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Influence of cover crops on the presence and transfer of pesticides in agricultural soils

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Abbreviation	Meaning
AHDB	The British Agriculture and Horticulture Development Board
AMPA	Aminomethylphosphonic acid
CEC	Cationic Exchange Capacity
CRA-W	Centre wallon de Recherches agronomiques
DOC	Dissolved Organic Carbon
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GUS	Groundwater Ubiquity Score
HPLC	High-Performance Liquid Chromatography
IPBES	Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services
K_H	Henry's law constant
LC-MS/MS	Liquid Chromatography with tandem Mass Spectrometry
MOCA	Mineral & Organic Chemical Analysis
OM	Organic Matter
PCA	Principal Component Analysis
PSW	Public Service of Wallonia
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SPGE	Société publique de gestion de l'eau

1. Introduction

Pesticides play a significant role in modern agriculture, by eliminating pests and boosting crop yields (Benton et al., 2021). However, the extensive use of these compounds presents a serious risk to human health and the environment. They persist in soils and water bodies, causing contamination and ecological damage (Defourny, 2022; Silva et al., 2019). Pesticides undergo many processes in the soil, including retention, degradation, and transfer to other reservoirs, influenced by numerous factors (Tudi et al., 2021). Understanding these processes is crucial to mitigate the persistence and toxicity of pesticides and their associated risks (IPBES, 2018).

The utilisation of cover crops, defined as non-cash crops grown to benefit soil, is becoming increasingly prevalent in agriculture (Kathage et al., 2022). Their primary function is to cover bare soil between cash crops. The use of cover crop species, such as winter barley, phacelia, and clover, has been demonstrated to provide multiple benefits, including nitrogen fixation, improved soil health, and reduced soil erosion (Alletto et al., 2010; Justes et al., 1999).

The utilisation of cover crops has the potential to mitigate the leaching of pesticides into waterways by improving soil parameters and reducing the transport of pesticides. Catch crops, which are primarily used to catch residual nitrates, have been demonstrated to have the capacity to also mitigate the impact of pesticides (Vogeler et al., 2023). Empirical evidence suggests that the use of cover crops can significantly reduce the concentrations of nitrates and pesticides in water (Gu et al., 2021). They enhance soil porosity, water retention, and microbial activity, thereby influencing soil parameters in a manner that decreases runoff and promotes biodegradation of the molecules (Calegari et al., 2020; Malik et al., 2000; Unger & Vigil, 1998).

The objective of this thesis is to assess the impact of winter cover crops on the presence and transfer of pesticides in agricultural soils. To achieve it, the impact of a living winter cover crop on the residual pesticide levels from the previous growing season in agricultural soils will be evaluated. To this end, two experimental approaches were developed: (i) a field experiment, followed by (ii) a greenhouse experiment.

The initial *in-situ* experiment was conducted on an agricultural plot, using a field setup. The placement of rhizons at two distinct soil depths permitted to follow the observation of pesticide transfer within the rhizosphere. The results indicated that pesticide concentrations were below the limit of quantification within the first month. This rapid decline may be attributed to the heavy autumn rainfall, which accelerated the leaching of the pesticide residues. Ultimately, this configuration was deemed unsuitable and was thus abandoned.

Subsequently, the field trial was transformed into a three-month greenhouse experiment, conducted from January to April 2024. A total of 19 pesticides (11 herbicides, 5 fungicides, 1 insecticide and 2 safeners), encompassing diverse range of physicochemical properties, were applied and analysed for concentration measurement. The concentrations were then compared over time under three conditions: (i) bare soil as a control modality; (ii) a winter cash crop (a winter cereal crop); and (iii) a winter catch crop (a multi-species cover crop). The comparisons were conducted in 10-litre pots containing agricultural soil.

2. Literature review

2.1 Pesticides in the environment

This initial section presents a comprehensive overview of pesticides employed in agricultural practices and their persistence in the environment. The following order is observed in the presentation of the topics: (i) the use of pesticides in agriculture; (ii) the risks associated with their presence for human and ecosystem health; and (iii) their occurrence in the environment and the processes that cause this presence.

2.1.1 Pesticides and phytopharmaceutical products

In recent decades, agriculture has undergone significant innovation, resulting in a substantial increase in crop yields (Benton et al., 2021). In Western Europe, this has resulted in a 68% increase in agricultural production since 1960 (FAO, 2005).

Several factors have contributed to the increase in agricultural productivity, which has been a consequence of both the industrial and the green revolutions. The most significant of these factors are varietal improvement, mechanisation, irrigation, and the application of synthetic fertilisers and pesticides (Alliot et al., 2022). This thesis will focus on pesticides as a standalone topic.

The term *pesticide* is derived from the English word *pest* and the Latin suffix *-cide*. The term *pest* encompasses any living pathogen, including fungi, insects, rodents, and plants, that causes harm to crops or livestock (Oxford Dictionary, 2023). The Latin verb *caedere*, meaning *to kill*, gave rise to the suffix *-cide*. The term *pesticide* is employed to describe a substance that targets and eliminates a crop pest. Consequently, pesticides are applied to crops with the objective of protecting them and increasing their yield (Tudi et al., 2021).

In the context of the agricultural sector, the term *phytopharmaceutical product* encompasses all synthetic pesticides. They may be composed of different chemical compounds, which are collectively referred to as active substances. These are the molecules that are chemically active and act on the plant physiology. Formulants are added to active substances to ensure stability and efficacy. Examples of such formulants include antifoam and antifreeze. The collective of the active substances and the formulants constitute the formulated product (Phytoweb, 2016). For example, Roundup® is a formulated product. The active substance is glyphosate, which is combined with several formulants, including ammonium sulphate (Mertens et al., 2018).

Active substances are typically classified according to their target organism (Dejardin, 2023). This encompasses herbicides (utilised for the control of weeds and grasses), fungicides, insecticides, and molluscicides (including snails and slugs). Phyto-protectants, such as safeners, or growth regulators lack a specific target organism. Conversely, they exert their effects on plant physiology, influencing processes such as growth and protection.

Pesticides can also be categorised according to their chemical nature (Dixon, 2004). This encompasses organic compounds (e.g., glyphosate, triazine, organochlorine compounds, organophosphorus compounds), mineral compounds (e.g., sulphur or copper-based molecules such as the Bordeaux mixture), and organo-mineral compounds.

It is also important to note that pesticides can also be classified according to other criteria, such as their degree of toxicity, their formulation format (granules, powders, etc.), and whether they are natural or synthetic (FAO, 2022).

2.1.2 Pesticide application on farmlands

The agricultural sector accounts for 85% of the total mass of pesticide usage, with the remaining amount being used to protect urban areas, such as gardens, public parks, and railroads (Kim et al., 2017). Global pesticide consumption in agriculture is estimated to be approximately three million tons annually (Tudi et al., 2021). The American continent accounts for the highest proportion of global pesticide consumption, representing over half of the total quantity consumed. Asia and Europe collectively account for 20% of global pesticide consumption, representing the second-largest consumer region after North America. Finally, Oceania and Africa collectively account for 5% of global pesticide consumption.

The average pesticide consumption in the 27 countries of the European Union (EU) is 3.3 kg ha^{-1} a^{-1} on farmlands, as reported by the Food and Agriculture Organization of the United Nations (FAO, 2022). Belgium is the fourth-largest user of pesticides, with an average application rate of 6.3 kg ha^{-1} a^{-1} applied (Figure 2-1), following the Netherlands, Cyprus, and the Republic of Ireland.

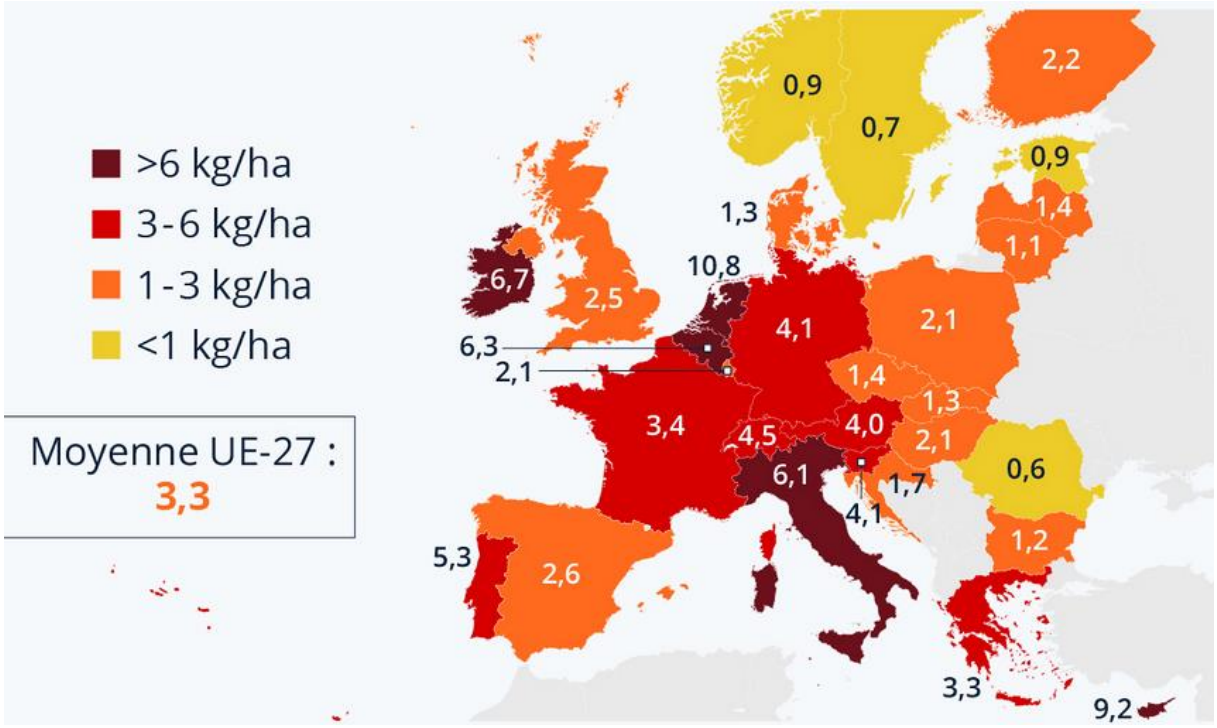

Figure 2-1: Mean pesticide consumption in 2020 [kg/ha of farmland] (FAO, 2022)

On a global scale, when the applied pesticide quantities are classified according to their target organism, herbicides are by far the most prevalent category used worldwide, with almost half of all products used (Figure 2-2; FAO, 2022). Glyphosate-based herbicides represent the most consumed substances globally, with an estimated annual consumption of over 800,000 tons of the active substance (Gandhi et al., 2021). Secondly, insecticides and fungicides/bactericides represent 25% of all pesticide use, respectively.

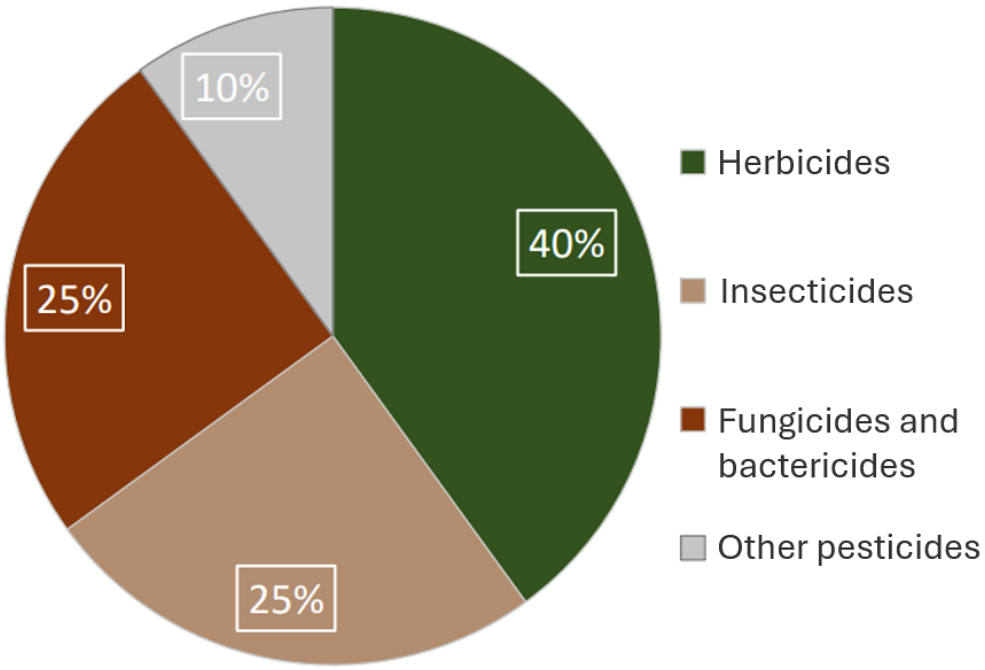

Figure 2-2: Proportion of distinct categories of pesticides applied worldwide in 2020 (FAO, 2022)

2.1.3 Environmental occurrence of pesticides

Given that pesticides are predominantly applied to agricultural soils, it is of paramount importance to ascertain their occurrence and behaviour in the environment. In accordance with the scientific literature, the term *residue* will be employed in this subsection, which refers to any remaining portion of pesticides and their metabolites in the environment (Silva et al., 2019). This subsection describes three environmental reservoirs: agricultural soils, surface waters and groundwater bodies (aquifers).

Agricultural soils

In 2019, Wageningen University conducted a scale analysis of EU agricultural soils using 317 topsoil samples. The findings indicated that 83% of agricultural soils exhibited the presence of between one and ten residues (Silva et al., 2019). Glyphosate is the residue most frequently detected, followed by its main metabolite, aminomethylphosphonic acid (AMPA; Figure 2-3).

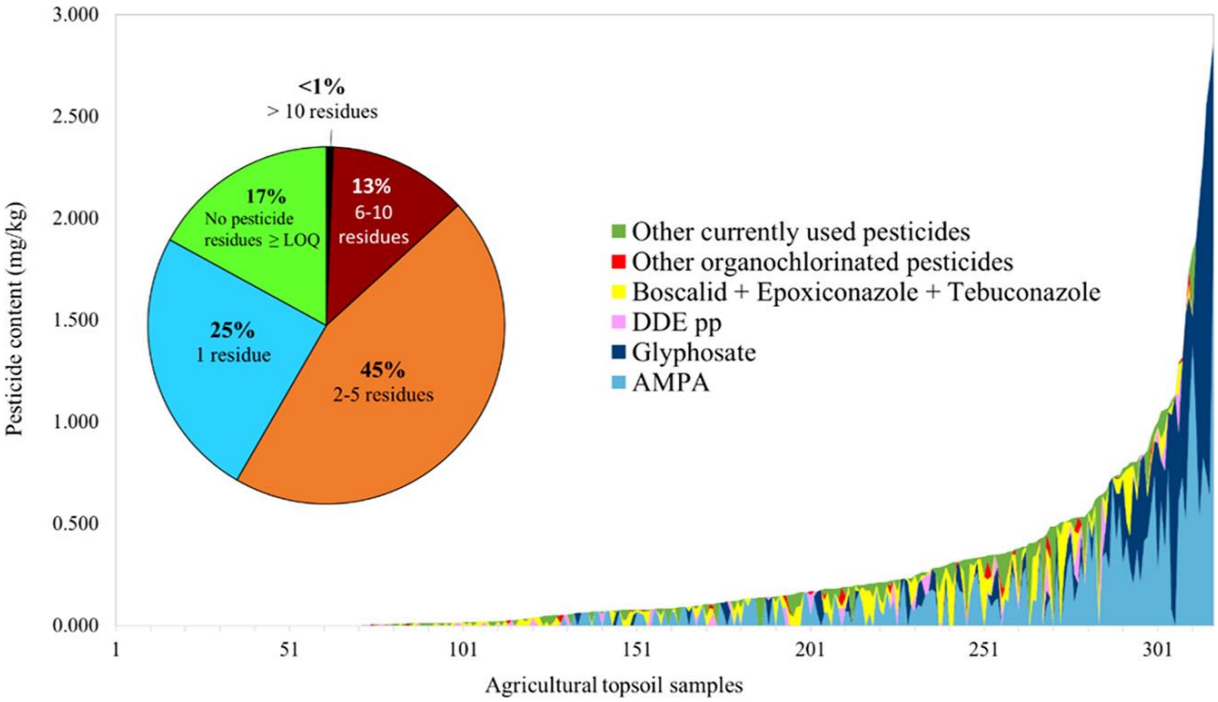

Figure 2-3: Pesticide distribution across the 317 EU agricultural topsoil samples. Topsoil samples (numbered from 1 to 317) were organized by increasing total pesticide content (Silva et al., 2019).

Furthermore, the European Commission has published a survey on soil analysis (Vieira et al., 2023). The study revealed that residues are present in 75% of the European agricultural soils (Vieira et al., 2023). Among the contaminated soils, 57% of the assessed sites contain mixtures of several pesticides, and 11% have been monitored with more than 10 different substances. Given that agriculture accounts for 38% of the total land area in the EU (157 million hectares), the survey concludes that over 28% of European land is contaminated with residues (Eurostat, 2022; Vieira et al., 2023).

Surface waters

According to Canopea, a Belgian environmental organisation, over 50% of lakes and rivers in Europe exhibit pesticide levels that can harm freshwater ecosystems (Defourny, 2022). At the European level, it is estimated that 7% of surface water fails to achieve good chemical status. This failure occurs when pesticide concentrations exceed the EU freshwater quality standards (Alliot et al., 2022). The threshold is reached when the quantity of the substance in question may cause a *disturbance to the environment*.

The Public Service of Wallonia (PSW) has published the ecological status of the Walloon surface waterbodies (SPW, 2020). The map presents the status of waterbodies in a colour-coded format, with blue representing a very good status and red representing a very poor status (Figure 2-4). It is notable that the regions with the highest levels of agricultural land use, such as Northern Wallonia, exhibit an overall poor status (yellow, orange, and red on the map).

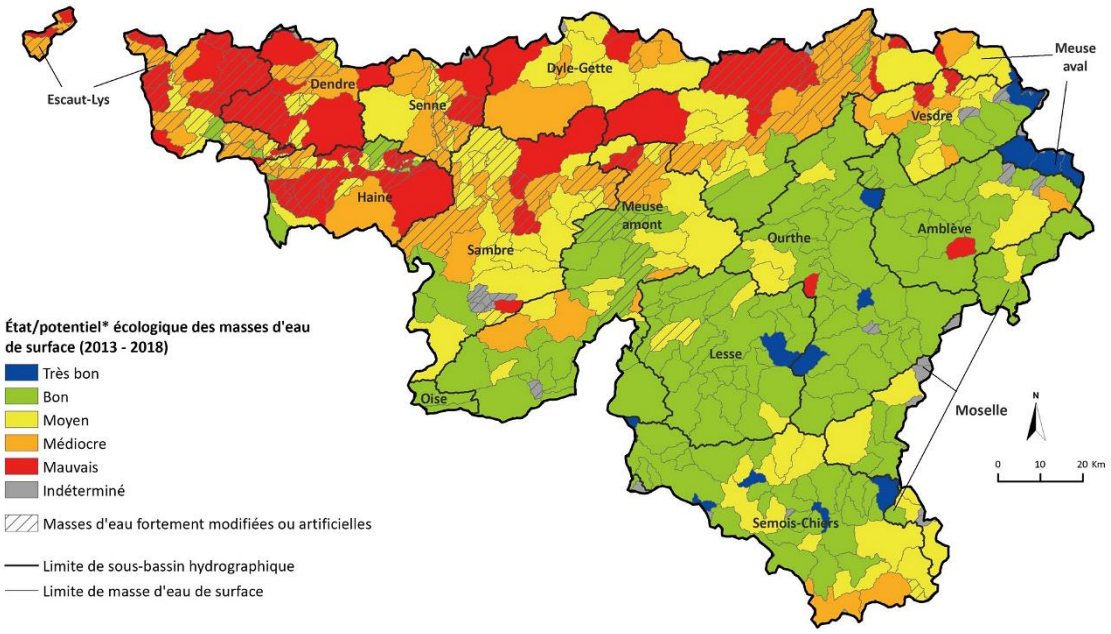

Figure 2-4: Ecological status of the 352 surface waterbodies in Wallonia between 2013 and 2018 (SPW, 2020).

Groundwater bodies

The primary sources of diffuse pollution of groundwater bodies were identified as nitrates and pesticides (Defourny, 2022). To illustrate, in the United States, Toccalino et al. (2014) discovered pesticides in 53% of the groundwater samples they tested.

In comparison to the EU, 6.5% of groundwater fails to meet the standards for good chemical status (see Surface waters, Alliot et al., 2022). In England, over 10% of groundwater sites used for drinking water were found to have pesticide concentrations exceeding the EU threshold (Swartjes & Van der Aa, 2020). Moreover, the same researchers demonstrated that pesticide concentrations in the Netherlands exceed 75% of the drinking water standards in 34% of total resources for drinking water.

In Wallonia, pesticides are responsible for more than 50% of the instances where groundwater body standards are exceeded (SPW, 2022). Specifically, 14 of the 34 groundwater bodies in Wallonia exhibit poor-quality status. The correlation between intensive farming in Wallonia and poor groundwater quality is analogous to that observed with freshwater quality (Figure 2-5, SPW, 2022).

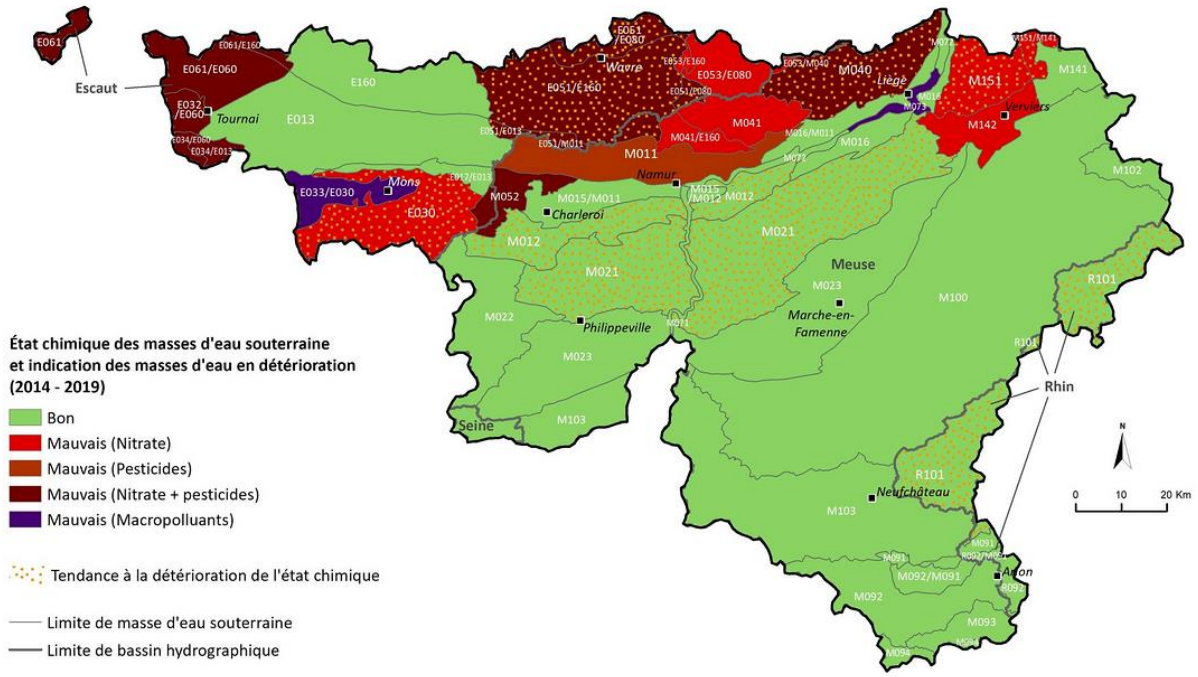

Figure 2-5: Chemical status of the 34 groundwater bodies in Wallonia between 2014 and 2019 (SPW, 2020).

2.1.4 Environmental risks associated with pesticide use

As previously demonstrated, pesticides applied to crops may persist in the environment as residues. The concentration of these pesticides in soil, water and ecosystems has been identified as a significant environmental risk factor due to their toxicity. The contamination of the environment generates social costs, which may be defined as negative externalities (Baumelle et al., 2023).

A case study conducted in China revealed that the annual externalities associated with pesticide application in rice systems amounted to up to US$1.4 billion (Pretty, 2007). These substantial costs are attributable to the adverse effects on human health and biodiversity.

Alliot et al. (2022) estimated that the social costs associated with the use of synthetic pesticides in France in 2017 ranged from €372 million to €8.205 billion. Among the three distinct sources of these hidden costs, environmental costs are by far the most significant (Figure 2-6).

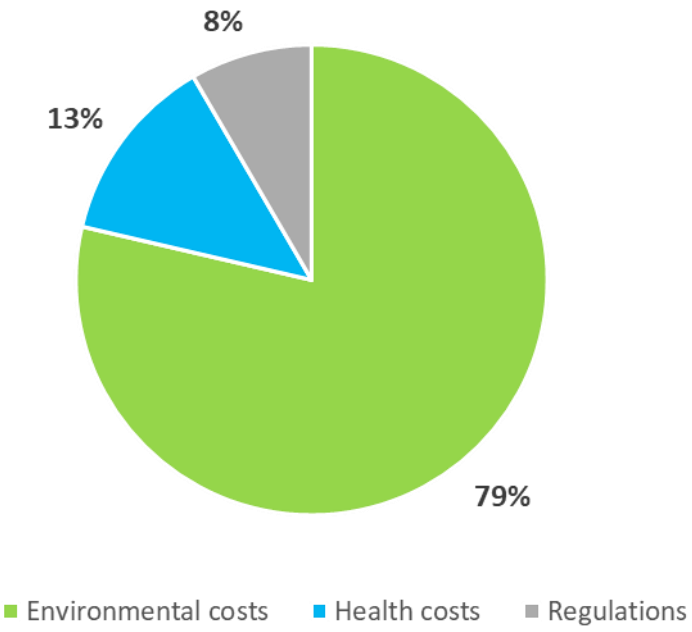

Figure 2-6: Sources of social costs due to synthetic pesticide use in France (Alliot et al., 2022).

Human health

Pesticides, which are often classified as toxic chemicals, have been observed to behave as endocrine disruptors, which can lead to the development of dangerous pathologies. These include disorders of the reproductive system, cancer, and neurodegenerative diseases. A *hazard* is defined as a potential source of harm, while a *risk* is the probability of a hazard causing harm (EFSA, 2016). It can be reasonably assumed that most farmers are at significant risk due to their chronic exposure to hazards, as a hazard becomes a risk when there is exposure. In France, Parkinson's disease is one of the diseases recognised as occupational among farmers (Defourny, 2022).

Biodiversity loss

The pervasive use of pesticides has become a significant threat to the health of ecosystems. These chemicals have been identified as a significant contributing factor in recent biodiversity loss (Baumelle et al., 2023).

A study by Dudley et al., (2017) found that over 40% of all invertebrate pollinators are at risk of extinction due to pesticide use. Consequently, the yields of crops that rely on these pollinators are diminished, and the agricultural economy is negatively impacted. Indeed, the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) estimates that approximately US$400 billion of crop value is directly dependent on pollinator populations (IPBES, 2018). Furthermore, a 75% decline in aerial insect biomass has been observed in Germany, largely attributed to agricultural intensification and augmented pesticide usage (Hallmann et al., 2017).

A total of 4,000 European monitoring sites have been investigated to ascertain the potential risks associated with organic chemicals for freshwater ecosystems. The toxicity of these chemicals to sensitive fish, invertebrate, and algal species has been demonstrated.

