THESE DE DOCTORAT DE
L'UNIVERSITE PIERRE ET MARIE CURIE

Spécialité
Ecologie du sol
Ecole doctorale Diversité du Vivant 0392

Présentée par

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Pour obtenir le grade de

DOCTEUR de l'UNIVERSITÉ PIERRE ET MARIE CURIE

Sujet de la thèse :

Participation des organismes ingénieurs à l'agrégation des sols:
analyse des patrons et mise en évidence des interactions

Date de soutenance le 1 décembre 2011
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Remerciements

J'exprime tout d'abord ma grande reconnaissance au professeur Patrick Lavelle qui m'a fait découvrir et aimer l'écologie du sol. Je le remercie de m'avoir guidé tout au long de ces trois années de doctorat ainsi que des corrections de mes textes, articles et du manuscrit de thèse. Merci également d'avoir bien voulu échanger et partager avec moi ses idées lors de diverses discussions, sans oublier ses encouragements tout au long de mes études. Au-delà de l'encadrement de la thèse, j'exprime à Patrick et Elena mes sentiments de gratitude pour leur gentillesse, leur chaleureux accueil en Colombie et leur dévouement pour me faire découvrir ce merveilleux pays!

Je tiens à remercier tout particulièrement Christophe Hissler pour son intérêt à l'égard de mon travail, sans lequel cette thèse n'aurait pas été réalisable! Je lui suis également très reconnaissant pour son constant soutien, pour sa disponibilité, ses conseils et son aide.

Un grand merci à Pascal Jouquet et Manuel Blouin pour leurs conseils, leur soutien, les diverses discussions et leurs corrections de protocoles expérimentaux.

Je remercie Vincent Hallaire et Jean-François Ponge d'avoir accepté la tâche de rapporteurs, ainsi que Jean-Yves Dubuisson et Eric Blanchart qui ont bien voulu participer au jury de thèse.

Au Luxembourg,

je suis très reconnaissante à Jean François Iffly pour m'avoir aidé à réaliser mes expériences au CRPGL. Je remercie également François Barnich, Jérôme Juilleret et Quoc Nguyen pour leur disponibilité et leur aide au quotidien au CRP-GL. Un grand merci également à Lucien Hoffmann pour l'intérêt à l'égard de mon travail.

Ma gratitude va aussi à Simone Marx de l'ASTA pour avoir réalisé les analyses de texture au dernier moment.

Merci à Jim Meisch pour les photos réalisées de mes expériences au CRP-GL.

A Paris,

un grand merci à Nuria Ruiz pour m'avoir aidé à identifier la macrofaune collectée sur les sites de terrain au Luxembourg, pour ses conseils et son constant soutien au cours de cette thèse.

Je remercie également tout particulièrement Florence Dubs pour ses conseils, ses aides au niveau des statistiques, les discussions sur mon travail et son constant soutien. Au délà de la thèse je la remercie également pour ses encouragements dans les moments difficiles de cette thèse et pour son amitié!

Merci aussi à Raphaël Marichal pour ses conseils, ses corrections et son soutien.

Je remercie également Cornélia Rumpel pour sa collaboration et pour les analyses réalisées à l'INRA de Grignon.

Un grand merci également à Pierre Alain Maron pour avoir accepté de me faire les analyses ARISA, ainsi que pour ses conseils dans la rédaction du protocole experimental.

A l'IRD je remercie Anne Pando, Sandrine Rieu et Annabelle Mellot de leur aide et leur disponibilité, sans oublier Fatima, Chi, Sylvain, Zeeshan, Charlène, Gaël et Thuy pour les bons moments passés à Bondy.

Je suis reconnaissante à Marie Donatien et Gaëlle Boutin, de l'école doctorale Diversité du Vivant, pour leur aide au niveau administratif.

Je remercie le Fond National de la Recherche (Luxembourg), l'Université Pierre et Marie Curie, le CRP-GL, l'IRD et le CIAT pour leurs appuis logistiques, administratifs et financiers.

En Colombia,

agradezco el apoyo, la colaboración y amistad de Sandra Loaiza y Pilar Hurtado. Agradezco a Elena Velàsquez por sus ayudas en los analisis estadisticos. Agradezco a Josefa y Hernan por su ayuda en la realización de las experiencias en los invernaderos y agradezco Steven Fonten por sus consejos, sus sugerencias y su ayuda en la correcta escritura en ingles. Un agradecimiento especial a Jorge Benalcazar por proporcionarme la tierra que me permitio realizar mis experiencias en colombia.

Agradezco a Sebastian y a su familia por el apoyo y la paciencia que me dieron durante todo mi tiempo en Colombia y por hacerme descubrir su bonita cultura y su hermoso pais.

An och iech een decken Merci Mam, Pap, Michel, Joël, Bomi a Bopi fier all är léif Ennerstëtzung, ouni déi ech ni esou wäit komm wier!

Résumé

Participation des organismes ingénieurs à l'agrégation des sols:
analyse des patrons et mise en évidence des interactions

Les organismes ingénieurs sont des acteurs majeurs de la macroagrégation, un processus indispensable à la production de services écosystémiques par les sols. L'ignorance de l'origine réelle des différents types d'agrégats trouvés dans les sols, de leurs transformations au cours du temps et de leurs positions dans la matrice du sol, empêche la description et la modélisation de la dynamique de la macroagrégation du sol et des processus associés.

Nous avons montré que la spectroscopie dans le proche infra-rouge (NIRS) permet d'identifier l'origine des macroagrégats construits par différents ingénieurs écosystémiques, les vers de terre et les plantes, dans différents milieux. Nous avons retrouvé dans les signatures spectrales de macroagrégats, collectés en surface et dans un bloc de sol sur le terrain, les signatures de référence de structures biogéniques obtenues dans des conditions contrôlées en microcosmes au laboratoire. Des expériences complémentaires réalisées en serre ont permis d'étudier les interactions entre racines et vers de terre dans la production de macroagrégats. Les structures biogéniques produites en présence d'une espèce de plante et d'une espèce de ver de terre ont révélé des effets additifs des deux espèces dans leurs signatures spectrales montrant une interaction plutôt que la production de structures isolées spatialement. Une dernière étude réalisée en laboratoire a montré que les effets, causés par le vieillissement de turricules sur leurs teneurs en MO, sont suffisants pour affecter les signatures spectrales des turricules et ainsi permettent de dater l'âge d'apparition d'un turricule.

Mes travaux de thèse proposent une nouvelle méthodologie pour analyser les origines des macroagrégats du sol, pour quantifier l'apport relatif des ingénieurs écosystémiques à l'agrégation du sol et pour évaluer la dynamique des macroagrégats dans la structure du sol.

***Mots-clés** : Agrégation des sols, Organismes ingénieurs, Spectroscopie dans le proche infrarouge, Vieillissement de turricules, Interactions entre vers de terre et racines*

Abstract

Participation of ecosystem engineers to soil aggregation:
analysis of patterns and interactions

Soil ecosystem engineers are major actors of soil macroaggregation, a process that drives the production of ecosystem services by soils. However, our inability to identify the origins of different types of macroaggregates found in soils, their transformations during aging and their positions in the soil matrix, is an obstacle for describing and modeling their dynamics and associated processes.

We showed that near infrared spectroscopy allows identifying origins of macroaggregates produced by different ecosystem engineers, earthworms and plants, in different environments. We found in spectral signatures of macroaggregates, collected at soil surface and in blocks of soil extracted in the soil matrix, reference signatures of biogenic structures obtained under controlled conditions in microcosms in a laboratory experiment. Complementary experiences realized in a greenhouse allowed us to study interactions between roots and earthworms in macroaggregate production. Biogenic structures, produced in presence of a plant species and an earthworm species, revealed additive effects in their spectral signatures, showing an interaction rather than a production of structures in different microenvironments. A last study realized in laboratory conditions demonstrated that OM modifications, caused in aging casts, are large enough to get detected by NIRS in macroaggregates and to estimate a cast's age.

My study proposes a new method to analyse soil macroaggregates origins, to quantify the relative contribution of ecosystem engineers to soil aggregation and to evaluate soil macroaggregate dynamics in the soil structure.

***Keywords**: Soil Macroaggregation, Ecosystem engineers, Near infrared spectroscopy, Cast aging, Earthworm-root interactions*

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Introduction

I. Introduction générale

Partout dans le monde, l'agriculture intensive a provoqué un appauvrissement des sols en matière organique, une perte d'une grande partie de leur biodiversité, sans parler des dégâts causés par l'érosion (MEA, 2005). Il est urgent de mettre en place des stratégies de gestion qui optimisent simultanément les fonctions de production, de régulation climatique, de stockage et d'épuration de l'eau, tout en garantissant le maintien de la biodiversité. Pour définir de tels systèmes de gestion des ressources naturelles, nous avons besoin de meilleures connaissances des dynamiques de la matière organique du sol et de son interaction avec la structure du sol, la clé de la conservation de l'intégrité des fonctions du sol (Pulleman, 2004). La matière organique du sol joue un rôle fondamental en assurant sa fertilité et sa productivité. Etudier sa dynamique est donc d'un intérêt majeur afin de maintenir ou de rétablir la qualité des sols.

L'unité basique de structure du sol est appelée agrégat du sol. Ce dernier est défini comme une association de particules du sol, qui ont une cohésion interne, inter-particulaire plus élevée que les particules autours (Lavelle et Spain, 2001). Dans la classification des agrégats du sol on différencie principalement entre les agrégats "biogéniques" et "physicogéniques" en fonction de leurs formes. Les agrégats biogéniques sont des macro-agrégats de forme arrondie qui sont visuellement identifiés comme des turricules ou comme d'anciens turricules, tandis que les agrégats physicogéniques sont des macroagrégats angulaires ou subangulaires qui ne peuvent pas être identifiés comme des turricules ou des vieux turricules (Bullock et al., 1985; Pulleman et al., 2005). Tisdall et Oades (1982) ont proposé une classification des agrégats principalement en microagrégats (<250 µm) et macoragrégats (>250 µm). Au cours de ce travail de thèse nous avons considéré uniquement les macroagrégats du sol.

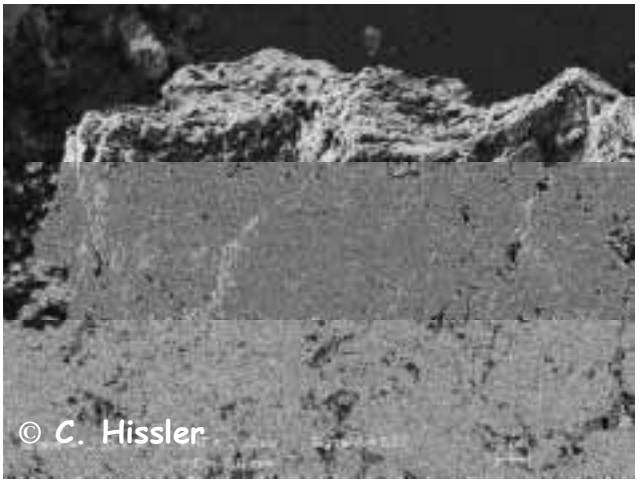

Figure 1: Microagrégats

Figure 2: Macroagrégat biogénique (>250µm)
Turricule de vers de terre

Figure 3: Macroagrégats biogéniques (>250µm)
Agrégats racinaires

Figure 4: Macroagrégats physicogéniques (>250µm)

L’agrégation remplit de nombreuses fonctions dans les sols. Les agrégats du sol sont souvent considérés comme des unités fonctionnelles qui contrôlent la dynamique de la matière organique du sol (MOS) et le cycle des éléments nutritifs (Tisdall et Oades, 1982; Beare et al., 1994; Chevallier et al., 2004; Bossuyt et al., 2005). L'agrégation du sol est également de première importance dans la régulation de l'écoulement de l'eau dans le sol (Prove et al., 1990) et la limitation de la diffusion de l’oxygène (Sexstone, 1985). Elle contrôle l'adsorption et le relargage d'éléments nutritifs (Linquist et al., 1997; Wang et al., 2001) ainsi que l'impact des eaux de ruissellement et l'érosion des sols (Barthès et Roose, 2002). Les agrégats influencent également les structures des communautés microbiennes (Hattori, 1988). Tous ces processus ont des effets profonds sur les dynamiques de la matière organique (MO) du sol et sur le cycle nutritif.

II. Développement des connaissances sur les mécanismes de l'agrégation

Depuis la première moitié du 20ème siècle le lien entre l'activité des organismes du sol, la décomposition et stabilisation de la matière organique du sol (MOS) et la dynamique de l'agrégation dans le sol ont été reconnus et étudiés. A cette époque il était déjà clair que 5 facteurs majeurs influencent l'agrégation du sol: 1. la faune du sol; 2. les microorganismes; 3. les racines; 4. des agents liants inorganiques et 5. les variables environnementales (Six et al. 2004). Vers 1960, plusieurs études pionnières, qualitatives, ont été menées pour comprendre l'influence de ces 5 facteurs dans l'agrégation. Martin et al. (1955) décrivent en détail l'impact positif des résidus organiques, de l'activité microbienne et des cations échangeables sur l'agrégation du sol.

Dans la période des années 50 jusqu'en 1984 des théories fondamentales sur les mécanismes de l'agrégation sont publiées dans 4 travaux majeurs. Le premier était celui d'Emerson (1959) qui propose un premier modèle d'un soil crumb formé de domaines de particules d'argiles et de quartz orientées. Ensuite Edwards et Bremner (1967) présentent la théorie selon laquelle une réaction en phase solide entre des minéraux d'argile, des cations polyvalents et la MO du sol est le processus majeur menant à la formation de microagrégats. En 1982, Tisdall et Oades proposent le concept de la hiérarchie des agrégats qui décrit une l'échelle spatiale des agrégats (micro- et macroagrégats) et comment les mécanismes de leur formation dépendent de cette échelle spatiale. Cette théorie explique comment les différents agents liants des agrégats agissent à différents niveaux de la hiérarchie des agrégats. Des agents liants, interagissant dans la formation et la stabilisation des agrégats, de nature persistante ou temporaire, ont été décrits. Des particules primaires libres et des petits agrégats de la taille du limon (<20µm) sont liés ensemble en microagrégats (20-250 µm) par des agents persistants (MO humifiée et des complexes de cations métalliques polyvalents), des oxydes et des aluminosilicates. Ces microagrégats à leur tour sont liés en macroagrégats (>250 µm) par des agents liants temporaires (hyphes fongiques et racines) par compactions locales du sol par un réseau d'hyphes ou de racines ou par des polysaccharides d'origine microbienne ou de plantes. Selon l'ordre hiérarchique, les microagrégats sont considérés comme plus stables que les macroagrégats. En 1984, Oades améliore la théorie de 1982 en modifiant le concept de la hiérarchie des agrégats et précisant que les microagrégats sont formés au centre des macroagrégats. Les racines et hyphes, formant les macroagrégats, ne persistent pas au cours du temps et se décomposent, entrainant la fragmentation des macroagrégats. Ces fragments revêtus de mucilages, résultant de la décomposition des tissus d'hyphes et de racines morts, se recouvrent d'une croûte d'argiles ce qui mène à la formation de nouveaux microagrégats.

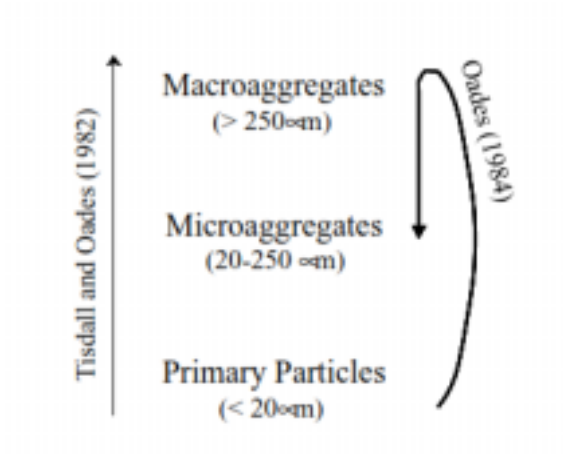

Figure 5: La chronologie de la formation des agrégats selon les ordres hierachiques du modèle de Tisdall and Oades (1982) vs. amélioré par Oades (1984). Adapté de Six et al. (2004)

Elliott et Coleman (1988) adoptent le concept de formation de microagrégats dans les macroagrégats d'Oades (1984) et attribuent cette formation de microagrégats aux conditions anaérobies du centre des macroagrégats. Il propose également un concept hiérarchique de 4 catégories de pores: 1. les macropores, 2. l'espace des pores entre les macropores, 3. les pores entre les microagrégats dans les macroagrégats et 4. les pores dans les microagrégats. Cette théorie a permis de comprendre comment le réseau des pores détermine le lien entre le réseau alimentaire du sol entre les organismes du sol; les microarthropodes vivant dans les macropores, les nématodes se déplacent entre les pores des macropores; les protozoaires et petits nématodes ainsi que les filaments mycéliens habitent l'espace des pores entre les microagrégats et les bactéries sont protégées dans les pores des microagrégats.

Le modèle d'Oades (1984) a été validé par de nombreuses études (Beare et al. (1994a), Gale et al. (2000), Six et al. (1999a, 2000). Les macroagrégats (250-2000 µm) sont en premiers formés autour de la matière organique fraîche. Si les macroagrégats ne sont pas perturbés les résidus se décomposent et se fragmentent en particules plus fines qui progressivement sont enveloppées par une couche de particules d'argile et de produits microbiens ce qui provoque la formation de microagrégats au sein des macroagrégats. Quand les macroagrégats sont déstabilisés, par la dégradation des agents liants, ils se divisent en microagrégats stables. Ces microagrégats forment ensuite les unités de base pour la formation de nouveaux macroagrégats.

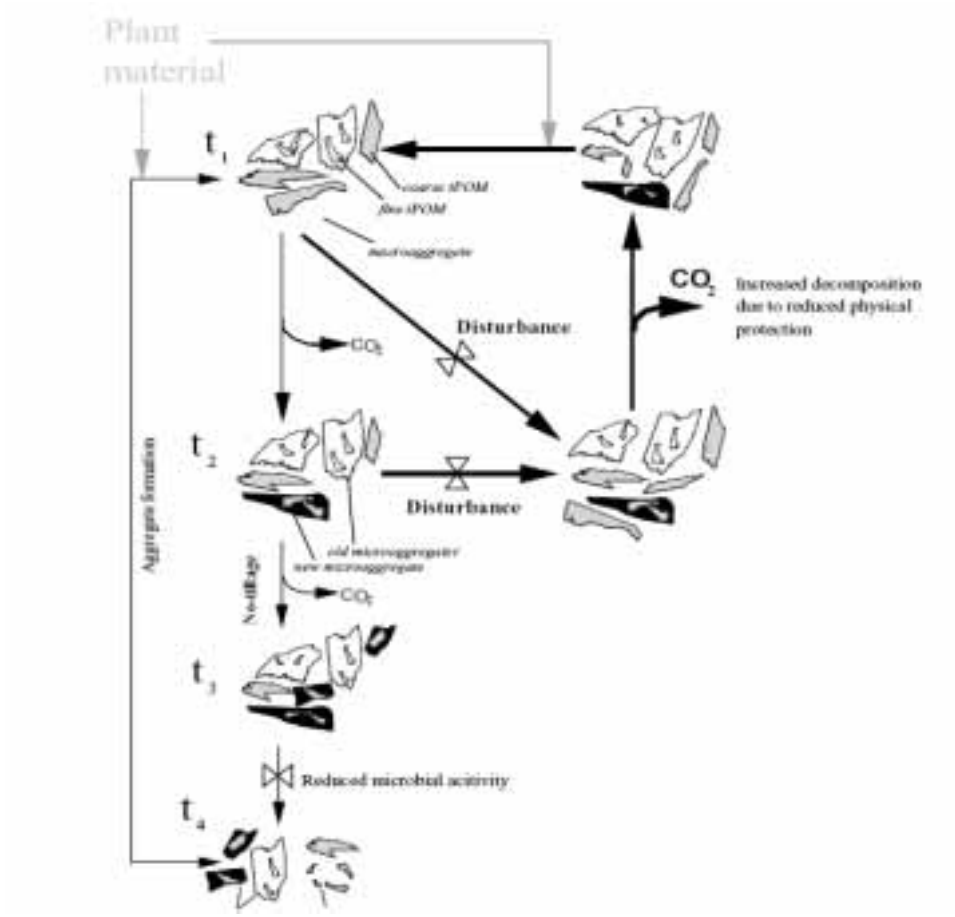

Figure 6 : Ce modèle du "cycle de vie" d'un macroagrégat illustre la formation de nouveaux microagrégats et leur accumulation dans les macroagrégats ainsi que la mineralisation du C organique associé aux agrégats. Figure adoptée de Six et al. 2000.

Plante et Mc Gill (2002a et b) ont présenté un nouveau modèle de l'agrégation où ils suggèrent que la relation entre la protection physique de la MOS et le turnover des macroagrégats diffère en fonction de la nature de la MO incorporée, c'est-à-dire entre résidus de MO frais et de MO stabilisée. Pour la MO stabilisée, plus le turnover serait lent plus la protection de la MO serait élevée. La séquestration du C dans le sol serait maximale pour un turnover d'agrégats à vitesse intermédiaire.

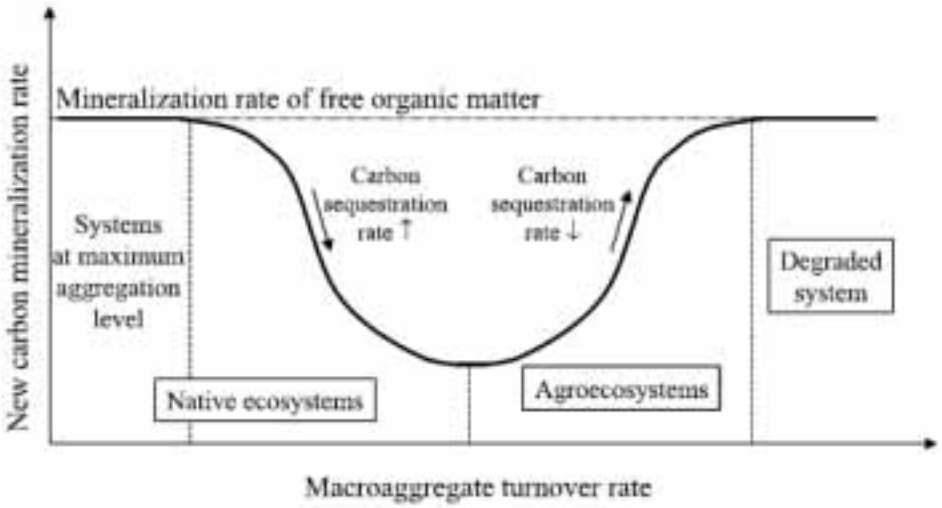

Figure 7: Changement du turnover des agrégats et minéralisation du C frais additionné à travers des écosystèmes. Figure adaptée de Plante and McGill (2002a).

Basées sur ces théories fondamentales, de nombreuses autres études on été menées pour comprendre le rôle de l'agrégation sur la fertilité et la structure du sol (Angers et al. 1997; Jastrow et Miller, 1998; Six et al. 1998, Puget 2000, Wander et Yang 2000, Puget et Drinkwater 2001). Cette approche développée au laboratoire se caractérise par l'ignorance complète du rôle de la macrofaune dans l'évolution de la matrice du sol.

Shipitalo and Protz (1989) présentent un premier modèle sur les mécanismes de l'agrégation par les vers de terre par la formation de microagrégats au sein des macroagrégats. Ils décrivent comment les vers de terre fragmentent la structure préexistante de fragments de litière et du sol durant la digestion du sol et de la MO ingérée. Durant le passage dans le tube digestif les minéraux d'argiles et de matière organique sont intimement mélangés et sont recouverts d'une couche de mucus et ainsi forment un nouveau nucléus pour des microagrégats. Au sein des turricules produits, le séchage et le vieillissement des turricules (thyxotropic hardening) facilitent le renforcement de liens entre des matériaux organiques, du mucus et des minéraux pour stabiliser les microagrégats nouvellement formés.

Cette théorie de Shipitalo et Protz (1989) sera reprise est approfondie par Barois et al. dans une étude en 1993. Par des analyses de scanning electron microscopy elle permet de visualiser comment le sol ingéré par les vers de terre est complètement déstructuré au cours du passage dans l'intestin du ver de terre et comment dans la partie postérieure du tube digestif le sol est restructuré en microagrégats. Les micro-organismes auparavant inactifs, reprennent la minéralisation de la fraction organique du sol au moment du passage dans le tube digestif,

stimulés par le mucus facilement dégradable produit par le ver de terre et sont remis au contact de particules organiques par le malaxage (1986). En comparaison à l'étude de Shipitalo et Protz, Barois et al. précisent que les microagrégats reformés dans la partie postérieure de l'intestin sont à la base des colonies de microorganismes qui sont ensuite couvertes par des particules d'argiles et de mucus de vers. Ces travaux permettent de mettre en évidence comment les éléments organiques du sol, initialement protégés dans les microagrégats, seront dégradés pour certains tandis que d'autres seront protégés dans les nouveaux microagrégats. Cette étude décrit également le rôle des populations microbiennes dans le tube digestif des vers. (détails des mécanismes sont décrits dans le paragraphe 2.2.3.)

En 1991, Martin et al. ont mis en évidence l'importance de l'assimilation de la MO dans les turricules de vers de terre (de 2 à 18%) et de la dynamique de la MO dans les turricules à court et à long terme. Des différences significatives de la distribution de la MO entre les tailles des fractions de particules ont été observées lors de la digestion du ver de terre. La concentration des fractions grossières diminuait fortement tandis qu'augmentait celle des fractions plus fines. De telles études de l'effet des vers de terre sur la dynamique du C restent rares (Martin et al, 1991; McCartney et al. 1997; Gilot-Villenave et al., 1996; Pashani et al., 1996) et leur résultats semblent contradictoires. Un point commun à toutes ces études est le stockage (plus ou moins importante en fonction des études) du C à long terme dans les turricules.

Plusieurs études publiées montrent que la stabilité structurale des turricules de vers de terre est plus élevée que celle des autres agrégats (Monnier et Jeanson, 1964; Van Rhee, 1977; De Vleesschauwer et Lal, 1981; Mc Kenzie et Dexter, 1988; Marinissen, 1994). Shipitalo et Protz (1988) mettent également en évidence que les turricules de vers de terre contiennent des microagrégats plus stables que les microagrégats contenus dans le sol environnant. Cependant les activités d'agrégation par les vers de terre permettent uniquement une amélioration de la stabilité structurale des agrégats si les turricules ont pu vieillir ou sécher (Shipitalo et Protz, 1988; Marinissen et Dexter, 1990). Alors que la stabilité structurale des turricules dépend du comportement alimentaire des vers de terre et de la qualité de MO ingérée (Shipitalo et Protz, 1988), la stabilité structurale dépend également des microorganismes qui prolifèrent dans les matériaux ingérés dans le tube digestif des vers de terre (Parle, 1963; Arthur, 1965) et dans les turricules (Brown, 1995; Edwards et Bohlen, 1996; Brown et al. 2000). Des effets opposés des vers de terre sur la dynamique de l'agrégation et son impact sur la structure du sol ont été mis en évidence par une série de travaux de Blanchart et al. (1993, 1997 et 1999) dans des écosystèmes tropicaux (voir sous chapitre 2.3.d).

En 2001, Jongmans et al. observent l'incorporation de matière organique fine, dans les premiers stades de sa décomposition, dans la microstructure dans les turricules de vers de terre. Ils concluent que la formation de microagrégats au sein des turricules est importante pour protéger la MO labile du sol. Ensuite une série d'études mettent en évidence que les vers de terre facilitent l'incorporation de résidus frais de MO dans les microagrégats des turricules (Bossuyt et al. 2004; Fonte et al. 2007) et que ce C est efficacement protégé contre la minéralisation (Pullmann et al. 2004; Marinissen, 2004 et Bossuyt et al. 2005).

III. Problématiques

Jusqu'à présent la recherche s'est principalement focalisée sur l'évaluation globale des mécanismes de l'agrégation du sol par des mécanismes physiques ou microbiens, délaissant ainsi quelque peu l'agrégation par la macrofaune. La stabilisation des agrégats par l'intermédiaire de processus physiques ou par l'action des racines et de la microfaune du sol ont été mis en évidence (Tishdall et Oades 1982, Plante et Mc Gill 2002 (a et b)). Le lien entre matière organique et agrégation du sol a été souligné par de nombreux auteurs. On a décrit certains mécanismes par lesquels ces débris organiques incorporés dans les sols par labour favorisent l'agrégation du sol : soit comme ressource pour des bactéries qui produisent des polysaccharides extracellulaires (Chenu 1993), ou pour des champignons mycorhiziens qui produisent de la glomaline (un autre composé fongique agrégeant) (Rillig et al, 2001 a et b), ou par la formation de réseaux denses de filaments qui retiennent les particules de sol.

Cependant, peu d'attention a été accordée à la formation de macroagrégats par les organismes ingénieurs de l'écosystème et leur dynamique dans le sol. Ces structures biogéniques ont généralement des durées de vie beaucoup plus longues que les agrégats d'origine physique et peuvent représenter un pourcentage considérable dans la structure du sol (Marinissen, 1994). Les travaux réalisés jusqu'à présent ne considèrent pas l'origine (biogénique ou physique) des agrégats, ni leur dynamique temporelle et spatiale, ni la dynamique de leur vieillissement conduisant à leur destruction.

Par ailleurs, un grand obstacle pour la description et la modélisation des dynamiques des macro-agrégats du sol et des processus associés, est l'ignorance de l'origine réelle des différents types d'agrégats trouvés dans les sols, de leur recyclage et de leurs positions dans la matrice du sol. L'identification des origines des agrégats trouvés dans le sol par analyse de leurs signatures spectrales est une approche nouvelle.

IV. Les mécanismes de l'agrégation dans le sol

A. L'agrégation par les microorganismes

La contribution de l'activité microbienne dans la formation d'agrégats et leur stabilisation a été résumée dans les revues de Lynch et Bragg (1985), Oades (1993) et Degens (1997) et Six et al. (2004). Bactéries et mycéliums (mycorrhizés ou saprophytes) participent aux mécanismes de la formation d'agrégats dans le sol. Il reste cependant difficile de séparer la contribution des deux acteurs à l'agrégation.

Le mycélium fongique a été décrit comme un "sticky string bag" à cause de ces réseaux d'hyphes qui encerclent des particules du sol et forment des macroagrégats par compaction du sol (Tisdall and Oades, 1982; 1993 ; 1997; Bossuyt et al. 2001; Plante and Mc Gill 2002 (a et b)). Les particules sont ensuite collées ensemble et stabilisées par des polysaccharides extracellulaires produits par les hyphes (Oades et Waters, 1991, Chenu, 1989). Cependant ces agrégats ne sont stables qu'aussi longtemps que le réseau d'hyphes est maintenu (Tisdall and Oades, 1982 ; Oades 1984 ; Plante and Mc Gill 2002 (a et b)). Parmi les mycéliums, les mycorhizes à arbuscules (MA) sont considérées par Rillig et al. (2001) comme plus actives que les mycéliums saprophages dans l'agrégation des sols en raison de leur biomasse dominante dans la rhizosphère. Les mycorhizes à arbuscules forment des associations mutualistes avec les racines de la majorité des plantes développées, à l'exception notable des Crucifères. Miller et Jastrow (2000), Rillig et al. (2001, 2002 et 2004) expliquent la formation de macroagrégats par les VAM par une production de glomaline, une glycoprotéine qui colle les particules minérales et la OM du sol ensemble. Des études menées par Wright and Upadhyaya (1998) et Rillig et al. (2001) montrent des corrélations positives entre le pourcentage de macroagrégats et la concentration de glomaline dans le sol. Mais si le rôle de la glomaline dans l'agrégation est bien identifié, l'origine de la molécule reste controversée. Récemment Gillespie et al. 2010 ont découvert que la glomaline ne montre aucune similitude avec des protéines ou de l'ADN d'origine de mycélium, ce qui met en question l'origine de la glomaline trouvée dans les agrégats. La minéralogie du sol influence également les mécanismes microbiens qui contribuent à l'agrégation d'un sol. Dans les sols à texture sableuse les hyphes fongiques sont les seuls microorganismes capables d'agréger les particules de sable en macroagrégats. .La biomasse bactérienne n'est que très peu relié à l'agrégation (Degens et al. 1994 ; Degens et Sparling 1996). Cependant dans les sols à texture argileuse, bactéries et mycéliums, jouent tous les deux des rôles importantes. Même si le rôle des

mycéliums dans la stabilisation des macroagrégats est bien identifié, il a aussi été mis en évidence un effet inverse des mycéliums sur les macroagrégats.

Les micro-organismes d'origine bactérienne contribuent également à l'agrégation de particules du sol. Chenu (1993) a mis en évidence la production de polysaccharides extracellulaires, qui forment une gaine organo-minérale à la surface extracellulaire bactérienne, composée d'un collage de polysaccharides et de particules minérales (majoritairement d'argile). Ce sont ces gaines d'argile et de polysaccharides qui contribuent à la liaison de particules dans l'agrégation.

