Effets des amendements organiques exogènes sur la composition de la matière organique et le stockage du carbone d'un sol dégradé par l'érosion dans le Nord du Vietnam

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THÈSE

PRÉSENTÉE A

L'UNIVERSITÉ PIERRE ET MARIE CURIE

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Par : Thi Phuong NGO

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SPÉCIALITÉ : Science du sol et l'environnement

Effets des amendements organiques exogènes sur la composition de la matière organiques et le stockage du carbone d'un sol dégradé par l'érosion dans le Nord du Vietnam

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Soutenue le 06 Mai 2014

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Chapitre 4: L’impact de différents amendements sur la composition chimique et le stock de carbone de sol tropical dégradé par érosion au Vietnam

Introduction générale

Situé à l’extrême Est de la péninsule indochinoise, le Vietnam qui est un pays agricole avec une densité de population de 267 personnes/km² (une des densités les plus fortes au monde) exerce une pression importante sur ses terres cultivables. La fréquence et la force des pluies caractéristiques du climat subtropical humide, combinés à l’utilisation intense des engrais et des pesticides mettent à mal la durabilité des systèmes de culture. De plus, l’exploitation agricole des terrains sur pentes dans le Nord du Vietnam augmente l’érosion et diminue la fertilité des sols.

La perte de fertilité est étroitement associée avec la diminution des teneurs en matière organique des sols (MOS). Il apparaît de ce fait urgent de trouver une alternative durable à la gestion conventionnelle des terres agricoles de pente. Dans ce cadre, la fertilisation organique des sols apparaît comme une alternative crédible, du point de vue économique, environnemental et sociétal. L’apport des amendements organiques exogènes (AOEs) aux sols peut assurer la réhabilitation de la qualité des sols et maintenir la qualité des eaux (Francou et al., 2005). Dans le nord de Vietnam, les substrats organiques peu couteux et abandonnement disponibles sont : le lisier de buffle et le biochar issus de la pyrolyse du bambou ou de la paille du riz (charbon vert).

La qualité de l’AOE apportée au sol est de la plus haute importance. Un AOE facilement minéralisable permet une rapide biodisponibilité des éléments minéraux, mais ne permet pas une augmentation durable des propriétés physiques des sols (stabilité structurale et résistance à l’érosion, infiltration de l’eau…), alors qu’un AOE « stable » permettra une séquestration sur le moyen ou long terme de la MOS, mais rendra disponible au fil du temps les éléments minéraux pour la plante (Parton et al., 1987).

Au Vietnam, le lisier de buffle issu de l’élevage est en général stocké puis épandue plusieurs fois par an comme engrais organique sur les sols. Cependant, l’absence de traitements préalables participent à une acidification des sols et une perte rapide des nutriments minéraux (Bouman et al., 1995). La stabilisation des lisiers par compostage avant son épandage sur les sols apparaît donc comme une alternative crédible afin d’améliorer la qualité de l'environnement et augmenter la durabilité des pratiques culturales.

Dans une perspective d’ingénierie écologique, les vers de terre épigés peuvent être utilisés pour transformer les lisiers et ainsi former un substrat appelé ‘lombricompost’ (ou vermicompost en anglais). Cette technique est en effet plus pratique par rapport au compostage, car il n’est plus nécessaire de retourner le compost durant le compostage. Grâce aux activités des vers, la matière organique se décompose plus vite et le temps nécessaire pour que le lombricompost soit mature est donc plus court que pour le compost. En outre, le lombricompost est probablement plus homogène et plus stable que le compost traditionnel (Atiyeh etal., 2000).

Récemment, les biochars, issus de la pyrolyse de la biomasse végétale ont été introduits comme un amendement organique permettant d’améliorer la qualité des sols (CEC, pH, teneurs en nutriments) et la croissance des plantes tout en diminuant les apports d’engrais (rétention de nutriments) et en séquestrant le carbone (Lehmann, 2007). L’enfouissement des biochars dans les sols a le potentiel de devenir l’une des techniques majeures d'intensification écologique de l'agriculture du 21ème siècle. Toutefois, le biochar n’est pas un engrais en tant que tel et possède peu d’éléments nutritifs biodisponibles. De ce fait, il est nécessaire de l’associer avec des engrais pour assurer une productivité satisfaisante des cultures (Steiner et al., 2007).

Pour assurer l’amélioration de plusieurs fonctions des MOS, il semble donc judicieux de mélanger plusieurs types d’intrants organiques. Pour mon travail de thèse, j’ai fait l’hypothèse, que l'application de biochars en mélange avec du compost ou du lombricompost pourrait être une solution pour créer un amendement organique à coût modéré améliorant la qualité des sols et la croissance des plantes en réduisant l'utilisation d'engrais minéral, tout en séquestrant massivement du carbone.

L’objectif général de cette thèse est d’évaluer, (1) l’utilité des quatre substrats organiques : lisier, compost, lombricompost et biochar seuls ou en association pour séquestrer du carbone, et (2) l’effet de l’apport des quatre substrats organiques seuls ou en association sur la quantité et qualité des MOS d’un sol agricole tropical dégradé typique du Nord de Vietnam.

Pour répondre à cet objectif, j’ai:

(1) caractérisé la nature chimique des amendements organiques disponibles au Vietnam (lisier, compost, lombricompost et biochar),

(2) évalué leur stabilité chimique et biologique en laboratoire et via une expérience sur le terrain,

(3) étudié leurs effets sur la composition chimique et le stockage du carbone dans un sol tropical dégradé.

L'ensemble des recherches menées durant cette thèse est présenté dans quatre chapitres. Le premier chapitre est une synthèse de la bibliographie existante, qui présente (1) le contexte de ma thèse, (2) les différents AOE, leur intérêt pour améliorer la qualité des sols et (3) les fonctions des MOS dans un contexte tropicale. A la fin du chapitre le lecteur trouvera la présentation du site expérimental et des AOE utilisés dans ma thèse. Les chapitres suivants sont présentés sous forme d'articles scientifiques. Ils sont articulés autour de trois parties. La **première partie** a pour but d'étudier la nature chimique du lisier de buffle, le compost et lombricompost qui sont produits à partir de lisier, et du biochar produit à partir de bambou. Dans une **seconde partie**, nous nous intéresserons à la stabilité des différents amendements organiques. La stabilité chimique a été évaluée par la résistance du matériel envers une attaque chimique. La stabilité biologique a été évaluée au laboratoire par une incubation et dans des conditions de terrain via une expérimentation avec des sachets de litières. La **troisième partie** présente une évaluation de l'impact des différents amendements organiques sur la composition des MO et le stock de carbone dans un sol tropical dégradé typique du Nord au Vietnam. Nous avons analysé ce sol après 3 ans d'expérimentations en mésocosmes cultivés avec du maïs dans des conditions de pluie naturelle.

Chapitre 1 : Contexte et synthèse bibliographique

1. Présentation du Vietnam et du contexte de la thèse

1.1. Situation démographique et économique

Le 1er Novembre 2013, le Vietnam a salué son 90 millionième citoyen. Le Vietnam est désormais le 14^{e} pays le plus peuplé au monde et donc le 3^{e} en Asie du Sud-Est. Cet événement représente pour le Vietnam « une structure démographique d'or », où la population active est du double de celle économiquement dépendante. Cette structure est particulièrement favorable au développement socio-économique, car elle donne d'abondantes ressources humaines jeunes, facteur important de l'attrait des investisseurs étrangers. Toutefois, avec une population de 90 millions d'habitants, le Vietnam doit pour ne pas atteindre le stade critique de surpopulation, intensifier durablement ces agrosystèmes, pour sauvegarder son autosuffisance alimentaire, ce qui représente un défi majeur. De plus cette démographie galopante induit une pression des zones urbaines en expansion constante sur les zones agricoles les plus fertiles. Cette prédation de l'espace urbain sur l'espace rural, impose aux paysans de mettre en culture de nouvelles terres et/ou d'intensifier leur utilisation des zones en pente de montagne. Ces zones étaient jusque là laissées à l'espace forestier du fait de leur faible fertilité ou utilisés en alternant culture et période de jachère.

1.2. Erosion et dégradation des sols dans le Nord du Vietnam

L'érosion des sols est un phénomène qui a des conséquences préoccupantes en Asie du Sud-Est, tant au niveau économique et social, qu'environnemental. L'érosion est un fléau particulièrement important au Nord du Vietnam. Il y a 2 types de facteurs liés à l'érosion. Le premier est un facteur anthropique dû à l'occupation des terres par l'homme. Les sols sont laissés nus sans zones d'écoulement préférentiel, ce qui conduit au phénomène de ruissellement, et à l'exportation de particules de sol du haut vers le bas des parcelles. Le deuxième facteur est environnemental et concerne les événements pluvieux torrentiels caractéristiques de la période de mousson qui, associés à une topographie en pente, favorisent des vitesses d'écoulement importantes. Les sols de pentes sont situés dans des zones particulièrement vulnérables aux phénomènes d'érosion. En effet, le Nord du Vietnam est caractérisé par un climat subtropical humide avec une saison des pluies très marquée qui engendre des dégâts importants du type érosion et glissement de terrain lorsque les pratiques culturales ne sont pas adaptées.

Les conséquences de l'érosion sont (1) la perte des MO qui se trouvent dans les couches supérieures du sol, associée à (2) la diminution de la fertilité des sols, ce qui conduit à (3) la réduction des surfaces agricoles. De plus, l'érosion des sols engendre également la pollution des eaux de surface (Lal, 2004 ; Nguyen et al., 2008).

Dans le Nord du Vietnam, les élevages bovins et porcins sont fréquents afin de répondre à la demande en viande des villes, mais également pour diversifier les revenus des agriculteurs. Traditionnellement, les petites exploitations agricoles nourrissent leurs animaux à partir de ressources végétales locales, comme l'herbe, les feuilles d'arbres ou la paille. Par contre, le développement de l'élevage peut entraîner d'autres problèmes environnementaux liés à la gestion de ces résidus. En effet, lorsque les déjections animales sont directement épandues sur les sols comme amendement, ils conduisent à une minéralisation rapide de la MOS, à l'acidification des sols et à une perte rapide de leurs éléments minéraux (Bouman et al., 1995). Leur épandage sans prétraitement amplifie donc les phénomènes de lixiviation et d'érosion, mais favorise également le maintien et la dissémination de parasites humains affectant les populations rurales (Phuc et al., 2006). Ainsi, l'utilisation des résidus d'élevage non transformé ne permet pas l'établissement de systèmes agricoles durables et des conditions sanitaires satisfaisantes pour les populations locales. Les Vietnamien sont donc aujourd'hui face au défi de trouver des amendements peu couteux des sols permettant d'augmenter leur teneur en matières organiques et ainsi leur fertilité. De plus, en zone rurale, les Vietnamiens doivent trouver un traitement pour les déchets issus de l'élevage. C'est le contexte, qui a conduit à une collaboration entre le Vietnam et la France en ce qui concerne le développement des pratiques agricoles durables. C'est ainsi, qu'une unité de l'IRD a été basée à Hanoi et que j'ai commencé ma thèse en me focalisant sur les solutions d'ingénierie écologique pour transformer les lisiers de buffle en un substrat permettant d'améliorer la qualité des sols tropicaux dégradés du Nord du Vietnam.

2. Synthèse bibliographique

2.1. Les amendements organiques exogènes (AOEs)

Les amendements organiques exogènes (AOEs) qu'on peut apporter aux sols sont des déchets organiques de diverses origines valorisés en agriculture. Ces AOEs sont en général des déchets issus d'activités agricoles, urbaines ou industrielles ou des produits issus de leur traitement (Peltre et al., 2011). L'apport des AOEs est une des actions possibles pour entretenir ou restaurer, voire augmenter les teneurs en MOS pour leur permettre de remplir différentes fonctions (voir ci-dessous).

2.1.1. Compost

Dans les zones rurales du Nord-Vietnam, les déchets disponibles potentiellement utilisables pour améliorer les sols provient essentiellement du lisier de buffle. Le compostage du lisier de buffle avant son épandage sur les sols a été proposé par les agents de l'IRD comme un prétraitement adéquat afin d'améliorer la valeur du lisier de buffle avant de son épandage aux sols, permettant d'améliorer la qualité des sols et d'augmenter la durabilité des pratiques culturales.

2.1.1.1. Processus de compostage

Chimiquement, le compostage est un processus de décomposition et de synthèse de molécules organiques stables. Celles-ci permettent après épandage une augmentation de la fertilité des sols, et en particulier ceux initialement appauvris en MOS (Caravaca et al., 2002). Le compostage passe par 4 phases: La phase mésophile (jusque 40°C), la phase thermophile (pouvant atteindre plus de 70°C) et enfin celle de refroidissement et de maturation (Fig. 1.1).

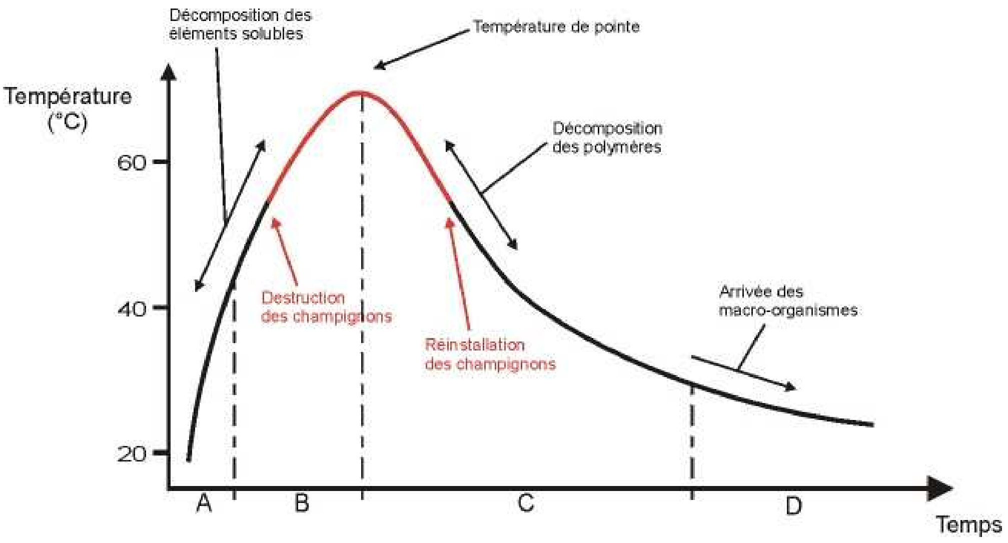


Figure 1.1 : Le processus du compostage (source : *compostage.info*)

Pendant la phase mésophile, les bactéries et champignons consomment les molécules simples et labiles comme les sucres, les protéines et les lipides. Par la phase thermophile, la température augmente en lien avec l'activité microbienne intense et l'émission de CO_2. La température élevée et la compétition microbienne exercent une sélection sur la diversité microbiologique et permettent une diminution des microorganismes pathogènes du produit composté par une destruction des micro-organismes pathogènes. Pour favoriser la biodégradation, un retournement est nécessaire afin de maintenir la quantité le l’oxygène et les conditions aérobies. Lors de la phase de refroidissement, l’activité microbienne et la température diminuent progressivement à cause de la diminution de la quantité de matières organiques facilement dégradables. Au cours de cette phase, les polymères comme la lignine, la cellulose… peuvent être dégradés. Suit la phase de maturation où la stabilisation des composts augmente. Un compost est considéré comme mature quand il est stable et donc résistant à la biodégradation (Francou et al., 2005).

2.2.1.2. Les effets du compost sur la qualité des sols

En général, les composts sont physiquement plus homogènes que les déchets dont ils sont issus. Ce sont des amendements qui peuvent contribuer de façon significative au maintien, voire à l’augmentation du stock des MOS (Leshermes et al., 2009) et qui jouent un rôle important dans la durabilité de la fertilité, et donc au développement de pratiques agricoles durables. Le compost est également une source d'azote, de phosphore et de potassium pour les cultures (Baldi et al., 2010). Le compost rend au sol ces nutriments, prolongeant ainsi leur présence dans le sol pour nourrir les végétaux pendant une plus longue période de temps.

Par conséquence, les végétaux cultivés dans un sol contenant du compost ont un meilleur rendement (Diacono et Montemurro., 2010). Le compost apporte aux sols non seulement de la matière organique et des nutriments minéraux tels que N, P et K mais aussi des éléments présents en faibles quantités tels que le fer, le manganèse, le cuivre, le zinc et le bore, nécessaires à la croissance des végétaux (Larchevêque et al., 2005). Il a été également démontré que certains composts permettent de faire disparaitre les odeurs désagréables, la destruction des germes pathogènes et des parasites animaux, peuvent donc réduire l’incidence de certaines maladies chez les végétaux et augmentent la production de la biomasse.

Par d’ailleurs, de nombreuses recherches ont en effet montré un effet bénéfique de l'apport des composts sur les propriétés des sols comme une augmentation de la CEC, une amélioration de la structure du sol ainsi que la quantité et qualité de la MOS (Rivero et al., 2004). Celik et al (2004) ont aussi démontré que le compost est capable d'augmenter la conductivité hydraulique du sol. Enfin, l’apport du compost permet une amélioration de la stabilité des agrégats de sol, et donc une meilleure résistance à l’érosion (Abiven et al., 2007).

2.1.2. Lombricompost

2.1.2.1. Processus de lombricompostage

Le lombricompost est un type particulier de compost. Il résulte de l’interaction entre les vers épigés et les microorganismes qui décomposent la MO dans des conditions non thermophiles (Pramanick, 2006). Les deux principaux types de vers utilisés pour le lombricompostage sont *Eisenia andrei* et *Perionyx excavatus*. Les deux sont souvent appelés « vers rouges » ou « vers du fumier ». L’action des vers de terre dans ce processus est à la fois physique (fragmentation et aération) et biochimique (minéralisation, humification). Ces vers peuvent consommer pratiquement tous les types de déchets organiques et peuvent consommer quotidiennement plus que leur propre poids (FAO, 2005). Les déjections des vers de terre sont riches en nitrates, et en formes disponibles de P, K, Ca et Mg. La consommation des substrats organiques par les vers de terre favorise également la croissance des bactéries et des actinomycètes.

Comme les vers de terre ont besoin d'une température allant de 0 à 35˚C (la température optimale étant de 25˚C), les systèmes de lombricompostage prévus pour fonctionner toute l'année doivent être conçus en fonction du climat. Le processus ne dégage pas d'odeurs et il ne produit pas de chaleur (Dominguez et al., 2004). Le lombricompostage est abandonnement utilisé dans les pays tropicaux comme l’Inde pour la transformation des déchets organiques.

2.1.2.2. Les effets du lombricompost sur la qualité des sols

Comme le compost, le lombricompost a un grand potentiel pour améliorer la qualité des sols et les rendements des cultures. Le lombricompost est un produit plus homogène avec une structure plus fine, et plus riche en éléments nutritifs solubles et disponibles pour les végétaux, en comparaison avec les composts traditionnels (Edwards and Burrows, 1988 ; Ndegwa and Thompson, 2001). Du point de vue strictement chimique, Ndegwa (2001) a montré que les excréments de vers de terre sont plus riches en nitrates et autres éléments fertilisants majeurs sous leurs formes solubles, comme le phosphore, le potassium, le magnésium et le calcium que les matières organiques de départ. Selon Albanell et al. (1988), le lombricompost possède un taux de sels solubles inférieurs, une capacité d'échange cationique supérieure et un plus fort taux de substances humiques par rapport aux matériaux initiaux. De plus, les nutriments y sont présents sous une forme plus facilement assimilable, ce qui laisse supposer un intérêt particulier pour la fertilisation organiques des cultures.

Il a été démontré par des expérimentations effectuées en serre, ainsi que via des essais au champ que le lombricompost peut augmenter le taux de croissance des plantes (Atiyeh et al., 2000 ; Acevedo and Pire, 2004). Canella et al. (2002) confirma la présence d'hormones de croissance végétales en relation avec l'humus du lombricompost et remarqua que ces dernières améliorent la croissance des racines et la formation de sites d'initiation de racines latérales chez le maïs exposé aux acides humiques issus du lombricompost du fumier de vache.

2.1.3. Les biochars

2.1.3.1. Processus de charbonisation

Les biochars proviennent de la pyrolyse de la biomasse faiblement valorisable d'origine végétale ou animale. Ce substrat a le potentiel d'améliorer la qualité des sols et la croissance des plantes tout en diminuant les apports d'engrais et en séquestrant le carbone (Lehmann, 2007). En général, ils se présentent sous forme de petits fragments charbonneux de couleur noir, très poreux et composés en majeur partie de carbone. La composition des biochars varie et dépend fortement de la nature de la biomasse utilisée et du processus de pyrolyse utilisé (Schimmelpfennig and Glaser., 2012).

Il existe plusieurs types de productions artisanales et industrielles du biochar mais la technique traditionnelle appelée « pyrolyse lente » est la plus populaire à travers le monde. Cette technique se traduit par la conversion de la biomasse végétale à une température de pyrolyse modérée (environ 500°C) et un temps de résidence long (plus d'une journée) (Photo ci-dessous).

Photo: Fabrication de bambou biochar au Vietnam (source P. Lamballe)

2.1.3.2. Effets du biochar sur la qualité des sols

L'utilisation du biochar est fort ancienne et remonte aux origines de l'agriculture en Europe (Sigault, 1975) comme en Amazonie (Glaser, 2002). La redécouverte des *Terra Preta* (Terre noire en Portugais) extrêmement fertile, qui est considéré aujourd'hui comme un des sols les plus fertiles au monde avec un rendements bien supérieur à celui des Oxisols adjacents (Steiner., 2008). Ces 'terres noires' sont le résultat de l'accumulation des résidus de combustion lente et des déchets organiques des communautés villageoises en bord du fleuve Amazone. Les études archéologiques ont montrées que dans certains cas cette accumulation a pu avoir lieu sur plusieurs millénaires, prouvant la stabilité de ce type de charbon (Glaser et al., 2001). L'application des biochars en agriculture en Asie est aussi bien documentée. C'est à la fin du 17^{e} siècle au Japon que les plus anciens écrits décrivant l'utilisation traditionnelle de cette pratique ont été retrouvés. Les biochars étaient produits à partir de la balle du riz, c'est-à-dire des enveloppes entourant les graines de riz (Jindo et al., 2012).

Les intérêts d'ajouter le biochar aux sols comme un amendement sont bien documentés ces dernières décennies. Les avantages de l'utilisation du biochar sont les suivants:

- *Séquestration de carbone*

Le biochar est un produit riche en carbone aromatique et caractérisé par sa récalcitrance à la décomposition par les micro-organismes (Kuzyakov et al., 2009 ; Zimmerman et al., 2011). L'enfouissement des biochars dans les sols a donc le potentiel d'augmenter les stocks de carbone des sols (Lehmann et al., 2003).

