Transformation and transport of inorganic and synthetic pesticides in soils of agricultural catchments

Fatima Meite

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ÉCOLE DOCTORALE des Sciences de la Terre et de l'Environnement (ED 413)

Laboratoire d'Hydrologie et de Géochimie de Strasbourg (LHYGES) UMR 7517

(Université de Strasbourg / ENGEES – CNRS)

THÈSE

Présentée par :

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Discipline / Spécialité : Géochimie de l'environnement

Transformation et transport des pesticides inorganiques et de synthèse dans les sols de bassins versants agricoles

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Curriculum Vitae

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GC-C-IRMS
MC-ICP-MS
GC-MS
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Hobbies

Reading, yoga, cooking and preparation of natural organic products

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2014 - 2018 **Ph.D. thesis in environmental geochemistry**
Laboratory of Hydrology and Geochemistry of Strasbourg (LHYGES)
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2012 - 2014 **Master in aquatic systems and water management**
Ecole des Ponts et Chaussées
University Paris Diderot
Paris, FRANCE

2010 - 2012 **Bachelor's degree in chemistry**
University Paris Diderot
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2008 - 2010 **Higher national diploma in chemistry**
High school Galilée
Gennevilliers, FRANCE

Research experience

Oct. 2014 -Sept. 2018 **Ph.D. student**

Ph.D. thesis: "Transformation and transport of synthetic and inorganic pesticides in agricultural soils" (Advisor: Dr. Gwenaël Imfeld)
Laboratory of Hydrology and Geochemistry of Strasbourg (LHYGES), UMR 7517 CNRS/ENGEES, University of Strasbourg, FRANCE
Laboratory experimentations:

- Assessing the dissipation and (bio)degradation of synthetic pesticides in soils exposed to several temperatures, water contents and microbial activities (GC-MS, GC-C-IRMS)
- Temporal monitoring of synthetic pesticides and metals speciation among the grain-size fractions of soils and the chemical fractions of soils (GC-MS, GC-C-IRMS, ICP-AES, MC-ICP-MS)
- Assessing of synthetic pesticides and metals transport via runoff and leaching waters from soils exposed to rainfalls with changing characteristics (SPE, GC-MS, ICP-AES)

Field experimentations: assessing pesticides and metals cycles in a vineyard catchment (SPE, GC-MS, GC-C-IRMS, ICP-AES) - 2015 and 2016 agricultural seasons

- Monitoring of the concentration of pollutants in soils and synthetic pesticides (bio)degradation
- Monitoring of particulate and dissolved export of pollutants in runoff and leaching waters

Mars - Aug. 2014 **Master internship**

Internship thesis: "Copper speciation and isotope fractionation along biogeochemical gradients formed in the Winogradsky columns" (Advisors: Dr. Gwenaël Imfeld, PhD student Izabella Babcsányi)
Laboratory of Hydrology and Geochemistry of Strasbourg (LHYGES), UMR 7517 CNRS/ENGEES, University of Strasbourg, FRANCE
Evaluation of the potential of Cu isotope analysis to understand Cu fate and the processes undergone by Cu in sediments and other wetlands with high redox gradients:

- Physico-chemical and microbial characterizations of supernatant waters sampling in Winogradsky columns made with sediments coming from a vineyard catchment

- Core sampling of the Winogradsky columns, realization of sequential chemical extraction and purification via chromatography and resin columns of Cu
- Cu analysis and measurement of Cu isotope fractionation (ICP-OES, MC-ICP-MS)

April - Aug. 2012

Bachelor's degree internship
Internship thesis: "Study of the French technical norm NF T 90 210 and characterization of Kjeldahl nitrogen and total phosphorus in water treatment plant sludge with sequential analysis" (Advisor: Laure Menjou)
SPAF DDP Laboratory (Direction of development and prospective laboratory of Public water treatment of Paris urban area, previously SIAAP), Colombes, FRANCE

- Development of a solid matrix pre-treatment procedure
- Assessment of quantification limits, yields and interferences
- Assessment of exactitude and uncertainties (norm NF T 90 220)

June - July 2009

Higher national diploma internship
Internship thesis: "Synthesis of DNA methyl-transferase inhibitor" (Advisor: Dr. Ludovic Halby)
CNAM Laboratory of chemical and pharmaceutical transformations, Paris, FRANCE

- Synthesis and purification of DNA methyl-transferase inhibitor by preparative HPLC
- Mass and NMR spectroscopic analyses

Publications and conferences

Meite F., Wiegert C., Guyot B., Imfeld G. Effect of soil moisture and temperature on the dissipation of a pesticide mixture. Chemosphere, *in preparation*

Meite F., Wiegert C., Granet M., Imfeld G. Aging of pesticides, Cu and Zn in agricultural soils: insights from CSIA of pesticides, Cu and Zn stable isotopes. Chemosphere, *in preparation*

Meite F., Guyot B., Payraudeau S., M., Imfeld G. Impact of rainfall patterns on the exports of Cu, Zn and polar pesticides from a vineyard catchment. Chemosphere, *in preparation*

Meite F., Alvarez-Zaldívar P., Crochet A., Wiegert C., Payraudeau S., Imfeld G., 2018. Impact of rainfall patterns and frequency on the export of pesticides and heavy-metals from agricultural soils. Sci. Total Environ. 616-617, 500–509

Alvarez-Zaldivar P., Payraudeau S., **Meite F.**, Masbou J., Imfeld G., 2018. Pesticide degradation and export losses at the catchment scale: insights from compound-specific isotope analysis (CSIA). Water Research, *accepted*

Masbou J., **Meite F.**, Guyot B., Imfeld G., 2018. Enantiomer-specific stable carbon isotope analysis (ESIA) to evaluate degradation of the chiral fungicide Metalaxyl in soils. Hazardous Material, *accepted*

Babcsányi I., **Meite F.**, Imfeld G., 2017. Biogeochemical gradients and microbial communities in Winogradsky columns established with polluted wetland sediments. FEMS Microbiology Ecology 93 (8): fix089

Babcsányi I., Chabaux F., Granet M., **Meite F.**, Payraudeau S., Duplay J., Imfeld G., 2016. Copper in soil fractions and runoff in a vineyard catchment: Insights from copper stable isotopes. Sci. Total Environ. 557–558, 154–162

Meite F., Wiegert C., Alvarez-Zaldivar P., Imfeld G., Compound-specific isotope analysis (CSIA) to evaluate the effect of temperature and soil water content on pesticides degradation in agricultural soils. JESIUM, 4-9 September 2016, Ghent, Belgium / Poster

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Chapitre 1 : Introduction

Le premier chapitre de ce manuscrit informe sur les problématiques liées à l'utilisation de pesticides de synthèse et inorganiques. Ainsi, le devenir et la spéciation de ces polluants dans les sols agricoles sont décrits, suivi d'un état de l'art présentant les facteurs contrôlant la transformation et la mobilité des pesticides en contexte agricole, mais aussi les méthodes et approches permettant d'étudier ces points. Ensuite, les impacts : i) des pesticides sur les cycles biogéochimiques, et ii) des pratiques agricoles sur la transformation et la mobilité de ces polluants seront présentés En fin de ce chapitre seront présentés les verrous scientifiques qui persistent, les questions de recherches et objectifs de ce travail de thèse.

1. Pesticides de synthèse et pesticides inorganiques : définition et problématique

Les pesticides, ou produits phytosanitaires, regroupent un ensemble de molécules inorganiques et de synthèse utilisées pour prévenir et lutter contre la présence d'adventices, d'insectes, ou de champignons. Les pesticides visent en général à : i) permettre la protection de plantes d'intérêt contre les maladies induites par des agents phytopathogènes, avant ou après leur récolte, ii) influencer significativement leur développement, ou iii) inhiber ou prévenir le développement de plantes indésirables ou de certains tissus végétaux constitutifs de l'espèce cultivée. Il existe plusieurs catégories de pesticides suivant leurs usages (Kelessidis and Stasinakis, 2012; Komárek et al., 2010; Urbaniak et al., 2016). Ces produits sont appliqués de façon régulière sur de nombreuses cultures de céréales, de fruits et de légumes mais également dans un cadre paysager lors de la culture de fleurs et plantes décoratives en milieu urbain.

Divers produits d'origine naturelle et minérale ont été utilisés avant l'apparition de pesticides de synthèse pour prévenir et lutter contre la présence d'insectes et de champignons, comme souligné notamment dans la Figure 1-1.

Figure 1-1 : Héritage historique de l'utilisation des pesticides (d'après Wiegert et Imfeld)

En effet, en agriculture étaient utilisés jusqu'au 4ème siècle différents sels minéraux et notamment des produits à base de soufre (S), de mercure (Hg), d'arsenic (As), mais aussi des graisses d'animaux (i.e. chats, oiseaux), des cendres, de la craie ou encore des fumigations faites en brulant des excréments d'animaux (i.e. gazelles) ou du soufre (Banaszkiewicz, 2010). Jusqu'au milieu des années 1880 des décoctions de feuilles de tabac, des extraits de fleurs de plantes, du chlorure de mercure et différentes chaux possiblement additionnées de sulfate de Cu (début de la bouillie Bordelaise) étaient principalement utilisés (Banaszkiewicz, 2010). Dès 1892 et la synthèse du premier herbicide de synthèse (le dinitro-ortho-cresol, DNOC), commença l'ère des pesticides de synthèse. Ont suivi alors l'apparition de différentes classes de fongicides, insecticides et herbicides avec pour commencer le fongicide Tiram en 1934. Puis ont alors été mis sur le marché les composés hydrocarbonés et polychlorés (1940s), le premier insecticide de synthèse phosphoré (Parathion, 1943), les herbicides 2,4-D (acide 2,4-dichlorophénoxyacétique, 1944) et MCPA (acide 2-méthyl-4-chlorophénoxyacétique, 1947), ou encore le DDT (dichlorodiphenyltrichloroethane, dont les propriétés insecticides ont été découvertes dans les années 1940). Des produits comme le Carbaryl (premier insecticide de la famille des carbarine, 1950), l'Atrazine (1957), ou encore le Glyphosate (1974) ont alors été synthétisés (Banaszkiewicz, 2010).

Ces composés appartiennent à différentes classes de pesticides. Ces classes se sont succédées en raison du retrait de leurs composés par les autorités suite aux problèmes environnementaux et toxicologiques posés par les pesticides mis sur le marché (Banaszkiewicz, 2010; Chauvel et al., 2012; Moreau, 1992). La Figure 1-2 illustrent le renouvellement des pesticides sur le marché.

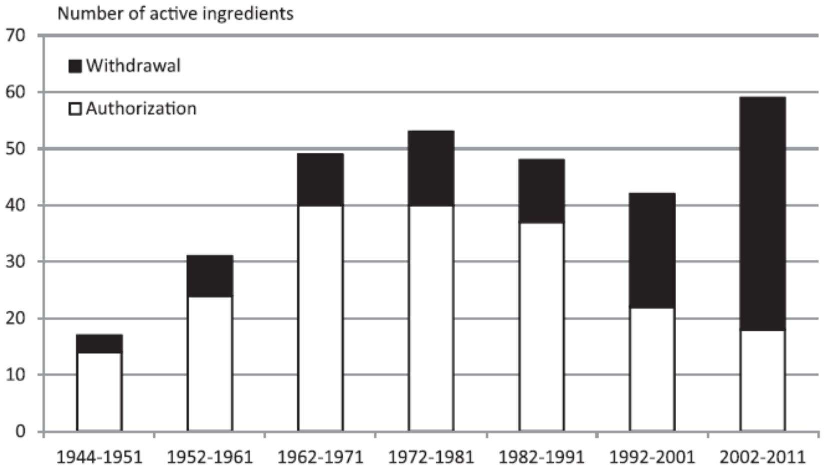


Figure 1-2 : Ingrédients actifs autorisés (carrés blancs) et retirés (carrés noirs) en France entre 1944 et 2011 (Chauvel et al., 2012)

Les premières générations de pesticides présentaient des effets néfastes pour la santé humaine et étaient également responsable de la baisse de diversité et de qualité des milieux aquatiques et terrestres (Chauvel et al., 2012). Le DNOC présentait une forte toxicité envers la faune. L'atrazine présentait une forte toxicité envers les organismes aquatiques, mais aussi des effets sur le système hormonal. Le DDT quant à lui s'accumulait dans la chaîne alimentaire et les graisses humaines provoquant ainsi des problèmes de fertilité chez les poissons et oiseaux, et des problèmes hormonaux chez les hommes (Banaszkiewicz, 2010).

Les pesticides appliqués sur les cultures sont des formulations composées d'un ou plusieurs principes actifs mélangés à des adjuvants (composés souvent appelés inertes par l'industrie) facilitant leur application ou leur efficacité (Mesnage et al., 2014). Ces adjuvants (i.e. solvant naphte et éthoxylates d'alcool gras dans la formulation Opus) peuvent être des émulsifiants (permettent aux ingrédients non solubles dans l'eau de rester sous forme d'émulsions uniformes), des agents mouillants (améliorent l'angle de contact des ingrédients avec les surfaces cibles comme les insectes ou feuilles des végétaux), des agents collants (permettent aux formulations d'adhérer à leurs cibles), des agents stabilisants (augmentent la durée de vie des formulation au cours de leur stockage), mais aussi des surfactants, solvants et agents contre la dérive qui limitent les pertes de produits au cours de leur application sur les cultures (Plimmer, 1990).

Les principes actifs utilisés aujourd'hui sont d'origine minérale (i.e. Cu, principe actif de la bouillie Bordelaise) ou synthétique (i.e. epoxiconazole ou glyphosate, principes actifs respectifs de l'Opus et du Roundup GT+). On parle alors de pesticides inorganiques et de pesticides de synthèse. De nos jours, les principales classes de pesticides utilisées sont les herbicides, les fongicides et les insecticides (Atwood and Paisley-Jones, 2017) comme le souligne la Figure 1-3 présentant les quantités de pesticides de synthèse utilisés aux Etats-Unis en 2012.

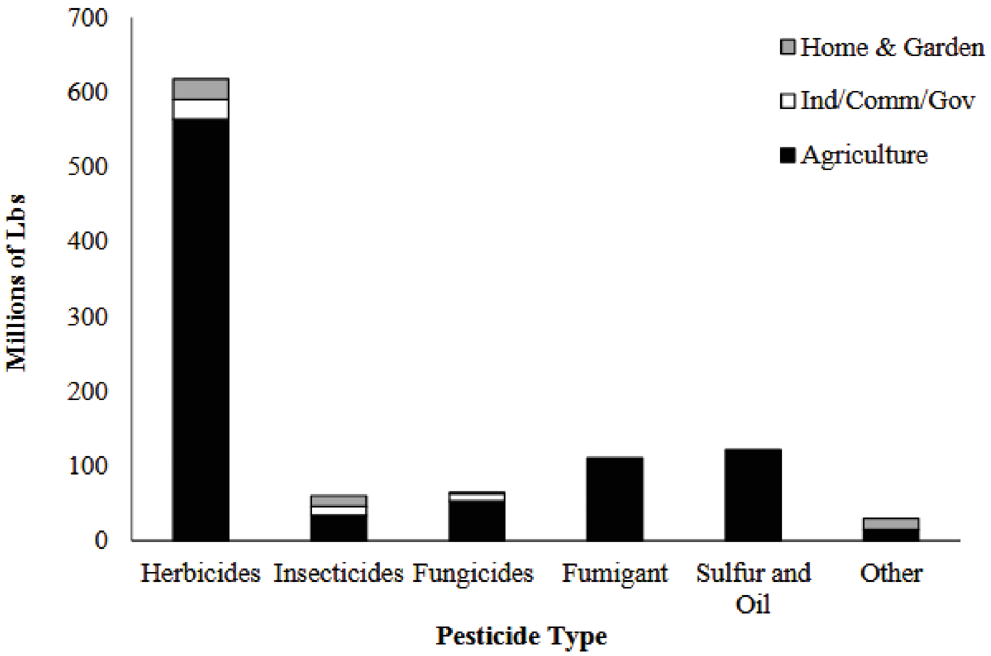


Figure 1-3 : Estimations en 2012 des usages de pesticides conventionnels aux Etats-Unis par type de pesticide et secteur marchand (Atwood and Paisley-Jones, 2017)

Les modalités d'application des pesticides sont variées en fonction de l'objectif visé : i) sur le sol pour lutter contre l'apparition d'adventice, ou ii) sur les parties aériennes des cultures : feuillage, tiges, tronc, fruits pour lutter contre les maladies, champignons ou insectes. Ces modes d'applications de pesticides conduisent à un phénomène appelé dérive qui contribue à ce qu'une proportion variable de pesticides se perde dans l'environnement sans atteindre les parties ciblées. La dérive des pesticides est définie comme le déplacement de gouttelettes, particules sèches ou pulvérisation de pesticides en dehors de la zone ciblée au cours de l'application ou immédiatement après celle-ci (Carlsen et al., 2006). Le phénomène principal est la « dérive de pulvérisation » dans lequel une partie du produit est projetée hors de la zone ciblée ou la quitte avant d'atteindre le sol. Il a été montré qu'après un traitement des cultures, la majorité des pesticides appliqués (jusque 75 %) est dispersée dans l'atmosphère (Barbash, 2014). Ces composés finissent par retomber sur les sols des cultures ou encore à proximité sur les routes comme illustré avec la Figure 1-4. La déperdition dans le sol intervient également, les produits étant notamment entraînés par les eaux de ruissellement (cf. par exemple Lefrancq et al., 2013). Ainsi, les applications de pesticides entrainent une pollution de différents compartiments environnementaux parfois très éloignés de la zone d'application.

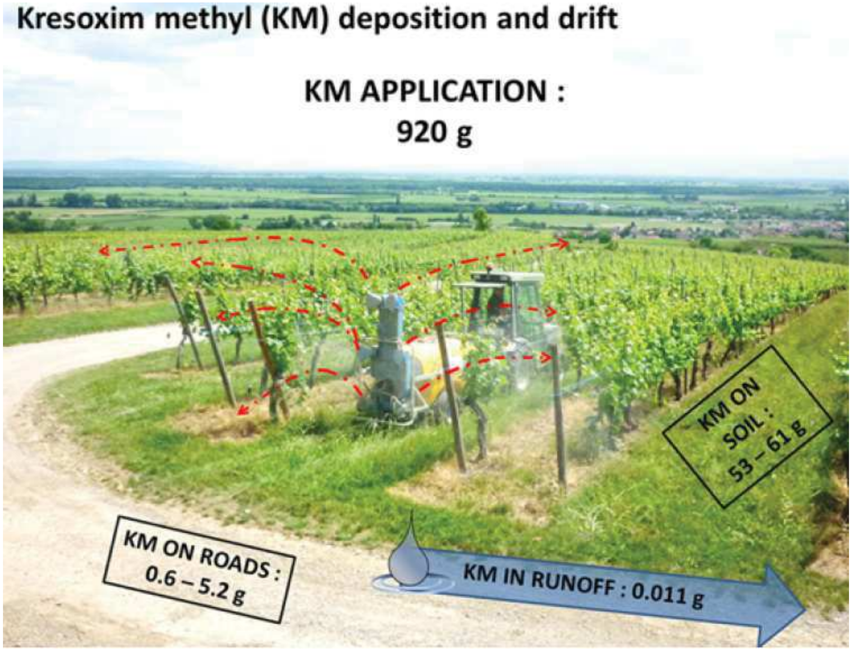


Figure 1-4 : Exemple de bilan de masse du dépôt et de la dérive (drift en anglais) du krésoxim-méthyle appliqué dans un bassin versant viticole (Lefrancq et al., 2013)

Une fois appliqués sur les sols, les pesticides subissent de nombreuses transformations, sont mobilisés ou séquestrés selon leurs propriétés physico-chimiques, mais aussi selon les propriétés du sol et les évènements climatiques. Ces produits peuvent être transportés par les eaux de ruissellement jusqu'aux milieux aquatiques menaçant alors ces écosystèmes.

Il a été montré que la présence de pesticide impacte la diversité écologique et qu'elle peut largement influencer les communautés microbiennes initialement présentes (Imfeld and Vuilleumier, 2012; Lo, 2010; Wyszkowska et al., 2012). Une étude récente montre notamment l'évolution de divers microorganismes aquatiques suite à une pollution avec un mélange d'insecticides (malathion, carbaryl, permethrin) et d'herbicides (atrazine, glyphosate) (Muturi et al., 2017). Leurs travaux montrent que les insecticides réduisent la diversité et la richesse microbienne mais aussi (suivant l'insecticide considéré) la structure des communautés microbiennes.

Les pesticides représentent également une menace pour l'être humain (Nicolopoulou-Stamati et al., 2016). Ces composés sont inhalés suite à leur présence dans l'air, mais aussi ingérés lors de la consommation d'eau contaminée. Une fraction non négligeable de pesticides se retrouve également dans les produits cultivés et entre donc dans la chaîne alimentaire, jusqu'à notre alimentation. Comme démontré notamment par Belenguer et al. (2014) et les fortes concentrations d'éthyle azinphos (composé présentant une forte bioaccumulation, $K_{OW} = 3,18$) de respectivement 86,17 $ng.L^{-1}$ et 65,64 $ng.L^{-1}$ dans du JUC-III et JUC-IV, deux types de poissons échantillonnés dans la rivière Llobregat (Catalogne). La présence de pesticides dans l'alimentation a également été suivie en mesurant les concentrations de produits de dégradation de pesticides organophosphorés (plus précisément de dialkylphosphate, DAP) dans les urines d'un groupe de volontaires se nourrissant à base de produits provenant d'une agriculture conventionnelle ou biologique (Curl et al., 2015). Cette étude montre que significativement plus de résidus de pesticides organophosphorés sont présents dans les urines des consommateurs ingérant des produits conventionnels (163 nmol DAP / g créatine, $p < 0,05$) par rapport à ceux se nourrissant de produits biologiques (121 nmol DAP / g créatine, $p < 0,02$).

Les pesticides retirés par les autorités compétentes mais aussi ceux actuellement sur le marché présentent des impacts sur la santé humaine et les milieux naturels qu'il reste important de maitriser. Il est donc nécessaire d'évaluer et de comprendre les mécanismes contrôlant l'évolution des pesticides appliqués sur les cultures et leurs transferts jusqu'aux milieux aquatiques.

2. Devenir et spéciation des pesticides de synthèse et pesticides inorganiques dans les sols

Les pesticides et métaux subissent une multitude de processus dans les sols. Des processus de sorption-désorption, dilution, ruissellement, infiltration et captage par les organismes et la végétation impactent à la fois les pesticides et les métaux (Arias-Estévez et al., 2008; Roberts et al., 2005). Les pesticides peuvent aussi se volatiliser, et la dérive sèche ou humide représente une fraction importante des exports vers les organismes non ciblés lors de l'application (Barbash, 2014; Carlsen et al., 2006). L'ensemble de ces processus n'altère cependant pas la structure des pesticides et sont donc des processus non-destructifs. Contrairement aux métaux qui subissent uniquement des processus non-destructifs, les pesticides subissent des processus destructifs qui modifient leur structure et leur composition moléculaire. Ces réactions de transformation sont des réactions de conjugaison, réarrangement ou dégradation (Coats, 1991). Les dégradations peuvent être de nature physique, chimique (abiotiques) ou biologique (d'origine biotique, directe ou indirecte). La Figure 1-5 résume les principaux processus rencontrés par les pesticides et métaux dans les bassins versants agricoles. Il a été montré que les principaux processus qui contrôlent le devenir des pesticides et métaux dans les sols sont les processus de sorption-désorption et de dégradation, en lien avec la biodisponibilité (Ma et al., 2006b; Ruggiero et al., 2002).

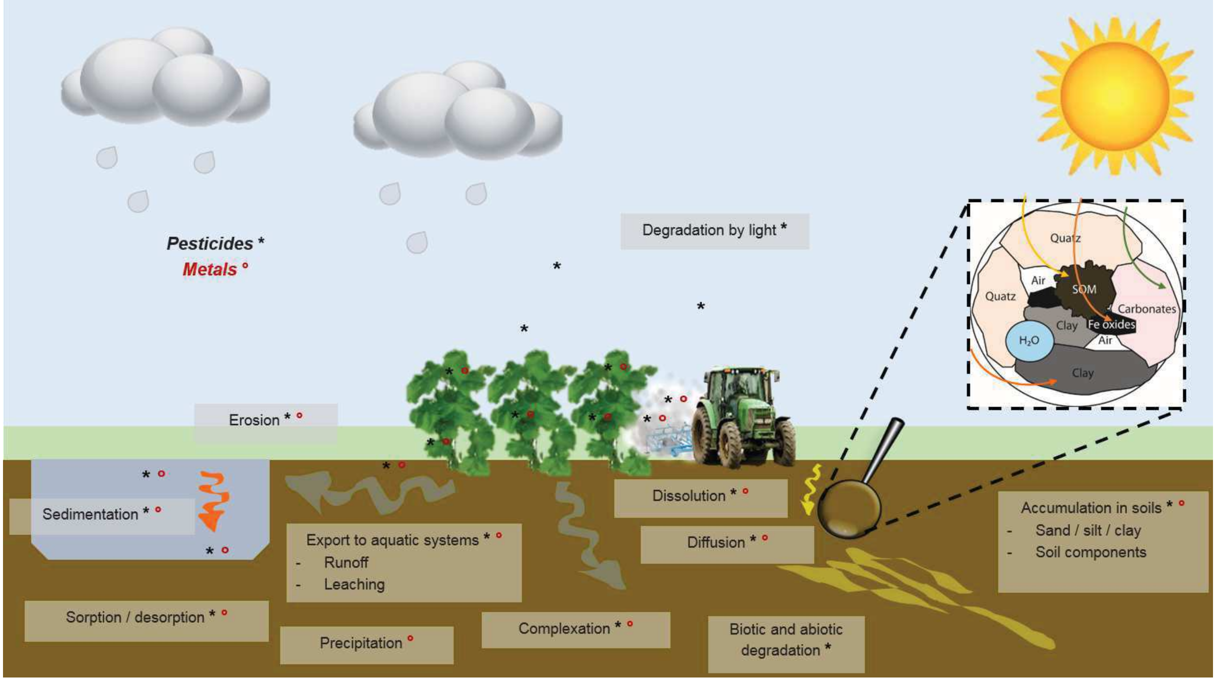


Figure 1-5 : Processus subis par les pesticides et métaux après leur application dans les bassins versants agricoles

Dans les sols, les pesticides et les métaux sont présents sous forme dissoute dans la solution de sol et sous forme particulaire dans la matrice solide. La sorption de ces composés est le passage de ceux-ci de la solution de sol à la matrice solide. Cela entraine la diminution de la disponibilité et de la mobilité des pesticides et métaux. Le partage de ces composés entre ces deux phases dépend d'un grand nombre de réactions, comme schématisé avec les Figures

1-6 et 1-7. Les pesticides peuvent être adsorbés à la matière organique des sols et aux phases minérales par liaison ionique, liaison hydrogène, forces de Van der Waals, échange de ligands, formation de complexes à transfert de charges, partage hydrophobe, liaison covalente ou encore séquestration (Gevao et al., 2000). Les pesticides peuvent également diffuser dans les micropores des constituants solides (Ruggiero et al., 2002). Les pesticides associés à la phase solide des sols peuvent être divisés en trois pools : les pesticides extractibles, non-extractibles et liés (ECETOC, 2013). Quant aux métaux, ils vont former des liaisons électrostatiques avec les hydroxydes et la matière organique présents dans la phase solide du sol. S'en suit alors une adsorption ou co-précipitation avec les carbonates avant que les métaux se retrouvent adsorbés sur les hydroxydes comme ceux de fer (Fe), manganèse (Mn) et aluminium (Al) (Rao et al., 2008). Les métaux peuvent aussi se complexer ou précipiter avec la matière organique et le S présent sous forme réduite. Enfin, une adsorption et une occlusion des métaux peuvent se produire au sein des argiles du sol.

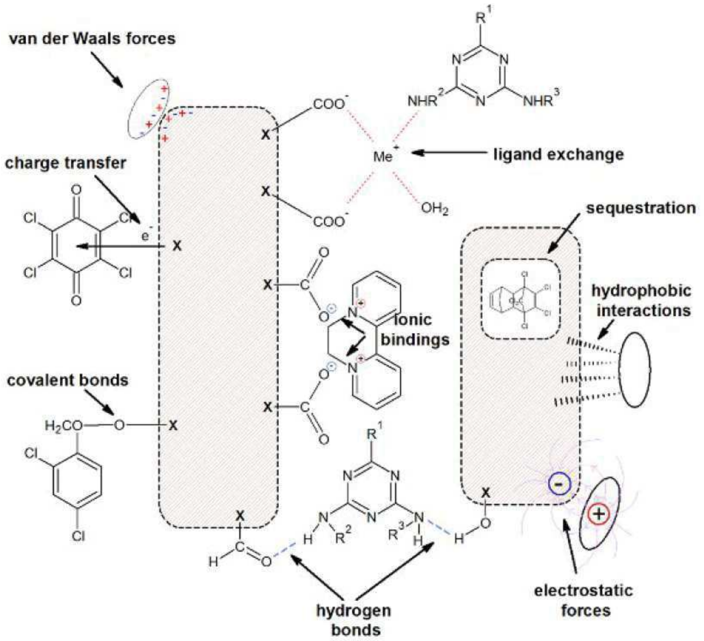


Figure 1-6 : Processus à l'origine de la sorption des molécules organiques dans les sols (ECETOC, 2013)

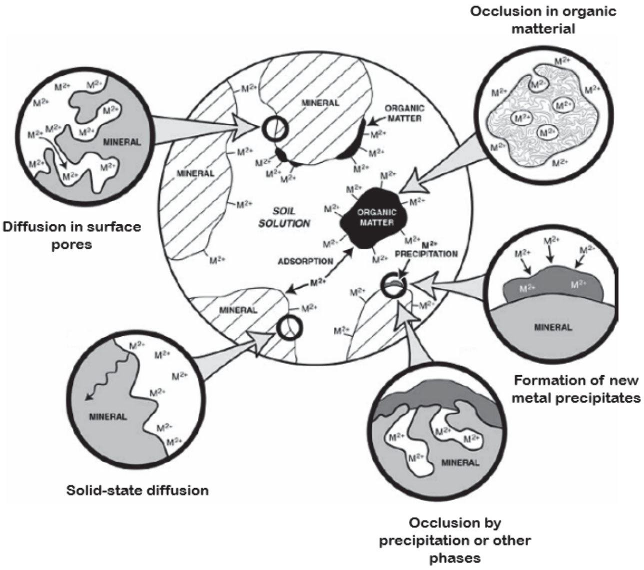


Figure 1-7 : Interactions entre les métaux et les constituants du sol (adapté de Buekers, 2007)

Le partage des polluants entre la phase solide des sols et la solution de sol dépend également des caractéristiques des polluants (i.e. l'hydrophobicité, la polarité, ou encore le coefficient de partage carbone organique / eau) mais aussi de celles des sols (i.e. la composition minéralogique, le taux de matière organique, ou encore le pH) (Komárek et al., 2010). Par exemple, alors que les pesticides non chargés, tels que l'atrazine et l'isoproturon, peuvent s'adsorber sur la kaolinite pure (Clausen et al., 2001), les pesticides ont généralement une forte affinité avec la matière organique. Lorsque le taux de matière organique est supérieur à 5 %, les pesticides sont principalement adsorbés sur la matière organique (Atwood and Paisley-Jones, 2017). Dès l'application de solutions de métaux et pesticides sur les sols, une rapide diminution de la disponibilité et de la mobilité de ces polluants est observée. Une étude conduite sur la disponibilité du Cu par Ma et al. (2006) montre une importante baisse de mobilité du Cu (diminution du pourcentage de Cu échangeable de 30 %) en 15 jours. Nkedi-Kizza et al. (2006) ont montré dans les sols une rapide sorption de l'Atrazine et du Diuron dans les trois premières heures et un équilibre atteint au bout de 24 h. Cette rapide diminution de la mobilité des pesticides et métaux est due à l'établissement de l'équilibre de sorption-désorption. Cette phase est accompagnée à plus long terme d'une baisse progressive de la disponibilité et de la mobilité de ces polluants souvent désignée par le terme « aging » (Gevao et al., 2000; Komárek et al., 2010; Y. B. Ma et al., 2006b).

Les coefficients de partage K sont déterminés expérimentalement ou théoriquement et permettent d'évaluer la proportion des pesticides et métaux entre phases liquide et solide. Les coefficients de partage entre l'eau et l'air K_{AW}, l'octanol et l'eau K_{OW} mais encore l'eau et la matière organique (carbone organique) dissoute K_{DOC} ou le sol K_D et K_{OC} sont des paramètres clés qui permettent d'approcher la capacité de volatilisation, de dissolution dans la solution de sol ou d'absorption d'un pesticide sur les constituants solides du sol (Boethling et al., 2004). La polarité des pesticides est également une caractéristique importante à prendre en compte lors des études de sorption dans les sols (Đurović et al., 2009). Pour les métaux, en plus des coefficients de partage entre l'eau et le sol ou certains de ces constituants (argiles, oxydes, carbonates, matière organique), le pH s'avère être un paramètre essentiel (Fike, 2001). La sorption du Cu augmente avec le pH en raison de la formation de charges négatives et de précipités. Des organismes comme l'USEPA recommandent pour ces raisons de maintenir à des pH supérieurs à 6,5 les sols recevant de forts apports massiques de métaux (Fike, 2001).

Les pesticides de synthèse subissent des réactions de transformation dont des dégradations physiques (photodégradation), chimiques (hydrolyse) et biologiques (biodégradation). Comme illustré avec la Figure 1-8, au cours de la dégradation les molécules des pesticides sont clivées menant à l'apparition de produits de dégradation ou métabolites ayant des caractéristiques différentes des pesticides originaux (aussi appelés produits parents). Les produits de dégradation sont souvent plus solubles et parfois plus toxiques que les pesticides eux-mêmes (Giacomazzi and Cochet, 2004; Shahgholi and Gholamalizadeh Ahangar, 2014). Lorsque la dégradation est complète, les pesticides sont entièrement minéralisés, leurs atomes de carbone sont alors transformés en CO_2. Différents facteurs sont impliqués lors des processus de dégradations. La lumière, la température (dégradation physique), le pH de l'eau (hydrolyse), l'oxygène, les constituants du sol (dégradation chimique), la faune, les virus, les algues, les enzymes, les levures, mais surtout les bactéries, champignons et actinomycètes (biodégradation) (Coats, 1991; Ruggiero et al., 2002). Même s'il est fréquemment reporté la prévalence de la dégradation biotique chez les pesticides, les dégradations abiotiques peuvent dominer dans des conditions où l'activité biologique est intense (Ruggiero et al., 2002). Les réactions de dégradations abiotiques peuvent être des réactions d'oxydation, d'hydrolyse ou de réduction (Ruggiero et al., 2002). Comme les constituants du sol favorisant les dégradations biotiques (i.e. les microorganismes) et abiotiques (i.e. les minéraux et matière organique du sol) sont souvent impliqués dans les mêmes réactions de dégradation, il peut être difficile de dissocier les mécanismes de dégradations biotiques des mécanismes de dégradations abiotiques.

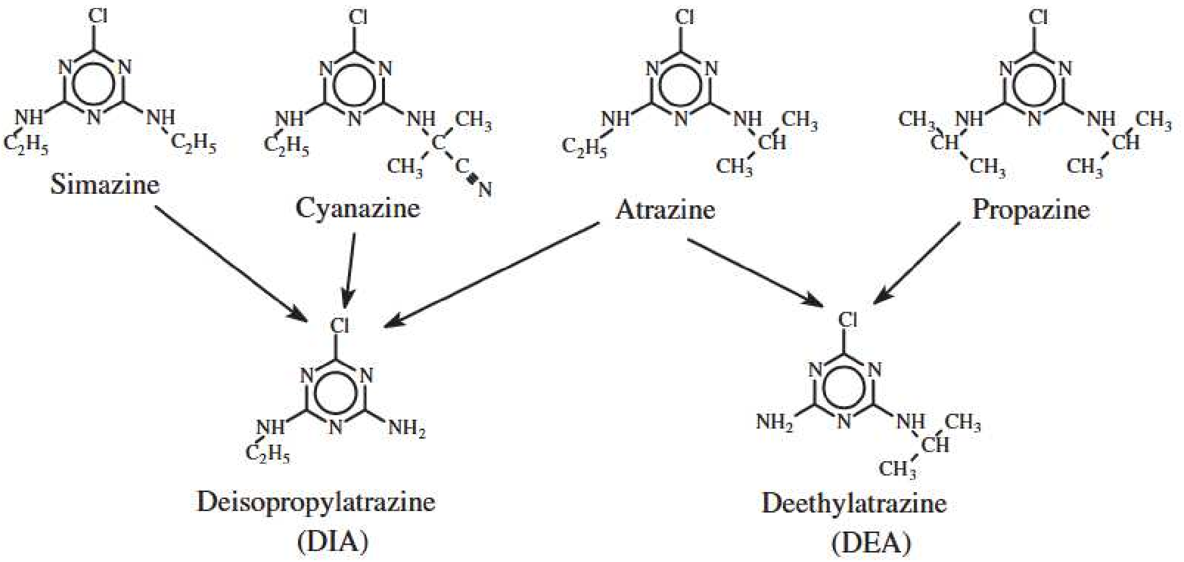


Figure 1-8 : Voies de dégradation biotique de l'atrazine, la cyanazine, la propazine et la simazine en dééthylatrazine et/ou déispropylatrazine (Thurman et al., 1994)

3. Facteurs contrôlant la transformation et la mobilité des polluants dans les sols agricoles

L'exportation de polluants par ruissellement ou lixiviation est également contrôlée par des facteurs, tels que les propriétés physico-chimiques du polluant (Gevao et al., 2000), l'hydrodynamique et les caractéristiques du sol (Buekers, 2007; Green and Ampt, 1911). En particulier, la disponibilité et la mobilité des polluants dans les sols tendent à diminuer au fil du temps en raison de la diffusion et l'adsorption des polluants dans les fractions minérales et organiques, dans un processus appelé "vieillissement" ou « aging » (Komárek et al., 2010; Y. B. Ma et al., 2006b). L'aging peut contrôler la fraction de polluants mobilisée et transportée. En effet, l'aging influe sur la proportion de polluants présents dans la phase dissoute, ou associés à des particules du sol et des colloïdes (Gevao et al., 2000; Huang et al., 2015; Jalali and Khanlari, 2008; Sauvé et al., 2000; Tang et al., 2006). L'aging influence donc la disponibilité du polluant pour sa transformation, à savoir la spéciation des métaux et la dégradation des polluants organiques. L'importance de la séquestration de polluants non dégradés peut alors influer sur l'ampleur de l'exportation de polluants accumulés dans les sols.

De plus, la couche superficielle (0-5 cm) des sols joue un rôle essentiel dans le contrôle de l'eau et des flux de polluants (Cerdan et al., 2002; Le Bissonnais et al., 2005), en particulier dans le cas des applications de pesticides en pré-levée sur des sols dénudés, ou de cultures pérennes, souvent caractérisées par des sols non végétalisés et une accumulation de métaux lourds (Tóth et al., 2016). Comprendre le rôle des facteurs extrinsèques (en particulier les caractéristiques des précipitations), en lien avec les facteurs intrinsèques, y compris les caractéristiques du sol ou des polluants, s'avère nécessaire pour estimer les voies d'export des polluants à partir du sol de surface et pour prédire cet export.

3.1. Facteurs intrinsèques au sol contrôlant la transformation et la mobilité des métaux et des polluants organiques

3.1.1. Métaux lourds

Les métaux sont immobilisés dans les sols par sorption, échange cationique, complexation et précipitation (carbonates, phosphates) (Buekers, 2007). Lorsque les teneurs en métaux sont faibles, l'immobilisation de ces derniers sur les sols est faite par sorption spécifique (complexe de sphère interne). Quand les concentrations en métaux dans les sols sont bien plus élevées et supérieures au produit de solubilisation, l'immobilisation des métaux est due à leur précipitation (Buekers, 2007).

La sorption des métaux chargés positivement peut se faire sur les hydroxydes, la matière organique et les argiles car ces constituants présentent des surfaces chargées négativement (Buekers, 2007). On définit alors la capacité d'échange cationique (CEC) d'un sol comme la quantité de cations que celui-ci peut retenir sur son complexe adsorbant à un pH donné. Ainsi, la fixation des métaux sera plus grande dans des sols présentant de fortes CEC (Du Laing et al., 2009). Suivant le pH, la sorption des métaux sur les minéraux et matière organique des sols diffère. Dans des sols aux pH faibles à modérés, le principal adsorbant des métaux est la matière organique. Tandis que dans des sols aux pH alcalins, les hydroxydes seront les minéraux où l'adsorption des métaux sera majoritaire (Buekers, 2007). Le pH est un facteur très important vis-à-vis de la sorption des métaux dans les sols et une remobilisation des métaux adsorbés sur la matrice solide des sols peut survenir suite à un changement de pH.

La spéciation chimique des métaux change graduellement dès leur application sur les sols. La spéciation d'un élément correspond à sa distribution entre des espèces chimiques définies (Clough et al., 2013). Dans les sols les fractions suivantes sont habituellement définies pour décrire la spéciation chimique des métaux : les fractions échangeable, acido-soluble, réductible, oxydable et résiduelle. Les métaux échangeables forment des liaisons électrostatiques avec les hydroxydes et la matière organique du sol. La fraction acido-soluble décrit les métaux qui s'adsorbent ou co-précipitent avec les carbonates. Les métaux adsorbés sur les hydroxydes du sol constituent la fraction réductible. Alors que les métaux peuvent complexer ou précipiter principalement avec la matière organique dans la fraction oxydable. Les métaux adsorbés ou piégés après occlusion dans les réseaux silicatés des argiles sont les métaux dits résiduels. Ces interactions entre les métaux et la phase solide des sols sont résumés dans la Figure 1-9. Après leur application sur les sols, la sorption des métaux s'intensifie avec le temps (Jalali and Khanlari, 2008). Ainsi les proportions de métaux échangeables (mais aussi les métaux liés aux carbonates) diminuent rapidement et les concentrations de métaux dans les fractions réductible, oxydable et résiduelle augmentent (Jalali and Khanlari, 2008).

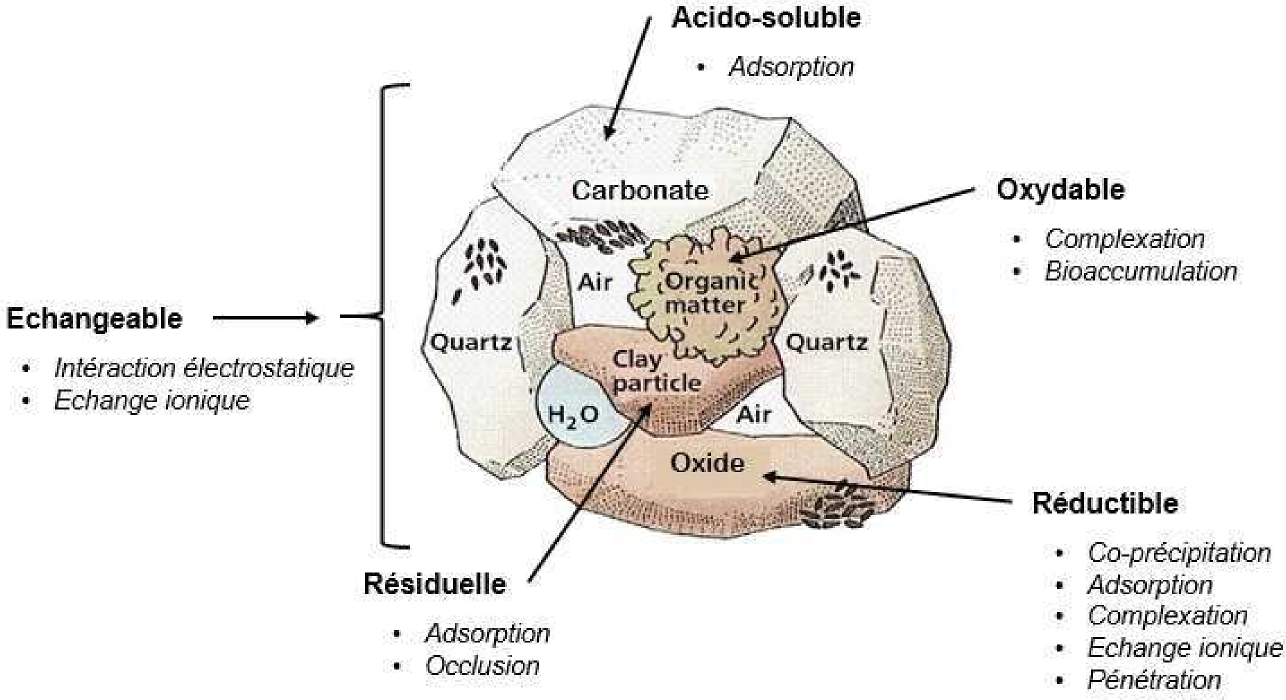


Figure 1-9 : Fractions chimiques des sols et interactions des métaux avec les constituants des sols

Par exemple, dans les sols viticoles, le Cu peut subir différentes transformations en raison de processus i) chimiques, tels que la formation de complexes avec la matière organique ou encore la précipitation de carbonates de cuivre $CuCO_3$ (Komárek et al., 2010), et ii) biologiques, par le prélèvement par la végétation (Duplay et al., 2014) et les microorganismes (algues, bactéries) (Andreazza et al., 2010).

3.1.2. Polluants organiques

Tout comme pour les métaux, la composition des sols impacte fortement le devenir des molécules de pesticides de synthèse après leur application. Ces polluants peuvent interagir avec les microorganismes présents dans les sols comme la matière organique mais aussi les minéraux.

Les teneurs en minéraux mais surtout en matière organique dans les sols contrôlent le type d'adsorption des pesticides et la nature des réactions de transformation qu'ils subissent. Au-delà de 5 % de matière organique, celle-ci constitue généralement la principale source d'adsorption pour les pesticides (hors pesticides ioniques). Tandis qu'en dessous de 5%, les argiles semblent principalement contrôler l'adsorption des pesticides dans les sols (Đurović et al., 2009). Les pesticides polaires et ioniques (cationiques principalement) s'adsorbent facilement sur les argiles hydrophiles et chargées négativement. Dans une moindre mesure, les pesticides hydrophobes et non-polaires peuvent aussi être adsorbés sur les argiles grâce à des microsites hydrophobes présents sur ces dernières (Cornejo et al., 2008).

Les argiles, mais aussi les hydroxydes métalliques, sont à l'origine de réactions de transformation abiotiques des pesticides. Ces minéraux peuvent catalyser des réactions d'hydrolyse, d'oxydation et de polymérisation des pesticides dans les sols (Ruggiero et al., 2002). La catalyse de ces réactions dépend de la structure des minéraux, de la réactivité de leurs surfaces. Parmi ces minéraux, les oxydes de manganèse jouent un rôle important dans le devenir des pesticides en raison de leur fort pouvoir oxydant. Les oxydes de manganèse peuvent être à l'origine de dégradation de pesticides comme l'Atrazine, le Glyphosate ou encore le 2-mercaptobenzothiazole (Remucal and Ginder-Vogel, 2014). Le quartz et les carbonates majoritairement présents dans les sols jouent cependant généralement un rôle mineur sur l'adsorption et la transformation des pesticides en comparaison aux argiles (Clausen et al., 2001).

La matière organique du sol peut également être à l'origine de réactions de dégradations abiotiques des pesticides. Différentes réactions ont été mises à jour comme l'hydroxylation de chloro-s-triazine suite à la formation de liaisons H entre la triazine et un groupement carboxylique protoné d'acides humiques, ou encore la déhalogénation de pesticides catalysée par des argiles après la sorption de ces pesticides sur des substances humiques (Ruggiero et al., 2002).

Le pH du sol est un paramètre crucial dans la dégradation des pesticides dans les sols. Cette dégradation est souvent la plus effective pour des pH proches de la neutralité. L'effet du pH sur la dégradation des pesticides est principalement dû à leur capacité à subir des hydrolyses catalysées en milieux alcalin ou acide (Shahgholi and Gholamalizadeh Ahangar, 2014). Le pH impacte également l'adsorption des pesticides, les échanges ioniques au sein des sols, leur potentiel d'oxydo-réduction, les processus de complexation ou encore les précipitations (Ruggiero et al., 2002).

Parmi les possibles voies de dissipation des pesticides dans les sols, les dégradations induites par les microorganismes sont les plus reconnues. Les microorganismes métabolisent les pesticides pour en assimiler les différents éléments compris dans leurs molécules et comme source d'énergie. Les pesticides peuvent également être minéralisés par les microorganismes par des réactions de cométabolismes, polymérisation (ou conjugaison) et accumulation. Les pesticides peuvent également être dégradés par les microorganismes de manière indirecte, lorsque l'activité microbienne génère des changements de paramètres du sol impactant les réactions de transformation des pesticides (Ruggiero et al., 2002).

Les conditions au sein des sols impactent grandement les microorganismes et la dégradation biotique des pesticides. La croissance des microorganismes est stimulée suivant les taux de nutriments présents dans les sols dont notamment le carbone, l'azote, l'oxygène, l'hydrogène et le phosphore. L'humidité des sols est également un facteur important dans la transformation des pesticides. La teneur en eau des sols contrôle le fonctionnement des microorganismes et la disponibilité des pesticides vis-à-vis des microorganismes. L'humidité des sols impacte également la diffusion de l'oxygène de l'air dans les sols provoquant des conditions aérobies ou anaérobies impactant la dégradation des pesticides (Shahgholi and Gholamalizadeh Ahangar, 2014).

Le nombre de réaction de transformation affectant les pesticides dans les sols augmente avec la température. En revanche, en cas de température trop élevée, un changement fonctionnel majeur des communautés microbiennes des sols peut survenir et impacter alors la dégradation biotique des pesticides de synthèse.

3.1.3. Importance de la fraction colloïdale

Comme abordé dans les paragraphes précédents, la sorption des pesticides et métaux dans les sols est fortement liée aux caractéristiques de ces sols comme leur teneur en eau et valeurs de pH. La sorption de ces contaminants ainsi que l'aging dans les sols impacteront fortement leur capacité à être mobilisés suite aux évènements pluvieux.

Pendant longtemps le transport des contaminants dans les sols a été étudié par le prisme d'un système à deux phases uniquement. Les polluants étant soit dissous en solution, soit fixés sur la phase solide des sols. Cependant des particules de faibles dimensions (colloïdes) peuvent être libérées à partir des sols et sont extrêmement importantes vis-à-vis du transport des contaminants (comme les pesticides de synthèse et les métaux) des sols vers les milieux aquatiques (Bin et al., 2011; Lapworth et al., 2005; McGechan and Lewis, 2002; Zhang et al., 2005). Les polluants peuvent être associés dans les sols à des particules colloïdales suite à leur adsorption ou précipitation avec ces particules. Les colloïdes sont des particules possédant au moins une dimension inférieure à 1 µm et présentent une surface spécifique très importante de 10 à 8000 $m^2.g^{-1}$. La mobilité des colloïdes est telle que ces particules formées dans ou à la surface des sols sont retrouvées dans les eaux de surfaces et les eaux souterraines suite aux évènements pluvieux (Kretzschmar and Schäfer, 2005). Ces colloïdes sont des particules composées : i) de minéraux inorganiques comme des argiles, hydroxydes métalliques, carbonates et phosphates, ii) de phases organiques (acides humiques et fulviques, polysaccharides et autre), mais aussi iii) de microorganismes comme des virus ou encore des bactéries. Les colloïdes sont libérés de la matrice solide des sols suite à des variations de teneur et de flux d'eau dans ces sols mais aussi en raison des caractéristiques physico-chimiques de la solution de sol. Parmi ces caractéristiques figurent les valeurs de pH ou la force ionique de la solution de sol (Kretzschmar and Schäfer, 2005).

En plus de la floculation/dispersion des colloïdes, un autre paramètre important dans la mobilité de ces derniers et donc le transport des polluants est la dimension des pores des sols. Les colloïdes sont plus facilement retenus par la phase solide des sols quand celle-ci comporte des micropores et non des macropores comme illustré avec la Figure 1-11. Les macropores sont fréquents dans les sols agricoles suite à la présence d'organismes et de racines de végétaux (McGechan and Lewis, 2002). Les teneurs en argiles des sols impactent également la formation et la dimension des pores. Les argiles enrobent les particules plus larges comme les sables et les limons. Ces différentes particules s'associent, formant alors des micro-agrégats puis des agrégats stabilisés par la matière organique et les composants inorganiques. La formation de ces agrégats impacte possiblement la porosité des sols, tandis que la rétention de l'eau dans les sols dépend en grande partie de la composition et de la stabilité de ces agrégats (Ruggiero et al., 2002).

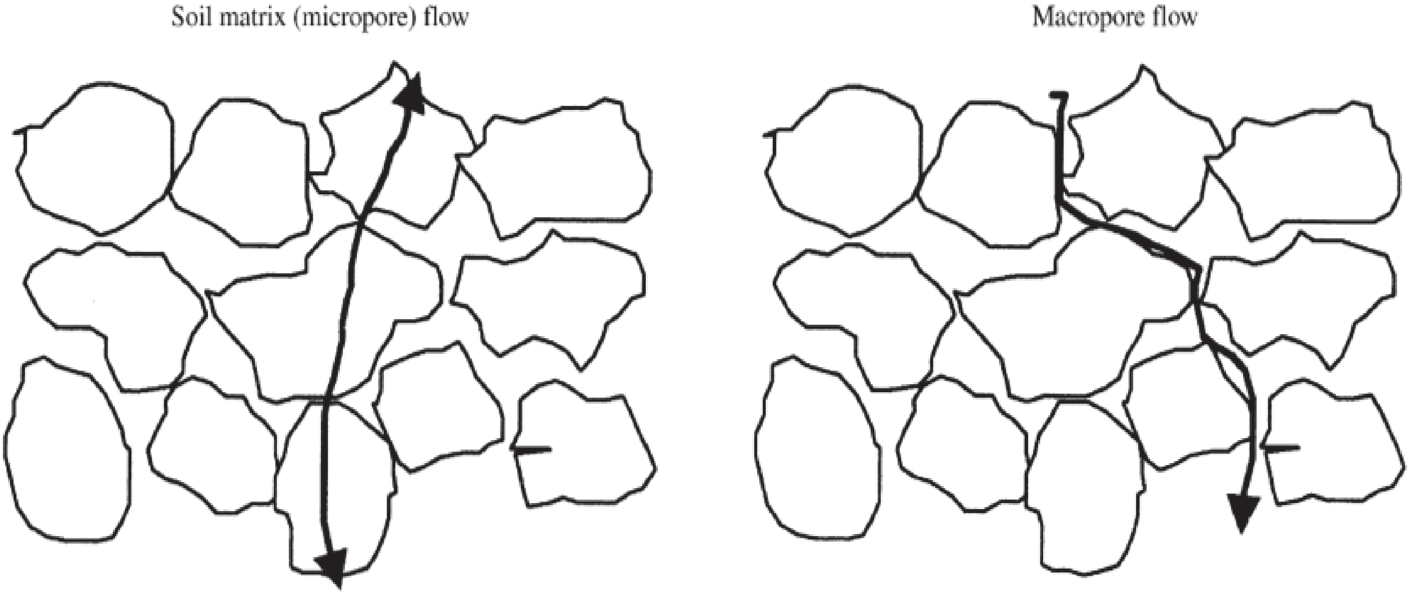


Figure 1-11 : Transport de particules et de colloïdes dans les sols en fonction de la porosité (McGechan and Lewis, 2002)

3.1.4. Co-contamination des sols agricoles

Les sols agricoles comme plusieurs compartiments environnementaux sont pollués à la fois aux pesticides et aux métaux. La présence simultanée de ces deux types de polluants peut interférer dans les processus qu'ils subissent comme mis en avant par plusieurs études dont celle conduite par Tariq et al. (2016).

La mesure de concentrations de différents métaux et pesticides dans des sols provenant de la culture de coton, a révélé que des corrélations existaient entre certains métaux et pesticides. Des trois pesticides qu'ils ont étudiés (imidaclopride, acétamipride et emamectine), les résultats montrent que le Cu stabilise l'acétamipride tandis que le Cr et le Ni favorisent la dégradation de l'acétamipride et de l'imidaclopride dans les sols (Tariq et al., 2016).

La présence de métaux impacte la stabilité des pesticides dans les sols en raison de la capacité des métaux à catalyser les réactions subies par les pesticides comme des réactions de photolyse ou hydrolyse (Tariq et al., 2016; Wang et al., 2007). La disponibilité des pesticides peut également être modifiée suite à leur complexation avec les métaux présents dans les sols, diminuant alors leur dégradation (Dousset et al., 2007; Helal et al., 2006; Tariq et al., 2016). De plus, des changements peuvent survenir au sein des communautés microbiennes dans les sols lorsque ces dernières se trouvent en présence de métaux (Tariq et al., 2016; Wang et al., 2007).

La co-contamination des sols aux pesticides et métaux peut induire des compétitions dans la sorption de ces deux types de contaminants sur les différentes phases solides des sols. Ainsi une modification de la sorption/désorption des pesticides et métaux peut survenir dans les sols. Différentes études montrent l'impact de la présence simultanée de polluants organiques et minéraux dans les sols sur les mobilités respectives de ces composés dont les travaux de Lalah et al. (2009) mais aussi ceux de Helal et al. (2006). En effet la présence de métaux dans les sols peut diminuer la sorption des pesticides sur ces sols. Comme l'ont démontré Lalah et al. (2009) pour le DDT en présence d'un gradient de concentrations de métaux. Au sein de ce sol limoneux-argileux, l'adsorption du DDT a été diminuée de 6,1 à 15,4 % lorsque ce dernier est mis en présence métaux de 10 à 100 $\mu g.g^{-1}$ de sol (Lalah et al., 2009). Suivant la nature des pesticides et métaux considérés, l'effet inverse peut être observé comme ce fut le cas dans l'étude conduite par Helal et al. (2006). Leurs travaux sur l'aldicarb et le methomyle en présence de cobalt, zinc et strontium montrent que les pesticides de synthèse étudiés présentent de plus grandes affinités avec les acides humiques et fulviques de la matière organique que le Co, Zn et Sr. La complexation de ces pesticides avec la matière organique entraine le relargage du Co, Zn et Sr de la matière organique du sol vers la solution de sol (Helal et al., 2006).

3.2. Facteurs extrinsèques contrôlant la transformation et la mobilité des métaux et des polluants organiques

L'aging, la transformation et la mobilité des polluants sont contrôlés par des facteurs extrinsèques non inhérents aux sols. Le laps de temps entre l'application de polluants (notamment les pesticides) et les événements de pluie, les caractéristiques de ces pluies (Huang et al., 2015; Nolan et al., 2008), les conditions climatiques, comme la température, ou encore la végétation sont des facteurs clés connus pour influencer le devenir des contaminants (Nolan et al., 2008), sans pour autant que leur action combinée sont bien connue.

3.2.1. Caractéristiques des pluies

Les pesticides et les métaux peuvent être exportés du sol vers les eaux de surface par ruissellement, incluant l'accumulation d'eau de surface par saturation du sol (« ponding ») puis l'écoulement de surface (« runoff ») (Lefrancq et al., 2014; Schwarzenbach and Westall, 1981), mais aussi la lixiviation de ces derniers lorsque l'eau de pluie transporte des polluants dissous à travers le profil du sol.

L'intensité des précipitations et leur durée affectent principalement la mobilisation des polluants et l'exportation du sol. Il a été démontré que l'intensité des pluies est directement proportionnelle à la lixiviation de l'Isoproturon à partir des colonnes de sol (Beulke et al., 2002).

De plus, l'infiltration de pesticides et métaux dans des sols peut être diminuée suite à la succession de périodes sèches et humides (Goldreich et al., 2011; Thouin et al., 2017). Par exemple, il a été montré par Goldreich et al. (2011) qu'une succession de pluie induisait une infiltration deux fois plus importante de Métolachlore. La succession de périodes sèches et humides impacte la libération de colloïdes et donc le transport de contaminants (Klitzke and Lang, 2007).

3.2.2. Humidité des sols

Les teneurs en eau des sols diffèrent suivant le volume généré par les pluies, leurs successions, le laps de temps écoulé entre ces pluies, la végétation, mais aussi la nature des sols et les températures (Korres et al., 2015). En effet, l'accumulation d'eau dans les sols se produit lorsque l'intensité des précipitations surmonte la capacité d'infiltration de la couche arable. En fonction de la pente du terrain et de la connectivité hydrologique amont-aval, l'eau peut générer un ruissellement atteignant les écosystèmes aquatiques.

La teneur en eau des sols impacte fortement la transformation des pesticides de synthèse. Suivant l'humidité des sols, les conditions aérobies évoluent tout comme l'activité des microorganismes mais aussi leurs capacités à dégrader les pesticides. Dans leur étude sur l'influence des teneurs en eau des sols sur la dégradation de trois pesticides (Linuron, Isoproturon et Métolachlore), Accinelli et al. (2005) ont obtenu deux tendances contraires. Tandis que le Linuron et l'Isoproturon se dissipaient respectivement 1,8 et 1,4 fois moins vite dans des sols saturés en eau, le Métolachlore était quant à lui dissipé plus rapidement avec un temps de demi-vie de 24,1 jours contre 32,2 jours dans des sols non saturés en eau.

La saturation en eau des sols peut être synonyme de remobilisation des métaux dans les sols et potentiels risques de pollution des milieux aquatiques vers lesquels les métaux peuvent alors être transportés, suivant le pH des sols et les conditions réductrices induites par la présence d'eau dans ces sols. En effet, dans de telles conditions la spéciation des métaux change. Ces derniers, pourtant adsorbés sur les constituants des sols, peuvent alors être solubilisés. C'est ce que montre l'étude de Weber et al. (2009) au cours de laquelle ils ont suivi le devenir de plusieurs métaux (Cu, Cd, Pb, Zn et Ni) dans des microcosmes réalisés avec un sol saturé. Leurs travaux montrent que ces métaux ont été mobilisés suite à la dissolution réductrice des hydroxydes de Fe(III) et de Mn(IV, III).

3.2.3. Couvert végétal

Le développement du couvert végétal à la surface des sols impacte également la transformation et la mobilité des contaminants notamment en raison de la proximité des racines des plantes dans la rhizosphère (compartiment du sol sous l'influence des racines).

Des études faites sur des bassins versants instrumentalisés comme des colonnes de sol réalisées au laboratoire montrent que la présence de plantes sur les sols réduit l'export par infiltration et ruissellement de métaux (Duplay et al., 2014), mais aussi de pesticides et leurs produits de dégradation (Diez et al., 2015; Sayyad et al., 2010). La diminution du transport de contaminants en présence d'un couvert végétal peut être due au prélèvement d'eau et de contaminants par les plantes, au phénomène d'évapotranspiration mais aussi à l'adsorption dans la rhizosphère des polluants sur les racines de plantes et à la baisse de l'érosion des sols (Duplay et al., 2014; Sayyad et al., 2010).

La rhizosphère abrite un grand nombre de microorganismes et est décrite comme étant fortement dynamique (Philippot et al., 2013). De nombreux processus se produisent dans la rhizosphère et changent les caractéristiques du sol. Les teneurs en sucres, acides aminés et organiques, mucilages, nutriments et eau évoluent suite à leur production ou leur prélèvement par les racines (Philippot et al., 2013). L'évolution de ces paramètres impactent également les valeurs de pH dans cette région des sols. Tous ces facteurs affectent les communautés microbiennes disponibles notamment pour la biodégradation des pesticides (Diez et al., 2015; Duplay et al., 2014).

Cependant, dans les cas où les pesticides appliqués atteignent directement la surface des sols, ce sont les processus de transformation des polluants directement au niveau de la couche arable et non dans la rhizosphère qui contrôlent la fraction de polluants exportée soit par accumulation, soit par ruissellement ou par lixiviation. Dans l'agrosystème végétalisé, la fraction lessivée de la pollution peut ainsi atteindre la rhizosphère. Tandis que la transformation des polluants dans la rhizosphère est relativement bien décrite (Imfeld et al., 2009), la mobilisation des polluants lors du forçage hydrologique s'avère moins bien connue. Il est pertinent d'étudier la mobilisation des métaux présents dans les sols. Après leur application sur les cultures, les métaux peuvent s'accumuler en surface des sols. Ils peuvent ensuite être transportés vers d'autres compartiments réactifs, tels que la rhizosphère (Babcsányi et al., 2016; Duplay et al., 2014).

En dépit de l'importance de ces facteurs, la contribution des facteurs intrinsèque et extrinsèque sur l'export horizontal (ruissellement) et vertical (infiltration) des polluants organiques et inorganiques des sols ont été, à ce jour, rarement quantifiés (Beulke et al., 2002; Nolan et al., 2008; Sauvé et al., 2000).

3.3. Méthodes et approches d'étude de la transformation et de la mobilité des contaminants dans les sols

3.3.1. Transformation des métaux

La quantification des métaux (concentrations totales) adsorbés, précipités et piégés dans les minéraux et la matière organique des sols ne peut se faire qu'après avoir complètement minéralisé les sols. Cette étape de minéralisation permet de passer l'ensemble des constituants solides des sols en solution à l'aide de mélanges d'acide nitrique (HNO_3), chlorhydrique (HCl), fluorhydrique (HF), et perchlorique ($HClO_4$) principalement (Hseu et al., 2002). S'en suit alors des mesures par spectrométrie comme la spectrométrie d'absorption atomique (AAS en anglais) ou la spectrométrie d'émission atomique mais aussi de masse à plasma à couplage inductif (ICP-AES ou -MS). La spectrométrie de fluorescence des rayons X peut également être utilisée pour mesurer les teneurs de métaux (Roberts et al., 2005).

Cependant la mesure des concentrations totales de métaux dans les sols ne suffit pas à évaluer l'évolution de ces contaminants dans l'environnement. Les métaux naturellement présents dans les sols comme ceux d'origine anthropique sont simultanément quantifiés après une digestion totale des sols. La quantification totale des métaux dans les sols ne permet cependant pas de distinguer si les métaux sont faiblement (liaisons électrostatiques avec la matière organique, adsorption sur divers minéraux) ou fortement (occlusion dans les argiles) associés aux constituants des sols et quels processus ils ont subi depuis leur application sur les sols. Il est donc également nécessaire de réaliser des extractions sélectives simples ou séquentielles afin d'évaluer les proportions de métaux présents sous les différentes formes spécifiques (liés, précipités ou piégés dans des minéraux définis) (Rao et al., 2008). Des protocoles d'extractions sélectives séquentielles comme celui de Tessier et al. (1979) ou encore celui du Bureau Communautaire de Référence (BCR) (Ure et al., 1992) ont été développé dans ce but de caractérisation fine des métaux dans les sols. Cependant, les solvants nécessaires à l'extraction des métaux dans les fractions chimiques ciblées ne sont pas toujours sélectifs, notamment dans le cas de la procédure de Tessier et al. (1979). L'utilisation de chlorure de magnésium par exemple pour extraire les éléments échangeables entraine une dissolution des carbonates et un relargage du Mg présent dans ces carbonates (Tessier et al., 1979). La dissolution des carbonates avec de l'acide acétique pour évaluer la fraction de métaux associés à ces derniers peut mener à la dissolution d'autres phases et la surestimation des métaux acido-soluble. Enfin, lors de l'extraction des métaux liés à la matière organique à l'aide de d'eau oxygénée, une mobilisation des métaux liés aux sulfites peut survenir.

En parallèle des quantifications sélectives, une mesure des isotopes stables des métaux peut s'avérer pertinente pour tracer l'histoire des métaux dans le sol.

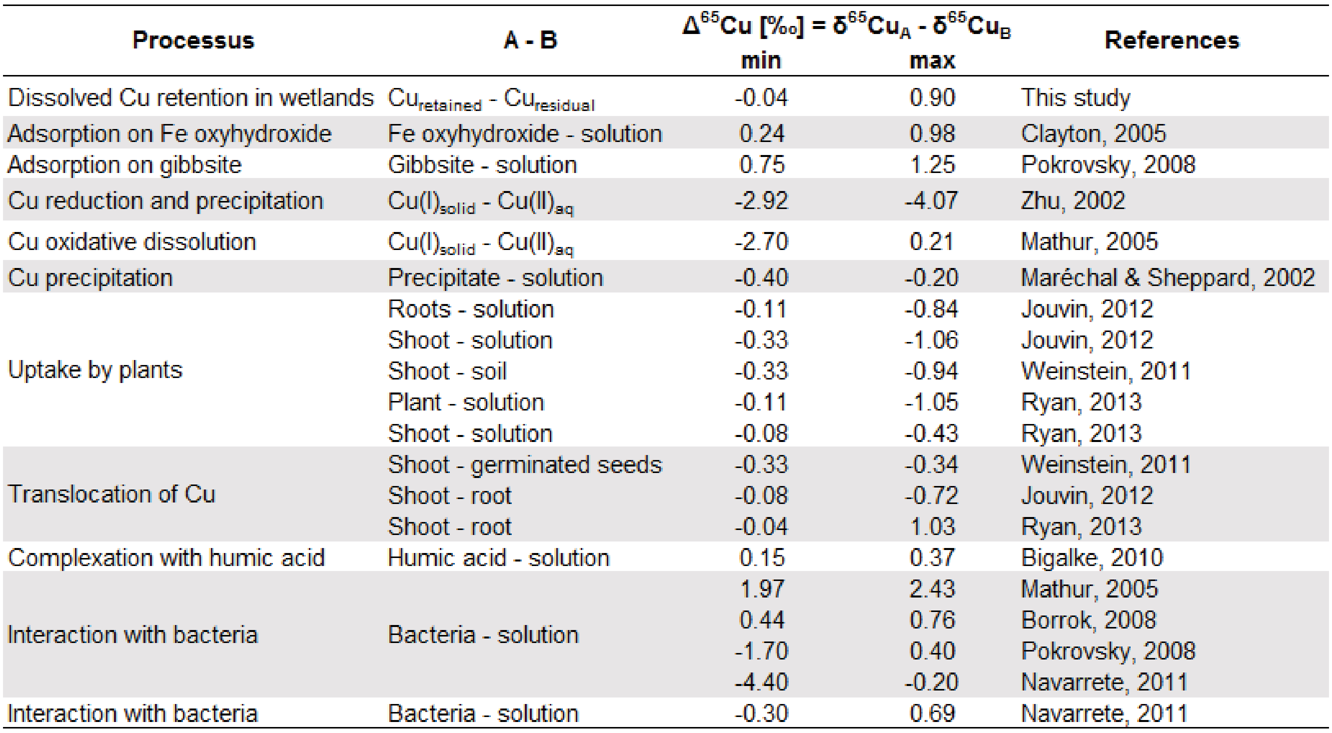
Des isotopes d'un élément chimique correspondent à des éléments qui diffèrent uniquement par leur nombre de neutrons, ainsi leur masse atomique varie. A l'inverse des isotopes radioactifs, les isotopes stables n'engendrent aucune radioactivité car leur noyau est énergétiquement stable et ne subit aucune modification de sa structure nucléaire. Tandis que la décroissance radioactive des isotopes radioactifs peut être utilisée pour estimer l'âge de divers objets, les isotopes stables sont utilisés comme traceurs de processus biogéochimiques et/ou de source (Weiss et al., 2008).

La proportion des isotopes d'un même élément (composition isotopique) peut se modifier pendant le passage de cet élément d'un état physique ou d'une composition chimique à une autre. Ceci entraîne une répartition différente des isotopes entre les réactifs et les produits lors des réactions produisant ainsi un fractionnement isotopique. Les fractionnements isotopiques peuvent être à l'équilibre (dans des systèmes à l'équilibre physique ou chimique) ou cinétiques (dans les réactions (bio)chimiques à sens unique) (Wiederhold, 2015; Young et al., 2002). Presque tous les fractionnements isotopiques qui surviennent dans l'environnement dépendent de la masse. La distribution isotopique d'une réaction à l'équilibre thermodynamique est strictement dépendante de la différence de masse relative entre les isotopes d'un élément donné. Ce fractionnement peut ainsi être utilisé pour suivre les processus en cours dans les systèmes eau-sol-organismes. Afin d'illustrer l'utilisation des isotopes stables de métaux pour évaluer les processus, le Cu est choisi ci-dessous en exemple.

Le cuivre possède deux isotopes stables le ^{63}Cu et le ^{65}Cu ayant chacun 29 protons, 29 électrons, mais possèdent 34 (^{63}Cu) et 36 neutrons (^{65}Cu) dans leur noyau. L'abondance moyenne dans la nature de l'isotope ^{63}Cu est 69,17 % contre 30,83 % pour l'isotope ^{65}Cu (Pérez Rodríguez et al., 2013). La composition isotopique du δ^{65}Cu dans les sédiments, le matériel biologique et les minéraux secondaires varie entre -3.0 ‰ and 5.7 ‰ (Weiss et al., 2008). La valeur isotopique initiale du Cu est en moyenne de 0 ‰ dans les sols provenant de roches-mères (Li et al., 2009).

L'adsorption du Cu sur les minéraux, sa complexation avec la matière organique, les réactions oxydoréduction, sa précipitation et son prélèvement par la végétation ou les microorganismes induisent des différences de valeurs isotopiques. Le fractionnement des isotopes du Cu a été observé suivant ces processus au sein des organismes, des sols, des milieux aquatiques mais aussi des sédiments. La Table 1-1 résume diverses études faites sur les fractionnements isotopiques du Cu générés par plusieurs processus dans l'environnement. Cette table présente notamment les travaux de (Pokrovsky et al., 2008) qui ont étudié l'impact de la sorption du Cu sur des hydroxydes de fer et ont mesuré des enrichissements isotopiques du Cu (Δ^{65}Cu(solide-solution)) de l'ordre de 1,0 ± 0,25 ‰ (gibbsite) et 0,78 ± 0,2 ‰ (goethite). De plus, une étude conduite par (Babcsányi et al., 2014) montre que le Cu dissous est appauvri en isotope lourd (^{65}Cu) suite à son adsorption sur des hydroxydes d'aluminium (et de fer) et de la matière organique présents dans le sédiment d'un bassin d'orage. Les argiles et la matière organique produisent un fractionnement isotopique significatif, de même que la transformation biotique du cuivre (passage par des racines, translocation dans les plantes). Enfin, au cours d'une expérience de sorption et d'incorporation du Cu faite avec différentes bactéries, (Navarrete et al., 2011) ont obtenu un fractionnement isotopique du Cu (Δ^{65}Cu(solution-solide)) allant de 0,2 à 2,6 ‰.

Table 1-1 : Fractionnement isotopique du Cu observé dans les zones humides et valeurs minimales et maximales de fractionnement isotopique expérimental du Cu lors de processus dans ces zones (d'après Babcsányi et al., 2014)



Processus	**A - B**	**Δ^{65}Cu [‰] = $\delta^{65}Cu_A$ - $\delta^{65}Cu_B$**		**References**
		min	**max**	
Dissolved Cu retention in wetlands	$Cu_{retained}$ - $Cu_{residual}$	-0.04	0.90	This study
Adsorption on Fe oxyhydroxide	Fe oxyhydroxide - solution	0.24	0.98	Clayton, 2005
Adsorption on gibbsite	Gibbsite - solution	0.75	1.25	Pokrovsky, 2008
Cu reduction and precipitation	$Cu(I)_{solid}$ - $Cu(II)_{aq}$	-2.92	-4.07	Zhu, 2002
Cu oxidative dissolution	$Cu(I)_{solid}$ - $Cu(II)_{aq}$	-2.70	0.21	Mathur, 2005
Cu precipitation	Precipitate - solution	-0.40	-0.20	Maréchal & Sheppard, 2002
Uptake by plants	Roots - solution	-0.11	-0.84	Jouvin, 2012
	Shoot - solution	-0.33	-1.06	Jouvin, 2012
	Shoot - soil	-0.33	-0.94	Weinstein, 2011
	Plant - solution	-0.11	-1.05	Ryan, 2013
	Shoot - solution	-0.08	-0.43	Ryan, 2013
Translocation of Cu	Shoot - germinated seeds	-0.33	-0.34	Weinstein, 2011
	Shoot - root	-0.08	-0.72	Jouvin, 2012
	Shoot - root	-0.04	1.03	Ryan, 2013
Complexation with humic acid	Humic acid - solution	0.15	0.37	Bigalke, 2010
Interaction with bacteria	Bacteria - solution	1.97	2.43	Mathur, 2005
		0.44	0.76	Borrok, 2008
		-1.70	0.40	Pokrovsky, 2008
		-4.40	-0.20	Navarrete, 2011
Interaction with bacteria	Bacteria - solution	-0.30	0.69	Navarrete, 2011

3.3.2. Transformation des polluants organiques

Suivant les caractères hydrophile et volatil des pesticides mais aussi suivant leur polarité, il est possible de mesurer leurs concentrations par chromatographie liquide ou gazeuse couplée à de la spectrométrie de masse (respectivement LC-MS et GC-MS). L'analyse des concentrations de pesticides dans les sols au cours du temps permet de suivre leur dissipation globale, sans distinction entre les processus non-destructifs (dilution ou la sorption) et les processus destructifs (dégradation) (Fenner et al., 2013).

De façon similaire aux métaux, il est possible d'extraire séquentiellement les différents pools de pesticides (extractibles, non-extractibles et liés) adsorbés sur les sols afin de comprendre comment l'aging des pesticides se mets en place dans le temps. Différents protocoles existent pour ce faire et ont en commun l'utilisation de trois solutions d'extractions différentes (EFSA panel on plant protection products and their residues, 2015). Dans un premier temps les sols étudiés sont extraits avec une solution aqueuse de chlorure de calcium ($CaCl_2$) à 0,01 M. Puis ces sols sont mis en présence de solvants organiques dits doux ou forts. L'extraction douce se fait avec un mélange d'eau, de solvant dipolaire et d'acide organique comme par exemple une solution d'acétonitrile, d'eau et d'acide acétique. L'extraction forte se fait avec un mélange d'eau et d'acétone.

Comme décrit auparavant, la dégradation des pesticides peut être biotique ou abiotique. Afin de déconvoluer les deux voies, une stérilisation des sols peut être effectuée à l'aide de diverses méthodes comme l'autoclavage avant l'application de pesticides sur les sols (Sinegani and Hosseinpur, 2010). Pour évaluer les voies de transformation des pesticides il est important de réaliser des quantifications de leurs produits de dégradation (Figure 1-8).

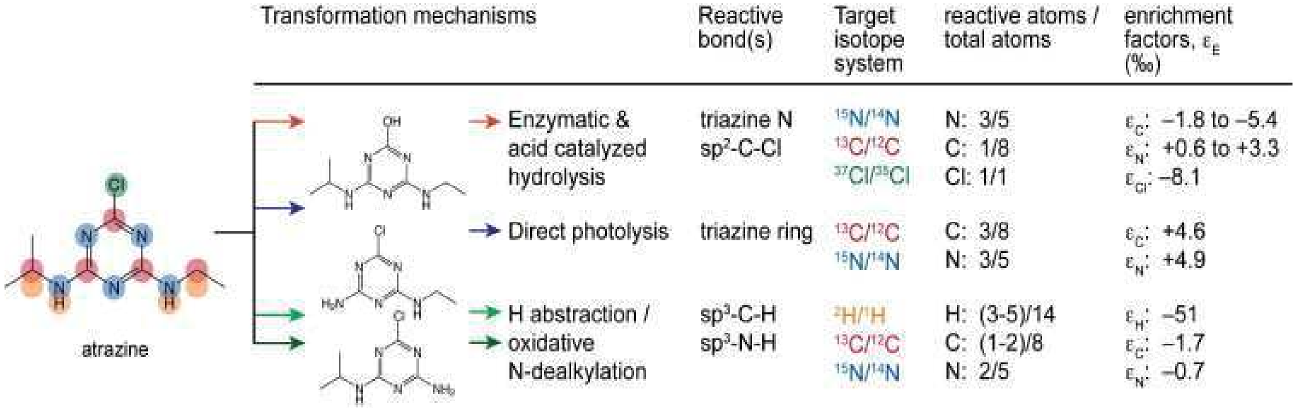
En plus de mesure des concentrations de pesticides parents et produits de dégradation, des analyses isotopiques par composés spécifiques (CSIA en anglais) peuvent également être réalisées.

Les pesticides contiennent des isotopes stables des éléments chimiques qui les composent dans une certaine proportion fixe (e.g. $^{15}N/^{14}N$, $^{13}C/^{12}C$, D/H). Les proportions des isotopes stables des différents éléments chimiques dans ces molécules est généralement peu variable, par exemple 98,93 % du carbone naturel est sous forme ^{12}C alors que 1,07 % est sous forme ^{13}C.

Les fractionnements isotopiques peuvent être à l'équilibre (dans des systèmes à l'équilibre physique ou chimique) ou cinétiques (dans les réactions (bio)chimiques à sens unique) (Young et al., 2002). Les pesticides subissent des processus destructifs et non-destructifs. Les fractionnements isotopiques générés par les processus non-destructifs sont à l'équilibre et très faibles contrairement aux processus destructifs qui sont bien plus importants et peuvent être suivis par CSIA (Elsner and Imfeld, 2016). Déterminer la proportion de chaque isotope stable dans une molécule a permis de réaliser de grandes avancées dans l'évaluation de la biodégradation de polluants *in situ* et la caractérisation fonctionnelle des communautés microbiennes impliquées (Manefield et al., 2002; Meckenstock et al., 2004; Schmidt et al., 2004). Plusieurs méthodes utilisent la chimie isotopique, couplée ou non à des approches biologiques, pour étudier le devenir des polluants. Parmi ces techniques, l'une des plus appliquées dans la caractérisation des sites pollués est la CSIA.

L'analyse CSIA consiste à caractériser et utiliser les signatures isotopiques des éléments au sein des molécules d'intérêt, ici les pesticides. Chaque polluant est en effet caractérisé par des ratios isotope lourd / isotope léger (e.g. $^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{15}N/^{14}N$) qui lui sont propres. Cette signature isotopique dépend du processus chimique de synthèse et/ou de l'origine du contaminant. Par exemple, l'origine des nitrates, provenant de fertilisants minéraux, des déjections animales ou de rejets de stations d'épuration, ont ainsi pu être différenciés en fonction de leur ratio $^{15}N/1^{4}N$ (Nestler et al., 2011 ; Fenech et al., 2012 ; Ohte, 2013), ouvrant ainsi la voie à des plans d'actions ciblés par usage.

L'analyse CSIA peut fournir des informations complémentaires aux analyses physico-chimiques et biomoléculaires sur la dégradation de polluants. Cette approche peut être en effet utilisée pour i) identifier la mise en place de la biodégradation, ii) quantifier son ampleur et iii) identifier les voies de dégradation prédominantes, comme illustré pour l'Atrazine dans la Figure 1-10.



Transformation mechanisms	Reactive bond(s)	Target isotope system	reactive atoms / total atoms	enrichment factors, ε_E (‰)
Enzymatic & acid catalyzed hydrolysis	triazine N sp^2-C-Cl	$^{15}N/^{14}N$ $^{13}C/^{12}C$ $^{37}Cl/^{35}Cl$	N: 3/5 C: 1/8 Cl: 1/1	ε_C: −1.8 to −5.4 ε_N: +0.6 to +3.3 ε_{Cl}: −8.1
Direct photolysis	triazine ring	$^{13}C/^{12}C$ $^{15}N/^{14}N$	C: 3/8 N: 3/5	ε_C: +4.6 ε_N: +4.9
H abstraction / oxidative N-dealkylation	sp^3-C-H sp^3-N-H	$^{2}H/^{1}H$ $^{13}C/^{12}C$ $^{15}N/^{14}N$	H: (3-5)/14 C: (1-2)/8 N: 2/5	ε_H: −51 ε_C: −1.7 ε_N: −0.7

Figure 1-10 : Mécanismes de transformation de l’Atrazine, liaisons réactives, systèmes isotopiques stables suivis pour l’analyse des processus de dégradation et nombre hypothétique d’atomes réactifs dans la molécule. La dernière colonne liste des facteurs d’enrichissement typiques obtenus pour les différentes réaction de dégradation (Hofstetter and Berg, 2011)

La CSIA a été largement développée et appliquée pour l'analyse du devenir des polluants organiques industriels dans l'environnement (Ahad et al., 2000; Griebler et al., 2004; Fischer et al., 2007; McKelvie et al., 2007; Bombach et al., 2010; Mundle et al., 2012). Un certain nombre d'études ont utilisé cette approche pour caractériser la biodégradation des solvants chlorés dans les aquifères pollués (Vieth et al., 2003; Meckenstock et al., 2004; Sherwood Lollar et al., 2010; Courbet et al., 2011; Bashir et al., 2015; Fischer et al., 2016). Cependant, cette approche reste très peu utilisée pour l'étude des pesticides et micropolluants dans les sols agricoles en raison de plusieurs verrous analytiques (purification et faibles concentrations initiales des analytes) et conceptuels (évaluation des sources de polluants diffus, transposition des applications communes dans les aquifères vers les sols et les bassins versants) (Elsner and Imfeld, 2016).

Les méthodes pour quantifier les pesticides et métaux dans les sols sont résumées dans la Table 1-2.

Table 1-2 : Quantification des métaux, pesticides et métabolites dans les sols

	Polluants	**Méthodes et solvants**	**Quantifications**
Sol total	Métaux	Mise en solution : *Acide nitrique (HNO_3)* *Acide chlorhydrique (HCl)* *Acide fluorhydrique (HF)* *Acide perchlorique ($HClO_4$)*	AAS ICP-AES et -MS RX
	Pesticides Métabolites	Extraction solide-liquide	GC- et LC-MS
Fractions « chimiques »	Métaux	Extraction solide-liquide sélective ou séquentielle : • *Echangeable* *Solution de chlorure ou nitrate diluée (< 1 M)* • *Acido-soluble* *Acide acétique* • *Réductible* *Chlorure d'hydroxylammonium* • *Oxydable* *Pyrophosphate de sodium ou potassium* *Acide nitrique (HNO_3) / acétate de sodium / eau oxygénée* • *Résiduel* *Acide nitrique (HNO_3)* *Eau régale ($^1/_3$ HNO_3 + $^2/_3$ HCl)*	AAS ICP-AES et -MS
	Pesticides	Extraction solide-liquide séquentielle : • *Extractible* *$CaCl_2$, 0,01 M* • *Non-extractible* *Eau / acétonitrile / acide acétique* • *Lié* *Eau / acétone*	GC- et LC-MS

3.3.3. Mobilité des polluants organiques et métaux

La mobilité des pesticides et métaux peut être suivie grâce à la mesure des concentrations dans les solutions de sol récupérées par centrifugation quand l’humidité des sols est suffisante. Dans le cas contraire, différents protocoles existent pour évaluer les teneurs de polluants dans cette phase. Généralement, cela s’effectue par extraction des sols en phase aqueuse ou des solutions salines faiblement concentrées (EFSA panel on plant protection products and their residues, 2015; Rao et al., 2008). La dissociation des pesticides et métaux dissous de ceux sous forme colloïdale s'effectue en isolant les colloïdes à l'aide de techniques d'ultrafiltrations et d'ultracentrifugations (Gooddy et al., 2007; Zhang et al., 2005).

D'autres méthodes peuvent être appliquées pour étudier la mobilité des pesticides et métaux dans les sols. L'utilisation de colorants fluorescents est en plein essor pour simuler notamment la mobilité des pesticides de synthèses dans des milieux naturels dont les sols en raison de leur comportement similaire (capacité à s'adsorber sur les sols et leur dissipation en présence de communautés microbiennes) (Lange et al., 2017). La méthode dite de « dilution isotopique » peut être utilisée pour évaluer la mobilité des métaux dans les sols. Cette méthode permet de suivre la quantité de métal en équilibre dynamique avec la solution de sol (Buekers, 2007). Des isotopes radioactifs de métaux sont appliqués dans des suspensions de sols. Après centrifugation, les quantités d'isotopes stables et radioactifs des métaux sont mesurées dans la solution obtenue. Le rapport d'isotopes radioactifs sur stables au cours du temps est alors déterminé et permet de suivre la mobilité des isotopes stables (et donc du métal étudié) dans les sols. Puis la proportion d'isotopes radioactifs par rapport à celle d'isotopes stables est suivie au cours du temps (Ma et al., 2006a).

Ces méthodes sont pertinentes pour l'évaluation des risques de transfert de pesticides ou de métaux dissous vers les plantes, les organismes vivants et les milieux aquatiques. Néanmoins lors de l'export horizontal, les eaux de ruissellement transportent les pesticides et métaux dissous, sous forme colloïdale mais aussi particulaire. Suivant la force des évènements pluvieux et la cohésion des agrégats à la surface des sols, ces derniers peuvent se rompre et diverses quantités de particules de sols (sables, limons, argiles) peuvent être entrainées dans les eaux de ruissellement. Les eaux de surfaces sont alors alimentées par des proportions multiples de particules du sol. Comme évoqué précédemment, les pesticides de synthèse et les métaux présentent des affinités différentes avec chacune de ces fractions granulométriques et leur export vers les milieux aquatiques représentent donc différents risques de pollution. Pour ces raisons, l'étude de la mobilité des polluants doit comprendre également le suivi des teneurs en pesticides et métaux dans les fractions granulométriques des sols (Babcsányi et al., 2016; Wang and Keller, 2008).

4. Impact des pesticides de synthèse et pesticides inorganiques dans les sols sur les cycles biogéochimiques des sols (et les organismes non-cibles)

Le métabolisme des plantes et des microorganismes est affecté lorsque les concentrations de métaux au sein des sols excèdent celles des fonds biogéochimiques (Lugauskas et al., 2005; Wyszkowska et al., 2012), mais aussi suite à l'introduction de pesticides de synthèse dans les sols (Imfeld and Vuilleumier, 2012; Lo, 2010).

Les végétaux cultivés sur des sols pollués aux pesticides et métaux présentent des croissances faibles en présence de fortes teneurs de pesticides ou suite à des pollutions métalliques. Les plantes ainsi cultivées peuvent avoir une transpiration réduite, floraison retardée mais aussi des maladies sur leurs feuilles, ou encore un système racinaire déformé (Borowik et al., 2017; Wyszkowska et al., 2012). Le prélèvement d'éléments comme les métaux par les plantes diffère suivant la nature des éléments présents et des possibles compétitions vis-à-vis de leur prélèvement (Tran and Popova, 2014). Les prélèvements par les plantes diffèrent également suivant les caractéristiques des sols mais aussi le niveau de contamination des sols. Avci and Deveci (2013) ont notamment montré de plus grands facteurs de transfert du sol aux végétaux du Cu, Zn et Mo (de 0,3 à 0,5) que de Ni, Cr, Co, Cd ou encore Pb (de 0,002 à 0,1) dans des maïs et autres plantes cultivées. Le prélèvement par les plantes d'éléments toxiques génère une perturbation des processus physiologiques au sein des plantes. La phytotoxicité des métaux envers les plantes repose sur cette perturbation (Wyszkowska et al., 2012). De plus, après l'application de pesticides est souvent observée une augmentation de la production d'exsudats par la couverture végétale recouvrant les sols (Diez et al., 2015).

L'impact de la pollution des sols aux pesticides et aux métaux diffèrent en fonction des microorganismes, notamment suivant leurs caractéristiques physiologiques, morphologiques et génétiques (Lo, 2010; Wyszkowska et al., 2012). Il a été observé un dérèglement des fonctions physiologiques des microorganismes, une dénaturation de leurs protéines et une destruction de leurs membranes cellulaires (Wyszkowska et al., 2012). Les effets variables des pesticides sur les communautés microbiennes peuvent dépendre du nombre d'application successive de pesticides mais aussi de conditions comme la température et la teneur en eau des sols (Shahgholi and Gholamalizadeh Ahangar, 2014). La mort des microorganismes sensibles aux polluants et la production de nouveau microorganismes avec le temps peut également expliquer la variabilité de la réponse des microorganismes après l'application de pesticides (Borowik et al., 2017).

En plus d'affecter les microorganismes des sols, les pesticides et métaux impactent également les enzymes produites par ces microorganismes. Les enzymes sont à l'origine d'un grand nombre de fonctions importantes dans les sols comme la dégradation des résidus de plantes ou la transformation des composés à base de N, P et S. Les métaux à l'état de trace sont importants pour le fonctionnement des enzymes dont les propriétés catalytique, structurale et régulatrice en dépendent. Les enzymes sont affectées par les métaux quand ceux-ci sont en forte concentration dans les sols. L'activité des enzymes mais aussi leur biosynthèse peuvent être impactées par les pollutions métalliques (Wyszkowska et al., 2012). Les pesticides et leurs métabolites affectent et surtout inhibent l'activité d'un grand nombre d'enzymes en modifiant les processus physiologiques des microorganismes comme la lyse des cellules ou encore la modification de la membrane des cellules (Borowik et al., 2017).

Les apports de pesticides et métaux dans les sols perturbent le fonctionnement de ces milieux. Ces deux types de contaminants affectent les microorganismes des sols et les végétaux qui a leur tour impactent le devenir de ces contaminants. L'activité microbienne peut être i) atténuée, ii) ne subir aucun effet, ou encore iii) être augmentée ponctuellement par cette double pollution (Imfeld and Vuilleumier, 2012). Ces effets impactent alors la dégradation des pesticides, la spéciation des métaux et/ou la production de matière organique à partir des résidus de végétaux. Ces derniers voient également leur croissance affectée et peuvent répondre en modifiant leurs productions d'exsudats impactant alors les formes de pesticides et métaux ainsi que leur transport dans les sols. La présence simultanée de pesticides et de métaux impacte également leurs fonctionnements respectifs avec notamment des compétitions pour les sites d'absorption suite à leur présence simultanée et affinité pour les mêmes constituants des sols (Lalah et al., 2009).

5. Effet des pratiques agricoles sur les polluants organiques et inorganiques dans les sols

La conversion des vignobles conventionnels vers l'agriculture biologique augmente (+121 % d'exploitations et +101 % de surface de 2007 à 2014 en France, Figure 1-12) et s'accompagne d'une augmentation du nombre de pratiques agricoles, comme le désherbage mécanique pour contrôler les adventices et l'application de produits phytosanitaires naturels (Agence Bio, 2015). En plus de préserver la qualité du vin et des produits agricoles, l'un des objectifs de l'agriculture biologique est de préserver la qualité des eaux et du sol. Cependant, peu d'études se sont penchées sur l'impact des pratiques viticoles en termes de fonctionnement hydrologique et biogéochimique (i.e. les processus cycliques de transfert d'éléments chimiques de l'environnement, tels que nutriments, éléments traces et polluants). L'agriculture conventionnelle utilise des produits chimiques tels que des fertilisants minéraux et des pesticides de synthèse, tandis que l'agriculture biologique (AB) autorise uniquement des fertilisants biologiques et minéraux naturels et quelques pesticides non synthétiques (Briar et al., 2007). En AB, l'application d'herbicides de synthèse est remplacée par une rotation pluriannuelle et diversifiée et un contrôle mécanique des adventices (travail du sol ou fauche), avec comme effet potentiel de limiter la perturbation liée à la pratique agricole sur l'environnement et notamment le sol. López-Piñeiro et al. (2013) ont notamment mis en évidence que la gestion d'une végétation naturelle améliore la qualité du sol par rapport au travail du sol en augmentant le carbone organique du sol, la teneur en eau, la biomasse et la biodiversité des populations microbiennes du sol. En effet, la plupart des sols viticoles sont considérés comme dégradés dû à l'érosion et à la diminution des réserves de nutriments (Martínez-Casasnovas et al., 2009), à l'accumulation de métaux (dont le Cu et le Zn) et de polluants organiques (Babcsányi et al., 2016; Komárek et al., 2010) ou de la compaction résultant des passages répétés de tracteurs (Lagacherie et al., 2006). Ces pratiques, liées à des itinéraires techniques évoluant en fonction des changements climatiques, peuvent affecter directement la mobilisation des métaux et des pesticides de synthèse à partir des sols vers les eaux.

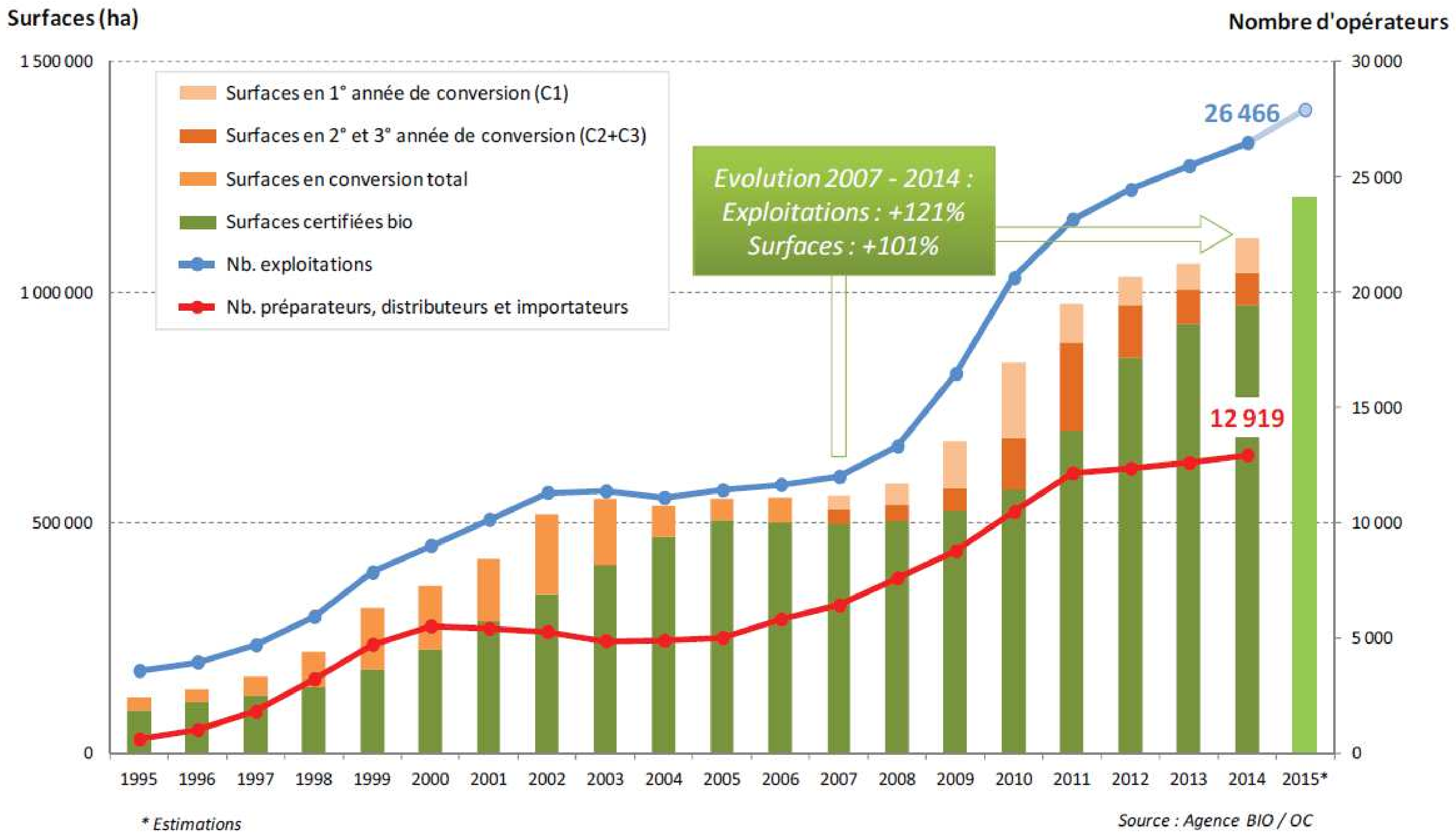


Figure 1-12 : Evolution en France depuis 1995 du nombre de producteurs et autres opérateurs bio ainsi que des surfaces en mode de production biologique (Agence Bio, 2015)

Cependant, très peu de recherches combinent des approches hydrologiques, géo- et hydro-chimiques et biologiques avec l'étude du développement de la vigne (alimentation, état sanitaire) pour évaluer globalement le fonctionnement des vignes et leur impact sur la qualité du vignoble. L'étude des données climatiques et des mesures *in situ* des termes hydrologiques (infiltration, ruissellement, érosion) permettent d'approcher le bilan hydrique des parcelles (Lefrancq et al., 2014). Le bilan hydrique peut être couplé à des indicateurs chimiques (quantité de matière organique, disponibilité de nutriments et de contaminants) afin d'estimer les stocks et les flux de solutés disponibles pour la croissance de la vigne et/ou impactant la qualité du sol (Bora et al., 2015; Duplay et al., 2014) ou des eaux (Gregoire et al., 2010).

