Organic matter contribution to aggregate stability in silty loam cultivated soils. carbon input effects.

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INSTITUT NATIONAL AGRONOMIQUE PARIS – GRIGNON
ECOLE DOCTORALE ABIES

THESE

présentée par

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pour l'obtention du grade de

DOCTEUR DE L'INSTITUT NATIONAL AGRONOMIQUE PARIS – GRIGNON

CONTRIBUTION DES MATIERES ORGANIQUES À LA STABILITE DE LA STRUCTURE DES SOLS LIMONEUX CULTIVES. EFFET DES APPORTS ORGANIQUES A COURT TERME

Soutenue publiquement le 15 de Décembre 2006 devant le jury composé de :

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Al cuidado del suelo del mundo

Avant-propos

Cette thèse a été accueillie par l'Unité de Science du Sol du centre de recherche de l'INRA Versailles-Grignon, par l'Unité Mixte de Recherche EGC (Environnement et Grandes Cultures) dans l'équipe Sol (INRA Versailles-Grignon) et par l'Unité Mixte de Recherche BIOEMCO (Biogéochimie et Ecologie des milieux continentaux, UPMC - CNRS - INRA - ENS - ENSCP - INA-PG). Elle a été dirigée par Claire Chenu.

Cette thèse a été financée par une bourse pre-doctorale INRA et a profité du cadre du projet GESSOL 2 : « Mise au point d'outils de prévision de l'évolution de la stabilité de la structure de sols sous l'effet de la gestion organique des sols ».

Remerciements

Je suis sud-américain.
Je viens du bout du monde. Je suis un inconnu.

Cependant, un jour comme n'importe quel jour, quelqu'un qui habite à 15000 km de chez moi m'a présenté sa petite famille. Et son quartier. Et le marché de Versailles. Et m'invite à travailler avec lui. Du coup je découvre la chance que j'ai eue.
Car cette personne est hors norme, hors série. Et j'essaie de suivre son hurricane d'idées, des projets, son hyperactivité, son organisation et son esprit sérieux face au travail.
Sa qualité humaine.
Je remercie de tout mon cœur Claire, Claire Chenu, pour l'honneur qu'elle m'a fait d'être la boussole de mon séjour en France.
Je la remercie encore car elle cristallise aussi la générosité des institutions et du peuple français pour m'avoir donné la possibilité de m'enrichir et de m'immerger dans sa culture.

Cette thèse a été aussi une aventure de vie. Car cachés entre les chiffres et les graphiques il y a des heures de discussions, des mails, d'apprentissage et des voyages. Et du stress et des paysages magnifiques et des délicieuses Mont d'Or et Cheval Blanc. Et de la fatigue. Mais aussi de l'Armagnac de l'arrière grand-père de François !
Et des gens surprenants. Et des bêtises, bien sûr.
N'oubliez pas, je suis sud-américain,
du sud de chez sud.
Yo soy de la
cruz del sur.
(où le froid arrive en juillet)

Je remercie très chaleureusement l'INRA et l'Université de Buenos Aires pour leur support et leur confiance.

Une partie des expérimentations présentées dans cette thèse ont été faites à Angers, à l'INH, où Jean-Charles Michel et Elodie m'ont chaleureusement accueilli. Un grand merci.
Je tiens également à exprimer mes sincères remerciements à Paul Hallett du SCRI, Dundee, Scotland (UK) for hosting me and guided my beautiful visit. Thanks you Paul.
Egalement mes sincères remerciement à Pauline Défossez et Daniel Boitez de l'INRA de Laon pour leur accueil.

La réalisation de cette thèse a été stimulée par des réunions de comité de pilotage qui ont été très intéressantes. Ma reconnaissance va à Jean-Charles Michel, Yves Le Bissonnais et Pauline Défossez pour leur participation et leur intérêt pour mon travail.

Toute ma reconnaissance à l'ensemble des membres du jury pour avoir accepté de se pencher sur ce mémoire et avoir jugée mon travail : les rapporteurs Jean Caron et Bernard Nicolardot, et les examinateurs Pascal Boivin, Paul Hallett et Safya Menasseri.

Mon périple m'a fait connaître d'abord l'Unité de Science du Sol de Versailles (aujourd'hui PESSAC). Je tiens à remercier Daniel Tessier d'avoir accepté être mon co-

encadrant et de m'avoir montré la passion pure pour la science du sol. J'ai commencé à me sentir à l'aise en France grâce à l'amitié de Jean Pierre Pétraud (merci pour ton aide fondamentale avec la méthode de stabilité !!!), Anne Jaulin, Laure Vieublé et May Balabane. Aussi à Christelle Fernandez, Christelle Dere et Laetitita Citeau. Je remercie très spécialement Mathieu Chevalier pour les discussions scientifiques, Grégory Lesturgez pour me faire découvrir l'oedomètre, Eliane Huard pour sa générosité, Nelly Wolf pour les séances de microscopie électronique, l'adorable Christiane et à le couple inoubliable Sébastien Breuil – Julien Sébastia.

Et puis j'ai déménagé à Grignon. L'équipe Sol de l'UMR Environnement et Grandes Cultures m'a accueilli. Et ici je remercie spécialement Enrique Barriuso pour m'avoir supporté et pour sa bonne humeur et pour m'avoir montré son exceptionnel travail de direction qu'il fait. La bande de Grignonnais est grande et inoubliable. Mes collègues de bureau biensûr : Michel Bettache, Laure Mamy, Mohamed Annabi, Jérémy Doublet, Ariel Rampoldi, Julien Moeys, Muriel Jolly. Les hors bureau mais pas moins heureux d'avoir fait leur connaissance: Sabine Houot, Yves Coquet, Véronique Etievent, Marjolaine Deschamps, Nathalie Bernet, Liliana Botero, Valérie Bergeaud, Pierre Vachier, Valérie Pot, Hélène Jean-Jaques, Marie France Rousseau, Christophe Labat, Marie-Josée L'Ostelier, Jean Noël Rampon, Guillaume Bodineau, Simone Poncelet, Christophe Labat, Dalila Hadjar, Clotilde Legrand et Emmanuelle Vaudour (merci pour ton bureau !). A tous mes remerciements.
Je remercie Patricia Garnier pour son intérêt et pour les discussions scientifiques autour des modèles.

J'ai connu encore une autre bande de gens superbe. La bande de Bioemco. J'ai eu beaucoup de plaisir à travailler avec Elsa Coucheney et de profiter de la connaissance de Christophe Moni, Haïthem Bahri, Mercedes Mendez, Naoise Nunan, Cyril Girardin (gracias por tu ayuda el día « J »), Marie Alexis, Thomas Lerch, Iñigo Virto, Liliane Guerrier, Valérie Pouteau, Cornelia Rumpel, Gérard Bardoux, Nicolas Pechot et Marie-France Dignac.

Los Quesos d'Auvergne c'est la bande de personnages qui m'a le plus aidé à diminuer le stress. Merci Florence Devienne-Barret, Pierre Benoît, Céline Décuq, Jérémy encore, Gwenaëlle Lashermes, Sébastien Schneider et Clément.

Au plan scientifique mes remerciements infinis aux chercheurs que j'ai rencontré par ailleurs et qui m'ont donné gentiment de leur temps et leur avis sur mon travail : Denis Angers, Tony Dexter, Michelle Wander, Iain Young et Alvin Smuckler.

Un fuerte agradecimiento a Miguel A. Taboada por el magnífico pase-gol que me hizo y que jamás olvidaré.

Finalement, ma gratitude à la belle ville de Jouy-en-Josas. Elle a été très importante dans le succès de l'intégration de ma petite famille en France donc de la mienne. Chers amis : François et Anne, Nelly et Ludo, Yves et Marie-Pierre, Cosimo et Sylvie, Coralie et Giovanni, Alexia et Thierry, je vous remercie infiniment. Jouy restera dans mon sang toute ma vie.
Tout mon amour et ma gratitude à Valeria qui a laissé derrière ses ambitions personnelles pour m'accompagner dans cette extraordinaire aventure avec Martina, Mercedes et Manuel. Cette thèse lui est dédiée.

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Chapitre Premier

Introduction

Motivation de la thèse. Contexte.

Objectif général de la thèse

Organisation du mémoire

Structure du Sol. Etat de connaissances.

Objectifs spécifiques et démarche de la thèse

1 Motivation de la thèse. Contexte.

Parmi les menaces pesant sur les sols et identifiées par la communauté européenne figurent l'érosion et la diminution de teneur en matières organiques (MO), menaces qui sont étroitement liées du fait du rôle des matières organiques dans la stabilité de la structure du sol.

Cinq millions d'hectares sont concernés en France dont 3,4 millions en sols limoneux. La carte d'aléa érosif établie par l'INRA et IFEN (2002) est étroitement corrélée à la carte des textures de sols et à la carte des teneurs en MO des sols du territoire français. Les zones à aléa érosif le plus élevé sont les sols limoneux cultivés à faible teneur en matières organiques. Ces sols se caractérisent en effet par une faible stabilité structurale. La stabilité de la structure est l'aptitude des agrégats du sol à résister à l'action désagrégeante de l'eau lors d'épisodes pluvieux. Elle dépend des caractéristiques constitutives du sol, en particulier texture, minéralogie, matières organiques, ainsi que des conditions climatiques. Les sols limoneux ont une grande fragilité constitutive et les matières organiques y sont le principal agent agrégeant (Le Bissonnais & Arrouays, 1997; Tessier *et al.*, 1998).

Bien que la relation entre stabilité structurale et texture ou teneur en matières organiques du sol soit reconnue depuis longtemps et fasse l'objet de très nombreuses publications, il n'existe pas aujourd'hui de relation statistique, ou fonction de pédotransfert (FPT), permettant de prévoir la dynamique de la stabilité structurale de sols.

Plusieurs causes expliquent la difficulté de prévoir l'évolution de la stabilité de la structure de sols cultivés:

a. Sa variation intra-annuelle liée aux conditions climatiques.

Le climat affecte la stabilité de la structure notamment via son effet sur l'histoire hydrique du sol et sur l'activité et l'abondance de la biomasse microbienne et de la faune, agents de la stabilité de la structure.

b. Sa variation intra-annuelle liée à l'apport de MO.

Les matières organiques ont un rôle de premier plan dans la stabilité de la structure des sols de texture intermédiaire.

c. Méthodologique.

Il n´existe pas de protocole normalisé pour la mesure de la stabilité structurale au plan international.

2 Objectif général de la thèse

L'analyse de ce contexte et de la bibliographie correspondante montrent la nécessité d'avoir des relations quantitatives entre l'effet du climat et l'apport de la MO sur la stabilité structurale à court terme (semaines, mois) pour arriver à comprendre, à quantifier et donc à prévoir sa variation intra-annuelle sur le terrain. L´objectif général de cette thèse est donc, approfondir la connaissance du déterminisme de la variabilité intra-annuelle de la stabilité de la structure du sol causée par l´apport de matières organiques et la variation de la teneur en eau du sol. L'enjeu est de contribuer à la construction d'un modèle prédictif de l'évolution temporelle de la stabilité structurale suite à un apport de MO au sol.

3 Organisation du mémoire

Le mémoire commence avec une revue bibliographique sur la problématique de la stabilité de la structure où on expose le concept de stabilité de la structure, les mécanismes impliqués dans la macroagrégation, les méthodes de mesure, sa dynamique à court terme et ses relations quantitatives avec l´apport de MO. Puis, les objectifs spécifiques et la démarche scientifique qui ont guidé cette thèse sont présentés. Ensuite les résultats sont présentés sous la forme de chapitres indépendants en anglais, chaque chapitre faisant l´objet d´un article publié ou en voie de publication. Suivront:

- Un chapitre qui concerne l´effet et la quantification des variables biologiques sur la dynamique de la stabilité de la structure suite à un apport de résidus de culture et sa modélisation.
- Un chapitre qui compare trois méthodes de mesure de l`hydrophobie du sol, afin d`évaluer l´impact de l´apport de résidus de culture sur cette propriété.
- Un chapitre qui analyse les relations entre les variables physiques élémentaires qui déterminent la stabilité de la structure et l´apport de matière organique.

- Un chapitre qui porte sur l´impact des cycles de réhumectation et dessiccation sur la dynamique de la stabilité de la structure et les variables biologiques qui la déterminent après un apport organique.

Finalement, des conclusions générales et des perspectives terminent ce rapport.

4 Structure du Sol. Etat de connaissances.

4.1 Un carrefour complexe du fonctionnement du sol

J. Letey, notable professeur de l'Université de Californie (USA), a exprimé, dans sa présentation au symposium national « Advances in soil science » (Australie) (1991), deux concepts fondamentaux qui concernent profondément l'étude de la structure du sol.

Premièrement, l'arrangement architectural des particules d'un sol à un moment donné est le résultat entre interactions des facteurs physiques, chimiques, minéralogiques et biologiques. Ces interactions rendent les études sur la structure du sol très complexes et aussi limitent le transfert d'informations déjà acquises.