- *Augmentation du rendement de la culture*

Le biochar est capable d'augmenter le rendement agricole grâce à l'augmentation de la résistance aux maladies des végétaux (Glaser et al., 2013). De plus, la présence de biochar diminue le lessivage des sols et augmente la quantité des éléments nutritifs disponibles (Lehmann et al., 2002 ; Steiner, 2007) qui sont nécessaires à la croissance des végétaux. Major et al (2010) ont montrés que les sols amendés avec le biochar sont plus riches en Ca et Mg disponibles pour les plantes. Le biochar est également connu pour sa capacité à synthétiser de l'éthylène (Spokas et al., 2010) dont l'action comme phytohormone est importante même à faible concentration (Arshad and Frankenberger., 2002). Il peut être un mécanisme potentiel pour augmenter la densité de racine, favoriser la croissance des graines, et augmenter la production de biomasse végétal (Spokas et al., 2010 ; Abeles et al., 1992).

- *Paramètres physicochimiques des sols*

Plusieurs études ont montrées que la présence de biochar permet d'améliorer la rétention en eau du sol (Glaser et al., 2002), le pH des sols acides (Spokas et al., 2011) et de diminuer des substances toxique des sols (Steiner et al., 2007).

2.1.4. Intérêt d'application conjointe des biochars et d'autres AOE

Amender des surfaces agricoles avec du carbone pyrogénique, biologiquement beaucoup plus stable que les résidus végétaux de nature cellulosique (Baldock et Smernik, 2002), est considéré comme une approche permettant de séquestrer massivement du carbone dans le sol (Lehmann et al., 2003). Les travaux sur l'utilisation du biochar dans les agrosystèmes ont portés essentiellement sur l'apport unique de biochar (Glaser et al., 2002; Lehmann et

Rondon, 2005., Spokas et al., 2009) ou en mélange avec des engrais minéraux (Steiner et al., 2007 ; Sohi et al., 2010 ; Liang et al., 2010). Cependant, le biochar est considéré comme pauvre en éléments nutritifs (Glaser et al., 2002 ; Glaser et Birk, 2012) alors que les matériaux compostés sont des sources durables de nutriments disponibles pour les plantes. Afin de restaurer la fertilité des sols fortement dégradé, une approche inspiré des techniques de l'ancienne agriculture précolombienne d'Amazonie (*Terra preta*) consisterait à associer les biochars avec des composts ou directement avec des résidus d'élevage.

Ces dernières années, cette pratique est devenue le centre d'intérêt de nombreuses équipes à travers le monde (Jindo et al., 2012a, 2012b ; Bolan et al., 2012 ; Liu et al., 2012). Tous ces auteurs ont ajouté les biochars au cours du compostage. Ce procédé semble diminuer le lessivage de l'azote du substrat (Steiner et al., 2010), augmenter le degré d'humification du produit final (Dias et al., 2010), changer la composition des communautés microbiennes impliquées dans le processus de compostage et diminuer la quantité de carbone soluble à l'eau (Jindo et al. 2012). Peu d'études ont été effectuées sur l'application conjointe de compost et de biochar. Il semble, que l'application conjointe du lombricompost et du biochar augmente la quantité des bactéries dans les sols ainsi que les eaux de ruissellement (Doan et al., 2013). Toutefois, les mécanismes permettant aux biochars d'améliorer la fertilité des sols tout en stockant du carbone à long terme restent largement inconnus. On dispose également de trop peu de données scientifiques pour conclure définitivement que l'association du biochar au compost permet d'augmenter le stockage du C dans les sols et particulièrement les sols tropicaux soumis à de très fortes précipitations.

2.2. Les matières organiques du sol

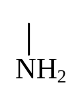
2.2.1. Définition et rôles des matières organiques du sol dans des sols tropicaux

Les matières organiques du sol (MOS) sont composées de l'ensemble des matériaux organiques morts qui ont été fabriqués par les végétaux, faune du sol ou micro-organismes (Kögel-Knabner., 2014). Elles sont constituées de tout le continuum entre litières mortes et substances organiques complètement transformées (Kögel-Knabner, 1993). Elles ne représentent que quelques pourcents du poids du sol mais jouent un rôle indispensable dans le comportement global du sol. Tout d'abord, les MOS participent à la stabilité de la structure du sol, et sa protection contre l'érosion via une augmentation des forces de liaison internes des agrégats (Le Bissonais et al., 2004). Ces forces de liaison peuvent être dues aux interactions électrostatiques entre les particules minérales du sol et certains composés organiques telle que les polysaccharides, qui participent à la formation des complexes organo-minéraux (Puget et al., 1995). De plus, les MOS assurent le stockage des éléments minéraux nutritifs pour les plantes et les microorganismes (Fontaine et al., 2011). Ainsi, les MOS constituent le principal déterminant de l'activité biologique en fournissant aux communautés microbiennes, leur principale source d'énergie et de nutriments. Par ailleurs, les MOS ont un rôle fondamental au niveau environnemental en retenant les micropolluants organiques et inorganiques (Vermeer, 1996 ; Tan and Binger, 1986). Cependant, les sols tropicaux se caractérisent généralement par de faibles quantités de MOS, en raison des conditions climatiques (humidité, haute température) favorisant un turnover rapide des MOS. En conséquence, la dégradation rapide de la litière impact directement la composition des MOS, se caractérisant par une forte proportion de composé d'origine microbienne interagissant avec la phase minéral, au détriment de composé dérivant directement des plantes (Kiem and Kögel-Knabner, 2003). Les minéraux des sols tropicaux sont souvent en majorité des kaolinites ayant une capacité moindre d'adsorption (Khawmee et al., 2013) et de protection des MOS. De plus, la réduction des périodes de jachère et la culture sur brulis augmentent la teneur en charbons des sols tropicaux (Rumpel et al., 2006) mais favorise l'érosion et les pertes en MOS.

2.2.2. Les grandes classes de molécules organiques de la MOS

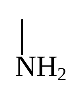
2.2.2.1. Les composés azotés.

Les protéines sont les composés azotés les plus abondants dans le sol (Knicker, 2011). Ils sont formés par la condensation d'acides aminés. Ceux-ci sont constitués d'une dizaine à une cinquantaine d'atomes qui ont la particularité de porter à la fois un groupement acide (-COOH) et un groupement amine ($-NH_2$). En effet, on distingue plusieurs types d'acides aminés selon la situation du groupe – COOH et des groupes $-NH_2$ comme α –aminés, β-aminés, δ-aminés, γ-aminés. Le type α –aminés est rencontré souvent dans le monde vivant et il participe aux processus biologiques les plus importants (Phan Tong Son, 1999).



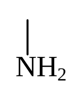
$$\mathrm{R{-}\underset{\substack{|\\ NH_2}}{CH}{-}COOH}$$

α –aminés



$$\mathrm{R{-}\underset{\substack{|\\ NH_2}}{CH}{-}CH_2{-}\ COOH}$$

β- aminés



$$\mathrm{R{-}\underset{\substack{|\\ NH_2}}{CH}{-}\ CH_2\ {-}CH_2\ {-}COOH}$$

γ -aminés

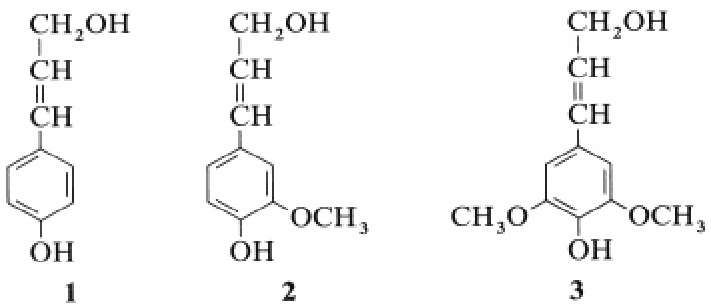
R : chaîne latérale de nature variable.

Les protéines composantes des matières organiques du sol sont essentiellement d’origine microbienne, car intrinsèquement labile à la dégradation. Leur persistance dans les sols pourrait s’effectuer grâce à une interaction avec la phase minérale (Kleber et al., 2007).

2.2.2.2. Les composés aromatiques.

Les composés aromatiques des MOS peuvent provenir des lignines compte tenu de leur rôle comme constituant important des plantes vasculaires. Les lignines sont des polymères aromatiques de poids moléculaire élevé, ce que leur confère une forte résistance à la biodégradation. Elles ont été donc considérées longtemps comme un composant important des MOS en étant en particulier responsable du stockage du carbone dans les sols (Haider, 1991).

La lignine résulte de la polymérisation oxydative de trois alcools phénoliques : l’alcool coniférylique, l’alcool sinapylique et l’alcool p-coumarylique (Ho, 2004)



3-(4-Hydroxyphenyl)-2-propen-1-ol (alcool p-coumarylique) **(1)**

3-(3-methoxy-4-hydroxyphenyl)-2-propen-1-ol (alcool coniférique) **(2)**

3-(3,5-dimethoxy-4-hydroxyphenyl)-2-pro-pen-1-ol(alcool sinapylique) **(3)**

La biosynthèse de ces alcools au sein de la plante s’effectue en une série d’étapes initiées par les enzymes. Les copolymères obtenus contiennent donc des unités de base parahydroxyphényles, (p-coumaryles) guiacyles (vanillyles) et syringyles.

Les lignines sont des biomarqueurs végétales. Récemment, il a été monté, que les lignines ne sont pas préservés dans les sols plus que quelques années (Thévenot et al., 2010).

Des autres composés aromatiques beaucoup plus stables dans les sols que les lignines sont des charbons provenant des feux et donc de la combustion de la biomasse. Egalement les biochars sont des composés aromatiques faisant partis des MOS. La persistance de ces composées aromatiques pyrogènes est due à leur structure intrinsèquement stable. En conséquence leur temps de résidence dans les sols peut atteindre plusieurs milliers d'années (Schmidt et al., 2011) en région tempéré. En région tropicale, il semble qu'il font partis des MOS stabilisés avec des temps de résidence de plusieurs centaines d'années (Rumpel et al., 2008).

2.2.2.3. Les sucres

Les sucres présentent de 5 à 25% de la MO des sols (Stevenson, 1982). Les sucres libres dans la solution du sol sont des monosaccharides tels que les sucres neutres, amino sucres, sucres acides (acides uroniques). Elles forment la cellulose (constituant de la paroi des cellules végétales) et l'hémicellulose, résultent de la condensation entre elles d'un grand nombre de sucres simples (Kögel-Knabner, 2002). En général, les sucres peuvent être facilement dégradés par les microorganismes, qui synthétisent à leur tour des polysaccharides (parois et mucilages). Par conséquence, les sucres dans les sols sont d'origine végétale (cellulose et hemicellulose) et microbienne (hemicellulose). La complexité de leurs structures, leurs adsorptions sur des composés minéraux et leurs liaisons avec des substances humiques peuvent leur conférer une certaine résistance à la biodégradation (Kiem and Kögel-Knabner, 2003). Leurs propriétés dépendent de la longueur de leurs chaines et de l'importance des ramifications. Leurs groupes fonctionnels hydrophiles permettent d'atténuer l'effet du mécanisme d'éclatement des agrégats lorsque l'eau s'adsorbe dans la porosité du sol (Puget et al., 1995).

2.2.2.4 Les composés aliphatiques

On distingue deux types des lipides: les lipides libres d'origine végétale et les lipides liés d'origine microbienne (Grasset et Alblès., 1998). Les lipides libres sont les composés solubles dans les solvants organiques, alors que les lipides liés sont piégés dans les réseaux des macromolécules de la MOS et non-extractibles directement par les solvants organiques. Dans le sol, les lipides sont essentiellement sous forme de graisses, cires et résines (Kögel-Knabner, 2002).

2.2.3. Méthodes chimiques de caractériser la MOS et l'AOE

Les MOS, bien que facilement quantifiable globalement, de par leur grande complexité chimique restent difficile à caractériser. A l'heure actuelle, il y a plusieurs méthodes pour étudier les MOS mais chaque méthode a des avantages et aussi des points faibles. Il est donc nécessaire de combiner différentes méthodes afin d'obtenir une information plus compète des MOS (Kögel-Knabner, 2000).

2.2.3.1. La résonance magnétique nucléaire du ^{13}C (RMN)

La spectroscopie RMN ^{13}C est une méthode non-destructive et largement utilisée pour caractériser la structure de la MOS (Kögel-Knabner et al., 1997). Elle consiste à soumettre l'échantillon à l'action conjointe de deux champs électromagnétiques, l'un intense et constant, l'autre faible et oscillant. L'absorption et le relargage par certains noyaux atomiques de l'échantillon de fréquences présentes dans la source électromagnétique servent à générer le spectre RMN. On mesure sur les spectres des intensités du signal pour les différentes fréquences de résonances correspondant à des structures chimiques. Ces valeurs sont exprimées en « déplacement chimique » de fréquence par rapport à la fréquence de vibration d'un standard δ en ppm. En science du sol, on subdivise les spectres RMN de ^{13}C en quatre régions. Chaque région de valeur de déplacement chimique correspond à un type de groupes fonctionnels, et il est possible de quantifier l'abondance de ces groupes dans le matériel analysé. On peut ainsi distinguer des régions du spectre correspondant aux groupes carboxyle / amide, C aromatique, C alkyle ou O, N alkyle (Wilson, 1981).

Cette technique nous informe sur la composition chimique globale des MOS. Le rapport alkyl C/O-alkyl C est utilisé pour suivre la biodégradation de la MO. Ce ratio augmente avec le degré de dégradation de la MOS (Baldock et al., 1997). Cependant, cette méthode à faible résolution entre les différentes formes chimiques ne permet pas d'étudier la caractérisation de différentes molécules qui composent les MOS.

2.2.3.2. Fractionnement biochimique des MOS

Le fractionnement biochimique de la matière organique exogène est inspiré du fractionnement Van Soest (1967). Il est abandonnement utilisé pour caractériser la qualité des matières organiques pures, comme les litières ou les composts (Peltre et al., 2010). Il s'agit d'un fractionnement de la matière organique en quatre familles biochimiques, sur la base de leur solubilisation successive dans des réactifs d'extraction :

- La fraction soluble (SOL): fraction soluble à l'eau bouillante et extrait par un détergent neutre à chaud (DNF). Cette fraction augmente au cours du compostage lié avec la maturation de compost (Francou et al., 2008)

- La fraction équivalente à l'hémicellulose (HEM): fraction solubilisée avec un détergeant acide à chaud après élimination de la fraction SOL. En général, cette fraction diminue au cours du compostage (Peltre et al., 2010).

- La fraction équivalente à la cellulose (CEL): fraction solubilisée avec l'acide sulfurique concentré à froid (ADL) après élimination des fractions SOL et HEM. Comme la fraction HEM, la fraction CEL diminue au cours du compostage (Francou et al., 2008).

- La fraction équivalente aux lignines et cutines (LIC) : correspond au résidu de l'extraction avec ADL. Cette fraction est considérée comme stable et augmente généralement au cours du compostage.

Le fractionnement biochimique est une technique simple, peu coûteuse, qui a été fréquemment utilisé dans les pays en voie de développement en région tropicale pour caractériser la qualité des litières. Bien qu'utile pour les AOEs, son application pour caractériser les MOS est moins pertinente.

2.2.3.3. Pyrolyse couplée à la chromatographie en phase gazeuse et à la spectrométrie de masse (GC/MS)

La pyrolyse est une méthode d'analyse qualitative qui a pour but de préciser la proportion des macromolécules organiques. Elle consiste en une dégradation thermique des composés organiques à haute température (entre 650 à 750°C) en absence d'oxygène. Lors de la pyrolyse, les macromolécules non-volatiles sont détruites et leurs produits sont ensuite libérés, puis volatilisés, et séparés par chromatographie gazeuse. L'utilisation d'un spectromètre de masse permet ensuite d'identifier des fragments produits qui sont des familles moléculaires tels que les méthoxyphénols dérivés des lignines (LIG), les cétones cycliques et furanes venant des polysaccharides (PS), des composés azotés venant des protéines (N), des lipides (LIP). Les composés qui n'ont pas de source biochimique distincte sont considérés comme non-spécififiques (Divers) (Dignac et al., 2006 ; Parnaudeau et Dignac., 2006).

Il y a deux types de colonnes utilisées pour la chromatographie en fonction des composants qu'on souhaite identifier: une colonne polaire et une non polaire. Les colonnes polaires sont utilisés pour quantifier des composés de type polysaccharides, lignines et protéines (Rumpel et al., 2008). A l'inverse, les composés les moins polaires tels que les alcanes et les alkenes sont moins retenus par ce type de colonne.

2.2.3.4. Analyse des lignines par oxydation cuivrique (CuO)

L'analyse de la lignine par hydrolyse alcaline au CuO est la méthode la plus utilisée ces dernières années pour l'étude des lignines des sols. L'hydrolyse alcaline oxydative de la lignine libère une série de composés phénoliques, dont l'identification les apparente aux types vanillique (V), syringique (S) ou cinnamique (C) (Bahri et al., 2006). Les acides, les aldéhydes et les cétones des unités V, S et C ont été quantifiés par chromatographie à phase gazeuse. La somme des unités V, S et C présente la concentration de la lignine dans les sols ou dans les amendements organiques. Le rapport acides/aldéhydes des dérivés des unités vanillyl et syringyl obtenus est utilisé comme indicateur du degré de dégradation de la lignine. Ce rapport diminue au cours de la dégradation (Thevenot et al., 2010).

2.2.3.5. Analyse des sucres non cellulosiques après hydrolyse à l'acide trifluoroacétique (TFA)

L'analyse des sucres du sol au niveau moléculaire implique une hydrolyse à l'acide trifluoroacétique (TFA) et une purification ainsi que la dérivation des monosaccharides, et sont ensuite analysés par chromatographie en phase gazeuse. L'analyse les sucres non-cellulosiques parait intéressante pour détecter l'origine végétale ou microbienne des sucres. Les rapports C6/C5 (Mannose+galactose) / (xylose+arabinose) et deoxy/C5 (Rhamnose+fucose) / (xylose+arabinose) sont des indicateurs qui peuvent aider à comprendre leur origine (polymères de structure des végétaux ou polysaccharides synthétisés par les microorganismes) (Rumpel et Dignac, 2006).

3. Site d'étude et matériel (AOEs et biochar)

3.1 Présentation la situation et le climat de la région de Hanoi

Les travaux réalisés durant cette thèse ont été mis en place à Hanoi. Hanoi est situé au Nord du Vietnam dans un climat subtropical humide. La pluviométrie atteint 1500 à 1800 mm par an en moyenne, avec 85% des précipitations entre les mois d'Avril et Octobre et avec une intensité variant de 70 à 200 mm h^{-1} (Fig. 1.1). L'humidité de l'air est toujours élevée, entre 75 et 100% (Tran Duc et al., 2004, Orange et al.,2007). Le sol utilisé dans cette étude venait du site d'étude du projet MSEC (Managing Soil Erosion Consortium résultant d'un partenariat entre l'IRD et le SFRI). Les sols dans ce site sont des Acrisols (FAO, 1998) avec une texture argileuse (50-60% d'argile en moyenne, Kaolinite majoritairement), avec un faible pH et une faible CEC (<10 cmol kg sol^{1}), poreux avec une densité d'environ 1.0 g cm^{-3} (Jouquet et al., 2008; Podwojewski et al., 2008). Le sol utilisé dans notre étude est un sol tropical dégradé venant d'un bassin versant où les pentes varient de quelques degrés à plus de 120% et la pente moyenne est située aux alentours de 45%.

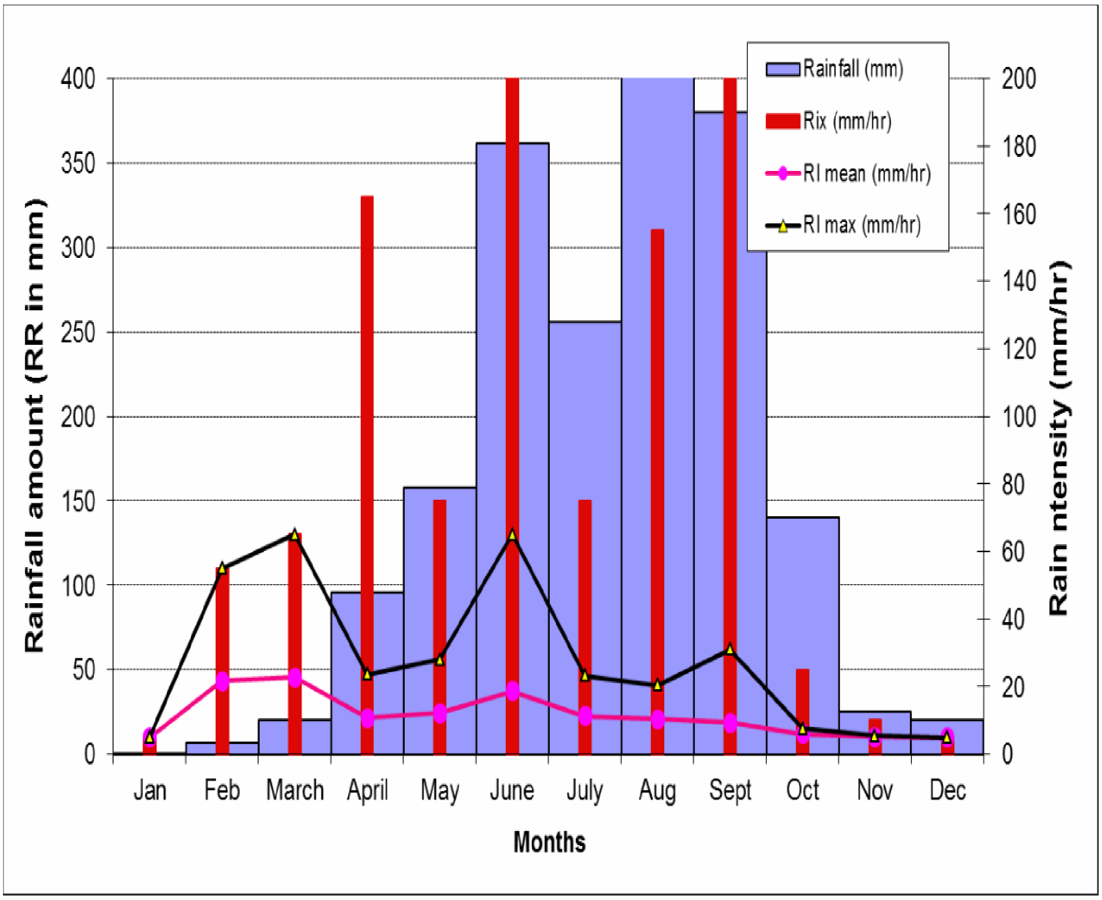


Figure 1.1 : Diagramme de la précipitation moyenne par mois de Hanoi sur l'année 2013 (source : Pham Dinh Rinh)

3.2. Fabrication du compost, du lombricompost et du biochar

Dans cette étude, le compost a été fabriqué à partir du fumier de buffle. Ce dernier a été échantillonné dans l'étable d'une ferme et placé durant 3 mois dans des bacs en béton d'une capacité de 500 L. Les bacs ont été couverts par un toit, de manière à conserver l'humidité nécessaire à l'activité microbienne, éviter le lessivage du compost par les pluies, limiter la volatilisation de l'azote. Le compost a été retourné toutes les 2 semaines environ afin d'homogénéiser le processus de dégradation et favoriser l'aération du substrat. A la fin des 3 mois, le compost a été stocké dans des bacs jusqu'à utilisation.