Dans ce contexte, les expériences contrôlées en laboratoire peuvent également aider à contraindre et hiérarchiser les facteurs contrôlant l'exportation des polluants et à faciliter le développement conceptuel d'approches de modélisation, potentiellement utilisées sur le terrain par la suite (Banzhaf and Hebig, 2016).

6. Verrous scientifiques, questions de recherche et objectifs

Les pesticides de synthèse sont massivement utilisés sur les cultures pour contrôler les pathogènes comme le mildiou, les adventices ou encore les insectes ravageurs (Kelessidis and Stasinakis, 2012; Komárek et al., 2010; Urbaniak et al., 2016). Les boues de stations d'épuration des eaux contenant des métaux lourds sont également appliquées sur les cultures comme fertilisants (Hussain, 2002; Komárek et al., 2010), tandis que les intrants métalliques, tels que les fongicides cupriques, sont massivement utilisés en viticulture et arboriculture. Comme souligné précédemment, ces pesticides et ces métaux peuvent s'accumuler dans les sols agricoles à des concentrations toxiques pour les organismes non-cibles, et réagir avec l'eau des sols en relation avec les fractions granulométriques (sable, limon, argile) et les carbonates, oxydes, phyllosilicates, matière organiques ou microorganismes contenus dans les sols. Avec le temps, les pesticides et métaux introduits dans les sols subissent une série de processus qui diminuent leur disponibilité et mobilité (McGrath et al., 2010). Les pesticides subissent des processus de sorption/désorption, dégradation physique, chimique ou microbienne (Elsner, 2010). Les métaux quant à eux peuvent diffuser dans les pores des sols, entrer dans la phase solide des sols par précipitation en surface ou co-précipitation avec les oxydes métalliques et les carbonates (Schosseler et al., 1999).

Suivant les conditions hydrologiques et les pratiques agricoles, les pesticides et les métaux peuvent être exportés des sols vers les eaux de surface et les eaux souterraines par érosion de particules de sol et dissolution de pesticides et métaux adsorbés sur ces particules dans la phase aqueuse du ruissellement surfacique ou de sous-surface. Bien que les pesticides subissent des tests avant leur mise sur le marché, leur détection (notamment celle d'herbicides) dans les eaux souterraines souligne la difficulté d'extrapoler ces tests menés au laboratoire aux conditions réelles du terrain. L'état de l'art présenté ci-dessus reflète plusieurs limites des concepts et des approches couramment utilisées pour étudier, notamment :

(i) La dégradation des pesticides dans les sols sur de longues périodes et dans des conditions de variation hydro-climatique,

(ii) L'identification des zones et des périodes d'export et de dissipation préférentielle des pesticides et métaux à l'échelle du bassin versant,

(iii) L'effet de la co-contamination des sols par des pesticides de synthèse et des métaux.

Ainsi, il est nécessaire d'évaluer en détail les processus de dissipation *in situ* contrôlant le devenir des pesticides largement utilisés et les facteurs qui les régulent. Comme l'hydrologie influe fortement sur la biogéochimie des sols et est en lien avec les changements de régimes climatiques, comprendre l'impact des patrons de pluie sur la dissipation et l'export des polluants comme les pesticides et les métaux lourds dans les sols est également fondamental.

Ce travail de thèse a donc pour but d'améliorer la compréhension de la transformation et du transport des pesticides et métaux dans les sols agricoles en combinant une conceptualisation des processus à partir d'expériences en microcosmes de laboratoire et en faisant le lien avec l'observation à l'échelle du bassin versant. Cela passe notamment par l'étude spécifique de : i) la cinétique de distribution des pesticides et métaux entre les fractions granulométriques des sols et les processus à l'origine de ces distributions, ii) l'impact de la teneur en eau et de la température des sols sur le devenir des pesticides de synthèse, iii) l'impact des patrons hydrologiques sur l'export simultané de pesticides de synthèse et de métaux lourds dans les sols. L'influence des patrons hydrologiques sur le cycle des polluants sera finalement abordée par une étude de terrain menée sur le bassin versant expérimental de Rouffach (SOERE Recotox), particulièrement adapté et équipé pour l'étude du lien entre le forçage hydrologique et la mobilisation conjointe de métaux et de pesticides, du sol vers les eaux, à l'échelle du bassin versant.

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Chapitre 2 : Méthodologie

Ce second chapitre porte sur les approches générales et méthodologiques de cette thèse. Après une présentation des bassins versants étudiés, les techniques et analyses utilisées seront détaillées. A savoir, l'extraction et la quantification des pesticides et métaux, l'étude des spéciations physique et chimique des polluants grâce à des séparations granulométriques et extractions chimiques séquentielles, la mesure des signatures isotopiques des pesticides et métaux, ainsi que les étapes préalables nécessaires à l'obtention de ces mesures isotopiques.

1. Approche générale et méthodologique de la thèse

Comme présenté dans l'introduction de ce manuscrit, le devenir et le transfert des pesticides et métaux dans les compartiments environnementaux dépendent de leurs caractéristiques physico-chimiques, de la qualité des sols et des organismes présents *in-situ*. Le couvert végétal, les conditions climatiques et les pratiques agricoles sont aussi des facteurs « externes » importants pour appréhender le devenir de ces molécules ubiquistes. Afin d'améliorer la compréhension de la transformation et du transport des pesticides et métaux dans les sols agricoles, des expériences à l'échelle du laboratoire (i.e. microcosmes ou mésocosmes) et un suivi expérimental sur le terrain ont été combinés. Au laboratoire, des expériences en microcosmes ont permis de mieux appréhender les processus subis par les pesticides et les métaux dans les sols agricoles. Le suivi expérimental sur le terrain a permis quant à lui d'observer et de quantifier les flux de pesticides et métaux à deux échelles : de la parcelle agricole (quelques centaines de m^2) au bassin versant (quelques km^2).

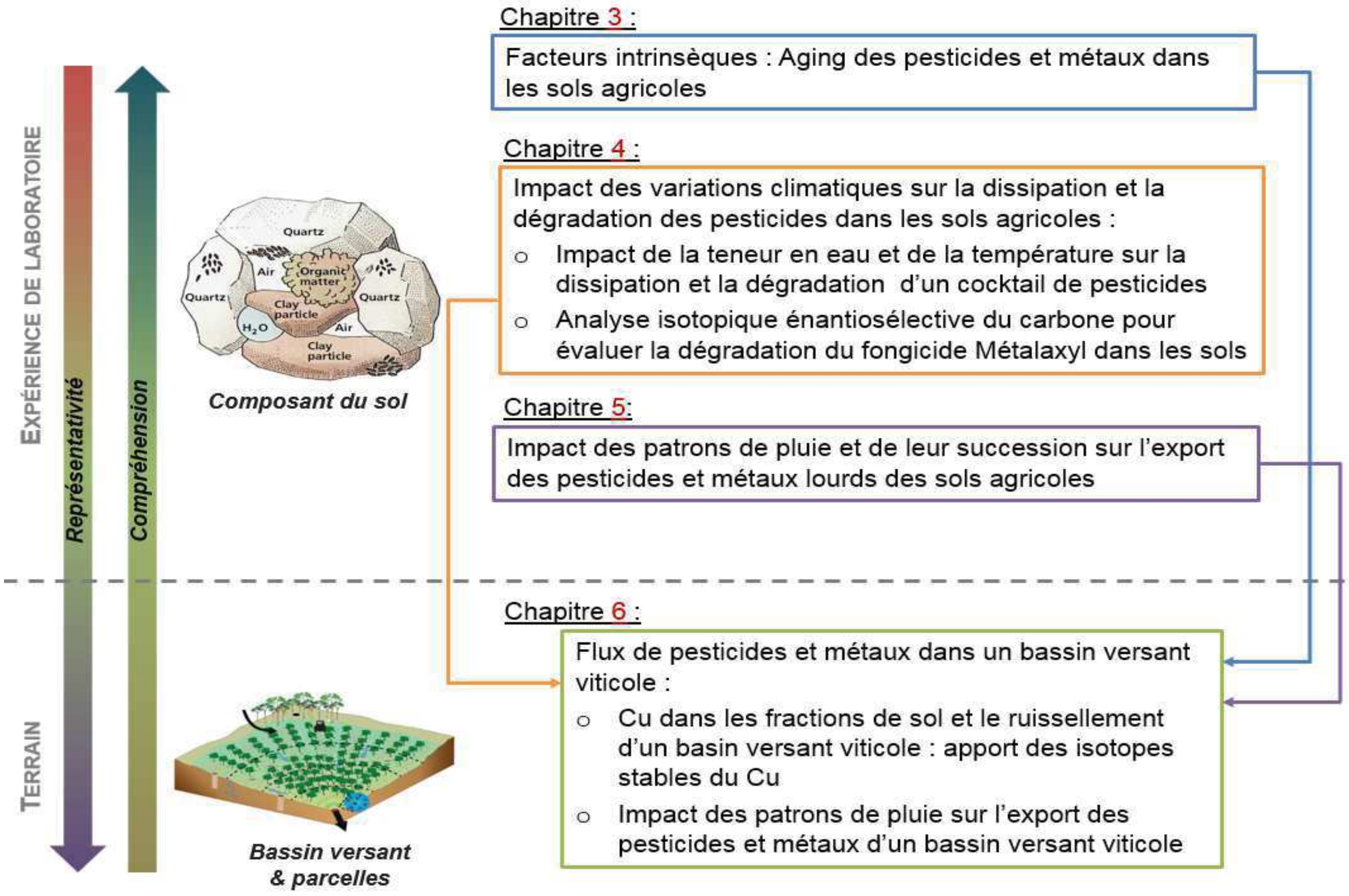
Les sites étudiés au cours de cette thèse et les méthodes utilisées sont présentés dans ce deuxième chapitre. Les chapitres suivants décrivent les études de laboratoire et de terrain qui ont été réalisées. Ainsi, l'impact des facteurs intrinsèques au sol sur la transformation des pesticides et métaux au cours du temps est discuté dans le chapitre 3. Plus précisément, il est présenté l'expérience d'aging de ces polluants dans deux sols agricoles et leur partage dans les fractions granulométriques et chimiques des sols. L'aging des polluants est suivi grâce à des mesures des concentrations (GC-MS et ICP-AES) mais aussi des signatures isotopiques des pesticides et métaux (CSIA à l'aide de GC-C-IRMS, $\delta^{65}Cu$ et $\delta^{66}Zn$ grâce au MC-ICP-MS).

L'objectif de ce chapitre est de souligner l'importance des constituants des sols (matière organique etc…) et de la présence simultanée de polluants organiques et minéraux (effet de la co-contamination) sur le devenir des pesticides et métaux dans les sols agricoles (Chapitre 3).

Dans le chapitre 4, l'impact des conditions et paramètres climatiques sur la persistance des pesticides de synthèse dans les sols agricoles est discuté en deux parties. Dans un premier temps est présenté l'impact de la teneur en eau des sols et la température sur la dissipation et la dégradation d'un mélange de pesticides. Une attention particulière est faite sur l'apport et le potentiel de l'analyse isotopique par composés spécifiques (CSIA) des pesticides pour évaluer, dans le futur, leur dégradation *in situ*, dans les sols. Dans une seconde partie, nous nous concentrons sur l'un des pesticides du mélange de pesticide utilisé : le fongicide chiral Métalaxyl. Pour ce composé, une analyse isotopique énantiosélective du carbone (ESIA) a été développée pour suivre précisément la dégradation énantiosélective de ce fongicide dans les sols (Chapitre 4).

Dans le chapitre 5, la dernière étude au laboratoire de ce travail de thèse est présentée. Cette expérience a été réalisée avec des microcosmes, plus précisément des colonnes de sols recevant une pluie « synthétique » afin d'évaluer l'impact des patrons de pluie et de leur succession sur l'export des pesticides et métaux des sols agricoles (Chapitre 5).

Les flux de pesticides et de métaux dans un bassin versant viticole sont évalués au cours du chapitre 6. Deux angles d'approches ont été privilégiés. Premièrement, une description précise du Cu dans les différentes fractions de sol et les ruissellements d'un bassin versant viticole est effectuée. Ce premier screening permet de tenter de répondre à la question « quelles informations apportent la mesure des isotopiques stables du Cu ? ». Ensuite, l'impact des patrons de pluie sur l'export des pesticides et des métaux en dehors de ce même bassin versant est évalué (Chapitre 6). Le schéma général de la thèse résumant l'ensemble des expériences effectuées est présenté dans la Figure 2-1, tandis que la Figure 2-2 présente l'approche méthodologique qui a été suivie au cours de ce travail de thèse.



Chapitre 3 :

Facteurs intrinsèques : Aging des pesticides et métaux dans les sols agricoles

Chapitre 4 :

Impact des variations climatiques sur la dissipation et la dégradation des pesticides dans les sols agricoles :

- Impact de la teneur en eau et de la température sur la dissipation et la dégradation d'un cocktail de pesticides
- Analyse isotopique énantiosélective du carbone pour évaluer la dégradation du fongicide Métalaxyl dans les sols

Chapitre 5:

Impact des patrons de pluie et de leur succession sur l'export des pesticides et métaux lourds des sols agricoles

Chapitre 6 :

Flux de pesticides et métaux dans un bassin versant viticole :

- Cu dans les fractions de sol et le ruissellement d'un basin versant viticole : apport des isotopes stables du Cu
- Impact des patrons de pluie sur l'export des pesticides et métaux d'un bassin versant viticole

Figure 2-1 : Approche générale de la thèse

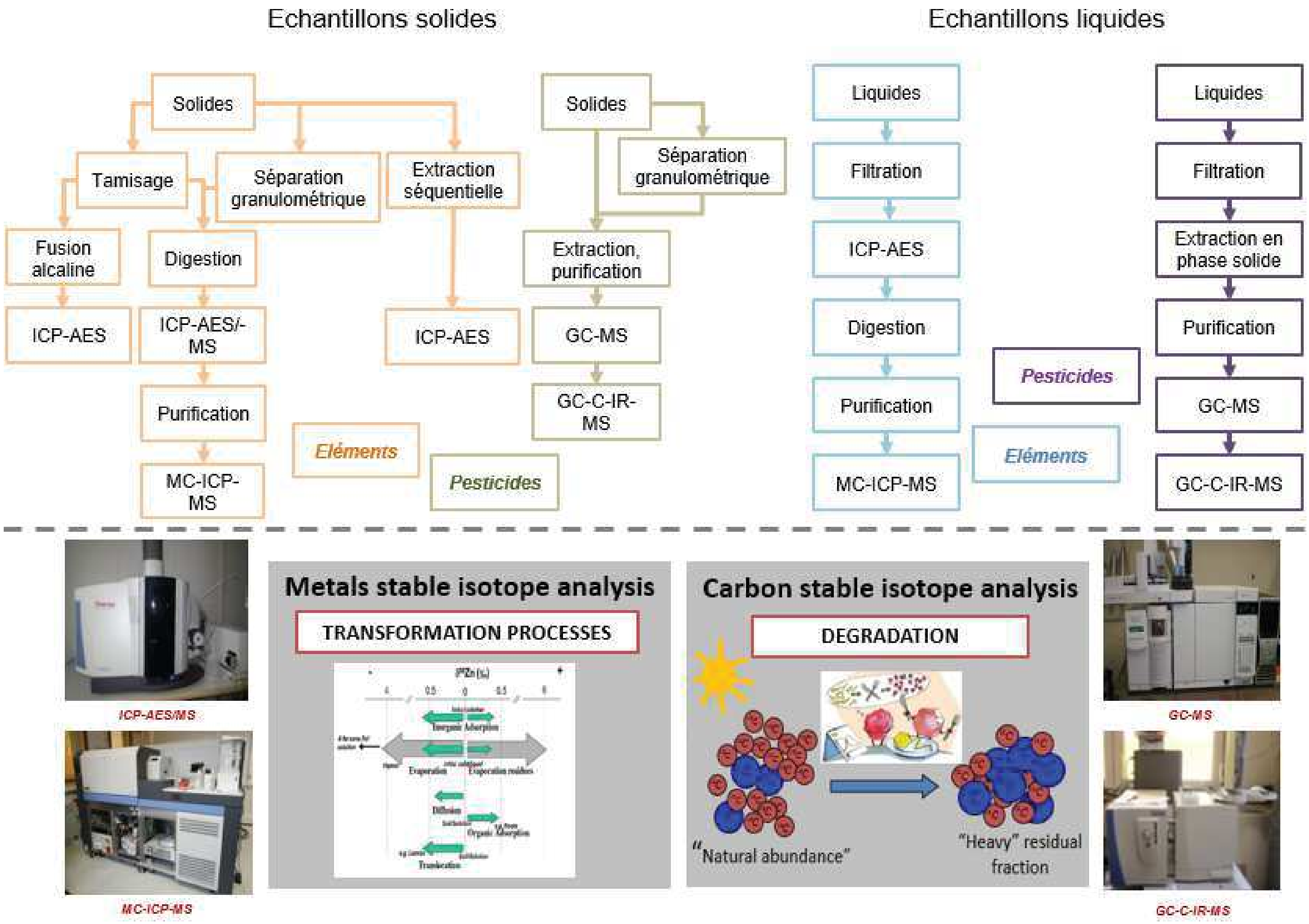


Figure 2-2 : Approche méthodologique suivie au cours de la thèse

Reprenant les résultats principaux des chapitres précédents, une conclusion générale du travail de thèse est réalisée dans le chapitre 7, à la fin duquel des perspectives de travaux futurs sont présentées (Chapitre 7). A la fin du manuscrit, en perspective également, une dernière étude à laquelle j'ai contribuée développe l'étude du devenir des métaux après leur export de bassins versants agricoles, suite à leur sédimentation dans des systèmes aquatiques (Chapitre 8).

2. Bassins versants étudiés

2.1. Bassin versant viticole

Le site étudié est un bassin versant viticole de 42,7 ha situé dans le piémont alsacien (Haut-Rhin, France; 47°57′9 N, 07°17′3 E) (Figure 2-3) (Gregoire et al., 2010). Ce bassin versant est recouvert de vignes (59 %), forêt et pâturages (29 %), bandes enherbées et fossés (7 %), mais aussi de routes et chemins (5 %) (Lefrancq et al., 2013). Après tamisage, le sol moyen présente une densité de 1.05 $g.cm^{-3}$. Ce sol contient 8.4 % de sables, 23.1 % de limons et 68.5 % d'argiles. Il est de type Cambisol Hypereutic Clayic et Cambisol Calcaric Siltic (Duplay et al., 2014). Ce sol calcaire limoneux argileux a un pH de 8.1 et contient 271 $g.kg^{-1}$ de $CaCO_3$, 16.7 $g.kg^{-1}$ de matière organique, pour une capacité d'échange cationique de 15.4 $cmol^{+}.kg^{-1}$. La pente moyenne de ce bassin versant est de 15 %. L'érosion au niveau des parcelles viticoles est limitée grâce à des bandes enherbées à l'aval et un enherbement tous les deux rangs. Pour augmenter l'infiltration de l'eau dans les sols, le sol des bandes non enherbées est labouré chaque année sur 15 cm (Babcsányi et al., 2016).

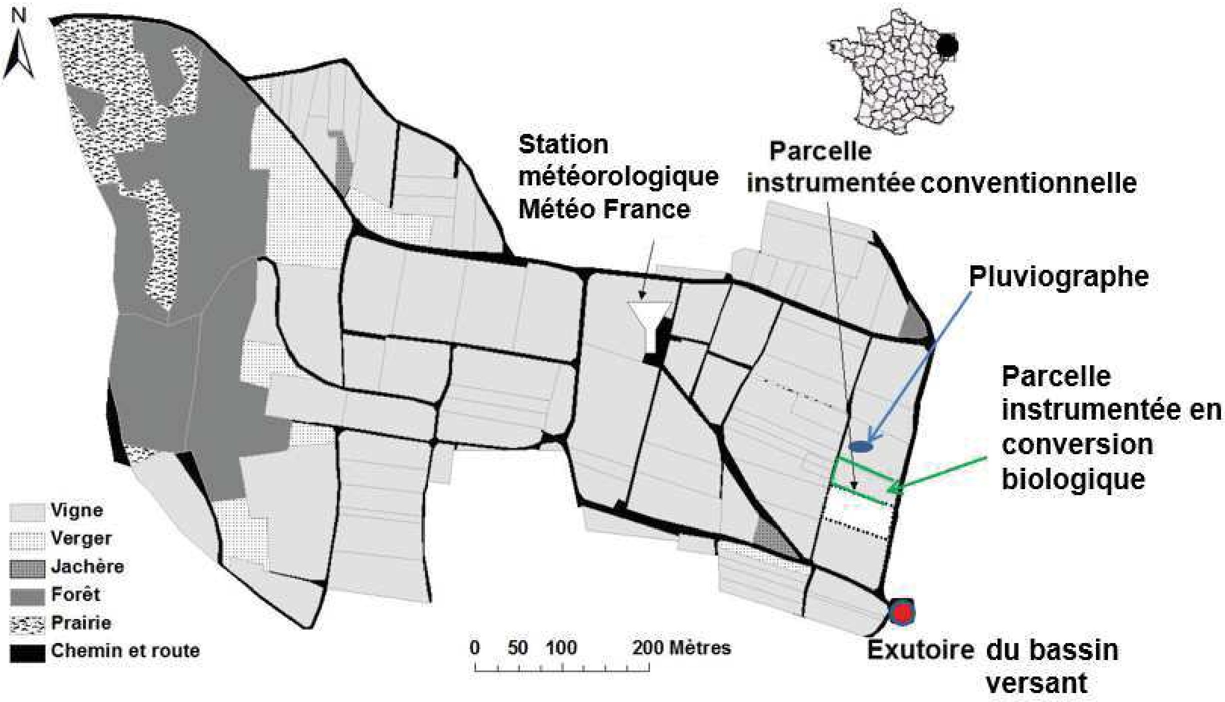


Figure 2-3 : Caractéristiques et occupation du sol du bassin versant viticole étudié

Une station météorologique présente sur le bassin permet de suivre précisément le vent, les températures et les évènements pluvieux. Les pluies entre avril et octobre sont en moyenne de 438 ± 86 mm (1998-2016 ; 372 mm en 2015). Les évènements pluvieux ne génèrent pas de ruissellement permanent sur ce bassin versant. En 2015, un coefficient de ruissellement moyen de 1 ± 0.86 % a été obtenu sur ce bassin. Le processus de ruissellement est de type Hortonien lorsque l'intensité des pluies dépasse la capacité d'infiltration des routes, chemin ou parcelles. Le ruissellement est contrôlé par le réseau routier lorsque les pluies sont de faible intensité (i.e. < 6 mm h^{-1}) (Lefrancq et al., 2014). Lorsque l'intensité des précipitations dépasse la conductivité hydraulique à saturation du sol (soit 58 mm h^{-1}, $n = 48$) le ruissellement observé sur le bassin versant provient majoritairement des parcelles de vigne (Tournebize et al., 2012).

Le dispositif suivant permet de suivre les eaux de ruissellement aux parcelles et à l'exutoire du bassin versant :

L'exutoire de ce bassin versant est instrumenté d'un canal venturi muni d'une canne de bullage, le tout relié à un débitmètre (Hydrologic, Sainte-Foy, Canada). Cette installation permet de mesurer les débits d'eau qui ruissellent à l'exutoire du bassin versant. A ce dispositif est relié un préleveur réfrigéré (4010 Hydrologic automatic sampler, Sainte-Foy, Canada) muni de carrousels. Ce préleveur est asservi au débitmètre. Ainsi il est possible de programmer le préleveur pour que 3 échantillons simultanés de 100 mL soient prélevés tous les 6 m^3 d'eau de ruissellement en sortie du bassin versant. Ceci représente l'asservissement mis en place au cours des campagnes d'échantillonnage. Les carrousels présents dans le préleveur permettent de discrétiser les évènements ruisselants.

Deux parcelles de ce bassin versant, une parcelle conventionnelle (applications de pesticides, herbicides, fongicides cupriques et soufrés) et une seconde en conversion biologique (où seuls des fongicides cupriques et soufrés sont utilisés) sont instrumentées. En détails, ces parcelles sont séparées au sol par une bordurette en polyéthylène (PE) d'une hauteur de 15 cm et sur 15 cm de profondeur. Des gouttières en béton à l'amont des parcelles permettent de protéger les parcelles suivies du ruissellement en tête du bassin versant. Ces parcelles sont munies, comme à l'exutoire du bassin versant, de canaux venturi, de débitmètres (DLK 102 ISMA, Forbach, France) et de préleveurs réfrigérés munis de carrousels (ISCO, Ponsel, Caudan, France). L'asservissement suivi au cours de la campagne a été de 100 mL d'eau de ruissellement prélevé tous les 3 L. A ce dispositif s'ajoute des plaques lysimétriques en Téflon (Conceptseal, Ernolsheim, France) et des sondes de teneur en eau (CR200X, Campbell Scientific, Anthony, France). Les plaques lysimétriques permettent de collecter l'eau du sol. Les sondes de teneur en eau permettent de suivre l'évolution de l'humidité des sols mais aussi les températures au sein de celui-ci tout au long des campagnes d'échantillonnage. Les plaques lysimétriques comme les sondes de teneur en eau sont installées sur chaque parcelle à 40 et 80 cm de profondeur. Les installations permettant de suivre les eaux de ruissellement aux parcelles sont présentés dans la Figure 2-4.

Figure 2-4 : Instrumentation présente sur les parcelles conventionnelle et biologique du bassin versant viticole. A) barrière en polyéthylène entre les parcelles, B) gouttière interceptant l'eau de ruissellement aux parcelles, C et D) canaux venturi et bidons de récupération des eaux de ruissellement, E) préleveur réfrigéré et débitmètre

En parallèle du prélèvement des eaux de ruissellement et d'infiltration (1 fois par semaine), sont échantillonnés le sol de surface de la parcelle biologique (1 fois par mois), le sol de surface de la parcelle conventionnelle mais aussi le sol de l'ensemble du bassin versant (1 fois par semaine). Des quantifications de pesticides, éléments majeurs et traces dont les métaux sont réalisés sur ces échantillons. Le sol de ce site a également été prélevé pour conduire des expériences en microcosmes au laboratoire. Environ 50 kg de sol de la parcelle conventionnelle suivie ont été échantillonnés le long des rangs non-enherbés, tous les deux rangs sur 7 rangs.

2.2. Bassin versant de grande culture

Le sol d'un second bassin versant agricole a été échantillonné pour conduire des expériences en microcosmes au laboratoire. Il présente des caractéristiques différentes (i.e.

physico-chimie, historique d'application des pesticides, etc…) du sol viticole permettant une comparaison. Situé à 30 km au nord-est de Strasbourg (Bas-Rhin, France; 48 47 19,56 N; 7 35 2,27 E), ce bassin versant de 47 ha présente une pente de 6,7 % (Figure 2-5) (Lefrancq et al., 2017b). De la betterave sucrière (70 %) et du maïs (18 %) sont principalement cultivés sur ce bassin versant. Le réseau de routes occupe quant à lui 3,5 % de la surface totale. Le sol de ce bassin a une densité de 0.99 g.cm^{-3} après tamisage et est composé de 10.3 % de sable, 61.5 % de limons et 28.3 % d'argiles. Trois types de sol sont présents sur ce bassin. Haplic Cambisol Calcaric Siltic et Cambisol Eutric Siltic au nord et au sud du bassin versant. Cambisol Colluvic Eutric Siltic au centre du bassin. Le sol du bassin versant contient en moyenne 8.1 g.kg^{-1} de $CaCO_3$, 55.2 g.kg^{-1} de matière organique, un pH de 6.97 et une CEC de 9.1 cmol^{+}.kg^{-1}. L'eau ruisselle jusqu'à l'exutoire du bassin versant et entre dans une conduite de 50 cm de diamètre instrumentée d'un débitmètre « Doppler » (2150 Isco, Lincoln, Nebraska, USA) et d'un préleveur (ISCO, Ponsel, Caudan, France) dont les instruments sont stockés sur le bassin versant dans une cabane prévue à cette effet (Figure 2-6). Pour réaliser les microcosmes en laboratoire, environ 50 kg de sol ont été échantillonnés entre les rangs des parcelles du bassin versant, tous les deux rangs sur 25 rangs.

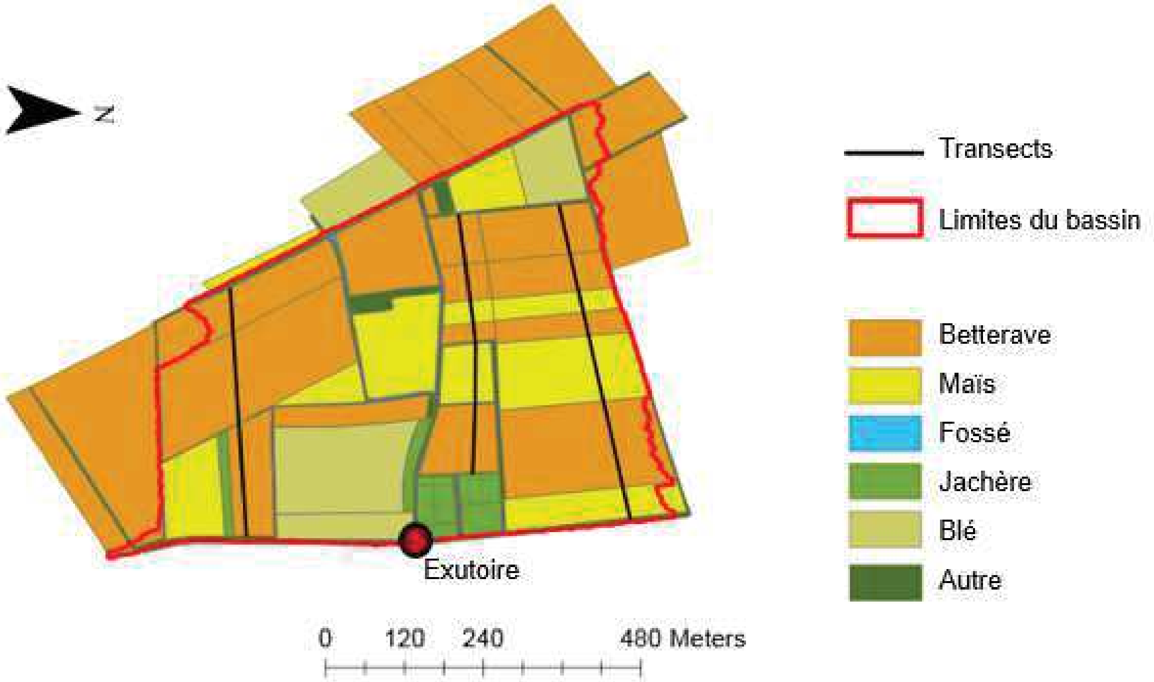


Figure 2-5 : Caractéristiques et occupation du sol du bassin versant de grande culture étudié

Figure 2-6 : Instrumentation présente sur le bassin versant de grande culture

Les caractéristiques des sols des bassins versants de grande culture et viticole sont résumés dans le tableau suivant (Table 2-1).

Table 2-1 : Caractéristiques des sols agricoles utilisés

Parameter	Unit	Crop soil	Vineyard soil
Bulk density* (ρ_b)	[g.cm^{-3}]	0.99	1.05
Clay	[%]	28.3	68.5
Silt	[%]	61.5	23.1
Sand	[%]	10.3	8.4
OM Loss on ignition	[%]	5.52	1.67
OC Black & Walkley	[%]	1.18	0.84
Al_2O_3	[%]	11.2	7.49
$CaCO_3$	[%]	0.81	27.1
Fe_2O_3	[%]	6.66	3.86
MnO	[%]	0.14	0.1
Cu	[mg.kg^{-1}]	48	171
Zn	[mg.kg^{-1}]	77	81
Metalaxyl	[mg.kg^{-1}]	0.21	0
S-metolachlor	[mg.kg^{-1}]	0.40	0.41
CEC	[cmol^{+}.kg^{-1}]	9.1	15.4
pH	[-]	6.97	8.1

*Après tamisage

3. Extraction et quantification des pesticides et métaux

3.1. Pesticides

La quantification des pesticides dans les eaux est réalisée après extraction en phase solide des pesticides dissous avec un système AutroTrace 280 SPE (Dionex®, CA, USA) permettant de traiter simultanément 6 échantillons. Brièvement, les échantillons d'eau (de 100 mL à 1 L suivant ruissellements obtenus sur le terrain) passent sur des cartouches SolEx C18 (Dionex®, CA, USA) remplies avec 1 g de silice. Ces cartouches sont préalablement lavés avec 5 mL d'éthanol (EtOH) suivi de 5 mL d'éthyle acétate (EtAc), puis conditionnées avec 5 mL d'eau déminéralisée. Puis les échantillons d'eau sont injectés sur les cartouches retenant les molécules d'intérêts en fonction de leurs polarités. Pour une meilleure extraction, la cartouche est ensuite séchée sous flux d'azote (10 min) et l'élution des pesticides étudiés se fait ensuite par 5 mL d'EtAc et 5 mL d'acétonitrile (ACN). Les extraits obtenus sont ensuite concentrés par évaporation du solvant (mixture EtAc + ACN) sous flux d'azote jusqu'à la dernière goutte. Enfin ces extraits concentrés sont mis en solution dans 500 à 1000µL d'acétonitrile (ACN) (Elsayed et al., 2014). Les extraits sont conservés à -18°C en attendant leur analyse.

Les pesticides présents dans la phase solide des sols sont quantifiés après une extraction solide-liquide adaptée de précédentes études (Anastassiades et al., 2003; Ivdra et al., 2014). Brièvement, 5-10 g de sol (brut, sans préparation préalable) est mis en présence d'EtAc dans un tube Falcon (50 mL) suivant un ratio 2:1 sol:solvant. Ce mélange est agité 15 s à l'aide d'un agitateur-vortex puis placé dans un bain à ultrasons pendant 5 min. Une quantité d'eau ultra-pure est ensuite ajoutée pour atteindre un sol avec une teneur en eau de 80 % (définie selon la teneur initiale du sol). Après une nouvelle agitation de 1 min, le mélange est centrifugé pendant 5 min à 5000 tours par minute (rpm) permettant la séparation et le prélèvement de la phase liquide organique. L'opération est répétée deux fois sans nouvel ajout d'eau ultra-pure. Les phases liquides organiques sont « poolées » et concentrées sous flux d'azote jusqu'à la dernière goutte, remises en solution dans 500-1000 µL d'ACN et mises en contact de sulfate de magnésium $MgSO_4$ anhydre pour en assécher les possibles traces d'eau. Une étape supplémentaire de purification des échantillons est effectuée avec l'ajout de 12,5 mg d'amine (PSA, primary secondary amine) et 75 mg de $MgSO_4$ anhydre aux 500 µL d'échantillons, agités

30 s et centrifugés 1 min à 5000 rpm. Les extraits sont conservés à -18°C en attendant leur analyse.

Un mélange de 20 pesticides a été étudié au cours de cette thèse. Ce mélange est composé de 9 fongicides (azoxystrobin, carbendazim, cymoxanil, cyprodinil, dimethomorph, krésoxim-méthyle, metalaxyl, pyrimethanil et tetraconazole) 10 herbicides (atrazine, diuron, isoproturon, isoxaben, oryzalin, pendimethalin, pyraflufen-éthyle, simazine, S-metolachlor et terbuthylazine) et 1 insecticide (flufenoxuron). En dehors du S-metolachlor, ces pesticides ont été choisis car ils ont été régulièrement utilisés par les viticulteurs du bassin versant viticole étudié. L'herbicide S-metolachlor est couramment utilisé par les agriculteurs du bassin versant de grande culture étudié. Suivant la polarité de ces composés, certains ont été suivis en GC-MS, d'autres en LC-MS.

La quantification de l'atrazine (ATR), azoxystrobin (AZO), cyprodinil (CYP), diméthomorph (DIM), krésoxim-méthyle (KM), métalaxyl (MTY), pendimethalin (PEN), pyraflufen-éthyle (PRY), pyriméthanile (PYR), simazine (SIM), terbuthylazine (TER), tétraconazole (TZL) et S-métolachlore (SMET) est réalisée par chromatographie gazeuse (Trace 1300, Thermo Fisher Scientific) couplée à de la spectrométrie de masse (ISQ, Thermo Fisher Scientific) (GC-MS), équipée avec une colonne TG-5MS (30 m × 0.25 mm de diamètre interne et une épaisseur de 0.25 μm), avec de l'hélium comme gaz vecteur à un débit de 1.5 mL.min^{-1}. Le four du GC est maintenu à 50 °C pendant 1 min, mis à 160 °C à 30 °C.min^{-1}, puis 220 °C à 4 °C.min^{-1} et 300 °C pendant 1 min à 30 °C.min^{-1}. 1,5 µL d'échantillon est alors injecté dans un injecteur split/splitless fonctionnant en mode split avec un flux de split à 6,0 mL.min^{-1} et maintenu à 280 °C. La ligne de transfert et la source de la masse sont chauffées à 320 °C. Le metolachlor d11 est utilisé comme standard interne. Les limites de détection et de quantification des composés suivis au GC-MS sont résumées dans la table suivante (Table 2-2). Les scans et rendements d'extraction de ces composés sont résumés dans la Table 2-3.

Table 2-2 : Limites de détection (LD) et quantification (LQ) instrumentale des pesticides suivis au GC-MS

	LD [µg.L^{-1}]	**LQ [µg.L^{-1}]**
ATR	11.5	34.8
AZO	7.7	23.4
CYP	4.3	13.0
DIM	24.9	75.8
KM	5.3	16.0
MTY	6.6	20.0
PEN	4.5	13.8
PRY	9.4	28.5
PYR	12.7	38.5
SMET	10.1	30.7
SIM	4.4	13.5
TER	9.3	28.2
TZL	6.3	19.3

Table 2-3 : Temps de rétentions et rendements d'extractions des pesticides quantifiables par GC-MS

	RT [min]	**Recovery [%]**	**SD [%]**
ATR	17.26	61.1	1
AZO	32.88	21.6	2
CYP	24.34	33.8	12
DIM	32.96	51.4	14
KM	26.22	2.2	1
MTY	22.43	67	3
PEN	24.53	5.7	3
PRY	27.44	3.6	1
PYR	18.74	55.4	6
SMET	16.96	23	9
SIM	23.48	23	2
TER	18.18	13.1	2
TZL	23.96	16.5	6

Les concentrations de carbendazim (CAR), cymoxanil (CYM), diuron (DIU), flufenoxuron (FLU), isoproturon (ISP), isoxaben (ISX) et oryzalin (ORY) ont été mesurées par LC-MS (TSQ Quantum Access Max, Thermo Scientific, Waltham, Massachusetts, USA). La phase mobile (0.3 ml.min^{-1}) est constituée d'un gradient d'ACN (30 to 95 %) et d'eau (70 to 5 %) à 4500 V. La dernière étape est maintenue pendant 3 min avant le retour à l'état initial pendant 6 min. A chaque fois, 20 µL de chaque échantillon est injecté dans une colonne EC 150/3 Nucleodur C18 Pyramid 3 µm. L'isoproturon d6 est utilisé comme standard interne. Les Tables 2-4 et 2-5 résument les scans, rendements mais aussi les limites de détection et quantification.

Table 2-4 : Temps de rétention et rendements des pesticides étudiés au LC-MS

	Parent mass	**Transition 1**	**Transition 2**	**RT [min]**	**Recovery in soil [%]**	**Recovery in water [%]**
CAR	192	160	132	2	23	41
CYM	199	128	83	7.3	29	56
ISP	207	72	165	13.9	105	98
DIU	233	72	188	14.3	80	91
ISX	333	165	107	17.4	23	48
ORY	347	288	305	17.8	30	82
FLU	489	158	141	21.5	172	33
ISP*d6*	213	78	171	13.8	-	-

Table 2-5 : Limites de détections et de quantification des pesticides étudiés au LC-MS

	LOD in soil [µg.g^{-1}]	**LOQ in soil [µg.g^{-1}]**	**LOD in water [µg.L^{-1}]**	**LOQ in water [µg.L^{-1}]**
CAR	0.17	0.5	0.01	1
CYM	0.17	0.5	0.01	1
ISP	0.17	0.5	0.003	0.01
DIU	0.17	0.5	0.003	0.01
ISX	0.17	0.5	0.01	1
ORY	0.17	0.5	1	5
FLU	0.5	5	0.01	25
ISP*d6*	-	-	-	-

Les formules des pesticides étudiés sont présentées dans la Figure 2-7. Les caractéristiques de ces composés sont résumées dans le Table 2-6.

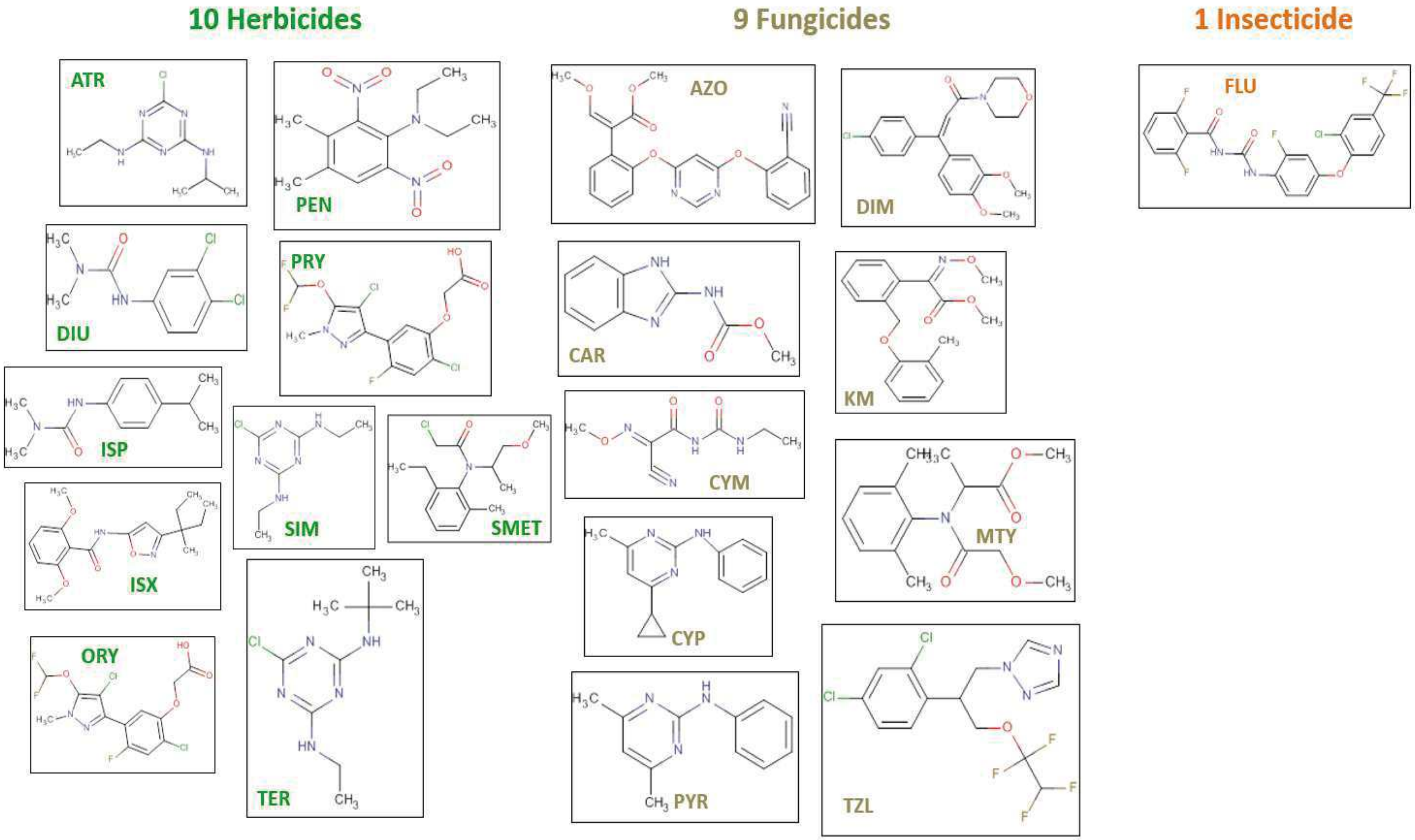


Figure 2-7 : Formules des pesticides du mélange utilisé pour doper les sols des microcosmes

Table 2-6 : Caractéristiques physico-chimiques principales des pesticides présents dans le mélange utilisé pour doper les sols des microcosmes

			Solubility [mg.L^{-1}]	**K_{oc}** [mL.g^{-1}]	**$K_{d\ crop}$** [mL.g^{-1}]	**K_{d} vineyard** [mL.g^{-1}]	**$DT_{50,th}$** [days]
Sorptive and hydrophobic	FLU	$C_{21}H_{11}ClF_6N_2O_3$	0.0043	157643	1863	1319	42
	PRY	$C_{13}H_9Cl_2F_3N_2O_4$	0.1	1949	23	16.3	0.3
Hydrophobic	PEN	$C_{13}H_{19}N_3O_4$	0.3	15744	186	132	90
	ORY	$C_{12}H_{18}N_4O_6S$	1.1	807	9.5	6.8	45
	ISX	$C_{18}H_{24}N_2O_4$	0.9	351	4.1	2.9	105
	SIM	$C_7H_{12}ClN_5$	5	340	4	2.8	60
	TER	$C_9H_{16}ClN_5$	6.6	219	2.6	1.8	75
	ATR	$C_8H_{14}ClN_5$	35	93	1.1	0.8	75
	DIU	$C_9H_{10}Cl_2N_2O$	35.6	499	5.9	4.2	76
Moderately hydrophobic	ISP	$C_{12}H_{18}N_2O$	70.2	122	1.4	1.02	12
	DIM	$C_{21}H_{22}ClNO_4$	29	1360	16.1	11.4	57
	CYP	$C_{14}H_{15}N_3$	13	1470	17.4	12.3	37
	PYR	$C_{12}H_{13}N_3$	121	301	3.6	2.5	55
	AZO	$C_{22}H_{17}N_3O_5$	6.7	581	6.9	4.9	78
	KM	$C_{18}H_{19}NO_4$	2	437	5.2	3.7	16
	CAR	$C_9H_9N_3O_2$	8	223	2.6	1.9	40
Sorptive and hydrophilic	TZL	$C_{13}H_{11}Cl_2F_4N_3O$	157	4680	55.3	39.2	61
	SMET	$C_{15}H_{22}ClNO_2$	480	185	2.2	1.5	15
Hydrophilic	CYM	$C_7H_{10}N_4O_3$	780	44	0.5	0.4	0.7
	MTY	$C_{15}H_{21}NO_4$	7100	163	1.9	1.4	42

3.2. Métaux

Contrairement aux molécules organiques, la quantification des métaux se fait après digestion des sols. Une préparation préalable des sols est nécessaire et consiste en leur séchage à l'étuve à 60 °C puis broyage et tamisage à 100 µm. La digestion de la matrice solide des sols est réalisée sous hotte à flux laminaire en salle blanche (classe ISO 6) dont l'air est filtré grâce à une batterie de filtres (Campfil 14743700, G4 ; 13687200, F7 et 3484.10.40, H14, USA).

Environ 100 mg d'échantillons de sol sec sont introduits dans des récipients en Téflon, Savilex préalablement lavés avec des bains successifs de 1:5 HCl concentré : eau ultra-pure (1 x 24 h) et d'eau ultra-pure (2 x 24 h). 1 mL d'acide nitrique distillé HNO_3 (15N) et 3 mL d'acide fluorhydrique HF (X N) supra-pur sont ajoutés dans ces récipients. Ces mélanges sont mis dans un bain à ultrasons pendant 15 min avant d'être placés sur plaque chauffante à 100 °C pendant 12 h afin de digérer la matrice silicatée des sols. Puis 1 mL d'acide perchlorique distillé est introduit dans les échantillons avant évaporation des acides HNO_3 et HF à 70 °C pendant 24 h. Pour digérer la matière organique présente dans les sols, les échantillons sont chauffés à 120 °C pendant 3 h puis à 150 °C pendant 48 h. Pour finir la digestion, 1 mL d'acide borique H_3BO_3 et 4 mL d'acide chlorhydrique HCl à 3 M sont introduits dans les échantillons. Ces mélanges sont chauffés à 100 °C pendant 5 h. Après vérification visuelle de l'absence de particules solides dans les solutions obtenues, les échantillons sont évaporés à 120 °C pendant 4 h puis 150 °C le temps nécessaire (environ 12 h). Ensuite 5 mL d'acide chlorhydrique à 7 M sont introduits sur les résidus secs pour les solubiliser. Les échantillons sont alors mis dans un bain à ultrasons pendant 30 min puis mis sur plaque chauffante à 70 °C pendant 12 h. Le temps global de l'étape de minéralisation des sols est de 5 jours. Les éléments majeurs et traces dont les métaux présents dans ces échantillons sont quantifiés par ICP-AES (ICAP6500, Thermo Fisher Scientific) après dilution de 100 µL de ces échantillons dans 10 mL d'acide nitrique HNO_3 à 0.5 M. Pour contrôler la procédure de digestion, les standards de roches BCR-2 (US Geological Survey, Reston, VA, USA) et SCL-7003 (Analytika, Prague, Czech Republic) sont digérés en même temps que les échantillons (Babcsányi et al., 2014). Une incertitude analytique de ± 5 % est obtenue comprenant la précision et la reproductibilité. Les limites de détection pour le Cu et le Zn sont quant à elles de respectivement 3 et 1 $\mu g.L^{-1}$.

4. Spéciation physique (classes granulométriques)

Les contaminants dont les pesticides et les métaux sont exportés sous forme dissoute et particulaire dans les eaux de ruissellement. Ces eaux transportent des particules de différentes classes granulométriques (sables, > 50 µm ; limons, 2-50 µm ; et argiles, < 2 µm) suite à l'érosion des sols au cours des évènements pluvieux (Martínez-Casasnovas et al., 2002; Mohamadi and Kavian, 2015). Or les pesticides et métaux présentent des affinités différentes avec les minéraux constituant les sables, limons et argiles (Clausen et al., 2001; Cornejo et al., 2008; Đurović et al., 2009; Ma and Uren, 1998; Pokrovsky et al., 2008; Remucal and Ginder-Vogel, 2014).

Pour ces raisons, il semble important de séparer les fractions granulométriques des sols et d'en étudier les proportions de pesticides et métaux. Pour ce faire, plusieurs protocoles ont été développés comprenant des étapes de tamisage, sédimentation à l'aide de la loi de Stokes (méthode de la pipette) (Buchan et al., 1993). De plus, le recours à la sonification est couramment utilisé pour aider à la séparation des classes granulométriques (Genrich and Bremner, 1974). Cependant, cette technique est source de dommages physiques et chimiques suivant l'énergie appliquée au cours de la procédure (Kaiser and Asefaw Berhe, 2014).

Dans le cadre de cette thèse, les fractions sableuses (> 50 µm), limoneuses (de 2 à 50 µm) et argileuses (< 2 µm) contenues dans nos sols ont été séparés d'après le protocole de Wang and Keller (2008). La Figure 2-8 résume ce protocole.

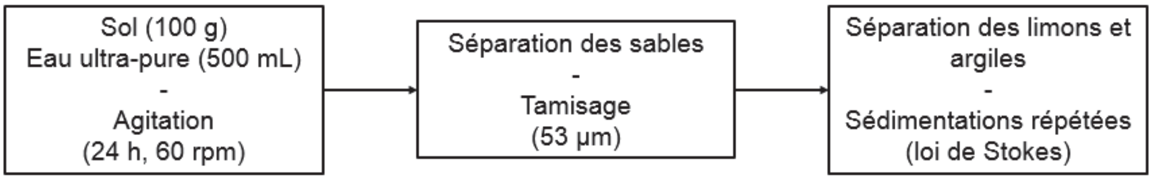


Figure 2-8 : Protocole suivi pour séparer les fractions granulométriques des sols agricoles étudiés, d'après Wang and Keller (2008)

Brièvement, une suspension de 100 g de sol et 500 mL d'eau ultra-pure a été agitée à 60 rpm durant 24 h. La fraction sableuse a alors été isolée en tamisant cette suspension à 53 µm. Les fractions limoneuses et argileuses ont alors été séparées à l'aide de sédimentations répétées dont la durée a été calculée avec la loi de Stokes. La suspension restante eau-limons-argiles est introduite dans des bouteilles Nalgène. Après centrifugation de ce mélange, l'eau surnageante contenant les argiles est mise de côté et de l'eau ultra-pure est introduite dans la bouteille Nalgène. Le culot est remis en suspension après agitation et une nouvelle centrifugation est réalisée pour sédimenter les limons. Cette opération est répétée jusqu'à l'obtention d'un surnageant clair. Pour finir la fraction argileuse en suspension dans les eaux surnageantes mises de côté ont également été sédimentées. Les fractions sableuses, limoneuses et argileuses ont alors été conservées au congélateur (-4 °C) avant d'en extraire les pesticides et mettre en solution les métaux en vue de quantifier ces polluants. La durée totale de la séparation granulométrique d'une série de 4 sols est de 5 jours.

5. Spéciation chimique

La spéciation chimique du Cu et du Zn a également été déterminée. Le partage des éléments majeurs et traces dans les fractions chimiques des sols est étudié depuis plus de quarante ans, notamment sur la base du protocole établi par Tessier et al. (1979). Ce protocole s'intéresse à cinq fractions : i) échangeable, ii) acido-soluble, iii) réductible, iv) oxydable et v) résiduelle. Ce protocole reste majoritairement utilisé même si au cours des années, un grand nombre de protocole de séparation chimique séquentielle ont été développé. Parmi ces protocoles, celui du Bureau Communautaire des Références (BCR) (Ure et al., 1992) est également largement utilisé. Ce protocole comporte un nombre de fractions chimiques restreint : i) fractions échangeable et acido-soluble confondues, ii) fractions réductible et oxydable confondues et iii) résiduelle. Récemment, Rao et al. (2008) ont proposé une synthèse des différents protocoles existant.

D'après ces travaux, un protocole a été développé permettant de déterminer la spéciation chimique des métaux associés à huit fractions chimiques: i) facilement échangeable, ii) moins échangeable, iii) acido-soluble, iv) réductible-Mn (associée aux oxydes de manganèse), v) réductible-Fe amorphes (associée aux oxydes de Fe amorphes), vi) réductible-Fe cristallisés (associée aux oxydes de Fe cristallisés), vii) oxydable (associés à la matière organiques) et enfin viii) résiduels.

Brièvement, environ 1 g de sol est mis en contact avec 25 mL de différentes solutions d'extraction suivant la fraction chimique à extraire. Une solution de chlorure de baryum $BaCl_2$ est utilisée pour extraire les éléments facilement échangeables. Pour l'extraction des éléments moins échangeable et acido-soluble, des solutions de nitrate de plomb $PbNO_3$ et d'acétate de sodium CH_3COONa (en présence d'acide acétique pour obtenir un pH de 5) respectivement sont utilisées. Les fractions réductibles ont été extraites à l'aide de chlorure d'hydroxylamine $NH_2OH{\cdot}HCl$ (oxyde de Mn), d'oxalate $H_2C_2O_4$ (oxyde de Fe amorphe) et d'oxalate d'ammonium $(NH_4)_2C_2O_4$ (en présence d'oxalate, oxyde de Fe cristallisé). Une solution de pyrophosphate de sodium $Na_4P_2O_7$ a servi à l'extraction des éléments associés à la matière organique. Des temps d'agitation compris entre 30 min et 24 h ont été appliqués après l'ajout des solutions d'extraction suivant les fractions à extraire. Les extractions des fractions associées aux oxydes de Fe amorphes et cristallisés ont été conduites respectivement à l'obscurité et sous rayons ultraviolets (UV). Chaque extraction a été suivi d'une centrifugation à 5000 rpm pendant 30 min afin de sédimenter les sols étudiés et récupérer la solution d'extraction et les éléments extraits. L'ensemble de ce protocole est résumé en Table 2-7.

Entre chaque étape d'extraction, les échantillons de sol ont subi 5 min d'agitation avec de l'eau ultra-pure. Pour finir, la fraction résiduelle a été dissoute en y ajoutant i) 10 mL de HNO_3 distillé et chauffant à 200 °C pendant 24 h sur plaque chauffante et ii) 10 mL de HCl distillé et 10 mL de HF supra-pur. Les échantillons ont alors été portés à 100 °C pendant 24 h. Après une évaporation des acides à 120 °C, les échantillons ont été solubilisés dans 5 mL de HCl 7 M grâce à un bain à ultrasons pendant 5 minutes et un chauffage sur plaque pendant 12 h.

Table 2-7: Protocole d'extraction chimique séquentielle

Fraction chimique	**Solution d'extraction**	**Concentration**	**Temps d'extraction**
Facilement échangeable	$BaCl_2$	0,1 M	2 h
Moins échangeable	$PbNO_3$	0,05 M	16 h
Acido-soluble	CH_3COONa (et CH_3COOH, pH 5)	1 M	6 h
Réductible-Mn (associé aux oxydes de Mn)	$NH_2OH \cdot HCl$	0,01 M	30 min
Réductible-Fe amorphe (associé aux oxydes de Fe amorphes)	$H_2C_2O_4$	0,1 M	4 h, à l'obscurité
Réductible-Fe cristallisé (associé aux oxydes de Fe cristallisés)	$(NH_4)_2C_2O_4$ (et $H_2C_2O_4$)	0,175 M (0,1 M)	3 h, sous UV
Oxydable (associé à la matière organique)	$Na_4P_2O_7$	0,1 M	24 h
Résiduelle	HNO_3, HCl / HF	16 M, 12 M / 29 M	24 h, 24h

6. Analyse isotopique par composé spécifique (CSIA) appliquée aux sols

L'utilisation du fractionnement isotopique dans l'étude de la biodégradation de composés organiques est basée sur le fait que, parmi les processus participant à la dissipation de ces composés, certains processus n'affectent pas les ratios entre isotopes stables (e.g. volatilisation, sorption, transport). A l'inverse, certains processus tels que la biodégradation et les réactions chimiques abiotiques s'accompagnent d'un fractionnement isotopique.

Les variations en isotopes stables sont généralement notées δ et exprimées en pour mille relatif à un standard international de référence de composition isotopique connue (e.g. Standard

Vienna-Pee-Dee Belemnite (PDB) pour le carbone) selon l'équation suivante (Eq. 2-1), pour l'analyse des isotopes stables ^{12}C et ^{13}C :

$$\delta^{13}C_{éch.} = \frac{R_{éch.} - R_{standard}}{R_{standard}} \tag{2-1}$$

où $R_{éch.}$ et $R_{standard}$ représentent respectivement les ratios $^{13}C/^{12}C$ de l'échantillon mesuré et du standard. Ainsi, des valeurs de δ positives représentent un enrichissement en ^{13}C par rapport au standard, tandis que des valeurs négatives représentent un appauvrissement en ^{13}C.

La mesure du rapport isotopique (R) du carbone (C) (cette mesure peut également être faite pour les éléments C, H, O, N, ou encore S) est réalisée par chromatographie en phase gazeuse couplée à un module de combustion et à un spectromètre de masse à rapport d'isotopes stables (GC-C-IRMS). Dans ce système séquentiel, les molécules sont vaporisées et poussées par un flux d'hélium dans une colonne chromatographique. Puis les constituants de l'échantillon sont séparés par chromatographie en phase gazeuse (GC). Les molécules séparées entrent alors dans un four de combustion (C) et subissent une phase d'oxydation/réduction au cours de laquelle les composés sont transformés en CO_2, H_2O et NO_x. Après diverses phases de piégeage en fonction des analyses souhaitées, les flux résultants en CO_2, H_2O et/ou NO_x sont ensuite introduits dans la source d'ionisation du spectromètre de masse (IRMS). Enfin, les flux résultants sont envoyés sur un système classique de spectrométrie de masse séparant les composés en fonction du ratio masse/charge dans un champ électromagnétique perpendiculaire au flux d'ions, pour la détermination des rapports isotopiques ($^{13}C/^{12}C$) individuels (Figure 2-9).

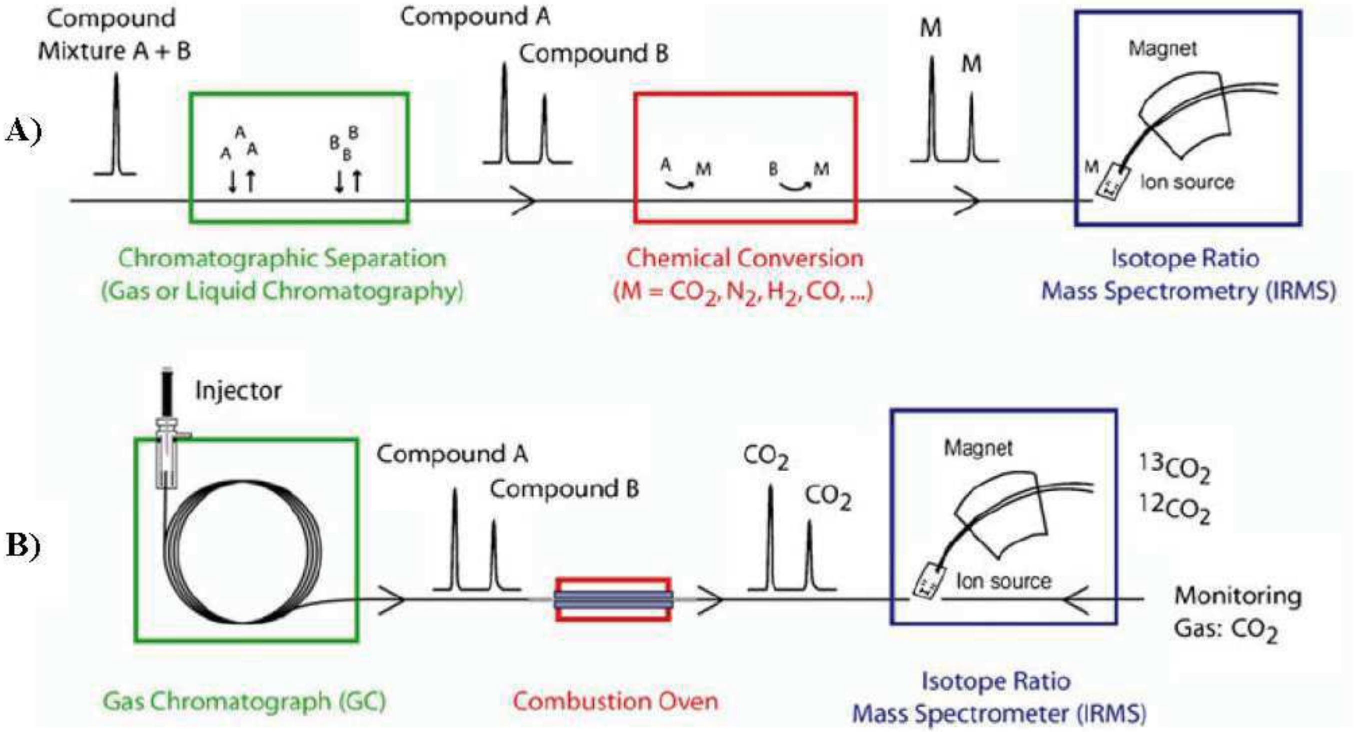


Figure 2-9 : Principe (A) et instrumentation (B) d'une chromatographie en phase gazeuse couplée à un module de combustion et à un spectromètre de masse à rapport d'isotopes stables (GC-C-IRMS) (Elsner et al., 2012)

Les isotopes « lourds » (^{13}C, ^{15}N, deutérium) présentent des liaisons plus fortes que les isotopes légers du même élément, et présentent ainsi une plus haute énergie d'activation (Elsner et al., 2005). Au cours du clivage d'une liaison, les isotopes les plus légers sont donc plus facilement mobilisés et ainsi les liaisons entre atomes légers seront plus rapidement dissociées, induisant un enrichissement en isotopes lourds dans la fraction de composé de départ. Cet enrichissement isotopique donne lieu un facteur d'enrichissement isotopique (α) dépendant de la réaction, plus souvent exprimé sous la forme ε_{bulk} selon l'équation (Eq. 2-2):

$$\varepsilon_{bulk} = (\alpha - 1) \times 1000 \tag{2-2}$$

L'enrichissement isotopique s'exprime à l'aide de l'équation de Rayleigh (Eq. 2-3) :

$$ln\left(\frac{\delta^{13}C_x + 1000}{\delta^{13}C_{in} + 1000}\right) = \frac{\varepsilon_{bulk}}{1000} \times ln\left(\frac{C_x}{C_{in}}\right) \tag{2-3}$$

où $\delta^{13}C$ désigne la composition isotopique $^{13}C/^{12}C$ du composé restant, $\delta^{13}C$ désigne le rapport isotopique initial, C_x/C_{in} désigne le ratio entre la concentration de substrat au début (C_x) et à la fin (C_{in}) de la réaction, et ε_{bulk} représente le facteur d'enrichissement isotopique.

A l'aide de l'équation de Rayleigh, des facteurs d'enrichissement isotopiques (ε) peuvent être obtenus lors d'études en laboratoire pour différents composés et réactions de dégradation catalysées par différentes souches microbiennes (Fletcher et al., 2011). Ils reflètent les variations de concentration observées pendant la dégradation d'un composé par rapport aux changements de la composition isotopique de ce composé. Ces facteurs peuvent être utilisés pour quantifier le degré de biodégradation d'un composé *in situ* à condition de connaître les facteurs d'enrichissement appropriés aux réactions de biodégradation de ce composé s'établissant sur site (Braeckevelt et al., 2012; Meckenstock et al., 2004; Richnow et al., 2003).

Afin d'aider à l'évaluation des sources de polluants mais aussi des voies de dégradation que subissent les pesticides dans l'environnement, il est possible de mesurer les rapports isotopiques de deux éléments à la fois. Cette technique, l'analyse isotopique par composé spécifique en deux dimensions (2D-CSIA) donne plus d'information que le CSIA en une dimension. L'utilisation du 2D-CSIA a notamment permis à Rosell et al. (2012) de déconvoluer les différentes voies de dégradation possible pour le méthyl tert-butyl éther (MTBE) (Figure 2-10) et l'impact de différentes cultures bactériennes sur ces dégradations.

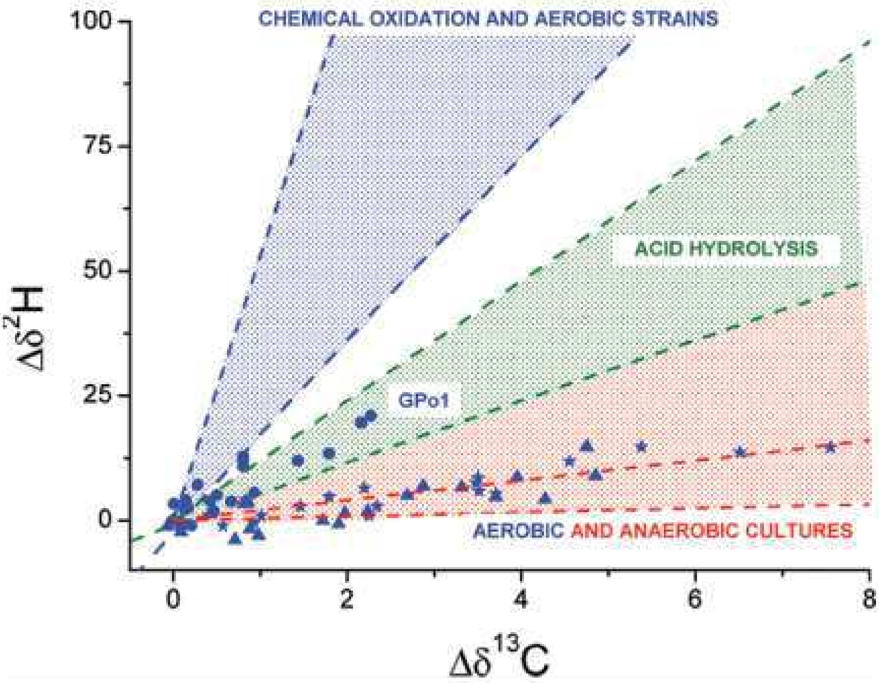


Figure 2-10: Apport du 2D-CSIA dans la compréhension des mécanismes de dégradation du MTBE (Rosell et al., 2012)

Il est également possible de déterminer l'effet cinétique isotopique apparent (AKIE en anglais) à partir de l'enrichissement isotopique ε_{bulk}. Cette conversion permet de comparer les fractionnements isotopiques du C avec ceux reportés dans la littérature. L'équation suivante (Eq. 2-4) est utilisée pour calculer les AKIE (Elsner et al., 2005).

$$AKIE \approx \frac{1}{1 + z \times \left(\frac{n}{x}\right) \times \frac{\varepsilon_{bulk}}{1000}} \qquad (2\text{-}4)$$

où n est le nombre d'atome d'un élément donné, x est le nombre de réactions réactives non distinguables, et z est le nombre de positions en compétition au sein de la molécule.

7. Analyse isotopique des métaux appliquée aux sols

7.1. Purification du Cu et du Zn

Des protocoles de double purification du Cu et du Zn adaptés respectivement des travaux de Marechal et al. (1999) pour le Cu (Babcsányi et al., 2014) et Moynier et al. (2006) pour le Zn ont été appliqués.

La Table 2-8 présente les protocoles de purification du Cu et du Zn, qui doit atteindre 100% pour effectuer une analyse isotopique, sous peine d'un fractionnement isotopique lié à la procédure. Des colonnes en polypropylène (PP) (Poly-prep chromatography columns, BioRad) sont remplies des résines échangeuses d'anions AGMP1 (100–200 mesh, chloride form, BioRad) pour isoler le Cu des échantillons et AG1X8 (100–200 mesh, chloride form, BioRad) pour la purification du Zn. Les volumes de résines à introduire varient selon la matrice (sol, eau) mais aussi le nombre de purifications (Table 2-8). La purification des éléments étudiés est réalisée en cinq étapes : i) lavage de la résine, ii) conditionnement de la résine, iii) chargement de l'échantillon, iv) élution de la matrice, et pour finir v) élution du Cu ou du Zn.

Le lavage de la résine se fait à l'aide de HNO_3 0,5 M et d'eau ultra-pure uniquement dans le cas du Zn, alors que l'utilisation de HCl 7M est également nécessaire pour laver la résine AGMP1 pour la purification du Cu. Les résines sont alors conditionnées avec les acides de reprise des échantillons à purifier après leur digestion. Les échantillons sont ensuite chargés sur les colonnes de résine avant l'élution de la matrice. Enfin, l'élution du Cu et du Zn est réalisée.

Chaque purification de Cu est suivie par une étape de minéralisation avec 1 mL de HNO_3 distillé de la matière organique éluée au cours de la procédure suite à l'utilisation de la résine AGMP1. Les échantillons et standards purifiés sont mis en solution dans le volume d'acide nitrique dilué (0,5 M HNO_3) nécessaire pour atteindre des concentrations de Cu et Zn respectivement de 100 et 200 $\mu g.L^{-1}$.

Table 2-8 : Protocole de purification du Cu et du Zn présents dans des échantillons d'eau et de sol

Element to purify	**Sample**	**Resin type**	**Resin volume**	**Resin wash**	**Conditioning**	**Sample load**	**Matric elution**	**Target element elution**
Cu	Solid (1st purification)	AGMP1	3 mL	3×8 mL HNO_3 0.5 M alternating with 8 mL H_2O, 8 mL HCl 7 M, 8 mL H_2O	10 mL HCl 7 M + H_2O_2 0.01 %	1 mL HCl 7 M + H_2O_2 0.01 %	6 mL HCl 7 M + H_2O_2 0.01 %	45 mL HCl 7 M + H_2O_2 0.01 %
Cu	Solid (2nd purification)	AGMP1	2 mL	3×7 mL HNO_3 0.5 M alternating with 7 mL H_2O, 7 mL HCl 7 M, 7 mL H_2O	8 mL HCl 7 M + H_2O_2 0.01 %	1 mL HCl 7 M + H_2O_2 0.01 %	8 mL HCl 7 M + H_2O_2 0.01 %	26 mL HCl 7 M + H_2O_2 0.01 %
Cu	Water (x 2)	AGMP1	1.6 mL	3×7 mL HNO_3 0.5 M alternating with 7 mL H_2O, 7 mL HCl 7 M, 7 mL H_2O	6 mL HCl 7 M + H_2O_2 0.01 %	1 mL HCl 7 M + H_2O_2 0.01 %	4 mL HCl 7 M + H_2O_2 0.01 %	26.5 mL HCl 7 M + H_2O_2 0.01 %
Zn	Solid, water (x 2)	AG1X8	1 mL	3×5 mL HNO_3 0.5 M alternating with 5 mL H_2O	3 mL HBr 1.5 M	1mL HBr 1.5 M	5 mL HBr 1.5 M	5 mL HNO_3 0.5 M

7.2. Analyse des isotopes du Cu et du Zn

Les analyses isotopiques du Cu ($^{65}Cu/^{63}Cu$) est du Zn ($^{66}Zn/^{64}Zn$) ont été rendues possibles grâce au développement du spectromètre de masse à source à plasma et à multi-collection Finnigan-Neptune MC-ICP-MS (Thermo Scientific, Waltham, MA, USA) (Figure 2-11).

Le MC-ICP-MS est un appareil composé d'un plasma à haute température comme source d'ions, d'un analyseur de masse et d'une unité de détection. Après une phase d'ionisation, les ions quittent la source puis sont focalisés et accélérés sous une différence de potentiel. Ensuite, un aimant sépare les ions suivant leur rapport masse sur charge (m/z). L'unité de détection (cages de Faradays) est un ensemble de collecteurs capable de mesurer les faisceaux d'ions de plusieurs isotopes à la fois. Cette unité de détection permet d'atteindre une précision de ± 0.001 % (Weiss et al., 2008).

Les rapports des isotopes du Cu et du Zn sont mesurés au MC-ICP-MS en mode plasma sec et humide. Des standards de Ni (NIST SRM 986) et de Cu (NIST SRM 976) sont utilisés respectivement pour les mesures des isotopes du Cu et du Zn. La correction du biais de masse de l'appareil est corrigé à l'aide des standards de Cu (NIST SRM 976) et de Zn (IRMM 3702) comme précédemment décrit par Li et al. (2009). Une mesure des rapports des isotopes du Cu consiste en 40 cycles de 8 s d'intégration. Pour le Zn, 30 cycles de 8 s sont nécessaires. L'incertitude analytique pour l'ensemble de la procédure (digestion, purification et analyse isotopique) est ≤ 0.05 ‰ (2σ, n=15) pour les valeurs de $\delta^{65}Cu$ et ≤ 0.09 ‰ (2σ, n=15) pour les valeurs de $\delta^{66}Zn$. Ces incertitudes sont déterminées à l'aide du standard géologique CRM-7003 ($\delta^{65}Cu = 0.18 \pm 0.05$ ‰, and $\delta^{66}Zn = -0.25 \pm 0.09$ ‰; incluant 3 échantillons digérés séparément et 15 mesures isotopiques).

A l'instar des pesticides, les variations en isotopes stables des métaux sont également notées δ et exprimées en pour mille relatif à un standard international de référence de composition isotopique connue (i.e. NIST 986 Ni pour le Cu ou NIST 976 Cu pour le Zn) selon l'équation suivante (Eq. 2-5), pour l'analyse isotopique du Cu :

$$\delta^{65}Cu_{éch.} = \frac{R_{éch.} - R_{standard}}{R_{standard}} \qquad (2\text{-}5)$$

où $R_{éch.}$ et $R_{standard}$ représentent respectivement (dans le cas du Cu) les ratios $^{65}Cu/ ^{63}Cu$ de l'échantillon mesuré et du standard. Ainsi, des valeurs de δ positives représentent un enrichissement en ^{65}Cu par rapport au standard, tandis que des valeurs négatives représentent un appauvrissement en ^{65}Cu.

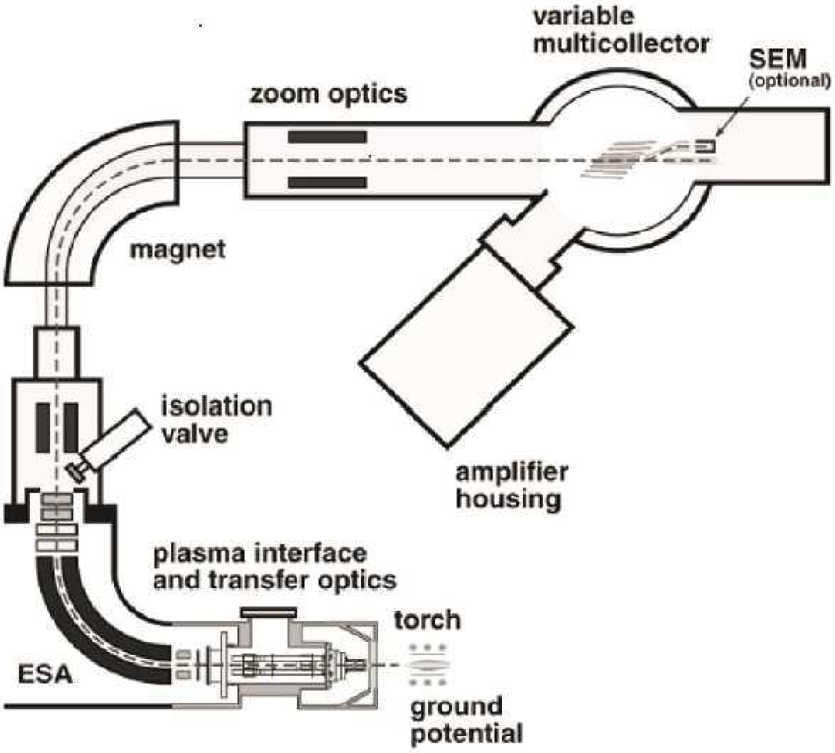
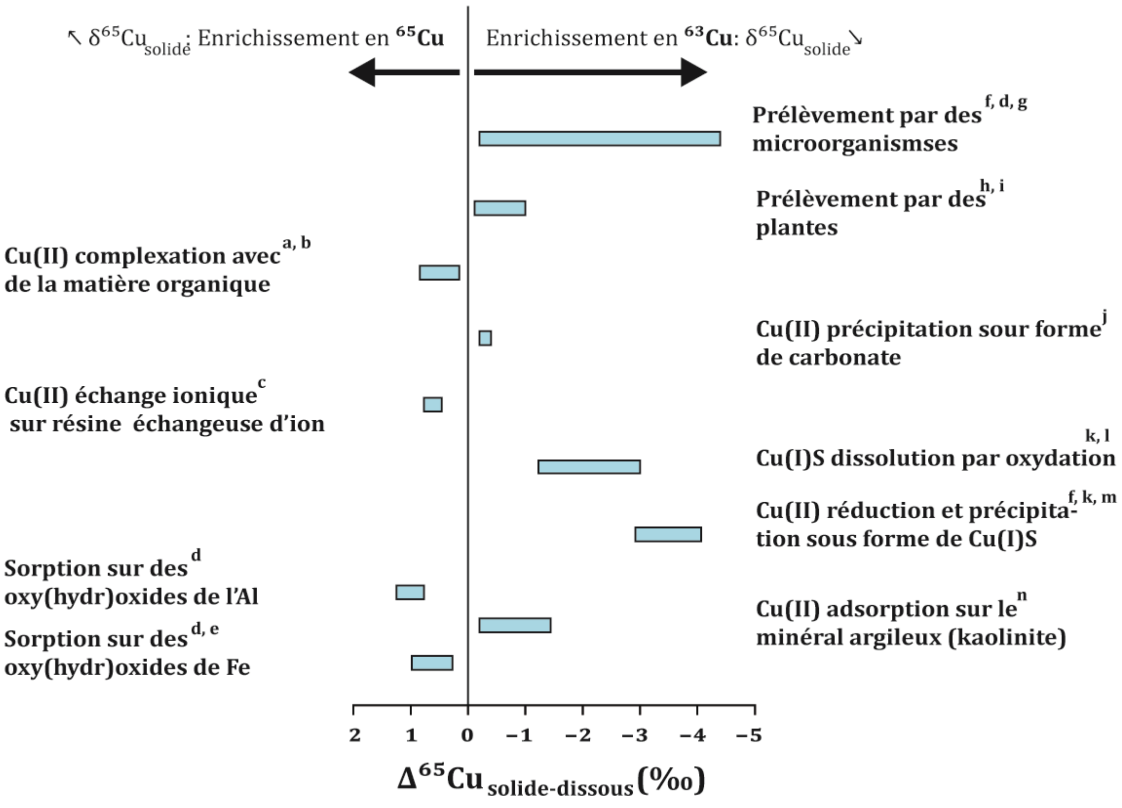


Figure 2-11 : Composition d'un spectromètre de masse à source à plasma et à multi-collection (Goldstein and Stirling, 2003)

Ainsi, l'étude du rapport des isotopes stables du Cu et du Zn peut être adaptée au suivi du cycle de ces éléments à l'échelle des agrosystèmes et des zones tampons humides afin de mieux comprendre les mécanismes qui contrôlent sa séquestration / mobilisation et son transport.

Le fractionnement des isotopes du Cu a été observé au cours de plusieurs processus au sein des organismes, des sols, des milieux aquatiques mais aussi des sédiments. L'adsorption du Cu sur les minéraux, sa complexation avec la matière organique, les réactions oxydoréduction, sa précipitation et son prélèvement par la végétation ou les microorganismes induisent des différences de valeurs isotopiques (Figure 2-12). En effet, diverses études montrent que la réduction/oxydation du Cu produit un fractionnement distinct. Pokrovsky et al., (2008) ont étudié l'impact de la sorption du Cu sur des hydroxydes de fer et ont mesuré des enrichissements isotopiques du Cu (Δ^{65}Cu(solide-solution)) de l'ordre de 1,0 ± 0,25 ‰ (gibbsite) et 0,78 ± 0,2 ‰ (goethite). Une étude conduite par (Babcsányi et al., 2014) montre que le Cu dissous est appauvri en isotope lourd (^{65}Cu) suite à son adsorption sur des hydroxydes d'aluminium (et de fer) et de la matière organique présents dans le sédiment d'un bassin d'orage. Les argiles et la matière organique produisent un fractionnement significatif, de même que la transformation biotique du cuivre (passage par des racines, translocation dans les plantes). Enfin, au cours d'une expérience de sorption et d'incorporation du Cu faite avec différentes bactéries, (Navarrete et al., 2011) ont obtenu un fractionnement isotopique du Cu (Δ^{65}Cu(solution-solide)) allant de 0,2 à 2,6 ‰.



a: Ryan et al., 2014, **b**: Bigalke et al., 2010, **c**: Maréchal et Albarède, 2002, **d**: Pokrovsky et al., 2008,
e: Balistrieri et al, 2008, **f**: Zhu et al., 2002, **g**: Navarrete et al., 2011, **h**: Jouvin et al., 2012, **i**: Ryan et al., 2013,
j: Maréchal et Sheppard, 2002 , **k**: Mathur et al., 2005, **l**: Kimball et al., 2009, **m**: Ehrlich et al., 2004,
n: Li et al., 2015

Figure 2-12 : Fractionnements isotopiques rencontrés pour le Cu au cours de différents processus

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Chapitre 3 : Aging du S-metolachlor, metalaxyl, Cu et Zn dans les sols agricoles ; apport des extractions séquentielle et analyses des isotopes stables

Dans le chapitre 3 est discuté l'impact des facteurs intrinsèques au sol sur la transformation des pesticides et métaux au cours du temps. En effet, ce chapitre présente l'expérience de vieillissement (aging en anglais) de ces polluants dans des sols agricoles ainsi que leur partage dans les fractions granulométriques et chimiques de ces sols. Le vieillissement des polluants est suivi grâce à des mesures des concentrations mais aussi des signatures isotopiques des pesticides et métaux. Au cours de ce chapitre est soulignée l'importance des constituants des sols (matière organique etc…) et de la présence simultanée de polluants organiques et minéraux (effet de la co-contamination) sur le devenir des pesticides et métaux dans les sols agricoles.

*Fatima Meite, Charline Wiegert, Mathieu Granet and Gwenaël Imfeld**

1. Abstract and highlights

The mobility of pesticides and metals and their transport towards aquatic systems depend on their partitioning among the soil grain-size particles over time. Although synthetic pesticides and metals may be applied simultaneously, their aging in soils is rarely and often independently evaluated. Here soils from experimental vineyard and crop catchments were spiked with a mixture of the fungicide metalaxyl (MTY, used in vineyard catchments) and the herbicide *S*-metolachlor (SMET, used in crop catchments) being two aromatic acetanilide compounds, as well as Cu and Zn (metals respectively used as fungicides in vineyard and fertilizer in several crop catchments), and incubated for 0, 10, 100 or 200 days in living or sterile soils to evaluate pollutant aging in relation to pollutant transformation. Pollutant proportioning was evaluated in the grain-size fractions using sequential chemical extractions of metals and stable isotope fractionation (^{65}Cu and ^{66}Zn, and pesticide CSIA). MTY and SMET were mainly released in the water used to separate the grain-size fractions of soils whatever the incubation period, the soil type and the occurrence of living microorganisms. The soil physical speciation revealed that organic matter in the particle-size fractions of soils mainly controls MTY and SMET proportioning. No clear effect of pesticide aging among soil fraction could be observed based on CSIA data of MTY and SMET. Cu and Zn proportioning followed a silt > clay > sand pattern, although chemical speciation of Cu and Zn differed and slightly changed during the first 10 days of incubation. Under abiotic condition, the soluble fraction of Cu release increased over time. Cluster analysis of Cu and Zn chemical speciation patterns suggests that the presence of pesticides may affect the speciation. δ^{65}Cu and δ^{66}Zn values varied between biotic and abiotic conditions, especially in the water used to separate the grain-size fractions and in the clay fraction. The stable isotope composition data suggest that Cu and Zn binding with the humic acid of SOM, oxides and sorption by soil microorganisms. This study shows that physical and chemical speciation can be efficiently coupled with stable isotope analysis of metals and CSIA of pesticides to study pollutant aging of both pesticides and metals in agricultural soils.

Highlights:

- MTY and SMET were mainly associated with sands and silts fractions of agricultural soils
- MTY and SMET aging could not be observed combining physical partitioning and CSIA
- Speciation Cu and Zn among particle-size fractions did not change after 10 days of aging
- Pesticides and metals were significantly associated with the sand fraction
- δ^{65}Cu and δ^{66}Zn values suggest binding with SOM, oxides and microorganisms

2. Introduction

Disposal of metal contaminated industrial effluent, sewage sludge and application of fertilizers, pesticides and municipal wastes has made agricultural soils sinks of pollution, which may adversely affect non-target soil organisms (Imfeld and Vuilleumier, 2012; Muturi et al., 2017; Wyszkowska et al., 2012). Pesticides and heavy-metals may sorb on the soil matrix according to their characteristics (Komárek et al., 2010; Swarcewicz and Gregorczyk, 2012; Zhang et al., 2014), and with time, pollutants are redistributed among porewater, minerals and organic matter, including organisms. The process responsible for this redistribution decreases pollutant availability and is known as aging (Komarek, 2010; Ma, 2006). With aging, pollutant sorption onto the soil matrix increases, thereby enhancing their persistence. Aging also impact transport of sorbed pollutants from agricultural soil towards aquatic ecosystems. Indeed, pollutant export during rainfall-runoff events depends on the particle-size fractions (Bullard et al., 2018; Martínez-Casasnovas et al., 2002; Mohamadi and Kavian, 2015; Renschler et al., 1999), which are mobilized as a function of the rainfall pattern (Babcsányi et al., 2016; Meite et al., 2018). While aging control persistence and mobilization of pollutants, knowledge on the physical and chemical proportioning of pollutant mixtures over time remains very scarce.

In soils, processes of dissolution, (co-)precipitation, complexation, sorption, diffusion and/or entrapment control the aging of metals and pesticides. Specific and non-specific bounding are involved in the sorption and complexation of metals and pesticides on the various soil compartments. The nature of soil components onto which pesticides sorb in soils mainly depends on the content of organic matter (OM). Pesticides often bound to the organic matter in soils with > 5 % OM by hydrophobic sorption, while they mainly bind to oxides, and especially clay minerals, in soils with < 5 % OM (Clausen et al., 2001; Cornejo et al., 2008; Đurović et al., 2009; Helal et al., 2006). Metals are fixed by the slow diffusion of metals into Fe-oxides (Bruemmer et al., 1988), hydrous oxides of Al and Mn (Trivedi and Axe, 2000), clay minerals (Ma and Uren, 1998) and by diffusion or precipitation in carbonates (Nakhone and Young, 1993). Metals such as Cu and Zn are relatively labile in soils and present high affinities with organic matter (Duplay et al., 2014; Néel et al., 2007; Quenea et al., 2009). With aging, Cu and Zn are expected to sorb onto oxides and are mainly retained by clay minerals (Komárek et al., 2010; Ma et al., 2006). Besides, in calcareous soils, Cu may precipitate as malachite $Cu_2CO_3(OH)_2$ (Ma et al., 2006). However, pesticides and metals are generally studied separately, and the effect of the co-contamination of soils on the respective speciation of these pollutants is thus not accounted for.

Both stable isotope analysis of metals (such as $\delta^{65}Cu$ and $\delta^{66}Zn$ measurements) and pesticides ($\delta^{13}C$ measurement of pesticides by compound specific isotope analysis, CSIA) can help assessing pollution sources and transformation processes. Cu isotope fractionation was observed during Cu adsorption onto Fe and Al oxy(hydr)oxides (Balistrieri et al., 2008; Pokrovsky et al., 2008), clay minerals (Li et al., 2015), as well as oxidation-reduction (Ehrlich et al., 2004; Zhu et al., 2002), uptake by plants (Jouvin et al., 2012; Ryan et al., 2013) or microorganisms (Navarrete et al., 2011), precipitation as Cu mineral phases (Ehrlich et al., 2004; Maréchal and Sheppard, 2002), and complexation with organic matter (Bigalke et al., 2010b; Ryan et al., 2014). Cu associated with DOM in the dissolved phase of runoff was isotopically heavier (El Azzi et al., 2013; Vance et al., 2008), and the distribution of Cu in particle-size fractions (i.e., sand, silt, clay) can also influence Cu mobilization from the soil during rainfall-runoff events (Roussiez et al., 2013; Wang et al., 2014). Recently, it was observed that δ^{65}Cu values significantly differed between particle-size soil fractions, suggesting that Cu isotope fractionation was primarily associated with the clay and fine clay fractions that include both SOM and mineral phases.

Several studies gave insights into the extent of Zn isotopes fractionation following the interaction of Zn with soil constituents. Especially, the fractionation of Zn isotopes was studied after Zn adsorption onto carbonates (Schott et al., 2014) and several oxides: goethite and ferrhydrite Fe oxides (Juillot et al., 2008), birnessite and pyrolusite Mn oxides but also Al oxides (Pokrovsky et al., 2005). The impact of Zn complexation with the humic acids on Zn isotopes fractionation was also studied (Jouvin et al., 2009), such as the impact of Zn adsorption and incorporation on bacteria (Kafantaris and Borrok, 2014). While Zn uptake by grass (*Agrostis capillaris* L.) did not fractionate Zn isotopes, heavier Zn isotopes were retained in the root of *A. capillaris*. Besides, the presence of plants changed the soil Zn pool mobilized in leaching. Isotopically heavier Zn was leached in presence of *A. capillaris* than in soil columns without vegetation (Houben et al., 2014).

Compound-specific isotope analysis (CSIA) of pesticides in soils may help to disentangle degradative (biotic or abiotic) from non-degradative processes, such as sorption, dilution, and offsite transport (Elsner and Imfeld, 2016). Pesticides present light and heavy stable isotopes, e.g., ^{12}C and ^{13}C, and molecules are degraded at slightly different rates depending on the strength of the atomic bounds (^{12}C-^{12}C bounds requiring less energy than ^{13}C-^{12}C or ^{13}C-^{13}C bounds). An enrichment of the heavier isotopes in the residual, non-degraded fraction of the pollutant can thus be observed during the degradation. In contrast, non-degradative attenuation processes are generally considered not to result in significant isotope fractionation (Schmidt et al., 2004). CSIA has been used to evaluate pesticide degradation, such as lindane (Badea et al., 2009), isoproturon (Penning et al., 2010), atrazine (Meyer et al., 2008), 2,6-dichlorobenzamide (BAM) (Reinnicke et al., 2012), and a metabolite of dichlobenil and phenoxy acid herbicides (Maier et al., 2013) in pure bacterial/fungal cultures. CSIA has also been employed to characterize the degradation of some chloroacetanilide herbicides in wetlands (Elsayed et al., 2014) and at catchment scale (Lutz et al., 2017). However, to our knowledge, the application of CSIA and isotopic signature of Cu and Zn to better understand aging in soil fractions is currently lacking.

Therefore, the purpose of this study was to evaluate: (i) the distribution of Cu, Zn, metalaxyl (MTY) and *S*-metolachlor (SMET) in soil physical and chemical fractions over time, (ii) the effect of soil characteristics and sterilization on pollutant proportioning among soil fractions, and (iii) the applicability of $^{65}Cu/^{63}Cu$, $^{66}Zn/^{64}Zn$ fractionation, as well as CSIA of MTY and SMET to study their aging in soils. We used laboratory experiments with soils from an experimental vineyard catchment (Duplay et al., 2014) or a crop catchment (Lefrancq et al., 2017b) to cover relevant agricultural land uses receiving pesticides and heavy metals. Widely used anilide pesticides, i.e., the fungicide metalaxyl (used in vineyard catchments) and the herbicide *S*-metolachlor (used in crop catchments), as well as Cu and Zn (being two metals respectively used as fungicides in vineyard and fertilizer in several agricultural contexts) were used as model pollutants in a mixture. The soils were spiked with these pollutants and incubated for 10, 100 or 200 days prior to separation of the grain-size fractions of soils, sequential chemical extractions of metals and stable isotope analyses of pollutant in the soil fractions.

3. Material and methods

3.1. Chemicals

Metalaxyl ($C_{15}H_{21}NO_4$, methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate) and *S*-metolachlor ($C_{15}H_{22}ClNO_2$, (S)-2-Chloro-N-(2-ethyl-6-methyl-phenyl)-N-(1-methoxypropan-2-yl)acetamide) were purchased from Sigma-Aldrich (St. Louis, MO, USA), with purity of 99.8 and 98.2 % respectively. Copper chloride ($CuCl_2$), zinc chloride ($ZnCl_2$) and salts used for the preparation of the artificial rainwater were purchased from Sigma-Aldrich (St. Louis, MO, USA) with purity ≥ 97 %. As presented in the Chapter 2, part 3, the water solubility, octanol-water partition coefficient K_{ow} (log) and organic carbon partition coefficient K_{oc} are 7100 mg.L^{-1}, 1.7 and 163 L.mg^{-1} for metalaxyl, and 480 mg.L^{-1}, 3.0 and 185 L.mg^{-1} for *S*-metolachlor, respectively (Table 2-6) (Kegley et al., 2016; PPDB Pesticide Properties DataBase, 2009). The soil/water partition coefficient (log K_d) of Cu and Zn used in this study were 2.7 and 3.1 L.mg^{-1}, respectively (Table 2-6) (Allison and Allison, 2005).

The extraction of the chemical fractions of soils were made with ultrapure water and $BaCl_2$ (0.1 M), $PbNO_3$ (0.05 M), CH_3COONa (1 M), $NH_2OH·HCl$ (0.01 M), $H_2C_2O_4$ (0.1 M), $Na_2C_2O_4$ (0.175 M) and $Na_4P_2O_7$ (0.1 M). These products were purchased from Sigma-Aldrich.

3.2. Soils studied

The characteristics of the soils studied are summarized in the Chapter 2, part 2. The Figures 2-3 and 2-5 show field occupation of the catchments. The Table 2-1 summarizes the physico-chemical properties of the soils.

3.3. Experimental set-up

The soil microcosms consisted of about 60 g of air dried soil in 100 mL ethanol-cleaned PE bottles. To maintain aerobic conditions in the microcosm atmosphere while limiting water loss and avoiding contamination, a 0.2 µm polytetrafluoroethylene (PTFE) syringe filter (Rotilabo®, Carl Roth®, France) was mounted on a syringe tip, which was stuck through the bottle cap (Figure 3-1). The MTY and SMET in DCM were spiked in distilled water and stirred for 6 h until solvent evaporation at 5 µg.g^{-1} of soil (environmental concentration). Cu and Zn were spiked at 1000 and 260 µg.g^{-1} of soil, respectively (concentrations highly toxic for soil microorganisms). The aqueous solutions were filter-sterilized with 0.2 µm syringe filters for soil spiking.

