UNIVERSITÉ DU QUÉBEC À MONTRÉAL

IMPACT DU TAUX D'ÉPANDAGE D'HERBICIDES À BASE DE GLYPHOSATE ET DES RÉGIES DE CULTURE SUR LES RENDEMENTS ET LA PSEUDO-PERSISTANCE DU GLYPHOSATE ET DE L'AMPA DANS UN SOL DE GRANDES CULTURES EN MONTÉRÉGIE

MÉMOIRE

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Pour le Chapitre 1, j'ai fait la majorité des analyses des teneurs de glyphosate et d'AMPA. Benoît Terrié et Jérôme Bernier Brillon ont effectué une partie des analyses des teneurs de glyphosate et d'AMPA. Matthieu Moingt a supervisé les analyses des teneurs de glyphosate et d'AMPA.

Pour le Chapitre 2, j'ai effectué les analyses des teneurs de glyphosate et d'AMPA. Matthieu Moingt a supervisé les analyses des teneurs de glyphosate et d'AMPA. Les taux de recouvrement ont été récoltés par les stagiaires Ariane Bernier et Myriam Fontaine. Les rendements ont été calculés par le centre de recherche sur les grains (CÉROM) et les analyses physico-chimiques du sol ont été réalisées par l'institut de recherche et de développement en agroenvironnement (IRDA).

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AM	Agricultural managements
AMPA	Aminomethylphosphonic acid
CC	Cover crops
CÉROM	Centre de recherche sur les grains
DS	Direct seeding
DT-50	Half-life
GBH	Glyphosate-based herbicide
GC-ECD	Gas chromatograph - electron capture detector
GR	Glyphosate-resistant
IRDA	Institut de recherche et de développement en agroenvironnement
LOD	Limit of detection
LOQ	Limit of quantification
TFAA	Trifluoroacétique anhydre
TFE	Trifluoroéthanol

LISTE DES SYMBOLES ET DES UNITÉS

cm	Centimetre
°C	Degree Celsius
g	Gram
ha	Hectare
kg	Kilogram
L	Liter
log	logarithm
m	Meter
mL	Milliliter
mm	Millimeter
M	Molar mass
µg	Microgram
µL	Microliter
µm	Micrometer
%	Percentage
rpm	Revolutions per minute
ρ	Spearman correlation

RÉSUMÉ GÉNÉRAL

L'Agriculture de Conservation promeut, entre-autres, une rotation des cultures, le semis direct (SD) et les cultures de couverture (CC). Cependant, ces pratiques requièrent l’usage répété de grandes quantités d'herbicides à base de glyphosate (HBG), ce qui peut entrainer la pseudo-persistance du glyphosate et de son principal produit de dégradation, l’acide aminométhylphosphonique (AMPA), dans les sols. La pseudo-persistance survient lorsque l'ajout d'un composé dans l'environnement est supérieur à sa capacité de dissipation. L'utilisation des CC est prônée pour maintenir et restaurer les fonctions du sol. De plus, certains auteurs arguent que les CC peuvent aussi faire compétition aux adventices et contribuer à réduire l’usage des herbicides. L'objectif de ce mémoire est d'évaluer l'impact de différentes pratiques agricoles sur les teneurs de glyphosate et d'AMPA du sol ainsi que sur les rendements des cultures commerciales. Pour ce faire, une rotation maïs-soya-blé, trois doses différentes d'application d'HBG et la présence ou l'absence de CC ont été testées sur quatre ans (2018-2021). Les moyennes de teneurs de glyphosate et d'AMPA présentes dans le sol sont comparables aux études de par le monde ayant obtenu les teneurs les plus faibles. Au fil du temps, aucune tendance d'accumulation de ces molécules n'a été observée. Par contre, la relative constance des teneurs de glyphosate et d'AMPA au fil du temps suggère la pseudo-persistance de ces molécules. Les différentes doses d'application d'HBG ainsi que la présence de CC ne semblent pas avoir impacté les teneurs en glyphosate ou en AMPA présentes dans la première couche du sol, alors que celles-ci ne diffèrent pas selon les doses appliquées et la présence de CC. Les rendements ont été significativement moins élevés avec la dose annuelle d'application d'HBG la plus faible (0,84 L/ha), alors que ceux avec la plus grande dose d'HBG (3,3 L/ha) ont été similaires aux rendements avec la dose intermédiaire (1,67 L/ha), cette dernière étant la dose recommandée par les fabricants pour une application d’HBG. Il n'y a pas eu de tendance observée entre les rendements et les teneurs de glyphosate, d'AMPA ou des éléments du sol. Ainsi, l'absence d'impact des pratiques agricoles sur les teneurs de glyphosate et d'AMPA suggère que les mécanismes de dissipation de ces molécules varient selon la dose d'HBG appliquée. Cela soulève des inquiétudes par rapport à la pollution diffuse que peuvent engendrer les applications d'HBG et concorde avec l'émergence d'études faisant état de l'augmentation de la quantité et de la fréquence de détection de ces molécules dans les écosystèmes environnants. Enfin, les rendements de maïs-grain et de soya découlant de la dose d'application la plus faible montrent, comme il était attendu, qu'une dose minimale d'HBG est requise afin de contrôler efficacement les adventices pour maintenir des rendements compétitifs. En revanche, les rendements similaires obtenus pour le maïs-grain entre la dose d'application intermédiaire et la dose la plus élevée suggèrent, dans notre cas, qu'une réduction de l'utilisation des HBG, avec des pratiques agricoles adaptées (CC), peut permettre un maintien de rendements compétitifs pour cette culture. Analyser l'impact de l'utilisation des HBG sur les rendements et sur la dynamique de dissipation du glyphosate et de l'AMPA dans un milieu ouvert et sur un cycle complet de rotation des cultures est d'autant plus important considérant les inquiétudes face à la pérennité de ce modèle agricole et à l'impact de l'utilisation intensive des HBG sur la santé humaine et environnementale.

Mots clés : herbicide à base de glyphosate, cultures de couverture, semis direct, glyphosate, AMPA, réduction de l'utilisation des herbicides à base de glyphosate

INTRODUCTION GÉNÉRALE

La stratégie de gestion des adventices la plus communément utilisée en grandes cultures consiste en l'utilisation d'herbicides à base de glyphosate (HBG) combinée à des cultures ayant été génétiquement modifiées pour leur résister (Maggi *et al.*, 2020). Cette stratégie de production en monoculture a permis de réduire les coûts de production et d'augmenter considérablement les rendements (Brookes *et al.*, 2017). Par contre, l'utilisation exhaustive des HBG entraine l'émergence de plantes ayant naturellement développé une résistance à ces herbicides, ce qui amoindrit leur efficacité. En 2023, dans le monde, on dénombrait 58 espèces d'adventices ayant développé divers mécanismes de résistance (Heap, 2023) alors qu'une recrudescence des adventices ayant des résistances à plusieurs herbicides est également constatée (Peterson *et al.*, 2018; Walsh *et al.*, 2004). Conséquemment, il y a une augmentation de l'utilisation des HBG et de l'utilisation de mélange d'herbicides pour maintenir un contrôle adéquat des adventices, ce qui augmente les risques pour la santé humaine et environnementale (Baek *et al.*, 2021; Benbrook, 2016; Myers *et al.*, 2016). Les quantités et la fréquence de détection du glyphosate et de son principal produit de dégradation, l'acide aminométhylphosphonique (AMPA), sont également en croissance dans l'environnement (Battaglin *et al.*, 2014; Giroux, 2022; Maccario *et al.*, 2022; Silva *et al.*, 2019; Singh, S. *et al.*, 2020). Ainsi, de plus en plus d'études font état des impacts de ces composés sur des écosystèmes et espèces non-cibles (Gomes *et al.*, 2014; Kanissery *et al.*, 2019; Myers *et al.*, 2016; Peillex et Pelletier, 2020; Samson-Brais *et al.*, 2021). Les préoccupations par rapport à l'usage de ce type d'herbicide sont donc grandissantes. Au Québec, la transition écologique des grandes cultures tente de répondre à cet enjeu et est soutenue par le plan d'agriculture durable 2020-2030 du gouvernement du Québec (PAD) (MAPAQ, 2020). Le PAD reconnait, entre autres, la nécessité d'améliorer la santé et la conservation des sols ainsi que de réduire l'utilisation des intrants chimiques.

Le glyphosate peut se retrouver dans l'eau, dans l'air et dans les précipitations et son temps de demi-vie (DT50), dans l'environnement, est variable, pouvant aller de 4 à 110 jours selon le cas (Grandcoin *et al.*, 2017; Myers *et al.*, 2016). Lorsque le glyphosate atteint le sol, il peut soit être dégradé et/ou lixivié ou persister. Le glyphosate est principalement dégradé par les microorganismes du sol qui l'utilise comme source de carbone (Andréa *et al.*, 2003). Cette dégradation produit majoritairement de l'AMPA (Sviridov *et al.*, 2015), un composé moins hydrosoluble que le glyphosate et donc moins mobile et plus persistant que ce dernier (Grandcoin *et al.*, 2017). Par exemple, le *Pesticide Properties Database* a établi le DT50 de l'AMPA à 234 jours (PPDB, 2022). Les mécanismes et taux de dissipation sont influencés par les conditions climatiques et par les propriétés physico-chimiques et biologiques du sol. Des paramètres tels que la nature et la quantité d'argile, le contenu en phosphore, les cations, la température, le pH et la matière organique influencent la persistance de ces molécules (Cassigneul *et al.*, 2016; Kanissery *et al.*, 2019; Sidoli *et al.*, 2016). Par exemple, bien que l'AMPA soit généralement considéré comme plus persistant que le glyphosate, dans un sol à forte teneur argileuse, le glyphosate peut présenter, dans certains cas, un DT50 plus grand que celui de l'AMPA (Bergström *et al.*, 2011; Grandcoin *et al.*, 2017). Le glyphosate va persister dans le sol lorsqu'il est adsorbé à la matrice de sol (Okada *et al.*, 2016; Simonsen *et al.*, 2008). Lorsqu'adsorbées, ces molécules sont moins biodisponibles à la dégradation microbienne et sont également moins mobiles, ce qui augmente leur persistance dans le sol (Bergström *et al.*, 2011; Duke *et al.*, 2012; Kanissery *et al.*, 2019). Dans certains cas, tel que lors de fortes précipitations ou lorsque ces molécules sont faiblement adsorbées à la matrice du sol, un déplacement par érosion ou un transport par lixiviation ont été observés (Hearon *et al.*, 2021; Padilla et Selim, 2020; Vereecken, 2005; Zhao *et al.*, 2009).

Alors que le glyphosate est considéré comme un composé non persistant (PPDB, 2023), dans certains cas, les pratiques agricoles peuvent entrainer sa pseudo-persistance dans les sols. Ce terme fait référence à une molécule qui, en soi, n'est pas considérée comme persistante, car elle est rapidement dissipée. Par contre, lorsque ces molécules sont ajoutées dans un écosystème plus rapidement que leur capacité de dissipation, elles sont alors qualifiées de pseudo-persistantes (Grenni *et al.*, 2013). La pseudo-persistance du glyphosate peut survenir lorsque la fréquence et les doses d'application d'HBG sont supérieures au taux de dissipation de la molécule (Primost *et al.*, 2017).

Le glyphosate et l'AMPA peuvent former des complexes avec des éléments du sol (Cu, Fe, Mn, N et Zn), ce qui réduit leur biodisponibilité pour les plantes. Certains de ces nutriments sont essentiels à leurs fonctions et à leur résilience face aux pathogènes (Duke *et al.*, 2012; Mertens *et al.*, 2018). La proportion des éléments complexés serait toutefois négligeable compte tenu de leurs teneurs élevées dans les sols selon Duke *et al.* (2012). Le glyphosate et l'AMPA peuvent également impacter les microorganismes du sol en provoquant un changement dans la composition microbienne, par exemple en augmentant les populations spécialisées dans la métabolisation de ces molécules (Andréa *et al.*, 2003; Lancaster *et al.*, 2010; Samson-Brais *et al.*, 2021; Zaidi et Khan, 2005). L'AMPA peut également affecter la physiologie des plantes résistantes au glyphosate en réduisant le contenu en chlorophylle des feuilles, en induisant la génération d'espèces réactives de l'oxygène (ROS) ou en diminuant la conductance stomatique (Reddy *et al.*, 2004; Smedbol *et al.*, 2019).

Pour améliorer l'impact des pratiques agricoles, les principes de l'Agriculture de Conservation sont mis de l'avant (Lucas *et al.*, 2018; MAPAQ, 2020). Sommairement, ce concept repose sur une perturbation minime ou absente du sol, une couverture permanente du sol et une diversification des espèces de culture (Kassam *et al.*, 2022; Scopel *et al.*, 2013). Pour ce faire, le semis direct (SD) et les cultures de couverture (CC) sont généralement utilisés. Ces pratiques sont bénéfiques pour le maintien des fonctions du sol et elles peuvent permettre de réduire la dégradation, la compaction et l'érosion des sols (Lucas *et al.*, 2018; Scopel *et al.*, 2013). Les CC sont utilisées pour couvrir le sol avant, pendant ou après la culture commerciale et faire compétition aux adventices (Osipitan *et al.*, 2019), décompacter le sol (Pott *et al.*, 2020) et augmenter l’activité biologique, la biodiversité (Martínez-García *et al.*, 2018), l’apport en azote du sol (Vincent-Caboud *et al.*, 2017) et le stockage de carbone (García-González *et al.*, 2018). Elles ont donc le potentiel de permettre une réduction de l'utilisation des HBG et de certains engrais. Ainsi, avoir une bonne implantation de CC, sans affecter la croissance des cultures d'intérêts, serait bénéfique pour le système agricole.

L'objectif de ce mémoire est d'évaluer l'impact de l'utilisation de CC et de trois différentes doses d'application d'HBG sur les teneurs de glyphosate et d'AMPA présentes dans la première couche de sol ainsi que sur les rendements dans un contexte de grandes cultures au Québec. Pour répondre aux objectifs de recherche, 48 parcelles expérimentales ont été installées en 2018 dans un champ agricole situé en Montérégie, au centre de recherche sur les grains (CÉROM). Les parcelles, en rotation maïs-soya-blé et cultivées en SD, ont été suivies pendant quatre années, soit jusqu'en 2021. Les quatre traitements de gestion des adventices testés étaient: avec CC et l'une des trois doses d'HBG (c.-à-d. 0,84 L/ha; 1,67 L/ha; 3,3 L/ha) et sans CC et la dose la plus élevée d'HBG (c.-à-d. 3,3 L/ha).