Le lombricompostage (ou 'vermicompost' en anglais) a été conduit en utilisant le fumier de buffle et les vers *Eisenia andreï*. L'ensemble a été laissé dans les bacs au repos durant 3 mois, avec quelques retournements légers afin de vérifier l'humidité et l'état de santé des vers de terre. Comme pour le compost, le lombricompost a été placé dans des bacs jusqu'à son utilisation. Les vers ont été récupérés et peuvent être réemployés pour un nouveau cycle.

Au Vietnam, le biochar peut être fabriqué principalement à partir de la paille du riz ou des résidus de bambou. Dans cette étude, le biochar est produit à partir du bambou Luong (*Dendrocalamus* barbatus Munro) couvrant environ 1.4 million ha de forêt et peut être donc une source importante disponible pour l'industrie du biochar. Ce biochar a été produit dans un four en briques en condition anaérobie. Le bambou préalablement séché a été introduit dans le four, puis chauffé lentement pendant 48 h (précarbonisation). La carbonisation s'effectue à une température de 500 à 600 °C pendant 72-96 h, et le refroidissement est réalisé à température ambiante pendant 3-5 jours. C'est une méthode de production artisanale, car la température n'est pas également répartie à l'intérieur du four à briques.

Chapitre 2 : Caractérisation de la nature chimique du lisier de buffle, du compost, du lombricompost et du biochar

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Ngo, P-T[a,c]., Rumpel, C[a]., Dignac, M-F[a]., Billou, D[a]., Tran, D.T[c]., Jouquet, P[b]., 2011. Transformation of buffalo manure by composting or vermicomposting to rehabilitate degraded tropical soils. *Ecological Engineering* 37, 269-276.

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1. Introduction

The nature of exogenous organic matter (EOM) used as organic amendment is of primary importance in the regulation of soil ecological functions (Morlat 2008). Compost is widely used as EOM. This is especially true in degraded soils in Northern Vietnam where it improves soil quality and plant growth (Doan et al., 2013). One interesting process for improving the quality of EOM is composting in presence of earthworms (Edwards et al. 2004; Edwards & Arancon 2004). The resulting material called vermicompost was reported to be a stabilized product compared to compost with higher contents of most nutrients in plant available forms (e.g., Edwards *et al.* 2004; Jouquet et al., 2011) and larger numbers and types of microorganisms such as plant growth promoting rhizobacteria (e.g., Edwards and Arancon 2004; Arancon *et al.* 2008; Adi and Noor 2009). Earthworms were found to accelerate compost maturation (Atiyeh et al., 2000), probably due to their interaction with the decomposer community (Sampedro and Dominguez, 2008). Vermicomposted manure was found to be processed into a much more homogenous product compared to composted manure that remained in compact clumps (Atiyeh et al., 2000). The use of EOM, such as compost and vermicompost may be particularly interesting as a source of P (Busato et al., 2012). Phosphorus is essential for plant growth but it is a non-renewable resource (Wang et al., 2012) and a limiting element of tropical terrestrial ecosystems.

In recent years the application of biochar has also gained attention as an environmental friendly, low cost and renewable soil amendment (Atkinson et al., 2010). Biochar is the product of the thermal degradation of organic materials in absence of air (pyrolysis). It is distinguished from charcoal by its use as a soil amendment (Lehmann and Joseph, 2009). It has been widely studied as a soil conditioner (Lehmann and Sohi., 2008), and may be especially useful to increase soil C sequestration (Naisse et al., 2014).

In order to assess the functions and stability of EOM and biochar in soil, information on their chemical composition is needed. Up to now most studies interested in the changes occurring during composting and/or the effect of compost on soil organic matter were based on chemical fractionation (Albrecht et al., 2008; Spaccini and Picolo, 2007; Som et al., 2009). However, such fractions are poorly related to biogeochemical processes operating in soil (Skjemstad et al., 1996). Therefore, the evaluation of the molecular composition of bulk compost and amended soil should be more useful to understand the biogeochemical processes during composting and vermicomposting and the influence of the incorporation of these organic amendments into SOM (Spaccini et al., 2009).

In this study, the nature of the four organic materials was evaluated by analyses of their elemental and isotopic composition. In addition, we evaluated their chemical composition with solid state ^{13}C NMR spectroscopy and analytical pyrolysis and their biochemical composition using different detergents (Van Soest fractionation). Their lignin component was characterised by CuO oxidation and their phosphorus forms were characterised using different chemical extractants (Hedley fractionation). We hypothesised that the contribution of stable compounds, such as aromatic, aliphatic compounds and lignin would increase during composting and vermicomposting compared to manure. The aims of the study were (1) to investigate the chemical and biochemical nature of the three EOM and biochar (2) to identify the chemical and biochemical changes occurring during their formation process and (3) to study the form of phosphorus in these four substrates in order to evaluate the fertilizer value of these substrates for P deficient tropical soils.

2. Methods

2.1. Elemental and isotopic analysis

Organic carbon (OC), nitrogen (N) content of buffalo dung, compost, vermicompost and biochar were measured using a CHN auto-analyser (CHN NA 1500, Carlo Erba). Hydrogen and oxygen contents were measured with an elemental analyser at the Service Central d’Analyse (SCA) of the CNRS in Solaize, France. Four replicates were analysed.

Stable isotope (^{15}N, ^{13}C) content of compost, vermicompost and manure were measured in a single analysis using a CHN auto-analyser (CHN NA 1500, Carlo Erba) coupled with an isotope ratio mass spectrometer. Stable carbon and nitrogen isotope ratios of the samples were expressed in parts per thousand (‰) using the delta notation:

$$\delta (X) = [R_{sample} / R_{Standard} - 1] \times 1000 \qquad (1)$$

where X was ^{13}C or ^{15}N and R was the corresponding ratio ^{13}C/^{12}C or ^{15}N/^{14}N. The isotopic ratios were calculated relative to the Pee Dee Belemnite Standard (PDB) for C and relative to atmospheric N_2 for nitrogen.

2.2. Solid-state ^{13}C nuclear magnetic resonance spectroscopy

The chemical composition of the four substrates was characterized by solid-state ^{13}C NMR spectroscopy. Spectra were recorded using a Bruker DSX 200 NMR spectrometer with a resonance frequency of 50.32 MHz, and a 7 mm zirconium rotor. The cross polarization magic angle spinning technique (CP-MAS) was applied with a spinning speed of 6.8 kHz, using a contact time of 1 ms and a pulse delay of 300 ms. Depending on the material, single scans were accumulated until reasonable signal to noise ratio was achieved. The chemical shift was referenced to the TMS scale and adjusted with glycine as an external reference.

2.3. Analytical pyrolysis

Analytical pyrolysis was achieved with a coil probe pyrolyser (CDS Pyroprobe 5150) coupled to a Hewlett Packard HP-5890 gas chromatograph and a Hewlett Packard HP- 5889 mass spectrometer (electron energy 70 eV) as reported by Rumpel et al. (2009). Prior to analysis soil samples were demineralised using 10% hydrofluoric acid. This treatment was shown to increase the detectability of organic compounds without major alterations on the organic matter itself (Rumpel et al., 2006). Briefly, 0.5-1 mg of ground demineralised sample was loaded in a quarz tube and heated to 650°C in 10s (30 s hold). The pyrolysis products were transferred to the GC system through a split/splitless injector operated in splitless mode, using He as carrier gas. A fused silica column (60 m, 0.32 mm i.d., film thickness 0.25 mm) coated with a polar Polyethyleneglycol (PEG) phase (column SolGelWax, SGE) was used to separate the pyrolysis products. The contribution of polysaccharide-derived and lignin-derived compounds as well as N-containing compounds to compost and vermicompost may be indicative of their potential degradability in soil. We were interested in those compounds and therefore chose a polar column (Dignac et al., 2006). The temperature of the GC oven was programmed from 30°C to 280°C at 2°C/min. The final temperature was hold for 15 min. Compounds were assigned on the basis of their mass spectra, GC retention times and comparison with library mass spectra (Wiley). The peak areas were integrated on the Total Ion Current (TIC) trace using the GC ChemStation program (Agilent Technologies). The total area was calculated as the sum of the areas of the identified peaks. The proportion of each area to the total area was calculated. These area proportions do not represent the proportion of the different pyrolysis products, for which MS responses might be very different. Analytical pyrolysis is not a quantitative method; however peak integration allows comparing the proportions of the areas of specific compound families in different samples.

2.4. Van Soest fractionation

The biochemical composition of EOM was determined using the Van Soest method (Vansoest and Wine, 1967) as modified by Linères and Djakovitch (1993). The soluble (SOL), hemicelulose-like (HEM) cellulose-like (CEL) and lignin + cutin-like (LIC) fractions were separated. One-gram of each substrate was mixed with 2 g calcinated sand and extracted with 100 mL hot water for 30 min followed by extraction with neutral detergent for 60 min (extraction of the SOL fraction, NDF residue), hot acid detergent for 60 min (extraction of the HEM fraction, ADF residue) and 180 min in cold, 72% sulphuric acid (extraction of the CEL fraction, ADL residue). The residual material corresponded to the LIC fraction and may contain lignin as well as cutin, suberin and other biomacromolecules. After each extraction step, the OM content was determined in the residues by loss on ignition at 480 °C. All biochemical fractions were expressed as a percentage of total OM according to the following equations: SOL = 100 − NDF, HEM = NDF − ADF, CEL = ADF − ADL, and LIC = ADL.

2.5. CuO oxidation

This method gives information on lignin content and composition of plant material, which may directly be related to the decomposition process. Alkaline cupric oxide (CuO) oxidation of dried and ground samples was performed at high temperature. CuO products were purified (Kögel and Bochter, 1985) and quantified as trimethylsilyl derivatives by gas chromatography (GC) with a HP gas chromatograph (HP GC 6890) equipped with a flame ionisation detector (FID) and a SGE BPX-5 column (50 m length, 0.25 mm inner diameter, 0.32 µm coating). Samples were injected in split mode (10:1). The GC oven temperature was programmed at 100°C during 2 min, then from 100°C to 172°C at 8°C/min, from 172 to 184°C at 4°C/min, and from 184 to 300°C at a rate of 10°C/min.

CuO oxidation yields a suite of single-ring phenol compounds (V-vanillyl, S-syringyl and C-p-coumaryl) with their aldehyde, ketone and acid side chains. Acid-to-Aldehyde ratios (Ac/Al) were calculated for the lignin monomers V and S. The sum of all units (V+S+C) was considered to represent the total lignin content of the sample. With increasing decomposition, VSC is usually decreasing, whereas the Ad/Al ratios of V and S units are increasing (Thevenot et al., 2010).

2.6. Phosphorus fractionation

The four substrates were subjected to the Hedley P fractionation scheme (Hedley et al., 1982) and modified by Ivanoff et al. (1998). Triplicate samples (0.5 g, passed through a 2-mm sieve) were put in 50 mL polyethylene tubes and sequentially extracted with 30 ml distilled water, 0.5 M $NaHCO_3$ (pH 8.5), 1.0 M HCl and 0.1 M NaOH by shaking the samples for 16 h. Thereafter, the suspensions were centrifuged and then filtered through 0.45 µm filters. The NaOH extract was adjusted with concentrated HCl to pH 0.2 to separate the humic acid-P fraction and fulvic acid- P fraction. Finally, the residual fraction was determined by ashing the residues from the NaOH extraction at 550°C for 1 h, followed by dissolution in 1 M H_2SO_4 for 24 h.

The inorganic P (Pi) concentration in previously neutralized (using p-nitrophenol as an indicator) extracts was determined colorimetrically (Murphy and Riley 1962). Total P (Pt) concentration in the extracts was determined after a continuous digestion with potassium persulfate, according to the method of Bowman (1989), and modified by Thien and Myers (1992). The concentration of organic P ($P_{o)}$ was calculated as the difference between those of Pt and Pi.

In our experiment NaOH-Pi represents the non labile inorganic P, HCl-Pi is the moderately labile whereas $NaHCO_3$-Pi and H_2O-Pi are labile Pi and usually assumed to be available to microbial and vegetation communities in the short term (Cross and Schlesinger, 1993). Organic forms of (Po) can be separated into different fractions: labile Po that can be extracted with H_2O and $NaHCO_3$, moderately labile Po that is extracted by HCl, and NaOH-$P_{fulvic,}$ moderately resistant Po (NaOH-P_{humic}) and highly resistant Po (residue-Po) (Ivanoff et al. 1998) that is thought to be available only on a long-term basis (Cross and Schlesinger, 1993). Water and $NaHCO_3$ extracted readily soluble compounds, including phosphate, phospholipids, DNA, and simple phosphate monoesters (Turner and Leytem 2004). Phosphate in the soil solution P pool is immediately available to plant and microbial.

2.7. Statistical analyses

Before analyses, data were tested for homogeneity of variance using the Levene’s test. Data were analyzed by analysis of variance (ANOVA). When effects were significant at a level of 0.05, means were tested with LSD test. A Principal Component Analysis (PCA) was carried out to differentiate the three EOMs based on their pyrolysis signature. Briefly, we used a matrix of 9 samples and the relative contribution of the five types of pyrolysis products previously described. All statistical calculations were carried out using R (R Development Core Team, 2008). Differences among treatments were declared at the <0.05 probability level of significance.

3. Results and discussion

3.1. Composition of organic amendments

(i) Exogenous Organic Matter

Elemental and isotopic composition of buffalo dung, compost and vermicompost

Carbon and nitrogen content as well as stable isotope ratios of C and N are shown in Table 2.1. The carbon content decreased strongly during composting of buffalo dung with and without earthworms. Compost and vermicompost were characterised by similar carbon contents, while higher nitrogen contents were found for vermicompost, leading to a lower C/N ratio. The C/N ratio of 11 recorded for vermicompost was similar to that of SOM, suggesting that vermicompost is more strongly humified than compost. Nitrogen enrichment during vermicomposting was reported before (e.g. Suthar, 2007) and is in line with the observation that earthworms tend to accelerate the maturation of organic wastes (Atiyeh et al., 2000). A similar low C/N ratio for vermicompost was recorded by Sen and Chandra (2007), who concluded, that a suitable degree of stabilisation was reached. The stable isotope ratios of carbon and nitrogen both increased significantly after composting compared to the original buffalo manure (Table 2.1). Our data are in the range of values reported by Lunch et al. (2006) for dairy manure and compost. Increasing stable isotope ratios are in line with the decreasing C/N ratio suggesting microbial processing of the material. Increasing $\delta^{13}C$ during microbial processing may be ascribed to accumulation of ^{13}C enriched compounds, such as microbial-derived amino acids and the preferential decomposition of ^{13}C depleted plant-derived compounds, such as lignins. Moreover, isotopic fractionation during microbial respiration could be another mechanism leading to ^{13}C enrichment. Van Dam et al. (1997) reported ^{13}C enrichment of 3‰ due to microbial respiration. ^{15}N enrichment during composting is only small and may have been caused by NH_3 volatilization and microbial isotope discrimination during N turnover (Lunch et al., 2006).

Table 2.1: *Carbon (OC), nitrogen (N), oxygen (O), hydrogen (H), C/N mass ratio, O/C and H/C atomic ratios as well as stable C and N isotopic ratios of buffalo dung, compost, vermicompost and soil before amendment. Different letters within the same column are related to significant differences (n=4)*

	C	N	C/N	O	H	H/C	O/C	$\delta^{13}C$	$\delta^{15}N$
	%	%		%	%			‰	‰
Manure	32.9^a	1.7^a	19^a	29.3^a	4.6^a	$1,8^a$	$0,7^b$	-26.2^a	5.2^a
Compost	19.7^b	1.3^b	14^b	$25,8^b$	3.5^b	$1,8^a$	$0,8^a$	-24.6^b	7.0^b
Vermicompost	19.4^b	1.7^a	11^c	23.6^c	3.1^b	$1,8^a$	$0,8^a$	-25.4^c	6.6^c

The oxygen and hydrogen content as well as O/C and H/C atomic ratios of the three EOM are also presented in Table 2.1. The oxygen and hydrogen content decreased significantly during composting of manure with and without earthworms. The hydrogen contents of compost and vermicompost were similar but higher oxygen contents were found for compost. These results are in line with many others and confirm the degradation of OM during composting and vermicomposting and a more important degradation when composting is done in the presence of earthworms (Campitelli and Ceppi., 2008; Ngo et al., 2012; Fornes et al., 2012). However, no differences in H/C ratios were measured between the three EOM. The O/C and H/C atomic ratios are often used to visualize OM composition (vanKrevelen., 1950). In our study, H/C was approximately 0.8 indicating rather fresh OM compounds. O/C was more variable than H/C ranging between 0.7 and 0.9. O/C ratio of manure was significant lower than the other EOM. In general H/C ratios are decreasing and O/C ratios are increasing during decomposition due to loss of carbohydrates and accumulation of aromatic compounds (Baldock et al., 2002). Similar elemental composition of manure, compost and vermicompost is somewhat surprising, suggesting that the chemical structures present in the three EOM were similar.

Chemical composition

Results of elemental analysis were confirmed by solid-state ^{13}C CPMAS NMR spectra of EOM indicating similar signals, dominated by a peak in the chemical shift region between 90 and 110 ppm (Fig. 2.1, which is usually assigned to acetal and ketal groups present in carbohydrates (Kögel-Knabner, 1997). A second important peak was observed in the aliphatic region (0-45 ppm) of all three spectra. Signals in this region may be assigned to lipids, cutin, suberin and other aliphatic biomacromolecules (Golchin et al., 1997). Small peaks can be observed in the 110-160 ppm region, which are due to C-C and phenol bonds. An additional peak may be observed in the chemical shift region between 160 and 190 ppm, which is most likely assigned to carboxylic groups (Mathers et al., 2003).

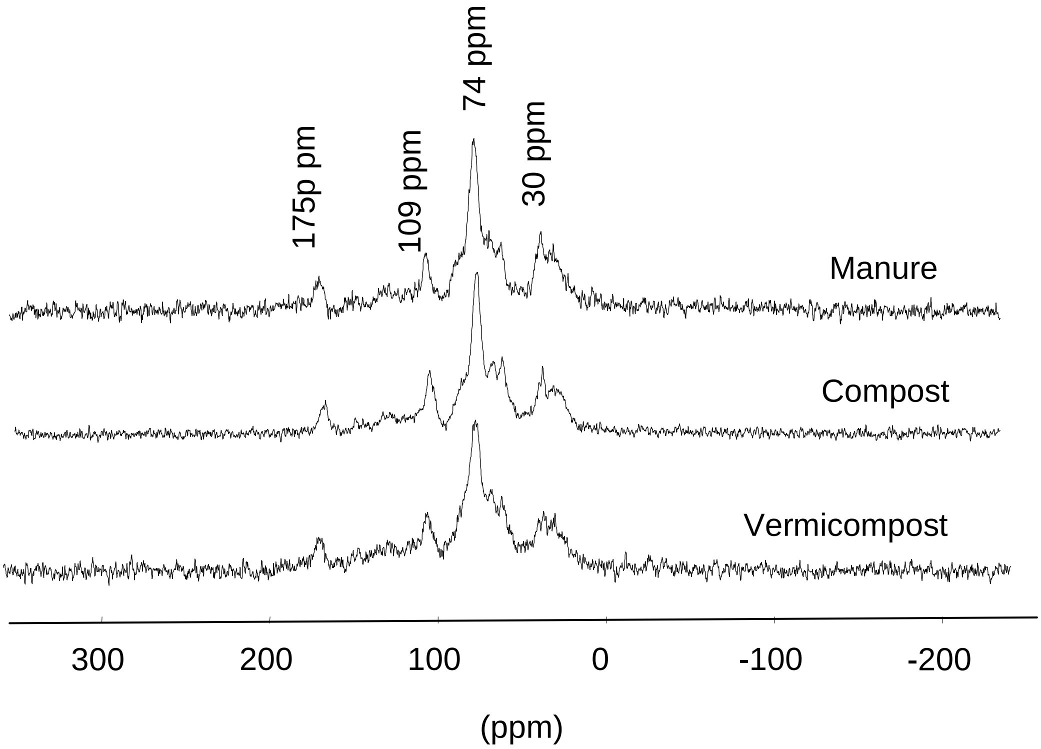

Figure 2.1: *Representative NMR spectra of buffalo manure, compost, vermicompost*

More detailed information on the molecular composition of buffalo manure, compost and vermicompost was obtained by analytical pyrolysis. This technique gives an overview of the bulk molecular composition of organic samples. Pyrograms of buffalo dung, compost and vermicompost showed contributions of polysaccharide-derived, lignin-derived, N-containing, aliphatic and unspecific pyrolysis products. The identified peaks represent for the five compound classes (Table 2.2).

Table 2.2: *Relative contribution of the areas of the pyrolysis products derived from polysaccharides (PS), lignin (LIG), aliphatic (A), N-containing (N) and unknown (U) compounds in buffalo manure, compost and vermicompost. Different letters within the same column are related to significant differences (n=5).*

	PS	**U**	**N**	**LIG**	**A**
			% of total area		
Manure	32.6 ± 2.2^{a}	29.3 ± 4.9^{a}	15.7 ± 1.1^{a}	17.1 ± 5.0^{a}	5.2 ± 0.7^{a}
Compost	22.9 ± 1.5^{b}	32.3 ± 2.1^{a}	24.7 ± 5.4^{ab}	14.0 ± 4.5^{a}	6.0 ± 0.2^{a}
Vermicompost	18.1 ± 5.4^{b}	32.5 ± 5.5^{a}	31.2 ± 3.6^{b}	11.7 ± 1.0^{a}	6.4 ± 1.8^{a}

Polysaccharide-derived and lignin-derived pyrolysis products were relatively more abundant in buffalo dung compared to compost and vermicompost, which showed higher contribution of N-containing and unspecific compounds (Table 2.2). The contribution of polysaccharide- and lignin-derived compounds to total area of identified pyrolysis products was lower and those of N-containing compounds higher for vermicompost compared to compost. These data are in line with the elemental and isotopic data (see above). The proportion of aliphatic pyrolysis products was similar in both organic amendments. Principal component analysis (PCA) explained in total 82.2 % of the data variation. In the PCA plan buffalo manure, compost and vermicompost are clearly distinguished by their chemical composition as seen by analytical pyrolysis (Fig 2.2).

