate-based formulation was applied.

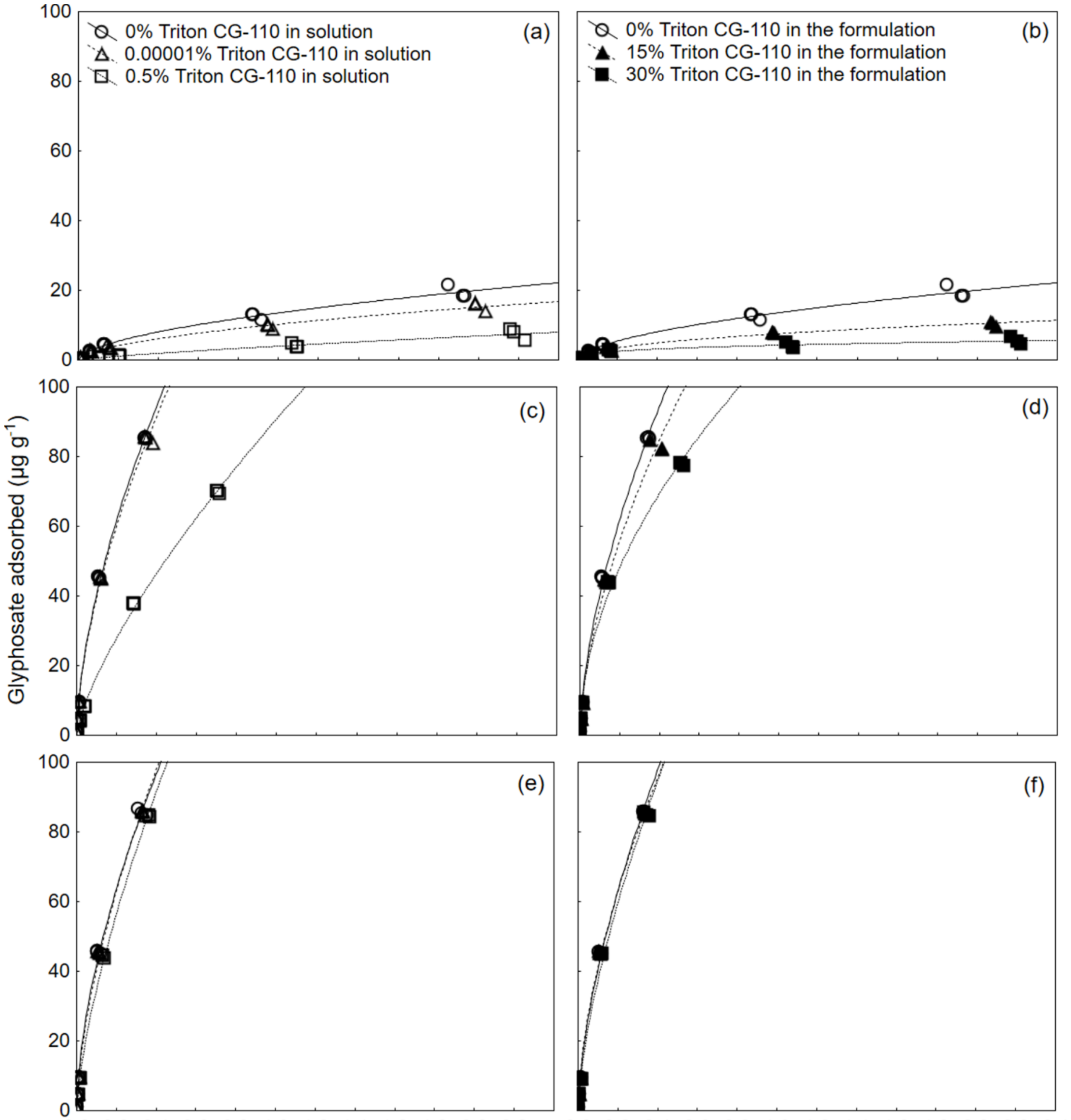

Fig. 7.3 Freundlich adsorption isotherms of glyphosate in (a) WS with 0, 0.00001 and 0.5 % of Triton CG-110 in the solution; (b) WS with 0, 15 and 30% of Triton CG-110 in the formulation; (c) SS with 0, 0.00001 and 0.5% of Triton CG-110 in the solution; (d) SS with 0, 15 and 30% of Triton CG-110 in the formulation; (e) CS with 0, 0.00001 and 0.5% of Triton CG-110 in the solution; (f) CS with 0, 15 and 30% of Triton CG-110 in the formulation. The lines represent the fitting curves and symbols the observed values.

