Fate of ^{14}C-S-metolachlor in crop residues and underlying soils.

Jules Hatier

Norwegian University of Life Sciences
Department of Plant and Environmental Sciences (IPM)
Master Thesis 30 credits 2012

Acknowledgements:

I wish to thank all the INRA Grignon soil team for its cordial welcome, its availability and its support in this experiment. I spend a really rewarding time doing this master thesis with them.

I wish to thank more particularly Valérie Bergeaud, Pierre Benoit, Valérie Dumeny, Jean-Noël Rampon and Christophe Labat for their supervision and their help in the laboratory.

I also really appreciate to work on the field with Simon Guliano and his colleagues.

Finally, I wish to give a particular thank to Lionel Alletto, for his trust, his rightness and his sense of human in the supervision of this thesis.

Table of contents

List of abbreviations and acronyms

l: litre

ml: millilitre

µl: microlitre

h: hour

ha: hectare

m: meter

cm: centimetre

nm: nanometre

min: minute

N: normal

%: pro cent

°C: Celsius degree

^{14}C: radioactively labelled carbon

$T_{1/2:}$ half-life

C-Org: organic carbon

HPLC: high precision liquid chromatography

List of illustrations

Figure:

Table:

1. Introduction

Water protection and water quality measures are currently under review by the European Union (European directive on water, October 2000), which has direct consequences on the reduction of negative externalities associated with agriculture on water quality. This reduction is concerned mainly with the use of nitrogen fertilizer and pesticides.

This European directive has been applied in France as the “loi sur l’eau” (law on water) in 2006. This political aim has been reinforced by the “grenelle de l’environnement” from 2007 and local targets to reach higher water quality standards have been defined in order to reach the goal of having two thirds of good quality water resources by 2015. In the case of non-compliance there would be a state fine.

Research projects working in this area, particular those with an agronomic approach are generally considering the risk evaluation specific to one crop included or not included in one cropping system. For the most part, research projects have focussed on reducing pesticides needs by limiting pest pressure and improving spreading techniques.

In addition to a reduction in pesticide use, cropping practices or landscape modifications (hedges for example) could be developed in order to limit water contamination by the pesticides already spread.

However, the actual knowledge of the behaviour of pesticides spread on fields is limited, as is the knowledge of more sophisticated technical solutions (cropping practices or landscape modification).

Along this same vein, it could be necessary to develop new technical solutions and to evaluate more deeply existing ones in order to go further in the reduction of the water contamination.

Cover cropping is an effective techniques for protecting soil against erosion (Malik et al., 2000), increase soil structure and soil fertility (Unger and Vigil, 1998), to control weeds, pests and pathogens (Worsham, 1991; Swanton et al., 1999) and to limit nitrogen loss (Ritter et al., 1998; Justes et al., 1999).

Cover crops generally allows for an increase of the organic material content and the soil biological activity (Reeves, 1994). Depending on the species and management, cover crops disturb water availability for the following crop by drying the soil profile (Unger and Vigil,

1998; Currie and Klocke, 2005) or by maintaining higher water availability compared to bare soil (Drury et al., 1999).

Moreover, these biophysical modifications could modify the water retention, the degradation and the transport of pesticides sprayed on the field (Alletto et al., 2010). For example, maintaining cover-crop residues at the soil surface, as in no-till agriculture, induces an increase of the interception and the retention of the pesticides sprayed (Reddy et al., 1995; Sadeghi and Isensee, 1997). The degradation of pesticides is also modified in the presence of cover-crops; they can increase the mineralisation of the 2.4-D in subsurface horizons (Bottomley et al., 1999). Along the opposite lines, research studies have showed a slowdown in the degradation of some herbicides depending on the nature of the residues (Zablotowicz et al., 1998; Gaston et al., 2001).

More generally, most research studies on cover crops are interested in the Nitrogen dynamic and very few references are present about the links between cover cropping and pesticide behaviour.

Recently, Potter et al. observed that summer cover crops can significantly reduce aquifer contamination by Atrazine and DEA (Potter et al., 2007). White et al. obtained similar results with Metolachlor (White et al., 2009). They posit the hypothesis that the use of cover crops induced faster dissipation and/or a less leaching of the molecules. They have not concluded about the relative importance of each one of these two hypotheses on the contamination reduction by the molecule.

Metolachlor is a widely used chlorocetanilide herbicide (Rivard L., 2003) which has been replaced in 2003 by the S-Metolachlor, composed of 80% of the isomer S of the metolachlor, the more active form of the molecule. S-Metolachlor is used as a pre-emergence and post-emergence weed control in a variety of crops including maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean (*Glycine max* L.) (Ahrens, 1994).

Metolachlor is commonly found in surface and groundwater where this product is used (Gilliom, 2007) and generally forms metabolites which present relatively high stability and/or environmental mobility. The detection of the S-metolachlor metabolites is common with a concentration often exceeding the parent molecule concentration (Kolpin et al., 1998).

All these characteristics designate the S-metolachlor as a highly desirable molecule to study. The aim of this Master's thesis is to study the influence of four different winter cover crop residues, one preceding crop residues and one mix of different crop residues on the fate of the S-metolachlor in laboratory conditions and to evaluate the potential benefits of these residues for the environment.

2. Materials and methods

For this experiment, we selected four winter cover crop species: the winter turnip rape (*Brassica rapa* L. *subsp. Oleifera*), the oats (*Avena Sativa* L.), the red clover (*Trifolium rubens* L.) and the phacelia (*Phacelia tanacetifoli* L.); one preceding crop specie, the maize (*Zea mays* L.) and one mix treatment.

The mix treatment was composed by one half maize residues, one sixth oat residues, one sixth clover residues and one sixth of phacelia residues.

The same total amount of dry material (2 g) was used for all the experimental units.

2.1. Field sites

The soil came from a 15-ha research station located in the alluvial corridor of the Garonne River (Lamasquère, France) cultivated with wheat and sunflower for the past 10 years. The slope across the field was low and ranged from 1 to 2 %. According to the World Reference Base for Soil Resources (ISSS-ISRIC-FAO, 1998), the soil was a clay loam soil. The substratum was an alluvial pebbly layer appearing at around 100 cm. The tillage system consisted of a conventional tillage: the field has been ploughed in the late autumn in order to limit the effect of the preceding crop (wheat), harvested in July of the previous year. No chloroacetanilide chemical (chemical from the S-metolachlor family) had been applied on the field sites for the last eight years.

The maize residues were sampled from a grain maize field located next to the research station and harvested in October 2010.

Cover crops species were cultivated in small experimental plots located at the engineering school of Purpan (Toulouse). These plots were also located in the alluvial corridor of the Garonne River and with similar weather conditions.

2.2. Sampling procedure

Soil

The surface layer of the soil (0-5 cm) was sampled regularly across the field. Samples were collected in small cylinders and put in plastic bags. All the samples were stored at 4°C.

Cover crops

The aerial part of the living cover-crops was cut as close as possible to the ground with scissors.

Maize residues

Maize residues were hand collected at the field surface four months after the grain harvest.

2.3. Samples conditioning

Soil

Soil samples were air-dried for 48 h and sieved at 5mm for the purpose of homogenization. Then, the soil samples were mixed together in order to create the same soil substrate used for all the modalities of the experiment.

Small amounts of this soil substrate (80 g) were dried in an oven at 105°C for 48 h to determine soil water content.

Cover crop and maize residues

The cover-crops residues were dried directly after the harvest in a ventilated oven at 40°C for 48 h. Then, cover crop residues were cut into small pieces of 1cm by 1cm. The dried residues were stored in closed plastic bags at ambient temperature. Small amounts of residues were dried in an oven at 105°C for 48 h to determine residual water content.

2.4.Water retention in soils and mulches

Incubation experiments for monitoring pesticide degradation in the laboratory were done with specific water content, corresponding to the water holding capacity (WHC) of the soil and mulche. This water holding capacity was considered similar to the soil water content at field capacity. In this study, field capacity was taken equivalent to a soil water matric potential of -330 hPa (pF 2,5). This value was chosen in order to reliable water availability conditions to micro-organisms.

The water content of the soil substrate was measured using pressure plates (Klute, 1986). The gravimetric water content of sieved samples, similar to those used for the incubation experiments, was measured after re-wetting to saturation at -330 hPa matric potentials.

The corresponding amount of water was ad to soils simultaneously with the building of the cylinders by two successive injections from the top of the cylinders.

To answer the question of the high water repulsion of dried organic residues, we used an alternative process for introducing the water to the crop residues. A mass of dried crop residues corresponding to the 2 g of dry material was introduced first in an independent receptacle. Then, we introduced the target amount of water, we stirred with a spatula, and we waited some minutes. Finally, we transfer the humidified residues and the non adsorbed water to the top of the cylinders.

