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POTENTIALITÉ DE STOCKAGE DE CARBONE DANS LES SOLS PAR APPORT DE MATIÈRES ORGANIQUES ÉXOGENES

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Résumé:

L’apport au sol de matières organiques exogènes (MOEs) d’origine résiduaire issues d’activités agricoles, urbaines ou industrielles permet d’augmenter les teneurs en matière organique du sol (MOS) et d’améliorer leur fertilité. De plus l’apport au sol de MOEs pourrait contribuer à la limitation des émissions nettes de gaz à effet de serre en séquestrant du C dans les sols. Le développement d’outils de prédiction est nécessaire pour une meilleure estimation du devenir à long terme des MOEs apportées au sol. De plus, il est important de savoir sous quelles formes la matière organique (MO) des MOEs est incorporée dans la MO du sol, ceci conditionnant la pérennité des accumulations de C après apports répétés de MOEs.

Cette étude qui vise à mieux comprendre et prédire la dynamique d’incorporation de la MO de MOEs dans la MOS s’est déroulée en trois étapes : (i) le développement et l’amélioration de méthodes de caractérisation de laboratoire permettant d’estimer le devenir des MOEs dans le sol, (ii) la fourniture de coefficients de partition des MOEs dans le modèle RothC pour simuler l’accumulation de C dans un sol suite à l’apport de MOEs de types divers et (iii) l’étude des changements de composition de la MOS suite à des apports répétés de MOEs.

La composition et la biodégradabilité de la fraction soluble (SOL) du fractionnement biochimique par la méthode Van Soest ont tout d’abord été étudiées en utilisant une méthode d’extraction alternative permettant la caractérisation des MO extraites. Cette fraction de la MO augmente avec le degré de stabilité d’un compost ne peut être assimilée à un compartiment facilement biodégradable comme dans les résidus végétaux. La caractérisation des fractions extraites et des résidus d’extraction a révélé que la fraction SOL évoluait au cours du compostage. Cette fraction de MO est très riche en composés polysaccharidiques en début de compostage et s’enrichit en MO riches en composés azotés au cours du compostage qui pourraient être stabilisées par complexation avec des ions métalliques.

Le potentiel de la spectroscopie proche infra-rouge (SPIR) pour la caractérisation de MOEs et de leur devenir dans le sol a ensuite été étudié. Un jeu de données regroupant 300 MOEs représentatives de la diversité des MOEs utilisées en agriculture à été utilisé. Des prédictions satisfaisantes ont été obtenues pour les teneurs en C et N des MOEs ainsi que pour les fractions biochimiques Van Soest des MOEs à l’exception de la fraction hémicellulose. L’indicateur de stabilité de la MO (ISMO), estimateur de la fraction résiduelle de MO des MOEs à long terme dans les sols, a été prédit de manière satisfaisante. Généralement, les variables liées à la minéralisation du C au cours d’incubation à long terme ont été moins bien prédites, à l’exception de l’indicateur ISMO basé sur des caractéristiques analytiques bien définies, probablement en raison de l’augmentation de la complexité et des interactions entre les facteurs gouvernant la minéralisation des MOEs en fonction de la durée d’incubation.

Puis, la potentialité de stockage de C à long terme dans un sol soumis à des apports répétés de MOEs a été étudiée en utilisant des résultats d’essais au champ de moyenne à longue durée. Les résultats de l’essai au champ Qualiagro ont tout d’abord été utilisés pour étudier l’accumulation du C dans un sol limoneux suite à des apports répétés de 1998 à 2009 d’un compost d’ordures ménagères résiduelles (OMR), d’un compost de déchets verts + boue (DVB), d’un compost de biodéchets (BIOD) et d’un fumier (FUM), apportés à une dose cible de 4 Mg $C.ha^{-1}$ tous les deux ans. Des augmentations significatives des stocks de C du sol ont été mesurées après 11 ans d’apport, allant jusqu’à 19.6 Mg $C.ha^{-1}$ pour 27 Mg $C.ha^{-1}$ de compost DVB apportés. Une reproduction satisfaisante des cinétiques d’accumulation du C du sol a été obtenue avec RothC après l’ajustement des coefficients de partition du C des MOEs dans les compartiments d’entrée labile (DPM), résistant (RPM) et humifié (HUM) du modèle (f_{DPM}, f_{RPM} et f_{HUM}, respectivement). L’extrapolation des simulations a permis de calculer des rendements d’accumulation du C dans le sol (% des apports de C additionnels comparés au témoin accumulés dans le sol) après 20 ans d’apport de MOE de 20, 34, 37 et 40% pour OMR, FUM, DVB et BIOD, respectivement. La quantité de C contenue dans la fraction de MOS > 50 µm a été préférentiellement augmentée par les apports de composts alors que l’apport de fumier a entrainé des proportions équivalentes de C accumulées dans les fractions de MOS de taille > 50 µm et 0-50 µm. Une bonne correspondance a été trouvée entre les proportions de C accumulées dans les compartiments DPM+RPM et BIO+HUM de RothC et les proportions de C dans les fractions de taille > 50 µm et 0-50 µm, respectivement. Les données de trois autres essais (Askov, Danemark, 31 ans, SERAIL, France, 14 ans et Ultuna, Suède, 51 ans) ont été ajoutées aux données de l’essai Qualiagro afin de développer des équations de régression pour prédire les coefficients de partition du C des MOEs dans le modèle RothC (f_{DPM}, f_{RPM} et f_{HUM}) à partir de l’indicateur ISMO calculé avec des valeurs de caractérisations des MOEs au laboratoire. L’utilisation des coefficients de partition ainsi prédits dans RothC a permis de simuler de façon satisfaisante l’accumulation du C suite aux apports de MOEs dans les différents essais au champ.

Enfin, la composition chimique de la MO du sol est modifiée par les apports de MOEs. Dans l’essai Qualiagro, des techniques de pyrolyse-CG/SM et de spectroscopie moyen infra-rouge en réflexion diffuse (DRIFT) montrent un enrichissement de la MO particulaire en lignine dans les parcelles amendées avec DVB et BIOD et FUM comparé aux parcelles témoin du aux apports de MOEs mais aussi à l’augmentation des résidus de culture et en composés azotés dans les parcelles amendées avec le compost BIOD, en relation avec la forte proportion de ces composés dans les MOEs épandues. Des changements dans la composition des MO de taille 0-50 µm ont également été détectées, présentant moins de relations directes avec la composition des MOEs épandues.

Abstract:

The application of Exogenous Organic Matters (EOMs) of residual origin issued from agricultural, urban or industrial activities on croplands allows to increase soil organic matter (SOM) content and to improve soil fertility. Additionally, it could play a significant role in the mitigation of the net emissions of greenhouse gazes through C sequestration in soil. The development of predictive tools is necessary to better estimate the fate of EOMs in soil in the long-term. Additionally it is important to better know under which form the organic matter (OM) is accumulated into SOM after EOM application, this conditioning the potential reversibility of C sequestration in soil.

This study aims at better understanding and predicting the dynamics of incorporation of the OM from EOMs into SOM. It has been carried out in tree steps: (i) the development and improvement of laboratory characterizations allowing to estimate the fate of EOMs in soil, (ii) the providing of partition coefficients of EOMs in the RothC model to simulate C accumulation in soil following application of various types of EOMs and (iii) the study of the changes in SOM composition after repeated applications of EOMs.

The composition and the biodegradability of the soluble (SOL) fraction of the Van Soest fractionation was first investigated using an alternative extraction method allowing to characterize the extracted organic compounds. This fraction of OM increases with compost maturity and stabilization, thus cannot be assimilated as easily biodegradable as in plant residues. We applied this method to a municipal solid wastes compost at two stages of maturity. The SOL fraction contains a large proportion of polysaccharide at the beginning of composting then forms of OM rich in N-containing compounds are formed during composting which could be stabilized by the formation of complexes with metal ions.

The potential of near infra-red spectroscopy (NIRS) to characterize the composition of EOMs and their behaviour in soil have been studied. A dataset including 300 EOMs representative of the broad range of EOMs applied to cultivated soils has been used to develop NIRS calibrations. Satisfactory predictions were obtained for the C and N contents as wall as for the Van Soest biochemical fractions of EOMs, except the hemicellulose fraction. The indicator of potentially remaining organic C in soil (I_{ROC}) was predicted with a good accuracy. Generally, variables related to the long-term C mineralization of EOM in soil during incubations were not predicted accurately, except for I_{ROC} which was based on analytical and well-identified characteristics, probably because of the increasing interactions and complexity of the factors governing EOM mineralization in soil as a function of incubation time.

In a second part, the potentiality of C sequestration in soil following repeated EOM applications have been studied using results from mid to long-term field experiments. The results of the Qualiagro experiments were first used to study the C accumulation in a loamy soil after the application from 1998 to 2009 of green wastes and sludge compost (GWS), municipal solid waste compost (MSW), biowaste compost (BIOW) and farmyard manure (FYM) with the target dose of 4 Mg C.ha^{-1} applied every two years. Significant increases of soil organic C stocks were measured after 11 years of EOMs applications, up to 19.6 Mg C.ha-1 for 27 Mg C.ha^{-1} of GWS compost applied. The RothC model successfully reproduced the C accumulation kinetics after fitting the partitioning coefficients of EOM C into the labile (DPM), resistant (RPM) and humified (HUM) entry pools of the model (f_{DPM}, f_{RPM} and f_{HUM}, respectively). The extrapolation of model simulation makes it possible to calculate yields of C accumulation in soil (% of total additional C inputs compared to control plots accumulated in soil) after 20 years of cultivation of 20, 34, 37 and 40% for MSW, FYM, GWS and BIOW, respectively. Compost applications preferentially increased the C amount in the fraction of SOM > 50 µm, whereas the FYM increased almost in similar proportion the C in SOM of size > 50 µm and 0-50 µm. A good correspondence was found between the proportions of C accumulated in the DPM+RPM and in the BIO+HUM RothC pools and the C proportions in the fractions of SOM of size > 50 µm and 0-50 µm, respectively. Data from tree others field experiments (Askov K2 experiment, Denmark, 31 years, SERAIL experiment, France, 14 years and Ultuna experiment, Sweden, 51 years) were added to the data of the Qualiagro experiment to develop regression equations to predict the partitioning coefficients of EOM C in RothC (f_{DPM}, f_{RPM} and f_{HUM}) from the I_{ROC} indicator calculated from laboratory characterizations values of EOMs. The use of the so predicted partitioning coefficients allowed to satisfactorily simulate the C accumulation after EOM application in the different field experiments.

Finely, changes in the chemical composition of SOM contained in particle size and density fractions of SOM after 8 years of EOMs applications in the Qualiagro experiment was evidenced using pyrolysis-GC/MS and DRIFT spectroscopy. The particulate OM > 50 µm of soils amended with GWS, BIOW and FYM were enriched in lignin compared to control plots both related to the large proportion of lignine in the MOEs but also to the larger amounts of crop residues produced in the amended plots. The particulate OM from the BIOW compost amended plots was enriched in N-containing compounds related to the high proportions of N-containing compounds in the BIOW compost. Changes in the composition of the SOM of size 0-50 µm were also detected, showing less direct relationships with the composition of the applied EOMs.

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Sommaire

Introduction générale

Dans les sols cultivés d'Europe de l'Ouest, des pratiques culturales intensives et l'abandon progressif des pratiques de polyculture - élevage par la spécialisation des entreprises agricoles ont conduites à des diminutions importantes des teneurs en matières organiques des sols (MOS) (Le Villio et al., 2001; Van-Camp et al., 2004; Ciais et al., 2010). Or la quantité et la qualité des MOS sont des composantes majeures de la fertilité des sols : fertilité chimique (disponibilité des éléments fertilisants), physique (amélioration de la porosité, augmentation de la rétention en eau, stabilité de la structure) et biologique (stimulation de l'activité microbienne) (Loveland & Webb, 2003; Annabi et al., 2007; Clapp et al., 2007). Une diminution des teneurs en MOS a pour conséquences, en plus de la perte de fertilité, leur sensibilisation à l'action dégradante des pluies et à l'érosion (Le Bissonnais, 1996; Abiven et al., 2009). Pour ces raisons, la commission européenne, dans un projet de directive cadre sur les sols, a récemment identifié la diminution des teneurs en MOS comme un des risques majeurs qui pèsent sur les sols (2006).

L'hypothèse d'un changement climatique induit par le dégagement de gaz à effet de serre (GES) par les activités humaines fait l'objet d'un consensus de plus en plus général. Le sol représente le plus grand réservoir de C de la biosphère continentale contenant environ deux fois le stock de C atmosphérique et trois fois le stock de C contenu dans la végétation (IPCC, 2001). Dans ce cadre, une augmentation des stocks de C dans les sols cultivés pourrait jouer un rôle significatif dans la limitation du flux net de GES vers l'atmosphère (Lal et al., 2007). L'article 3.4 du protocole de Kyoto prévoit la prise en compte de puits de carbone tels que le sol dans les bilans globaux de GES. Plusieurs expertises ont été réalisées à l'échelle nationale (Arrouays et al., 2002) et européenne (Schils et al., 2008) afin d'estimer le potentiel de stockage de C dans les sols et le rôle induit des sols dans la lutte contre le changement climatique. Ces expertises concluent à un bénéfice non négligeable du stockage de C dans les sols compte tenu du faible coût d'application des pratiques stockantes et des bénéfices environnementaux et agronomiques annexes qu'elles entrainent

L'apport au sol de matières organiques exogènes (MOEs) est une des principales actions permettant de maintenir voire d'augmenter les stocks de C des sols. Les MOEs sont définies comme l'ensemble des matières organiques appliquées au sol dans le but de fertiliser, d'amender ou de restaurer les sols. Les MOEs incluent les résidus organiques issus des activités agricoles, urbaines et industrielles ainsi que les produits issus de leur traitement mais comprend également les résidus de culture produits in situ du fait de la similarité de leur valeur amendante et des méthodes permettant de les caractériser (Marmo et al., 2004). Environ 1.6 milliard de tonnes de MOEs sont produites chaque année dans l'union européenne, réparties en 61% de MOEs d'origine animale, 25% de résidus de culture, 7% de déchets urbains (boues d'épuration, bio-déchets et déchets verts) et 7% de déchets industriels. Plus de 90% des MOEs (hors résidus de culture) épandues sur les sols sont d'origine animale, indiquant un potentiel important d'augmentation du retour au sol des MOEs d'origines urbaines et industrielles (Marmo, 2008) qui devrait être accentué par des mesures récentes prises au niveau européen visant à augmenter la part des bio-déchets recyclée en agriculture via la production de composts ou de digestats de méthanisation (European Commission, 2010). En effet en France 11 millions de tonnes des déchets organiques d'origine urbaine et industrielle produits chaque année sont traitées par voie biologique alors que 32 millions de tonnes le sont potentiellement (ADEME, 2006).

Les effets des apports de MOEs sur les stocks de C des sols sont très variables et des MOEs épandues dans des conditions similaires lors d'expériences au champ de longue durée peuvent entrainer des cinétiques très distinctes d'accumulation du C dans le sol (Christensen & Johnston, 1997; Gerzabek et al., 1997; Bipfubusa et al., 2008). Ces résultats variables sont dus à des conditions pédoclimatiques contrastées, à des compositions chimiques différentes des MOEs. L'incertitude importante des quantités de C accumulées après des apports de MOEs ainsi que la méconnaissance des formes sous lesquelles ces MOEs sont incorporées aux MOS conditionnant la réversibilité du stockage rendent la prise en compte de ces pratiques difficiles dans des bilans globaux.

La capacité des MOEs à augmenter les stocks de C du sol est principalement liée à leur nature chimique ainsi qu'à leur degré de transformation si les MOEs subissent un traitement avant épandage (Thuriès et al., 2002; Francou, 2003; Jensen et al., 2005). La stabilité des MOEs, définie comme leur résistance à la biodégradation, peut être évaluée par différentes méthodes de laboratoire telles que la mesure du potentiel de minéralisation du C des MOEs au cours d'incubation en conditions contrôlées (AFNOR, 2009b) ainsi que par le fractionnement biochimique des MOEs par solubilisations successives selon la méthode Van Soest (Van Soest & Wine, 1967; AFNOR, 2009a). Cependant, ces méthodes demeurent longues à mettre en oeuvre et le fractionnement biochimique des MO a soulevé certaines interrogations dans l'interprétation qui peut être faite de la nature des fractions séparées dans le cas de MO compostées. Une meilleure caractérisation et le développement de méthodes et d'indicateurs plus rapides à mettre en oeuvre pourraient permettre d'améliorer l'estimation du devenir des MOEs dans les sols.

Le recours à des expérimentations au champ de longue durée est indispensable pour appréhender en conditions réelles les effets d'apports répétés de MOEs sur la dynamique de la MO du sol. En effet, les effets des apports de MOEs sur les sols ont souvent été étudiés à court terme ou en conditions de laboratoire mais peu d'informations sont disponibles sur les processus d'incorporation des MOEs aux MO des sols en conditions réelles à long terme.

La complexité des mécanismes et la multiplicité des facteurs affectant l'évolution du C du sol sous l'effet de différentes conditions pédoclimatiques et différentes pratiques culturales impliquent l'utilisation de modèles simulant la dynamique du C pour prévoir l'évolution à long-terme du C dans les sols, en particulier en cas d'apport de MOEs. Les modèles de dynamique du C tels que RothC ou Century (Jenkinson & Rayner, 1977; Parton et al., 1987) ont montré leur efficacité sous de nombreuses conditions climatiques, pour différents types de sols (Smith et al., 1997). Néanmoins ces modèles nécessitent la calibration du partitionnement des MOEs dans les différents compartiments d'entrée de MO dans les sols pour qu'ils puissent être utilisés pour simuler des accumulations potentielles de C dans les sols suite à des apports de MOEs de compositions diverses.

L'objectif général de ce travail de thèse vise à mieux comprendre et prévoir la dynamique d'incorporation de MOEs à la MO d'un sol au fur et à mesure de leurs apports.. Il se décompose en 3 sous-objectifs :

- le développement d'outils de caractérisation de laboratoire permettant de préciser la nature chimique des MOEs et de mieux estimer leur devenir dans les sols.

- l'approfondissement des connaissances sur les formes sous lesquelles ces MOEs sont incorporées dans la MO du sol au travers de l'étude de l'incorporation de composts urbains dans un dispositif au champ de longue durée.

- l'adaptation d'un modèle de dynamique du C du sol afin de le rendre opérationnel pour simuler le stockage potentiel de C dans les sols suite à des apports répétés de MOEs.

Cette thèse s'appuie sur le site expérimental Qualiagro mis en place en 1998 afin d'étudier les effets d'apports de composts urbains sur un sol limoneux de la région parisienne (Houot et al., 2002). Elle a été financée par une allocation de recherche du ministère de l'enseignement supérieur et de la recherche. La partie expérimentale a été financée Véolia Environnement Recherche et Innovation dans le cadre du programme de recherche Qualiagro en collaboration avec l'INRA. Elle a été réalisée au sein de l'équipe « sol » de l'unité mixte de recherche INRA / AgroParisTech Environnement et Grandes Cultures à Thiverval-Grignon.

Ce manuscrit de thèse est divisé en six chapitres, le premier chapitre propose une synthèse de la bibliographie existante autour du sujet et aboutit à l'énoncé des objectifs de la thèse ainsi qu'à la justification des approches choisies pour y répondre. Les chapitres suivant, rédigés sous forme d'articles scientifiques, sont répartis en trois parties. La première partie propose des améliorations d'outils et de méthodes permettant d'estimer le devenir des MOEs dans les sols. La seconde partie présente l'étude de l'accumulation du C dans les sols suite à des apports répétés de MOEs. La troisième partie propose une étude des modifications de la composition chimique de la MO du sol après apports répétés de composts urbains et de fumiers. Les conclusions et les perspectives de ce travail de thèse sont enfin énoncées.

Chapitre 1. Synthèse bibliographique

1.1. Utilisation des matières organiques exogènes en agriculture

1.1.1. Gisement de matières organiques exogènes

Les MOEs ont été définies comme l'ensemble des matières organiques appliquées au sol dans le but de fertiliser, d'amender ou de restaurer les sols. Les MOEs incluent les résidus organiques issus des activités agricoles, urbaines et industrielles ainsi que les produits issus de leur traitement (ensemble de MOEs rassemblées sous l'appellation générique de produits résiduaires organiques, PRO) et comprend également les résidus de culture produits in situ du fait de la similarité des processus impliqués dans leur devenir, de leurs effets sur la fertilité des sols et des méthodes permettant de les caractériser (Marmo *et al.*, 2004).

Au niveau de l'Europe, environ 1.6 milliard de tonnes de MOEs sont produites chaque année, réparties en 61% de PRO d'origine animale, 25% de résidus de culture, 7% de déchets urbains (boues d'épuration, bio-déchets et déchets verts) et 7% de déchets industriels. Cependant, plus de 90% des MOEs appliquées au sol (hors résidus de culture) sont d'origine animale (Marmo, 2008). En France, Le gisement annuel de MOEs comprend une majorité d'effluents d'élevage (150 Mt, ADEME, 2006) qui s'élève en réalité à 300 Mt mais dont la moitié sont produits in situ et ne rentrent donc pas dans une logique d'épandage. Les effluents d'élevage étant déjà en totalité restitués au sol, leur potentiel de stockage de C additionnel dans les sols peut être considéré comme nul. Le gisement annuel de déchets organiques d'origine urbaine et industrielle théoriquement susceptible d'être concernés par une filière de gestion biologique en vue de leur retour au sol est de plus de 32 Mt, essentiellement d'origine urbaine. Ces chiffres ne prennent pas en compte les effluents liquides agroalimentaires et la part des déjections animales (ADEME, 2006). Sur ce gisement, environ 11 Mt sont recyclées par retour au sol à travers différentes voies:

- Le compostage avec 5Mt de déchets entrant pour 2.5 Mt de composts produits,
- La méthanisation qui est une pratique nouvelle en essor,
- L'épandage direct des boues (urbaines ou industrielles) concernant 5 à 6 Mt de matière brute, soit 0.5 Mt de matière sèche et correspondant à environ 50 à 60% des boues produites.

Les quantités de déchets d'origine urbaine et industrielle présentent un fort potentiel d'augmentation de retour au sol et peuvent constituer une source localement importante de matière organique (MO) pour les sols en zones périurbaines, particulièrement là où l'élevage a disparu.

1.1.2. Traitement des MOEs par compostage

Le compostage est le procédé majeur de traitement biologique des déchets en vue de leur retour au sol. Il s'agit d'un procédé de décomposition et de transformation contrôlées de déchets organiques biodégradables, sous l'action de populations microbiennes diversifiées vivant en milieu aérobie, qui aboutit à un produit organique stabilisé, enrichi en composés humiques, le compost (ITAB, 2002).

Le compostage se caractérise par:

- une production de chaleur au début du compostage (couramment comprise entre 40 et 70 °C) liée à l'activité des micro-organismes aérobies décomposeurs (oxydations exothermiques),

- une perte de masse et de volume (environ 50 %) liée à la minéralisation de la MO par les micro-organismes, à la perte d'eau par évaporation et au tassement (perte de structure),

- une transformation chimique des matières premières organiques par voies chimique, biochimique et physique (changement de couleur, d'aspect, de granulométrie) notamment avec l'humification des MO,

- une production de chaleur liée à l'activité microbienne intense.

Le compostage aboutit à :

- une plus grande homogénéité du compost par rapport à celle des déchets initiaux,

- la stabilité des matières organiques du compost, au sens de résistant à la minéralisation, d'autant plus importante qu'il est évolué, ou mature, (riche en composés humiques)

- l'hygiénisation du compost par destruction des germes pathogènes, des parasites animaux, des graines et des organismes de propagation des végétaux.

- l'absence d'odeurs désagréables.

Le compostage est souvent caractérisé par l'évolution de la température au cours du procédé. Quatre phases sont distinguées :

> - **la phase mésophile**: pendant cette phase les micro-organismes mésophiles indigènes (bactéries et champignons) dégradent les molécules simples (sucres simples, acides aminés, alcools…) et transforment une partie des polymères (protéines, acides nucléiques, amidon, pectine, hémicellulose, cellulose…). L'activité des microorganismes lors de cette phase entraîne une montée rapide en température et un important dégagement de CO_2.
>
> - **La phase thermophile**: au cours de cette phase, la température atteint des températures élevées, (60 à 70 °C) auxquelles ne résistent que les micro-organismes thermo-tolérants. Au cours de cette phase, on observe une perte d'azote minéralisé sous forme amoniacale (NH_4^+) et une évaporation d'eau plus importante. La forte activité microbienne au cours de cette phase entraîne une diminution de la teneur en O_2 dans l'andain de compostage. Pour maintenir cette disponibilité en O_2 et les conditions aérobies, les andains sont retournés. Après un

retournement, on observe un regain d'activité microbienne et une remontée de la température. La bonne aération des andains peut également être assurée par insufflation d'air à travers les andains. Cependant le procédé de compostage doit comporter au moins un retournement qui permet la réincorporation des matières présentes en bordure au centre du tas.

- **La phase de refroidissement**: lors de cette phase, on observe une diminution progressive de l'activité microbienne, liée à la diminution de biodégradabilité du compost, se traduisant par une diminution de la température du compost.

- **La phase de maturation**: l'activité des micro-organismes est faible, le compost est recolonisé par des champignons pouvant dégrader la lignine.

La phase thermophile du compostage est suivie d'une diminution de la température liée à la diminution de l'activité microbienne. Ce ralentissement est lié à la stabilisation de la MO du compost qui peut être définie comme l'augmentation de sa résistance à la biodégradation.

La stabilisation de la MO des composts se poursuit durant la phase de maturation. Le degré de maturité d'un compost est défini par le niveau de stabilisation de sa MO. Un compost mature, c'est-à-dire dont la MO est stabilisée, a une biodégradabilité très faible proche de celle de la MO du sol. Francou (2003) a établi une gamme de stabilité des matières organiques des composts suivant leur taux de minéralisation du C organique en mélange dans un sol et en conditions contrôlées (28°C pendant 108 jours). Au cours du compostage, les composts évoluent vers des produits aux caractéristiques de plus en plus proches, indépendantes de leurs origines, mais les vitesses de stabilisation dépendent du type de déchets compostés et des procédés de compostage. Dans le cas des déchets verts, par nature riches en lignine, le mélange initial peut déjà être lentement minéralisable et l'évolution de la MO au cours du compostage peut atteindre rapidement une certaine stabilité puis évoluer très lentement, que le procédé soit accéléré ou non. A l'opposé, la prédominance dans les déchets initiaux de papiers par nature riches en cellulose, requiert une durée de compostage plus longue pour arriver à des composts stabilisés, sans doute en raison d'un déficit en azote. De ce fait, les composts d'ordures ménagères, plus riches en papiers-cartons, sont encore instables au bout de trois mois et nécessitent au moins six mois de compostage, avec retournements réguliers, pour se stabiliser (Francou *et al.*, 2005).

Les composts réalisés à partir de déchets urbains peuvent être répartis en 4 catégories :

- **les composts de déchets verts** obtenus à partir des résidus d'élagage, de tonte, et d'entretien des jardins publics ou privés ;

- **les composts de biodéchets** obtenus à partir de la fraction fermentescible des ordures ménagères collectée sélectivement, ou à partir des déchets de Marché d'Intérêts Nationaux (M.I.N). En pratique, le compostage de biodéchets requiert l'incorporation de déchets verts structurants en début de procédé ;

- **les composts d'ordures ménagères résiduelles** obtenus à partir de la fraction résiduelle des ordures ménagères après collecte sélective des emballages propres et secs ;

- **les composts de boues de station d'épuration** obtenus à partir des boues de station d'épuration mélangées à des co-substrats servant de structurants.

Les évolutions des quantités de composts urbains produits en France montrent une croissance toujours soutenue pour les déchets verts, une croissance très forte pour les boues d'épuration, un développement lent du compostage de biodéchets triés à la source. Le compostage des ordures ménagères montre un creux dans l'évolution des quantités produites mais pourrait reprendre dans des conditions de procédés actualisés et orientés vers la production de composts valorisables. Aujourd'hui, environ 3.6 Mt de déchets urbains (OM résiduelles, biodéchets et déchets verts) sont compostées alors qu'environ 16 Mt sont potentiellement compostable (ADEME, 2006).

1.1.3. La méthanisation

La méthanisation, également appelée digestion anaérobie, est une technologie basée sur la dégradation de la matière organique par des microorganismes en milieu anaérobie qui aboutie à la production :

- de biogaz composé d'environ 50 à 60% de CH_4 et de 20 à 50 de CO_2, pouvant être utilisé sous différentes formes : combustion pour la production de chaleur, et d'électricité, production de carburant.

- De digestat : produit humide, riche en matière organique, partiellement stabilisé qui peut être retourné au sol directement ou après compostage.

La méthanisation présente l'avantage d'une double valorisation de l'énergie et de la matière organique. De plus le traitement de certains déchets graisseux ou très humides non compostable est possible. Comparé au compostage, la méthanisation présente d'avantage de contraintes techniques et requiert une technicité accrue. La méthanisation est peu adaptée aux déchets ligneux ou ayant une MO stable. Toute MO est susceptible d'être décomposée par méthanisation (excepté des composés très stables), cependant la méthanisation est particulièrement adaptée aux déchets riches en eau ayant une MO facilement biodégradable et facilement pompable pour permettre un fonctionnement en continue. Les déchets sont généralement traités en mélange (ADEME, 2006).

1.2. Méthodes de caractérisation des MOEs, indicateurs de leur devenir dans les sols

Un nombre important de méthodes peuvent être utilisées pour caractériser les MOEs. Le Tableau 1.1 présente une synthèse des principales méthodes qui peuvent être utilisées. Certaines de ces méthodes sont à la base utilisées pour la caractérisation de la MO des sols comme l'IRTF, la Pyrolyse-CG/SM, la RMN ou le fractionnement granulo-densimétrique et peuvent être également utilisées pour la caractérisation des MOEs. Les sections suivantes présentent une description de certaines de ces méthodes.

Tableau 1.1. Méthodes de caractérisation des matières organiques de matières organiques exogènes (adapté d'après Muller et al., 2003 et Doublet, 2008).

Méthodes	Principe	Type d'informations	Avantages et inconvénients
Minéralisation Incubation	Suivi la minéralisation du carbone au cours d'incubation de mélange sol-compost. (méthode normalisée)	Biodégradabilité résiduelle de la MO du compost (opposé de la stabilité).	**Avantages:** - Traduit le comportement d'un compost après incorporation dans du sol. - L'ajustement de fonctions mathématiques permet d'estimer les proportions de C facilement minéralisable et résistant face à la biodégradation. - Utilisable comme estimateur du K1, coefficient isohumique ou proportion de la MO des MOEs s'incorporant à la MO des sols. **Inconvénients:** - Durée de manip longue pour accéder précisément aux proportions résistante et facilement minéralisable
Fractionnement humique	Extraction des acides fulviques (soluble dans base et acide) et acides humiques (soluble dans base et précipité par acide) pouvant être suivi par une purification des substances obtenues.	Traduit la concentration en composés plus ou moins solubles. Traduit le degré d'humification lorsque l'extraction est suivie d'une purification.	**Avantages:** - Réalisation rapide - Utilisation du rapport AH/AF comme indicateur de stabilité **Inconvénients:** - Les substances assimilées aux substances humiques peuvent aussi être des biomolécules présentant la même réactivité aux extractants.
Fractionnement biochimique	Attaques successives d'un échantillon à l'eau chaude, au détergent neutre chaud, au détergent acide chaud et à l'acide concentré à froid permettant de séparer la MO en différentes fractions. (méthode normalisée)	Donne les proportions de MO sous forme de MO soluble, hémicellulose, cellulose et lignine.	**Avantages:** - Donne la concentration des principaux constituants de la MO. - Permet de calculer des indices de stabilité des MOEs. **Inconvénients:** - Méthode assez lourde. - Présence d'autres composés dans les différentes fractions (lipides dans le soluble, protéines, substances humiques dans la fraction lignine,…)
Spectroscopie IRTF	L'échantillon est soumis à un rayonnement électromagnétique dans la gamme du moyen infrarouge (400-4000 cm-1). Les fréquences absorbées correspondent aux fréquences de vibration des liaisons chimiques.	Présence de classes de groupes chimiques : composés aromatiques ou aliphatiques, amides, …	**Avantages:** - Donne des informations sur la nature chimique des constituants. - Méthode rapide **Inconvénients:** - Interprétation des spectres difficile sur MO complexes - Vibration des minéraux à certaines longueurs d'onde - Pollution par l'humidité ambiante de la pastille utilisée - Préparation des pastilles - Broyage fin nécessaire (< 200 µm) - Appareillage.
Spectroscopie DRIFT	Même principe que l'IRTF mais avec un module supplémentaire permettant d'utiliser la réflexion diffuse.	Idem IRTF	**Avantages:** - Mêmes avantages que L'IRTF - Préparation de l'échantillon plus simple (permet l'analyse de poudres). - Non destructif - Utilisable pour développer des modèles de prédiction **Inconvénients:** - Moins précis que l'IRTF - Interprétation des spectres difficile. - Broyage fin nécessaire (< 200 µm) - Vibration des minéraux à certaines longueurs d'onde - Non quantitatif - Appareillage.
RMN	Absorption par certains noyaux atomiques de fréquences présentes dans le champ électrique oscillant auquel est soumis l'échantillon.	Caractérisation structurelle des composés.	**Avantages:** - Non destructif - Pas d'extraction préalable indispensable **Inconvénient:** - Appareillage - Non quantitatif
Pyrolyse	Dégradation thermique de l'échantillon entre 650℃ et 750℃ en absence d'oxygène couplée à un chromatographe et un spectromètre de masse.	Caractérisation de macromolécules organiques.	**Avantages:** - identification des macromolécules organiques qui ne sont pas directement chromatographiables - Pas de traitement chimique préalables de l'échantillon **Inconvénients:** - Sélective pour composés volatils - Origine commune possible de certains composés - Co-élution de certains pics - Interprétation des chromatogrammes fastidieuse - Non quantitatif - Appareillage.
Fractionnement granulométrique	Désagrégation en milieu aqueux de la matière organique et estimation de sa distribution dans des classes granulométriques	Distribution granulométrique de la matière organique	**Avantages :** - Séparation physique de la MO - Permet d'estimer la contribution des classes granulométriques dans la stabilisation de la MO - Analogie avec la MO des sols par rapport aux problématiques de stockage du C **Inconvénients :** - Solubilisation d'une partie de la matière organique dans l'eau
SPIR	L'échantillon est soumis à un rayonnement électromagnétique dans la gamme du proche infrarouge (800-2500 nm). Les longueurs d'onde absorbées correspondent aux fréquences de vibration des liaisons chimiques.	Information chimique sur le matériel utilisable grâce à des méthodes d'analyse de données multivariées (chimiométrie) pour prédire des caractéristiques des MOEs obtenues avec d'autres méthodes	**Avantages :** - Rapide - Peu de préparation de l'échantillon (broyage < 1mm) - Une seule analyse pour prédire diverses caractéristiques **Inconvénient :** - Interprétation qualitative des spectres difficile - Appareillage.

1.2.1. Mesure du potentiel de minéralisation du C organique des MOEs

La stabilité des MOEs, c'est-à-dire leur résistance à la biodégradation, est classiquement caractérisée par la mesure de leur potentiel de minéralisation du C organique. La mesure est réalisée au cours d'incubation d'un mélange sol / MOE en conditions contrôlées de laboratoire en conditions d'azote et d'oxygène non limitantes. Le CO_2 dégagé est le plus souvent piégé par de l'hydroxyde de sodium (NaOH) et dosé par colorimétrie ou par une méthode équivalente (AFNOR, 2009b). Les incubations sont réalisées sur une période habituelle de 91 jours, dans des conditions théoriquement optimales d'activité microbienne du sol. La température est fixée à 28℃, l'humidité du sol est équivalente à la capacité de rétention en eau à pF 2.8 et les MOEs sont finement broyées (< 1mm) afin de favoriser le contact avec le sol. Les MOEs montrent des potentiels de minéralisation contrastés en fonction de leur composition et de leur degré de stabilisation. Bernal et al. (1998) ont reporté des proportions de C de compost minéralisées allant de 7 à 77% en fonction de la composition et de la mixture initiale des déchets du temps et du procédé de compostage.

Il semble cependant que la minéralisation du C des MOEs soit plus faible au cours d'incubations de laboratoire comparé à leur minéralisation au champ (Lashermes *et al.*, 2009). Ceci pourrait être due au travail du sol ou aux alternances de dessiccation – réhumectation qui ont lieu au champ et non au laboratoire où l'humidité est maintenue à un niveau constant. La dessiccation du sol ou de composts entraîne la mort des microorganismes non adaptés mais augmente la disponibilité des ressources pour les organismes survivant (Mondini *et al.*, 2002). Apres réhumectation, des nutriments provenant des microorganismes morts ou rendus accessibles par la désagrégation physique du sol sont utilisés par les microorganismes survivants. Plusieurs études montrent qu'une réhumectation rapide du sol entraîne un dégagement soudain de CO_2 pouvant durer 2 à 6 jours (Pulleman & Tietema, 1999; Franzluebbers et al., 2000; Fierer & Schimel, 2003). Miller et al (2005) montrent que le CO_2 cumulé dégagé lors d'incubations en conditions contrôlées à 20℃ durant 16 semaines est plus important pour des sols ayant subi des alternances d'humectation – dessiccation répétées que pour le sol témoin maintenu à humidité constante.

Le choix d'une température d'incubation constante relativement élevée (28℃), différente de la température moyenne au champ s'explique par la possibilité de réduire la durée des incubations pour représenter un même intervalle de temps au champ. Pour cela, des fonctions de température sont utilisées. Elles permettent de calculer des équivalents degrés – jours et ainsi de représenter la minéralisation au champ par des incubations plus courtes sans besoin d'extrapoler les cinétiques. Différentes fonctions de températures sont utilisées par les modèles simulant la dynamique du C et de N dans les sols (Rodrigo *et al.*, 1997).

La mesure du potentiel de minéralisation des MOEs s'avère en définitive être une méthode reflétant fidèlement le niveau de stabilité des MOEs mais l'extrapolation des résultats de laboratoire au champ nécessite la prise en compte de l'influence sur les résultats des conditions de préparation des échantillons étudiés et d'incubations (fonction de température et d'humidité, carence en N…).

1.2.2. Fractionnement biochimique des MOEs par solubilisations successives selon la méthode Van Soest

La fractionnement biochimique de Van Soest a été mis au point pour l'analyse de la digestibilité des fourrages (Van Soest, 1963; Van Soest & Wine, 1967; Van Soest et al., 1991). Linères & Djakovitch (1993) ont adapté cette méthode à la caractérisation de la biodégradabilité des MOEs. La méthode de fractionnement est normalisé en France selon la norme XP U 44-162 (AFNOR, 2009a).

Le fractionnement Van Soest permet de séparer 4 fractions de MO dont les tailles sont déterminées par différence pondérale puis exprimées en % de la MO totale ou en % du C organique total des MOEs. Ces fractions peuvent être assimilées à des substances biochimiques équivalentes. Les fractions séparées sont :

- La fraction « soluble »: séparée par extraction à l'eau bouillante suivie d'une extraction à chaud par un détergent neutre. Le résidu obtenu, nommé NDF (Neutral Detergent Fiber), contient la majeure partie des parois végétales. Les substances solubilisées contiennent théoriquement des sucres, les acides organiques, les pectines, les lipides, l'amidon, des tannins, la majorité des protéines et de l'azote non protéique. La norme précise toutefois que le résidu peut contenir diverses substances (protéines, amidon, tannins) fortement liées aux polymères non solubilisés. Cette fraction peut augmenter en proportion de la MO avec la maturité des composts (Thuriès et al., 2002; Annabi et al., 2007; Francou et al., 2008) ce qui met en cause son assimilation à des composés facilement biodégradables. La séparation de la matière organique solubilisée par l'eau bouillante et par le détergent neutre est possible et permet d'affiner la caractérisation de la distribution de la matière organique (Morvan et al., 2006; Doublet et al., 2010).

- La fraction équivalent hémicellulose: solubilisée à chaud par un détergent acide après élimination de la fraction qualifiée comme soluble. Le résidu obtenu, appelé ADF (Acid Detergent Fiber), contient théoriquement la cellulose et la lignine. La fraction hémicellulose est calculée par la différence NDF-ADF. La fraction hémicellulose Van Soest diminue généralement en proportion au cours du compostage (Francou et al., 2008; Doublet et al., 2010).

- La fraction équivalent cellulose: solubilisée à froid par de l'acide sulfurique concentré après élimination des fractions qualifiées de soluble et hémicellulose. Le résidu obtenu, appelé ADL (Acid Detergent Lignin), contient théoriquement la lignine. La fraction cellulose est calculée par la différence ADF- ADL. La cellulose est dégradée pendant les phases mésophile, thermophile et de refroidissement par des champignons, des bactéries et des actinomycètes. La fraction cellulose Van Soest diminue généralement en proportion au cours du compostage (Francou et al., 2008; Doublet et al., 2010).

- La fraction équivalent lignine et cutine: correspond au résidu ADL de l'extraction par l'acide sulfurique concentré, qui contient théoriquement la somme des lignines et des cutines, il peut contenir par ailleurs des composés fortement liés à ces polymères comme des composés azotés. La fraction lignine augmente généralement en proportion au cours du compostage (Francou et al., 2008; Doublet et al., 2010).

De nombreuses études ont mis en évidence des relations entre le contenu de ces fractions et la minéralisation du C et de N des MOEs au cours d'incubations en conditions contrôlées (Henriksen & Breland, 1999; Trinsoutrot *et al.*, 2000; Thuriès *et al.*, 2002; Parnaudeau *et al.*, 2004; Bruun *et al.*, 2005; Jensen *et al.*, 2005; Morvan *et al.*, 2006; Lashermes *et al.*, 2009). Cependant, la fraction dite soluble ne semble pas bien représenter la biodégradabilité des composts pour lesquels elle pourrait contenir à la fois des composés facilement biodégradables et des composés secondaires stabilisés (Thuriès et al., 2002; Parnaudeau et al., 2004; Annabi et al., 2007; Francou et al., 2008; Morvan & Nicolardot, 2009).

1.2.3. Pyrolyse-CG/SM

La pyrolyse couplée à la chromatographie en phase gazeuse et à la spectrométrie de masse (pyrolyse-CG/SM) constitue un outil puissant pour caractériser la MO du sol (**MOS**) (Ceccanti et al., 1986; Schulten & Leinweber, 1996; González-Vila et al., 1999) et des MOEs (Ayuso et al., 1996; González-Vila et al., 2001; Dignac et al., 2005). La pyrolyse permet de casser de larges molécules en fragments caractéristiques de plus petites tailles, qui sont séparés sur une colonne chromatographique sous gaz vecteur (chromatographie en phase gazeuse : CG). (Wampler, 1999). L'utilisation d'un spectromètre de masse (SM) permet ensuite la reconnaissance des fragments produits. La pyrolyse des MO complexes génère un large éventail de produits de propriétés chimiques variées liées à leur origine biochimique (composés aliphatiques, methoxyphénols dérivés des lignines, cétones cycliques et furanes venant des polysaccharides, composés azotés provenant de protéines, acides organiques…) (Gonzales Vila et al, 2001 ; Dignac et al, 2005).

L'étude de la MO de composts à l'aide de cette méthode a montré des compositions variables en fonction des déchets compostés (Dignac et al, 2005). Parnaudeau (2005) a montré la possibilité d'utiliser les données semi-quantitatives issues de pyrolyse-CG/SM pour paramétrer le modèle CANTIS de dynamique du C et N dans les sols (Garnier et al., 2003). Dans cette étude, les simulations utilisant les données issues de pyrolyse ont montré une amélioration par rapport à celles utilisant des données du fractionnement biochimique de Van Soest.

Des rapports spécifiques d'aires de pics de produits lors de la pyrolyse peuvent être utilisés comme indicateurs des propriétés des MO:

- L'humification de la MO peut être estimée par le rapport entre furfural, produit de pyrolyse provenant des polysaccharides, sur pyrrole, dérivé de composés azotés, de MO humifiée et parois cellulaires microbiennes (Ceccanti et al., 1986) ou encore par le rapport acide acétique, dérivé de produits biodégradables, sur pyrrole (Garcia et al., 1993). Ces rapports montrent une diminution avec l'humification des MO (Ceccanti et al., 1986; Garcia et al., 1993; Dignac et al., 2005).

- La présence de polymères synthétiques peut être évaluée par le rapport toluène / styrène (Fabbri et al., 1998). Le toluène et le styrène sont des composés produits à la fois par la pyrolyse de polymères naturels et synthétiques mais le rapport de leurs contribution est supérieur à 1 pour des échantillons à dominance naturelle et inférieur à 1 pour des échantillons ayant une majorité de polymères synthétiques (Fabbri et al., 1998).

1.2.4. La Résonnance magnétique nucléaire du 13C (RMN)

La Résonnance magnétique nucléaire du ^{13}C est basée sur le nombre de spin des noyaux C. Un champ magnétique extérieur peut interagir avec le moment magnétique de spin, un peu comme le champ magnétique terrestre fait tourner l'aiguille d'une boussole. les noyaux C peuvent prendre différentes orientations sous l'effet d'un champ magnétique. Il est alors possible de détecter la résonance du noyau entre deux niveaux d'énergie. En pratique, l'environnement électronique du noyau le protège du champ magnétique appliqué, le champ magnétique réel nécessaire à la résonance dépend de cette protection. La protection conférée par l'environnement électronique des noyaux est différente entre les différents groupes fonctionnels. On mesure sur les spectres des intensités du signal pour les différentes valeurs de « déplacement chimique » de fréquence par rapport à la fréquence de vibration d'un standard δ en ppm. Chaque intervalle de valeur de déplacement chimique correspond à un type de groupes fonctionnels, et il est possible de quantifier l'abondance de ces groupes dans le matériel analysé. On peut ainsi distinguer des régions du spectre correspondant aux groupes carboxyle / amide, C aromatique, C alkyle ou O, N alkyle. La RMN du ^{13}C est très utilisée dans l'étude de la MO des sols (Baldock & Skjemstad, 2000; Kögel-Knabner, 2002) et a été utilisée pour caractériser des composts (Inbar et al., 1989; Preston et al., 1998; González-Vila et al., 1999).

1.2.5. Spectroscopie moyen infrarouge à transformée de Fourier (IRTF)

La spectroscopie moyen infrarouge à transformée de Fourier est largement utilisée pour l'étude des MOS (Chen et al., 2002; Ellerbrock & Kaiser, 2005; Gerzabek et al., 2006; Du & Zhou, 2009) et a été utilisée pour la caractérisation des MOEs (Smidt & Meissl, 2007; Francou et al., 2008). La spectroscopie IRTF est basée sur la mesure de l'absorption d'un rayonnement par l'échantillon analysé à différentes longueurs d'onde du spectre infrarouge moyen compris entre 400 et 4000 cm^{-1} (équivalent à 2500-25000 nm). L'absorption du rayonnement est modulée par les vibrations d'élongation ou de déformation des liaisons chimiques présentes dans l'échantillon ainsi que par la géométrie des molécules. Dans l'infrarouge moyen, les longueurs d'onde que l'échantillon absorbe, sont caractéristiques des groupes chimiques présents dans le matériel analysé. L'analyse d'un spectre permet de rapprocher l'abondance de certains pics à l'abondance de composés organiques (Tableau 1.2).

Tableau 1.2. Principales bandes d'absorption dans l'infrarouge moyen d'intérêt dans l'étude de la MO des sols et des MOEs, évolution au cours de la dégradation des MO (d'après Ouatmane *et al.*, 2000; Francou, 2003; Gerzabek *et al.*, 2006; Smidt & Meissl, 2007; Francou *et al.*, 2008).

Nombre d'onde (cm^{-1})	Assignation	Vibration	Commentaires	Evolution
3700-3050	OH de l'eau, N-H	Elongation	Large bande difficilement interprétable, influencée par l'humidité de l'échantillon	↑↓
2925	C-H aliphatiques,	Elongation		↓
2850	C-H aliphatiques,	Elongation		↓
2520	carbonates $CaCO_3$			↑
1720	C=O des acides carboxyliques	Elongation		↓
1650-1600	C=C aromatiques C=O amides I et quinones N-H amines primaires et amides	Vibration du squelette Elongation Déformation		↑ (Smith & Meissl 2007) → (Francou et al. 2008)
1505-1515	C=C aromatiques des lignines	Vibration du squelette		
1425-1460	CH_2	Déformation, élongation	Fréquence absorbée par les carbonates CaCO3	
	COO^- Acides carboxyliques			↓
1384	Nitrates NO_3	Déformation	Pic très étroit	↑(apparition dans les composts stabilisés)
1320-1250	C-N amine I et II aromatiques			↑↓
	C-O des acides carboxyliques	Elongation		↓
	C-N des amides III			
1040-1050	C-O polysaccharides éthers aromatiques	Elongation	Absorption par la silice Si	↓
870-600	Minéraux		Massif non interprétable (absorption par des minéraux)	

↑ : Augmentation, ↓ : diminution, ↑↓ : augmentation ou diminution, → constance

1.2.6. *Spectroscopie moyen infrarouge par réflexion diffuse à transformée de Fourier (DRIFT)*

La spectroscopie infrarouge par réflexion diffuse à transformée de Fourier (diffuse reflectance infrared Fourier Transform spectroscopy : DRIFT) est une technique proche de l'IRTF à la différence que l'analyse ne se fait pas en transmission mais par réflexion diffuse. Un rayon infrarouge est projeté sur l'échantillon et réfléchi dans toutes les directions. Une partie de l'énergie est absorbée par l'échantillon, le reste est dispersé et collecté généralement par un miroir sphérique et redirigé vers un détecteur. La DRIFT a l'avantage d'être très rapide, non destructive et permet l'analyse de poudres sans préparation particulière hormis un broyage très fin (< 200 µm) et dans certains cas un saupoudrage avec du KBr pour améliorer la dispersion.

La DRIFT a été utilisée pour caractériser la composition de MOEs et pour suivre des changements dans la composition de la MOS suite à des apports de MOEs (Sohi et al., 2001; Adani et al., 2007; Francioso et al., 2007). Elle a également été utilisée pour prédire divers constituants organiques de la MOS (Zimmermann *et al.*, 2007a; Bornemann *et al.*, 2010) et des MOEs (Reeves & Van Kessel, 2002) grâce à des modèles de régression multivariée. La précision de la DRIFT est inférieure à celle de l'IRTF mais permet une cadence d'analyse plus rapide. En outre, la DRIFT permet de scanner une surface correspondant à une quantité de matériel plus importante qui peut palier à des problèmes d'hétérogénéité des échantillons.

1.2.7. *Spectroscopie proche infrarouge (SPIR)*

La spectroscopie proche infrarouge (SPIR) est basée sur l'absorption d'un rayonnement proche infrarouge par le matériel analysé. Comme pour la spectroscopie dans l'infrarouge moyen, l'absorption est modulée par les vibrations des liaisons chimiques de l'échantillon. Dans le domaine du proche infrarouge (800 – 2500 nm), les absorptions ne sont pas dues aux vibrations fondamentales des molécules mais à des vibrations harmoniques et à des vibrations de combinaisons. Il en résulte des bandes d'absorption très larges et un fort chevauchement des bandes d'absorption des différentes liaisons chimiques. Il est donc difficile d'assigner des bandes d'absorption à des caractéristiques chimiques précises de l'échantillon comme dans l'infrarouge moyen. La SPIR n'est donc pas une technique de caractérisation à proprement parler mais trouve son intérêt dans l'utilisation de méthodes d'analyses de données multivariées pour extraire et utiliser les informations sur la composition chimique du matériel analysé (méthodes chimiométriques). Des régressions multivariées telle que la régression des moindres carrés partiels (partial least square regression : PLS) permettent de construire des modèles de régression entre les spectres proche infrarouge et des valeurs de référence (par exemple la teneur en C). Une fois les calibrations des modèles de prédiction réalisée, ils peuvent être utilisés pour prédire divers constituants d'échantillons dont les valeurs sont inconnues.

Les avantages de cette technique sont sa rapidité (env. 150 échantillons par jour), et son faible coût par rapport aux analyses de référence. De plus la SPIR est une technique non destructrice et qui nécessite peu ou pas de préparation de l'échantillon.

La SPIR est un outil de plus en plus répandu dans pour l'étude de la MOS et des MOEs. La SPIR a été utilisée pour prédire des teneurs en C et N du sol (Barthès et al., 2006; Terhoeven-Urselmans et al., 2006; Brunet et al., 2007), pour estimer la composition élémentaire et biochimique de matériaux végétaux (Gillon *et al.*, 1999; Stuth *et al.*, 2003; Stenberg *et al.*, 2004; Thuriès *et al.*, 2005), de matériaux compostés (Michel et al., 2006; Albrecht et al., 2009; Vergnoux et al., 2009) et de fumiers (Malley et al., 2002; Reeves & Van Kessel, 2002; Sørensen et al., 2007), ainsi que pour prédire la minéralisation du C et de N au cours d'incubations de matériaux végétaux (Bruun et al., 2005; Shepherd et al., 2005; Borgen et al., 2010) ou la minéralisation de la MO du sol (Thomsen *et al.*, 2009) ou encore pour la maîtrise du processus de compostage (Ben-Dor et al., 1997; Albrecht et al., 2008).

La difficulté dans l'étude de MO complexes et hétérogènes comme les MOEs en SPIR est la nécessité d'ensembles d'échantillons relativement homogènes et couvrant à la fois des plages de valeurs des données de référence suffisamment étendues pour réaliser une calibration précise (Thuriès *et al.*, 2005). Les modèles de prédiction par SPIR pour les MOEs trouvés dans les littératures ont souvent été calibrés en utilisant des MOEs relativement homogènes, ce qui permet d'obtenir des prédictions assez précises. Cependant peu d'études ont testé la possibilité de construire des calibrations utilisables pour des échantillons hétérogènes, cette approche peut pourtant être intéressante car les modèles SPIR sont valables uniquement pour des échantillons similaires à ceux utilisés pour construire les calibrations, ce qui limite l'intérêt de modèles développés sur des échantillons trop homogènes. De plus, dans l'étude des MOEs, il peut s'avérer difficile de collecter un nombre suffisant d'échantillons du même type et avec des valeurs de données de référence suffisamment étendues pour construire des calibrations satisfaisantes.

1.2.8. Indicateurs du devenir des MOEs dans les sols

Différents indicateurs ont été proposés pour estimer la part des MOEs potentiellement incorporée à la MOS à long terme à partir de leur composition biochimique. Ces indicateurs ont été construits par régression entre les fractions biochimiques et des taux de carbone résiduel déterminés par extrapolation de cinétiques de minéralisation réalisées en conditions contrôlées.

L'indice de Stabilité Biologique (ISB) (Linères & Djakovitch, 1993) permet d'estimer la part des MOEs potentiellement incorporée à la MOS à long terme à partir des fractions soluble, équivalents hémicellulose et lignine et cutine (SOL, HEM et LIC, respectivement) du fractionnement biochimique par la méthode Van Soest et la fraction de cellulose brute (CEW) déterminée par la méthode de Wende (AFNOR, 2005) selon l'équation :

ISB = 2,112 – (0,02009 SOL) – (0,02378 HEM) + (0,00840 LIC) – (0,02216 CEW)

l'indicateur Tr^{CBM} défini comme le taux de C restant au sol après l'incorporation de la MOE à long terme est calculé à partir des fractions soluble, équivalents hémicellulose, cellulose et lignine et cutine (SOL, HEM, CEL et LIC, respectivement) et prend en plus en compte l'effet de la fraction minérale (MM) de la MOE, et donc de sa teneur en MO (Robin, 1997).

Tr^{CBM} = + (0,3221 SOL - 0,7155 HEM + 0,6717 CEL + 1,8919 LIC) MO 10^{-2} + 0,0271 MM

Avec MM = 100 – teneur en MO

Ces deux indicateurs, présentent l'inconvénient d'avoir été construits à partir d'un faible nombre de produits et s'avèrent donc peu robustes. Pour y remédier, une base de donnée rassemblant des caractéristiques de dégradation en conditions contrôlées et des caractéristiques chimiques de la MO (fractions biochimiques Van Soest) de plus de 400 MOEs a été construite et a permis la construction d'un nouvel Indicateur de Stabilité de la Matière Organique (ISMO) des MOEs qui représente la proportion de C organique des MOEs s'incorporant à long terme à la MO des sols (Lashermes *et al.*, 2009). L'ISMO a été construit à partir de l'extrapolation de cinétiques de minéralisation du C à long terme et peut être calculé à partir de données de fractionnement biochimique de la MO Van Soest et de la proportion du C minéralisé après 3 jours d'incubation de 83 MOEs (boues, composts, fumiers, végétaux et engrais organo-minéraux). L'ISMO, normalisé (AFNOR, 2009a), est calculé selon l'équation :

ISMO = 44.5 + 0.5 SOL – 0.2 CEL + 0.7 LIC – 2.3 C_{3j}

Avec SOL, CEL et LIC les fractions Van Soest soluble, équivalents cellulose et lignine et cutine exprimées en % de la MO totale et C_{3j} la proportion de C de la MOE minéralisée après 3 jours d'incubation en conditions contrôlées à 28°C (en % du C organique total). L'ISMO s'avère plus robuste que les indicateurs précédents (ISB et TR^{CBM}) mais la comparaison de l'indicateur ISMO avec des données au champ semble indiquer une surestimation du stockage du C, ce qui pourrait être du à une minéralisation plus faible lors des incubations de laboratoire qu'au champ (Lashermes *et al.*, 2009).

Thuries et al. (2002) proposent le calcul du compartiment stable (Ps) d'un modèle de décomposition ajusté sur des cinétiques de minéralisation du C de MOEs au cours d'incubations à partir de la fraction lignine et cutine du fractionnement Van Soest et de la teneur en matière minérale MM selon:

Ps = 1.61 LIC + 0.62 MM pour des engrais organiques et des MOEs d'origine animale

P_S = 3.60 LIC pour des MOEs d'origine végétale.

Ces indicateurs constituent une première estimation du devenir des MOEs dans les sols. L'utilisation de modèles plus complexes de dynamique du C dans les sols est nécessaire pour des estimations plus précises de la dynamique du C à long terme après des apports de MOEs.

1.3. Dynamique du C dans les sols après des apports de MOEs

1.3.1. Etats des stocks de C des sols cultivés, potentiel du stockage de C pour limiter l'émission nette de gaz à effets de serre

Le C organique des sols (COS) représente le plus grand réservoir de C de la biosphère continentale. La quantité de C contenue dans le premier mètre du sol a été estimée entre 1500 et 2000 Gt C (Figure 1.1) soit environ trois fois plus que le C contenu dans l'atmosphère sous forme de CO_2 (environ 750 Gt C) et environ deux fois plus que le C contenu dans la végétation (environ 500 Gt C), l'océan renfermant quelques 38 000 GtC (IPCC, 2001). Une augmentation même minime du stockage dans les sols pourrait donc jouer un rôle significatif dans la limitation du flux net de gaz à effet de serre (GES) vers l'atmosphère

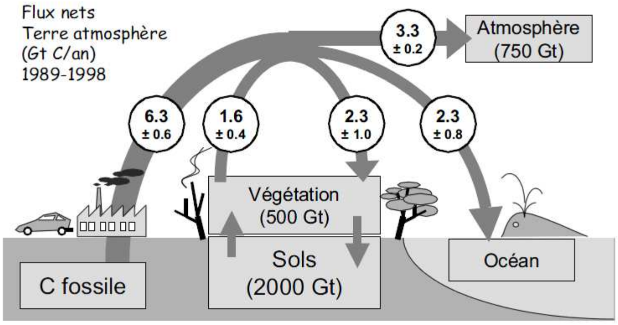


Figure 1.1. Cycle global du C à l'échelle planétaire : réservoirs et flux. (Arrouays et al. 2002, d'après IPCC, 2001).

Les flux nets de C vers l'atmosphère peuvent être positifs (sources) ou négatifs (puits). En ce qui concerne les sols cultivés, des estimations récentes à l'échelle européenne rapportent des pertes moyenne de C du sol de 17 g $C.m^{-2}.an^{-1}$ ces vingt dernières années (Ciais *et al.*, 2010). Ces estimations soufrent toutefois d'un manque d'harmonisation des méthodes de mesure. Globalement, les sols agricoles à l'echelle mondiale auraient perdu environ 55 Gt C au cours du siècle dernier du fait du développement, de la modernisation et de l'intensification de l'agriculture (IPCC, 2000).

Les sols européens sont riches en C organique dans les pays scandinaves ainsi qu'en Irlande et dans le Nord du Royaume-Uni qui totalisent environ 50% des stocks de C des sols européens, largement composés de C des tourbières (20% du stock de C des sol européens) (Schils *et al.*, 2008). Les teneurs en C sont moyennes dans les sols d'Europe centrale et faibles dans les sols du sud de l'Europe et du pourtour méditerranéens (Figure 1.2). Ces disparités Nord-Sud sont principalement dues aux différences de températures et de drainage des sols influençant les vitesses de décomposition des MO du sol (IFEN, 2007).