Figure 8. Associations entre polysaccharides et argiles (Chenu et al. 1993)

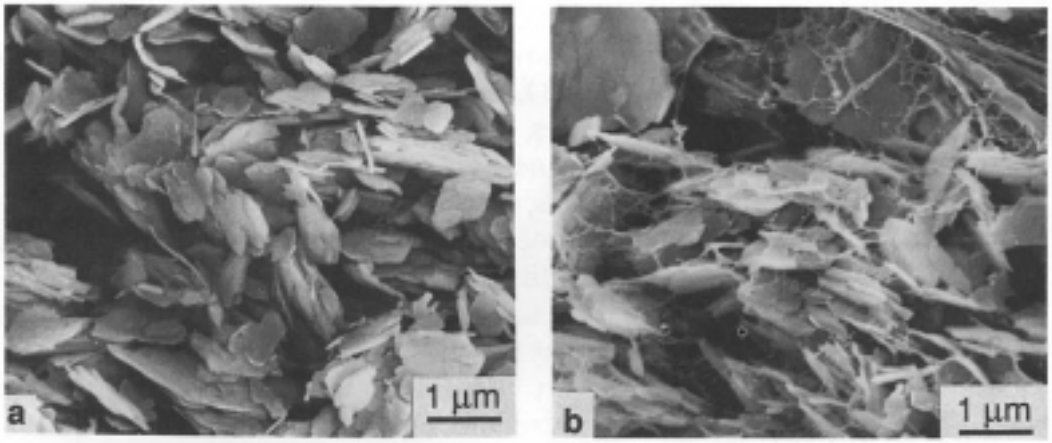

Figure 8: Microstructures d'association entre argiles et polysaccharides. (a) Cryo-SEM kaolinite, - 0.0032 MPa. (b) Cryo-SEM kaolinite-xanthan; contenu de polysaccharides extracellulaires 2,5% w/w argile, -0.0032 MPa.

B. L'agrégation par les organismes ingénieurs du sol

a. Les organismes ingénieurs du sol

Les ingénieurs écosystémiques sont définis par Jones *et al.* (1994 et 1997) comme des organismes qui modulent directement ou indirectement la disponibilité des ressources à d'autres espèces, en causant des changements d'état physique de matériaux biotiques ou abiotiques. Dans le sol, les vers de terre, les plantes (avec leurs racines), les termites et les fourmis sont considérés comme les principales espèces ingénieurs, jouant un rôle dans la dynamique de l'agrégation du sol. Ces organismes sont également responsables de l'altération

des dynamiques de l'écosystème à travers la modification, maintenance et/ou création d'habitats pour d'autres organismes de l'écosystème (Jouquet et al., 2006) dans le sol.

Chaque organisme ingénieur possède des traits de vie bien spécifiques qui caractérisent leurs actions sur leur environnement. Chez les vers de terre, les attributs des traits varient même fortement au cours de la vie, car les jeunes peuvent être 100 fois plus petits que les adultes de la même espèce (Lavelle 1983). Les traits biologiques jouent un rôle important dans la composition des communautés et dans l'action qu'ont celles-ci sur leur environnement (par exemple l'action des vers de terre sur le sol). Ainsi chaque organisme ingénieur produit des macroagrégats avec des caractéristiques propres à son espèce ou à sa communauté. Par conséquent ces agrégats ont des dynamiques différentes et aussi des impacts différents sur le sol.

b. Les différents groupes fonctionnels de vers de terre

Trois grandes catégories de vers de terre sont définies d'après des critères morphologiques, physiologiques et l'impact fonctionnel (Bouché 1977; Lavelle and Spain 2001) – Fig. 2:

Les **épigés** sont les plus petites espèces (1 à 5 cm) ; ils évoluent dans les premiers centimètres du sol, brassant et fractionnant la matière organique.

Les **endogés** (1 à 20 cm) ne viennent jamais à la surface (ou n'y vienne que rarement). Vivant constamment dans le sol, ils créent des réseaux de galeries horizontaux très ramifiés et se nourrissent de matière organique déjà dégradée. Les endogés sont divisés en trois sous catégories en fonction de leur utilisation des ressources (Lavelle, 1981) : Les polyhumiques ingèrent sélectivement des particules de OM dans le sol et vivent à l'interface du sol et de la litière. Les mésohumiques se nourrissent du sol dans les premiers 10-15 cm de la matrice du sol et n'ingèrent pas sélectivement des particules de MO. Les oligohumiques vivent dans des couches profondes du sol et se nourrissent de ressources alimentaires très basses en énergie.

Les **anéciques,** dont le lombric commun fait partie, sont les plus grandes espèces : 10 à 110 cm. Ils se déplacent dans des galeries verticales qui peuvent descendre jusqu'à 3 m. Ils mélangent la matière organique à

la matière minérale et rejettent leurs déjections à la surface du sol, sous forme de turricules. Ces vers de terre se reconnaissent à leur pigmentation antéro-dorsale.

Seuls les vers de terre anéciques et endogés sont considérés comme des ingénieurs de l'écosystème du fait de leur impact particulier sur l'ensemble du sol.

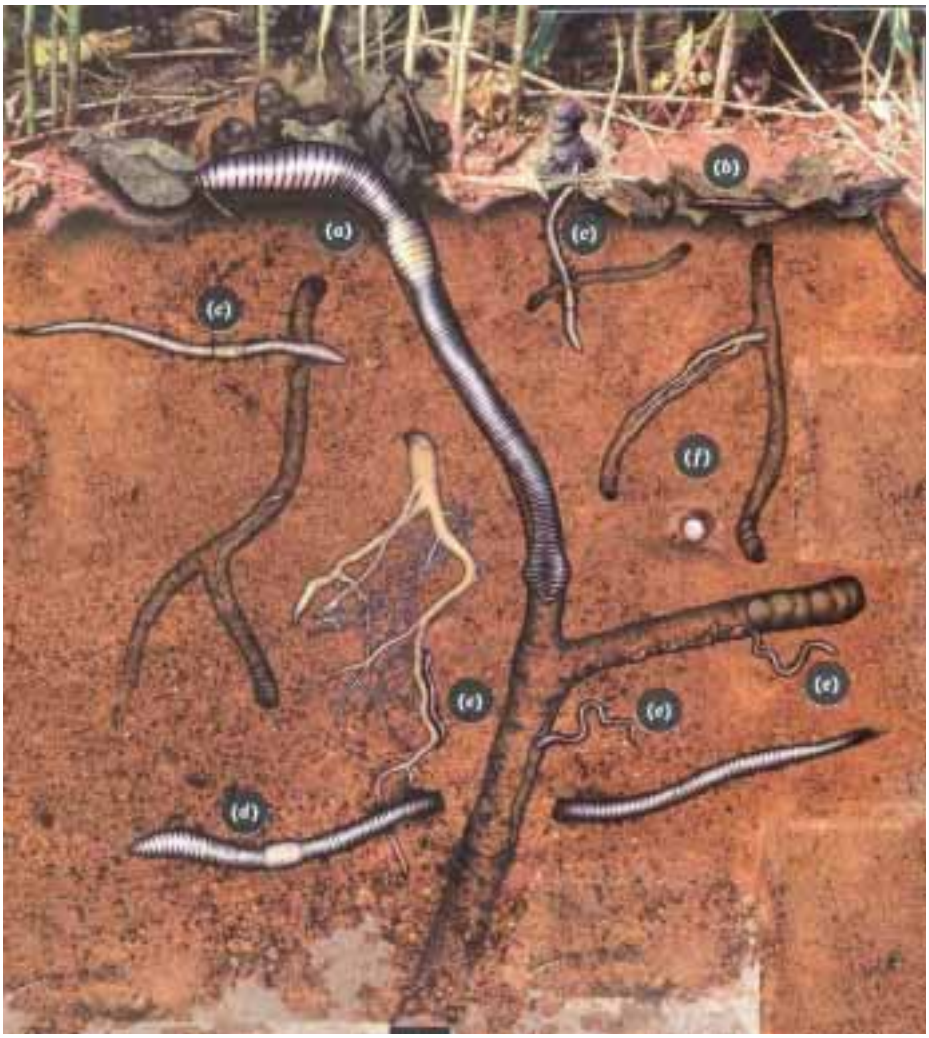

Figure 9: Illustration montrant les différentes stratégies écologiques et la diversité fonctionnelle des vers de terre. (a) ver anécique dans sa galerie verticale semi-permanente; (b) ver épigé, habitant de la litière ; (c), (d) et (e) différents vers endogés dans des galeries subhorizontales éphémères ; (f) cocon de ver. Adapté de Lavelle et al. (1998)

c. Processus digestifs et agrégation du sol par les vers de terre

Les turricules des espèces épigées et anéciques se distinguent principalement des turricules produits par les espèces de vers endogés en raison des comportements alimentaires distincts dans les trois groupes fonctionnels. Les espèces épigées et anéciques consomment un mélange de litière et de sol en proportions variant d'une espèce à l'autre, montrant un gradient des espèces épigés (turricules plus riches en MO) aux espèces anéciques. Généralement les espèces anéciques ingèrent des débris plus larges, mais plus décomposés par la mésofaune et la microflore, alors que les espèces épigés consomment des débris de litière plus petits, en raison de leur petite taille (Lavelle et Spain, 2001). Les espèces endogées, considérées comme des agents majeurs de l'agrégation et de la stabilisation de la MO du sol (Lavelle et Spain, 2001), se nourrissent de sol enrichi par de la MO.

Caractéristiques de l'agrégation par les espèces épigés

Les espèces épigées vivent au dessus de la surface minérale du sol, principalement dans la couche de litière couvrant le sol. En se nourrissant directement de la litière du sol, les espèces épigées produisent des turricules fortement enrichi en MO. Les espèces épigés possèdent un système digestif avec une capacité enzymatique assez diverse, incluant entre autre des cellulases, qui leur permet de se nourrir directement de la litière morte des plantes (van Gansen, 1962; Parle, 1963; Neuhauser et Hartenstein, 1978). Les agrégats de ces espèces restent très peu étudiés de nos jours. Une des raisons pour ce manque de connaissances et notamment la taille réduite des turricules produits.

Caractéristiques de l'agrégation par les espèces anéciques

Les vers de terre anéciques enfouissent des feuilles mortes de la litière dans leur galeries et les ingèrent quelques semaines plus tard mélangées à du sol, après que l'interaction avec la microflore les a rendu plus digestible pour les vers de terre (Wright, 1972; Cooke, 1983, Cortez *et al.*, 1989). D'autres espèces accumulent la litière à la surface du sol dans l'orifice de leur galerie ou autour. Ils créent ainsi des "middens" ou apparaît une forte activité de la microflore et de la mésofaune à la surface du sol (Nielsen and Hole, 1964; Dozsa-Farkas, 1978; Hamilton and Sillman, 1989). Les espèces anéciques combinent une forme de digestion, basée sur une

production de quelques enzymes basiques, avec une digestion mutualiste qui intervient en combinaison avec la microflore du sol ingéré (Lavelle and Spain, 2001). Certaines espèces anéciques, comme *L. terrestris* (Bouché 1983), ré-ingèrent la litière contenue dans leurs turricules plusieurs fois.

Parmi les macroagrégats produits par les espèces anéciques se distinguent deux natures de biostructures différentes, les turricules produits en surface du sol ou dans les galeries et les galeries produites creusées dans le sol (Brown et al. 2000). Lors de la formation de galeries les vers de terre exercent de la pression sur le sol environnant et du mucus externe est déposé sur les parois externes (Edwards et Bohlen 1996). Ils peuvent aussi creuser en ingérant et rejetant le sol. Les parois des galeries ont des contenus moins élevés en MO que les turricules mais se distinguent clairement du sol environnant. Les 2 premiers millimètres de la paroi des galeries ont une concentration en microflore très élevée, 5 à 25% de la microflore totale du sol (Lavelle et Spain 2001). Les parois des galeries sont tapissées à chaque passage du vers par du mucus. Les turricules de surface des vers de terre anéciques se caractérisent par une teneur en MO relativement élevée, avec un contenu important de larges particules de MO de plantes, en comparaison à des turricules produits par des espèces de vers de terre endogées (Graff, 1971, Aldag et Graff, 1975). Cependant les proportions de débris de plantes dans les turricules peuvent varier beaucoup suivant que les vers de terre se nourrissent directement de la litière à la surface ou dans les galeries ou ingèrent du sol pour creuser des galeries en profondeur. Il a aussi été montré par Bolton et Philipson (1976) que les turricules d'espèces anéciques sont plus riches en éléments minéraux fins que le sol environnant. Finalement il a aussi été observé par Kanyonyo ka Kajando (1984) que les espèces tropicales ingèrent des proportions de litière plus faibles que les espèces anéciques des régions tempérées. Cependant la structure, la composition et l'abondance des turricules internes, produits dans les galeries, n'ont pas été mesurées jusqu'à présent.

Caractéristiques de l'agrégation par les espèces endogés

Les espèces de vers de terre endogés peuvent être classées en deux grands groupes en fonction de leur comportement alimentaire : endogés polyhumiques, mésohumiques et oligohumiques (Lavelle 1983). Leurs turricules produits peuvent avoir des compositions assez variables, notamment en concentration en MO du sol, en fonction du comportement alimentaires des vers de terre.

1) Les endogés polyhumiques ont un comportement alimentaire intermédiaire entre celui des anéciques et celui des endogés. Ces espèces entraînent de la litière de la surface du sol dans la matrice et la mélangent au

sol. Des proportions de débris de plantes assez importantes peuvent être retrouvées dans leur tube digestif (jusqu'à 50-70%). Ces espèces forment également des galeries majoritairement subhorizontales (Kretzschmar, 1982) et des turricules peuvent être produits à la surface du sol et dans la matrice.

2) Les endogés mésohumiques et oligohumiques sont exclusivement géophages, se nourrissent de la MO du sol et ne produisent pas de réseaux de galeries semi permanentes comme les anéciques. L'analyse du contenu digestif a montré qu'ils se nourrissent entre autres d'une sélection de tissus de plantes mortes.

La plupart des vers de terre endogés, sélectionnent avec plus ou moins d'intensité les particules organiques et minérales qu'ils ingèrent. Dans les régions tempérées, Bouché et Kretzschmar (1974) on trouvé des concentrations de débris de plantes allant jusqu'à 15% dans les turricules d'espèces endogés. Lee (1985) et de nomberux auteurs à sa suite (Barois et al., 1999) ont mesuré des teneurs en MO plus élevées dans les turricules que dans le sol environnant. Ces résultats confirment que les vers de terre sélectionnent des débris de plantes dans le sol au lieu de simplement ingérer le sol tel qu'il est. Cependant le taux de concentration relative en MO dans les turricules varie en fonction des espèces (Barois *et al.*, 1999), ce qui permet de distinguer les mésohumiques qui ingèrent le sol superficiel (0-10 cm) sans opérer de sélection notable, des plyhumiques qui ingèrent un sol nettement enrichi et des oligohumiques qui à l'inverse ingèrent le sol très pauvre en MO des horizons plus profonds du sol . Ces concentrations en MO restent cependant toujours plus faibles que dans les turricules des espèces anéciques et épigés. Les espèces endogées sélectionnent également des particules minérales d'une certaine classe de taille dans le sol, ce qui varie aussi en fonction de l'espèce et de la nature du sol. Barois et al. (1999) mettent en évidence des taux d'argiles significativement plus élevés dans les turricules que dans des sols environnants pauvres en argile, avec aussi des cas d'enrichissement des turricules en particules de sable dans des sols riches en argile.

Le sol ingéré par les vers de terre est complètement dispersé au cours du passage dans l'intestin. Dans la partie postérieure du tube digestif le sol est restructuré en microagrégats (Barois et al. 1987, 1993; Shipitalo et Protz 1989), avant de former les macroagrégats stables représentés par les turricules ayant subi un cycle de séchage. Généralement dans la phase de digestion active dans l'intestin, les espèces endogées assimilent la MO par une digestion mutualiste avec des microorganismes, combinaison d'un processus de digestion direct incluant des enzymes produits par le ver de terre et d'un processus mutualiste utilisant les enzymes produits par la microflore du sol ingéré, stimulée de façon sélective. Les communautés microbiennes contenues dans le tube digestif des vers de terre endogés sont proches de celles du sol environnant par leur

composition spécifique mais la biomasse est significativement plus élevée dans les turricules frais. Dans des études sur la digestion du sol par *Pontoscolex corethrurus,* Barois et al. (1986, 1993) décrivent comment les populations microbiennes du tube digestif de *Pontoscolex corethrurus* diminuent clairement dans la partie très antérieure du tube digestif lors de la digestion du sol par le vers. Ensuite toujours dans la partie antérieure, il se produit une activation sélective d'une microflore qui va participer à la digestion (Lavelle et al. 2005). Dans la partie moyenne et postérieure de l'intestin l'activité microbienne est clairement stimulée. Finalement dans les turricules fraîchement produits la biomasse microbienne chute dans les 24h qui suivent le dépôt.

Scheu *et al.* (1987) et Lavelle *et al.* (1992) trouvent que la biomasse microbienne des turricules frais est significativement plus élevée que dans le sol environnant. L'intestin des vers et les turricules frais peuvent être considérés comme des microhabitats spécifiques favorables aux microorganismes, avec une forte teneur en eau, un pH proche de la neutralité et des substrats organiques dispersés dans ce milieu liquide (Lavelle et Spain, 2001). La biomasse microbienne diminue clairement jusqu'à des valeurs proches du sol contrôle dans les deux premiers jours suivant la production de turricule (Lavelle et al. 1992). Dans cette deuxième phase de la digestion le sol ingéré est restructuré en microagrégats (Barois et al. 1987, 1993; Shipitalo et Protz 1989).

d. Classification des espèces de vers de terre endogés tropicaux en vers de terre "compactants" et "décompactants" (Blanchart et al., 1997)

Lee et Foster (1991) ont suggéré qu'un mélange d'espèces anéciques et endogées est le plus bénéfique pour la structure du sol. Dans les agro-écosystèmes tropicaux l'interaction entre espèces endogées avec différentes influences sur la structure du sol semble être nécessaire pour un maintien de la fertilité physique du sol (Blanchart *et al.* 1997). Blanchart *et al.* (1997, 1999) ont observé dans les écosystèmes tropicaux deux comportements différents de vers endogés avec des effets contradictoires sur les propriétés physiques du sol. Une nouvelle nomenclature des vers endogés est proposée par Blanchart *et al.* (1997) en espèces « compactantes », les endogés de grande taille, et des espèces « décompactantes », souvent –mais pas toujours- de petite taille. La classification des vers de terre dépend de leur effet sur la densité apparente du sol et la macroagregation. Les espèces « compactantes » augmentent la densité apparente dans le sol, en augmentant la proportion de grands macroagrégats dans le sol, leurs turricules de grande taille et stables à long terme. L'effet des espèces compactantes sur les caractères physiques du sol dépend aussi de l'abondance et de la nature des argiles présentes dans le sol. Les macroagrégats des espèces « compactantes » forment des microsites

anaérobies où du C minéralisable peut être protégé. Dans les agroécosystèmes de Yurimaguas (Pérou), la production intense de turricules de surface par les espèces « compactantes », *Pontoscolex corethrurus*, en l'absence de résidus organiques à la surface du sol, forme une couche compacte, croûte de battance qui diminue l'infiltration de l'eau dans le sol (Pashanasi *et al.,* 1992). Les espèces « décompactantes » se nourrissent en partie des turricules des vers « compactants » qu'ils mélangent probablement avec des débris organiques et produisent de petits turricules moins stables. Ils diminuent la densité apparente du sol et le nombre de grands macroagrégats dans le sol en augmentant ainsi l'infiltration et la porosité du sol alors qu'ils diminuent la capacité de rétention d'eau dans le sol. En ingérant les macroagrégats de grande taille, les espèces « décompactantes » accélèrent la minéralisation de la MO protégée dans les macroagrégats des espèces « compactantes ».

e. L'agrégation par les racines

Les racines influencent la structure physique du sol par 5 processus principaux (Angers et Caron, 1998); Degens ,1997),: 1. la pénétration des racines, 2. le changement de régime hydrique dans la rhizosphère, 3. L'exsudation racinaire, 4. la décomposition de racines mortes et 5. les compactions locales du sol par les racines. Cependant il reste difficile de séparer l'influence du processus de compaction racinaire locale de celui de l'exsudation et vice versa. L'effet des Mycorhizes à arbuscules (MA), souvent associés aux systèmes racinaires, sur l'agrégation du sol complique encore la distinction entre les effets de l'agrégation par les racines et l'agrégation par les MA. Finalement des différences de l'architecture et de la morphologie des racines entre les espèces déterminent l'effet global des racines sur la structure du sol et l'agrégation racinaire.

Dans la rhizosphère la densité apparente du sol peut être de 12 à 35% supérieure en raison de la compaction locale au contact de la racine (Young, 1998), Cette augmentation s'observe surtout dans la zone de 50 à 200 µm autour des racines, où se forment des microagrégats (Dorioz et al., 1993). Par contre la pénétration des racines dans la matrice du sol diminue les macroagrégats.

Les racines peuvent également former des macroagrégats, très instables, par leur mécanisme d'absorption d'eau. Dans ce cas, l'assèchement local du sol au contact de la racine facilite la liaison d'exsudats racinaires aux particules d'argile (Reid and Goss, 1982).

Par le mécanisme d'exsudation racinaire, les racines provoquent la macroagrégation du sol directement et indirectement par stimulation de l'activité microbienne de la rhizosphère (Rillig, 2002). Des mucilages produits par les racines peuvent aussi coller les particules du sol directement ensemble sans intervention de mécanismes microbiens (Morel et al., 1991). Baldock and Kay (1987) ont observé que les racines stabilisent les agrégats à travers l'exsudation de matériaux, majoritairement des polysaccharides. Les racines influencent également indirectement l'agrégation du sol par stimulation des microorganismes (Mycorhizes à arbuscules (MA, bactéries ou autres mycéliums) par rhizodéposition (voir mécanismes décrits dans le sous chapitre D) (Tisdall and Oades 1982; 1993; Plante and Mc Gill 2002, Miller and Jastrow 2000; Rillig et al. 2002). Rillig et al. (2002) ont même mis en évidence que l'agrégation racinaire est principalement due à l'excrétion de la protéine glomaline par AMF. Le degré d'influence des racines sur l'agrégation est très variable et dépend de la quantité d'exsudats produits par la plante (Six 2004). Celui ci est régulé par: 1. le régime hydrique dans le sol (par exemple peu d'eau dans le sol stimule la production de mucilage par le maïs (Watt et al., 1994 et Czames et al, 2000)); 2. les espèces de plantes; 3. l'âge de la racine (une production plus forte a été observée dans les premières étapes du développement racinaire (Gransee and Wittenmayer, 2000)); 4. la profondeur des racines (plus d'agrégats sont observés proche de la surface du sol (Merbach et al., 1999)).

Durant la décomposition de racines mortes, la macroagrégation est induite notamment par la stimulation de l'activité microbienne autour des racines. L'impact de cet effet varie en fonction de la quantité de matériel et de la décomposabilité du matériel (Robinson et Jacques, 1958). Il a même été observé par Gale et al. (2000) que la plupart des macroagrégats stables sont formés après la sénescence des racines et non pendant la phase végétative.

Dans plusieurs études par Tisdall et Oades (1982), par Miller et Jastrow (1990) et Jastrow et al. (1998) il a été mis en évidence que les racines peuvent former des macroagrégats par des mécanismes de compactions locales des particules et les stabiliser ensuite par l'addition d'exsudats racinaires, comme les mucilages. Ces macroagrégats ne sont cependant stables que tant que les racines sont vivantes. Au moment de la décomposition des racines, ces macroagrégats se dispersent.

f. L'agrégation par les termites

. Certains termites influencent la stabilité structurale du sol, spécialement la microstructure (Lobry de Bruyn et Conacher, 1990; Holt et Lepage, 2000; Bignell et Holt, 2000). Les espèces géophages forment des microagrégats à travers leur digestion, en produisant des pelotes fécales, ou mélangeant du sol avec de la salive en utilisant leurs mandibules (Jungerius et al.,1999; Bignell et Holt, 2002). Même si la plupart des microstructures stables sont trouvées dans les termitières, elles sont ensuite dispersées suivant des mécanismes encore inconnus. Certains auteurs affirment que l'accumulation au cours du temps de ces microagrégats très stables explique la structure très favorable des sols des Cerrados brésiliens ou de certaines régions du Kenya (Wielemaker 1984 ; Balbino (2001) ; Eschenbrenner (1988). Dans ces sols très argileux, les particules fines sont regroupées en particules plus grandes, des pseudosables, qui gardent les propriétés electrochimiques favorables des argiles, mais acquièrent les propriétés physiques plus favorables des sables, notamment la présence de pores texturaux relativement grands qui retiennent une partie de l'eau utile du sol. C'est ainsi que les sols des Cerrados brésilines ont des densités apparentes relativement faibles en comparaison de la zone homologue des savanes des llanos Orientales de Colombie où la densité apparente est le plus souvent supérieure à 1,6. Par contre aucune information n'existe sur la dispersion de ces biostructures dans la matrice du sol autour des termitières (Holt et Lepage, 2000). Beaucoup d'études supplémentaires seront nécessaire pour comprendre la structuration du sol par les termites.

V. Contexte théorique

De nos jours un grand obstacle pour la description et la modélisation de la dynamique des agrégats du sol et leur impact sur l'érosion, est l'ignorance de l'origine réelle des différents types d'agrégats trouvés dans les sols. Le rôle des organismes ingénieurs du sol (Lavelle, 1997 ; Jouquet et al., 2006) dans l'agrégation du sol est encore insuffisamment connu. Les travaux réalisés jusqu'à présent ne considèrent pas l'origine des agrégats, ni leur dynamique temporelle et spatiale.

Cependant deux nouvelles méthodes, développées récemment, devraient permettre une meilleure description des origines des agrégats. L'analyse morphologique initiée avec les travaux minutieux de Ponge (1990) et continuée avec les approches plus rapides de Topoliantz et al (2000), et finalement de Velasquez et al (2007) ont permis de séparer de la matrice du sol, les agrégats physiques, racinaires et biogéniques (produits par l'activité de la macrofaune du sol). L'utilisation de la spectroscopie dans le proche infrarouge (NIRS) a montré une grande spécificité dans les signatures spectrales des biostructures du sol en fonction de leurs origines (Hedde et al. 2005; Velasquez et al. 2007; Zhang et al. 2009). Les signatures spectrales reflètent la quantité et la qualité de la matière organique accumulée dans chaque biostructure. Les invertébrés ont des comportements alimentaires spécifiques qui les amènent à ingérer des éléments organiques différents. Durant le passage du sol à travers le tube digestif, l'addition de salive ou de mucus intestinal stimule de manière sélective l'activité de certaines communautés microbiennes. La production d'enzymes durant la digestion et la qualité des matériaux non-assimilés contribuent à la formation de différentes signatures spectrales.

Dans le présent travail la méthode d'analyse morphologique de Velasquez et al. (2007) est utilisée. Elle propose une quantification de l'agrégation du sol basée sur l'identification de l'origine des agrégats par leur forme et leur signature spectrale au moyen de la spectrométrie infrarouge (NIRS). Cependant l'analyse des signatures des agrégats est plus précise, puisque nous mesurons la signature spectrale pour chaque agrégat isolé, et non pour un mélange de biostructures de même morphologie, prélevés en un même site (méthode proposée par Velasquez et al., 2007).

Dans le **premier chapitre** nous testons sur divers terrains l'identification des agrégats suivant leur origine en utilisant ces deux méthodes. L'origine des macroagrégats est déterminée en comparant les signatures spectrales de macroagrégats, collectés dans un bloc de sol, à des signatures de référence de macroagrégats

obtenues en conditions contrôlées au laboratoire. Le chapitre répond aux questions suivantes : Comment identifier l'origine des macroagrégats du sol? Comment quantifier l'apport relatif des ingénieurs écosystémiques à l'agrégation du sol?

Hyp1: La NIRS permet de caractériser les macroagrégats frais produits par les ingénieurs écologiques, à la surface du sol et dans la matrice, par une signature spectrale spécifique.

Hyp2: La NIRS permet de caractériser chaque macroagrégat séparé de la matrice du sol, par une signature spectrale spécifique.

Dans un deuxième et troisième chapitre sont réalisées des expériences en conditions de laboratoire pour tester les facteurs qui peuvent influencer la variabilité intra-spécifique des signatures spectrales.

Le **deuxième chapitre** analyse la variation de la signature NIRS au cours du vieillissement de turricules de vers de terre endogés, incubés en conditions contrôlées au laboratoire. Ce chapitre répond à la question suivante: Y-a-t'il une variation temporelle des signatures spectrales à court et à moyen terme?

Hyp1: La signature spectrale d'un agrégat évolue au cours de son vieillissement. Il sera donc possible de pouvoir dater l'apparition d'un turricule.

Hyp2: **Les variations de la signature NIRS s'expliquent par les variations des paramètres physico-chimiques C et N liés à la dynamique de la minéralisation.**

Hyp3 : Le processus de séchage et de réhumectation des agrégats peut avoir un impact sur la protection physique des macroagrégats et par conséquence influencer la signature spectrale.

Le **troisième chapitre** présente trois expériences réalisées en serre, testant les interactions entre les vers de terre et les racines, dans la constitution des macroagrégats. Le chapitre permet de répondre aux questions suivantes: Y-a-t'il des interactions entre organismes ingénieurs dans la constitution des agrégats? Les agrégats d'origines mixtes, s'il en existe, ont-ils des signatures spectrales spécifiques? De quelle nature sont ces interactions?

Hyp1: **Lors de la formation d'un agrégat biogénique, les racines et les vers de terre peuvent additionner leurs effets.**

Hyp2: Les agrégats d'origines mixtes ont des signatures spectrales spécifiques.

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Chapitre 1:

Participation of ecosystem engineers to soil macroaggregation

A. Zangerlé, C. Hissler, P. Lavelle

Abstract

Nowadays our inability to identify the origins of different types of macroaggregates found in soils is an obstacle to describing and modelling aggregates dynamics and associated processes (C sequestration; hydraulic properties).

In this field study, Near Infrared Spectroscopy (NIRS) was tested as a tool to discriminate between origins of macroaggregates, collected at the soil surface and in the soil matrix. Root macroaggregates and casts were identified by comparing their NIR spectral signatures to the signatures of macroaggregates, produced by the same ecosystem engineers in laboratory conditions, living in the same soil. NIRS analyses allowed us to characterize freshly produced macroaggregates of each species by a specific spectral signature because of quantitatif and qualitatif differences of OM in macroaggregates between the species that produced them.

Morphological analysis of soils allowed to characterize the soil matrix composition by four aggregate classes (casts, root and physical macroaggregates and non-aggregated soil).

NIRS spectral signatures of grown and homogenized blocks of soil of an agricultural parcel field site were compared to soil samples containing non aggregated soil and an increasing proportion of cast-originating soil (0, 25, 50, 75 and 100% of cast soil). PCA analyses showed cast mixes and root macroaggregates to be significantly differentiated from the non aggregated soil and grown blocks of soil to contain approximately 50 to 75% of casts.

Methodological improvements achieved in the first two steps were then used to try and identify the contribution of aggregates of different origins to the soil matrix in soil blocks taken from different field situations. NIR spectra of macroaggregates of the matrix of the forest site exhibited high variability inside groups of macroaggregates and PCA projections did not allow to visualize a clear superimposition of spectral signatures of unknown macroaggregates taken from the soil matrix with structures of known origin produced in laboratory cultures.

This study confirms that NIRS can be used as a tool to identify freshly produced macroaggregate in field sites. However the method turned out to be limited as the origins of all macroagregates from the soil matrix still cannot be identified by this method.

Keywords*: Soil Macroaggregation, Ecosystem engineers, Near infrared spectroscopy, Earthworm cast, Soil matrix*

I. Introduction

The process of soil macroaggregate formation by ecosystem engineers —roots, earthworms, termites, ants and a few other invertebrates— has been documented by numerous studies (Plante & McGill, 2002; Six *et al*., 2004; De Gryze *et al*., 2006; Blanchart et al. 1999; Zhang et al. 2010; Zangerlé et al. 2011). Biogenic structures produced by invertebrates through their bioturbation and other mechanical activities, soon become highly stable macroaggregates that comprise a large proportion of upper soil horizons. Macroaggregates formed by roots by entanglement of particles (Tisdall and Oades, 1982; Miller and Jastrow, 1990; Jastrow et al., 1998), are further stabilized by mucilages and other exudates that hold particles together. Arbuscular mycorrhizal fungi, which form mutualistic associations with roots of most higher plants, contribute to root aggregation by entangling particles in temporary nets creating fragile short lived aggregates (Tisdall and Oades 1982; 1993; Plante and Mc Gill 2002), and/or stick them together with glomaline (Miller and Jastrow 2000, Rillig et al. 2002; Rillig 2004). However, the origin of glomalin has been questioned recently by Gillespie et al. (2010) who found no homology with proteins or DNA of mycorrhizal origin.

Past research on soil aggregation mostly focussed on the resistance of these structures to different levels and kinds of physical stresses (Le Bissonnais 1996). Little attention was paid to the mechanisms that produced these aggregates (Pulleman et al., 1995; Bossuyt et al., 2004; Velásquez et al., 2007). A major obstacle to the description and modelling of macroaggregate dynamics and associated processes, is an almost complete ignorance of the origin of the different types of aggregates found in soils, their turnover times and distribution in the soil matrix.Virtually no studies consider aggregate ageing processes, the dynamics of aggregation as a result of physical and biogenic processes, and disaggregation through ageing and physical destruction (Blanchart 1999; Decaëns et al., 1999; Le Bayon et al., 2002). This gap in our knowledge makes it difficult to understand the roles of the different actors involved in macroaggregate formation and to model the consequences on soil aggregation and associated functions of fluctuations in the populations and communities of the ecosystem engineers that produce biogenic aggregates.