The first factor, which explains 55 % of the total variability, seems to be related to the relative contribution of area of polysaccharide-derived pyrolysis products to the total ion chromatogram. Vermicompost and to a lesser extent compost are correlated in negative direction with the polysaccharide contribution to the TIC, indicating disappearance of these compounds compared to buffalo manure and increase of the relative importance of N-containing, aliphatic and unspecific pyrolysis products. This is in agreement with observations by ^{13}C CPMAS NMR spectroscopy during composting of sewage sludge and green wastes, which showed a degradation of polysaccharides (Albrecht et al., 2008; Spaccini and Piccolo, 2007). However, unlike our study, these authors noted an increase of the relative proportions of lignin and aliphatic compounds during composting. These differences may be explained by the higher content of lignocellulosic biomass in greencomposts. In the rumen of buffalos, these structures are processed by bacteria to a certain extent (Kuhad et al., 1997). Due to the anaerobic conditions in the rumen, lignin mineralisation may be incomplete (Colberg, 1988). Thus buffalo manure is probably prone to microbial transformation, which leads to the accumulation of microbial-derived N containing compounds.

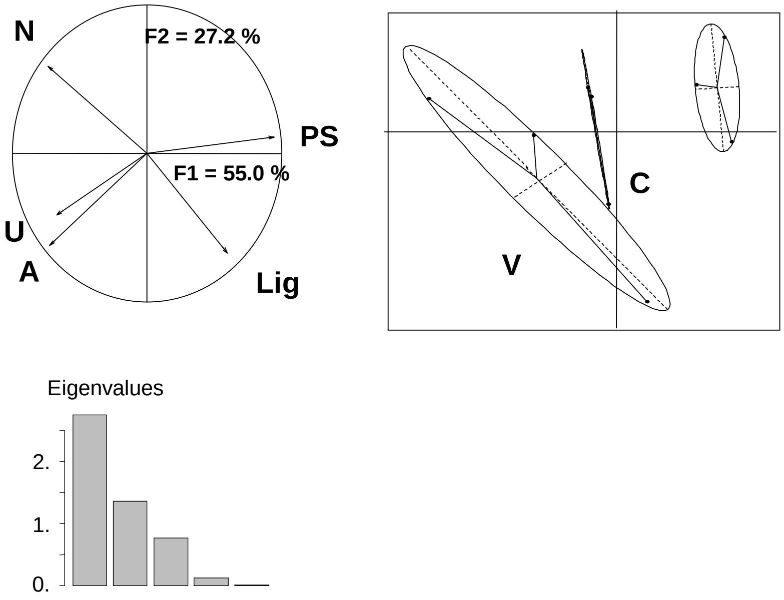

Fig. 2.2: *PCA analysis of the relative areas of the pyrolysis products grouped according to their probable origin (polysaccharide-derived PS, lignin-derived Lig, N-containing N, aliphatic A and unknown U compounds) released from buffalo manure (M), compost (C) and vermicompost (V)*

Biochemical composition

Table 2.3 presents the biochemical composition of the four organic substrates obtained after Van Soest fractionation. Between 50 to 60% of the EOM biomass were found in the soluble fraction (SOL fraction). This fraction increased during composting and was higher in vermicompost than compost. An increase of soluble compounds with regards to the precursor material is usually recorded for composted material (Morvan and Nicolardot., 2009; Francou et al. 2008; Annabi et al. 2007). This might be related to degradation of structural compounds and formation of soluble N-containing compounds, during composting, through microbial activity (Peltre et al., 2010).

Table 2.3: *Biochemical fractions of the buffalo manure (M), compost (C), vermicompost (V): soluble (SOL), cellulose (CEL), hemicellulose (HEM), lingnin and cutin (LIC), LIC/CEL ratio. Different letters within the same column are related to significant differences (n = 3).*

Substrates	Hot water soluble C (%TOC)	Fraction (% MS)				
		SOL	HEM	CEL	LIC	CEL/LIC
M	$17.47^{a}\pm0.3$	$51.3^{c}\pm0.2$	$14.3^{b}\pm0.4$	$16.0^{a}\pm1.2$	$15.4^{b}\pm0.1$	$1.0^{a}\pm0.03$
C	$13.5^{b}\pm0.7$	$57.7^{b}\pm0.0$	$16.2^{a}\pm0.5$	$13.0^{b}\pm0.5$	$13.1^{c}\pm0.1$	$1.0^{a}\pm0.02$
V	$13.4^{b}\pm0.3$	$61.4^{a}\pm0.1$	$13.2^{c}\pm0.2$	$10.4^{c}\pm1.5$	$15.0^{b}\pm0.1$	$0.69^{b}\pm0.04$

The cellulose fraction decreased from 16% of OM in buffalo manure to 13% in compost and 10% in vermicompost. This is in line with the elemental analyses and several studies (e.g., Komilis and Ham, 2003; Paradelo et al., 2012), showing that cellulose is the most readily biodegradable fraction during composting, after water soluble fraction. The CEL/LIC ratio reached 0.69 for vermicompost and was lower than for manure and compost ($p > 0.05$). This suggests that vermicompost is more biologically stable than the two others EOM types because of the high contribution of the residual Van Soest fraction, which is most probably composed of lignin as well as other non soluble aromatic and aliphatic molecules. The CEL/LIC ratio being an indicator of the evolution of organic waste quality during composting, is relatively high compared to other composts (Komilis and Ham, 2003; Paradelo et al., 2012). This may be related to the fact, that the cellulose content of manure has already been reduced by about 70% compared to plant litter because of its digestion in the rumen in contrast to the lignin component, which is usually preserved (Russell et al., 2009). However, the residual Van Soest fraction is not exclusively related to lignin and does not allow for the characterization of the lignin molecule. The changes of the lignin content and composition occurring during composting and vermicomposting were analysed after CuO oxidation.

CuO oxidation

As little is known about the degradation of lignin in compost (Tuomela et al., 2000) and because pyrolysis is not quantitative, we analysed in more detail the degradation of lignin during composting with and without earthworms. For this purpose, we used CuO oxidation, which releases a suite of phenolic monomers of vanillyl, syringyl and p-coumaryl type with acid and aldehyde side chains.

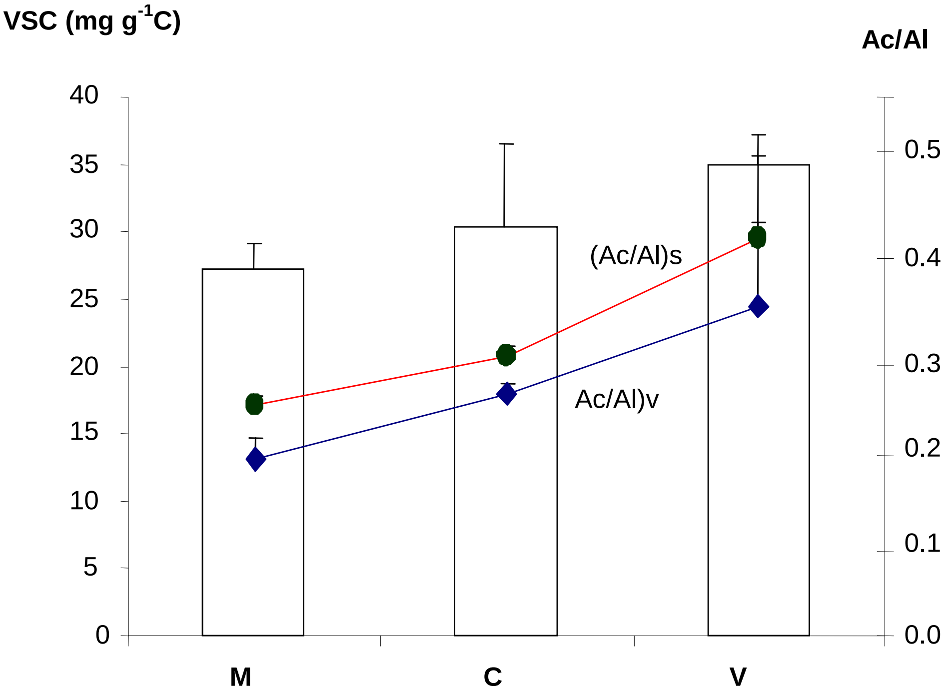

Fig. 2.3: *Lignin signature obtained by CuO oxidation of buffalo manure (M), compost (C) and vermicompost (V). The total concentrations of lignin (VSC, mg/gC) are represented by white bars on the left axis, the acid-to-aldehyde ratios (Ac/Al) indicative of the state of degradation of lignin are represented by the dots (left axis); Data are presented as means and standard deviation (n=5).*

The sum of CuO oxydation products (VSC) allows for the estimation of the lignin content. Its degree of degradation was assessed by the acid to aldehyde ratios of vanillyl and syringyl units, which tend to increase upon degradation (Hedges et al., 1988). Lignin content (VSC) of buffalo manure, compost and vermicompost ranged between 27 and 36 g kg^{-1} C (Fig. 2.3). Lignin content was highest in vermicompost followed by compost and buffalo dung. These differences were however not significant. The acid to aldehyde ratios of vanillyl and syringyl ranged between 0.16 and 0.4. It increased in the order buffalo manure < compost < vermicompost. Our results suggest that lignin is oxidised and transformed during the process. The stronger transformation during vermicomposting may be related to activation of fungal growth by earthworm grazing (Aira et al., 2006).

(ii) Biochar

As shown several times before (Schimmelpfennig et al., 2012 and references therein), biochar is characterized by a higher C/N ratio and far lower H/C and O/C ratios (0.4 and 0.1 respectively) than the others organic substrates (Table 2.4), due to hydrogen and oxygen loss and carbon enrichment during combustion. These low ratios indicate a high carbonization degree and hence probably a high stability in soils (Baldock et al., 2012).

Table 2.4: *Carbon (OC), nitrogen (N), oxygen (O), hydrogen (H), C/N mass ratio, O/C and H/C atomic ratios of the bamboo biochar.*

	N (%)	C (%)	C/N	O (%)	H (%)	H/C	O/C
Biochar	0.87±*0.02*	74.16±*1.92*	84,6±*2.0*	9.48±*0.45*	2.42±*0.32*	0.39±*0.03*	0.10±*0.02*

In accordance with the elemental results ^{13}C CPMAS NMR spectra of biochar shows only one broad signal centered at about 128 ppm, which may be assigned to aromatic C (Mathers et al., 2003). Peaks at 30, 75 and 240 ppm are derived from spinning sidebands.

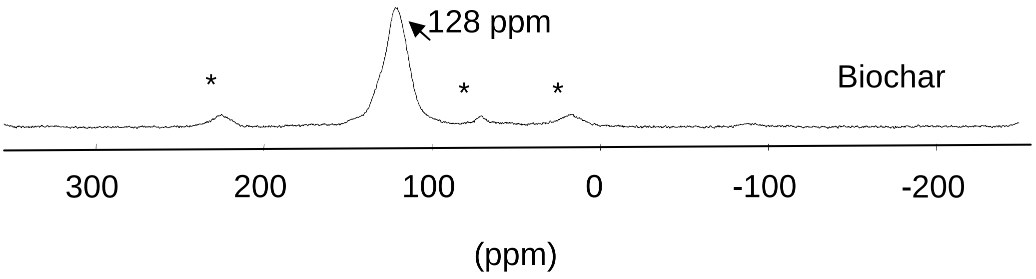

Figure 2.4: *Representative NMR spectra of Bamboo biochar (B). Spinning sidebands are indicated by **

In agreement with elemental and structural analyses, the biochemical fractionation showed that biochar was much more resistant to the reagents (Table 2.3) than the three EOM. However, despite its recalcitrant nature biochar yielded 30% of extractable material (SOL + CEL + HEM fractions). The SOL fraction that is usually considered as a labile carbon fraction represented 17.8% of OM. The HEM and CEL fractions of biochar were small with a contribution of less than 15%. The HEM fraction was near to zero and the CEL was a minor faction (12.2%) with high variability. Generally, heat-induced decomposition of hemicellulose and cellulose in biomass occurs rapidly at low temperatures, between 230 and 400°C (Alexis et al., 2010). The LIC fraction of biochar showed the highest contribution (70% of OM) leading to a very low CEL/LIC ratio (0.17). Once again, this result confirms the highly recalcitrant nature of this material.

Table 2.5: *Biochemical fractions of the bamboo biochar: soluble (SOL), cellulose (CEL), hemicellulose (HEM), lingnin and cutin (LIC), LIC/CEL ratio.*

Substrats	Hot water soluble C (%TOC)	Fraction (% MS)				
		SOL	HEM	CEL	LIC	CEL/LIC
Biochar	1.8±0.4	17.8 ±0.2	0.5±0.5	12.0±6.0	69.7±0.2	0.17±0.08

3.2. Phosphorus forms in organic amendments

The distribution of different phosphorus fractions obtained from the sequential extraction of P is presented in Table 2.6. Phosphorus exists in organic (Po) and inorganic (Pi) forms. Pi represented the majority of the extractable P from all 4 substrates. Highest contribution of Pi was observed for biochar with 93% of Pt and lowest Pi contribution was recorded for manure (59% of Pt). Composting and vermicomposting increased the contribution of Pi by 20%. The $NaHCO_3$-Pi was the most important Pi fraction in manure, compost and vermicompost with approximately 2.7 mg g^{-1}. Contents of H_2O-extractable P_i of EOM were around 0.5 mg g^{1}. The H_2O-Pi of vermicompost was significantly lower than for manure and compost. This difference may be explained by incorporation of soluble P into the solid OM matrix (Busato et al., 2012).

The H_2O-Po was very low in all three EOM, ranging from 0.02 to 0.06 mg g^{-1}, and the $NaHCO_3$–Po reached 0.42 mg g^{-1}. Despite the different C content of the three EOM (Table 2.6), no major differences were observed in these Po fractions that are usually considered mobile and easily mineralizable in soil (Roboredo et al., 2012). These fractions can have environmental relevance even at relatively small concentrations (Turner and Leytem 2004). Labile Po content may depend on manure type, as we observed lower concentrations in our study with buffalo manure compared to pig manure (Ajiboye et al., 2004). The total labile P fraction (Pi+Po) of EOM, represented by water soluble and $NaHCO_3$ extractable P, accounted for more than 50% of total P. This is in agreement with previous studies on animal manure (McDowell and Stewart., 2005), compost (Gagnon et al., 2012) and vermicompost (Solis-Mejia et al., 2012), confirming the high value of EOM as P fertiliser.

Composting and vermicomposting changed the contribution of moderately labile P fractions extractable with HCl. Most HCl-P of manure was extractable in organic form, whereas composting and vermicomposting led to a strong decrease of HCl-Po (from 1.4‰ for manure to 0.6 and 0.4 ‰ for compost and vermicompost, respectively) and an increase of HCl-Pi (from 0.03‰ for manure to 1.7‰ for compost and vermicompost). These results indicate that organic P was mineralized by microbial activity and thus transformed into plant available form by composting and vermicomposting.

Table 2.6: *Phosphorus fractions of the buffalo manure (M), compost (C), vermicompost (V) and bamboo biochar (B): H_2O (H_2O extractable P), NaHCO3 (NaHCO3 extractable P), HCl-P (HCl-extractable P), NaOH (NaOH extractable P), Residual-P. Different letters within the same column are related to significant differences (n = 3).*

	H2O		NaHCO3		HCl		NaOH			Residual
Sample	Pi (mg.g^{-1})	Po (mg.g^{-1})	Pi (mg.g^{-1})	Po (mg.g^{-1})	Pi (mg.g^{-1})	Po (mg.g^{-1})	Pi (mg.g^{-1})	Pfulvic (mg.g^{-1})	Phumic (mg.g^{-1})	Po (mg.g^{-1})
M	0,54^{a}±0.02	0,06^{a}±0.02	2,54^{a}±0.08	0,42^{a}±0.03	0,30^{c}±0.01	1,44^{a}±0.07	0.03^{b}±0.01	0,70^{a}±0.01	0,03^{b}±0.00	0,42^{a}±0.03
C	0,58^{a}±0.01	0,02^{a}±0.01	2,73^{a}±0.10	0,42^{a}±0.02	1,77^{a}±0.02	0,61^{b}±0.03	0.02^{b}±0.01	0,68^{a}±0.02	0,16^{a}±0.01	0,33^{b}±0.02
V	0,46^{b}±0.02	0,03^{a}±0.02	2,81^{a}±0.5	0,43^{a}±0.03	1,58^{a}±0.02	0,36^{c}±0.01	0.02^{b}±0.01	0,68^{a}±0.01	0,14^{a}±0.02	0,32^{b}±0.04
B	0,28^{c}±0.03	0,02^{a}±0.01	0,36^{b}±0.03	0,03^{b}±0.01	0,68^{b}±0.01	0,12^{d}±0.03	0.40^{a}±0.04	0,00^{b}±0.00	0,00^{c}±0.00	0,20^{c}±0.02

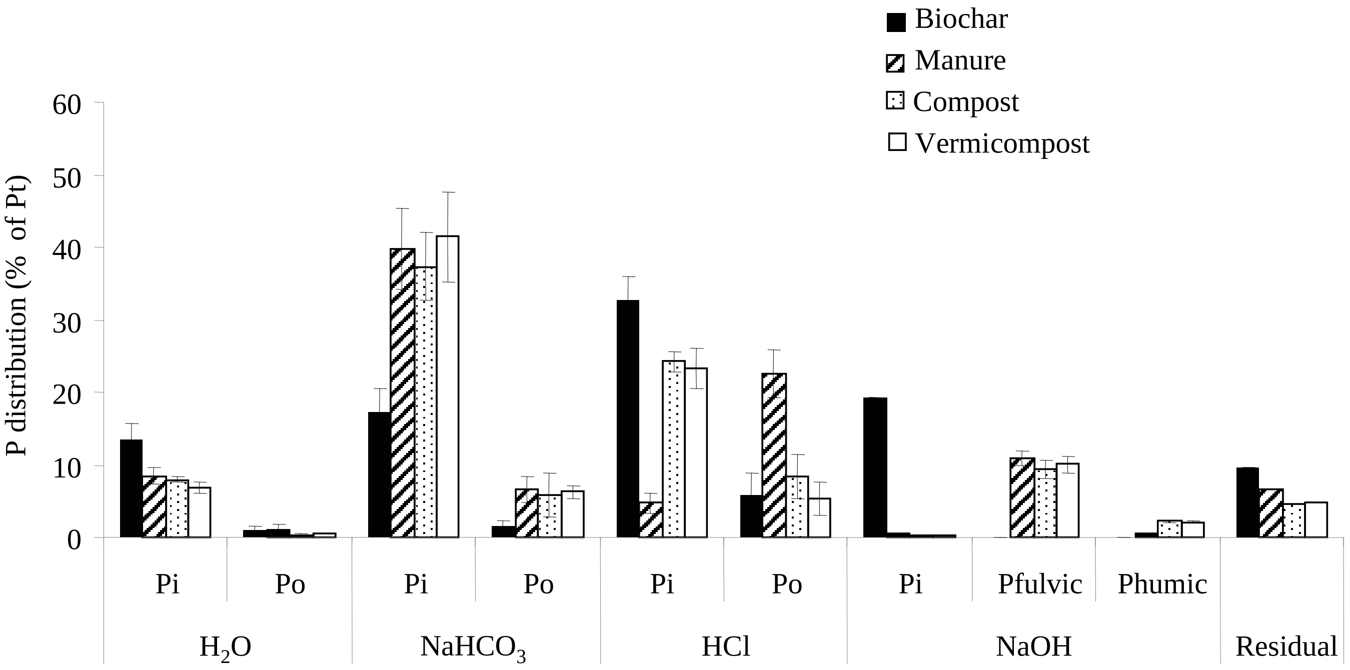

Figure 2.5: Distribution of phosphorus in the diffirence fractions (% Pt) of of bamboo biochar (B), buffalo manure (BM), compost (C), vermicompost (V)

Po extracted by stronger solutions (e.g., NaOH) consists mainly of phytic acids (Turner and Leytem, 2004). The NaOH–Po corresponds to the sum of the fulvic acid–Po and humic acid–Po. It has been reported as an important phosphorus source for plants after mineralization (Huo et al., 2011). Although no difference was observed in the NaOH-P_{fulvic} fraction between EOMs, our study shows that NaOH-P_{humic} of compost and vermicompost (0.16 and 0.14 ‰ respectively) were significantly higher than that of the manure (0.03‰). Similar results were recorded by Zvomuya et al. (2006) for composted and fresh cattle manure. Our results suggest that application compost and vermicompost from buffalo manure could lead to the soils enrichment with Po, considered as moderately available.

Non-labile Po corresponds to the residual P fraction. This fraction is of minor importance and presents around 5-7 % of total EOM P (Fig. 2.5). Interestingly, residual-P was higher in manure (0.42‰) than in compost and vermicompost (0.32‰), suggesting that composting led to mobilization of this non-labile P form.

The P content of biochar was much lower than in the three EOM, and mainly inorganic (83% of the extraction, Fig. 2.5). These results suggest that (i) the bamboo residues were poor in organic P or/and (ii) organic P compounds have been transformed to the inorganic form by the pyrolysis process. The labile P fraction represented 33% of the total P. P was mainly in the form of moderately resistant inorganic P as HCl-Pi and NaOH-Pi with 0.68 and 0.4 mg/g, respectively. These two forms are usually associated with Fe and Al or Ca, that present in the biochar (Wang et al., 2012).

4. Conclusion

The elemental composition and chemical structure of manure, compost, and vermicompost were similar. Only molecular methods and VanSoest fraction based on solubility of organic matter in different detergents were able to identify their different natures. Composting and vermicomposting increased the contribution of soluble biochemical fractions and led to decrease in CEL/LIC ratios. Vermicompost was depleted in polysaccharides and enriched in N-containing compounds compared to compost. CuO oxidation showed that vermicompost is enriched in transformed lignin. Therefore, composting of buffalo manure in the presence of earthworms led to a more microbially processed end product compared to regular composting which could influence its function after addition to soil. Biochar produced from bamboo by pyrolysis is characterised by highly condensed aromatic structure confirming several previous researches. However, up to 30 % of this material may be more easily degradable, as it was found in the neutral detergent soluble fraction. All organic subtracts may have fertilizer value as 90% envrion of total phosphorus was extractable by chemical reagent.