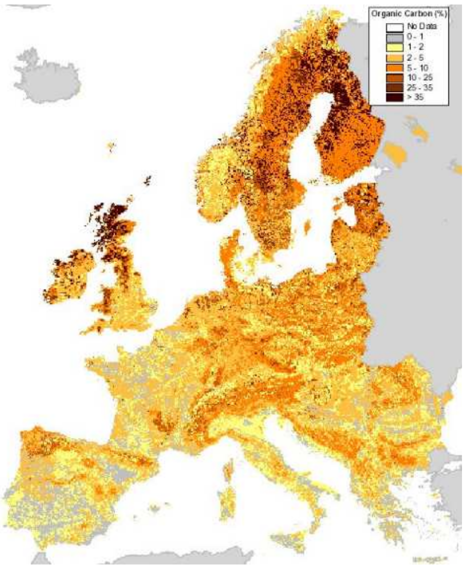
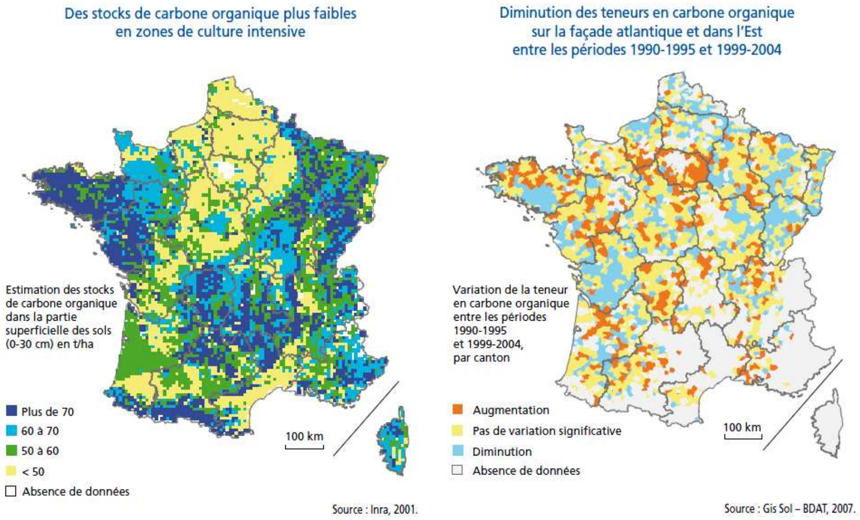


Figure 1.2. Teneur en C organique de l'horizon de surface des sols européens (source : European soil portal 2010: http://eusoils.jrc.ec.europa.eu/)

A l'échelle de la France, le stock de C des sols cultivés a diminué entre les périodes 1990-1995 et 1999-2004 (Figure 1.3). Ce déstockage est de l'ordre de 6 Mt C par an soit 0.2% de leur stock initial (IFEN, 2007).


Des stocks de carbone organique plus faibles en zones de culture intensive

Source : Inra, 2001.

Diminution des teneurs en carbone organique sur la façade atlantique et dans l'Est entre les périodes 1990-1995 et 1999-2004

Source : Gis Sol – BDAT, 2007.

Figure 1.3. Etat et évolution des stocks de C dans les sols cultivés en France (d'après IFEN, (2007)

Il a été estimé que le potentiel de stockage de C additionnel total du sol à l'échelle de la France était de l'ordre de 1 à 3 Mt C/an sur 20 ans. Cela correspond seulement à 1 à 2% des émissions de gaz à effet de serre annuels cependant, cela peut représenter une part importante de l'effort à consentir pour respecter les engagements pris par l'union Européenne dans le cadre du protocole de Kyoto de réduire de 8% les émissions nettes de GES durant la période 2008-2012 par rapport à l'année de référence 1990 (la France s'engageant à maintenir ses émissions au niveau de l'année 1990) (Arrouays *et al.*, 2002).

Le stockage de C dans les sols est beaucoup plus lent que le déstockage qui a lieu lors de l'abandon des pratiques stockantes ou d'un changement d'usage des terres. En conséquence, les accumulations de C constituées par des pratiques stockantes ne peuvent être conservées à long terme qu'à condition de conserver des usages des terres et des pratiques agricoles similaires à celles qui ont conduit à l'augmentation des stocks de C (Arrouays *et al.*, 2002).

Les expertises sur le potentiel des sols pour stocker du C et diminuer ainsi les émissions nettes de gaz à effet de serre (GES) vers l'atmosphère réalisées à l'échelle française (Arrouays *et al.*, 2002) et européenne (Schils *et al.*, 2008) concluent à un potentiel non négligeable du stockage de C dans les sols du fait du faible coût d'application des pratiques stockantes ainsi que des bénéfices environnementaux et agronomiques que cela entraine. Le stockage de C dans les sols ne représente toutefois pas une solution « miracle » et doit être combiné avec d'autres pratiques. Il est également nécessaire de prendre en compte les dégagements de GES sur l'ensemble des filières en prenant en comptes les émissions des autres gaz à effets de serre que le CO_2 (N_2O, CH_4).

Concernant les MOEs, l'expertise de l'INRA conclut que les composts issus de déchets urbains constituent un potentiel non négligeable de stockage de C dans les sols (0.6 tC/ha/an par exemple pour des composts de déchets verts + boues d'épuration), les surfaces concernées restent limitées mais l'utilisation de ces MOEs peut être localement intéressante en zone périurbaine (Arrouays *et al.*, 2002).

1.3.2. Turnover des matières organiques du sol (MOS)

Les stocks de C organique dans les sols naturels présentent un équilibre entre les entrées de C par les résidus carbonés d'origine végétale ou animale et leur biodégradation par les microorganismes du sol aboutissant à leur minéralisation sous forme de CO_2 libéré dans l'atmosphère. La MOS est composée d'environ 50 % de C organique. Elle a une composition complexe et hétérogène. Les composés organiques constituant la MOS sont constitués de résidus végétaux partiellement décomposés sous forme particulaire, de micro-organismes impliqués dans les processus de décomposition, de sous-produits issus de l'activité microbienne, d'exsudats racinaires, de matières organiques plus ou moins stabilisées, ces composés ayant des temps de résidence contrastés dans les sols (Figure. 1.4).

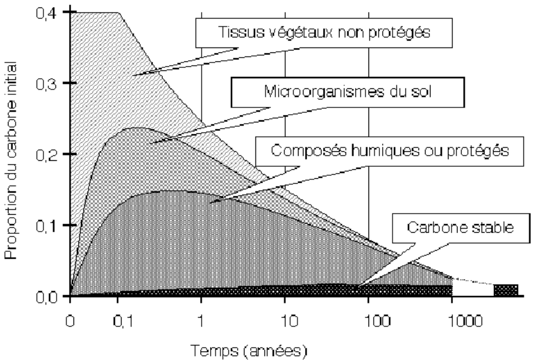


Figure 1.4. Représentation qualitative et quantitative du devenir du carbone organique incorporé par les végétaux dans le sol, dans un agrosystème cultivé français moyen (d'après Arrouays et al. 2002).

La **décomposition** des MOS peut être définie comme la combinaison de trois processus (Baldock & Skjemstad, 2000):

- **la transformation** : conversion du C organique d'une structure chimique à une autre suite aux attaques enzymatiques et à des réactions chimiques,
- **l'assimilation** : incorporation du C organique dans les tissus des organismes décomposeurs,
- **la minéralisation** : conversion du C organique en CO_2 via la respiration.

La décomposition du C organique est un processus complexe qui dépend d'un nombre important de facteurs notamment de la qualité des MOE apportées au sol, des propriétés du sol, notamment sa teneur en argiles et en carbonates, du climat (température, humidité), des pratiques culturales (travail du sol…). La première phase de décomposition est relativement rapide dans le cas d'apport de MOEs non transformées et aboutit à la perte de un quart à deux tiers des apports organiques en un à deux ans (Jenkinson & Rayner, 1977). Suit une phase de décomposition plus lente où environ 90% de la MO est décomposée en une période de 10 à 100 ans. Puis une troisième phase où la totalité de la MO est décomposée avec un turnover de 100 à 1000 ans. Il n'existe donc pas ou presque pas de stockage de C définitif dans les sols car toute MO ou presque est à terme minéralisée.

La MOS est une entité dynamique qui évolue de manière continue au cours du temps. Le renouvellement du C (turnover) est défini comme le bilan des entrées et sorties dans un compartiment de MOS (pool) donné. Le turnover du C d'un compartiment est en général quantifié par son temps moyen de résidence (TMR). Le TMR d'un compartiment est défini soit par le temps moyen durant lequel un composé réside dans le compartiment à l'équilibre ou par le temps moyen requis pour renouveler totalement le contenu du compartiment à l'équilibre. Le turnover des matières organiques est le plus souvent décrit par l'utilisation d'équations différentielles d'ordre 1:

$$\frac{dCi}{dt} = I - kiCi$$

où Ci est la quantité de C dans le compartiment i, I est l'apport de C organique, t est le temps et ki est la vitesse de décomposition du compartiment i. A l'équilibre, le TMR peut être calculé comme :

$$TMR = \frac{1}{ki}$$

La MOS peut ainsi être vue comme un ensemble de compartiments ayant des turnovers différents (Figure 1.5). Cette subdivision de la MOS en compartiments cinétiques est à la base de la formulation de la majorité des modèles décrivant la dynamique du C (modèles multi-compartimentaux) tels que RothC ou CENTURY (Jenkinson & Rayner, 1977; Parton et al., 1987).

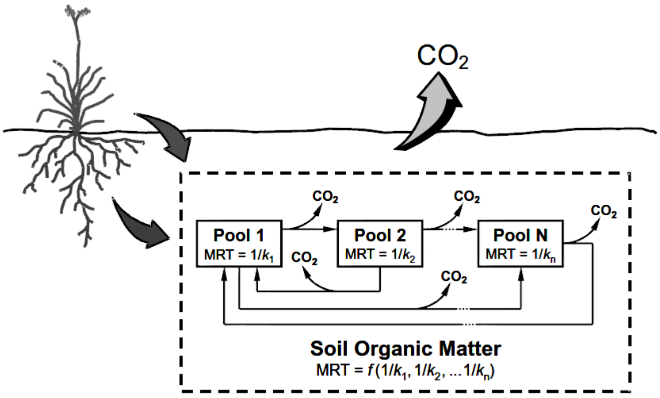


Figure. 1.5. Représentation de la MOS en compartiments (pools) ayant des temps moyens de résidence (mean residence time: MRT) propres (d'après Six et Jastrow, 2002).

Pour des sols cultivés, le temps moyen de résidence du COS a été estimé à 61 ± 9 ans pour une fourchette allant de 18 à 165 ans pour une synthèse de 10 études différentes (Six & Jastrow, 2002).

1.3.3. Facteurs naturels influençant la dégradation de la MOS

Il existe une multitude de facteurs influençant la vitesse de dégradation de la MOS. Ces facteurs agissent en général de manière combinée. Parmi les plus importants, on peut citer :

- **La température** : Une augmentation de la température de 10℃ diminue le temps de résidence d'un facteur 2 à 3 (Raich & Schlesinger, 1992). De nombreuses fonctions de température ont été mises au point pour moduler l'intensité de la biodégradation en fonction de la température dans les modèles (Parton et al., 1987; Jenkinson et al., 1991).

- **La texture du sol** : les argiles fournissent une protection physique et physico-chimique de la MO (Baldock & Skjemstad, 2000). Une augmentation des teneurs en argile diminue donc la vitesse de décomposition de la MOS. La teneur en argile fait varier les temps de résidence de la MOS dans la gamme 1-2.5 (Arrouays *et al.*, 1999). Il a été mis en évidence des saturations en C des sols plus ou moins rapides en fonction de leur texture (Six *et al.*, 2002).

- **L'humidité du sol** : L'humidité du sol favorise la biodégradation et peut faire varier le temps de résidence du C dans un facteur de 1 à 2 (Rodrigo *et al.*, 1997). L'activité biologique et la minéralisation seraient maximales pour une saturation en eau de la porosité de l'ordre de 50 à 70% (Scott *et al.*, 1996).

On peut également mentionner l'influence du pH et des cations du sol, ainsi que l'influence de la macrofaune pour son rôle de fragmentation et de brassage de la MO.

1.3.4. Mécanismes de stabilisation de la MOS

Le C stabilisé dans les sols, à temps de résidence long est primordial dans une problématique de stockage du C. La stabilisation des MO peut être définie comme l'augmentation de leur résistance à la dégradation. Les mécanismes de stabilisation de la matière organique dans les sols ne sont pas encore totalement élucidés et font encore l'objet de nombreuses études (von Lützow et al., 2006; Sollins et al., 2007). La théorie de l'humification, aujourd'hui remise en cause, explique la stabilité des MOS par la formation de structures condensées et très aromatiques qui leur confèreraient une fort potentiel de résistance à la biodégradation (Stevenson, 1994). De nombreuses études ont depuis souligné que les MO stabilisées dans les sols contiennent des quantités importantes de composés intrinsèquement biodégradables tels que des polysaccharides ou des produits dérivés de protéines (Gleixner et al., 2002; Kiem & Kögel-Knabner, 2003; Rillig et al., 2007). D'autres études ont mis en évidence l'importance des complexes organo-minéraux dans la stabilisation des MO (Ludwig et al., 2003; Kleber et al., 2007). Les mécanismes de stabilisation apparaissent donc complexes et généralement combinés, ils peuvent être différenciés en plusieurs classes de mécanismes comme proposé par von Lützow et al. (2006) :

- **Préservation sélective (récalcitrance)**, aboutissant à l'accumulation relative des molécules récalcitrantes, du fait de leurs caractéristiques moléculaires intrinsèques leur conférant une résistance à la biodégradation.

- **Inaccessibilité spatiale (protection physique)**, la localisation des MO empêche leur accès aux microorganismes et aux enzymes. L'inaccessibilité est causée par l'occlusion de la MO par l'agrégation, la disposition à l'intérieur de phyllosilicates, l'hydrophobicité et l'encapsulation à l'intérieur de macromolécules.

- **Interactions avec des surfaces minérales ou organiques et des ions métalliques (protection physico-chimique),** les MO sont protégées de la minéralisation par des interactions inter-moléculaires entre la MO et des composants inorganiques ou d'autres MO. Les interactions possibles incluent les échanges de ligands, la formation de ponts cationiques, des interactions faibles, ou la complexation avec des métaux.

Ces mécanismes de stabilisation n'on pas la même importance et n'interviennent pas sur les mêmes échelles de temps. La récalcitrante des MO, dans le cas d'apport de résidus végétaux non transformés est surtout importante les premières années. Durant les phases suivantes de la décomposition, l'inaccessibilité des MO aux organismes décomposeurs ainsi que les interactions organo-minérales semblent être les processus dominants (von Lützow *et al.*, 2006). Toute MO est à terme minéralisable et il n'existe pas de récalcitrance absolue des MO.

1.3.5. Méthodes de fractionnement des MOS

La MOS peut être fractionnée dans le but de séparer des compartiments de MO ayant des turnovers différents. Deux grandes familles de séparation des MO peuvent être distinguées : les séparations chimiques et physiques.

La séparation « chimique » des MOS consiste à extraire ou solubiliser une partie des MOS par action de réactifs chimiques. La séparation des MO en acides humiques, acides fulviques et humines a été très utilisée dans l'étude des MOS (Stevenson, 1994; Piccolo, 1996).

- Les **acides humiques** sont solubles dans une solution alcaline (NaOH ou pyrophosphate de sodium) puis précipitées par acidification de l'extrait alcalin.

- les **acides fulviques**, sont également soluble dans une solution alcaline mais restent solubles lors de l'acidification de l'extrait alcalin.

- les **humines** constituent les substances insolubles dans une solution alcaline.

Les diverses études sur les substances séparées montrent une variabilité importante dans la composition de ces fractions, néanmoins, les acides fulviques sont généralement plus riches que les acides humiques en fonctions COOH et en hydrates de C. Les acides humiques seraient essentiellement composés de composés hydrophobes possédant des chaines aliphatiques. La composition des humines est moins connue du fait de la difficulté de séparer les humines de la matière minérale du sol (Stevenson, 1994; Piccolo, 1996). Cette méthode de fractionnement est cependant controversée, Duchaufour (1970) montrait déjà que les fractions séparées n'offrent pas de propriétés biochimiques définies. Les acides humiques et l'humine en particulier peuvent être eux même subdivisés en composés jeunes, peu polymérisés, faciles à dégrader ou au contraire en composés vieillis très polymérisés, résistant à la biodégradation (Zbytniewski & Buszewski, 2005).

L'hydrolyse acide a également été utilisée pour séparer une fraction non hydrolysable résistante de la MOS (Paul et al., 1997; Augris et al., 1998). Balesdent (1996) a cependant rapporté l'impossibilité de séparer chimiquement des composés organiques ayant des âges différents par extraction alcaline, hydrolyse acide, oxydation thermique, oxydation par H_2O_2 ou par pyrolyse. Eusterhues et al. (2003) ont montré la possibilité de séparer des MOS ayant des âges différents par oxydation au $Na_2S_2O_8$ séparant une MO chimiquement récalcitrante et par déminéralisation du sol par attaque au HF qui libèrerait les composés organiques protégés par occlusion ou par interaction avec des minéraux.

Les méthodes de **fractionnement granulométrique et densimétrique** de la matière organique du sol permettent d'isoler des compartiments ayant des dynamiques différentes. Ce type de fractionnement est basé sur l'idée que la stabilité des MO dans les sols ne dépend pas seulement de leur composition intrinsèque mais également et des associations avec les constituants du sol au travers de l'agrégation par la protection physique de MO et par les interactions avec les minéraux (Christensen, 1992; Chenu & Plante, 2006). Le fractionnement granulo-densimétrique consiste à disperser le sol de façon plus ou moins forte afin de casser les agrégats et séparer les MO libres, les MO protégées physiquement et les MO liées physico-chimiquement. Les fractionnements granulo-densimétriques présentent l'avantage de séparer les MO sans modifier leur composition. Le sol est d'abord dispersé soit par agitation avec des billes de verre soit par l'application d'ultrasons (Balesdent *et al.*, 1991; Christensen, 1992). Les particules sont en suite séparées selon leur taille par tamisage sous eau ou par sédimentation. La séparation des MO légères peut être réalisée par flottation dans de l'eau ou à l'aide de liqueurs de densités différentes. L'utilisation de liqueurs densimétriques présente toutefois le risque de contaminer ou de dénaturer les MO.

Les MO associées aux fractions grossières du sol (> 50µm), appelées MO particulaires (MOP) sont composées en majorité de débris végétaux figurés plus ou moins fragmentés, peu associés aux minéraux et ayant un turnover rapide (Christensen, 1992; Balesdent, 1996). Pour des sols limoneux cultivés avec une transition de culture de blé à maïs plus ou moins récente, Balesdent (1996) calculait, en utilisant l'abondance naturelle en ^{13}C, des vitesses de renouvellement de 3 ans pour les fractions 200 - 2000 µm et de 18 ans pour les fractions 50 – 200 µm.

Les MO associées aux fractions fines (0-50µm) sont des MO plus anciennes avec un turnover de 63 ans mesuré par Balesdent (1996). Elle sont associées à des micro-agrégats assurant une protection des MO par adsorption sur des surfaces minérales et par occlusion (Chenu & Plante, 2006). Les particules de taille 0-50 µm peuvent être subdivisées en particules de la taille des limons grossiers (20-50µm), particules de la taille des limons fins (2-20 µm) et particules de la taille des argiles (< 2 µm).

1.3.6. Effet d'apports de MOEs sur les stocks de C du sol

Les apports de MOEs permettent généralement d'augmenter les teneurs en MO des sols (Haynes & Naidu, 1998; Edmeades, 2003). Cependant, peu de données sont publiées sur les évolutions des stocks de C. Une synthèse des données bibliographiques réalisée par Muller *et al.* (2004) à montré que sur 128 articles faisant état de changement de la MOS suite à des apports de MOEs, seulement 23 permettaient de déterminer l'effet des apports de MOEs sur les stocks de C du sol. Les données nécessaires pour déterminer l'efficacité des MOEs à augmenter les stocks de C du sol sont :

- Les quantités de C apportées par les MOEs et leur fréquence d'apport
- La profondeur d'enfouissement des MOEs
- L'évolution des teneurs en COS
- Des mesures de la masse volumique apparente du sol pour les différents traitements, y compris témoin
- Pouvoir comparer des masses de sol identiques entre les traitements et tout au long de l'essai. En effet, il est fréquemment observé une modification de la densité du sol suite à des apports de MOEs (souvent une baisse). Il faut donc prendre en compte une partie de l'horizon sous jacent en compte ce qu'il est nécessaire d'avoir des mesures des teneurs en C de l'horizon sous jacent (Ellert & Bettany, 1995; Lee et al., 2009).

Les rendements d'accumulation du C après des apports répétés de MOEs peuvent ainsi être calculés selon :

$\mathrm{Rdt} = \Delta C_i / C_{cum}$

où ΔC_i est la quantité de C accumulée après un nombre d'années données (différence de stock par rapport à un traitement témoin sans apport) et C_{cum} est la quantité totale de C apportée par les MOEs pendant le nombre d'années considérées. Ces rendements sont dépendants de la durée de l'essai, en effet le taux d'accumulation du C après des apports de MOEs tend à décroitre avec le temps (ΔCi/ nombre d'années) (Thomsen & Christensen, 2010). Ils sont également influencés par les conditions climatiques et la nature du sol de l'essai. Le guide pour l'inventaire des gaz à effet de serre de l'IPCC (1997) préconise d'utiliser une durée standardisée de 20 ans pour la comparaison de différentes pratiques permettant le stockage de C dans les sols.

La synthèse réalisée par Muller et al. (2004) propose la comparaison des rendements d'accumulation du C du sol par des apports de différentes MOEs (Tableau 1.3).

Tableau 1.3. Synthèse des rendements d'accumulation du C du sol générés par des apports de matières organiques exogènes (d'après Muller *et al.*, 2004)

	Nombres de MOEs	Moyenne (± ecart type)	Fourchette	Références
Boues d'épuration	20	0.30 ± 0.15	0.09-0.70	(Stark & Clapp, 1980; Robertson et al., 1982; Mbagwu & Piccolo, 1990; McGrath et al., 2000; Gerzabek et al., 2001; Bergkvist et al., 2003)
Boues de papeterie	3	0.17 ± 0.01	0.08-0.28	(Chantigny *et al.*, 1999)
Compost d'ordures ménagères et biodéchets	7	0.37 ± 0.13	0.15-0.58	(Giusquiani *et al.*, 1995; Leita *et al.*, 1999)
Composts de boues	4	0.37 ± 0.05	0.32-0.42	(Tester, 1990; McGrath et al., 2000)
Fumiers	21	0.25 ± 0.09	0.16-0.39	(Delas & Molot, 1983; Ndayegamiye & Cote, 1989; Persson & Kirchmann, 1994; Gerzabek et al., 1997; Aoyama et al., 1999; Paré et al., 1999; McGrath et al., 2000; Manjaiah & Dhyan, 2001; Dorado et al., 2003; Hao et al., 2003)
Lisiers	4	0.20 ± 0.15	0.02-0.40	(Ndayegamiye & Cote, 1989; Min *et al.*, 2003)

Une très forte variabilité des résultats est constatée en raison de conditions (température, type de sol, climat) et des durées différentes des essais, du manque de mesures des densités des sols qui faussent les estimations des stocks de C, et du regroupement sous une même appellation de MOEs en réalité très différentes. On peut toutefois noter des rendements supérieurs pour les composts et relativement faibles pour les lisiers et les boues de papeteries. Le recours à la modélisation est nécessaire pour une meilleure comparaison de l'efficacité des MOEs à augmenter les stocks de C pour différents sites et différentes durées d'apport.

Beaucoup d'études on mis en évidence des relations quantitatives entre la composition biochimique des MOEs et leur dégradabilité dans les sols qui pour certaines ont abouti à la construction d'indicateurs de leur devenir dans les sols (c.f. partie 1.2.8.) (Linères & Djakovitch, 1993; Robin, 1997; Henriksen & Breland, 1999; Trinsoutrot et al., 2000; Thuriès et al., 2002; Parnaudeau et al., 2004; Bruun et al., 2005; Jensen et al., 2005; Morvan et al., 2006; Lashermes et al., 2009). Cependant, ces études sont basées sur des rapprochements entre la composition biochimique des MOEs et la mesure de la minéralisation du C et de N au cours d'incubations en conditions contrôlées. Peu d'études ont présenté des rapprochements qualitatifs ou quantitatifs entre la qualité des MOEs et les augmentations des stocks de C qu'elles entrainent lors d'expérimentations au champ de longue durée.

1.3.7. Effet des apports de MOEs sur la composition de la MOS

Le fractionnement granulo-densimétrique de la matière organique a été fréquemment utilisée pour décrire les effets d'apports de MOEs sur la MOS. La fraction de MO particulaire (MOP) peut être considérée comme un indicateur précoce des modifications de la MOS totale qui se manifestent à plus long terme (Haynes, 2005). Une augmentation préférentielle des MOP a fréquemment été observée suite à des apports de MOEs au sol (Gerzabek *et al.*, 2001; Kong *et al.*, 2005; Sleutel *et al.*, 2006; Zhou *et al.*, 2010). Zhou et al. (2010) suggèrent que l'accumulation des MOP suite à des apports de fumiers serait due à leur récalcitrante plus qu'à leur protection physique ou physico-chimique. Cependant le C contenu dans les MOP ne représente qu'une faible proportion du C total du sol, mesurée entre 7 et 9% du C total pour un sol sans apport de MOEs et un sol avec apport de fumier par Gerzabek *et al.* (2006) et entre 11 et 22% du C total dans l'étude de Sleutel *et al.* (2006). En conséquence même si le C des MOEs est préférentiellement accumulé dans les MOP à l'échelle de la dizaine d'années (Sleutel *et al.*, 2006), le C accumulé à plus long terme est majoritairement contenu dans les fractions de la taille des limons et des argiles (Gerzabek *et al.*, 2001; Kirchmann *et al.*, 2004).

Les effets des apports de MOEs sur l'augmentation des teneurs en C et N et l'augmentation de la biomasse microbienne sont bien connus et bien documentés dans la littérature (Houot & Chaussod, 1995; Haynes & Naidu, 1998; de Araujo *et al.*, 2010). En revanche, les résultats concernant la composition chimique détaillée de la MOS suite à des apports de MOEs sont peu nombreux et parfois contradictoires en raison de la diversité des MOEs apportées, des méthodes utilisées et des conditions d'expérimentation différentes.

Une augmentation des proportions de C aliphatique d'origine microbienne a souvent été rapportée après des apports de fumiers dans des essais à long terme (Gerzabek *et al.*, 2006; Monaco *et al.*, 2008; Zhou *et al.*, 2010), ainsi qu'une augmentation de composés dérivés de lignine probablement issus des pailles apportées avec les fumiers (Schulten & Leinweber, 1996; Lima *et al.*, 2009).

Concernant des apports de composts, Spaccini et al. (2009) rapportent une persistance de résidus ligneux non décomposés un an après un apport de compost dont le type n'est pas précisé. Une augmentation de la proportion de composés issus de lignine a également été trouvée par Leifeld *et al.* (2002) au cours d'incubations sur une durée de 18 mois pour un compost de biodéchets peu stabilisé alors qu'un compost stabilisé n'entrainait pas d'augmentation des composés issus de lignine. Leifeld *et al.* (2002) rapportent également une augmentation des proportions de C aromatique déterminées par RMN du ^{13}C dans les sols amendés avec des composts ainsi qu'une dégradation préférentielle des polysaccharides des composts et des sols. Gonzalez-Vila et al. (1999) rapportent que la seule modification de la MOS suite à un apport d'un compost d'ordures ménagères à court terme est une modification de la qualité et de la quantité de lipides du sol et concluent que des apports de composts ne peuvent pas contribuer à l'accumulation de forme stable du C. Enfin, Lima et al. (2009) rapportent une augmentation des proportions de dérivés de protéines et de glucides suite à des apports répétés et à long terme (30 ans) de composts d'ordures ménagères.

Devant une telle variabilité des résultats, il apparait que les effets des apports de composts sur la MOS sont encore peu documentés et que les processus en jeu ne sont pas encore bien compris. Certaines études relatent des effets à court terme mais dans une problématique de stockage de C il est important d'évaluer les effets à long terme lors d'essais en conditions réelles au champ. Les résultats semblent indiquer une diversité des effets sur la composition de la MOS en fonction de la composition des MOEs apportées.

1.4. Modélisation de la dynamique du C des MOEs apportées au sol.

1.4.1. Modèles existant de dynamique du C

Des modèles généraux de dynamique du C du sol peuvent être utilisés pour simuler le devenir du C des MOEs. Il existe un grand nombre de modèles de dynamique de la MOS, 36 d'entre eux ont été recensés dans le cadre du réseau SOMNET (soil organic matter network : www.rothamsted.bbsrc.ac.uk/aen/somnet/index.htm) du GCTE (Global Change and Terrestrial Ecosystems; www.gcte.org). La majorité des modèles de dynamique du C répartissent la MOS en compartiments ayant des vitesses de dégradation différentes. La prise en compte des variables affectant la dynamique du C telles que le climat, la texture du sol et les pratiques culturale dans des modèles est nécessaire pour prévoir leur dynamique dans le sol.

Le modèle de Henin et Dupuis est un des premiers modèle de dynamique du C mis au point (Henin & Dupuis, 1945). Il décrit la dynamique du C avec un seul compartiment de MOS et deux paramètres :

- K1 : la fraction de l'apport annuel de C au sol qui entre dans la MOS, le reste étant supposé être minéralisé instantanément. Le paramètre K1, nommé coefficient isohumique, est spécifique pour chaque MOE en fonction de sa composition.

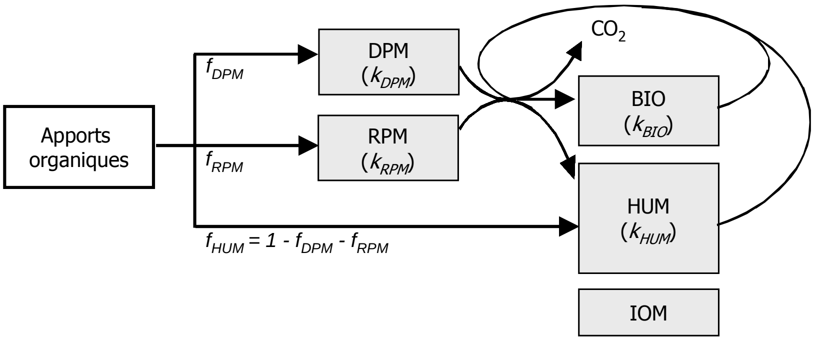
- Le paramètre K2 (an^{-1}) est le coefficient de minéralisation de la MOS. Le K2 peut être calculé en fonction de la température moyenne annuelle, de la teneur en argile et de la teneur en $CaCO_3$ du sol (Rémy & Marin-Laflèche, 1976). Le modèle de Henin et Dupuis est largement utilisé dans la pratique par les utilisateurs et les décideurs. Il a notamment été utilisé lors de l'expertise de l'inra pour estimer les potentialités de stockage dans les sols agricoles Français (Arrouays et al., 2002) et par Le Villio et al. (2001) pour estimer les quantités de MOE pour restaurer et entretenir les niveaux de MO des sols limoneux français. Le modèle AMG (Andriulo *et al.*, 1999) est une évolution du modèle de Henin et Dupuis, subdivisant le compartiment de MOS en un compartiment de C actif et un compartiment de C stable qui ne se dégrade pas. Ces deux modèles ont un pas de temps annuel et sont valables uniquement pour des prévisions supérieures à 5 ans.

Les modèles multi-compartimentaux plus complexes permettent une description plus précise de la dynamique du C. Les variations annuelles et saisonnières peuvent être prises en compte grâce à des compartiments de MO transitoires à turnover rapides. Ils permettent également une meilleure prise en compte des variables pédo-climatiques du milieu. Leurs compartiments sont essentiellement définis par des turnovers différent mais se rapprochent également de caractéristiques fonctionnelles (biomasse microbienne, C actif ou structural, C stabilisé par protection physique, physico-chimique ou en raison de leur récalcitrante).

Neuf de ces modèles ont été comparés sur des données de sept essais de long terme (Smith *et al.*, 1997) parmi eux on peut citer CENTURY (Parton *et al.*, 1987) qui simule la dynamique du C, la croissance des plantes, et les cycles de N, P et S, DAISY (Hansen *et al.*, 1991) qui contient des sous-modèles hydrologiques, de dynamique de l'azote et du C, et de croissance des plantes. Le modèle NCSOIL (Molina *et al.*, 1983) qui simule la dynamique couplée C et N, et le modèle RothC (Jenkinson & Rayner, 1977; Coleman & Jenkinson, 1999) qui simule uniquement la dynamique du C. Certains modèles représentent une distribution continue de la qualité de la MOS (Bosatta & Agren, 1985) mais nécessitent le développement de méthodes pour mesurer la distribution continue de qualité de la MOS pour vérifier leur résultats (Bruun *et al.*, 2010). Le choix d'un de ces modèles dépend de la problématique traitée, les modèles les plus utilisés dans la problématique du stockage du C sont les modèles RothC et CENTURY, qui ont des structures relativement similaires.

1.4.2. Le modèle RothC

Le modèle RothC est le pionnier des modèles multi-compartimentaux (Jenkinson & Rayner, 1977). Il décrit la dynamique de la MOS en la répartissant en cinq compartiments ayant des turnovers différents (Figure 1.6). Chaque compartiment excepté celui de MO inerte suit une cinétique de décomposition du premier ordre. Les apports de C sont répartis dans les compartiments d'entrée de MO labile (DPM), résistante (RPM) et humifiée, c'est-à-dire stabilisée (HUM) par des coefficients de partition qui dépendent de la nature des apports. A chaque pas de temps mensuel, une partie de chaque compartiment est décomposée suivant la vitesse de décomposition propre du compartiment (k). Une partie est minéralisée sous forme de CO_2 et le reste est transféré dans les compartiments BIO (biomasse microbienne) et HUM (humifié). La proportion qui est transformée en CO_2 et en BIO + HUM est déterminée par la teneur en argile du sol. Le rapport CO_2 / (BIO + HUM) est calculé selon l'équation : CO_2 / (BIO + HUM) = 1.67 (1.85 + 1.60 exp(-0.0786 % argile)). La part transférée dans BIO + HUM est répartie en 46% de BIO et 54% de HUM. Les constantes de vitesses sont modifiées à chaque pas de temps par trois facteurs multiplicatifs, dépendant de la température, du déficit hydrique du sol, de la présence/absence de végétation (Coleman & Jenkinson, 1999).



Compartiments	**Temps moyens de residence (=1/k)**
Decomposable plant material (DPM)	1.2 mois
Resistant plant material (RPM)	3.3 ans
Microbial biomass (BIO)	1.5 ans
Humified organic matter (HUM)	50 ans
Inert organic matter (IOM)	Infini
Données d'entrée	
Etat initial des compartiments	
Couvert végétal (présence / absence)	
Flux d'apport de C	
Coefficients de partition (f_{DPM}, f_{RPM}, f_{HUM}) des MO apportées	
Teneur en argile du sol	
Température moyenne mensuelle de l'air	
Pluviométrie cumulée mensuelle	
Evaporation open pan	
Données de sortie	
Evolution des stocks de C du sol et de la taille des compartiments, respiration	

Figure 1.6. Présentation des compartiments du modèle RothC et de leurs constantes de vitesse d'évolution, données d'entrée nécessaires et de sortie (d'après Coleman & Jenkinson, 1999)

1.4.3. Mesure de la taille des compartiments des modèles de dynamique du C

Une étape délicate dans l'utilisation des modèles de dynamique du C est l'estimation de la taille initiale des compartiments qui a une importance considérable sur le résultat des simulations, il est donc utile de disposer de méthodes capables de séparer et de quantifier le contenu de chaque compartiment. Mesurer la taille des compartiments permet également la vérification des résultats des modèles ainsi qu'une meilleure interprétation des mécanismes impliqués dans le stockage du C en rendant les modèles plus mécanistes. Ainsi de nombreuses études ont tenté de relier la taille des compartiments de modèles à des fractions de MOS mesurables ou de développer de nouveaux modèles ayant des compartiments basés sur des fractions mesurables (Balesdent, 1996; Christensen, 1996; Elliott *et al.*, 1996; Puget *et al.*, 2000; von Lützow *et al.*, 2007; Zimmermann *et al.*, 2007b).

Concernant le modèle RothC, Balesdent (1996) a établi des corrélations satisfaisantes entre la fraction de MO particulaire > 50 µm et le compartiment RPM de RothC dans un sol limoneux cultivé sous maïs et entre la fraction 0-50 µm et les compartiments BIO + HUM + IOM. L'auteur conclut que la majorité du C du compartiment RPM est contenu dans la fraction > 50 µm même si une partie se trouve dans la fraction 0-50 µm. De même, Ludwig et al. (2005) ont montré pour un sol limoneux sous maïs une correspondance entre la fraction 0-63 µm et les compartiments BIO + HUM + IOM. Skjemstad et al. (2004), dans une étude sur des sols australiens, ont montré une correspondance entre la fraction de MO particulaire > 53µm et le compartiment RPM. Un compartiment de C sous forme de charbon était corrélé au compartiment IOM (les sols étudiés étant riches en charbons) et la différence entre carbone total, compartiment labile et charbon correspondait au compartiment HUM. Zimmermann et al. (2007b), dans une étude sur 123 sols de surface agricoles en Suisse, ont montré une correspondance entre les fractions de C organique particulaire > 63 µm et de C organique dissous avec les compartiments DPM + RPM, une correspondance entre les fractions de C associées à la somme argiles + limons (< 63 µm) oxydable au NaOCl et les compartiments HUM + BIO et une correspondance entre le C organique résistant à une oxydation au NaOCl et le compartiment IOM. Le fractionnement granulo-densimétrique apparaît donc être une méthode satisfaisante pour séparer des fractions de MOS correspondant aux compartiments du modèle RothC.

1.4.4. Simulation de l'accumulation du C suite à des apports de MOEs.

Les modèles multi-compartimentaux peuvent être utilisés pour simuler le devenir du C provenant spécifiquement des apports de MOEs et de la production additionnelle de résidus de cultures liée aux augmentations de production végétale après épandage par rapport à des parcelles témoin. Cela est rendu possible par la linéarité de ces modèles, les cinétiques de dégradation y étant décrites par des équations différentielles d'ordre 1. Ce qui implique:

- que le devenir de X kilogrammes d'apport est X fois le devenir d'un kilogramme;
- le devenir d'une portion du carbone est indépendant du reste du carbone;
- toutes choses égales par ailleurs, le différentiel de carbone simulé entre un sol avec et sans apport de MOE est égal au devenir simulé de la MOE seule.

La simulation de l'accumulation du C par les MOEs permet de s'affranchir de l'étape délicate d'estimation de la taille initiale des compartiments de MOS. Cependant la linéarité des modèles implique un certain nombre de simplifications, notamment la non prise en compte de l'influence du « priming effect » c'est-à-dire de la modification de la vitesse de décomposition de la MO native du sol par des apports de MOE (Fontaine et al., 2003). Une approche non linéaire est possible mais plus compliquée à mettre en œuvre et nécessite une description dépendante de l'échelle et de la localisation du C (Monga *et al.*, 2008).

1.4.5. Prise en compte de la qualité des MOEs dans les modèles de dynamique du C

La qualité des MOEs dans les modèles multi-compartimentaux de dynamique du C est prise en compte par leur répartition dans les compartiments d'entrée suivant des coefficients de partition ou par la modification des vitesses de dégradation des compartiments. Ces paramètres ont souvent été déterminés empiriquement pour quelques MOEs par ajustement sur des données d'essais de long terme. Le modèle RothC propose des coefficients de partition pour les résidus de culture (f_{DPM} = 59%, f_{RPM} = 41%, c.f. Figure 1.6) et les fumiers (f_{DPM} = 49%, f_{RPM} = 49%, f_{HUM} = 2%).

Certains modèles proposent des méthodes pour déterminer les paramètres prenant en compte la qualité des MOEs. Dans le modèle CANTIS de dynamique couplée du C et N (Garnier *et al.*, 2001), la qualité des MOEs est décrite par leurs fractions biochimiques selon la méthode Van Soest. Gabrielle *et al.* (2004) proposent une détermination des paramètres prenant en compte la qualité des MOEs dans le modèle NCSOIL à partir de l'indicateur de stabilité biologique calculé à partir de fractions biochimiques des MOEs (ISB, voir 1.2.8). Dans le modèle CENTURY, la qualité des MOEs est décrite par la rapport lignine / N (Parton *et al.*, 1987) et dans le modèle STICS, par le rapport C / N (Nicolardot *et al.*, 2001). Cependant, le rapport C / N n'est pas adapté pour décrire la qualité de tous les types de MOEs car des formes de N stabilisées non assimilables par les microorganismes sont en général formées au cours du compostage (Paré *et al.*, 1998) ce qui modifie l'interprétation qui peut être faite du rapport C / N. De même, le rapport lignine / N pourrait ne pas être représentatif de tous les types de MOEs du fait de l'apparition de N stabilisé et de la dégradation partielle de la lignine au cours du compostage et de l'apparition de composés récalcitrants secondaires qui peuvent être retrouvés dans la fraction soluble du fractionnement Van Soest (Thuriès *et al.*, 2002; Morvan & Nicolardot, 2009). Enfin, l'indicateur ISB a montré des limites du fait qu'il a été calibré sur un faible nombre de MOEs.

Il est donc nécessaire de développer des fonctions de transfert permettant de déterminer les paramètres des modèles à partir de mesures fiables de leur biodégradabilité au laboratoire. L'indicateur ISMO estimant la part du C des MOEs potentiellement accumulée à long terme dans le sol a montré sa robustesse pour une grande variété de MOEs et il pourrait être utilisé comme indicateur de la qualité des MOEs dans les modèles.

1.5. Conclusions, objectifs et démarche du travail

La capacité des MOEs à augmenter les stocks de C du sol dépend en premier lieu de la composition de leur MO. De nombreuses études ont établi des relations entre les fractions séparées par le fractionnement biochimique par la méthode Van Soest et le potentiel de minéralisation du C des MOEs mesuré au cours d'incubations en conditions contrôlées. Ces méthodes permettent de calculer des indicateurs de stabilité des MOEs tels que l'indicateur de stabilité biologique (ISB) et plus récemment l'indicateur de stabilité de la MO (ISMO) calibré sur un plus grand nombre de MOEs. Ces indicateurs ont pour vocation d'estimer la proportion du C des MOEs potentiellement accumulable dans le sol à long terme. Ces méthodes et indicateurs s'avèrent donc très utiles pour l'estimation du devenir des MOEs dans les sols. Cependant, ces méthodes demeurent longues à mettre en place, couteuses et génèrent des déchets chimiques polluants, il serait souhaitable de les compléter de méthodes plus rapides comme la spectroscopie proche infra-rouge (SPIR) qui est de plus en plus utilisée pour prédire diverses caractéristiques des sols et des MOEs à partir d'une mesure rapide et peu couteuse. Plusieurs études ont établi des modèles de prédiction par SPIR de diverses caractéristiques des MOEs. Ces modèles ont généralement été développés en utilisant des MOEs relativement homogènes et ne sont pas extrapolables à d'autres types de MOEs. La capacité de la SPIR pour prédire la part de C potentiellement résiduelle dans les sols après épandage des MOEs a peu été étudiée. Par ailleurs, la composition et la biodégradabilité des fractions séparées par la méthode Van Soest restent encore mal connues ce qui nuit à l'interprétation des résultats fournis par cette méthode. La fraction dite « soluble » du fractionnement Van Soest séparée à l'eau chaude puis par un détergent neutre contient théoriquement des composés rapidement biodégradés dans les sols mais plusieurs études ont remis en cause la nature facilement biodégradable de cette fraction qui dans le cas de composts ou de MOEs très transformées pourrait contenir également des formes de C stabilisées sans qu'elles aient pu être mises en évidence.

Une fois apportées au sol, les MOEs permettent généralement d'augmenter les stocks de C du sol. Ces augmentations sont très variables et des MOEs d'un même type épandu dans des conditions similaires lors d'expériences au champ de longue durée peuvent entrainer des cinétiques distinctes d'accumulation du C dans le sol. Ces différences sont dues aux conditions pedo-climatiques propres à chaque site d'étude, en particulier la température et la teneur en argile des sols, ainsi qu'à une composition chimique variable de MOEs. En effet, des MOEs portant la même appellation peuvent regrouper en réalité des MOEs ayant des compositions chimiques très diverses. Bien que de nombreuses études aient fait le lien entre la qualité biochimique des MOEs mesurable au laboratoire et leur potentiel de minéralisation au cours d'incubations en conditions contrôlées, peu d'études ont tenté de relier les quantités de C accumulées au cours d'essais au champ à la composition chimique des MOEs apportées. De plus, les formes sous lesquelles les MOEs sont accumulées à long terme dans les sols sont encore mal connues, en particulier dans le cas de composts fabriqués à partir de déchets urbains où des effets à court terme ou au cours d'incubations de laboratoire sont plus souvent rapportés, du fait du manque d'expérimentations au champ et à long terme portant sur ce type de MOEs.

L'utilisation de modèles de dynamique du C permet de prendre en compte les effets des variables pédoclimatiques et des pratiques culturales qui influencent la dynamique du C ainsi que de bâtir divers scénarios d'apport. Cela en fait des outils incontournables dans l'étude du stockage de C par des apports de MOEs. Il n'existe pas à notre connaissance de modèle simulant spécifiquement la dynamique du C des MOEs dans les sols. Des modèles généraux de dynamique du C du sol multi-compartimentaux peuvent être utilisés pour simuler l'accumulation du C provenant spécifiquement des MOEs et du surplus de résidus de culture que leur apport peut entrainer. Le modèle RothC est l'un des plus utilisés pour des problématiques de stockage de C dans les sols, il présente l'avantage d'avoir été testé dans une grande diversité de situations, de décrire la dynamique du C avec relativement peu de paramètres et de nécessiter peu de données d'entrée. Les paramètres permettant de prendre en compte la composition chimique des MOEs dans les modèles ont souvent été déterminés empiriquement pour un nombre limité de MOEs (cas du modèle RothC). Pour certains modèles, des méthodes existent pour déterminer ces paramètres à partir de caractéristiques des MOEs mesurables au laboratoire (rapport C/N, lignine/N…), cependant ces indicateurs semblent insuffisants pour pouvoir s'appliquer à une grande diversité de MOEs, notamment à des MOEs compostées.

Au vu de ces considérations, **les objectifs du travail de thèse ont été :**

- **De développer des outils de caractérisation de laboratoire permettant de préciser la nature chimique des MOEs et de mieux estimer leur devenir dans les sols.**
    - En précisant la nature chimique et la biodégradabilité de la fraction soluble du fractionnement Van Soest dans le cas d'un compost à différents stades de maturité.
    - En développant des modèles de prédiction du devenir des MOEs dans le sol par spectroscopie proche infrarouge (SPIR).
- **D'adapter le modèle RothC de dynamique du C du sol afin de le rendre opérationnel pour simuler le stockage potentiel de C dans les sols suite à des apports répétés de MOEs.**
    - En paramétrant le modèle RothC sur les données de différents essais de longue durée où des MOEs sont apportées et en reliant les paramètres ajustés à des caractéristiques mesurables des MOEs.
- **D'approfondir les connaissances sur les formes sous lesquelles ces MOEs sont incorporées dans la MO du sol.**

- En s'appuyant sur un essai au champ de longue durée où des composts urbains et un fumier de ferme sont apportés (essai Qualiagro).

La démarche générale adoptée pour ce travail est synthétisée dans la **Figure 1.7**.

Les résultats de ce travail de thèse sont présentés dans cinq chapitres, correspondant chacun à un projet d'article. Ces chapitres sont répartis en trois parties thématiques.

La **Partie I** présente des développements de méthodes et d'indicateurs permettant d'estimer le devenir des MOEs dans les sols. Elle regroupe le **Chapitre 2** et le **Chapitre 3.**

Le **Chapitre 2** présente une approche visant à préciser la nature chimique et la biodégradabilité de la fraction soluble du fractionnement biochimique Van Soest. La fraction soluble de la méthode Van Soest est extraite à l'eau chaude puis par un détergent neutre. Ce détergent contenant lui-même du C, son utilisation empêche la caractérisation des composés organiques séparés. Une méthode alternative d'extraction a été mise au point afin d'extraire des composés organiques similaires à ceux extraits par la méthode Van Soest mais exempt de produit carbonés. Le protocole d'extraction ainsi développé a été appliqué à un compost d'ordures ménagères résiduelles à deux stades de maturité (immature et mature). Les fractions séparées ont été caractérisées par pyrolyse-CG/SM et spectroscopie IRTF, le potentiel de minéralisation du C au cours d'incubations des fractions solubles à l'eau chaude et des résidus après les différentes phases d'extraction a été mesuré. Ces analyses ont permis de préciser la nature chimique et la biodégradabilité de la fraction soluble Van Soest dans le cas d'un compost à différents stades de maturité.

Le **Chapitre 3** présente le potentiel de la SPIR pour prédire diverses caractéristiques des MOEs: teneur en C, N, fractions biochimiques par la méthode Van Soest, minéralisation du C au cours d'incubations ainsi que la proportion de C stable des MOEs potentiellement accumulable dans les sols à long terme. Le développement des modèles SPIR s'appuie sur un large jeux de données regroupant 300 MOEs des types différents (fumiers, composts, matériaux végétaux…) dont la plupart ont été collectés dans le cadre d'une précédente étude ayant abouti à la construction d'une base de données regroupant des caractérisations de MOEs provenant de différentes équipes de recherche et ayant servi à la construction de l'indicateur de stabilité de la MO: ISMO.

La **Partie II** présente l'étude de l'accumulation de C dans le sol suite à des apports répétés de MOEs en conditions réelles au champ. Elle regroupe le **Chapitres 4** et le **Chapitre 5** :

Dans un premier temps (**Chapitre 4**), l'accumulation du C du sol suite à des apports réguliers de composts urbains et de fumiers a été étudiée dans l'essai au champ Qualiagro (Feucherolles, 78) mis en place en 1998. La dynamique du C organique total du sol a été étudiée ainsi que sa répartition dans des fractions granulo-densimétriques après 8 années d'apport. Les résultats ont été mis en relation avec la composition chimique et biochimique des composts et fumiers apportés (fractionnement Van Soest, potentiel de minéralisation du C, indicateur ISMO). Le partitionnement du C total des MOEs dans les différents compartiments d'entrée du modèle RothC a été optimisé pour reproduire les cinétiques d'accumulation du C suite aux apports de composts et de fumier. Les résultats ont confirmé la possibilité d'utiliser le modèle RothC pour simuler l'accumulation du C dans le sol après des apports de MOEs. La possibilité de prédire les paramètres de RothC décrivant la qualité des MOEs à partir de caractérisations des MOEs au laboratoire à été testé, montrant un réel potentiel mais demandant d'être confirmé par des résultats d'autres essais au champ où d'autres MOEs sont apportées.

Des données d'autres essais au champ ont été collectées afin de calibrer le modèle RothC pour une plus grande diversité de sites et de MOEs (**Chapitre 5**). L'objectif était de calibrer le modèle RothC pour un nombre suffisant de MOEs pour pouvoir établir une fonction de transfert entre les paramètres ajustés de RothC et des caractéristiques des MOEs mesurables au laboratoire. Les paramètres prenant en compte la qualité des MOEs dans RothC (coefficients de partition dans les compartiments d'entrée) ont ainsi été ajustés sur les données de quatre essais au champ de longue durée où différentes MOEs sont apportées : L'essai d'Askov (Danemark), l'essai Qualiagro (Feucherolles, 78), l'essai de la station expérimentale SERAIL (Brindas, 69) et l'essai d'Ultuna (Uppsala, Suède). Les caractéristiques mesurées au laboratoire des MOEs épandues dans les différents essais on été utilisées pour prédire les paramètres ajustés de RothC à partir de l'indicateur de stabilité de la MO (ISMO).

la **Partie III,** est constituée du **Chapitre 6** dans lequel les modifications de la composition chimique de la MO contenue dans des fractions granulo-densimétriques du sol de l'essai Qualiagro sont étudiées après 8 années d'apport de composts et de fumier. Les fractions de la MO du sol ainsi que les MOEs épandues ont été caractérisées par pyrolyse-CG/SM et par spectroscopie DRIFT, ce qui a permis de mettre en évidence des modifications de la MO du sol après des apports répétés de composts et de fumier et de relier ces modifications à la composition chimique des MOEs épandues.

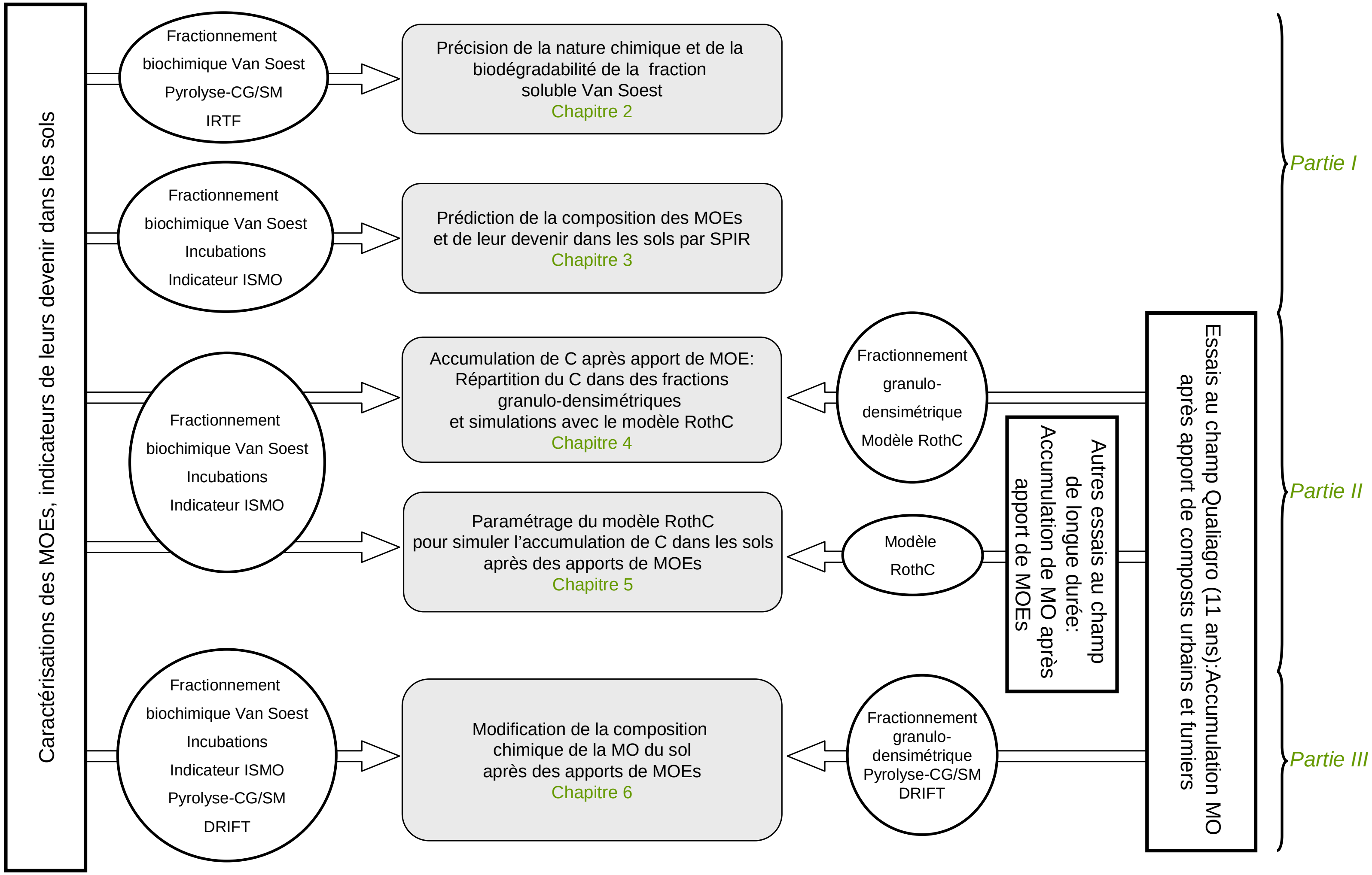


Figure 1.7. Schéma de la démarche générale adoptée pour ce travail de thèse.

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Partie I. Amélioration d’outils et de méthode de caractérisation des matières organiques exogènes et d’estimation de leur devenir dans les sols

Chapitre 2. Change of the chemical composition and biodegradability of the Van Soest soluble fraction during composting: A study using a novel extraction method

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2.1. Abstract:

Van Soest fractionation is widely employed to characterize exogenous organic matter. The soluble fraction of Van Soest fractionation (SOL, extracted using hot water and then neutral detergent) often increases in line with compost maturity, although it is generally considered as labile. We have developed an alternative extraction method that comprises four successive steps (extraction using hot water, sodium tetraborate, dichloromethane/methanol and chelating resin) in order to clarify the chemical nature of the SOL fraction and explain its biodegradability. This method was tested on municipal solid waste compost sampled during the thermophilic phase (MSWi) and after 8 months of composting (MSWm). Both methods extracted similar proportions of organic matter. The composition of the residues was similar in MSWm although differences were noted for the extraction of polysaccharides and lipids in the case of MSWi. The hot water extractable fraction decreased during composting. Its high biodedegradability in MSWi was linked to the high polysaccharide content revealed by pyrolysis-GC/MS and FTIR spectroscopy. The increase in the sodium tetraborate extractable fraction mainly explained the increase in the SOL fraction during composting. This was made up of N-containing compounds, polysaccharides and lipids in the immature compost, and a majority of N-containing compounds in the mature compost. During composting, the stabilization of organic matter in the SOL fraction extractable by sodium tetraborate and EDTA might principally involve N-containing structures through the formation of complexes of organic matter with metal ions, especially Ca^{2+}, which may be broken down during extraction of the Van Soest soluble fraction. These mechanisms still need to be investigated.

Keywords:

Van Soest soluble, neutral detergent fraction, municipal solid waste compost degradability, pyrolysis, FTIR, incubation.

2.2. Introduction

On the loamy soils of western Europe, intensive cropping practices can result in a decrease in soil organic matter (SOM), particularly in areas where animal breeding has disappeared (Le Villio et al., 2001), thus increasing soil sensitivity to the risk of erosion and inducing a loss in soil fertility. This loss of SOM has thus been identified by the European Commission as one of the major threats to soils (2006).

The treatment of biological waste by composting is a growing practice that enables the production of organic amendments rich in stabilized organic matter (OM). The application of compost to the soil contributes to increasing SOM content and improving soil fertility (Lal et al., 2007). In suburban areas, composts produced using urban waste are an important and potentially increasing source of OM, and their use on cultivated soils may make it possible to reconcile waste recycling and an increase in SOM levels (Hargreaves et al., 2008).

The efficiency of exogenous OM in increasing SOM is dependent on its degradation kinetics following its application on soils. This efficiency can be determined through a biochemical characterization of exogenous OM using the Van Soest fractionation procedure. This method has been applied to the characterization of exogenous OM such as animal manure or compost (Charest and Beauchamp, 2002; Thuriès et al., 2002; Morvan et al., 2005; Francou et al., 2008) and is now standardized in France (AFNOR, 2005). An indicator of the fraction of added OM that might remain in soils has been defined, based both on the quantification of Van Soest biochemical fractions and on the mineralization of added carbon during short-term incubations (Lashermes et al., 2009).

The Van Soest biochemical fractionation method was initially developed to evaluate forage digestibility (Van Soest, 1963; Van Soest and Wine, 1967) and was then adapted to the characterization of exogenous OM (Linères and Djakovitch, 1993). This method enables the step-wise chemical extraction of organic materials that are assimilated to “soluble”, “cellulose-”, “hemicellulose-” and “lignin- and cutin-” like compounds. During composting, cellulose- and hemicellulose-like fractions generally decrease, while the lignin- and cutin- like fraction increases (Charest and Beauchamp, 2002; Thuriès et al., 2002; Annabi et al., 2007; Francou et al., 2008). Changes to the “soluble” fraction during composting remain unclear. According to the modified standardized procedure (Linères and Djakovitch, 1993; AFNOR, 2005), this fraction is obtained after two extraction steps, one in hot water and the second in a mixture of neutral detergents. This soluble fraction is generally considered as being made up of labile compounds and is thus expected to decrease during composting (Thuriès et al., 2002). However, this fraction has been shown to increase in line with compost stability (defined as compost resistance to biodegradation) in various urban waste composts (Annabi et al., 2007; Francou et al., 2008). The organic fractions extracted by cold and hot water and then neutral detergents (SOL fraction) from manures with differing degrees of stability displayed marked differences in terms of carbon (C) and nitrogen (N) availability (Morvan and Nicolardot, 2009). Thus the SOL fraction of stabilized composts may contain slowly biodegradable organic compounds. However, the chemical composition of this “soluble” fraction remains poorly understood since it cannot be analyzed directly because of the organic chemicals present in the neutral detergent used for its extraction.