Two recent methodological developments, however, should enable a better description of the origins of soil macroaggregates. While visual morphological analyses allow separating physical from invertebrate and root biogenic macroaggregates, the use of near infrared (NIR) spectral signals seem to allow discriminating macroaggregates according to the process or organism that created them (Hedde et al. 2005; Velasquez et al.

2007; Zhang et al. 2009; Zangerlé et al. 2011). NIRS signatures actually reflect the amount and nature of organic matter accumulated in specific ways in each kind of biogenic or physical structure. Invertebrates have selective feeding habits that result in the ingestion of preferred organic items. Additions of saliva or intestinal mucus selectively enhance microbial populations during gut transit. Enzyme production as part of the digestion process and the quality of assimilated and non-assimilated materials contributes to the formation of different signatures. Soil chemical and physical analyses of structures and the assessment of their microbial communities and enzymatic activities support this observation (Mora et al. 1991; Hedde 2005).

The next step is then to test whether Near Infrared Spectroscopy (NIRS) allows discriminating, in a natural soil matrix, freshly produced soil macroaggregates according to the organism or physical mechanisms that created them. To test this hypothesis, we collected freshly produced earthworm casts at the soil surface or from the soil matrix according to visual criteria. The species that produced these casts was then determined by comparing NIRS signal with signals of casts produced by individuals of the earthworm species taken from the field, in controled laboratory conditions. This approach should allow to evaluate the respective contribution of different ecosystem engineers to the macroaggregated phase of the soil matrix.

We then tested the hypothesis that a systematic assessment of NIR signature of all macroaggregated structures might allow quantifying the relative contributions of ecosystem engineers to the soil matrix? Below ground macroaggregates produced by plant roots and earthworms were first quantified with a visual method of aggregate separation (Velasquez et al. 2007). We then quantified the proportion of soil comprised by a given type of aggregate in a block of natural soil, based on NIRS-signatures assessed with pure structures (Jouquet et al. (2010)). Different proportions of earthworm casts were mixed to non aggregated soil in order to generate samples with variable proportions of cast soil.

Methodological improvements achieved in the first two steps were then used to try and identify the contribution of aggregates of different origins to the soil matrix in soil blocks taken from different field situations.

II. Materials and methods

Our study was conducted at three different field sites in Luxemburg. Earthworm sampling was first done to identify earthworm species present in the field sites and quantify their populations. We then made cultures of all earthworms and a locally dominant plant species, in laboratory microcosms to collect the macroaggregates that they produce. A reference database of NIR spectral signatures was established. In a last step, macroaggregates were collected at the soil surface and in the soil matrix at the field site. NIR spectral signatures of macroaggregates of unknown origin, collected in the field sites, were than compared to those of the reference database to identify their origins. Macroaggregates identified in this study were all casts of different earthworm species, except the root aggregates collected in the agricultural field site.

Study sites

The study was carried out in three different field sites in Luxemburg, with different soils, plant covers and earthworm communities. Sampling of earthworms, root macroaggregates and casts took place in april 2010.

The deciduous forest at Rumelange had a dense ivy (*Hedera helix*) understory and the highest diversity of earthworms, with 3 anecic (*Apporectodea longa*, *Lombricus terrestris* L., *Nicodrilus nocturnus)*, 3 endogeic (*Octolasium cyaneum*, *Apporectodea caliginosa* (Savigny 1826), *Allolobophora chlorotica* (Savigny)) and 1 epigeic species (*Lumbricus rubellus (*Hoffmeister, 1843)). Soil was loamy on top of a limestone bedrock, with a 22.8% sand, 59.58% silt and 17.62% clay in the upper 10 cm of soil. Total average C and N contents were 2.7% and 0.2%, respectively.

The meadow site at Kopstal was part of a rotation and came after a winter wheat culture. The earthworm community was comprised of 3 anecic (*Apporectodea longa*, *Lombricus terrestris* L., *Nicodrilus nocturnus*) and 2 endogeic species *(Apporectodea caliginosa* (Savigny 1826), *Allolobophora chlorotica* (Savigny)). Soil was sandy on top of a calcareous sandstone bedrock and exhibited low organic matter contents . Soil in the upper 10

cm of soil had 63.75% sand, 24.74% silt and 11.51% clay while respective C and N contents were 1.8% and 0.2%.

The agricultural plot next to Luxemburg city was cropped to rape seedlings at the period of sampling and winter wheat has been grown the previous season. The plot had the lowest diversity of earthworm species with only 1 anecic (*Nicodrilus nocturnus)* and 2 endogeic species *(Apporectodea caliginosa* (Savigny 1826), *Allolobophora chlorotica* (Savigny)). Soil was loamy on top of a marl bedrock with 16.62% sand, 51.31% silt and 32.07% clay contents and total C and N contents of 1.5% and 0.2%, respectively, in the upper 10cm of the soil.

Soil macrofauna sampling

Soil macrofauna was sampled using the standard Tropical Soil Biology and Fertility (TSBF) method (Anderson and Ingram, 1993) at 15 locations regularly distributed 1m apart on a regular grid (4*2m). Each sample consisted of a block of soil, 25*25*30 cm deep. A 0.2% formaldehyde solution was first applied at the soil surface to extract earthworms from the soil. Non extracted soil earthworms were then collected from the soil blocks by hand sorting. Earthworms were stored in 4% formaldehyde and identified to the species level.

Macroaggregate separation

Live earthworm from all species were collected in each field site and kept for 3 weeks in laboratory microcosms to get casts for each soil/species combination. Winter wheat was also grown from seed during 6 weeks to get root macroaggregates. Soil of the upper 15 cm layer was collected at each site, air dried, sieved at 2 mm and homogenized. One litre plastic pots were filled with 800g of soil and each was inoculated with a single earthworm. Soil moisture was maintained at field capacity throughout the experiment. Capillary *per ascensum* diffusion through small holes drilled at the bottom of the pots was preferred in order to prevent physical soil aggregation by surface splash effect.

When microcosms were dismantled the earthworms were collected and weighed individually after carefully breaking soil into large clods. Soil was slowly air dried (over seven days) in order to harden

aggregates and facilitate separating them from soil that had not been macro-aggregated by specific earthworm- or root-associated processes.

Soil was then shaken and passed through a 5mm mesh sieve to disperse weak aggregates created during drying and handling and to retain resistant aggregates built by the activity of earthworms and roots. Casts and root macroaggregates were analysed by NIR infrared spectroscopy to create a reference database of NIR spectral signatures of all macroaggregtes.

Macroaggregate assessment at field sites

Fresh surface casts were collected at each field site. Soil blocks 5*5*10cm in size were taken from the first 10 cm layer of the soil and air dried for several days. Macroaggregates removed from these blocks were first characterized according to their supposed origin (root macroaggregates, casts or physical) based on their morphology and size following the Velásquez et al. (2007) procedure. Surface casts, internal casts and root macroaggregates were then analysed by NIR infrared spectroscopy. We only selected fresh surface and internal casts that were clearly identified visually and had a spherical shape and a smooth surface for the first experiment. When evaluating the proportion of casts in a given soil volume from the Rumelange field site, all macroagregates were extracted in the blocks and later characterized by NIRS spectral analyses.

Only Rumelange site had been chosen for comparing NIRS spectra of all macroaggregates, resulting from a bloc of soil, to NIRS spectra of casts obtained in laboratory cultures. Despite an ivy understory on the field site, no root macroaggregates have been found in the soil matrix by morphology analyses. This allows us to avoid a presence of root macroaggregates in soil blocks of species which could not been raised in laboratory cultures. Kopstal field had not been chosen for these analyses because of the many plant species that could not been raised in laboratory cultures. Luxemburg field site was neither suitable for this analyses because of its high soil heterogenity.

The contribution of earthworms casts to the composition of the soil matrix was evaluated by comparing the NIR signal of natural soil to non aggregated soil added with different proportions of fresh cast material following Jouquet et al (2010) method. Casts of the 3 earthworm species of Luxemburg study site, produced in laboratory conditions, were thus mixed with non aggregated soil in order to generate samples with increasing (0,

25, 50, 75 and 100% respectively) proportions of cast-originating soil. Cast originating soil was composed by one third of casts per each of the 3 specis found in Luxemburg field site (*Nicodrilus nocturnus, Apporectodea caliginosa* (Savigny 1826), *Allolobophora chlorotica* (Savigny)). Soil blocks 5*5*5 cm in size were taken in the field dried, grinded, sieved at 200 µm and homogenized. Spectral signatures of the samples with increasing proportions of cast material were then compared to spectral signatures of the soil blocks to quantify the proportion of cast present in the soil blocks. Luxemburg field site had been chosen for these analyses because of the low diversity of earthworm species found in this field site.

NIR Spectral Signatures

The average weight of macroaggregate samples for NIRS analysis was 5g (SE=1.41). Each aggregate was crushed and passed through a 200 µm-mesh sieve to obtain homogeneous preparations of all samples. Roots were separated from aggregates during crushing to avoid contamination of the spectral signal by solid plant material. Samples were packed in a quartz-glass container and placed in a spectrophotometer (FOSS NIR SYSTEM 6500, Silver Spring, MD, USA) with a 1100-2500 nm spectral range. The reflectance measurements were made at two nanometer intervals. Reflectance (R) was converted to absorbance (A) using the equation: $A = \log (1 / R)$ and further transformed to second derivative according to general procedures recommended for the treatment of this particular type of signal. Finally, average values were calculated for 10 nm intervals in order to reduce the number of variables processed. Data analyses were conducted using Winisi, Version 1.5, software system.

Mineral N and C were determined from conventional CHN analyses (Truespec CHNS, LECO).

Statistical Treatments

NIR spectra of macroaggregates of different origins were analysed separately for each field site and grouped into homogenous clusters by a hierarchical cluster analysis (CAH). The discrimination threshold of the CAH was adjusted to the number of different earthworm species that had been used in laboratory experiments per field site including the control treatment. For each field site we run a principal component analyses (PCA) of NIR spectral variables of field collected macroaggregates and casts obtained in laboratory conditions. The table of data to be treated had 139 columns—the secondary derivatives of the spectral signals for each of the 139 wavelengths measured each 10 nm—and number of lines was equal to the number of macroaggregates

analysed by NIRS for each field site. Permutation tests on PCA coordinates allowed testing for differences among treatments.

Differences in C and N contents among macroaggregates obtained in each treatment were evaluated using parametric analysis of variance (ANOVA). Comparisons between means were tested with Tukey's test. Differences among treatments were evaluated at the <0.05 probability level of significance.

All statistical analyses were performed with R software (Ihaka and Gentleman, 1996; R-Development-Core-Team, 2009) and the package ade4 for multivariate analysis (Chessel et al., 2004; Drayand Dufour, 2007; Drayetal., 2007).

III. Results

NIRS as a tool to separate macroaggregates according to their origins

PCA analysis compared NIRS signals of field collected macroaggregates from Rumelange site and casts produced in laboratory conditions with the same soil. The 20 different groups were significantly separated ($p<0.001$, Figure 1) and the treatment effect explained 25.5% of total variance. Of the 20 different groups, 10 groups were composed by macroaggregates and non aggregated soil resulting from the field site Rumelange (treatments with labels in numbers on figure 1), previously regrouped in treatments by a CAH analyses, and 10 groups were composed by casts obtained in laboratory conditions (treatments with labels in characters on figure 1) including also non aggregated soil. The first factor (39.7% of total variance explained) clearly separated macroaggregates according to their C and N contents ($p<0.05$, Table 1). Structures obtained in laboratory conditions, used to create the reference database of spectral signatures of casts, showed rather small intra treatment variability as compared to NIR spectra of unknown casts from the field site. Projections of homogeneous groups of field structures separated by CAH largely coincided with projections of casts of known origin obtained in laboratory cultures (Figures 1). This suggests that field casts isolated by CAH had been made by the ecosystem engineer that had structures projected in the same area of the factorial plane. However several treatments of the reference spectral signatures were not overlapping with a spectral signature of an unknown group formed by CAH analyses*: L. longa, N. nocturnus, L. rubellus* and treatments 3, 4 and 6 that were not overlapping. The anecic species, *L. rubellus* and *L. terrestris,* showed the highest contents in OM in casts, significantly different from those of the endogeic species, *O. cyaneum ,* and *A. chlorotica*. Contrary to our expectations, the anecic species *A. longa* and *N. nocturnus* highlighted just lightly higher C and N contents than the control soil.

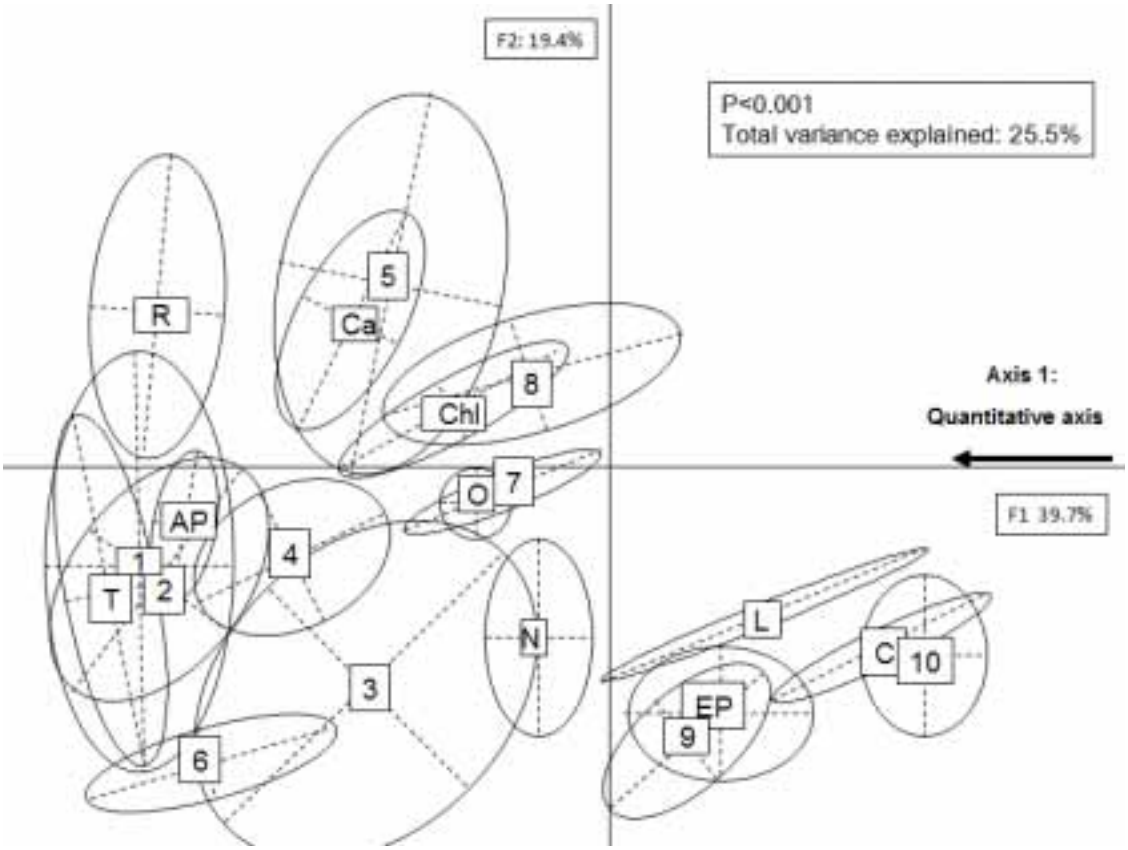

Figure 1:

Projection of barycenters of NIRS spectra of macroaggregates resulting from the field site Rumelange (treatments with labels in numbers) , previously regrouped in treatments by a CAH analyses, and NIRS data composed by casts obtained in laboratory conditions (treatments with labels in characters).

C: *non aggregated soil; Ca: Apporectodea caliginosa*; R: *Lumbricus rubellus*; L: *Lombricus terrestris;* N: *Nicodrilus nocturnus;* O: *Octolasium cyaneum*; Chl: *Allolobophora chlorotica*; L: *Apporectodea longa;* AP: Anecic Praeadults; EP: Endogeic Praeadults

	C		N	
	p<0.05	*SE*	p<0.05	*SE*
C	2,72 [d]	*0,20*	0,22 [c]	*0,021*
EP	2,81 [c]	*0,25*	0,24 [b]	*0,021*
L	2,87 [c]	*0,20*	0,24 [b]	*0,021*
N	3,08 [b]	*0,22*	0,24 [bc]	*0,010*
O	3,09 [b]	*0,23*	0,25 [b]	*0,004*
Chl	3,10 [b]	*0,25*	0,25 [b]	*0,028*
Ca	3,21 [ab]	*0,23*	0,26 [ab]	*0,030*
R	3,45 [a]	*0,20*	0,28 [a]	*0,019*
AP	3,47 [a]	*0,09*	0,26 [ab]	*0,023*
T	3,56 [a]	*0,06*	0,28 [a]	*0,017*

Table 1.

Total C and N contents of macroaggregates obtained in laboratory conditions for each species of Rumelange field site.

C: *non aggregated soil; Ca: Apporectodea caliginosa*; R: *Lumbricus rubellus*; L: *Lombricus terrestris;* N: *Nicodrilus nocturnus;* O: *Octolasium cyaneum*; Chl: *Allolobophora chlorotica*; L: *Apporectodea longa;* AP: Anecic Praeadults; EP: Endogeic Praeadults

PCA analysis of NIR spectra of the meadow rotation site, in Kopstal, showed a significant separation among the groups of macroaggregates, 8 sets of structures produced in laboratory conditions by identified earthworms and the 8 groups of undefined field structures separated by CAH ($p<0.001$, Figure 2). Factor 1 accounted for 43.6% of the explained variance and factor 2 for 22.7%. The treatment effect explained 33.1% of total variance. The first factor clearly separated macroaggregates, according to total C and N contents (Table 2, $p<0.05$). Despite a rather high intra variability of groups formed by field collected casts, almost each treatment of the reference spectral signatures was totally overlapping an unknown group separated by the CAH analyses. Like at the Rumelange field site, the spectral signatures of *A. longa* and *N. nocturnus* did not overlap with any other treatments. *L. terrestris, A. caliginosa* and *A. chlorotica* showed the highest C and N amounts with rather similar values. *A. longa* and *N. nocturnus* had low contents in OM in their casts.

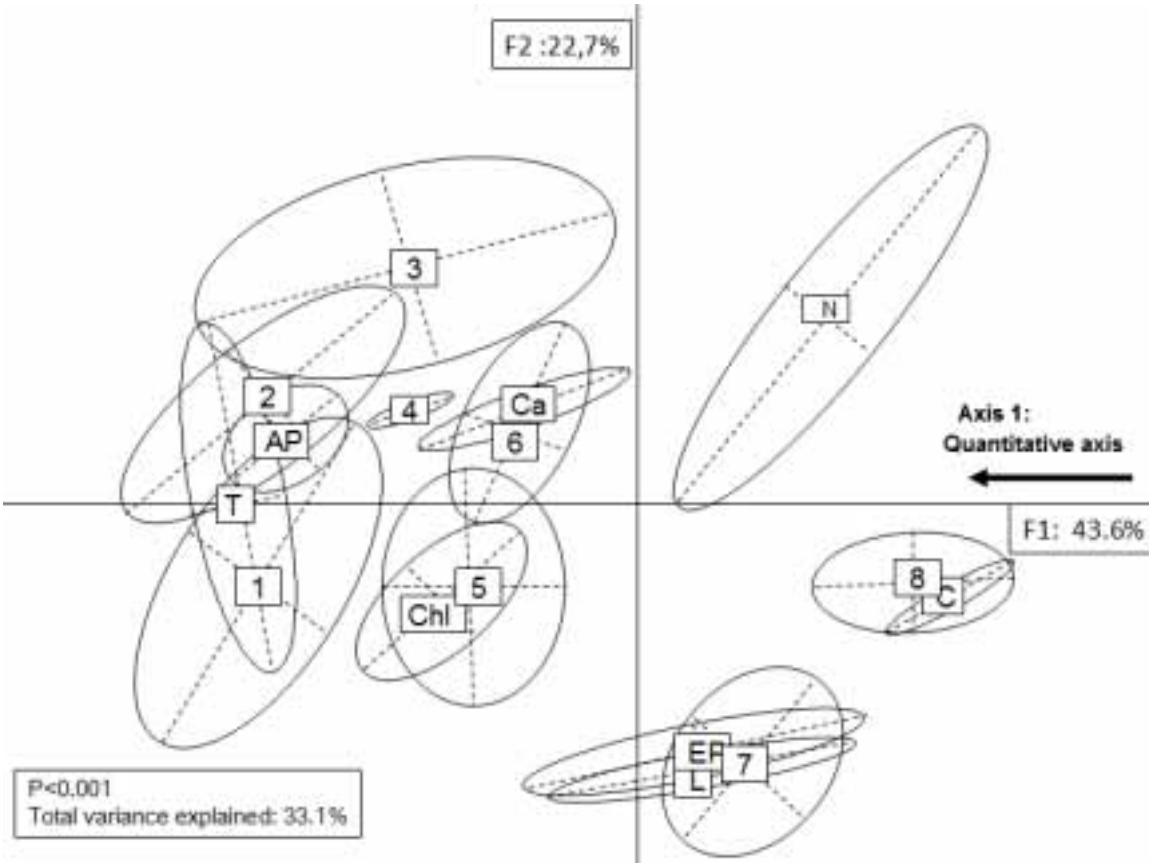

Figure 2:

Projection of barycenters of NIRS spectra of macroaggregates resulting from the field site Kopstal (treatments with labels in numbers) , previously regrouped in treatments by a CAH analyses, and NIRS data composed by casts obtained in laboratory conditions (treatments with labels in characters).

C: *non aggregated soil; Ca: Apporectodea caliginosa*; L: *Lombricus terrestris;* N: *Nicodrilus nocturnus;* Chl: *Allolobophora chlorotica*; L: *Apporectodea longa;* AP: Anecic Praeadults; EP: Endogeic Praeadults

	C		N	
	p<0.05	*SE*	p<0.05	*SE*
C	1,80 [c]	*0,23*	0,20 [b]	*0,014*
L	2,16 [b]	*0,19*	0,23 [a]	*0,022*
N	2,19 [b]	*0,25*	0,23 [a]	*0,026*
EP	2,21 [b]	*0,15*	0,23 [a]	*0,020*
Ca	2,42 [ab]	*0,22*	0,24 [a]	*0,012*
Chl	2,55 [ab]	*0,30*	0,24 [a]	*0,029*
AP	2,62 [a]	*0,28*	0,24 [a]	*0,015*
T	2,63 [a]	*0,21*	0,24 [a]	*0,028*

Table 2.

Total C and N contents of macroaggregates obtained in laboratory conditions for each species of Kopstal field site.

C: *non aggregated soil; Ca: Apporectodea caliginosa*; L: *Lombricus terrestris;* N: *Nicodrilus nocturnus;* Chl: *Allolobophora chlorotica*; L: *Apporectodea longa;* AP: Anecic Praeadults; EP: Endogeic Praeadults

PCA analysis of NIR spectra of the agricultural parcel, in showed a significant separation among the 12 different treatments ($p<0.001$, Figure 3); although intra treatment variability of each treatment was rather large. The treatment effect explained 19.2% of total variance and the first factor explained 31.9% and the second 18.5% of the total explained variance. The first factor separated macroaggregates in two main groups, one with casts from the earthworm species, *N. nocturnus, A. caliginosa, A. chlorotica* and three treatments of field collected macroaggregates and a second group of casts from endogeic preadults, control soil, root macroaggregates of winter wheat and three other treatments of unknown macroaggregates. Total C and N contents of macroaggregates explained treatment segregation along the first axis (Table 3, $p<0.05$). High intra treatment variability did not allow a clear separation of each single reference treatments. Accordingly the PCA did not show a clear overlapping of unknown treatments with reference treatments. In this case, PCA analyses of the NIRS data does not allow to identify aggregates of unknown origins.

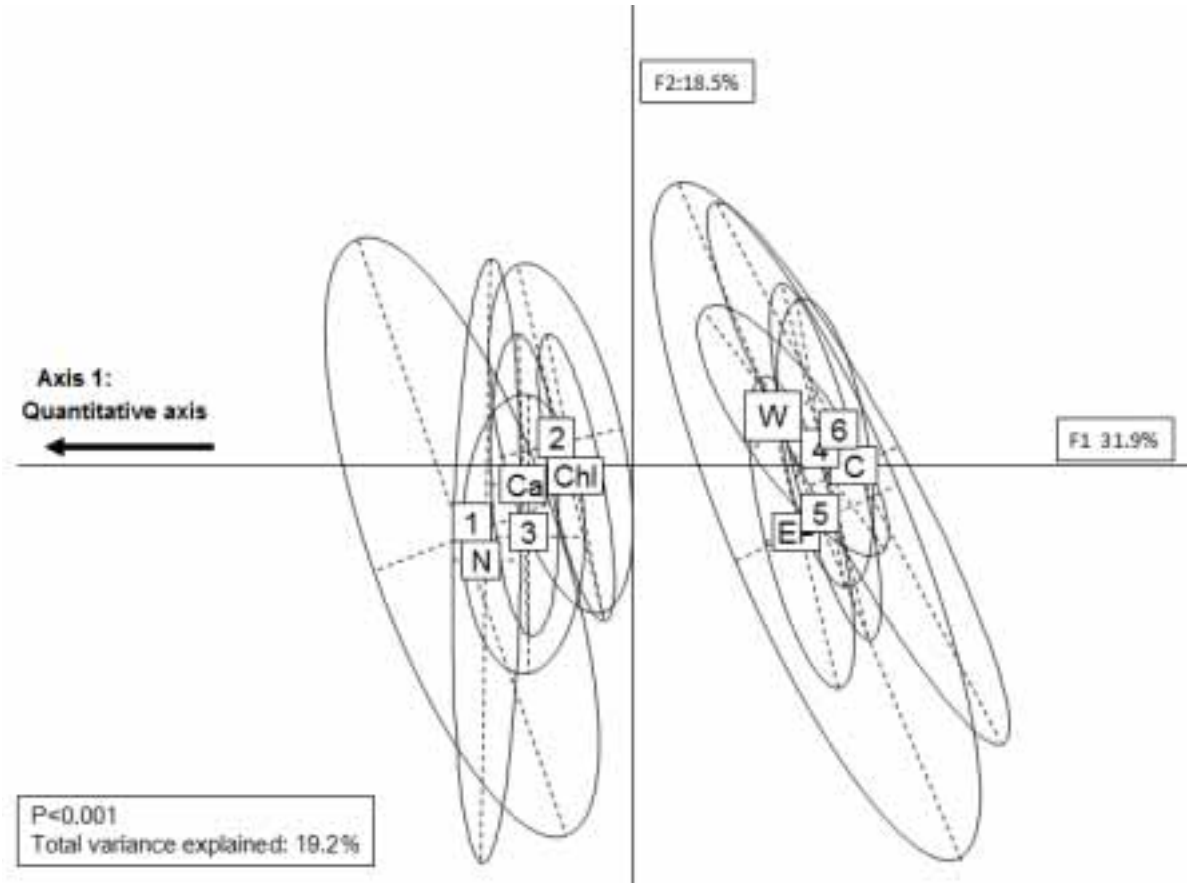

Figure 3:

Projection of barycenters of NIRS spectra of macroaggregates resulting from the field site Luxembourg (treatments with labels in numbers) , previously regrouped in treatments by a CAH analyses, and NIRS data composed by casts obtained in laboratory conditions (treatments with labels in characters).

C: *non aggregated soil; Ca: Apporectodea caliginosa*; N: *Nicodrilus nocturnus;* Chl: *Allolobophora chlorotica*; L: *Apporectodea longa;* EP: Endogeic Praeadults; W: Winter weath

C: *non aggregated soil; Ca: Apporectodea caliginosa*; N: *Nicodrilus nocturnus;* Chl: *Allolobophora chlorotica*; L: *Apporectodea longa;* EP: Endogeic Praeadults; W: Winter weath

	C		N	
	p<0.05	*SE*	p<0.05	*SE*
C	1,52 [b]	*0,39*	0,17 [b]	*0,028*
W	1,59 [b]	*0,42*	0,18 [b]	*0,035*
EP	1,81 [b]	*0,37*	0,18 [b]	*0,041*
Chl	2,34 [a]	*0,35*	0,22 [a]	*0,044*
Ca	2,38 [a]	*0,42*	0,22 [a]	*0,026*
N	2,37 [a]	*0,40*	0,23 [a]	*0,010*

Table 3.

Total C and N contents of macroaggregates obtained in laboratory conditions for each species of Luxemburg field site.

C: *non aggregated soil; Ca: Apporectodea caliginosa*; N: *Nicodrilus nocturnus;* Chl: *Allolobophora chlorotica*; L: *Apporectodea longa;* EP: Endogeic Praeadults; W: Winter weath

Evaluation of the contribution of each ecosystem engineer to the macroaggregate composition of the soil matrix

Morphological analysis of soils allowed to separate the macroaggregated part of the soil matrix into four classes (casts, root and physical macroaggregates and non-aggregated soil). Generally the forest site of Rumelange differed from the other two sites with the highest amount of casts and physicogenic macroaggregates, 41.2% and 35.5% respectively, and only 18.9% of non aggregated soil, the lowest value recorded among the three sites, and no root aggregates. At the pasture site of Kopstal, with a sandy soil with low OM content, we found the lowest amount of casts and the highest proportion of non aggregated soil (Table 4). Macroaggregates classified according to their origin (casts, root macroaggregates and physicogenic macroaggregates) by the morphological analysis were analysed by NIR spectroscopy analysis.

Table 4.

Label	root aggregates	casts	physicogenic aggregates	non aggregated soil	organic matter debris
Kopstal	14,9%	25,1%	22,4%	37,2%	0,4%
Rumelange	0,0%	41,2%	35,3%	18,9%	4,6%
Luxembourg	13,6%	30,7%	30,8%	24,8%	0,0%

Table 4.

Results of morphological analysis, which show dry weights of soil samples, classified in four macroaggregate classes (casts, root and physical macroaggregates and non-aggregated soil) and organic matter debris found in the soil samples.

PCA analysis of NIR spectra of the pasture site did not show any significant separation among samples of the different morphological classes ($p>0.05$). PCA analysis of the other two field sites, on the opposite, the forest site and the agricultural parcel, exhibited significant differences among morphological classes ($p<0.001$). However total variance explained was rather low with respective values of only 9.01% and 13.5%. PCA analyses of NIR spectra did not confirm the results highlighted by the morphology analysis.

NIRS spectral signatures of average soil of the crop field site were compared to soil samples containing non aggregated soil added with increasing proportions of cast material soil (a mix of *Nicodrilus nocturnus, Apporectodea caliginosa* (Savigny 1826) and *Allolobophora chlorotica* (Savigny) casts) (0, 25, 50, 75 and 100% of cast soil). PCA analyses showed that all mixed soil samples with increasing proportions of cast material and root macroaggregates were significantly different from the non aggregated soil. Soil samples with different proportions of cast material, root macroaggregates and non aggregated soil from the field side were clearly separated along the first axis, which explained 51.9% of the total variability. Separation between treatments was significant ($p<0.001$) and the treatment effect explained was 23.1% of total variance (Figure 4). The average, homogenized soil was projected in the part of the factorial plane corresponding to 50 to 75% casts ($p<0.001$, Figure 4); although with a large intragroup variability in the average soil.

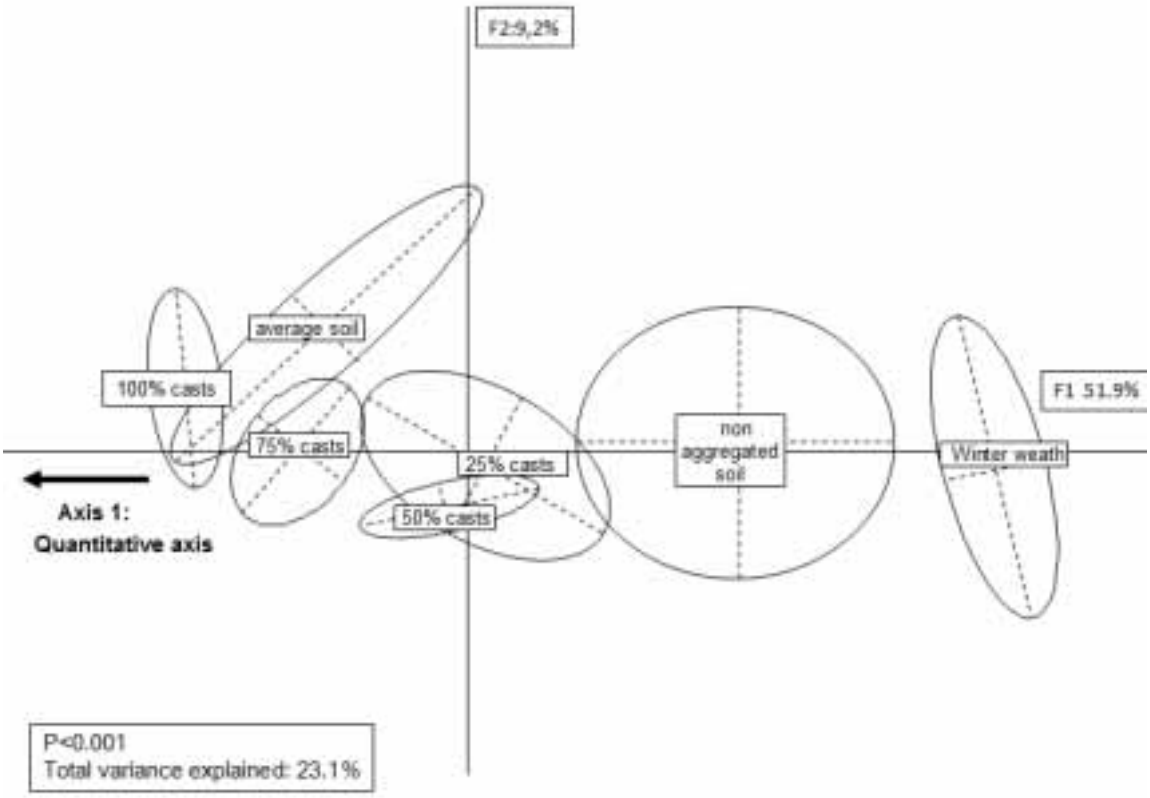

Figure 4:

Projection of barycenters of NIRS spectral signatures of grown and homogenized blocs of soil of Luxemburg field site (average soil) were compared to soil samples containing non aggregated soil and an increasing proportion of cast-originating soil (0, 25, 50, 75 and 100% of cast soil) and root macroaggregates of winter weath.

