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**Persistence à long terme des matières organiques dans  
les sols : caractérisation chimique et contrôle  
minéralogique**

Suzanne Lutfalla

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Spécialité de doctorat : Sciences de l'Environnement

Par

**Madame Suzanne Lutfalla**

Long-term persistence of soil organic matter: chemical characterization and  
mineralogical control

-

Persistence à long terme des matières organiques dans les sols : caractérisation  
chimique et contrôle minéralogique

**Thèse présentée et soutenue à Paris, le 19 novembre 2015 :**

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## **Foreword**

2015 is a year that has the power to change the way we deal with climate change. The last IPCC report, published earlier this year, draws conclusions that are ever more alarming and is reminding the whole world that climate change is one of the biggest challenges humankind is facing in the 21<sup>st</sup> century. Furthermore, the COP21 conference is currently under preparation, with the aim to finally succeed in reaching a legally binding global agreement on greenhouse gas emissions. Last but not least, 2015 is also the International Year of Soils as announced by the United Nations Food and Agriculture organization.

Finishing my PhD during this same year and contributing, even a little bit, to this global synergy is an honor.





## Abbreviations

AFES	<i>Association française pour l'étude du sol</i>
BF	Bare fallow
BPCA	Benzene polycarboxylic acid
C	Carbon
CEC	Cation exchange capacity
CLS	Canadian Light Source
COP21	Conference of Parties n°21 (United Nations)
DOC	Dissolved organic carbon
FTIR	Fourier Transform InfraRed
INRA	<i>Institut national de la recherche agronomique</i>
IPCC	Intergovernmental panel on climate change
IR	Infrared
MRT	Mean residence time
N	Nitrogen
NASA	National aeronautics and space administration
NEXAFS	Near edge X-ray absorption fine structure
OC	Organic carbon
OM	Organic matter
PCA	Principal component analysis
POM	Particulate organic matter
Py-GC-MS	Pyrolysis Gas chromatography Mass spectrometry
PyOC	Pyrogenic organic carbon
SD	Standard deviation
NMR	Nuclear magnetic resonance
SGM	Spherical grating monochromator
SIMS	Secondary ion mass spectrometry
SOC	Soil organic carbon
SOM	Soil organic matter
STXM	Scanning transmission X-ray microscopy
TOC	Total organic carbon
UV	Ultraviolet
VIS	Visible
XANES	X-ray absorption near edge structure
XAS	X-ray absorption spectroscopy

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# PART 0: RESUME SUBSTANTIEL EN LANGUE FRANCAISE

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## **Persistance à long terme des matières organiques dans les sols : caractérisation chimique et contrôle minéralogique**

### **Préambule**

2015 est une année qui peut voir évoluer la façon dont nous appréhendons et répondons au changement climatique. Le dernier rapport du Groupe d'experts intergouvernemental sur l'évolution du climat (GIEC) publié en 2014 présente des conclusions de plus en plus alarmantes et nous rappelle à quel point le changement climatique fait partie des enjeux actuels majeurs. Cette année, la COP21 (*21<sup>st</sup> Conference of Parties*) se déroulera dans le but de parvenir à un accord universel contraignant qui vise à mettre en place un plan de lutte contre le changement climatique. Enfin, les Nations Unies ont désigné 2015 Année Internationale des Sols.

Conclure mon travail doctoral sur le carbone dans les sols en 2015 et participer ainsi à cette synergie internationale est un honneur pour moi.

### **1. Introduction**

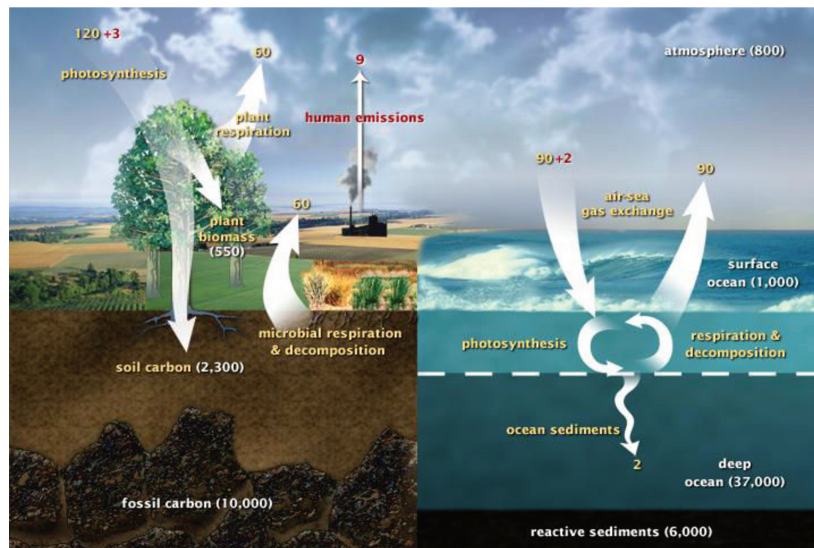
#### **1.1. Les sols dans le cycle global du carbone**

Le changement climatique est principalement dû à l'émission d'origine anthropique de gaz à effet de serre dans l'atmosphère. Les principaux gaz concernés sont le dioxyde de carbone (CO<sub>2</sub>), le méthane (CH<sub>4</sub>), le protoxyde d'azote (N<sub>2</sub>O) et la vapeur d'eau (H<sub>2</sub>O). Le dioxyde de carbone est le gaz qui a le plus d'effet, aujourd'hui, sur le changement climatique. Sa concentration atmosphérique est passée d'un niveau préindustriel de 280 parties par millions (ppm) en 1850 à plus de 390 ppm en 2014. Les effets du changement climatique se feront sentir à de nombreux niveaux : sécurité alimentaire menacée, montée du niveau des eaux, perturbation du cycle hydrologique et des précipitations, écosystèmes fragilisés... C'est pour cela que de nombreux scientifiques ont coopéré pour rassembler et analyser les données scientifiques afin d'informer les gouvernements sur la nature et l'ampleur du changement climatique (GIEC). Par ailleurs, des institutions ainsi que des personnalités internationales ont appelé à la mise en œuvre d'un plan de lutte contre le changement climatique. Enfin, des initiatives locales sur ce sujet voient le jour un peu partout dans le monde.

Comprendre la science liée au changement climatique est un prérequis à la prise de décision et à la lutte efficace contre le changement climatique. L'élément chimique le plus important dans ce phénomène est le carbone (présent dans 2 des 4 gaz à effet de serre les plus importants). Il est donc primordial d'étudier et de comprendre le cycle du carbone, c'est à dire de comprendre l'ensemble des flux entre les différents réservoirs de carbone sur Terre.



Le carbone est échangé entre cinq réservoirs majeurs : la biosphère, la géosphère, la pédosphère, l'hydrosphère et l'atmosphère. En termes de flux naturels, à l'échelle de l'année au siècle, les principaux échanges de carbone se font entre la biosphère et l'atmosphère – par la photosynthèse et la respiration de la biomasse-, entre l'atmosphère et l'hydrosphère – par l'équilibre de la dissolution des carbonates dans les océans- et entre les couches profonde et superficielle des océans. Le développement des activités industrielles humaines et les changements massifs d'usage des sols ont conduit à l'émission de carbone depuis la biosphère, la pédosphère et la géosphère vers l'atmosphère.



**Figure 1 : Représentation des différents réservoirs de carbone, de leurs stocks et des flux qui les relient et qui constituent le cycle. Globale du carbone. (Source : NASA).**

En termes de quantité de carbone contenu dans les différents réservoirs (Figure 1), le sol –la pédosphère- contient environ trois fois plus de carbone que l'atmosphère. On estime que le premier mètre de sol contient environ 1500 Gt de carbone organique (Post et al., 1982, Jobbagy and Jackson, 2000) ; ce qui en fait le réservoir continental le plus important (devant la biomasse, qui en contient environ 560 Gt).

Le carbone organique du sol se trouve principalement (i) dans les tissus vivants des plantes, des animaux et des microorganismes du sol (ii) dans les matières organiques issues des êtres vivants dans ou à la surface du sol et qui sont incorporées au sol (Van-Camp et al., 2004). La matière organique du sol (en excluant les êtres vivants dans le sol) peut avoir trois origines : les plantes, les animaux (faune et micro-faune) et le feu. Les plantes sont la principale source de carbone organique dans le sol. En effet, lorsqu'une plante pousse, ses racines grandissent dans le sol et exsudent sans cesse des composés organiques. Enfin, quand la plante meurt, ces racines restent dans le sol et la partie aérienne de la plante (feuilles, tiges) peut être incorporée dans le sol, ce qui vient alimenter le compartiment de matière organique du sol (Rasse et al., 2005). Les animaux et microorganismes contribuent de la même manière à la matière organique du sol. Enfin, la combustion de la biomasse produit du carbone organique pyrogénique qui peut également être incorporé à la matrice du sol.

Une fois le carbone présent dans la matière organique du sol (MOS), il existe plusieurs phénomènes qui peuvent conduire à sa sortie du sol : la biodégradation et la minéralisation, la photo-oxydation, l'érosion ou la lixiviation. La **biodégradation** d'une matière organique est le résultat de plusieurs étapes : (i) la fragmentation et l'enfouissement des débris organiques dans le sol par la faune, sa colonisation et sa modification par les microorganismes du sol et son utilisation comme substrat par les microorganismes. Quand cette utilisation comme substrat conduit à l'émission de  $\text{CO}_2$  on dit qu'il y a eu **minéralisation** de la matière organique. La photo-oxydation est un processus d'oxydation de la matière organique en



**Figure 2 : Représentation schématique des principales entrées et sorties de carbone du sol.** (Source mentionnée sur l'image).

présence de lumière et d'oxygène, des études se sont intéressées à la photo-oxydation de la matière organique dissoute (Judd et al., 2007) mais peu d'études existent sur la photo-oxydation des MOS. La lixiviation est un processus qui entraîne la perte de matières organiques par lessivage, elle est due aux flux d'eau dans le sol et résulte en un déplacement de la matière organique vers des couches du sol situées en profondeur, voire même à sa sortie du sol vers d'autres réservoirs (nappes phréatiques, rivières...) (Montagne et al., 2009). Enfin, l'érosion peut être responsable du transport physique de la matière organique du sol en dehors du sol par le vent et les précipitations (Gregorich et al., 1998).

Les entrées et sorties de carbone du sol sont schématisées dans la figure 2. Afin de comprendre et prédire la dynamique du C dans le sol, des modèles ont été développés –les plus connus sont RothC et Century (Parton et al., 1988, Coleman and Jenkinson, 1996). Avec ces modèles, la notion de temps de résidence moyen du carbone dans le sol a été définie, c'est la durée moyenne qui va s'écouler entre l'entrée d'une molécule de C dans le sol et sa sortie du système. Pour des raisons de praticité et de simplification, les modèles définissent en général plusieurs réservoirs de matière organique du sol qui présentent des temps de résidence contrastés : un réservoir labile (ou rapide), un réservoir lent et un réservoir passif. Ces modèles sont basés sur des données montrant l'existence de matières organiques d'âges contrastés dans les sols.

### 1.2.La persistance du carbone correspond-elle a de la stabilisation ?

L'utilisation de la technique de datation au carbone 14 (ou radiocarbone), bien qu'ayant des limites analytiques importantes, a permis de mettre en évidence l'existence de matières organiques du sol d'âges très élevés (Torn et al., 1997, Jenkinson et al., 2008). En effet,

certaines matières organiques persistent dans les sols depuis des centaines voire des milliers d'années.

Les trois mécanismes physicochimiques responsables de cette persistance des matières organiques dans les sols sont (i) la récalcitrance chimique, (ii) la protection physique et (iii) la protection physicochimique (von Lutzow et al., 2006, Sollins et al. 1996, 2007, Six et al., 2002).

Le processus de **récalcitrance chimique** est fondé sur l'idée que la vitesse de biodégradation et la persistance d'une matière organique dans le sol dépend de sa nature chimique. Il existe en effet des études expérimentales qui montrent que pour un écosystème donné, des molécules simples comme des monosaccharides (glucose par exemple) sont biodégradées plus vite que des molécules complexes comme des polysaccharides (cellulose par exemple) (Kogel-Knabner et al., 2002, Johnson et al., 2007). Cependant, il a été démontré dans plusieurs études que des composés supposés récalcitrants (la lignine par exemple) étaient biodégradés rapidement (Dignac et al., 2005, Marschner et al., 2008). Il est maintenant de plus en plus accepté que les microorganismes responsables de la biodégradation des MOS sont capables de biodégrader tous les types de matières organiques du sol d'origine naturelle (Dungait et al., 2012). En revanche, certains travaux semblent montrer que le carbone pyrogénique –très condensé (donc riche en fonctions aromatiques)- pourrait appartenir à la catégorie des molécules récalcitrantes (Schmidt et al., 2002, Skjemstad et al., 2002) et persisterait dans les sols. La récalcitrance chimique ne peut donc pas expliquer à elle seule la persistance à long terme de certaines MOS (von Lutzow et al., 2006) sauf peut-être pour le carbone pyrogénique (Schmidt et al., 2011) ce qui explique une forte attention sur ce type de matières organiques de la part de la communauté scientifique.

La **protection physique** des matières organiques du sol intervient quand il y a une barrière physique entre la matière organique – le substrat- et les microorganismes. Dans ce cas, l'organisme décomposeur ne peut pas atteindre la matière organique et elle est ainsi protégée. C'est ce qui peut arriver au sein des agrégats du sol. Ces assemblages sont à la base de la structure physique du sol et de nombreuses propriétés d'un sol en dépendent. Des études ont montré que la biodégradation augmentait lorsque les agrégats de sols étaient détruits (Mueller et al., 2012, Six et al., 2000, Balesdent et al., 2000). Les derniers résultats sur l'étude de l'influence de l'agrégation sur la persistance des matières organiques montrent que la protection est plus importante et plus efficace au sein de petits agrégats (micro agrégats, inférieurs à 0.25 mm de diamètre) (John et al., 2005, Virto et al., 2008, Chenu and Plante, 2006). Néanmoins, dans un sol, les agrégats sont régulièrement détruits et reformés, la matière organique du sol est donc régulièrement mise en contact avec les microorganismes. Ce mécanisme de protection ne peut pas non plus, à lui seul, expliquer la persistance à long terme des matières organiques du sol (Baldock and Skjemstad, 2000, Virto et al., 2010).

La **protection physicochimique** correspond à l'interaction entre débris organiques et surfaces minérales. Elle intervient à des échelles fines, en général dans la classe granulométrique des argiles (particules de diamètre inférieur à 2 $\mu$ m). Plusieurs types d'interaction peuvent exister entre une molécule organique et une surface minérale : liaison covalente, complexation,

interaction d'énergie faible (hydrogène, Van der Waals,...). Ces liaisons sont caractérisées par des énergies qui correspondent à l'énergie qu'un microorganisme devra fournir pour pouvoir biodégrader la matière organique du sol, l'interaction agit donc comme une barrière contre la biodégradation. De nombreuses études ont montré une corrélation entre l'âge des matières organiques du sol et la quantité d'argiles d'un sol (Sharpenseel and Becker-Heidmann, 1992, Torn et al., 1997, Paul et al., 2001). De plus, Feng et al. (2011) ont montré une corrélation entre la quantité d'argiles et de limons fins (2-20  $\mu\text{m}$ ) et la quantité de carbone en effectuant une méta-analyse de la littérature. Si de nombreuses études ont été consacrées au rôle de la granulométrie des argiles (particules < 2  $\mu\text{m}$ ), le rôle respectif des différents phyllosilicates sur la protection de la matière organique du sol n'a pas été résolu (Barré et al. 2014).

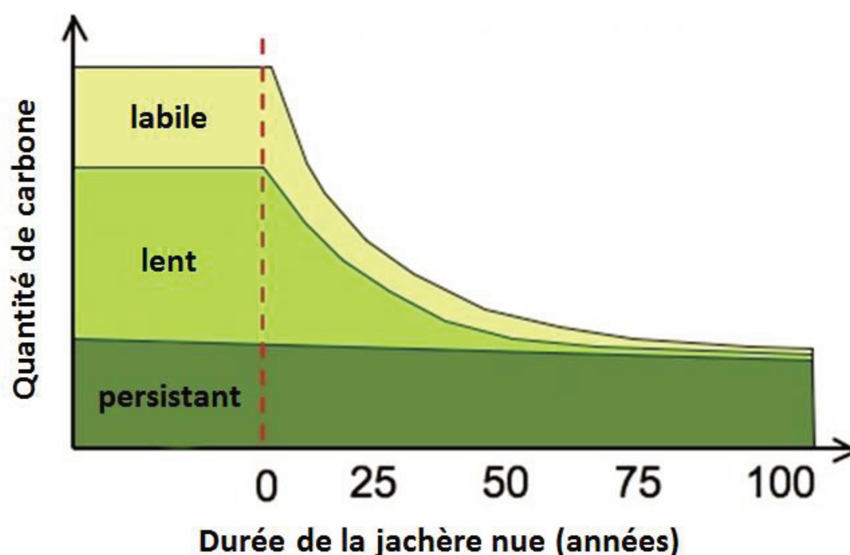
Ces trois mécanismes de protection ont été identifiés, il s'agit désormais de comprendre leurs interactions et leur importance relative, c'est l'objet de ce travail de thèse. D'autres facteurs peuvent influencer la biodégradation des matières organiques du sol mais ne sont pas au cœur de ce projet de recherche : le climat, la ressource en eau, le pH, les communautés microbiennes peuvent tous avoir un rôle sur la biodégradation.

## 2. Etudier les matières organiques du sol avec des outils novateurs et des échantillons historiques

L'étude de la persistance des matières organiques du sol est actuellement limitée par deux difficultés : (i) l'interaction avec le reste du sol (la matrice minérale) rend difficile l'utilisation de nombreuses techniques d'analyse et (ii) il est presque impossible d'isoler expérimentalement le carbone persistant du carbone total. Mon projet de thèse lève ces deux verrous techniques grâce à l'utilisation combinée de techniques d'analyse de pointe et d'échantillons uniques permettant l'accès au carbone persistant : les jachères nues de longue durée.

### 2.1. Les jachères nues de longue durée

Les échantillons de jachères nues de longue durée proviennent de parcelles maintenues vierges de toute végétation dans lesquelles, au fur et à mesure de la biodégradation, la quantité totale de carbone diminue, entraînant un enrichissement relatif en carbone persistant (Figure 3 et 4) (Ruhlmann, 1999).



**Figure 3 : Evolution temporelle de la quantité de carbone d'un sol de jachère nue. (Adapté d'une communication personnelle de Claire Chenu).**

De nombreux travaux se sont déjà intéressés aux échantillons de jachères nues (par exemple Wagai et al., 2013, Hirsch et al., 2009, Lefevre et al., 2014, Barré et al., 2010). Dans cette étude, les échantillons utilisés pour une partie des analyses proviennent des archives historiques de cinq sites d'un réseau européen de jachères nues de longue durée : Askov (Danemark), Ultuna (Suède), Rothamsted (Royaume Uni), Grignon et Versailles (France). Pour chacun de ces sites, des échantillons ont été régulièrement prélevés et stockés. Pour l'autre partie des analyses qui visait à étudier le contrôle minéralogique de la persistance des matières organiques du sol, des échantillons de la plus longue jachère nue (79 ans, site de Versailles) ont été sélectionnés et ont été fractionnés afin d'isoler des fractions



granulométriques présentant des minéralogies contrastées au sein de la fraction argile (particules de diamètre inférieur à 2  $\mu\text{m}$ ).

### **2.2.Présentation du travail de thèse**

Le but de ce travail de thèse est de (i) caractériser chimiquement les matières organiques persistantes dans le sol, grâce aux échantillons de jachères nues, progressivement enrichis en carbone persistant – c'est l'objet de la section 3 de ce résumé- et (ii) identifier l'influence de la minéralogie sur la persistance observée– c'est l'objet de la section 4 de ce résumé.



**Figure 4 : Photographie des 42 parcelles de la jachère nue de Versailles (France). Crédit : O. Crouzet.**

### 3. Signature chimique de l'évolution de la MOS vers des composés persistants

#### 3.1. Les oxydations chimiques ne permettent pas d'isoler le carbone persistant

Nous nous sommes intéressés aux techniques d'oxydations chimiques et à leur efficacité à isoler une proportion du carbone persistant des sols. En effet, ces méthodes ont été utilisées en science du sol pour tenter d'isoler le carbone persistant selon l'hypothèse que le carbone du sol résistant à l'oxydation chimique serait aussi celui résistant à la biodégradation (Helfrich et al. 2007).

Nous avons utilisés des échantillons de la jachère nue la plus longue du réseau, Versailles, où les derniers apports de carbone ont eu lieu il y a 80 ans afin de tester si les méthodes d'oxydation chimique permettent d'isoler le carbone persistant à l'échelle séculaire. Nous avons étudié l'effet de deux réactifs usuels – le peroxyde d'hydrogène,  $H_2O_2$ , et l'hypochlorite de sodium,  $NaOCl^-$  sur la quantité totale de carbone après 0 et 79 ans de jachère nue (Jagadamma et al., 2010, Bruun et al., 2008). Les échantillons sont notés  $LTBF_0$  et  $LTBF_{79}$  avant oxydation, après oxydation ils sont notés  $LTBF_0+H_2O_2$ ,  $LTBF_0+NaOCl$  et  $LTBF_{79}+H_2O_2$ ,  $LTBF_{79}+NaOCl$  selon l'oxydant utilisé. Si ces méthodes isolent efficacement le carbone séculaire, alors la quantité de carbone résistant à l'oxydation dans les deux échantillons (après 0 et 79 ans de jachère nue) devrait être la même que dans l'échantillon de 79 ans de jachère nue non oxydé (figure 5).

Les résultats sont présentés dans le tableau 1 et la figure 5. Les quantités de carbone après oxydation sont très faibles, autour de  $1 \text{ mgC.g}^{-1}$  sol, il y a donc peu de carbone résistant au traitement chimique. La comparaison entre les échantillons traités et l'échantillon après 79 ans de jachère nue non oxydé montre une grande différence ; il y a environ cinq fois plus de carbone dans l'échantillon non oxydé. Les traitements oxydatifs testés ici n'ont donc pas réussi à isoler le carbone persistant dans les sols sur une échelle séculaire.

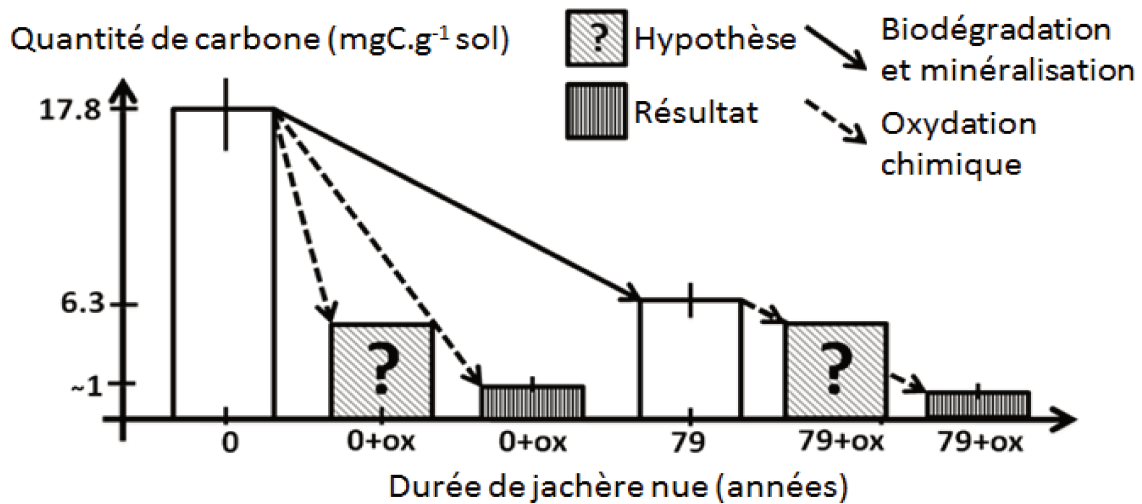


Figure 5 : Effet d'une oxydation chimique sur un sol de jachère nue : hypothèse et résultats (les deux réactifs utilisés conduisent à des résultats similaires). (source Lutfalla et al., 2013)

Tableau 1 : Quantité de carbone avant et après oxydation chimique par  $H_2O_2$  ou  $NaOCl$  sur des échantillons initiaux et finaux de jachère nue.

Echantillon	Quantité de carbone ( $mgC\ g^{-1}\ sol$ )					Carbone restant après traitement	% de la quantité de carbone initiale ( $LTBF_0$ )
	Plot 13	Plot 30	Plot 32	moyenne	écart type	%	%
$LTBF_0$	19.417	15.834	18.194	<b>17.82</b>	1.82	100	100
$LTBF_0+H_2O_2$	2.016	1.426	1.540	<b>1.66</b>	0.31	9.3	9.3
$LTBF_0+NaOCl$	1.261	1.057	1.064	<b>1.13</b>	0.12	6.3	6.3
$LTBF_{79}$	6.788	5.340	6.790	<b>6.31</b>	0.84	100	35.4
$LTBF_{79}+H_2O_2$	1.295	0.884	0.952	<b>1.04</b>	0.22	16.6	5.8
$LTBF_{79}+NaOCl$	0.983	0.774	0.910	<b>0.89</b>	0.11	14.1	5.0

Nous avons aussi testé l'hypothèse que les oxydations chimiques ont en fait isolé le carbone beaucoup plus persistant qu'à l'échelle du siècle. Cette hypothèse pourrait expliquer que la quantité de carbone résistante à l'oxydation soit beaucoup plus faible que la quantité de carbone après 79 ans de jachère nue. L'hypothèse n'est néanmoins pas vérifiée, en effet la quantité résistante sur les échantillons après 0 ans de jachère nue est significativement différente de la quantité résistante sur l'échantillon après 79 ans.

La conclusion de ce travail est que les deux méthodes d'oxydation testées ici ne permettent pas d'isoler de façon représentative ni le carbone persistant à l'échelle du siècle ni à des échelles temporelles plus grandes.

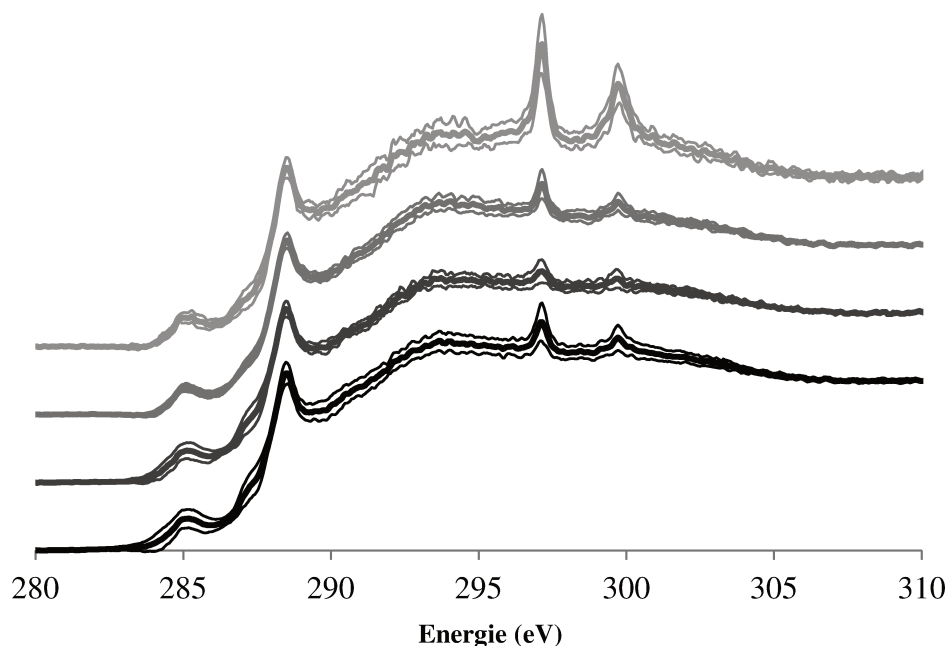


### 3.2. Les signatures chimiques du carbone persistant et du carbone initial sont similaires

La nature chimique du carbone persistant est très mal caractérisée, ceci pour deux raisons : (i) il n'y a pas de technique expérimentale qui permette de l'isoler du reste de la matière organique, (ii) la matière organique persistante est souvent associée à la matrice minérale, ce qui complique sa caractérisation.

Dans cette partie de ma thèse, nous avons surmonté ces difficultés en (i) utilisant des échantillons uniques provenant des jachères nues et en (ii) les caractérisant par la technique de pointe utilisant le rayonnement synchrotron NEXAFS (*Near-edge X-ray absorption fine structure*). Premièrement, les jachères nues de longue durée permettent l'étude du carbone persistant grâce à l'absence d'entrées de carbone et la biodégradation progressive des composés les plus labiles (Barré et al., 2010). La matière organique des jachères nues devient donc progressivement enrichie en composés persistants. Deuxièmement, la technique NEXAFS permet de caractériser la spéciation du carbone sans nécessiter de préparation de l'échantillon et avec peu de bruit provenant de la matrice minérale (Lehmann et al. 2009, Leinweber et al., 2013). Toutes les mesures NEXAFS de cette section ont été réalisées au *Canadian Light Source* (CLS, Saskatoon, Canada) (Régier et al., 2007) et ont consistés en l'obtention du spectre d'émission de fluorescence au seuil K du carbone (280 eV), les échantillons analysés correspondent aux échantillons initiaux, finaux et intermédiaires des cinq jachères nues du réseau européen.

L'évolution de la quantité de carbone avec le temps montre une décroissance du carbone pour tous les sites de jachères nues, ce qui correspond à l'absence d'apport de carbone et à la biodégradation du carbone initialement présent dans les parcelles. Les spectres obtenus par



**Figure 6 : Spectres NEXAFS au seuil du carbone de la matière organique contenue dans différents échantillons de jachère nue. Du plus foncé (en bas) au plus clair (en haut) : Versailles, Askov, Rothamsted, Ultuna. Les spectres sont normalisés et moyennés (toutes les dates et tous les répliqués ensemble) et l'écart type est représenté de part et d'autre de la moyenne.**

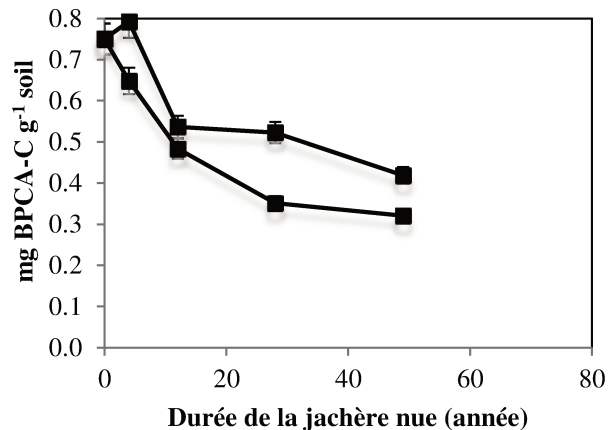
NEXAFS montrent que la signature chimique du carbone est la même pour tous les sites et pour toutes les dates (Figure 6). Une étude statistique plus fine de l'évolution temporelle met en évidence des changements significatifs qui se retrouvent sur plusieurs sites. Ces évolutions montrent que plus le carbone est persistant, plus il est enrichi en composé oxydés –détecté par une augmentation de la proportion d'acides carboxyliques avec le temps- ce qui peut être interprété comme une protection préférentielle de ces composés par adsorption sur les surfaces minérales.

**Deux conclusions découlent de ces résultats. La première est que la signature chimique du carbone persistant est proche de celle du carbone total et la deuxième est que l'évolution chimique modeste de la signature du carbone conduit à un enrichissement relatif en composés carbonés oxydés.**

### 3.3. Le carbone pyrogénique des sols n'est pas particulièrement persistant

Le carbone pyrogénique du sol est une forme de matière organique qui découle de la combustion partielle de biomasse, c'est une forme de charbon. Il peut avoir de nombreuses formes chimiques : il peut être peu condensé ou il peut être très aromatique, selon la température et les conditions de la combustion (Wiedemeier et al., 2015). Il est actuellement considéré comme la matière organique du sol la plus persistante, qui pourrait persister dans les sols plusieurs siècles sans être biodégradée (Forbes et al., 2006). Dans le cadre de la lutte contre le changement climatique, la stratégie qui consisterait à stocker du carbone pyrogénique dans le sol sous forme de biochar (résidu de la pyrolyse de biomasse) a déjà été proposée plusieurs fois et fait l'objet de nombreuses études (Sohi et al., 2012, Lehmann, 2007).

Afin d'étudier la persistance du carbone pyrogénique à l'échelle de la décennie et du siècle et de la comparer à la persistance du reste des composés carbonés, nous avons dosé la quantité de carbone pyrogénique dans les échantillons du réseau européen de cinq sites de jachères nues à différentes dates afin d'étudier l'évolution temporelle de la quantité de carbone pyrogénique. Pour chaque site de jachère nue, nous avons sélectionné les échantillons initiaux, finaux et nous avons sélectionné trois dates intermédiaires afin d'obtenir une évolution temporelle comprenant cinq dates. La quantité et la qualité du carbone pyrogénique dans les échantillons ont été caractérisées par la méthode BPCA – *benzene polycarboxylic acids*- qui se base sur la synthèse puis l'analyse de cinq marqueurs moléculaires du carbone pyrogénique, les acides carboxyliques benzéniques (Glaser et al., 1998, Wiedemeier et al., 2013).



**Figure 7 : Evolution de la quantité de carbone pyrogénique vue par la méthode BPCA dans la jachère nue de Rothamsted (2 parcelles). (Source : Lutfalla et al. en révision pour la revue *Nature Climate Change*).**

Les résultats montrent que la quantité de carbone pyrogénique, analysée par BPCA, décroît avec le temps dans tous les sites (exemple des résultats obtenus pour un site en Figure 7). La comparaison entre les échantillons initiaux et finaux de chaque site montre que la jachère nue entraîne une perte comprise entre 19.8 et 57,4% du carbone pyrogénique initial. Le carbone pyrogénique n'est donc pas inerte à l'échelle de la décennie (la durée écoulée entre échantillon initial et échantillon final varie selon les sites, elle est comprise entre 27 and 79 ans). Dans le même temps, le reste du carbone des échantillons décroît aussi, mais plus vite. La quantité finale de carbone est comprise entre 32 et 68% de la quantité initiale. Le carbone pyrogénique bien que n'étant pas inerte est donc plus persistant que le carbone non pyrogénique.

**Tableau 2 : Temps de résidence moyen (TRM) du carbone pyrogénique (COPy) et du carbone non pyrogénique (COS) calculés à partir du jeu de données et estimations trouvées dans la littérature. A chaque valeur de temps de résidence est associée une incertitude.**

Nous avons estimé le temps de résidence moyen du carbone pyrogénique et du carbone non pyrogénique pour chacun des sites et chacun des répliqués afin de pouvoir comparer

Parcelle	TRM COPy (années)		TRM COS (années)		Ratio TRM COPy/COS
	Valeur	Incertitude	Valeur	Incertitude	
Askov1	60	9	46	8	1.3
Askov2	76	10	77	11	1.0
Rothamsted1	42	7	31	5	1.4
Rothamsted2	76	15	36	6	2.1
Ultuna1	183	11	101	3	1.8
Ultuna2	179	18	96	13	1.9
Grignon1	183	22	114	25	1.6
Grignon2	169	31	110	19	1.5
Versailles1	214*	53	72*	8	3.0*
Versailles2	106*	27	71*	4	1.5*
Versailles3	80	9	49	6	1.6
Versailles4	141*	25	55*	6	2.6*
Moyenne	116 (126**)	15 (20**)	73 (72**)	10 (10**)	1.58 (1.77**)
Singh et al., 2012	291 (353***)		Ø		
Lehmann et al., 2015	300 – 6000		Ø		
Forbes et al., 2006	>1000		Ø		

\* données polluées lors de la 2ème guerre mondiale

\*\* en utilisant les données polluées

\*\*\* pour échantillons de terrain

quantitativement les persistances de ces différentes formes de la matière organique du sol. Les résultats sont présentés dans le tableau 2, en moyenne le temps de résidence moyen du carbone pyrogénique est de 116 ans et celui de la matière organique du sol est de 73 ans, soit 1.6 fois supérieur. Cela signifie que le carbone pyrogénique est environ deux fois plus

persistant que le carbone non-pyrogénique, beaucoup moins que ce qui est actuellement considéré.

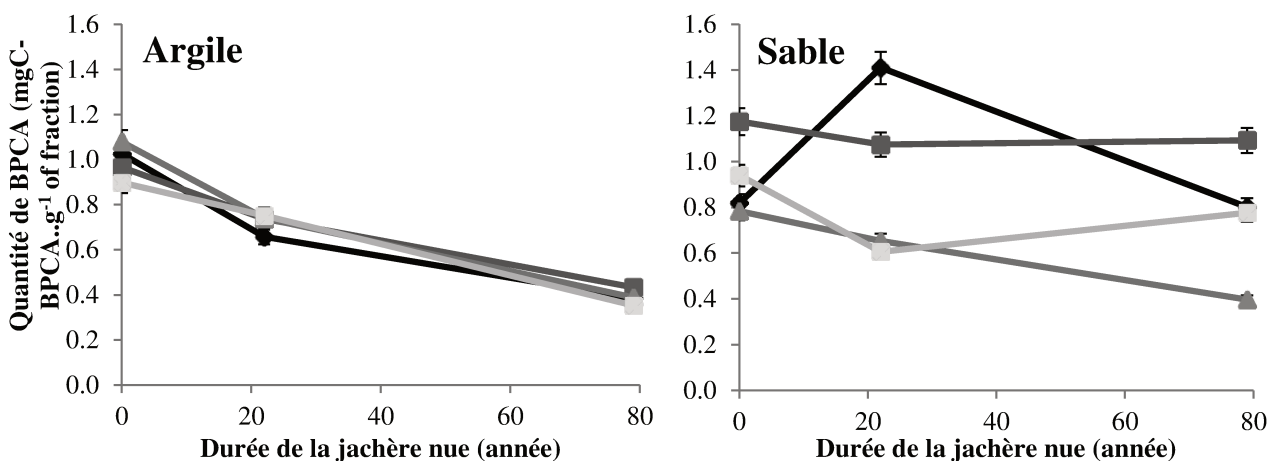
**Nos résultats suggèrent que le potentiel de stockage à long terme de carbone dans le sol grâce au carbone pyrogénique est plus faible que ce qui est actuellement considéré.**

### 3.4. Le carbone pyrogénique associé à différentes fractions granulométriques présente des caractéristiques très contrastées

La carbone pyrogénique est une forme de matière organique présente dans tous les sols, sa persistance n'est pas encore complètement caractérisée. Dans le but d'améliorer la compréhension des mécanismes de protection du carbone pyrogénique nous avons étudié les propriétés et la dynamique du carbone pyrogénique dans les différentes fractions granulométriques d'un sol de jachère nue.

Nous avons étudié la jachère nue la plus longue, celle de Versailles où les apports de carbone ont été stoppés il y a 80 ans. Quatre réplicats ont été étudiés et trois dates ont été sélectionnées : 0, 22 et 79 ans de jachère nue. Pour chaque réplicat et chaque date, nous avons effectué un fractionnement granulométrique afin de séparer la fraction sable et la fraction argile. Nous avons ensuite analysé la quantité et la qualité du carbone pyrogénique (méthode BPCA) pour chaque date et chaque réplicat dans trois fractions : sol total, fraction sable (particules de diamètre supérieur à 50  $\mu\text{m}$ ), fraction argile (particules de diamètre inférieur à 2  $\mu\text{m}$ ).

Les résultats montrent que les fractions sable et argile contiennent du carbone pyrogénique très différent (Figure 8). Le carbone pyrogénique contenu dans les argiles est peu condensé et a une dynamique temporelle quasi identique à celle du carbone non pyrogénique de la fraction. En effet, après 79 ans de jachère nue plus de 60% du carbone pyrogénique et du carbone non pyrogénique ont disparu. Au contraire, le carbone pyrogénique contenu dans la fraction sable est très condensé et sa quantité ne diminue pas significativement avec le temps.



**Figure 8 : Evolution temporelle de la quantité de carbone pyrogénique dans deux fractions granulométriques de la jachère nue de Versailles (mgBPCA-C.g<sup>-1</sup> fraction, quatre parcelles). Les barres d'erreur représentent la variabilité entre deux réplicats techniques (n=2).**

Ces résultats suggèrent que dans un même sol, deux types de carbone pyrogénique peuvent

coexister et présenter des origines et des caractéristiques très contrastées. Le carbone pyrogénique contenu dans les argiles correspond vraisemblablement aux résidus de combustion de biomasse créés lors d'un feu naturel alors que celui contenu dans la fraction sable provient sans doute de charbon carbonifère. Fait intéressant, pour les deux fractions, l'évolution chimique des charbons avec le temps montre un enrichissement en structures plus condensées.

**La principale conclusion de cette étude est que le carbone pyrogénique présent dans la fraction argile présente la même dynamique temporelle de dégradation que le carbone non pyrogénique de cette fraction, pendant 80 ans de biodégradation continue. Il n'y donc pas de persistance particulière du carbone pyrogénique. Ceci est sans doute dû à la nature chimique peu condensée du carbone pyrogénique dans cette fraction.**

## 4. Contrôle minéralogique de la persistance à long terme du carbone dans les sols

Il est en général admis que les minéraux de la fraction granulométrique argile (diamètre inférieur à 2  $\mu\text{m}$ ) peuvent protéger les molécules organiques de la biodégradation par la formation d'associations organominérales. Cependant, le contrôle minéralogique de la persistance des matières organiques du sol est encore mal compris. En effet, deux verrous inhérents au système étudié –le sol- empêchent l'étude systématique des minéraux fins des sols et de leur influence sur la protection des matières organiques. D'une part, on ne peut extraire les matières organiques du sol sans les perturber fortement, ce qui rend les résultats ainsi obtenus souvent obsolètes. Il est donc primordial de développer des techniques de visualisation et d'analyse *in situ*, c'est ce que nous proposons dans cette étude. D'autre part, les sols sont des milieux de constitution très hétérogène qui contiennent une grande diversité d'espèces minéralogiques sous la forme d'une multitude de particules de très petite taille. Il est donc difficile de les séparer et de les observer. Enfin, cette hétérogénéité de constitution à des échelles très faibles conduit à une grande hétérogénéité spatiale qu'il faut prendre en compte quand on étudie le système sol, quelle que soit l'échelle étudiée.

Les phyllosilicates, une espèce minéralogique particulière souvent présente dans les sols, peuvent protéger les matières organiques du sol, comme cela a été démontré dans les travaux de Chevalier et al. (2003) et Pronk et al. (2014). Cependant, l'impact de la minéralogie des phyllosilicates sur la protection des matières organiques du sol n'est pas encore élucidé (Barré et al. 2014), c'est l'objectif de cette étude.

### 4.1. Présentation du protocole et des analyses

Dans cette partie du projet de recherche, nous nous sommes intéressés à la persistance du carbone au sein de la fraction argile de la plus longue jachère nue du réseau européen (Versailles). Nous avons sélectionné 5 dates (0, 10, 22, 52 et 79 de jachère nue), nous avons ensuite effectué un fractionnement granulométrique pour isoler la fraction argile à chacune de ces dates, puis nous avons effectué un fractionnement au sein de la fraction argile pour isoler trois sous-fractions : les argiles fines (0-0,05  $\mu\text{m}$ ), les argiles intermédiaires (0,05-0,2  $\mu\text{m}$ ) et les argiles grossières (0,2-2  $\mu\text{m}$ ).

L'obtention de diffractogrammes des rayons X pour chacune des trois sous-fractions argiles nous a donné la minéralogie des argiles contenues dans les différentes fractions. L'analyse des échantillons avec la technique synchrotron NEXAFS nous a permis d'étudier la spéciation chimique du carbone au sein des différentes fractions. Enfin, la combinaison de la technique synchrotron NEXAFS avec la microscopie STXM (*Scanning transmission X-ray microscopy*) nous a permis d'étudier la localisation du carbone au sein des sous-fractions et la nature des associations organominérales. Toutes les analyses synchrotron ont été réalisées au Canadian Light Source (CLS, Saskatoon, Canada) au seuil du carbone (280 eV) pour la technique NEXAFS seule, et du seuil du carbone au seuil de l'azote (280-450 eV) pour la technique NEXAFS couplée au STXM, une technique particulièrement innovante pour l'étude d'échantillons naturels.

#### 4.2. Minéralogie des argiles

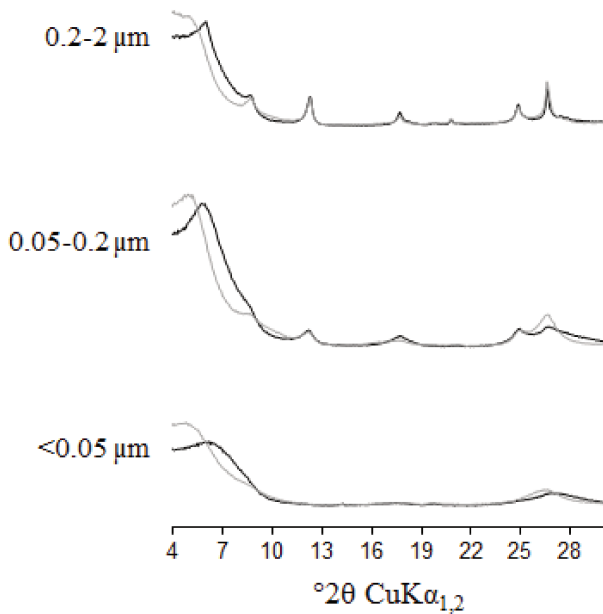


Figure 9 : Diffractogrammes RX des sous-fractions argiles, séchées à l'air (ligne noire) et dans le glycol (ligne grisée). De haut en bas : argiles grossières (0.2-2  $\mu\text{m}$ ), argiles intermédiaires (0.05-0.2  $\mu\text{m}$ ), argiles fines (<0.05

La figure 9 présente le résultat de l'étude de la minéralogie des sous-fractions obtenues par fractionnement granulométrique. On observe deux types de minéralogie. Le premier type correspond à la minéralogie des argiles fines et intermédiaires, ces deux sous-fractions contiennent des smectites et des minéraux interstratifiés illite/smectite. Le deuxième type correspond à la minéralogie observée pour les argiles grossières qui contiennent les mêmes pics de smectite et de minéraux interstratifiés illite/smectite auxquels s'ajoutent des pics caractéristiques de kaolinite et d'illite. Nous avons donc pu étudier l'influence de la minéralogie sur la persistance du carbone car nous avons obtenu des fractions granulométriques de minéralogie contrastée.

#### 4.3. Dynamique temporelle du carbone dans les sous-fractions

L'évolution temporelle de la quantité de carbone au sein des sous-fractions argiles met en évidence une décroissance cohérente avec l'arrêt des apports de carbone dû à la mise en place de la jachère nue (Figure 10).

