Évaluation des changements quantitatifs et qualitatifs du stock de carbone du sol après l'application d'effluents d'élevage

Thèse

Émilie Maillard

Doctorat en sols et environnement
Philosophiae doctor (Ph.D.)

Québec, Canada

Résumé

Il est important de quantifier la réponse des stocks de carbone (C) du sol à l'application d'effluents d'élevage pour des enjeux agronomiques et environnementaux. Les objectifs de cette thèse étaient de : 1/ Mesurer la réponse des stocks de C du sol à l'application d'effluents d'élevage à partir d'études individuelles du monde entier et d'évaluer l'impact de différents facteurs (climat, propriétés du sol, utilisation des terres, caractéristiques de l'effluent) sur cette réponse; 2/ Évaluer l'influence du type de travail du sol et du système de culture sur la réponse des stocks de C après l'application de lisier; 3/ Déterminer l'impact à long terme de l'application de lisier sur le stock de C du sol et sur des formes de C de différents degrés de protection. Les résultats de la méta-analyse (chapitre 1) montrent qu'en moyenne à l'échelle globale, l'application d'effluents d'élevage mène à des stocks de C du sol plus élevés comparativement à la fertilisation minérale ou à un témoin non amendé. L'amplitude de la réponse du stock du C du sol à l'application d'effluents dépend principalement de la quantité de C apportée par l'effluent. Le climat a également influencé la réponse du stock de C mais son effet pourrait être confondu avec celui de la quantité totale de C apportée par l'effluent. À une échelle plus locale, 15 et 21 années d'application de lisier de bovins ont mené à des stocks de C plus élevés comparativement à un témoin non amendé (chapitre 3) ou à la fertilisation minérale (chapitres 2 et 3). Pour les deux sites, l'effet du lisier était limité à la surface du sol (20 et 30 premiers centimètres). Sur le site de Normandin (QC), la réponse du C du sol à l'application de lisier était différente selon la séquence de cultures avec une réponse plus forte pour la rotation à base de plantes pérennes que pour la monoculture de céréales. Sur le site d'Agassiz (CB), l'amplitude de la réponse du C du sol à l'application du lisier dépendait de la quantité de C apportée et cette réponse était atténuée lorsque cette application était combinée avec celle de la fertilisation minérale. De plus, l'application de lisier sur ce site a favorisé l'incorporation de C dans des fractions de matière organique relativement protégées.

Abstract

For both agronomic and environmental purposes, it is important to quantify the response of soil organic carbon (SOC) stocks to animal manure application. The objectives of this PhD thesis were : 1/ To quantify the response of SOC stocks to manure application from a large worldwide pool of individual studies, and to assess the impact of explanatory factors such as climate, soil properties, land use and manure characteristics; 2/ To determine the influence of tillage and cropping systems on the response of SOC stocks to the application of liquid dairy manure (LDM); 3/ To determine the impact of LDM on SOC stocks in the whole soil and specific physical fractions corresponding to different levels of protection. The meta-analysis (chapter 1) suggests that overall, at the global scale, animal manure application results in significantly larger SOC stocks compared to mineral fertilization or unamended control. The magnitude of SOC stock response to manure application depends mainly on the cumulative manure-C input. Climate also influenced the SOC stock response but its effect could not be decoupled from that of manure-C input. At a local scale, 15 and 21 years of LDM application resulted in significantly higher SOC stocks compared to an unamended control (chapter 3) or mineral fertilization (chapters 2 and 3). For both sites, the LDM effect was limited to the topsoil (down to 20- or 30-cm). In Normandin (QC), the magnitude of the SOC stock response to LDM was dependent on crop sequence, with a much greater effect of LDM application in SOC stocks in the perennial-based rotation than in the cereal monoculture (chapter 2). In Agassiz (BC), the magnitude of the SOC stock response was dependent on manure C input and this response seemed to be smaller with the combined application of LDM and mineral fertilization (chapter 3). In addition, LDM application favoured the incorporation of C in relatively protected fractions of organic matter.

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C : carbone, carbon
CO_2 : dioxyde de carbone
FLF: free light fraction
GHG : greenhouse gas
GIEC : Groupe d'experts Intergouvernemental sur l'Évolution du Climat
HCl : acide chlorhydrique
HF: heavy fraction
H_2O_2 : peroxide d'hydrogène
IALF: intra-aggregate light fraction
IPCC : Intergouvernmental Panel on Climate Change
LDM : liquid dairy manure
LF : light fraction
MO : matière organique
N : nitrogen
NaOCl : hypochloride de sodium
$Na_2S_2O_8$: peroxodisulphate disodium
NH_4^+ : ions ammonium
NO_3^- : ions nitrates
OC : organic carbon
PO_4^{3-} : ions phosphates
SCHF: silt+clay-size heavy fraction
SHF: sand-size heavy fraction
SO_4^{2-} : ions sulfates
SOC : soil organic carbon
TFA : acide fluoroacétique

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L'accomplissement de ce projet de doctorat n'aurait sans doute pas été possible sans le soutien et la collaboration de nombreuses personnes que je voudrais remercier ici.

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Avant-propos

Cette thèse comporte trois chapitres rédigés en anglais sous forme d'articles scientifiques pour publication. Je suis la première auteure de chacun de ces chapitres et j'ai réalisé seule ou en collaboration les étapes suivantes : 1/ la définition des enjeux et objectifs pour ces différents chapitres; 2/ la recherche bibliographique, la collecte de données, les analyses de laboratoire, le traitement des données et les analyses statistiques; 3/ l'interprétation et la discussion des résultats et 4/ la rédaction des manuscrits et la réalisation des tableaux et figures.

Le premier chapitre est une méta-analyse sur l'effet de l'application des effluents d'élevage sur les stocks de carbone du sol. Cet article a été rédigé en collaboration avec Denis Angers, chercheur au Centre de recherche et de développement sur les sols et les grandes cultures (Agriculture et Agroalimentaire Canada) à Québec, et professeur associé au département des sols et du génie agroalimentaire de la faculté des sciences de l'agriculture et de l'alimentation à l'Université Laval. Pour ce chapitre, j'ai effectué la recherche de littérature, collecté les données nécessaires pour la méta-analyse, réalisé les analyses statistiques, et enfin rédigé plusieurs versions de cet article en interprétant et discutant les résultats observés. Denis m'a guidée dans ce travail d'interprétation et de discussion et a corrigé plusieurs versions de cet article afin de l'améliorer constamment. Ce chapitre a été publié en 2014 dans la revue « Global Change Biology » sous le titre « Animal manure application and soil organic carbon stocks: a meta-analysis ».

Le deuxième chapitre concerne les effets de pratiques agricoles comme le travail du sol et l'implantation de cultures pérennes sur la réponse des stocks de carbone du sol à l'application de lisier de bovins sur le site de Normandin (Qc). Cet article a été rédigé en collaboration avec Denis Angers, Martin Chantigny, Jean Lafond, Denis Pageau, Philippe Rochette, Gabriel Lévesque, Nicole Bissonnette et Léon-Étienne Parent. Martin Chantigny et Philippe Rochette sont chercheurs au Centre de recherche et de développement sur les sols et les grandes cultures (Agriculture et Agroalimentaire Canada) à Québec. Gabriel Lévesque est adjoint de recherche au même endroit. Nicole Bissonnette était biologiste de recherche au Centre de recherche et de développement sur les sols et les grandes cultures (Agriculture et Agroalimentaire Canada) à Québec mais est aujourd'hui retraitée. Jean Lafond et Denis Pageau sont respectivement biologiste de recherche et chercheur scientifique au Centre de recherche et de développement sur les sols et les grandes cultures (Agriculture et Agroalimentaire Canada) à Normandin. Léon-Étienne Parent est professeur-chercheur au département des sols et du génie agroalimentaire de la faculté des sciences de l'agriculture et de l'alimentation à l'Université Laval. Pour ce chapitre, j'ai effectué une grande partie de la compilation des données, j'ai réalisé les analyses statistiques et rédigé plusieurs versions de cet article en interprétant et discutant les résultats observés.

Denis Angers m'a guidé dans cette étape d'interprétation et de discussion, a corrigé plusieurs versions de cet article pour l'améliorer et a notamment traduit en anglais toute la partie de « Résultats et Discussion ». Martin Chantigny a apporté des corrections à l'article et émis plusieurs suggestions pour l'améliorer. Léon-Étienne Parent a également effectué une révision de l'article et émis plusieurs commentaires pour l'améliorer. Nicole Bissonnette et Gabriel Lévesque ont réalisé les échantillonnages du sol à Normandin avec l'aide de Jean Lafond. Gabriel Lévesque a aussi effectué les analyses de carbone total au laboratoire. Nicole avait commencé la compilation des données avant son départ à la retraite. Philippe Rochette, Jean Lafond et Denis Pageau effectueront des révisions de l'article dans les prochaines semaines pour améliorer encore l'article avant une future soumission pour publication.

Le troisième chapitre concerne les effets de l'application de lisier de bovins sur les stocks de carbone du sol entier et dans des fractions physiques de différents degrés de protection, sur le site d'Agassiz (C.B.). Cet article a été rédigé en collaboration avec Denis Angers, Martin Chantigny, Shabtai Bittman, Philippe Rochette, Gabriel Lévesque, Derek Hunt et Léon-Étienne Parent. Shabtai Bittman et Derek Hunt sont respectivement chercheur scientifique et biologiste de recherche au Centre de recherches agroalimentaires du Pacifique (Agriculture et Agroalimentaire Canada) à Agassiz. Pour ce chapitre, j'ai effectué une bonne partie des analyses en laboratoire avec l'aide de Gabriel Lévesque et de deux étudiants. J'ai réalisé le travail de compilation des données, les analyses statistiques et j'ai ensuite rédigé plusieurs versions de cet article en interprétant et discutant les résultats observés. Denis Angers m'a guidée dans cette étape d'interprétation et de discussion et a corrigé plusieurs versions de cet article pour l'améliorer. Martin Chantigny a révisé deux versions de cet article et émis plusieurs suggestions de façon à l'améliorer. Léon-Étienne Parent a également effectué deux révisions de l'article et émis plusieurs commentaires pour l'améliorer. Shabtai Bittman a communiqué toute l'information nécessaire sur le site expérimental. Il a aussi corrigé l'anglais et a émis plusieurs commentaires pour améliorer l'article. Gabriel Lévesque a réalisé l'échantillonnage du sol à Agassiz avec l'aide de Derek Hunt et Shabtai Bittman. Philippe Rochette effectuera une révision de l'article dans les prochaines semaines. Ce chapitre sera soumis très prochainement pour publication dans la revue « Agriculture, Ecosystems and Environment ».

Introduction générale

Introduction

Étude de la réponse du carbone du sol à l'application d'effluents d'élevage : des enjeux agronomiques et environnementaux

Les effluents d'élevage (fumiers, lisiers, composts de ferme) sont utilisés couramment par les agriculteurs en tant qu'engrais. Par exemple, au Québec, environ 31 millions de tonnes d'engrais de ferme sont épandues chaque année sur les sols agricoles (Hébert *et al.*, 2010). Les agriculteurs peuvent également apporter des fumiers ou composts de ferme afin d'entretenir le niveau de matière organique (MO) (Clément *et al.*, 2010), étant donné que dans un agroécosystème, la plupart de la biomasse végétale est exportée et que les stocks de MO peuvent ainsi diminuer dans le temps.

Les amendements organiques améliorent, par la MO qu'ils apportent, l'ensemble des propriétés du sol et donc la fertilité et la qualité du sol (Soltner, 2000). En effet, la MO joue un rôle important dans le sol par ses multiples propriétés et fonctions (Stevenson, 1994). Sa couleur noire peut faciliter le réchauffement du sol par absorption des rayons solaires. Elle possède une forte rétention en eau et contribue ainsi à prévenir l'assèchement. Elle interagit avec les minéraux argileux, favorisant la formation d'agrégats. Elle peut ainsi améliorer les échanges gazeux, stabiliser la structure du sol et augmenter la perméabilité à l'eau. Elle tamponne la disponibilité des éléments mineurs pour les plantes supérieures. La MO tamponne également le pH du sol. Elle augmente la capacité d'échange cationique du sol. La décomposition de la MO permet le relâchement de dioxyde de carbone (CO_2), ainsi que des ions ammonium (NH_4^+), nitrates (NO_3^-), phosphates ($H_2PO_4^-$ et HPO_4^{2-}) et sulfates (SO_4^{2-}). Ainsi, la MO représente une source d'éléments nutritifs pour la croissance des plantes et augmente la diversité microbienne. Étant donné le lien entre les engrais de ferme et la MO, il existe donc un enjeu agronomique à déterminer l'impact de l'application d'effluents d'élevage sur le carbone (C) du sol, principal composant de la MO.

Selon le Groupe d'experts Intergouvernemental sur l'Évolution du Climat (2007), la concentration en CO_2 atmosphérique était de 379 ppm en 2005. Ce niveau n'avait jamais été atteint durant les 450 000 dernières années (Janzen, 2004). De plus, le taux d'accumulation moyen du CO_2 dans l'atmosphère semble augmenter ces dernières années. En effet, le taux est passé de 3.2 Gt C/an dans les années 1990 à 4.1 Gt C/an entre 2000 et 2005. L'augmentation rapide de la concentration en CO_2 atmosphérique ainsi que son niveau élevé laissent penser que le cycle du C est perturbé. Ces perturbations s'expliquent en grande partie par l'ajout de quantités élevées de CO_2 dans l'atmosphère suite au changement de l'utilisation du paysage et à la combustion de C fossile (Scholes *et al.*, 1999). La majeure partie de l'augmentation de la température moyenne du globe observée depuis le milieu du XX^{ième} siècle est très probablement attribuable à l'augmentation des concentrations de gaz à effet de serre de sources anthropiques (GIEC, 2007). L'augmentation du stockage de C dans les puits naturels tels que la végétation et les sols est une des mesures envisagées pour atténuer l'accumulation de CO_2 atmosphérique (Janzen, 2004). Parmi l'ensemble des réservoirs de C, les sols représentent un réservoir non négligeable. Il s'agit en effet du troisième réservoir après les océans et le réservoir géologique (Lal, 2004). Étant donné que les sols contiennent environ trois fois plus de C que le réservoir atmosphérique (Lal, 2004), une augmentation relativement mineure de ce réservoir pourrait contrebalancer de façon significative l'augmentation de la concentration en CO_2 atmosphérique (Fontaine *et al.*, 2004). À l'opposé, une diminution du réservoir pourrait augmenter de manière assez conséquente la concentration en CO_2 atmosphérique. Dans ce contexte, il apparaît donc pertinent de déterminer l'effet des pratiques agronomiques sur le C du sol afin d'identifier celles pouvant mener à une augmentation ou à une diminution des stocks de C du sol. De plus, il y a aussi un intérêt à connaître la persistance du stockage de C dans le sol.

Limites des estimations des stocks de C après l'application d'effluents d'élevage

Le contenu en C organique du sol est traditionnellement exprimé en concentration (g C kg^{-1} sol) ou en stock (kg C m^{-2} ou Mg C ha^{-1}). La connaissance de l'impact de l'application d'effluents d'élevage sur les stocks de C du sol est pertinente au niveau agronomique pour l'estimation de coefficients de rétention du C des effluents notamment dans les calculs de bilans humiques. L'agronome ou l'entreprise agricole peut avoir besoin de réaliser un bilan humique du sol afin de calculer la quantité d'amendements organiques nécessaire pour maintenir les sols à la teneur en MO désirée (Clément *et al.,* 2010). Cette approche nécessite l'utilisation de coefficients isohumiques (ou coefficients de rétention) de la MO ajoutée. Hénin et Dupuis (1945) ont défini le coefficient isohumique comme la fraction de la MO apportée qui a été convertie en MO plus stable. Ils proposaient alors un coefficient de 0.30 pour le fumier. Différentes méthodes sont utilisées pour estimer les coefficients d'humification des résidus de plantes : expériences à long terme, techniques d'abondance naturelle des isotopes ^{13}C, incubations avec des techniques de marquage ^{13}C et ^{14}C, estimations indirectes d'après les propriétés biochimiques des résidus apportés (Kätterer *et al.,* 2011). Ces méthodes peuvent aussi être utilisées dans le cas des effluents d'élevage. Par exemple, certains auteurs ont calculé un coefficient de rétention du C à partir d'expériences de longue durée (Triberti *et al.*, 2008). Dans le guide de référence en fertilisation du centre de référence en agriculture et agroalimentaire du Québec, des coefficients isohumiques sont proposés (Tableau I) (Clément *et al.,* 2010). Ces coefficients ont déjà été présentés par Soltner (2000). Mais la méthode de détermination de ces coefficients n'est pas décrite. De plus, les coefficients sont classés selon l'origine animale des effluents mais ce facteur n'est probablement pas le seul à influencer le coefficient de rétention. Qu'en est-il par exemple du climat, du sol, de la dose d'application de l'effluent, de l'alimentation du bétail et de la composition biochimique de l'effluent ? Les connaissances actuelles sur les coefficients isohumiques des effluents paraissent donc assez limitées.

Au niveau environnemental, la connaissance de la réponse des stocks de C du sol à l'application d'effluents d'élevage est également nécessaire à une plus grande échelle afin d'améliorer la comptabilisation des stocks de C dans le cadre des inventaires de gaz à effet de serre. Le GIEC (2007) propose une méthode simple de comptabilisation des stocks de C pour les pays qui ne disposent pas d'un programme spécifique d'inventaire des gaz à effet de serre. Cette méthode est bien décrite par Ogle *et al.* (2005) dans leur méta-analyse sur les impacts des pratiques agricoles sur le stockage du C organique du sol. Le stock de C du sol est calculé selon l'équation suivante : SOC = RC * BF * TF * IF * LA, où RC correspond au stock référence de C, BF au coefficient de base (stockage relatif de C par rapport à un système naturel), TF est le coefficient du travail du sol, IF est le coefficient d'apport de C et LA, la superficie pour un système d'utilisation du sol particulier. Plus précisément, RC est la quantité de C organique du sol stocké sous des conditions naturelles. Le coefficient BF représente le changement dans le stock du C du sol occasionné par la mise en culture de ces terres pendant au moins 20 ans. Parmi les coefficients additionnels utilisés pour modifier le stockage du C dans un sol cultivé, les coefficients d'entrée de C (IF) permettent d'estimer l'effet relatif d'une augmentation ou d'une diminution des apports de C au sol. Ainsi, le GIEC propose des coefficients d'augmentation des stocks de C organique du sol suite à l'apport de MO et en particulier d'effluents d'élevage variant de 1.37 à 1.41 (Tableau II). Ogle *et al.* (2005) avaient proposé des estimés similaires pour l'effet de l'apport d'amendements organiques. Ils estimaient que des rotations avec des apports élevés d'amendements organiques augmentaient le stockage dans les premiers 30 cm du sol d'un coefficient de 1.34±0.08 et 1.38±0.06 pour des climats sec et humide, respectivement. Les estimés du GIEC (2007) ou de Ogle *et al.* (2005) présentent certaines limites. Ils sont proposés pour différents climats mais en considérant l'erreur, on s'aperçoit que la différence n'est pas significative, ce qui revient en fait à proposer un seul coefficient quel que soit le climat. De plus, ces estimés reposent sur moins de 10 études, offrant une faible représentativité des conditions existant dans le monde. Ensuite, la dose d'application et plus précisément la quantité de C apporté par l'effluent n'est pas considérée.

Il apparaît donc nécessaire de raffiner les données utilisées actuellement, que ce soit pour les bilans humiques ou pour les inventaires de gaz à effet de serre. Pour cela, il est nécessaire d'étudier les sources de variabilité des effets de l'application d'effluents d'élevage sur les stocks de C du sol et de réaliser des études portant sur l'application d'effluents d'élevage dans différents contextes.

Variabilité des impacts de l'application d'effluents d'élevage sur les stocks de C du sol

Dans la littérature, les impacts de l'application d'effluents d'élevage sur les stocks de C du sol sont variables en direction et en amplitude. Pour la plupart des études, une augmentation des stocks de C a été constatée dans les parcelles soumises à une application répétée d'effluents en comparaison aux parcelles recevant la fertilisation minérale recommandée ou aux parcelles témoins. Par exemple, après 10 ans d'application de litière de volaille, le contenu en C organique était plus élevé de 3.2 Mg C/ha dans la couche superficielle 0-20 cm par rapport au contenu des parcelles soumises à la fertilisation minérale recommandée dans le sud-est des États-Unis (Sainju *et al.*, 2008a). Au Népal, Gami *et al.* (2009) ont observé que les stocks de C de la couche 0-30 cm étaient en moyenne plus élevés de 19.1 Mg C/ha dans les parcelles ayant reçu des applications annuelles de fumier de bovins (12 Mg/ha/an sur une base de matière sèche) pendant 25 années consécutives par rapport aux parcelles témoins non amendées. En Chine, après 22 années d'application de fumier de porcs à une dose de 15 Mg/ha/an (sur une base de matière fraîche), les stocks de C dans la couche 0-15 cm du sol étaient supérieurs de 3.8 Mg C/ha par rapport à ceux des parcelles sous fertilisation minérale (Huang *et al.*, 2010a). Au Kenya, Kamoni *et al.* (2007) ont observé une différence de 8 Mg C/ha entre les stocks de C dans la couche 0-20 cm des parcelles ayant reçu un fumier de bovins et ceux des parcelles soumises à la fertilisation minérale après 19 années. L'augmentation des stocks de C dans le sol peut être le résultat d'un ajout direct de C provenant de l'effluent lui-même ou d'un apport indirect de C suite à une éventuelle augmentation du rendement des racines et des résidus de culture (Aoyama *et al.,* 1999, Bhattacharyya *et al.*, 2010). En observant les résultats des études rapportant une augmentation des stocks de C après l'application d'effluents d'élevage, on remarque qu'il existe une grande variabilité dans l'amplitude de l'augmentation. Parmi les études rapportant une augmentation des stocks de C du sol, certaines avaient testé l'effet de doses d'application d'effluents très supérieures aux doses utilisées dans la pratique (Benbi and Senapati, 2010, Gami *et al.*, 2009).

Quelques études ont noté une absence d'augmentation des stocks de C, voire même une diminution de ces stocks. Dans une expérience menée aux États-Unis, Franzluebbers *et al.* (2001) ont émis l'hypothèse que l'absence d'effet de l'application de litière de volaille sur les stocks de C du sol était liée au faible taux d'application (5.4 t/ha sur une base de matière sèche). Au Québec, Angers *et al.* (2010) ont supposé que l'absence d'effet sur les stocks de C du sol après l'application de lisier de porcs était liée à la composition de ce lisier, riche en acides gras volatiles rapidement minéralisés. Ils ont également avancé l'hypothèse que l'ajout de lisier a pu accélérer la décomposition du C déjà présent dans le sol (« priming effect »). Cette dernière hypothèse avait également été avancée par Morari *et al.* (2006) après leur observation d'une baisse des stocks de C du sol en remplaçant le fumier solide de ferme par du lisier.

L'absence d'effet significatif après l'application d'effluents peut également être en lien avec une grande variabilité des stocks de C à l'intérieur d'un même dispositif (Viaud *et al.,* 2011).

De façon générale, la quantité de C organique du sol va varier selon la quantité de MO qui retourne au sol (Johnston *et al.,* 1989, Mandal *et al.*, 2008), le taux de dégradation de cette MO et du C existant (Johnston *et al.,* 1989), le climat (Dersch and Bohm, 2001, Johnston *et al.*, 1989, Mandal *et al.,* 2008, Triberti *et al.*, 2008) et les propriétés du sol (Mandal *et al.*, 2008, Triberti *et al.,* 2008). Plus précisément, certains auteurs rapportent un effet de la dose d'application de l'effluent animal. À une faible dose, il peut y avoir absence d'effet d'application d'effluents sur les stocks de C (Franzluebbers et al. 2001). Dans leur revue de littérature sur l'effet de l'application d'effluents d'élevage, Khaleel *et al.* (1981) ont conclu qu'il était difficile d'établir une relation simple entre la variation de C organique du sol et le C appliqué. Les résultats sont variables après l'augmentation d'une dose donnée d'application d'effluent. En effet, en doublant la dose de fumier de bovins dans une expérience menée aux États-Unis, Lee *et al.* (2007) ont observé une différence deux fois plus élevée entre les stocks de C des parcelles recevant le fumier et ceux des parcelles soumises à une fertilisation minérale. Par contre, Sainju *et al.* (2008a) ont remarqué une absence d'augmentation de l'accumulation de C du sol après le doublement de la dose d'application de litière de volaille dans une expérience aussi menée aux États-Unis. L'augmentation des stocks de C avec la dose d'effluent n'est donc pas forcément linéaire. D'autres caractéristiques de l'effluent (origine animale, type : solide ou liquide) pourraient également expliquer une partie de la variabilité de l'effet de l'application d'effluents. On a vu précédemment que l'absence d'effet sur les stocks de C du sol après l'application de lisier de porcs pouvait être attribuée au contenu de ce lisier riche en acides gras volatiles rapidement minéralisés (Angers *et al.*, 2010). Le fumier représente un mélange d'excréments avec de la paille ou d'autres matériaux de litière. Le fumier contiendrait alors plus de composés de C récalcitrants que le lisier qui est un mélange des excréments avec de l'eau (Bertora *et al.,* 2009). Dans une expérience menée en Italie, Grignani *et al.* (2007) ont observé que le lisier de bovins augmentait moins le contenu en C organique du sol que le fumier de bovins. De même, Triberti *et al.* (2008) ont avancé l'hypothèse que la MO du lisier de bovins se dégraderait plus rapidement que celle du fumier de bovins pour expliquer les coefficients de rétention du C de l'effluent dans le sol de 3.8% pour le lisier et 8.1% pour le fumier.

Quelques auteurs ont aussi observé un effet des propriétés du sol (texture, teneur initiale de C dans le sol) sur la capacité d'accumulation de C après l'application d'effluents. Gami *et al.* (2009) ont observé des stocks de C plus élevés dans le dispositif de Bhairahawa au Népal que dans celui de Parwanipur. Ils ont avancé l'hypothèse que ce résultat était lié à la différence de texture de sol entre les sites, le sol de Bhairahawa contenant plus d'argiles. Dans une expérience menée en Italie, Morari *et al.* (2006) ont observé que le coefficient de rétention du C était plus élevé dans un sol argileux que dans un sol sableux. En

Autriche, Dersch et Bohm (2001) ont observé une augmentation moyenne du C organique du sol, suite à l'application additionnelle de fumier, plus élevée sur le site où le niveau de C initial était le plus faible.