At the forest site, NIR spectra of all macroaggregates extracted from the blocks of soil, from the upper 10cm, were analysed by a hierarchical cluster analysis (CAH). The discrimination threshold of the CAH was defined as the same number of groups as the number of different earthworm species found at the site and kept in laboratory cultures. Groups identified by the hierarchical cluster analysis were then compared to NIRS signals of casts obtained in laboratory conditions, in a PCA projection. Separation between groups of aggregates was significantly different ($p<0.001$) but total variance explained was rather low (8.7%). Because of high intra treatment variability, the PCA did not allow to demonstrate a clear overlap among projections of spectral signatures of unknown macroaggregates with signatures of casts obtained in laboratory cultures.

IV. Discussion

NIRS allowed identifying soil macroaggregates according to their origins in the forest sites of Rumelange and in the meadow rotation in Kopstal, although with a relatively large variance in all results. PCA analyses showed relatively clear overlapping among projections of unknown macroaggregates collected from the field and fresh casts obtained in laboratory cultures in most groups (Figures 1 and 2). Results however do not allow clear identifications for all groups. Heterogeneity in non aggregated soil in the field place probably increases intra-group variability, which avoids a visually clear separation between species on PCA projections. In our study, non aggregated soil from the annual crop field site revealed a relatively high heterogeneity probably due to agricultural practices like mineral fertilisation. For field sites with very heterogeneous soils, PCA analysis revealed not to be the appropriate statistical approach for analysing NIR spectral signatures. Different statistical approaches should be tested in further studies. An additional limit of our proposed methodology, observed at Rumelange field site, was the size of casts. *L. rubellus* casts, collected in laboratory conditions, where rather small, which probably was the reason why no *L. rubellus* casts could be found at the field site. None of the groups isolated by the CAH analysis, did overlap with spectral signatures of *L. rubellus* on the PCA projection.

Spectral signatures of casts of *N. nocturnus* and *A. longa* obtained in laboratory conditions exhibited relatively low total C and N contents, close to levels measured in non aggregated soil. Literature however, regularly mentions higher amounts in OM in casts produced by anecic than by endogeic species (Graff, 1971; Aldag and Graff, 1975; Lavelle and Spain, 2001). This actually has been confirmed in casts of *L. terrestris* and anecic preadults in our study. Low OM amounts in *N. nocturnus* and *A. longa* casts, may be explained by their feeding behaviour in microcosm conditions. Positions of *N. nocturnus* and *A. longa* spectral signatures close to the non aggregated soil on the PCA projection suggests that unlike expected for their ecological classification, these earthworms did not feed on OM litter debris, (Lavelle and Spain, 2001). In the aim of preventing a too high heterogeneity on NIR spectral signatures, no plant litter debris had been added to microcosms in the laboratory. In the absence of plant litter, anecic species like *N. nocturnus* and *A. longa* ingested soil only because they could not feed on additional plant debris. In these conditions, the individuals from both species lost weight, but the difference was not significant. This observation seems to confirm that both species did not show their usual feeding behaviour in captivity. That no treatments, resulting from the CAH analyses, overlapped with spectral signatures of *N. nocturnus* and *A. longa* on the PCA projection, might be explained

by the expected differences of feeding behaviour of both species. In similar conditions, L. terrestris, an epianecic species according to Bouché (1972) managed to accumulate organic matter in their casts.

Morphological analyses allowed us to quantify the below ground macroaggregation activities of plant roots and earthworms. However, NIRS signatures of these structures were not significantly different in all cases and even when they were, the total variance explained remained limited.

The comparison of the average signal of a block of soil to an artificial mixture of non aggregated soil with increasing proportions of cast-originating soil, allowed to estimate the contribution of earthworms to the soil matrix composition. The method allowed to estimate the quantity of cast present in the crushed blocks of soil, to be approximately 50 to 75%. However this approach did not allow a precise quantification of the contribution of each single species to the soil matrix. In any case, this method provides a much larger figure for the contribution of earthworm casts to the soil volume (50-75%) than the morphological method (30.7%). This suggests that a great part of the aggregates identified as "physical" may have been earthworm casts that had lost their particular shape and that another large part of the "non aggregated" soil came from unstable cast material. Even macroaggregates identified as root aggregates in morphological analysis might in reality be casts that have been produced in the rhizosphere.

These results call for more methodological developments, especially, exploring a larger range of field situations, looking at interactions among roots and earthworms in the construction of macroaggregates and the constitution of their NIR signals, and also looking at possible changes in NIR signature during ageing of the aggregates.

NIR spectra of macroaggregates of the matrix of the forest site in Rumelange exhibited high variability inside groups of macroaggregates and PCA projections did not allow to visualize a clear superimposition of spectral signatures of unknown macroaggregates taken from the soil matrix with structures of known origin produced in laboratory cultures. Such high intra group variability could be explained by different scenarios. First, ageing of macroaggregates may affect the spectral signature of casts in the soil matrix. A second reason for the high variability inside groups separated by the morphological approach, observed in the forest site matrix, might be the presence of macroaggregates which have been produced by ecosystem engineers others

than earthworms. Macroaggregates, classified as physical macroaggregates in morphology analysis, may have been produced by roots or fungal hyphae. Spectral signatures of this physical macroaggregates could differ from control soil and could explain the high intra treatment variability.

In conclusion, the use of NIRS as a tool to identify biostructures in the soil matrix had already be confrimed by Velasquez et al(2007) and Hedde et al. (2005) in field conditions and by Zhang et al. (2009) in laboratory conditions. This study confirms that NIRS can be used as a tool to identify freshly produced macroaggregate in field sites. However the method turned out to be limited as the origins of all macroagregates from the soil matrix still cannot be identified by this method. The method seems finally to be limited by soil heterogenity of the field side. Sampling in field sites should be realized in a very small area to reduce as much as possible the heterogenity of the non aggregated soil. In further studies we need to test if modifications occurring in ageing casts would explain the differences observed. We also need to check whether macroaggregates are always produced by single actors as was the case so far in our experiments, or id several actors, namely roots and earthworms, might collaborate thus creating structures with mixed signals.

Conclusion Chapitre 1

Au cours de ce premier chapitre est présentée une approche de terrain, testant l'hypothèse que la spectroscopie dans le proche infrarouge (NIRS) permet d'identifier les origines de macroagrégats frais produits par des organismes ingénieurs du sol. L'origine des macroagrégats est déterminée par comparaison des signatures spectrales des macroagrégats frais, collectés dans un bloc de sol sur le terrain et à la surface du sol, à des signatures de référence de macroagrégats obtenus sous conditions contrôlées au laboratoire.

L'hypothèse 1 est validée par notre étude, menée sur 3 terrains, se différenciant par les natures de leurs sols, par leurs couvertures végétales et par la diversité et densité des organismes ingénieurs. Le comportement alimentaire des espèces de vers de terre anéciques (*A. longa* et *N. nocturnus*) a affecté la qualité de la MO des turricules produits en captivité. Par conséquence les signatures spectrales des turricules obtenus en captivité n'ont plus permis l'identification des turricules produites sur le terrain par ces espèces. La taille des turricules produits par les espèces de vers de terre sur le terrain semble aussi représenter un facteur limitant de notre approche.

Des analyses de la morphologie du sol nous ont permis de quantifier la macroaggregation par les racines et les vers de terre dans la matrice du sol. Cependant, les signatures spectrales de ces structures n'ont pas été signativement différentes dans toutes les classes d'agrégats.

Les données spectrales de mélange de turricules avec du sol non agrégé (à des proportions allant de 0% à 100%) ont été comparés à du sol de matrice broyé et du sol non agrégé. Des analyses ACP nous ont révélé que le sol de matrice de la parcelle agricole contenait environ entre 50 à 75 % de turricule.

Les macroagrégats contenus dans des blocs de sol, extraits dans la matrice du sol du terrain de forêt, ont été analysés par NIRS. Des analyses ACP révèlent que la comparaison des signatures spectrales de macroagrégats, extraits dans les blocs de sol, à des signatures de référence ne montrent pas de superpositions claires entre les signatures spectrales inconnues et celles provenant du laboratoire. Cette étude ne sera pas suffisante pour valider l'hypothèse 2 du premier chapitre (Hyp 2 : La NIRS permet de caractériser chaque macroagrégat séparé de la matrice du sol, par une signature spectrale spécifique).

Dans ce chapitre est confirmé que la NIRS peut être utilisée comme outil pour identifier les origines des macroagrégats frais de la matrice du sol. Cependant la méthode ne paraît pas suffisamment précise pour identifier tous les macroagrégats extraits dans la matrice du sol. Les résultats obtenus au cours de ce chapitre exigent des analyses supplémentaires pour une meilleure compréhension des mécanismes qui pourraient être à l'origine de la grande variabilité des signatures spectrales des macroagrégats trouvés dans la matrice du sol. Dans ce travail de thèse deux études supplémentaires seront menées en laboratoire, dans des conditions contrôlées, pour tester deux mécanismes susceptibles d'avoir un impact sur les signatures spectrales des macroagrégats:

- le vieillissement des macroagrégats du sol
- les interactions entre organismes ingénieurs

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Chapitre 2:

Near infrared spectroscopy (NIRS) to estimate macroaggregates age

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Abstract

Earthworms, as ecosystem engineers, are major actors of soil aggregation, a process that drives the production of ecosystem services by soils. However lifetimes, degradation rates and the role in soil organic matter (SOM) and nutrient dynamics of these structures remain poorly known. In this experiment, NIR spectral signatures were measured in internal casts of the endogeic earthworm, *Aporrectodea caliginosa*, incubated in controlled laboratory conditions for different periods of time and dynamics of total C and N amounts were assessed in ageing casts. Our study demonstrated for the first time that OM modifications, caused in aging casts, are large enough to get detected by NIRS in macroaggregates produced in laboratory conditions by a single species. The PCA of NIR spectral analysis highlighted three main stages of maturation, evolving each one at different speeds; 1. a stage of fast evolution of NIR spectral signatures during the first 48 hours, 2. a maturation period from days 3 to 30 with a progressive evolution of NIR spectral signatures, 3. a last stage of maturation (days 45 to 90), visually hardly separated from the control soil. C (respective N) contents in casts remained greater than in control soil during the hole incubation time (respective during 14 days).

Keywords: Earthworms; Laboratory experiment; Cast ageing; Near Infrared Spectroscopy; C and N kinetics; Aporectodea caliginosa

I. Introduction

Earthworms, as ecosystem engineers, are major actors of soil aggregation, a process that drives the production of ecosystem services by soils. The casts they produce are actually recognised to have a great importance in the regulation of soil processes (Lavelle et al., 1997). However lifetimes, degradation rates and the role in soil organic matter (SOM) and nutrient dynamics of these structures remain poorly known. This gap in knowledge makes it difficult to determine the overall effect of earthworms in C sequestration and hydrological soil functions that are enhanced when aggregates are stable and long lived.

Casts are microsites where microbe and small invertebrate activities are selected and regulated at specific scales of time and space (Marinissen and Bok, 1988; Hamilton and Sillman, 1989: Hamilton and Scheu; 1990; Lloyd, 1991; Lavelle et al., 1997; Loranger et al., 1998). When compact and large, aggregates comprise extended volumes of soil, where anaerobic conditions and the paucity of porous space may reduce microbial activity to almost nil (Martin 1991), thus enhancing C sequestration. A few counter examples, however, suggest that not all aggregates behave this way (Mc Inerney et al. 2001), possibly due to coarse textural conditions: sand may leave a larger amount of pores suitable as bacterial habitats.

Time scales considered in experiments may also explain contrasted effects of aggregates on C sequestration since fresh casts tend to loose C whereas conservation is maximum in aged ones (Lavelle and Martin, 1992). In the short-term, large quantities of nutrients, easily assimilable by plants, are found in fresh depositions (Blair et al., 1995; Chapuis-Lardy et al., 1998). Most of these nutrients derive from earthworm excretion of urine and from the mutual digestion (between earthworm and microorganisms in the earthworm's gut) of the ingested OM (Barois and Lavelle, 1986; barois 1987). Fresh earthworm casts incubated in laboratory conditions generally exhibit high rates of microbial respiration in the few days that follow their egestion (Lavelle et al. 1992b). Casts produced by endogeic earthworms in a laboratory experiment required approximately 30 days to decrease their C microbial biomass (Scheu 1987) and 16 days to diminish N microbial biomass (Lavelle 1992b) down to control soil levels. However Mariani et al. (2007) showed that in Colombian savannas internal casts of the anecic earthworm *Martiodrilus sp*. had a stable total C amount during 30 days. NH_4 contents showed a sharp decrease since the first days. They were initially at levels several times higher than the bulk soil and decreased to similar bulk soil values with ageing. In a laboratory study with endogeic earthworm *Martiodrilus sp.,* Martin (1991) showed that after 100 days C contents stabilized in casts and that

after 1 year C contents in casts contained 10% more C than the control soil. This experiment showed the importance of time in the dynamics of aggregates and their effects on soil function. The dynamics of aggregate production and destruction over time is therefore important to their function as microsites for carbon stocking (Elliot et al. 1988; Six et al. 2000).

Past research has mainly assessed soil aggregation by measuring resistance of these structures to different levels and kinds of physical stresses (Le Bissonnais 1996). Little attention has been paid to the mechanisms of aggregation, whether physical or biogenic (Pulleman et al., 1995; Bossuyt et al., 2004; Velásquez et al., 2007). Few studies consider aggregate ageing processes, the dynamics of aggregation as a result of physical and biogenic processes, and disaggregation through ageing and physical destruction (Blanchart 1999; Decaëns et al., 1999; Le Bayon et al., 2002; Mariani et al. 2007; Jouquet et al. 2009).

Our inability to identify the origins, turnover time and within the soil matrix of the different types of aggregates has long been a major obstacle to describing and modeling macroaggregate dynamics in soils and associated processes. Two recent methodological developments should enable better description of the origins and dynamics of soil macroaggregates. Morphological analyses allow separating aggregates according to the process that produced them, physical (formation of fissures and cracks) or biological (invertebrate or root activities). On the other hand, near infrared (NIR) spectral signatures allow discriminating aggregates according to the process or organism that created them (Hedde et al. 2005; Velasquez et al. 2007; Zhang et al. 2009). NIR spectral signatures reflect the amount and nature of organic matter that are specific to every kind of biogenic or physical structure. Invertebrates have selective feeding habits that result in the specific ingestion of organic items. Addition of saliva or intestinal mucus selectively enhances microbial populations and enzyme production during gut transit (Lattaud et al. 1994). The qualities of assimilated and non-assimilated materials further contribute to the formation of different signatures. Soil chemical and physical analyses of structures and the assessment of their microbial communities and enzymatic activities support this observation (Mora et al. 1991; Hedde 2005). We do not know yet, however, if NIRS spectra change during the ageing processes of aggregates. If it were the case, the age of an aggregate could be identified by assessing the NIR spectral signature.

In the present study NIR spectral signatures were measured in internal casts of the endogeic earthworm, *Aporrectodea caliginosa*, incubated in controlled laboratory conditions for different periods of time (from 0 to 90 days). Dynamics of chemical properties (total C and N amounts) were assessed in ageing casts to test if their changes could be linked to corresponding modifications of NIR spectral signatures. A series of fresh casts were

dried and remoistened three days later to test the hypothesis that no change occurs in NIR signal as long as casts are dry. This will allow us to test if physical protection of the OM in casts would get affected by the drying/wetting process of casts.

II. Materials and methods

A 3-month laboratory experiment was set up to monitor changes in NIR spectral signatures of casts with ageing. Casts were incubated for different times in controlled experimental conditions. At 16 dates, we analysed 2 samples of incubated casts and their respective non ingested control soil, each replicated three times.

Soil and Earthworms

The earthworm species used for the experiment was *Aporrectodea caliginosa* (Savigny 1826), a soil feeding endogeic, commonly found in pastures and low-input cropping systems in many temperate areas of the world (Bouché 1972). Further studies have shown that this earthworm actually adds small amount of decomposed litter to the ingested soil since they cannot grow on soil alone. Earthworms and soil were collected in a deciduous forest in Rumelange (Luxembourg) in the upper 15 cm layer of the soil profile. Soil was a clay-loam with textural B horizon on top of a limestone bedrock (pH(H_2O) was 6.25 and pH($CaCl_2$) 5.76). Total C and N average contents were 2.65% and 0.24%, respectively.

Production and ageing of casts

The soil was air dried, sieved at 2 mm and homogenized. Earthworms were kept for 2 days in the experimental soil to empty their guts of the soil they had previously fed on. They were then kept in a plastic box (1 x 1 x 0.5 m) filled with the experimental soil moistened at field capacity.

At each sampling date earthworms were set for 24 h in boxes filled with 500 g experimental soil, which had been previously moistened at field capacity. Ten adult worms were inoculated in each culture box. During this time, they would produce ca. 10g fresh casts inside the soil volume. After 24 h earthworms were gently removed from the boxes without disturbing the soil matrix. Culture boxes, containing soil and casts, were then hermetically closed and incubated at 15°C. Control soil was incubated in similar conditions for comparison.

Samples of casts, non ingested soil and control soil were taken after 12 hours, 1, 2, 3, 4, 5, 6, 7, 10, 14, 21, 30, 60 and 90 days.

A series of casts was dried just after removal of earthworms and remoistened three days later to check the hypothesis that no change occurs in NIR signal as long as casts are dry.

Figure 1: Aporrectodea caliginosa

Figure 2: Culture box filled with homogenized soil and casts

NIRS spectral analysis and CHN analysis

Three cast samples were taken from each culture box, each treatment was then replicates three times resulting in a total number of 144 samples for each the two sets of experiments (moist and dried/remoistened) respectively. The average weight of samples was 2g (SE=0.31). Each sample was crushed and passed through a 200 µm-mesh sieve to obtain a homogeneous preparation of all samples. Samples were packed in a quartz-glass container and placed in a spectrophotometer (FOSS NIRSystems 5000, Silver Spring, MD, USA) with a 1100-2500 nm spectral range. The reflectance measurements were made at two nanometer intervals. Reflectance (R) was converted to absorbance (A) using the equation: A = log (1 / R) and further transformed to second derivative according to general procedures recommended for the treatment of this particular type of signal. Finally, average values were calculated for 10 nm intervals in order to reduce the

number of variables processed. Data analyses were conducted using the ISI software system (Shenk and Westerhaus 1991).

Mineral N and C were determined from conventional CHN analyses (Truespec CHNS, LECO).

Statistical Treatments

Principal components analyses (PCA) were performed using the R package, with ADE4 library for multivariate analyses [R Development Core Team, 2004; ADE-4 software (Thioulouse et al. 1997)]. Permutation tests on PCA coordinates allowed for testing differences among treatments.

Differences among amounts of total C and N content of aggregates were evaluated using parametric analysis of variance (ANOVA). Comparisons between means were tested with Tukey's test. Differences among treatments were evaluated at the <0.05 probability level of significance.

III. Results

Incubation time of casts and control soils greatly affected their total C and N contents ($p<0.001$; $p<0.001$, figures 1 and 2) and their NIR spectral signatures ($p<0.001$ figure 3).

Effect of incubation time on total C contents

Earthworm casts exhibited great differences in C contents and kinetics compared with the control treatments. Total C content was greatest in fresh casts with a mean value of 4.6%, (SE) i.e., 1.74 times the control. C contents in casts remained greater than in control during the hole incubation time (Figure 1, Table 2, $p<0.05$). After 90 days incubation C contents in casts had dropped down to 2.42% that is 52.6% of the initial content. The concentration decreased sharply in the first 24hours, later decreased regularly until the 3rd day. With increasing time of incubation the total C concentration of casts tended to decrease slightly, but not significantly, until day 45. Days 60 and 90 showed significant lower total C contents but concentrations still did not tended to stabilize after 90 days of incubation (Figure 1, Table 1, $p<0.05$). Control has an initial content equal to 2.65% and exhibit a significant but very regularly and small decrease in total C contents until 2.25% of total C, during the 90 days of incubation.

Figure 3.

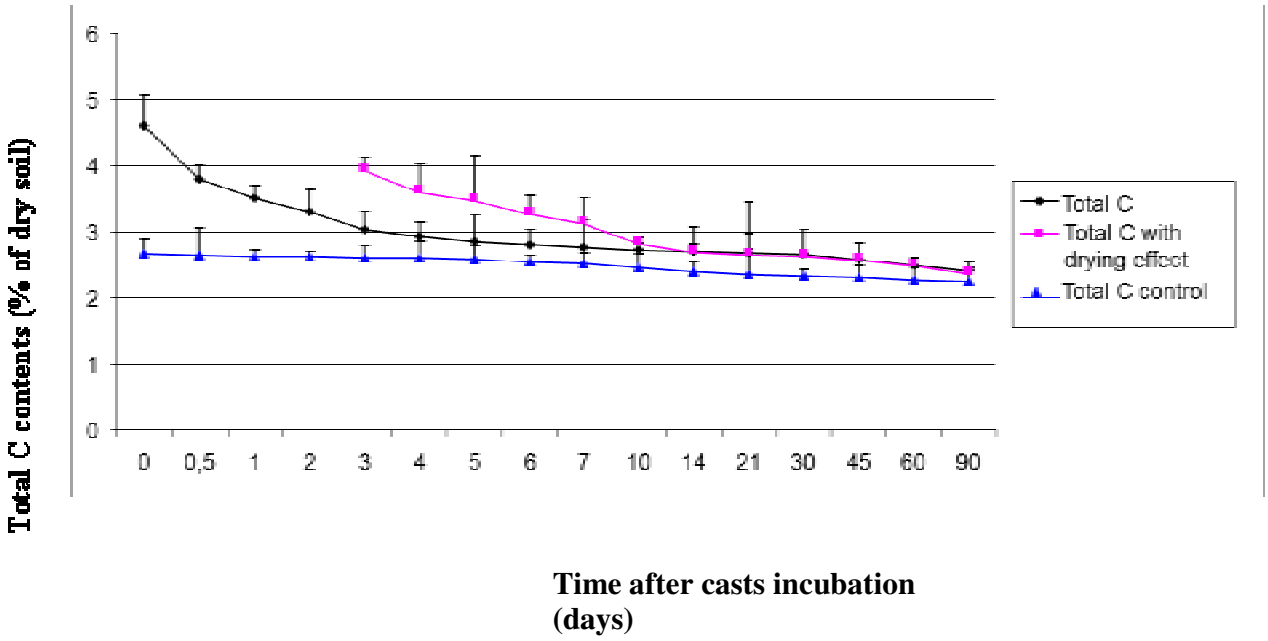

Figure 3.
Evolution over time of total C concentrations in simple cast treatments, control soil treatments and casts exposed to drying/wetting effects treatments.

Table 1.

	T	12H	D1	D2	D3	D4	D5	D6	D7	D10	D14	D21	D30	D45	D60	D90
Ct	a	b	c	d	e	e	e	e	e	e	e	ef	ef	ef	f	f
Cd					a	b	c	cd	d	d	de	de	de	e	e	e
Cc	a	a	a	a	a	a	a	a	a	ab	ab	ab	ab	ab	b	b

Table 2.

	T	12H	D1	D2	D3	D4	D5	D6	D7	D10	D14	D21	D30	D45	D60	D90
Ct	a	a	a	a	b	b	b	b	b	a	a	a	a	a	a	a
Cd					a	a	a	a	a	a	a	a	a	a	a	a
Cc	b	b	b	b	c	c	c	c	c	b	b	b	b	b	b	b

Table 1.
Table representing results of comparisons between means of total C analysis between each day, tested separately for each series of samples (simple casts, control soil or drying/wetting casts) (tested with Tukey's test). Differences among treatments were evaluated at the <0.05 probability level of significance. (T = freshly produced casts; 12H = casts aged 12hours; D1-D90 = casts aged 1-90 days; ct = simple casts, cc = control soil and cd = drying/wetting casts)

Table 2.
Table representing results of comparisons between means of total C analysis between the 3 series of samples (simple cast treatments, control soil treatments and drying/wetting treatments) per day (tested with Tukey's test). Differences among treatments were evaluated at the <0.05 probability level of significance. (T = freshly produced casts; 12H = casts aged 12hours; D1-D90 = casts aged 1-90 days; ct = simple casts, cc = control soil and cd = drying/wetting casts.)

Effect of incubation time on total N contents

N concentrations was greatest in fresh casts (0.39%, 1.63 times the control), and decreased down to 0.23% in casts incubated for 90 days. Concentrations decreased sharply down to 0.26% during the first 48 hours. Later total N contents decreased slightly (Figure 2, Table 3, $p<0.05$). In control soils N decreased slightly and regularly during the 90 days (from 0.24% to 0.22%), but did not tended to stabilize (Figure 2, Table 3, $p<0.05$). N contents in casts remained greater than in control during the first 21 days of the incubation time (Figure 2, Table 4, $p<0.05$).

Effect of drying and rewetting on total N and C kinetics in casts

Drying and rewetting fresh casts after three days significantly influenced their overall total C and N contents (Figure 1 et 2, $p<0.05$). C concentrations of casts that experienced this treatment were significantly higher than simple cast groups of the same incubation in dates (3,4,5). However after 3 days, differences were no longer significant (Figure 1, Table 2, $p<0.05$). N concentrations remained significant higher from the simple cast groups only one single day (day 3) (Figure 2, Table 4, $p<0.05$).

C amounts of cast groups DH4, DH5 and DH6 (C: 3.9, 3.6, 3.5) are lower than the concentrations in cast groups aged 0H, 1day and 2 days (C: 4.6, 3.8, 3.5 and N: 0.35, 0.28, 0.26 respectively) but showed a similar sharp decrease as observed in simple cast treatments during the first 3 days of incubation. This means that samples from drying/wetting treatments have C contents equivalent to simple cast groups that have been produced 3 days later. Cast groups, exposed to drying and rewetting, showed a lower initial total N content than simple cast groups with only 0.28% compared to 0.39%. N contents in drying/wetting treatments remained only one day significantly higher than in simple cast groups (Figure 2, Table 4 $p<0.05$).

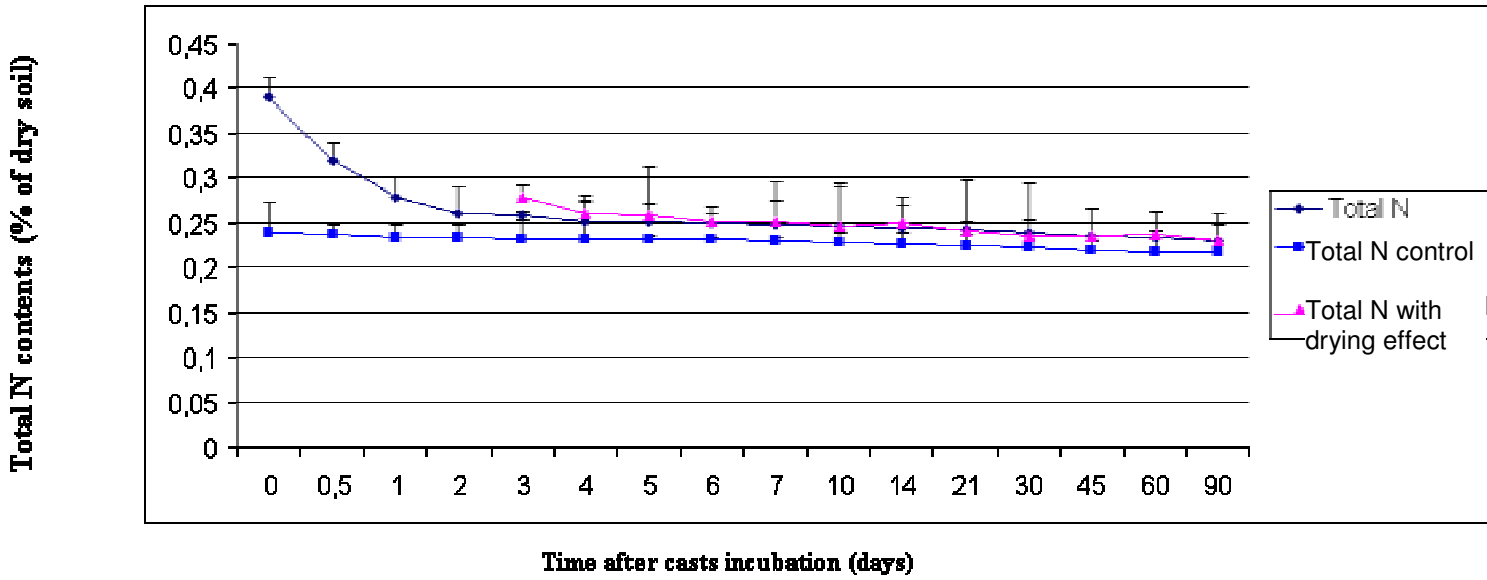

Figure 4.
Evolution over time of total N concentrations in simple cast treatments, control soil treatments and casts exposed to drying/wetting effects treatments.

Table 3.

	T	12H	D1	D2	D3	D4	D5	D6	D7	D10	D14	D21	D30	D45	D60	D90
Nt	a	b	c	cd	cd	d	d	d	de	de	de	de	de	e	e	e
Nd					a	b	b	b	bc	bc	bc	bc	bc	c	c	c
Nc	a	a	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	b	b	b

Table 4.

	T	12H	D1	D2	D3	D4	D5	D6	D7	D10	D14	D21	D30	D45	D60	D90
Nt	a	a	a	a	b	a	a	a	a	a	a	a	a	a	a	a
Nd					a	a	a	a	a	a	a	a	a	a	a	a
Nc	b	b	b	b	c	b	b	b	b	b	b	a	a	a	a	a

Table 3.
Table representing results of comparisons between means of total N analysis between each day, tested separately for each series of samples (simple casts, control soil or drying/wetting casts) (tested with Tukey's test). Differences among treatments were evaluated at the <0.05 probability level of significance. (T = freshly produced casts; 12H = casts aged 12hours; D1-D90 = casts aged 1-90 days; nt = simple casts, nc = control soil and nd = drying/wetting casts)

Table 4.
Table representing results of comparisons between means of total N analysis between the 3 series of samples (simple cast treatments, control soil treatments and drying/wetting treatments) per day (tested with Tukey's test). Differences among treatments were evaluated at the <0.05 probability level of significance. (T = freshly produced casts; 12H = casts aged 12hours; D1-D90 = casts aged 1-90 days; nt = simple casts, nc = control soil and nd = drying/wetting casts)

NIR Spectral Signatures

PCA of the 135 variables describing NIR spectra showed a significant separation among the 17 different treatments (Figure 3a, $p<0.001$), although variability was rather large inside some treatments. The treatment effect explained 33.4% of total variance ($p<0.001$).

Projections on axes 1 and 2 of the PCA showed three separate cast groups: fresh casts (0 to 2 days), intermediate (3 to 30 days) and oldest casts (45-90 d). The first factor (46.3% of total variance explained) clearly separated aggregates according to their time of incubation since deposition. The second factor (31% of total variance explained) separated mostly fresh casts (cast groups aged 0H, 12H, day 1 and day 2) from older casts.

The correlation circle among wavelengths and axes of the PCA showed an association of the first axis with high reflectance in NIR wavelengths 1100-1190, 1200-1210, 1300-1310, 1360-1390, 1430-1470, 1650-1880, 1900-1970, 1990-2000, 2050-2120, 2250-2280, 2310-2370, 2440nm (Fig. 3b). Projection of C, N and C:N data, as additional variables in the plane, defined by axes 1 and 2, shows that the first factor of the PCA is clearly associated to the C and C:N variables and the second axis to the N variable (Fig. 3b). Just a few NIR wavelengths (1510-1530, 1550, 1640, 2170, 2230, 2410-2430) are correlated with the second axis, which separates mostly the youngest samples of incubation days T0 to day 2 from the others.

Figure 5a.