The effects on adsorption were similar when Triton CG-110 was applied proportionally to the glyphosate amount. Glyphosate adsorption in WS and SS soil was reduced with the increasing percentage of Triton CG-110 in the formulation, whereas no significant effects were detected in CS soil (Fig. 7.3b, 7.3d, 7.3f). In WS, the $K_f$ value observed in the case of 30% of Triton in the formulation (4.2 $\mu g^{1-1/n}$ $(ml)^{1/n}$ $g^{-1}$) was lower than those observed with 15% (7.6 $\mu g^{1-1/n}$ $(ml)^{1/n}$ $g^{-1}$) and 0% of Triton. Similarly, in SS the $K_f$ value was lower with 30% of Triton in the formulation (112.3 $\mu g^{1-1/n}$ $(ml)^{1/n}$ $g^{-1}$) than those observed with 15% (146.8 $\mu g^{1-1/n}$ $(ml)^{1/n}$ $g^{-1}$) and 0% (Table 7.2). Thus, although no significant effect was seen in the CS soil, in the WS and SS soils the glyphosate adsorption was reduced in the presence of Triton CG-110 when glyphosate and surfactant was applied at realistic field rates. However, the expected rapid and complete biodegradability of the APG-based surfactant, as reported in the Closed Bottle Test (OECD 301 D) results, where a mineralization level of 88% over a 28-day period with an initial concentration of 2 mg $L^{-1}$ has been described, should be taken into account (EEC, n.d.). The observed effect on glyphosate adsorption is therefore most likely transient, and should gradually decrease as the surfactant degrades, reducing the competition of glyphosate with surfactant for soil adsorption sites. The effects on glyphosate adsorption on WS and SS soil appeared to be unrelated to the CMC of Triton CG-110. This suggests that the observed effect is not related to the formation of micelles. The pH values of the soils decreased with Triton CG-110 addition, but this cannot explain the observed decreased adsorption. The decrease of pH level at high Triton CG-110 concentrations should have decreased the negative charge of both glyphosate and soil surfaces, and would be expected to increase rather than decrease adsorption (Gimsing et al., 2004a; Vereecken, 2005). It is more likely that competitive adsorption mechanisms are at play. It is known that glyphosate adsorption occurs mainly on the mineral phase of the soil, in particular, iron and aluminium oxides (Morillo et al., 2000). Metal oxides play an important role in the adsorption not only of glyphosate but also of APG surfactants (Al-Rajab and Hakami, 2014; Balzer and Luders, 2000; Borggaard and Gimsing, 2008; Vereecken, 2005). The adsorption of Triton CG-110 would make the soil surface more hydrophobic, and may have led to direct competition with glyphosate for similar adsorption sites, thereby reducing the glyphosate adsorption. This effect is more pronounced in WS than in SS soil, and only barely noticeable in CS soil. Clay minerals are also responsible for the adsorption of glyphosate (Dion et al., 2001; Glass, 1987; Sprankle et al., 1975) therefore, in the CS soil, glyphosate should have been less affected by this competition with surfactant due to a greater number of available adsorption sites.

Table 7.2 Freundlich parameters ($K_f$, 1/n and $R^2$ value) for WS, SS and CS adsorption of glyphosate with different Triton CG-110 concentrations (± SE).