Et deuxièmement, il est impossible de mesurer quantitativement la structure du sol. Cette affirmation nous amène à une frustration scientifique du fait qu'en science un phénomène doit pouvoir être quantifié et reproduit par quelqu'un d'autre. Ainsi, les index qui essaient de quantifier la structure du sol dépendent de la méthode de mesure et donc, l'étude de la structure du sol peut être considéré, selon Letey (1991) plutôt comme un art plutôt qu`une science.

Tout en étant le résultat de nombreuses interactions entre différents types de facteurs, la structure du sol conditionne un éventail de processus physiques et biogéochimiques dans les environnements naturels et agronomiques. Quelques processus sont listés au Tableau I-1.

Les processus biogéochimiques généralement ne fonctionnent pas de manière isolée, ils sont très connectés entre eux. La distribution des unités structurales d'un sol contrôle la disponibilité de l`oxygène, de l'eau et donc la résistance à la pénétration de racines de plantules en lit de semences. Le contact sol – grain et donc l`imbibition de la

graine et sa germination dépendent de la taille et disposition des fragments du sol (Díaz-Zorita et al., 2002).

En plus d'être la cause la plus importante de la formation des croûtes de battances, la rupture de la structure est responsable de la production de microagrégats et particules qui sont facilement transportés par le splash et l`érosion hydrique (Le Bissonnais, 1996; Leguedois & Bissonnais, 2004). La formation de croûtes de battance est très importante pour l`établissement et la productivité d'une culture et dépends de ce fait de la structure du sol.

Tableau I-1. Processus biologiques, chimiques et physiques influencées par la structure du sol, modifié d`Amézteka (1999) et de Díaz-Zorita et al. (2002).

Nature de Processus	Processus
Biologique	Habitat de la microflore et mésofaune du sol Stocke et cycle des nutriments (dénitrification, séquestration de C, etc.) Imbibition de graines et émergences des plantules Croissance de plantes et racines Production de cultures
Chimique	Sorption et désorption de composés inorganiques et organiques Transport de solutés Pollution
Physique	Rétention d'eau et évaporation Formation de macropores et macroporosité Infiltration et mouvement de l'eau, aération Croûte de battance Erosion hydrique et éolienne Tassement, densité

L'autre axe fondamental de l`influence de la structure de sol sur ses propriétés est l`influence sur les processus de transport d'eau et de solutés. Etant donné que la structure du sol détermine la disposition des solides d'un sol et donc au même temps la disposition de l'espace poral (i.e. taille de pores, connectivité, tortuosité, etc.), elle contrôle la conductivité hydraulique du sol et la rétention d'eau (Brady, 1990). Elle affecte donc la

rétention et la biodégradation des produits chimiques dans le sol (i.e. pesticides, fertilisants).

L'architecture du sol a une profonde influence sur le microorganismes du sol et ses processus car ils habitent dans un réseau poral qui est complexe, tortueux, plus au moins interconnecté et rempli d'eau et d'air (Young & Ritz, 2000). Parallèlement, en agrégeant les particules de sol autour d'eux, les microorganismes modifient leur environnement. L'action agrégeante peut être interprétée comme la création d`un habitat plus favorable ou peut être vue comme un effet collatéral de la production de diverses biomolécules ayant d'autres fonctions pour les microorganismes (Chenu & Cosentino, 2007). En termes d'habitat, un sol agrégé offre une diversité plus grande de tailles de pores et donc d'habitats physiques et offre aussi une quantité plus importante de pores non saturés et de micropores qui sont situés à une distance courte des pores non saturés (Parry et al., 1999). L'activité microbienne et sa survie sont fortement affectées par la taille des pores (Chenu & Stotzky, 2002).

Ainsi, la structure du sol est un facteur clé du fonctionnement d'un sol, de sa capacité à supporter la vie des animaux et plantes et à affecter la qualité de l'environnement (Fig. I-1) (Holland, 2004), particulièrement la séquestration de carbone et la qualité de l'eau (Bronick & Lal, 2005). La structure de sol a été proposé comme un indice de la qualité du sol (Amézketa, 1999). Selon Bronick & Lal, (2005) elle a en effet un rôle vital sur la production durable d'aliments et le bien-être de l'ensemble de la société.

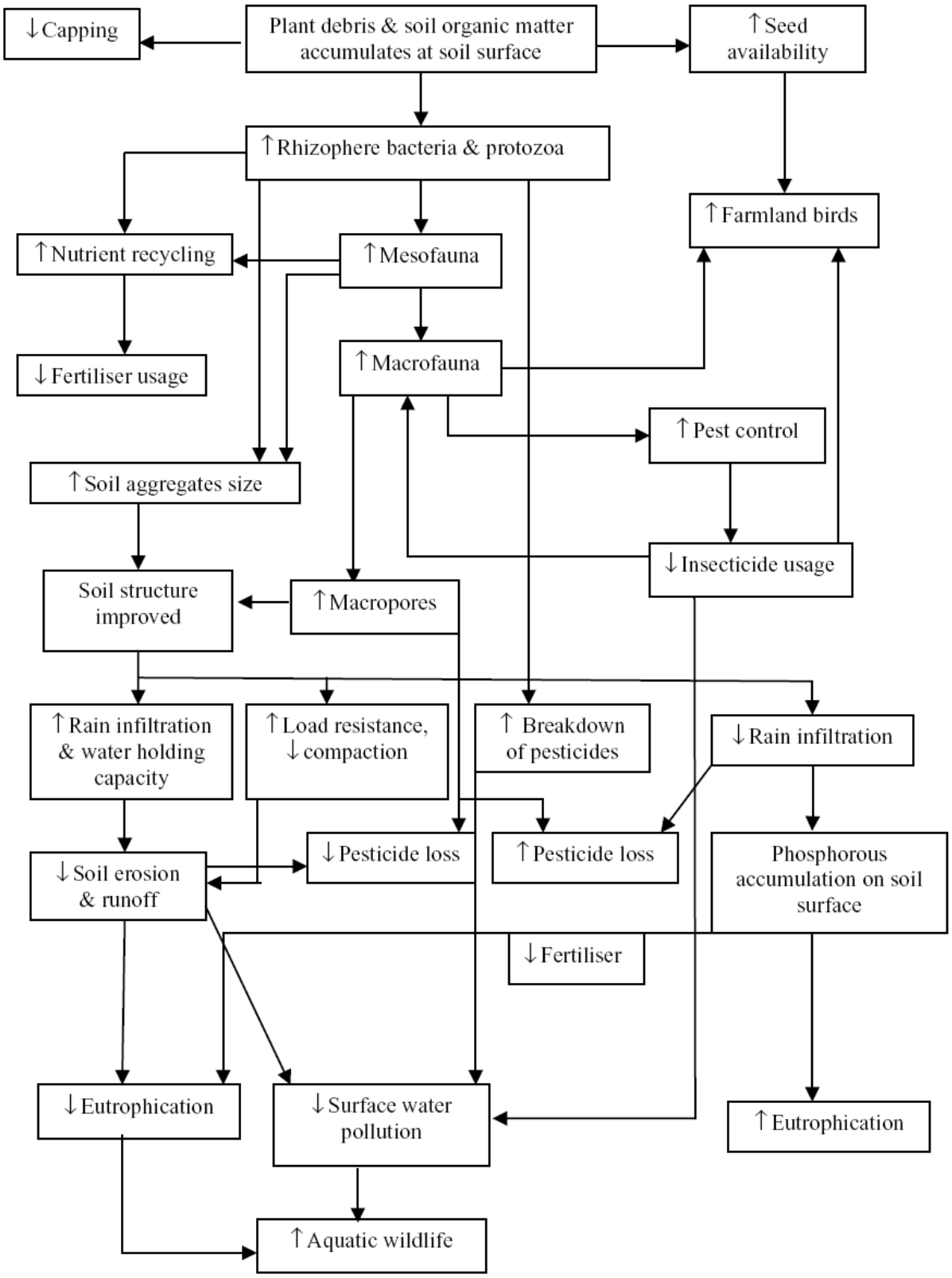

Fig. I-1. Impact de la stabilité de la structure sur l'environnement (Holland, 2004).

4.2 Définitions

Il n'existe pas une définition unique et universelle de la structure du sol. La structure du sol est un concept qualitatif, qui fait référence à une propriété du sol intégrative. La définition la plus acceptée est celle de Dexter (1988) qui est très générale mais prend en compte les différents aspects de la structure qui se manifestent à différentes échelles :

« La structure de sol est l'hétérogénéité spatiale des différents constituants ou propriétés du sol ».

Dexter (1988) propose donc que la structure est un synonyme de l`arrangement de particules ou propriétés à toutes les échelles.

La structure du sol peut être vue à partir de la phase solide comme un ensemble de particules qui forment les agrégats de différentes tailles et formes (point de vue « agrégats »), ou du point de vue de la phase des vides comme un réseau des pores de différents tailles, formes et connectivités. Les deux aperçus décrivent la même complexité dans la gamme des échelles spatiales de nanomètre au mètre. La différence la plus importante entre le point de vue poral et le point de vue de la phase solide (agrégats) est le concept de stabilité structurale. Ainsi, Kay (1990) fait appel à trois termes pour essayer de mieux définir la structure du sol : la forme, la stabilité et la résilience.

4.2.1 Forme structurale

Selon Kay (1990) le terme de « forme structurale » s'applique au groupe de caractéristiques qui décrit l'arrangement hétérogène des solides et des vides existant dans le sol en un moment donné. Cette définition est très proche à celle proposé par Dexter (1988) et met en valeur l'arrangement de particules et propriétés du sol. Des exemples de caractéristiques de cette forme structurale sont la porosité total, la distribution des tailles de pores, l'organisation des zones de fractures, etc.

4.2.2 Stabilité structurale

Le terme de stabilité structurale décrit la capacité d'un sol à conserver son arrangement de particules solides et des vides lorsqu'il est exposé à différents stress. Les

stress naturels sont principalement l'action disruptive de l'eau et les stress anthropiques, l'impact des outils de travail du sol. Dans la littérature scientifique, le terme de stabilité de la structure fait généralement référence à la stabilité vis-à-vis de l'eau.

4.2.3 Résilience structurale

Ce terme définit la capacité d'un sol à récupérer sa forme structurale via des processus naturels lorsque les stress sont réduits ou s'arrêtent. Des exemples des processus naturels qui aident à récupérer la forme structurale sont les cycles de dessiccation et réhumectation, les cycles de gel et dégel, l'endurcissement de la structure et l'activité biologique, i.e. le développement des racines ou l'activité de faune du sol.

Kay (1998) ajoute un 4ème terme, la « vulnérabilité », qui reflète la combinaison de la stabilité et de la résilience (Fig I-2). Les sols peu stables et peu résilients sont les sols les plus vulnérables.

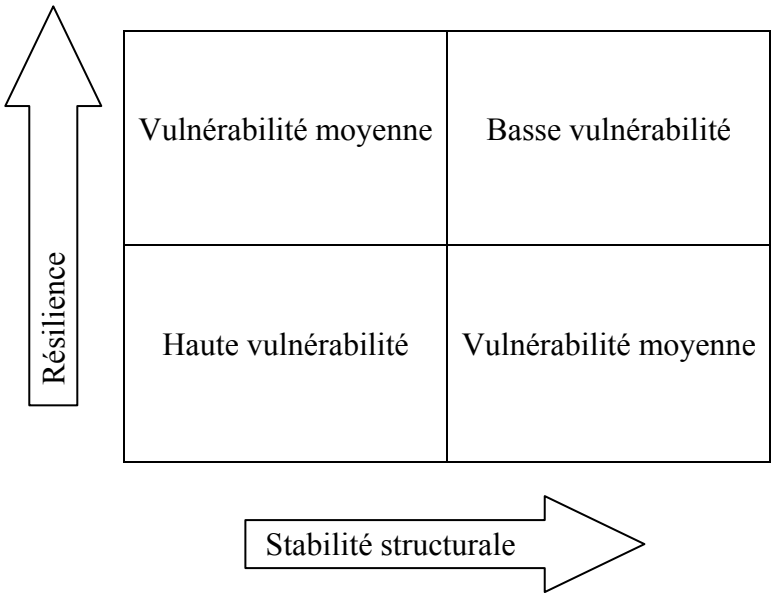

Fig. I-2. Variation de la vulnérabilité avec la stabilité structurale et la résilience (Kay, 1998).

La structure de sol est donc à la fois une propriété statique et dynamique. Les changements de la stabilité de la structure, i.e. l'acquisition ou perte d'une forme structurale, ont lieu à diverses échelles de temps et peuvent être fréquents.