Les argiles fines contiennent proportionnellement à leur masse une plus grande quantité de C initiale – 147,8  $\text{mgC.g}^{-1}$  fraction- que les argiles grossières et intermédiaires – respectivement 46,6 et 35,9  $\text{mgC.g}^{-1}$  fraction. Les argiles totales (somme des trois sous-fractions) contiennent environ 40% du carbone total de l'échantillon. Ces 40% se répartissent dans les trois sous-fractions : environ 65% de cette proportion se trouvent dans les argiles grossières, 27% dans les argiles intermédiaires et 8% dans les argiles fines. Ce sont également les argiles fines qui perdent le plus de carbone au cours de la jachère nue arrivant à 49,2  $\text{mgC.g}^{-1}$  fraction après 79 ans de jachère nue. Enfin, pour toutes les sous-fractions, il n'y a pas d'évolution significative de la quantité de carbone entre 52 et 79 ans de jachère nue. La décroissance de la quantité de carbone a donc principalement lieu pendant les 50 premières années de la jachère nue. Il n'y a pas de relation claire entre l'évolution de la quantité de carbone et la minéralogie des différentes fractions.



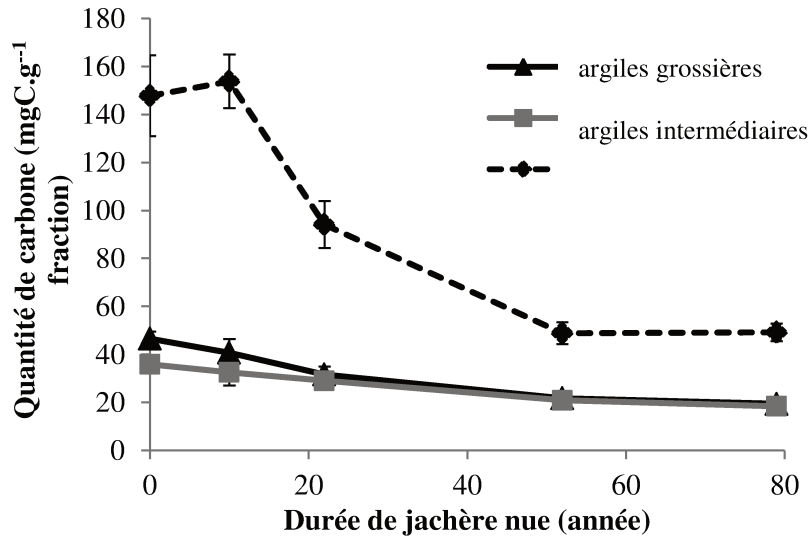


Figure 10 : Evolution temporelle de la quantité de carbone ( $\text{mgC.g}^{-1}$  fraction) dans les trois sous-fractions argiles du sol de Versailles (les barres d'erreur représentent l'écart type entre les quatre réplicats).

#### 4.4. Evolution du C/N

Le rapport C/N (proportion relative des quantités de carbone et d'azote au sein d'un échantillon) des matières organiques contenues dans les différentes sous-fractions nous renseigne sur l'origine probable de ces matières organiques. Le C/N des sous-fractions est très faible, il prend des valeurs comprises entre 4,3 –à la fin de la jachère nue- et 7,9 –au début de la jachère nue. Dans toutes les fractions le rapport C/N diminue avec le temps, cela signifie que lorsque la matière organique du sol devient plus persistante, elle s'enrichit en composés azotés.

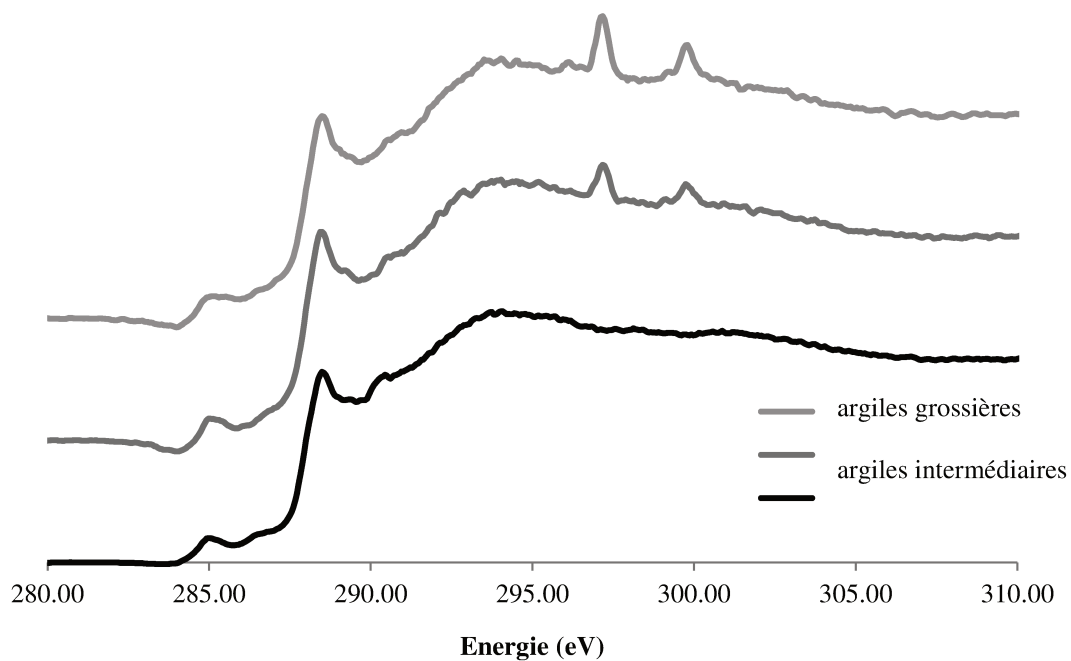
#### 4.5. Signature NEXAFS

La spéciation du carbone vue par la technique NEXAFS est similaire dans les trois sous-fractions et pour toutes les dates (Figure 11). Il y a donc peu de différences entre les sous fractions et peu d'évolution de la chimie de MOS avec le temps. Une analyse statistique poussée montre que les trois sous-fractions sont en fait légèrement différentes et qu'avec le temps, il y a de plus en plus de composés oxydés. Nous n'avons pas détecté d'influence de la minéralogie dans ces résultats.

#### 4.6. Données STXM-NEXAFS

L'étude de la spatialisation des matières organiques du sol et des espèces minérales par NEXAFS couplé au STXM nous permet d'identifier différentes associations organominérales et d'étudier leur évolution avec le temps. Les associations organominérales entre matière organique et smectite et illite/smectite paraissent persistantes alors que les associations matière organique et illite semblent disparaître avec le temps et avec l'apparition progressive d'illite sans matières organiques.





**Figure 11 : Spectres normalisés NEXAFS au seuil du carbone des trois sous-fractions argiles d'un réplikat prélevé au début de la jachère nue.**

**Ces résultats suggèrent un potentiel de protection contrasté selon la minéralogie des phyllosilicates. Les smectites et minéraux interstratifiés illite/smectite protégeraient mieux la matière organique du sol que les illites. Il s'agit maintenant de généraliser ce résultat et de proposer une explication à cette différence. Cela pourrait être dû à une différence de propriétés de surface qui favoriserait l'adsorption de molécules à la surface des smectites.**

## 5. Conclusions et perspectives

Nous avons pu montrer grâce aux résultats expérimentaux obtenus sur les jachères nues de longue durée que :

- (i) Les méthodes d'oxydation chimique ne peuvent pas être utilisées pour isoler et étudier des compartiments cinétiques de la matière organique du sol.
- (ii) La protection physicochimique est prédominante dans l'explication de la persistance à long terme des matières organiques du sol. Il faut donc prendre en compte les propriétés physiques et minéralogiques (granulométrie, minéralogie, texture,...) d'un sol pour estimer le degré de protection des matières organiques. Il découle aussi de ce résultat que la nature chimique des molécules organiques importe peu quand il s'agit de stocker du carbone à l'échelle du siècle.
- (iii) La technique de visualisation NEXAFS/STXM *in situ* à l'échelle submicrométrique permet d'étudier l'influence de la minéralogie sur la persistance des matières organiques du sol.

De plus, ce travail de thèse fournit des preuves expérimentales provenant d'échantillons naturels de l'importance de la chimie de surface et de la minéralogie dans la persistance des matières organiques du sol. Nous avons pu montrer que le carbone persistant est enrichi en composés azotés et oxydés. Ce qui souligne la plus grande importance des processus d'adsorption à la surface des minéraux. Ces résultats sont de ce fait en accord avec le modèle en couche d'oignon qui vise à représenter conceptuellement l'interaction de la matière organique sur les surfaces minérales (Kleber et al. 2007) et avec les concepts actuels de dynamique de la matière organique du sol présentés dans la publication de Kleber et Lehmann (2015, Nature).

En termes de mécanismes, il y aurait donc coexistence de deux mécanismes physicochimiques qui expliquent la persistance à long terme des matières organiques du sol : la protection physique au sein des agrégats et la protection physicochimique par adsorption sur les surfaces minérales. Les agrégats se formant et se détruisant régulièrement, il y aurait peu à peu une prédominance des phénomènes d'adsorption, détectée expérimentalement par un enrichissement relatif en composés oxydés et azotés, et que nous avons observé dans nos échantillons. Ces résultats constituent les premières preuves expérimentales du modèle proposé dans un article de perspectives publié dans la prestigieuse revue scientifique internationale Nature en 2015.

De plus, les observations à l'échelle de la décennie au siècle montrent que la récalcitrance chimique ne joue qu'un rôle mineur sur la persistance à long terme du carbone dans les sols. Plus particulièrement, nous avons pu démontrer la relativement faible persistance du carbone pyrogénique dans le sol, notamment dans les fractions fines. Ces résultats sont pionniers car ils correspondent à la première étude de l'évolution à long terme du carbone pyrogénique et ils montrent que le carbone pyrogénique, contrairement à ce qui est considéré dans la littérature, n'est pas inerte. Ce résultat a des implications concrètes sur un secteur industriel, la production de biochar, et aura des répercussions sur la mise au point de stratégies de lutte contre le changement climatique (volet mitigation).



# PART 1: THE PERSISTENCE OF SOIL ORGANIC CARBON NEEDS TO BE STUDIED

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## 1. Soils and the global carbon cycle

### 1.1. Global carbon cycle and global changes

Climate change is the result of the natural interaction between solar radiation and the molecules present in the atmosphere: the heat trapping property of carbon dioxide and other greenhouse gases (CO<sub>2</sub>, N<sub>2</sub>O, CH<sub>4</sub>, H<sub>2</sub>O) is proven and is part of the natural processes that occur in the atmosphere (see Box below). We talk about climate change today because, even though it is based on natural processes, the high concentration of greenhouse gases in the atmosphere, mainly due to human activities, is starting to have important climatic consequences. Among the different greenhouse gases, CO<sub>2</sub> has the largest effect on global climate. It is the gas whose atmospheric content increased the most from the preindustrial era

**BOX 1: Greenhouse effect of the atmosphere, a natural process that makes life possible on Earth**

Cause of climate change: “when solar radiation (sunlight) passes through the atmosphere it warms the Earth’s Surface. This heat is radiated back under the form of Infra-Red radiation toward space. Most of the out-going heat (around 90%) is absorbed by greenhouse gas molecules and re-emitted in all directions, warming the surface of the Earth and the lower atmosphere” this is the Greenhouse effect of our atmosphere, climate change is due to the increase of this natural effect due to human activities, i.e. release of more of these greenhouse gases through (for CO<sub>2</sub>): deforestation, land use changes, cements and burning fossil fuels.

to today. Atmospheric CO<sub>2</sub> concentrations have risen from approximately 280 parts per million (ppm) prior to 1850, to 381.2 ppm in 2006<sup>1</sup>. Levels keep increasing and for the first 7 months of 2015, the average CO<sub>2</sub> concentration is 401.7 ppm (Scripps institute, observatory<sup>2</sup>). The symbolic threshold of 400 ppm is often mentioned in daily news around the world (Le Monde<sup>3</sup>, The Guardian<sup>4</sup>, The New York Times<sup>5</sup>)

The increased atmospheric contents of greenhouse gases are mainly due to human activities, through direct contribution like emissions of greenhouse gases by burning of fossil fuels (approximately two-thirds of the total increase in atmospheric CO<sub>2</sub>) or through indirect contributions like global land use changes that induce the release of greenhouse gases in the atmosphere (the clearing of forests and the cultivation of land for food production for

<sup>1</sup> Link: [https://www.wmo.int/pages/mediacentre/press\\_releases/documents/GHG\\_Bulletin\\_No.8\\_en.pdf](https://www.wmo.int/pages/mediacentre/press_releases/documents/GHG_Bulletin_No.8_en.pdf)

<sup>2</sup> Link: <http://co2now.org/images/stories/data/co2-atmospheric-mlo-monthly-scripps.pdf>

<sup>3</sup> Link: [http://www.lemonde.fr/planete/article/2013/05/02/vives-inquietudes-autour-d-une-unite-de-mesure-du-rechauffement-climatique\\_3170141\\_3244.html?xtmc=400\\_ppm\\_co2&xtcr=8](http://www.lemonde.fr/planete/article/2013/05/02/vives-inquietudes-autour-d-une-unite-de-mesure-du-rechauffement-climatique_3170141_3244.html?xtmc=400_ppm_co2&xtcr=8)

<sup>4</sup> Link: <http://www.theguardian.com/environment/2015/may/06/global-carbon-dioxide-levels-break-400ppm-milestone>

<sup>5</sup> Link: [http://www.nytimes.com/aponline/2015/05/06/science/ap-us-sci-carbon-dioxide.html?\\_r=0](http://www.nytimes.com/aponline/2015/05/06/science/ap-us-sci-carbon-dioxide.html?_r=0)

instance) (Lal, 2004). Evidence for rapid climate change already exists: sea level rise (around 17 centimetres in the last century), global temperature rise, warming oceans, shrinking ice sheets, declining Arctic sea ice, glacial retreat, extreme events, ocean acidification, decreased snow cover, etc. (see detailed review by NASA<sup>6</sup>).

Through its effects on the environment we live in, climate change can and will have serious impacts on our society. Food security is likely to be threatened, countries such as Vanuatu Islands will gradually lose ground because of rising sea levels, locally rising temperatures will threaten ecosystems (coral reefs) and species (phytoplankton, the base of our food chain). That is why scientists from all around the world have cooperated to gather the available scientific data and alert politicians (IPCC, 2014), international organisations are calling for action<sup>7</sup> and local initiatives are sprouting everywhere<sup>8</sup>.

Scientists can take part on several levels: they can help to better understand the causes and effects of climate change, design models to try and predict the amplitude of the global changes and the impact of different politics, offer possible mitigation and adaptation solutions and help raise the awareness of the general public.

Understanding the science behind climate change is a prerequisite for all of the above actions. It all starts with the global carbon cycle. In terms of chemical elements, the main greenhouse gases being carbon dioxide -CO<sub>2</sub>- and methane -CH<sub>4</sub>- (with N<sub>2</sub>O and H<sub>2</sub>O), carbon is one of the most important elements responsible for climate change. Carbon is the building block of all living organisms; it is ubiquitous on our planet and is perpetually cycling, taking up different chemical forms along the way. The global carbon cycle encompasses all the pools and fluxes of organic and mineral carbon. The most important aspects of the global carbon cycle for climate change related issues are the fluxes, i.e. the quantities of C exchanged between the different pools. Global carbon reservoirs have been defined by scientists in order to simplify the study of the cycle of this element on Earth. Carbon is exchanged among five main carbon pools on Earth: the biosphere, the pedosphere, the geosphere, the hydrosphere and the atmosphere. On short timescales (years to century) the main natural exchanges occur first between the biosphere and the atmosphere, through photosynthesis and respiration, then between atmosphere and hydrosphere, through the equilibrium of the dissolution of

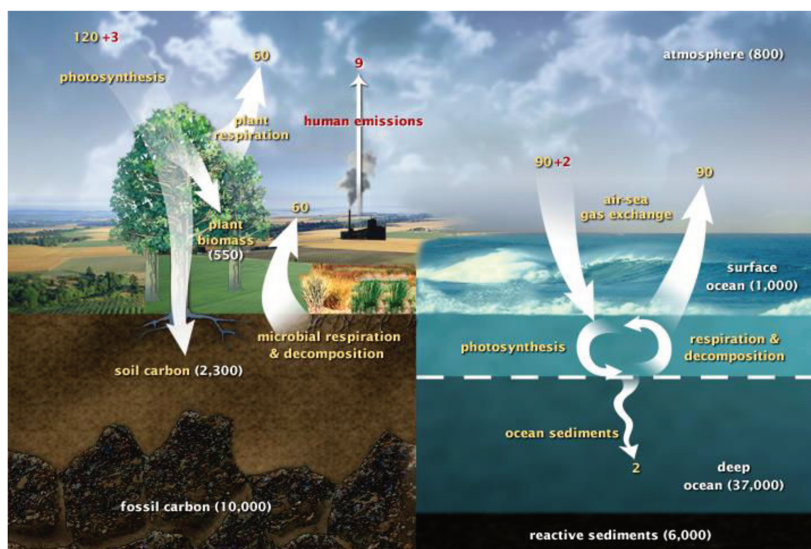
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<sup>6</sup> Link: <http://climate.nasa.gov/evidence/>

<sup>7</sup> Link: [http://www.lemonde.fr/paris-climat-2015/article/2015/08/26/sur-le-climat-nous-n-avons-pas-de-temps-a-perdre-estime-ban-ki-moon\\_4736798\\_4527432.html](http://www.lemonde.fr/paris-climat-2015/article/2015/08/26/sur-le-climat-nous-n-avons-pas-de-temps-a-perdre-estime-ban-ki-moon_4736798_4527432.html)

<sup>8</sup> See for instance Alternatiba. Link : <https://alternatiba.eu/en/>

carbonates in the oceans and finally inside the hydrosphere pool, between deep and surface layers of the oceans. The development of industrial activities and the massive land use changes, which men are responsible for, causes carbon emissions from the biosphere, the pedosphere and the geosphere to the atmospheric pool.



**Figure 1: Representation of the different carbon reservoirs, their carbon content and the fluxes that link them which define the global carbon cycle. (Source: NASA).**

Looking at the amounts of carbon present in the different pools (Figure 1), we can see that there is three times more carbon in the soil than in the atmosphere, making it the largest terrestrial carbon reservoir. Total C in terrestrial ecosystems is approximately 3170 gigatons (Gt; 1 Gt = 1 petagram = 1 billion metric tons). Nearly 80% (2500 Gt) of this amount is found in soil (Lal, 2008). Estimations on the quantity of carbon contained in soils are not very precise (Lorenz and Lal, 2005). Nevertheless, available data show that soil carbon can be either organic (1550 Gt) or inorganic carbon (950 Gt). Inorganic carbon consists of elemental carbon and carbonate materials such as calcite or dolomite (Lal, 2004). Most of the soil organic carbon is found in the top meter of the soil: 1500 Gt (Post et al., 1982, Jobbágy and Jackson, 2000). The amount of carbon found in terrestrial biomass (living plants and animals) is comparatively small relative to that found in soil (560 Gt). The soil carbon pool is approximately 3 times larger than the atmospheric pool of 800 Gt (Oelkers and Cole, 2008). Only the ocean has a larger carbon pool, at about 38,000 Gt of C, mostly in inorganic forms (Houghton, 2007). Understanding the fluxes of carbon in and out of the soil is therefore crucial, to better understand the effect of global changes on soils in terms of carbon budget. When considering the total organic carbon of a soil, inputs come mainly from the deposition



of plant residues and losses are dominated by the mineralization of organic matter (Baldock and Skjemstad, 2000).

## 1.2. Soil is a complex system which contains a lot of carbon

“the thin layer of minerals, living microorganisms, dead plants and animals blanketing the planet is the mother of all terrestrial life and every nation’s most strategic resource” (Montgomery, 2010)

### **Definition**

“A definition is neither true nor false; it is useful or useless” (H. Saner, student of Karl Jaspers, german philosopher)

By definition soil is the interface between the geological world (the earth’s crust) and the biological world (plants and animals). It is a critical zone, fragile and essential. Soil formation is a slow process for the human scale (around 1 cm per century) (Chesworth, 1992, Fyfe et al., 1983). There is no fixed definition of a soil; each definition will depend on the field of study. For instance, a geologist could consider a soil as the product of weathering, reworking and arrangement of the upper layers of the earth’s crust under the action of life, the atmosphere and the exchanges of energy that are manifested by it (Aubert and Boulaine, 1980).

According to Brady and Weil (2008), a soil is (1) a dynamic natural body composed of minerals and organic solids, gases, liquids and living organisms which can serve as a medium for plant growth. (2) the collection of natural bodies occupying parts of the Earth’s surface that is capable of supporting plant growth and has properties resulting from the integrated effects of climate and living organisms acting upon parent material, as conditioned by topography, over periods of time.

The French association for the study of soil (AFES) has published their own definition, which has been designed to be understandable by a wide public. Here is my attempt at translating it.

The soil is a volume which extends from the surface of the Earth to the depth where a little-weathered solid rock, hard or loose and little affected by pedogenesis, appears. The depth of a soil can therefore vary from a few centimeters to tens of meters (or more). It is, locally, a part of the pedogenetic cover which extends on all the surface of the Earth. It generally consists of several horizons corresponding to the organisation of organic and mineral constituents. This

organisation is the result of pedogenesis and weathering of the parent material. An intense biological activity is taking place in the soil (roots, fauna, microorganisms)<sup>9</sup>.

### **Formation**

Soil formation is the result of the interaction between minerals and living organisms under given environmental conditions. These constant interactions and the diversity of possible environmental conditions explain the huge variety of soils that can be found on Earth. Soil formation is a dynamic process which never stops. The number of factors that can influence soil formation is nevertheless relatively small:

- The bed rock or parent material, three characteristics of the parent material will influence soil formation: its chemical and mineralogical compositions will influence the type of minerals which will be present in the soil and its structure will partly determine the speed at which the soil is formed.
- The organisms which live in, on or outside the soil and the organic matter they produce. The fauna (macro or micro) and the flora will trigger the formation of the soil, they will have an impact on the structure of the soil (porosity, formation of aggregates, bioturbation) and on the weathering of minerals.
- Climate will have an impact through temperatures (freezing/thawing episodes) and rain events (water content, local anaerobic conditions, vertical transports of elements). Climate can also affect rock weathering and biological activity.
- Topography, soil formation also depends on the slope and the local topography.
- Humankind, humans have had an influence on soils and soil formation for a very long time. They can prevent soil formation, stop it or shape it by choosing specific uses for the land that surrounds them. Agriculture, forestry, and urban development, are all practices that have an impact on the formation of soil.
- Time, soil formation is a slow and never ending process, and it is because of the temporality of all of the above mentioned parameters, long or short, from nanoseconds to millennia, that soil formation occurs.

### **Composition**

A soil is a mix of mineral, living, and organic material. In terms of volume, soil is around 20-30% air (pore space), 20-30 % water, 45% mineral and 5% living and organic materials.

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<sup>9</sup> Link to the definition (in French) : [http://afes.fr/afes/docs/AFES\\_d%C3%A9finition\\_SOL.pdf](http://afes.fr/afes/docs/AFES_d%C3%A9finition_SOL.pdf)

The **mineral part** of a soil is composed of a wide variety of minerals which depend on the bedrock the soil has been created on, *i.e.* the parent material. Several nomenclatures exist which give a classification of the minerals in soils: by composition or by size. **Living material** encompasses all kinds of organisms, animal and vegetal. Animals include moles, earthworms, ants, termites which belong to the meso and macrofauna. On a smaller scale,

### **BOX 2: Soil diversity and classification**

Several parameters influence the composition and characteristics of a soil: the bedrock, the climate, the processes, the plants, human activities. Different processes take place during the formation of a soil. First, there is the physical and chemical weathering of the bedrock, which explains the texture of the soil. Then, there is the incorporation of organic matter thanks to the activity of living organisms, this activity also includes bioweathering of the mineral matrix and leads to a first spatial organization of the soil in layers (organomineral layers under a surface organic layer). Finally, physical transport of mineral phases and organic compounds leads to the organization of a soil in differentiated horizontal layers: the soil horizons. Each horizon is a homogeneous morphological unit.

The nature and organization of the different soil horizons constitute the profile of a soil. Horizons are generally horizontal layers which are superposed one on top of the other, their thickness can go from a few centimeters to meters. They are the reference unit for the description of soils and they are used to develop soil classifications (reference and taxonomy). Horizons depend on soil pedogenesis. They are usually organized in 4 families:

- Holorganic horizons (called O) mainly composed of plant debris more or less decomposed and still recognizable (usually forest soils);
- Organomineral horizons (A, L) which can be differentiated from the first one (O) by more decomposed organic material and a majority of mineral constituents;
- Mineral horizons (S, B), in these horizons geochemical alteration, leaching and accumulation processes occur;
- Deep horizons (C, M) correspond to the underlying bedrock which can be more or less altered.

microorganisms populate every part of the soil, several taxonomic classes can be found: archea, bacteria, actinomycete, fungi. In terms of plants all kinds of organisms can also be found from old gigantic redwoods trees in Oregon to miniature birch in Iceland and all kinds of grass, flowers, bush... All of these living organisms have coexisted and coevolved in soils for millions of years (Lavelle et al., 2006).

The **organic material** is composed of the tissues and molecules which formerly belonged to living organisms, it contains most of the carbon of the soil (cf next 2 sections of this part).

## Complexity

Soil is one of the most complex systems in science (as highlighted by the 2004 special issue of *Science*, Figure 2), it is composed of thousands of different molecules, mixed together with billions of living organisms (most of which have never been cultured) and mineral particles of all sizes. It is so complex that we cannot use the standard tools used in other fields like chemistry, physics, and biology. In order to try to understand the whole system, soil scientists have either attempted to have a global view of the system (holistic approach) or they have tried to develop techniques and experimental setups aimed at simplifying, extracting, isolating, and testing only one or few

parameters (the reductionist approach). This is the reason for chemical extractions, physical fractionation, spectroscopies and long term field experiments that will be presented at a later stage of this manuscript. The complexity of soil functioning has led authors to considering soil's organization as stochastic, this has been refuted by Webster (2000), while authors like Lavelle et al. (2006) have developed the representation of a self-organised system (Perry, 1995) where ecosystem engineers -plant roots, macrofauna and microorganisms- work on maintaining the functions of the soil. According to some authors, soil would even be a living organism (Phillips, 2009).

## Functions

Soils **functions** are multiple: the main function (for mankind) is agriculture, indeed soil is the environment which produces most of the terrestrial biomass. Modern agriculture tended to reduce soil function to being solely the medium on which plants grew, but it is clear that soil should rather be considered as a multifunctional crossroads as developed by Gobat et al. (2004) or as a biogeochemical hub. Soils are indeed involved in intricate issues of tremendous importance such as food security, water cycle, social value, and carbon cycling.



**Figure 2: Special issue of *Science*: “Soils, The Final Frontier” (2004). Source: *Science*.**

Soil is the basis for most human activities, it is the source of agricultural and forestry productions, it is a source of raw material, it can be a storing place for waste (and possibly for carbon), it is an important landscape component and it holds the memory of our civilizations and our cultures.

### Threats

Nowadays there are several **threats** to the soil, one explanation for the importance of these threats is that they would involve a rapid destruction of the soil (days/months/year) compared to the rate at which a soil grows (order of magnitude of 1cm/century). A non-exhaustive list of these threats includes land use change, urban growth, nutrient depletion, pollution, erosion.



#### **BOX 3: The international year of Soils**

The Food and Agriculture Organisation of the United Nations has declared 2015 to be the International Year of Soil. Events and meetings have been going on all year to raise awareness about the issues concerning soils for food security, agriculture, mitigation of climate change, poverty alleviation, and sustainable development. Final events will take place in December 2015. More info on: <http://www.fao.org/soils-2015/en/>

### Potentials

Today, many people look at the soil with new ideas and new perspectives. Solving the problem of starvation (by growing crops more efficiently), providing new medicine (by looking at the microorganisms that are present in the soil), preventing diseases (specific sorption properties of viruses and toxins on some minerals in the soil), depollution, bioremediation, and helping mitigate climate change (through carbon uptake).

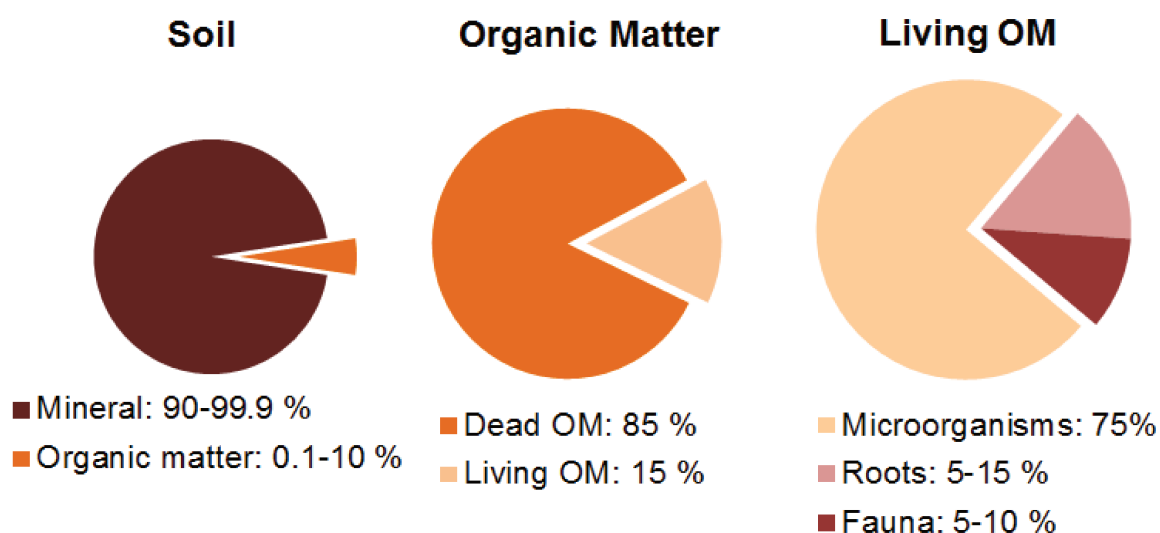
#### **1.3. Carbon inputs to the soil**

As previously mentioned, soil carbon can be either organic or inorganic. Inorganic carbon consists of elemental carbon and carbonated minerals such as calcium carbonate ( $\text{CaCO}_3$ ). Here we will focus on the organic forms of carbon in soils. Soil organic carbon is contained in the soil organic matter which is composed of both the living material present in the soil, the organic matter they produce and the dead organisms, formerly living in, on, or outside the soil (Van-Camp et al., 2004). Soil organic matter is a continuum of molecules (Bosatta and Ågren,

1999) which represents only a small fraction of a soil (in weight) and which is composed of both living and dead tissues (Figure 3).

When living biomass is excluded, soil organic matter is a complex mixture of compounds present in the soil and which comes from formerly living organisms. In terms of elemental composition, soil organic matter is roughly 50% (in weight) carbon, 40% oxygen, 5% nitrogen, 4% hydrogen and traces of other atoms such as Phosphorus, Sulfur, Potassium... The origin of SOM is diverse, it can come from plants, animals, microorganisms, fire, and air.

## Organic Matter in soils



**Figure 3: Distribution of different kinds of organic matter in soils (% dry weight). Adapted from Van Camp et al. (2004)**

### Plant-derived OM

The main source of carbon (or organic matter) in soils is plants (Figure 4). Indeed, as plants grow on the soil their roots grow in the soil; this generates two sources of C. First, roots exudate organic compounds in the soil and second, when a plant dies, its roots remain in the soil and become part of the SOM. The aerial biomass is also a source of SOM as plant debris (like fallen leaves, crop residues, or when the plant dies) that fall on the soil are incorporated into the soil.

The most visible incorporation of OM into soils happens at the surface, as in forests for instance, where the litter is progressively degraded by the surface fauna and incorporated into the first horizons of the soil. Nevertheless, roots contribute to a great extent to the OM found in soils (Rasse et al., 2005, Clemmensen et al., 2013).

### **Fauna and microorganisms**

Microorganisms are responsible for part of the carbon of the soil. The generic term “microorganisms” encompasses a wide variety of organisms: fungi, algae, bacteria, archaea, etc. Microorganisms are ubiquitous in soils, it is usually estimated that 1 gram of soil contains around  $10^9$  bacteria and the equivalent of 10-1000 m of fungal hyphae. Microorganisms contribute to soil organic carbon by their biomass but also by the extracellular polymeric substances they exudate.

Other sources of carbon come from the soil fauna which can include vertebrates, nematodes and arthropods of varying sizes. Examples of soil vertebrates include mice or moles; examples of the arthropods are ants, termites, or beetles. Nematodes include the common “earthworms”.

### **Other sources of OM include fires and wind deposition**

Another process that creates SOM is fire, natural or man-made. The thus created organic carbon is referred to as pyrogenic organic carbon (PyOC). Fire-derived organic carbon encompasses a wide variety of compounds, which go from highly condensed char materials to amorphous and almost non aromatic compounds (Hammes et al., 2007, Wiedemeier et al., 2015, Kleber, 2010). PyOC stocks in soils can go from a few percents to up to 50% of SOC (Schmidt et al., 1999, Czimczik et al., 2005).

Finally, atmospheric deposition can also be significant in places where there are strong winds or in places which are close to sources of atmospheric carbon (incinerators, industries;...) (Schmidt et al., 1996). SOM deposited by wind and rainwater can come from any of the sources previously presented (plant, animal or fire).



**BOX 4: a few words about nitrogen**

Other elements are necessary for the development of living organisms on soils: nitrogen for instance. The main source of nitrogen for the biosphere is the atmosphere (under the gaseous form  $N_2$ ). But, as such, it is not readily usable by most organisms which need nitrogen combined in organic or mineral compounds. Some microorganisms are able to directly uptake this form of nitrogen, and some create symbiosis with plants. But for most living organisms, nitrogen uptake can only be done when nitrogen is under the form of ammonia or nitrates ( $NH_3$ ,  $NO_3$ ). Nitrogen stocks mostly contain nitrogen under an organic form, this nitrogen needs to be mineralized (to produce  $NH_3$  and  $NO_3$ ) in order for plants to be able to use it. Microorganisms are responsible for this nitrogen mineralization. There are usually two steps involved: nitrification and ammonification. Once the nitrogen is mineralized, there is competition between living organisms to consume it (plants and microorganisms). Nitrified nitrogen can also be further mineralized in  $N_2$  or in  $NO_2$  under gaseous forms. Microorganisms are thus responsible for the fluxes of nitrogen in the soil: gains come from the fixation of gaseous  $N_2$ , losses come from the denitrification processes. Other abiotic processes complement these biotic processes: water or irrigation, fertilizers, adsorption of gaseous ammonia, losses by volatilization of ammonia, leaching, erosion...

**1.4. Fate of carbon molecules in soils**

Carbon molecules in soils will ultimately be biodegraded, mineralized, photo-oxidized, eroded or leached (Figure 4).

**Biodegradation and mineralization processes.**

Biodegradation can be considered a **destructive process** which disassembles dead materials into their basic components. It is the result of a biological process which involves the progressive fragmentation and burial of fresh organic material in the soil (by the soil fauna), its colonization and alteration by microorganisms and its utilization as a substrate for microorganisms. When this utilization leads to the emission of  $CO_2$  gases, the process is referred to as mineralization and the emission of gas is called respiration.

Biodegradation can also be considered a **constructive process** which leads to the formation of new living organisms.

The biological decomposition of SOM depends on many factors: temperature, water content, nutrient availability, quantity and nature of the SOM... This will lead to some SOM being quickly biodegraded and some that remain in soils for longer periods of time. It is now fairly accepted that the soil biotic community is able to decompose any organic matter of natural origin (von Lützow et al., 2006, Dungait et al., 2012). Every SOM could be decomposed,



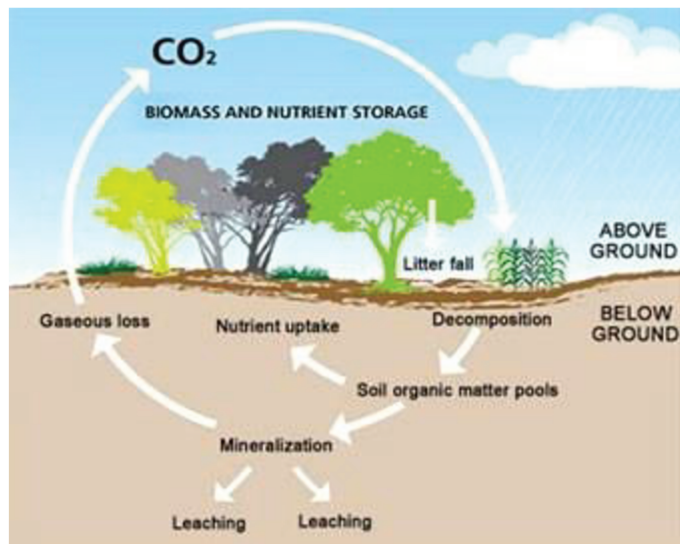
however all SOM does not end up being biodegraded and other processes can lead to the transformation and export of carbon molecules.

### Photo-oxidation

Photo-oxidation is a natural process that happens when molecules are subject to radiant light (sunlight for instance) in the presence of oxygen. It is the oxidation caused by the action of light. Evidence for photo-oxidation of SOM is scarce but it is a process which has been studied for dissolved organic matter (DOM) (Opsahl and Benner, 1998, Judd et al., 2007) or for organic matter present in snow and frozen environments (Grannas et al., 2004).

### Leaching

Leaching refers to the loss of soluble material. It can concern mineral constituents, nutrients and organic molecules. It is caused by the flows of water in the soil profile. As the soil is composed of different horizons, compounds that leave an upper horizon through leaching processes can either end up in one of the lower horizons or leach through the whole soil profile (Montagne et al., 2009). As previously mentioned it will have an influence on the water soluble part of SOM, also called dissolved organic matter (DOM).



**Figure 4: Cycle of the carbon and associated SOM in and out of the soil.** Source: <http://conta11.my3gb.com/carbon/images/soil-potential-for-carbon-sequestration-2.jpg>

### Erosion

Physical transport of material can also occur for OM that is not necessarily soluble. Erosion is caused by rainfall and wind and can explain the export of OM from soils (Gregorich et al., 1998, Pimentel et al., 1995). For instance, in regions with strong winds and where soils are not protected, they might lose their upper horizons which are rich in OM. Erosion also concerns soils located on slopes, this can lead to high variations of carbon content along a topography (Lavelle et al., 2006).

### **BOX 5: the functions of SOM**

Soil organic matter is essential for terrestrial ecosystems, they are a trading place (or stock exchange market) for the cycle of many chemical elements. SOM contributes to the physical qualities of a soil through its influence on the water retention capacity, the structure and the stability of a soil. They therefore act as a barrier against erosion. SOM will also have an impact on the color of a soil and therefore on albedo and warming properties. SOM contributes to the chemical quality of soils (fertility) through its role as an ion reservoir for plants and also thanks to their Cation Exchange Capacity (CEC). Water quality also depends on SOM as they can act as a filter which will retain metal ions and pollutants. They are the main feedstock for plants and a variety of organisms and as such they determine the biological fertility of a soil and contribute to soil biodiversity.

Sources: Tisdall and Oades, 1982, Stephenson, 1994.

### **Turnover times and models**

These degradation and export processes will not necessarily happen straight after a carbon molecule enters the soil. Carbon molecules will consequently display contrasted turnover times. And that is why the conceptual notion of mean residence time of molecules in soils has been defined: it is the average amount of time a molecule will stay in the soil before being altered or exported (by any of the mechanisms introduced earlier).

Models have been established to simulate carbon dynamics in soils with the goal of predicting future productivity of ecosystems or soil carbon changing conditions. Modelling each molecule individually is too complex and time consuming. Moreover there is no experimental way to know the mean residence time of an individual molecule. Models therefore usually simplify the problem by grouping SOM in functionally homogeneous compartments of similar mean residence times.

Most models for carbon dynamics are linear, the most famous being Century and RothC (Parton et al., 1988, Coleman and Jenkinson, 1996) where soil carbon organic matter decomposes at different rates following first-order kinetics according to the conceptual pool it has been divided into. SOC is usually divided into active, slow, and passive pools. With the idea that the active pool would somehow be more biodegradable, whereas the passive pool would be more recalcitrant, possibly because of the chemical complexity of its molecules or because its molecules are physically protected from biodegradation [see next section]. However, there is still no successful way to link these conceptual SOM pools to actual SOM fractions which can be experimentally isolated from soils. But there is **evidence of contrasted turnover times for carbon in soils.**

## 2. Carbon persistence in soils: is it stabilisation?

### 2.1. Evidence of carbon persistence in soils: radiocarbon ages

Radiocarbon dating has been used on soils to estimate the age of soil organic carbon. Indeed as soil organic carbon is derived from living organisms (plants, animals, microorganisms, fungi...) we can measure the ratio of the isotopes of carbon (in this case:  $^{14}\text{C}/^{12}\text{C}$ ) and have an estimate of how long the organic matter has been in the soil (see BOX below).

Radiocarbon measurements of SOC have been performed in many studies, either to characterize individual samples or along soil profiles and pedosequences (Torn et al., 1997, Baisden et al., 2002). Soil profile measurements, as reported in Jenkinson and Coleman (2008), Trumbore (2009), Rumpel et al. (2002), indicate that radiocarbon ages increase with soil depth (see also Trumbore, 2000, Torn et al., 2002). Jenkinson et al. (2008) showed that along a soil profile from an arable field, ages in the top layer were 1192 years (0-23 cm) and increased along the soil profile to reach 12 090 years in the deepest collected layer at 206-229 cm.

The ages thus measured are really contrasted, they range from a few centuries to several thousand years. Ages of 253 years in unfertilized grassland or 340 years in the top layer of an arable field were detected by Jenkinson et al. (2008). Torn et al. (1997), using radiocarbon measurements on a soil chronosequence, modelled turnover times of SOC slower than 20 000 years.

There are several major limitations to the use of this technique for the study of soil organic carbon dynamics. Firstly, the radiocarbon measurement of a soil sample gives an average value for the age of SOC although SOC is contained in a complex mixture of different SOM with contrasted turnover times (Bruun et al., 2008). Secondly, evidence for old radiocarbon ages does not necessarily mean that the compound is stabilized or belongs to a pool of carbon with really slow turnovers (which can be referred to as passive, or inert). Indeed, a single measurement of SOC cannot give information on the dynamics of the compounds. Imagine a situation where sometime in the past an important input of freshly dead material entered the soil and started being decomposed for the most part with a relatively “quick” rate, because there was so much material initially, we are still able to find some and to trace it back to very old radiocarbon ages. The temptation to say that this is evidence for very persistent material is high. Another limitation is that the material could have been dead before entering the soil and

the radiocarbon ages would thus not represent the actual duration of its persistence in soils. Moreover, microorganisms which feed on very old material will display very old radiocarbon ages too even though there is an internal turnover of the SOC (Kramer and Gleixner, 2008). Finally, since the 1950's, anthropic activities have altered the  $^{14}\text{C}$  content of the atmosphere, increasing it with nuclear bomb testing and decreasing it with the burning of  $^{14}\text{C}$  deprived fossil fuels. It is therefore difficult to define a standard for solid comparison.

Despite these limitations, the experimental evidence for very old radiocarbon ages of SOC is undisputable. This proves that some carbon has been standing in the soil for hundreds to thousands of years. This carbon can be referred to as persistent. Understanding this long-term persistence of carbon is crucial for several reasons: it will help scientists build more accurate models to predict the reaction of soil organic carbon to global environmental changes and it could lead the way to the design of potential climate change adaptation and mitigation strategies.

#### **BOX 6: Radiocarbon dating technique**

Radiocarbon dating is a technique that was invented in the late 1940's and is based on the properties of a radioactive isotope of carbon, namely radiocarbon (or  $^{14}\text{C}$ ). It was first used in the field of archeology in order to determine the age of an object containing organic material. It uses the fact that every living organism exchanges carbon with its surrounding environment (i.e. the atmosphere), therefore, as long as it is living it will contain a steady amount of  $^{14}\text{C}$  which depends on the level of  $^{14}\text{C}$  present in the atmosphere. When the organism dies, it no longer exchanges carbon with its environment and the  $^{14}\text{C}$  cannot be renewed. From the moment of the death of the organism, this initial stock of  $^{14}\text{C}$  can only decrease through radioactive decay. Knowing the levels of  $^{14}\text{C}$  in the environment at the moment of death, we can measure the remaining  $^{14}\text{C}$  and calculate how long the organism has been dead (indeed, this calculation is easy as radioactive decay follows an exponential decay).

$$N = N_0 e^{-\lambda t}$$

Where, N: number of radioactive atoms at time t;  $N_0$  initial number of radiocarbon atoms, t: time of measurement,  $\lambda$ = reciprocal of the half-life (i.e. the average time a given atom will survive before undergoing radioactive decay, it depends on the isotope studied).

## **2.2. Three physicochemical mechanisms are invoked to explain the persistence of carbon in soils**

Three main physicochemical mechanisms are invoked to explain this measured persistence of SOM and are presented in this section of the manuscript. The first one is usually referred to as chemical recalcitrance, it is based on the concept that biodegradation of a molecule will depend its their chemical composition. The second and third mechanisms rely on the mineral matrix of the soil. The second mechanism is physical protection, and ensues from spatial inaccessibility of the decomposers to the molecules, due to their entrapment in aggregates of different sizes. Finally, the third mechanism corresponds to the protection of molecules from biodegradation because of their interactions with mineral particles (adsorption for instance). They have been reviewed in von Lützow et al. (2006), Sollins et al. (1996, 2007) and Six et al. (2002). Even though they are well identified and commonly accepted by the community, studying their relative importance and their interactions remains a challenge both conceptually and technically.

### **2.2.1. Chemical recalcitrance i.e. how the chemical nature of a molecule explain its persistence in soils**

The idea that certain classes of molecules are more persistent than others solely because of the chemical composition is tempting and can be understood easily. Indeed, one of the exit routes for SOC is biodegradation by microorganisms, it is therefore understandable to hypothesize that some compounds are more easily decomposed than others. For instance, very “simple” molecules in terms of chemical composition and structure, like sugars, would be easily decomposable, whereas complex polymeric-like structures, like lignin, are more complex and could be more difficult to mineralize. Therefore, if a microorganism were facing these two options, it might choose to decompose the easiest one, namely the monosaccharide, before the other one. That is the concept of chemical recalcitrance.