Indeed, acute lethal and chronic long-term effects are estimated at 14% and 42% of the sites, respectively (Malaj et al., 2014). Among the various classes of organic chemicals, three categories of pesticides—insecticides, fungicides, and herbicides—contribute significantly to the toxicity of these chemicals to freshwater ecosystems (Figure 2-7).

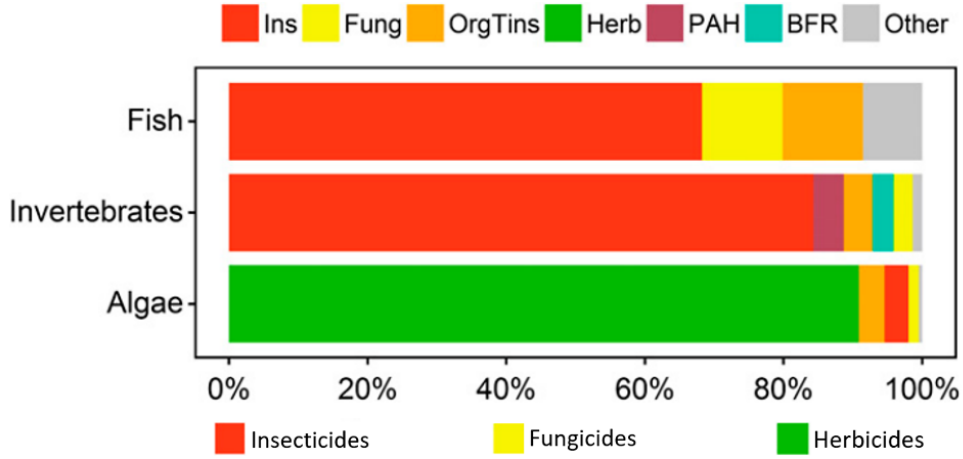

Figure 2-7: Proportion of sites acutely affected by different chemical groups (Malaj et al., 2014).

2.1.5 Environmental fate of pesticides

An appreciation of the environmental persistence of pesticides necessitates an evaluation of their behaviour. Pesticide residues can undergo numerous processes that control their environmental fate. The following subsection will present a detailed analysis of three key processes: soil retention, soil degradation, and transfer towards other reservoirs (Figure 2-8).

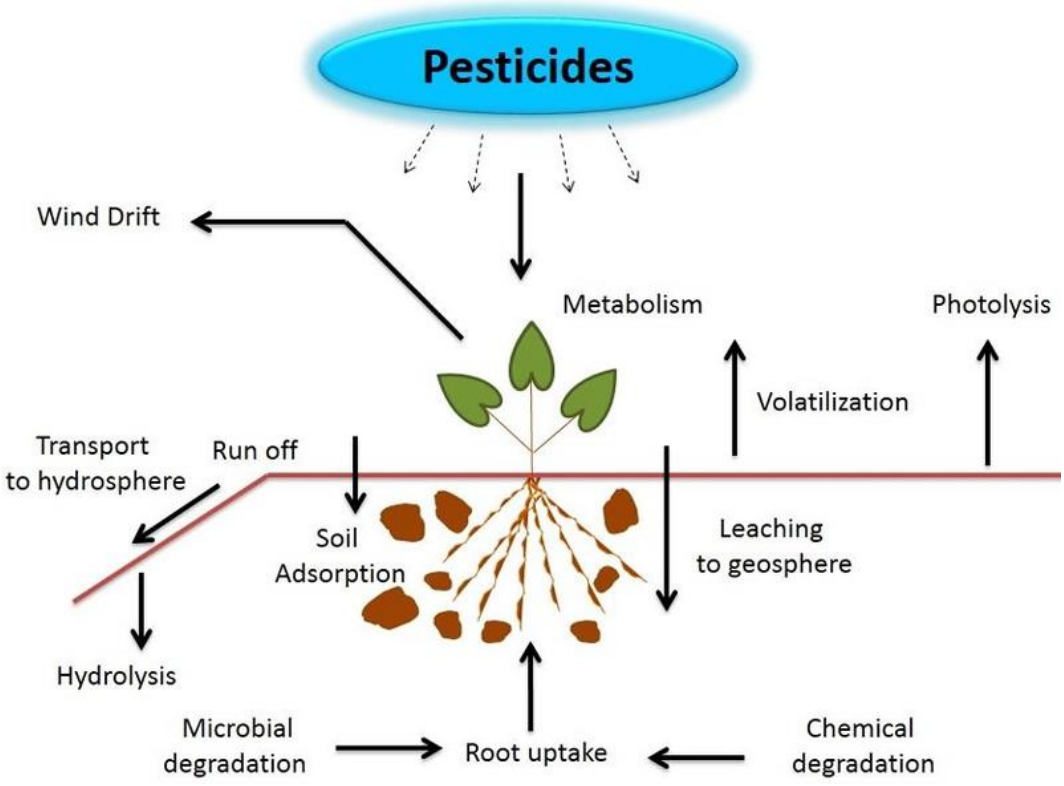

Figure 2-8: Environmental fate of pesticides in soils (Ahemad & Khan, 2013)

Soil retention

One of the principal processes by which pesticides may be retained in the soil is adsorption. Charged molecules have the capacity to adsorb onto soil particles, including organo-mineral complexes. The degree of sorption is contingent upon two distinct groups of factors: the soil parameters (pH, soil organic matter (SOM) content, cationic exchange capacity (CEC), etc.) and certain agricultural practices (such as mulch and vegetative cover). Research has demonstrated that SOM content is the parameter that exerts the greatest influence on pesticide retention in soil (Agnan & Pelletier, 2019; Alletto et al., 2010; Gu et al., 2021). Consequently, it has been observed that pesticide sorption is higher in soils with vegetative cover and organic amendments.

Degradation

In addition, pesticides can be degraded in the soil (Tudi et al., 2021). The three degradation types are as follows: (i) biodegradation (biolysis); (ii) chemical degradation; and (iii) photo-degradation (photolysis; Table 2-1).

Table 2-1: Types of pesticide degradation in the soil (Tudi et al., 2021).

	Biodegradation	*Chemical degradation*	*Photo-degradation*
Degrading agent	Soil microorganisms	Water, ions, molecules	Sunlight rays
Influencing factors	Soil moisture, temperature, oxygen, soil pH, SOM content, and soil porous structure	Soil temperature, soil moisture, soil pH, and pesticide binding onto soil particles	Intensity of light, length of exposure, and the half-life properties of the pesticide

In general, the composition of a substance and the external environment will determine the type of degradation that occurs in soil (Table 2-1). For example, a climate characterised by elevated levels of both sunlight and precipitation will result in a greater propensity for hydrolysis and photolysis. It can be further postulated that solar radiation will also elevate soil temperature, microbial activity, and thus biolysis.

When regard to the differing degradation processes observed in soil of varying depth, biodegradation is the principal degradation type observed in rhizosphere and deeper soils, while surface soil predominantly exhibits chemical and photo-degradation (Tudi et al., 2021).

Transfer

The final major process in the fate of pesticides in the environment is the transfer of molecules to other reservoirs than soil (Tudi et al., 2021). Following the application of pesticides to crops, they can be transferred to the atmosphere, surface waters, deeper soil and groundwater bodies (Ahemad, 2013). This transfer mechanism can be also classified into three types: (i) volatilisation, (ii) surface runoff, and (iii) leaching (Table 2-2).

Table 2-2: Types of pesticide transfer after application (Ahemad, 2013).

	Volatilisation	*Surface runoff*	*Leaching*
Mechanism	Conversion of a liquid or solid into a gas	Movement in water over a sloping surface	Vertical leaching towards soil depths
Landing reservoir	Atmosphere	Surface waters	Groundwater bodies
External influencing factors	Air: temperature, sunlight, evapotranspiration rate, humidity, air movement. Soil: texture, moisture, and SOM content	Area slope, soil texture, soil moisture, amount and timing of rainfall, irrigation	Air: evapotranspiration rate, temperature, and rainfall Soil: permeability, texture, moisture, and SOM content
Intrinsic influencing factors	Vapour pressure K_H	Water solubility, DT50	GUS leaching index, water solubility, DT50, K_{OC}

In contrast to degradation processes influenced by soil and environmental factors, the types of transfer will be rather induced by the pesticide's intrinsic properties (Table 2-2). Indeed, several of its physicochemical traits will give rise to a specific behaviour. For instance, a high vapour pressure indicates volatility and thus a tendency to volatilise. Furthermore, a high solubility, leachability, and half-life may result in increased leaching towards deep soil and groundwater.

Leaching and surface runoff are considered the most significant transfer types in terms of pollution (Alletto et al., 2010). These processes result in a reduction of groundwater quality and contamination of surface waters (Defourny, 2022).

2.2 Cover crops in agriculture

A definition of cover crops and their use in agriculture will be provided, followed by an examination of the benefits they offer to soils. Subsequently, a specific type of cover crop will be introduced: catch crops. The issue of excess nitrate fertilisers has already been addressed in this context; it may therefore be possible to extend the definition and use of cover crops to the case of pesticides.

2.2.1 Cash crop or cover crop?

In the context of agriculture, the term *cash crop* is defined as a primary crop cultivated for the purpose of generating profit (Eurostat, 2023). For example, common cereal cash crops include maize, wheat, rice and barley (FAO, 2005). Nevertheless, regarding the subject matter of this thesis, the focus will be on cover crops rather than cash crops.

The British Agriculture and Horticulture Development Board (AHDB, 2024), defines *cover crops* as non-cash crops that provide benefits to a rotation. It is not their purpose to generate profit; their primary function is to cover the bare soil during the period between two principal cash crops. Although the state of the cover crop may change over time, its role in covering the soil remains constant. The living biomass may die off and turn into dead litter, which will still physically protect the bare soil (Calegari et al., 2020).

2.2.2 Cover crop use in agriculture

The EU has observed an increase in the utilisation of cover crops on agricultural land, from 6.5% in 2010 to 8.9% in 2016 (Borrelli & Panagos, 2020). This proportion of European agricultural land remains relatively low, but there has been a slight increase (Kathage et al., 2022). With regard to the distribution of cover crops across Europe, some countries exhibit regions where the proportion of cover crops is considerable (Figure 2-9; Fendrich et al., 2023). The northern regions of France, the Benelux countries, Germany, and Denmark are at the vanguard of this trend. Conversely, the southern and eastern regions of Europe lag in terms of the area dedicated to cover crops.

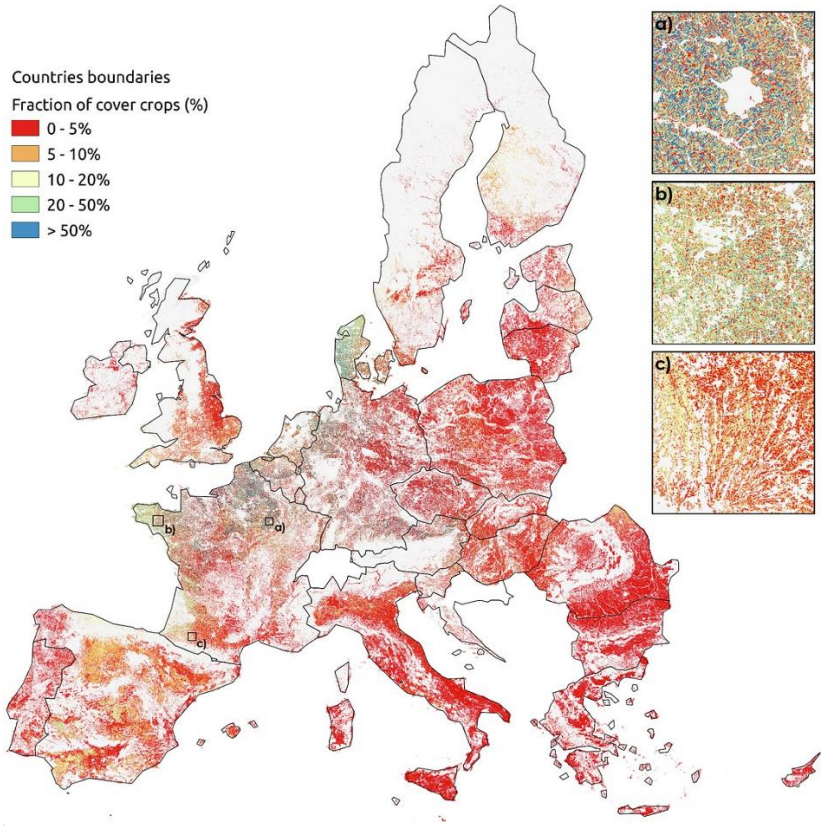

Figure 2-9: Left: model predictions of the occurrence of cover crops in Europe. Right: Three zooms: Predictions on the East, West and South of France (a, b, and c, respectively, Fendrich et al. 2023).

Winter cover crop species

Some cover crops can be sown in the summer between two cash (or forage) crops, whereas others are utilised in the winter between the autumn harvest and the next spring crop. Two distinct categories of winter cover crops can be identified: inter-cash crops (which also serve as soil cover) and non-cash cover crops (AFDA, 2021).

On the one hand, multiple non-cash crops are commonly used as winter cover crops, including winter barley (*Hordeum vulgare*), winter wheat (*Triticum aestivum*), oats (*Avena sativa*), rye (*Secale cereale*) and triticale (*× Triticosecale*), among others. These cash crops are typically cultivated in monoculture as soil cover. Conversely, numerous non-cash crops are also commonly found, including phacelia (*Phacelia tanacetifolia*), white mustard (*Sinapis alba*), hairy vetch (*Vicia villosa*), red clover (*Trifolium incarnatum*), black radish (*Raphanus sativus*), and winter peas (*Pisum sativum*), among others. Non-cash cover crops may be cultivated either individually (e.g., phacelia) or in a multi-species mixture (e.g., mustard with vetch and clover).

2.2.3 Benefits of cover cropping

It can be demonstrated that the use of cover crops has a positive effect on soil health and fertility. Among the numerous advantages of cover cropping, three aspects are considered here: (i) Nitrogen fixation; (ii) soil physical and biological health; and (iii) soil geochemistry.

Nitrogen fixation

The incorporation of cover crops into a farming rotation confers a primary advantage in the form of nitrogen fixation. This is a significant reason why farmers frequently incorporate cover crops into their rotations (SPW, 2024). When this function is ensured, the term *green manure* is often employed (SPGE, 2024). Nitrogen-fixing legumes are typically cultivated as green manure, with hairy vetch (*Vicia villosa*), crimson clover (*Trifolium incarnatum*), and winter peas (*Pisum sativum*) representing common examples.

Nitrogen-fixing cover crops convert atmospheric nitrogen into an aqueous form that is available to plants in the soil (Burris & Roberts, 1993). The organic nitrogen is accumulated and applied to the soil as a fertiliser. As this nutrient will be utilised by the subsequent cash crop, the utilisation of green manure species in rotation enables farmers to reduce their synthetic fertiliser inputs. Indeed, research has demonstrated that a 25 to 35% reduction in chemical nitrogen fertilisers applied to oilseed rape crops could be achieved by intercropping with green manure (Gu et al., 2021).

Soil physical and biological health

The implementation of cover cropping has been demonstrated to enhance soil health in a multitude of ways. The most significant benefits are those related to particle retention, soil erosion, water retention, and biological activity (Unger & Vigil, 1998).

Firstly, cover crops provide a mechanical protection to the soil against rain, wind, and solar radiation. Moreover, they restrict its temperature fluctuations (Calegari et al., 2020). Secondly, the reduction of soil erosion using cover crops has been widely demonstrated. Some research has demonstrated a positive effect of different cover crop species on soil erosion. For example, perennial ryegrass, crimson clover, lespedeza, and tall fescue have been demonstrated to reduce soil erosion by approximately 64, 61, 51, and 37%, respectively (Malik et al., 2000).

Furthermore, the extensive root systems of cover crops facilitate the opening of soil layers. This aeration increases the infiltration potential and permeability of the soil, thereby increasing its water-holding capacity (Calegari et al., 2020). Finally, the biomass is provided and will eventually decay to be left as dead litter. This process of organic matter (OM) decomposition will stimulate soil biological activity.

Soil geochemistry

The principal effects of cover crops on soil geochemistry are nutrient cycling, increased soil organic carbon (SOC) content, reduced leaching and nutrient losses, and enhanced plant uptake of water and nutrients (Figure 2-10; Luz et al., 2023).

Moreover, the uptake of nutrients and the sorption of pesticides are both enhanced due to an increase in CEC (Calegari et al., 2020; Rector et al., 2020).

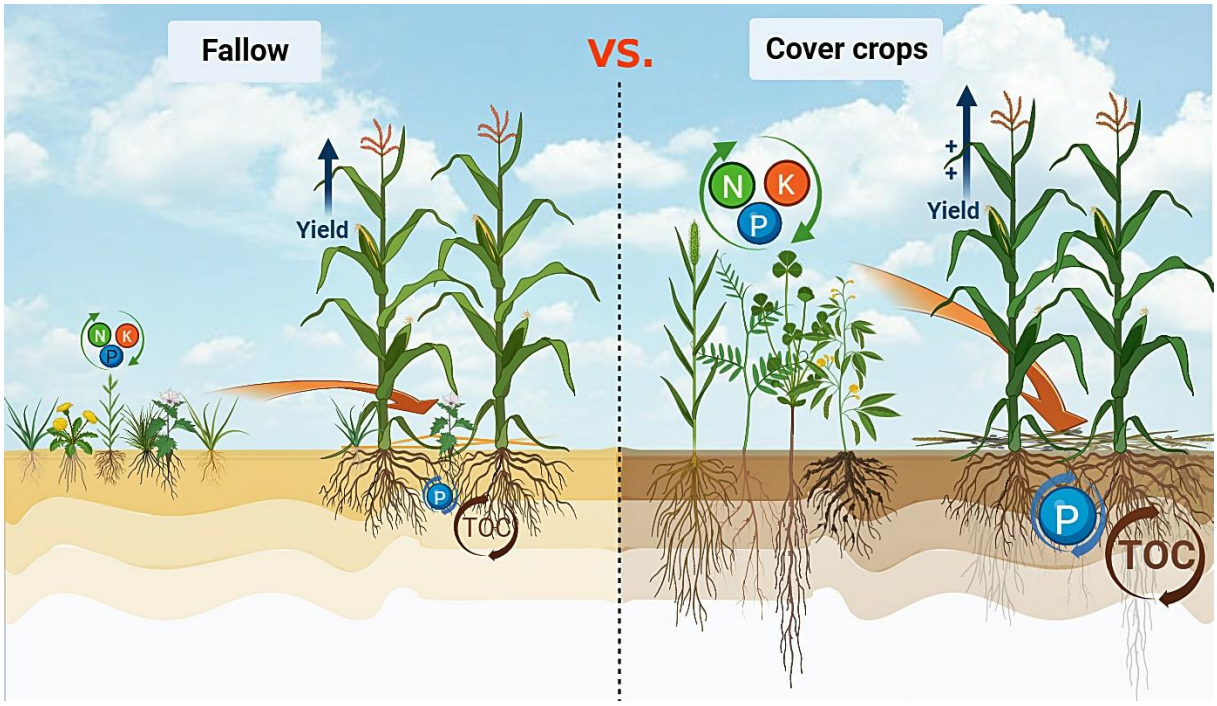

Figure 2-10: Agronomic impacts of cover crops in the context of corn cultivation in Brazil (Luz et al., 2023).

2.3 Potential of cover crops to mitigate pesticide leaching

The introduction of cover crops and pesticides will be used as a point of departure to examine the relationship between the two. The objective of this study is to demonstrate the potential of cover cropping to address concerns related to pesticide use in agriculture. The focus of this thesis is on two processes in agricultural soils: (i) pesticide persistence and (ii) pesticide leaching.

2.3.1 Pesticide catch crops

To minimise the leaching of pesticide residues, the cultivation of catch crops between cash crops can be employed. The term *catch crop* is employed to describe a subset of cover crops that have been specifically designed to mitigate soil compound leaching by capturing them (AHDB, 2024). In most cases, this refers to nitrate catch crops, which are cultivated with the objective of preventing nitrate leaching into aquifers (SPGE, 2024). In Wallonia, the sowing of nitrate catch crops is mandatory following the application of mineral nitrogen to a field (SPW, 2024).

Several cover crop species are eligible for the designation of nitrate catch crops, including phacelia (*Phacelia tanacetifolia*) and certain legumes (Figure 2-11). For example, an article published by Justes et al. (1999) demonstrated that the presence of a radish (*Raphanus sativus*) cover crop for one year resulted in a 50% reduction in nitrate concentration in drained water, in comparison to a bare soil modality.

Although the concept of catch crops is well established in the context of excess nitrogen fertilisers (nitrates), it is not yet the case for excess pesticides, which remain in the environment as residues. Nevertheless, as scientific research demonstrates comparable positive outcomes in reducing pesticide leaching through cover crops, the term pesticide catch crops may soon emerge and become part of agricultural legislation. For example, Potter et al. (2007) demonstrated that summer cover crops were able to significantly reduce atrazine (herbicide) leaching to shallow groundwater in Southern Florida. A further study demonstrated that the use of cover crops resulted in a 41% reduction in the dissipation and leaching of metolachlor (herbicide; White et al., 2009). This prevented some of the metolachlor and its metabolites from reaching groundwater. In this instance, the utilisation of cover crops may prove beneficial in maintaining the quality of groundwater.

Figure 2-11: Phacelia is a typical cover crop used as a nitrate catch crop in rotations (Gaenssler, 2003).

2.3.2 Influence of catch crops on soil parameters

The objective of this study is to examine the potential of cover cropping, and more specifically catch crops, to reduce pesticide leaching. The process of reducing leaching is related to the modification of certain soil parameters by catch crops. In addition to the benefits of cover crops for soil health (see 2.2.3), three main soil properties, among others, are of particular importance: water holding capacity, microbial activity, and SOM content.

Firstly, the increased soil porosity created by the root systems of the cover crop will promote water retention in the soil pores. The retention of water in the soil results in a reduction in runoff. Given that many pesticides are transported in water, they are less likely to leach when there is less water drainage. A study led by Alletto et al. (2012) estimated that the use of cover crops reduced the leaching of isoxaflutole (herbicide) by a factor of 1.8 compared to its bare soil counterpart.

Secondly, it has been demonstrated that cover crops, like any other vegetation, increase microbial activity in the soil through several processes (Lange et al., 2015). These processes include the introduction of dead litter to the topsoil, the creation of aeration and porosity in the soil by the roots, the exchange of compounds in the rhizosphere, and so forth. Similarly, the enhanced microbial activity facilitates the biodegradation of pesticides, thereby reducing their leaching (Cassigneul, 2019). This process is primarily observed in the upper soil layers.

Thirdly, the aerial biomass from cover crops will eventually produce dead litter, which will be converted into organic matter and incorporated into the soil. This case study demonstrates that a higher SOM content promotes pesticide retention in the topsoil layers (Agnan & Pelletier, 2019; Alletto et al., 2010). As previously indicated, greater retention results in reduced leaching, which in turn leads to enhanced degradation.

For the three aforementioned reasons, it can be postulated that the cultivation of cover crops on a non-productive crop field left as bare soil may result in a reduction in the concentration and transfer of pesticide residues in the soil (Figure 2-12).

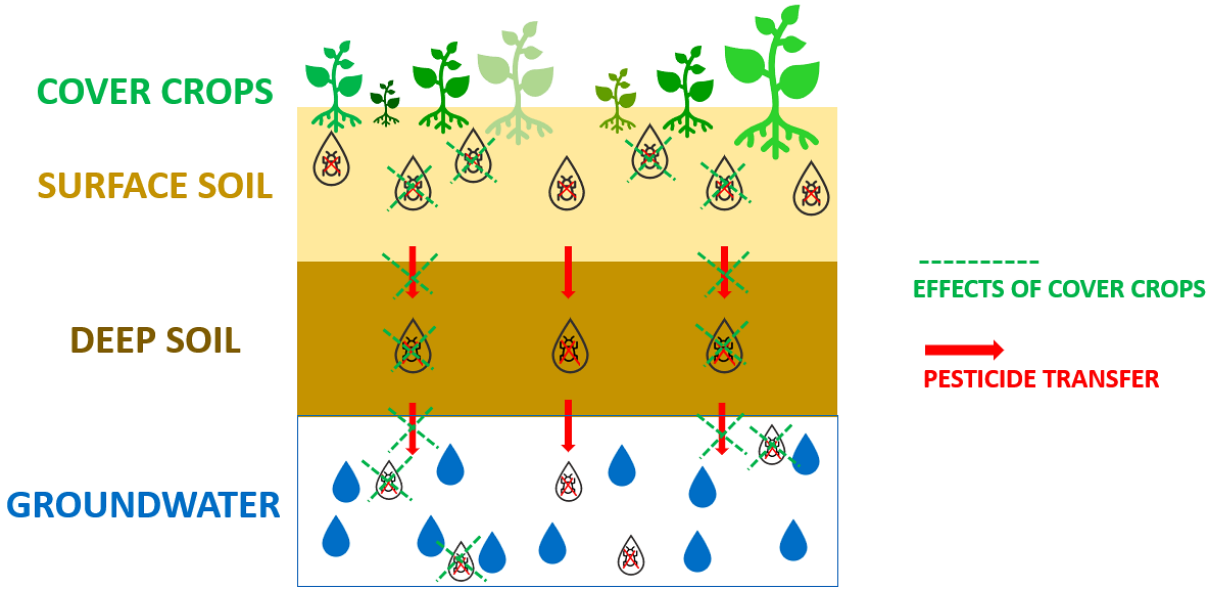

Figure 2-12: Potential reduction of pesticide leaching induced by cover cropping.

3. Objectives and approach

The current scientific literature on the utilisation of cover crops primarily concerns its impact on the nitrogen cycle, specifically its capacity to absorb excess nitrates and reduce their leaching. It is also important to also assess the effects of cover crops on the behaviour of pesticides in the soil (Alletto et al., 2012). Among the various processes that can be assumed to be induced by a cover crop, two main processes can be identified: (i) Reduced leaching due to reduced water drainage, induced by increased evapotranspiration in the presence of a cover crop, and (ii) enhanced pesticide biodegradation due to an increase in soil biological activity.

The objective of this thesis is to assess the influence of winter cover crops on the presence and transfer of pesticides in agricultural soils. The variety of the substances applied results in a wide range of physicochemical properties, which consequently give rise to different behaviours in the soil. Two distinct types of cover crops are employed in this study. For the purposes of this work, the term *cover crop* encompasses any vegetative cover sown in the experimental pots. The research is conducted in a agricultural context, with the crops used in the experiment representing those commonly employed in current crop rotations in Wallonia.

The research comprises a three-month experiment conducted in a greenhouse, between January and April 2024. The levels of pesticides in pots containing agricultural soil will be monitored under three soil cover modalities: (i) bare soil, serving as a control; (ii) a winter cash crop (a winter cereal crop); and (iii) a winter catch crop (a multi-species cover crop). A pesticide mixture comprising 19 distinct molecules is applied to all the modalities. Subsequently, soil and soil solution are sampled from the pots and analysed in the laboratory (Figure 3-1). During the three-month experimental period, soil solution samples will be collected on two occasions: after two months and after three months. The solid soil is sampled on three occasions: at the outset (sowing), and after two and three months.