4.3 Agrégats vs structure

Les agrégats sont des sous-ensembles de la structure de sol et ont été définis par Martin et al. (1955) et par Kemper & Chepil (1966) comme des assemblages naturels des particules primaires liées entre elles d'une manière plus forte qu'avec les particules qui les entourent. Les agrégats peuvent être facilement observés, par exemple, dans la rhizosphère d'une prairie ou grâce à l'application d'une force ou un stress externe sur des zones de faible cohésion comme dans la couche labourée d'un sol cultivé. Les agrégats peuvent être définis à différentes échelles de micrométrique à centimétrique, selon les méthodes d'observation disponibles ou les énergies mises en jeu.

Young et al. (2001) critiquent le point de vue « agrégats », car ils considèrent que les agrégats ne représentent pas le sol in situ. Selon ces auteurs, les mesures sur des agrégats ne renseignent pas sur la distribution spatiale des processus ou constituants du sol et ne sont pas liées à la connectivité, tortuosité ou l'hétérogénéité spatiale de pores en 3D. De nouvelles méthodologies comme la tomographie de rayons X et l'utilisation des modèles numériques intégratifs permettent de mettre en évidence la disposition spatiale des agrégats.

Amézketa (1999) considère que pour une caractérisation complète de l'agrégation du sol la structure et sa stabilité doivent être analysées au niveau de macro et microagrégats (< 250 μm) car différentes tailles d'agrégats ont différentes stabilités et répondent différemment aux conditions environnementales et aux pratiques culturales. Cependant, pour les sols cultivés où l'étude de la stabilité de la structure prend de l'importance, les stress externes les plus importants (i.e. la pluie, le vent, l'irrigation, les pratiques culturales) on un impact sur les macroagrégats. Ainsi l`étude des agrégats de taille millimétriques devient importante car ils sont les plus sensibles au mode d`occupation du sol et la désagrégation à cette échelle est responsable de la formation de la croûte de battance, du ruissellement et de l`érosion.

L'analyse à l'échelle de microagrégats, où les agents inorganiques permanents sont les responsables de l'agrégation (i.e. aluminosilicates amorphes, oxydes et polymères organiques adsorbés, floculation), est particulièrement d'importance pour les sols affectés par la dispersion physico-chimique.

4.4 Mécanismes impliqués dans la macroagrégation

La dynamique des agrégats est caractérisée par trois phases : la formation à partir de matériel non agrégé, la stabilisation et la destruction des agrégats (Tisdall & Oades, 1982). Les trois phases se produisent d'une manière séquentielle et peuvent être simultanés. La séparation des phénomènes est difficile car, par définition, les agrégats ont une certaine cohésion et donc leur séparation implique l'application d'un stress externe.

4.4.1 Mécanismes de destruction des agrégats ou désagrégation

La désagrégation à cause de l'eau peut être le résultat de plusieurs mécanismes physico-chimiques et physiques intervenant à différentes échelles spatiales depuis celle des particules d'argile à celle des macroagrégats (Le Bissonnais, 1996).

Quatre principaux mécanismes ont pu être identifiés et sont résumés par Le Bissonnais (1996) : l'éclatement, la microfissuration par gonflement différentiel, la désagrégation mécanique sous l'impact des gouttes de pluie et la dispersion physico-chimique (Figure I-3).

Les mécanismes se différentient par la nature des liaisons interparticulaires et l'énergie impliquée dans la désagrégation, par les conditions physiques et chimiques requises pour la désagrégation, par la cinétique des processus, par les propriétés de sol qu'influencent le mécanisme et enfin par la nature et distribution de la taille des produits de désagrégation (Le Bissonnais, 1996) (Tableau I-2).

4.4.1.1 L'éclatement

C'est la désagrégation par compression de l'air piégé lors de l'humectation. La pression interne créée par l'air piégé produit la rupture de l'agrégat si elle est supérieure à la cohésion interne de l'agrégat (Panabokke & Quirk, 1957). L'effet de l'air piégé dépend du volume d'air dans les agrégats, la vitesse d'humectation (hydrophobie) et la résistance à la traction des agrégats humides donc de leur cohésion (Concaret, 1967). L'éclatement diminue avec l'augmentation de la teneur en eau initiale des agrégats jusqu'à saturation (Panabokke & Quirk, 1957; Le Bissonnais, 1988). Les fractions résultantes de l'éclatement sont principalement des microagrégats et leur taille augmente avec la teneur en argile. Ce mécanisme est particulièrement important en sols limoneux.

Tableau I-2. Caractéristiques de principaux mécanismes de désagrégation (Le Bissonnais, 1996).

Mécanisme de désagrégation	**Eclatement**	**Désagrégation par gonflement différentiel**	**Désagrégation mécanique**	**Dispersion physico-chimique**
Nature des forces en jeu	Pression interne de l'air piégé lors de l'humectation	Pression interne de l'air par gonflement différentiel de l'argile	Pression externe par l'impact des gouttes de pluie	Forces d'attraction internes entre les particules colloïdales
Propriétés du sol qui contrôle le mécanisme	Porosité mouillabilité cohésion interne	Gonflement potentiel conditions d'humectation cohésion	Cohésion à l'état humide (argile, MO et oxydes)	Statut ionique minéralogie de l'argile
Fractions résultantes	Microagrégats	Macro et microagrégats	Particules élémentaires	Particules élémentaires
Intensité de la désagrégation	Forte	Limitée	Cumulative	Totale

4.4.1.2 La microfissuration par gonflement différentiel.

Le gonflement et retrait différentiel des argiles dans les agrégats entraînent des stress internes qui produisent des fissurations et peuvent détruire les agrégats.

L`éclatement de sols initialement humides et le gonflement et retrait différentiel produisent des microagrégats de mêmes caractéristiques (Le Bissonnais, 1988). Néanmoins, la désagrégation par éclatement diminue avec la teneur en argile d'un sol, tandis que la microfissuration par gonflement différentiel augmente (Chan & Mullins, 1994).

4.4.1.3 La désagrégation mécanique sous l'impact des gouttes de pluie.

L'énergie cinétique des gouttes de pluie disperse mécaniquement les particules. La désagrégation mécanique est importante principalement lorsque le sol est humide car les agrégats ont alors une faible cohésion interne. La désagrégation mécanique peut aussi être provoquée par les outils de travail du sol, notamment avec un sol humide.

4.4.1.4 La dispersion physico-chimique.

Elle est le résultat de la réduction des forces attractives entre les colloïdes du sol lors de l'humectation (Emerson, 1967). La stabilité à la dispersion dépend des caractéristiques ioniques des cations présents. Les cations monovalents causent la dispersion physico-chimique due à des stress osmotiques. Ainsi, ce mécanisme dépend fortement du pourcentage de sodium échangeable et c'est le mécanisme le plus destructif car il produit des particules élémentaires.

Ainsi, n'importe quel processus qui augmente la cohésion entre les particules du sol va améliorer la résistance de la structure à tous les mécanismes de désagrégation. De plus, la diminution du gonflement des argiles devrait limiter l'ampleur de la destruction des agrégats par microfissuration. Finalement, en diminuant la vitesse d'entrée d'eau, l'air piégé dans l'agrégat a plus des possibilités de sortir des agrégats et donc, la pression interne d'air diminue et les tensions mécaniques dues au gonflement et à l'éclatement se distribuent pendant plus de temps. En conséquence, la stabilité structurale à l'éclatement et à la microfissuration augmentent (West et al., 1987).

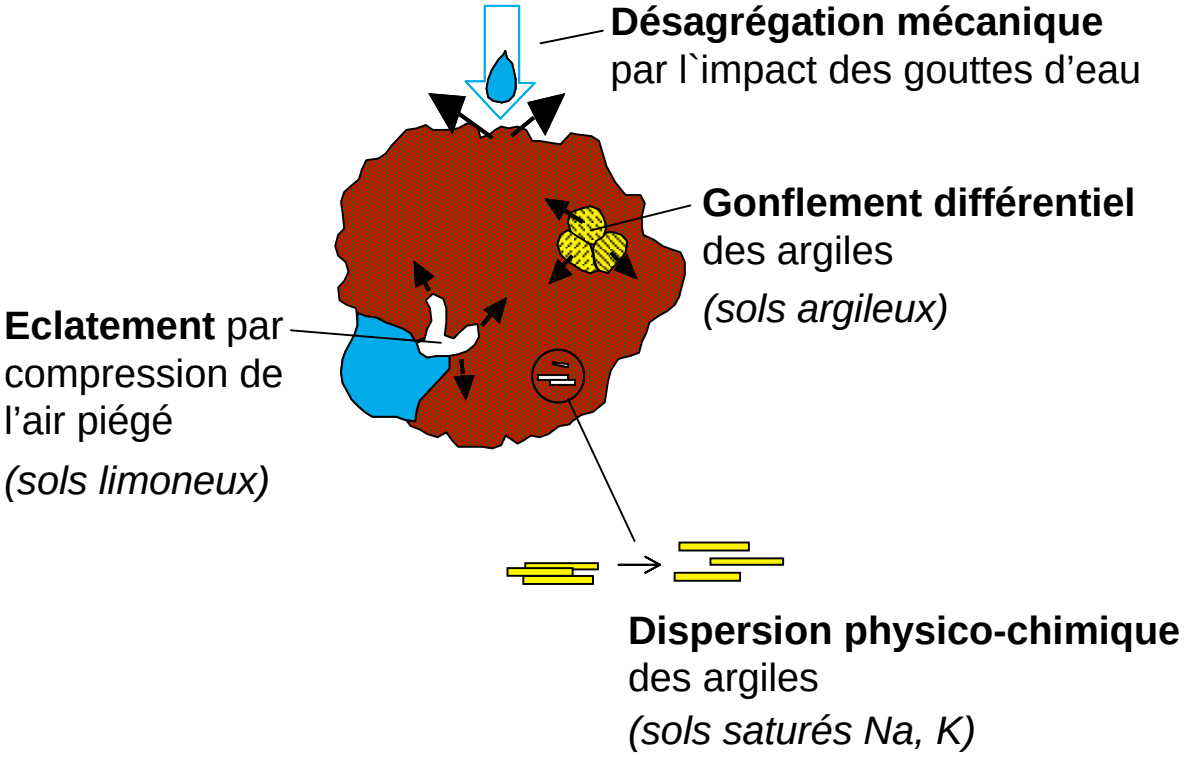

Fig. I-3. Mécanismes de désagrégation à l'eau (Chenu & Cosentino, 2007).

4.4.2 Mécanismes de stabilisation des macroagrégats

Un des moteurs principaux de la dynamique des macroagrégats est la matière organique et sa liaison indissociable avec l`activité microbienne. La MO est la source d`énergie des microorganismes hétérotrophes donc le moteur de leur croissance et développement. Cette croissance physique et la production de sous-produits microbiens associés impliquent des modifications des propriétés physiques qui gèrent la dynamique des agrégats. Les microorganismes sont donc des agents importants de la formation, de la stabilisation et de la destruction des agrégats. Ainsi, la dynamique des microorganismes de sol est étroitement liée à la dynamique de l'agrégation du sol (Golchin et al., 1998). Ils agissent par différents mécanismes que nous présenterons ici.

4.4.2.1 Collage « Gluing »

Une grande proportion des microorganismes a la capacité d'adhérer à particules solides, organiques ou minérales. Même si la quantification directe de l'adhésion est compliquée, l'observation directe de bactéries, algues et champignons adhérant au particules de sol est possible (Chenu & Stotzky, 2002). La force d'adhésion des microorganismes peut être très importante (Kendall & Lower, 2004) et explique largement la cohésion d'édifices argile fine - cellules microbiennes. Mais c'est grâce à la production de polysaccharides extracellulaires que les microorganismes ont un fort impact sur la stabilité des agrégats. Cette relation a été mise en évidence (i) en ajoutant des polysaccharides purs au sol ou aux constituants de sol et vérifiant que la stabilité structurale augmente immédiatement (Chenu, 1989; Chenu et al., 1994; Czarnes et al., 2000), (ii) en stimulant leur production in situ (Amellal et al., 1998) ou (iii) avec des corrélations entre la stabilité des agrégats et les polysaccharides extractibles à l'eau chaude, fraction qui a été montrée comme étant d'origine microbienne ou racinaire (Haynes & Swift, 1990) (Tableau I-3).

Les effets positifs des polysaccharides extracellulaires sur la stabilité de la structure sont doubles: ils ont une tendance à s'adsorber aux surfaces minérales grâce à leur réactivité de surface et ils établissent un pontage entre particules minérales (Chenu, 1989). Les exopolysaccharides sont de macromolécules de poids moléculaire élevé (10^5 à $>10^6$ Daltons) et l'adsorption sur des argiles et silicates est de grand affinité et peu réversible (Khandall et al., 1992). Grâce à leur conformation linéaire, à leur poids moléculaire et à

leur capacité à former des liaisons intermoléculaires, les exopolysaccharides forment des fibres de quelques nm d'épaisseur et > 100 nm de longueur et des réseaux plurimoléculaires (McIntire & Brant, 1997).