Le climat (température et humidité) est souvent avancé pour expliquer la variabilité des effets de l'application d'effluents d'élevage. Les sols des régions tropicales, subtropicales, arides ou semi-arides dégageraient plus de CO_2 par unité de C organique du sol comparativement aux sols de régions plus froides ou tempérées, en raison notamment des plus fortes températures (Mandal *et al.,* 2007). En effet, le taux de décomposition de la MO du sol et de la MO appliquée serait favorisé. De même, pour Triberti *et al.* (2008), dans les climats les plus chauds, on peut s'attendre à une décomposition accélérée de la MO du sol mais seulement en présence d'une disponibilité en eau suffisante. En Inde, Benbi et Senapati (2010) ont observé une faible rétention du C appliqué malgré des doses d'application du fumier 2 à 3 fois supérieures aux doses utilisées par les producteurs. Ils ont relié ce résultat au climat très chaud et humide de la région qui a pu favoriser l'oxydation rapide du C organique du sol. Su *et al.* (2006) ont remarqué que la séquestration du C organique du sol après l'application de fumier était plus importante en régions arides qu'en régions humides. Dans une revue de littérature sur l'effet de l'apport d'effluents d'élevage sur le C du sol, Franzluebbers et Doraiswamy (2007) soulignent que le régime de température semble avoir plus d'impact sur la rétention du C que le régime de précipitation. En effet, ils ont calculé que 23±15% du C appliqué provenant d'un effluent animal était retenu au sol dans les régions tempérées ou froides et seulement 7±5% était retenu dans les régions chaudes. Le sol des régions humides retenait 8±4% du C appliqué alors que le sol des régions sèches en retenait 11±14%.

Certains paramètres liés à la culture (nombre de cultures par année, espèces cultivées) peuvent occasionner une variabilité de l'impact de l'application d'effluents d'élevage. En effet, la quantité de C organique du sol dépend également du renouvellement annuel des résidus de racines, de leurs exsudats et des chaumes (Regmi *et al.,* 2002). Kukal *et al.* (2009) ont observé un taux de séquestration du C plus élevé dans un système riz-blé que dans un système maïs-blé. Ils expliquaient ce résultat par la production de biomasse plus importante du riz. On peut supposer que l'effet de la culture devrait être limité dans le cas où le taux de séquestration du C est calculé par rapport à une référence qui aurait un rendement équivalent au rendement des parcelles recevant l'effluent. Il faut également souligner l'effet de la culture du riz dans des conditions aquatiques sur l'accumulation de C dans le sol. Ces conditions pourraient retarder l'oxydation et entraîner une accumulation de la MO (Mandal *et al.*, 2008).

D'autres facteurs peuvent faire varier l'effet de l'application de l'effluent sur les stocks de C du sol : profondeur de sol prise en compte pour le calcul des stocks de C, effluent appliqué seul ou avec fertilisation minérale, choix de la référence pour calculer les différences de stocks de C (témoin ou fertilisation minérale). Certaines pratiques comme le travail du sol ou l'implantation de cultures pérennes peuvent également avoir un impact sur la réponse des stocks du C du sol à l'application d'effluents d'élevage. Des stocks de C plus élevés à la surface du sol sont généralement observés après un travail du sol réduit en comparaison à un travail conventionnel avec labour (West and Post, 2002). Globalement, des systèmes de culture basés sur des plantes pérennes mènent à des augmentations de C organique du sol (Bell *et al.,* 2012), alors que les monocultures conventionnelles peuvent occasionner une perte de matière organique du sol (Fageria, 2012). Cependant, peu d'études de longue durée se sont intéressées aux effets combinés de l'application d'effluents d'élevage et d'autres pratiques agricoles comme le travail du sol (Sainju *et al.,* 2008b, Viaud *et al.*, 2011) ou le système de culture (Holeplass *et al.,* 2004).

Il est pertinent de relever aussi que peu d'attention est accordée aux couches profondes (>30 cm) du profil de sol dans les études sur les effets de pratiques agronomiques sur stocks de C du sol (Syswerda *et al.*, 2011). Le C des couches plus profondes peut avoir différentes origines : MO dissoute, produits racinaires, particules transportées depuis la surface (Schmidt *et al.*, 2011). Le labour peut contribuer à la redistribution du C de la surface vers les horizons plus profonds (Angers and Eriksen-Hamel, 2008). L'étude des horizons plus profonds est particulièrement pertinente dans le cas des lisiers. En effet, l'application de lisier peut augmenter le C soluble (Angers *et al.,* 2006, Briceño *et al.*, 2008) qui pourrait ensuite migrer dans les horizons les plus profonds (Royer *et al.*, 2007).

Stabilité du C du sol et fractions de la MO

L'étude de la réponse des stocks de C du sol à l'application d'effluents d'élevage ne renseigne pas sur la stabilité et la persistance du C dans le sol. Le C organique du sol n'est pas homogène en termes de propriétés et de stabilité et il peut être divisé conceptuellement en différentes fractions avec des durées de vie variables (Heitkamp *et al.*, 2011). Il y a pourtant un intérêt à connaître l'effet de l'application d'effluents d'élevage sur les formes de C du sol et en particulier sur les formes relativement stables dans le contexte environnemental.

La stabilité de la MO et du C organique est liée à trois caractéristiques de la MO qui sont sa récalcitrance, ses interactions intermoléculaires et son accessibilité (Sollins *et al.,* 1996). La récalcitrance réfère à la complexité des caractéristiques moléculaires d'un composé organique. Les interactions intermoléculaires sont établies entre des composés organiques, ou entre des composés organiques et inorganiques. L'accessibilité d'un composé organique réfère à sa localisation par rapport à celle des microorganismes et des enzymes. La stabilité augmente avec la récalcitrance et les interactions intermoléculaires, ces deux caractéristiques ayant tendance à diminuer le taux de dégradation des composés organiques par les microorganismes et les enzymes. La stabilité diminue avec l'accessibilité. En effet, plus la MO est accessible aux microorganismes et aux enzymes et plus elle risque d'être dégradée. La contribution relative de ces différents facteurs (associations avec les minéraux, récalcitrance, piégeage dans les agrégats) à la stabilisation de la MO est encore inconnue (Mikutta *et al.*, 2006). Mais de récentes découvertes semblent indiquer que la capacité de maintien de la MO (stabilité) dans le sol pendant des millénaires ne serait pas tant une propriété moléculaire de la MO mais plutôt une propriété de l'écosystème (Schmidt *et al.,* 2011). En effet, la MO pourrait se maintenir dans le sol en raison de facteurs physico-chimiques et biologiques de l'environnement qui réduiraient la probabilité de décomposition. Six *et al.* (2002) ont proposé un modèle conceptuel des dynamiques de la MO dans le sol reposant sur trois différents processus de stabilisation (Figure I). La stabilisation chimique consiste en la formation de liaisons chimiques ou physico-chimiques entre la MO et les particules minérales du sol. Il y a donc établissement d'interactions intermoléculaires lors de cette stabilisation. La stabilisation physique consiste en la formation d'agrégats avec séparation de la biomasse microbienne et de la MO, et avec changement qualitatif de la MO (préservation des composés organiques les plus récalcitrants). Il y a donc établissement d'interactions et diminution de l'accessibilité des composés organiques. Enfin, la stabilisation biochimique représente la complexification de la composition chimique des composés organiques (propriété du matériel végétal d'origine ou propriété atteinte au cours de la décomposition). Cela fait référence à la récalcitrance de la MO. Plusieurs réservoirs de stabilité variable sont représentés sur la figure I : C du sol non protégé, C du sol physiquement protégé (C associé aux micro-agrégats, C associé aux limons et argiles), C du sol biochimiquement protégé (non hydrolysable). On peut considérer aussi que le C associé aux limons et argiles est chimiquement protégé. Les principaux mécanismes de protection et de libération de la MO du sol sont : formation et dégradation des agrégats, adsorption et désorption, condensation et complexation (Six *et al.*, 2002). Les différents réservoirs peuvent se chevaucher. En effet, la fraction non hydrolysable peut ne pas être seulement stabilisée biochimiquement et être également partiellement stabilisée par association avec les particules minérales. La MO liée aux particules minérales peut aussi être incorporée dans des agrégats.

Six *et al.* (2002) ont établi un schéma de fractionnement précis en laboratoire pour mesurer ces différents réservoirs. En réalité, il existe plusieurs méthodes de fractionnement de la MO du sol permettant d'isoler des fractions de MO plus ou moins stables. Le fractionnement physique du sol par densité ou par la taille des particules permet d'isoler respectivement la fraction légère de la MO et la MO particulaire non liées aux particules minérales du reste de la MO qui serait, elle, protégée physiquement (Gregorich and Beare, 2007). Suivant la teneur en argile du sol, on peut également fractionner le sol selon différentes tailles d'agrégats et s'intéresser à la teneur en C dans ces différents agrégats. La MO au sein des agrégats du sol aurait un taux de décomposition plus faible que la MO à l'extérieur (Li and Zhang, 2007). La fraction de C protégée physiquement par des associations avec limons et argiles dans les micro- (<250 µm) et macro-agrégats (>250 µm) peut également être étudiée. Dans la littérature, il existe une grande variabilité des protocoles de fractionnement physique suivant les objectifs des articles. Par exemple, Laganière *et al.*

(2011) ont proposé un protocole de fractionnement physique permettant de comparer la protection physique du C dans le sol de différents peuplements (Figure II). Ce protocole combinait un fractionnement par la taille des agrégats, un fractionnement densimétrique et un fractionnement granulométrique. Les agrégats étaient également dispersés afin de récupérer la MO intra-agrégats. Virto *et al.* (2008) ont utilisé un protocole plus complexe afin d'analyser les formes de C dans des micro-agrégats inférieurs à 50 µm (Figure III). Leur protocole de fractionnement physique par taille et par densité a permis de mesurer dans un premier temps le C de ces micro-agrégats et après la dispersion de ces agrégats, ils ont pu isoler le C de la MO piégée dans ces micro-agrégats et le C de la MO adsorbée aux surfaces des particules minérales de la taille d'argiles et de limons. Sohi *et al.* (2001) ont proposé un protocole de fractionnement densimétrique et granulométrique moins complexe permettant d'isoler des fractions de MO du sol correspondant à différents niveaux de protection physique de C du sol. Cette méthode permet d'isoler une fraction légère de la MO intra-agrégats et permet aussi de séparer la fraction lourde du sol.

Des fractionnements chimiques de la MO peuvent également être réalisés dans le but d'isoler une fraction de C biochimiquement protégé : hydrolyse avec l'acide fluoroacétique (TFA) ou l'acide chlorhydrique (HCl), traitements d'oxydation avec le peroxyde d'hydrogène (H_2O_2), le peroxodisulphate de sodium ($Na_2S_2O_8$) ou l'hypochlorite de sodium (NaOCl) (Helfrich *et al.,* 2007). L'objectif de ces fractionnements est d'obtenir une fraction de C organique stable analogue au réservoir inerte de MO des principaux modèles de dynamique du C organique dans le sol. Helfrich *et al.* (2007) ont comparé l'efficacité de plusieurs méthodes de fractionnement chimiques à isoler un réservoir de C organique stable du sol. Aucune méthode n'a permis d'obtenir une fraction de C analogue au réservoir inerte du modèle de carbone de Rothamsted. Cependant, la fraction de C isolée était plus âgée, plus stable et les traitements avec les oxydants chimiques H_2O_2 et $Na_2S_2O_8$ étaient les plus efficaces. Ces traitements d'oxydation seraient semblables à la biodégradation en enlevant de façon préférentielle le C organique moins protégé et plus récent et en sauvegardant des composés plus vieux et plus associés aux surfaces minérales. Cependant, les fractionnements physiques de la MO pourraient être des indicateurs plus efficaces de différents réservoirs fonctionnels (Wander and Traina, 1996) et sont beaucoup moins destructifs que dans le cas des méthodes chimiques classiques de fractionnement (Assis *et al.*, 2012).

La MO particulaire et la fraction légère de la MO sont considérées comme des fractions labiles du C (Yan *et al.*, 2007). Comparativement à ces fractions, le C de la MO piégée dans les agrégats serait physiquement protégé de la dégradation microbienne. À l'intérieur des agrégats, le C associé aux particules minérales serait encore mieux protégé (Laganière *et al.,* 2011). On suppose donc que ces fractions vont être plus stables que les fractions labiles.

Des impacts de l'application d'effluents d'élevage mesurés majoritairement sur les fractions labiles du C du sol

L'effet de l'application d'effluents d'élevage sur les fractions non protégées du C (MO particulaire, fraction légère libre) a été relativement bien étudié, étant donné la relation étroite entre ces fractions et la qualité et la fertilité des sols. Dans une expérience menée en Inde, la concentration en C organique particulaire était plus élevée après 32 ans d'applications combinées de fumier de ferme et de fertilisants minéraux, en comparaison aux parcelles témoins (Rudrappa *et al.,* 2006). En Chine, l'application de fumier de ferme pendant 25 ans a également augmenté la concentration en C de la MO particulaire par rapport aux parcelles sous fertilisation minérale et témoins (Yan *et al.,* 2007). Par contre, ces derniers n'ont pas observé d'accumulation du C de la fraction légère de la MO. D'autres auteurs ont pourtant remarqué une augmentation du C de la fraction légère de la MO. Par exemple, dans une autre expérience menée en Chine, la masse de C de la fraction légère a augmenté avec l'application de fumier de ferme pendant 20 ans (Yang *et al.,* 2007). Dans une autre expérience menée en Chine, la concentration en C organique dans la fraction légère fut la plus élevée dans les parcelles recevant du fumier de ferme pendant 20 ans (Wu *et al.*, 2004). Peu d'études se sont intéressées aux effets des effluents d'élevage sur les fractions plus stables comme la MO associée aux minéraux ou celle piégée dans les micro-agrégats (Hai *et al.*, 2010, Huang *et al.*, 2010a, Huang *et al.,* 2010b, Wu *et al.,* 2005). Des études sur le C et l'agrégation ont soulevé des questions sur les mécanismes de stabilisation du C après l'application d'effluents d'élevage. Plusieurs études ont montré que le C organique était préférentiellement piégé dans les macro-agrégats plutôt que dans les micro-agrégats avec l'application d'effluents d'élevage. Par exemple, l'apport de fumier de ferme pendant 26 ans a significativement augmenté les concentrations et les stocks de C dans les macro-agrégats dans une expérience menée en Chine (Huang *et al.*, 2010b). En Afrique, Adesodun et Odejimi (2010) ont observé qu'il y avait plus de C dans les macro-agrégats que dans les micro-agrégats après 3 années d'application de compost porcin quel que soit le taux d'application. En Chine, la plupart du C organique du sol était stocké dans les macro-agrégats dans les parcelles recevant du fumier de ferme pendant 22 ans (Su *et al.,* 2006). En Inde, après 7 années d'application de fumier de ferme, l'accumulation de C dans les agrégats du sol était plus importante de façon générale mais elle était plus élevée dans les macro-agrégats que dans les micro-agrégats (Benbi and Senapati, 2010). Lors de l'incorporation du fumier au sol, la MO particulaire se décomposerait graduellement pour produire de la biomasse microbienne, des produits métaboliques et des substances humiques associées aux particules de limons et d'argiles (Aoyama *et al.,* 1999). Pendant la décomposition, la MO particulaire serait progressivement incorporée dans le réservoir associé aux minéraux. En utilisant le marquage avec les isotopes ^{13}C et ^{15}N, Angers *et al.* (1997) ont montré que les composés de C nouvellement incorporés se retrouvaient très vite dans les macro-agrégats.

Au final, l'effet de l'application d'effluents d'élevage sur les fractions labiles a été beaucoup étudié. Ces fractions labiles sont importantes pour la qualité du sol, les dynamiques de la MO et l'activité microbienne. Et globalement, étant donné que la plupart des fractions labiles semblent augmenter avec l'application d'effluents d'élevage, l'apport d'effluents entretient bien la qualité du sol. Cependant, l'effet des effluents sur le C des fractions intermédiaires et stables a été moins étudié, alors que ces fractions ont un intérêt dans le contexte environnemental puisque ce C pourrait être piégé pendant plus longtemps. La connaissance des effets de l'application d'effluents d'élevage sur les différentes fractions de la MO pourrait permettre d'émettre des hypothèses sur les mécanismes de stabilisation de la MO dans le sol.

Problématique

Plusieurs limites sur les connaissances actuelles des effets de l'application d'effluents d'élevage sur le stockage du C du sol et sur sa stabilité relative peuvent être soulevées. Les effets de l'application d'effluents d'élevage sont variables dans le sens et dans l'amplitude. Plusieurs facteurs comme les caractéristiques de l'effluent d'élevage (dose, composition, type : solide/liquide, …), les propriétés du sol, le climat, etc. peuvent influencer les effets de l'application d'effluents d'élevage. Le nombre d'études permettant d'estimer les coefficients isohumiques n'est pas connu et moins de 10 études ont contribué à l'estimation des coefficients d'augmentation des stocks de C dans les recommandations du GIEC (2007). À une échelle globale, il paraît nécessaire de raffiner ces coefficients pour des enjeux agronomiques et environnementaux en identifiant les facteurs les plus importants agissant sur la réponse des stocks de C à l'application d'effluents. À une échelle plus locale, il est important d'approfondir les connaissances sur les facteurs de variation en étudiant notamment l'effet de pratiques réalisées en combinaison avec l'application d'effluents d'élevage comme par exemple le travail du sol ou le type de cultures sur la réponse du C du sol. De plus, les couches les plus profondes devraient être considérées, particulièrement dans le cas des lisiers. Ensuite, peu d'études se sont intéressées à l'effet de l'application d'effluents d'élevage sur les formes stables alors que ces formes sont très pertinentes au niveau environnemental et que leur réponse à l'application d'effluents peut permettre de mieux comprendre les mécanismes de stabilisation du C de l'effluent.

En réponse à ces questions soulevées, les objectifs de cette thèse de doctorat sont de :

1/ Mesurer la réponse des stocks de C du sol à l'application d'effluents d'élevage à partir d'études individuelles du monde entier et évaluer l'impact de différents facteurs (climat, propriétés du sol, utilisation des terres, caractéristiques de l'effluent) sur cette réponse.

2/ Évaluer l'influence du type de travail du sol et du système de culture sur la réponse des stocks de C après l'application de lisier de bovins.

3/ Déterminer l'impact à long terme de l'application de lisier de bovins sur le stock de C du sol et sur des fractions physiques spécifiques du C du sol de différents degrés de protection.

Principales hypothèses

L'objectif 1 sera abordé au chapitre 1. Les hypothèses suivantes peuvent être posées :

1.1. En moyenne, à l'échelle globale, l'application d'effluents d'élevage augmente les stocks de C comparativement à un témoin non amendé et à la fertilisation minérale.

1.2. Le stock de C du sol augmente de façon linéaire avec la quantité de C apportée par l'effluent.

1.3. L'augmentation des stocks de C est plus importante dans les régions froides ou tempérées que dans les régions chaudes.

1.4. L'augmentation des stocks de C est plus élevée après l'application d'effluents d'origine bovine qu'après l'application d'effluents d'origine porcine étant donné les différences de régime alimentaire et de digestion.

L'objectif 2 sera abordé au chapitre 2. Les hypothèses suivantes peuvent être posées :

2.1. L'augmentation des stocks de C du sol après l'application de lisier de bovins est plus élevée avec un travail du sol réduit qu'avec un travail du sol conventionnel.

2.2. L'augmentation des stocks de C du sol après l'application de lisier de bovins est plus élevée avec l'implantation de cultures pérennes qu'avec des cultures annuelles continues.

L'objectif 3 sera abordé au chapitre 3. Les hypothèses suivantes peuvent être posées :

3.1. Les stocks de C du sol sont plus élevés suite à l'application de lisier de bovins comparativement au témoin non amendé et à la fertilisation minérale.

3.2. Le stock de C du sol augmente avec la dose d'application du lisier.

3.3. Les stocks de C dans les fractions de MO relativement stables sont plus élevés suite à l'application de lisier comparativement au témoin et à la fertilisation minérale.

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Tableau I : Coefficients isohumiques de différents effluents d'élevage (adapté de Clément *et al.,* 2010)

Source de MO	Coefficient isohumique (%)
Purin de bovins	5
Lisier de porcs	10
Lisier de volailles	10
Lisier de bovins	15
Fumier peu décomposé	25
Fumier moyennement décomposé	40
Fumier bien décomposé	50

Tableau II : Facteurs de variation des stocks relatifs de C organique du sol (sur 20 ans) suite à l'apport de MO (adapté de Lasco *et al.*, 2006)

Degré d'intensité d'apport de C	Régime de température	Régime hygrométrique	Valeur par défaut GIEC	Erreur	Description
Moyen	Tous	Sec et humide / pluvieux	1.00	SO	Cultures annuelles avec céréales et retour des résidus. Apport d'engrais minéraux ou rotations de cultures fixatrices d'azote.
Élevé (avec effluent animal)	Tempéré / boréal et tropical	Sec	1.37	±12%	Entrées de C beaucoup plus élevées que pour les systèmes culturaux à entrées moyennes, en raison de l'apport supplémentaire d'effluent animal.
		Humide / pluvieux	1.44	±13%	
	Tropical montagnard		1.41	±50%	

SO : sans objet

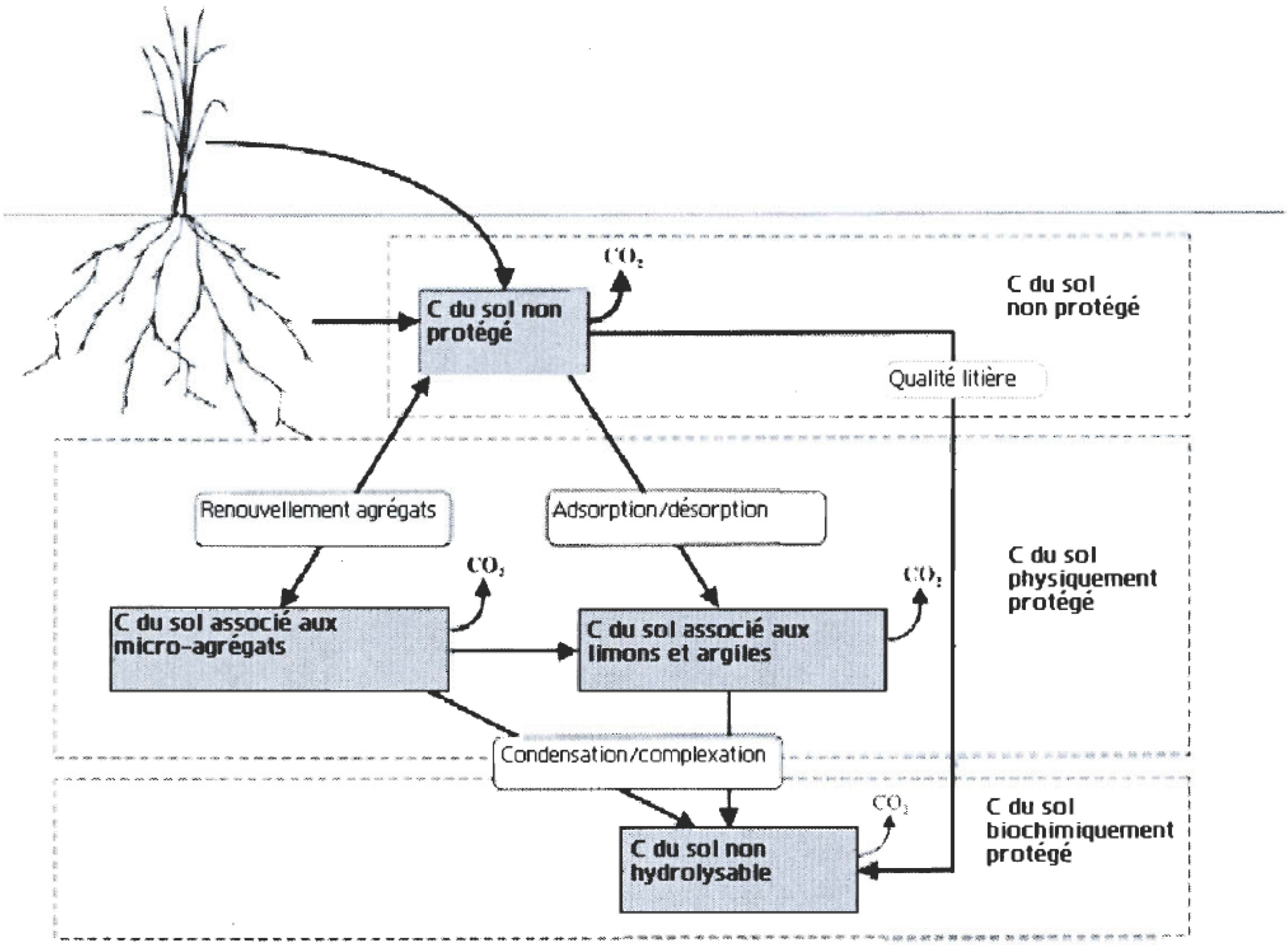

Figure I : Modèle conceptuel des dynamiques de MO du sol (adapté de Six et al. 2002)

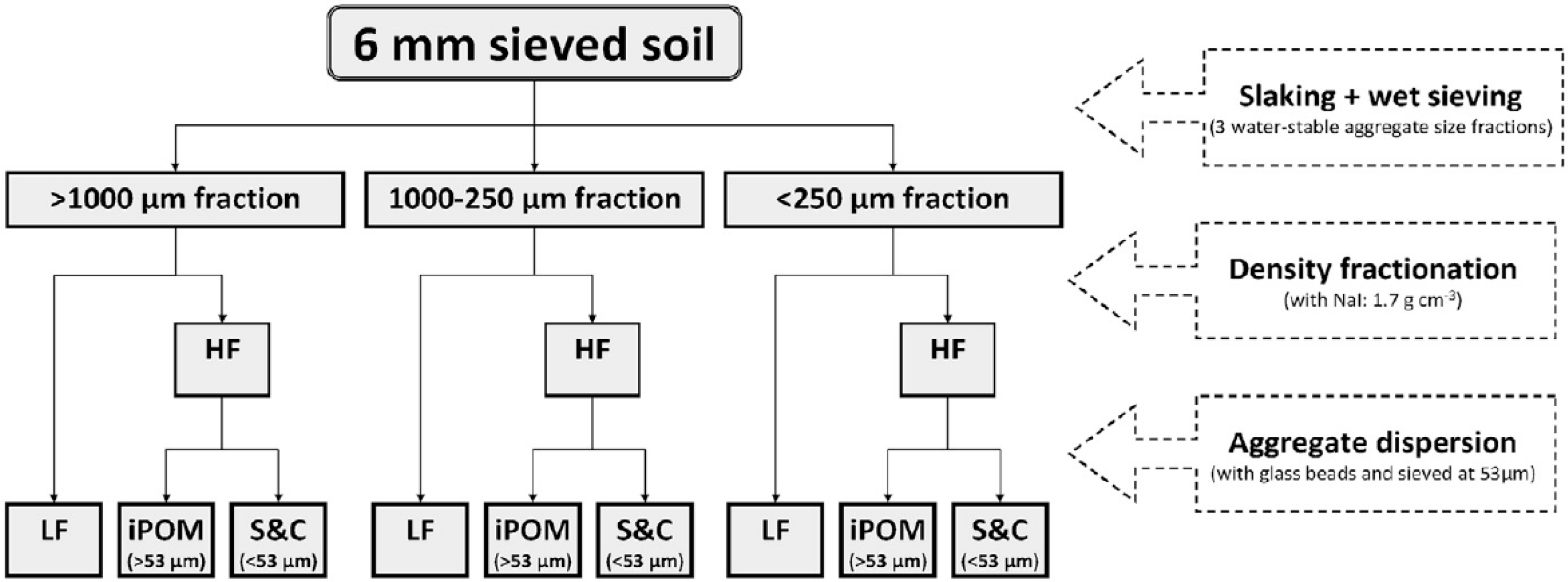

Figure II : Schéma de fractionnement physique de la matière organique (HF : fraction lourde, LF : fraction légère, iPOM : MO particulaire intra-agrégat, S&C : MO associée aux argiles et limons) (Laganière et al. 2011)