Le premier chapitre traite de l'évolution des teneurs de glyphosate et d'AMPA du printemps 2018 au printemps 2021 et également de la tendance de ces teneurs au courant d'une saison. L'objectif était de déterminer si l'utilisation de différentes doses d'HBG et de CC allaient influencer les teneurs observées dans les sols et d'analyser si les variables à l'étude exerçaient une influence sur la dynamique de dissipation de ces composés sur une échelle de temps plus courte. Dans cette étude, nous émettons l'hypothèse que : (I) les doses d'application la plus élevée et intermédiaire d'HBG présenteront des teneurs en glyphosate et en AMPA qui suggéreront leur pseudo-persistance dans le sol et (II) les parcelles avec CC auront des teneurs en glyphosate et en AMPA plus faibles que celles sans CC.

Le deuxième chapitre traite des rendements des trois cultures à la troisième année du projet. L'objectif était de voir l'impact des divers taux d'application d'HBG et de la présence des CC sur les rendements de chaque culture. Ces rendements étaient également analysés selon les teneurs des éléments du sol, de glyphosate et d'AMPA en plus des taux de recouvrement des CC, des cultures d'intérêts et des adventices. Nous émettons l'hypothèse que les parcelles avec des CC et une dose annuelle d'HBG de 1,67 L/ha auront des rendements similaires à ceux des parcelles sans CC et avec une application annuelle de 3,3 L/ha d'HBG, car les CC pourraient compenser la réduction du taux d'application de GBH.

CHAPITRE 1

INFLUENCE OF GLYPHOSATE-BASED HERBICIDE APPLICATION DOSES AND COVER CROPS ON GLYPHOSATE AND AMPA PSEUDO-PERSISTENCE IN FIELD CROP TOPSOIL

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1.0 Abstract

Conservation Agriculture promotes, inter alia, crop rotation, direct seeding (DS) and cover crops (CC). However, those practices require high and frequent usage of glyphosate-based herbicide (GBH), which can lead to glyphosate and aminomethylphosphonic acid (AMPA), its main degradation product, pseudo-persistence in soils. Those molecules can impact non-target species, but also cash crops by affecting their nutrition and disease resilience. CC can allow reducing GBH use by competing with weeds while also maintaining and restoring soil functions. This study conducted in southern Quebec (Canada) comprises 48 plots installed in 2018 and monitored until spring of 2021. Those plots have been managed with a corn/soybean/wheat rotation in DS and three different GBH (*Roundup Weathermax®* 540 g a.i./L) application doses: 0.84, 1.67 and 3.3 L/ha. To evaluate the GBH doses impact, CC and cash crop on glyphosate and AMPA soil contents, four agricultural managements (AM) were tested: with CC and each of the three GBH doses, and without CC but with the highest GBH dose (i.e. 3.3 L/ha). Soil samples were collected three times a year. Mean glyphosate and AMPA contents are 0.08 ± 0.09 and 0.15 ± 0.14 µg/g, respectively, whereas their contents tendencies from 2018 to 2021 are not different between the AM. Interestingly, those contents do not differ between different AM at any sampling campaign. Moreover, the pseudo-persistence of these compounds is observed and could be linked to the adsorption saturation capacity of the soil matrix.

Keywords: glyphosate, AMPA, pseudo-persistence, soil dissipation, cover crops, direct seeding, glyphosate-based herbicide, field crop.

1.1 Introduction

Glyphosate-based herbicides (GBH) combined with glyphosate-resistant (GR) crops constitutes the most common weed management strategy in field crop agriculture worldwide (Maggi *et al.*, 2020). Over time, GBH efficiency has decreased, mainly due to the emergence of glyphosate-resistant weeds (Heap et Duke, 2018). Consequently, GBH spraying doses and frequencies have increased over time (Baek *et al.*, 2021; Benbrook, 2016; Myers *et al.*, 2016). The extensive use of GBH is of growing concern, glyphosate and aminomethylphosphonic acid (AMPA), its main by-product, detection frequency as well as contents in soils and in the environment have increased over time (Battaglin *et al.*, 2014; Giroux, 2022; Silva *et al.*, 2019). Glyphosate and AMPA can have deleterious effects on non-target organisms (Gomes *et al.*, 2014; Kanissery *et al.*, 2019; Myers *et al.*, 2016; Peillex et Pelletier, 2020; Samson-Brais *et al.*, 2021). These compounds can also impact cash crops by reducing micronutrients bioavailability and, therefore, affect crop nutrition and diseases resistance (Kanissery *et al.*, 2019; la Cecilia et Maggi, 2018; Myers *et al.*, 2016; Swart et Wolmarans, 2014). Those drawbacks can be caused or enhanced by glyphosate and AMPA pseudo-persistence in soil (Primost *et al.*, 2017; Singh, S. *et al.*, 2020; Van Bruggen *et al.*, 2018). This term refers to a molecule considered as non-persistent because of its rapid dissipation, but when it is added to an ecosystem more rapidly than the ecosystem dissipation capacity, that molecule will then be considered as pseudo-persistent (Grenni *et al.*, 2013).

Once glyphosate reaches the soil, its fate and dissipation rate are influenced by soil physicochemical and biological properties as well as climate conditions (Kanissery *et al.*, 2019). Glyphosate degradation in soil mostly occur through microbial processes and mainly produces AMPA (Sviridov *et al.*, 2015). Microbes use an oxidoreductase enzyme that cleaves the glyphosate C-N bond, which produces AMPA. AMPA can also be degraded by soil microbes which use the C-P lyase enzyme (Sviridov *et al.*, 2015). Glyphosate and AMPA degradations in soil are strongly influenced by their adsorption potential (Duke *et al.*, 2012). This adsorption is due to glyphosate and AMPA hydrogen bond and their interactions with metal ions through complexation (Sen et Chattoraj, 2021; Vereecken, 2005). There is a negative correlation between the adsorption of both compounds in soils and pH and available phosphate (Okada *et al.*, 2016; Sidoli *et al.*, 2016; Zhao *et al.*, 2009) and a positive correlation with aluminum, iron oxide and clay contents as well as with cation exchange capacity (Bergström *et al.*, 2011; Okada *et al.*, 2016; Sidoli *et al.*, 2016; Vereecken, 2005). When adsorbed on soil, glyphosate and, to a higher extent, AMPA, are less available to microorganisms and their mobility is also reduced (Bergström *et al.*, 2011; Okada *et al.*, 2016). This results in the pseudo-persistence of these compounds in soils (Bergström *et al.*, 2011; Grandcoin *et al.*, 2017; Mertens *et al.*, 2018; Okada *et al.*, 2016). However, vertical transport of glyphosate was reported after heavy rainfall (Vereecken, 2005) or when soil factors provide low adsorption capacity (Zhao *et al.*, 2009). Glyphosate dissipation can also be affected by agricultural managements (AM). For example, direct seeding (DS) combined with cover crops (CC) can impact glyphosate and AMPA dissipation. Glyphosate degradation into AMPA and global mineralization can be higher in bare soils compared to CC systems as plant residues offer sorption sites for glyphosate, which can reduce or delay glyphosate transfer to soil or its runoff in the surrounding environment (Cassigneul *et al.*, 2015; Cassigneul *et al.*, 2016; Napoli *et al.*, 2016). Furthermore, CC can impact soil microorganisms, organic matter content, soil humidity and erosion (Giusti *et al.*, 2023; Locke *et al.*, 2008; Martínez-García *et al.*, 2018; Scopel *et al.*, 2013).

Considering the multiples factors influencing glyphosate and AMPA dissipation, their reported half-life (DT50) in soils is variable. The Pesticide Properties Database views glyphosate as non-persistent with a DT50 of 16 days (PPDB, 2023), while it views AMPA as persistent with a DT50 of 234 days (PPDB, 2022). But DT50 of those molecules has also been reported to range between 4 and 110 days for glyphosate and between 26 and 98 days for AMPA (Grandcoin *et al.*, 2017) (Table 1.1). Most studies on glyphosate and AMPA contents in soils were conducted in Europe and South America (Aparicio *et al.*, 2013; Bento *et al.*, 2019; Giard *et al.*, 2022; Karasali *et al.*, 2019; Laitinen *et al.*, 2009; Primost *et al.*, 2017; Silva *et al.*, 2019). Following the trends through time of glyphosate and AMPA contents in soils, the study of the impact of

GBH doses, CC and crop rotation, requires multi-year monitoring. To our knowledge, this type of studies remains inexistent in northern regions. In this study, we hypothesize that: (I) the higher and average GBH application doses will show persistent glyphosate and AMPA soil contents through time suggesting their pseudo-persistence and (II) plots with CC will have lower glyphosate and AMPA soil contents. This study took place in southern Quebec (Canada) and reports the influence of three GBH doses and CC in an experimental field cultivated with a corn-soybean-wheat rotation on glyphosate and AMPA contents and trends from spring 2018 to spring 2021. To do so, four AM have been applied and glyphosate and AMPA contents in the soil first 20 cm have been measured three times per growing season.

Table 1.1 Glyphosate and AMPA half-life (DT50) measured in open field studies

Articles	Country	Soil type	Glyphosate (days)	AMPA (days)
Bento *et al.* (2019)	Argentina	Loess	6	55
Bergström *et al.* (2011)	Sweden	Sandy	17	60
		Clay	110	35
Simonsen *et al.* (2008)	Denmark	Loam	9	32

1.2 Material and methods

1.2.1 Experimental field

The open field experiment took place from spring 2018 to spring 2021 in a heavy clay soil at the Grain Research Center (CEROM) at Saint-Mathieu de Beloeil (Quebec, Canada) (45°34'58.9"N 73°14'15.1"W). The first 20 cm soil horizon contains on average (n = 16) 3.47 ± 0.52% of organic matter, 27.75 ± 0.96% of silt and 72.25 ± 0.96% of clay. Main mineral contents are 10.68 ± 3.07 mg kg^{-1} P, 2914.09 ± 175.85 mg kg^{-1} Ca, 798.44 ± 42.84 mg kg^{-1} Mg, 1047.63 ± 23.62 mg kg^{-1} Al, 317.38 ± 22.04 mg kg^{-1} K, 219.19 ± 13.10 mg kg^{-1} Fe, 21.55 ± 4.66 mg kg^{-1} Mn, 11.43 ± 0.55 mg kg^{-1} Cu and 2.63 ± 0.27 mg kg^{-1} Zn. Prior setting the experiment, the field was cultivated with corn without GBH application (2016) and roundup ready soybean (2017). At the very beginning of the experiment, in 2018, the experimental field was treated with a uniform 3.3 L/ha application of GBH *Roundup Weathermax*® (540 g of active ingredient (a.i.)/L).

The experimental field was subdivided into 48 plots of 9x20 m each, disposed according to a random pattern depending on their cash crop and AM (Annexe A). All plots were cultivated with DS with a corn (*Zea mays L.*)-soybean (*Glycine max [L.] Merr*.)-wheat (*Triticum aestivum L.*) asynchronous rotation. From 2018 to 2021, the three crop rotations were: wheat-corn-soybean-wheat (WCSW), corn-soybean-wheat-corn (CSWC) and soybean-wheat-corn-soybean (SWCS). The AM setting included CC and three distinct GBH (*Roundup Weathermax*® 540 g a.i./L) application doses: 0.84 L/ha (454 g a.i./ha, referred to as CC_0.84 in the text), 1.67 L/ha (902 g a.i./ha, referred to as CC_1.67 in the text) and 3.3 L/ha (1782 g a.i./ha, referred to as CC_3.3 in the text) as well as without CC along with 3.3 L/ha GBH dose (referred to as DS_3.3 in the text). Each combination of crop and AM setting was replicated in four plots. GBH doses were applied in two times during the growing season. The first GBH application on soybean and corn was made at plant growth stage V2. The second one was made at stages R1 for soybean and V4 for corn (Table 1.2). Wheat plots did not receive a GBH application, but a uniform *Embutox*® (2.25 L/ha) application in 2019 and an *Infinity*® (0.83 L/ha) application in 2020 and 2021 (Table 1.2).

1.2.2 Soil sampling

In spring 2018, before the uniform GBH application, an initial sampling campaign (C0) of 16 samples was realized. Then, soil samples were taken three times each year, specific dates are presented in Table 1.2. The sampling campaign 1 (C1) took place at the pre-emergence stage, before the first GBH application. Campaign 2 (C2) took place in the days following the second GBH application, which was approximately one month after the first one. The last sampling, campaign 3 (C3), was realized at the end of the cash crop growing season. Following, the last two digits of the year combined with the campaign abbreviation would be used, e.g. 2019 campaign 1 is referred to as 19C1. On each plot, three soil cores (0-20 cm) were taken with a 7 cm diameters manual soil auger, pooled and homogenized before being placed in a cooler. Soil samples were stored at -20 °C.