2.5.Incubation

Pre-incubation

Samples were pre-incubated 6 days in the dark, at a temperature of 28 °C before the ^{14}C-S-metolachlor introduction to allow the adaptation of the microorganisms to incubation conditions, and to give the residues conditions as close as possible to field conditions. Indeed, referring to a previous study (Ashlam, 2010), the speed of degradation is 3 to 4 times faster in laboratory conditions than in the field. Along these lines, 6 days were corresponding approximately to the 3 weeks periods in between the cover crops being destroyed and the first pre-emergence treatment.

^{14}C-S-metolachlor introduction

A solution of ^{14}C ring labelled S-metolachlor [S-2-chloro-N-(2-ethyl-6-methylphenyl) acetamide] corresponding to the agronomical dose of 980 grams per hectare, was carefully spread over the bare soil and the crops residues from the top of the cylinders at day 0.

Incubation

Degradation of ^{14}C-S-metolachlor was monitored for 84 days, in the dark, at a temperature of 28 °C. Duplicate samples were run for five incubation periods: 0, 7, 22, 49 and 84 days. For each incubation periods, we used three duplicate samples for each modality.

2.6.Organic carbon and ^{14}C-S-metolachlor mineralisation measure

Cylinders were placed in 2 litre hermetic jars with a vial of 10 ml of water to keep the relative humidity constant and a vial of 20 ml of 1N NaOH to trap the CO_2 and the $^{14}CO_2$ evolved during incubation. The volume of the jars was large enough to prevent anoxic conditions within the soil. Trapping solutions were periodically replaced and analysed for CO_2 and $^{14}CO_2$ concentrations.

Measure of the organic carbon

The CO_2 content in the NaOH solution was determined colorimetrically on a Skalar autoanalyser (Skalar Analytique, 75015, Paris, France). Measurements of organic carbon released from bare soil samples were used to normalise data from the other samples to estimate the amount organic carbon released by the cover residues.

Measure of the ^{14}C-S-metolachlor mineralisation

$^{14}CO_2$ concentration were analysed by adding 10 mL of scintillating liquid (Ultima Gold XR Packard) and after dark adaptation to reduce chemiluminescence, solutions were counted 10 min in a Tri-Carb 2100TR scintillation counter (Perkin-Elmer Ins., Courtaboeuf, France±0.5).

2.7. ^{14}C-S-metolachlor extractions

For each incubation period, mulch residues and the first centimeter of the top soil were removed from the cylinders and placed separately into polypropylene centrifuge tubes (250 ml). Regarding to results obtained in similar conditions, the rest of the soil contained in the cylinder was considered as radioactivity free and was excluded from the analyses. Indeed, less than 5% radioactivity was found after the first centimeter of soil (Ashlam, 2010).

A sequence of four extractions with end-over-end shaking was done at room temperature. To obtain the water-extractable fraction of ^{14}C, the first 24 h extraction was done by adding 40 ml of aqueous 0.01 M $CaCl_2$ to the mulch residues and 75 ml to the soil. Tubes were then centrifuged at 10000 RCF for the mulch residues and at 6000 RCF for the soil for 10 min. ^{14}C content of the supernatant was measured by scintillation counting. The supernatant was further replaced by the same amount of methanol (40 ml and 75 ml), tubes were shaken for 8 h and then centrifuged.

Fractions of ^{14}C-S-metolachlor extractible were characterised according to the strength of ^{14}C molecules links with the substrate. The $CaCl_2$ fractions, considered as being the easiest to extract, contained the ^{14}C molecules weakly adsorbed by the soil and cover crops residues. The methanol fraction contains molecules strongly adsorbed. The remaining none-extractible ^{14}C molecules were considered to be strongly bond to their substrate (cover crops residues or soil).

2.8. HPLC analyses

Methanol extracts were concentrated by evaporation under a vacuum at 50 °C using Rotavapor R-200 (Büchi, Champigny, France). The residues were filtered through regenerated cellulose disc filters (0,45 µm, Alltech France, Templemars). Samples were analysed using a waters (Milford, MA, USA) HPLC appliance (600 E Multisolvent Delivery System, 717 Autosampler) equipped with a 996 Photodiode Array Detector (Waters) and a radioactive flow detector (Packard-Radiomatic Flo-One A 550). Analysis were performed on a Nova-Pak C18 column from Waters (250 mm *4,6 mm ID, 4 µm particle size, spherical particles, 60 A pore size endcapped). The mobile phase was 45/55 (v/v) methanol/water with a flow rate of 1 ml min^{-1} and a detection wavelength of 254 nm. The duration of each analysis was 45 min. The injected sample volume varied between 300 and 400 µl. The time of appearance of S-metolachlor metabolites in the HPLC report were normalised by synchronizing the S-metolachlor peaks.

Overall pattern of metabolite concentration variation over the period of the experiment was estimated by cumulating the concentration of each metabolite measured in each fraction extracted and across the different samples comprising a cover for each time point. The pattern of those metabolites was estimated for the bare soil sample by cumulating metabolite concentration measured in each fraction extracted directly from this sample.

2.9.Half-life calculation

Half-life ($T_{1/2}$) values can be derived from standard laboratory dissipation studies to provide a numerical indication of pesticide persistence in soils (Beulk and Brown, 2001). In this study, the decrease of concentrations over time was described according to a first-order kinetics fitted to untransformed data, $C_t = C_0 e^{-kt}$ where C_t is the measured soil concentration in S-metolachlor at time t, C_0 is the initial concentration measured immediately after application, and k is the first-order rate constant. The time at which the concentration reaches half the initial concentration is referred to as the half-life in days ($T_{1/2}=\ln 2/k$).

We choose to use a double exponential approach as described by Beulke and Brown (Beulke and Brown, 2001). We considered that this approach was more suitable to describe the decline of measured concentrations of ^{14}C-S-metolachlor. Indeed, using two exponential equations showed stronger correlation between the measured data point and their estimation than when a single equation was used. For most cover crops the correlations obtain by this technique were greater than 99%. Only winter turnip rape and red clover had slightly lower R^2 of 92% and 97% respectively. Indeed, the rate of degradation of the S-metolachlor molecules reached a steady stage after 49 days.

3. Results and discussion

3.1.Evolution of the humidity rate in crops residues and soils

The relative humidity of the crop residues and soils were measured all long the experiment.

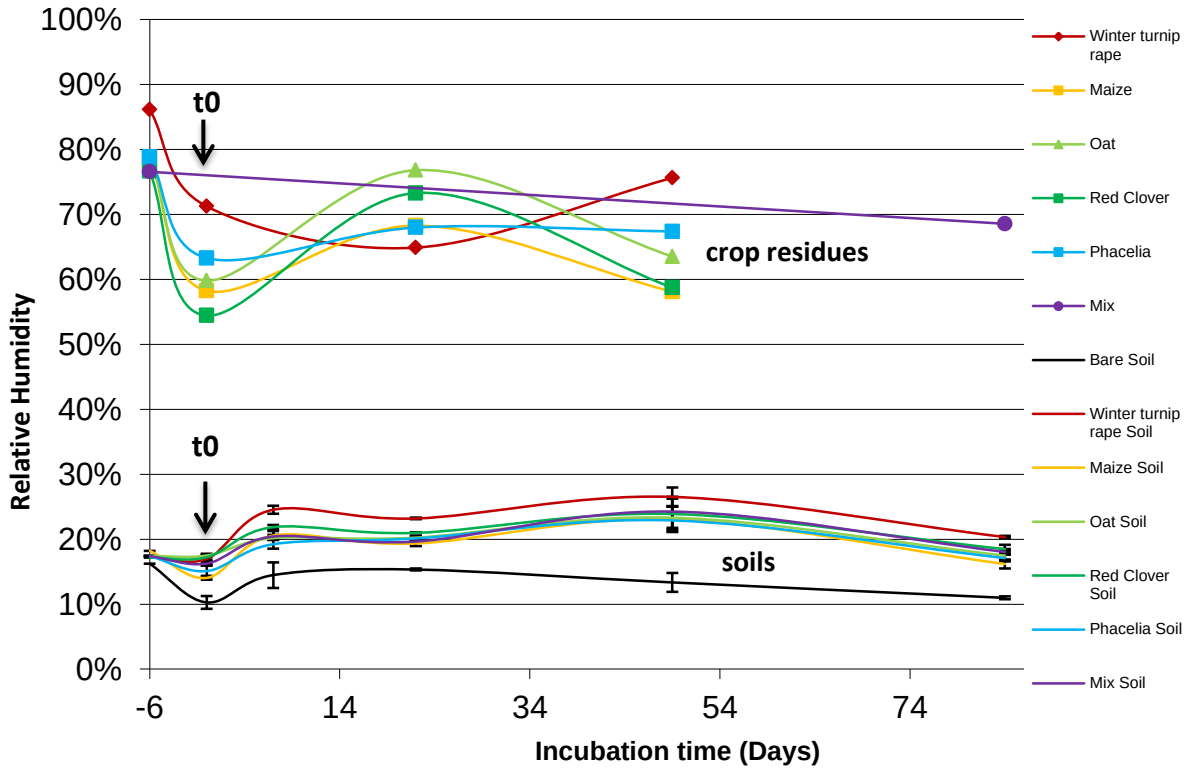

Figure 1: Change in relative humidity in crop residues and soil during 90 days. *Samples were contained in a 2.5L sealed atmosphere. The relative humidity was defined as the proportion of water in the wet material.*

Relative humidity of all the crop residues ranged from 55% to 85% between day -6 to day 49. The only relative humidity recorded after 49 days in cover residues was the mix one. Since the mix is composed of residues similar to the one present in the other treatments, it is assumed in this experiment that all other cover would have followed the same pattern in change in relative humidity and would approximate 70% at the end of the experiment.