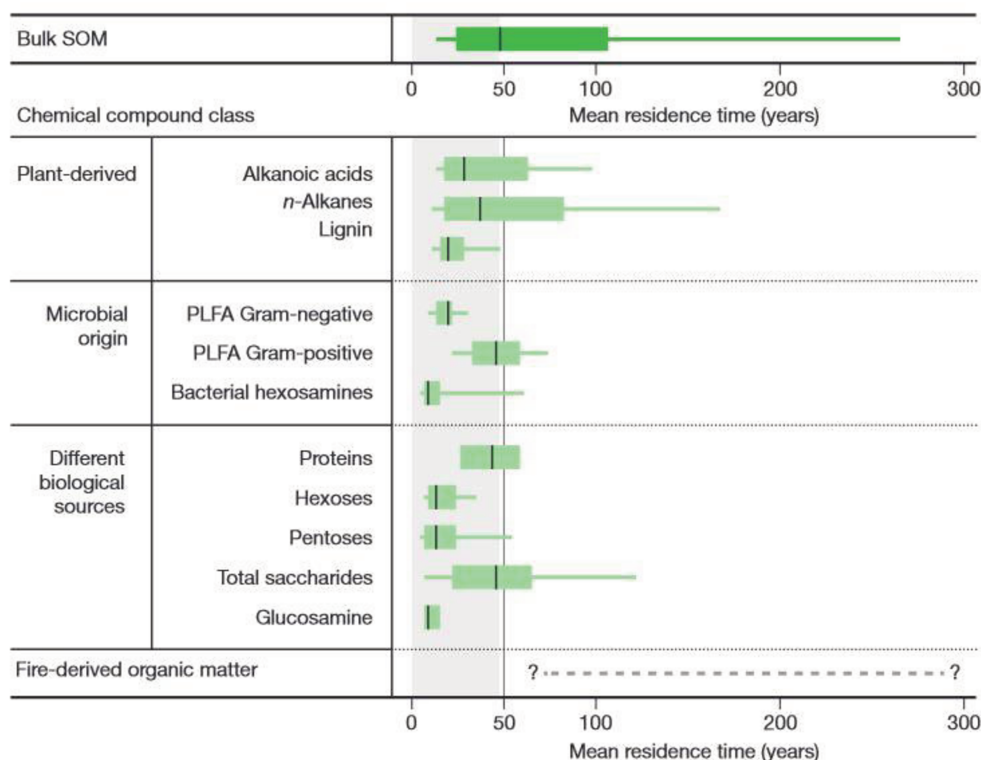
The old historical model of SOM envisioned two kinds of SOM: on the one hand there were compounds still recognizable as plant and microbial metabolites, on the other hand there would be humic substances which would correspond to very large polyaromatic structures created through abiotic processes (Schulten et al., 1998, Brady and Weil, 2008). These complex polymers were considered recalcitrant in soils. But these structures are now considered as being mostly artefacts of extraction procedures and there has been a shift in the way soil scientists conceptualize SOM. SOM is now viewed as a mixture of organic

molecular fragment derived from the depolymerization and oxidation of standard biomolecules (Sollins et al., 2007).

The influence of chemistry on the biodegradability of SOC has been demonstrated in incubations and in the field (Kögel-Knabner, 2002, Johnson et al., 2007).

But contradictory results have also been found. Compounds which were considered recalcitrant such as lignin (Derenne and Largeau, 2001) have been found to degrade quickly (Dignac et al., 2005, Thevenot et al., 2010, Marschner et al., 2008). Old organic matter has been found to contain so called labile molecules such as proteins and microbial polysaccharides (Amelung et al., 2002, Kiem and Kögel-Knabner, 2003, Knicker, 2004, Kleber et al., 2011).

Amelung et al. (2008) summarized these findings in their review and showed that all the extracted compounds from the soil so far had turnover times shorter than the average turnover of bulk SOC, even for supposedly recalcitrant compounds.



**Figure 5: Mean residence time estimations for bulk SOC and isolated compounds (Schmidt et al. 2011).**

Schmidt et al. (2011) adapted this table and added the uncertainty linked to the turnover time of pyrogenic material, to offer a possible explanation to this observed discrepancy (See Figure 5). Indeed, if many compounds which were considered recalcitrant have been proven to

degrade more quickly than anticipated, there still remain doubts about the degradability of pyrogenic, i.e. fire-derived, material (Marschner et al., 2008). The wide variety of charred compounds created during a fire event and their particularly condensed structure would make them highly recalcitrant. The microbial communities would not have developed the right set of enzymes to biodegrade them. Additionally, there has been evidence for long radiocarbon ages of charcoal pieces and many soils contain such material (Schmidt et al., 2002, Skjemstad et al., 2002, Dai et al., 2005, Jenkinson et al., 2008). Residence time for Pyrogenic organic carbon has been estimated to be in the order of 500–10 000 years in soils (Skjemstad et al., 1998, Schmidt et al., 2002, Lehmann et al., 2015, Forbes et al., 2006). Due to this apparent long residence time, the formation of charred OM is regarded as a carbon stabilization process that could result in effective carbon retention in the soil (Swift, 2001). Moreover, decomposition rates have been found to decrease with increasing charring temperature (Baldock and Smernik, 2002).

However, given the frequency of fire and the vegetation worldwide, if pyrogenic carbon were inert, the earth would be covered by several meters of ashes and charcoal pieces. Moreover, fire events have happened, either naturally or because of humankind, for way too long (Glasspool et al., 2004) for microbial communities not to have developed the enzymatic steps necessary to biodegrade pyrogenic material. And finally, there are more and more studies showing the biodegradability of pyrogenic organic carbon (Hamer et al., 2004, Hammes et al., 2008, Cheng et al., 2008, Cheng et al., 2006, Bird et al., 1999, Ohlson et al., 2009) and evidencing relatively short turnover times for pyrogenic organic carbon.

Stretching the concept of chemical recalcitrance to its maximum, if recalcitrance were the only driver of biodegradation, the degradation rate of “recalcitrant” material would be little altered by changes in the environmental conditions. Whereas the contrary has been found, degradation rates of a given molecule can vary greatly depending on factors like clay content, pH and microbial community composition, all of which are not related to the molecular composition of the compound in question (Kleber, 2010).

Therefore, one straightforward conceptual drawback of such a mechanism is that it looks at the SOC cycle without taking into account the environment in which this cycle takes place, the main component being the mineral matrix in which both the substrate (SOC) and the decomposing organisms are located. Thus, identical compounds in terms of chemical composition might turnover at different rates according to the conditions they are in (position in the soil profile, location in an aggregate, characteristics of the microbial community

present, water and temperature conditions, presence of other compounds,...). And finally, similarly to PyOC, decomposers have been in contact with SOM for millennia, and have developed the enzymatic pathways to biodegrade all natural compounds (Ekschmitt et al., 2005, Dungait et al., 2012).

As a result, chemical recalcitrance cannot explain long-term persistence of organic carbon in soils. Chemical composition cannot render a compound resistant to biodegradation, however the steps involved in this decomposition can be of varying complexities. It is now generally accepted that the chemistry can affect decomposition rates, but not as an absolute property and more as a relative one. In that regard, it can explain differences in the early phases of decomposition.

“The view that OM stabilization is dominated by the selective preservation of recalcitrant organic components that accumulate in proportion to their chemical properties can no longer be accepted.” von Lützow et al. (2006).

Nowadays it is fairly accepted among the SOM community that chemical recalcitrance plays a role on the short-term persistence of SOC but that other mechanisms, which involve the mineral matrix of the soil, are predominant on longer timescales (several decades).

### **2.2.2. Two mechanisms of protection by the minerals of the soil**

The mineral part of a soil is as diverse as the organic part. It is composed of particles of different sizes, with varying chemical compositions and structural organizations.

Soil scientists have really quickly designed methods to isolate different fractions of the mineral matrix of a soil. The easiest fractionation technique is size fractionation, which is obtained by the use of sieves for big particles and centrifugation for smaller particles. It usually involves the isolation of different **reference size fractions which are not purely arbitrary but have been defined to reflect the changes of characteristics of the particles and the kind of properties they confer to a soil (see Figure 6). The distribution in the different size fractions will define the texture of a given soil:**

- the sand fraction ( $50 \mu\text{m} < d < 200 \mu\text{m}$ ): these particles are usually visible to the naked eye, they are mainly composed of a single mineral, quartz ( $\text{SiO}_2$ ) and sometimes from other primary silicates. Because of their chemical composition, sand particles do not constitute a significant potential source of nutrients for plants. Because of their size, sand particles have low specific surface area and thus have



little water and nutrients holding capacity and do not stick together to form a coherent mass.

- silt fraction (coarse:  $20 < d < 50 \mu\text{m}$  and fine:  $2 < d < 20 \mu\text{m}$ ) : silt particles are similar in shape and mineral composition to the sand particles but there can also be occurrences of phyllosilicates minerals which are commonly found in clay size fractions. The smaller size of the minerals gives them better water holding capacities, and the minerals are more easily weathered.
- clay fraction ( $d < 2 \mu\text{m}$ ). Clay size fraction can be really diverse in terms of mineralogy and should not be confused with “clay minerals” i.e. phyllosilicates. Soil clay-size fractions can contain six main types of minerals: phyllosilicates (known as clay minerals), metallic oxides and hydroxides (ferrihydrite, goethite ...), primary minerals (quartz, feldspars ...), and in some soils carbonates, gypsum or short-range order alumina-silicate minerals (allophane, imogolite). Their small size gives them high specific surface areas and they have high holding capacities. Unlike sand and silt particles, clays are not shaped like grains but more like flakes or platelets. Specific properties will depend on the kind of clay mineral present in the soil. Thanks to their retention capacities, Clay size minerals retain pollutants or toxic compounds in the soil and prevent their further spreading in the environment (in rivers and ground water) (Dixon and Weed, 1989, Lagaly, 2001).

Another fractionation method exists; it relies on the different densities of soil particles. The fractionation is usually performed using sodium polytungstate solutions which allows separation between the uncomplexed light fraction (which would mainly consist of poorly decomposed OM) and the heavy occluded fraction, considered made of organomineral associations (Derenne and Quénéa, 2015).

The difference between these two main fractionation techniques is conceptual: one

will isolate different sizes and the other will be isolate more functional fractions of the soil (they can be more easily linked to mechanistic processes) (Moni et al., 2012).



**Figure 6: Distribution of particles by size in a traditional American snack. Much like a soil, the different classes of particles display contrasted characteristics (here it is flavor for instance), and much like organic matter in the soil, it is caramel which binds the different particles together in aggregate-like structures. (Source: the Internet).**

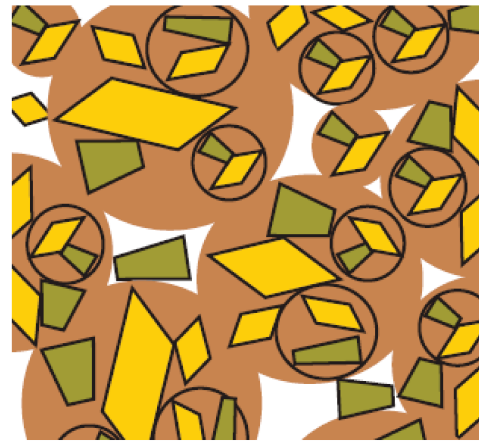
Soil structure comes from the different assembling possibilities of these mineral particles, glued together by organic material, these units are called aggregates. Aggregation is the main process which explains soil structure; aggregates will be responsible for soil properties such as porosity (size, connectivity, and tortuosity). Aggregates are composed mainly of minerals (responsible for the general shape of the aggregate) and organic material (responsible for the properties and the cohesion of the structure) and can be of different sizes (from nanometers to centimeters). Aggregates are formed through the process of humidification/dessication, by the activity of plant roots and soil fauna (hyphae and earthworms). It is thus clear that the minerals play a role in the dynamics of decomposition of SOC as there is an intimate link between soil organic carbon and the mineral matrix. For instance, soil texture has been shown to influence the composition of microbial communities (Gelsomino et al., 1999, Johnson et al., 2003) and could therefore have an effect on SOC turnover. There has even been evidence

of a greater impact of the texture than of the nature of plants growing on soils (Groffman et al., 1996).

Different scales and different mechanisms can explain SOC persistence in soils thanks to minerals. The possible interactions between SOC and minerals depend on the chemical nature of both the mineral particles and the SOC and on the three dimensional arrangement of the different constituents.

### 2.2.2.1. Physical protection in aggregates

Aggregates, due to their spatial organization can isolate some material from the rest of the soil; that is why the concept of protection of organic material inside aggregates was developed (Figure 7). Indeed, if organic molecules are trapped inside an aggregate, microorganisms (and their enzymes) cannot reach them and they will remain in the soil, untouched. Moreover, microbial activity can be decreased in aggregates because of environmental constraints such as lack of oxygen or lack of nitrogen (Angers and Chenu, 1997).



**Figure 7: Simplified representation of the structure of an undisturbed soil. Aggregates (brown regions) are made up of mineral particle (yellow zones) and organic matter (green zones), some of which becomes inaccessible. Adapted from Chenu et al. (in press).**

There has been experimental evidence of the importance of aggregates in biodegradation. Indeed SOC mineralization rates have been found to increase after the disruption of aggregates (Mueller et al., 2012, Tebrügge and Düring, 1999, Six et al., 2000, Balesdent et al., 2000). Moreover, several studies based on density fractionation have found that the organic carbon contained in the occluded fractions (VS free fraction) had longer radiocarbon ages than bulk SOM (Leifeld et al., 2009, Schulze et al., 2009).

Soil structure is defined by aggregation and is an important driver of SOM biodegradation (VanVeen and Kuikman, 1990). Soil structure has an impact on soil water (retention capacities, evaporation, diffusion), on aeration, on nutrient and enzyme transport and even on erosion (Leguédouis and Bissonnais, 2004, Díaz-Zorita et al., 2002). And because the poral system is the habitat for microorganisms (Young and Ritz, 2000), they will be impacted by soil structure (Carson et al., 2010, Ruamps et al., 2011). Microorganisms though of tremendous importance for the processes that take place in soils, cannot significantly move in

the soil matrix, this characteristic has been referred to as the « sleeping beauty paradox » (Lavelle et al., 2006). Soil structure will therefore determine the localization of microorganisms in the poral network and the accessibility of SOM to these decomposers (Ladd et al., 1996, Six et al., 2000, Ritz et al., 2004, Yoo et al., 2006) .

The dynamics of SOC in macroaggregates have been studied a lot, because of the sensitivity of macrostructure to land use changes (John et al., 2005, Puget et al., 1995). But it has been found that changes in the macrostructure did not induce differences in the carbon content of soils in the long term (Paradelo et al. submitted). Moreover, it has been found that, protection is more efficient in small aggregates than in larger structures (John et al., 2005). Franzluebbers and Arshad (1997) have shown that carbon mineralization was more important in bigger aggregates (diameter above 0.25 mm). Furthermore, there is evidence that the smaller the mineral particles involved in the aggregate the better the protection, this would also be linked to the presence of iron and aluminum oxides (Duiker et al., 2003) and would isolate the SOC from the decomposers and their enzymes (von Lützow et al., 2006). Nowadays, the main hypothesis is that soil microaggregates can protect SOC from biodegradation either at the silt size (Virto et al., 2008) or at finer scales (Chenu and Plante, 2006, Calabi-Floody et al., 2011, Dümig et al., 2012). These microstructures can act as a barrier between SOC and microorganisms and they can create anoxic conditions which can in turn inhibit biodegradation (Balesdent et al., 2000, Kemmitt et al., 2008). Chenu and Plante (2006) have evidenced such structures in soils with Transmission electronic microscopy, the images display aggregates of sizes around one micrometer containing intact plant debris, or bacteria.

The necessary construction/destruction cycles of the aggregates in soils mean that the protected OM is periodically unprotected. This protection mechanism can therefore only be temporary and for some authors it cannot explain carbon persistence in soils after several decades (Baldock and Skjemstad, 2000, Virto et al., 2010) .

However, when looking at such small structures, it is difficult to differentiate between this protection mechanism and the protection of SOC by adsorption on the mineral surfaces. A recent study, relying on a specific fractionation protocol, estimates that in silt-sized aggregates, around half of the protected carbon would be protected because of spatial

inaccessibility and half of it would be protected through adsorption on the mineral surfaces (Moni et al., 2010).

### **2.2.2.2. Physicochemical protection by organomineral interactions**

Physicochemical protection corresponds to the adsorption of SOC at the surface of specific mineral particles.

The possibility of such organomineral interaction is understandable, indeed the characteristics of the mineral surfaces make several types of covalent and noncovalent interactions possible: ligand exchange, polyvalent cation bridges (i.e. coordination of anionic or polar functional groups of OM to adsorbed polyvalent inorganic cations), interaction of metal ions with organic substances (complexation), electrostatic bonds, and weak interactions like Van der Waals, hydrogen bonding or hydrophobic interactions.

The nature of the interaction will depend on:

- (i) the type, abundance and charge characteristics of the functional groups at the surface of OM and mineral particles;
- (ii) the size, shape and topography of the surface;
- (iii) the degree of aggregation.

Each kind of interaction can be characterized by an energy, and because of this energy it will be more difficult for microorganisms to biodegrade the organic compounds involved in organomineral associations. The energy required to break these bonds and access the protected SOC would range from 0 to 80 kJ.mol<sup>-1</sup> (Enell et al., 2005). Because clay-sized minerals display higher specific surface areas and because of their particular chemical composition, this protection mechanism occurs mainly at the clay size. Apart from gypsum, all the minerals found in clay size fractions have been shown to efficiently protect SOM from decomposition (Sollins et al., 2009, Basile-Doelsch et al., 2009, Torn et al., 1997, Mikutta et al., 2006, Baldock and Skjemstad, 2000, Fernández-Ugalde et al., 2011, Romanyà and Rovira, 2011). Furthermore, different mineral species have been shown to interact with SOM: poorly crystallized aluminosilicates such as allophanes (Torn et al., 1997), metallic oxi-hydroxides (Wiseman and Püttmann, 2005, (Skjemstad et al., 1990, Kleber et al., 2005) and phyllosilicates (Pronk et al., 2013).

**BOX 7: Phyllosilicates in soils**

Soil phyllosilicates can have three different origins: they can be inherited from parent materials, transformed from other minerals in water–rock interaction or neosynthesized. Two groups of phyllosilicate layers can be distinguished: the 1:1 and the 2:1 layers. The basic structure of 1:1 layers is made of one tetrahedral sheet of covalently linked cations to oxygen ions and one octahedral sheet. The 2:1 layers contain one octahedral sheet of covalently linked cations in-between two tetrahedral sheets.

In tetrahedral sheets the cations are  $\text{Si}^{4+}$ , they can alternatively be substituted by  $\text{Al}^{3+}$  ions. In octahedral sheets, the cations can be trivalent ( $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$ ) or bivalent ( $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ ). The substitution of a given cation by a cation which has less positive charges ( $\text{Si}^{4+}$  substituted by  $\text{Al}^{3+}$  for instance) results in a net negative charge of the sheet. This negative charge is most often compensated by cations or hydroxy-cations adsorbed on the surface of phyllosilicate layers. Interlayer cations are bound with water molecules in the case of expandable clay layers (e.g. smectite) or anhydrous such as in illite particles.

Isomorphic cation substitutions are frequent for 2:1 clay layers and much less common for 1:1 layers. As a result, 1:1 layers are not permanently charged. Kaolinite is an example for 1:1 layer phyllosilicate whereas illite, chlorite, smectite or vermiculite are 2:1 layer phyllosilicates commonly found in soils. Both 2:1 and 1:1 clay layers have pH-dependent charges corresponding to surface hydroxyls situated at the broken edges of clay layers that can be ionized depending on pH conditions.. Phyllosilicates do not occur in soils as individual layers, but as stacks of several layers, either of similar clay layers, e.g. illite or kaolinite, or many types of interstratified minerals. Illite/smectite mixed layered minerals seem to be the most frequent, especially in temperate climate soils.

Types of mineral surfaces encountered on phyllosilicates:

- (1) single coordinated hydroxyl can be found at the edges of layer silicates;
- (2) siloxane groups with permanent layer charge (negative) due to isomorphic substitution. In soils, these siloxane groups are present on some 2:1-layer-type phyllosilicates, such as vermiculite, illite, and smectite; and
- (3) siloxane groups without layer charge. This type occurs in 1:1-type minerals (kaolinite group).

Adapted from Barré et al. (2014).

Evidence for stabilization of OM by binding to mineral surfaces comes from the fact that soil OM in fine silt and clay fractions has been found to be older (Wattel-Koekkoek et al., 2003, Quideau et al., 2001, Eusterhues et al., 2003) or to have longer turnover times (Balesdent, 1996, Ludwig et al., 2003). Kalbitz et al. (2005) showed that sorption of soluble OM to subsoil material (Bw horizon) decreased OM mineralization by 20–30% compared with mineralization in the soil solution. Scharpenseel and Becker-Heidmann (1992), Torn et al. (1997), Paul et al. (2001) all detected a direct relationship between radiocarbon age and clay content.

Moreover, Feng et al. (2011) found a positive correlation between the quantity of particles in the fine fraction and carbon content.

The nature of clay minerals should have an impact on (i) the type of protected SOC, (ii) the strength of the protection, as seen in contrasted turnover times of protected SOC. Here we will exclusively focus on the interaction with phyllosilicates which are dominant in the clay-size fractions of our main study site. Evidence for an influence of the type of phyllosilicate on the persistence of SOM is scarce. The studies which looked at the influence of phyllosilicates on the protection of SOC suggested that the nature of the mineral phase influences the quantity of protected carbon, in terms of particular clay types (1:1 or 2:1) as was demonstrated by Feng et al. (2011). Wattel-Koekkoek et al. (2001 and 2003) found that smectites seem to adsorb more aromatic compound and less polysaccharides than kaolinite minerals, and that OM bound to kaolinite had a turnover time of 360 years whereas smectite interactions led to turnover times of 1100 years. A review by Schulten and Leinweber (2000) showed that phyllosilicate mineralogy seemed to have an influence on the quantity of Carbon stored (see Figure 8).

However, few studies have investigated the nature of the SOM protected by organomineral interactions (Sollins et al., 2009) and the evolution of this protected SOM. The composition of OM stabilized by specific minerals has also been suggested by analyzing OM in

Reference	Clay (%)	Predominant clay minerals	C <sub>org</sub> (%)
Kyuma et al. (1969)	60	Smectite	2
	39	Kaolinite and smectite	1–2
	32	Allophane	13–14
Lowe and Parasher (1971)	6	Smectite, less vermiculite	6
	17	Chlorite (vermiculite, illite)	11
Tate and Churchman (1979)	3–15	Illite (vermiculite)	4–16
	8	Smectite	15
Cameron and Posner (1979)	17–18	Kaolinite	2–3
Young and Spycher (1979)	8–45	Chlorite-mixed layers	1–20
Spycher and Young (1979)	45	Smectite	2
Turchenek and Oades (1979)	17	Kaolinite and illite	2–6
	45	Smectite and illite	7–9
	46	Palygorskite	3–7
Leinweber and Reuter (1988)	69	Smectite	1–2
	8	Illite, less smectite	4–6
	9	Chlorite-mixed layers	8–11
Christensen (1988)	9	Illite (quartz, feldspars, smectite)	8–9
Leinweber (1995)	2	Smectite	6–17
Randall et al. (1995)	28	Smectite/vermiculite	2–5

**Figure 8: Link between clay contents (%), predominant clay type and content of organic carbon data compiled from the literature (ranges originate from different clay subfractions or management practices). From Schulten and Leinweber (2000).**



mineralogical diverse density or size fractions of soils (Laird et al., 2001, Kahle et al., 2003, Kleber et al., 2004).

It has been shown that with time the molecules involved in organomineral associations form new covalent bonds (Kaiser et al., 2007). However, this protection mechanism is not indefinite, recent data suggests that SOM previously protected by their belonging to organomineral associations could be unprotected by the compounds usually present in root exudates (Keiluweit et al., 2015). Indeed, these compounds (oxalic acid) would induce the disruption of organomineral interactions and lead to higher quantities of accessible SOM.

### **2.3. Should we talk about stability or persistence?**

The concept of stability of SOM has emerged from the experimental evidence of carbon persisting in soils for long periods of time even though it is not thermodynamically stable and even though it should be biodegraded by microorganisms. Some carbon would thus be stabilized in soils. We first thought that super macromolecular structures could explain this perceived stability. Then we developed the concepts of chemical recalcitrance and protection mechanisms. But as we have seen, none of the three mechanisms can set the fate of individual organic molecules in soils; none of them is an absolute protection against biodegradation. Therefore, as much as we say that each protection mechanism is relative and not absolute, stability will not be an intrinsic and absolute property of SOM. Moreover, stability is not a measurable property as it depends on too many variables.

We are thus left with the only measurable information available: persistence, that is, how long a given molecule has been standing in the soil. And as we have seen, it is not always easy to measure this property, and, even when we can, the techniques to measure this persistence are not exempt of disadvantages and limitations...

However, understanding the dynamics of carbon in soils is more crucial than ever, and in order to further our knowledge and to improve our experimental designs, soil scientists have developed conceptual models.

There are different views on the dynamics of SOM, there is for instance the concept of “First In/Last Out” or the temporary stabilization process view.

The “First in / last out” model is based on the idea of SOC storage being like a storage unit for furniture, the first SOC molecules to enter the soil will be stored at the “back” of the unit and will therefore be the last one to be utilized by microorganisms, it agrees with the onion model



developed by Kleber et al. (2007) and which has been summarized in Kogel-Knabner and Amelung, 2014).

Another model for SOC stabilization, the temporary stabilization process, is based on the concept of SOC being protected from decomposition because they are in aggregates. Due to the regular formation and destruction of aggregates, this process can lead to the biodegradation of either old or young carbon, with no preference for either. With this model, some SOC will remain for very long periods of time because the aggregate it is located in will remain intact for a very long time, whereas other carbon molecules will be biodegraded very quickly because their aggregate is destroyed quickly.

## **2.4. Limitations of the approach**

### **2.4.1. Should we try to store carbon in soils?**

Storing C in soils is considered a win-win solution, as it could absorb CO<sub>2</sub> from the atmosphere and it would improve soils. Indeed, it is generally accepted that many soils have experienced decreases of their C contents with the development of agriculture because of exportation of plant material that would normally have been returned to the soil (harvest). There would therefore be room for carbon “storage” (more like recovering of previous C contents), with the help of C conserving practices for instance. However, some authors argue that organic matter is not *per se* an improvement of soils, the improvement coming from the decomposition of organic matter and not from the presence of organic matter itself (Janzen, 2006). According to the authors, there is thus a dilemma between the will to sequester carbon in soils and the will to improve soil quality. However, I do not think these two ideas are mutually exclusive, because there is strong evidence that soil fauna can biodegrade any organic compound from natural origin and that soil carbon contents can be higher than what is currently observed, there is room for both an improvement of soil quality and an increase in soil carbon stocks.

### **2.4.2. Looking at the persistence of SOC from a soil physicochemist’s point of view**

Because soil is such a complex system, involving highly contrasted components, many disciplines have studied it. When studying soils, one can encounter and collaborate with geologists, hydrogeologists, climatologists, physicists, theoretical physicists, chemists, geochemists, biogeochemists, biologists, microbiologists, ecologists, pedologists, etc.

This has not helped soil science to establish itself as an individual discipline and that is partly the reason why soil science units are usually attached to other departments and do not have their own institutes. A striking example is that the three main soil science laboratories where I have performed experiments during my PhD work were attached to three different types of institutions: one was in a Geology Laboratory, the other in an Institute for the Environment and the third belonged to a Geography Department.

I am a chemist and therefore have been looking at the soil with a physicochemical point of view. There are advantages to my background in chemistry: I believe I have a strong technical understanding of the methods used, I have had to develop an interdisciplinary approach, not having followed any soil science course I was able to see the problem with a “fresh” eye, and I have, whenever possible, tried to use concepts from other fields (first in last out, flow reactors).

But it can also lead to serious limitations when one realizes that explanation for SOM decomposition relies in three main controls: the environment (physicochemical mechanisms), the substrate (physicochemical mechanisms) but also the organisms that can degrade C in soils. So C is protected by chemical and physical mechanisms but also biological mechanisms in the biological activity of microorganisms, which has not been the focus of my PhD project.

### 2.4.3. What other factors can influence the persistence of SOC?

The three previously presented mechanisms for SOC protection from biodegradation – chemical recalcitrance, physical protection and physicochemical protection- are not the only factors which have a significant role in SOC turnover. A summary of these factors is presented in Figure 9. For instance, SOC biodegradation is also dependent on the microbial activity of soils. This research project does not aim at assessing the impact of climatic conditions or microorganisms on the degradation of SOC but it is important to acknowledge these effects and that is why the factors involving environmental conditions and living organisms will be briefly presented in this part of the manuscript.

Controls of soil organic carbon biodegradation		
Environment	Substrate	Decomposers
<b>Drivers:</b> <ul style="list-style-type: none"> <li>• Moisture</li> <li>• Radiation (IR, VIS, UV)</li> </ul>	<b>Energy source:</b> <ul style="list-style-type: none"> <li>• Bioenergetic potential</li> <li>• Oxidation state</li> </ul>	<b>Functionality:</b> <ul style="list-style-type: none"> <li>• Grazers / Predators</li> <li>• Mycorrhizal fungi</li> <li>• Free-living fungi</li> <li>• Bacteria/archaea</li> </ul>
<b>Structure:</b> <ul style="list-style-type: none"> <li>• Total pore space</li> <li>• Pore size distribution</li> <li>• Preferential flow path</li> <li>• Mineral surface chemistry</li> </ul>	<b>Carbon/nutrient source:</b> <ul style="list-style-type: none"> <li>• Molecular stoichiometry (C:N:P)</li> <li>• S, Ca, Mg, K content</li> <li>• Micronutrient content</li> </ul>	<b>Microbial diversity:</b> <ul style="list-style-type: none"> <li>• Evenness</li> <li>• Richness</li> <li>• Abundance</li> </ul>
<b>Resources:</b> <ul style="list-style-type: none"> <li>• Oxygen availability</li> <li>• Alternate e-acceptors</li> <li>• Proton concentration</li> <li>• Catalytic metals</li> <li>• Organic co-metabolites</li> <li>• Nutrients on minerals</li> </ul>	<b>Assimilability:</b> <ul style="list-style-type: none"> <li>• Aqueous solubility</li> <li>• Molecular size</li> </ul>	<b>Activity:</b> <ul style="list-style-type: none"> <li>• Dormancy</li> <li>• Competitive interactions</li> <li>• Growth stage</li> </ul>
	<b>Accessibility:</b> <ul style="list-style-type: none"> <li>• Location in pore space</li> <li>• Coprecipitated with minerals</li> <li>• Adsorbed</li> <li>• Encapsulated in aggregates</li> </ul>	<b>Ability to use O<sub>2</sub>:</b> <ul style="list-style-type: none"> <li>• Aerobes</li> <li>• Obligate anaerobe</li> <li>• Facultative anaerobe</li> <li>• Microaerophile</li> </ul>

**Figure 9: Drivers of the biodegradation of soil organic carbon. Adapted from Kleber, personal communication.**

For further information on these topics, the reader is advised to read other research work conducted under the supervision of Prof. Claire Chenu (Ruamps, Juarez, Lefevre).

#### **2.4.3.1.Climate**

Climate is an important driver for organic matter decomposition as it will influence the biological activity of soil mainly through water availability and temperature.

Microorganisms have different responses to temperature changes (Rothschild and Mancinelli, 2001, Standing and Killham, 2006). The sensitivity of SOC degradation to temperature has received a lot of attention (see review by Conant et al., 2011 and work by Lefèvre et al., 2014) Water availability and water dynamics will influence SOC biodegradation because it is the main medium for the diffusion of nutrients, enzymes, dissolved gaz. Moreover, water will also have an impact on the dispersion and the connectivity of microbial communities (Jocteur Monrozier et al., 1993, Ranjard and Richaume, 2001).

#### **2.4.3.2.Soil pH**

Soil pH depends on the nature of the bedrock (Standing and Killham, 2006). Changes in pH have been proven to affect the composition, structure and diversity of soil microbial communities (Bååth et al., 1995, Fierer and Jackson, 2006, Lauber et al., 2008) and will thus have an impact on SOC biodegradation. Moreover, studies have also evidenced a correlation between pH and fungi/bacteria ratio (Bååth and Anderson, 2003, Högberg et al., 2006).

#### **2.4.3.3.Soil fauna**

Macrofauna and mesofauna have an impact on the dynamics of SOC. On the one hand, soil fauna participates in the biodegradation of SOC through the fragmentation of plant debris which will thus be more easily degraded by microorganisms. On the other hand, soil fauna is partly responsible for soil structure: the digestion process of earthworm participates in the formation of aggregates by intimately mixing soil organic matter and mineral phases (Lavelle et al., 2006, De Gryze et al., 2005, Plante and McGill, 2002). This mechanism will protect part of the SOM from biodegradation (second and third mechanisms of protection) (Jiménez and Lal, 2006).

#### **2.4.3.4.Microorganisms**

Every SOM can be biodegraded (Dungait et al., 2012), but two main microbial characteristics can influence the degradation dynamics of SOC.

Several studies have looked at the impact of microbial diversity on SOM decomposition, using different approaches (Griffiths et al., 2000, Nannipieri et al., 2003, Bell et al., 2005, Degens et al., 2001). Their results seem to indicate that microbial communities are

characterized by a strong functional redundancy which means that perturbations leading to modifications of the structure or the diversity of microbial communities do not lead to a loss of functions (Nannipieri et al., 2003).

Another mechanism of microbial origin has been shown to have an impact on SOM persistence, it has been referred to as priming effect. It was discovered as a side effect of studies looking at the degradation of substances added to soil. An increased release of CO<sub>2</sub> from the SOC was often detected compared to what was observed without additions of external substances. It has been extensively studied (Kuzyakov et al., 2000) and is interpreted as an increased microbial activity due to the addition of fresh material in the system. Another mechanism involving fresh organic compounds has been evidenced very recently by Keiluweit et al. (2015). Using artificial roots, they have shown that the root exudate composed of standardized compounds could liberate previously protected SOC by disrupting the preexisting organomineral interactions. According to the study, this mechanism could have a greater magnitude than standard priming effect.

## 2.5. Scientific questions

Keeping in mind the focus of this research project and the limitations it bears, we can highlight several scientific questions that arise from the presentation of these protection mechanisms.

- What is the relative importance of the different protection mechanisms? Is there one predominant mechanism?
- What is the chemical nature of persistent carbon?
  - Which compounds? Which chemical functions in interaction with which minerals?
  - How does the chemical nature of the C evolve as biodegradation occurs?
  - Is there a physical or physicochemical protection?
- Can we isolate the persistent C? How can we characterize the nature of soil C?

The next part of this manuscript will present different analytical tools to study soil organic matter and the experimental set ups that have been designed to isolate and/or study persistent carbon.

PART 2: STUDYING PERSISTENT  
CARBON WITH STATE-OF-THE-ART  
TECHNIQUES AND RELEVANT  
HISTORICAL FIELD EXPERIMENTS,  
THE LONG-TERM BARE FALLOWS

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There are two major obstacles to the study of persistent carbon in soils: (i) the interaction with the mineral matrix makes it difficult to use some experimental techniques and interpret the data thus obtained and (ii) it is nearly impossible to experimentally isolate persistent carbon from total organic carbon.

Here, we overcome these two obstacles by choosing emerging analytical tools and studying unique long-term bare fallow samples allowing access to persistent carbon. These long-term bare fallow experimental plots have been kept free of vegetation by manual or chemical weeding for several decades and have been regularly sampled and stored. With time, biodegradation occurs and samples get enriched in persistent carbon.

This part of the manuscript aims at presenting the different experimental ways that have been developed in soil science in order to study persistent carbon and will then briefly introduce the different analytical tools which have been used in this research project.



## 1. Studying persistent carbon with long-term bare fallows

Because it has strong implications for soil carbon dynamics and for the response of soil organic carbon stocks to global changes (Schuur et al., 2008), the persistence of carbon in soils has been extensively studied. In order to gain insights on this persistent carbon pool, defined by the age of the SOM, several techniques have been developed, some of which are briefly presented here. Among those, only long-term bare fallows provide the opportunity to efficiently isolate persistent carbon from total organic carbon.

### 1.1. Presentation of the different techniques to study the persistence of SOC

#### 1.1.1. Studying without isolating

##### 1.1.1.1. Datation techniques

This technique relies on the  $^{14}\text{C}$  radioactive isotope of carbon and has already been presented in the first part of this manuscript (Section 2.1 of Part 1).

##### 1.1.1.2. Incubations

Incubations are laboratory experiments in which small samples of soils (usually between 30 and 300 grams of soil) are taken from the field and placed in closed containers. They are then placed in controlled conditions (moisture, temperature, addition of substrate, inoculation with microorganisms...) for given amounts of time. Selected soil characteristics are monitored throughout the duration of the incubation (*e.g.*  $\text{CO}_2$  respiration, evolution of pH, microbial biomass, etc.). There are numerous examples of soil incubation experiments; they have been used to study a wide array of factors of biodegradation and microbial activity.

Evolution of the mineralization rates of organic carbon in soil can be measured with long-term laboratory incubations (several years). The common underlying hypothesis being that the most labile organic matter will be biodegraded at the beginning of the experiment and towards the end of the incubation the compounds mineralized are less biodegradable. Mineralization rates at the beginning and at the end of the incubation would therefore characterize different pools of soil organic carbon (Conant et al., 2008, Lefèvre et al., 2014).

One limitation of this approach for the study of the persistence of SOC is that incubations do not last more than a few years, at most, and can therefore not be used to study decadal to

centennial persistence of SOC. One thing to also keep in mind is that they create artificial conditions for the evolution of soils.

### 1.1.1.3.C3/C4 experiments

**This technique relies on the distinct  $^{13}\text{C}$  isotopic signatures of C3 vs. C4 plants, which are preserved upon organic matter decomposition (Balesdent et al., 1987).**

C3/C4 chronosequences are fields (natural or experimental) which have undergone a change of vegetation between plants which have a C3 metabolic pathway (which involves the Calvin cycle; this category of plant includes almost all temperate species and all trees) and plants which have a C4 metabolic pathway (which involves an additional cycle called the Hatch-Slack pathway, examples of this category of plants include corn, sugar cane, and sorghum) (Sage et al., 2011).

These metabolic differences lead to different isotopic compositions of the carbon compounds created by photosynthesis and therefore to a different isotopic signature of the organic matter inputs to the soil. The  $^{13}\text{C}$  signature of carbon compounds produced by C3 plants varies between -22 and -40 ‰ with an average value of -27 ‰ whereas it ranges from -9 and -19 ‰ for the carbon compounds created by C4 plants, averaging at -12 ‰ (Balesdent et al., 1987) (standard expression of  $^{13}\text{C}$  content by comparison with the Vienna Pee Dee Belemnite VPDB international reference). As a comparison, atmospheric  $\text{CO}_2$  has an average  $^{13}\text{C}$  isotopic signature of -7‰. These differences make it possible to study the change of the  $^{13}\text{C}$  isotopic signature of the soil samples and infer information about the turnover of the C3 (or C4) SOM.

Because we are able to differentiate the OM from before and after the vegetation change, we can therefore estimate SOM turnover. Several studies have used it as an *in situ* label for total organic carbon dynamics (e.g. Balesdent et al., 1988, Rasse et al., 2006), specific compounds ( e.g. Bahri et al., 2006, Derrien et al., 2006), or size-fractions of the SOC (e.g. Balesdent et al., 1987, Fernandez-Ugalde et al., 2016). Nevertheless, one major limitation of this method is that it does not isolate carbon, it only allows for a partial study of the dynamics of SOC. Furthermore, the only change between the OM before and after the vegetation change is isotopic, therefore, only techniques which give access to isotopic information can be used in order to study carbon dynamics in these C3/C4 setups.

## 1.1.2. Isolate to study

### 1.1.2.1. Laboratory techniques

As previously mentioned, models which try to explain soil organic carbon dynamics and turnover have relied on the definition of conceptual pools of SOC that no protocol has been able to successfully isolate yet. “Measuring the modelable” has not been successful so far, but several attempts have been made at taking the problem from the other end and “model the measurable” (Christensen, 1996, Magid et al., 1996, Sohi et al., 2001). Protocols have been developed in order to try and isolate different pools of SOC, some of which are briefly discussed in the following paragraphs: chemical extractions, physical fractionation, chemical fractionation. For more in depth presentations of the existing fractionation methods, the reader is redirected to the reviews by von Lützow et al. (2007) or by Moni et al. (2012).

It was first believed that chemical extractions would isolate “recalcitrant”, older, more persistent carbon fractions containing the macromolecular superstructure that were supposed to be a recalcitrant form of SOC. Alkali solutions, such as NaOH or pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7$ ) were used to solubilize large amounts of SOM. The organic molecules thus solubilized by alkali are named humic substances. The material insoluble in alkali is named humin. Lowering the pH below 2, by using HCl, isolates two fractions of the humic substances by triggering the precipitation of a part of the dissolved compounds. On the one hand the precipitate is named humic acid extracts, on the other hand, the soluble part is referred to as fulvic acid. These chemical extractions led to the concept of persistent organic carbon being progressively integrated in macromolecular structures which would be difficult to biodegrade. However, alkaline extractions present serious artifacts, as detected by Baldock and Nelson (2000). It is now rejected by most of the SOM scientific community and considered more as an operational definition (Kleber and Johnson, 2010, Schmidt et al., 2011) than as a representation of *in situ* SOM. Indeed, there is overwhelming evidence that SOM and as a consequence persistent SOM is composed of relatively simple molecular structures (Lehmann et al., 2008, Kelleher and Simpson, 2006, Piccolo, 2002, Sutton and Sposito, 2005, Sollins et al., 2007).

Physical fractionation has also been extensively used in order to try and isolate different pools of SOC with contrasted turnover times. With the goal to “model the measurable”, Sohi et al. (2001) developed a physical fractionation protocol aimed at isolating pools suitable for modeling purposes, with a reference to the Roth C model. In their study, several steps of

ultrasonic dispersion and density fractionation are used in order to isolate three main pools: (i) the free organic matter (or light fraction) isolated before the disruption of water stable aggregates, (ii) the intra aggregate “free” organic matter (occluded light fraction) obtained after ultrasonic dispersion of water stable aggregates, and (iii) the organic matter linked to the mineral particles (organomineral fraction). These three pools are hypothesized to display contrasted turnover times, the light fraction would be composed of fresh plant material with high reactivity and hence high turnover rates, the occluded free organic matter would contain more degraded material (intermediate turnover) and the organomineral fraction would comprise the more persistent compounds (slow turnover). There are three limitations to these approaches. First, they usually infer turnover times from the supposed biological origin of the OM and from the state of decomposition and potential association to minerals; whereas it is neither the chemical nature nor the biological origin of the OM which will define its persistence in an ecosystem. Second limitation, mineral-bound OM is not only composed of persistent OM. Finally, the third limitation is that none of the thus-isolated fractions is homogeneous in terms of kinetics.

Chemical fractionation is one of the approaches which have been developed to easily remove biodegradable organic matter and thus isolate a more persistent fraction. Several factors influence the conditions of the chemical fractionation: the chemical conditions of the reaction (pH, temperature, duration), and the soil's characteristics (mineralogy, type of organic matter). Several chemical treatments have been studied (Jagadamma and Lal, 2010): alkaline dissolution (Stevenson, 1994), photo-oxidation (Skjemstad et al., 1993), acid hydrolysis (Silveira et al., 2008) and chemical oxidations (Mikutta et al., 2005b). Chemical oxidations have been considered particularly efficient in isolating persistent carbon as they would efficiently mimic the natural processes occurring during biodegradation. The reagents used for chemical oxidations differ according to the studies. The three most accepted are hydrogen peroxide ( $H_2O_2$ ) (Eusterhues et al., 2005), sodium peroxodisulfate ( $Na_2S_2O_8$ ) (Lorenz et al., 2006) and sodium hypochlorite ( $NaOCl$ ) (Zimmermann et al., 2007). Paul et al. (1997) have found that the fraction of OM resisting chemical oxidation had older radiocarbon ages than bulk OM. However, several studies have shown that chemical oxidations do not efficiently isolate persistent carbon (Grasset et al., 2009, Lutfalla et al., 2014). More material on chemical oxidations can be found in Part 3 (Section 1) of this manuscript.

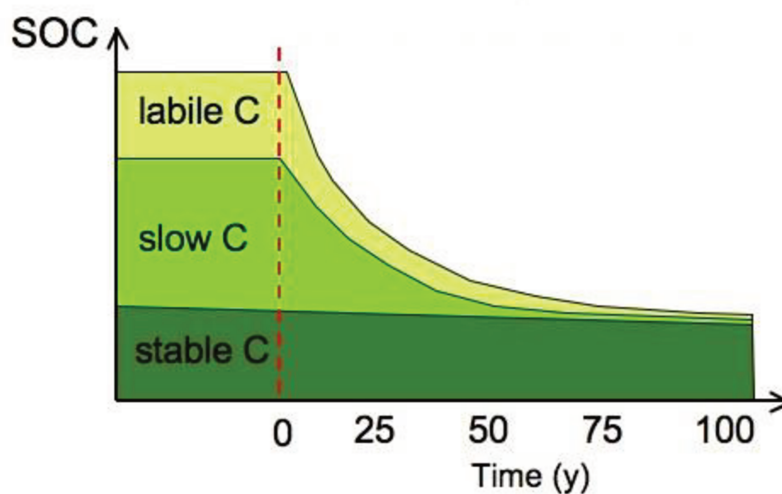
### 1.1.2.2. Long-term bare fallows

One naive way to assure that only persistent C is present in a soil is to stop C inputs and await the more labile compounds to decompose. Long-term bare fallow experiments (LTBF) provide such conditions. Bare fallow plots are field experiment in which the soils have been kept free of plants by manual and/or chemical means for several decades. Carbon inputs in soils come mainly from plants and thus these particular bare fallow plots can be considered as receiving no inputs of carbon. With time, biodegradation occurs and the soils get enriched in persistent carbon.

## 1.2. Long-term bare fallows

### 1.2.1. Concept

Long-term bare fallows are field experiments in which plots are kept free of vegetation. Samples of the plots are taken at regular intervals, air-dried and stored in archive collections. As the duration of the bare fallow increases, biodegradation occurs, the carbon content of the plots decreases and the samples get enriched in persistent carbon (see Figure 10) (Rühlmann, 1999). Although C inputs are not theoretically eliminated in bare fallow plots, due to atmospheric deposition, wind-blown particles and occasional weeds, such inputs can be considered as negligible as long as the bare fallow is suitably monitored and atmospheric deposition has been observed to be low.



**Figure 10: Conceptual dynamics of soil organic carbon in long-term bare fallows. Adapted from Chenu, personal communication.**

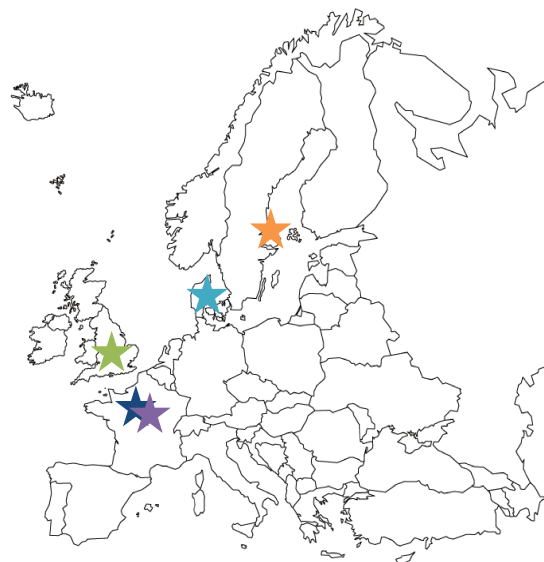
Bare fallows with different durations exist, and the possibility to access the kinetics of C has already been exploited in several studies. For instance, Kiem and Kögel-Knabner (2003) and Wagai et al. (2013) have studied bare fallows with durations up to 20 years.