Table 1.2 Dates and specifications of sampling campaign, herbicide application, sowing and harvest for each crop (corn, soybean and wheat) and each year (2018-2021)

2018-07-02: Initial sampling campaign (C0) 2018-07-05: GBH application of 3.3 L/ha on all plots								
Corn			Soybean			Wheat		
Action	Date	Specification	Action	Date	Specification	Action	Date	Specification
2018								

Sowing	11-May	Elite E65G82R (COOP)®	Sowing	30-May	Altitude R2 (SECAN)®	Sowing	10-May	Moka (Semican)®
GBH1	12-May	Weathermax®	GBH1	03-Jun	Weathermax®	CC sowing	10-May	Berseem and crimson clover
GBH2	03-Jun	Weathermax®	GBH2	27-Jun	Weathermax®	Harvest	10-Aug	
Campaign 2	06-Jun		Campaign 2	04-Jul		CC sowing	13-Aug	CC mix^d
CC sowing	13-Jun	CC mix^a	Campaign 3	04-Oct		Campaign 3	04-Oct	
Campaign 3	04-Oct		Harvest	05-Oct				
CC sowing	26-Oct	Rye	CC sowing	06-Oct	Winter wheat			
Harvest	12-Nov							
2019								
Sowing	08-May	P9188AM®	Campaign 1	09-May		Sowing	08-May	Hoffman®
Campaign 1	09-May		Sowing	18-May	Altitude R2®	Campaign 1	09-May	
GBH1	12-May	Weathermax®	GBH1	18-May	Weathermax®	CC sowing	12-May	Berseem and crimson clover
GBH2	13-Jun	Weathermax®	GBH2	24-Jun	Weathermax®	Herbicide	06-Jun	Embutox®
CC sowing	17-Jun	CC mix^b	Campaign 2	02-Jul		Harvest	15-Aug	
Campaign 2	20-Jun		CC sowing	06-Sept	Winter wheat	Herbicide	23-Aug	Roundup®
Campaign 3	19-Sept		Campaign 3	19-Sept		CC sowing	27-Aug	CC mix^d
Harvest	29-Oct		Harvest	15-Oct		Campaign 3	19-Sept	
CC sowing		Not sowed						
2020								
Sowing	14-May	P9188AM®	Campaign 1	20-May		Sowing	25-Apr	Hoffman HRF®
Campaign 1	20-May		Sowing	26-May	Altitude®	Campaign 1	20-May	
GBH1	24-May	Weathermax®	GBH1	02-Jun	Weathermax®	Herbicide	24-May	Infinity®
GBH2	15-Jun	Weathermax®	GBH2	03-Jul	Weathermax®	CC sowing	01-Jun	Berseem and crimson clover
CC sowing	19-Jun	CC mix^c	Campaign 2	14-Jul		Campaign 2	22-Jun	
Campaign 2	22-Jun		Campaign 3	24-Sept		Harvest	10-Aug	
CC sowing	08-Sept	Rye	CC sowing	06-Oct	Lexington wheat	CC sowing	14-Aug	CC mix^d
Campaign 3	24-Sept		Harvest	31-Oct		Campaign 3	24-Sept	
Harvest	18-Oct							
2021								
Sowing	07-May	P9188AM Hybride®	Campaign 1	12-May		Sowing	19-Apr	Hoffman®
Campaign 1	12-May					Campaign 1	12-May	

Abbreviations: GBH1, glyphosate-based herbicide first application; GBH2, glyphosate-based herbicide second application; CC, cover crops; CC mix^a, rye, common vetch, crimson clover, radish and turnip; CC mix^b, rye, common vetch, radish and turnip; CC mix^c, radish, turnip and crimson clover; CC mix^d, buckwheat, sunflower, broad bean, radish, phacelia, peas and oat.

1.2.3 Glyphosate and AMPA contents quantification

Soil samples were freeze-dried for 48 hours at -50 °C, then crushed and sieved with a 2 mm sieve. Then, glyphosate and AMPA were extracted using a slightly modified version of the method described by Alferness et Iwata (1994). Five grams of soil and 40 mL of Alferness solution (0.125M NH_4OH and 0.05M

KH_2PO_4) were put in a 50 mL falcon for extraction. The falcon was then mixed with a vortex mixer and placed on a rotating wheel for 45 min at 50 rpm. Subsequently, samples were centrifuged for 20 min at 3500 rpm followed by a 2 mL filtration with a 0.22 µm nylon filter. Then, 40 µL was transferred in an injection vial before being evaporated to dryness with a nitrogen stream. The extract was derivatized following the method described by Deyrup *et al.* (1985). The samples were derivatized by adding 1000 µL of trifluoroacetic anhydride (TFAA), 500 µL of trifluoroethanol (TFE) and heated for 60 min at 100 °C. Afterward, vials were cool down to room temperature before being evaporated to dryness with a stream of nitrogen. Samples were then dissolved in 1000 µL of isopropyl acetate prior to the GC-ECD injection. The injection was done using a Varian GC 3800 gas chromatograph combined with a Zebron ZB-1 GC columns (phenomenex) (30 m × 0.25 mm ID, 0.25 µm). The temperatures were set at 280˚C and 300˚C for the injector and the detector, respectively. The initial oven temperature was 70˚C for 1 min, then a 1˚C per min increase to 84˚C, a 4˚C per min increase to 120˚C and a 80˚C per min increase to 250˚C, held for 7 min. The total run time was 32.63 min. High-purity hydrogen was used as a carrier gas at a flow of 1.4 mL per min and the injection volume was 1 µL. A calibration curve was built from six standards (0, 1, 2, 3, 4, 6 µg/L for AMPA and 0, 0.5, 1, 1.5, 2, 3 µg/L for glyphosate) by spiking the extract of a nearby soil located outside the crop field that has never received any GBH application. This curve was used to obtain glyphosate and AMPA concentrations which was then converted to contents. The limit of detection (LOD) and limit of quantification (LOQ) were, respectively, 0.02 and 0.05 µg/g for glyphosate and 0.03 and 0.09 µg/g for AMPA and were determined using Mocak *et al.* (1997) method (Samson-Brais *et al.*, 2022).

1.2.4 Statistical analyses

Results below the LOD or between the LOD and the LOQ were assigned values of half of the LOD or the LOQ, respectively, in line with other studies (Beecraft et Rooney, 2021; McGuire, M. K. *et al.*, 2016). As such, glyphosate and AMPA results below the LOD were set to 0.01 and 0.02 µg/g, respectively. Glyphosate and AMPA results between LOD and LOQ were set to 0.02 and 0.05 µg/g, respectively.

All data were taken into account in the statistical analyses. Data normality was tested with the Shapiro-Wilk test and the homogeneity of variance was tested with the Bartlett test. Glyphosate and AMPA contents were not normally distributed, so a non-parametrical test was used. Glyphosate and AMPA contents for each year were compared with the non-parametrical Kruskal-Wallis test to determine if there was significant difference in glyphosate and AMPA contents between years.

The second series of statistical tests was to analyze if there was a difference in glyphosate and AMPA soil contents between AM for a specific campaign (C1, C2 and C3) and crop rotation (CSWC and WCSW) during the 2019 and 2020 growing seasons. To determine if significant difference could be observed, the Kruskal-Wallis test was used. Also, to evaluate if there was significant difference of glyphosate and AMPA contents between campaign (C1-C2-C3) of a specific year (2019 and 2020) and crop rotation (CSWC and WCSW), the Kruskal-Wallis test was run between campaigns for each year and crop. When the Kruskal-Wallis test was significant, the Wilcoxon post hoc test was applied (p-value < 0.05).

1.3 Results

1.3.1 Glyphosate and AMPA soil contents

Average glyphosate and AMPA soil contents from the initial sampling campaign (C0) (n = 16) are 0.05 ± 0.04 µg/g and 0.11 ± 0.13 µg/g, respectively. Following C0, glyphosate and AMPA soil contents range from below the LOD to 0.84 µg/g and from below the LOD to 0.93 µg/g, respectively. Glyphosate and AMPA means and standard deviations from all data collected during this experiment (n = 367) are 0.08 ± 0.09 µg/g and 0.15 ± 0.14 µg/g, respectively. Glyphosate and AMPA contents from 2018 to 2021 according to the AM used, for each crop rotation, are presented in Figures 1.1, 1.2 and 1.3. The SWCS (p-value = 3.8e^{-11}), CSWC (p-value = 1.5e^{-09}) and WCSW (p-value = 4.2e^{-08}) rotations show that year 2019 exhibits significantly higher AMPA contents than the other years.

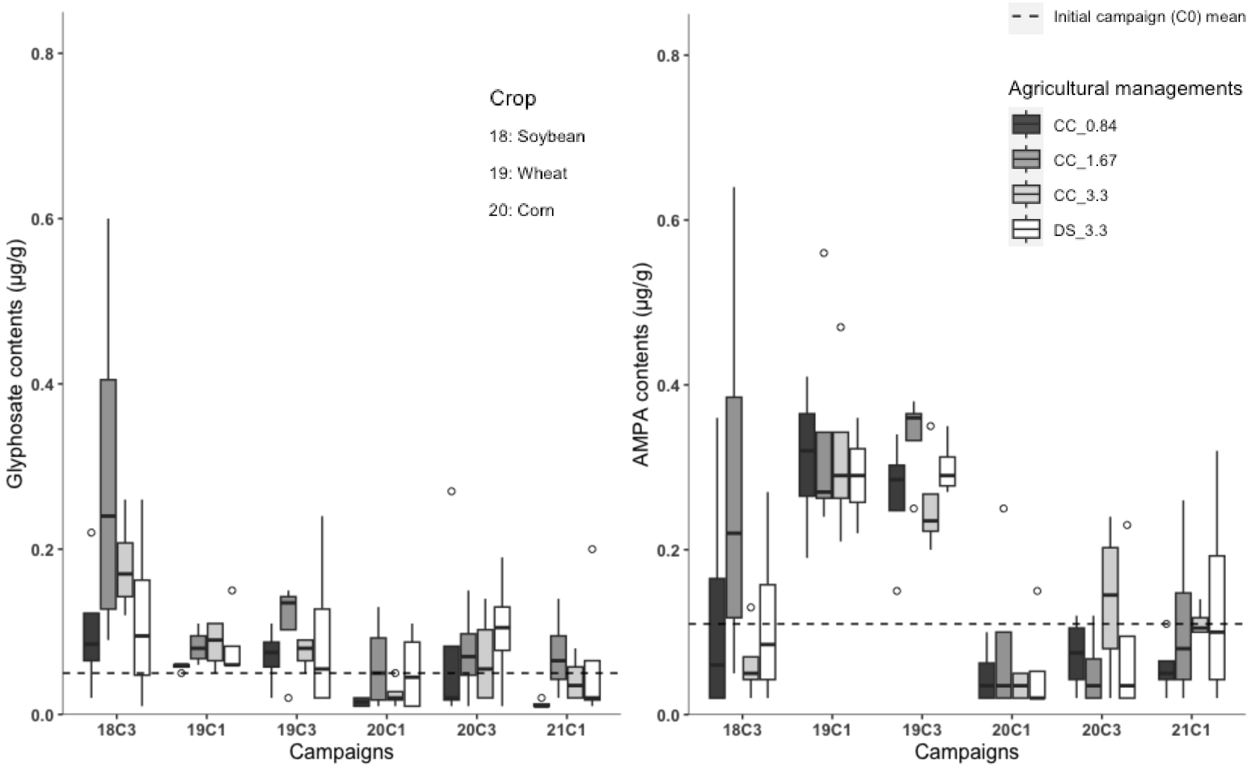

Figure 1.1 Glyphosate and AMPA soil contents (0-20 cm) trends for the soybean-wheat-corn-soybean (SWCS) rotation plots. Box plots are for each agricultural managements (AM), years (2018-19-20-21) and campaign (C1-C2-C3). DS = direct seeding, CC = cover crops, CC_0.84 = DS+CC with a 0.84 L/ha glyphosate-based herbicide (GBH) dose, CC_1.67 = DS+CC with a 1.67 L/ha GBH dose, CC_3.3 = DS+CC with a 3.3 L/ha GBH dose and DS_3.3 = DS with a 3.3 L/ha GBH dose. For each box plot, n = 4. Box plots represent the 25 and 75 % quantiles, the horizontal line inside is the median and the circles ◦ represent outliers.

1.3.2 Cash crops and AM effect on glyphosate and AMPA soil contents

Glyphosate and AMPA soil contents between the AM of two crop rotations (CSWC and WCSW) (Figures 1.2 and 1.3) of each sampling campaign (C1-C2-C3) in 2019 and 2020 were analyzed. No significant difference is observed when comparing glyphosate or AMPA contents between the AM of each sampling campaign of a specific crop and year. The SWCS could not be compared, as samples of the 2019 C2 campaign were not analyzed.

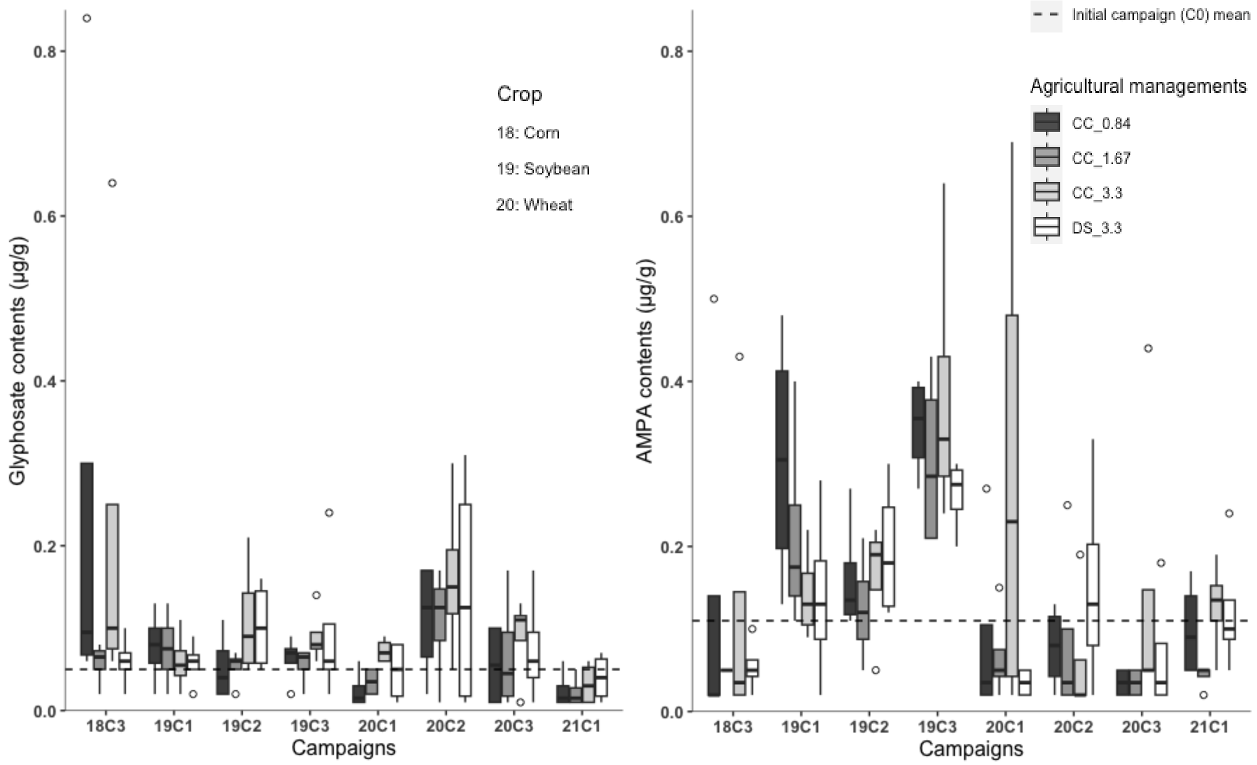

Figure 1.2 Glyphosate and AMPA soil contents (0-20 cm) trends following the corn-soybean-wheat-corn (CSWC) rotation plots. Box plots are for each agricultural managements (AM), years (2018-19-20-21) and campaign (C1-C2-C3). DS = direct seeding, CC = cover crops, CC_0.84 = DS+CC with a 0.84 L/ha glyphosate-based herbicide (GBH) dose, CC_1.67 = DS+CC with a 1.67 L/ha GBH dose, CC_3.3 = DS+CC with a 3.3 L/ha GBH dose and DS_3.3 = DS with a 3.3 L/ha GBH dose. For each box plot, n = 4. Box plots represent the 25 and 75 % quantiles, the horizontal line inside is the median and the circles ◦ represent outliers.