Even though the pattern in change of relative humidity of the winter turnip rape residues was slightly different than the ones of the other crop residues, it was comprised within the same range: 55% to 85%.

Variations in relative humidity of the soil samples were small and all the samples except the one without residues (bare soil) reached a steady state of 20% of relative humidity from day 7 to day 84. Lost in relative humidity of the bare soil sample was greater than in any other samples reaching a minimum of 10% at day 84. Data suggest that water lost from the soil by evaporation was minimized in samples with crop residues.

It was considered that water availability was not a limiting factor for the growth and survival of the micro-organisms present both in the soil and in the crop residues because the relative humidity calculated for the beginning of the experiment (-6 day) in all the samples was corresponding to the water holding capacity (WHC) of the crop residues and soils and because relative humidity remained over 10% and 50% throughout the experiment in the soil and in the crop residues respectively.

3.2.Organic carbon mineralisation

We followed the mineralisation of the crop residues organic carbon during the 90 days of the experiment for the mix and during the 55 first days for the others cover treatments.

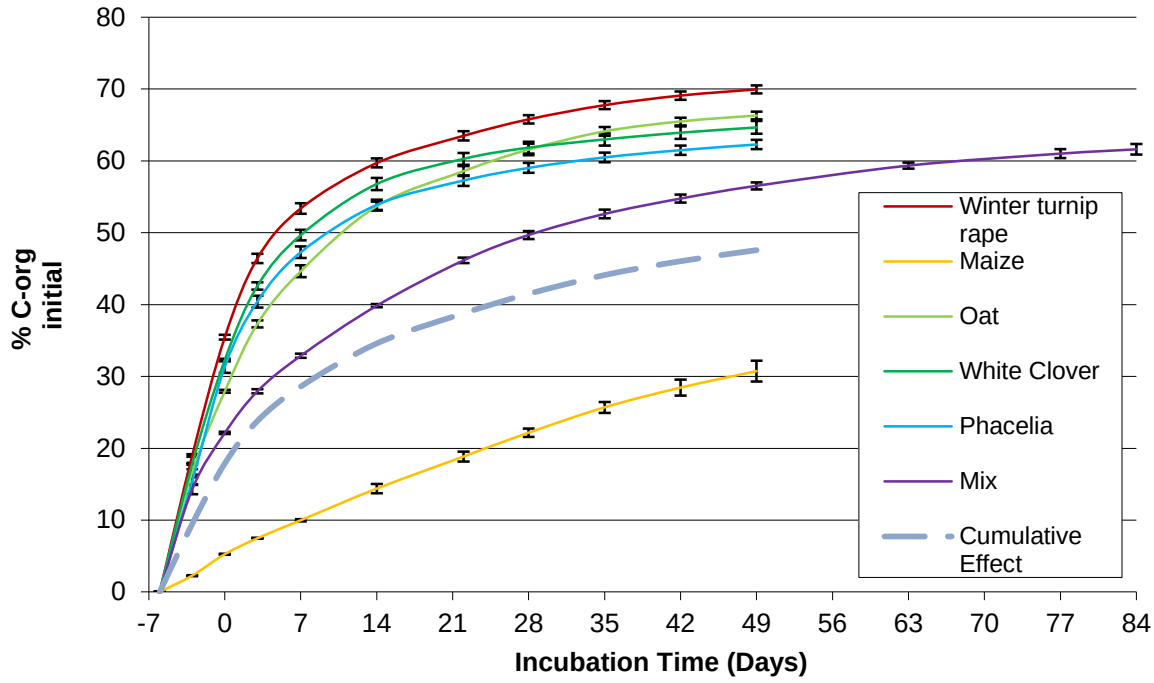

Figure 2: Mineralisation of organic carbon of the crop residues. *The initial organic carbon was calculated from the data of Chuette (Chuette, 2011).*

Measurements of organic carbon release through mineralization processes are considered to be good indicators of the degradation of the crop residues and of the total microbial activity (Martinez-Salgado et al., 2010).

Cumulative change in organic carbon lost by mineralization from the maize and the mix residues were on average lower than in any other cover by 35% and 10% respectively after 49 days.

Unlike, the cover crop residues, the maize residues were harvested after four months maturation in the field respectively to what would happen in agricultural system. This could explained the difference observed in organic carbon mineralisation.

The reduced cumulative change in organic carbon of the mix residues could only partially been explained by the 50% maize contained in this mix. Effectively, the change in organic carbon that should theoretically be obtained from such mix, when attributing a fraction of the change in organic carbon to each portion that compose the mix (50% maize residues, 17% phacelia, 17% red clover and 17% oats), should be approximately 10% lower than the one measured in the mix.

We postulate that increase rate of degradation of the mix residues could be attributable to an increased degradation of the maize fraction from a more suitable growth media. It is possible that factors such as presence of certain chemicals, maintenance of pH or presence of enzymes in the mix could enhance the degradation of the maize.

3.3.^{14}C-S-metolachlor mineralisation

We followed the mineralisation of the ^{14}C-S-metolachlor during the 84 days of the experiment.

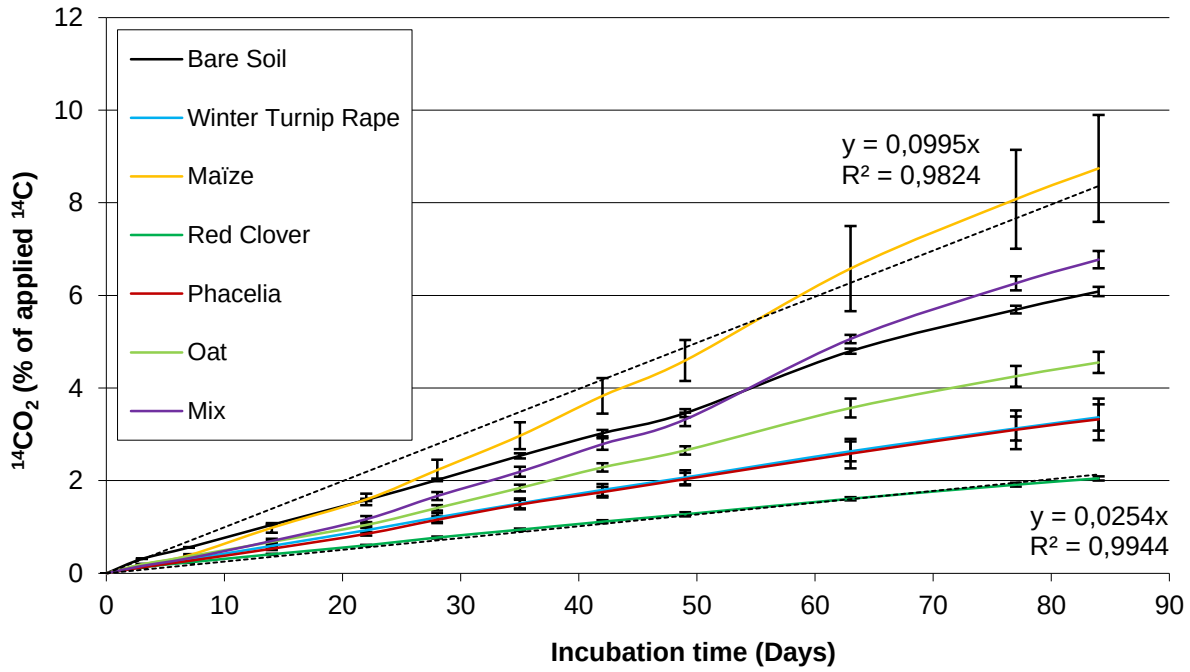

Figure 3: Mineralisation of ^{14}C-S-metolachlor during 84 days.

Mineralisation is considered to be the last step of pesticide degradation, leading to its complete removal from the soil (Alletto et al., 2010).

No chloracetanilide chemicals (chemicals of the S-metolachlor family) were applied in the soil used in this experiment for the past eight years and yet, there were no lag time observed in the ^{14}C-S-metolachlor mineralisation. This suggests that no adaptation period was needed for the micro-organisms to do a complete degradation of ^{14}C-S-metolachlor molecules therefore that no specific micro-organisms were needed for ^{14}C-S-metolachlor mineralisation.

The mineralisation was relatively constant throughout the experiment suggesting that none of factors that could potentially inhibit this process become limiting throughout the experiment.