### 1.2.2. European network

A network of five long-term bare fallows was established a few years ago, the five sites cover contrasted conditions with differences in soil textures, climatic conditions, carbon contents, history of the sites and duration of bare fallow (see Table 1, more detailed information on the sites can be found in Barré et al. (2010)).

Each site in the network fulfils the following criteria: 1) The experimental plots have been kept bare (vegetation-free) and without organic amendments for more than 25 years; 2) there have been several measurements of SOC in the

plough layer; and 3) there is a soil archive with soil samples taken at intervals since the establishment of the experiment. The five sites in the study are located at Ultuna (Sweden), Askov (Denmark), Rothamsted (United Kingdom) and Versailles and Grignon (France) (see Figure 11).



**Figure 11: Five sites of the LTBF Network. Light blue: Askov, dark blue: Grignon, green: Rothamsted, Orange: Ultuna, purple: Versailles.**

#### 1.2.2.1. Askov

The Askov bare fallow experiment started in 1956 on the Askov Lermarken site. Previous land use was cultivation (from 1800). The soil classifies as an Orthic Luvisol (FAO, 1989) of coarse sandy loam texture, the clay fraction is dominated by illite, smectite and kaolinite. The pH of the topsoil is kept at 5.5 to 6.5 by liming every 4 to 5 years. Soil bulk density is 1.50 g cm<sup>-3</sup>. The plot size of the Askov LTBF was 11.7m×9.4m (110m<sup>2</sup>), with eight replicates. The plots were kept free of vegetation by frequent tillage to 20 cm depth but received annual dressing of mineral NPK fertilizers. The experiment was terminated in 1985, and further details on the experiment can be found elsewhere (Christensen and Johnston, 1997, Bruun et al., 2003).

**Table 1: Selected characteristics of the five LTBF sites.**

	<b>Askov (DK)</b>	<b>Grignon (FR)</b>	<b>Rothamsted (UK)</b>	<b>Ultuna (SW)</b>	<b>Versailles (FR)</b>
Coordinates	55°28N 9°07 E	48°51N 1°55 E	51°82N 0°35 E	59°49N 17°38 E	48°48N 2°08 E
Starting date	1956	1959	1959	1956	1928
Last sampling for this study	1983	2007	2008	2009	2008
Duration (years)	27	48	49	53	79
Previous land use	arable	grassland	grassland	arable	grassland
Mean annual temperature (°C)	7.8	10.7	9.5	5.5	10.7
Annual precipitation (mm)	862	649	712	533	628
Soil texture and type	Coarse sandy loam Orthic Luvisol	Silty loam Luvisol	Flinty silty clay loam Chromic Luvisol	Clay loam Eutric Cambisol	Silty loam Luvisol
Texture (sand/silt/clay)	82/11/7	16/54/30	7/68/25	23/41/36	26/57/17
Sampling depth (cm)	20	25	23	20	25
Initial SOC (g kg <sup>-1</sup> )	17.1	13.5	29.0	15.7	20.0
Final SOC (g kg <sup>-1</sup> )	11.6	8.6	10.0	10.6	6.5

### 1.2.2.2. Grignon

The Grignon LTBF experiment started in 1959 in the gardens of the “Chateau de Grignon” (France). In this experiment, the bare fallow treatment was replicated 6 times in 6 blocks. Previous land use was unmanaged grassland since before 1875. The soil is a silty loam classified as Luvisol (FAO, 1989) and developed within a colluvial carbonated deposit, with pH (in water) of 8.0–8.3. The size of the plots is 3.2×3.2m<sup>2</sup>; all plots are dug by hand twice a

year to 25 cm depth, and are kept free from vegetation by hand weeding and herbicide treatment. Further details on the experiment can be found elsewhere (Houot et al., 1989).

### **1.2.2.3.Rothamsted**

The Rothamsted Bare Fallow was started in 1959 on a site which had been in managed grassland since 1838. The soil is a flinty silty clay loam classified as a Chromic Luvisol (FAO, 1989). The area consists of four sub-plots, each 12.5×7 m<sup>2</sup>. Soils were sampled in December 1959 (immediately before the site was first ploughed) to a depth of 23 cm. Last sampling session occurred in 2008. In December 1959, organic C in the soil was 29.0 gC kg<sup>-1</sup> and pH (in water) was 6.3; in 2008, organic C had declined to 10.0 gC kg<sup>-1</sup> and pH to 5.2. The site is kept free from weeds by ploughing/cultivating 2–4 times per year to 20– 22 cm depth; herbicides are used occasionally.

### **1.2.2.4.Ultuna**

The “Ultuna continuous soil organic matter experiment” was started in 1956, most probable previous land use is agriculture for the last few centuries. The soil has been classified as a Eutric Cambisol (Kirchmann et al., 1996). The experiment consists of 15 treatments replicated in four blocks, 60 plots in total. The size of each plot is 2×2 m<sup>2</sup>. Since 1956, all cultivation has been performed manually with a spade to 20 cm depth. Earlier time series of C data from the bare fallow as well as other treatments have been used for calibrating and validating different soil C models (Andrén and Kätterer, 1997, Hyvonen et al., 1996, Petersen et al., 2005).

### **1.2.2.5.Versailles**

The Versailles LTBF experiment forms part of the larger 42-plot experiment that was initiated in 1928 and is now kept by INRA. Located in the gardens of the 'Chateau de Versailles', this experiment was designed to study the effects of long-term application of fertilizers and amendments on the composition and physical properties of loess soils (Burgevin and Hénin, 1939). Previous land use was unmanaged grassland and forest before the 17th century. Ten of the 42 plots were used as bare fallow reference plots. The soil is a silty loam Luvisol (FAO, 1989), developed within the aeolian loess cover that characterizes the Paris Basin. In 1929 the soil had a pH of 6.4 and a CEC of 15.3 cmol+ kg<sup>-1</sup> (Cobaltihexamine method). Seventy years later (in 1999), the pH had decreased to 5.6 and the CEC to 8.7 (Pernes-Debuyser and Tessier, 2002). The size of the plots is 2×2.5 m<sup>2</sup>. All plots are dug by hand twice a year to 25 cm depth, and are kept free from vegetation by hand weeding and herbicide treatment.



### 1.2.3. Previous studies on the LTBF

#### 1.2.3.1. From the LTBF Network

Initial carbon concentrations are diverse, but soil organic C is decreasing with time at all sites (Barré et al., 2010) (see Figure 12). Previous history of the site (arable or grassland) has an impact on the dynamics of C, sites previously used for grassland showed steeper initial carbon declines whereas sites previously in arable cropping showed a more constant decrease in soil C during the years that followed introduction of bare fallow. During the 10 first years, the annual soil C decrease rates averaged less than  $1.6 \text{ gC kg}^{-1}$  for sites previously under arable cropping and were above  $3.5 \text{ gC kg}^{-1}$  for sites on former grassland. At the last sampling, between 32 and 68% of the initial SOC is lost. Observed decrease rates are in agreement with those observed in C3/C4 experiments (see next section of manuscript) and those found in models.

In their study using four sites of LTBF Network, Menichetti et al. (2014) have investigated the  $^{13}\text{C}$  signature of the organic carbon as bare fallow occurs. They have evidenced an increase of the  $\Delta^{13}\text{C}$  value in all sites, with mean annual enrichment ranging between 0.008–0.024 ‰. The dynamics of this increase were similar enough to suggest that site characteristics were not the main factor explaining this increase. Previous land use (arable or grassland) was detected to have an impact on isotope abundance and fractionation.

Using samples from 4 out of the 5 sites, Lefèvre et al. (2014) have shown that more persistent C (final samples) has a higher temperature sensitivity than total C (initial samples), as evidenced by the calculation of apparent activation energies for similar losses of  $\text{CO}_2$  during incubations at different temperatures. Their results provide strong evidence for a relationship between temperature sensitivity and protection of the soil organic carbon.

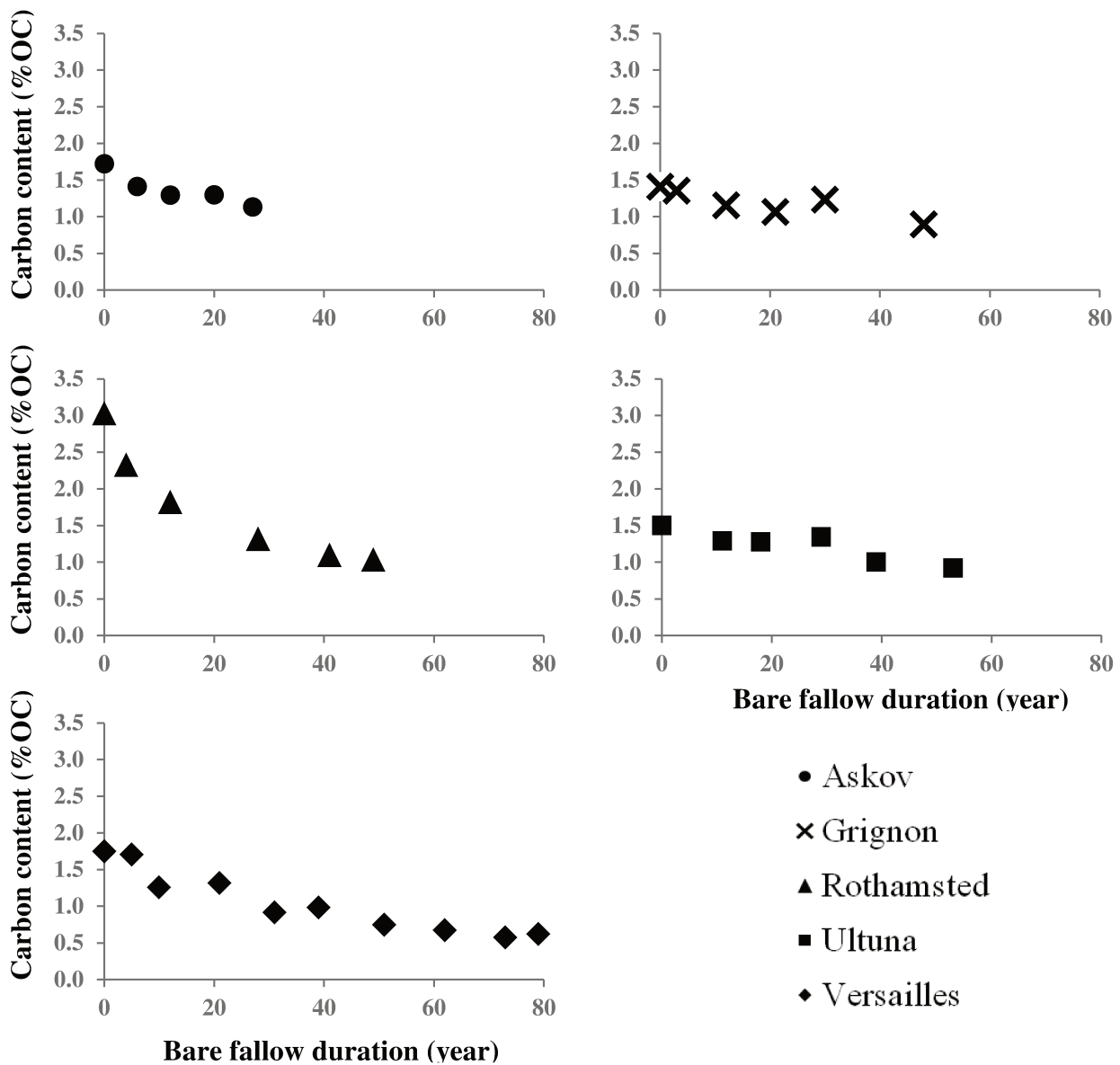


Figure 12: Carbon dynamics in the five sites of the LTBF Network

### 1.2.3.2. From the longest LTBF: Versailles

The longest bare fallow of the network is Versailles, with 79 years of treatment at the last sampling session (2008). This experiment has been extensively studied, as shown in the following studies: Plante et al., 2004, 2005, Balabane and Plante, 2004, Guenet et al., 2011, Guenet et al., 2012, Paradelo et al., 2013, Lutfalla et al., 2014. Persistent organic carbon in these plots has been found to contain no particulate organic matter and to be enriched in carbon associated with clays (Vasilyeva et al., in prep). Persistent SOC also displays a particular thermal behavior, it is less thermally labile than organic matter from an adjacent

cultivated field (Plante et al., 2005). Moreover, the chemical signature of the SOC as seen by Curie-point pyrolysis shows no particular enrichment in lipids (Grasset et al., 2009).

In terms of protection mechanisms, Balabane and Plante (2004) have shown that location in macroaggregates cannot explain the observed long-term persistence of organic carbon in these soils.

Priming effects on multidecadal persistent carbon have been investigated by Guenet et al. (2012) and have shown that persistent carbon is similarly sensitive to the addition of fresh organic material in bare fallows and in adjacent cultivated fields. This raises the question of the similarity of C dynamics between long-term bare fallows and vegetated soils. Making rough estimates of decomposition rates in the field from incubation data, they concluded that overall turnover rates of C were likely not very different.

Furthermore, another study by Guenet et al. (2011) confirmed the results of Hirsch et al. (2009) and found that in the LTBF of Versailles, after 79 years of BF, microorganisms are less abundant but have kept the same metabolic capacities than cultivated soils receiving fresh organic matter.



**Figure 13: Picture of the LTBF of Versailles (Photo by O. Crouzet).**

#### **1.2.4. Comparison with C3/C4 experiment and proof of concept**

Long-term bare fallows are the only technique which allow the isolation of a more persistent part of SOC. The price to pay for this isolation is high: no new carbon inputs mean no vegetation on the soil. The surface is therefore bare (see Figure 13) and, for a given location, it will experience harsher conditions than an adjacent planted soil: increased temperature during the hot months, colder temperatures in winter (albedo, and no cover from trees and plants), water will run differently along the soil profile as there are no roots, ... Some people have even referred to long term bare fallows as experiments which “starve” the soil (Hirsch et al., 2009). Without going as far, these experiments can be considered as caricatures. But they

are necessary because there are no other satisfactory options for the study of multidecadal carbon dynamics in soils. They can be considered a large scale incubation under field conditions and as such they already constitute an incredible asset. However, the applicability of the results obtained should be discussed.

One of the ways to verify if what we measure in these experimental plots represent in any way what would happen under “plant” conditions is to compare the degradation dynamics obtained in LTBF with dynamics obtained with other long-term field experiments that also give information about turnover and persistence of C in soils, namely C3/C4 chronosequences. In order for the results to be comparable, both experiments (LTBF and C3/C4) should be subject to the same conditions (soil type and climatic conditions).

The LTBF of Versailles is located next to a C3/C4 experiment. The adjacent Les Closeaux field experiment is a C3/C4 chronosequence which started in 1993 and consists in a series of chronosequences of C4 plants (maize) planted on a C3 soil. This study area, has thoroughly been described in Dignac et al. (2005), it consists of 32 research plots which were randomly defined in 1993. Before this time, the whole field had been cultivated with C3 plants only. Four control plots remained under winter wheat (0 year of maize) and four other plots were cultivated with maize since 1993. The rest was progressively converted to maize and will not be used for this comparison. The last sampling session took place in 2012, 19 years after the maize conversion and at a depth of 25 cm. Because the isotopic signatures of C3 and C4 plants are different, we are able to measure how much of the soil organic carbon present in 2012 comes from the initial carbon pool (C3 plants) and how much comes from the new input of C4 plants.

**Table 2: Carbon content and estimated mean residence time of TOC in soils which have undergone a C3/C4 vegetation change. Remaining C3-C was calculated from measuring the delta <sup>13</sup>C of TOC.**

Vegetation type and sampling date	Wheat 1993	Wheat 2012	Maize 2012
Duration with C4 plants	0	0	19
TOC (mgC g <sup>-1</sup> soil)	na	12.8 (0.3)	11.2 (0.4)
Remaining C3-C (mgC. g <sup>-1</sup> sol)	-	-	9.90 (na)
Decay rate of C3-C (mgC.g <sup>-1</sup> .y <sup>-1</sup> )	-	-	0.153
MRT (Init Vs Fin) (years)	-	-	<b>74</b>

**Table 3: Carbon content and estimated mean residence time of TOC in a LTBF soil (Versailles, France)**

<b>BF duration (years)</b>	<b>LTBF _ 0</b>	<b>LTBF _ 79</b>
Duration with C4	0	0
TOC (mgC g <sup>-1</sup> soil)	18.49	6.29
Rate of decay (mgC g <sup>-1</sup> . y <sup>-1</sup> )		0.154
MRT (Init VS Fin) (year)		<b>74</b>

Initial carbon contents differ for the two sites, the LTBF site has an initial C content of 18.49 mgC.g<sup>-1</sup> and the C3/C4 has an initial C content of 12.8 mgC.g<sup>-1</sup> (Table 2 and 3).

The first way of comparing the two sites is to calculate decay rates (Table 2 and 3), both are very similar with an average value of 0.153 and 0.154 mgC.g<sup>-1</sup>.y<sup>-1</sup> respectively. This provides first evidence for similar dynamics. The obvious limitation of such a comparison comes from the fact that calculating yearly decay rates relies on the hypothesis that we can model the evolution of TOC contents by (i) only looking at initial and final values, (ii) assuming that the decay of SOC is linear (constant each year), (iii) comparing dynamics over different durations (19 years for C3/C4, 80 years for LTBF).

However, as previously mentioned in this manuscript, carbon turnover in soils is not linear, simplest models rely on mono exponential decays (first order kinetics). This approach allows us to compare different durations as they take into account this variation of the decay rate with time. Comparing Mean residence times obtained with one pool model of exponential decay (initial vs final) (Table 2 and 3), we obtain similar MRT -74 years for both C3/C4 and LTBF). Additionally, when we take into account intermediate dates -one intermediate date for C3/C4 and three for LTBF- we also find similar MRT (data not shown).

These comparisons provide evidence that long-term bare fallows, though they are caricatures of soil systems, display similar dynamics to those observed in cultivated environments. As such, they constitute a unique opportunity to study the mechanisms explaining long-term persistence of carbon in soils.

## 1. Presentation of the analytical tools

As previously explained, Soil organic carbon is contained in a complex and heterogeneous mixture of organic material intimately bound with mineral phases. This renders its characterization arduous. Numerous techniques have been used to decipher the chemical structure of SOC, each only yield partial information on the precise composition of SOM.

Different analytical approaches have been developed, a non-exhaustive list includes:

- spectroscopy (NMR, Raman, FTIR, X-ray fluorescence) which gives information on the nature of the chemical functions,
- mass spectrometry which provides molecular identification of complex soluble organic mixtures,
- secondary ion mass spectrometry (SIMS) gives insights on spatial elemental and isotopic analysis,
- degradation followed by spectrometry (Py-GC-MS for instance) provides information on the molecules present through the cleavage of SOM.

Methods such as Nuclear Magnetic Resonance (NMR), Fourier Transform Infra-red spectroscopy (FTIR), Pyrolysis coupled with gas chromatography and mass spectrometry (Py-GC-MS) have been extensively used to study soil organic matter. They have limitations which have been highlighted in several studies (Poirier et al., 2005). One of the limitations of these techniques is the influence of the mineral matrix (so called “matrix effects”) on the data obtained. NMR signals can be hampered by the presence of paramagnetic compounds (such as Iron or Copper) (Kögel-Knabner, 1997). FTIR displays both the information about the minerals and the OM of a sample (Haberhauer et al., 1998, Jordá et al., 2015). Thermal treatments such as pyrolysis look promising for some aspects (reviewed in Derenne and Quénéa, 2015) however several important limitations exist. For instance, the cracking of OM during Py-GC-MS can be influenced by the mineral matrix of the soil and can create cross-linked polymeric structures which were not present in the initial sample (Schulten et al., 1996, Huang et al., 1998).

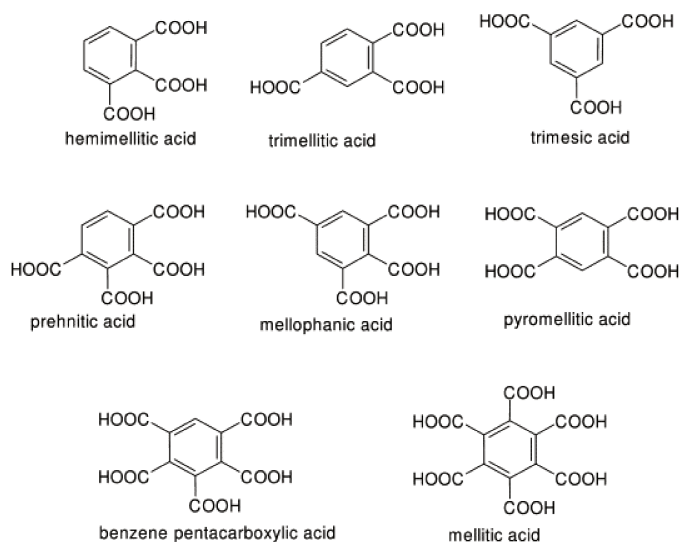
This section of the manuscript will present the main techniques which have been used in this research project: the benzene polycarboxylic acids (BPCA) method to characterize the pyrogenic material contained in LTBF samples, Near-edge X-ray absorption fine structure (NEXAFS) spectroscopy to elucidate the chemical signature of SOM in LTBF samples,

Scanning transmission X-ray microscopy (STXM) coupled with NEXAFS spectroscopy to get information on both the chemical nature and the localization of organic matter and mineral material.

### 1.1. Studying pyrogenic carbon with the BPCA method

Pyrogenic material is ubiquitous in soils, it comes from natural or man-made fires. Studying it is complicated, many different techniques exist and they have been well presented in the review paper by Hammes et al. (2007). In this work, we have chosen to use the benzene polycarboxylic acids (BPCA) method to study the pyrogenic material in long-term bare fallow samples.

It is based on the creation and further analysis of molecular markers of pyrogenic carbon, the



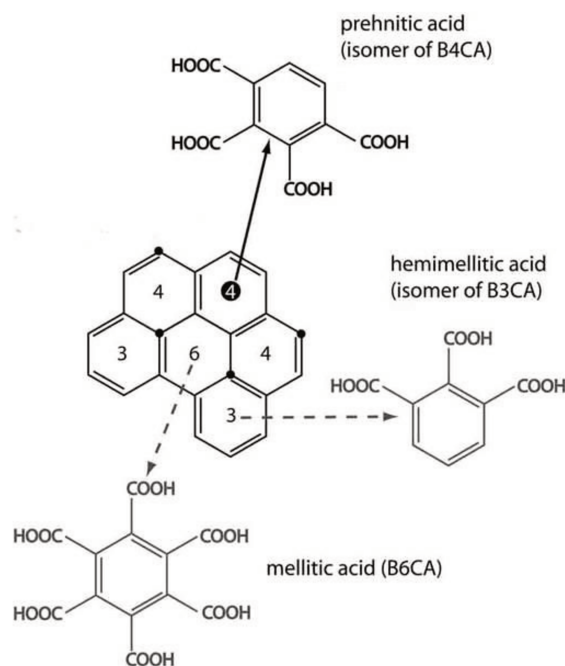
Benzene PolyCarboxylic Acids (BPCA) (see Figure 14 for chemical composition). This method was first developed by Glaser et al. (1998) and has been progressively improved by Schneider et al. (2011b) and Wiedemeier et al. (2013). It now consists in an acid attack on milled soil samples with nitric acid (under pressure and at 170°C for 8h) the BPCA are created at this stage and the next steps aim at purifying the mix to make a chromatography analysis

**Figure 14: 8 BPCAs are created and analyzed during the BPCA analysis of PyOC. Source: Brodowski et al. (2005).**

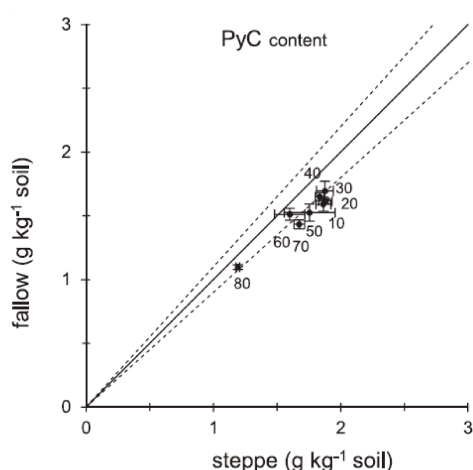
possible (with an HPLC). The nitric acid breaks down the pyrogenic matrix and creates monomers of the benzene arrangements (see Figure 15) which are classified according to the number of acid moieties linked to the benzene ring. From less substituted to more substituted, we find the B3CAs –hemimellitic, trimellitic and trimesic BPCA-, the B4CAs -prehnitic, mellophanic, pyromellitic BPCA-, one B5CA –benzene pentacarboxylic acid- and one B6CA –mellitic acid. This yields a large variety of BPCAs and the number of COOH groups on each benzene ring indicates how many other rings a particular ring was originally connected to (Dittmar, 2008). This method therefore gives information on the quality of the PyOC material (Schneider et al., 2010, Wiedemeier et al., 2013, Wiedemeier et al., 2015, Ziolkowski et al., 2011).



Like other methods, BPCA has limitations. The first drawback is presented in Glaser et al. (1998), it is based on the fact that it is a destructive method: in order to create one BPCA monomer, at least 5 benzene units have to be destroyed. There is therefore a consistent underestimation of the quantity of PyOC material in the samples. A corrective factor has been proposed by Glaser et al. but will not be used in this work as it is not proven that it renders the calculations of exact PyOC content accurate (Brodowski et al., 2005). Other studies have found that BPCA could be artificially created during the procedure or originate from melanoidin or other non PyOC material (Glaser and Knorr, 2008, Allard et al., 1997). These artifacts have been attributed to the different pre-treatment steps (Brodowski et al., 2005, Dittmar, 2008) which have been removed in the recently revised method.



**Figure 15: Creation of individual BPCAs during the acid attack. (Source: geo.uzh.ch).**



**Figure 16: PyOC content in the fallow soil and in an adjacent steppe (adapted from Vasilyeva et al., 2011).**

Few studies using the recently revised method have been published (Wiedemeier et al., 2013). Soil samples have been studied with the previous method presented in Schneider et al. (2011b). For instance, Schneider et al., (2010) have looked at the changes in the distribution of the different BPCA molecular markers induced by the change in temperature used to pyrolyse wood. They found that with increasing pyrolysis temperature (from 200°C to 1000°C) the proportion of B6CA increased, probably reflecting the increased condensation of the material. Using a space-for-time approach, Schneider et al. (2011) found no significant changes in the quality of pyrogenic material with time (increasing duration since last fire input). They also did not detect any significant changes in the quantity of PyOC but acknowledged the fact that this lack of evolution could be due to the large spatial heterogeneity of PyOC stocks in the plots



they studied. Vasilyeva et al. (2011) have found that a lack of vegetation does not interact with the persistence of PyOC in soils. This study compared a 55 years bare fallow soil to soil from an adjacent native grass steppe. Fire history was identical for both soils and it was found that changes in PyOC contents were similar for long-term bare fallow and vegetated soil (Figure 16). In all of these studies, the stability of PyOC is attributed for a large part to the organomineral interaction of the pyrogenic material and the mineral surfaces.

## 1.2. New imaging and spectroscopy techniques

In the past decade, new techniques have emerged that allow the characterization of SOM. More precisely, imaging techniques have been developed and submicron images are now available to an ever increasing number of soil scientists. These techniques have been presented in Rennert et al. (2012). They are giving new insights on the mechanisms of the persistence of SOC. NanoSIMS for instance can give information on the chemical and isotopic composition of both organic and mineral materials (Slodzian et al., 1992) without disrupting their spatial organization (Herrmann et al., 2007) and is only starting to be used in soil science (Remusat et al., 2012, Mueller et al., 2012, Hatton et al., 2012).

Synchrotron-based spectroscopies have also been adapted to study environmental samples. The advantage of these techniques comes from the fact that soil samples can be studied without major pretreatment and extraction. And contrary to most other methods, they are relatively sensitive and display good resolution. One drawback is that although these techniques can give good results concerning the gross chemical composition and the distribution of functional groups, individual molecules cannot be identified.

### 1.2.1. NEXAFS Bulk

#### **Concept**

Near-edge X-ray absorption fine structure (NEXAFS) spectroscopy is also called X-ray absorption fine structure (XANES), it gives information on the functional groups within the organic matter by enabling the study of the carbon and nitrogen atoms (among others). This method belongs to the X-ray absorption spectroscopies (XAS) which probe the local bonding and coordination environment of elements in whole samples. Bulk XAS techniques permit for high throughput, the study of whole soils, and high sampling density.

It is based on the excitation of core-level electrons to unoccupied or partially occupied orbitals, the method analyses the phenomena associated with this excitation to get information on the chemistry of the element probed: absorption of energy, fluorescence, emission of

photon (Hitchcock et al., 2008). The method has been developed in the 1980s and first uses to characterize SOC date from the mid-1990s (Kirz et al., 1995, Urquhart and Ade, 2002). Since the core electron energies of different elements are unique, specific elements can be probed in a complex sample by scanning the X-ray beam energy across the binding energies of the core electrons in the elements of interest. Promotion of these core level electrons into unoccupied (i.e., valence) electron orbitals occurs when the incident X-ray energy coincides with an accessible transition energy, resulting in the resonant absorption of the X-ray photons. Observations of subtle changes in the resonant absorption energies can be used to differentiate oxidation states, bonding environments, and neighboring atoms. By looking at the energy corresponding to the excitation of carbon atoms (around 290 eV), the method classifies carbon into functional groups based on spectral features corresponding to specific compound classes.

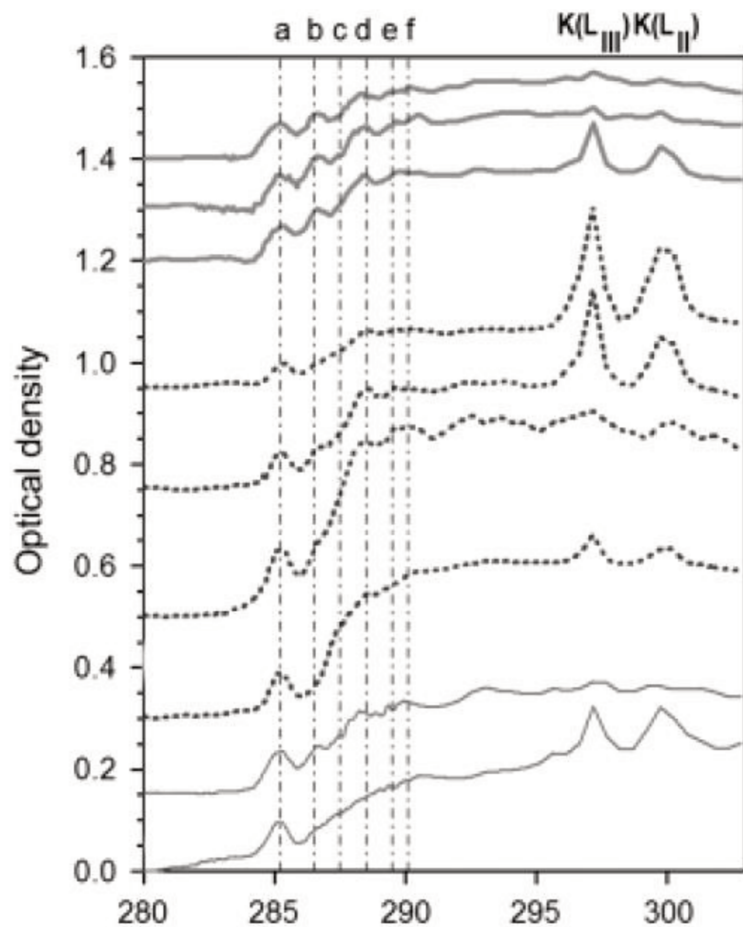
This technique allows one to characterize *in situ* soil organic matter without extraction. Soil organic matter extraction techniques are controversial, and may cause alterations and artifacts. It is also not necessary, to enrich organic matter concentrations by separating it from the mineral fraction or to digest samples in hydrofluoric acid (HF) to remove minerals, paramagnetic materials, or other components for the analysis to proceed. One of its limitations is that it is only semi quantitative (in that regard it is similar to NMR, Baldock et al., 2004).

### **Selected results**

Since 1995, many studies have been carried out using NEXAFS spectroscopy to gain insights on the chemistry of SOM.

Lehmann et al. (2008) showed that different soils had similar chemical signatures. Samples from five very different soils in New Zealand, USA, Brazil, Kenya and Panama showed almost identical spectral features. NEXAFS data has also evidenced subtle but persistent changes of SOC functional groups as a result of cultivation (Solomon et al., 2005, 2007). Using NEXAFS and a combination of other methods, Kleber et al. (2011) showed that there was no correlation between age and chemistry of SOM. They compared the chemistries of OM which had different ages and showed that the oldest OM displayed less aromatic groups than the younger OM, even though aromatic structures are usually considered very persistent in soils.

Looking at subarctic soils, Gillespie et al. (2014) showed that buried soil containing high levels of C contained C resisting decomposition because of chemical modification of the C. A particular accumulation of ketone moieties and the loss of carbohydrates, phenolic and carboxylic compounds were detected by NEXAFS spectroscopy of buried horizons. The authors use this finding to propose that ketone be used as biomarkers for microbially transformed SOM. In the same study they find that SOM in permanently frozen mineral soils was more susceptible to biodegradation than that in buried mineral and organic soils in the annually frozen active layer. And, in accordance with their previous finding, the C signature of these permanently frozen horizons did not show ketone signals but instead showed strong phenolic content.



**Figure 17: NEXAFS spectra of nanoaggregates taken from the A horizons of an Inceptisol (adapted from Kleber et al. 2011). Functional groups: (a) aromatic, (b) phenolic, (c) aliphatic, (d) amide and carboxyl C, (e) O-alkyl, (f) carbonyl groups.**

In another study, Gillespie et al. (2013) found that long-term N addition to soils resulted in an enrichment in proteinaceous compounds. In comparison, soils which did not receive N addition displayed a different chemical signature and were specifically enriched in plant-derived compounds such as aromatics, phenolics, carboxylic acids and aliphatic compounds. They conclude that it suggests that the decomposition of plant residues is limited by nitrogen availability. Kleber et al. (2011) have investigated the NEXAFS signature of nanoaggregates from different soils (Figure 17). They have grouped the spectra in three different types (contrasted spectral features) and attributed potential origins for the OM: kerogen-like material and organic material during the first stages of decomposition, and material progressively decomposed (more and more dominated by microbial characteristics).

### 1.2.2. STXM/NEXAFS

Not only is the chemical signature of OM important but also its spatial distribution, and its specific association with minerals. Hence, there is a need to directly investigate the associations of OM with specific minerals in natural soils. However, this is challenging because there are few techniques that can simultaneously characterize soil organic matter and soil minerals. High spatial resolution and state-of-the-art spectroscopic techniques can provide such insights. Scanning Transmission X-ray Microscopy (STXM), combined with Near Edge X-ray Absorption Fine Structure spectroscopy (NEXAFS) is a powerful method for analyses of C functional group distribution in soils at the submicrometric scale (Lehmann et al., 2008, Wan et al., 2007, Schumacher et al., 2005). This method can also detect and analyse mineral parts of the sample, depending on the energy range of the synchrotron beamline.

#### **Concept**

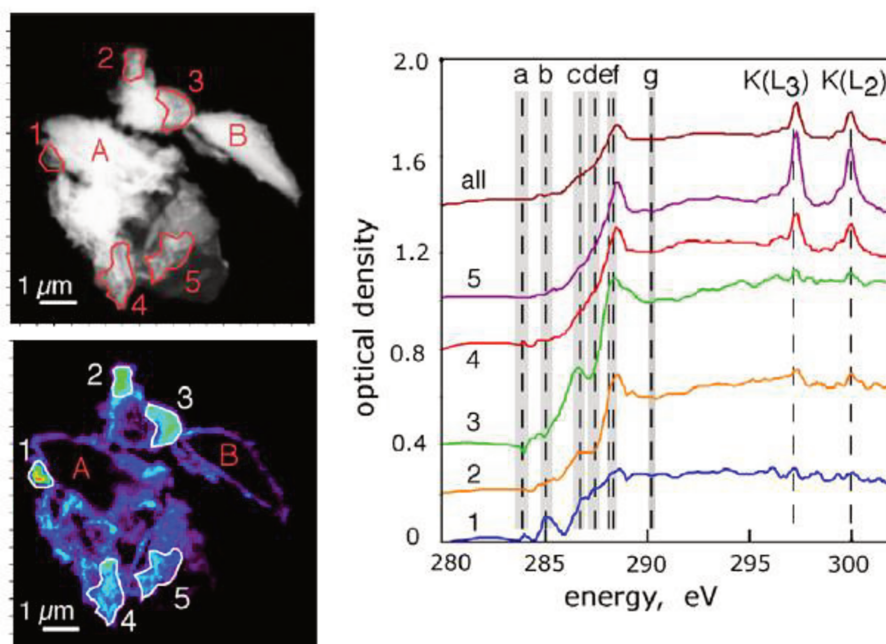
Scanning transmission X-ray microscopy is a synchrotron-based technique that allows the visualization and chemical mapping of thin samples. The transmission image of the X-ray has a spatial resolution of a few tens of nanometers. The information on the type of element present is inferred from the characteristic energy levels of atoms (Thieme et al., 2007, Lehmann et al., 2009, Thieme et al., 2010). When it is coupled with NEXAFS, depending on the energy range which is used, it can give information on both the chemical nature and the localization of organic matter and mineral material.

In a nutshell, this technique is spatially resolved (around 10 nm resolution), does not damage too much the sample (little beam damage), allows to study all “low energy” elements (Carbon, nitrogen, sulphur, oxygen...), does not require pretreatment that will alter the information, is not destructive of the sample (possible to use several method on the same sample for cross confirmation) but has the problem of “the needle in the haystack” (how can it be representative).

#### **Selected results**

The combination of these two methods has allowed detecting SOM at submicrometric scales, either under the form of discrete particles or as coatings on soil mineral particles. Distinct chemical signatures of SOM have also been detected in a same sample. Kinyangi et al. (2006) have studied micro and nanoaggregates isolated from a Kenyan Oxisol and were able to characterize two types of OM: the OM located on the exterior of the aggregate, adsorbed to

the mineral surface, displayed spectral features corresponding to oxidized material (carboxylic moieties) whereas the interior of the aggregate contained more diverse OM which mainly corresponded to aromatic and aliphatic functional groups and which was attributed a less processed, more plant-derived origin. The SOC coating minerals contained more carboxylic groups whereas poral SOC seemed more aromatic and aliphatic. Increased proportions of carboxylic groups for SOC bound to minerals was also reported in Lehmann et al. (2007). Additionally, Solomon et al. (2012) and Wan et al. (2007) have shown that the heterogeneity of the distribution of OM also depends on the mineral phases it is absorbed on (Figure 18). Chen et al. (2014) have detected strong correlations between Ca and OM, evidencing a preferential collocation of calcium and OM. The calcium did not come from carbonates and they conclude that calcium plays a strong role in the formation of organomineral associations at the nanoscale. Lehmann et al. (2008) provided first insights on the stark contrast between similar chemical signatures at the bulk scale (NEXAFS spectroscopy) and large spatial variability at the submicrometer scale. Another study, carried out by Keiluweit et al. (2012), looked at the association between nitrogen-rich microbial compounds and mineral surfaces. They found that nitrogen rich compounds could coat mineral particles such as iron oxides and hydroxides relatively quickly. Moreover, the chemical signature of the carbon in their samples seems to indicate a strong microbial origin of the OM associated with minerals. Their results seem to indicate that certain chemical compounds are selectively preserved through their adsorption on mineral surfaces (aliphatic compared to aromatic moieties for instance).



**Figure 18: Example of Carbon NEXAFS spectra obtained from different regions of a microaggregate (cambisol) and the average of the spectra for the whole sample. Regions A and B are too thick and no transmission signal was retrieved. Labeled regions correspond to the numbered spectra on the right. Six functional groups are highlighted: (a) quinonic, (b) aromatic, (c) phenolic, (d) aliphatic, (e) peptidic, (f) carboxylic, (g) carbonates. The potassium peaks are also detected. From Wan et al. (2007).**

## 2. Outline of the work

The goal of this PhD project is to characterize multidecadal persistent carbon and to investigate the mechanisms responsible for this persistence. Using appropriate analytical tools and unique samples coming from long term-bare fallows we hope to contribute to a better characterization of SOM which can improve model developments and further our fundamental understanding of mechanisms that explain the long-term persistence of C in soils.

The first part of the manuscript aimed at introducing the research project and placing it in a global context.

The second part of the manuscript introduced the experimental design that enables us to study persistent carbon and the analytical tools that were used to characterize persistent carbon.

The third part of the manuscript seeks to investigate the chemical nature of persistent carbon and gives insights on the relatively small importance of chemical recalcitrance.

The first section of the third part of the manuscript contains work that has been done in order to test the efficiency of chemical oxidations in isolating a persistent pool of carbon. By using initial and final samples from the longest bare fallow of the Network (Versailles) and carrying out chemical oxidations we wanted to see the effect of chemical oxidation on both. By testing this we wanted to investigate the efficiency of oxidation methods to isolate centennial soil organic carbon, but also to see whether it could be isolating a much more persistent pool of carbon (mean residence time of several centuries or millennia).

The second section of the third part presents the chemical evolution of soil organic carbon in LTBF. We used samples from four sites of the LTBF Network and analyzed them with synchrotron spectroscopy (NEXAFS at the SGM beamline, CLS, Canada). Our aim was (i) to see whether persistent carbon had a specific chemical signature and (ii) to characterize the chemical evolution of organic carbon as biodegradation occurs.

The third section of this part of the manuscript contains the results of our work carried out to specifically investigate the long-term persistence of Pyrogenic carbon. We used samples coming from the five sites of the LTBF Network and analyzed them with the BPCA technique (University of Zurich, Switzerland). Our goal was to see if Pyrogenic material was particularly persistent on multidecadal timescales.

The fourth section of this third part of the manuscript presents the result of our in-depth study of the persistence of pyrogenic carbon in the longest bare fallow (Versailles). We wanted to understand what was driving the apparent short persistence of PyOC detected in the previous section of the work. We analyzed the pyrogenic carbon content in particle size fractions of the soil over the whole duration of the bare fallow. Our goal was to see if pyrogenic carbon displayed the same dynamics and the same characteristics in the coarse fraction (sand size) and in the fine fraction (clay size) of the same soil.

The fourth part of this PhD project seeks to characterize organomineral interactions. Three clay size fractions with contrasted mineralogies were isolated from samples of the longest bare fallow of the LTBF Network (Versailles). Our goal was to isolate different minerals contained in the same soil in order to study the dynamics of the organic matter associated with these different mineral fractions. We carried out elemental and synchrotron analyses (NEXAFS and STXM/NEXAFS) in order to characterize both the carbon speciation and the type of organomineral associations present in the clay subfractions as well as their evolution over the duration of the bare fallow.

The fifth part of the PhD project draws the conclusions of the different parts and proposes perspectives for further research.





## PART 3: CHEMICAL SIGNATURE OF THE EVOLUTION OF SOM TOWARDS MORE PERSISTENT COMPOUNDS

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## 1. Are chemical oxidation methods relevant to isolate a soil pool of centennial carbon?

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### Abstract

Despite its relevance to long term carbon sequestration in soils, there is to date no successful experimental way to isolate all the stable pool of soil organic carbon (SOC), i.e., SOC that has a residence time of centuries to millennia.

Long term bare fallows (LTBF) offer a unique opportunity to study stable SOC, as without carbon inputs and with continuing biodegradation and mineralization, SOC becomes progressively enriched in its most stable components. Here, we took advantage of the 42-plots LTBF experiment of Versailles (France), where C inputs stopped 80 years ago, to test the relevance of chemical oxidation methods to isolate a pool of carbon stable at the centennial timescale. To do so, we studied the effect of two oxidizing reagents -hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>, and sodium hypochlorite, NaOCl- on soil total organic carbon (TOC) content after 0 and 79 years of LTBF. If these methods can isolate centennial stable C, then chemical oxidation resistant-C from 0 and 79 years of LTBF should equate, in amount, with TOC after 79 years of LTBF.

Results showed that chemical oxidation strongly decreased TOC in both soils. Oxidation-resistant OC accounted for ca.1 mgC.g<sup>-1</sup> soil, which is five times smaller than TOC in the untreated LTBF<sub>79</sub> soils, after 79 years of biological oxidation. Moreover, the amount of oxidation-resistant OC was significantly lower in LTBF<sub>79</sub> compared to LTBF<sub>0</sub>. We conclude that neither centennial stable carbon nor older stable carbon can be successfully isolated in representative amounts by chemical oxidation methods.

### Keywords

Soil organic matter, bare fallow, stable carbon, stabilization, chemical oxidation

### Abbreviations

INRA: Institut national de la recherche agronomique; LTBF: Long Term Bare Fallow; SD: standard deviation; SOC: soil organic carbon; TOC: total organic carbon.

## 1.1. Introduction

$^{14}\text{C}$  measurements (e.g. Trumbore et al., 1997, Jenkinson et al., 2008) and long-term C3/C4 vegetation changes (Balesdent et al., 1988) showed that there is SOC with turnover times of hundreds of years to millennia in soils, often called stable carbon, which represents a significant part of total SOC. To date, there is still no successful experimental way to isolate this stable pool of SOC, although it is crucial as it would enable soil scientists to better understand the stabilization mechanisms of soil organic matter which would then help in predicting the effect of climate or land use changes on SOC dynamics. Isolating this stable pool of carbon would also be useful for SOC dynamic models, as experimental results on its size and nature would enable a better parameterization of the stable C compartment size in models such as RothC and Century (Helfrich et al., 2007, von Lützow et al., 2007, Falloon and Smith, 2000).

Different experimental techniques have been developed to isolate and quantify the stable carbon pool: acid hydrolysis, physical fractionation, photo-oxidation, chemical oxidation, thermal treatments (e.g., Bruun et al., 2008, Jagadamma and Lal, 2010). In this paper we focus on chemical oxidation methods which are said to be the most efficient way to isolate OM with long turnover times as they can supposedly imitate the natural microbial oxidative processes which happen during biodegradation of SOC (Helfrich et al., 2007). Two widely used chemical oxidizing agents were selected: hydrogen peroxide –  $\text{H}_2\text{O}_2$ – (Eusterhues et al., 2005, Righi et al., 1995) and sodium hypochlorite –  $\text{NaOCl}$ – (Zimmermann et al., 2007, Siregar et al., 2005, Mikutta et al., 2005b, Mikutta and Kaiser, 2011). Results on the efficiency of such methods are contrasted as some studies measured older radiocarbon ages for OC resisting oxidation (Theng et al., 1992, Kleber et al., 2005, Helfrich et al., 2007, Jagadamma et al., 2010) whereas others did not isolate older OC (Bruun et al., 2008, Balesdent, 1996). Furthermore, the ability of these methods to quantify a stable OC pool, i.e. to isolate the whole fraction of centennial to millennial organic matter and not a subset of it, was not determined.