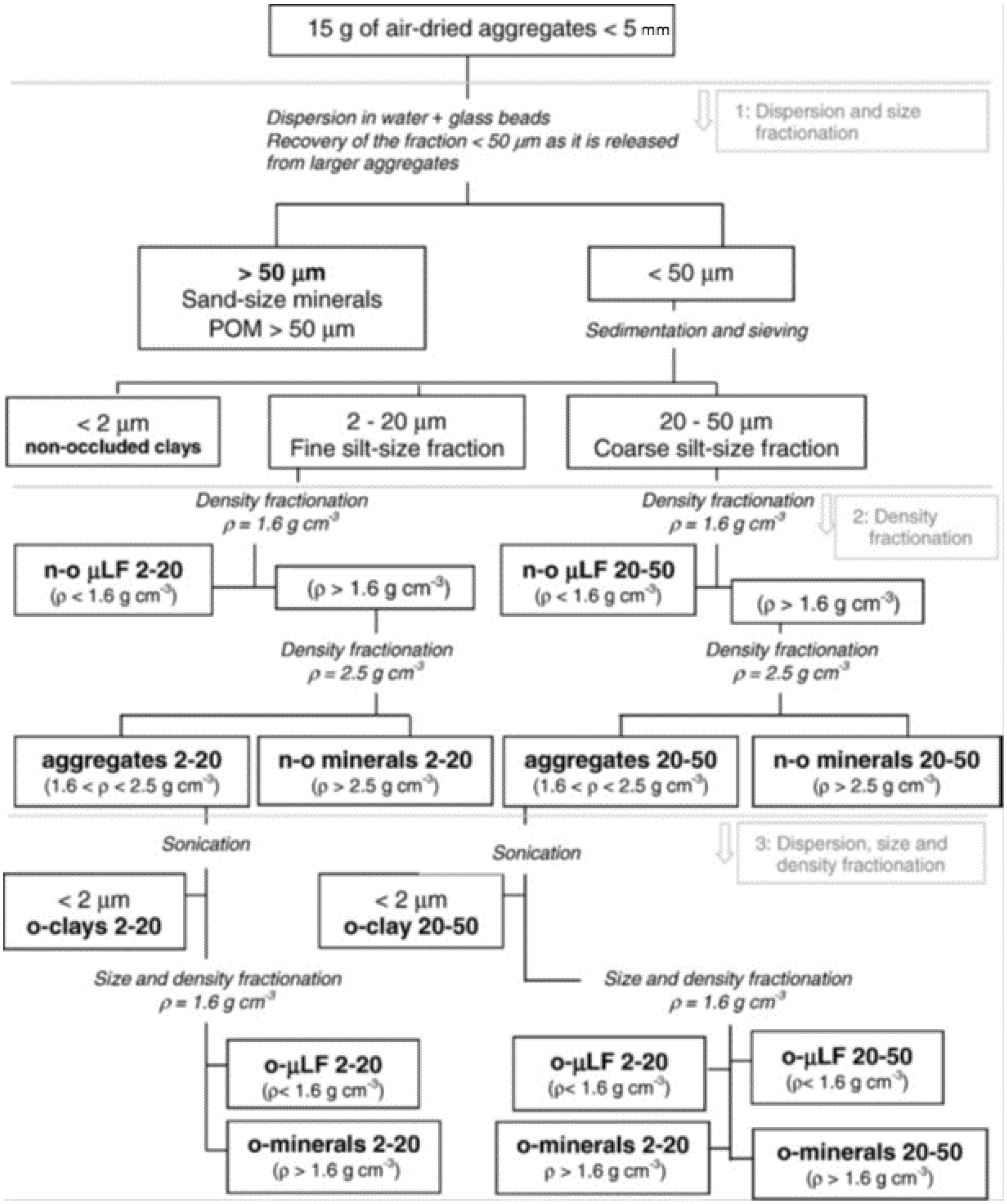

Figure III : Protocole de fractionnement de la matière organique utilisé par Virto et al. (2008)

Chapter 1: Animal manure application and soil organic carbon stocks - a meta-analysis

Chapitre 1 : Méta-analyse des changements de stocks de carbone du sol suite à l'application d'effluents d'élevage

ÉMILIE MAILLARD and DENIS ANGERS

Résumé

L'étude des changements de stocks de carbone (C) du sol après l'application d'effluents d'élevage présente à la fois des intérêts agronomiques et environnementaux. De plus, il y a un besoin spécifique à quantifier les changements de stocks de C du sol pour une utilisation dans les inventaires nationaux d'émissions de gaz à effet de serre. Nous avons quantifié la réponse des stocks de C du sol à l'application d'effluents d'élevage à partir d'études individuelles du monde entier et déterminé l'influence de facteurs explicatifs comme le climat, les propriétés du sol, l'utilisation des terres et les caractéristiques de l'effluent. Notre étude est basée sur une méta-analyse de 42 articles de recherche totalisant 49 sites et 130 observations dans le monde. Un effet dominant de l'apport total de C par l'effluent sur la réponse des stocks de C du sol a été observé, puisque ce facteur expliquait au moins 53% de la variabilité des différences de stocks de C du sol par rapport à la fertilisation minérale ou à un témoin non fertilisé. Les effets d'autres facteurs explicatifs n'étaient pas évidents à partir de notre jeu de données. Un coefficient de rétention global du C de l'effluent d'une valeur de 12% ±4 (Intervalle de confiance de 95%, IC) a pu être estimé pour une durée moyenne des études de 18 années à partir de la régression linéaire entre les apports totaux de C par l'effluent et la différence des stocks de C du sol. Selon une approche comparable à celle du Groupe d'experts Intergouvernemental sur l'Évolution du Climat (GIEC), nous avons estimé un facteur relatif de changements de stocks de 1.26 ±0.14 (IC 95%) qui était aussi lié à l'apport total de C par l'effluent. Nos résultats offrent des perspectives pour le raffinement des coefficients de rétention des effluents utilisés dans les guides de bonnes pratiques agricoles et pour l'amélioration des facteurs de changement des stocks de C du sol dans les inventaires nationaux de gaz à effet de serre par la prise en compte de l'apport de C par l'effluent. Finalement, cette étude souligne aussi le besoin de documenter davantage l'effet à long terme de caractéristiques de l'effluent comme l'espèce animale (essentiellement porcs et volaille) et le système de gestion de l'effluent (en particulier l'entreposage liquide vs. solide).

Abstract

The impact of animal manure application on soil organic carbon (SOC) stock changes is of interest for both agronomic and environmental purposes. There is a specific need to quantify SOC change for use in national greenhouse gas (GHG) emission inventories. We quantified the response of SOC stocks to manure application from a large worldwide pool of individual studies and determined the impact of explanatory factors such as climate, soil properties, land use and manure characteristics. Our study is based on a meta-analysis of 42 research articles totaling 49 sites and 130 observations in the world. A dominant effect of cumulative manure-C input on SOC response was observed as this factor explained at least 53% of the variability in SOC stock differences compared to mineral fertilized or unfertilized reference treatments. However, the effects of other determining factors were not evident from our dataset. From the linear regression relating cumulative C inputs and SOC stock difference, a global manure-C retention coefficient of 12% ± 4 (95% Confidence Interval, CI) could be estimated for an average study duration of 18 years. Following an approach comparable to the Intergovernmental Panel on Climate Change, we estimated a relative SOC change factor of 1.26 ± 0.14 (95% CI) which was also related to cumulative manure-C input. Our results offer some scope for the refinement of manure retention coefficients used in crop management guidelines and for the improvement of SOC change factors for national GHG inventories by taking into account manure-C input. Finally, this study emphasizes the need to further document the long-term impact of manure characteristics such as animal species, especially pig and poultry, and manure management systems, in particular liquid vs. solid storage.

Introduction

As animal manure contains organic matter, an increase in soil organic carbon (SOC) content is generally expected following its land application, as reported in many individual studies. For example, in the southeastern United States, Sainju *et al.* (2008a) observed an increase of soil surface (0-20 cm) SOC stock of about 3.2 Mg C ha^{-1} after 10 years of poultry litter application in comparison to mineral fertilizer plots. In Nepal, after 25 annual cattle manure applications, surface (0-30 cm) SOC stocks were higher by about 19.1 Mg C ha^{-1} than control (unfertilized) plots (Gami *et al.*, 2009). In China, after 22 years of pig manure application, the surface soil layer (0-15 cm) accumulated 3.8 Mg C ha^{-1} more than mineral fertilizer alone (Huang *et al.*, 2010a). However, some studies report no significant or even negative change of SOC stocks following manure application (Angers *et al.*, 2010, Franzluebbers *et al.*, 2001). Obviously, there is great variability in the magnitude of SOC stocks change after manure application.

Individual studies and a few review articles have attempted to relate the variability in the magnitude of SOC change to various explanatory factors such as climate (Triberti *et al.*, 2008), manure application rate (Franzluebbers *et al.*, 2001), manure management system (farmyard manure/slurry) (Grignani *et al.*, 2007), soil texture (Gami *et al.*, 2009), initial SOC concentration (Dersch & Bohm, 2001), land use, and time of application. Few global studies have attempted to quantify the effects of these explanatory factors from all the available literature. Ogle *et al.* (2005) and Franzluebbers & Doraiswamy (2007) focused solely on the impact of climatic zone on SOC change after manure application. The former study used probably < 5 experimental sites, and the latter probably < 15. Moreover, the data presented suggest that the effect of climatic zone on SOC change after manure application was not statistically significant.

Due to the close relationship between SOC and soil quality (Gregorich *et al.*, 1994), there is strong interest in developing tools to advise farmers on the effect of manure on SOC. For that purpose, some fertilizer and crop management guidelines report retention coefficients for manures of different animal species or decomposition degree (e.g. Clément *et al.*, 2010, Soltner, 2000). The retention or isohumic coefficient is defined as the fraction of applied organic matter which is "transformed" into soil organic matter (Hénin & Dupuis, 1945). The determination of manure C retention coefficient is also of interest for C modelling as C retention coefficient for crop residues is a substrate quality parameter used in most SOC models (Kätterer *et al.*, 2011).

The global quantification of SOC change after manure application is also relevant for national greenhouse gas (GHG) emission inventories. The Intergovernmental Panel on Climate Change (IPCC) provides default SOC stock change factors for manure application for C accounting for national GHG inventories (IPCC, 2006). These factors represent the effect on SOC stocks after regular addition of animal manure for a period of at least 20 years (IPCC, 2006). We understand that these estimates were generated from probably 5 individual studies, and the amount of manure-C input and management activity categories such as animal species or manure management system were not taken into account. Such considerations would represent a major improvement in estimate accuracy.

Emission factors for CH_4 and N_2O are already proposed for specific animal species and manure management systems (IPCC, 2006).

There is therefore a need to refine global quantification of SOC changes following manure application from both agronomic and environmental perspectives. The objective of our work was to quantify the response of SOC stocks to manure application from a large worldwide pool of individual studies, and to assess the impact of explanatory factors such as climate, soil properties (texture, initial SOC concentration), land use and manure characteristics (C input, animal species, manure management system).

Materials and methods

Literature search and study selection

We searched literature published up to the end of 2011 using two bibliographic databases: CAB Abstracts and Scopus. Specific keywords describing animal category (*animal, pig, cattle, hog, poultry, sheep, horse, livestock*), manure (*compost, mud, sludge, ooze, effluent, waste, manure, dung, slurry, muck slurry, farmyard manure*) and soil carbon (*soil carbon sequestration, soil carbon accumulation, soil carbon content, soil carbon quantity, soil carbon concentration, soil carbon density, soil carbon stocks*) were combined. From > 1000 articles containing these keywords, we selected those which met the following criteria:

1) Agronomic field experiments on cropland or grassland were included, but greenhouse, forest and mine soil reclamation experiments were excluded;

2) The experimental design should be replicated;

3) The soil C stocks (Mg C ha^{-1}) should be available or computable from soil C concentrations (g C kg^{-1} or %) and bulk density values. In studies which reported soil organic matter concentration instead of C, we estimated C as 58% of the organic matter;

4) Experiments should include at least one treatment with animal manure and a reference treatment which could be a control (without any fertilization) and/or a mineral fertilization treatment. Based on this criterion, two datasets (REF-zero; REF-min) were computed to evaluate SOC response to manure application in comparison to an unfertilized control and a mineral fertilization treatment, respectively. Studies could be part of one or both dataset(s). Mineral fertilization had to include nitrogen (N). For the REF-min dataset, manure could be applied alone or combined with mineral fertilization to carry additional N, P and K to approach mineral supply of the mineral fertilization treatment.

5) Study duration should be at least 3 years. When there were more than one article on the same experiment, the latest was considered if soil C stocks (Mg ha^{-1}) were reported in both articles. If only one reported soil C stocks, this article was selected even if it was not the latest.

Soil C stocks were recorded or computed to 30-cm depth when possible. Stocks for soil depths of < 15 cm were not retained. For one site, soil C stocks were given for a whole profile of 100 cm (Srinivasarao *et al.*, 2011). Only two sites reported soil C stocks on an equivalent mass basis (Kätterer *et al.*, 2011, Viaud *et al.*, 2011). Standard deviations for each treatment were collected or evaluated from standard errors or critical value of comparison test (e.g. LSD). The sample size was also collected. The DataThief software (Tummers & van der Laan, 2006) was used to estimate data from graphs. In addition, for each experimental site, we compiled metadata that would be used as explanatory factors of the effect of manure application: i.e. climatic data, soil properties (texture, initial SOC concentration), land use, manure properties (application rate, C concentration, C input, animal species, management system: liquid or solid storage) and study duration. If the data were not available in the article, the authors were requested to provide missing information. IPCC climate zone was determined for each experimental site from geographic coordinates and the world map of IPCC climate zones (European Comission, 2012). We also considered precipitation and temperature data in the articles to confirm the climate zone given by the world map. For two sites, cumulative manure-C input was known for a shorter study duration and was extended for the whole period of application (Bandyopadhyay *et al.*, 2011, Holeplass *et al.*, 2004, Nayak *et al.*, 2012, Singh *et al.*, 1998). For two sites, manure-C input was evaluated with manure-C concentration which was available only for one year (Gami *et al.*, 2009, Grandy *et al.*, 2002).

Choice of effect size index

An effect size is a value reflecting the magnitude of the treatment effect in comparison to a reference treatment (Borenstein *et al.*, 2009). For each observation (comparison between manure and reference treatment), we calculated two effect size indices to evaluate SOC response to manure application. A first index corresponded to the difference between the SOC stock in the plot receiving manure and the SOC stock in the reference plot (Mg C ha^{-1}) thereafter referred to as *SOC stock difference*. As mentioned earlier, two datasets were created according to the reference plot i.e. a REF-zero dataset when the reference plot was a control and a REF-min dataset when the reference plot was a plot receiving mineral fertilization. Finally, a second index corresponded to the ratio of the SOC stock in the plot receiving manure to the SOC stock of the plot receiving mineral fertilization thereafter referred to as *relative SOC change*. This index would be comparable to the default relative SOC change factors provided by IPCC (2006) and to factors determined by Ogle *et al.* (2005) from experiments which lasted at least 20 years. This index was estimated on a reduced REF-min dataset including only studies with duration of 20 years or more to be consistent and comparable with the IPCC approach.

Statistical analysis

Three categorical factors (climate, animal species, and land use) and three continuous factors (cumulative manure-C input, clay and silt content, and initial SOC concentration (g kg^{-1})) were retained for the analysis. Three climate classes were constituted from the IPCC climate classification: cool temperate, warm temperate and tropical. Three land use classes were tested: annual crops, perennial crops and rice paddies. For animal species, cattle and poultry manure had sufficient observations for statistical analysis of both datasets. The effect of pig manure could only be determined for the REF-zero dataset and the effect of goat manure could not be tested due to lack of observations. The effect of manure management system (solid vs liquid) could not be tested due to too few observations for liquid manure.

A mixed model was used by including a random variable for the site, in order to take into account the dependency of several effect sizes at a same site. Since standard deviations were rarely available to weight by the inverse of variance, the analysis was weighted by sample size. Statistical significance of each explanatory factor (cumulative manure-C input, animal species, clay and silt content, initial SOC concentration, climate, and land use) was tested independently from the others for the two indices as datasets were not complete for all explanatory factors. The assumptions of homogeneity of variance and normality of the residuals were verified graphically. For normality, Shapiro-Wilk and Kolmogorov-Smirnov tests were also verified. Heterogeneity was corrected either by modeling a different variance for each level of a categorical factor or by modeling the variance as a power of the mean. Log transformations were carried out for some cases. The statistical significance between means of the different levels of categorical factors was given by least square differences. For continuous factors, R-Squared was calculated by using a method recommended for mixed models (Edwards *et al.*, 2008). As datasets were not complete for all categorical and continuous factors, the number of observations was specified on the figures. A Student test was used to test the statistical significance of global means for both indices. All statistical analyses were performed using the SAS software, version 9.2 (SAS Institute, Cary, NC). Statistical results were considered to be significant at the 0.05 α level.

Results and discussion

General results

By applying our selection criteria, 42 articles were retained for 49 sites in the world (Table 1). Among these 49 replicated sites, 40 were randomized, 8 were presumed to be randomized based on the description of the experimental design, and one was not randomized. The soil depth considered varied from 15 to 100 cm, with an average of 26 cm. Duration of the studies ranged from 3 to 82 years, with an average of 18 years (18 years also for studies with known cumulative manure-C input).

The 130 observations from the 49 sites were separated into REF-zero and REF-min datasets to evaluate the SOC response to manure application in comparison to an unfertilized control and to mineral fertilization, respectively. The *SOC stock difference* (1st index) was calculated for every observation of both datasets. In total for this effect size, 36 sites (57 observations) were available for the REF-zero dataset and 43 (73 observations) for the REF-min dataset. Among the 43 sites included in the REF-min dataset, 22 (28 observations) had duration of ≥ 20 years and were used to analyze the *relative SOC change* (2nd index).

Overall effect of manure application on SOC stocks compared to a reference

Overall, as expected, the positive response in the *SOC stock difference* (1st index) indicated a significantly larger SOC stock in plots receiving animal manure than in both reference treatments (Table 2). The higher SOC stock in manured treatments could be due to direct C input by the manure itself and indirect C input through increased net primary production (including roots and crop residues) (Aoyama *et al.*, 1999, Bhattacharyya *et al.*, 2010, Whalen & Chang, 2002). The average SOC response to manure application was larger when using control plots as the reference (9.4 Mg C ha^{-1}) than mineral fertilizer (5.6 Mg C ha^{-1}). If we assume that in most studies of the REF-min dataset the amount of C added as roots and crop residues was similar in the manure treatment and in the mineral fertilizer reference treatment, thus the positive SOC response following manure application can probably be mainly attributed to direct C input by manure.

Factors influencing the SOC stock difference

Our meta-analysis showed a significant linear relationship between the *SOC stock difference* (1st index; Mg C ha^{-1}) and cumulative manure-C input (Mg C ha^{-1}) for the REF-zero ($p<0.0001$; R^2 = 0.53) and the REF-min ($p<0.0001$; R^2 = 0.59) datasets (Fig. 1.1a,b). In this analysis, cumulative manure-C input is a combination of annual manure-C application rate and study duration. Positive linear relationships between cumulative amount of added organic matter and SOC stocks or stock changes associated with treatments involving various amounts of manure or other organic C sources have often been observed in individual studies (e.g. Kong *et al.*, 2005, Majumder *et al.*, 2008, Thomsen & Christensen, 2004). As far as we know, our study is the first one reporting such a relationship between *SOC stock difference* compared to a reference treatment and cumulative animal manure-C input from a worldwide set of studies. It is noteworthy that despite widely variable sources of data (soil, climate, study duration, animal species, land use etc.), we can explain at least 53% of the variability in *SOC stock difference* by cumulative manure-C input. For comparison purposes, we added eight individual observations (not included in our meta-analysis due to lack of replications) from seven long-term sites in the United Kingdom (Jenkinson *et al.*, 1990, Jenkinson & Johnston, 1977), Germany (Ludwig *et al.*, 2011), Canada (Grant *et al.*, 2001) and China (Guo *et al.*, 2007) (Fig. 1.1a,b). These points clearly fit in the same general linear relationship as the data retained in our meta-analysis. Our results corroborate linear increases in SOC stocks with increasing organic C inputs shown by most current models of SOC dynamics (e.g., RothC (Coleman & Jenkinson, 1995), Century (Parton *et al.*, 1987)). Indeed, the relationship between C input and *SOC stock difference* is linear up to very high levels of cumulative C input.

The group analysis indicated a strong trend towards an effect of climatic zone on the *SOC stock difference* for both the REF-zero (p=0.062) and the REF-min (p=0.060) datasets (Fig. 1.2a,b). The *SOC stock difference* was lower in tropical climate than in warm temperate climate for the REF-zero (p=0.027) and REF-min (p=0.052) datasets. In addition, for the REF-min dataset, the *SOC stock difference* tended to be lower in tropical climate than in cool temperate climate (p=0.078) (Fig. 1.2b). This observation is consistent with the general understanding that soils in warmer climates, where decomposition is faster, may accumulate C slower than soils in colder climates (Freibauer *et al.*, 2004). However, it should be noted that for tropical climate, a significant number of observations (10 of 21 for the REF-zero dataset and 12 of the 33 for the REF-min dataset) actually had a cumulative manure-C input < 45 Mg C ha^{-1} and that cumulative manure-C input was unknown for the other observations. Therefore, from our meta-analysis, we cannot rule out that the lower SOC stocks under tropical climate may simply be due to relatively lower cumulative manure-C inputs. This clearly emphasizes the importance of documenting C inputs in agronomic studies reporting SOC change.

The effect of animal species on the *SOC stock difference* was not statistically significant (Fig. 1.3a,b). However, in both REF-zero and REF-min datasets, cattle manure showed a slight trend to higher values than the others, and confidence intervals suggested a significant positive *SOC stock difference* after cattle manure application but not after pig and poultry manure application. This significant response of SOC after cattle manure application would be consistent with the idea that its organic matter is more stable than that from poultry and pig manure (Velthof *et al.*, 2000). However it should be underlined that compared to cattle, the number of observations was very low and the variability very high for pig and poultry manures, which limits the interpretation. In addition, similar to climate, we cannot rule out that the low values for pig and poultry manure may also be related to a relatively low cumulative manure-C input associated with these studies (<45 Mg C ha-1, for all relevant studies except one). Clearly, more studies on the impact of pig and poultry manure are required, and these should include C input data.

We found no effects of land use, clay and silt content, or initial SOC concentration on the *SOC stock difference*. This is partly due to missing observations in the case of initial SOC concentration (Table 1.1) or to the limited number of studies involving “perennial” and “paddy” cropping systems in the case of land use. In addition, these explanatory factors may be of minor significance compared to the overwhelming effect of cumulative manure-C input.

Estimation of manure-C retention coefficient

Overall, our global analysis showed that cumulative manure-C input was the main factor explaining SOC response to manure application. Many studies have estimated C retention coefficients from the slope of linear relationships between cumulative C input and SOC stocks or stock changes associated with experimental treatments including various amounts of manure or crop residues (Benbi & Senapati, 2010, Bhogal & Shepherd, 1997, Buyanovsky & Wagner, 1998, Campbell *et al.*, 1991, Kong *et al.*, 2005, Mandal *et al.*, 2007, Thomsen & Christensen, 2004). We outlined previously that in the case of the REF-min dataset, the positive SOC response to manure application can be mainly attributed to C input by manure. Thus, the slope of the linear relationship between the cumulative manure-C input (Mg C ha^{-1}) and the *SOC stock difference* (1^{st} index; Mg C ha^{-1}) for the REF-min dataset (Fig. 1.1b) can be considered as an approximation of the manure-C retention coefficient, which represents the average proportion of manure-C remaining in soil. Our estimated global manure-C retention coefficient is 12% ± 4 (95% Confidence Interval, CI) for an average study duration of 18 years. As described earlier, by adding eight individual observations from seven long-term sites (without replicates) in the United Kingdom (Jenkinson *et al.*, 1990, Jenkinson & Johnston, 1977), Germany (Ludwig *et al.*, 2011), Canada (Grant *et al.*, 2001) and China (Guo *et al.*, 2007), the slope remains at 12% ± 2 (95% CI).

To the best of our knowledge, this is the first time that a C retention coefficient of animal manure can be deduced from a worldwide set of studies offering a wide range of cumulative C input levels (10-175 Mg C ha^{-1} for observations included in the meta-analysis; 3-402 Mg C ha^{-1} with additional observations from unreplicated designs). Indeed, all of the studies cited previously involved individual sites. Moreover, the C input estimates in these studies included either crop residues only or a mix of crop residues and manure. Using 14 long-term field experiments in Europe, Smith *et al.* (1997) established a linear relationship between yearly relative changes in SOC stock compared to a reference treatment and the amount of fresh organic manure added annually. However, the absence of manure dry matter and C contents did not allow us to calculate the cumulative manure-C input and consequently a C retention coefficient from their data.

Our estimated manure-C retention coefficient is lower than the value of 23% reported for animal manure in a study from 7 different sites in the United Kingdom (Bhogal *et al.*, 2007). However their 95% CI was 9 to 37 which overlaps with ours. In addition, Bhogal *et al.* (2007) pointed out that the average increase of SOC change representing 23% of manure-C could only be regarded as the initial rate of SOC increase, as SOC accumulation rates decline over time, and consequently their retention coefficient could be overestimated. Moreover, their value was measured by calculating the difference in SOC between manured and unmanured treatments without specifying if the unmanured treatments included mineral fertilization or not. Thus, their manure-C retention coefficient could also be overestimated if some C in soils originated from crop residues rather than manure.

Our manure-C retention coefficient can be compared to those estimated for crop residues. After 18 years of annual straw addition, Thomsen & Christensen (2004) observed a retention coefficient of 14% for straw C. Campbell *et al.* (1991) reported a C retention coefficient of 6% for crop residues on a 30-year old site. After 10 years, Kong *et al.* (2005) observed a value of 8% across ten different cropping systems. Manure was also applied in one of their cropping systems. From Buyanovsky & Wagner (1998), we calculated that 6 and 7% of C applied as crop residues, and additionally from manure in some plots, were retained in soil during a very long period of "humification" extending to 100 years. Therefore, our value of manure-C retention coefficient (12% ± 4), estimated for an average study duration of 18 years, is comparable to those for crop residues determined in the medium to long-term (10-100 years), and supports Buyanovsky & Wagner (1998) who argued that there was no reason to consider that manure was more effective for SOM enhancement than plant residues. However, retention of C added as manure may be greater than crop residues in the short-term. For instance, in an *in situ* decomposition study, Thomsen & Christensen (2010) observed a higher retention of C added in the form of sheep faeces (30%) than C directly applied as crop residues (19%) after 9 annual additions. Likewise, in a laboratory incubation,14% of C applied in feed was retained in soil after 1 to 2 years, against 48% of C applied as faeces (Thomsen *et al.*, 2013). Taken together, our results and the above-cited literature suggest that despite apparently faster early decomposition of crop residues compared to manure, longer term stabilization may be relatively similar for both.