Cumulative mineralisation of ^{14}C-S-metolachlor reached a maximum of about 9% after 84 days (≤5 % after 49 days) for the maize and around 6% (≥3% after 49 days) for the bare soil. The lower values of cumulative mineralisation were found in red clover residues with about 2% after 84 days (≥1.3 % after 49 days) and phacelia and winter turnip rape with about 3% after 84 days (around 2% after 49 days). The oat covers had a cumulative mineralisation greater than 4.5 % after 84 days (≥2.6 % after 49 days) and the mix one a cumulative mineralisation greater than 6.8% after 84 days (≥3.3% after 49 days).

This was consistent with most of the literature (Staddon et al., 2001; Rice et al., 2002; Miller, 1997). To our knowledge only two studies showed greater cumulative mineralisation; Kotoula-Syka et al. reported a cumulative mineralisation of ^{14}C-S-metolachlor after 52 days greater than 8% (Kotoula-Syka et al., 1997) and Krutz et al. reported a cumulative mineralization greater than 10% in cultivated soil and greater than 23% in vegetated filter strip soil after 60 days of incubation (Krutz et al., 2006).

Mineralisation of the bare soil sample was greater than in any others treatments with crop residues except for the maize and partially for the mix ones. This suggests that the presence of fresh cover crop residues inhibit ^{14}C-S-metolachlor mineralisation process.

3.4. Fate of the ^{14}C in the soils and crops residues

We synthesized the evolution of all the fractions characterising the fate of the ^{14}C in the crop residues and underlaying soils during the 84 days of the incubation in one single graphic per treatment.

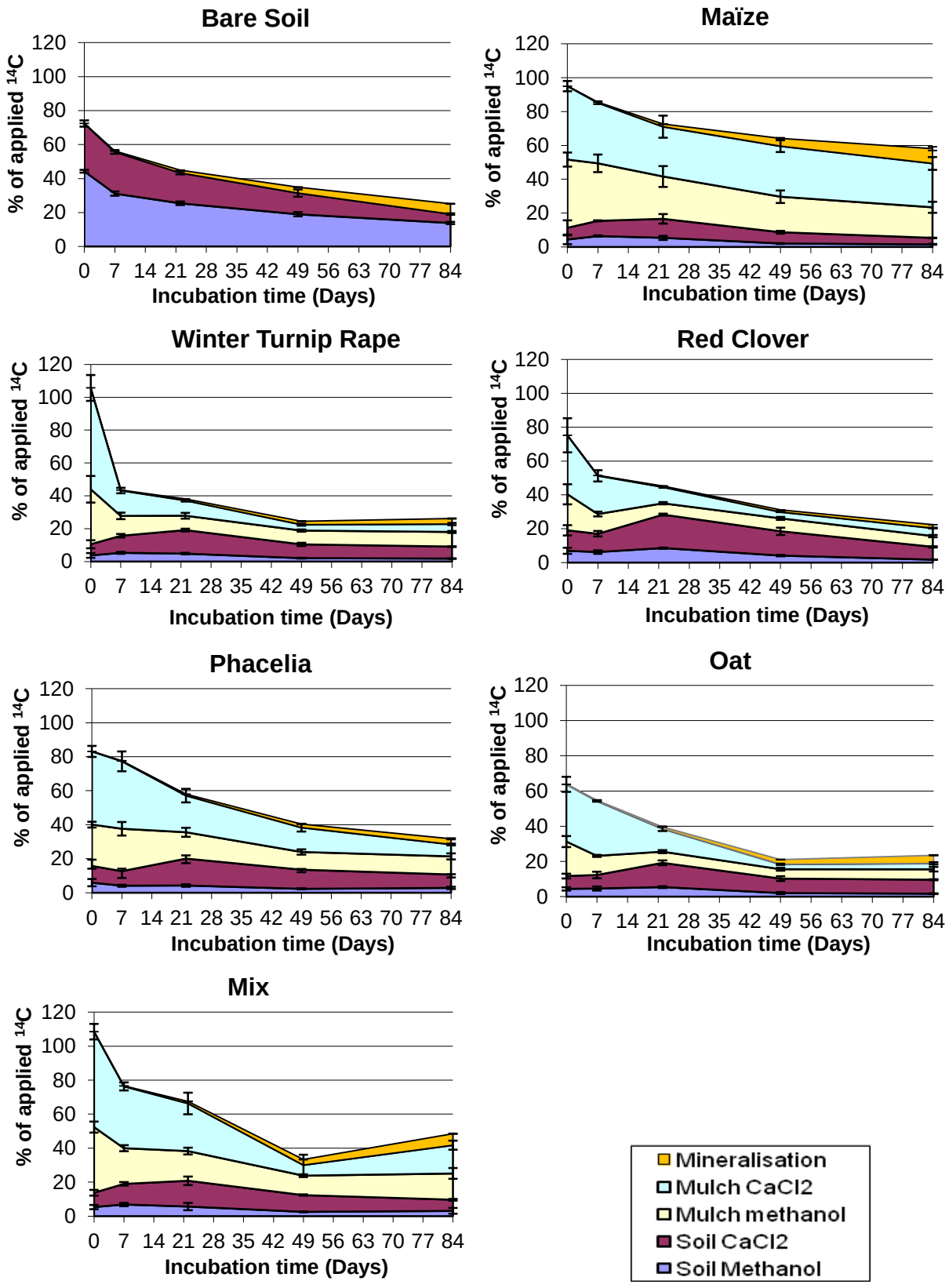

Figure 4: Fate of the ^{14}C molecules in the bare soil and the crop residues and soils during the 84 days.

Interception

All crop residues had similar interception capacity in this experiment. Most of the radioactivity was intercepted by the crop residues at day 0. Indeed, the sum of the extractible fraction of radioactivity found in soils under the covers residues ranged between 10% and 20% of the applied ^{14}C suggesting that the residues intercepted up to 80% of the ^{14}C-S-metolachlor.

These results are similar to those found in the literature (Locke, 1992; Novak et al., 1996; Reddy and Locke, 1998; Reddy et al., 1997; Zablotowicz et al., 2000). In the same vein, it has been demonstrated by (Bank and Robinson, 1986) that a soil cover with a amount of residues greater than 4.5t per ha could intercept more than 90% of the Metolachlor.

It was also suggested that interception of pesticides by crop residues could be up to 60% greater than by the soil (Boyd et al., 1990; Reddy et al., 1995).

Over time however, part of the ^{14}C molecules were transferred from the crop residues to the top layer of the soil. This was showed by an increase of radioactivity in soil extractible fraction after 22 days suggesting that a possible desorption of the ^{14}C molecules occurred and were transferred gravitationally to the subjacent soil layers. It has been suggested by Mazzonici et al (1998) that crop residues could increase retention time of pesticide by accumulating them (Mazzonici, 1998). However when residues degradation occur later on, part of the pesticide previously accumulated are gradual released by desorption.

We suggest in this experiment that the total amount of ^{14}C molecules present in the subjacent soil layers was constantly increasing. Indeed, this quantity was under evaluated because a portion of these molecules become strongly bond to the soil substrate and would become non extractable.

Retention

^{14}C molecules were considered to be relatively weakly adsorbed at day 0. This was shown by the fact that most of the extractable radioactivity was in the $CaCl_2$ fraction in both the bare soil samples and the treatments with crop residues.

Throughout the experiment, the fate of ^{14}C molecules followed generally the same patterns for all the cover crops. The fraction of ^{14}C molecules extractable consistently decreased from 80% to 30% on average across the treatments. This diminution of extractable fraction over time suggests a proportional increase of the non extractable fraction because the total amount of radioactivity remains constant through the experiment. The non extractible fractions were considered to be mainly located in the mulch residues rather than in the soil. Effectively, it has been shown by Dao (1991) that most of the metribuzine molecules were physically entrapped by the cellulose microfibrils embedded in a lignin-hemicelluloses matrix within cell wall structure (Dao, 1991). Moreover, we supposed that the bond residues were forms along we the degradation of the ^{14}C-S-metolachlor. Indeed, the formation of bound residues is often correlated with the degradation half-life of the molecules (Gaston and Locke, 2000; Lavorenti et al., 2003; Zablotowicz et al., 2000).

The slower diminution of extractible fractions found in both the maize and the mix residues suggest that there were less bond residues in those substrates than the cover crop residues.

3.5.The ^{14}C-S-metolachlor degradation.

The ^{14}C-S-metolachlor quantity, stated in function of the applied ^{14}C, was determined as the ^{14}C-S-metolachlor molecules present in the CaCl2 and Methanol solutions for each extraction dates. We measured it declined during the experiment (Figure 5 and 6).