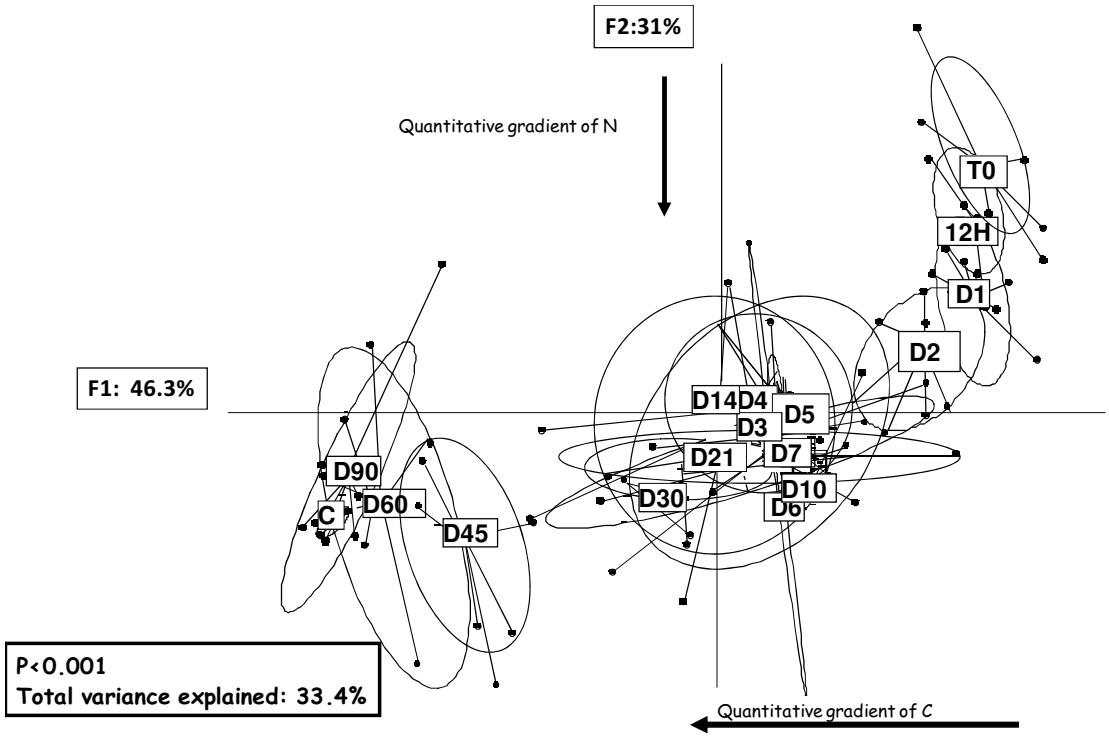

Figure 5a.

Projection of barycenters of NIRS spectra of casts and control soil, incubated at 16 different dates, in planes defined by factors 1 and 2 of PCA. Solid lines indicate major effects observed along factorial axes. (C = control soil; T0 = freshly produced casts; 12H = casts aged 12 hours; D1-D90 = casts aged 1-90 days.)

Figure 5b.

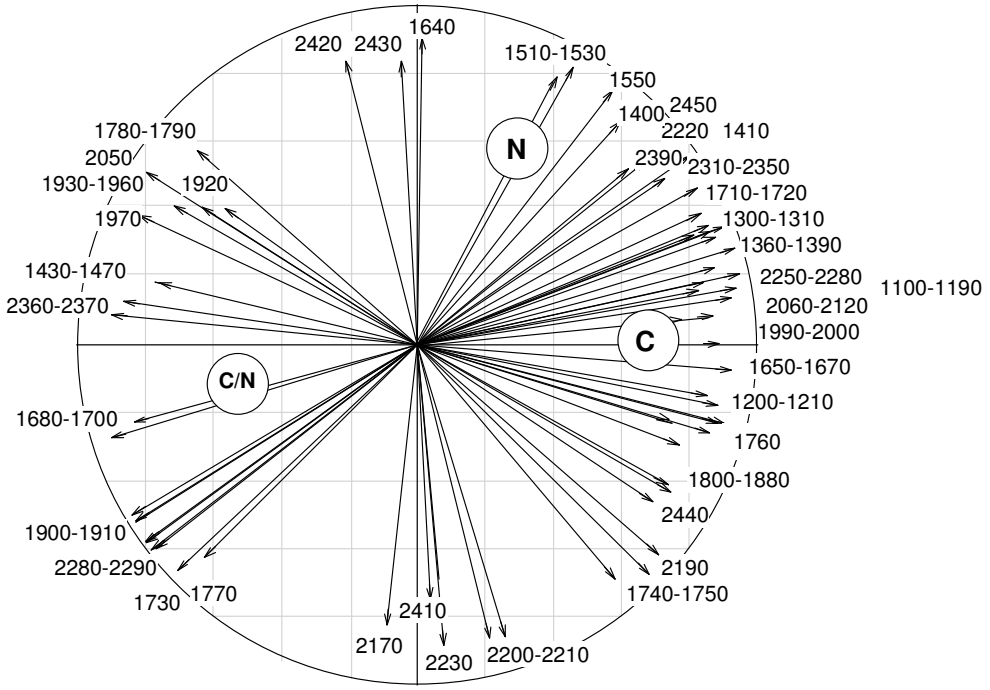

Figure 5b.

Correlation circle among NIR wavelengths and factors. C, N, and C:N variables projected as additional variables not taken into account in calculations. For the correlation circle, variables were selected that had respective weights on axes 1 and 2 equal to at least half maximum values, respectively, calculated for these axes (Velásquez 2007).

Effect of drying on NIR Spectral Signatures

The PCA of four drying/wetting treatments and continuously wet cast groups (T0, D1-5 and D3H, D4H, D5H, D6H) showed a significant separation of the two sets of data (21.9% total variance explained , $p<0.001$) (Figure 4). The treatment effect explained 21.9% of total variance. The first factor (38.23% of variance explained), clearly separated aggregates according to their total C content. The second factor (21.64% of variance explained) separated cast groups according to their total N content.

Samples of drying/wetting treatments and always moist cast groups, produced 3 days after the first one's, showed equivalent C and N contents and manifest very close positions in the PCA's factorial plan.

Figure 6.

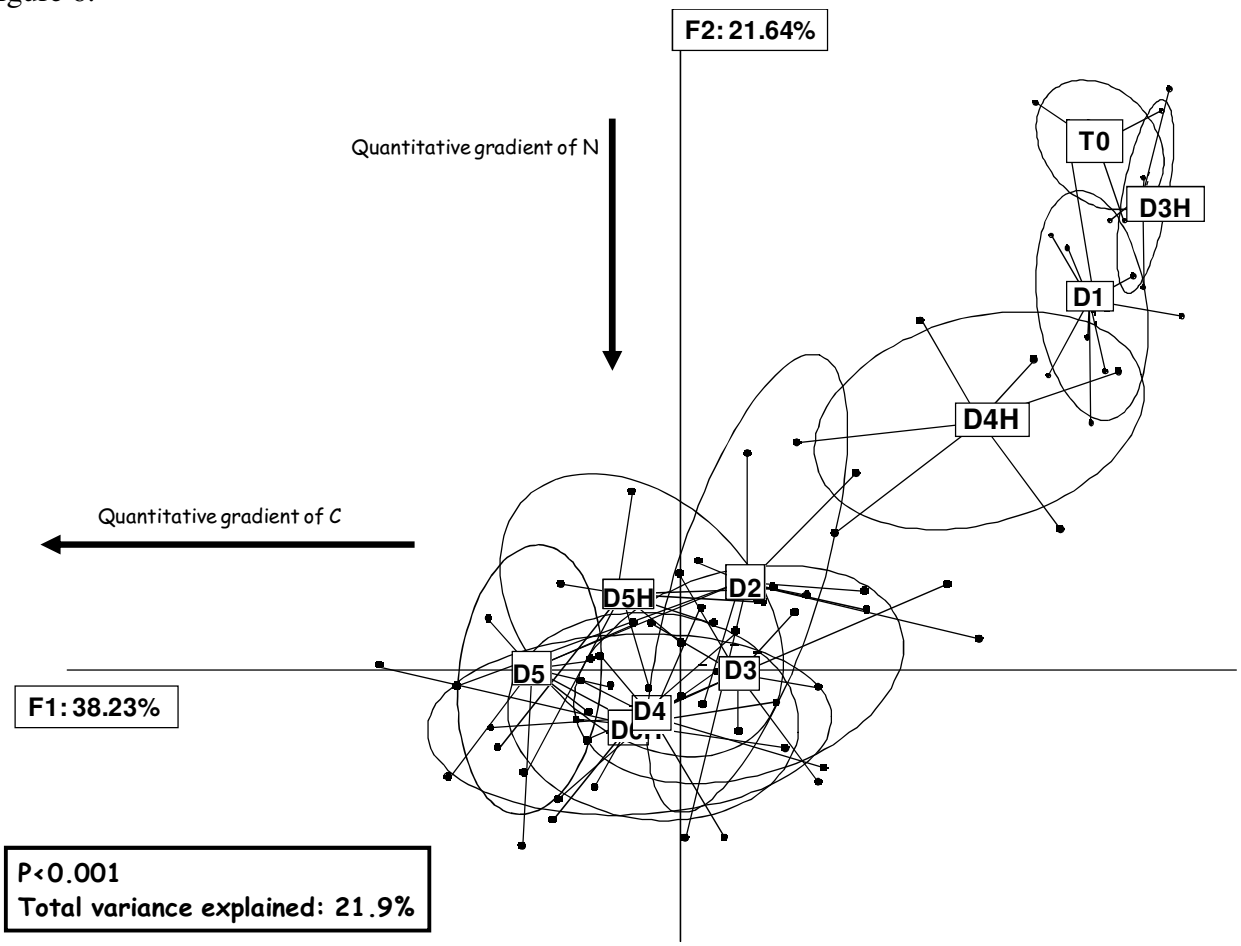

Figure 6.
Projection of barycenters of NIRS spectra of four drying/wetting treatments and six simple cast treatments in planes defined by factors 1 and 2 of PCA. Solid lines indicate major effects observed along factorial axes. (T0 = freshly produced casts; D1-5 = simple cast treatments and D3H, D4H, D5H, D6H = drying/wetting treatments)

IV. Discussion

NIRS as a tool to identify aggregate's age

NIR spectral analyses have been used to identify structures produced by different ecosystem engineers (Hedde et al. 2005, Velasquez et al. 2007 and Zhang et al. 2009; Zangerlé 2011, in press). Modifications of soil OM, caused by ecosystem engineering, result in species-specific organic fingerprints in their respective biostructures. Our study demonstrated for the first time that OM modifications, caused in aging casts, are large enough to get detected by NIRS in macroaggregates produced in laboratory conditions by a single species. The PCA of NIR spectral analysis highlighted three main stages of maturation, evolving each one at different speeds. A fast modification of spectral signatures during the first 48 hours allowed a precise dating of freshly produced casts. During a maturation period from day 3 to 30, the casts spectral signature evolves relatively progressive, which can be associated mostly to the first axis of the PCA. Spectral signatures of samples of the last stage of cast's maturation, collected between days 45 to 90, were visually hardly separated from each other.

The second factor of the PCA, which separated mostly cast groups aged 0H, 12H, 1 day and 2 days from each other and was correlated to total N contents of casts, was associated with just a few NIR wavelengths (1510-1530, 1550, 1640, 2170, 2230, 2410 and 2430). Most of these wavelengths correspond to those that Murray and Williams (1990) found to be correlated with NH4+ (1510-1650nm). Similar to our results Lavelle et al. (1992) observed sharp decreases of NH4+, NO3- and total N contents in casts during the first 48 hours after fresh cast production in a laboratory experiment by NH4+ volatilization, denitrification of NO3- process and loss of microbial biomass. Thereby the second axis of the PCA projection might be associated to a strong NH4+ loss and a loss of microbial biomass in fresh casts during the first 48 hours.

Barycentres of cast groups collected between days 3 to 90, projected of in the PCA's factorial plane, were rather close. The results of total C analysis and the

projection of the C and C:N data, as additional variables in the PCA projections, showed that the first factor of the PCA projection is correlated to differences in the organic matter content of the aggregates. Thereby, small differences of C contents between incubation days 3 to 90 might be an explanation why these barycentres are just lightly separated from each other on the PCA projection. Also, control soils and aggregates, of incubation days 45 to 90, have very similar spectroscopic signatures, which can be probably explained by their equal C contents (Table 1).

Several NIR wavelengths (1100, 1120-1170, 1780-1790, 1970, 2050, 2250-2280, 2310-2350, 2390, 2430nm) of the total range of variables correlated to the first axis have been associated to C and N contents in literature. Al-Abbs et al. (1972) found that wavelengths ranging from1500 to 1800 contributed significantly to the prediction of the soil OM. Dalal and Henry (1986) used certain wavelengths (1744, 1870 and 2052nm) to calibrate the equation for predicting organic C. Henderson et al. (1992) found a correlation between the organic C content and wavelengths (1065, 1085-1105, 1125-1165, 1955-1965, 2215, 2265, 2285-2295, 2315, 2335-2365, 2385-2415, 2435 and 2495 nm).

C and N dynamics in ageing casts

During 90 days, the total C content loss of 52,6% and total N content loss of 58,97% in incubated casts are comparable with the few similar values available from endogeic species experiments in laboratory conditions (Lavelle and Spain 2001).

During the 90 days of incubation, casts had significantly higher contents in C than control soils. Both soils continued to loose C data sustained rate until day 90 when the experiment was stopped. Martin (1991) showed that after 100 days, C contents stabilised in casts and control soil, after casts had exhibited a sharp decrease in the first 2 days in their contents from the time of their deposition and later decreased slightly during 100 days. After one year, casts contained 10% more C than the control. Mariani et al (2006), observed that total C contents in casts remain significant higher than in bulk soil after 60 days in field conditions. These studies confirm our results since they highlighted a sharp decrease in C contents during the

first 3 days of incubation and later a very slight decrease from days 4 to 90. Like observed by Martin et al. (1991) and Mariani (2006), aggregates did not reach the bulk soil total C level after 90 days in our experiment. Previous results of the literature showed that microbial N biomass in casts produced by endogeic earthworms decreased down control soil after 16 days (Lavelle 1992b). N microbial biomass results observed by Lavelle (1992b) are rather similar to our results since total N contents of casts reach control soil between days 14 to 21. However compared to results of Martin et al (1991), total C contents of casts decreased much faster in our experiment during the first 3 days. We observed a loss of 1.56 % of total C of casts during the first 3 days compared to a loss of 0.1% showed by Martin et al. Total N concentration decreased from 0.39% to 0.26% in our experiment and only from 0.72% to 0.69% in the experiment of Martin et al. This could be related to leakage of soluble and microbial C and N from the casts to the surrounding soil. In Martin (1991) experiment, casts had been separated from the surrounding soil and no such leakage was possible. On the other hand, Decaëns (2001), observed higher contents in mineral N in soil at the contact of casts of Martiodrilus carimaguensis in field conditions which suggests that leakage occurs. The very fast decrease in mineral N and microbial biomass observed in the experiment of Lavelle et al (1992), where casts were kept in contact with non ingested soil, show similar trends.

Higher levels of total C in casts compared to bulk soil are generally explained by the feeding behaviour of earthworms. The selective ingestion of elements with high C and N contents such as roots, casts of other earthworms, coarse organic particles and fine textured particles with high C contents may explain higher C and N concentrations in fresh casts.

Total C concentration in casts decreased sharply in the first 24 hours of the experiment. In the short-term, large quantities of nutrients, easily assimilable by plants, are found in fresh depositions of earthworms (Blair et al., 1995). Fresh casts, incubated in laboratory conditions, generally exhibit high rates of microbial respiration in the few days that follow their egestion (Lavelle et al. 1992b). A rapid mineralisation of these easily degradable nutrients, derive from the mutual digestion (between earthworm and microorganisms in the earthworm's gut) of the ingested OM

(Lattaud et al., 1994; Barois et Lavelle, 1986; Barois et al, 1987), could explain this sharp loss of C during the first hours.

Similar results as in our experiment, Lavelle et al. (1992) observed of a sharp decrease in N concentrations of Pontoscolex corethrurus casts, during the first 48 hours following deposition. The N rich nutrients, mineralised in the first hours, derive from earthworm N natural excretion (urine) and microbial activity enhanced during gut transit (Barois et Lavelle (1986); Blair et al. (1995)).

Effect of drying on NIR Spectral Signatures

The drying/wetting effect, applied to some casts, showed a similitude in NIR spectral signatures and C concentrations between aggregates that have been produced with an interval of 3 days and with an interval of 24h in N concentrations. The drying process of fresh aggregates lasted approximately 12 hours. But casts got rehumected again at field capacity just after 3 days. Microbial activity has been stopped during over 50 hours, which could explain the observed indeed latecomer of 3 days in C mineralisation of easily degradable nutrients. The 12 hours of drying required until the microbial activity had been stopped were probably sufficient for a significant N content loss. Indeed dry/wetting treatment casts showed a significant lower N concentration than simple cast groups at the first day of their incubation.

Conclusion

Further experiments, with different earthworm species and in different soils, will show if NIR spectroscopic separation demonstrated in this experiment is sufficient to allow identifying aggregates, produced by a single species, according to their age. A second step will be the validation of this method under uncontrollable field conditions. This novel method could present a great step towards the understanding and modelling of the temporal dynamics of aggregation in soils.

Conclusion du chapitre 2

Ce deuxième chapitre nous a permis de vérifier l'hypothèse proposée en conclusion du premier chapitre : Les effets, causés par le vieillissement de macroagrégats sur leurs teneurs en MO, sont suffisants pour affecter les signatures spectrales des turricules. Nous avons voulu tester par cette étude si le facteur temps pouvait expliquer la forte variabilité intra-traitements des signatures spectrales des macroagrégats des blocs de sol, prélevés dans la matrice du sol au cours de l'expérience présentée au chapitre 1. La première hypothèse du deuxième chapitre, disant que les variations des signatures spectrales des turricules causées au cours de leur vieillissement permettraient le datage de l'apparition d'un turricule, est validée par les analyses ACP des spectres NIRS obtenus. Ces derniers ont permis de mettre en évidence trois phases principales de maturation. Les barycentres regroupés selon les 3 phases, sont séparés selon le premier axe de l'ACP, corrélé aux teneurs en C des agrégats. Les trois phases se caractérisent par différentes vitesses d'évolution de la signature spectrale. Une première phase courte, évolution rapide, sur les deux premiers jours regroupe les traitements frais et âgés de 12, 24 et 48 heures. Les échantillons sont séparés nettement selon le deuxième axe, corrélés aux taux de N des macroagrégats. La deuxième phase est la phase de maturation des macroagrégats, à vitesse plus lente, et regroupe les échantillons des jours 3 à 30, très peu séparés. Une dernière phase de maturation regroupe les échantillons des jours 45 à 90 et le traitement contrôle. Les longueurs d'ondes corrélées fortement aux axes 1 et 2 de l'ACP, sont décrites dans la littérature comme étant associés aux teneurs en MO, C total et C organique pour le premier axe et teneurs en NH_4^+, NO_3^- and total N pour le second axe. Ainsi notre étude nous a également permis de valider la deuxième hypothèse, que les variations de la signature NIRS s'expliquent par les variations des paramètres physico-chimiques C et N, liés à la dynamique de la minéralisation. Des chutes rapides des teneurs en C et N ont pu être mises en évidence sur les 3 premiers jours, suivies par des diminutions plus lentes des concentrations sans aboutir à des stades de stabilisation. Des analyses NIRS de turricules soumis à des effets de séchage et réhumectation à différentes dates, ont permis de vérifier l'hypothèse 3, qui n'a été que partiellement validée. Les analyses ont pu mettre en évidence que la protection

physique du C dans les macroagrégats n'est pas affectée par ces processus de séchage/ réhumectations alors que les teneurs totales de N chutent significativement au cours des premières heures du séchage des agrégats.

Notre étude a donc permis de démontrer pour la première fois que les modifications de la MO, qu'ont lieu au sein des turricules au cours de leur vieillissement, sont suffisantes pour être détectées par la NIRS dans les macroagrégats produits sous conditions contrôlées en laboratoire par une seule espèce.

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Chapitre 3:

Interactions entre organismes ingénieurs dans la macroagrégation du sol

Article a:

Do Earthworms and Roots Cooperate to Build Soil Macroaggregates?

A Microcosm Experiment

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Article accepté dans Geoderma, septembre 2011 (réf. GEODER_10821)

Highlights

- NIRS analysis of macroaggregates allows to identify the organisms that created them
- Plant roots and earthworms interact in macroaggregate building
- *P. lanceolata* and *T. pratense* produced root aggregates in common
- Ingestion rates of *A. caliginosa* and *A. chlorotica* decreased in common treatments

Abstract

Soil ecosystem engineers are major actors of soil macroaggregation, a process that drives the production of ecosystem services by soils. However, our inability to identify the origins of different types of macroaggregates found in soils is an obstacle to describing and modeling their dynamics and associated processes (C sequestration; hydraulic properties). This laboratory study investigated mechanisms of biological soil macroaggregation by two different earthworm species (*Apporectodea caliginosa* (Savigny) and *Allolobophora chlorotica* (Savigny) and two plant species (*Trifolium pratense, Plantago lanceolata* L.), in isolation and in all possible combinations. Near infrared (NIR) spectral analysis significantly discriminated macroaggregates according to the organisms that created them since each organism produced macroaggregates with distinct NIR signals ($p<0.001$). The largest departure from the control signal was observed with *T. pratense* whereas earthworms and *P. lanceolata* specific signals were less contrasted. Macroaggregates formed in the presence of more than one ecosystem engineers had mixed signals showing that several actors had participated in their construction.–This means that roots and earthworms did not produce macroaggregates in isolation and rather added their effects in building structures of mixed origins. Further studies based on the present methodology will tell us more on below ground behaviors of ecosystem engineers and their interactive building of soil habitats.

Keywords*: Earthworm-root interactions, Earthworm casts, NIR spectral signature, Soil Macroaggregation*

I. Introduction

This paper describes soil macroaggregate formation by roots and earthworms, the main ecosystem engineers in most soils, under laboratory experimental conditions. Different combinations of two earthworm and two plant species were tested to assess the role of each organism in macroaggregation and describe the results of their interactions.

Microorganisms are given a prominent role in current models of soil aggregation (Tisdall and Oades 1982; Tisdall, 1994; Six et al. 2002). While bacteria in laboratory experiments create microaggregates by glueing particles with polysaccharides (Chenu 1993), fungal hyphae entangle particles in temporary nets creating fragile short lived aggregates (Tisdall and Oades 1982; 1993; Plante and Mc Gill 2002), and/or stick them together with glomaline (Rillig et al. 2002; Rillig 2004). Larger organisms, classified as soil ecosystem engineers—roots, earthworms, termites, ants and a few other invertebrates—also participate in soil aggregation. Roots produce mucilages and other exudates that hold particles together, which had already been compacted by root pressure and local drying of soil (Miller and Jastrow 1990). Many invertebrates build biogenic structures that may become highly stable macroaggregates and comprise a large proportion of upper soil horizons. For example, earthworms from the Lamto savanna in the Ivory Coast annually egest 800 to 1000 Mg dry weight casts per ha (Lavelle 1978). Once these casts have dried they become compact and can remain largely intact for several years if not broken by physical processes, like effects of repeated wetting-drying cycles or agricultural machinery or ingested by other invertebrate ecosystem engineers (Shipitalo and Protz 1988; Blanchart et al. 1999). Compaction during gut transit probably explains the high cohesion of these assemblages due to simple attraction forces (Barois and Lavelle 1986). Some authors consider, however, that microbial-induced aggregation eventually gives these aggregates their high resistance to external forces (Bossuyt et al. 2004). A diverse array of aggregate consolidation processes may be expected to operate given the wide range of soil composition and diversity in feeding habits and digestion processes exhibited by soil ecosystem engineers (Lavelle et al. 1997). Physical processes that create fissures and cracks in soils, the role of texture, and the

effects of specific types of clay minerals must also be taken into account (Lavelle and Spain 2001). While the former tend to isolate discrete blocks of particles that they pack together, fine textured elements may cement larger particles inside these blocks or biological constructions ensuring the stability of the structure. High contents in fine silt and clay minerals, especially of the 2:1 category thus increase the stability of aggregates.

Past research has mainly focused on soil aggregation by physical methods and the resistance of these structures to different levels and kinds of physical stresses (Le Bissonnais 1996). Little attention has been paid to the mechanisms of aggregation, whether physical or biogenic (Pulleman et al., 1995; Bossuyt et al., 2004; Velásquez et al., 2007). Virtually no studies consider aggregate ageing processes, the dynamics of aggregation as a result of physical and biogenic processes, or disaggregation through ageing and physical destruction (Blanchart 1999; Decaëns et al., 1999; Le Bayon et al., 2002).

Our inability to identify the origins of the different types of aggregates found in soils, their turnover time, and their array within the soil matrix has long been a major obstacle to describing and modeling macroaggregate dynamics in soils and their associated processes. Two recent methodological developments, however, should enable better description of the origins of soil macroaggregates. While morphological analyses allow separating physical from invertebrate and root biogenic macroaggregates, the use of near infrared (NIR) spectral signature allows discriminating macroaggregates according to the process or organism that created them (Hedde et al. 2005; Velasquez et al. 2007; Zhang et al. 2009). NIRS signatures reflect the amount and nature of organic matter accumulated in specific ways in each kind of biogenic or physical structure. Invertebrates have selective feeding habits that result in the ingestion of preferred organic items. Additions of saliva or intestinal mucus selectively enhance microbial populations during gut transit. Enzyme production in digestion and the quality of assimilated and non-assimilated materials contribute to the formation of different signatures. Soil chemical and physical analyses of structures and the assessment of their microbial communities and enzymatic activities support this observation (Mora et al. 1991; Hedde 2005). We expected that each macroaggregate, from a given soil, should have a specific recognizable signature depending on its origin. We can also possibly identify situations where different organisms, e.g. roots and earthworms, have combined their

effects in building structures. In this case, we expect the spectra to have compounds originating from both sources.

While many papers acknowledge the role of aggregation as a soil attribute and function, the origin of soil macroaggregates has been largely ignored so far. This gap in our knowledge makes it difficult to understand the roles of the different actors involved in macroaggregate formation and to model the consequences on soil aggregation and associated functions of fluctuations in the populations and communities of these ecosystem engineers.

While roots and earthworms are each considered significant actors of soil macroaggregation, no research has been done on their possible interactions. Simple observations of soil aggregates often show earthworm casts attached to the roots. What this means is not clear. Do earthworms feed on root material and leave their casts where they fed? Or do roots grow in freshly deposited casts or in an accumulation of old casts?

In the following laboratory study we first characterized macroaggregates according to their origin (biological or physical) based on their morphology and size following the Velásquez et al. (2007) procedure. NIRS allowed analyzing spectral signatures of macroaggregates formed by worms or roots cultivated in isolation. We then analyzed NIR signals of macroaggregates produced in different combinations of earthworm and plant species to search for interactions between both species. Different scenarios can be considered: 1. No interaction: roots and worms produce macroaggregates independently in different microenvironments and possibly compete for space to produce their respective structures. The quantity of macroaggregates produced by earthworms and plants combined is a simple additive function of the amount of macroaggregates produced by earthworms and roots in isolation. NIRS signatures of macroaggregates then belong to two separate groups and are similar to signatures from single species treatments. 2. Interaction: Roots and worms combine their effects on macroaggregate formation. Interaction affects the amount of macroaggregates created (increases or decreases) and their respective signatures. Signatures may either be intermediate or express the enhancement of one or another actor in macro-aggregation.

II. Materials and methods

The two plant species used for the experiment were, red clover (*Trifolium pratense*), a fast growing species expected to favor bacteria rather than fungi in its rhizosphere (Bardgett et al. 2005; De Vries et al. 2006), and *P. lanceolata* L., which on the other hand, is expected to host a larger proportion of fungi in its rhizosphere. These characteristics should result in different NIRS signatures of macroaggregates formed by roots of the two species. We also expected different kinds of interactions with earthworms that associate better with bacteria than with fungi for their digestion (Barois and Lavelle 1986)

A laboratory microcosm experiment was set up to measure and assess macroaggregates (>1cm in diameter) produced by two different species of earthworms and two species of plants respectively, in isolation and in combination (Table 1). There were a resulting 16 treatments, each replicated three times (Table 1).

Plants, earthworms and soil

Earthworm species used for the experiment were *Apporectodea caliginosa* (Savigny 1826) and *Allolobophora chlorotica* (Savigny), commonly found in pastures and low-input cropping systems in many temperate areas of the world. Both are classified as soil feeding endogeic species (Bouché 1977). *A. caliginosa* is known to ingest a small amount of decomposed litter debris mixed in the soil; while *A. chlorotica* has a more typically geophagous regime.

Plant species used for the experiment were red clover (*Trifolium pratense*) and ribwort plantain (*Plantago lanceolata* L.).

Individuals of *A. caliginosa* were collected at Bondy (a permanent grassland in a suburb of Paris); while *A. chlorotica* were collected in the Fontainebleau forest (50 km south of Paris). Soil was collected at Bondy from the upper 15 cm layer, air dried and sieved at 1.85 mm. The Bondy soil is sandy (with 47% and 17% coarse and fine sand, respectively), and had a 15% silt and 21% clay content; C and N average content were 3.74% and 0.25%, respectively, and the C:N ratio was 14.85. Semi-

decomposed poplar litter was air dried, crushed, sieved at 2mm, and applied to the soil surface of the microcosms as additional food for earthworms.

Figure 1: *Trifolium pratense*

Figure 2*: Plantago lanceolata* L

Microcosms

Plastic pots (15cm in diameter, 20cm deep) were filled with 800g of dry soil and covered with a thin layer of 5g sieved, leaf litter to serve as food for the earthworms. Plants were sown at the beginning of the experiment (20 seeds per pot). Four earthworms per pot (ca. 2.4g fresh weight on average) were inoculated five weeks later, after plants had grown and developed root systems. Microcosms were assigned randomly to avoid confounding effects due to uneven lighting

Soil moisture was maintained at field capacity throughout the experiment. Capillary *per ascensum* diffusion through small holes drilled at the bottom of the pots was preferred in order to prevent physical soil aggregation by surface splash effect.

Microcosms were dismantled eight weeks after the beginning of the experiment. The aboveground part of the plants was first cut and removed. Earthworms were collected and weighed individually after carefully breaking soil into large clods. Soil was slowly dried (over seven days; 40°C) in order to harden macroaggregates and facilitate separating them from soil that had not been macro-aggregated by specific earthworm- or root-associated processes.

Soil was then shaken and passed through a 5mm mesh sieve to disperse weak macroaggregates created during drying and handling and to retain resistant macroaggregates built by the activity of earthworms and roots. The resistant macroaggregates were visually separated according to their origin; biogenic aggregates, produced by earthworms (generally round shaped), and root aggregates stuck to the roots (with variable external shapes).

NIRS Spectral Signatures

A total of 240 macroaggregates from five macroaggregates sampled randomly from each replicate pot were analyzed. Average weight of samples was 4.46g (SE=1.41). Each macroaggregate was crushed and passed through a 200 µm-mesh sieve to obtain homogeneous preparations of all samples. Roots were separated from macroaggregates during crushing to avoid contamination of the spectral signal by solid plant material. Samples were packed in a quartz-glass container and placed in a spectrophotometer (FOSS NIRSystems, Silver Spring, MD, USA) with a 1100-2500 nm spectral range. The reflectance measurements were made at two nanometer intervals. Reflectance (R) was converted to absorbance (A) using the equation: $A = \log (1 / R)$ and further transformed to second derivative according to general procedures recommended for the treatment of this particular type of signal. Finally, average values were calculated for 10 nm intervals in order to reduce the number of variables processed. Data analyses were conducted using the ISI software system (Shenk and Westerhaus 1991).

Statistical Treatments

Principal components analyses (PCA) were performed using the R package, with ADE4 library for multivariate analyses [R Development Core Team, 2004; ADE-4 software (Thioulouse et al. 1997)]. Permutation tests on PCA coordinates allowed for testing differences among treatments. The table of data to be treated had 139 columns—the secondary derivatives of the spectral signal for each of the 139 successive wavelengths—and 240 lines—the 240 macroaggregates (five samples per three replicates of the 16 treatments).

Differences among total macroaggregate production, earthworm biomass and plant above ground biomass obtained in each treatment were evaluated using parametric analysis of variance (ANOVA). Comparisons between means were tested with Tukey's test. Differences among treatments were evaluated at the <0.05 probability level of significance.

III. Results

Growth of earthworm and plant and aggregate production

Earthworms had a reasonably high rate of survival (70.8%); and their biomass was generally stable during the experiment. No significant difference in earthworm biomass was observed at the end of the experiment among treatments ($p>0.1$. Table 1). Plants grew well. *T. pratense* achieved higher biomasses than *P. lanceolata* L. in all treatments ($p<0.001$. Table 1). There was no significant effect of earthworms on above plant ground biomass by the end of the experiment ($p>0.1$ Table 1).

Macro-aggregation was important in most experimental units, with 142.13 to 336.74g dry weight of macro-aggregated soil and with significant differences among treatments ($p<0.02$). Production of macroaggregates decreased significantly in the presence of both earthworm species (treatment AC; $p <0.02$, Table 1) compared to single species treatments; although earthworms did not exhibit weight loss during the experiment. Fewer macroaggregates were produced in the presence of *P. lanceolata* L. than *T. pratense* roots. Soil macroaggregation decreased significantly in treatments containing a mixture of *P. lanceolata* L. and an earthworm species— ($p<0.02$). This decrease in soil macroaggregation was not observed in treatments containing a mixture of *T. pratense* and one earthworm species.

Table1.