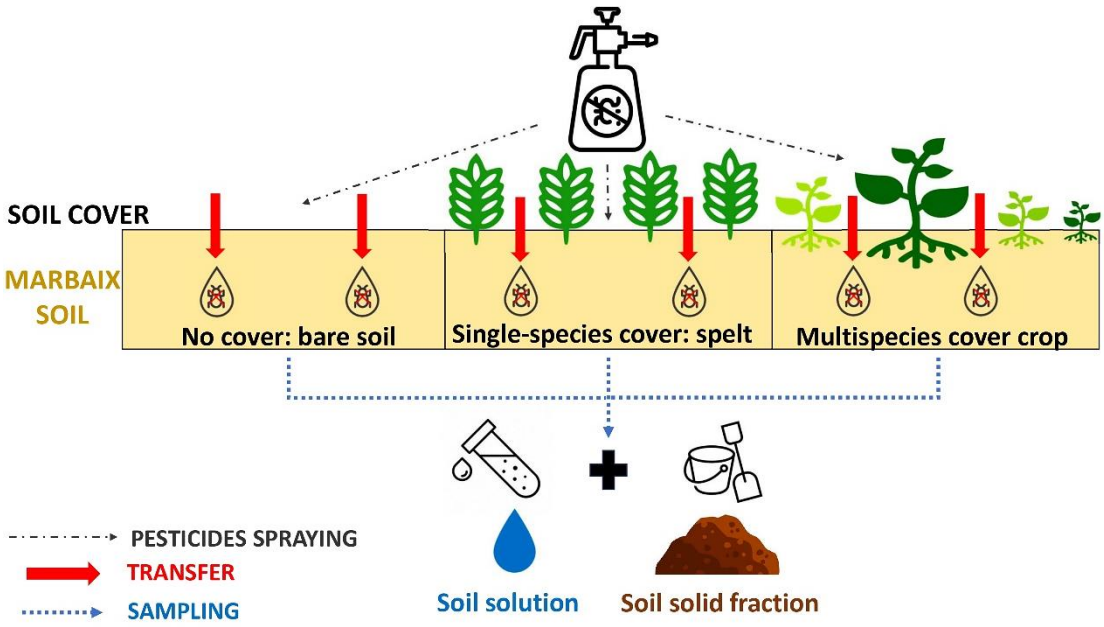

Figure 3-1: Summary of the experimental approach and setup.

4. Materials and methods

4.1 Study site

4.1.1 Geographical and climatic context

The soil utilised in the experiment was sourced from an arable field at the Alphonse de Marbaix centre (UCLouvain experimental farm), situated in the commune of Chaumont-Gistoux, province of Walloon Brabant, Belgium (50.668 °, 4.639 °; Figure 4-1).

The Marbaix site is characterised by a temperate oceanic climate, with mild and rainy winters and cool and humid summers. The average temperature is 10.5 °C, with an average annual rainfall of 790 mm (IRM, 2024).

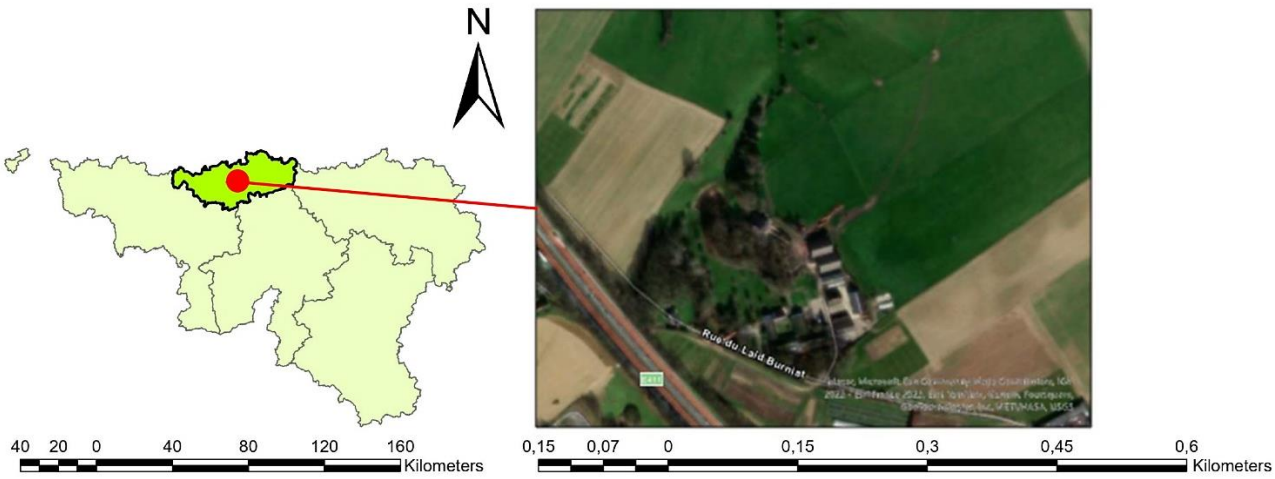

Figure 4-1: Location of the study site, Wallonia, Belgium.

4.1.2 Geological and pedological context

The Marbaix site is situated within the silty region of Brabant, on the *Sint-Huibrechts-Hern* formation (a fine sand formation, covered by a thick loess deposit; Rekk et al., 2017). The soils are silty and exhibit moderate or imperfect natural drainage (SPW, 2024).

The pH of the soil is slightly acid pH (6.5), with a total carbon content of 1.3%, and a CEC of 11 $cmol_c\ kg^{-1}$ (Thami, 2021). The soil granulometry is composed of 47% silt, 34% sand, and 19% clay, thus qualifying it as a silty soil with textural B horizon and phase with thin A horizon (SPW, 2021).

4.2 Description of the experiment

4.2.1 Preparation of the pots

The top 10 cm of the soil was collected during the week of 18 December 2023. The harvested soil was unpacked and placed in the experimental pots on 22 December. A total of 35 pots, each with a volume of 10 litres and a surface area of 0.07 m^2, were filled. 10 pots were allocated to each soil cover modality (see 4.2.3), with an additional five pots serving as a control group to be analysed one month after the commencement of the experiment.

4.2.2 Spraying of the pesticide mixture

The pesticide mixture was prepared by combining all the molecules: (i) that were available at the experimental farm, and (ii) that could be analysed by the Walloon Agricultural Research Centre (CRA-W) laboratories. The resulting mixture was a solution of 14 commercial products (19 active substances: 10 herbicides, 6 fungicides, 1 insecticide and 2 safeners; Table 4-1). The quantity of each molecule sampled was calculated to reach the maximum authorised dose in agriculture in Belgium when spraying on the pots (Phytoweb, 2016).

Table 4-1: Quantity of each molecule sprayed per pot (Phytoweb, 2016).

Active substance	*Formulated product(s) containing the substance*	*Sprayed quantity (µg/pot)*
Clopyralid	Bofix®	560
Cloquintocet-mexyl	Axial ®, Capri®, Frimax®	284
Fenpicoxamid	Aquino®	700
Flonicamid	Afinto®	1121
Florasulam	Primus®	35
Fluroxypyr	Bofix®, Frimax®	2101
Fluxapyroxad	Mizona®, Revytrex®	1822
Glyphosate	Barclay® Gallup Super 360	10086
Halauxifen-methyl	Frimax®	44
Iodosulfuron-methyl-sodium	Mesiofis Pro®	21
MCPA	Bofix®	5603
MCPB	Butizyl®	14009
Mefenpyr-diethyl	Mesiofis Pro®	315
Mefentrifluconazole	Revytrex®	1402
Mesosulfuron-methyl	Mesiofis Pro®	105
Pinoxaden	Axial ®	420
Pyraclostrobin	Comet New®, Mizona®	6304
Pyroxsulam	Capri®	137
Tebuconazole	Tebusip®	5253

On 22 December, the pesticide mixture was applied uniformly to all pots. The 500 mL solution comprised 13 mL of the formulated product mixture and 537 mL of distilled water.

With regard to the subsequent watering of the plants, the pots were supplied with water on a regular basis (two volumes of 0.5 L of rainwater per week) to ensure a homogeneous distribution of the pesticide solution throughout the soil in the pots.

4.2.3 Sowing of the cover crops

The pots were allocated into three soil cover modalities: the first is bare soil, which serves as a control, while the two others are different cover crops. The cover crop seeds were sown on 5 January:

1) Large winter spelt (*Triticum spelta*) was used as the second modality, with an average mass per hectare of 191 kg $ha^{-1}$of seeds sown,
2) A multi-species mix was used as the third modality, with an average seed mass per hectare of 148 kg ha^{-1} of seeds sown. This melliferous mix from *Semailles* comprises seeds of buckwheat (*Fagopyrum esculentum*), phacelia (*Phacelia tanacetifolia*), vetch (*Vicia villosa*), and white mustard (*Sinapis alba*).

The distribution of the pots into the different modalities can be observed in the greenhouse setup (Figure 4-2).

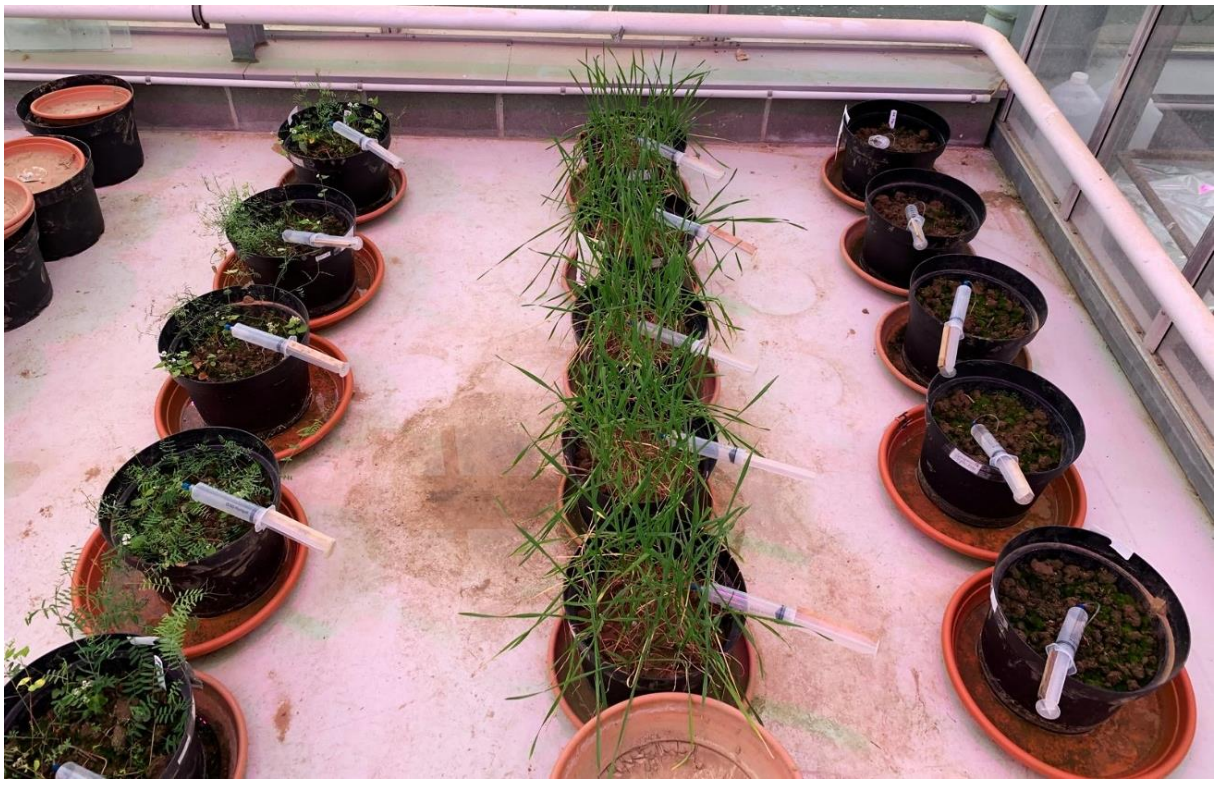

Figure 4-2: Greenhouse setup of the pots spread into three modalities (15 remaining pots among the 30). From left to right, multi-species mix, winter spelt, and bare soil modality.

4.3 Sampling

Three distinct materials were subjected to sampling: solid soil, soil solution, and the aerial biomass of the cover crops (Figure 4-3).

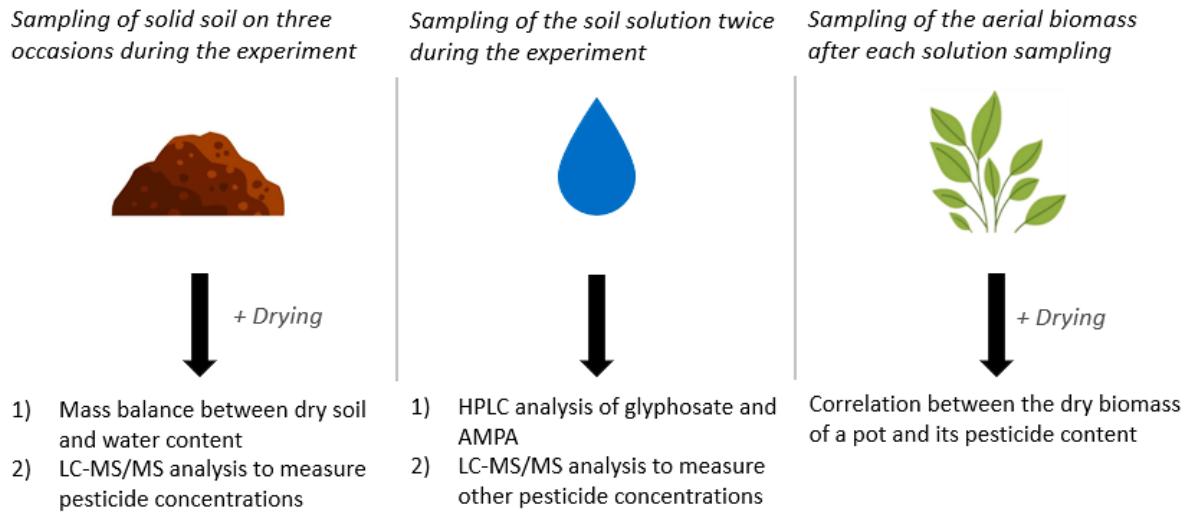

Figure 4-3: Summary of the sampling of three different materials: soil, soil solution, and vegetation.

Initially, the five control pots were collected two weeks after the pesticide mixture was sprayed for soil sampling. Subsequently, every pot was sampled for both soil and soil solution (Figure 4-4). A total of 10 pots from each of the three experimental modalities were collected at two distinct periods: the first five on 19 February (with 3 modalities x 5 pots = 15 pots harvested), and the last five on 25 March (with the 15 remaining pots).

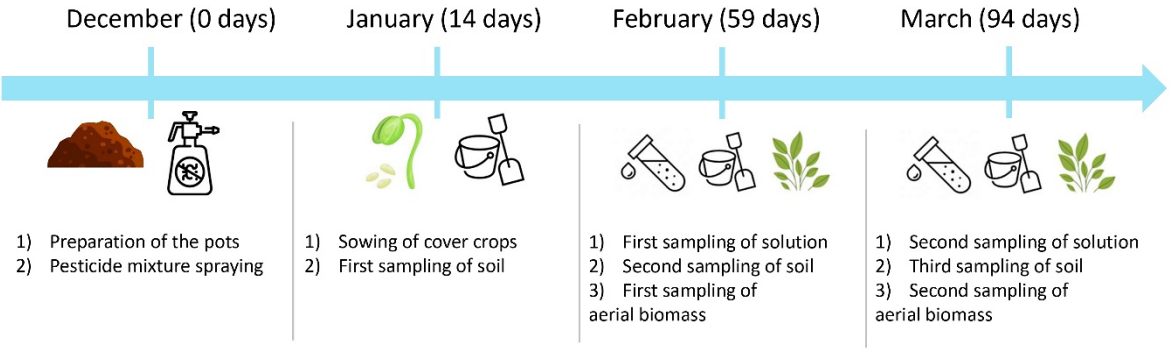

Figure 4-4: Timeline of the major experimental steps in the greenhouse.

The initial soil sampling was conducted concurrently with the sowing process on 5 January, which was 14 days after the spray application on 22 December (approximated to one month, as indicated in the remainder of this manuscript). The initial general sampling of both matrices was conducted on 19 February, which was 59 days after the spraying (approximated to two months in the remainder of this manuscript). The second general sampling was conducted on 25 March, which was 94 days after the spraying (approximated to three months in the remainder of this manuscript).

4.3.1 Soil sampling

For each soil sample, the corresponding pots were emptied, the soil content was weighed, and the pots were removed from the experiment. Sub-samples were sent to the CRA-W laboratory for pesticide quantification, while others were retained for solution content determination (by weighing before and after drying). Sampling in January (soil from the five control pots) and February (first time for both soil and soil solution) was conducted in accordance with a previously established protocol: 1 kg was collected to be sent to the CRA-W laboratory, while 500 g was sampled for oven drying (Figure 4-5, A). There was a slight change for the March sampling (second time for both soil and soil solution) 750 g of soil were collected, of which only 250 were sent to the CRA-W.

The initial mass of fresh soil was compared to the mass of dried soil to estimate the fresh soil moisture content. To achieve this, the soil samples were subjected to drying in an oven at a temperature of 60 °C for a period of 24 to 48 hours (Figure 4-5, B).

Figure 4-5: Oven in the MOCA laboratories (ELI, UCLouvain; A). Dried soil sample after 24 hours in the oven (B).

4.3.2 Soil solution sampling

The soil solution was sampled with micro-rhizons (Figure 4-6, A), which employed hydrophilic polyether sulfone membranes. The micro-rhizons are 10 mm in length and 1 mm in diameter, with an average pore size of 0.15 µm (19.21.21 F, Rhizosphere, Wageningen, The Netherlands). The micro-rhizons were placed vertically in the first 10 cm of the soil in each pot and the solution from the soil was collected using a 60 mL polypropylene syringe (BD Plastipak luer lock; Figure 4-6, B).

Figure 4-6: Micro-rhizon (**A**). Syringe connected to a micro-rhizon to collect a pot soil solution (**B**).

4.3.3 Cover crop sampling

Sampling of the cover crops was conducted by cutting the aerial biomass of the cover crops at the collar of the plant shoots. The fresh biomass was then dried in the oven at a temperature of 60 °C for a period of 24 hours, after which it was weighed.

4.4 Quantification of pesticide residues

Two distinct analytical techniques were employed to quantify the concentrations of pesticides in the samples. A preliminary analysis was conducted in the laboratories of the Department of Soil Sciences, with the assistance of the *Mineral and Organic Chemical Analysis* platform (MOCA). In addition, the analysis of all other molecules, for both soil and soil solution samples, was conducted by the CRA-W, based in Gembloux (CRA-W, 2024).

Regarding the molecules analysed, from the initial 19, a total of 20 molecules will be monitored, given that glyphosate is also measured with its main metabolite, AMPA. It is important to note that not all the 20, but 18 substances were analysed from each matrix: (i) Glyphosate and AMPA are not measured in the solid soil samples, while (ii) fenpicoxamid and pinoxaden are not measured in the soil solution samples.

4.4.1 Analysis of glyphosate and AMPA in soil solution samples: MOCA laboratories

This subsection provides a summary of the analytical protocol described in the methodology of Bemelmans et al. (2023). The analysis of glyphosate and AMPA was conducted in the MOCA laboratories of the faculty. The manipulations were conducted by Igor and Noé, with the assistance of Nathan Bemelmans and Hélène Dailly.

Reagents used and sample preparation

A solution of a borate buffer at pH 9 was prepared to allow derivatisation between FMOC (fluorenymethoxycarbonyl) and glyphosate and AMPA. This was done to prevent complexation between divalent cations and glyphosate and AMPA during analysis.

A 200 µg L^{-1} solution of FMOC chloride was employed for the analysis. This fluorescent compound allows the detection of glyphosate by forming a bond between FMOC and glyphosate. This was achieved by the dissolution of 10 mg of FMOC chloride in 50 mL of acetonitrile. Standard solutions of glyphosate and AMPA were prepared at concentrations of 0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 µg L^{-1}. This was achieved by dissolving 10 mg of glyphosate and AMPA in 500 mL of Milli-Q[1] water and diluting several times. The purpose of these standards is to calibrate the instrument. The signal measured for these solutions of known concentration allowed the unknown concentration of the samples to be calculated from their emitted signal.

Finally, the analytical samples were prepared by mixing 1 mL of solution (from the sample or the standard), 100 µL of borate buffer solution (pH 9) and 200 µL of FMOC solution. A blank was also prepared by replacing the sample solution with 1 mL of Milli-Q water. Any signal emitted by this solution (which was assumed to have a concentration of zero by the instrument) would be considered as noise and subtracted from all other samples.

HPLC analytical method

The analysis of glyphosate and AMPA from the soil solution samples was conducted using high-performance liquid chromatography (HPLC; 1200 series, Agilent Technologies Santa Clara, CA, USA) to quantify glyphosate and AMPA in the soil solution. This method employed a separation and quantification process for organic molecules present in a sample (Figure 4-7).

[1] Milli-Q® water is ultra-purified through a filter/resin/membrane system and has a resistivity of 18.2 MΩ cm at 25°C.

Figure 4-7: HPLC in the laboratories of the MOCA platform (ELI, UCLouvain).

The mobile phase eluents consisted of a 10 mmol L^{-1} pH 7 phosphate buffer (A) and a 45:45:10 acetonitrile-methanol-Milli-Q solution (B). The stationary phase comprised a Merck Lichrocart C18 column (150mm × 4.6 mm) with a 5 µm particle size and a temperature of 35°C. The glyphosate-FMOC and AMPA-FMOC molecules were detected using a fluorescence detector with an excitation wavelength of 260 nm and an emission wavelength of 310 nm.

Upon injection of the sample into the mobile phase, a ratio of 75% eluent A and 25% eluent B is maintained from minute 0 to minute 1. Upon reaching the column, the glyphosate-FMOC and AMPA-FMOC molecules exhibit a heightened affinity for the column, resulting in their retention. From minute 1 to 10, the proportion of eluent B in the mobile phase increases linearly until it reaches 100% at minute 10. The glyphosate-FMOC and AMPA-FMOC molecules enter the mobile phase when their affinity for the mobile phase is greater than that of the column. Subsequently, the complexes are identified by fluorescence. From minute 17 to 22, the quantity of eluent B is reduced to 25% to initiate a new cycle (Manning & Bootman, 2022).

Quality of HPLC data

For each analysis campaign of glyphosate and AMPA in solution, the limit of quantification (LOQ) is determined using the glyphosate and AMPA calibration curve. The concentration of the first standard for which the measured signal agrees with the calibration curve is defined as the limit of quantification. The limit of detection (LOD) is then defined as one-third of the LOQ (Table 4-2).

Table 4-2: LOD and LOQ of glyphosate and AMPA from the soil solution analyses for the two campaigns.

	First campaign (22/02/2024)		*Second campaign (08/04/2024)*	
	Glyphosate	**AMPA**	**Glyphosate**	**AMPA**
LOD (µg L^{-1})	0.03	0.03	0.07	0.07
LOQ (µg L^{-1})	0.1	0.1	0.2	0.2

4.4.2 Analysis of other pesticides: CRA-W laboratories

This subsection provides a summary of the Sol-Phy-Ly protocol, which is described in greater detail in the CRA-W report (Vandenberghe et al., 2021). A more detailed methodology can be found in the appendices (see 8.2.1). All subsequent analyses were conducted entirely by Alodie Blondel and her team at the CRA-W. The samples were sent to their laboratories on the day of the pot collection.

Reagents used and preparation of the solid soil samples

The reagents employed in this study included 5 g of fresh soil sieved to 2 mm and stored at – 18 °C. The solvents acetonitrile and ULC-MS grade methanol were obtained from Biosolve (Dieuze, France). The standards of ≥ 97% purity for the compounds under study were obtained from Sigma-Aldrich (Darmstadt, Germany) and HPC Standards GmbH (Cunnersdorf, Holland).

The initial stage of the sample preparation process involved the placement of 5 g of fresh soil in a Falcon tube, followed by the addition of 5 mL of water. The mixture was then manually agitated and allowed to macerate for 30 minutes. Subsequently, 10 mL of acetonitrile acidified with 1% formic acid was added to the tube, which was then agitated and left to macerate for a further 30 minutes. A sachet of QuEChERS salt containing 4 g $MgSO_4$, 1 g NaCl, 0.5 g sodium citrate dibasic sesquihydrate and 1 g sodium citrate tribasic dihydrate was added to the sample to dry and separate the aqueous and organic phases (Figure 4-8). The tube was then manually vortexed for one minute and centrifuged at 4800 rcf for five minutes. Afterwards, the supernatant (organic phase) was filtered through a 0.2 µm PTFE filter.

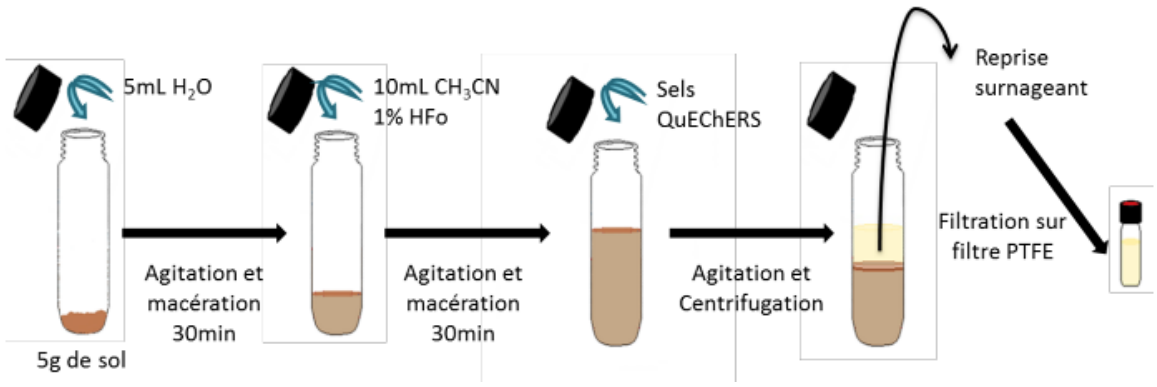

Figure 4-8: Pesticide extraction in a solid soil sample with the QuEChERS method (Vandenberghe et al., 2021).

Reagents used and preparation of the soil solution samples

For the analysis of the soil solution samples, the following reagents and solvents were used: UPLC-MS grade methanol from Biosolve (Dieuze, France) and ultrapure water obtained using a Milli-Q system from EMD Millipore (USA). The compound standards of ≥97% purity were procured from Sigma-Aldrich (Darmstadt, Germany) and HPC Standards GmbH (Cunnersdorf, Netherlands).

For instrument calibration, stock solutions of each molecule were prepared in acetone or methanol and stored frozen at -18 °C for up to three months. The intermediate solutions were prepared by dilution in water immediately prior to use. Prior to analysis, water samples were stored in a refrigerator at 4 °C. The samples were then centrifuged for 15 minutes at 4 °C and 4800 rcf to limit the presence of suspended solids, after which they were transferred to a vial for injection.

LC-MS/MS analytical method

The resulting solution from the sample preparation was transferred to a vial and analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). All technical details concerning the chromatographic and spectrometric parameters of the LC-MS/MS are presented in the appendices (see 8.2.1 for soil samples and 8.2.2 for soil solution samples).

Quality of LC-MS/MS data

The LOQ provided by the CRA-W for the LC-MS/MS analysis varies according to the molecule and the matrix of the sample (solid soil or soil solution). The range of permissible limits is provided in Table 4-3.

Table 4-3: Value ranges of LOD and LOQ from the CRA-W analyses: soil and soil solution.

	Soil samples		*Soil solution samples*	
	LOD (µg kg^{-1})	**LOQ (µg kg^{-1})**	**LOD (µg L^{-1})**	**LOQ (µg L^{-1})**
Min	0.07	0.2	0.01	0.025
Max	1.67	5	0.5	1.5

A comprehensive table in the appendices provides the LOD and LOQ for each of the pesticides analysed, both for solid soil and soil solution samples (see 8.2.3).

4.5 Data processing

All data processing was conducted using the open-source software *RStudio* (version 2024.4.1.748; RStudio Inc., Boston, USA), with *R* 4.3.3 (R Foundation for Statistical Computing, Vienna, Austria) employed as the statistical computing environment. Furthermore, *Microsoft Excel®* 2016 (version 2404; Microsoft®, Washington, USA) was employed to facilitate the visualisation of data tables and the plotting of certain graphs.