A variety of molecular techniques can be used to describe the chemical composition of complex OM. Pyrolysis coupled with gas chromatography and mass spectrometry (pyrolysis-GC/MS) is a powerful tool to study compost OM (Gonzalez-Vila et al., 1999; Dignac et al., 2005). It provides global and precise fingerprinting of pyrolysable organic structures as a result of gas chromatographic (GC) separation of the volatile compounds released following pyrolysis and their identification by mass spectrometry (MS). In addition, Fourier Transformed Infra-Red (FTIR) spectroscopy has often been used to assess compost maturity (Ouatmane et al., 2000; Francou et al., 2008).

The aim of the present study was to demonstrate how composting affects the chemical composition of the Van Soest SOL fraction obtained using hot water extraction followed by neutral detergent extraction. Based on knowledge of the potential action of the chemicals present in the neutral detergent mixture, we were able to develop a novel extraction procedure to isolate a fraction devoid of contaminating organic chemicals and only containing organic constituents comparable to those extracted with the neutral detergent mixture. The new procedure was applied to two composts produced using the same composting process and sampled at two different levels of maturity. The extracted OM and extraction residues were characterized by pyrolysis-GC/MS and FTIR spectroscopy. Their potential biodegradability was assessed during incubations with soil under laboratory-controlled conditions.

2.3. Materials and Methods

2.3.1. Composts

Municipal solid waste (MSW) compost was used during this study. The initial mixture contained residual municipal solid wastes obtained after the selective collection of recyclable materials (paper and cardboard, glass, plastic and metal containers). Details on the composting process can be found in Annabi et al. (2007). The compost was sampled from the same industrial pile after one month of composting, at a time when the compost OM was still highly biodegradable (**immature compost MSWi**), and after 8 months of composting when the compost OM was stabilized (**mature compost MSWm**). The two compost samples were sieved at 10 mm, air dried and finely ground (<1 mm) before analysis. Total organic C and N contents were determined by dry combustion with a CHN autoanalyzer (Micromass NA 1500 NC). The total organic C contents of the immature and mature composts were 252.9 ± 2.8 and 134.1 ± 0.8 g.kg^{-1} dry matter, respectively. The total N contents of the immature and mature composts were 14 ± 0.3 and 15.1 ± 0.3 g.kg^{-1} dry matter, respectively.

2.3.2. Extraction of the Van Soest soluble fraction

The Van Soest soluble fraction was obtained using the standardized method (AFNOR, 2005) derived from the classic Van Soest procedure (Van Soest and Wine, 1967) as modified by Linères and Djakovitch (1993). The two-step extraction procedure included an initial extraction in hot water at 100℃ (water extractable fraction: **WE** fraction) followed by a second extraction in a neutral detergent solution at 100℃ for 60 minutes (**SOL-ND**). In the standardized procedure, the extraction is performed in glass crucibles. However, flasks were used in the present work to make it possible to easily recover the soluble extracts and to fractionate larger samples necessary for the further characterizations.

The WE fraction was extracted from 9-g sub-samples of ground compost covered with 150 mL of hot water for 30 minutes. Several sub-samples were extracted in multi-position heating mantles (6 x 250 ml) with reflux columns. Under the standardized Van Soest procedure, the sample is mixed with inert sand. So that the process would be as similar as possible to Van Soest conditions, 2g of sand (previously heated at 500℃) was added to the compost sample before extraction, which probably exerted a mechanical influence on the boiling process. The soluble fraction was separated from the solid residue by centrifugation at 6000 g for 15 min in 500-mL polypropylene copolymer (PPCO) flasks, and then filtered through glass crucibles (porosity 40-90 µm) used for standard Van Soest fractionation. The extractions were performed on three batches of six flasks for MSWi and four batches of six flasks for MSWm. Extracts from the same batch were then pooled in order to obtain sufficient material for subsequent characterizations, and then freeze-dried.

Three of the residues of the hot water extraction for MSWi, and six for MSWm, were extracted using 150 mL of neutral detergent solution (for 1 L distilled water: 30 g sodium lauryl sulphate, 18.61 g sodium dihydrogen ethylene-diaminetetraacetic, 6.81 g sodium tetraborate decahydrate; 4.56 g sodium hydrogen phosphate; and 10 mL triethylene glycol; pH between 6.9 and 7.1) for 60 min, after adding 10 drops of octanol to prevent foaming. After centrifugation as described above, the residues (Neutral Detergent Fibre residue: **NDF residue**) were rinsed 10 times with 100 mL of hot distilled water (10 min agitation then centrifugation as previously) and finally dried for 48 h at 40℃.

2.3.3. A novel alternative fractionation procedure

The neutral detergent mixture contains organic chemicals, which could be a problem during chemical characterization of the extracted OM. We thus developed an alternative extraction procedure where C-containing chemicals were replaced by C-free chemicals or resins, based on the identified actions of the original chemicals in the neutral detergent solution. Sodium tetraborate and sodium hydrogen phosphate solubilizing OM at pH 7 were retained in the new fractionation procedure because they do not contain C. Sodium dodecyl sulphate is a surfactant that extracts lipids, and was replaced by dichloromethane-methanol (2:1 v/v), which is classically used to extract lipids (Cequier-Sanchez et al., 2008). Sodium ethylene-diamine-tetracetate (EDTA) is a chelating agent that solubilizes OM complexed to metals. This was replaced by a chelating resin (Amberlite IRC-748). The new stepwise fractionation method was divided into three successive steps following hot water extraction, which are presented in Figure 2.1. and detailed below.

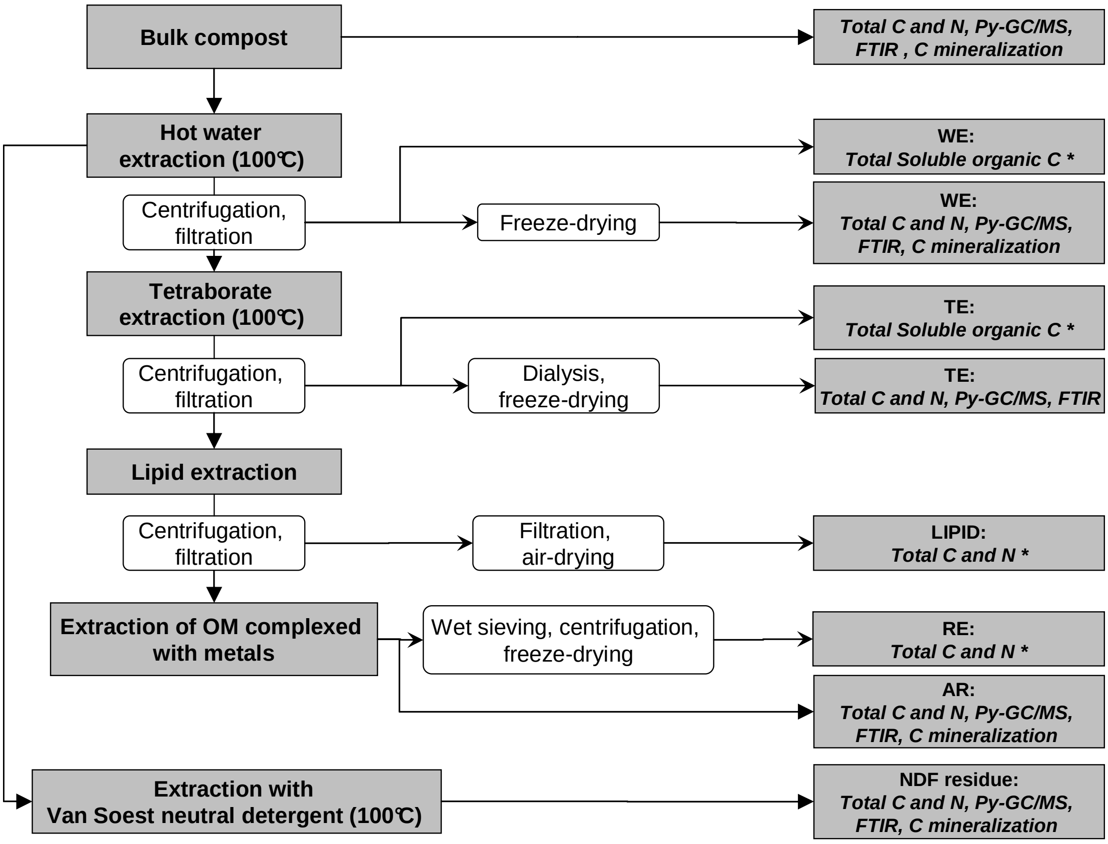

Figure 2.1. Scheme of the novel alternative extraction procedure and of the classical Van Soest extraction of the soluble fraction. Description of the analysis performed on each extract and residue. **WE**: hot water extract, **TE**: tetraborate extract, **LIPID**: lipid extract, **RE**: "resin" extract, **AR**: residue of the novel alternative procedure, **NDF residue**: final residue after neutral detergent extraction under the classical Van Soest procedure.

2.3.3.1. Tetraborate extraction (100℃)

Residues remaining after hot water extraction (six residues for MSWi and six for MSWm) were boiled for 60 min with a tetraborate solution (for 1 L distilled water: 6.81 g sodium tetraborate decahydrate, 4.56 g sodium hydrogen phosphate; pH adjusted to 7 with 1.93 mL 85% phosphoric acid). The extracts were recovered after centrifugation (10 min, 6000 g) and filtered as in neutral detergent extraction. The residues were rinsed 10 times with 100 mL hot distilled water (10 min agitation and then centrifugation as previously).

Soluble C was measured in each extract and then all replicates corresponding to one compost were pooled. Aliquots of 50 mL of the extracts (the so-called "tetraborate" extract: **TE**) were dialysed against distilled water to remove sodium tetraborate for 48 h at 4℃ using a Spectra/Por® membrane with a molecular weight cut-off point of 1000 Da, and then freeze-dried.

2.3.3.2. Lipid extraction

After tetraborate extraction, two residues (duplicate extraction) for MSWi and MSWm were shaken for 4 h in a dichloromethane-methanol mixture (2:1 v/v, 30 mL per g residue). The extracts were recovered by centrifugation for 15 min at 1950 g in corex glass tubes and the extraction process was repeated for 12 h. The lipid extracts were filtered through a glass fibre filter (porosity: 1.2 µm, Wathman GF/C) to eliminate residual particles. The residues recovered after filtration were then air-dried.

2.3.3.3. Extraction of OM complexed with metals

The ethylene-diamine-tetracetate (EDTA) present in the neutral detergent solution was replaced by a chelating resin (Amberlite IRC 748, previously cleaned by shaking 50 g of resin twice in 300 mL Milli-Q water in a PPCO flask). Each replicate of the residue after lipid extraction (two for MSWi and two for MSWm) was shaken with 200 mL Milli-Q water with resin (4 g resin per g residue) in PPCO flasks. After 5 h, the solid residue and the extract were separated from the resin by wet sieving. Finally, after centrifugation (15 min at 6000 g), the extract was separated (so-called “resin” extract **RE**) and the residue (residue of the alternative method: **AR**) dried for 48 h at 40℃. Before any further characterizations, all extraction residues were ball milled to less than 200 µm (Retsch PM100).

2.3.4. Elemental composition

The total soluble organic C content was determined in the six water and tetraborate extracts by infrared detection after combustion using a total organic C (TOC) autoanalyzer (TOC Schimadzu 5050 A). The total C and N contents were measured in all freeze-dried residues obtained during the different extraction steps of the alternative method, in the NDF residues of the original Van Soest fractionation, in the freeze-dried water extracts (three replicates for WEi and four replicates for WEm), in tetraborate extracts (one replicate for TEi and TEm), in air-dried lipid extracts (two replicates for each compost) and in resin extracts (two replicates for REi and REm) by dry combustion with a CHN autoanalyzer (Micromass NA 1500 NC). The C mass balance of the alternative extraction procedure was calculated using the measured C concentrations in the water and tetraborate soluble extracts, in the air-dried lipid extracts and in the freeze-dried resin extracts (**RE**).

2.3.5. Laboratory incubations

The mineralization of organic C was measured during laboratory incubations of mixtures of soil and total compost, freeze-dried WE extracts, NDF residues and alternative method residues (AR). The soil was sampled from the upper layer (0-28 cm) of a loamy soil (clay, 15.6%; silt, 77.9%, sand, 6.5%, C, 10 g kg^{-1}), sieved at 4mm before use and kept moist in a cold room until use. Dry masses of ground organic materials equivalent to 120 mg of organic C were added to the equivalent of 40 g of dry soil. The mixtures were incubated in 0.5 L hermetically-sealed jars for 21 days at 28°C. The water content of the mixtures was adjusted to 22.5% (v/m) (corresponding to 80% of the field capacity) with Milli-Q water and then checked during the incubation period. A control incubation was run with soil alone. In each jar, carbon mineralized as CO_2 was trapped in 10 mL 1M NaOH which was replaced after 1, 4, 7, 14, 21 days of incubation and colorimetrically analyzed for C content on a continuous flow analyzer (Skalar, the Netherlands). With the jar opening to replace the NaOH traps, it was possible to run the incubations under aerobic conditions. All incubations were performed in triplicate. Three additional empty jars were incubated with NaOH traps only in order to measure the initial CO_2 level in the jars and the carbonate content of NaOH.

2.3.6. Curie-point pyrolysis-gas chromatography-mass spectrometry (Pyrolysis-GC/MS)

For pyrolysis-GC/MS, 0.5-3 mg samples were loaded into tubular ferromagnetic wires. The wires were heated inductively to their curie temperature of 650°C in 0.15 s (10 s hold) using a pyrolysis unit (Fisher GSG Curie point Pyrolyser 1040 PSC). Pyrolysis products were separated using a Hewlett Packard HP-5890 gas chromatograph (GC) on a 60 m fused silica SolGelWax capillary column (SGE, 0.32 mm i.d, film thickness 0.5 µm), with helium as the carrier gas. They were identified with a Hewlett Packard HP-5889 mass spectrometer (electron energy: 70 eV, ion source temperature: 250 °C). The temperature of the GC oven was set to rise from 30°C to 280°C (2°C/min), after which the temperature was maintained at 280°C for 15 min. The different compounds were identified on the basis of their mass spectra, GC retention times and comparison with the Wiley mass spectrum library. Biochemical families were defined according to the probable origin of the pyrolysis products: polysaccharide-derived compounds, lignin-derived compounds, N-containing compounds and unspecified compounds (Dignac et al., 2005; Parnaudeau & Dignac, 2007). Peak areas were obtained by integrating the peaks of the total ion current trace (TIC) using the MSD Chemstation (version G1701DA D.01.00). The relative abundances of peaks and biochemical families were calculated by dividing the surface areas of individual peaks and the sum of the surface areas of peaks originating from a biochemical family by the total surface area of all identified peaks, and expressed as a percentage of the total surface area of the identified peaks. All pyrolysis were performed in one replicate. For graphical comparisons, all pyrograms were displayed with toluene having approximately the same peak height. In order to highlight relationships between pyrolysates, similitude indexes *Sij* were calculated. The twenty most abundant identified peaks of each pyrogram were selected first of all. Between these twenty main peaks, the relative abundances of the common peaks between two pyrolysates were compared according to the formula: $Sij = \sum_{k=1}^{n}(\min(Ii^k, Ij^k)/\max(Ii^k, Ij^k))$, where the most abundant of the two pyrolysis products compared is always placed in the denominator; I^k is the relative abundance of the pyrolysis product k, *i* and *j* are the pyrolysates being compared and n is the number of peaks common to the twenty most abundant pyrograms (adapted from Ceccanti et al., 1986). This index enabled assessment of the similarity between two pyrolysates in terms of both the number of main peaks they had in common and the similarity of the abundance of those peaks. This index increased in line with the increase in similarity, with a maximum of twenty meaning that the twenty most abundant peaks of two pyrolysates being compared were identical and that their relative abundances were equal.

2.3.7. Fourier-Transform Infra-Red (FTIR) spectroscopy

About 2 mg of a sample were mixed with 200 mg KBr in an agate mortar. KRr pellets were formed under a pressure of 2 tonnes for 5 minutes. Infrared spectra were recorded in the mid-infrared range at 4000-400 cm^{-1} with average spectra of 32 scans and a resolution of 1 cm^{-1} using a Bruker IFS-48 spectrophotometer. Relative absorbance values *rA* were computed according to the formula: $rA = (Ai / \sum_{i=3000cm-1}^{400cm-1} Ai) * 100$ where *Ai* is the absorbance at wave number *i*. The O-H band between 4000 and 3000 cm^{-1} was not included in this sum because of its sensitivity to water content.

2.3.8. Statistical analysis

Relationships between total compost organic matter and organic fractions were revealed by Principal Component Analysis (PCA) performed using SPAD 5.0 software (CISIA-CERESTA, 2001), and a hierarchical classification method was used to define clusters (using Ward's algorithm) that grouped the most similar samples.

2.4. Results

2.4.1. Quantification of organic matter fractions obtained using the Van Soest and alternative procedures

The proportions of total organic C recovered after the successive steps of the alternative fractionation procedure are presented in Table 2.1. Most organic C was extracted with hot water and tetraborate. The total C extracted after the 4-step alternative procedure represented 42% of the total compost C in immature compost and 48% in mature compost, while the proportions obtained using the standard

Van Soest method were 40% and 48%, respectively. The proportions of carbon present in the WE and lipid fractions decreased from the immature to the mature compost. By contrast, the proportion of tetraborate extractable C and of resin extractable C increased from the immature to the mature compost.

When expressed in terms of compost dry mass, the total amount of extracted C was lower in the more mature compost. The amounts of C extracted by tetraborate and resin remained fairly constant during composting while the lipid and water extractable C contents were higher in MSWi compost than in MSWm compost. The TE fractions had similar C contents in the two composts but a higher N content in the more mature compost. As expected for products soluble in a non-polar solvent, the lipid fraction displayed very high C/N ratios in both composts.

Table 2.1. Distribution of C and N in the fractions (mean ± standard deviation) of immature (**MSWi**) and mature (**MSWm**) composts obtained after the four steps of the novel fractionation procedure and extracted using the classic hot water + neutral detergent combination of the Van Soest procedure (**SOL-Van Soest**). NA: non-attributed standard deviation value because of lack of replicates.

	g C kg C^{-1} of bulk compost		g C kg^{-1} of bulk compost		g C kg^{-1} of fraction		g N kg^{-1} of fraction		C / N	
	MSWi	MSWm	MSWi	MSWm	MSWi	MSWm	MSWi	MSWm	MSWi	MSWm
Water Extractable (WE)	196 ± 1	148 ± 1	48.8 ± 0.5	19.7 ± 0.1	343.3 ± 3.5	225.4 ± 2. 9	38.8 ± 0.4	50.1 ± 1.2	8.9 ± 0.1	4.5 ± 0.1
Tetraborate Extractable (TE)	107 ± 2	214 ± 2	27.0 ± 0.6	28.7 ± 0.3	329.6 ± NA	339.1 ± NA	36.4 ± NA	54.5 ± NA	9.1 ± NA	6.2 ± NA
LIPID	74 ± 13	43 ± 10	19.2 ± 3.3	5.9 ± 1.4	736.6 ± 6.0	683.9 ± 15 3.8	5.5 ± 0.3	6.5 ± 1.4	134.8 ± 6.2	105.5 ± 10.8
Resin Extractable (RE)	46 ± 9	79 ± 2	8.4 ± 2.3	10.6 ± 0.3	110.1 ± 2.6	60.9 ± 1.5	5 .1 ± 0.4	5.1 ± 0.1	21.4 ± 1.2	11.9 ± 0.1
Total alternative method	**423 ± 45**	**483 ± 10**	**103.4 ± 4.1**	**64.9 ± 1.5**						
SOL-Van Soest	**405 ± 10**	**481 ± 20**	**102.4 ± 9.5**	**64.5 ± 2.6**						

2.4.2. Mineralization of organic C in the fractions

More C was mineralized during incubation (Figure. 2.2) in the immature compost (35.4% of TOC mineralized after 21 days) than in the mature compost (7.9% of TOC mineralized). The proportions of C mineralized in the WE fractions were higher than the proportions mineralized in total composts (42.8% and 23.0% of TOC in WE fractions of immature and mature composts, respectively). The proportions of C in the NDF residues of the standard Van Soest procedure mineralized after 21 days (30.6% and 10.0% of TOC in immature and mature NDF residues, respectively) were close to the proportions of C mineralized in the residues of the alternative extraction procedure (31.7% and 13.2% of TOC in residues from immature and mature composts, respectively). The C mineralization was also much higher in the residues obtained from the immature compost than from the mature compost. The proportion of C mineralized in the residues of immature compost (30.6% and 31.7% for NDFi and ARi residues, respectively) was lower than that mineralized from total compost (35.4% of TOC). By contrast, in mature compost, mineralization was slightly higher in extraction residues (10.0% and 13.2% of TOC in the NDFm and ARm residues, respectively) than in bulk compost (7.9% of TOC).

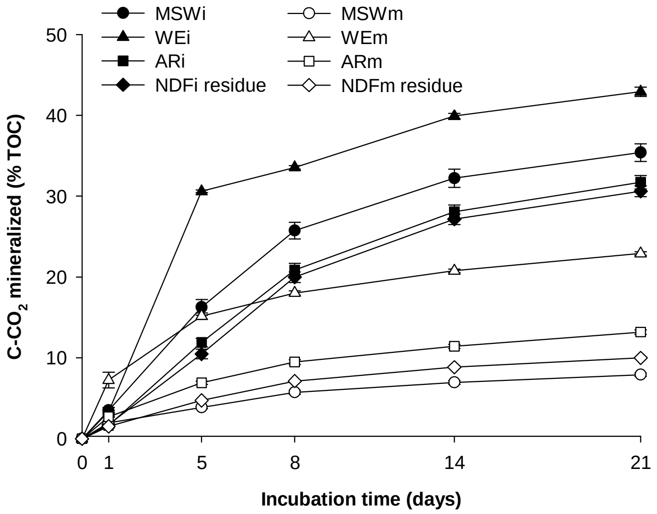

Figure 2.2. Organic carbon mineralization during soil incubation of bulk composts (**MSW**), hot water extracts (**WE**), and residues after neutral detergent extraction under the classic Van Soest procedure (**NDF residue**) and after the novel alternative procedure (**AR**) for the immature (**i**) and mature (**m**) composts. The results are presented ± standard errors calculated from three replicates.

2.4.3. Characterization of composts, fractions and residues using pyrolysis-GC/MS

The chemical compositions of the immature (Figure. 2.3) and mature (Figure 2.4) composts and their fractions (WE, TE fractions) and residues (NDF and AR residues) were characterized using pyrolysis-GC/MS. Pyrolysis products and their principal biochemical origins are presented in Table 2.2. The compounds identified were grouped into five biochemical families: N-containing compounds (N), compounds originating from polysaccharides (PS), lignin-derived products (LIG) and compounds of unspecified origin (U). The sum of the relative surface areas of identified peaks of the different biochemical families are presented in Table 2.3. Some carboxylic acids, probably derived from wax lipids, were also present in the pyrograms for the immature compost. However, it was not possible to integrate the poorly resolved and very large peaks observed at the ends of the pyrograms. For this reason, fatty acids were not taken into account when calculating the proportions of biochemical families. Pyrolysis-GC/MS is not adapted to characterization of fatty acids, and lignin derived compounds might be underestimated by Pyrolysis-GC/MS. These compounds would be better determined using thermochemolysis with TMAH (Gonzalez-Vila et al., 1999).

2.4.3.1. Pyrolysates of bulk composts

The pyrolysate of bulk MSWi compost was dominated by compounds derived from polysaccharides (36% of identified peaks Figure 2.3, Table 2.3), with 1-hydroxypropan-2-one (PS3) being the principal polysaccharide-derived compound thus identified. By contrast, the pyrogram of bulk MSWm compost (Figure 2.4 and Table 2.3) displayed only a few polysaccharide-derived products (11%). The main lignin-derived compounds in both compost pyrolysates were 2-methoxy-phenol (LIG1) and 2-methoxy-4-vinyl-phenol (LIG5). A higher proportion of unspecified products was observed in MSWm (63%) than in MSWi (47%), mainly because of the larger proportion of styrene (U9), which was a dominant peak in both pyrograms (16% and 38% of all identified peaks in MSWi and MSWm, respectively).

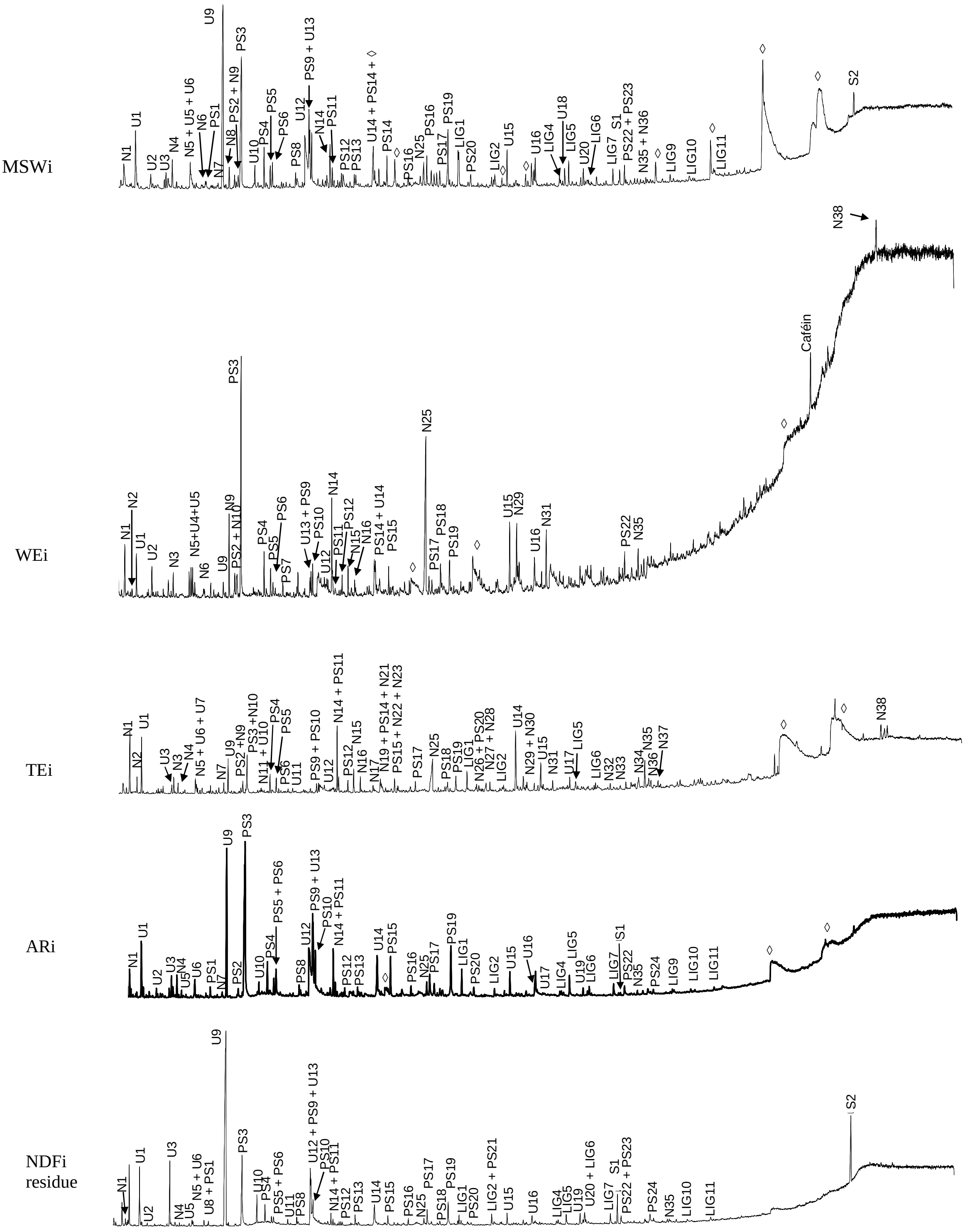

Figure 2.3. Pyrograms of total immature compost **(MSWi)**, hot water extract (**WEi)**, tetraborate extract (**TEi)**, the residue of the alternative method (**ARi)** and the residue after extraction with neutral detergent under the standard Van Soest procedure (**NDFi residue**). The names of the compounds are presented in Table 2.2. ◊: carboxylic acids.

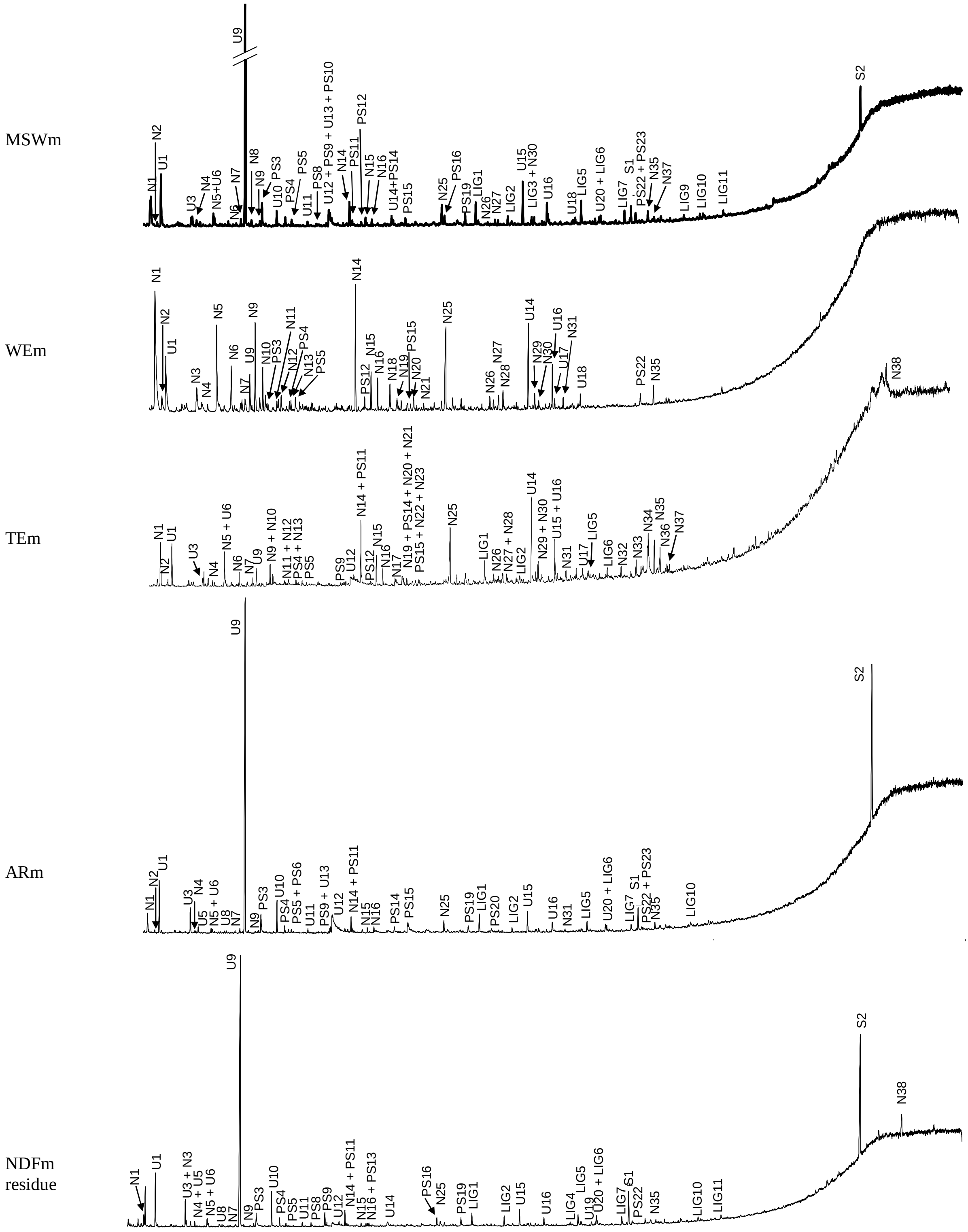

Figure 2.4. Pyrograms of total mature compost (**MSWm**), hot water extract (**WEm**), tetraborate extract (**TEm**), the residue of the alternative method (**ARm**) and the residue after extraction with neutral detergent under the standard Van Soest procedure (**NDFm residue**). The names of the compounds are presented in Table 2.2.

Table 2.2. Pyrolysis products identified by pyrolysis-GC/MS and classified in terms of their probable biochemical origin in bulk composts (**MSW**), hot water extracts (**WE**), tetraborate extracts (**TE**), alternative method residues (**AR**), residues after extraction with neutral detergent under the standard Van Soest procedure (**NDF residue**) for the immature (**i**) and mature (**m**) composts.

No.	Name
Compounds derived from polysaccharides	
PS1	2-Methyl-furan
PS2	3-Hydroxy-2-butanone
PS3	1-Hydroxy-propan-2-one
PS4	2-Cyclopenten-1-one
PS5	2-Methyl-2-cyclopenten-1-one
PS6	1-Hydroxy-2-butanone
PS7	2-Nonanone
PS8	3-Furancarboxaldehyde
PS9	2-Furancarboxaldehyde
PS10	2-Propanone
PS11	3-Methyl-2-cyclopenten-1-one
PS12	2,3-Dimethyl-cyclopent-2-en-1-one
PS13	5-Methyl-2-furancarboxaldehyde
PS14	Dihydro-2(3H)-furanone
PS15	2-Furanmethanol
PS16	3-Methyl-2(5H)-furanone
PS17	1,3-Cyclopentanedione
PS18	2(3H)-Furanone, dihydro-4-methyl
PS19	1,2-Cyclopentanedione, 3-methyl
PS20	2-Hydroxy-3-ethyl- cyclopenten-1-one
PS21	4H-Pyran-4one, 3-hydroxy-2-methyl
PS22	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose
PS23	2,3-Dihydro-benzofuran
PS24	2-Furancarboxaldehyde, 5-(hydroxymethyl)
Compounds derived from lignins	
LIG1	2-Methoxy-phenol
LIG2	2-Methoxy-4-methyl-phenol (Me-guaiacol)
LIG3	Phenol, 4-ethyl-2-methoxy-
LIG4	2-Methoxy-4-(2-propenyl)-phenol (eugenol)
LIG5	2-Methoxy-4-vinyl-phenol
LIG6	2,6-Dimethoxy-phenol
LIG7	2-Methoxy-4-(1-propenyl)-phenol (isoeugenol)
LIG8	2,5-Dimethoxy-4-(2-propenyl)-phenol
LIG9	2,6 Dimethoxy 4-vinyl-phenol
LIG10	1-(4-Hydroxy-3-methoxyphenyl)-ethanone
LIG11	2,6-Dimethoxy-4-(2-propenyl)-phenol
N-containing compounds	
N1	Acetonitrile
N2	Propanenitrile
N3	3-Methyl-butanenitrile
N4	1-Methyl-1H-pyrrole
N5	Pyridine
N6	3-Methyl-pyridine
N7	4-Methyl-pentanenitrile
N8	2,5-Dimethyl-1H-pyrrole-1
N9	2-Methyl-pyridine
N10	4-Methyl-pyridine
N11	2,5 -Dimethyl-pyridine
N12	2,4 -Dimethyl-pyridine
N13	2,3 -Dimethyl-pyridine
N14	1H-Pyrrole
N15	2-Methyl-1H-pyrrole
N16	1-Methyl-1H-pyrrole
N17	2,5-Dimethyl-1H-pyrrole2
N18	Benzonitrile
N19	2-ethyl-1H-pyrrole
N20	2,4dimethyl-1H-Pyrrole
N21	Acetamide, N-methyl
N22	1-ethyl-1H-pyrrole
N23	2-ethyl-4-methyl-1H-Pyrrole
N24	3-Methyl-benzonitrile
N25	Acetamide
N26	2-Pyridinamine
N27	Butanamide, 3 methyl
N28	Benzeneacetonitrile
N29	2-Pyrrolidinone
N30	Benzenepropanenitrile
N31	2-Piperidinone
N32	2,5-pyrrolidinedione, 2-methyl
N33	2,5-pyrrolidinedione, 2-methyl
N34	2-Hydroxy-pyridine
N35	1H-Indole
N36	2,5-Pyrrolidinedione
N37	3-Methyl-1H-indole
N38	Diketopiperazine
Compounds of unspecific origin	
U1	Benzene, methyl- = toluene
U2	Hexanal
U3	Ethyl-benzene
U4	Benzene, 1,2-dimethyl
U5	1-Butanol
U6	Cyclopentanone
U7	2-Heptanone
U8	Benzene, propyl
U9	Styrene
U10	Methyl-styrene
U11	Benzene, 2-propenyl
U12	Acetic acid
U13	Propanoic acid
U14	1,2-Ethanediol
U15	Phenol
U16	4-Methyl-phenol
U17	3-Methyl-phenol
U18	4-Ethyl-phenol
U19	m/z: 57, 69, 85 : non identified
U20	Benzene, 1,1’-(1,3-propanediyl)bis
Compounds derived from synthetic polymers	
S1	Styrene dimer
S2	Styrene trimer

2.4.3.2. Pyrolysates of hot water extracts

The pyrolysate of the WE fraction of immature compost (WEi, Figure 2.3) was dominated by N-containing and polysaccharide-derived compounds (respectively 40% and 34% of the total surface area of identified peaks, Table 2.3). The pyrogram was dominated by peaks of acetamide (N25, 14.6% of the identified peak area), an N-containing compound which might derive from amino-sugars (Boon et al., 1981), glycin-containing proteins (Tsuge and Matsubara, 1985) or from peptidoglycan present in bacterial cell walls (Eudy et al., 1985), and hydroxypropanone (PS3, 13.7% of the total area of identified peaks) that probably originates from cellulose (Helleur et al., 1985). The pyrolysate of the MSWm water extract (WEm, Figure 2.4) was dominated by N-containing compounds (77% of the identified peak area) and only a few polysaccharide-derived peaks remained (4% of the identified peak area). The two principal peaks were acetonitrile (N1, 17.5% of the identified peak area), an N-containing compound of ambiguous origin, and acetamide (N25, 7.5% of the identified peak area). In both pyrolysates of WEi and WEm, compounds of unspecified origin made a small contribution (26% and 19% of the identified peak areas for WEi and WEm, respectively) when compared with their contribution to the pyrograms of bulk compost. This was mainly due to the small contribution of styrene (U9 representing 1% and 3% of the identified peak areas for WEi and WEm, respectively). No lignin-derived products were detected in the pyrolysates of either WE.

Table 2.3. Relative surface areas represented by pyrolysis products originating from different biochemical families (as a percentage of the sum of the surface areas of all identified peaks). **PS**: polysaccharide-derived products, **N**: N-containing products, **LIG**: lignin-derived products, **U**: products of unspecific origin. Pyrolysis products that were integrated but not identified (**NI**, as a % of the sum of the areas of all integrated peaks) were not included in calculations of these proportions. The proportions are presented for the bulk composts (**MSW**), hot water extracts (**WE**), tetraborate extracts (**TE**), residues after alternative extraction (**AR**), and residues after extraction with neutral detergent under the standard Van Soest procedure (**NDF residue**) for the immature (**i**) and mature (**m**) composts.

	LIG	N	PS	--------------U-------------		NI	PS/N
				total	styrene		
	-------------% of total area of identified peaks-----------------					% of total area of integrated peaks	
MSWi	6	11	36	47	16	19	3.4
MSWm	10	16	11	63	38	7	0.8
WEi	0	40	34	26	1	15	0.8
WEm	0	75	5	20	3	4	0.1
TEi	4	51	19	27	3	11	0.4
TEm	3	65	3	29	1	12	0.1
ARi	6	6	43	45	10	8	7.1
ARm	6	9	9	77	45	4	1.0
NDFi residue	5	3	25	67	34	10	8.0
NDFm residue	8	8	9	75	50	7	1.2

2.4.3.3. Pyrolysates of tetraborate extracts

The tetraborate extract of MSWi (TEi, Figure 2.3) released high proportions of N-containing and polysaccharide-derived compounds during pyrolysis (respectively 51% and 19% of the total areas of identified peaks, Table 2.3). The two principal peaks were acetamide (N25, 8.8% of the total area of identified peaks) and phenol (U15, 6.8% of the total area of identified peaks). Phenol is an ubiquitous pyrolysis product, which may originate from tyrosine-containing peptides and proteins (Tsuge and Matsubara, 1985), as well as from lignins (Saiz-Jimenez and De Leeuw, 1986), tannins (Galletti and Reeves, 1992) or polysaccharides (Pouwels et al., 1987). In the pyrolysate of the MSWm tetraborate extract (TEm, Figure 2.4), the principal peaks were 2-hydroxy-pyridine (N34, 11.7% of the total area of identified peaks) probably deriving from alanine-containing proteins and peptides (Chiavari and Galletti, 1992) and phenol (U15, 8.2% of the total area of identified peaks). The pyrolysate of TEm was dominated by N-containing products (64% of the total area of identified peaks) and displayed only a few polysaccharide-derived compounds (3% of the total area of identified peaks, Table 2.3). Large and unresolved peaks of lipid-derived carboxylic acids were detected at the end of the pyrogram for TEi (Figure 2.3) but not that for TEm (Figure 2.4). A few lignin-derived compounds (LIG1, LIG2, LIG5 and LIG6) were identified in the pyrograms of TEi and TEm, although lignins were not expected to be solubilized in tetraborate. Pyrrole (N14), an ubiquitous compound derived from proteins and peptides, microbial cells (Ceccanti et al., 1986) or pigments like chlorophyll was present at high proportions in the pyrolysates of both TEi and TEm. A small quantity of styrene (U9) was detected in these pyrolysates (3% and 1% of the identified peak areas for TEi and TEm, respectively).

2.4.3.4. Pyrolysates of extraction residues

In MSWi compost, polysaccharide-derived products dominated the pyrolysates of both residues after neutral detergent extraction or alternative procedure (NDFi and ARi, respectively, Figure 2.3 and Table 2.3). They accounted for 25% and 43% of total areas of identified peaks in the pyrolysates of NDFi and ARi, respectively, large proportion as observed in the pyrolysate of bulk immature compost (36%). The carboxylic acid peaks observed at the end of the pyrogram for bulk MSWi compost were not present in the pyrolysate of the NDFi residue but was observed in the pyrolysate of ARi although some were extracted using tetraborate.

In MSWm compost, both residues (ARm and NDFm) presented similar characteristics of their pyrolysates with fewer polysaccharide-derived compounds and higher proportions of unspecified compounds, and particularly more styrene (45% and 50% of the total area of identified peaks respectively, Table 2.3) when compared to the pyrolysates of extraction residues of the immature compost (ARi and NDFi, respectively). Fewer lignin-derived, nitrogenous compounds and more ubiquitous compounds were present in the pyrolysates of ARm and NDFm compared with the pyrolysate of the bulk MSWm compost.

2.4.4. FTIR spectroscopy

The FTIR spectra of bulk composts (MSWi and MSWm) and of the hot water (WEi and WEm) and tetraborate (TEi and TEm) extracts are presented in Figure 2.5. Interpretation of these spectra was based on data in the literature (Ouatmane et al., 2000; Francou et al., 2008, Smidt and Meissl, 2007). We focused our interpretation on the height of several bands of interest: absorption bands at 2925 and 2850 cm^{-1} (aliphatic C-H stretch), the band at 1600-1650 cm^{-1} (aromatic C=C, in addition to quinines, conjugated carboxyls and ketones and the C=O stretch of primary amide), a narrow peak at 1384 cm^{-1} (N-O stretch of nitrates) and the band at 1040 cm^{-1} (C-O stretch of polysaccharides and aromatic ether). The aliphatic bands at 2925 cm^{-1} and 2850 cm^{-1} were higher for MSWi than for MSWm. Regarding the intensity of the aliphatic bands in the FTIR spectra, alkanes and alkenes peaks should be observed in the pyrolysates although they were not detected. This was probably due to the GC polar column used, non-polar column being more suitable for the characterization of aliphatic structures producing alkanes and alkenes upon pyrolysis (Dignac et al., 2006).

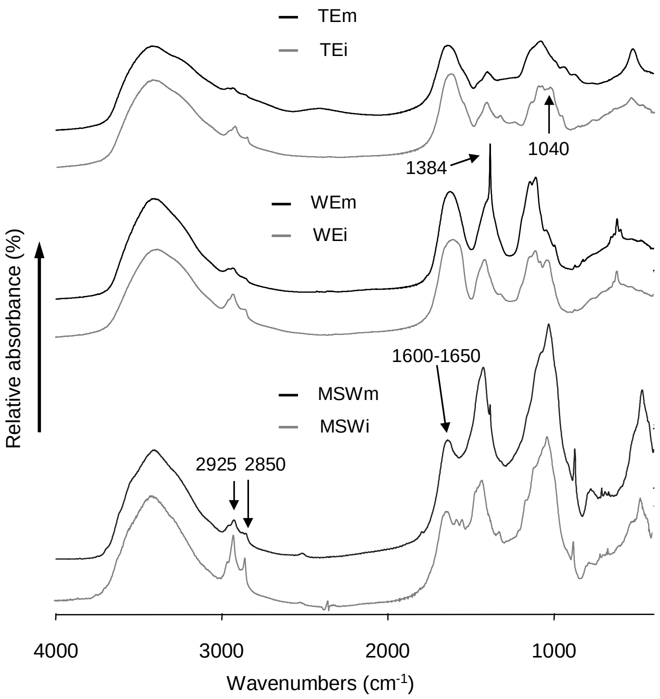

Figure 2.5. Fourier-transform infrared spectra of bulk composts (**MSW**), hot water extracts (**WE**) and tetraborate extracts (**TE**) for the immature (**i**) and mature (**m**) composts.

The FTIR spectra of WEi displayed more intense aliphatic bands (2925 and 2850 cm^{-1}) than those seen for WEm. The nitrate band at 1384 cm^{-1} was very intense in WEm spectra but not visible in WEi spectra which was consistent with its presence in MSWm spectrum and not in MSWi one. By contrast, the band at 1040 cm^{-1} was present in the spectra of WEi but not in that of WEm, reflecting the higher levels of polysaccharides in the WEi extract which was consistent with the results found in pyrolysis.

Aliphatic bands (2925 and 2850 cm^{-1}) were visible in TEi spectra although they were less intense than in WEi spectra and very weak in TEm spectra. The band at 1040 cm^{-1} was present in TEi but not in TEm spectra.

2.4.5. Principal Component Analysis (PCA)

The data used for PCA were the relative surface areas of pyrolysis products originating from the different biochemical families: polysaccharide-derived products (PS), N-containing products (N), lignin-derived products (LIG) and products of unspecified origin (U) as a percentage of the total area of identified peaks, the C/N ratio of the residues and WE and TE extracts. The proportion of organic C mineralized after 21 days of incubation (C21) was used as illustrative variable because data were not available for TE extracts. The plane defined by the two first principal components accounted for 88% of total inertia (Figure 2.6). The first principal component was significantly and positively correlated with the proportion of N-containing compounds released during pyrolysis (N, r = 0.98, $p<0.01$), significantly and negatively correlated with the proportion of compounds of unspecified origin (U, r = -0.87, $p<0.01$), the proportion of lignin-derived compounds (LIG, r = -0.80, $p<0.01$) and the C/N ratio (r = -0.75, $p<0.05$). The second principal component was significantly and negatively correlated with polysaccharide-derived compounds (PS, r = -0.88, $p<0.01$). The C21 variable was strongly, negatively correlated to the second principal component (r = -0.93, $p<0.01$). The second component thus represented the degree of biodegradability of the samples.

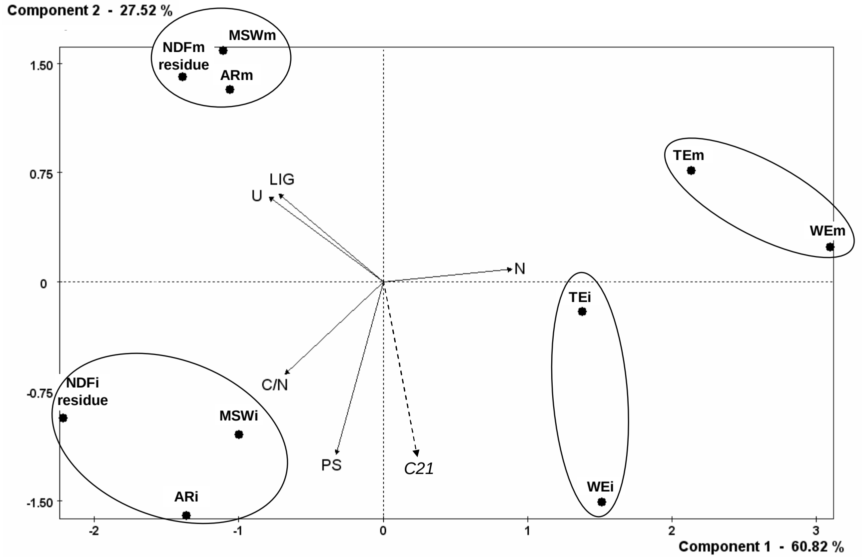

Fig. 6. Projection of samples onto the plane defined by the two first principal components of PCA. Pyrolysates of bulk composts (**MSW**), hot water extracts (**WE**), tetraborate extracts (**TE**), alternative method residues (**AR**), residues after extraction with neutral detergent under the standard Van Soest procedure (**NDF residue**) for the two levels of compost maturity: immature (**i**) and mature (**m**) and projection of the variables. **PS**: proportions of polysaccharide-derived compounds identified by pyrolysis-GC/MS, **N**: proportions of N-containing compounds, **LIG**: proportions of lignin-derived compounds, **U**: compounds of unspecific origin, **C/N** ratio of total organic C content divided by total N

content, **C21**: proportion of organic C mineralized after 21 days of incubation, used as an illustrative variable.

The proportion of polysaccharide-derived compounds was significantly correlated with C21 (r = 0.79, $p < 0.05$). The proportion of compounds of unspecified origin was significantly and negatively correlated to the proportion of N-containing compounds (r = -0.84, $p < 0.01$) and positively to the proportion of lignin-derived compounds (r = 0.79, $p < 0.01$). The C/N ratio contrasted with the proportion of N-containing compounds with a significant negative correlation (r = -0.70, $p < 0.05$).

The organic samples studied under PCA were represented on the plane defined by the first two components and grouped into clusters after statistical classification (Figure 2.6). Bulk MSWi compost and the two extraction residues of this immature compost were grouped in cluster 1 characterized by high proportions of polysaccharide-derived compounds in their pyrolysates, a high C/N ratio and a high C21 value. Cluster 2 grouped the extraction residues and bulk sample of the mature compost characterized by high proportions of lignin-derived and unspecified compounds, low proportions of polysaccharide-derived products in their pyrolysates and low C21 values. Cluster 3 grouped WEi and TEi, the pyrolysates of which were both rich in N-containing and polysaccharide-derived compounds. WEm and TEm were situated in cluster 4, with high proportions of N-containing compounds but lower proportions of polysaccharide-derived compounds than immature compost extracts.

Clusters 3 and 4, corresponding to the extracts arising from MSWi and MSWm, had very different position in the projection plane. WEi was situated at the very bottom of the plane because of the high proportion of polysaccharides in the pyrolysate that explained its high biodegradability (C21). By contrast, WEm was situated in the right-hand part of the plane because of the high proportion of N-containing products in the pyrolysate. Their C/N ratios were also very different (8.9 and 4.5, respectively). Tetraborate extracts from immature and mature composts were also situated in different parts of the PCA plane but differed less markedly than the hot water extracts. The tetraborate extract of immature compost had a higher C/N ratio than that of mature compost (9.1 and 6.2, respectively).

2.5. Discussion

2.5.1. Evaluation of the alternative fractionation procedure

The Van Soest procedure has been widely employed to assess the stability (i.e. resistance to biodegradation) and predict the efficiency at increasing SOM (Thuriès et al., 2002; Lashermes et al., 2009) of exogenous OM from various sources. The soluble fraction obtained using the Van Soest method is generally related to labile compounds (Thuriès et al., 2002). However, the chemical nature of the organic molecules present in the soluble fraction may change during composting and the proportion of C contained in this fraction often increases in line with compost maturity (Annabi et al., 2007; Francou et al., 2008). The aim of the novel extraction method developed during this study was thus to extract an organic fraction (i) that as far as possible was comparable to that obtained with the neutral detergent solution used in the classical Van Soest procedure, and (ii) in which organic constituents could be characterized without interference from the substances used for extraction. The two extraction methods were compared in terms of the proportion of total organic carbon extracted, the biodegradability of residues after extraction and the chemical characterization of OM remaining in the extraction residues by pyrolysis. Both methods extracted similar proportions of TOC (Table 1.1) and the residues after extractions displayed similar C mineralization patterns (Figure 2.2).

The pyrolysate of the ARi residue differed slightly from that of the NDFi residue; 12 of their 20 main peaks were common and a similitude index of 8.2 was determined (Table 2.4). A higher relative proportion of polysaccharide-derived compounds was identified in ARi when compared to the NDFi residue.

Table 2.4. Similarity indexes between pyrolysates calculated using the 20 main peaks of each pyrogram (calculation detailed in Materials and Methods section). Values in parentheses are the number of common peaks between two pyrolysates out of the 20 main peaks of each pyrogram. The indexes are presented for the bulk composts (**MSW**), hot water extracts (**WE**), tetraborate extracts (**TE**), residues after alternative extraction (**AR**), and residues after extraction with neutral detergent under the standard Van Soest procedure (**NDF residue**) for the immature (**i**) and mature (**m**) composts.

	MSWi	ARi	NDFi residue	WEi	TEi	MSWm	ARm	NDFm residue	WEm
MSWi	20.0 (20)								
ARi	14.0 (18)	20.0 (20)							
NDFi residue	7.8 (11)	8.2 (12)	20.0 (20)						
WEi	7.0 (11)	6.3 (10)	5.6 (8)	20.0 (20)					
TEi	5.8 (11)	4.6 (10)	3.4 (6)	7.3 (12)	20.0 (20)				
MSWm	6.4 (11)	5.1 (11)	7.2 (12)	7.2 (11)	5.5 (11)	20.0 (20)			
ARm	6.7 (10)	6.7 (12)	9.5 (13)	5.0 (10)	4.0 (11)	11.2 (17)	20.0 (20)		
NDFm residue	6.2 (12)	1.9 (6)	8.9 (14)	3.7 (8)	3.4 (10)	10.6 (16)	12.7 (16)	20.0 (20)	
WEm	2.4 (8)	2.9 (6)	1.3 (4)	3.7 (11)	6.7 (10)	3.1 (8)	1.8 (7)	2.2 (8)	20.0 (20)
TEm	4.4 (8)	5.6 (8)	1.9 (3)	6.2 (11)	9.2 (12)	4.8 (9)	4.1 (9)	3.2 (8)	4.7 (9)

The large carboxylic acid peaks present at the end of MSWi pyrogram were probably due to lipids from greasy wastes. They were no longer present in MSWm after 8 months of composting. Carboxylic acids were present in the ARi pyrolysate but not in that of the NDFi residue (Figure 2.3), suggesting that the abundant lipids in immature compost were better extracted by the neutral detergent solution than by the alternative procedure.

For mature compost, the pyrogram of the residue after the alternative extraction procedure was very similar to that of the NDF residue, with 16 of the 20 main peaks being common to each pyrogram and a similitude index of 12.7. The new fractionation method could thus be used to replace extraction with a neutral detergent solution (Van Soest method) to characterize the corresponding extracted organic fraction and evidence its evolution during composting. Nevertheless, the results obtained on immature compost should be interpreted with caution because differences were observed regarding the extraction residues of this compost.

The abundant presence of styrene in the pyrolysates of MSW bulk compost and residues after the different extraction processes could be linked to the presence of plastics in the compost (Annabi et al., 2007). Styrene and toluene are released during the pyrolysis of both natural OM and synthetic polymers (Fabbri, 2001). When the toluene/styrene ratio is higher than one, their presence is of natural origin, but if the ratio is lower than one, the presence of styrene is mainly due to synthetic polymers (Dignac et al., 2005). The ratio was higher than one in the WE and TE extracts, which was thus indicative of the natural origin of styrene in the extracts. But the ratio was lower than one for all residues and the bulk composts. Furthermore, the presence of styrene dimer and trimer (S1 and S2) in the pyrolysates, specific to styrene polymers (Dignac et al., 2005), confirmed the synthetic origin of styrene in the pyrolysates of bulk MSW compost and residue after extraction. They were always more abundant in pyrolysates originating from MSWm than from MSWi and this was probably due to the lower abundance of products formed during the pyrolysis of mature compost.

2.5.2. Evolution during composting of the chemical nature and biodegradability of extracted organic fractions

The proportion and values for water extractable C were lower with the more mature compost (Table 2.1), in line with the findings of Said-Pullicino et al. (2007). However, higher proportions of water extractable C were measured during our study compared to the study of Said-Pullicino et al. (2007) because hot rather than cold water was used. Nonetheless, the proportion of TOC extracted in the WE remained high for mature compost because of its lower TOC content. Hot water extractable C may partly be composed of microbial products that continuously form and degrade during composting (Charest et al., 2004). Hot water extractable C has frequently been used to estimate potentially bioavailable C in soils (von Lutzow et al., 2007). As expected, the hot water extracts of both immature and mature composts displayed a higher degree of biodegradability than the respective bulk composts or extraction residues (Figure 2.2). Furthermore, the water extractable C of immature compost was twice as biodegradable as that of mature compost. The chemical composition of the water extracts of immature and mature composts differed markedly, as revealed by the very low similitude index of the pyrolysates (3.7 with only 11 common peaks, Table 2.4). Their contrasted biodegradability, C/N and biochemical compositions led to very different positions of the water extracts on the PCA plane (Figure 2.6). The principal difference in the chemical compositions of water extracts, as revealed by pyrolysis and FTIR, was the marked reduction in polysaccharide levels in mature compost when compared to immature compost. Water extractable polysaccharides are known to be easily biodegradable (Cheshire et al., 1974). The composting process probably led to a preferential decay of those easily biodegradable polysaccharides (Chefetz et al., 1998), thus explaining their lower proportion in the water extract of mature compost. The pyrolysate of the mature water extract, characterized by a lower C/N ratio, was dominated by N-containing products. N-containing compounds generally derive from intrinsically labile products such as proteins. However, recent studies have shown that N-containing products are important constituents of stabilized OM in soils (Rillig et al., 2007; Knicker, 2000), which may explain the lower biodegradability of the water extractable C from mature compost compared to that of immature compost.

Contrary to water extractable C, tetraborate extractable C levels were relatively similar in immature and mature composts. Tetraborate extractable C represented an increasing proportion of compost TOC due to the decrease of TOC content in bulk compost during composting. Tetraborate extracted C contained relatively more polysaccharide- and lipid-derived compounds in immature compost than in mature compost. The mature compost TE extract mostly displayed N-containing compounds that could be present in either labile or stabilized organic structures in the compost. The polysaccharides extracted with hot water and tetraborate theoretically corresponded to soluble bacterial exopolymers or soluble simple sugars, because cellulose and hemicelluloses are supposed to be extracted during later steps of the Van Soest method. However, some hemicelluloses and cellulose appeared to be present already in the so-called “soluble” fraction of the Van Soest method applied to compost, because some polysaccharide-derived compounds observed on the pyrograms of hot water and tetraborate extracts were known to originate from cellulose (hydroxypropanone, PS3) or hemicellulose (furanmethanol, PS9) (Helleur et al., 1985; Pouwels et al., 1987). The chemical nature of the hot water and tetraborate extracts differed more for mature compost (similitude index of 4.7 with nine common major peaks, Table 2.4) than for immature compost (similitude index of 7.3 with 12 common major peaks). On the other hand, the two water extracts (WEi and WEm) also differed more than the two tetraborate extracts (similitude index of 3.7 with 11 common major peaks and 9.2 with 12 common major peaks, respectively).

2.5.3. Change of the chemical nature and biodegradability of the fractions extracted using the alternative method indicative of changes to the biodegradability of the “soluble” Van Soest fraction during composting

The stabilization of bulk compost during composting was attested by the decrease of its potential C mineralization during laboratory incubations. In addition, the increase of the ratio of the FTIR bands at 1650 cm^{-1} and 2925 cm^{-1} from 1.2 for MSWi to 1.7 for MSWm reflected the increase in aromaticity during composting (Ouatmane et al., 2000; Francou et al., 2008) and the nitrate FTIR band at 1384 cm^{-1} proposed by Grube et al. (2006) as an indicator of compost maturity was visible in the spectra of MSWm but not in the spectra of MSWi.

The NDFi and ARi residues were less biodegradable than bulk immature compost (MSWi) which may have been the result of extraction by the neutral detergent solution or, under the alternative method, of the most biodegradable fraction of OM. By contrast, bulk mature compost (MSWm) tended to be less biodegradable than the NDFm and ARm residues, suggesting the extraction of more stabilized materials in the Van Soest “soluble” extract, or with the alternative method, when applied to mature compost.

The proportions of hot water extractable C and lipid fractions decreased when compost maturity increased. Meanwhile, the proportions of tetraborate and resin extractable C increased and were responsible for the increase in the “soluble” fraction in line with compost maturity. These two fractions almost doubled in mature composts when compared to immature composts, and the tetraborate fraction represented 21.4% of TOC in the mature compost.

The biodegradability of the OM extracted with tetraborate could not be measured during incubation because insufficient material could be collected. However, if we advance the hypothesis that more polysaccharide is related to higher degradability (as observed for hot water extracts), the tetraborate extracted OC of immature compost might be more biodegradable than that of mature compost because of its higher polysaccharide content.