| Soil type | Triton CG-110 in solution (%) | $K_f$ ($\mu g^{1-1/n}$ $(ml)^{1/n}$ $g^{-1}$) | 1/n | $R^2$ |
|---|---|---|---|---|
| WS | 0 | 13.5 (± 0.36) | 0.57 (± 0.035) | 0.983 |
| WS | 0.00001 | 10.3 (± 0.26) | 0.56 (± 0.034) | 0.984 |
| WS | 0.5 | 4.0 (± 0.30) | 0.79 (± 0.102) | 0.953 |
| SS | 0 | 165.4 (± 4.32) | 0.61 (± 0.018) | 0.997 |
| SS | 0.00001 | 159.5 (± 4.51) | 0.61 (± 0.019) | 0.996 |
| SS | 0.5 | 90.8 (± 1.10) | 0.73 (± 0.015) | 0.998 |
| CS | 0 | 175.1 (± 4.55) | 0.64 (± 0.017) | 0.997 |
| CS | 0.00001 | 167.3 (± 6.41) | 0.60 (± 0.025) | 0.995 |
| CS | 0.5 | 173.3 (± 3.91) | 0.70 (± 0.016) | 0.998 |
| Soil type | Triton CG-110 in the formulation (%) | $K_f$ ($\mu g^{1-1/n}$ $(ml)^{1/n}$ $g^{-1}$) | 1/n | $R^2$ |
| WS | 15 | 7.6 (± 0.17) | 0.46 (± 0.025) | 0.985 |
| WS | 30 | 4.2 (± 0.20) | 0.32 (± 0.040) | 0.999 |
| SS | 15 | 146.8 (± 5.67) | 0.60 (± 0.028) | 0.992 |
| SS | 30 | 112.3 (± 2.88) | 0.53 (± 0.020) | 0.994 |
| CS | 15 | 166.3 (± 4.41) | 0.60 (± 0.017) | 0.997 |
| CS | 30 | 174.5 (± 6.05) | 0.67 (± 0.024) | 0.995 |

### *7.3.3 Effect on glyphosate leaching*

The preliminary tests conducted with WS and SS soil showed that no glyphosate was detected in the leachate from the SS-columns at a flow rate of 0.2 ml $min^{-1}$ within the experimental duration of three PV. Considering the extensive glyphosate adsorption in SS soil detected in the adsorption experiments, it is likely that virtually all the glyphosate initially applied on SS soil columns was retained by the soil matrix. The leaching experiment was therefore conducted only with WS. The setup of the column leaching experiment is likely to represent a worst-case scenario. The system simulates continuous heavy rainfall (188 mm during 8 hours) on water-saturated sand with no organic matter or biological activity that could adsorb or degrade either the glyphosate or surfactant. The duration of the experiment was ≈ 4.4 PV. The non-linear regression of the cumulative leaching data yielded good fits to the Gompertz equation (Fig. 7.4) and information on inflection points and shapes of the curves (parameters, $R^2$ values and cumulative leaching after 75 mm of leachate are presented in Table 7.3).

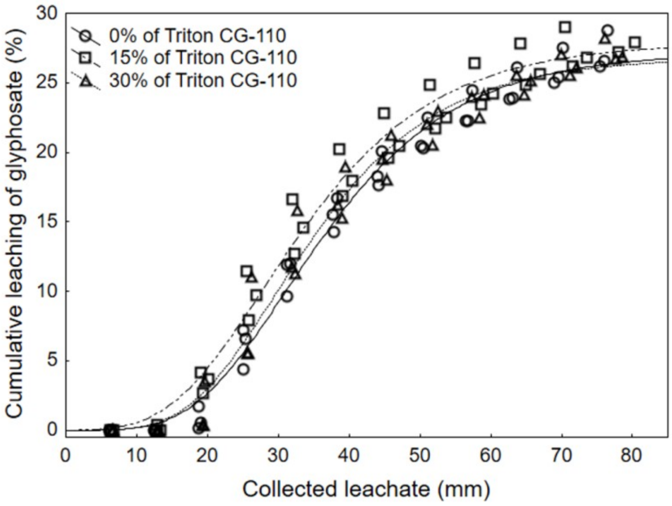

Fig. 7.4 Cumulative glyphosate leaching in WS with different Triton CG-110 concentrations. The lines represent the fitting curves and symbols the observed values.

Glyphosate is generally considered a non-mobile compound. One of the main mechanisms involved in glyphosate leaching is the preferential flow through macropores and cracks between soil aggregates. Glyphosate leaching is therefore often found to be lower in non-structured sandy soils due to the lack of macropores (Borggaard and Gimsing, 2008; de Jonge et al., 2000; Stone and Wilson, 2006). Glyphosate leaching was moderate (< 30% of the applied dose), but substantially higher than that measured in previous studies with washed sand (Cederlund et al., 2017; Cederlund and Börjesson, 2016), and this can be attributed to the smaller size of the soil columns used in this study, which were chosen precisely for this purpose. The maximum glyphosate concentration was detected after 1.4 to 1.8 PV and ranged between 0.75 and 1.05 mg $L^{-1}$. Cumulative leaching after 75 mm of leachate was 26.1, 27.1 and 26.2 with 0%, 15% and 30% of Triton CG-110 in the formulation, respectively. The analysis of variance yielded no significant differences between treatments, indicating that glyphosate leaching was unaffected by the Triton CG-110 concentrations tested. Since the ANOVA result did not yield an overall statistically significant difference in group means, no post hoc test was performed.