4.4.2.2 Enrobage par des microorganismes filamenteux («Physical Entanglement»)

Plusieurs études ont montré que les champignons forment des réseaux d'hyphes sur la surface des agrégats (Harris, 1972; Tisdall & Oades, 1982; Marinissen & Dexter, 1990; Guggenberger *et al.*, 1999). Des observations à l'œil nu ou en microscopie électronique ont mis en évidence l'enrobage physique de particules de sol par des hyphes de champignons (Tisdall & Oades, 1982; Dorioz *et al.*, 1993; Degens *et al.*, 1996; Angers & Chenu, 1998). Ces observations visuelles ont été corroborées par de nombreuses corrélations significatives entre la longueur des hyphes fongiques et la stabilité structurale (Tableau I-3).

Bien que beaucoup d'articles scientifiques font référence à l'enrobage de champignons comme un mécanisme fréquent et important pour l'agrégation, il n'existe pas une investigation en science du sol sur la biophysique ou biomécanique du mécanisme (Ritz & Young, 2004).

La force d'adhésion des hyphes fongiques aux particules de sol peut être très élevé, en particulier en présence d'exopolysaccharides et peut varier avec l'âge de mycélium (Li et al., 2002). Il est attendu qu'elle varie également avec les espèces, le diamètre des hyphes, etc. Le pontage par les champignons a le potentiel d'augmenter la résistance à la traction des agrégats.

Tableau I-3. Corrélations entre la stabilité structurale et plusieurs variables microbiennes (Chenu & Cosentino, 2007).

Référence	Sol : type ou texture	COT*	Polysaccharides Totaux	Polysaccharides solubles à l'acide dilué	Polysaccharides extractibles à l'eau chaude	Biomasse microbienne	Longeur d'hyphes
				coefficients de détermination stabilité structurale versus variable			
Angers & Mehuys (1989)	Clay loam			0,63			
Angers et al. (1993)	Clay loam	0,001ns		0,28	0,43	0,33	
Angers (1992)	Loam	0,6				0,4 ns	
Angers et al. (1999)	Sandy loam, Podzol	0,77		0,8		0,57	
Ball et al.(1996)	Loam	0,83	0,97	ns	0,98		
Bethlenfalvay et al.(1999)	Silt loam						0,565
Bissonnette et al.(2001)	Silty clay	0,95		0,89		0,9	
Capriel et al.(1990)	20% Clay 63% Silt					0,82	
Carter (1992)	Sandy loam, Podzol	0,942				0,947	
Carter et al. (1994)	Sandy Loam	ns		< 0,25	< 0,25	ns	
Chan & Heenan (1999)	Oxic Paleustalf	ns				0,64	
Chantigny et al. (1997)	SiClLoam and Clay Loam			0,42/0,05		ns	
Degens et al. (1994)	Sandy loam	ns		ns	0,05	ns	ns
Degens & Sparling (1996)	Sandy, Podzol			ns	ns	0,83-0,54	
Degens et al. (1996)	Sandy						0,41
Drury et al. (1991)						0,26	
Denef & Six (2005)	Loam, illitic					0,59	
	Clay loam , ferralsol					0,21	
Haynes & Swif (1990)	Silt loam	0,58	0,57 ns		0,74		
Haynes et al. (1991)	Sandy loam	0,66	0,67	0,56ns	0,83		
	Silt loam	0,77	0,75	0,76	0,84	0,957*	
	Clay loam	0,72	0,76	0,6ns	0,79		
Haynes & Tregurtha (1999)	Silt loam	0,92			0,72 ns	0,8	

* Carbone organique totale

Tableau I-3. Corrélations entre la stabilité structurale et plusieurs variables microbiennes (continuation).

Référence	**Sol : type ou texture**	**COT***	**Polysaccharides Totaux**	**Polysaccharides solubles à l'acide dilué**	**Polysaccharides extractibles à l'eau chaude**	**Biomasse microbienne**	**Longeur d'hyphes**
				coefficients de détermination stabilité structurale versus variable			
Haynes (2000)	Silt loam	0,61	0,66		0,99		
Jastrow et al.(1998)	Silt loam	0,43			0,55	0,65	0,89
Kiem & Kandeler (1997)	6 – 30% clay	0,34				0,59 – 0,87	
Kinbursky et al. (1989)	Sewage sludge (clay 3 à 44%)				0,58 to 0,85		
Lax & Garcia-Orenes (1993)	Clay loam		0,88				
Metzger et al. (1987)	Sandy clay loam		0,5		0,68		0,74
Perfect et al. (1990)						0,11	
Roberson (1991)	Loam	ns				0,71	
Roberson et al. (1995)	Sandy loam	ns				ns. en été: 0,52	
Roldan (Roldan et al., 1994)	Loamy clay			0,66-0,28			
Tisdall & Oades (1980)		0,93					0,77
Ibrahim & Shindo (1999)							0,95
Spaccini (2002)	Dif textures	0,9		0,3-0,8			

* Carbone organique totale

4.4.2.3 Hydrophobie

A partir du modèle hiérarchique de la structure proposé par Tisdall et Oades (1982) et de sa validation sur sols tempérés par plusieurs auteurs, l'accent a été mis sur la formation et la stabilisation des macroagrégats par des exsudats microbiens et par l'enrobage par des hyphes fongiques. Cependant, un autre mécanisme qui peut expliquer la stabilisation de la structure et le développement de l'hydrophobie, qui reçoit maintenant une attention croissante dans la littérature.

La réduction de la vitesse d'infiltration de l`eau dans les sols a été associé tôt à l'augmentation de la stabilité de la structure (Concaret, 1967). Les sols vierges, stables et riches en matières organiques ont des vitesses d'infiltration plus lentes (Caron *et al.*, 1996; Hallett *et al.*, 2001a) que les sols cultivés.

Des fortes corrélations on été trouvées entre composants des matières organiques (fractions aliphatiques et acides humiques) et la stabilité structurale (Chaney & Swift, 1984; Capriel *et al.*, 1990). Par ailleurs, les exsudats des racines et des microorganismes peuvent produire des films hydrophobes, particulièrement après une dessiccation (Hallett et al 2001). Néanmoins, les articles qui ont essayé de établir des relations cause – effet entre l`hydrophobie d`origine microbienne et l`agrégation ont montré des résultats contradictoires. Lorsqu'un substrat est apporté au sol et donc, lorsque l'activité microbienne est stimulée, la sorptivité à l'eau diminue et la répulsion augmente (Hallett & Young, 1999; De Gryze *et al.*, 2004) et le temps de pénétration de la goutte d'eau diminue (Cosentino *et al.*, 2006b). Cependant, De Gryze et al. (2006a) ont observé augmentation de l'hydrophobie après un apport de 1% de paille de blé sur un seul sol de prairie sur cinq de différentes textures. Par ailleurs, l'hydrophobie, l`activité microbienne et la formation des agrégats n'étaient pas corrélées, ce qui montre, pour eux, que l'importance des mécanismes de stabilisation dépend du type de sol.

Le développement de l'hydrophobie dans les sols a tôt été associé à la présence de champignons (Savage et al., 1969) et ils sont considérés par Hallett et al. (2001a) comme le principal groupe responsable de l'hydrophobie sub-critique. Néanmoins, l'effet de différents espèces de champignons sur l`hydrophobie du sols est variable (McGhie & Posner, 1980).

Les champignons exsudent de molécules spécifiques, les hydrophobines, qui dans le sol peuvent former membranes amphiphiliques très insolubles. Tandis que la présence

d'hydrophobines n'a jamais été étudie dans le sol, il est probable qu'elles y soient présentes car beaucoup de champignons ont la capacité de les exsuder in vitro.

Les mycorhizes exsudent des protéines insolubles, les glomalines, qui ont été bien corrélées avec la stabilité de la structure (Wright & Upadhyaya, 1998). Néanmoins, Feeney et al. (2004) n'ont pas trouvé de relations spécifiques entre la présence de glomaline et l`hydrophobie du sol.

Les lipides extracellulaires peuvent aussi être responsables de la répulsion à l'eau d'origine microbienne (Capriel et al., 1990). L'addition de lipides et acides humiques au sol a augmenté immédiatement l'agrégation (Harris *et al.*, 1966), mettant en évidence leur effet direct, sans doute via une augmentation de l`hydrophobie.

Un facteur qui contrôle le développement de l'hydrophobie est l'occurrence de cycles de dessiccation et réhumectation et l`amplitude et la durée de la période de dessiccation. En effet, les substances hydrophobes d'origine microbienne sont généralement amphiphiliques, elles ont tendance à exposer leurs parties hydrophobes vers les pores après une dessiccation (Michel et al., 2001).

La plupart des sols se caractérisent par une hydrophobie sub-critique, i.e. un niveau de répulsion de l`eau qui permet encore au sol de s'humecter (Tillman *et al.*, 1989; Wallis & Horne, 1992; Hallett *et al.*, 2001a). L`hydrophobie sub-critique a d'importantes conséquences sur les propriétés du sol. Elle affecte la conductivité hydraulique et de solutés du sol, augmente le ruissellement et diminue la désagrégation pour éclatement. La répulsion sub-critique est donc une propriété importante par son impact sur la stabilité de la structure et elle est fortement affectée par l'activité des microorganismes (Hallett & Young, 1999; White *et al.*, 2000; Feeney *et al.*, 2004).

4.4.2.4 Système poral

Un impact physique des matières organiques et de l`activité microbienne associée est le changement du réseau poral du sol. Il a été démontré que l'addition de substrats organiques modifie la connectivité, densité et hétérogénéité de pores et fissures à différentes échelles du µm (Dorioz et al., 1993) au cm (Preston et al., 1999). Une explication possible est que les zones riches en polysaccharides d'origine microbienne ont une capacité de gonflement – retrait contrasté par rapport aux zones sans activité microbienne (Chenu, 1993). Les événements de dessiccation – réhumectation ne sont pas une condition nécessaire, car les

champignons peuvent aussi créer de pores comme a été suggéré par Emerson & McGarry (2003). Ces auteurs proposent que l'incrément relatif de pores > 30 µm avec l'apport de matières organiques au sol a été dû aux vides laissés par les hyphes fongiques dont les parois ont été stabilisées par des polysaccharides extracellulaires.

La modification du système poral joue un rôle important sur la stabilité des agrégats. L'éclatement est un mécanisme de destruction d'agrégats très important en sols limoneux. Concaret (1967) a montré que la pression interne qui se produit lorsque les agrégats sont humectés dépend fortement de la possibilité de l'air à s'échapper. Ainsi, le changement du système poral du sol affecte la stabilité de la structure.

4.5 Méthodes d'évaluation de la stabilité de la structure

Des douzaines de méthodes d'évaluation de la stabilité des agrégats on été utilisées à partir de 1930 (Le Bissonnais, 1996) (Tableau I-4).

Les méthodes les plus utilisées pour expliquer la sensibilité des sols à l'érosion par leur stabilité structurale sont celles qui déterminent la distribution de la taille des agrégats après l'application d'un stress à l'eau, mécanique ou les deux (Amézketa, 1999). La distribution de la taille des agrégats après tamisage dans l'eau est la méthode la plus courante pour étudier les effets de l'eau sur la macroagrégation. Généralement, ces méthodes impliquent l'humectation des échantillons et la séparation par tamisage sous l'eau. Initialement, des méthodes multi-tamis on été utilisées pour la détermination de la stabilité structurale, mais, Kemper et Rosenau (1986) on montré que les résultats obtenus avec un seul tamis étaient aussi bien corrélés avec les phénomènes en plein champ, que les méthodes multi-tamis. L'avantage des méthodes à un seul tamis est leur simplicité et donc la possibilité de traiter un grand nombre d'échantillons en peu de temps. C'est ainsi que la méthode de Kemper Rosenau (1986) est devenu la méthode la plus utilisée pour l'évaluation de la stabilité de la structure (Amézketa, 1999).

Même en utilisant la méthode de Kemper Rosenau (1986), les résultats peuvent être affectés par des petites variations dans la procédure depuis le prélèvement de l'échantillon jusqu'au résultat. Ainsi, la standardisation devrait prendre en compte : (i) le prélèvement et la préparation de l'échantillon ; (ii) le conditionnement physique d'échantillon avant l'application du test (taille d'agrégats, teneur en eau) ; (iii) les traitements à appliquer

(méthode d'humectation, vitesse d'humectation) ; (iv) la mesure de la désagrégation et (v) l`expression de résultats (Amézketa, 1999).

Tableau I-4. Caractéristiques de principales méthodes d'évaluation de la stabilité structurale (adapté de Le Bissonnais, 1996).