The data processing primarily involved the assessment of the geochemistry of the molecules, and the analysis of the molecule concentrations in the experimental samples. The processing involved three distinct steps: (i) principal component analyses, (ii) mass balance calculations, and (iii) a statistical comparison of modalities.

4.5.1 Principal component analyses

Geochemistry of the molecules

To study the molecules, their physicochemical properties were extracted from the pesticide database *PPDB*, which was established by the University of Hertfordshire (PPDB, 2024). This database is frequently updated (Lewis et al., 2016). A total of nine properties of interest were selected for the study, namely the molecular mass, the groundwater ubiquity score (GUS), the solubility in water (s), the n-octanol-water partition coefficient (K_{OW}), the organic carbon-water partition coefficient (K_{OC}), Henry's law constant (K_H), the vapour pressure, the half-life in soil (DT50_soil), and the half-life in water (DT50_water).

A first principal component analysis (PCA) was conducted on the properties, using the *FactoMineR* and *factoextra* packages. The data were transformed by the logarithmic function, then centred and reduced.

Furthermore, the pesticides were grouped into clusters using the K-means clustering method (Datanovia, 2018), following their separation by the PCA. This was coded using the *kmeans()* function from the *stats* package.

Pesticide concentrations in the analytical samples

A second PCA was conducted based on the pesticide concentrations observed in the samples. The variables were subsequently centred and reduced by a centred log-ratio transformation. This was accomplished using the *clr()* function of the *compositions* package.

4.5.2 Calculation of the mass balances

Mass balances

The second part of the data processing primarily focused on the pesticide content in the pots and their partitioning between the solid soil and the solution, regardless of any soil cover modality. The bare soil control was therefore subjected to a separate analysis to identify any patterns of molecule partitioning between the two matrices.

To this end, the resulting concentrations were converted into masses in *Excel* to perform mass balances for each pot. The quantities of pesticide in each matrix were calculated by multiplying the concentration with the mass of the matrix.

For the solid soil, the mass factor was the entire pot as the concentrations in soil samples provided by the CRA-W were expressed in µg kg^{-1} of fresh soil:

$$quantity\ in\ the\ soil = [soil\ sample] * mass\ of\ the\ pot \qquad [mg/pot]$$

For the soil solution, the mass factor was the mass of water in the pots, obtained by weighing and drying fresh soil samples (see 4.3.1):

*quantity in the solution = [solution sample] * mass of water in the pot* [mg/pot]

Relationships between pesticide properties and mass losses

A brief investigation of the relationship between the physicochemical properties of the pesticides and their mass losses in soil and soil solution was conducted (see 5.3.2). A correlation matrix was constructed in *RStudio*, using the *rcorr()* function from the *Hmisc* package. Coefficients were plotted, and p-values extracted to search for any correlation between the variables in question.

4.5.3 Statistical test for comparisons between parameters

To compare the samples according to their modality, a hypothesis testing procedure was employed to ascertain whether any significant differences existed. A 95% confidence interval was employed, applied to any p-value resulting from a test. All the functions employed during the statistical testing were included in the *R* package *stats*.

Data distribution: normality and homogeneity

If the distribution of the data is normal (Shapiro-Wilk pre-test with the *shapiro.test()* function), a second pre-test was performed to assess homogeneity (Bartlett's pre-test with the *bartlett.test()* function). If the distribution is homogeneous, that is, if the population variances are equal, a one-way ANOVA could then be performed using the *anova()* function.

Furthermore, two post-hoc tests were conducted to ascertain which modalities exhibited significant differences from one another. The first of these is a Dunnett's test, employed to compare every modality with the bare soil control (*DunnettTest()* function). The second is a Tukey HSD test (*TukeyHSD(aov())* function).

Data distribution: normality and heterogeneity

If the distribution was found to be normal (Shapiro-Wilk) but not homogeneous (Bartlett), it could be concluded that the population variances are not equal. Consequently, a Welch's ANOVA was employed, utilising the *welch.test()* function.

Following the application of a Welch's ANOVA, two post-hoc tests were conducted: (i) a Dunnett's test, analogous to that employed in the case of a one-way ANOVA, and (ii) pairwise t-tests between all modalities.

Data distribution: no normality

If the distribution did not exhibit any normality, a non-parametric Kruskal-Wallis test was therefore applied, using the *kruskal.test()* function. Subsequently, two post-hoc tests were also conducted to complete the analysis: (i) a usual Dunnett's test, and (ii) a Dunn's test using the *Bonferroni* adjustment method (*dunnTest()* function), which is a more robust approach to controlling the familywise error rate.

5. Results and discussion

This chapter is divided into four sections: (i) the geochemistry of the pesticides applied and monitored during the experiment; (ii) a global insight into the concentrations of the molecules; (iii) the characterisation of the behaviour of the pesticides in the bare soil control; and (iv) the comparison between the modalities (influence of the cover crop).

5.1 Geochemistry of the studied molecules

The objective of this initial section is to characterise and classify the studied molecules in terms of their physicochemical properties. This classification will facilitate the grouping of molecules based on their geochemical similarity. A selection of specific molecules from the initial 20 will then be made to ensure a more representative analysis.

5.1.1 Classification of the molecules

To classify the active substances according to their environmental behaviour, a PCA was performed on the physicochemical properties of the molecules. Of the nine properties detailed in the methodology (see 4.5.1), only the six most contrasting were retained: the molecular mass, the groundwater ubiquity score (GUS), the solubility (s), the n-octanol-water partition coefficient (K_{OW}), the vapour pressure, and the half-life in soil (DT50_soil; Figure 5-1). Furthermore, only molecules that were measured in both soil and soil solution were selected, thereby excluding three of the 19 molecules initially sprayed (Table 4-1): glyphosate, fenpicoxamid, and pinoxaden. The remaining 16 molecules were retained for the purpose of performing the PCA (Figure 5-1).

The results indicate that the first principal component (43.2% of the data variance) allowed the grouping of certain herbicides with positive scores (correlated with GUS and solubility), and most fungicides with negative scores (correlated with K_{OW} and DT50_soil). The properties in question demonstrate that principal component 1 is primarily driven by aqueous mobility. It is correlated with GUS and solubility, and anti-correlated with hydrophobicity (K_{OW}) and persistence in soil (DT50_soil).

The second principal component (27.9% of the data variance) exhibited a contrasting pattern, with mesosulfuron-methyl presenting the highest positive score (correlated with molecular mass) and MCPB showing the highest negative score (correlated with vapour pressure). Principal component 2 is observed to be driven by persistence in the pot. It is correlated with low degradability (molecular mass), and anti-correlated with volatility (vapour pressure).

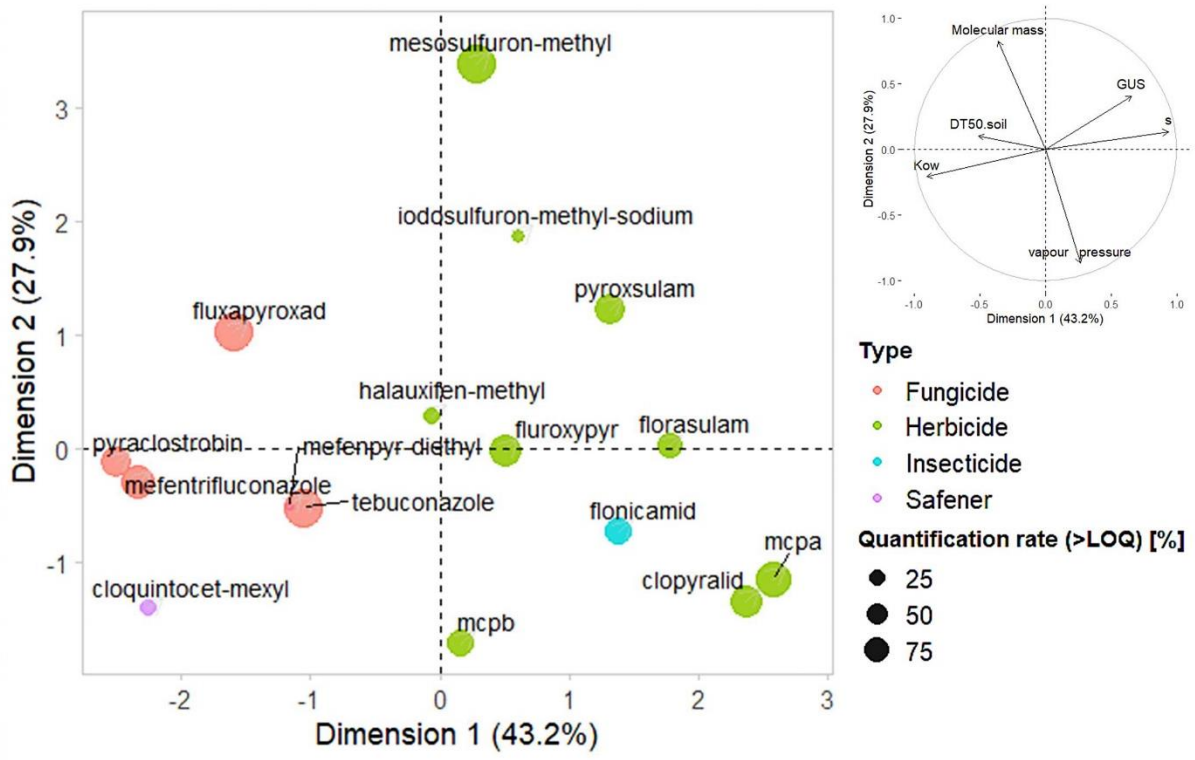

Figure 5-1: PCA based on the physicochemical properties of the 16 retained molecules: representation by molecule (left) and by physicochemical properties (upper right). The colour represents the families of molecules according to their target and the size represents the quantification rate in the measurements (> LOQ).

5.1.2 Grouping of molecules based on their physicochemical nature

To group pesticides with a similar physicochemical nature, a K-means clustering method was applied to the PCA, resulting in the formation of four groups of pesticides (Figure 5-2).

The order of the groups is indicative of their size, with the number of molecules in each group as follows (Table 5-1): group 1 (6 molecules), group 2 (5 molecules), group 3 (3 molecules), and group 4 (2 molecules).

Table 5-1: Classification of the molecules into four groups by K-means clustering.
The quantification rate (>LOQ) given in bold ranks the molecules of each group.

Group 1	*Group 2*	*Group 3*	*Group 4*
Fluxapyroxad **(99%)**	Fluroxypyr **(70%)**	Mesosulfuron-methyl **(99%)**	MCPA **(80%)**
Tebuconazole **(99%)**	MCPB **(46%)**	Pyroxsulam **(61%)**	Clopyralid **(70%)**
Mefentrifluconazole **(77%)**	Flonicamid **(46%)**	Iodosulfuron-methyl-Na **(10%)**	
Pyraclostrobin **(60%)**	Florasulam **(37%)**		
Cloquintocet-mexyl **(14%)**	Halauxifen-methyl **(14%)**		
Mefenpyr-diethyl **(7%)**			

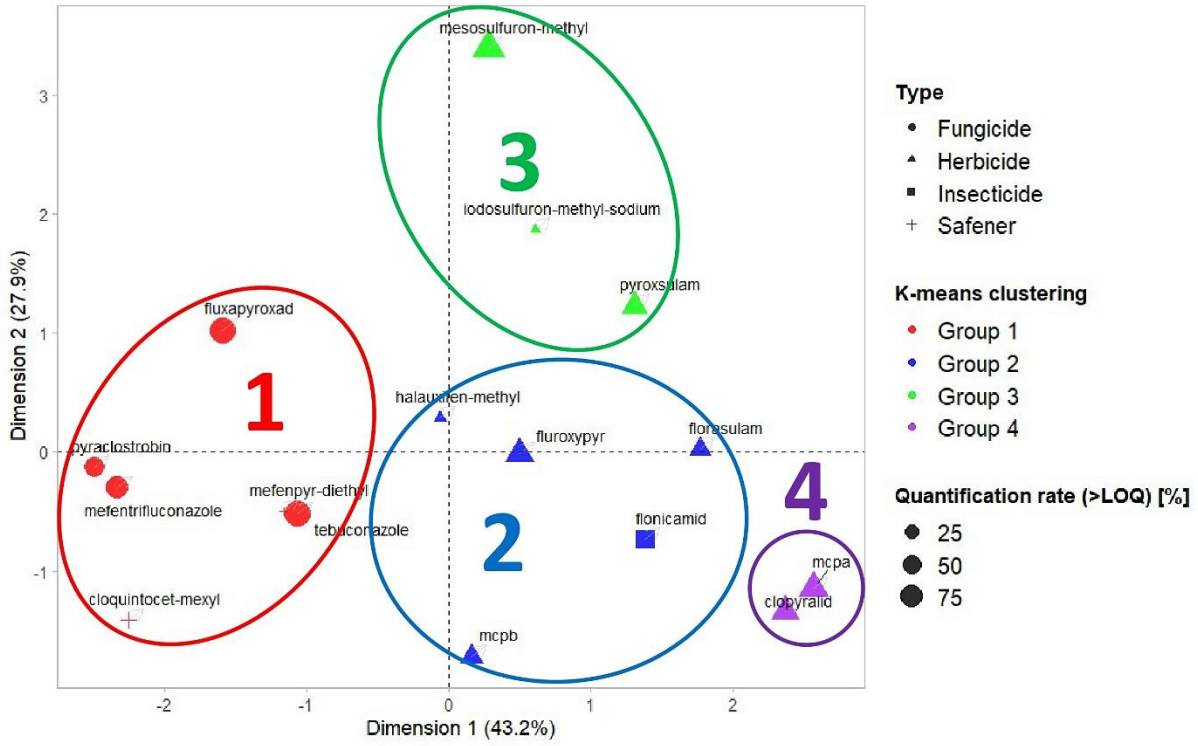

Figure 5-2: K-means clustering performed on the PCA based on the properties of the 16 retained molecules. The colour represents the groups formed by the clustering and the size represents the quantification rate in the measurements (> LOQ).

5.1.3 Environmental behaviour of reference molecules

For the sake of clarity, an illustrative example of a molecule that is representative of each group is presented, accompanied by its corresponding spider diagram (Figure 5-3). The most quantified molecules are selected, even if they are not the most contrasted in the initial PCA (Figure 5-1).

The diagrams illustrate the physicochemical nature of the four groups. The differences between the four groups are readily apparent. Among their most contrasted properties, fluxapyroxad (group 1; quantified at 99%) exhibits a high DT50_soil, mesosulfuron-methyl (group 3; quantified at 99%) demonstrates a very high GUS, and MCPA (group 4; quantified at 80%) displays a high solubility and vapour pressure.

Finally, it can be observed that fluroxypyr does not exhibit any significant degree of contrast. Nevertheless, given that this weak contrast is similar to most molecules within its group (group 2; Figure 5-1). Moreover, it is the most frequently quantified molecule of group 2 (70%; Table 5-1) and is consequently an adequate reference molecule.

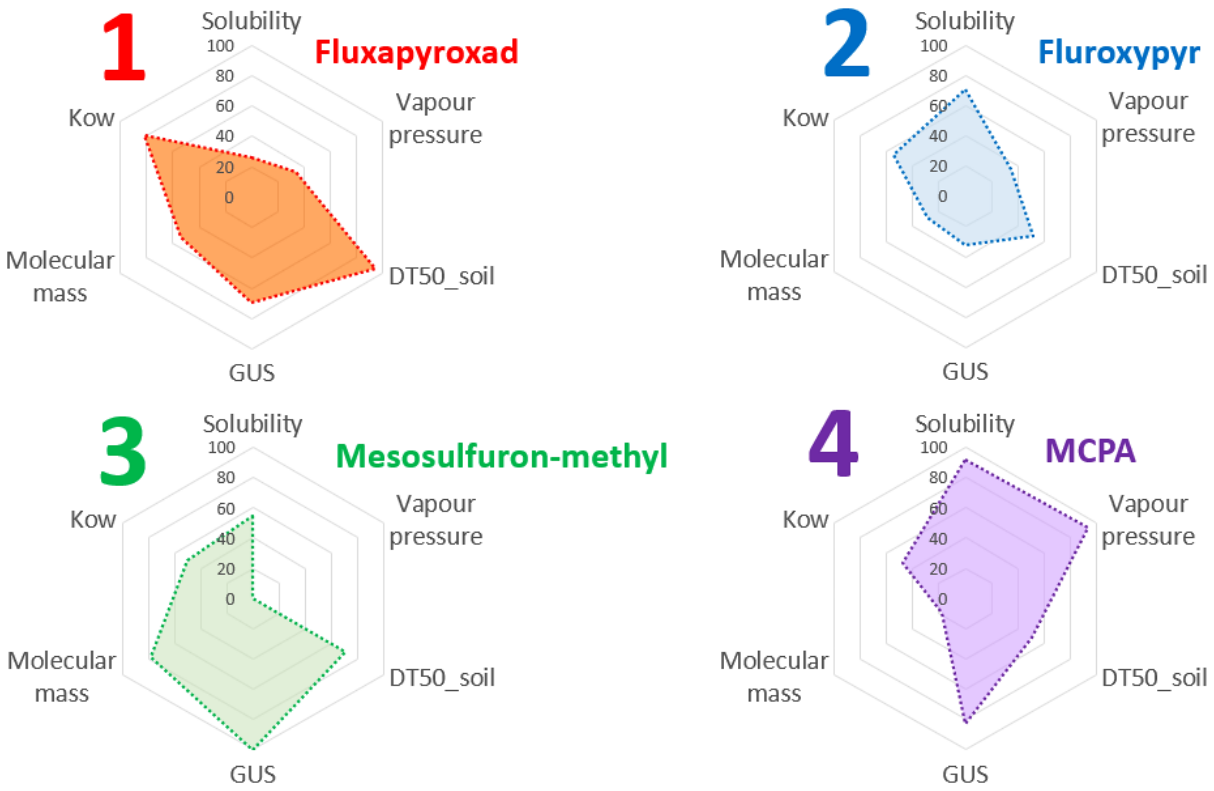

Figure 5-3: Spider diagrams of reference molecules for each group. Properties are shown in relative values, based on a range between the minimal and the maximal value among all molecules [%].

5.2 Global overview of the pesticide concentrations

Having completed the classification of pesticides according to their geochemistry, this section presents a comprehensive overview of all pesticide concentrations. The objective of this section is to identify any noteworthy patterns, which will then be subjected to further analysis in the subsequent sections.

5.2.1 Pesticide concentrations in the samples

Soil samples

To facilitate the visualisation of the temporal evolution of the concentrations, these were grouped into ranges. All samples were grouped irrespective of the modality (Figure 5-4). The vertical axis displays the number of measurements per concentration range, expressed in µg kg^{-1} of fresh soil. The colour code indicates the ranges in which each molecule is found. The quantification ranges from light green (≤ LOQ) to dark red (high concentrations). Green indicates a detection without quantification, while dark green indicates an absence of detection (< LOD). As exemplified with glyphosate and AMPA in soil samples, white indicates an absence of measurement of the molecule in the matrix in question.

A general decrease in concentration can be observed between the initial samples (Figure 5-4, **A**) and the subsequent month (Figure 5-4, **B**). These observations can be attributed to four assumed processes: (i) atmospheric volatilisation of the pesticides, (ii) uptake by the cover crops, (iii) microbial degradation, and (iv) leaching along with the water given to the pots.

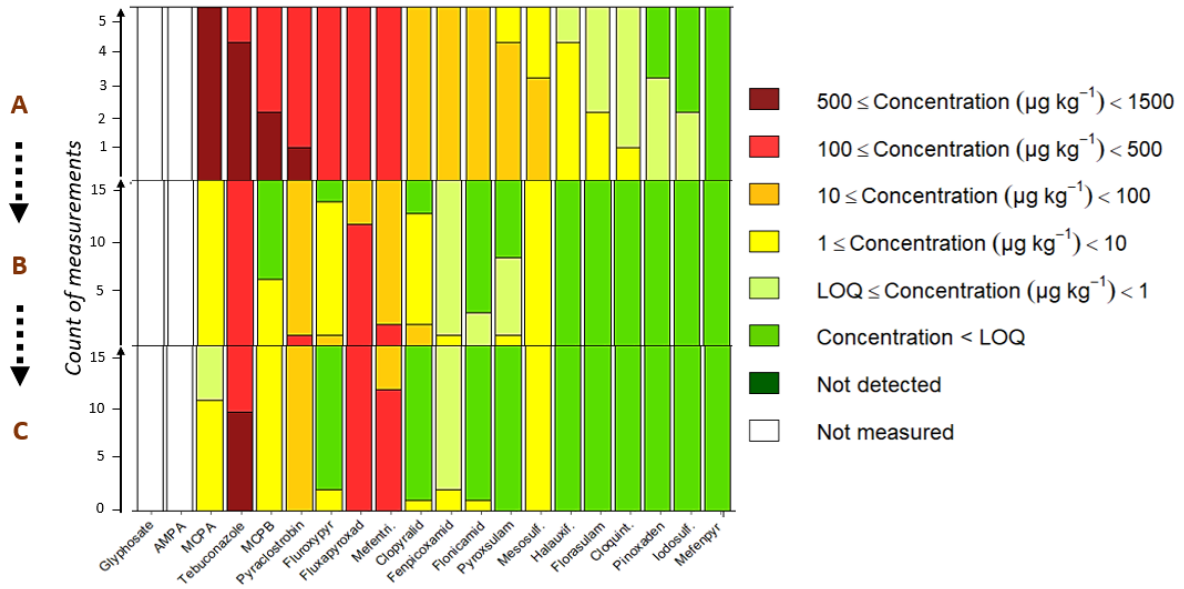

Figure 5-4: Number of measurements per concentration range for the 20 molecules: soil samples after one (**A**), two (**B**), and three months (**C**) of experiment [µg kg^{-1} fresh soil].

Soil solution samples

The approach of plotting the soil samples according to concentration ranges was also applied to the soil solution samples. As the soil solution from the pots was only sampled on two occasions (not in January), the number of measurements is only displayed for the second- (Figure 5-5, **A**) and third-month samplings (Figure 5-5, **B**).

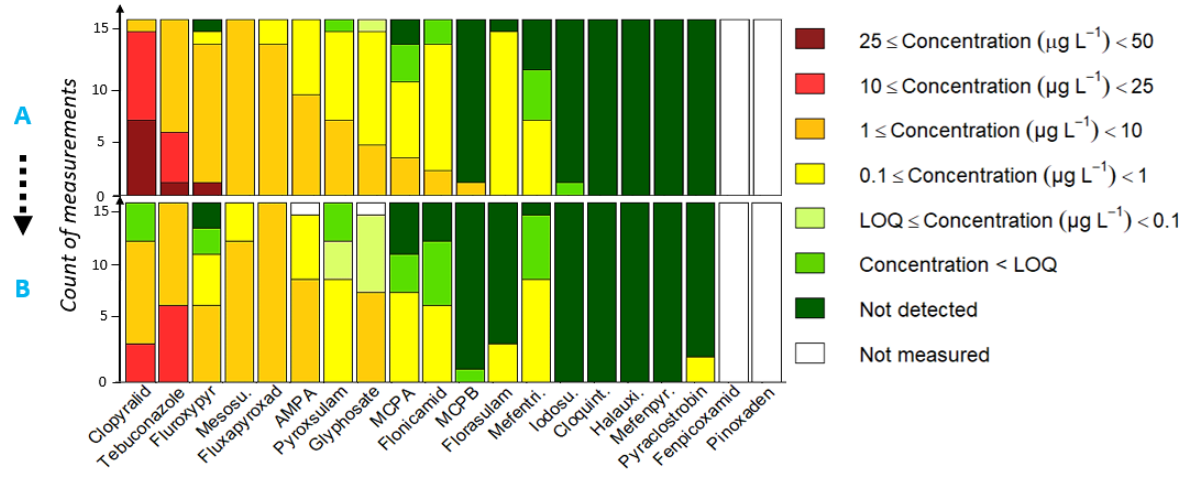

Figure 5-5: Number of measurements per concentration range for the 20 molecules: soil solution samples after two (A) and three (B) months of experiment [µg L^{-1} of soil solution].

Visualisation of the two matrices

As the molecules are less quantified in the samples over time (Table 5-2), only the second-month samples will be examined to select the most representative molecules for further analysis.

Table 5-2: Average quantification rate in the samples for all molecules. The colour represents the matrix.

Sampling time	> LOQ (soil)	> LOQ (solution)
1 month	89%	/
2 months	54%	60%
3 months	46%	51%

To facilitate a direct comparison, the concentrations in both matrices were plotted alongside each other (Figure 5-6). The names of the molecules are written in the colour of the group to which they belong.

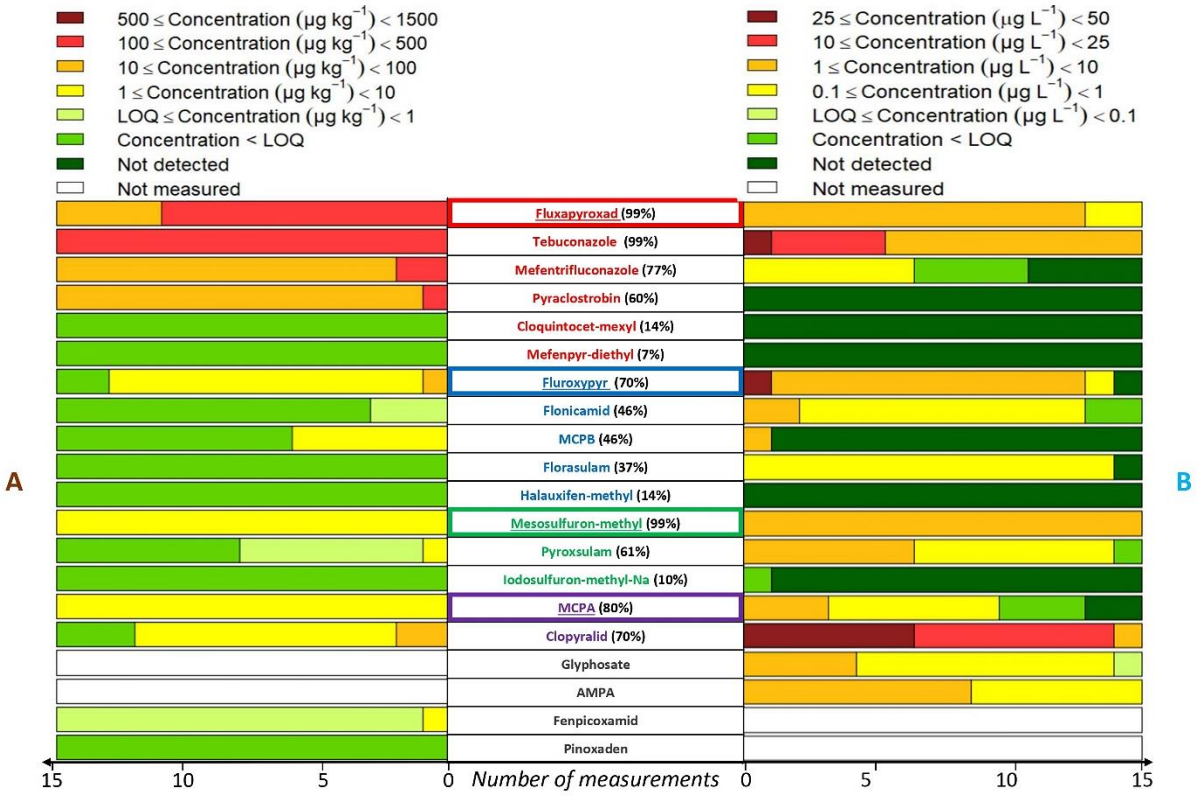

Figure 5-6: Number of measurements per concentration range for the 20 molecules (coloured by group): soil (**A**) and soil solution (**B**) samples after two months of experiment (Group 1 in red; group 2 in blue; group 3 in green; group 4 in purple; no group in black). The representative molecule of each group is framed. The quantification rate (>LOQ) is given in bold for each molecule and ranks them within each group.