1.3.3 Glyphosate soil contents trends during the growing seasons

Comparing glyphosate contents of each campaign (C1-C2-C3) for a growing season of a given cash crop shows that, for the CSWC rotation, no difference is observed between sampling campaigns of the 2019 soybean plots (Table 1.3). The 2020 wheat plots present significantly higher glyphosate contents at C2 than C1 (*p*-value = 0.04), while C3 is not different than C1 or C2 (Table 1.3). For the WCSW rotation, 2019 corn plots have significantly higher glyphosate contents at C2 than C1 (*p*-value = 0.0003) and C3 (*p*-value = 0.0008) and the 2020 soybean plots have significantly higher glyphosate contents at C2 than at C1 (*p*-value = 0.002), while C3 is not different than C1 or C2 (Table 1.3).

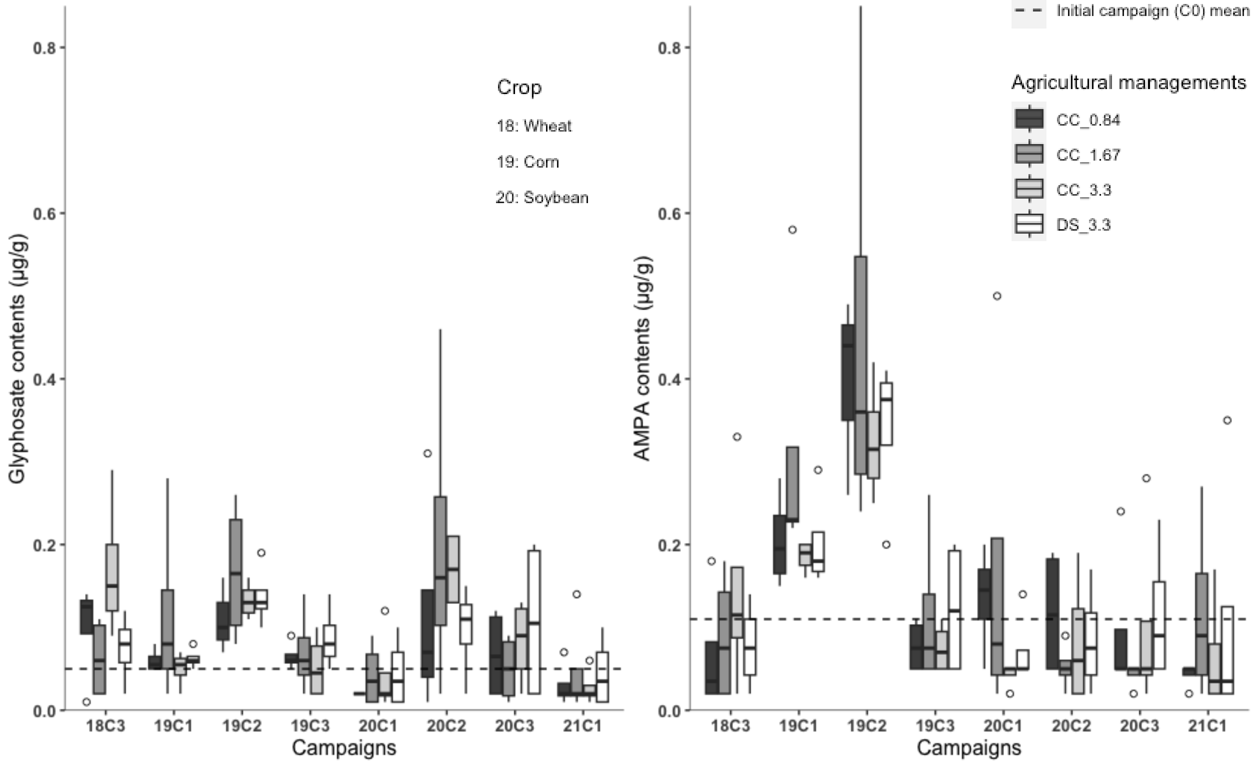

Figure 1.3 Glyphosate and AMPA soil contents (0-20 cm) trends following the wheat-corn-soybean-wheat (WCSW) rotation plots. Box plots are for each agricultural managements (AM), years (2018-19-20-21) and campaign (C1-C2-C3). DS = direct seeding, CC = cover crops, CC_0.84 = DS+CC with a 0.84 L/ha glyphosate-based herbicide (GBH) dose, CC_1.67 = DS+CC with a 1.67 L/ha GBH dose, CC_3.3 = DS+CC with a 3.3 L/ha GBH dose and DS_3.3 = DS with a 3.3 L/ha GBH dose. For each box plot, n = 4. Box plots represent the 25 and 75 % quantiles, the horizontal line inside is the median and the circles ◦ represent outliers.

Table 1.3 Average glyphosate contents (µg/g) for each sampling campaign (C1-C2-C3) following two crop rotation: corn-soybean-wheat-corn (CSWC) and wheat-corn-soybean-wheat (WCSW)

Rotation	Year	Crop	C1	C2	C3
CSWC	2019	Soybean	0.07 ± 0.04 A	0.08 ± 0.05 A	0.08 ± 0.05 A
	2020	Wheat	0.05 ± 0.03 A	0.13 ± 0.10 B	0.07 ± 0.06 AB
WCSW	2019	Corn	0.07 ± 0.06 A	0.14 ± 0.05 B	0.07 ± 0.04 A
	2020	Soybean	0.04 ± 0.04 A	0.15 ± 0.12 B	0.08 ± 0.06 AB

Notes: The Kruskal-Wallis (p-value < 0.05) test was used to test for differences in glyphosate contents between campaign of each specific year and crop. Significant differences are displayed with the letters A and B.

1.3.4 AMPA soil contents trends during the growing seasons

Comparing AMPA contents of each campaign over a growing season (C1-C2-C3) for a specific crop shows that, for the CSWC rotation, 2019 soybean plots have significantly higher AMPA contents at C3 than C1 (*p*-value = 0.02) and C2 (*p*-value = 0.0002), while no difference is observed between sampling campaigns of the 2020 wheat plots (Table 1.4). For the WCSW rotation, the three sampling campaigns of the 2019 corn plots are all significantly different, with higher contents at C2 than at C1 (*p*-value = 0.0008) and higher contents at C1 than at C3 (*p*-value = 0.0004) (Table 1.4). No difference is observed between sampling campaigns of the 2020 soybean plots (Table 1.4).

Table 1.4 Average measured AMPA contents (µg/g) for each sampling campaign (C1-C2-C3) following two crop rotation: corn-soybean-wheat-corn (CSWC) and wheat-corn-soybean-wheat (WCSW)

Rotation	Year	Crop	C1	C2	C3
CSWC	2019	Soybean	0.20 ± 0.13 A	0.16 ± 0.07 A	0.32 ± 0.11 B
	2020	Wheat	0.12 ± 0.19 A	0.09 ± 0.10 A	0.07 ± 0.11 A
WCSW	2019	Corn	0.23 ± 0.10 A	0.38 ± 0.17 B	0.10 ± 0.07 C
	2020	Soybean	0.11 ± 0.12 A	0.08 ± 0.06 A	0.09 ± 0.08 A

Notes: The Kruskal-Wallis (*p*-value < 0.05) test was used to test for differences in glyphosate contents between campaign of each specific year and crop. Significant differences are displayed with the letters A, B and C

1.4 Discussion

1.4.1 Glyphosate and AMPA soil contents

Glyphosate and AMPA soil contents measured in this study, 0.08 ± 0.09 µg/g and 0.15 ± 0.14 µg/g, for glyphosate and AMPA, respectively, are of the same order of magnitude to those reported in other studies run in northern climate (Laitinen *et al.*, 2009; Maccario *et al.*, 2022; Silva *et al.*, 2018) (Table 1.5). Glyphosate and AMPA agricultural soil contents reported so far in the literature vary depending on soil type, AM and climate. For example, in Argentina, where high glyphosate and AMPA contents are reported, field crops can receive up to six GBH applications per year (equivalent to 6.4 L/ha, representing up to 3564 g a.i./ha) (Aparicio *et al.*, 2013; Bento *et al.*, 2019), as compared to usually two GBH applications per growing season in Quebec (3.3 L/ha, representing up to 1782 g a.i./ha) (Maccario *et al.*, 2022).

Table 1.5 Glyphosate and AMPA soil contents measured by other studies in agricultural field

		Glyphosate contents (µg/g)	AMPA contents (µg/g)
Articles	Country	Mean	Mean
Aparicio *et al.* (2013)	Argentina	0.40	0.69
Bento *et al.* (2019)	Argentina	0.35	1.50
Laitinen *et al.* (2009)	Finland	0.11	0.09
Maccario *et al.* (2022)	Canada	0.07	0.30
Silva *et al.* (2018)	Europe	0.14*	0.15*

*Medians

1.4.2 Anomaly of AMPA contents observed in 2019

In this study, the 2019 AMPA contents are significantly higher than the other years for all crop rotations (Figures 1.1, 1.2 and 1.3). The AMPA contents increase between 18C3 and 19C1 is unexpected since no glyphosate input happened between these two samplings campaigns. In this study, the average March and April temperature in 2019 is 0.9 °C while it is 2.6 °C in 2020 (ECCC, 2023), meaning a slower ice melt and longer snow covering the soil in 2019. AMPA degradation can be slowed down by colder temperatures (Bento *et al.*, 2019; Muskus *et al.*, 2019) and low oxygen or anoxic condition (Li *et al.*, 2016), leading to higher soil contents. However, this observation still cannot fully explain the higher contents observed in this study. The AMPA increase must be due to a content input, which remains unexplained.

1.4.3 Glyphosate and AMPA adsorption capacity by soils

At each campaign of the 2019 or 2020 seasons, no significant difference in glyphosate or AMPA contents is observed between the AM of a given cash crop. This result is unexpected considering a single to fourfold difference in GBH doses among the various AM. It is also worth noting that 2020 was the third year of installation of the experimental plots and that the various AM impacts could have been consolidated over time (Hill *et al.*, 2021). Those observations (Figures 1.1, 1.2 and 1.3) contradict Samson-Brais *et al.* (2022)'s findings, who reported that soybean plots presented significant differences in glyphosate and AMPA contents along with different AM. However, the later study did not use CC and the 1.67 L/ha (902 g a.i./ha) GBH dose was applied in a single application, compared to two in our study. Samson-Brais *et al.* (2022) also showed that a 3.3 L/ha vs. a 1.67 L/ha GBH dose increased glyphosate soil contents but not AMPA ones. On the other hand, in Argentina, under heavy GBH usage (up to 6 applications of 902 g a.i./ha), Peruzzo *et al.* (2008) and Primost *et al.* (2017) showed that glyphosate and AMPA soil contents were correlated with GBH application doses. In the present study, fairly similar glyphosate and AMPA contents between the AM of each sampling campaign could be explained by the adsorption capacity of the soil matrix. While adsorption is influenced by multiple factors, glyphosate and AMPA residual contents could represent the adsorption saturation capacity of the soil matrix (Sidoli *et al.*, 2016). The fractions of glyphosate and AMPA not constituting the residual pool would therefore be degraded or transferred away from the 20 cm soil horizon. Indeed, Andréa *et al.* (2003) have shown quick glyphosate mineralization right after GBH application, while Hearon *et al.* (2021); Padilla et Selim (2020); Rampazzo Todorovic *et al.* (2014) have shown the potential high mobility of non-adsorbed glyphosate and AMPA. Following Lancaster *et al.* (2010) findings, our observations (e.g. no difference in glyphosate nor AMPA content between our lowest GBH application dose and the highest) would mean that the proportion of glyphosate and AMPA dissipated beyond the first 20 cm of soil is different depending on the GBH dose applied. Likewise, the soil adsorption sites available for glyphosate would be quickly saturated and the exceeding amount of glyphosate would be dissipated. The fact that glyphosate and AMPA contents are not significantly different between GBH application doses corroborates the hypothesis of quick soil saturation and thus glyphosate dissipation. This observation is of concern regarding the potential impacts of those molecules on human health and the environment (Myers *et al.*, 2016). It would also corroborate other studies pointing out an increase in glyphosate and AMPA concentrations in surface waters of field crop regions (Battaglin *et al.*, 2014; Giroux, 2022).

1.4.4 Glyphosate and AMPA soil contents trends over the course of a year

Two years and two crop rotations provided four glyphosate (Table 1.3) and four AMPA (Table 1.4) contents trends, where, for each situation, the three sampling campaigns were compared between them. For glyphosate contents, three out of four trends present C2 contents higher than C1 (Table 1.3). This was expected considering that C2 was run around one week following the second GBH application (Table 1.2). However, one of those three trends have wheat, which did not receive GBH application during the growing season. The increase of glyphosate soil contents in wheat plots could, at least partially, be explained by the high adsorption capacity of crop residues. Studies have shown that plant residues delay glyphosate transfer to soil (Cassigneul *et al.*, 2015; Mamy *et al.*, 2016). In the crop rotation of this study, soybean cultivation predated wheat cultivation. Part of applied glyphosate could have been adsorbed on soybean and CC residues in 2019 and delayed its transfer into the soil until 2020. While corn residues weakly adsorb glyphosate (Aslam *et al.*, 2013), soybean residues present a higher adsorbance capacity (Rampoldi *et al.*, 2011).