A recent study showed that long term bare fallows (i.e., vegetation free fallows) offer a unique opportunity to study the stable compartment of SOC (Barré et al., 2010), as without carbon inputs and with continuing biodegradation and mineralization, SOC becomes progressively enriched in its most stable components (Rühlmann, 1999). Using first order kinetics, it was shown that in the INRA 42 plots experiment that was initiated in 1928 in

Versailles, more than 80 % of total SOC is stable (Barré et al., 2010) at the centennial timescale.

The goal of this paper was to test the efficiency of chemical oxidation in isolating and quantifying a pool of stable carbon, using two widely used oxidizing agents:  $H_2O_2$  and  $NaOCl$ . We studied the amount of C remaining after these chemical oxidations on soils sampled in the same experimental plots at different stages of LTBF: at the beginning of the experiment (LTBF<sub>0</sub> samples) and after 79 years of LTBF (LTBF<sub>79</sub> samples). If these chemical methods were efficiently isolating a centennial carbon pool, then oxidized-LTBF<sub>0</sub> samples should contain roughly 80% of the carbon present in untreated-LTBF<sub>79</sub> samples. Furthermore, the oxidation treatment should only remove about 20% of the C of LTBF<sub>79</sub> samples (Figure

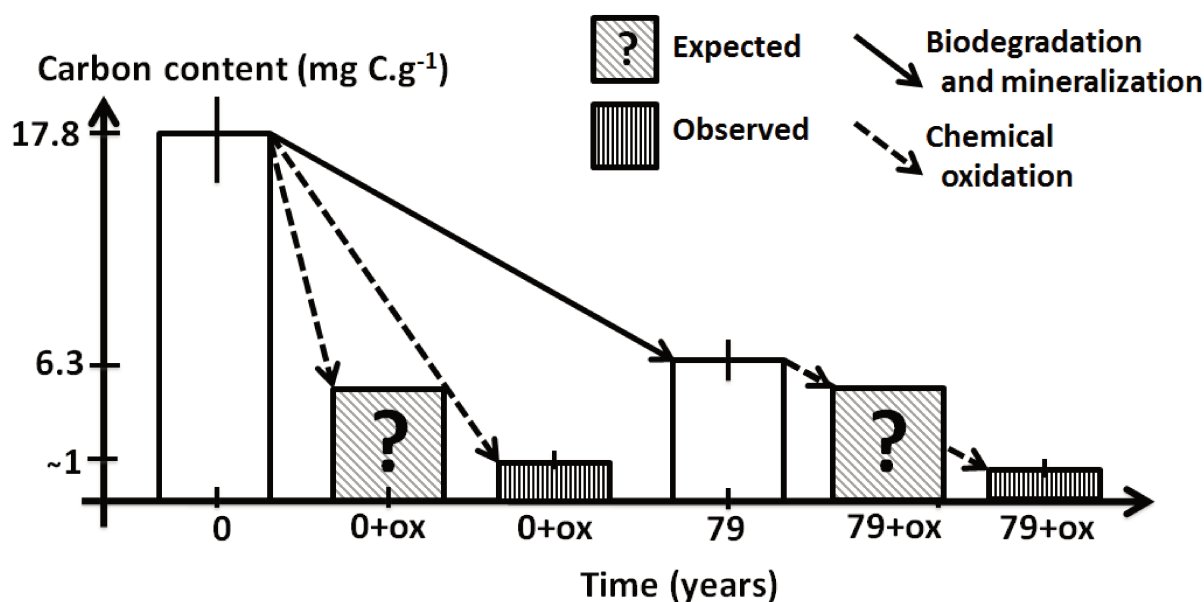


Figure 19 : Effect of chemical oxidation on soils from a LTBF: expected results and obtained results (both reactants lead to similar results).

19).

## 1.2. Materials and methods

### 1.2.1. Site description

The INRA (Institut National de la Recherche Agronomique) 42 Plots experiment is a long term bare fallow experiment which was started in Versailles (France) in 1928 to test the impact of fertilizers and amendments on soil structure (Burgevin and Hénin, 1939). Here, we

focused on 3 of the 10 control plots. Since 1929, the soil has not been cropped and has been weeded (by hand or with the help of herbicides) and plowed (to the depth of 25 cm) twice a year. It is a non-carbonated brown soil (silty loam Luvisol: 16 % clay, 57 % silt, 27 % sand) and the control plots have an average pH value of 6.3 in 1929 and of 5.2 in 2008 (Pernes-Debuyser and Tessier, 2002, and Paradelo et al., 2013).

We studied soils from the beginning of the experiment, sampled in 1929 at 0-25 cm depth and archived since then, which will be referred to as LTBF<sub>0</sub>, and soils from the last sampling session in 2008 (0-20 cm), after 79 years of bare fallow, which will be referred to as LTBF<sub>79</sub>. We used samples issued respectively from plots 13, 30 and 32 as replicates.

### 1.2.2. Soil sampling and processing

#### Soil fractionation

Decomposing plant debris (particulate organic matter) is a fraction that has a strong impact on SOC characteristics, and decays within 2 to 3 decades. In order to focus on SOC with longer residence time we discarded it through physical fractionation to study the finest fraction (particles < 50 µm). In LTBF<sub>79</sub> soils, the plant debris have disappeared therefore most of the SOC is contained in the fraction < 50 µm.

For each of the three replicates of LTBF<sub>79</sub>, 20 g of bulk soil were fractionated using a wet sieving protocol (Balesdent et al., 1991). The soils were shaken in 100 mL of deionized water with 10 glass beads for 16 hours, which allows complete dispersion of > 50 µm aggregates. The samples were then sieved through two successive sieves, one with a 200 µm mesh and the other with a 50 µm mesh. For each of the three replicates of the LTBF<sub>0</sub> samples, the same fractionation protocol was followed but with 5 g of bulk soil, 25 mL of deionized water and 5 glass beads. The size fraction less than 50 µm diameter was freeze-dried and weighed. Mass balance of the fractionation was around 99.5 % of recovery, which is consistent with published results (Balesdent et al., 1998).

#### Soil analyses

##### *H<sub>2</sub>O<sub>2</sub> oxidation*

H<sub>2</sub>O<sub>2</sub> treatment followed the method of Jagadamma et al. (2010). For each sample, 1g of the fraction <50 µm was wetted with 10 mL of deionized water, and 30 mL of H<sub>2</sub>O<sub>2</sub> (10 % weight) was added. The mixture was heated at 50°C with a water bath and left to react during 2 to 3 days with agitation. Once cooled, the reaction batch was centrifuged at 2500g for 15

minutes. The supernatant is discarded and the solid residue was kept. Oxidation was repeated twice more. At the end of the third oxidation, the solid residue was washed three times with 40 mL of deionized water, centrifuged, freeze-dried and weighed.

#### *NaOCl oxidation*

The protocol followed the method of Bruun et al. (2008) with a solution of 6% NaOCl but with higher centrifugation speed (2500g instead of 1400g). For each sample, 1g of the fraction < 50  $\mu\text{m}$  was mixed with 250 mL of NaOCl (concentration of 6 % in weight). The pH was adjusted to pH=8 with 37 % concentrated HCl (+/- 10 drops). The reaction occurred at room temperature, for 6 hours under agitation. The suspension was then centrifuged at 2500g for 15 minutes; the solid residue was separated from the supernatant for another round of oxidation. The oxidation was repeated twice more. After the third oxidation, the solid residue was treated the same way as for H<sub>2</sub>O<sub>2</sub> oxidation.

#### **1.2.3. Elemental analysis**

The organic carbon content of samples was measured by dry combustion in a CHN autoanalyser (Carlo Erba NA 1500). The soil contains no carbonates, so total C contents are equivalent to total organic carbon contents.

#### **1.2.4. Statistical analysis**

The statistical analyses were conducted using the free software environment for statistical computing R (<http://www.r-project.org>). The comparison between C remaining after H<sub>2</sub>O<sub>2</sub> and NaOCl treatments on soil coming from the same plot and sampled at the same date was assessed using pairwise t-tests. The comparison of C remaining after oxidation treatments on LTBF<sub>0</sub> and LTBF<sub>79</sub> soils sampled on the same plot was assessed using pairwise t-test. A level of significance of P = 0.05 was considered for all analyses.

### **1.3. Results and discussion**

As can be seen in Table 4, there was more carbon remaining for H<sub>2</sub>O<sub>2</sub> treated samples than for NaOCl treated samples, showing that NaOCl systematically oxidized more carbon than H<sub>2</sub>O<sub>2</sub>. The levels of carbon removal for soils treated with H<sub>2</sub>O<sub>2</sub> were consistent with previous studies using the same method (Plante et al., 2004, Grasset et al., 2009, Helfrich et al., 2007, Jagadamma and Lal, 2010), whereas NaOCl oxidation seemed to remove more carbon than previous results published by Bruun et al. (2008). Differences in soil texture (sandy vs loamy) or POM content could explain the differences in carbon removals. Other studies using NaOCl



used less oxidizing conditions and consistently obtained a larger resisting pool of carbon (Jagadamma et al., 2010, Mikutta et al., 2005a).

**Table 4 : Carbon contents before and after chemical oxidations with H<sub>2</sub>O<sub>2</sub> or NaOCl**

Treatment	Carbon content (mgC g <sup>-1</sup> fraction)					Carbon remaining after the treatment	% of initial LTBF <sub>0</sub> carbon
	Plot 13	Plot 30	Plot 32	mean	SD	%	%
<b>LTBF<sub>0</sub></b>	19.417	15.83	18.194	<b>17.82</b>	1.82	100	100
		4					
<b>LTBF<sub>0</sub>+H<sub>2</sub>O<sub>2</sub></b>	2.016	1.426	1.540	<b>1.66</b>	0.31	9.3	9.3
<b>LTBF<sub>0</sub>+NaOCl</b>	1.261	1.057	1.064	<b>1.13</b>	0.12	6.3	6.3
<b>LTBF<sub>79</sub></b>	6.788	5.340	6.790	<b>6.31</b>	0.84	100	35.4
<b>LTBF<sub>79</sub>+H<sub>2</sub>O<sub>2</sub></b>	1.295	0.884	0.952	<b>1.04</b>	0.22	16.6	5.8
<b>LTBF<sub>79</sub>+NaOCl</b>	0.983	0.774	0.910	<b>0.89</b>	0.11	14.1	5.0

As was expected, more carbon was removed from LTBF<sub>0</sub> soils than from LTBF<sub>79</sub> soils (Table 4), as LTBF<sub>0</sub> soils are supposedly richer in labile carbon than LTBF<sub>79</sub> ones, after 79 years of biodegradation. If oxidation methods had efficiently isolated a centennial stable pool of carbon, i.e. isolated all SOC with centennial turnover times, then oxidized-LTBF<sub>0</sub> samples would have contained roughly 80% of the carbon present in untreated-LTBF<sub>79</sub> samples. But such was not the case, oxidized-LTBF<sub>0</sub> samples had carbon contents ranging from 1.13 to 1.66 mgC g<sup>-1</sup> (depending on the reactant), which is 18-26% of the LTBF<sub>79</sub> carbon content, much lower than the 80% expected. Moreover, oxidations should have removed only 20% of the C from LTBF<sub>79</sub> samples (according to Barré et al., 2010). Nevertheless, 83-86% (depending on the reactant) of the carbon was removed. In the light of these results, we conclude that natural biodegradation at the centennial scale cannot be reproduced by the two tested oxidative treatments.

However, one can hypothesize that the oxidation treatment is efficient at extracting C stable at a much longer timescale. The amount of C remaining after chemical oxidation was significantly higher in oxidized-LTBF<sub>0</sub> compared to oxidized-LTBF<sub>79</sub> samples (paired t-tests,  $p < 0.05$  for both H<sub>2</sub>O<sub>2</sub> and NaOCl). This shows that chemical oxidation using the two classical tested oxidizing agents did not extract a C pool that is inert at the scale of the bare fallow duration such as the Roth-C IOM pool (Coleman and Jenkinson, 1996).

If we make the usual assumption that soil C pools follows a first order decay kinetic  $C(t)=C(0)*\exp(-t/\tau)$ , we can calculate the turnover time ( $\tau$ ) of the C pool isolated by oxidation. In this case, we would have  $C_{ox}(LTBF_{79})=C_{ox}(LTBF_0)*\exp(-79/\tau)$ . After calculation, the isolated C pools would have turnover times equal to  $169\pm 8$  and  $359\pm 130$  years respectively for  $H_2O_2$  and NaOCl. Such C pools would have turnover times in the same range than the slower pools in models such as Century (passive pool, Parton et al., 1988), or Daisy (slow pool, (Bruun et al., 2003). However, in these models, the passive or slow pools usually represent ca. 30% of SOC (Kelly et al., 1997). In our case, oxidation isolates at best 9.3% of initial SOC. We can conclude that the two tested oxidizing agents can, at best, extract a small part of the C pool with a turnover time of a few centuries.

#### 1.4. Conclusion

We conclude that chemical oxidation using the two classical tested oxidizing agents does not allow to quantitatively isolate a stable C pool with centennial turnover times. There is still an important need for research in this field and we therefore highly recommend more research to be continued in order to improve our understanding of SOC stabilization mechanisms operating at the centennial scale.

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Section 1: Are chemical oxidation methods relevant to isolate a soil pool of centennial carbon?

## 2. Persistent carbon has a similar chemical signature as total soil organic carbon

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### Abstract

Though it is of crucial importance in order to better understand carbon persistence in soils, the chemical nature of stable soil organic matter (SOM) is poorly characterized. This is explained by two major difficulties: (1) there is still no successful experimental way to isolate the pool of SOM that has a pluri-decennial residence time, and (2) SOM with high residence time is often associated to minerals, which complicates its characterization.

In this work, we overcame these two major difficulties by characterizing samples from long-term bare fallow (LTBF) experiments using the state-of-the-art synchrotron-based NEXAFS spectroscopy (Canadian Light Source, Saskatoon, Canada). Firstly, LTBF experiments offer a unique opportunity to study persistent SOM, as without carbon inputs and with continuing biodegradation and mineralization, SOM becomes progressively enriched in its most persistent components. Secondly, NEXAFS technique allows the characterization of Carbon speciation with no sample pre-treatments and little to no noise induced by the mineral part of the samples. In this work, the fluorescence emission spectra at the Carbon K-edge threshold (280 eV) were measured for samples taken up at the initiation of 5 different european LTBF experiments and several decades later.

Results show that all sites display similar spectra and that there is no drastic change in the global chemical signature with time. However subtle, the differences in chemical composition between different dates are significant and spectra are highly reproducible between field replicates. Trends shared by all sites show that as C gains persistence, it is

found in more oxidized forms (mainly carboxylic moieties) which is interpreted as preferential binding between such functional groups and mineral surfaces.

Two main conclusions can be drawn from these findings. First, the gross chemical signature of SOM, as seen by NEXAFS, does not drastically change during 3 to 8 decades of biodegradation. This is in agreement with the current concept that persistent organic matter and total organic matter are not very different chemically. Secondly, the modest changes which were observed are consistent across sites, and lead to more oxidized forms of organic carbon.

## 2.1. Introduction

In order to improve the accuracy of predictive models for the evolution of soil carbon stocks in a changing environment, many efforts have been done to understand the mechanisms that explain the long-term persistence of carbon in soils. Three main mechanisms are usually invoked to explain the protection of organic matter from biodegradation: chemical recalcitrance, entrapment in aggregates, and adsorption on minerals (von Lützow et al., 2006, Six et al., 2002, Sollins et al., 1996). Characterizing the chemistry of persistent carbon is one way to gain insights on the pool of persistent carbon. Information on the mechanisms can be inferred by comparing chemical signatures of different pools of OC. Highly contrasted chemical signatures between TOC and Persistent OC with selective preservation of some functional groups will point towards a predominant role of either the chemical recalcitrance mechanism or adsorption on mineral surfaces. On the contrary, similar distributions between different functional groups will provide evidence for preferential protection by entrapment in aggregates involving the mineral matrix

Chemical recalcitrance is now considered important in the initial phases of biodegradation (up to a few years), whereas occlusion in aggregates and organomineral associations are believed to explain longer term persistence of carbon in soils. Studies which have looked at persistent carbon have identified several patterns in the chemistry of this pool of C. Using  $^{13}\text{C}$  NMR on samples from different long term agroecosystems, Kiem et al. (2000) found that refractory organic carbon was enriched in aromatic moieties (attributed to an increase in aryl-C) and depleted in O-alkyl functional groups. Furthermore, refractory carbon was slightly more oxidized than the organic matter contained in labile pools. Their results show that all carbon species were affected by degradation processes but to different extents, they order the different functional groups from more affected to less affected: O/N-alkyl C > alkyl C > carboxyl C > aromatic C.

As for now, attempts to experimentally isolate the persistent pool of OC from TOC by oxidations or hydrolysis have not been successful (Plante et al., 2011, Lutfalla et al., 2014), and insights on the chemistry of persistent C are still needed.

For the present study, we used the unique opportunity provided by long-term bare fallow experiments to study the long-term persistence of carbon in soils under field conditions. These experimental plots have been kept free of vegetation since the experiment started (by weeding) and regularly sampled. As most C inputs in soils come from plants, total C inputs

during the bare fallow period can therefore be considered as negligible. With time, biodegradation occurs, carbon contents gradually decrease as there is no input of fresh organic carbon and samples are therefore enriched in persistent carbon (Rühlmann, 1999, Barré et al., 2010, Kiem et al., 2000, Kiem and Kögel-Knabner, 2002). Our study is based on archived soils sampled in five European long-term bare fallow (LTBF) experiments that allowed us to study the decay rates of SOC over several decades and under different temperate pedo-climatic settings (see Table 5). This plant-free experimental setup is one of the few possibilities to study *in situ* the long-term dynamics of SOC in agricultural soils by providing information on initial stocks and on the subsequent decadal scale changes with no interference from fresh C inputs.

In the past decade, new techniques have emerged that allow the characterization of SOM. More precisely, NEXAFS spectroscopy is giving new insights on the mechanisms of the persistence of SOC by elucidating the distribution of functional groups containing carbon (Lehmann et al., 2009, Leinweber et al., 2013). The advantage of these techniques comes from the fact that soil samples can be studied without major pretreatment and extraction. And contrary to most other methods, they are relatively sensitive and display good resolution. One drawback is that although these techniques can give good results concerning the gross chemical composition, specific compounds cannot be identified.

In this work, we want to investigate the changes in chemistries as biodegradation occurs over several decades. We hypothesize that:

- (a) the OC from the different sites will display similar chemical signatures (distribution of functional groups) as they all have an grassland or arable and have relatively similar initial carbon contents (except Rothamsted);
- (b) biodegradation will first lead to a relative enrichment in aromatic moieties during the first couple of decades of the BF;
- (c) after the first decades, biodegradation of the preserved aromatics will occur and the resulting chemical signature will be similar to the initial patterns;
- (d) changes observed will lead to more oxidized forms of C and a relative enrichment in polar moieties as these forms of SOC are expected to preferentially bind to mineral surfaces (through adsorption).

## 2.2. Materials and Methods

### 2.2.1. Sites description

The soil samples were from a European network of differently sited LTBF experiments. The characteristics of the five sites involved in this study have already been described (Barré et al. 2010) and are summarized in Table 5. For each LTBF experiment, we selected two adjacent field plot replicates (four replicates at Versailles). For analyses, we used archived soils sampled at the start of the bare fallow treatment and after different fallow periods. For Askov, four sampling dates were selected (years 0, 6, 12 and 27 of bare fallow), for Rothamsted, five dates were selected (years 0, 4, 12, 28, 49), five dates for Ultuna (years 0, 11, 18, 39, 53) and ten dates for Versailles (0, 5, 10, 21, 31, 39, 51, 62, 73, 79).

**Table 5: Characteristics of the four LTBF sites.**

	<b>Askov (DK)</b>	<b>Rothamsted (UK)</b>	<b>Ultuna (SW)</b>	<b>Versailles (FR)</b>
Coordinates	55°28N 9°07 E	51°82N 0°35 E	59°49N 17°38 E	48°48N 2°08 E
Starting date	1956	1959	1956	1928
Last sampling	1983	2008	2009	2008
Duration (years)	27	49	53	79
Previous land use	arable	grassland	Arable	grassland
Mean annual temperature (°C)	7.8	9.5	5.5	10.7
Annual precipitation (mm)	862	712	533	628
Soil texture and type (FAO, 1989)	Coarse sandy loam Orthic Luvisol	Flinty silty clay loam Chromic Luvisol	Clay loam Eutric Cambisol	Silty loam Luvisol
Texture (Sand/silt/clay)	82/11/7	7/68/25	23/41/36	26/57/17
Sampling depth (cm)	20	23	20	25
Initial SOC (g kg <sup>-1</sup> )	17.1	29.0	15.7	20.0
Final SOC (g kg <sup>-1</sup> )	11.6	10.0	10.6	6.5



### 2.2.2. CHN analysis

The contents of SOC and of nitrogen in the samples were measured by dry combustion employing a CHN Autoanalyser (Carlo Erba NA 1500). The soils contained no carbonates, therefore total C is equivalent to organic C contents.

### 2.2.3. Synchrotron NEXAFS spectroscopy

The 'bulk' carbon speciation of particle size fractions was investigated by Near edge X-ray absorption fine structure (NEXAFS) spectroscopy at the carbon K-edge (270 – 330 eV) using the 11-ID-1 Spherical Grating Monochromator (SGM) beamline located at the Canadian Light Source (CLS – Canada) (Regier et al., 2007). The CLS storage ring is operated at 2.9 GeV and between 250 and 150 mA current. Two horizontal deflecting mirrors and one vertical focusing mirror direct the light from the 45mm planar undulator through the entrance slit and onto a 1700 lines/mm grating (low energy grating) which diffracts the light through a movable exit slit that has been set at 25  $\mu\text{m}$  for the present experiments. A pair of toroidal refocusing mirrors are used to focus the beam onto the samples in the endstation chamber under a working pressure of about  $10^{-7}$  Torr. A 100 nm thick titanium filter is used to remove the contribution of second order light. The beam spot size is approximately 1 mm  $\times$  100  $\mu\text{m}$  with a flux (normalized to 100 mA) greater than 1012 photons at 250 eV. This beamline measures the fluorescence yield of samples exposed to the incident beam as a function of its energy with a spectral resolution of about 0.1 eV. When normalized to the scattering intensity from a clean gold blank, fluorescence yields are directly comparable to absorption data, especially as the SGM uses an Amptek silicon drift detector placed at exactly 90 degrees to the incident beam allowing getting rid of scattering light. For the present study, dry particle size fractions were pressed onto gold foils (99.99 % purity – Sigma Aldrich) which were then mounted on a copper sample holder. Calibration in energy was done using a set of pure reference materials (calcium carbonate, benzoic acid and histidine (> 99 % purity – Sigma Aldrich)). Each spectrum reported in the present study corresponds to the average of about 50 measurements. Of note, only the first 100 nanometers of the sample surface are probed using the SGM setup. Spectra have been averaged, background subtracted and normalized using the Igor Pro software.

Spectra were normalized to total carbon content using the Athena software package for X-ray absorption spectroscopy (Ravel and Newville, 2005). Edge step normalization was performed using an E0 value of 291.5 eV. NEXAFS spectra were normalized across the full recorded range. A semi-quantitative analysis of C 1s NEXAFS spectra was carried out by peak

deconvolution using the Athena software. The deconvolution procedure was applied using Gaussian transitions with parameters. Peak magnitude and energy for all Gaussian transitions were constrained, only the height of the Gaussian was free to vary.

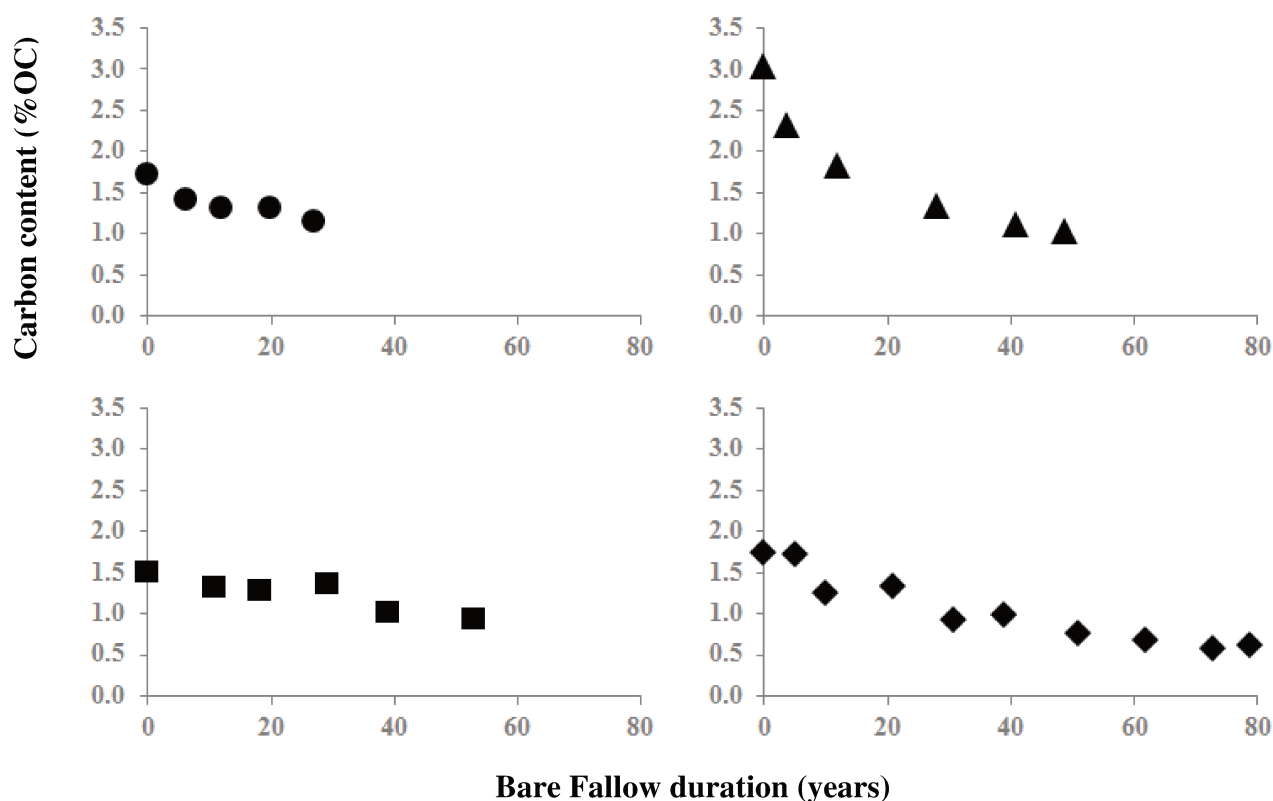
#### **2.2.4. Statistical analysis**

The statistical analyses were conducted using the free software environment for statistical computing R (<http://www.r-project.org>). The significance of the difference between C remaining after 79 years of BF in the three particle size fractions was assessed using pairwise t-tests. The significance of the evolution of the relative proportion of each functional group (result of the deconvolution of the spectra) with time was assessed by testing the significance of time in a mixed model (mixed model, with random effect of field replicates). A level of significance of  $P = 0.05$  was considered for all analyses.

## 2.3. Results

### 2.3.1. Dynamics of Total Organic Contents

As previously described (Barré et al., 2010), initial carbon concentrations are diverse, but soil organic C is decreasing with time at all sites (see Figure 20). Previous history of the site (arable or grassland) seems to have an impact on the dynamics of C, sites previously used for grassland showed steeper initial carbon declines whereas sites previously in arable cropping showed a more constant decrease in soil C during the years that followed introduction of bare fallow. During the 10 first years, the annual soil C decrease rates averaged less than  $1.6 \text{ gC kg}^{-1}$  for sites previously under arable cropping and were above  $3.5 \text{ gC kg}^{-1}$  for sites on former grassland. At the last sampling, between 32 and 68% of the initial SOC is lost.

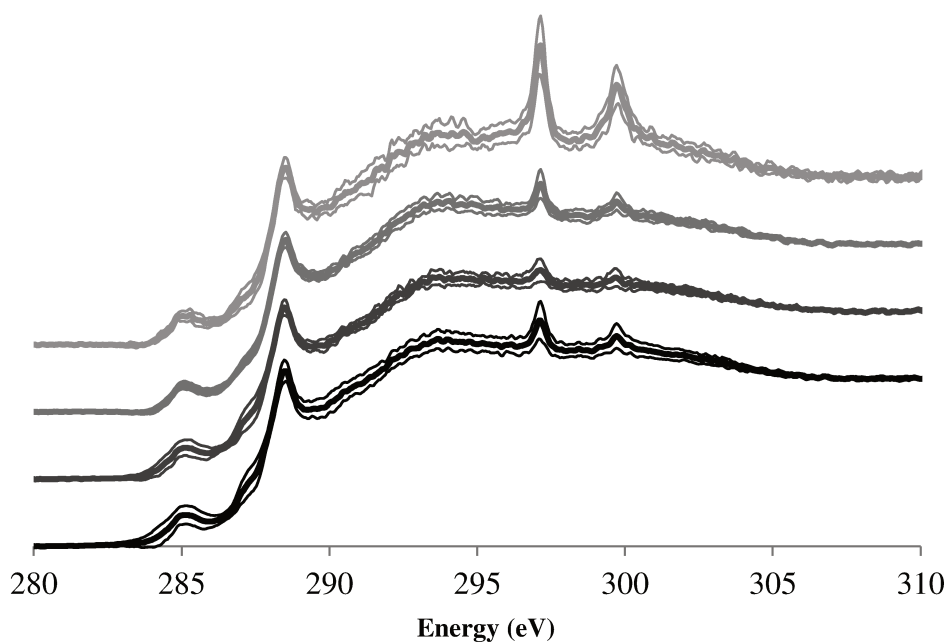


**Figure 20: Carbon dynamics in the five sites of the LTBF Network. From left to right, top row: Askov, Rothamsted, bottom row: Ultuna, Versailles.**

### 2.3.2. NEXAFS spectra

The distribution of the OC in different functional groups is similar between sites (Figure 21). The chemical signature is dominated by carboxyl moieties (288.6 eV). Averaged and

normalized spectra share the following patterns, a peak around 285 eV corresponding to aromatic and C=C bonds, a sharp peak around 288.6 eV corresponding to carboxylic functional groups. The energy range around 286-287 eV displays more inter and intra site variability, this region corresponds to phenol and ketones. All the sites show the potassium L edge peaks at 297.1 and 299.7 eV but with different intensities which correlate well with the clay content. Clay contents in Askov, Versailles, Rothamsted and Ultuna are 7, 17, 25, and 36 % (weight) respectively and the higher the clay content, the more intense the potassium peaks are. As can be seen in the graphical representation of the standard deviation (Figure 21), there is no drastic changes with time in the patterns observed. Main evolutions with time take place in the three energy ranges previously mentioned: aromatic and C=C double bonds around 285 eV, ketones and phenols around 287 eV and carboxylic moieties at 288.6 eV.



**Figure 21: NEXAFS spectra of bulk organic matter. From darker (bottom) to lighter (top) color: Versailles, Askov, Rothamsted, Ultuna. Normalized and averaged spectra (all dates and all replicates) and standard deviation are displayed.**

### 2.3.3. Evolution of the distribution of functional groups

In order to refine our analysis and detect possible trends in the small detected variability with time, we performed data treatment as described in Materials and Methods. The deconvolution of the signal with Gaussian curves allowed us to compare the relative

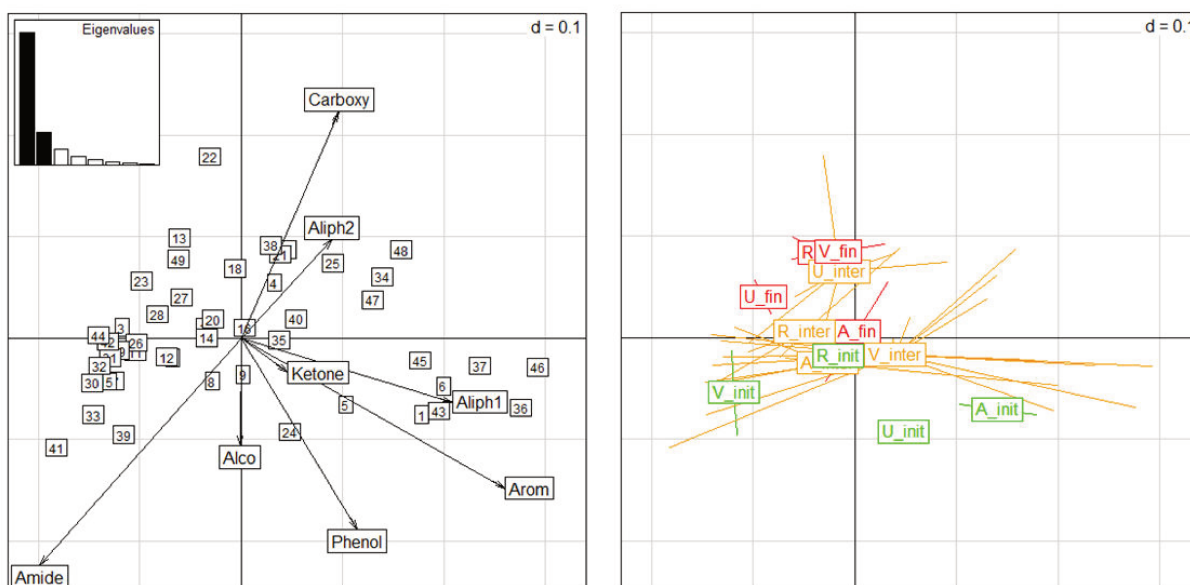
proportions of different moieties. Initial and final distributions of C in the different functional groups are contrasted as shown in Table 6. Seven functional groups are thus studied: aromatics and double C=C bonds (between 284.4 and 285.5 eV), ketones/carboxyamides (286.1-286.7 eV), phenols/ketones (287.1 eV), aliphatics (287.7 eV), amides/peptides (288.2 eV), carboxylics (288.6 eV), alcohols (289.2 eV). Comparison between initial and final values show that aromatic and double C=C bonds decrease at all sites except at Versailles. Ketones and carboxyamides and aliphatics show variable evolutions, their relative proportion tends to decrease in Askov, Rothamsted and Ultuna but is increasing in Versailles. Phenolic and ketone moieties decrease everywhere but initial and final values are similar. Amides and peptides increase everywhere except at Versailles. Carboxylics increase everywhere and display the highest variations. Alcohols display different evolutions: decreasing in two sites (Rothamsted and Versailles) and increasing in the others (Askov and Ultuna).

**Table 6: Proportions of the different chemical functional groups for initial and final samples. Each value corresponds to the average over the two field replicates of normalized gaussian area over the seven groups selected, coefficients of variation are in parenthesis. A: Askov; R: Rothamsted; U: Ultuna; V: Versailles.**

	Aromatics	Ketones	Phenols	Aliphatics	Amides	Carboxylics	Alcohols
<b>A_init</b>	10.70 (0.17)	5.29 (0.11)	10.88 (0.01)	7.86 (0.08)	14.56 (0.11)	19.30 (0.01)	15.51 (0.00)
<b>A_Fin</b>	6.98 (0.17)	4.96 (0.12)	9.41 (0.13)	5.63 (0.09)	17.45 (0.19)	22.03 (0.09)	16.04 (0.05)
<b>R_init</b>	9.18 (0.50)	4.80 (0.10)	9.48 (0.11)	6.31 (0.37)	16.23 (0.23)	20.55 (0.10)	16.76 (0.09)
<b>R_Fin</b>	4.67 (0.24)	3.05 (0.02)	8.44 (0.13)	5.57 (0.13)	17.58 (0.06)	24.59 (0.07)	16.53 (0.04)
<b>U_init</b>	9.04 (NA)	4.65 (NA)	11.45 (NA)	5.20 (NA)	17.97 (NA)	19.65 (NA)	15.48 (NA)
<b>U_Fin</b>	5.82 (0.17)	3.94 (0.00)	7.59 (0.10)	2.57 (0.08)	19.81 (0.04)	23.85 (0.02)	18.25 (0.08)
<b>V_init</b>	5.57 (0.04)	4.42 (0.22)	9.27 (0.33)	4.55 (0.30)	22.64 (0.03)	20.24 (0.08)	16.55 (0.03)
<b>V_Fin</b>	8.10 (0.27)	4.92 (0.04)	8.27 (0.21)	6.65 (0.27)	14.44 (0.24)	23.79 (0.02)	14.16 (0.08)

Further statistical analysis shows that the evolutions detected by the deconvolution follow small but consistent trends (Figure 22). Main evolution happens on the second axis of the PCA, which explains only a small amount of the total variability (around 20%). As previously seen, the relative proportion of carboxylics increased in all sites (significant for two sites: Rothamsted and Versailles), relative decrease of aromatics was detected in all

sites except Versailles (significant for one site: Ultuna). In Versailles samples, aromatics showed a small but significant increase in aromatics. Finally, we detected a possible depletion in amide compounds which was significant in one site (Versailles).



**Figure 22: Principal component analysis (PCA) of the areas of the gaussians corresponding to functional groups. On the left, composition of the first two PCA axis in terms of functional groups. On the right, position of the different sites at different dates (initial sampling date, intermediate sampling dates, final sampling date), axis 1 is horizontal, axis 2 is vertical.**

## 2.4. Discussion

### 2.4.1. The OC from the different sites display similar chemical signatures

The spectra of the different sites display similar patterns. As previously reported in literature (Lehmann et al., 2008, Solomon et al., 2005, Solomon et al., 2007, Purton et al., 2015), all spectra present aromatic double C=C bonds features, variable amounts of ketones and phenolic moieties and amides/peptides and carboxylic compounds. Our results show that the OM contained in these samples is composed of a complex mixture of functional groups, with slightly varying relative proportions. Such similarities between sites has already been detected and has been justified by the fact that the heterogeneity of OM happens at finer scales (nanometers) and that the bulk signature of OM will display an averaged distribution which will always contains similar information (Lehmann et al., 2008). Looking at what has been published so far on analysis of SOM with NEXAFS spectroscopy, our spectra correspond to patterns usually found either in dead OM which already started being biodegraded (VS living

tissue) and OM found in organomineral interactions (Keiluweit et al., 2012) or microbially processed OM (Kleber et al., 2011).

#### **2.4.2. With time and decrease in carbon content, chemical signature is stable**

Attempts to isolate compound classes from the SOM pool that would display longer persistence than bulk SOM, like lignin, have not been successful so far (Schmidt et al., 2011, Amelung et al., 2008). In accordance with such data, our results show that there is no particular functional group which is clearly selectively preserved in our soils. During the first decades of the BF, no site showed the selective preservation of “recalcitrant” organic matter. We hypothesize that the labile pool of these soils is too small and therefore the selective preservation of compounds belonging to this pool during the early stages of degradation cannot be detected in the signal of total organic matter. Small differences are detected over several decades of bare fallow. They show losses of aromatic C and double C=C bonds which have been considered persistent in soils (Derenne and Largeau, 2001) and show a relative enrichment in oxidized forms of C which will be discussed later. The Versailles site shows different dynamics for aromatic and double C=C compounds, a significant enrichment in these functional groups is observed, which can be interpreted as an effect of the bombing episode reported in Lutfalla et al. (under review). Indeed, there have been reports of a bombing event near the location of the bare fallow which would have created significant quantities of charred organic material, and a corresponding increase of the pyrogenic organic carbon content was observed in these plots using BPCA analysis (Lutfalla et al., under review). The pyrogenic organic carbon content of the other sites was also assessed in the aforementioned study and no significant increases were detected during the bare fallow. The enrichment of aromatics detected in the present study at Versailles constitutes further evidence of this bombing event. In the other sites, the decrease in aromatics and C=C bonds is in agreement with the fact that plant-derived lignin, cutin and suberin compounds have been shown to decrease faster than bulk SOM (Amelung et al., 2008, Schmidt et al. 2011).

Considering that the observed soil carbon losses range from one third to more than two thirds of the initial C between the first and the last sampling, the OM signature is remarkably stable, showing modest differences as C content decreases. We can interpret this relative stability of the SOM signature as evidence for the fact that compounds considered “recalcitrant” plant-derived compounds such as lignins, cutins and suberins actually decompose relatively quickly

in soils (Dignac et al., 2005). Using similar long term experiments, Kiem et al. (2000) detected a small but significant enrichment in aromatic C for bare fallows samples, more specifically aryl-C that they interpret as evidence of the refractory nature of aromatic C which we are not detecting here.

Similar to the observations of the present study, Purton et al. (2015) looked at samples from a climosequence and samples from soils under different uses (grassland, arable, forest) and found very similar spectra at the Carbon K edge with NEXAFS spectroscopy. They interpret this data as evidence for the little influence of climate and land use changes on the global distribution of functional groups in SOM.

Nevertheless, the information given by NEXAFS spectroscopy is not characterizing individual molecules but is showing the distribution of carbon in different functional groups, the molecular repartition of these functional groups is likely to depend on the site, type of previous land-use and biological activity of the sites. Therefore, our results do not show that molecules in soils are static. Indeed, because we only detect functional groups, we have no way of knowing what the composition of molecules in the soil is. As biodegradation occurs, molecular rearrangements take place with only modest influence of the distribution of functional groups.

This observed chemical signature also gives insights on the mechanisms of persistence that are taking place in the soils. Indeed, no selective preservation of particular functional groups means that chemical recalcitrance is not a dominant protection mechanism at the multidecadal timescale. This is in accordance with the current consensus of chemical recalcitrance not impacting long term dynamics and preservation of soil organic carbon (von Lützow et al., 2006, Kleber et al., 2011).

#### **2.4.3. The modest changes observed lead to more oxidized forms of C**

The chemical changes we detected were modest but consistent across most sites. The main feature of this evolution was that as C becomes more persistent in soils, it is found in more and more oxidized forms as evidenced by the observed relative increase of carboxylic moieties. Similarly, Kiem et al. (2000) have found a slight increase in the oxidation state of persistent carbon from bare fallows compared to adjacent vegetated plots containing labile organic matter. Gregorich et al. (2015) detected a similar chemical signature of soils from a bare fallow compared with adjacent arable and grassland sites, with a relative increase in carboxylic functional groups and decrease in aromatic and amide moieties. On the contrary,



looking at subarctic soils with NEXFAS spectroscopy, Gillespie et al. (2014) showed that buried soil contained high levels of C resisting decomposition because of chemical modification of the C leading to a particular accumulation of ketone moieties and the loss of carbohydrates, phenolic and carboxylic compounds in these buried horizons.

The oxidized nature of persistent carbon has already been documented (von Lützow and Kögel-Knabner, 2010). The oxidation state of carbon can vary widely, from **-4** (in CH<sub>4</sub>) to **+4** (such as in CO<sub>2</sub>). Several interpretations can be put forward for the relative enrichment in oxidized compounds that we observed. First, these bare fallow soils have not received inputs of fresh plant-derived organic matter for several decades, the soil organic matter is therefore enriched in microbially transformed material, which is more oxidized (Gregorich et al., 2015). Secondly, oxidized moieties such as carboxylic functional groups will more easily bind to minerals than reduced forms of C (aliphatic chains for instance). Indeed the energy of the interaction between polar (and possibly charged) carboxylic moieties and mineral surfaces will be higher than in the case of apolar aliphatic groups. Carboxylic groups will interact strongly with multivalent cations and protect the molecule from biodegradation (Baldock and Skjemstad, 2000, Oades, 1988). In addition, Sollins et al. (2009) detected a good correlation between increase in the oxidation state and increased MRT in the organic matter of several density fractions. Our results therefore seem to point towards protection through interaction with minerals.

## 2.5. Conclusion

In the long-term field experiments studied, on average, more than half of the initial carbon is gone after several decades of interrupted carbon inputs. The study of the remaining carbon (i.e. persistent C) and of the evolution of the chemical signature of the C in these plots showed that no drastic chemical changes are taking place as several decades of biodegradation occur. The same mix of functional groups can be found in the initial TOC and in the persistent C. Evidencing that chemical recalcitrance is not the main mechanism explaining long-term carbon persistence. Small trends in the evolution of the relative proportion of chemical moieties show an increase of oxidized form of C across the sites and no specific persistence of aromatic and C=C bonds. We interpret such results as a preferential binding of carboxylic chemical groups to mineral surfaces, favoring their persistence in soils.

### 3. Soil pyrogenic organic carbon lacks long-term persistence

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#### Abstract

Pyrogenic organic carbon (PyOC) derived from incomplete burning of biomass is considered the most persistent fraction of soil organic carbon (SOC), being expected to remain in soil for centuries. Climate change mitigation strategies thus include C sequestration in soil by addition of PyOC in the form of biochar, a solid pyrolysis residual of plant biomass. Based on a unique set of European long-term field experiments, we provide the first extensive comparison between PyOC and SOC persistence in soils. We found that PyOC contents decrease rapidly in soils, and that the mean residence time of native PyOC is just 1.6 times longer than that of SOC. Our results suggest that the potential for long-term C storage in soil by PyOC amendments is less than currently anticipated.

### 3.1. Introduction

Soil organic matter stores three times more carbon (C) than the atmosphere and enhanced C storage in soil is considered a valid climate change mitigation strategy. Thus, studies of soil organic carbon (SOC) dynamics are crucial to deepen our insight into the global C cycle and its interaction with climate change (Ciais et al., 2013).

Pyrogenic Organic Carbon (PyOC) is a charred residue derived from incomplete burning of biomass and is a native component of most soils. Stocks of PyOC can vary from a few percent to up to 45% of the SOC (Schmidt et al., 1999, Czimeczik et al., 2005) depending on past frequency of fire (natural or man-made), vegetation history and soil geological origin. PyOC has been long considered to be almost inert in soils, intact charcoal fragments and other forms of PyOC being found at depth in soils where SOC residence times exceed thousands of years (Glaser and Amelung, 2003, Gouveia et al., 2002, Preston and Schmidt, 2006, Schmidt et al., 2002). More recent reviews suggest that the persistence of PyOC in soil may range from just a few centuries to millennia (Singh et al., 2012, Lehmann et al., 2015).

PyOC is nowadays investigated as a climate change mitigation strategy under the name of biochar (solid residue from thermal conversion of biomass, used as a soil amendment (Ciais et al., 2013)). Biochar efficiency as a mitigation technique relies on its long-term persistence, which determines its C offset value (Forbes et al., 2006, Woolf et al., 2010). However its dynamic is difficult to assess experimentally. Direct evidence underpinning its long-term persistence is still lacking and results published so far are primarily from short term (< 5 years) laboratory incubations which elucidate only the initial phases of PyOC decay (Singh et al., 2012b, Zimmerman, 2010).

As far as we know, existing assessments of the longer-term fate of PyOC are relying on “space for time” approaches, where PyOC contents between plots which experienced their last fires at different dates are compared (Schneider et al., 2011a). However, soil spatial variability, differences in past land management and the lack of knowledge about the initial conditions of the experiment render the extrapolation of these results uncertain. Further, the effective long-term gain in the overall C storage from biochar additions can only be evaluated by comparing the residence times of corresponding PyOC and SOC pools in the very same samples. Such evaluations have been made for other specific SOC components (Amelung et al., 2008) but not for PyOC.