In each group, a single molecule, previously depicted in Figure 5-3, is selected as reference for further investigation, due to its optimal quantification rate within the group (highlighted in coloured frames in Figure 5-6). As the rationale for their selection was previously provided, fluxapyroxad is selected for group 1, fluroxypyr for group 2, mesosulfuron-methyl for group 3, and MCPA for group 4.

5.2.2 Visualisation of the experimental samples

A second PCA, based on the pesticide concentrations measured in the samples, is presented in Figure 5-7. The results indicate that the first principal component (60.4% of the data variance) allowed the grouping of solution samples with positive scores (mostly correlated with soluble and volatile molecules), and soil samples with negatives scores (correlated with hydrophobic and persistent molecules). The molecules in question demonstrate that principal component 1 is primarily influenced by the matrix of the sample, as it is correlated with solubility and vapour pressure, and anti-correlated with persistence in soil (DT50_soil) and hydrophobicity (K_{OW}).

The second principal component (28.1% of the data variance) exhibited a contrasting relationship mainly between two molecules: (i) iodosulfuron-methyl-sodium (highest molecular mass) with the highest positive score (correlated with the third-month solution samples), and (ii) and MCPA (high GUS and vapour pressure) with the highest negative score (correlated with the first-month soil samples). Principal component 2 is observed to be driven by the age of the samples, which indicates the persistence of certain molecules. Indeed, more recent solution samples (third month) with persistent pesticides exhibit a positive correlation, while earlier soil samples (first month) with transient molecules are anti-correlated.

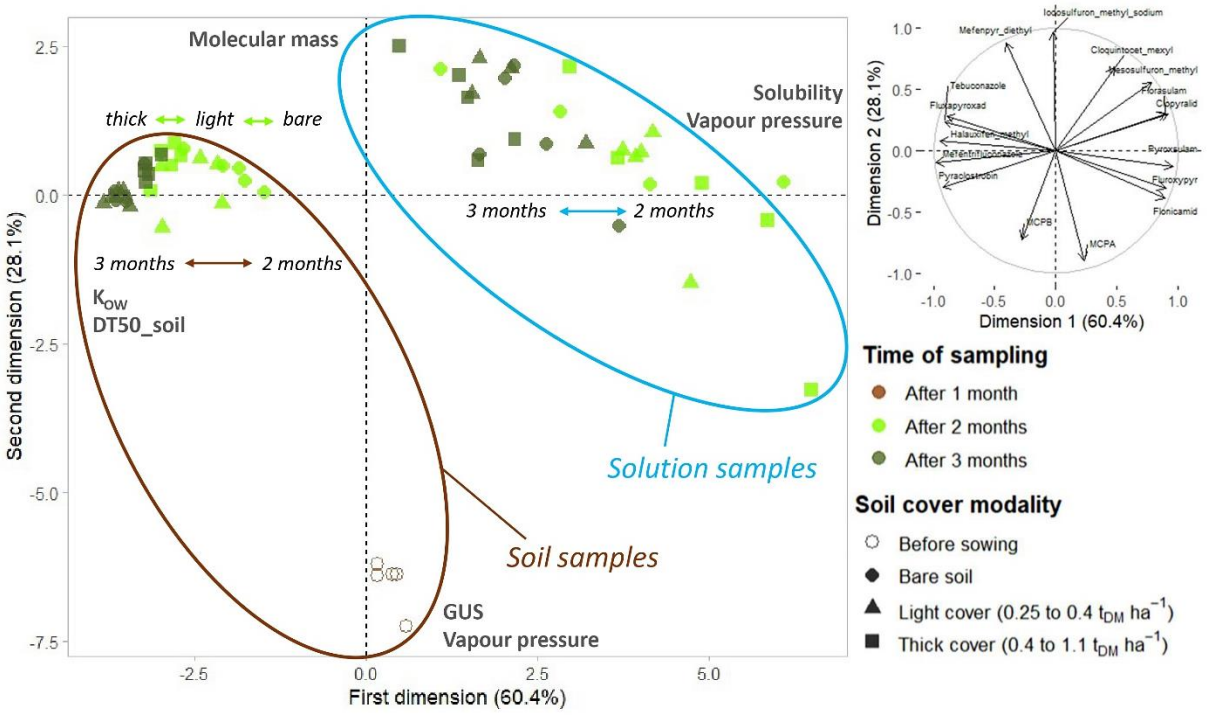

Figure 5-7: PCA based on pesticide concentrations in the samples: representation by samples (left) and by molecule (upper right).

5.3 Pesticide content in bare soil

Following the presentation of the data on the molecule concentrations, the bare soil control will be analysed. This will be followed by a comparison between the different modalities.

Several questions can be posed, including: Does the evolution of pesticide concentrations in the pots, regardless of their modality, exhibit a discernible pattern? Does the quantity of pesticide in the pots decrease over time? What is the distribution of pesticides between the soil and the soil solution? Does the partitioning of the molecules intro the soil and soil solution depend on their properties?

5.3.1 Temporal evolution of pesticide mass

As previously outlined in subsection 5.1.3, the four reference molecules are now subject to further investigation (Figure 5-3). The initial step involved the observation of the mass loss of those molecules in bare soil over time. Given the considerable disparity in the initial sprayed quantities among the molecules, the mass losses are presented as relative percentages. To provide a more intuitive visualisation of the loss, the relative remaining mass is plotted, from the spraying until the second- and the third-month samplings (Figure 5-8).

Fluxapyroxad

Given the unexpected increase observed in the soil for fluxapyroxad (group 1 with high DT50_soil and K_{OW}), this compound will be discussed first. The relative mass plotted after three months is found to be slightly higher than the initial sprayed quantity, reaching 114%.

This anomalous increase can be attributed to a sampling bias, which may have resulted in errors in the concentration values (Figure 5-4). Indeed, a comparison of the 1 kg sampling (after two months; see 4.3.1) with the 250 g sampling (after three months) revealed that the latter yielded a greater proportion of drier, finer soil particles. This may have resulted in an overestimation of molecules showing a higher hydrophobicity and affinity for soil particles. This assumption is consistent with the observations made on fluxapyroxad, which exhibited a high hydrophobicity (high K_{OW}) and a high persistence in soil (high DT50_soil; Figure 5-3).

Mesosulfuron-methyl

The remaining three molecules exhibit certain common patterns. Firstly, the mass exhibits a slight decrease (on rare occasions, a statistically significant decrease) in both matrices between the two samplings, in contrast to fluxapyroxad. This can be attributed to the fact that the three molecules exhibit a lower hydrophobicity (K_{OW}) and persistence in soil (DT50_soil) than the overestimated fluxapyroxad. These differences in properties may therefore justify the conclusion that the molecules are less subject to the aforementioned sampling bias.

Nevertheless, this mass decrease is rarely significant for all molecules and the relative content exhibited by the molecules differs (Figure 5-8). Consequently, they will be analysed separately.

The highest remaining mass in soil was observed for mesosulfuron-methyl (group 3 with a high GUS and DT50_soil), with values up to 30% after two months. The temporal evolution of this matrix exhibits a slight decrease to 22% after three months (no significance). In the soil solution, a slight reduction is also observed from 7 to 3%, between the two months (no significance).

The GUS index is indicative of solubility and leachability towards groundwater, while the DT50_soil is indicative of persistence in soil. It can therefore be postulated that group 3 molecules are stable and tend to remain in soil and soil solution for an extended period.

Fluroxypyr

Subsequently, fluroxypyr (group 2 with a moderately high vapour pressure and K_{OW}) exhibits, after two months, a weak remaining mass of 2.4% in soil and 0.7% in soil solution. Over time, although the soil solution exhibits a certain stability (with a non-significant slight decrease to 0.1%), one of the rare significant reductions is observed in the soil, reaching 0.8%.

Notwithstanding the lack of distinctive properties exhibited by fluroxypyr, most group 2 molecules are characterised by a moderately high volatility (vapour pressure) and hydrophobicity (K_{OW}). This can account for the pronounced mass loss observed after two months for both matrices, as well as for the significant decrease in soil between the second and the third months. It can be reasonably assumed that the hydrophobicity of the molecule in question may result in a greater transfer and retention of the substance within the soil matrix, in contrast to the soil solution. In addition, the volatility of the molecule may result in a significant loss within the soil.

MCPA

Finally, the lowest remaining mass was observed for MCPA (group 3 with high solubility and vapour pressure) after two months. The relative mass of the compound in soil was found to be 0.3% and in soil solution 0.04%. After three months, a slight decrease is observed, reaching 0.2% in soil and 0.01% in soil solution (no significance).

This stability in the two matrices is consistent with the physicochemical nature of group 3 molecules. Indeed, given that they exhibit a low affinity for soil particles (high solubility) and a very high volatility (vapour pressure), the majority of their mass is dispersed into the atmosphere before reaching the second month. Consequently, the subsequent decreases observed in the second and the third months are minimal and not statistically significant.

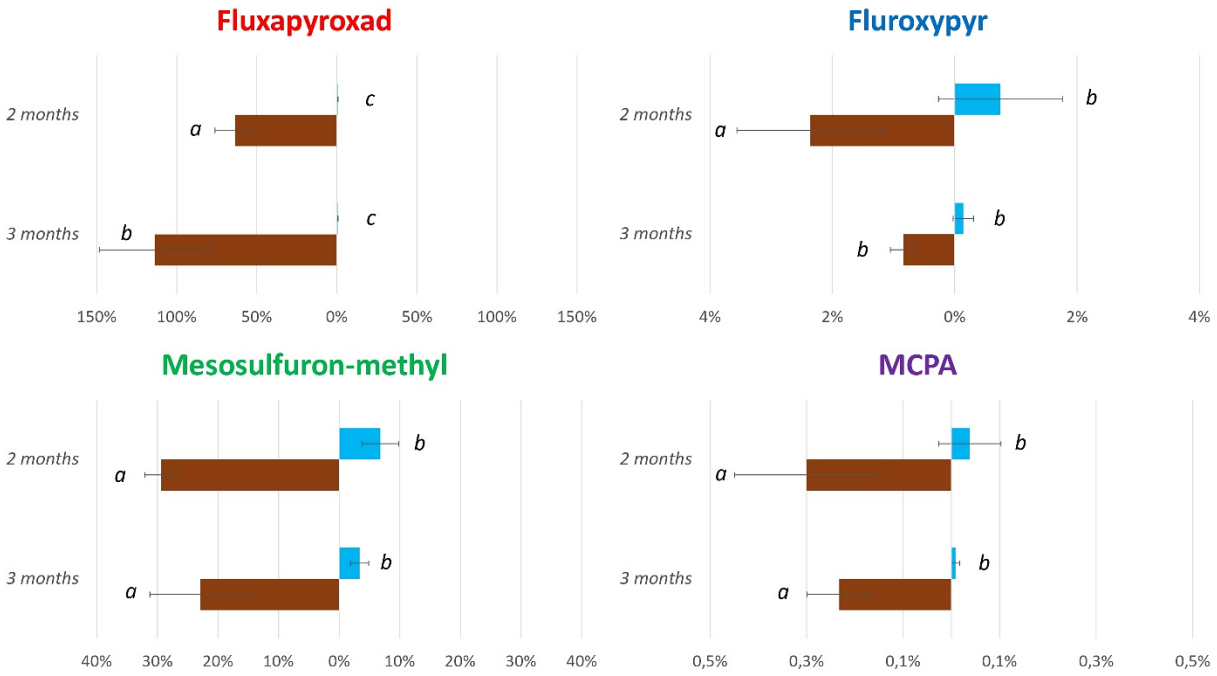

Figure 5-8: Average relative remaining mass [%] from spraying until second-month and third-month sampling for bare soil modality pots: comparison between soil (brown bars) and soil solution (blue bars) of the 4 reference molecules.

5.3.2 Influence of properties on the mass loss of pesticides

To observe potential relationships between the properties of the molecules and their mass losses, a correlation matrix was constructed. Two categories of variables are compared in this study: (i) Physicochemical properties (the same as those used for the first PCA; Figure 5-1), and (ii) the pesticide mass losses between the second and third month (soil, soil solution, and the ratio between the two).

In relative terms, no correlation coefficient exceeds 50% in either the positive or negative ranges (Table 5-3). Furthermore, none of the p-values corresponding to these coefficients fall below the conventional significance threshold of 0.05 (as indicated in bold in Table 5-3). Consequently, it is challenging to conclude that there is a linear relationship between specific properties and patterns of mass losses.

It is possible that there may be more complex connections, such as non-linear relationships or linear combinations including multiple variables. Indeed, several other parameters, which were not included in the analysis (e.g., evapotranspiration rate and modality), may also be of interest. Consequently, further analysis is required, which, due to time constraints, will not be carried out as part of this thesis.

Table 5-3: Coefficients of the correlation matrix between pesticide properties and masses (Pearson method). Positive coefficients (correlation) are coloured in green, and negative coefficients (anti-correlation) in red. The asymptotic corresponding p-values are given in bold.

Properties	*Soil*	*Soil solution*	*Solution/Soil*
Molecular mass	-14% **(0.60)**	2% **(0.93)**	-1% **(0.96)**
Solubility	-45% **(0.08)**	13% **(0.64)**	23% **(0.39)**
Kow	32% **(0.22)**	-23% **(0.39)**	-29% **(0.27)**
Vapour pressure	23% **(0.39)**	-5% **(0.84)**	19% **(0.48)**
DT50_soil	25% **(0.35)**	4% **(0.88)**	-37% **(0.15)**
GUS	-21% **(0.44)**	23% **(0.38)**	13% **(0.63)**

5.4 Influence of cover crops on pesticide dynamics

In accordance with the thesis objectives, this fourth section presents a comparison of the modalities. The objective of this section is to address key questions, including whether the presence of cover crops affects the evolution of pesticide content in the pots.

In addition to the matrix distinction on the second PCA (Figure 5-7), the shapes of the graph points differentiate the modalities. To ascertain whether the pesticide masses vary depending on the modality, an analysis will be conducted utilising an indicator: the aerial biomass of the cover crops. Following drying and weighing, the cover crops exhibited varying densities. Throughout the course of the experiment, the pots exhibited a growth in their aerial biomass over time. Consequently, to obtain the most contrasting results, only the third-month samples, with the highest biomass, will be analysed (Figure 5-9).

To facilitate clarity in this section, the modalities will be renamed in accordance with their tonnage per hectare:

- The designation of the *bare soil* control will remain unchanged, given that it has no aerial biomass.
- The winter spelt, which demonstrated a growth from 0.4 t_{DM} ha^{-1} after two months (±0.04) to 1.1 t_{DM} ha^{-1} after three months (±0.02), will be considered as *thick cover*.
- The multi-species mix, which exhibited a growth from 0.25 t_{DM} ha^{-1} after two months (±0.08) to 0.40 t_{DM} ha^{-1} after three months (±0.09), will be considered as *light cover*.

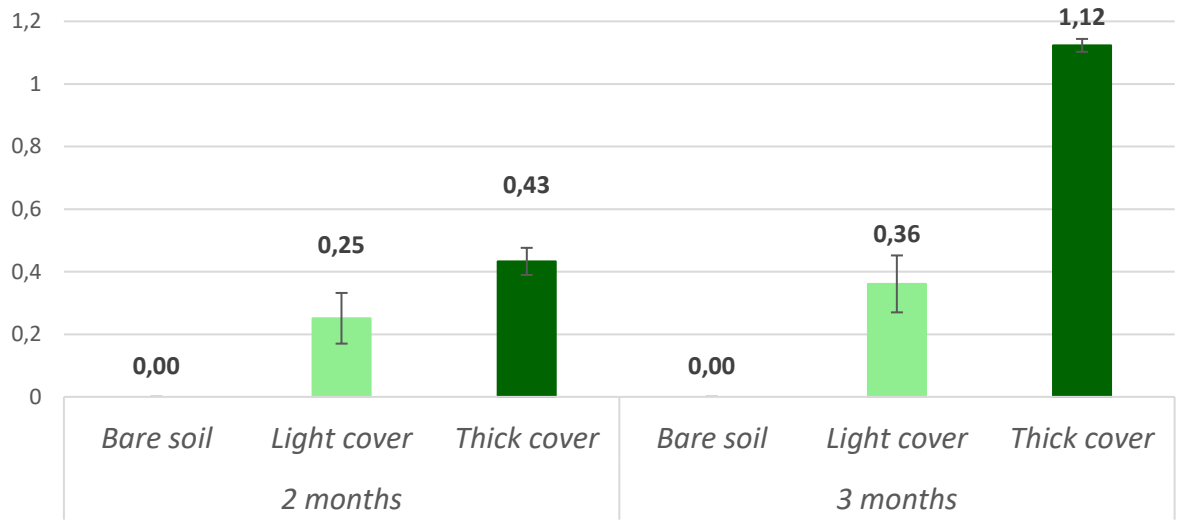

Figure 5-9: Average aerial biomass of modalities after 2 and 3 months [t ha^{-1}]. The multi-species mix is represented as light cover, while the winter spelt is represented as thick cover.

5.4.1 Comparison between the modalities

Summary of the assumptions concerning the effects of cover crops

As hypothesised in the earlier chapters, the presence of a dense cover crop is assumed to induce three main processes on pesticides:

1) Uptake by the cover crop,
2) Increased biodegradation due to greater microbial activity in the rhizosphere,
3) Increased retention in the rhizosphere due to greater evapotranspiration, which would promote biodegradation.

In the rhizosphere, biodegradation is considered to be the most influential process, as research has demonstrated on multiple occasions that biological decomposition is the most important natural way to remove pesticides from the environment (Briceño et al., 2007; Tudi et al., 2021).

Observation of the pesticide mass of the four reference molecules

The average mass per pot was compared for each matrix separately for the four reference molecules (Figure 5-3). The soil was initially analysed (Figure 5-10), followed by the soil solution (Figure 5-11).

When a significant difference is observed, it is consistently exhibited by a lower mass under the thick cover crop. Indeed, for all molecules and matrices, the light cover crop is never found to be significantly different from the control (Figure 5-10; Figure 5-11). Consequently, it can be postulated that there is an anti-correlation between the mass of the pesticide and the aerial biomass density of the cover crop. That is to say, the higher the aerial biomass, the lower the pesticide mass.

Based on the observed anti-correlation, it can be also postulated that the aerial biomass of the cover crop exerts a more significant influence on the ageing rate of pesticide residues than its specific diversity. Indeed, the thick cover, consisting of a single-species crop (winter spelt), exhibits a lower pesticide content than the light cover, comprising four distinct crop species (buckwheat, phacelia, vetch and white mustard). Given that root biomass is typically proportional to aerial biomass, this can be attributed to the enhanced biodegradation in a more developed rhizosphere (Akman et al., 2017).

With regard to the observed reduction in mass resulting from the thick cover, three distinct patterns can be discerned: (i) a decrease of pesticide mass in both soil and soil solution (shown by mesosulfuron-methyl), (ii) a decrease of pesticide mass in soil only (shown by fluxapyroxad and MCPA), and (iii) an absence of any differences of pesticide mass between the modalities (shown by fluroxypyr). These three behaviours will be discussed in more detail after Figure 5-11.

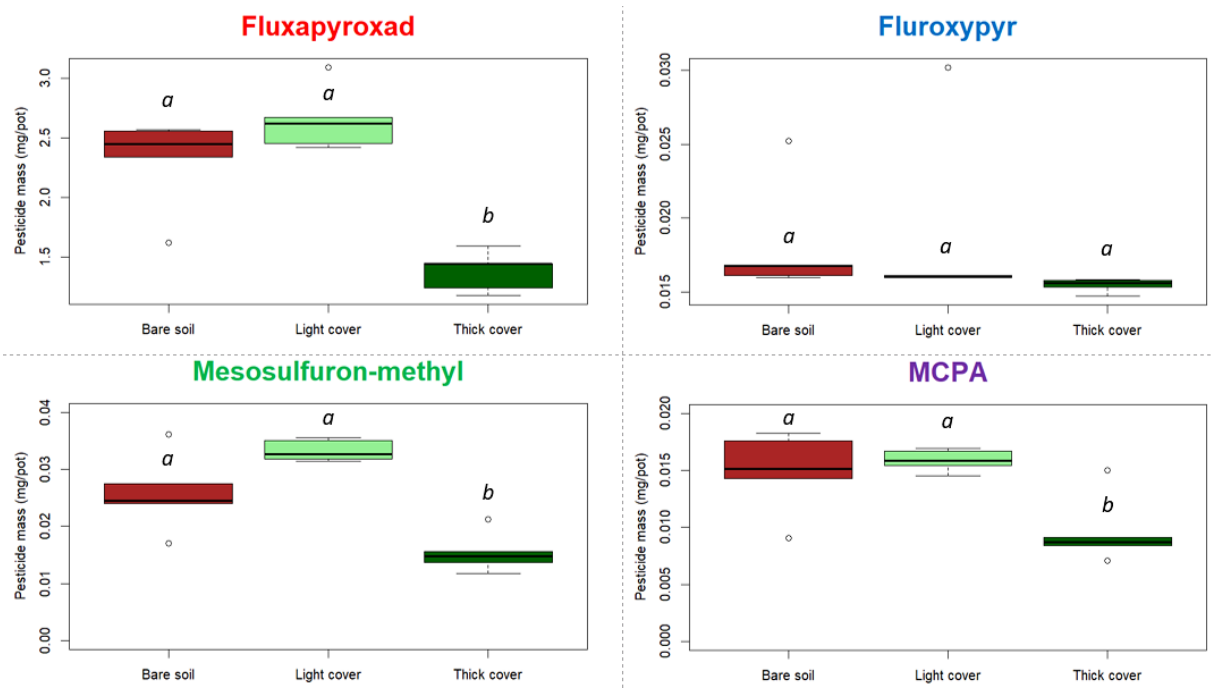

Figure 5-10: Average mass of the four reference molecules in the soil after 3 months [mg/pot]. The letters show a statistically significant difference between modalities (p-value < 0.05).

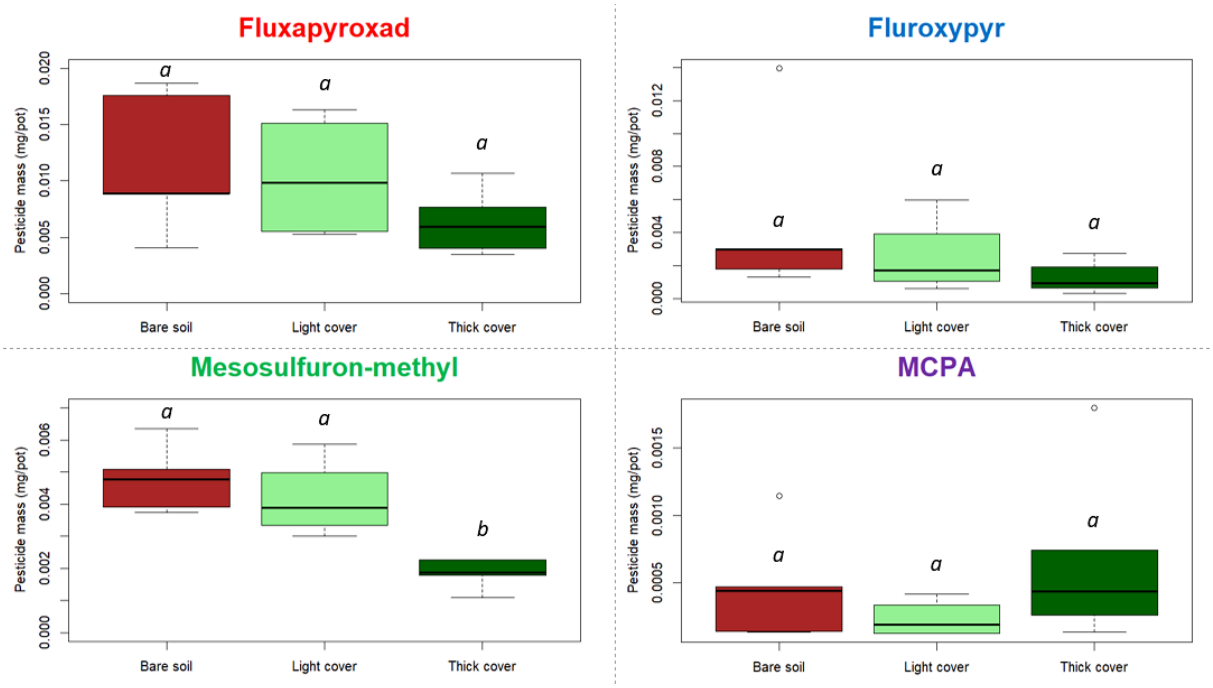

Figure 5-11: Average mass of the four reference molecules in the soil solution after 3 months [mg/pot]. The letters show a statistically significant difference between modalities (p-value < 0.05).

5.4.2 Influence of the thick cover: decrease of pesticide mass in soil and soil solution

The only molecule to undergo a reduction in mass in both soil and soil solution under the thick cover modality is mesosulfuron-methyl. It is distinguished by a markedly high leaching potential (GUS), a relatively high persistence in soil (DT50_soil), a moderate solubility, and a very low volatility (vapour pressure).

The high leachability (GUS) may be a contributing factor to this strong subject to cover crop influence. Given that GUS is an empirical index proportional to persistence in soil (DT50_soil) and inversely proportional to adsorption to soil particles (K_{OC}; Wauchope et al., 1992), a high value of this index implies that mesosulfuron-methyl is likely to undergo mobility in soil and transfer towards deeper soil layers or groundwater bodies (Gustafson, 1989). By reducing the leaching of the molecule, the thick cover may enhance microbial biodegradation and plant uptake to a lesser extent. (Cassigneul, 2019; Potter et al., 2007).

Furthermore, the high mobility of mesosulfuron-methyl in both soil and water (high GUS and moderate solubility) can be attributed to its ability to transfer between soil and soil solution (Araya et al., 2024). Given the well-established presence of this molecule in the two matrices, it is unsurprising that its quantity was observed to be affected in both soil and solution.

Other group 3 molecules exhibit a similar physicochemical nature to that of mesosulfuron-methyl (Figure 5-12). Consequently, it can be expected that they will exhibit an analogous pattern regarding the influence of modalities. The same reasoning that was applied to mesosulfuron-methyl can therefore be used to justify those patterns.

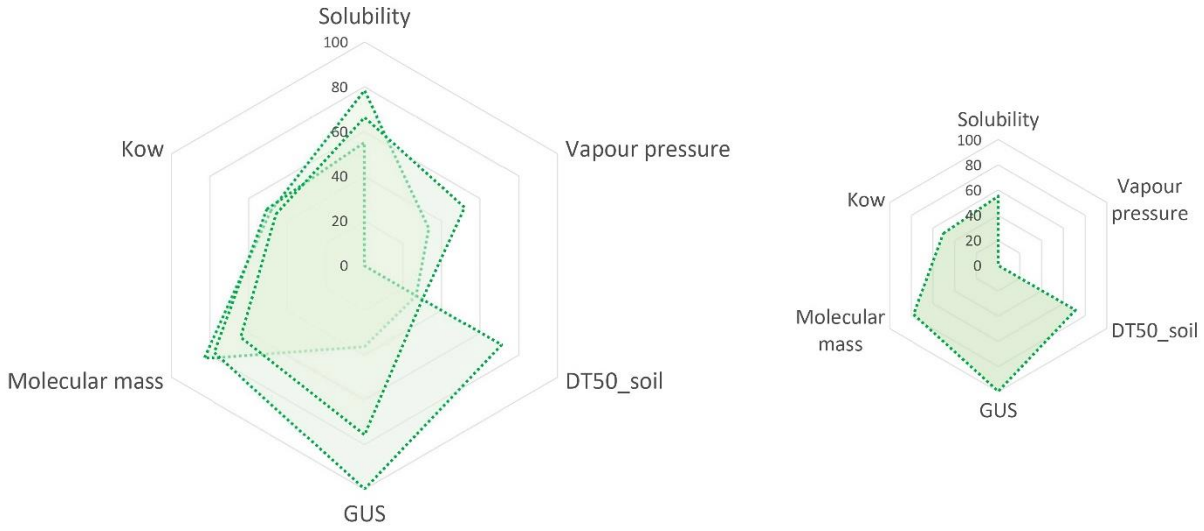

Figure 5-12: Comparison between the spider diagram of the properties of all group 3 molecules (left) and the one of mesosulfuron-methyl alone (right).