Treatment	Aggregates produced (g) (AP)	Initial earthworm biomass (g)	Final earthworm biomass (g)	Final plant above ground biomass (g)
	pValue<0.02	pValue>0.1	pValue>0.1	pValue<0.001
A	307.90 [a]	1.37	1.41	
	(45.00)	*(0.33)*	*(0.24)*	
C	312.03 [a]	1.15	1.10	
	(41.09)	*(0.12)*	*(0.07)*	
AC	203.57 [c]	1.70	1.73	
	(64.86)	*(0.1)*	*(0.23)*	
Tr	217.10 [c]			1.02 [a]
	(75.38)			*(0.11)*
Pl	142.13 [d]			0.32 [b]
	(96.42)			*(0.40)*
TrPl	194.9 [cd]			0.68 [ab]
	(31.23)			*(0.29)*
TrA	292.13 [a]	2.29	2.45	0.98 [a]
	(14.23)	*(0.15)*	*(0.18)*	*(0.25)*
TrC	308.6 [a]	1.78	1.79	1.06 [a]
	(19.82)	*(0.17)*	*(0.14)*	*(0.51)*
PlA	256.53 [b]	1.90	1.92	0.23 [b]
	(23.88)	*(0.06)*	*(0.05)*	*(0.17)*
PlC	277.83 [b]	1.19	1.17	0.18 [b]
	(28.59)	*(0.12)*	*(0.03)*	*(0.12)*
Tr A C	315.5 [a]	2.06	2.04	1.03 [a]
	(113.08)	*(0.34)*	*(0.41)*	*(0.25)*
Pl A C	274.03 [b]	0.78	0.94	0.13 [b]
	(45.99)	*(0.16)*	*(0.20)*	*(0.05)*
TrPlA	336.74 [a]	3.1	3.06	0.67 [ab]
	(36.37)	*(0.24)*	*(0.22)*	*(0.23)*
TrPlC	325.53 [a]	2.32	2.26	0.68 [ab]
	(45.19)	*(0.26)*	*(0.22)*	*(0.37)*
Tr Pl A C	283.87 [ab]	2.65	2.65	0.75 [ab]
	(35.02)	*(0.31)*	*(0.43)*	*(0.21)*

Table 1.
Total macroaggregate production, earthworm biomass and plant aboveground biomass obtained in each treatment. AP: total dry weight of macro aggregated soil produced during our experiment. (A = *Apporectodea caliginosa* (Savigny), Tr = *Trifolium pratense*, C = *Allolobophora chlorotica* (Savigny), Pl = *Plantago lanceolata* L., Tr = *Trifolium pretense*)

Figure 4: Casts of *Aporrectodea caliginosa*

Figure 3: Root aggregates of *Trifolium pratense*

Figure 5: Casts of *Allolobophora chlorotica*

NIR Spectral Signature

General results

The PCA of the 139 variables describing NIR spectra showed a significant separation among the 16 different treatments (Fig. 1a); although intra treatment variability was rather large. The treatment effect explained 43.8% of total variance. This effect was significant ($p<0.001$). The PCA of Figure 1a regroups the barycenters in two plots, one plot centered on *T. pratense* and combinations of *T. pratense* and *P. lanceolata* and a second plot of single combinations excluding *T. pratense*. The first factor (50.3% of total variance explained) clearly separated macroaggregates produced in both plots in presence or absence of *T. pratense.* The second factor (13.7% of total variance explained) contrasted mixed treatments with plants and earthworms with single species treatments. Treatments with one single ecosystem engineer, the earthworms *A. caliginosa* (A) and *A. chlorotica* (C) and plants *T. pratense* (Tr) or *P. lanceolata* (Pl)) or combinations of a single type of organism (AC and TrPl), generally departed less from the control than treatments associating plants and earthworms.

Figure 6a.

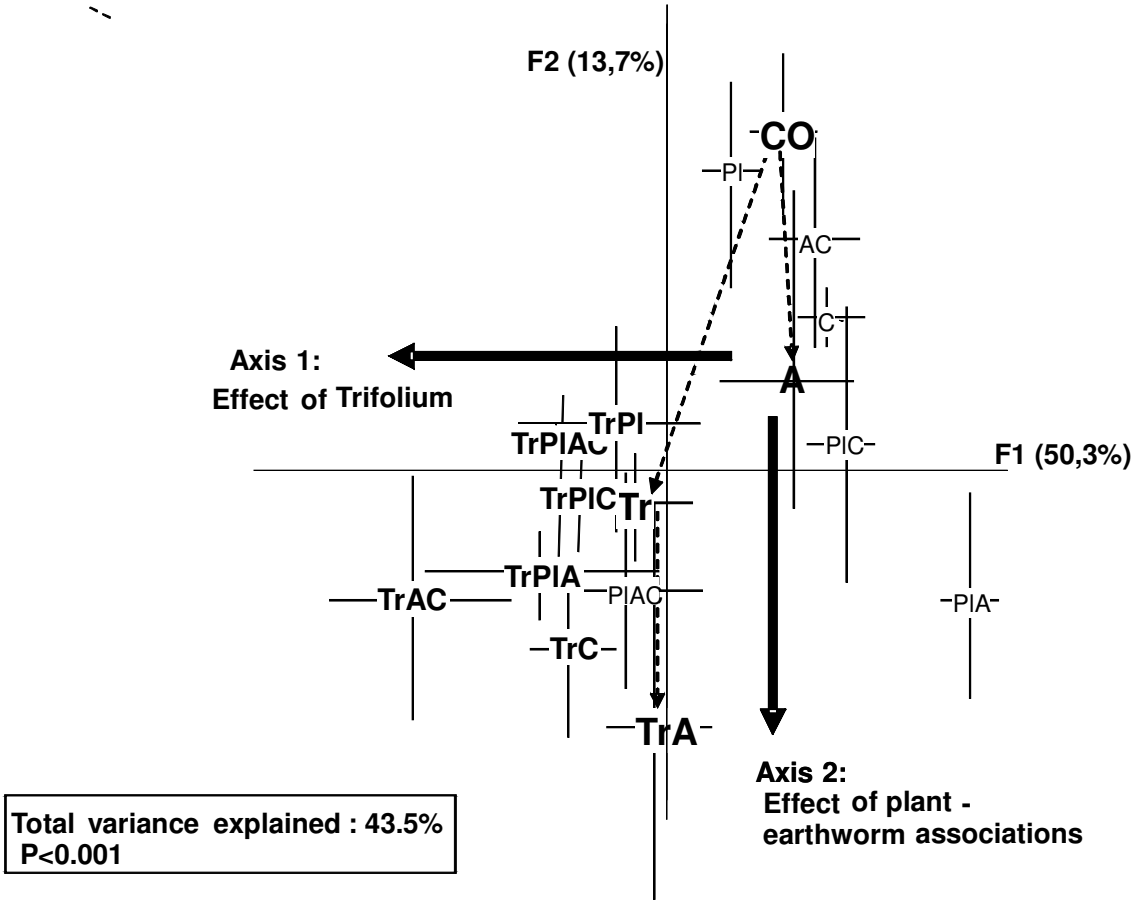

Figure 6a:

Projection of barycenters of NIRS spectra of aggregates produced in the 16 treatments, in planes defined by factors 1 and 2 of PCA analysis (15 replicates per treatment). Solid lines indicate major effects observed along factorial axes; dotted lines underline the additive influences of clover (Tr) and *A. caliginosa* (A) in NIRS signal of treatment TrA. C: *Allolobophora chlorotica* (Savigny); A: *Apporectodea caliginosa* (Savigny); Tr: *Trifolium pratense*; Pl: *Plantago lanceolata* L.; Co: control.

Carbon (C) and nitrogen (N) contents of macroaggregates were always higher than those of the control soil ($p < 0.05$): while the C:N ratio of macroaggregates was lower than that of the control soil ($p < 0.001$). The specific effect of plant and earthworm associations on organic matter quality, which separates treatments on axis 2 of Figure 1a (13.7% of total variance explained) is associated to the C, N and C:N variables on the correlation circle (Fig. 1b). Just a few NIR wavelengths (1120, 1400, 2170, 2220-2230, 2450) can be associated with C and N on Figure 1b. Most of the NIR wavelengths, close to the first axis, differentiate a biotic footprint independent of C and N.

The correlation circle of Figure 1b shows an association of the first axis with high reflectances in NIR wavelengths 1170-1190, 1200-1210, 1300-1310, 1360-1390, 1420-1470, 1650-1760, 1780-1880, 1900-1970, 1990-2000, 2020-2120, 2250-2290, 2310-2370, 2420-2440 (Fig. 1b). Control and earthworm single treatments on axis 2 were associated to high reflectances in wavelengths 1120-1140, 1770, 2170, 2230, 2410-2430 (Fig. 1b). Mixed earthworm plant treatments were associated along axis 2 with high values in wavelengths 1410, 1550, 2190, 2210, 2450.

Table 2 shows that earthworm treatments (A, C and AC) were characterized by specific reflectances in wavelengths 1770, 2350, 2420-2430 and 2460. Plant treatments (Tr, Pl an TrPl) were associated to high reflectances in wavelengths in 1120-1140, 1810, 1820, 1930, 1950, 2050, 2080, 2140, 2190-2200, 2220-2230, 2410 and 2440.

Figure 7b.

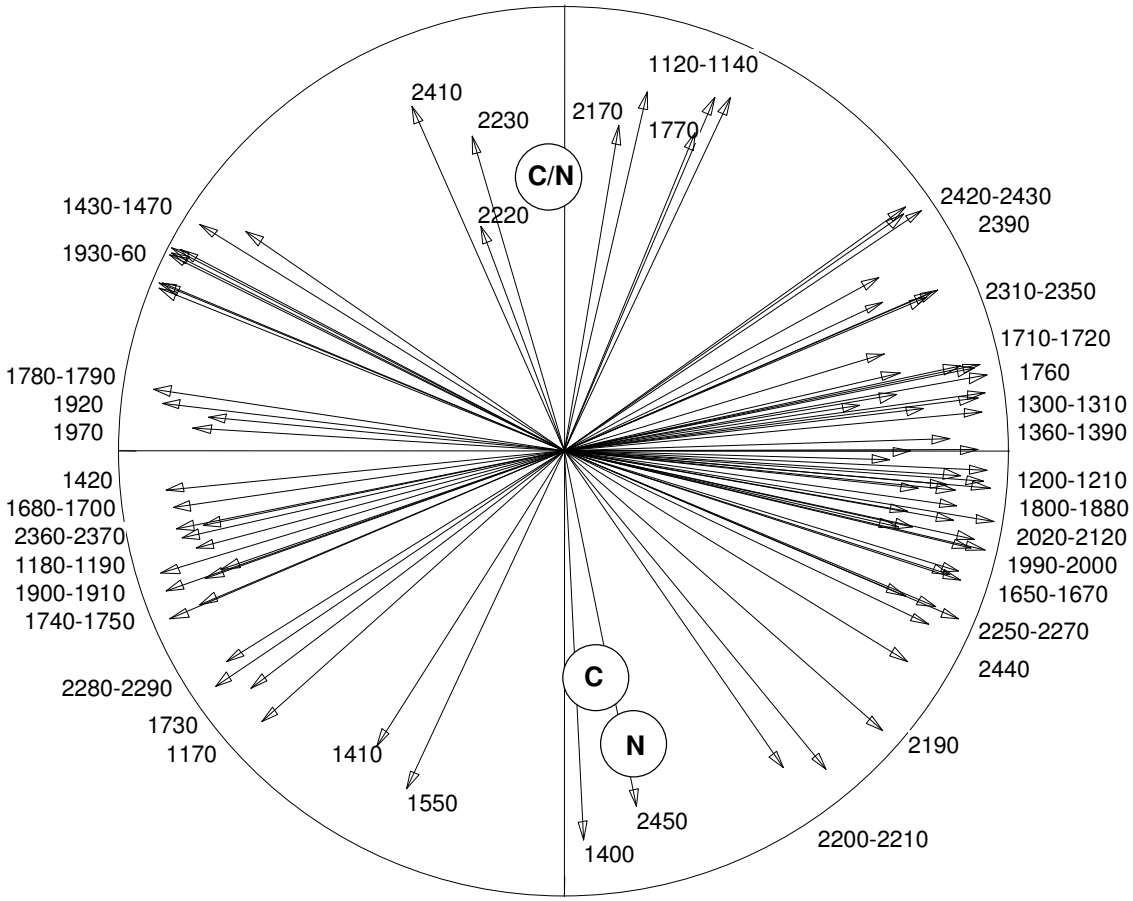

Figure 7b.

Correlation circle among NIR wavelengths and factors. C, N, and C:N variables projected as additional variables not taken into account in calculations. For the correlation circle, variables were selected that had respective weights on axes 1 and 2 equal to at least half maximum values, respectively, calculated for these axes (Velásquez 2007).

Data from the earthworm and plant groups were treated separately to better describe specific effects and provide clues to the interpretation. Analyzed data sets comprised repetitions of control, plus relevant parts of the global data set previously analyzed.

Earthworm signatures

PCA showed a significant separation between *A. caliginosa* and *A. chlorotica* treatments (Table 2). Each earthworm species was characterized by a specific spectral signature ($p<0.001$), despite a rather large dispersion of projections within the same treatment. The C:N ratio in macroaggregates decreased from control soil to the A (*A. caliginosa* alone) treatment ($p<0.001$) on axis 2.

Plant signatures

Macroaggregates produced in rhizospheres of *T. pratense* and *P. lanceolata* L. had rather different spectral signatures ($p<0.001$) (Fig. 2). *P. lanceolata* was very close to the control in the plane defined by factorial axes 1 and 2. Soil enrichment in organic matter was rather low in macroaggregates formed in plant rhizospheres and not significant ($p<0.4$).

Figure 8.

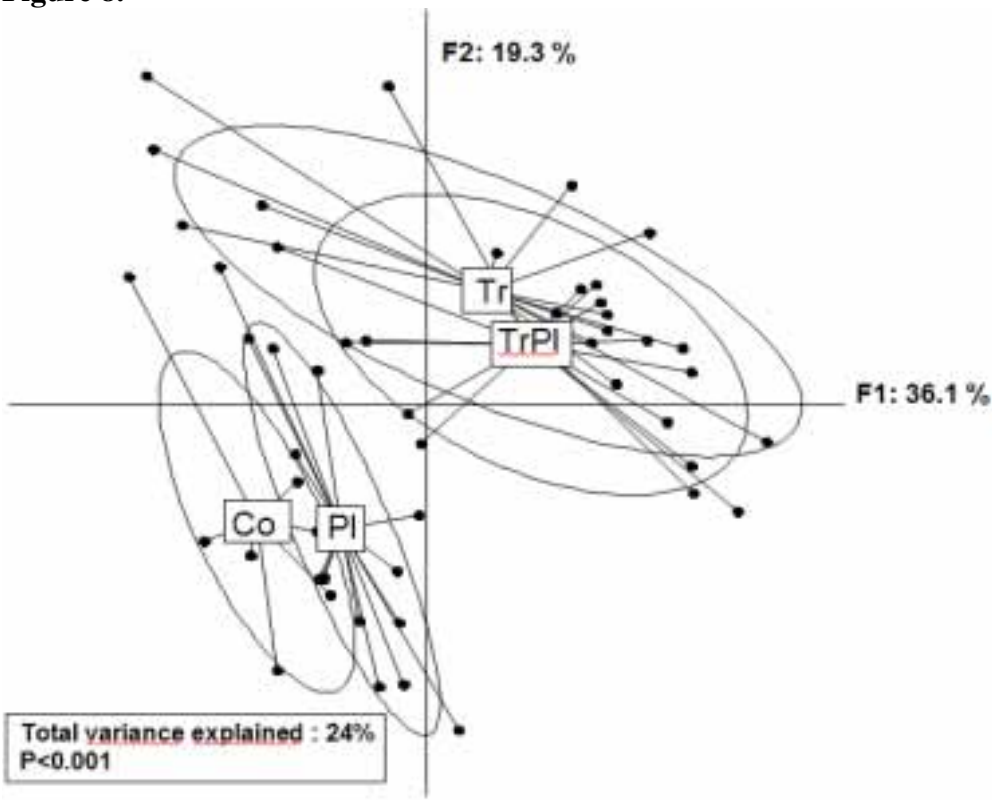

Figure 8:

Projection of barycenters of NIRS spectra of aggregates produced in four treatments, in a plane defined by factors 1 and 2 of the PCA analysis (15 replicates per treatment). Pl: *Plantago lanceolata* L.; Tr: *Trifolium pratense*; Co: control.

Mixed treatments signatures

The respective positions in factorial plane 1-2 of barycenters of treatments with earthworms and plants (TrA, TrC, PlA and PlC (cf table 1)) clearly suggested additive effects (Fig.1a). This was particularly evident in the TrA (cf table 1) treatment where the segment that links Tr to TrA is exactly equal to the segment that links the control (Co) to the A treatment (Fig. 1a). Additive effects are also suggested in other cases, although with less demonstrative results.

Interactions occured in more complex treatments, although the effects were not as clearly understandable. *T. pratense,* when present, always exerted a strong effect on signatures; while other organisms had less marked influence.

Table 2.

Analysed data	Total variance explained by treatment	P value	Factor 1 (% of the explained variance)	Factor 2 (% of the explained variance)	Characteristic NIR wavelengths	Specific NIR wavelengths
Treatments containing only earthworm species (A, C, AC)	10.25	<0.001	35.3	16.0	1170-1180, 1360-1390, 1410-1460, 1650-1690, 1710-1770, 1830-1880, 1900-1920, 2000-2040, 2090-2120, 2250-2260, 2280-2350, 2370, 2420-2430, 2450-2460	1770, 2350, 2420-2430, 2460
Treatments containing only plant species (Tr, Pl, TrPl)	23.53	<0.001	36.1	19.3	1120-1140, 1170-1180, 1360-1470, 1550-1560, 1660-1670, 1690, 1710-1740, 1760, 1780-1790, 1810-1880, 1900-2050, 2080-2120, 2140, 2190-2230, 2250-2340, 2410-2440	1120-1140, 1810, 1820, 1930, 1950, 2050, 2080, 2140,2190-2200, 2220-2230, 2410, 2440
Treatment containing only control (Co)					1170-1180, 1200, 1280, 1310, 1370-1380, 1400-1440, 1550-1560, 1650-1680, 1700, 1720-1730, 1750, 1850-1940, 1960, 2020, 2040, 2210, 2260-2270, 2300, 2370, 2450	

Table 2.

Analysis of NIR spectra for the separate data sets; for earthworm treatments (A, C and AC) and for plant treatments (Tr, Pl and TrPl) (Total variance explained by PCA analysis, variance explained by factor 1 and 2, P value calculated with permutation test). Characteristic wavelengths are variables of the correlation circle that were selected for having respective weights on axes 1 and 2 at least equal to half maximum values, respectively, calculated for these axes (Velásquez 2007). Specific wavelengths are selected characteristic wavelengths that are unique for plant or earthworm treatments.

IV. Discussion

Soil Macro-aggregation by Ecosystem Engineers

Soils were significantly macro-aggregated by plants and earthworms – i.e., our "ecosystem engineers". In only two months, from 17.8 % to 42.1 % of total soil was transformed into macroaggregates, a result that confirms the ability of these organisms to transform large amounts of soil in relatively short periods of time. This observation - previously recorded for earthworms in several studies - had not been recorded for roots. The finding confirms that soil macro-aggregation by ecosystem engineers may be very fast when conditions are favorable to their activity.

In the conditions of our experiments, more aggregates were produced by earthworms than by the roots . *A. caliginosa* produced an average 2.6g dry soil g-1 fresh weight earthworm biomass day-1 and A. chlorotica 3.37g dry soil g-1 fresh weight earthworm biomass day-1 . Since earthworms do not normally re-ingest their own casts, this amount of macroaggregates is equivalent to their soil ingestion rate.

Comparison of the amount of macroaggregates formed by a single engineer (a plant or an earthworm) or two associated engineers suggest greater macroaggregate production with more engineers. An average of 245g of dry soil was produced in one component treatments, 260g in a two components treatment, 314g with three, and 283g with four components.

In only one case, the presence of the two earthworm species (AC) formed significantly fewer macroaggregates than the single earthworm species acting alone. Since earthworm biomass at the end of the experiment remained at the same level as in the single species treatments, we conclude that decrease in macroaggregate formation was the result of a positive interaction between the earthworms: one species probably fed on casts of the other species which resulted in a lower amount of macroaggregates formed. *A. chlorotica,* known to have a rather more geophagous regime than *A. caliginosa,* was presumably the one feeding on the enriched and partly digested litter debris in *A. caliginosa* casts.

NIRS as a Tool to Identify Origin of Aggregates

This study confirmed the ability of NIR spectrometry to discriminate soil macroaggregates according to the organisms that produced them in their respective root rhizosphere and earthworm drilosphere (Hedde et al. 2005; Velásquez et al. 2006; Zhang et al. 2009). Signatures of macroaggregates departed from the non-aggregated soil control to different extents and in different directions according to the organisms and their respective associations in the treatments. In spite of some degree of intra variability in NIRS signals, differences between treatments were highly significant. We thus confirm that it is possible to identify the origin of a single macroaggregate from its NIR spectral signature.

Aggregation Processes

Our results provide some clues and raise novel questions on the way soil ecosystem engineers—in this study, plant roots and earthworms— macro-aggregate the soil. Macroaggregates generally have higher C and N contents than bulk soil, a finding that indicates that enrichment in organic matter is associated to the macro-aggregation process. *T. pratense* mixed and single species macroaggregates showed significant higher C and N contents than *P. lanceolata* which could explain that highly contrasted departures of NIRS signals from control soil in root macroaggregates possibly indicate much lower rhizodeposition by *P. lanceolata* than by *T. pratense*. If confirmed by direct measurement, this observation would mean that some wave lengths of the NIR spectra, measured in similar conditions, would be markers of specific compounds of root exudates. Their absorbance would then be used as a proxy to exudate production. Other analyses should identify macro-aggregation processes in the respective rhizospheres of *P. lanceolata* and *T. pratense* and identify microbial contributions to the process. A hypothesis to test would be the existence of significant differences in contents of microbial byproducts, like glomaline or bacterial extracellular polysaccharides, in macroaggregates of P. lanceolata and T. pratense that could explain differences observed in spectral signatures?

The greatest part of the variability in NIR signals is explained by the quality of organic matter: organic enrichment in macroaggregates was mainly observed along

axis 2 of the PCA (Fig. 1). In this respect, *T. pratense* exudates seem to have a much different chemical composition than organic products associated to *P. lanceolata* or earthworm activities.

Interactions among Ecosystem Engineers in Soil Aggregation

Plant-plant interactions

Little is known of interactions among root systems in soils. The most generally accepted view is still that of competitive exclusion that would predict strictly separated rooting systems (Tilman 1982). This approach predicts the formation of separate macroaggregates in treatments with two different plant species. Such a result would have been associated with an NIR signature of each species. Our results showed that this was not the case. The PCAs performed on the plant treatments (Fig. 2) showed that no single macroaggregate of the TrPl treatment associating the two plant species had its projection in the ellipse that comprises the Pl or Tr alone treatments, thus showing that most macroaggregates are probably a common production of roots of both species.

Plant-earthworm interactions

Relationships among roots and earthworms are still largely unknown. Root feeding by earthworms has been very seldom reported; and most earthworms seem to avoid eating root litter, at least in the early stages of root decomposition. Roots have often been observed in ageing earthworm casts, however; and intense fine root colonization of freshly deposited casts has been observed (Decäens et al., 2001), which shows that roots often take advantage of nutrients available in casts. Our results clearly show interactions in macroaggregate formations given that macroaggregates formed in the presence of two different engineers were significantly different from macroaggregates formed in the presence of only one organism (Fig. 1a). Samples projected in factorial plane 1/2 of PCA of NIR spectral variables form a continuum that suggests additive effects. This shows that several actors had participated in their building. Juxtaposition of aggregates of different origins with signals of only one ecosystem engineer was not observed. The effect is especially clear for the *T.*

pratense and *A. caliginosa* treatment (Fig. 1a) where the vector that links the control (Co) to the TrA treatments is almost an exact geometric sum of vectors Co-A and Co-Tr. More organic material is present in macroaggregates than in control soil (as indicated by location of the TrA treatment on the side of axis 2 where C and N variables are projected), which seems to indicate that rhizodeposition was stimulated by earthworm activity and earthworms probably fed upon root exudates and rhizodeposition, since earthworms are known to feed preferably on organic materiel enriched soils. Another interpretation of the additive effect may be that macroaggregates formed by earthworms were further colonized by roots, which in turn added their rhizodeposition to the specific organic compounds mixed by the worm in the soil. A lower production of rhizodeposition in the *P. lanceolata* rhizosphere would make it less attractive to earthworms resulting in less clear indications of additive effects in NIR signatures of mixed plant/earthworm treatments.

Significance as Regards Soil Function

This experiment showed how interactions below ground can lead to the macro-aggregation of high volumes of soil in a very short period of time. Interpretation of the results, however, was rather complicated because we lack knowledge of root biology and interaction processes in root systems, as well as on earthworm-root interactions. Our observations show that interactions may be rather diverse; and that these interactions suggest different mechanisms of cooperation that should be considered in future models.

Our experiment was limited in time and space and involved a completely new system where soil macrostructure had been eliminated. These are conditions of an early phase succession process (phase alpha according to Gunderson and Holling's model, 2002) where systems are reorganized in somewhat unpredictable manners. Soil volume was also rather limited, which favored the coexistence of organisms, although experiments conducted in similar conditions have shown negative interactions and mutual avoidance of earthworms and/or evidence of disturbed behaviors when antagonistic species were introduced together (Felten and Hemmerling 2009; Decäens et al. 2008, Hedde et al. 2007).

Further, we need to explore, in natural field conditions, the capacity of NIRS to indicate which ecosystem engineer was responsible for the formation of a given

macroaggregate. This would allow doing 3-D descriptions of the array of structures of different origins as a basis for modeling and for the formulation of new Soil Ecology theories.

Acknowledgement

The authors are very grateful to Didier Brunet for NIRS analyses and to Max Sarrazin for CHN analyses. We would like to thank Florence Dubs and Elena Velasquez for their advice on statistical analyses and constructive discussions. We would like to thank as well Steven Fonte and Alexandra Walter for the linguistic proofreading and comments. This project was supported financially by IRD (unit research UMR137-BIOSOL and UMR-211-BIOEMCO).

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Conclusion de l'article 3

Ce travail nous confirme les résultats obtenus au chapitre 1. La NIRS permet de caractériser des macroagrégats de chaque organisme ingénieur par une signature spectrale propre. Les macroagrégats des plantes et des vers de terre ont montré des réflexions spécifiques dans les longueurs d'ondes (1770, 2350, 2420-2430 et 2460) pour les vers et (120-1140, 1810, 1820, 1930, 1950, 2050, 2080, 2140, 2190-2200, 2220-2230, 2410 et 2440) pour les plantes. Cependant cette étude répond aussi à l'hypothèse : Lors de la formation d'un macroagrégat biogénique, les deux acteurs, racines et vers de terre, peuvent additionner leurs effets. Dans un milieu complètement homogène et non structuré, ont été introduit des graines de plantes et des vers de terre afin de tester comment, lors de la structuration du sol, se forment la rhizosphère et la drilosphère. L'expérience valide les hypothèses 1 et 2. Une interaction entre les deux acteurs, plante et ver de terre, a pu être mise en évidence par une signature spectrale propre aux macroagrégats produits dans des microcosmes, contenant plantes et vers de terre. Ceci nous montre que les racines et vers de terre n'ont pas produit des macroagrégats en isolation mais qu'ils ont additionné leurs effets dans la construction d'un macroagrégat aux origines mixtes. La projection des spectres dans le plan factoriel d'une ACP révèle un effet additif visible entre les projections des barycentres de macroagrégats monospécifiques de plantes et de vers de terre et des macroagrégats aux origines mixtes. Les longueurs corrélées au premier axe de l'acp sont associées à des différences qualitatives de la MO des macroagrégats et non à des différences quantitatives en C et N. C'est le deuxième axe qui est corrélé aux variables teneur total de C et N. Ces dernières sont associées aux longueurs d'ondes (1120, 1400, 2170, 2220-2230, 2450).

Hors sur l'ACP de cette expérience, il a aussi été montré que les effets additifs entre espèces de plante et espèces de ver de terre apparaissent plus ou moins important. En conséquence deux expériences supplémentaires ont été réalisées afin de tester si les interactions observées peuvent être considérées comme un mécanisme général ou s'il s'agit d'une observation unique. Nous avons également essayé de

comprendre si les intensités observées varient avec les espèces de plantes et/ou des vers de terre? Les deux expériences suivantes ont été réalisées avec des espèces de vers de terre ayant des comportements alimentaires différents et des espèces de plantes appartenant à des groupes fonctionnels différents (légumineuses et graminées). Les expériences ont été conduites dans des conditions climatiques différentes, avec des espèces de plantes et des vers de terre typiques les uns de régions tempérées et les autres typiques de régions tropicales. L'utilisation du traceur naturel ^{13}C devrait nous fournir plus d'informations sur la nature du marqueur de l'activité des plantes. Si la rhizodéposition ou des fragements de tissu de plante sont à l'origine du marqueur racinaire, identifié dans les signatures spectrales des macroagrégats racinaires, alors nous devrons pouvoir discriminer la MO d'une plante C4 dans ces macroagrégats (produits dans du sol ayant une trace de plantes en C3). Si cette hypothèse ne sera pas validée, nous pourrons conclure que le marqueur n'est pas de nature de plantes. Des analyses du traceur naturel ^{13}C seront faites dans l'expérience suivante. Elles devraient nous fournir une réponse claire à la question si les traceurs de l'activité racinaires, identifiés dans les macroagrégats monospécifiques et mixtes, sont d'origine de plante ou non.

Article b :

General patterns in soil macroaggregate formation by roots and earthworms. A mesocosm approach.

Zangerlé A. , Hissler C., Hurtado P., Loaiza S., Lavelle P.

Abstract

This laboratory study investigated mechanisms of soil macroaggregate formation by plants and earthworms of two different functional groups, under laboratory experimental conditions by near infrared spectroscopy analyses. This approach allowed us to test if interactions among ecosystem engineers are a once observed pattern or if it is a generally applicable pattern. Natural abundances of ^{13}C of C4 plants were used as a marker of root activity in macroaggregates.

Near infrared (NIR) spectral analysis significantly discriminated macroaggregates according to the organisms that created them since each organism produced macroaggregates with distinct NIR signals ($p<0.001$). Macroaggregates formed in the presence of legumes and earthworms had mixed NIR spectral signals showing that several actors had participated in their construction in a same microenvironnement. However additive effects on spectral signatures are not observed for all plant-earthworm combinations, since grasses did not show interaction effects with earthworms in macroaggregate building.

Results of natural ^{13}C abundance of *Brachiaria hybrid Mulato 2* showed that root activity markers comprise of plant material.

A significant increase of shoot biomass, root biomass and shoot/root ratio of plant species *Brachiaria hybrid Mulato 2* and *Trifolium ruttinova* in the presence of earthworms have been highlighted by this study.

In further studies NIR spectral analysis of direct measurement of potential root and earthworm activity markers need to be explored, to associate specific wave lengths of the NIR spectra to root macroaggregates and casts. Their absorbance could be used as a marker of specific compounds of root or earthworm activity.

Keywords: Earthworm-root interactions, Earthworm casts, NIR spectral signature, Soil Macroaggregation

I. Introduction

The process of soil macroaggregate formation by ecosystem engineers — roots, earthworms, termites, ants and a few other invertebrates— has been documented by numerous studies (Plante & McGill, 2002; Six *et al*., 2004; De Gryze *et al*., 2006; Blanchart *et al.* 1999; Zhang *et al.,* 2009; Zangerlé *et al*., 2011). This study describes soil macroaggregate formation by plants and earthworms of two different functional groups, under laboratory experimental conditions. Different combinations of earthworms and plant species were tested to assess the role of each organism in macroaggregation and describe the results of their interactions.

Biogenic structures are produced by invertebrates through their bioturbation and other mechanical activities. They become highly stable macroaggregates that comprise a large proportion of upper soil horizons. Roots form macroaggregates by entanglement of particles (Tisdall and Oades, 1982; Miller and Jastrow, 1990; Jastrow et al., 1998), which are stabilized by produced mucilages and other exudates that hold particles together. Arbuscular mycorrhizal fungi, which form mutualistic associations with roots of the majority of higher plants, contribute to root aggregation activity by entangling particles in temporary nets creating fragile short lived aggregates (Tisdall and Oades 1982; Oades, 1993; Plante and Mc Gill 2002), and/or stick them together with glomaline (Miller and Jastrow 2000, Rillig et al. 2002; Rillig 2004). However, lately the origin of glomalin has been questioned by Gillespie et al. (2010) which documented that glomalin itself showed no homologies with proteins or DNA of mycorrhizal origin.

While many papers acknowledge and measure its role of aggregation as a soil attribute and function, the origin of soil macroaggregates has been largely ignored so far. This gap in our knowledge makes it difficult to understand the roles of the different actors involved in macroaggregate formation and to model the consequences on soil aggregation, on associated functions of fluctuations in the populations and communities of these ecosystem engineers and on C sequestration in soils. Morphological analyses and Near Infrared Spectroscopy should enable better description of the origins of soil macroaggregates. While morphological analyses allow separating physical from invertebrate and root biogenic macroaggregates, the

use of near infrared (NIR) spectral signature allows discriminating macroaggregates according to the process or organism that created them (Hedde et al. 2005; Velasquez et al. 2007; Zhang et al. 2009; Zangerlé et al. 2011). NIRS signatures reflect the amount and nature of organic matter accumulated in specific ways in each kind of biogenic or physical structure. Several mechanisms explain these differences. First, invertebrates have selective feeding habits that result in the ingestion of preferred organic items. Then additions of saliva or intestinal mucus selectively enhance microbial populations during gut transit. Enzyme production in digestion and the quality of assimilated and non-assimilated materials further contribute to the formation of different signatures. Soil chemical and physical analyses of structures and the assessment of their microbial communities and enzymatic activities support this observation (Mora et al. 1991; Hedde 2005).