For AMPA soil contents, the 2019 trends in both corn and soybean plots (Table 1.4) are opposed (Figures 1.2 and 1.3), suggesting that cash crops may influence AMPA dissipation, which was also observed by Samson-Brais *et al.* (2022). However, the AMPA soil contents trend in soybean plots was not repeated in 2020 (Table 1.4). In fact, both 2020 trends present no content difference between sampling campaigns. Therefore, those results suggest that AM and cash crops are not the dominant variables influencing soil AMPA contents and would follow Guijarro *et al.* (2018), who pointed out that soil is a complex and dynamic environment and multiple factors influence GBH dissipation in soil.

1.4.5 Pseudo-persistence of glyphosate and AMPA in soil

Despite GBH application doses of up to 3.3 L/ha (1782 g a.i./ha), CC, three crops rotations and monitoring from 2018 to 2021, none of the AM tested have showed accumulation trends over time of glyphosate or AMPA contents in topsoils (Figures 1.1, 1.2 and 1.3). This contradicts Primost *et al.* (2017)'s findings in various Argentinian farms with no-till practices and crops rotation. After five years, these authors have estimated a glyphosate accumulation of 1 ug/g after every five spraying events, with a mean glyphosate application per spraying event equivalent to our 1.67 L/ha and 2.0 to 5.8 GBH applications per year (Primost *et al.*, 2017). However, in Argentina, Villarreal *et al.* (2020) showed that in a corn-soybean/wheat rotation, CC, no-till and three GBH applications per year (GBH doses not specified), no glyphosate accumulation in soil was observed after ten years. Guijarro *et al.* (2018) and Villarreal *et al.* (2020) have pointed out that glyphosate accumulation highly depends on soil properties. Indeed, studies have shown that soil clay content was positively correlated with glyphosate persistence (Bergström *et al.*, 2011; Okada *et al.*, 2016). However, while our study was conducted in heavy clay soil, we do not find a progressive glyphosate accumulation in soils. Although, in an open field study, many factors like soil water content, microbial activity and soil nutrients contents can also impact glyphosate dissipation, making it vastly complex to observe the AM direct impacts on glyphosate dissipation (Bergström *et al.*, 2011; Rampazzo Todorovic *et al.*, 2014; Villarreal *et al.*, 2020).

Despite the absence of accumulation trends, glyphosate and AMPA pseudo-persistence in soils was observed by their relatively stable contents throughout this study (Figures 1.1, 1.2 and 1.3). This appears to reinforce the idea that soil adsorption sites available for glyphosate and/or AMPA can be easily saturated resulting in the dissipation of the exceeding applied glyphosate in the environment. In addition, three out of four seasonal trends of glyphosate and AMPA contents do not show significant decrease between C2 and C3, which are three months apart (Tables 1.3 and 1.4). These observations go along with the pseudo-persistence concept (Daughton, 2003; Grenni *et al.*, 2013; Primost *et al.*, 2017), meaning that GBH application doses, even at the lowest one (0.84 L/ha), were higher than glyphosate dissipation rates.

1.4.6 Cover crops impact on glyphosate and AMPA soil contents

Over four years, glyphosate and AMPA soil contents in AM including CC are not significantly different from those without CC (Figures 1.1, 1.2 and 1.3). These observations would not follow studies that have shown CC impacts on soil functions and soil microbiology, like reducing erosion and increasing OM and soil humidity, which can influence glyphosate and AMPA dissipation (Cassigneul *et al.*, 2016; Giusti *et al.*, 2023; Napoli *et al.*, 2016; Scopel *et al.*, 2013). Therefore, our results suggest that, in our experiment, CC did not significantly influence glyphosate or AMPA dissipation. Considering that the full establishment of CC in a northern climate is challenging (Lapierre *et al.*, 2022), it could take longer to highlight CC impacts. As such, Hill *et al.* (2021) have pointed out that CC beneficial effects on soil health can take more than five years, while Singh, J. *et al.* (2021) did not observe CC impact after four seasons.

1.5 Conclusion

Our results show that higher GBH annual doses do not necessarily translate into higher glyphosate or AMPA soil contents. Along with a four-year monitoring of three crops asynchronous rotations using three different GBH annual doses and the presence or not of CC in the plots, glyphosate and AMPA contents in the soil first 20 cm are not significantly different between the AM. While no glyphosate nor AMPA accumulation trend is observed for all AM tested, their potential pseudo-persistence in crop topsoil is pointed out. Our results suggest that with the soil characteristics, climatic conditions and AM of this study, GBH application doses were higher than glyphosate dissipation rates, which is congruent with the pseudo-persistence concept. Finally, the similar contents of glyphosate and AMPA that have persisted in soil despite the different GBH doses suggest that dissipation rates of glyphosate and AMPA may vary according to the AM employed. It would then suggest that the exceeding content is mineralized by microorganisms or is moved away from the 0-20 cm soil horizon. It also means that the fractions of glyphosate and AMPA not constituting the pseudo-persistent pool are potentially highly mobile in soil. These observations are of concern considering the impact that glyphosate may have on non-target species and humans, especially via the contamination of water sources. This corroborates, at least in part, the increase in the detection frequency of glyphosate and AMPA. Gathering knowledge on glyphosate and AMPA dissipation in soils over time, along with various AM, is crucial to understand the persistence dynamic of these compounds in soils and their potential impact on the environment and cash crops, as the future of industrial agriculture cropping systems is at stake.

CHAPITRE 2

INFLUENCE OF GLYPHOSATE-BASED HERBICIDE APPLICATION DOSES AND COVER CROPS ON CORN, SOYBEAN AND WHEAT YIELDS

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2.0 Abstract

The industrial cropping system perennity could be adversly impacted by the vast use of glyphosate-based herbicide (GBH). The emergence of glyphosate-resistant weeds is reducing GBH efficacity while glyphosate and its main by-product, aminomethylphosphonic acid (AMPA), could limit crop yields by affecting plants nutrition and soil microorganisms. Cover crops (CC) are often proposed to reduce GBH usage and restore soil functions while maintaining high yields. This study took place in southern Quebec (Canada) on 48 plots installed in 2018 with a corn/soybean/wheat rotation and direct seeding (DS). Different agricultural managements (AM), CC and three different GBH (540 g a.i./L) doses: 0.84, 1.67 and 3.3 L/ha, were tested for their impacts on yields. Soil elementary composition, glyphosate and AMPA contents, plants cover rates, measured after the second GBH application, and yields were analyzed after three years of cultivation. Yields were significantly lower in plots using the 0.84 L/ha dose while they were similar from plots with the 1.67 and 3.3 L/ha doses. No correlation was observed between yields and elements, glyphosate or AMPA soil contents, while weeds cover adversely impacted soybean and wheat yields, but not corn yields. While the 0.84 L/ha GBH dose did not efficiently control weeds to allow high yields, similar high yields using 1.67 and 3.3 L/ha annual doses of GBH highlight the potential to use reduced GBH doses when using DS, CC and a crop rotation.

Keywords: field crops, yields, agricultural managements, cover crops, glyphosate-based herbicide, glyphosate, AMPA.

2.1 Introduction

Since their introduction in the 1990s, glyphosate-based herbicides (GBH), combined with glyphosate-resistant (GR) crops, have allowed a considerable boost in field crops yields as well as a decrease in production cost (Brookes *et al.*, 2017). The translocation of glyphosate from leaves to other plant tissues is conveyed by adjuvants contained in GBH. The active ingredient (a.i.) inactivates the 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) enzyme, which is essential to the shikimic acid pathway (Herrmann et Weaver, 1999). GBH low cost and ease of use have led to its world-wide use but, consequently, to the emergence of glyphosate-resistant (GR) weeds (Baek *et al.*, 2021). As a matter of fact, both GBH spraying doses and application frequencies have increased over time (Baek *et al.*, 2021; Benbrook, 2016; Heap et Duke, 2018). Glyphosate and aminomethylphosphonic acid (AMPA), the main microbial degradation product of glyphosate in soils, detection frequencies in the environment have increased over time (Battaglin *et al.*, 2014; Giroux, 2022; Maccario *et al.*, 2022; Silva *et al.*, 2019; Singh, S. *et al.*, 2020). AMPA is considered more persistent than glyphosate in soils (Bergström *et al.*, 2011; Grandcoin *et al.*, 2017; Simonsen *et al.*, 2008) and can be phytotoxic, even for GR plants (Reddy *et al.*, 2004; Smedbol *et al.*, 2019). Glyphosate fate and behavior in the environment are dictated by multiple processes. One of them, sorption, has a strong influence on glyphosate transport, plant uptake and degradation (Duke *et al.*, 2012). Moreover, glyphosate and AMPA, can form complexes with metals and other soil components (Bärwald Bohm *et al.*, 2014; Duke *et al.*, 2012; Sidoli *et al.*, 2016). Consequently, studies have pointed out their adverse effects on nutrients bioavailability, which could affect crops nutrition and disease resilience, potentially reducing crops yields (Johal et Huber, 2009; Kanissery *et al.*, 2019; Mertens *et al.*, 2018; Swart et Wolmarans, 2014). Crop yields can also be impacted by soil microorganisms, which play a critical role in soil functions (Lancaster *et al.*, 2010; Zaidi et Khan, 2005). While glyphosate can negatively affect soil microorganism richness, activity or diversity (Andréa *et al.*, 2003; Gimsing *et al.*, 2004; Lancaster *et al.*, 2010; Samson-Brais *et al.*, 2021), it can also have a positive effect or no effect at all (Bärwald Bohm *et al.*, 2014; Haney *et al.*, 2000; Schlatter *et al.*, 2017). Agricultural managements (AM) like cover crops (CC), crop rotation and fertilization have also been reported to impact microbial structural and functional diversity (Berg et Smalla, 2009; Dunn *et al.*, 2021; Giusti *et al.*, 2023; Lauber *et al.*, 2008).

Some of the negative impacts arising from industrial agriculture could be mitigated by using CC in AM. Indeed, CC can reduce soil erosion, degradation and compaction while enhancing soil water conservation, organic matter content, plant diversity and ecosystem services (Blanco-Canqui *et al.*, 2015; Hill *et al.*, 2021; Iglesias et Garrote, 2015; Lapierre *et al.*, 2022; McGuire, A., 2021; Murrell, 2017; Osipitan *et al.*, 2019; Pott *et al.*, 2020). Also, CC could allow a usage reduction of fertilizers and herbicides by increasing soil nitrogen contents and reducing weeds presence by competing for spaces and resources while releasing allelochemicals (Lapierre *et al.*, 2022; Osipitan *et al.*, 2019; Teasdale *et al.*, 2007). Despite CC potential positive impacts, concerns arise from resources competition between them and cash crops (Basche *et al.*, 2016; Blanco-Canqui *et al.*, 2015; Sanders *et al.*, 2018). However, CC impact on soil water availability seems compensated by the fact that CC facilitate water infiltration and improve soil hydraulic proprieties (Blanco-Canqui *et al.*, 2015; García-González *et al.*, 2018).

Reducing GBH annual application doses in order to provide positive impacts for the agricultural system and the environment while maintaining high yields has become nowadays a fundamental issue. In this study, an experimental field was cultivated during three years with a corn (*Zea mays L.*)-soybean (*Glycine max [L.] Merr*.)-wheat (*Triticum aestivum L.*) asynchronous rotations using four different AM, i.e. plots receiving three different GBH annual application doses (i.e. 0.84 L/ha; 1.67 L/ha; 3.3 L/ha) along with CC and plots with a 3.3 L/ha annual dose without CC, in order to evaluate the impact of GBH reduction as well as CC on yields. The influence of glyphosate, AMPA and soil elements contents in soil on yields were also tested. We hypothesize that plots using AM with CC and a GBH annual dose of 1.67 L/ha will give yields similar to plots without CC and with 3.3 L/ha GBH annual applications, as CC could compensate the GBH application rate reduction. Also, yields will be negatively correlated with glyphosate and AMPA soil contents.

2.2 Material and methods

2.2.1 Field experiment

The experimental plots were installed in 2018 at the Grain Research Center (CEROM) at Saint-Mathieu de Beloeil (Quebec, Canada) (45°34'58.9"N 73°14'15.1"W) in a field characterized with a heavy clay soil type. Prior setting the experiment, the field was cultivated with corn without GBH application (2016) and Roundup Ready soybean (2017). At the very beginning of the experiment, in 2018, the experimental field was treated with a uniform 3.3 L/ha application of GBH *Roundup Weathermax*® (540 g a.i./L). More details, including soil composition and main mineral contents of the first 20 cm soil horizon, are presented in the first chapter of this thesis.

2.2.2 Experimental design

The field was subdivided into 48 plots of 9x20 m each, disposed according to a random pattern depending on their cash crop and AM (Annex A). Each combination of crop and AM setting was replicated in four plots. All plots were cultivated with direct seeding (DS) with a corn-soybean-wheat asynchronous rotation. The three different annual GBH doses of *Roundup weathermax*® used in this study were applied in two separate and equal applications during the growing season (Table 2.1). The AM setting included the seeding of CC (see Table 2.1 for the species list) with three distinct annual GBH application doses: 0.84 L/ha, 1.67 L/ha and 3.3 L/ha (referred to as CC_0.84, CC_1.67 and CC_3.3, respectively in the text) as well as without CC and 3.3 L/ha GBH dose (referred to as DS_3.3 in the text). The first GBH application on soybean and corn was made at plant growth stage V2. The second one was made at stages R1 for soybean and V4 for corn. Wheat plots did not receive a GBH application, but a uniform *Infinity*® (0.83 L/ha) herbicide application. Details on AM activities are presented in Table 2.1.