In a previous study, Cederlund and Börjesson (2016) observed a dose-dependent increase in glyphosate leaching caused by the addition of an APG surfactant (Cederlund and Börjesson, 2016). However, the surfactant amounts applied in that study, equal to or above the recommended use rate of APG as a foaming agent for hot water weed control, were about 30 to 160 times greater than the highest amount used in our experiment (30% of Triton CG-110 in the formulation). The absence of a significant effect observed in our study suggests that the presence of up to 30% of Triton CG-110 in the glyphosate-based formulation is unlikely to cause any problems in terms of glyphosate leaching when the product is applied at realistic field rates.

Currently, little is known about the impact of other surfactants contained in glyphosate-based formulations on glyphosate environmental fate, but some studies about the influence of surfactants on the leaching of other pesticides have been published. Results show that the interactions between surfactant, pesticide and soil can be complex and depend on the properties and concentration of both pesticide and surfactant. Sánchez-Camazano et al. (1995) and Iglesias-Jiménez et al. (1996) observed that the addition of Tween 80, a non-ionic surfactant, decreased the mobility and increased the adsorption of hydrophobic pesticides diazinon and atrazine and ethofumesate, presumably because they are adsorbed by the surfactant that is previously adsorbed by the soil. By contrast the anionic surfactant sodium dodecyl sulphate (SDS) was found to significantly decrease adsorption of the same pesticides by the soil, particularly at high concentrations where micelles were formed (Iglesias-Jiménez et al., 1996; Sánchez-Camazano et al., 1995). The mobility of the more water-soluble pesticide acephate, that is chemically more similar to glyphosate, was generally less affected by either surfactant. Huggenberger et al. (1973) studied the effects of two non-ionic surfactants, an alkylpolyoxyethylene ethanol and a mixture of a polyoxyethelene ester and a polyoxyethylene ether, and reported reduced mobility of lindane and diuron at moderate concentrations but significantly increased mobility at higher concentrations. However, they concluded additions of surfactants to pesticide sprays would be unlikely to increase leaching because higher concentrations of surfactants would readily be diluted as they are leached down the soil profile.

Table 7.3 Model parameters for glyphosate leaching (± SE) and cumulative leaching after 75 mm of leachate (mean value ± SE, n = 3) in WS with different Triton CG-110 concentrations.

| Triton CG-110 in the formulation (%) | a | b | $x_0$ | $a_{75}$ | $R^2$ |
|---|---|---|---|---|---|
| 0 | 27.2 (± 0.61) | 13.1 (± 0.93) | 31.1 (± 0.59) | 26.1 (± 0.42) | 0.994 |
| 15 | 27.9 (± 0.72) | 13.2 (± 1.18) | 28.0 (± 0.76) | 27.1 (± 0.46) | 0.989 |
| 30 | 26.8 (± 0.68) | 12.4 (± 1.10) | 29.7 (± 0.71) | 26.2 (± 0.29) | 0.990 |

### *7.3.4 Effect on glyphosate mineralization*

$^{14}C\text{-}CO_2$ evolution in SS and CS followed first order kinetics with an $R^2$ greater than 0.99 for all the treatments (Fig. 7.5b, 7.5c and Table 7.4), while the mineralization of glyphosate in WS was better described by a logistic model with an $R^2$ greater than 0.98 (Fig. 7.5a, Table 7.5). One-way ANOVA performed on the kinetic parameters revealed that there were no significant differences in glyphosate mineralization between the Triton CG-110 concentrations tested in any of the soils. Since the ANOVA result did not yield an overall statistically significant difference in group means, no post hoc test was performed. This suggests that a concentration of APG-based surfactant of up to 30% in the glyphosate formulation should not represent a relevant factor influencing active ingredient mineralization in the soil. The lack of an effect on mineralization despite a significant effect on glyphosate adsorption to WS and SS suggests that availability was not limiting for degradation in these cases, while in the clay soil, where adsorption was stronger, it was not significantly affected by the surfactant addition. Other possible ways in which surfactants could influence the mineralization vary from the surfactant being used as preferential substrate (Thiem, 1994) to surfactant toxicity (Aronstein et al., 1991; Thiem, 1994). However, since no differences were observed, we can assume that the tested surfactant does not compete with glyphosate for microbial degradation. Regarding the toxicity, as mentioned above, APG-based surfactants are generally considered to have a low toxicity and are easily biodegradable in the environment (von Rybinski and Hill, 1998). A similar result was observed by Simões et al. (2019) when testing the effect of another and more toxic surfactant, the polyethoxylated tallow amine (POEA). Degradation rate of glyphosate was similar when this compound was applied as a pure active ingredient or as a formulated product POEA, suggesting that POEA does not significantly affect the degradation rate of glyphosate in the soil.