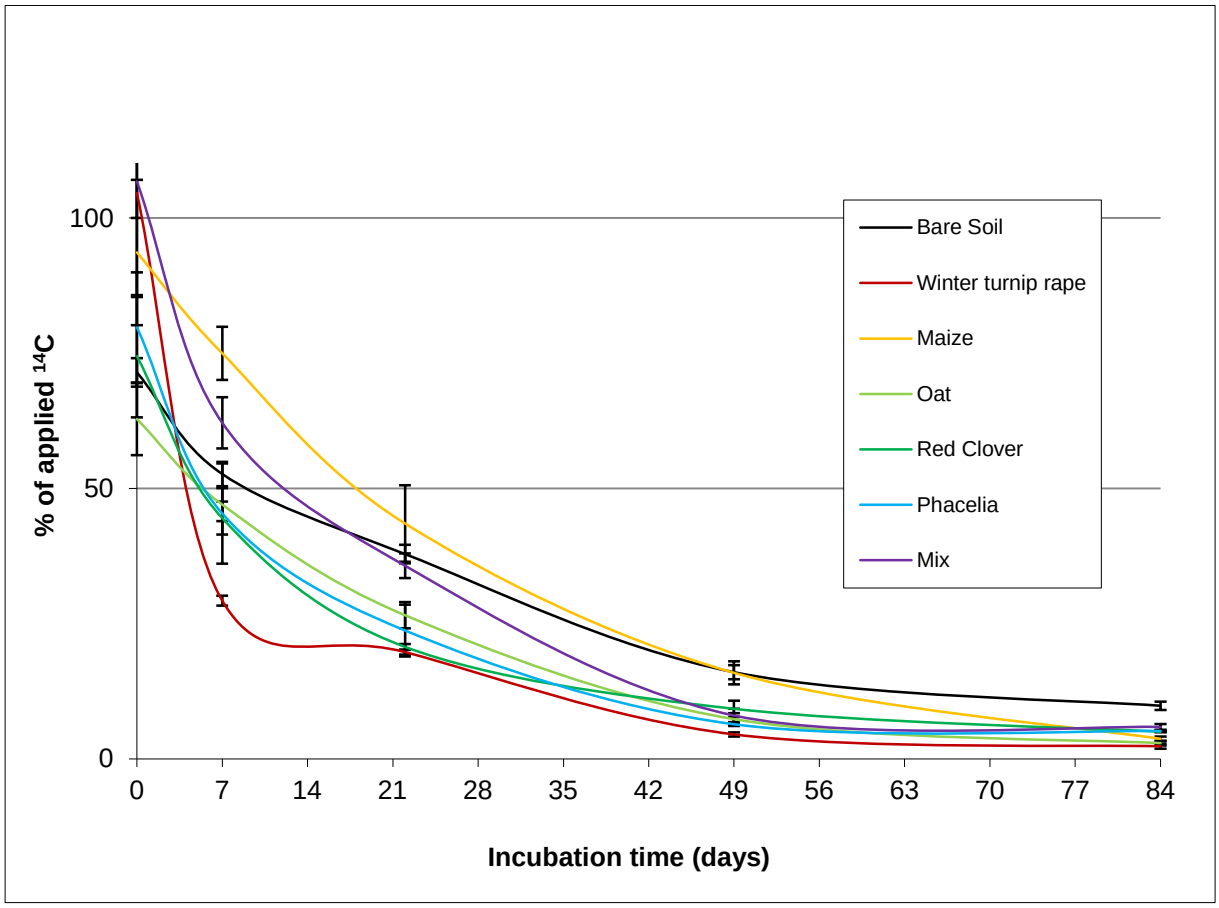

Figure 5: Degradation kinetics of ^{14}C-S-metolachlor over 84 days (crop residues and soil effect cumulated).

Data suggest some inaccuracies in measurements of ^{14}C-S-metolachlor degradation proper to our experimental approach. Effectively some values observed were slightly over 100% which would theoretically be impossible. This lack of precision was also by relatively high standard deviations, especially for the winter turnip rape data.

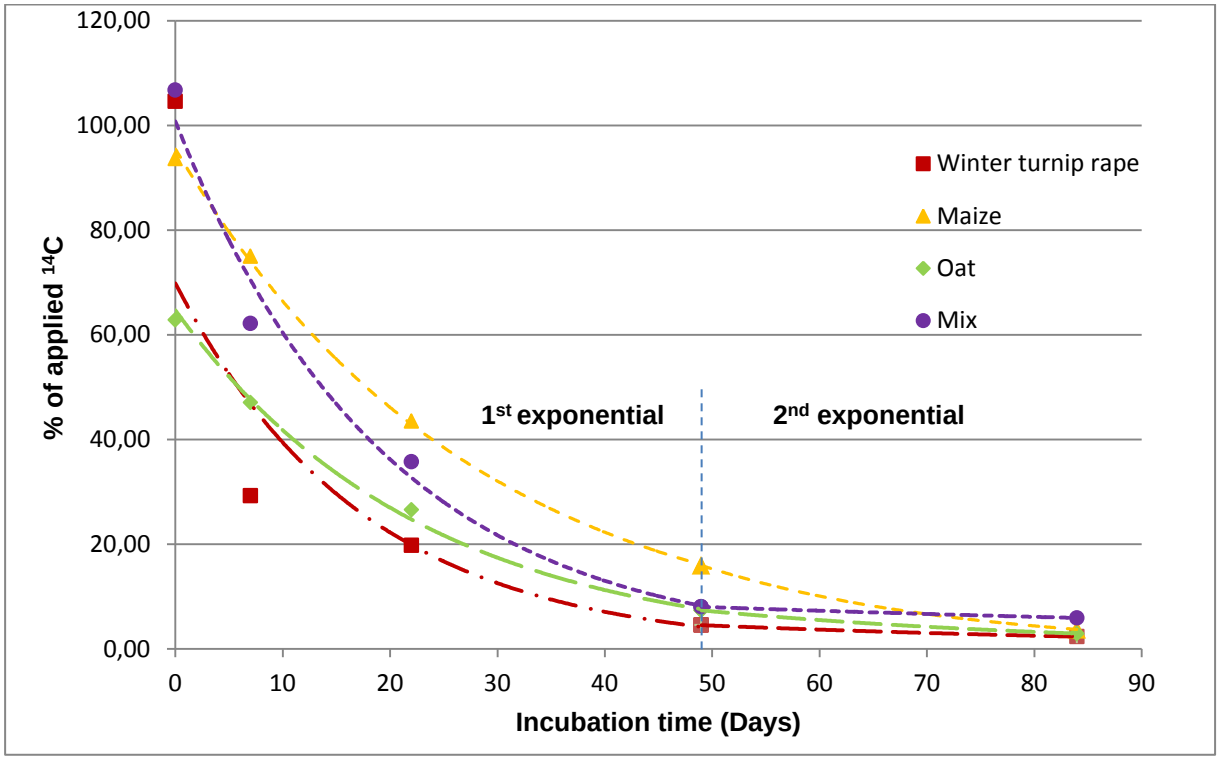

Figure 6: Correlation between the simulated evolution of C_{14} S-metolachlor degradation using an exponential model and data points measures from the different crop residues over the 84 days of the experiment. *Curves were fitted with first-order kinetics ($C_{(t)} = C_0 e^{-kt}$) represented by the discontinued lines. The quantity of ^{14}C-S-metolachlor in the different treatments were represented by the points.*

Table 1: Estimated half-life (DT50) of the ^{14}C-S-metolachlor for each substrate

Type of cylinder	Period concerned	exponential approach (C=C0exp(-kt))	Correlation coefficient	DT50 t = ln2/k
Bare Soil	[0;49]	y=69,289exp(-0,0296t)	r^2=0,99	24
Winter turnip rape	[0;49]	y=69,843exp(-0,0572t)	r^2=0,92	12
Maize	[0;49]	y=95,492exp(-0,0364t)	r^2=0,99	19
Oat	[0;49]	y=64,688exp(-0,0438t)	r^2=0,99	16
Red Clover	[0;49]	y=63,091exp(-0,0412t)	r^2=0,97	17
Phacelia	[0;49]	y=71,951exp(-0,0498t)	r^2=0,99	14
Mix	[0;49]	y=100,76exp(-0,0512t)	r^2=0,99	14
Bare Soil	[49;84]	y = 31,805exp(-0,014t)		50
Winter turnip rape	[49;84]	y = 11,493exp(-0,019t)		37
Maize	[49;84]	y = 122,22exp(-0,042t)		17
Oat	[49;84]	y = 26,841exp(-0,026t)		27
Red Clover	[49;84]	y = 21,782exp(-0,017t)		41
Phacelia	[49;84]	y = 8,6793exp(-0,006t)		116
Mix	[49;84]	y = 12,324exp(-0,009t)		77
Bare Soil	[0;84]	y=63,851exp(-0,0238t)	r^2=0,97	29
Winter turnip rape	[0;84]	y=56,349exp(-0,0419t)	r^2=0,90	17
Maize	[0;84]	y=98,317exp(-0,0385t)	r^2=0,99	18
Oat	[0;84]	y=59,249exp(-0,0375t)	r^2=0,99	19
Red Clover	[0;84]	y=54,713exp(-0,0311t)	r^2=0,94	22
Phacelia	[0;84]	y=56,809exp(-0,033t)	r^2=0,90	21
Mix	[0;84]	y=80,605exp(-0,0353t)	r^2=0,92	20

DT 50 calculated in the different samples ranged between 12 and 23 days. Metolachlor is considered to be moderately persistent in different soil types (U.S. EPA, 1997), and has an average DT 50 of 114 days (Kollman and Segawa, 2000). However, DT 50 values can be influenced by soil type and environmental conditions and DT 50 values have been reported to range between 15 and 132 days (USDA, 1995; Extoxnet, 2000; Kollman and Segawa, 2000).