Figure 3-1: Experimental set-up

The soil microcosms were prepared under laminar flow hood with sterile material. Successive layers of soils (about 30 g) were added in the 100 mL bottle, each layer was then spiked with the pesticide and/or metal solution or distilled water (control). The water volume of each microcosm was adjusted to reach final volumetric water content of 20 %. The soil was finally thoroughly mixed on a Vortex mixer (30 seconds at room temperature).

Seven sets of soil microcosm experiment were prepared in triplicates for the crop and the vineyard soils: (i) biotic soils spiked with MTY and SMET only, (ii) biotic soils spiked with Cu and Zn only, (iii) biotic soils spiked with MTY, SMET, Cu and Zn, (iv) autoclaved soils spiked with MTY and SMET only, (v) autoclaved soils spiked with Cu and Zn only, (vi) autoclaved soils spiked with MTY, SMET, Cu and Zn, and (vii) autoclaved soils adjusted with sterile distilled water only as control soils. Sterile soils were autoclaved three times successively in glass bottles at 125 °C for 15 min. Soils were stored at room temperature for 12 hours between each autoclave run. All experiments were incubated for 200 days in the dark at 20 °C.

Sampling was carried out on days 0, 10, 100, and 200. In total, 64 experiments, which includes 16 spiked non-sterilized (biotic) microcosms, 16 spiked sterilized (abiotic) microcosms, 16 non-sterilized and 16 sterilized non-spiked (control) microcosms (see Table 3-3 for the detailed experimental design) were prepared in triplicates.

Table 3-3: Detailed scheme of analyses for each incubation period (0, 10, 100 and 200 days)

					Analysis type			
Soil	**Sterilization**	**Compound**	**Spike [µg g^{-1}]**	**Replicates**	**Concentration (ICP-AES)**	**Concentration (GC-MS)**	**Stable isotopes (MC-ICP-MS)**	**CSIA (GC-C-IRMS)**
Crop	No	None	0	3	√	√	√	√
		Cu, Zn	1000, 260	3	√		√	
		MTY, SMET	5, 5	3		√		√
		All	a.d.	3	√	√	√	√
	Yes	None	0	3	√	√	√	√
		Cu, Zn	1000, 260	3	√		√	
		MTY, SMET	5, 5	3		√		√
		All	a.d.	3	√	√	√	√
Vineyard	No	None	0	3	√	√	√	√
		Cu, Zn	1000, 260	3	√		√	
		MTY, SMET	5, 5	3		√		√
		All	a.d.	3	√	√	√	√
	Yes	None	0	3	√	√	√	√
		Cu, Zn	1000, 260	3	√		√	
		MTY, SMET	5, 5	3		√		√
		All	a.d.	3	√	√	√	√

Total spiked non-sterilized samples: 16

Total spiked sterilizes samples: 16

Total non-spiked non-sterilized samples: 16

Total non-spiked sterilized samples: 16

a.d. denotes all doses

3.4. Procedure of sequential chemical extraction of Cu and Zn

The procedure to extract metals from the chemical fractions of soils is explained in Chapter 2, part 5 and Table 2-7 summarizes the different steps.

3.5. Physical separation of the particle-size fractions of soils

The separation of the particle-size fractions of soils is explained in Chapter 2, part 4. The Figure 2-8 show the different steps of the procedure.

3.6. Elemental analyses

The procedure to analyze the chemical composition analysis (including Cu and Zn) of soils is presented in Chapter 2, part 3.2.

3.7. Metalaxyl and S-metolachlor analysis

Pesticides extraction and analysis is explained in Chapter 2, part 3.1. The Table 2-3 summarizes the extraction recoveries obtained in GC-MS for MTY and SMET.

3.8. Carbon isotope analysis, notation and calculation

The analysis of carbon isotope composition is presented in Chapter 2, part 6. Reference carbon isotope composition values of standards of the pesticides studied were obtained using an elemental analyzer-isotopic ratio mass spectrometer (EA-IRMS) coupled isotope ratio mass spectrometer (Table 3-6). The reproducibility of triplicate measurements was ≤ 0.2 ‰ (1σ). The reported $\delta^{13}C$ errors correspond to the standard deviation (1σ) calculated from triplicate measurements of duplicate tests. The mean $\delta^{13}C$ shifts ($\Delta\delta^{13}C$) associated with the extraction method from crop and vineyard soil confirmed the absence of significant fractionation effects (Table 3-6).

Table 3-6: Carbon isotope composition of SMET and MTY standards and **$\Delta\delta^{13}C$** caused by the extraction protocol

	$\Delta\delta^{13}C$ [‰] *vs.* VPDB [a]		
	EA-IRMS *vs.* GC-C-IRMS	**GC-C-IRMS extracted (5 g of soil) *vs.* non-extracted**	
		Crop	**Vineyard**
SMET	0.6 ± 0.3	0.3 ± 0.5	0.7 ± 0.6
MTY	0.8 ± 0.2	-0.4 ± 0.6	-0.1 ± 0.3

[a] The error given for the $\Delta\delta^{13}C$ values was calculated via error propagation based on ± 1 SD of mean $\delta^{13}C$ values from $n \geq 3$ measurements for each sample. $\Delta\delta^{13}C$ values were obtained on the difference between the measured $\delta^{13}C$ value of a given compound in the extract and the mean $\delta^{13}C$ value of the corresponding standard obtained by replicate injection in the GC-C-IRMS system at optional conditions.

3.9. Cu and Zn isotopes analysis, notation and calculation

The procedure needed to analyze the Cu and Zn isotopes is presented in Chapter 2, part 7. The Table 2-8 gives the purification steps of Cu and Zn from soil and water matrices.

3.10. Data analysis

To visualize dissimilarities between MTY, SMET, Cu and Zn profiles of distribution among the particle-size fractions of soils or Cu and Zn distribution profiles among chemical fractions, profiles were separately classified by cluster analysis. The distance between profiles was calculated based on the Bray-Curtis dissimilarity index. A hierarchical cluster analysis was performed on the resulting dissimilarity matrix using the Ward's method that minimizes information loss on merging clusters (Ward 1963).

4. Results and discussion

4.1. Dissipation of MTY and SMET in the agricultural soils

MTY and SMET were mainly found in the water used for the separation of the grain-size fractions of soils, independently of incubation period, the type of soils and their biotic state (Figure 3-2). This result reflects not only the water solubility of MTY (7100 mg.L^{-1}) and SMET (480 mg.L^{-1}) but also their water-soil partitioning coefficient K_d. MTY had K_d of 1.9 and 1.4 mL.g^{-1}, respectively, in the crop and vineyard soil, while SMET have K_d of 2.2 and 1.5 mL.g^{-1}, respectively, in the crop and vineyard soil (Kegley et al., 2016; PPDB Pesticide Properties DataBase, 2009). When mixing 100 g of soil spiked at 5 µg.g^{-1} in 500 mL of water during 24 h, about 480 µg (96%) of MTY and SMET were released into the water phase. At equilibrium, after 200 days of incubation, MTY and SMET in the dissolved phase were, putatively in the form of colloids-associated pesticides. Altogether, these results emphasize the potential mobilization of MTY and SMET from soil into runoff water, even long after the application of those pesticides on agricultural soils.

With mass balances of the separated particle-size fractions coming from 97 to 99 %, the procedure used to separate the particle-size fractions of our soils was validated. Pesticides may sorb on oxides and especially clay minerals or be entrapped in them (Clausen et al., 2001; Cornejo et al., 2008; Moyo et al., 2014; Remucal and Ginder-Vogel, 2014). However, pesticides have less specific affinities with quartz and calcite (Clausen et al., 2001). In our case, MTY and SMET were mainly associated to silt and, to a lesser extent, to clay (Figure 3-2). Indeed, MTY and SMET partitioning consistently followed a silt > sands > clay pattern, whereas a dominant partitioning of MTY and SMET to silt and clay fractions was expected. This supports the idea that the extent and characteristics of soil organic matter (SOM) in the particle-size fractions may explain the distribution of MTY and SMET.

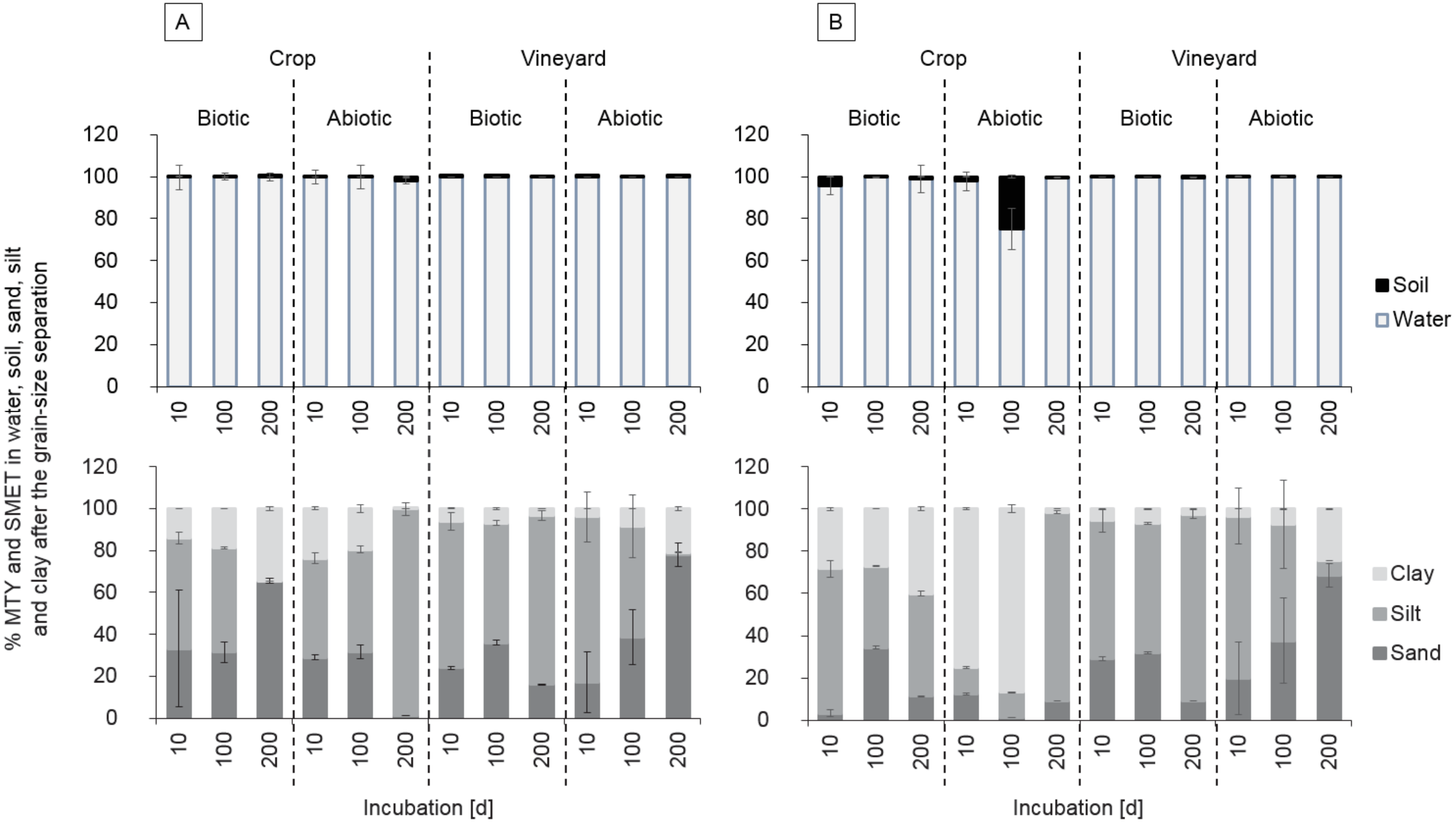

Figure 3-2: Partitioning of MTY (A) and SMET (B) among the crop and vineyard soils spiked with all contaminants, the waters used to separate the particle-size fractions of soils, the sand, silt and clay fractions in biotic and abiotic conditions. Error bars denote 95 % confidence intervals

SOM contains different functional groups such as alkyl-C, O-alkyl-C, aromatic-C, carboxylic-C or ketonic/aldehyde-C enabling specific specific and non-specific bounds with pesticide molecules (Gevao et al., 2000; Guo et al., 2010; Komárek et al., 2010; Schmidt and Kögel- Knabner, 2002). The quality POM and MOM may largely vary among the soil fractions and affected the partitioning of MTY and SMET. SOM can be divided into particulate organic matter (POM, > 50 µm) and mineral-associated fraction of soil organic matter (MOM, < 50 µm) (Benoit et al., 2008; Guo et al., 2010; Moyo et al., 2014; Tirloni et al., 2012). While sand samples obtained after separation of particle-size fractions of soils likely contains POM, the silts and clays are expected to mainly contain MOM. POM is known as a light-density organic fraction and the most accessible SOM fraction for pesticides, while SOM content increases with decreasing particle size (Guo et al., 2010; Quenea et al., 2009; Schmidt and Kögel- Knabner, 2002). Interactions with soil minerals and especially minerals contained in the smallest particle-size fractions may stabilized it in the form of organo-mineral complexes (Mertz et al., 2005; Poirier et al., 2005; Schmidt and Kögel- Knabner, 2002). Hence, less degraded SOM in the coarser fractions (silt) and more altered SOM in the clay fractions (Besnard et al., 2001; Parat et al., 2002; Quenea et al., 2009) may have altered the partitioning patterns of SMET and MTY in the soils.

The carbon isotope composition of SMET and MTY slightly changed during the 200 days of incubation Figure 3-3. Larger $\Delta\delta^{13}C$ values were expected after 10 day of incubation to reflect biodegradation before aging of SMET and MTY in the soils. Indeed, during biological reactions such as biodegradation the lighter isotope is very often enriched in the reaction product relative to the substrate (Hoefs, 2015), which tend to get heavier during pesticide degradation (Elsayed et al., 2014). In the both soils, $\Delta\delta^{13}C$ values increased with time in the biotic experiment with both SMET and MTY ($\Delta\delta^{13}C > 1$ ‰), whereas the carbon isotope composition did not change significantly in the abiotic experiments (Figure 3-3). In the biotic experiment, SMET and MTY amounts in the different soil fractions was below the quantification limits, which did not allow CSIA. This emphasizes that the initial mass of SMET and MTY was significantly depleted due to biodegradation, whereas the isotope composition of both SMET and MTY could still be measured in the soil fractions after 200 days of incubation in the abiotic experiments.

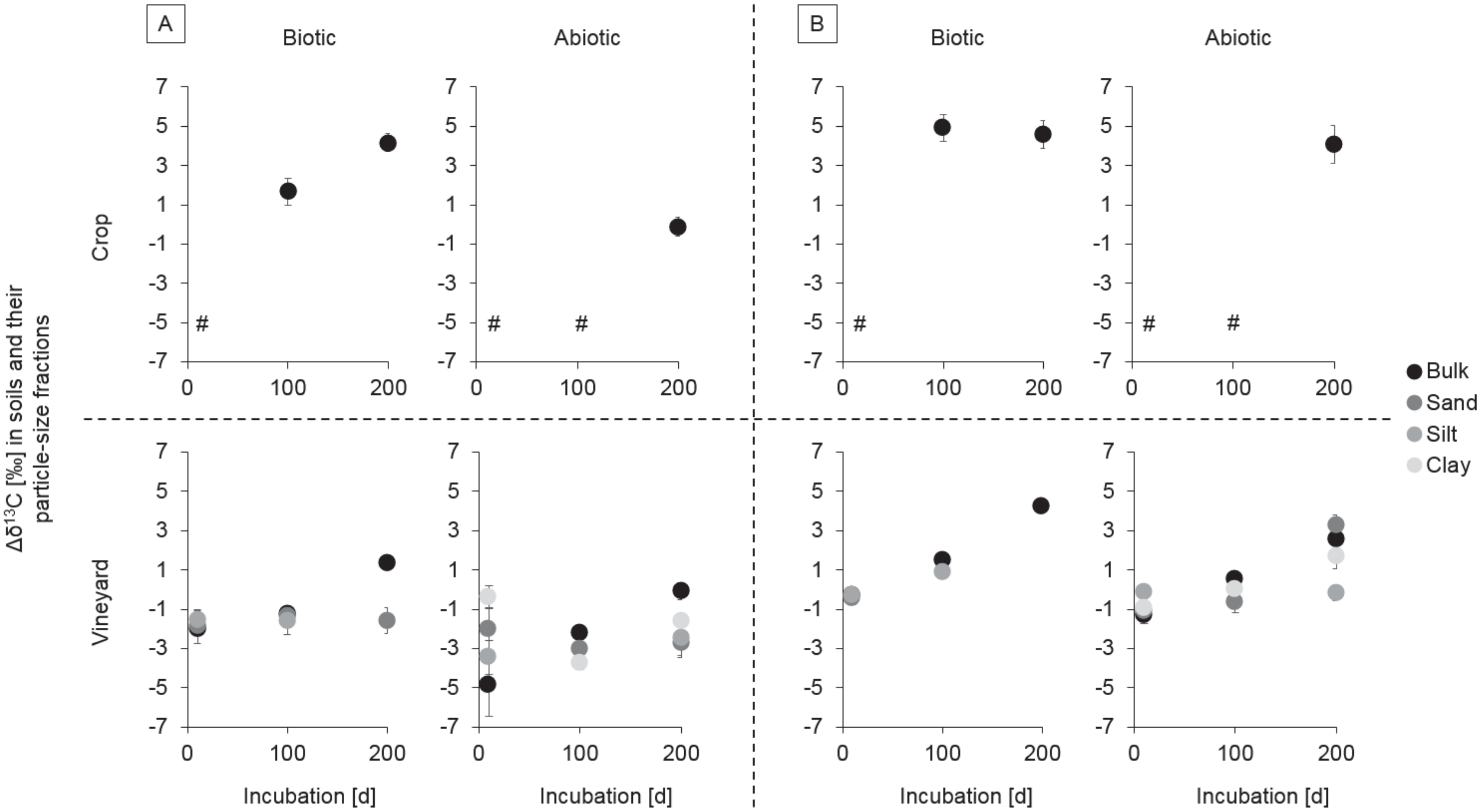

Figure 3-3: Evolution of the C isotope ratios Δδ^{13}C [‰] of MTY (A) and SMET (B) among the bulk crop and vineyard soils and their particle-size fractions compared to the ratio of the standard pesticide molecules (Δδ^{13}C = $\delta^{13}C_{sample,t}$ - $\delta^{13}C_{standard}$). Error bars denote standard deviations. "#" denotes that no significant values were obtained

Among the various abiotic degradation pathways, chemically mediated transformations such as hydrolysis, oxidation, reduction, and photolysis are reported to cause carbon isotopic shifts of organophosphorus pesticides and glyphosate (Mogusu et al., 2015; Wu et al., 2014). Although photolysis can occur under field conditions, it was not relevant in our case since experiments were incubated in a dark chamber. Abiotic hydrolysis was likely not a major dissipation pathway in the soil as half-lives for SMET and MTY are higher than 100 days (pH 1 - 9) (Extoxnet: Extension Toxicology Network, 2000; Zheng and Ye, 2001). Reported half-life time of 116 days for MTY suggests, however, that hydrolysis may contribute to the slight change of isotope composition observed for MTY after 200 day ($\Delta\delta^{13}C > 1.5$ ‰). In the case of slow or no hydrolysis, other chemical reactions, e.g., oxidation, can affect the chemical transformation of pesticides (Tomizawa, 1975). Therefore, the occurrence of oxidation as an abiotic degradation process can be assumed in the aerobic biotic and abiotic experiments leading to the slight isotopic shifts of SMET (~ 1.5‰ in the abiotic experiments) over 200 days. The role of Fe (III) and Mn (III/IV), as naturally occurring soil oxidants, in the oxidation of various herbicides, including metolachlor, and also anilide compounds has been observed previously (Pignatello and Baehr, 1994; Remucal and Ginder-Vogel, 2014; Ruggiero et al., 2002). In addition large K_{oc} values of MTY and SM and relatively high OM contents of our soils emphasize that hydrophobic sorption may have led to the formation of non-extractable residues. Sorption thus has to be considered as the main non-degradative process affecting the extracted amount and apparent dissipation rates of MTY and SMET in the abiotic experiments. Overall, a comparison of the dissipation rates and the isotope fractionation, MTY and SMET in the biotic and abiotic experiments indicates that dissipation in the biotic experiments results from biodegradation, abiotic degradation, and non-degradative processes. The contribution of each mechanism may, however, largely depend on soil water content, pesticide properties, or, to the application doses.

In the future, it may be worth to include sequential pesticides extraction from soils to follow the pesticide aging and processes of soil binding versus destructive processes. Pesticides associated to the solid phase of soils can be divided in three pools: extractible, less extractible and bound pesticides (ECETOC, 2013). To access to these different pools of pesticides, the use of different extractant is needed: (i) 0.01 M calcium chloride ($CaCl_2$) solution – extractible pesticides, (ii) water, in addition to dipolar solvent and organic acid – less-extractible pesticides, and (iii) water in addition to acetone – bound pesticides (EFSA panel on plant protection products and their residues, 2015).

4.2. Partitioning of Cu and Zn among grain-size fractions of the soils

Only a minor fraction of Cu and Zn was released in the water phase. Cu and Zn partitioning among the grain-size fractions was similar and followed a silts > clays > sands pattern (Figure 3-4). Cu partitioning to soil was higher in the biotic experiments and increased with time (Figure 3-4A). Zn partitioning was similar in the biotic and in the abiotic experiments as well as in the crop and vineyard soils (Figure 3-4B). This partitioning slightly evolve with the incubation periods (Figure 3-4). The cluster analysis revealed major change in the Cu distribution among the particle-size fractions between the biotic and the abiotic experiments (Figure 3-5). In contrast, Zn distribution patterns did not change much and appeared to be mainly related to time (one cluster mostly comprising experiments collected after 10 days of incubation). A similar trend could be observed for Cu in the vineyard biotic soil experiments. Nevertheless, minor changes in the partitioning of Zn were seen between the biotic and abiotic experiment, between the soil and the water fraction and between the soil particle-size fractions (Figure 3-4B). Indeed, Zn desorption in soils could be counteract by Fe-oxides preventing Zn from desorption by providing to this metal an additional clay-sized phase to sorb (Antoniadis and Golia, 2015).

The particle-size fractions of soils are composed of different minerals in addition to different soil organic matter. From a mineralogical point of view, the sand fraction is mainly composed of quartz, while the most content of oxides and secondary silicate minerals as phyllosilicates are contained respectively in the silt and the clay fractions (Nenadović et al., 2010). With decreasing particle size, decreasing amounts of SiO_2 and increasing amounts of Al_2O_3 could be observed, whereas Fe_2O_3 was most contained in the silt fraction of soils with decreasing particle size.

In our experiment, Cu sorption in biotic soils was higher than in abiotic experiments, although partitioning among the particle-size fractions did not significantly change (Figure 3-4A). However, soil autoclaving seem to affect organic matter and favor export of Cu into the water phase. Autoclaving soils suppress the activity of their microorganisms, while favoring decomposition of organic matter. However, metals can equally be sorbed on both living and dead microorganisms. Kafantaris and Borrok (2014) studied the sorption of Zn onto different living and dead bacterial species and obtained equal sorption of Zn onto living and dead (12 h of ultraviolet radiations) *Pseudomonas mendocina*. However, the activity of soil microorganisms impacts the sorption of metals onto soil surfaces. soil microorganisms, including eubacteria (i.e. cyanobacteria or gram-positive bacteria), unicellular eukaryotes (i.e. yeast, diatoms, protozoa) and multicellular eukaryotes as fungi (Buscot and Varma, 2005), are part of the SOM can interact with soil minerals and especially clay minerals. As SOM, microorganisms may influence the speciation of metals in soils. Microorganisms can transform metals, uptake (intracellular accumulation) them or change their sorption onto the soil matrix (via bio-sorption, sorption on organic and inorganic precipitates as oxalate, carbonates, phosphates, oxides and sulfides or formation of them) (Gadd, 2010; Ledin, 2000).

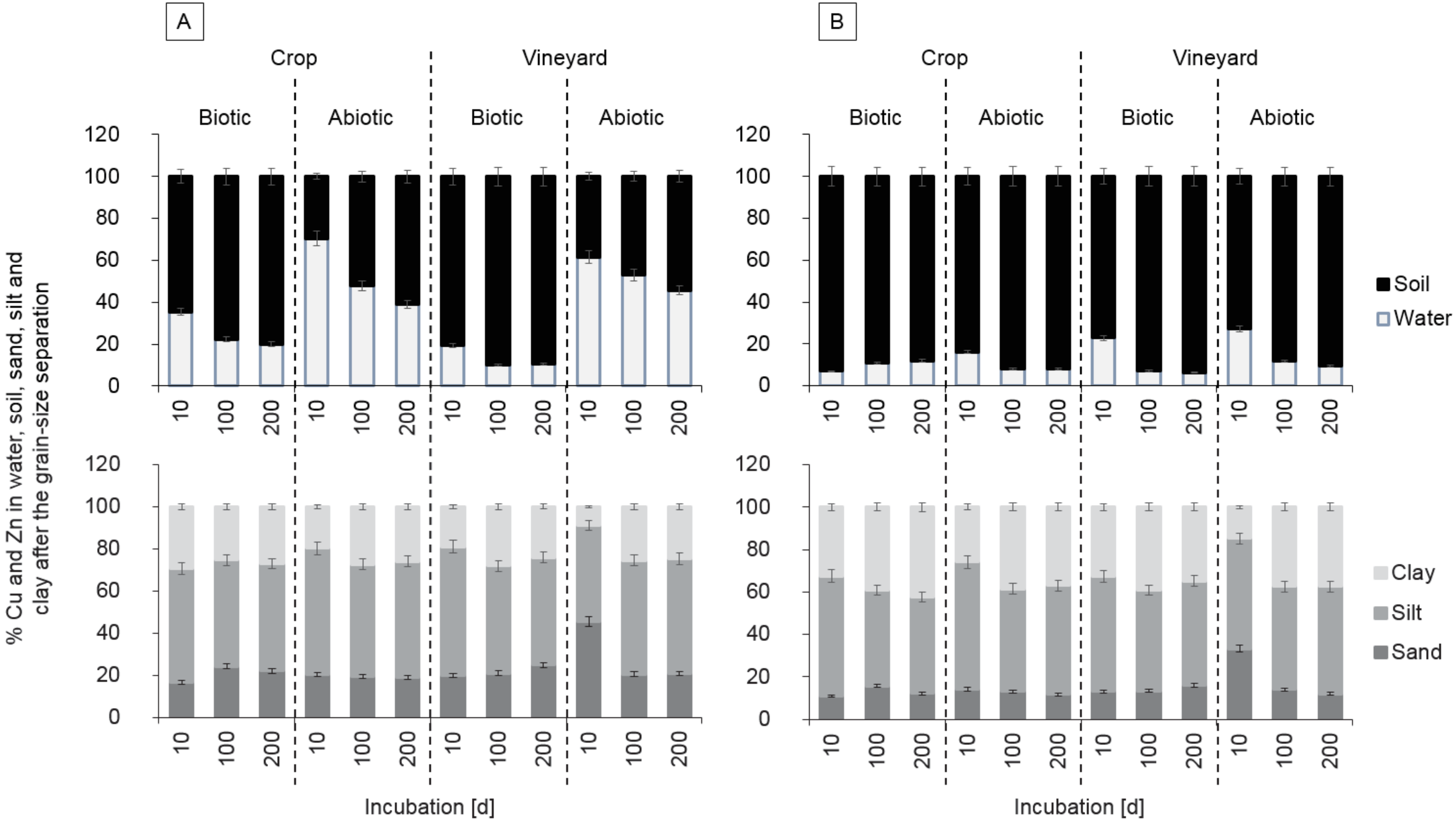

Figure 3-4: Partitioning of Cu (A) and Zn (B) among the crop and vineyard soils spiked with all the contaminants, the waters used to separate the particle-size fractions of soils, the sand, silt and clay fractions in biotic and abiotic conditions. Error bars denote 95 % confidence intervals

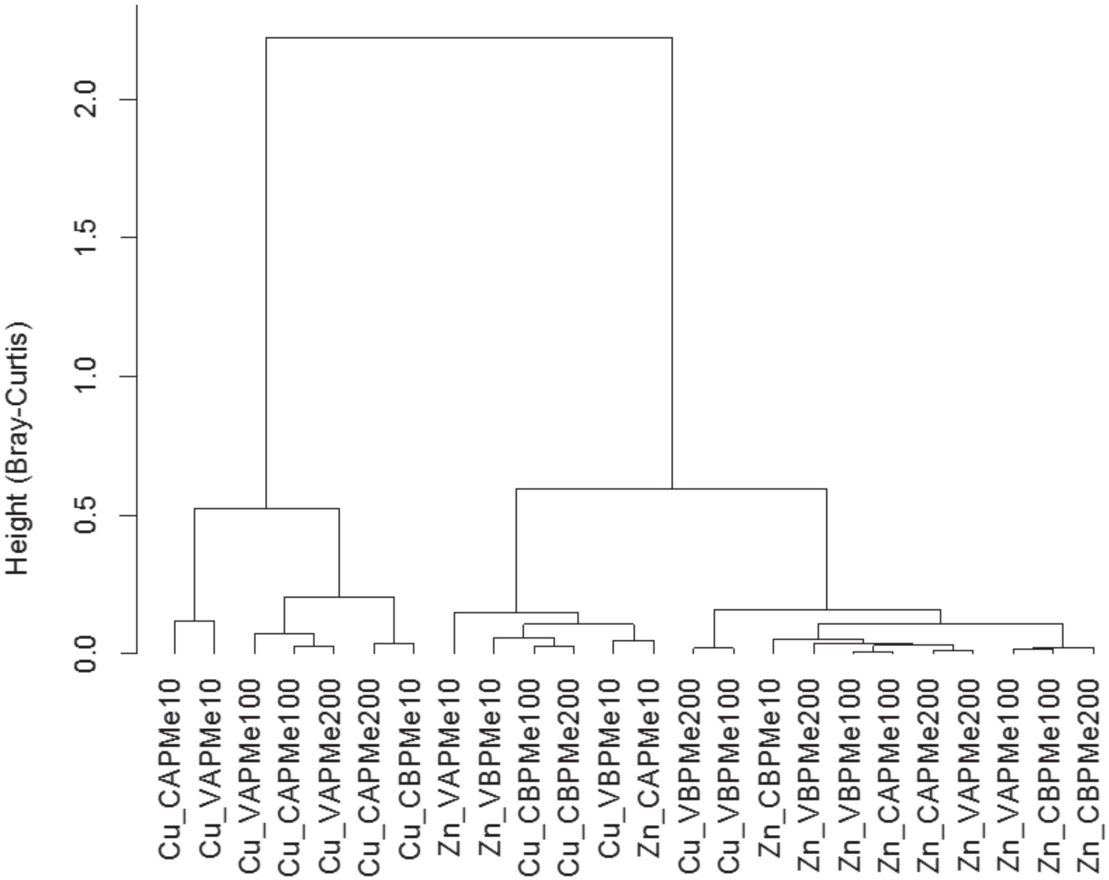

Figure 3-5: Hierarchical cluster analysis (Ward's method) of data of Cu and Zn distribution in the particle-size fractions (water, soil, sand, silt, clay) in crop (C) or vineyard (V) abiotic (A) and biotic (B) soils incubated with both pesticides and metals (PMe) and incubated up to 200 days with experimental sacrifices at day 10, 100 and 200

4.3. Chemical partitioning of Cu and Zn

In the vineyard soil, Cu was mainly and constantly associated to the LExch and AFeOx fractions (Figure 3-6). Contrasting with the vineyard soil, chemical partitioning of Cu in the crop soils largely changed during the ten days after its spiking in the soil. During the first 10 days, LExch-Cu amount dramatically decreased, Acid-Cu remained constant and the other fraction of Cu increased. From 10 days onwards, the LExch-, MnOx-, Org- and CFeOx-Cu amounts reached a steady state contrary to Acid-Cu, which slightly increased with time. The partitioning of Cu did not change after 100 days on incubation in the crop soil spiked with metals only (Figure 3-6). These results reflect the predominance of Cu in the silt and clay fractions of soils.

In the vineyard soil, Zn followed a similar pattern as Cu, although a large proportion of Zn was also found in the Res fraction (Figure 3-6). The chemical partitioning of Zn in the crop soil particularly changed between its application and 10 days of incubation. No exchangeable forms of Zn occurred in the soil after 10 days, while MnOx-, Org-Zn, and especially Res-Zn fraction increased. The speciation of Zn did not significantly change after 100 days (Figure 5), which also reflected the partitioning of Zn among the grain-size fractions of soils. Indeed, Zn predominated in the clay fraction and less in the sand fraction, which contrasted with Cu.

Overall, both the physical and chemical speciation of Cu and Zn changed less between 10 and 200 days of incubation than between day 0 and day 10 (Figures 3-4 and 3-6). The chemical speciation of these metals mainly changed during the first 10 days of incubation, as previously observed (e.g. Ma et al., 2006a ; Ma et al., 2006b). Within the 10 first days after spiking, LExch-Cu amount dramatically decreased, whereas Acid-Cu remained constant and the other fraction of Cu increased in the crop soil. From 10 days onwards, the LExch-, MnOx-, Org- and CFeOx-Cu amounts reached a steady state contrary to the Acid-Cu fraction, which slightly increased with time (Figure 3-6). In comparison, no exchangeable forms of Zn occurred in the soil after 10 days, while MnOx-, Org-Zn, and especially Res-Zn fraction increased (Figure 3-6).

The cluster analysis of Cu and Zn patterns of chemical partitioning revealed major changes according to the type of soil (Figure 3-7). Indeed, the first cluster was only composed of samples from the crop soil, whereas the second cluster was mainly composed of vineyard soil samples. However, chemical partitioning of both Cu and Zn differed, as emphasized by distinct Cu and Zn patterns. The Cu and Zn partitioning obtained among the chemical fractions of soils were consistent with the literature and the known behavior of these metals with the minerals of soils. Indeed, Cu and Zn are known to be associated to carbonates but especially oxides and clays minerals after processes of adsorption, (co-)precipitation, diffusion in micropores, occlusion (Al-Qunaibit et al., 2005; Ma et al., 2006; Schosseler et al., 1999). The minerals within the sand fraction are relatively inert. The presence of Cu and Zn in sands is not caused by the interaction of these metals with quartz but mainly with the POM contained in the sand fraction (Quenea et al., 2009).

However the results of the sequential chemical extraction of Cu and Zn should be considered with caution because extractants used to assess the amounts of metals in the chemical fractions of soil may lack of selectivity. For instance, the use of magnesium chloride to extract exchangeable elements may dissolve carbonates. Hence a release of Mg from carbonates may occur. Besides the use of acetic acid may lead to the dissolution of soil other phase and an overestimation of metals bound to carbonates (Tessier et al., 1979).

In a lesser extent, patterns of Zn and Cu chemical partitioning also differed in presence and absence of pesticides (Figure 3-6), mainly in the crop soil (Figure 3-7). Indeed, in soils spiked with metals only, major changes could be observed after 10 days of incubation, while the Cu and Zn speciation did not evolve between 10 and 200 days in the presence of pesticides. In the vineyard soil, Cu and Zn were associated to the LExch and AFeOx fractions, although a larger proportion of Zn occurred in the residual fraction (Figure 3-6). The addition of both metals and pesticides decreased the Lexch fraction of Cu, whereas the AFeOx-Cu fraction increased over time. However, CFeOx-Zn and Res-Zn fractions slightly increased with pesticide addition (Figure 3-6). This suggests that the redistribution of Cu and Zn between the LExch and the AFeOx fractions from the vineyard soil could be due to the preferential sorption of pesticides to organic and inorganic sites where metals were initially sorbed. Indeed, the LExch fraction is made of metals bound by covalent forces to organic and inorganic sites, whereas the AFeOx fraction metals are associated to amorphous Fe-oxides after processes of co-precipitation, adsorption, surface complex formation, ion exchange and/or penetration into the mineral (Rao et al., 2008). Metals present in the Res fraction are strongly bound or entrapped into minerals and especially clays and oxides (Clausen et al., 2001; Đurović et al., 2009, 2009; Remucal and Ginder-Vogel, 2014; Ruggiero et al., 2002).

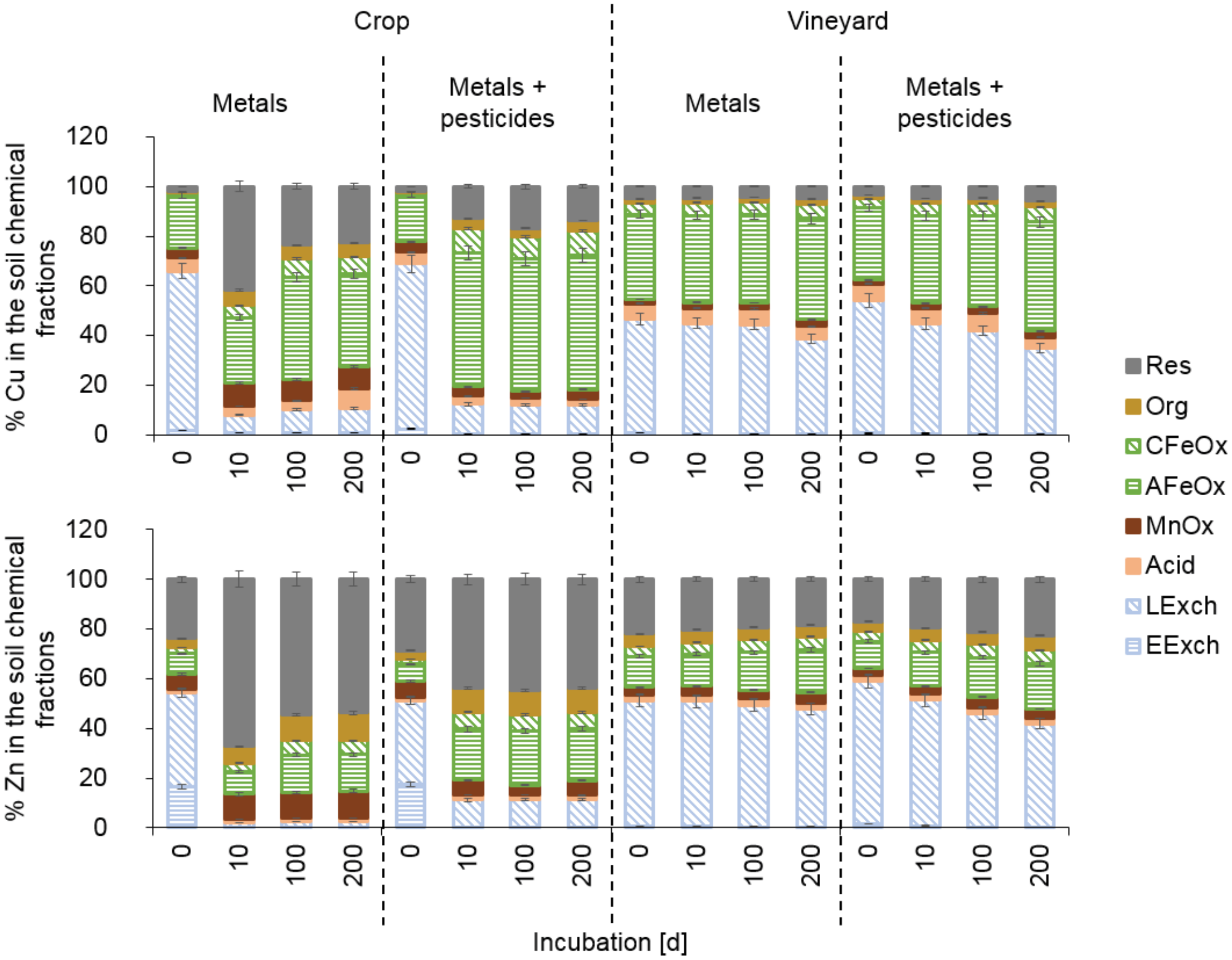

Figure 3-6: Speciation of Cu and Zn among the chemical fractions of the crop and vineyard soils when only metals were spiked or both metals and pesticides. Error bars denote 95 % confidence intervals

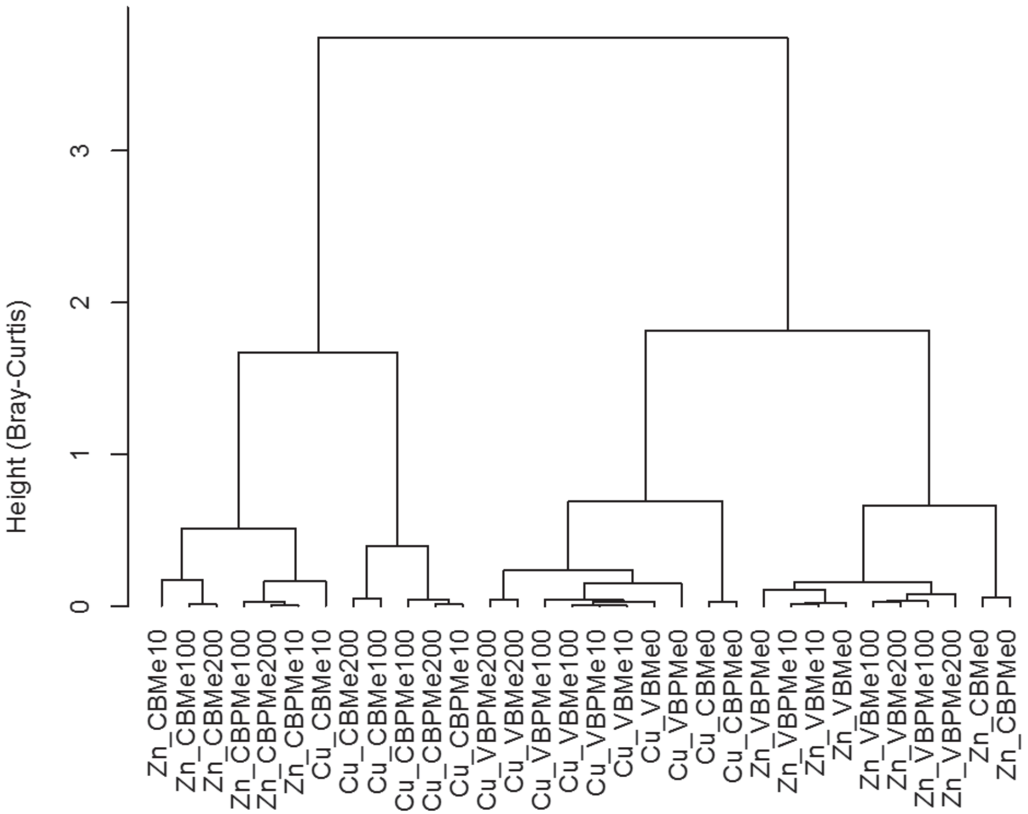

Figure 3-7: Hierarchical cluster analysis (Ward's method) of data of Cu and Zn distribution in the chemical fractions of soils (EExch, LExch, Acid, MnOx, AFeOx, CFeOx, Org and Res) in crop (C) and vineyard (V) biotic (B) soils spiked with metals (Me) or spiked with both metals and pesticides (PMe) and incubated up to 200 days with experimental sacrifices at day 0, 10, 100 and 200

In the crop soil spiked with both metals and pesticides, chemical partitioning did not change much between 10 and 100 days of incubation, while the AFeOx fraction was systematically lower at day 10 without addition of pesticides (Figure 3-6). This may be due to the redissolution of sorbed metals (after precipitation/nucleation processes) with decreasing amounts of metals in solution after continual diffusion into micropores of soil solids (Ma et al., 2006b). This trend was not observed in the presence of pesticides. This suggests that sorption of pesticides onto clay minerals limited their interaction with metals in the soil solution, which altered metal redissolution and distribution in the soil.

4.4. δ^{65}Cu and δ^{66}Zn values in particle-size fractions

The δ^{65}Cu and δ^{66}Zn values mainly varied among (i) particle-size fraction, (ii) the crop and vineyard soils, (iii) the incubation periods, and (iv) the sterilization state of soils (Figures 3-8 and 3-9). Minor changes were obtained in the physical and chemical partitioning of Cu and Zn in both the particle-size and chemical fractions of soils. Therefore, differences in the Cu and Zn isotope composition may reflect different binding patterns of Cu and Zn with the particle-size fractions of soils over time.

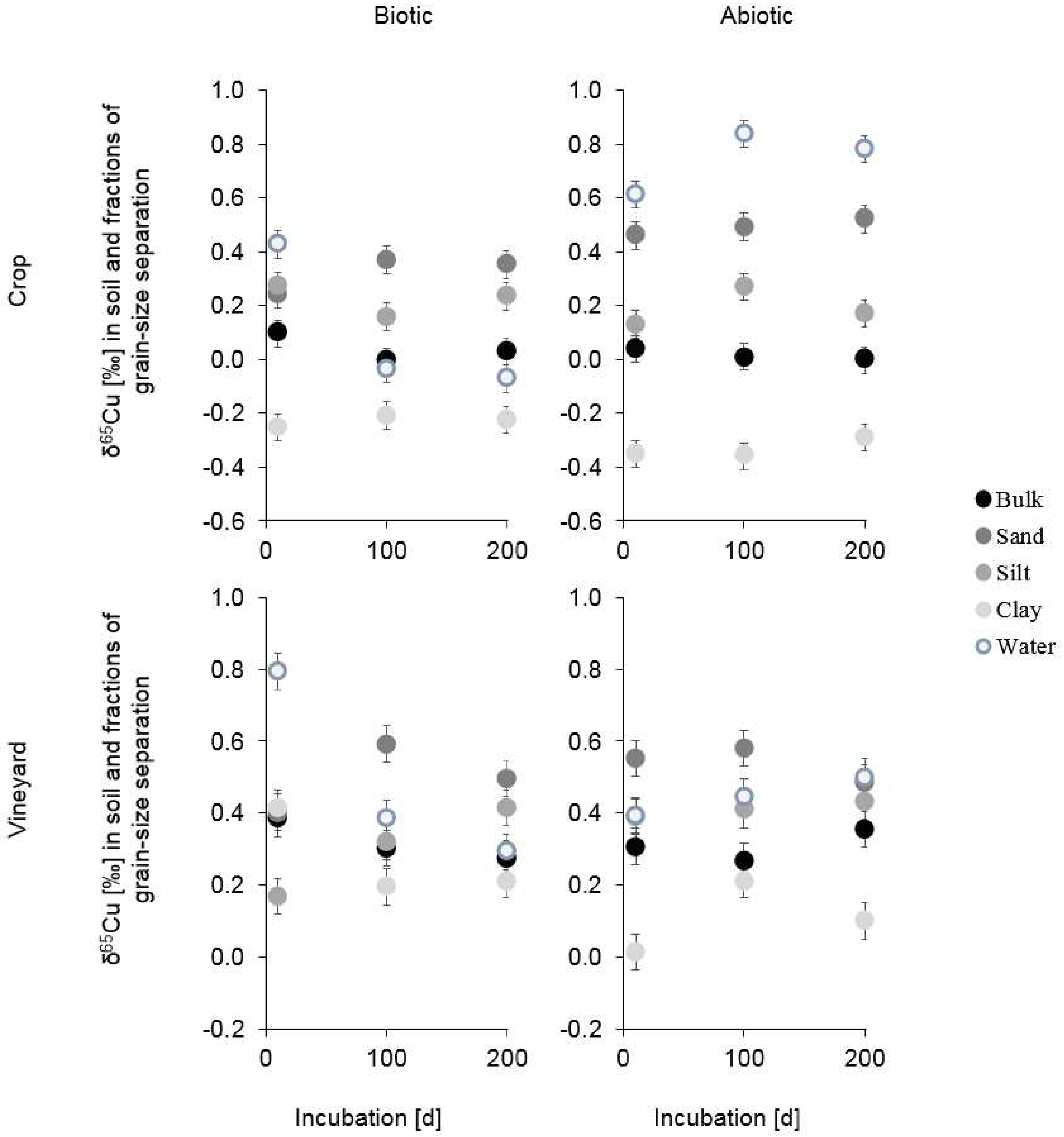

Figure 3-8: Temporal change of δ^{65}Cu values [‰] among the bulk crop and vineyard soils, their particle-size fractions and the water used during the separation protocol. Error bars denote analytical uncertainty of ± 0.05 ‰ (including precision and reproducibility)

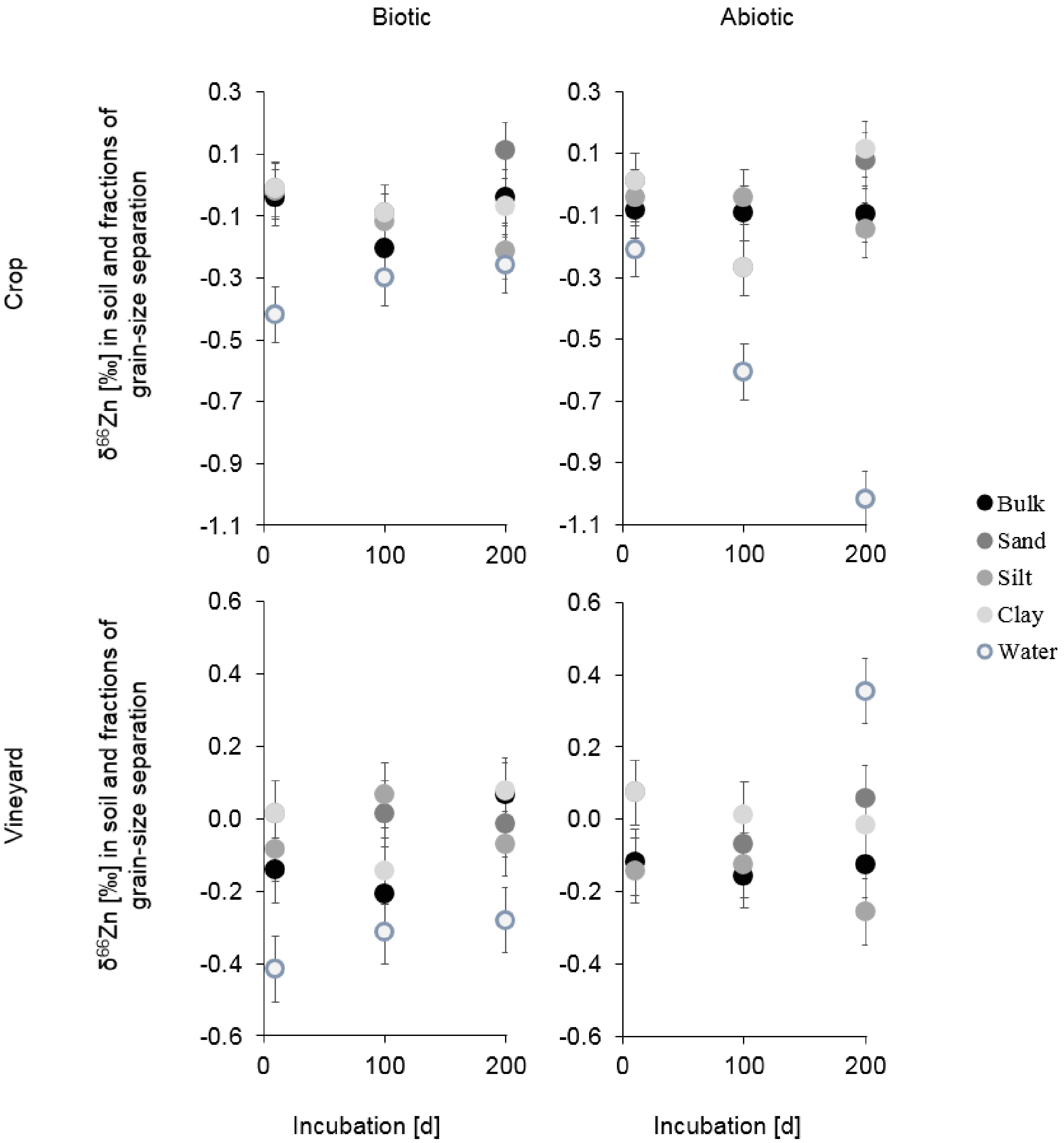

Figure 3-9: Temporal change of δ⁶⁶Zn values [‰] among the bulk crop and vineyard soils, their particle-size fractions and the water used during the separation protocol. Error bars denote analytical uncertainty of ± 0.08 ‰ (including precision and reproducibility)

Cu isotopic signatures in the silt fraction of the biotic crop soil changed from 10 to 100 days ($\Delta_{10\text{-}100}\delta^{65}Cu_{silt\text{-}biotic\text{-}crop}$ = 0.12 ± 0.05 ‰), but not during the 100 following days ($\Delta_{100\text{-}200}\delta^{65}Cu_{silt\text{-}biotic\text{-}crop}$). This suggests that processes controlling Cu sorption changed within the silt fraction. Indeed, the complexation of Cu with organic matter is known to generate a fractionation of Cu isotope $\Delta^{65}Cu_{humic\text{-}acid\text{-}solution}$ ranging from 0.15 to 0.37 ‰ (Bigalke et al., 2010). The silt fraction is mainly composed of oxides (Nenadović et al., 2010) but also quartz, to a lesser extent silicates and phyllosilicates and organic material. The smaller the particle size is, the lower are OM degradation state and availability, and the higher is OM and OC contents (Quenea et al., 2009; Schmidt and Kögel- Knabner, 2002). A major mechanism of Cu sorption in soil may be complexation via the formation of inner sphere surface complexes as revealed by extended X-ray absorption fine structure (EXAFS) and X-ray absorption near-edge structure (XANES) spectroscopy (Flogeac et al., 2004). Among the soil particles, silica and/or smectite fraction, coated by organic matter are essentially involved in Cu(II) retention process. However, Cu surface precipitation may be excluded. Metals can form complexes with the organic matter thanks to their carboxyl (-COOH), carbonyl (C=O) and phenolic groups (Bradl, 2004). Hence, we hypothesized that during the first stage of the experiment (up to 100 days), the speciation of Cu was mainly controlled by its complexation with organic matter coating the particles of the silt fraction of the crop soil.

From day 100 to 200, microorganisms seemed to be also involved the fate of Cu in the biotic soil experiments. Kinetic effects may have mostly caused isotopic variations in most biological systems. During biological reactions (e.g. photosynthesis, bacterial processes) the lighter isotope is usually enriched in the reaction product relative to the substrate (Hoefs, 2015). Besides, most of the fractionations in biological reactions generally take place during the so-called rate determining step, which is the slowest step (Hoefs, 2015). Typically, Cu isotope fractionation generated by Cu sorption onto soil microorganisms differed from that generated by Cu complexation with the humic substances of OM. $\Delta^{65}Cu_{bacteria\text{-}solution}$ is known to range (in case of living microorganisms) from -4.4 to 2.43 ‰ (Mathur et al., 2005; Navarrete et al., 2011; Pokrovsky et al., 2008), which may compensate the effect of Cu sorption to humic substances ($\Delta^{65}Cu_{humic\text{-}acid\text{-}solution}$).

Processes in the silt fraction of the crop soil seemed to act in an opposite way in the absence of soil microbial activity. In the abiotic experiment, Cu isotope composition during the first 100 days yielded $\Delta_{10\text{-}100}\delta^{65}Cu_{silt\text{-}abiotic\text{-}crop}$ = -0.14 ± 0.05 ‰, which is in the same range than change in Cu isotope composition between day 100 and 200 ($\Delta_{100\text{-}200}\delta^{65}Cu_{silt\text{-}abiotic\text{-}crop}$ = 0.10 ± 0.05 ‰). Soil autoclaving not only led microbial death in the abiotic experiments, but also may have altered SOM quality and favor mineralization of organic carbon (OC) and organic nitrogen (ON) within the SOM. According to $\Delta\delta^{65}Cu$ values, Cu complexation with the organic matter may have occurred mainly between 100 and 200 days of incubation, before 100 days of incubation, Cu was putatively sorb onto microbial debris. Indeed The extent of the corresponding Cu isotope fractionation $\Delta^{65}Cu_{bacteria\text{-}solution}$ range from -0.3 to 0.69 ‰ (Navarette et al., 2011).

In the vineyard soil, no clear temporal trends were observed in the silt fraction ($\Delta_{10\text{-}100}\delta^{65}Cu_{silt\text{-}biotic\text{-}vineyard}$ = -0.15 ± 0.05 ‰ and $\Delta_{100\text{-}200}\delta^{65}Cu_{silt\text{-}biotic\text{-}vineyard}$ = -0.09 ± 0.05 ‰). Besides, no significant change in the $\delta^{65}Cu$ values was observed in the silt fraction of the abiotic vineyard soil. Microorganisms may play a dominant role in Cu sorption occurring in the silt fraction of the vineyard soil that contains less oxides and organic matter than the crop soil (Table 2-1). Similarly to the silt fraction, changes in Cu isotope composition in the clay fraction of vineyard soil were observed with time ($\Delta_{10\text{-}100}\delta^{65}Cu_{clay\text{-}abiotic\text{-}vineyard}$ = -0.20 ± 0.05 ‰ and $\Delta_{100\text{-}200}\delta^{65}Cu_{clay\text{-}abiotic\text{-}vineyard}$ = 0.11 ± 0.05 ‰), and according to the biotic state of the soil ($\Delta_{10\text{-}100}\delta^{65}Cu_{clay\text{-}biotic\text{-}vineyard}$ = 0.22 ± 0.05 ‰ and $\Delta_{10\text{-}100}\delta^{65}Cu_{clay\text{-}abiotic\text{-}vineyard}$ = -0.20 ± 0.05 ‰). In soils, microorganisms associate themselves with soil components surfaces, such as oxides and clay minerals (Ruggiero et al., 2002). Clay mineral may influence the concentration of nutrients, protect microorganisms against predators and can also lead to the immobilization of microbial cells (Ruggiero et al., 2002). The largest content of secondary silicate minerals, such as phyllosilicates, are in the clay fraction (Nenadović et al., 2010). Therefore, it can be assumed that clay contains higher content of microorganisms than the other size fractions. This may partly explain $\delta^{65}Cu$ values in the clay fraction of the vineyard soil. Indeed, the kinetic isotopic fractionations of biologically-mediated processes vary in magnitude, depending on reaction rates, concentrations of products and reactants, but also according to the environmental conditions (Kendall and Caldwell, 1998).

Changes in $\delta^{66}Zn$ values were also observed during the experiment, especially in the clay fractions of vineyard (biotic) soil and crop (abiotic) soil experiments. In the vineyard soil, $\delta^{66}Zn$ values largely varied over time ($\Delta_{10\text{-}100}\delta^{66}Zn_{clay\text{-}biotic\text{-}vineyard}$ = 0.16 ± 0.09 ‰ and $\Delta_{100\text{-}200}\delta^{66}Zn_{clay\text{-}biotic\text{-}vineyard}$ = -0.22 ± 0.09 ‰), and a similar trend could be observed in the crop soil ($\Delta_{10\text{-}100}\delta^{66}Zn_{clay\text{-}abiotic\text{-}crop}$ = 0.28 ± 0.09 ‰ and $\Delta_{100\text{-}200}\delta^{66}Zn_{clay\text{-}abiotic\text{-}crop}$ = -0.38 ± 0.09 ‰). Changes of the $\delta^{66}Zn$ values could also be recorded in the sand fraction of the abiotic crop soil

($\Delta_{10\text{-}100}\delta^{66}Zn_{sand\text{-}abiotic\text{-}crop}$ = 0.28 ± 0.09 ‰ and $\Delta_{100\text{-}200}\delta^{66}Zn_{sand\text{-}abiotic\text{-}crop}$ = -0.35 ± 0.09 ‰) and vineyard soils ($\Delta_{10\text{-}100}\delta^{66}Zn_{sand\text{-}abiotic\text{-}vineyard}$ = 0.14 ± 0.09 ‰ and $\Delta_{100\text{-}200}\delta^{66}Zn_{silt\text{-}abiotic\text{-}vineyard}$ = 0.13 ± 0.09 ‰). However, no significant shift could be recorded in the sand fraction of the biotic soils, except between 100 and 200 days for the crop soil ($\Delta_{100\text{-}200}\delta^{66}Zn_{sand\text{-}biotic\text{-}crop}$ = -0.20 ± 0.09 ‰). This suggests that similar Zn sorption processes occur over time, whatever the biotic state of soil in the sand fraction.

As for Cu, Zn speciation in soils largely depends of the OM content in the particle-size fractions of soils. The Zn isotope signature measurements revealed than until 100 days, Zn remained mainly associated to SOM, certainly by complexation. Indeed, Zn sorption and complexation with organic material in soils favor the heavy Zn isotopes (Jouvin et al., 2009; Kafantaris and Borrok, 2014; Opfergelt et al., 2017). For instance, Jouvin et al. (2009) obtained Zn isotope fractionation ($\Delta^{66}Zn_{humic\text{-}acid\text{-}solution}$) ranging from 0.21 ± 0.09 ‰ to 0.28 ± 0.10 ‰. However, at 200 days the Zn isotope shifts indicated that another process controlled the speciation of Zn. In soils, Zn may also be associated to oxides and clay minerals (Opfergelt et al., 2017). We hypothesize that Zn was preferentially associated to oxides over time. Indeed, according to the Zn isotope fractionation in the literature, depletion in $\delta^{66}Zn$ in soils is mainly due to its association with oxides (Juillot et al., 2008; Pokrovsky et al., 2005) and among them Mn and Al oxides. Our soils contained few amounts of Mn oxides (0.12 % on average), Fe oxides (6.66 % in the crop soil and 3.86 % in the vineyard soil) but higher Al oxides contents (11.2 and 7.49 % respectively in the crop and vineyard soils) (Table S2). Positive Zn isotope fractionation were obtained after the association of Zn with Fe oxides (Juillot et al., 2008; Pokrovsky et al., 2005). In contrast, negative Zn isotope fractionation has been observed when Zn is associated with Al oxides (Pokrovsky et al., 2005), indicating the impact of Al oxides in the speciation of Zn in the studied soils. Besides, varying extent of fractionation was observed among soils and among fractions, which may reflect different type of Al oxides and content of Al oxides. The nature and amount of sorption sites of minerals very likely affect Zn isotopic fractionation (Guinoiseau et al., 2016). Indeed, $\delta^{66}Zn$ values largely differed according to the sorption sites of clay minerals (edge site: $\Delta^{66}Zn_{adsorbed\text{-}solution}$ = 0.49 ± 0.06 ‰, basal site: $\Delta^{66}Zn_{adsorbed\text{-}solution}$ = 0.18 ± 0.06 ‰).

The $\delta^{66}Zn$ values also changed over time in the silt fraction of the vineyard biotic soil ($\Delta_{10\text{-}100}\delta^{66}Zn_{silt\text{-}biotic\text{-}vineyard}$ = -0.15 ± 0.09 ‰ to $\Delta_{100\text{-}200}\delta^{66}Zn_{silt\text{-}biotic\text{-}vineyard}$ = 0.13 ± 0.09 ‰). The most content of oxides are contained in the silt fraction of soils (Nenadović et al., 2010). Based on $\delta^{66}Zn$ values, we can assume that during the first 30 days of the experiment Zn was mainly associated to oxides in the silts and especially Al oxides, while during the remaining 100 days of the experiment, Zn was mainly associated to the OM of the silt fraction.

However, the effect of microorganism on δ^{66}Zn values is unlikely based on our dataset. This is in agreement with the cluster analyses that revealed different chemical partitioning patterns in the biotic experiments for Cu only.

5. Conclusion

To evaluate aging of pesticides and metals in agricultural soils soil experiments were incubated up to 200 day and particle-size separation, sequential chemical extraction, quantification of MTY, SMET Cu and Zn but also stable isotope analyses were combined.

During the particle-size separation procedure, most of the MTY and SMET were exported in water. The distribution of the remaining part of MTY and SMET among particle-size fractions highlighted the importance of organic matter associated to sand, silt and clay fractions. This is relevant as the intensity of rainfall events may control the type of soil particle-size fractions eroded by runoff waters (Martínez-Casasnovas et al., 2002; Mohamadi and Kavian, 2015). Besides, the volume of water generated by rainfall events may impact the dissolved loads of pesticides and metals export by runoff and leaching (Meite et al., 2018). In this experiment, MTY and SMET were mainly measured in the water fraction used to separate the grain-size fractions of soils while metals were mainly measured in soil particles. According to the amounts of MTY, SMET, Cu and Zn found among the grain-size fractions of soils, the silt fraction mainly controlled the speciation of these contaminants in soils. The speciation the pesticides and metals studied was similar in the studied soils with high content of silts (crop soil, 61.5 %; Table 2-1) as in soils with lower content of silts (vineyard soil, 23.1 %; Table 2-1). Therefore, in case of intense rainfalls, mobilization of mainly silt-bound pesticides and metals and transport by surface runoff is expected. In the case of longer events with lower intensity, leaching of pesticides and metals may be expected as well as higher export of dissolved pesticides and metals in the dissolved phase of runoff.

CSIA of MTY and SMET highlighted the occurrence of biodegradation in the biotic experiments. The speciation of Cu and Zn among the particle-size fractions of soils remained constant over time. The δ^{65}Cu and δ^{66}Zn values enabled to suggest dominant processes driving sorption of Cu and Zn in the particle-size fractions of soils, including complexation with the humic acid of SOM, binding to oxides and sorption onto microorganisms. The comparison between biotic and abiotic experiment was particularly useful to highlight dominant processes occurring during Cu and Zn aging. For both pesticides and metals, the sand fraction accounted most than expected in the speciation of these pollutants.

To confirm the trends observed in the evolution of the δ^{65}Cu and δ^{66}Zn values, intermediate or longer incubation period could be investigated in the future. In addition, similar studies could be conducted with soils with different properties and different qualities of organic matter to evaluate the impact of SOM degradation state of the organic matter within the grain-size fractions of soils on the speciation of both pesticides and metals. Overall, this study shows that physical and chemical speciation can be efficiently coupled with stable isotope analysis of metals and CSIA of pesticides to study pollutant aging of both pesticides and metals in agricultural soils.

6. References

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Chapitre 4 : Impact des variations climatiques sur la dissipation et la dégradation des pesticides dans les sols agricoles

Deux études sont présentées dans le chapitre 4. Ce chapitre discute de l'impact des conditions hydro-climatiques sur la persistance des pesticides de synthèse dans les sols agricoles. Dans un premier temps est présenté l'impact de la température et la teneur en eau sur la dissipation et la dégradation d'un mélange de pesticides et l'apport de l'analyse isotopique par composés spécifiques (CSIA) pour évaluer la dégradation in situ de ces polluants dans les sols. Enfin, l'accent est mis sur l'un des pesticides du mélange de pesticides utilisé : le fongicide chiral Métalaxyl et l'analyse isotopique énantiosélective du carbone (ESIA) développée pour suivre la dégradation énantiosélective de ce fongicide dans les sols.

Partie 4.1. Effet de la teneur en eau des sols et de la température sur la dissipation d'un mélange de pesticides

Fatima Meite, Charline Wiegert, Benoit Guyot and Gwenaël Imfeld

1. Abstract and highlights

Synthetic pesticides and their degradation products can persist in soils following pesticide application. The fraction of pesticide mobilised after rainfall events may strongly vary according to both soil properties and pesticide degradation. However, further informations are needed in our actual knowledge of the effect of temperature or soil moisture on the persistence of pesticides in soils. We evaluated the effect of soil water contents (20 and 40 %) and temperatures (20 and 30°C) on degradation kinetics of pesticides applied as a mixture on a crop and a vineyard soil and incubated for 200 days. The soils were spiked at environmental concentrations (5 $\mu g.g^{-1}$) in a mixture of 9 fungicides, 10 herbicides and 1 insecticide typically used by farmers in the studied catchments. The single first-order (SFO) or bi-exponential (BEXP) model revealed that dissipation kinetics of pesticides in the soils largely differed according to soil type > temperature > soil water content. Pesticide dissipation increased with higher temperature, and to a lesser extent, with soil water content. CSIA enabled to evaluate transformation reactions of pesticides, which may involve either nuclephilic substitution reactions S_N1 or S_N2, depending on soil and temperature conditions. Our results emphasise the wide spectrum of half-life times that can be expected for individual pesticide in a mixture and under different soil conditions, and also underline the potential of CSIA to quantify the extent of pesticide degradation and elucidate reaction mechanisms in contaminated soils.

Highlights:

- Half-live times of pesticides degradation in a mixture often differed from typical DT_{50}
- The soil physico-chemical properties mainly impacted pesticide dissipation
- Higher temperature and soil water content tended to favor dissipation of pesticides
- Models emphasised pesticide dissipation according to soil > temperature > water content
- CSIA suggests different degradation reactions with changing soil conditions

2. Introduction

Herbicides, fungicides and insecticides are often applied simultaneously or in specific succession throughout the agricultural season. This extensive use of pesticides may lead to severe soil pollution (Swarcewicz and Gregorczyk, 2012; Zhang et al., 2014) when large amounts of pesticides reach the soils (Barbash, 2014). Once on the soil, pesticides interact with soil organic matter and mineral constituents. This leads to dissipation of pesticides resulting from both biotic and abiotic processes (Khandelwal et al., 2014; Ruggiero et al., 2002; Zhang et al., 2014). The extent and contribution of dissipation processes are crucial for pesticide exports toward aquatic ecosystems because, following rainfall events, freshly applied or remaining pesticide fractions can be mobilized in runoff and leaching waters (Lefrancq et al., 2017a; Meite et al., 2018). However, further informations are needed in our actual knowledge of the effect of soil moisture and temperature on the biotic and abiotic dissipation of individual pesticides in a mixture .

Pesticide dissipation kinetic may largely differs according to intrinsic factors, such as its chemial properties, or extrinsic factors, including the occurrence of other pesticides, soil composition, moisture or temperature (Long et al., 2014; Shahgholi and Gholamalizadeh Ahangar, 2014). While in soils with organic matter content > 5 %, non-polar pesticide sorption is mainly controlled by the organic material, in soils with less organic matter content (< 5 %), pesticide sorption occurred onto clay minerals and oxides (Đurović et al., 2009). The sorption of pesticides on the soil matrix impacts their microbial and chemical degradation. Microorganisms do not easily access to sorbed pesticides as they do not take up directly surface-sorbed compounds (Ruggiero et al., 2002). The adsorbed pollutants are in equilibrium in trace amounts with the soil solution, which renders them available for biodegradation. Soil moisture impact this equilibrium as well as the activity of microorganisms (Shahgholi and Gholamalizadeh Ahangar, 2014). Variations in soil water content can change the amounts of pesticides dissolved in the soil (Arias-Estévez et al., 2008), the concentration of nutrients in the soil solution but also alter microbial communities (Ruggiero et al., 2002). Besides, temperature also highly influences the pesticide degradation, as the number of the biochemical reactions involved in degradation processes rise with the temperature rise. However above an optimal temperature, the microbial cells decease (Shahgholi and Gholamalizadeh Ahangar, 2014).

Compound specific isotope analysis (CSIA) enable to assess pesticides degradation over long time scales without analyses of degradation products (Elsner and Imfeld, 2016). During chemical transformation, lighter isotopes (e.g., ^{12}C) exhibit lower activation energy, generally resulting in faster reaction times relative to their heavier counterparts (e.g., ^{13}C). This leads to an enrichment of the heavier isotopologues in the non-degraded pesticide fraction remaining in environmental samples (Elsner, 2010). In contrast, abiotic aparent dissipation processes in soils, such as hydrophobic sorption or dilution, is expected to resulting in non-significant or low isotope fractionation (Imfeld et al., 2014; Kopinke et al., 2017). As a result, the average isotope value (e.g., ^{13}C) of the non-degraded fraction can then be used to quantify degradation. This concept is modeled in laboratory experiments by the Rayleigh equation that relates isotopic ratios to the extent of biodegradation by isotopic fractionation factors ε (Breukelen and Prommer, 2008). Magnitude of ε depends on many factors, including the number of carbon atom in the molecule, the type of bond broken (e.g., C–H or C–O), the effects of non-reacting positions and intramolecular competition (Elsner et al., 2005). However reference experiments using pesticide CSIA to evaluate factors controlling pesticide dissipation in the soil are mostly lacking so far.

Therefore, the purpose of this study was to evaluate the effect of (i) the microbial activity of soils (using soil sterilization and comparing biotic versus abiotic soils), (ii) the soil properties (soil with high clay levels versus high organic matter and oxides levels), (iii) the soil temperature (20 and 30 °C), and (iv) the soil (volumetric) water content (20 and 40 %) on the dissipation kinetics (half-life times) of individual pesticides in a mixture and the contribution of degradative processes. Two agricultural soils were spiked at environmental concentrations (5 $\mu g.g^{-1}$) with a mixture composed of 9 fungicides (anilinopyrimidine, benzimidazole, cyanoacetamide oxide, morpholine, phenylamide and strobilurin ones), 10 herbicides (benzamide, chloroacetamide, dinitroaniline, phenylpyrazole, phenylurea, triazine and urea ones) and 1 benzoylurea insecticide. These compounds were selected as they are typically used by farmers in well-referenced experimental Rouffach and Alteckendorf catchments (France) from which soils were collected. Soil microcosms were incubated for 200 days under the different conditions. Pesticide dissipation kinetics and rates were retrieved using single first-order (SFO) or bi-exponential (BEXP) models. CSIA methods for carbon istopes ($\delta^{13}C$) measurements were adapted from previous studies to evaluate the usefulness of carbon CSIA to determine degradation and potential mechanisms in soils, relying on isotopic enrichment facors (ε_{bulk}) and carbon apparent kinetic isotopic effect factors ($AKIE_C$) calculations.

3. Material and methods

3.1. Chemicals

Nine fungicides (azoxystrobin, carbendazim, cymoxanil, cyprodinil, dimethomorph, kresoxim-methyl, metalaxyl, pyrimethanil and tetraconazole), ten herbicides (atrazine, diuron, isoproturon, isoxaben, oryzalin, pendimethalin, pyraflufen-ethyl, simazine, *S*-metolachlor and terbuthylazine) and one insecticide (flufenoxuron) were purchased from Sigma–Aldrich (St. Louis, MO, USA) with reported purity ranging from 96 to 99.9 % (analytical grade). The chemical properties of selected pesticides are provided in Table 2-6. Stock solutions were prepared at 500 $mg.L^{-1}$ in acetonitrile (purity of 99.9% minimum) as individual pesticide and were stored at -20°C. These pesticides were selected for the mixture because of their utilization by farmers in the studied catchments.

3.2. Soils

The characteristics of the soil used and their origin are presented in Chapter 2, part 2. The soil properties are summarized in Table 2-1.

3.3. Experimental set-up

The soil microcosms consisted of about 20 g of air-dried soil in sterile 30 mL crimp glass vials, with sterile silicone/natural PTFE caps (Interchim®, France). To maintain aerobic conditions in the microcosm atmosphere while limiting water loss and avoiding contamination, a 0.2 µm syringe filter (Rotilabo®, Carl Roth®, France) was mounted on a syringe tip, which was stuck through the vial cap (Figure 4.1-1). The pesticide mixture solutions in DCM were spiked in distilled water and stirred until solvent evaporation. The pesticide aqueous solutions were filter-sterilized with 0.2 µm syringe filters for soil spiking.

Figure 4.1-1: Experimental set-up

The soil microcosms were prepared under laminar flow hood with sterile material. Successive layers of soils (about 5 g) were added in the 30 mL glass vials, each layer was then spiked with the pesticide solution (at environmental concentrations of 5 $\mu g.g^{-1}$ of soil) or distilled water (control), and the mixture was finally thoroughly mixed on a Vortex mixer (30 seconds at room temperature). The water volume of each microcosm was adjusted to reach final volumetric water content of 20 or 40 % to evaluate the effect of soil moisture on pesticide dissipation. Three sets of soil microcosm experiment were prepared with: (i) non-sterilized (biotic) soils spiked with the pesticide mixture, (ii) sterile (autoclaved) soils spiked with the pesticide mixture in order to evaluate abiotic herbicide dissipation, and (iii) non-sterilized soils adjusted with sterile distilled water only as control soils. Sterile soils were autoclaved three times successively in glass bottles at 125 °C for 15 min. Soils were stored at room temperature for 12 hours between each autoclave run.

All experiments were incubated for 200 days in the dark at 20 or 30°C to evaluate the effect of the temperature on pesticide dissipation. Sampling was carried out based on a sacrificial approach on days 0, 10, 50, 100, and 200. In total, 120 experiments including 40 non-sterilized (biotic) microcosms, 40 microcosms with sterile (autoclaved) soils spiked with the pesticide mixture, and 40 non-sterilized and non-spiked control soils were set-up in triplicates (See Table 4.1-3 for the detailed experimental design).

Table 4.1-3: Detailed experimental design per incubation period. Five incubation periods were applied: 1, 10, 50, 100, and 200 days

Soil	**Sterilization**	**Spike**	**Dose [µg g^{-1}]**	**Replicates**	**Analysis type**		
					Concentration (GC-MS)	**Concentration (LC-MS)**	**CSIA (GC-C-IRMS)**
Crop	No	Water	/	3	√	√	√
		Pesticide mixture	5	3	√	√	√
	Yes	Pesticide mixture	5	3	√	√	√
Vineyard	No	Water	/	3	√	√	√
		Pesticide mixture	5	3	√	√	√
	Yes	Pesticide mixture	5	3	√	√	√

Total non-spiked samples (background): 40

Total spiked and sterilized samples (control): 40

Total spiked samples: 40

3.4. Pesticides analysis

The procedure of extraction and analysis of pesticides is explained in Chapter 2, part 3.1. The extraction recoveries of pesticides with GC- and LC-MS are given respectively in Table 2-3 and Table 2-4.

3.5. Mathematical models used to assess the dissipation's kinetics of pesticides

3.5.1. Single first-order (SFO) model

For this model, a simple first-order differential equation is needed and integrated as follows:

$$\frac{dX}{dt} = -k_1 X \quad (4.1\text{-}2)$$

$$X = X_0 e^{-k_1 t} \quad (4.1\text{-}2)$$

Where, *X* is the pesticide concentration ($\mu g.kg^{-1}$) at time *t* (d) after application, X_o is the initial concentration ($\mu g.kg^{-1}$), and k_1 is the first-order rate constant (d^{-1}).

The first-order dissipation half-life is equal to the time required for half of the initial residues to dissipate (DT_{50}). This is calculated by setting $X = 0.5X_o$ in the Equation 4.1-2.

3.5.2. Bi-exponential (BEXP) model

For the BEXP model, the Equations 4.1-3, 4.1-4 and 4.1-5 describe respectively the concentration-time relationship, the integrated form and the solving equation for *X*:

$$\frac{dX}{dt} = -(k_1 X + k_2 X^2) \quad (4.1\text{-}3)$$

$$e^{-k_1 t} = \frac{X(k_1 + k_2 X_0)}{X_0(k_1 + k_2 X)} \quad (4.1\text{-}4)$$

$$X = \frac{k_1 X_0}{(k_1 + k_2 X_0)e^{k_1 t} - k_2 X_0} \quad (4.1\text{-}5)$$

Where, k_1 is the first-order rate constant ($kg.\mu g^{-1}.d^{-1}$), k_2 is the second-order rate constant ($kg.\mu g^{-1}.d^{-1}$), *t* is the time, *X* is the concentration at time t, and X_0 is the concentration at t = 0. By setting $X = 0.5\ X_0$, the DT_{50} can be derived from Equation 4.1-5.

3.6. Carbon isotope analysis

The analysis of the carbon isotope composition of the thirteen pesticides quantified by GC-MS is explained in Chapter 2, part 6. Reference carbon isotope composition values of standards of the pesticides studied were obtained using an elemental analyzer-isotopic ratio mass spectrometer (EA-IRMS) coupled isotope ratio mass spectrometer (Table 4.1-6). The reproducibility of triplicate measurements was ≤ 0.2 ‰ (1σ). The $\delta^{13}C$ values were calibrated using a two-point calibration against the Vienna Pee Dee Belemnite (V-PDB) standard. Minor shifts due to the experiment were obtained in the isotopic signatures of pesticides as summarized in Table 4.1-6.

Table 4.1-6: Carbon isotope composition of ATR, PYR, SMET and MTY standards and $\Delta\delta^{13}C$ caused by the extraction protocol

	$\Delta\delta^{13}C$ [‰] *vs.* VPDB [a]		
	EA-IRMS *vs.* GC-C-IRMS	**GC-C-IRMS extracted (5 g of soil) *vs.* non-extracted**	
		Crop	**Vineyard**
ATR	-0.6 ± 0.6	o.p.[b]	o.p.
PYR	-0.3 ± 0.5	o.p.	o.p.
SMET	0.6 ± 0.3	0.3 ± 0.5	0.7 ± 0.6
MTY	0.8 ± 0.2	-0.4 ± 0.6	-0.1 ± 0.3

[a] The error given for the $\Delta\delta^{13}C$ values was calculated via error propagation based on ± 1 SD of mean $\delta^{13}C$ values from n ≥3 measurements for each sample. $\Delta\delta^{13}C$ values were obtained on the difference between the measured $\delta^{13}C$ value of a given compound in the extract and the mean $\delta^{13}C$ value of the corresponding standard obtained by replicate injection in the GC-C-IRMS system at optional conditions.

[b] Data treatment on process.

3.7. Carbon isotope notation and calculation

In addition to carbon isotope ratios, bulk isotope enrichment factors (ε_{bulk}) and apparent kinetic isotope effect (AKIE) values, we assessed the extent of biodegradation [%] on the dissipation of pesticides as the follows (Eq. 4.1-6):

$$B = \left[1 - \left(\frac{\delta^{13}C_x + 1000}{\delta^{13}C_{in} + 1000}\right)^{\left(\frac{1000}{\varepsilon_{bulk}}\right)}\right] \times 100 \qquad (4.1\text{-}6)$$

4. Results and discussion

4.1. Dissipation kinetics and DT_{50} values in the biotic experiments

In the biotic experiment, dissipation kinetics of TZL, ISP, DIM, CYP, AZO, PRY and ORY better fitted a SFO model ($r_{SFO}^2 > 7$, Table 4.1-7), while those of SMET, MTY, CAR, SIM, TER, ATR, PYR, DIU and ISX better fitted a BEXP model ($r_{BEXP}^2 > 7$, Table 4.1-7).

Unfortunately, dissipation kinetics for FLU and PEN could not be obtained due to poor extraction from the soil, which can be related to their higher K_{oc} values (Table 2-6).

Accordingly, pesticides were divided in two sub-groups. In the SFO sub-group, the amount of pesticides was assumed to decrease as a function of the amount of remaining substrate and the logarithm of the remaining amount over time is linear (Srinivasan et al., 2014). In the BEXP group, initial fast pesticide dissipation may be privileged at the water-soil interface, where microorganisms may easily access to the pesticides (Ruggiero et al., 2002; Srinivasan et al., 2014). Except for CYM, dissipation decreased during the experiment, and particularly after day 50, suggesting prevailing biodegradation in the early stage. In a second phase, a slower dissipation of pesticides bound to soil may occur as slow desorption-diffusion processes govern pesticide dissipation. When pesticide dissipation kinetics better fit a BEXP model, oxidative reactions (O- and N-dealkylations) catalyzed by soil oxides and clays at the soil-water interface may prevail (Ruggiero et al., 2002). This suggests that the selection of a dissipation kinetic model should account for possible degradation reactions to retrieve accurate half-life times.

The DT_{50} obtained for pesticides in our experiment generally did not correspond to dissipation kinetics reported in the literature (see Table 2-1). The half-lives of SMET, MTY, CAR, TER, PYR, TZL, ISP, DIM, CYP, PRY and ORY were higher while those of SIM, ISX, ATR, DIU and AZO were lower than typically reported half-life times (Tables 4.1-8, 4.1-9, 4.1-10 and 4.1-11). TZL was highly persistent in our case (DT_{50} = 740 ± 5 days (Table 4.1-8) in our study, which is one order of magnitude higher than reported values (DT_{50} =61 days) (Kegley et al., 2016; PPDB Pesticide Properties DataBase, 2009). This fungicide is known to persist (Alam et al., 2013; Zhang et al., 2014). The dissipation of KM was very slow and began only at 100 days to reach total degradation only after 200 days (Figure 4.1-2), whereas disappearance of KM after more than 20 days in spiked-soil experiments has been reported (Khandelwal et al., 2014).

Table 4.1-7: Correlation coefficients obtained with the SFO and BEXP models in the biotic crop systems

Groups		**20 °C / 20 % w.c.**		**20 °C / 40 % w.c.**		**30 °C / 20 % w.c.**		**30 °C / 40 % w.c.**	
		$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$	$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$	$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$	$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$
	PRY	0.73	0.42	0.08	0.89	0.20	0.24	0.07	0.21
Hydrophobic	ORY	0.94	0.92	0.88	0.88	0.94	0.89	0.15	0.64
	ISX	0.80	0.93	0.73	0.76	0.83	0.34	0.80	0.28
	SIM	0.82	0.90	0.68	0.24	0.55	0.66	0.56	0.65
	TER	0.91	1.00	0.61	0.98	0.83	0.85	0.49	0.73
	ATR	0.85	0.90	0.87	0.93	0.77	0.77	0.65	0.69
	DIU	0.79	0.92	0.90	0.98	0.87	0.89	0.87	0.95
	ISP	0.84	0.65	0.91	0.85	0.94	0.33	0.87	0.90
Moderately hydrophobic	DIM	0.94	0.43	0.63	0.46	0.88	0.24	0.85	0.43
	CYP	0.98	0.24	0.39	0.24	0.96	0.24	0.35	0.24
	PYR	0.83	0.86	0.90	0.90	0.76	0.79	0.67	0.60
	AZO	0.93	0.24	0.72	0.24	0.94	0.24	0.61	0.24
	KM	0.65	0.24	0.63	0.80	0.13	0.59	0.14	0.81
	CAR	0.74	0.96	0.62	0.24	0.84	0.88	0.78	0.87
Sorptive and hydrophilic	TZL	0.98	0.24	0.38	0.24	0.96	0.24	0.81	0.24
	SMET	0.79	0.80	0.85	0.86	0.77	0.54	0.73	0.70
Hydrophilic	CYM	0.03	0.24	0.02	0.98	0.02	0.96	0.02	0.99
	MTY	0.91	0.92	0.64	0.89	0.87	0.54	0.82	0.79

Table 4.1-8: SFO modelled half-lives and dissipation rates of pesticides in the biotic crop systems

Groups		**20 °C / 20 % w.c.**		**20 °C / 40 % w.c.**		**30 °C / 20 % w.c.**		**30 °C / 40 % w.c.**	
		DT$_{50}$ [d] (SE)	**k^d [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k^d [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k^d [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k^d [d^{-1}]**
	PRY	29 (1)	0.03	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hydrophobic	ORY	75 (0)	0.009	49 (0)	0.01	76 (0)	0.009	n.a.	n.a.
	ISX	44 (2)	0.02	45 (4)	0.01	41 (0)	0.02	49 (3)	0.01
	SIM	45 (1)	0.02	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	TER	93 (3)	0.007	n.a.	n.a.	56 (3)	0.01	n.a.	n.a.
	ATR	50 (1)	0.01	56 (2)	0.01	36 (1)	0.02	n.a.	n.a.
	DIU	46 (1)	0.01	114 (4)	0.006	46 (0)	0.02	178 (10)	0.005
	ISP	49 (1)	0.01	78 (2)	0.009	70 (2)	0.01	104 (8)	0.008
Moderately hydrophobic	DIM	98 (2)	0.01	n.a.	n.a.	67 (1)	0.01	159 (15)	0.01
	CYP	112 (1)	0.006	n.a.	n.a.	89 (1)	0.008	n.a.	n.a.
	PYR	41 (0)	0.02	197 (7)	0.003	37 (2)	0.02	n.a.	n.a.
	AZO	73 (1)	0.007	21 (1)	0.005	55 (5)	0.01	n.a.	n.a.
	KM	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	CAR	40 (3)	0.02	n.a.	n.a.	43 (0)	0.02	70 (6)	0.009
Sorptive and hydrophilic	TZL	740 (5)	0.001	n.a.	n.a.	452 (5)	0.001	676 (13)	0.0008
	SMET	30 (1)	0.02	41 (0)	0.02	28 (1)	0.02	30 (1)	0.02
Hydrophilic	CYM	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	MTY	54 (1)	0.01	n.a.	n.a.	44 (2)	0.02	143 (11)	0.006

n.a.: half-lives and dissipation rates could not be retrieved due to poor and non-significant correlations

Table 4.1-9: SFO modelled half-lives and dissipation rates of pesticides in the biotic vineyard systems

Groups		**20 °C / 20 % w.c.**		**20 °C / 40 % w.c.**		**30 °C / 20 % w.c.**		**30 °C / 40 % w.c.**	
		DT$_{50}$ [d] (SE)	**k^{d} [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k^{d} [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k^{d} [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k^{d} [d^{-1}]**
	PRY	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hydrophobic	ORY	n.d.	n.d.	70 (2)	0.01	74 (1)	0.009	71 (1)	0.01
	ISX	n.d.	n.d.	n.a.	n.a.	72 (4)	0.009	n.a.	n.a.
	SIM	n.a.	n.a.	51 (5)	0.01	90 (9)	0.009	n.a.	n.a.
	TER	70 (1)	0.01	n.a.	n.a.	43 (0)	0.02	n.a.	n.a.
	ATR	46 (1)	0.01	50 (4)	0.01	33 (1)	0.02	n.a.	n.a.
	DIU	n.d.	n.d.	141 (3)	0.005	46 (1)	0.02	91 (1)	0.008
	ISP	n.d.	n.d.	113 (2)	0.006	42 (1)	0.02	64 (1)	0.01
Moderately hydrophobic	DIM	53 (9)	0.008	n.a.	n.a.	41 (6)	0.01	n.a.	n.a.
	CYP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	PYR	38 (0)	0.02	146 (6)	0.004	36 (1)	0.02	99 (8)	0.006
	AZO	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	KM	182 (1)	0.004	55 (0)	0.01	n.a.	n.a.	n.a.	n.a.
	CAR	n.d.	n.d.	78 (8)	0.008	49 (3)	0.01	n.a.	n.a.
Sorptive and hydrophilic	TZL	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SMET	29 (0)	0.02	43 (4)	0.00	27 (0)	0.03	n.a.	n.a.
Hydrophilic	CYM	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	MTY	73 (2)	0.01	106 (3)	0.007	54 (0)	0.01	95 (3)	0.008

n.a.: half-lives and dissipation rates could not be retrieved due to poor and non-significant correlations

Table 4.1-10: BEXP modelled half-lives and dissipation rates of pesticides in the biotic crop systems

Groups		**20 °C / 20 % w.c.**			**20 °C / 40 % w.c.**			**30 °C / 20 % w.c.**			**30 °C / 40 % w.c.**		
		DT_{50} [d] (SE)	**k^{d}_{1} [d^{-1}]**	**k^{d}_{2} [d^{-1}]**	**DT_{50} [d] (SE)**	**k^{d}_{1} [d^{-1}]**	**k^{d}_{2} [d^{-1}]**	**DT_{50} [d] (SE)**	**k^{d}_{1} [d^{-1}]**	**k^{d}_{2} [d^{-1}]**	**DT_{50} [d] (SE)**	**k^{d}_{1} [d^{-1}]**	**k^{d}_{2} [d^{-1}]**
	PRY	n.a.	n.a.	n.a.	16 (11)	0.07	-0.9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hydrophobic	ORY	17 (12)	0.08	-0.04	17 (9)	0.06	-0.03	15 (12)	0.08	-0.04	n.a.	n.a.	n.a.
	ISX	29 (14)	0.06	-0.04	5 (7)	0.2	-0.06	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SIM	23 (7)	0.04	-0.008	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	TER	91 (4)	0.01	-0.008	62 (5)	0.02	-0.006	26 (4)	0.03	-0.02	6 (7)	0.1	-0.06
	ATR	23 (7)	0.04	-0.02	37 (5)	0.03	-0.007	21 (3)	0.04	-0.01	n.a.	n.a.	n.a.
	DIU	46 (3)	0.02	-0.003	118 (1)	0.007	-0.0005	35 (4)	0.03	-0.006	135 (2)	0.003	0.002
	ISP	n.a.	n.a.	n.a.	77 (5)	0.004	0.006	n.a.	n.a.	n.a.	19 (9)	0.06	-0.03
Moderately hydrophobic	DIM	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	CYP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	PYR	57 (14)	0.04	-0.02	221 (8)	-0.005	0.005	41 (2)	0.01	0.004	n.a.	n.a.	n.a.
	AZO	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	KM	n.a.	n.a.	n.a.	6 (8)	0.2	-1.2	n.a.	n.a.	n.a.	8 (9)	0.1	-1.5
	CAR	44 (6)	0.02	-0.004	n.a.	n.a.	n.a.	21 (7)	0.05	-0.01	15 (10)	0.08	-0.01
Sorptive and hydrophilic	TZL	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SMET	39 (1)	0.02	0.002	20 (7)	0.05	-0.02	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hydrophilic	CYM	n.a.	n.a.	n.a.	255 (7)	0.01	-0.03	111 (1)	0.006	0.001	146 (2)	0.007	-0.006
	MTY	76 (1)	0.008	0.0008	106 (5)	0.001	0.004	n.a.	n.a.	n.a.	91 (9)	-0.002	0.008

n.a.: half-lives and dissipation rates could not be retrieved due to poor and non-significant correlations

Table 4.1-11: BEXP modelled half-lives and dissipation rates of pesticides in the biotic vineyard systems

Groups		**20 °C / 20 % w.c.**			**20 °C / 40 % w.c.**			**30 °C / 20 % w.c.**			**30 °C / 40 % w.c.**		
		DT$_{50}$ [d] (SE)	**k$^{d}_{1}$ [d^{-1}]**	**k$^{d}_{2}$ [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k$^{d}_{1}$ [d^{-1}]**	**k$^{d}_{2}$ [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k$^{d}_{1}$ [d^{-1}]**	**k$^{d}_{2}$ [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k$^{d}_{1}$ [d^{-1}]**	**k$^{d}_{2}$ [d^{-1}]**
Hydrophobic	PRY	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	14 (12)	0.09	-0. 6	n.a.	n.a.	n.a.
	ORY	n.d.	n.d.	n.d.	16 (11)	0.07	-0.04	12 (13)	0.1	-0.06	16 (11)	0.07	-0.04
	ISX	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	17 (13)	0.08	-0.08	n.a.	n.a.	n.a.
Moderately hydrophobic	SIM	24 (7)	0.04	-0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	TER	20 (12)	0.07	-0.08	40 (1)	0.02	-0.002	6 (7)	0.2	-0.1	n.a.	n.a.	n.a.
	ATR	27 (7)	0.04	-0.01	40 (3)	0.02	-0.004	6 (7)	0.1	-0.05	15 (4)	0.05	-0.01
	DIU	n.d.	n.d.	n.d.	142 (8)	-0.004	0.006	n.a.	n.a.	n.a.	82 (0)	0.009	-0.0004
	ISP	n.d.	n.d.	n.d.	99 (2)	0.005	0.002	n.a.	n.a.	n.a.	32 (9)	0.04	-0.02
	DIM	19 (13)	0.02	-0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	CYP	14 (12)	0.09	-0.2	107 (5)	0.001	0.01	32 (8)	0.04	-0.05	83 (8)	-0.0006	0.02
	PYR	22 (6)	0.04	-0.02	157 (13)	-0.01	0.01	n.a.	n.a.	n.a.	110 (14)	-0.01	0.01
	AZO	86 (8)	0.07	-0.09	64 (5)	0.006	0.008	22 (8)	0.05	-0.04	n.a.	n.a.	n.a.
	KM	n.a.	n.a.	n.a.	16 (12)	0.08	-0.4	17 (11)	0.07	-0.4	6 (8)	0.1	-1.2
	CAR	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	17 (11)	0.07	-0.03	23 (9)	0.05	-0.008
Sorptive and hydrophilic	TZL	165 (6)	0.01	-0.01	70 (6)	0.003	0.01	127 (2)	0.007	-0.003	n.a.	n.a.	n.a.
Hydrophilic	SMET	26 (1)	0.03	0.002	39 (2)	0.02	0.002	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	CYM	n.d.	n.d.	n.d.	264 (8)	-0.006	0.02	158 (3)	0.001	0.007	330 (2)	-0.0002	0.007
	MTY	69 (4)	0.006	0.003	100 (10)	-0.005	0.007	37 (1)	0.02	-0.002	77 (9)	-0.0009	0.008

n.a.: half-lives and dissipation rates could not be retrieved due to poor and non-significant correlations

n.d.: half-lives and dissipation rates could not be retrieved due to experimental issue

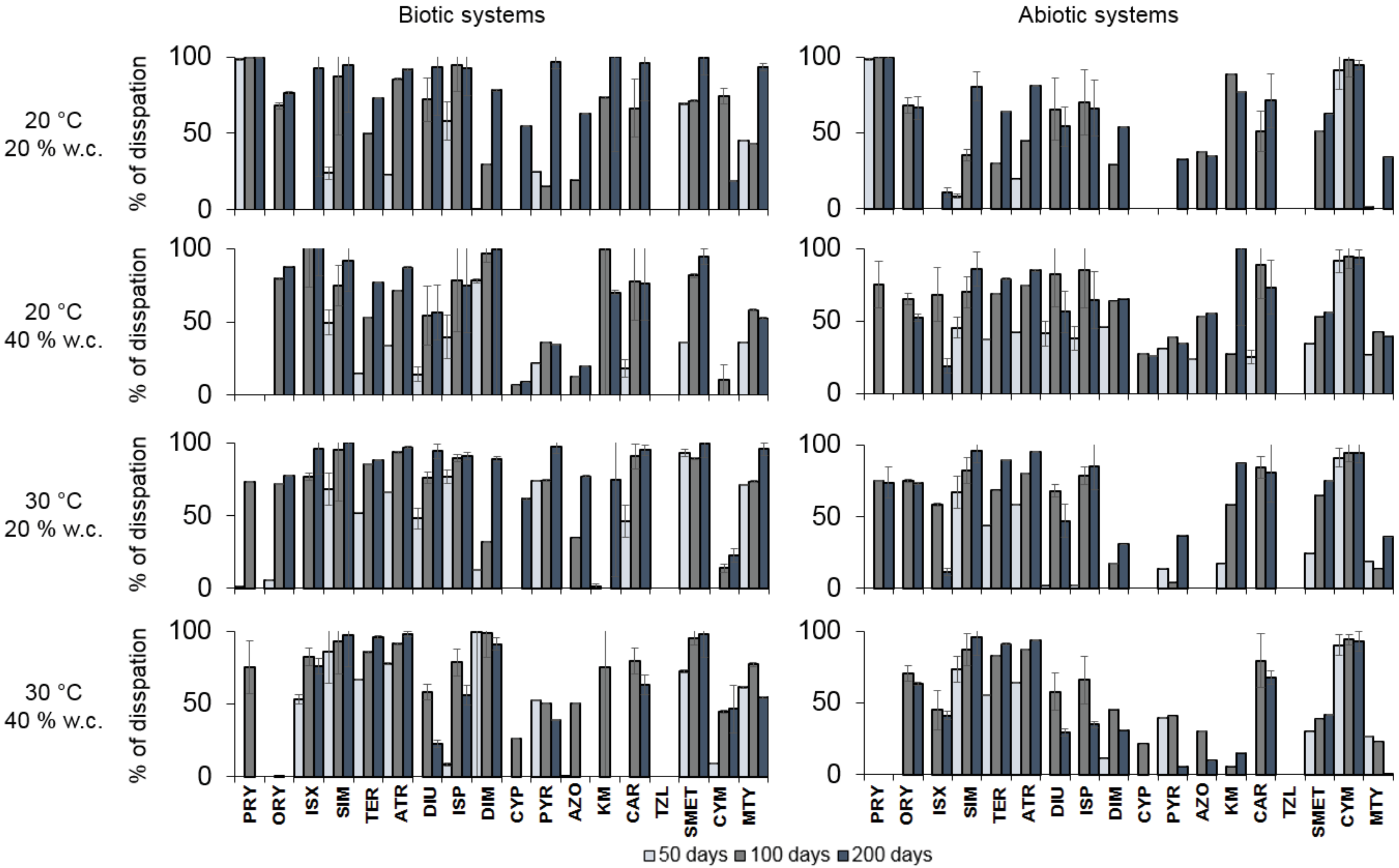

Figure 4.1-2: Dissipation at 50, 100 and 200 days of pesticides in crop systems. Error bars denote 95% confidence intervals

Comparison of DT_{50} obtained in this study with those from the literature suggests that the discrepancy between observed and reported DT_{50} values for individual pesticides may be related to the simultaneous use of pesticides in the mixture. The input of several pesticides in soils may generate competition for soil matrix sorption sites according to the characteristics of the mixture of pesticides (Swarcewicz and Gregorczyk, 2012). Accordingly, changes in the bioavailable fraction of pesticides may affect microbial communities, at both a taxonomic and functional level (Zhang et al., 2014), which in turn may alter pesticides biodegradation and soil functioning.