5.4.3 Influence of the thick cover: decrease of pesticide mass in soil only

The findings of the study indicated that fluxapyroxad and MCPA exhibited comparable differences between the two modalities. While no discernible difference was observed in the soil solution, a notable reduction in soil was evident under the thick cover treatment. However, given the stark contrast in the physicochemical nature of the two molecules, they will be discussed separately.

Case of the fluxapyroxad: high persistence and low mobility in the soil

Throughout the experiment and for any modality, fluxapyroxad exhibited a weak but relatively constant concentration in soil solution (Figure 5-5; Figure 5-8). This behaviour can be supported by its nature, which presents a remarkably high degree of persistence in soil (DT50_soil), a weak solubility, and a substantial hydrophobicity (K_{OW}). Given that fluxapyroxad is predominantly found in the soil, it can be postulated that its quantity in soil solution may be insufficient to exhibit any significant difference between the modalities. It is possible that the thick cover crop may have exerted an effect on the soil solution too, had the pesticide mass in this matrix would have been more significant.

Given that fluxapyroxad is a highly persistent molecule in soil, it is not immediately apparent why any significant differences should be observed between the modalities. Nevertheless, three potential explanations for these findings can be proposed.

Firstly, the structure of pesticides, and particularly their molecular mass and chemical and spatial structure, plays an important role in facilitating faster biodegradation (Bose et al., 2020). Consequently, despite its physicochemical nature being considered persistent and recalcitrant, fluxapyroxad exhibits a relatively low molecular mass (Figure 5-3). This characteristic implies that this light molecule could be subjected to higher biodegradation than expected, in the case of sufficiently high microbial activity (for example, in the presence thick cover crop with a developed root system and an active rhizosphere). Nevertheless, given that MCPA and fluroxypyr have similar molecular masses to fluxapyroxad, this argument of differentiation would not be a sufficient explanation in isolation. Consequently, the chemical and spatial structure may also be implicated in this phenomenon (Bose et al., 2020).

Moreover, the rate of degradation is also contingent upon the availability of specific microbial populations and enzymes required for the biodegradation of specific molecules (Gavrilescu, 2005). This appears to be the case here, where fluxapyroxad has been subjected to a higher degree of degradation than fluroxypyr. For an identical modality, microbial and enzymatic conditions may have favoured fluxapyroxad degradation.

It is generally accepted that soil-sorbed pesticides are thought to be unavailable for biodegradation (Ogram et al., 1985). Nevertheless, some studies have indicated results that are contrary to this consensus. It has been demonstrated that persistent compounds, such as fluxapyroxad, may also be degraded by microorganisms (Li et al., 2015; Park et al., 2001).

The behaviour of fluxapyroxad can be extrapolated to the other group 1 molecules, given their equivalent properties (Figure 5-13). The extremely high persistence in soil (high DT50_soil and K_{OW} and low solubility and GUS) indicates a presence that is favoured in the soil rather than in the soil solution. This is evidenced by the influence of the thick cover, which is mainly observed in soil samples.

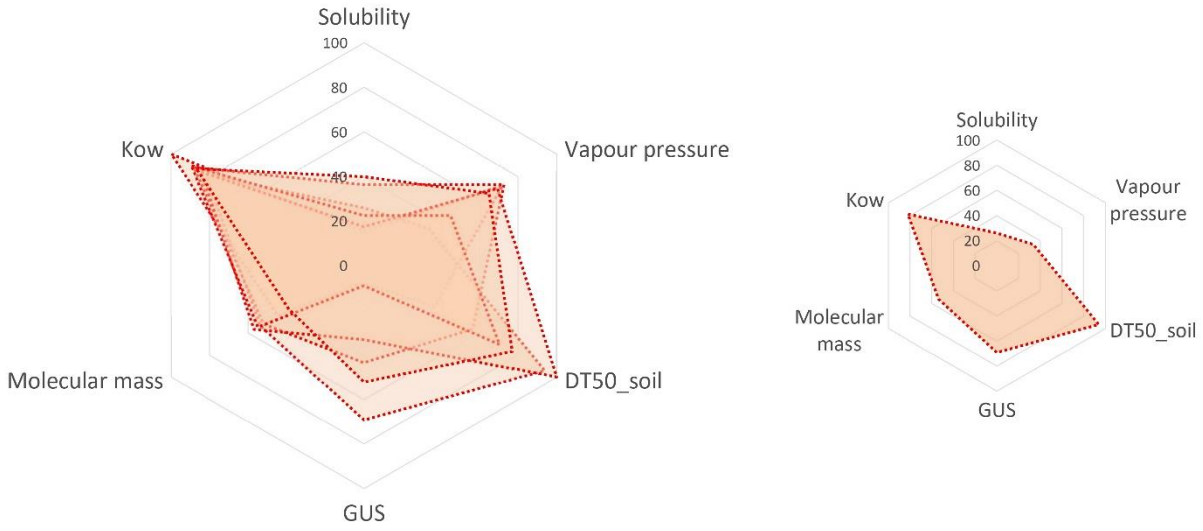

Figure 5-13: Comparison between the spider diagram of the properties of all group 1 molecules (left) and the one of fluxapyroxad alone (right).

It is acknowledged that the rationale provided to explain the fluxapyroxad results is not particularly robust. Further investigation is required to ascertain the reasons behind this non-intuitive behaviour. Additional parameters and processes may be required for a better understanding.

Case of the MCPA: low persistence and high mobility in the soil

With regard to the results presented in Figure 5-10 and Figure 5-11, the rationale for their explanation is more straightforward in the case of MCPA than in the fluxapyroxad example. Indeed, the physicochemical nature exhibited by MCPA provides a more intuitive explanation for the mass differences between soil and soil solution.

Firstly, due to its high volatility (vapour pressure), MCPA exhibits an exceptionally low persistence in the environment, regardless of the matrix. Indeed, the second PCA performed, based on the pesticide concentrations in the samples (Figure 5-7), contrasts the first-month samples quite markedly from the other samples. This distribution is driven by a single molecule, which is the focus of this case study (MCPA). This suggests that the residence time of MCPA in soil and soil solution was relatively brief due to its high volatility.

Furthermore, to elucidate the distinction between modalities, namely the mass reduction induced by the thick cover crop, the three initial assumptions can be leveraged. Indeed, the higher evapotranspiration rate, induced by the dense biomass, inhibited the leaching of MCPA, which then tended to remain longer in the rhizosphere (White et al., 2009). Consequently, the uptake of the molecules by the crop roots may have been enhanced due to a high aqueous mobility (high GUS and solubility). Indeed, research has demonstrated that the more hydrophilic a pesticide is, the less time it takes for it to reach a steady-state concentration in the xylem (Sicbaldi et al., 1997). For a highly soluble compound such as MCPA, which is highly hydrophilic, it can be therefore expected that the pesticide will be absorbed by the plant roots (Behrendt & Brüggemann, 1993; Liu et al., 2021).

It is anticipated that an increase in microbial degradation will occur in conjunction with a reduction in leaching. This process has already been elucidated and previously referenced.

AMPA, which is the other group 4 molecule, exhibits properties that are highly analogous to those of MCPA (Figure 5-14). Consequently, the same outcomes are anticipated for AMPA, which can be explained by a similar rationale as that of MCPA.

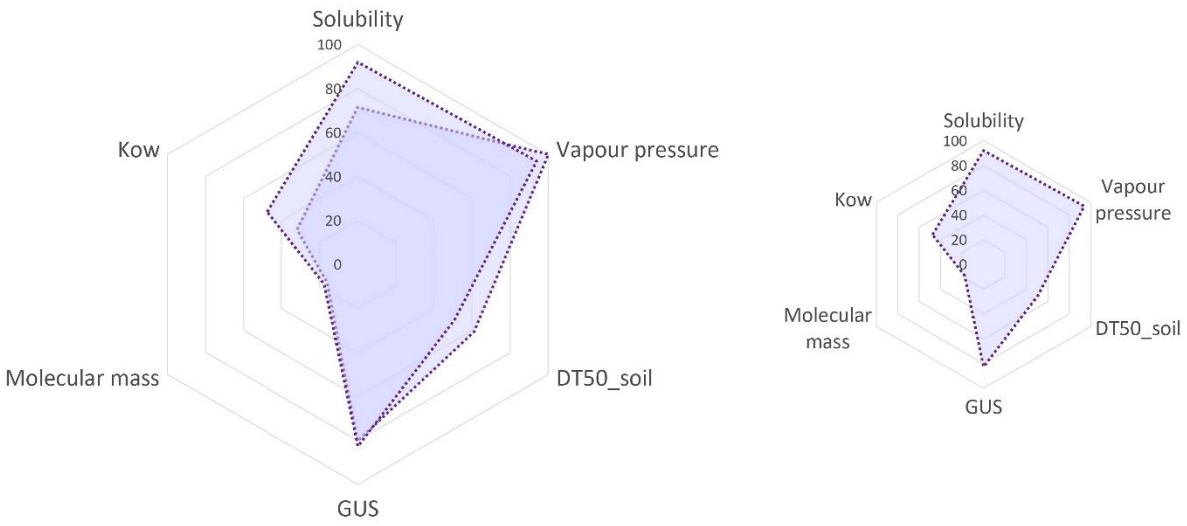

Figure 5-14: Comparison between the spider diagram of the properties of all group 4 molecules (left) and the one of MCPA alone (right).

5.4.4 Absence of differences between the modalities

Fluroxypyr is the only molecule to demonstrate no significant differences between the modalities. Fluroxypyr exhibits a low DT50_soil and a moderately high solubility, resulting in high concentrations at the commencement of the experiment. As illustrated in subsection 5.3.1, the substantial content was followed by notable decreases in both soil and soil solution over time (Figure 5-8).

The absence of significant differences between the modalities (insufficient) low quantity remaining after three months. When considering the third-month samples, this rationale is relevant for the soil, but not for the soil solution. On the one hand, all soil samples exhibited concentrations below LOQ. These extremely low results make it reasonable to assume that the content is unlikely to result in differences between modalities. The soil solution samples, on the other hand, revealed concentrations significantly above LOQ. Consequently, it would be irrelevant to extend the justification of the soil concentration to the soil solution samples. Further research may be required in this instance.

It is notable to mention that observing the average physicochemical nature of the other group 2 molecules provides a more comprehensive understanding of the results exhibited by fluroxypyr (Figure 5-15). It is evident that group 2 molecules exhibit a high degree of hydrophobicity, which results in a preference for the solid phase of the soil over the soil solution (low solubility and GUS and high K_{OW}). Moreover, they also exhibit low persistence in the environment (high vapour pressure and low DT50_soil).

When the two aforementioned property traits are coupled, it becomes evident that the thick cover effect on soil only can be explained intuitively. Initially, molecules were transferred from the soil solution to accumulate in the solid phase of the soil. Thereafter, they were either volatilised (caused by the high vapour pressure) or biodegraded (caused by the thick cover-induced microbial activity).

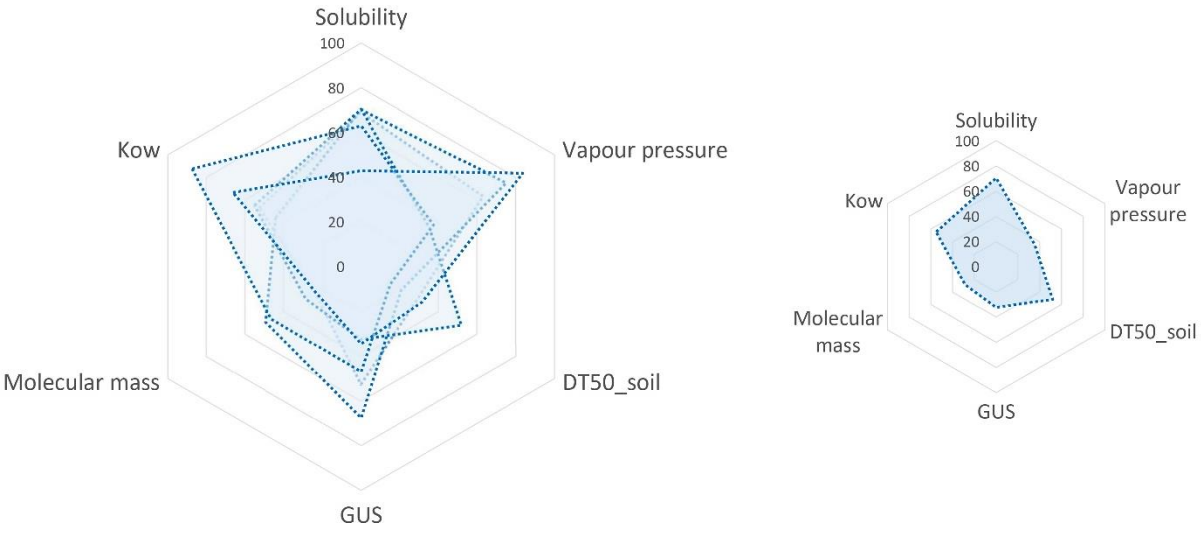

Figure 5-15: Comparison between the spider diagram of the properties of all group 2 molecules (left) and the one of fluroxypyr alone (right).

6. Conclusion and perspectives

Summary of the context and objectives

In modern agriculture, pesticides are essential for protecting crops and enhancing yields. Yet, their persistent residues present a significant risk to human health and ecosystems, primarily through groundwater contamination, surface runoff, and atmospheric dispersion.

Cover crops, and particularly (nitrate) catch crops, offer many benefits by mitigating nitrate leaching and potentially reducing pesticide residues in agricultural soils. Although nitrate catch crops are already legislated in Wallonia, pesticide catch crops have yet to be legally considered.

The objective of this thesis was to evaluate the influence of cover crops on the presence and transfer of pesticides in agricultural soils, through a three-month greenhouse experiment. The study investigated three soil cover modalities: bare soil (control), a winter cash crop (winter spelt), and a winter catch crop (multi-species cover crop), analysing 19 pesticides over time.

Summary or the results

Prior to undertaking an analysis of the pesticide concentrations, it was necessary to examine the physicochemical nature of the pesticides. The diverse properties of the molecules were employed to cluster the pesticides into groups with similar natures.

A comprehensive overview of pesticide concentrations revealed distinct distributions. Persistent and hydrophobic molecules predominated in solid soil, while soluble and volatile ones were the most common in soil solution. A temporal analysis highlighted a remanence of persistent molecules over time.

The examination of the bare soil control revealed a variety of temporal mass reductions among different molecules, with a few unexpected increases that may have been influenced by sampling biases. The distribution dynamics were found to reflect properties such as solubility, volatility, and persistence.

In the comparison of the modalities, only the winter spelt (mono-species cover) exhibited a statistically significant reduction in pesticide content in the pots. This outcome was attributed to the biomass density of the spelt, which was three times greater than that of the multi-species mix (t ha^{-1}). In the three-month context of the greenhouse conditions, the density of the cover crops (aerial and root biomass) appeared to be a more influential factor than their diversity in reducing the quantity of pesticide residues in the soil pots.

Recommendations for the implementation of winter cover crops in agricultural practices

The findings of this study may offer valuable insights that could benefit agricultural practices. It is recommended that cover crops be sown as soon as possible after the previous crop harvest, to ensure that the soil is adequately covered (i.e. a sufficiently developed cover crop) before the high leaching risks, associated with autumn and winter rainfall, become apparent.

The key factor in the effectiveness of a cover crop in reducing pesticide leaching is its biomass density. This density plays a dual role: (i) on the aerial level, it increases evapotranspiration and

reduces drainage, and (ii) on the root level, it enhances microbial degradation in a well-developed rhizosphere.

This lesson was also learned during the failure of the initial experimental trial, which was conducted in a crop field. Indeed, the initial field results indicated that pesticide concentrations were below the LOQ within the first month. This rapid decline was attributed to the heavy autumn rainfall, which may have accelerated the leaching of the pesticide residues.

Limitations

It is first necessary to acknowledge that the greenhouse conditions present notable limitations:

- The experiment yielded insights into mass balance, yet it did not simulate the leaching conditions typically found in crop fields.
- The absence of direct solar radiation in the greenhouse raises questions about the impact of albedo on pesticide behaviour, which warrants further investigation.
- The mild indoor temperatures did not reflect outdoor winter conditions: it would be beneficial to consider outdoor temperatures and their fluctuations in future studies.

Moreover, the LOD and LOQ set measurement thresholds without confirming a total absence of pesticides. This raises concerns about the potential for soil and aquifer contamination.

Finally, the limited duration of the experiment may have resulted in a bias results towards the stronger effects of mono-species cover crops over those of a more diverse nature. Over time, the specific diversity could have a greater influence on outcomes than biomass density.

Perspectives

Further investigation may be warranted in the following areas:

- Conducting larger-scale, *in-situ* field experiments, incorporating more pedoclimatic parameters.
- Extending the study duration to observe the long-term effects of cover crops, particularly the influence of multi-species root systems.
- Employing diverse cover crop densities and species to gain a comprehensive understanding of ecosystem dynamics.
- Monitoring pesticide transfer across soil depths and plant tissues is recommended.
- Integrating a hydrological study to explore water regimes, leaching pathways, and evapotranspiration rates.
- Analysing soil parameters in conjunction with pesticide quantification, with the objective of enhancing the understanding of their biological impacts: pH, SOM, dissolved organic carbon (DOC), CEC, nutrients, and nitrogen dynamics.

These perspectives aim to address current limitations and extend the understanding of pesticide dynamics in agricultural ecosystems, paving the way for more comprehensive and environmentally sound practices.

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8. Appendices

8.1 Pesticide data

8.1.1 Formulation and maximum authorised dose of the formulated products

Table 8-1: Formulation and maximum authorised dose of the pesticides (L ha^{-1} in blue or kg ha^{-1} in brown).

Formulated product	*Active substance(s)*	*Formulation*	*Maximum authorised dose*
Afinto®	Flonicamid	500	0.32
Aquino®	Fenpicoxamid	50	2
Axial®	Cloquintocet-mexyl	12.5	1.2
	Pinoxaden	50	1.2
Barclay® Gallup Super 360	Glyphosate	360	4
Bofix®	Clopyralid	20	4
	Fluroxypyr	40	4
	MCPA	200	4
Butizyl®	MCPB	400	5
Capri®	Cloquintocet-mexyl	75	0.25
	Pyroxsulam	75	0.25
Comet New®	Pyraclostrobin	200	2.5
Frimax®	Cloquintocet-mexyl	12	0.5
	Fluroxypyr	280	0.5
	Halauxifen-methyl	12.5	0.5
Mesiofis Pro®	Iodosulfuron-methyl-sodium	2	1.5
	Mefenpyr-diethyl	30	1.5
	Mesosulfuron-methyl	10	1.5
Mizona®	Fluxapyroxad	30	2
	Pyraclostrobin	200	2
Primus®	Florasulam	50	0.1
Revytrex®	Fluxapyroxad	66.7	3
	Mefentrifluconazole	66.7	3
Tebusip®	Tebuconazole	250	3

8.1.2 Physicochemical properties of the applied molecules

All the properties of pesticides were found in the pesticide database built by the University of Hertfordshire (PPDB, 2024). The missing data is left empty by means of consistency because many other sources often give various values.

Table 8-2: Physicochemical properties of the studied molecules (PPDB, 2024).

Molecule	Type	Molecular mass	Solubility	log(K_{OW})	Vapour pressure	K_H	BCF	DT50 in soil	DT50 in water	log(K_{OC})	GUS
AMPA	Metabolite	111.04	1466561	-1.63	0.02	0.16	3.16	234.00	121	7.60	1.56
Clopyralid	Herbicide	192.00	7850	-2.63	1.36	0.00	1.00	23.20	1000	1.61	3.02
Cloquintocet-mexyl	Safener	335.80	0.59	5.20	0.01	0.00	621.00	5.00	139	9.20	0.00
Fenpicoxamid	Fungicide	614.64	0.04	4.40	0.00	0.00	18.36	3.50	0.92	10.88	-0.29
Flonicamid	Insecticide	229.16	5200	-0.24	0.00	0.00	1.00	3.10	1000	0.47	1.87
Florasulam	Herbicide	359.28	6360	-1.22	0.01	0.00	1.50	1.85	1000	3.09	2.50
Fluroxypyr	Herbicide	255.03	6500	0.04	0.00	0.00	62.10	13.10	223		1.03
Fluxapyroxad	Fungicide	381.31	3.44	3.13	0.00	0.00	36.00	183.00	185		2.57
Glyphosate	Herbicide	169.10	100000	-6.28	0.01	0.00	0.50	16.11	1000	7.26	0.29
Halauxifen-methyl	Herbicide	345.16	1830	3.76	0.00	0.00	217.00	1.30	155	7.26	1.64
Iodosulfuron-methyl-Na	Herbicide	529.24	25000	-0.70	0.00	0.00	1.00	2.70	1000		1.19
MCPA	Herbicide	200.62	250000	-0.81	0.40	0.00	1.00	12.07	1000	4.30	3.13
MCPB	Herbicide	228.67	60.40	1.33	0.05	0.00	1.00	3.65	1000	4.65	1.12
Mefenpyr-diethyl	Safener	373.23	20.00	3.83	0.01	0.00		17.50	41	6.45	1.49
Mefentrifluconazole	Fungicide	397.78	0.81	3.40	0.00	0.00		268.00	1000		1.06
Mesosulfuron-methyl	Herbicide	503.51	483	-0.48	0.00	0.00	1.00	43.50	253		3.85
Pinoxaden	Herbicide	400.51	200	3.20	0.00	0.00	1.00	0.50	14.90		-0.32
Pyraclostrobin	Fungicide	387.82	1.90	3.99	0.00	0.00	706.00	41.90	1000	9.14	0.05
Pyroxsulam	Herbicide	434.35	3200	-1.01	0.00	0.00	1.00	3.30	1000	3.50	2.84
Tebuconazole	Fungicide	307.82	36	3.70	0.00	0.00	78.00	63.00	1000		1.86

Spider charts of the pesticides

A spider diagram is built for each molecule individually. Only the properties kept in the first PCA are plotted: the molecular mass, the GUS, the solubility, the know, the vapour pressure, and the soil half-life (DT50_soil). The values of the properties are relative: they are percentages built from all the values of the 20 molecules.

The charts are grouped by pesticide clusters, following their alphabetical order. The diagram colour represents the families of molecules according to their target: herbicides in green, fungicides in grey, insecticides in yellow, metabolites in light green, and safeners in blue. The four pesticides that do not belong to any cluster due to a preliminary exclusion (glyphosate, AMPA, fenpicoxamid, and pinoxaden) are plotted after the four groups.

The following charts show the spider diagrams of pesticides from group 1.

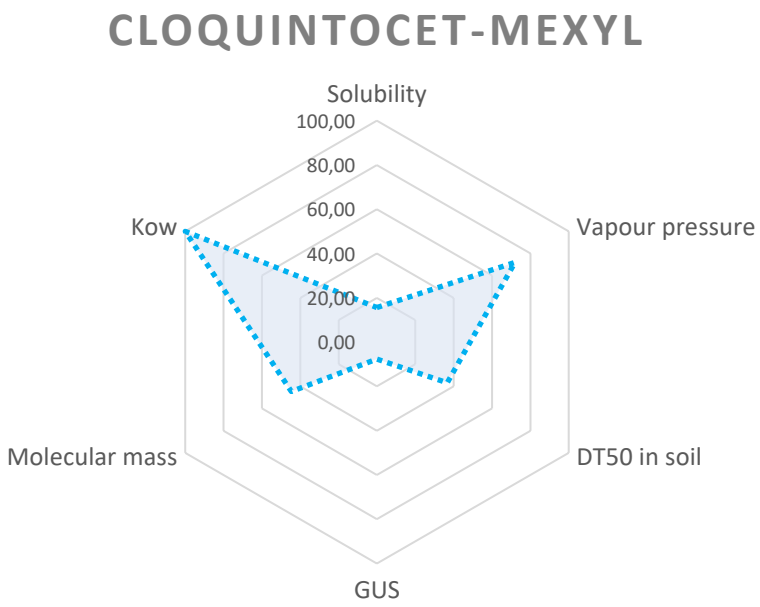

Figure 8-1: Spider chart showing the main physicochemical properties of cloquintocet-mexyl.

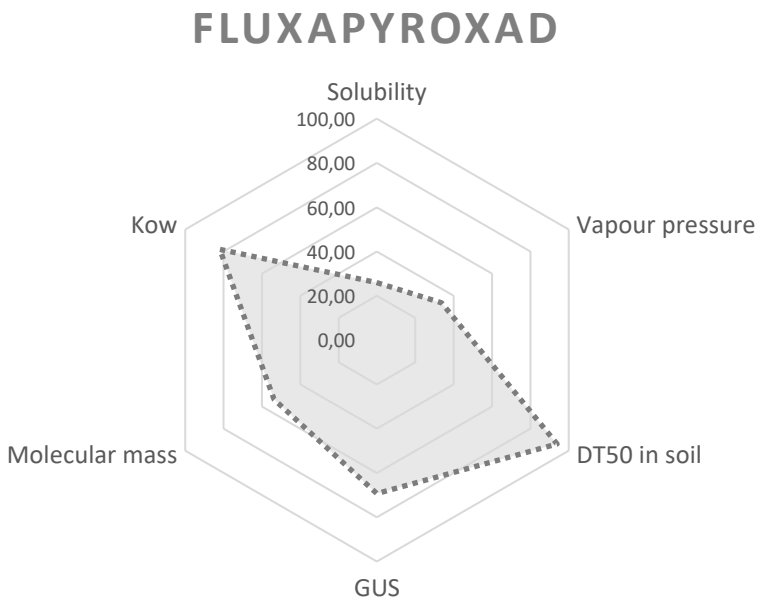

Figure 8-2: Spider chart showing the main physicochemical properties of fluxapyroxad.

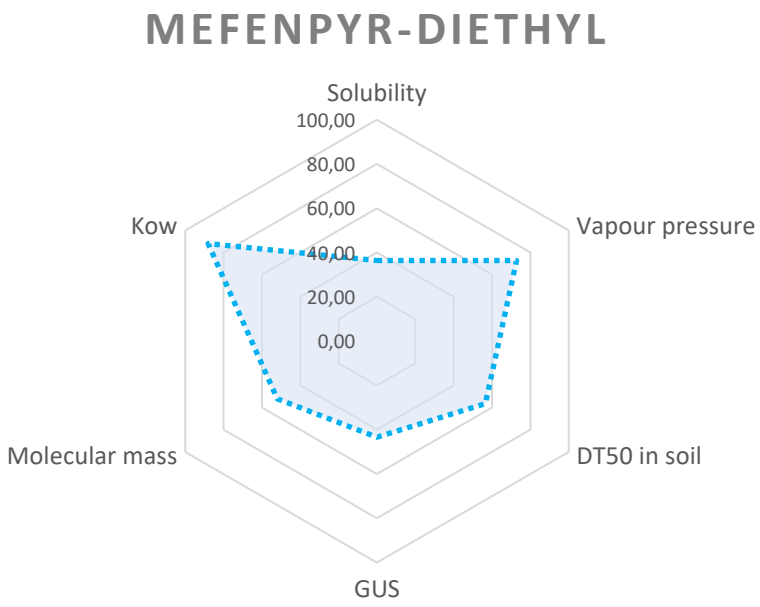

Figure 8-3: Spider chart showing the main physicochemical properties of mefenpyr-diethyl.