NIR spectral analysis allowed Zangerlé et al. (2011) to observe interactions among plants and earthworms in macroaggregate formations. Nirs spectral signatures of macroaggregates, formed in the presence of more than one ecosystem engineer, revealed additive effects on signals, which showed that both added their effects in building structures of mixed origins. Additive effects on signals however appeared more or less strong depending on plant species. In this study we would like to test if these interactions among ecosystem engineers are a once observed pattern or if it is a generally applicable pattern? We also try to understand if the intensities of the observed interactions vary with plant and earthworm species?

To test this hypothesis, four plant species of different functional groups, legumes and grasses, and four earthworm species with two different feeding behaviours were chosen to replicate the mesocosm experiment of Zangerlé et al. under tropical and temperate climate conditions. Earthworm species introduced by Zangerlé et al. (2011) in their experiment were soil feeding endogeic and polyhumic endogeic earthworm species. In our experiment beside the endogeic and polyhumic endogeic species, *L. terrestris* was used, an anecic species, which feeds on plant litter at the soil surface. By introducing these two species we tested if litter feeding species, in combination with plants, might produce macroaggregates which NIRS spectral signals differs to the geophage species spectral signals.

Macroaggregates were characterized according to their origin (biological or physical) based on their morphology and size following the Velásquez et al. (2007) procedure. NIRS allowed analyzing spectral signatures of macroaggregates formed by worms or roots cultivated in isolation. We then analyzed NIR signals of macroaggregates produced in different combinations of earthworm and plant species to search for interactions between both species.

Nowadays very little is known of mechanisms behind this root-earthworm cooperation in aggregate building and the nature of the observed markers of root activities. Natural abundance of ^{13}C of *Brachiaria hybrid Mulato 2* (C4 plant) was used as a marker of root activity in macroaggregates. If rhizodeposition or fragments of plant tissue comprise the organic marker identified in NIR spectral signals, we should be able to discriminate soil organic matter of a C4 plant in the macroaggregates. If this hypothesis is not validated, we shall conclude that the marker has no plant origin. It may be microorganisms on which earthworms would feed like bacteria, mycoriza or rhizobia, or even bacterial or fungal byproducts like extracellular polysaccharides or glomalin. Analysis of ^{13}C and ^{15}N contents should give us a clear answer to the question if the observed organic marker has a plant origin.

II. Materials and Methods

Two separate laboratory mesocosm experiments were set up to measure and assess macroaggregates (>1cm in diameter) produced by different species of earthworms and plants, in isolation and in combination. Each experiment had 16 different treatments, representing all representing all possible combinations of plants and earthworm species, plus the respective controls.

Soil, Plants and Earthworms

In a first experiment, realised under temperate climate conditions, we introduced *Aporrectodea caliginosa* (Savigny), a soil feeding polyhumic endogeic earthworm species, and *Lumbricus terrestris* (L.), an anecic species. Plant species used were red clover *Trifolium ruttinova* and the common grass *Lolium perenne* L., both grown from seed. In a second experiment, common tropical endogeic earthworm species *Pontoscolex corethrurus* (Muller) and *Amynthas corticis* (Kinberg) and seeds of plant species *Brachiaria hybrid Mulato 2* and *Phasealus vulgaris* were used.

Individuals of *A. caliginosa* were collected in a permanent grassland in a suburb of Luxemburg city; while *L. terrestris* was bought from a fishing shop. *P. corethrurus* (Muller) and *A. corticis* were collected in permanent grasslands close to Cali (Colombia). Soils were both extracted in permanent grassland from the upper 15 cm layer, air dried, sieved at 2 mm and homogenized. Soil used for the first experiment was poor in organic matter, with 40.13% sand, 45.31% silt and 14.56% clay contents respectively and had average total C and N contents of 1.60% and 0.12%, respectively, and a C:N ratio of 13.33. Soil of the second experiment was a sandy soil (with 47.33% sand, 16.28% silt and 36.39% clay), rich in organic matter (total C and N content in average were 3.98% and 0.32%, respectively, and the C:N ratio was 12.53).

Figure 1: *Trifolium ruttinova*

Figure 2: *Lolium perenne* L.

Figure 3: *Phasealus vulgaris*

Figure 4: *Brachiaria hybrid Mulato 2*

Figure 5*: Lumbricus terrestris*

Figure 6: Aporrectodea caliginosa

Figure 7: *Pontoscolex corethrurus* (de petite taille sur la photo)
Amynthas corticis (de grande taille sur la photo)

Mesocosms

For each experiment, Plastic pots of 1 litre in the first experiment (5 litres for the second experiment) were filled with respective 800g and 5000g of dry, homogenized soil. Plants were sown at the beginning of the experiment (20 seeds per pot). Four earthworms per pot were inoculated five weeks later, after four selected plants had grown and developed root systems. Mesocosms were assigned randomly to avoid confounding effects due to uneven lighting

Method

Soil moisture was maintained at field capacity throughout the experiment. Capillary *per ascensum* diffusion through small holes drilled at the bottom of the pots was preferred in order to prevent physical soil aggregation by surface splash effect.

Mesocosms were dismantled eight weeks after the beginning of the experiment. The aboveground part of the plants was first cut and removed. Earthworms were collected and weighed individually after carefully breaking soil into large clods. Soil was slowly air dried (over seven days) in order to harden aggregates and facilitate separating them from soil that had not been macro-aggregated by specific earthworm- or root-associated processes.

Soil was then shaken and passed through a 5mm mesh sieve to disperse weak aggregates created during drying and handling and to retain resistant aggregates built by the activity of earthworms and roots. The resistant aggregates were visually separated according to their origin; biogenic aggregates, produced by earthworms (generally round shaped), and root aggregates stuck to the roots (with variable external shapes).

NIRS Spectral Signatures

A total of 240 aggregates from five aggregates sampled randomly from each replicate pot were analyzed. Average weight of samples was 4.46g (SE=1.41). Each aggregate was crushed and passed through a 200 µm-mesh sieve to obtain homogeneous preparations of all samples. Roots were separated from aggregates during crushing to avoid contamination of the spectral signal by solid plant material.

Samples were packed in a quartz-glass container and placed in a spectrophotometer (FOSS NIR SYSTEM 6500, Silver Spring, MD, USA) with a 1100-2500 nm spectral range. The reflectance measurements were made at two nanometer intervals. Reflectance (R) was converted to absorbance (A) using the equation: A = log (1 / R) and further transformed to second derivative according to general procedures recommended for the treatment of this particular type of signal. Finally, average values were calculated for 10 nm intervals in order to reduce the number of variables processed. Data analyses were conducted using Winisi, Version 1.5, software system.

Mineral N and C were determined from conventional CHN analyses (Truespec CHNS, LECO).

Statistical Treatments

Principal components analyses (PCA) were performed using were performed with R software (Ihaka and Gentleman, 1996; R-Development-Core-Team, 2009) and the package ade4 for multivariate analysis (Chessel et al., 2004; Drayand Dufour, 2007; Drayetal., 2007). Permutation tests on PCA coordinates allowed for testing differences among treatments. The table of data to be treated had 139 columns—the secondary derivatives of the spectral signal for each of the 139 successive wavelengths—and 240 lines—the 240 aggregates (five samples per three replicates of the 16 treatments). In a second step, NIR spectra was analysed by a hierarchical cluster analysis (CAH). The discrimination threshold of the CAH was defined as the number of treatments of the experiment (16).

Differences among total macroaggregate production, earthworm biomass and plant above ground biomass obtained in each treatment were evaluated using parametric analysis of variance (ANOVA). Comparisons between means were tested with Tukey's test. Differences among treatments were evaluated at the <0.05 probability level of significance.

III. Results

Earthworm survival

In the two experiments, treatments containing two earthworm species showed a loss of earthworm biomass for both earthworm species. The presence or absence of plants in mixt earthworm treatments did not influence the loss of earthworm biomass. *P. corethrurus* and *L. terrestris* gained fresh biomass in each single species treatment, in presence of plants, but lost biomass in the absence of plants. However *A. corticis* lost fresh biomass in all treatments. *A. caliginosa* gained fresh biomass in all treatments in presence or absence of plants. *P. corethrurus* produced cocoons in each single species treatment, but did not reproduce in presence of *A. corticis* (Table 1)

In single species treatments earthworms had a reasonably high rate of survival (*A. corticis* 91%, *P. corethrurus* 96.75%, *L. terrestris* 68.5% and *A. caliginosa* 100%). In treatments containing two earthworm species we observed a relatively high loss of individuals; for *P. corethrurus* an average loss of 70.5%, for *L. terrestris* 68.5% and for *A. corticis* 66%. Only *A. caliginosa* did not show a significant loss of individuals in polycultures (95.5%). (Table 1)

Figure 8: Cocoon (marked by the white arrow) and individuals of earthworm species P*ontoscolex corethrurus.*

Table 1.

Amynthas corticis						
Treatments	Initial biomass (g fresh weight)		Final biomass (g fresh weight)		Survival of individuals	
	pValue>0.05	*SE*	pValue<0.01	*SE*		
Co	1.45	*0.20*	1.13 [a]	*0.20*	88%	
P	1.31	*0.05*	0.62 [b]	*0.06*	60%	
Ph	1.32	*0.02*	1.22 [a]	*0.07*	94%	
B	1.43	*0.04*	1.14 [a]	*0.15*	88%	
BPh	1.33	*0.04*	1.23 [a]	*0.10*	94%	
P+Ph	1.46	*0.01*	1.03 [a]	*0.09*	72%	
P+B	1.47	*0.11*	0.81 [ab]	*0.18*	66%	
P+BPh	1.43	*0.07*	0.76 [ab]	*0.24*	66%	
Pontoscolex corethurus						
Treatments	**Initial biomass (g fresh weight)**		**Final biomass (g fresh weigt)**		**Survival of individuals**	**Cocoons (number)**
	pValue>0.05	*SE*	pValue<0.05	*SE*		
Co	0.91	*0.03*	0.84 [a]	*0.04*	93%	22.3
A	0.90	*0.23*	0.56 [b]	*0.06*	61%	1
Ph	0.92	*0.04*	0.97 [a]	*0.13*	100%	12
B	0.92	*0.06*	0.90 [a]	*0.02*	94%	6
BPh	0.89	*0.12*	0.95 [a]	*0.04*	100%	17
A+Ph	0.91	*0.09*	0.72 [ab]	*0.13*	94%	0
A+B	0.89	*0.01*	0.54 [b]	*0.08*	61%	0
A+BPh	0.90	*0.16*	0.66 [ab]	*0.23*	66%	0.6

Lumbricus terrestris					
Treatments	**Initial biomass (fresh weight)**		**Final biomass (fresh weight)**		**Survival of individuals**
	pValue>0.05	*SE*	pValue<0.01	*SE*	
Co	4.58	*0.550*	4.47 [a]	*1.087*	64%
C	3.49	*0.270*	3.10 [b]	*0.191*	50%
Tr	3.72	*0.733*	4.64 [a]	*0.288*	94%
R	3.33	*0.387*	4.68 [a]	*1.050*	88%
RTr	3.46	*0.520*	4.62 [a]	*0.910*	100%
C+Tr	3.87	*0.066*	3.03 [b]	*0.468*	64%
C+R	4.30	*0.181*	3.81 [ab]	*1.800*	88%
C+RTr	4.55	*0.084*	3.75 [ab]	*0.947*	72%
Aporrectodea caliginosa					
Treatments	**Initial biomass (fresh weight)**		**Final biomass (fresh weight)**		**Survival of individuals**
	pValue>0.05	*SE*	pValue<0.01	*SE*	
Co	0.57	*0.023*	0.61 [a]	*0.141*	100%
T	0.55	*0.010*	0.38 [b]	*0.040*	88%
Tr	0.54	*0.034*	0.59 [a]	*0.080*	100%
R	0.53	*0.019*	0.58 [a]	*0.051*	100%
RTr	0.57	*0.011*	0.60 [a]	*0.132*	100%
T+Tr	0.50	*0.012*	0.45 [ab]	*0.050*	94%
T+R	0.57	*0.008*	0.49 [ab]	*0.036*	100%
T+RTr	0.60	*0.007*	0.59 [a]	*0.182*	100%

Table 1.

Effects of plant species (R= *Ray-Grass hybride,* Tr= *Trifolium ruttinova,* B= *Brachiaria hybrid Mulato 2* and Ph= *Phasealus vulgaris*) on the initial biomass (average fresh weight per individual), final biomass (average fresh weight per individual) and survival rate of *Aporrectodea caliginosa* (Savigny), *Lumbricus terrestris L., Pontoscolex corethrurus* (Muller), *Amynthas corticis* (Kinberg)

Plant growth

Trifolium ruttinova achieved higher biomasses than *Ray-Grass hybride* in all treatments (P<0.001 Table 2). But any of both species gained higher biomasses in TrR[1] treatment than in isolation. In the second experiment *Brachiaria* reached shoot and root biomasses (shoot: 1.85g (*P:0.64*), root: 0.75g (*P:0.39*)) that were about twice as high as *Phasealus vulgaris* biomasses (shoot:0.82g (*P:0.23*), root:0.19g (*P:0.06*)) (P<0.001 Table 2). In the BPh[2] treatment *Brachiaria* achieved significant higher biomasses than in isolation. (Table 2)

Effect of earthworms on plant biomass

Statistical models (Table 2) showed that shoot biomass, root biomass and shoot/root ratio of *Brachiaria* and *Trifolium ruttinova* were significantly affected by the presence of earthworms. However this earthworm effect on plant growth of *Trifolium ruttinova* varied with earthworm species. *L. terrestris* had a significant effect on plant growth of *Trifolium ruttinova,* but no significant effect of *A. caliginosa* on plant growth was found. Shoot and root biomasses, in presence of *L. terrestris,* were only significant in absence of *A. caliginosa* (P<0.001 and P<0.005 Table 2). Root biomass and root:shoot ratio of *Ray-Grass hybride* decreased clearly in presence of *L. terrestris* (P<0.005 Table 2). In experience 2 root and shoot biomass of *Brachiaria* were clearly increased by the presence of the earthworm species, *P. corethrurus* and *A. corticis*, in monocultures and polycultures. Earthworms increased root and shoot biomass of *Brachiaria* in each treatment (P<0.001 Table 2). Especially cultures with two earthworm species had a significant higher root and shoot biomass than cultures with just a single species (P<0.001 Table 2). BPhAP[3] had a root biomass of 2.67g (*P: 0.86*), 3.7 times the control, and shoot biomass of 4.20g (*P:0.59*), 2.3 times the control. On the other hand *A. corticis* and *P. corethrurus* had any significant effect on *Phasealus vulgaris* growth rate (Table 2).

Table 2.

Brachiaria hybrid Mulato 2						
Treatments	Shoot biomass (g dry weight)		Root biomass (g dry weight)		Root:shoot ratio	
	pValue<0.001	*SE*	pValue<0.001	*SE*	pValue<0.001	*SE*
Co	1.85 [d]	*0.64*	0.75 [d]	*0.39*	0.41 [d]	*0.10*
Ph	2.24 [cd]	*1.24*	1.18 [c]	*0.70*	0.53 [c]	*0.18*
A	2.45 [cd]	*1.40*	1.13 [c]	*0.58*	0.43 [d]	*0.14*
P	2.61 [c]	*0.46*	2.01 [b]	*0.65*	0.82 [a]	*0.16*
AP	3.58 [ab]	*1.26*	2.25 [ab]	*1.16*	0.63 [b]	*0.29*
A+Ph	3.40 [b]	*0.63*	2.09 [b]	*0.46*	0.61 [b]	*0.11*
P+Ph	3.41 [b]	*0.60*	2.07 [b]	*0.76*	0.61 [b]	*0.19*
AP+Ph	4.20 [a]	*0.59*	2.67 [a]	*0.86*	0.64 [b]	*0.21*
Phaseolus vulgaris						
Treatments	Shoot biomass (g dry weight)		Root biomass (g dry weight)		Root:shoot ratio	
	pValue>0.05	*SE*	pValue>0.05	*SE*	pValue>0.05	*SE*
Co	0.82	*0.23*	0.19	*0.06*	0.22	*0.06*
B	0.80	*0.30*	0.19	*0.08*	0.23	*0.08*
A	0.82	*0.15*	0.21	*0.02*	0.25	*0.04*
P	0.80	*0.14*	0.21	*0.08*	0.26	*0.03*
AP	0.81	*0.15*	0.18	*0.06*	0.21	*0.06*
A+B	0.82	*0.18*	0.17	*0.04*	0.22	*0.04*
P+B	0.83	*0.18*	0.19	*0.05*	0.21	*0.05*
AP+B	0.81	*0.16*	0.18	*0.06*	0.22	*0.04*
Trifolium ruttinova						
Treatments	Shoot biomass (g dry weight)		Root biomass (g dry weight)		Root:shoot ratio	
	pValue<0.001	*SE*	pValue<0.05	*SE*	pValue<0.005	*SE*
Co	0.54 [b]	*0.087*	0.12 [b]	*0.022*	0.22 [b]	*0.017*
R	0.53 [b]	*0.118*	0.13 [b]	*0.033*	0.25 [ab]	*0.031*
C	0.55 [b]	*0.168*	0.13 [b]	*0.016*	0.24 [b]	*0.057*
T	0.68 [a]	*0.061*	0.19 [a]	*0.041*	0.28 [a]	*0.071*
CT	0.53 [b]	*0.032*	0.12 [b]	*0.032*	0.23 [b]	*0.072*
C+R	0.53 [b]	*0.072*	0.12 [b]	*0.024*	0.22 [b]	*0.046*
T+R	0.65 [a]	*0.116*	0.15 [ab]	*0.025*	0.23 [b]	*0.056*
CT+R	0.48 [b]	*0.182*	0.11 [b]	*0.072*	0.22 [b]	*0.068*
Ray-Grass hybride						
Treatments	Shoot biomass (g dry weight)		Root biomass (g dry weight)		Root:shoot ratio	
	pValue>0.05	*SE*	pValue<0.005	*SE*	pValue<0.001	*SE*
Co	0.37	*0.011*	0.07 [a]	*0.014*	0.2 [a]	*0.006*
Tr	0.39	*0.048*	0.08 [a]	*0.026*	0.21 [a]	*0.019*
C	0.4	*0.097*	0.09 [a]	*0.016*	0.22 [a]	*0.072*
T	0.4	*0.048*	0.02 [b]	*0.01*	0.04 [b]	*0.043*
CT	0.4	*0.061*	0.03 [b]	*0.005*	0.08 [b]	*0.048*
C+Tr	0.41	*0.072*	0.09 [a]	*0.024*	0.23 [a]	*0.048*
T+Tr	0.39	*0.076*	0.03 [b]	*0.015*	0.07 [b]	*0.048*
CT+Tr	0.43	*0.132*	0.03 [b]	*0.057*	0.06 [b]	*0.048*

Table 2.

Effects of earthworms (C = *Aporrectodea caliginosa* (Savigny), T= *Lumbricus terrestris L.,* P= *Pontoscolex corethrurus* (Muller), A= *Amynthas corticis* (Kinberg)) and plant species on root (dry weight), shoot (dry weight) and shoot/root ratio of *Ray-Grass hybride, Trifolium ruttinova, Brachiaria hybrid Mulato 2 and Phasealus vulgaris.*

Aggregate production

In the experiment under tropical conditions, macroaggregation was very important in most experimental units, with 670.5g (*P: 44*) to 2785g (*P:139*) dry weight of macroaggregated soil and with significant differences among treatments ($p<0.05$, Table 3). Macroaggregation was as well important in most treatments of the second experiment, with 224g (*P: 41*) to 598g (*P:21*) dry weight of macroaggregated soil and with significant differences among treatments ($p<0.05$ Table 3). Production of macroaggregates decreased significantly in treatments with two earthworm species (AP and CT; $p <0.05$) compared to single species treatments; despite earthworms did exhibit a significant weight loss during the experiment in AP and CT treatments. In the first experiment fewer macroaggregates were produced in the presence of *Phasealus vulgaris* than *Brachiaria* roots and in the second experiment ray gras produced significant less root macroaggregates than *Trifolium ruttinova*. *P. corethrurus* tended to have a more important macroaggregation rate than *A. corticis*, however the differences were significant only in presence of *Brachiaria. A. caliginosa* had a higher macroaggregation rate than *L. terrestris* in each single species treatment.

Table 3.

	Aggregates produced (g) AP	
	pValue<0.05	*SE*
T	247[c]	*49*
C	598[a]	*28*
TC	260[c]	*12*
TTr	404[bc]	*49*
TR	224[c]	*41*
CTr	598[a]	*21*
CR	461[b]	*51*
TTrR	377[bc]	*16*
CTrR	591[a]	*51*
TCTr	414[b]	*59*
TCR	433[b]	*25*
TCTrR	512[ab]	*17*
R	424[b]	*65*
Tr	425[b]	*10*
RTr	478[ab]	*22*

	Aggregates produced (g) AP	
	pValue<0.05	*SE*
A	1375[c]	*158*
P	1593[c]	*136*
AP	671[d]	*44*
B	1900[b]	*158*
Ph	1355[c]	*82*
BPh	1823[bc]	*122*
APh	1686[bc]	*142*
AB	2234[b]	*113*
PPh	1869[b]	*102*
PB	2785[a]	*139*
ABPh	2135[ab]	*110*
PBPh	2402[ab]	*156*
APPh	1847[bc]	*131*
APB	2427[ab]	*86*
APBPh	2260[ab]	*197*

Table 3.
Total macroaggregate production obtained in each treatment. AP: total dry weight of macro aggregated soil produced during our experiment. (R= *Ray-Grass hybride,* Tr= *Trifolium ruttinova,* B= *Brachiaria hybrid Mulato 2,* Ph= *Phasealus vulgaris* , C = *Aporrectodea caliginosa* (Savigny), T= *Lumbricus terrestris L.,* P= *Pontoscolex corethrurus* (Muller), A= *Amynthas corticis* (Kinberg)).

Figure 9 and **10**: *Brachiaria hybrid Mulato 2* root aggregates

Figure 11: Mixt origin aggregate (*Brachiaria hybrid Mulato 2* and *Pontoscolex corethrurus*)

Figure 12: Root agregates *Trifolium ruttinova*

Figure 13: Root agregates *Ray-Grass hybride*

Figure 14: Cast of *Aporrectodea caliginosa*

Figure 15: Casts of *Lumbricus terrestris*

^{13}C marking to detect plant root activities in macroaggregates

In experience 2, natural abundance of ^{13}C of macroaggregates of *Brachiaria*, in the single plant treatment or in presence of *A. corticis*, showed an overall average of −20.7‰ to -20.9‰ which was significant higher than control soil (-21,12‰) (Figure 1, $p<0.05$). All macroaggregates produced in presence of *A. corticis* in mixed or single treatments, except PhA, showed (−20.7‰ to -20.9‰) significant lower ^{13}C contents than control soil (Figure 1, $p<0.05$). The results confirm our hypothesis that a plant activity was observed in macroaggregates produced in presence of earthworms and *Brachiaria.* However just BA was significantly higher than the control soil (Figure 1, $p<0.05$) whereas BP showed a same tendance but was not significantly different of the control soil.

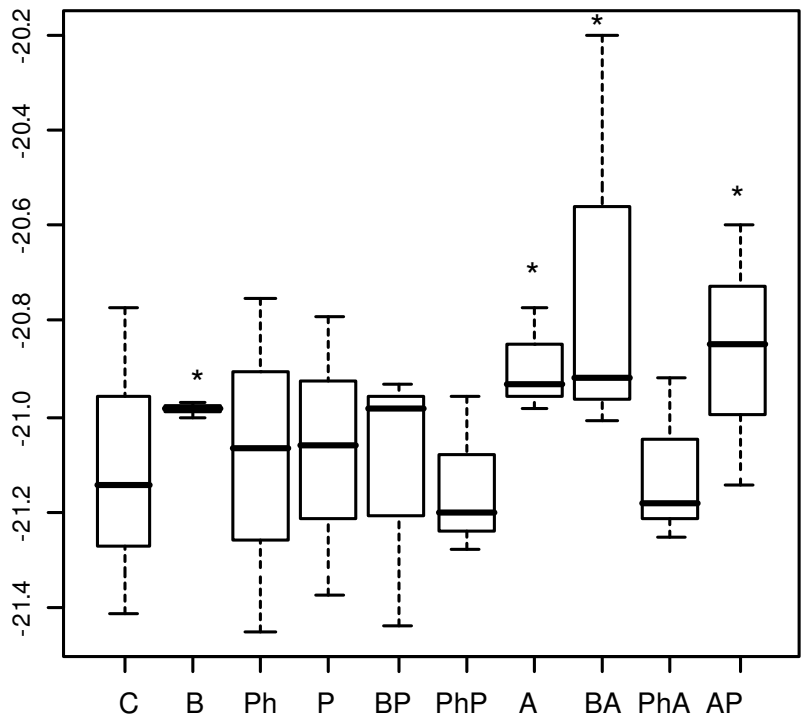

Figure 16.

Abundances of ^{13}C of macroaggregates of each treatment of experience 2. * indicate treatments that are significantly different from control soil. (B = *Brachiaria hybrid Mulato 2,* Ph = *Phasealus vulgaris* , P = *Pontoscolex corethrurus* (Muller), A = *Amynthas corticis* (Kinberg), C: control).

NIR Spectral Signature

General results

The PCA of the 139 variables, describing NIR spectra of the first experience, showed a significant separation among the 16 different treatments (Figure 2). The treatment effect explained 41.5% of total variance. This effect was significant ($p<0.001$). The first factor (52.9% of total variance explained) clearly separated macroaggregates produced in two main plots characterized by a presence or absence of *Trifolium ruttinova* and called "Effect of Trifolium" in Figure 2. Total C and N analysis showed that the first axis explained differences in C and N contents of aggregates (Table 4, $p<0.05$). The second factor (17.3% of total variance explained) contrasted mixed treatments with plants and earthworms with single species treatments; except for R treatments. The PCA of Figure 2 seperated the barycenters in 4 plots. The furthest from the control was centered on *Trifolium ruttinova – L. terrestris* and their combinations with the other species. A second plot comprised combinations of *Trifolium ruttinova* and *A. caliginosa* in absence of *L. terrestris* and a third plot is composed of *Ray-Grass hybride* combinations excluding *Trifolium ruttinova*. Treatments with one single ecosystem engineer, earthworms *A. caliginosa* (C) and *L. terrestris* (T) and their combination (TC) generally departed less from the control than treatments of single plant species and treatments of plants and earthworms associations.

The correlation circle among wavelengths and axes of the PCA showed an association of the first axis with high reflectance in NIR wavelengths 1180-1190, 1360-1380, 1420-1460, 1600-1840, 1900-1960, 1990-2000, 2020-2120, 2250-2290, and 2310-2360. Projection of C, N and C:N data, as additional variables in the plane, defined by axes 1 and 2, shows that the first factor of the PCA is clearly associated to the C, N and C:N variables. Just a few NIR wavelengths 1100-1170, 2170-2180, 2240 and 2410-2430 are correlated with the second axis.

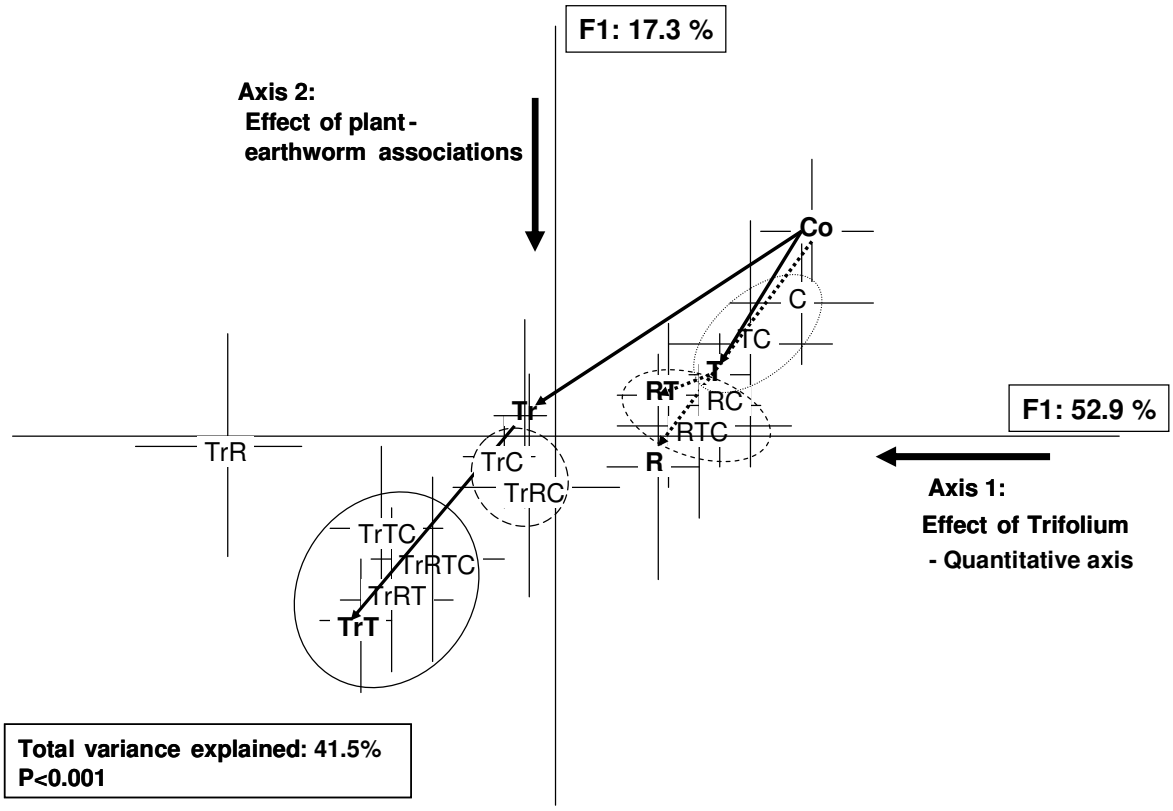

Figure 17.

Projection of barycenters of NIRS spectra of aggregates produced in the 16 treatments of experience 1, in planes defined by factors 1 and 2 of PCA analysis (15 replicates per treatment). Large vertical and horizontal arrows indicate major effects observed along factorial axes. Small solid lines underline the additive influences of Tr and T in NIRS signal of treatment TrT. Dotted lines highlight signals of RT in an intermediate position between the single species treatments R and T. No additive influences between actors have been observed. (R = *Ray-Grass hybride,* Tr = *Trifolium ruttinova,* C = *Aporrectodea caliginosa* (Savigny), T = *Lumbricus terrestris L.,* Co: control).

In the second experiment the PCA of the NIRS spectra showed a significant separation among the 16 different treatments (Figure 3) with a first factor that explained 45.6% and the second 11.2% of the total variance. The treatment effect was significant ($p<0.001$) and explained 37.1% of total variance. The first factor clearly separated macroaggregates in three main plots, characterized by the presence or absence of *Phaseolus vulgaris* and *Brachiaria.* As in the first experiment, total C and N contents of macroaggregates explained treatment separation on the first axis (Table 4, $p<0.05$). The second factor contrasted mixed treatments of plants and earthworms with single species treatments. Treatments with only earthworm species, *P. corethrurus* and *A. corticis* and their combination (TC), departed less from the control than treatments of single plant species and treatments of plant and earthworm associations. Factor 1 and 2 of the PCA showed almost no departure of the plant species combination treatment, (BPh), from the control soil. However the third axis of the PCA separated very clearly the BPh treatment from the remaining macroaggregates.

The correlation circle of Figure 3 showed an association of the first axis with high reflectances in NIR wavelengths 1350-1390, 1420-1470, 1600-1820, 1900-1910, 1930-1960, 1980-2000, 2020-2120, 2250-2270, 2310-2320 and 2340-2360. Projection of C, N and C:N data, as additional variables in the plane, defined by axes 1 and 2, showed that the first factor of the PCA is clearly associated to the C, N and C:N variables. The second axis was correlated to the wavelengths 1100-1170, 2170-2180, 2240 and 2410-2430.

NIRS data of earthworm and plant macroaggregates of both experiences were treated each separately with control soil in a PCA analyses to obtain correlation circles that allowed a better characterizing of plant and earthworm activities by specific reflectances in wavelengths. Earthworms of experience 1 showed specific reflectances in wavelengths 1770, 2320-2340, 2450-2460. Earthworm activity, in experience 2, showed very similar results with specific correlations in reflectances of wavelengts 1770, 2320-2350, 2420, 2450-2460. Plant treatments of experience 1 were associated to high reflectances in wavelengths in 1120-1140, 1820, 1930, 1960, 2050, 2080, 2140, 2190-2210, 2220-2240, 2410-2420 and 2450 and plants of experience 2 were correlated to high reflectances in wavelengths 1510-1520, 1530-1540, 1640, 1955-1980, 2330-2380, 2440 and 2480.