Our study is based on archived soils sampled in five European long-term bare fallow (LTBF) experiments that allowed us to study corresponding decay rates of PyOC and SOC over several decades and under different temperate pedo-climatic settings (Barré et al., 2010) (see Table 7). The LTBF soils have been without organic amendments and kept free from plants by weeding and herbicides for up to 80 years. As most C inputs in soils come from plants, total C inputs during the bare fallow period can therefore be considered as negligible. This plant-free experimental setup is one of the few possibilities to study in situ the long-term dynamics of both PyOC and SOC in agricultural soils by providing information on initial stocks and on the subsequent decadal scale changes with no interference from fresh C inputs.

**Table 7: Selected characteristics of the five LTBF sites.**

	<b>Askov (DK)</b>	<b>Grignon (FR)</b>	<b>Rothamsted (UK)</b>	<b>Ultuna (SW)</b>	<b>Versailles (FR)</b>
Coordinates	55°28N 9°07 E	48°51N 1°55 E	51°82N 0°35 E	59°49N 17°38 E	48°48N 2°08 E
Starting date	1956	1959	1959	1956	1928
Last sampling for this study	1983	2007	2008	2009	2008
Duration (years)	27	48	49	53	79
Previous land use	arable	grassland	grassland	arable	grassland
Mean annual temperature (°C)	7.8	10.7	9.5	5.5	10.7
precipitation (mm.y <sup>-1</sup> )	862	649	712	533	628
Soil texture and type	Coarse sandy loam Orthic Luvisol	Silty loam Luvisol	Flinty silty clay loam Chromic Luvisol	Clay loam Eutric Cambisol	Silty loam Luvisol
Texture (sand/silt/clay)	82/11/7	16/54/30	7/68/25	23/41/36	26/57/17
Sampling depth (cm)	20	25	23	20	25
Initial SOC (g kg <sup>-1</sup> )	17.1	13.5	29.0	15.7	20.0
Final SOC (g kg <sup>-1</sup> )	11.6	8.6	10.0	10.6	6.5

## 3.2. Results

### 3.2.1. PyOC contents decrease rapidly

Revealing the dynamics of PyOC requires a reliable method for its determination in soil. Several methods have been proposed (Hammes et al., 2007). The Benzene Polycarboxylic Acid (BPCA) molecular marker method (Glaser et al., 1998, Wiedemeier et al., 2013) has the

PART 3: CHEMICAL SIGNATURE OF THE EVOLUTION OF SOM TOWARDS MORE PERSISTENT COMPOUNDS

advantage of targeting only pyrogenic derived molecular markers, thus yielding conservative but quantitative estimates of PyOC and information about its quality (Wiedemeier et al., 2015a).

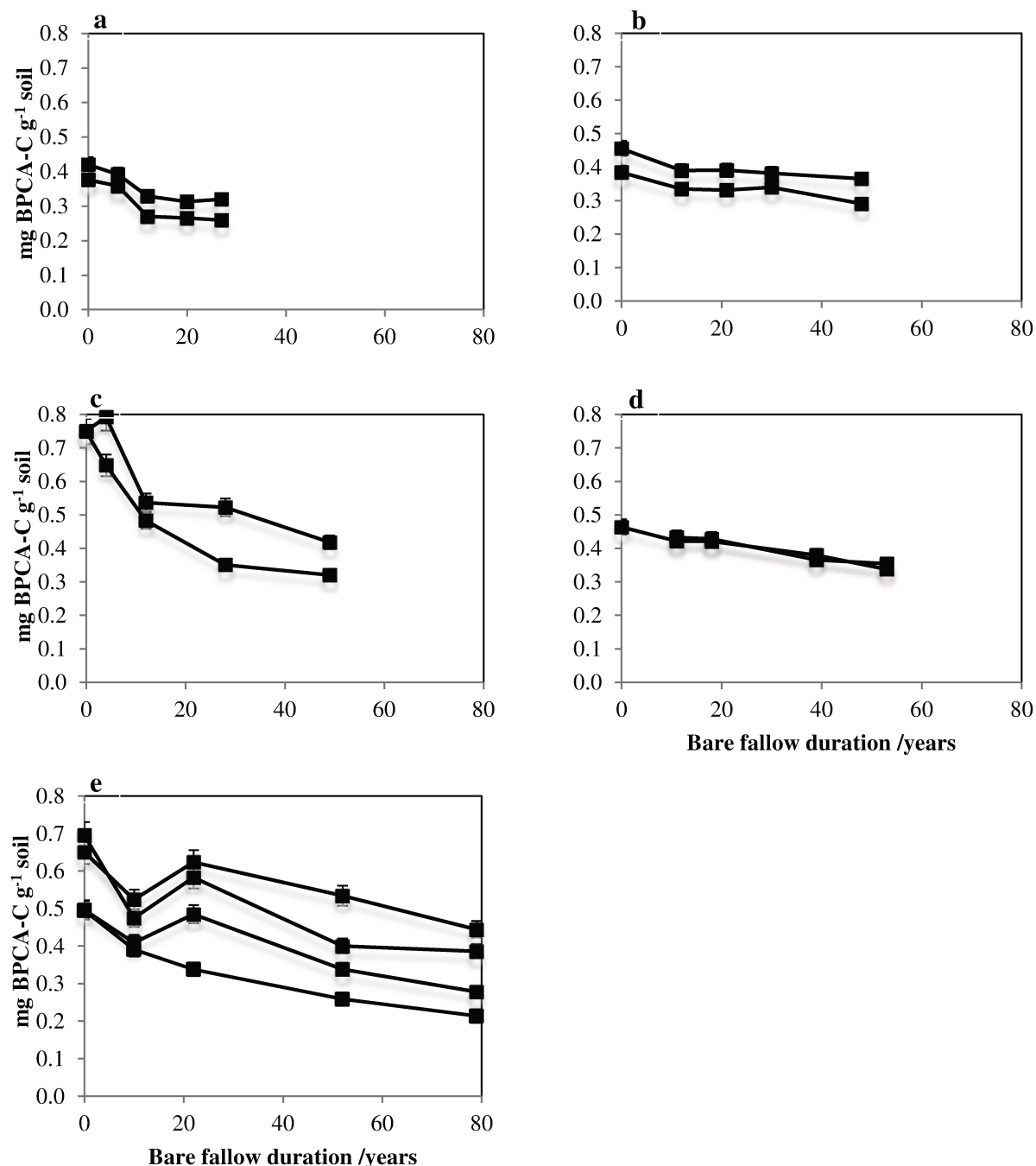


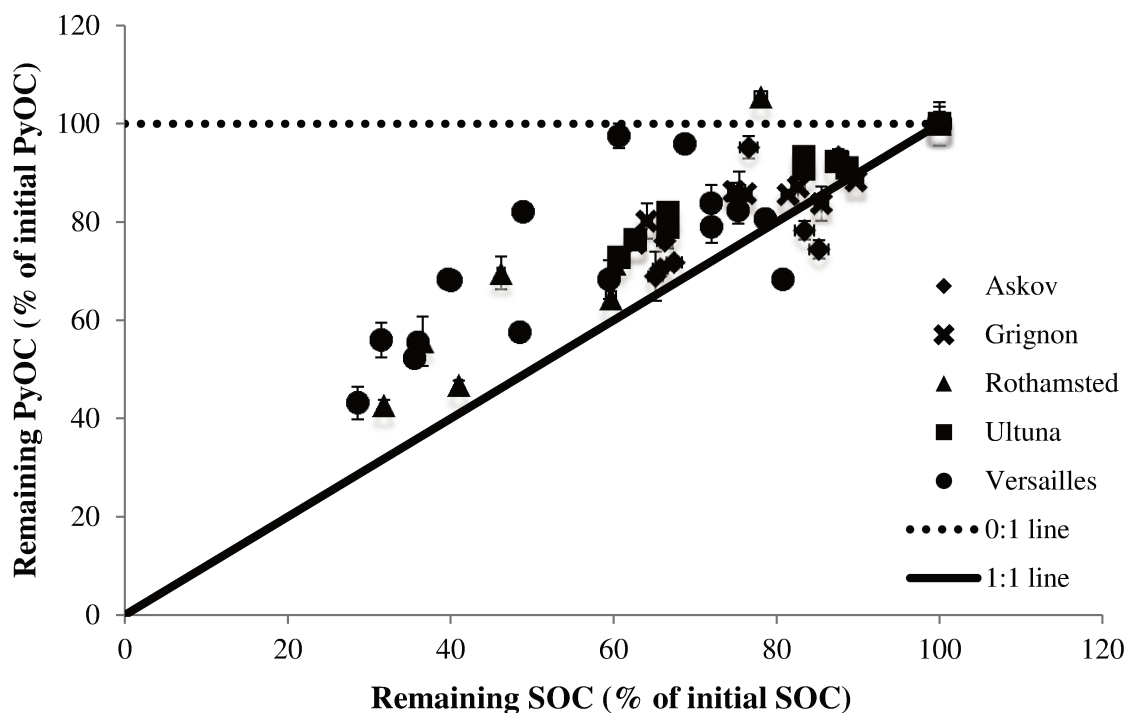
Figure 23: Contents of native pyrogenic organic carbon (PyOC) in soil decrease with time at all LTBF sites and for all field replicates (mg BPCA-C g<sup>-1</sup> soil, time in years): Askov (A), Grignon (B), Rothamsted (C), Ultuna (D), and Versailles (E). Each line corresponds to an experimental plot. Error bars represent PyOC variability within analytical replicates (always < 10 %).

We found that contents of PyOC in soil decreased surprisingly fast at all LTBF sites (Figure 23, and Table 11 in Supplementary material). Initial concentrations varied between 0.38 mg PyOC-C g<sup>-1</sup> soil (plots Grignon1 and Askov1) and 0.75 mg PyOC-C g<sup>-1</sup> soil (Rothamsted1 and 2) corresponding to 2 and 4% of the SOC. This is in line with results from other studies using a similar method (Glaser et al., 1998, Schneider et al., 2011b). For all LTBF sites, the final PyOC concentrations were significantly lower than initial ones (p-value < 0.001). The loss of PyOC between the first and the last soil sampling ranged from 19.8 to 57.3% of the initial PyOC content but was not related to the length of the bare fallow period. At Versailles and Rothamsted, PyOC contents showed steep initial declines, with 20 to 35 % of the initial PyOC being lost during the first decade, corresponding to average annual losses of 1.35% at Versailles and 2% at Rothamsted. The PyOC increased between year 10 and year 22 in soil from the Versailles bare fallow, probably due to the impact of nearby bombing during the Second World War.

### 3.2.2. PyOC and SOC loss differs little

As previously reported (Barré et al., 2010), SOC contents decreased at all LTBF sites due to the absence of C inputs and the continued decomposition of SOC formed during the pre-fallow period. At the last sampling, between 32 and 68% of the initial SOC was lost (Table 7).

The PyOC and SOC pools showed similar dynamics (Figure 24) and the PyOC loss patterns were consistent across the range of pedo-climatic settings. Loss rates of PyOC were significantly smaller than those observed for SOC (p-value < 0.001). The difference in PyOC and SOC loss rates was reflected in a relative PyOC enrichment at all LTBF sites; the initial PyOC content ranged from 2 to 4% of the SOC while final PyOC values ranged from 2 to 6% of the SOC. At Rothamsted, one sample fell above 100% of PyOC (Figure 24) but was within the 10% variation. This outlier is ascribed to spatial heterogeneity.



**Figure 24: PyOC losses occur more slowly than SOC losses (1:1 line), far from the concept of PyOC being inert (0:1 line). Values are expressed as % of initial SOC and PyOC contents. Time series start at the 100% values for both PyOC and SOC, vertical error bars represent PyOC variability within analytical replicates (< 10 %), horizontal error bars represent analytical errors on SOC measurements (< 3 %). The average annual loss of SOC ranged from 0.7% at Ultuna to 1.4% at Rothamsted while losses of PyOC varied from 0.4% at Versailles to 1.2% at Rothamsted.**

### 3.2.3. PyOC residence time ranges from 42 to 183 years

The mean residence time (MRT) for PyOC and SOC was estimated using first order kinetics in order to compare the relative persistence of PyOC and SOC (Table 8) (Barré et al., 2010). We applied a one pool model with mono-exponential decay that assumes PyOC to be one homogeneous pool and is frequently used to describe SOC dynamics (Parton et al., 1988). Although simplistic, this model allows transparent and robust comparisons between PyOC and SOC dynamics and between our dataset and previously published data.



Excluding only data from the Versailles plot distorted by bombing impact, the MRT of PyOC was found to range from 42 to 183 years, with an average of 116 years across all sites and a standard deviation of 15 years. The average MRT for PyOC (116 years) is 1.6 times longer than the MRT estimated for SOC (73 years). This difference is significant (p-value < 0.001) and falls in the low end of estimates -decades to centuries- given in the latest report of the Intergovernmental Panel on Climate Change (IPCC)(Ciais et al., 2013).

**Table 8: Mean residence time (in years) for PyOC and SOC calculated from the whole data set and estimates from previous studies. The significance of the model is shown in the standard deviation (SD) of each MRT estimates (in years).**

	MRT PyOC (years)		MRT SOC (years)		Ratio MRT PyOC/SOC
	mean	SD	mean	SD	
Askov1	60	9	46	8	1.3
Askov2	76	10	77	11	1.0
Rothamsted1	42	7	31	5	1.4
Rothamsted2	76	15	36	6	2.1
Ultuna1	183	11	101	3	1.8
Ultuna2	179	18	96	13	1.9
Grignon1	183	22	114	25	1.6
Grignon2	169	31	110	19	1.5
Versailles1	214*	53	72*	8	3.0*
Versailles2	106*	27	71*	4	1.5*
Versailles3	80	9	49	6	1.6
Versailles4	141*	25	55*	6	2.6*
Average	116 (126**)	15 (20**)	73 (72**)	10 (10**)	1.58 (1.77**)
Singh et al., 2012	291 (353***)		Ø		
Lehmann et al., 2015	300 – 6000		Ø		
Forbes et al., 2006	>1000		Ø		

\* data impacted by WW2 bombing event at Versailles

\*\* including hampered data

\*\*\* for field studies

The ratio between MRT of PyOC and SOC differed among LTBF sites. For the Askov2 plot, the MRT of PyOC and SOC are the same, evidencing no specific PyOC persistence, while for Versailles1 plot the MRT of PyOC was three times longer than that of SOC. Despite large differences in pedo-climatic conditions among the LTBF sites, no relationship could be

established between PyOC loss and general site characteristics such as soil type and climate. However, sites with a pre-fallow history of permanent grasslands (Rothamsted and Versailles) showed a steeper initial decline in PyOC than sites with a pre-fallow history of arable use (Askov and Ultuna).

### 3.3. Discussion

The small difference in PyOC and SOC persistence contrasts with current concepts on this topic. SOC MRT in the long term bare fallow plots are similar to those estimated from other experimental set-ups like C3-C4 sequences for similar pedo-climatic conditions (Dignac et al., 2005, Rasse et al., 2006). Whereas compared to previously reported estimates, the PyOC MRT calculated for soil from our five North European locations is three to ten times shorter than MRT values reported in the literature (Singh et al., 2012a, Forbes et al., 2006).

A previous study showed that a lack of vegetation does not interact with the persistence of PyOC in soils (Vasilyeva et al., 2011). This study compared a 55 years bare fallow soil to soil from an adjacent native grass steppe. It was found that changes in PyOC contents were similar for long term bare fallow and vegetated soil. So we can hypothesize that the absence of organic inputs did not alter PyOC dynamics in the LTBF soils.

Losses of PyOC can be due to biotic (microbial degradation) and abiotic mechanisms (photo-oxidation, erosion, and leaching). We consider that the consistent loss of PyOC observed across the contrasted pedo-climatic settings is mainly due to biotic processes. Except for Rothamsted, no significant soil bulk density changes were observed in the different LTBF sites, the same soil layers were therefore consistently sampled over the duration of the experiments (see Material and Methods). Photo-oxidation impacts charred materials situated at the soil surface, but can only be of minor importance in our study since soil samples were extracted from the Ap-horizon (0-20 or 0-30 cm soil depth depending on the site). Further, the experimental setup of the LTBF experiments ensured that losses of PyOC and SOC by horizontal transport (e.g. erosion) were insignificant during the fallow period. Leaching mechanism can take place in this setup, however previous studies focusing on the potential losses of PyOC by solubilisation concluded to the very minor role of this process as compared to the biological degradation (Maestrini et al., 2014, Abiven et al., 2011). Finally, similar PyOC molecular qualitative changes across sites (Figure 37 in Supplementary material) cannot be explained by either erosion or leaching but support the notion of biological decay being the primary driver for loss of PyOC.

Our results challenge the current concepts of PyOC being extremely persistent in soil. We did find that PyOC was slightly more persistent than total SOC and as such, our results align with the current notion of PyOC being the most persistent fraction of SOC (Schmidt et al., 2002, Amelung et al., 2008). However the relatively short MRT observed for PyOC in the LTBF soils suggests that its persistence has been greatly overestimated in previous reports. This observation carries severe implications for current concepts of soil C sequestration and should be taken into account when developing biochar technologies for C storage in soils.

### **3.4. Methods**

#### **3.4.1. European network of long term bare fallows (LTBF)**

The soil samples were from a European network of differently sited LTBF experiments. The characteristics of the five sites involved in this study have already been described (Barré et al., 2010). For each LTBF experiment, we selected two adjacent field plot replicates (four replicates at Versailles). For analyses, we used archived soils sampled at the start of the bare fallow treatment and after different fallow periods (samples are air-dried and kept in the dark). Except at Rothamsted, there are no significant changes in the bulk density of the soils. Precise fire history at those sites is not known but measured PyOC most probably comes from burning of grass and low vegetation during the century before the fallow treatment (based on the history of the sites and the distribution of the different BPCA molecular markers).

#### **3.4.2. PyOC assessment**

PyOC contents were assessed with the BPCA method, a previously described state-of-the-art molecular marker approach for PyOC analysis in environmental samples (Wiedemeier et al., 2013). Samples were analyzed in duplicate and analytical error was always < 10%. For each analytical batch of soils, a laboratory reference soil (Chernozem) was included to ensure the quality of the analytical procedures. The BPCA method is known to provide a conservative assessment of PyOC in soil. It targets pyrogenic molecular markers only, which was also the focus of the current study. The BPCA method is consistent but probably underestimates the absolute amount of PyOC (Glaser et al., 1998), and a conversion factor of 2.2717 has been proposed to convert “BPCA-PyOC” to “total-PyOC”. We refrained from this and present unconverted PyOC-values because only relative changes in the PyOC content were of interest.

### 3.4.3. SOC assessment

The content of SOC in the samples was measured by dry combustion employing a CHN Autoanalyser (Carlo Erba NA 1500). Except for Grignon, the soils contained no carbonates, therefore total C is equivalent to organic C contents. The Grignon soils were treated with HCl to remove soil carbonates (Harris et al., 2001).

### 3.4.4. Statistical analysis

The statistical analyses were conducted using the free software environment for statistical computing R (<http://www.r-project.org>).

### 3.4.5. One pool model

This model assumes that the loss of PyOC can be described by first order kinetics:

$$C(t) = C(0) \cdot \exp(-t/\text{MRT})$$

Where  $C(t)$  is concentration of PyOC ( $\text{mg PyOC-C g}^{-1}$  soil) at time  $t$  (years after start of bare fallow),  $C(0)$  is the initial PyOC concentration ( $\text{mg PyOC-C g}^{-1}$  soil), and MRT is Mean Residence Time of the PyOC (years). The same model was used to assess the MRT of SOC. The Least Square method was used to estimate model parameters.

## Acknowledgments

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## Author contributions:

S.L., C.C., P.B. and S.A. designed the study and wrote the paper; S.L. carried out analyses; D.W. developed the improved BPCA method and provided technical advice; F.v.O, A.M., T.K., S.H. and B.T.C. provided the LTBF soil samples. All authors subsequently reviewed and amended the manuscript.

### SECTION 3: Pyrogenic organic carbon lacks long-term persistence

## 4. PyOC displays contrasted multidecadal dynamics in particle size fractions of a silty loam luvisol

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### Abstract

Pyrogenic organic carbon is ubiquitous in terrestrial ecosystems and there is still a lot of uncertainty regarding its persistence in soils. A better understanding of the mechanisms explaining its dynamics and a comparison between total organic carbon (TOC) and pyrogenic organic carbon (PyOC) are crucial as they will help characterizing the true potential of pyrogenic material for possible storage of carbon in soils as a climate change mitigation strategy.

Here we used a long-term bare fallow experiment which has lasted for 79 years to study the multidecadal dynamics of both TOC and PyOC. Three sampling dates were selected to characterize the initial, intermediate and final states of the bare fallow experiment (year 0, 22 and 79 of the bare fallow, respectively) on four field replicates. We carried out a size fractionation on each sample and performed BPCA analysis in order to study the persistence of PyOC and to see if pyrogenic material was homogeneous in the different size fractions. Sand and clay size fractions displayed contrasted PyOC. PyOC associated to the clay fraction displayed dynamics almost equal to the dynamics of TOC. After 79 years of bare fallow, more than 60% of both TOC and PyOC were gone in this fraction. The chemistry of this PyOC suggests an origin from natural fires of biomass (trees, grass). PyOC associated with sand fractions did not significantly decrease over the duration of the experiment. Because TOC of the fraction decreases with time, the relative proportion of TOC in the form of PyOC almost doubled after 79 years of bare fallow. This PyOC was really condensed, the most probable origin is carboniferous coal used in heating systems.

Overall, PyOC was not inert in the size fractions, the chemical signature of the BPCA molecular markers showed consistent increases in the condensation of the PyOC with time. This study is, to our knowledge, the first long-term assessment of the dynamics of PyOC in size fractions of a temperate soil. Our results constitute unprecedented evidence of equivalent persistence of TOC and PyOC in the clay size fraction. This absence of specific persistence is likely due to the interaction of two factors which influence the biodegradability of PyOC: the size and the surface substitution of the individual particles of PyOC (below 2 $\mu$ m) which make organomineral interactions possible and the origin of the pyrogenic material which will determine the degree of condensation.

#### 4.1. Introduction

Soils store three times more carbon than the atmosphere and as such they constitute the biggest terrestrial carbon reservoir (Jobbágy and Jackson, 2000). Because there is evidence of persistence of SOC for hundreds to thousands of years (Torn et al., 1997, Jenkinson et al., 2008), there have been many attempts at trying to identify specific compounds which would display long persistence and could therefore be used to store carbon in soils. Pyrogenic organic carbon is a particular class of SOC which is naturally occurring in soils and has already been proposed as a potential climate change mitigation strategy (Lehmann, 2007), under the form of industrially produced biochar which would be used as soil amendments (Sohi, 2012, Stewart et al., 2013). Their particular chemical structure would protect them from biodegradation. Pyrogenic carbon is a complex mixture of compounds (Schmidt and Noack, 2000, Keiluweit et al., 2010, Spokas, 2010). Depending on the degree of aromatization, substitution, pyrogenic organic carbon (PyOC) can display a wide range of contrasted properties (Hammes et al., 2007, Wiedemeier et al., 2015a). And therefore, existing studies on the persistence of PyOC find a large range of persistence time for PyOC and biochar (Singh et al., 2012a), it is still not properly understood which processes are responsible for the persistence of PyOC in soils.

Chemical quality of PyOC is often proposed as being responsible for long term residence of PyOC in soils. The review of Spokas (2010) showed that the O:C ration of pyrogenic material seemed to constitute a good proxy for the persistence of PyOC. According to this study, a ratio below 0.2 seems to provide a persistence of PyOC above 1000 years. Nevertheless, the quality of PyOC is also characterized by the aromaticity and the degree of aromatic condensation of the pyrogenic material. The BPCA method can describe both (Wiedemeier et al., 2015a). Organo-mineral associations have been proposed as an important mechanism for SOC persistence in soil (von Lützow et al., 2006) either through entrapment in aggregates or through preferential adsorption of SOM in mineral surfaces. One major characteristic of PyOC is its high surface area and sorption capacity. It has been shown that PyOC can be rapidly associated to minerals and in large amount (Singh et al. 2014).

Here, we investigated chemical (PyOC biomarkers) and physicochemical (size fractionation) persistence mechanisms on archive soil samples from a long-term field experiment in order to compare and quantify the relative importance of these two mechanisms on the PyOC mean residence time. The particular experimental setup of the long-term bare fallow allowed us to compare the biodegradation dynamics of both the PyOC pool and the TOC pool. Indeed,



long-term bare fallow plots have been kept free of vegetation since the experiment started (by weeding) and regularly sampled. As most C inputs in soils come from plants, total C inputs during the bare fallow period can therefore be considered as negligible. With time, biodegradation occurs, carbon contents gradually decrease as there is no input of fresh organic carbon and samples are therefore enriched in persistent carbon (Rühlmann, 1999, Barré et al., 2010). Moreover, it has already been shown that size could efficiently discriminate between different pools of PyOC displaying contrasted chemical characteristics and reactivities (Nocentini et al., 2010).

We hypothesize that:

- (1) The chemistry of PyOC will be contrasted in the different particle size fractions
- (2) The dynamics of PyOC will be contrasted in the different particle size fractions

## 4.2. Materials and method

### 4.2.1. Soil description and sampling

The INRA (Institut National de la Recherche Agronomique) 42 Plots experiment is a long-term bare fallow experiment which was started in Versailles (France) in 1928 to test the impact of fertilizers and amendments on soil structure (Burgevin and Hénin, 1939). Here, we focused on 4 of the 10 control plots. Since 1929, the soil has not been cropped and has been weeded (by hand or with the help of herbicides) and plowed (to the depth of 25 cm) twice a year. It is a non-carbonated brown soil (silty loam Luvisol: 16 % clay, 57 % silt, 27 % sand) and the control plots have an average pH value of 6.3 in 1929 and of 5.2 in 2008 (Pernes-Debuyser and Tessier, 2002 and Paradelo et al., 2013).

We studied soils sampled at 3 different dates to represent the initial, intermediate and final states of the bare fallow: 1929 (year 0 of the BF), 1951 (22 years of BF), and 2008 (79 years of BF). The samples have been archived (stored air-dried and in the dark) since then. Sampling depth was 0-25 cm for 1929 and 1951 samples and 0-20 cm for the last sampling in 2008. We used samples issued respectively from plots 13, 21, 22 and 32 as replicates.

### 4.2.2. Size fractionation

Soil samples were subjected to a combination of a two-step physical dispersion and a particle-size fractionation adapted from Balesdent et al. (1998) and Fernandez-Ugalde et al. (2016) to separate the following organo-mineral particle-size fractions: sand fraction ( $>50\ \mu\text{m}$ ), two silt fractions (coarse: 20-50  $\mu\text{m}$ , fine: 2-20  $\mu\text{m}$ ), and clay fraction. Approximately 50 g of soil were shaken overnight with 20 glass beads in 180 ml of deionized water to break aggregates  $>50\ \mu\text{m}$ . The resultant suspension from dispersion was then passed through a 50- $\mu\text{m}$  sieve to separate the fraction  $>50\ \mu\text{m}$ , i.e. sand and free particulate organic matter. This step prevents the disruption of particulate organic matter during the subsequent ultrasonic dispersion (Balesdent et al., 1991). The suspension  $<50\ \mu\text{m}$  was sonicated in an ice bath for a total input of  $320\ \text{J mL}^{-1}$  using a digital sonifier, max. output = 120 W, probe with a flat tip of 2.5 cm diameter, operating at 20 kHz (Sonics model 500W). The dispersion conditions allowed recovering a clay-size fraction ( $<2\ \mu\text{m}$ ) equivalent in proportion to that achieved during standard particle-size fractionation (Balesdent et al., 1991, 1998). At that stage, the solution was centrifuged at 79g for 12 minutes to first separate the clay fraction (0-2 $\mu\text{m}$ ) then at 5g for 1 minute to separate the fine silt fraction (2-20 $\mu\text{m}$ ) and the coarse silt (20-50 $\mu\text{m}$ ) fraction.

Sand and coarse silt fractions were oven-dried at 60°C and weighed and fine silt and clay fractions were freeze-dried and weighed.

#### **4.2.3. Elemental analysis**

The organic carbon content of samples was measured by dry combustion in a CHN autoanalyser (Carlo Erba NA 1500). The soil contains no carbonates, so total C contents are equivalent to total organic carbon contents.

#### **4.2.4. PyOC analysis with the BPCA method**

PyOC contents were assessed with the BPCA method, a previously described state-of-the-art molecular marker approach for PyOC analysis in environmental samples (Wiedemeier et al., 2013). Here we performed the BPCA analysis on milled bulk (300mg/analysis), sand (300mg/analysis) and clay (100mg/analysis) fractions at the three dates and on the four field replicates. Samples were analyzed in duplicate and analytical error was always < 10%. For each analytical batch of soils, a laboratory reference soil (Chernozem) was included to ensure the quality of the analytical procedures. The BPCA method is known to provide a conservative assessment of PyOC in soil. It targets pyrogenic molecular markers only, which was the focus of the current study. The BPCA method is consistent but probably underestimates the absolute amount of PyOC (Glaser et al. 1998), and a conversion factor of 2.2717 has been proposed to convert “BPCA-PyOC” to “total-PyOC”. We refrained from this and present unconverted PyOC-values because only relative changes in the PyOC content were of interest.

We estimated the content and evolution of PyOC in the silt fraction by difference between on the one hand bulk PyOC and on the other hand clay and sand PyOC.

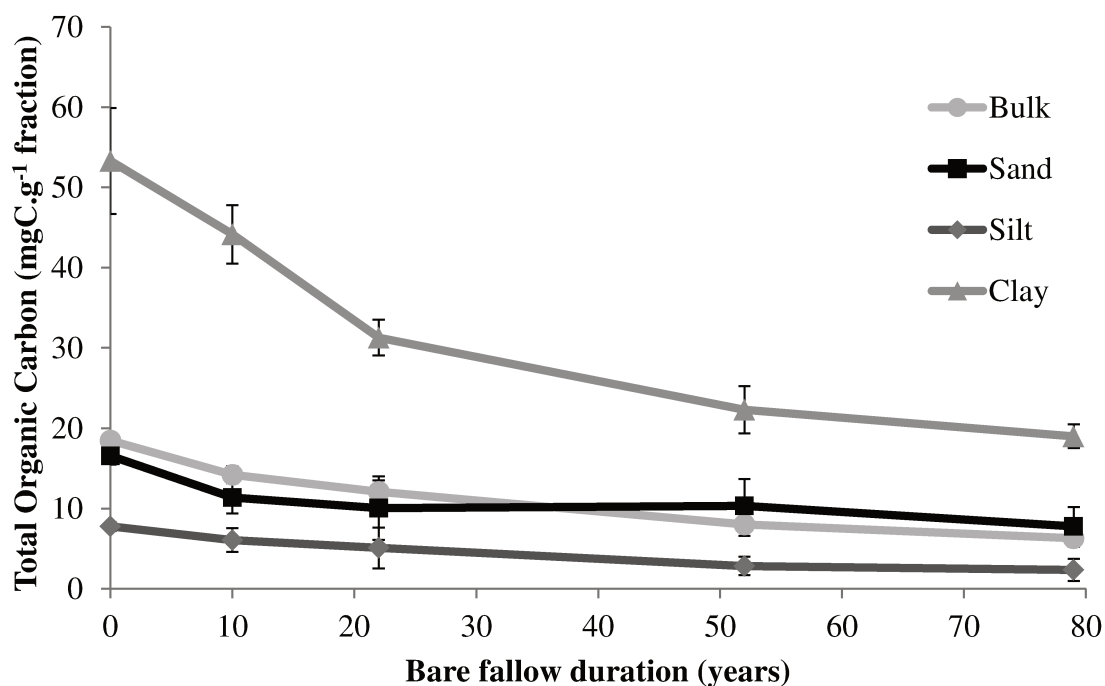
#### **4.2.5. Statistical analyses**

The statistical analyses were conducted using the free software environment for statistical computing R (<http://www.r-project.org>). The significance of the difference between TOC and PyOC contents between different dates was assessed using pairwise t-tests. A level of significance of  $P = 0.05$  was considered for all analyses.

### 4.3. Results

#### 4.3.1. Dynamics of TOC in the bulk and in the size fractions

As expected, total organic carbon (TOC) content decreased with time in all field replicates and in all size fractions (Figure 25). Variability between field replicates was low. For bulk samples, the initial average C content was  $18.5 \text{ mgC.g}^{-1}$  soil and final value was  $6.29 \text{ mgC.g}^{-1}$ , almost 66% of the initial C was lost after 8 decades of bare fallow. For sand and silt, initial carbon contents were  $16.6$  and  $7.8 \text{ mgC.g}^{-1}$  fraction respectively; final contents were  $7.8$  and  $2.3 \text{ mgC.g}^{-1}$  fraction, which corresponds to 53 and 69.9 % loss over the 80 years of biodegradation, respectively. Clay samples had the highest initial and final C content,  $53.3$  and  $19 \text{ mgC.g}^{-1}$  fraction respectively and they experienced a total C loss similar to the bulk (64.4 %).



**Figure 25:** Evolution of C content in the size fractions and bulk samples. Error bars represent the variability between field replicates.

### 4.3.2. Dynamics of PyOC in the fractions

PyOC content decreased in the bulk samples of the four plots from an average of 0.58 mgC-PyOC.g<sup>-1</sup> soil to 0.33 mgC-PyOC.g<sup>-1</sup> soil, which corresponds to a 43% decrease (Figure 26). The variability between the four plots was high (standard deviation ranges from 18 to 31%). The observed decrease of PyOC in bulk samples was lower than the SOC decrease.

PyOC in the sand fraction (represented in Figure 26) showed a much higher variability between field replicates (standard variation ranges from 19 to 41%) and there was no significant evolution of the PyOC quantity in this fraction during the 80 years of LTBF (paired t-test initial/final: p-value=0.13), the average PyOC content over the four field replicates and the three dates was 0.87 mgC-PyOC.g<sup>-1</sup> fraction.

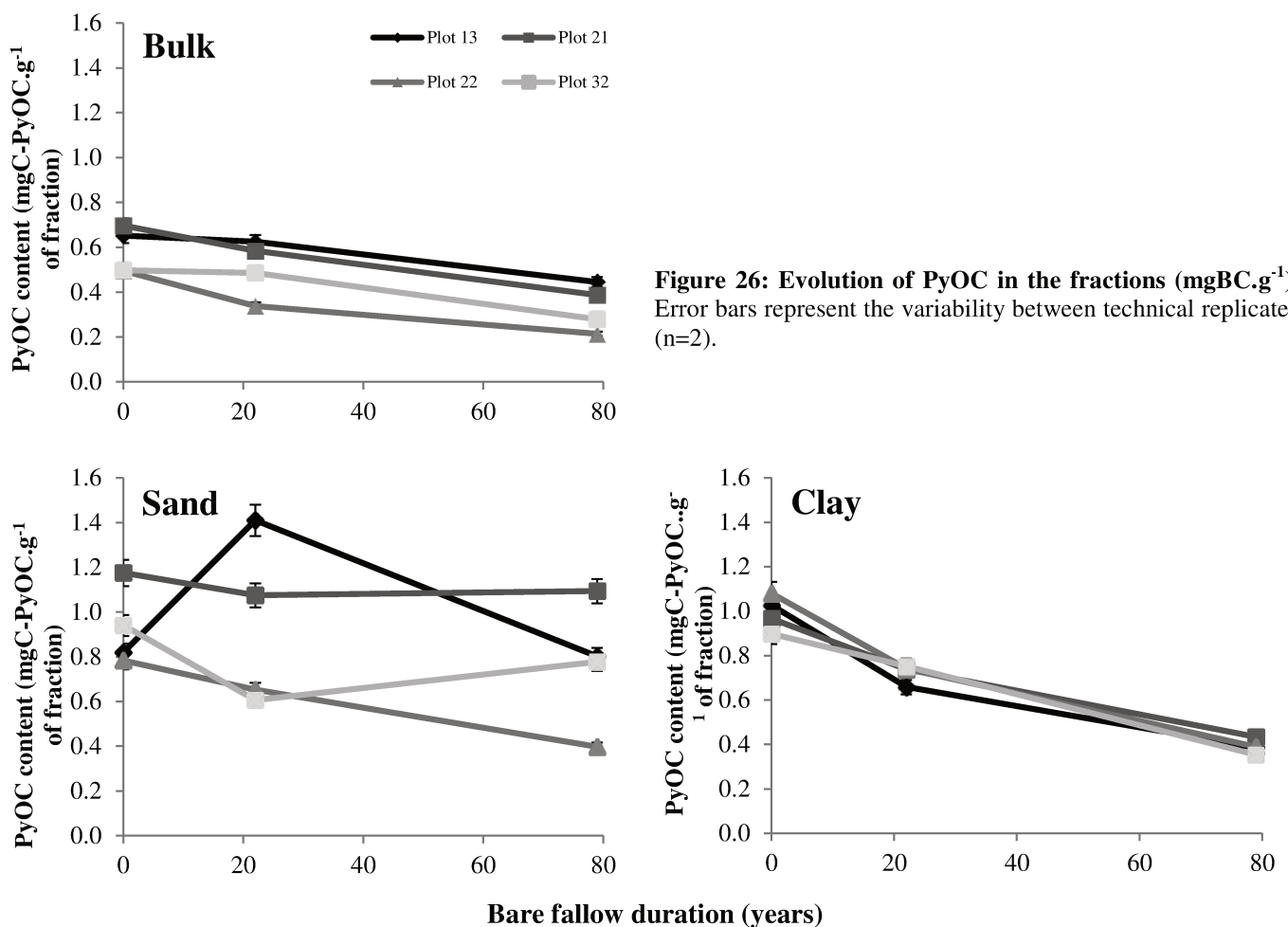
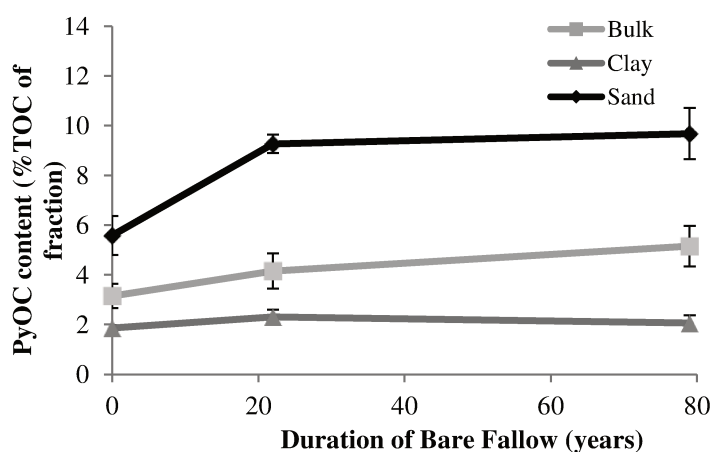


Figure 26: Evolution of PyOC in the fractions (mgBC.g<sup>-1</sup>). Error bars represent the variability between technical replicates (n=2).

PyOC in the clay fraction decreased significantly over the 80 years (Figure 26, p-value=0.0005), from an average of 0.99 mgC-PyOC.g<sup>-1</sup> soil to 0.39 mgC-PyOC.g<sup>-1</sup> soil, which corresponds to a 60% decrease. The variability between the four plots was low (below 10%). The rate of this decrease was similar for PyOC and SOC in the clay fraction.

Looking at the proportion of C in the form of PyOC in each of the analysed fractions we detected an important increase in the proportion of PyOC during the first 22 years of bare fallow (Figure 27). The proportion stabilized after and did not significantly evolve in the sand and clay fractions between 22 and 79 years of the BF. PyOC in the sand represented a much higher proportion of TOC than in clays.

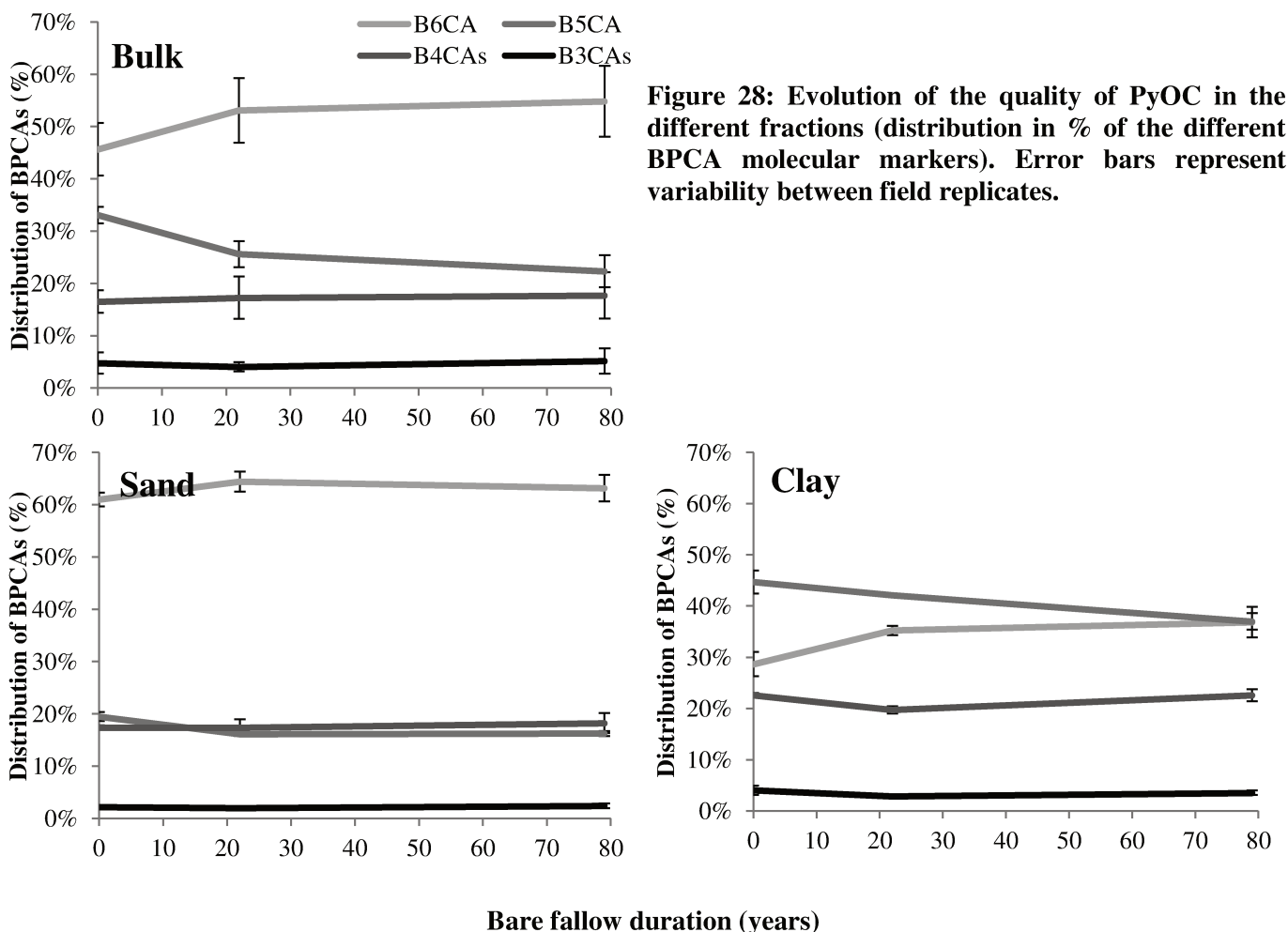
Clay and Sand fractions accounted for approximately 70% of total PyOC, thus, PyOC in the silt fraction represented approximately 30% of total PyOC. This proportion remained stable over the whole duration of the experiment.



**Figure 27: Evolution of PyOC in the fractions (% of TOC of fraction). Error bars represent the variability between field replicates (n=4).**

### 4.3.3. Distributions of the different BPCA molecular markers

The BPCA method also yielded information about the quality of the PyOC material. Four types of molecular markers were identified, indicating different degrees of aromaticity, from the most condensed B6CA to the less condensed B3CAs. The isolated size fractions displayed PyOC with contrasted chemical signatures (Figure 28), PyOC in the bulk had an intermediate distribution (around 50% of B6CA), PyOC in the sand fraction was the most condensed with more than 60% of B6CA whereas PyOC in clay fraction was less condensed (around 30-35% of B6CA). In the sand fraction, further BPCA analysis on individual pieces of the pyrogenic material isolated under a microscope showed even higher condensation of the material (B6CA distribution ranged between 68 and 72%).



By difference, PyOC in the silt fraction displayed an average distribution between the different BPCAs as follows: 42% of B6CA, 37% of B5CA, 15% of B4CAs and 6% of B3CAs

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in 1929 and 56% of B6CA, 22% of B5CA, 17% B4CAs and 6% B3CAs in 2008. These distributions resembled the distribution of the bulk soil.

In all three fractions, there was a significant evolution over 80 years of LTBF towards more condensed material (paired t.test initial/final, p-value<0.05). In terms of variability, size fractions had really low variability between field replicates, evidencing that PyOC isolated in these fractions was homogeneously found in all tested plots.



## 4.4. Discussion

### 4.4.1. PyOC in the size fractions display contrasted persistence

Results show that size fractionation efficiently isolated two different types of PyOC associated to different size fractions of the soil. In the sand fraction, the PyOC is persistent as we did not detect any significant evolution of the absolute content of PyOC associated with this fraction. Nevertheless, there have been reports of inputs of pyrogenic material during the bare fallow experiment of Versailles (Lutfalla et al. under review) during WW2 as a result of a bomb exploding nearby. This input was not detected in the bulk samples presented here because of the date selection (it happened between year 10 and 22 of the bare fallow, data shown in Lutfalla et al.). This input was not detected in the clay fraction either. We hypothesize that it consisted of sand-size fragments. Therefore, the apparent persistence of the PyOC material in the sand fraction could be misleading.

The PyOC present in the clay fraction decreased with time and its dynamics were almost identical to the dynamics of TOC in this fraction. As a result, the PyOC detected in bulk samples show an intermediate persistence: significant decrease occurred over the duration of the BF but the dynamics led to a lower persistence than the persistence of TOC. Overall PyOC was not inert; here we isolated different size fractions associated with PyOC displaying contrasted persistence. Because the bare fallow experiment prevented any inputs of fresh organic carbon through the absence of plants, TOC decreased with time in all fractions. The contrasted persistence of PyOC in the two size fractions led to different relative enrichments in PyOC as a proportion of TOC. The relative proportion of PyOC compared to TOC almost doubled in the sand fraction over the 8 decades of BF (from 5.6% to 9.7%) whereas there was no significant enrichment in the proportion of PyOC in the clay size fraction (2.0% on average).

The equivalent persistence of TOC and PyOC in the clay fraction detected in this study contradicts most literature that states that PyOC is more persistent than SOC (Forbes et al., 2006). Latest analysis of published data on the persistence of PyOC has shown that its persistence is not as long as previously thought. Field-based studies show mean residence times ranging from 90 to 750 years, estimated by a one-pool decay model (Singh et al., 2012a). Using simple mono exponential decay model on the present data, we find that the average MRT for TOC in the clay is 63 years (SD: 14y) and 81 years (SD: 12y) for PyOC (Table 12 in Supplementary material). Therefore, PyOC is only 1.3 times more persistent than

TOC in this fraction. Incubations have already demonstrated that pyrogenic material can decrease at similar rates than TOC in the first phases of biodegradation (Hilscher et al., 2009), but our results constitute first evidence of the possibility of an equivalent long-term persistence of TOC and PyOC.