Type de traitement	Echantillon initial	Expression du résultat	Auteurs
Tamisage à l'eau	3 - 5 mm	DMP*	Yoder (1936)
	< 2 mm	% > 200 µm	Hénin et al. (1958)
	Sol entier	ΔDMP	De Leenheer & De Boodt (1959)
	1 – 2 mm	% > 250 µm	Kemper & Rosenau (1984)
	2 – 3.4 mm	DMP	Churchman & Tate (1987)
	1 - 2 mm	% > 250 µm	Pojasok & Kay (1990)
Gouttes de pluie	4 – 5 mm	Temps à la désagrégation	Low (1967)
	2 – 9 mm	DMP	Young (1984)
	5 – 8 mm	Temps à la désagrégation	Farres (1987)
	Sol entier	% < 125 µm	Loch (1994)
Ultrasons	4 – 5 mm	Taux de dispersion	Edwars & Bremner (1967)
	4 – 5 mm	Porosité inter-aggrégats	Grieve (1980)
Immersion	3 – 5 mm	Qualitatif	Emerson (1967)
Tamisage à sec	< 4 mm	DMP	Kemper & Chepil (1965)
Plusieurs	3 – 5 mm	DMP	Le Bissonnais (1996)

* DMP: diamètre moyen pondéré.

La principal limitation de la méthode de Kemper et Rosenau (1986) est l'impossibilité de distinguer entre différents mécanismes de désagrégation. Le traitement qui s'applique à l'échantillon (réhumectation lente) et la mesure de la désagrégation ne sont pas clairement séparés, et la mesure de désagrégation (tamisage à l'eau) est même destructrice.

La diversité de méthodes empêche toute synthèse quantitative des résultats. En France, le test de stabilité structurale mis au point par Hénin et al. (1958) a été à la base à de nombreux travaux, et servait de méthode de référence pour porter un jugement sur la stabilité structurale de sols. Cette méthode n'est aujourd'hui plus utilisée dans les laboratoires (abandon du benzène comme prétraitement, développement des recherches montrant la nécessité de faire évoluer ce test).

Le Bissonnais (1996) a proposé un cadre méthodologique unifié pour la mesure de la stabilité des agrégats de sol incluant les aspects les plus intéressants des méthodes préexistantes. La méthode est une modification de celle de Hénin et al. (1958) avec des aspects de Yoder (1936), Grieve (1980), Kemper & Rosenau (1986) et autres. Ainsi, la méthode propose de mesurer le diamètre moyen pondéré (DPM) des agrégats tamisés à l'éthanol après avoir appliqué trois pré traitements indépendants : réhumectation rapide, réhumectation lente et agitation mécanique dans l'eau après réhumectation à l'éthanol (Fig. I-4).

L'éthanol, étant donné sa faible tension superficielle et sa constante diélectrique vis-à-vis de l'eau, évite l'éclatement et le gonflement des agrégats de sol. C'est grâce à cet effet que la méthode peut séparer l'impact des différents mécanismes de désagrégation sur la stabilité de la structure (Amézketa, 1999). Le DPM des agrégats après la réhumectation rapide mesure l'éclatement. Le DPM après la réhumectation lente mesure la désagrégation due à la microfissuration par gonflement différentiel d'argiles et le DPM après l'agitation mécanique des agrégats étant pré humectés à l'éthanol mesure la cohésion interparticulaire indépendamment de l'éclatement et le gonflement différentiel.

Un autre avantage de la méthode est la séparation de l'application du traitement (stress externe) et la mesure de la désagrégation, car le tamisage est fait sous l'éthanol, donc, il n'y a pas d'énergie annexe impliquée. L'éthanol évite aussi la réagrégation qui se produit pendant le séchage de sol préhumecté à l'eau. C'est le point de vue mécanistique de la méthode que nous a amené à la choisir pour la démarche de ce travail.

Il faut cependant préciser que, quelle que soit la méthode utilisée, l'estimation de la stabilité structurale reste un test et un indicateur, plus qu'une mesure *stricto sensu*. La stabilité structurale n'est pas sanctionnée par une grandeur physique précise, mais indirectement par la masse résultant d'une contrainte. Cette contrainte n'est pas non plus définie par une énergie précise (Abiven, 2004).

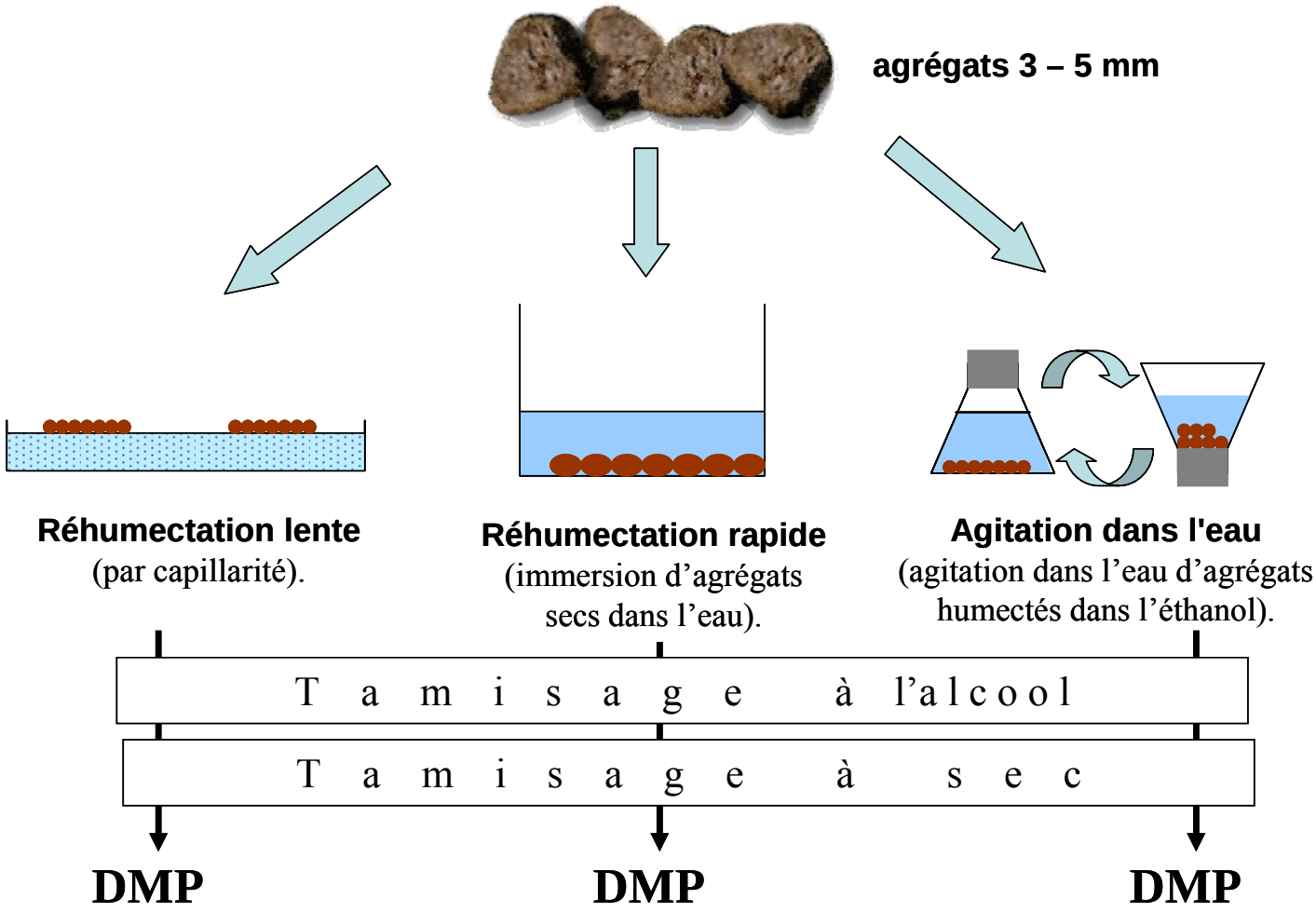

Fig. I-4. Méthode de stabilité structurale proposée par Le Bissonnais (1996)

4.6 Dynamique de la stabilité de la structure à court terme

4.6.1 Les cycles de dessiccation – réhumectation.

La structure du sol dépend de nombreux facteurs qui ont été revus par Amézteka (1999) qui les a classifié en : (i) facteurs intrinsèques ou internes du sol, liés à des caractéristiques primaires et (ii) facteurs exogènes ou externes. Les facteurs intrinsèques sont la texture, la minéralogie, la teneur en matières organiques, le pH, la concentration d'électrolytes, les oxydes etc. Les facteurs exogènes sont les variables climatiques, biologiques, les systèmes de culture, le temps, etc. Bien que les premiers facteurs soient liés à la stabilité de la structure, ils représentent la variabilité d'ordre pédologique qui évolue lentement. En revanche, les facteurs exogènes sont plus dynamiques à court terme. En conséquence, la stabilité structurale exhibe fréquemment une grande variabilité interannuelle et saisonnière. Cette variation est généralement plus importante que les différences de stabilité de la structure entre type de sols ou systèmes de culture (Perfect et al., 1990; Angers et al., 1999). Le climat modifie directement la stabilité de la structure principalement par l'action sur la teneur en eau du sol et indirectement par la stimulation saisonnière de l'activité microbienne du sol. Parmi les effets directs, la teneur en eau au moment de prélèvement (Perfect et al., 1990) et la teneur en eau antécédente (Caron et al., 1992) ont été montré être des modificateurs importants de la structure. Cependant, il n'y a pas de relation simple entre les cycles de dessiccation – réhumectation et la dynamique de macroagrégats car ils affectent au même temps des processus chimiques ou physiques et l'activité microbienne (Denef et al., 2001). L'absence des cycles de réhumectation peut influencer aussi la stabilité de la structure par endurcissement (*aging*). L'augmentation de la cohésion dans le temps a été observé par des ingenieurs civils et nomée « *thixotropic age hardening* » (Molope et al, 1985). Des processus physiques liés aux rearrangements des particules d'argiles semblent être les résponsables (Dexter et al, 1988).

4.6.2 L'étroite relation des dynamiques des matières organiques, de l'activité de microorganismes et de la stabilité de la structure.

A partir de l'article de Tisdall & Oades (1982), l'idée que l'origine de la structure du sol est due à différentes forces à différents échelles spatiales s'est installé. A une échelle moléculaire, les forces électrostatiques et de van der Waals sont responsables des

microstructures comme les particules argileuses. Progressivement, à des échelles plus grandes, l'agrégation est dominée par des microorganismes (bactéries et champignons) qui exsudent des substances agglutinantes et enrobent la matrice du sol ou puis par des racines qui lient les agrégats et particules entre eux (Young & Crawford, 2004). Le modèle implique fortement l'activité microbienne dans la genèse de la structure du sol et a donné la base de plusieurs modèles conceptuels de la dynamique du complexe sol-microorganismes à court terme (Angers & Chenu, 1998; Six *et al.*, 2002a).

L'incorporation de MO peut largement contribuer à la variation intra-annuelle de la structure. C'est grâce à la stimulation de microorganismes que la stabilité de la structure augmente rapidement après apports organiques.

Cette dynamique à court terme a été mise en avant par des modèles conceptuels qu'intègrent la dynamique de la MO avec la formation, stabilisation et la désagrégation de la structure du sol. Ces modèles ont été développés progressivement pendant les 10 dernières années par plusieurs groupes de recherche dans le monde et ont été discuté dans plusieurs articles et revues (Angers & Chenu, 1998; Golchin *et al.*, 1998; Jastrow *et al.*, 1998; Balesdent *et al.*, 2000; Six *et al.*, 2002a; Six *et al.*, 2004). Brièvement, l'idée en commun des modèles est que quand une matière organique fraîche (active) est incorporée au sol (e.g. incorporation de résidus ou racines en décomposition) elle devient le noyau pour la formation et la stabilisation des agrégats car elle stimule localement l'activité microbienne et donc, les mécanismes agrégeants présentés en 1.4.1. Lorsque la matière organique labile se décompose l'activité microbienne diminue concomitamment et les agrégats formés deviennent de moins en moins stables. C'est ainsi que la dynamique microbienne, de la matière organique et des agrégats sont finalement couplées (Fig. I-5).

4.7 Relations quantitatives entre l'apport de la MO, l'activité microbienne et la stabilité de la structure à court terme.

Très tôt dans les recherches sur la stabilité structurale, Monnier (1965) proposait un model conceptuel dans lequel la dynamique de la stabilité structurale après un apport de MO était liée à la qualité de la MO apportée (Fig. I-6). Son schéma montre la complexité de la dynamique temporelle qu'il faudrait prédire pour évaluer l'effet à court terme d'un apport de MO au sol.

Plusieurs causes expliquent donc la difficulté à prévoir l'évolution de la stabilité de la structure de sols cultivés : sa variation intra-annuelle est liée à la fois aux conditions climatiques et à l'apport de MO (quantité et qualité) et l´absence d´un protocole normalisé pour la mesure de la stabilité structurale a limité jusqu'ici l'intégration des nombreux travaux de la littérature.

Jusqu'a présent les modèles quantitatifs de la dynamique de la stabilité des agrégats sont très peu nombreux. Néanmoins, de tels modèles sont nécessaires pour pouvoir prédire la dynamique de la structure associée à pratiques culturales (modalités de travail du sol, rotation, apport d'amendements organiques ...). Par ailleurs, les modèles de la dynamique de MO doivent prendre en compte aussi l'évolution de la structure car elle influence la biodégradation par le mécanisme de protection physique.