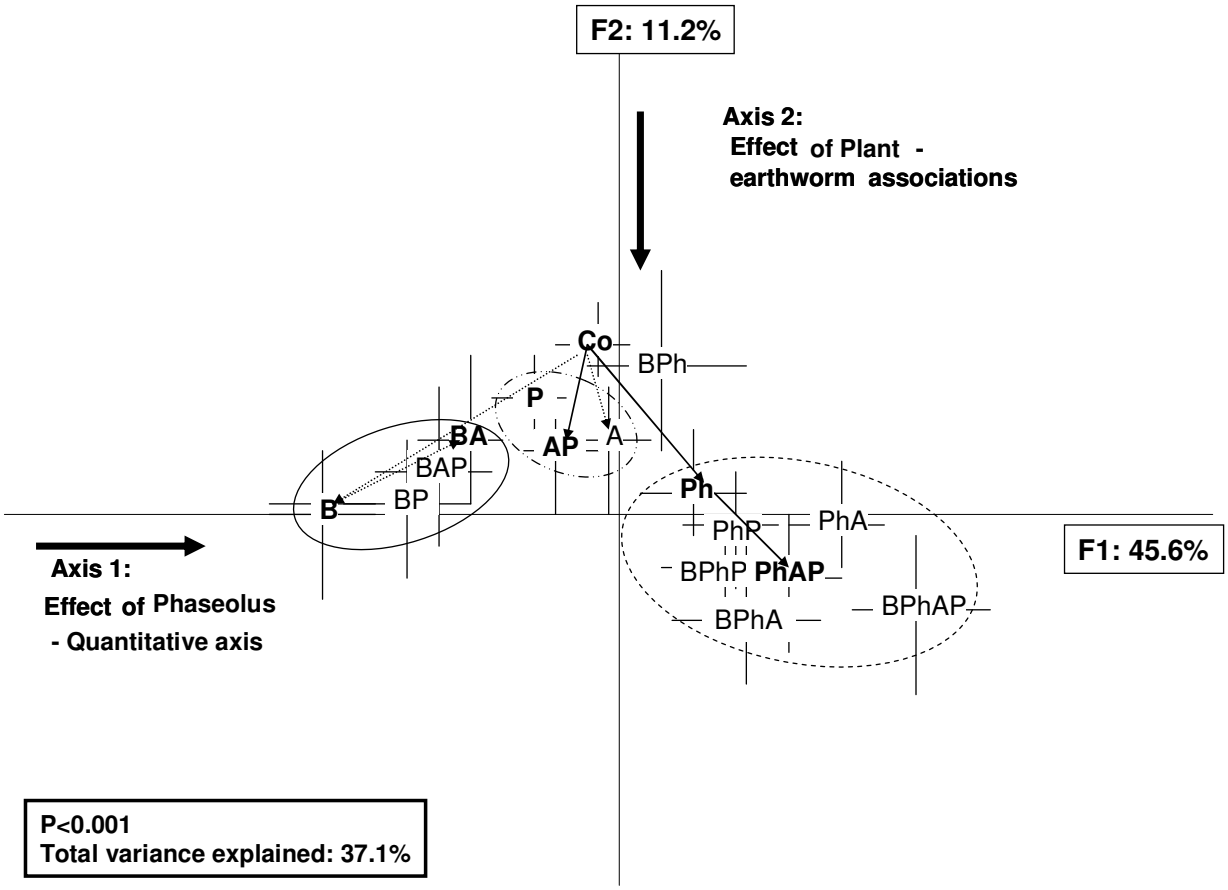

Figure 18.

Projection of barycenters of NIRS spectra of aggregates produced in the 16 treatments of experience 2, in planes defined by factors 1 and 2 of PCA analysis (15 replicates per treatment). Big solid lines indicate major effects observed along factorial axes. Small solid lines underline the additive influences of Ph and AP in NIRS signal of treatment PhAP. Dotted lines highlight signals of BA in an intermediate position between the single species treatments B and A. No additive influences between actors have been observed. (B = *Brachiaria hybrid Mulato 2,* Ph = *Phasealus vulgaris* , P = *Pontoscolex corethrurus* (Muller), A = *Amynthas corticis* (Kinberg), Co: control).

In both experiments the positions on the PCA of several treatments, with earthworms and plants in combination (TrC, TrT, PhA and PhP) clearly suggested an addition of each type of signature, earthworm or plant, in any single macroaggregate (Figure 1 and Figure 2.). However additive effects on signals appeared more or less strong depending on the earthworm species. For example *A. caliginosa* departed much less from the control soil than *L. terrestris* in the first experience. And consequently, the mixed origin treatment TrC was much less contrasted from the single plant treatment Tr as TrT. The results obtained in both experiments show us only interactions of legumes with earthworm species irrespective on the earthworms feeding behaviour. Signals of macroaggregates formed in the presence of grasses and earthworms, had an intermediate position between the single species treatments. NIRS signatures of macroaggregates belong to two separate groups and are similar to signatures from single species treatments. NIR spectra of macroaggregates was analysed by a hierarchical cluster analysis (CAH) to check if roots and earthworms did produce aggregates in isolation or if mixed aggregates have been produced but additive signals are not shown by the PCA. Results of the CAH analyses reveal that macroaggregates resulting of mixed treatments are classified in a separate group than macroaggregates produced in single treatments. This results show us that macroaggregates produced in mixt plant-earthworm treatments, in presence of *Brachiaria* or *Ray-Grass hybride* and an earthworm species, have the same origins. We conclude that probably mixed origins explain this result of the CAH analyses even if no additive signals of plant species (*Brachiaria* or *Ray-Grass hybride)*, are shown on Figure 2 and 3.

C and N contents of root aggregates

Macroaggregates produced in rhizospheres of plants, in single species treatments and mixed plant species treatments, had all significant different spectral signatures ($p<0.001$) (Figure 2 and Figure 3). Soil enrichment in organic matter was mostly high in root macroaggregates and significantly different for each plant species. *Trifolium ruttinova* and *Ray-Grass hybride,* in the mixed plant treatments, produced macroaggregates with the highest C and N contents of experiment 1. However spectral signatures of macroaggregates of *Phasealus vulgaris* and *Brachiaria* in mixed

plant treatments differed hardly from the control soil and their C and N contents where not significantly different from the control soil. (Table 4)

Table 4.

Experiment 1							**Experiment 2**						
label	**C**		**N**		**C/N**		**label**	**C**		**N**		**C/N**	
	pValue<0.05		pValue<0.05		pValue<0.05			pValue<0.05		pValue<0.05		pValue<0.05	
TrR	1.89 [a]	*0.10*	0.15 [a]	*0.01*	12.60 [d]	*0.14*	**BPhAP**	4.43 [a]	*0.11*	0.35 [a]	*0.02*	12.66 [ab]	*0.27*
TrT	1.78 [b]	*0.09*	0.13 [b]	*0.01*	13.69 [c]	*0.15*	**PhA**	4.31 [b]	*0.04*	0.36 [a]	*0.05*	12.00 [b]	*1.44*
TrRT	1.77 [b]	*0.14*	0.13 [b]	*0.01*	13.70 [c]	*0.21*	**PhAP**	4.31 [b]	*0.07*	0.33 [b]	*0.01*	13.06 [a]	*0.54*
TrTC	1.76 [b]	*0.11*	0.13 [b]	*0.01*	13.62 [cd]	*0.13*	**BPhA**	4.23 [c]	*0.26*	0.33 [b]	*0.04*	12.81 [a]	*0.19*
TrRTC	1.74 [bc]	*0.12*	0.13 [b]	*0.01*	13.69 [c]	*0.23*	**PhP**	4.13 [d]	*0.26*	0.35 [a]	*0.06*	11.69 [b]	*1.42*
TrRC	1.73 [c]	*0.11*	0.13 [b]	*0.01*	13.73 [c]	*0.13*	**BPhP**	4.10 [d]	*0.19*	0.33 [b]	*0.01*	12.48 [ab]	*0.31*
TrC	1.71 [c]	*0.15*	0.12 [b]	*0.01*	13.86 [b]	*0.28*	**Ph**	4.08 [d]	*0.28*	0.32 [b]	*0.04*	12.68 [ab]	*0.72*
Tr	1.70 [c]	*0.10*	0.12 [b]	*0.01*	13.82 [bc]	*0.19*	**BPh**	4.02 [de]	*0.09*	0.32 [b]	*0.02*	12.48 [ab]	*0.43*
R	1.66 [d]	*0.10*	0.12 [bc]	*0.01*	13.87 [ab]	*0.27*	**C**	3.98 [e]	*0.10*	0.32 [b]	*0.01*	12.53 [ab]	*0.64*
RTC	1.65 [d]	*0.08*	0.12 [b]	*0.01*	13.84 [b]	*0.25*	**A**	3.96 [e]	*0.03*	0.35 [a]	*0.01*	11.45 [c]	*0.45*
RT	1.65 [d]	*0.07*	0.12 [bc]	*0.01*	13.75 [c]	*0.12*	**AP**	3.95 [ef]	*0.50*	0.32 [b]	*0.03*	12.46 [ab]	*0.66*
RC	1.64 [d]	*0.15*	0.12 [b]	*0.01*	13.91 [b]	*0.18*	**P**	3.91 [f]	*0.04*	0.31 [b]	*0.01*	12.45 [ab]	*0.26*
T	1.64 [d]	*0.12*	0.12 [bc]	*0.01*	13.66 [c]	*0.17*	**BA**	3.87 [f]	*0.11*	0.31 [b]	*0.01*	12.27 [b]	*0.43*
TC	1.63 [de]	*0.12*	0.12 [bc]	*0.02*	13.58 [c]	*0.29*	**BAP**	3.62 [g]	*0.45*	0.30 [bc]	*0.05*	12.24 [b]	*0.58*
C	1.59 [e]	*0.11*	0.11 [bc]	*0.02*	14.45 [a]	*0.29*	**BP**	3.21 [g]	*1.32*	0.25 [c]	*0.10*	12.60 [ab]	*0.26*
Te	1.56 [f]	*0.16*	0.11 [c]	*0.02*	14.18 [a]	*0.16*	**B**	2.84 [g]	*1.79*	0.23 [c]	*0.14*	12.46 [ab]	*0.12*

Table 4.

Total C and N contents and C/N ratio of macroaggregates of each treatment of both experiments.

IV. Discussion

Effect of earthworms on plant growth

Earthworms can greatly enhance grassland productivity, in temperate (Stockdill 1982) and tropical regions (Blakemore 1997; Brown et al. 1999a), by improving soil physical and chemical conditions and acting directly on plant physiology and growth through hormone like products (Mackay et al. 1982; Syers and Springett 1984). Brown et al. (1999a) showed how *P. corethrurus* was an important activator of plant production in several sites throughout the tropics by having a high potential for increasing yields, particularly in nutrient-poor soils, and on perennial plants. Endogeic, soil-feeding earthworms, such as *P. corethrurus,* develop mutualistic relationships with soil microorganisms in their guts and digest soil organic matter, thus stimulating the mineralization and recycling of nutrients, particularly N (Lavelle et al.1992) and P (López-Hernández et al. 1993) in their cast. In our study we show how the two geophagous species *P. corethrurus* and *A. corticis* in combination or isolation increase biomass of *Brachiaria* in single species cultures. This increase in *Brachiaria* biomass, due to *P. corethrurus*, confirms Brown et al. study even if the soil, with a content of total C and N content of 3.98% and 0.32% respectively cannot be considered a nutrient-poor soil. Reasons for the significant higher increase of *Brachiaria* shoot and root biomass, due to a positive interactive effect of the two geophagous species, *A. corticis* and *P. corethrurus,* are still not clear. A scenario which could explain an improve in plant growth of *Brachiaria* biomass in presence of *A. corticis* and *P. corethrurus* could be that both species feed on a different, or partly different, C pool in the soil, which would result in a better mineralisation of the soil OM in presence of both species. This scenario could also explain why *P. corethrurus* only produced cocoons in treatments in absence of *A. corticis.* A negatif effect of both species on each other would result in a partial overlapping of food niches.

In the second experiment, realised in a nutrient rich soil, only *L. terrestris* showed significant effects on *Trifolium ruttinova* shoot and root biomass. Our results contradict the hypothesis that legumes are less responsive to earthworms than grasses since they are not limited by nitrogen (Brown et al. 2004; Wurst et al. 2005). A

similar effect of *L. Terrestris* on *Trifolium dubium* has been showed by Laossi et al. (2009), which suggests that the observed *L. terrestris* effect on legumes might not be due to an enhancement of N mineralization. No significant interaction between the anecic and endogeic earthworms, *L. Terrestris* and *A. caliginosa,* were found in this study contrary to our expectations. *A. caliginosa* might inhibit the positif effect of *L. terrestris* on *Trifolium ruttinova*'s growth rate.

L. terrestris decreased root biomass and root:shoot ratio of *Ray-Grass hybride.* The most immediate explanation is that plant invested less in root production due to improved nutrient availability.We cannot however discard the less likely hypothesis of significant root consumption by earthworms or hormone like effects on plant development (Blouin 2005).

^{13}C marking to detect plant root activities in macroaggregates

The soil, used for experiment 2, was taken in a grassland, covered by C4 plants for the last 6 years, after the original dry forest vegetation (C3 plants) had been cleared. As a result the control soil had an intermediate abundance of ^{13}C between C3 and C4 plants. We suppose that litter fragments present in soil might mostly come from C4 plants.

^{13}C abundances in *Brachiaria* root macroaggregates were significantly higher than control soil, which confirms that the natural abundance of ^{13}C in *Brachiaria* was sufficient to demonstrate a plant activity in root macroaggregates. Root activity markers, highlighted on spectral signatures of NIR analysis, comprise of plant material, like plant debris of roots and plant litter or root exudates.

^{13}C abundances in *A. corticis* casts in single species treatments were significant higher than control soil. This observation highlightconfirms that *A. corticis* feeds on C4 plant debris, with higher contents in 13C. Barois et al. (1999) showed that *A. corticis* feed preferably on small plant fragments in soil and that they develophighly efficient mutualistic interactions with the soil microflora, which allows

them to digest organic matter in soils. The results confirm our hypothesis that plant products were incorporated to macroaggregates produced in presence of *A. corticis* and *Brachiaria*.

Zhang et al. (1993) previously suggested that *P. corethrurus*, an endogeic mesohumic species, feed specifically on fungal structures and small, degraded and humified plant fragments in the rhizosphere. This observation could confirm that *P. corethrurus* casts did not show a significant difference with the control soil. *P. corethrurus* probably fed on small, humified fragments of C3 plants, in the soil, and on fungal structures in our experiment. Analysing the natural abundance of ^{13}C in all soil particle size fractions of macroaggregates and earthworm tissue, might give us more informartions about the earthworms feeding behaviour.

Interactions among ecosystem Engineers in Soil Aggregation

Interaction between plants and earthworms

Observations of interaction effects between plant species, *T. pratense* or *P. lanceolata,* and earthworms species, *A. caliginosa* or *A. chlorotica,* in macroaggregate building in NIR spectral signatures, suggested by Zangerlé et al (2011), have been confirmed and more general laws can be perceived. Plants and earthworms can build macroaggregates of common origines in a same microenvironnement. However contribution of both species to the NIR signature may not be equivalent. Additive effects on spectral signatures are not observed for all plant-earthworm combinations. The results seems to depend on the plant species (with opposite patterns for legumes and grasses) and not on earthworm species. However examination of NIR spectral signals are not sufficient to describe the exact scenario that could explain the absence of additive effects between grasses and earthworms in mixed treatments. CAH analyses of NIR spectra reveal that macroaggregates resulting of mixed treatments, in presence of *Brachiaria* or of *Ray-Grass hybride* and an earthworm species, have mixed origins but show no additive signals of plant species (*Brachiaria* or *Ray-Grass hybride)* in their spectral signatures. These results tend to reject the scenario of roots and earthworms that produce aggregates in isolation, independently in different

microenvironments and possibly compete for space or occupy different portions of the soil matrix to produce their respective structures. CAH analysis might confirm the hypothesis that macroaggregates were built in the same microenvironment by plants and earthworms in common but without expressing an additive effect in NIR spectral signatures. The results of the CAH analyses are supported by ^{13}C contents analyses of macroaggregates produced in presence of *Brachiaria* and *A. corticis*. They revealed that organic matter of plant was found in macroaggregates probably produced by both actors in common in the same microenvironment. Different scenarios could explain the presence or absence of an expression of additive effects in interactions between plants and earthworms in macroaggregation on spectral signatures. Observed additive effects in spectral signals means that the plant adds her specific signal to the earthworms specific signal. Roots have often been observed in ageing earthworm casts, and intense fine root colonization of freshly deposited casts has been observed by Decäens et al. (2001), which shows that roots often take advantage of nutrients available in casts. A colonization of casts is a scenario that could explain an addition between plant and earthworm specific effects. The loss of plants specific signal in root-earthworm interactions in macroaggregation might be explained by a partial digestion of the specific component of one actor by the second actor. Geophageous earthworm species could feed on rhizosphic soil, containing the plant activity marker, like products resulting from rhizodeposition or root tissue debris, and as a consequence a partial digestion would alter the root activity marker. The digestion of rhizospheric soil could also result in a stimulation of the microbial activity of the rhizosphere, which could degrade an activity marker of one of both actors. Anecic earthworm species, feeding on plant litter debris, might also alter the plant specific marker by the digestion of the OM.

Feeding behaviour of earthworm species

NIR spectral analysis of both experiments confirm that casts and macroaggregates produced with presence of different plant and earthworm species are characterized by specific spectral signatures (Figure 2 and 3, $p<0.001$). C and N analysis of macroaggregates, which associated to the first axis of the PCA, show that organic matter contents of casts differ according to the species that produced them (Table 3, $p<0.05$). In experiment 1, *L. terrestris* produced casts with a significant

higher content in organic matter than *A. caliginosa* (Table 3, $p < 0.05$). These results suggest different feeding behaviours of the two earthworm species. *L. terrestris*, an anecic species, feeds partly on fresh plant litter, while *A. caliginosa*, an endogeic polyhumic species, feeds on soil added with humified plant fragments (Bouché and Kretzschmar 1974). Distances between spectral signatures of RT and RC barycentres are less marked than between TrT and TrC barycentres (Figure 2, $p<0.001$). These differences in spectral signatures might be explained by a more important litter production of *Trifolium ruttinova* than *Ray-Grass hybride.* The same trend is observed, in experiment 2, where species specific signatures of casts and macroaggregates of mixed origins are separated along axis 1 of the PCA (Figure 3, $p<0.001$) according to their respective C and N contents. These results seem to confirm that *A. corticis* fed on fresher organic matter debris and *P. corethrurus* rather on small humified plant fragments.

Species specific markers in spectral signatures

We still completely ignore which organic markers of root and earthworm activities influence spectral signals and how they resist to decomposition in the soil environment. Markers may originate from rhizodeposition products, like root exudates, or fragments of roots and/or plant litter. However markers also comprise in living microbial biomass, like rhizobia and arbuscular mycorrhizal fungi, or bacterial by products, like extracellular polysaccharides (Chenu et al. 1993) or glomaline. NIR spectral analysis of isolated root exudates, fresh root tissue and intestinal mucus of earthworms, performed in similar conditions, would allow to associate specific wave lengths of the NIR spectra to root or earthworm activity. Their absorbance could be used as a marker of specific compounds of root or earthworm activity.

Plant-plant interactions

Like Zangerlé et al. (2011), we found that mixed plant species macroaggregates are probably a common production of roots of both species. PCAs performed on the plant treatments (Fig. 1a and Fig. 2a) highlighted that no single macroaggregate of treatments associating the two plant species had its projection in

the ellipse that comprises single plant treatments which excludes the option of spatially independent constructions. We also confirmed by NIR spectral analysis and C and N contents that *Trifolium ruttinova* and *Ray-Grass hybride* produced a higher rhizodeposition of both plant species in mixed plant species treatments. A different pattern was observed in experiment 2, as *Phasealus vulgaris* and *Brachiaria* produced mixed macroaggregates with very low C and N contents, and NIR spectral signatures rather different from the control soil on the first two axis of the PCA. However PhB barycentre is clearly distinguishable from all treatments on the third axis of the PCA (Figure 3, $p<0.001$). Both plant species might produce a specific organic marker of root activity in presence of both plant species.

Conclusion

Interpretation of the results was rather complicated because we lack knowledge of root biology and interaction processes in root systems, as well as on earthworm-root interactions. Our observations show that interactions may be rather diverse; and that these interactions suggest different mechanisms of cooperation that should be considered in future models. Further, we need to explore NIR spectral analysis of direct measurement of potential root and earthworm activity markers may allow to associate specific wave lengths of the NIR spectra to root macroaggregates and casts. Their absorbance could be used as a marker of specific compounds of root or earthworm activity.

Conclusion chapitre 3

Les deux dernières expériences ont eu comme but de tester si l'interaction entre vers de terre et racines de plantes, observée au cours de la première expérience du chapitre, peut être considérée comme un mécanisme général, ou s'il s'agit d'une observation unique. Les hypothèses 1 et 2, testées avec l'expérience de l'article précédent, ont de nouveau été validées. En effet les macroagrégats formés en présence de légumineuses et de vers de terre ont eu des signatures spectrales mixtes, montrant que les deux acteurs ont participé à leur formation dans le même microenvironnement. Lors de la structuration des sols, la rhizosphère et la drilosphère n'ont pas été séparées spatialement et ont formé un système unique. Cependant les effets additifs entre signatures spectrales n'ont pas été observés en présence de toutes les espèces de plantes. *Lolium perenne* L. et *Brachiaria hybrid Mulato 2* n'ont pas affiché d'effets additifs entre les signatures spectrales. L'analyse statistique CAH nous laisse supposer qu'il y a bien eu une interaction entre les deux acteurs dans la formation de macroagrégats même si des effets additifs n'ont pas pu être observés. Un scénario de ségrégation spatiale, c'est-à dire la formation de macroagrégats par les deux acteurs dans des microenvironnements séparés, n'a pas été mis en évidence dans cette étude.

L'utilisation du traceur naturel ^{13}C nous a révélé que la nature du traceur de l'activité racinaire, observé dans les macroagrégats racinaires, est de nature racinaire. Les résultats nous laissent supposer que la rhizodéposition et/ou des fragments de tissu de plante sont à l'origine du marqueur racinaire, puisque ce dernier a été identifié dans les signatures spectrales des macroagrégats racinaires.

Une augmentation significative de la biomasse sèche des espèces de plantes *Brachiaria hybrid Mulato 2* and *Trifolium ruttinova* en présence de vers de terre a également pu être mise en évidence au cours de cette étude.

Les trois études réalisées en laboratoire au cours de ce chapitre ont permis de montrer des interactions entre les ingénieurs de l'écosystème, vers de terre et racines de plantes, dans la formation de macroagrégats. Ces interactions affichent dans certains cas des effets additifs entre signatures spectrales des acteurs et dans certains

cas non. Cependant les natures de ces interactions trophiques et des traceurs de l'activité des espèces, mis en évidence dans les projections spectrales, restent inconnus. Dans le futur, des études supplémentaires doivent être menées pour mesurer directement les spectres des marqueurs potentiels de l'activité racinaire ou des vers de terre. Leurs spectres obtenus permettront éventuellement une mise en évidence d'une forte absorbance à des longueurs d'ondes spécifiques aux vers de terre ou aux plantes. Leurs absorbances pourraient être utilisées comme marqueurs de composés spécifiques de l'activité des vers de terre ou des plantes.

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Discussion générale et perspectives

Les travaux de recherche réalisés jusqu'à présent, essayant d'expliquer les mécanismes de formation des macroagrégats du sol, ne considèrent pas suffisamment l'origine (biogénique ou physique) des macroagrégats, ni leur dynamique temporelle et spatiale, ni la dynamique de vieillissement conduisant à leur destruction. Cette ignorance des origines diverses des agrégats du sol, de leur cycle de vie et de leurs positions dans la matrice du sol, constitue un grand obstacle pour la description et la modélisation de la dynamique des macroagrégats du sol et des processus associés. Un premier objectif de cette thèse était de proposer des outils et une méthodologie permettant d'identifier l'origine des macroagrégats trouvés dans le sol. L'approche novatrice proposée dans ce travail confirme la capacité de la spectroscopie dans le proche infra rouge (NIRS) à discriminer l'origine des macroagrégats du sol. Contrairement à toutes les approches proposées dans les travaux antérieurs, cette méthode permet d'analyser l'origine de chaque macroagrégat isolé et non plus d'un mélange de structures répondant de façon homogène aux agressions physiques (Le Bissonais, 1996) ou de biostructures se ressemblant morphologiquement (Hedde et al. 2005, Velasquez et al. 2007).

Nous avons montré à travers les différents chapitres de cette thèse que la spectroscopie dans le proche infrarouge (NIRS) permet de caractériser les macroagrégats de chaque organisme ingénieur par une signature spectrale propre, en conditions contrôlées mais aussi dans les conditions plus hétérogènes du terrain. Dans le premier chapitre, qui analyse les structures collectées sur le terrain, nous avons testé une méthode permettant d'identifier les origines de turricules de vers de terre et de macroagrégats racinaires, en comparant leurs signatures spectrales à des signatures spectrales de référence obtenues dans les conditions contrôlées du laboratoire. Cette approche est validée par des résultats très clairs montrant un chevauchement entre signatures spectrales de turricules frais collectés sur le terrain et les signatures spectrales de références des macroagrégats issus de l' élevage des espèces rencontrées sur le terrain. L'interprétation des principaux facteurs des ACPs, analysant les

spectres NIRS, a révélé que ce sont principalement des différences quantitatives de teneurs en MO qui permettent de différencier visuellement entre les origines et l'âge des macroagrégats. Cette première étude de terrain a aussi révélé que les macroagrégats plus anciens collectés sur le terrain dans un bloc de sol ne sont plus directement comparables à des signatures spectrales de macroagrégats monospécifiques obtenus en laboratoire. Deux nouvelles questions sont alors posées à la fin de ce chapitre : 1. est-ce la transformation de la MO, contenue dans les macroagrégats, au cours du vieillissement des macroagrégats, qui différencie leur signature spectrale ou 2. est-ce que plusieurs acteurs intervenant dans la formation d'un macroagrégat lui donnent un signal NIRS différent des structures produites par un seul acteur ?

Le changement du signal NIRS au cours du vieillissement des macroagrégats et les interactions entre organismes ingénieurs dans la production de macroagrégats « mixtes » ont été étudiés dans des expériences en microcosmes (chapitres 2 et 3). Dans une première étude, nous montrons pour la première fois que les effets causés par le vieillissement de macroagrégats sur leurs teneurs en MO, sont suffisants pour affecter les signatures spectrales des turricules (chapitre 2). Dans les conditions de l'expérience, le signal du turricule converge progressivement vers celui du sol moyen qu'il rejoint au bout de 90j. Cette observation montre ainsi la possibilité de repérer dans la matrice du sol les structures jeunes, avec en perspective la mesure de la production des macroagrégats au cours du temps et par déduction, de leur disparition. Cette approche pourrait ainsi permettre de connaître la durée de vie des macroagrégats et ainsi mesurer le temps durant lequel le C est stocké dans ces structures à l'abri des organismes minéralisateurs.

Dans le troisième chapitre est abordée une question jamais encore posée dans la littérature : existe-il des interactions entre organismes ingénieurs dans la formation de macroagrégats ou leur formation est-elle un processus individuel? Jusqu'à présent tous les modèles, proposant des mécanismes de l'agrégation, considèrent les macroagrégats d'origine biogénique, comme ayant de fait, des origines monospécifiques (Shipitalo et Protz, 1988 ; Barois et al., 1986 ; Rillig et al. ; 2002). Trois expériences, réalisées sous conditions contrôlées en laboratoire, ont permis d'étudier les mécanismes d'interactions entre vers de terre et racines pour la formation

des macroagrégats par analyses NIRS. Les analyses spectrales mettent en évidence des signaux intermédiaires, propres aux macroagrégats d'origines mixtes, montrant l'existence d'interactions entre les deux acteurs dans la formation de macroagrégats lorsqu'ils sont réunis dans un microcosme. Validés par plusieurs expériences, testant les interactions entre plantes et vers de terre appartenant à différents groupes fonctionnels, les mécanismes d'interactions observées peuvent être considérés comme des mécanismes généraux. Cependant des effets additifs entre signatures spectrales des vers de terre et des plantes n'ont pas été observés avec toutes les espèces de plantes. Dans les expériences du troisième chapitre des réflectances importantes dans des longueurs d'ondes caractérisant spécifiquement l'activité des plantes ou des vers de terres dans l'agrégation ont été mises en évidence.

Divers auteurs ont observé la présence de racines dans des turricules en vieillissement, (Decäens et al., 2001 ; Lavelle et al., 2007), montrant que les racines ont tendance à coloniser les turricules à la recherche de nutriments. Cette observation fournit une explication plausible au mécanisme d'interaction observé au cours des expériences du troisième chapitre. L'origine monospécifique de macroagrégats de plantes et de vers de terre a seulement été montrée sur le terrain pour des macroagrégats fraichement produits. Les interactions entre organismes ingénieurs dans l'agrégation se sont donc probablement produites dans les jours suivants l'apparition des macroagrégats. Les analyses NIRS, faites pour identifier les origines mixtes de macroagrégats dans les expériences au cours du troisième chapitre, ont seulement pu être testées sur des turricules âgés de trois semaines en moyenne, ce qui ne permet pas de dater précisément le moment de l'apparition de la signature mixte des macroagrégats. Notre étude ne permet donc pas de dire de manière définitive s'il s'agit d'un mécanisme de Co-construction par les vers de terre et plantes au moment de la formation de l'agrégat ou d'une formation en deux temps, dans l'hypothèse où le signal typique de la racine s'ajoute lorsque la racine atteint le turricule nouvellement formé et le colonise. Une simple expérience en laboratoire comportant l'extraction journalière de macroagrégats dans les microcosmes, contenant vers de terre et plantes, permettrait sans doute une meilleure compréhension des interactions entre acteurs au cours du temps.

Jusqu'à aujourd'hui on ignore les natures de ces interactions trophiques entre vers de terre et racines de plantes dans la production de macroagregats. Les origines des traceurs de l'activité des plantes ou des vers de terre, mis en évidence dans les projections spectrales des macroargégats, restent inconnues. Des produits résultants de la rhizodéposition, comme les exsudats ou débris racinaires ou de la biomasse microbienne vivante (comme par exemple des rhizobiums) ou des produits bactériens ou mycéliens (comme par exemple des polysaccharides extracellulaires ou la glomaline) peuvent être à l'origine des traceurs de l'activité racinaire dans les macroagrégats. Dans le futur, des études supplémentaires doivent être menées pour mesurer directement les spectres des marqueurs potentiels de l'activité racinaire ou des vers de terre, isolés auparavant. Par exemple les spectres NIRS, mesurés directement sur des exsudats racinaires ou de colonies microbiennes isolées auparavant, permettront éventuellement de faire des liens entre des fortes absorbances à des longueurs d'ondes spécifiques aux vers de terre ou aux plantes. Leurs absorbances pourraient être utilisées comme marqueurs de composés spécifiques de l'activité des vers de terre ou des plantes.

Nos résultats pourraient aussi servir à proposer un premier modèle de la dynamique des macroagrégats et de leur impact sur la séquestration du C dans les sols. Jusqu'à présent aucun modèle ne considère la dynamique temporelle de l'agrégation et moins encore une description de la dynamique de leur vieillissement conduisant à leur dispersion. L'utilisation de la NIRS qui signale l'apparition des macroagrégats au cours du temps, pourrait être testée pour analyser la dynamique d'une population de macroagrégats sur le terrain. En premier lieu des signatures spectrales de références de macroagrégats de différents âges, jusqu'à au moins 90 jours, seraient établies. Ensuite des macroagrégats seraient extraits de la matrice du sol, à intervalles de temps réguliers. Une estimation du vieillissement de la population des macroagrégats serait alors possible en comparant les signatures spectrales des macroagrégats du terrain avec les signatures de références. Cette approche nous permettrait également d'évaluer le turnover des macroagrégats de ce site de terrain, en considérant les proportions de macroagrégats appartenant à différentes phases du vieillissement à chaque date d'échantillonnage. Dans nos travaux (chapitre 2) nous avons pu mettre en évidence que durant leur vieillissement, les signatures spectrales des turricules passent par trois états principaux. Ces phases se différencient par les

vitesses d'évolution de la signature spectrale et sont corrélées aux différences en teneurs de C des macroagrégats. En faisant des analyses à des dates d'échantillonnages réguliers, les proportions de macroagrégats, appartenant aux différentes phases du vieillissement, pourraient donner des renseignements sur les turnovers des macroagrégats d'une population sur un site de terrain. L'utilisation conjointe de la méthode de séparation morphologique de Velasquez et al (2007) et de la spectrométrie NIR permet ainsi d'explorer le domaine jusque à l'inabordable de la dynamique interne des macroagrégats dans le sol.

En conclusion mes travaux de thèse proposent une nouvelle méthodologie pour analyser les origines des macroagrégats du sol, pour quantifier l'apport relatif des ingénieurs écosystémiques à l'agrégation du sol et pour évaluer la dynamique des macroagrégats dans la structure du sol. Cette méthode a montré que la co construction des macroagrégats par les vers de terre et les plantes est un fait fréquent sinon général. Des expériences simples au laboratoire devraient vérifier ce fait en testant l'interaction d'un nombre assez large d'espèces de vers de terre et de plantes, représentatives des principaux groupes fonctionnels des uns et des autres.

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