Chapitre 3 : Stabilité chimique et biologique du lisier de buffle, du compost, du lombricompost et du biochar

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Ngo, P-T[1, 2], Rumpel, C[1]., Alexis, M.A[1]., Ngo, Q.A[3]., Vargas, G.V [4], Mora Gil, M.L[4]., Dang, D.K[2]., Pascal Jouquet[5]., 2013. Biological and chemical reactivity and phosphorus forms of buffalo manure compost, vermicompost and their mixture with biochar. *Bioresource Technology 148, 401-407*

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1. Introduction

Exogenous organic matter (EOM) amendments may be used for the rehabilitation of degraded soils, often characterized by low carbon contents (Pascual et al., 1998). The quality of EOM applied to soil as an amendment is very important, because it affects physical, chemical and biological soil properties (Baldi et al., 2010). For example, easily mineralizable OM allows for a high bioavailability of mineral nutrients but does not allow for a sustainable rehabilitation of soil physical properties (structural stability and resistance to erosion, water infiltration...) or long-term increase of soil carbon storage. Therefore, the development of an EOM amendment allowing for long-term sequestration of organic matter is of great interest. This is even more the case for tropical countries, where organic material is subjected to intense microbial degradation processes (Zech et al., 1987). Numerous studies have shown that composts produced from animal manure can successfully increase both the quality and quantity of soil organic matter in temperate environment (Gabrielle at al., 2005; Lashermes et al., 2009). In the tropical condition, Rivero et al (2004) found that the addition of compost to soil can increase the quality of humic acid with more functional groups and aromacity (Rayak et al., 2007). However, quantitative information on carbon storage under tropical conditions induced by EOM, in particular related to their stability is scarce.

Under tropical conditions, intense mineralization and humification processes may affect especially fresh EOM, such as animal manure. Composting may result in stabilized material. Recently it has been shown that composting in the presence of earthworms, the so-called vermicomposting technology, under tropical conditions further reduced biodegradability of the final product (Sierra et al., 2013). Another possibility to reduce the microbial degradation of EOM could be its joint application with biochar, which was shown to increase organic matter stabilization through negative priming effects (Zimmermann et al., 2011). Biochar is the product of the thermal degradation of organic materials in absence of air (pyrolysis). It is distinguished from charcoal by its use as a soil amendment (Lehmann and Joseph, 2009). Biochar has been widely studied as a soil conditioner (Lehmann and Sohi., 2008), and may be especially useful to increase soil C storage. However, the effect of biochar on the chemical and biological stability of EOM has not been quantified up to now.

In the present study, we used buffalo manure, its compost and vermicompost, bamboo biochar and their mixtures and subjected them to chemical attack and microbiological degradation in order to assess their stability. Chemical stability was evaluated after oxidation with acid dichromate solution. Additionally, we used laboratory incubation as well as litterbags incubation under the natural rainfall condition to access their biological stability. The aim of our study was to compare (1) the chemical and biological stability of the three EOMs and (2) to evaluate the effect of biochar on these properties. We hypothesized that the presence of biochar would increase the stability of the three EOMs in a tropical degraded soil.

2. Materials and methods

2.1. Organic amendments

We used the buffalo manure, its compost and vermicompost and biochar produced from bamboo for analyses. Their origin and fabrication processes are reported in the chapter 1.

2.2. Dichromate oxidation

Chemical recalcitrance of substrates and their mixtures was estimated using a $K_2Cr_2O_7$ acid oxidations following the method of Bird and Gröcke (1997). Acid dichromate ($K_2Cr_2O_7$) is a strong oxidant, which changes its colour during the reaction. The handling of acid dichromate requires special care as the reagent is toxic, mutagen, carcinogen, and can cause serious problems when entering the drinking water supply. 1g of dry sample was mixed with 20 ml of 0.1M $K_2Cr_2O_7$ in 2M H_2SO_4 in a capped centrifuge tube and placed in a water bath at 80°C. The reagent was changed when the color had changed from orange to green. After 0.5h, 1.5h, 3h, 7h, 12h, and 35 h, the residues were separated from solution by centrifugation. Thereafter, the residues were cleaned 4 times with 40ml distilled water, free-dried and weighed. Their C content was recorded using an elemental analyzer and the amount of C lost calculated by mass balance.

The biochar effect was calculated by comparing the Coxidised of EOM and biochar alone and their mixtures using the formula:

$$\delta = \frac{\mathrm{Cmix} - (\mathrm{Cb} + \mathrm{Co})/2}{(\mathrm{Cb} + \mathrm{Co})/2} \times 100\%$$

where: Cmix is Carbon oxidized from mixture, Cb is carbon oxidized from biochar, Co is carbon oxidized from EOM (manure, compost or vermicompost)

If δ is negative, the biochar has reduced the chemical oxidation of the EOM, while biochar enhanced chemical oxidation of EOM if δ is positive.

2.3. Incubation experiment in laboratory

Incubations of EOM-alone and EOM-biochar mixtures (1:1) were carried out in triplicate for each treatment in sterilized 50 ml borosilicate vials with rubber septa, in a temperature controlled room (28°C) for 7 months. The water was adjusted corresponding to 50% of the field capacity. At the beginning, the flask's atmosphere was free of CO_2. The decomposition of organic amendments was measured by monitoring the release of CO_2 using respiration flasks with a MICROGC (Agilent, Santa Clara, USA). After each CO_2 measurement, all flasks were flushed with reconstituted, moistened and CO_2 free-air.

The biochar effect has been calculated by comparing the mineralization of EOM and biochar alone and their mixtures using the formula below:

$$\delta = \frac{\text{Cmix} - (\text{Cb} + \text{Co})/2}{(\text{Cb} + \text{Co})/2} \text{ x } 100\%$$

Where: Cmix is Carbon mineralized from the mixture, Cb is carbon mineralized from biochar, Co is carbon mineralized from EOM (manure, compost or vermicompost)

If δ is negative, the biochar has reduced the mineralization of the EOM, while its mineralization was stimulated by biochar if δ is positive.

2.4. Litterbags experiment

100 g of the different substrates (buffalo manure, compost, vermicompost, biochar) and their mixture (EOM:Biochar 1:1) was placed into individual nylon litterbags (20 x 20 cm with 1mm mesh). 4 replicates were prepared for each treatment. The litterbags were exposed on the surface of soil under the natural condition during one year in Hanoi. Four bags of each treatment were collected after 0, 4, 16, 48 weeks of incubation. Upon harvest, samples were removed from the bags, air dried during 72h, weighed and stored for chemical analysis.

2.5. Elemental analysis

Organic carbons (OC), nitrogen (N) content of samples from littterbags experiment were measured using a CHN auto-analyser (CHN NA 1500, Carlo Erba). Four replicates were analysed.

2.6. Statistical analysis

The results of the incubations and dichromate oxidations were analyzed by repeated ANOVA with treatment (manure, compost, vermicompost, biochar and their interactions) as independent variables and time as the within subject factors. Differences among treatments were afterwards analyzed through Tukey’s multiple comparison test. Statistical analyses were performed using the R software (R Development Core Team, 2008). Differences among treatments were declared at the <0.05 probability level of significance.

3. Results and discussion

3.1. Chemical stability

Figure 3.1 shows the amount of carbon lost after chemical oxidation with acid potassium dichromate, which is a strong reagent, inducing quick loss of pure organic materials. This chemical reaction is usually used to measure the degree of biochar carbonization, and may be used to estimate the labile fraction of C in biochar (Calvelo et al., 2011; Naisse et al., 2013). In this study, we used this method to determine if it reflects the biological degradation of EOM and their mixtures with biochar (biochar:EOM ratio 1:1). The kinetic curves of oxidized C during the oxidation of compost, vermicompost and manure were similar. After 30 min, around 75% of manure, compost and vermicompost TOC and 1.6 % biochar TOC were oxidized. After 3.5 h of oxidation, C loss reached around 90% for the three EOM and 13.4% for the biochar. This value did not much increase over time and no significant difference was observed between the three EOM, whereas lowest carbon oxidizidation was noted for biochar.

The interaction between biochar and the three EOM led to significant reduction of the oxidation of the substrates (~40%, Table 3.1). Oxidation of EOM in mixture with biochar was initially high and after 35 h reached a threshold of around 32% of TOC mineralized, without significant difference between them. Biochar particles most probably reduced the energy of free electrons thus reducing their oxidation efficiency leading to preservation of EOM, when oxidized in mixture. Moreover, biochar could have absorbed EOM into its matrix rendering them unaccessible to the chemical reagent.

Table 4: *Effect of biochar (B) on the organic carbon mineralization and oxidation (% of initial) of buffalo manure (M), compost (C) and vermicompost (V)*

Treatments	7 months of incubation	35h of oxidation
M+B	11,82% ± 0.44	-33.89% ± 3.43
C+B	-7,69% ± 0.46	-42.68% ± 4.39
V+B	12,08% ± 0.83	-40.48% ± 5.42

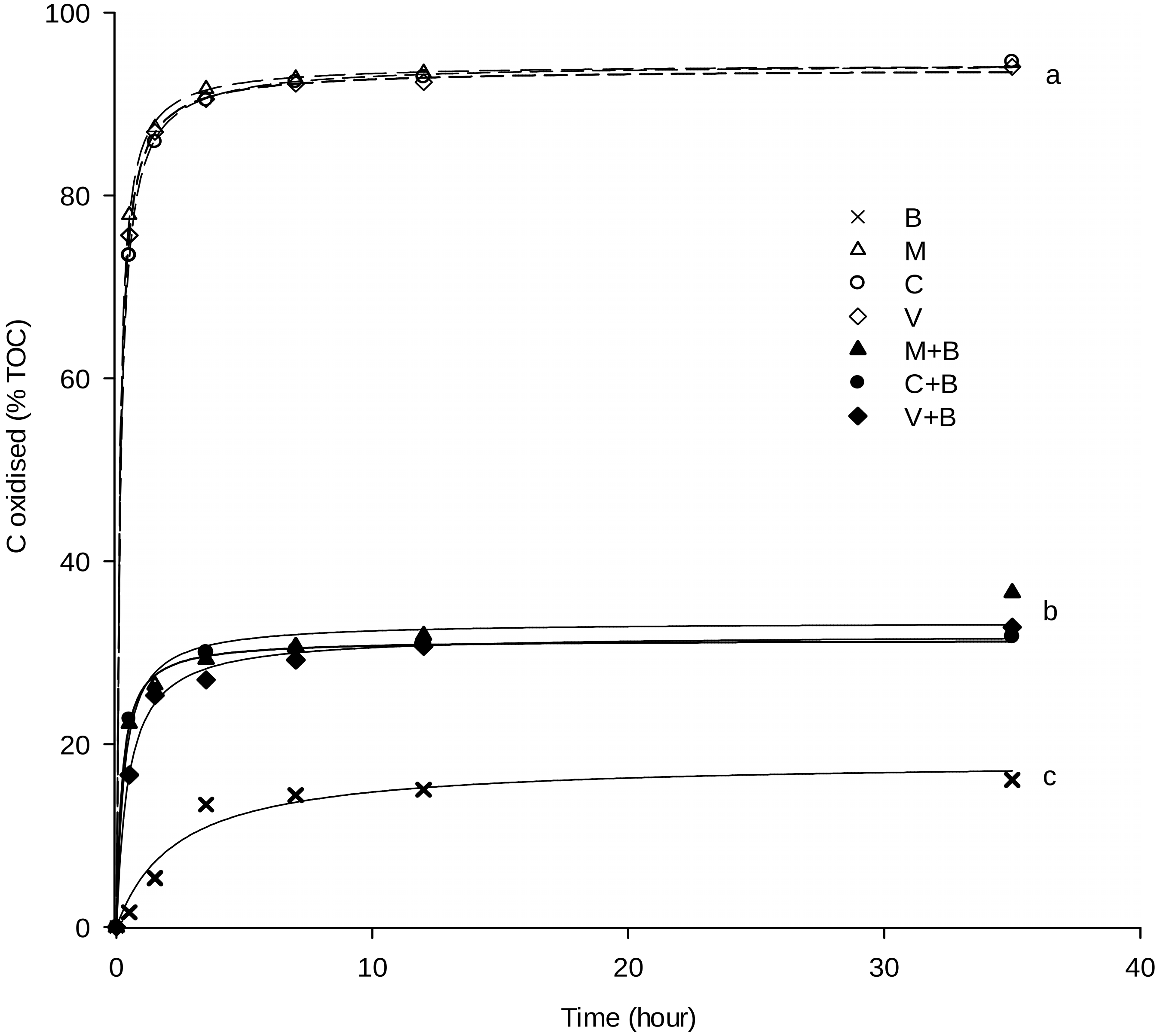

Fig 3.1: *Carbon oxidized (%TOC) during 35h of the dichromate oxidation of bamboo biochar (B), buffalo manure (M), compost (C), vermicompost (V) and their mixtures with biochar. Values with the same letters are not different at P = 0.05 (n=3)*

Although handling of acid dichromate requires some care due to its toxic nature, it should be noted that the oxidation method is an easy, quick and affordable method able to determine the labile carbon fractions. After 35h of the oxidation, more biochar was oxidized chemically than biologically after 7 months. However, conversely to the biological incubation, the oxidation did not show any differences among three EOM and their mixture with biochar. Thus, this method is not able to reflect the biological degradation at short timescales. We hypothesize that the amount of carbon remaining after chemical oxidation may roughly be related to the long-term survival potential of organic substrates. According to our results this would indicate that all three EOM are not stable in the long-term and therefore if applied alone would not be suited to increase the carbon sequestration potential of soils. However, our study also indicates that the utilization of biochar may increase their lifetime in soil.

3.2. Biological stability

(i) Laboratory incubation

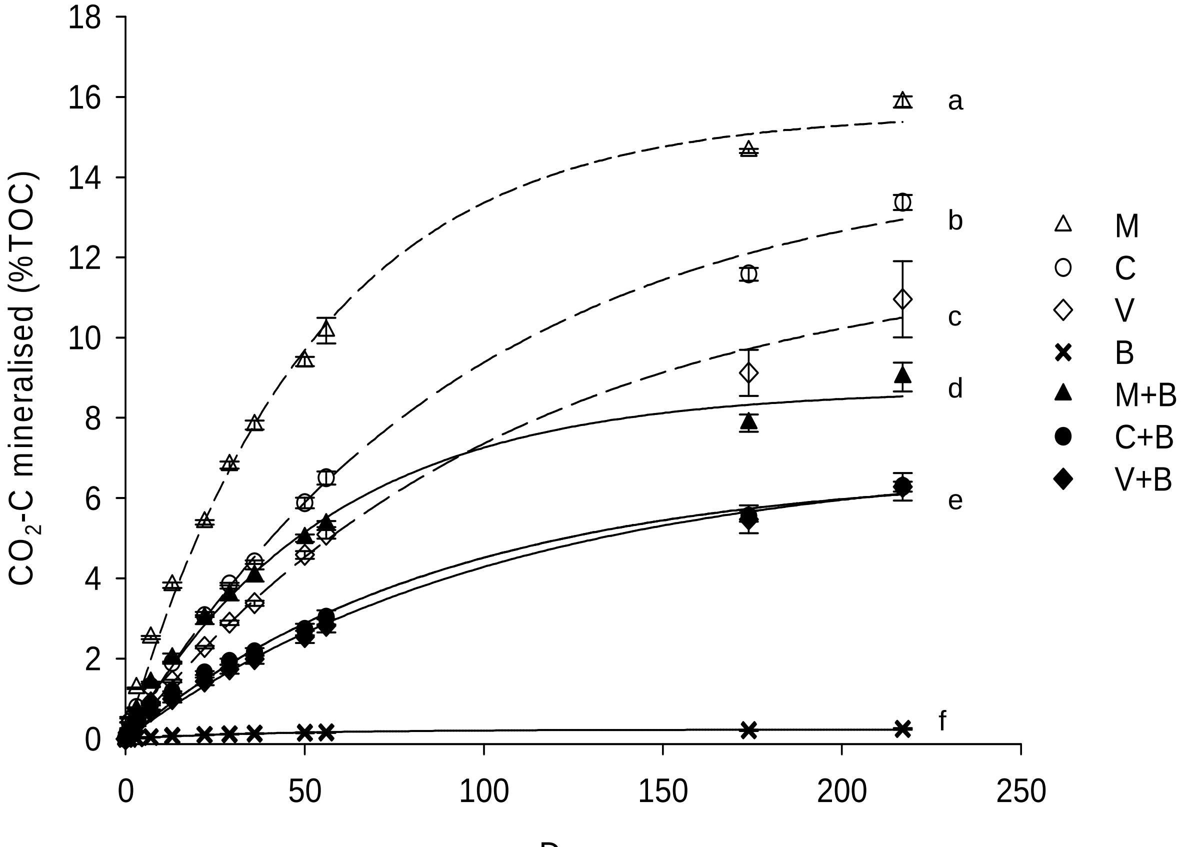

Figure 3.2 : Organic carbon mineralised during the soil incubation of bamboo biochar (B), buffalo manure (BM), compost (C), vermicompost (V) and their mixtures with biochar. Values with the same letters are not different at P = 0.05 (n=3)

Figure 3.2 shows the percentage of carbon mineralized during the 7 months of incubation. Two-way ANOVA showed a significant influence of the EOM's origin ($F_{2.39}$ = 17.6, p<0.001) and significant interaction between EOM and biochar ($F_{2.39}$ = 4.3, p = 0.01). At the end of the incubation experiment, the strongest mineralization was observed for manure and the lowest was recorded for biochar. Total quantity of C mineralized ranged between 0.25 to 15.87 % of TOC. Most C was mineralized from manure (15.9% of TOC) followed by compost (13.4% of TOC) and vermicompost (10.9% of TOC). Only 0.25% of TOC of biochar had been mineralized at the end of the incubation period. The much higher rate of decomposition for manures and composts compared to biochars may be easily explained by their chemical nature (see above). Lower mineralization of vermicompost and compost as compared to manure was observed by others (Morvan and Nicolardot., 2009; Hartz et al., 2000) and may be explained by their higher content of hot water extractable C (%TOC) (Fig. 3.2), which represents potentially bioavailable organic carbon (Lutzow et al., 2007; Said-Pullicino et al., 2007; Peltre et al., 2010). It is usually considered most accessible to microorganisms. The first mineralization phase of organic substrates is generally controlled by their content of soluble material (Sanaullah et al., 2010).

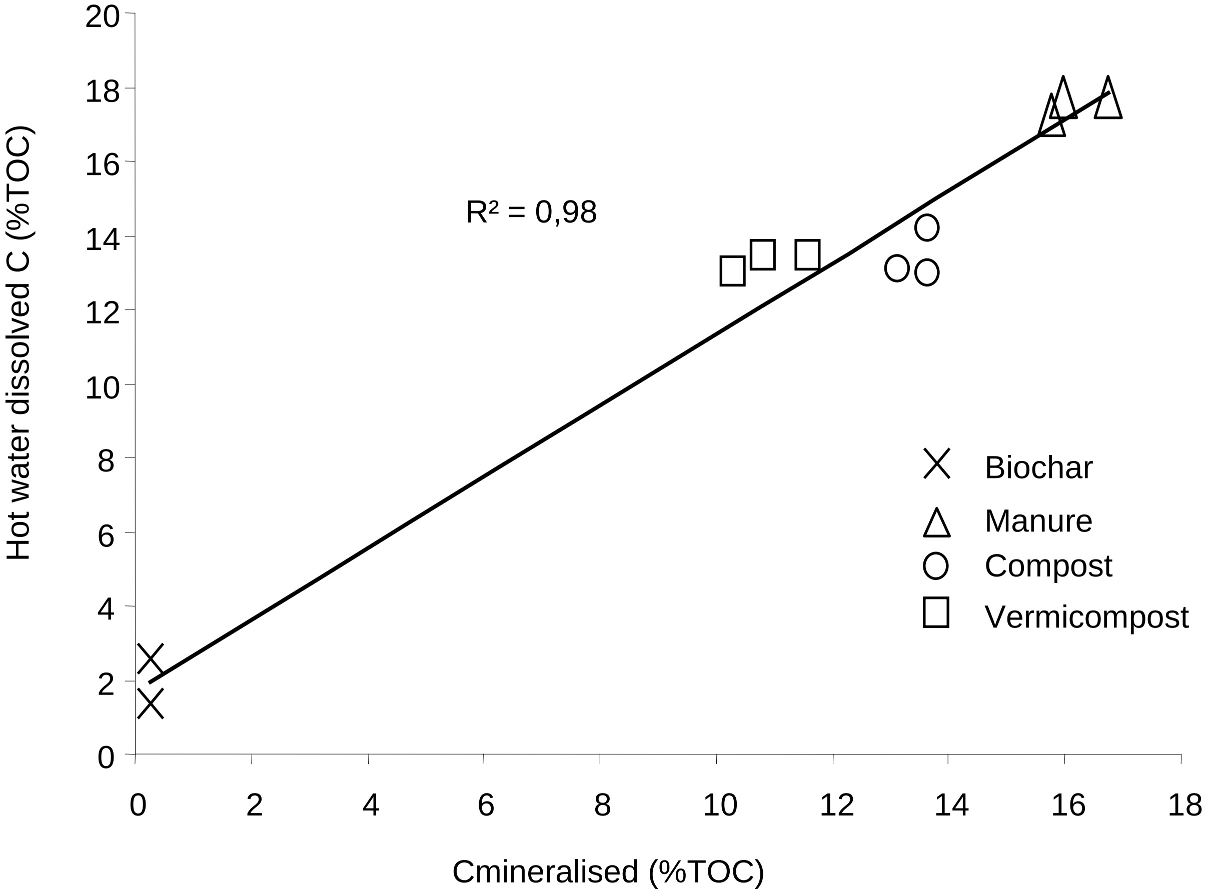

Figure 3.3: *Relationship between hot water dissolved organic carbon (%TOC) and mineralized C (%TOC) during incubation of four substrates*

The C mineralization of the mixtures (biochar:EOM ratio 1:1) ranged from 6.28% for compost and vermicompost, to 9.01% for manure. Although the amount of mineralized carbon was significantly different for compost and vermicompost, it was similar when these two substrates were incubated in the presence of biochar. This suggests that the presence of biochar reduced the effect of the pre-treatment (composting vs. vermicomposting). Table 3.1 presents the effect of biochar on the organic carbon mineralization of EOM. The presence of biochar increased the mineralization of manure and vermicompost but reduced the mineralization of compost. These results are somewhat surprising and our study does not allow us to pinpoint the exact reason of this negative interaction between biochar and manure and vermicompost on one side and positive interaction between biochar and compost on the other side. These differences could suggest the presence or stimulation of specific microorganisms in the case of the compost and/or the reduction of their activity in interaction with biochar in the case of the manure and vermicompost.

(ii) Litterbags incubation

Fig 3.4 presents the percentage of carbon remaining during one year of incubation in litterbags under field condition. The C losses from litterbags are probably due to CO_2 emissions during decomposition of substrates as well as leaching of soluble compounds (Sanaullah et al., 2010).