Perfect & Kay (1990) ont proposé un modèle linéaire où la stabilité structurale relative dépendait de l`abondance relative des agents agrégeants mais qu'il n'a pas été plus développé.

Plus récemment, De Gryze et al. (2005) ont quantifié en conditions contrôlées l'influence de différentes quantités de résidus de cultures apportées au sol sur la formation d'agrégats et ont testé les prédictions de 4 différents modèles mathématiques (déterministes et mécanistes). Ils ont montré que la variable la plus explicative de l'évolution de la formation d'agrégats à partir de sol déstructuré était la production de CO_2 cumulée au cours des incubations de 21 jours. Les auteurs se sont intéressées seulement à la prédiction de la formation de la structure qui se produit rapidement après l'apport de MO (premier pic du schéma de Monnier, 1965, Fig. I-6). Abiven (2004) a développé le premier modèle qui prévoit l`augmentation initiale de la stabilité structurale et la diminution qui la suit à partir de caractéristiques biochimiques de résidus de cultures apportés et de paramètres empiriques qui décrivent la forme de la courbe de la stabilité structurale au cours du temps, par une approche de régressions mutiples. Cependant ce modèle est très dépendant des matières organiques utilisées pour le développer et apparaît assez difficile à transposer à d'autres situations. L`enjeu scientifique est donc de développer un modèle prédictif de la totalité de la courbe de la dynamique de la stabilité structurale à court terme à partir de toutes les variables qui la modifient : quantité de MO apportée, qualité biochimique des MO, type de sol, teneur en eau antécédente, etc., etc. La seule approche possible pour arriver à un modèle complexe et intégratif est une approche mécanistique qui donne la possibilité de sortir des conditions locales dans lesquelles les expérimentations ont été faites.

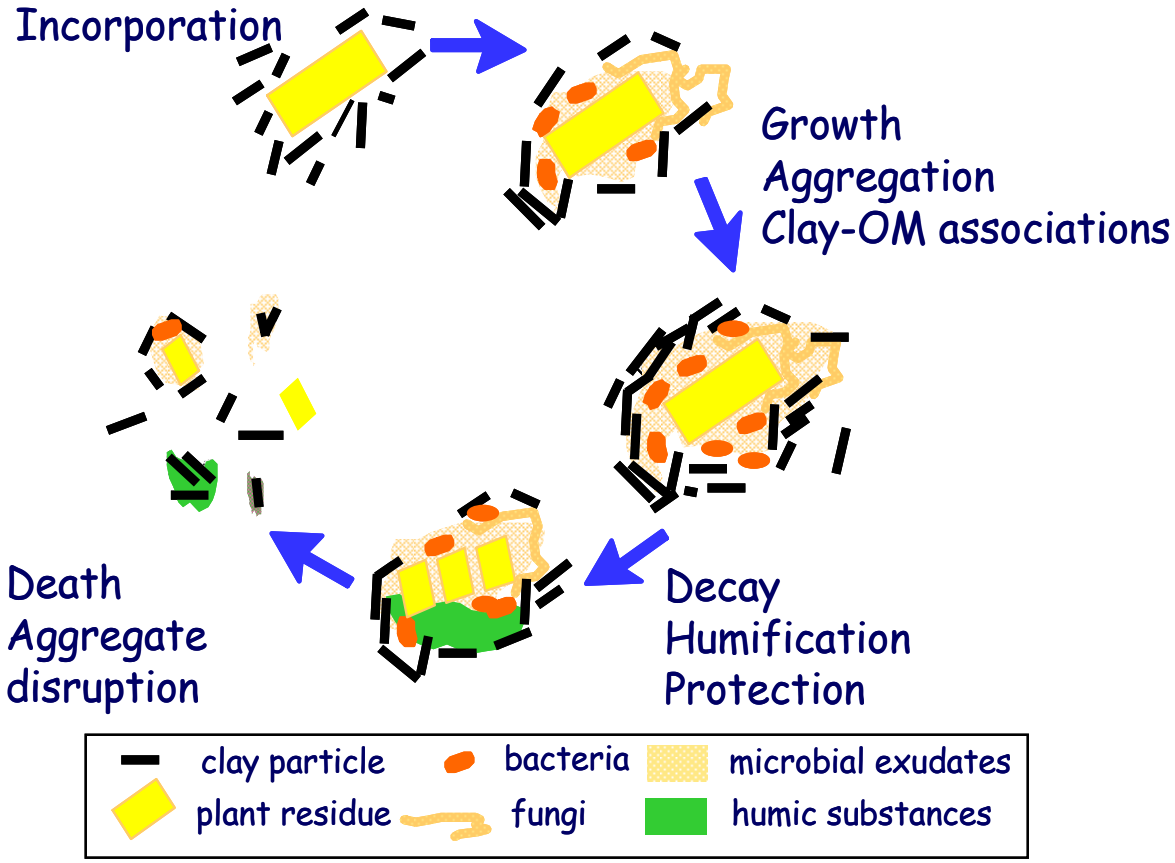

Fig. I-5. Schéma de l'interaction entre la décomposition de la matière organique et la formation et destruction des agrégats (Golchin et al. 95; Puget 97, Chenu et al. 98, Balesdent et al., 99; Six et al. 98) in Chenu et al (2002).

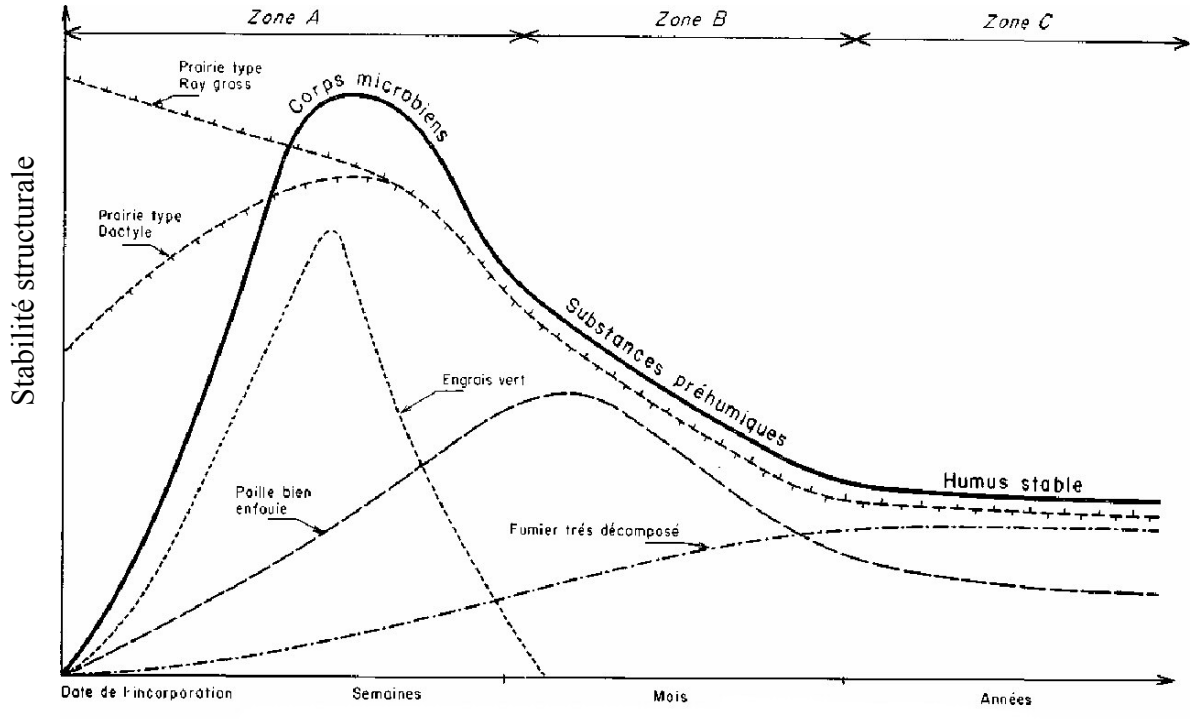

Fig. I-6. Modèle conceptuel de l'impact de différent types de matières organiques apportés sur la stabilité des agrégats (Monnier, 1965).

5 Objectifs spécifiques et démarche de la thèse

5.1 Objectifs

Afin de mieux prévoir l´évolution de la stabilité structurale à court terme suite à un apport de matières organiques, nous avons choisi une approche mécaniste. Nous avons étudié l'effet de deux facteurs : (i) la quantité de MO incorporée au sol et (ii) les alternances d'humectation - dessiccation. Dans cette approche, nous nous sommes fixé les objectifs suivants:

- A.- Évaluer les effets de différentes doses de matières organiques apportées sur (i) la stimulation de l´activité microbienne, (ii) les propriétés physiques élémentaires qui sont à la base de la stabilité structurale: cohésion interparticulaire, hydrophobie, porosité et (iii) la stabilité structurale aux différents mécanismes de désagrégation.
- B.- Analyser les effets couplés de cycles de dessiccation - réhumectation et de l'activité microbienne sur la stabilité structurale suite à un apport de MO.

L´étude bibliographique que nous avons faite nous a conduit à développer un modèle conceptuel mécaniste de relations entre l´apport de MO et la stabilité structurale. Ce modèle a été décisif pour concevoir les expérimentations prioritaires et pour décider des mesures de laboratoire et il sert de fil conducteur à cette thèse. Dans ce modèle, on peut diviser le jeu d´influences de l´apport de C sur la stabilité de la structure en trois niveaux (Fig. I-7). Tout d´abord les microorganismes du sol sont stimulés grâce à l´entrée de C dans le système et leur biomasse et la production de biomolécules augmentent. Cette augmentation modifie certaines propriétés physiques élémentaires du sol qui contrôlent la stabilité de la structure. Ce contrôle est exercé en modifiant l´impact de trois mécanismes de désagrégation sur la stabilité structurale : l´éclatement, la microfissuration et la désagrégation mécanique. Nous avons essayé de quantifier directement ou indirectement différentes parties ou compartiments du modèle conceptuel. Pour différentes concentrations de C apporté et pour l`existence ou non de cycles de dessiccation et réhumectation, nous avons quantifié les contributions relatives de chacun à la stabilité structurale dans le temps.

Nous avons divisé, donc, l`objectif A en trois objectifs spécifiques qu'on peut situer sur le schéma (Fig. I-7) de la façon suivante:

- A.1. Analyser les relations temporelles entre l´addition du C au sol, la croissance et l'activité microbienne et la stabilité de la structure. Construire un modèle qui prévoit l´évolution de la stabilité de la structure à court terme. De 1 à 3 sur la Fig. I-7.
- A.2. Etant donné l´importance de l´hydrophobie dans la stabilité à l´éclatement nous avons voulu comparer différentes méthodes permettant sa mesure et examiner sa relation quantitative avec la stabilité structurale. Niveau 2 sur la Fig. I-7.
- A.3. Quantifier la contribution relative des propriétés physiques élémentaires à l´amélioration de la stabilité structurale grâce à l´apport de MO. De 2 à 3 sur la Fig. I-7.

5.2 Démarche

Etant donné la fragilité des sols limoneux vis-à-vis de la désagrégation par l'eau, nous avons choisi ce contexte et apporté des résidus de culture à un sol limoneux cultivé. Afin de contrôler au mieux l´influence de l´apport de C sur la stabilité des agrégats nous avons choisi de réaliser des expérimentations en laboratoire. Nous avons travaillé avec un dispositif simple et standardisé ayant pour but une grande homogénéité de départ des paramètres physiques (potentiel hydrique, taille des agrégats, contact avec la paille) et biologiques. Le dispositif consistait en des incubations des agrégats calibrés de sol avec des résidus de culture avec un potentiel hydrique et une température contrôlés. Notre choix d´utiliser des agrégats naturels (déjà formés) et calibrés entre 3 – 5 mm était guidé par la nécessité d´éviter la superposition de deux processus qui ont lieu simultanément dans le sol, la formation et la stabilisation des agrégats, et pour pouvoir utiliser un protocole standardisé d´évaluation de la stabilité structurale.

Comme nous souhaitions mettre en évidence exclusivement l´effet de l´apport de C sur la stabilité de la structure nous avons exclu l´effet des plantes et de plus nous nous sommes placés en conditions non limitantes en azote.