An increase in compost stability during composting is often explained and quantified by a reduction in aliphatic structures and an increase in aromaticity (Ouatmane et al., 2000; Francou et al., 2008; Som et al., 2009). It may result from a selective preservation of aromatic structures and a more rapid degradation of aliphatic structures which is known to be relevant in soils during the initial phase of plant decomposition (von Lutzow et al., 2006). During our study, the FTIR spectra of the tetraborate extract of mature compost displayed very low aliphatic peaks (2925 and 2850 cm^{-1}) and polysaccharide peak (1040 cm^{-1}, Figure 2.5) compared to the tetraborate extract of immature compost and of hot water extracts, suggesting that biodegradability was probably lower.

Another factor that might explain the higher proportion of tetraborate extractable C in more mature compost was that although Van Soest and Wine (1967) described the use of sodium tetraborate and hydrogen phosphate in the neutral detergent solution as a buffer to control pH, sodium tetraborate also has complexing properties and has been used at pH 9.7 to extract organo-metallic complexes from soils (Bruckert, 1982). Sodium tetraborate mainly extracts organic molecules by breaking ionic bonds leading to the ionisation of acidic and phenolic functional groups and forming complexes with metals, especially aluminium. Sodium tetraborate is nevertheless a weaker extraction product than the widely-employed sodium pyrophosphate that can break covalent bonds or sodium hydroxide that can break all kinds of organo-mineral bonds (Bruckert, 1982). The OC extracted with the chelating resin used to replace EDTA was also complexed with metals. The complexation of soil organic matter or soil-dissolved organic matter with metal ions, especially Ca^{2+}, Al^{3+} and Fe^{3+}, is known to exert a stabilizing effect on biodegradation (Baldock and Skjemstad, 2000). On the other hand, the MSW composts studied contained substantial amounts of $CaCO_3$ (8.1% and 11.7% of dry matter in MSWi and MSWm, respectively), Al (1.4% and 1.8% of dry matter in MSWi and MSWm) and Fe (0.8% and 1.1 % of dry matter in MSWi and MSWm, respectively) (Annabi, 2005). In both immature and mature composts, the organic C extracted with tetraborate and chelating resin could originate from C stabilized against biodegradation because of complexing with metals. Further study of metal speciation in these composts will be necessary to validate this hypothesis.

2.6. Conclusions

A new extraction method has been proposed for OM extraction which is comparable to that extracted using the neutral detergent solution of the Van Soest method, but allows chemical characterization of the extracted fractions. Both methods extracted similar proportions of OM and the composition of the residues was similar in mature compost although differences are noted in the case of the immature compost. The hot water soluble and lipid fractions decreased when compost maturity increased, whereas the OM extracted with sodium tetraborate and chelating resin increased. During composting, easily degradable polysaccharides extractable in hot water were probably degraded preferentially, whereas part of the N-containing compounds became stabilized against biodegradation while remaining extractable in the Van Soest soluble fraction. The mechanisms leading to this stabilization may involve the formation of complexes of OM with metal ions, especially Ca^{2+}, which may be broken down during extraction of the Van Soest soluble fraction. Further studies are required to gain a clearer understanding of these mechanisms. This new extraction method will be used in further studies to determine whether stabilization of organic matter during composting is always related to similar chemical evolution of soluble fraction whatever the initial waste mixture or composting process.

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Chapitre 3. Near infrared reflectance spectroscopy: a tool to characterize the composition of different types of exogenous organic matter and their behaviour in soil

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3.1. Abstract

In addition to total organic carbon and nitrogen, potential organic carbon mineralization under controlled laboratory conditions and indicators such as the indicator of remaining organic carbon in soil (I_{ROC}), based on Van Soest biochemical fractionation and short-term carbon mineralization in soil, are used to predict the evolution of exogenous organic matter (EOM) after its application to soils. The purpose of this study was to develop near infrared reflectance spectroscopy (NIRS) calibration models that could predict these characteristics in a large dataset including 300 EOMs representative of the broad range of such materials applied to cultivated soils (plant materials, animal manures, composts, sludges, etc.). The NIRS predictions of total organic matter and total organic carbon were satisfactory ($R^2 = 0.80$ and 0.85, ratio of performance to deviation, RPD = 2.2 and 2.6, respectively), and prediction of the Van Soest soluble, cellulose and holocellulose fractions were acceptable ($R^2 = 0.82$, 0.73 and 0.70, RPD = 2.3, 1.9 and 1.8, respectively) with coefficients of variation close to those of the reference methods. The NIRS prediction of carbon mineralization during incubation was satisfactory and indeed better regarding the short-term results of mineralization ($R^2 = 0.78$ and 0.78, and RPD = 2.1 and 2.0 for 3 and 7 days of incubation, respectively). The I_{ROC} indicator was predicted with fairly good accuracy ($R^2 = 0.79$, RPD = 2.2). Variables related to the long-term C mineralization of EOM in soil were not predicted accurately, except for I_{ROC} which was based on analytical and well-identified characteristics, probably because of the increasing interactions and complexity of the factors governing EOM mineralization in soil as a function of incubation time. This study demonstrated the possibility of developing NIRS predictive models for EOM characteristics in heterogeneous datasets of EOMs. However, specific NIRS predictive models still remain necessary for sludges, organo-mineral fertilizers and liquid manures.

Keywords

Carbon mineralization, characterisation, compost, degradability, indicators, manure, near infrared reflectance spectroscopy, soil organic matter, Van Soest fractionation

Research Highlights

- NIRS can be used for predicting various characteristics of exogenous organic matters (EOMs) on heterogeneous sample sets.
- The indicator of remaining organic C from EOMs in soil (I_{ROC}) is satisfactorily predicted with NIRS.
- Variables related to short-term C mineralization during incubations of EOMs are generally better predicted than variables related to long-term C mineralization.

3.2. Introduction

A decline in soil organic matter content has been identified as one of the major threats to soil by the Commission of the European Communities (2006). Exogenous organic matter (EOM) is an important and potentially growing source of material that could be applied to cultivated soils to improve their fertility and increase their organic matter content, thus also potentially contributing to the prevention of global warming through carbon sequestration (Marmo et al., 2004). Exogenous organic matter includes all organic matter arising from external sources such as urban areas, municipalities, agriculture, forestry and industry. It also comprises crop residues as they may have amending properties as urban, industrial or animal EOMs and can be characterized using the same methods (Marmo et al., 2004). Tools to predict the fate of EOM in soil would enable better account to be taken of their contribution to global environmental balances.

Various characteristics of EOM have been used to predict its transformation in soils. Van Soest biochemical fractions (Van Soest, 1963; Van Soest & Wine, 1967; AFNOR, 2005a) and the potential mineralization of total organic carbon (TOC) contained in EOM (EOM-TOC) during soil incubation under controlled laboratory conditions have been standardized (AFNOR, 2005a, b) and are useful tools to predict the fate of EOM in soil (Henriksen & Breland, 1999; Trinsoutrot et al., 2000; Thuriès et al., 2002; Francou et al., 2008). The most stable part of EOM remaining in soil over the long-term can be estimated from incubation data by extrapolating the kinetics of EOM-TOC mineralization or fitting a stable pool of EOM-TOC decomposition models (Thuriès et al., 2001; Sleutel et al., 2005; Lashermes et al., 2009). It can also be estimated with the indicator of remaining organic C in soil (**I_{ROC}**, Lashermes et al., (2009) calculated as a function of soluble, cellulose- and lignin+cutin-like fractions of Van Soest fractionation (SOL, CEL and LIC, respectively), and the proportion of EOM-TOC that is mineralized after three days of incubation in soil (C_{3d}). The equation used to calculate I_{ROC} was based on a partial least square (PLS) regression between the analytical characteristics of EOM and the EOM-TOC remaining in soil over the long-term extrapolated from the kinetics of C degradation during long-term incubations (more than 156 days). In both cases (fitting of mineralization kinetics or calculation of the I_{ROC} indicator) the estimation of remaining organic C requires quite costly and time-consuming analytical methods.

Near infrared reflectance spectroscopy (NIRS) measures the reflectance of samples in the spectral range between 800 and 2500 nm. The quantification of analytical characteristics based on NIRS requires multivariate statistical methods, referred to as chemometric methods, to fit the spectral response to sample properties in order to build predictive models. Once calibration has been developed, NIRS is a rapid, inexpensive and non-destructive method for sample characterization which does not require any consumables. It has already been used to determine the total organic C and nitrogen (N) contents of soils (Barthès et al., 2006), to assess the elemental and biochemical composition of plant materials (Stuth et al., 2003; Stenberg et al., 2004; Thuriès et al., 2005), composted materials (Michel et al., 2006; Albrecht et al., 2009; Vergnoux et al., 2009) and animal manures (Malley et al., 2002; Reeves & Van Kessel, 2002; Sørensen et al., 2007), to predict the C and N mineralization of plant materials (Bruun et al., 2005; Shepherd et al., 2005; Borgen et al., 2010) or the mineralization of soil organic matter (Thomsen et al., 2009). However, NIRS predictive models for the composition and long-term degradability of EOM in soils have generally been built using relatively homogeneous EOM sets that include either plant materials or animal manures or composts etc., and rarely with heterogeneous EOM sets.

The objectives of this study were to evaluate the potential of NIRS: (1) to determine organic C and total N contents and Van Soest biochemical fractions in a sample set of 300 EOMs representing the broad diversity and heterogeneity of the EOMs applied in agriculture, and (2) to predict the proportion of EOM-TOC remaining in soil in the long-term after EOM applications, either by predicting the stable pool of a decomposition model fitted to the kinetics of EOM-C mineralization during laboratory incubations or by predicting the I_{ROC} indicator.

3.3. Materials and Methods

3.3.1. EOM dataset

The dataset included 300 EOM samples collected and analyzed in the context of different research programmes (Lashermes et al., 2009) and covering a broad range of fresh and transformed organic materials applied in agriculture. These included 32% of urban composts (17% of municipal solid waste composts, 7% of co-composts of sludge and green wastes, 7% of biowaste composts, 1% of green waste composts), 10% of composted animal manures, 16% of non composted sludges, 14% of farmyard manures, 10% of plant materials, 6% of anaerobically-digested wastes, 4% of liquid manures, 3% of organic and organo-mineral fertilizers, 2% of non-composted urban wastes and 3% of screening refuse and organic amendments. The 124 composts included in the dataset were selected from a larger database of 270 composts on basis of the representativeness of their NIR spectra (see *3.3.4.1*) in the planes of a principal component analysis (PCA) using the standardized Mahalanobis distance H between paired samples. This procedure was performed using the SELECT function of WinISI software version 1.63 (Foss NIRSystems, Infrasoft International, State College, PA, USA) (Shenk & Westerhaus, 1991). During the SELECT procedure, the algorithm identifies and selects spectra by discarding their most closely neighbouring spectra. This process is repeated until the desired number of samples is reached.

3.3.2. Chemical and biochemical characterisation of EOM

All EOM samples were oven-dried at 40°C and finely ground (<1mm) before analysis. Total organic matter content (**TOM**) was determined by loss on ignition at 480°C, total organic C content (**TOC**) by dry combustion using an Elemental Analyser after the dissolution of carbonates with hydrochloric acid (AFNOR, 1995), and total N content (**totN**) also by dry combustion (AFNOR, 1998). Mineral N (NH_4^+

and NO_3^-) contents were determined on dry EOM after KCl extraction by spectrophotometry on a continuous flow analyzer (AFNOR, 1996, 1997). The organic N content (**orgN**) was calculated as the difference between total N and mineral N. The biochemical composition of EOM was determined using the Van Soest method (Van Soest, 1963; Van Soest & Wine, 1967) as modified under the French standard XP U 44-162 (AFNOR, 2005a). The soluble (**SOL**), hemicellulose-like (**HEM**) cellulose-like (**CEL**) and lignin+cutin-like (**LIC**) fractions were separated. One-gram-samples of ground EOM were mixed with 2 g calcinated sand and extracted with 100 mL hot water for 30 min and then neutral detergent for 60 min (extraction of the SOL fraction, NDF residue), hot acid detergent for 60 min (extraction of the HEM fraction, ADF residue) and 180 min in cold, 72% sulphuric acid (extraction of the CEL fraction, ADL residue). The residual material (ADL) corresponded to the LIC fraction. After each extraction step, the OM content was determined in the residues by loss on ignition at 480°C. All biochemical fractions were expressed as a percentage of TOM according to the following equations: SOL = 100 – NDF, HEM = NDF – ADF, CEL = ADF – ADL, and LIC = ADL. The holocellulose fraction (**HOL**) was calculated as HOL = HEM + CEL.

The C mineralization of EOM (expressed as a percentage of TOC) was measured during 91-day incubations of soil+EOM mixtures in hermetically sealed jars under controlled conditions at 28°C with a soil moisture level corresponding to 75% to 100% of the soil water holding capacity. Because the incubations were being run in the context of various research programmes, different cultivated soils taken from temperate areas were used. It was assumed that the effect of soil type on the EOM-TOC mineralization would be negligible when compared with the impact of EOM quality (Coppens, 2005). C-CO_2 was trapped in 10 mL 0.5M NaOH which was periodically replaced, and then determined using colorimetry (AFNOR, 2005b).

3.3.3. Stable organic C in EOM

The indicator of remaining organic carbon (**I_{ROC}**) estimates the stable pool of EOM-TOC potentially remaining in soil over the long-term after application. It is calculated using the SOL, CEL and LIC fractions of Van Soest fractionation and the proportion of EOM-TOC mineralized after 3 days of incubation (C_{3d}), according to the formula:

$$I_{ROC} = 44.5 + 0.5\ SOL - 0.2\ CEL + 0.7\ LIC - 2.3\ C_{3d} \qquad [1]$$

It is expressed as a percentage of TOC. This indicator was based on extrapolating long-term C mineralization kinetics during incubation for more than 156 days (Lashermes et al., 2009).

In the present study, it was not possible to realize similar extrapolations of mineralization kinetics since the available C mineralization kinetics were measured during a shorter period (91 days). Instead, we estimated the stable pool of EOM-TOC by fitting the kinetics of C mineralization to a simplified three-pool decomposition model as proposed by Thuriès et al. (2002). In this simplified model, only the size of the labile and stable pools need to be fitted, the pools having similar degradation rates for all EOMs. It could be written as:

$$MC(t) = 100 - (L.e^{-0.4t} + (100 - L - S).e^{-0.012t} + S) \qquad [2]$$

where MC(t) is the cumulated amount of C mineralized at time t (expressed as % of TOC), L is the labile pool of EOM-TOC, S the stable pool of EOM-TOC recalcitrant to mineralization and (100 – L – S) the resistant pool of EOM partially degraded during the incubation period. A sub-set of 291 EOM samples was used since only short-term mineralized C was measured for the nine remaining EOM samples. The fittings were performed using the non linear least squares (nls) function implemented in R version 2.9.0 (R Development Core Team, 2009). The model was well adjusted to the incubation data ($R^2 = 0.98$ and RMSE = 1.9). Our estimation of the stable C in EOM was thus based either on the I_{ROC} indicator or on the stable pool (S) of the fitted decomposition model.

3.3.4. NIRS analysis

3.3.4.1. Spectrum acquisition and detection of spectral outliers

The NIR spectra were recorded on a NIRSystems 6500 spectrophotometer (Foss, Silver Spring, MD, USA) with an initial spectral range of 400 to 2498 nm with 2 nm intervals. Only the range 1100–2498 nm (NIR) was used subsequently because preliminary tests showed that this produced more accurate predictions than the full range (visible and NIR), possibly because the visual range is highly sensitive to moisture and variable grinding conditions (Bruun et al., 2005). This was probably the case regarding our sample set as it originated from different research programmes. The samples were oven-dried at 40℃ and ground to pass a 1 mm screen, but without additional drying to remove residual moisture prior to NIRS analysis as this has been shown to have little effect on NIRS calibration results (Lovett et al., 2005). The EOM samples were packed in 5-cm ring cups and scanned in reflectance mode. Two different spectra (each averaged from 30 scans) were recorded for each EOM on independent cup fillings, and determined in terms of absorbance (logarithm of the inverse of reflectance). Both absorbance spectra were then averaged when repeatability was considered to be good, according to the root mean square (RMS); i.e. when the RMS between two sample spectra was smaller than 2.5 times the average of all RMS calculated for each sample of the dataset. Otherwise, spectrum acquisition was repeated.

The spectra were processed using The Unscrambler 9.8 software (CAMO Software AS, Oslo, Norway). The spectra were transformed: (1) using the Savitzky Golay second derivative and smoothing calculated over four data points on both sides, which reduces baseline variation and enhances spectral features (Reeves et al., 2002), and (2) using a full multiplicative scatter correction (MSC) that corrects light scattering for different particle sizes (Geladi et al., 1985). Preliminary tests showed that these pre-treatments produced better results than standard normal variate and detrend transformation (SNVD).

Principal component analysis (PCA) was then performed to check the spectral homogeneity of the dataset. The Hotelling T^2 statistic was used to identify spectral outliers. This statistic determines the distance of a spectrum from the average spectrum and compares this distance with a critical limit according to an F-test in order to identify outliers.

3.3.4.2. NIRS calibration, cross-validation and external validation

Seventy five percent of the set (211 EOM samples) were randomly selected for calibration and the remaining samples were used for external validation (70 EOMs). Calibration was performed by partial least square (PLS) regression on transformed absorbance spectra (Geladi & Kowalski, 1986).

Leave-one-out cross-validation was used to determine the optimum number of PLS components required to calibrate the models and then calculate the predicted values on the calibration subset in order to assess the robustness of the models. Each sample in the calibration set was predicted successively with a PLS model developed using all other samples, thus supplying an independent prediction for each sample. The residuals of cross-validation predictions were pooled to calculate the root mean square error of cross-validation ($RMSE_{CV}$). At the end of this first cycle, samples with large residuals (i.e. a difference between prediction and measurement more than 3 times the RMSE value calculated using all calibration samples) and samples with a strong influence (leverage) on the model (leverage >5 times the average leverage of all samples) were discarded as calibration outliers and cross-validation was repeated. This cycle of cross-validation and outlier removal was performed twice. In addition, two cycles were performed to eliminate non-significant wavelengths (i.e. with a high degree of uncertainty of coefficients during leave-one-out cross-validation) to render the final model more reliable (Martens & Martens, 2000). A final cycle of cross-validation then defined the optimum number of components required for the final model, calculated using all samples minus calibration outliers. The optimum number of components was attained when the addition of a supplementary component led to an increase of less than 6% in the explained variance (default value in The Unscrambler).

The predictive accuracy and robustness of the models was then evaluated on the EOM-subset of validation.

3.3.4.3. Statistics

The statistical methods used to evaluate goodness of fit included the coefficient of determination and the root mean square error calculated for predicted values of the entire calibration set (**R^2_C** and

$RMSE_C$, respectively) or during cross-validation (**R^2_{CV}** and **$RMSE_{CV}$**, respectively) after spectral and calibration outlier removal, and for predicted values of the validation set minus spectral outliers (**R^2_P** and **$RMSE_P$**, respectively). In order to compare the quality of calibrations for variables with different units and ranges, the ratio of performance to deviation for cross-validation on the calibration set (**RPD_{CV}**) and for the validation set (**RPD_P**) were calculated as the ratio between standard deviations (SD) of measured data and $RMSE_{CV}$ or $RMSE_P$, respectively. The coefficients of variation of $RMSE_{CV}$ and $RMSE_P$ compared to the means of calibration and validation sets (**CV_{CV}** and **CV_P** in %) were calculated as CV = RMSE * 100 / $\bar{y}$ where $\bar{y}$ is the average of measured values. These coefficients of variation could be compared with the CV of reproducibility of the reference analytical results (CV_R), calculated as $CV_R = \sqrt{SD_L{}^2 + SD_r{}^2} * 100 / \bar{y}$ where SD_L is the inter-laboratory standard deviation and SD_r is the standard deviation of repeatability (intra-laboratory), thus providing information on possibilities for model improvement (Dardenne, 2010).

3.4. Results and Discussion

3.4.1. Analytical characteristics of EOM

Table 3.1 presents the basic statistics for the entire dataset (n = 300) regarding all the reference analytical characteristics of EOM that would be further predicted using NIRS, including I_{ROC} indicators and potential C mineralization after 3, 7, 28 and 91 days of incubation in soil (n = 291). The optimized parameters of the fitted model [2] (the labile (L) and stable (S) pools) are also presented. Information on N content was not available for 12% of the samples. All determined parameters covered broad ranges of values related to the considerable diversity of EOM types and origins in the dataset. Most variable distributions presented skewness and excess kurtosis close to 0, corresponding to a normal distribution. Positive skewness indicates a distribution concentrated at low values with relatively few high values, the reverse being true for negative skewness. Positive excess kurtosis is indicative of a more acute distribution peak around the mean whereas negative excess kurtosis indicates a lower and broader distribution peak around the mean. The total N and organic N contents displayed distributions that were far from Gaussian, with high skewness and excess kurtosis due to the relatively small number of samples with totN and orgN contents higher than 4% of dry weight. The hemicellulose-like fraction, the proportions of C mineralized after 3 and 7 days of incubation (C_{3d} and C_{7d}), and the labile pools (L) mostly displayed low values and consequently a fairly high skewness >1.

Table 3.1. Summary of analytical characteristics of the dataset on exogenous organic matter. Total organic C: TOC, total organic matter: TOM, total N: totN, organic N: orgN, Van Soest biochemical fractions (soluble: SOL, hemicellulose-like: HEM, cellulose-like: CEL, lignin+cutin-like: LIC and holocellulose like: HOL), exogenous organic C mineralized after 3, 7, 28 and 91 days of incubation (C_{3d} C_{7d}, C_{28d} and C_{91d}), labile and stable pools (L and S) of model [2], indicator of remaining organic C: $I_{ROC,}$. n: number of available data for the variable considered (n=300 for the whole dataset), SD: standard deviation, Min: minimum value, Max: maximum value, DW: dry weight.

Variables	Unit	n	Mean	SD	Min.	Max.	Skewness	Kurtosis excess
TOC	%DW	300	29.8	10.7	3.5	73.4	0.1	0.2
TOM	%DW	300	57.8	20.7	8.0	99.0	0.0	-0.9
totN	%DW	265	2.3	2.0	0.3	14.3	3.0	11.6
orgN	%DW	264	2.2	1.9	0.0	13.5	3.0	11.9
SOL	%TOM	300	40.0	16.4	3.7	87.1	0.5	-0.1
HEM	%TOM	300	12.6	8.1	0.7	49.6	1.5	3.1
CEL	%TOM	300	24.3	12.7	0.4	58.5	0.3	-0.6
LIC	%TOM	300	23.0	12.3	1.2	62.4	0.4	-0.2
HOL	%TOM	300	36.9	14.2	6.7	85.5	0.6	0.3
C_{3d}	%TOC	300	6.7	6.2	0.4	30.2	1.5	1.8
C_{7d}	%TOC	291	12.5	10.3	0.8	51.4	1.1	0.5
C_{28d}	%TOC	291	20.8	13.9	1.8	71.7	0.7	-0.1
C_{91d}	%TOC	291	27.8	16.1	0.9	81.7	0.6	0.0
L	%TOC	291	11.9	10.6	0.0	48.4	1.0	0.3
S	%TOC	291	61.6	21.1	0.0	98.1	-0.5	-0.4
I_{ROC}	%TOC	300	60.4	17.0	8.1	91.5	-0.5	-0.4

Some variables were correlated (Table 3.2). As expected, total organic C (TOC) and total organic matter (TOM) contents were strongly correlated (r = 0.90), as were totN and orgN (r = 0.98). The total N content was positively correlated with the SOL fraction and the proportion of C mineralized after 3 days of incubation (C_{3d}). The SOL and CEL fractions were negatively correlated, as found previously by other authors (Stenberg et al., 2004). The LIC fraction was negatively correlated with C mineralization during incubation (C_{3d} C_{7d}, C_{28d} and C_{91d}) and the labile pools of the fitted models (L), and positively correlated with I_{ROC}, and with the stable pool of the model (S). The relationships between mineralization behaviour and biochemical fractions for our dataset containing partly degraded EOM such as composts differed from those found by Jensen et al. (2005) for non-degraded plant residues. In such untransformed organic materials, the potential C mineralization during incubations was better correlated with the SOL and CEL fractions than with the LIC fraction, while a reverse tendency was found here. Parameters describing the kinetics of potential C mineralization were all inter-correlated, sometimes with very strong correlation coefficients, such as between the proportions of C mineralized after seven days of incubation (C_{7d}) and the labile pools of the mathematical model (L), or between the stable pool of the model (S) and the proportion of C mineralized after 28 days of incubation (C_{28d}). The stable pool of the model (S) and the I_{ROC} indicator were correlated (r = 075) and both were similarly correlated with the proportion of C mineralized after 91 days of incubation (C_{91d}), thus meaning that they provided similar information about the potentially remaining organic C long time after EOM application. The characteristics of the calibration and validation sets of samples (results not shown) differed little from those of the entire dataset, thus indicating a satisfactory random separation of samples in the calibration and validation sets.

Table 3.2. Pearson correlation coefficients (r) between analytical characteristics. Total organic C: TOC, total organic matter: TOM, total N: totN, organic N: orgN, soluble : SOL, hemicellulose-like: HEM, cellulose-like: CEL, lignin+cutin-like fractions: LIC and holocellulose-like: HOL, exogenous organic C mineralized after 3, 7, 28 and 91 days of incubation (C_{3d} C_{7d}, C_{28d} and C_{91d}), labile and stable pools of model [2] (L and S), indicator of remaining organic C: I_{ROC}. With 300 samples, the correlations are significant ($P < 0.001$) from $r = 0.19$, for more relevant interpretations, only r values > 0.5 are in bold.

Variables	TOC	TOM	totN	orgN	SOL	HEM	CEL	LIC	HOL	C_{3d}	C_{7d}	C_{28d}	C_{91d}	L	S
TOC	1.00														
TOM	**0.90**	1.00													
totN	0.25	0.25	1.00												
orgN	0.31	0.29	**0.98**	1.00											
SOL	-0.28	-0.29	**0.56**	**0.52**	1.00										
HEM	0.32	0.41	0.31	0.37	-0.05	1.00									
CEL	0.33	0.36	**-0.50**	-0.47	**-0.74**	-0.13	1.00								
LIC	-0.18	-0.27	-0.45	-0.45	**-0.54**	-0.47	0.04	1.00							
HOL	0.48	**0.56**	-0.27	-0.22	**-0.69**	0.46	**0.82**	-0.23	1.00						
C_{3d}	0.10	0.10	**0.61**	**0.62**	**0.56**	0.15	-0.29	**-0.56**	-0.17	1.00					
C_{7d}	0.19	0.19	0.46	**0.50**	0.38	0.23	-0.05	**-0.60**	0.08	**0.82**	1.00				
C_{28d}	0.25	0.27	0.40	0.43	0.28	0.30	0.06	**-0.63**	0.22	**0.73**	**0.96**	1.00			
C_{91d}	0.29	0.30	0.44	0.47	0.28	0.30	0.04	**-0.61**	0.21	**0.81**	**0.81**	**0.86**	1.00		
L	0.15	0.16	0.48	**0.50**	0.41	0.24	-0.09	**-0.61**	0.05	**0.82**	**0.99**	**0.95**	**0.78**	1.00	
S	-0.35	-0.36	-0.28	-0.33	-0.13	-0.33	-0.18	**0.56**	-0.35	**-0.59**	**-0.83**	**-0.93**	**-0.88**	**-0.79**	1.00
I_{ROC}	-0.36	-0.42	-0.38	-0.43	-0.14	-0.37	-0.24	**0.70**	-0.43	**-0.81**	**-0.81**	**-0.81**	**-0.87**	**-0.80**	**0.75**

3.4.2. NIRS prediction of the elemental composition of EOM

A total of 19 samples were eliminated from further analysis as spectral outliers. They included 12 sludges (out of 48 in the entire dataset) and five organic and organo-mineral fertilizers (out of eight in the entire dataset).

Good calibration and validation results were obtained for TOC, TOM and totN contents according to the criteria reported by Saeys et al. (2005) with R^2_{CV} >0.82 and RPD_{CV} >2.5 (Table 3.3, Figure 3.1). The quality of calibration and validation regarding orgN was less good than for totN.

Increasing the diversity of samples in a dataset generally causes a reduction in calibration accuracy (Stenberg et al., 2004; Thuriès et al., 2005; Brunet et al., 2007; Galvez-Sola et al., 2010). As a consequence, and in view of the broad heterogeneity of our EOM dataset, the accuracy of our predictions was sometimes poorer than that reported in studies dealing with more homogenous types of EOMs (Malley et al., 2002; Sørensen et al., 2007; Vergnoux et al., 2009), which were furthermore analysed by one laboratory, whereas in the present study they were analysed by different laboratories. Nevertheless, our results were sometimes as good as those generated by studies performed on more homogenous sets of products for TOC (Thuriès et al., 2005) and for totN (Reeves & Van Kessel, 2002; Michel et al., 2006). The variability of TOC and TOM predictions (CV_p = 13.3% and 16.9%, respectively) was close to the CV of the reproducibility CV_R (i.e. inter- and intra-laboratory CV) of the reference method used in French standards: 16.7% and 9.4% for TOC and TOM, respectively (AFNOR, 1995, 2000). Possibilities to increase the accuracy of predictions for TOC and TOM might thus be limited. For the total N content, the difference between CV_P and CV_R was larger, i.e. 31.1% vs. 10.4% for NIRS and the reference method, respectively (AFNOR, 1998), suggesting a greater possibility to enhance the accuracy of predictions for totN than for TOC and TOM. However, most totN values were low (high skewness, Table 3.1) resulting in a relatively low mean of measured values and leading to high CV_P and CV_R. As a result, the CV could only be usefully interpreted for variables with a skewness near to zero.

The calibration outliers for TOM, TOC, totN and orgN were mainly sludges, organic or organo-mineral fertilizers and liquid manures, which represented the products that differed from the rest of the samples in terms of their spectral relationships with the variables.

Table 3.3. NIRS calibration and validation statistics for the determination of EOM properties. Total organic C: TOC, total organic matter: TOM, total N: totN, organic N: orgN, Van Soest biochemical fractions (soluble: SOL, hemicelluloses-like: HEM, cellulose-like: CEL, lignin+cutin-like: LIC and holocellulose like: HOL), exogenous organic C mineralized after 3, 7, 28 and 91 days of incubation (C_{3d} C_{7d}, C_{28d} and C_{91d}), labile and stable pools of model [2] (L and S), indicator of residual organic C: I_{ROC},. DW: dry weight. See part 3.3.4.3 for a description of the statistics. n_C: number of samples used for calibration, n_P: number of samples used for validation. Out_C: number of outliers discarded during calibration. Mean, standard deviation (SD) and root mean square error (RMSE) are expressed in the same units as the variable considered. The coefficient of variation CV is expressed as a percentage.

		Calibration (211 samples)											Validation (70 samples)						
Variables	Unit	n_C	Out_C	Mean	SD	$RMSE_C$	F	R^2_C	$RMSE_{CV}$	R^2_{CV}	RPD_{CV}	CV_{CV}	n_P	Mean	SD	$RMSE_P$	R^2_P	RPD_P	CV_P
TOC	%DW	195	16	30.3	9.4	2.9	5	0.91	3.1	0.89	3.0	10.4	70	30.2	10.5	4.0	0.85	2.6	13.3
TOM	%DW	201	10	58.8	19.7	6.4	3	0.89	6.9	0.88	2.9	11.7	70	58.4	21.7	9.9	0.80	2.2	16.9
totN	%DW	173	10	2.0	1.3	0.4	5	0.92	0.4	0.90	3.1	20.9	65	1.7	1.2	0.5	0.81	2.2	31.1
orgN	%DW	170	13	1.9	1.0	0.4	5	0.84	0.5	0.80	2.3	24.6	62	1.6	1.0	0.6	0.75	1.7	36.3
SOL	%TOM	200	11	38.8	14.3	6.3	3	0.80	6.6	0.78	2.1	17.1	70	35.2	15.7	6.7	0.82	2.3	19.2
HEM	%TOM	204	7	11.9	6.7	3.7	4	0.69	3.9	0.65	1.7	33.2	70	11.7	7.9	5.7	0.49	1.4	48.3
CEL	%TOM	202	9	24.9	12.5	4.5	3	0.87	4.6	0.86	2.7	18.5	70	26.1	12.4	6.4	0.73	1.9	24.6
LIC	%TOM	195	16	22.2	10.5	3.7	5	0.87	4.0	0.85	2.6	18.1	70	27.0	12.4	7.1	0.69	1.7	26.4
HOL	%TOM	199	12	37.4	13.7	4.8	4	0.88	5.2	0.86	2.6	13.9	70	37.8	14.3	8.0	0.70	1.8	21.2
C_{3d}	%TOC	202	9	5.9	5.2	2.0	6	0.84	2.3	0.80	2.2	39.5	70	5.4	4.5	2.1	0.78	2.1	39.4
C_{7d}	%TOC	189	16	10.9	8.8	2.8	5	0.90	3.0	0.88	2.9	27.8	67	10.0	7.3	3.7	0.78	2.0	36.5
C_{28d}	%TOC	185	20	18.6	11.9	4.4	4	0.87	4.7	0.84	2.5	25.4	67	17.9	10.7	6.2	0.67	1.7	34.5
C_{91d}	%TOC	190	15	25.7	14.2	6.7	4	0.78	7.2	0.74	2.0	28.2	67	25.0	13.1	8.6	0.58	1.5	34.2
L	%TOC	190	15	10.2	9.1	2.9	5	0.90	3.2	0.88	2.8	31.7	67	9.3	7.6	3.8	0.77	2.0	40.8
S	%TOC	188	17	63.7	19.2	8.4	4	0.81	9.2	0.77	2.1	14.4	67	64.7	17.6	13.2	0.50	1.3	20.4
I_{ROC}	%TOC	196	15	62.0	15.7	5.4	4	0.88	5.9	0.86	2.7	9.5	70	63.4	13.7	6.3	0.79	2.2	9.9

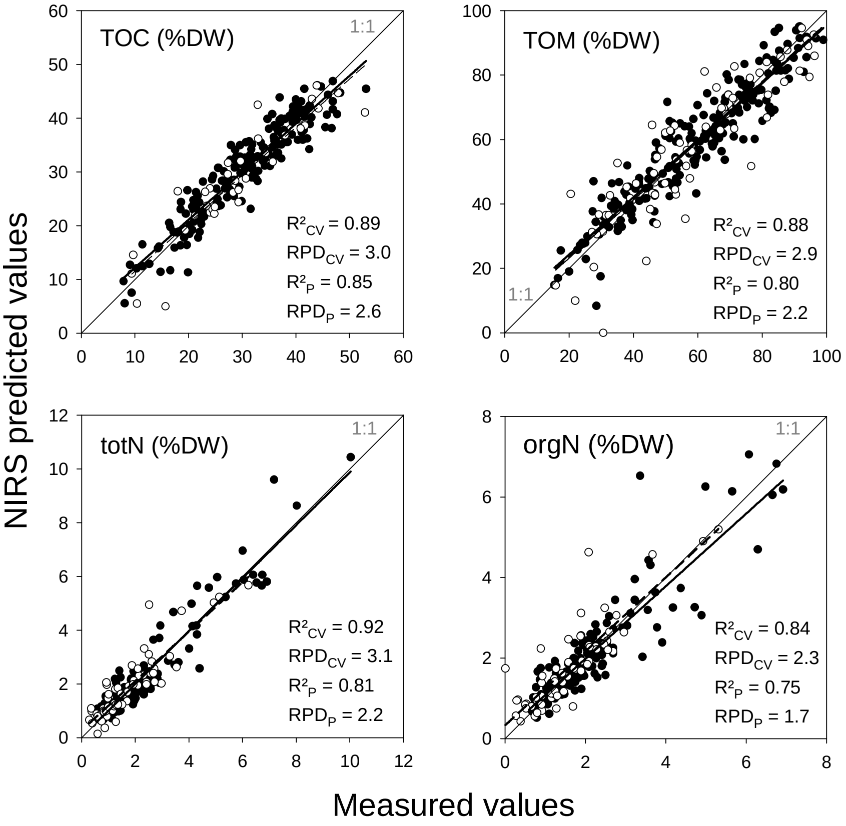

Figure 3.1. Predicted vs. measured values for total organic C (TOC), total organic matter (TOM), total N (totN), organic N (orgN) as a percentage of dry weight (%DW). Predictions on the calibration set during cross-validation (black circles, continuous regression lines, see part 3.3.4.2) and predictions on the independent validation set (white circles, dashed regression lines).

3.4.3. NIRS prediction of Van Soest biochemical fractions of EOMs

Calibration of the CEL and LIC fractions was quite satisfactory according to the criteria reported by Saeys et al. (2005) (Table 3.3, Figure 3.2), but the differences between the calibration and prediction results were quite large. Calibrations of the SOL and HEM fractions were less accurate, but the prediction results of the SOL fraction were good. Some studies with more homogenous groups of EOMs analysed by one laboratory obtained better results for the prediction of SOL and CEL fractions (Stenberg et al., 2004; Thuriès et al., 2005; Vergnoux et al., 2009). Nevertheless, prediction of these fractions appeared to be quite satisfactory when compared to the CV_R (AFNOR, 2005a; SOL: CV_R = 19.4%, CV_P = 19.2%, CEL: CV_R = 14.5%, CV_P = 24.6%), suggesting a limited opportunity to improve these predictions for EOMs analysed by different laboratories. The predictions were less good for LIC, as was the difference regarding reproducibility of the reference method (CV_R = 4.9%, CV_P = 26.4%). Poor prediction of the HEM fraction has been reported elsewhere (Stenberg et al., 2004; Thuriès et al., 2005); furthermore, we found a considerable difference between the CV_P (41.4%) and the reproducibility of the reference method (CV_R = 12.5%; AFNOR, 2005a). The holocellulose fraction, corresponding to the sum of the hemicellulose and cellulose fractions (HOL = HEM + CEL) gave results similar to the prediction of the CEL fraction, suggesting that the Van Soest procedure may be relatively inaccurate in separating the CEL and HEM fractions.

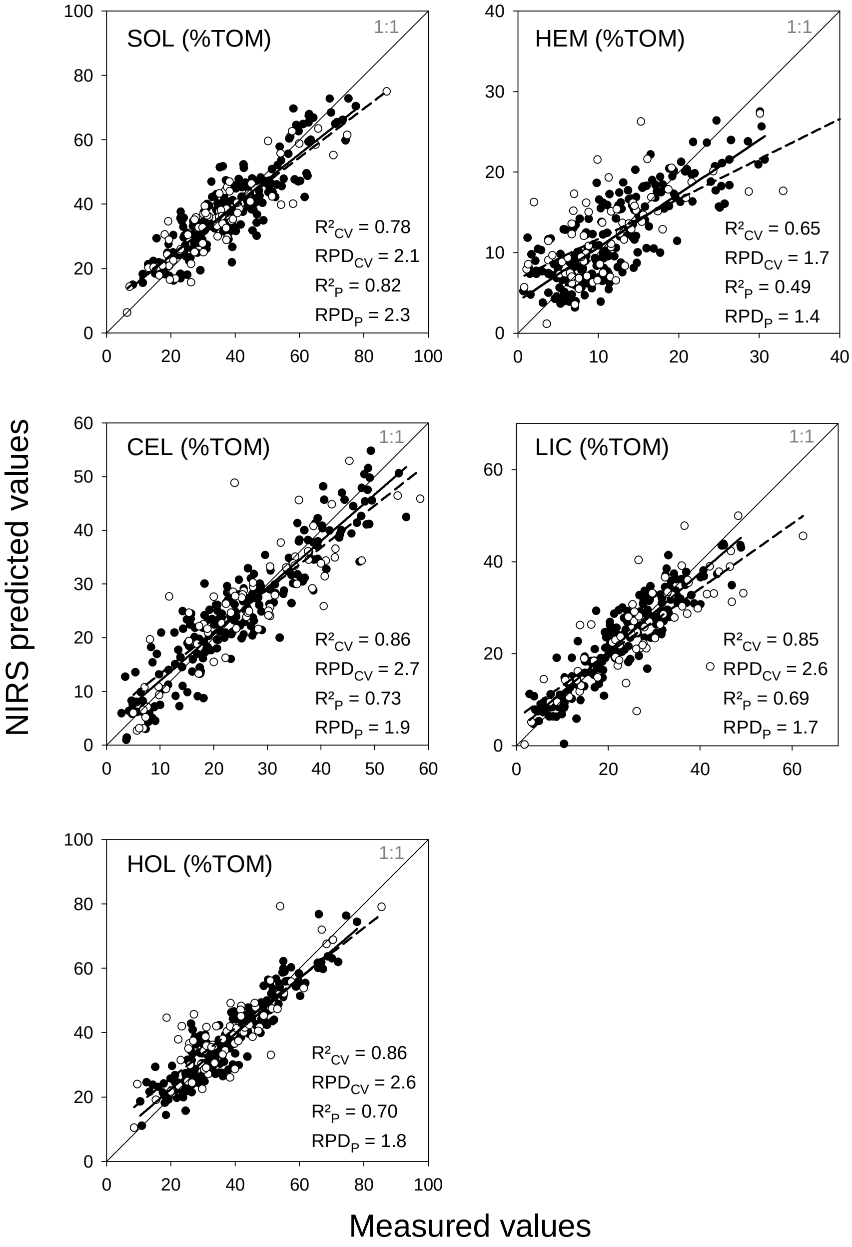

Figure 3.2. Predicted vs. measured values of soluble (SOL), hemicellulose-like (HEM), cellulose-like (CEL), lignin+cutin-like (LIC) and holocellulose-like (HOL = HEM + CEL) fractions of Van Soest fractionation expressed as a percentage of total organic matter (%TOM). Predictions on the calibration set during cross-validation (black circles, continuous regression lines, see part 3.3.4.2) and predictions on the independent validation set (white circles, dashed regression lines).

3.4.4. NIRS prediction of EOM-C mineralized during laboratory incubations

Both the experimental data on cumulated mineralized TOC during incubation and the parameters of the mathematical model used to simulate TOC mineralization kinetics (L and S, equation [2]) were predicted from NIR spectra. The L and S pools predicted by NIRS were used to calculate EOM-TOC mineralization data. The accuracies of both EOM-TOC mineralization predictions were then compared.

The calibration accuracy of the proportions of mineralized EOM organic carbon decreased in the order: $C_{7d} > C_{28d} > C_{3d} \geq C_{91d}$ (Table 3.3, Figure 3.3). Similar results had been found for C_{28d} by Shepherd et al. (2005) on plant materials from tropical regions. Bruun et al. (2005) also observed an increase in predictive accuracy up to 7 days of incubation, followed by a decrease until 217 days of incubation. The better calibration of C_{7d} could be related to broader variability at the beginning of the incubation (C_{3d}). The larger CV_{CV} of C_{3d} was probably related to the small number of high values when compared with the number of low values (skewness = 1.6, Table 3.1). The accuracy of prediction was poorer for external validation with the exception of C_{3d}, and remained better for C_{3d} and C_{7d} than for the other dates.

Calibration and validation statistics were good for the L (labile) pool. The S (stable) pool was fairly well calibrated but poorly validated ($RPD_p = 1.3$). Such prediction of model parameters would make it possible to determine the whole kinetics of C mineralization and enable their easy extrapolation. Direct NIRS predictions of C_{3d}, C_{7d}, C_{28d} and C_{91d} were compared with the C_{3d}, C_{7d}, C_{28d} and C_{91d} values calculated using NIRS predictions of the L (labile) and S (stable) pools and produced similar results, with slightly better accuracy using the direct prediction approach; this was contrary to the findings of Bruun et al. (2005) who reported a better prediction of mineralized C using the NIRS prediction of previously fitted parameters from decomposition models than with the direct NIRS prediction of mineralized C. Thus the whole kinetics of C mineralization and their extrapolation could be approached using NIRS predictions of the parameters of a fitted C mineralization model. However, variables relative to long-term incubation results (C_{28d}, C_{91d} and S pool) were less accurately predicted. This could be related to the increase in interactions and the complexity of the factors governing EOM mineralization in soil as a function of the incubation period, when compared to short-term mineralization that might be better related to well-identified compounds (i.e. simple sugars, polysaccharides etc.) with distinct chemical bonds that vibrate in the NIR range.

In our case, the outliers mainly consisted of sludges, organic or organo-mineral fertilizers and liquid manures; i.e. products that differed from the other samples in terms of their matrix. In most cases, we were able to exclude the hypothesis that they were classified as outliers because of incorrect laboratory measurements, as the same samples were found to be outliers during the prediction of most analytical variables.

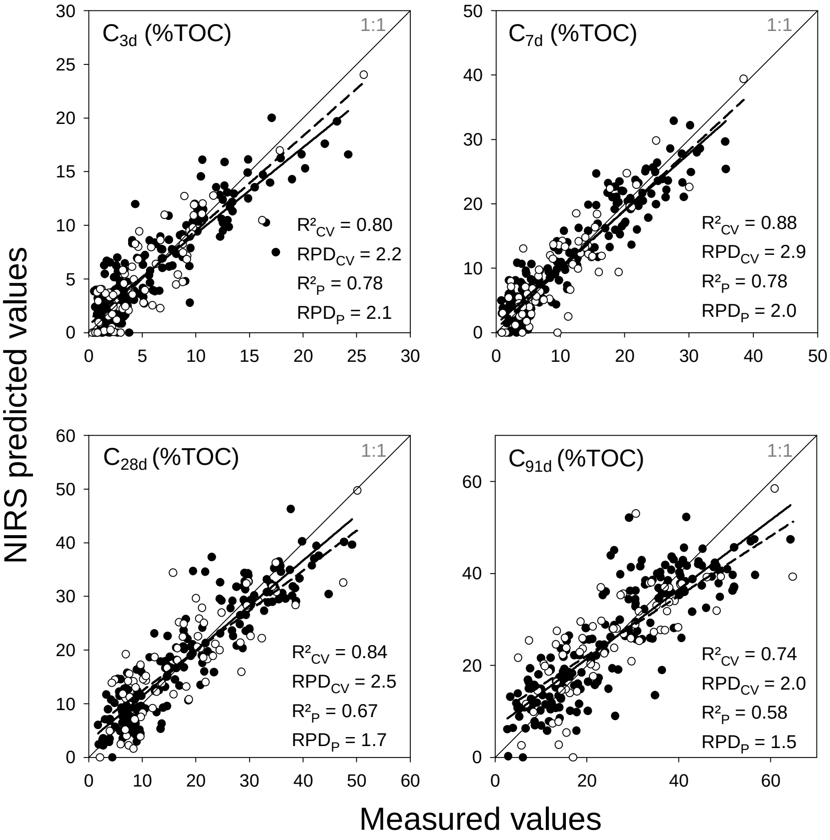

Figure 3.3. Predicted vs. measured values of the proportions of TOC mineralized after 3, 7, 28 and 91 days of incubation at 28℃ (C_{3d}, C_{7d}, C_{28d} and C_{91d}), expressed as a percentage of total organic C (%TOC). Predictions on the calibration set during cross-validation (black circles, continuous regression lines, see part 3.3.4.2) and predictions on the independent validation set (white circles, dashed regression lines).

3.4.5. NIRS prediction of the stable organic C in EOM

The stable pool (S) of model [2] estimated the proportion of stable organic C potentially remaining in soil after EOM applications. Acceptable results were obtained for the NIRS calibration of the S pool (R^2_{CV} = 0.77, RPD_{CV} = 2.1). However the validation results indicated that the model could not produce a satisfactory prediction of stable organic C in EOMs (Table 3.3, Figure 3.4a).

The I_{ROC} indicator was better predicted under both cross-validation and independent validation, with fairly good robustness of the prediction model (R^2_{CV} = 0.86, RPD_{CV} = 2.7, R^2_P = 0.79, RPD_P = 2.2, respectively; Table 3.3 and Figure 3.4b). We hypothesised that NIRS was better able to predict I_{ROC} than the S pool because it was calculated from laboratory measurements of well identified biochemical fractions and short-term mineralization data, all easily predictable with NIRS. Inversely, the S pool was less accurately predicted because it was linked to mineralization data at the end of the incubation period and due to the increasing degree of interactions governing EOM mineralization in soil in line with increasing incubation times, as discussed previously. The possibility to enhance I_{ROC} prediction using separate NIRS-predicted values for SOL, CEL, LIC and C_{3d} in order to re-calculate I_{ROC} using equation [1] was tested, but this did not improve the I_{ROC} prediction when compared to the direct NIRS method.

Thus, the I_{ROC} indicator and its NIRS-prediction provide a better estimation of the proportion of stable organic C in EOMs when only data of EOM-TOC mineralization during medium-term incubations (91 days) are available since it has been calibrated using incubations data over a longer period (> 156 days). When no mineralization data are available, the I_{ROC} indicator is the only option for the estimation of the proportion of stable organic C in EOMs. Nevertheless, long-term incubation data probably remained more accurate to determine the proportion of stable organic C in EOMs more precisely and it should be interesting to test the potential NIRS-calibration of stable organic C in EOMs based on enough long-term incubation data.

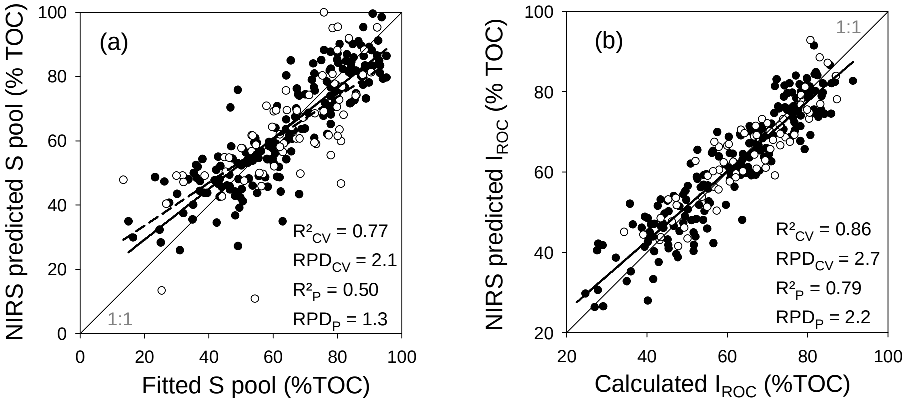

Figure 3.4. Predicted vs. reference values of: **(a)** the fitted stable pool of model [2] (S, see part 3.3.3) and **(b)** the calculated indicator of remaining organic C in soil (I_{ROC}). Values expressed as a percentage of total organic C (%TOC). Predictions on the calibration set during cross-validation (black circles, continuous regression lines, see 3.3.4.2) and predictions on the independent validation set (white circles, dashed regression lines).

3.5. Conclusions

The aim of this study was to investigate the potential of NIRS to predict the chemical and biochemical characteristics of EOM, the proportions of TOC easily mineralized during laboratory incubations and the proportions of stable organic C in EOM, either through the prediction of the stable pool of a decomposition model fitted to the kinetics of TOC mineralization during incubations or by predicting an indicator of remaining organic C in soils (I_{ROC}) calculated from the experimental results of biochemical fractions and short-term mineralization of organic C. We used a dataset of 300 EOM samples which as far as possible covered the diversity of EOMs applied in agriculture. Satisfactory predictions were achieved for the C content, N content and Van Soest fractions of EOMs. The predictions of EOM-TOC mineralization during laboratory incubations were satisfactory, with a better prediction of short-term rather than long-term mineralization results and similar accuracy for the direct NIRS prediction of mineralized C and for mineralized C calculated using NIRS predictions of fitted pools in a decomposition model. The NIRS prediction of the stable (S) pool was insufficiently accurate to satisfactorily predict the stable proportion of organic C in EOM. The prediction of I_{ROC} was fairly satisfactory in light of the heterogeneity of the dataset and might be better suited to predicting stable organic C in EOM than the S pool. Variables related to the long-term mineralization of EOM in soil were almost impossible to predict using NIRS, except for I_{ROC} which was calculated from biochemical fractions and short-term incubation results. This may have been due to the greater complexity and interactions governing EOM mineralization in soil, associated with increasing incubation periods. Short-term mineralization in soil was better predicted with NIRS, probably because it was more closely linked to well defined molecules and chemical bonds more easily measurable with NIRS. This study thus demonstrated that NIRS could be used to rapidly determine important characteristics of EOMs using predictive models valid for a highly heterogeneous dataset of EOMs, including various composts, solid manures, etc. However, there remains a need for specific NIRS predictive models for the agro-industrial and sewage sludges, organic and organo-mineral fertilizers and liquid manures that constituted the majority of the outliers found during the present study.

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Partie II. Accumulation de C dans le sol suite à des apports répétés de MOEs: Paramétrage du modèle RothC en vue de simuler le devenir de MOEs dans les sols

Chapitre 4. Carbon accumulation in soil following repeated applications of different organic amendments: distribution within particle size and density fractions and simulation with the RothC model.

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4.1. Abstract

The application of exogenous organic matter (EOM) of residual origin in arable soils can help to maintain or increase soil organic carbon (SOC) levels. However, it is necessary to better understand under which form EOM carbon accumulates into SOC. We used the mid-term (11 years) Qualiagro experiment to study the SOC dynamics following the application of green waste and sludge compost (GWS), municipal solid waste compost (MSW), biowaste compost (BIOW) and farmyard manure (FYM). Repeated applications of EOMs led to significant increases of SOC stocks. The proportion of the additional C inputs compared to reference plots accumulated into the soil (yield of C accumulation) after 11 years decreased in the order: BIOW (64%) > GWS (58%) > FYM (50%) > MSW (28%). The RothC model successfully reproduced the C accumulation patterns after fitting the partition coefficients of EOM-C into the model entry pools. The proportions of C accumulated in the DPM+RPM and in the BIO+HUM RothC pools corresponded to the proportions of C accumulated in the fractions >50 µm and 0-50 µm of SOC, respectively. Compost applications preferentially increased the C contained in the fraction of SOC >50 µm, whereas the FYM increased in similar proportion the >50 µm and 0-50 µm fractions. The extrapolation of the RothC simulation predicted yields of C accumulation after 20 years of application of 20, 34, 37 and 40 % for MSW, FYM, GWS and BIOW. The EOM applications led to fairly large C accumulation in soil compared with other practices leading to C sequestration in soil.

4.2. Introduction

The application of exogenous organic matters (EOMs) of residual origin on cultivated soils (farmyard manures, composts, sludges, digestates etc.) can help to maintain or increase soil organic matter contents (Marmo, 2008), especially in intensively managed soil of western Europe depleted in organic C and where animal breeding has disappeared (Le Villio et al., 2001; Van-Camp et al., 2004).

Increasing soil organic matter (SOM) contents has many positive effects on soil quality: reducing soil erosion, enhancing biological activity, improving tilth and workability etc. (Marmo et al., 2004). In addition, it could play a significant role in mitigation of the net emissions of greenhouse gases in the atmosphere through carbon sequestration in soil (Lal et al., 2007; Marmo, 2008).

Only part of the EOM applied to the soil will eventually become part of SOM. The yields of C accumulated in soil reported in the literature are highly variable and depend on the chemical nature of the EOMs, the frequency of EOM application, the pedoclimatic conditions etc. (Gerzabek et al., 1997; Kong et al., 2005). The high uncertainty of C accumulation over the long-term makes that C storage in soil following application is difficult to take into account in global balances (Smith et al., 2007; Marmo, 2008). It is thus necessary to better characterise under which form the remaining EOM accumulate into soil and describe the dynamics of incorporation of EOM into SOM. As a matter of fact, the mean residence time of the accumulated OM conditions the reversibility of C storage. For this purpose, long-term field experiments provide essential data and the Qualiagro field experiment has been initiated in 1998 to study the behaviour in soil of various EOMs in a loamy soil initially poor in soil organic carbon (SOC) and cultivated under a maize-wheat rotation.

The size and density fractionation of SOM can be used to describe the localisation of SOM in different dynamic pools. It separates OM with fast turnover localised in coarse size fractions from OM with slow turnover contained in finer fractions (Christensen, 1992; Balesdent, 1996). In a field cultivated under maize, Balesdent (1996) calculated mean residence times of 3 and 18 years for OM in 200-2000 µm and 50-200 µm size fractions, respectively and a mean residence time of 63 years for OM in the 0-50 µm size fraction. In addition, multi-compartment C dynamics models are useful tools to simulate the C stocks evolution in soil. Many C dynamics models have been reported in the literature, the most widely used to study C sequestration in soil being the RothC model (Jenkinson & Rayner, 1977; Coleman & Jenkinson, 1999) and the CENTURY model (Parton et al., 1987).

The advantage of RothC is that it describes C dynamics with few parameters and need few input data compared with CENTURY. In the RothC model, total organic C (TOC) from EOM (EOM-TOC) is split into a labile a resistant and possibly a fraction of humified pool (DPM, RPM and HUM, respectively) according to partition coefficients (f_{DPM}, f_{RPM}, $f_{HUM} = 1 - f_{DPM} - f_{RPM}$, respectively).

The distribution of EOM-TOC in RothC entry pool has been originally determined for the most common EOMs (crop residues and farmyard manure) by fitting against long-term field data in the

Rothamsted long-term field experiments (Coleman & Jenkinson, 1999). The proposed initial EOM-TOC distribution in the RothC entry pools was 59% DPM and 41% RPM for crop residues and 49% DPM, 49% RPM and 2% HUM for farmyard manure (Coleman & Jenkinson, 1999). However, it remains necessary to determine other sets of EOM-TOC partition coefficients in RothC entry pools for other types of EOMs which has not been investigated yet as far as we know.

The objectives of the present study were: (i) to describe the dynamics of EOM incorporation into SOM in the Qualiagro field experiment by studying the C accumulation in the amended plots compared to reference plots; (ii) to relate these dynamics of EOM incorporation into SOM with the initial characteristics of EOMs; (iii) to simulate the soil organic carbon accumulation following EOM application using the RothC model.

We first looked at the composition of the applied EOMs, then we used size and density fractionation to describe the distribution of EOMs in dynamic pools of SOM. The partition coefficients of EOM-TOC into the RothC entry pools were then optimised by fitting the simulated kinetics of soil C accumulation to the experimental ones. The relationship between separated size and density fractions and RothC pools was discussed.

4.3. Materials and methods

4.3.1. Qualiagro field experiment

The ongoing Qualiagro experiment, located in Feucherolles (Île-de-France), approximately 50 km west of Paris (48°52’ N, 1°57’ E), was initiated in 1998 . The soil is a Luvisol (FAO) with 78.7% loam, 15.2% clay and 6.2% sand in the ploughed layer (0-29 cm). Mean annual temperature was 11.0 ± 0.5 °C and mean annual precipitations were 643 ± 128 mm during the studied period (1998 – 2009). Three urban composts are compared to a farmyard manure (**FYM**) used as reference amendment and to reference plots without organic applications (**Ref**). The applied composts include: (i) a compost derived from co-composting of green waste with sewage sludge (**GWS**), (ii) a municipal solid waste compost obtained from composting of residual solid wastes after sorting and separate collection of dry and clean packagings (**MSW**), and a biowaste compost produced by the co-composting of green wastes and source separated organic fraction of municipal solid wastes (**BIOW**). Detailed information on the composting processes can be found in Annabi et al. (2007). The experiment has been divided into two parts with distinct N fertilisation treatments: optimum and minimum mineral N fertilisation (reffered as +N and -N, respectively). The five treatments (four EOMs amendments and a reference treatment without EOM) are distributed in each half of the field experiment according to a randomised block design with four blocks. The plots are 10 * 45 m and are separated from adjacent plots by 6-m bands. The four blocks are 25 m apart to prevent cross-contamination during EOM applications. The field is cultivated with a wheat–maize rotation. The wheat residues are exported but the maize residues are ploughed into the soil after harvest. The grain yields and the dry mass of plant residues are measured every year from manual plant sampling in each plot before mechanical harvesting. The composts and

FYM are applied every second year in September on wheat stubbles with a target dose of 4 Mg C ha^{-1}. The amounts of EOM really applied are measured at each application. Barley was cultivated in 2007 due to a regional attack of *Diabrotica virgifera* avoiding maize cultivation. As a result, EOMs were exceptionally applied two consecutive years in 2006 and 2007.

4.3.2. Chemical and biochemical characterisation of EOMs

The EOMs were sampled at each application, air dried and finely ground (< 1mm) before analysis. Total organic matter (TOM) was determined by loss on ignition at 480℃. Total organic C (TOC) and total N (totN) were determined by dry combustion using an Elemental Analyser after additional grinding (< 0.2mm) and dissolution of carbonates with hydrochloric acid (AFNOR, 1995; 1998). The biochemical composition of EOMs was determined on 1-mm ground samples using the Van Soest method (Van Soest, 1963; Van Soest & Wine, 1967) as modified in the French standard XP U 44-162 (AFNOR, 2009a). The C mineralisation of EOMs (1-mm ground samples, expressed as percent of TOC) was measured during 91-day incubations of soil-EOM mixtures in hermetically sealed jars and controlled conditions at 28℃ with soil moisture corresponding to 85% of soil water holding capacity. The C-CO_2 was trapped into 10 ml of 0.5 M NaOH periodically replaced, and determined by colorimetry (AFNOR, 2009b). The indicator of residual organic carbon (I_{ROC}) evaluating the proportion of EOM-TOC potentially remaining in soil after application over the long-term (Lashermes et al., 2009) was calculated using the soluble (SOL), cellulose-like (CEL) and lignin + cutin-like (LIC) fractions of the Van Soest fractionation and the proportion of EOM-TOC mineralized after 3 days of incubation (C_{3d}), according to the formula:

$$I_{ROC} = 44.5 + 0.5\ SOL - 0.2\ CEL + 0.7\ LIC - 2.3\ C_{3d} \qquad [1]$$

with SOL, CEL, LIC expressed as % of TOM and C_{3d} as % of TOC.