Pesticides could be classified in five groups according to their persistence in the soils: (i) extremely persistent (CYP and TZL), (ii) persistence for more than 100 days (ISX, AZO and CYM), (iii) weak dissipation within 50 days (ORY, TER and KM), (iv) pronounced dissipation within 50 days (SIM, ATR, DIU, ISP, DIM, PYR, CAR, SMET and MTY) and (v) not persistent (PRY). Hence, pesticides belonging to the three first groups may accumulate in soils and may be transported to aquatic ecosystems associated to soil particles during surface runoff. In contrast, less persistent pesticides (fourth and fifth groups) may generate degradation products in the soil, which can then be exported in the dissolved phase of surface runoff.

4.2. Effect of soil sterilization by autoclaving

The half-life times obtained in the abiotic experiments for both the crop (Figure 4.1-2) and the vineyard soils (Figure 4.1-3) were generally up to one order of magnitude higher than those obtained in the biotic experiments (see Tables 4.1-12 and 4.1-13 and Table 4.1-14 for corresponding correlation coefficients).

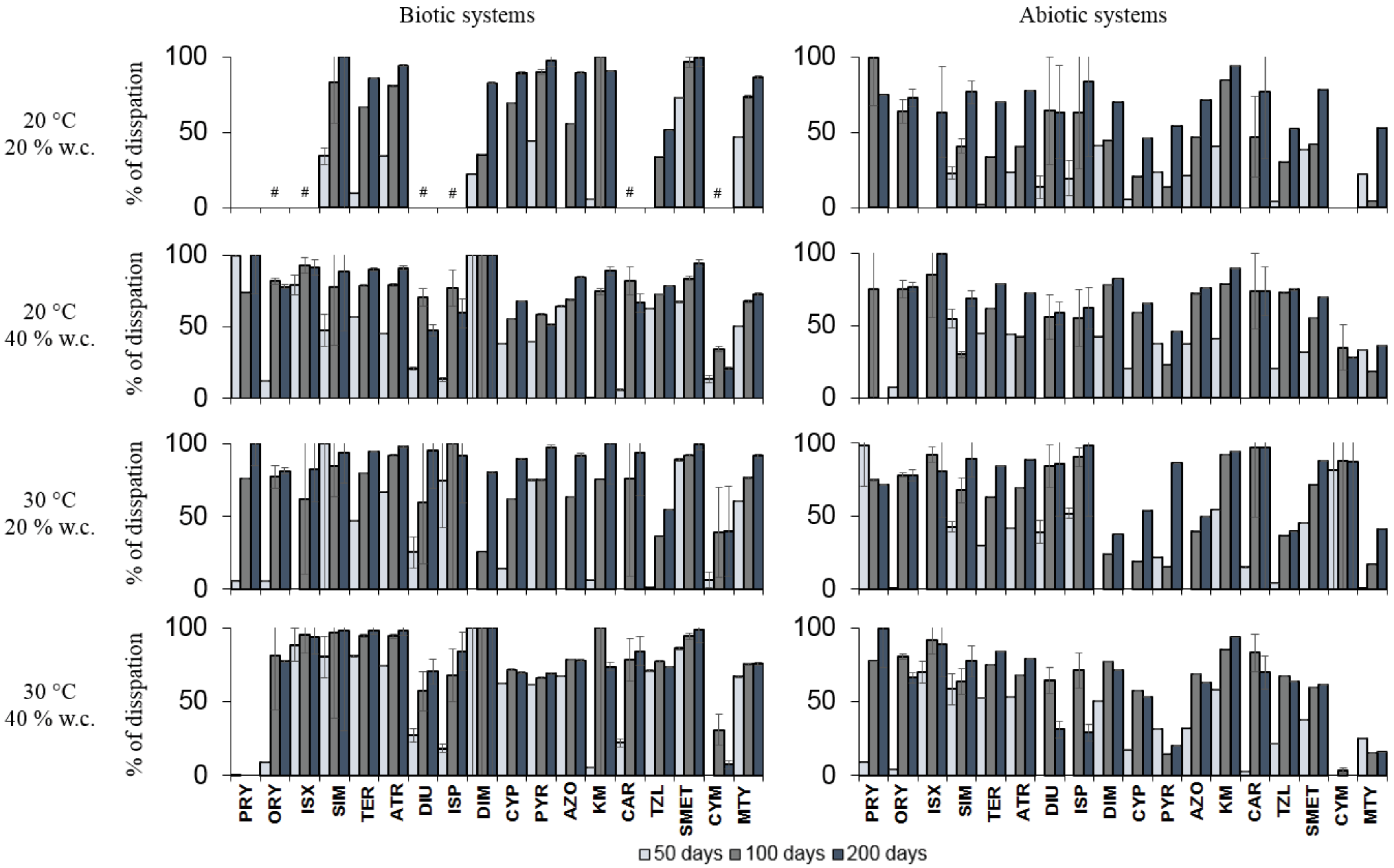

Figure 4.1-3: Dissipation at 50, 100 and 200 days of pesticides in vineyard systems. Error bars denote 95% confidence intervals. "#" indicates that dissipation rates could not be retrieved due to experimental issue

Table 4.1-12: SFO modelled half-lives and dissipation rates of pesticides in the abiotic crop systems

Groups		**20 °C / 20 % w.c.**		**20 °C / 40 % w.c.**		**30 °C / 20 % w.c.**		**30 °C / 40 % w.c.**	
		DT_{50} [d] (SE)	**k^d [d^{-1}]**	**DT_{50} [d] (SE)**	**k^d [d^{-1}]**	**DT_{50} [d] (SE)**	**k^d [d^{-1}]**	**DT_{50} [d] (SE)**	**k^d [d^{-1}]**
	PRY	61 (0)	0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hydrophobic	ORY	91 (0)	0.008	106 (0)	0.007	75 (0)	0.009	81 (0)	0.009
	ISX	146 (0)	0.005	86 (7)	0.007	103 (3)	0.007	70 (2)	0.01
	SIM	84 (0)	0.008	59 (6)	0.01	42 (2)	0.02	n.a.	n.a.
	TER	123 (0)	0.006	n.a.	n.a.	55 (2)	0.01	n.a.	n.a.
	ATR	81 (0)	0.009	60 (4)	0.01	42 (1)	0.02	43 (4)	0.01
	DIU	118 (0)	0.006	104 (5)	0.006	131 (6)	0.006	178 (12)	0.005
	ISP	94 (0)	0.007	92 (3)	0.007	59 (2)	0.01	164 (14)	0.005
Moderately hydrophobic	DIM	131 (0)	0.006	n.a.	n.a.	262 (17)	0.005	126 (14)	0.004
	CYP	409 (0)	0.002	n.a.	n.a.	651 (8)	0.001	n.a.	n.a.
	PYR	302 (0)	0.002	186 (11)	0.003	227 (4)	0.003	n.a.	n.a.
	AZO	122 (0)	0.005	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	KM	60 (0)	0.01	n.a.	n.a.	52 (0)	0.01	n.a.	n.a.
	CAR	83 (0)	0.008	n.a.	n.a.	58 (2)	0.01	67 (4)	0.01
Sorptive and hydrophilic	TZL	448 (0)	0.002	211 (9)	0.003	525 (10)	0.001	n.a.	n.a.
	SMET	123 (0)	0.006	110 (8)	0.005	83 (0)	0.008	151 (9)	0.004
Hydrophilic	CYM	42 (0)	0.02	46 (1)	0.02	44 (1)	0.02	48 (3)	0.02
	MTY	413 (0)	0.002	207 (4)	0.004	273 (3)	0.002	n.a.	n.a.

n.a.: half-lives and dissipation rates could not be retrieved due to poor and non-significant correlations

Table 4.1-13: BEXP modelled half-lives and dissipation rates of pesticides in the abiotic crop systems

Groups		20 °C / 20 % w.c.			20 °C / 40 % w.c.			30 °C / 20 % w.c.			30 °C / 40 % w.c.		
		DT_{50} [d] (SE)	k^d_1 [d^{-1}]	k^d_2 [d^{-1}]	DT_{50} [d] (SE)	k^d_1 [d^{-1}]	k^d_2 [d^{-1}]	DT_{50} [d] (SE)	k^d_1 [d^{-1}]	k^d_2 [d^{-1}]	DT_{50} [d] (SE)	k^d_1 [d^{-1}]	k^d_2 [d^{-1}]
Hydrophobic	PRY	n.a.	n.a.	n.a.	9 (9)	0.1	-1.4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	ORY	16 (12)	0.0770	-0.04	85 (4)	0.01	-0.004	14 (12)	0.08	-0.04	15 (12)	0.08	-0.04
	ISX	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Moderately hydrophobic	SIM	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	48 (5)	0.009	0.004	n.a.	n.a.	n.a.
	TER	122 (6)	0.01	-0.01	57 (1)	0.01	-0.002	48 (2)	0.02	-0.007	31 (3)	0.03	-0.006
	ATR	83 (4)	0.01	-0.004	53 (1)	0.01	-0.001	33 (2)	0.02	-0.005	31 (1)	0.02	-0.002
	DIU	106 (1)	0.006	0.0004	110 (12)	-0.008	0.01	102 (1)	0.006	0.0006	n.a.	n.a.	n.a.
	ISP	77 (2)	0.01	-0.002	92 (8)	-0.002	0.009	17 (10)	0.07	-0.03	111 (1)	0.008	-0.001
	DIM	109 (1)	0.008	-0.002	76 (0)	0.002	0.007	n.a.	n.a.	n.a.	150 (5)	-0.0003	0.005
	CYP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	PYR	297 (13)	0.02	-0.01	231 (12)	-0.01	0.007	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	AZO	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	KM	8 (8)	0.1	-0.4	n.a.	n.a.	n.a.	6 (7)	0.2	-0.5	n.a.	n.a.	n.a.
	CAR	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	13 (11)	0.08	-0.02	n.a.	n.a.	n.a.
Sorptive and hydrophilic	TZL	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hydrophilic	SMET	116 (2)	0.009	-0.002	120 (6)	-0.0005	0.004	67 (2)	0.01	-0.002	175 (8)	-0.005	0.004
	CYM	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	MTY	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a.: half-lives and dissipation rates could not be retrieved due to poor and non-significant correlations

Table 4.1-14: Correlation coefficients obtained with the SFO and BEXP models in the abiotic crop systems

Groups		**20 °C / 20 % w.c.**		**20 °C / 40 % w.c.**		**30 °C / 20 % w.c.**		**30 °C / 40 % w.c.**	
		$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$	$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$	$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$	$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$
	PRY	0.91	0.25	0.04	0.81	0.17	0.22	0.22	0.24
Hydrophobic	ORY	0.96	0.92	0.97	0.99	0.94	0.89	0.95	0.90
	ISX	0.98	0.24	0.77	0.24	0.97	0.24	0.89	0.24
	SIM	0.95	0.24	0.70	0.24	0.78	0.76	0.56	0.24
	TER	0.97	1.00	0.53	0.93	0.86	0.92	0.50	0.85
	ATR	0.94	0.99	0.81	0.91	0.82	0.84	0.71	0.80
	DIU	0.97	0.96	0.87	0.75	0.94	0.95	0.79	0.24
	ISP	0.96	0.96	0.91	0.80	0.92	0.89	0.71	0.98
Moderately hydrophobic	DIM	0.97	0.98	0.63	0.86	0.87	0.24	0.76	0.93
	CYP	1.00	0.24	0.46	0.24	0.93	0.24	0.36	0.24
	PYR	1.00	0.86	0.77	0.80	0.95	0.99	0.38	0.24
	AZO	0.98	0.24	0.53	0.94	0.68	0.24	0.60	0.24
	KM	0.91	0.81	0.48	0.24	0.89	0.76	0.62	0.24
	CAR	0.95	0.24	0.63	0.24	0.92	0.85	0.83	0.24
Sorptive and hydrophilic	TZL	1.00	0.24	0.83	0.24	0.87	0.24	0.32	0.24
	SMET	0.97	0.99	0.81	0.89	0.94	0.95	0.81	0.88
Hydrophilic	CYM	0.84	0.60	0.88	0.61	0.87	0.55	0.88	0.61
	MTY	1.00	0.24	0.98	0.24	0.98	0.24	0.46	0.24

This emphasizes that soil sterilization dramatically reduced the activity of soil microorganisms, which were mainly involved in pesticide transformation (Ruggiero et al., 2002). In abiotic experiments, pesticide sequestration may operate thanks to their physical entrapment in soil matrix sites, while hydrophobic sorption may act in bounding pesticides to soil constituents (Ruggiero et al., 2002). In addition, soil clays and oxides may play a major role in oxidative reactions such as O- and N-dealkylations (Ruggiero et al., 2002). Noteworthy, sterilization of soil may impact its quality, and in particular the amounts of soil organic matter (Berns et al., 2008), which in turn may affect sorption and hydrolytic processes. However, destructive processes such as hydrolysis are expected to be limited in soils for the studied compounds given their high half-life times for hydrolysis (Table 2-6). While pesticides were generally less dissipated in the abiotic experiments, large variations could be observed for the five groups of persistence defined above.

Experiments carried out with TZL revealed that application of TZL on soils decreases activity soil microorganisms (Zhang et al., 2014). Dissipation kinetics of TZL in crop soil were larger than 200 days in our case, and did not change much in the vineyard soil (from 165 ± 6 days in biotic condition (Table 4.1-11) to 178 ± 4 days in abiotic condition (Table 4.1-15) using the BEXP model. In the abiotic experiments, and contrary to the biotic experiments, MTY, SIM, PYR, CAR, AZO, KM and ISX better fitted the SFO model, whereas SMET, TER and ATR fitted the BEXP model. This may reflect the impact of soil microorganisms on the dissipation of MTY, SIM, PYR, CAR, AZO, KM and ISX. While the SFO model only describes a linear dissipation, the BEXP model describes i) a rapid dissipation occurring at the solid-water interphase where microorganisms easily access to pesticides and ii) a slower dissipation occurring at the surface of soil particles and governed by desorption-diffusion processes (Srinivasan et al., 2014). The degradation of PYR was higher in biotic than abiotic condition. This compound was highly dissipated in biotic condition while less than 60% of PYR was degraded in the abiotic experiments (Figure 4.1-2 and 3, Table 4.1-9 and Table 4.1-16). In contrast, CYM dissipation was higher in the abiotic soil. KM dissipation was similar in the biotic and abiotic experiments (91 – 94%) (Figure 4.1-3).

Table 4.1-15: BEXP modelled half-lives and dissipation rates of pesticides in the abiotic vineyard systems

Groups		**20 °C / 20 % w.c.**			**20 °C / 40 % w.c.**			**30 °C / 20 % w.c.**			**30 °C / 40 % w.c.**		
		DT$_{50}$ [d] (SE)	**k$^{d}_{1}$ [d^{-1}]**	**k$^{d}_{2}$ [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k$^{d}_{1}$ [d^{-1}]**	**k$^{d}_{2}$ [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k$^{d}_{1}$ [d^{-1}]**	**k$^{d}_{2}$ [d^{-1}]**	**DT$_{50}$ [d] (SE)**	**k$^{d}_{1}$ [d^{-1}]**	**k$^{d}_{2}$ [d^{-1}]**
Hydrophobic	PRY	8 (8)	0.1	-1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	6 (8)	0.1	-1.3
	ORY	17 (12)	0.07	-0.04	19 (10)	0.06	-0.03	14 (12)	0.09	-0.04	18 (9)	0.06	-0.02
	ISX	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Moderately hydrophobic	SIM	99 (4)	0.01	-0.003	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	TER	109 (8)	0.02	-0.02	75 (2)	0.007	0.002	66 (3)	0.01	-0.009	44 (1)	0.02	-0.001
	ATR	95 (4)	0.01	-0.004	101 (3)	0.003	0.002	56 (1)	0.01	-0.002	61 (2)	0.009	0.003
	DIU	84 (0)	0.009	-0.0003	79 (3)	0.01	-0.003	22 (7)	0.05	-0.01	23 (8)	0.04	-0.01
	ISP	40 (6)	0.03	-0.009	20 (12)	0.06	-0.03	26 (5)	0.03	-0.01	78 (1)	0.01	-0.0009
	DIM	94 (1)	-0.002	0.009	63 (0)	0.02	-0.005	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	CYP	175 (2)	0.006	-0.006	98 (1)	0.006	0.001	137 (6)	0.01	-0.02	124 (4)	0.0009	0.01
	PYR	205 (11)	0.02	-0.008	203 (8)	-0.006	0.004	80 (14)	0.04	-0.02	n.a.	n.a.	n.a.
	AZO	129 (6)	0.009	-0.003	39 (3)	0.01	0.0002	137 (1)	0.007	-0.003	73 (2)	0.008	0.003
	KM	22 (6)	0.04	-0.1	29 (5)	0.03	-0.03	57 (8)	0.004	0.1	21 (4)	0.04	-0.04
	CAR	23 (12)	0.06	-0.02	n.a.	n.a.	n.a.	10 (9)	0.1	-0.02	n.a.	n.a.	n.a.
Sorptive and hydrophilic	TZL	178 (4)	0.008	-0.007	28 (8)	0.04	-0.02	219 (2)	0.001	0.004	87 (2)	0.006	0.003
Hydrophilic	SMET	107 (0)	0.006	0.0000	96 (2)	0.005	0.001	77 (5)	0.003	0.007	104 (6)	-0.0002	0.004
	CYM	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	337 (4)	0.006	-0.01
	MTY	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a.: half-lives and dissipation rates could not be retrieved due to poor and non-significant correlations

Table 4.1-16: Correlation coefficients obtained with the SFO and BEXP models in the biotic vineyard systems

Groups		**20 °C / 20 % w.c.**		**20 °C / 40 % w.c.**		**30 °C / 20 % w.c.**		**30 °C / 40 % w.c.**	
		$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$	$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$	$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$	$\mathbf{r^2_{SFO}}$	$\mathbf{r^2_{BEXP}}$
	PRY	0.19	0.24	0.49	0.56	0.46	0.89	0.01	0.21
Hydrophobic	ORY	n.a.	n.a.	0.90	0.88	0.93	0.88	0.91	0.89
	ISX	n.a.	n.a.	0.44	0.66	0.86	0.95	0.39	0.57
	SIM	0.67	0.91	0.70	0.24	0.82	0.54	0.49	0.57
	TER	0.94	0.94	0.48	0.86	0.85	0.77	0.42	0.67
	ATR	0.84	0.92	0.77	0.89	0.75	0.72	0.65	0.72
	DIU	n.a.	n.a.	0.95	0.86	0.88	0.55	0.96	0.95
	ISP	n.a.	n.a.	0.98	0.94	0.86	0.70	0.93	0.96
Moderately hydrophobic	DIM	0.78	0.99	0.46	0.55	0.91	0.24	0.50	0.55
	CYP	0.55	0.91	0.38	0.89	0.54	0.96	0.38	0.79
	PYR	0.82	0.87	0.88	0.76	0.79	0.53	0.77	0.70
	AZO	0.56	0.95	0.38	0.82	0.60	0.91	0.46	0.51
	KM	0.98	0.24	0.89	0.90	0.69	0.90	0.66	0.80
	CAR	n.a.	n.a.	0.72	0.24	0.89	0.91	0.68	0.92
Sorptive and hydrophilic	TZL	0.43	0.99	0.34	0.81	0.42	0.99	0.35	0.70
	SMET	0.75	0.71	0.71	0.79	0.73	0.62	0.64	0.64
Hydrophilic	CYM	n.a.	n.a.	0.01	0.91	0.00	0.96	0.03	0.98
	MTY	0.95	0.86	0.97	0.78	0.90	0.85	0.96	0.76

4.3. Effect of the soil type

Overall, pesticide dissipation in the vineyard soils was larger than in the crop soil in both biotic and abiotic experiments (Figures 4.1-2 and 4.1-3). This trend was particularly pronounced for the moderately hydrophilic pesticides (Figure 4.1-4).

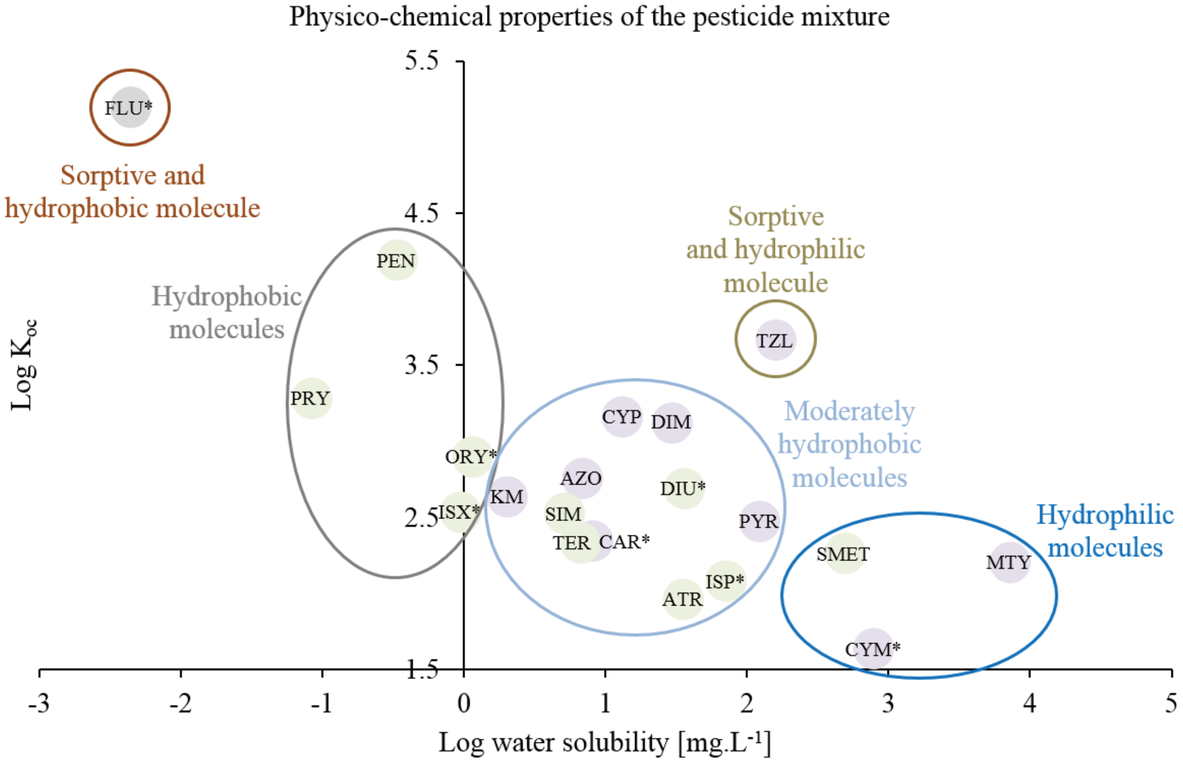

Figure 4.1-4: Representation of pesticide clusters made according to the log of water solubility and log of sorption capacities of pesticides to the soil organic matter.

Differences in the dissipation kinetics in the vineyard and the crop soil can be attributed to both physico-chemical and microbial characteristics. The dissipation extent and the half-life times (SFO DT_{50} in Table 4.1-9 and BEXP ones in Table 4.1-11) of SMET and MTY were quite similar between the two soils, suggesting few effects of the soil type. TZL did not undergo significant dissipation in the crop soil and the SFO model was retained to assess its half-life time, while the BEXP model was used for the vineyard soil ($r_{SFO}^2 < 0.7$, Table 4.1-16), as TZL was dissipated within 100 days. The dissipation of TZL may reflect its affinity to organic matter. The crop soil contains 3.3-fold more organic matter than the vineyard soil and the persistence of TZL in the crop soil may be related to soil organic content.

A similar behavior could be observed for CYP (half-life time of 112 ± 1 days in the crop soil (Table 4.1-8) against 14 ± 12 days in the vineyard soil (Table 4.1-11). SIM, TER, ATR and DIM had similar dissipations in the crop and in the vineyard soils (Figures 4.1-2 and 4.1-3). However, DT_{50} values for TER were higher in the vineyard soil (91 ± 4 days) compared to the crop soil (70 ± 1 days) (Table 4.1-10 and Table 4.1-9). PYR dissipation differed in the vineyard and the crop soil (DT_{50} (BEXP model): 57 ± 14 days in the crop soil (Table 4.1-10) and 22 ± 6 days in the vineyard soil (Table 4.1-11). DT_{50} of KM could not be evaluated in the crop soil but it reached 182 ± 1 days (Table 4.1-9) in the vineyard soil (reported DT_{50} average 16 days (PPDB Pesticide Properties DataBase, 2009). The DT_{50} of AZO was higher in the vineyard soil (86 ± 8 days) compared to the crop soil (73 ± 1 days) (Table 2). Although PRY was completely dissipated in the crop soil, it was not significantly dissipated in the vineyard soil. The result obtained in the vineyard soil for PRY is surprising because the persistence of PRY has not been reported (Baker et al., 2002).

Besides sorption to soil organic matter, pesticides can form bound residues onto clay minerals as revealed in studies on soil mineral and pesticide interactions (Cornejo et al., 2008; Đurović et al., 2009). The vineyard soil contains 2.4-fold more clays than the crop soil (Table 2-1). In addition, extraction recoveries are sensitive to clay content, and lower extraction yield were obtained with increasing proportion of clay (Đurović et al., 2009). This may explain higher DT_{50} values for the hydrophobic pesticides studied here. The crop soil contains 1.5-fold, 1.7-fold and 1.4-fold more Al, Fe(III) and Mn(II) oxides respectively (Table 2-1). This highlights the putative impact of soil oxides on oxidative degradation pathways. For instance, N-dealkylation reactions can be undergone by ORY, ISX, SIM, TER, ATR, DIU, ISP, PYR, CAR and MTY.

Differences in the dissipation kinetics in the vineyard and the crop soils can also be partly attributed to distinct microbial communities, at both the taxonomic and functional levels. Pesticides may affect non-target soil organisms, including microorganisms in their modes and extent of responses to pesticides (Imfeld and Vuilleumier, 2012). Hence, the history of a soil and hence the record of pesticide application can affect pesticides half-lives (Rouchaud et al., 2000). However, change of the soil bacterial communities is likely to be controlled by the soil type in our experiment. Indeed, combined effects of variables such as soil properties, e.g., texture and pH, treatment records, and vegetation generally influence bacterial communities more strongly than pesticide treatment (Brockett et al., 2012; Buckley and Schmidt, 2003; Girvan et al., 2004; Nielsen et al., 2010; Tsiknia et al., 2014). Both soil properties and treatment records can considerably alter the habitats and processes of substrate usage in soils. This in turn may determine transformation pathways and microbial composition (Smith and Harris, 2007). Although soil type largely affect dissipation kinetics in the studied soil, soil temperature and moisture may also alter pesticide dissipation.

4.4. Effect of temperature

Globally, pesticide dissipation was larger at 30°C than at 20°C (Figures 4.1-2 and 4.1-3). The effect of temperature was particularly pronounced up to 50 days of incubation and in the abiotic experiments. After 100 days of incubation, the relative dissipation with increasing temperature decreased. Only ISP and DIM in biotic and abiotic crop experiments, respectively, were not significantly affected by the temperature. AZO, TZL, CYM and MTY presented similar trends in the biotic and the abiotic vineyard soil experiments (Figure 4.1-3). In the latter experiments, dissipation ratios of experiments at 20 °C to experiments at 30 °C ranged from 0.6 to 0.8.

Temperature is known to impact several soil components and reactions. In particular, the soil temperature influences the microbial content and activity (Cessna et al., 2017; Reedich et al., 2017; Su et al., 2017), while higher temperature may decrease the abundance of microorganism (Shahgholi and Gholamalizadeh Ahangar, 2014). Besides, soil temperature may enhance transformations (Long et al., 2014; Shahgholi and Gholamalizadeh Ahangar, 2014) and especially abiotic ones by increasing the rate of abiotic reactions such as hydrolysis and oxidation (Cessna et al., 2017). The attenuation of the impact of temperature with time could affect some other factors involved in pesticides' dissipation. Indeed, a multitude of factors can simultaneously impact the dissipation of pesticides, such as with time depletion of essential nutrient contents for microbial communities or pesticides concentrations (Shahgholi and Gholamalizadeh Ahangar, 2014). Besides, increases in temperature might also impact pesticide sorption as rising water solubilities are expected with an increase of temperature (Cessna et al., 2017). This can in turn increase pesticides bioavailability (Ruggiero et al., 2002).

4.5. Effect of soil water content

The effect of soil water contents (20 % versus 40 %) on the pesticides dissipation was less pronounced than that of temperature (Figures 4.1-2 and 4.1-3). Besides, no clear temporal trend in pesticide dissipation could be observed as a function of soil water content.

Overall, the dissipation of SMET and MTY was lower at 40 % water content compared to 20 %, and dissipation ratios of experiment at 20 % to experiments at 40 % ranged from 0.6 to 1 after 200 days of incubation. Dissipation of TZL increased at 40 % water content in the vineyard soil by 1.6 in the biotic experiments and 1.5 in the abiotic experiments but tended to decrease with time. In contrast, the effect of water content on the dissipation of moderately hydrophobic pesticides appeared to be weak. Only DIM dissipation increased by 30% at 40 % water content compared to the experiment with 20 % of water content. The dissipation extent of PYR and AZO decreased by about 2-fold at 40 % water content, whereas KM dissipation only decreased by 1.3-fold. Under abiotic conditions, dissipation PYR, AZO and KM increased by 1.7-fold at 40 % water content compared to corresponding 20 % experiments. Under biotic conditions, dissipation of DIU, ISP and CYP decreased by, respectively, 1.6, 1.2 and 2.6 in experiments at 40 % water content in the crop soil. A similar trend could be observed in the vineyard soil: CYP and PYR dissipation also decreased by 1.3 and 1.7, respectively. In contrast, dissipation of TER and CYP increased in the abiotic experiments.

For more hydrophilic pesticides, the soil water content may largely influence the partitioning of pesticides among the solid and liquid phases of soils, as well as pesticide diffusion, movement and availability to microorganisms. Water-soluble pesticides are directly impacted by soil moisture variations. Soil moisture can also affect microbial communities by controlling nutrient availability (Long et al., 2014; Ruggiero et al., 2002; Shahgholi and Gholamalizadeh Ahangar, 2014) and exposure to pesticides. Hence, with the increase of soil water content, pesticide dissipation is expected to increase as they undergo more microbial degradation. However, water saturation in soils can increase the proportion of anaerobic zone, and thus alter microbial composition and pesticide transformation pathways

Regarding hydrophobic molecules, the effect of soil water content on pesticides dissipation was significant mainly in the early stages of incubation, whereas for moderately hydrophobic molecules, no significant impact of the soil water content on the dissipation of

PRY, ORY and ISX could be noticed. Alike for the effect of temperature, the effect of soil water content may decrease with time as hydrophobic sorption prevails.

4.6. Insights from pesticide CSIA ($\delta^{13}C$)

ε_{bulk} and AKIE values could be reliably retrieved only for PYR, MTY, SMET and ATR in our experiments ($R^2 > 0.6$, $p < 0.05$; $n = 5$), whereas poor Rayleigh fittings hampers to retrieve enrichment factors for the other pesticide. As the experiment was carried out with natural soil samples spiked at environmental concentrations, the CSIA focused on carbon because as sufficient amount of carbon enabled reliable stable isotope measurements.

The isotopic enrichment factors (ε_{bulk}) of PYR in the biotic crop soils at 20 °C for both 20 and 40 % of soil water content as well as at 30 °C/ 20 % of water content were -2.25 ± 0.24 ‰ (corresponding $AKIE_C$ of 1.028 ± 0.003 ‰), -4.98 ± 0.78 ‰ ($AKIE_C$ = 1.064 ± 0.010 ‰) and -1.13 ± 0.09 ‰ ($AKIE_C$ = 1.014 ± 0.001 ‰), respectively (Table 4.1-17). ε_{bulk} for the biotic vineyard soil experiment at 30 °C and 40 % of water content reached -0.98 ± 0.12 ($AKIE_C$ of 1.012 ± 0.001 ‰) (Table 4.1-18). No enrichment factors could be retrieved under abiotic conditions, which highlights the role of biodegradation in the dissipation of PYR. The degradation pathway of PYR may involve N-dealkylations, addition of -NO2 groups and substitution of amine by hydroxyl groups (JMPR, 2007). Comparison of $AKIE_C$ values with those of the literature suggests that PYR degradation may occur via S_N1 ($AKIE_C$ from 1.00 and 1.03 ‰) or S_N2 ($AKIE_C$ from 1.03 and 1.09 ‰) nucleophile substitution (Elsner et al., 2005). The values are comprised between for S_N2 and between for S_N1 nucleophile substitution. CSIA revealed that S_N2 nucleophile substitution of PYR may predominate at 20°C, whereas S_N1 may prevail at 30°C for both soil water contents.

The ε_{bulk} for MTY in crop experiments reached -2.214 ± 0.437 ‰ ($AKIE_C$ = 1.034 ± 0.007 ‰) and -3.309 ± 0.615 ‰ ($AKIE_C$ = 1.052 ± 0.010 ‰) in the experiments with 20% water content at 20 °C and 30 °C, respectively, (Table 4.1-17). In the vineyard experiments incubated at 20 °C and 20 % of water content, MTY had ε_{bulk} of -1.126 ± 0.092 ‰ hence yielding an $AKIE_C$ of 1.017 ± 0.001 ‰ (Table 4.1-18). No enrichment factors could be assessed for MTY in abiotic conditions neither with the crop soil, nor with the vineyard soil experiments. Similarly to PYR, CSIA clearly show that microbial degradation was a prevailing dissipation pathway for MTY. In soils, MTY transformation may be due to N-dealkylations and hydrolyses of carboxyl esters (FAO and Organization, 2005), which are consistent with $AKIE_C$ values comprised in the range of reported values for S_N1 and S_N2 nucleophilic substitution reactions. However, the potential degradation mechanisms could not be related to the experimental temperature in the case of MTY.

Table 4.1-17: Enrichment factors (ε_{bulk}) of the pesticides, apparent kinetic isotope effect of C ($AKIE_C$) and shifts with pesticides' standards for the biotic crop systems

		20 °C n.a. 20 % w.c.			**20 °C n.a. 40 % w.c.**			**30 °C n.a. 20 % w.c.**			**30 °C n.a. 40 % w.c.**		
Groups	**Compound**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**
Moderately hydrophobic	ATR	2.136 (2.150)	n.a.	n.a.	2.165 (0.435)	n.a.	n.a.	4.316 (0.823)	n.a.	n.a.	1.770 (0.414)	n.a.	n.a.
		1.355 (2.012)			4.814 (0.703)			-4.770 (0.698)			-3.096 (0.693)		
		1.711 (2.423)			3.375 (0.320)			6.839 (0.311)			-3.2185 (0.210)		
	PYR	0.001 (0.865)	-2.256 (0.242)	1.028 (0.003)	-0.322 (0.676)	-4.988 (0.778)	1.064 (0.010)	2.270 (0.278)	-1.129 (0.087)	1.014 (0.001)	-0.052 (0.149)	n.a.	n.a.
		1.154 (0.920)			1.261 (0.957)			1.752 (0.402)			0.916 (0.380)		
		7.301 (0.437)			1.132 (0.816)			4.256 (0.515)			1.536 (0.138)		
Hydrophilic	SMET	4.496 (0.544)	-1.370 (0.257)	1.021 (0.004)	1.770 (0.347)	-1.840 (0.256)	1.028 (0.004)	3.690 (0.890)	n.a.	n.a.	3.060 (0.794)	n.a.	n.a.
		3.328 (0.626)			3.468 (0.583)			1.640 (0.967)			1.484 (0.837)		
		6.780 (0.488)			4.940 (0.762)			4.018 (1.093)			2.353 (0.880)		
	MTY	1.253 (0.702)	-2.214 (0.437)	1.034 (0.007)	0.657 (0.492)	n.a.	n.a.	1.218 (0.214)	-3.309 (0.615)	1.052 (0.010)	1.025 (0.533)	n.a.	n.a.
		3.602 (0.617)			1.918 (0.269)			6.220 (0.265)			3.271 (0.251)		
		6.112 (0.551)			2.300 (0.241)			10.910 (0.593)			3.037 (0.352)		

n.a.: ε_{bulk} and $AKIE_C$ could not be retrieved due to poor and non-significant calculations ($p < 0.05$)

Table 4.1-18: Enrichment factors (ε_{bulk}) of the pesticides, apparent kinetic isotope effect of C ($AKIE_C$) and shifts with pesticides' standards for the biotic vineyard systems

		20 °C n.a. 20 % w.c.			**20 °C n.a. 40 % w.c.**			**30 °C n.a. 20 % w.c.**			**30 °C n.a. 40 % w.c.**		
Groups	**Compound**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**
Moderately hydrophobic	ATR	0.283 (0.723) -0.857 (0.931) 5.743 (1.071)	n.a.	n.a.	0.547 (2.028) 0.274 (1.823) 12.158 (2.185)	n.a.	n.a.	0.159 (0.582) 0.250 (0.497) 3.779 (0.612)	n.a.	n.a.	4.107 (0.431) -4.473 (2.386) -6.301 (0.206)	n.a.	n.a.
	PYR	1.790 (1.076) 12.032 (4.048) -2.905 (1.477)	n.a.	n.a.	1.620 (0.804) -1.395 (0.460) -0.700 (0.180)	n.a.	n.a.	2.904 (0.879) 1.997 (1.203) -1.410 (0.829)	n.a.	n.a.	1.315 (0.457) 1.670 (1.002) 1.655 (0.387)	-0.98 (0.12)	1.012 (0.001)
Hydrophilic	SMET	6.019 (0.823) 3.193 (3.848) 2.843 (0.264)	n.a.	n.a.	1.781 (0.736) 2.847 (0.488) 4.751 (0.852)	n.a.	n.a.	7.522 (0.580) 7.984 (0.491) -0.566 (0.412)	n.a.	n.a.	3.315 (0.617) 4.333 (0.597) 2.965 (0.576)	n.a.	n.a.
	MTY	0.225 (0.602) 0.888 (0.697) 1.984 (0.781)	-1.13 (0.09)	1.017 (0.001)	1.071 (0.270) 0.044 (0.549) 0.913 (0.313)	n.a.	n.a.	0.696 (0.177) 2.991 (0.394) 2.258 (0.618)	n.a.	n.a.	1.813 (0.250) 1.746 (0.240) -0.700 (0.679)	n.a.	n.a.

n.a.: ε_{bulk} and $AKIE_C$ could not be retrieved due to poor and non-significant calculations ($p < 0.05$)

The carbon enrichment factors for SMET in the biotic crop soil experiment were -1.37 ± 0.26 ‰ ($AKIE_C$ = 1.021 ± 0.004 ‰) and -1.84 ± 0.26 ‰ ($AKIE_C$ = 1.028 ± 0.004 ‰) for 20% and 40 % of soil water content, respectively (Table 4.1-17), and -1.16 ± 0.24 ‰ ($AKIE_C$ = 1.018 ± 0.004 ‰) in the abiotic SMET (30°C, 20 % w.c.) (Table 4.1-19). The $AKIE_C$ obtained for SMET revealed S_N1 nucleophilic substitutions whatever the temperature or the soil water content. The transformation reported for SMET can be mainly attributed to microbial degradation and comprised, among other, reductive dehalogenation or addition of $-SO_3H$ groups (Zemolin et al., 2014) that may be followed with carbon isotope analysis (Elsayed et al., 2013). The abiotic soils were autoclaved, and the involvement of microorganisms is unlikely. In the case of slow or no hydrolysis (expected given the soil pH values), other chemical reactions, e.g., oxidation and reduction, can affect the chemical transformation of pesticides (Tomizawa, 1975). Therefore, the occurrence of oxidation as an abiotic degradation process can be assumed in the abiotic soils leading to the slight isotopic shifts over 200 days. It has been proved that metolachlor can be susceptible to oxidation in the presence of soil oxidants (Pignatello and Baehr, 1994). To see the extent of the microbial degradation in our conditions we calculate the extent of biodegradation (B) using previously obtained enrichment factors (Elsayed et al., 2013). After 200 days of incubation, we found biodegradation extent of SMET reaching 99.4 % and 93.7 % in biotic crop soils incubated at 20 °C and containing respectively water content of 20 % and 40 %.

For ATR, isotopic data could only be obtained in abiotic experiments exposed to 20 °C and 20 % of volumetric water content. In the crop soil experiment, ε_{bulk} of ATR reached -3.89 ± 0.31 ‰ ($AKIE_C$ of 1.032 ± 0.003 ‰) (Table 4.1-19), whereas ATR ε_{bulk} reached -4.16 ± 0.18 ‰ ($AKIE_C$ of 1.034 ± 0.002 ‰) in the vineyard soil (Table 4.1-20). Transformation mechanisms leading to the degradation of ATR are enzymatic and acid catalyzed hydrolysis and N-dealkylation (Hofstetter and Berg, 2011). ε_{bulk} of ATR are in agreement with those retrieved previously for hydrolysis of ATR (Meyer and Elsner, 2013), as biotic oxidative dealkylation could be reasonably excluded in the autoclaved soils. Indeed, in the crop and vineyard abiotic soils experiment degradation of PYR, MTY and SMET leading to significant carbon isotope fractionation could be observed. Hydrolysis may have been enhanced through autoclaving that altered soil organic matter. The mechanisms responsible for the acid catalysis of ATR is known for long, which may involve soil organic matter and functional groups of fulvic acids (Gamble and Khan, 1985).

Table 4.1-19: Enrichment factors (ε_{bulk}) of the pesticides, apparent kinetic isotope effect of C ($AKIE_C$) and shifts with pesticides' standards for the abiotic crop systems

Groups	**Compound**	**20 °C n.a. 20 % w.c.**			**20 °C n.a. 40 % w.c.**			**30 °C n.a. 20 % w.c.**			**30 °C n.a. 40 % w.c.**		
		$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**
Moderately hydrophobic	ATR	-1.966 (1.071)	-3.891 (0.307)	1.032 (0.003)	-2.242 (0.339)	n.a.	n.a.	1.651 (0.645)	n.a.	n.a.	2.524 (0.595)	n.a.	n.a.
		-0.360 (1.063)			-4.131 (0.541)			-0.669 (0.870)			-2.145 (1.079)		
		2.800 (1.426)			-1.842 (8.720)			-0.998 (1.276)			-3.611 (0.786)		
	PYR	0.456 (0.512)	n.a.	n.a.	-0.613 (0.534)	n.a.	n.a.	-0.004 (0.330)	n.a.	n.a.	0.323 (0.579)	n.a.	n.a.
		1.873 (0.456)			3.184 (0.461)			2.547 (0.621)			3.303 (0.671)		
		1.108 (0.416)			0.392 (0.425)			1.880 (0.646)			1.106 (0.402)		
Hydrophilic	SMET	-0.612 (1.070)	n.a.	n.a.	-0.221 (0.705)	n.a.	n.a.	0.536 (0.385)	-1.159 (0.236)	1.018 (0.004)	0.529 (0.554)	n.a.	n.a.
		1.041 (0.780)			0.547 (0.683)			1.209 (0.722)			-0.358 (1.144)		
		0.401 (0.801)			0.180 (0.865)			1.559 (0.994)			-0.377 (0.402)		
	MTY	0.705 (0.757)	n.a.	n.a.	-0.541 (0.158)	n.a.	n.a.	0.620 (0.382)	n.a.	n.a.	0.094 (0.278)	n.a.	n.a.
		1.828 (0.704)			0.370 (0.153)			0.864 (0.205)			0.129 (0.197)		
		1.919 (0.683)			0.322 (0.584)			1.888 (0.419)			0.978 (0.357)		

n.a.: ε_{bulk} and $AKIE_C$ could not be retrieved due to poor and non-significant calculations ($p< 0.05$)

Table 4.1-20: Enrichment factors (ε_{bulk}) of the pesticides, apparent kinetic isotope effect of C ($AKIE_C$) and shifts with pesticides' standards for the abiotic vineyard systems

		20 °C n.a. 20 % w.c.			**20 °C n.a. 40 % w.c.**			**30 °C n.a. 20 % w.c.**			**30 °C n.a. 40 % w.c.**		
Groups	**Compound**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**	**$\Delta\delta^{13}C$ [‰] (SD) 50 / 100 / 200 d**	**ε_{bulk} [‰] (SE)**	**$AKIE_C$ [‰] (SE)**
Moderately hydrophobic	ATR	0.319 (0.564)	-4.162 (0.176)	1.034 (0.002)	0.301 (1.442)	n.a.	n.a.	1.607 (0.733)	n.a.	n.a.	2.280 (0.911)	n.a.	n.a.
		1.344 (0.846)			-3.842 (1.114)			0.438 (0.806)			-2.817 (0.661)		
		5.066 (0.787)			1.802 (1.457)			-5.543 (3.135)			5.265 (0.556)		
	PYR	1.289 (0.474)	n.a.	n.a.	-0.646 (0.849)	n.a.	n.a.	0.151 (0.299)	n.a.	n.a.	0.233 (0.408)	n.a.	n.a.
		2.282 (0.780)			2.380 (0.758)			2.467 (0.319)			3.269 (0.578)		
		2.887 (0.693)			-0.060 (0.945)			-2.341 (0.631)			1.936 (0.665)		
Hydrophilic	SMET	1.532 (0.289)	n.a.	n.a.	0.839 (0.539)	n.a.	n.a.	2.448 (0.337)	n.a.	n.a.	0.585 (0.143)	n.a.	n.a.
		1.248 (0.735)			1.347 (0.431)			1.248 (0.679)			-0.285 (0.217)		
		2.812 (0.326)			0.639 (0.709)			2.830 (0.333)			-0.450 (0.544)		
	MTY	1.478 (0.150)	n.a.	n.a.	0.560 (0.526)	n.a.	n.a.	0.766 (0.476)	n.a.	n.a.	0.419 (0.694)	n.a.	n.a.
		2.326 (0.091)			1.651 (0.676)			1.289 (0.300)			0.209 (0.634)		
		2.359 (0.182)			1.095 (1.065)			1.623 (0.716)			0.895 (0.993)		

n.a.: ε_{bulk} and $AKIE_C$ could not be retrieved due to poor and non-significant calculations ($p< 0.05$)

5. Conclusion

The effect of soil characteristics (crop and vineyard soils), temperature and water content on the persistence and dissipation of pesticides in soils have rarely been investigated. Therefore, we evaluated here the effect of the activity of the microorganisms of soil, soil properties, as well as soil temperature and volumetric water content on the dissipation kinetics of individual pesticides in a mixture and the contribution of degradative processes.

Half-life times of pesticides widely varied in the soils compared to typical DT_{50}. The half-life times obtained in the abiotic experiments for both the crop and the vineyard soils were generally up to one order of magnitude higher than those obtained in the biotic experiments. This emphasizes that pesticide dissipation could be mainly attributed to biodegradation. However, pesticide dissipation in the vineyard soils was generally larger than in the crop soil in both biotic and abiotic experiments, which could be related to soil organic content. This trend was particularly pronounced for the moderately hydrophilic pesticides. Temperature appeared to enhance dissipation of individual pesticides of mixture, while the effect of soil water contents (20 % versus 40 %) on the pesticides dissipation was less pronounced than that of temperature. Compound-specific isotope analysis (CSIA) enabled to specificially evaluate in situ degradation of PYR, MTY, SMET and ATR, and to suggest putative transformation reactions that may be attributed to changing soil and temperature conditions.

Further analysis of degradation product will help to elucidate specific degradation pathways for each soil conditions. In the future, multi-element stable isotope analysis (C, N, H) may be attempted to specifically track prevailing transformation pathways and quantify the degradation extent of pesticides in the soil. Further, the impact of soil conditions on the microbial communities exposed to a mixture of pesticides could be evaluated with our set-up using archaeal and bacterial 16S rRNA surveys (Illumina Miseq). Such approaches, combined with detailed follow-up of pesticide dissipation, may be useful to study the response of microbial communities exposed to mixtures of agrochemicals in soils.

These results provide a step forward towards predicting pesticide degradation in relation to soil characteristics, temperature and soil water content. This is crucial in the actual context of climate change as global warming and extreme weather events may lead to the overall rise of temperature, as well as the rise of the number of drought and flooding (Wang et al., 2006). These rises may change the overall transformation and the release of pesticides in soil by altering the wet-dry patterns and the mean annual temperature.

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Partie 4.2. Analyse isotopique énantiosélective du carbone pour évaluer la dégradation du fongicide Métalaxyl dans les sols

Jérémy Masbou, Fatima Meite, Benoît Guyot, Gwenaël Imfeld

1. Abstract and highlights

Chiral pesticides are often degraded enantioselectively in soils leading to disparity among enantiomers that display potentially different toxicity levels. Underlying degradation extents and processes remain out of reach in the field by conventional bulk and enantiomer concentrations analysis. Enantioselective stable carbon isotope analysis (ESIA) combines compound specific isotope analysis (CSIA) and enantioselective analysis, and bears potential to distinguish enantiomer degradation from non-destructive dissipation. We developed ESIA of the fungicide Metalaxyl, providing the first $^{13}C/^{12}C$ ratios for S-Metalaxyl and R-Metalaxyl separately, and applied it to follow degradation in agricultural soil incubation experiments. Similar enantioselective degradation ($k_{S\text{-MTY}}$ = 0.007-0.011 day^{-1} > $k_{R\text{-MTY}}$ = 0.03-0.07 day^{-1}) was associated with isotope fractionation ($\Delta\delta^{13}C_{S\text{-MTY}}$ ranging from 2 to 6 ‰). While R-Metalaxyl degradation was rapid ($T_{1/2}\approx$10 days), concomitant enrichment in heavy isotopes of the persistent S-Metalaxyl occurred after 200 days of incubation ($\varepsilon_{S\text{-}Metalaxyl}$ ranging from -1.3 to -2.7‰). In contrast, initial racemic ratios and isotopic compositions were conserved in abiotic controls, which indicates the predominance of microbial degradation in soils. Degradation products analysis and apparent kinetic isotope effect (AKIE) suggested hydroxylation as a major enantioselective degradation pathway in our soils. Altogether, our study underscores the potential of ESIA to evaluate the degradation extent and mechanisms of chiral micropollutants in soils.

Highlights:

- Enantioselective stable isotope analysis (ESIA) of chiral fungicide Metalaxyl in soils
- Biodegradation causes enantiomeric and carbon isotopic fractionation of Metalaxyl
- Hydroxylation of Metalaxyl is a major degradation pathway in soils

2. Introduction

Chiral pesticides, i.e. pesticides with at least two enantiomers which are non-superimposable mirror images of each other, may constitute more than 25% of currently used pesticides worldwide [1, 2]. Enantiomers of chiral pesticides have identical physico-chemical properties, and transport processes or abiotic reactions appear not changing enantiomers proportions [3]. In contrast, degradation of chiral pesticides may be mainly microbiologically-mediated and enantioselective [4-6]. Enantiomers may also differ in terms of efficacy against plant pathogens, toxicity, and persistence [2]. As a results, active enantiomers in commercial formulations have been partially but not systematically enriched to improve pesticide efficiency on target organisms [7]. Novel approaches to improve the fate of chiral pesticides and their environmental monitoring, in particular in soils receiving large amounts of pesticides, are thus required to evaluate risks associated with enantioselective degradation.

Metalaxyl [methyl-N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate] is an acylalanine chiral fungicide used worldwide and in several crops to control plant diseases caused by pathogens of the Oomycota division [5]. Metalaxyl (MTY) has a chiral carbon and consists of a pair of enantiomers, (+)-S-Metalaxyl (S-MTY) and (–)-R-Metalaxyl (R-MTY, Figure 4.2-1). The fungicidal activity almost entirely originates from the R-enantiomer [6]. As a result, commercial formulations have been progressively enriched in the R-enantiomer in some regions [7]. It remains, however, essential to evaluate the persistence and degradation of racemic MTY (rac-MTY) because it is applied worldwide, and conversion from S-MTY converting to R-MTY and vice-versa have been reported [8]. With the advent of enantioselective chromatography techniques, behavior of chiral pesticides in the environment, including MTY, have been increasingly studied [2, 9].

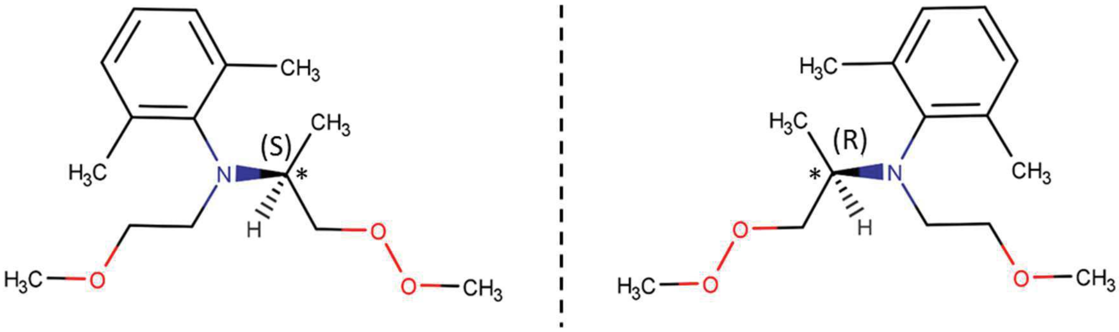

Figure 4.2-1: Chemical structures of Metalaxyl enantiomers

Degradation of chiral pesticides in soil may be mainly microbiologically-mediated and enantioselective [10-12]. Various fungi, bacteria, and actinomycetes have been described to degrade MTY in soils [13-15]. Driven specific bacterial communities, soil pH and oxygen concentration parameters have been shown to control both the extent and the direction of enantioselective biodegradation of MTY [7, 16-19]. Stereoselective enzymatic reactions during degradation mainly depends on the three-dimensional structure of the enzyme and its interaction with the substrate driven by steric constrains, which may preferential transform and deplete an enantiomer among the others [20, 21]. Another process that may involve enzyme enantioselectivity is the active transport into the cell, where the degradation reaction occurs. Because rates differences among enantiomers often results in deviation from racemic ratios [22], changes in the enantiomeric fraction (EF) have been widely used as an indicator of degradation [23]. However, biodegradation may occur without marked enantioselectivity, which may limit the use of EF values to evaluate biodegradation of some chiral molecules [24].

In this case, analogous to enantiomeric ratios, bulk compound-specific isotope analysis (CSIA) may help evaluating *in situ* biodegradation of chiral pesticides [25-27]. Because kinetic isotope effects (KIE) typically favor transformation of light isotopes (e.g., ^{12}C), heavy isotopes (^{13}C) become enriched in the non-degraded pesticide fraction. Increasing $^{13}C/^{12}C$ isotope ratios in a parent compound thus provide direct evidence of its degradation. This concept is modeled in lab experiments by the Rayleigh equation that links isotopic ratios to the extent of biodegradation by isotopic fractionation factors ε [28]. Analogously, the Rayleigh concept can also be applied to model enantioselective biodegradation [29]. Magnitude of ε_{bulk} depends on many factors, including the number of carbon atom in the molecule, the type of bond broken (e.g., C–H or C–O), the reaction mechanism, the effects of non-reacting positions and intramolecular competition [30]. Derived from ε values, apparent kinetic isotope effect (AKIE) values have been introduced to account for these factors [30] and pathway specific AKIEs have been referenced for various experimental reactions [27, 30]. Despite a high potential to disentangle degradation mechanisms, especially when isotope systems ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$) are combined, bulk CSIA approach remains limited for chiral molecules as enantiomers undergo various degradation routes.

Therefore, combining CSIA and enantioselective analysis techniques, enantiomeric stable isotope analysis (ESIA) may greatly enhance the evaluation of sources and transformation processes chiral compounds in the environment [24]. Firstly applied to α-hexachlorocyclohexane (α-HCH)[31-33], ESIA have also been developed for polar herbicides (phenoxy acids) [34, 35], synthetic polycyclic musk galaxolide (HHCB)[36], and phenoxyalkanoic methyl herbicides [37]. In these pioneer studies, ESIA have demonstrated particular benefits to characterize biodegradation of these chiral contaminants and to differentiate abiotic and biotic transformation in the field [33]. However, concepts, methods and application of ESIA of chiral pesticides in environmental compartments, such as soils, are mostly missing so far.

The purpose of this study was to explore the applicability of ESIA as a tool to assess the biodegradation process of MTY enantiomers in soils. A gas chromatography combustion isotope ratio mass spectrometry (GC–C–IRMS) method was developed for stable carbon isotope analysis of MTY enantiomers in environmental soils. The novel method was applied to evaluate the *in situ* biodegradation of MTY in agricultural soil experiments. Both abiotic and biotic crop and vineyard soils were tested to explore the sensitivity of carbon isotope fractionation and the influence of various soil characteristics. Special attention have been paid to consider these first reference values for potential environmental applications.

3. Material and methods

3.1. Chemicals and Rac-Metalaxyl standard stock solution preparation

Analytical grade standards (purities, >99%) of rac-MTY, Metalaxyl-M (corresponding to R-MTY enantiomer), Metolachlor-d_{11} and alachlor-d_{13}, solvents (dichloromethane and ethyl acetate; HPLC grade purity >99.9%) and sulfate magnesium ($MgSO_4$, ReagentPlus® ≥99.5%) were purchased from Sigma Aldrich (St. Louis, USA). Primary secondary amide (PSA) bonded silica was purchased from Supelco (Bellefonte, USA). For analytical development and reproducibility check, two stock solutions of rac-MTY and M-Metalaxyl of 1 $mg.mL^{-1}$ were prepared in dichloromethane (DCM) and stored at -18°C until analysis.

3.2. Soil extraction procedure

The extraction of pesticides from soils is explained earlier in the Chapter 2, part 3.1. Prior to experiment, possible isotope artifacts of the soil extraction and concentration procedure on the enantiomer fractionation and carbon isotope fractionation was evaluated. Briefly, rac-MTY standard was spiked into 5 g of uncontaminated soil from the crop field site to concentrations in the range of 20-50 $\mu g.g^{-1}$ dried-soil. After 24 h of equilibration time, three experiments (20, 30 and 50 $\mu g.g^{-1}$ dried-soil) in triplicate undergone extraction, clean-up and concentration procedure before GC-C-IRMS measurements.

3.3. Bulk-Metalaxyl and degradation products analysis

The quantitative analysis of bulk-MTY is presented in Chapter 2, part 3.1. Carboxylic acid Metalaxyl, hydroxyMetalaxyl, demethyl-Metalaxyl and didemethyl-Metalaxyl degradation products were analysed by LC/MS as previously described [40] (Table 4.2-1, Figure 4.2-2).

Table 4.2-1: Mass spectrometry parameters for detection of metalaxyl degradation products by LC-MS

Metabolite	Retention time [min]	Parent mass [amu]	Fragment mass [amu]	SRM collision Energy [V]	Tube Lens [V]	Polarity	Reference
Carboxylic Acid Metalaxyl (GCA 10906)	3.5	294.0	204.1	28	63	-	In house developments
Demethylmetalaxyl (GCA 62826)	7.8	264.0	192.2	18	83	-	
Hydroxymetalaxyl	7.3	296.0	146.0	29	46	+	[1]
Didemethylmetalaxyl	7.2	252.0	206.0	15	10	+	

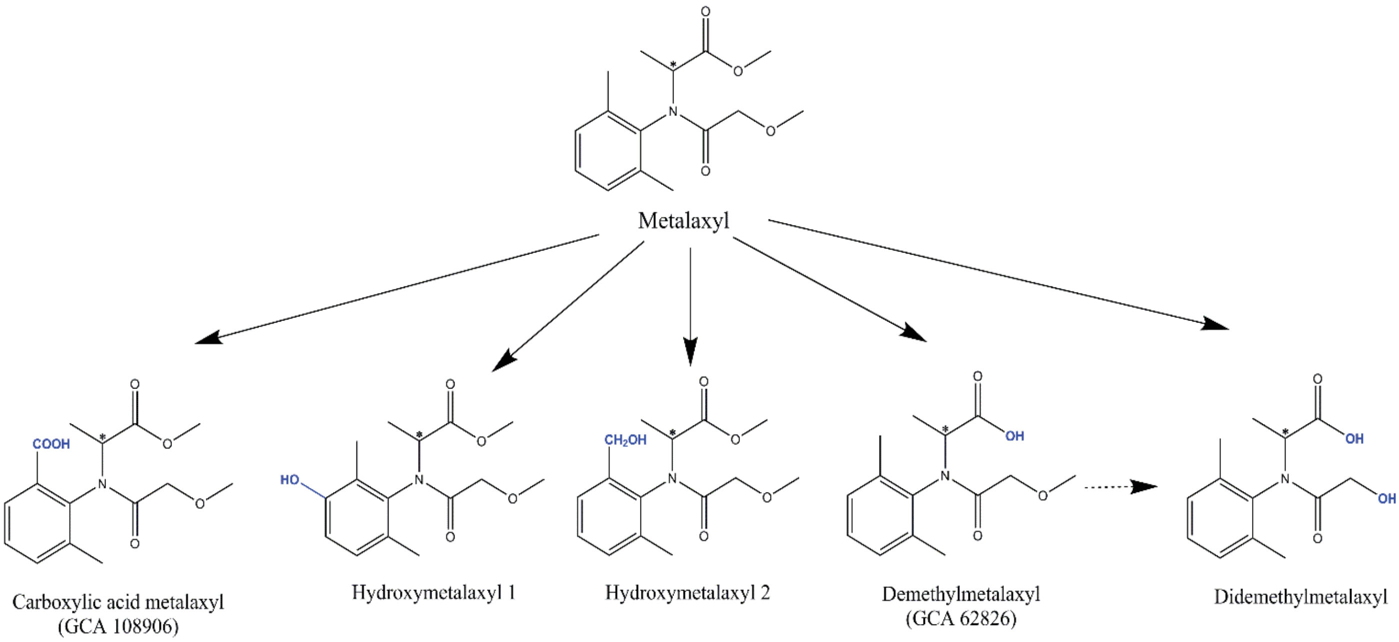

Figure 4.2-2: Proposed Metalaxyl degradations pathways in soils. Carboxylic acid metalaxyl (m/z 294), hydroxymetalaxyl 1 and 2 (m/z 296), demethylmetalaxyl (m/z 266) and dimethylmetalaxyl (m/z 252) are the degradation products. Adapted from [1]

3.4. Enantiomer-specific stable carbon isotope analysis

The carbon isotope composition of MTY enantiomers was determined using a GC-C-IRMS system consisting of a gas chromatograph (Trace 1310, Thermo Fisher Scientific) coupled via a GC IsoLink/Conflo IV interface (Thermo Fisher Scientific) to an isotope ratio mass spectrometer (DeltaV plus, Thermo Fisher Scientific). The oxidation furnace of the GC-Isolink interface was set to a temperature of 1000 °C. A BGB-172 column with 20% tert-butyldimethylsilyl-beta-cyclodextrin phase (30 m × 0.25 mm, 0.25 µm film thickness, BGB Analytik, Adliswil, Switzerland) was used for chromatographic separation, with helium as the carrier gas at a flow rate of 2.0 mL min^{-1}. The column was held at 50 °C for 1 min, heated at a rate of 10 °C min^{-1} to 150 °C, then up to 200 °C at 1 °C min^{-1} and finally heated at 20 °C min^{-1} to 220 °C and held for 1 min. Samples (3 µL) were injected using a Combi PAL GC Autosampler (CTC Analytics) into a split/splitless injector operated in splitless mode and held at 250 °C.

Metalaxyl was fully resolved into its enantiomers (enantiomer resolution, R ≈ 1). As previously observed with similar β-cyclodextrin phase column [17], the enantiomer elution sequence was S-MTY as first-eluted at 51.1 min and R-MTY as second-eluted at 51.7 min. Injection of single-enantiomer R-MTY standard confirmed this elution sequence. Typical chromatogram is given in Figure 4.2-3.

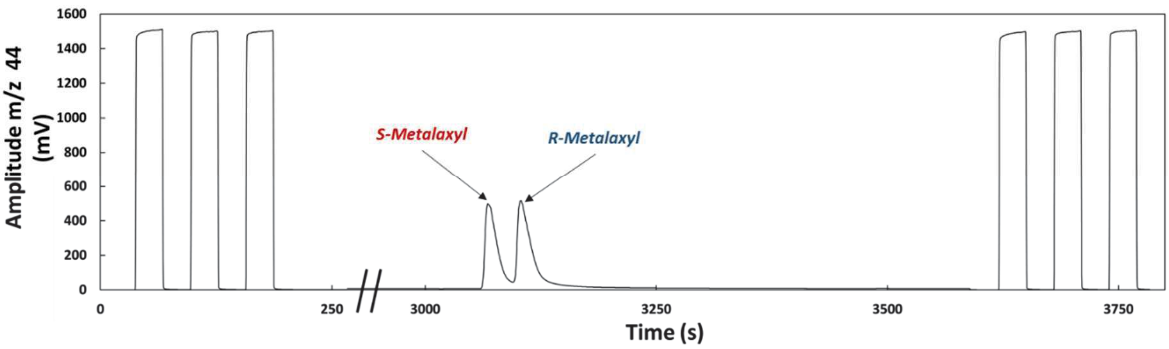

Figure 4.2-3: Typical GC-IRMS chromatogram (m/z 44) of a metalaxyl racemic mix. Examples with an injection volume of 3 µL corresponding to an amount of carbon injected of 25 ngC per enantiomer

The $\delta^{13}C$ values were calibrated using a three-point calibration using international reference material AIEA-600, USGS-40, and USGS-41 ($\sigma < 0.05$ ‰) and reported in parts per thousand (‰) against the Vienna Pee Dee Belemnite (VPDB) standard, according to the following equation (Eq. 4.2-1):

$$\delta^{13}C_{X-MTY} = \left(\frac{(^{13}C_{X-MTY}/^{12}C_{X-MTY})_{sample}}{(^{13}C_{CO2}/^{12}C_{CO2})_{ref}} - 1\right) \times 1000 \qquad (4.2\text{-}1)$$

with *X*=bulk, X=S or X=R

A caffeine reference material (IAEA-600) was measured every nine injections to control the quality of the measurements. Values of $\delta^{13}C=-28.1\pm0.4$‰ (σ, n=15) were consistent with $\delta^{13}C=-27.8\pm0.1$‰ (σ) determined by EA-IRMS in inter-laboratory calibrations [41]. For accurate rac-MTY consensus mean determination, long-term reproducibility was evaluated by measuring an in-house rac-MTY standard (50-100 ngC injections) every 10 GC-C-IRMS injections. In the absence of chiral certified standard material, this in-house standard was used for detecting analytical bias during sample batch sessions. Results of 70 injections are displayed in Table 4.2-2 and Figure 4.2-4. Consensus means of $\delta^{13}C_{S\text{-}MTY}$ of -31.7 ± 1.0 ‰ (Mean ± 2σ, n=70), similar to $\delta^{13}C_{R\text{-}MTY}=-31.8 \pm 0.9$ ‰ (Mean ± 2σ, n=70), and $\delta^{13}C_{Bulk\text{-}MTY}=-31.7 \pm 0.4$ ‰ (Mean ± 2σ, n=70) were obtained. The reference carbon isotope composition ($\delta^{13}C_{Bulk\text{-}MTY}$) of rac-MTY standard was obtained using an elemental analyzer-Isolink CN (Thermo Fisher Scientific) coupled to an isotope ratio mass spectrometer (DeltaV plus, Thermo Fisher Scientific).

Table 4.2-2: ESIA validation tests on rac-Metalaxyl standard

	Linearity tests		*Long-term reproducibility*		*Soil extraction tests*	
Enantiomeric fractionation	**EF$_{S\text{-}MTY}$**	**2σ (*n*)**	**EF$_{S\text{-}MTY}$**	**2σ (*n*)**	**EF$_{S\text{-}MTY}$**	**2σ (*n*)**
	0.44	0.03 (9)	0.46	0.04 (70)	0.47	0.02 (3)
Isotopic fractionation	**$\delta^{13}C$ [‰]**	**2σ (*n*) [‰]**	**$\delta^{13}C$ [‰]**	**2σ (*n*) [‰]**	**$\delta^{13}C$ [‰]**	**2σ (*n*) [‰]**
S-MTY	-31.7	0.8 (6)	-31.7	1.0 (70)	-31.5	0.5 (3)
R-MTY	-31.4	0.7 (8)	-31.8	0.9 (70)	-32.1	0.5 (3)
Bulk-MTY Double-Peak Integration	-31.6	0.3 (9)	-31.7	0.4 (70)	-31.6	0.3 (3)
Bulk-MTY Mass balance	-31.6	0.4 (9)	-31.7	0.6 (70)	-31.8	0.2 (3)
EA-IRMS	-31.3	0.1 (3)	-31.3	0.1 (3)	-31.3	0.1 (3)
$\Delta\delta^{13}C_{Bulk\text{-}MTY}$ EA-IRMS vs GC-C-IRMS	0.3	0.1	0.4	0.1	0.3	0.1

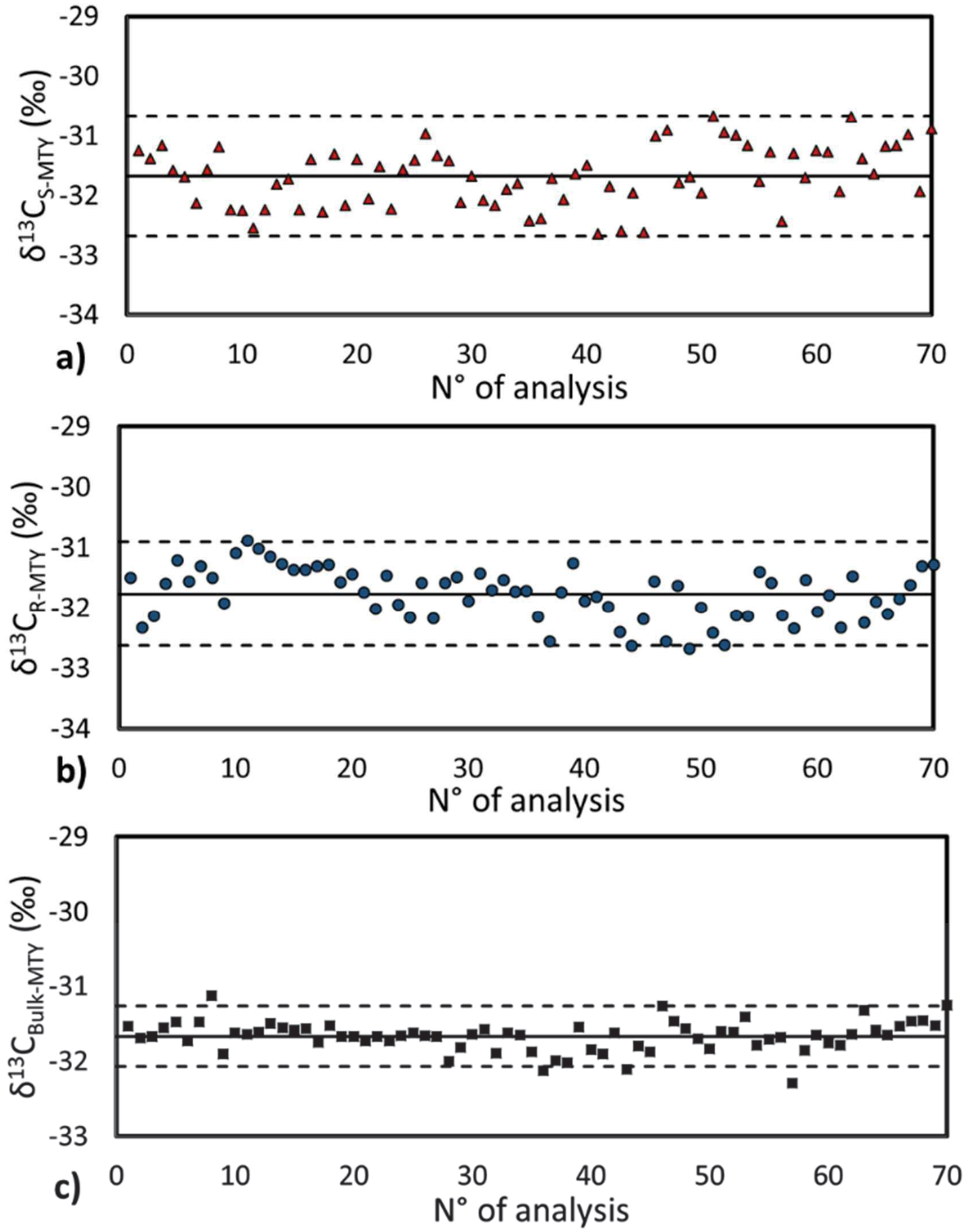

Figure 4.2-4: Long-term $\delta^{13}C$ reproducibility of a) S-MTY, b) R-MTY and c) Bulk-MTY. The solid and dotted lines represent the mean and ±2SD respectively

3.5. Data analysis

3.5.1. Enantiomeric fractionation and carbon isotope calculations

Relative change in the proportion of each enantiomer was calculated by $EF_{S\text{-}MTY}$ and $EF_{R\text{-}MTY}$ enantiomeric fractionation according to (Eq. 4.2-2):

$$EF_{S-MTY} = \frac{Peak\ area_{S-MTY}}{Peak\ area_{S-MTY} + Peak\ area_{R-MTY}} \qquad (4.2\text{-}2)$$

Rac-MTY mixture then displays $EF_{S\text{-}MTY}$=0.5, while an enriched S-MTY solution yields an $EF_{S\text{-}MTY}$> 0.5.

The isotopic composition of bulk-MTY ($\delta^{13}C_{Bulk-MTY}$) from the isotope ratios of the individual MTY enantiomers determined by GC-C-IRMS was obtained by mass-balance calculation [31]:

$$\delta^{13}C_{Bulk-MTY} = EF_{S-MTY} \times \delta^{13}C_{S-MTY} + EF_{R-MTY} \times \delta^{13}C_{R-MTY} \qquad (4.2\text{-}3)$$

To validate the mass balance, bulk-MTY $\delta^{13}C_{bulk\text{-}MTY}$ was also determined by integration of both R and S-MTY peaks (Figure 4.2-3) using the ISODAT application software (Thermo Scientific), considering each peak independently. Comparison of the mass balance and double-peak integration approaches is reported in the Table 4.2-2.

Bulk isotope enrichment factors (ε_{bulk}) were calculated from the logarithmic linearization using the Rayleigh equation (Eq. 4.2-4):

$$\ln\left(\frac{\delta^{13}C_x + 1000}{\delta^{13}C_0 + 1000}\right) = \frac{\varepsilon_{bulk}}{1000} \times \ln\left(\frac{C_x}{C_0}\right) \qquad (4.2\text{-}4)$$

where $\delta^{13}C_0$ and $\delta^{13}C_x$ are the measured carbon isotope ratios at the beginning (t=1 day) and at t=x days from the beginning of the experiment, and C_0 and C_x are the corresponding concentrations. Enrichment factors for S- and R-MTY were calculated in the same way. The error of the isotope enrichment factor was given as the 95% confidence interval (CI) and determined using regression analysis as described elsewhere [42].

To compare observed carbon isotope fractionation with fractionation reported in the literature, the apparent kinetic isotope effect (AKIE) values were calculated using Eq. 4.2-5 [43] :

$$AKIE \approx \frac{1}{1+z\times(\frac{n}{x})\times\frac{\varepsilon_{bulk}}{1000}} \qquad (4.2\text{-}5)$$

where n is the number of atoms of a given element, x is the number of indistinguishable reactive positions, and z is the number of positions in intramolecular competition. For AKIE calculations, n=15 carbon atoms, x=1 indistinguishable reactive position, and z=2 positions in intramolecular competition for ring-methyl hydroxylation. The uncertainty of the AKIE was estimated by error propagation as described elsewhere [44].

3.5.2. Statistics

Statistical analyses were performed using JMP software (SAS Institute, USA) and the function “Data Analysis” from the Add-In “Analysis Tool Pack” of Microsoft Excel. Isotopic enrichment factors were calculated from linear regressions of Eq. 4.2-4 and rate constants (or half-life parameters) were determined using Single First-Order Rate Model (SFO) from plots of ln (C/C_0) versus time.

3.6. Soil degradation experiments: Soils description and preparation

The description of the soils used for the degradation experiment and the preparation of these soils are presented in Chapter 2, part 2 (details of soils physico-chemical characteristics are provided in Table 2-1).

3.7. Soil degradation experiments: Experimental set-up, sampling and spiking

The soil degradation experiment is explained in Chapter 4, part 4.1. The Figure 4.1-1 shows the experimental set-up used. The pesticide mixture used for this experiment was composed of rac-MTY, 8 other fungicides, 10 herbicides and 1 benzoylurea insecticide (see Table 2-6 for details).

4. Results and discussion

4.1. Validation of Metalaxyl ESIA

Linearity tests to validate the method was firstly performed on rac-MTY standard (Tables 4.2-1 and 4.2-2). The overall uncertainty of MTY-ESIA on triplicate injections (2σ=0.5-0.8 ‰) was in the same range than that of other studies (S.-L. Badea & Danet, 2015; Maier et al., 2013). For accurate analysis, we suggest a minimum injection of 5 $ngC_{R\text{-}MTY}$ and 30 $ngC_{S\text{-}MTY}$,,and a maximum injection of 90 ngC for each enantiomer. In these conditions, analytical precision of 2σ<0.3‰ for amplitude >90mV (m/z 44) on each enantiomers (>5ngC) are consistent with typical bulk-CSIA measurements [27]. In term of accuracy, S-MTY $\delta^{13}C_{S\text{-}MTY}$ of -31.7 ± 0.8 ‰ (Mean ± 2σ, n=6) was similar to $\delta^{13}C_{R\text{-}MTY}$=-31.4 ± 0.7 ‰ (Mean ± 2σ, n=8) and $\delta^{13}C_{Bulk\text{-}MTY}$=-31.6 ± 0.3 ‰ (Mean ± 2σ, n=9) and did not significantly differ from the $\delta^{13}C_{Bulk\text{-}MTY}$=-31.3 ± 0.1 ‰ (Mean ± 2σ, n=3) obtained by EA-IRMS for rac-MTY standard. This cross-calibration of rac-MTY confirmed the absence of significant isotopic fractionation bias associated with MTY-ESIA.

Prior to applying MTY-ESIA on soil degradation experiment, possible isotope artifacts of the soil extraction and concentration procedure on the enantiomer fractionation and carbon isotope fractionation was evaluated. Given the uncertainties, enantiomeric fractionation $EF_{S\text{-}MTY}$=0.47±0.02 (Mean ± 2σ, *n*=3) and $\delta^{13}C$ values $\delta^{13}C_{Bulk\text{-}MTY}$=-31.6 ± 0.3 ‰ (Mean ± 2σ, *n*=3) obtained for rac-MTY standards extracted from soils overlap those of the non-extracted standard (Table 4.2-2). The absence of enantiomeric fractionation caused by sorption and extraction processes is consistent with previous observations [46]. Non-significant shift of EF and $\Delta\delta^{13}C_{Bulk\text{-}MTY}=0.3\pm0.1‰$ with EA-IRMS measurements indicates the absence of enantiomer and carbon isotope fractionation during the soil extraction method used in this study, and thus the suitability of the method for MTY-ESIA in soil samples.

4.2. Kinetics of Bulk-Metalaxyl dissipation in soils

Concentrations of bulk-MTY (GC-MS) and MTY enantiomers (derived from EF) were followed-up in soils degradation experiments during 200 days (Figure 4.2-6 and Table 4.2-5). Degradation patterns fitted first-order kinetics using $R^2>0.8$ ($p^*< 0.05$) as a criteria. In biotic conditions, the dissipation of bulk-MTY was relatively fast with $T_{1/2}$ values ranging from 50±10 days to 65±10 days (SE, n=5) for crop and vineyard soils respectively (Table 4.2-6), which is similar to previously reported [7]. In contrast, bulk-MTY was stable ($T_{1/2}>200$ days) for both soils under abiotic conditions, highlighting the predominence of biollogically mediated MTY dissipation processes in soils.

At this stage, no significant bulk-MTY kinetic differences could be pointed out between the crop and the vineyard soil. However, pollution history differs between the two soils considered in this study, potentially leading to different microbial adaptative response of microorganisms, including pesticide degradation [47]. While the vineyard was regularly treated with Ridgold F® (R-MTY mixture), MTY was not used in the crop field. With *a priori* MTY-adapted microbial communities, higher dissipation rates would be expected in the vineyard soil. However, similar dissipation kinetics and extent leading to 5% and 10% of remaining Bulk-MTY were observed after 200 days for crop and vineyard experiments, respectively (Tables 4.2-5 and 4.2-6). This result emphazises the limited influence of previous MTY amendments on soil-microbial degradation abilities, as suggested previously [13, 48]. However, anilides molecules, such as the herbicide S-metolachlor were also regularly applied on the crop soil. Because MTY and metolachlor have been shown to be both degraded by a similar microflora (e.g *Trichoderma sp. and Bacillus sp.* isolated from soil with no history of MTY/Metolachlor applications) [13], similar enzymes may be involved in their metabolism.

Although biotic dissipation process has been monitored by changes in bulk-MTY concentrations, evidence of MTY molecular degradation is not given at this stage. Since degradation processes have shown to be enantioselective [10-12], MTY enantiomers analysis has been further applied to define the observed dissipation process.

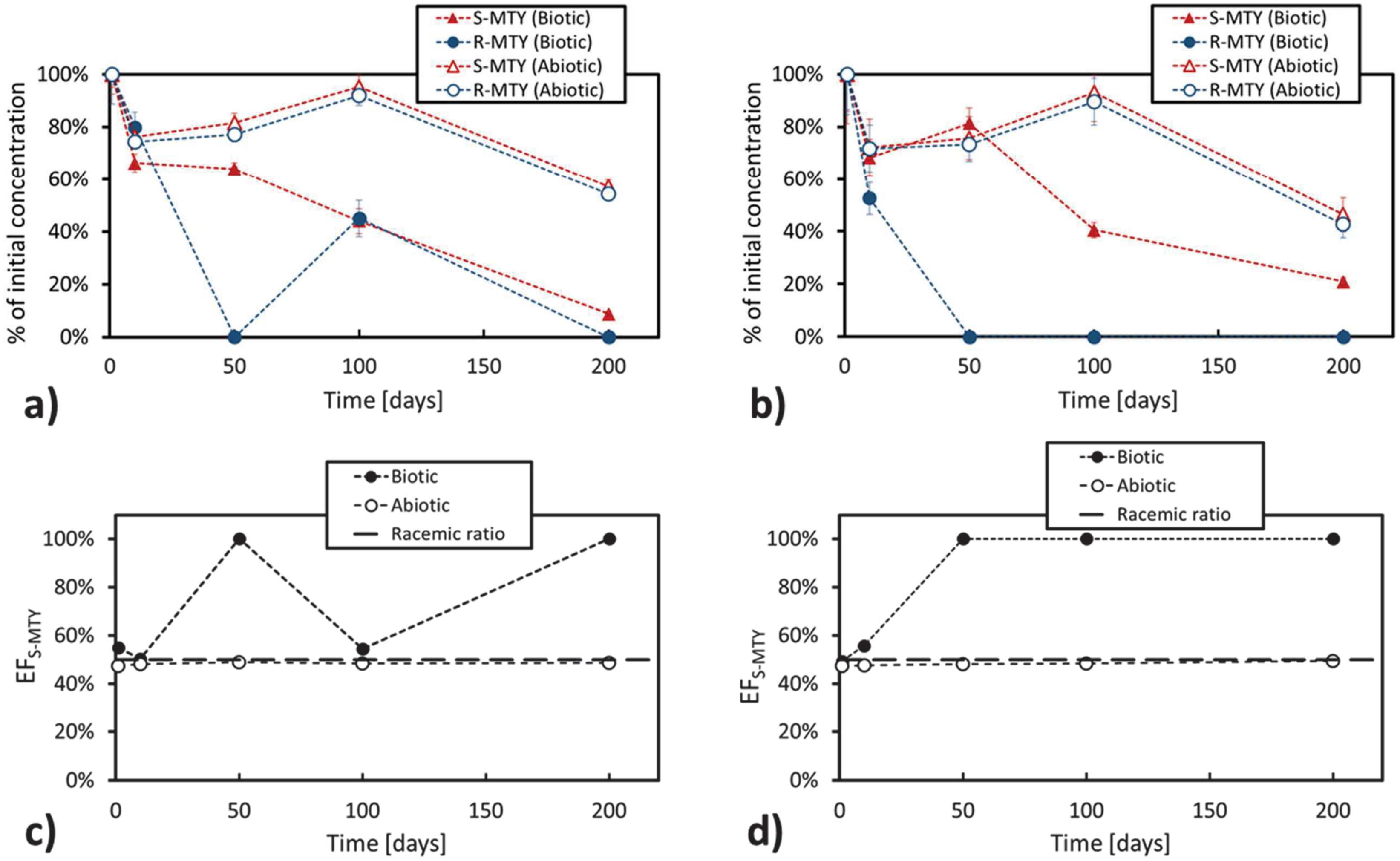

Figure 4.2-6: S-MTY and R-MTY dissipation (% of initial concentration) during soil incubation experiments and corresponding enantiomeric enrichment factors ($EF_{S\text{-}MTY}$, %) for the crop soil (a) and (c), and the vineyard soil (b) and (d). Error bars denote the total analytical uncertainty (± 2SD, %), which incorporates both accuracy and reproducibility of n=3 measurements

Table 4.2-5: Mean and standard deviations of metalaxyl enantiomeric concentrations and stable carbon isotope ($\delta^{13}C$) triplicate measurements for the two soils degradation experiments and their autoclaved controls associated. With overall uncertainties of 2σ=0.6 and 0.8‰ on $\delta^{13}C_{S\text{-}MTY}$ and $\delta^{13}C_{R\text{-}MTY}$ (n=3) respectively, uncertainties are comparable to those obtained in standard stock solution analysis (Table 1). Due to baseline artifacts in some samples, uncertainties range are however higher (2σ from 0.2 to 1.7 ‰)

Sampling site	Experimental conditions	Sampling time days	[Bulk-MTY] µg.g^{-1}	EF	2σ	[S-MTY] µg.g^{-1}	2σ µg.g^{-1}	[R-MTY] µg.g^{-1}	2σ µg.g^{-1}	$\delta^{13}C_{S\text{-}MTY}$ ‰	2σ ‰	$\delta^{13}C_{R\text{-}MTY}$ ‰	2σ ‰	$\delta^{13}C_{Bulk\text{-}MTY}$ ‰	2σ ‰
Alteckendorf (wheat and maize crops)	Not autoclaved	1	5.00	0.55	0.01	2.75	0.11	2.25	0.13	-31.19	0.39	-30.53	0.86	-30.91	0.24
		10	3.62	0.50	0.00	1.82	0.03	1.80	0.03	-30.78	0.20	-30.70	0.59	-30.67	0.45
		50	1.76	1.00	0.00	1.76	0.00	0.00		-30.48	0.34	<LOD	<LOD	-30.48	0.34
		100	2.22	0.54	0.02	1.21	0.08	1.01	0.10	-27.77	1.12	-29.53	1.72	-28.68	0.31
		200	0.24	1.00	0.00	0.24	0.00	0.00		-25.19	1.19	<LOD	<LOD	-25.19	1.19
	Autoclaved	1	5.00	0.47	0.01	2.37	0.06	2.63	0.05	-32.00	0.63	-31.64	0.19	-31.73	0.53
		10	3.76	0.48	0.00	1.81	0.03	1.95	0.03	-31.63	0.42	-31.94	0.27	-31.68	0.38
		50	3.97	0.49	0.00	1.94	0.03	2.03	0.03	-31.28	0.75	-31.75	0.89	-31.55	0.35
		100	4.68	0.48	0.01	2.26	0.05	2.42	0.05	-31.13	0.58	-31.63	0.84	-31.32	0.18
		200	2.78	0.49	0.01	1.36	0.03	1.43	0.03	-30.73	0.67	-31.92	0.07	-31.36	0.25
Rouffach (Vineyard)	Not autoclaved	1	5.00	0.49	0.02	2.47	0.17	2.53	0.17	-31.15	0.60	-31.74	0.79	-31.41	0.22
		10	3.02	0.56	0.01	1.68	0.05	1.33	0.06	-30.95	0.53	-29.99	1.13	-30.40	0.58
		50	2.01	1.00	0.00	2.01	0.00	0.00		-30.54	0.35	<LOD	<LOD	-30.54	0.35
		100	1.00	1.00	0.00	1.00	0.00	0.00		-30.09	0.58	<LOD	<LOD	-30.09	0.58
		200	0.52	1.00	0.00	0.52	0.00	0.00		-29.03	0.38	<LOD	<LOD	-29.03	0.38
	Autoclaved	1	5.00	0.47	0.02	2.37	0.22	2.63	0.20	-31.83	0.12	-31.88	0.98	-31.93	0.72
		10	3.59	0.48	0.01	1.71	0.10	1.88	0.09	-31.20	0.88	-31.87	0.81	-31.63	0.24
		50	3.72	0.48	0.00	1.79	0.03	1.93	0.02	-31.38	1.11	-31.76	0.30	-31.61	0.43
		100	4.56	0.48	0.01	2.21	0.06	2.36	0.05	-31.46	0.37	-31.32	0.39	-31.36	0.13
		200	2.22	0.50	0.01	1.10	0.05	1.12	0.05	-31.28	0.39	-31.84	0.78	-31.38	0.80

Table 4.2-6: Summary of k and $T_{1/2}$ values obtained for S-MTY, R-MTY and bulk-MTY

Sampling site	Experimental conditions	S-MTY				R-MTY				Bulk-MTY			
		k day^{-1}	SE day^{-1}	$T_{1/2}$ day	SE day	k day^{-1}	SE day^{-1}	$T_{1/2}$ day	SE day	k day^{-1}	SE day^{-1}	$T_{1/2}$ day	SE days
Alteckendorf (Crop)	Not autoclaved	0.011	0.002	62	10	0.007^{a,b}	-	94$^{a, b}$	-	0.014	0.03	50	10
	Autoclaved	0.002^{c}	-	358^{c}	-	0.002^{c}	-	327^{c}	-	0.002^{c}	-	341^{c}	-
Rouffach (Vineyard)	Not autoclaved	0.007	0.001	94	16	0.071^{b}	-	10^{b}	-	0.011	0.002	65	10
	Autoclaved	0.003^{c}	-	250^{c}	-	0.003^{c}	-	218^{c}	-	0.003	-	233^{c}	-

[a] value biased by the outlier value obtained at t=100 days. Removing this values gives k≈0.025 day^{-1} and DT_{50}≈28 days
[b] uncertainty not given (n≤3)
[c] slope obtained from Equation S2 not significant (p>0.05)

4.3. Enantioselective degradation in soils

In autoclaved controls, S-MTY and R-MTY concentrations remain constant during the experiment (Figure 4.2-6), exept for the last sampling point where concentration slightly decrease to $[S\text{-}MTY]_{t=200}/[S\text{-}MTY]_{t=1}=57\%$ and $[S\text{-}MTY]_{t=200}/[S\text{-}MTY]_{t=1}=46\%$ for crop and vineyard soils respectively. The low dissipation rates ($T_{1/2}>200$ days, $k\leq0.003$ day^{-1}, Table 4.2-6) coincide with non significant enantioselectivity ($EF_{S\text{-}MTY}=0.48\pm0.01$, σ) over the experiments. This is in line with previous sorption experiments with rac-MTY, for which no significant difference in the K_d values was observed for both S- and R- enantiomers [49]. In constrast, in non-autoclaved soil, dissipation of both R- and S-MTY emphasised that degradation was the prevailing MTY dissipation process in our experiment [7, 49]. Degradation of rac-MTY was enantioselective in both the crop and the vineyard soils, with the R-enantiomer being systemically degraded faster than the S-enantiomer. In the vineyard soil experiments, R-MTY could not be detected at t=50 days, yielding an EF=1 until 200 days. Half-life time of S-MTY ($T_{1/2}$ = 94±16 days, SE, n=5) was higher than that of R-MTY ($T_{1/2}$ ≈10 days, n=2, Table 4.2-6). A similar pattern could be observed in the crop soil, although R-MTY was detected at t=100 days (1.01±0.10 μg.g^{-1} and EF=0.54±0.02, n=3). This may reflect heterogeneity in the soil highlighted by sample collection using a sacrificial approach. While this outlier value did not allow precise determination of $T_{1/2}$ (R-MTY), $T_{1/2}$ for S-MTY of 62±10 days (±SE, n=5) suggests faster degradation for S-MTY in the crop soil. These results agree with those obtained in similar soil incubation experiments, indicating faster degradation of the R-MTY ($T_{1/2}$=12-68 days, $k_{R\text{-}MTY}$=0.01-0.06 day^{-1}) compared to the S-MTY ($T_{1/2}$=35-99 days, $k_{S\text{-}MTY}$=0.007-0.02 day^{-1} [49]).

As experiments were carried out with neutral or slightly alkaline soils (6.9<pH<8.1, Table 2-1), our results are in agreement with previous findings, insofar degradation of R-MTY was faster than that of S-MTY in aerobic soils at pH > 5 [7]. R-MTY may thus be preferentially degraded by microorganisms, while the activation/inhibition of enantiomer-specific enzymes may be controled by pH [7]. The similar enantioselective biodegradation patterns for both crop and vineyard soils suggests that the systematic use of R-MTY mixture in Rouffach vineyard did not result in the enrichment of active R-MTY degrading microorganisms.

Besides, soils physicochemical characteristics may influence MTY degradation rate to a greater extent than microbial community composition [50]. Then, for circumneutral pH soils, the extent and enantioselectivity of MTY biodegradation may be soil-dependent, and occur more slowly and with less enantioselectivity in finer-textured soil than in the coarser-textured soils [49], possibly reflecting MTY bioavailability in soils. In our case, granulometry vary from 28% to 69% of clay for the crop and the vineyard soils, respectively (Table 2-1). The large proportion of fine minerals associated with higher amount of organic matter (4.8%) in the vineyard soil could have led to slighlty higher sorption process revealed by MTY ageing at the later stage of incubation. The higher bulk-MTY dissipation at t=200 days in the abiotic controls supports this idea. MTY sorption might thus influence its bioavailability, which is in agreement with higher bulk-MTY (i.e S-MTY, EF=1) resilence at=200 days in biotic vineyard soil compared to crop soil experiment.

Altogether, the bulk and MTY enantiomers results highlight that biodegradation is the major dissipation process in the studied soils and is strongly enantioselective. However, at this stage, the influence of soil physico-chemical properties and microbial degradation may not be disentangled, especially in the later stage of incubation, because both biotic and abiotic degradation of MTY may be enantioselective and occur simultaneously in the living soil experiments. Since stable isotope analysis have shown abilities for approaching biodegradation extent and mechanisms [27], MTY-ESIA was further applied.

4.4. Carbon stable isotope fractionation of Metalaxyl enantiomers

In the soil experiments, both MTY enantiomers display significant carbon isotope fractionation (Figure 4.2-7 and Table 4.2-5). For S-MTY, carbon isotope composition changed from $\delta^{13}C_{S\text{-}MTY}$=-31.2±0.4‰ and $\delta^{13}C_{S\text{-}MTY}$=-31.2±0.6‰ (t=1 day) up to $\delta^{13}C_{S\text{-}MTY}$=-25.2±1.2‰ and $\delta^{13}C_{S\text{-}MTY}$=-29.0±0.4‰ (t=200 days) in the crop and the vineyard soils, respectively. In autoclaved controls, carbon isotopic fractionation was insignificant for both MTY enantiomers after 200 days (Figure 4.2-7). In the vineyard soil, carbon isotopic ratios remain constant for S-MTY (from $\delta^{13}C_{S\text{-}MET}$=-31.8±0.1‰ at t=1 day to $\delta^{13}C_{S\text{-}MET}$=-31.0±1.1‰ at t=200 days) and for R-MTY (from $\delta^{13}C_{R\text{-}MET}$=-31.9±1.0‰ to $\delta^{13}C_{R\text{-}MET}$=-31.8±0.8‰).