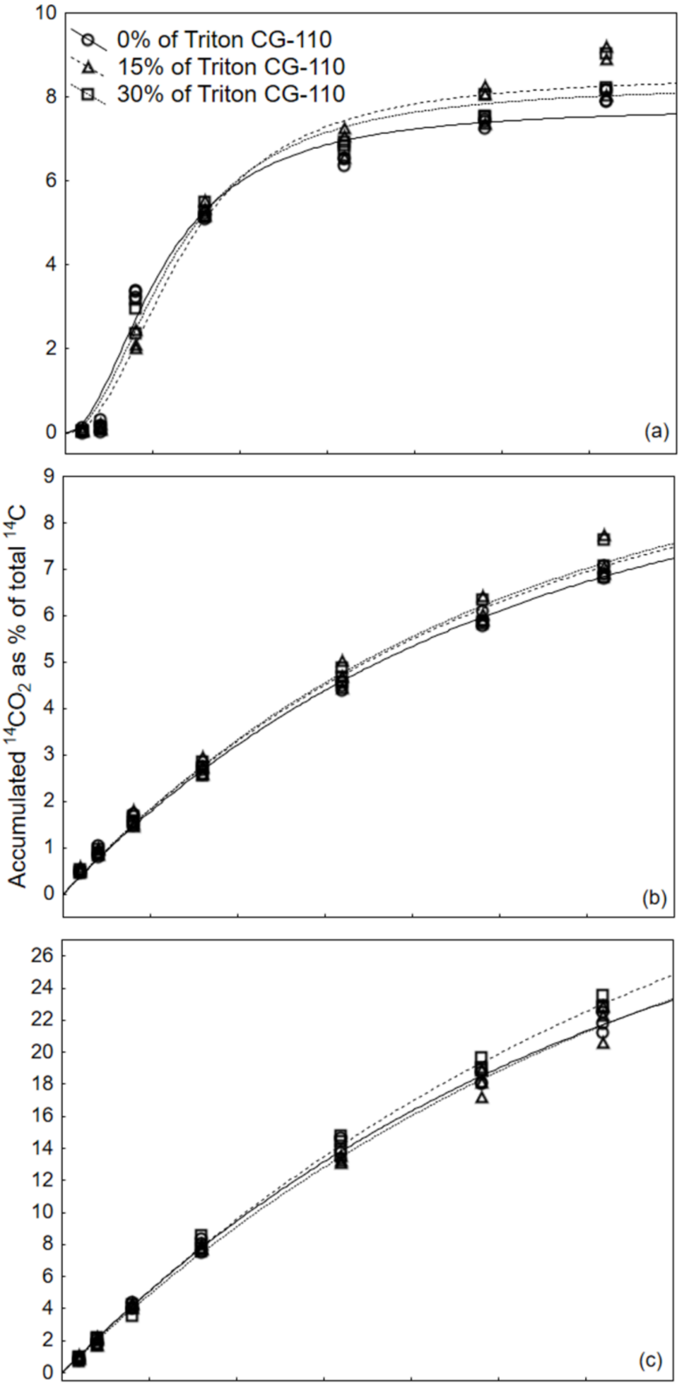

Fig. 7.5 Cumulative glyphosate mineralization in WS a), SS b) and CS c) soil with different Triton CG-110 concentrations, expressed as accumulated $^{14}CO_2$ as the percentage of total $^{14}C$ applied. The lines represent the fitting curves and symbols the observed values.