4.3.3. Soil sampling and C stocks

The ploughed layer (0-29 cm) was sampled before each EOM application (ten individual samples pooled in each plot). Total organic C (TOC) was determined on air dried, 2-mm sieved and 200-µm ground soil by dry combustion using an elemental analyser after dissolution of carbonates with hydrochloric acid (AFNOR, 1995). Detailed results are presented in Table S1 of supporting information.

The bulk density of the ploughed layer was measured in each plot using the cylinder method in 1998 (start of the experiment), 2004 and 2009. The initial bulk density (1998) differed between the two parts of the field experiment (1.26 and 1.30 Mg m^{-3} in the part of the experiment with optimum and minimum N fertilisation, respectively). The bulk density significantly increased in the reference plots to 1.39 and 1.41 Mg m^{-3} in 2009 in the +N and –N parts of the experiment, respectively. In the plots with EOM application, the bulk density slightly increased (mean values of 1.32 and 1.34 Mg m^{-3} in the +N and –N parts, respectively without significant differences between the organic treatments). The evolution of bulk density in the ploughed layer was interpolated in between 1998 and 2004, then between 2004 and 2009 (Table S2 of supporting information).

To accurately quantify the changes in C stocks during the experiment, equivalent soil masses must be compared in all treatments and over the time (Ellert & Bettany, 1995; Lee *et al.*, 2009). To account for the changes in soil bulk densities in the ploughed layer, we calculated the amount of C in an "equivalent" soil mass in all treatments of both +N and –N parts of the experiment. The additional soil thickness required to reach this equivalent soil mass in treatments with lighter ploughed layers was calculated as described by Ellert and Bettany (1995):

$$T_{add} = ((M_{soil,equiv} - M_{soil,surf}) * 10^{-4}) / \rho_{b\ subsurface} \qquad [2]$$

where T_{add} is the additional thickness of subsurface layer expressed in cm required to attain the equivalent soil mass, $M_{soil,\ equiv}$ is the equivalent soil mass of the heaviest horizon in Mg ha^{-1}. In our study, the heaviest 0-29 cm horizons were in the reference plots in 2009 with bulk densities of 1.39 and 1.41 Mg m^{-3} in the +N and –N parts, respectively which give equivalent soil masses $M_{soil,\ equiv}$ of 4029 and 4095 Mg ha^{-1} for the +N and –N parts, respectively. $M_{soil,\ surf}$ is the soil mass in the surface (ploughed) layer of the treatment considered (in Mg ha^{-1}) and $\rho_{b\ subsurface}$ is the bulk density of the subsurface layer (in Mg m^{-3}).

The amounts of C per hectare in equivalent soil masses were calculated by summing the C in surface layers and in the additional subsurface layers necessary to attain the equivalent soil mass:

$$M_{C,\ equiv} = M_{C,\ surf} + M_{C,\ Tadd} \qquad [3]$$

where $M_{C,\ equiv}$ is the amount of C per hectare in the equivalent soil mass (Mg ha^{-1}), $M_{C,\ surf}$ is the amount of C in the surface layer (Mg ha^{-1}) and $M_{C,\ Tadd}$ is the amount of C in the additional subsurface layer calculated with the equation [2] (in Mg C ha^{-1}).

The bulk density and the C content of the subsurface horizon (29 – 35 cm) were measured in each plot in September 2004. The bulk density of the subsurface layer was not significantly different between treatments and average values were used in all treatments for each N fertilisation level of the experiment (1.44 Mg m^{-3} and 1.48 Mg m^{-3} for the +N and –N levels, respectively). These values were assumed to be constant from 1998 to 2009. The C content of the subsurface horizons sampled in 2004 showed significant differences between treatments. We assumed that the C content of the subsurface horizon was constant from 1998 to 2009 in the reference plots (9.4 and 9.2 g C kg^{-1} for the +N and –N parts, respectively). Those values were attributed to all treatments at the beginning of the experiment in 1998. The C content in the subsurface horizon was then interpolated between 1998 and 2004 when the C contents in the subsurface horizon were measured in all plots (ranging from 9.6 g C kg^{-1} for the MSW-N treatment to 11.1 g C kg^{-1} for the BIOW+N treatment). After 2004, it was calculated as 0.91 (average ratio between the C contents in the 29-35 and 0-29 cm depth horizons measured in 2004) multiplied by the C content of the 0-29 horizon in the given treatment. The details of C stocks contained in the topsoil and subsoil horizons are given in Table S3 of supporting information.

4.3.4. C inputs from crop residues

The wheat residues were exported except for stubbles with a height of about 17 cm accounting for about 30% of the measured dry matter of above ground residues (Y_S, in Mg ha^{-1}). The maize residues were entirely returned to the soil. Stem bases of wheat and maize were estimated as accounting for 5% of the total above ground production (i.e. Y_S + dry matter grain yield: Y_G in Mg ha^{-1}) as reported by Kätterer et al. (1993).

The total C from roots (C_R, in Mg C ha^{-1}) were estimated according to the equation given by Bolinder et al. (2007) assuming a C concentration of all plant part of 0.45 g C g^{-1} as:

$$C_R = ((Y_G + Y_S)/S:R)*0.45 \quad [4]$$

where Y_G is the dry matter yield of grain yield in Mg ha^{-1}, S:R is the shoot to root ratio (7.4 for wheat, 5.6 for maize). The extra-root C ($C_{E,}$ in Mg C ha^{-1}), including root exudates and other material derived from root turnover were estimated from Bolinder et al. (2007) as:

$$C_E = 0.65*C_R \quad [5]$$

Because we considered only the ploughed layer, the values of root C and extra-root C (C_R and C_E) were corrected using the vertical root distribution function proposed by Jackson et al. (1996):

$$Y_R = 1 - \beta^d \quad [6]$$

where Y_R is the cumulative root fraction, *β* is the "extinction coefficient" (β = 0.961 for crops) and *d* is the depth of the studied soil layer (in cm) calculated to reach equivalent soil mass in all treatments as explained previously (Y_R = 0.68 for 29 cm).

Finely, total C inputs from crop residues (C_{res}, in Mg C ha^{-1}) can be expressed for wheat and maize for a given treatment *i* and a given year *k* as:

$$C_{res}(wheat)_{ik} = 0.35*0.45*Y_{S(ik)} + (C_{R(ik)} + C_{E(ik)})*Y_{R(ik)} \quad [7]$$

$$C_{res}(maize)_{ik} = 1.05*0.45*Y_{S(ik)} + (C_{R(ik)} + C_{E(ik)})*Y_{R(ik)} \quad [8]$$

The additional C inputs from crop residues compared to reference plots (ΔC_{res}) used as entry data in RothC were calculated by subtracting the C_{res} in reference plots from the C_{res} of the treatments with

EOM application. The cumulated additional C inputs from crop residues for the period 1998-2009 ($\Delta C_{res\text{-}11y}$) were calculated by summing the ΔC_{res} of all years between 1998 and 2009.

4.3.5. Yields of C accumulation

The yields of C accumulation in soil (in % of total additional C inputs) from EOM application and additional C inputs from crop residues after 11 years of EOM application compared to reference plots were calculated according to:

$$Y_{11y} = \Delta C_{11y} / (C_{EOM-11y} + \Delta C_{res-11y}) * 100 \qquad [9]$$

where ΔC_{11y} is the measured differences between C stocks in the plots receiving EOMs and C stocks in the reference plots after 11 years of EOM application, $C_{EOM\text{-}11y}$ is the cumulated C inputs from EOMs for the period 1998-2009 and $\Delta C_{res\text{-}11y}$ is the additional C inputs from crop residues as described previously.

4.3.6. Size and density fractionation of soil organic matter

The ploughed layers were sampled in September 2006 before EOM application in all plots of three blocks in the optimum N fertilisation part (+N) of the experiment (3 field replicates). The soil was fractionated in size and density fractions: light particulate organic matter (POM) >50 µm, heavy fraction >50 µm and organo-mineral fraction 0-50 µm according to Balesdent et al. (1991). Briefly, 100 g of air dried soil were shaken overnight with 360 ml of demineralised water and 20 glass beads to break the aggregates. After disaggregation, the soil was sieved under water at 5 mm to remove coarse materials. Two successive wet sieving at 200 µm then at 50 µm were performed. The light and heavy fractions of the material retained at 200 µm and 50 µm were separated by flotation in water in a 150 ml beaker. The light and heavy fractions separated at 200 µm and 50 µm were gathered to obtain light and heavy fractions >50 µm. The separated fractions were ball milled and their total C content was measured by dry combustion using an Elemental Analyser (CHN autoanalyser, Micromass NA 1500 NC).

4.3.7. Simulation of C accumulation in soil with RothC

The RothC 26.3 model (Coleman & Jenkinson, 1999) was used to simulate the C accumulation in soil after repeated EOM applications compared with non-amended reference plots in the Qualiagro experiment. The RothC model describes soil C dynamics considering five organic C pools: a labile pool (DPM: "decomposable plant material", mean residence time: MRT, of 1.2 month), a resistant pool (RPM: "resistant plant material", MRT of 3.3 yrs), a humified pool (HUM, MRT of 50 yrs) a microbial biomass pool (BIO, MRT 1.5 yrs) and an inert OM pool (IOM, MRT infinite). When EOM is added to the soil, it is split into the DPM pool, the RPM pool and possibly the HUM pool according to partition coefficients f_{DPM}, f_{RPM} and $f_{HUM} = 1 - f_{DPM} - f_{RPM}$. Then, at each monthly time step a fraction of each pool is decomposed according to the specific decay rate constants of each pool and either mineralised into CO_2 or transferred into the humified (HUM) or the microbial biomass pools (BIO). The proportion of C mineralized into CO_2 or transferred into BIO+HUM is determined by the clay content of soil. Then the part transferred into BIO+HUM is separated into 46% BIO and 54% HUM. Rate modifying factors are applied to the decay rate constants to account for soil humidity, air temperature and soil cover (covered or not covered). The input data of RothC included: the soil clay content, monthly climatic data (potential evapotranspiration, air temperature, cumulated rainfalls), soil cover, C inputs from EOMs, additional C inputs from crop residues, stem bases, roots and root exudates compared with reference plots. All simulations were run as difference with the reference treatment (i.e. only the C accumulation compared to reference plots was simulated). Thus the initial sizes of the soil organic pools were set to zero, including the size of the inert OM pool (IOM). This was possible because in the RothC model, the kinetics of C dynamics of each pool are described with first order kinetics $dCi/dt = -kiCi$ where Ci is the C stock of the given pool at time t and ki its degradation rate. The fate of total soil C is the sum of the fate of C of the different pools and the difference of C evolution in soils with and without EOM application corresponds to the evolution of the additional input of OM in the amended soil including the EOM application and the additional crop residue produced related to the increase in crop yield. The priming effect of EOM applications on native SOM degradation is thus neglected.

The monthly cumulated potential evapotranspiration was calculated from daily climatic data measured in the experiment (solar radiation, air temperature, wind velocity and relative humidity) according to Penman (1948).

The partition coefficients of EOM-TOC into the DPM, RPM and HUM entry pools of RothC (f_{DPM}, f_{RPM}, $f_{HUM} = 100\% - f_{DPM} - f_{RPM,}$ with values expressed as % of EOM-TOC) were adjusted to fit the kinetics of C stock accumulation in soil. For each EOM, the partition coefficients were considered as identical for each application year. The f_{DPM} and f_{RPM} coefficients were set to be comprised between 0 and 100% of EOM-TOC and the f_{HUM} coefficient was set to be ≤20% of EOM-TOC, This constraint on f_{HUM} was set to prevent unrealistic estimates of the coefficients, e.g. EOM having high f_{DPM} and f_{HUM} coefficients but an f_{RPM} coefficient equal to zero. The other parameters of the model were conserved as in the original publication (Coleman & Jenkinson, 1999). The experimental results of C stock evolution measured in both parts of the field experiment (with optimum and minimum N fertilisation) were used simultaneously to optimise the partition coefficients of each EOM by minimising the residual sum of squares between measured and simulated values using the Excel solver with the Newton method. Several standard statistical indicators were used to evaluate the goodness of fit (Smith *et al.*, 1997b). The Spearman coefficient of correlation (r) was used to assess whether simulated values follow the same patterns as the measured ones. The efficiency of the model was calculated as:

$$EF = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$$

where y_i and $\hat{y}_i$ are the measured and predicted values for the sample i, $\bar{y}$ is the mean of measured values and n the number of samples. The model efficiency expresses the proportion of total variance of the measured data explained by the model. The root mean square error (RMSE) was used to evaluate the difference between measured and predicted values and the coefficient of variation of the RMSE (CV(RMSE) in %) was calculated as RMSE / $\bar{y}$*100 to evaluate the relative error between measured and predicted values.

4.3.8. Simulation of potential sequestration of C through EOM applications

A duration of 20 years has been proposed by IPCC (1997) for the evaluation of potential C storage in soil related with a given land-use or crop practice. To compare the potential storage of organic C after 20 years of the different EOM applications, we took into account both the C input through EOM application but also the C input through additional crop residue production after EOM application compared to reference treatment. The accumulated C values were determined for September 2018, after 20 years of application. The additional C inputs from crops compared to reference treatment were calculated for the period 2008-2018 from the average excess yields of the last three maize (2003, 2005 and 2008) and wheat crops (2004, 2006 and 2007). The resulting cumulated additional C inputs from crop residues for the period 1998-2018 ($\Delta C_{res\text{-}20y}$) were calculated by summing the ΔC_{res} of the different years between 1998 and 2018. The C input corresponding to each EOM application were set to 4 Mg C ha^{-1} applied every second year in September during the period 1998-2018 with a total of 40 Mg C ha^{-1} applied after 20 years. The cumulated C inputs from EOM are written $C_{EOM\text{-}20y}$ for the period 1998-2018.

The yields of C sequestration after simulation of 20 years of application using RothC were calculated by replacing ΔC_{11y} in equation [9] by the simulated cumulated differences between C stocks in the plots receiving EOMs and reference plots after and 20 years of EOM application ($\Delta C_{20y\text{-}sim}$). The resulting simulated yields of C accumulation after 20 years can then be expressed as:

$$Y_{20y-sim} = \Delta C_{20y-sim} / (C_{EOM-20y} + \Delta C_{res-20y}) * 100 \qquad [10]$$

The amount of C that would be released as CO_2 after stopping the EOM applications was then simulated over the period 2018-2028 by setting all C input to zero in RothC. The percentage of C accumulated after 20 years of EOM applications (i.e. in 2018) that would be released during ten years after stopping the applications has been calculated.

4.3.9. Statistical analysis

The results of C stock, size and density fractionation were compared according to the non-parametric Kruskal-Wallis analysis of variance on ranks. The Kruskal-Wallis test was followed by pairwise multiple comparisons (all pairwise) and by a multiple comparison versus reference plots using the Dunn's method. The changes in C stock with time in a same treatment were tested using the Friedman repeated measures analysis of variance on ranks. All statistical tests were performed using the SigmaPlot 11.0 software (Systat Software Inc. 2008).

4.4. Results and Discussion

4.4.1. Characteristics of the EOMs applied

The EOMs applied in the Qualiagro experiment had contrasted chemical compositions. The GWS and BIOW composts had low C contents, low C:N ratios and high proportions of LIC fractions compared to the MSW compost and the FYM (Table 4.1). The indicator of residual organic C in soil (I_{ROC}) was higher for GWS and BIOW than for MSW and intermediate for FYM. The low I_{ROC} value of MSW (45.0% of TOC) was due to its larger proportions of C mineralized during incubation in soil (C_{3d}) and of cellulose fraction (CEL), related to an important proportion of paper and cardboard in the initial wastes before composting leading to a lower maturity of this compost. The FYM had higher proportions of HEM fraction and slightly lower proportions of SOL fraction compared to the composts. These analytical characteristics slightly differed between years for a same type of amendment (see Table S4 of supporting information). However no trend could be identified except for MSW compost which tended to be less stabilized from 1998 to 2009 as shown by a regular decrease of the I_{ROC}, and increase of the proportions of C mineralized after 3, 7 and 91 days of incubation (C_{3d}, C_{7d} and C_{91d})..

Table 4.1. Mean analytical characteristics of the EOMs applied from 1998 to 2009 in the Qualiagro experiment: total organic matter (TOM), total organic C (TOC), total N (totN) expressed as % of dry matter at 105 ℃ (DM), TOC to totN ratio (C:N), soluble, hemicellulose-, cellulose-, and lignin + cutin- like fractions (SOL, HEM, CEL, LIC) expressed as % of TOM, proportions of EOM-TOC mineralized after 3, 7 and 91 days of laboratory incubation in soil at 28 ℃ (C_{3d}, C_{7d} and C_{91d}) expressed as % of TOC and indicator of residual organic C in soil (I_{ROC}) also expressed as % of TOC and calculated with equation [1]. Mean values ± standard deviation of the seven EOMs applied from 1998 to 2009. Detailed analysis are presented in Table S1 of supplementary material.

Bulk EOM	TOC	totN	C : N	SOL	HEM	CEL	LIC	C_{3d}	C_{7d}	C_{91d}	I_{ROC}
	% DM	% DM		--------------------------------%TOM--------------------------------				%TOC	%TOC	%TOC	%TOC
GWS	27.1 ± 6.2	2.3 ± 0.3	12 ± 3	45.7 ± 9.4	5.1 ± 2.1	20.8 ± 10.1	28.5 ± 11.9	1.8 ± 0.9	2.9 ± 1.1	12.0 ± 5.1	78.9 ± 10.5
MSW	31.9 ± 4.7	1.7 ± 0.3	19 ± 5	42.5 ± 8.7	6.4 ± 2.3	35.8 ± 10.3	15.2 ± 2.4	10.6 ± 2.9	22.0 ± 5.5	44.3 ± 8.6	45.0 ± 12.8
BIOW	18.6 ± 2.4	1.6 ± 0.4	12 ± 2	44.4 ± 7.7	4.2 ± 2.8	20.3 ± 2.3	31.1 ± 8.9	2.7 ± 1.3	3.8 ± 1.6	12.6 ± 7.1	78.3 ± 5.8
FYM	32.1 ± 5.3	2.3 ± 0.3	14 ± 4	39.1 ± 5.9	12.0 ± 2.9	25.5 ± 7.7	23.4 ± 2.5	2.8 ± 0.3	5.0 ± 1.4	16.6 ± 4.5	68.9 ± 6.0

4.4.2. Evolution of field C stocks after EOM application

Detailed results of TOC and bulk density evolutions in the different treatments are presented in Table S1 and S2 in supporting information, respectively. The C stock was maintained to a constant level between 1998 and 2009 in the reference plots with optimum N fertilisation (Ref+N) (41.7 ± 1.0 Mg C ha^{-1}, P = 0.624, Figure 4.1) while it decreased significantly in the reference plots with minimum N fertilisation (Ref-N) (41.6 to 38.9 Mg C ha^{-1}, P = 0.012). The C stocks significantly increased between 1998 and 2009 in all treatments with EOM application. The changes of C stocks increased regularly during the 11 years of the experiment and could be adjusted in all treatments with linear regressions having R^2 ranging from 0.94 to 0.97 (detailed result not shown) indicating that equilibrium between C inputs and soil C mineralisation had not been reached. The soil of the Qualiagro experiment was depleted in OM at the beginning of the experiment (C content = 10.4 ± 0.17 g C kg^{-1} soil in 1998), resulting in an important C sink capacity of the soil.

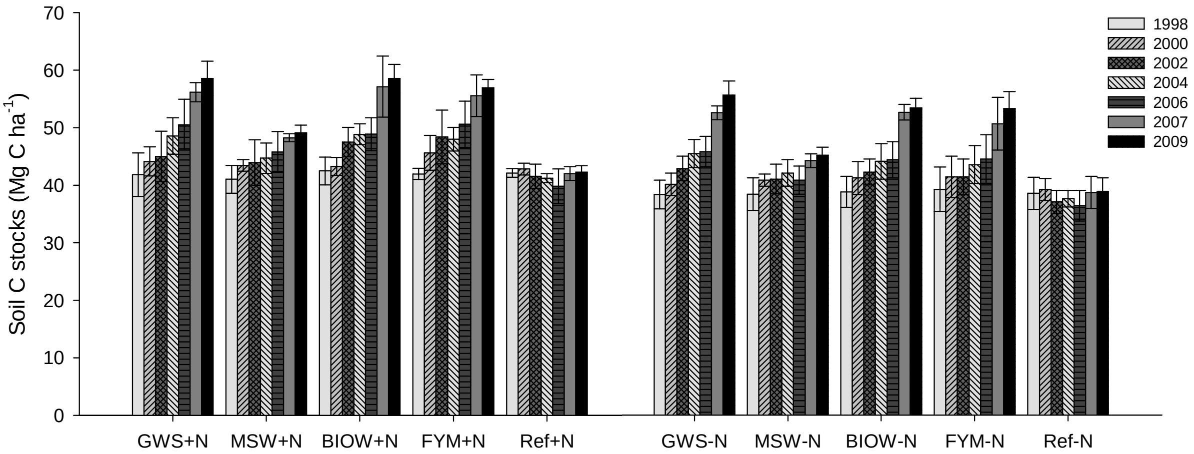

Figure 4.1. Evolution of C stocks in the Qualiagro experiment during the period 1998-2009 in the plots with application of green waste and sludge compost (GWS), municipal solid waste compost (MSW), biowaste compost (BIOW), farmyard manure (FYM) compared to reference plots without EOM application (Ref) for two modalities of N fertilisation (with optimum and minimum N fertilisation: +N and –N, respectively). Mean values, errors bars are standard deviations, n = 4 field replicates.

The most important increases of C stock after 11 years of experiment (ΔC_{11y}, Table 4.2) were observed in the GWS amended plots (accumulation of 16.3 and 19.6 Mg C ha^{-1} measured in 2009 for GWS amended plots with optimum and minimum N fertilisation: GWS+N and GWS-N, respectively). Nevertheless the BIOW had the highest yield of C accumulation indicating that the BIOW compost was the most efficient at increasing C stocks (Y_{11y} of 56.2% and 63.1% for GWS and BIOW respectively, mean of the +N and -N parts). This was due to the smaller cumulated amounts of C really applied ($C_{EOM-11y}$) in the BIOW amended plots than in the GWS amended plots ($C_{EOM-11y}$ = 22.7 and 26.7 Mg C ha^{-1} for BIOW and GWS respectively). The smallest yields of C accumulation were observed in the MSW amended plots (28.4%) and were intermediate in the FYM amended plots (49.8%).

Table 4.2. Cumulated additional C inputs via EOM application and increased crop residues compared to reference plots after 11 years (1998-2009) of cultivation ($C_{EOM-11y}$, $\Delta C_{res-11y}$, respectively) expressed in Mg C ha^{-1}. Accumulated C in soil compared to reference plots measured after 11 years of cultivation in 2009 ($\Delta C_{11y-mes}$) also expressed in Mg C ha^{-1}. Yields of C accumulation in soil, i.e. proportion of total additional C inputs accumulated in soil after 11 years of experiment ($Y_{11y-mes}$ expressed in % of cumulated C inputs). Annual rates of C accumulation calculated over the 11 years ($C_{11y-mes}/11$). Results are given for the treatments with application of green waste and sludge compost (GWS), municipal solid waste compost (MSW), biowaste compost (BIOW) and farmyard manure (FYM) for the parts of the experiment with optimum and minimum N fertilisation (+N and –N, respectively).

Treatment	Total added C with EOM 1998-2009	Total additional C from crop residues 1998-2009	Total accumulated C in soil measured in 2009	Yield of C accumulation after 11 years	Mean C accumulation rate over 11 years
	$C_{EOM-11y}$	$\Delta C_{res-11y}$	ΔC_{11y}	Y_{11y}	$\Delta C_{11y}/11$
	--------------------------------Mg C ha^{-1}-------------------------------			% total additional C inputs	Mg C ha^{-1} year^{-1}
GWS+N	26.7	1.9	16.3	57.0	1.48
GWS-N	26.7	6.9	19.6	58.2	1.78
MSW+N	23.2	1.0	6.8	28.2	0.62
MSW-N	23.2	6.7	8.6	28.6	0.78
BIOW+N	22.7	1.8	16.3	66.2	1.48
BIOW-N	22.7	4.7	17.2	62.7	1.56
FYM+N	26.6	2.8	14.7	49.9	1.33
FYM-N	26.6	7.9	17.1	49.7	1.56

The rate of C accumulation in soil usually decreases with the duration of the experiment (Six *et al.*, 2002; Chung *et al.*, 2008; Thomsen & Christensen, 2010). Other factors like soil type and climate also affect the C accumulation in soil. As a result comparisons with other studies are difficult because the yields of C accumulation must be compared between experiments with equivalent duration and cumulated amounts of C added by EOMs. Two studies were found with approximately equivalent duration but with slightly higher amounts of added C with animal manure. Delas and Molot (1983) reported a C accumulation yield of 32% after 12 years of cultivation for a total amount of 31.7 Mg C added with farmyard manure. Thomsen and Christensen (2010) found a yield of 30% for a total amount of 32 Mg C sheep faeces after 9 years of application. The lower yields found in those two studies may be attributed to the sandy soil used in the study of Delas and Molot (1983) (76% of sand) because sandy soils have less ability to protect SOM from biodegradation than clay or loamy soils (Six et al., 2002; von Lützow et al., 2006). The higher yields of accumulated C in the FYM amended plots in our study may be due to a higher degree of stability of the applied farmyard manure that was usually stored at least one month in the field before application. This suggests that one type of EOM may in reality gather products having distinct levels of stability. The higher stability of the applied farmyard manure compared to others studies is well reflected by the large value of I_{ROC} (68.9%, Table 4.1) and by the low proportion of C mineralized after 91 days of incubation in soil at 28 ℃ (16.6%, Table 4.1). As a comparison, Morvan and Nicolardot (2009) reported decreasing proportion of C mineralized after 100 days of incubation from 40% for fresh farmyard manure to 10% for composted farmyard manure. The yield of accumulated C calculated for plots amended with GWS (56.2%, mean of GWS+N and GWS-N) were close from the value found by Tester (1990) although with different experiment duration (5 years of GWS compost) and different soil texture (53% sand). The most efficient EOMs at increasing SOM (BIOW and GWS composts) were characterised by large LIC fraction and low CEL fraction (Table 4.1). The yield of C accumulation in soil tended to increase with the I_{ROC} indicator and the LIC fraction (no significant relationship) and decreased when the CEL fraction and the proportion of C mineralized after 3, 7 and 91 days of incubation (C_{3d}, C_{7d} and C_{91d}) increased.

4.4.3. Distribution of SOC in size and density fractions following EOM application

Table 4.3 shows the proportions of soil mass in size and density fractions and their C content in soils sampled in September 2006 before EOM application. The mass recoveries after fractionation yielded 98.9% to 99.7%. The EOM application significantly changed the masses of light fraction >50 µm (particulate organic matter: POM) in soil that were larger in the GWS+N and BIOW+N amended plots than in the reference plots. Inversely, the mass of heavy fraction >50 µm and of organo-mineral fraction 0-50 µm were not significantly different between treatments.

The C recovery after fractionation was comprised between 93% and 108% of TOC in the bulk soil C. The C content of the bulk soil sampled in 2006 was significantly different between the organic treatments. The C concentrations of POM (in g C kg^{-1} of fraction) and the amount of C in the POM fraction (in g C kg^{-1} of soil) were significantly increased by EOM applications. The C amount in the POM fraction was significantly higher in the GWS+N and BIOW+N treatments than in the reference treatment. The amount of C in the heavy fraction >50 µm was highly variable, depending on the quantity of residual OM left in the heavy fraction during the separation step by flotation because priority was given to have POM without mineral particles rather than heavy fractions without residual OM. To circumvent the variability of C amount in the heavy fraction, we calculated the amount of C in the entire fraction of size >50 µm by summing the POM and heavy fractions >50 µm. We assumed that the OM composition in the light and heavy fractions >50 µm was fairly similar as very few amount of charcoals were present in this soil as visually observed.

The EOM applications tended to increase C amount in the organo-mineral 0-50 µm fraction compared to the reference treatment, especially in the BIOW+N and FYM+N amended plots. The C present in the 0-50 µm fraction is particularly important for long-term C sequestration, since characterized by a longer turnover time in soil (Christensen, 1992; Balesdent, 1996). In our case, the C amounts in the 0–50 µm fractions tended to increase in line with the yields of C accumulation in soil Y_{11y} ($r = 0.93$) although this was not significant because calculated with only 4 points.

Table 4.3. Distribution of mass and C in size and density fractions of soil sampled in September 2006 after 8 years of cultivation with EOMs application (4 applications) and just before the fifth application. (Means ± standard deviation, n = 3 field replicates). Soils were sampled in the part of the experiment with optimum N fertilisation (+N) in all treatments with application of green waste and sludge compost (GWS+N), municipal solid waste compost (MSW+N), biowaste compost (BIOW+N), farmyard manure (FYM+N) and reference (Ref+N).

Treatment	Bulk soil	>50 µm			0-50 µm	Recovery
		Light fraction (POM)	Heavy fraction	Light + heavy fraction		%
Mass: g kg^{-1} bulk soil						
Ref+N		10.6 ± 0.6 (a)	55.6 ± 0.9 (a)	66.2 ± 1.3 (a)	929.1 ± 1.9 (a)	99.5
GWS+N		14.5 ± 1.0 (b)†	58.7 ± 1.4 (a)	73.2 ± 1.1 (a)	923.5 ± 1.5 (a)	99.7
MSW+N		13.2 ± 0.5 (ab)	64.7 ± 3.6 (a)†	77.9 ± 3.8 (a)†	916.0 ± 2.8 (a)	99.6
BIOW+N		14.1 ± 1.3 (ab)†	57.1 ± 7.3 (a)	71.2 ± 8.6 (a)	917.9 ± 9.8 (a)	98.9
FYM+N		12.7 ± 0.5 (ab)	58.9 ± 1.6 (a)	71.6 ± 1.1 (a)	922.0 ± 4.1 (a)	99.4
		(P = 0.025)	*(P = 0.109)*	*(P = 0.088)*	*(P = 0.138)*	
C content in fractions: g C kg^{-1} fraction						
Ref+N		170.0 ± 4.1 (a)	10.6 ± 11.0 (a)		8.52 ± 0.31 (a)	
GWS+N		229.6 ± 6.0 (b)	11.1 ± 4.9 (a)		9.44 ± 0.42 (a)	
MSW+N		193.1 ± 5.9 (ab)	10.4 ± 6.8 (a)		8.97 ± 0.21 (a)	
BIOW+N		206.8 ± 9.8 (ab)	18.3 ± 5.0 (a)		9.56 ± 0.50 (a)	
FYM+N		201.2 ± 4.7 (ab)	12.7 ± 3.6 (a)		9.49 ± 0.40 (a)	
		(P = 0.017)	*(P = 0.468)*		*(P = 0.045)*	
C content: g C kg^{-1} bulk soil						
Ref+N	9.81 ± 0.92 (a)	1.80 ± 0.11 (a)	0.59 ± 0.63 (a)	2.39 ± 0.63 (a)	7.92 ± 0.28 (a)	105.1
GWS+N	12.99 ± 1.08 (a)†	3.32 ± 0.22 (b)†	0.65 ± 0.27 (a)	3.97 ± 0.37 (a)	8.72 ± 0.39 (a)	97.7
MSW+N	11.15 ± 1.14 (a)	2.55 ± 0.01 (ab)	0.66 ± 0.39 (a)	3.21 ± 0.39 (a)	8.24 ± 0.22 (a)	102.8
BIOW+N	12.47 ± 0.62 (a)	2.92 ± 0.34 (ab)†	1.04 ± 0.26 (a)	3.95 ± 0.52 (a)	8.78 ± 0.43 (a)	102.1
FYM+N	12.90 ± 0.00 (a)†	2.55 ± 0.04 (ab)	0.74 ± 0.19 (a)	3.29 ± 0.23 (a)	8.75 ± 0.36 (a)	93.4
	(P = 0.048)	*(P = 0.013)*	*(P = 0.541)*	*(P = 0.068)*	*(P = 0.070)*	
Distribution of total C within the fractions: %						
Ref+N		17.5 ± 1.9	5.5 ± 5.5	23.0 ± 4.4	77.0 ± 4.4	
GWS+N		26.2 ± 0.6	5.1 ± 2.0	31.3 ± 1.7	68.7 ± 1.7	
MSW+N		22.3 ± 1.3	5.7 ± 3.1	28.0 ± 2.2	72.0 ± 2.2	
BIOW+N		22.9 ± 1.2	8.1 ± 1.9	31.0 ± 2.2	69.0 ± 2.5	
FYM+N		21.2 ± 1.2	6.2 ± 1.9	27.3 ± 2.5	72.7 ± 2.5	

† Significant difference with the reference treatment (Dunn’s multiple comparison with a reference group, see 4.3.6.)

The C in the coarse POM fraction (>50 µm) is preferentially affected by higher C inputs in the time scale of decades (Gerzabek et al., 2001; Kong et al., 2005; Sleutel et al., 2006). This fraction was clearly affected after 8 years of EOM application. The lowest proportion of C contained in the fraction >50 µm was found in the reference treatment (23.0%) and the largest in the GWS+N and BIOW+N treatments (31.3% and 31.0% for GWS+N and BIOW+N, respectively) that accumulated the highest amounts of C in soil amongst all the treatments.

Calculating the distribution of accumulated C within the fractions, we found that 73.6%, 67.5% and 65.0% of the TOC accumulated in the MSW+N, GWS+N and BIOW+N treatments, respectively were in the coarse fractions >50 µm and inversely 26.4%, 32.5% and 35.0% in the 0-50 µm fraction. In the FYM amended plots, the C was accumulated in almost similar proportions in the SOM fractions of size >50 µm and 0-50 µm (53.1% and 46.9%, respectively). Zhou et al. (2010) suggested that the C accumulation in POM fractions after manure application could be due to higher recalcitrance of POM.. The higher incorporation of OM in the fine 0-50 µm fraction with FYM application could be due to both the stimulation of microbial activity following farmyard manure application as reported by several authors (Angers & N'Dayegamiye, 1991; Houot & Chaussod, 1995; Haynes & Naidu, 1998) and to the direct incorporation of a stable fraction of FYM into SOM as indicated by the fairly high level of stability of the FYM (high I_{ROC} and C_{91d} values, Table 4.1).

4.4.4. Simulation of C stock evolutions using RothC

The RothC model successfully reproduced the C accumulation patterns in soil following successive EOM applications after fitting the partition coefficients of EOM-TOC between the DPM, RPM and HUM pools of the model (Table 4.4). The RMSE between measured and simulated C stocks varied between 0.69 and 1.61 Mg C ha^{-1} and the CV(RMSE) between 13.3 and 17.0%. The proportions of RPM pools decreased in the order BIOW>GWS>FYM>MSW. For BIOW, GWS and FYM the maximum allowed proportions of HUM (20%) were reached. The distribution for FYM fitted on the Qualiagro experiment differed from that proposed in the RothC model that was previously fitted on results of the Rothamsted experiments (49% DPM, 49% RPM, 2% HUM, Coleman and Jenkinson, 1999). As mentioned previously, the farmyard manures applied in the Qualiagro experiment were probably more stabilised against biodegradation than those applied in the Rothamsted experiment. The MSW compost had a contrasted distribution within the RothC entry pools compared to the other EOMs, with 62.3% of total organic C in the DPM pool and 37.7% in the RPM pool.

Table 4.4. Fitting of the partition coefficient of EOM total organic C (TOC) in the RothC entry pools (f_{DPM}, f_{RPM} and f_{HUM}) and quality of the simulations of C accumulation dynamics after fitting. RMSE: root mean square error between measured and simulated C stocks, r: coefficient of correlation, CV(RMSE): coefficient of variation of the RMSE calculated as RMSE / mean of measured C stocks * 100, EF: model efficiency. Results for the treatments with optimum and minimum N fertilisation (+/- N) with application of green waste and sludge compost (GWS +/-N), municipal solid waste compost (MSW +/-N), biowaste compost (BIOW +/-N) and farmyard manure (FYM +/-N).

	DPM	RPM	HUM	RMSE	CV(RMSE)	r	EF
	-------------------------	% TOC	-------------------------	Mg C ha^{-1}	%		%
GWS +/- N	15.3	64.7	20.0	1.30	13.3	0.98	0.95
MSW +/- N	62.3	37.7	0.0	0.69	13.9	0.97	0.92
BIOW +/- N	0.0	80.0	20.0	1.61	17.0	0.97	0.92
FYM +/- N	30.1	49.9	20.0	1.24	13.3	0.98	0.93

The Figure 4.2 shows the RothC simulations of the kinetics of C accumulation under the different EOM treatments. The simulations showed a rapid increase of C accumulated in the RPM pool that tended to reach equilibrium after approximately ten years of application. The HUM pool also regularly increased during simulation. A small proportion of total organic C was present in the BIO pool compared to the RPM and HUM pools and rapidly reached equilibrium (0.5 to 0.7 Mg C ha^{-1}). The C contained in the DPM transitory pool was degraded within few months after EOM applications. A larger proportion of C accumulated in the BIO pool was measured in the plots amended with the MSW compost compared to the other organic treatments FYM, GWS and BIOW (7.6, 3.9, 3.5 and 3.2%, respectively in September 2009 in the plots with optimum N fertilisation). The MSW compost more enhanced soil microbial activity than other EOMs due to its higher biodegradability, as reflected by its high proportion of C mineralized after 3, 7 and 91 days of incubation (C_{3d}, C_{7d} and C_{91d}) and its low I_{ROC} value (Table 4.1). The proportions of C accumulated in the HUM pools increased in the order MSW<BIOW<GWS (36, 41 and 46%, respectively in September 2009 in the plots with optimum N fertilisation). The HUM pool were directly fed by the HUM fraction of the GWS and BIOW composts whereas the application of MSW compost increased the HUM pool through a higher stimulation of soil microbial activity due to its higher biodegradability. The application of FYM led to a slightly higher proportion of C accumulated in the HUM pool (52% in September 2009 in the part with optimum N fertilisation) resulting both from the incorporation of humified OM of FYM (f_{HUM} = 20%, Table 4.4) and a stimulation of biological activity by a labile fraction of FYM (f_{DPM} = 30%).

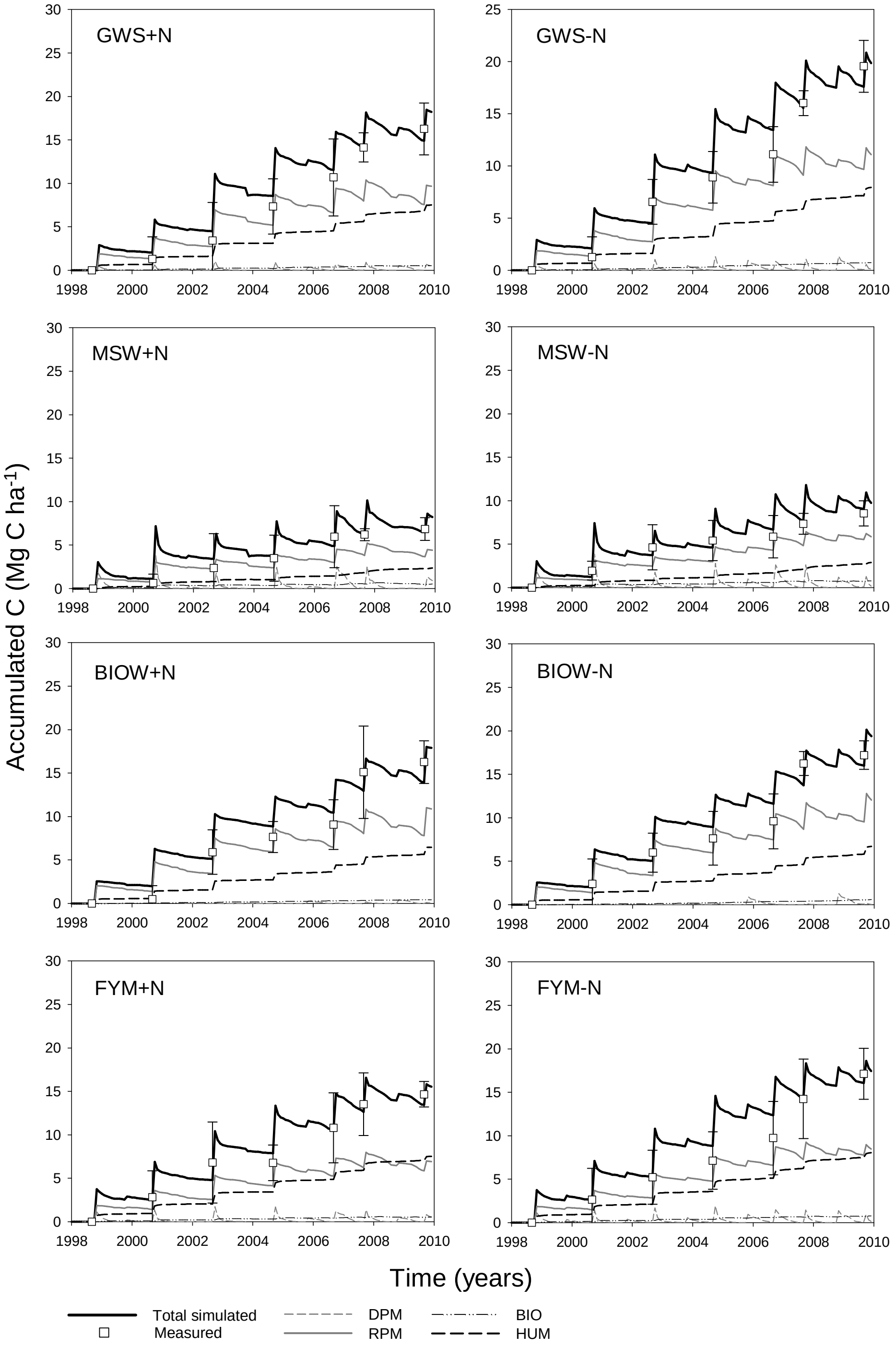

Figure 4.2. Simulation of C accumulation kinetics with RothC after fitting of the partition coefficients (f_{DPM}, f_{RPM} and f_{HUM}) in both parts of the experiment with optimum and minimum N fertilisation (+N and –N, respectively). Measured accumulated C compared to reference plots, total accumulated C simulated and distribution of total simulated accumulated C in RothC pools: DPM (decomposable), RPM (resistant), BIO (microbial biomass) and HUM (humified). Results given for the organic treatments: GWS: green waste and sludge compost, MSW: municipal solid waste compost, BIOW: biowaste compost, FYM: farmyard manure.

4.4.5. Extrapolation of RothC simulation

The RothC simulations were extrapolated to calculate yield and annual rates of accumulated C after 20 years of application (Table 4.5). The extrapolation showed that a lower proportion of the total additional C inputs following EOM applications compared to reference treatment was accumulated in soil after 20 years than after 11 years (Table 4.2). Gerzabek et al. (1997) found a yield of C accumulation equivalent to 27.3% of a total of 70 Mg C ha^{-1} applied with farmyard manure after 37 years of experiment in the Ultuna long-term experiment in Sweden. In our case, RothC extrapolation until reaching similar amounts of added FYM (72 Mg C ha^{-1} applied after 36 years) gave a similar yield of C accumulation of 27.4% although climatic conditions were milder in Île-de-France than in Sweden (mean annual temperature of 5.5℃ in Ultuna, Sweden compared to 11.0℃ in the Qualiagro experiment). This was probably due to the low biodegradability of the FYM used in our experiment.

Table 4.5. Cumulated additional C inputs via EOM applications and increased crop residues compared to reference plots after 20 years of cultivation (1998-2018) ($C_{EOM\text{-}20y}$, $\Delta C_{res\text{-}20y}$, respectively). Accumulated C in soil compared to reference plots extrapolated with RothC after 20 years of cultivation ($\Delta C_{20y\text{-}sim}$). Yields of C accumulation in soil extrapolated with RothC after 20 years of cultivation ($Y_{20y\text{-}sim}$). Annual rates of C accumulation calculated over 20 years with extrapolated values. Results are given for the treatments with application of green waste and sludge compost (GWS), municipal solid waste compost (MSW), biowaste compost (BIOW) and farmyard manure (FYM) and for the part of the experiment with optimum and minimum N fertilisation (+N and –N, respectively).

Treatment	Total additional C from crop residues 1998-2018	Total accumulated C in soil extrapolated in 2018	Yield of C accumulation after 20 years	Mean C accumulation rate over 20 years	accumulated C released 10 years after interrupting EOM applications
	$\Delta C_{res\text{-}20y}$ Mg C ha^{-1}	$\Delta C_{20y\text{-}sim}$ Mg C ha^{-1}	Y_{20y_sim} % total additional C inputs	$\Delta C_{20y\text{-}sim}/20$ Mg C ha^{-1} yr^{-1}	% of accumulated C
GWS+N	4.0	17.1	38.8	0.85	25.8
GWS-N	18.3	20.4	35.0	1.02	27.8
MSW+N	1.5	8.0	19.3	0.40	32.6
MSW-N	15.4	11.3	20.5	0.57	34.5
BIOW+N	4.4	18.4	41.4	0.92	29.0
BIOW-N	12.3	20.4	39.1	1.02	30.2
FYM+N	5.9	16.3	35.4	0.81	23.2
FYM-N	17.8	19.2	33.1	0.96	25.0

We compared the C accumulation rates (in Mg C ha^{-1} yr^{-1}) resulting from the EOM applications with these obtained with other agricultural practices leading to C sequestration in soil. Because the C doses applied in the Qualiagro experiment were approximately two times the doses usually applied in practice, we divided by two the accumulation rates presented in Table 4.5 to compare the values with those given in the literature. We obtained in average for the +N and -N parts of the experiment: 0.24, 0.44, 0.47 and 0.49 Mg C ha^{-1} yr^{-1} for MSW, FYM GWS and BIOW applications, respectively for 20 Mg C ha^{-1} of EOMs applied, 0.8 to 3.0 Mg C ha^{-1} applied via additional crop residues in the +N part for MSW and FYM applications, respectively and 6.1 to 9.2 Mg C ha^{-1} in the -N part for BIOW and GWS applications, respectively. Those rates fell into the range given by IPCC (2000) based on data of Smith et al (1997a) of 0.2-1.0 Mg C ha^{-1} yr^{-1} for organic amendments (biosolids, manure and straw). In comparison, conservation tillage could lead to SOC accumulation at the rate of 0.34 Mg C $ha^{-1}.yr^{-1}$ as estimated by Smith et al. (1998) or at the rate of 0.20 Mg C ha^{-1} yr^{-1} as estimated by Arrouays et al. (2002). Green manure cultivated between two harvested crops could lead to SOC accumulation at the rate of 0.16 Mg C ha^{-1} yr^{-1} (Arrouays *et al.*, 2002) and ley-arable farming at the rate 0.54 Mg C ha^{-1} yr^{-1} (Smith *et al.*, 1997a).

The evolution of C stocks after interruption of EOM application was simulated with RothC for the period 2018-2028 (Table 4.5, Figure 4.3). This scenario showed that the release of accumulated C through mineralisation increased in the order FYM<GWS<BIOW<MSW with 24, 27, 30 and 34% of the accumulated C released as CO_2 10 years after stopping EOM applications, respectively (in average of the +N and -N part). The intensity of C release was related to the proportion of accumulated C in the RPM and BIO pools, rapidly degraded whereas the C contained in the HUM pool still increased of 5 to 10% compared to 2018 due to the C input related to the degradation of the others pools.

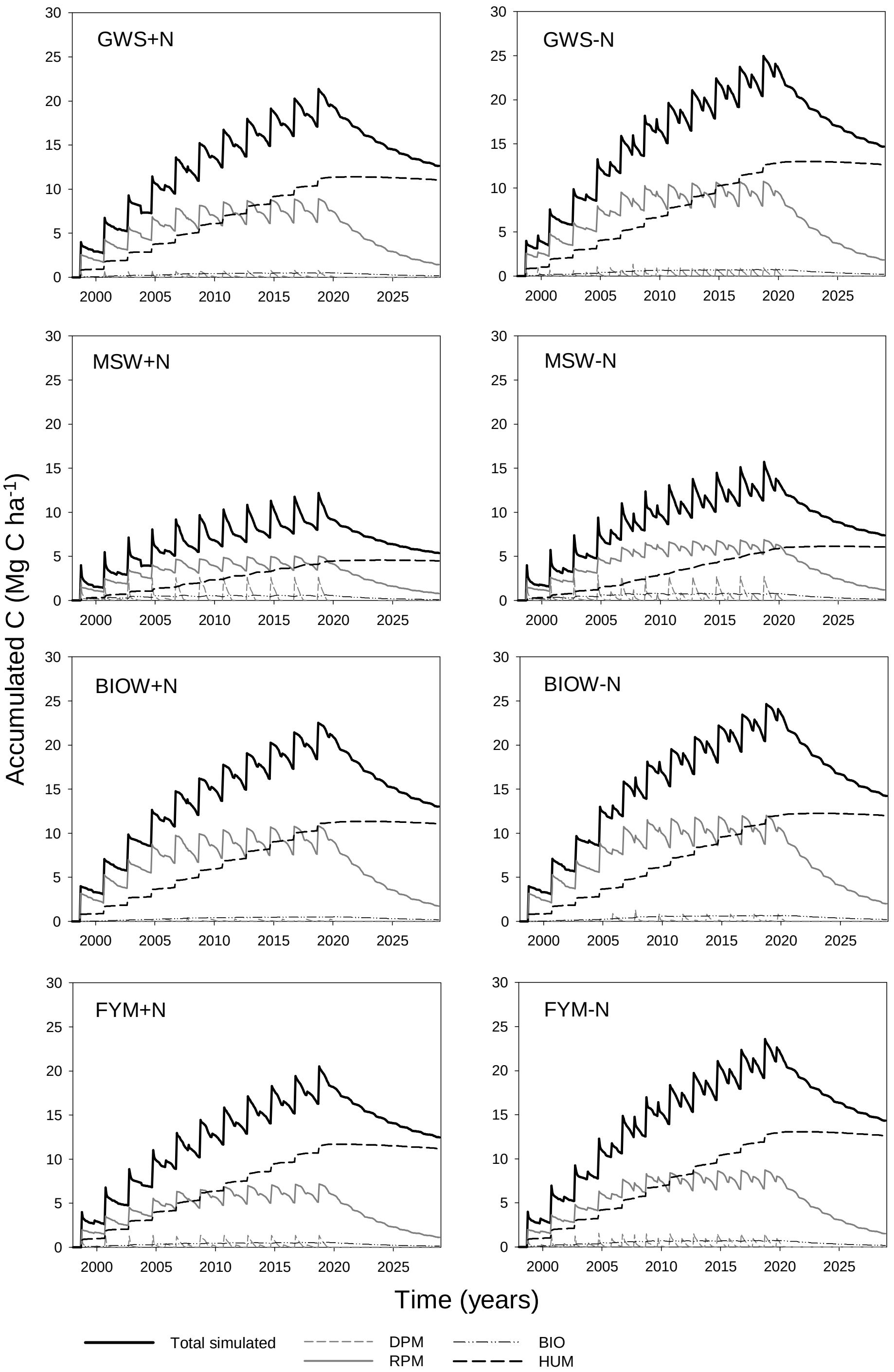

Figure 4.3. Scenario of EOMs application using the partition coefficients of EOMs in RothC entry pools previously fitted to measured values in field extrapolated to 20 years (2018) followed by interruption of the applications from 2018 to 2028. Total accumulated C simulated and distribution of total simulated accumulated C in RothC pools: DPM (decomposable), RPM (resistant), BIO (microbial biomass) and HUM (humified). Results given for the organic treatments: GWS: green waste and sludge compost, MSW: municipal solid waste compost, BIOW: biowaste compost, FYM: farmyard manure for the parts of the experiment with optimum and minimum N fertilisation (+N and -N, respectively).

4.4.6. Comparison of particle size fractions and model pools

We studied the relationships between additional C compared to reference plots in the size fractions of SOM and in the model pools. Other studies previously reported relationships between RothC pools and size and density fractions of SOM (Balesdent, 1996; Ludwig *et al.*, 2003; Zimmermann *et al.*, 2007). The amounts of C accumulated in the model pools were previously converted from Mg C ha^{-1} to g C kg^{-1} soil taking into account and subtracting the proportion of accumulated C in the subsoil layer accounting for 14, 21, 16 and 14% of the accumulated C in the GWS+N, MSW+N, BIOW+N and FYM+N treatments, respectively then by dividing by the mass of soil per ha in the 0-29 cm soil layer for each treatment.

Good relationships were found between the amounts of additional C in the SOC fraction >50 µm (light POM+heavy fraction >50 µm) and the DPM+RPM pools of RothC and between the 0-50 µm fraction and the BIO+HUM pools for the GWS+N, MSW+N and BIOW+N treatments (Figure 4.4, a). The accumulated C were also distributed in similar proportions in the size fractions and in the model pools for the GWS+N, MSW+N and BIOW+N treatments (Figure 4.4, b), being comprised into the standard errors of the repartition of the size fractions.

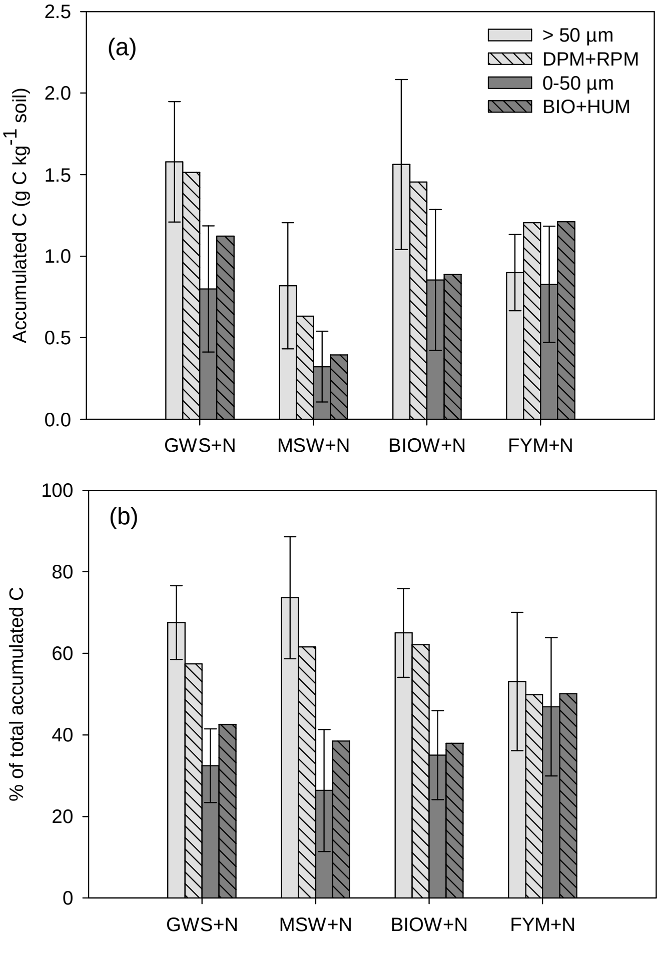

Figure 4.4. Comparison between the distribution of the accumulated C following EOM application in particle size fractions and in RothC pools expressed as additional soil C content compared to reference plots (in g C kg^{-1} soil) (a), and as proportions of the total accumulated C (b).

For the FYM+N treatment, the total amount of accumulated C in 2006 given by the model was much higher than the total C amount recovered with size fractionation (2.4 and 1.7 g C kg^{-1} soil, respectively. Nevertheless when compared as proportions of C accumulated (Figure 4.4, b), the RothC pools and the size fractions for the FYM+N treatment were also similar.

The compost applications preferentially increased the C amounts in the fraction of SOM >50 µm whereas the FYM increased in similar proportions the C in SOM of size >50 µm and 0-50 µm, corresponding to the DPM+RPM and the BIO+HUM pools, respectively. It might be explained by the composition of FYM that both contains a pool of labile OM (f_{DPM}=30%, Table 4.4) that stimulates microbial activity and thus lead to the alimentation of the humified fraction of SOM with degradation by-products, and a pool of humified OM (f_{HUM}=20%) alimenting directly the humified fraction of SOM.

4.5. Conclusions

Repeated applications of EOMs in the Qualiagro experiment from 1998 to 2009 led to significant C accumulation in soil (up to 19.6 Mg C ha^{-1} compared to the reference treatment in the GWS amended plots). The C accumulation patterns were well reproduced by the RothC model after fitting the EOM partition coefficients into the entry pools (f_{DPM}, f_{RPM}, f_{HUM}). The BIOW and GWS composts were the most efficient at increasing C stocks (64 and 58% of the additional C inputs accumulated in soil after 11 years, respectively) followed by the FYM and the MSW compost (50 and 28% of additional C inputs accumulated) which was related to the lower stability of EOM-TOC as measured in the laboratory. However a simulation with RothC of C release after interruption of EOM applications predicted that the C from the FYM would be released less rapidly than C accumulated with the others EOMs due to fact that it accumulated higher proportions of C in the stabilised fraction of SOM than the composts, both through the stimulation of microbial activity and through the direct incorporation of a humified fraction of FYM into SOM.

The C concentrations of the POM >50 µm fraction and the C amount contained in this fraction were significantly increased by the EOM applications. The C contained in the 0-50 µm fraction tented to increase after EOM applications in a lesser extent. Compost applications preferentially increased the C contained in SOM >50 µm whereas FYM applications increased in similar proportions the C contained in SOM fraction of size >50 µm and 0-50 µm. The C accumulated in the particles size fraction >50 µm and 0-50 µm corresponded well to the C accumulated the DPM+RPM and BIO+HUM pools of RothC respectively.

The extrapolation of the RothC simulation predicted yields of C accumulation after 20 years of application of 22, 34, 37 and 40% of the cumulated inputs for MSW, FYM, GWS and BIOW. The levels of C accumulated were fairly large compared with values reported in the literature for other practices leading to C sequestration in soil like reduce tillage or green manure crops. The application of compost and farmyard manure seems to be efficient to store C in soil but special attention must be paid to the composition of the C accumulated which determines the reversibility of C storage.

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4.7. Supporting Information

Table S1. C contents in the topsoil (in g C kg^{-1} soil) measured in September before each EOM application. Results given for the organic treatments: GWS: green waste and sludge compost, MSW: municipal solid waste compost, BIOW: biowaste compost, FYM: farmyard manure and for the reference treatment: Ref, for the parts of the experiment with optimum and minimum N fertilisation (+N and -N, respectively). Mean ± standard deviation, n = 4 field replicates.