In the biotic experiment, systematic enrichment of lighter isotopes in the remaining MTY fraction relate kinetic isotope effect typicaly observed in biodegradation processes [27]. Enrichment factors observed in the vineyard soil ($\varepsilon_{S\text{-}MTY}$=-1.3±0.3‰) were smaller than those retrieved from the crop soil ($\varepsilon_{S\text{-}MTY}$=-2.7±0.5‰). Since this is the first report for MTY bulk or enantiomeric isotope fractionation, no enrichment factors values are available for comparison. Enrichment factors values are, however, similar to those of structurally close chloroacetanillide herbicides retrieved from lab-scale wetlands experiments ($\varepsilon_{alachlor}$= −2.0 ± 0.3‰, $\varepsilon_{acetochlor}$= −3.4 ± 0.5‰, [51]). With fewer data available ($n \leq 3$) due to fast R-MTY degradation ($T_{1/2}$(R-MTY)≈10 days), isotopic fractionation associated to biodegradation was less sensitive for R-MTY. Similarly to S-MTY, slight enrichment in $\delta^{13}C_{R\text{-}MTY}$ was observed (Figures 4.2-7a and 4.2-7b). Carbon isotopic ratios changed from $\delta^{13}C_{R\text{-}MTY}$=-30.5±0.9 ‰ (t=day 1) to $\delta^{13}C_{R\text{-}MTY}$=-29.5±1.7‰ (t=50 days), and from $\delta^{13}C_{R\text{-}MTY}$=-31.7±0.8‰ (t=day 1) to and $\delta^{13}C_{R\text{-}MTY}$=-30.0±1.1‰ (t=10 days) in the crop and the vineyard soil, respectively. However, with $n \leq 3$ and unsignificant Rayleigh relationships ($p>0.05$), accurate enrichment factor could not be retrieved. No isotope enrichment factors could also be retrieved for the abiotic control due non-significant Rayleigh correlation ($p>0.05$ and $r^2<0.8$, Table 4.2-7).

In order to further interpret the observed fractionation and provide indication of prevailing degradation mechanisms, we calculated AKIE values for MTY degradation in the soil experiments. AKIE values are associated with isotope effect of the specific bond cleavage [30]. Little information is available on the degradation pathways of MTY in soils. However, in cell suspension of plants and in human liver microcosm, MTY undergoes mainly ring-methyl or phenyl ring hydroxylation and (di)demethylation on the ester and ether group [52, 53]. More scarcely, hydroxylation at the meta-position on the phenyl ring may occur (Figure 4.2-2). AKIE values range from 1.007 to 1.014 for bulk-MTY, from 1.010 to 1.021 for S-MTY and 1.011±0.004 for R-MTY in our biotic experiments (Table 4.2-7). These values fit in the range of experimentally derived $AKIE_C$=1.01-1.03 obtained in the litterature of for oxidation of C-H bonds [43]. With typically $AKIE_C$=1.03-1.07, SN_2 C-O cleavage (esther/ether demethylation) is unlikely to be predominent in our case [43]. Thus, isotope fractionation of S- and R-MTY suggests the ring or meta-position methyl hydroxylation as the main degradation process in our biotic soil experiments. This hypothesis should be confirmed the analysis of MTY degradation products.

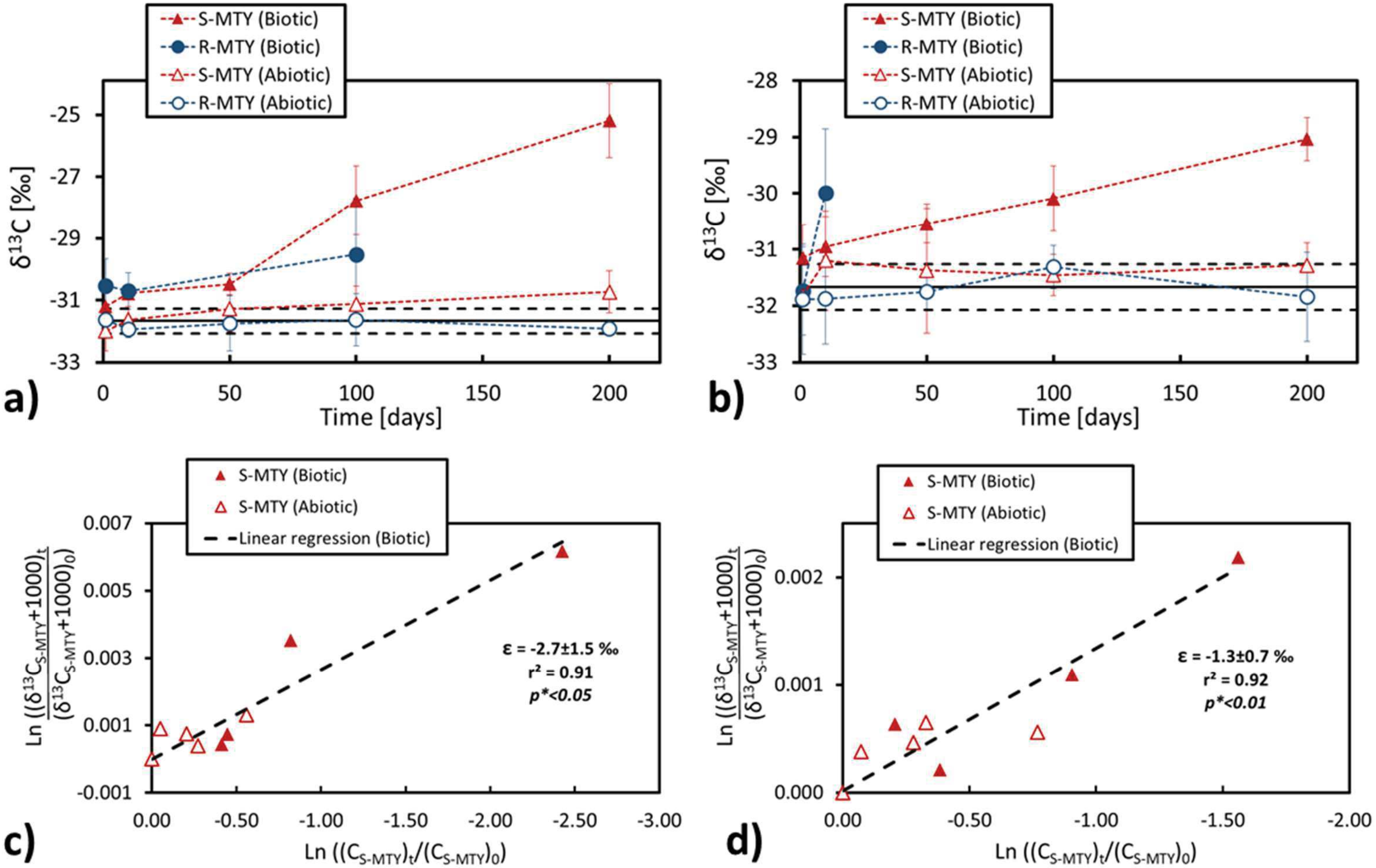

Figure 4.2-7: δ^{13}CS-MTY and δ^{13}CR-MTY for a) the crop soil and b) the vineyard soil. Only S-MTY Rayleigh plots are displayed in the figure c) and d) respectively. The overall isotope fractionation data are given in the Table 4.2-7. Error bars denote the total analytical uncertainty (± 2SD, %), which incorporates both accuracy and reproducibility of n=3 measurements

Table 4.2-7: Metalaxyl enantiomeric enrichment factors (ε) and associated AKIE retrieved from the crop and vineyard soil experiments. The error of the isotope enrichment factor was given as the 95% confidence interval (CI) and determined using regression analysis as described elsewhere [42]. The uncertainty of the AKIE was estimated by error propagation as described elsewhere [44]. For AKIE calculations, n=15 carbon atomes, x=1 indistinguishable reactive position, and z=2 positions in intramolecular competition for ring-methyl hydroxylation

	Crop soil				*Vineyard soil*			
	Biotic		**Abiotic**		**Biotic**		**Abiotic**	
	ε ± 95% CI [‰]	**AKIE ± 95% CI**	**ε ± 95% CI [‰]**	**AKIE ± 95% CI**	**ε ± 95% CI [‰]**	**AKIE ± 95% CI**	**ε ± 95% CI [‰]**	**AKIE ± 95% CI**
S-MTY	-2.7±1.5	1.020±0.011	n.a*	n.a*	-1.3±0.7	1.010±0.005	n.a*	n.a*
R-MTY	n.a*	n.a*	n.a*	n.a*	n.a**	n.a**	n.a*	n.a*
Bulk-MTY	-2.0±1.3	1.015±0.010	n.a*	n.a*	-0.9±0.5	1.007±0.004	n.a*	n.a*

*Rayleigh slope not significant ($p>0.05$ *and* $r^2<0.8$)
** $n \leq 2$

4.5. Metalaxyl degradation pathways in the soils

In addition to CSIA, MTY degradation product analysis (Figure 4.2-2) revealed the systematic occurrence of hydroxy-Metalaxyl (hydroxy-MTY) in biotic experiments after 50 days of incubation (Figure 4.2-8). This is consistent with both the biologically mediated hydroxylation of MTY predicted by AKIE calculations, and the large detection of benzyl alcool derivatives in cell suspensions of lettuce and potatoes following treatment with rac-MTY [52]. In addition, the expression of the hydroxylation pathway may be at the origin of the observed pH dependance on the stereoselective degradation of MTY [7]. Indeed, a reverse enantioselectivity in MTY degradation was previously observed at pH<4. While hydroxylation is likely enhanced at neutral/basic pH, this suggests that another degradation mechanism with different enantioselectivity occured in acidic conditions.

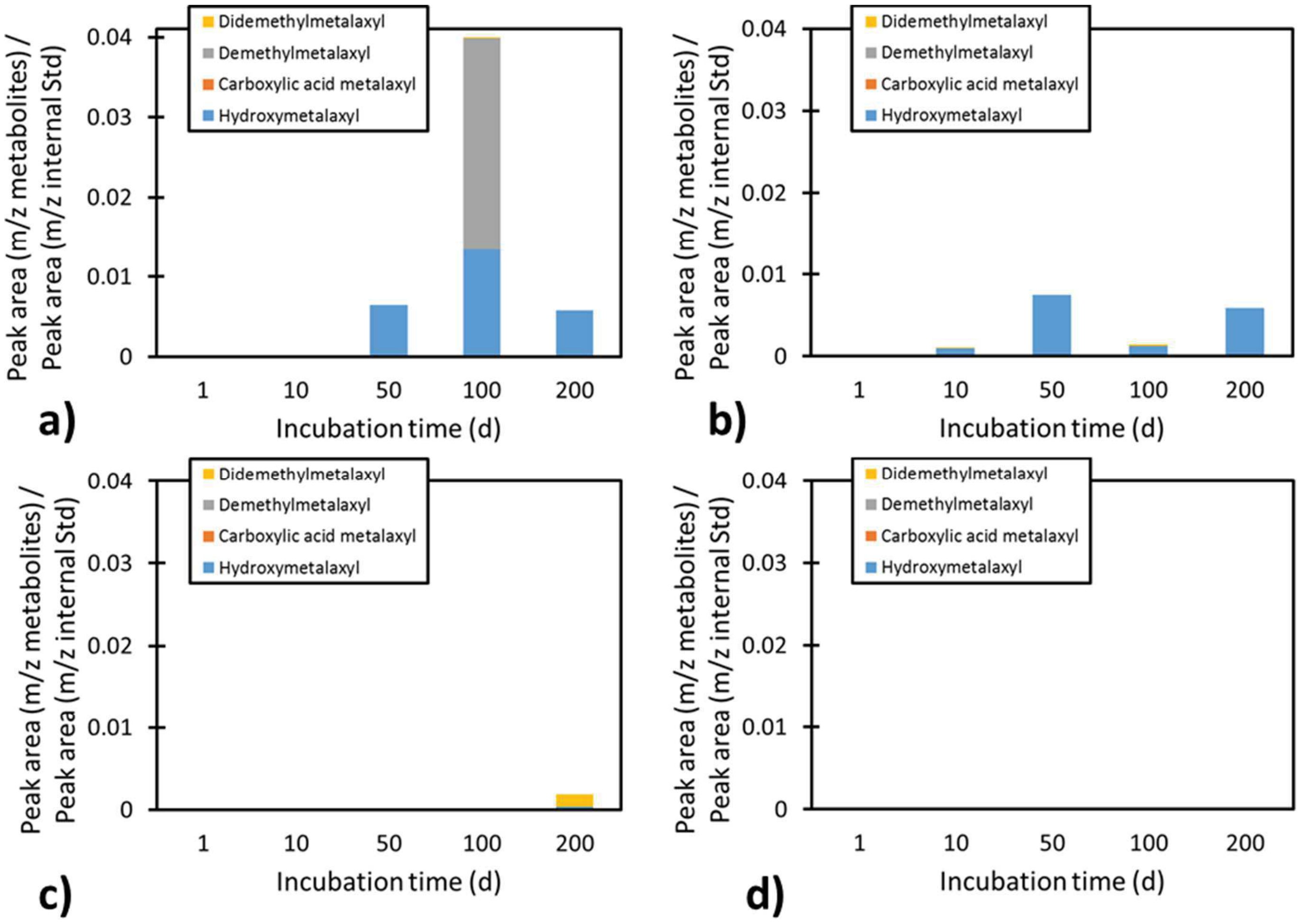

Figure 4.2-8: Normalized metabolites peak areas as a function of incubation time for a) crop and b) vineyard soil experiments. Associated abiotic controls are displayed in c) and d)

The lack of commercially available standards for the two hydroxy-MTY metabolites displaying similar m/z values did not enable to differenciate phenyl ring (meta position) from ring-methyl hydroxylation. While AKIE calculation and transformation product detection were consistent with hydroxylation in both soils (Table 4.2-7 and previous section), closer inspection of AKIE values may help to circumvent this bottleneck. Recently, microbial hydroxylation of phenol, cresols and ethylbenzene have shown distinct $AKIE_C$=1.003-1.010 for ring hydroxylation and $AKIE_C$=1.014-1.033 for side chain hydroxylation [54, 55]. With AKIE=1.007-1.021, our data overlapp the two value ranges, which emphasise that both reactions possibly occurred. Concomittant ring and side chain hydroxylation of MTY have been previously suggested by ^{1}H-NMR analysis of MTY in pure culture with the fungus *Syncephalastrum racemosum* [56]. Here, $AKIE_{S\text{-}MTY}=1.021\pm0.004$ in the crop soil was consistant with ring-methyl hydroxylation, whereas $AKIE_{S\text{-}MTY}=1.010\pm0.002$ in the vineyard soil suggests the prevalence of phenyl-ring hydroxylation.

The detection of hydroxy-MTY in association with S-MTY enantiomer and the absence of the R enantiomer indicates that the hydroxylation reaction was enantioselective. Steric constrains likely influence the enzyme discrimination of the hydrogen atom abstracted from the substrate, which cause kinetic degradation rates between enantiomers. Similar constrains have been suggested to explain ethylbenzene enantioselectivity in a theoritical study including modelling [57]. Interestingly, hydroxy-MTY was associated with larger production of demethyl-Metalaxyl at t=100 days (≈66% of total MTY metabolites, Figure 4.2-8) in the crop soil in the absence of significant enantiomer shift (EF=0.54±0.02, 2σ, Table 4.2-5). The simultaneous occurrence of demethylated and hydroxylated products in this sample coincided with the observed EF "outlier" value (Figure 4.2-6c), and suggests another degradation pathways for MTY. The Rayleigh plot (Figure 4.2-7c) shows a possible higher carbon isotope fractionation at t=100 days, yielding an estimated $\varepsilon\approx-4.3‰$ (AKIE≈1.033, n=2) consitent with $AKIE_C$=1.03-1.07 observed in SN_2 C-O cleavage (esther/ether demethylation) [30]. Finally, this singular sample points out the limits of single enantioselective approach. In fact, EF≈0.5 observed for this sample is not consistent with biodegradation revealed by simultaneous drastic MTY concentration decrease, metabolites detection and carbon isotopic fractionation parameters.

Noteworthy is the absence of detection of transformation products in the abiotic controls during the experiments. The change in MTY concentrations for both enantiomers observed at t=200 days (Figures 4.2-6a, 4.2-6b) likely reflected enhanced sorption and aging process rather than degradation. The absence of significant isotopic fractionation and the insignificant ε observed in the abiotic controls (Table 4.2-7) confirm that sorptive processes prevailed after 200 days of incubation [58]. In addition, our results thus support the idea that sorption is not enantioselective as suggested previously [49]. In the later study, sorption was assumed to indirectly control MTY enantioselectivity during leaching, as it determined MTY residence time within the soil column, and thus the extent and enantioselectivity of its degradation.

While the detection of degradation products have proven relevance, additional insights onto mechanisms of bond cleavage could be obtained using multi-element CSIA of nitrogen [27]. Unfortunately, dual isotope fractionation analysis to couple carbon with of nitrogen isotope analysis in ESIA approaches to potentially discriminate more finely hydroxylation mechanisms, could not be attemped here due to the small contribution of nitrogen to the molar mass of a MTY molecule (≈5%) and the amount required for analysis (30 ng of nitrogen on column). The environmentally-relevant initial concentration of the studied soils (5 $\mu g.g^{-1}$) was ten times lower than that necessary for subsequent nitrogen CSIA.

5. Conclusion

In this study, ESIA emergent analytical method was successfully developed and applied for Metalaxyl chiral fungicide in soils. Absence of carbon isotope fractionation from soil extraction to GC-IRMS measurements raise the potential of MTY-ESIA for tracking soil degradation enantioselective processes. With lower sensitivity by a factor of two (2 enantiomers) and minimum of 5 $ngC_{R\text{-}MTY}$ and 30 $ngC_{S\text{-}MTY}$ injected, further developments in sample preparation (e.g purification by HPLC) should be considered for enhancing environmental applications. Incubation experiments confirmed that under aerobic conditions and circumneutral pH values, the MTY degradation in soil is enantioselective. Here we go further indicating the enantiospecific ε factors, which combined with MTY-ESIA in field soil samples, could help approaching *in-situ* enantiospecific biodegradation extent since the chiral molecule application. Most importantly, we demonstrated that hydroxylation of MTY at various positions was a major biodegradation pathways of MTY degradation. The fate of the chiral-hydroxylated products in the environment and their potential toxicity on non-target soil and water organisms still have to be investigated.

In the future, it is envisioned that ESIA will be available for measuring nitrogen ($^{15}N/^{14}N$) and hydrogen ($^{2}H/^{1}H$) isotope signatures of MTY. Hence, it should be possible to apply the multi-element ESIA for a better characterization of transformation pathways, e.g., between diverse hydroxylation reactions. This novel approach offers perspectives for assessing the environmental fate of various chiral anilide pesticides.

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Chapitre 5 : Impact des patrons de pluie et de leur succession sur l'export des pesticides et métaux lourds des sols agricoles

Le chapitre 5 présente la dernière étude au laboratoire de ce travail de thèse. L'impact des patrons de pluie et de leur succession sur l'export par ruissellement et infiltration de deux pesticides de synthèse (Métalaxyl et S-métolachlore) et deux métaux (Cu et Zn) de sols agricoles est évalué grâce à des colonnes de sols recevant une pluie « synthétique ». Cette étude souligne comme les facteurs extrinsèques (caractéristiques des pluies) impactent fortement l'export des polluants comparés aux interactions entre les sols et les polluants à court terme.

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1. Abstract and highlights

The combined influence of soil characteristics, pollutant aging and rainfall patterns (i.e. intensity-duration-volume) on the export of pollutants from soils remains poorly understood. We used laboratory experiments and parsimonious modeling to examine the impact of rainfall patterns and successive rainfalls on the ponding and leaching of a pollutant mixture in crop and vineyard soils and with two pollutant aging stages (0 and 10 days). The pollutant mixture included the fungicide metalaxyl, the herbicide *S*-metolachlor, and inorganic copper (Cu) and zinc (Zn). Four rainfall patterns, which differed in their durations and intensities, were applied twice successively with a 7 days interval on each soil type. The global export of pollutants was significantly controlled by the rainfall duration and frequency ($p<0.01$). During the first rainfall event, the longest and most intense rainfall pattern yielded the largest export of metalaxyl (44.5 ± 21.5 % of the initial mass spiked in the soils), *S*-metolachlor (8.1 ± 3.1 %) and Cu (3.1 ± 0.3 %). Soil compaction observed after the first rainfall reduced in the second rainfall the leaching of remaining metalaxyl, *S*-metolachlor, Cu and Zn by 2.4-, 2.9-, 30- and 50-fold, respectively. In contrast, soil characteristics and aging did not predominantly influence pollutant export (i.e. pollutant mass exported via leaching or in ponding water out of initial pollutant mass in soils).

However, the leaching of Zn and Cu was significantly influenced by the soil type and aging, respectively. This underscores that extrinsic factors, such as rainfall characteristics, may prevail over soil-pollutants interactions to control pollutant export patterns when aging period is short. Overall, we anticipate our study to be a starting point for more systematic evaluation of the dissolved pollutant ponding/leaching partitioning and the export of pollutant mixtures from different soil types in relation to rainfall patterns.

Highlights:

- Combining soil experiments and modeling to examine rainfall impact on pollutant export
- Cu, Zn, metalaxyl and *S*-metolachlor leaching driven by rainfall duration and frequency
- Soil compaction reduced pollutant leaching after one rainfall and increased ponding
- Soil characteristics and aging had less influence on pollutant leaching and ponding
- Towards systematic evaluation of pollutant export in relation to rainfall patterns

2. Introduction

Pesticides are widely used to control pests while sludge from wastewater treatment plants (WWTP) containing heavy metals is often spread as fertilizers (McLaren et al., 2004). Pesticides and heavy-metals in sludge may thus accumulate in agricultural soils and, following rainfall-runoff events, eventually reach surface water and groundwater. Predicting the export of pollutants from soils into aquatic ecosystems is thus essential to limit transport risks and evaluate toxicological exposure (Arias-Estévez et al., 2008; Schäfer et al., 2012). Pesticides and metals are exported from the soil to surface water by runoff, i.e., ponding and overland flow (Lefrancq et al., 2014; Schwarzenbach and Westall, 1981; Shi et al., 2011). Ponding occurs when the rainfall intensity overcomes the top soil infiltration capacity. Depending of slope and upstream-downstream hydrological connectivity, ponded water will flow downstream generating runoff. Pollutants are also exported into sub-surface water by leaching when rainwater carries dissolved pollutants through the soil profile. However, the impact of rainfall characteristics on the export pathways of organic and inorganic pollutant mixtures from the soil remains largely unexplored.

Export of pollutants by ponding and runoff or leaching is controlled by intrinsic factors, such as the physico-chemical properties of the pollutant (Gevao et al., 2000) as well as the soil hydrodynamics and characteristics (Buekers, 2007; Green and Ampt, 1911). In particular, the availability and mobility of pollutants in soils tend to decrease over time due to pollutant diffusion and sorption into mineral and organic fractions, a process termed "aging" (Komárek et al., 2010; Ma et al., 2006a). Aging can control the fraction of pollutant mobilized and transported from the soil either in the freely-dissolved phase or associated with soil particles and colloids (Gevao et al., 2000; Huang et al., 2015; Jalali and Khanlari, 2008; Sauvé et al., 2000; Tang et al., 2006). Together with aging, pollutant transformation, including speciation of metals and degradation of organic pollutants, can influence the extent of pollutant export from soils. Pollutant aging and transformation are themselves controlled by soil-extrinsic factors, such as the time between an application and a rainfall event (Huang et al., 2015; Nolan et al., 2008). Whereas pollutant aging in soils may decrease pollutant export, rainfall frequency may increase it (Goldreich et al., 2011; Huang et al., 2015). In addition, rainfall intensity and duration may primarily affect pollutant mobilization and export from the soil. Larger rainfall intensity have been shown to increase leaching of isoproturon from soil columns (Beulke et al., 2002), while successive rainfall events doubled leaching of metolachlor from the soil at the second event (Goldreich et al., 2011). In agricultural catchments prone to Hortonian runoff, ponding/leaching water partitioning is mainly controlled by top-soil (0-5 cm) hydraulic properties, i.e. bulk density and saturated hydraulic conductivity (Armand, 2009; Chahinian et al., 2006). This latter can decrease from 2 orders of magnitudes between crop sowing and harvest due to rainfall event frequencies modifying the ponding/leaching threshold (Armand, 2009; Lefrancq et al., 2017). Landscape patchwork of top soil hydraulic properties also controls runoff connectivity (Harel and Mouche, 2014) with associated pollutants both in dissolved and eroded phases. For pre-emergent herbicides such as the *S*-metolachlor, this top-soil layer (0-5 cm) plays a critical role to reduce or enhance water and pesticides fluxes the first weeks before development of a significant root compartment. Understanding and predicting the temporal evolution of top-soil hydraulic compartment along rainfalls frequency is then of the utmost importance to better estimate water and pollutants fluxes reaching the deeper rhizosphere compartment and below, the groundwater table. If impacts of the deeper rhizosphere on pollutants leaching were identified (Crowley et al., 2001; Diez et al., 2015), lab-scale evidences on the boundary condition played by top-soil compartment on deeper water and solute leaching are still lacking. Besides, the contribution of intrinsic and extrinsic factors to the export of both organic and inorganic pollutants from soils has been, to date, rarely quantified (Beulke et al., 2002; Nolan et al., 2008; Sauvé et al., 2000). In this context, laboratory experiments can help to constrain and hierarchize factors controlling pollutant export to drive modeling approaches potentially used in the field (Banzhaf and Hebig, 2016).

The purpose of this study was to evaluate how rainfall patterns (i.e., intensity-duration-volume) influence the export of synthetic pesticides and heavy metals from soils in relation to i) soil characteristics, ii) rainfall frequency, and iii) short-term pollutant aging (ten days). We used laboratory experiments with soils from experimental vineyard (Duplay et al., 2014) or crop catchments (Lefrancq et al., 2017), which cover relevant agricultural land uses receiving pesticides and heavy metals. Widely used anilide pesticides, i.e., the fungicide metalaxyl and the herbicide *S*-metolachlor, as well as Cu and Zn were used as model pollutants in a mixture. To evaluate sensitive parameters controlling pollutant ponding and leaching, a parsimonious physically-based model was developed to derive key parameters (i.e, the saturated hydraulic conductivity Ksat and the organic carbon partition coefficient Koc) that regulate pollutant export.

3. Material and methods

3.1. Chemical and artificial rainwater

Metalaxyl ($C_{15}H_{21}NO_4$, methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate) and *S*-metolachlor ($C_{15}H_{22}ClNO_2$, (S)-2-Chloro-N-(2-ethyl-6-methyl-phenyl)-N-(1-methoxypropan-2-yl)acetamide) were purchased from Sigma-Aldrich (St. Louis, MO, USA), with purity of 99.8 and 98.2 % respectively. Copper chloride ($CuCl_2$), zinc chloride ($ZnCl_2$) and salts used for the preparation of the artificial rainwater were purchased from Sigma-Aldrich (St. Louis, MO, USA) with purity ≥ 97 %. The water solubility, octanol-water partition coefficient Kow (log) and organic carbon partition coefficient Koc are 7100 mg.L^{-1}, 1.7 and 163 L.mg^{-1} for metalaxyl, and 480 mg.L^{-1}, 3.0 and 185 L.mg^{-1} for *S*-metolachlor, respectively (Kegley et al., 2016; PPDB Pesticide Properties DataBase, 2009). The soil/water partition coefficient (log K_d) of Cu and Zn used in this study were 2.7 and 3.1 L.mg^{-1}, respectively (Allison and Allison, 2005).

The artificial rainwater was prepared according to the ERM-CA408 reference material (ERM certification report, 2010) with ultrapure water (10 L) and NH_4Cl (19.6 mg),

$Ca_2NO_3 \cdot 4H_2O$ (32 mg), $Mg_2NO_3 \cdot 6H_2O$ (28 mg), K_2HPO_4 (9 mg), $NaNO_3$ (4 mg), NaF (4.5 mg), Na_2SO_4 (22.2 mg) and $NH_4 \cdot H_2PO_4$ (11.2 mg). The targeted pH was 6.3 ± 0.6.

3.2. Soil collection

The collection of soils was made according to the Chapter 2, part 2. Particle size distribution, saturated hydraulic conductivity and bulk density as well as pressure-soil water content curve were measured by laser granulometry using a Beckmann Coulter LS230, head constant permeameter and direct measurement of soil cylinder, respectively (Table 5-1). The detailed physico-chemical characteristics of the soils are provided in Table 2-1. The vineyard soil contains more clays (68.5 %) and carbonates (27.1 %), while the crop soil was characterized by more organic matter (5.52 %), sand (10.3 %) and silt (61.5 %). The crop soil is neutral (pH 7.0) while the vineyard soil is more alkaline (pH 8.1).

Table 5-1: Methods and standards for soil analyses

Variables	Methods in brief	Norms and/or procedure
Soil water content	Drying sample in an oven set at 105°C	NF ISO 11465
pH	Electrometry in a soil–solvent suspension (1:2.5)	NF EN 12176
Organic matter	Mass loss by ignition at 375°C during 16 h	NF EN 12875
$CaCO_3$	CO_2 gas with hydrochloric acid	NF ISO 10693
CEC	Cobalt hexamine method	NF X 31-130
Granulometry	Laser granulometer	See Ertlen et al. (2010)
Saturated hydraulic conductivity	Head constant permeameter	See Amoozegar (1989)
Bulk density	Direct measurement of soil cylinder	See Madsen et al. (1986)
Pressure soil-water content curve	Direct measurement of soil cylinder	See Madsen et al. (1986)
Elementary composition	ICP-AES* after lithium tetraborate fusion	See Chabaux et al. (2013)

* Inductively Coupled Plasma Atomic Emission Spectroscopy

3.3. Rainfall patterns

To retrieve the four rainfall patterns, the Alsatian foothills region (France) was selected as a major cultural areas in temperate climate where orographic effect intensifies extreme rainfalls (i.e., the 99th percentile of precipitation during wet days or maximum amount of precipitation in five consecutive days) (Dankers and Hiederer, 2008). The selected rainfall station (47°57′9 N, 07°17′3 E, Rouffach, Haut-Rhin, France) is located in the experimental vineyard catchment from which the vineyard soil was collected. The catchment is characterized by intense events generating both pesticides and Cu runoff (Lefrancq et al., 2013; Babcsányi et al., 2016). A Gumbel law (Gumbel, 1958; Papalexiou and Koutsoyiannis, 2013) was applied on the 6 min MeteoFrance database (https://donneespubliques.meteofrance.fr/) from 1998 to 2016 to derive mean intensities for rainfall duration of 6, 12, and 30 minutes and return period of 2, 20 and 50 years. The four selected rainfall patterns differ in their intensities (from 30 to 135 mm.h^{-1}), durations (from 6 to 30 min), volume (from 23 to 58 mL) and return period (from 2 to 50 years) (Table 5-3). The rationale behind this selection is to (i) cover the range of rainfall patterns observed on the regional scale, (ii) represent a wide panel of possible patterns in temperate climate, and (iii) generate a sufficient water volume for further pollutant quantification.

Table 5-3: Characteristics of the rainfall patterns

	Return periods [years][a]	**Duration [min]**[a]	**Intensity [mm.h^{-1}]**[b]	**Rate [mL.mn^{-1}]**[c]	**Volume [mL]**[c]	**Description**
Pattern 1	2	30	30	1.1	31.5	Long-lasting pattern with low intensity
Pattern 2	2	12	55	1.9	23.1	Mid-lasting pattern with intermediate intensity
Pattern 3	20	30	55	1.9	57.8	Long-lasting pattern with intermediate intensity
Pattern 4	50	6	135	4.7	28.4	Short pattern with high intensity

[a] Selected return periods and rainfall durations
[b] Calculated intensities with Gumel law associated to return periods and durations
[c] Pump rate and associated rainfall volume using a soil column area of 7.07 cm²

3.4. Experimental set-up and operations

Soil columns consisted of filtration units (Steriflip, MerckMillipore, Billerica, MA, USA) and 50 mL polyethylene centrifugation tubes (Figure 5-1). The conical part of the centrifugation tubes were cut and tubes were screwed above the filtration units. Centrifugation tube caps (4 mm Ø digs) were drilled to insert a pipe connected to a multi-channel peristaltic pump (ICP-8, Ismatec, Wertheim, Germany). The pump delivered artificial rainwater on soil samples areas (7.07 cm²) at rates corresponding to the four selected rainfall patterns, i.e. from 1.1 to 4.7 mL.min^{-1} (Table 5-3).

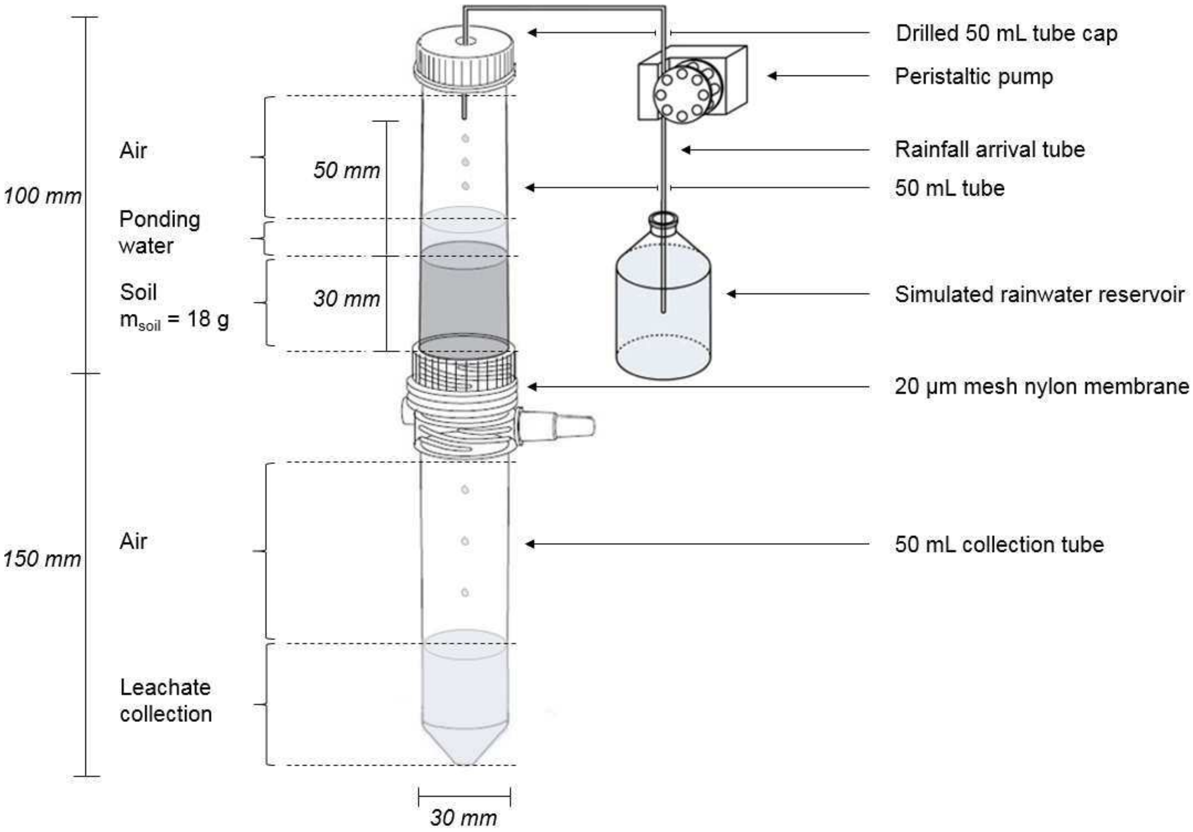

Figure 5-1: Experimental set-up

Soil samples were homogeneously spiked at 50 µg.g^{-1} with an aqueous mixture of metalaxyl, *S*-metolachlor, $CuCl_2$ and $ZnCl_2$ to reach a soil water content of 20 % (weight/weight). Control soils consisted of non-spiked soil with water content of 20 % (w/w). The upper part of each column was filled with 18 g of control or spiked soils to reach 3 cm height. The soil was retained in the column with a 20 µm mesh nylon membrane (Figure 5-1). Half of the soil columns were exposed to rainfall immediately after spiking. To evaluate the effect of pollutants aging in the soils on the extent of pollutant export, the other half of the columns was incubated in the dark at 20 °C for 10 days before exposition to rainfall.

The four rainfall patterns were applied separately, yielding a total of 16 experiments, i.e. 4 rainfall patterns, applied on 2 soils (spiked vineyard and crop soils), with 2 aging time (0 and 10 days), each run in triplicate, requiring a total of 48 columns. In parallel, rainfall patterns generating the less and the most water volumes (i.e., patterns 1 and 4, respectively; Table 5-3) were applied on vineyard and crop control soils without pollutant spiking to evaluate the background export of pollutants. To evaluate the influence of rainfall frequency on pollutant export, each experiment received two successive and identical rainfalls separated by a seven days interval.

At the end of each rainfall, leached and ponded waters (the latter collected by a syringe immediately after the rainfall event) obtained from each triplicate experiments were collected and pooled to obtain a homogeneous sample for each conditions and sufficient amount of pesticides and metals for further quantification. The soil from each triplicate systems were collected and pooled at the end of the second rainfall. The water and soil samples were immediately frozen at - 20 °C until further analyses. Soil samples were analyzed to quantify the remaining mass of pollutants after the second rainfall.

3.5. Elemental analyses

Dissolved organic carbon (DOC) in water samples was quantified using a carbon analyzer (Shimadzu TOC – VCPH). Acetate, major anions and cations (chloride, nitrate, sulfate, ammonium, sodium, potassium, magnesium and calcium ions) were quantified using ion chromatography (ICS 3000 Dionex, San Diego, CA, USA, ± 2%). The analysis of the chemical composition of soils including Cu and Zn is explained in Chapter 2, part 3.2.

3.6. Metalaxyl and *S*-metolachlor analysis

The volumes of leaching and ponding water were adjusted up to 25 mL with ultra-pure water prior to solid phase extraction (SPE). The extraction of Metalaxyl and *S*-metolachlor from water and soil samples is explained in Chapter 2, part 3.1.

3.7. Data analysis

During the first rainfall, the relative loads (*RL1*) of pollutants were estimated according to Eq. 5-1:

$$RL1_{(x,\ j,\ p,\ s,\ a)} = \frac{m_{x,\ j,\ p,\ s,\ a,\ r1}}{m_{init(x,\ s,\ a)}} \times 100 \qquad (5\text{-}1)$$

where $m_{x,\ j,\ p,\ s,\ a,\ r1}$ is the mass of pollutant x {metalaxyl, *S*-metolachlor, Cu or Zn} [M] exported via process j {ponding or leaching}, with a rainfall pattern p {1, 2,3 or 4, Table 5-3}, for the soil s {crop or vineyard}, with aging process a {no aging or 10 days aging} after the first rainfall r1 and m_{init} (x, s, a) the initial pollutant mass in soil for corresponding pollutant (x), soil (s) and aging process (a) [M]. For the experiments with 10 days of pollutant aging, m_{init}(x, s) was measured for metalaxyl and *S*-metolachlor before the first rainfall.

The relative loads during the second rainfall (*RL2*) expressed as a percentage [-] were calculated by mass balance adjustments and a first-order rate model (SFO) to account for pesticide degradation that occurred between the two successive rainfalls. Hence Eq. 5-1 is updated as follows to account for export losses during the first pulse and sinks associated to degradation in between the two pulses (Eq. 5-2):

$$RL2_{(x,\ j,p,s,a)} = \frac{m_{x,\ j,\ p,\ s,\ a,\ r2}}{\left(m_{init(x,\ s,\ a)} - \sum_{j=ponding}^{j=leaching} m_{x,\ j,\ p,\ s,\ a,\ r1}\right) \cdot e^{-k_x t}} \times 100 \qquad (5\text{-}2)$$

where, similar to Eq. 5-1 $m_{x,\ j,\ p,\ s,\ a,\ r2}$ is the mass [M] exported during the second rainfall r2. The dissipation rate constant k_x [T^{-1}] for pesticides x {metalaxyl or *S*-metolachlor} was calculated by $k_x = \ln(2)/DT_{50x}$ ($k_x = 0$ for metals), where DT_{50} [T] is the pesticide half-life obtained from the Pesticides Properties DataBase (2009).

To evaluate differences in exported pollutants across rainfall patterns (i.e. intensity- duration-volume), rainfall frequency, soils or aging time, nonparametric Kruskal-Wallis and Wilcoxon tests were applied at a significance level of 0.05 using the R program (Copyright 2005, The R Foundation for Statistical Computing, Version 2.15.1).

To visualize dissimilarities in patterns of pollutant export, two-dimensional nonmetric multidimensional scaling (NMDS) (Smith and Mather, 2012) based on Bray-Curtis dissimilarities of Hellinger-transformed data (square-root transformation of relative masses of exported pollutants) was used. The relationship between the pollutant export patterns and the experimental variables (i.e. soil type, aging, rainfall frequency, intensity, volume and duration) was investigated by fitting vectors a posteriori onto the NMDS. The significance of the vector fitting was assessed using a Monte-Carlo permutation test (1000 permutation steps).

3.8. Modeling of pollutant ponding and leaching

Partitioning between infiltration and ponding was determined using the Green & Ampt formalism (Green and Ampt, 1911; Mein and Larson, 1973), which simulates overland flow when rainfall intensity overcomes the soil infiltration capacity and given by Eq. 5-3:

$$f = \mathrm{K}_{\mathrm{sat}}\left(\frac{\omega\Delta\theta}{F}+1\right) \tag{5-3}$$

where f is infiltration [L], Ksat is saturated hydraulic conductivity [L.T^{-1}], ω is suction at the wetting front [L], $\Delta\Theta$ is the moisture difference between initial and saturation conditions [-] and F is the cumulative infiltration [L]. Parameter values are presented in Tables 5-4 and 5-5.

Table 5-4: Calibrated $\mathrm{K}_{\mathrm{sat}}$ used to fit all rainfall modalities simultaneously with the model during the second pulse. $\mathrm{K}_{\mathrm{sat}}$ during the first rainfall was measured (Table 5-1)

Parameter	**Unit**	**Value**				**Rainfall Frequency**
		Crop soil		Vineyard soil		
$\mathrm{K}_{\mathrm{sat,\ all\ patterns}}$	[cm.h^{-1}]	13.45		17.62		1 (measured)
		t = 0 day	t = 10 days	t = 0 day	t = 10 days	
$\mathrm{K}_{\mathrm{sat,\ pattern\ 1}}$	[cm.h^{-1}]	0.054	0.013	0.352	0.025	2 (calibrated)
$\mathrm{K}_{\mathrm{sat,\ pattern\ 2}}$	[cm.h^{-1}]	0.538	0.179	0.035	0.235	
$\mathrm{K}_{\mathrm{sat,\ pattern\ 3}}$	[cm.h^{-1}]	0.013	0.179	0.176	0.352	
$\mathrm{K}_{\mathrm{sat,\ pattern\ 4}}$	[cm.h^{-1}]	0.179	0.077	0.018	0.117	

Table 5-5: Green & Ampt input parameters (excepted $\mathrm{K}_{\mathrm{sat}}$ detailed in Table 5-4)

Parameter	**Unit**	**Crop soil**	**Vineyard soil**	**Source**
ω, suction at the wetting front	[mm]	1100	1100	Matrix suction determined based on previous evaluation of soils at source sites (Lefrancq et al., 2017)
$\Theta_{t=0}$, initial moisture	[-]	0.2	0.2	Experimental measurement
Θ_{sat}, saturation moisture	[-]	0.62	0.61	Experimental measurement

The retention and release of pesticides in the top-soil was conceptualized with a mixing layer model (Joyce et al., 2010; McGrath et al., 2008) (Eq. 5-6) and a diffusion model across soil-ponded water interface (Havis et al., 1992) (Eq. 5-8). The approach assumes linear equilibrium sorption where the initial pore water concentration (C_l) is given by:

$$C_l(t_0) = \frac{C_s(t_0)}{K_d} \tag{5-4}$$

$$K_d = K_{oc} \cdot f_{oc} \tag{5-5}$$

where C_s is the total concentration at the beginning of the experiment (t_0), K_d is the partition coefficient [V.M^{-1}]. To fit pollutant export across rainfall patterns, K_{oc} values for metalaxyl, *S*-metolachlor, Cu and Zn were calibrated within the range of values of the Pesticides Properties DataBase (2009). Organic carbon fractions (f_{oc}) [-] and bulk densities (ρ_b) [M.V^{-1}] for soils were measured (Table 2-1).

The total losses [M] considered for a rainfall event include mass loss due to leaching (M_{lch}) and mass loss due to ponding (M_{pnd}). Masses due to leaching are computed by subtracting initial pore water mass from the remaining mass in pore water at the end of an event of time Δt:

$$M_{lch} = C_l(t_0)\left(1 - e^{\left(\frac{-L_z \Delta t}{RZ\theta_{sat}}\right)}\right) Z\theta_{sat} \tag{5-6}$$

where $C_l(t_0)$ is initial concentration [M.L^{-1}] in pore water, L_z is leached depth [L], Z is the soil depth [L], θ_{sat} the saturated moisture and R the retardation factor [-], which is given by:

$$R = 1 + \frac{\rho_b + K_d}{\theta_{sat}} \tag{5-7}$$

Masses in ponding water were retrieved by calculating the mass flux across the top soil boundary based on the concentration gradient between soil pore and ponded water concentrations, such that:

$$M_{pnd} = \int_{t_p}^{\Delta t} K_L \left(C_l(t) - C_p(t) \right) dt \cdot A \tag{5-8}$$

where t_p is the time of ponding [T], K_L [L.T^{-1}] is the mass transfer coefficient that relates solute flux from the soil surface to the ponding water, C_p is the concentration in ponded water depth [M.L^{-1}] and A the soil-water boundary area [L]. The ability of the model to reproduce pollutant export by ponding or leaching was evaluated by comparing the observed and simulated ponded or leached volumes while maximizing the coefficient of determination (r^2).

4. Results and discussion

4.1. Partitioning of water ponding and leaching

Contrasted water flow paths were observed for the two successive rainfalls. The first rainfall generated only leaching without ponding, independently of the rainfall patterns, which indicates that the rainfall intensities never overcame the soil infiltration capacity. This is in agreement with high values of saturated hydraulic conductivity (K_{sat}) e.g. 135 and 176 mm.h^{-1} measured for the crop and vineyard soils, respectively. High values of K_{sat} were a consequence of the soil sieving step and can be observed in the field just after tillage (Zeng et al., 2013).

The observed volumes of ponded and leached water were compared to those simulated with the model to evaluate the ability of the experimental set-up to mimic water ponding and leaching. The Green & Ampt model predictions parametrized with the measured (before the first rainfall) and calibrated K_{sat} values (before the second rainfall) (Tables 5-4 and 5-5) fitted well the observed leaching and ponded volumes (mean $r^2 = 0.98$) (Table 5-6). K_{sat} values before the second event were calibrated as they could not be measured without disturbing the soil in the columns. The calibrated K_{sat} values to fit the observed volumes for the second event were one to two orders of magnitude lower than those measured before the first rainfall event (Table 5-4). Such decrease of K_{sat} values was also observed in the field as a result of aggregate breakdown by rainfall and clogging of larger pores (Armand, 2009; Chahinian et al., 2006). Soil depth (i.e., 30 mm) also decreased by 4 to 11 mm after the first rainfall event, which further indicated changes of soil porosity, bulk density and hydraulic conductivity. Overall, different ponding/leaching water partitioning between the first and the second rainfall events underlined that the threshold behaviour of ponding was controlled by top soil K_{sat} values, as observed in field conditions (Lefrancq et al., 2017; Zehe et al., 2005). The good fitting of simulated ponding and leaching water volumes by Green & Ampt underscored that soil matrix properties (i.e. K_{sat}, bulk density, soil moisture) controlled the hydraulic properties of the columns without significant preferential flows along the boundary between the column surface and the soil. This validation step on column hydraulic functioning allowed inferring the impact of rainfall patterns on pollutant export from soil matrix by leaching and ponding.

Table 5-6: Observed and simulated ponding and leaching water volumes

			Crop				**Vineyard**			
			Observed		**Modelled**		**Observed**		**Modelled**	
			$V_{leached}$ [mL]	**V_{ponded} [mL]**	**$V_{leached}$ [mL]**	**V_{ponded} [mL]**	**$V_{leached}$ [mL]**	**V_{ponded} [mL]**	**$V_{leached}$ [mL]**	**V_{ponded} [mL]**
Rainfall 1	Freshly spiked systems	Pattern 1	17.51	/	17.86	/	19.08	/	18.91	/
		Pattern 2	5.90	/	9.66	/	11.11	/	11.60	/
		Pattern 3	40.40	/	44.45	/	46.40	/	46.39	/
		Pattern 4	12.96	/	14.18	/	13.61	/	14.18	/
	Aged (t = 10 d) systems	Pattern 1	20.09	/	17.86	/	20.87	/	18.91	/
		Pattern 2	10.60	/	9.66	/	11.29	/	11.60	/
		Pattern 3	47.20	/	44.45	/	47.03	/	46.39	/
		Pattern 4	14.29	/	14.18	/	17.71	/	14.18	/
Rainfall 2	Freshly spiked systems	Pattern 1	9.38	22.62	9.28	21.19	26.74	3.72	24.14	6.32
		Pattern 2	19.00	3.91	19.24	3.95	3.67	17.73	3.80	18.03
		Pattern 3	2.47	28.91	3.54	23.85	18.60	39.35	19.03	38.96
		Pattern 4	8.25	20.94	8.40	19.96	0.00	26.63	1.10	25.79
	Aged (t = 10 d) systems	Pattern 1	3.39	27.31	3.57	26.39	6.20	22.83	5.94	24.56
		Pattern 2	11.41	7.31	11.74	11.45	12.99	8.03	13.22	9.97
		Pattern 3	20.29	33.48	19.42	38.56	29.23	23.69	27.24	30.74
		Pattern 4	5.47	19.04	5.47	22.89	6.55	19.35	6.70	21.66

4.2. Impact of rainfall patterns on pollutant export

No pesticides and metals could be detected in leachates from the control soils (non-spiked soils), the metalaxyl, *S*-metolachlor, Cu and Zn concentrations in the crop and vineyard control soils are provided in Table 2-1. This confirmed that the experimental set-up enabled to evaluate the mass export of freshly spiked and short-term aged pollutants. While rainfall patterns did not significantly impact the ponding/leaching ratios (i.e. $RL2_{\text{ponding}}/RL2_{\text{leaching}}$) of pesticides ($p>0.1$), concentrations of heavy metals in ponding waters were too low to evaluate their ponding/leaching partitioning (<1 and <3 $\mu g.L^{-1}$ for Zn and Cu, respectively). Only up to 1.2 ± 1.2 % and 0.2 ± 0.1 % of metalaxyl and *S*-metolachlor, respectively, was exported in ponding water (i.e. $RL2_{\text{ponding}}$, crop, vineyard and aged systems confounded).

Several processes may control the transfer of dissolved pesticides into ponding water, including i) diffusion (induced by concentration gradients), ii) ejection of solution from the soil surface by raindrops, iii) desorption of pesticides sorbed onto soil, and iv) desorption of pesticides bound to soil particles eroded by raindrops (and surface flow) (Shi et al., 2011). In our case, the main processes driving pesticides transfer in ponding water were desorption of pesticides from soils and diffusion. Ejection of solute pesticides solution from the soil surface and bound pesticides desorption of soil particles eroded may not explain dissolved pesticide loads in ponding waters. Indeed, our experimental set-up, unlike in field conditions, did not allow for rain drops to approximate terminal velocities lowering the kinetic energy expected to drive solute entrainment (Assouline and Mualem, 1997; Gao et al., 2005). Therefore, the impact of simulated raindrops on the transfer of solute pesticides to ponding water was likely limited in our case. Instead, transfer to ponding was likely primarily driven by pesticide physico-chemical properties. Ponding/leaching ratios (i.e. $RL2_{\text{ponding}}/RL2_{\text{leaching}}$) for *S*-metolachlor, for example, were twice higher than those for metalaxyl (Figure 5-2), which can be related to the higher solubility of metalaxyl (7100 $mg.L^{-1}$) compared to metolachlor (480 $mg.L^{-1}$).

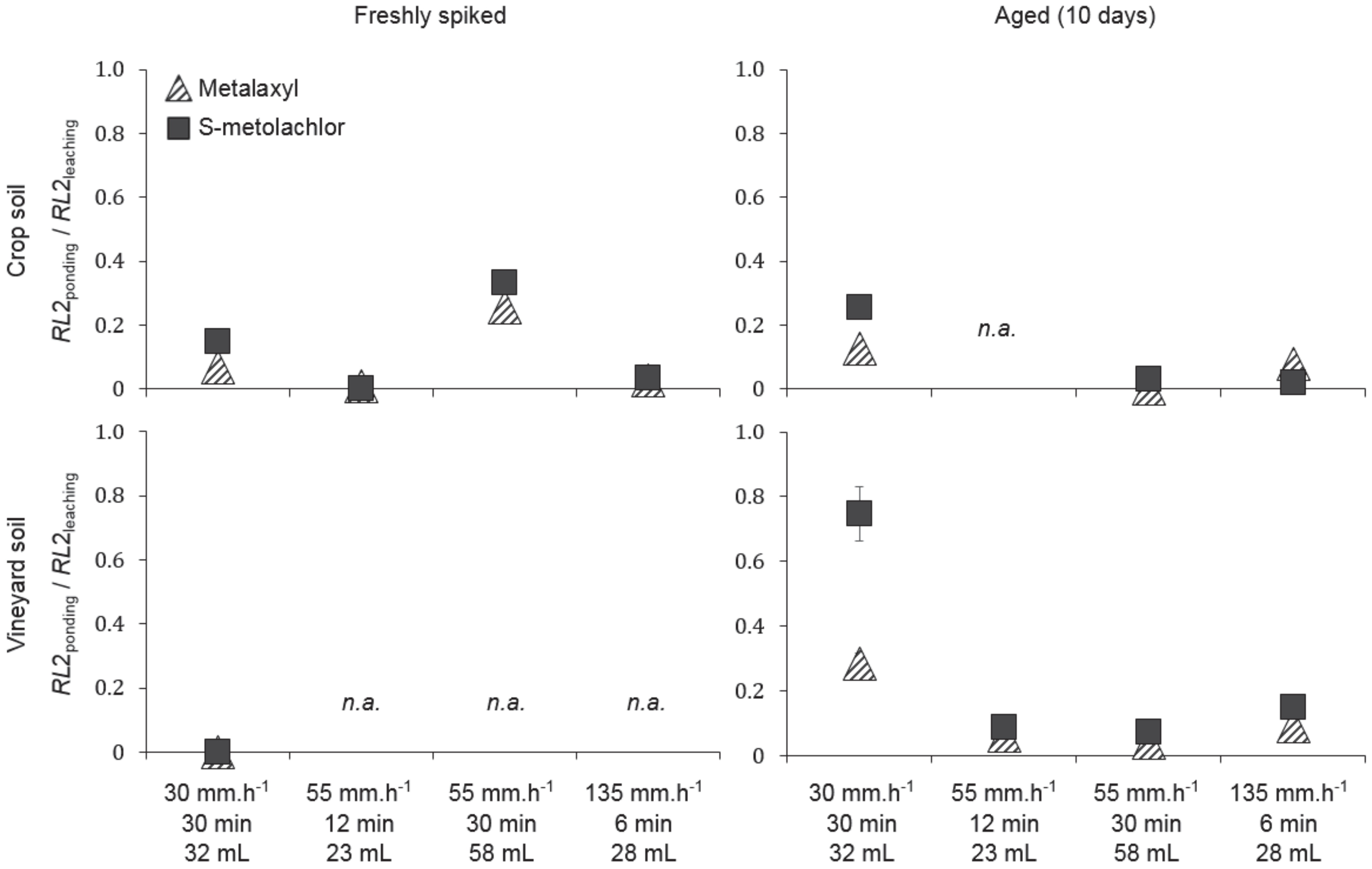

Figure 5-2: Ratios of ponding to leached metalaxyl and *S*-metolachlor exports (second rainfall cycle only, $RL2_{\text{ponding}}/RL2_{\text{leaching}}$). Error bars denote 95 % confidence intervals. “n.a.” denotes the absence of value when no leaching or ponding water was produced

In contrast, rainfall patterns significantly impacted leaching of metalaxyl ($p<0.01$), *S*-metolachlor ($p<0.05$) and Cu ($p<0.05$) during the first rainfall cycle. Changes in pollutant leaching profiles were visualized by the NMDS ordination of metalaxyl, *S*-metolachlor, Cu and Zn leached loads from the vineyard and the crop soils exposed to the four rainfall patterns in two successive events (Figure 5-3). The ordination showed that leaching profiles produced by the third rainfall pattern (long-lasting pattern with intermediate intensity; Table 5-3) during the first rainfall significantly differed from the other profiles ($p<0.001$) (Figure 5-3). The third rainfall pattern induced the largest leached loads for both pesticides and metals. Amounts of metalaxyl, *S*-metolachlor, Cu and Zn leached (i.e. $RL1_{\text{leaching}}$ and $RL2_{\text{leaching}}$) during the third rainfall pattern reached 44.5 ± 21.5%, 8.1 ± 3.1%, 3.1 ± 0.3% and 2.2 ± 0.2% of the spiked dose, respectively (all systems confounded) (Figures 5-4 and 5-5).

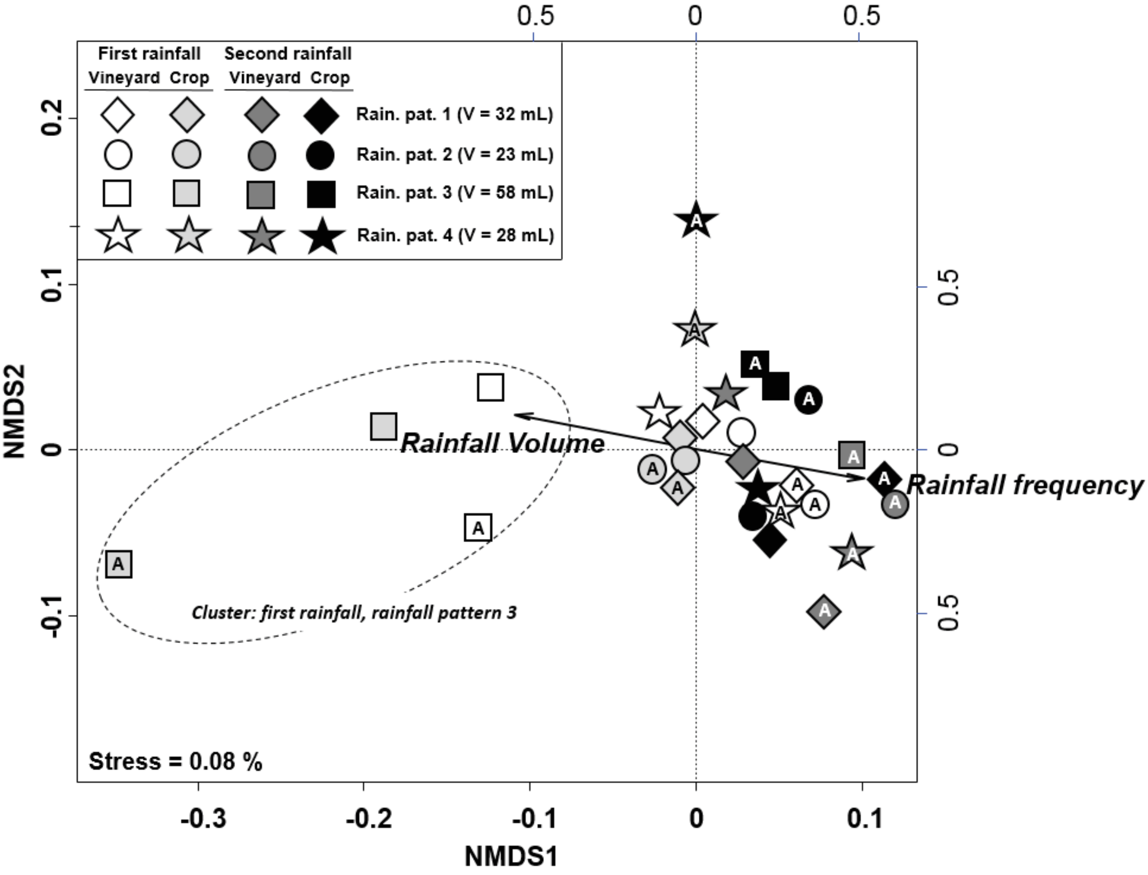

Figure 5-3: 2D-NMDS ordination of metalaxyl, *S*-metolachlor, Cu and Zn export ($RL1_{\text{leaching}}$ and $RL2_{\text{leaching}}$) profiles from the vineyard and the crop soils exposed to the four rainfall patterns, following pollutant aging (A) or not, and after the first and the second rainfall. Vectors that correspond to experimental variables (rainfall frequency, aging, rainfall intensity, volume and duration) and significantly correlated with pollutant export profiles are shown (i.e., volume and

cycles). The significance of fitted vectors was calculated by a posteriori permutation of variables at $p<0.001$. Vector arrows were fitted to the NMDS ordination depicting the direction and magnitude of change of the variable

To evaluate the relationship between the leaching profiles (i.e. $RL1_{\text{leaching}}$ and $RL2_{\text{leaching}}$), the rainfall patterns and the soil characteristics, the experimental variables (i.e., soil type, aging, rainfall frequency, intensity, volume and duration) were fitted onto the NMDS ordination of pollutant leaching profiles (Figure 5-3). The analysis revealed that pollutant leaching profiles mainly correlated with changes in rainfall volumes and rainfall frequency ($p<0.001$). This emphasizes the impact of rainfall volume on pollutant leaching from the soils. With the addition of water, the instantaneous sorption equilibria is shifted enabling higher leaching of pollutants to occur (Arias-Estévez et al., 2008). Overall, the leaching of metals and pesticides from the studied soils (Figures 5-4 and 5-5) were in agreement with previous observations. Cumulative leaching of metals from soils three years after their application were lower than 1% (McLaren et al., 2004), while up to 50 % of metolachlor was exported from 50 g soil columns (300 mm height x 59 mm inner diameter) initially spiked with atrazine and metolachlor applied drop wise at the soil surface (Kumar Ghosh and Singh, 2012).

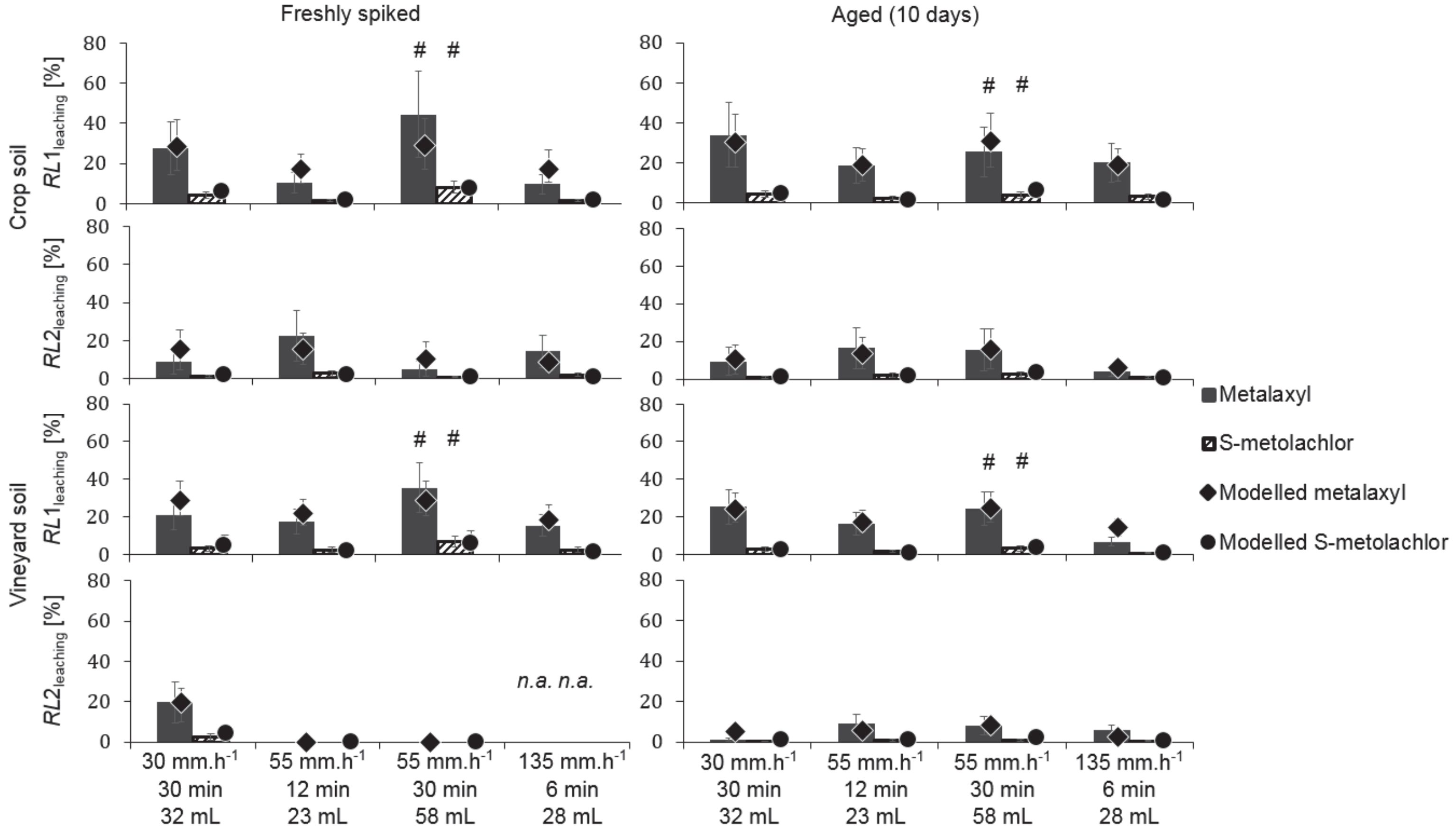

Figure 5-4: Experimental (bars) and modelled (points) percentages of metalaxyl and *S*-metolachlor leached ($RL1_{\text{leaching}}$ and $RL2_{\text{leaching}}$) from the freshly spiked and aged (10 days) crop and vineyard soils after 1 rainfall (cycle 1) and 2 rainfalls (cycle 2) with 7 days of interval. Error bars denote 95 % confidence intervals. “#” indicates the cluster “First rainfall – Rainfall pattern 3” of the NMDS ordination. “n.a.” denotes the absence of value when no leaching water was produced

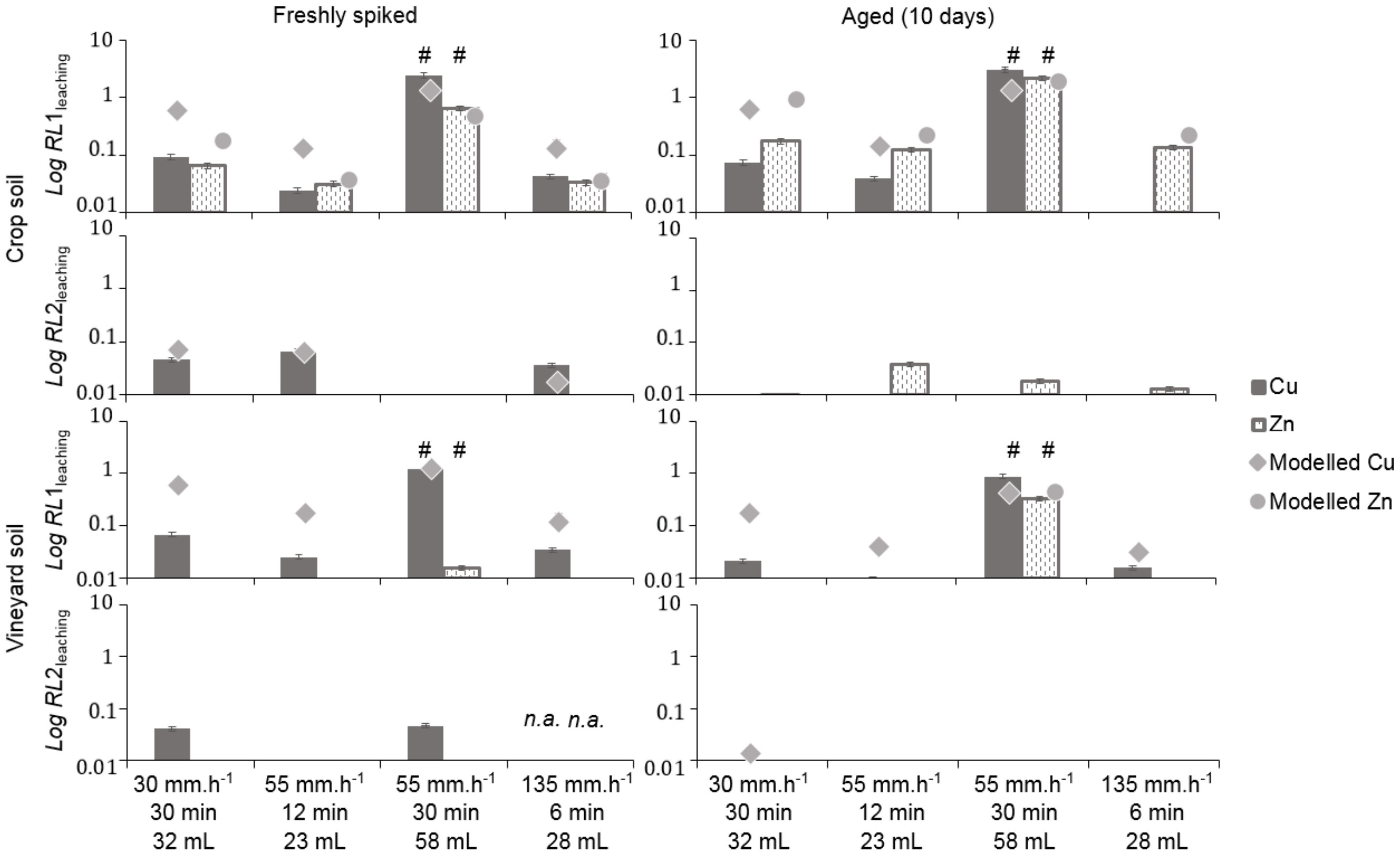

Figure 5-5: Experimental (bars) and modelled (points) logarithms of Cu and Zn leached (*RL1*$_{\text{leaching}}$ and *RL2*$_{\text{leaching}}$) from the freshly spiked and aged (10 days) crop and vineyard soils after 1 rainfall (cycle 1) and 2 rainfalls (cycle 2) with 7 days of interval. Error bars denote 95 % confidence intervals. ”#” indicates the cluster “First rainfall – Rainfall pattern 3” of the NMDS ordination. “n.a.” denotes the absence of value when no leaching water was produced

On the field, the impact of rainfall patterns on pollutant export from soil matrix also depends on the rainfall event frequency impacting soil compaction and crusting. Assessing the impact of rainfall frequency on pollutant export is thus needed to quantify the temporal evolution of export risk.

4.3. Impact of rainfall frequency on pollutant export

The same rainfall patterns were applied twice successively on the soil columns at seven days of interval to investigate the effect of successive rainfalls on pollutant leaching (i.e., $RL1_{\text{leaching}}$ and $RL2_{\text{leaching}}$ for all soils, freshly spiked and aged systems). The rainfall patterns had no significant impact during the second rainfall ($p>0.1$) when comparing the normalized leaching profiles (%) on the NMDS ordination (Figure 5-3). Leaching profiles generated by the second rainfall (i.e., $RL2_{\text{leaching}}$) event differed from those generated by the first rainfall event (i.e., $RL1_{\text{leaching}}$). In particular, rainfall frequency had the highest impact on metalaxyl and *S*-metolachlor leaching ($p<0.0005$) compared to Cu and Zn ($p<0.01$). Overall, the second rainfall leached 2.4-, 2.9-, 30- and 50-fold less metalaxyl, *S*-metolachlor, Cu and Zn, respectively, compared to the first rainfall (Figures 5-4 and 5-5). This supports the idea that distinct pools of pollutants within soil aggregates were mobilized from the soils after a seven days interval following the first rainfall event.

Indeed, the sieved soils used for the experiment had initially no crust, lower bulk density and higher K_{sat} values, which likely facilitated water percolation within the soils during the first rainfall (Vaezi et al., 2017), and thus enhanced pollutant mobilization. Soil aggregation is expected to decrease with successive rainfalls, which can release pesticides trapped on aggregates (Goldreich et al., 2011). In addition, wet-dry cycles have been observed to increased desorption of metolachlor from soils by up to a 2-fold factor (Goldreich et al., 2011). However, an opposite trend was observed in our experiment. Soil compaction occurred after the first rainfall, which likely caused dissociation of soil aggregates during the first rainfall event by slaking and wetting processes (Vaezi et al., 2017). Dissociation of soil aggregates likely then mobilized pollutants and enhanced pollutant leaching in a single rainfall. As a result, the occurrence of a pollutant pool available for leaching in a second rainfall, as previously reported (Goldreich et al., 2011), could not be observed in our case. In addition, soil compaction caused by the first rainfall clearly increased the ponding/leaching ratio in the second rainfall. This limited pollutants leaching during the second rainfalls and hid the impact of rainfall patterns on pollutant leaching during the second rainfall events. To some extent, our setup allowed exploring two extreme situations commonly observed in the field after 80 – 100 mm of rainfall over three weeks of an agricultural season. While the initial sieved soil mimicked the beginning of a crop season just after tillage (topsoil with low bulk density and high K_{sat}), soil compaction and crusting (especially for loamy soils) following the first rainfall was similar to that eventually observed in the field after a rainfall depth of 80 – 100 mm (Armand, 2009).

The model fitted well the observations of pollutant leaching without aging (average $r^2 = 0.80$ for the four pollutants, $RL1_{\mathrm{leaching}}$), although, for some experiments, it was necessary to use lower K_{oc} values than those retrieved from the literature (Table 5-7). This suggests that the time between pollutant spiking and the beginning of rainfall patterns (about 3 hours) was insufficient to reach complete sorption equilibrium, which is in agreement with previous observation (Gulkowska et al., 2016). The model showed limitations in predicting Cu and Zn loads in leaching waters for the first rainfall event and in ponding water for the second rainfall. This limitation may be due to the lack of representation in particle transport via erosion but also colloid-facilitated transport of metals. In addition, the low number of experiments generating Cu and Zn loads limited the ability to evaluate the adequacy of alternative models.

While extrinsic factors (i.e. rainfall patterns and frequencies) clearly controlled the risk of pollutant export from the soil matrix, intrinsic factors such as soil characteristic and aging process can also enhance or reduce pollutant mobilization.

Table 5-7: Calibrated K_{oc} used to fit pesticide and metal exports in the model

	Parameter	**Unit**	**Value**			
			Crop soil		Vineyard soil	
			t = 0 day	t = 10 days	t = 0 day	t = 10 days
Metalaxyl	$K_{oc,\ \mathrm{first\ rainfall}}$	[mL.g^{-1}]	15	14	25	30
	$K_{oc,\ \mathrm{second\ rainfall}}$	[mL.g^{-1}]	25	25	20	53
S-metolachlor	$K_{oc,\ \mathrm{first\ rainfall}}$	[mL.g^{-1}]	63	75	125	200
	$K_{oc,\ \mathrm{second\ rainfall}}$	[mL.g^{-1}]	125	125	98	196
Cu	$K_{oc,\ \mathrm{first\ rainfall}}$	[mL.g^{-1}]	248	249	466	933
	$K_{oc,\ \mathrm{second\ rainfall}}$	[mL.g^{-1}]	784	39	7841	1568
Zn	$K_{oc,\ \mathrm{first\ rainfall}}$	[mL.g^{-1}]	495	197	93041	933
	$K_{oc,\ \mathrm{second\ rainfall}}$	[mL.g^{-1}]	49	98951	49	99

4.4. Impact of soil characteristics and aging on pollutant export

Soil type and aging were not predominant factors for pollutant leaching compared to rainfall patterns and frequency (Tables 5-8 and 5-9). However, Zn and Cu leaching were also influenced by the soil type ($p<0.005$) and aging ($p<0.05$), respectively. Zn leaching ($RL1_{\text{leaching}}$ and $RL2_{\text{leaching}}$) from the vineyard soils were 9.4-fold smaller than that from the crop soils (Figures 5-4 and 5-5). In contrast, Cu, metalaxyl and *S*-metolachlor leaching ($RL1_{\text{leaching}}$ and $RL2_{\text{leaching}}$) from the vineyard and crop soils were similar. Larger Zn leaching from the crop soil may reflect both crop soil characteristics and different Zn speciation in the crop and the vineyard soils. Previous studies on the same vineyard soil showed that Zn was predominantly associated with the residual fraction (silicate matrix), secondly to carbonates, followed by organic matter and at last the oxide fraction, regardless of the soil type, depth or organic matter content (Duplay et al., 2014). Zn is known to diffuse into interlayers of silicates (Al-Qunaibit et al., 2005; Ma et al., 2006b; Ma and Uren, 1998) and also to remain fixed in the soil matrix after its diffusion into carbonates (Buekers, 2007; Ma et al., 2006). Clay and carbonate content of the crop soil were, respectively, 2 and 33 times lower compared to the vineyard soil (Table 2-1). An experiment carried out by Huang and teammates (2015) witnessed that even after one day of incubation, a fast process caused the distribution of freshly applied metals among the soil fractions (Huang et al., 2015). Also dealing with the leaching of pollutants (metals only: herein Cu, Zn and Cd) and their aging, Huang et al. (2015) got to that point after the monitoring of a specific metals soil fraction where metals are weakly electrostatic absorbed on the soil surface, can be released by ion-exchange processes and can be (co-)precipitated with soil carbonates. Therefore, we argue that Zn may be less retained in crop soil compared to the vineyard soil, thereby increasing Zn leaching during rainfall.

Table 5-8: Impacts of rainfall patterns, rainfall frequency, soil and 10-days aging on the ponding ($RL1_{\text{ponding}}$ and $RL2_{\text{ponding}}$), ponding/leaching ratios and leaching of metalaxyl, *S*-metolachlor, Cu and Zn ($RL1_{\text{leaching}}$ and $RL2_{\text{leaching}}$) as revealed by symbols indicating the obtained Kruskal-Wallis and Wilcoxon analyses p-values

	Ponding ($RL1_{\text{ponding}}$ & $RL2_{\text{ponding}}$)		**Leaching ($RL1_{\text{leaching}}$ & $RL2_{\text{leaching}}$)**				**Ponding/leaching**	
	Metalaxyl	*S*-metolachlor	Metalaxyl	*S*-metolachlor	Cu	Zn	Metalaxyl	*S*-metolachlor
Patterns	-[a]	-	-	-	-	-	-	-
Soils	-	-	-	-	-	**	-	-
Aging	-	-	-	-	*	-	-	-
Frequency	n.d.[b]	n.d.	***	***	**	**	n.d.	n.d.

[a] "-", "*", "**" and "***" denote, respectively, no correlation ($p>0.1$), and significant correlations $p<0.05$, $p<0.01$ and $p<0.001$.

[b] "n.d." indicates that no statistical analysis could be made (absence of water ponding during the first rainfalls, and absence of water leaching or pesticides/heavy metals leaching following the second rainfalls).

Table 5-9: Impacts of the intensity, duration and volume of rainfall patterns on the leaching of metalaxyl, *S*-metolachlor, Cu and Zn ($RL1_{\text{leaching}}$ and $RL2_{\text{leaching}}$) as revealed by symbols indicating the obtained rank-based Spearman test p-values

		Metalaxyl	***S*-metolachlor**	**Cu**	**Zn**
First and second rainfalls	Intensity	**[a]	*	-	-
	Duration	**	**	*	-
	Volume	*	*	*	.
First rainfall	Intensity	*	.	-	-
	Duration	***	***	***	.
	Volume	***	***	***	*

[a] “-”, “.”, “*”, “**” and “***” denote, respectively, no correlation (p>0.1), weak correlation (p<0.1), and significant correlations p<0.05, p<0.01 and p<0.001.

In contrast, organic matter likely controlled both Cu speciation and mobility in the vineyard and the crop soils. In the studied vineyard catchment, Cu speciation mainly depends on the soil type, depth and organic matter content (Duplay et al., 2014). Cu was mainly bound to organic matter in the study soil, except in sandy soils, where it was mainly associated to carbonates and silicate matrix (Duplay et al., 2014). Hence, Cu leaching was less impacted than Zn by differences in clays or calcite content among the studied soils.

Based on metalaxyl and *S*-metolachlor physico-chemical characteristics and organic matter content of the crop and the vineyard soils, pesticides can also interact with soil minerals, as previously reported (Clausen et al. 2001). While uncharged pesticides, such as atrazine and isoproturon, can sorb onto pure kaolinite (Clausen et al., 2001), pesticides generally have high affinity with organic matter. When TOC is larger than 0.1 %, organic matter becomes the main sorbent of pesticides in soils (Clausen et al., 2001; Schwarzenbach and Westall, 1981). Therefore, because organic matter content was similar in both soils, the soil type likely was not the main factor that controlled leaching of metalaxyl and *S*-metolachlor.

Aging strengthens over time the association of a chemical with the soil components, thereby lowering pollutant export with increasing contact time (Gevao et al., 2000). In our case, the aging phase of ten days prior to rainfall applications only had an impact on Cu leaching. Cu speciation may change for the 10-days of aging, as the distribution of a freshly applied heavy metal in soils is expected to follow a multi-steps sorption. First of all, metals are rapidly sorbed onto mineral and organic surfaces. This stage is followed by a slower aging phase, characterized by the diffusive penetration or chemisorption of surface-sorbed heavy metals into the soil constituents (which are Fe-oxides, hydrous oxides of Al and Mn, clay minerals), as well as diffusion or precipitation into carbonates (Jalali and Khanlari, 2008; Tang et al., 2006). During aging, Cu most probably undergone a diffusive penetration and interacted with oxides, clays and carbonates of soils, which slightly decreased Cu export in the aged experiments compared to the freshly spiked experiments. Huang et al. (2015) found that the decrease of the labile forms in soils over 10 days were higher for Cu than for Zn (Huang et al., 2015). In parallel, they observed an important increase of the soils Cu reducible fraction corresponding to the Cu mainly associated with the soil oxides (Huang et al., 2015). As Zn was mainly associated to clays and carbonates (Duplay et al., 2014), its mobility did not significantly change during the 10-days aging period. In comparison, a decrease in Zn leaching was observed after a 63-days aging phase, as it might be remobilized from exchangeable sites to stronger ones at 65 % (Sayen and Guillon, 2014).

Aging of metalaxyl and *S*-metolachlor occurs differently. In addition to the degradation that pesticides can undergo with time, increasing contact time with soils may create stronger bonds to soil particles (Gevao et al., 2000; Komárek et al., 2010). However, metalaxyl and *S*-metolachlor had relatively weak affinity for the soils, as emphasized by K_d values lower than 2 $L.kg^{-1}$ in our soils (estimated based on the foc of the soils and the K_{oc} of metalaxyl and *S*-metolachlor). Indeed, K_d values of about 100 $L.kg^{-1}$ or more indicate strongly sorbed pesticides (Wauchope et al., 2002). The lower apparent K_d values calibrated in the model for the aged soils compared to those calculated from the K_{oc} values retrieved from databases (Kegley et al., 2016) emphasizes the low impact of a ten day aging period on pesticides and Zn leaching in the soil experiments. A longer contact time between the soil matrix and pollutants and a more systematic soil type investigation, covering the diversity of agricultural lands, is thus required to address the conditions for which extrinsic factors (i.e. rainfall patterns and frequencies) predominate on intrinsic characteristics such soil type and aging period.

5. Conclusion

This study revealed the primary influence of extrinsic factors, such as rainfall frequency, rainfall duration but also volume and revealed the secondary influence of soil composition and aging on pollutant export by leaching and ponding from agricultural soils. The rainfall volume particularly affected the pollutants' export during the first rainfall event after their application. The adequate hydrological functioning of the experimental design, i.e. without significant preferential flows, was confirmed by simulation of ponding and percolated waters using a parsimonious numerical model. The model was capable to generally reproduce the mass balance for both metalaxyl and *S*-metolachlor leaching through the soil matrix. The rainfall volumes and rainfall frequency, through their impact on the soil compaction, were the main factor controlling pesticide leaching. However, Cu and Zn leaching were also controlled by the aging period and the soil type, respectively.

Most importantly, the rainfall patterns significantly impacted the leaching of pesticides and Cu during the first rainfall, in both freshly spiked and aged soils. One significant implication is that the first rainfall following the field application of pesticides may be critical for pesticide export. In field conditions, preferential flow in macropores could additionally increase pesticide transport. As a result, leaching of pre-emergence pesticides applied just after tillage and sowing at the beginning of the growing season may represent a specific risk. In contrast, mobilization of metals will hardly occur over the seasons as low desorption may happen, provided that pH and redox conditions remain constant. Only few rainfall patterns may generate significant metal leaching, as showed here for the long-lasting events with low and intermediate rainfall intensities. Such rainfall patterns may particularly induce metal leaching at the beginning of the agricultural season, before progressive soil compaction over the season. Our results also underscore the importance of soil characteristics with respect to metal speciation for the export of metals by leaching.

Although initial soil conditions and characteristics influence the runoff transport of pesticides, their assessment at the catchment scale remains often difficult due to their heterogeneity. Therefore, systematic knowledge of the impact of rainfall characteristics on pollutant export is required to improve the prediction of prevailing flow path in small catchments. By refining the expected exports of pesticides and metals in different conditions, we anticipate this study to be a preliminary step to more systematically evaluate the impact of rainfall patterns on pollutant export in benchmark soil tests.

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Chapitre 6 : Flux de pesticides et métaux dans un bassin versant viticole

Le chapitre 6 traite en deux parties des flux de pesticides et de métaux au sein d'un bassin versant viticole. Une évaluation du Cu dans les fractions de sol du bassin versant et dans les eaux de ruissellement à l'exutoire de ce bassin sera présenté, suivi de l'étude de l'impact des patrons de pluie sur l'export des pesticides et des métaux en dehors de ce même bassin versant.

Partie 6.1. Teneur en Cu dans les fractions de sol et les eaux de ruissellement d'un bassin versant viticole : apport des isotopes stables du Cu

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1. Abstract and highlights

Understanding the fate of copper (Cu) fungicides in vineyard soils and catchments is a prerequisite to limit the off-site impact of Cu. Using Cu stable isotopes, we investigated Cu retention in soils and runoff transport in a vineyard catchment (Rouffach, France) in relation to the use of Cu fungicides and the hydrological conditions. The δ^{65}Cu values of the bulk vineyard soil varied moderately through the depth of the soil profiles (-0.12 to 0.24 ‰ ± 0.08 ‰). The values were in the range of those in the fungicides (-0.21 to 0.11‰) and included the geogenic δ^{65}Cu value of the untreated soil (0.08 ‰). However, δ^{65}Cu values differed between particle-size soil fractions (-0.37 ± 0.10 ‰ in fine clays and 0.23 ± 0.07 ‰ in silt). Together with the soil mineralogy, the results suggest that Cu fungicides primarily sorb to clay minerals, although Cu isotope patterns could be partly explained by Cu aging in the soil. Cu export by runoff from the catchment accounted for 1 % of the applied Cu mass, 84 % of which was Cu bound to suspended particulate matter (SPM). The runoff displayed δ^{65}Cu values from 0.52 to 1.35 ‰ in the dissolved phase (< 0.45 µm) compared to -0.34 to -0.02 ‰ in the SPM phase, indicating that mainly particles of the clay fraction contributed to the export of solid-bound Cu. This study

shows that Cu stable isotopes enable to identify Cu distribution and retention processes in soils and to trace Cu at the catchment scale.

Highlights:

- We investigated Cu sorption processes in vineyard soils and runoff transport
- Cu export by runoff from the catchment accounted for 1 % of the applied Cu mass
- δ^{65}Cu values differed between the particle-size soil fractions
- The clay soil fraction controlled sorption and runoff export of Cu fungicides
- Cu stable isotopes enabled to trace Cu distribution in soils and runoff transport

2. Introduction

Copper (Cu) is a trace element that can adversely impact soil biota and fertility when released to the environment (Komarek et al., 2010). Vineyard soils are frequently contaminated by Cu due to the use of Cu fungicides since the end of the 19th century (EC/889/2008; MacKie et al., 2012). Vineyards are prone to soil erosion, therefore Cu can be transported downstream to aquatic ecosystems vulnerable to Cu due to its potential toxicity towards aquatic organisms (El Azzi et al., 2013; Fernández-Calviño et al., 2008; Flemming and Trevors, 1989). However, knowledge of the fate of anthropogenic Cu in the soils and Cu transport from agricultural catchments to predict the off-site impact of Cu remains scarce.

In addition to traditional methods, the fate and transport of anthropogenic Cu in the environment can be studied using stable Cu isotopes (^{65}Cu/^{63}Cu) (Babcsányi et al., 2014; Bigalke et al., 2013; Bigalke et al., 2010a; El Azzi et al., 2013; Pérez Rodríguez et al., 2013; Petit et al., 2013; Thapalia et al., 2010). Cu isotope fractionation may occur during Cu adsorption onto Fe and Al oxy(hydr)oxides (Balistrieri et al., 2008; Pokrovsky et al., 2008), and clay minerals (Li et al., 2015), oxidation-reduction (Ehrlich et al., 2004; Zhu et al., 2002), uptake by plants (Jouvin et al., 2012; Ryan et al., 2013) or microorganisms (Navarrete et al., 2011), precipitation as Cu mineral phases (Ehrlich et al., 2004; Maréchal and Sheppard, 2002), and complexation with organic matter (Bigalke et al., 2010b; Ryan et al., 2014). In vineyard soils, Cu fungicides can sorb to clays, iron (Fe), manganese (Mn) and aluminum (Al) oxy(hydr)oxides, carbonates, (co)precipitate with secondary minerals and complex with soil organic matter (SOM) (Komarek et al., 2010). SOM efficiently sorbs Cu, while dissolved organic matter (DOM) complexes with and maintains Cu in solution, thus favoring its mobilization by rainfall (Komarek et al., 2010). Off-site export of Cu in vineyards was recently evaluated using Cu isotope analysis (El Azzi et al., 2013). Cu was found in rivers mainly associated with Fe oxy(hydr)oxides in the suspended particulate matter (SPM), while Cu fungicides were mainly bound to SOM (El Azzi et al., 2013). The SPM-bound Cu was found to be isotopically lighter compared to the dissolved Cu, which may be related to isotopically heavier Cu associated with DOM in the dissolved phase (El Azzi et al., 2013; Vance et al., 2008). However, the distribution of Cu in particle-size fractions (i.e., sand, silt, clay) can also influence Cu mobilization from the soil during rainfall-runoff events (Roussiez et al., 2013; Wang et al., 2014). Cu can accumulate in the coarse particulate SOM (Besnard et al., 2001; Parat et al., 2002) as well as in finer soil particles that are preferentially mobilized by runoff (Di Stefano and Ferro, 2002). However, the Cu isotope distribution in the particle-size fractions of the soil and in runoff following Cu mobilization by rainfall is currently unknown.

We hypothesized that Cu isotopes can be used to evaluate anthropogenic Cu retention and distribution in particle-size fractions of agricultural soils as well as Cu export during rainfall-runoff events. We combined a Cu mass balance approach at the scale of a vineyard catchment, particle-size separation of bulk vineyard soils, mineralogical and Cu isotope analysis to investigate i) the retention and distribution of Cu in particle-size fractions of the vineyard soil, and ii) the mobilization and transport by runoff of the dissolved and SPM-bound Cu in relation to Cu-fungicide use and rainfall patterns. The δ^{65}Cu values of the Cu-fungicides, ^{65}Cu/^{63}Cu fractionation upon Cu retention and distribution in the soils, and Cu transport in runoff were evaluated during the period of Cu fungicide use (May to July). Together with the soil mineralogy, the results show that particles of the clay fraction mainly control the sorption and runoff transport of Cu at the catchment scale.

3. Material and methods

3.1. Study catchment and soils

The general presentation of the study site is made in chapter 2, part 2. The rainfall was monitored by a meteorological station located in the catchment (Lefrancq et al., 2013). Rainfall between May and July averaged 187 ± 64 mm (1998-2011) and was 157 mm during the study period (May 11 to July 20). The mean runoff coefficient in the catchment was 1.61 % during the study period. The rainfall-runoff events do not generate a permanent stream in the catchment. Discharge at the catchment's outlet is driven by Hortonian overland flow. The road network mainly contributes to the discharge during low rainfall intensity (i.e. > 6 $mm.h^{-1}$) (Lefrancq et al., 2014), while the vineyard plots contributes when the rainfall intensity overcomes the saturated hydraulic conductivity (i.e. 58 $mm.h^{-1}$ ± 50, n=48) (Tournebize et al., 2012). The saturated hydraulic conductivity threshold can be reduced by one order of magnitude on plots when top soil sealing appears. The overland flow on plots is reduced by the 2 to 3 m grass cover and strips at the plot edges (Lefrancq et al., 2014). Hortonian overland flow prevailed, as indicated by fast hydrological response to rainfall events (i.e. 6 to 12 min between rainfall and runoff peaks) and fast recession period (less than 1 h) with total discharge cessation at the outlet. The contribution of baseflow is thus unlikely as steep slope (i.e 15 %) and high vertical saturated hydraulic conductivity prevent downslope lateral flows on saturated area.

The soils are calcareous clay loam with a soil bulk density of 1.4 g cm^{-3} developed on a loess basement (Tournebize, 2001). The soil types are Cambisol (Hypereutric Clayic) (site 1) and Haplic Cambisol (Calcaric Siltic) (site 2) (IUSS Working Group WRB, 2006) (Duplay et al., 2014). The vineyard plots are permanently covered by grass in every second inter-row to limit soil erosion, and weeded inter-rows are ploughed to a depth of 15 cm to increase water infiltration. The grass cover was implemented 3 to 4 years after the planting of grape vines. Vine growing started in 1973 at the site 1 (previously grassland) and in 1962 at the site 2 (Figure 6.1-1). The width between grass-covered vine inter-rows is 170 cm at site 1 (as well as on 77 % of the vineyard catchment), 240 cm at site 2, while the width between weeded inter-rows is 140 cm. Surface soils at site 1 have less carbonates, and slightly higher OM compared to site 2 (Figure 6.1-1 and Table 6.1-1). Soils are mainly composed of calcite, quartz, and minor amounts of feldspars, dolomite and clay minerals (Table 6.1-2) (Duplay et al., 2014). The A horizon extends until 30/45 cm depth, the B horizon until 80/100 cm, and the C horizon until the bedrock (loess) situated at ~210 cm.