Metolachlor half-life values similar to the one of our experiment have been reported in soils permanently covers by plants or plants residues It has been shown for example, that the metolachlor half-lives was only 10 days in a grass buffer strip soil, 23 days in the adjacent bare soil, 18 days in a hairy vetch filter strip soil and 38 days in the corresponding cultivated soil (Staddon et al., 2001). Otto et al., 1997 also reported a half life value of 9 days for metolachlor in a no till soil and a half life value of 29 days for the adjacent cultivated soil (Otto et al., 1997).

It has been suggested that the rate of pesticides breakdown could be influenced by factors such temperature, relative humidity, microbial activity, process of nitrification, oxygen availability, radiation, leaching and the nature of the growth media (Extoxnet, 2000). In this experiment, samples differed only by the nature of their crop residues. Differences in pesticides breakdown were therefore attributable to the differences in the nature of the growth media.

Data suggest that the presence of crop residues enhanced the rate of degradation of the ^{14}C-S-metolachlor. This was shown by lower values of DT50 in all samples with crop residues when compare to the bare soil one. This was consistent with the founding of Teasdale et al. showing an acceleration of metolachlor degradation between 1.5 and 3 times greater in vetch residues covers than in bare soil (Teasdale et al., 2003).

For most treatments, the increase in speed of mineralisation of the organic carbon seems to be associated to a shorter half-life (DT50) of degradation of the ^{14}C-S-metolachlor. This result suggested that the degradation of the crop residues enhanced the degradation of the ^{14}C-S-metolachlor.

Only the mix and the red clover did not follow this pattern. These differences in trends could be explained by the specificity of the unique chemical mix present in the mix residues and possibly by the particularly high level of Nitrogen present in the red clover residues. Effectively, red clover are known to be particularly efficient in fixing Nitrogen (Fageria, 2005).

For the second period, day 49 to day 84, exponential approaches were calculated from only two values, which mean that no coefficient of correlation could be established. Consequently, the half life values should be considered with caution.

Longer half-life were found in the 49 to 84 days period for all treatments when compared to the first calculated DT50, except for the maize cover which remained similar to the first DT 50.

3.6.^{14}C-S-metolachlor metabolites

Analyse of HPLC reports highlighted the predominance of certain metabolites. We presented the most frequent one in the following table (table 2). We characterized the main metabolites by their C18 column residence time, their date of appearance, meaning the first extraction date in which the metabolite was identified, their number of appearances and the relative proportion of each metabolite compare to the sum of all the identified metabolites.

Table 2: Identification and quantification of the ^{14}C-S-metolachor associated metabolites.

Identified Metabolites	C18 column residence time	Date of appearance	Number of appearance	Quantitative Importance (all HPLC analyses cumulated)	Relative Proportion
all metabolites (SMOC excluded)	[2,70;38,80]		422	4026,94	100
Xa	[28,60;29,00]	d7 (d0 for 2 values)	79	1162,07	28,86
Xb	[25,80;26,00]	d7	35	305,2	7,58
Xc	[28,30;28,40]	d7 (d0 for 2 values)	19	259,49	6,44
Xd	[27,90;28,10]	d22 (d7 for 1 value)	28	214,32	5,32
Xe	[24,20;24,40]	d22 (d7 for 1 value)	14	84,37	2,10
Xf	[14,50;14,60]	d22	10	82,32	2,04
Xg	[26,20;26,30]	d7	11	72,02	1,79
Key periods					
A1	[27,60;29,30]		142	1821,81	45,24
A2	[25,40;26,90]		69	500,27	12,42
A3	[23,70;24,80]		25	150,21	3,73
A4	[11,50;17,30]		107	1048,3	26,03
A5	[3,60;5,40]		34	263,09	6,53

The major breakdown process of metolachlor in the soil is considered to occur through aerobic and anaerobic microorganisms activities and the two by-products generally identified through this degradation pathway are metolachlor ESA-(ethane sulfonic acid) metolachlor OA – oxanilic acid (Barbash et al., 1999). However, in this experiment, only few peaks were observed during the 12-14 minutes period characteristics of the Metolachlor ESA and Metolachlor OA metabolites. This suggests that the S-metolachlor degradation pathway described in this experiment could be different than the one usually found in the literature.

The existence of at least one alternative pathway of the S-metolachlor degradation has been published in an experiment using sandy loam soil, showing the (2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl) amino oxo-acetic acid as the primary by-product of this degradation process (WSSA, 1994). Further investigation would be necessary to identify whether the pathway observed in our experiment would be similar to the one presented by WSSA (1994).

We represented the evolution of the concentration of the four main metabolites during the experiment in the cover crops and in the bare soil in the figure 7 and 8.

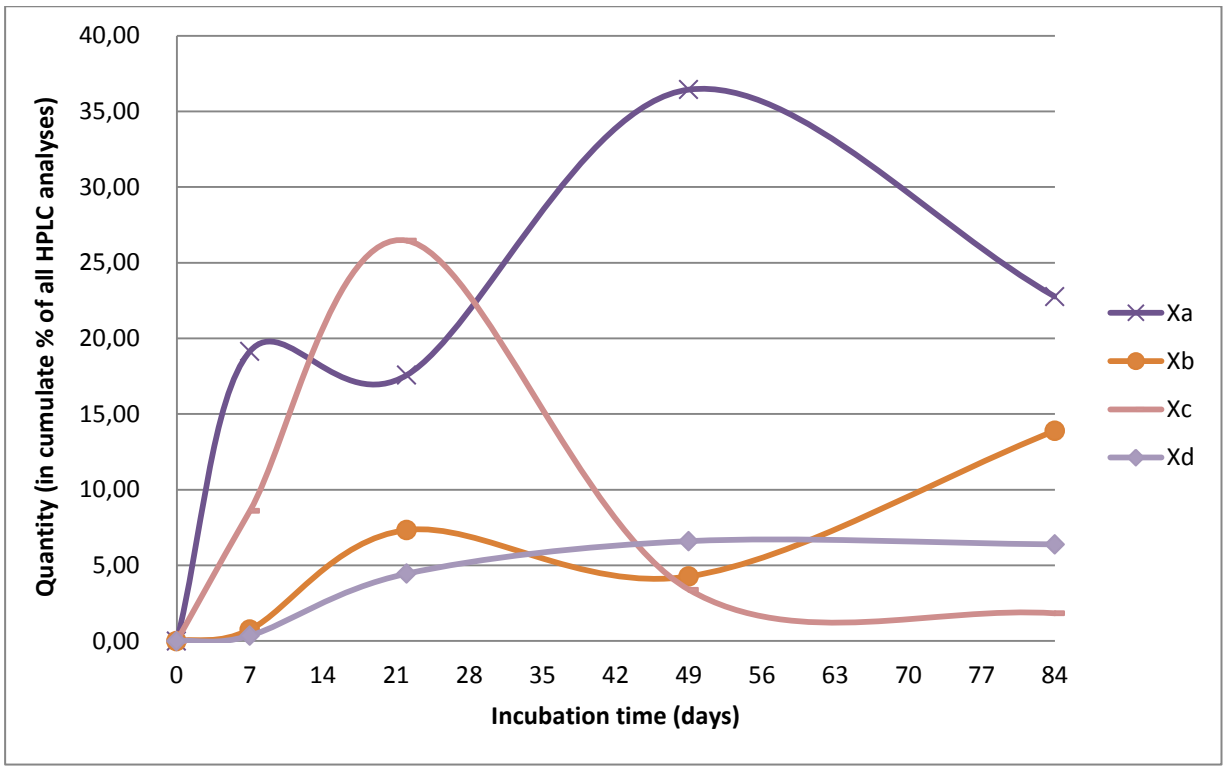

Figure 7: Change in total concentration of Xa, Xb, Xc, Xd metabolites produced across all the samples with crop residues over the 84 days period.