Treatment	C content in the topsoil (0-29 cm) g C kg^{-1} soil						
	1998	2000	2002	2004	2006	2007	2009
GWS+N	10.5 ± 1.0	11.1 ± 0.7	11.2 ± 1.2	12.1 ± 0.8	12.6 ± 1.2	13.8 ± 0.4	14.6 ± 0.8
MSW+N	10.3 ± 0.7	10.9 ± 0.3	11.0 ± 1.1	11.2 ± 0.7	11.4 ± 0.9	12.0 ± 0.2	12.3 ± 0.3
BIOW+N	10.7 ± 0.7	10.8 ± 0.4	11.9 ± 0.7	12.2 ± 0.5	12.2 ± 0.8	14.0 ± 1.4	14.6 ± 0.6
FYM+N	10.5 ± 0.3	11.5 ± 0.8	12.1 ± 1.2	12.0 ± 0.5	12.6 ± 1.1	14.3 ± 0.9	14.2 ± 0.4
Ref+N	10.6 ± 0.2	10.7 ± 0.3	10.4 ± 0.6	10.3 ± 0.2	9.9 ± 0.8	10.5 ± 0.3	10.5 ± 0.3
GWS-N	10.2 ± 0.7	10.6 ± 0.5	11.2 ± 0.6	11.8 ± 0.6	11.9 ± 0.7	13.1 ± 0.3	14.4 ± 0.6
MSW-N	10.2 ± 0.7	10.8 ± 0.3	10.7 ± 0.7	11.0 ± 0.6	10.6 ± 0.6	11.4 ± 0.3	11.7 ± 0.4
BIOW-N	10.3 ± 0.7	10.9 ± 0.8	11.1 ± 0.6	11.5 ± 0.8	11.5 ± 0.8	13.6 ± 0.4	13.8 ± 0.4
FYM-N	10.4 ± 1.0	10.9 ± 1.0	10.8 ± 0.8	11.3 ± 0.9	11.5 ± 1.1	13.6 ± 1.2	13.8 ± 0.8
Ref-N	10.2 ± 0.7	10.3 ± 0.5	9.6 ± 0.5	9.6 ± 0.4	9.1 ± 0.7	9.6 ± 0.7	9.5 ± 0.6

Table S2. Bulk density of the topsoil (0 – 29 cm) measured in 1998, 2004 and 2009 and interpolated between 1998 and 2004 and between 2004 and 2009. Results given for the organic treatments: GWS: green waste and sludge compost, MSW: municipal solid waste compost, BIOW: biowaste compost, FYM: farmyard manure and for the reference treatment: Ref, for the parts of the experiment with optimum and minimum N fertilisation (+N and -N, respectively)

Treatment	Bulk density of the topsoil in Mg m^{-3}						
	1998	2000	2002	2004	2006	2007	2009
GWS+N	1.26	1.27	1.29	1.30	1.31	1.31	1.32
MSW+N	1.26	1.27	1.29	1.30	1.31	1.31	1.32
BIOW+N	1.26	1.27	1.29	1.30	1.31	1.31	1.32
FYM+N	1.26	1.27	1.29	1.30	1.31	1.31	1.32
Ref+N	1.26	1.28	1.30	1.32	1.35	1.36	1.39
GWS-N	1.30	1.31	1.32	1.33	1.33	1.33	1.34
MSW-N	1.30	1.31	1.32	1.33	1.33	1.33	1.34
BIOW-N	1.30	1.31	1.32	1.33	1.33	1.33	1.34
FYM-N	1.30	1.31	1.32	1.33	1.33	1.33	1.34
Ref-N	1.30	1.32	1.34	1.36	1.38	1.39	1.41

Table S3. C stocks in the topsoil layers (0 – 29 cm) in Mg C ha^{-1} and C amounts from the subsoil layers added to the C stocks of the topsoil to reach equivalent soil masses corresponding to the mass of topsoil in 2009 reference plots (4029 and 4095 Mg ha^{-1} for the part with optimum and minimum N fertilisation: +N and –N, respectively). Results given for the organic treatments: GWS: green waste and sludge compost, MSW: municipal solid waste compost, BIOW: bio-waste compost, FYM: farmyard manure and for the reference treatment: Ref for the +N and -N parts of the experiment. Mean ± standard deviation, n = 4 field replicates.

Treatment	1998	2000	2002	2004	2006	2007	2009
	-------------------------------C stocks in the topsoil (0 – 29 cm) in Mg C ha^{-1}--						
GWS+N	38.3 ± 3.8	40.8 ± 2.5	41.9 ± 4.4	45.7 ± 3.2	47.8 ± 4.4	53.4 ± 1.7	56.0 ± 3.0
MSW+N	37.5 ± 2.4	40.2 ± 1.0	41.0 ± 3.9	42.1 ± 2.6	32.5 ± 3.6	45.8 ± 0.7	47.0 ± 1.3
BIOW+N	38.9 ± 2.4	39.9 ± 1.6	44.3 ± 2.6	46.0 ± 1.8	46.3 ± 2.9	54.3 ± 5.3	56.0 ± 2.4
FYM+N	38.4 ± 1.0	42.3 ± 3.0	45.2 ± 4.7	45.1 ± 2.0	47.9 ± 4.0	52.7 ± 3.6	54.5 ± 1.5
Ref+N	38.6 ± 0.8	39.8 ± 1.0	39.1 ± 2.1	39.2 ± 0.8	38.6 ± 3.0	41.2 ± 1.2	42.3 ± 1.1
GWS-N	38.4 ± 2.5	40.1 ± 1.9	42.9 ± 2.1	45.5 ± 2.5	45.8 ± 2.7	52.6 ± 1.2	55.7 ± 2.5
MSW-N	38.4 ± 2.8	40.9 ± 1.1	41.1 ± 2.6	42.1 ± 2.3	40.9 ± 2.4	44.3 ± 1.2	45.2 ± 1.4
BIOW-N	38.8 ± 2.7	41.3 ± 2.9	42.3 ± 2.2	44.1 ± 3.1	44.4 ± 3.2	52.7 ± 1.4	53.4 ± 1.7
FYM-N	39.3 ± 3.9	41.4 ± 3.6	41.4 ± 3.1	43.6 ± 3.3	44.5 ± 4.2	50.7 ± 4.6	53.3 ± 2.9
Ref-N	38.6 ± 2.8	39.2 ± 1.9	37.1 ± 2.0	37.6 ± 1.4	36.4 ± 2.7	38.7 ± 2.8	38.9 ± 2.3
	----------C amount from subsoil added to the topsoil to attain the equivalent soil mass in Mg C ha^{-1}---------						
GWS+N	3.6	3.4	3.2	2.9	2.7	2.8	2.6
MSW+N	3.6	3.3	2.9	2.6	2.4	2.4	2.2
BIOW+N	3.6	3.4	3.2	2.9	2.6	2.8	2.6
FYM+N	3.6	3.4	3.2	2.9	2.7	2.9	2.5
Ref+N	3.6	3.0	2.5	2.0	1.2	0.8	0.0
GWS-N	3.0	2.9	2.7	2.5	2.6	2.7	2.8
MSW-N	3.0	2.8	2.6	2.4	2.3	2.4	2.3
BIOW-N	3.0	2.9	2.8	2.6	2.5	2.8	2.7
FYM-N	3.0	2.9	2.8	2.7	2.5	2.8	2.7
Ref-N	3.0	2.5	2.0	1.4	0.9	0.6	0.0

Table S4. Analytical characteristics of the EOMs applied between 1998 and 2007 in the Qualiagro field experiment: total organic C (TOC), total N (totN), TOC to totN ratio (C/N), total organic matter (TOM), soluble, hemicellulose-, cellulose-, and lignin- and cutin- like fractions (SOL, HEM, CEL, LIC), proportions of EOM TOC mineralized after 3, 7 and 91 days of laboratory incubation in soil at 28℃ (C_{3d}, C_{7d} and C_{91d}) and indicator of residual organic C in soil (I_{ROC}) calculated with equation [1]. % DM: % of dry matter at 105℃. GWS: green waste and sludge compost, MSW: municipal solid waste compost, BIOW: biowaste compost, FYM: farmyard manure.

Sample	TOC	totN	C/N	SOL	HEM	CEL	LIC	C_{3d}	C_{7d}	C_{91d}	I_{ROC}
	% DM	% DM		--------------% TOM----------------				% TOC	% TOC	% TOC	% TOC
GWS 98	27.23	2.83	9.63	48.80	5.77	13.80	31.70	0.99	1.92	6.41	86.04
GWS 2000	18.70	1.85	10.12	40.90	1.90	7.40	49.80	1.30	3.19	10.90	95.34
GWS 2002	34.93	2.23	15.69	33.77	4.97	30.80	30.50	0.97	1.53	7.45	74.35
GWS 2004	33.59	2.23	15.09	38.50	8.20	34.07	19.20	2.18	3.27	20.02	65.36
GWS 2006	24.47	2.50	9.79	55.47	5.96	20.03	18.53	3.34	4.47	12.13	73.51
GWS 2007	23.83	2.33	10.23	56.47	3.80	18.47	21.27	2.24	3.14	15.34	78.78
MSW 1998	30.37	2.02	15.04	50.80	6.13	29.40	13.70	6.43	17.01	32.78	58.81
MSW 2000	31.30	2.07	15.16	48.70	5.00	26.90	19.40	7.83	16.09	36.21	59.05
MSW 2002	27.74	1.60	17.29	46.80	3.77	34.53	14.90	10.16	18.51	41.77	48.06
MSW 2004	27.04	1.60	16.85	45.93	10.50	27.03	16.57	13.40	24.17	51.74	42.83
MSW 2006	39.00	1.53	25.49	31.85	6.85	47.87	13.42	12.33	27.27	51.40	31.89
MSW 2007	35.93	1.51	23.79	31.10	6.37	49.30	13.23	13.16	28.91	52.04	29.19
BIOW 1998	15.75	1.63	9.64	47.10	4.56	19.50	28.90	2.70	4.80	11.58	78.16
BIOW 2000	18.40	1.19	15.45	30.20	1.70	18.80	49.30	0.34	0.65	3.48	89.58
BIOW 2002	17.75	1.36	13.10	43.70	9.20	19.47	27.70	2.75	3.89	11.35	75.52
BIOW 2004	17.35	1.36	12.80	44.20	4.83	23.20	27.73	3.62	5.29	24.55	73.06
BIOW2006	19.60	1.55	12.65	52.96	3.05	17.63	26.36	4.09	4.48	15.69	76.50
BIOW 2007	22.87	2.25	10.16	48.53	1.60	23.10	26.77	2.67	3.66	8.69	76.75
FYM 1998	28.65	2.32	12.34	41.70	14.30	20.70	23.30	2.83	5.22	18.60	71.00
FYM 2000	41.30	1.84	22.45	29.30	12.60	35.60	22.50	3.10	6.66	20.66	60.65
FYM 2002	34.46	2.57	13.41	40.57	14.50	18.87	26.13	2.36	3.55	13.26	73.89
FYM 2004	33.08	2.57	12.87	36.07	8.37	34.10	21.43	3.03	6.74	21.74	63.75
FYM 2006	27.80	2.27	12.25	46.75	8.47	18.27	26.51	2.87	3.67	10.21	76.18
FYM 2007	27.47	2.08	13.21	40.23	13.83	25.50	20.43	2.58	4.26	14.90	67.88

Chapitre 5. Carbon accumulation in soil after repeated applications of different organic amendments evaluated by applying the RothC model to four long-term field experiments.

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5.1. Abstract

Multi-compartment soil carbon (C) models such as RothC are widely used for simulating changes in C stocks in arable soils. However, rigorous routines for establishing entry pools that can account for the broad diversity of exogenous organic matter (EOM) applied to croplands are still lacking. Data on C increases in stocks after repeated EOM applications were obtained from four long-term experiments (LTE): Askov K2 (Denmark, 31 yrs), Qualiagro (France, 11 yrs), SERAIL (France, 14 yrs) and Ultuna (Sweden, 52 yrs). The adjustment of the partition coefficients of total organic C in EOM (EOM-TOC) into the labile, resistant and humified entry pools of RothC (f_{DPM}, f_{RPM}, f_{HUM}, respectively) allowed to successfully reproduce the observed C accumulation kinetics in the LTEs. The fitted coefficients were related to characteristics of the EOM, including Van Soest biochemical fractions, EOM-TOC and the lability of the EOMs in short-term laboratory incubations. An indicator of EOM-TOC retained in soil (I_{ROC}), calculated from C in the soluble, lignin+cutin-like and cellulose-like Van Soest fractions, and the proportion of EOM-TOC mineralized during 3 days of incubation was used to predict the f_{DPM} and f_{RPM} coefficients estimated from field data simulations. Using the predicted partition coefficients for the different EOMs resulted in RothC simulations with only slightly higher errors compared to those using fitted partition coefficients. However, the partition coefficients could not be predicted for some EOMs causing a very high C accumulation in soil such as peat. According to the simulations, between 14 (green manure) and 59 % (compost) of the total EOM-TOC was retained in the soils after 20 years when simulations were adjusted to temperate climate conditions.

Keywords

Animal manure; organic amendments; crop residues; composts; C storage; analytical characterization; RothC model

Research highlights

- The RothC model successfully reproduced the kinetics of C accumulation in soil after exogenous organic matter (EOM) applications during four long-term field experiments after adjustment of the EOM partition coefficients in the entry pools of RothC.
- The partition coefficients of EOMs in RothC could be predicted from laboratory characterizations of EOMs.
- C accumulation in soil following EOM applications was correctly predicted using partition coefficients of EOMs predicted from laboratory characterizations.

5.2. Introduction

In most soils, declining soil organic C (SOC) content leads to reduced soil fertility, less biological activity and greater susceptibility to erosion (Matson *et al.*, 1997; Ciais *et al.*, 2010), and SOC decrease has been identified as a major threat to the quality of the soil resource (European Commission, 2006). In addition, soils account for one of the largest terrestrial C reservoirs and small but consistent increases in SOC stocks mitigate climate change effects by storing atmospheric CO_2-C in soil organic matter (Lal *et al.*, 2007).

Annual application of exogenous organic matter (EOM) to cultivated land may lead to long continued accumulation of SOC (Marmo *et al.*, 2004). In this study, EOM is defined as plant residues, animal manures, and organic wastes from urban areas, forestry and industry as these materials are subject to similar transformations in soil and similar management methods (Marmo *et al.*, 2004). In Europe, recycling of organic biodegradable wastes is encouraged and is expected to increase in the future (European Commission, 2010).Various EOMs differ in their contribution to stable SOC, depending on their origin and degree of transformation before being added to soil (Christensen and Johnston, 1997; Gerzabek *et al.*, 1997; Bipfubusa *et al.*, 2008). Since C stocks change slowly, long-term field experiments are needed to evaluate the effects of repeated applications of EOM (IPCC, 1997).

Multi-compartment models of C turnover in soil (Jenkinson and Rayner, 1977; Parton *et al.*, 1987; Andren and Kätterer, 1997; Bruun *et al.*, 2003) accurately simulate SOC dynamics in long-term field experiments under different climatic conditions and soil types (Smith *et al.*, 1997b). RothC is one of the most widely used models that simulates SOC dynamic based on relatively few parameters and input data. In RothC, total organic C in EOM (EOM-TOC) is distributed into pools of labile (DPM), resistant (RPM) and humified (HUM) organic matter according to partition coefficients (f_{DPM}, f_{RPM} and f_{HUM}, respectively). The RothC model was initially calibrated with results from several Rothamsted long-term experiments to establish partition coefficients for crop residues (f_{DPM} = 59% and f_{RPM} = 41% of EOM-TOC) and farmyard manure (f_{DPM} = 49%, f_{RPM} = 49% and f_{HUM} = 2% of EOM-TOC) (Coleman and Jenkinson, 1999). However, EOMs applied on cultivated land exhibit contrasting chemical composition and decomposability in soil (Thuriès *et al.*, 2002; Lashermes *et al.*, 2009). Using RothC to simulate long-term changes in SOC after successive applications of EOM requires a methodological approach that allows relevant partition coefficients to be established.

Some SOC turnover models propose methods to determine the EOM quality parameters from chemical characteristics. In the CENTURY model, EOM-TOC is split into two entry pools based on the lignin:N ratio (Parton *et al.*, 1987), while with the STICS model the behaviour of plant residues is based on their C:N ratio (Nicolardot *et al.*, 2001). However, these approaches have been found to be inadequate to characterize EOMs such as composts and sludge (G. Lashermes, pers. comm.). The stabilized form of N in composts violates the biological meaning of the C:N ratio (Paré *et al.*, 1998). Moreover, the lignin content may not be a valid approximation of the recalcitrant fraction of composted

EOM because lignin is partly degraded during the composting process while other secondary and biologically recalcitrant compounds are recovered in the soluble fraction (Morvan and Nicolardot, 2009; Peltre *et al.*, 2010). Similarly, Hyvönen et al. (1996) argued that the lignin in EOM was not a valid indicator of EOM quality in a model of continuous quality distribution of soil organic matter.

Lashermes et al. (2009) recently presented an indicator of the fraction of EOM-TOC that potentially remains in soil in the long term (I_{ROC}). The I_{ROC} indicator is calculated from analytical characteristics of the EOM and has been calibrated against long-term C mineralization kinetics in laboratory incubations for a wide range of EOMs including plants materials, animal manures and various types of fresh or composted wastes. We surmise that this indicator can be used to estimate the partition of EOM-TOC in the RothC model.

Our objectives were (i) to isolate the partition coefficients of the RothC entry pools for contrasting EOMs, using data from long-term field experiments and assuming that the decay rate for a given pool is the same regardless of EOM, (ii) to estimate these partition coefficients using easily available analytical EOM characteristics, and (iii) to simulate the C storage in soil amended with EOMs of contrasting origin and biological stability.

5.3. Materials and methods

5.3.1. Field experiments

Data was assembled from four differently sited long-term field experiments (LTE) where various types of EOMs had been applied regularly and covering different soil types, climatic conditions and management practices. The LTEs included the Askov K2 (Christensen and Johnston, 1997), Qualiagro (Houot *et al.*, 2002), SERAIL (Berry *et al.*, 2008) and Ultuna (Gerzabek *et al.*, 1997). Table 5.1 summarizes selected characteristics of the LTEs.

Table 5.1. Principal characteristics of the field experiments.

	Askov K2 experiment	Qualiagro experiment	SERAIL experiment	Ultuna experiment
Location	Askov, Denmark	Feucherolles, France	Brindas, France	Uppsala, Sweden
Duration	30 yrs	11 yrs	14 yrs	52 yrs
Clay content (%)	2.5	15.2	16.8	37.0
Initial C content (%)	0.3	1.0	1.2	1.5
Mean annual temperature (℃)	7.4 ± 0.7	11.0 ± 0.5	12.9 ± 0.5	5.7 ± 1.0
Mean annual rainfall (mm)	972 ± 160	643 ± 128	822 ± 118	541 ± 93
Crop succession	Spring barley, fiber flax, winter cereals, silage maize	Wheat, maize	Vegetables rotation	62% spring cereals, 15% silage maize, 33% rape and other crops
EOMs applied	Mature straw, sawdust, farmyard manure, peat	Municipal solid waste compost, farmyard manure, green waste and sludge compost, biowaste compost	Dehydrated farmyard manure, fresh farmyard manure, enriched bark compost, enriched compost with coffee cake	Straw, green manure, sawdust, farmyard manure, sewage sludge, peat

5.3.1.1. The Askov K2 experiment

The Askov K2 experiment was situated at Askov Experimental Station, Denmark (55°28' N, 9°55' E). The experiment was initiated in 1956 and terminated in 1986. The sand soil was retrieved from the 50 -100 cm soil depth and held 0.3 % organic C, 95 % sand, 2.5 % clay, 2.4 % silt when sampled and adopted as plough layer (0-25 cm) in large concrete cylinders (diam. 0.986 m; area 0.76 m^2; depth 0.50 m). Mean annual temperature and precipitation was 7.4 ± 0.7 ℃ and 972 ± 160 mm, respectively, during the experimental period. The experiment grew a four-course rotation of spring barley, fibre flax, winter cereals (wheat and rye), and silage maize. Four different EOMs of known C content were applied at an annual rate of 6.5 Mg ha^{-1}: physiologically mature cereal straw (**STR-Ask)**, sawdust (**SAW-Ask)**, dry farmyard manure (**FYM-Ask)** and sphagnum peat (**PEA-Ask)**. Each treatment was in two replicates and reference plots without EOM amendment were included. All plots received additional mineral N fertilization. At harvest, all above ground plant parts were removed. Soils were sampled from 0-25 cm soil depth every four years and analysed for C content. The depth of the soil layer amended with EOM (0-25 cm) was deliniated by a coarse-meshed net to ensure a constant amount of soil into which the EOM was incorporated and from which soil samples were taken. A soil bulk density of 1.5 Mg m^{-3} was adopted (Bruun et al., 2003). Further details of the experiment are given by Christensen and Johnston (1997).

5.3.1.2. The Qualiagro experiment

The ongoing Qualiagro experiment, located in Feucherolles (Île-de-France) approximately 50 km west of Paris (48°52’ N, 1°57’ E), was initiated in 1998 . In this study we extract data from the period 1998-2009. The soil is a Luvisol (FAO) with 78.7 % silt, 15.2 % clay and 6.2 % sand in the plough layer (0-29 cm). The initial C content was 1.0%. Mean annual temperature was 11.0 ± 0.5°C and precipitation 643 ± 128 mm during 1998-2009. The soil is under a wheat/maize rotation. Four types of EOMs are applied to separate plots (10 x 45 m^{-2}): municipal solid waste obtained from composting of residual solid wastes after removing dry and clean packaging (**MSW-Qua**), farmyard manure (**FYM-Qua)**, compost derived from co-composting green waste with sewage sludge (**GWS-Qua),** and a biowaste compost produced by co-composting green wastes with a source separated organic fraction of municipal solid wastes (**BIOW-Qua**). Each treatment plot is separated from adjacent plots by 6 m wide strips. The experiment is divided into two sections with separate N fertilization treatments: optimum and minimum mineral N fertilization (referred as **+N** and **-N**, respectively). The five treatments (four EOM amendments and a reference treatment without EOM) occur under each N fertilization regime in a randomized bloc design with four blocks, the blocks being placed 25 m apart to prevent cross-contamination during EOM applications. The EOMs are applied every second year in September after wheat harvest at a target rate of 4 Mg C ha^{-1}, the actual amount of EOM applied being measured after application. Wheat straw is removed after harvest whereas maize harvest residues are incorporated into the soil. Annual dry matter yields of grain and plant residues are determined by manual samplings before harvest. Barley was grown in 2007 due to prognoses of a regional attack of *Diabrotica virgifera* to maize, causing EOMs to be applied in two consecutive years (2006 and 2007). Soil was sampled from the plough layer prior to each EOM application and analysed for C content. Bulk density of the plough layer was measured plot-wise in 1998 (start of the experiment), 2004 and 2009. Additionally, bulk density and C content of the subsoil (29-35 cm) was measured in September 2004.

5.3.1.3. The SERAIL experiment

The ongoing SERAIL experiment, located in Brindas, near Lyon, France (45°43’ N, 4°42’ E), was started in 1995. In this study, we extract data from the period 1995 - 2009. The plough layer (0-30 cm) is a sandy loam with 66.0 % sand, 17.2 % silt and 16.8 % clay and an initial organic C content of 1.2 %. Mean annual temperature was 12.9 ± 0.5°C and precipitation 822 ± 118 mm during 1995-2009. The soil was under a rotation of vegetables (lettuce, turnip, spinach, leek, carrot, cabbage, Swiss chard, celery). Five different EOMs are applied: dried farmyard manure (Fumeterre, **FMT-Ser**), fresh farmyard manure (**FYM-Ser**), enriched bark compost (Algoforestier) produced by co-composting bark with poultry manure, liquid manure and algae (**ALG-Ser**), compost (Vegethumus) produced by co-composting coffee cake (90%), sheep manure and wool waste (**VGH-Ser**), and green waste compost (**GWC-Ser**). The EOMs are applied every year in the spring before crop planting at rates equivalent to the amount of C in 30 Mg ha^{-1} of the fresh farmyard manure applied the given year (referred to as “C equivalent”: EqC). Since 1999, a supplementary addition has been based on the amount of “stable” C in 30 Mg ha^{-1} of the fresh farmyard manure (referred to as “humus equivalent”: EqH). This addition was calculated by multiplying the amount of C contained in the manure by a biological stability index BSI (Linères and Djakovitch, 1993). The BSI was calculated as BSI = 2.112 – 2.009 SOL – 2.378 HEM – 2.216 CEW + 0.840 LIC where SOL, HEM and LIC are the soluble-like, hemicellulose-like and lignin+cutin-like fractions of the Van Soest biochemical fraction (Van Soest and Wine, 1967) and CEW is the raw cellulose fraction determined using the Wende method (AFNOR, 2005) with the BSI and biochemical fractions expressed as g g^{-1} of total organic matter content (TOM). This field experiment has a randomized block design with 3 blocks. Each block includes all organic treatments (5 EOMs * 2 doses) and a reference plot without organic amendments in 12 * 1.4 m plots. Soil is sampled each year from the ploughed layer (0-30 cm) before EOM application and determination of the C content. A fixed value of 1.40 Mg m^{-3} has been used for bulk density throughout the experiment, corresponding to the average bulk density measured in 2009 in the ploughed layer prior to EOM application and assumed to remain constant during the experiment as no differences in bulk density have been found between treatments.

5.3.1.4. The Ultuna experiment

The ongoing Ultuna experiment, located near Uppsala, Sweden (59°49' N, 17°38' E) was started in 1956. In this study, we extract data from the period 1956-2007. The soil is an Eutric Cambisol (FAO) containing 22% sand, 41% silt and 37% clay in the ploughed layer (0-20 cm), with an initial carbon content of 1.5% C. The average annual temperature was 5.7 ± 1.0°C and average annual cumulated precipitations were 541 ± 93 mm during 1956-2007. The cultivated crops were predominantly spring cereals until 1999 and silage maize thereafter. Six different EOMs were applied: straw (**STR-Ult)**, green manure (grass) (**GM-Ult)**, sawdust (**SAW-Ult)**, farmyard manure (**FYM-Ult)**, anaerobically-digested sewage sludge (**SLU-Ult)** and sphagnum peat (**PEA-Ult)**. The different treatments are laid out in a randomized block design in 2 * 2 m plots (four replicates) separated by wooden frames. For the straw, sawdust and peat treatments, plots without and with mineral N fertilization (referred as -N and +N, respectively) were investigated. Plots receiving applications of green manure, farmyard manure and sewage sludge did not received mineral N fertilization. An additional treatment corresponded to the application of FYM specially enriched in P (referred as FYM + P -N). Approximately 4 Mg C ha^{-1} of the EOMs were applied in the autumn of 1956, 1960, 1963 and every second year thereafter, and worked into the upper 20 cm of the soil with a spade. The topsoil (0-20cm) was sampled for an analysis of the C content before the EOM applications in 1956, 1967, 1974, 1975, 1977, 1979, 1983, every two years from 1985 to 2001, in 2005 and 2007. The bulk density of the ploughed layer was measured in 1956, 1975, 1991 and 1997. The C content of the subsoil layer (20-25 cm) was measured under each treatment in 1991. A more complete description of the experiment can be found in Kirchmann *et al.* (1994).

5.3.2. C stocks

Soil C concentrations were converted into C stocks (in Mg C ha^{-1}) contained in the topsoils using soil bulk densities. C stocks were calculated at a fixed depth in the Askov K2 and SERAIL experiments (25 and 30 cm, respectively) as soil bulk densities were assumed to be constant in these cases (1.50 and 1.40 Mg m^{-3}, respectively). For the Qualiagro and Ultuna experiments, the C stocks were calculated by considering an equivalent soil mass, as described by Ellert and Bettany (1995) and Lee et al. (2009) because significant changes in bulk densities between sampling years and between treatments were measured during these experiments. The mass of soil contained in the ploughed layer at a fixed depth was calculated as:

$$M_{top} = BD_{top} * T_{top} * 10^2 \qquad [1]$$

where M_{top} is the dry soil mass (Mg ha^{-1}), BD_{top} the bulk density (Mg m^{-3}) and T_{top} the thickness of the ploughed layer (in cm). The amount of C in the ploughed layer was calculated as:

$$C_{top} = conc_{top} * M_{top} \qquad [2]$$

where C_{top} is the mass of C in the ploughed layer (Mg C ha^{-1}) and $conc_{top}$ is the C concentration in the ploughed layer (g C g^{-1}).

The amounts of C in an equivalent soil mass for each treatment and throughout the experiments were calculated for the Qualiagro and Ultuna experiments using the equations:

$$M_{sub} = M_{equiv} - M_{top} \qquad [3]$$

$$C_{equiv} = C_{top} + conc_{sub} * M_{sub} \qquad [4]$$

where M_{equiv} is the selected equivalent soil mass (Mg ha^{-1}), M_{sub} the mass of additional soil from the subsoil layer added to reach an equivalent soil mass and $conc_{sub}$ is the C concentration in the subsoil layer (g C g^{-1}). The equivalent soil mass corresponded to the heaviest soil horizon under all treatments and at all time points.

During the Qualiagro experiment, the bulk density of reference plots increased significantly from 1.26 Mg m^{-3} in 1998 to 1.39 Mg m^{-3} in 2009 in the section under optimum N fertilization, and from 1.30 to 1.41 Mg m^{-3} in the section under minimum N fertilization. In plots receiving EOM applications, bulk density increased slightly (mean values of 1.26 to 1.32 Mg m^{-3} in the section under optimum N fertilization, and of 1.30 to 1.34 Mg m^{-3} in the section receiving minimum N fertilization). To calculate the amount of C in the topsoil layer (C_{top}), equations [1] and [2] were used, with BD_{top} values interpolated linearly between the BD_{top} values measured in 1998 and 2004 and between the BD_{top} values measured in 2004 and 2009. The interpolations were calculated separately for the optimum and minimum N fertilization sections of the experiments and for reference and amended plots. The C contents in the equivalent soil mass were then calculated using equations [3] and [4] where the equivalent soil masses (mass of soil in the heaviest ploughed layer) corresponded to the masses of soil in the reference plots measured in 2009 (4029 and 4095 Mg ha^{-1} in the field sections under minimum and optimum N fertilization, respectively). The C concentration in the subsurface horizon (conc$_{sub}$ in [4]) was measured in 2004 in the 29-35 cm layer and significant differences were found between treatments. The C concentration was considered to be constant in the reference plots between 1998 and 2009 (9.4 g C kg^{-1} and 9.2 g C kg^{-1} under optimum and minimum N fertilization, respectively), values which had been attributed to all plots at the beginning of the experiment in 1998. The conc$_{sub}$ values were then interpolated between 1998 and 2004 until they attained those measured in 2004 with each treatment (ranging from 9.6 g C kg^{-1} for MSW under minimum N treatment to 11.1 g C kg^{-1} for BIOW under optimum N treatment). The conc$_{sub}$ values between 2004 and 2009 were calculated as 0.91 (average ratio between conc$_{sub}$ and conc$_{top}$ measured in 2004) multiplied by the conc$_{top}$ value for the given treatment and the given year.

During the Ultuna experiment, BD$_{top}$ values decreased over time under all treatments, the equivalent soil mass corresponding to the heaviest ploughed soil layer (0-20 cm) during the experiment being the soil mass in the 0-20 cm layer at the beginning of the experiment (2880 Mg ha^{-1} in 1956). The bulk density of the ploughed layer was measured in 1956, 1975 and 1991 and 1997 (Kirchmann *et al.*, 1994; Kirchmann and Gerzabek, 1999). BD$_{top}$ values between 1956 and 2007 were calculated from linear regressions computed using the four measured BD$_{top}$ values under each treatment (average $R^2 = 0.79$). Equations [1] to [4] were used to calculate the C mass in the equivalent soil mass for each treatment and year. The C concentration of the subsurface horizon was sampled at a depth of between 20 and 25 cm in 1991. The ratio between conc$_{top}$ and conc$_{sub}$ was calculated using the values measured in 1991. The value of this ratio for reference plots (0.72) was used to set the initial conc$_{sub}$ values for all treatments in 1956. The conc$_{sub}$ values were then linearly interpolated between 1956 and 1991. The ratio of conc$_{top}$ / conc$_{sub}$ calculated with each treatment in 1991 were then used to calculate conc$_{sub}$ values between 1991 and 2007 from the conc$_{top}$ values measured during each sampling year.

5.3.3. C inputs from crop residues

During the Qualiagro, Ultuna and SERAIL experiments, crop yields differed in amended and reference plots. These differences were greater in plots with minimum or no additional N fertilization (Ultuna and Qualiagro). Crop yields were not measured during the Askov experiments, but differences in crop yields between amended and reference plots were nevertheless expected to be small because all plots received mineral N fertilization (23.4 g N m^{-2} for maize and 10.2 g N m^{-2} for other crops) and all aboveground plant parts were removed.

During the Qualiagro experiment, all wheat crop residues were removed except for stubble about 17 cm tall, which accounted for about 30% of the measured dry matter yield of above-ground residues (Y_S, in Mg ha^{-1}). Maize residues were all returned to the soil. During the Ultuna experiment, all plant parts were removed except for stem bases and roots. The stem bases of cereal and rape crops were estimated to account for 5% of total aboveground production (i.e. Y_S + dry matter grain yield: Y_G in Mg ha^{-1}), as reported by Kätterer et al. (1993).

Total C from roots (C_R, in Mg C ha^{-1}) was estimated according to the method described by Bolinder et al. (2007), assuming a C concentration of all plant parts of 0.45 g C g^{-1} as follows:

$$C_R = ((Y_G + Y_S)/S:R)*0.45 \qquad [5]$$

where Y_G is the dry matter grain yield in Mg ha^{-1}, S:R is the shoot to root ratio (7.4 for wheat, barley and oats, 5.6 for maize, 11.8 for fodder rape and 5.2 for oilseed rape). extra-root C ($C_{E,}$ in Mg C ha^{-1}), including root exudates and other materials derived from root turnover, were estimated according to Bolinder et al. (2007) as:

$$C_E = 0.65 * C_R \qquad [6]$$

Because we only considered the ploughed layer, values for root C and extra-root C (C_R and C_E) were corrected using the vertical root distribution function proposed by Jackson et al. (1996):

$$Y_R = 1 - \beta^d \qquad [7]$$

where Y_R is the cumulative root fraction, β is the “extinction coefficient” (β = 0.961 for crops) and d is the depth of the soil layer studied (29 cm and 20 cm in the Qualiagro and Ultuna experiments, respectively). For the SERAIL experiment, the C derived from vegetable crops roots and root exudates was estimated to account for 10% of total aboveground production, the proportion of root C contained in the ploughed layer of soil (0-30 cm) being estimated as equal to 90% of total root C (J.M. Machet, personal communication). In addition, 20% and 10% of aboveground production were returned to the soil in the cases of cabbage and Swiss chard, respectively.

When compared to reference plots (ΔC_{res}), the additional C input from crop residues used as entry data in RothC was calculated by subtracting the C_{res} for reference plots from the C_{res} for plots receiving EOM applications.

5.3.4. RothC model

The RothC 26.3 model describes soil C dynamics by considering five organic C pools: a labile pool (DPM: “decomposable plant material”; mean residence time of 1.2 months), a resistant pool (RPM: “resistant plant material”; mean residence time of 3.3 yrs), a humified pool (HUM;mean residence time of 50 yrs), a microbial biomass pool (BIO; mean residence time 1.5 yrs) and a pool of inert organic matter that is not degraded (IOM) (Coleman and Jenkinson, 1999). When EOM is added to the soil, it is split between the DPM, RPM and possibly HUM pools according to the partition coefficients f_{DPM},

f_{RPM} and $f_{HUM} = 1 - f_{DPM} - f_{RPM}$. Then, at each monthly time step, a fraction of each pool is decomposed according to the specific decay rate constants of each pool and either mineralized into CO_2 or transferred into the humified (HUM) or microbial biomass pools (BIO). The proportion that is converted into CO_2 and BIO+HUM is determined by the clay content of soil. Rate modifying factors are applied to the decay rate constants to account for soil humidity (topsoil moisture deficit), air temperature and soil cover (covered or not covered). All C pools except the inert organic matter pool degrade following first order linear differential equations: $dC_i/dt = -k_iC_i$, where C_i is the C stock of a given pool at time t and k_i its decay rate constant. This formalism implies that the fate of total soil C equals the sum of the different pools and that the difference in C dynamics between soils with or without EOM applications corresponds to the C dynamics resulting from EOM applications, assuming a negligible priming effect of EOM applications on native SOC degradation. It was therefore possible to run the model on differences in C stock evolution compared to a reference treatment (i.e. only the C accumulation compared to reference was simulated). As a result, the initial sizes of the different pools did not need to be estimated and were set to zero. The entry data for RothC included: the clay content of soils, monthly climatic data (potential evapotranspiration, air temperatures, cumulated rainfall), soil cover (covered or not covered), C inputs from EOMs, additional C inputs from crop residues including stem bases, roots and roots exudates compared to reference plots (when higher crop yields were measured in amended plots). Potential evapotranspiration was calculated from daily climatic data collected at meteorological stations situated near the experimental sites (solar radiation, air temperature, wind velocity and relative humidity) according to the method described by Penman (1948).

The partition coefficients of EOM-TOC into the entry pools DPM, RPM and HUM (f_{DPM}, f_{RPM}, $f_{HUM} = 100\% - f_{DPM} - f_{RPM}$, respectively, expressed as % of EOM-TOC) were adjusted to fit the kinetics of C stock accumulation in soil. The f_{DPM} and f_{RPM} fractions were set so that they were between 0 and 100% of EOM-TOC, and f_{HUM} was set at ≤ 20% of EOM-TOC. This constraint on f_{HUM} was set to prevent any unrealistic estimates of the coefficients, e.g. EOM having high f_{DPM} and f_{HUM} coefficients but an f_{RPM} coefficient equal to zero which is not realistic and would prevent linking the partition coefficients with the chemical composition of EOMs. The other model parameters were conserved, as in the original publication (Coleman & Jenkinson, 1999). Model fitting was performed on plots with, without or with minimum N fertilization, simultaneously for each EOM treatment (Ultuna and Qualiagro experiment) and on plots with EqC and EqH doses simultaneously for the SERAIL experiment by minimizing the residual sum of squares between measured and simulated values (using Excel solver with the Newton method). Several standard statistical indicators were used to evaluate the goodness of fit (Smith *et al.*, 1997b). The root mean square error (RMSE, in Mg C ha^{-1}) was used to evaluate the difference between measured and predicted values, and the coefficient of variation of the RMSE (CV(RMSE) as a %) was calculated as RMSE / $\bar{y}$ * 100 where $\bar{y}$ was the mean of measured values, in order to evaluate the relative error between measured and predicted values. The Spearman coefficient of correlation (r) was used to determine whether the simulated kinetics displayed the same pattern as measured kinetics. The efficiency of the model was calculated using:

$$EF = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$$

where y_i and $\hat{y}_i$ are the measured and predicted values for the sample *i*, $\bar{y}$ is the mean of measured values and *n* is the number of samples. Model efficiency expresses that part of the variance in measured data which is explained by the model. A positive EF value indicated that the simulated values described the trend seen in the measured data better than the mean of measured values, whereas a negative EF value indicated that the simulated values described the data less well than a mean of measured values.

5.3.5. Yields of C accumulation in soil

The rate of C accumulation in soil tended to decrease in line with the duration of the experiment (Six *et al.*, 2002; Chung *et al.*, 2008; Thomsen and Christensen, 2010). When compared to reference plots, the amounts of C accumulating in soil and resulting from EOMs and additional crop residues were thus compared over a similar period of 20 years with all treatments, as suggested by the IPCC guidelines for greenhouse gas inventories (1997). The yields of C accumulation in soil (as a % of total additional C inputs) were calculated using the differences between C stocks in amended and reference plots (ΔC_{20y}) simulated using RothC according to:

$$Y_{20y} = \Delta C_{20y} / (C_{EOM-20y} + \Delta C_{res-20y}) * 100 \qquad [8]$$

where $C_{EOM-20y}$ are the cumulated C inputs from EOMs after 20 years of application and $\Delta C_{res-20y}$ are the cumulated additional C inputs from crop residues compared to reference plots after 20 years. Because the Qualiagro and SERAIL experiments lasted less than 20 years, RothC simulations were extrapolated to obtain values of accumulated C after 20 years of EOM application with the RothC input data estimated as described below. In the Qualiagro experiment, average additional C inputs from crops compared to reference plots were calculated from the measured yields of the last three harvests of the experiment for maize (2003, 2005 and 2008) and wheat (2004, 2006 and 2007) when differences in crop yields between amended and reference plots could be clearly established. The accumulated C values were determined for September 2019, after 21 years of application, in order to conserve the interval of two years following the last EOM application. The resulting cumulated additional C inputs from crop residues for the period 1998-2019 ($\Delta C_{res-20y}$) were calculated by summing the ΔC_{res} of the different years between 1998 and 2019. The C inputs from EOMs for the period 2009-2019 were set to be 4 Mg C ha^{-1} applied every two years in September. In the SERAIL experiment, RothC simulations were extrapolated from 2009 to 2015 to obtain values of accumulated C after 20 years (1995-2015). Data for the period 2009-2015 were obtained by applying the same vegetable rotations as during the period 1995-2001. Average climatic data measured during the experimental periods where applied to the extrapolated periods (1998-2009 and 1995-2009 for the Qualiagro and SERAIL experiments, respectively).

5.3.6. Laboratory characterization of EOMs

EOMs were fractionated into soluble, hemicellulose-like, cellulose-like and lignin+cutin-like fractions using the Van Soest method (Van Soest, 1963; Van Soest and Wine, 1967) as modified in the French XP U 44-162 standard (AFNOR, 2009a). The C mineralization of EOMs was measured after 3 days of incubation of soil-EOM mixtures in hermetically sealed jars under controlled conditions at 28°C (C_{3d}, expressed as a % of TOC) with soil moisture corresponding to 75% of the soil water holding capacity. The C-CO_2 was trapped in 10 mL of 0.5 M NaOH and determined by colorimetry (AFNOR, 2009b).

Total organic C (TOC) was determined by dry combustion using an elemental analyser after the dissolution of carbonates with hydrochloric acid (AFNOR, 1995). The indicator of remaining organic carbon (I_{ROC}) evaluating the proportion of EOM potentially remaining in the soil after application over the long-term (Lashermes et al., 2009) was calculated using the SOL, CEL and LIC fractions of Van Soest fractionation and the proportion of EOM-TOC mineralized after 3 days of laboratory incubation (C_{3d}), according to the formula:

$$I_{ROC} = 44.5 + 0.5\ SOL - 0.2\ CEL + 0.7\ LIC - 2.3\ C_{3d} \qquad [9]$$

with SOL, CEL and LIC and C_{3d} expressed as a % of TOM and TOC, respectively.

For the Qualiagro experiment, characterizations were performed on EOMs sampled before each application between 1998 and 2007 (6 applications), so that the values presented were average values of six samples. For the SERAIL experiment, the analytical characteristics were the average values for EOMs applied in 2005 and 2009 because values for short-term C mineralization during incubations were only available for the EOMs sampled during those years. For the farmyard manure and dehydrated farmyard manure treatments (FYM-Ser and FMT-Ser), only 2005 data were used because FYM-Ser and FMT-Ser were particularly stable in 2009 and were thus not representative of the farmyard manures applied throughout the experiment. For the Ultuna experiment, three pooled samples were constituted with the EOMs applied in 1975+1979, 1991+1993+1995 and 2005+2007 in order to obtain sufficient material to perform the characterizations, assuming a certain homogeneity between EOMs applied during a short period. The values presented for the Ultuna experiment were thus the average values of three samples. The EOMs applied in the Askov K2 experiment were only analyzed for their C content (Christensen and Johnston, 1997). For sawdust and peat, we assigned the values measured for sawdust and peat during the Ultuna experiment. For farmyard manure, we used an average value of all the farmyard manures applied during the Qualiagro, SERAIL and Ultuna experiments, while for straw, we used the results from Djakovitch (1988) because the straw applied in Askov was mature and could be assumed to be less degradable than the straw applied during the Ultuna experiment.

5.3.7. Calculation of linear regressions

Linear regressions to predict the partition coefficients previously fitted with field data were calculated using the I_{ROC} indicator as an independent variable. Leave-one-out cross-validations were calculated by predicting each sample successively with the regression model calibrated with the rest of the samples. Regressions and cross validations were calculated using R (R Development Core Team 2010). The statistics used to evaluate the quality of regression models included the root mean square error (RMSE), the coefficient of variation of the RMSE: CV(RMSE) and the coefficient of determination R^2. These statistics were calculated using the values determined with the final regression equations (RMSE, CV(RMSE), R^2) and the values predicted during cross-validation (i.e. not used for model development: $RMSE_{CV}$, CV($RMSE_{CV}$), R^2_{CV}).

5.4. Results and Discussion

5.4.1. Characteristics of the EOMs applied

Table 5.2 shows the characteristics of all EOMs applied during the long-term field experiments (LTEs), covering a broad range of biochemical compositions. Non-transformed EOMs (i.e. non-degraded plant materials such as straw, green manure and sawdust) had a high TOC content (> 40% of DM), a high cellulose content (> 35% of EOM-TOC) and a low soluble fraction (< 30% of EOM-TOC) compared to composted EOMs with a lower TOC content (< 35% of DM), a lower cellulose content (< 25% of EOM-TOC except for the MSW compost) and a higher soluble fraction (> 40% of EOM-TOC, except for the VGH compost), which was in line with the range of values reported by Lashermes et al. (2009) on plant materials and composted materials. The farmyard manures had intermediate biochemical values as they corresponded to OM partly degraded during feed digestion and storage. Peat had a biochemical composition and TOC content close to those of non-transformed plant materials but probably contained stabilized substances, as indicated by its low C mineralization after 3 days of incubation (C_{3d} = 0.7% of TOC). Non-transformed plant materials displayed contrasted proportions of mineralized C after 3 days of incubation, ranging from sawdust (2.6%) to green manure (18.8%). Composts had low C_{3d} values, except for the ALG and MSW composts. The I_{ROC} indicator calculated from the SOL, CEL, LIC and C_{3d} values using equation [9] provided an estimation of the EOM-TOC potentially remaining in the soil in the long-term after application. I_{ROC} values ranged from 11.0% for green manure to 92.2% for the GWC compost. The highest I_{ROC} values were found for composts, except for the MSW compost which had a low I_{ROC} value (45.0%). The farmyard manures had contrasted I_{ROC} values, ranging from 49.3% for the FYM applied during the SERAIL experiment to 68.9% for the FYM applied during the Qualiagro experiment. The I_{ROC} value of the sewage sludge applied during the Ultuna experiment fell within the range of values reported by Lashermes et al. (2009) for sewage sludges, although the high standard deviation of I_{ROC} (61.2 ± 15.6%) indicated a marked variability in the composition of the sludges applied during the Ultuna experiment, mainly resulting from their marked differences in degradability during short term incubations (C_{3d} = 7.4 ± 4.5%).

Table 5.2. Analytical characteristics of the EOMs applied during the Askov K2 (Ask), Qualiagro (Qua), SERAIL (Ser) and Ultuna (Ult) experiments: total organic C (TOC), expressed as a % of dry matter at 105℃ (DM), soluble, hemicellulose-, cellulose-, and lignin+cutin- like fractions (SOL, HEM, CEL, LIC) as determined using the Van Soest fractionation method, proportions of EOM-TOC mineralized after 3 days of laboratory incubation in soil at 28℃ (C_{3d}) and the indicator of remaining organic C in soil (I_{ROC}) determined using equation [9] (see part 5.3.6). Mean values ± standard deviation.

	TOC	SOL	HEM	CEL	LIC	C_{3d}	I_{ROC}
	% DM	% TOC					
STR-Ask	44.6	14.1	31.2	46.9	8.0	5.5	35.2
SAW-Ask	49.9 ± 0.1	8.2 ± 0.3	11.7 ± 2.3	56.1 ± 3.0	24.1 ± 3.6	2.6 ± 2.3	48.3 ± 3.7
FYM-Ask	33.8 ± 5.9	34.3 ± 8.3	16.8 ± 7.2	28.5 ± 7.5	20.4 ± 5.2	3.9 ± 1.4	61.4 ± 11.2
PEA-Ask	47.7 ± 1.5	28.3 ± 4.4	11.2 ± 4.1	38.1 ± 6.2	22.5 ± 6.3	0.7 ± 0.2	65.2 ± 2.9
MSW-Qua	31.9 ± 4.7	42.5 ± 8.7	6.4 ± 2.4	35.8 ± 10.3	15.2 ± 2.4	10.6 ± 2.9	45.0 ± 12.8
FYM-Qua	32.1 ± 5.3	39.1 ± 5.9	12.0 ± 2.9	25.5 ± 7.7	23.4 ± 2.5	2.8 ± 0.3	68.9 ± 6.0
GWS-Qua	27.1 ± 6.2	45.7 ± 9.4	5.1 ± 2.1	20.8 ± 10.1	28.5 ± 11.9	1.8 ± 0.9	78.9 ± 10.5
BIOW-Qua	18.6 ± 2.4	44.4 ± 2.7	4.2 ± 2.8	20.3 ± 2.3	31.1 ± 8.9	2.7 ± 1.3	78.3 ± 5.8
FMT-Ser	38.5	27.6	27.4	29.0	16.0	6.3	49.3
FYM-Ser	37.2	32.0	27.2	29.2	11.5	5.9	49.3
VGH-Ser	34.3	32.6 ± 7.8	3.4 ± 7.1	22.9 ± 4.5	41.1 ± 5.4	6.8 ± 2.4	69.4 ± 4.5
ALG-Ser	15.7	45.0 ± 6.7	5.5 ± 2.2	18.5 ± 10.9	31.0 ± 2.0	1.0 ± 0.3	82.7 ± 6.3
GWC-Ser	22.2	48.0 ± 4.4	3.5 ± 1.7	10.1 ± 0.5	38.3 ± 5.6	0.5 ± 0.4	92.2 ± 2.5
STR-Ult	45.6 ± 0.0	11.8 ± 2.1	31.2 ± 7.1	49.6 ± 6.7	7.6 ± 2.5	8.0 ± 1.1	27.4 ± 2.0
GM-Ult	45.6 ± 1.4	26.7 ± 6.4	30.9 ± 4.2	36.8 ± 2.8	5.6 ± 0.6	18.8 ± 0.7	11.0 ± 4.8
SAW-Ult	49.9 ± 0.1	8.2 ± 0.3	11.7 ± 2.3	56.1 ± 3.0	24.1 ± 3.6	2.6 ± 2.3	48.3 ± 3.7
FYM-Ult	35.9 ± 8.2	25.6 ± 6.0	23.1 ± 5.6	34.1 ± 5.6	17.2 ± 5.2	5.3 ± 0.4	50.3 ± 7.2
SLU-Ult	26.2 ± 6.9	46.6 ± 11.2	17.3 ± 6.7	16.6 ± 4.0	19.5 ± 1.7	7.4 ± 4.5	61.2 ± 15.6
PEA-Ult	47.7 ± 1.5	28.3 ± 4.4	11.2 ± 4.1	38.1 ± 6.2	22.5 ± 6.3	0.7 ± 0.2	65.2 ± 2.9

STR : straw, SAW : sawdust, FYM : farmyard manure, PEA : peat, MSW : municipal solid waste compost, GWS : green waste and sludge compost, BIOW : biowaste compost, FMT : dehydrated FYM, VGH : enriched coffee cake compost (Vegethumus), ALG : enriched bark compost (Algoforestier), GWC : green waste compost, GM : green manure, SLU : sewage sludge.

5.4.2. Simulation of C accumulation in soil with RothC

The partition coefficients of EOM-TOC in the DPM, RPM and HUM entry pools of RothC (namely f_{DPM}, f_{RPM}, $f_{HUM} = 1 - f_{DPM} - f_{RPM}$) were fitted to reproduce the kinetics of C accumulation during the four LTEs, the other parameter values being kept as in the original RothC model.

Fitting the partition coefficients for each type of EOM satisfactorily reproduced the C accumulation kinetics of the Askov K2 experiment (CV(RMSE) of 8.6 to 17.5%, Table 5.3), with simulated values remaining close to the standard deviations of the measured values. During this experiment, the best fit was obtained for the FYM treatment with good CV(RMSE), r and EF values related to the regularity of measured C accumulation kinetics. The treatment involving peat applications led to a large amount of

C accumulating in the soil and required a f_{HUM} higher than 20% of EOM-TOC to be accurately simulated with RothC.

Good reproductions of C accumulation kinetics were also obtained for the Qualiagro experiment after the fitting of partition coefficients (CV(RMSE) of 13.3 to 17.0%, Table 5.3). The same sets of fitted coefficients enabled a satisfactory simulation of C accumulations in plots with both minimum and optimum N fertilization and amended with the same EOM. The best simulation results were obtained for the treatment with GWS compost applications, while the simulations were slightly less accurate for plots with BIOW compost applications. With all treatments used during the Qualiagro experiment, the simulated values remained between, or very close to, the standard deviation of the measured values.

The quality of the simulations was poorer for the SERAIL experiment when compared to the other LTEs (CV(RMSE) of 35.2 to 65.8%) because of the greater variability in measured C stocks during this experiment. However, the simulations reproduced C accumulation trends fairly accurately, as shown by the acceptable correlation coefficients obtained, except for plots amended with VGH which displayed highly variable C stocks for the EqC dose but for which the C accumulation in the EqH dose plot was quite well reproduced. The fitted coefficients in the SERAIL experiment were thus considered as a valuable reflection of the quality of the EOMs applied during this experiment.

C accumulation kinetics were well reproduced for the Ultuna experiment (CV(RMSE) of 12.3 to 24.2%, Table 5.3), but C stocks during the Ultuna experiment appeared to reach saturation starting in the years between 1995 and 2000, depending on the treatments; the model failed to reproduce these values, except with the sawdust amended treatment. The differences between measured and simulated values were accentuated by the particularly large additional C inputs (compared to reference plots) from maize residues cultivated between 2000 and 2007, especially in plots without N fertilization. The differences between measured and simulated values at the end of the simulated time period in the Ultuna experiment induced low model efficiency values (EF, Table 5.3) whereas the measured values were accurately simulated in the period prior to soil C saturation.

Table 5.3. Fitting of EOM partition coefficients in the RothC labile, resistant and humified entry pools (f_{DPM}, f_{RPM}, f_{HUM}, expressed as a % of EOM total organic C: TOC) and measurements of best fit between simulated and measured C stock dynamics. RMSE: root mean square error between measured and simulated accumulated C values, CV(RMSE): coefficient of variation of the RMSE, r: coefficient of correlation, EF model efficiency. Results for the Askov K2 (Ask), Qualiagro (Qua), SERAIL (Ser) and Ultuna (Ult) experiments.

Treatment	f_{DPM}	f_{RPM}	f_{HUM}	RMSE	CV(RMSE)	r	EF
	-----------------------	% TOC	-----------------------	Mg C ha^{-1}	%		
STR-Ask	58.5	38.4	3.1	1.6	13.4	0.94	0.89
SAW-Ask	56.9	31.5	11.6	2.8	17.5	0.89	0.77
FYM-Ask	37.7	42.3	20.0	1.3	8.6	0.98	0.96
PEA-Ask	18.8	38.9	42.4	4.0	12.9	0.97	0.93
MSW-Qua	62.3	37.7	0.0	0.7	13.9	0.97	0.92
FYM-Qua	30.1	49.9	20.0	1.2	13.3	0.98	0.93
GWS-Qua	15.3	64.7	20.0	1.3	13.3	0.98	0.95
BIOW-Qua	0.0	80.0	20.0	1.6	17.0	0.97	0.92
FMT-Ser	71.2	28.8	0.0	2.0	36.2	0.74	0.55
FYM-Ser	70.1	29.9	0.0	3.4	65.8	0.62	0.38
VGH-Ser	39.2	60.8	0.0	3.9	61.5	0.33	-0.16
ALG-Ser	8.9	82.4	8.7	3.8	41.2	0.65	0.39
GWC-Ser	2.0	49.2	48.8	4.1	35.2	0.83	0.68
STR-Ult	88.2	11.8	0.0	2.3	24.2	0.81	0.14
GM-Ult	90.3	9.7	0.0	2.5	22.8	0.84	0.07
SAW-Ult	53.2	44.5	2.3	1.7	12.3	0.95	0.84
FYM-Ult	49.2	40.6	10.2	3.3	17.2	0.91	0.59
SLU-Ult	0.0	85.3	14.7	6.7	22.4	0.82	0.11
PEA-Ult	0.0	57.8	42.2	5.8	16.7	0.91	0.72

STR : straw, SAW : sawdust, FYM : farmyard manure, PEA : peat, MSW : municipal solid waste compost, GWS : green waste and sludge compost, BIOW : biowaste compost, FMT : dehydrated FYM, VGH : enriched coffee cake compost (Vegethumus), ALG : enriched bark compost (Algoforestier), GWC : green waste compost, GM : green manure, SLU : sewage sludge.

5.4.3. Proportion of the cumulated C inputs remaining in soil

Table 5.4 presents by comparison with reference plots the cumulated additional amounts of C from EOMs and crop residues after 20 years of cultivation: $C_{EOM\text{-}20y}$ and $\Delta C_{res\text{-}20y}$, respectively, and the proportion of C from EOM and additional C from crop residues that accumulated in the soil (yields of C accumulation in soil after 20 years Y_{20y}, simulated with RothC). The EOMs displayed variable efficiency in increasing SOC stocks with Y_{20y} ranging from 17% to 72.9% of additional C inputs accumulated in the soil with the dehydrated farmyard manure of the SERAIL experiment and the peat of the Ultuna experiment, respectively. The Y_{20y} values calculated separately for plots with or without N fertilization and receiving EOM treatment during the Qualiagro and Ultuna experiments were similar, because the lower C stocks in reference plots without N fertilization compensated for the higher C inputs from crop residues (compared to references) in plots without N fertilization. The differences between climatic conditions, soil types and cumulated amounts of C applied from EOMs during each experiment make it difficult to compare Y_{20y} values between sites. These differences were particularly important for the Ultuna experiment because of its colder climate (mean annual temperature of 5.7°C) which led to a slower degradation of SOC and thus to higher SOC accumulation. The Y_{20y} values were fairly well correlated with the I_{ROC} indicator when the values of different treatments were compared site by site (r > 0.90) but not when all experiments were included in the calculation (r = 0.43), because of the differences in site-specific conditions (climate, soil type, rates of EOM application). In order to successfully compare the potential C accumulation in soil after EOM applications, the yields of C accumulation after 20 years of EOM applications were calculated under standard conditions for all EOMs applied during the different LTEs using the RothC results obtained with the previously fitted partition coefficients of EOM-TOC in the entry pools of the model (f_{DPM}, f_{RPM}, f_{HUM}). Contrasted climates were chosen: a Mediterranean climate (San-Giuliano, Corsica, France; average annual temperature: 16.0°C, average cumulated annual precipitations: 822 mm), a medium temperate climate (Feucherolles, Île-de-France, France; average annual temperature: 11.0°C, average cumulated annual precipitations: 643 mm) and a Nordic climate (Ultuna, Sweden; average annual temperature: 5.7°C, average cumulated annual precipitations: 541 mm). The C inputs from EOMs were set at 2 Mg C ha^{-1} applied every two years in September, with a total of 20 Mg C ha^{-1} applied over 20 years, corresponding to the dose commonly applied in practice. Additional C inputs from crop residues (compared to reference plots) were assumed to be null, considering plots with mineral fertilization. The clay content of the soil was set at 15%. Table 5.5 presents the results of these calculations. Climate exerts a strong influence on the proportion of EOM-TOC accumulating in soils. The yields of C accumulation were on average 1.6 times higher under the Nordic climate than under the Mediterranean climate, with the C accumulation being intermediate under the temperate climate. The different EOMs had contrasted efficiencies in increasing SOC stocks, with yields of C accumulation in the soil ranging from 12.1% to 53.4% for GM-Ult and GWC-Ser, respectively, under the Mediterranean climate and from 17.5% to 72.6% for GM-Ult and GWC-Ser under the Nordic climate. The annual rates of C accumulation ranged from 0.12 to 0.54 Mg C ha^{-1} yr^{-1} for GM-Ult and GWC-Ser, respectively, under the Mediterranean climate, from 0.14 to 0.60 Mg C ha^{-1} yr^{-1} for GM-Ult and GWC-Ser, respectively, under the temperate climate and from 0.18 to 0.73 Mg C ha^{-1} yr^{-1} for GM-Ult and GWC-Ser, respectively, under the Nordic climate. These rates fell within the range given by the IPCC (2000) based on data from Smith et al. (1997a) proposing 0.2-1.0 Mg C ha^{-1} yr^{-1} for organic amendments (biosolids, manure and straw). By comparison, conservation tillage could lead to SOC accumulation at a rate of 0.34 Mg C ha^{-1} yr^{-1}, as estimated by Smith et al. (1998), or at a rate of 0.20 Mg C ha^{-1} yr^{-1} as estimated by Arrouays et al. (2002). Green manure cultivated between two harvested crops can lead to SOC accumulation at a rate of 0.16 Mg C ha^{-1} yr^{-1} (Arrouays *et al.*, 2002).

Table 5.4. Additional cumulative C inputs via EOMs and additional crop residues compared to reference plots after 20 years of cultivation ($C_{EOM-20y}$, Δ $C_{res-20y}$, respectively). Accumulated C in soil compared to reference plots simulated with RothC after 20 years of cultivation (ΔC_{20y}). Yields of C accumulation in soil after 20 years of cultivation (Y_{20y}, see part 5.3.5 for calculation). Results for the Askov K2 (Ask), Qualiagro (Qua), SERAIL (Ser) and Ultuna (Ult) experiments. -N : no or minimum N fertilization, +N: with mineral N fertilization. EqC: carbon equivalent dose, EqH : humus equivalent dose (see part 5.3.1.3).