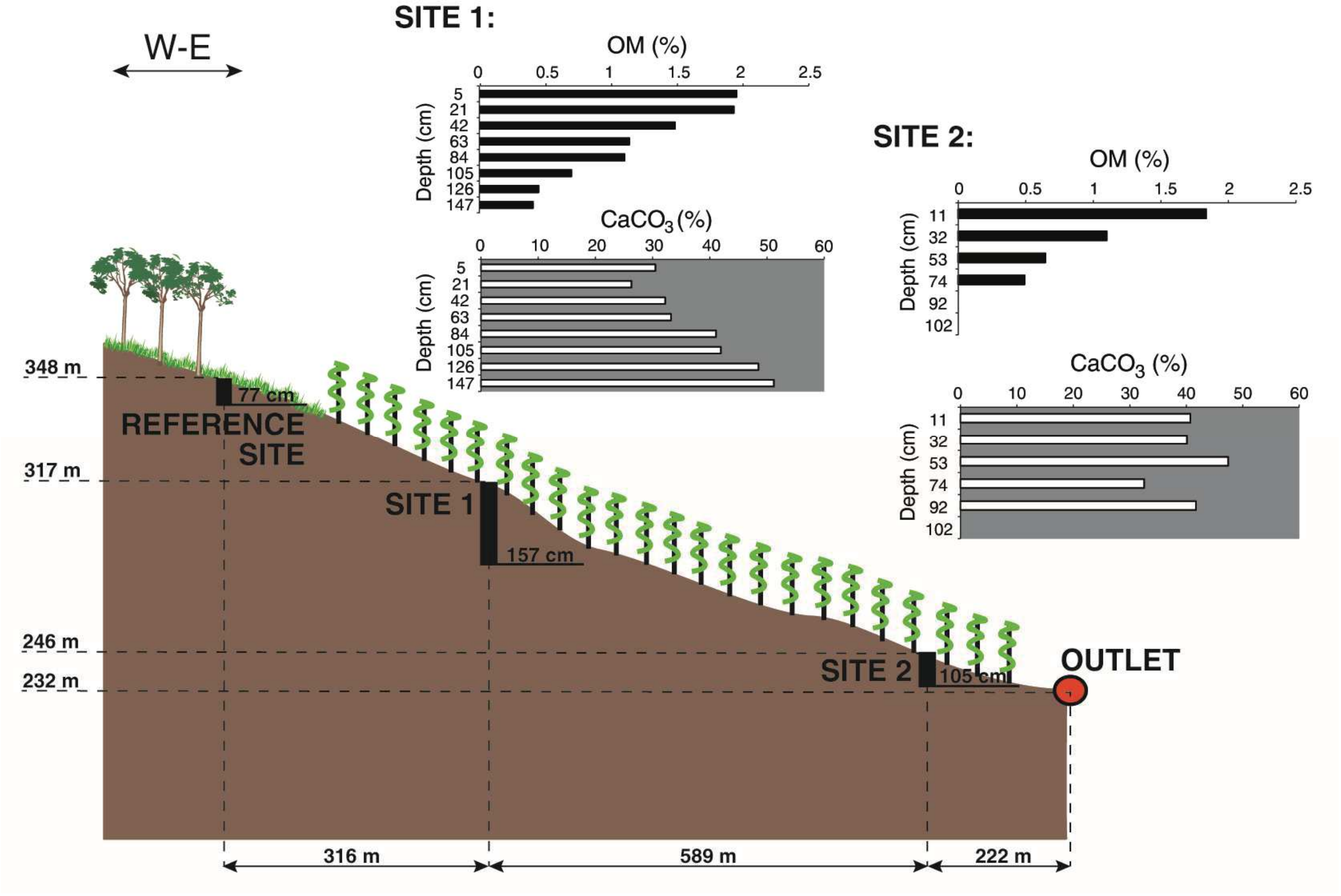

Figure 6.1-1: Cross-section of the vineyard catchment at Rouffach (Alsace, France) and location of the soil profiles (site 1 and 2) and the reference site. The depth variations of the carbonate ($CaCO_3$) and the organic matter (OM) concentrations under weeded inter-rows are provided for sites 1 and 2

Table 6.1-1: The physico-chemical characteristics of the soil depth profiles in the studied vineyard catchment, in the composite top-soils and in the reference soil (Rouffach, Alsace, France) and Cu enrichment factors (EF)

							%										mg kg^{-1}				
	Depth [cm]	%OM (LOI)	%Corg	%OM (WBC)	%CaCO$_3$	pH$_{H2O}$	SiO$_2$	Al$_2$O$_3$	MgO	CaO	Fe$_2$O$_3$	MnO	TiO$_2$	Na$_2$O	K$_2$O	P$_2$O$_5$	S	Cu	Ni	Zn	EF (Cu/Fe)
Site 1, soil under grass	0-18	6.1	nd	nd	31.6	7.80	47.7	8.16	1.18	15.5	3.47	0.09	0.51	0.44	1.80	0.29	723.4	39.8	21.9	74.9	4.6
	36-54	4.5	0.626	1.251	34.7	7.80	48.2	8.66	1.34	17.5	3.77	0.10	0.54	<dl	2.26	0.26	1125.8	28.9	27.9	64.3	3.1
Site 1, soil under weed row	0-10	6.6	0.975	1.949	30.5	7.60	46.9	8.30	1.37	15.7	3.56	0.10	0.52	0.37	2.39	0.31	753.2	74.2	33.6	99.7	8.4
	10-31	4.2	0.965	1.929	26.3	8.15	48.6	8.95	1.32	16.7	3.81	0.10	0.54	0.71	1.96	0.21	742.9	72.5	45.0	111.2	7.6
	31-52	4.7	0.740	1.480	32.2	7.70	47.6	9.08	1.51	16.2	3.85	0.09	0.56	0.43	2.38	0.26	749.7	27.2	36.6	66.2	2.8
	52-73	3.5	0.567	1.134	33.2	8.24	40.7	9.65	1.49	19.7	3.95	0.08	0.53	0.44	2.10	0.16	nd	12.0	29	49	1.2
	73-94	4.0	0.549	1.097	41.1	7.80	36.3	10.13	1.63	23.0	3.75	0.06	0.53	0.15	2.30	0.15	1336.3	14.2	17.5	42.4	1.5
	94-115	3.1	0.346	0.692	41.9	8.23	32.5	9.85	1.94	24.3	3.37	0.05	0.52	<dl	3.00	0.17	996.7	16.6	30.5	45.9	2.0
	115-136	3.0	0.221	0.442	48.5	nd	28.3	9.26	1.70	27.1	3.78	0.06	0.46	<dl	2.10	0.13	nd	14.0	35	43	1.5
	136-157	3.1	0.199	0.399	51.2	7.80	28.6	9.59	1.73	27.4	3.67	0.06	0.47	<dl	2.34	0.13	792.7	11.5	35.2	45.9	1.3
Site 2, soil under grass	0-18	5.0	0.822	1.645	38.7	7.80	37.5	6.83	1.48	22.1	3.87	0.07	0.38	0.37	1.81	0.46	1427.1	175.3	18.5	77.4	18.2
	30-42	4.0	0.578	1.157	39.0	8.00	37.7	6.86	1.70	22.7	3.80	0.07	0.37	0.43	2.45	0.36	664.4	129.1	46.2	70.3	13.6
Site 2, soil under weeded row	0-21	4.8	0.917	1.834	40.7	8.00	40.3	7.34	1.74	20.4	3.83	0.08	0.42	0.59	2.58	0.47	690.4	230.7	35.6	93.8	24.2
	21-42	3.7	0.549	1.098	40.1	8.10	41.4	8.29	1.63	20.4	4.30	0.08	0.45	0.40	2.09	0.36	655.7	150.6	29.9	78.1	14.1
	42-63	3.1	0.322	0.645	47.4	8.10	36.4	7.26	1.89	23.5	4.20	0.06	0.41	0.05	2.38	0.23	916.8	43.5	23.6	56.3	4.2
	63-85	2.5	0.245	0.490	32.5	nd	26.2	5.21	1.29	33.6	3.32	0.05	0.28	0.13	1.24	0.15	1419.5	29.1	40.2	56.1	3.5
	85-98	2.2	nd	nd	41.7	nd	30.7	6.74	1.45	28.4	3.97	0.06	0.36	<dl	1.57	0.18	nd	36.0	28	49	3.6
	98-105	nd	nd	nd	nd	nd	23.4	4.60	1.56	33.9	2.99	0.05	0.24	<dl	1.08	0.16	1110.2	32.1	20.4	43.1	4.3
Composite top-soil 06/04	0-5	nd	nd	nd	nd	nd	43.0	7.07	1.59	20.0	3.67	0.09	0.44	0.63	1.63	0.33	nd	104.5	36.0	95.3	11.4
Composite top-soil 11/05	0-5	nd	nd	nd	nd	nd	42.4	7.40	1.64	20.9	3.82	0.11	0.46	0.66	1.71	0.38	nd	105.8	38.0	98.9	11.1
Composite top-soil 08/06	0-5	4.8	0.837	1.674	32.23	8.03	46.9	7.99	1.53	17.7	4.08	0.10	0.49	0.60	1.80	0.35	nd	72.1	41.0	103.6	7.1
Reference soil	67-77	nd	nd	nd	nd	nd	38.4	6.90	0.73	25.1	4.62	0.06	0.407	0.05	1.56	0.13	885.6	11.5	39.0	50.7	1.0

Table 6.1-2: Mineral phases detected by XRD in bulk soil and the different particle-size soil fractions

Samples	Mineral phases
Bulk soil	calcite, quartz, minor amounts of feldspars, dolomite, clay minerals
Silt	quartz, calcite, dolomite, feldspars (albite, microcline, orthoclase), traces of clay minerals (illite, chlorite and/or kaolinite)
Clay	kaolinite, illite, traces of chlorite and fine fractions of quartz, calcite, goethite and feldspars (albite, microcline, orthoclase)
"Fine clay"	illite, kaolinite and interstratified organic matter?

3.2. Cu fungicide applications

Cu fungicide applications were estimated based on surveys addressed to the wine producers of the catchment. Cu was sprayed directly on the vine canopy to combat downy mildew (MacKie et al., 2012). Cu fungicides were applied almost on all vineyards (> 97%) in the catchment from May to August in quantities of 2.2 ± 0.3 kg(of the fungicide preparation). $ha^{-1}.y^{-1}$ (2008-2011) in the forms of Cu-sulfate (Bordeaux mixture, $CuSO_4$ + $Ca(OH)_2$), Cu-oxide (Cu_2O), Cu-hydroxide ($Cu(OH)_2$) and Cu-oxychloride ($3Cu(OH)_2{\cdot}CuCl_2$). Application rates of the Cu-fungicides ranged from 0.09 to 1.50 kg ha^{-1} in 2011, depending on the vine-producers and the type of Cu-fungicide. The study was performed from May 11 to July 20, 2011, covering most of the Cu application period and corresponding to 63 % of the annual application of Cu. The estimated input of atmospheric Cu to the catchment (~83 g) represented $\leq$ 0.5 % of the amount of Cu fungicides during the study period (Chabaux et al., 2005) (Table 6.1-3).

Table 6.1-3: Estimates of annual Cu inputs, seasonal Cu fluxes (from May 11 to July 20, 2011) and Cu stocks in the upper soil and the grass biomass of the vineyard catchment (Rouffach, France; 42.7 ha, of which 25.3 ha of vineyards)

Stock in the catchment soil (top 5 cm)[1]	[kg]	1800
Stock in vineyard soils (top 5 cm)	[kg]	1670
Stock in the grass biomass of vineyards[2]	[kg]	5.2
Initial stock in the vineyard topsoils (top 5 cm)[3]	[kg]	200
Accumulation rate in the vineyard topsoils (top 5 cm)[4]	[kg ha^{-1} year^{-1}]	1.2
Fungicide applications	[kg]	16.5
	[kg ha^{-1} year^{-1}]	1.24
Atmospheric input[5]	[kg ha^{-1} year^{-1}]	6×10^{-3}
SPM-bound export by runoff	[kg ha^{-1} season^{-1}]	3×10^{-3}
Dissolved export by runoff	[kg ha^{-1} season^{-1}]	5×10^{-4}

[1] Estimated based on the mean Cu concentration in vineyard top-soil samples (top 5 cm of the soils sampled along 3 transects in the catchment) and the Cu concentration of the (uncultivated) reference soil (top 5 cm).
[2] Estimated based on the mean Cu concentrations in the grass shoot (22.8 mg.kg^{-1}) and the root following Cu applications (44.3 mg.kg^{-1}) (Duplay et al., 2014), a shoot to root biomass ratio of 1.75 (Bolinder et al., 2002) and a maximum dry grass biomass of 6.7 t.ha^{-1} (Tournebize, 2001).
[3] Estimated based on the Cu concentrations in the (uncultivated) reference soil.
[4] Estimated based based the initial stock in the vineyard topsoils (top 5 cm) and the actual stock in vineyard soils (top 5 cm) over the 50 years period of vine growing.
[5] Estimated based on the mean Cu concentration of the rainwater in the Alsatian basin (Chabaux et al., 2005) and the annual rainfall depth in the catchment in 2011 (502 mm).

3.3. Soil and runoff collection

Four soil cores were collected in two weeded and two grass-covered inter-rows, as well as one soil sample at a local forested reference site at 70 cm depth (Figure 6.1-1). Soil cores were collected down to a depth of 157 cm at site 1 and 105 cm at site 2 using a hand auger. The depth resolution of the soil sampling ranged from 10 to 20 cm. The soil physico-chemical characteristics were described elsewhere (Table 6.1-1) (Duplay et al., 2014). Briefly, the observed soils are alkaline, highly calcareous and display a SOM concentration ranging from 0.4 to 1.9 % (Table 6.1-1). Only four superficial soil horizons (of the soil profiles) under grass-covered inter-rows were selected for chemical composition and Cu isotope analyses. The soil samples were stored in polyethylene bags. Composite topsoil (0-5 cm) samples were also collected along three transects across the catchment (~50 g of soil was collected each ~10th vine-rows, corresponding to a distance of 15 to 20 meters) and pooled to one composite sample per sampling date on April 6, May 11 and June 8, 2011. A reference soil was collected in the forested area of the catchment (Figure 6.1-1), which was never cultivated, thus enabling a comparison with soils from sites 1 and 2. The reference soil was sampled at 70 cm, close to the bedrock and served for quantifying the geogenic Cu.

The water discharge at the outlet of the catchment was continuously monitored using a bubbler flow module (Hydrologic, Canada) combined with a Venturi channel. Flow-proportional samples of runoff water were collected during each runoff event (300 mL every 3 m^3) using a 4010 Hydrologic automatic sampler (Hydrologic, Canada) (Maillard and Imfeld, 2014), and samples were combined weekly into a single composite sample. Samples were refrigerated and transported to the laboratory.

3.4. Sample preparation and analysis

The preparation and mineralization of soil samples for elemental analysis is presented in Chapter 2, part 3.2.

A composite topsoil (0-5 cm) sampled on June 8, the weeded topsoils at site 1 (0-10 cm) and 2 (0-21 cm) and the reference soil were selected to determine the Cu distribution among particle size fractions (i.e., sand, silt, clay and fine clay) and analyzed in duplicate. Approximately 20 g of sieved soil (< 2 mm) were suspended in 200 mL of 18.2 MΩ water (Millipore, Billerica, MA, USA) and disaggregated by high intensity sonication as described previously (see the SI for the detailed protocol) (Genrich and Bremner, 1974). Mineral phases present in bulk soil and particle-size fractions were determined by X-ray diffraction (XRD) (Bruker D5000, Karlsruhe, Germany) (Duplay et al., 2014).

The runoff water samples were filtered using a 0.45 µm cellulose acetate membrane (Millipore) and the filtrate (named below 'dissolved phase') was acidified to pH ~2 using double-distilled nitric acid. Hydrochemical analyses were performed as described elsewhere (Lucas et al., 2010).

3.5. Cu isotope analysis

The procedure needed to perform Cu isotope analysis is explained in Chapter 2, part 7.

3.6. Data analysis

Changes in the Cu levels in the studied soils were evaluated using an enrichment factor (EF) that compared the Cu levels at site 1 and 2 to soil at 70 cm depth from the reference forested site upslope of the vineyards:

$$\mathrm{EF_{Cu}} = \frac{\left(\frac{\mathrm{Cu_{sample}}}{\mathrm{Fe_{sample}}}\right)}{\left(\frac{\mathrm{Cu_{reference}}}{\mathrm{Fe_{reference}}}\right)} \qquad (6.1\text{-}1)$$

where Cu and Fe are, respectively, the concentrations of Cu and Fe (mg kg^{-1} d.w.) in the vineyard soil sample and the reference soil (Figure 6.1-1). Fe was selected as the reference element due to its low variation coefficient over the soil profiles, thereby limiting EF variations due to local heterogeneities (Reimann and de Caritat, 2005).

A seasonal export coefficient was calculated as the ratio of the total Cu load exported from the vineyard catchment by runoff over the total Cu mass applied during the study period (May 11 to July 20, 2011).

The mean δ^{65}Cu value of the applied Cu fungicides was calculated by an isotopic mass balance accounting for each individual Cu fungicide:

$$\begin{aligned}\delta^{65}\mathrm{Cu_{mean}} &= \mathrm{f_{CuSO_4}} \times \delta^{65}\mathrm{Cu_{CuSO_4}} + \mathrm{f_{3Cu(OH)_2CuCl_2}} \times \delta^{65}\mathrm{Cu_{3Cu(OH)_2CuCl_2}} \\ &\quad + \mathrm{f_{Cu(OH)_2}} \times \delta^{65}\mathrm{Cu_{Cu(OH)_2}} + \mathrm{f_{Cu_2O}} \times \delta^{65}\mathrm{Cu_{Cu_2O}}\end{aligned} \qquad (6.1\text{-}2)$$

where f is the mass fraction of the Cu fungicides used and δ65Cu value is the Cu isotope composition of the fungicides.

Hydro-climatic data and chemical compositions of soils and runoff were compared using the paired non-parametric Spearman rank correlation test using R (www.r-project.org), with p-values set at 0.05.

4. Results

4.1. Cu fungicides

1.24 kg ha^{-1} Cu was applied on the catchment from May 5 until August 1, 2011 (i.e. 26.07 kg for the entire vine-growing season). 16.50 kg Cu was applied during the study period (May to July), which represented ~1% of the initial Cu stock in the top 5 cm of the vineyard soils (~1670 kg) (Table 6.1-3). The seven Cu fungicides obtained from local farmers (Kocide ($Cu(OH)_2$), Champ flo ($Cu(OH)_2$) and Cursor ($3Cu(OH)_2CuCl_2$) were not used in 2011) had δ^{65}Cu values ranging from -0.21 to 0.91 ‰. The δ^{65}Cu values of the four Cu fungicides used in 2011 (Bordeaux mixture ($CuSO_4$ + $Ca(OH)_2$), Nordox (Cu_2O), Syphal ($3Cu(OH)_2 \cdot CuCl_2$), Cuprofix ($CuSO_4$)) varied from -0.21 to 0.11 ‰ (Figure 6.1-2 & Table 6.1-4).

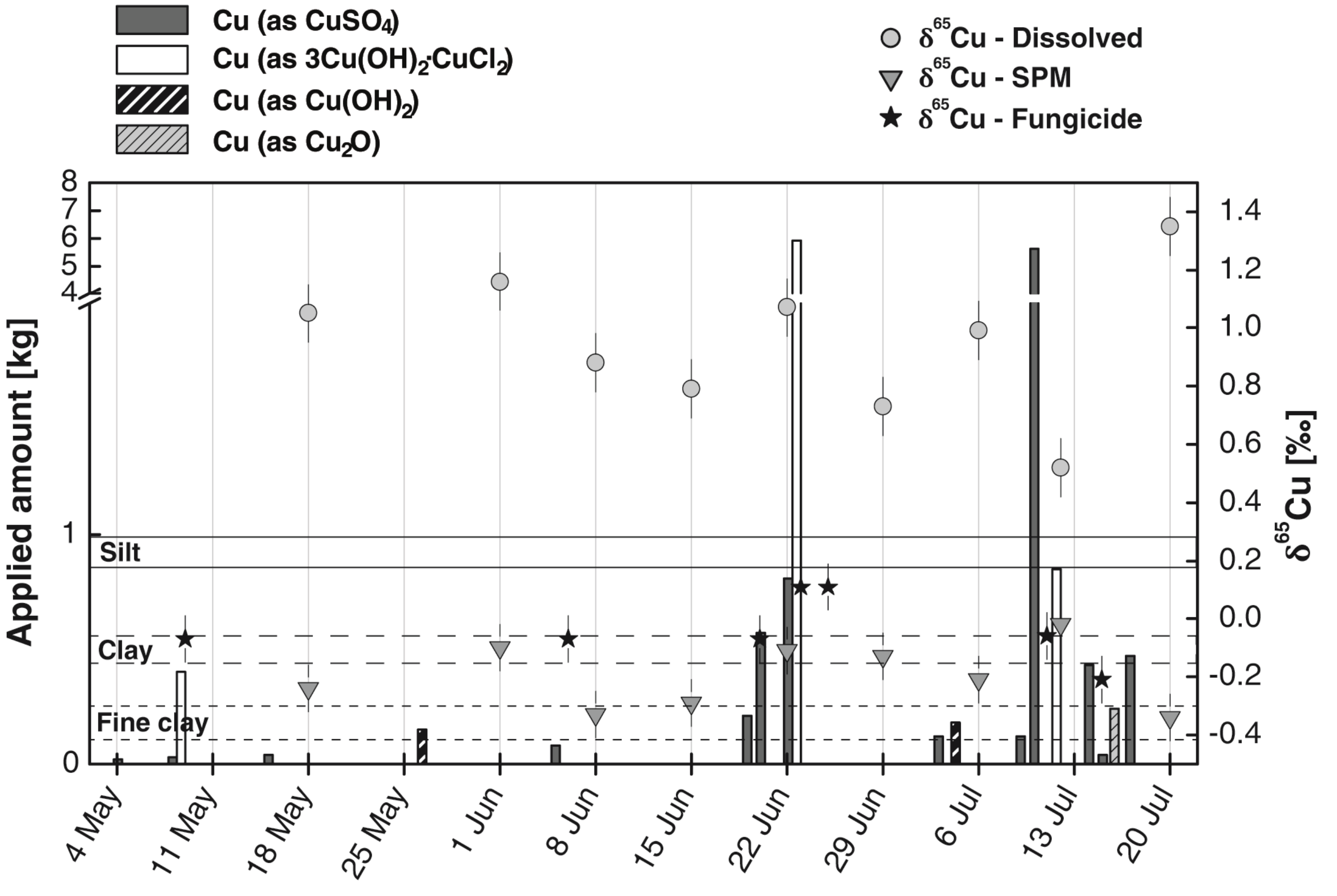

Figure 6.1-2: Amounts and δ^{65}Cu of the Cu fungicides applied on the vineyard catchment (Rouffach, France) and δ^{65}Cu of runoff-associated Cu in the dissolved and the SPM-bound phases from May 11 to July 20. Horizontal bars represent the range of δ^{65}Cu values of the soil size fractions (i.e. silt, clay, "fine clay"). Error bars correspond to the analytical uncertainty of the Cu isotope measurements (± 0.08 ‰)

Table 6.1-4: The δ^{65}Cu of the measured Cu-based fungicides applied in Rouffach during the vine-growing season in 2011 or earlier

Product name	Cu form	δ^{65}Cu (‰)	Applied in 2011
Champ flo	$Cu(OH)_2$	0.22	no
Kocide	$Cu(OH)_2$	0.91	no
Cursor	$3Cu(OH)_2 \cdot CuCl_2$	0.22	no
Bordeaux mixture	$CuSO_4 + Ca(OH)_2$	-0.06	yes
Nordox	Cu_2O	-0.21	yes
Syphal	$3Cu(OH)_2 \cdot CuCl_2$	0.11	yes
Cuprofix	$CuSO_4$	-0.07	yes

4.2. Cu accumulation and variations of δ^{65}Cu values in the vineyard soils

The Cu concentration of grass-covered topsoils (0-18 cm) of both sampling sites was lower than that of the weeded topsoils (0-10/21 cm) (Table 6.1-1). However, the δ^{65}Cu values of bulk grass-covered (0-18 cm) and weeded (0-10/21 cm) topsoils were similar. This suggests that grass cover did not affect Cu isotope fractionation in the bulk soil. Estimated Cu in the grass biomass (~5.2 kg) accounted for ~0.3 % of the total Cu stock in the first 5 cm of the vineyard soils, indicating relatively low Cu uptake in the grass (Table 6.1-3).

Cu accumulated in the surface soil layers (0 to 50 cm) (Figure 6.1-3). Concentrations in the top 20 cm at site 2 (231 mg.kg^{-1}) were higher than in the top 10 cm at site 1 (74 mg.kg^{-1}) (Table 6.1-1), and gradually decreased with depth down to 11.5 mg.kg^{-1}. Correspondingly, enrichment factors (EF) were higher at site 2 (4.0 to 27.6) compared to site 1 (1.4 to 9.6) (Figure 6.1-3), which reflects the longer period of Cu fungicide use (i.e., the vine-bearing grapes were planted 11 years earlier at site 2 than at site 1). The estimated Cu accumulation rate in the first 5 cm of the vineyard soils of the catchment during the 50 years of vine-growing (~1.2 kg.ha^{-1}.year^{-1}) corresponded to that of current Cu application rates (1.24 kg.ha^{-1}.year^{-1}) (Table 6.1-3). The δ^{65}Cu values in the soil profile ranged from -0.12 to 0.24 ‰ at site 1, and did not significantly vary at site 2 (-0.05 to 0.04 ‰) (Figure 6.1-3). The δ^{65}Cu value of the background Cu in the reference soil (0.08 ‰ at 67-77 cm depth) differed from that in the deepest soil horizon at site 1 (-0.12 ‰) (Figure 6.1-3).

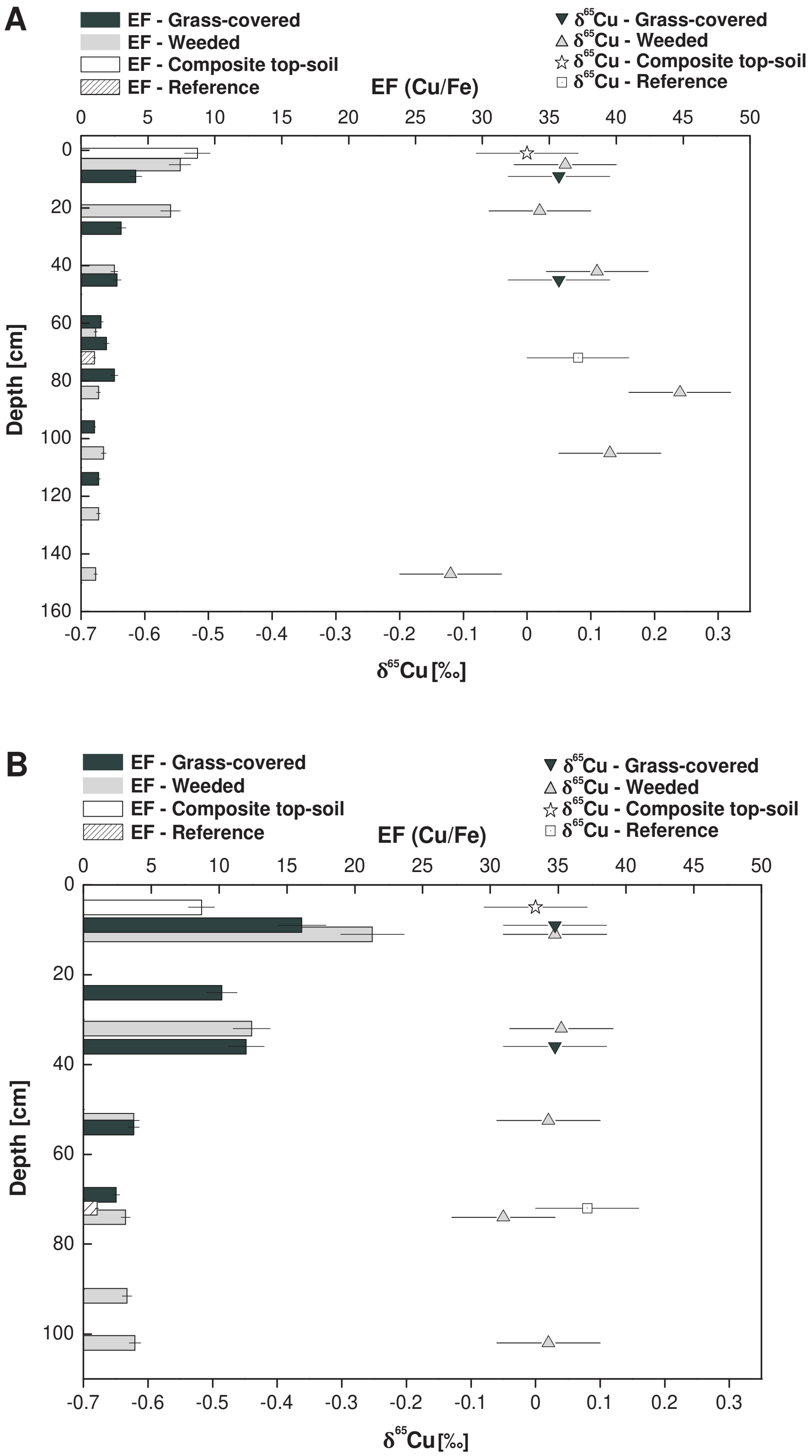

Figure 6.1-3: Cu enrichment factors (EF) and δ^{65}Cu values in the soil depth profiles at site 1 (A) and site 2 (B) of the vineyard catchment (Rouffach, France). The deepest soil horizon (67-77 cm) at an uncultivated reference site and the composite topsoil (0-5 cm) are also displayed

4.3. Cu distribution and δ^{65}Cu values in the soil particle-size fractions

The particle-size separation yielded qualitatively distinct soil fractions, i.e. silt containing mainly primary minerals and clays secondary minerals (Table 6.1-2). The fine clays and the clay fractions represented 26 to 31 % (total weight) of the bulk vineyard soils. Cu concentrations were twice as high in the fine clay and the clay fractions (111 to 467 mg.kg^{-1}) than in the bulk soils (Tables 6.1-1 and 6.1-5). Cu concentrations were higher in the fine clay fraction (76 to 408 mg.kg^{-1} Cu, accounting for 2.6 to 4.8 %) than in the silt fraction (62 to 154 mg.kg^{-1}), except at site 1. At site 1, the proportion of Cu in the silt fraction (55 %) was higher than that in the clay fraction (40 %), the fine clay fraction (3 %) and the soil solution (2 %) (Table 6.1-5). Topsoils at site 2 (0-21 cm) were coarser with less clay and larger sand fractions compared to site 1 (0-10 cm) (Table 6.1-5). At site 2, 51 % of Cu accumulated mainly in the clay fractions (47 % was in the clay and 4 % in the fine clay), and in lower proportions in the silt (27 %) and the sand (~20 %). Cu concentrations in the aqueous solution obtained from the clay separations varied from 5 to 32 µg.L^{-1} (corresponding to 1.5 to 4.1 mg.kg^{-1} in the bulk soil). The reference soil was composed of sand (30.0 %), silt (39.5 %), clay (23.5 %) and fine clay (0.1 %) fractions, with geogenic Cu concentrations of 11.7, 9.4, 16.6, 3.6 (i.e., 30.6 %, 23.3 %, 33.9 %, < 0.1 %), respectively, and 0.4 mg.kg^{-1} (i.e., 3.1 %) in the aqueous solution (Table 6.1-5).

Differences of Cu concentrations and δ^{65}Cu values in the soil fractions indicated Cu isotope fractionation in the vineyard soils (Table 6.1-5). The fine clay fraction was the most depleted in ^{65}Cu (δ^{65}Cu values from -0.42 to -0.32 ‰, $\bar{x} \pm 2\sigma$: -0.37 ± 0.10 ‰), followed by the clay fraction (-0.15 to -0.06 ‰; $\bar{x} \pm 2\sigma$: -0.08 ± 0.09 ‰). In contrast, the silt fraction was enriched in ^{65}Cu (0.19 to 0.24 ‰; $\bar{x} \pm 2\sigma$: 0.23 ± 0.07 ‰) (Figures 6.1-2 & 6.1-3, Table 6.1-5). Cu in the solution was also isotopically heavier (0.04 to 0.44 ‰). δ^{65}Cu values of the particle-size fractions of the reference soil (-0.09 ‰ in the silt, 0.18 ‰ in the clay and 1.28 ‰ in the solution) differed significantly from those of the vineyard soil. The δ^{65}Cu value of the fine clay fraction in the reference soil could not be analyzed due to insufficient Cu amounts.

Table 6.1-5: Proportions, organic matter content, Cu concentrations and δ^{65}Cu variations of the different particle-size soil fractions

		Weight % in the soil	% OM (LOI)	Cu [mg kg^{-1}]	δ^{65}Cu [‰]	2*sd	n
Composite top-soil 08/06 (1)	fine clays (> 0.45 µm)	3.1	nd	106.2	-0.42	0.13	3
	clay fractions (< 2 µm)	25.9	6.0	132.8	-0.07	0.11	3
	silt fraction (2 µm < x < 63 µm)	46.3	2.4	61.6	0.28	0.13	3
	Solution (< 0.45 µm)	-	nd	1.85	0.32	0.06	2
Composite top-soil 08/06 (2)	fine clays (> 0.45 µm)	4.8	nd	101.1	-0.32	0.07	2
	clay fractions (< 2 µm)	26.1	5.6	112.8	-0.06	0.07	2
	silt fraction (2 µm < x < 63 µm)	47.2	2.6	71.3	0.19	0.11	2
	Solution (< 0.45 µm)	-	nd	1.49	0.44	0.10	2
Surface soil at site 1	fine clays (> 0.45 µm)	2.6	nd	76.4	-0.33	0.08	3
	clay fractions (< 2 µm)	26.6	7.5	110.7	-0.06	0.13	3
	silt fraction (2 µm < x < 63 µm)	49.7	3.5	82.0	0.24	0.04	2
	Solution (< 0.45 µm)	-	nd	4.11	0.12	0.12	2
Surface soil at site 2	fine clays (> 0.45 µm)	3.3	nd	408.3	-0.40	0.07	2
	clay fractions (< 2 µm)	22.7	6.9	467.2	-0.15	0.07	2
	silt fraction (2 µm < x < 63 µm)	41.1	2.7	154.4	0.23		1
	Solution (< 0.45 µm)	-	nd	1.58	0.04	0.02	2

4.4. Cu export and δ^{65}Cu values in SPM-bound and dissolved runoff phases

A total of 43 rainfall events occurred during the study period that generated 27 runoff events. At least 1 rainfall-runoff event occurred weekly (weekly hydrological data are summarized in Table 6.1-6). Rainfall depth ranged from 0.2 to 23.9 mm with mean intensities from 0.3 to 14.0 mm.h^{-1}. The runoff volumes per event ranged from 2.5 to 195.4 m^3, amounting to a total volume of 1247.8 m^3 for the investigation period. SPM concentrations in runoff ranged from 110 to 2866 mg.L^{-1} (Table 6.1-6). SPM-bound Cu concentrations in runoff ranged from 90 to 366 mg.kg^{-1} ($\bar{x} \pm 2\sigma$: 164 ± 177 mg.kg^{-1}) and from 40.1 to 395.2 µg.L^{-1} ($\bar{x} \pm 2\sigma$: 118 ± 219.7 µg.L^{-1}) on a volumetric basis. Dissolved Cu (< 0.45 µm particle-size fraction) concentrations in runoff ranged from 7.7 to 32.0 µg.L^{-1} ($\bar{x} \pm 2\sigma$: 21.6 ± 16.0 µg.L^{-1}) (Table 6.1-7), which was 7 to 27 times higher than average global dissolved Cu concentrations in rivers (1.19 µg.L^{-1}) (Vance et al., 2008). The δ^{65}Cu values of the dissolved Cu in runoff ranged from 0.52 to 1.35 ‰ and from -0.02 to -0.34 ‰ in the SPM-bound Cu fraction (Figure 6.1-2). δ^{65}Cu values of SPM-bound Cu were similar to those of the clay and the fine clay fractions of the vineyard topsoils (0-10/21 cm). This suggests a preferential mobilization of fine soil particles during rainfall-runoff (Figure 6.1-2) in agreement with previous observations (Wang et al., 2014).

Altogether, 144 g of Cu was exported in runoff from the catchment during the investigation period, representing 0.9 % of the Cu mass applied (Table 6.1-3). Comparatively, estimates of Cu deposition onto non-target areas such as road surfaces prone to runoff ranged between 0.05 to 0.4 % of the applied Cu amount during the investigation period, assuming the same application mode and device as for the fungicide kresoxim methyl applied in the same catchment (Lefrancq et al., 2013), but with a higher volumetric rate (133 L.ha^{-1} versus 160 L.ha^{-1}). This indicates that Cu from the vineyard plots represented the major source of Cu exported from the catchment in surface runoff.

Table 6.1-6: Rainfall data monitored by the meteorological station situated at the study catchment (Rouffach, Alsace, France) and hydrological measurements at the outlet of the catchment

Date	Rainfall depth [mm]	Rainfall duration [mn]	Mean intensity [mm]/[h]	Max. intensity [mm]/[6mn]	Quiescent period [d]	Mean runoff discharge [L s^{-1}]	Max. runoff discharge [L s^{-1}]	Min. time between application and runoff [d]
18 May	12.7	420	1.70	0.8	3.89	2.60	4.64	3
01June	16.3	636	-	-	8.83	1.51	2.50	5
08 June	8.0	744	0.77	0.4	6.71	1.41	2.56	1
15 June	10.8	318	3.54	2.5	6.18	3.55	9.61	8
22 June	15.3	672	4.34	2.2	7.10	2.78	15.67	15
29 June	29.7	384	4.55	6.0	6.53	8.90	34.58	1
06 July	9.7	582	0.92	1.0	7.46	6.70	44.69	4
12 July	28.5	798	2.29	3.5	4.34	2.54	18.58	0
20 July	26.3	1668	1.10	4.2	5.63	1.80	18.36	0

Table 6.1-7: Chemical composition of SPM, Cu concentrations and δ^{65}Cu of runoff-associated Cu phases

		%									mg kg^{-1}									
Date	Dissolved Cu [µg L^{-1}]	Al	Mg	Ca	Fe	Mn	Ti	Na	K	P	Cu	Zn	S	EF (Cu/Fe)	% dissolved Cu in runoff	log Kd	δ^{65}Cu dissolved	δ^{65}Cu in SPM	δ^{65}Cu of total Cu in runoff	$\Delta^{65}Cu_{SPM-dissolved}$
18 May	7.7	3.41	0.69	3.4	2.27	0.039	0.185	0.22	0.54	0.157	135	279	1844	21	10	4.2	1.05	-0.24	-0.11	-1.29
01June	14.8	3.09	0.60	6.4	2.09	0.044	0.211	0.26	0.27	0.123	90	165	1142	15	23	3.8	1.16	-0.10	0.19	-1.26
08 June	32.0	5.71	1.02	4.2	3.99	0.092	0.273	0.25	1.70	0.319	366	455	2969	33	44	4.1	0.88	-0.33	0.21	-1.21
15 June	22.6	3.97	0.85	9.0	2.76	0.073	0.244	0.33	1.14	0.138	103	182	1357	13	12	3.7	0.79	-0.29	-0.16	-1.08
22 June	22.9	4.36	0.84	7.3	2.93	0.070	0.243	0.28	0.39	0.149	121	166	1180	15	23	3.7	1.07	-0.11	0.17	-1.18
29 June	30.8	3.72	0.92	10.0	2.73	0.077	0.279	0.43	0.15	0.140	138	144	1087	18	7	3.7	0.73	-0.13	-0.07	-0.86
06 July	13.8	4.74	0.96	9.9	3.07	0.087	0.297	0.47	0.59	0.162	126	166	1251	15	14	4.0	0.99	-0.21	-0.04	-1.20
12 July	24.2	5.62	0.84	6.95	3.25	0.082	0.270	0.33	1.51	0.178	146	152	1294	16	20	3.8	0.52	-0.02	0.09	-0.54
20 July	25.3	6.83	0.98	6.24	4.01	0.089	0.288	0.29	1.99	0.228	249	232	1755	22	21	4.0	1.35	-0.34	0.01	-1.69

5. Discussion

5.1. Evolution des concentrations de pesticides et métaux dans les fractions granulométriques des sols

In the vineyard, δ^{65}Cu values for the grass-covered (0-18 cm) were similar to those of weeded topsoils (0-10/21 cm) and Cu translocation from the soil to the grass was low (~0.3 %) (Table 6.1-3). This supports the idea that the vegetation did not affect the Cu isotope pattern in the vineyard soils. Indeed, uptake of Cu is expected to enrich in ^{63}Cu the plants, yielding a $\Delta^{65}Cu_{plant-nutrient}$ of -0.1 to -1 ‰ (Jouvin et al., 2012; Ryan et al., 2013). In addition, δ^{65}Cu values did not vary significantly with depth at site 2, which suggests that Cu uptake by grape vines is limited in calcareous vineyards (Chopin et al., 2008).

The depletion in ^{65}Cu with soil depth associated with lower Cu enrichments (EF $\leq$ 2) below 70 cm at site 1 concurs with the Cu isotope distribution patterns of geogenic Cu observed in oxic weathered soils (Bigalke et al., 2011). The variations in the δ^{65}Cu values below 70 cm at site 1 suggest that Cu isotope fractionation occurred during weathering of the bedrock and transport of geogenic Cu into the soil or loess deposits of different origins from which the soil layering developed (Bigalke et al., 2011). In addition, the vertical transport of isotopically lighter colloidal or solid-bound Cu during the rainwater infiltration may also contribute to the vertical distribution of Cu isotopes.

The bulk soils have similar δ^{65}Cu regardless of the level of Cu enrichment because the mean δ^{65}Cu value of Cu fungicides (0 ‰) encompassed that of geogenic Cu in the soil. The δ^{65}Cu values of the bulk vineyard soils thus did not indicate sorption and accumulation of anthropogenic Cu. However, the δ^{65}Cu and the Fe/Cu molar ratios for the particle-size fractions of vineyard soils were negatively correlated, whereas they did not correlate in the case of the reference soil (Figure 6.1-4). Fe is distributed not only in the Fe oxy(hydr)oxides, such as goethite (as detected by XRD analysis in the clay fractions), but also in the clay minerals, such as chlorite (Table 6.1-2). The relationship between the δ^{65}Cu values and the Fe/Cu molar ratios supports the idea that Cu isotope fractionation is associated with Cu distribution processes in the soil. Cu distribution processes in the calcareous vineyard soils include Cu precipitation as carbonates and (competitive) sorption of Cu to mineral and SOM phases.

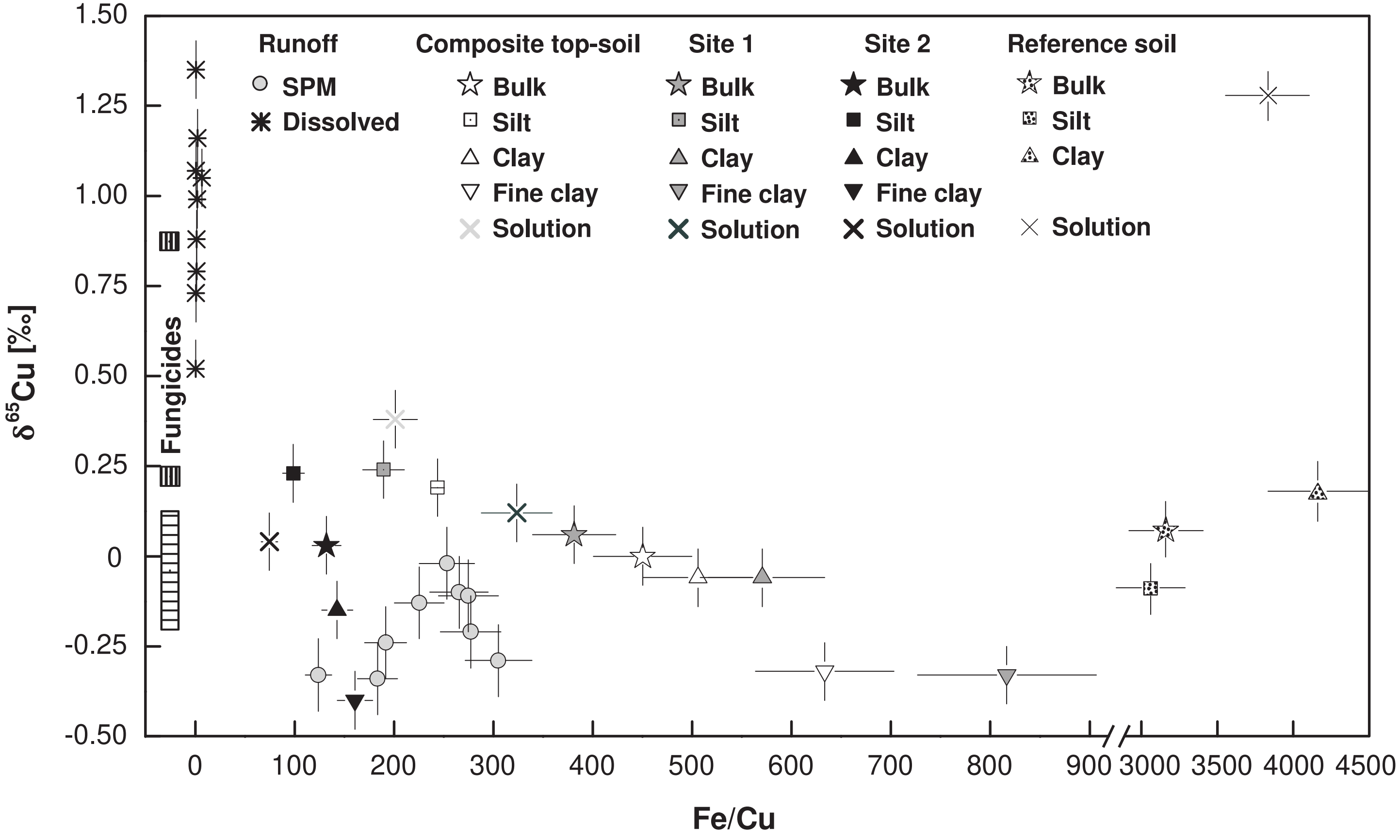

Figure 6.1-4: δ^{65}Cu values of the SPM-bound and dissolved Cu in runoff at the outlet of the catchment (Rouffach, France). The soil fractions of a composite top-soil (0-5 cm), the surface soils under weeded inter-rows at site 1 (0-10 cm) and 2 (0-21 cm) as well as the reference soil are displayed as a function of the Fe/Cu molar ratios. The vertical bars represent the range of δ^{65}Cu values of the Cu fungicides applied in 2011 (horizontal hatching) and not applied in 2011 (vertical hatching). Cu concentrations of the soils differed at site 1 and 2 (Fe concentrations are similar)

Precipitation of Cu as carbonates or hydroxides was previously noted as the prevailing process of Cu retention in calcareous soils (El Azzi et al., 2013; Komárek et al., 2009; Ma et al., 2006b; Ponizovsky et al., 2007). The precipitation of malachite is expected to enrich the precipitates in ^{63}Cu (Δ^{65}CuCu(II)aq-malachite = 0.17 to 0.38 ‰) (Maréchal and Sheppard, 2002). Hence, the relative enrichment in ^{63}Cu observed in the clay fractions as compared to the δ^{65}Cu values in the reference soil may indicate Cu carbonate precipitation. In this case, Cu carbonate precipitation would enrich in ^{65}Cu the residual, non-precipitated Cu in the silt and the sand fractions of vineyard soils. However, XRD data showed that carbonate phases (calcite, dolomite) prevailed in the silt fraction that was enriched in ^{65}Cu. Only traces of calcite in the clay and no carbonates were found in the fine clay fractions that were enriched in ^{63}Cu (Table 6.1-2). Cu precipitation as carbonate thus cannot explain the enrichment in ^{65}Cu in the silt fraction and that in ^{63}Cu in the clay fractions. Hence, Cu retention processes involving mineral and SOM phases should be considered.

Competitive sorption of Cu in the different mineral and SOM phases is first to be considered to explain the trends of δ^{65}Cu values variations in the grain-size fractions (Figure 6.1-2). Fe oxide (de Santiago-Martín et al., 2013; Sayen et al., 2009), clay minerals and organo-clay complexes (Besnard et al., 2001; Sipos et al., 2008) as well as the formation of SOM-Cu complexes (Strawn and Baker, 2009) control the Cu sorption and accumulation in soils. Cu sorption to Fe oxy(hydr)oxides (Δ^{65}CuFe oxy(hydr)oxides-Cu(II)$_{aq}$ = 0.24 to 0.98 ‰) (Balistrieri et al., 2008; Pokrovsky et al., 2008) and to SOM (Δ^{65}CuSOM-Cu(II)$_{aq}$ = 0.15 to 0.37 ‰) (Bigalke et al., 2010b) is expected to enrich the sorbed Cu in ^{65}Cu. In contrast, preferential sorption of ^{63}Cu onto clay minerals has been recently evidenced (Δ^{65}Cukaolinite-Cu(II)aq = -0.29 to -1.46 ‰) (Li et al., 2015). Based on the mean δ^{65}Cu value of Cu fungicides (~0 ‰) and the Δ^{65}Cu during sorption of Cu$_{aq}$ to SOM and the mineral phases, the enrichment in ^{63}Cu in the clay fractions indicates preferential Cu sorption onto clay minerals (Table 6.1-2), as Cu sorption to Fe oxides or SOM would have enriched the clay fractions in ^{65}Cu. The silt fraction contains essentially primary minerals to which Cu has lower affinity compared to the secondary mineral phases in the clay fractions (Table 6.1-2) (Komarek et al., 2010). Therefore, preferential sorption of Cu to SOM likely caused the enrichment in ^{65}Cu in the silt fraction. The higher SOM/Al and SOM/Fe ratios in the silt fraction than in the clay fractions also support the idea that Cu-SOM interactions prevailed in the silt fraction.

Alternatively, Cu diffusion into the mineral matrix during Cu ageing may also cause a sequential depletion in ^{63}Cu in the clay and the fine clay soil fractions, and reduce Cu mobility and availability in the soil (Ma et al., 2006a). However, the extent and the direction of Cu isotope fractionation during Cu aging in soils are currently unknown. Aging is the slower stage of Cu retention in soils that follows the rapid sorption of Cu (Ma et al., 2006b; Sayen et al., 2009) and involves Cu diffusion into the microstructure of clay minerals or organo-clay complexes. Such Cu redistribution from cation-exchange sites to stronger binding sites at the surface of clay minerals by aging has been observed in calcareous soils (Sayen et al., 2009).

Overall, Cu distribution processes in the vineyard soils involve preferential Cu sorption in the clay fractions with concomitant enrichment in ^{63}Cu and sorption to SOM causing enrichment in ^{65}Cu in the silt fraction. This pattern is also reflected in runoff from the vineyard catchment.

5.2. Cu distribution in runoff and export from the vineyard catchment

Previous studies have shown that the dissolved phase of Cu in rivers was enriched in ^{65}Cu compared to the suspended solids due to the equilibrium partitioning of Cu isotopes between the organically complexed Cu and the Cu sorbed to the SPM phase (El Azzi et al., 2013; Vance et al., 2008). In the case of competitive Cu sorption in the vineyard soils, δ^{65}Cu values of the grain-size fractions are congruent with those in the dissolved phase of runoff provided that Cu sorption to the soil proceeds through primary Cu sorption to clays and secondary sorption of the residual Cu to the silt fraction. In this case, Cu mobilized by the rainwater and dissolved in runoff consists in the residual, less-adsorbed pool of ^{65}Cu, possibly in the form of Cu-DOM complexes (Thompson and Ellwood, 2014; Vance et al., 2008). However, no significant correlation could be observed between DOM and Cu concentrations in runoff ($\rho= 0.18$, $p=0.63$, $n=9$) (Table 6.1-7). In addition, Cu(I) oxide fungicides (Nordox, δ^{65}Cu: -0.21 ‰) may be oxidized in the soil, which is expected to release isotopically heavier Cu(II) in solution as found in the case of Cu(I) sulfide re-oxidation (Mathur et al., 2014).

It can also be hypothesized that kinetic isotope fractionation during Cu ageing in the vineyard soil results in higher δ^{65}Cu values in the runoff. Higher dissolved δ^{65}Cu values in runoff than in the soil solutions following particle-size distribution supports the idea of kinetic isotope fractionation during Cu aging in the vineyard soils (Figure 6.1-4). In this case, δ^{65}Cu values of Cu dissolved in runoff reflect the stage of Cu ageing in the vineyard soils, with higher

δ^{65}Cu values corresponding to the mobilization of freshly added Cu and decreasing δ^{65}Cu values over time, during Cu aging. Freshly applied Cu is expected to be more mobile than older ('aged') Cu in the soil, as indicated by larger weekly dissolved loads of Cu in runoff following Cu fungicide applications (Figure 6.1-5). However, no correlation between the δ^{65}Cu values of Cu fungicides and δ^{65}Cu of runoff-associated Cu could be observed (Figure 6.1-2). The contribution of freshly applied Cu fungicides to the δ^{65}Cu values in runoff could be evaluated using flow-proportional sampling during a rainfall-runoff event.

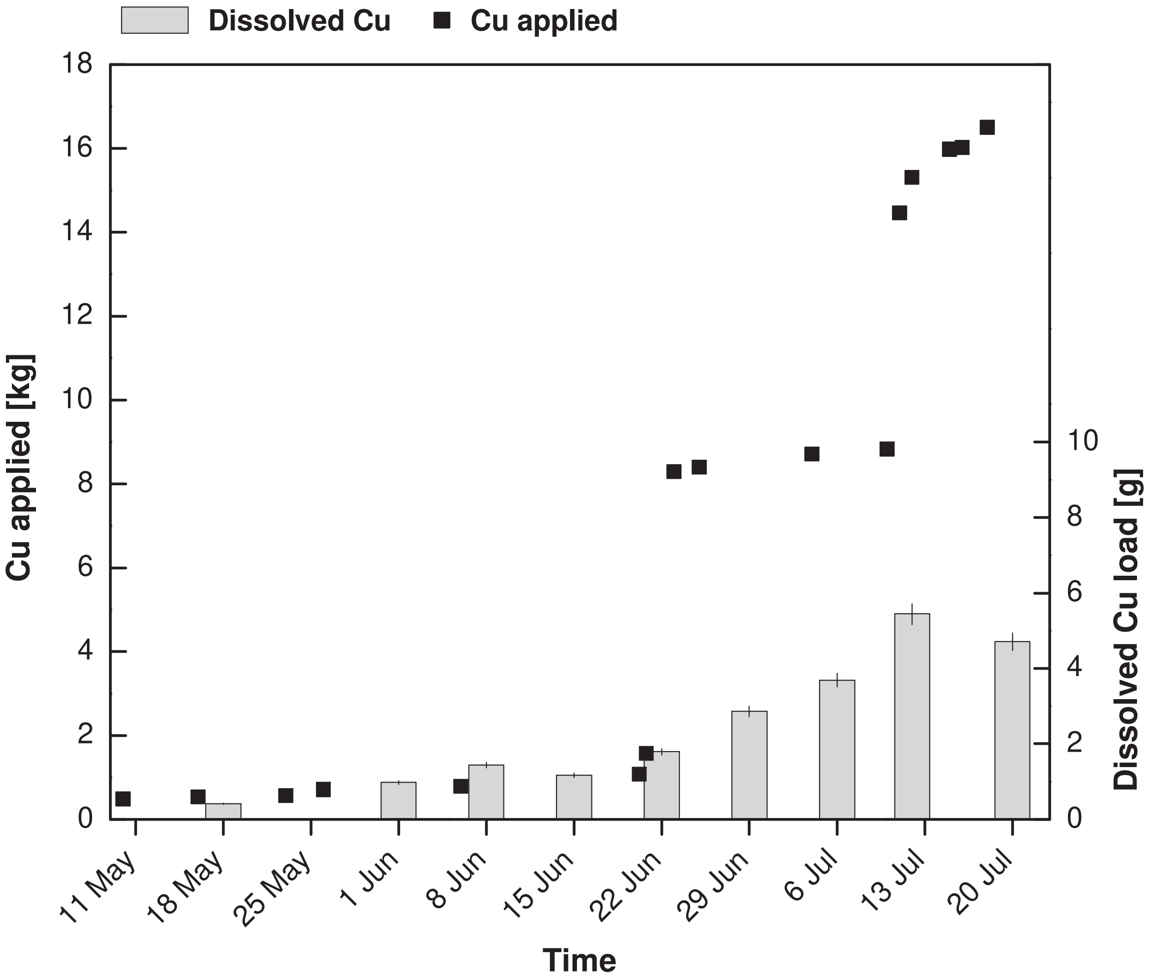

Figure 6.1-5: The relationship between Cu-based fungicide applications and weekly dissolved copper loads in runoff

The relatively small export of Cu by surface runoff (~1 % of the applied Cu amount), which included the Cu drifted on roads, emphasizes the low mass transfer of anthropogenic Cu from the vineyard soils. The Cu export in runoff was mainly associated with SPM (56 to 93 % depending on the rainfall-runoff events). This indicates that erosion was the major process mobilizing anthropogenic Cu during rainfall-runoff events from vineyards. The negative δ^{65}Cu values of the SPM-bound Cu in runoff suggest that Cu was mainly bound to the clay and fine clay fractions (Figure 6.1-2). The relationship between δ^{65}Cu values and the molar ratios of Fe/Cu in the SPM indicates a mixing between the clay fractions and the silt fractions of the topsoil (0-5 cm) as end members (Figure 6.1-4), and the high contribution of the clay soil fractions to the SPM-bound Cu exported by runoff.

Rainfall characteristics may also influence Cu mobilization from the soils through erosion processes and Cu export from the vineyards. Intense rainfall events resulted in higher concentrations of dissolved Cu in the runoff (r=0.96, p=0.003, n=7, without the June 8 sample) that may be due to the higher contribution of runoff generated on the vineyard soils. The Al/Ca ratios was used as an index for particle-size distribution in the SPM, as the silt contained high Ca (due to carbonates) and the clay minerals were enriched in Al (Chen et al., 2014). Al/Ca ratios indicate that SPM in runoff (0.5 to 2.0) consisted of a mixture of silt (0.2 to 0.4) and clay (2.2 to 2.8) (Figure 6.1-6). The Cu/Ca ratios show that during intense rainfall events, coarser materials containing less Cu (such as silt and sand) were mobilized along with Cu-enriched soil particles (such as clays) (Figure 6.1-6). The greater contribution of the coarser soil particles to the Cu export decreased the total concentration of SPM-bound Cu in runoff. The coarser soil particles increased the quantity of the exported SPM and thus that of SPM-bound Cu, but to a proportionally lower extent than if an equivalent mass of Cu-enriched clays was exported (Figure 6.1-7).

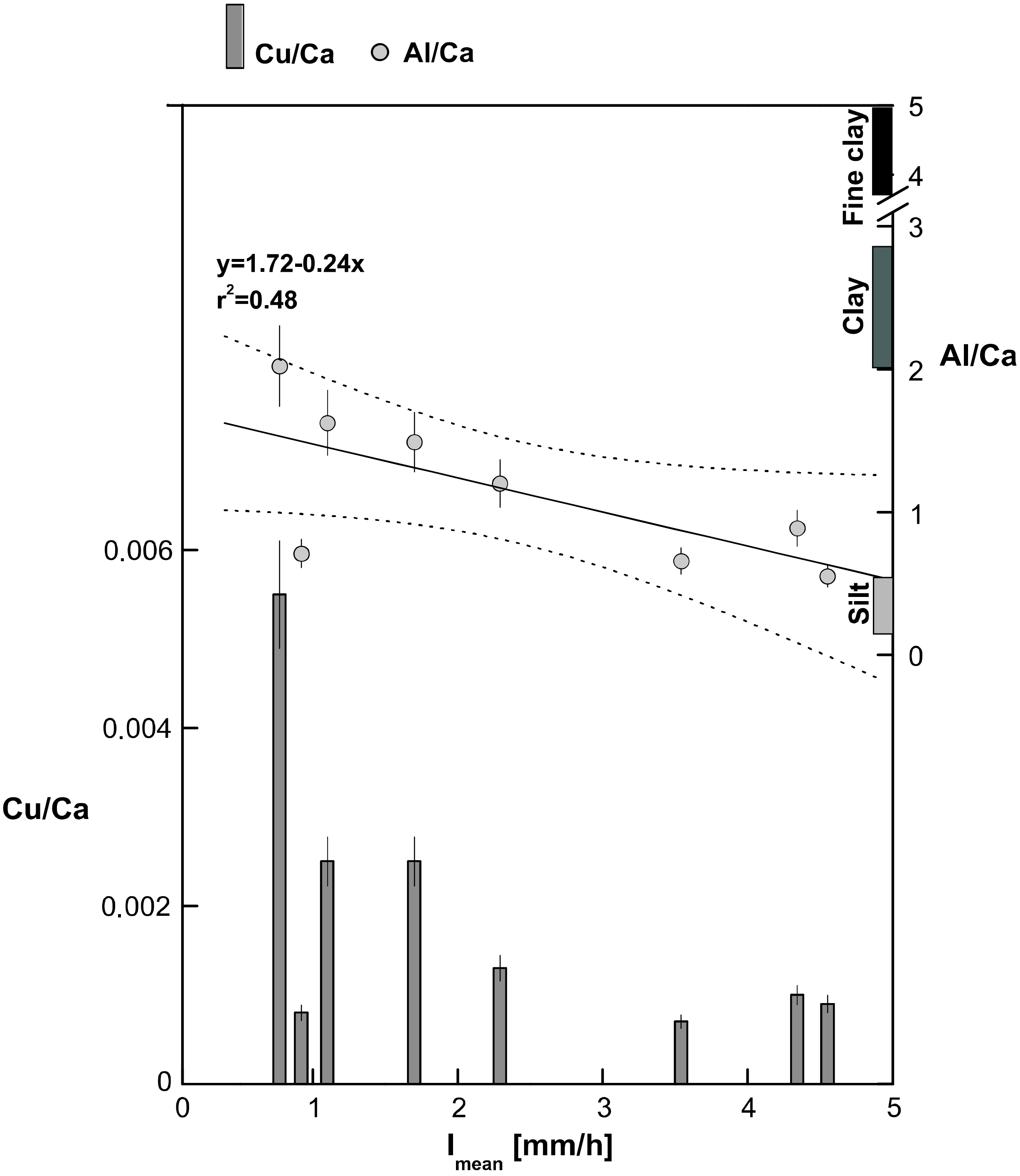

Figure 6.1-6: The figure shows how the rainfall intensity affects the erosion processes and the chemical patterns of SPM transported by runoff (Ca normalized Cu concentrations and Al to Ca molar ratios) at the outlet of the vineyard catchment (Rouffach, France). Error bars correspond to the propagated analytical uncertainties

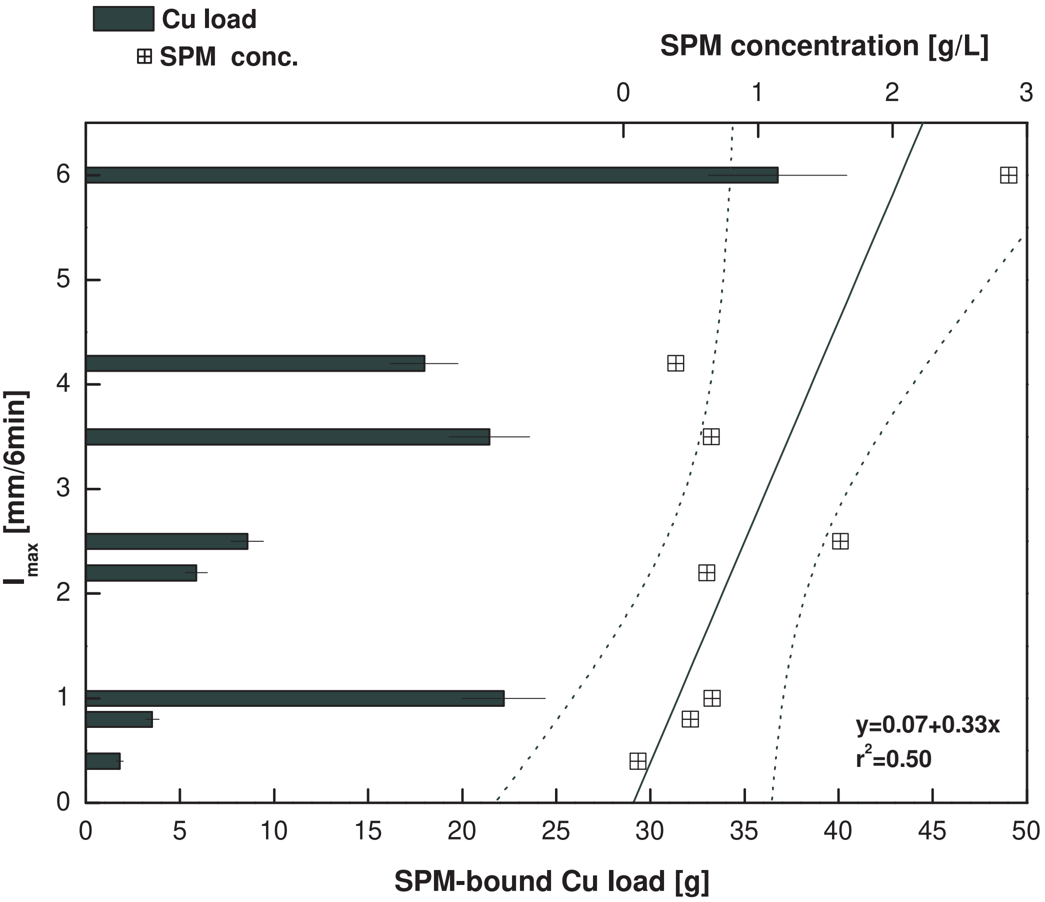

Figure 6.1-7: The export of SPM and SPM-bound Cu loads as influenced by the rainfall intensity

6. Conclusion

We investigated the distribution of Cu-fungicides in the grain-size fraction of vineyard soils and runoff transport at the catchment scale using Cu stable isotopes, mineralogical, hydrological and hydrochemical analyses. Although the δ^{65}Cu values in the bulk soil were in the same range as those of Cu fungicides and geogenic Cu, the δ^{65}Cu values differed between particle-size soil fractions. Together with the soil mineralogy, the δ^{65}Cu values in the soil fractions suggest that the clay fractions govern the Cu isotope distribution in the vineyard soils, either by equilibrium sorption to clay minerals or by kinetic isotope effects during the aging process. The distribution of Cu in the different soil fractions also influenced the Cu mobility and transport during rainfall-runoff events. Isotopically heavier dissolved Cu and lighter SPM-bound Cu in runoff may be generated the vineyard soils during Cu distribution between the soil constituents and Cu mobilization by rainfall. The negative δ^{65}Cu values and Al/Ca ratios of the SPM-bound Cu in runoff indicated that Cu was mainly bound to the clay and fine clay fractions, although coarser particles can also contribute to Cu export during intense rainfall events. Cu export by runoff from the catchment accounted for 1 % of the applied Cu mass on a seasonal basis, 84 % of which was Cu bound to SPM. Overall, the results show that Cu distribution processes occurring in soils of agricultural catchment may be relevant for interpreting the resulting δ^{65}Cu values in runoff and predicting the Cu load transported downstream to vulnerable aquatic ecosystems. The isotope fractionation upon individual processes influencing Cu distribution in soils, especially those driving the long-term Cu transformation (i.e., aging), may help in the future to better understand the fate of anthropogenic Cu in contaminated soils.

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Partie 6.2. Impact des patrons de pluie sur l'export des métaux et pesticides polaires d'un bassin versant viticole

Fatima Meite, Benoit Guyot, Sylvain Payraudeau, Gwenaël Imfeld

1. Abstract and highlights

Runoff is a major process of pesticide transport from agricultural land to downstream aquatic ecosystems. The impact of rainfall characteristics on the transport of runoff-related pesticide is crucial, although it is rarely evaluated at the catchment scale and during rainfall-runoff events. Here we examined the effect of rainfall patterns on concentrations and loads of copper (Cu, used as a fungicide), zinc (Zn, frequently used for wine growing) and common polar pesticides (carbendazim, cymoxanil, isoproturon, diuron, isoxaben, oryzalin and flufenoxuron) in runoff from a vineyard catchment. The characteristics of rainfall event (i.e. rainfall intensity, depth and duration) clustered in "small", "long", "moderate" and "intense" patterns controlled the transport of pesticide loads in runoff. Loads of pesticides and metals in runoff normalized by their respective soil concentrations significantly increased with rainfall depth, rainfall intensity and duration, as well runoff volumes and maximal discharge. In contrast, mean event concentrations were generally not impacted by rainfall or runoff characteristics. Small rainfall pattern differed from other patterns with respect to metals and pesticides export generated. This likely reflects that distinct zones of the vineyard catchment contributed to runoff. Although pesticide characteristics as well as the amount and the timing of applications are drivers for pesticide runoff, our results emphasise that rainfall patterns also determine pesticide transport in small headwater catchment. This suggests that the forecasted changes of pattern distribution of regional rainfalls may severely influence both metal and pesticide export from agricultural lands.

Highlights:

- Runoff loads of pesticides and metals significantly increased with rainfall depth, rainfall intensity and duration, as well runoff volumes and maximal discharge
- Mean event concentrations were generally not impacted by rainfall or runoff characteristics

- Small rainfall pattern differed from other patterns with respect to metals and pesticides export generated

2. Introduction

Surface runoff is a major process of contaminant transport from agricultural catchments. A significant portion of pesticides (0.1 to 5 %) applied to fields can move during rainfall-runoff events to downstream aquatic ecosystems (Liess and von der Ohe, 2005; Lefrancq et al., 2013; Lefrancq et al., 2017). Pesticide runoff may significantly threat drinking water resources and aquatic ecosystems (Liess and von der Ohe, 2005; Probst et al., 2005; Elsaesser et al., 2011). Transport of pesticides in runoff is controlled by the timing and amount of pesticide applications as well as by hydrological characteristics (Muller et al., 2003; Leu et al., 2004; Nolan et al., 2008; McGrath et al., 2010). Improving prediction of pesticide export from agricultural lands accounting for hydrological characteristics is crucial in the context of climate change. However, knowledge of the relationship between rainfall characteristics and pesticide runoff from agricultural lands remains scarce.

Pesticide concentrations and loads in runoff largely vary during the agricultural season and between years (Elsaesser et al., 2011; Grégoire et al., 2010; Maillard et al., 2011a, 2011b). Fungicides, including Cu, and herbicides are generally used throughout the growing season, and application dose and timing determine the pesticide pool that can be mobilized during rainfall-runoff events. Physico-chemical properties of pesticides and their persistence in soils mainly control pesticide mobilization and runoff during storm events (Ellis et al., 2010; Saison et al., 2010). For instance, vineyards with steep slopes are generally prone to soil erosion, transporting both dissolved and particulate-bound pesticides to downstream aquatic ecosystems (El Azzi et al., Babcsanyi et al., 2016). Metals and hydrophobic pesticides efficiently sorb to soil organic matter (SOM), while dissolved organic matter (DOM) complexes with metals and maintain them in the water phase, which may favour their mobilization by rainfall (Komarek et al., 2010). Export of pesticides in runoff from vineyard catchments is commonly observed, although it usually represents less than 1% of the applied amounts (Louchart et al., 2001; Grégoire et al., 2010; Rabiet et al., 2010; Maillard et al., 2011a, Babcsanyi et al., 2016). However, the effect of rainfall characteristics on both concentrations and loads of pesticides in runoff during the growing season is a poorly known (McGrath et al., 2010), in particular in small headwater catchments.

The impact of rainfall characteristics on nonpoint source pesticide contamination is typically investigated in large catchments (> 10 000 ha) (Kuivila and Foe, 1995; Domagalski, 1996; Kratzer, 1999; Zhang et al., 2008). Although rainfall depth seems to determine transport of runoff-related pesticides in larger catchments (Clark et al., 1999), the timing relative to pesticide application may be a prevailing factor in smaller and headwater catchments (< 10 000 ha) (Leu et al., 2004; Leu et al., 2005; Hively et al., 2011). Headwaters in agricultural areas receive proportionally higher pesticide inputs (Lorenz et al., 2016). In headwater catchments, pesticide export dynamics is generally discontinuous and complex, depending on rainfall occurrence and seasonal pesticide inputs (Stehle et al., 2012). Soil water contents, vegetation and the soil pool of pesticide available to runoff can alter pesticide export in runoff but remain difficult to predict at the catchment scale due to soil heterogeneity and temporal variation (Zehe and Bloschl, 2004; Payraudeau and Grégoire, 2011). In addition, large variability of rainfall-runoff characteristics requires a detailed design for storm sampling (Payraudeau and Grégoire, 2011; Coupe et al., 2012; Lefrancq et al., 2017) to quantitatively determine the effect of rainfall characteristics on pesticide runoff.

The purpose of this study was thus to evaluate the relationship between the rainfall patterns and the transport of runoff-related pesticides, Cu and Zn from a small (42 ha) headwater vineyard catchment. As runoff is a threshold process that is intimately linked with rainfall (Zehe et al., 2005; Spence, 2010), we hypothesized that rainfall patterns (i.e., variations in rainfall depth, intensity and duration) can influence runoff export of both synthetic pesticides and inorganic inputs (Cu and Zn) in small vineyard catchment. The influence of different rainfall characteristics on the runoff of Cu, Zn and selected polar synthetic pesticides observed during two hydrologically contrasted wine-growing seasons (2015 and 2016) was compared accounting for the initial pesticide pools in the soil. Widely-used fungicides cymoxanil and carbendazim, herbicides (isoxaben and isoproturon), a novel herbicide (oryzalin), as well as hazardous compounds, which are progressively forbidden in the EU (therbicide diuron and insecticide flufenoxuron), were selected for the present study. This selection was made based on their widespread former and current use and the high frequency of application and detection in runoff from the study catchment (Grégoire et al., 2010; Maillard et al., 2014).

3. Materials and methods

3.1. Vineyard catchment

A description of the vineyard catchment studied is made in Chapter 2, part 2. This catchment is represented in Figure 6.2-1 along with the zones where pesticides were applied in 2015 and 2016. Annual rainfall averaged 637 ± 112 mm (1998-2014) and was 521 and 733 mm in 2015 and 2016 respectively. From vineyard growing season, i.e. March to October rainfall averaged 441 ± 89 (1998-2014) with 375 and 434 mm for 2015 and 2016, respectively.

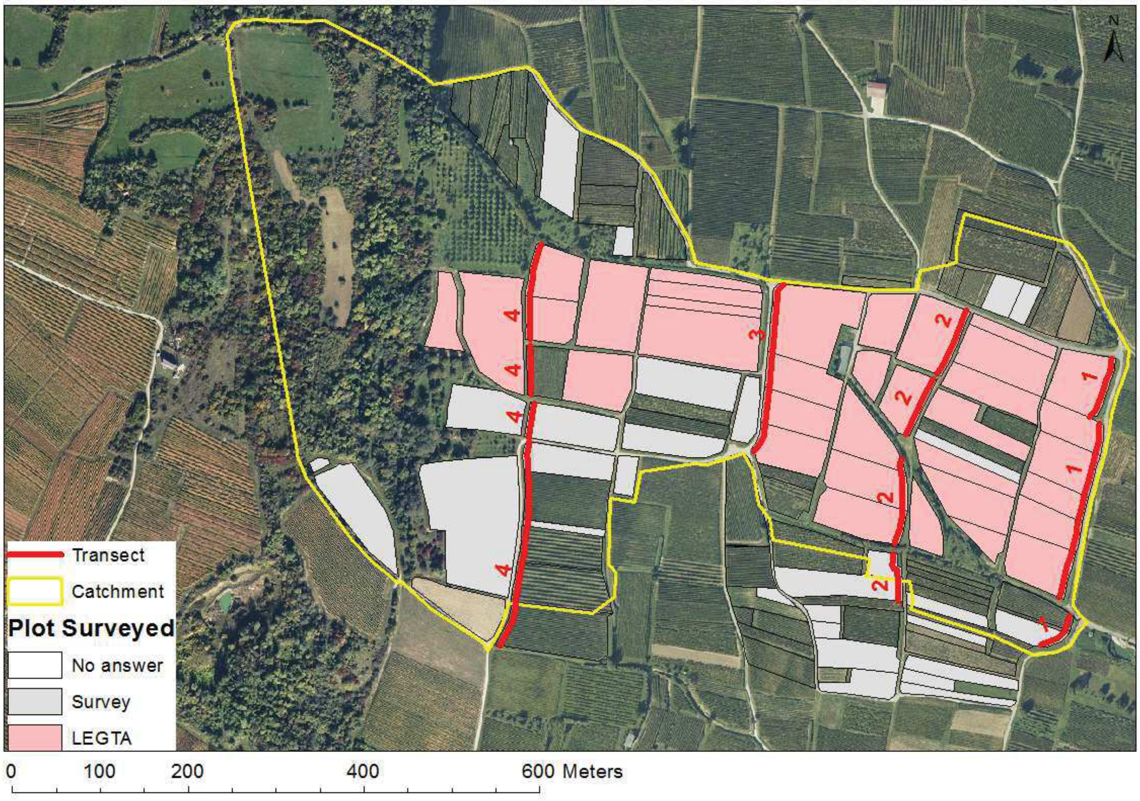

Figure 6.2-1: Rouffach catchment delineation with the 4 top soil transects. Pesticides applications were determined on the grey and pink plots (2015 and 2016)

The mean runoff coefficient in the catchment was 0.98 % during the study period (from March 26 to October 6 2015 and from March 30 to October 10 2016). The rainfall-runoff events do not generate a permanent stream in the catchment.

Discharge at the catchment's outlet is driven by Hortonian overland flow. The road network mainly contributes to the discharge during low rainfall intensity (i.e. > 6 mm.h^{-1}) (Lefrancq et al., 2014), while the vineyard plots contribute when the rainfall intensity overcomes the saturated hydraulic conductivity (i.e. 58 mm.h^{-1} ± 50, n = 48) (Tournebize et al., 2012). The saturated hydraulic conductivity can be reduced by one order of magnitude on plots when top soil sealing appears. Overland flow on plots is reduced by grass strips of 2 to 3 m width at the plot edges (Lefrancq et al., 2014). Hortonian overland flow prevails, as indicated by fast hydrological response to rainfall events (i.e. 6 to 12 min between rainfall and runoff peaks) and fast recession period (< 1 h) with total discharge cessation at the outlet. The contribution of baseflow is thus unlikely as steep slopes (i.e. 15 %), with high vertical saturated hydraulic conductivity prevent downslope lateral flow on saturated zones.

The type of soil and vegetation of this catchment are presented in Chapter 2, part 2.

3.2. Estimation of pesticide applications

Pesticide applications were estimated based on surveys addressed to the wine producers of the catchment. Cu fungicides are applied almost on all vineyards (> 97 %) in the catchment from May to August in quantities of about 2.2 ± 0.3 kg (of the fungicide preparation).ha^{-1}.y^{-1} (2008–2011). Targeted chemical properties as well as summary data of pesticide application and loss in surface runoff are provided in Table 6.2-1. Results of the field survey for the yearly application amounts of each chemical sorted by dates to evaluate the variation of applied amounts and application timing among pesticides and years are not available yet and could not be included in this thesis.

Table 6.2-1: Application, top-soil background and export coefficient of Cu, Zn and the seven pesticides

	Cu	Zn	Carbendazim	Cymoxanil	Isoproturon	Diuron	Isoxaben	Oryzalin	Flufenoxuron
Applied 2015 (kg)	58.72	2.21	n.a.	2.18	n.a.	n.a.	0.12	1.38	n.a.
Applied 2016 (kg)	w.s.	w.s.	n.a.	w.s.	n.a.	n.a.	w.s.	w.s.	n.a.
Initial topsoil (0-1 cm) conc. (2015) mg kg^{-1} dry soil	154	112	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Initial topsoil (0-1 cm) conc. (2016) mg kg^{-1} dry soil	120	80	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Initial topsoil (0-1 cm) mass (2015) kg	545.3	396.6	1.77	1.77	1.77	1.77	1.77	1.77	1.77
Initial topsoil (0-1 cm) mass (2016) kg	424.9	283.3	1.77	1.77	1.77	1.77	1.77	1.77	1.77
Load 2015 (kg for Cu and Zn ; g for pesticides)	1.45	1.63	0.013	11.47	0.068	2.47	0.013	2.60	0.242
Load 2015 (kg for Cu and Zn ; g for pesticides)	1.68	1.81	0.019	5.08	0.122	3.58	1.94	1.47	5.08
Export coef. of application only 2015 (%)	2.47	73.8	-	0.526	-	-	0.010	0.189	-
Export coef. of application only 2016 (%)	w.s.	w.s.	-	w.s.	-	-	w.s.	w.s.	-
Export coef. application and background 2015 (%)	0.24	0.41	0.001	0.29	0.004	0.139	0.001	0.083	0.014
Export coef. application and background 2016 (%)	w.s.	w.s.	0.001	w.s.	0.007	0.202	w.s.	w.s.	0.287

n.a.: not applied

w.s.: waiting results of survey

3.3. Top soil, rainfall and runoff collection

3.3.1. Rainfall collection

Rainfall data at a resolution of six minutes were recorded within the study catchment with tipping bucket (Meteo France, station n°68287003). According to the fast hydrological response time to rainfall event, a dried period of 2 h was selected to extract the rainfall events from the 6 minutes database.

3.3.2. Top soil and runoff collection

An average concentration in top soil (0-1cm) of Cu, Zn and the seven polar pesticides were determined by weekly sampling on four transects across the catchment (Figure 6.2-1) during the 2015 and 2016 growing seasons. The weekly sample was composed of 1 kg of top soil composed of sub-samples collected about each 20 m along the transects. This length-proportional weekly sample was placed on ice during transportation to the laboratory.

Automatic runoff measurement and water sampling was carried out at the outlet of the catchment (Figure 6.2-1) March 26th to October 6th 2015 and from March 30th to October 10th 2016). Runoff were monitored by measuring water depth using bubbler flow modules (Hydrologic, Sainte-Foy, Québec, Canada) combined with a Venturi channel. Flow proportional samples of runoff water were collected during each runoff event (300 mL every 18 m^3) using a 4010 Hydrologic automatic sampler (Hydrologic, Canada) (Maillard and Imfeld, 2014). Runoff water samples were collected in a 24-bottles sampler consisting of glass bottles for pesticides analysis (even numbers, e.g. 2, 4, 6,..), and PEHD bottles for Cu and Zn analysis (odd numbers, e.g. 1, 3, 5, …). Flow-proportional water samples were stored in the dark at 4 °C after each runoff event, and placed on ice during transportation to the laboratory. Water samples collected for pesticide measurements were extracted within 24 h for pesticide analysis. The detailed procedure of sample collection and storage ensuring reliable pesticide measurements was previously tested and described (Grégoire et al., 2010; Domange and Grégoire, 2006).

3.4. Chemical analyses

3.4.1. Pesticides

The extraction of pesticides from soil samples is presented in Chapter 2, part 3.1.

The runoff water samples were filtered using a 0.45 µm cellulose acetate membrane (Millipore). Pesticides were quantified from the filtrate water after solid phase extraction as explained in Chapter 2, part 3.1.

The quantification of pesticides with LC-MS was made according to Chapter 2, part 3.1. The pesticides recoveries, detection and quantification limits are summarized in Tables 2-4 and 2-5.

3.4.2. Cu and Zn

The treatment of samples and the quantification of elements including Cu and Zn is explained in Chapter 2, part 3.2.

3.5. Data analysis

3.5.1. Clustering analysis of rainfall events

The 82 rainfall events were classified by cluster analysis based on rainfall depth, maximum rainfall intensity and rainfall duration values. Rainfall data treatment was performed using the R software (*R Development Core Team, 2008;* Version 3.4.3). The values were standardized (Legendre and Gallagher, 2001) prior to calculating of the distance between rainfall events based on the Euclidean dissimilarity index. A hierarchical cluster analysis was performed on the resulting dissimilarity matrix using the Ward's method (Ward 1963). The optimal number of clusters was determined using the Spearman's rank correlations applied to the binary distance matrix from clusters (Becker et al., 1988). The clusters of rainfall patterns corresponded to different types of rainfall events displaying similar characteristics.

3.5.2. Rainfall return period

A Gumbel law (Gumbel, 1958; Papalexiou and Koutsoyiannis, 2013) was applied on the 6 min MeteoFrance database from 1998 to 2014 to derive the return period for the 82 rainfall events depending of the mean rainfall intensity and rainfall event duration. For each of the 82 events, the maximum return period was retrieved from the entire event to determine the most intensive period during the event. For example, a rainfall event with a total duration of 120 min can be characterized by a 2 year return period but present a maximum of 10 years return period for the most intensive 20 min.

3.5.3. Runoff-related Cu, Zn and pesticides

Event Load. For the calculations of the Event Loads (*EL*) of Cu, Zn and pesticides, each flow-proportional instantaneous concentrations was multiplied with the representative runoff volume monitored according to Eq. 6.2-1:

$$EL_p = \sum_{i=1}^{n} C_{pi} V_i \qquad (6.2\text{-}1)$$

Where EL_p is the event load for the pollutant *p* (Cu, Zn or pesticides, in [g] for Cu and Zn, and [mg] for the seven pesticides), C_{pi} is the instantaneous concentration of *p* (Cu, Zn or pesticides) of the ith sample (only even numbers for pesticides [µg L^{-1}] and odd numbers for Cu and Zn [mg L^{-1}], V_i is the representative runoff volume monitored (accounting for successive even and odd samples) [m^3]. Dissolve and particulate loads were computed for Cu and Zn and only dissolve loads were calculated for the seven pesticides.

Based on the weekly top soil concentrations of pollutant, a normalized EL_{norm} was calculated to integrate changes to the top soil pool of Cu, Zn and the seven pesticides. Hence co**m**parisons can be **m**ade even if dissipation of the pesticides occurs in soils or the re**m**aining **m**asses of pollutants differ due to important rainfall events. EL_{norm} is calculated according to Eq. 6.2-2:

$$ELnorm_p = EL_p \times \frac{Background\ concentration\ top\ soil_p}{Concentration\ top\ soil_{wp}} \qquad (6.2\text{-}2)$$

where Concentration in top soil$_{\mathrm{wp}}$ corresponds to the mean concentration of pollutant *p* (Cu, Zn and the seven pesticides) collected the week *w* during the wine-growing season before the rainfall-runoff event. The background concentration for pollutant *p* corresponds to the initial concentration at the beginning of the season for Cu and Zn and to the detection limit concentration for the seven pesticides.

Event Mean Concentration. The event mean concentration (EMC_p) of Cu, Zn and the seven pesticides was calculated as the ratio of the corresponding event load ($ELnorm_p$) on the total event runoff volume (*V*) according to Eq. 6.2-3:

$$EMC_p = \frac{ELnorm_p}{V} \qquad (6.2\text{-}3)$$

The EMC for each pollutant *p* (Cu, Zn and the seven pesticides) were expressed in mg L^{-1} for dissolve and particulate Cu and Zn and in µg L^{-1} for dissolve pesticides.

3.6. Statistical analysis of pesticide metrics

Non-parametric Kruskall-Wallis and pair-wise Wilcoxon rank sum tests were used to determine whether the type of rainfall events significantly affects runoff pesticide metrics ($p < 0.1$). Rank-based Spearman's test ($\alpha = 0.1$) was used to assess the correlation between rainfall-runoff characteristics and runoff pesticide metrics (i.e. event mean concentrations and dissolved event loads).

4. Results and discussion

4.1. Typology of the rainfall events

The hydrological dataset comprises all rainfall-runoff events monitored with at least one sample collected. Among the 107 rainfall-runoff events monitored during the two wine-growing seasons (Table 6.2-2), four were excluded due to low runoff volume (5.6 ± 4.2 m^3) below the threshold to activate the sampler and 21 due to sampler malfunction, e.g. clogging by floating vegetation (23.8 ± 22.7 m^3). Detailed hydrological data of the 82 storm events studied are provided in annex (Table 6.2-3).

Table 6.2-2: Characteristics of the rainfalls generating runoff collected from March to October 2015 and 2016. The rainfall generated runoff enabling to sample at least one water sample

		2015	**2016**
Total yearly rainfall depth	(mm)	521	733
Study period[1]			
Number of rainfall event	(-)	79	107
Number of rainfall-runoff events	(-)	48	59
Number of rainfall-runoff events with samples	(-)	32	50
Rainfall depth	(mm)	375	434
Rainfall depth associated to runoff events	(mm)	314	378
Rainfall depth associated to runoff events with samples	(mm)	239	348
Proportion of rainfall depth generating runoff	(%)	84	87
Proportion of sampled rainfall-runoff depth generating runoff	(%)	76	92

[1] March 26 to October 6 2015 and from March 30 to October 10 2016

Table 6.2-3: Characteristics of the 82 rainfall-runoff events including clustering-based topology in “small”, “long”, “moderate” and “intense” types

Event	**Date**	**Rainfall**			**Runoff**				**Runoff coefficient**	**Rainfall event type**
		Tot$_{rainfall}$	**I$_{max}$**	**t$_{rainfall}$**	**Q$_{mean}$**	**Q$_{max}$**	**V**	**t$_{runoff}$**		
		[mm]	[mm h^{-1}]	[mn]	[l s^{-1}]	[l s^{-1}]	[m^3]	[mn]	[%]	
3	29/03/2015 19:36	9.2	4	834	0.58	1.75	26.1	750	0.7	Long
5	31/03/2015 16:42	1.2	4	48	0.06	0.11	1.0	277	0.2	Small
11	02/04/2015 15:36	4.0	4	126	2.24	4.19	18.7	139	1.1	Small
12	03/04/2015 17:54	3.2	2	450	0.20	1.19	6.2	526	0.4	Moderate
13	04/04/2015 09:12	7.8	2	630	1.72	3.50	57.1	554	1.7	Moderate
22	01/05/2015 00:06	36.1	8	1554	3.11	12.89	289.6	1553	1.9	Long
23	02/05/2015 22:54	8.4	4	480	2.28	5.22	90.6	662	2.5	Moderate
25	03/05/2015 17:00	3.6	6	234	2.02	5.03	47.0	387	3.0	Moderate
44	14/06/2015 20:54	18.5	12	864	1.54	9.50	85.7	925	1.1	Long
45	15/06/2015 22:12	5.9	39	30	2.97	43.42	54.1	304	2.1	Intense
49	21/06/2015 05:18	1.2	4	144	0.47	1.08	0.9	31	0.2	Small
50	22/06/2015 21:18	4.0	6	186	2.14	3.69	14.9	116	0.9	Small
54	18/07/2015 01:36	1.4	6	78	0.06	1.17	1.2	334	0.2	Small
55	18/07/2015 09:36	3.2	4	66	0.49	3.19	10.0	340	0.7	Small
56	19/07/2015 07:06	2.4	10	30	1.57	2.69	5.7	61	0.6	Small
57	22/07/2015 13:48	24.2	84	36	9.99	102.47	130.7	218	1.3	Intense
58	22/07/2015 18:54	12.6	55	18	9.63	91.79	89.5	155	1.7	Intense
59	23/07/2015 01:36	1.8	10	6	0.34	0.74	0.9	45	0.1	Small
60	25/07/2015 02:06	3.8	18	12	0.39	5.92	7.1	301	0.4	Small
61	26/07/2015 18:54	1.4	4	42	0.02	0.23	0.3	296	0.1	Small
68	13/08/2015 21:12	4.4	4	258	0.16	1.00	5.2	537	0.3	Moderate
69	14/08/2015 16:12	1.0	2	42	0.09	0.81	1.6	306	0.4	Small
70	15/08/2015 23:30	3.4	2	672	0.19	0.89	7.2	616	0.5	Moderate
71	16/08/2015 14:00	1.0	2	96	0.05	0.78	1.0	353	0.2	Small
75	24/08/2015 07:36	8.6	14	138	2.50	7.39	20.7	138	0.6	Small
83	13/09/2015 19:24	23.3	57	522	3.38	41.67	166.4	820	1.7	Intense
86	16/09/2015 05:42	6.2	4	456	5.65	14.92	79.4	234	3.0	Moderate
88	17/09/2015 06:18	17.7	8	486	8.82	26.81	295.1	558	3.9	Long
100	04/10/2015 00:06	10.4	6	390	0.88	3.58	33.3	633	0.7	Moderate
102	05/10/2015 18:00	2.8	4	228	0.43	1.94	7.0	272	0.6	Moderate
103	06/10/2015 13:06	4.0	10	102	0.76	6.44	16.9	372	1.0	Small
104	06/10/2015 21:54	2.0	4	198	0.32	2.22	6.0	313	0.7	Moderate
108	01/04/2016 06:30	2.4	4	336	0.11	0.22	1.7	254	0.2	Moderate
109	04/04/2016 08:06	2.6	8	42	1.00	2.64	6.1	102	0.5	Small
111	05/04/2016 08:42	12.8	4	858	1.35	4.50	73.6	908	1.3	Long
114	12/04/2016 02:42	3.2	12	84	0.32	2.67	6.9	365	0.5	Small
115	12/04/2016 18:30	6.6	6	324	0.91	5.36	24.4	447	0.9	Moderate
116	13/04/2016 07:18	2.8	6	144	0.88	3.40	9.3	175	0.8	Small
117	13/04/2016 22:00	1.2	2	54	0.14	0.28	0.9	110	0.2	Small
118	15/04/2016 07:00	5.0	4	270	0.57	2.19	19.2	558	0.9	Moderate
119	15/04/2016 20:42	7.0	6	408	1.69	6.67	37.0	364	1.2	Moderate

122	16/04/2016 16:24	2.0	8	114	0.66	2.78	5.8	146	0.7	Small
123	16/04/2016 22:06	7.6	8	288	2.39	4.53	54.6	381	1.7	Moderate
124	17/04/2016 05:30	7.6	6	366	2.15	5.81	65.9	511	2.0	Moderate
125	17/04/2016 16:24	3.0	2	300	0.84	1.64	17.2	341	1.3	Moderate
126	18/04/2016 02:30	1.4	2	216	0.59	1.36	6.7	191	1.1	Moderate
127	18/04/2016 09:12	2.0	2	246	0.63	1.83	12.6	333	1.5	Moderate
129	23/04/2016 10:12	8.8	4	582	0.85	2.14	13.6	267	0.4	Moderate
134	26/04/2016 11:12	2.6	10	48	0.41	3.11	6.0	244	0.5	Small
135	26/04/2016 15:18	2.6	6	300	0.15	0.44	2.2	241	0.2	Moderate
136	26/04/2016 22:48	1.0	4	12	0.47	1.08	1.6	58	0.4	Small
139	30/04/2016 16:00	15.3	4	696	1.03	1.44	15.5	252	0.2	Long
142	01/05/2016 19:42	8.1	32	78	3.88	20.11	59.7	256	1.7	Intense
153	12/05/2016 01:36	17.7	18	354	3.56	11.97	80.6	377	1.1	Intense
155	13/05/2016 00:24	4.2	10	96	2.51	5.42	20.8	138	1.2	Small
156	13/05/2016 11:42	3.4	6	102	1.14	4.33	11.5	168	0.8	Small
157	13/05/2016 20:06	4.0	18	66	0.63	9.69	12.9	342	0.8	Small
159	14/05/2016 11:06	5.5	45	18	3.76	24.44	40.9	181	1.7	Intense
162	18/05/2016 21:48	5.6	10	72	1.95	4.92	11.8	101	0.5	Small
163	19/05/2016 01:30	4.4	16	48	0.99	10.61	20.6	348	1.1	Small
169	28/05/2016 22:30	14.0	51	240	4.29	66.89	116.1	451	1.9	Intense
175	02/06/2016 03:00	9.2	4	360	2.31	5.99	59.1	427	1.5	Moderate
176	03/06/2016 06:00	0.6	2	48	0.11	0.22	1.1	162	0.4	Small
177	03/06/2016 08:48	0.8	2	30	0.65	0.86	1.7	45	0.5	Small
179	04/06/2016 15:00	14.7	49	306	1.93	44.67	61.9	534	1.0	Intense
180	05/06/2016 15:48	1.2	4	24	0.13	0.48	1.0	138	0.2	Small
181	06/06/2016 01:30	1.4	8	18	0.43	0.81	2.3	88	0.4	Small
183	08/06/2016 12:54	56.3	114	630	18.32	166.47	773.1	703	3.2	Intense
186	11/06/2016 04:42	4.6	4	162	1.99	3.44	27.5	230	1.4	Small
187	11/06/2016 18:24	4.8	2	402	1.19	2.75	36.4	511	1.8	Moderate
188	12/06/2016 05:36	1.0	2	108	0.69	1.69	7.4	180	1.7	Small
189	12/06/2016 16:30	1.0	2	144	0.07	0.61	0.5	110	0.1	Small
191	13/06/2016 02:48	2.2	4	114	0.83	1.74	11.6	234	1.2	Small
192	14/06/2016 09:36	4.0	4	144	1.25	3.03	18.1	241	1.1	Small
196	15/06/2016 07:30	4.6	6	252	1.12	5.03	21.3	316	1.1	Moderate
208	24/06/2016 21:00	31.4	26	498	12.33	90.19	238.2	322	1.8	Intense
213	13/07/2016 08:54	1.6	6	54	0.77	2.03	3.1	68	0.5	Small
214	13/07/2016 13:00	3.8	14	174	0.23	2.85	2.3	166	0.1	Small
216	14/07/2016 07:24	2.8	6	90	0.77	2.50	5.3	114	0.4	Small
217	14/07/2016 12:24	2.6	6	120	0.56	1.69	2.7	79	0.2	Small
227	04/08/2016 16:30	16.7	18	306	0.72	6.75	24.1	562	0.3	Intense
245	18/09/2016 02:30	18.7	8	900	0.26	3.31	35.5	2299	0.4	Long

During the study period, the 107 rainfall-runoff events represented 84 % (2015) and 87 % (2016) of the total recorded rainfall (Table 6.2-2). The 82 rainfall-runoff events retained for further analysis covered 76 % (2015) and 92 % (2016) of the total rainfall depth generating runoff during wine-growing seasons (Table 6.2-2).

An intensity-duration-frequency analysis using the Gumbel law revealed that the 82 events covered return periods ranging from 1 to 45 years (Figure 6.2-2). For comparison, the four artificial rainfalls used in the lab-scale experiment (Chapter 5) were added in Figure 6.2-2 (Meite et al., 2018). Observed (field) and artificial (used in the laboratory) rainfalls followed similar negative power trends between rainfall duration and rainfall intensity with same return period range [1-50 years] because the same Rouffach data set (1998-2014) was used to design the four rainfalls (see Chapter 5).

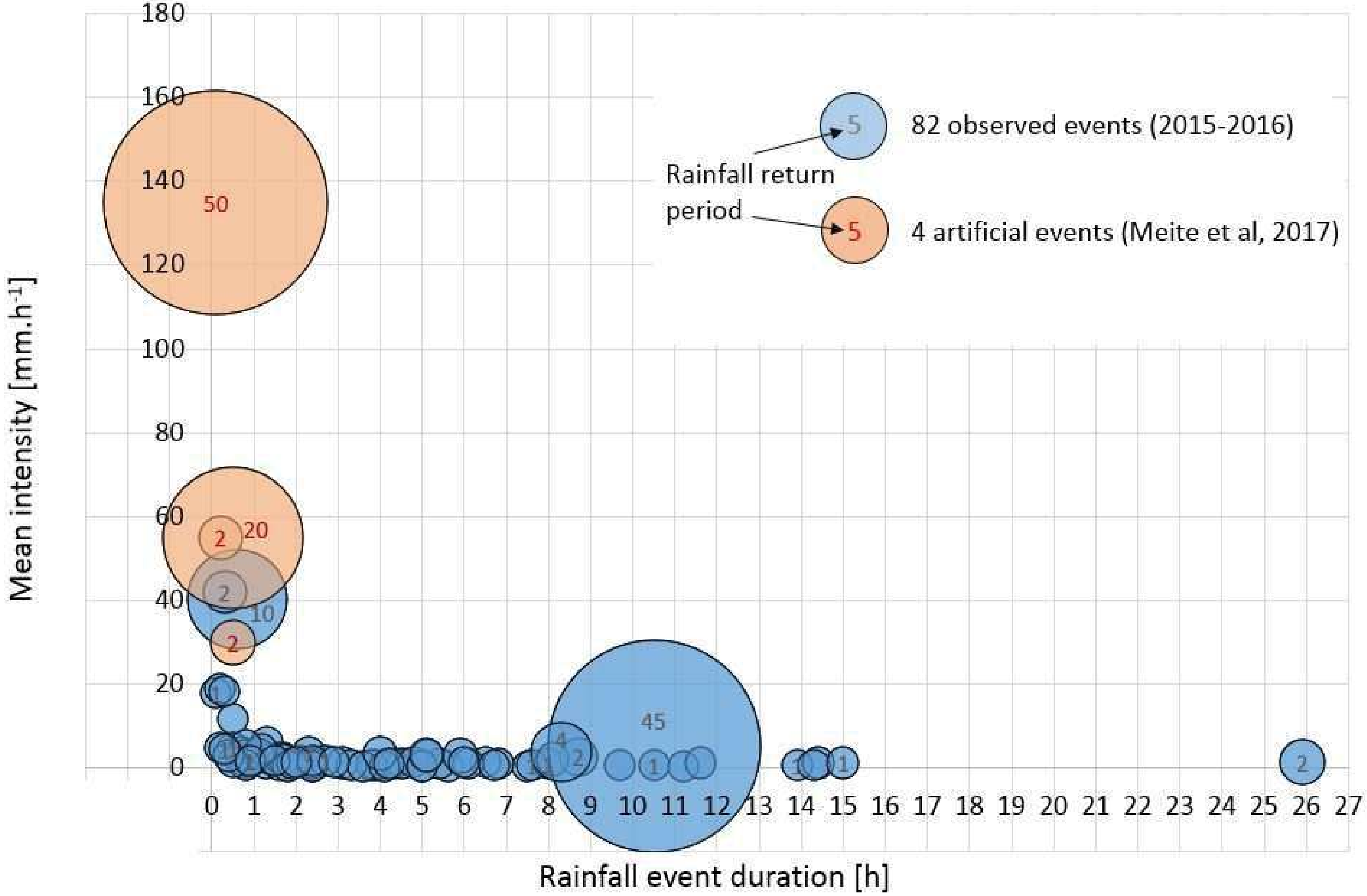

Figure 6.2-2: Return period of the 82 rainfall-runoff events monitored during the vineyard growing season in 2015-2016 (in blue), and of the 4 artificial rainfalls used in the lab-scale experiment (Chapter 5 and Meite et al., 2018)

The cluster analysis based on the 82 rainfall events enabled to extract four distinct rainfall with similar characteristics, hereby referred to as "small", "moderate", "intense" and "long" (Figure 6.2-3).