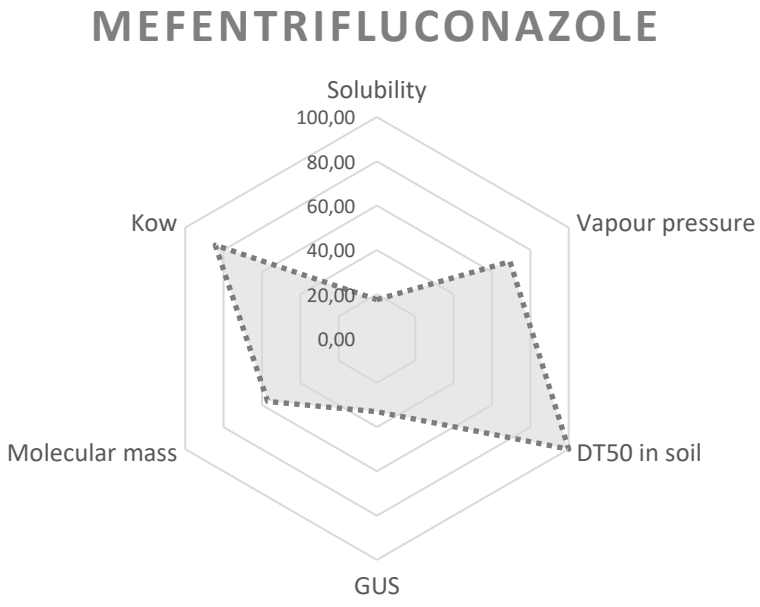

Figure 8-4: Spider chart showing the main physicochemical properties of mefentrifluconazole.

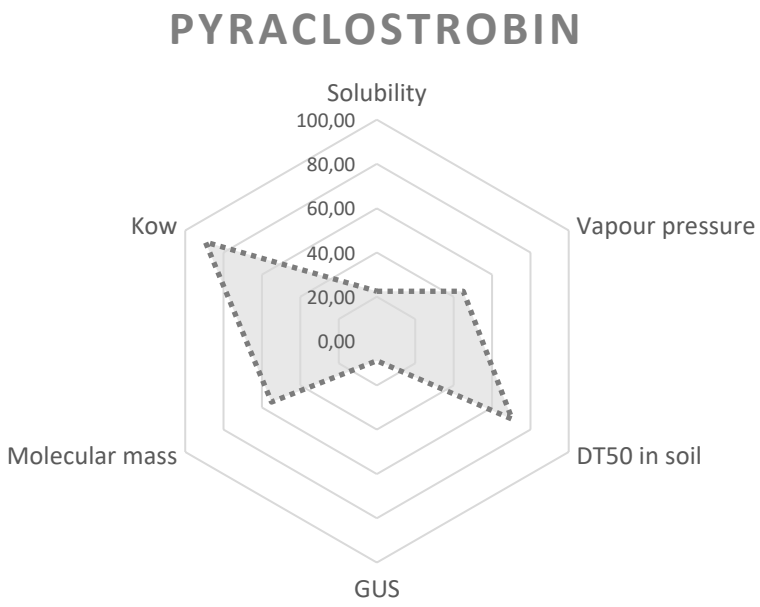

Figure 8-5: Spider chart showing the main physicochemical properties of pyraclostrobin.

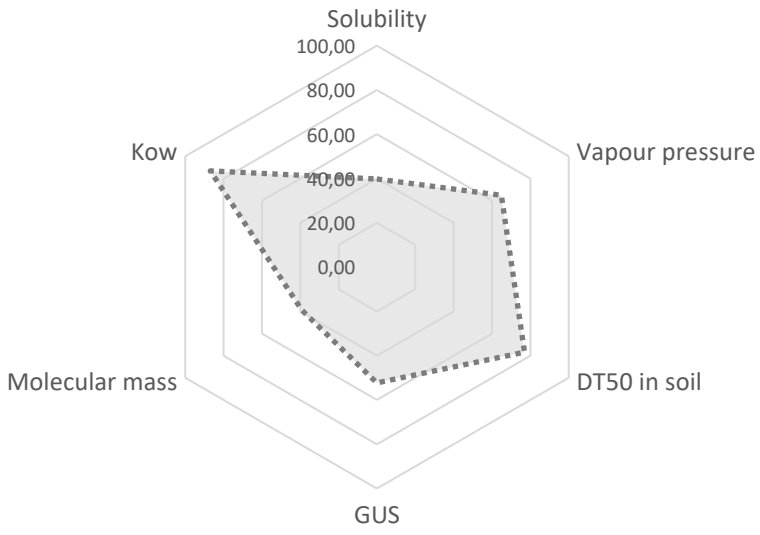

Figure 8-6: Spider chart showing the main physicochemical properties of tebuconazole.

The following charts show the spider diagrams of pesticides from group 2.

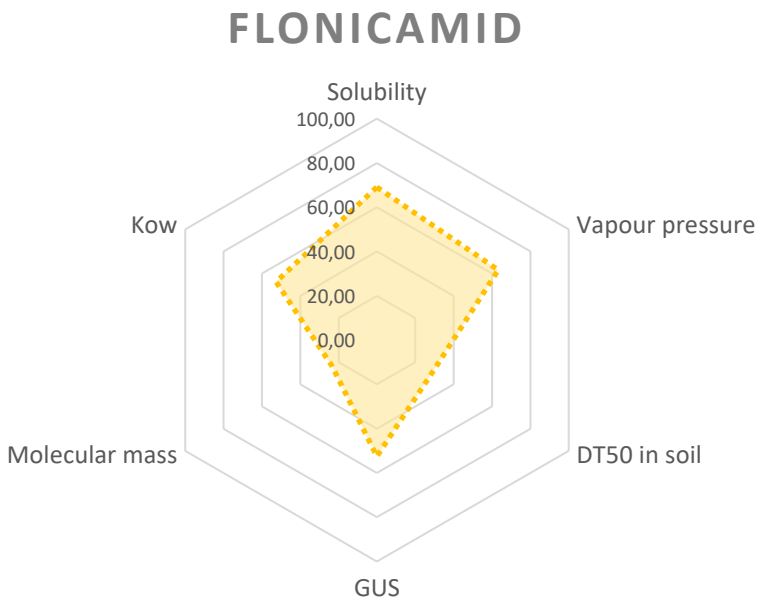

Figure 8-7: Spider chart showing the main physicochemical properties of flonicamid.

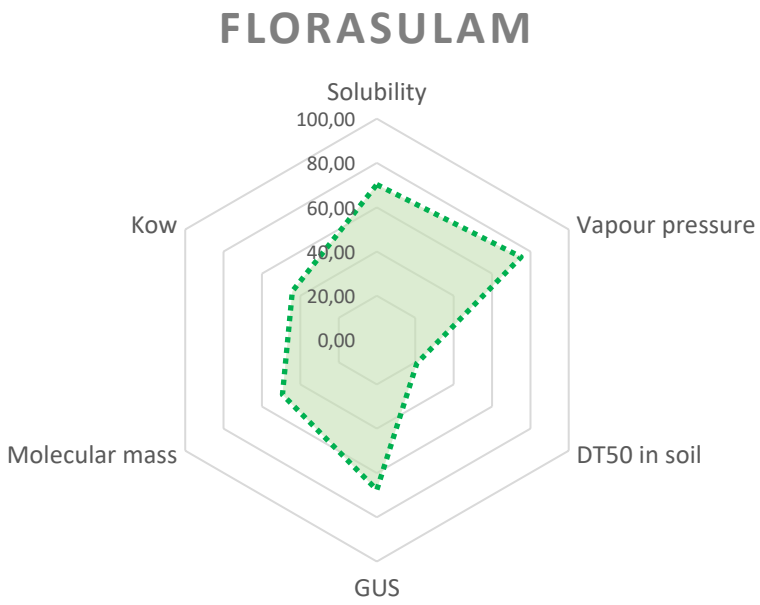

Figure 8-8: Spider chart showing the main physicochemical properties of florasulam.

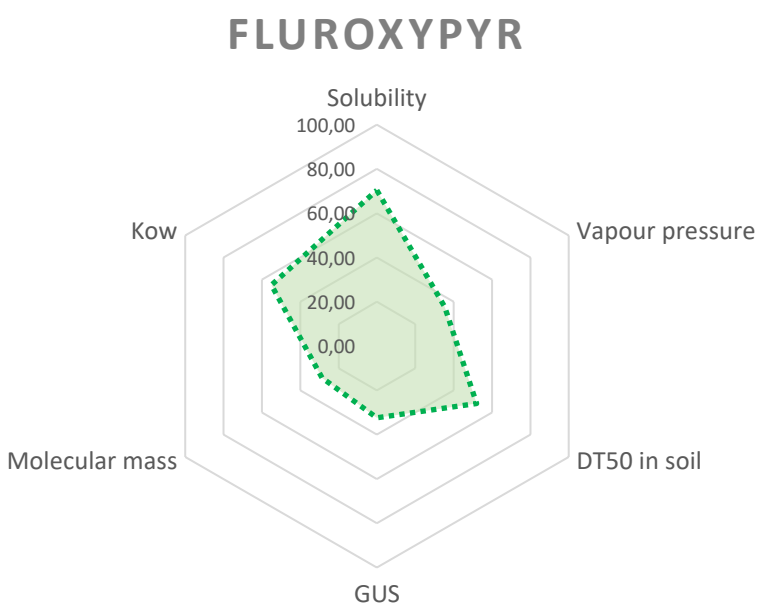

Figure 8-9: Spider chart showing the main physicochemical properties of fluroxypyr.

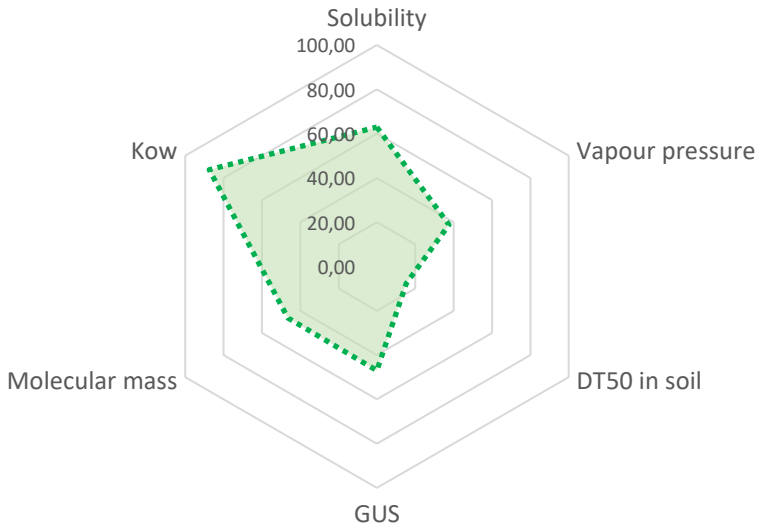

Figure 8-10: Spider chart showing the main physicochemical properties of halauxifen-methyl.

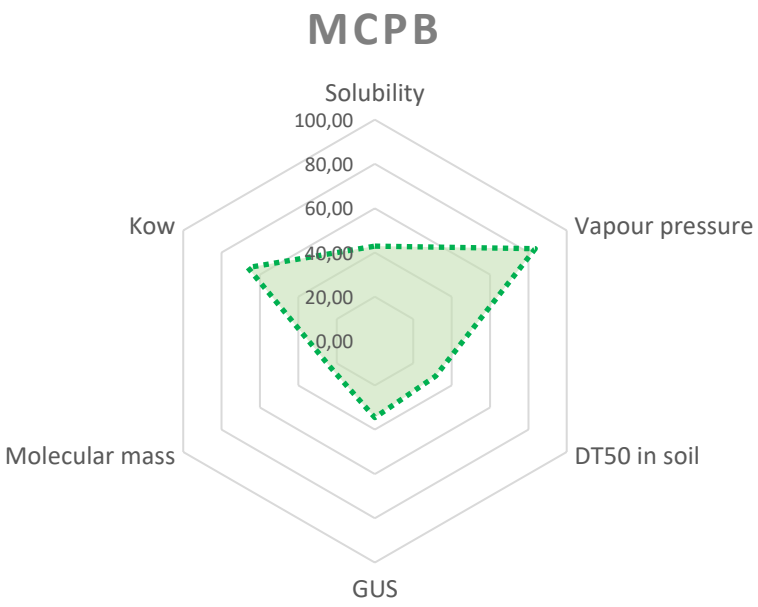

Figure 8-11: Spider chart showing the main physicochemical properties of MCPB.

The following charts show the spider diagrams of pesticides from group 3.

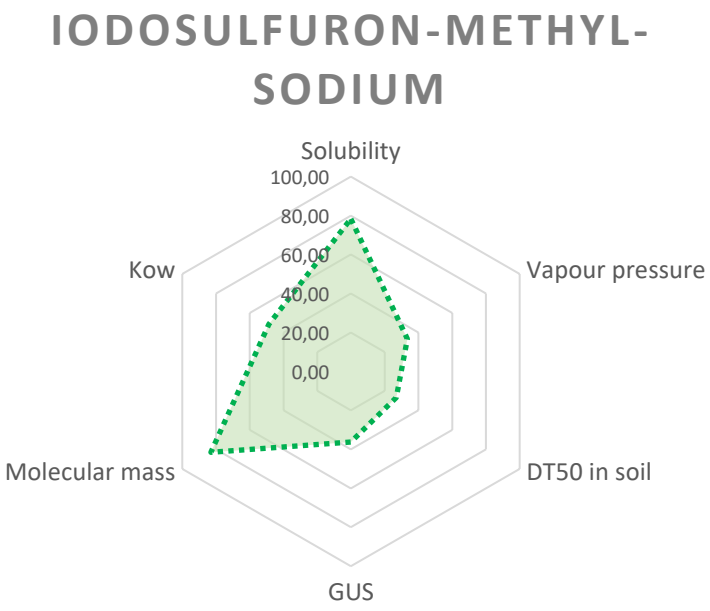

Figure 8-12: Spider chart showing the main physicochemical properties of iodosulfuron-methyl-sodium.

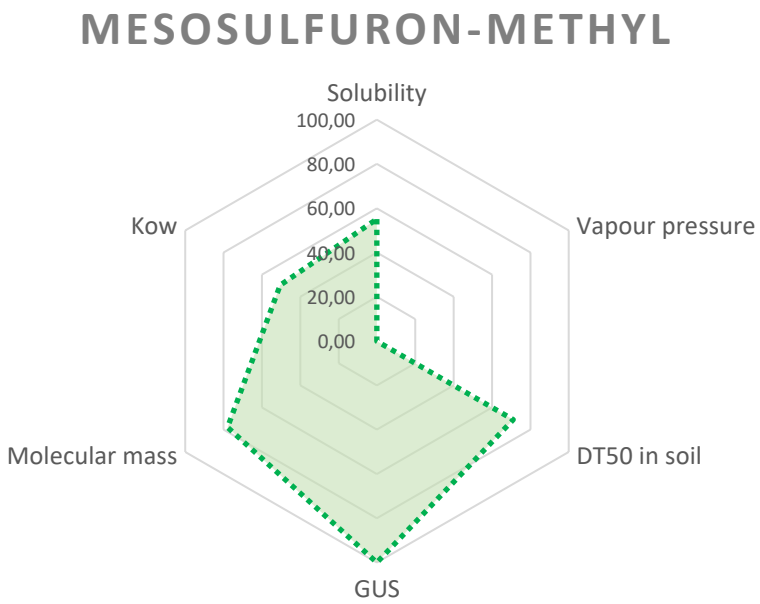

Figure 8-13: Spider chart showing the main physicochemical properties of mesosulfuron-methyl.

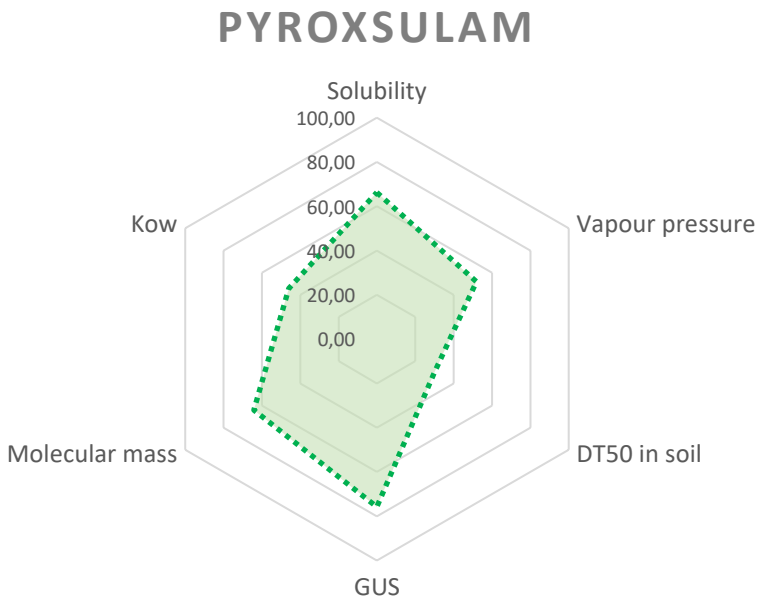

Figure 8-14: Spider chart showing the main physicochemical properties of pyroxsulam.

The following charts show the spider diagrams of pesticides from group 4.

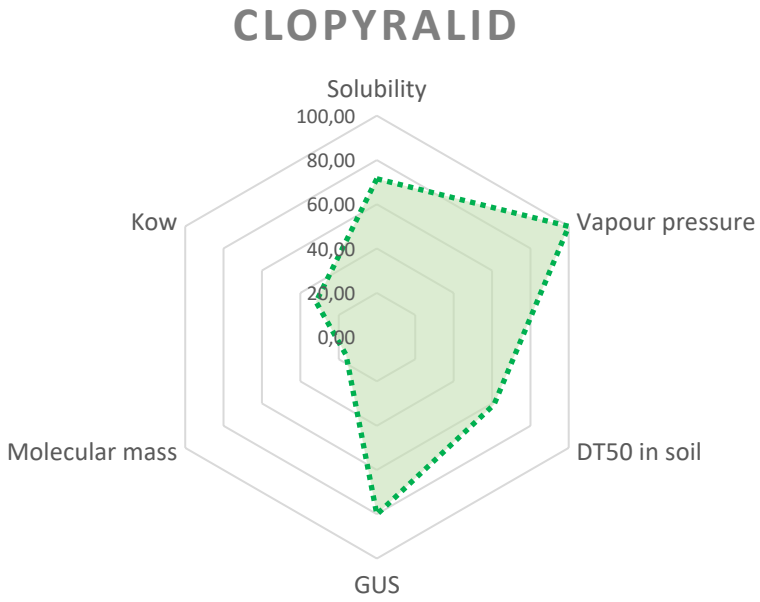

Figure 8-15: Spider chart showing the main physicochemical properties of clopyralid.

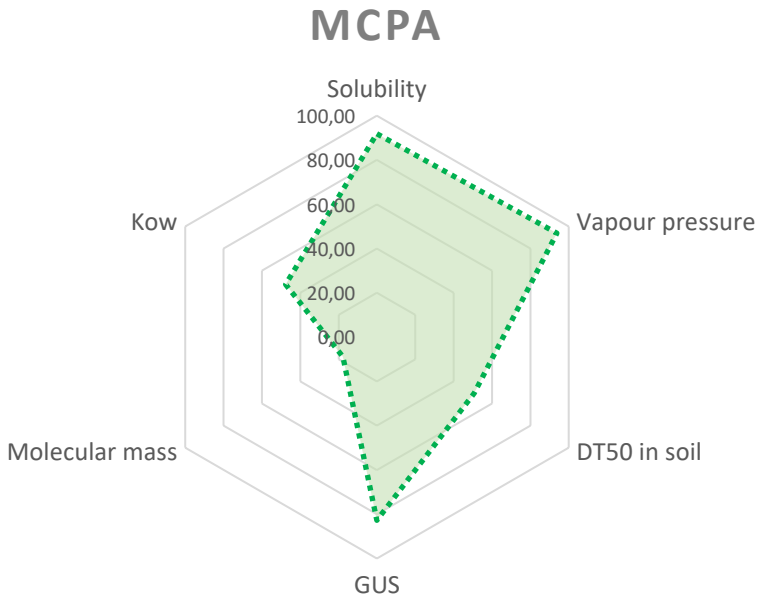

Figure 8-16: Spider chart showing the main physicochemical properties of MCPA.

The following charts show the spider diagrams of pesticides belonging to no group.

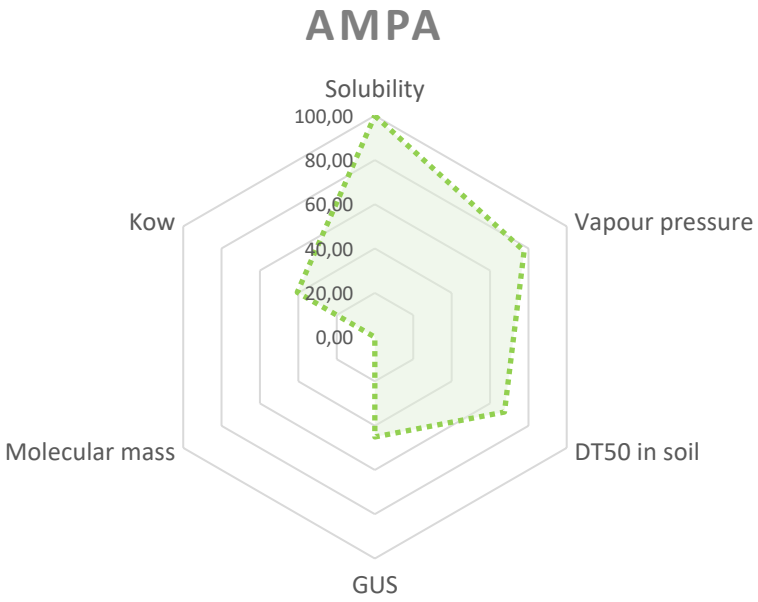

Figure 8-17: Spider chart showing the main physicochemical properties of AMPA.

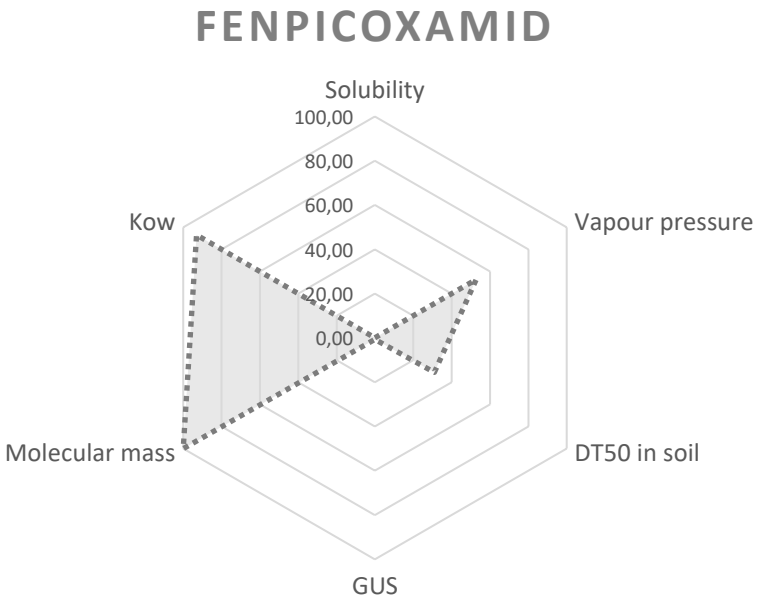

Figure 8-18: Spider chart showing the main physicochemical properties of fenpicoxamid.

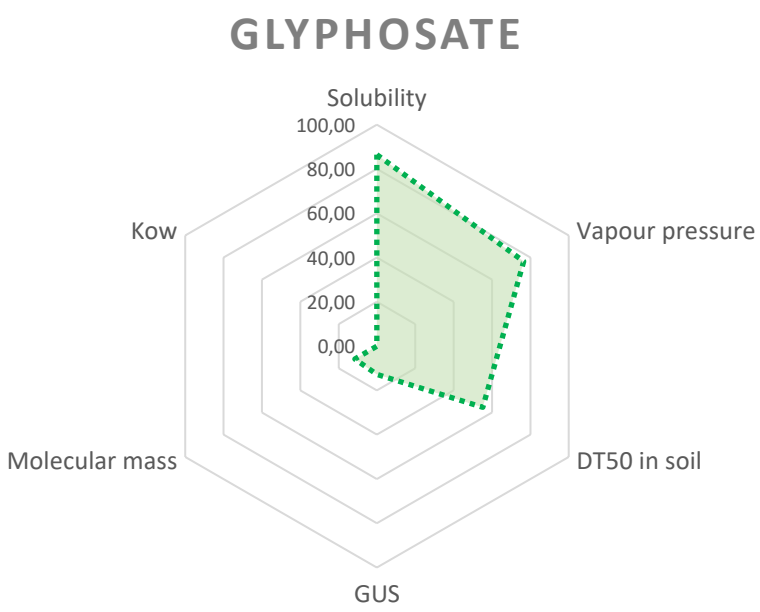

Figure 8-19: Spider chart showing the main physicochemical properties of glyphosate.

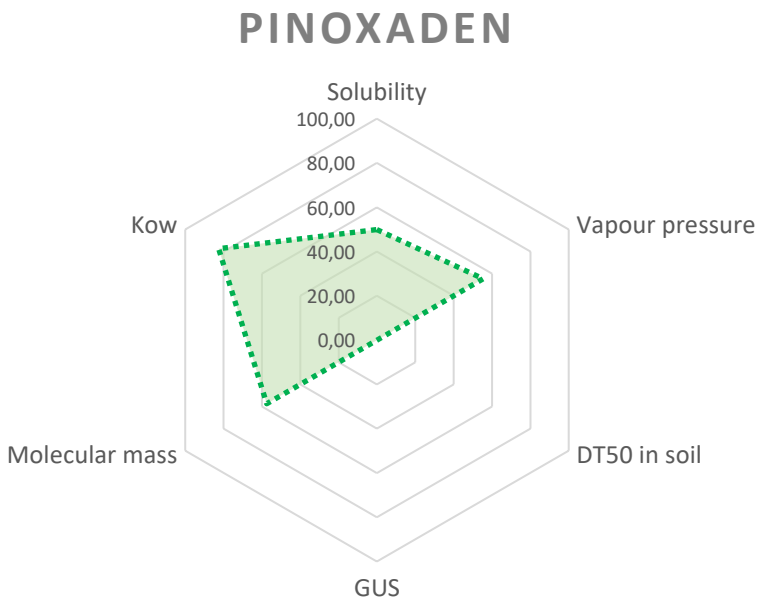

Figure 8-20: Spider chart showing the main physicochemical properties of pinoxaden.

8.2 Pesticide analysis at the CRA-W (LC-MS/MS)

8.2.1 Solid soil samples

Reagents used

Acetonitrile and ULC-MS grade methanol were purchased from Biosolve (Dieuze, France). Ultrapure water (resistivity 18.2 MΩ cm) was obtained using a Milli-Q system (EMD Millipore, USA). Standards for the compounds studied were purchased from Sigma Aldrich (Darmstadt, Germany) and HPC Standards GmbH (Cunnersdorf, Holland). They all have a purity of ≥97%. QuEChERS salt sachets (individual sachets containing 4 g MgSO4, 1 g NaCl, 0.5 g sodium citrate dibasic sesquihydrate, 1 g sodium citrate tribasic dihydrate) were purchased from Biosolve (France). Stock solutions were prepared in acetonitrile or methanol and stored in the refrigerator at 4°C for 3 months. Intermediate solutions were prepared by dilution in acetonitrile immediately before use.