Table 2.1 Dates and specifications of herbicide application, sowing and harvest for each crop and year

Corn			Soybean			Wheat		
Action	Date	Specification	Action	Date	Specification	Action	Date	Specification
				2018				
Sowing	11-May	Elite E65G82R (COOP)®	Sowing	30-May	Altitude R2 (SECAN)®	Sowing	10-May	Moka (Semican)®
GBH1	12-May	Weathermax®	GBH1	03-Jun	Weathermax®	CC sowing	10-May	Berseem and crimson clover
GBH2	03-Jun	Weathermax®	GBH2	27-Jun	Weathermax®	CC sowing	13-Aug	CC mix[d]
CC sowing	13-Jun	CC mix[a]	CC sowing	06-Oct	Winter wheat			
CC sowing	26-Oct	Rye						
				2019				
Sowing	08-May	P9188AM®	Sowing	18-May	Altitude R2®	Sowing	08-May	Hoffman®
GBH1	12-May	Weathermax®	GBH1	18-May	Weathermax®	CC sowing	12-May	Berseem and crimson clover
GBH2	13-Jun	Weathermax®	GBH2	24-Jun	Weathermax®	Herbicide	06-Jun	Embutox®
CC sowing	17-Jun	CC mix[b]	CC sowing	06-Sept	Winter wheat	Herbicide	23-Aug	Roundup®
						CC sowing	27-Aug	CC mix[d]
				2020				
Sowing	14-May	P9188AM®	Sowing	26-May	Altitude®	Sowing	25-Apr	Hoffman HRF®
GBH1	24-May	Weathermax®	GBH1	02-Jun	Weathermax®	Herbicide	24-May	Infinity®
GBH2	15-Jun	Weathermax®	GBH2	03-Jul	Weathermax®	CC sowing	01-Jun	Berseem and crimson clover
CC sowing	19-Jun	CC mix[c]	Sampling campaign 2	14-Jul		Sampling campaign 2	22-Jun	
Sampling campaign 2	22-Jun		CC sowing	06-Oct	Lexington wheat	Harvest	10-Aug	
CC sowing	08-Sept	Rye	Harvest	31-Oct		CC sowing	14-Aug	CC mix[d]
Harvest	18-Oct							

Abbreviations: GBH1, glyphosate-based herbicide first application; GBH2, glyphosate-based herbicide second application; CC, cover crops; CC mix[a], rye, common vetch, crimson clover, radish and turnip; CC mix[b], rye, common vetch, radish and turnip; CC mix[c], radish, turnip and crimson clover; CC mix[d], buckwheat, sunflower, broad bean, radish, phacelia, peas and oat.

2.2.3 Sampling campaign

The 2020 sampling campaign (C2) took place in the days following the second GBH application (Table 2.1). At each plot, three soil cores (0-20 cm) were taken with a 7 cm diameter manual soil auger, pooled and homogenized before being placed in a cooler. Soil samples were stored at -20 °C upon analyses. Weeds, cash crops and CC cover rates were measured using two 0.5 x 1 m quadra randomly put on the ground plot with a visual estimation of each species cover percentage. Data of glyphosate, AMPA and soil elementary composition contents as well as plants cover rates are all from C2.

2.2.4 Soil physicochemical analyses

Soil physicochemical analyzes were done by the *Institut de recherche et de développement en agroenvironnement* (IRDA) and obtained using the Mehlich 3 extraction method (Mehlich, 1984) and determined with and inductively coupled plasma-optical emission spectrometer (ICP-OES; Perkin Elmer Optima 4300DV, Shelton, CT, USA). Phosphorus (P) (LOD: 0.19 µg, LOQ: 0.49 µg), potassium (K) (LOD: 0.5 µg, LOQ: 1.7 µg), calcium (Ca) (LOD: 4.2 µg, LOQ: 8.7 µg), magnesium (Mg) (LOD: 1.1 µg, LOQ: 2.3 µg), aluminum (Al) (LOD: 0.7 µg, LOQ: 1.7 µg), copper (Cu) (LOD: 0.15 µg, LOQ: 0.35 µg), iron (Fe) (LOD: 0.45 µg, LOQ: 1.07 µg), manganese (Mn) (LOD: 0.05 µg, LOQ: 0.15 µg) and zinc (Zn) (LOD: 0.05 µg, LOQ: 0.12 µg) contents were measured from soil cores of soybean and corn plots collected at C2.

2.2.5 Yields

Crops were harvested at physiological maturity (Table 2.1). Only the middle rows of each plot were harvested to calculate yields and thus limiting border effects. Soybean and wheat were harvested with the HEGE harvester while corn was harvested with the DELTA harvester. Yields humidity were adjusted at 13.5%, 14.5% and 14% for soybean, corn and wheat, respectively.

2.2.6 Glyphosate and AMPA contents

Glyphosate and AMPA contents in the soil first 20 cm are from the soil cores collected at C2 and are provided in the first chapter of this thesis.

2.2.7 Statistical analysis

The Shapiro-Wilk test was used to evaluate the data distribution normality. To analyze if AM had a significant effect on yields, since yields data of each crop (n = 16) were normally distributed, an ANOVA test was done between AM and each crop yields. When the ANOVA test was significant, the multiple comparisons Tukey post hoc test (p-value < 0.05) was applied. Also, since the soil elementary composition contents (n = 16), plants cover rates (n = 48) and glyphosate and AMPA contents (n = 48) data sets were not normally distributed, a non-parametrical Spearman correlations analysis was performed between each crop yields and those data sets. Lastly, the non-parametrical Spearman correlations test (*p*-value < 0.05) was also applied between each soil elements contents and glyphosate and AMPA contents.

2.3 Results

2.3.1 Corn, soybean and wheat 2020 yields

Corn yields ranged between 4.24 and 9.79 t/ha, while the mean yield in 2020 for this region was 9.42 t/ha (PGQ, 2023). Significant corn yields differences were observed between AM (Figure 2.1). Corn yields were significantly lower in CC_0.84 plots. Yields in CC_1.67 plots were significantly lower than those in CC_3.3 plots but not different from DS_3.3 plots. Both yields for CC_3.3 and DS_3.3 plots were not different.

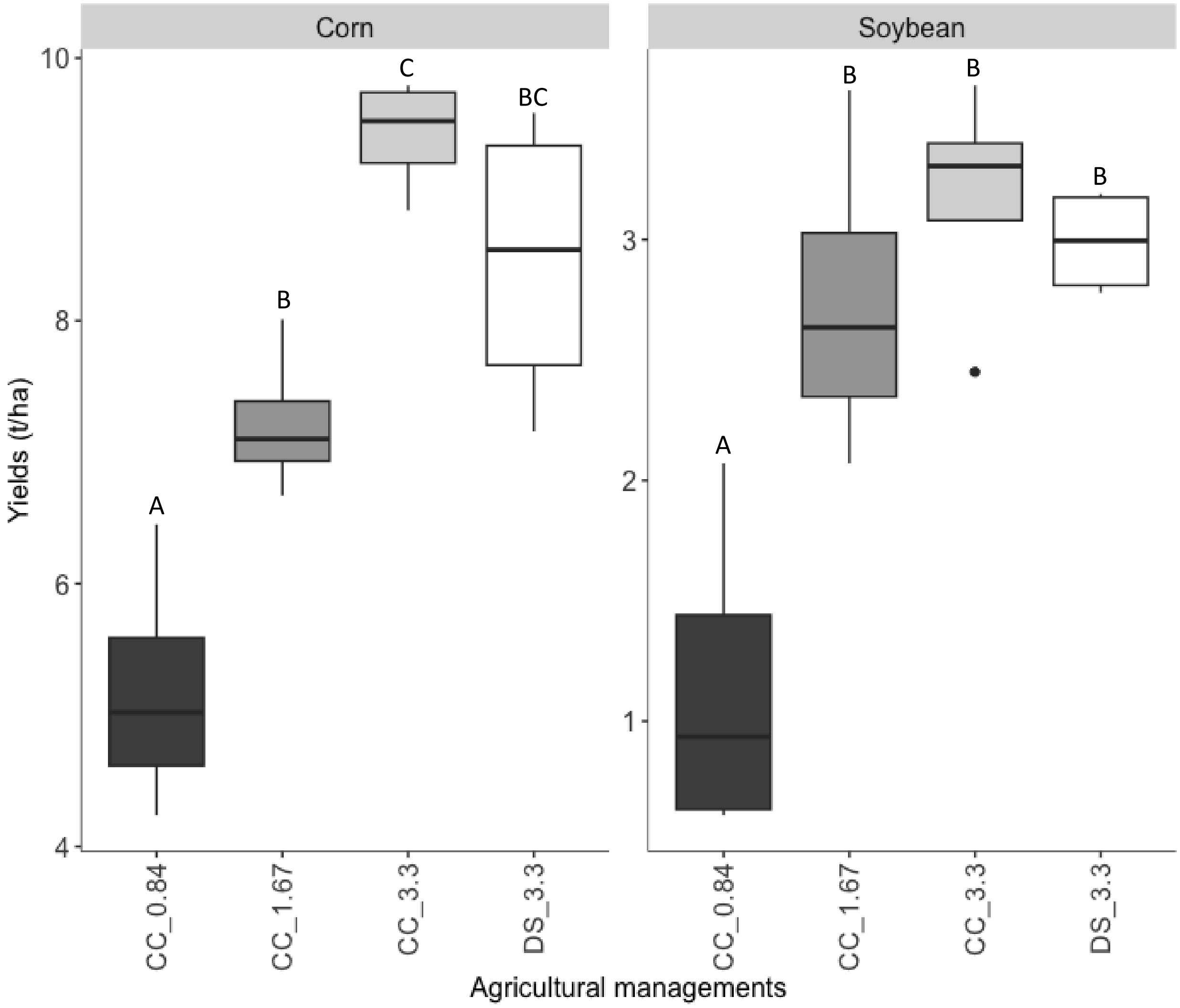


Figure 2.1 Box plots of corn and soybean yields for each agricultural management. DS = direct seeding, CC = cover crops, CC_0.84 = DS+CC with a 0.84 L/ha glyphosate-based herbicide (GBH) dose, CC_1.67 = DS+CC with a 1.67 L/ha GBH dose, CC_3.3 = DS+CC with a 3.3 L/ha GBH dose and DS_3.3 = DS with a 3.3 L/ha GBH dose. For each box plot, n = 4. Box plots represent the 25 and 75 % quantiles, the horizontal line inside is the median and the circles (•) represent outliers. The whiskers represent the minimum and maximum values. Yields significant differences were tested with the ANOVA test (*p*-value < 0.05) and displayed with the letters A, B and C.

Soybean yields ranged between 0.61 and 3.64 t/ha, while the mean yield for this region that year was 3.41 t/ha (PGQ, 2023). As observed for corn plots, soybean yields were significantly lower in CC_0.84 plots in comparison to plots employing other AM. However, soybean yields were similar between the three other AM (CC_1.67, CC_3.3 and DS_3.3) contrary to what was observed for corn plots (Figure 2.1).

Wheat yields ranged between 0.34 and 1.96 t/ha, while the mean yield for this region that year was 2.58 t/ha (PGQ, 2023). No significant wheat yields difference was observed between the AM legacy (Figure 2.2). The term legacy refers to the fact that wheat plots received a uniform application of herbicide other than GBH, therefore, distinction between GBH doses refers to the influence from the previous two seasons.

2.3.2 Soil elementary composition contents according to yields, glyphosate and AMPA contents

Means of soil elementary composition contents (P, K, Ca, Mg, Al, Cu, Fe, Mn and Zn) at C2 are presented in Table 2.2. No significant correlation was observed between corn or soybean yields and any of the soil elementary composition contents measured at C2 (Table 2.3). Soil elementary composition was not measured in wheat plots.

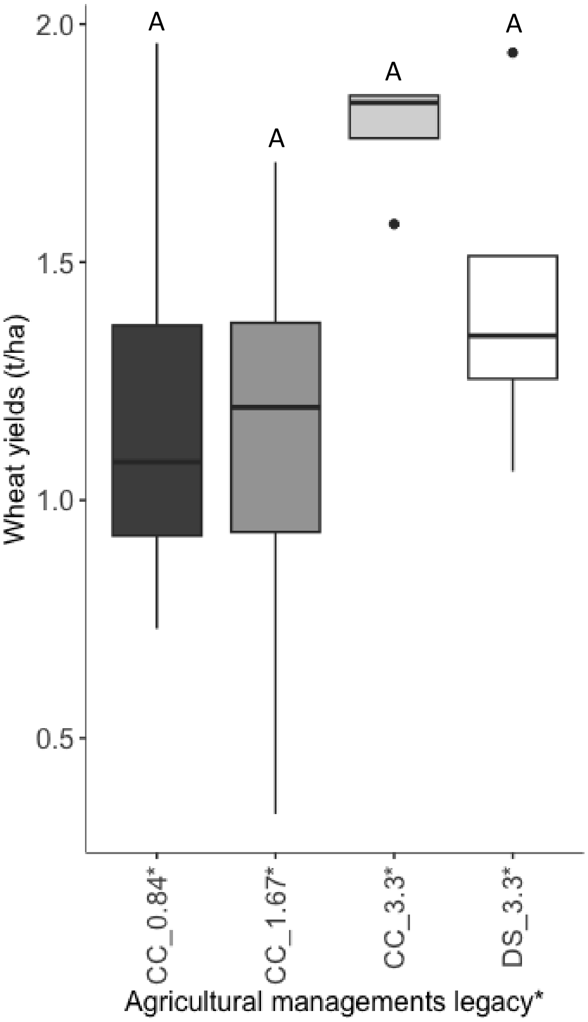


Figure 2.2 Box plots of wheat yields for each agricultural management legacy. DS = direct seeding, CC = cover crops, CC_0.84 = DS+CC with a 0.84 L/ha glyphosate-based herbicide (GBH) dose, CC_1.67 = DS+CC with a 1.67 L/ha GBH dose, CC_3.3 = DS+CC with a 3.3 L/ha GBH dose and DS_3.3 = DS with a 3.3 L/ha GBH dose. For each box plot, n = 4. Box plots represent the 25 and 75 % quantiles, the horizontal line inside is the median and the circles (•) represent outliers. The whiskers represent the minimum and maximum values. Yields significant differences were tested with the ANOVA test (*p*-value < 0.05). The same letter means that no significant differences were observed.

*Wheat plots did not receive GBH application (Table 2.1). However, GBH was applied in those plots for the past two years. The GBH dose distinction refers to their legacy impact on wheat yields.