Estimation of relative SOC change after manure application

The 2nd effect size index is defined as the ratio of SOC stock in plots receiving manure to SOC stock in plots receiving mineral fertilization. It represents a *relative SOC change* factor such as used by IPCC (2006) and Ogle *et al.* (2005), and estimated from experiments that lasted at least 20 years. The average value for the *relative SOC change* (2nd index) was 1.26 ± 0.14 (95% CI) for experiments that lasted at least 20 years (Table 1.2). This ratio was lower than the average of IPCC relative SOC change factors (1.41) proposed for high inputs with manure (IPCC, 2006) and the average of high input factors with amendments (1.36) proposed by Ogle *et al.* (2005). Nevertheless our estimation was based on a larger number of sites (22 sites for 28 observations) against probably 5 studies for IPCC (2006) and less than 5 for Ogle *et al.* (2005).

A significant linear relationship was also observed between the cumulative manure-C input (Mg C ha^{-1}) and the *relative SOC change* ($p=0.026$; $R^2 = 0.31$) (Fig. 1.4). Climatic zone did not affect the *relative SOC change* (Fig. 1.5). Similarly to the *SOC stock difference* (1st index), no effect of land use, clay and silt content or initial SOC concentration was observed. The reduced REF-min dataset was limited by the low number of observations for cumulative manure-C input, and some climate and animal species classes. Clearly, as mentioned previously, more data are necessary to elucidate the global impact of these explanatory factors.

The IPCC Guidelines for National Greenhouse Gas Inventories provide different values of soil C stock change after animal manure application in different climatic zones: 1.37 for temperate, boreal and tropical dry climates; 1.44 for temperate, boreal and tropical moist/wet climates; and 1.41 for tropical montane climates (IPCC, 2006). From a statistical standpoint, these values are not significantly different from each other. Moreover, the amount of C input by manure is not taken into account in these estimates. Our results offer some scope for refinement of these change factors by taking into account the amount of manure applied. However the relationship between the cumulative manure-C input (Mg C ha^{-1}) and the *relative SOC change* probably needs to be confirmed with more data in order to propose a new operational set of IPCC factors.

Limits and outlook

To the best of our knowledge, the present study is the first global analysis of factors determining SOC response to manure application which includes a significant number of studies. For this meta-analysis, we chose to work with high-quality data (replicated randomized experiments, values expressed as SOC stocks, C input from manure etc.). Consequently, our conclusions were constrained by missing data and the meta-analysis raises many points that need to be improved in future research.

Firstly, SOC expressed as stock or bulk density data necessary to compute SOC stock were missing in many articles that were scanned. This information is essential to examine the relationship between cumulative manure-C input and SOC change. Ideally, SOC stocks should also be expressed on an equivalent mass basis to avoid any bias in the estimation of changes in SOC stocks when the entire soil profile is not sampled (in case there would be a significant amount of SOC beneath the maximum depth of sampling) (VandenBygaart & Angers, 2006). In the studies considered in our analysis, only two reported SOC stocks on an equivalent mass (Kätterer *et al.*, 2011, Viaud *et al.*, 2011).

Another important point that limited our analysis was detailed information allowing estimation of manure-C input (C concentration, application rate, dry matter content) which was absent from many articles reporting SOC stocks. Consequently, manure-C input could be estimated in only 29 of the 49 sites, representing a total of 86 observations in both datasets. This information is necessary to analyze the relationship between SOC change and cumulative manure-C input and refine the estimation of our manure-C retention coefficient. Indeed, there is uncertainty associated with this relationship due to the substantial lack of observations with cumulative manure-C input greater than 75 Mg C ha^{-1} (Fig. 1.1a,b and Fig. 1.4). In addition, there is significant variability in our data for cumulative manure-C input <75 Mg C ha^{-1}. However, these data represent the current state of available information for studies on SOC response to manure application.

Other relevant information for our analysis such as animal species and initial SOC concentration was often missing in the scanned articles (Table 1.1). In addition, some classes of explanatory factors had a low number of observations compared to others. For example, fewer studies used poultry or pig manure compared to cattle manure.

Similarly, we did not find enough studies involving liquid manure compared to solid. Perennial cropping systems were less represented than annual systems. Another limitation was the lack of tropical sites outside of India. So extrapolating our results to other tropical regions should be done with caution. Most sites were located in Europe, Asia, or North America with South America, Africa and Oceania being under- or not represented.

The results of this meta-analysis show that at a global scale cumulative manure-C input has a dominant effect on SOC change following manure application. When more data become available, the effects of explanatory factors like climate, animal manure characteristics, soil clay and silt content, initial SOC concentration and land use could be studied further by determining their effect on the slope of the relationship between cumulative C input and SOC stock change using meta-regression with multiple explanatory factors.

In accordance with our selection criteria, many short-term studies (<3 years) addressing manure impact on SOC were omitted from the meta-analysis (Agbede, 2010, Bilalis & Karamanos, 2010, Islam *et al.*, 2011, Lynch *et al.*, 2005, Mellek *et al.*, 2010). Most of these studies mentioned specific information on manure characteristics including manure-C input (Agbede, 2010, Lynch *et al.*, 2005, Mellek *et al.*, 2010) which is encouraging for future meta-analyses, and we can only hope that these studies will be maintained.

In summary, our findings suggest that at a global scale, above animal species, land use or soil properties, the amount of applied manure-C was the dominant driver determining the extent of SOC increase following manure application. We also observed a strong tendency for *SOC stock difference* to be lower in tropical than other climatic zones, but this effect could not be decoupled from that of manure-C input. Our results for the relative change factors, but also for manure-C retention coefficient, offer some scope for the improvement of national GHG inventory methodologies. There were obvious gaps in the data especially with regards to animal species (especially pig and poultry manure), manure management system (e.g. liquid vs solid storage), and under-representation of some geographic regions. We emphasize that long-term field studies covering these gaps either be initiated or continued, and that these studies document important explanatory factors such as manure application rate and C concentration which were very often absent from research articles.

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Table 1-1: Summary of data for the sites included in the analysis

Location	Dataset	n[1]	Sampling depth (cm)	Duration (yrs)	Cumulative manure-C input (Mg C ha^{-1})	Animal species	IPCC climatic zone	Soil clay (%)	Soil silt (%)	Initial SOC (g kg^{-1})	Land use	Reference[2]
Saint-Lambert de Lévis, Qc, Canada	REF-zero	2	30	20	NA	pig	cool temperate	34	56	NA	perennial	(Angers *et al.*, 2010)
Ludhiana, Penjab, India	REF-zero, REF-min	4	15	7	28	NA	tropical	17	24	5	paddy	(Benbi & Senapati, 2010)
Almora, Uttarakhand, India	REF-zero, REF-min	2	30	8	13	cattle	warm temperate	34	56	7	annual	(Bhattacharyya *et al.*, 2007, Bhattacharyya *et al.*, 2009); pers. comm.
Meden Vale, Nottinghamshire, UK	REF-zero	5	30	4	6;13;19;25;32	poultry	cool temperate	6	8	11	annual	(Bhogal *et al.*, 2009, Bhogal & Shepherd, 1997)
Foulum, Denmark	REF-zero	1	30	11	2	pig	cool temperate	9	13	23	annual	(Chirinda *et al.*, 2010)
Koraput, Orissa, India	REF-min	4	15	3	NA	cattle	tropical	19	24	6	annual	(Dass *et al.*, 2008)
Waldviertel, Austria	REF-zero, REF-min	2	25	21	NA	NA	cool temperate	8	13	NA	annual	(Dersch & Bohm, 2001)
Alpenvorland, Austria	REF-zero, REF-min	2	25	21	NA	NA	cool temperate	50	40	NA	annual	(Dersch & Bohm, 2001)
Pingliang, Gansu, China	REF-zero, REF-min	2	20	26	20	cattle	warm temperate	34	43	5	annual	(Fan *et al.*, 2008); pers. comm.
Farmington, Georgia, USA	REF-min	4	20	12	29	poultry	warm temperate	13	18	NA	perennial	(Franzluebbers & Stuedemann, 2010); (Franzluebbers & Stuedemann, 2009); pers. comm.
Bhairahawa, Nepal	REF-zero, REF-min	2	30	25	NA	cattle	tropical	18	71	NA	paddy	(Gami *et al.*, 2009); pers. comm.
Tarahara, Nepal	REF-zero, REF-min	2	30	25	NA	cattle	tropical	11	58	NA	paddy	(Gami *et al.*, 2009); pers. comm.
Parwanipur, Nepal	REF-zero, REF-min	3	30	23	22	cattle	tropical	14	58	NA	paddy	(Gami *et al.*, 2009); pers. comm.
Presque Isle, Maine, USA	REF-min	1	15	6	26	cattle	cool temperate	NA	NA	NA	annual	(Grandy *et al.*, 2002)
Isfahan, Ispahan, Iran	REF-zero, REF-min	6	20	7	44;87;175	cattle	warm temperate	34	56	5	annual	(Hemmat *et al.*, 2010)
Akershus, Norway	REF-min	3	25	48	29	cattle	cool temperate	20	40	40	annual; perennial	(Holeplass *et al.*, 2004); (Singh *et al.*, 1998)
Nanchang, Jiangxi, China	REF-zero, REF-min	2	15	22	67	pig	warm temperate	26	NA	9	annual	(Huang *et al.*, 2010a); (Huang *et al.*, 2010b); pers. comm.
Prairie du Sac, Wisconsin, USA	REF-min	1	30	4	NA	cattle	cool temperate	15	60	NA	annual	(Jokela *et al.*, 2009)
Nairobi, Kenya	REF-zero, REF-min	4	20	19	20;39	cattle	warm temperate	67	22	20	annual	(Kamoni *et al.*, 2007); (Kapkiyai *et al.*, 1999); (Kibunja *et al.*, 2010)
Embu, Kenya	REF-zero	2	20	13	20;41	goat	tropical	31	13	NA	annual	(Kamoni *et al.*, 2007); (Kihanda *et al.*, 2005)
Uppsala, Sweden	REF-zero, REF-min	2	20*	53	101	cattle	cool temperate	37	41	15	annual	(Kätterer *et al.*, 2011); (Kirchmann *et al.*, 2004)
Taipei, Taiwan	REF-zero	2	20	3	NA	pig	tropical	14	66	14	annual	(Lee *et al.*, 2006)
Brookings, South Dakota, USA	REF-zero, REF-min	4	30	4	12;23	cattle	cool temperate	32	62	NA	perennial	(Lee *et al.*, 2007)

Therwil, Switzerland	REF-zero, REF-min	2	20	27	24;32	cattle	cool temperate	16	72	15	annual	(Leifeld *et al.*, 2009);(Fliessbach *et al.*, 2007)
Yangling, Shaanxi, China	REF-min	1	20	17	NA	cattle	warm temperate	17	52	6	annual	(Liang *et al.*, 2011, Zhao *et al.*, 2010)
Shenyang, Liaoning, China	REF-zero, REF-min	3	20	22	44;22	pig	cool temperate	25	58	9	annual	(Lou *et al.*, 2011)
Padova, Italy	REF-zero, REF-min	4	30	36	155;129	cattle	warm temperate	15	38	12	annual	(Morari *et al.*, 2006); pers. comm.
Parbhani, Maharastra, India	REF-min	2	15	7	NA	NA	tropical	53	16	5	annual	(More & Hangarge, 2003)
Chinon, France	REF-zero	2	30	28	23;46	cattle	warm temperate	9	5	8	perennial	(Morlat & Chaussod, 2008); pers. comm.
Cuttack, Orissa, India	REF-zero, REF-min	2	15	35	32	cattle	tropical	20	14	7	paddy	(Nayak *et al.*, 2009)
Kanpur, Uttar Pradesh, India	REF-min	1	30	25	NA	NA	tropical	18	35	3	paddy	(Nayak *et al.*, 2012)
Sabour, Bihar, India	REF-min	1	30	25	NA	NA	tropical	28	22	5	paddy	(Nayak *et al.*, 2012)
Kalyani, West Bengal, India	REF-min	1	30	23	12	cattle	tropical	50	30	9	paddy	(Nayak *et al.*, 2012); (Bandyopadhyay *et al.*, 2011); pers. comm.
Ludhiana, Penjab, India	REF-min	1	30	26	NA	cattle	tropical	18	28	3	paddy	(Nayak *et al.*, 2012); (Walia *et al.*, 2010)
Pendleton, Oregon, USA	REF-zero, REF-min	2	30	56	37	cattle	warm temperate	18	70	NA	annual	(Rasmussen & Parton, 1994)
Ludhiana, Penjab, India	REF-zero, REF-min	2	30	32	NA	NA	tropical	7	33	3	paddy	(Rasool *et al.*, 2007); (Kukal *et al.*, 2009)
Ludhiana, Penjab, India	REF-zero, REF-min	2	30	32	NA	NA	tropical	16	24	3	annual	(Rasool *et al.*, 2008); (Kukal *et al.*, 2009)
Almora, Uttarakhand, India	REF-zero, REF-min	8	15	3	34;27;20;14;22	cattle	warm temperate	34	56	11	annual	(Saha *et al.*, 2008)
Bhubaneswar, Orissa, India	REF-zero, REF-min	6	30	3	NA	cattle	tropical	10	25	NA	perennial	(Saha *et al.*, 2010b)
Umiam, Meghalaya, India	REF-min	1	30	5	NA	NA	tropical	34	34	NA	annual	(Saha *et al.*, 2010a)
Belle Mina, Alabama, USA	REF-zero, REF-min	4	20	10	11	poultry	warm temperate	27	58	12	annual	(Sainju *et al.*, 2008a); (Sainju *et al.*, 2010); (Sainju *et al.*, 2008b)
Varanasi, Uttar Pradesh, India	REF-zero, REF-min	3	15	9	22;11	NA	tropical	62	22	1	annual	(Sarkar *et al.*, 2003)
Akola, Maharastra, India	REF-zero, REF-min	3	15	19	NA	NA	tropical	NA	NA	NA	annual	(Sharma *et al.*, 2011)
Patna, Bihar, India	REF-zero, REF-min	3	15	4	NA	NA	tropical	74	9	5	paddy	(Singh *et al.*, 2001)
Sardar Krushinagar, Gujarat, India	REF-zero, REF-min	5	100	18	27;10	cattle	tropical	11	4	2	annual	(Srinivasarao *et al.*, 2011); pers. comm.
Zhangye, Gansu, China	REF-zero, REF-min	2	20	23	52	pig-cattle-sheep	cool temperate	20	52	12	annual	(Su *et al.*, 2006); (Hai *et al.*, 2010)
Lucknow, Uttar Pradesh, India	REF-zero, REF-min	2	30	5	23	NA	tropical	13	25	3	perennial	(Suman *et al.*, 2009); (Singh *et al.*, 2007)
Bignan, France	REF-min	1	30*	8	18	poultry	warm temperate	17	42	25	annual	(Viaud *et al.*, 2011); pers. comm.
Moystad, Norway	REF-zero, REF-min	4	20	82	NA	cattle	cool temperate	14	34	24	annual	(Yang *et al.*, 2007)

[1] n = number of observations; [2] for each site, the 1st cited reference included soil C stocks. The other references contained unavailable information in the 1st one; * Equivalent topsoil depth of the study reference treatment

Table 1-2: Global means of the two effect size indices with the 95% Confidence Interval (CI) and probability level corresponding to the Student test

Index	REF-zero dataset	REF-min dataset
1st: SOC stock difference (Mg C ha^{-1})	9.4 ± 4.1 (95% CI); p<0.0001	5.6 ± 2.8 (95% CI); p<0.0001
2nd: relative SOC change		1.26 ± 0.14 (95% CI); p<0.0001

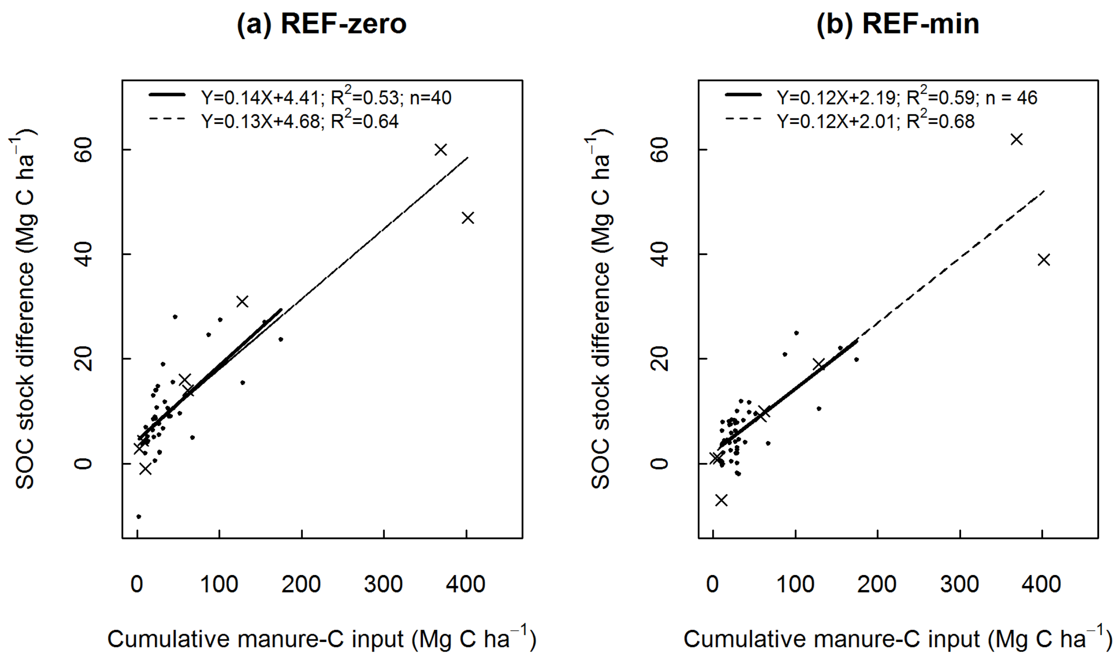

Figure 1-1 : Relationship between *SOC stock difference* and cumulative manure-C input for the REF-zero (a) and REF-min (b) datasets (•), and with additional observations from non- replicated sites (×).

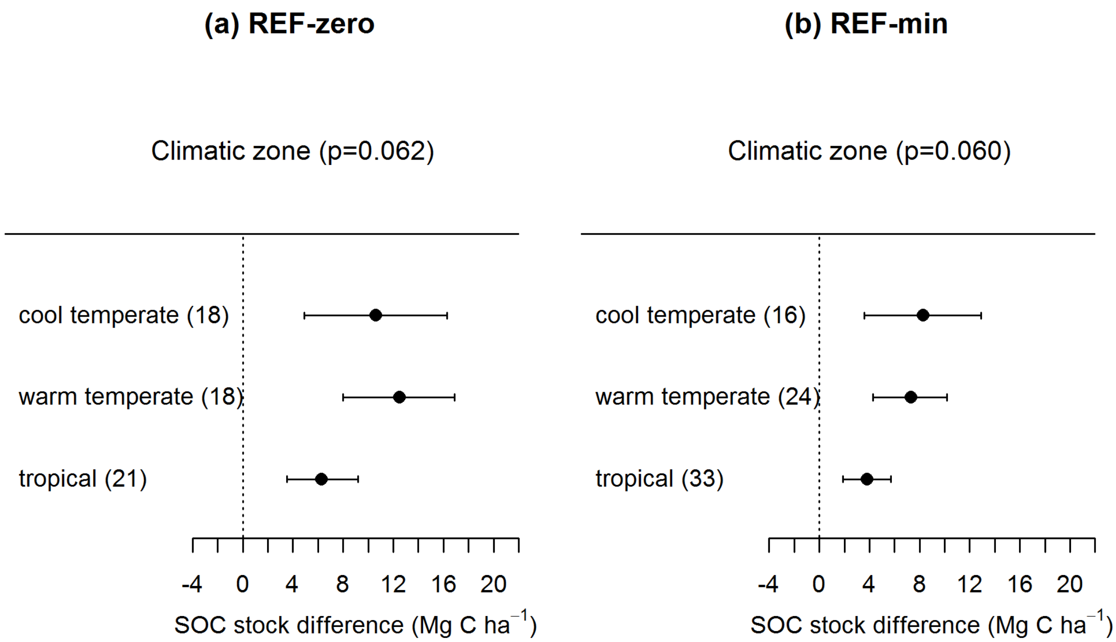

Figure 1-2 : Effect of climatic zone on the *SOC stock difference* due to manure application for the REF-zero (a) and REF-min (b) datasets (number of observations in each climate class in parenthesis, means and 95% Confidence Interval (CI)).

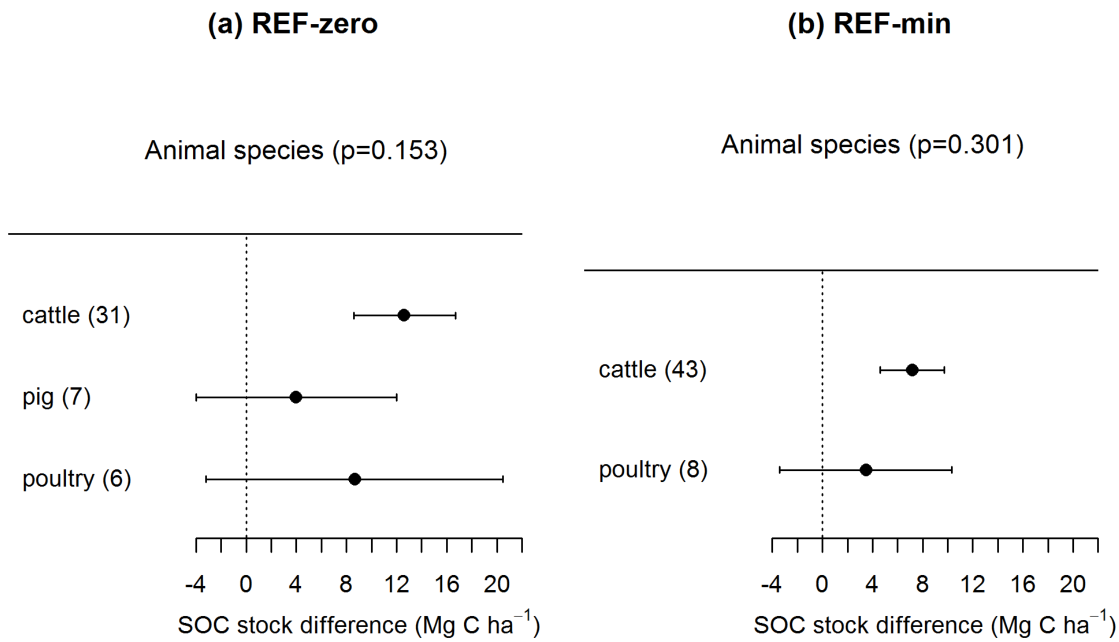

Figure 1-3 : Effect of animal species on the *SOC stock difference* due to manure application for the REF-zero (a) and REF-min (b) datasets (number of observations in each species class in parenthesis, means and 95% Confidence Interval (CI)).

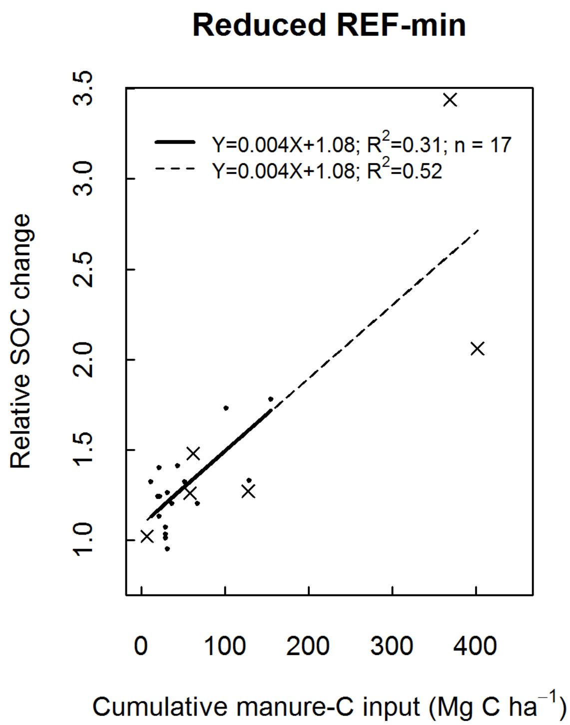

Figure 1-4 : Relationship between the *relative SOC change* and cumulative manure-C input for the reduced REF-min dataset (•), and with additional observations from non-replicated sites (×).

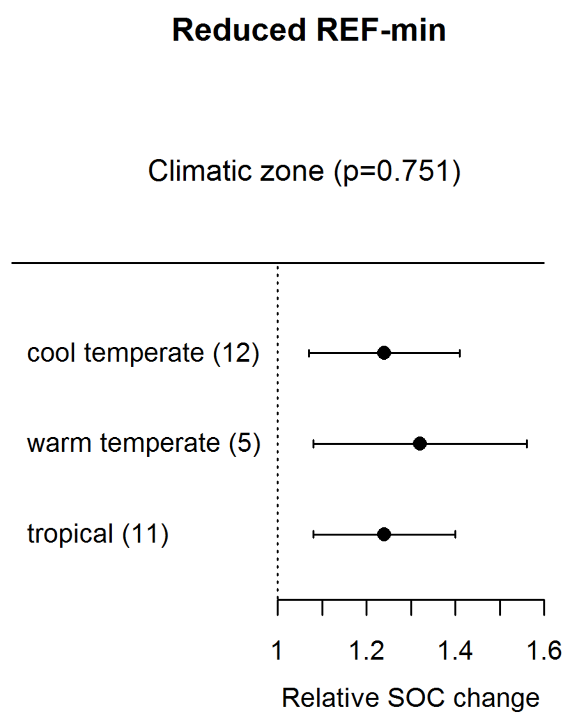

Figure 1-5 : Effect of climatic zone on the *relative SOC change* for the reduced REF-min dataset (number of observation in each climate class in parenthesis, means and 95% Confidence Interval (CI)).

Chapter 2: Management factors determining SOC stock changes following long-term application of liquid dairy manure

Chapitre 2 : Pratiques de gestion influençant les changements de stocks de carbone du sol suite à l'application de lisier de bovins

ÉMILIE MAILLARD, DENIS A. ANGERS, MARTIN CHANTIGNY, JEAN LAFOND, DENIS PAGEAU, PHILIPPE ROCHETTE, GABRIEL LÉVESQUE, NICOLE BISSONNETTE, and LÉON-ÉTIENNE PARENT

Résumé

Contrairement à l'effet positif bien reconnu des effluents d'élevage solides sur les stocks de carbone (C) du sol, l'effet des effluents liquides est moins clair. De plus, la réponse des stocks de C du sol à l'application d'effluents d'élevage peut varier en fonction des pratiques agricoles combinées habituellement avec cette application, comme le travail du sol ou des systèmes de culture incluant des plantes pérennes. Notre objectif était de déterminer la réponse des stocks de C du sol à l'application à long terme (21 années) de lisier de bovins combinée avec deux pratiques de travail du sol d'automne (labour conventionnel vs. chisel) et deux séquences de cultures (monoculture de céréales vs. rotation basée sur des cultures pérennes) le long d'un profil de sol (0-50 cm). Après 21 années d'application de lisier de bovins, les stocks de C du sol étaient plus élevés dans les 20 premiers centimètres comparativement à la fertilisation minérale. Aucun effet du lisier de bovins n'était observé en-dessous de 20 cm. L'amplitude des changements de stocks de C du sol induits par l'application de lisier dépendait de la séquence de culture, avec un plus grand effet du lisier sur les stocks de C dans la rotation basée sur les cultures pérennes que dans la monoculture de céréales. La rétention du C dérivé de l'application du lisier semblait plus forte dans la rotation à base de cultures pérennes que dans la monoculture. Le type de travail primaire du sol n'a pas influencé l'accumulation de C du sol après l'application d'effluents d'élevage. Il semble donc que la fréquence de labour plus faible dans la rotation à base de cultures pérennes (tous les trois ans) par rapport à la monoculture (annuelle) a favorisé l'accumulation de C dérivé de l'effluent dans le sol, alors que le type de travail du sol d'automne (labour conventionnel vs. chisel) a eu peu d'influence.