Sample preparation before analysis

The soil, sieved to 2 mm, was stored in a freezer at -18°C prior to analysis. The extraction procedure was as follows: 5 g of soil was placed in a Falcon tube to which 5 ml of water was added. This mixture was shaken manually and left to macerate for 30 minutes, then 10 mL of acetonitrile acidified with 1% formic acid was added. The sample was stirred again and left to macerate for 30 minutes. A sachet of QuEChERS salt was then added to dry and separate the aqueous and organic phases. The tube was then manually vortexed for one minute and centrifuged at 4800 rcf for 5 minutes. The supernatant (organic phase) was filtered through a 0.2 µm PTFE filter. This solution was then transferred to a vial and analysed by LC-MS/MS.

LC-MS/MS analysis method

- Chromatographic parameters
    - LC: Water Acquity UPLC
    - Column: Waters ACQUITY UPLC™ HSS T3, 1.7 µm, 100 x 2.1 mm i.d.
    - Mobile phase: Water/methanol (90/10, v/v) with 0.5% formic acid and 10 µN NH4Fo (mobile phase A) methanol 0.5% formic acid (mobile phase B)
    - Flow rate: 0.3 mL min^{-1}
    - Column temperature: 40°C
    - Injection volume: 10 µL
    - Gradient:

Temps	Phase mobile	
	% A	% B
0 min	95	5
0,50 min	95	5
8 min	0	100
9 min	0	100
9,50 min	95	5
11 min	95	5

Figure 8-21: Elution gradient used for the multi-residues analysis by LC-MS/MS.

- Spectrometric parameters
  - Mass spectrometer: Waters Acquity TQD (Tandem Quadrupole)
  - Ionisation mode: positive electrospray (ESI+)
  - Detection mode: Multiple Reaction Monitoring (MRM)
  - Cone gas flow (curtain) 80 L h^{-1} (nitrogen)
  - Source temperature: 130°C
  - Desolvation gas flow: 800 L h^{-1} (nitrogen)
  - Desolvation temperature: 350°C
  - Collision gas (CAD) 0.28 mL min^{-1} (Argon)
  - Integration software: QuantLynx

8.2.2 Soil solution samples

Reagents and solvents used

UPLC-MS grade methanol was purchased from Biosolve (Dieuze, France). Ultrapure water (resistivity 18.2 MΩ cm) was obtained using a Milli-Q system (EMD Millipore, USA). Standards for the compounds studied were purchased from Sigma Aldrich (Darmstadt, Germany) and HPC Standards GmbH (Cunnersdorf, Holland), all with a purity of ≥97%. Stock solutions were

prepared in acetone or methanol and stored frozen at -18°C for up to 3 months. Intermediate solutions were prepared by dilution in water immediately before use.

Sample preparation before analysis

Water samples are stored in a refrigerator at 4°C prior to analysis. They are centrifuged for 15 minutes at 4°C and 4800 rcf to limit suspended solids, then transferred to a vial prior to injection.

Calibration line

Each active ingredient was dissolved in 20 mL of acetone, except for hydroxybentazone, chloridazon, desphenylchloridazonle, 2-amino-4-methoxy-6-methyl-1,3,5-triazine and metazachlor ESA. These solutions are stored in a freezer at -18°C for 1 year.

Reference solvent and matrix solutions (water recovered from a lysimeter in the Hesbaye region) were injected and the peak areas compared. were injected and the peak areas compared. As there was no difference between the two, quantification of the quantification of the molecules present in the samples was performed using reference solutions prepared in prepared in Milli-Q water. For the calibration line, 100 µL of these solutions were diluted in 900 µL of Milli-Q water to obtain concentrations of 0.5, 0.2, 0.1, 0.05, 0.02, 0.01 and 0.005 µg mL^{-1}.

LC-MS/MS analytical method

- Chromatographic parameters
    - LC: Nexera X2TM Shimadzu
    - Autosampler: SIL-30AC Shimadzu
    - Pump: LC-30AD Shimadzu
    - Column oven: CTO-30AC Shimadzu
    - Detector: X500R Time-Of-Flight Mass Spectrometer (Q-TOF) ABSciex
    - Software of integration: Sciex OS 1.3.1
    - Column: Waters ACQUITY UPLC™ HSS T3, 100 mm x 2.1 mm i.d., 1.8 µm particle size
    - Mobile phase: A: water, U-LC reagent grade 90 %, methanol 10% with 0.1% formic acid and 5mM ammonium formate B: methanol with 0.1% formic acid, U-LC reagent grade
    - Tray temperature: 4°C
    - Flow rate: 0,3 mL min^{-1}
    - Gradient: see 0 (Figure 8-21)
    - Column temperature: 40 °C
    - Injection volume: 10 µL

Regarding spectrometric parameters, two types of electrospray ionisation are carried out: the first method is in positive electrospray, the second one in negative. The Table 8-3 provides information about which method(s) is/are used for each molecule.

- Ionisation: positive electrospray (ESI +)
    - Ion spray voltage: 5500 V
    - Curtain gas (cone gas): 20 psi (air)
    - Nebulisation gas flow (probe): 60 psi (air)
    - Desolvation gas flow (ceramic): 60 psi (air)
    - Temperature: 550°C
    - Declustering potential: 70 V
    - Collision energy (CE): 5 V
    - Collision energy spread: 0 V
    - TOF start mass (Da): 70
    - TOF stop mass (Da): 1000
    - Accumulation time (sec): 0.3
- Ionisation: negative electrospray (ESI -)
    - Ion spray voltage: -4500 V
    - Curtain gas (cone gas): 20 psi (air)
    - Nebulisation gas flow (probe): 50 psi (air)
    - Desolvation gas flow (ceramic): 50 psi (air)
    - Temperature: 550°C
    - Declustering potential: -80 V
    - Collision energy (CE): -5 V
    - Collision energy spread: 0 V
    - TOF start mass (Da): 100
    - TOF stop mass (Da): 500
    - Accumulation time (sec): 0.3

Table 8-3: Polarity of the electrospray ionisation (ESI) : positive (ESI+) and negative (ESI-).

ESI+	*ESI-*
Clopyralid	Clopyralid
Cloquintocet-mexyl	MCPA
Fenpicoxamid	MCPB
Flonicamid	
Florasulam	
Fluroxypyr	
Fluxapyroxad	
Halauxifen-methyl	
Iodosulfuron-methyl-sodium	
Mefenpyr-diethyl	
Mefentrifluconazole	
Mesosulfuron-methyl	
Pinoxaden	
Pyraclostrobin	
Pyroxsulam	
Tebuconazole	

8.2.3 Limits of quantification and detection of molecules

In the following table, the LOQ and LOD of all the molecules analysed by the LC-MS/MS at the CRA-W are provided (Sol-Phy-Ly protocol; Vandenberghe et al., 2021). The values are provided for each type of samples: (i) solid soil, and (ii) soil solution (Table 8-4).

Table 8-4: Limits of detection (LOD) and quantification (LOQ) of all the molecules analysed by the LC-MS/MS.

	Solid soil		*Soil solution*	
	LOD (µg kg^{-1})	**LOQ (µg kg^{-1})**	**LOD (µg L^{-1})**	**LOQ (µg L^{-1})**
Clopyralid	1.67	5	0.5	1.5
Cloquintocet-mexyl	0.07	0.2	0.03	0.1
Fenpicoxamid	0.07	0.2	/	/
Flonicamid	0.17	0.5	0.03	0.1
Florasulam	0.08	0.25	0.03	0.1
Fluroxypyr	0.83	2.5	0.17	0.5
Halauxifen-methyl	0.17	0.5	0.01	0.025
Iodosulfuron-methyl-Na	0.25	0.75	0.07	0.2
Mefenpyr-diethyl	0.33	1	0.05	0.15
Mefentrifluconazole	0.42	1.25	0.08	0.25
Mesosulfuron-methyl	0.17	0.5	0.03	0.1
Pinoxaden	0.07	0.2	/	/
Pyraclostrobin	0.33	1	0.07	0.2
Pyroxsulam	0.08	0.25	0.02	0.05
Tebuconazole	0.33	1	0.05	0.15
MCPA	0.07	0.2	0.07	0.2
MCPB	0.83	2.5	0.25	0.75

8.3 Raw results of the analyses

8.3.1 Pesticide concentrations in soil samples

The raw results of concentrations in soil samples were given in µg kg^{-1} of fresh soil. For a better representation of the soil matrix, they were converted into µg kg^{-1} of dry soil by a correction with the percentage of dry mass in the pots (%DM).

The following formula was used : *[dry soil] = [fresh soil] * %DM* [µg kg^{-1} of dry soil]

Table 8-5: Raw results of the concentrations in soil samples: first nine pesticides by alphabetical order.

id	rep.	modality	date	Clop.	Cloq.	Fenp.	Floni.	Flor.	Flur.	Flux.	Halau.	Iodo.
1	A	initial	t0	75,00	0,39	16,86	42,47	1,32	260,91	205,11	1,06	0,78
2	B	initial	t0	33,16	0,52	12,31	18,85	0,76	136,40	140,46	0,87	<LOQ
3	C	initial	t0	35,81	0,62	14,20	24,13	0,75	134,72	132,54	0,77	<LOQ
4	D	initial	t0	35,70	0,43	16,67	18,82	0,78	153,34	157,91	0,92	<LOQ
5	E	initial	t0	38,96	1,30	29,71	28,14	0,93	218,99	186,11	1,39	0,66
61	A	mix	t1	4,38	<LOQ	0,80	<LOQ	<LOQ	3,69	125,73	<LOQ	<LOQ
62	B	mix	t1	5,40	<LOQ	0,30	<LOQ	<LOQ	3,46	66,76	<LOQ	<LOQ
63	C	mix	t1	7,64	<LOQ	0,44	0,46	<LOQ	6,35	81,79	<LOQ	<LOQ
64	D	mix	t1	<LOQ	<LOQ	0,55	<LOQ	<LOQ	2,30	98,38	<LOQ	<LOQ
65	E	mix	t1	5,08	<LOQ	0,51	<LOQ	<LOQ	3,97	94,30	<LOQ	<LOQ
66	A	spelt	t1	5,48	<LOQ	0,74	<LOQ	<LOQ	2,56	127,15	<LOQ	<LOQ
67	B	spelt	t1	4,93	<LOQ	0,38	<LOQ	<LOQ	<LOQ	86,80	<LOQ	<LOQ
68	C	spelt	t1	6,64	<LOQ	0,46	<LOQ	<LOQ	3,33	103,25	<LOQ	<LOQ
69	D	spelt	t1	<LOQ	<LOQ	0,56	<LOQ	<LOQ	1,40	104,13	<LOQ	<LOQ
70	E	spelt	t1	4,61	<LOQ	0,50	<LOQ	<LOQ	<LOQ	108,63	<LOQ	<LOQ
71	A	bare	t1	<LOQ	<LOQ	0,47	<LOQ	<LOQ	2,74	86,09	<LOQ	<LOQ
72	B	bare	t1	6,92	<LOQ	0,39	<LOQ	<LOQ	4,54	83,62	<LOQ	<LOQ
73	C	bare	t1	6,91	<LOQ	0,38	<LOQ	<LOQ	5,99	68,35	<LOQ	<LOQ
74	D	bare	t1	10,34	<LOQ	0,35	0,47	<LOQ	8,57	74,29	<LOQ	<LOQ
75	E	bare	t1	8,59	<LOQ	0,55	0,41	<LOQ	4,82	68,86	<LOQ	<LOQ
76	A	mix	t2	<LOQ	<LOQ	0,79	<LOQ	<LOQ	<LOQ	221,05	<LOQ	<LOQ
77	B	mix	t2	<LOQ	<LOQ	0,67	<LOQ	<LOQ	2,62	213,62	<LOQ	<LOQ
78	C	mix	t2	<LOQ	<LOQ	0,76	<LOQ	<LOQ	<LOQ	219,07	<LOQ	<LOQ
79	D	mix	t2	<LOQ	<LOQ	0,65	<LOQ	<LOQ	<LOQ	201,97	<LOQ	<LOQ
80	E	mix	t2	<LOQ	<LOQ	0,84	<LOQ	<LOQ	<LOQ	259,79	<LOQ	<LOQ
81	A	spelt	t2	<LOQ	<LOQ	0,48	<LOQ	<LOQ	<LOQ	121,79	<LOQ	<LOQ
82	B	spelt	t2	<LOQ	<LOQ	0,37	<LOQ	<LOQ	<LOQ	108,20	<LOQ	<LOQ
83	C	spelt	t2	<LOQ	<LOQ	0,22	<LOQ	<LOQ	<LOQ	100,86	<LOQ	<LOQ
84	D	spelt	t2	<LOQ	<LOQ	0,26	<LOQ	<LOQ	<LOQ	133,34	<LOQ	<LOQ
85	E	spelt	t2	5,57	<LOQ	0,38	0,93	<LOQ	<LOQ	129,95	<LOQ	<LOQ
86	A	bare	t2	<LOQ	<LOQ	0,35	<LOQ	<LOQ	<LOQ	132,87	<LOQ	<LOQ
87	B	bare	t2	<LOQ	<LOQ	0,65	<LOQ	<LOQ	<LOQ	202,38	<LOQ	<LOQ
88	C	bare	t2	<LOQ	<LOQ	0,60	<LOQ	<LOQ	<LOQ	205,48	<LOQ	<LOQ
89	D	bare	t2	<LOQ	<LOQ	0,87	<LOQ	<LOQ	2,10	213,44	<LOQ	<LOQ
90	E	bare	t2	<LOQ	<LOQ	0,64	<LOQ	<LOQ	<LOQ	185,62	<LOQ	<LOQ

Table 8-6: Raw results of the concentrations in soil samples: last nine pesticides by alphabetical order.

id	rep.	modality	date	Mefenp.	Mefent.	Meso.	Pino.	Pyra.	Pyro.	Tebu.	MCPA	MCPB
1	A	initial	t0	<LOQ	154,05	11,66	<LOQ	418,19	16,61	617,10	915,30	555,27
2	B	initial	t0	<LOQ	112,94	5,62	0,16	286,56	6,18	409,77	408,44	170,22
3	C	initial	t0	<LOQ	101,32	5,73	0,15	229,80	8,53	386,59	431,63	254,79
4	D	initial	t0	<LOQ	124,44	7,93	<LOQ	324,61	8,62	492,38	492,24	165,90
5	E	initial	t0	<LOQ	148,64	10,96	0,47	379,43	10,27	556,32	608,17	497,61
61	A	mix	t1	<LOQ	87,51	2,39	<LOQ	77,78	0,20	329,51	3,22	4,05
62	B	mix	t1	<LOQ	34,04	1,78	<LOQ	26,49	0,19	153,52	1,58	<LOQ
63	C	mix	t1	<LOQ	51,70	2,13	<LOQ	47,54	0,45	204,88	1,88	2,17
64	D	mix	t1	<LOQ	65,87	2,34	<LOQ	54,99	<LOQ	250,54	1,15	1,99
65	E	mix	t1	<LOQ	54,07	2,62	<LOQ	51,06	0,28	230,03	0,92	<LOQ
66	A	spelt	t1	<LOQ	90,77	2,82	<LOQ	89,91	<LOQ	360,16	1,40	3,23
67	B	spelt	t1	<LOQ	55,81	2,69	<LOQ	45,08	<LOQ	220,71	1,12	<LOQ
68	C	spelt	t1	<LOQ	69,08	2,39	<LOQ	45,73	<LOQ	284,23	1,08	<LOQ
69	D	spelt	t1	<LOQ	62,94	2,78	<LOQ	52,93	<LOQ	277,97	0,87	2,00
70	E	spelt	t1	<LOQ	67,35	2,55	<LOQ	45,97	<LOQ	282,65	0,96	2,09
71	A	bare	t1	<LOQ	57,24	2,18	<LOQ	42,56	<LOQ	232,32	0,96	<LOQ
72	B	bare	t1	<LOQ	56,52	2,73	<LOQ	33,05	0,51	219,70	1,07	<LOQ
73	C	bare	t1	<LOQ	43,79	2,58	<LOQ	37,35	0,55	169,66	0,98	<LOQ
74	D	bare	t1	<LOQ	46,92	2,57	<LOQ	32,78	0,83	195,00	1,30	<LOQ
75	E	bare	t1	<LOQ	44,42	2,27	<LOQ	40,70	0,69	194,08	1,18	<LOQ
76	A	mix	t2	<LOQ	152,20	2,61	<LOQ	60,34	<LOQ	522,19	1,31	6,48
77	B	mix	t2	<LOQ	140,95	3,09	<LOQ	55,88	<LOQ	474,57	1,47	6,77
78	C	mix	t2	<LOQ	148,50	2,66	<LOQ	42,62	<LOQ	498,30	1,29	5,82
79	D	mix	t2	<LOQ	130,90	2,73	<LOQ	43,11	<LOQ	436,13	1,21	6,52
80	E	mix	t2	<LOQ	189,89	2,95	<LOQ	65,72	<LOQ	587,86	1,41	7,38
81	A	spelt	t2	<LOQ	69,78	0,99	<LOQ	25,45	<LOQ	242,57	0,73	4,82
82	B	spelt	t2	<LOQ	71,59	1,19	<LOQ	27,86	<LOQ	238,66	0,80	5,11
83	C	spelt	t2	<LOQ	56,03	1,26	<LOQ	17,94	<LOQ	214,43	0,60	4,04
84	D	spelt	t2	<LOQ	76,29	1,78	<LOQ	30,60	<LOQ	286,50	0,70	4,23
85	E	spelt	t2	<LOQ	81,10	1,40	<LOQ	24,62	<LOQ	297,14	1,35	4,37
86	A	bare	t2	<LOQ	80,50	1,40	<LOQ	23,80	<LOQ	281,96	0,74	4,11
87	B	bare	t2	<LOQ	134,20	1,99	<LOQ	51,39	<LOQ	442,03	1,46	6,26
88	C	bare	t2	<LOQ	132,88	1,97	<LOQ	50,11	<LOQ	474,49	1,15	6,17
89	D	bare	t2	<LOQ	142,30	3,01	<LOQ	48,32	<LOQ	467,55	1,26	7,17
90	E	bare	t2	<LOQ	115,43	2,19	<LOQ	35,84	<LOQ	427,96	1,45	6,88

8.3.2 Pesticide concentrations in soil solution samples

Table 8-7: Raw results of the concentrations in soil solution samples: first nine pesticides by alphabetical order.

id	rep.	modality	date	Glypho.	AMPA	Clop.	Cloq.	Floni.	Flor.	Flur.	Flux.	Halau.
61	A	mix	t1	0,37	0,86	14,81	ND	0,30	0,19	3,24	3,75	ND
62	B	mix	t1	0,21	0,76	17,30	ND	0,21	0,23	3,10	1,14	ND
63	C	mix	t1	0,44	2,02	22,38	ND	0,38	0,26	5,57	3,18	ND
64	D	mix	t1	2,47	1,30	20,66	ND	0,54	0,23	3,55	4,74	ND
65	E	mix	t1	0,35	2,69	32,91	ND	1,81	0,58	8,98	8,40	ND
66	A	spelt	t1	0,61	0,37	38,86	ND	0,38	0,28	8,55	2,69	ND
67	B	spelt	t1	0,56	1,91	16,12	ND	<LOQ	0,20	0,85	2,59	ND
68	C	spelt	t1	0,37	0,75	44,57	ND	4,61	0,75	42,02	2,15	ND
69	D	spelt	t1	8,90	0,34	21,66	ND	0,89	0,28	4,92	0,45	ND
70	E	spelt	t1	0,10	3,07	25,82	ND	0,41	0,23	2,45	5,07	ND
71	A	bare	t1	2,92	2,75	2,88	ND	<LOQ	ND	ND	4,56	ND
72	B	bare	t1	1,27	3,51	24,09	ND	0,20	0,23	1,35	9,01	ND
73	C	bare	t1	0,23	0,30	41,03	ND	0,48	0,39	9,88	0,46	ND
74	D	bare	t1	0,79	2,47	47,94	ND	0,67	0,40	5,29	7,49	ND
76	A	mix	t2	4,34	1,07	13,52	ND	0,30	0,17	2,94	8,05	ND
77	B	mix	t2	5,56	0,41	1,68	ND	<LOQ	ND	<LOQ	2,92	ND
78	C	mix	t2	5,13	1,29	4,55	ND	ND	ND	0,83	3,11	ND
80	E	mix	t2	0,10	0,46	3,63	ND	<LOQ	ND	0,98	7,40	ND
81	A	spelt	t2	6,33	1,42	<LOQ	ND	<LOQ	ND	<LOQ	1,83	ND
82	B	spelt	t2	0,10	0,46	3,63	ND	0,13	ND	1,48	4,17	ND
83a	C	spelt	t2	3,97	2,56	6,12	ND	0,26	ND	1,82	2,93	ND
83b	C	spelt	t2	5,12	2,41	2,20	ND	<LOQ	ND	ND	3,33	ND
84	D	spelt	t2	0,10	2,05	<LOQ	ND	ND	ND	ND	5,49	ND
85	E	spelt	t2	0,10	2,37	<LOQ	ND	<LOQ	ND	0,51	2,20	ND
86	A	bare	t2	7,03	1,68	15,36	ND	0,85	0,21	6,94	9,29	ND
87	B	bare	t2	0,10	0,55	12,99	ND	0,14	0,19	1,53	4,48	ND
88	C	bare	t2	0,10	0,87	8,65	ND	ND	ND	0,92	4,62	ND
89	D	bare	t2	0,10	0,76	7,18	ND	0,21	ND	1,52	8,95	ND
90	E	bare	t2	0,33	0,17	5,98	ND	<LOQ	ND	0,65	2,01	ND

Table 8-8: Raw results of the concentrations in soil solution samples: last nine pesticides by alphabetical order.

id	repl.	modality	date	Iodo.	Mefenp.	Mefent.	Meso.	Pyra.	Pyro.	Tebu.	MCPA	MCPB
61	A	mix	t1	ND	ND	<LOQ	2,77	ND	0,34	7,28	0,27	ND
62	B	mix	t1	ND	ND	<LOQ	1,63	ND	0,32	3,20	<LOQ	ND
63	C	mix	t1	ND	ND	0,28	2,08	ND	0,70	9,90	<LOQ	ND
64	D	mix	t1	ND	ND	<LOQ	2,78	ND	0,51	9,96	0,23	ND
65	E	mix	t1	ND	ND	0,63	4,46	ND	3,07	24,70	4,70	ND
66	A	spelt	t1	ND	ND	ND	3,53	ND	1,03	6,45	0,49	ND
67	B	spelt	t1	ND	ND	<LOQ	3,02	ND	0,19	7,65	ND	ND
68	C	spelt	t1	<LOQ	ND	ND	5,37	ND	5,83	7,39	5,37	3,49
69	D	spelt	t1	ND	ND	ND	3,79	ND	1,40	2,21	1,14	ND
70	E	spelt	t1	ND	ND	0,35	3,24	ND	0,80	14,81	0,25	ND
71	A	bare	t1	ND	ND	0,36	1,16	ND	<LOQ	14,42	ND	ND
72	B	bare	t1	ND	ND	0,57	6,78	ND	0,46	32,13	<LOQ	ND
73	C	bare	t1	ND	ND	ND	1,91	ND	1,75	1,21	0,21	ND
74	D	bare	t1	ND	ND	0,46	3,94	ND	1,75	24,48	0,31	ND
76	A	mix	t2	ND	ND	0,29	2,89	ND	0,42	12,80	0,20	ND
77	B	mix	t2	ND	ND	<LOQ	1,66	ND	0,07	4,46	ND	ND
78	C	mix	t2	ND	ND	<LOQ	2,21	ND	0,10	5,35	ND	ND
80	E	mix	t2	ND	ND	0,29	1,97	ND	0,14	13,04	<LOQ	ND
81	A	spelt	t2	ND	ND	ND	0,57	ND	<LOQ	6,04	<LOQ	ND
82	B	spelt	t2	ND	ND	0,37	0,97	ND	0,17	11,90	0,97	ND
83a	C	spelt	t2	ND	ND	<LOQ	1,11	ND	0,20	9,14	0,64	ND
83b	C	spelt	t2	ND	ND	<LOQ	0,85	ND	<LOQ	7,49	<LOQ	ND
84	D	spelt	t2	ND	ND	0,27	1,16	ND	<LOQ	15,30	ND	ND
85	E	spelt	t2	ND	ND	<LOQ	1,24	ND	0,06	8,10	0,24	ND
86	A	bare	t2	ND	ND	0,50	3,16	ND	0,87	16,09	0,57	<LOQ
87	B	bare	t2	ND	ND	0,53	1,97	0,26	0,22	9,33	0,23	ND
88	C	bare	t2	ND	ND	0,35	2,47	ND	0,20	8,74	ND	ND
89	D	bare	t2	ND	ND	0,68	2,59	0,48	0,19	16,62	0,22	ND
90	E	bare	t2	ND	ND	<LOQ	1,84	ND	0,07	4,61	ND	ND

8.4 Cover crop data

Table 8-9: Quantity of cover crop seeds sown in the experimental pots [kg ha^{-1}]. The values are extrapolated from the average mass of one seed and the surface area of one pot.

Average mass of one seed of spelt:	53,4 mg/seed
Surface area of a pot:	0,070 m^2
Typical mass of spelt per hectare:	175 kg ha^{-1}
Average mass of the spelt per hectare sown:	**191 kg ha^{-1}**
Typical mass of phacelia per hectare (mix):	10 kg ha^{-1}
Typical mass of buckwheat per hectare (mix):	35 kg ha^{-1}
Typical mass of mustard per hectare (mix):	8 kg ha^{-1}
Typical mass of vetch per hectare (mix):	20 kg ha^{-1}
Average mass of the mix per hectare sown:	**148 kg ha^{-1}**

Influence of cover crops on the presence and transfer of pesticides in agricultural soils

Igor Turine

Pesticides are an indispensable component of modern agriculture, serving for pest control and yield enhancement. However, their extensive use presents a significant risk to human health and the environment due to their persistence in soils and water bodies. The objective of this thesis is to evaluate the influence of winter cover crops on the presence and transfer of pesticides in agricultural soils, through a three-month greenhouse experiment.

This study examines three soil cover modalities: bare soil (control), a winter cash crop (winter spelt), and a winter catch crop (multi-species cover crop). A total of 19 pesticides, encompassing a range of physicochemical properties, were applied, and their concentrations were monitored over time.

The results demonstrate that pesticides exhibit distinct distributions, with persistent and hydrophobic molecules exhibiting a prevalence in soil, while soluble and volatile ones are prevalent in the soil solution. A temporal monitoring revealed the remanence of persistent molecules over time. The analysis of the control samples exhibited a range of mass reductions among different molecules, with a few unexpected increases that may be attributed to sampling biases.

A comparison of the modalities reveals that only winter spelt (monospecific cover) significantly reduces the pesticide content. This reduction can be attributed to the biomass density of the spelt, which was three times greater than that of the multi-species mix. In the experimental conditions, the density of the cover crop was found to be a more influential factor than its diversity in reducing pesticide residues. These findings indicate that high-biomass cover crops can play a crucial role in mitigating pesticide leaching.

To achieve the greatest efficacy, it is recommended that cover crops be sown as soon as possible after the previous crop harvest, to ensure adequate soil coverage before the onset of autumn and winter rainfall. To gain a more comprehensive understanding of the efficacy of diverse cover crop species and densities, as well as to enhance sustainable agricultural practices, it is necessary to conduct longer and larger-scale field trials. Moreover, a coupled study of soil geochemistry and hydrology is required to gain a deeper understanding of pesticide dynamics.

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