Table 2.2 Means of the soil elementary composition contents (µg/g) in the soil first 20 cm measured at the sampling campaign 2

Crop	P	K	Ca	Mg	Al	Cu	Fe	Mn	Zn
Corn	11.50	325.62	2775.50	761.12	1040.88	10.88	217.75	20.07	2.32
Soybean	10.96	330.38	2785.12	780.75	1035.25	10.84	218.12	21.30	2.36

Table 2.3 Non-parametrical Spearman correlations between yields and soil elementary composition contents, plants cover rates and glyphosate and AMPA soil contents

	Corn		Soybean		Wheat	
Elements	*ρ*	P	*ρ*	P	*ρ*	P
P	0.60	0.13	0.22	0.61		
K	0.63	0.09	-0.09	0.83		
Ca	0.14	0.75	0.17	0.69		
Mg	-0.29	0.50	0.19	0.65		
Al	0.43	0.30	-0.35	0.40		
Cu	0.64	0.10	-0.34	0.40		
Fe	0.31	0.46	0.23	0.59		
Mn	0.21	0.62	-0.01	0.98		
Zn	0.40	0.33	-0.11	0.80		
Covers						
Weeds	-0.42	0.11	-0.80	0.0002*	-0.60	0.02*
Cash crops	-	-	0.81	0.0004*	0.84	0.00005*
Contents						
Glyphosate	-0.09	0.75	0.52	0.04*	0.44	0.09
AMPA	-0.23	0.39	0.04	0.89	0.45	0.20

Notes: * P, *p*-value < 0.05. *ρ*, rho coefficient.

A negative correlation (*p*-value = 0.04, rho coefficient = -0.53) was observed between glyphosate soil contents and calcium (Ca) ones (Figure 2.3). No other significant correlation was observed between glyphosate contents and the other soil elementary composition contents. Also, AMPA contents was positively correlated with phosphorus (P) contents (*p*-value = 0.03, rho coefficient = 0.55) (Figure 2.4). No other significant correlation was observed between AMPA contents and the other soil elementary composition contents.

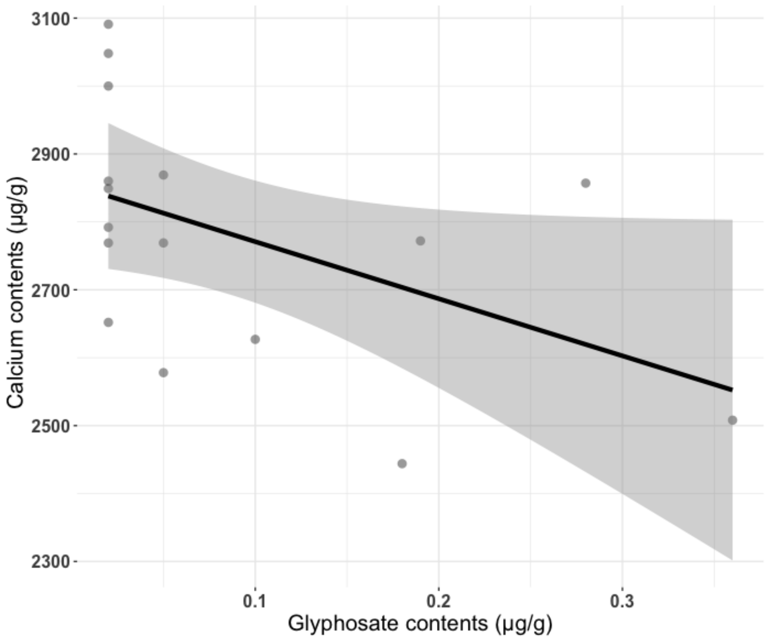


Figure 2.3 Correlation between glyphosate and calcium contents (μg) in the soil first 20 cm of soybean and corn plots (n = 16) measured at the sampling campaign 2. Spearman's rho coefficient = -0.53, *p*-value = 0.04

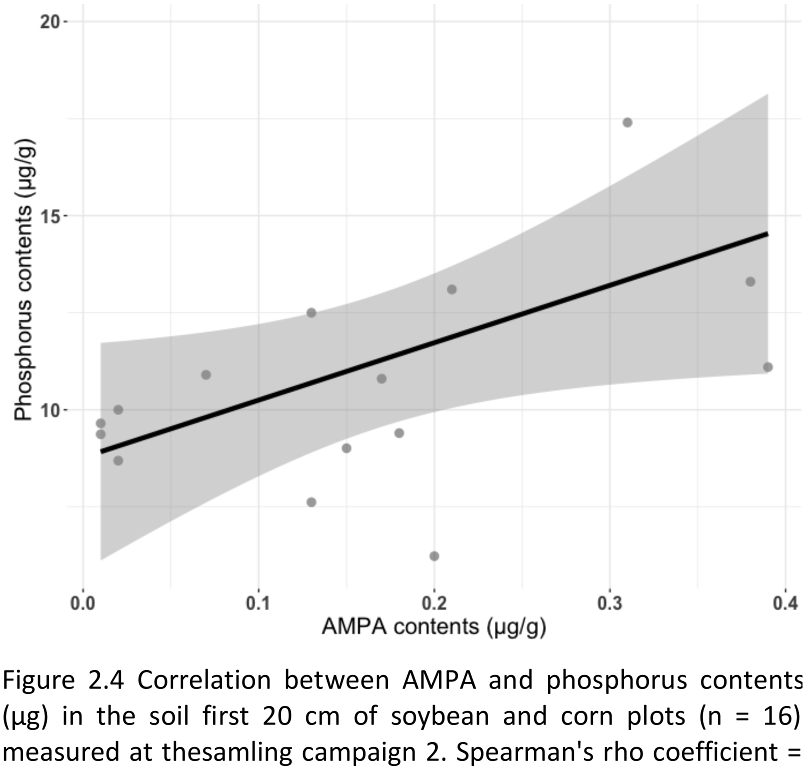


Figure 2.4 Correlation between AMPA and phosphorus contents (μg) in the soil first 20 cm of soybean and corn plots (n = 16) measured at thesamling campaign 2. Spearman's rho coefficient = 0.55, *p*-value = 0.03

2.3.3 Plants cover rates influence on yields

Means and standard deviations for weeds and cash crop cover rates of each crop are presented in Table 2.4. Soybean and wheat yields were negatively correlated with weeds cover rates, while no correlation between corn yields and weed cover rates were observed (Table 2.3). Cash crops cover rates had a positive correlation with soybean and wheat yields (Table 2.3). Corn cover rates had a mean of 2.56% at C2 (Table 2.4), so no correlation was made between corn cover rates and yields. Lastly, although perennial plants, CC sowed in 2019 did not survive the winter, therefore, correlations between CC cover rates measured at C2 and yields were not tested.

Table 2.4 Means and standard deviations (n = 16) of the plants cover rates (%) measured at the sampling campaign 2

Crop	Weeds	Cash crop
Corn	13.69 ± 5.69	2.56 ± 1.67
Soybean	13.19 ± 9.61	29.06 ± 15.60
Wheat	6.38 ± 6.23	31.62 ± 13.57

2.3.4 Glyphosate and AMPA contents impact on yields

Means and standard deviations of glyphosate and AMPA contents are presented in Table 2.5. Soybean yields were positively correlated with glyphosate soil contents (Table 2.3). No other significant correlation was observed between glyphosate or AMPA contents and yields.

Table 2.5 Means and standard deviations (n = 16) of glyphosate and AMPA soil contents (µg/g) in the soil first 20 cm measured at the sampling campaign 2

Crop	Glyphosate	AMPA
Corn	0.18 ± 0.12	0.09 ± 0.11
Soybean	0.15 ± 0.12	0.08 ± 0.06
Wheat	0.13 ± 0.10	0.09 ± 0.10

2.4 Discussion

2.4.1 Corn, soybean and wheat yields after three seasons using four distinct AM

After three growing seasons (2018-19-20), both corn and soybean yields are significantly lower with CC_0.84 than with the other AM (CC_1.67, CC_3.3 and DS_3.3) (Figure 2.1). These results are in agreement with Bärwald Bohm *et al.* (2014), who also observed lower yield when weeds were not properly controlled, and reaffirm the necessity of efficiently controlling weeds in order to favor high yields. In our case, a 0.84 L/ha GBH dose, applied in two times, is not sufficient to obtained high yields (Figure 2.1). While two GBH applications, rather than one, is usually considered more efficient to control weeds (Gower *et al.*, 2002; Swanton *et al.*, 2000), a single application of the lowest dose, 0.84 L/ha, when well-timed, can be preferable to control weeds. Timing is critical for weed control and an adequate timing of application could provide efficient weed control even with a lower dose (Mulugeta et Boerboom, 2000). In our case, agronomists determined the appropriate time for the second dose. However, caution needs to be taken with the use of low GBH application doses, as it can enhance the emergence of glyphosate resistance in weeds (Sammons et Gaines, 2014). In this study, soybean yields are negatively correlated with weeds cover rates measured at C2 (Table 2.3). GBH is used to supress weeds competing with cash crops for light, water and nutrients (Soltani *et al.*, 2017; van der Meulen et Chauhan, 2017). Those results suggest that the lowest GBH doses applied is not enough to reduce weeds to a point where the cash crop growth is optimized. However, weeds cover rates measured at C2 are not correlated with corn yields, which does not follow Birendra *et al.* (2013), Nurse *et al.* (2007) and Yeganehpoor *et al.* (2015) who have shown that a lower weeds presence enhances corn yields. However, our study was based on a three year crop rotation, consequently, it would follow Singh, J. *et al.* (2021) observations that a crop rotation can increased corn yields and Smith et Gross (2006) observations that corn yields could remain high even with higher presence of weeds but with crop rotation. Indeed, crop rotation enhances weeds diversity, which decreases their impact on cash crop by reducing their ecological interactions (Smith et Gross, 2006). Adeux *et al.* (2019) argued that not all weed species have a negative impact on crop yields and that enhanced weed diversity could reduce the abundance and probability of competitive species.

In this study's experimental field, corn and soybean yields obtained with the 1.67 L/ha GBH dose (CC_1.67) are not different from those obtained with the 3.3 L/ha GBH dose without CC (DS_3.3) (Figure 2.1). This suggests that in 2020, a 1.67 L/ha GBH dose was enough to control weeds and sustain high yields, which could be attributed to CC benefits. This observation for corn yields is congruent with Smedbol *et al.* (2020) and Samson-Brais *et al.* (2022) who also reported no corn yields difference between a 3.3 L/ha GBH dose and a 1.67 L/ha one. However for soybean yields, Smedbol *et al.* (2020) and Samson-Brais *et al.* (2022) reported significant lower yields with 1.67 L/ha dose as compared to 3.3 L/ha GBH one. Those two studies were done in the same experimental station as the present one, but not the same year. They were also done over only one year, without a crop rotation and without CC. Therefore, it is possible that, in our case, the AM used over three seasons including CC allows maintaining high soybean and corn yields with a 1.67 L/ha GBH dose instead of the 3.3 L/ha one. Those observations go along with Cholette *et al.* (2018) and Sabbagh *et al.* (2020) studies for North American field crop, which suggest that CC may have the capacity to reduce weeds presence while maintaining high corn and soybean yields, suggesting a potential reduction of GBH usage.

As no GBH application was done for wheat during the growing season, it is the AM legacy impact of the past seasons that could have influenced wheat yields. However, after two seasons (2018-19), no significant difference is observed between the wheat yields and AM employed (Figure 2.2). Thus, the poorer weeds control achieved with the 0.84 L/ha dose in the past years did not adversely impact wheat yields in the following year. Finally, it must be considered that, in this experiment, wheat crop was not optimal, the highest wheat yield obtained (1.96 t/ha) being lower than the mean wheat yield (2.58 t/ha) from this region that year (PGQ, 2023).

2.4.2 Cover crops influence on yields

In this experiment, CC sown in 2019 (Table 2.1), although perennial plants, did not survive the winter and consequently no CC covers were observed at C2 in 2020. The 2019-2020 winter climate conditions were harsh and a thick layer of ice provoked anoxic conditions, which has lead to the death of the CC. As observed in this study and as noted by Lapierre *et al.* (2022), in a northern climate, the short growing season and the winter are a challenge for the full establishment of CC. As CC benefits are mostly dependent on their establishment and biomass, their absence or weak presence will jeopardize their potential impacts (Wittwer *et al.*, 2017). However, since CC have been sown since 2018 in this experiment (Table 2.1), their impact on soil properties and functions could have influenced cash crops in 2020 after having been established for two years. As such, despite that the following studies did not use the same CC species as us, we can still note that Langeroodi *et al.* (2019) have pointed out that CC residues can impact weeds establishment and Lapierre *et al.* (2022) have reported that CC present in the fall can alter soil temperature during winter and, consequently, impact soil properties of the following year. The roots system of CC could also have benefited the cash crops by favoring water infiltration and reducing erosion and compaction

(Lucas *et al.*, 2018; Pott *et al.*, 2020; Scopel *et al.*, 2013). Consequently, the fact that yields obtained with the 1.67 L/ha GBH dose were similar to 3.3 L/ha GBH dose (Figure 2.1) could partially be attributed to CC beneficial impacts. Benefits from a change in AM may take time to show concrete impacts. For example, Bacq‑Labreuil *et al.* (2021) who studied structural soil changes following a change of AM, only found one parameter change after two years, while the other ones took approximately ten years. Hill *et al.* (2021) have pointed out that perennial CC beneficial effects on soil health could take more than five years. Therefore, AM impacts analyzed in this study might become more significant over the years. Lastly, implementing CC without affecting yields, as seen in this study and in Singh, J. *et al.* (2021)'s one is highly encouraging considering the ecological benefits that CC may offer.

2.4.3 Soil elementary composition contents

2.4.3.1 Effects of soil elementary composition contents on yields

The link between yields and soil elementary contents (P, K, Ca, Mg, Al, Cu, Fe, Mn or Zn) is well established (Eker *et al.*, 2006; Johal et Huber, 2009; Kanissery *et al.*, 2019). In our study, yields differences could not be linked to any soil elementary content (Table 2.3). Considering that glyphosate and AMPA can form complex with those elements in soils and, consequently, affect their bioavailability and transport (Barrett et McBride, 2006; Duke *et al.*, 2012; Sidoli *et al.*, 2016), our results indicate that the various AM tested have allowed adequate soil elements contents and bioavailability for the crops development, which has also been observed by Reddy *et al.* (2018).