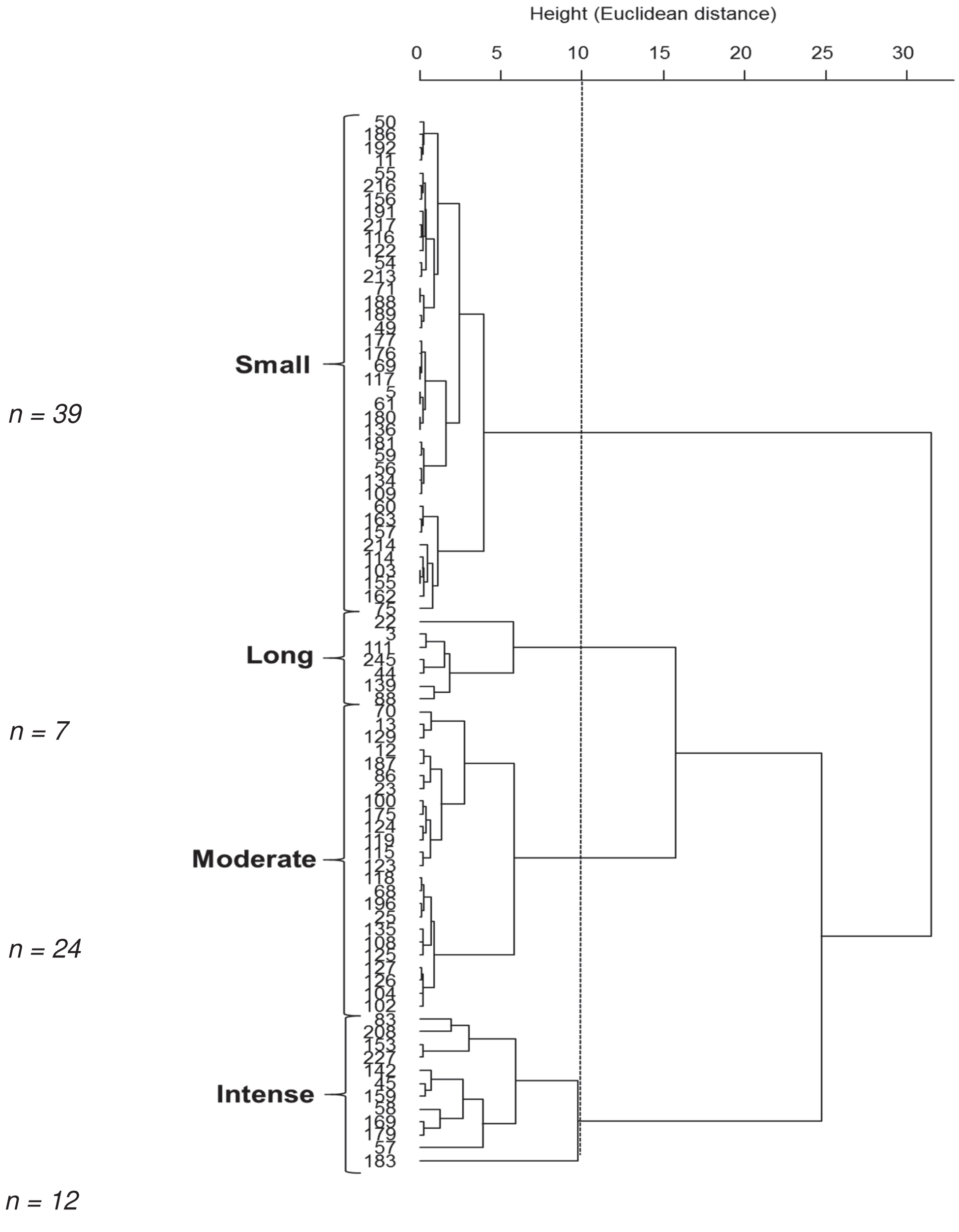

Figure 6.2-3: Patterns of rainfall events after cluster analysis based on rainfall depth, intensity and duration data. The distance between rainfall events was computed based on the Euclidean dissimilarity index. The hierarchical cluster analysis was performed on the resulting dissimilarity matrix using the Ward's minimum variance method. The analysis of optimal cluster number allowed identifying four clusters of rainfall events that differ among each other

A summary of hydrological data and characteristics of these four types of rainfall is provided in Table 6.2-4.

Table 6.2-4: Rainfall and runoff characteristics of the "small", "long", "moderate" and "intense" rainfall patterns

	Rainfall Event Type	**Range**	**Mean ± SD**	**Median**
Discharge volume (m^3)	Small	0.3 - 27.5	7.7 ± 7.3	5.8
	Long	15.5 - 295.1	117.3 ± 122.2	73.6
	Moderate	1.7 - 90.6	29.6 ± 26.2	20.3
	Intense	24.1 - 773.1	152.9 ± 204.3	85.0
Peak of discharge (L/s)	Small	0.1 - 10.6	2.9 ± 2.5	2.6
	Long	1.4 - 26.8	8.6 ± 9.1	4.5
	Moderate	0.2 - 14.9	3.6 ± 3.1	2.5
	Intense	6.7 - 166.5	59.2 ± 46.7	44.0
Mean discharge (L/s)	Small	0.02 - 2.51	0.79 ± 0.72	0.63
	Long	0.26 - 8.82	2.38 ± 2.98	1.35
	Moderate	0.11 - 5.65	1.22 ± 1.22	0.86
	Intense	0.72 - 18.32	6.23 ± 5.22	3.82
Duration of runoff event (mn)	Small	31 - 372	186 ± 106	162
	Long	252 - 2299	1035 ± 685	908
	Moderate	191 - 662	412 ± 140	384
	Intense	155 - 820	407 ± 212	350
Rainfall depth (mm)	Small	0.6 - 8.6	2.7 ± 1.6	2.6
	Long	9.2 - 36.1	18.3 ± 8.6	17.7
	Moderate	1.4 - 10.4	5.2 ± 2.7	4.7
	Intense	5.5 - 56.3	19.2 ± 14	15.7
Maximum of rainfall for 6 min (mm/h)	Small	2 - 18	7 ± 4.5	6
	Long	4 - 12	6.9 ± 3	8
	Moderate	2 - 8	4.2 ± 1.8	4
	Intense	18 - 114	49 ± 27.7	47
Mean rainfall intensity (mm/hour)	Small	0.4 - 19	3.1 ± 3.9	1.9
	Long	0.7 - 2.2	1.3 ± 0.5	1.3
	Moderate	0.3 - 1.6	0.9 ± 0.4	0.9
	Intense	2.7 - 42	11.9 ± 14.4	4.6
Duration of rainfall event (mn)	Small	6 - 186	82.8 ± 49.1	78
	Long	486 - 1554	884.6 ± 328.2	858
	Moderate	198 - 672	360.2 ± 130.9	330
	Intense	18 - 630	253 ± 219.2	273
Dry duration before rainfall (day)	Small	0.1 - 25	2.3 ± 4.5	0.7
	Long	0 - 12.7	4 ± 4.5	2.8
	Moderate	0 - 10.9	1.5 ± 2.4	0.5
	Intense	0.1 - 20.4	4 ± 5.9	1.9
Runoff coefficient (%)	Small	0.1 - 1.7	0.6 ± 0.4	0.5

	Long	0.2 - 3.9	1.4 ± 1.2	1.1
	Moderate	0.2 - 3	1.2 ± 0.8	1.1
	Intense	0.3 - 3.2	1.6 ± 0.7	1.7
TSS mass (kg)	Small	0.8 - 112.9	21.2 ± 23.5	16
	Long	28.6 - 792.5	213.6 ± 271.1	103
	Moderate	4.5 - 218.7	67.1 ± 65	40
	Intense	44.4 - 1544.1	476.5 ± 467.8	327
TSS concentration (g/L)	Small	0.8 - 5.5	21.2 ± 23.5	2.8
	Long	0.1 - 4.1	213.6 ± 271.1	2.8
	Moderate	0.1 - 5.3	67.1 ± 65	2.7
	Intense	1.7 - 5.4	476.5 ± 467.8	3.6
Return period of the event (year)	Small	1 - 1.027	1 ± 0.004	1
	Long	1.001 - 2.2	1.2 ± 0.4	1.09
	Moderate	1 - 1.004	1 ± 0.001	1
	Intense	1.005 - 45	5.8 ± 12.6	1.21
Maximum return period during the event (year)	Small	1 - 1	1 ± 0	1
	Long	1 - 1.4	1.1 ± 0.1	1.08
	Moderate	1 - 1	1 ± 0	1
	Intense	1 - 408.1	36.2 ± 117.1	1.42

"Small" rainfall encompasses short (82.8 ± 49.1 min), low mean intense (3.1 ± 3.9 $mm.h^{-1}$) events, with a rainfall depth lower than 8.6 mm. The "moderate" rainfall includes events that display slightly lower values for depth and mean intensity but with longer rainfall durations compared to "small" rainfalls (Table 6.2-4). The "intense" and "long" rainfall encompasses about 23 % of the monitored events. The "intense" rainfall is characterized by the highest maximum rainfall (49 ± 27.7 $mm.h^{-1}$) and mean intensity (11.9 ± 14.4 $mm.h^{-1}$) with a similar median rainfall depth as the "long" rainfall pattern. The "long" rainfall comprises events with long rainfall durations (884.6 ± 328.2 min), large rainfall depth (18.3 ± 8.6 mm), and relatively low maximum intensity (6.9 ± 3 $mm.h^{-1}$).

Wilcoxon pair-wise comparisons of rainfall characteristics revealed that maximal intensities, rainfall depths and rainfall durations differed significantly among the four types of rainfall ($p < 0.001$), which confirmed the robustness of the cluster analysis. Pair-wise comparisons also underlined significant differences among patterns for (1) the runoff volume (except between “long” and “intense” patterns), (2) the maximum discharge (except between “moderate” and “long” patterns) and (3) the runoff event duration (except between “moderate” and “intense”). The detailed Kruskal-Wallis test and pair-wise comparisons results are provided in the annex (Table 6.2-5 and 6.2-6). TSS concentrations also differed between “intense” (476.5 ± 467.8 $g.L^{-1}$) and the 3 other rainfall types (from 21.2 ± 23.5 $g.L^{-1}$ for “small” type to 213.6 ± 271.1 $g.L^{-1}$ for “long” type between underscoring higher erosion potential of the “intense” events, although concentration ranges overlapped. Altogether these results underscored that the four rainfall patterns generated contrasted runoff events, which may be produced by different landscape components (i.e., roads and/or vineyard plots).

Table 6.2-5: Comparison of hydrologic characteristics of sampled rainfall events among event types (i.e. “small”, “long”, “moderate” and “intense”)

	Characteristic[a]	H[b]	p-value[c]
Rainfall	$Tot_{rainfall}$	49	<0.001
	$I_{\max}$	40	<0.001
	$t_{rainfall}$	36	<0.001
Runoff	$Volume$	42	<0.001
	$Q_{\max}$	32	<0.001
	$Q_{duration}$	40	<0.001

[a] Abbreviations: $Tot_{rainfall}$, total rainfall; $I_{\max}$, event maximum intensity; $t_{rainfall}$, rainfall event duration ; $Volume$, event runoff volume; $Q_{\max}$, event maximum discharge; $Q_{duration}$, runoff event duration.

[b] Kruskal-Wallis rank sum statistic, $df = 3$; Kruskal-Wallis rank sum test of the null that the location parameters of the distribution of x are the same in each group. Alternative is that they differ in at least one.

[c] p-values for two-tailed tests.

Table 6.2-6: Pair-wise comparison of hydrologic characteristics of sampled rainfall-runoff events among rainfall event types (i.e. “small”, “long”, moderate” and “intense”)

	Characteristic	**Type**	W[b]	p-value[c]
Rainfall	$Tot_{rainfall}$	Small - Moderate	197	<0.001
		Small - Intense	4	<0.001
		Small - Long	0	<0.001
		Moderate - Intense	24	<0.001
		Moderate - Long	1.5	<0.001
		Intense - Long	36	0.673
	$I_{\max}$	Small - Moderate	634	0.015
		Small - Intense	2	<0.001
		Small - Long	127	0.780
		Moderate - Intense	0	<0.001
		Moderate - Long	39	0.028
		Intense - Long	84	<0.001
	$t_{rainfall}$	Small - Moderate	0	<0.001
		Small - Intense	155	0.083
		Small - Long	0	<0.001
		Moderate - Intense	184	0.179
		Moderate - Long	3	<0.001
		Intense - Long	3	0.001
Runoff	*Volume*	Small - Moderate	188	<0.001
		Small - Intense	12	<0.001
		Small - Long	8	<0.001
		Moderate - Intense	47	0.001
		Moderate - Long	36	0.022
		Intense - Long	58	0.190
	$Q_{\max}$	Small - Moderate	390	0.276
		Small - Intense	3	<0.001
		Small - Long	68	0.038
		Moderate - Intense	2	<0.001
		Moderate - Long	57	0.216
		Intense - Long	77	0.002
	$Q_{duration}$	Small - Moderate	102	<0.001
		Small - Intense	76	<0.001
		Small - Long	11	<0.001
		Moderate - Intense	156	0.670
		Moderate - Long	25	0.006
		Intense - Long	13	0.013

[a] Abbreviations: $Tot_{rainfall}$, total rainfall; $I_{\max}$, event maximum intensity; $t_{rainfall}$, rainfall event duration; Volume, event runoff volume; $Q_{\max}$, event maximum discharge; $Q_{duratio}n$, runoff event duration.

[b] Wilcoxon rank sum statistic (with continuity correction).

[c] p-values for two-tailed tests.

4.2. Impact of the rainfall type on Cu, Zn and polar pesticides in runoff

Cu and Zn were systematically detected in all runoff samples, both in the dissolved and particulate phases during the study period (Table 6.2-7). Carbendazim, cymoxanil, isoxaben, diuron and oryzalin were more frequently detected (> 50 %) compared to isoproturon and flufenoxuron (< 40 %) (Table 6.2-7). In top soil, Cu and Zn mean concentrations were (± σ), respectively, 125.8 ± 14.6 $mg.kg_{drysoil}^{-1}$ and 93.4 ± 11.8 $mg.kg_{drysoil}^{-1}$, and did not change much over the season or between years. The weekly frequencies of detection for top soil pesticides were 81 %, 69 %, 14 %, 67 %, 79 %, 76 % and 98 % for carbendazim, cymoxanil, isoproturon, diuron, isoxaben, oryzalin and flufenoxuron, respectively. The top soil concentrations of the seven pesticides were systematically below limits of quantification even after plot application. This is due to a dilution effect that reflects sample mixing over the fours transects. Hence, the obtained mean soil concentrations is reflect the pesticide pool at the vineyard catchment scale.

Consequently, the event loads (*EL*) (Eq. 6.2-1) were calculated without normalization of the event mean concentrations (*EMC*) by the initial pesticide concentrations in the vineyard soil. The total Suspended Solid concentrations and load, the *EMC* and the *EL* of pollutants (Cu, Zn and the seven pesticides) are summarized in Table 6.2-7. The detailed *EMC* and *EL* statistics for Cu, Zn and the seven pesticides for each rainfall type are provided in the annex (Table 6.2-8). Cu and Zn exported bound to TSS represented 98 % (Cu) and 97 % (Zn) of the total export for the 82 events, with a mean event particulate contribution of 95.1 ± 5.8 % (Cu) and 93.4 ± 6.9 % (Zn).

Table 6.2-7: Detection frequency, concentration (*EMC*) and loads (*EL*) of Cu, Zn and the seven pesticides for the 82 rainfall-runoff events

		Event Mean Concentration [mg L^{-1}][a] [µg L^{-1}][b]			Event Load [g][a] [mg][b]		
	Frequency	Range	Mean ± SD	Median	Range	Mean[a]	Median
TSS	-	0.11 - 5.45	2.97 ± 1.39	2.77	0.85 - 1544.1	117.7 ± 248.4	32.2
Dissolved Cu	100	0.01 - 0.07	0.017 ± 0.015	0.01	0.01 - 9.4	0.776 ± 1.735	0.169
Particulate Cu	100	0.03 - 6.47	0.941 ± 1.275	0.43	0.1 - 927.4	37.4 ± 112.1	6.24
Dissolved Zn	100	0.003 - 0.18	0.038 ± 0.05	0.02	0.004 - 25.3	1.424 ± 3.978	0.02
Particulate Zn	100	0.04 - 5.55	1.007 ± 1.152	0.47	0.09 - 922	40.6 ± 114.421	7.87
Carbendazim	83	0 - 0.01	0.008 ± 0.004	6.24	0 - 7.73	0.395 ± 0.979	0.116
Cymoxanil	62	0 - 29.56	4.774 ± 6.825	1	0 - 2632.9	201.8 ± 438.6	6.74
Isoproturon	39	0 - 1.37	0.086 ± 0.306	0	0 - 100.6	2.317 ± 12.419	0
Diuron	56	0 - 17.62	1.424 ± 3.028	0.01	0 - 1706.4	73.7 ± 226.7	0.128
Isoxaben	68	0 - 0.01	0.007 ± 0.005	0.01	0 - 7.73	0.344 ± 0.977	0.055
Oryzalin	59	0 - 14.86	1.337 ± 2.764	1	0 - 721.3	49.6 ± 126.3	2.89
Flufenoxuron	38	0 - 6.88	0.84 ± 1.9	0	0 - 773.1	26.6 ± 94.7	0

[a] for Cu and Zn

[b] for pesticides

Table 6.2-8: Summary statistics for Event Mean Concentration and loads among rainfall event types (i.e. “small”, “long”, moderate” and “intense”)

Compound	**Rainfall Event Type**	**Load [mg^a or g^b]**			**Mean Concentration [mg L$^{-1\ a}$ or µg L^{-1b}]**		
		Range	Mean±SD	Median	Range	Mean±SD	Median
Dissolved Cu	Small	0.01 - 0.69	0.13 ± 0.15	0.077	0.009 - 0.069	0.02 ± 0.02	0.013
	Long	0.14 - 5.61	1.72 ± 1.96	0.671	0.009 - 0.023	0.01 ± 0.01	0.010
	Moderate	0.02 - 1.48	0.35 ± 0.35	0.253	0.009 - 0.019	0.01 ± 0	0.009
	Intense	0.24 - 9.4	3.2 ± 3.3	1.875	0.009 - 0.069	0.02 ± 0.02	0.017
Particulate Cu	Small	0.1 - 45.9	5.94 ± 9.01	2.504	0.059 - 6.47	1.06 ± 1.56	0.300
	Long	2.15 - 273.62	74.96 ± 97.19	50.370	0.034 - 4.535	1.04 ± 1.58	0.684
	Moderate	0.34 - 73.61	15.22 ± 19.63	7.221	0.034 - 1.611	0.58 ± 0.49	0.434
	Intense	9.36 - 927.36	161.88 ± 252.95	112.197	0.046 - 3.893	1.21 ± 1.11	1.020
Dissolved Zn	Small	0 - 3.77	0.26 ± 0.63	0.124	0.003 - 0.182	0.04 ± 0.06	0.021
	Long	0.11 - 25.29	4.51 ± 9.21	1.484	0.003 - 0.086	0.02 ± 0.03	0.013
	Moderate	0.02 - 6.02	0.69 ± 1.21	0.352	0.003 - 0.177	0.03 ± 0.05	0.023
	Intense	0.17 - 17.82	4.89 ± 6.38	1.457	0.004 - 0.136	0.04 ± 0.05	0.014
Particulate Zn	Small	0.09 - 29.73	6.48 ± 8.19	2.901	0.1 - 5.546	1.07 ± 1.26	0.470
	Long	2.58 - 353.5	95.78 ± 124.78	72.366	0.051 - 5.546	1.28 ± 1.94	0.924
	Moderate	0.36 - 95.1	18.75 ± 25.97	8.685	0.046 - 1.579	0.72 ± 0.56	0.470
	Intense	4.94 - 922.01	162.99 ± 250.65	130.800	0.042 - 3.87	1.23 ± 1.14	1.157
Carbendazim	Small	0 - 0.27	0.07 ± 0.07	0.053	0 - 0.01	0.008 ± 0.004	0.01
	Long	0 - 2.95	1.12 ± 1.27	0.736	0 - 0.01	0.009 ± 0.004	0.01
	Moderate	0 - 0.91	0.27 ± 0.28	0.182	0 - 0.01	0.008 ± 0.004	0.01
	Intense	0 - 7.73	1.27 ± 2.1	0.608	0 - 0.01	0.007 ± 0.004	0.01
Cymoxanil	Small	0 - 612.22	62.97 ± 139.46	3.134	0 - 29.56	5.58 ± 8.04	1
	Long	0 - 2632.92	761.21 ± 1030	73.640	0 - 12.77	4.14 ± 5.07	1
	Moderate	0 - 823.45	164.65 ± 258.29	7.921	0 - 16.65	4.49 ± 6.09	1
	Intense	0 - 1764.19	400.81 ± 563.62	105.193	0 - 13.72	3.1 ± 4.71	1
Isoproturon	Small	0 - 15.68	0.67 ± 2.81	0	0 - 1.37	0.09 ± 0.31	0
	Long	0 - 100.62	21.46 ± 39.47	0	0 - 1.37	0.28 ± 0.53	0

	Moderate	0 - 2.33	0.11 ± 0.48	0	0 - 1.37	0.06 ± 0.28	0
	Intense	0 - 6.11	0.92 ± 1.69	0.541	0 - 0.01	0.005 ± 0.004	0.005
Diuron	Small	0 - 484.11	20.27 ± 78.57	0.057	0 - 17.62	1.44 ± 3.09	0.01
	Long	0 - 663.92	180.02 ± 296.92	0.736	0 - 6.53	1.46 ± 2.41	0.01
	Moderate	0 - 641.53	58.36 ± 137.24	0.009	0 - 17.62	1.48 ± 3.59	0.005
	Intense	0 - 1706.4	216.34 ± 479.35	47.621	0 - 7.16	1.23 ± 2.12	0.136
Isoxaben	Small	0 - 0.21	0.04 ± 0.06	0.012	0 - 0.01	0.006 ± 0.005	0.01
	Long	0 - 2.95	1.11 ± 1.27	0.736	0 - 0.01	0.008 ± 0.004	0.01
	Moderate	0 - 0.91	0.25 ± 0.29	0.131	0 - 0.01	0.007 ± 0.005	0.01
	Intense	0 - 7.73	1.08 ± 2.16	0.451	0 - 0.01	0.005 ± 0.005	0.006
Oryzalin	Small	0 - 220.87	14.96 ± 42.8	0.493	0 - 14.86	1.48 ± 3.42	0.913
	Long	0 - 721.3	157.15 ± 269.85	15.542	0 - 8.41	1.63 ± 3.03	1
	Moderate	0 - 372.45	34.93 ± 75.61	15.440	0 - 6.52	1.15 ± 1.71	1
	Intense	0 - 647.13	128.92 ± 201.4	50.268	0 - 7.55	1.08 ± 2.09	0.73
Flufenoxuron	Small	0 - 188.87	13.34 ± 37.69	0	0 - 6.88	1.24 ± 2.24	0
	Long	0 - 96.52	22.66 ± 35.82	0	0 - 1	0.25 ± 0.38	0
	Moderate	0 - 250.28	19.46 ± 57.77	0	0 - 6.88	0.64 ± 1.93	0
	Intense	0 - 773.09	86.43 ± 220.9	0	0 - 1	0.27 ± 0.42	0

[a] for Cu and Zn

[b] for the 7 pesticides

EMC significantly differed among rainfall types only for dissolved Cu (between the "small" and "Moderate" patterns, and the "Moderate" and "Intense" patterns) and Flufenoxuron ("small" - "Moderate" patterns) (see annex; Tables 6.2-9 and 6.2-10). Cu, Zn and pesticide concentrations in runoff may largely vary among rainfall events for the same land use (Nolan et al., 2008; Shipitalo and Owens, 2011, Babcsanyi et al., 2016). Although the amount and timing of pesticide application may determine the delivery of pesticides in runoff, the *EMC* also reflect the mobilisation of pesticides that occurs during intense rainfall. In the present study, different *EMC* among rainfall types could generally not be observed. In contrast, loads systematically differed ($p < 0.001$ to 0.1) among rainfall types for all pollutants, except for diuron and flufenoxuron in the annex (Table 6.2-9). Pesticide loads significantly differed between "small" events and more severe rainfall events, i.e. "long" or "intense" in the annex (Table 6.2-11). This is in agreement with previous studies showing that pesticide loss generally increases with increasing rainfall input (Louchart et al., 2001; Beulke et al., 2002; Nolan et al., 2008).

Table 6.2-9: Comparison of event mean concentrations and event loads among rainfall event type

Compound	**Event Mean Concentration**		***Event Load***	
	H[a]	*p*-value[b]	*H*	*p*-value
Dissolved Cu	10	0.019	39	<0.001
Particulate Cu	2	0.674	30	<0.001
Dissolved Zn	1	0.831	32	<0.001
Particulate Zn	1	0.895	28	<0.001
Carbendazim	1	0.689	21	<0.001
Cymoxanil	0.1	0.992	8	0.054
Isoproturon	6	0.107	12	0.008
Diuron	0.4	0.920	4	0.242
Isoxaben	1	0.733	16	<0.001
Oryzalin	2	0.595	8	0.052
Flufenoxuron	6	0.091	3	0.437

[a] Kruskal-Wallis rank sum statistic.
[b] *p*-values for two-tailed tests.

Such differences in pollutant runoff could also be related to specific runoff characteristics. For instance, "intense" events generated large loss of pesticides resulting in larger event loads in runoff. This was the case even some weeks after pesticides applications or years after pesticide ban (i.e. diuron was banned in 2008), although *EMC* generally remained low and did not differ significantly among the rainfall types. Intense rainfall events are characterized by larger raindrop impacts that increase the mixing of soil surface particulate, soil solution and runoff water (Jarvis, 2007). This phenomenon may enhance mobilization from the vineyard soil of more persistent and less-sorptive pesticides, such as flufenoxuron and diuron and thus their transport in runoff.

Table 6.2-10: Pair-wise comparison of Event Mean Concentrations in sampled storms among rainfall event types (i.e. “small”, “moderate”, “intense” and “long”)

Compound	**Type**	***W*[a]**	***p*-value[b]**
Dissolved Cu	Small - Moderate	629	0.019
	Small - Intense	174	0.186
	Small - Long	142	0.877
	Moderate - Intense	56	0.002
	Moderate - Long	63	0.297
	Intense - Long	53	0.372
Flufenoxuron	Small - Moderate	606	0.024
	Small - Intense	294	0.140
	Small - Long	166	0.328
	Moderate - Intense	127	0.466
	Moderate - Long	67	0.325
	Intense - Long	41	0.961

[a] Wilcoxon rank sum statistic (with continuity correction).
[b] *p*-values for two-tailed tests.

Table 6.2-11: Pair-wise comparison of Event Loads in sampled storms among rainfall event types (i.e. “small”, “moderate”, “intense” and “long”)

Compound	**Type**	***W***[a]	***p*-value**[b]
Dissolved Cu	Small - Moderate	253	0.002
	Small - Intense	8	<0.001
	Small - Long	17	<0.001
	Moderate - Intense	29	<0.001
	Moderate - Long	34	0.017
	Intense - Long	55	0.299
Particulate Cu	Small - Moderate	280	0.008
	Small - Intense	22	<0.001
	Small - Long	48	0.007
	Moderate - Intense	33	<0.001
	Moderate - Long	48	0.094
	Intense - Long	56	0.261
Dissolved Zn	Small - Moderate	225	<0.001
	Small - Intense	24	<0.001
	Small - Long	39	0.003
	Moderate - Intense	57	0.003
	Moderate - Long	55	0.182
	Intense - Long	50	0.536
Particulate Zn	Small - Moderate	274	0.006
	Small - Intense	36	<0.001
	Small - Long	46	0.004
	Moderate - Intense	44	<0.001
	Moderate - Long	49	0.104
	Intense - Long	52	0.432
Carbendazim	Small - Moderate	285	0.010
	Small - Intense	73	<0.001
	Small - Long	43	0.004
	Moderate - Intense	72	0.016
	Moderate - Long	46	0.079
	Intense - Long	42	1
Cymoxanil	Small - Moderate	406	0.367
	Small - Intense	128	0.016
	Small - Long	79	0.072
	Moderate - Intense	103	0.163
	Moderate - Long	58	0.215
	Intense - Long	39	0.831
Isoproturon	Small - Moderate	582	0.055
	Small - Intense	134	0.018
	Small - Long	117	0.521
	Moderate - Intense	63	0.001
	Moderate - Long	58	0.100
	Intense - Long	48	0.629

Diuron	Small - Moderate	287	0.009
	Small - Intense	128	0.017
	Small - Long	35	0.002
	Moderate - Intense	110	0.254
	Moderate - Long	43	0.056
	Intense - Long	34	0.523
Isoxaben	Small - Moderate	298	0.013
	Small - Intense	158	0.078
	Small - Long	96	0.200
	Moderate - Intense	113	0.305
	Moderate - Long	76	0.737
	Intense - Long	42	1
Oryzalin	Small - Moderate	253	0.002
	Small - Intense	8	<0.001
	Small - Long	17	<0.001
	Moderate - Intense	29	<0.001
	Moderate - Long	34	0.017
	Intense - Long	55	0.299

[a] Wilcoxon rank sum statistic (with continuity correction).
[b] *p*-values for two-tailed tests.

4.3. Relationship between hydrologic characteristics and pollutant transport

The study of the relationship between hydrologic characteristics and pollutant metrics showed contrasting results between *EMC* and *EL* (annex V; Tables 6.2-12 and 6.2-13). *EMC* of pollutants only rarely or weakly correlated with both rainfall and runoff characteristics (annex V Table 6.2-12). *EMC* of dissolved Cu and isoproturon positively correlated with maximum rainfall intensity (Spearman's rank correlation statistic, $\rho_s = 0.22$ to 0.25, $p < 0.05$). *EMC* of dissolved Zn and the runoff volume negatively correlated ($\rho_s < -0.25$, $p < 0.02$) or for the *EMC* of isoproturon and flufenoxuron and the duration of runoff event ($\rho_s = -0.18$, $p < 0.1$). This emphasized that long runoff events or large volumes tended to reduce the *EMC* of these pollutants. This may reflect fast flush of solutes that are readily available and, with time and after a significant rainfall volume, limited desorption due to moderate (isoproturon with K_d of 3078, PPDB Pesticide Properties DataBase, 2009) to high soil affinity (Zn with flufenoxuron with K_d of 3078, PPDB Pesticide Properties DataBase, 2009). This effect was, however, weak and not obvious for all pesticides.

Table 6.2-12: Pair-wise correlation between the Event Mean Concentrations and rainfall-runoff characteristics

Compound	**Rainfall**						Runoff					
	***Tot**$_{rainfall}$*		**$I_{\max}$**		**$t_{rainfall}$**		***Volume***		**$Q_{\max}$**		$D_{duration}$	
	ρ^b	p-value	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value
Dissolved Cu	0.07	0.485	0.25	0.021	-0.18	0.111	0.07	0.548	0.18	0.102	-0.02	0.859
Particulate Cu	0.03	0.749	0.13	0.248	-0.12	0.299	0	0.963	0.03	0.732	0.10	0.350
Dissolved Zn	-0.13	0.224	0.03	0.763	-0.18	0.08	-0.25	0.018	-0.15	0.168	-0.13	0.254
Particulate Zn	0	0.999	0.04	0.701	-0.07	0.478	-0.03	0.778	-0.03	0.804	0.15	0.166
Carbendazim	-0.02	0.844	-0.05	0.633	0.08	0.429	0.11	0.305	0.05	0.638	-0.16	0.132
Cymoxanil	0.13	0.254	-0.06	0.585	0.17	0.121	0.16	0.146	0.12	0.289	-0.09	0.436
Isoproturon	0	0.985	0.22	0.044	-0.12	0.259	-0.07	0.505	0	0.970	-0.19	0.081
Diuron	0.18	0.103	0.012	0.913	0.18	0.105	0.16	0.152	0.11	0.286	-0.05	0.642
Isoxaben	0	0.991	-0.01	0.922	0.10	0.371	0.03	0.803	-0.01	0.861	-0.08	0.497
Oryzalin	0.02	0.846	-0.13	0.260	0.23	0.036	0.07	0.483	0	0.979	-0.10	0.394
Flufenoxuron	-0.13	0.242	-0.09	0.399	0.02	0.882	-0.04	0.664	-0.11	0.326	-0.18	0.09

[a] Abbreviations: $Tot_{rainfall}$, total rainfall; $I_{\max}$, event maximum intensity; $t_{rainfall}$, rainfall event duration ; Q_{mean}, event mean runoff rate; $Q_{\max}$, event maximum event rate; V, event aggregate runoff volume; t_{runoff}, runoff event duration; *Cr*, event runoff coefficient.
[b] Spearman's rank correlation statistic.

Table 6.2-13: Pair-wise correlation between the Event Loads and rainfall-runoff characteristics

Compound	**Rainfall**						**Runoff**					
	$Tot_{rainfall}$		$I_{\max}$		$t_{rainfall}$		***Volume***		$Q_{\max}$		$Q_{duration}$	
	ρ^{b}	p-value	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value
Dissolved Cu	0.85	<0.001	0.47	<0.001	0.45	<0.001	0.92	<0.001	0.85	<0.001	0.55	<0.001
Particulate Cu	0.73	<0.001	0.45	<0.001	0.33	0.002	0.77	<0.001	0.74	<0.001	0.50	<0.001
Dissolved Zn	0.74	<0.001	0.39	<0.001	0.41	<0.001	0.74	<0.001	0.68	<0.001	0.53	<0.001
Particulate Zn	0.72	<0.001	0.42	<0.001	0.36	<0.001	0.76	<0.001	0.72	<0.001	0.54	<0.001
Carbendazim	0.65	<0.001	0.29	0.007	0.47	<0.001	0.79	<0.001	0.67	<0.001	0.38	<0.001
Cymoxanil	0.44	<0.001	0.13	0.232	0.34	0.002	0.48	<0.001	0.41	<0.001	0.17	0.134
Isoproturon	0.18	0.09	0.35	0.001	-0.02	0.887	0.11	<0.001	0.19	0.085	-0.07	0.498
Diuron	0.36	<0.001	0.09	0.383	0.30	0.005	0.37	<0.001	0.29	0.008	0.13	0.246
Isoxaben	0.50	<0.001	0.23	0.03	0.42	<0.001	0.55	<0.001	0.46	<0.001	0.13	0.247
Oryzalin	0.34	<0.001	0.05	0.606	0.41	<0.001	0.43	<0.001	0.46	<0.001	0.13	0.247
Flufenoxuron	0.07	0.534	0.01	0.896	0.16	0.130	0.16	0.148	0.06	0.544	0.13	0.247

[a] Abbreviations: $Tot_{rainfall}$, total rainfall; $I_{\max}$, event maximum intensity; $t_{rainfall}$, rainfall event duration ; Volume, event runoff volume; $Q_{\max}$, event maximum discharge; $Q_{duration}$, runoff event duration.

[b] Spearman's rank correlation statistic.

Exported loads of both dissolved and particulate Cu and Zn and pesticides, except flufenoxuron, positively correlated with rainfall depths ($\rho_s = 0.18$ to 0.85, $p < 0.001$). Pesticide loads also positively correlated with rainfall maximum intensities ($\rho_s = 0.23$ to 0.47, $p < 0.001$ to 0.03), except for cymoxanil, diuron, oryzalin and flufenoxuron, as well as with rainfall duration ($\rho_s = 0.33$ to 0.47, $p < 0.001$ to 0.02), except for isoproturon, diuron and flufenoxuron (Table 6.2-13). Similarly, exported loads of pollutants also positively correlated with the runoff volume generated ($\rho_s = 0.11$ to 0.92, $p < 0.001$) and the maximum runoff discharge ($\rho_s = 0.19$ to 0.85, $p < 0.001$ to 0.085), except for flufenoxuron. In contrast, runoff event duration correlated only for Cu and Zn dissolved and particulate EL and carbendazim ($\rho_s = 0.38$ to 0.55, $p < 0.001$). Though the amount and timing of chemical applications are expected to largely influence the magnitude of the pesticide loads in runoff, the results indicate that hydrologic characteristics, such as rainfall intensity, mean discharge and runoff volume, also control pesticide runoff in the study catchment. This trend seems, however, not to apply for formerly used or banned pesticides (e.g., isoproturon, diuron, flufenoxuron) likely in low concentration but persisting in soils, for which rainfall-runoff characteristics have less impact.

Altogether, the results also underlined different critical source area (Frey et al., 2009) for pollutant mobilization and transport depending on the rainfall patterns. Indeed, road networks are likely the only components involved in runoff generated during “small” events according to the maximum depth (< 8.6 mm), maximum intensity of 18 mm.h^{-1}, the saturated hydraulic conductivity of vineyard plots and the buffer impact of grass strips on upstream plot runoff (Lefrancq et al., 2014). For “moderate”, “long” and “intense” types, maximum runoff coefficients closed to 3% remained low but emphasize an extension of critical source areas from the road networks to the vineyard plots. Hence, pesticides were directly mobilized from the vineyard soil during these types of rainfall-runoff events. In addition, the variability of runoff coefficients for these three rainfall types (from 0.2 to 3.9 %) also highlight the possible interplay between initial soil water content, rainfall properties, and agricultural practices. However, no correlation between dry period between rainfall events and runoff coefficients could be noticed ($p > 0.5$).

From an operational point of view, despite of the variability on runoff genesis between and within rainfall types, strong correlations ($\rho_s = 0.72$ to 0.85, $p < 0.001$) between Cu and Zn dissolved and particulate loads and rainfall depth suggest that rainfall depth may be a major predictors of metal export from small vineyard catchments. Impacts of agricultural practices on Cu and Zn partitioning in soil, timing of pesticide application and associated export over the growing season in the same catchment (Duplay et al., 2014) may thus be confounded by primary hydrological signals, such as rainfall depth. This may be explained, on the one hand, by the relative homogeneity of Cu and Zn applications over time and background concentrations in the catchment, and strong sorption of Cu and Zn to SOM at the soil pH. In contrast, lower correlations between pesticides and rainfall or runoff characteristics ($\rho_s = 0.18$ to 0.65, $p < 0.001$ to 0.1) preclude the development of simple predictive tools based on hydrologic data, even if this strategy is still explored (Moser et al., open to discussion HESS). An alternative strategy would consist to use more sophisticated predictive tool to simulate temporal and spatial variability of dissipation and transport pathways (Payraudeau and Gregoire, 2012, Gassmann et al., 2014), accounting for persistence, and thus availability and physico-chemical properties of the molecules.

5. Conclusion

In this study, the impact of rainfall patterns on pesticides, Cu and Zn export in runoff was investigated through two wine-growing periods in a small vineyard catchment. The results support the hypothesis that, even in small catchment areas, the characteristics of rainfall event (i.e. depth, intensity and duration) may partially control the transport of Cu and Zn and seven polar pesticides loads in runoff. Although other factors, such as chemical characteristics as well as amount and timing of applications are important drivers for Cu, Zn and pesticide runoff, rainfall patterns determine pollutant transport from land to downstream aquatic ecosystems. In particular, our study underscored that Cu and Zn dissolved and particulate transport strongly correlates to rainfall depth. A major result of this study is that intense rainfall pattern enhance runoff generation from vineyard plots, top soil erosion and export of both dissolved and particulate pollutants. Together with the expected increase of maximum rainfall intensity in the coming decades (Larsen et al., 2009; Floris et al., 2010; Mailhot and Duchesne, 2010), this underscores the need to design best management practices reducing runoff, erosion and associated pollutant loads.

Overall, rainfall characteristics should be considered for the apportionment of pesticide applications and for developing management practices that can help to limit pesticide runoff from small agricultural catchments into aquatic ecosystems. Further studies are, however, needed to better understand and predict the impact of hydrological patterns on pesticide runoff from small agricultural catchments with respects to soil conditions and characteristics of pesticides application.

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Chapitre 7 : Conclusions et perspectives

Le chapitre 7 constitue la conclusion de ce travail de thèse. Dans un premier temps les facteurs contrôlant la transformation et la mobilité des pesticides dans les sols co-contaminés seront proposés, ainsi qu'une hiérarchisation de ces facteurs. Ces points seront suivis par une discussion sur les intérêts et limites des expériences de laboratoire pour évaluer les cinétiques de transformation et les processus in situ. Pour conclure ce chapitre seront présentées à court et long termes des perspectives à ce travail de thèse.

1. Introduction de ce chapitre

Suite aux pratiques agricoles de ces dernières décennies, des produits phytosanitaires composés de principes actifs minéraux ou synthétiques additionnés de divers adjuvants (pesticides inorganiques ou de synthèse) (Mesnage et al., 2014) ont massivement été appliqués sur les cultures. Après leur application sur les sols, les pesticides et métaux sont dilués dans la solution de sol, exportés par ruissellement ou infiltration, captés par les organismes et la végétation, mais surtout ils subissent des processus de sorption-désorption et dégradation, contrôlant leur spéciation (Arias-Estévez et al., 2008; Ma et al., 2006; Roberts et al., 2005; Ruggiero et al., 2002). Les pesticides de synthèse subissent également des réactions de transformation dont des dégradations physiques (photodégradation), chimiques (hydrolyse) et biologiques (biodégradation). Sont impliqués dans ces processus la lumière, la température (dégradation physique), le pH de l'eau (hydrolyse), l'oxygène, les constituants du sol (dégradation chimique), la végétation et les microorganismes (biodégradation) (Coats, 1991; Ruggiero et al., 2002). Le partage des pesticides et métaux entre la solution de sol et les phase solide dépend des caractéristiques des polluants (i.e. l'hydrophobicité, la polarité, ou encore le coefficient de partage carbone organique / eau) mais aussi de celles des sols (i.e. la composition minéralogique, le taux de matière organique, ou encore le pH) (Komárek et al., 2010). Ce partage entraine la diminution de la disponibilité et mobilité des polluants.

Ce travail de thèse s'inscrit dans ce contexte et vise notamment à traiter des questions scientifiques suivantes :

1. Comment évoluent les pools de pesticides de synthèse et métaux dans les sols agricoles notamment en fonction des conditions hydrologiques ?
2. Quel est l'impact des conditions hydro-climatiques mais aussi de l'activité des communautés microbiennes des sols agricoles sur la dissipation et les réactions de transformation de pesticides générées par ces communautés microbiennes ?
3. Comment s'opère au fil du temps le partage des pesticides de synthèse et des métaux comme le Cu et le Zn entre les fractions de sols agricoles ?
4. Quelles sont dans un contexte viticole les voies de transformation et de transport des pesticides de synthèse et des métaux ?

Ainsi, l'impact des facteurs intrinsèques sur la transformation des pesticides et métaux au cours du temps a été étudié (Chapitre 3), permettant de mettre en évidence l'importance de la matière organique vis-à-vis de la spéciation physique des pesticides et métaux. Afin de compléter ce travail, l'impact des conditions et paramètres climatiques sur la persistance des pesticides de synthèse dans les sols agricoles a été abordé en deux parties. D'une part l'impact de la teneur en eau des sols et la température sur la dissipation et la dégradation d'un mélange de pesticides ont été évalués (Chapitre 4, première partie). Ce travail a montré notamment une dissipation des pesticides plus rapide avec une augmentation de la température tandis qu'un changement de teneur en eau impacte faiblement la dissipation des pesticides. Les analyses CSIA ont également montré un changement de mécanismes dans les processus de transformation des pesticides en fonction du type de sol et des conditions de température et teneur en eau. La dissipation et transformation du fongicide chiral métalaxyl présent dans le mélange de pesticides suivi a ensuite été étudié plus en détail (Chapitre 4, deuxième partie). Cette étude a permis de mettre en évidence une dégradation plus rapide de l'énantiomère R du métalaxyl par rapport à l'énantiomère S montrant sa plus forte stabilité dans les sols. De plus, aucune évolution du rapport racémique et de la composition isotopique initiales du métalaxyl n'a été observée en condition abiotique démontrant l'implication des microorganismes des sols sur la dégradation de ce fongicide.

Suivant le type d'application effectué par les agriculteurs, le transferts des pesticides et des métaux en dehors des zones à traiter peut devenir prépondérant et engendrer une forte pollution des compartiments environnementaux (Barbash, 2014; Carlsen et al., 2006). Au sein de ces compartiments, les pesticides posent des problèmes toxicologiques et sanitaires importants vis-à-vis de l'homme et sa santé, la flore mais aussi la faune et leur diversité et fonctionnement (Imfeld and Vuilleumier, 2012; Muturi et al., 2017; Nicolopoulou-Stamati et al., 2016; Wyszkowska et al., 2012). De façon logique, les travaux suivants ont porté sur l'étude de la mobilité des polluants en nous intéressant particulièrement à l'impact des patrons de pluie et de leur succession sur l'export des pesticides et métaux des sols agricoles (Chapitre 5). Cette étude a montré que l'export des pesticides et métaux était principalement contrôlés par la durée et la fréquence des pluies. En comparaison des facteurs extrinsèques (caractéristiques des pluies), le type de sol et l'aging ont peu impacté l'export des polluants suivis. Enfin, les flux de pesticides et de métaux dans un bassin versant viticole ont été abordés sous deux angles : i) en évaluant les pools de Cu dans les sols en lien avec le ruissellement d'un bassin versant viticole en s'appuyant notamment sur l'analyse des isotopes stables du Cu (Chapitre 6, première partie), ii) en évaluant l'impact des patrons de pluie sur l'export des pesticides et métaux hors de ce même bassin versant, grâce à une étude statistique du lien entre les patrons de pluie (intensité-hauteur de pluie-durée) et les exports de polluants (Chapitre 6, deuxième partie). Ces deux études montrent un export du Cu principalement sous forme particulaire, dont la signature isotopique montre que ce Cu provient de la fraction argileuse des sols. Ces études montrent également que les teneurs de pesticides et métaux exportés par ruissellement augmentent avec la hauteur des pluies, leurs intensités et durées, mais aussi le volume d'eau ruisselante et leur débit. Cependant les concentrations moyennes de polluants lors des évènements n'étaient pas impactées par les caractéristiques des pluies et ruissellements.

Les principaux résultats de ces études sont donc discutés de façon transversale dans les sections suivantes, en mettant en particulier en relief : i) les facteurs contrôlant la transformation et la mobilité des pesticides et métaux dans les sols co-contaminés, ii) l'intérêt et les limites des expériences de laboratoire pour évaluer les cinétiques de transformation et les processus *in situ*, iii) la conceptualisation de la transformation et la mobilité des polluants dans des sols multi-contaminés sur la base de résultats de laboratoire, et iv) l'intérêt d'une méthodologie intégrée de l'étude des polluants des sols multi-contaminés, en allant notamment vers l'étude des interactions entre type de polluants dans les sols. Cette discussion peut se schématiser ainsi :

La Figure 7-1 schématise les conclusions principales de la thèse.

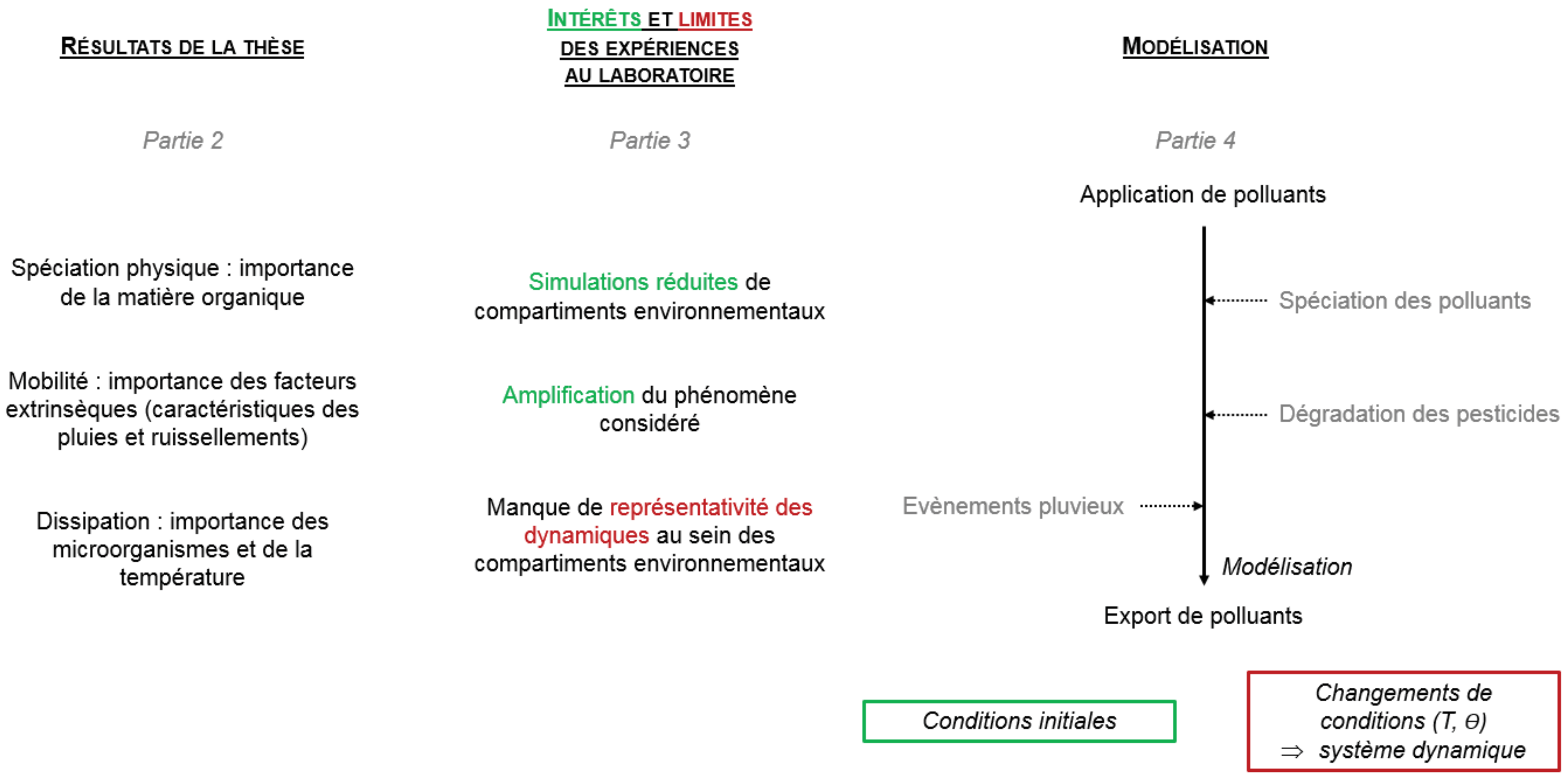


Figure 7-1 : Conclusions principales de la thèse

2. Facteurs contrôlant la transformation et la mobilité des pesticides dans les sols co-contaminés

La transformation et la mobilité des pesticides de synthèse et des métaux dans les sols sont contrôlées par des facteurs inhérents ou non aux sols. Après l'introduction de solutions de pesticides et métaux dans les sols, des interactions électrostatiques, échanges ioniques, forces de Van der Waals, liaisons H, ou encore des liaisons covalentes se forment entre les polluants (pesticides et métaux) et les phases solides constituant les sols (matières organiques et minéraux) (Buekers, 2007; Gevao et al., 2000; Komárek et al., 2010). Les pesticides et métaux peuvent également diffuser dans ces phases solides et y être séquestrés. Le rapport de teneur en matière organique par rapport aux minéraux contrôle la spéciation des pesticides (Đurović et al., 2009). L'adsorption des métaux sur les sols peut également être due à leur précipitation si les concentrations en métaux sont élevées. Avec le temps, le partage des pesticides et métaux entre les constituants du sol change et la force de l'association entre ces polluants et les phases s'intensifient. La disponibilité et la mobilité des polluants dans les sols tendent alors à diminuer. Ce processus est appelé "vieillissement" ou plus communément « aging » (Komárek et al., 2010; Ma et al., 2006b). Par rapport au 'vieillissement' des métaux et des pesticides de synthèse dans les sols, plusieurs questions restent en suspens, et notamment (i) Comment s'opère le partage des pesticides et métaux dans les fractions granulométriques des sols (spéciation physique) ? et (ii) Comment s'opère le partage des métaux dans les fractions chimiques des sols ?

Les études conduites au cours de ce travail de thèse ont tenté d'aborder ces questions. Ces études montrent entre autres que la composition des sols influence principalement la transformation des pesticides et métaux. La comparaison systématiquement de deux sols agricoles dont les propriétés physico-chimiques diffèrent et la comparaison de séries biotiques et des séries abiotiques de ces sols ont été conduites. Ces comparaisons montrent que la matière organique présente dans les sols contrôle principalement le partage des pesticides et métaux entre les fractions sableuses, limoneuses et argileuses. De plus les microorganismes au sein des sols sont principalement responsables de la dégradation des pesticides de synthèse. Les études conduites montrent également que cette dégradation est impactée par les teneurs en argiles et oxydes dans les sols. Les argiles en raison d'une possible forte sorption des pesticides sur ces dernières, et les oxydes suite aux réactions d'oxydation des pesticides à leur surface. Toutefois, nos études montrent que la composition des sols étudiés impacte peu l'export des polluants étudiés.

Les conditions physico-chimiques impactent la transformation et la mobilité des pesticides et métaux. Ainsi, suivant les valeurs de pH du sol, les charges de surface des matières organiques, oxydes ou encore minéraux argileux changent, ce qui modifie alors l'interaction des pesticides et métaux avec ces phases mais aussi l'importance de la dégradation des pesticides (Fike, 2001; Shahgholi and Gholamalizadeh Ahangar, 2014). La porosité, l'humidité ou encore les valeurs de pH peuvent induire un relargage important de colloïdes dans les sols (Kretzschmar and Schäfer, 2005). Ces particules, dont au moins une des dimensions est inférieure à 1 µm, sont très mobiles dans les sols et responsables de l'export des pesticides et métaux qui s'y associent par adsorption ou précipitation (Kretzschmar and Schäfer, 2005). Les études menées dans cette thèse ont souligné que la spéciation chimique des métaux évolue principalement dans les premiers jours (10 jours) suivant l'application de ces contaminants sur les sols.

L'étude simultanée des pesticides de synthèse et des métaux doit être approfondie, cette étude constitue un champ relativement récent de recherche. La présence simultanée de pesticides et métaux impacte la répartition respective de ces composés dans les sols en raison de leurs affinités avec les mêmes constituants des sols (Helal et al., 2006; Lalah et al., 2009). Ainsi des compétitions peuvent exister pour leur sorption sur les matières organiques. La dissipation des pesticides dans les sols est également impactée suite à la catalyse par les métaux des réactions de dégradation (Tariq et al., 2016; Wang et al., 2007) ou la modification des communautés microbiennes dans les sols induite par la pollution métallique (Tariq et al., 2016; Wang et al., 2007). Nos travaux suggèrent que la co-contamination avec des pesticides semble modifier la spéciation chimique du Cu et du Zn suivant le type de sol utilisé pour réaliser nos microcosmes. La co-contamination des sols peut donc être synonyme de relargage de pesticides ou métaux dans les solutions de sols suivant les quantités et caractéristiques des polluants, rendant alors ces polluants plus mobiles.

La transformation et la mobilité des pesticides et métaux dépendent également des conditions climatiques, météorologiques et du couvert végétal à la surface des sols. Ainsi, la température, les caractéristiques des évènements pluvieux, et leur impact sur la teneur en eau des sols influencent le devenir des pesticides et métaux. Les changements de température modifient la sorption des polluants sur les sols (Cessna et al., 2017). La température impacte également sur les microorganismes des sols (Cessna et al., 2017; Reedich et al., 2017; Su et al., 2017). Sur cet aspect, une question cruciale mais peu abordée dans la littérature, a été de mieux cerner quels sont les impacts de la température et la teneur en eau sur la dissipation des pesticides de synthèse. Nos travaux ont permis de mettre en évidence qu'une élévation de la température augmente la dissipation des pesticides de synthèse tandis qu'une élévation de la teneur en eau des sols n'a pas eu systématiquement cet effet. A ce titre, l'emploi d'une approche par analyse isotopique composée spécifique, dont le rôle premier a été de déceler l'occurrence d'une dégradation des pesticides de synthèse dans les sols, peut également permettre de mieux discriminer les processus de dégradation relevant du biotique de ceux relevant des mécanismes abiotique. Dans ce sens, cette étude pionnière pose possiblement les bases d'une application de terrain permettant d'évaluer l'hétérogénéité de de la dégradation in situ, dans les sols contaminés.

Suivant l'intensité et la durée des pluies, un export de polluants adsorbés à des particules sur les particules de sol érodées peut avoir lieu (Babcsányi et al., 2016; Oliver et al., 2012). Une question de cette thèse a été de déterminer quels sont les impacts de la succession des pluies et des périodes d'aging des polluants avant les pluies sur leur export. Nous avons ainsi montré dans une expérience de laboratoire que le volume d'eau apporté aux sols a effectivement un fort impact sur les quantités de pesticides de synthèse et métaux exportés de nos microcosmes par infiltration (Meite et al., 2018). Au-delà du volume d'eau généré par l'intensité et la durée des évènements pluvieux, la succession des pluies a été source de mobilisation supplémentaire des pesticides dans nos systèmes. De plus, suivant le volume généré par ces pluies et les capacités d'infiltration des sols, une forte humidité résiduelle peut s'installer dans les sols et modifier notamment les caractéristiques oxydo-réductrices des sols, les communautés microbiennes présentes mais aussi le développement du couvert végétal.

En effet, la spéciation de métaux comme le Cu dépend fortement des conditions oxydo-réductrice, de plus les microorganismes peuvent constituer des surface adsorbantes pour les métaux mais aussi dégrader les pesticides (Babcsányi et al., 2017; Navarrete et al., 2011; Shahgholi and Gholamalizadeh Ahangar, 2014). Quant aux végétaux à la surface des sols, ils peuvent être synonymes de prélèvement de pesticides et métaux par les racines, ou d'adsorption de ces polluants en surface des végétaux. Les végétaux et leur réseau de racines dans les sols (la rhizosphère) peuvent également modifier la spéciation et la dégradation des polluants (Diez et al., 2015; Duplay et al., 2014) par le biais d'un relargage ou d'un prélèvement de sucres, acides aminés et organiques, mucilages, nutriments et eau par les racines (Philippot et al., 2013).

Ce qui entraine notamment des modifications au sein des communautés microbiennes des sols et du pH dans le milieu. L'étude des changements de communautés microbiennes, tant taxonomiques que fonctionnels, lors des différentes modifications pourrait faire l'objet d'un travail futur permettant de mieux cerner l'impact des changements de conditions environnementales des sols sur les communautés bactériennes et fongiques.

3. Hiérarchisation des facteurs contrôlant la transformation et la mobilité des pesticides dans les sols co-contaminés

En combinant les informations obtenues au cours des différentes expériences menées dans cette thèse, une hiérarchisation des facteurs contrôlant (a) la transformation et (b) la mobilité peut être tentée.

- Transformation des pesticides et métaux

L'impact de trois variables sur la transformation des polluants a été testé. Suivant l'ampleur de la dissipation des pesticides de synthèse et l'évolution de la spéciation des pesticides et métaux, la hiérarchisation suivante est proposée : i) température (dissipation des pesticides constamment plus importante avec une augmentation de la température d'incubation), ii) teneur en eau (impact sur la dissipation des pesticides moins marqué que la température et non systématique), et iii) temps (spéciation (physique particulièrement) des pesticides et métaux relativement stable au cours du temps).

L'impact des constituants des sols dont leurs communautés microbiennes a également été investigué et suit l'ordre suivant : i) microorganismes (dégradation des pesticides principalement en présence de microorganismes), ii) matière organique (faible impact des phases minérales dans le partage des pesticides et métaux entre les fractions granulométriques des sols, contrôle de la matière organique lors de ce partage), iii) oxydes (favorisent des réactions d'oxydation des pesticides suspectés d'être contrebalancés par les argiles présentes dans nos sols), et iv) argiles (faussent l'étude de la dissipation des pesticides en raison de leurs capacité à sorber ces composés mais aussi de la gêne qu'ils peuvent provoquer lors de l'extraction des pesticides des sols).

- Mobilité des pesticides et métaux

Nos travaux montrent que la fréquence des pluies contrôle l'export des polluants dissous, suivi du volume généré par les pluies alors que l'intensité et la durée n'affectent pas l'export de polluants dissous. Tandis que la hauteur d'eau des pluies, leurs intensités et durées contrôlent l'export des polluants sous forme particulaire. Ainsi les impacts de ces variables sur la mobilité des pesticides et métaux peuvent être hiérarchisés comme suit : i) fréquence, ii) volume, et iii) intensité/durée des pluies. En comparaison des facteurs extrinsèques, les facteurs inhérents aux sols semblent peu affecter la mobilité des pesticides et métaux.

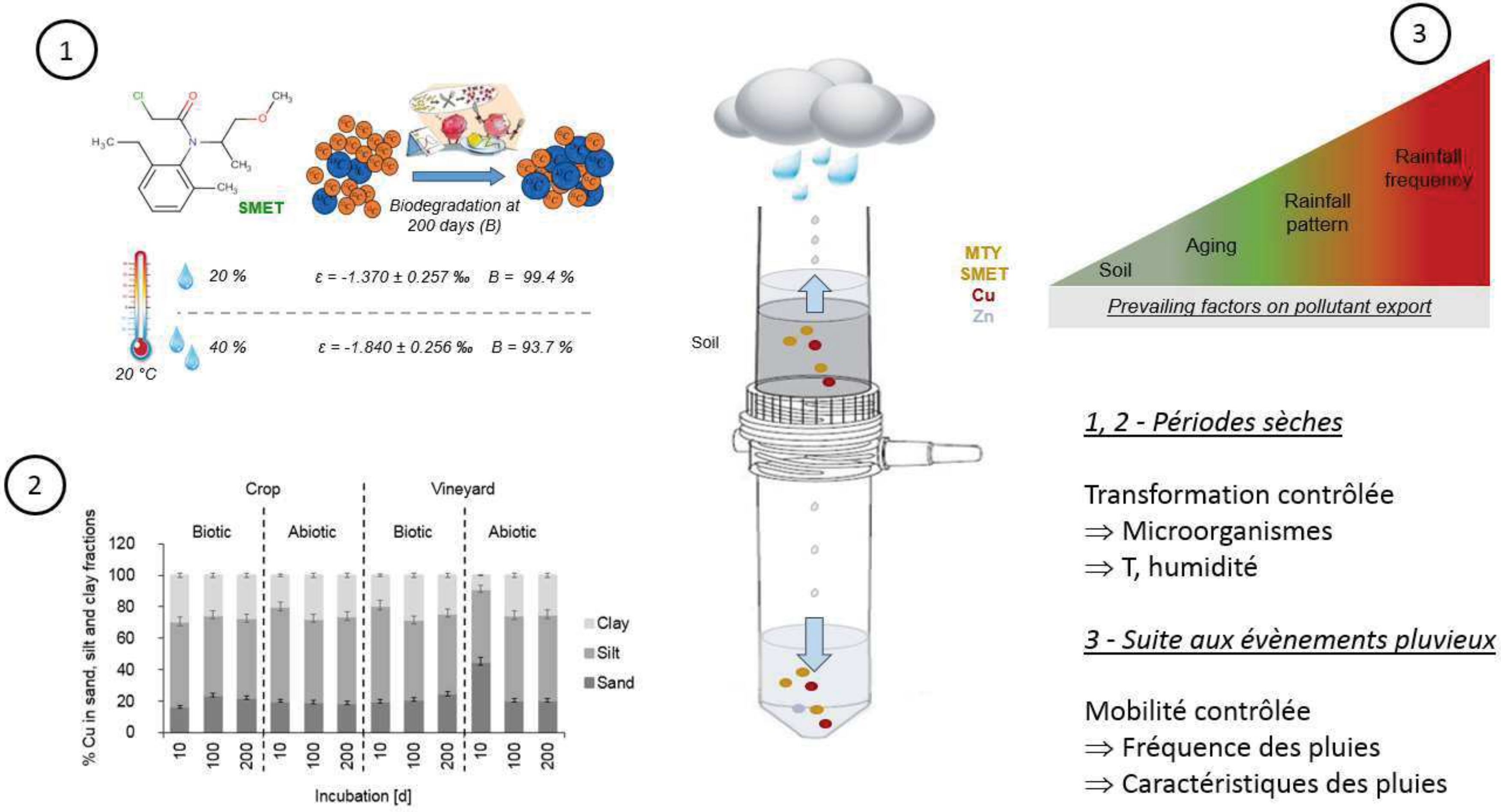


Figure 7-2 : Intégration des résultats des expériences de la thèse

La confrontation des résultats obtenus dans cette thèse avec les changements de conditions climatiques, hydrologiques et agricoles dans les agrosystèmes permet de souligner quelques implications, illustré ici par un scénario au cours de la saison agricole, qui dans le cas de cultures viticoles et céréalières s'étend en moyenne de mars à novembre, et un autre en cours en dehors de ces saisons, le reste de l'année.

- Saison agricole

Pendant les saisons agricoles, une grande variété de produits comprenant des pesticides et des métaux sont régulièrement appliqués sur les parcelles. Mes travaux ont particulièrement mis en évidence le rôle et l'importance de la matière organique pour la disponibilité des polluants organiques et inorganiques. En l'absence de génération ou d'application de matière organique supplémentaire par rapport à celle présente dans les sols pour aider au développement des cultures, et les rhizodépots mis à part, les pools de matière organique des sols se dégradent avec différentes cinétiques suivant leur association avec les minéraux des sols (Quenea et al., 2009; Schmidt and Kögel- Knabner, 2002). Les polluants appliqués sur les sols se retrouvent adsorbés à ces pools de matières organiques et dans une plus faible mesure aux minéraux présents dans les sols suivant les ratios de matières organiques par rapport aux minéraux (Đurović et al., 2009). Des microorganismes constituent également les stocks de matières organiques. Les pesticides et métaux peuvent être adsorbés sur ces microorganismes mais aussi être ingérés ou dégradés par ces derniers (Kafantaris and Borrok, 2014; Navarrete et al., 2011). En plus de l'aging et donc de la sorption progressive des pesticides et métaux aux phases solides des sols, entre chaque application de ces polluants s'opère leur export suite aux conditions météorologiques. L'ampleur de ces exports diffère suivant les intensités et durées des pluies, mais aussi l'étendue des processus d'aging qui ont alors réduit la disponibilité et la mobilité des polluants (Meite et al., 2018).

Au cours du ressuyage des sols, suite à chaque évènement pluvieux, les teneurs en eau au sein de ceux-ci diminuent progressivement. La teneur en eau est un paramètre important dans le fonctionnement des microorganismes, le développement de la végétation, la dégradation de la matière organique, le détachement des colloïdes mais aussi de multiples processus de transformation des pesticides et métaux (Ashworth and Shaw, 2006; Kretzschmar and Schäfer, 2005; Ruggiero et al., 2002; Shahgholi and Gholamalizadeh Ahangar, 2014; Sierra et al., 2015).

La dynamique de l'aging et du transfert des pesticides et métaux au cours du ressuyage des sols est relativement changeante et importante. Comme l'a montré ce travail de thèse, la succession d'évènement pluvieux rend cette dynamique récurrente dans les sols. Les variations de température peuvent accentuer ou au contraire, comme évoqué dans la littérature, ralentir cette dynamique en influant également sur les organismes vivants dans les sols et la sorption des polluants. Suivant la nature des applications de produits sur les parcelles cultivées, la sorption des pesticides peut évoluer tout comme leur dégradation, et le relargage des métaux des phases solides aux solutions de sol peut être accentué. Ces variations de teneur en pesticides et métaux dans les solutions de sol peuvent alors impacter les microorganismes des sols.

- Hors saison agricole

En dehors des saisons agricoles et en l'absence des applications de produits, les pools de contaminants en fin de saison évoluent principalement en fonction de l'aging, de l'enchainement de différents évènements pluvieux, des variations de température, en lien avec les teneurs en eau. En revanche en fin de saison agricole, un nouveau pool de matière organique se forme suite à la présence de débris de plantes récoltées à la surface des sols (Cotrufo et al., 2015). Un nouveau partage des pesticides et métaux peut alors s'établir progressivement dans les sols suite à la remobilisation de ces polluants par les volumes d'eau générés par les pluies, en fonction des propriétés physico-chimiques des polluants.

Ces deux scénarii montrent comment la dynamique des processus de transformation et de transport des pesticides et métaux interagissent en lien avec les facteurs inhérents ou non aux sols, rendant complexe le suivi et la compréhension *in situ* du cycle des polluants dans les agrosystèmes. Ces points complexifient également la prédiction de l'impact environnemental des pesticides et métaux sur les sols et sur les milieux aquatiques vers lesquels ils sont exportés tout au long de l'année. Ainsi, comprendre les phénomènes qui se produisent au sein des bassins versants agricoles suite à l'application de pesticides et métaux, et évaluer l'impact de chaque facteur intrinsèque et extrinsèque sur le devenir des pesticides et métaux souligne l'intérêt d'étudier les interactions entre un ou plusieurs processus élémentaires. Cela passe notamment par la conception d'expériences de laboratoire en conditions contrôlées, simulant le dynamisme rencontré dans les milieux naturels, et par un degré suffisant de conceptualisation des hypothèses et des scénarii testés lors de ces expériences.

4. Intérêt et limites des expériences de laboratoire pour évaluer les cinétiques de transformation et les processus *in situ*

Les microcosmes sont des simulations à faible échelle de compartiments environnementaux. Il est possible au sein de ces systèmes fermés de contrôler plusieurs conditions simultanément de sorte à retirer des lois phénoménologiques sur la base d'hypothèse. La réalisation de microcosmes permet ainsi d'étudier les interactions entre les processus dans les sols, en lien avec les variables environnementales (pluies, température, etc.) tout en en simplifiant la complexité existante sur le terrain. Ainsi, les paramètres supposés contrôler un phénomène peuvent être déterminés de façon précise, en minimisant l'influence d'autres paramètres qui rendraient ce phénomène peu ou non détectable sur le terrain. En ce sens, les expériences de laboratoire permettent d'amplifier le phénomène considéré, de sorte à l'appréhender et à mesurer son influence sur le système considéré.

Dans le cas de l'étude de la transformation et du transport de pesticides de synthèse et métaux dans les sols agricoles, les microcosmes réalisés ont permis de caractériser la dégradation de pesticides de synthèse à l'aide de mesure isotopiques (CSIA). De plus, ces microcosmes ont permis de maitriser : i) la durée de l'aging des polluants, ii) l'état biotique des sols, iii) les teneurs en eau et oxygène, iv) les températures mais également v) la qualité et les caractéristiques des pluies simulées pour évaluer la mobilité des polluants. En plus de ces conditions importantes dans le cadre de l'étude des processus subis *in situ* par les polluants et la cinétique de ces processus, des concentrations de pesticides et métaux suffisantes, mais proche des concentrations environnementales ont pu être appliqué dans les microcosmes afin de conduire l'ensemble des mesures nécessaires.

Cependant les microcosmes et expériences de laboratoire pour simuler les processus se produisant dans l'environnement présentent un certain nombre de désavantages. Les limites de ces systèmes peuvent être conceptuelles mais aussi méthodologiques.

En effet la dynamique générée par l'hydrologie, les conditions climatiques et le fonctionnement des organismes vivants observée sur le terrain n'est pas reproductible au laboratoire. De plus la réalisation de microcosmes peut être source d'autres problèmes de représentativité des agrosystèmes. Dans ces systèmes, les sols ne sont pas identiques en tout point, créant alors des problèmes d'hétérogénéité lors de l'échantillonnage des sols. Les prétraitements de sols nécessaires aux expériences de laboratoire comme le tamisage ou la stérilisation par autoclave, modifient la structure initiale des sols et le possible parcours de l'eau au sein de ces sols.

Si les expériences de laboratoire peuvent apporter beaucoup pour mieux comprendre les phénomènes complexes en cours sur le terrain, une meilleure prise en compte de la dynamique rencontrée par les agrosystèmes est cependant nécessaire dans les expérimentations conduites au laboratoire. Dans le cas de l'étude de la transformation et du transport de pesticides de synthèse et métaux dans les sols agricoles, les mesures suivantes pourraient être adoptée dans notre cas en fonction des résultats obtenus dans cette thèse. D'une part, augmenter le suivi temporel en multipliant les échantillonnages sacrificiels entre 0 et 50 jours des microcosmes pollués aux pesticides et métaux permettrait de mieux évaluer la spéciation de ces composés dans les premiers jours suivant leurs applications dans les sols. Afin d'améliorer le suivi de la sorption et de l'aging des pesticides de synthèse, une extraction séquentielle des pools de pesticides dans les sols (extractibles, moins facilement extractibles, adsorbés à la matrice solide) pourrait être menée. Il serait également nécessaire de développer un protocole de séparation de la matière organique et des minéraux contenus dans les fractions granulométriques des sols afin de connaitre les proportions de pesticides et métaux associés à chacune de ces phases en conditions contrôlées, représentant une sorte d'état d'équilibre par rapport aux distributions observées sur le terrain.

Finalement, les simplifications faites dans les expériences de laboratoire doivent aussi permettre d'aborder la complexité des interactions entre le sol, les formulations chimiques telles qu'utilisées sur le terrain et les caractéristiques hydrologiques. Cela permettrait d'augmenter la représentativité des expériences en utilisant les formulations réellement appliquées en contexte agricole et non uniquement les principes actifs de ces formulations.

5. Perspectives

5.1. Vers une conceptualisation de la transformation et de la mobilité des polluants dans des sols multi-contaminés

L'intégration des différents travaux menés dans cette thèse montrent comment un travail mené en amont intégrant une conceptualisation passant possiblement par la modélisation et combinée avec des expériences de laboratoire peut améliorer la compréhension de la transformation et du transport des polluants dans les sols multi-contaminés sur le terrain.

Les résultats de cette thèse permettront d'une part de fournir de nombreux paramètres sur les phénomènes régissant la transformation et la mobilisation de pesticides, du Cu et du Zn à partir de sol, en vue d'une modélisation prédictive. Les paramètres obtenus dans ce travail de thèse pourront être utilisés pour paramétrer un modèle numérique de devenir et de l'export des polluants dans les agrosystèmes. Notamment, il est prévu de travailler sur le modèle LISEM (Limburg Soil Erosion Model) qui est un modèle événementiel de prédiction des ruissellements et érosion à l'échelle d'un bassin versant (Lefrancq et al., 2017). Il est prévu d'utiliser les facteurs d'enrichissement isotopiques et les cinétiques de biodégradation des pesticides de synthèse déterminé au cours de ce travail de thèse ainsi que les cinétiques de dissipation. Ces données permettront de mieux contraindre par modélisation les quantités de ces composés exportables des sols agricoles jusqu'aux milieux aquatiques au cours des saisons agricoles.

De plus l'évolution des pools de pesticides réellement mobilisables de différents sols au cours d'une saison et suivant les conditions climatiques et hydrologiques pourra également être abordée grâce à nos expérimentations. Aujourd'hui, très peu de modèle intègrent les conditions initiales du sol et l'impact de l'évolution de ces conditions sur les taux et les voies de transformation des polluants dans les sols. Compte tenu de la variabilité de ces phénomènes reportés également dans les travaux de cette thèse, cela s'avère cependant indispensable si l'objectif est d'évaluer quantitativement le pool de matière exporté lors des événements pluvio-ruisselants. Nos travaux mettent également l'accent sur l'importance des phénomènes d'aging et de sorption au sens large dans le devenir à long terme des polluants et donc de l'enjeu majeur que constitue la prise en compte de la fraction particulaire lors de l'export. Là également, une meilleure prise en compte par les modèles de la fraction des polluants associés aux différents types de particules constitue une piste de recherche future.

D'autre part, notre expérience sur l'impact des patrons hydrologiques sur l'export des pesticides et métaux a permis de développer et de tester un formalisme afin d'évaluer le partage des polluants dissous dans une couche superficielle des sols (couche de mélange) entre l'export horizontal (eau surnageante à la surface des sols « ponding » pouvant entrer en ruissellement) et l'export vertical (infiltration). Ce partage est décrit à l'aide d'une constante appelée K_{film} (Figure 7-3). Nos résultats ont montré que l'export vertical dominait rendant impossible la détermination de K_{film} pour les polluants étudiés. Nos résultats ont également souligné que l'équilibre de sorption attendu pour les polluants étudiés n'était pas atteint rendant nécessaire la pondération du coefficient de partage de nos composés avec le sol K_d. Cet état de non-équilibre sur le terrain reste largement sous-estimé et très peu pris en compte dans les formalismes mathématique à l'heure actuelle. Il serait dès lors pertinent d'évaluer d'un point de vue mécaniste les facteurs principaux affectant l'atteinte du point d'équilibre et également de prendre ce phénomène en compte pour la prédiction quantitative d'événements survenant immédiatement ou proche d'une application de pesticides ou de métaux.

Dans ce sens, nos travaux ont également abordé la sorption des pesticides et métaux sur les sols sous l'angle de leur spéciation physique dans les sols au cours du temps. Ces travaux permettent d'entrevoir que les différents K_d peuvent être affinés et qu'il est possible de prendre en compte la répartition des pesticides et des métaux dans les fractions granulométriques des sols. Cela ouvre la voie vers l'étude des pools de polluants mobilisés ou non mobilisés suivant la dimension des agrégats et la porosité des sols.

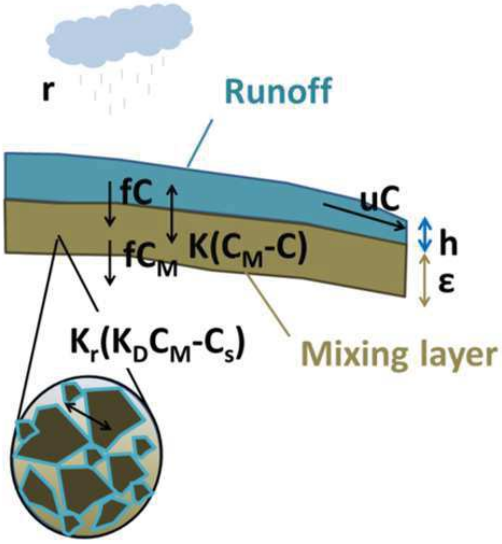
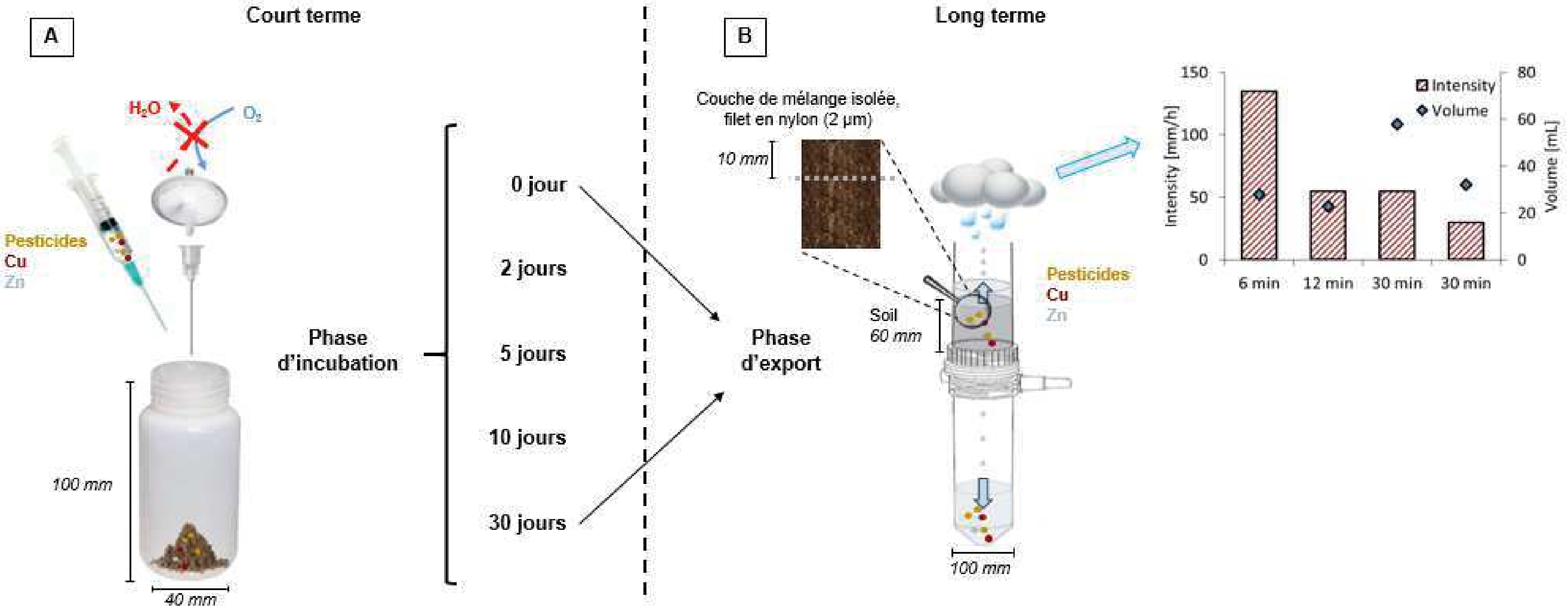


Figure 7-3 : Distribution des polluants dissous dans la couche de mélange (mixing layer) entre l'export horizontal (ruissellement) et l'export vertical (infiltration), la constante K (ou plus communément K_{film}) décrit cette distribution

La Figure 7-4 présente les expériences à court et long terme pouvant être générées sur la base des travaux menés. Afin d'aller plus loin sur l'étude du devenir des polluants dans les sols sur la base des enseignements tirés de cette thèse, une expérience simple à court terme et une autre de plus longue haleine sont proposés ci-dessous.



C

	P total	M total	CSIA	M isotopes	FTIR
Sols bruts	√	√	√	√	X
Fractions granulométriques	√	√	√	√	√
Eaux exportées – dissous	√	√	X	√	X
Eaux exportées – colloïdes	√	√	X	√	X

Figure 7-4 : Schéma des perspectives. A) Expérience à court terme. B) Expérience à plus long terme. C) Analyses à conduire sur les échantillons

- Expérience à court terme

Nos travaux ont mis en évidence que la matière organique joue un rôle important dans la sorption des pesticides et métaux, même au sein des fractions granulométriques. La spéciation des polluants, surtout celle des métaux, évoluent principalement dans les premiers jours (10 jours) après leur application sur les sols. Pour ces raisons il semble important d'évaluer, avec un pas de temps plus faible que celui défini dans nos travaux, la spéciation à court terme des pesticides et métaux entre les parts minérales et organiques des fractions granulométriques. D'autre part, l'accent pourrait être mis sur l'évaluation de la qualité de la matière organique dans ces fractions.

A l'instar de l'expérience sur l'impact de la teneur en eau et de la température sur la dissipation et la dégradation d'un mélange de pesticides (cf. Chapitre 4, partie 1), des microcosmes de sols agricoles sans modification de leur état biotique pourraient être artificiellement pollués. Trois types de pollutions seraient appliquées : i) un mélange de métaux uniquement, ii) un mélange de pesticides uniquement, iii) ces deux types de contamination. Les concentrations seraient 10 fois supérieures aux teneurs environnementales afin de pouvoir conduire l'ensemble des investigations prévues. Un nombre suffisant de microcosmes serait réalisé pour effectuer des échantillonnages sacrificiels à 0, 2, 5, 10 et 30 jours. Les fractions granulométriques d'une partie du sol de ces microcosmes seraient séparées avant qu'au sein de chaque fraction, la matière organique soit isolée de la fraction minérale. Les groupes fonctionnels au sein de la matière organique présente dans chaque fraction granulométrique seraient caractérisés à l'aide de spectrométrie infra-rouge à transformation de Fourrier (FTIR). Ces caractérisations permettront d'évaluer les interactions possibles entre ces matières organiques et les polluants. Les concentrations de pesticides et métaux dans le sol total de chaque système comme dans les portions minérales et organiques des fractions granulométriques seraient mesurées à chaque pas de temps. Pour finir, les facteurs d'enrichissement isotopique des pesticides seraient déterminés par GC-C-IR-MS et les signatures isotopiques des métaux seraient mesurées par MC-ICP-MS.

- Expérience à plus long terme

Afin de savoir quels pools de pesticides et métaux sont mobilisés dans les eaux d'infiltration et quelle est la part de la dégradation au cours de l'export vertical des pesticides dans une colonne de sol, l'expérience décrite dans le paragraphe précédent peut être prolongée comme suit.

Les systèmes incubés 0 et 30 jours du dispositif précédent seraient réalisés dans une proportion plus importante. A l'instar de l'expérience sur l'impact des patrons de pluie et de leur succession sur l'export des pesticides et métaux lourds des sols agricoles (cf. Chapitre 5), ces systèmes seraient soumis à quatre patrons de pluies aux caractéristiques (intensité [$mm.h^{-1}$]/durée [min]) suivantes : 30/30, 55/12, 55/30, 135/6. Pour cela, des dispositifs équivalents seraient utilisés mais présentant une largeur et une hauteur plus importante. De plus, dans chaque système la couche de mélange serait rendue facilement isolable et extractible du reste de la colonne de sol en plaçant un film en nylon (2 µm) à 1 cm de la surface du sol. En plus des séparations, extractions et mesures expliquées dans le paragraphe précédent, les concentrations de pesticides et métaux des fractions dissoute et colloïdale seraient quantifiées avant d'en mesurer les signatures isotopiques des métaux et du C des pesticides.

5.2. Vers une méthodologie intégrée d'étude des polluants des sols multi-contaminés : vers l'étude des interactions entre types de polluants dans les sols

Nos travaux ont mis en avant que l'étude conjointe des pesticides de synthèse et métaux dans les sols agricoles, mais aussi de l'impact de la présence de chacun de ces contaminants sur leur aging et leur mobilité, est nécessaire pour répondre aux enjeux rencontrés par les agrosystèmes subissant une contamination multiple, combinant souvent métaux et pesticides de synthèse. En effet, dans les sols, les pesticides et métaux peuvent s'adsorber sur les mêmes minéraux et matières organiques. La présence de ces polluants dans les sols impacte le fonctionnement et la diversité des microorganismes. De plus, les métaux présents dans les sols sont impliqués dans divers processus biotiques comme abiotiques. La co-contamination des sols peut alors altérer les phénomène de sorption/désorption ainsi que les transformations, favorisées par certains pesticides et/ou métaux (Helal et al., 2006; Lalah et al., 2009; Tariq et al., 2016; Wang et al., 2007).

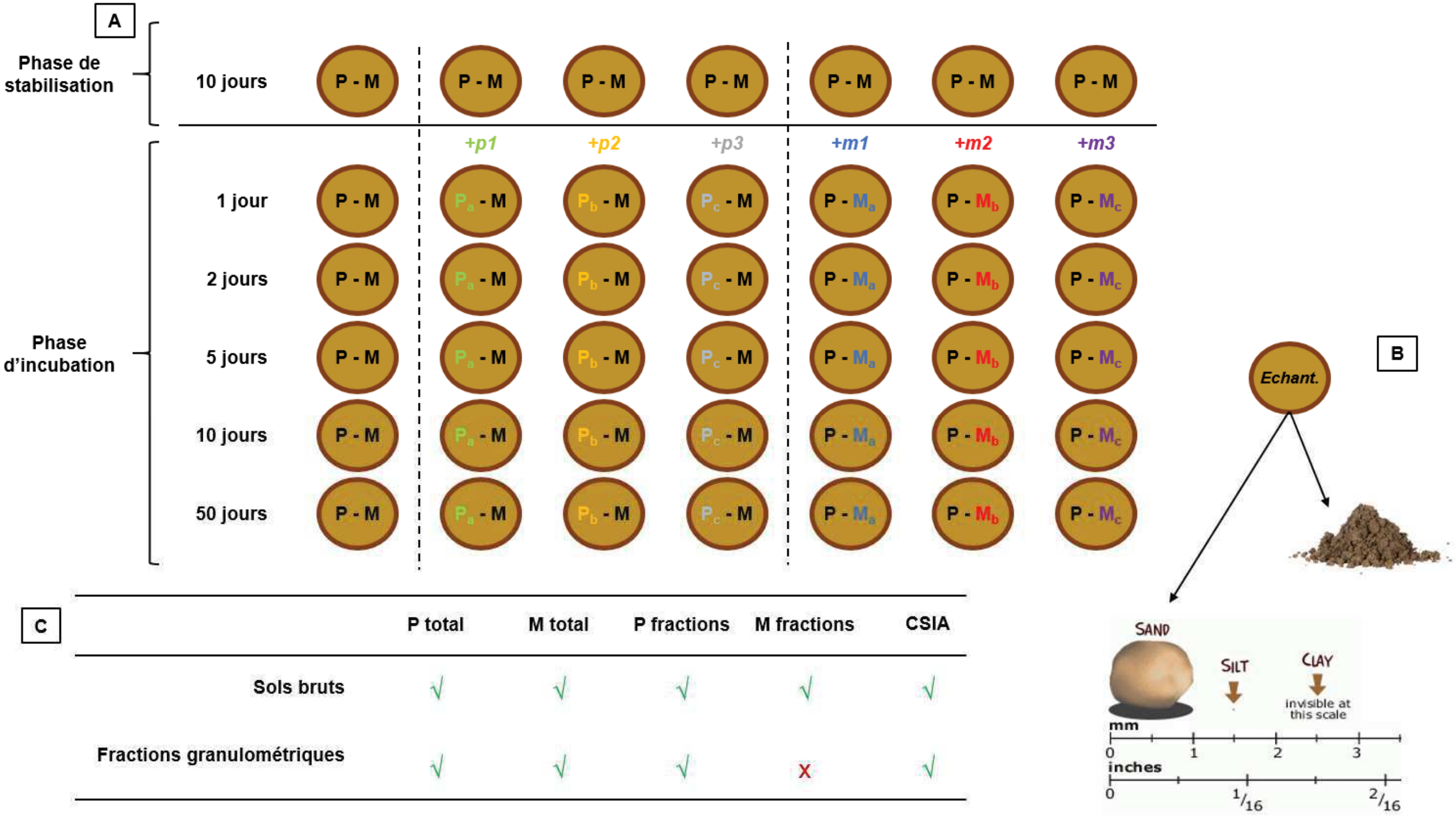
Concrètement, afin d'illustrer l'utilisation d'une méthodologie intégrée, l'expérience suivante pourrait être réalisée. Dans les études futures, tant réglementaires qu'académiques, cette dimension de co-contamination doit être davantage prise en compte. Ainsi, l'approche traditionnelle par microcosmes pourrait être davantage conduite sur la base de sols agricoles artificiellement pollués à la fois avec des pesticides et des métaux.

Un mélange pertinent de pesticides (neutres polaires, apolaires, cationiques) (ou en formulation commerciale pertinente) et métaux (Cu, Zn, Cd) serait introduit dans des microcosmes de sols agricoles. Ces systèmes constitueraient les échantillons « contrôles » de l'expérience. Un nombre suffisant de systèmes (35 en triplicatas) serait conçu pour réaliser des échantillonnages sacrificiels à 1, 2, 5, 10, 50 et 100 jours.

Après 10 jours d'une première phase d'incubation, des ajouts supplémentaires de pesticides mais aussi de métaux suivant trois concentrations (environnementale x5, - x10, - x100) seraient effectués sur 30 dans 35 systèmes pour évaluer l'impact de la co-contamination sur l'aging de ces polluants.

Après les échantillonnages sacrificiels, des extractions séquentielles des pesticides et métaux seraient réalisées. Ces extractions séquentielles seraient faites sur le sol total des contrôles comme des systèmes aux multiples pollutions à chacun des pas de temps de l'étude. Les pesticides facilement extractibles, extractibles et adsorbés constitueraient les pools séquentiellement extraits. Les métaux échangeables, liés aux oxydes, liés à la matière organique et résiduels seront séquentiellement extraits. De plus, les fractions granulométriques de chaque contrôle et système seraient séparées pour en évaluer les teneurs totales de pesticides et métaux. Pour finir, des analyses CSIA du ^{13}C seraient conduites sous tous les échantillons afin d'évaluer la dégradation des pesticides.

La Figure 7-5 résume ce dispositif expérimental. Cette étude permettrait de quantitativement estimer les changements de spéciation des pesticides et métaux dans des sols co-contaminés suivant les apports de chaque type de polluants.



			+p1	+p2	+p3	+m1	+m2	+m3
Phase de stabilisation	10 jours	P - M	P - M	P - M	P - M	P - M	P - M	P - M
Phase d'incubation	1 jour	P - M	P_a - M	P_b - M	P_c - M	P - M_a	P - M_b	P - M_c
	2 jours	P - M	P_a - M	P_b - M	P_c - M	P - M_a	P - M_b	P - M_c
	5 jours	P - M	P_a - M	P_b - M	P_c - M	P - M_a	P - M_b	P - M_c
	10 jours	P - M	P_a - M	P_b - M	P_c - M	P - M_a	P - M_b	P - M_c
	50 jours	P - M	P_a - M	P_b - M	P_c - M	P - M_a	P - M_b	P - M_c

	P total	M total	P fractions	M fractions	CSIA
Sols bruts	√	√	√	√	√
Fractions granulométriques	√	√	√	X	√

Figure 7-5 : Dispositif expérimental pour l'étude des interactions entre type de polluants (pesticides, P et métaux, M) dans les sols. Les inscriptions « *+px* » et « *+mx* » représentent les différentes concentrations à doper. A) nombre de systèmes à réaliser en triplicatas. B) Séparation granulométrique d'une partie du sol des systèmes. C) Analyses prévues

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Chapitre 8 : Gradients biogéochimiques et communautés microbiennes dans des colonnes de Winogradsky réalisées avec des sédiments humides pollués

Ce chapitre présente une étude réalisée sur un bassin d'orage présent à l'exutoire du bassin versant viticole étudié et permet de suivre l'impact de la pollution en pesticides sur les zones humides. En effet le but de cette étude est d'évaluer le comportement du Cu dans des milieux anoxiques grâce à la construction de quatre colonnes de sédiment provenant du bassin d'orage pollué, ce sont des colonnes de Winogradsky. Les colonnes de Winogradsky sont utilisées comme des écosystèmes miniatures, où une multitude de processus (bio)chimiques se produisent simultanément affectant notamment la spéciation des métaux comme le Cu. Cette étude souligne au cours du temps les changements biogéochimiques survenant à différentes profondeurs des zones humides.

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1. Abstract and highlights

A Winogradsky column is a miniature ecosystem prepared by incubating enriched sediments in clear cylinders that can be used to study the relationship between biogeochemical gradients, microbial diversity and pollutant transformation. Biogeochemical processes and microbial communities changed with time and depth in Winogradsky columns incubated with heavy-metal-polluted wetland sediments for 520 days. 16S rRNA surveys were complemented by geochemical analyses, including heavy metal proportioning, to evaluate biogeochemical gradients established in the mostly anoxic columns. Dissolved oxygen was depleted below the water-sediment interface (WSI), while NH_4^+, Fe^{2+}, S^{2-} and acetate generally increased by one order of magnitude at the bottom of the column. Microbial niche differentiation occurred by depth and from the light-exposed surface to the interior of the columns. Gradients resulting from nutrient uptake by algae, and from iron and sulphate reduction mainly drove diversification. In contrast, heavy-metal proportioning did not significantly influence microbial diversity as Cu and Zn were immobilized at all depths in the residual fraction and Pb was mainly found in the oxidisable fraction. However, Ni in porewater gradually increased with depth and was significantly associated with changes of microbial community. Proteobacteria were particularly abundant in the top water and the WSI layers, whereas Firmicutes and the class Bacteroida dominated down-core. Low diversity and richness of communities at the WSI and column bottom coincided with algal-derived carbon sources and cellulose fermentation, respectively. We anticipate this study to be a starting point to use Winogradsky columns to delineate microbial and geochemical dynamics in polluted sediments.

Highlights:

- Microbial communities and biogeochemical gradients in polluted Winogradsky columns
- Gradients formed by oxygen, iron and sulphate reduction drove microbial diversification
- Cu, Zn, and Ni mainly found in the residual fraction did not affect microbial communities
- Winogradsky columns to delineate biogeochemical dynamics in polluted sediments

2. Introduction

A Winogradsky column is a miniature ecosystem that can be used as a model system to study microbial communities involved in biogeochemical cycles and the transformation of pollutants. Over space and time, numerous, uncontrollable physico-chemical factors have an impact on microbial communities involved in the transformation of pollutants in natural sediments, which hampers study of their development (Lüdemann et al. 2000; Van der Gucht et al. 2007). By contrast, the Winogradsky sediment column expands the volume of natural processes, enabling microbial communities to grow in stratified ecosystems created by chemical gradients (Dworkin 2012; Winogradski 1888). As Winogradsky columns can be replicated and maintained under controlled conditions, they offer an opportunity to study how microbial communities and biogeochemical processes stratify and affect the pollutant transformation in sediments.