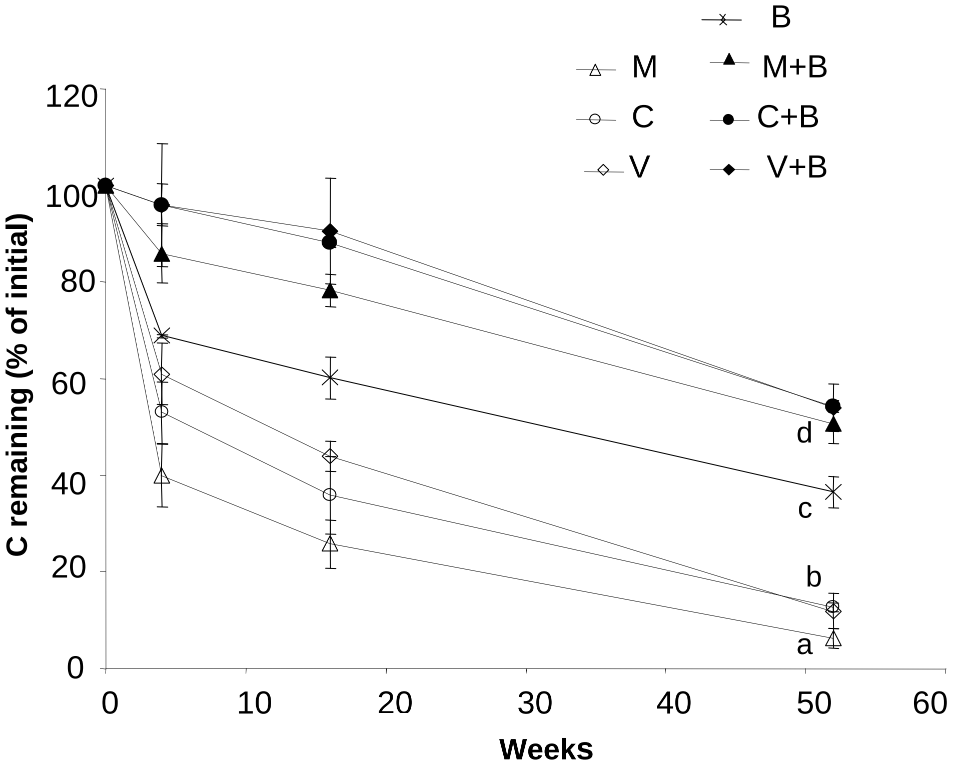

Fig 3.4: *Organic C remaining (% of total C) in buffalo manure (M), compost (V), vermicompost (V), biochar (B) and their mixture (M+B, C+B, V+B)*

After one year of incubation, only about 10% C of EOMs remained. The C loss from manure was higher than that of compost and vermicompost, confirming its lower resistant in the field. This result is in line with our laboratory incubation showing that manure was mineralized more quickly than the other substrates most likely because of its higher content of water soluble C. In the first period of the incubation, C remaining was slightly lower for compost than vermicompost but no significant difference was observed between them, especially at the end of the experiment. This result is not in line with that observed by laboratory incubation, showing that vermicompost mineralized less than compost. In reel conditions, C loss is not only C mineralized but also C leached and loss of fines particles by erosion.

More than 50% of biochar was lost at the end of the experiment. This loss is much higher than observed by laboratory incubation and may be explained especially by the lost of small particulates and dissolved compounds by leaching (Naisse et al., 2014).

The C remaining of the mixtures (biochar:EOM ratio 1:1) ranged from 54% for compost and vermicompost, to 50 % for manure. In according with the data of laboratory incubation, the C remaining curves of compost and vermicompost in presence of biochar were similar. C remaining of the substrates' mixtures was higher than the EOMs and surprising it was even greater than biochar alone. In the mixture, the fine particles of biochar could be trapped in the EOMs' matrices, thus protected to leaching. The biochar can also ameliorate the physical stability of EOMs as it can improve the structural cohesion between particles (Busscher et al., 2011). Therefore we suggest that there may be positive interactions in terms of C sequestration between the two substrates, which may also prevent physical loss once exposed under field conditions.

4. Conclusion

We analysed the composition of EOM as well as their chemical and biological reactivity alone or in mixture with biochar (1:1) by three different experiments. Our results showed that biological incubation may be more suitable than chemical oxidation to assess the short-term stability of organic matter. However, incubation may not be suited to represent all C losses occurring after field exposure as evidenced by our one-year litterbag experiment. In particular losses of fine particles from biochar due to physical fractionation, which may be significant under tropical rainfall conditions, are not accounted for.

Short-term mineralization and one year incubation with litterbags showed significant differences in C mineralisation between the three EOM, while no difference was noticed by chemical oxidation, suited to assess the long-term stability of organic substrates. The addition of biochar reduced the difference in biological stability of compost and vermicompost and decreased the chemical reactivity of all three EOM. In the field condition, the mixtures of biochar with other EOMs were significantly more resistance to C loss than substrates alone. Protection of biochar by EOM was probably due to physical protection. We therefore suggest that the mixture of EOM with biochar may be beneficial for increasing C sequestration due to mutual protection of EOM as well as biochar.

Chapitre 4 : L'impact de différents amendements sur la composition chimique et le stock de carbone de sol tropical dégradé par érosion au Vietnam

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1. Introduction

The degradation of soils by erosion with loss of organic carbon and fertility are amongst the greatest current threats for agriculture in the world. This is especially true in Southeast Asia where ecosystems are characterized by rapid biogeochemical cycling (Lal et al, 2005; Valentin et al., 2008). In Northern Vietnam, the cultivation of annual crops, such as maize and cassava on sloping lands is responsible for soil erosion (Podwojewski et al., 2008; Valentin et al., 2008). This results in a severe degradation of soils and off-site effects such as a pollution of water and siltation of water reservoirs.

Several technologies and agricultural practices have been suggested to rehabilitate these degraded soils. Amongst them, the incorporation of exogenous organic matter (EOM) into soil has been proposed to improve soil quality and reduce off-site effects (Ngo et al., 2011, Amossé et al., 2013). However, a major issue of this approach is the relatively fast degradation of fresh organic material applied to soil (Bolan et al., 2012), which requires the regular application of EOM and reduces the acceptability of this approach to farmers. A substantial way to improve the durability of EOM relies on composting organic matter in presence of epigeic earthworms, for obtaining so-called vermicompost (Dominguez et al., 2004). It is largely recognized that soils amended with vermicompost have higher water holding capacity and higher plant available nutrient contents (Arancon et al., 2008; Pramanik et al., 2007). An abundant literature also showed that this substrate has beneficial impacts on plant growth and yield (Canellas et al., 2002; Jouquet et al., 2010). The utilization of biochar is also an interesting strategy to increase SOM storage and improve soil fertility (Lehman et al., 2009), because it requires in most cases only one single application. Biochar is a pyrolysis product of biomass, extremely recalcitrant to micro- organisms and usually considered as soil conditioner (Lehmann et al., 2009). Therefore there has been increasing interest in the mixture of EOM with biochars in order to enhance C storage and at the same time improve soil quality and plant growth while reducing mineral fertiliser use (Bolan et al., 2012, 2013; Ngo et al., 2013). Indeed, it was shown recently, that the presence of bamboo biochar led to a protection of OM against biological and chemical oxidation, suggesting that it could increase the carbon sequestration potential of compost, vermicompost and manure, when applied to soil in mixture (Ngo et al., 2013).

There are several studies about the impact of EOM amendment as well as biochar on SOM, but the impact of their mixture on soil biogeochemical properties in degraded tropical soils under natural conditions remains unknown. To close this knowledge gap, we used an original mesocosm approach, allowing for the assessment of organic amendment effects on soil properties and plant growth under controlled conditions with natural climate. We used tropical degraded soil sampled in Northern Vietnam amended with buffalo manure, its compost and vermicompost as EOM and cultivated maize during three years. The EOM are available to the local population and have been shown to be valuable soil amendments (Ngo et al., 2011, 2013). Vermicompost was applied alone or in mixture with a unique dose of bamboo biochar during the first year. Our aim was to test the effect of the annual application of these EOMs and biochar on C storage and OM composition of degraded tropical soils. The specific objectives of this study were to evaluate (1) the effect of EOM on soil carbon storage and in particular the contribution of mineral bound C after 3 years of EOM amendment and maize cropping, (2) the effect of EOM on SOM composition assessed by carbohydrate signatures and analytical pyrolysis and (3) the influences of biochar on these effects. We hypothesised that the addition of vermicompost and biochar may be suited for increasing carbon storage and that there is a synergy effect of their joint application. All EOM treatments were supposed to change SOM composition as seen by the bulk molecular analyses and carbohydrate signatures. These biogeochemical characterisation of soil may allow us to detect alterations of SOM composition ultimately related to its origin (plant or microbial derived) and its quality in terms of nutrition function and/or carbon storage. We further hypothesised that mineral bound carbon would increase after three years in treatments with high C storage.

2. Materials and methods

2.1. Soil and organic amendments

The soil used in this study was sampled from the MSEC experimental catchment in Dong Cao village in north-east Vietnam, approximately 50 km south-west of Hanoi (20°57 N, 105°29 E). The soil was collected from the first 10cm of the soil surface, air-dried and afterwards sieved at 2 cm to discard stones and litter residues. It is an Acrisol (Fao., 1999) with an acidic pH (pH_{H2O}= 5.3) and contains ~43% sand, 34.28% silt, and 22.3% clay (Doan et al., 2013). Compost and vermicompost were produced by incubation of buffalo manure during 3 months for maturation in different and separate units. The procedure adopted was based on local farmer's knowledge. Briefly, buffalo manure was placed in 500 L bags and covered by a lid to prevent evapotranspiration and moisture addition due to rainfall, thereby conserving compost humidity and preventing anaerobic conditions due to rainwater addition. Every 1-2 weeks the compost was mixed thereby increasing aeration and favoring aerobic conditions. Vermicomposting was carried out in a similar way, the only difference being the addition of earthworms. The earthworm species used to produce the vermicompost was *Eisenia andrei.*

The biochar used in this study was provided by GRET (Hanoi) and produced on a farm in Cang Vallage, Xuan Phu commune, Thanh Hoa province, from bamboo Luong (*Dendrocalamus* barbatus Munro) by carbonizing bamboo at 600°C inside an airless (anaerobic) brick kiln and a specialized activation oven.

The elemental compositions of the different EOM and biochar are given in Table 4.1.

Table 4.1: *Carbon and nitrogen content (mg/g) of organic amendments. Data are presented as means and standard deviation (n=3)*

	Manure	**Compost**	**Vermicompost**	**Biochar**	**Degraded soil**
C (mg/g)	309.6 ±10,1	163.4 ±1,1	155.2 ±2.0	742.1 ±19.2	3.92 ±021
N (mg/g)	17.2 ±3.0	15.6 ±0.2	17.2 ±0.3	8.7 ±0.2	0.32 ±0.03

2.2. Experimental design

The experiment was carried out at the Soil and Fertiliser Research Institute in Hanoi, Vietnam with terrestrial mesocosms (1 m x 1 m x 2m high, see Doan et al. 2014 for more information on the experimental procedure). Each mesocosm was filled with 1m^3 of degraded soil from MSEC watershed and four maize seedlings were sown per mesocosm once a year during three years. The chemical nutrient inputs was urea CH_4N_2O, %N=46.3%, 40g m^{-2}, postash (K_2O, %K=60%, 16g m^{-2}) and phosphate (P_2O_5, %P=16%, 50 g m^{-2}). Plants received four different kinds/types of fertilizations: chemical nutrients (control-F) or the same amount of chemical nutrients plus organic fertilization (2 kg of buffalo manure (M), compost (C) or vermicompost (V). These amendments were applied before each cultivation, in total 3 times on the same location in mesocosmes during the experiment duration (3years). The influence of additional incorporation of biochar (700g) was tested for the control (F+B) and vermicompost (V+B) treatments. Biochar was added only one time at the beginning of experiment. Inorganic and organic amendments were added to the soil in four small holes which were then sown with maize seeds (25cm diam, 20cm depth). Four maize seedlings were grown per mesocosm without irrigation under natural rainfall conditions. More information on the experimental design can be found in Doan et al. (2014).

In total, 30 mesocosms were used for 6 treatments and the number of replicates per treatment was n=5. After three years of cultivation, the soil from the rhizosphere (25cm diam, 20cm depth) was collected and air-dried for the analysis.

2.3. Elemental analysis

Organic carbon (OC), nitrogen (N) content of difference treatments were measured in a single analysis using a CHN auto-analyser (CHN NA 1500, Carlo Erba) coupled with an isotope ratio mass spectrometer.

Determination of carbon stocks

Before incubation the carbon stocks of the initial and amended soils were calculated after determination of the OC contents by the following formula:

$$\text{Cstock initial} = S \times Csi + A \times Ca \times 3 + B \times Cb$$

where D is the density of soil, R is the diameters (25cm), h is the depth of amended soil (20cm). Csi is carbon content at the beginning of the experiment, A is the weight of the organic amendment and Ca is the carbon content of the amendment, B is the weight of the biochar, Cb is the carbon content of the biochar and where S is the weight of amended soil, calculated by the formula below:

$$S = D \times \pi \times (R/2)^2 \times h$$

Where D is the density of soil, R is the diameters (25cm), h is the depth of amended soil (20cm).

After three year we determined the carbon stocks of amended soil by the following formula:

$$\text{Cstock 3 yr} = S \times Cs$$

where S is the weight of soil, Cs is the carbon content of soil at the end of the experiment.

In all treatments, we calculated the soil C stock as percentage of the C stock of same soil at the beginning of the experiment.

2.4. Demineralisation with HF (hydrofluoric acid)

In this study, we used demineralisation with 10% HF to study the contribution of mineral bound carbon, which may represent a more stabilised C form in tropical soil [18]. Demineralisation of soil was carried out with 10% hydrofluoric acid (HF). The HF treatment was performed on three randomly selected replicated samples by adding 10 ml of 10% HF to 1 g of soil. Briefly, the suspensions were shaken for 2 h at room temperature, then centrifuged and the supernatant removed. This procedure was repeated five times. Then the residue was washed five times with deionized H_2O to remove salts and residual HF. Afterwards the samples were freeze-dried and weighed. The concentration of OC in the residue was determined and the amount of OC soluble upon this treatment was calculated by mass balance. The HF soluble C will be presented as percentage of carbon lost per total soil carbon content. Demineralised samples were also used for analytical pyrolysis as they are concentrated in C and do not contain minerals, which might interfere with the pyrolysis reaction.

2.5. Neutral non cellulosic carbohydrates

Sugars are labile compounds in soil organic matter which can be rapidly mineralized by soil microorganisms. Their degradation may be influenced by a stabilization of sugar by interaction with soil minerals and their incorporation into stable aggregates (Rumpel et al., 2010). The soil sugars analysis is a tool to understand their origin and function. Non cellulosic sugars were analyzed after trifluoroacetic acid (TFA) hydrolysis by gas chromatography (Amelung et al., 1996) as alditol acetates. We used the method developed by (Rumpel et aDignac., 2006) with modifications introduced by Eder et al. (2010). Briefly, 1g of soil was hydrolyzed using 10ml of 4M trifluoroacetic acid (TFA) at 110 °C for 4 h. After the hydrolysis, myoinositol (2g L^{-1}) was added as internal standard and the soil removed by filtration using a glass fiber filter (Whatman GF/F). After the evaporation of TFA by a speedvac, 0.5ml of myoinositol (internal standard) and 0.9ml of 0.2M EDTA were added to the samples. Thereafter, sugars were derivatised in screw-top test tubes with 1ml of $NABH_4$-DMSO solution in water bath at 40°C for 1.5hrs. These derivatised sugars were extracted by liquid–liquid extraction using 1ml dichloromethane. Gas chromatographic measurements of the monosaccharides were performed with a HP 6890 gas chromatograph equipped with a flame ionization detector. Separation of the monosaccharide units was achieved with a 60 m fused silica capillary column (BPX 70, 0.32 mm internal diameter, 0.25 mm film thicknesses) and the following temperature program: the temperature was raised from 200 to 250 °C at 3

°C min^{-1} and then kept isothermal for 15 min. The concentrations of individual neutral sugars were calculated based on the internal standard myoinositol's peak area. The sum of 8 monosaccharides gives the total non-cellulosic sugar content of the sample. We calculated the C6 carbohydrates as sum of galactose and mannose, and C5 as sum of arabinose and xylose. The C6/C5 ratio and rhamnose+fucose (desoxy)/C5 ratios are usually used to assess the contribution of plant- and microbial-derived sugars. Three out of five repetitions were chosen randomly and analysed for sugars content.

2.6. Analytical pyrolysis

Analytical pyrolysis was achieved with a coil probe pyrolyser (CDS Pyroprobe 5150) coupled to a Hewlett Packard HP-5890 gas chromatograph and a Hewlett Packard HP-5889 mass spectrometer (electron energy 70 eV). Briefly, 0.5–1mg of ground demineralised sample was loaded into a quartz tube and heated to 650 ◦C in 10 s (30 s hold). The pyrolysis products were transferred to the GC system through a split/splitless injector operated in splitless mode, using He as carrier gas. A fused silica column (60 m, 0.32mm i.d., film thickness 0.25mm) coated with a non-polar polyethyleneglycol (PEG) phase (column SolGelWax, SGE) was used to separate the pyrolysis products. The contribution of polysaccharide-derived and lignin-derived compounds as well as N-containing compounds, aliphatic and unspecific compounds to compost and vermicompost may be indicative of their potential degradability in soil. Compounds were assigned on the basis of their mass spectra, GC retention times and comparison with library mass spectra (Wiley). The peak areas were integrated based on the total ion current (TIC) trace using the GC ChemStation program (Agilent Technologies). The total area was calculated as the sum of the areas of the identified peaks. The proportion of each area to the total area was calculated.

2.. Statistic analysis

Before ANOVA, data were tested for homogeneity of variance using the Levene's test. Differences in elemental and chemical properties among control soil and amended soil with EOMs after 3 years were assessed by analysis of variance (one way ANOVA). A two way ANOVA was used to test the effect of the interaction between fertilization (control or vermicompost) and biochar (with or without) treatments. Differences among treatments were afterwards analyzed through Tukey's multiple comparison test. A Principal Component Analyses (PCA) was carried out to differentiate the treatments based on the chemical signature of the SOM. All statistical calculations were carried out using R (R Development

Core Team, 2008). Differences among treatments were declared at the 0.05 probability level of significance.

3. Results

3.1. Elemental analysis

Carbon and nitrogen contents of the soils before and at the end of the three year experiment are showed in Table 4.2. The lowest content was recorded for the soil before the experiment. Total soil organic carbon contents (TOC) increased during the 3 year experiment in all treatments and ranged from 9.41 mg C g^{-1} to 52.75 mg C g^{-1}. There was no significant difference among soils amended with buffalo manure, compost and vermicompost ($P>0.05$). However the addition of these amendments led to a strong increase of organic carbon compared with the control soil amended with mineral fertilisers only ($P < 0.05$). Carbon content of manure, compost, and vermicompost treatments was around 2 times higher than for control soil.

The highest carbon and nitrogen content was observed in the case of soil amended with vermicompost and biochar. The presence of biochar increased soil nitrogen and carbon contents in treatments with mineral fertiliser and vermicompost, although this effect was more pronounced in the case of soil amended with mineral fertilisers only. The presence of biochar also increased the C to N ratio of both treatments from 10 to 28 in the case of control soil and from 10 to 17 in the case of vermicompost one.

After 3 years of experiment, we recovered only 54% of all carbon that should be present in the manure treated soil taking into account all amendments and the carbon stock of the initial soil (Table 4.2). In contrast, soil treated with compost and vermicompost have stable carbon storage. An increase of 23% of C stock beyonds external C additions was observed in the case of vermicompost amended soil with biochar. Interestingly, the carbon content of the control soil was 3 times higher than initial soil, showing that this soil has as high C storage capacity.

Table 4.2: *Carbon and nitrogen content, C/N ratio and C stock (n=5) and HF soluble C (n=3) of amended soils before (soil T_0) and after incubation. The treatment with and without biochar are indicated by (+) and (-). Data are presented as means and standard deviation (n=5). Data with different lower-case letters indicate significant differences between fertilizer treatments (one way ANOVA for soil at T0, control soil, buffalo manure, compost and vermicompost treatments, Tukey test, P < 005). Different capital letters indicate differences between Fertilizer (control vs vermicompost) × Biochar (with vs without) treatments (two way ANOVA, Tukey test, P < 005).*

Fertilization	**Biochar**	**N content (mg/g)**	**C content (mg/g)**	**C/N**	**C Stock (kg/mesocoms)**	**C stock (% of initial**)**	**HF soluble C (% initial)**
Soil T_0	-	0.32^{a} ±0.03	3.92^{a} ±021	12^{b} ±1	0.14^{a} ±0.07	nd	nd
Fertiliser	-	0.94^{Ab} ±0.08	9.41^{Ab} ±078	10^{Aa} ±1	0.51^{Ab} ±0.03	283.1^{Cc}±3.1	45.9^{Aa} ±1.4
	+	1.40^{B} ±0.11	39.40^{C} ±3.64	28^{C} ±1	1.32^{B} ±0.39	97.4^{A}±4.3	45.7^{A} ±0.5
Manure	-	2.12^{c} ±0.10	21.87^{c} ±1.31	10^{a} ±0	0.90^{c} ±0.07	46.5^{a}±2.9	46.4^{a} ±3.8
Compost	-	2.33^{c} ±0.17	24.57^{c} ±1.69	10^{a} ±0	1.02^{c} ±0.05	91.5^{b}±2.0	40.5^{a}±1.0
Vermicompost	-	2.50^{Cc} ±0.49	25.36^{Bc} ±6.50	10^{Aa} ±1	1.05^{Bc} ±0.27	98.2^{Ab}±9.7	43.3^{Ba}±1.6
	+	3.22^{D} ±0.15	52.75^{D} ±5.49	17^{B} ±2	1.96^{C} ±0.16	123.5^{B}±4.4	49.4^{C} ±1.4

[*] nd: not determined

** Initial was calculated as explaining in the “Materials and methods”

3.2. Mineral bound OM

Mineral bound C content ranged from 40.6 to 49.5 % of TOC (Table 4.2). There was no significant difference among the treatments with chemical fertiliser, manure, compost and vermicompost. The presence of biochar led to a higher proportion of mineral bound C in the case of vermicompost amended soil but it has no effect in the control soil.

3.3. Sugar signature

Table 4.3 shows the total sugar content and the contents of five ring (C5) and six ring sugars (C6) in soils. The sugar content ranged from 2.63 to 8.25 mg.g^{-1} soil. It was lowest for the control soil and highest for soil amended with vermicompost and biochar. Biochar had no effect on the carbohydrates signature of mineral soil ($P > 0.05$ between control soil with and without biochar). The effect of biochar on the carbohydrate signature of soil was most obvious for vermicompost amended soils. Biochar increased the content of both plant-derived (C5) and microbial synthesized (C6) carbohydrates of soil amended with vermicompost (Table 4.3).