Abstract

As opposed to the well-known positive effect of solid animal manure on soil organic carbon (SOC) stocks, the effect of liquid animal manure is less clear. In addition, the response of SOC stocks to animal manure may vary with management practices usually combined with manure application, such as tillage or cropping system including perennial forages. The objective of our work was to determine the response of SOC stocks along a 0-50 cm soil profile to long-term (21 years) application of liquid dairy manure (LDM) in combination with two fall primary tillage practices (moldboard plowing *vs.* chisel plowing), and two crop sequences (cereal monoculture *vs.* cereal-perennial forage rotation). After 21 years of LDM application, SOC stocks were higher in the top 20 cm compared to mineral fertilization. No effects were observed below 20 cm. The magnitude of the manure-induced change in SOC was dependent on crop sequence, with a much greater effect of LDM application in SOC stocks in the perennial-based rotation than in the cereal monoculture. The retention of manure-derived C in SOC appeared to be much greater under the cereal-perennial forage rotation than under the cereal monoculture. To the contrary, the primary tillage practice did not influence the accumulation of manure-induced SOC. It therefore appears that the lower frequency of tillage in the perennial-based rotation (every three years) than in the monoculture (yearly operation) favoured the accumulation of manure-derived C in soil, whereas primary tillage practice (moldboard *vs.* chisel plow) had little influence.

Introduction

In addition to providing nutrients to agricultural crops, applying animal manure to land generally leads to increased soil organic matter content and improved soil quality. Quantifying the changes in soil organic carbon (SOC) induced by application of animal manure is therefore of interest for agronomic purposes and to address environmental issues such as climate change mitigation through SOC sequestration (Stockmann *et al.*, 2013). Even though general relationships between manure-C input and SOC change can be established at the global scale (Maillard and Angers, 2014), the influence of factors such as climate, animal manure characteristics, soil properties, and management practices that could affect this relationship are still poorly understood (Maillard and Angers, 2014). In agricultural regions where livestock and plant production co-exist, animal manure is applied in combination with other management practices such as tillage or in various cropping systems (e.g. annual vs. perennial). Higher SOC stocks at the soil surface are generally expected following conversion from conventional till to reduced tillage (reviewed by West and Post, 2002). Overall, perennial-based cropping systems are associated with increases in SOC (Bell *et al.*, 2012), whereas conventional monoculture can cause a loss of organic matter due to low levels of organic inputs and soil disturbance from frequent tillage (Fageria, 2012). As tillage practices and cropping systems determine the type, amount, and depth distribution of crop residues and soil organic matter (SOM), they may also have an influence on the fate of C derived from animal manure. However, relatively few long-term field studies have focused on the combined effects of manure application and other management practices, such as tillage practice (Sainju *et al.,* 2008, Viaud *et al.*, 2011) or cropping system (Holeplass *et al.*, 2004) on SOC stocks. In a meta-analysis in the Mediterranean Basin, Aguilera *et al.* (2013) have highlighted a significant beneficial effect on SOC stocks where animal manure was applied in combination with conservation management practices such as reduced- or no-tillage and cover crops.

Historically, in eastern Canada and elsewhere, dairy cattle manure has been managed in the solid form. However, in the past few decades there has been a significant shift towards management in the liquid or semi-solid form (e.g. VanderZaag *et al.*, 2013). Studies focusing on the response of SOC stocks to liquid animal manure are scarce compared to literature on solid manure (Maillard and Angers, 2014). In addition, response of SOC to liquid manure appears quite variable with positive (Lynch *et al.,* 2005, Mellek *et al.,* 2010), neutral (Angers *et al.*, 2010) or even negative effects (Angers *et al.,* 2010, Chirinda *et al.*, 2010, Jokela *et al.*, 2009).

Studies on the response of SOC stocks to management practices have historically mainly focused on the surface layers of the soil profile, ignoring or paying little attention to the subsoil (Syswerda *et al.,* 2011). With the objective of accounting for all SOC changes induced by management practices, it is critical to consider changes occurring in deeper horizons. Moreover, after liquid manure application, there is a possibility that manure-derived dissolved organic carbon (DOC) is transferred at depth. Indeed, there is usually an increase in soil DOC concentration following application of liquid manure (Angers *et al.,* 2006, Briceño *et al.,* 2008), and leaching of DOC has been observed in grassland soils fertilized with liquid swine manure (Royer *et al.,* 2007). In addition, several studies have shown that SOC content may be higher under mouldboard plowing than no-till at the bottom of the plow layer (Angers and Eriksen-Hamel, 2008), which underlines the importance of considering the subsoil in studies on SOC. Considering the whole soil profile is also particularly relevant in the case of comparison of annual with perennial systems, as there is a greater allocation of roots in deep soil layers with perennials (Bell *et al.,* 2012).

The objective of our work was to determine the response of SOC stocks to long-term (21 years) application of liquid dairy manure (LDM) in combination with 1) two primary tillage practices (conventional *vs.* reduced), and 2) two cropping systems (cereal monoculture *vs.* cereal–perennial forage rotation). We hypothesized that the accumulation of SOC following long-term LDM application would be greater in cropping systems involving reduced tillage and perennial crops than under continuous annual cropping and conventional tillage.

Materials and Methods

Site description

The field site is located at the Normandin Experimental Farm of Agriculture and Agri-Food Canada (48º50'N, 73º33'E) in Québec, Canada. The area is characterized by a cool continental climate with mean annual temperature of 1.1°C, and mean annual precipitation of 849 mm. The silty clay soil belongs to the Normandin series and is classified as a Humic Gleysol (Soil Classification Working Group 1998). At initiation of the study, the 0-20 cm soil layer had the following characteristics: pH-water (1:1) of 5.6, 26.1 g kg^{-1} organic C (0-7.5 cm), 490 g kg^{-1} clay and 80 g kg^{-1} sand. More details on the site, soil and experimental design of this study are presented in Bissonnette *et al.* (2001). A split-split plot experiment was initiated in 1989 with two crop sequences as main plots, two primary tillage practices as sub-plots, two nutrient sources as sub-sub-plots, and four replicates (plot size is 10 m by 5 m).

The crop sequences are a continuous spring barley (*Hordeum vulgare* L., 'Chapais') monoculture (M) and a 3-yr cereal-perennial forage rotation (R). From 1989 to 1999, the cereal-perennial forage rotation consisted of barley underseeded with a forage mix of timothy (*Phleum pretense* L. 'Champ') and red clover (*Trifolium pretense* L. 'Prosper'). Barley was harvested at the end of the 1st year, followed by two years of forage production. Since 2000, orchard grass (*Dactylis glomerata* L., 'Okay') has replaced timothy in the forage mix. The two fall primary tillage practices consist in chisel plow (CP) to a depth of 15 cm, and moldboard plow (MP) to a depth of 20 cm. The two cropping sequences involved different tillage frequencies: yearly in M, and at the end of the forage phase in R (i.e. once every three year).

The two nutrient sources were a complete mineral fertilizer (MIN) and liquid dairy manure (LDM). The fertilizers were broadcast and immediately incorporated to a depth of about 7.5 cm by one pass of a disk-harrow prior to seeding of barley on each year in the monoculture, and on the barley year in the rotation. Fertilizers were broadcast but not incorporated during the two forage years of the rotation system. According to local recommendations, the plots with the MIN treatment received 70 kg N ha^{-1} yr^{-1} as NH_4NO_3 for barley, and an average of 74 kg total N ha^{-1} yr^{-1} (range of 65 to 80 kg total N ha^{-1} yr^{-1}) for forage. For forage only, a second application of NH_4NO_3 after the first cut provided an additional average of 46 kg total N ha^{-1} yr^{-1} (range of 25 to 60 kg total N ha^{-1} yr^{-1}). Mineral P and K fertilizers were also added according to local recommendations. The LDM was obtained from a local dairy herd and was applied at 50 m^3 ha^{-1} yr^{-1} from 1990 to 2010. On the forage years of the rotation plots, 30 m^3 ha^{-1} of LDM was also applied after the first cut of hay from 1996 to 2010 (a supplemental application of 30 m^3 ha^{-1} of LDM was applied only in 2000 after the second cut of hay). The LDM provided an average of 152 kg total N ha^{-1} yr^{-1} (range of 73 to 242 kg total N ha^{-1} yr^{-1}) to plots under forage production, and 102 kg total N ha^{-1} yr^{-1} (range of 73 to 153 kg total N ha^{-1} yr^{-1}) to the barley plots. The LDM dry matter content was measured every year with an overall average of 49 g kg^{-1} for the 21 years. We estimated the C input from manure over 21 years by considering a mean C content of 390 g kg^{-1} dry matter. This estimate was calculated using the average manure-C content of the last six years (2007-2012) for which data were available. Barley was harvested in mid-August to mid-September, depending on the year, and gave an average grain yield of 3.5 Mg ha^{-1} yr^{-1}. Two cuts of hay were taken annually from the forage plots, which gave an average yield of 4.7 Mg dry matter ha^{-1} yr^{-1}. The annual C input to soil consisted of crop residues in the MIN plots, and crop residues and manure in LDM plots. The C inputs derived from the cereal and the forage crops were estimated using allocation coefficients proposed by Bolinder *et al.* (2007) for crops in eastern Canada. The C inputs derived from plant biomass and LDM are presented for all treatments in Table 2.1.

Soil sampling

In 2010, three randomly located pits were dug to 60 cm in each experimental plot, and one soil sample was collected at each of 6 soil layers in each pit: 0-5, 5-10, 10-20, 20-30, 30-40, and 40-50 cm. The three samples from each soil layer in each plot were combined to obtain a composite sample for each soil layer of each plot. Soil was sieved at 6 mm directly in the field and air-dried at room temperature in the laboratory. Soils were also sampled to determine the bulk density of each layer. Stainless cylinders of 5.5-cm diameter and 3-cm height were used for the top 10 cm, and stainless cylinders of 5-cm diameter and 5-cm height were used for the deeper layers. Soil cores were dried at 105ºC to determine bulk density.

Carbon analysis and SOC stock calculations

Soil C concentration (g kg^{-1}) was measured by dry combustion with a CNS analyzer (LECO, TRUSPEC, Leco Corp., St-Joseph, MI, USA) on air-dried soil, ground and sieved at 0.5 mm. To account for differences in soil mass between treatments, SOC stock (Mg ha^{-1}) was calculated by using the equivalent soil mass approach (Ellert and Bettany, 1995). For the cumulative soil layers 0-10, 0-20, 0-30, 0-40 and 0-50 cm, we calculated the C stock in the respective equivalent soil masses: 65, 130, 260, 390, 520 and 650 kg m^{-2}. For lighter soil layers, the C stock in the equivalent soil mass was calculated by summing C stock in the considered soil layer (C stock at fixed depth), plus those in the additional thickness of the subsurface layer required to attain the equivalent soil mass, as described in detail in Ellert and Bettany (1995). For heavier soil layers, the C stock in the equivalent soil mass was calculated by subtracting those in the excess mass of soil (Ellert *et al.,* 2006). Layer by layer (10-20, 20-30, 30-40 and 40-50 cm) C stocks were obtained by subtracting the cumulative C stocks.

Statistical analysis

Crop sequence, tillage practice, nutrient source and their interactive effects on C stocks were analyzed by applying an ANOVA with the mixed procedure of SAS (version 9.2; SAS Institute, Cary, NC) for each soil layer separately. The split-split plot design was considered in the ANOVA comprising the following terms and their associated degrees of freedom in parentheses: block (3); crop sequence (1); whole plot error (3); tillage practice (1); crop sequence × tillage practice (1); sub-plot error (6); nutrient source (1); crop sequence × nutrient source (1); tillage practice × nutrient source (1); crop sequence × tillage practice × nutrient source (1); sub-subplot error (11) (instead of 12 due to a missing value). Assumptions of homogeneity of variance and normality of residuals were verified graphically. Normality of residuals was also tested using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Log transformation was required in some cases to ensure homogeneity of variance. Tables and figures present least-square means and standard errors of non-transformed data. Statistical results were considered significant at the $P<0.05$ level, but effects with $P<0.10$ were also discussed. The Tukey's test was used to compare treatment means. We used the "pdmix800.SAS" program to assign Tukey letters for treatments (Saxton, 1998).

Results and discussion

Overall, nutrient source had a significant effect on SOC stocks in the 0-20 cm soil layer (Table 2.2). More specifically, in the 0-10 cm layer, there was a significant interaction between nutrient source and crop sequence as SOC stocks were 22% greater with LDM than MIN in the cereal-forage rotation, but only 7% (not significant) greater with LDM than MIN in the cereal monoculture (Table 2.3, Figure 2.1). In the 10-20 cm layer, SOC stocks were significantly greater with manure than mineral fertilizer regardless of tillage practice or crop sequence treatments. In deeper soil layers, SOC stocks decreased sharply and there were no effects of nutrient source, which does not support the possibility that liquid manure affects SOC stocks below the plow layer. The only significant treatment effect at depth was that of tillage practice, which will be discussed later.

As opposed to the well-known positive effect of solid animal manure on SOC, the effect of liquid manure is less clear (Aguilera *et al.*, 2013). Despite a few studies that report positive effects of liquid manure on SOC (Lynch *et al.*, 2005, Mellek *et al.,* 2010), it is more commonly acknowledged that liquid manures have little effects on SOC (Aguilera *et al.*, 2013). The absence of an effect, or even a decrease in SOC stocks (Angers *et al.*, 2010, Chirinda *et al.,* 2010, Jokela *et al.,* 2009) is generally attributed to the high decomposability of the C compounds and the generally low C input (Plaza *et al.,* 2004), and a possible priming effect on the decomposition of SOC induced by liquid manure (Fangueiro *et al.*, 2007, Plaza *et al.,* 2004). In our study, we did observe a significant positive effect of LDM on SOC stocks. It is important to note that many studies reporting a negative effect involved pig manure (Angers *et al.,* 2010, Chirinda *et al.,* 2010, Plaza *et al.*, 2004). The manure organic matter originating from pig production would contain more degradable compounds than that of cattle manure due to differences in feeding and digestion systems, and bedding management (Velthof *et al.*, 2000). Indeed, the ruminant's diet generally presents a much higher fiber content than that of pigs, which are mainly fed grains, and consequently their feces contain a much higher fibre content (Velthof *et al.*, 2000).

One of the most interesting results of this study is the much greater effect of LDM application in the perennial-based rotation than in the cereal monoculture, particularly in the top 10 cm (Table 2.3). When considering the cumulative SOC stocks for increasing portions of the soil profile, we observed a significant crop sequence x nutrient source interaction down to 30 cm (Table 2.4). However, the differences between the treatments were already apparent in the top 10 cm (Table 2.3) and remained similar down to 50 cm (Table 2.5). When considering the top 30 cm, LDM application increased SOC stocks by 16% in the perennial-based rotation, and only by 0.5% (not significant) in the cereal monoculture (Table 2.5). Conditions prevailing in the perennial-based rotation appeared conducive to the stabilization of manure-derived C. One of the main factors that can explain this effect is the difference in C input induced by manure application, either directly or indirectly through its influence on crop yields (Table 2.1). Indeed, compared to the mineral fertilization, LDM total C inputs induced by manure application were on average 89% higher in the rotation, as opposed to 38% in the monoculture. On average, estimates of C retention coefficients (see footnote of Table 2.6 for details of calculations) for the whole-soil profile (0-50 cm) reveal that 36% of the C derived from LDM was retained as SOC in the perennial-based rotations, whereas it was slightly negative at 7% in the monoculture (Table 2.6). This generally means that manure-induced C (direct and indirect) was retained in the rotation at a fairly high rate and lost in the monoculture. These results are consistent with the very variable effect of liquid manure generally observed in the literature as mentioned earlier. In their meta-analysis, Maillard and Angers (2014) also highlighted the large variability in the retention of C induced by manure, and suggested that more studies are needed to determine the influence of climate, soil type, manure type and origin, and other management factors. Our results provide field-based observations confirming that manure-induced changes in SOC can be strongly modulated by other management practices such as crop rotations.

The differential effect of LDM on SOC retention in the two crop sequences could be explained by differences in the frequency of tillage, which occurred only once every three years in the perennial-based rotation, whereas it was performed each year in the monoculture. Following manure application, organic matter is incorporated into stable soil aggregates (Aoyama *et al.*, 1999, see also chapter 3). More frequent tillage operations can lead to aggregate disruption, which exposes intra-aggregate organic matter to microbial decomposition (Angers *et al.*, 1992, Six *et al.,* 2000). However, it is interesting to note that the primary tillage practice (moldboard *vs.* chisel plowing) did not affect the retention of manure-derived C. This is in agreement with Viaud *et al.* (2011) who reported no interactive effect on SOC stocks between tillage practices and animal manure application. In our study, it appears that it is more the frequency of tillage than the tillage practice itself that influenced the response of SOC stocks to LDM application.

In the broader context, these results can also provide valuable information for regional- and national-based assessment of management practices favouring soil C sequestration. In a European-based assessment, Smith *et al.* (1997) hypothesized that the effect of manure on SOC would be greater in arable soil than in grassland. They put forward that because arable soils contain less organic matter, they would be more prone to accumulate C following manure application. Our results tend to contradict this hypothesis and rather suggest that manure-induced changes in SOC are greater in grassland-based systems than in annually tilled arable cropping systems.

In addition to the manure effects, our study also provides interesting results concerning the impact of tillage practices on SOC in these systems. In the top 10 cm of soil, there was a significant tillage practice x crop sequence interaction (Table 2.2) with a greater beneficial effect of reduced tillage on SOC stocks in the rotation than in the monoculture (Table 2.3, Figure 2.2). The effect of tillage practice was not significant in the following soil layer (10-20 cm), but greater SOC stocks were found in the 20-30 and 30-40 cm layers of soils under moldboard plow than in soils under reduced tillage, regardless of crop sequence or nutrient source (Table 2, Table 3, Figure 2.2). The analysis of the effect of tillage practice on SOC stocks in the 10-20 cm soil layer could be improved with a finest soil sampling in this layer (10-15 and 15-20 cm) to consider the differential depths of tillage practices (15 cm for chisel plow and 20 cm for moldboard plow). The positive effect of reduced tillage on surface SOC, especially in the rotation system, is consistent with the literature (e.g. review by West and Post, 2002). However, in the monoculture system, where tillage operations take place every year, it seems that reduced tillage did not bring much benefit to SOC. The greater SOC content at depth under inversion (moldboard) tillage compared to no-till or reduced tillage has been noticed in soils of eastern Canada (Angers *et al.,* 1997, Carter, 2005, Poirier *et al.*, 2009), and in many situations around the world (Angers and Eriksen-Hamel, 2008, Luo *et al.*, 2010). When considering cumulative SOC stocks, the positive effect of reduced tillage was observed for the 0-20 cm layer, but the effect became not significant when considering the 0-30 or the 0-50 cm profile (Tables 2.4 and 2.5). Overall, as observed by others in eastern Canada (Angers *et al.,* 1997, Carter, 2005, Poirier *et al.,* 2009) and elsewhere (Angers and Eriksen-Hamel, 2008, Blanco-Canqui and Lal, 2008, Luo *et al.*, 2010), the greater SOC stocks at depth under plowing compensate for the greater values found at the surface under reduced tillage, resulting in no net effect of tillage on SOC stocks when considering the whole soil profile.

Conclusion

Overall, our study provides a new contribution to clarify the impact of animal manure on SOC stocks, and in particular liquid manure for which previous literature shows contradictory results. Our study shows that in the long term (21 years), yearly application of LDM results in an increase in SOC stocks in the top 20 cm of soil. No effects were observed at depth (>20 cm). Interestingly, our results clearly show that the magnitude of the manure-induced change in SOC is dependent on crop sequence. Indeed, the retention of manure-derived C in SOC was much greater under a cereal-perennial forage rotation than under a cereal monoculture. To the contrary, the primary tillage practice did not influence the retention of manure-C in soil. It therefore appears that the lower frequency of tillage in the perennial-based rotation (every three years) than in the monoculture (yearly operation) favoured the retention of manure-derived C in soil, whereas primary tillage practice (moldboard *vs.* chisel plow) had little influence. Our study also provides additional data supporting previous studies showing that reduced tillage influences SOC distribution in the profile, but has little effect on the total SOC stocked in the whole soil profile (e.g. 0-50 cm).

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Table 2-1: Estimated annual C inputs from manure and plant biomass returned to soils in the different management systems.

Management system$^{\gamma}$	Annual manure-C input (Mg C ha^{-1} yr^{-1})	Annual plant biomass-C input (Mg C ha^{-1} yr^{-1})$^{\Delta}$	Total C input (Mg C ha^{-1} yr^{-1})
M-CP-MIN		1.8	1.8
M-CP-LDM	0.9	1.7	2.6
M-MP-MIN		1.9	1.9
M-MP-LDM	0.9	1.6	2.5
R-CP-MIN		1.8	1.8
R-CP-LDM	1.2	2.0	3.2
R-MP-MIN		1.6	1.6
R-MP-LDM	1.2	1.9	3.1

$^{\gamma}$M, barley monoculture; R, barley-forage rotation; CP, chisel plowing; MP, moldboard plowing; MIN, mineral fertilization; LDM, liquid dairy manure.

$^{\Delta}$Plant biomass C inputs were evaluated by considering a C content of 45% in the plant tissues, shoot:root ratio and plant C allocation coefficients proposed by Bolinder et al. (2007). 15% of above-ground parts were considered to return to the soil as litter fall and harvest losses for barley and for forage in the 2nd year of the rotation. An additional 10% was considered for forage in the 3rd year of the rotation.

Table 2-2: Probabilities of the treatment effects on SOC stocks in individual (0-10, 10-20, 20-30, 30-40 and 40-50 cm) soil layers.

Source of variance	Soil layers				
	0-10 cm	10-20 cm	20-30 cm	30-40 cm	40-50 cm
Crop sequence	**0.006**	**0,023**	0,224	0,565	0,436
Tillage practice	**0.000**	0.907	**0.002**	**0.021**	0.294
Nutrient source	**0,000**	**0,021**	0,577	0,378	0,339
Crop sequence × Tillage practice	**0.014**	0.305	0.346	0.214	0.473
Crop sequence × Nutrient source	**0,011**	0,278	0,078†	0,831	0,533
Tillage practice × Nutrient source	0.490	0.936	0.408	0.652	0.284
Crop sequence × Tillage practice × Nutrient source	0.617	0.709	0.328	0.767	0.630

†p<0,1

Table 2-3: Means (standard errors) for SOC stocks (kg m^{-2}) in individual (0-10, 10-20, 20-30, 30-40 and 40-50 cm) soil layers. Values are only shown for statistically significant effects.

Treatment	Soil layers				
	0-10 cm	10-20 cm	20-30 cm	30-40 cm	40-50 cm
Crop sequence					
R	3.53 (0.20)	2.87 (0.19)			
M	2.67 (0.20)	2.41 (0.19)			
Tillage practice					
CP	3.43 (0.19)		1.43 (0.20)	0.69 (0.11)	
MP	2.77 (0.19)		2.02 (0.20)	0.85 (0.11)	
Nutrient source					
MIN	2.88 (0.19)	2.51 (0.19)			
LDM	3.31 (0.19)	2.76 (0.19)			
Crop sequence × Tillage practice					
R-CP	4.00 (0.21) a				
R-MP	3.05 (0.21) b				
M-CP	2.85 (0.21) bc				
M-MP	2.49 (0.21) c				
Crop sequence × Nutrient source					
R-MIN	3.87 (0.21) b				
R-LDM	3.18 (0.21) a				
M-MIN	2.58 (0.21) c				
M-LDM	2.76 (0.21) bc				

M, barley monoculture; R, barley-forage rotation; CP, chisel plowing; MP, moldboard plowing; MIN, mineral fertilization; LDM, liquid dairy manure.

Table 2-4: Probabilities of the treatment effects on SOC stocks in the cumulative (0-20, 0-30, 0-40 and 0-50 cm) soil layers.

Source of variation	Soil depths			
	0-20 cm	0-30 cm	0-40 cm	0-50 cm
Crop sequence	**0.007**	**0.012**	**0.015**	**0.017**
Tillage practice	**0.015**	0.852	0.727	0.621
Nutrient source	**0.002**	**0.042**	0.084†	0,127
Crop sequence × Tillage practice	0.075†	0.097†	0.092†	0.095†
Crop sequence × Nutrient source	0.063†	**0.053**	0.073†	0,089†
Tillage practice × Nutrient source	0.709	0.913	0.982	0.949
Crop sequence × Tillage practice × Nutrient source	0.658	0.483	0.496	0.497

†$p<0,1$

Table 2-5: Means (standard errors) for SOC stocks (kg m^{-2}) in the cumulative (0-20, 0-30, 0-40 and 0-50 cm) soil layers. Values are only shown for statistically significant effects.

Treatment	Soil depths			
	0-20 cm	0-30 cm	0-40 cm	0-50 cm
Crop sequence				
R	6.40 (0.38)	8.21 (0.57)	9.00 (0.66)	9.58 (0.72)
M	5.08 (0.38)	6.71 (0.57)	7.47 (0.66)	8.02 (0.72)
Tillage practice				
CP	6.06 (0.38)			
MP	5.41 (0.38)			
Nutrient source				
MIN	5.39 (0.38)	7.15 (0.57)	7.95 (0.66)	
LDM	6.08 (0.38)	7.77 (0.57)	8.52 (0.66)	
Crop sequence × Tillage practice				
R-CP	6.92 (0.40) a	8.50 (0.60) a	9.24 (0.69) a	9.81 (0.75) a
R-MP	5.87 (0.41) b	7.92 (0.61) ab	8.75 (0.70) a	9.34 (0.76) a
M-CP	5.20 (0.40) b	6.47 (0.60) c	7.11 (0.69) b	7.61 (0.75) b
M-MP	4.96 (0.40) b	6.95 (0.60) bc	7.82 (0.70) ab	8.42 (0.75) ab
Crop sequence × Nutrient source				
R-MIN	5.87 (0.40) b	7.61 (0.61) b	8.41 (0.69) ab	9.00 (0.75) ab
R-LDM	6.93 (0.41) a	8.81 (0.60) a	9.58 (0.70) a	10.15 (0.76) a
M-MIN	4.91 (0.40) c	6.70 (0.60) b	7.48 (0.69) b	8.05 (0.75) b
M-LDM	5.24 (0.40) bc	6.73 (0.60) b	7.46 (0.69) b	7.98 (0.75) b

M, barley monoculture; R, barley-forage rotation; CP, chisel plowing; MP, moldboard plowing; MIN, mineral fertilization; LDM, liquid dairy manure.

Table 2-6: SOC stocks in the whole (0-50 cm) soil profile for the different management systems, total C input (plant biomass + LDM) for the 21 years of study, and retention coefficients of LDM-derived C.