In aquatic ecosystems, heavy metals are usually bound to particles and settle to bottom sediments, where they accumulate (Machado et al. 2016). Steep physicochemical gradients formed locally over time in sediments have an impact on microbial communities and biogeochemical processes, thereby controlling pollutant transformation and transport to other compartments of the aquatic system (Borch et al. 2010; Pedersen et al. 2015). Heavy metals precipitate under reducing conditions as metal-sulphide species or mixed heavy metal-iron-sulphides (Machado et al. 2016; Morse & Luther 1999). Depending on the prevailing redox conditions, heavy metals can be released in the form of colloidal metal and sulphide particles (Weber et al. 2009) during the reductive dissolution of metal-sorbing oxy(hydr)oxides or oxidation of reduced metal species (Gounou et al. 2010; Simpson et al. 2012). However, little is known about the interaction between biogeochemical processes and microbial diversity along redox gradients established in heavy metal-polluted sediments.

In the Winogradsky column, photosynthetically active organisms at the column surface use light, and nutrient cycling is sustained as the structured microbial ecosystem develops. Redox gradients from the top to bottom and surface to interior of the columns are established as algae produce oxygen in the overlying water and aerobic microorganisms gradually consume oxygen downwards in the column. In the anoxic sediments down-core, sulphate-reducing microorganisms produce H_2S that diffuses upwards in the column and create a sulphide gradient. Winogradsky columns have been used previously to study photosynthetic sulphur and non-sulphur bacteria (Loss et al. 2013a; Martinez-Alonso et al. 2006), spatial changes in the bacterial communities of flooded paddy soil (Lüdemann et al. 2000) or the treatment of textile dyes (de Sousa et al. 2012). Recently, 16S rRNA gene surveys using high-throughput sequencing revealed diverse microbial communities within Winogradsky columns (Abbasian et al. 2015), which were structured over time (Esteban et al. 2015) or according to the sediment sources and depth (Rundell et al. 2014). Later studies suggest that the Winogradsky microbial community establishes a founder effect followed by diversification and enrichment over time of specific or rare taxa. To our knowledge, Winogradsky columns have not been evaluated using both geochemical and microbial approaches after a long incubation period (>500 days) and in the context of sediment polluted by heavy metals.

Therefore, the purpose of this study was to explore the diversification of biogeochemical processes and microbial communities by depth and from the interior to the surface of the Winogradsky columns established with heavy-metal polluted sediments. The zonation of biogeochemical processes was delineated after 520 days of incubation with chemical composition analyses of porewater and sediment samples, including sulphur stable-isotope analysis and the geochemical proportioning of Cu, Ni, Pb and Zn using sequential chemical extractions. In parallel, bacterial and archaeal 16S rRNA surveys were conducted to evaluate the diversity of microbial communities in the stratified habitats formed by geochemical gradients in the columns.

3. Materials and methods

3.1. Wetland sediments

Fifty kilograms of surface sediment (1-5 cm) was collected on 5th December 2012 from five zones of a shallow (<1 m depth) artificial stormwater wetland (Rouffach, Alsace, France; 47°57’9 N, 07°17’3 E). The wetland is situated at the outlet of the 42.7-ha vineyard catchment that collects stormwater and suspended solids that are mainly polluted by Cu and Zn; the vineyard is used for wine growing (Babcsányi et al. 2014; Maillard & Imfeld 2014). The vegetation covered 80% of the wetland area with *Phragmites australis* (Cav.) Steud., which represented 90% of the total vegetation cover. The sediment was sieved (<2 mm) to remove large debris, thoroughly homogenized with a mixer and stored at 4°C until the columns were set up.

3.2. Column set-up

Duplicate glass columns were successively rinsed with 20% HCl, 18 MΩ water (Millipore, Billerica, MA, USA) and ethanol (96%). Relatively large glass columns (Ø: 15 cm, height: 65 cm) were used to collect large sediment samples (>240 cm^3) and reduce the effect of heterogeneity when comparing samples. Planar oxygen sensor spots (Presens, Regenburg, Germany) were deployed in the inner part of the glass column at 15, 28, 38, 47 and 56 cm below the water surface for *in situ* measurement of dissolved oxygen. A PVC stand with a fixed perpendicular Teflon stick was placed at the bottom of the columns before packing the sediments to facilitate the extraction of an intact sediment core at the end of the experiment (see the Sampling section and Figure S1).

The physico-chemical characteristics of the collected sediment are provided in Table S1. The two columns were filled to 42 cm with sediment mixed to homogeneity with 4.5% (by weight) Na_2SO_4, 0.2% Na_2CO_3, 0.5% K_2HPO_4, and 0.5% NH_4SO_4 (Loss et al. 2013). Pure cellulose (0.7%) was added only to the bottom 19 cm of the column as a supplemental organic carbon source to establish a steep sulphide gradient within the column and favour fermentation.

Air bubbles were removed using a spatula. The packed sediment was overlaid with 19 cm of distilled water layer (3.4 L) at the top of the sediment column (42 cm of total sediment height). The columns were covered with a transparent Plexiglas disk secured with a rubber band to limit atmospheric contamination and water evaporation. The columns were incubated at 20°C with 12 h d^{-1} illumination using 150 W tungsten-halogen lamps for 520 days (74.3 weeks).

3.3. Sampling

The overlying water was collected after 520 days of incubation using plastic syringes and Teflon® needles. For algal analysis, 100 mL of water was filtered at <12 µm and preserved by adding 0.25% (v:v) glutaraldehyde. For microbial DNA extraction, 250 mL of water was filtered through sterile 0.2 µm nitrate cellulose filters (Millipore, Billerica, MA, USA), which were then stored in sterile 50 mL Falcon tubes at −20°C until further processing. The top and bottom water layers (~1.6 L each) of the overlying water were collected separately for hydrochemical analyses.

Intact sediment cores were extracted from the middle of each column by sinking a PVC tube (Ø: 12 cm, height: 65 cm) into the sediments up to the PVC support placed at the bottom of the column (Figure S1). The sediment core in the PVC tubes was then raised and kept intact with the help of the Teflon stick fixed to the PVC support placed in the columns before sediment packing (Figure S1). The intact cores were immediately frozen at −20°C. In addition, light-exposed sediment samples were collected along the column wall at 3, 8, 16 and 28 cm below the water-sediment interface (WSI) to evaluate changes in the microbial community in a latitudinal gradient from the light-exposed surface to the interior of the column (Figure S1). The frozen sediment cores collected in the middle of the columns were sliced into 2-cm layers (top 10 cm) and 6-cm layers (bottom section) with a tungsten saw, as steeper gradients were expected in the top 10-cm (Figure S1). Half of the layer was kept frozen until geochemical and sulphur isotope analyses. The other half-layers were thawed in a glove box (Jacomex BS531, Dagneux, France) under N_2 atmosphere (<1 ppm O_2). After homogenization, 1 g of each layer was collected for DNA extraction and stored in sterile tubes at −20°C until further processing. The thawed sediment layers were centrifuged separately at 1,700 g for 30 min (Jouan B4, Thermo Electron Corp., Waltham, MA, USA) to retrieve the porewater. Then, 2.5 mL of the porewater was filtered (<0.2 µm) using syringe filters under a nitrogen atmosphere for immediate Fe^{2+} analysis. The remaining porewater was filtered using 0.2-µm PTFE membranes, acidified with double-subboiled HNO_3 and stored at 4°C until hydrochemical analyses.

3.4. Analyses

3.4.1. Algae

Enumeration, taxonomic identification and biomass determination of algae in the overlying water (50:50 pooled sample from the top and bottom water layers sub-samples) was carried out at the GreenWater Laboratory (Palatka, FL, USA) following standard protocols (Examination of Water and Wastewater Method 10200 F and Hillebrand et al., 1999). Algal counts were carried out using an inverted microscope with phase contrast optics (Nikon Eclipse TE200, Tokyo, Japan).

3.4.2. Hydrochemistry

The pH, organic/inorganic carbon, alkalinity, conductivity, anions and major and trace elements of the filtrates were quantified using the FR EN ISO laboratory procedures, as described elsewhere (Lucas et al. 2010). Sulphide ions (S^{2-}) were quantified with Arrow probes (Lazar Research Laboratories, Inc., Los Angeles, CA, USA) under a N_2 atmosphere with an uncertainty of ± 0.5%. Ferrous iron (Fe^{2+}) was measured using an UV spectrophotometer (Shimadzu UV-1700, Pharmspec, Kyoto, Japan) with an uncertainty of ± 3%. Acetate was quantified by ion chromatography (ICS 3000 Dionex, San Diego, CA, USA) with an uncertainty of ± 2%.

3.4.3. Geochemistry

Sediment organic matter (OM) (SOL-0401), organic carbon (C_{org}) (NF ISO 10694), inorganic carbon (C_{inorg}) (NF ISO 10694), pH (in water) (NF ISO 10390), total carbon (C_{tot}) (NF ISO 10694), total nitrogen (N_{tot}) (NF ISO 13878), $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ (SOL-1402) were measured according to the NF ISO standards and procedures.

For chemical composition analysis, dried sediments were powdered using an agate disk mill (<63 µm) prior to alkaline fusion and total dissolution by acids and measurements by ICP-AES and ICP-MS using the geological standards BCR-2 (US Geological Survey, Reston, VA, USA) and SCL-7003 (Analytika, Prague, Czech Republic) for quality control (Babcsányi et al. 2014). Fractionation of Zn, Cu, Pb, Ni and Fe was quantified using a sequential extraction procedure adapted from previous methods (McKeague 1966; Rauret et al. 2000; Tessier et al. 1979) and detailed elsewhere (Semhi et al. 2014). Five fractions were sequentially extracted: (1) exchangeable (cation exchange), (2) acid-soluble (carbonate bound), (3) reducible (Fe- and Mn-oxide bound), (4) oxidisable (organic matter and sulphide bound) and (5) residual (silicate matrix and refractory sulphur species). Samples were treated under a N_2 atmosphere until the oxidisable stage to avoid oxidation of reduced sulphides. Special care was taken to avoid losing sediment material. Residues obtained at each extraction stage were washed for five minutes with 20 mL of 18 MΩ water (Millipore) using an end-over-end shaker and centrifuged (1,700 g, 30 min). The residues recovered in the washing solutions were added to the previously extracted fraction. All solutions were acidified and stored at 4°C in polyethylene vials until analysis by ICP-AES and -MS. Based on separate triplicate extractions and measurements, the uncertainty of the fractionation data was <10% (Semhi et al. 2014).

Mineral phases in the initial sediment and selected sediment layers were identified by X-ray diffraction (XRD) analysis (Brüker D5000, Brüker Corp, Billerica, MA, USA) (3–65° 2θ scanning angle, 1 s–0.02° upwards step, Cu anticathode, wavelength λ $K\alpha 1 = 1.54056$ Å, 30 mA 7 current 40 kV voltage and 30 mA).

3.4.4. S stable isotope analysis

Dried and powdered sediment samples were acidified with 1 M HCl, left overnight to remove inorganic carbon, neutralized by successive washing with distilled water and oven-dried at 60°C. The sulphur isotope composition was analysed using an EA-IRMS system. The furnace was held at 1,080°C. Sediment plus a vanadium pentoxide catalyst was combusted in the presence of oxygen, and combusted gases were swept over combustion catalysts (tungstic oxide/zirconium oxide) and reduced with high-purity Cu wires. Sulphur dioxide was resolved from N_2 and CO_2 on a packed GC column at 32°C. For quality control, tin capsules of an IA-R061 in-house standard and the IAEA-SO-5 standard were regularly measured during the sequence. The analytical uncertainty that incorporates both accuracy and reproducibility for the mean δ^{34}S-values was <0.3‰. The δ^{34}S values [‰] were expressed relative to the Vienna Canyon Diablo Troilite (VCDT).

3.4.5. DNA extraction

Total DNA was extracted from the filters and the sediment with a PowerSoil® DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) following the manufacturer's instructions. The concentrations of DNA were determined using a Qubit® Fluorometer and Qubit® dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA).

3.4.6. Illumina's MiSeq sequencing

Sequencing was performed at the Research and Testing Laboratory (Lubbock, TX, USA) using Illumina MiSeq. The 16S rRNA gene spanning hypervariable region V4 was amplified in a two-step process. The study compares the concordance of results and overall performance of the primer sets 515F/806R (5′-GTGCCAGCMGCCGCGGTAA-3′/5′-GGACTACHVGGGTWTCTAAT-3′) (Walters et al. 2011), traditionally used by the Earth Microbiome Project (EMP; http://www.earthmicrobiome.org/emp-standard-protocols/16s/), and 519wF/909R (5′-CAGCMGCCGCGGTAA-3′/5′-TTTCAGYCTTGCGRCCGTAC-3′) (Klindworth et al. 2013) for partial bacterial and archaeal 16S rRNA gene amplification.

The forward and reverse primers were constructed with the Illumina i5 (5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′) and the Illumina i7 (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′) sequencing primers, respectively. Sequences were generated by PCR in 25 µl reactions with the Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), 1 µl of each 5 µM primer and 1 µl of template. Reactions were performed on ABI Veriti thermocyclers (Applied Biosytems, Carlsbad, California) under the following thermal profile: 95°C for 5 min, then 25 cycles of 94°C for 30 sec, 54°C for 40 sec, 72°C for 1 min, followed by one cycle of 72°C for 10 min and 4°C hold.

Products from the first stage amplification were added to a second PCR. Primers for the second PCR were designed based on the Illumina Nextera PCR primers as follows: Forward - AATGATACGGCGACCACCGAGATCTACAC[i5index]TCGTCGGCAGCGT and Reverse -CAAGCAGAAGACGGCATACGAGAT[i7index]GTCTCGTGGGCTCGG. The second stage amplification was run in the same conditions as in the first stage except for 10 cycles. Amplification products were visualized using eGels (Life Technologies, Grand Island, New York). Products were pooled equimolar and each pool was size selected in two rounds using Agencourt AMPure XP (BeckmanCoulter, Indianapolis, Indiana) in a 0.7 ratio for both rounds. Size selected pools were quantified using the Quibit 2.0 fluorometer (Life Technologies) and loaded at 10 pM on an Illumina MiSeq (Illumina, Inc. San Diego, California) 2×300 flow cells.

3.5. Data analysis

3.5.1. Processing of Illumina’s MiSeq data

Denoising, chimera checking, generation of operational taxonomic units (OTUs) and taxonomic classification were performed using the custom-scripted bioinformatics pipeline of the Research and Testing Laboratory (Lubbock, TX, USA). Briefly, denoising and OTUs’ generation were accomplished after conversion into FASTA formatted sequences and quality files using USEARCH (Edgar 2010) and UPARSE OTU for OTU selection (Edgar 2013). Chimera checking was performed using UCHIME algorithms executed in de novo mode (Edgar et al. 2011). Sequences were clustered into OTUs at different levels of sequence identity using the UPARSE algorithm. The centroid sequence from each cluster was then run against the USEARCH global alignment algorithm against a highly curated database compiled by Research and Testing Laboratory and originating from NCBI (http://nbci.nlm.nih.gov). Based on the sequence identity percentage derived from BLASTn, sequences with identity scores to known or well-characterized 16S sequences >97% identity (<3% divergence) were resolved at the species level, >95% to 97% at the genus level, >90% to 95% at the family level, >80% to 90% at the order level, >80 to 85% at the class level and between 77% – 80% at the phylum level. Any match below this level of identity was not used in taxonomical analysis. Matrices of taxonomic data were further used to visualize changes in community structures.

3.5.2. Microbial diversity and structure analysis

To visualize dissimilarities in bacterial and archaeal community structures, two-dimensional nonmetric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities of Hellinger-transformed data (square-root transformation of relative abundances) was used. The relationship between the community profiles and biogeochemical variables was investigated by fitting environmental vectors *a posteriori* onto the NMDS, and their significance was assessed with a Monte-Carlo permutation test (1000 permutation steps).

To calculate the diversity and richness indices, the Illumina MiSeq sequences were re-analysed using MOTHUR version 1.36.1 (http://www.mothur.org) starting from denoised and chimera-checked sequences, aligned, and clustered to define OTUs at 97% sequence identity. A subsample of sequences was then randomly selected to obtain equally sized datasets according to the standard operating procedure (Schloss et al. 2009). The resulting datasets were used to calculate the diversity indices using R and for rarefaction analysis. Shannon's diversity index (H') was calculated as $H' = -\sum p_i \ln p_i$, and the inverse Simpson's diversity index (S) was calculated as $S = 1/(1 - D)$ with $D = \sum p_i^2$, where p_i is the relative abundance of species i. The Chao1 richness estimate was calculated as $S_{chao1} = S_{obs} + {f_1}^2/_{2\times f_2}$, where S_{obs} is total number of OTUs in a sample, f_1 is the number of OTUs with only one sequence (i.e., "singletons") and f_2 is the number of OTUs with only two sequences (i.e., "doubletons").

4. Results

4.1. Algal analysis

In overlying water, green algae (*Chlorophyta*) prevailed. In particular, the *Tetracystis*/*Chlorococcum* sp. genera were present in unicellular and colonial forms, with a diameter ranging from 5.0 to 14.5 µm (Figure S3). The algal and volumetric biomass concentrations were larger in column 2 (232×10^3 cells mL^{-1}; 30×10^6 μm^3 mL^{-1}) than in column 1 (124×10^3 cells mL^{-1}; 13×10^6 μm^3 mL^{-1}). Lower algal concentrations in column 1 corresponded to a larger accumulation of dead algal matter at the WSI, as indicated by the higher concentrations of total and dissolved organic carbon, as well as total nitrogen (Figure 1).

4.2. Biogeochemical gradients

The vertical profiles of the geochemical parameters for both porewater and sediments showed that the Winogradky columns had stratified after 520 days of incubation (Figures 1, 2 and S2). Globally, similar biogeochemical patterns were obtained for the duplicate columns. The abrupt increase in the average C:N ratios from ~11 at the WSI to ~25 down-core in the sediment (Figure 1) can be attributed to a shift from mainly algal to vascular plants (i.e., *Phragmites australis*) dominance of organic carbon (Meyers & Ishiwatari 1993). Profiles of pH, inorganic and organic carbon, acetate and calcium showed carbonate dissolution with concomitant accumulation of organic-rich material and acetate production at the WSI (Figure 1). In the zone beneath the WSI (18 to 24 cm below the water surface), depletion of organic matter (OM) and the increase of inorganic carbon (Figure 1) suggest intense OM degradation. Dissolved O_2 was depleted in the first 10 cm below the WSI, while the NH_4^+, Fe^{2+}, S^{2-} and acetate concentrations increased down core in the sediment, underscoring the gradients of terminal electron-accepting processes (TEAP).

The increase of alkalinity and S^{2-} with depth emphasized SO_4^{2-} reduction. Both the increase in dissolved S^{2-} (up to 210 µM) below 50 cm (Figure 2) and decrease in Fe^{2+} indicate the formation of FeS (black precipitates were observed in the columns, Figure S2). XRD analysis revealed the formation of pyrite in the column 2 whereas pyrite could not be detected in the initial sediment (Table S2). The stratification of SO_4^{2-} reduction in the columns was also emphasized by the abrupt change in the isotopic composition of sulphur ($\delta^{34}S$) at the WSI. The $\delta^{34}S$ values were constant below the WSI (-18.6 ± 1.0 ‰), which is consistent with active SO_4^{2-} reduction occurring in deeper anoxic zones. However, the $\delta^{34}S$ values abruptly decreased at the WSI (-25.7 ± 1.7 ‰), which suggests the oxidation to SO_4^{2-} of isotopically light S^{2-} diffusing upwards from the underlying zones of the columns.

4.3. Metal proportioning

While Cu, Zn and Pb concentrations in the sediment porewater were generally below the detection limit and low (<40 µg.L^{-1}), Ni concentrations gradually increased with depth up to 362 µg.L^{-1} (Table S3). The average bulk sediment content (± SE) of Cu, Zn, Ni and Pb over depth was 121 ± 22 µg.g^{-1}, 131 ± 26 µg.g^{-1}, 30 ± 5 µg.g^{-1}, and 36 ± 9 µg.g^{-1}, respectively. Changes in heavy metals concentrations in the sediment are provided relative to thorium (Th) as a conservative element to account for dilution/concentration effects. The Th normalized concentrations emphasise slight enrichments in Fe, Cu and Zn (+30 to 60% compared to the initial sediment) from 4 to 10 cm below the WSI after 520 days of sediment incubation (Figure 2 and 3).

Different sequential extraction patterns for Cu, Zn, Ni and Fe reflected different sorption capacities of the metals on the wetland sediment. After 520 days of incubation, 85 to 95% of Cu, Zn and Ni was associated with the residual fraction (silicate matrix and refractory sulphur species), whereas Pb (20% to 70% of total content) dominated in the oxidisable fraction (organic matter and sulphur-bound) below 40 cm (Figures 2 and 3). In addition to the residual fraction, Cu was also found in the oxidisable fraction (5 to 15%), while a minor proportion of Zn and Ni was associated with the acid-soluble and reducible fractions (2 to 5%). Fe was mainly associated with the acid-soluble fraction and to a minor extent with the reducible fraction (≤2 %) (Figure 2). Together with the minor proportion of the reducible fractions of Cu, Zn, Ni and Pb, this emphasizes that Fe oxy(hydr)oxides did significantly influence metal dynamics in the Winogradsky columns.

4.4. Diversity of microbial communities

A total of 834,283 (with 515F/806R primers, including 96.7% bacterial tags, 1.1% archaeal tags and 2.2% no hits tags) and 796,426 (with 519wF/909R, including 95.3% bacterial, 2% archaeal and 2.6% no hits tags) high-quality sequences (>~250 bp) were obtained from the 30 sediment samples. The mean number of tags per samples was 27,665 (515F/806R) and 26,218 (519wF/909R). The tags covered 27 phyla, 68 classes, 143 orders, 300 families and 488 genera. Although the sequencing depth did not systematically allow a survey of the full extent of microbial diversity (see Figure S5 for rarefaction curves), the patterns of beta diversity and the overall taxon relative abundances of the dominant lineages can be inferred (Bates et al. 2011). The rarefaction curves of diversity indices reached asymptotes (Figure S6), indicating that sampling was sufficient to capture the diversity of microbial communities.

The column communities were dominated by members of two main phyla (Proteobacteria and Firmicute) that represent an average of 50% of each sample (515F/806R) (71% using 519wF/909R). Previous reports have also suggested that Proteobacteria are dominant in metal-contaminated sediments (Gillan et al. 2015; Hemme et al. 2010). Unknown Bacteria accounted for 2 to 43% (mean of 19%) of the total communities. More than 50% of genera composing the microbial communities in the Winogradsky columns were greater than 1% abundance (excluding the contribution of unknown Bacteria). This underscores that several abundant genera composed the communities. By contrast, only approximately 20% of the genera were rare (< 1% abundance) and 4% were very rare (< 0.1% abundance) (Figure 4 and S6).

In terms of the metrics of microbial diversity, Shannon and inverse Simpson diversity, unlike the S_{chao1} abundance-based richness estimator, differed significantly with depth (Figure 4 and S7). Compared to the initial sediment, microbial diversity in both columns was higher in samples of the middle of the sediment profile (depth: 30 to 48 cm) and lower in samples from the WSI and bottom zone (below 48 cm).

4.5. Diversification and composition of microbial communities

Changes in microbial assemblages were visualized by the NMDS ordination of Illumina MiSeq data (Figure 5). The microbial community of the initial sediment clearly differed from all other samples, which emphasizes niche differentiation from the founding populations to clearly distinct communities. Column sediment samples were mainly discriminated according to depth. Water and WSI samples differed from sediment samples (p=0.01). Similar patterns were observed for the two columns, and the datasets obtained with the 515F/806R and 519wF/909R primers pairs yielded concordant results and overall performance.

The Winogradsky columns were characterized by major differences in the abundance of bacterial and archaeal taxonomic groups with depth and from the surface to interior of the columns (Figure S8 and S9). Proteobacteria, which are often linked to the oxic zones of redox gradients (Brune et al. 2000), were specifically abundant in the overlying water layer and the first sediment layers. In contrast, Firmicutes and class Bacteroida dominated down-core, below 30-cm depth (or 12 cm below the WSI). Changes in the microbial community composition are discussed below in relation to the diversification of biogeochemical processes observed along the Winograsky columns, including changes in nutrients, redox-sensitive species and metal proportioning.

5. Discussion

In this study, high-throughput sequencing was complemented by elemental and metal proportioning analyses to provide a holistic view of the relationship between chemical conditions and microbial communities along biogeochemical gradients stabilized for 520 days in Winograsky columns. To evaluate this relationship, the biogeochemical parameters (i.e., nutrients, carbon, metals, ions, metal fractions) were fitted *a posteriori* onto the NMDS ordination of community profiles from the core sediment samples (Figure S10). It revealed that the microbial community composition correlated best with changes in S^{2-}, NH_4^+, Na^+, K^+, acetate, PO_4^{3-}, HCO_3^-, alkalinity ($p<0.001$), and to a minor extent with changes in NO_3^-, Ca^{2+}, Mg^{2+}, CO_3^{2-}, total iron and Ni in porewater ($p<0.01$). This is in agreement with the composition of microbial assemblages that were not specific to polluted environments, but rather reflected nutrient and redox gradients as well as geochemical zonation.

The differentiation of microbial niches in the Winogradsky column and their metabolic activity as well as the algal nutrient uptake resulted in elemental and nutrient gradients. Decreasing Na^+ and K^+ concentrations from the bottom to the top of the column and slightly higher concentrations in the water layer (Table S3) indicated nutrient diffusion from the sediment to the water. The assimilation of PO_4^{3-} and NH_4^+ by algal cells in the water and CO_2 consumption during photosynthesis altered the concentration profiles of these nutrients in the porewater beneath the WSI. The proliferation of algae in the overlying water suggests that Cu, Zn and Ni released from the sediments (0.13 to 0.27 µM) did not greatly influence algal development (Moenne et al. 2016). In the overlying water layer, Proteobacteria populations were dominated by the class Alphaproteobacteria, and genera such as *Parvibaculum* and *Tistlia*, known to develop under aerobic conditions. Although algal colonization along the column wall was associated with oxygen production (Figure 1), oxygen was probably depleted radially towards the interior of the sediment layers by the consumption of aerobic heterotrophic microorganisms. These include Betaproteobacteria, which were particularly abundant at the WSI. Similarly, microbial oxygen consumption with depth is expected to exceed diffusive oxygen supply from algae and overlying water, and thus favour the use of alternative TEAs, such as Fe^{3+} and sulphate.

Carbon cycling in Winogradsky columns is mainly controlled by the mineralization of sediment organic matter (OM) and CO_2 incorporation into the biomass of autotrophic microorganisms. The intense OM mineralization observed in the zone below the WSI (Figure 1) can be related to the broad diversity of organic carbon sources derived from both sediment and algae as well as the lower concentrations of toxic H_2S diffusing upwards in the column. Higher acetate concentrations at and below the WSI (Figure 1) suggests lower consumption rate of acetate by Fe^{3+} and/or sulphate-reducing microorganisms due to the occurrence of more favorable TEA, such as oxygen and nitrate. In addition, carbonate dissolution in the zone below the WSI can be attributed to pH decrease induced by pyrite oxidation or/and CO_2 production during heterotrophic breakdown of OM and algal respiration (Cirkel et al. 2014, du Laing et al., 2009). Molar ratios of ~0.3 to ~0.4 of Ca+Mg over HCO_3^- (Table S3) support the idea that CO_2 production, rather than pH decrease (pH was buffered), caused carbonate dissolution at the WSI and below.

Interestingly, OM mineralization and carbonate dissolution at and below the WSI was associated with relatively low microbial diversity and richness and a microbial composition differing from that of the overlying water and the bottom sediments. This may be due to the selection pressure exerted by prevailing algal-derived carbon sources with a low C:N ratio. Similarly, supplemental cellulose (0.7% w:w) in the bottom layers (<19 cm) likely reduced diversity while favouring fermentative processes (Rundell et al. 2014). Overall, the low relative proportion of rare (<20%) and very rare (<4%) taxa found in the Winogradsky columns after 520 days of incubation contrasts from previous results showing that 61-78% of genera were very rare after 126 days of incubation (Rundell et al. 2014). This suggests that longer incubation periods can enhance drastic changes in both nutrients and accumulation of metabolic products, and favour the dominance of slow growing microorganisms over time while reducing rare genera. Changes in the chemical reactions in the columns through the development of depth-specific microbial metabolism can also gradually increase the abundance of more slowly growing organisms while decreasing that of initially active ones found in the freshly collected sediments. For instance, Cyanobacteria remained abundant only in the light-exposed zones (WSI and light-exposed sediment) compared to the initial sediment (Figure S8). Cyanobacteria (>12%) but also Betaproteobacteria (>35%) (order Rhizobiales) mostly appeared in the WSI layers, as well as Chloroflexi of the non-phototropic class Anaerolinea and free-floating filamentous cyanobacteria of the *Arthrospira* genus and Alphaprotebacteria, including the strictly aerobic genus *Phenylobacterium*. Overall, Betaproteobacteria increased in the WSI while Firmicutes increased by one fold at the bottom of the columns after 520 days of incubation. In the lower depths, Firmicutes (Clostridia, genera *Clostridiisalibacter* and *Candidatus*), but also Bacteroidetes (order Bacteroidales and Cytophagales) and the ammonia-oxidizing *Thaumarchaeota* (genus *Nitrososphaera*) generally increased in abundance with depth.

Iron cycle in the Winogradsky columns was mainly evidenced by microbial Fe^{3+} reduction with local Fe mobilization (see the Th normalized depth profile of sediment-bound Fe, Figure 2), and pyrite oxidation at the WSI. In addition, pyritization following Fe^{3+} reduction was evidenced by XRD analysis (Table S2). Increase of total and ferrous iron concentrations as well as acetate in the porewater of the bottom sediments (i.e., below 48 cm) indicates that microbial Fe reduction contributed to the diagenesis of organic matter. Fe enrichment between 4 and 10 cm below the WSI (Figure 2) coincided with higher OM mineralization. Pyrite re-oxidation at the WIS as well as Fe mobilization from the bottom sediment layers (Figure 2) during dissimilatory Fe^{3+}-reduction (Lovley, 2004) can both contribute to Fe re-distribution with depth. Iron reducing microorganisms in sediments are phylogenetically diverse. Proteobacteria and Firmicutes, which were particularly abundant down-core below the WSI, are typically the dominant groups among the iron-reducing communities in sediments (e.g., Li et al., 2011). However, the presence of *Thiocapsa* in the light-exposed sediment below the WSI, which can oxidize sulphides that diffuse upwards from underlying zones to the WSI, supports the idea that pyrite oxidation occurred at the WSI. This is also in agreement with the sulphur stable isotope analysis.

The trend in S isotopic composition reflects a mostly closed reaction system with continued SO_4^{2-} reduction. The remaining SO_4^{2-} and S^{2-} in the sedimentary porewater became progressively enriched in ^{34}S when the rate of SO_4^{2-} reduction and precipitation of FeS exceeded that of diffusion (Canfield et al. 1992; Ryu et al. 2006). However, gypsum precipitation may also have contributed to the consumption of dissolved sulphate because the porewater was saturated with regards to gypsum (K_{sp}= $2.5*10^{-5}$). Based on the sulphate mass balance after 520 days of incubation, sulphate consumption rates were estimated from 0.30 µmol SO_4^{2-} cm^{-3} d^{-1} in the WSI zone up to 0.49 µmol SO_4^{2-} cm^{-3} d^{-1} at the bottom of the columns. Although such high rates are rarely found in natural lake sediments, they are similar to those observed in sulphate-rich lake sediments (0.30 µmol SO_4^{2-} cm^{-3} d^{-1}) or in seawater sediments (Holmer and Storkholm, 2001; Habicht and Canfiled, 1997). These results are in agreement with the occurrence of several strictly anaerobic sulfate-reducing bacteria along the Winogradsky columns, including genera such as *Desulfatitalea*, *Desulfosarcina*, *Desulfobulbus*, *Desulfocapsa*, *Desulfofustis*, *Desulfofaba* and *Desulfococcus* of the family Desulfobacteraceae (Proteobacteria).

Overall, changes in microbial communities were mainly associated with the nutrients, the oxidation and reduction of iron and sulphur rather than with heavy metal proportioning. Despite the known limitation of the sequential extraction procedures (Tessier et al. 1979; Rauret et al. 2000), proportioning of heavy metals in sediments allows to evaluate the influence of organo-mineral phases on their availability and mobility in sediments. However, it does not provide direct information about their toxicity in multi-polluted sediments (Baran & Tarnawski 2015), which may be obtained by probing the microbial response (Imfeld and Vuilleumier, 2012). After 520 days of incubation, the potentially mobile fraction of heavy metals in the Winogradsky column (i.e., including the carbonate-bound, Fe/Mn oxides and organic matter-bound fractions) was low in all sediment strata (≤12 %) (Figure 3). Release of heavy metals and response of microbial communities are thus expected to be limited unless dramatic changes (e.g., pH or redox) in the physico-chemical conditions of the sediment occur with time or column depth. Nevertheless, the correlation analysis suggests that changes in microbial communities may be associated with porewater concentrations of Ni that increased with the column depth. While nickel homeostasis has been recognized as a general microbiological concern, to date, the mechanisms of nickel toxicity in microorganisms remain poorly understood (Macomber and Hausinger, 2011).

of metals associated with the residual fraction in sediments increased over the 520 days of incubation owing to the migration of metals to higher-energy binding sites (Du Laing et al. 2009; Ma et al. 2006). For instance, more than 50% of Cu in the initial sediment was in the acid-soluble (carbonate-bound), reducible (Fe- and Mn-oxide bound) and oxidisable fractions (Figures S4), whereas Cu was mainly associated with the residual fraction after 520 days of incubation (Figure 3). Changes of Cu proportioning over time (Figure S4) suggest that microbial communities responded to heavy metals in the early stage of the incubation, but diversified according to the zonation of biogeochemical activities in the latter incubation stages.

The slight enrichments of Cu, Zn, Ni and Pb in the zone from 4 to 10 cm below the WSI indicate metal migration between sediment layers during the 520 days of incubation (Figure 3). In anoxic sediments, the Cu, Zn, Ni and Pb concentrations can be affected by the degradation of OM and carbonate dissolution, with the concomitant release of sediment-bound metals, the formation/dissolution of Fe and Al-oxy(hydr)oxides or redox-induced changes of sulphur forms and subsequent sequestration/mobilization of reduced metal sulphides (Du Laing et al. 2009). Both OM degradation and calcite dissolution occurred below the WSI as well as the partial re-oxidation of metal sulphides at the WSI likely contributed to the depth re-distribution of Cu, Ni, Zn and Pb. In addition, Fe enrichment below the WSI and changes in Cu proportioning during sediment aging suggest the release of calcite- and OM-bound metals with concomitant formation of more refractory metal sulphide phases, such as pyrite (included in the residual fraction). In the bottom of the columns, the formation of soluble bisulphide and polysulphide complexes with Cu, Ni and Zn at high sulphide concentrations (<30 µmol L^{-1}) (Huerta et al., 1998) likely increased metal concentrations in porewater.

6. Conclusion

This study delineates the spatial distribution of bacteria and archaea with depth and from light-exposed surfaces to the interior of the sediment cores using Winogradsky columns established with metal-polluted sediments incubated during 520 days. It is postulated that niche differentiation occurred mainly by depth in the mature columns, while diversification was driven by biogeochemical gradients and depth-specific carbon sources at the WSI and the bottom of the columns. Such reproducible differentiation processes in Winogradsky columns can help to understand how a diverse microbial community develops over time and adapts to biogeochemical fluctuations in polluted sediments. Changes of the chemical composition of the initial sediments along biogeochemical gradients in the Winogradsky columns highlight the role of microorganisms and related TEAPs in the cycling of Fe and S. One significant finding is that the distribution of microorganisms with depth was mainly associated with potential niches of prevailing TEAP rather than heavy-metal distribution in the sediments. As expected, a very large proportion of heavy metals was immobilized in the residual fraction, thereby limiting the community response induced by toxic metals, with the exception of Ni in porewater. If physico-chemical conditions change with time or depth, metals may be progressively released. Winogradsky column may thus serve to evaluate risks of heavy-metal mobilization and release and its potential impact on organisms when biogeochemical conditions vary in dynamic environments. In particular, delineating the distribution of microbial communities and pollutant mobility at steep gradients in the WSI and over time may be crucial in the future for predicting the WSI functioning in polluted aquatic ecosystems.

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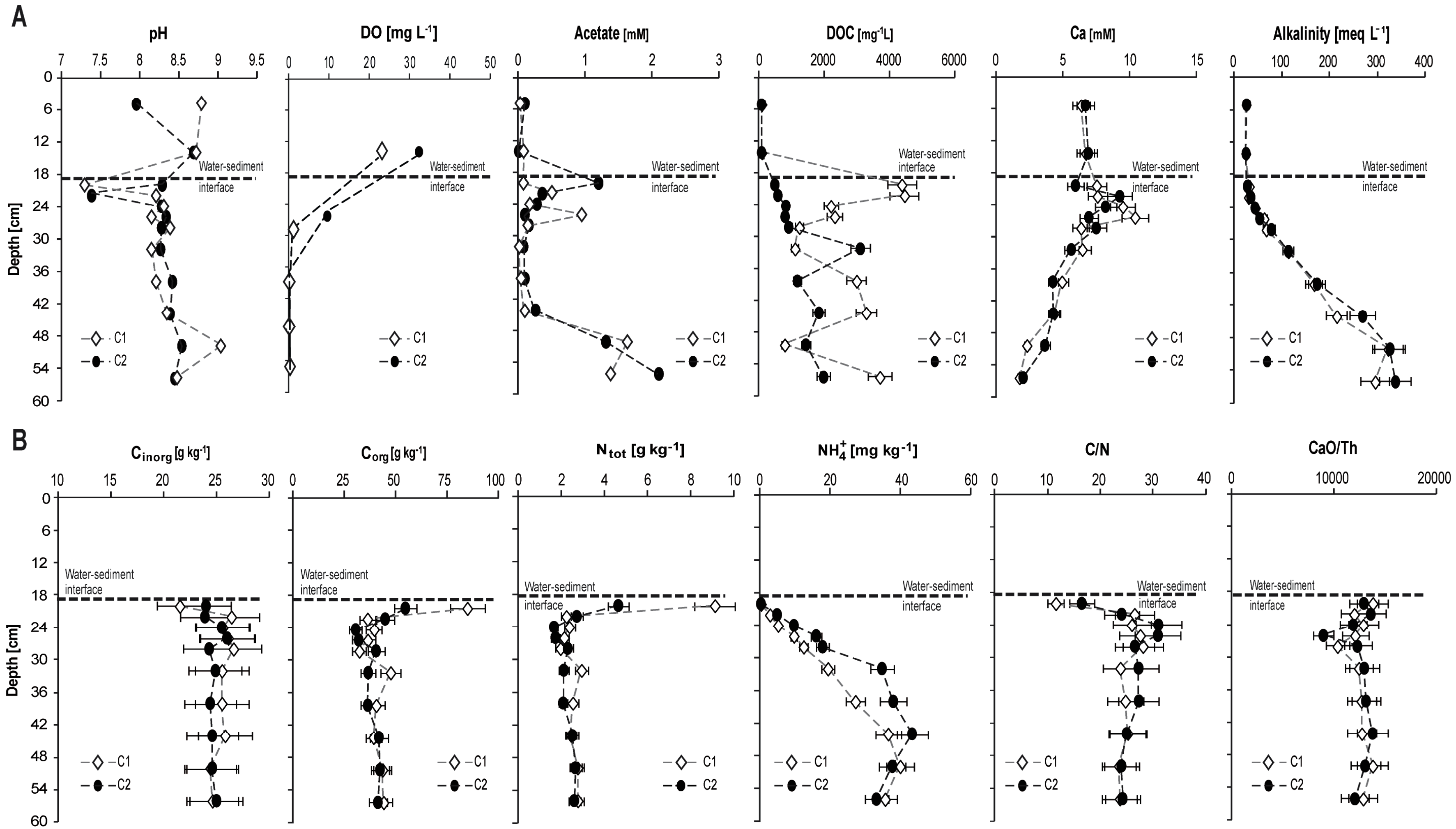

Figure 1. Vertical biogeochemical profiles in the Winogradsky pore water (A) and in the sediments (B).

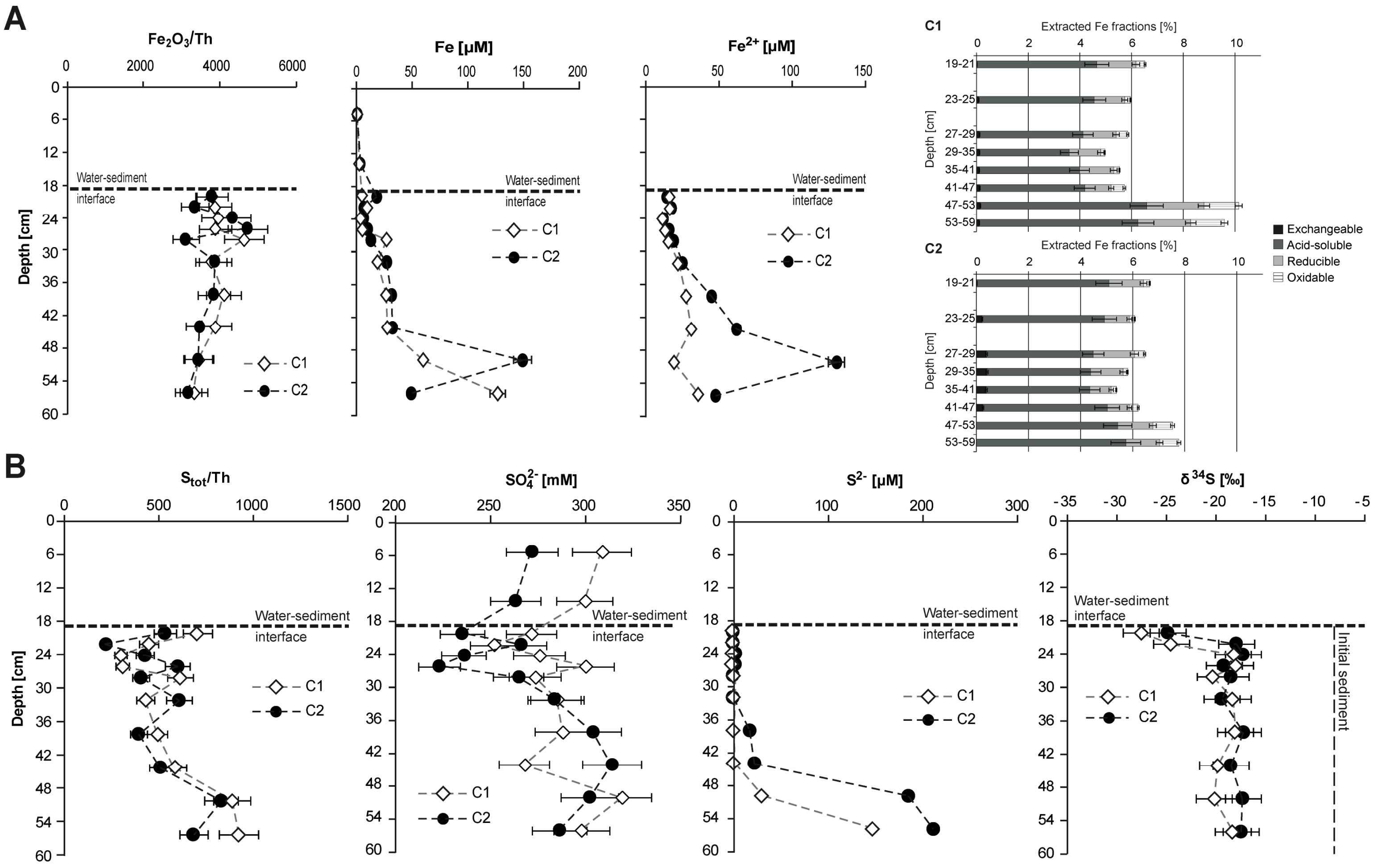

Figure 2. Iron (A) and sulphur (B) biogeochemistry in the Winogradsky columns. Changes in iron and total sulphur concentrations along the profiles were evaluated accounting for dilution/concentration effects and are provided relative to thorium (Th) as a conservative element.

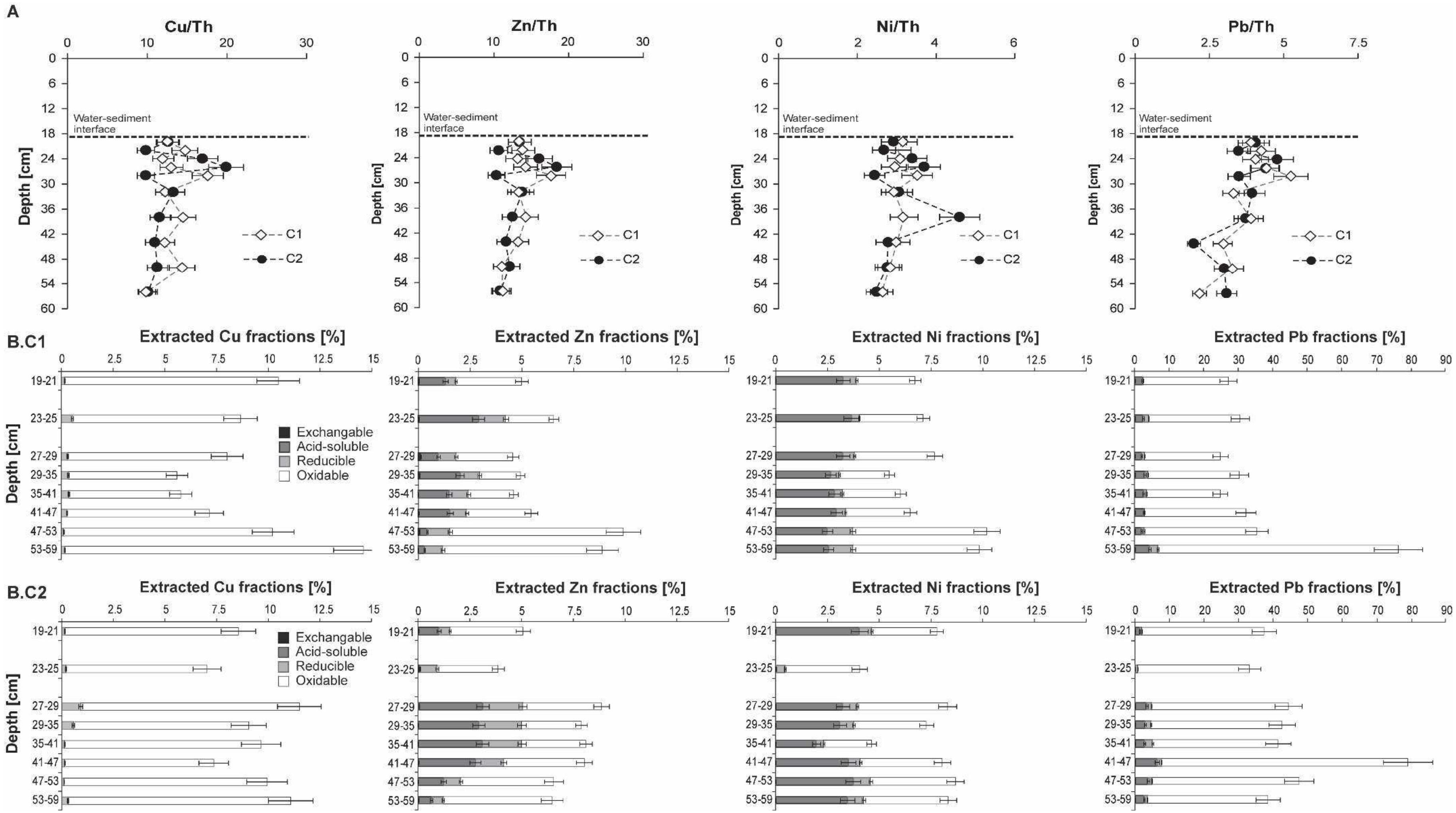

Figure 3. Vertical profiles of heavy metals in pore water (A) and relative proportions (% by weight) of non-residual fractions of Cu, Zn, Ni and Pb in column 1 (B.C1) and column 2 (B.C2). The residual fraction of heavy metals accounts for the remaining % (not displayed).

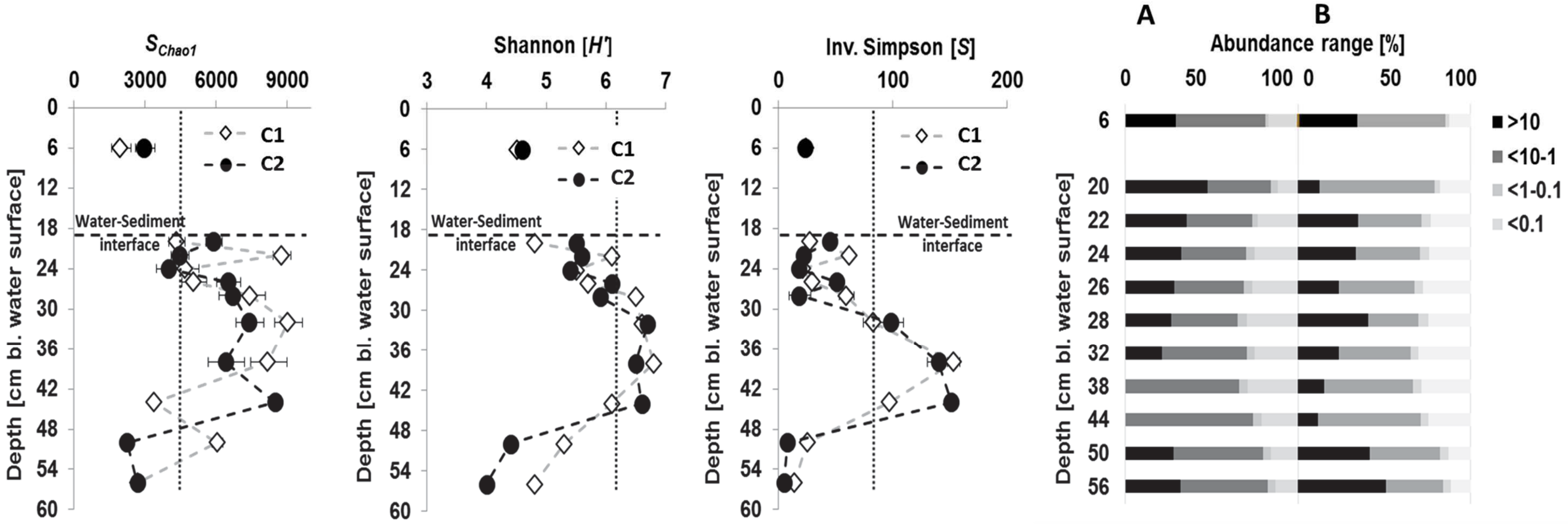

Figure 4. Richness, diversity and distribution of bacterial genera (OTUs at 97% sequence identity; using bacterial 515F/806R primers; 10117 sequences sampled). The error associated with the Chao 1, Shannon and Simpson indexes was <15%, <1.7%, and <11% of the value, respectively. The vertical dashed lines indicate richness and diversity values of the initial sediment. On the right, the relative distribution of abundant and rare genera is shown for column 1 (A) and column 2 (B).

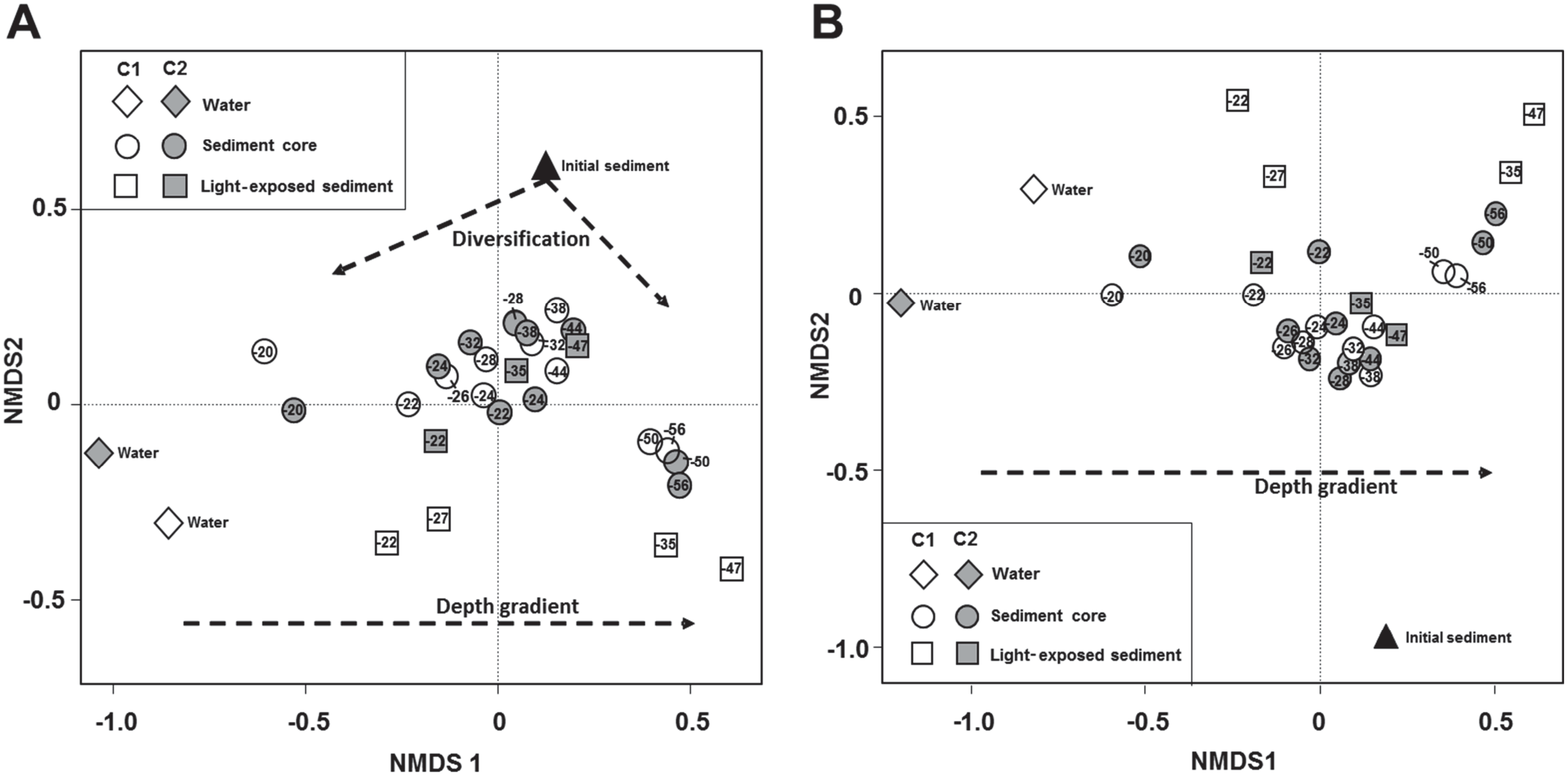

Figure 5. 2D-NMDS ordination of community profiles of Illumina MiSeq-sequenced samples of the Winogradsky columns obtained with the (A) 515F/806R and (B) 519wF/909R primer pairs. Numbers in the objects indicate the depth [cm] below the water surface. Plot stress for (A) and (B) = 0.08.

Table S1. Physico-chemical characteristics of the sediments used in the Winogradsky columns.

pH	**[-]**	7.85
Org. matter	**[g kg^{-1}]**	77
N tot		n.a.
N-NO_3	**[mg kg^{-1}]**	n.a.
N-NH_4		n.a.
SiO2	**[%]**	50.26
Al_2O_3		7.59
MgO		1.57
CaO		13.1
Fe_2O_3		3.21
MnO		0.074
TiO_2		0.44
Na_2O		1.17
K_2O		2.20
P_2O_5		0.31
Stot		0.15
Cu	**[mg kg^{-1}]**	120
Zn		114
Ni		26.0
Pb		31
Th		9.83

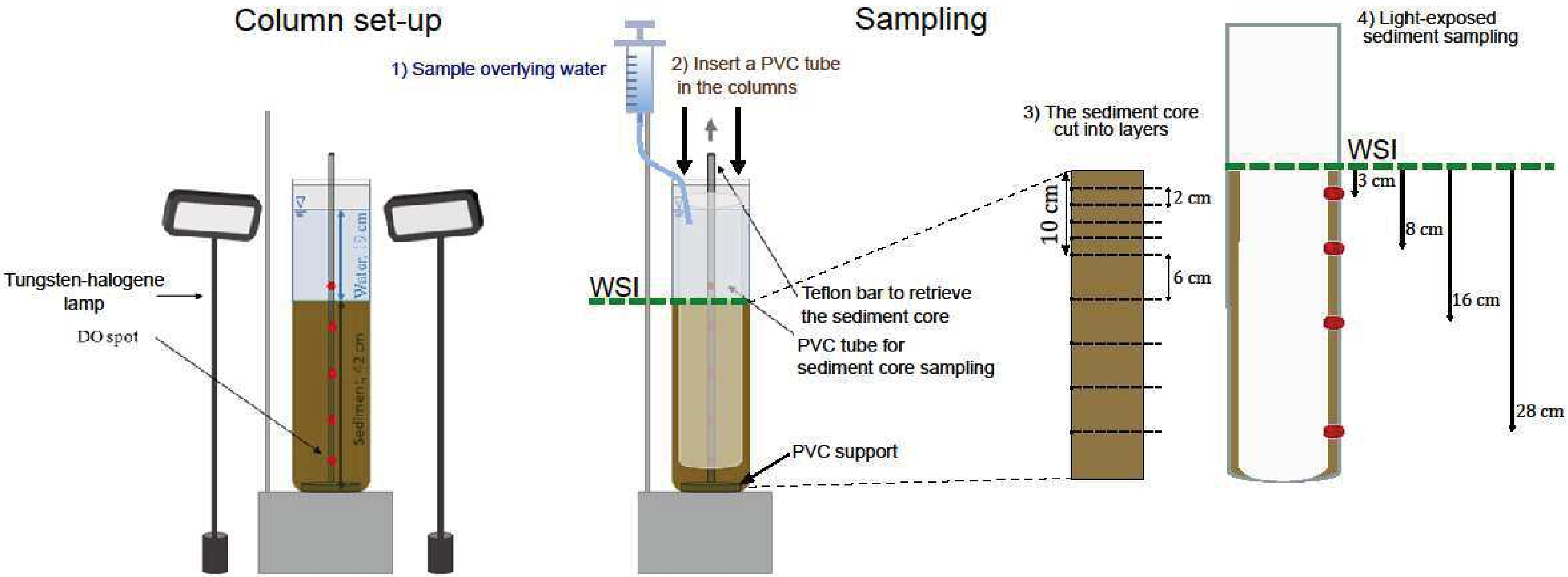

Figure S1: Schematic of the Winogradsky columns showing the deployment of dissolved oxygen spots and the sampling devices. **(1)** The overlying water was collected after 520 days of incubation using plastic syringes and Teflon® needles. **(2)** Intact sediment cores were extracted from the middle of each column by sinking a PVC tube (Ø: 12 cm, height: 65 cm) into the sediments up to a PTFE support placed at the bottom of the column. The cores in PVC tubes were then raised and kept intact with the help of a PTFE stick fixed to the bottom support, which was placed in the columns before addition of sediment. **(3)** The intact cores were immediately frozen at –20°C. The frozen sediment cores collected in the middle of the columns were sliced into 2-cm layers (top 10 cm) and 6-cm layers (bottom section) with a tungsten saw, as steeper gradients were expected in the top 10-cm. **(4)** Light-exposed sediment samples were collected along the column wall at 3, 8, 16 and 28 cm below the water-sediment interface (WSI) to evaluate changes in the microbial community in a latitudinal gradient from the light-exposed surface to the interior of the column (Figure S1).

Table S2. Mineral phases detected by the XRD analysis in the initial sediment and specific layers of column 1 and column 2.

Samples		
	Depth in the whole column	**Phases detected by XRD**
Initial sediments		Quartz, calcite, dolomite, feldspars, clay minerals (illite, chlorite and/or kaolinite)
C1	27-29 cm	Quartz, calcite, dolomite, feldspars (albite, microcline), chlorapatite, clay minerals (illite, chlorite and/or kaolinite)
	53-59 cm	Quartz, calcite, dolomite, feldspars (albite, microcline), clay minerals (illite, chlorite and/or kaolinite)
C2	25-27 cm	Quartz, calcite, dolomite, feldspars (albite, microcline), apatite (chlorapatite and/or fluorapatite), **pyrite**, clay minerals (illite, chlorite and/or kaolinite)
	53-59 cm	Quartz, calcite, dolomite, feldspars (albite, microcline), chlorapatite, **pyrite**, clay minerals (illite, chlorite and/or kaolinite)

Table S3. Physico-chemical characteristics of pore water along the Winogradsky columns. WSI = water-sediment interface. Pb was detected neither in the overlying water, nor in the pore water.

	Depth [cm below water surface]	**pH**	**NH_4^+ [mM/l]**	**Na^+ [mM]**	**K^+ [mM]**	**Mg^{2+} [mM/l]**	**Ca^{2+} [mM/l]**	**Si [mM/L]**	**Acetate [mM/l]**	**Cl^- [mM/l]**	**SO_4^{2-} [mM/l]**	**PO_4^{3-} [mM/l]**	**DOC [mg/l]**	**CO_3^{2-} [mq/l]**	**HCO_3^- [mq/l]**	**Alkalinity [mq/l]**	**Fe [µM/L]**	**Al [ppm]**	**Cu [ppm]**	**Ni [ppm]**	**Zn [ppm]**
Column 1	5 (water)	8.8	0.001	584.2	32.61	1.91	6.17	0.42	0.02	0.61	310.5	0.67	89	3.8	20.0	24.7	0.39	0.000	0.017	0.009	0.000
	14 (water)	8.72	0.00	571.2	31.71	1.93	6.51	0.41	0.09	0.94	301.4	0.69	89	2.8	20.5	24.2	1.24	0.002	0.019	0.009	0.001
	20 (WSI)	7.31	1.39	524.8	25.71	1.99	7.32	0.29	0.08	0.66	272.7	0.82	5200	0.5	29.3	31.4	4.91	0.027	<ld	0.089	0.039
	22	8.23	1.46	486.1	25.62	1.90	7.38	0.39	0.51	0.73	252.8	1.16	5300	1.5	27.0	29.5	8.91	<ld	<ld	0.023	0.022
	24	8.32	4.34	536.3	27.65	2.28	9.27	0.35	0.19	0.60	277.1	1.02	2610	3.8	39.0	43.9	4.29	0.023	0.018	0.029	0.024
	26	8.17	9.05	596.2	31.11	2.72	10.18	0.41	0.99	0.56	301.6	1.05	2750	1.7	58.8	62.2	4.70	0.007	<ld	0.043	0.012
	28	8.40	11.88	565.2	28.69	2.44	6.15	0.41	0.15	0.57	274.8	0.95	1460	5.8	60.0	66.6	26.79	0.472	<ld	0.058	0.011
	32	8.17	22.00	611.4	31.00	2.96	6.25	0.51	0.03	0.65	286.5	1.36	1300	6.5	105.0	112.7	18.63	0.032	<ld	0.041	0.010
	38	8.22	32.58	660.2	33.39	2.80	4.73	0.58	0.03	0.62	289.4	1.70	3550	11.8	153.0	166.5	26.40	0.063	<ld	0.046	0.004
	44	8.35	36.72	677.3	34.10	2.57	4.17	0.66	0.09	0.63	269.2	1.88	3900	20.0	192.0	213.8	27.32	0.058	<ld	0.065	0.008
	50	9.06	17.46	879.5	47.02	1.42	2.12	0.50	1.63	0.65	321.0	2.89	925	76.0	244.0	321.0	59.67	0.256	0.018	0.239	0.015
	56	8.47	43.75	799.4	39.97	1.46	1.56	0.31	1.38	0.68	299.3	2.80	4400	32.0	262.0	294.0	126.48	0.370	<ld	0.359	0.027
Column 2	5 (water)	7.97	0.00	517.3	30.18	1.87	6.49	0.44	0.10	0.57	273.1	0.60	84	5.3	19.8	25.8	0.32	0.002	0.008	0.006	0.000
	14 (water)	8.70	0.00	501.7	29.64	1.88	6.71	0.42	0.01	0.64	264.3	0.60	85	3.3	21.0	24.7	3.04	0.004	0.008	0.010	0.033
	20 (WSI)	8.30	1.47	451.7	24.17	2.29	5.75	0.41	1.20	0.68	235.8	1.05	567	0.8	25.0	27.4	18.01	<ld	<ld	0.048	0.026
	22	7.40	2.64	521.1	29.11	2.37	9.05	0.41	0.36	0.56	267.3	1.11	680	2.8	31.9	34.7	6.39	<ld	0.009	0.047	0.014
	24	8.29	6.02	462.9	25.77	2.22	7.99	0.38	0.28	1.08	237.1	1.05	971	3.6	38.9	43.4	6.19	0.137	<ld	0.040	0.012
	26	8.35	9.70	444.7	23.95	2.27	6.75	0.48	0.10	0.66	223.6	1.09	943	3.5	47.4	53.3	9.82	<ld	<ld	0.028	0.019
	28	8.29	17.59	552.5	29.73	2.75	7.30	0.45	0.16	0.71	266.2	1.03	1076	6.0	70.6	77.4	12.70	0.034	<ld	0.019	0.004
	32	8.28	26.64	603.3	32.36	3.09	5.44	0.47	0.08	0.70	285.0	1.40	3690	8.8	104.0	113.2	27.01	<ld	<ld	0.031	0.018
	38	8.43	32.11	698.5	37.88	3.08	4.07	0.78	0.10	0.69	305.7	2.33	1390	18.5	154.6	173.2	31.34	0.044	0.030	0.093	0.012
	44	8.40	44.00	794.4	43.24	2.65	4.10	0.73	0.26	0.66	315.9	3.12	2180	30.0	238.0	268.2	32.16	0.082	0.020	0.063	0.012
	50	8.55	40.11	832.0	45.11	1.87	3.47	0.76	1.31	0.68	303.9	2.81	1700	42.0	282.0	325.4	148.79	0.138	0.023	0.298	0.035
	56	8.46	46.42	812.8	43.58	1.45	1.85	0.47	2.10	1.58	287.8	3.25	2350	36.0	300.0	336.7	48.89	0.719	0.026	0.362	0.038

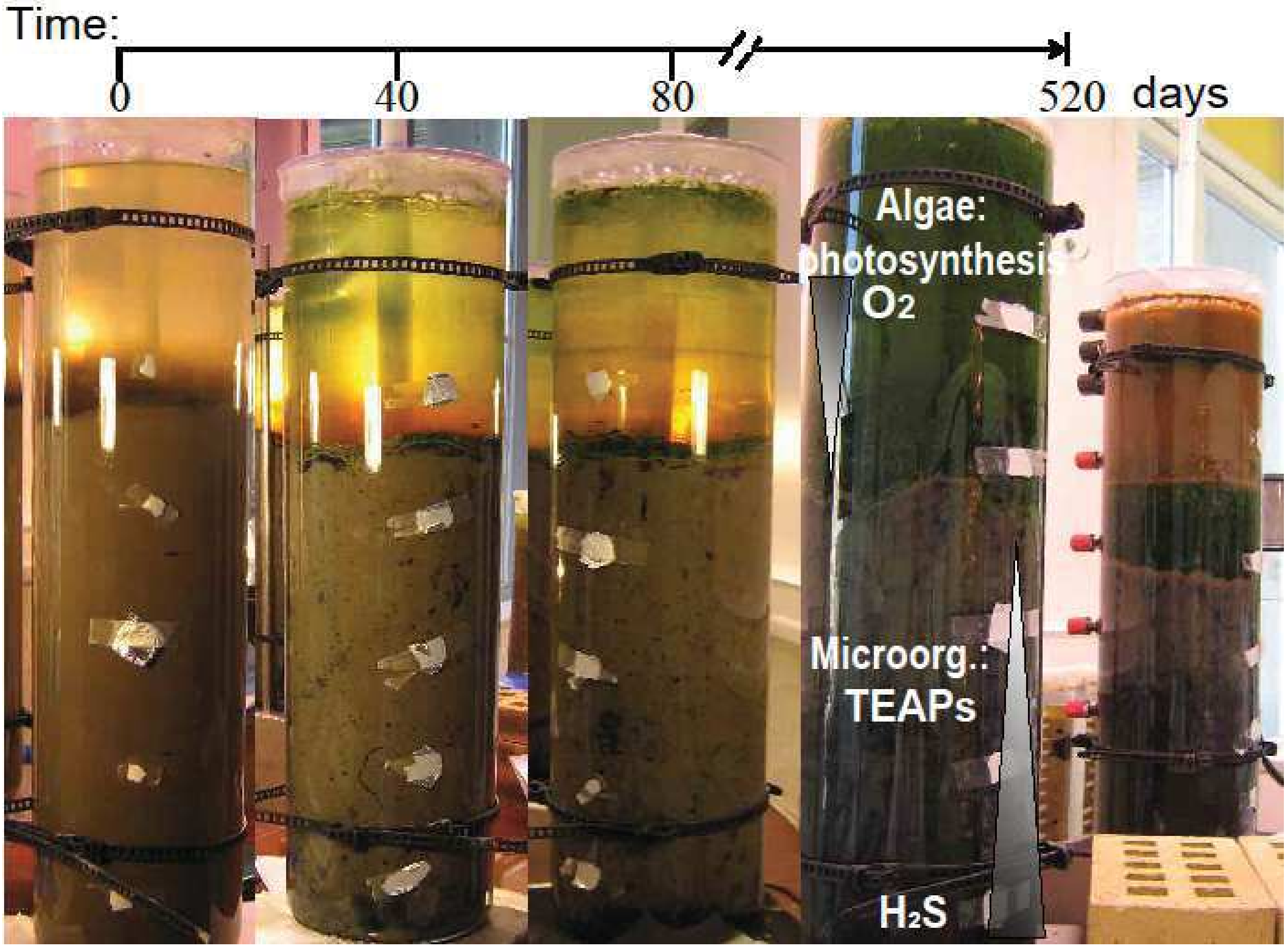

Figure S2. Winogradsky columns at day 40, 80 and 520 of incubation.

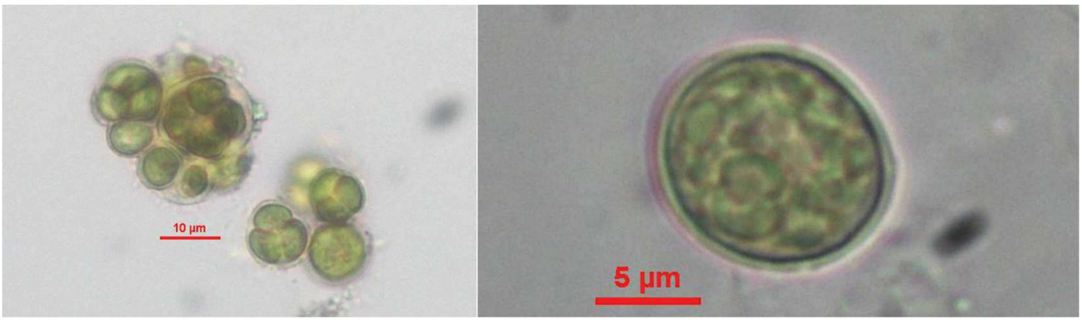

Figure S3: Colonial and unicellular forms of *Tetracystis/Chlorococcum* sp. in the water layer of the Winogradsky columns.

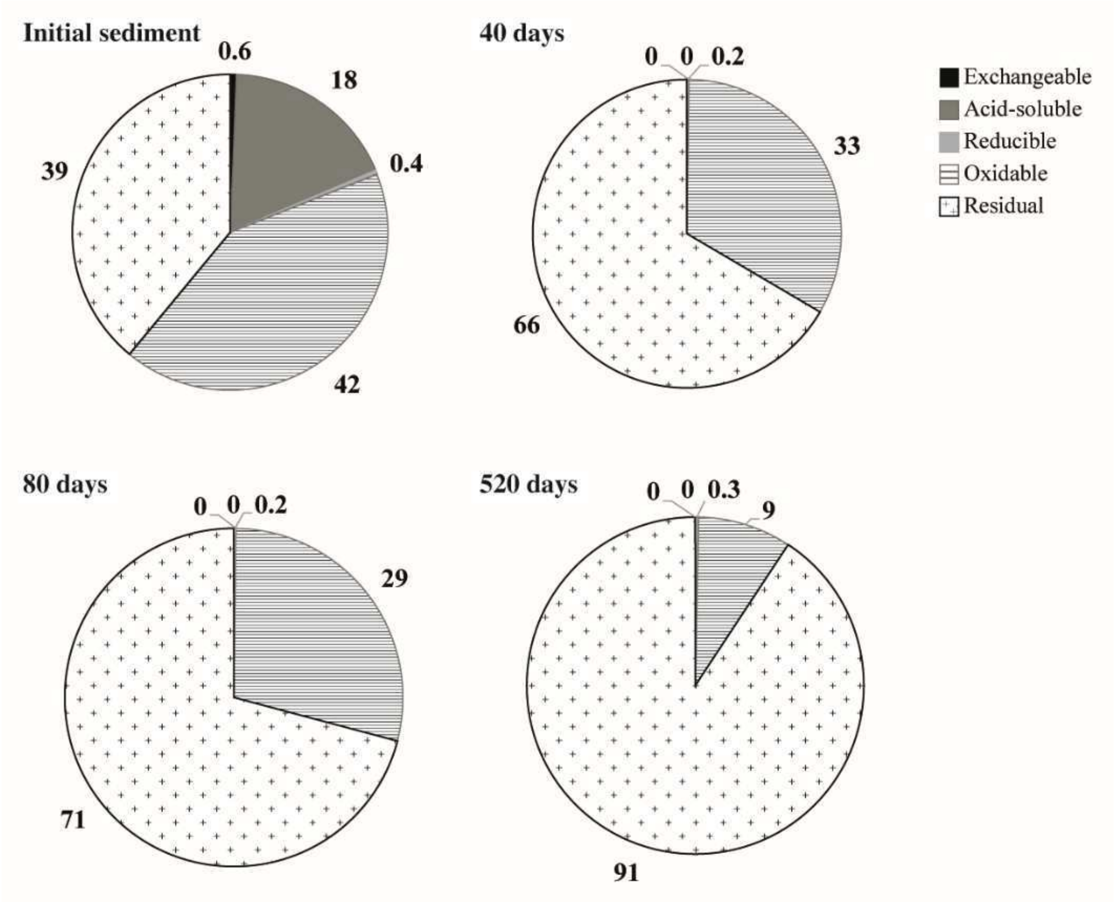

Figure S4: Temporal changes in the proportioning of Cu as assessed by sequential chemical extractions of the initial sediment and after 40 days, 80 days and 520 days of incubation in Winogradsky columns.

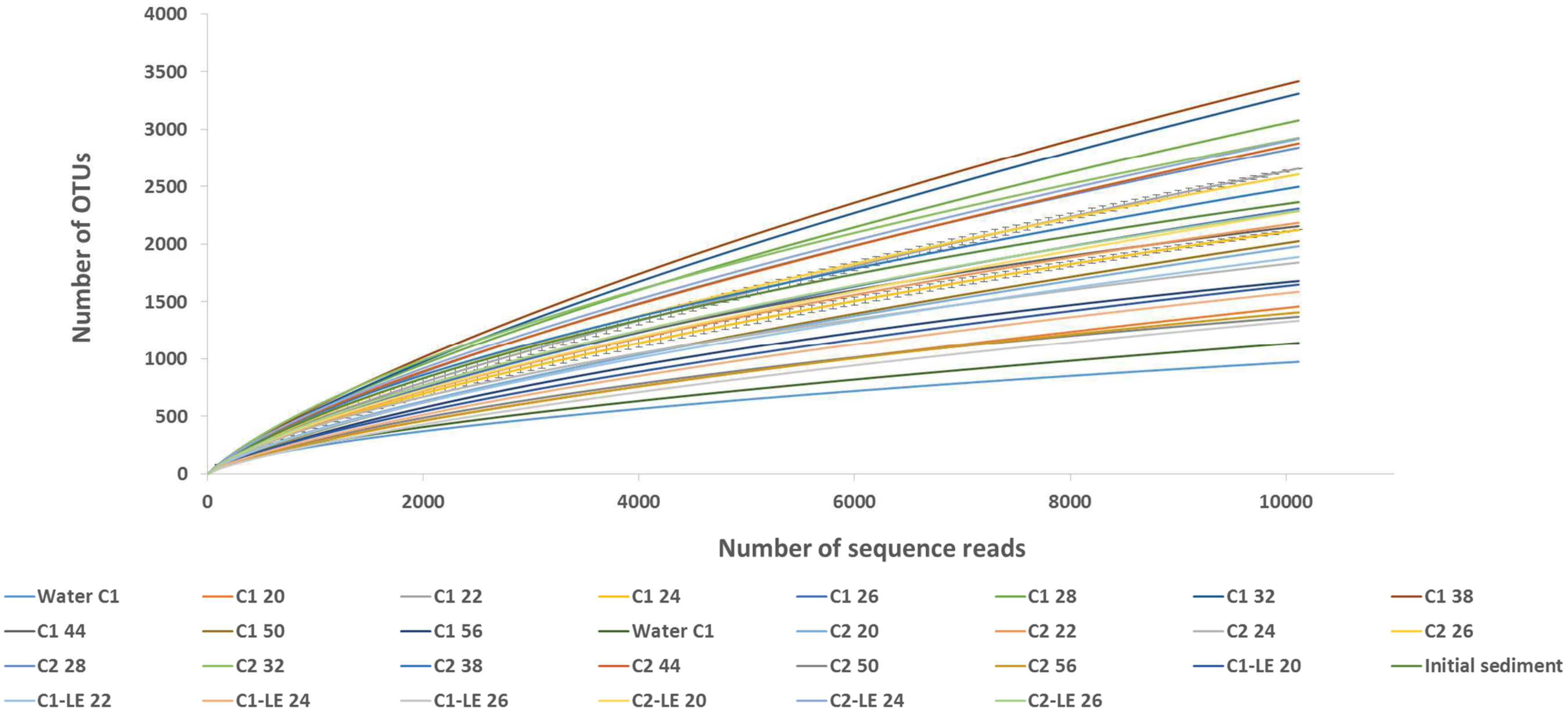

Figure S5. Rarefaction curves for bacterial OTUs clustering at 97% sequence identity. Curves are shown for depth-specific sediment samples from the Winogradsky columns after 520 days of incubation.

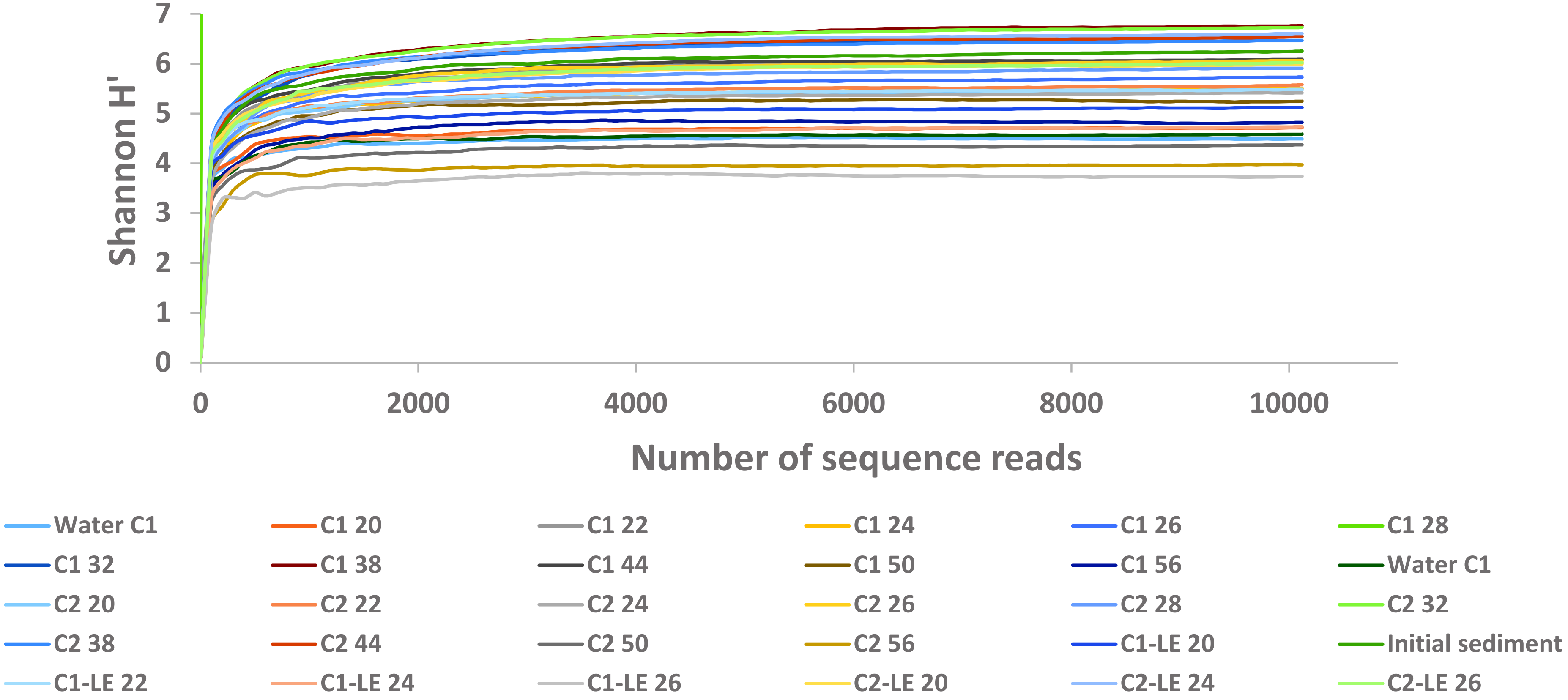

Figure S6. Rarefaction curves for Shannon H'diversity (97% sequence identity). Curves are shown for depth-specific sediment samples from the Winogradsky columns after 520 days of incubation. Similar results were obtained for Chao1 and Simpson diversity indexes (data not shown).

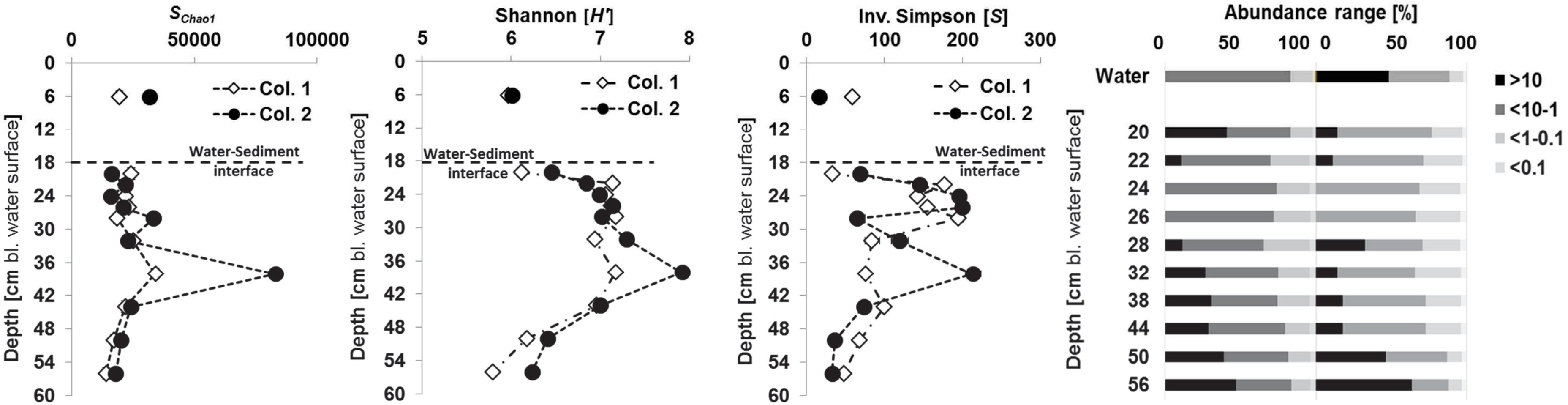

Figure S7. Richness, diversity and distribution of archaeal genera (OTUs at 97% sequence identity; using 519wF/909R primers; 10,050 sequences sampled). On the right, the distribution of abundant and rare genera is shown for column 1 (A) and column 2 (B). The error associated with the Chao 1, Shannon and Simpson indexes was <15%. <1.7%. and <11% of the value, respectively.

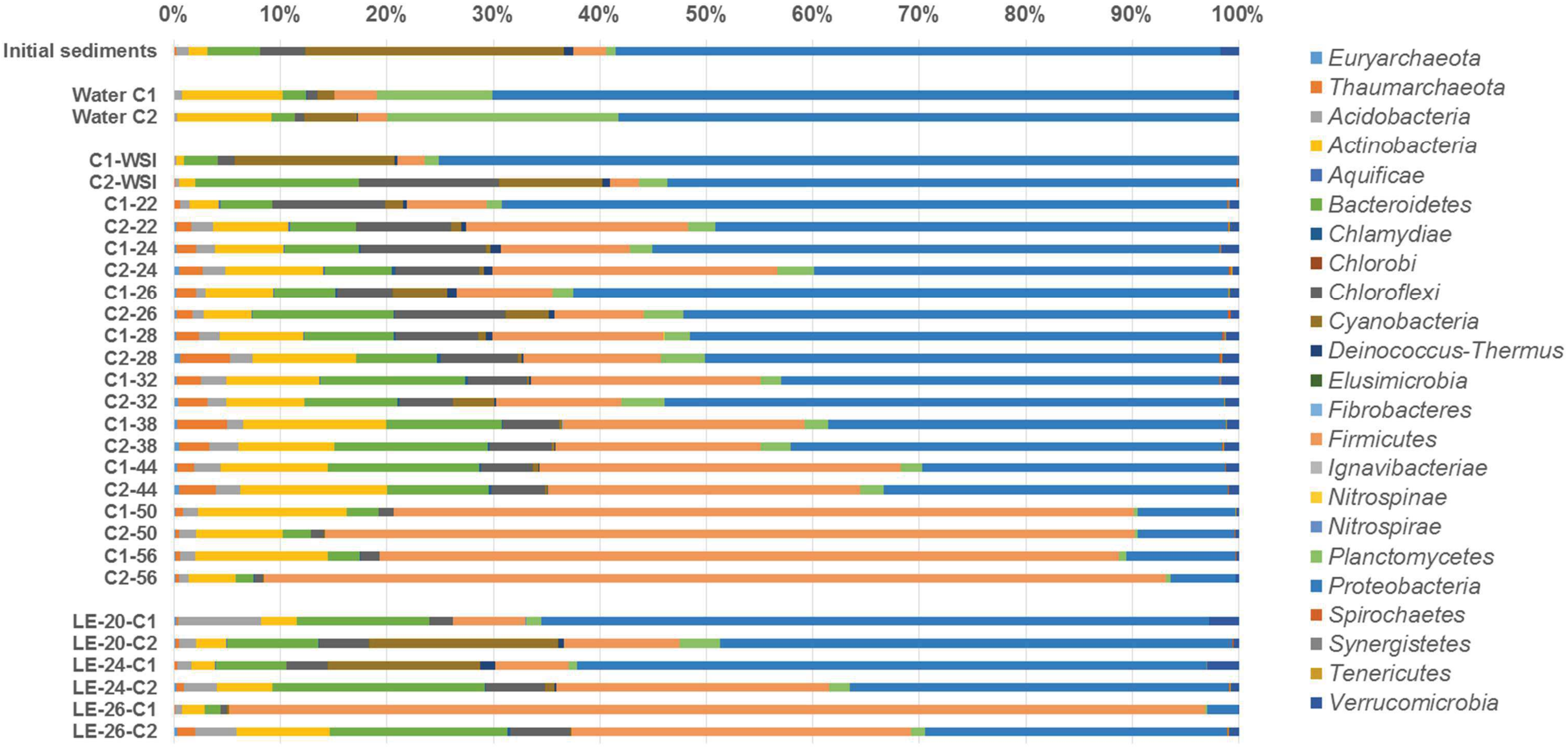

Figure S8. Relative abundance [%] of bacterial phyla (defined at 77% sequence identity using the 515F/806R primer pair) in Winogradsky column 1 (C1) and 2 (C2) in the initial sediment, the top-water layer (Water) and down-core in the sediment, from the water-sediment interface (WSI) up to the bottom of the columns (56 cm below water surface). LE = light-exposed samples (20, 24 and 26 cm below the water surface).

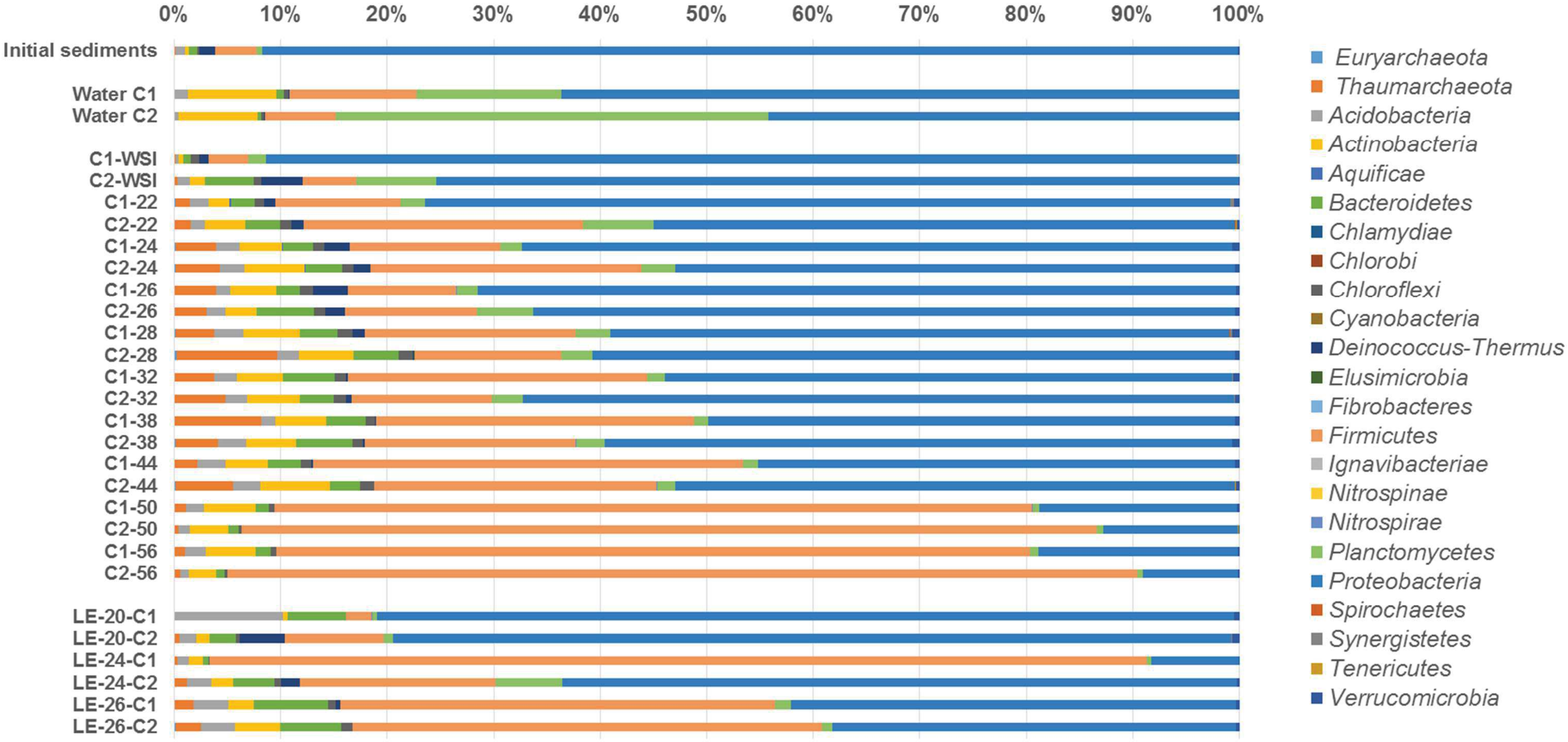

Figure S9. Relative abundance [%] of archaeal phyla (defined at 77% sequence identity, using the 519wF/909R primer pair) in Winogradsky column 1 (C1) and 2 (C2) in the initial sediment, the top-water layer (Water) and down-core in the sediment, from the water-sediment interface (WSI) up to the bottom of the columns (56 cm below water surface). LE = light-exposed samples (20, 24 and 26 cm below the water surface.

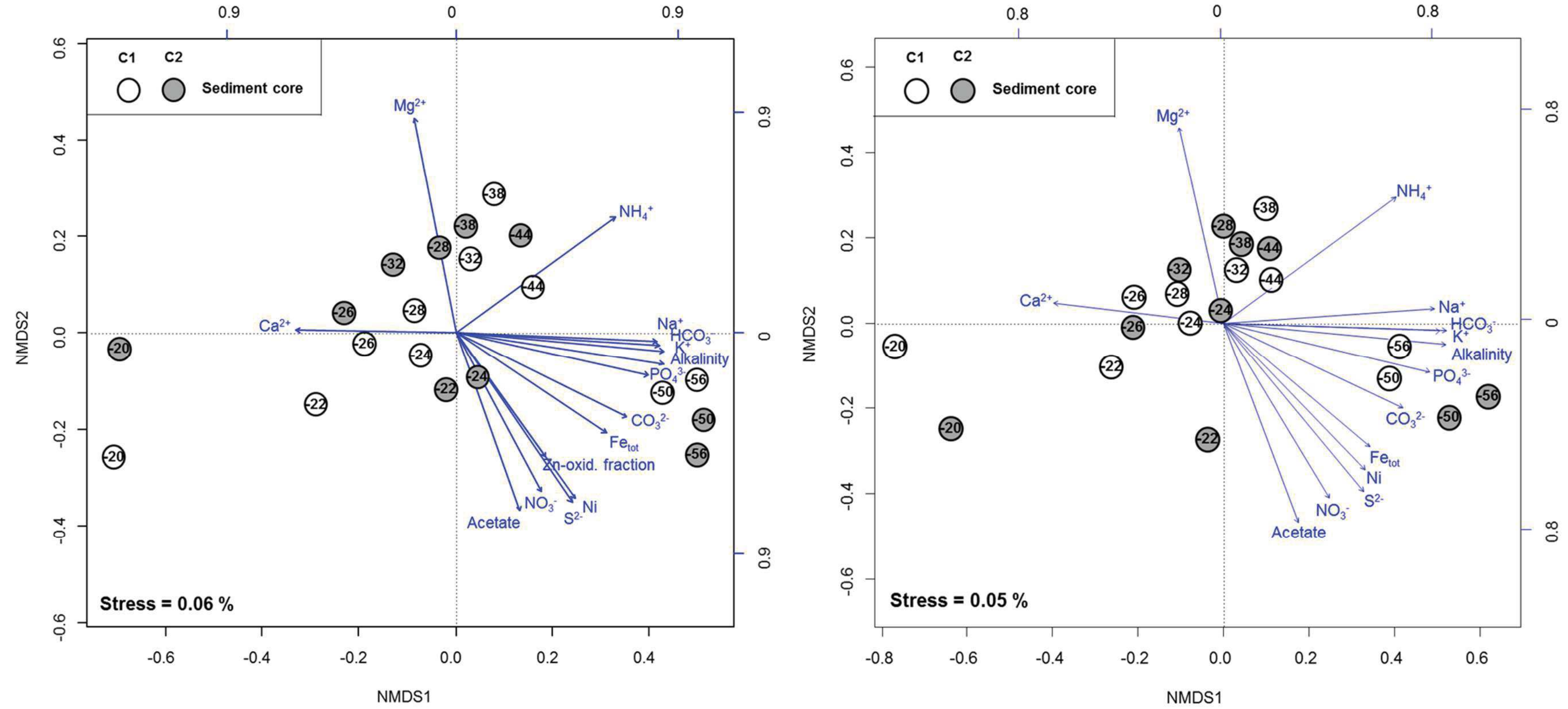

Figure S10. 2D-NMDS ordination of community profiles of Illumina MiSeq-sequenced sediment samples of the Winogradsky columns obtained with the (right) 515F/806R and (left) 519wF/909R primer pairs. Numbers in the objects indicate the depth [cm] below the water surface. Vectors that correspond to chemical variables significantly correlated with microbial community structure are shown. The significance of fitted vectors was calculated by a posteriori permutation of variables at $p < 0.01$. Vector arrows were fitted to the NMDS ordination depicting the direction and magnitude of change of the variable.

Université de Strasbourg

Fatima MEITE

Ecole et Observatoire des Sciences de la Terre

Transformation et transport des pesticides inorganiques et de synthèse dans les sols de bassins versants agricoles

Résumé

Malgré les tests d'homologation des pesticides et métaux, des teneurs alarmantes de ces produits sont relevées dans l'environnement. Une meilleure connaissance des processus subis par ces produits dans les agrosystèmes est nécessaire afin de réduire les pollutions liées à leur utilisation.

Cette thèse vise à améliorer la compréhension de la transformation et du transport des pesticides et métaux pas l'étude détaillée de ces polluants dans les sols agricoles durant 200 jours, en fonction des conditions hydro-climatiques et l'analyse isotopique des pesticides (par composés spécifiques, CSIA) et des métaux.

Ces travaux révèlent l'importance de la matière organique sur la spéciation des polluants, les relations entre les conditions, la dissipation et les mécanismes de transformation des pesticides, mais aussi le contrôle de l'infiltration des polluants par la durée et la fréquence des pluies. Ces résultats permettent d'améliorer la prédiction du transport des polluants dans les agrosystèmes.

Mots clés : Pesticides, métaux, sols agricoles, spéciation, dégradation, CSIA, signature isotopique des métaux, infiltration, ruissellement

Résumé en anglais

Despite homologation tests of pesticides and metals, alarming concentrations are measured in the environment. A better knowledge of the transformation processes undergone by these products in agrosystems is needed to predict and limit the pollution.

This PhD. thesis allowed to understand the transformation and transport of pesticides and metals in agricultural soils during 200 days and with respect to different hydro-climatic conditions using sequential extraction and isotopic fractionation of pesticides (compound specific isotopic analyses, CSIA) and metals.

This work revealed the relevant of soil organic matter on the speciation of pollutants, the relationship between hydro-climatic conditions, the dissipation and the transformation mechanisms of peticides, and how pollutant infiltration is controlled both by rainfall length and frequency. These resuls will improve the transport models of pesticides in agrosystems.

Keywords: Pesticides, metals, agricultural soils, speciation, degradation, CSIA, isotopic signatures of metals, leaching, runoff