Treatment	$C_{EOM-20y}$ Mg C ha^{-1}	$\Delta C_{res-20y}$ Mg C ha^{-1}	ΔC_{20y} Mg C ha^{-1}	Y_{20y} %	Average Y_{20y} %
STR-Ask	62.8	0.0	14.1	22.5	22.5
SAW-Ask	66.6	0.0	18.4	27.6	27.6
FYM-Ask	48.0	0.0	17.9	37.3	37.3
PEA-Ask	68.0	0.0	37.2	54.6	54.6
MSW-Qua -N	41.2	15.4	11.4	20.2	
MSW-Qua +N	41.2	1.8	8.1	18.9	19.6 ± 0.9
FYM-Qua -N	44.7	18.4	20.5	32.5	
FYM-Qua +N	44.7	6.4	17.6	34.4	33.5 ± 1.3
GWS-Qua -N	46.1	17.1	22.1	34.9	
GWS-Qua +N	46.1	4.6	19.0	37.4	36.2 ± 1.7
BIOW-Qua -N	42.8	12.7	21.4	38.6	
BIOW-Qua +N	42.8	4.7	19.3	40.7	39.6 ± 1.5
FMT-Ser EqC	51.9	4.4	9.8	17.3	
FMT-Ser EqH	50.9	4.4	9.4	17.0	17.2 ± 0.3
FYM-Ser EqC	51.9	5.3	10.1	17.6	
FYM-Ser EqH	49.8	5.3	9.5	17.3	17.4 ± 0.2
VGH-Ser EqC	51.9	7.1	13.5	22.8	
VGH-Ser EqH	29.1	7.1	8.1	22.5	22.6 ± 0.3
ALG-Ser EqC	51.9	5.7	18.9	32.7	
ALG-Ser EqH	38.7	5.7	14.0	31.5	32.1 ± 0.9
GWC-Ser EqC	51.9	3.3	31.1	56.3	
GWC-Ser EqH	31.4	3.3	18.9	54.5	55.4 ± 1.3
STR-Ult -N	31.0	0.0	6.6	21.2	
STR-Ult +N	31.6	0.2	6.7	21.2	21.2 ± 0.0
GM-Ult -N	31.1	2.0	7.0	21.1	21.1
SAW-Ult -N	31.6	-0.9	11.0	35.9	
SAW-Ult +N	31.6	0.0	11.3	35.7	35.8 ± 0.1
FYM-Ult -N	30.0	1.4	12.8	40.8	
FYM-Ult +N	30.0	1.1	12.8	40.9	40.9 ± 0.1
SLU-Ult -N	28.8	3.3	19.5	60.7	60.7
PEA-Ult -N	31.9	0.0	23.0	72.2	
PEA-Ult +N	35.9	0.7	26.9	73.6	72.9 ± 1.0

STR : straw, SAW : sawdust, FYM : farmyard manure, PEA : peat, MSW : municipal solid waste compost, GWS : green waste and sludge compost, BIOW : biowaste compost, FMT : dehydrated FYM, VGH : enriched coffee cake compost (Vegethumus), ALG : enriched bark compost (Algoforestier), GWC : green waste compost, GM : green manure, SLU : sewage sludge.

Table 5.5. Yields of C accumulation in soil and annual rates of C accumulation after 20 years of cultivation (Y_{20y}, see part 5.3.5 for calculation) calculated with RothC using standard conditions and different climates for all EOMs, with EOMs applied at a dose of 2 Mg C ha^{-1} every two years (20 MgC ha^{-1} in 20 years). Results for the Askov K2 (Ask), Qualiagro (Qua), SERAIL (Ser) and Ultuna (Ult) experiments.

	Y_{20y} (% of the total C inputs)			Rate of C accumulation (Mg C ha^{-1} yr^{-1})		
Treatment	Mediterranean climate	Temperate climate	Nordic climate	Mediterranean climate	Temperate climate	Nordic climate
STR-Ask	18.2	21.8	31.2	0.18	0.22	0.31
SAW-Ask	23.5	27.0	35.4	0.24	0.27	0.36
FYM-Ask	31.2	35.7	46.5	0.31	0.36	0.47
PEA-Ask	47.3	52.4	63.4	0.48	0.52	0.64
MSW-Qua	15.8	19.2	28.4	0.16	0.19	0.29
FYM-Qua	32.2	37.2	49.4	0.32	0.37	0.50
GWS-Qua	34.1	40.2	55.2	0.34	0.40	0.55
BIOW-Qua	36.1	43.2	61.2	0.36	0.43	0.61
FMT-Ser	14.6	17.5	25.0	0.15	0.18	0.25
FYM-Ser	14.7	17.7	25.4	0.15	0.18	0.26
VGH-Ser	18.8	23.9	37.4	0.19	0.24	0.38
ALG-Ser	28.1	34.9	52.9	0.28	0.35	0.53
GWC-Ser	53.4	59.4	72.6	0.54	0.60	0.73
STR-Ult	12.4	14.1	18.3	0.13	0.14	0.19
GM-Ult	12.1	13.6	17.5	0.12	0.14	0.18
SAW-Ult	18.4	22.4	33.0	0.19	0.23	0.33
FYM-Ult	23.7	27.8	37.9	0.24	0.28	0.38
SLU-Ult	32.9	40.2	58.9	0.33	0.40	0.59
PEA-Ult	49.6	56.1	70.5	0.50	0.56	0.71

STR : straw, SAW : sawdust, FYM : farmyard manure, PEA : peat, MSW : municipal solid waste compost, GWS : green waste and sludge compost, BIOW : biowaste compost, FMT : dehydrated FYM, VGH : enriched coffee cake compost (Vegethumus), ALG : enriched bark compost (Algoforestier), GWC : green waste compost, GM : green manure, SLU : sewage sludge.

5.4.4. Relationship between the analytical characteristics of EOMs and C accumulation in the soil

Close relationships were found between the chemical and biochemical characteristics of EOMs, the values of their fitted partition coefficients in RothC and the yields of C accumulation in soil after 20 years of application calculated under different climates (Y_{20y}) (Table 5.6). Positive significant correlations ($p < 0.01$) were found between the labile pool fraction (f_{DPM}) and the TOC content, hemicellulose-like fraction (HEM) and proportion of EOM-TOC mineralized after 3 days of incubation (C_{3d}). Negative significant correlations were found between f_{DPM} and the lignin+cutin like fraction (LIC) and with the indicator of remaining C in the soil (I_{ROC}). Inversely, the resistant pool fraction (f_{RPM}) was positively correlated with the I_{ROC} indicator and LIC fraction and negatively correlated with the TOC content, HEM fraction and C_{3d}. The humified pool fraction (f_{HUM}) was correlated with the I_{ROC} indicator ($p < 0.01$) and negatively correlated with C_{3d} ($p < 0.05$). Correlations between EOM characteristics and

f_{HUM} were weaker than those found for f_{DPM} and f_{RPM} due to the constraint applied to fit this coefficient (f_{HUM} < 20%). The peat in the Askov K2 and Ultuna experiments and the green waste compost in the SERAIL experiment could not be fitted with the constraint on the f_{HUM} value and were therefore not included in the correlations. The sewage sludge in the Askov K2 experiment was also discarded from the correlation because it led to an unexpectedly large C accumulation in soil, reflected by a high f_{RPM} value, by contrast with its composition and degradability determined in the laboratory (high C_{3d} and fairly low I_{ROC} values, Table 5.2) which may have been due to a high variability of the composition and degradability of the sludges applied during this experiment, as discussed above.

The yield of C accumulation in soil calculated for the scenario of EOM application over 20 years in RothC (Y_{20y}, see 5.4.3) under different climates (Mediterranean: Med, Temperate: Temp and Nordic: Nord) also showed close relationships with the composition of the EOMs applied, and especially with C_{3d} (negative correlations, $p < 0.001$) and with the I_{ROC} indicator (positive correlations, $p < 0.001$).

The EOMs with the highest f_{DPM} coefficients were green manure and straw in the Ultuna experiment. The EOMs with the highest f_{RPM} coefficients corresponded to composted EOMs: enriched bark compost in the SERAIL experiment, biowaste compost and green waste and sludge compost in the Qualiagro experiment (ALG-Ser , BIOW-Qua and GWS-Qua, respectively). The farmyard manures (FYMs) applied during the four LTEs displayed contrasted partition coefficients. In the SERAIL experiment, 70% of the FYM carbon applied was allocated to the labile pool (f_{DPM}) and FYM displayed a high residual biodegradability when compared to those applied in the other experiments, as indicated by its high proportion of C mineralized after 3 days of laboratory incubation (C_{3d}), its low proportion of the lignin+cutin- like fraction (LIC) and its low I_{ROC} value (Table 2). In the Qualiagro experiment, the FYM applied had larger fractions of total C in the resistant and humified pools (50 and 20% of EOM-TOC, respectively) related to its lower C_{3d}, higher LIC and higher I_{ROC} values compared with FYM-Ser. The coefficients obtained for FYMs in the different experiments also differed from those parameterized in the Rothamsted experiment (f_{DPM} = 49%, f_{RPM} = 49%, f_{HUM} = 2%, Coleman and Jenkinson, 1999).

The sewage sludge in the Ultuna experiment led to a high C accumulation in soil, contrary to what might have been expected from its characteristics measured in the laboratory (high C_{3d}, fairly low I_{ROC} value, Table 5.2). Witter et al. (1993) measured a smaller microbial biomass and a lower base respiration in sewage sludge amended plots in Ultuna compared to the other treatments. Microbial activity may have been inhibited because of the low pH and excessively high heavy metal concentrations in the sludge.

The broad variability of the analytical results suggests that taking account of the real composition of each EOM applied would enable a better simulation of their C accumulation in soil as a broad range of partition coefficients was found for the different EOMs, including between EOMs of the same type (FYM).

Table 5.6. Pearson correlation coefficients between EOM characteristics and RothC partition coefficients and yields of C accumulation under different climatic conditions. Total organic C content (TOC), soluble, hemicellulose-, cellulose- and lignin+cutin-like fractions (SOL, HEM, CEL and LIC, respectively), proportion of EOM-TOC mineralized after 3 days of laboratory incubation at 28℃ (C_{3d}), indicator of remaining organic C in soil (I_{ROC}), partition coefficients of EOM-TOC into the labile, resistant and humified pools of RothC (f_{DPM}, f_{RPM}, f_{HUM}, respectively). Yields of C accumulation of EOM-TOC in soil after 20 years of application simulated with RothC with the previously fitted partition coefficients under a Mediterranean, temperate and Nordic climate (Y_{20y}-Med, Y_{20y}-Temp, Y_{20y}-Nord, respectively)

	TOC	SOL	HEM	CEL	LIC	C_{3d}	I_{ROC}	f_{DPM}	f_{RPM}	f_{HUM}	Y_{20y}-Med	Y_{20y}-Temp
TOC	1											
SOL	-0.90^{***}	1										
HEM	0.59^{*}	-0.54	1									
CEL	0.88^{***}	-0.92^{***}	0.41	1								
LIC	-0.54^{*}	0.41	-0.88^{***}	-0.48	1							
C_{3d}	0.37	-0.11	0.49	0.16	-0.59	1						
I_{ROC}	-0.79^{***}	0.65^{**}	-0.79^{***}	-0.68^{**}	0.85^{***}	-0.78^{***}	1					
f_{DPM}	0.80^{***}	-0.63^{*}	0.78^{***}	0.62^{*}	-0.79^{***}	0.71^{**}	-0.93^{***}	1				
f_{RPM}	-0.82^{***}	0.62^{*}	-0.80^{***}	-0.63^{*}	0.83^{***}	-0.67^{**}	0.92^{***}	-0.96^{***}	1			
f_{HUM}	-0.49	0.43	-0.47	-0.39	0.43	-0.58^{*}	0.64^{**}	-0.75^{**}	0.55^{*}	1		
Y_{20y}-Med	-0.66^{**}	0.55^{*}	-0.63^{*}	-0.52^{*}	0.61^{*}	-0.68^{**}	0.81^{***}	-0.91^{***}	0.76^{***}	0.96^{***}	1	
Y_{20y}-Temp	-0.70^{**}	0.57^{*}	-0.67^{**}	-0.55^{*}	0.67^{**}	-0.70^{**}	0.85^{***}	-0.94^{***}	0.82^{***}	0.93^{***}	0.99^{***}	1
Y_{20y}-Nord	-0.76^{**}	0.61^{*}	-0.74^{**}	-0.59^{*}	0.73^{**}	-0.71^{**}	0.90^{***}	-0.98^{***}	0.90^{***}	0.86^{***}	0.97^{***}	0.99^{***}

*, ** and *** indicate significance at $p < 0.05$, 0.01 and 0.001, respectively.

5.4.5. Simulation of C accumulation using partition coefficients predicted from laboratory characterizations of EOMs

5.4.5.1. Prediction of partition coefficients from laboratory characterizations

Regressions were calculated in order to predict the previously fitted partition coefficients from the laboratory characterizations of EOMs. Because the I_{ROC} indicator was the laboratory-based variable that was the most strongly correlated with RothC partition coefficients (Table 5.6), it was used as a predictor for the f_{DPM} and f_{RPM} coefficients (expressed as a % of EOM-TOC), so that f_{HUM} was calculated as f_{HUM} = 100% - f_{DPM} - f_{RPM}. The results of the regressions presented in Table 5.7 and Figure 5.1 showed a satisfactory accuracy for the prediction of f_{DPM} and f_{RPM}, R^2_{CV} being calculated with leave-one-out cross-validation results of 0.84 and 0.80 for f_{DPM} and f_{RPM}, respectively. The slope of the f_{DPM} and f_{RPM} regression models (a coefficients, Table 5.7) differed significantly from 0 ($p < 0.001$) and were close to 1 for the f_{RPM} regression model. The small differences between the calibration and cross-validation results indicated a fairly good robustness of the regression models. The predictive ability of the partition coefficients of RothC using all EOM biochemical fractions and the proportions of EOM-TOC mineralized after 3 days of incubation as predictors instead of the I_{ROC} indicator was tested, and produced a similar accuracy of prediction as that using the I_{ROC} indicator as the predictor (detailed results not shown). However, use of the partition coefficients predicted in this way produced less accurate simulations of C accumulation in the field when compared to the simulation using the partition coefficients determined with the I_{ROC} indicator.

Table 5.7. Linear regression between the RothC partition coefficients f_{DPM} and f_{RPM} as dependent variables and the indicator of remaining organic C in soil (I_{ROC}) as the independent variable (predictor). Slope (a) and intercept (b) of the regression line presented ± standard deviation. Root mean square error, coefficient of variation of the RMSE, coefficient of determination given for the calibration results (RMSE, CV(RMSE), R^2) and for the cross-validation results ($RMSE_{CV}$, $CV(RMSE_{CV})$, R^2_{CV}).

Dependent variable	Predictor	Regression coefficients		RMSE	CV(RMSE)	R^2	$RMSE_{CV}$	$CV(RMSE_{CV})$	R^2_{CV}
		a	b	%TOC	%		%TOC	%	
f_{DPM}	I_{ROC}	-1.254 ± 0.14	115.922 ± 7.78	9.40	19.29	0.87	10.58	21.70	0.84
f_{RPM}	I_{ROC}	0.979 ± 0.12	-8.928 ± 6.59	8.08	18.57	0.85	9.34	21.45	0.80

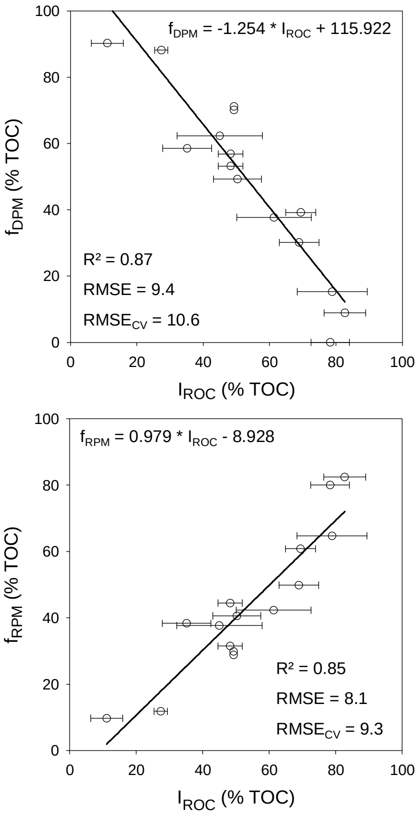

Figure 5.1. Relationship between the indicator of remaining organic C (I_{ROC}, error bars = standard deviations) and partition coefficients into the labile and resistant pools of RothC (f_{DPM} and f_{RPM}). Lines are regression lines, determination coefficient (R²), root mean square error calculated using the entire sample set and using cross-validation predictions (RMSE and $RMSE_{CV}$, respectively).

5.4.5.2. Simulations of C accumulation in soil using predicted partition coefficients

The RothC partition coefficients predicted by cross-validation (i.e. each sample predicted with a regression model calculated with the rest of the samples) were used in RothC to simulate the C accumulation in soil in the LTEs. Table 5.8 presents the values of the predicted partition coefficients along with statistics to assess the goodness of fit of the simulations of C accumulation in soil after EOM applications using those predicted coefficients. In Figure 5.2 to Figure 5.5, we compare the measured and simulated kinetics of C accumulation in soil using either the fitted or predicted partition coefficients of the four LTEs. The differences between the simulation results obtained with fitted and predicted coefficients were assessed by calculating the Δ CV(RMSE), calculated as the difference between the CV(RMSE) values obtained with the predicted and fitted partition coefficients.

Table 5.8. Predictions of EOM partition coefficients in the RothC labile, resistant and humified entry pools (f_{DPM}, f_{RPM}, f_{HUM}) with the regression models presented in Table 5.7 and the quality of simulations of C accumulation dynamics with RothC using the predicted partition coefficients. RMSE: root mean square error between measured and simulated accumulated C values, CV(RMSE): coefficient of variation of the RMSE, Δ CV(RMSE): difference of the CV(RMSE) values using the predicted and fitted partition coefficients, r: coefficient of correlation, EF model efficiency. Results for the Askov K2 (Ask), Qualiagro (Qua), SERAIL (Ser) and Ultuna (Ult) experiments.

Treatment	f_{DPM}	f_{RPM}	f_{HUM}	RMSE	CV(RMSE)	Δ CV(RMSE)	r	EF
	-----------------	% TOC	-----------------	Mg C ha^{-1}	%	%		
STR-Ask	73.7	23.7	2.6	3.1	25.4	12.0	0.95	0.61
SAW-Ask	55.3	38.9	5.9	3.3	21.0	3.5	0.87	0.68
FYM-Ask	39.1	51.9	9.0	3.4	22.2	13.6	0.97	0.71
MSW-Qua	59.3	34.9	5.8	0.9	17.7	3.8	0.97	0.87
FYM-Qua	29.5	59.6	11.0	1.4	15.5	2.1	0.97	0.91
GWS-Qua	17.4	69.1	13.5	1.5	15.3	2.0	0.98	0.93
BIOW-Qua	21.5	65.1	13.4	2.7	28.6	11.6	0.97	0.76
FMT-Ser	52.8	40.1	7.1	3.0	55.3	19.1	0.74	-0.06
FYM-Ser	52.9	40.0	7.1	3.9	75.1	9.4	0.61	0.19
VGH-Ser	27.6	58.8	13.6	4.6	73.3	11.8	0.33	-0.64
ALG-Ser	13.2	69.2	17.6	3.9	42.1	0.9	0.65	0.37
STR-Ult	80.0	19.3	0.6	2.7	28.1	3.9	0.82	-0.15
GM-Ult	100.0	0.0	0.0	2.8	25.7	2.9	0.82	-0.18
SAW-Ult	55.5	37.9	6.6	2.0	14.4	2.1	0.95	0.79
FYM-Ult	53.1	40.3	6.6	3.6	19.2	2.0	0.91	0.48

STR : straw, SAW : sawdust, FYM : farmyard manure, MSW : municipal solid waste compost, GWS : green waste and sludge compost, BIOW : biowaste compost, FMT : dehydrated FYM, VGH : enriched coffee cake compost (Vegethumus), ALG : enriched bark compost (Algoforestier), GM : green manure.

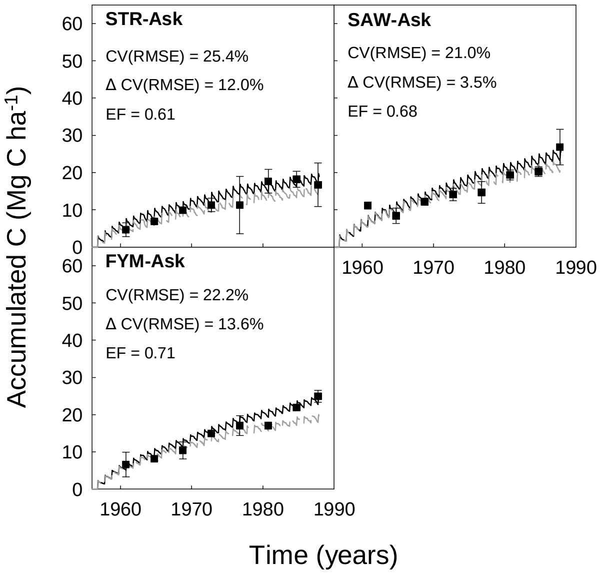

Figure 5.2. Accumulation of soil C compared to reference plots after EOM applications during the Askov K2 experiment. Measured values (■, errors bars = standard deviation) and values simulated with RothC using partition coefficients fitted with field data (black lines) or predicted using the I_{ROC} indicator (grey dashed lines). STR: straw, SAW: sawdust, FYM: farmyard manure, PEA: peat. Coefficient of variation of the RMSE (CV(RMSE)) and model efficiency (EF) of the simulations using the predicted partition coefficients, Δ CV(RMSE): difference between the CV(RMSE) values obtained using the predicted and fitted partition coefficients.

For the Askov K2 experiment (Figure 5.2) the simulation using predicted coefficients remained satisfactory for the straw amended plot, despite a relatively high Δ CV(RMSE) value, and the simulated values remained close to the standard deviation of measured values. Small differences were found for the sawdust amended plots using fitted and predicted coefficients, whereas for the farmyard manure (FYM-Ask) amended plots the use of predicted coefficients led to an underestimation of C accumulation at the end of the kinetic. This underestimation could be attributed to the use of average I_{ROC} values for the FYM applied in the other experiments, the compositions of which varied markedly (Table 5.2).

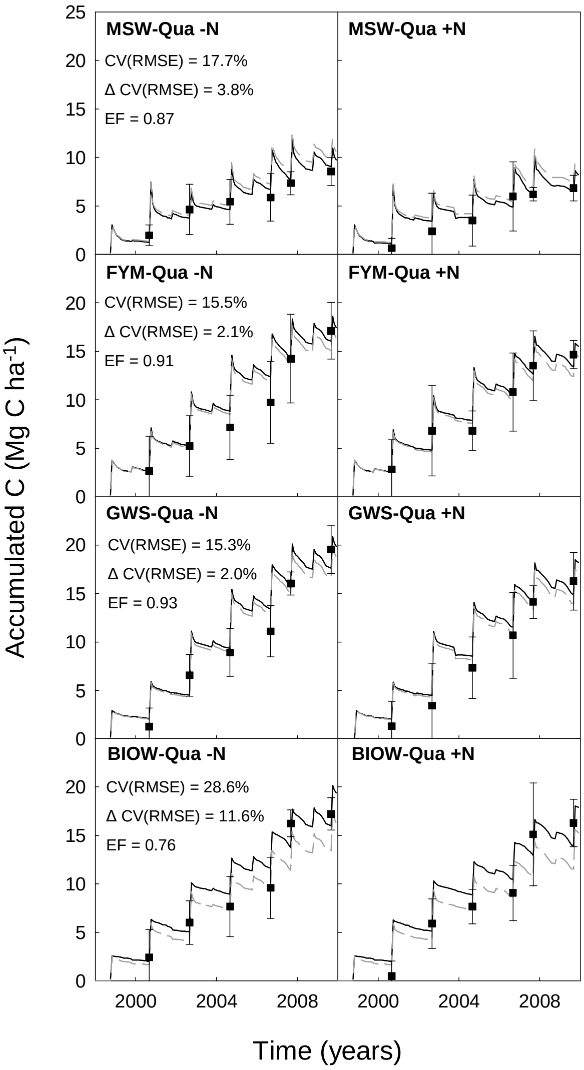

Figure 5.3. Accumulation of soil C compared to reference plots after EOM applications during the Qualiagro experiment. Measured values (■, errors bars = standard deviation) and values simulated with RothC using partition coefficients fitted with field data (black lines) or predicted using the I_{ROC} indicator (grey dashed lines). MSW: municipal solid waste compost, FYM: farmyard manure, GWS: green waste and sludge compost, BIOW: biowaste compost. -N: plots with minimum mineral N fertilization, +N: plots with optimum mineral N fertilization. Coefficient of variation of the RMSE (CV(RMSE)) and model efficiency (EF) of the simulations using the predicted partition coefficients calculated for the +N and -N parts of the experiment taken together, Δ CV(RMSE): difference between the CV(RMSE) values obtained using the predicted and fitted partition coefficients.

Successful simulations were achieved for the Qualiagro experiment using the predicted partition coefficients for plots amended with MSW compost, FYM and GWS compost, with small Δ CV(RMSE) values between the simulations using fitted and predicted coefficients and good model efficiency values (0.87 to 0.93, Table 5.8, Figure 5.3). Simulations with the predicted coefficients slightly underestimated the measured values for the plots amended with the BIOW compost.

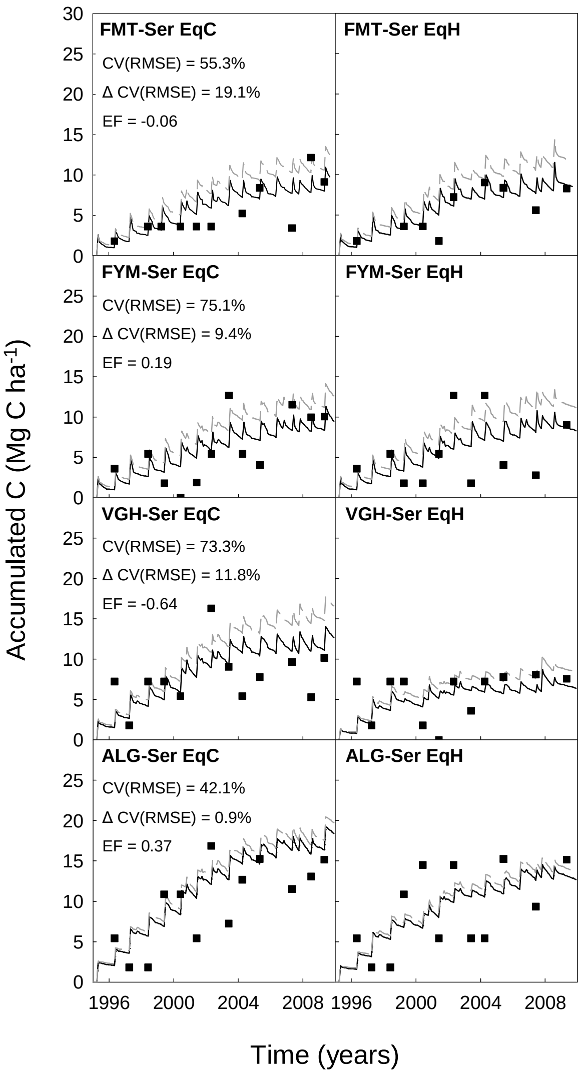

Figure 5.4. Accumulation of soil C compared to reference plots after EOM applications during the Serail experiment. Measured values (■) and values simulated with RothC using partition coefficients fitted with field data (black lines) or predicted using the I_{ROC} indicator (grey dashed lines). FMT: dehydrated farmyard manure, FYM: farmyard manure, VGH: enriched coffee cake compost (Vegethumus), ALG: enriched bark compost (Algoforestier), GWC: green waste compost. EqC: "C equivalent" dose, EqH: "humus equivalent" dose (see 5.3.1.3). Coefficient of variation of the RMSE (CV(RMSE)) and model efficiency (EF) of simulations using the predicted partition coefficients calculated for the EqC and EqH parts of the experiment taken together, Δ CV(RMSE): difference between the CV(RMSE) values obtained using the predicted and fitted partition coefficients.

As for the results of the SERAIL experiment (Figure 5.4), small differences were found using the predicted and fitted partition coefficients for plots amended with FYM, VGH compost and ALG compost (Δ CV(RMSE) of 9.4%, 11.8% and 0.9%, respectively). A higher Δ CV(RMSE) value was found for plots amended with dehydrated farmyard manure, but the CV(RMSE) and correlation coefficient values remained better for the FMT treatment than for the other treatments used during this experiment because simulations using fitted coefficients were better for this treatment than for the others.

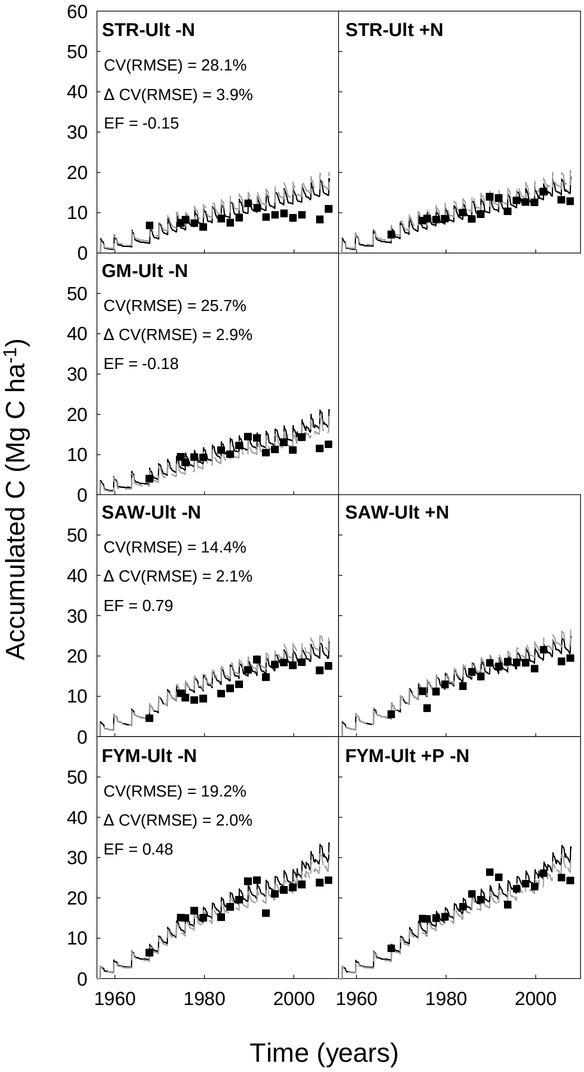

Figure 5.5. Accumulation of soil C compared to reference plots after EOM applications during the Ultuna experiment. Measured values (■) and values simulated with RothC using partition coefficients fitted with field data (black lines) or predicted using the I_{ROC} indicator (grey dashed lines). STR: straw, GM: green manure, SLU: sewage sludge, SAW: sawdust, FYM: farmyard manure, PEA: peat. -N: plots without mineral N fertilization, +N: plots with mineral N fertilization. Coefficient of variation of the RMSE (CV(RMSE)) and model efficiency (EF) of the simulations using the predicted partition coefficients calculated for the +N and -N parts of the experiment taken together, Δ CV(RMSE): difference between the CV(RMSE) values obtained using the predicted and fitted partition coefficients.

The results obtained for the Ultuna experiment (Figure 5.5) indicated small differences between the simulated values obtained using the fitted and predicted coefficients (Δ CV(RMSE) ranging from 2.0% to 3.9%). The correlation coefficients indicated relatively good reproducibility of the trend of C accumulation in soil. However, low model efficiency values were obtained for plots amended with straw and green manure because the model failed to simulate the soil C saturation observed at the end of the experimental period, as previously discussed.

5.5. Conclusions

Fitting of the partition coefficients of exogenous organic matters (EOMs) in the entry pools of the RothC model (labile, resistant and humified pools) satisfactorily reproduced the kinetics of SOC accumulation after repeated EOM applications during four long-term field experiments, without modifying the other model parameters. The EOMs displayed different efficiencies for increasing SOC stocks ranging from 0.14 to 0.60 Mg C $ha^{-1}.yr^{-1}$ accumulated in soil over a period of 20 years, with 2 Mg C ha^{-1} of EOMs applied every two years for green manure and green waste compost, respectively, under a temperate climate. The EOMs applied during field experiments covered a broad range of materials for which contrasted values for fitted partition coefficients were obtained, related to their chemical composition. The partition coefficients previously fitted could be predicted using the indicator of potentially remaining organic C in soil (I_{ROC}) calculated from the biochemical fractions of Van Soest fractionation and the proportions of C mineralized after three days of laboratory incubation. Use in RothC of the partition coefficients predicted from laboratory characterizations enabled a simulation of SOC accumulation after EOM applications with relatively small differences (an average increase of 6.7% in the coefficient of variation of the RMSE) compared to the use of fitted partition coefficients. The approach thus proposed could be used to simulate C accumulation under other field conditions and with different types of EOMs. However, further research is needed to predict partition coefficients for EOMs such as peat that will lead to very high levels of C accumulation in soil.

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Partie III. Composition chimique de la MO du sol après des apports répétés de matières organiques exogènes

Chapitre 6. Modifications to the chemical composition of organic matter in particle size and density fractions after 8 years of compost and manure applications in a loamy soil

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Projet de manuscrit pour soumission à Organic Geochemistry.

6.1. Abstract

Changes to the chemical composition of SOM after repeated applications of composts and farmyard manure were investigated. The soil was sampled from a field experiment (Qualiagro experiment, Île-de-France) after 8 years of applications of green waste and sludge compost (GWS), municipal solid waste compost (MSW), biowaste compost (BIOW) or farmyard manure (FYM). The soil was fractionated into particulate organic matter >50 µm (POM), a heavy fraction >50 µm and a 0-50 µm fraction demineralized with hydrofluoric acid (HF). All soil fractions and the organic amendments applied were analyzed for their C and N contents, and the amendments, POM fractions and HF-treated 0-50 µm fractions were characterized using pyrolysis-GC/MS and DRIFT spectroscopy. Compost and manure applications significantly increased the C and N contents of the POM fractions, but increased the C and N content of the 0-50 µm fraction to a lesser extent. A lower ratio of (furfural+acetic acid) / pyrole pyrolysis products was found in the POM fractions of amended plots, indicating a higher degree of recalcitrance compared with the POM from reference plots. Pyrolysis-GC/MS and DRIFT spectroscopy revealed an enrichment in lignin in the POM from soils amended with GWS, BIOW and FYM. The pyrolysate of the POM fraction from the soil receiving MSW compost revealed the presence of plastics originating from the MSW compost in this fraction. Higher proportions of N-containing pyrolysis compounds in the POM fraction, and of benzene pyrolysis products in the 0-50 µm fraction, were found in the soil amended with BIOW compost, probably originating from the humified OM of the compost. DRIFT spectroscopy showed relative enrichment in aliphatic compounds of the 0-50 µm fractions from amended plots when compared to reference plots.

Keywords

Soil organic matter, organic amendments, manures, composts, particle size fractionation, pyrolysis-GC/MS, DRIFT spectroscopy

Research Highlights

- Compost and manure applications significantly increased the C and N contents of the particulate organic matter (POM) fractions.

- The POM of amended plots displayed a higher degree of recalcitrance compared to the POM from reference plots.

- The POM of plots amended with green waste + sludge compost, biowaste compost and farmyard manure were enriched in lignin when compared to the POM from reference plots.

- A relative enrichment in aliphatic compounds was found in the 0-50 µm fractions from amended plots when compared to reference plots.

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6.2. Introduction

In the cultivated soils of western Europe, intensive crop management can lead to a loss of soil organic matter (SOM) (Ciais *et al.*, 2010), principally due to a decrease of organic matter (OM) inputs into the soil and to a modification of SOM decomposition rates (Sleutel *et al.*, 2006). The loss of SOM causes a variety of environmental problems, such as a decrease in soil biological activity and chemical fertility, and enhanced susceptibility to erosion, water pollution, etc. (Matson *et al.*, 1997). For these reasons, a reduction in SOM has been identified as one of the major threats to the quality of the soil resource (Commission of the European Communities, 2006).

The application of exogenous organic matter (EOM) to the soil can help to maintain or increase SOM stocks and contribute to limiting global warming by storing C in soils (Marmo *et al.*, 2004). EOM is defined as all organic matter arising from external sources such as urban areas, municipalities, agriculture, forestry and industry that can be returned to soils to improve soil fertility or restore degraded land. It also includes crop residues as they behave in soil as urban, industrial or animal EOM and can be characterized using the same methods (Marmo et al., 2004).

The efficiency of EOM at increasing SOM stocks is highly variable and EOM applied at the same rate under the same conditions during long-term field experiments can yield very distinct SOM accumulation patterns (Christensen & Johnston, 1997; Gerzabek et al., 1997). The chemical and biochemical characteristics of EOM govern their fate once applied to soil (Bernal et al., 1998; Trinsoutrot et al., 2000; Thuriès et al., 2002; Jensen et al., 2005). Increases in the proportions of non-decomposed lignins in soils have been observed after biowaste and green waste compost applications (Leifeld *et al.*, 2002; Spaccini *et al.*, 2009), together with modifications to the quantity and quality of soil lipids (González-Vila *et al.*, 1999; Spaccini *et al.*, 2009). Increases in the protein and sugar contents of SOM after the long-term application of household waste composts have also been reported (Lima *et al.*, 2009). After manure applications, increases in aliphatic C of microbial origin have often been observed (Gerzabek *et al.*, 2006; Monaco *et al.*, 2008; Zhou *et al.*, 2010), as well as increases in lignin-derived products probably arising from the straw supplied with manure (Schulten & Leinweber, 1996; Lima et al., 2009).

Results in the literature are often difficult to compare because of the diversity of factors governing SOM decomposition and the different methods used to characterize the chemical composition of SOM. Studying the effects of EOM applications on SOM composition during medium- or long-term field experiments is a relevant approach because such effects cannot be fully reproduced under laboratory conditions and only appear over the long-term. Only a few studies have described changes to the chemical composition of SOM following the application of EOM made with urban wastes during long-term field experiments (Lima et al., 2009). The Qualiagro field experiment was initiated in 1998 to study the effects of three composts of urban origin: a green waste and sludge compost (GWS), a biowaste compost (BIOW) and a municipal solid waste compost (MSW); these were compared with farmyard manure as the reference amendment and with reference plots without amendment. Urban waste is an important and potentially increasing source of organic matter. The recycling of its organic fraction by agriculture has been encouraged, particularly in urbanized regions from which animal breeding has disappeared (European Commission, 2010).

Detecting changes to SOM composition following organic amendments requires the use of complementary analytical techniques that can detect modifications of the macromolecules or functional groups making up OM. Among the most used, pyrolysis coupled with gas chromatography and mass spectroscopy (pyrolysis-GC/SM) has been found to be sufficiently powerful to determine precisely the composition of SOM (e.g. Ceccanti et al., 1986; Schulten & Leinweber, 1996; González-Vila et al., 1999) or compost OM (e.g. Ayuso et al., 1996; González-Vila et al., 1999; Dignac et al., 2005b) by isolating pyrolytic fragments of identifiable biochemical origins (polysaccharides, lignin, N-containing etc.). In addition, diffuse reflectance infra-red Fourier transform (DRIFT) spectroscopy is a rapid and non-destructive technique that has been utilized to characterize EOM composition and changes in the functional groups of SOM following EOM applications (Sohi et al., 2001; Adani et al., 2007; Francioso et al., 2007).

Separating SOM fractions with a different turnover in soil can help to relate the changes to the chemical composition of SOM induced by organic amendments to SOM dynamics. Of the SOM fractionation methods available, particle size and density fractionation is one of the most relevant to isolate SOM fractions with distinct turnovers in soil, without altering SOM composition. It enables the separation of particulate OM associated with sand size particles >50 µm with mean soil residence times ranging from a few months to a few years from SOM associated with clay and silt sized particles (0-50 µm) with mean soil residence times of several decades (Balesdent, 1996).

This study aimed to investigate changes to SOM distribution and chemical composition in particle size and density fractions resulting from repeated EOM applications in the Qualiagro field experiment, and to relate these changes to SOM behaviour and to the initial chemical composition of the EOM applied. The soil was sampled eight years after starting the experiment, which was two years after the last EOM application. From the soil we separated particulate organic matter >50 µm (POM), a heavy fraction of > 50 µm and a 0-50 µm fraction. The latter fraction was demineralized with hydrofluoric acid (HF). The separated fractions were analyzed for their C and N contents and the POM and HF-treated 0-50 µm fractions were characterized using pyrolysis-GC/MS and DRIFT spectroscopy, as were the composts and farmyard manure applied.

6.3. Material and methods

6.3.1. Field experiment and soil sampling

The Qualiagro field experiment is located in the Île-de-France region, 50 km west of Paris, on a Luvisol (FAO soil classification) containing 78.7% silt, 15.2% clay and 6.2% sand in the ploughed layer

(0-29 cm). The mean annual temperature was 11.0 ± 0.5 °C and mean precipitations were 643 ± 128 mm during the period 1998-2009. Three urban composts are being compared to a farmyard manure (**FYM**) as the reference amendment and to a reference treatment without organic amendment (**Ref**). The composts applied are: a compost made from the co-composting of green waste with sewage sludge (approximately 80% and 20% of fresh weight, respectively) (**GWS**), a municipal solid waste compost made from the composting of residual solid wastes after the removal of dry and clean packaging (approximately 17% green waste, 25% kitchen waste, 25% paper and cardboard, 20% glass and plastics and 13% other waste, Annabi et al., 2007) (**MSW**) and a biowaste compost resulting from the co-composting of green wastes and the source-separated organic fraction of municipal solid wastes (approximately 80% and 20%, respectively) (**BIOW**). The treatments (four organic amendments and the reference) were distributed according to a randomized block design with four blocks. The plots were 10 * 45 m and were separated from adjacent plots by 6-m bands. The four blocks were 25 m apart to prevent contamination during EOM spreading. The field was cultivated with a wheat–maize rotation. The composts and farmyard manure were applied every other year in September on wheat stubbles with a target dose of 4 Mg C ha^{-1}. All plots received additional mineral N to meet plant demands. The ploughed layer (0-29 cm) of the soil was sampled in 2006 after four EOM applications and, two years after the last EOM application (ten separate samples pooled in each plot). Total organic C (TOC) was determined on air dried, 2-mm sieved and 200-µm ground soil by dry combustion using an Elemental Analyser, carbonate content is close to zero in this soil.

6.3.2. Chemical and biochemical characterization of EOMs

The EOMs applied in 1998, 2000, 2002 and 2004 were sampled, air dried and finely ground (<1mm) before analysis. Total organic matter (TOM) was determined by loss on ignition at 480°C, and total organic C (TOC) by dry combustion using an Elemental Analyser after the dissolution of carbonates with hydrochloric acid (AFNOR, 1995), while total N (totN) was also determined by dry combustion (AFNOR, 1998). The biochemical composition of all EOMs was determined using the Van Soest method (Van Soest, 1963; Van Soest & Wine, 1967) as modified in the French standard XP U 44-162 (AFNOR, 2009a). The soluble (SOL), hemicellulose-like (HEM), cellulose-like (CEL) and lignin- and cutin-like (LIC) fractions were separated (expressed as a % of TOM). The C mineralization of EOM (expressed as a percentage of TOC) was measured during 91-day incubations of soil-EOM mixtures in hermetically sealed jars under laboratory controlled conditions at 28°C with a soil moisture corresponding to 85% of the soil water holding capacity. C-CO_2 was trapped in 10 ml of periodically replaced 0.5 M NaOH, and determined using colorimetry (AFNOR, 2009b). The Indicator of Residual Organic Carbon **(**I_{ROC}**)** evaluates the proportion of EOM that might remain in soil after application over the long term (AFNOR, 2009a; Lashermes et al., 2009). It is calculated using the SOL, CEL and LIC fractions of Van Soest fractionation and the proportion of EOM-TOC mineralized after 3 days of incubation (C_{3d}), according to the formula:

$$I_{ROC} = 44.5 + 0.5\ SOL - 0.2\ CEL + 0.7\ LIC - 2.3\ C_{3d} \qquad [1]$$

with SOL, CEL and LIC values expressed as a % of TOM and I_{ROC} and C_{3d} expressed as a % of TOC.

6.3.3. Size and density fractionation of soil organic matter

The topsoil sampled in September 2006 was fractionated into size and density fractions: light particulate organic matter (POM) >50 µm, a heavy fraction >50 µm and an organo-mineral fraction at 0-50 µm, according to the method described by Balesdent et al. (1991). Briefly, 100 g of air dried soil were shaken overnight with 360 ml demineralised water and 20 glass beads to break down the macro-aggregates >50 µm. After disaggregation, the soil was sieved under water at (5 mm sieve) to remove coarse material. This was followed by wet sieving at 200 µm then at 50 µm. The light fractions of the material retained at 200 µm and 50 µm were separated from the heavy ones by floating them in water in a 150 ml beaker. The fractions separated at 50 and 200 µm were collected to obtain light and heavy fractions >50 µm. The separated fractions were ball milled and their C and N contents were measured by dry combustion using an Elemental Analyser (CHN autoanalyser, Micromass NA 1500 NC). Fractionations were performed on three field replicates for each treatment, corresponding to three out of the four replicate plots of the field experiment.

6.3.4. Hydrofluoric acid (HF) treatment

The organic part of organo-mineral fractions sized 0-50 µm was isolated by demineralization with HF. Briefly, the 0-50 µm fractions of the three field replicates were grouped together and 20 g of this fraction was submerged in 200 ml 10% HF and shaken five times for two hours. After each treatment, the soil suspension was centrifuged and the supernatant removed. Residual HF was eliminated from the final material by washing five times with distilled water. After freeze drying, the materials were weighed and homogenised with ball mill. Their C and N contents were measured as described previously.

6.3.5. Pyrolysis-gas chromatography-mass spectrometry (pyrolysis-GC/MS)

Samples of approximately 10 mg were loaded into quartz tubes. The tubes were heated rapidly by means of platinum filaments to 650°C in 0.15 s (30 s hold) using a pyrolysis unit (Pyroprobe 5150, CDS Analytical Inc.). Pyrolysis products were separated using a Hewlett Packard HP-5890 gas chromatograph (GC) on a 60 m fused silica SolGelWax capillary column (SGE, 0.32 mm i.d, film thickness 0.5 µm), with helium as the carrier gas. They were identified with a Hewlett Packard HP-5889 mass spectrometer (electron energy: 70 eV, ion source temperature: 250°C). The temperature of the GC oven was set to rise from 30°C to 280°C at a rate of 2°C min^{-1}, after which the temperature was maintained at 280°C for 15 min. The different compounds were identified on the basis of their mass spectra, GC retention times and comparison with the Wiley mass spectrum library. Biochemical families were defined according to the probable origin of the pyrolysis products: polysaccharide-derived compounds (PS), lignin-derived compounds (LIG), N-containing compounds (N), lipid-derived compounds (LIP), phenols (Ph), aromatic compounds of unspecific origin (U-Ar) and other compounds of unspecific origin (U) (Dignac et al., 2005b; Parnaudeau & Dignac, 2007; De la Rosa et al., 2008). Peak areas were obtained by integrating the peaks of the total ion current trace (TIC) using the MSD Chemstation (version G1701DA D.01.00). The auto-integration function was used to define the optimum integration parameters for each pyrogram. Selection of the 50 main peaks of each pyrogram was based on their absolute abundance, and the relative abundances of peaks were calculated by dividing the surface areas of individual peaks by the total surface area of identified peaks. The results were expressed as a percentage of the total surface area of the 50 main peaks of the pyrogram. Selection of the 50 main peaks of each pyrogram was performed to prevent any bias linked to the variable number of peaks integrated in each pyrogram due to the variable concentration of material pyrolysed that could not be completely controlled.

To explain changes in OM composition, ratios of the abundance of pyrolysis products were calculated. The ratios of furfural (originating from polysaccharides) to pyrrole (derived from nitrogenous compounds, humified organic matter and microbial cell walls) and acetic acid (derived from easily biodegradable products) to pyrrole had previously been shown to decrease as a function of increases in the degradation and humification of pyrolysed material (Ceccanti *et al.*, 1986; Garcia *et al.*, 1993). Because furfural and acetic acid were co-eluted, we calculated the (furfural + acetic acid) ratio to pyrrole (written as (Fu + Ac) / Py). The toluene to styrene ratio (written as To / Sty) was used as an indicator for the presence of synthetic polymers in the pyrolysed material. Toluene and styrene are released during pyrolysis of both natural OM and synthetic polymers (Fabbri *et al.*, 1998) but the ratio of their respective contributions has been shown to be greater than one for natural samples and lower than one for synthetic polymers (Fabbri *et al.*, 1998).

Pyrolysis was performed on the three field replicates for the POM fraction, on three replicates of the same sample for the HF-treated 0-50 µm fraction and on three replicate samples of the EOMs applied in 2004.

6.3.6. Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy

Samples ground at <200 µm were packed into multiple-well plate (17 wells per plate) and scanned in the mid infra-red region (4000 – 400 cm^{-1}) under diffuse reflectance with a scanning window of 6 mm diameter and using a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific inc.). Spectra were obtained by averaging 32 scans with a resolution of 4 cm^{-1}. The spectra were then processed with Essential-FTIR software (V. 1.5, Operant LLC, 2009). The 750-400 cm^{-1} part of the spectra was removed because of poor signal resolution in this region and also because this region mainly contains band related to the vibrations of mineral structures. The spectra were then smoothed over five data points on both sides and converted into Kubelka-Munk units to compensate for the differences between DRIFT spectra and their transmission equivalents. This conversion created a more linear relationship between peak heights and concentrations in the corresponding functional groups.

Interpretation of the spectra was based on other published works (Ouatmane et al., 2000; Gerzabek et al., 2006; Smidt & Meissl, 2007; Francou et al., 2008). We focused our interpretation on the heights of several peaks of interest: the absorption peak at 2925 cm^{-1} (aliphatic C-H stretch), the peak at 1650 cm^{-1} (aromatic C=C, in addition to quinones, conjugated carboxyl groups and ketones and the C=O stretch of primary amides) and the peak at 1513 cm^{-1} characterizing the aromatic skeleton of lignin. Corrected peak heights were obtained for each peak according to the following parameters: base 1/peak/base 2 (in cm^{-1}); i.e. 3000/2925/2800, 1825/1650/1530, 1530/1513/1490. To enable comparisons between spectra, relative peak heights were calculated by dividing each peak height by the sum of the peak heights at 2925, 1650 and 1513 cm^{-1}.

6.3.7. Statistical analysis

The differences between treatments were tested using non-parametric Kruskal-Wallis analysis of variance on ranks. To isolate treatments that differed from the others, we used pairwise multiple comparisons according to Dunn’s method. In addition, multiple comparisons versus the reference group (Dunn’s method) were applied to compare each treatment involving an EOM application directly with the reference treatment. All Kruskal-Wallis and Dunn’s tests were performed with SigmaPlot V. 11.0 software (Systat Software Inc. 2008).

6.4. Results

6.4.1. Characteristics of the EOMs applied

The EOMs applied in 1998, 2000, 2002 and 2004 were characterized separately, and average values (n = 4) were calculated (Table 6.1). The EOMs differed significantly in terms of their C and N contents. The BIOW compost had the lowest C and N contents (17.3% and 1.4%, respectively), FYM the highest C content (34.4%) and the GWS compost and FYM the highest N contents (2.3%). The GWS and BIOW composts had lower C:N ratios than the MSW compost and FYM, although the differences were not statistically significant. The LIC fraction was the only Van Soest fraction to reveal significant differences between EOMs (P = 0.018). Larger LIC fractions were measured in the GWS and BIOW composts and the smallest LIC fraction was found in the MSW compost. In addition, MSW displayed the highest potential C mineralization during laboratory incubations, as revealed by the significantly higher proportions of C mineralized after 3, 28 and 91 days of incubation at 28°C (C_{3d}, C_{28d} and C_{91d}, respectively). The differences in potential mineralization were more pronounced at day 28 (C_{28d}) than after 3 and 91 days of incubation (C_{3d} and C_{91d}). The indicator of potential residual organic C in soil calculated from the Van Soest fractions and C_{3d} (I_{ROC}, Lashermes et al., 2009, equation [1]) was significantly higher for the GWS and BIOW composts (80.3% and 79.1%, respectively) than for the

MSW compost (52.2%), indicating a potentially larger amount of organic C remaining in the soil after the application of GWS and BIOW composts than after the MSW compost; FYM had an intermediate I_{ROC} value (67.3%).

Table 6.1. Analytical characteristics of the EOMs applied between 1998 and 2004 in the context of the Qualiagro experiment. Total organic C: TOC, total N: totN, TOC : totN ratio: C : N, Van Soest biochemical fractions (soluble: SOL, hemicellulose-like: HEM, cellulose-like: CEL, lignin-and cutin-like: LIC), organic C mineralized after 3, 28 and 91 days of incubation in soil (C_{3d}, C_{28d}, C_{91d}), indicator of potentially residual organic C: I_{ROC}. Values are presented for the green waste and sludge compost: GWS; municipal solid waste compost: MSW; biowaste compost: BIOW and farmyard manure: FYM. Mean ± standard deviation (n = 4), DM: dry matter at 105℃. P-value from the Kruskal-Wallis test.

	TOC	totN	C : N	SOL	HEM	CEL	LIC	C_{3d}	C_{28d}	C_{91d}	I_{ROC}
	g kg^{-1} DM			%TOM				%TOC			
GWS	286 ± 74 (ab)	23 ± 4 (ab)	12.6 ± 3.2 (a)	40.5 ± 6.3 (a)	5.2 ± 2.6 (a)	21.5 ± 12.9 (a)	32.8 ± 12.7 (b)	1.4 ± 0.6 (a)	5.1 ± 1.9 (a)	11.2 ± 6.2 (a)	80.3 ± 13.1 (b)
MSW	291 ± 20 (ab)	18 ± 3 (ab)	16.1 ± 1.2 (a)	48.1 ± 2.2 (a)	6.3 ± 2.9 (a)	29.5 ± 3.6 (a)	16.1 ± 2.5 (a)	9.5 ± 3.0 (b)	30.4 ± 3.9 (b)	40.6 ± 6.3 (b)	52.2 ± 8.1 (a)
BIOW	173 ± 11 (a)	14 ± 2 (a)	12.8 ± 2.4 (a)	41.3 ± 7.6 (a)	5.1 ± 3.1 (a)	20.2 ± 2.0 (a)	33.4 ± 10.6 (b)	2.4 ± 1.4 (ab)	6.4 ± 3.5 (a)	12.7 ± 8.7 (ab)	79.1 ± 7.3 (b)
FYM	344 ± 52 (b)	23 ± 3 (b)	15.3 ± 4.8 (a)	36.9 ± 5.6 (a)	12.4 ± 2.8 (a)	27.3 ± 8.8 (a)	23.3 ± 2.0 (ab)	2.8 ± 0.3 (ab)	12.9 ± 3.1 (ab)	18.6 ± 3.8 (ab)	67.3 ± 6.2 (ab)
	(P = 0.019)	*(P = 0.018)*	*(P = 0.292)*	*(P = 0.084)*	*(P = 0.053)*	*(P = 0.277)*	*(P = 0.018)*	*(P = 0.011)*	*(P = 0.006)*	*(P = 0.016)*	*(P = 0.010)*

6.4.2. Total organic C and N in bulk soil and separated fractions

EOM applications increased the C and N contents in topsoil (Table 6.2), but more in GWS, BIOW and FYM amended soils than in MSW amended soil. The C:N ratio of bulk soil was around 10 but tended to be higher in amended plots.

The amounts of POM isolated tended to be higher in amended plots compared to the reference (Table 6.3), which was significant in GWS and BIOW amended plots. The C and N contents of POM fractions significantly differed among treatments, and tended to be higher in EOM amended plots compared to the reference (Table 6.2). The greatest increase was measured in the POM of GWS-treated plots. The C:N ratio of the POM tended to be lower after EOM applications when compared to the reference plots. Significant differences were found in C and N levels when expressed as g of C and N per kg of soil (Table 6.3) between the amended and reference treatments.

The C content of the 0-50 µm organo-mineral fraction (before HF treatment) was significantly higher following treatments with EOM applications (Table 6.2). The total N content of the 0-50 µm fraction, although not statistically significant, tended to be higher in treatments with EOM applications when compared to the reference treatment. The highest N content in the 0-50 µm fractions was observed in GWS amended plots. When expressed as a percentage of total N (Table 6.3), the proportion of N in the 0-50 µm fraction was tended to be higher in the reference treatment than in amended treatments, which was significant between the GWS and the reference treatment. The C:N ratio of organo-mineral 0-50 µm fractions varied significantly as a function of treatments. The lowest C:N ratio was found in MSW amended soil and the highest in FYM amended soil.

Table 6.2. Total organic carbon (TOC), total nitrogen (totN) contents and C:N ratio in bulk topsoil, soil particulate organic matter >50 µm (POM), soil heavy >50 µm fraction, soil 0-50 µm fraction and HF-treated 0-50 µm fraction under the treatments of the field experiment: reference: Ref; green waste and sludge compost: GWS; municipal solid waste compost: MSW; biowaste compost: BIOW and farmyard manure: FYM. Mean ± standard deviation (n = 3 replicate plots for bulk soils, POM >50 µm, heavy >50 µm and 0-50 µm fractions, n = 3 replicates of the same sample for the HF-treated 0-50 µm fraction). P-value from the Kruskal-Wallis test, † : treatment significantly different from the reference treatment.

	TOC (g kg^{-1})	totN (g kg^{-1})	C:N
Bulk topsoil sampled in 2006			
Soil-Ref	9.81 ± 0.92 (a)	1.03 ± 0.05 (a)	9.6 ± 0.5 (a)
Soil-GWS	12.99 ± 1.08 (a)†	1.26 ± 0.08 (a)	10.3 ± 0.4 (a)
Soil-MSW	11.15 ± 1.14 (a)	1.13 ± 0.11 (a)	9.9 ± 0.1 (a)
Soil-BIOW	12.47 ± 0.62 (a)	1.25 ± 0.07 (a)	10.0 ± 0.4 (a)
Soil-FYM	12.81 ± 1.23 (a)	1.23 ± 0.06 (a)	10.4 ± 0.5 (a)
	(P = 0.090)	*(P = 0.086)*	*(P = 0.365)*
POM >50 µm			
POM-Ref	170.0 ± 4.1 (a)	11.3 ± 0.3 (a)	15.0 ± 0.2 (a)
POM-GWS	229.6 ± 6.0 (b)	15.9 ± 0.5 (b)	14.5 ± 0.8 (a)
POM-MSW	193.1 ± 5.9 (ab)	13.5 ± 0.8 (ab)	14.3 ± 0.5 (a)
POM-BIOW	206.8 ± 9.8 (ab)	13.9 ± 0.5 (ab)	14.9 ± 0.2 (a)
POM-FYM	201.2 ± 4.7 (ab)	13.9 ± 0.5 (ab)	14.4 ± 0.2 (a)
	(P = 0.017)	*(P = 0.024)*	*(P = 0.209)*
Heavy fraction >50 µm			
Heavy-Ref	10.6 ± 11.0	0.3 ± 0.1	27.8 ± 22.4
Heavy-GWS	11.1 ± 4.9	0.6 ± 0.2	17.6 ± 6.4
Heavy-MSW	10.4 ± 6.8	0.5 ± 0.1	18.2 ± 8.1
Heavy-BIOW	18.3 ± 5.0	0.8 ± 0.1	23.1 ± 2.3
Heavy-FYM	12.7 ± 3.6	0.9 ± 0.2	14.3 ± 1.5
	(P = 0.827)	*(P = 0.056)*	*(P = 0.563)*
0-50 µm fraction			
0-50-Ref	8.52 ± 0.31 (a)	0.98 ± 0.04 (a)	8.7 ± 0.0 (ab)
0-50-GWS	9.44 ± 0.42 (a)	1.09 ± 0.04 (a)	8.7 ± 0.1 (ab)
0-50-MSW	8.97 ± 0.21 (a)	1.04 ± 0.03 (a)	8.6 ± 0.0 (a)
0-50-BIOW	9.56 ± 0.50 (a)	1.05 ± 0.06 (a)	9.1 ± 0.1 (ab)
0-50-FYM	9.49 ± 0.40 (a)	1.02 ± 0.04 (a)	9.3 ± 0.1 (b)
	(P = 0.045)	*(P = 0.079)*	*(P = 0.022)*
HF-treated 0-50 µm fraction			
HF-0-50-Ref	99.4 ± 4.4	9.7 ± 1.8	10.3 ± 0.2
HF-0-50-GWS	155.2 ± 16.7	15.6 ± 1.0	9.9 ± 0.0
HF-0-50-MSW	139.7 ± 32.4	14.2 ± 4.0	9.8 ± 0.0
HF-0-50-BIOW	147.0 ± 30.7	14.6 ± 2.6	9.9 ± 0.0
HF-0-50-FYM	147.0 ± 19.4	14.4 ± 2.4	10.2 ± 0.1

Table 6.3. Results of particle size and density fractionation: Mass, total C and total N amounts in particle size and density fractions and proportion of total recovered C and N in the fractions. The fractions include: particulate organic matter > 50 µm (POM), heavy fraction >50 µm, fine fraction 0-50 µm. Results are given for all the treatments: Reference: Ref; green waste and sludge compost: GWS; municipal solid waste compost: MSW; biowaste compost: BIOW and farmyard manure: FYM. Mean ± standard deviation (n = 3 replicates from plots). P-value from the Kruskal-Wallis test, † : treatment significantly different from the reference treatment.