The mineralization kinetics of glyphosate in WS differed from that in SS and CS soils. All treatments in WS showed an initial lag phase of a few days followed by more intense mineralization for about 5 days, which gradually slowed. The lag phase observed in WS likely corresponds to the increase of specific microbial populations responsible for the metabolic degradation of glyphosate, since the starting microbial population in the washed sand should be very low (Kubiak et al., 1995; Perrin-Ganier et al., 2001). Theoretically, the WS is a clean substrate with virtually no organic matter, and the added glyphosate and surfactant should be the only carbon, nitrogen and phosphorus sources available. The chemical analysis did not show a significant amount of carbon, but low amounts of nitrogen and phosphorus (Table 7.1). However, we detected a low background respiration in the untreated washed sand, suggesting that there were still some carbon sources available for mineralization. For SS and CS soils, the absence of a lag phase would indicate that, prior to mineralization, no adaptation of the soil microflora was necessary.

The overall glyphosate mineralization in WS after 31 days of incubation was higher than expected, and it slightly exceeded the mineralization in SS. However the maximum $^{14}$C mineralized (% of applied herbicide), expressed by the kinetic parameter *a*, was higher in SS than in WS. CS samples exhibited a higher glyphosate mineralization capacity than both SS and WS. After 31 days, when the mineralization experiment was discontinued, the average percentage of the initial total $^{14}$C applied to the soil recovered as $^{14}$C-CO$_2$ was 22.2% in CS and 8.4 and 7.1% in WS and SS, respectively.

The higher glyphosate mineralization observed in the CS samples corresponded with a higher basal respiration activity compared to the other soils (Table 7.6). The basal respiration rates increased with increasing amount of surfactant added, suggesting that the surfactant was mineralized, but were not significantly higher in the samples treated with glyphosate alone compared with glyphosate + Triton CG-110 (Table 7.6).

Table 7.4 Model parameters for glyphosate mineralization in SS and CS soil with different Triton CG-110 concentrations in the formulations (± SE).

| Soil type | Triton CG-110 in the formulation (%) | a (%) | k (day$^{-1}$) | R$^2$ |
|---|---|---|---|---|
| SS | 0 | 9.4 (± 0.40) | 0.042 (± 0.0029) | 0.998 |
| SS | 15 | 9.8 (± 0.57) | 0.041 (± 0.0039) | 0.997 |
| SS | 30 | 9.9 (± 0.65) | 0.041 (± 0.0044) | 0.996 |
| CS | 0 | 34.6 (± 1.77) | 0.032 (± 0.0024) | 0.999 |
| CS | 15 | 40.9 (± 2.25) | 0.027 (± 0.0021) | 0.999 |
| CS | 30 | 37.5 (± 2.96) | 0.028 (± 0.0031) | 0.998 |

Table 7.5 Model parameters for glyphosate mineralization in WS with different Triton CG-110 concentrations in the formulations (± SE).

| Soil type | Triton CG-110 in the formulation (%) | a (%) | $x_0$ (days) | b | $R^2$ |
|---|---|---|---|---|---|
| WS | 0 | 7.8 (± 0.30) | 5.50 (± 0.439 ) | -2.01 (± 0.263) | 0.988 |
| WS | 15 | 8.3 (± 0.31) | 6.16 (± 0.451) | -2.05 (± 0.245) | 0.991 |
| WS | 30 | 8.5 (± 0.30) | 6.70 (± 0.439) | -2.19 (± 0.253) | 0.992 |

Table 7.6 Values of basal respiration for WS, SS and CS samples in mineralization study.

| Triton CG-110 in the formulation (%) | Basal respiration (µg $CO_2$-C $g^{-1}$ $day^{-1}$) WS | SS | CS |
|---|---|---|---|
| Non-treated | 1.27 (± 0.184) | 2.14 (± 0.047) | 4.40 (± 0.125) |
| 0 | 1.34 (± 0.107) | 2.34 (± 0.037) | 4.75 (± 0.210) |
| 15 | 1.73 (± 0.092) | 2.35 (± 0.189) | 5.01 (± 0.093) |
| 30 | 1.79 (± 0.025) | 2.51 (± 0.053) | 5.14 (± 0.207) |

## 7.4 Conclusions

In this study, the influence of an APG-based surfactant, Triton CG-110, on adsorption, leaching and mineralization of glyphosate in different soils was tested. A moderate reduction in glyphosate adsorption was observed only in washed sand and in sandy soil both above and below the critical micellar concentration, whereas in clay soil the adsorption remained unaffected. Despite the effect observed on adsorption, glyphosate leaching in washed sand was not significantly affected by the Triton CG-110 level, with glyphosate and the surfactant applied at realistic proportions and recommended field rates. Regarding mineralization, this study showed that the presence of up to 30% of Triton CG-110 in glyphosate-based formulations is unlikely to have a significant effect on glyphosate mineralization in the soil.