2.4.3.2 Effects of glyphosate and AMPA soil contents on soil elementary composition contents

In this experiment, most soil elementary contents are not correlated with glyphosate or AMPA contents, which is congruent with other studies (Moreira *et al.*, 2016; Reddy *et al.*, 2018). Nonetheless, there are two significant correlations observed. The positive correlation between AMPA and phosphorus (Figure 2.4), which is mainly found in its phosphate form in soils, could be explained by the fact that, in soil, phosphorus can induce the remobilization of bound glyphosate by taking its place at binding sites, making it readily available to be degraded in AMPA (Bott *et al.*, 2011; Grandcoin *et al.*, 2017; Mertens *et al.*, 2018). Also, Zhan *et al.* (2018) have pointed out that, although AMPA is difficult to access by soil microorganisms due to its strong adsorption to the soil matrix, it still represents a potential source of phosphorus (P) for soil microorganisms and can be degraded. The presence of readily available phosphate in soil can therefore inhibit AMPA degradation as representing a more energy efficient P source. The second correlation is a negative one between glyphosate and calcium contents (Figure 2.3). Calcium was shown to form poorly soluble complex with glyphosate, which reduces its mobility and plant uptake (Bott *et al.*, 2011; Cakmak *et al.*, 2009; Mertens *et al.*, 2018). Therefore, a positive correlation between both compounds would have been expected. However, an analysis of the proportion of calcium that is complexed, which was not made in this study, could have clarified the role of that element in the glyphosate complexion. While GBH have the potential to impact soil elementary contents, in an open field study, other factors like growth conditions, soil fertility and stage of the plant development also have an impact on those contents (Bärwald Bohm *et al.*, 2014; Reddy *et al.*, 2018; Rosolem *et al.*, 2010) and could have also influenced our observations. It was expected that a lower elementary bioavailability would result in a lesser plant uptake of those elements, consequently transposing to a higher soil element contents (Sebiomo *et al.*, 2012). However, Barrett et McBride (2006) have shown that the adsorption of glyphosate on soil elements could enhance its mobility potential. Consequently, when looking only at the soil first 20 cm, a higher mobility could have dissimulated the GBH impact on soil elements bioavailability.

2.4.4 Glyphosate and AMPA soil contents impact on yields

Glyphosate and AMPA can also affect soil microorganisms (Andréa *et al.*, 2003; Lancaster *et al.*, 2010; Samson-Brais *et al.*, 2021) and plants physiology (Reddy *et al.*, 2004; Smedbol *et al.*, 2019), which could ultimately impact crop yields. However, our results do not show a negative correlation between yields and glyphosate or AMPA soil contents (Table 2.3). Consequently, these results suggest that glyphosate and AMPA contents measured at C2 were not high enough to adversely affect yields. As a matter of fact, physiological plant damage may not lead to a yield decrease (Duke *et al.*, 2012). Furthermore, Schlatter *et al.* (2017), Guijarro *et al.* (2018) and Bärwald Bohm *et al.* (2014), with a yearly GBH application of up to 2x 960 g a.i. ha^{-1}, which is similar to our 3.3 L/ha dose, found minimal or no GBH impact on soil microorganisms.

2.5 Conclusion

Our study highlights that a 1.67 L/ha (902 g a.i./ha) annual dose coupled to the implementation of CC and crop rotation leads to high yields, similar to those obtained along with the3.3 L/ha (1782 g a.i./ha) GBH annual dose with or without using CC. A possible decrease in GBH application doses when CC are implemented is a great observation. On the other hand, a 0.84 L/ha (454 g a.i./ha) GBH annual dose is insufficient to efficiently control weeds in field crop and consequently affects corn and soybean yields, showing that the GBH application dose cannot be reduced too much. We argue that, in our case, proper

AM based on CC and crop rotation for several years (three growing years in our study) may contribute to maintain high yields while significantly limiting the usage of GBH and drawbacks associated to it. This study also showed that soil elementary composition, which can be linked to crop nutrition, is not correlated with yields, which suggests that the AM used in this study did not affect plant nutrient uptake. As impacts of different AM may take several years to be perceptible, a longer monitoring could provide a better understatement of the AM potential impacts. Since their introduction to the field crop domain, GBH have contributed to a considerable boost in crop yields and a reduction in production cost. But, nowadays, the sustainability of that cropping systems may be threatened by multiple potential adverse impacts of GBH. High yields could thus be maintained over time by adopting agricultural practices based on CC and crop rotation while limiting GBH usage. There is however an implementation plan and choice of species that need to be developed for every region.

CONCLUSION GÉNÉRALE

Le but de cette étude a été d'évaluer l'impact de différentes pratiques agricoles sur la dynamique de dissipation du glyphosate et de l'AMPA ainsi que sur les rendements dans un contexte de grandes cultures au Québec. L'influence de trois doses d'HBG, de la présence ou l'absence de CC, des teneurs de la composition élémentaire du sol, du glyphosate et de l'AMPA et des taux de recouvrement des plantes ont été analysés.

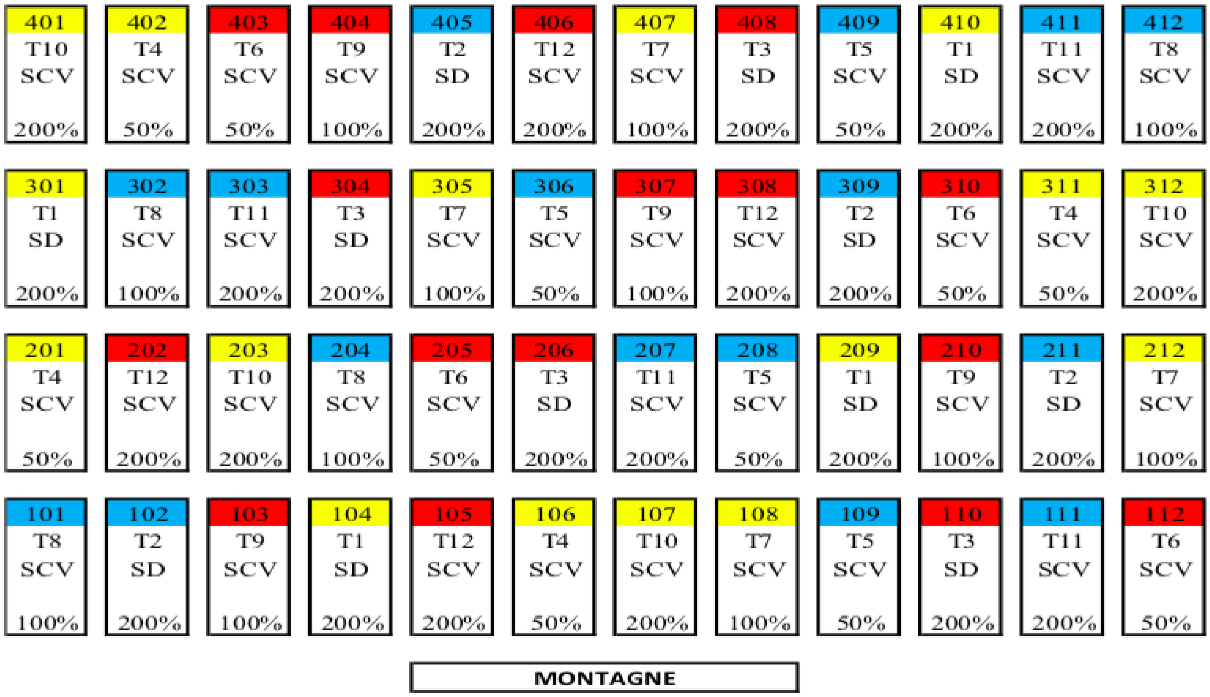
Le Chapitre 1 a permis d'évaluer les tendances de dissipation des teneurs de glyphosate et d'AMPA au courant d'une saison et également sur une perspective de quatre années. La comparaison des teneurs aux différentes campagnes d'échantillonnage entre les régies de cultures testées, soit trois doses d'HBG et la présence de CC, a permis de constater que celles-ci n'ont pas impacté les teneurs de glyphosate ou d'AMPA de la première couche de sol, et ce, pour aucune des campagnes d'échantillonnage d'aucune des cultures (maïs, soya ou blé) en rotation sur trois ans. Cette observation suggère que des teneurs relativement similaires, indépendamment de la dose d'HBG appliquée, persistent dans la première couche de sol, alors qu'une grande proportion de celle-ci semble se dissiper dans l'environnement. Également, bien qu'une accumulation de ces molécules au fil des saisons n'ait pas été observée, une relative constance des teneurs de glyphosate et d'AMPA, au fil des campagnes, a été observée. La dynamique des tendances des teneurs de ces composés au courant d'une saison et sur la durée totale de l'étude suggère la pseudo-persistance de ces composés pour toutes les régies de culture testées. Dans un milieu ouvert, de nombreux facteurs vont influencer la dynamique de dissipation du glyphosate. Approfondir les connaissances par rapport à la dynamique de dissipation du glyphosate pourrait permettre de limiter la pollution diffuse associée aux HBG, d'optimiser et de réduire leur utilisation et, ainsi, limiter leurs impacts indésirables.

Le Chapitre 2 a permis d'analyser l'impact des différentes régies de culture sur les rendements. Les teneurs de la composition élémentaire du sol, du glyphosate et de l'AMPA ainsi que les taux de recouvrement des plantes ont également été mis en lien avec les rendements des différentes cultures. Ce chapitre a démontré la nécessité d'utiliser une dose d'HBG suffisamment élevée pour permettre un contrôle efficace des adventices afin d'obtenir des rendements compétitifs. De plus, dans cette expérience, la dose d'application intermédiaire avec CC a présenté des rendements similaires à la dose la plus élevée sans CC, dose couramment utilisée dans les champs québécois. Cette observation, qui pourrait être attribuée à l'utilisation du SD, des CC et d'une rotation des cultures, est encourageante dans une perspective de réduction de l'utilisation des intrants chimiques dans les pratiques agricoles. Elle concorde également avec le plan d’agriculture durable 2020-2030 du gouvernement du Québec (PAD) (MAPAQ, 2020) qui reconnait, entre autres, la nécessité d’améliorer la santé et la conservation des sols ainsi que la réduction de l'utilisation des intrants chimiques. Par ailleurs, les teneurs de la composition élémentaire du sol n'ont présenté aucune corrélation avec les rendements, alors que les teneurs en glyphosate ou AMPA n'ont pas eu d'effet néfaste sur les rendements. Développer les connaissances sur les pratiques culturales permettant de réduire l'impact des grandes cultures sur leur environnement est essentiel pour contribuer à la pérennité des grandes cultures.

La richesse de cette étude provient de son design expérimental ayant permis de suivre des parcelles dans un milieu ouvert et sur un cycle complet de rotation de cultures (maïs, soya, blé). Les deux chapitres qui en découlent ont permis l'étude de l'impact de quatre régies de culture sur la dynamique de dissipation du glyphosate dans la première couche de sol ainsi que sur les rendements, et ce, dans un contexte de grandes cultures en Montérégie, région à forte vocation agricole. Les aspects ayant été étudiés ont une importance considérable pour la pérennité des grandes cultures. L'étude complémentaire de la profondeur 20-40 cm serait grandement pertinente pour enrichir les résultats présentés dans ce mémoire. Mieux comprendre l'herbicide le plus utilisé au monde et élaborer des alternatives permettant de réduire ses impacts tout en maintenant des rendements compétitifs est essentiel. Les changements climatiques entraineront des conditions nécessitant d'adapter les pratiques culturales afin de rendre les cultures plus résilientes.

ANNEXE A

Design expérimental du champ au CEROM



🟨 401 T10 SCV 200%	🟨 402 T4 SCV 50%	🟥 403 T6 SCV 50%	🟥 404 T9 SCV 100%	🟦 405 T2 SD 200%	🟥 406 T12 SCV 200%	🟨 407 T7 SCV 100%	🟥 408 T3 SD 200%	🟦 409 T5 SCV 50%	🟨 410 T1 SD 200%	🟦 411 T11 SCV 200%	🟦 412 T8 SCV 100%
🟨 301 T1 SD 200%	🟦 302 T8 SCV 100%	🟦 303 T11 SCV 200%	🟥 304 T3 SD 200%	🟨 305 T7 SCV 100%	🟦 306 T5 SCV 50%	🟥 307 T9 SCV 100%	🟥 308 T12 SCV 200%	🟦 309 T2 SD 200%	🟥 310 T6 SCV 50%	🟨 311 T4 SCV 50%	🟨 312 T10 SCV 200%
🟨 201 T4 SCV 50%	🟥 202 T12 SCV 200%	🟨 203 T10 SCV 200%	🟦 204 T8 SCV 100%	🟥 205 T6 SCV 50%	🟥 206 T3 SD 200%	🟦 207 T11 SCV 200%	🟦 208 T5 SCV 50%	🟨 209 T1 SD 200%	🟥 210 T9 SCV 100%	🟦 211 T2 SD 200%	🟨 212 T7 SCV 100%
🟦 101 T8 SCV 100%	🟦 102 T2 SD 200%	🟥 103 T9 SCV 100%	🟨 104 T1 SD 200%	🟥 105 T12 SCV 200%	🟨 106 T4 SCV 50%	🟨 107 T10 SCV 200%	🟨 108 T7 SCV 100%	🟦 109 T5 SCV 50%	🟥 110 T3 SD 200%	🟦 111 T11 SCV 200%	🟥 112 T6 SCV 50%

MONTAGNE

Dimension des parcelles: 9*20m

2018	2019	2020	2021
🟨 Maïs	🟨 Soya	🟨 Blé	🟨 Maïs
🟥 Blé	🟥 Maïs	🟥 Soya	🟥 Blé
🟦 Soya	🟦 Blé	🟦 Maïs	🟦 Soya

Applications
200% = 3,3 L/ha en 2 applications
100% = 1,67 L/ha en 2 applications
50% = 0,84 L/ha en 2 applications

Coordonnées
750 ch. Trudeau, St-Mathieu-de-Beloeil
Tél: 450-464-2715

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