Management system[α]	SOC stock (kg C m^{-2})	SOC stock change induced by LDM[β] (kg C m^{-2})	Total C input for the 21 yrs[γ] (kg C m^{-2})	Difference in C input induced by LDM[Δ] (kg C m^{-2})	LDM-derived C retention coefficient[ε] (%)	Average LDM-derived C retention coefficient[η] (%)
M-CP-MIN	7.52 (0.82)[θ]		3.81			
M-CP-LDM	7.70 (0.82)	0.18	5.47	1.66	11	-7
M-MP-MIN	8.58 (0.82)		4.03			
M-MP-LDM	8.26 (0.82)	-0.32	5.34	1.31	-24	
R-CP-MIN	9.34 (0.82)		3.71			
R-CP-LDM	10.28 (0.82)	0.94	6.77	3.07	31	36
R-MP-MIN	8.66 (0.82)		3.39			
R-MP-LDM	10.01 (0.86)	1.35	6.63	3.24	42	

[α] M, barley monoculture; R, barley-forage rotation; CP, chisel plowing; MP, moldboard plowing; MIN, mineral fertilization; LDM, liquid dairy manure.

[β] SOC stock difference between LDM and MIN; [γ] Total C input includes plant biomass and direct input from manure; [Δ] Difference in total C input between LDM and MIN; [ε] LDM-derived C retention coefficient represents the proportion of manure-derived C input (direct and plant biomass) retained as SOC relative to mineral fertilization. It was calculated as the ratio of the SOC stock change induced by LDM to the difference of C input induced by LDM; [η] Average of the two tillage practices for each crop sequence.

[θ] Least square means (standard errors).

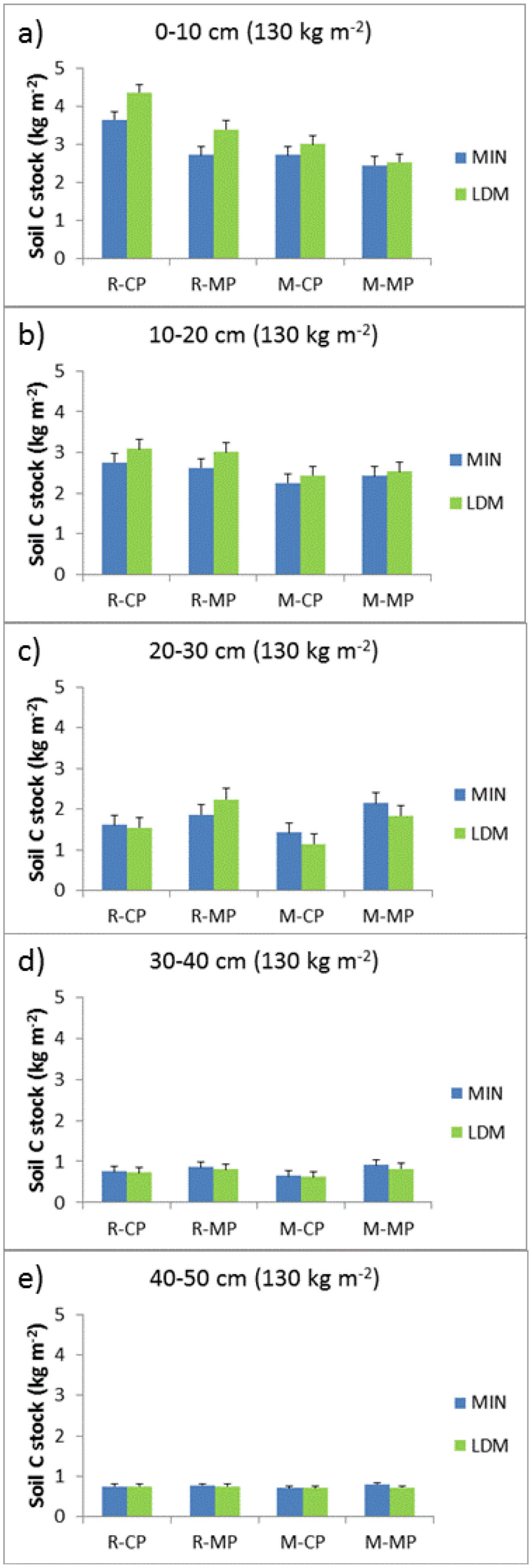

Figure 2-1: SOC stocks in the different management systems in the individual (0-10, 10-20, 20-30, 30-40 and 40-50 cm) soil layers. Least square means and standard errors are presented.

Chapter 3: Carbon accumulates in organo-mineral complexes after long-term liquid dairy manure application

Chapitre 3 : Accumulation du carbone dans des complexes organo-minéraux suite à l'application de lisier de bovins

ÉMILIE MAILLARD, DENIS A. ANGERS, MARTIN CHANTIGNY, SHABTAI BITTMAN, PHILIPPE ROCHETTE, GABRIEL LÉVESQUE, DEREK HUNT, and LÉON-ÉTIENNE PARENT

Résumé

Il est important de quantifier et de comprendre l'impact de l'application d'effluents d'élevage sur les stocks de carbone organique (CO) du sol pour répondre à des enjeux agronomiques et environnementaux. L'effet des effluents liquides sur les stocks de CO du sol a été moins étudié et est plus variable que celui des effluents solides. De plus, peu d'études ont analysé les effets des effluents d'élevage sur des fractions relativement stables du CO du sol. Notre objectif était de quantifier le CO dans le sol entier et dans des fractions physiques spécifiques de la matière organique (fractions légères libre et intra-agrégats, fractions lourdes de la taille des sables et de la taille des limons-argiles) le long d'un profil de sol (0-50 cm) après 15 années d'application de lisier de bovins et de fertilisation minérale. Les deux types de fertilisants étaient appliqués à des doses équivalentes à 200 et 400 kg de N minéral ha^{-1} an^{-1} (doses faible et forte, respectivement) à une prairie de fétuque élevée (*Festuca arundinacea* Schreb. var. festorina) sur un sol limoneux (série Monroe) d'un climat maritime à Agassiz (Colombie Britannique, Canada). Le stock de CO du sol entier était significativement plus élevé à la surface du sol (jusqu'à 20 cm) après l'application du lisier de bovins comparativement à la fertilisation minérale ou au témoin non amendé. Il n'y avait pas d'effet sur le CO dans les couches profondes (en-dessous de la profondeur de labour), ce qui peut être lié à l'absence de transfert de CO ou à un apport de CO insuffisant pour contrebalancer un possible effet accélérant de la minéralisation du CO du sol induit par le lisier. La fertilisation minérale a mené à des stocks de CO plus élevés mais seulement dans les 5 premiers centimètres du sol. Par rapport à la fertilisation minérale, l'effet du lisier de bovins était plus profond, vraisemblablement en relation avec des apports directs de CO par l'effluent. L'application de lisier de bovins a favorisé l'incorporation de CO dans des complexes organo-minéraux primaires et secondaires essentiellement. L'incorporation dans la fraction légère libre de la matière organique était plus faible et non significative. La séparation granulométrique de la fraction lourde (densité > 1.8 Mg m^{-3}) du sol a mis en évidence la présence de complexes organo-minéraux de la taille des sables très sensibles aux traitements, et particulièrement à l'application de lisier de bovins. Cette fraction lourde de la taille des sables devrait être considérée davantage afin de détecter de façon plus fine les effets des changements de pratiques sur les stocks de CO du sol.

Abstract

Quantifying and understanding the impact of animal manure on soil organic carbon (OC) is important for agronomic and environmental purposes. The influence of liquid manures on soil OC stocks has been less studied and was found to be more variable than that of solid manures. In addition, only a few studies have analyzed the effects of animal manure on relatively stable fractions of soil OC. Our objective was to quantify OC in the whole soil and in specific physical fractions of organic matter (free and intra-aggregate light fractions, and sand-size, silt+clay-size heavy fractions) along a 0-50 cm soil profile after 15 years of applications of liquid dairy manure (LDM) and mineral fertilizers. Manure and mineral fertilizer were both applied at nominally 200 and 400 kg mineral-N ha^{-1} yr^{-1} rates (low and high rates, respectively) to tall fescue (*Festuca arundinacea* Schreb. var. festorina) grown on a Monroe silt in a maritime climate in Agassiz (British Columbia, Canada). The whole-soil OC stock was significantly higher in the top soil (down to 20-cm depth) amended with LDM as compared to mineral fertilizers or unamended top soil. There was no effect on OC in the subsoil (below the tillage depth), which may be linked to the absence of C transfer or to an insufficient C input to offset a possible LDM-induced priming effect on soil OC mineralization. Mineral fertilization led to higher OC stocks but only in the top 5 cm of soil. In comparison to mineral fertilization, the effect of LDM on soil C was deeper, likely due to the direct C inputs from LDM. LDM application favoured the incorporation of C into primary and secondary organo-mineral complexes and less into the free light fraction of soil organic matter. The size separation of the heavy (density > 1.8 Mg m^{-3}) soil fraction revealed the presence of sand-size organo-mineral complexes that were sensitive to treatments, and particularly to LDM application. This sand-size heavy fraction should be given more scrutiny to detect more precisely the effect of management changes in soil OC stocks.

Introduction

Animal manure is a significant source of organic matter which is important for soil fertility through its effects on physical, biological and chemical properties (Stevenson, 1994). In addition, in the context of climate change, storage of carbon (C) in soils may help to reduce the rate of accumulation of atmospheric C dioxide (Janzen, 2004). Hence, it is relevant to identify the agronomic practices that may lead to changes in soil C stocks. Studies focusing on the impact of liquid animal manure on soil OC stocks are relatively scarce compared to those looking at solid manure (Maillard and Angers, 2014). In addition, its effect appears quite variable with positive (Lynch *et al.*, 2005, Mellek *et al.*, 2010), neutral (Angers *et al.*, 2010) or even negative impacts (Jokela *et al.*, 2009, Angers *et al.*, 2010, Chirinda *et al.*, 2010).

Evaluation of manure-induced changes in soil total OC stocks does not inform on the nature or persistence of soil OC. Resistance to decomposition of OM depends on its association with minerals, occlusion within aggregates, and its inherent recalcitrance (Mikutta *et al.*, 2006). The soil OC pool is not homogeneous in terms of stability and it can be conceptually divided into fractions with different residence times (Heitkamp *et al.*, 2011). A classification of soil OC into three conceptual pools with increasing recalcitrance and mean residence time is usually proposed (Schwendenmann and Pendall, 2008, Von Lützow *et al.*, 2008): a 'labile' or 'active' pool, a 'slow' or 'intermediate' pool and a 'passive' or 'resistant' pool. Quantification of these conceptual pools (Von Lützow *et al.*, 2007, Heitkamp *et al.*, 2011) is usually approached using either chemical or physical fractionation methods but remains a challenge. It has been argued that particle size and density fractions of organic matter might be more effective indicators of functionally distinct organic matter pools than classical chemically based methods (Wander and Traina, 1996). These methods are also less destructive than chemical methods (Assis *et al.*, 2012). The response of 'hypothetically labile' fractions, such as microbial biomass, particulate organic matter, or light organic matter, to animal manure application has been largely studied (Houot and Chaussod, 1995, Min *et al.*, 2003, Rudrappa *et al.*, 2006, Yan *et al.*, 2007, Yang *et al.*, 2007, Sainju *et al.*, 2008, Hai *et al.*, 2010), particularly because these fractions are viewed as indices of soil quality and fertility. The C content and concentration in these fractions were generally found to be greater with animal manure application than with mineral fertilizers or in unfertilized control. Fewer studies analyzed the effects of animal manure on more stable fractions of soil OC, such as mineral-associated organic matter or organic matter occluded in micro-aggregates (Wu *et al.*, 2005, Hai *et al.*, 2010, Huang *et al.*, 2010a, Huang *et al.*, 2010b). Sohi *et al.* (2001) proposed a combined density and particle-size separation scheme to isolate specific soil organic matter fractions corresponding to different levels of physical protection. This method distinguishes an intra-aggregate light fraction of organic matter from its free counterpart (Sohi *et al.*, 2010). Another feature of this method is the size separation of the heavy fraction. Studying the effect of animal manure on specific fractions of different levels of physical protection may help understanding the stabilization mechanisms of manure-derived organic matter in soils.

Studies on the impact of management practices on soil OC stocks have historically focused mainly on the surface layers of the soil profile, ignoring or paying little attention to the subsoil (Syswerda *et al.*, 2011). Main sources of organic matter input into subsoil are dissolved organic carbon (DOC), plant roots and root exudates, and transport of particulates from the surface, which can be enhanced by bioturbation (Rumpel and Kögel-Knabner, 2011). Consequently, animal manure application or mineral fertilization could affect the C stock in the subsoil, and this response may be different than that in the topsoil. Studying soil OC stocks in the whole profile seems to be particularly relevant in the context of liquid manure application, as there may be significant downward transfer of manure-derived DOC. Indeed, there is usually an increase in soil DOC concentration following application of liquid manure (Angers *et al.*, 2006, Briceño *et al.*, 2008), and leaching of DOC has been observed in grassland soils fertilized with liquid swine manure (Royer *et al.*, 2007). Therefore, to account for all soil OC changes induced by a treatment, it is important to consider changes that may occur in the subsoil horizons. Moreover, as many studies addressing the mechanisms of soil OC stabilization in subsoil layers have been carried out in the laboratory, it is necessary to evaluate the significance of these mechanisms with *in situ* long-term experiments (Schmidt *et al.*, 2011).

The objective of our work was to determine the long-term impact of liquid dairy manure (LDM) and mineral fertilizer application on OC stocks in the whole soil and its physical fractions in the 0-50 cm profile of a soil cropped to tall fescue (*Festuca arundinacea* Schreb.) in a mild maritime climate. We hypothesized that after 15 years of LDM application, (i) soil OC stocks are larger than under mineral fertilization or in an unamended control, (ii) soil OC stocks are greater in more stable OC fractions, such as intra-aggregate light fraction and heavy fraction of organic matter, after LDM applications, and (iii) that the response of soil OC stock and all of its fractions to treatments differs between topsoil and subsoil.

Material and methods

Site description

The field site is located at the Pacific Agri-Food Research Centre at Agassiz in south coastal British Columbia, Canada (49°17'N 121°45'W). The climatic zone is warm temperate fully humid with warm summer according to Köppen-Geiger climate classification. The silt to silt loam soil belongs to the Monroe series and is classified as a moderately well to well-drained Eutric Eluviated Brunisol (Soil Classification Working Group, 1998). The soil contains 97 g kg^{-1} sand and 166 g kg^{-1} clay (30 cm-depth) and the pH water (1:1) is 5.6. More details on the soil and site characteristics are available in Bittman *et al.* (2007). A stand of tall fescue (*Festuca arundinacea* Schreb. var. festorina) was established in 1993. A complete randomized block design with four replications was established in 1994 on this stand. To improve the stand, tall fescue was reseeded in 2003 following moldboard plowing to 25 cm; the field was harvested but no measurements were taken as yields were very low.

Six treatments were considered in this study: an unfertilized control (Control), two liquid dairy manure (LDM) treatments applied at target rates of 50 (Man-low) and 100 kg ha^{-1} (Man-high) of NH_4-N (total ammonium nitrogen) per application, two mineral (ammonium nitrate) fertilizer treatments applied at rates of 50 (Fert-low) and 100 kg N ha^{-1} (Fert-high) per application, and a treatment with alternating applications of manure at rates of 100 kg NH_4-N ha^{-1} and fertilizer at rates of 100 kg N ha^{-1} (Alt) within each year. These treatments were applied to plots measuring 3 × 65 m from 1996 to 2010 (15 years). Applications were carried out generally four times each year, in early spring and after each hay cut, except for the final cut in the fall. There were just three applications in 1994. No treatments were applied in 2003 as the stand was ploughed.

The LDM with wood shavings for bedding, mean dry matter (DM) content of 67 g kg^{-1} and C content of 407 g kg^{-1} DM was obtained from typical local high producing dairy farms. Manure was surface banded with a sliding-shoe applicator. We estimated that over 16 years a total of 57 Mg C ha^{-1} was applied to the Man-low and 110 Mg C ha^{-1} was applied to the Man-high. The C inputs derived from the grass stand were estimated using allocation coefficients proposed by Bolinder *et al.* (2007). Forage DM yields and C inputs derived from plant biomass and manure are presented for all treatments in Table 3.1.

Soil sampling

In March 2011, three randomly located pits were dug to 60 cm in each experimental plot, and three soil samples were collected at different locations in the pit at each of 6 depths: 0-5, 5-10, 10-20, 20-30, 30-40 and 40-50 cm. The three samples from each soil layer in each plot were combined. Soil samples were passed through a 6 mm sieve in the field and air-dried at room temperature in the laboratory. For bulk density determination, soils were sampled for each layer using stainless steel cylinders (5-cm diam.; 5-cm height) and dried at 105ºC.

Soil organic matter fractionation

We used a soil organic matter fractionation method adapted from Sohi *et al.* (2001) and also available online (http://www.rothamsted.ac.uk/aen/CarbonCycling/pdf/Fractionation_manual.pdf). The method combines density fractionation in sodium iodide aqueous solution (NaI, 1.8 g ml^{-1}), and particle size fractionation (Fig. 3.1). Fifteen grammes of air-dried soil were placed in a 250-mL centrifuge bottle and moistened with 7 ml of NaI overnight. The next day, 90 mL of NaI solution were added and the bottle was gently shaken by hand to suspend soil particles. The soil suspension was then centrifuged for 45 minutes at 4100 g. The NaI with all material floating or in suspension was collected by siphoning. The free light fraction (FLF) of organic matter was recovered by filtering at 0.45 µm using a vacuum filtration unit fitted with a nylon membrane filter (Nylaflo, Pall Corporation, Ann Arbor, NI, USA) and washed with about 200 mL of distilled water. The organic matter was removed from the filter by rinsing it with distilled water and scraping it with the edge of a razor, and then transferred into a plastic cup. The NaI collected during the first filtration was returned to its original bottle. The bottle was shaken to re-suspend the soil residue, and soil aggregates in the suspension were dispersed using an ultrasonic generator (Model Q500, QSONICA, Newton, Connecticut, USA) fitted with a 6-mm diameter probe. A dispersion energy of 750 J g^{-1} was applied, corresponding to a rate of 47 W (calibrated by temperature change in water) for 240 seconds. The instrument was adjusted between 69 and 70% of amplitude to obtain 47 W of calorimetrically determined power dissipation. The intra-aggregate light fraction (IALF) of the organic matter was recovered after centrifugation, as described above for the FLF. The FLF and IALF samples were dried at 50ºC for 48h then weighed. The residual soil containing the heavy fraction of organic matter was rinsed three times by pouring 90 mL of distilled water, centrifuging for 15 min at 2600 g, and siphoning the water above the soil residue. Finally, 100 mL of distilled water was added to the soil residue and the mixtures was shaken for at least 3 hours using a reciprocal shaker before washing through a 53 µm-sieve to separate the sand-size heavy fraction (SHF) from the silt+clay-size heavy fraction (SCHF). Each fraction was collected with distilled water in a 600-mL beaker, dried at 50 ºC for 5 days then weighed. For the subsoil layers (30-40 cm and 50-60 cm), two sub-samples of 15 g were used to maximize the quantity of recovered light organic matter. One sample of LDM and samples of FLF, IALF and SHF selected from three unreplicated treatments (Control, Man-high, Fert-high), and three sampling depths (0-5, 10-20, 40-50 cm) were examined under a binocular microscope (Model Axio Scope A.1, Zeiss).

C and N analyses and soil OC stocks calculation

Subsamples of whole soil, SHF and SCHF were ground with a ball mill in preparation for C and N analysis whereas the FLF and IALF samples were analyzed for C and N without additional preparation. Total C and N concentrations in both whole soil and soil organic matter fractions were determined with a CNS analyzer (Model TRUSPEC, LECO Corp., Saint-Joseph, MI, USA). To determine the C concentration of each soil organic matter fraction on a whole soil basis (g C kg^{-1} soil), C concentration of the fraction (g C kg^{-1} fraction) was multiplied by the mass of the fraction samples (g), then divided by the mass of the whole soil sample (g). On average, 99.6% of the initial soil mass and 97.4% of soil OC were recovered from fractionated samples. The OC stock (kg m^{-2}) for a fixed depth in whole soil and in soil fractions was computed by multiplying the C concentration (g C kg^{-1} soil) by the sampling depth (m) and the related bulk density (g cm^{-3}). To account for different soil bulk density between treatments, C stock was corrected by using the equivalent soil mass approach (Ellert and Bettany, 1995). For the cumulative soil layers 0-5, 0-10, 0-20, 0-30, 0-40 and 0-50 cm, we calculated the C stock in the respective equivalent soil masses: 50, 100, 200, 300, 400 and 500 kg m^{-2}. For lighter soil layers, the OC stocks in the equivalent soil mass were calculated by summing C stock in the considered soil layer (C stock at fixed depth), plus those in the additional thickness of the subsurface layer required to attain the equivalent soil mass, as described in detail in Ellert and Bettany (1995). For heavier soil layers, the C stocks in the equivalent soil mass were calculated by subtracting those in the excess mass of soil (Ellert *et al.*, 2006). Layer by layer (0-5, 5-10, 10-20, 20-30, 30-40 and 40-50 cm) C stocks were obtained by subtracting the cumulative C stocks.

Statistical analysis

Treatment effects within a soil layer and the whole 0-50 cm soil profile for all soil C variables (whole-soil OC stocks and OC stocks in the different fractions) were analyzed using a randomized complete block design and the Mixed procedure of SAS (SAS Institute, Cary, NC). Assumptions of homogeneity of variance and normality of residuals were verified graphically. Normality of residuals was also tested using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Log transformation was required in some cases to ensure homogeneity of variance. Tables and figures present least-square means and standard errors of non-transformed data. Tukey's HSD test was used to compare means between treatments ($\alpha = 0.05$). We used the program "pdmix800.SAS" to assign Tukey letters for treatments (Saxton, 1998). Finally, linear regression analyses were performed to explore the relationships between C inputs and soil OC stocks

Results and discussion

Whole-soil OC

A positive effect of liquid dairy manure

Overall, OC stocks in the 0-10 cm soil layer were significantly greater for the LDM or ALT treatments than control or Fert treatments (Fig. 3.2.a). However, for the 10-20 cm soil layer and for the 0-50 cm layer, only Man-high OC stock was significantly greater than mineral fertilization and control (Fig. 3.2.a, Table 3.2). Previous studies showed that the effect of liquid manure application on soil OC can be quite variable with positive (Lynch *et al.*, 2005, Mellek *et al.*, 2010), neutral (Angers *et al.*, 2010) or even negative impacts (Jokela *et al.*, 2009, Angers *et al.*, 2010, Chirinda *et al.*, 2010). The higher soil OC with LDM application may be due to the direct input of C by manure (Aoyama *et al.*, 1999a, Whalen and Chang, 2002, Bhattacharyya *et al.*, 2010), as dairy manure generally contains fibers from undigested forages and from bedding, which in this case contained wood chips (Fig. 3.3). The LDM may have also indirectly increased soil OC stocks by increasing the aboveground and belowground C inputs (litter fall, harvest losses, root exudates) to the soil through the increase in plant growth (Table 3.1) as was previously reported (Aoyama *et al.*, 1999a). A closer linear regression was observed between whole-soil OC stock in the 0-50 cm soil profile and total (plant biomass + LDM) C inputs than between OC stocks and plant biomass C inputs (Table 3.3). This supports the idea that manure-C contributed directly to soil OC stocks in plots receiving LDM application in addition to plant biomass-C inputs. It should be kept in mind that plant C inputs were calculated by using allocation coefficients (Bolinder *et al.*, 2007) assuming that belowground C inputs were proportional to aboveground C inputs.

A minor effect of mineral fertilization

Mineral fertilization did not significantly affect soil-whole OC when considering the 0-50 cm soil profile (Table 3.2), but it was 16% higher under the high mineral fertilization rate than under the unamended control in the 0-5 cm soil layer (Fig. 3.4.a). When considering the 0-10 cm layer, the difference in favour of mineral fertilization varied from 3 to 8%, but was not statistically significant. This percentage of change was similar to those estimated in recent global meta-analysis reporting an impact of nitrogen (N) fertilization on soil OC. Indeed, Lu *et al.* (2011) reported an average 3.5% increase in C storage after N addition in agricultural ecosystems while Ladha *et al.* (2011) estimated an average soil OC increase of 8% following the application of synthetic fertilizer N. Mineral fertilization probably increase the soil OC stock in comparison to an unamended control by increasing plant yield (Lu *et al.*, 2011) which was observed in our study (Table 3.1).

A deeper effect of liquid dairy manure application compared to mineral fertilization

Overall, the positive effect of LDM on soil OC was observed deeper in the soil profile than that of the mineral fertilization, down to 20-cm depth for LDM instead of 5 cm for mineral fertilization (Fig. 3.2.a, Fig. 3.4.a). The presence of soil OC deeper in the profile in the LDM than Fert treatments, can be explained by: 1) the physical transfer of C from above- and belowground plant biomass and LDM to underlying layers following the single tillage operation at 25 cm performed in 2003 and by bioturbation, and 2) the possible migration of soluble C from LDM itself or from the product of its decomposition through leaching in this grassland and its adsorption onto soil mineral particles in the underlying soil layer. Royer *et al.* (2007) hypothesized that higher DOC concentration in tile-drain (90-cm depth) water under perennial forage than under corn was caused by preferential flow through a network of vertical macropores, originating from soil cracks, fissures, root channels, and earthworms burrows. However, we did not observe significant effects of LDM on OC stocks in the subsoil as we originally hypothesized. The absence of an effect of mineral fertilization below the 5 cm soil layer and of LDM below the 20 cm soil layer could be explained as follows: 1) C was not physically transferred to the deeper layers; 2) Increased above-ground yields following mineral N fertilization and LDM may not result in proportional increase in root growth at depth, as N addition may increase the plant shoot: root ratio (Lu *et al.*, 2011); 3) Increased belowground C inputs (after mineral fertilization and LDM application) or direct C input from LDM may have been just sufficient to offset a possible priming effect on the decomposition of plant residues and native soil organic matter (Plaza *et al.*, 2004, Fangueiro *et al.*, 2007, Ladha *et al.*, 2011).

The soil OC stocks under Man-low and Alt treatments were higher than in the control and lower than under Man-high only in the 0-10 cm soil layer, whereas the effect of Man-high was apparent down to 20-cm (Fig. 3.2.a). Thus, the LDM application rate seemed to affect the intensity and the depth of soil OC response. As suggested earlier, C derived from Man-low and Alt treatments did not migrate below the 10-cm soil layer, or the C input with these lower manure application rates was not sufficient to offset a possible priming of soil C mineralization. Absence of change in soil OC stock or even depressed OC stocks following application of liquid manure have been observed previously (Angers *et al.*, 2010, Chirinda *et al.*, 2010).