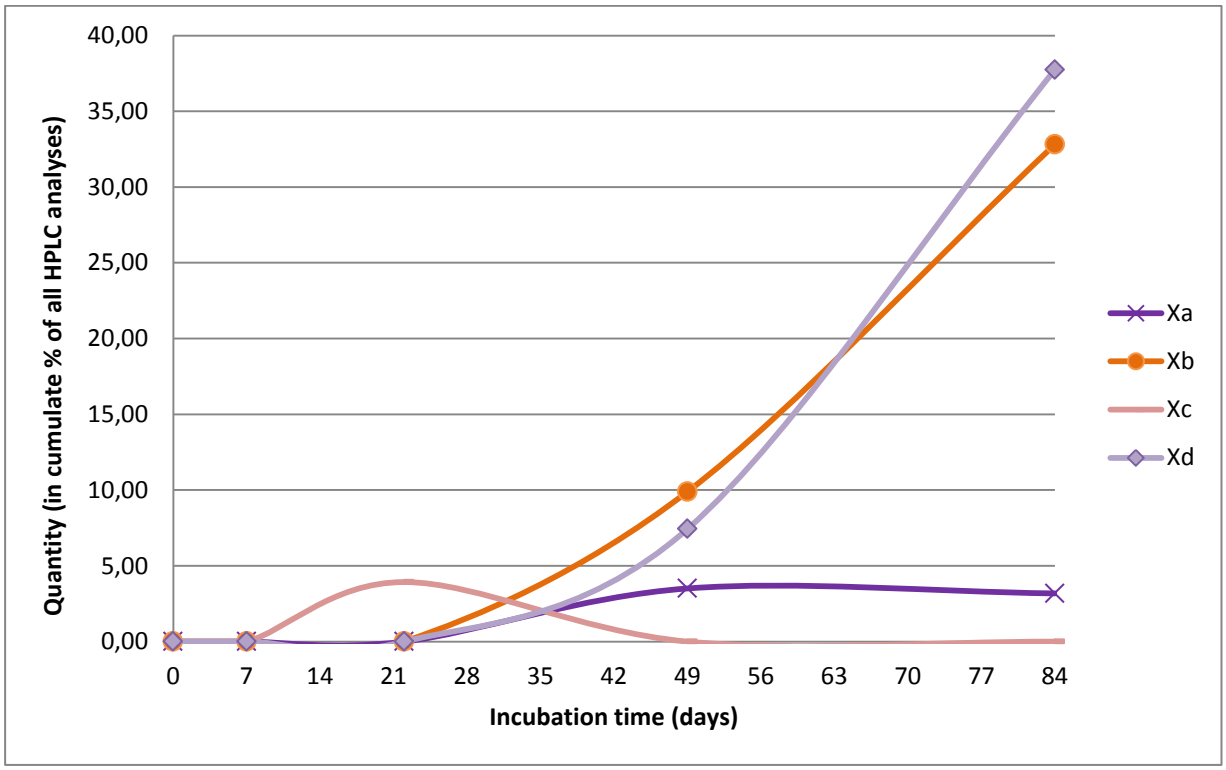

Figure 8: Change in total concentration of Xa, Xb, Xc, Xd metabolites produced by the bare soil sample over the 84 days period.

Differences in time of appearance of the different metabolites concentration appears between the treatments with crop residues and the bare soil samples. It seems that late increase in the relative concentration of Xb and Xd metabolites could be specific to the extracts collected from the bare soil sample. Early production of Xa and Xc metabolites seems to be associated to the extracts collected from samples with crop residues. This suggests that the degradation of ^{14}C-S-metolachlor could results from two chronological and special steps processes with an early degradation of metolachlor into Xa and Xc occurring in the treatments with crop residues and a further degradation, possibly after leaching in the bare soil into Xb and Xd.

4. Conclusion

This laboratory study showed the following:

1- the mineralisation of the ^{14}C-S-metolachlor was increased with the maize residues and reduced with the cover crops residues (Figure 3)

2- the crop residues proved to be efficient in intercepting ^{14}C-S-metolachlor (Figure 4)

3- the cover crop residues fixed a large amount of ^{14}C residues under the form of bond residues (Figure 4)

4- the cover crop residues and in more limited way the maize residues, enhanced the degradation of the ^{14}C-S-metolachlor (Figure 5,6 & Table 1)

The contrasting high degradation and low mineralisation of the C_{14}S-metolachlor in the cover crop residue samples suggest that the degradation of ^{14}C-S-metolachlor in these systems is only partial. Effectively, the ^{14}C benzene circle present in the ^{14}C-S-metolachlor molecule is the most stable structural feature of this molecule. It is possible that the microorganism populations present in the rich fresh residues were only efficient in partially but rapidly degrading the ^{14}C-S-metolachlor molecule, yet preserving the integrity of the ^{14}C benzene circle and therefore preventing its mineralization. In bare soil however, where microorganisms grow on poorer substrate and must be more efficient in extracting metabolite to survive, it is possible that the ^{14}C benzene circle was more efficiently degraded and the ^{14}C-S-metolachlor mineralisation completed.

This suggests that the presence of crop residues at the soil surface could potentially have beneficial effects by reducing the environmental impact of pesticides used in agriculture and that the benefits seems larger with fresh cover crop residues compare to aged maize residues. However, further investigation in the field would be necessary to validate the founding of this study. Our experimental model could be made more realistically correlated with what is happening in the field by simulating rain falls. It has been suggested that differences observed between experiment carried out in the field and those carried out in laboratories could be explained by enhancing leaching that naturally occurs in the field following a rainfall event (Gaston et al., 2001).

5. References

Ahrens, W. H., 1994. Herbicide handbook**.** 7.ed., 352.

Alletto L., Coquet Y., Benoit P., Heddadj D., Barriuso E., 2010. Tillage management effects on pesticide fate in soils. A review. Agron. Sustain. Dev. 30. 367–400.

Aslam, S. 2010. Impact of organic mulch on pesticide dynamics under conservation agriculture. Unpublished data.

Banks P.A., Robinson E.L., 1986. Soil reception and activity of acetochlor, alachlor, and metolachlor as affected by wheat (Triticum aestivum) straw and irrigation, Weed Sci. 34, 607-611.

Barbash, Jack E., Gail P. Thelin, Dana W. Kolpin, and Robert Gilliom. 1999. Distribution of major herbicides in ground water of the United States. U. S. Geological Survey, Water-Resources Investigations Report, 98 – 4245.

Beulke, S., Brown, C.D., 2001. Evaluation of methods to derive pesticide degradation parameters for regulatory modelling. Biol. Fertil. Soils 33, 558-564.

Bottomley, P.J., Sawyer, T.E., Boersma, L., Dick, R.P., Hemphill, D.D., 1999. Winter cover crop enhances 2,4-D mineralization potential of surface and subsurface soil. Soil Biol. Biochem. 31, 849-857.

Boyd S.A., Xiangean J., Lee J.F., 1990. Sorption of nonionic organic compounds by com residues from a no-tillage field, J. Environ. Qual. 19, 734–738.

Chuette, D., 2012. Etude des variations biochimiques et des propriétés de mouillabilité de mulchs de différents couverts végétaux au cours de leur décomposition. Unpublished data.

Currie, R.S., Klocke, N.L., 2005. Impact of a terminated wheat cover crop in irrigated corn on atrazine rates and water use efficiency. Weed Science 53, 709-716.

Dao T.H., 1991. Field decay of wheat straw and its effects on metribuzin and S-ethyl metribuzin sorption and elution from crop residues, J. Environ. Qual. 20, 203-208.

Drury, C.F., Tan, C.S., Welacky, T.W., Oloya, T.O., Hamill, A.S., Weaver, S.E., 1999. Red clover and tillage influence on soil temperature, water content, and corn emergence. Agron. J. 91, 101-108.

Extoxnet : Extension Toxicology Network, 2000. Pesticide information profile : metolachlor

Fageria N. K., 2005. Role of Cover Crops in Improving Soil and Row Crop Productivity. Communications in Soil Science and Plant Analysis. 36, 2733-2757.

Gaston, L.A., Boquet, D.J., Bosch, M.A., 2001. Fluometuron wash-off from cover crop residues and fate in a loessial soil. Soil Sci. 166, 681-690.

Gaston L.A., Locke M.A., 2000. Acifluorfen, sorption, degradation, and mobility in a Mississippi delta soil, Soil Sci. Soc. Am. J. 64, 112–121.

Gilliom, R. J., 2007. Pesticides in the Nation's streams and ground water. Envirn. Sci. Techno. 41 (10), 3408-3414.

Isss-Isric-Fao, 1998. World Reference Base for Soil Resources. In: Spaargaren, O.C.(Ed.) ISSN: 0532-0488 Wageningen/Rome.

Justes, E., Mary, B., Nicolardot, B., 1999. Comparing the effectiveness of radish cover crop, oilseed rape volunteers and oilseed rape residues incorporation for reducing nitrate leaching. Nutr. Cycl. Agroecosyst. 55, 207-220.

Klute, A., 1986. Water retention: laboratory methods. In: Klute, A. (Ed.), Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods. Agronomy Monograph, vol. 9. ASA and SSSA, Madison, WI. 635-652.

Kolpin , D.W., Thurman, E.M., Linhart, S.M., 1998. The environmental occurrence of herbicides; the importance of degrades in ground water. Arch. Environ. Contamin. Toxicol. 35, 385-390.

Kollman, W., R. Segawa, 2000. Pest Chemistry Database. Environmental Hazards Assessment Program. Californian Department of Pesticide Regulation. Sacramento, CA.

Kotoula-Syka E., Hatzios K.K., Berry D.F., Wilson H.P., 1997. Degradation of acetanilide herbicides in history and non-history soils from Eastern Virginia. Weed Technol 11, 403–409.