Treatment	> 50 µm		0-50 µm	Recovery %
	POM	Heavy fraction		
Mass : g kg^{-1} bulk soil				
Ref	10.6 ± 0.6 (a)	55.6 ± 0.9 (a)	929.1 ± 1.9 (a)	99.5
GWS	14.5 ± 1.0 (b)†	58.7 ± 1.4 (a)	923.5 ± 1.5 (a)	99.7
MSW	13.2 ± 0.5 (ab)	64.7 ± 3.6 (a)†	916.0 ± 2.8 (a)	99.6
BIO	14.1 ± 1.3 (ab)†	57.1 ± 7.3 (a)	917.9 ± 9.8 (a)	98.9
FYM	12.7 ± 0.5 (ab)	58.9 ± 1.6 (a)	922.0 ± 4.1 (a)	99.4
	(P = 0.025)	*(P = 0.109)*	*(P = 0.138)*	
C amount : g C kg^{-1} bulk soil				
Ref	1.80 ± 0.11 (a)	0.59 ± 0.63 (a)	7.92 ± 0.28 (a)	105.1
GWS	3.32 ± 0.22 (b)†	0.65 ± 0.27 (a)	8.72 ± 0.39 (a)	97.7
MSW	2.55 ± 0.01 (ab)	0.66 ± 0.39 (a)	8.24 ± 0.22 (a)	102.8
BIO	2.92 ± 0.34 (ab)†	1.04 ± 0.26 (a)	8.78 ± 0.43 (a)	102.1
FYM	2.55 ± 0.04 (ab)	0.74 ± 0.19 (a)	8.75 ± 0.36 (a)	94.0
	(P = 0.013)	*(P = 0.541)*	*(P = 0.070)*	
N amount : g N kg-1 bulk soil				
Ref	0.12 ± 0.004 (a)	0.02 ± 0.007 (a)	0.93 ± 0.008 (a)	103.7
GWS	0.22 ± 0.002 (b) †	0.03 ± 0.009 (a)	0.98 ± 0.021 (a)	97.7
MSW	0.18 ± 0.003 (ab)	0.03 ± 0.009 (a)	0.95 ± 0.036 (a)	102.2
BIOW	0.20 ± 0.020 (ab)	0.04 ± 0.009 (a)	0.96 ± 0.050 (a)	96.2
FYM	0.18 ± 0.003 (ab)	0.05 ± 0.012 (a) †	0.94 ± 0.034 (a)	95.4
	(P = 0.016)	*(P = 0.065)*	*(P = 0.157)*	
Proportion of total recovered organic C in fractions: %				
Ref	17.5 ± 1.9 (a)	5.5 ± 5.5 (a)	77.0 ± 4.4 (a)	
GWS	26.2 ± 0.6 (b) †	5.1 ± 2.0 (a)	68.7 ± 1.7 (a)	
MSW	22.3 ± 1.3 (ab)	5.7 ± 3.1 (a)	72.0 ± 2.2 (a)	
BIOW	22.9 ± 1.2 (ab)	8.1 ± 1.9 (a)	69.0 ± 2.5 (a)	
FYM	21.2 ± 0.7 (ab)	6.2 ± 1.6 (a)	72.7 ± 2.0 (a)	
	(P = 0.017)	*(P = 0.615)*	*(P = 0.068)*	
Proportion of total recovered N in fractions: %				
Ref	11.5 ± 0.9 (a)	1.8 ± 0.5 (a)	86.7 ± 1.1 (b)	
GWS	18.1 ± 0.7 (b) †	2.9 ± 0.6 (ab)	79.0 ± 1.0 (a) †	
MSW	15.3 ± 0.3 (ab)	3.0 ± 0.6 (ab)	81.7 ± 0.5 (ab)	
BIOW	16.3 ± 0.7 (ab)	3.7 ± 0.7 (ab)	80.0 ± 1.3 (ab)	
FYM	15.1 ± 0.5 (ab)	4.4 ± 1.0 (b) †	80.5 ± 1.4 (ab)	
	(P = 0.014)	*(P = 0.048)*	*(P = 0.042)*	

The C contents of the 0-50 µm fractions after HF treatment were 11.7 to 16.4 times higher than before treatment; and the N contents were 9.9 to 14.4 times higher than before HF treatment (enrichment factors calculated as the C or N content after HF treatment / C or N content before HF treatment, Table 6.4). HF treatment removed 95% to 96% of the sample mass, 35% to 41% of the TOC and 40% to 48% of total N (Table 6.4). The 0-50 µm fraction from the reference treatment displayed less mass loss compared to the other treatments, although its C and N losses were similar or higher, resulting in lower C and N enrichments. The C and N enrichment factors in HF-treated 0-50 µm fractions were similar for all organic treatments. More N than C was lost during the HF treatment. This resulted in an increase in the C:N ratio under all treatments. The differences in C:N ratios before and after HF treatment decreased in the order: Ref > GWS ≥ MSW > FYM > BIOW.

Table 6.4. Losses of mass, C and N, C, N and C:N enrichment factors, (calculated as C or N content or C:N ratio after HF treatment / C or N content or C:N ratio before HF treatment), after HF treatment of the 0-50 µm fractions with all treatments

	mass loss %	C loss (%)	C enrichment	N loss (%)	N enrichment	C:N enrichment factor
HF-0-50-Ref	94.7	38.3	11.7	47.6	9.9	1.18
HF-0-50-GWS	96.2	36.8	16.4	44.7	14.4	1.14
HF-0-50-MSW	96.2	40.9	15.6	48.1	13.7	1.14
HF-0-50-BIOW	95.7	34.7	15.4	39.6	14.2	1.08
HF-0-50-FYM	95.9	36.6	15.5	42.1	14.1	1.10

6.4.3. Characterization using pyrolysis-GC/MS of the bulk EOMs applied and of the soil fractions

6.4.3.1. Pyrolysis-GC/MS of EOMs

The pyrolysates of the EOMs applied in 2004 (two years before soil sampling) displayed contrasting compositions (Figure 6.1a). The pyrolysate of GWS compost was dominated by polysaccharide-derived compounds (PS, 29% of identified peaks) that were present at a higher proportion compared to other EOMs. The GWS pyrolysate also displayed relatively large proportions of lignin-derived (LIG) and unspecific compounds (U). The pyrolysate of the MSW compost was rich in unspecific aromatic compounds (U-Ar), mostly because of the great abundance of the styrene peak. The MSW compost was also rich in lipid-derived compounds (LIP) and had a lower proportion of lignin-derived compounds than the other EOMs. The pyrolysate of BIOW compost contained significantly higher proportions of N-containing compounds and a lower proportion of polysaccharide-derived compounds when compared to the other EOMs.

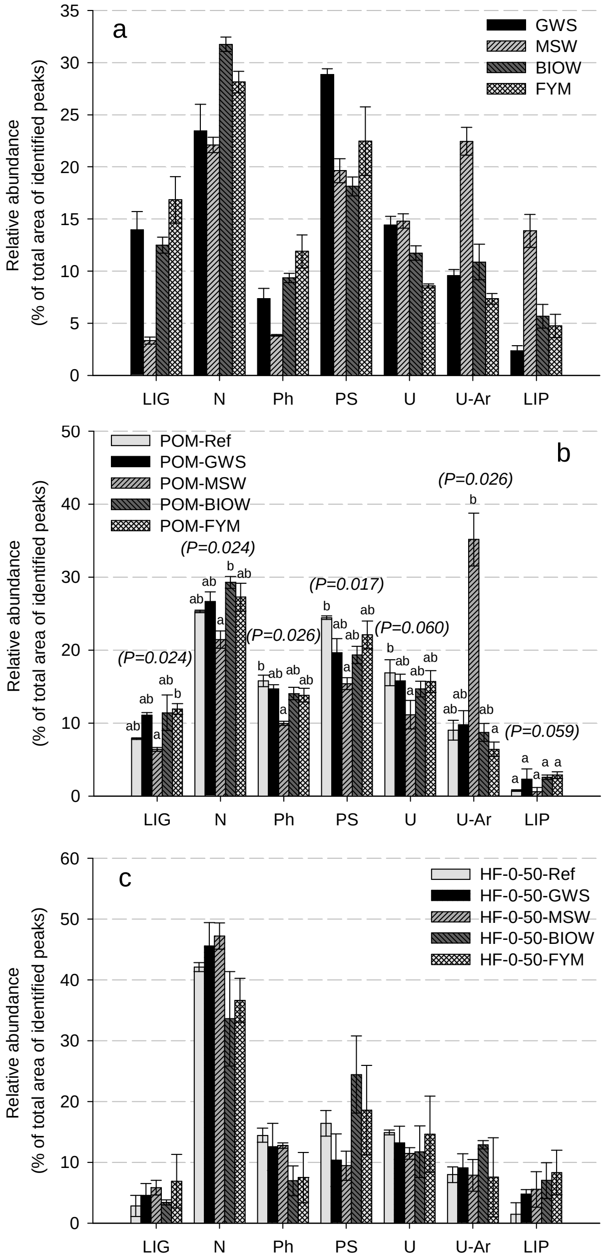

Figure 6.1. Relative surface area corresponding to pyrolysis products originating from the biochemical families identified: LIG: lignin-derived compounds; N: N-containing compounds; Ph: phenols; PS: polysaccharide-derived compounds; U: products of unspecific origin; U-Ar: aromatic products of unspecific origin; LIP: lipid-derived compounds. The proportions are presented for the bulk EOMs applied in September 2004 (a), soil POM fractions >50 µm (b) and HF-treated 0-50 µm fractions (c) of soils under the different treatments in the field experiment: reference: Ref; green waste and sludge compost: GWS; municipal solid waste compost: MSW; biowaste compost: BIOW and farmyard manure: FYM. Mean ± standard deviation (n = 3 replicates of the same sample for bulk EOMs and HF-treated 0-50 µm fractions, n = 3 replicates from plots for POM >50 µm fractions). P-value from the Kruskal-Wallis test, letters corresponds to homogenous groups according to pairwise comparisons with the Dunn's method. The percentage of the total area of the 50 main peaks of each programme not identified accounted for between 3.0% and 7.6% of the bulk EOMs, for 1.4% to 2.1% of the POM fractions and for 4.3% to 9.7% of the 0-50 µm fractions.

6.4.3.2. Pyrolysis-GC/MS of POM fractions

The pyrolysis-GC/MS of POM fractions revealed distinct chemical compositions as a function of the organic treatment (Figure 6.1b). The pyrolysate of the POM of reference plots (POM-Ref) had the highest proportions of polysaccharide-derived and unspecific compounds (24% and 17%, respectively).

The POM fraction of the MSW treatment displayed the highest proportion of aromatic compounds of unspecific origin (35%), because of the high proportions of styrene derivatives in this pyrolysate (styrene and methyl styrene accounting for 13%). As a result, the peak areas of pyrolysis products from the other biochemical families could be as abundant as with the other treatments but the proportions were low due to the high contribution of U-Ar compounds.

In the pyrolysate of POM-GWS, POM-BIOW and POM-FYM, slightly higher proportions of lignin-derived compounds were found in comparison with the pyrolysates of the POM of reference plots. All pyrolysates of POM fractions (except for MSW due to excess proportion of U-Ar compounds) also contained a relatively high proportion of phenol compounds, which probably also derive partly from lignin. The pyrolysate of the POM fraction of the BIOW treatment was slightly enriched in N-containing compounds (29%).

The proportions of lignin-derived and N-containing compounds in POM increased in line with the proportions of lignin-derived and N-containing compounds in the pyrolysates of the the EOMs applied in the order MSW<BIOW<GWS<FYM for lignin-derived compounds and MSW<GWS<FYM<BIOW for N-containing compounds (Figure 6.1, a and b).

6.4.3.3. Pyrolysis-GC/MS of HF-treated 0-50 fractions

The composition of the pyrolysates of the HF-treated 0-50 µm fraction revealed differences between the treatments in terms of the proportions of N-containing and polysaccharide-derived compounds (Figure 6.1c). Unlike the POM fractions, these differences did not seem to be correlated with the composition of the pyrolysates of bulk EOMs.

The HF-treated 0-50 µm fraction of the BIOW and FYM plots markedly differed from the HF-treated 0-50 µm fractions of the other treatments. They were depleted in N-containing and phenolic compounds and enriched in polysaccharide- and lipid-derived compounds when compared to the three other treatments.

6.4.4. Characterization using DRIFT spectroscopy of the bulk EOMs applied and the size and density fractions of soil

The EOMs applied over the period 1998-2004 (four applications), the POM fraction and the HF-treated 0-50 µm fractions were characterized using diffuse reflectance infrared Fourier transform (DRIFT). The relative corrected peak heights (expressed as a percentage of the sum of peak heights) of the selected peaks of interest at 2925 cm^{-1}, 1650 cm^{-1} and 1513 cm^{-1} (see 6.3.6), are presented in Figure 6.2.

The proportion of the 2925 cm^{-1} peak was significantly higher for the MSW compost (39%) compared to the BIOW compost. The BIOW and GWS composts had the lowest values (16% and 18%, respectively) and the FYM intermediate value (23%) (Figure 6.2a). The 1650 cm^{-1} peak heights (aromatics, carboxylic acids and amides) also differed significantly, between treatments. The 1650 cm^{-1} peak heights of the BIOW compost (82%) was significantly different from that of the MSW treatment (61%). The peak at 1513 cm^{-1} characterizing the aromatic rings of lignin was significantly higher for FYM (12%) compared with MSW (0%).

The spectra of the POM fraction in soil from the MSW treatment tended to differ from the other treatments although not significantly with slightly higher proportion of the aliphatic peak at 2925 cm^{-1} and slightly lower proportion of the 1513 cm^{-1} peak (Figure 6.2b). The peak at 1650 cm^{-1} was found at similar proportions in the POM fractions of all treatments. The proportions of 1513 cm^{-1} in soil POM fractions tended to increase in the same order as that observed for bulk EOMs.

The proportions of the different DRIFT peaks in the HF-treated 0-50 µm fraction showed clear differences when the reference plot was compared to amended plots. Under the reference treatment, this fraction contained a lower proportion of aliphatic and lignin peaks (2925 and 1513 cm^{-1}) and a higher proportion of the aromatics, carboxylic acids and amides peak at 1650 cm^{-1} compared to amended treatments (Figure 6.2c). The HF-treated 0-50 µm fraction of the MSW treatment showed a slightly higher proportion of the peak at 2925 cm^{-1} compared to other amended treatments. The proportions of the lignin peak (1513 cm^{-1}) increased in the same order as that of the bulk EOMs and POM fractions of amended soils (GWS > FYM > BIOW > MSW).

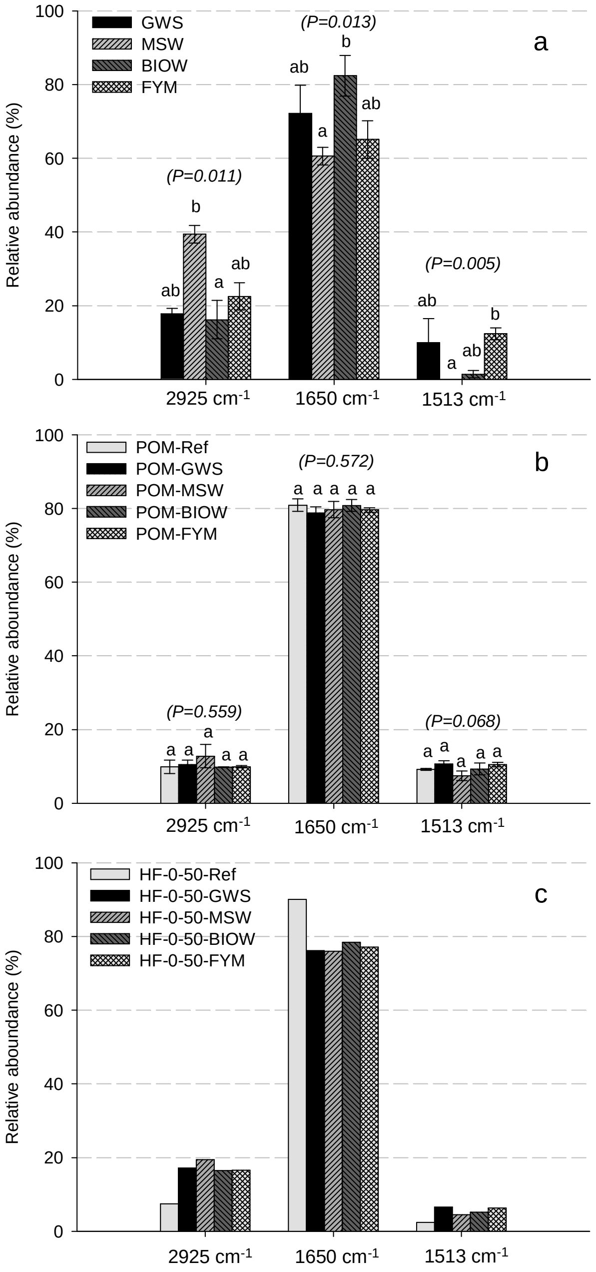

Figure 6.2. Mean relative absorbance as a percentage of the sum of corrected heights of selected peaks, for the EOMs applied in 1998, 2000, 2002 and 2004 (a); POM >50 µm (b) and HF-treated fractions (c) of soil after 8 years of the different treatments: (n = 4 for bulk EOMs, n = 3 replicate plots for the POM fractions, n = 1 for the HF-treated 0-50 µm fraction). P-value from the Kruskal-Wallis test.

6.4.5. Relationships between analytical variables

The proportions of lignin-derived products identified by pyrolysis-GC/MS, and the proportions of the DRIFT peak at 1513 cm^{-1} (aromatic skeleton of lignins) were correlated (r = 0.67, P < 0.01, Table 6.5) confirming that both parameters characterized the same ligneous structures. Likewise, lipid-derived pyrolysis compounds and the abundance of the 2925 cm^{-1} DRIFT peak were correlated (r = 0.86, P < 0.001, Table 6.5), confirming that both parameters characterized the same aliphatic structures.

Table 6.5. Pearson's correlation coefficients between analytical variables: total organic C and N contents (TOC and totN), C:N ratio, lignin-derived pyrolysis compounds: LIG; N-containing pyrolysis compounds: N; phenol compounds: Ph; polysaccharide-derived compounds: PS; compounds of unspecified origin: U; aromatic compounds of unspecified origin: U-Ar; lipid-derived compounds: LIP; (furfural + acetic acid) / pyrrole ratio: (Fu + Ac) / Py; toluene / styrene ratio: To / Sty; DRIFT spectroscopy peaks at 2925 cm^{-1} (aliphatics), 1650 cm^{-1} (aromatics, amides) and 1513 cm^{-1} (lignin).

Variables	totC	totN	C:N	LIG	N	Ph	PS	U	U-Ar	LIP	(Fu + Ac) / Py	To / Sty	2925 cm^{-1}	1650 cm^{-1}
totC	1.00													
totN	0.87^{***}	1.00												
C:N	0.71^{**}	0.28	1.00											
LIG	0.63^{*}	0.56^{*}	0.49	1.00										
N	-0.66^{**}	-0.34	-0.84^{***}	-0.42	1.00									
Ph	-0.29	-0.44	0.07	0.20	0.24	1.00								
PS	0.49	0.41	0.41	0.46	-0.68^{**}	-0.21	1.00							
U	-0.21	-0.39	0.16	-0.18	-0.17	0.25	0.24	1.00						
U-Ar	0.13	-0.03	0.30	-0.32	-0.47	-0.42	-0.13	-0.23	1.00					
LIP	0.20	0.28	-0.06	-0.31	0.06	-0.76^{**}	-0.09	-0.13	0.09	1.00				
(Fu + Ac) / Py	-0.33	-0.47	0.07	-0.23	-0.25	0.30	0.29	0.48	0.09	-0.36	1.00			
To / Sty	-0.19	-0.26	0.01	0.24	0.11	0.65^{*}	0.32	0.36	-0.69^{**}	-0.42	0.44	1.00		
2925 cm^{-1}	0.53	0.56^{*}	0.19	-0.15	-0.15	-0.74^{**}	-0.02	-0.28	0.29	0.86^{***}	-0.50	-0.58^{*}	1.00	
1650 cm^{-1}	-0.80^{***}	-0.81^{***}	-0.39	-0.19	0.34	0.57^{*}	-0.19	0.29	-0.18	-0.65^{*}	0.38	0.39	-0.88^{***}	1.00
1513 cm^{-1}	0.41	0.36	0.34	0.67^{**}	-0.32	0.47	0.40	0.04	-0.26	-0.58^{*}	0.33	0.48	-0.42	-0.05

* significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

6.5. Discussion

6.5.1. Comparison of the chemical composition of OM in soil fractions

The POM fractions in soils of all treatments were characterized by high proportions of lignin-derived pyrolysis compounds and DRIFT peaks at 1513 cm^{-1} (characteristic of lignin), a high C:N ratio and a relatively high proportion of polysaccharide-derived pyrolysis compounds. These features suggest that POM fractions were mostly composed of non-degraded POM originating from crop residues, which is supported by their high (furfural + acetic acid) / pyrrole ratio compared with the ratio of the bulk EOMs, reflecting a low degree of OM recalcitrance (Table 6.6). POM fractions had a higher C:N ratio than 0-50 µm fractions, as generally observed (Leinweber & Reuter, 1992; Guggenberger et al., 1994; Schulten & Leinweber, 2000; Leifeld & Kögel-Knabner, 2005; Gerzabek et al., 2006). The relative enrichment in N linked to the decrease in particle size was supported by the relative enrichment in N-containing pyrolysis compounds in the HF treated 0-50 µm fraction when compared to the POM fraction (Figure 6.1). This N enrichment might reflect the greater stabilization of OM in the 0-50 µm fraction, since several studies have evidenced the major contribution of N-containing products in stabilized SOM (Knicker, 2000; Rillig *et al.*, 2007). Schulten and Leinweber (1996) reported a similar enrichment in N-containing pyrolysis compounds in line with decreasing particle size, and Balesdent (1996) calculated a longer turnover time of the 0-50 µm fraction compared to the coarser fractions of SOM, indicating greater stability of the 0-50 µm fraction. Some OM have been lost during the HF treatment, and were not characterized in our study. HF is known to solubilize preferentially OM associated to minerals (Eusterhues *et al.*, 2003; Rumpel *et al.*, 2008), probably corresponding to the most stabilized SOM fraction, especially in the clay and silt sized fraction (von Lützow *et al.*, 2006). The OM lost during HF treatment might mostly correspond to OM associated to minerals and thus to the most stabilized SOM fraction.

We observed a relative depletion in lignin-derived pyrolysis products in line with decreasing particle size, as previously reported (Guggenberger et al., 1994; Schulten & Leinweber, 1996; Thevenot et al., 2010), suggesting that the selective preservation of lignin from degradation in the long term was not particularly relevant in our case. In a review of the literature, Thevenot et al. (2010) suggested that only some lignin may be stabilized in the fine fraction of SOM by interaction with clay minerals, whereas other lignin pools may have a more rapid turnover than that of total SOM (Dignac et al., 2005a; Heim & Schmidt, 2007).

Table 6.6. Ratio of characteristic pyrolysis products calculated for the bulk EOMs applied in 2004, for particulate OM (POM) >50 µm, and for the HF-treated 0-50 µm fraction of soils sampled under the different treatments of the field experiment. GWS: green waste and sludge compost; MSW: municipal solid waste compost; BIOW: biowaste compost; FYM: farmyard manure. Mean ± standard deviation (n = 3 replicates of the same sample for bulk EOMs and HF-treated 0-50 µm fractions, n = 3 replicates from plots for POM >50 µm fractions). P-value from the Kruskal-Wallis test, † : treatment significantly different from the reference (Ref) treatment.

	Humification ratio (furfural+acetic acid) / pyrrole	Toluene / styrene
Bulk EOMs applied in 2004		
GWS	3.8 ± 0.39	1.9 ± 0.23
MSW	3.4 ± 0.49	0.5 ± 0.07
BIOW	2.0 ± 0.05	2.1 ± 0.77
FYM	2.9 ± 0.61	3.6 ± 0.84
POM >50 µm		
POM-Ref	13.3 ± 0.82 (b)	5.0 ± 1.64 (a)
POM-GWS	9.4 ± 0.31 (ab)	2.9 ± 1.13 (a)
POM-MSW	8.7 ± 0.88 (ab)	0.5 ± 0.05 (a)†
POM-BIOW	6.0 ± 1.04 (a)†	3.1 ± 0.30 (a)
POM-FYM	7.7 ± 0.91 (ab)	5.1 ± 0.59 (a)
	(P = 0.017)	*(P = 0.035)*
HF-treated 0-50 µm fraction		
HF-0-50-Ref	5.7 ± 0.40	3.6 ± 0.28
HF-0-50-GWS	4.1 ± 1.64	2.9 ± 0.16
HF-0-50-MSW	3.5 ± 0.36	1.6 ± 1.08
HF-0-50-BIOW	10.0 ± 4.55	3.2 ± 0.46
HF-0-50-FYM	8.6 ± 2.94	2.8 ± 0.37

6.5.2. Effects of EOM applications on the chemical composition of POM

The chemical composition of the POM fractions of soil was significantly modified after eight years of EOM applications. Under all EOM treatments, we observed an increase in C and N contents in the POM fraction when compared to the reference treatment, along with a decrease in the pyrolysis humification ratio (Table 6.6) indicating a higher degree of POM decomposition in amended plots. Lignin-derived pyrolysis products were more abundant in the pyrolysates of POM from soils amended with GWS, BIOW and FYM, in line with the high proportions of lignin-derived compounds in the pyrolysates of the corresponding bulk EOMs. This suggests a direct incorporation of lignin from GWS, BIOW and FYM into POM, as reported in previous studies relative to biowaste and green waste compost (Leifeld *et al.*, 2002; Spaccini *et al.*, 2009) or farmyard manure applications (Lima *et al.*, 2009). This result was supported by the slightly higher abundances of the DRIFT peak at 1513 cm^{-1} (aromatic skeleton of lignin) in the POM from plots amended with GWS, BIOW and FYM, also fairly well correlated with the abundances of the 1513 cm^{-1} peak in bulk EOMs (r = 0.89, Table 6.5). The lignin enrichment in POM fractions may result from: 1) the large proportion of green wastes in the initial waste mixtures of GWS and BIOW composts (approximately 80% of fresh weight) and the cereal straw supplied with FYM, and/or 2) the higher inputs of crop residues due to an increase in crop yields in amended soils that might contribute to the lignin enrichment of the POM in treated plots when compared to the reference plot. Indeed, crop yields during the period 1998-2006 (dry weight of grain + straw measured each year) increased by 3%, 5%, 6% and 9% compared to the reference plot in GWS, BIOW, MSW and FYM amended plots, respectively. Lignin enrichment was high in both GWS and FYM amended plots, which respectively had the smallest and largest increases in crop yields compared to the reference. Thus, the hypotheses of both lignin enrichment of the POM due to the incorporation of lignin from GWS compost, or the higher incorporation of crop residues, may have been relevant.

Pyrolysis-GC/MS and DRIFT spectroscopy revealed that POM from soil amended with MSW compost was not enriched in lignin when compared with the POM of the reference and other EOM treatments, which may have been related to the small proportion of lignin-derived compounds in the pyrolysate of bulk MSW compost and its low proportion in the 1513 cm^{-1} peak. Green waste represented only a small part of the initial waste mixture of the MSW compost (approximately 17% of fresh weight, Annabi at al., 2007), which contained more paper and cardboard (approximately 25% of fresh weight).

Of all the EOMs applied, bulk MSW composts contained the highest proportion of lipid-derived pyrolysis compounds and the highest proportion of the 2925 cm^{-1} peak. This high proportion of the 2925 cm^{-1} peak in MSW compost was paired with a high proportion of the 2925 cm^{-1} peak in the POM fraction of MSW amended plots. However, the POM fraction of MSW amended plots showed the lowest proportion of lipid-derived pyrolysis compounds that tended to be larger in soils amended with GWS, BIOW and FYM compared with the reference treatment. González-Vila et al. (1999) found that changes in lipid composition and quantities were the principal effect of MSW compost application on SOM quality. In our case, the increase in lipid derived compounds was not detected with pyrolysis-GC/MS, maybe because direct pyrolysis is not the best method to study lipids, which would be better characterized using thermochemolysis with TMAH (González-Vila *et al.*, 1999; Spaccini *et al.*, 2009).

The particularly high proportion of U-Ar compounds in the pyrolysate of POM of the MSW treatment (35%, Table 6.4), due to the greater abundance of styrene and methyl styrene in this pyrolysate (16 ± 2% of identified peaks, detailed results not shown), influenced the proportions of the other biochemical families. It possibly explained why the increase in the proportion of the aliphatic DRIFT peak at 2925 cm^{-1} in the MSW treatment was not paired with an increase in the proportion of lipid-derived compounds under pyrolysis-GC/MS. The proportions of compounds in the pyrolysates of POM fraction in the MSW treatment were more comparable to the other treatments after recalculation without the styrene and methyl styrene peaks (8%, 26%, 12%, 18%, 13%, 23% and 1% for LIG, N, Ph, PS, U, U-Ar and LIP, respectively) but confirmed that POM from plots amended with MSW were not enriched in lignin-derived or lipid-derived pyrolysis compounds when compared to the other treatments.

The higher proportion of N-containing pyrolysis compounds in POM from plots amended with BIOW composts was probably due to the incorporation of stabilized OM from the BIOW compost enriched in N-containing pyrolysis compounds. Indeed, the BIOW compost displayed low biodegradability during laboratory incubations and high I_{ROC} values (Table 6.1), indicating the high degree of OM stabilization during composting.

The higher proportion of styrene in the pyrolysate of POM from the MSW treatment was probably related to the presence of inert synthetic polymers such as plastics originating from the MSW compost (Dignac et al., 2005b), as reflected by the significantly lower toluene to styrene ratio in the POM fraction of soil amended with MSW compared with the other POM fractions. It also suggests that plastic fragments from the compost remained in this soil fraction for at least two years after compost application.

Contrary to the results reported by other authors (Leifeld et al., 2002; Gerzabek et al., 2006), no differences were found in the abundance of the DRIFT peak at 1650 cm^{-1}. This peak corresponds to the vibration of either aromatic C, quinones, conjugated carboxyl groups and ketones and primary amides, it may not reflect well the degree of aromaticity of the sample. This assumption is confirmed by the low correlation between this peak and the aromatic structures identified upon pyrolysis (lignin-derived pyrolysis compounds and aromatic compounds of unspecific origin, Table 6.5).

The POM fractions of the GWS, BIOW and FYM treatments were characterized by higher proportions of lignin (lignin-derived pyrolysis compounds and 1512 cm^{-1} peak) compared to the POM fraction of the reference and MSW treatments. More differences were found between the chemical composition of the POM of MSW amended soil and of other treatments, related to the high proportion of aromatic pyrolysis compounds of unspecific origin (mainly styrenes).

6.5.3. Effects of EOM applications on the chemical composition of the 0-50 µm fraction

EOM applications significantly increased the C contents of 0-50 µm soil fractions and slightly increased their N contents. A significant effect of EOM treatments was found on the C:N ratio of the 0-50 µm fraction, contrary to the findings in the review by Christensen (1992) who reported no significant effects of various agronomic practices on the C:N ratio of clay and silt size fractions. Higher C and N enrichment factors were found in HF-treated 0-50 µm fractions from amended soils when compared with the reference soil. HF is known to solubilize preferentially OM associated with minerals (Eusterhues *et al.*, 2003; Rumpel *et al.*, 2008). Because larger amounts of OM were solubilized in the reference soil, this suggests that it contained more OM associated with minerals when compared to the amended soils. This assumption was supported by the findings of Gerzabek et al. (2006), who argued that most additional C from manure in the silt-sized fraction (2-63 µm) was not involved in mineral associations.

EOM amendments increased the contribution in 0-50µm fractions of the aliphatic peak at 2925 cm^{-1} and increased the lignin proportions, as revealed by pyrolysis-GC/MS and DRIFT spectroscopy.

The different EOMs also exerted some specific effects on the composition of the 0-50 µm fraction. The pyrolysate of the 0-50 µm fraction of soil amended with MSW compost was enriched in N-containing pyrolysis compounds when compared to the other EOM treatments. This may have been related to the greater stimulation of soil microbial activity by the MSW compost, as indicated by its higher potential C mineralization in soil during laboratory incubations (Table 6.1) and as reported in the literature (Annabi *et al.*, 2007; Hargreaves *et al.*, 2008). This stimulation of soil microbial activity could have resulted in a greater release of N-containing organic compounds of microbial origin in soil. These N-containing compounds are known to be well stabilized against degradation in soils (Knicker, 2000; Kleber et al., 2007; Rillig et al., 2007). The MSW compost also tended to increase the proportion of aliphatic structures in the 0-50 µm soil fraction, as revealed by the stronger intensity of the 2925 cm^{-1} DRIFT peak suggesting an increase in lipid content of the fraction that was not paired with an increase in lipid-derived pyrolysis products, as previously found for the POM fraction.

The GWS compost slightly increased the proportion of N-containing compounds in the pyrolysate of this fine fraction. This increase probably resulted from the incorporation in soil of the fine fraction of GWS compost, enriched in N-containing products from sludge that have been shown to be stabilised rapidly in soil (Doublet *et al.*, 2010).

The application of BIOW compost led to an increase in the proportion of aromatic compounds of unspecific origin (U-Ar) in the pyrolysate of the 0-50 µm fraction. These aromatic compounds of unspecific origin were made up of benzene and styrene pyrolytic derivatives. In this case, the increase in U-Ar compounds was due to an increase in benzenes of natural origin, as indicated by the low toluene / styrene ratio. Benzenes mainly derive from aromatic structures of stabilized OM (Ceccanti et al., 1986; Ayuso et al., 1996). The increase in the proportion of benzenes in the 0-50 µm fraction of soil amended with BIOW probably reflected the incorporation of aromatic structures from stabilized OM originating from this compost.

A slightly higher C:N ratio was found for the 0-50 µm fraction of soil amended with FYM when compared with other amended soils and the reference treatment. This higher ratio was probably related to the high C:N ratio of FYM (Table 6.1), the slightly higher proportion of polysaccharide-derived pyrolysis compounds and the lower proportion of N-containing pyrolysis compounds compared with the reference, GWS and MSW plots.

6.6. Conclusions

This study evidenced changes in the chemical composition of the organic matter of a soil after eight years of repeated exogenous organic matter applications. These changes were detected in soil sampled two years after the last EOM application. The changes in chemical composition of the POM fraction were directly related to the composition of the applied EOMs as evidenced by pyrolysis-GC/MS and DRIFT spectroscopy. The POM from amended plots showed a higher degree of recalcitrance when compared to the POM from reference plots. The POM from soils amended with GWS, BIOW and FYM were enriched in lignin when compared to the reference soil, which could be due to the accumulation of lignin from EOMs and to higher inputs of lignin from crop residues resulting from the higher crop yields of amended plots. The POM from soil amended with the BIOW compost was enriched in N-containing pyrolysis compounds, in line with the high proportion of N-containing compounds in BIOW composts. The pyrolysate of the POM fraction of soil receiving MSW compost revealed the presence of plastics originating from the compost in this fraction. Chemical changes were also detected in the 0-50 µm size fraction after EOM applications but these changes were less directly related to the composition of the applied EOMs. The GWS compost modified the composition of the 0-50 µm fraction by slightly increasing the proportion of N-containing compounds, which probably derived from the sludge. MSW composts also increased the proportion of N-containing compounds in the 0-50 µm fraction, which was probably due to a stimulation of microbial activity related to the high biodegradability of this compost. The BIOW compost increased the proportion of benzene pyrolysis compounds, possibly reflecting the incorporation of aromatic benzene units from stabilized OM in the BIOW compost. FYM slightly increased the C:N ratio and the proportion of polysaccharide-derived compounds in the 0-50 µm fraction. Further investigation should be conducted to elucidate the processes involved in the incorporation of EOM in SOM. Such study could be conducted using ^{13}C labeled composts with pyrolysis-GC/MS coupled with isotope ratio mass spectrometry (IRMS) to follow compost incorporation into SOM, or by performing particle size and density fractionation of the EOM applied.

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Conclusion générale et perspectives

La diminution des teneurs en matière organique (MO) dans les sols cultivés d'Europe de l'Ouest a entrainé une diminution de leur fertilité biologique, physique et chimique se traduisant, entre autres, par une diminution de la disponibilité en éléments fertilisants, une baisse de l'activité biologique et une augmentation de leur sensibilité à l'érosion. Le maintien ou l'augmentation des niveaux de MO dans les sols (MOS) est donc nécessaire pour le maintien de leur qualité. De plus une augmentation des stocks de C des sols cultivés pourrait jouer un rôle significatif dans la limitation des émissions nettes de gaz à effet de serre vers l'atmosphère. L'apport au sol de matières organiques exogènes (MOEs) est une des actions permettant de maintenir voire d'augmenter les stocks de C des sols. Les MOEs d'origine urbaine et industrielle représentent une source importante et en potentielle augmentation de MO pour les sols, notamment dans des zones périurbaines où les pratiques de polyculture-élevage ont disparu, systèmes de culture qui assurent classiquement la fourniture de MO pour les sols via les effluents d'élevage.

La capacité des MOEs à augmenter les stocks de C organique d'un sol dépend en premier lieu de la composition chimique de leur MO. Cette qualité de MO peut être évaluée par la caractérisation des MOEs au laboratoire avec des méthodes telles que la mesure du potentiel de minéralisation du C des MOEs au cours d'incubations en conditions contrôlées ou le fractionnement biochimique de leur MO par la méthode Van Soest. Ces méthodes permettent une première évaluation de la proportion de matière organique des MOEs potentiellement accumulable dans les sols à long terme par le biais de calcul d'indicateurs. Cependant ces méthodes restent longues à mettre en œuvre, coûteuses et génératrices de déchets polluants. De plus, la composition chimique exacte des fractions extraites par le fractionnement biochimique Van Soest reste mal connue. En particulier, la fraction dite soluble extraite à l'au chaude puis par un détergent neutre pourrait contenir des composés organiques stabilisés dans le cas de composts ou de MOEs très transformées.

Le recours à des expérimentations au champ de longue durée est nécessaire pour appréhender en conditions réelles les effets d'apports répétés de MOEs sur la dynamique de la MO du sol. Peu d'études ont tenté de relier les quantités de C accumulées dans les sols suite à des apports de MOEs au cours d'essais au champ de longue durée à la composition chimique des MOEs apportées. De plus, les formes sous lesquelles les MOEs sont accumulées à long terme dans les sols sont encore mal connues, en particulier dans le cas de composts fabriqués à partir de déchets urbains où des effets à court terme ou au cours d'incubations de laboratoire sont plus souvent rapportés.

Les modèles de dynamique de la MOS sont des outils incontournables dans l'étude du stockage de C dans les sols suite à des apports de MOEs. Cependant, ces modèles restent peu paramétrés pour simuler la dynamique du C dans les sols suite à des apports de MOEs de natures très diverses.

L'objectif de ce travail de thèse était de mieux comprendre et prévoir la dynamique d'incorporation de la matière organique de MOEs à la MO d'un sol au fur et à mesure de leurs apports. Il a été mis en œuvre en 3 étapes :

(i) Etape méthodologique avec le développement et l'amélioration d'outils de caractérisation au laboratoire permettant d'estimer le devenir des MOEs dans les sols ;

(ii) Etape de modélisation avec la fourniture de parametres du modèle RothC permettant de simuler l'accumulation du C dans les sols suite à des apports de MOEs de compositions diverses ;

(iii) Etape de caractérisation des changements de la MO d'un sol après des apports répétés de MOEs afin de mieux comprendre les formes sous lesquelles est incorporée dans le sol la MO de MOEs d'origine urbaine en comparaison avec un fumier de ferme dont la dynamique d'incorporation à la MO d'un sol est mieux connue. Ce travail a été réalisé en s'appuyant sur le dispositif au champ Qualiagro.

1. Développement d'outils de caractérisation des MOEs

Le travail méthodologique a d'abord porté sur une des méthodes les plus couramment utilisées pour évaluer le devenir dans les sols de MOEs après apport : le fractionnement Van Soest. On observe classiquement l'augmentation de la fraction « soluble » au cours du traitement par compostage de déchets. Elle ne peut sans doute plus être assimilée à une fraction labile comme c'est le cas quand le fractionnement est appliqué à des matières organiques non transformées comme les résidus de culture. L'évolution au cours du compostage de la nature chimique et de la biodégradabilité de la fraction « soluble » a été étudiée dans le cas d'un compost d'ordures ménagères résiduelles échantillonné à différents stades de maturité (immature et mature). Le détergent neutre servant à extraire cette fraction contenant lui-même du C, son utilisation empêchait toute caractérisation des composés organiques extraits ce qui nous a mené à mettre au point une nouvelle méthode d'extraction dans le but d'extraire des composés organiques similaires à ceux extraits par le détergent neutre mais permettant leur caractérisation chimique. La nouvelle méthode mise au point à permis d'extraire des proportions de MO similaires à celles extraites avec la méthode d'origine pour le compost mature alors que de légères différences entre les proportions extraites par les deux méthodes ont été trouvées pour le compost immature. Les proportions de MO soluble à l'eau et de lipides diminuent pendant que les proportions de MO extraites au tétraborate de sodium et des complexées avec des métaux augmentent quand la maturité du compost augmente..

Au cours du compostage, on observe une dégradation préférentielle des polysaccharides extractibles à l'eau chaude, accompagnée de l'apparition de formes de MO stabilisées contenant des proportions importantes de composés azotés. Ces formes stabilisées de MO restant toutefois extractibles par le détergent neutre du fractionnement Van Soest, elles pourraient être stabilisées par la formation de complexes avec des ions métalliques présents dans le compost, notamment des ions Ca^{2+} présents en forte proportion dans le compost étudié. Cette MO stabilisée par complexation serait solubilisée sous l’action des réactifs complexant présents dans le détergent neutre (tétraborate de sodium et EDTA).

Il apparait donc que la fraction soluble du fractionnement Van Soest contient des composés organiques de nature différente entre un compost mature et immature et qui correspondraient dans le cas du compost mature à des MO stabilisées contre la biodégradation, plutôt en raison d’interactions physico-chimiques se développant aux cours du compostage plutôt qu’en raison de l’apparition de molécules récalcitrantes à la dégradation.

La seconde partie du travail méthodologique avait pour but de tester l’utilisation potentielle de la spectroscopie proche infra-rouge (SPIR) pour prédire la composition chimique et biochimique des MOEs ainsi que la proportion du C des MOEs potentiellement accumulable dans les sols à long terme. Un jeu de données regroupant 300 MOEs couvrant autant que possible la diversité des MOEs épandues sur les sols cultivés à été utilisé.

Les prédictions des teneurs en C organique total, en MO et en azote des MOEs sont satisfaisantes ainsi que celles des fractions biochimiques Van Soest à l’exception de la fraction hémicellulose.

Les proportions de C facilement minéralisable des MOEs sur des durées courtes d’incubation sont mieux prédites que celles potentiellement minéralisables à plus long terme mesurées au cours d’incubations plus longues. Ces différences sont sans doute liées à la plus grande homogénéité des fractions organiques chimiquement identifiables et impliquées dans les résultats de minéralisation en temps court, celles-ci pouvant être détectables dans la réponse spectrale des MOEs. En revanche, la minéralisation à plus long terme fait intervenir des fractions plus nombreuses et plus hétérogènes qui sont sans doute plus difficilement identifiables en spectroscopie proche infrarouge.

La proportion de C stable des MOEs potentiellement accumulable dans les sols à long terme a été estimée par le calcul de l’indicateur de stabilité de la MO des MOEs (ISMO, Lashermes et al. 2009) et par la proportion de C des MOEs allouée au compartiment de C stable (S) d’un modèle de décomposition ajusté sur les cinétiques de minéralisation du C des MOEs au cours d’incubations. L’indicateur ISMO est prédit de façon satisfaisante contrairement au compartiment S du modèle cinétique. La meilleure prédiction de l’indicateur ISMO pourrait à nouveau être due au fait que cet indicateur est calculé à partir de fractions biochimiques et de proportions de C minéralisé après 3 jours d’incubation ayant des propriétés chimiques et biochimiques bien définies, facilement prédictibles par SPIR contrairement au compartiment S dont l’estimation est basée sur la proportion de C minéralisable à long terme au cours d’incubations.

Les résultats ont montré qu’il est possible de calibrer des modèles de prédiction par SPIR valables pour une grande diversité de MOEs. Toutefois, des calibrations spécifiques restent nécessaires pour certaines MOEs telles que les boues d’épuration et agro-industrielles et des engrais organo-minéraux.

2. Potentialité de stockage de C via des apports de MOEs dans les sols : fourniture de paramètres du modèle RothC en vue de simuler le devenir de MOEs dans les sols

La seconde étape de la thèse était dédiée à l'étude de la potentialité de stockage de C à long terme dans un sol soumis à des apports répétés de MOEs au champ. Pour cela les résultats d'essais au champ de longue durée ont été utilisés.

Dans un premier temps, seul l'essai au champ Qualiagro a été utilisé. Dans cet essai, des apports répétés de trois composts d'origine urbaine (compost d'ordures ménagères résiduelles : **OMR**, compost de déchets verts + boue de station d'épuration : **DVB**, compost de biodéchets : **BIOD**) et un fumier (**FUM**) à une dose d'environ 4 Mg C.ha^{-1} appliqués tous les deux ans sur la période 1998-2009 ont entrainé des accumulations significatives de C dans le sol par rapport au traitement témoin, allant jusqu'à 19.6 Mg C.ha^{-1} dans le traitement avec apport de 27 Mg C.ha^{-1} de compost de déchets vert + boue. Les cinétiques d'accumulation du C dans le sol ont pu être reproduites de manière satisfaisante par le modèle RothC après l'ajustement des coefficients de partition du C des MOEs dans les compartiments d'entrée du modèle (f_{DPM}, f_{RPM}, f_{HUM}). Des valeurs contrastées de coefficients de partition ont été trouvées pour les différentes MOEs, avec f_{DPM} = 0, 15, 30 et 62%, f_{RPM} = 80, 65, 50 et 38% et f_{HUM} = 20, 20, 20 et 0% pour BIOD, DVB, FUM et OMR, respectivement.

Les simulations des dynamiques d'accumulation du C par le modèle RothC ont été extrapolées jusqu'à 20 ans afin de faciliter la comparaison des résultats avec la littérature. L'extrapolation des simulations a permis de calculer des rendements d'accumulation du C dans le sol de 20, 34, 37 et 40 % après 20 années d'apport pour OMR, FUM, DVB, BIOD, respectivement (pour un total de 40 Mg C.ha^{-1} apportés pour tous les traitements). Des taux annuels de C accumulé dans le sol ont également été calculés à partir de ces simulations pour des doses équivalentes à celles couramment utilisées dans la pratique (équivalentes à la moitié des doses appliquées dans l'essai Qualiagro soit un total de 20 Mg C.ha^{-1} apportés dans tous les traitements). Les taux ainsi calculés ont été de 0.24, 0.44, 0.47 et 0.49 Mg C.ha^{-1}.an^{-1} pour OMR, FUM, DVB, BIOD, respectivement. Ces taux représentent des accumulations de C relativement importantes comparées à celles rapportées dans la littérature pour d'autres pratiques stockantes telles que le travail du sol réduit (0.34 Mg C.ha^{-1}.an^{-1}) ou l'utilisation d'engrais verts, c'est-à-dire de cultures intermédiaires entièrement restituées au sol (0.16 Mg C.ha^{-1}.an^{-1}).

Les niveaux d'accumulation du C dans le sol des composts et du fumier mesurés au champ dépendent de leur stabilité déterminée par la mesure du potentiel de minéralisation du C des MOEs au cours d'incubations en conditions contrôlées ou par le calcul de l'indicateur de stabilité de la MO des MOEs (ISMO).

Le fractionnement granulo-densimétrique de la MOS permet d'isoler des compartiments de MO ayant des dynamiques différentes. Le sol de l'essai Qualiagro prélevé dans les différents traitements après 8 années d'apport de MOEs (4 apports, sol prélevé 2 ans après le dernier apport) a été fractionné en MO >50 µm légère (MO particulaire : MOP) et lourde et en fraction organo-minérale de taille 0-50 µm.

Les quantités de C sous forme de MOP ayant un temps moyen de résidence dans les sols rapide augmentent dans les sols recevant les MOEs (double augmentation des quantités de MOP et de leur concentration en C). Les quantités de C contenues dans les fractions de MO de taille 0-50 µm, ayant un temps moyen de résidence dans les sols plus long augmentent également mais dans une moindre mesure. L'apport de fumier entraine une proportion plus importante du C accumulé dans la fraction de taille 0-50 µm comparé aux apports de composts. Une bonne correspondance a été trouvée entre le C accumulé dans la fraction de MOP et les compartiments DPM+RPM de RothC ainsi qu'entre le C accumulé dans la fraction de MO de tailles 0-50 µm et les compartiments BIO+HUM de RothC.

Afin de développer des équations de prédiction des coefficients de partition du modèle RothC à partir de caractéristiques des MOEs mesurées au laboratoire, les résultats de trois autres essais au champ de longue durée ont été agrégés à ceux de notre essai Qualiagro, ceci permettant d'augmenter la diversité des MOEs prises en compte. : essai d'Askov (Danemark : 31 ans d'apport Christensen & Johnston, 1997); essai de la SERAIL (Bindas, France, 14 ans d'apport, Berry et al., 2008) et essai d'Ultuna (Suède, 52 ans d'apport, Gerzabek et al., 1997).

L'ajustement des coefficients de partition des MOEs dans les compartiments d'entrée de RothC a permis de reproduire de manière satisfaisante les cinétiques d'accumulation du C suite aux apports de MOEs mesurées au champ. Les valeurs des coefficients ajustés sont reliées à leurs caractéristiques chimiques et biochimiques mesurées au laboratoire.

Des équations de régression ont été développées afin de prédire les coefficients de partition des MOEs dans les compartiments labile et résistants de RothC (f_{DPM} et f_{RPM}), à partir de l'indicateur de stabilité de la MO (ISMO) calculé à partir de fractions biochimiques des MOEs par la méthode Van Soest et de la proportion de C des MOEs minéralisée après trois jours d'incubation en conditions contrôlées. L'utilisation des coefficients de partition ainsi prédits permet de simuler les cinétiques d'accumulation du C des MOEs dans les différents essais au champ avec des différences acceptables comparé à l'utilisation des coefficients ajustés sur les cinétiques d'accumulation du C mesurées (augmentation de 6.7% du coefficient de variation en moyenne). Ces équations de prédiction des coefficients de partition du C des MOEs dans RothC peuvent donc être utilisées pour simuler des cinétiques d'accumulation du C dans le sol suite à des apports MOEs de types divers et dans diverses situations. Le développement d'une autre approche reste toutefois nécessaire pour des MOEs entrainant des accumulations très importantes de C dans le sol telles que les tourbes apportées dans l'essai d'Askov et d'Ultuna ou le compost de déchets vert apporté dans l'essai de la SERAIL qui n'ont pas pu être intégrées pour le développement des équations de régression.

3. Qualité de la matière organique accumulée

La dernière étape du travail visait à étudier les modifications de composition chimique de la MOS suite aux apports répétés de MOEs. Les fractions granulo-densimétriques séparées précédemment (cf point 2) ainsi que les composts et le fumier épandus ont été caractérisés par pyrolyse-CG/SM et par spectroscopie moyen infra-rouge en réflexion diffuse (DRIFT). Une augmentation des teneurs en C et N des MOP a été mesurée dans les parcelles amendées. La caractérisation des MOP révèle une influence directe de la composition des MOEs apportées sur la composition des MOP. Les MOP des sols amendés avec les composts de déchets verts + boue et de biodéchets et avec le fumier sont plus enrichis en lignine que les MOP des parcelles témoin, ce qui peut être due à une accumulation de lignine provenant des MOEs ou provenant d'une quantité plus importante de résidus de culture retournés au sol en raison de l'augmentation des rendements des cultures dans les parcelles amendées. Les MOP des parcelles amendées avec le compost de biodéchets sont enrichies en composés azotés liée à la proportion importante de composés azotés mesurée pour le compost de biodéchet apporté. Le pyrolysat de la fraction de MOP des sols amendés avec le compost d'ordures ménagères résiduelles révèle la présence de plastiques provenant du compost.

Des modifications de la composition chimique des fractions de MOS de taille 0-50 µm déminéralisées au préalable par attaque à l'acide fluorhydrique (HF) ont également été détectées. Contrairement au MOP, ces modifications de compositions chimiques n'ont pas montré de relation directe avec la composition chimique des MOEs apportées. La fraction 0-50 µm des parcelles amendées avec le compost de déchets verts + boue est enrichie en composés azotés, provenant probablement des boues d'épuration généralement riche en N. Celle-ci reste peu visible dans la caractérisation par pyrolyse-GC/MS du compost total mais leur présence préférentielle dans les fractions fines des composts a été démontrée (Doublet et al., 2010). L'apport de compost d'ordures ménagères résiduelles entraine aussi une augmentation de la proportion de composés azotés dans la fraction 0-50 µm probablement en raison d'une forte stimulation l'activité microbienne liée à la biodégradabilité importante de ce compost telle que mesurée lors d'incubations en conditions contrôlées. La fraction 0-50 µm du sol amendé avec le compost de biodéchets est enrichie en composés benzéniques formés durant la pyrolyse reflétant l'incorporation d'unités benzéniques aromatiques provenant probablement de MO stabilisées du compost de biodéchets apporté. L'apport de fumier entraine une légère augmentation du rapport C : N et une augmentation des composés dérivés de polysaccharides dans la fraction 0-50 µm.

En conclusion, les résultats acquis au cours de ce travail confirment la possibilité d'utiliser des méthodes analytiques et des approches cinétiques similaires à ceux définis à la base pour des études sur la dynamique du C dans le sol où les apports organiques sont essentiellement des résidus de cultures pour l'étude de la dynamique du C dans le sol suite à des apports de MOEs d'origines diverses. L'hypothèse sous jacente est que les compartiments du modèle RothC, qui peuvent être approchés par des fractions granulo-densimétriques de la MOS >50 µm et 0-50 µm ont des compositions et des comportements cinétiques comparables quelle que soit la nature des MOEs apportées. Cette hypothèse permet de décrire et de modéliser correctement l'accumulation du C dans le sol après des apports répétés de MOEs. Il en va de même pour les fractions séparées par le fractionnement biochimique Van Soest qui sont supposées de dégradabilité relativement homogène, ce qui permet le calcul d'indicateurs tels que l'indicateur de stabilité de la MO (ISMO) qui permet effectivement de décrire correctement le degré de stabilité des MOEs et qui peut être utilisé pour déterminer les paramètres décrivant la qualité des MOEs dans le modèle RothC.

Cependant, l'étude de la composition chimique de la MO accumulée dans ces fractions a révélé des différences notables de composition suivant les MOEs apportées indiquant que les apports de MOEs n'aboutissent pas tous à l'accumulation d'une MOS de dégradabilité semblable. Il en résulte des conséquences qui peuvent être importantes sur le niveau de réversibilité du stockage entrainé par les apports de MOEs qui peut être plus ou moins rapide suivant la composition et le niveau de stabilisation des MO accumulées dans le sol. Les MOP des sols ayant reçu des composts montrent un degré de récalcitrance supérieure par rapport aux MOP du sol témoin. La dégradabilité des MOP pourrait donc être influencée par la composition des MOEs apportées. De même la fraction soluble du fractionnement Van Soest ne présente pas la même degradabilité suivant qu'il s'agit d'un compost peu stabilisé ou d'un compost stabilisé. Il convient donc de rester prudent dans l'interprétation de ces compartiments et fractions.

Les résultats acquis dans le cadre de ce travail de thèse ont permis d'identifier plusieurs perspectives de recherches :

L'étude menée sur la biodégradabilité de la fraction soluble du fractionnement Van Soest suggère une extraction de MO stabilisée par complexation avec des métaux et éventuellement avec la phase minérale du compost mature (stabilisé). Aucune étude n'a été trouvée dans la littérature portant sur de tels mécanismes de stabilisations des MO au cours du compostage. Pourtant les interactions entre minéraux et MO sont reconnues comme étant des mécanismes majeurs de stabilisation des MO dans les sols. Il serait intéressant d'étudier précisément le rôle de ces interactions entre métaux, matière minérale et MO dans les composts d'autant plus qu'au cours du compostage, la matière minérale est concentrée au fur et à mesure de la dégradation de la MO jusqu'à atteindre près de 70% de la matière sèche du compost, il est donc possible que cette matière minérale joue un rôle dans la stabilisation de la MO des composts.

La nouvelle méthode mise au point pour extraire des composés similaires à ceux extraits dans la fraction soluble de la méthode Van Soest pourrait être utilisée pour d'autres type de composts afin de déterminer si la stabilisation de la MO au cours du compostage est liée à des évolutions similaires de la fraction soluble quelque soit la nature de déchets compostés ou quelle que soit le procédé de compostage.

La spectroscopie proche infra-rouge a montré un potentiel certain pour prédire diverses caractéristiques des MOEs et leur devenir dans les sols. Il est cependant difficile de déterminer à partir de quelles structures chimiques les caractéristiques des MOEs sont prédites. L'utilisation de la spectroscopie moyen infra-rouge par réflexion diffuse permet une meilleure interprétation des structures chimiques influentes dans les modèles de prédiction. Utilisée seule ou couplée à des spectres proche infra-rouge, la spectroscopie moyen infra-rouge pourrait permettre d'améliorer l'interprétation des liaisons chimiques influentes dans les modèles de prédiction. L'amélioration de la qualité de ces modèles serait alors facilitée. L'utilisation de la spectroscopie proche ou moyen infra-rouge pour la prédiction de la dégradabilité des MO dans les sols, ainsi que la prédiction directe de paramètres de modèles de dynamique des MO dans les sols sont des applications qu'il serait intéressant de développer. La spectroscopie proche ou moyen infra-rouge pourrait notamment être un moyen de mesurer une qualité continue de la matière organique du sol afin de vérifier les résultats de modèles de dynamique de la MOS s'appuyant sur ce type d'approche qui promettent une description améliorée de la dynamique de la MOS avec un nombre réduit de paramètres ayant une signification biologique mieux définie (Bruun et al., 2010). L'utilisation de la spectroscopie infra-rouge de terrain pour prédire, entre autre, les teneurs en C organique des sols est également un domaine en développement. Il serait intéressant de relier les informations spectrales obtenues sur le terrain aux spectres infra-rouge des MOEs épandues mesurés au laboratoire.

Le paramétrage du modèle RothC a permis de simuler de manière satisfaisante la dynamique du C du sol après des apports de MOEs et de développer des équations permettant de prédire les coefficients de partition des MOEs dans les compartiments d'entré du modèle à partir de caractérisations des MOEs au laboratoire. L'ajustement des paramètres a été réalisé à partir d'une méthode classique de minimisation des carrés des écarts entre valeurs mesurées et valeurs prédites. Ces ajustements pourraient être améliorés en appliquant des méthodes de calibration Bayesiennes permettant de prendre en compte l'incertitude liée aux mesures dans le processus de calibration afin de determiner l'incertitude liée aux paramètres ajustés et aux résultats des simulations. D'autre part, l'utilisation de modèles non linéaires permettrait une description plus précise de la dynamique de la MOS après des apports de MOEs en prenant en compte l'échelle et la localisation du C. Ce type de modèle permettrait notamment de prendre en compte les phénomènes de modification de la vitesse de décomposition de la MO native du sol par des apports de MOE (priming effect).

Les résultats obtenus dans le cadre de la caractérisation chimique des MO particulaires et des MO de taille 0-50 µm de la MOS ont montré des compositions chimiques différentes en fonction des MOEs apportées dans le cadre de l'essai Qualiagro. On peut déduire de ces compositions chimiques contrastées des niveaux de stabilité des MO contenues dans ces fractions également différentes mais qui n'ont pu être mesurés directement. Il serait intéressant de mesurer la minéralisation potentielle de ces fractions au cours d'incubations en fonction des différents types de MOEs apportées. Par ailleurs, l'utilisation d'autres techniques de caractérisation telle que la RMN pourrait permettre de préciser et de vérifier les résultats trouvés à l'aide de la pyrolyse-CG/SM et de la spectroscopie moyen infra-rouge en réflexion diffuse. D'autre part, les changements de composition chimique des fractions granulo-densimétriques des sols après apports de MOEs pourraient être comparés à la composition chimique de fractions-granulo-densimétriques des composts et des fumiers épandus. Une méthode de fractionnement sous eau adaptée de la méthode utilisée pour le fractionnement de la MOS mais optimisée pour le fractionnement des composts pourrait être utilisée. Cette méthode permet de séparer des fractions ayant des vitesses de dégradation différentes (Doublet et al., 2010). Cette comparaison entre composition chimique des fractions granulo-densimetriques des sols et des MOEs apportées devrait permettre de préciser les processus en jeu dans l'incorporation de la MO des MOEs dans la MO du sol.

Notre étude s'est limitée à la dynamique du C dans les sols, cependant, il semble important de considérer également la dynamique du N qui conditionne la dynamique du C dans les sols. L'ensemble des méthodes utilisées et développées dans le cadre de ce travail de thèse pourraient être adaptées pour étudier la dynamique du N dans les sols suite à des apports de MOEs.

La calibration du modèle RothC proposée pourrait être utilisée pour tester différents scénarios d'accumulation du C suites à des apports des MOEs de types divers à l'échelle de la parcelle agricole ou à l'échelle d'un territoire en relation avec les ressources de MOEs disponibles dans ce territoire. Les simulations obtenues pourraient être spatialisés à l'échelle de la région ou du territoire.

Il serait utile d'intégrer ces résultats dans des analyses de cycles de vie des pratiques de recyclage des déchets afin d'améliorer la prise en compte de la part de stockage de carbone potentiel dans les sols suite à des apports de MOEs et ainsi améliorer l'estimation du bilan environnemental du retour au sol des MOEs par rapport à d'autres modes de gestion.

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