Overall, considering all mineral and LDM treatments, our results suggest that the greater the total C inputs, the greater the soil OC stock, and the deeper the treatment effect (Table 3.1, Table 3.2, Fig. 3.2). However, one exception was with the Alt treatment. Even though Alt and Man-low treatments involved similar quantity of manure-C input, crop yield was significantly greater with the Alt treatment (Table 3.1). Despite this higher crop yield, the OC stock was not greater than in Man-low, and was even slightly lower (not significant). Moreover, the OC stock under Alt treatment was closer to that under Fert-high despite a greater C input derived from higher yield and LDM itself. Taken together, these results suggest that mineral fertilization primed the decomposition of soil OC or other C pools. As pointed out earlier, N fertilization can promote a priming effect on decomposition of plant residues and native soil organic matter (Ladha *et al.*, 2011). However, it was not possible in our field study to identify which of the OC pools (native soil OC, plant C, or LDM C) was primed by mineral N fertilization.

OC in soil physical fractions

Visual description of fractions

The visual appearance of the light fractions in the surface soil layer (Fig. 3.5.a.b) suggests that this fraction is mainly composed of semi-decomposed plant and animal residues as previously reported (Gong *et al.*, 2009, Assis *et al.*, 2012). Both light fractions (FLF and IALF) also contained spores and small pieces of charcoal. The length of plant residues varied from 1 to 2 mm. The IALF, freed by sonication, was most probably occluded within aggregates which are secondary organo-mineral complexes according to Christensen (2001). The IALF was marked by an apparently greater degree of decomposition than the FLF and by the presence of charcoal of varied sizes (Fig. 3.5.b). For the manured plots, we also observed coarse woody debris in both light fractions, which likely derived from the wood chips used in beddings (Fig. 3.3).

Overall, we found only a few studies that have separated the heavy fraction into different particle size fractions (e.g. Gregorich *et al.*, 2009 , Huang *et al.*, 2010a,Tong *et al.*, 2014). Using the microscope, we observed that the sand-size heavy fraction (SHF) in the surface soil layers was composed of fine particulate organic matter, small pieces of charcoal, and individual sand particles (Fig. 3.5.c). The length of organic residues (from 50 to 500 µm) was smaller than in the light fractions, and their nature and origin were difficult to establish. This fine particulate organic matter seemed to correspond to plant tissues in an advanced stage of decomposition, and was clearly coated with very small mineral particles (Fig. 3.5.d). In the subsoil layers, there were fewer organic particles compared to sand particles, and we observed some small mineral concretions (from about 300 to 700 µm) that resisted sonication and likely contained organic matter.

The presence of particulate organic matter of sand-size in the heavy fraction was not expected. The high degree of coating of this fine particulate organic matter with mineral particles probably explains its presence in the heavy fraction. For a given thickness and chemical composition per unit of mineral coatings, the surface area (and thus the size) of the organic particles would determine the net density of the mineral-organic association (Wagai *et al.*, 2009). The smaller size of the particulate organic matter could have resulted from processes associated with decay or might be an artefact of the sonication treatment (Wagai *et al.*, 2009). However, the C:N ratios of FLF, IALF, SHF and SCHF were, respectively, 41, 34, 16 and 11, which would confirm a higher degree of decomposition and of microbial colonization for the SHF and SCHF than both light fractions, as the C:N ratio of the soil microbial biomass is around 6 to 8 (Stevenson and Cole, 1999). This is in agreement with our current understanding of organic residue decomposition where organic C is oxidized by heterotrophic microbes, whereas N is relatively conserved in the microbial biomass owing to microbial N demand (Wagai *et al.*, 2009).

Occlusion of OC in aggregates following liquid dairy manure application

The OC stock in the IALF was significantly higher under the Man-high treatment than under the mineral fertilization and unamended treatments in the 0-10 cm soil layer and in the whole 0-50 cm soil profile (Fig. 3.6.b, Fig. 3.7.b, Table 3.2). However, there was no significant effect of either LDM treatment on the FLF of any of the soil layers or the whole soil profile (Fig. 3.6.a, Fig. 3.7.a, Table 3.2). These results suggest that LDM more than the control or Fert treatments favours the occlusion of uncomplexed organic matter into soil aggregates (secondary organo-mineral complexes). Aoyama *et al.* (1999b) also observed an increase in the amount of protected C (g kg^{-1} soil) in aggregates (250-1000 μm) after solid manure but not after mineral fertilizer application. Extracellular polysaccharides or mucus derived from microbe or decomposer activity could promote encrustation of partially decomposed plant debris with mineral particles (Golchin *et al.*, 1994). As a source of energy and nutrients for soil microbes, manure may stimulate the production of extracellular polysaccharides, thereby indirectly promoting aggregate formation (Sleutel *et al.*, 2006). In addition, manure may also be a direct source of polysaccharides and aliphatic and aromatic compounds that can bind to soil particles (Sleutel *et al.*, 2006).

A positive effect of LDM and mineral fertilization in heavy fractions

Mineral fertilization and LDM increased OC stocks in both heavy fractions (Fig. 3.2.b, Fig. 3.4.b, Fig. 3.6.c, Fig. 3.7.c). Similarly to whole-soil OC, the effects were greater and apparently deeper in the profile with LDM than with mineral fertilizer. The observation of a closer linear regression between OC stocks in both SHF and SCHF in the 0-50 cm soil profile and total C inputs (plant C + LDM C) than between OC stocks and plant C inputs (Table 3.3), supports the idea that manure-C contributed both directly and indirectly to OC stocks of these heavy fractions.

Overall, our observations clearly show that C derived from LDM application is essentially recovered in the heavy fraction, which suggests that it is in a relatively stable form. Stabilization mechanisms of OC in soils associated with LDM application have to consider its composition and physical nature. Both solid and liquid phases of LDM contain C. The soluble C present in the LDM itself and derived from its decomposition may adsorb directly on clay- and silt-size particles. Soluble C is well known to directly bind to mineral particles (Neff and Asner, 2001). In addition, as described for plant residues (Golchin *et al.*, 1994) and for solid manure (Aoyama *et al.*, 1999a), the solid phase of LDM (mainly undigested plant fibers and beddings) would be progressively fragmented and coated with minerals, resulting in organic matter particles such as those observed in the SHF (Fig. 3.5.c.d). This coating of organic residues with minerals would be triggered by the input of labile compounds from the LDM. Indeed, Cotrufo *et al.* (2013) underlined the importance of labile organic compounds in the formation of stable soil organic matter. Since labile organic compounds are utilized more efficiently by microbes, they are perceived as a main source of microbial products, which promote bonding to mineral particles and aggregate formation. Further, as suggested by Aoyama *et al.* (1999a), during the gradual decomposition of the solid phase, the degradation products would become progressively associated to the clay- and silt-size particles and, therefore, recovered in the SCHF. These different mechanisms are summarized in Figure 3.8.

A sand-size heavy fraction responsive to treatments

The sand-size heavy fraction seemed to be very responsive to treatments in our study. Changes in OC stocks were proportionately greater in the SHF compared to the whole soil or any other fraction. For instance, increased OC stocks in the SHF was 38 to 170% higher for LDM (Fig. 3.6.c) than in the control and mineral fertilization but only 13 to 39% for the whole-soil OC (Fig.3.2.a). In addition, the effect of treatments on OC stocks in the SHF was observed deeper in the soil profile. The great sensitivity of this heavy fraction in our study contrasts with frequent reports that the light fraction is most sensitive to changes in cropping systems such as reducing tillage or including perennial crops in arable rotations (Janzen *et al.*, 1992, Janzen *et al.*, 1998, Bolinder *et al.*, 1999). As described earlier, this is likely due to the physical nature and composition of LDM, which triggers its incorporation in the heavy fractions directly through its soluble fraction and by providing solid C substrate serving as nucleus for stable aggregate formation and C stabilization (Fig. 3.8).

Conclusion

Fifteen years of LDM application favoured soil OC accumulation in topsoil (down to 20- 30-cm depth) but not in the deeper layers, probably due to limited C transfer or to an insufficient C input to offset a possible priming effect on soil OC mineralization. Mineral fertilization also led to higher soil OC stocks but its effect was limited to the top 5 cm. The relatively deeper effect of LDM vs. mineral fertilization was probably linked to the direct C inputs from LDM. LDM application favoured the incorporation of C in primary and secondary organo-mineral complexes which suggests that LDM-derived soil OC would be relatively stable. We hypothesize that this is due to the nature and composition of the LDM which is a source of both labile and undecomposed organic residues that stimulate aggregation processes and consequent OC stabilization. From a methodological point of view, the presence of sand-size organo-mineral complexes in the heavy fraction that were very responsive to treatment effects emphasizes the relevance of separating this fraction by size in order to accurately detect management-induced changes in soil OC.

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Table 3-1: Dry matter yields for each treatment with corresponding manure C inputs, biomass C inputs (using allocation coefficients proposed by Bolinder et al. (2007) and 15% of above-ground parts which returned to the soil as litter fall and harvest losses), and total C inputs. (There was no manure treatment and no yield recorded in 2003)

treatment	DM yield ($Mg\ ha^{-1}\ yr^{-1}$)	Manure C input ($Mg\ ha^{-1}\ yr^{-1}$)	Biomass C input to soil ($Mg\ ha^{-1}\ yr^{-1}$)	Total C input to soil ($Mg\ ha^{-1}\ yr^{-1}$)
Control	5.5		1.6	1.6
Fert-low	9.9		2.9	2.9
Fert-high	11.0		3.2	3.2
Alt	13.8	3.8	4.0	7.8
Man-low	11.5	4.1	3.4	7.5
Man-high	13.7	7.9	4.0	11.9

Table 3-2: Whole-soil OC stocks and OC stocks in the FLF (free light fraction), IALF (intra-aggregate light fraction), SHF (sand-size heavy fraction) and the SCHF (silt&clay-size heavy fraction) in the whole 0-50 cm soil profile

Treatment	Whole-soil		FLF	IALF		SHF		SCHF	
	(kg C m^{-2})								
Control	13.24	B	0.43	0.58	B	1.00	D	10.77	B
Fert-low	13.34	B	0.40	0.59	B	1.16	CD	10.73	B
Fert-high	13.68	B	0.46	0.54	B	1.32	BC	11.03	AB
Alt	13.70	B	0.45	0.60	B	1.37	BC	10.76	B
Man-low	14.64	AB	0.44	0.66	AB	1.52	B	11.42	AB
Man-high	16.09	A	0.54	0.76	A	2.05	A	12.30	A
P	0.001^{***}		0.662^{NS}	0.005^{**}		0.0001^{***}		0.027^{*}	
Standard error	0.39		0.07	0.04		0.06		0.35	

^{NS}non-significant; *p<0.05; **p<0.01; ***p<0.001

A different letter means a significant difference at P=0.05 (Tukey's test).

Table 3-3: Regression coefficients (R^2) between soil OC stocks in the 0-50 cm soil profile and plant biomass C inputs and total C inputs (plant biomass + LDM)

	Plant biomass	Total (plant biomass + LDM)
C-FLF	0.30^{NS}	0.65^{NS}
C-IALF	0.24^{NS}	0.76^{*}
C-S	0.57^{NS}	0.88^{**}
C-SC	0.25^{NS}	0.66^{*}
Whole-soil OC	0.37^{NS}	0.80^{*}

^{NS}non-significant; $^{*}p<0,05$; $^{**}p<0,01$;
$^{***}p<0,001$

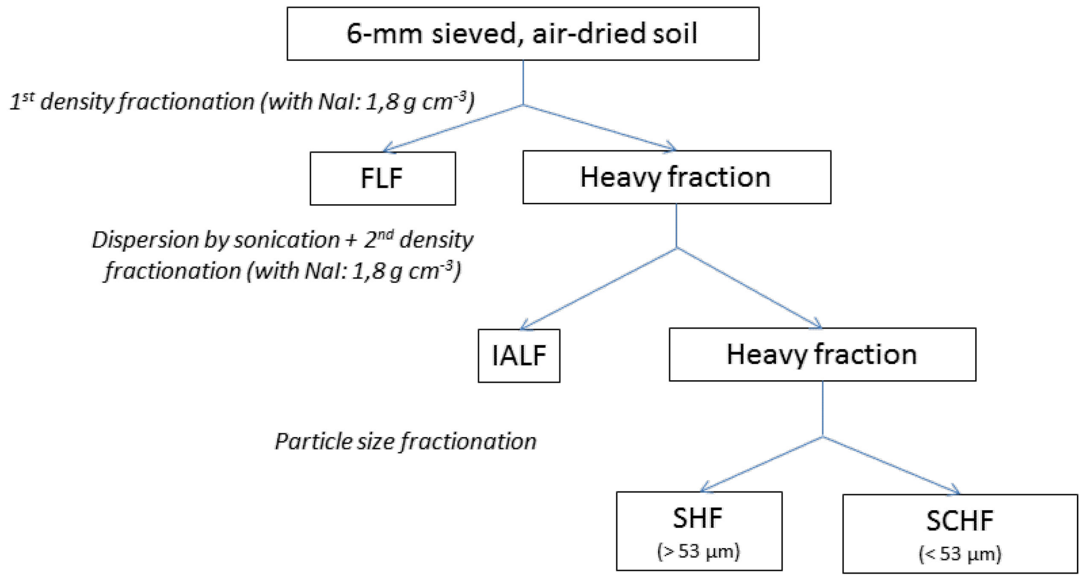

Figure 3-1: Scheme of the soil organic matter fractionation adapted from a method developed by Sohi et al. (2001). FLF = free light fraction; IALF = intra-aggregate light fraction; SHF = sand-size heavy fraction; SCHF = silt + clay-size heavy fraction

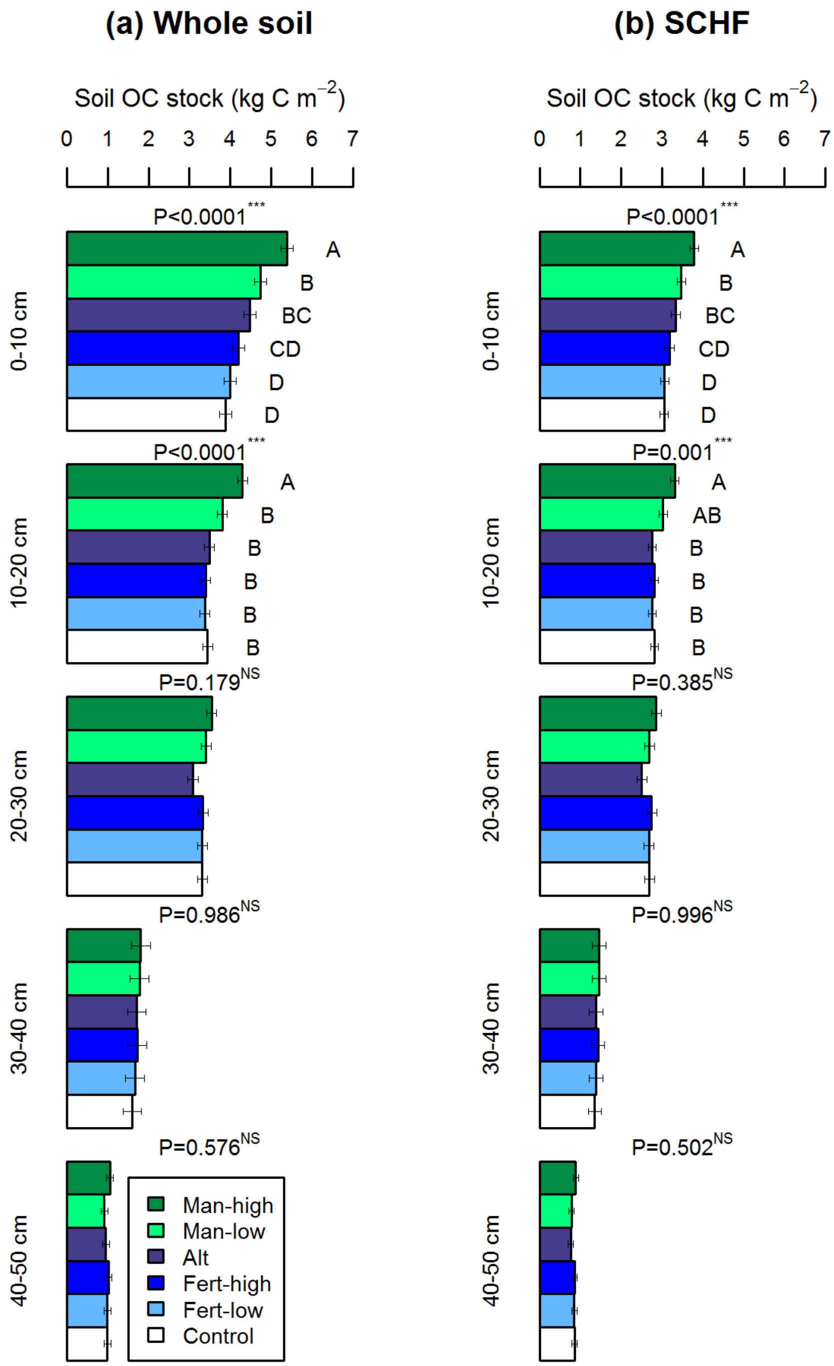

Figure 3-2 : Effect of treatments on whole-soil OC stocks, and on OC stocks in the silt & clay heavy fraction (SCHF) in the 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm soil layers. The soil equivalent mass for each soil layer is 100 kg m^{-2}. Least square means and standard errors are presented. Different letters within a given fraction and depth indicates significant difference at P=0.05 (Tukey's test). (^{NS}non-significant; *p<0,05; **p<0,01; ***p<0,001)

Figure 3-3 : Photographs of dried liquid dairy manure

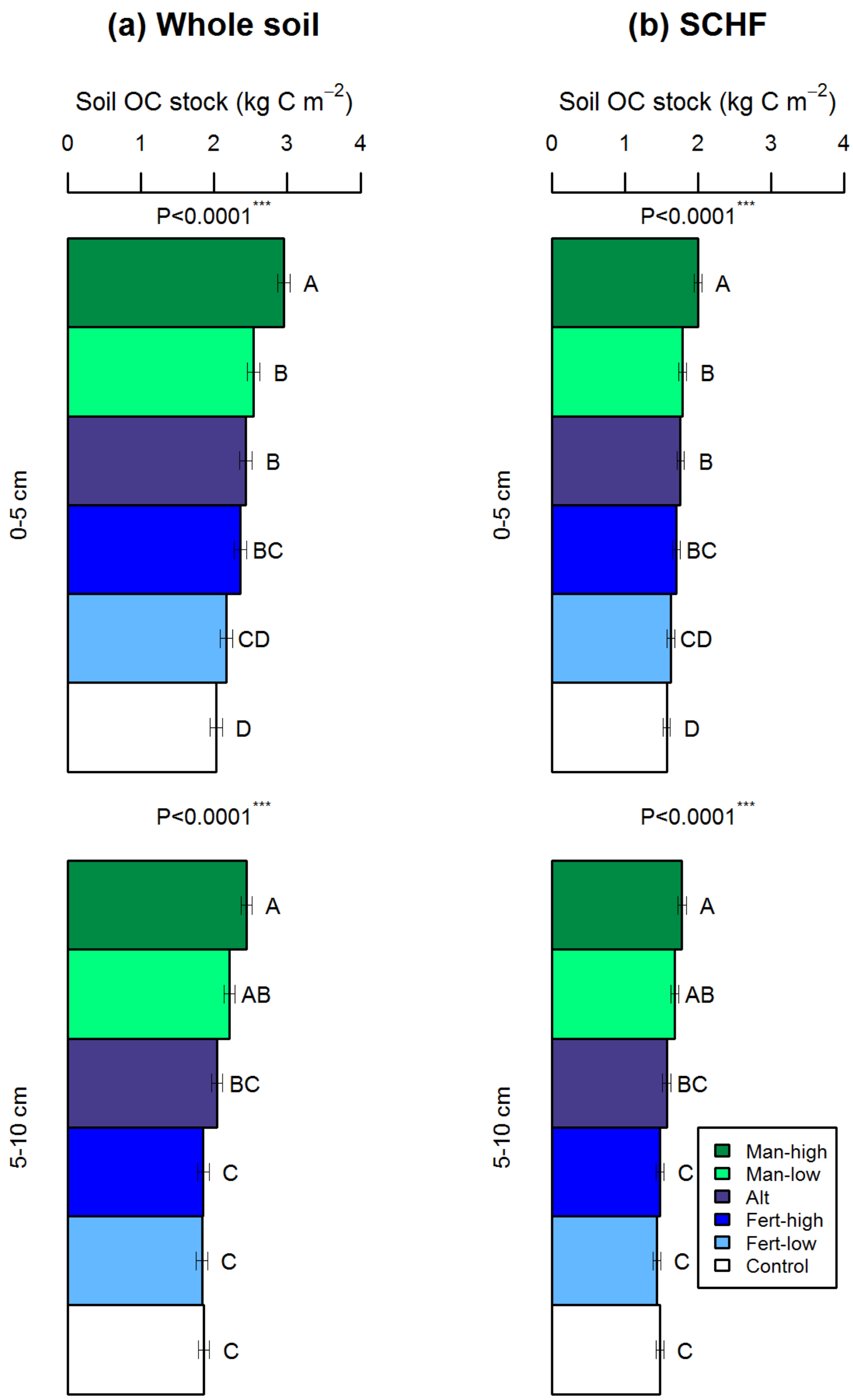

Figure 3-4 : Effect of treatments on whole-soil OC stocks, and on OC stocks in the silt & clay heavy fraction (SCHF) in the 0-5 cm and 5-10 cm soil layers. The soil equivalent mass for each soil layer is 50 kg m^{-2}. Least square means and standard errors are presented. Different letters within a given fraction and depth indicates significant difference at P=0.05 (Tukey's test). (^{NS}non-significant; *p<0,05; **p<0,01; ***p<0,001)

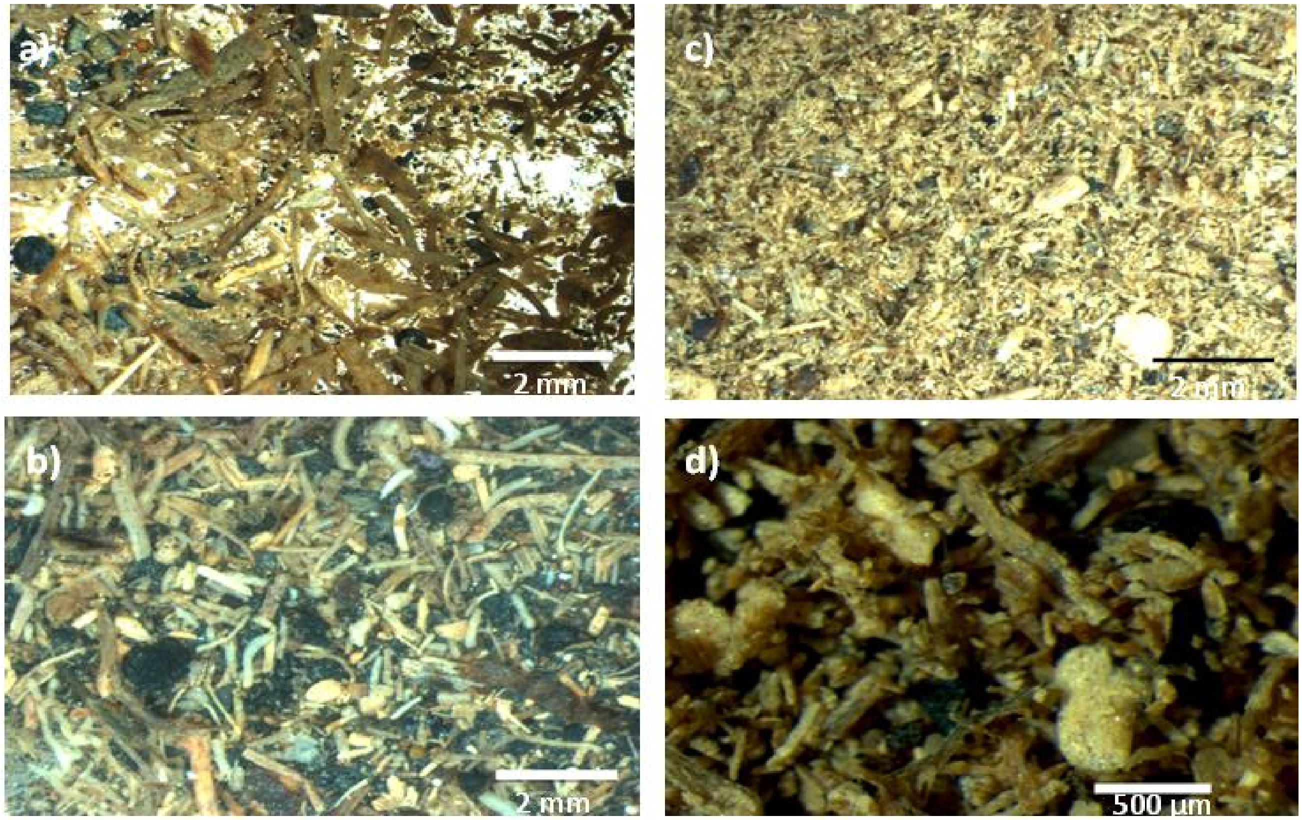

Figure 3-5 : Microscope photographs of the free light fraction (a), intra-aggregate light fraction (b), and sand-size heavy fraction (c,d).