A principal component analysis (PCA) was carried out using all indicators of the carbohydrates signature (Fig. 4.1). Soil samples were clearly separated on axes 1 and 2 that explained 78 and 13% of the total variance, respectively. The first axis was negatively related to all individual carbohydrates and positively to C6/C5 ratio. The second axis was associated with the deoxy/C5 ratio. Manure treatment was positively correlated with the deoxy/C5 ratio. Soils treated with vermicompost with or without biochar were clearly differentiated (Fig. 4.1). However, the control soil with and without biochar were similar and correlated in the negative direction with all carbohydrates.

Table 4.3: *Concentration of neutral sugars, xylose plus arabinose (C5), mannose plus galactose (C6) as well as C5/C6 ratio of control soil and soil amended with vermicompost, manure and compost. Data are presented as means and standard deviation (n=3). Data with different lower-case letters indicate significant differences between fertilizer treatments (one way ANOVA for control soil, buffalo manure, compost and vermicompost treatments, Tukey test, P < 005). Different capital letters indicate differences between Fertilizer (control vs vermicompost) × Biochar (with vs without) treatments (two way ANOVA, Turkey test, P < 005).*

Amendment	Biochar	Sugar total (mg g^{-1})	C5 mg g^{-1} soil	C6 mg g^{-1} soil	C6/C5
Fertiliser	-	2.63^{Aa} ±0.23	0.50^{Aa} ±0.09	1.30^{Aa} ±0.08	2.64^{Cb} ±0.34
	+	2.96^{A} ±0.66	0.53^{A} ±0.14	1.42^{A} ±0.27	2.70^{C} ±0.02
Manure		5.47^{b} ±0.30	1.39^{b} ±0.10	2.03^{b} ±0.10	1.46^{a} ±0.03
Compost		6.73^{c} ±0.84	1.75^{c} ±0.16	2.35^{b} ±0.15	1.34^{a} ±0.07
Vermicompost	-	5.63^{Bb} ±0.05	1.40^{Bb} ±0.16	1.94^{Bb} ±0.23	1.39^{Ba} ±0.05
	+	8.25^{C} ±1.42	2.33^{C} ±0.49	2.79^{C} ±0.35	1.18^{A} ±0.06

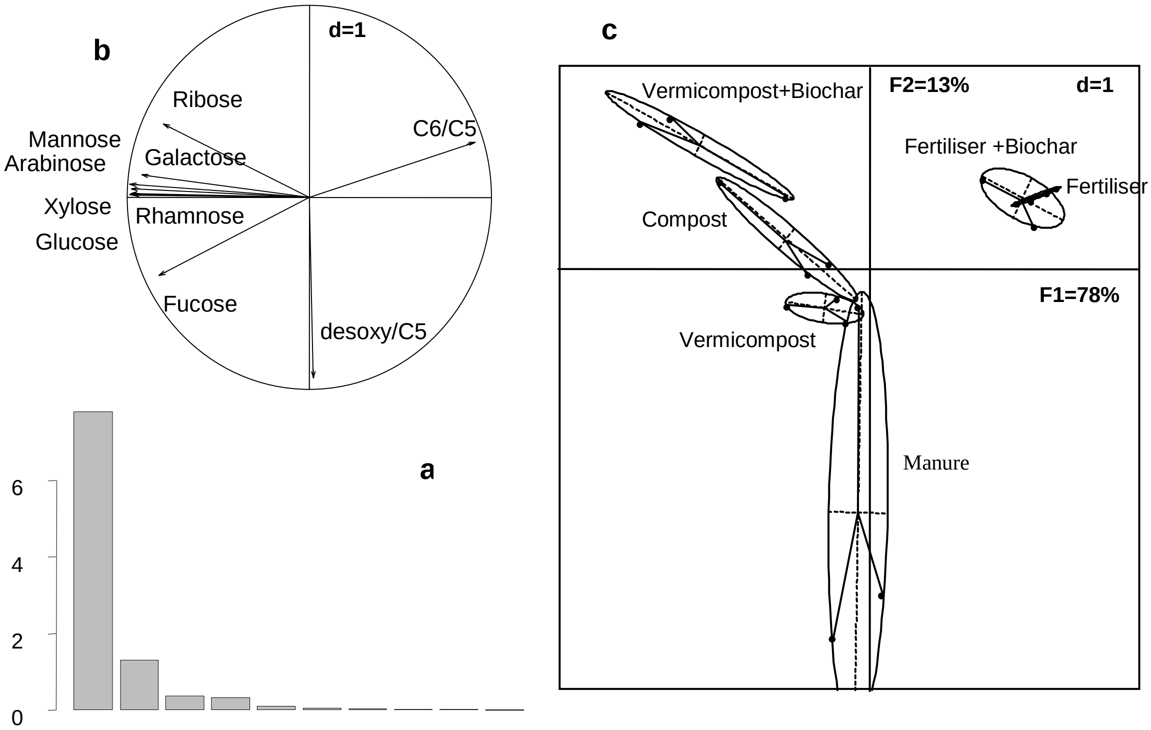

Fig 4.1: *Principal component Analysis (PCA) of sugar signatures of soil amended with mineral fertiliser, vermicompost with and without biochar, compost and buffalo dung. (a) Eigenvalues diagram, (b) Correlation circle on F1-F2 plane, (c) Ordination of the samples in the plane defined by axes 1 (F1) and 2 (F2) of the PCA.*

3.3. Pyrolysis GC/MS

Figure 4.2 and 4.3 show the pyrograms of 6 amended soils after 3 year of incubation. The main compounds identified after pyrolysis were grouped according to their origins into three major classes: compounds originating from the pyrolysis of polysaccharides (PS), lignin-derived products (LIG) and N-containing compounds (N). Compounds that could have resulted from the pyrolysis of several distinct biological sources were designated unspecific (U). These compounds are listed in Table 3. Their contribution to amended soils at the end of experiment is presented in the Table 4.4 and 4.5.

Table 4: *Pyrolysis products in soils amended with manure, compost, vermicompost and mineral fertiliser with (+B) or withour (-B) biochar*.

No	Name	Fertiliser mineral		Manure	Compost	Vermicompost	
		-B	+B	-B	-B	-B	+B
Compounds derived from polysaccharides							
PS1	Furan 3 Methyl	+	+	+	+	+	+
PS 2	Furan 2 Methyl				+		
PS3	Furan 25 di Methyl		+	+	+	+	+
PS4	3 Furandehyde						+
PS5	2 Furan carboxaldehyde	+	+	+	+	+	+
PS6	2 Cyclopentene 1 one 2 Me		+	+	+	+	+
PS7	2 Furan methanol					+	+
PS8	2 Furan carboxaldehyde 5 Me	+	+	+	+	+	+
PS9	2 Cyclo pentene 1 one 3 Me	+	+	+	+	+	+
PS10	2 Cyclobutene 12 dione 34 dihydroxy			+		+	+
PS11	2 Cyclopenten 1 one; 2 hydoxy 3 Me	+	+	+	+	+	+
PS12	4H pyran 4 one 3 hydroxy 2 Me					+	+
PS13	Benzo furan 23 dihyrdo	+	+	+	+	+	+
N-containing compounds							
N1	1H Pyrolle 1 Me	+	+	+	+	+	+
N2	Pyridine	+	+	+	+	+	+
N3	1 H Pyrole			+	+		
N4	Pyridine 2 Me	+	+	+	+	+	+
N5	1H Pyrole 2 Me		+	+	+	+	
N6	Et Benzene		+		+	+	+
N7	Pyridine 4 Me	+	+				
N8	Benzo nitrile	+	+		+		+
N9	Indole				+	+	
N10	Benzene acetone nitrile	+	+	+			
N11	Benzene propane nitrile	+	+	+			
N12	1H Indene 3 Me			+	+	+	

No	Name	Fertiliser mineral		Manure	Compost	Vermicompost	
		-B	+B	-B	-B	-B	+B
N14	Indole					+	+
N15	1H pyrole	+	+		+		+
N16	1H pyrrole 15 dione		+				+
Compounds derived from lignin							
LIG1-G	Phenol 2 methoxy 4 Me			+	+	+	+
LIG2-G	Benzaldehyde 4 hydroxy 3 methoxy			+			+
LIG3-G	5Et Methoxy phenol					+	+
LIG4-G	2 methoxy 4 vinyl phenol	+	+	+	+	+	+
LIG5-S	Phenol 26 dimethoxy	+		+	+	+	+
LIG6-S	45 di methoxy 2 Me phenol			+	+	+	+
LIG7-G	Phenol 4 methoxy 4 (1propenyl)				+	+	+
LIG8-G	Ethanone 1(4 dydroxy 3 methoxyphenyl)				+	+	+
LIG9-G	Acetamide N (24 dihydro 3 methoxy phenyl)						+
LIG10-G	4 Hydroxy 3 methoxy phenyl Et alcohol						+
LIG11-S	Phenol 26 dimethoxy 4(2 propenyl)						+
LIG12-G	26 DiMe 3 MethoxyMe p bezoquinone						+
LIG13-S	Phenol 45 dimethoxy 2(2 propenyl)		+		+	+	+
Compounds of unspecific origin							
U1	Benzene		+	+	+	+	+
U2	Butanal 2 Me	+	+	+	+	+	+
U3	Benzene Me	+	+	+	+	+	+
U4	p Xylene			+	+		
U5	Styrene	+	+	+	+	+	+
U6	Phenol	+	+	+	+	+	+
U7	Phenol 2 methyl		+	+	+		
U8	Phenol 4 methyl	+	+	+	+	+	+
U9	Phenol 4 ethyl		+	+	+	+	+
U10	Phenol 24 dimethyl			+			

A series of n-alkene/n-alkane doublets were obtained for soil amended with manure, compost and vermicompost (Fig. 4.2) as we used non-polar column (Dignac et al., 2005). These three pyrograms were rather similar but significantly different from control soil (Fig. 4.3). A number of unspecific pyrolysis products could be identified (Table 4). The major pyrolysis products in the amended soils were low molecular weight components, such as benzenic (U2, U3), and phenolic (U6, U7, U8, U9) compounds which can arise from difference sources (Figure 4.2). Soils amended with EOMs are much more abundant in LIG products than the control soil amended with mineral fertiliser only (Table 4.5). The presence of biochar led to an increase of LIG compounds in the case of vermicompost but no significant effect was found for the control soil. Two types of lignin derived compounds can be distinguished: -G for guaiacyl-type (bearing a single methoxy group on carbon 3 of the aromatic ring) and –S for syringyl type unit (with methoxy groups on carbon atoms 3 and 5). The ratio LIG-G/LIG-S was calculated using the integrated area of LIG-G and LIG-S compounds. It was highest for the soil amended with vermicompost and biochar (2.78) and lowest for control soil (1.21). Soil amended with compost has lower LIG-G/ LIG-S ratio (1.3) than soil with manure (2.6) or vermicompost (2.5).

In the soils amended with EOMs, polysacchraide pyrolysis products such as furan related species and cyclopentenone are also abundant and clearly visible in the pyrogram. Vermicompost amended soil was more abundant in polysaccharide derived compounds than the other two soils amended with EOMs. In this soil, PS products represented the highest fraction of the pyrogram (35%, Table 4.5).

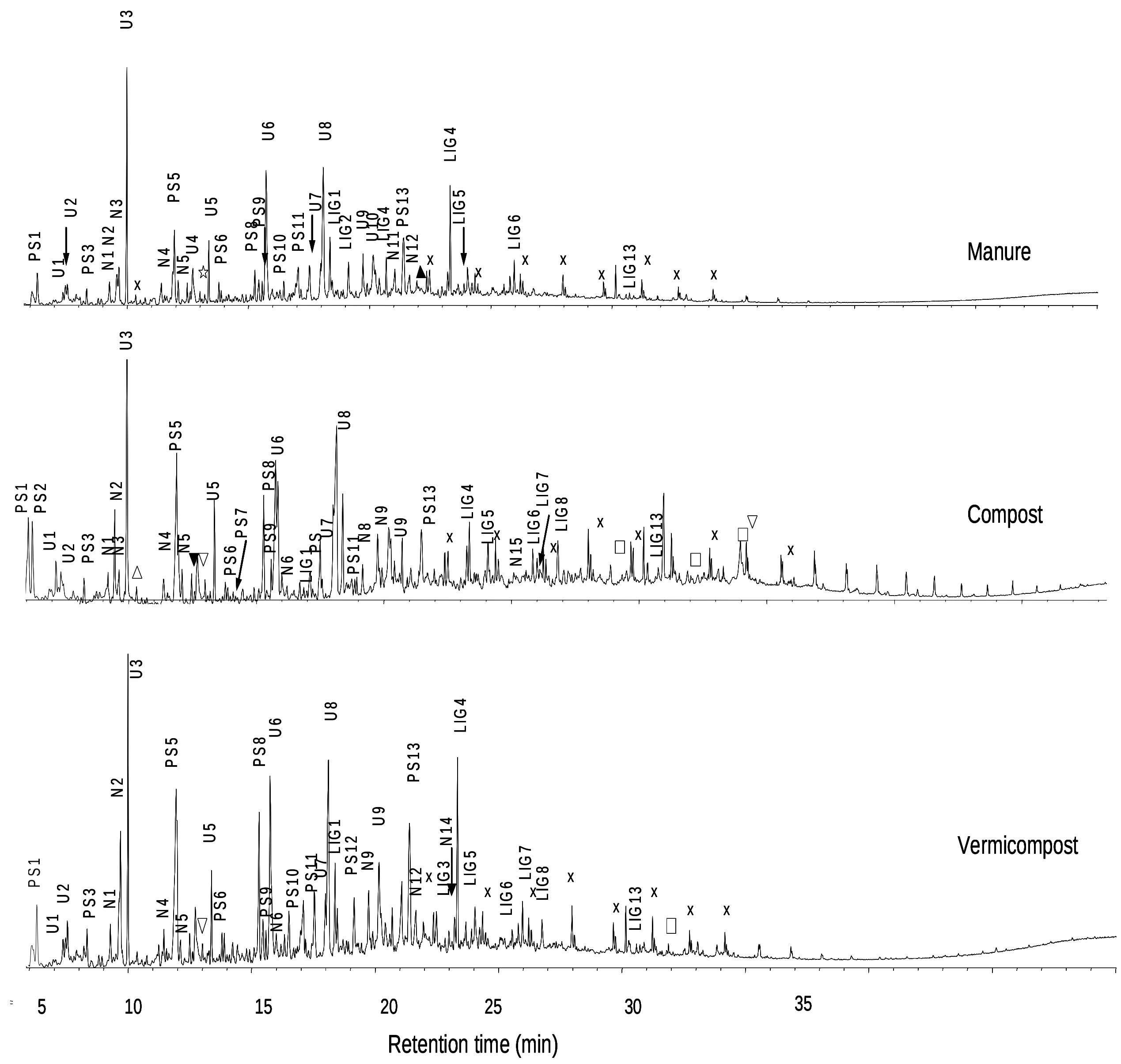

Figure 4.2: *Pyrogram of soils amended with manure compost or vermicompost Name of compounds are listed in Table 4 Others compounds: (□) aliphatic carboxylic acids (x) n-alkene/n-alkene doublet (∆) alkane or alkene (▼) alkyl benzene*

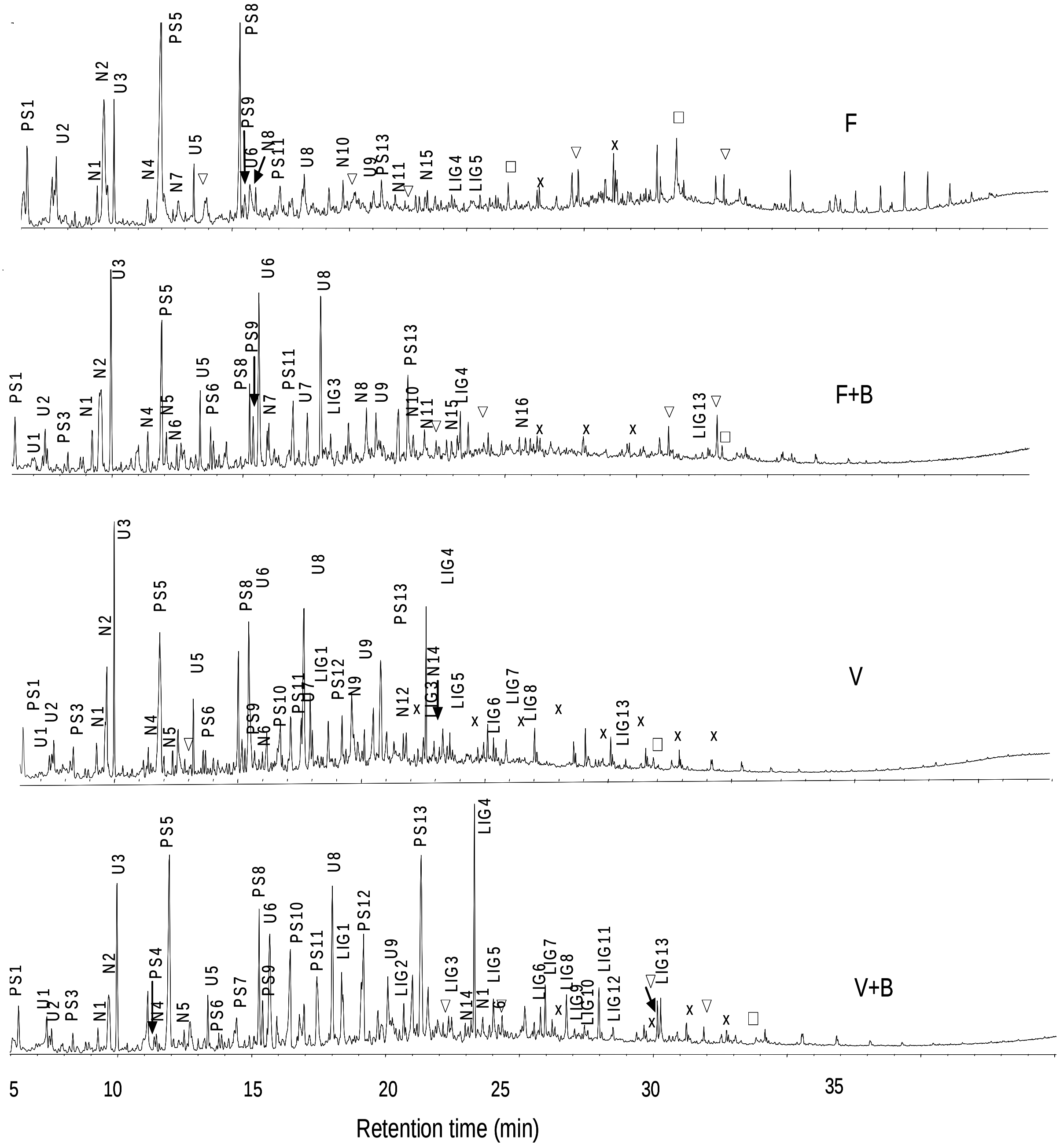

Figure 4.3: *Pyrogram of soil amended with mineral fertiliser (F) and with vermicompost (V) in present of absence of biochar (B) Name of compounds are listed in Table 4 Others compounds: (□) aliphatic carboxylic acids (x) n-alkene/n-alkene doublet (Δ) alkane or alkene (▼) alkyl benzene*

Table 4.5: *Relative contribution of the pick areas of the pyrolysis products derived from polysaccharides (PS), lignin (LIG), N-containing (N) and unknown (U) compounds as well as ratio of pick area of LIG-G and LIG-S in amended soils*

Amendment	**Biochar**	**PS**	**N**	**LIG**	**U**	**LIG-G/LIG-S**
			% of total identified peaks area			
Fertiliser	-	50.3^{Bc}±3.3	28.2^{Cb}±4.6	24.9^{Ba}±6.1	19.1^{Ab}±3.8	1.21^{Aa}±0.18
	+	29.1^{A}±4.2	30.3^{C}±3.6	38.8^{C}±4.5	26.7^{B}±2.9	2.49^{B}±0.26
Manure	-	26.0^{a}±5.6	23.3^{ab}±2.9	15.2^{a}±3.5	35.5^{a}±4.7	2.64^{b}±0.12
Compost	-	24.9^{a}±2.1	18.8^{a}±3.4	17.9^{a}±2.2	38.4^{a}±2.7	1.31^{a}±0.30
Vermicompost	-	$35.^{3Ab}$±3.8	15.3^{Ba}±2.4	14.4^{Aa}±2.5	35.0^{Cb}±3.2	2.50^{Bb}±0.24
	+	44.4^{B}±6.2	7.8^{A}±1.8	23.1^{B}±1.6	24.8^{B}±4.1	2.78^{B}±0.21

4. Discussion

4. 1. Effect of manure, compost and vermicompost

i) Soil carbon storage

Soil carbon storage development during our experiment may be influenced by addition of EOMs as well as plant litter input. Our experimental setup did not allow distinguishing between those sources. Our results indicate that carbon storage was increased by organic amendments with regards to the control soil, which is in line with other authors showing that the exogenous organic matter such as compost (Gabrielle et al., 2009) and vermicompost (Ngo et al., 2012) can enhance soil C storage. This increasing C storage probably results from C additions due to the organic amendment or indirectly from the development of roots in soil. After three years of incubation, the treatment with manure lost 54% of carbon taking into account all manure additions as well as the soil C stocks at the beginning of the experiment. This loss was much higher than C loss in soil amended with compost or vermicompost, indicating that organic matter from manure was strongly decomposed during the experiment and/or the occurrence of intensive priming effects due to the addition of labile material. The rate of decomposition of organic materials as well as the priming effects induced by their addition to soil are generally controlled by soluble organic carbon and increase with increasing in soluble carbon of the organic amendments (Peltre et al., 2010; Bolan et al., 2013). Another possibility would be less root C inupt in this treatment compared to the other amendments. Therefore, our results are in line with the observation of higher hot water soluble C content in buffalo manure compared with their compost and vermicompost (Ngo et al., 2013). However, in contrast to our initial hypothesis, these differences did not seem to affect the amount of C stabilised by mineral interactions as we recorded similar contribution of HF soluble C (Table 4.2).

ii) Molecular characteristics of soil organic matter after 3 year of experiment

Neutral sugars are labile compounds, which are usually rapidly metabolised by the soil microbial biomass as a source of energy (Solomon et al., 2000). Their content and composition may be influenced by a stabilization of sugar by interaction with soil minerals and their incorporation into stable aggregates (Oades., 1967). The soil sugars analysis is a tool to understand their origin and function. In this study, the sugar content of EOM amended soils was two times higher than for control soil. This might be explained by a contribution of carbohydrates from organic amendment, root biomass and/or microbial biomass due to higher activity. The sugar content of soil amended with compost was higher than the sugar content of soil amended with manure and vermicompost. Sugars can be plant or microbial-derived. The ratios C6/C5 and deoxy/C5 are usually used to discriminate between carbohydrates derived from plants and microbial input (Oades., 1967). These ratios usually increase with increasing contribution of microbial-derived carbohydrates. The soils amended with EOM have a lower C6/C5 ratio compared to the mineral treatment. The C5 content of EOM amended soil was 3 times higher than control soil, indicating an increasing contribution of plant, probably root-derived sugars. This is in agreement with Solomon et al. (2000) indicating that the higher proportions of plant derived sugars were indicators of the direct input of organic residues into soil. The lower C6/C5 ratio of compost amended soil compared to the ones ameliorated with manure and vermicompost indicates lower contribution of microbial derived sugars. This result is in line with Ngo et al., (2012) and could indicate lower microbial activities in compost amended soils compared to vermicompost amended ones.