A previous study showed that a lack of vegetation does not interact with the persistence of PyOC in soils (Vassilyeva et al., 2011). This study compared a 55 years bare fallow soil to soil from an adjacent native grass steppe. It was found that changes in PyOC contents were similar for long term bare fallow and vegetated soil. So we consider that the absence of organic inputs did not alter PyOC dynamics in the LTBF soils.

Different mechanisms can explain the decrease of PyOC in the bulk and size fractions: biotic degradation, photo-oxidation, leaching or erosion. We consider that the loss of PyOC observed in this study is mainly due to biotic processes, because, first, photo-oxidation impacts charred materials situated at the soil surface, but can only be of minor importance in our study since soil samples were extracted from the Ap-horizon (0-20 cm soil depth). Second, the experimental setup of the LTBF experiment ensured that losses of PyOC and SOC by horizontal transport (e.g. erosion) were insignificant during the fallow period, as all plots are fenced with plastic fences preventing erosion. Third, leaching can take place in this setup, however previous studies focusing on the potential losses of PyOC by solubilisation concluded to the very minor role of this process as compared to the biological degradation (Maestrini et al., 2014, Abiven et al., 2011).

#### **4.4.2. Clay-PyOC and sand-PyOC have contrasted chemistries**

Bulk PyOC was clearly more heterogeneous and coherently displayed more variability, as it resulted from the combination of contrasted PyOC from different size fractions. Looking at the distribution of the different BPCA molecular markers and comparing it with distributions of BPCA obtained on reference char material (Wiedemeier et al., 2015a, Schneider et al., 2010) we characterized the type of material in each size fraction. Bulk samples displayed the mixture of different pyrogenic material associated to the size fractions. Therefore, PyOC analyzed from bulk samples displayed an intermediate distribution of BPCA, i.e. it ranged from 46 to 53% of B6CA. These values correspond to reference chars from wood and grass produced at charring temperatures around 600 °C. PyOC associated with sand fractions was consistently more condensed. The proportion of B6CA in this fraction was comprised between 60 and 65% which would correspond to charring temperature exceeding 700 °C. The

abundant and extremely condensed individual pieces of char which were analyzed specifically showed that the material present in these fractions was probably not created during wildfires, as the temperature in these natural events is around 450 °C or 500°C (Turney et al., 2006, Campbell et al., 2007). Clay size fraction contained much less condensed PyOC, the proportion of B6CA ranged from 29 to 37 %, which is characteristic of chars created in the 350-500 °C temperature range (Wiedemeier et al., 2015a). We therefore hypothesized that the origins of the pyrogenic material associated to the different size fractions were contrasted. On the one hand, PyOC in the clay fraction would correspond to material created through natural burning of biomass (wood and grass) which is likely to have occurred on this experimental site a couple of centuries before the start of the bare fallow experiment. The last input probably occurred during the 19th century (before the construction of the buildings surrounding the experimental site, which happened during the second half of the 19th century). On the other hand, PyOC in the sand fraction could come from pieces of carboniferous coal used in heating systems and the input to this particular soil would have happened in the second half of the 19<sup>th</sup> century.

Evolution with time of the chemistry of the pyrogenic material was similar in all fractions. We observed a consistent evolution of PyOC quality towards more condensed forms.

#### **4.4.3. Protection mechanisms**

In terms of chemistry, we observed two types of PyOC, a condensed one, which was very persistent and another one, less condensed and less persistent, which had the same decomposition rate than SOC. This result is in line with the literature which finds good correlations between persistence and degree of condensation of pyrogenic material (Lehmann et al., 2015, Nguyen et al., 2010, Singh et al., 2012a). In terms of quantity, there was more PyOC in the sand fraction than in the clay fraction. This is in contradiction with most studies which find that coarse PyOM (found in the sand fraction) disappears more quickly than fine PyOC, either because of fragmentation or because of preferential protection of PyOC in microaggregates in the finer fractions (Kuzyakov et al., 2009, Hilscher and Knicker, 2011). Nocentini et al. (2010) found results similar to ours but with different size fractions. They studied four size fractions of pyrogenic material created during a wildfire and found that the smaller particles were less condensed and more reactive than the coarser particles.

The apparent persistence of pyrogenic material in the sand fraction is hiding an input of pyrogenic material during the bare fallow. However, as hypothesized, sand-PyOC still seems

to be more persistent than clay-PyOC. This is probably partly due to its high condensation which can make it more difficult to degrade but also to its physical properties. Indeed, it has been found that fragmentation of PyOC explains the rapid disappearance of PyOC from coarse size fractions (Kuzyakov et al., 2009, Hilscher and Knicker, 2011). Here the sand fraction displays the highest absolute and relative PyOC contents. Specific size and physical properties of the material could explain its resistance to both fragmentation and degradation.

In the clay fraction, most of the PyOC should be found in organomineral associations (Brodowski et al., 2005, Cheng et al., 2008, Hilscher and Knicker, 2010, Hilscher and Knicker, 2011). PyOC is initially hydrophobic, but very quickly negative surface charges appear and favor interaction with mineral surfaces. It is usually considered that PyOC in fine fraction is protected against biodegradation because of its interaction with mineral surfaces (Nguyen et al., 2009, Schnieder et al., 2011a, Vasilyeva et al. 2011). The results presented here contradict this finding by showing equivalent TOC and PyOC dynamics on the multidecadal timescale. We interpret this as a potential effect of the low-condensation of the material which would explain why the protection mechanisms similarly affect the long-term persistence of TOC and PyOC.

#### 4.5. Conclusion

The combination of long-term bare fallow samples and size fractionation procedure allowed us to study long-term dynamics of contrasted PyOC material in a same soil and to compare it with equivalent TOC persistence. We characterized a condensed and relatively persistent kind of PyOC in the sand fraction and a less condensed and easily decomposable PyOC in the clay fraction (same persistence as TOC in this fraction). PyOC persistence in the coarser fractions is explained by particular chemistry (chemical recalcitrance). PyOC persistence in the finer fractions is explained by interactions with the mineral particles (physicochemical protection against biodegradation). These values are the first long-term assessment of the dynamics of PyOC in size fractions of a temperate soil and they show that under certain conditions PyOC is not more persistent than TOC on centennial time scales.



PART 4: MINERALOGICAL CONTROL  
OF SOIL ORGANIC MATTER  
PERSISTENCE

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## **Submicrometric observations of multidecadal persistent organic matter in soil**

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## Abstract

Assessing how mineral surfaces, especially at small scale, can protect soil organic carbon (SOC) from biodegradation is crucial. The questions we address in this work are (1) does the pluri-decadal persistent soil organic matter stabilized by minerals have a specific chemical composition and (2) do different mineral species lead to different organo-mineral interactions and stabilize different quantities of soil organic carbon and different types of soil organic matter.

Here we used the unique opportunity offered by long-term bare fallows to study *in situ* SOC dynamics in several fine fractions of a silty loam soil. With no vegetation, i.e. no external input of fresh C, the plant-free soil of the Versailles 42 Plots (INRA, France) has been progressively enriched in persistent SOC during the 80 years of BF. Soil samples from 0, 10, 22, 52, and 79 years after the beginning of the BF (four field replicates per date) were selected. We carried out a particle size fractionation on all samples in order to isolate three sub-fractions in the clays: fine clay (0 - 0.05  $\mu\text{m}$ ), intermediate clay (0.05 - 0.2  $\mu\text{m}$ ), and coarse clay (0.2 - 2  $\mu\text{m}$ ). X-ray diffraction analyses showed contrasted mineralogies in the fine and intermediate clay (smectite and mixed layered illite/smectite) as opposed to the coarse clay (smectite, illite, kaolinite and mixed layered I/S). We performed elemental analysis and synchrotron-based spectroscopy and microscopy (NEXAFS bulk and STXM at the carbon K edge of 280 eV, CLS Saskatoon, Canada) to study the dynamics, the distribution and the chemical speciation of SOC in these fractions.

The quantity of SOC appeared to be stabilized after 50 years of bare fallow in all three subfractions, even though individual dynamics were contrasted. Coarse and intermediate clays had the same final C content. Fine clay experienced the highest C losses and displayed the highest final C content suggesting that it contained both more labile C and more persistent C. In all subfractions, C:N ratios were really low (below 8) and were decreasing with time, evidencing the dominant presence of N-enriched microbial SOC, especially in the finest subfractions. NEXAFS data showed that SOC became enriched in carboxylic and phenolic functions with BF duration. STXM-NEXAFS data showed that, in the smectitic fine and intermediate subfractions, during the first 50 years of bare fallow, all mineral particles were associated with SOC. On the contrary, in the coarse clays, SOC displayed more diversity: the chemical signature was more diverse and illite mineral particles that were not associated with SOC appeared faster in the chronosequence. Smectitic minerals (including both smectite and interstratified illite/smectite minerals) were therefore associated to more persistent carbon and seemed to display higher protective capacities than illite minerals. This may be due to higher chemical interactions between smectite particles and microbially-derived N- and carboxylic-enriched compounds.

## 1. Introduction

Soils represent an important carbon reservoir on the global scale as they store three times more carbon than the atmosphere (Batjes, 1996). The organic carbon in soils is found in molecules that mainly come from plants and microorganisms and that have different turnover rates. There is experimental evidence that some organic molecules have been present in soils for hundreds to thousands of years (Torn et al., 1997, Jenkinson et al., 2008).

Three main mechanisms have been invoked to explain this long-term persistence (Six et al., 2002, von Lützow et al., 2006): chemical recalcitrance, physical protection and physicochemical protection. Two of them involve the mineral matrix and are recognized as major protection processes. The first of these two mechanisms is physical protection of SOC through the entrapment of SOC within the soil matrix. Due to the complex 3-D architecture of soil porosity, microorganisms cannot access the SOC and it is therefore protected from biodegradation. The scale at which this protection is taking place is micrometric to nanometric (Chenu and Stotzky, 2002). The second of these two mechanisms is physicochemical protection of SOC through the sorption of SOC on mineral surfaces (by bonds of different energies: hydrogen, Van der Waals, complex-ligand...). The energy required to break these bonds acts as a protection from biodegradation (Baldock and Skjemstad, 2000, Sollins et al., 2006, Chevallier et al., 2003). Several studies have evidenced good correlations between the content of fine particles and the carbon content of a soil (Feng et al., 2013, Martin et al., 2011). This correlation could be explained by the preferential protection of SOC by mineral phases (Hassink, 1997, Six et al., 2002) though a lot remains to be done in order to understand the nature of the interaction between SOM and mineral phases. Phyllosilicates have been shown to protect SOM efficiently (e.g. Chevallier et al., 2003, Pronk et al., 2014). The studies which looked at the influence of the minerals on the protection of SOC showed that the nature of the mineral phase influences the quantity of protected carbon, in terms of particular clay types (1:1 or 2:1) for instance, as was demonstrated by Feng et al. (2013). The 2:1 minerals display higher surface areas and more precisely higher external surface areas than 1:1 minerals. Ordered by decreasing external surface areas we usually find: Ca-smectite > illite > kaolinite (e.g. Girard et al., 2005). But it is still not clear if and how the mineralogy of phyllosilicates impacts soil organic carbon persistence in soils (Barré et al., 2014).

Several studies have shown that particle-size fractionation allows for isolation of relevant pools displaying contrasted mineralogies (Laird et al., 1991, Hubert et al., 2012, Fernandez-Ugalde et al., 2016) which can then be used to study specific organomineral associations

(Laird et al., 2001, Gonzalez and Laird, 2003). However, few studies have investigated the nature of the SOM protected by organomineral interactions (Sollins et al., 2009) and the evolution of this protected SOM.

One major obstacle to the study of persistent carbon in soils is that it is nearly impossible to experimentally isolate persistent carbon from total organic carbon. In this work, we use the unique opportunity provided by long-term bare fallow experiments to study the long-term persistence of carbon in soils under field conditions. These experimental plots have been kept free of vegetation since the experiment started (by weeding) and regularly sampled. With time, biodegradation occurs, carbon contents gradually decrease as there is no input of fresh organic carbon and samples are therefore enriched in persistent carbon (Rühlmann, 1999, Barré et al., 2010).

Another obstacle to the study of SOC is the interaction with the mineral matrix which makes it difficult to use some experimental techniques and interpret the data thus obtained. Here, we isolated specific particle-size organomineral fractions from LTBF samples and studied them with synchrotron-based spectroscopy, namely Near edge X-ray absorption fine structure (NEXAFS) and scanning transmission X ray microscopy coupled with NEXAFS (STXM/NEXAFS). These two techniques constitute promising tools for the study of the influence of phyllosilicates in the protection of organic C at the nanoscale as highlighted in the review of Barré et al. (2014). They have already been used to characterize the nature of organic C in soil samples and to study specific organomineral interactions, in aggregates for instance (Lehmann et al., 2005, Wan et al., 2007, Keiluweit et al., 2012, Gillespie et al., 2015).

Using such techniques, Solomon et al. (2012) and Wan et al. (2007) detected an influence of the adsorbent mineral phase on the heterogeneity of OM distribution. Other studies looked at localization of specific OM and found heterogeneity in the distributions of compounds (Kinyangi et al., 2006, Wan et al., 2007, Lehmann et al., 2008, Solomon et al., 2012).

Combining LTBF samples and synchrotron-based spectroscopy we address three questions: (1) what is the chemical nature of long-term persistent SOM associated to minerals? (2) do different mineral phases protect different quantities of SOC? (3) does the SOC protected by different mineral phases have the same chemical composition and can we detect different organomineral associations?

## 2. Materials and method

### 2.1. Soil description and sampling

The INRA (Institut National de la Recherche Agronomique) 42 Plots experiment is a long-term bare fallow experiment which was started in Versailles (France) in 1928 to test the impact of fertilizers and amendments on soil structure (Burgevin and Hénin, 1939). Here, we focused on 4 of the 10 control plots. Since 1929, the soil has not been cropped and has been weeded (by hand or with the help of herbicides) and plowed (to the depth of 25 cm) twice a year. It is a non-carbonated brown soil (silty loam Luvisol: 16 % clay, 57 % silt, 27 % sand) and the control plots have an average pH value of 6.3 in 1929 and of 5.2 in 2008 (Pernes-Debuyser and Tessier, 2002, and Paradelo et al., 2013).

We studied soils sampled at 5 different dates and archived (stored air dried and in the dark) since then: 1929 (year 0 of the bare fallow), 1939, 1951, 1981 and 2008. Sampling depth was 0-25 cm for the 4 first dates and 0-20cm for the last date. We used samples issued respectively from plots 13, 21, 22 and 32 as replicates.

### 2.2. Particle size fractionation

Soil samples were subjected to a combination of a two-step physical dispersion and a particle-size fractionation adapted from Balesdent et al. (1998) and Fernandez Ugalde et al. (2016) to separate the following organo-mineral particle-size fractions: sand fraction ( $>50 \mu\text{m}$ ), two silt fractions ( $20\text{-}50 \mu\text{m}$ ,  $2\text{-}20 \mu\text{m}$ ), and three clay fractions ( $0.2\text{-}2 \mu\text{m}$ ,  $0.05\text{-}0.2 \mu\text{m}$ , and  $<0.05 \mu\text{m}$ ) (Laird et al., 1991; Hubert et al., 2012). Approximately 50 g of soil were shaken overnight with 20 glass beads in 180 ml of deionized water to break aggregates  $>50 \mu\text{m}$ . The resultant suspension from dispersion was then passed through a  $50\text{-}\mu\text{m}$  sieve to separate the fraction  $>50 \mu\text{m}$ , i.e. sand and free particulate organic matter. This step prevents the disruption of particulate organic matter during the subsequent ultrasonic dispersion (Balesdent et al., 1991). The suspension  $<50 \mu\text{m}$  was sonicated in an ice bath for a total input of  $320 \text{ J}\cdot\text{mL}^{-1}$  using a digital sonifier, max. output = 120 W, probe with a flat tip of 2.5 cm diameter, operating at 20 kHz (Sonics model 500W). The dispersion conditions allowed recovering a clay-size fraction ( $<2 \mu\text{m}$ ) equivalent in proportion to that achieved during standard particle-size fractionation (Balesdent et al., 1991). At that stage, the solution was centrifuged at 79g for 12 minutes to first separate the clay fraction ( $0\text{-}2\mu\text{m}$ ) then at 5g for 1 minute to separate the fine silt fraction ( $2\text{-}20\mu\text{m}$ ) and the coarse silt ( $20\text{-}50\mu\text{m}$ ) fraction. Sand and coarse silt

fractions were oven-dried at 60°C and weighed and fine silt and clay fractions were freeze dried and weighed.

The size fractionation of the clay was carried out on a subsample of 2 g of clays which were resuspended in water and sonicated at 320 J.mL<sup>-1</sup>. The sonicated suspension was then sequentially centrifuged at 23056 g for 61 minutes to separate the fraction <0.05 µm, and at 5764 g for 15 minutes 22 seconds to separate the fraction 0.05-0.2 µm and the fraction 0.2-2 µm. All clay subfractions were freeze-dried and weighed.

Total mass recovery was 99.1 % (SD=0.44%) for the first step of the fractionation, mass recovery for the clay fractionation was 98.3 % (SD=1.70%). Particle size distribution results (results not shown) showed no evolution with time. In terms of distribution of the different sizes, coarse silt and sand dominated the texture. Loss of organic C during fractionation procedures was small, total carbon recovery was 96.4% (SD=10%) on average.

### **2.3. Clay mineralogy analyses**

The mineral composition of particle-size fractions 2-20 µm, 0.2-2 µm, 0.05-0.2 µm, and <0.05 µm was determined by X-ray diffraction (XRD) analyses. Oriented deposits of an approximate density of 10 mg cm<sup>-2</sup> were prepared with the fractions by using the filter transfer method (Moore and Reynolds, 1997). The air-dried, oriented deposits were analyzed with a RIGAKU UltraX18HF X-ray diffractometer (RIGAKU, Tokyo, Japan). The XRD patterns were accumulated at 0.05° step intervals for 3 s counting time in the range from 3 to 35° 2θ by using Cu Kα radiation. The oriented deposits of the fractions separated in one replicate at all the selected sampling dates were then exposed to ethylene glycol vapor over 16 hours at 60°C. The XRD patterns of glycolated deposits were acquired in the same conditions as the air-dried samples.

### **2.4. Elemental analysis**

The total organic carbon (C) and nitrogen (N) contents of all particle-size fractions were measured by dry combustion in a CHN autoanalyser (Carlo Erba NA 1500). The soil contains no significant amounts of carbonates, so total C contents are equivalent to total organic carbon contents.

## 2.5. Synchrotron analyses

### 2.5.1. NEXAFS BULK

The ‘bulk’ carbon speciation of particle size fractions was been investigated by Near edge X-ray absorption fine structure (NEXAFS) spectroscopy at the carbon K-edge (270 – 330 eV) using the 11-ID-1 Spherical Grating Monochromator (SGM) beamline located at the Canadian Light Source (CLS – Canada) (Regier et al., 2007). The CLS storage ring is operated at 2.9 GeV and between 250 and 150 mA current. Two horizontal deflecting mirrors and one vertical focusing mirror direct the light from the 45mm planar undulator through the entrance slit and onto a 1700 lines/mm grating (low energy grating) which diffracts the light through a movable exit slit that has been set at 25  $\mu\text{m}$  for the present experiments. A pair of toroidal refocusing mirrors are used to focus the beam onto the samples in the endstation chamber under a working pressure of about  $10^{-7}$  Torr. A 100 nm thick titanium filter is used to remove the contribution of second order light. The beam spot size is approximately 1 mm  $\times$  100  $\mu\text{m}$  with a flux (normalized to 100 mA) greater than 1012 photons at 250 eV. This beamline measures the fluorescence yield of samples exposed to the incident beam as a function of its energy with a spectral resolution of about 0.1 eV. When normalized to the scattering intensity from a clean gold blank, fluorescence yields are directly comparable to absorption data, especially as the SGM uses an Amptek silicon drift detector placed at exactly 90 degrees to the incident beam allowing getting rid of scattering light. For the present study, dry particle size fractions were pressed onto gold foils (99.99 % purity – Sigma Aldrich) which were then mounted on a copper sample holder. Calibration in energy was done using a set of pure reference materials (calcium carbonate, benzoic acid and histidine (> 99 % purity – Sigma Aldrich)). Each spectrum reported in the present study corresponds to the average of about 50 measurements. Of note, only the first 100 nanometers of the sample surface are probed using the SGM setup. Spectra have been averaged, background subtracted and normalized using the Igor Pro software.

### 2.5.2. NEXAFS STXM

For NEXAFS STXM analysis, each clay fraction was finely ground with an agate mortar, suspended in filter-sterilized and deionized water (Milli-Q), applied to a  $\text{Si}_3\text{N}_4$  window with a micropipette, and air-dried. STXM analysis of zones identified by standard microscopes ( $\times 10$ ,  $\times 20$ ) was performed on the CLS. XANES data were collected on beamline 10ID-1 (SM beamline – Kaznatcheev et al., 2007) at the Canadian Light Source (CLS). Beamline 10ID-1 (CLS) works in the soft X-ray energy range (130–2500 eV) and is based on an elliptically

polarized undulator (EPU) which provides circularly polarized light (up to 1000 eV) and linearly polarized light (130- 2500 eV).. The CLS storage ring was operated at 2.9 GeV and between 100 and 250 mA current. The microscope chamber was first pumped down to 100 mTorr after sample insertion and back-filled with He. Rationale for the use of STXM to study biominerals can be found in Cosmidis et al. (2014). Energy calibration was accomplished using the well-resolved 3p Rydberg peak at 294.96 eV of gaseous CO<sub>2</sub> for the C K-edge. Chemical imaging and NEXAFS spectra were obtained using image sequence scans (i.e., stacks) over a range of photon energies at a specific element edge. The C 1s, K 1, Ca 2p, N 1s data were collected (in that order) over the same areas in order to identify the associations of OC with specific soil mineral phases. The stack measurements for each element were obtained with dwell times of 1-1.5 ms and with pixel sizes from 40-80 nm. Clean areas of the Si<sub>3</sub>N<sub>4</sub> membrane were used to normalize the transmission signal obtained from analyzed regions of interest. Transmission images at energies below and at the relevant absorption edge energies were converted into optical density (OD) images [OD = ln(I<sub>0</sub>/I), with I<sub>0</sub> being the incident photon flux and I the transmitted flux]. Image sequences (also called “stacks”) acquired at multiple energies spanning the relevant absorption edge (from 275 to 450 eV, 275-330 eV for C 1s edge, 390–440 eV for N 1s edge) were used to extract NEXAFS spectra. Alignment of images of stacks and extraction of XANES spectra were done using the aXis2000 software (v.2.1n). Normalization and assessment of spectral peak positions were determined using the Athena software package (Ravel and Newville, 2005). Extensive databases of reference C-XANES spectra were available from the literature (e.g., Solomon et al., 2009).

### **2.5.3. NEXAFS Data processing and deconvolution**

For STXM/NEXAFS, stack images were aligned via a spatial cross-correlation analysis, clean areas of the Si<sub>3</sub>N<sub>4</sub> membrane were used to normalize the transmission signal obtained from analyzed ROIs, and NEXAFS spectra were extracted from groups of pixels of ROIs using the aXis 2000 software package. Extracted C 1s NEXAFS spectra for the Bulk NEXAFS and STXM/NEXAFS were normalized using the Athena software package for X-ray absorption spectroscopy (Ravel and Newville, 2005). Edge step normalization was performed using E<sub>0</sub> values of 291.5 eV for C. NEXAFS spectra were normalized across the full recorded range. Peak assignments can be found in the figure captions. A semi-quantitative analysis of C 1s NEXAFS spectra (either extracted from distinct ROIs in the image for STXM/NEXAFS or on the bulk spectra for Bulk NEXAFS) was carried out by peak deconvolution using the Athena

software. The deconvolution procedure was applied using Gaussian transitions with parameters. Peak magnitude and energy for all Gaussian transitions were constrained, only the height of the Gaussian was free to vary.

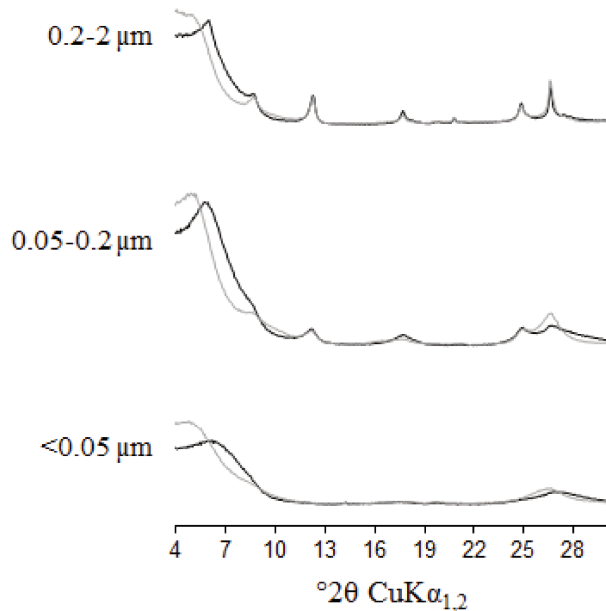
### **2.6. Statistical analyses**

The statistical analyses were conducted using the free software environment for statistical computing R (<http://www.r-project.org>). The significance of the difference between C contents or C:N ratios was assessed using pairwise t-tests. A level of significance of  $P = 0.05$  was considered for all analyses.



### 3. Results

#### 3.1. Mineralogies of the clay subfractions

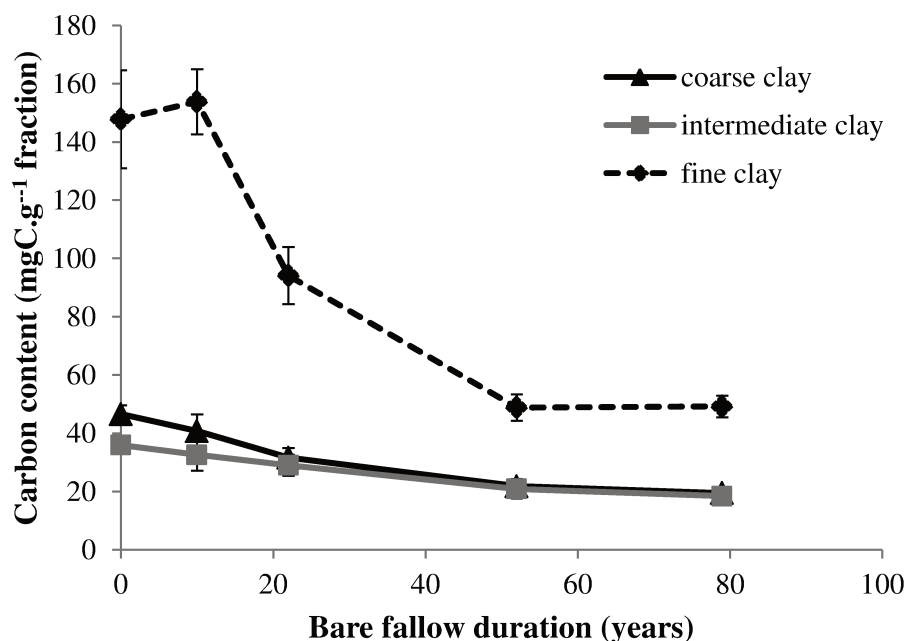


**Figure 29:** X-ray diffraction patterns, air-dried (dark line) and glycolated (grey line), of particle-size fractions in one of the replicated plot of LTBF sampled at the beginning of the BF. From top to bottom: coarse clay fraction (0.2-2  $\mu\text{m}$ ), medium clay fraction (0.05-0.2  $\mu\text{m}$ ), fine clay fraction (<0.05  $\mu\text{m}$ ).

XRD patterns were obtained for the three clay subfractions and showed no evolution during the 80 years of bare fallow (Figure 29). As expected from previous work on an adjacent and similar soil (Hubert et al., 2009, Fernandez Ugalde et al. 2016), the three clay subfractions displayed contrasted mineralogies, all dominated by phyllosilicates (Figure 29). On the one hand, coarse clay contains discrete smectite, illite and kaolinite. On the other hand, fine and intermediate clay XRD patterns display discrete smectite, illite and kaolinite as well as mixed-layered illite and smectite.

#### 3.2. Carbon and nitrogen dynamics are different in three subfractions

As expected, carbon content decreases with time in all three fractions and for the four replicates (see Figure 30), indeed the plant-free set-up prevents any significant carbon input during the bare fallow experiment and as time goes by biodegradation occurs and carbon is biodegraded, hence the observed decrease in carbon content. However, the initial carbon contents and ensuing dynamics differed between the three fractions (see Figure 30 and Table 9). The three clay fractions displayed contrasted initial C contents (paired t.test,  $p$ -value<0.05). Intermediate clays had the lowest initial carbon content,  $35.9 \pm 2.19 \text{ mgC.g}^{-1}$  fraction, coarse clay had an intermediate initial carbon content of  $46.6 \pm 2.95 \text{ mgC.g}^{-1}$  fraction and fine clay displayed the highest initial clay content of  $147.8 \pm 16.9 \text{ mgC.g}^{-1}$  fraction. This high amount of initial carbon could be partly due to the presence of dissolved organic carbon in this fraction (DOC). No evolution of the TOC content was detected between year 52 and year 79 of the bare fallow in all subfractions (significant for fine clays and intermediate



**Figure 30: Carbon content (mgC.g<sup>-1</sup> fraction) in the three different particle size fractions (error bars represent the standard deviation observed over the 4 field replicates)**

clays). In all three clay fractions, more than half of the initial carbon content was gone after 79 years of bare fallow (significant decrease, paired t.test initial VS final). Overall, coarse, intermediate, and fine clays have lost 59%, 49% and 67% of their initial carbon content. As a comparison bulk TOC decreased from 18 to 6 mgC. g<sup>-1</sup>, which corresponds to a loss of 67% of carbon over the 8 decades of bare fallow and bulk TOC still significantly decreased between year 52 and 79 (paired t.test).

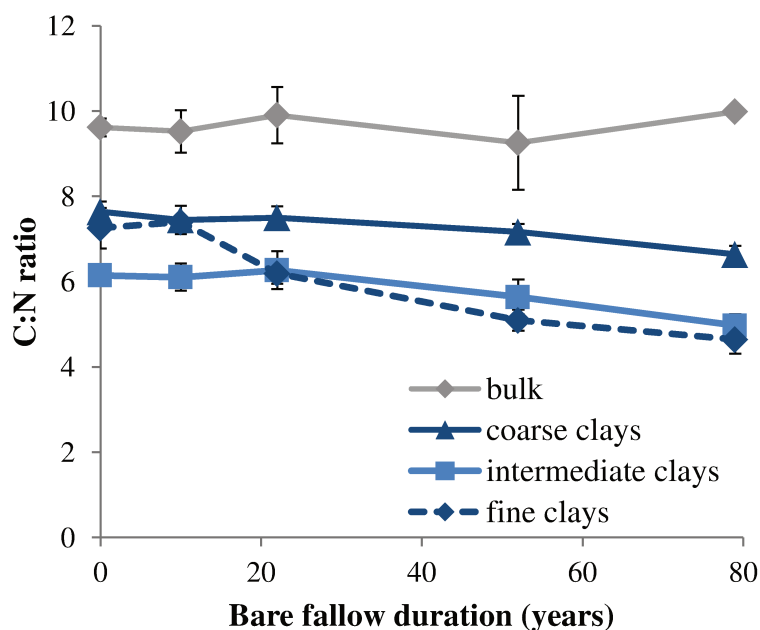
**Table 9: Initial and final carbon contents and corresponding loss of organic carbon in each of the three clay particle size fractions. Standard deviations are indicated between brackets.**

Fraction	Coarse	Intermediate	Fine	Bulk Soil
Size	0.2-2μm	0.05-0.2 μm	0-0.05 μm	
% weight of each fraction	66 (3.1)	25 (3.2)	7.2 (0.9)	-
Initial C content (mgC.g <sup>-1</sup> fraction)	46.6 (2.9)	35.9 (2.2)	147.8 (16.9)	18
Final C content (mgC.g <sup>-1</sup> fraction)	19.4 (1.2)	18.4 (2.2)	49.2 (3.7)	6
% lost initial vs final	59%	49%	67%	67%



### 3.3. Evolution of C:N ratios with time in all particle size fractions

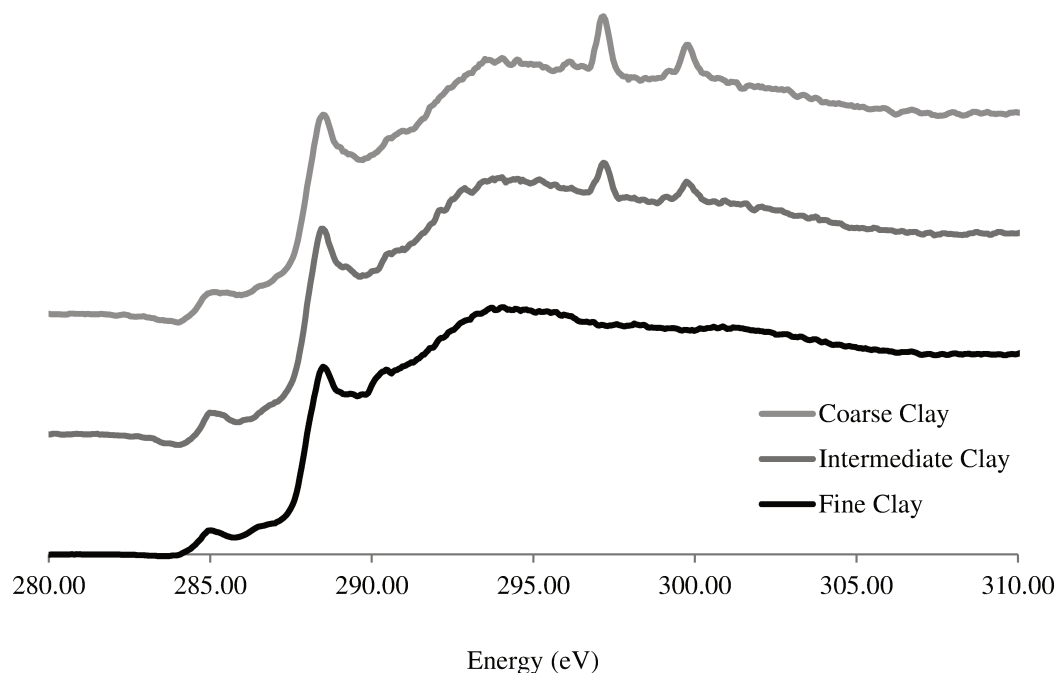
C:N ratio in the clay fractions was low, with individual values ranging from 4.3 and 7.9. Values were significantly lower than C:N in the bulk (Paired. t.test, p-value < 0.05)(Figure 31). C:N ratio decreased with time in all fractions and final C:N ratios were significantly lower than initial values (paired t.test, p-value < 0.05). Fine clay experienced the strongest decrease in C:N ratio: from  $7.3 \pm 0.5$  to  $4.6 \pm 0.3$  in 79 years of bare fallow.



**Figure 31: C:N ratio in the three particle size fractions and in total soil.**

### 3.4. Chemical composition of SOM associated to clay fractions as analyzed with NEXAFs spectroscopy

All C NEXAFS spectra of clay fractions consisted of peaks representing signals of aromatics and double C=C bonds (between 284.4 and 285.5 eV), ketones/carboxyamides (286.1-286.7 eV), phenols/ketones (287.1 eV), aliphatics (287.7 eV), amides/peptides (288.2 eV), carboxylics (288.6 eV), alcohols (289.2 eV) (Figure 32).



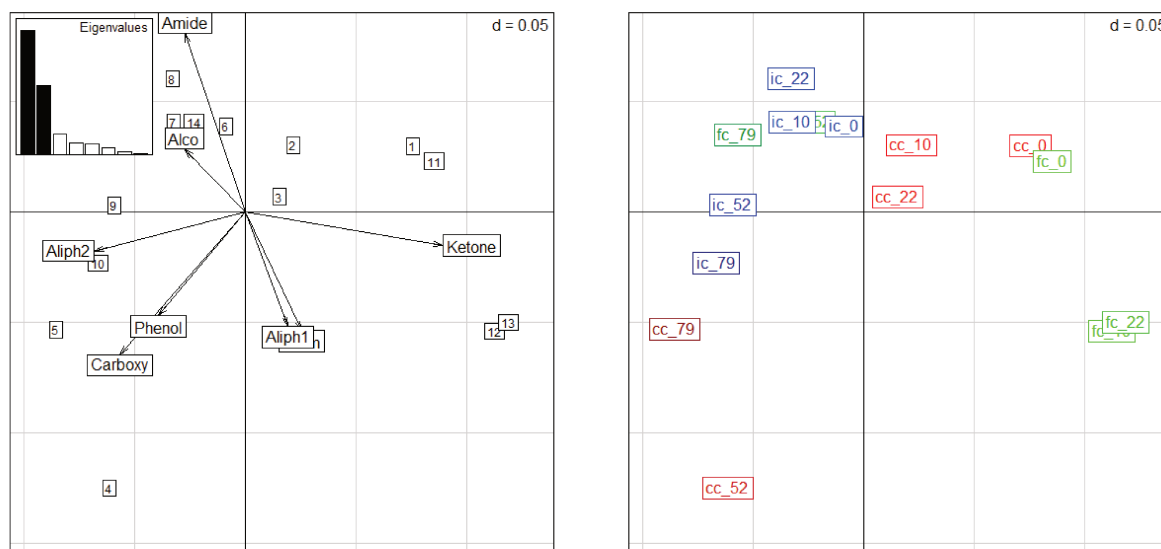
**Figure 32: Normalized Near edge X-ray absorption fine structure (NEXAFS) spectra for one of the four replicates of the BF at the initial sampling date (year 0 of bare fallow) and for the three clay subfractions.**

The three sub fractions displayed very similar NEXAFS spectra and there were no striking changes with time (see Figure 32). One slight difference between the three fractions came from the presence of a peak around 289.2 eV in the intermediate fraction which is attributed to the presence of alcohol and hydroxyl functions. The potassium peaks (297.1 and 299.7 eV) were visible in the coarse clay fraction, they appeared with a smaller height in the intermediate clay and were not detected in the fine clay which is the fraction with the highest carbon content.

Deconvolution of the data and PCA on the relative quantity of the different selected chemical functions showed that the three fractions had slight differences (Figure 33).

The evolution with time was coherent for coarse and intermediate clays, with a negative correlation to both the first and second axis in a PCA analysis. This translated a net increase in the proportion of phenolic and carboxylic groups and a small decrease in the proportions of ketones moieties with time. The fine clay fraction displayed a different evolution with time, which was not consistent across the 8 decades of the BF. It first consisted in an increase in

ketones, aliphatic and aromatic moieties and then a relative increase in alcohols and amides (see Figure 33).



**Figure 33: Results of PCA analysis for all samples of the three particle size fractions. Labels indicate the fraction (cc: coarse clay; ic: intermediate clay; fc: fine clay) and duration of BF at the sampling date (0, 10, 22, 52, 79). Axis 1 is horizontal, axis 2 is vertical.**

### 3.5. STXM/NEXAFS observations of clay fractions recovered after 0, 10, 22, 52, and 79 years of bare fallow

The energy range of the STXM/NEXAFS images allowed us to investigate the presence and chemical environment of four elements: carbon (K edge), potassium (L edge), calcium (L edge) and nitrogen (K edge). Based on the presence of these 4 elements, we defined five kinds of regions of interest (ROIs) in the images obtained, as can be seen in Figure 34: (1) regions where only carbon and nitrogen are detected (no K nor Ca), referred to as region R1, (2) regions where carbon, nitrogen and potassium appear (no Ca), referred to as region R2, (3) regions where carbon, nitrogen and Ca are detected (no K), region R3, (4) regions where all four elements are present (C, N, K, and Ca), referred to as region R4, (5) and regions where neither carbon nor calcium are present but only K and nitrogen are detected, region R5. We did not detect any regions containing only Ca, it was only detected in combination with organic carbon.

Calcium and Potassium are the signature of mineral phases in the clays. They are present in the interlayer and surface sites of phyllosilicates, potassium being found in illites and calcium

in swelling clays or in very discrete regions containing calcium carbonates (the soil is not carbonated). Furthermore, no significant amounts of feldspar were detected in the XRD patterns. Carbon is present only in organic matter, and nitrogen can be present under both mineral and organic forms ( $\text{NH}_4^+$  or nitrogen rich organic matter). Indeed, ammonium ions can be also be found in phyllosilicates interlayer sites, mostly replacing potassium ions in high charge (illite) clay layers.

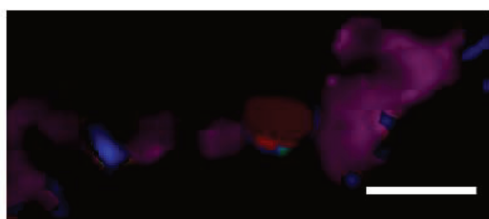
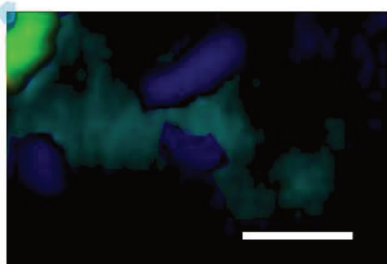
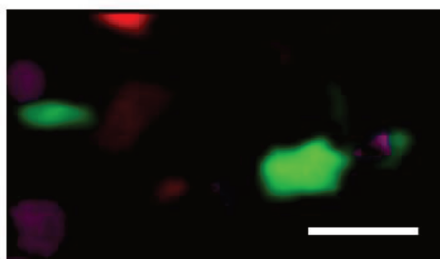
We analysed all the pixels in our STXM images and normalized the distribution of these pixels between the five ROIs. The summary of the occurrence and relative importance of the different regions for each date and each clay subfraction is presented in Table 10. Region 1 occurred only twice, showing that, with our resolution (~50 nm) most of the OM appeared to be adsorbed on mineral surfaces. Region 1 was only observed in coarse clay fractions. At the beginning of the bare fallow, when the carbon contents were still high in all fractions, we only detected regions of interest showing colocation of minerals and organic matter (Regions R2, R3 and R4). The first occurrence of a mineral signal without significant amounts of associated organic C (region R5) occurred after 22 years of bare fallow, in the coarse clay. At the last sampling, after 79 years of bare fallow, the three clay fractions displayed R5 regions.

**Table 10: Occurrence and importance of the different assemblages of organic matter and minerals (distribution of pixels which display STXM/NEXAFS signal in the different regions defined in the text).**

Sample	Region 1 OM (%)	Region 2 OM +K (%)	Region 3 OM + Ca (%)	Region 4 OM + K + Ca (%)	Region 5 K (%)	Total (%)
1929 coarse clay				100		100
1929 intermediate clay				100		100
1929 fine clay			100			100
1939 coarse clay		100				100
1939 intermediate clay				100		100
1939 fine clay				100		100
1951 coarse clay	50			26	24	100
1951 intermediate clay				100		100
1951 fine clay				100		100
1981 coarse clay	4	28		68		100
1981 intermediate clay				100		100
1981 fine clay			6	94		100
2008 coarse clay		60			40	100
2008 intermediate clay		82			18	100
2008 fine clay		89			11	100

Spectra for region 1 had a really low signal:noise ratio and are not represented here. They differed between the two occurrences: in 1951, the OM spectrum was dominated by a sharp peak at 288.2 eV (amide moieties) whereas the OM in 1981 displayed a broad peak around 285.5 eV (aromatic and C=C double bonds).

Region 2 is a region that displays organomineral interactions involving potassium-containing minerals and organic matter. The chemical signature of this organic matter, was relatively similar across fractions and dates, showing a strong aromatic signal around 285 eV and a sharp carboxylic peak (288.6 eV) (Figure 35).



**Figure 34: Optical density map of particle size fractions with colored regions of interest (n=5) (white line is 1  $\mu\text{m}$ ).**

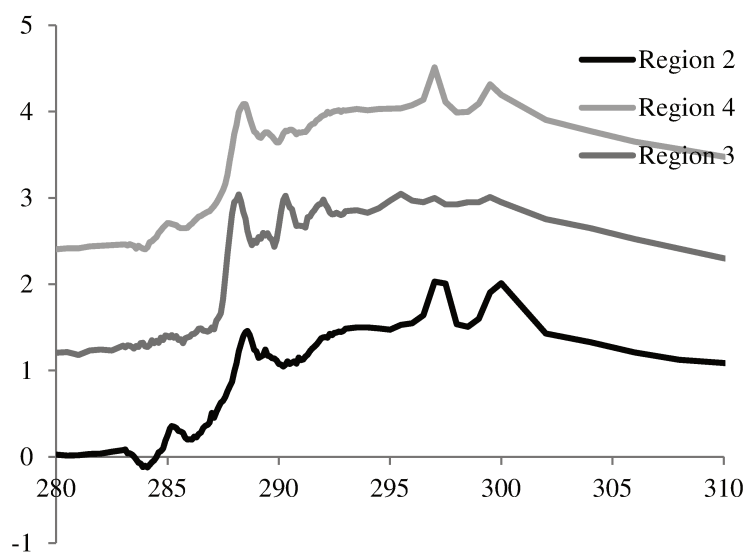
Region 3 corresponds to the organomineral association of Calcium compounds with organic matter. The chemical signature of the associated organic matter was different than in regions involving potassium. No specific aromatic or C=C bonds were detected and the spectra was dominated by a amide/peptide peak at 288.2 eV. The peak at 290.3 eV evidenced the presence of very discrete regions containing calcium carbonate. The absence of peaks at 297.1 and 299.7 eV confirmed the absence of potassium (Figure 35).

Region 4 contains organomineral associations involving both calcium and potassium minerals and organic matter. The corresponding C spectra was similar than the one of region 2: a peak around 285 eV corresponding to aromatic and C=C bonds, a sharp peak at 288.6 eV corresponding to carboxylic functional groups.

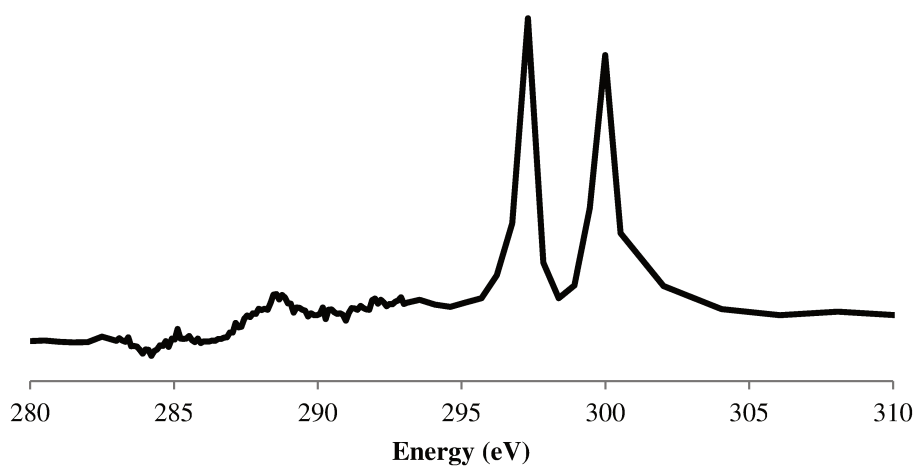
We detected the two potassium peaks at 297.1 and 299.7 eV and we did not detect any significant peak at 190.3 eV showing no significant amounts of calcium carbonates (Figure 35). This ROI was only observed for fine clays.