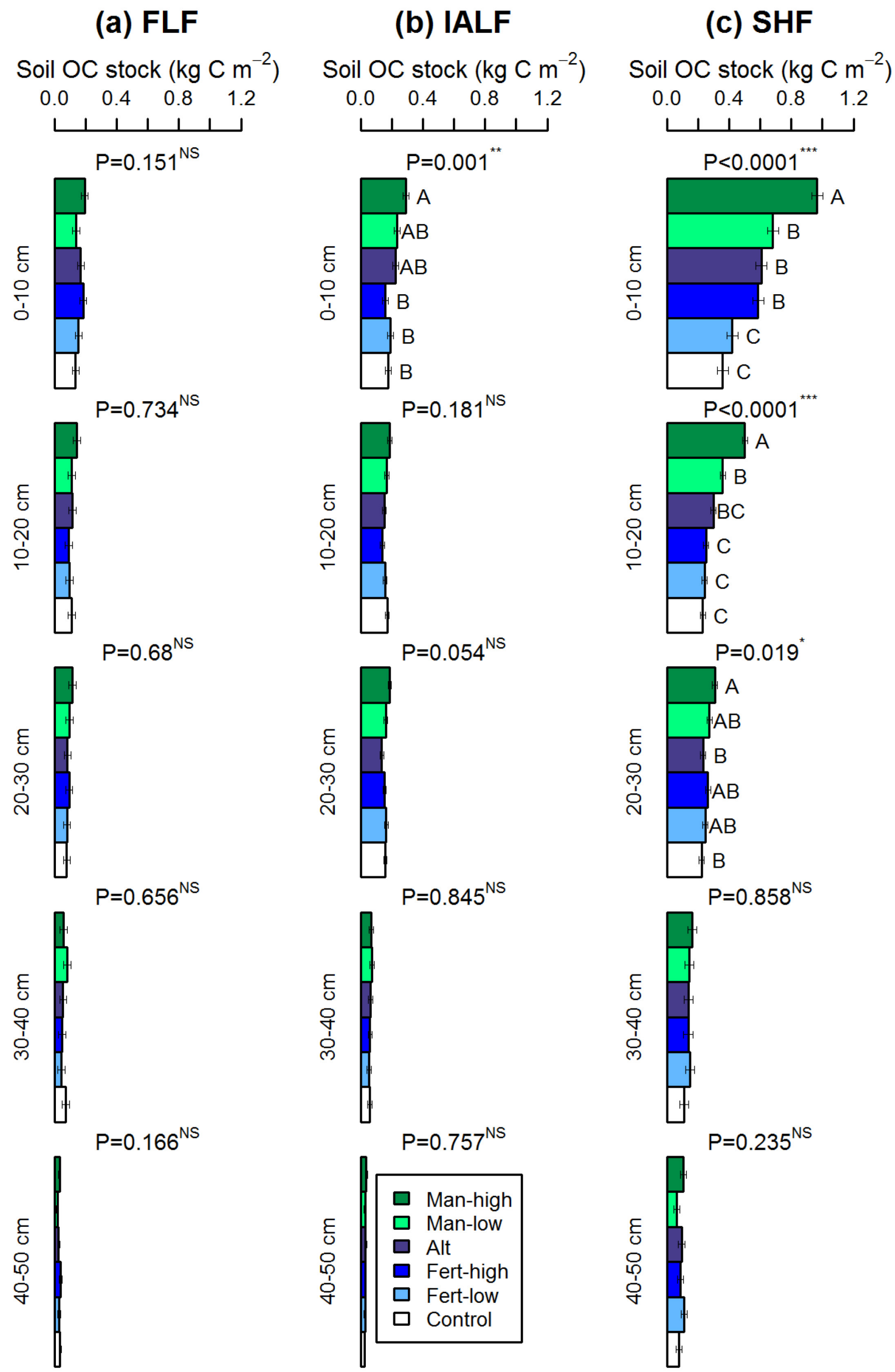

Figure 3-6 : Effect of treatments on OC stocks in the free light fraction (FLF), intra-aggregate fraction (IALF) and sand-size heavy fraction (SHF) in the 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm soil layers. The soil equivalent mass for each soil layer is 100 kg m^{-2}. Least square means and standard errors are presented. Different letters within a given fraction and depth indicates significant difference at P=0.05 (Tukey's test). (^{NS}non-significant; *p<0,05; **p<0,01; ***p<0,001)

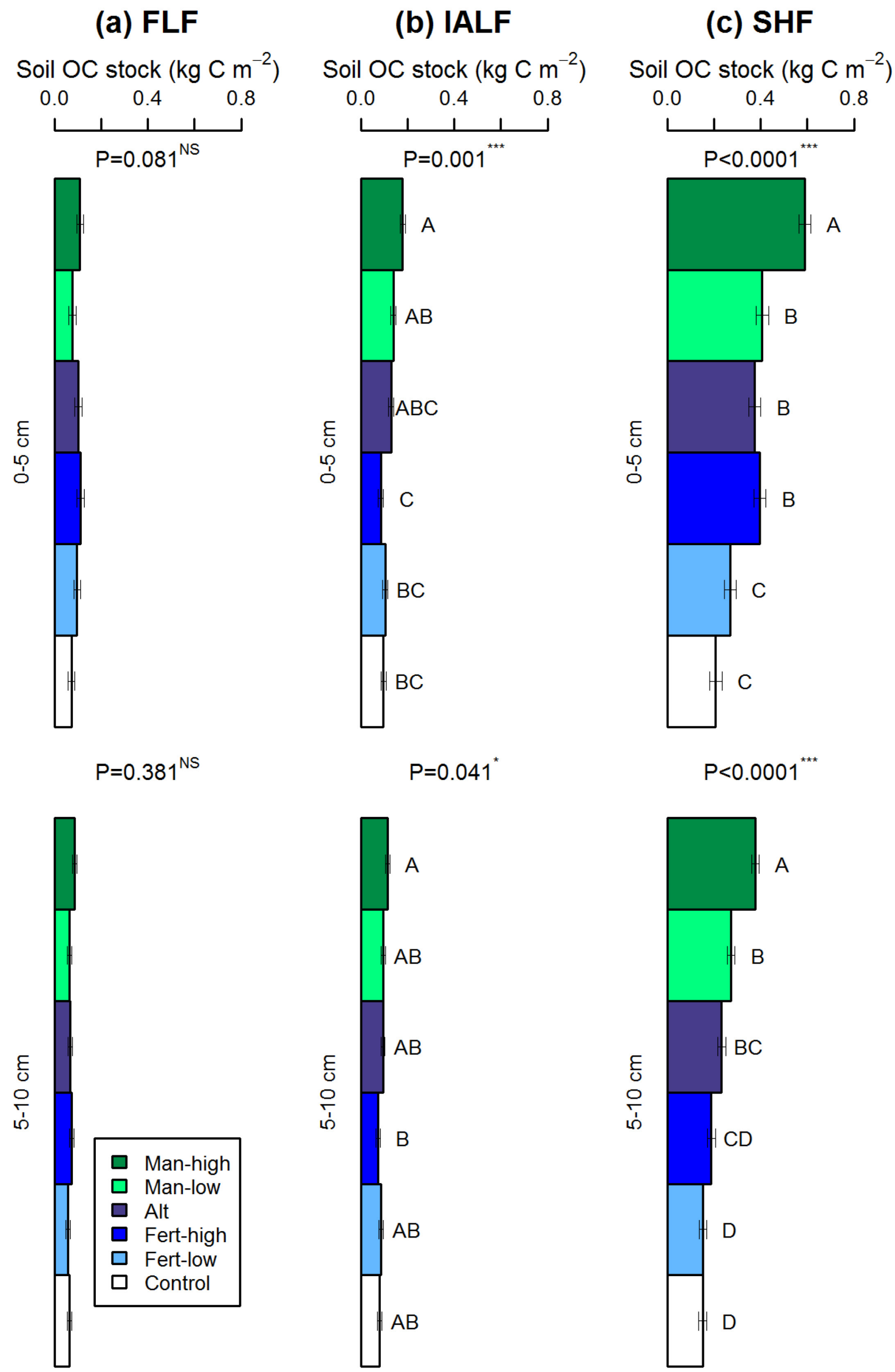

Figure 3-7 : Effect of treatments on OC stocks in the free light fraction (FLF), intra-aggregate fraction (IALF) and sand-size heavy fraction (SHF) in the 0-5 cm and 5-10 cm soil layers. The soil equivalent mass for each soil layer is 50 kg m^{-2}. Least square means and standard errors are presented. Different letters within a given fraction and depth indicates significant difference at P=0.05 (Tukey's test). (^{NS}not significant; *p<0,05; **p<0,01; ***p<0,001)

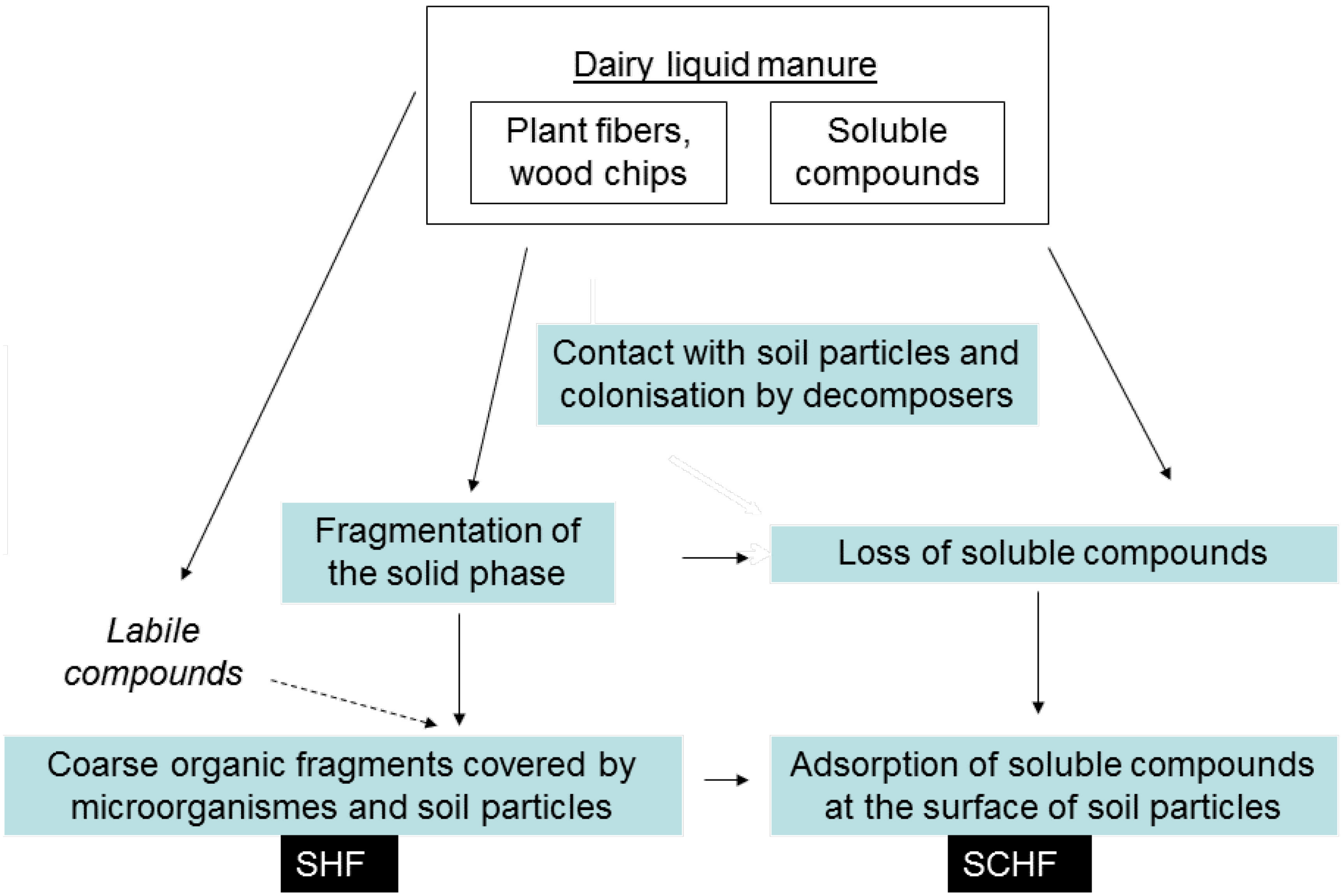

Figure 3-8 : Schematic diagram of the fate of liquid dairy manure (LDM) in the sand-size heavy fraction (SHF) and the silt & clay heavy fraction (SCHF).

Conclusion générale

Principaux résultats

Les résultats de la méta-analyse (chapitre 1) montrent qu'en moyenne à l'échelle globale, l'application d'effluents d'élevage mène à des stocks de C du sol significativement plus élevés comparativement à la fertilisation minérale ou à un témoin non amendé. L'amplitude de la réponse du stock du C du sol à l'application d'effluents dépend principalement de la quantité de C apportée par l'effluent. Le climat a également influencé la réponse du stock de C mais son effet pourrait être confondu avec celui de la quantité totale de C apportée par l'effluent. À une échelle plus locale, 15 et 21 années d'application de lisier de bovins ont mené à des stocks de C significativement plus élevés comparativement à un témoin non amendé (chapitre 3) ou à la fertilisation minérale (chapitres 2 et 3). Pour les deux sites, l'effet du lisier de bovins était limité à la surface du sol (20 et 30 premiers centimètres). Sur le site d'Agassiz (CB), l'amplitude de la réponse du C du sol à l'application du lisier dépendait de la quantité de C apportée et cette réponse semblait être atténuée lorsque l'application de lisier était combinée avec celle de la fertilisation minérale. Sur le site de Normandin (QC), la réponse du C du sol à l'application de lisier était différente selon la séquence de cultures avec une réponse plus forte pour la rotation à base de plantes pérennes que pour la monoculture de céréales.

Sur le site de Normandin, il a été estimé que 36% du C dérivé de l'application de lisier de bovins (incluant le C du lisier lui-même et le C des apports de plantes) était retenu dans le sol de la rotation à base de plantes pérennes comparativement à la fertilisation minérale. Ce coefficient était négatif (-7%) dans la monoculture. Sur le site d'Agassiz, 18% et 21% du C dérivé de l'application de lisier de bovins était retenu dans le sol par rapport à la fertilisation minérale respectivement pour la dose faible et la dose forte. Si on compare la rotation à base de plantes pérennes du site de Normandin avec la prairie du site d'Agassiz, on constate que pour une même quantité de C apportée par le lisier de bovins (apports directs + indirects), la proportion de C qui est retenue par rapport à la fertilisation minérale est plus importante pour le site de Normandin. Ce résultat peut être lié à la différence de climat entre les deux sites (tempéré chaud pour Agassiz et continental froid pour Normandin). En effet, les sols des climats chauds, où la décomposition est plus rapide, pourraient accumuler le C plus lentement que les sols des climats froids (Freibauer *et al.*, 2004). Ces deux sites diffèrent également par la texture de leur sol (limon à loam limoneux pour Agassiz et argile limoneuse pour Normandin) (Figure i). Le sol du site de Normandin, plus riche en argiles, pourrait favoriser la rétention du C après l'application d'effluents. Des études ont en effet noté une augmentation de la rétention du C dans des sols à texture fine (Ladd *et al.* 1995, Liang *et al.* 1998). Clément *et al.* (2010) rapportent un coefficient isohumique de 15% pour un lisier de bovins. En considérant que le C représente 40% de la matière organique du lisier et 58% de la MO du sol, cela équivaut un coefficient de rétention pour C du lisier de bovins de 22%. Nos valeurs pour les sites d'Agassiz et de Normandin sont donc comparables à ce coefficient. À fin de comparaison, les observations des sites d'Agassiz et de Normandin ont été ajoutées sur la relation globale entre le changement de stock de C induit par l'application d'effluent (vs. fertilisation minérale) et la quantité de

C apportée par l'effluent observée dans la méta-analyse du chapitre 1 (Figure ii). Il faut noter qu'une partie du C du sol accumulé par rapport à la fertilisation minérale est attribuable à un effet indirect de l'application du lisier à travers l'augmentation du rendement en plus de l'effet direct du lisier. Cependant, l'apport indirect de C par les résidus représente une faible proportion de l'apport direct de C par le lisier (environ 17% en moyenne pour les deux sites). Sur le graphique, on observe que pour une quantité équivalente de C accumulée dans le sol par rapport à la fertilisation minérale, la quantité de C apportée avec le lisier est beaucoup plus forte à Agassiz (dose faible du lisier) comparativement à la rotation à base de pérennes du site de Normandin (Figure ii). Ceci est en accord avec ce qui a été discuté précédemment quant à l'effet du climat et de la texture du sol. Il faut noter que dans cette comparaison, ces effets semblent ici primer sur l'effet de la fréquence du travail du sol, puisque malgré un seul labour pour le site d'Agassiz, l'apport d'une quantité plus importante de lisier mène à une réponse du C du sol similaire à la rotation à base de pérennes du site de Normandin occasionnant un travail du sol tous les trois ans. Malgré une certaine variabilité pouvant être due à plusieurs facteurs (climat, texture du sol, fréquence du travail du sol, etc.), les quatre points de ces deux sites s'intègrent quand même assez bien aux données de la méta-analyse, soulignant ainsi que la relation linéaire observée au niveau global est relativement robuste.

La stabilité relative du C accumulé après l'application de lisier de bovins a pu être appréciée spécifiquement sur le site d'Agassiz. L'application de lisier de bovins a favorisé l'incorporation du C dans des fractions de matière organique relativement protégées. Cela peut être mis en relation avec le fait que le lisier utilisé ici était une source de composés labiles et de résidus organiques non décomposés qui pourraient favoriser grandement les processus d'agrégation biologiques et par conséquent une stabilisation relative du C.

Principales contributions

Les résultats observés dans le chapitre 1 offrent des perspectives de raffinement des méthodologies proposées pour les inventaires nationaux de gaz à effet de serre, soulignant la nécessité de prendre en compte la quantité de C apportée par l'effluent. Ces résultats soulignent également le manque d'études sur les effets des caractéristiques de l'effluent comme l'espèce animale (porc et volaille particulièrement) et l'entreposage de l'effluent (solide vs. liquide) sur la réponse des stocks de C du sol à l'application d'effluents d'élevage. Dans cette optique, les résultats observés sur les sites de Normandin et d'Agassiz des chapitres 2 et 3 sont donc une nouvelle contribution aux connaissances sur la réponse des stocks de C du sol après l'application d'effluents liquides. De plus, ces résultats apportent des réponses intéressantes aux producteurs laitiers qui s'interrogent sur l'empreinte environnementale de leurs pratiques. En effet, l'application de lisier de bovins peut favoriser l'accumulation de C dans les couches de surface du sol (20-30 premiers centimètres) comparativement à la fertilisation minérale. De plus, comme le suggèrent les résultats du site d'Agassiz, le C pourrait être accumulé dans des fractions relativement stables et pas seulement dans des fractions labiles.

Enfin, une des originalités des résultats du site d'Agassiz était la mise en évidence de matières organiques grossières (de la taille des sables) enduites de particules minérales très sensibles aux traitements. Il semble donc être relativement pertinent de séparer cette fraction pour détecter plus précisément les changements de stocks de C du sol induits par des modifications de pratiques agronomiques. De plus, ces résultats soulèvent un questionnement sur la nature de la matière organique particulaire (POM). Cette fraction est souvent qualifiée de « labile » alors qu'elle pourrait inclure de la matière organique grossière enduite de minéraux et donc relativement protégée.

Limites et perspectives

Au niveau de l'estimation globale de la réponse des stocks de C à l'application d'effluents d'élevage, plusieurs limites ont été soulevées au niveau de la disponibilité des données, en plus du manque d'études concernant les effluents porcins, de volaille et les effluents liquides soulevés dans le paragraphe précédent. Les deux limites les plus importantes étaient probablement 1/ le faible nombre d'études exprimant le C du sol en terme de stocks ou fournissant la densité apparente en plus de la concentration en C du sol et 2/ le manque d'informations nécessaires à l'estimation du C apporté par l'effluent animal. Ces informations sont indispensables pour examiner la relation entre l'apport de C par l'effluent et la réponse du stock de C du sol.
Il demeure aussi une grande variabilité dans les données au niveau global en particulier pour l'estimation du coefficient de rétention de l'effluent. Les résultats du site de Normandin vont également dans ce sens avec l'observation d'une réponse plus importante du stock du C du sol à l'application de lisier de bovins dans un système combinant l'utilisation de cultures pérennes et la faible fréquence du travail du sol. De plus, les résultats des études individuelles réalisées sur les sites d'Agassiz et de Normandin sont spécifiques aux conditions des sites et aux types de lisiers. Par exemple, sur le site d'Agassiz, l'accumulation de C dans des fractions relativement stables après l'application de lisier de bovins a été mise en relation avec la nature même de ce lisier qui était une source à la fois de résidus non décomposés (fibres non digérées ou résidus de litière) et des composés solubles. L'application de lisier porcin pourrait ne pas favoriser un tel stockage de C.

Devant l'ensemble de ces limites, il paraît donc pertinent d'initier ou de continuer les études au champ avec application d'effluents d'élevage à long terme sur les thèmes suivants : effet de l'interaction de l'application d'effluents avec le travail du sol, effet de l'application d'effluents sur des prairies, comparaison de la réponse du stock de C du sol après l'application de lisier de bovins et de lisier de porcs, influence de la présence et du type de litière dans le lisier sur la réponse du stock de C du sol, …

Au niveau de la nature et de la stabilité relative du C accumulé après l'application d'effluents d'élevage, il serait intéressant d'étudier davantage la fraction lourde du sol de la taille des sables, qui contenait de la matière organique grossière. Les observations visuelles et le rapport C:N de cette fraction nous laissent supposer que les débris végétaux grossiers sont enduits de particules minérales liées par des composés microbiens. Cette hypothèse pourrait être vérifiée par l'analyse des glucides du sol qui permettent de quantifier de façon relative l'origine végétale ou microbienne des glucides (Chantigny and Angers, 2007, Martins *et al.,* 2012).

Afin de raffiner l'évaluation du coefficient de rétention du C issu des effluents d'élevage, il serait intéressant de suivre le C apporté directement par l'effluent dans le sol et de le distinguer du C apporté indirectement par les résidus végétaux suite à l'augmentation de rendement. Il est possible de distinguer le C du sol de celui provenant de l'effluent en ayant recours au marquage isotopique (Dungait *et al.*, 2010). Le marquage isotopique pourrait également être utilisé pour estimer la présence ou non d'un effet « priming » suite à l'application d'effluents (Angers *et al.*, 2007, Bol *et al.*, 2003). Grâce au marquage, on pourrait différencier si les pertes de C provenant du sol correspondent à une décomposition du C de l'effluent ajouté ou à une décomposition du C natif du sol.

Enfin, de façon plus générale, les coefficients de stockage de C développés dans cette thèse tant à l'échelle globale (chapitre 1) que régionale (chapitres 2 et 3) pourraient être considérés dans le bilan « carbone » des exploitations agricoles, dans les inventaires nationaux de gaz à effet de serre ou dans l'évaluation environnementale de la filière « élevage ».

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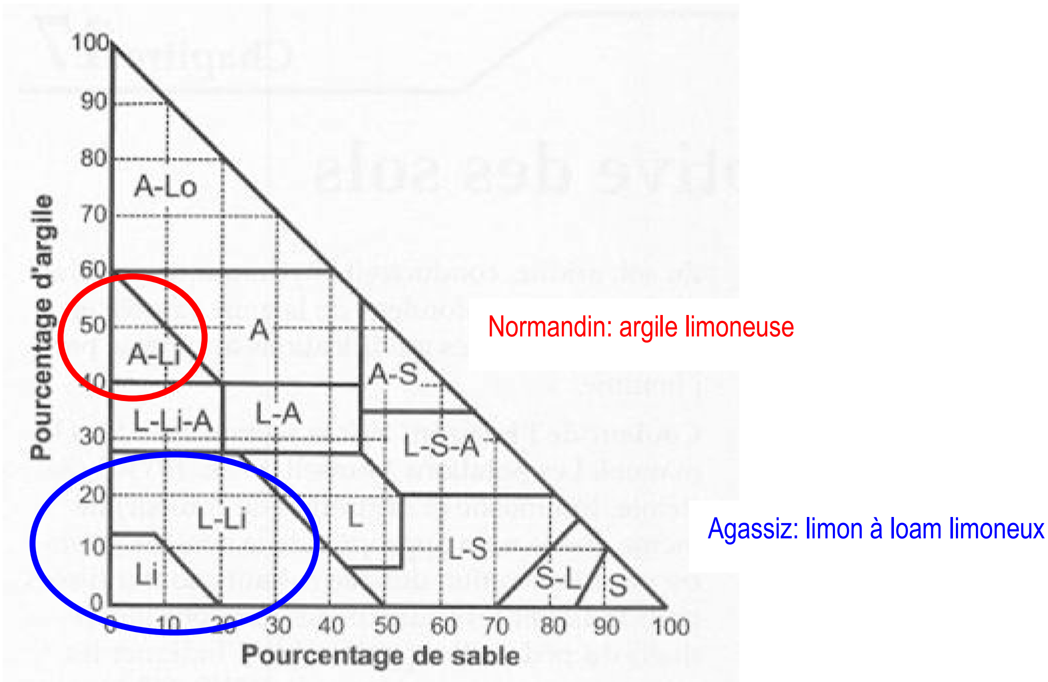

Figure i : Texture du sol sur les sites de Normandin et Agassiz

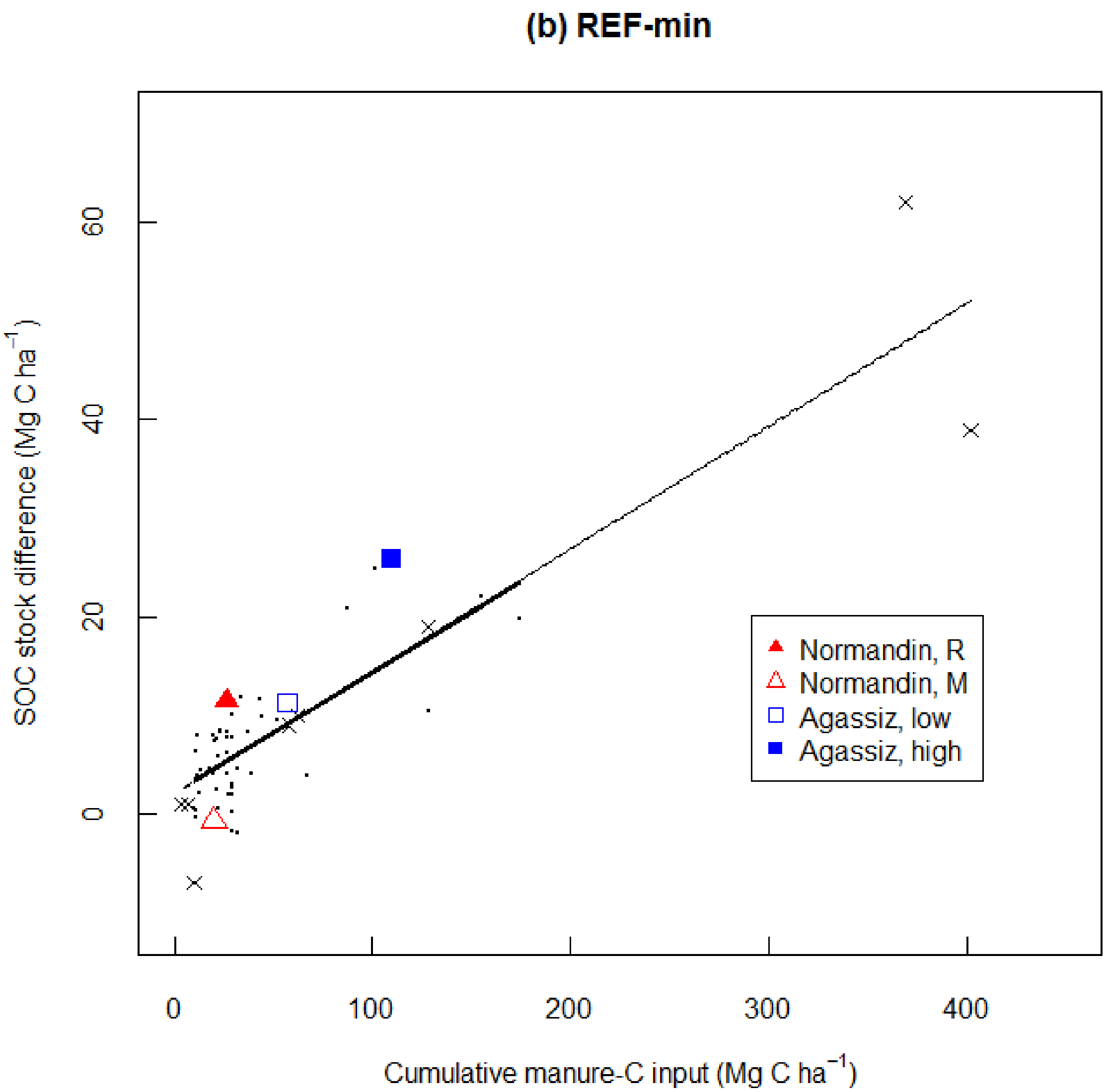

Figure ii : Observations des sites de Normandin et Agassiz ajoutées à la relation entre l'apport direct de C par l'effluent et le changement de stock de C (vs. Fertilisation minérale) observée dans la méta-analyse du chapitre 1. (●) Observations des sites inclus dans le jeu de données REF-min de la méta-analyse; (×) Observations additionnelles de sites dont le dispositif ne comportait pas de répétitions.