Krutz L.J., Gentry T.J., Senseman S.A., Pepper I.L., Tierney D.P., 2006. Mineralisation of atrazine, metolachlor and their respective metabolites in vegetated filter strip and cultivated soil. Pest Management Science. 62, Issue 6, 505–514.

Lavorenti A., Rocha A.A., Prata F., Regitano J.B., Tornisielo V.L., Pinto O.B. , 2003. Reactions of diclosulam in a Rhodic Hapludox soil under no-till and conventional tillage systems, Rev. Bras. Cienc. Solo 27, 183–190.

Locke M.A., 1992. Sorption-desorption kinetics of alachlor in surface soil from two soybean tillage systems, J. Environ. Qual. 21, 558–566.

Malik, R.K., Green, T.H., Brown, G.F., Mays, D., 2000. Use of cover crops in short rotation hardwood plantations to control erosion. Biomass Bioenerg. 18, 479-487.

Martinez Salgado M.M., Gutiérrez Romero V., Jannsens M., Ortega Blu R. 2010. Biological soil quality indicators: a review. Microbiology Series Nº 2. VOL. 2. 319-328.

Mazzoncini M., Lorenzi R., Risaliti R., Sorce C., Ginanni M., Curadi M., Pini R., 1998. Diclofop-methyl dissipation in clay soil under different tillage systems in central Italy, Soil Till. Res. 46, 241–250.

Miller J.L., Wollum A.G., Weber J.B., 1997. Degradation of 14C-atrazine and ^{14}C-metolachlor in soil from four depths. J. Environ Qual. 26, 633–638.

Novak J.M., Watts D. W., Hunt P.G., 1996. Long-term tillage effects on atrazine and fluometuron sorption in coastal plain soils, Agr. Ecosyst. Environ. 60, 165-173.

Otto S., Riello L., During R. A., Hummel H. E., Zanin G., 1997. Herbicide dissipation and dynamics modelling in three different tillage systems, Chemosphere 34, 163–178.

Potter, T.L., Bosch, D.D., Joo, H., Schaffer, B., Munoz-Carpena, R., 2007. Summer cover crops reduce atrazine leaching to shallow groundwater in southern Florida. Journal of Environmental Quality 36, 1301-1309.

Reddy K.N., Locke M.A., 1998. Sulfentrazone sorption, desorption, and mineralization in soils from two tillage systems, Weed Sci. 46, 494–500.

Reddy K. N., Locke M. A., Wagner S. C., Zablotowicz R. M., Gaston L. A., Smeda R. J., 1995. Chlorimuron ethyl sorption and desorption kinetics in soils and herbicide- dessicated cover crop residues, J. Agr. Food Chem. 43, 2752–2757.

Reddy K. N., Locke M. A., Gaston L. A., 1997. Tillage and cover crop effects on cyanazine adsorption and desorption kinetics, Soil Sci. 162, 501-509.

Reeves, D.W., 1994. Cover crops and rotations. In: Hatfield, J.L., Stewart, B.A. (Eds.), Advences in soil science: Crops residue management. Lewis, Boca Raton, FL.

Rice P.J., Anderson T.A. and Coats J.R., 2002. Degradation and persistence of metolachlor in soil : effects of concentration, soil moisture, soil depth and sterilization. Environ Toxicol Chem. 21, 2640-2648.

Ritter, W.F., Scarborough, R.W., Chirnside, A.E.M., 1998. Winter cover crops as a best management practice for reducing nitrogen leaching. J. Contam. Hydrol. 34, 1-15.

Rivard, L. , 2003. Environmental fate of metolachlor. Environmental Monitoring Branch. Department of Pesticide Regulation. Califomia, 14 .

Sadeghi, A.M., Isensee, A.R., 1997. Alachlor and cyanazine persistence in soil under different tillage and rainfall regimes. Soil Sci. 162, 430-438.

Staddon W.J., Locke M.A., Zablotowicz R.M., 2001. Microbiological characteristics of a vegetative buffer strip soil and degradation and sorption of metolachlor. Soil Sci Soc Am J 65, 113–1142.

Swanton, C.J., Shrestha, A., Roy, R.C., Ball-Coelho, B.R., Knezevic, S.Z., 1999. Effect of tillage systems, N, and cover crop on the composition of weed flora. Weed Science 47, 454-461.

Teasdale J. R., Shelton D. R., Sadeghi A. M., Isensee A. R., 2003. Influence of hairy vetch residue on atrazine and metolachlor soil solution concentration and weed emergence, Weed Sci. 51, 628–634.

Unger, P.W., Vigil, M.F., 1998. Cover crop effects on soil water relationships. Journal of Soil and Water Conservation 53, 200-207.

U. S. Department of Agriculture Alternative Crops and Systems Lab., 1995. ARS Pesticide Properties : Metolachlor.

U. S. Environmental Protection Agency : Office of Pesticide Programs, 1997. The acetanilide pesticides : alachlor, metolachlor, and acetochlor.

White, P.M., Potter, T.L., Bosch, D.D., Joo, H., Schaffer, B., Munoz-Carpena, R., 2009. Reduction in metolachlor and degradate concentrations in shallow groundwater through cover crop use. J. Agric. Food Chem. 57, 9658 - 9667.

Weed Science Society of America, 1994. Herbicide Handbook 7th Edition, Champaign, IL. 197–200.

Worsham, A.D., 1991. Role of cover crops in weed management and water quality. In: Hargrove, W.L. (Ed.), Cover Crops for Clean Water. Soil and Water Conservation Society, Ankeny, Iowa. 141-145.

Zablotowicz, R.M., Locke, M.A., Smeda, R.J., 1998. Degradation of 2,4-D and fluometuron in cover crop residues. Chemosphere 37, 87-101.

Zablotowicz R. M., Locke M. A., Gaston L. A., Bryson C. T., 2000. Interactions of tillage and soil depth on fluometuron degradation in a Dundee silt loam soil. Soil Till. Res. 57, 61–68.

.

Confidential: No

Author: Jules Hatier **Year: 2012**

Department:

(Do not write in this box)

Fate of ^{14}C-S-metolachlor in crop residues and underlying soils

Key-words : interception, retention, mineralisation, degradation
Mots clés: interception, rétention, minéralisation, dégradation

Résumé:

Le S-métolachlore est un herbicide de la famille des chlorocétanilides largement utilisé en désherbage pré-levée et post-levée sur une diversité de céréales comprenant le maïs (*Zea mays* L.), le coton (*Gossypium hirsutum* L.) ou encore le soja (*Glycine max* L.). Le S-métolachlore et ses métabolites sont couramment retrouvés dans les eaux de surface et les eaux souterraines situées à proximité de cultures où cette molécule est appliquée. Cette étude cherche à évaluer l'effet de résidus de couverts végétaux et de résidus d'une culture précédente sur l'interception, la rétention, la dégradation et la minéralisation du S-métolachlore. Dans ce but, nous avons sélectionné quatre types de résidus de couverts végétaux : la navette (*Brassica rapa* L. *subsp. oleifera*), l'avoine (*Avena Sativa* L.), le trèfle rouge (*Trifolium rubens* L.) et la phacélie (*Phacelia tanacetifoli* L.) ; un type de résidus de culture, le maïs (*Zea mays* L.), une modalité composée d'un mélange de quatre de ces cinq espèces ainsi qu'une modalité témoin de sol nu. Les résidus de couverts végétaux et de maïs se sont montrés efficaces dans l'interception du S-métolachlore et ont permis d'accélérer sa dégradation. Les résidus de couverts végétaux ont également stabilisé une part importante des métabolites du S-métolachlore sous forme de résidus liés. Ces résultats suggèrent que la présence d'une couche de résidus végétaux en surface a un effet positif sur l'environnement en limitant l'impact du S-métolachlore utilisé en agriculture. Cependant, des études aux champs sont nécessaires pour valider les résultats obtenus en condition de laboratoire.

Abstract:

S-metolachlor is a chlorocetanilide herbicide widely used as a pre-emergence and post-emergence weed control in a variety of crops such as maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L.). S-metolachlor and its metabolites are commonly found in surface and groundwater close to fields where this molecule is applied. This study aimed to evaluate the effect of crop residues on the interception, retention, degradation and mineralisation of ^{14}C-S-metolachlor. One bare soil modality, four modalities with cover crop residues from one single species such as winter turnip rape (*Brassica rapa* L. *subsp. oleifera*), oat (*Avena Sativa*), red clover (*Trifolium rubens* L.) and phacelia (*Phacelia tanacetifoli* L.), one modality with four months aged maize residues (*Zea mays* L.), and one modality with a mix of residues of four species were used in this study. The crop residues were efficient in intercepting ^{14}C-S-metolachlor and enhancing its degradation. The fresh cover crop residues were also fixing a large amount of ^{14}C-S-metolachlor residues under the form of bond residues. This suggests that the presence of cover crops residues could potentially have a beneficial effect and reduce the environmental impact of the S-metolachlor used in agriculture. However, further investigation in the field would be necessary to validate the founding of this study.

Total number of volumes: 1
Number of pages of the main document: 39