Spectra for region 5 showed the characteristic peaks of Potassium at 297.1 and 299.7 eV and the nitrogen step, no other peaks are detected (Figure 36).



**Figure 35: Average spectra at the Carbon edge of 3 regions of interest containing both organic and mineral material.**



**Figure 36: NEXAFS spectra of region 5 containing potassium and no significant amount of OM.**

## 4. Discussion

### 4.1. Contrasted carbon dynamics in the three clay fractions

The three clay fractions displayed contrasted carbon degradation dynamics that cannot be clearly related to their mineralogy but which seem related to the presence of microaggregates in the coarse clay fraction. Fine, intermediate and coarse clays lost 67, 49 and 59% of their SOC, respectively. Coarse and intermediate clays lost less C compared to bulk soil whereas fine clays lost as much C as the bulk soil. Fine clays displayed the highest final C contents and were therefore associated to the highest amounts of both labile and persistent SOC. It can be partly explained by the presence of labile dissolved organic carbon in the finest fraction, as explained by Balesdent et al. (1998) and Tiessen and Stewart (1983), which is favored by the fractionation protocol as highlighted by Laird et al. (2001), and Fernandez-Ugalde et al. (2016). This hypothesis explains why the finest fraction lost more carbon during the first 50 years of bare fallow (67%). In turn, the higher specific surface area of the minerals in the fine fraction can explain the higher amount of carbon in the final samples. This result is in agreement with the fact that the smectitic minerals contained in this fraction display higher specific surface areas and are thus able to bind with more organic carbon as proposed by Kaiser and Guggenberger (2000) or Kahle et al. (2004). Coarse and intermediate clay fractions had similar C content after 80 years of bare fallow. As evidenced by Chenu and Plante (2006), stable micro-aggregates can persist in fractionated soils and are likely still present in the coarse clay fraction. These microaggregates explain why coarse clays, although displaying smaller specific surface areas have a similar C content as intermediate clay fractions after 80 years of bare fallow. The mass distributions between the different subfractions are remarkably constant with time, showing that the microaggregates persist with time, at least during 79 years.

Although their dynamics are different, the three subfractions seem to have reached relatively stable carbon contents after 50 years of bare fallow. Indeed no significant differences were observed between the carbon content of each fraction after 50 and 80 years of bare fallow treatment. Two explanations can be put forward to explain such stability in C contents: minimum protective capacity of the clay minerals and stable microbial biomass. Several studies have hypothesized that clay minerals have a maximum protective capacity which would correspond to the amount of C they could bind and protect from biodegradation (Six et al., 2002, Feng et al., 2011). In our case, stable carbon contents after several decades of BF could represent a “minimum protective capacity”, i.e. the amount that will keep being

protected after drastic conditions and biodegradation of most of the organic carbon. Another process that participates in the stabilization of the carbon content is microbial activity. It can indeed be rightly hypothesized that after several decades of continuing biodegradation, most of the organic carbon found in the small particle-size fractions comes from microbial origin: either as a part of the living microbial biomass or as part of formerly living microorganisms and exudates of said biota. In that case, we can imagine that the system has evolved from an initial steady-state –characterized by continuing inputs of fresh carbon- to a new steady-state where inputs are mostly microbial. In this supposed newly reached steady-state, the microorganism communities would have reached stable reduced population sizes which would be sustained by the small inputs of OM from the coarser particle-size fractions. This is in accordance with the observation that bulk soil organic carbon continues to decrease whereas the clays have reached equilibrium. The next years and decades of this long-term bare fallow will provide precious insights on these microbial ecosystems.

#### **4.2. The chemical signature of the OM in the three fractions shows an enrichment in nitrogen-rich compounds and in more oxidized forms of OC**

As hypothesized, C:N ratios decreased with time in all subfractions, reaching particularly low values. In accordance with the classical result of increasing C:N ratio with increasing sizes of particle-size, fractions clay fractions display lower C:N ratio than the bulk (Christensen, 1992, Balesdent, 1987, Balesdent et al., 1998). C:N ratio in the fine clay displays the sharpest decrease, in agreement with the initial presence of high C:N DOC which is biodegraded in the first years of the bare fallow. C:N ratios had similar final values in the smectitic fine and intermediate fractions and were lower than in the coarse fraction. It can be due to the presence of plant-derived organic material with higher C:N ratios entrapped in microaggregates in the coarse clay. This provides further support for the likely presence of microaggregates in the coarse clay. Similarly, Chenu and Plante (2006) and Chenu et al. (2009) observed fine plant debris within < 2  $\mu\text{m}$  aggregates. This less processed plant-derived material would participate in the higher global C:N ratio observed in the coarse clays. Additionally, Laird et al. (2001) observed a decrease of C:N with decreasing clay-size and increasing smectite content in a soil with a great content of smectitic minerals.

The decrease of C:N ratio can be explained by both a more microbial OM and a selective enrichment in nitrogen-rich compounds (Gleixner et al., 2002). On the one hand, no fresh inputs of high C:N ratio compounds from plants will lead to a decrease in C:N ratio. Moreover, final C:N ratios are really low (from 4.3 to 6.9) and seem to point towards

predominance of bacteria over fungi (Sterner and Elser, 2002, Frey et al., 2004, Högberg et al., 2006). On the other hand, as nitrogen moieties bind easily to mineral surfaces they can be selectively preserved. This finding can also be interpreted as an experimental evidence for the conceptual work by Kleber et al. (2007). Indeed, SOM protected by minerals should be enriched in nitrogen as, when we isolate SOM from fine fractions, we are able to isolate larger proportion of SOM located in the contact zone where amphiphilic N-rich molecules are linked by electrostatic bonds to the mineral surfaces.

In terms of carbon speciation of the OM, NEXAFS analysis of the samples did not detect significant differences between the three fractions. They all display patterns representative of microbial organic matter (Keiluweit et al., 2012, Kleber et al., 2011). However, with time, the chemical signature of the fractions shifted towards more oxidized forms of carbon. Indeed, coarse and intermediate fractions showed a clear evolution toward increased proportions of carboxylic moieties as evidenced by the PCA analysis on the deconvoluted NEXAFS spectra. The fine fraction displayed more irregular evolution with time, attributed to the presence of the labile DOC at the beginning of the bare fallow. The oxidized nature of persistent carbon has already been documented (von Lützow and Kögel-Knabner, 2010). We interpret this relative increase in oxidized moieties as evidence for the preferential binding of these functional groups to the mineral surfaces of the clay fractions. Indeed, these chemical structures bind to minerals more easily than reduced forms of C (aliphatic chains for instance). Carboxylic groups will interact strongly with multivalent cations and protect the molecule from biodegradation (Baldock and Skjemstad, 2000, Oades, 1988).

### **4.3. Identifying the different organomineral interactions**

#### **4.3.1. Microaggregates**

The localization of the OM and its interaction with mineral species was contrasted between the different size fractions. In the coarse clay, four out of the five regions defined were found over the 8 decades of bare fallow, whereas the other fractions displayed only three. The diversity of organic forms detected was more important in the coarse fraction (Table 10). Moreover, the coarse fraction was the only fraction which displayed OM without minerals (Region 1). We interpreted this finding as evidence for the presence and persistence of micro and nanoaggregates entrapping particulate or amorphous OM in the coarse clay fraction as observed in Chenu and Plante (2006). Such result also means that different mechanisms of

persistence are occurring in the different clay fractions: organic matter found in fine and intermediate clays would be almost exclusively protected by adsorption with the mineral surfaces, while coarse clays would provide additional protection by occlusion in aggregates as was already proposed in Balesdent et al. (1987). This additional protection mechanism could compensate for the supposed lower specific surface area of the coarse clay and explain the similar final C contents of coarse and intermediate fractions. Furthermore, the presence of OM without minerals even after 50 years of bare fallow indicates that such nanoaggregates can persist in soils for long periods of time as was proposed by Fernandez-Ugalde et al. (2016).

### **4.3.2. Different organomineral interactions**

#### *4.3.2.1. Variability*

Five types of organomineral interactions were detected in the size fractions of the chronosequence. Pure organic matter showed strong aromatic and carboxylic moieties, which are characteristic of plant-derived material such as lignin, cutin or suberin. The only minerals detected without organic matter were displaying a strong potassium signal and no signal for calcium. Illite clays are the only minerals corresponding to this signature in the soil studied.

The three organomineral interactions involving both organic and mineral material displayed contrasted characteristics. Aromatic structures were much more present in the OM associated to potassium only, i.e. OM in interaction with illites. The carboxylic peak detected in this interaction with illites was not present in the OM associated with calcium, i.e. smectite clays. Indeed, in these organomineral associations, the energy is shifted and corresponds to amide and peptide functional groups (288.2 eV). This finding points towards a strong influence of mineralogy on the nature of the OM which interacts with mineral surfaces. Nevertheless, further research work is needed in order to confirm this result, as the small number of pixels analyzed for two of the three regions involving both mineral and organic material prevents us from drawing definitive conclusions.

The chemical nature of this increasingly present nitrogen is not completely elucidated. Indeed, STXM/NEXAFS data of these samples showed no definite peaks. Such imprecise patterns for nitrogen have already been observed in organomineral associations (P. Nico, not published) and the explanations are still speculative. Nitrogen could be contained in nitrogen-rich compounds (microbially-derived, peptides, amides...) and thus be preferentially adsorbed on

mineral surfaces. Nevertheless, the evidence for STXM regions containing nitrogen and potassium without any significant amounts of carbon seems to point towards the additional presence of ammonium held on clay and not on organic matter, as already proposed in Bremner (1959) and reported by Jenkinson et al. (2008).

#### 4.3.2.2. *Evolution with time*

With time, new types of organomineral interactions appeared. Indeed, during the first 20 years of bare fallow, only organomineral associations involving organic matter and minerals were detected. The first occurrence of minerals without significant amounts of OM appears after 20 years of bare fallow. At the last sampling session, after almost 80 years of biodegradation, all subfractions displayed minerals without OM. As mentioned in the results, our data show that calcium is always found associated with OM. Specific interactions between calcium and organic matter has been reported in the literature, either under the form of carbonates or under the form of calcium salts (Duchaufour, 1976, Muneer and Oades, 1989, Baldock and Skjemstad, 2000). Furthermore, multivalent cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) can create bridges between mineral surfaces and organic matter and haven been used in conceptual models in explaining organomineral interactions (Kleber et al., 2007). Colocation of calcium and organic matter has already been detected using STXM/NEXAFS (Benzerara et al., 2004, Chen and Sparks, 2015). In the present study, the soil studied is non-carbonated, but we detected spectral patterns characteristic of calcium carbonates in one of the regions (region 3). The other region containing calcium (region 4) did not show these spectral patterns and therefore we interpret the presence of calcium as evidence for the presence of swelling clays such as smectites.

#### 4.3.2.3. *Different protection capacities*

As mentioned in the introduction, very few studies have looked at the influence of the type of phyllosilicates on the persistence of OM, and those who have often acknowledged that they did not know if total dispersion had been attained (Barré et al., 2014).

Our data suggest that mineralogy significantly influence organic C stabilization by direct adsorption. Indeed, phyllosilicates containing potassium (illite) were detected without significant OM loading (regions 5) and the relative abundance of their surfaces increased over time. On the other hand, smectites probed by their calcium were always found in association with organic matter over the chronosequence. Furthermore, fine clays –dominated by smectitic minerals- protected a larger amount of carbon than the two other subfractions. We

then conclude that smectite is more efficient at storing organic carbon by adsorption than is illite. This is a clear experimental evidence of what is generally considered, as stated in Bruun et al. (2010): among phyllosilicates, smectites have a higher protection capacity than illite and kaolinite.

Based on these results, the protection capacities of the different clay minerals present in the soil studied rank, from more efficient to less efficient: smectite and interstratified illite and smectite > illite.

## 5. Conclusion

In this study we analyzed the long-term dynamics of carbon degradation in size fractions of contrasted mineralogies. The combination of size fractionation and nanoscale visualization techniques allowed us to study individual organomineral associations *in situ* over eight decades of biodegradation in a long-term bare fallow experiment.

Our study showed that the persistence of organic matter found in organomineral associations favours N-rich compounds and carboxylic functional groups, probably because of their specific binding properties. We detected particulate organic matter in the coarser subfraction which was interpreted as the presence of microaggregates. Organic matter in the coarse clays was therefore protected by both adsorption and entrapment in microaggregates. Organic matter in the fine and intermediate clays was mainly protected by adsorption. As the chemical signature of POM and OM protected by adsorption is relatively similar, we were not able to detect these different protection mechanisms by NEXAFS spectroscopy on the fractions.

We found that the carbon speciation of the OM associated to different size fractions is relatively similar, whereas the evolution of the carbon content with time differs in the three subfractions. We detected five different types of organomineral associations, from pure organic matter to pure minerals. Furthermore, the organic material found in association with different minerals displayed contrasted chemical signature depending on the type of mineral it was bound to.

Finally, our results provide first insights in the apparent longer persistence of OM bound to smectitic phyllosilicates and show that nanoaggregates are structures that can persist and protect SOM from biodegradation in soils for several decades. We showed that smectite and interstratified illite and smectite seem to have higher protection capacities than illite. Further research work is needed in order to confirm these exciting new findings.





## PART 5: CONCLUSIONS, TAKE HOME MESSAGES AND PERSPECTIVES

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## 1. Global context of the work

Estimations of the quantity of carbon stored in soils show that there would be approximately 1500 Gt of carbon in the top meter of soils worldwide (Jobbágy and Jackson, 2000). They are the biggest terrestrial carbon reservoir, containing more carbon than the biomass. In comparison to the atmospheric pool of carbon, soils contain three times more carbon than the atmosphere. As such they are of tremendous importance when studying the global carbon cycle.

Soil organic carbon has three main possible origins: (i) it can belong to the living organisms that populate the soil, (ii) it can be part of the dead organic matter, i.e. belong to the organic tissues of formerly living organisms which populated the soil or the surface of the soil, (iii) it can be created by fire and be part of the pyrogenic organic carbon pool.

The fate of organic matter in soils is to eventually be biodegraded by microorganisms or exit the system through abiotic processes. Nevertheless, there is experimental evidence that soil organic carbon can persist in soils for very long periods of time. Radiocarbon dating has evidenced that some soil organic matter has persisted in the soil for hundreds to thousands of years (Trumbore, 1997, Jenkinson, 2008). The reasons for this persistence are still discussed in our scientific community and this work aimed at furthering our understanding of long-term persistence of organic carbon in soils.

Three main mechanisms are invoked to explain the persistence of carbon in soils. They act as a protection against biodegradation. The first mechanism is chemical recalcitrance, it relies on the intuitive concept that chemical complexity (size, shape, functional groups) of the SOM will affect the rates of biodegradation of individual compounds in soils. The more complex a molecule, the more “recalcitrant” it is and the longer it will persist in soils. This mechanism has received a lot of attention but it is now accepted that it only affects the first stages of biodegradation and cannot explain long-term persistence of carbon in soils. The second mechanism is physical protection through spatial inaccessibility. Organic carbon entrapped inside aggregates is protected from biodegradation because microorganisms cannot access it. However, the structure of a soil is not a static property. There is evidence that macroaggregates are regularly broken down and reformed and hence, protection by entrapment of organic matter in macroaggregates cannot explain long-term persistence in soils. Research is now focusing on the study of microaggregates at the silt and clay-size in order to see to which extent these microstructures can protect carbon from biodegradation in

the long term. The third mechanism also involves the mineral matrix, it is the physicochemical protection through organomineral interactions. Adsorption and other interactions between the surface of mineral particles and organic molecules are characterized by bonding energies. Microorganisms need to spend this extra energy in order to biodegrade the molecules interacting with mineral surfaces. That energetic barrier acts as a protection against biodegradation. New imaging techniques and spectroscopic tools provide precious information on this mechanism by enabling the submicrometric visualization of organomineral interfaces.

Though these three mechanisms have been identified, conceptualized and evidenced in experimental studies, a lot remains to be done in order to understand their relative importance, and their interactions. The present doctoral work provided insights on the different protection mechanisms by looking at the characteristics of organic carbon progressively enriched in its most persistent components.

The main findings of this work are summarized here, put into perspective and enlarged to future research prospects.

## **2. Chemical oxidations cannot isolate centennial carbon**

The first part of this manuscript aimed at determining if common oxidation techniques could efficiently mimic biotic degradation of the organic matter. NaOCl and H<sub>2</sub>O<sub>2</sub> have been used as oxidizing reagents in order to isolate “refractory” organic matter. Several studies have used this as a way to gain insights on the particular chemistry of the pool of carbon resisting oxidation. Extrapolation of these results to the “stable” pool is frequent; here, we wanted to use the unique opportunity offered by long-term bare fallows to test the validity of such extrapolations. Using samples from the longest bare fallow of the LTBF Network (Versailles), we tested the effect of two reagents on the carbon contents of initial and final samples. These samples displayed contrasted carbon contents and we compared the carbon contents of the organic matter resisting the oxidation protocol.

Results showed that chemical oxidation using the two tested oxidizing agents (NaOCl and H<sub>2</sub>O<sub>2</sub>) did not allow to quantitatively isolate a stable C pool with centennial turnover times. Further analysis of the data using simple one-pool mono-exponential decay models to characterize the kinetics of biodegradation showed that both reagents can, at best, isolate a small part of the C pool with a turnover time of a few centuries (equivalent to the slow pool of the Century or the Daisy model)..

Overall, we have shown that chemical oxidation creates conditions which are too harsh to be compared to biotic degradation. Eight decades of long-term bare fallow led to the loss of two thirds of the initial carbon in the samples. In comparison, 18 to 180 hours of contact between the oxidizing agent and soil samples removed between 83 and 94% of the initial carbon.

Although, it is tempting to try and reproduce the natural processes happening during biodegradation of organic matter in soils, it is more and more accepted that chemical oxidations are not efficient in that matter. Furthermore, these methods only deal with oxidation processes whereas the impact of microbes on soil organic matter through biodegradation and decomposition also involves a progressive evolution of other characteristics: the nitrogen contents of the organic matter evolves, there is a progressive incorporation of the OM in the soil structure which will in turn affect the protection mechanisms and the likelihood of persistence or biodegradation.

### **3. Modest but consistent changes in the bulk chemical signature of OM after several decades of biodegradation**

In the second part of the present work, we aimed at studying the chemical characteristics of SOM as it becomes more persistent in order to gain insights on the mechanisms explaining long-term persistence of SOC. Using synchrotron-based NEXAFS spectroscopy, we analyzed samples of bulk soil from four sites of the LTBF Network. Results show that, with the increase in the proportion of persistent carbon, the gross chemical signature modestly shifts towards more oxidized functional groups. Indeed, all sites displayed an increased proportion of carboxylic moieties with time. This is interpreted as an evidence of preferential adsorption of polar groups to mineral surfaces which acts as a protection against biodegradation.

NEXAFS spectroscopy allowed us to detect the modest changes occurring in the chemical signature of SOM when it is subject to several decades of biodegradation. Though requiring little sample preparation and being relatively little impacted by the presence of the mineral phase, this technique is not without drawbacks. Indeed, it provides information on the proportion of the different functional groups present in the SOM but does not highlight the molecular structure of the SOM. Organic molecules undertake a highly dynamic journey when they persist in soil; they will react in the soil solution, they will be incorporated in living organisms, be exudated, cut, rearranged, adsorbed, solubilized, mineralized... In that regard, NEXAFS seems to provide oddly passive information about the characteristics of SOM;

giving only indirect cues about the global environment of OM (living organism, plant debris, individual compounds adsorbed on minerals,...).

To my opinion, in order to further our understanding of the long-term persistence in soils we need to bring together several methods. Each analytical technique has limitations and we therefore need a multi-methodological approach when examining SOM chemistry (Leinweber et al., 2013). Indeed, no single technique used to characterize SOM contains enough information about the nature of SOM to characterize the processes happening on multidecadal timescales. Getting information about these dynamic rearrangements of the OM is crucial and we need to develop a more comprehensive understanding of these processes as results from a single analytical technique may be misleading and provide only a limited understanding of SOM chemical quality.

Analytical tools to characterize SOM can be classified in four different groups: techniques which provide information on the chemical structure at the molecular scale (Derenne and Quénéa, 2015), techniques which characterize the gross chemical signature in terms of functional groups (Kögel-Knabner, 1997, Lehmann et al., 2009), techniques which give insights on thermal and energetic properties of the OM (Plante et al., 2009, Saenger et al., 2013, Disnar et al., 2003, Sebag et al., 2006). All of these constitute different pieces of the puzzle and the results they bring need to be considered together in order to elucidate the long-term persistence of SOC.

Although outside of the scope of this doctoral work, analyses corresponding to the different categories presented above have been carried out on samples from the LTBF Network. Like NEXAFS spectroscopy, they also highlight consistent trends in the evolution of the SOM and complement nicely the present study. For instance, Rock-Eval 6 pyrolysis and Thermogravimetry – Differential scanning calorimetry (TG- DSC) were used on samples from the LTBF Network and show that as biodegradation occurs, the OM becomes more thermally stable and generates less energy when combusted. Furthermore, Curie Point Pyrolysis coupled with Gas chromatography and mass spectrometry (Py-GC-MS) on TMAH-treated samples evidence a consistent evolution for three out of the four sites studied, analysis of the results show a global decrease in plant-derived compounds and a relative increase in acid and amino acid moieties. This is in agreement with the fact that no fresh organic matter inputs occur and that OM becomes enriched in polar and oxidized compounds.

It is only through the combination of these pieces of information that we can start building a better understanding of what drives long-term persistence of carbon in soils. All in all, it seems to point towards the fact that persistence of OM is the result of a high energy cost and low energy gain that come with the biodegradation of this material. Moreover, the observed energetic and thermal properties of persistent OM are consistent with an enhanced protection by organomineral interactions.

Further research should therefore involve the use of a combination of different analytical tools. On the particular topic of NEXAFS analysis, comparing spectra from soil samples and spectra from reference material mixed with pure minerals could allow a better assessment of the sensitivity of the method. Additionally, comparisons of the results obtained on LTBF samples and on equivalent vegetated experiments should be carried out. Finally, useful information could be gained from the analysis of samples taken deeper in the soil profile. Indeed, all samples analyzed here come from the top layer (25-30 cm depth depending on the site).

#### **4. PyOC is not particularly persistent in soils**

In this part of the present study, our goal was to shed light on the assertion of several scientific publications, which suggest that long-term soil carbon storage with pyrogenic carbon is potentially possible. The specific chemical nature of this type of soil organic carbon is thought to be protecting it from biodegradation. If this hypothesis was to be verified, climate change mitigation techniques could be designed through massive and sustainable carbon storage in soils. I have analyzed samples from the five sites of the LTBF Network using the benzene polycarboxylic acids (BPCA) molecular markers method. Results confirm that pyrogenic carbon stocks decrease quickly in all soils tested. Estimated MRT for PyOC are, on average 1.6 times longer than MRT for SOC.

Though this study was not focusing on the ability of biochar to store carbon in soils, it showed that natural pyrogenic carbon displays dynamics which are similar to total organic carbon. As such, it is providing the field of research related to biochar useful information on the possible evolution of this material when it is added to soils.

Further research should aim at confirming this observed dynamic. Studies such as the one carried out by Vasilyeva et al. (2011) could be performed on samples from the LTBF Network. Vasilyeva et al. compared the PyOC contents of two adjacent plots with the same



fire history, one under bare fallow and the other vegetated and showed that there was no influence of the bare fallow.

Another inspiring perspective is the generalized use of BPCA coupled with  $^{14}\text{C}$  analysis to better characterize the origin and age of the pyrogenic material in environmental samples (see Gierga et al., 2014). Additionally, the development of techniques such as Hy-Py (hydrogen pyrolysis) constitutes a promising step in the enhanced characterization of pyrogenic material yielding more quantitative information (Wurster et al., 2012, 2013, Zimmermann et al., 2012). Finally, a better understanding of the mechanisms explaining the dynamics of PyOC could be achieved through a more in-depth analysis of the persistence of PyOC, in different size-fractions of a soil for instance. That was the aim of the next part of this work.

## **5. Bulk PyOC characteristics are the result of a combination of contrasted types of PyOC located in different size fractions of the soil**

In this part of the study, we wanted to investigate the relative importance and persistence of PyOC in different size fractions of the longest bare fallow of the LTBF network, i.e. Versailles. We analyzed both the PyOC and TOC contents in bulk, sand and clay fractions at different stages of the bare fallow. Results show that the persistence observed in the bulk is actually the result of a combination of contrasted persistence in the sand and clay fractions. This persistence seems to be linked to differences in chemistry and physical properties. Indeed, PyOC in the sand fraction is highly condensed and seems to resist fragmentation; its persistence is higher than the bulk persistence and it is representing a significant part of the TOC of this fraction. PyOC in the clay fraction is less condensed and it decreases at a rate equivalent to the TOC in this fraction. The importance of PyOC in the clay fraction is stable through time and has a low value (a few percent of the TOC). This equivalent decrease between TOC and PyOC has never been observed and goes against the general belief that organomineral interactions selectively preserve PyOC in the fine fractions.

This work showed that PyOC in the fine fractions, given that it is relatively little condensed, has an equivalent reactivity and persistence than TOC. To confirm this result, the same protocol could be applied to other soils of the LTBF Network, or other long-term field experiments (with or without plants). In addition, it could be interesting to carry out a study of the existing literature to review the estimated proportions of PyOC in fine fractions over a wide range of soils and conditions. Finally, studying the fine fraction with STXM/NEXAFS

could provide insights on the mechanisms that explain this short persistence of PyOC in our soils, by trying to identify and specifically study PyOC at the nano- and micro- scales.

## **6. Different minerals provide different protection potentials for SOM**

As mentioned on several occasions throughout this manuscript, adsorption of organic matter to mineral surfaces acts as protection from biodegradation. In the fifth part of this work, we wanted to investigate the impact of the nature of mineral surfaces on the long-term persistence of organic carbon in soils. This protection mechanism happens in the fine fractions of soils. We focused on phyllosilicates as they were the predominant mineral species in the clay fraction of the soil studied. Here we studied samples from the longest bare fallow of the LTBF Network (Versailles), selected 5 dates to cover the eight decades of biodegradation and performed a first size fractionation on the samples to isolate the clay fraction. Further fractionation of the clay was carried out to isolate three size-fractions: coarse clay, intermediate clay and fine clay. We analysed the dynamics of carbon and nitrogen contents, as well as characterized the gross chemical signature of the organic matter of the size fractions and obtained spatially resolved chemical information on the organomineral associations present in the three size fractions of the clay.

Overall, we evidenced an effect of the mineralogy on the persistence of SOC. Based on the appearance of illite particles without OM and on the fact that fine and intermediate clays -rich in smectitic minerals- protected more carbon, we conclude that the protective capacity of smectite and interstratified illite/smectite minerals is higher than the protective capacity of illite. Furthermore, the observed enrichment in polar functional groups and in nitrogen points towards an important protection through enhanced adsorption on mineral surfaces.

In this work we have decided to study the influence of phyllosilicates on the persistence of SOC by looking at the respective influence of the different mineral species of a given soil by fractionation. Different approaches have also been developed to study the influence of phyllosilicates on the protection of organic carbon (Barré et al., 2014): (i) studying the impact of pure phyllosilicates on the biodegradation of OM in suspension, (ii) studying how the addition of pure phyllosilicates to soils affects SOM biodegradation, (iii) comparing the SOM associated to soils displaying contrasted phyllosilicate mineralogies. The first two methods give insights on the properties of phyllosilicates but do not represent natural ecosystems. The third method resembles the method selected in our work in that it studies existing natural systems. However, the results obtained by such methods present limitations as several factors

are still covarying with phyllosilicate mineralogy (e.g. climate, pH and oxide content, which provide a different chemical environment, also land uses, the type and quantity of organic matter entering the soil, and even soil texture).

The next step to further our understanding of the influence of phyllosilicates on the long-term protection of organic carbon is to perform a more precise separation of the different mineral species. Protocols using sodium cations achieve a better dispersion of the microstructures and would ensure a more complete dispersion of the clays to break the microaggregates and obtain a better mineralogical contrast between size fractions. This would enable a better differentiation of the two protection mechanisms: entrapment and adsorption. Moreover, the presence of soluble organic matter in the finest fraction impeached the proper analysis of the first stages of the degradation, removing them during the fractionation procedure could be a way to further our understanding of the mineralogical control of OM.

Our results point towards the importance of nitrogen in the long-term persistence of SOM through interactions with minerals. However, STXM/NEXAFS yielded poorly defined spectra at the nitrogen edge, which could be due to the presence of mineral phases. Future work should focus on elucidating the reasons for this technical obstacle and should try to characterize more precisely the dynamics of nitrogen in the SOM in terms of chemistry, origin and age of the nitrogen-containing molecules.

Additionally, further research must include an in-depth work on the representativity of the results of submicrometric visualization techniques. Here we look at images of a few micrometers, selected because of their characteristics (thin enough so that there is transmission through the sample). Upscaling is necessary and is still one of the challenges that these nanoscale visualization techniques have to meet.

The present study suggests that different minerals are associated with different kinds of OM. More data on the different kinds of organomineral associations is needed to better characterize the type of OM associated to the different minerals. Another exciting perspective lies on the dating of the OM associated to the different minerals. Focusing on the existing samples, other techniques could yield more information on the nature of both the organic and the mineral part of the fractions. For instance, we could look at the same samples with Transmission Electronic Microscopy to have access to the localization of other elements. Furthermore, the mineralogy could be better elucidated and quantified by using a model like the one developed by Hubert et al. (2009, 2012) on the DRX data. And finally, these results could be confirmed

by performing similar combinations of fractionation and visualization techniques on other sites of the LTBF Network.

## 7. Conclusions and perspectives for long-term bare fallows

### 7.1. Long-term bare fallows are an incredible asset to study persistent carbon

Long-term bare fallows have been presented in the introduction part of this manuscript. Here I wish to highlight once again the incredible asset they constitute. They provide unprecedented insights on the long-term dynamics of carbon in soils. The results presented here were all obtained on samples coming from LTBF. Even though a section of this manuscript is dedicated to the comparison between the dynamics of C in LTBF and C3/C4 experiments and showed that the kinetics are similar, I want to address here a few of the critics which have been coming up regularly when I presented my results in order to go a step further in demonstrating the applicability of my results to soil ecosystems in general.

#### **Leaching**

As explained in the introduction, leaching is an abiotic process which is one of the exit routes of SOM. Indeed, as water runs down the soil profile it can solubilize and take organic matter which will leave its location and either go down in the profile or exit the soil. One can argue that after several decades without plants and without inputs of fresh organic carbon, the observed decrease in TOC is mostly due to leaching. If leaching was the dominant explanation for decrease of TOC, then the dynamics observed in the 5 sites of the LTBF Network would be contrasted. Indeed, leaching is dependent on the texture of the soil. The five sites have highly contrasted texture; the clay content of the soils used range from 7% at Askov (D) to 36% to Ultuna (SW). Still, the observed dynamics (carbon, pyrogenic material) are similar. Therefore, leaching is not the main driver of the observed decay in TOC and PyOC in the LTBF.

#### **No plants**

The absence of plants for several decades in a soil is of course changing the ecosystem, there is no doubt about that. The question is rather if that change is going to prevent us from deducing information on the persistence of organic carbon. We hypothesize that we can derive information on this topic through the results obtained on LTBF. The first argument that confirms our hypothesis is that modelling the dynamics of SOC in LTBF requires as much fitting as is required for modelling for any other soil, not more, not less. Secondly, as was presented in this manuscript, the observed dynamics of the longest LTBF and the adjacent

C3/C4 experiment are similar. Thirdly, the information on microbial communities shows that there is no loss of the main properties of the biota after several decades of LTBF. Overall, the hypothesis that we can infer information on the dynamics and the nature of persistent carbon with LTBF is verified.

In order to see how the particular BF land use affected the inputs of OM in soils. Several BF plots of the Rothamsted experimental station were replanted to see study the reverse dynamics of SOC. Further research on the evolution of these plots will provide precious information on this topic.

## **7.2. Prospects for long-term bare fallows**

Even though these LTBF are "caricatures that starve the soils for the sole benefit of furthering scientific knowledge" as some could put it, I, personally, am really excited and looking forward to the next decades of research on these long-term field experiments. Is the carbon content at Versailles going to keep on decreasing, but with smaller and smaller decay rates? Is it going to stabilize around this low level, being sustained by the "negligible" carbon inputs which are not so negligible anymore? How is this mainly microbial system going to evolve? How will the organomineral associations evolve? Are more and more mineral surfaces going to become free of organic matter?

All of these questions and many more will be addressed in the coming years.

## **8. General conclusion**

Looking at the different results obtained during this doctoral work and trying to combine them in order to try and see a global pattern is arduous. From where I stand, I think that this study shows that there is still a lot to be done in order to understand the processes driving long-term persistence of carbon in soils. Nevertheless, here are a few results of the work I have carried out during my PhD.

This work provides unprecedented field-based evidence for the importance of the surface chemistry for the dynamics of SOM. It has shown that persistent carbon is enriched in nitrogen-rich compounds and in more oxidized forms of carbon. The results of the work seem to be in agreement with the onion layer model for the interaction of SOM on mineral surfaces (Kleber et al. 2007). It is showing that the long-term persistence of carbon is mainly driven by organomineral interactions. Multidecadal observations presented in this study confirm that

chemical recalcitrance only plays a role in the first years of biodegradation. Furthermore, aggregates at the macroscale do not seem to explain long-term persistence of SOC. Protection mechanisms can happen at different scales according to the material studied: PyOC persistence was detected in coarse fractions and TOC persistence relies on micro- and nanoscale processes. At these scales, the interactions with minerals become dominant and drive the dynamics of OM. Another finding of the present work is that microaggregates seem to be very persistent in soils. Understanding more about the dynamics of these microstructures is crucial. And finally, coming up with a way to efficiently distinguish between the two mechanisms involving the mineral matrix (entrapment VS adsorption) is necessary. Bulk information (NEXAFS and C:N ratios), show that the shift in gross chemical signature is pointing towards a higher importance of the adsorption over the entrapment. Overall, this is the order of importance of each protection mechanism I would propose, based on a broad extrapolation of the findings of this work:

Adsorption of minerals > entrapment in aggregates > chemical recalcitrance

Chemical recalcitrance would be the short-lived influence of chemistry during the first stages of decomposition. Entrapment would correspond to the progressive incorporation of OM in the soil structure and would influence the persistence of OM on longer time scales, but as macroaggregates are regularly broken down and reassembled the OM involved in these structures is regularly “unprotected”. Once the dynamics of microaggregates elucidated, this leaves adsorption as the mechanism explaining persistence on longer timescales. With adsorption as the main driver for long-term persistence, we can understand why SOM becomes progressively enriched in nitrogen and in polar compounds such as carboxylic acids. The dynamic contact-layer between mineral surfaces and OM leads to a progressive enrichment in compounds which are more strongly bound to the minerals, hence the observed increases in nitrogen and carboxylic moieties.



## PART 6: REFERENCES

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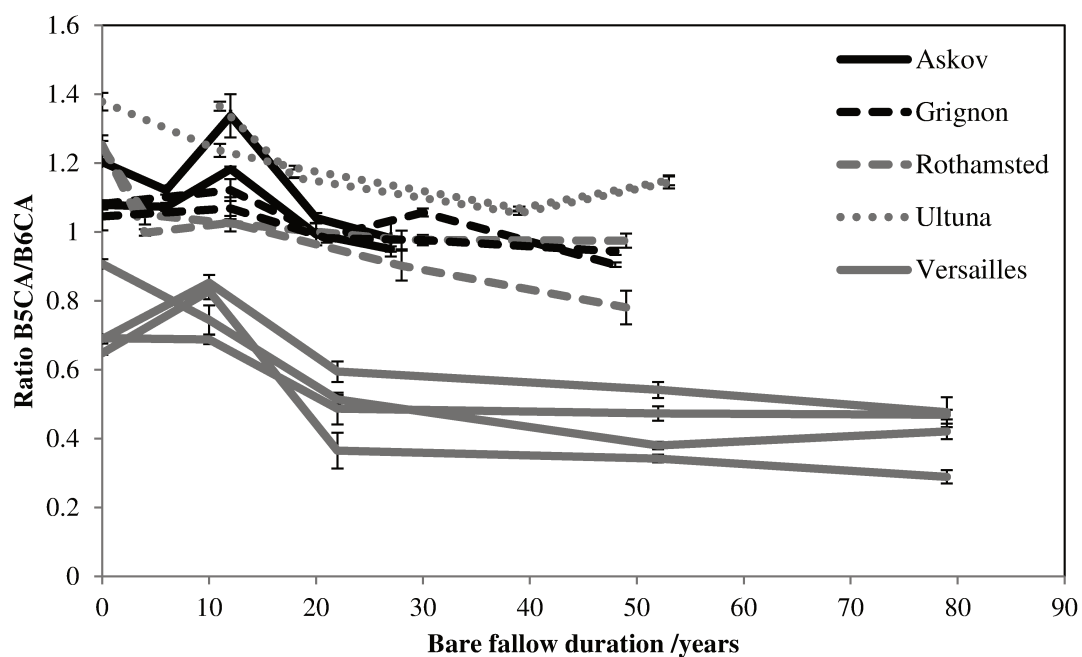
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# PART 7: SUPPLEMENTARY MATERIAL

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**Figure 37: Consistent qualitative evolution of PyOC at all LTBF sites. Evolution of the ratio between the quantities (mg PyOC-C g<sup>-1</sup>soil) of two molecular markers, B5CA and B6CA, analysed with the BPCA method. Error bars represent the variability observed between technical replicates (Supplementary material).**

**Table 11: PyOC contents (mg PyOC.g<sup>-1</sup> soil) and coefficient of variation CV (%) for all samples and all sites (Supplementary material).**

Time under bare fallow (years)		0	6	12	20	27
Askov1	PyOC content (mg PyOC.g <sup>-1</sup> soil)	0.38	0.36	0.27	0.27	0.26
	Coefficient of variation (CV, %)	2.17	4.58	1.35	1.62	10.00
Askov2	PyOC content	0.42	0.39	0.33	0.31	0.32
	CV	0.34	3.10	3.77	3.73	2.64
Time under bare fallow (years)		0	12	21	30	48
Grignon1	PyOC content	0.38	0.34	0.33	0.34	0.29
	CV	0.43	6.85	3.50	1.07	2.04
Grignon2	PyOC content	0.46	0.39	0.39	0.38	0.37
	CV	2.64	1.03	0.84	6.96	7.19
Time under bare fallow (years)		0	4	12	28	49
Rothamsted1	PyOC content	0.75	0.65	0.48	0.35	0.32
	CV	4.26	7.51	2.93	1.72	2.07
Rothamsted2	PyOC content	0.75	0.79	0.54	0.52	0.42
	CV	2.92	2.07	0.75	6.63	10.00
Time under bare fallow (years)		0	11	18	39	53
Ultuna1	PyOC content	0.46	0.42	0.42	0.38	0.34
	CV	8.80	0.77	3.23	2.73	3.61
Ultuna2	PyOC content		0.43	0.43	0.37	0.35
	CV		0.76	1.99	1.67	8.72
Time under bare fallow (years)		0	10	22	52	79
Versailles1	PyOC content	0.65	0.52	0.62	0.53	0.44
	CV	6.98	1.86	1.26	0.53	2.65
Versailles2	PyOC content	0.70	0.47	0.58	0.40	0.39
	CV	2.81	0.48	7.51	0.40	0.39
Versailles3	PyOC content	0.49	0.39	0.34	0.26	0.21
	CV	4.79	6.49	7.91	0.74	6.66
Versailles4	PyOC content	0.50	0.41	0.48	0.34	0.28
	CV	0.17	5.37	4.92	3.02	7.12

**Table 12 : MRT of PyOC and SOC in the size fractions. Model used: mono exponential decay and one pool model (Supplementary material).**

One pool	MRT TOC (y)	SD (y)	MRT PyOC (y)	SD (y)	Ratio PyOC/TOC
Clay13	54	11	70	11	1.29
Clay21	61	11	93	5	1.53
Clay22	54	18	71	7	1.32
Clay32	84	13	90	9	1.08
<b>Clay- Average</b>	<b>63</b>	<b>14</b>	<b>81</b>	<b>12</b>	<b>1.28</b>
Sand13	131	30	Not possible	Not possible	Not possible
Sand21	111	49	874	454	7.85
Sand22	40	14	119	1	2.94
Sand32	63	34	254	220	4.06
<b>Sand- Average</b>	<b>86</b>	<b>42</b>	<b>416</b>	<b>403</b>	<b>4.82</b>
Bulk13	72	8	214	53	2.97
Bulk21	71	4	106	27	1.49
Bulk22	49	6	80	9	1.63
Bulk32	55	6	141	25	2.56
<b>Bulk- Average</b>	<b>62</b>	<b>12</b>	<b>135</b>	<b>58</b>	<b>2.19</b>

A simple one pool model (exponential decay) applied to the data leads to values of MRT for both TOC and PyOC. Using the same data set for TOC and PyOC, that is to say 3 dates and 4 field replicates (5 dates for the bulk), we find an average bulk MRT of 62 years for TOC (with a standard deviation of 12 years) and of 135 years for PyOC (overestimated because of WW2 event at Versailles, see 1<sup>st</sup> paper on PyOC), bulk PyOC is therefore twice more persistent than TOC.

PyOC in the clay fraction is only slightly more persistent than TOC (ratio of 1.28) (paired t.test, p-value=0.0453).

Persistence of the PyOC in the sand fraction cannot be estimated, the error on the estimation is as high as the estimated value. Except for one plot (the same one that has not been impacted by WW2 on the bulk measurements), for which PyOC in the sand fraction has a persistence of 119 years, 3 times more than TOC of the fraction (40 years).





**Titre :** Persistance à long terme des matières organiques dans les sols : caractérisation chimique et contrôle minéralogique

**Mots clés :** matière organique du sol, mécanismes de protection, argiles, carbone pyrogénique, NEXAFS, cycle du carbone

**Résumé :** Les sols stockent trois fois plus de carbone que l'atmosphère sous la forme d'un mélange de molécules, la matière organique des sols (MOS). Certaines de ces molécules sont présentes dans le sol depuis des centaines voire des milliers d'années. Trois mécanismes de protection sont utilisés pour expliquer cette persistance à long terme des matières organiques dans les sols : (i) la récalcitrance chimique, (ii) la protection physique dans les agrégats et (iii) la protection physicochimique par adsorption sur les surfaces minérales. Le but de ce projet de thèse est d'améliorer la compréhension de ces processus de protection et de leur importance relative. Mon projet de thèse utilise des échantillons permettant l'accès au carbone persistant : les jachères nues de longue durée (5 sites en Europe). Il s'agit de parcelles maintenues vierges de toute végétation dans lesquelles, au fur et à mesure de la biodégradation, la quantité totale de carbone diminue, entraînant un enrichissement relatif en carbone persistant. La première étude expérimentale de ce travail de thèse vise à tester l'efficacité des méthodes d'oxydation chimique. Les deux réactifs les plus couramment utilisés -l'hypochlorite de sodium NaOCl et le peroxyde d'hydrogène H<sub>2</sub>O<sub>2</sub>- ont été testés sur des échantillons de la plus longue jachère nue (Versailles). Il est conclu que les méthodes d'oxydation chimique n'arrivent pas à isoler efficacement un réservoir de carbone persistant à l'échelle du siècle.

En termes de mécanismes de persistance, les résultats obtenus montrent que la récalcitrance chimique n'est pas le principal mécanisme de protection. En effet, sur la durée de la jachère nue, la composition chimique de la MOS, caractérisée par spectroscopie NEXAFS, ne présente que peu de changements. Un enrichissement en composés présentant des groupements acides carboxyliques est détecté pour tous les sites testés. Une étude poussée de la persistance spécifique du carbone pyrogénique des sols a aussi été réalisée, ces composés sont actuellement considérés cinq fois plus persistants que le carbone total. Les résultats montrent que le carbone pyrogénique est moins persistant que prévu. En effet, le temps de résidence moyen du carbone pyrogénique obtenu par la méthode BPCA (116 ans) est seulement 1,6 fois supérieur à celui de la MOS (73 ans). L'étude du contrôle minéralogique de la persistance des MOS montre que les argiles contenant du potassium (illite) protègent moins de carbone que les argiles smectitiques. Le rapport C/N décroît avec le temps dans toutes les fractions argiles, ce qui prouve que les composés riches en azote sont préférentiellement préservés. Enfin, la présence de microagrégats dans la fraction grossière des argiles implique la coexistence de deux mécanismes de protection : la protection physique et la protection par adsorption sur les minéraux

**Title :** Long-term persistence of soil organic matter: chemical characterization and mineralogical control

**Keywords :** soil organic matter, protection mechanisms, clays, pyrogenic carbon, NEXAFS, carbon cycle

**Abstract:** Soils store three times more carbon than the atmosphere, under the form of a complex mixture of molecules called soil organic matter (SOM). Some of these molecules have been standing in the soil for hundreds to thousands of years. Three main mechanisms are invoked to explain this long term carbon persistence in soils, (i) chemical recalcitrance, (ii) physical protection in aggregates and (iii) protection by adsorption on mineral surfaces. One of the major challenges in SOM science is to better understand the relative importance of each mechanism, that is the aim of this PhD project.

Here, we use samples from by long term bare fallows (5 sites across Europe). These experimental plots have been kept free of vegetation by manual or chemical weeding for several decades and have been regularly sampled and stored. As the duration of the bare fallow increases, biodegradation occurs and samples get enriched in persistent carbon

First experiments consisted in testing the efficiency of chemical oxidations (two reagent were tested, sodium hypochlorite -NaOCl- and hydrogen peroxide -H<sub>2</sub>O<sub>2</sub>) on the longest bare fallow. We concluded that oxidation

methods were not able to efficiently isolate a pool of persistent carbon at the centennial timescale.

In terms of mechanisms of persistence, the obtained results show that chemical recalcitrance does not seem to be the major mechanism. Indeed, over the duration of the bare fallow, the chemical composition of SOM, as seen by synchrotron based NEXAFS spectroscopy, shows little changes. There is a consistent increase in carboxylics for all sites (12% increase on average) though it is significant for 2 out of the 4 selected sites. We also studied the particular persistence of soil pyrogenic carbon, which is thought to be at least five times more persistent than bulk SOM. Results show that pyrogenic carbon lacks long term persistence. Indeed the BPCA-estimated mean residence time of pyrogenic carbon (116 years) is on average 1.6 times longer than MRT for bulk SOM (73 years).

Finally, the study of mineralogical control of the persistence of SOC showed that clay minerals containing potassium (illite) seemed to protect less carbon. As seen by NEXAFS-STXM, more mineral surfaces with very little SOM appear with the duration of bare fallow. C:N ratio decreased in all clay fractions, suggesting a preferential persistence of N-rich compounds. Presence of microaggregates in the coarser clay fraction led to the coexistence of two protection mechanisms: adsorption and physical protection.