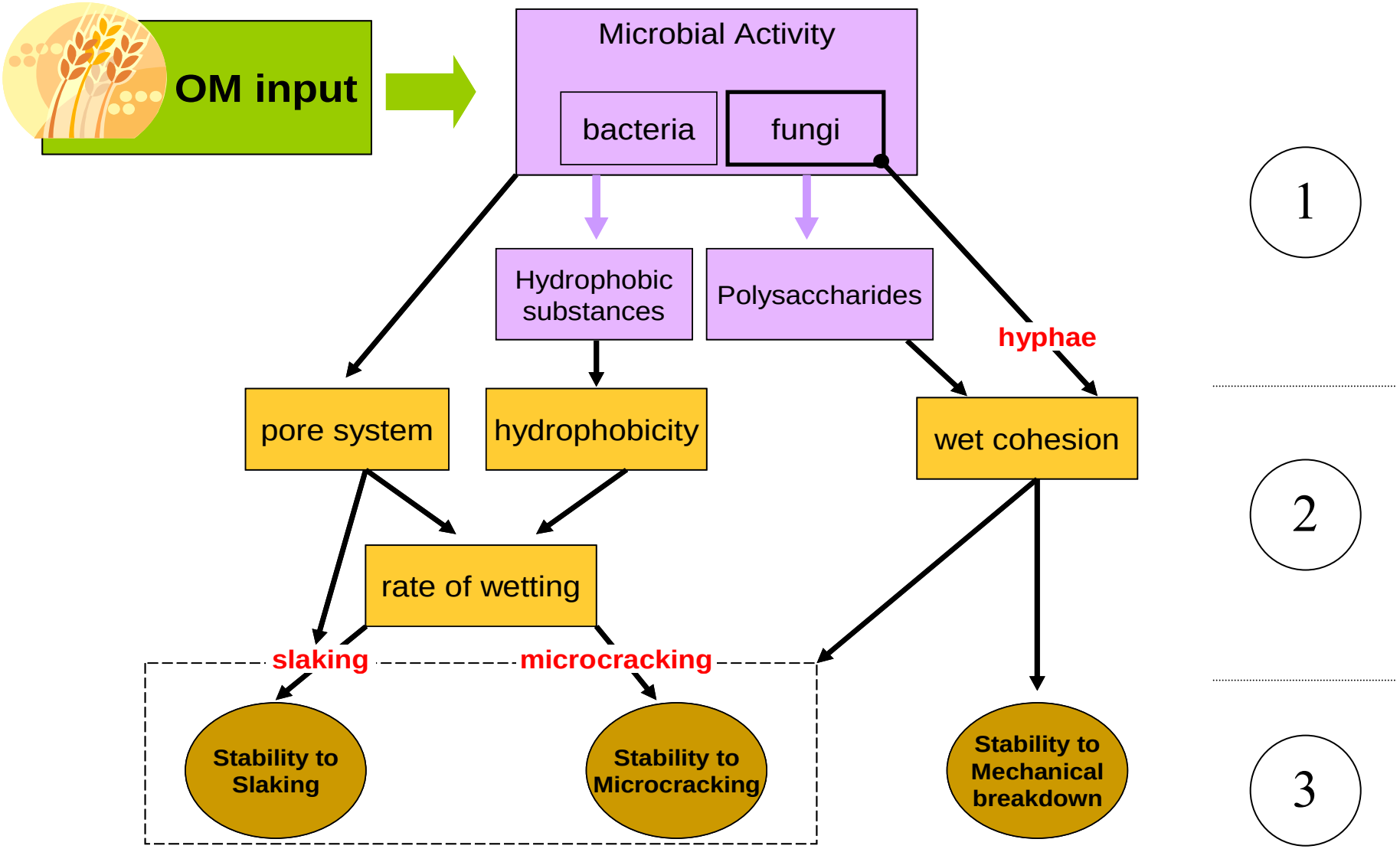

Fig. I-7. Modèle conceptuel mécaniste de relations entre l´apport de MO et la stabilité structurale. Cas d´un apport de MO végétale sur un sol instable limoneux.

Chapitre 2

L´effet et la quantification des variables biologiques sur la dynamique de la stabilité structurale

Short-term aggregate stability dynamics after a carbon input: quantitative relationships and modelling [1]

Résumé

La stabilité structurale des sols vis à vis de stress externes est une propriété clé pour le fonctionnement du sol, et sa prédiction est donc essentielle. Cependant, il n`existe pas aujourd´hui de manière de la prévoir ou de déterminer les pratiques culturales améliorantes.

Le but de ce travail a été (i) d'établir des relations quantitatives entre l'apport de matière organique au sol (des résidus de culture) et la stabilité structurale à trois mécanismes de désagrégation et (ii) de développer un modèle prédictif. Nous avons incubé des macroagrégats de sols pendant 8 mois après l'apport d'une large gamme de doses de pailles de maïs. Nous avons mesuré les principaux agents microbiens impliqués dans l'agrégation : la biomasse microbienne-C, l'activité microbienne (respiration de CO_2), les champignons (teneur en ergosterol) et les polysaccharides extracellulaires (polysaccharides solubles à l'eau chaude). Nous avons utilisé trois tests de stabilité qui mettent en évidence différents mécanismes de désagrégation (l`éclatement, la microfissuration et la désagrégation mécanique) pour analyser la dépendance des relations agent microbien-stabilité de la structure aux mécanismes de désagrégation.

Dans nos conditions d'incubation, la réponse microbienne à l`apport carboné a été linéaire et l'activité microbienne et ses sous-produits étaient les principaux déterminants de la stabilité de la structure qui augmentait rapidement après l'apport organique, puis diminuait lentement. Il n`y a pas eu de réponse différentielle de la stabilité de la structure vis à vis de l`éclatement, la microfissuration et la désagrégation mécanique. La stabilité structurale était bien décrite par une fonction linéaire de la biomasse microbienne et de la respiration cumulée. Nous proposons un nouveau modèle dans lequel un modèle mécaniste de transformations du carbone (CANTIS) simuler la biomasse microbienne et le CO2 dégagé en fonction de la qualité et de la quantité des résidus incorporés, est couplé à la fonction statistique que nous avons développée. Ce couplage prédit bien la dynamique de la stabilité de la structure observée et doit maintenant être testé pour des conditions expérimentales plus larges.

***Mots- clés** : stabilité structurale, la méthode de Le Bissonnais, CANTIS, variables biologiques, modèle, CO_2, C de la biomasse microbienne.*

[1] Ce chapitre est constitué d'un article en préparation pour *European Journal of Soil Science* — Cosentino, D.; Chenu, C; and Garnier, P. Short-term aggregate stability dynamics after a carbon input: quantitative relationships and modelling.

Abstract

Given the importance of soil structure and of its stability to external stresses for soil functioning, prediction of aggregate stability changes with time is a critical issue. However, there is still little capacity neither to predict aggregation nor to implement management practices of soil to favour it.

In silty soils the organic matter dynamics plays an essential role in aggregation by stimulating microorganisms. Thus, the dynamics of microbial activity, organic compounds and aggregation are intimately associated. In this work we aimed to establish predictive quantitative relationships between the addition of C to soil as plant residues and the aggregate stability to three breakdown mechanisms and to develop a predictive model on a mechanistic basis.

We developed an 8-month incubation experiment and we measured the main biological variables involved in aggregation and the aggregate stability of natural pre-existing aggregates after the addition of a range of maize residues. We used three aggregate stability tests, each relating to a different breakdown mechanism (slaking, microcracking and mechanical breakdown) to test whether the analyzed relationships were dependent on the breakdown mechanism.

In optimum controlled conditions when C is added to soil as a plant residue the response in microbial activity and it by-products was linear. Microbial activity and by-products were the main determinants of aggregate stability in the short-term and indicators of living microorganisms were responsible determining aggregate stability very early after the C input. Aggregate stability to slaking, microcracking and mechanical breakdown responded similarly to stimulated microorganisms.

In constant conditions, a mechanistic and linear model based on biological variables simulated reasonably well the whole dynamics of aggregate stability over several months. This allowed coupling this linear model with a pre-existing C transformations model (CANTIS) that may simulate biological variables in wider conditions than our experimental set-up.

Keywords: *aggregate stability, Le Bissonnais method, CANTIS, biological variables, model, CO_2, microbial biomass-C.*

1 Introduction

Soil structure is a highly dynamic soil property that varies with land use and cropping systems on a pluri-annual scale but also varies within a growing season, in particular in relation with organic matter additions to soil (Bullock et al., 1988; Perfect et al., 1990; Blackman, 1992). Given the importance of soil structure and of its stability to external stresses for soil functioning, prediction of aggregate stability changes with time is a critical issue.

There is no universal link between soil microorganisms' dynamics and soil aggregation. Their relationship is very soil type dependent (Six et al., 2002b). If we

consider silty soils, the relationship becomes relatively less complex because microorganisms play a more important role on aggregation compared to the soil mineral phase. The organic matter dynamics plays an essential role in aggregation by stimulating microorganisms, being the main source of energy for heterotrophs and acting as a primary binding agent itself. However, even considering one type of soil, there is still very little capacity to predict microbially-mediated aggregation nor deliberate management practices of soil to favour it (Chenu & Cosentino, 2007).

The dynamics of microbial activity, organic compounds and aggregation are intimately associated. This fact was crystallized in several fairly similar conceptual models (Angers & Chenu, 1998; Golchin et al., 1998; Six et al., 2002a) that emphasize the role of fresh organic matter acting as nuclei for aggregate formation and stabilization because it locally stimulates microbial activity and thus microbial binding and stabilizing mechanisms. Hence, when a fresh organic source is available, microbial activity becomes the main responsible for the three phases of the aggregate dynamics: formation, stabilization and destruction. Thus, formation and stabilization increase after the addition of organic matter. These two processes can be simultaneous, working in initially disaggregated soil focuses in aggregate formation (CaesarTonThat & Cochran, 2000; Bossuyt et al., 2001) and taking pre-existing aggregates concentrates on stabilization (Cosentino *et al.*, 2006a). Dealing with pre-existing aggregates or with soil in which the structure had been previously destroyed, impacts on the rate of microbial activity, thus complicating comparisons among models and the prediction of a real field situation.

Another problem that contributes to a certain degree of confusion is that there is not a single way to measure soil structure (so the aggregate stability) and, in addition, the term is not completely objective and express a qualitative concept that can only be evaluated using direct or indirect measurements procedures (Díaz-Zorita et al., 2002). Dozens of methods for measuring aggregate stability have been developed and the lack of a satisfactory standard methodology is a problem in this field (Le Bissonnais, 1996). As an integrative soil property that depends on several mechanisms, aggregate stability should not be evaluated with a single test but by assessing aggregate stability to different stresses.

Ideally, we should know the relative role of each aggregation agent and its dynamics to obtain mechanistic models that allow predicting aggregation in different conditions. Thus, for instance, we could predict the consequences of different qualities and quantities of C inputs in soils submitted at various temperatures and water contents on soil

aggregate stability in the short-term. Therefore, longer term predictions in aggregation will become reachable. However, the attempts in finding a hierarchy of aggregation agents were not always successful, since many variables have seldom been compared in one study and various microbially-mediated aggregation mechanisms are simultaneously at play and may be additive or create synergisms, e. g. increasing the cohesion, increasing hydrophobicity, modifying the pore system, etc. (Chenu & Cosentino, 2007).

Another way to reach the objective of predicting aggregate dynamics is through an integrative approach. Since most microorganisms having an effect on soil structure are C-source dependent, establishing a quantitative direct relationship between soil structure and carbon inputs is potentially promising. Nevertheless, this implies a big effort in measuring numerous particular combinations of plant residues (decomposition and amounts) and soil conditions. Abiven (2004) related the initial biochemical characteristics of several plant residues (from cereals to vegetables) to the shape parameters for changes in aggregate stability with time. Modulator functions (temperature, C/N, humidity) were used to apply this model in field conditions. Since soil respiration is an indicator of general soil microbial activity and is strongly associated with the rates and decomposability of C incorporated, it can be considered also an integrative variable to predict short-term aggregation. In this way, De Gryze et al. (2005), tested the performance of different mathematical models in predicting aggregate formation after the addition of several wheat residues rates for three different soils, taken CO_2 respiration as the unique driver. Their models performed quite well, but unfortunately they were aimed to predict the observed increase in aggregation at a very short time (21 days) been not able to predict the decrease in aggregation that occurs later on.

We aimed to establish predictive relationships between the addition of C to soil as plant residues and the aggregate stability to three breakdown mechanisms and to develop a predictive model on a mechanistic basis. Detailed objectives were:

- to analyze the quantitative relationship between the amount of plant residues added to soil and aggregate stability, and identify, if any, a threshold over which aggregate stability does not increase with residue addition;
- to analyze the relationship between biological variables related to aggregating agents, and aggregate stability over time;

- to propose and test a new model for predicting the temporal evolution of aggregate stability after OM addition to soil based on the coupling of a decomposition model with an aggregate stability function derived from (ii).

We developed an 8-month incubation experiment and we measured the main biological variables involved in aggregation and the aggregate stability of natural pre-existing aggregates after the addition of a range of maize residues. We used three aggregate stability tests, each relating to a different breakdown mechanism, to test whether the analyzed relationships were dependent on the breakdown mechanism.