APG-based surfactants are used as comparatively non-toxic co-formulants added to glyphosate-based formulations in order to increase herbicide uptake by the plants. As indicated by our data, the presence of this surfactant, at the percentages contained in the currently available glyphosate-based formulations, is unlikely to cause a significant effect on the environmental fate of glyphosate in soil.

## 7.5 Supplementary material

Table 7.S1 Results of the heteroscedastic t-tests on the $K_f$ values of glyphosate.

| Soil type | Triton CG-110 in solution (%) | $K_f$ | 95% confidence interval | | Difference[1] | | p-value[2] |
|---|---|---|---|---|---|---|---|
| WS | 0 | 13.5 | 12.8 | 14.3 | 0 - 0.00001 | -3.3 | 0.000 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| WS | 0.00001 | 10.3 | 9.7 | 10.8 | 0.00001 - 0.5 | -6.3 | 0.000 |
| WS | 0.5 | 4.0 | 3.4 | 4.6 | 0 -0.5 | -9.5 | 0.000 |
| SS | 0 | 165.4 | 165.3 | 184.9 | 0 - 0.00001 | -5.9 | 0.177 |
| SS | 0.00001 | 159.5 | 153.4 | 181.3 | 0.00001 - 0.5 | -68.8 | 0.000 |
| SS | 0.5 | 90.8 | 164.8 | 181.8 | 0 -0.5 | -74.7 | 0.000 |
| CS | 0 | 175.1 | 156.1 | 174.6 | 0 - 0.00001 | -7.8 | 0.165 |
| CS | 0.00001 | 167.3 | 149.8 | 169.2 | 0.00001 - 0.5 | 5.9 | 0.782 |
| CS | 0.5 | 173.3 | 88.4 | 93.2 | 0 -0.5 | -1.9 | 0.379 |

| Soil type | Triton CG-110 in the formulation (%) | $K_f$ | 95% confidence interval | | Difference[1] | | p-value[2] |
|---|---|---|---|---|---|---|---|
| WS | 0 | 13.5 | 12.8 | 14.3 | 0 - 15 | -6 | 0.000 |
| WS | 15 | 7.6 | 9.7 | 10.8 | 15 - 30 | -3.3 | 0.000 |
| WS | 30 | 4.2 | 3.4 | 4.6 | 0 - 30 | -9.3 | 0.000 |
| SS | 0 | 165.4 | 165.3 | 184.9 | 0 - 15 | -18.6 | 0.007 |
| SS | 15 | 146.8 | 153.4 | 181.3 | 15 - 30 | -34.5 | 0.000 |
| SS | 30 | 112.3 | 164.8 | 181.8 | 0 - 30 | -53.1 | 0.000 |
| CS | 0 | 175.1 | 156.1 | 174.6 | 0 - 15 | -8.8 | 0.089 |
| CS | 15 | 166.3 | 149.8 | 169.2 | 15 - 30 | 8.2 | 0.858 |
| CS | 30 | 174.5 | 88.4 | 93.2 | 0 - 30 | -0.6 | 0.468 |

[1]: Difference between $K_f$ values.

[2]: p-values for the null hypothesis of no significant difference (heteroscedastic t-test).

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# General conclusions

This PhD thesis aimed to investigate the environmental fate of four widely used herbicides (glyphosate, terbuthylazine, metolachlor, and mesotrione), to evaluate the application of buffer strips as a mitigation measure, and to assess the role of conservation agriculture about surface runoff and soil loss. Analyzing the findings of the experiments presented in the different chapters, some general conclusions may be summarized.

Conservation agriculture has several positive outcomes, and the improvement of soil structure is one of the most important since it can lead to a reduction in runoff and soil erosion. Although the non-tilled field we monitored was still in the transition period from conventional to conservation agriculture, conservation agriculture was beneficial for reducing runoff and soil erosion, promoting soil and water conservation. The surface micro-topographical structure of the non-tilled soil, with rougher surfaces and more pronounced concavities and convexities, played a role in reducing the runoff water volume and storing more water and sediments, compared to the tilled soil. The reduction of runoff and sediment yield with conservation agriculture could be translated into a lower risk of herbicide losses from the field to surface waters, having important benefits for the sustainability of this agricultural practice.