The highest C6/C5 ratio was observed for control soil, meaning the highest contribution of microbial-derived sugars compared to plant-derived ones. This was further evidenced by PCA analyses, which differentiated the control soil based on its high C6/C5 ratio and may be explained by the fact that tropical soils with low C content contain highly stabilised organic matter with a high contribution of microbial material stabilised by mineral interactions (Larre-Larrow et al., 20003; Miltner et Zech., 1998). Under such conditions, microbial sugars may be stabilised preferentially compared to those derived from plants (Rumpel et al., 2010). On the other hand it is unlikely that the lower C6/C5 ratio can be explained by plant-derived sugars in manure, compost and vermicompost treatment, as those substrates are highly degraded and rich in microbial derived sugars (Ngo et al., 2011).

The PCA analysis further showed that also manure-amended soil was positive correlated with a higher contribution of microbial-derived carbohydrates. However, the microbial derived carbohydrates of manure may be different than the ones found in soil due to the contrasting indicator (higher desoxy/C5 ratio instead of C6/C5). This is in line with the difference in microbial community composition of manure amended soil compared with compost and vermicompost amended soil (Doan et al., 2013). Manure in contrast to soil and other EOMs may also have a higher contribution of soluble carbon that is generally labile thus was available for micro organisms (Ngo et al., 2013). This could induce a strong priming effect (Tuomela et al., 2002) and thus explain the highest C decrease during the experiment (see above).

Information on the bulk molecular composition of soil amended with organic materials was obtained by analytical pyrolysis. With this method very detailed changes in molecular composition can be assessed, which allow for the evaluation of the origin and state of degradation of SOM. Our results show that SOM of control soil was enriched in carbohydrates whereas soil treated with EOM had a higher relative contribution of lignin derived products, which are constitute biopolymers of plants (Tab. 4.4). This could indicate the incorporation of lignin originating from EOMs and/or the greater root development in these treatments. Buffalo manure may still contain lignin, as this component is poorly degraded during digestion in the animals' rumen (Susmel et al., 1993). As observed for PS products, the presence of LIGs is less abundant in manure amended soil than the compost and vermicompost amended soil. This could be explained by the fact that compost and vermicompost are richer in lignin than manure (Ngo et al., 2011). Lignin is a chemically recalcitrant compound, thus resistant to the mineralization and concentrated in the first stages of biodegradation during compositing as well as after incubation of EOM in soil. Moreover, biomass production and therefore most likely root development and plant litter input is most likely higher in soils amended with compost and vermicompost compared to the soil amended with manure. Higher lignin could therefore in addition to input with the amendment be related to higher plant litter input.

The Lig-G/Lig-S ratio may reflect the degree of lignin biodegradation (Theverot et al., 2010). This ratio increases upon the decomposition because the Lig-G are more resistant than Lig-S (Dignac et al., 2006). The presence of many more G than S type lignins in all of the pyrogram indicates a preferential degradation of S type during experiment (Table 4.3). Our results show that the LIG-G/LIG-S ratio of compost amended soil was two times lower than the one of manure and vermicompost amended soil. This is in line with the results of sugar content (see above), indicating that compost amended soil may have lower microbial activity and thus lower decomposition rates than soils amended with the other EOMs. It is also in accordance with Ngo et al., (2013) showing that manure has more labile C than other subtracts, thus easier degraded in the soil, leading to a higher LIG-G/LIG-S ratio.

4.2. Biochar effect

iii) Soil carbon storage

The enhancement of soil carbon storage following biochar addition is evident for both the control and vermicompost treatments. Our data show that in the case of joint application with vermicompost soil C storage was enhanced beyond the external C additions (Table 4.2). This is in accordance with many studies showing that biochar can successfully increase the carbon storage under temperate (Malghani et al., 2013) as well as tropical conditions (Lemann et al., 2003). Biochar is rich in aromatic carbon, thus it is highly recalcitrant to decomposition in natural environments and may have a long residence time in soils. Ngo et al. (2013) showed that the bamboo biochar is very stable and mineralised only 0.23% TOC after 7 months of laboratory incubation under optimum conditions. In addition, several studies have shown that biochar induces a negative priming effect, meaning that it can enhance soil C storage via the processes of OM sorption to biochar and physical protection of native soil organic matter (Zimmerman et al., 2011; Kuzyakov et al., 2009; Naisse et al., 2014; Liang et al., 2010). The increase of carbon storage in the soil containing biochar in addition to other amendments may be also due to an increase of root biomass, especially in the case of vermicompost. Both vermicompost (Jouquet et al., 2010) and biochar (George et al., 2012) can ameliorate the soil physical property such as aggregate stability, porous structure. These factors are favourable for the development of root systems. In addition, both vermicompost and biochar may both stimulate root growth. Cenellas et al., (2002) found that plant growth hormones in vermicompost improved root growth and lateral roots initiation in maize, whereas better root growth in the presence of biochar was ascribed to ethylene production (Spokas et al., 2011). The presence of biochar also seemed to increase the amount of mineral bound C measured as HF soluble C of the vermicompost amended soil, indicating that this soil contains higher amounts of carbon stabilised by mineral interactions (Kaser et al., 2002). It is well know that biochar has a high CEC, which could be beneficial for creating an interaction between SOM and soil mineral phase.

ii) *Molecular characteristics of soil organic matter after 3 year of experiment*

The presence of biochar increased significantly the abundance of lignin and polysaccharide derived compounds of soil amended with vermicompost, while no effect was found for soils amended with chemical feriliser. Both soils amended with mineral fertilizers are depleted in LIG compounds. However, the pyrogram of soil amended with vermicompost and biochar was dominated by pyrolysis products originating from lignin and polysaccharides (Table 4.4, 4.5). In addition, the presence of biochar has also significant effects on the neutral sugar signatures of vermicompost amended soil (Table 4.3). Soil with and without biochar are clearly distinguished in the PCA plane. Vermicompost amended soil with biochar has a higher sugar, C5 and C6 content than vermicompost amended soil without biochar. These results can be explained by many factors. This may be due to (i) the strong adsorption of carbohydrates from initial soil and vermicompost into biochar matrix, which can protect the organic matter against the microbial decay (Keith et al., 2011). The increase of plant-derived sugar in amended soil with biochar can also be due to (ii) an increasing of root biomass as discussed above and (iii) an increase of the microbial derived carbohydrate in micro-pores of biochar (Jindo et al., 2012).

However, no significant difference on lignin derived compound abundance, C5, C6 as well as total sugar content was found between soil amended with mineral fertiliser in presence or absence of biochar. This difference between chemical fertilized soil and vermicompost amended soil may be explained by the fact that amended soils with organic amendment are rich in organic matter and micro organisms. In contrast, soil with mineral fertiliser is poor in organic matter and also has a low microbial abundance and diversity. In addition, bamboo biochar produced at high temperature is probably depleted in micro-organisms and has very low labile C content (Bolan et al., 2013). Thus, soil amended with mineral fertiliser has not the same benefit from biochar addition as observed for vermicompost amendment. In vermicompost amended soil on the other hand the addition of biochar may change SOM composition and have a positive impact on the contribution of recalcitrant as well as easily decomposable SOM compounds.

5. Conclusion

Buffalo manure, compost and vermicompost have positive effect on the soil carbon storage and also the composition of the organic matter of tropical degraded soils. Both compost and vermicompost amendment lead to higher soil C storage than buffalo manure addition. In terms of total C budget after addition to soil compost and vermicompost are more favourable than manure. They also change SOM quality through their impact on the contribution of polysaccharide and lignin compounds. Vermicompost amended soil seems to be characterised by high microbiological activity and most probably also greater plant litter input.

Bamboo biochar increased the carbon storage in soils amended with mineral fertiliser or vermicompost. This may be ascribed to its high resistance in soil as well as protection of labile organic matter. The presence of biochar changed SOM quality when applied in addition to vermicompost, whereas it has not effect when applied in addition to mineral fertiliser.

Conclusion générale

L’apport des amendements organique exogènes (AOEs) est une option crédible permettant de limiter l’impact de l’agriculture sur l’environnement, tout en permettant d’augmenter la fertilité des sols. L’utilisation des AOEs est d’autant plus intéressante dans les pays du sud, permettant ainsi de maintenir un bon niveau de productivité des agrosystèmes, voire de réhabiliter les sols dégradés. De plus, l’apport des AOEs au sol permet de stocker du C dans les sols, et contribue ainsi à limiter les émissions carbonées vers l’atmosphère. Au Vietnam, le lisier de buffle, son compost et son lombricompost, ainsi que le biochar de bambou sont des amendements organiques disponibles. Dans cette thèse, nous avons combiné des travaux au laboratoire et d’autres en conditions semi-contrôlées (via des mésocosmes) afin d’évaluer le potentiel de ces substrats à séquestrer du C dans les sols.

(i) Compost vs lombricompost

L’impact des AOEs sur les propriétés des sols est très contrasté en fonction de leurs origines et leurs modes de production. Dans la première partie de ces travaux, nous avons caractérisé la nature chimique des amendements organiques. Aucune différence de structure entre compost et lombricompost n’a été observée par résonance magnétique du ^{13}C à l’état solide (RMN). Cependant, les analyses élémentaires et isotopiques ainsi que les analyses moléculaires montrent que le compostage et le lombricompostage impactent différemment la matière organique. Une augmentation du ^{13}C a été observée au cours du compostage et du lombricompostage. Ceci peut être expliqué par une décomposition des composés pauvres en ^{13}C telle que la lignine, comme observé par pyrolyse GC-MS. Ces résultats conduisent à présenter le lombricompost comme un AOE plus stable que le compost, confirmé en cela par les mesures de carbone soluble à l'eau chaude et de composition chimique par la proportion de composés azotés et de polysaccharides. Ainsi, nous suggérons que le lombricompost est plus stable que le compost et donc restera plus long temps dans le sol.

L’incubation au laboratoire à 28°C pendant 7 mois confirmait les résultats précedents, méttant en évidence la stabilité biologique supérieur du lombricompost comparée au compost, c’est à dire plus récalcitrant à la dégradation microbienne. Cependant, l’expérimentation mené au Vietnam au cours d’une exposition d’un an en conditions environnementale avec des sachets de litières, présenté des résultats contrasté avec ceux obtenus en laboratoire, ne montrant aucune différence entre le compost et le lombricompost. Ceci peut être expliqué par le lessivage et l’érosion de C considérable survenant au cours des épisodes de pluies intenses. Par ailleurs, les résultats de stabilité chimique par oxydation à l’acide dichromate des trois AOEs présentent des résultats similaires, indiquant que leurs stabilité à long terme (dixième années) potentiellement similaire (Naisse et al., 2014).

Nous avons vérifié les observations faites au laboratoire par une expérimentation avec des mésocomes pendant 3 ans en conditions tropicaux humides à Hanoi (chapitre 4). Nos résultats montrent qu’à la fois le compost et le lombricompost ont un effet positif sur le stockage de carbone dans le sol ainsi que la composition de la MOS. Cependant, aucune différence signifiante entre les traitements avec compost et lombricompost n’a été observée. Cette expérience réalisée dans des conditions semi-controlées, ne nous a également pas permis de conclure à une plus grande stabilité du lombricompost. Ceci permet met en évidence la nécessiter de confronter les résultats obtenus en laboratoire dans des conditions contrôlées à la complexité des processus opérant en milieu naturel. Toutefois, dans les sols tropicaux, l’apport de lombricompost peut diminuer la quantité d’éléments lessivés et d’augmenter la production de biomasse par rapport compost (Jouquet et al., 2010 ; Doan et al., 2013). Le lombricompostage ne doit pas être seulement vu comme une technique pour protéger la MO et augmenter les stocks de MO. Il est également nécessaire de prendre en compte d’autres aspects comme l’augmentation non négligeable de la fertilité, ainsi que la réduction du lessivage de nutriments, diminuant ainsi l’impact sur la qualité de l’eau, et enfin sa pertinence économique, ainsi que son acceptabilité sociale.

(ii) Le biochar et son intérêt de l’associer avec les MO

Le biochar a une structure complètement différente de celles des AOEs précédentes (chapitre 2). Nos travaux ont confirmé plusieurs études précédentes, montrant la récalcitrance tant chimique (Naisse et al., 2013), que biologique (Zimmeman et al., 2011) du biochar. Pour cette raison, l’amendement de biochar a souvent été considéré comme solution pour séquestrer massivement du carbone dans les sols à large échelle (Lehmann et al., 2003). Ainsi nos résultats suggèrent l’intérêt de combiner le biochar avec les AOEs pour améliorer la fertilisation des sols et le stock de carbone en même temps. Cependant, les mécanismes contrôlant les interactions entre biochar et AOEs sont encore peu clairs. Nous avons utilisé plusieurs approches afin de déterminer la stabilité chimique et biologique des trois AOEs en association avec le biochar dans des conditions différentes. Nos résultats de stabilité ont montré clairement des interactions entre les AOEs et le biochar. La stabilité des AOEs ainsi améliorée, met en évidence l'intérêt des associations biochar / AOEs. Cette protection de la MO est d'autant plus importante dans les sols tropicaux où la dynamique de la MO est supérieure à celle des écosystèmes tempérés.

Le dernier chapitre a montré l'influence positive du biochar sur la MOS après 3 ans d'expérimentation. L'utilisation de biochar entraînait une augmentation du stock de carbone de sol amendé avec le fertilisant minéral ou avec le lombricompost. De plus, nous avons observé des différences de production végétale (Doan et al, en préparation). Le lombricompost ainsi que le biochar ont tendance à augmenter la production végétale. Nous estimons que l'augmentation du stock de carbone dans le traitement avec biochar pourrait être en partie accrut par la stimulation de la biomasse racinaire par la synthèse de phytohormone tel que l'éthylène. En ce sens, la présence de biochar a augmenté l'abondance de composés dérivés de la lignine, et de polysaccharides d'origines végétales et microbiennes. Le mélange du lombricompost et du biochar semble l'association la plus adapté afin d'augmenter la fertilité et le stock de carbone des sols tropicaux dégradés

Perspectives:

Les principales perspectives de cette étude sont les suivantes :

- Les résultats de cette étude ont été obtenus à partir d'un sol collecté dans le Nord du Vietnam. Pour généraliser ces résultats, il conviendrait d'étudier plus en détail plusieurs types de sols avec des propriétés pédo-climatiques contrastés représentatifs de l'ensemble du Vietnam.

- Il serait également intéressant d'étudier la composition et la dynamique de la MO dans différent compartiment de la matière organique des sols amendés (fractions physiques).

- Cette étude s'est focalisée sur l'effet des amendements organiques sur le stockage du carbone et la composition de la matière organique des sols. Il conviendrait également d'implémenter cette étude d'une étude de la dynamique des nutriments tel que le phosphore et l'azote.

- Plusieurs études ont montré que le biochar peut augmenter l'activité microbienne de la MOE (Bolan et al., 2013, Khan et al., 2014). Ce résultat nous suggère le rôle intéressant des biochars, dans la fabrication de nouveau compost utilisant le lisier de buffle et le biochar comme matériaux initiaux. Ainsi il serrait intéressant d'étudier le co-compostage permettant de diminuer le temps du compostage en accélérant la minéralisation par les microorganismes. De plus, la présence du biochar peut réduire l'émission de NH_3 et augmenter la nitrification (Khan et al., 2014), permettant ainsi de réduire les pertes en éléments azotées. L'apport de vers de terre durant ce compostage pourrait permettre à développer un amendement plus performant capable de remplir de multiples fonctions, tant du point de vue environnemental qu'agronomique.

- Une méthode d'évaluation physicochimique simple et rapide tel que l'analyse thermogravimétrique (Plante et al., 2011) semble prometteuse afin de statuer sur la stabilité de matière organique tel que le compost (Fernandez et al., 2012). Une perspective intéressante à ce travail serait de confronter de telles données obtenues pour nos AOEs avec les données provenant de nos incubations, ainsi qu'avec les AOEs dans le sol provenant de l'expérimentation en mésocosmes.

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Anexe

Publications

Ngo P.T, Rumpel C, Doan T.T., Henry-des-Tureaux T, Dang D.K, Jouquet P., in Press. Use of organic substrates for increasing soil organic matter quality and carbon sequestration of tropical degraded soil (a 3 years mesocosms experiment). *Carbon Management*.

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Communications

Ngo P.T, Jouquet P, Doan T.T., Dang D.K, Rumpel C., 2013. Long-term impact of organic amendments (compost, vermicompost and biochar) on soil organic matter quality. *RAMIRAN 5th international conference, 3-5 June 2013, Versailles, France*. **Oral presentation**

Ngo P.T, Jouquet P, Doan T.T., Dang D.K, Rumpel C., 2013. The use of organic substrates to increase soil fertility and carbon sequestration of degraded soil in Northern Vietnam.

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Résumé

Une stratégie pour améliorer durablement la fertilité des sols dégradés par érosion au Nord du Vietnam consiste à apporter des amendements organiques exogènes (AOEs). L'application du lisier de buffle, son compost et lombricompost, ainsi que le biochar permettraient à faible coût d’améliorer la qualité des sols et la croissance des plantes en réduisant l'utilisation d'engrais, mais également de séquestrer du carbone. L’objective de cette thèse est d’évaluer (1) l’utilité des quatre AOEs seuls ou en association pour séquestrer du carbone, et (2) l’effet de l’apport des quatre substrats organiques seuls ou en association sur la quantité et qualité des matières organiques d’un sol agricole tropical dégradé typique du Nord du Vietnam. Pour répondre à cet objectif, nous avons effectué des incubations au laboratoire, en sachet de litière dans des conditions de terrain et suivi pendant trois ans une expérience en mesocosme avec ajouts des AOEs au sol cultivé en conditions de terrain.

Nos résultats montrent que le compostage en présence de vers de terre conduit à une plus forte transformation de la matière organique issue du lisier de buffle par rapport au compostage traditionel. Le lombricompost était enrichi en composés azotés et appauvri en polysaccharides, et se caractérisait par une altération plus importante des lignines en comparaison avec le compost. La stabilité biologique de ces AOEs a été liée à leur teneur en carbone soluble, diminuant suivant l’ordre : lisier de buffle > compost > lombricompost, alors que leur réactivité chimique était similaire. Le biochar, constitué de carbone aromatique, se caractérisait par une très forte stabilité biologique et chimique. L’association du biochar avec les autres AOEs a influencé leur stabilité chimique et biologique, en protégeant la matière organique labile contre l'oxydation chimique et la dégradation biologique.

Notre expérience de trois ans en mésocosmes en conditions naturelles a montré que tous les AOEs ont un effet positif sur le stockage du carbone dans le sol ainsi que sur la qualité de la matière organique de sol. L’association de biochar et de lombricompost en augmenttant l’abondance des composés dérivés de la lignine ainsi que la teneur en sucre provenant des plantes et des microorganismes, semble montrer un effet synergique de cette association sur la fertilité des sols (activité microbienne, prospection racinaire). Comparé aux résultats d’incubation en sachet de litières, ces résultats indiquent un effet significatif et contrasté des AOEs sur la croissance des plantes en particulier sur l’apport racinaire.

En conclusion, l’association de biochar et de lombricompost semble la meilleure solution pour améliorer la qualité de sol dégradé par l'érosion dans le Nord du Vietnam. Ces résultats devront être confirmés par des études à long terme, tant du point de vue de la qualité physicochimique que biologique des sols amendés par ces AOEs.

Mots clés : sol tropical, compost, lombricompost, biochar, stock de carbone, matière organique de sol

Abstract

Intensification of agricultural use of tropical soils in Northern Vietnam leads to acidification associated with organic matter loss, rapid decline of their fertility and high erosion rates. Such negative effects may be counteracted by exogenous organic amendments (EOMs). The application of buffalo manure, its compost and vermicompost and biochar alone or in mixture with other EOMs is promising to improve at low cost soil quality, plant growth and carbon sequestration. The aims of this study were to evaluate (1) the usefulness of the four EOM alone or in mixture to sequester carbon and (2) the effect of these amendments on quantity and quality of soil organic matter of a tropical soil degraded by erosion. To anwer to this objective we carried out incubations in the laboratory, in litterbags under field conditions and we monitored a mesocom experiment, where the EOM were added and subjected during three years to cultivation and natural rainfall conditions.

Our results showed that composting in the presence of earthworms led to stronger transformation of buffalo manure than regular composting. Vermicompost was enriched in N-containing compounds and depleted in polysaccharides. It further contained stronger modified lignin compared to regular compost. Biological reactivity of these AOEs was related to their soluble organic matter content, which decreased in the order buffalo manure > compost > vermicompost, whereas their chemical reactivity was similar. Bamboo biochar was enriched in aromatic carbon and characterized by a high biological and chemical recalcitrance. The presence of biochar influenced the biological as well as chemical reactivity of the other organic amendments. It led to a protection of organic matter against chemical oxidation and changed their susceptibility to biological degradation.

Our experience with mesocosmes during 3 year under the natural conditions showed that all organic amendments had a positive effect on soil carbon storage and significantly influences soil organic matter quality. Biochar could increase the soil carbon sequestration potential, when applied in mixture with vermicompost. The presence of biochar increased lignin derived compounds abundance as well as both plant and microbial sugar content of soil amended with vermicompost but it had no effect in the case of control soil. Compared to our litterbag experiment these results show that EOM have a strong contrasting effect on plant growth, in particular root litter input. In conclusion, the incorporation of biochar and vermicompost seems to be the best solution to improuve the quality of degraded soil in Northem Vietnam. More studies about the impacts of this amendment on soil physico-chemical and biological properties in long-term are needed to comfirm these results.

Keys words: Tropical degraded soil, compost, vermicompost, biochar, carbon sequestration, soil organic matter quality