Special focus was given to the analytical aspect in order to develop methods that would allow the rapid, accurate, and reliable determination of glyphosate and AMPA in aqueous and soil samples. Satisfactory results were obtained in the method validation for both water and soil samples. The application of the method to runoff water allowed us to assess the satisfactory performance of a 6 m wide buffer strip with two rows of trees and shrubs in reducing the loss of glyphosate and AMPA from a non-tilled field in the transition from conventional to conservation agriculture. The efficacy of different types of buffer strips in reducing herbicides runoff from a non-tilled field during the transition phase was also investigated with three different herbicides – terbuthylazine, metolachlor, and mesotrione – applied in a mixture as a formulated product. Buffer strips demonstrated to be a very effective tool for reducing herbicides runoff and mitigate agricultural pollution. A synergistic action between the two mitigation measures (buffer strips and conservation tillage) was also hypothesized.

The developed method was applied to the analysis of soil samples to investigate the dissipation of glyphosate and the formation and dissipation of AMPA in tilled and non-tilled soil at two different depths. Crop residues, distributed on the surface of the NT field, intercepted a significant amount of glyphosate at the time of application, thus reducing the amount of herbicides that reached the soil surface in NT soil compared to CT. This study showed that conservation agriculture could slow down the dissipation of glyphosate in comparison with conventional agriculture, but no clear effect was observed for AMPA.

Soil texture and depth were identified as factors that influence the dissipation of terbuthylazine and S-metolachlor applied in mixture with mesotrione. The dynamics of these herbicides under field conditions are mostly dominated by their microbial degradation, while soil texture, organic matter, and soil moisture play an important role in their decay rate. None clear effect on the dissipation of mesotrione was observed in different soil textures and depths. Sewage amendment slightly increased the persistence of terbuthylazine and S-metolachlor applied in a mixture in the soil, and it had a slight effect on the formation and dissipation dynamics of desethyl-terbuthylazine. A higher persistence of these compounds in the soil may extend the risk of water contamination instead of reducing it. This suggests that the sewage amendments should be applied with particular caution in areas where the water is already contaminated by these herbicides. Agronomic practices aiming to reduce the applied amount of herbicide should be adopted.

The effect of the alkyl polyglucoside surfactant Triton CG-110 on the environmental fate of glyphosate in the soil was investigated. The presence of this surfactant, at the percentages contained in the currently available glyphosate-based formulations, did not produce significant effects except for a moderate reduction in glyphosate adsorption in washed sand and sandy soil, which is likely to be a temporary effect given the rapid biodegradability of the surfactant. Therefore, this co-formulant should not cause a significant effect on the environmental fate of glyphosate in soil, and given its low toxicity and high biodegradability, it should not raise additional concerns.

The insights gained in this PhD research about the environmental fate of these widely used herbicides in the soil are important if we consider the widespread and recurrent use of these agrochemicals all over the world, which is resulting in high social concerns about environmental and human safety. This calls for the application of effective mitigation measures to avoid serious soil and water contamination and consequent off-site risk. In addition to the mitigation of pollution, there is a need for more sustainable agricultural management. The application of buffer strips and the adoption of conservation agriculture, though being beneficial from many points of views, must always be accompanied by correct and sustainable use of herbicides.

The environmental fate of herbicides can vary greatly, even in the same type of soil and within the same geographical area, and some compounds, particularly AMPA, might be accumulating in the soil. Moreover, climatic variables interact with management variables leading to site- and herbicide-specific effects on the environmental behavior of these pollutants. Therefore, a number of topics remain to be investigated and assessed, namely:

- Deeply investigate the environmental fate of the studied herbicides in other soil types and crop systems especially in light of the ongoing climate change;

- Controlled studies on how residues from different crops and cover crops intercept and adsorb the applied glyphosate, and how much could be washed off during rainfall events;
- Comparative studies that combine the thoroughness of the small-scale studies with the wideness of broad-scale assessments in the monitoring of runoff and soil erosion in conservation and conventional tillage;
- Frequent monitoring of soil hydrological properties of tilled and non-tilled soil and multi-temporal topographical survey in order to monitor the surface changes throughout the seasons:
- Controlled studies with a broad range of glyphosate-based formulations containing different co-formulants and in a wide range of soils varying in texture and characteristics, to gain more comprehensive insight and identify the formulations that are less harmful to the environment. Furthermore, it could be useful to scale up the system to investigate behavior under conditions closer to the field;
- Field studies to investigate on a possible synergistic action between buffer strips and conservation tillage as runoff mitigation measures.