2 Materials and methods

2.1 Study area, site description and sampling

The soil used in this experiment was a silt loam Luvisol sampled at the experimental site of the Institut National de la Recherche Agronomique – INRA – (48°48'29"N, 2°04'58"E), at Versailles, France. The climate is temperate with an annual rainfall of 639 mm yr^{-1} (1928-2003) and 10.5°C annual mean temperature. The texture was of 167 g kg^{-1} clay, 562 g kg^{-1} silt and 271 g kg^{-1} sand, with a total carbon content of 9.0 g kg^{-1}, Ct/Nt: 9.3 and pH (H_2O) of 7.0. It had been cultivated for more than 50 years with conventional tillage (mouldboard plow at 0-30cm) with a rotation based on wheat (Triticum aestivum L.), colza (Brassica napus L.) and pea (Pisum sativum L.).

We carefully sampled the soil from six points of the parcel at 0-20 cm depth (Ap horizon) with shovels to keep the natural structure of the soil as much as possible. The soil was sampled at a water content of 0.19 g H_2O g^{-1} soil. At the laboratory, the larger clods were gently crumbled by hand at field moisture along their natural fissures and sieved to obtain an adequate amount of aggregates between 3.15 and 5 mm. Great care was taken to avoid damaging the natural aggregates. After sieving, coarse organic matter (free roots and plant debris) was removed with tweezers and the soil was then stored in the dark in plastic boxes at 4°C for one month (~0.16g H_2O g^{-1} soil). To avoid any compaction the aggregates were spreaded in a 2 cm layer. The aggregates were preincubated at 20°C for twelve days before incubation to minimize the variations in microbial activity due to changes in temperature conditions.

2.2 Added organic matter

We used maize (Zea mays L.) residues from stems and leaves, dried at 40°C for one week and ground < 500 µm. It had a carbon (C) content of 429.8 g C kg^{-1}; a nitrogen (N) content of 6.29 g N kg^{-1} and a C/N of 68.3 which were measured on a Carlo Erba Instruments (NA 1500 series 2) element analyser. Straw composition was assessed on an Extractor for raw fiber determination (FIWE 6, Velp Scientifica, Italy) according to the Van Soest fractionation and Weende methodology. Residue used had 26.69 % soluble organic compounds (SOL); 31.39 % hemicelluloses (HEM); 37.7 % celluloses by Weende (CEW) and 3.42 % lignin + cutin (LIC). A biological stability index (BSI) of 0.02 was calculated (Association-française-de-normalisation, 2004) from: BSI = 2.112 – (0.02009 SOL) – (0.02378 HEM) + (0.0084 LIC) – (0.02216 CEW). This means that 2 % of the maize straw we used was potentially stable to the biodegradation.

2.3 Incubation procedure

We incubated soil aggregates in optimum conditions for biological degradation: water, nitrogen and oxygen availability were ensured. Three to five mm aggregates were incubated at 20°C ± 0.5 °C for 8 months with different concentrations of added maize straw: 0, 2.5, 5, 10, 15 and 20 g C kg^{-1} soil.

The equivalent of 160 g dry sol (105°C, 24h) was placed in glass jars and we added 0, 0.93, 1.86, 3.72, 5.58 and 7.45 g of straw to get the concentrations described above. The samples with added maize straw were mixed carefully and immediately sprayed with a solution of NO_3NH_4 to adjust the soil+straw sample C/N ratio to 10 and its water potential (Ψ) to -10 kPa simultaneously. Thus, the corresponding water contents to the different added straw concentrations were: 0.20, 0.21, 0.23, 0.25, 0.27 and 0.29 (g/g). The samples with no straw addition (controls) were treated in the same way, but with deionized water. All treatments were kept at field capacity (Ψ = -10 kPa) during all the incubation. A beaker without direct contact with soil containing 20 ml of deionized water was also added to the jars to minimize desiccation. At the end of incubation the jars had lost < 1 % (g/g soil) of water content. In treatments (concentrations of added straw) designated to be sampled at the 8^{th} month, a second beaker with NaOH was also placed in the jars to trap and measure the CO_2 produced during incubation by respiration of microorganisms.

2.4 Soil analyses

The evolved C-CO_2 trapped in 30 ml NaOH (1N) was used to estimate soil respiration, daily the first week and increasing the intervals thereafter. CO_2 trapped in NaOH was evaluated by colorimetric determination with a continuous flux Skalar (Breda, Nederlands). CO_2 is volatilized by adding 1N H_2SO_4 and then injected in Na_2CO_3 buffered solution with phenolphthalein. Discoloration of phenolphthalein provoked by CO_2 concentration is measured by spectrophotometry at 550 nm.

We calculated the amount of C mineralized from the straw from the difference with the control treatment. We thereby assumed the absence of any priming effect.

At days 0, 3, 7, 14, 28, 63, 135 and 253 of incubation three replications per concentration of C added were sampled. We determined immediately in a moist subsample the microbial biomass-C by fumigation-extraction according to Vance et al. (1987). The difference between C extracted with 0.03 M K_2SO_4 from chloroform-fumigated and unfumigated soil samples were measured with the TOC-5050A Shimadzu elemental analyzer using a Kc = 0.45.

Another subsample was taken and frozen to measure within a month the ergosterol content with a technique modified from Djajakirana et al., (1996) and Gong et al., (2001) developed by Annabi (2005). Ergosterol is a biomarker of living fungi. The ergosterol was estimated with a Waters 2695 HPLC using 3.5 g of moist soil extracted with 120 ml ethanol agitated with glass beads for 30 min.

The rest of the sample was dried during at least 72 h at 40 °C in a ventilated oven. A 1 g aliquot was taken to estimate the hot-water extractable carbohydrate-C (HWEC) (Puget et al., 1999). The aliquot was suspended in 20 ml of hot deionized water (80 °C) for 24 h and the carbohydrate content of the extract was analysed by the H_2SO_4/phenol method (Dubois et al., 1956).

To assess aggregate stability, we carefully re-sieved the samples into aggregates from 3.15 to 5 mm (hereinafter referred to 3-5 mm). Almost no energy was applied and only attached aggregated > 5 mm were separated by hand. The mean size proportion of aggregates from 3-5 mm respect to the entire sample (3-5 mm/0-5 mm) was 89 % with a standard deviation of 3.9 %.

Aggregate stability and hot-water extractable polysaccharides were measured on 0, 5, 10, and 20 g C kg^{-1} soil added maize straw.

2.5 Aggregate stability tests

In non-dispersed soils there are 3 main breakdown mechanisms, slaking, microcracking or breakdown by differential swelling, and mechanical breakdown by raindrop impact (Le Bissonnais, 1996). We measured the soil aggregate stability according to Le Bissonnais (1996). The entire method is performed on 3 – 5 mm aggregates. It involves three pre-treatments with different subsamples before sieving in alcohol at 50 µm and dry sieving of the resulting fraction (> 50 µm).

Each pre-treatment attempts to separate different breakdown mechanisms: in the slow-wetting test, 6 g of aggregates are capillary rewetted with water on a tension table at a potential of – 0.3 kPa for > 60 minutes; in the fast-wetting test, 6 g of aggregates are immersed in deionized water for 10 min and in the stirring after prewetting test, the aggregates are saturated in ethanol for 30 min, then manually agitated in deionized water in an Erlenmeyer end over end for 20 times. Dry-sieving was performed by hand with a nest of six sieves (2,000, 1,000, 500, 200, 100 and 50 µm) and the mean weight diameter (MWD) was calculated as the sum of the mass fraction remaining on each sieve after sieving, multiplied by the mean aperture of the adjacent sieves.

2.6 Modelling residue decomposition with CANTIS

The CANTIS model (carbon and nitrogen transformations in soils) (Garnier et al., 2003) simulates the transformations of carbon and nitrogen including the decomposition of organic matter, mineralization, immobilization, nitrification and humification (Fig. II-1). Soil organic matter is divided into three non-living organic pools (fresh, soluble and humified organic matter) and two living pools. The microbial population is divided into an autochthonous biomass that decomposes humified organic matter and a zymogenous biomass that decomposes fresh and soluble organic matter.

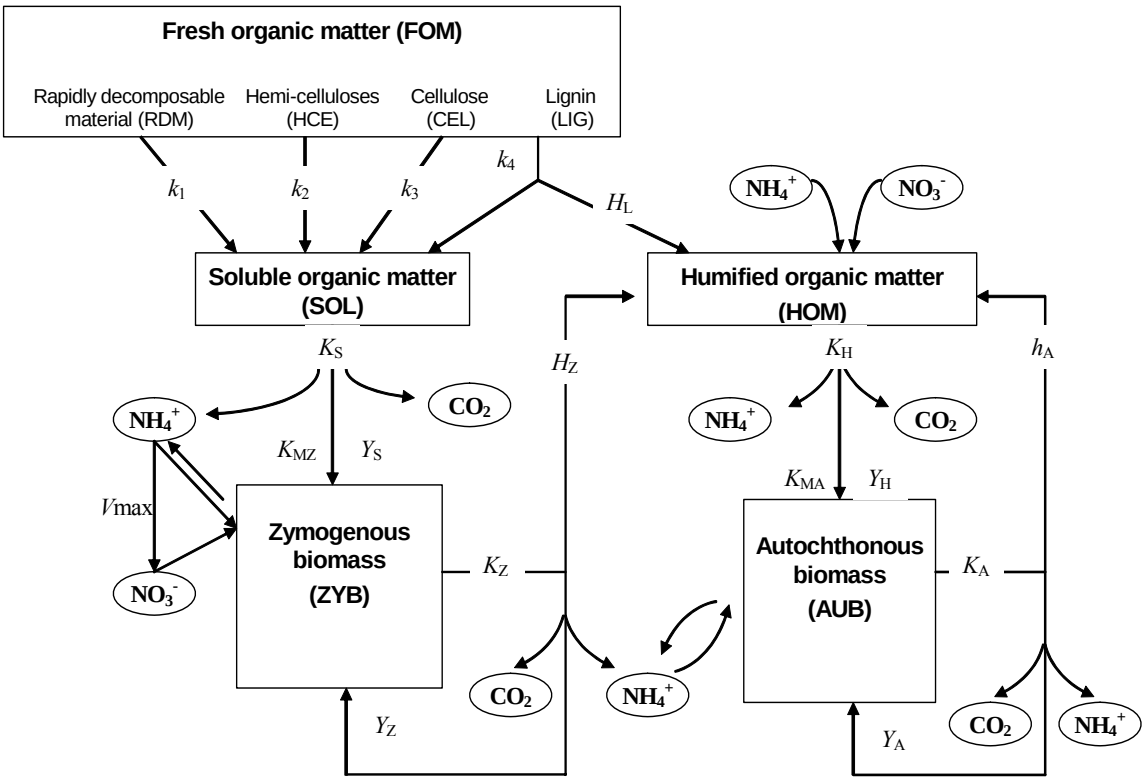

Fig. II-1. Flow diagram of the CANTIS model.

Decomposition of fresh and soluble organic matter is assumed to follow first-order kinetics as:

$$\frac{dCi}{dt} = -k_i \, C_i \, f_T f_W f_N f_B \qquad (1)$$

where C_i is the carbon content of the organic matter pool i, k_i is the decomposition rate constant of that pool, f_T is the temperature function, f_W is the moisture function, f_N is the nitrogen limitation function, and f_B is the biomass-dependent function. The first three functions have been described previously by Garnier et al. (2001). The function f_B relative to the zymogenous biomass is calculated as follows:

$$f_B = \frac{B_Z}{K_{MZ} + B_Z} \qquad (2)$$

where B_Z is the carbon content in the zymogenous biomass, and K_{MZ} is an empirical factor connected to the size of the zymogenous biomass. The f_B function enables takes into account a limitation of decomposition by microbial colonization of the substrate, which may be due either to the specificity of the substrate or to a reduced contact area between soil and substrate.

We calibrated CANTIS with the data from the control sample and the straw addition sample at 5 g C kg^{-1} soil rate. CANTIS was fed with the initial conditions of the control treatment (no added straw) in our incubation experiment: T° and water potential, C, N, CO_2 and microbial biomass-C of soil. Then we optimized 3 CANTIS parameters (h_A, k_A and k_H) (Table II-1). Thereafter, some model parameters (k_2, k_3, k_Z and h_Z) were optimised to obtain the best fit between microbial biomass-C and CO_2 simulated by CANTIS and observed in laboratory at 5 g C kg^{-1} soil added residue rate. Parameters and values of fresh organic matter pool used are listed in Table II-1. The selected k_2 value of 0.04 day^{-1} was close to that proposed by Cheshire et al. (1974)* (0.03 day^{-1}) and that Simonart & Mayaudon (1958)* (0.08 day^{-1}). The selected value for k_3 of 0.01 day^{-1} was in the same range as that suggested by Simonart & Mayaudon (1958)* (0.02 day^{-1}) and by Minderman (1968)* (0.03 day^{-1}). All others parameters values were used according to Garnier et al. (2001).

The optimized parameters at 5 g C kg^{-1} soil were then used to test the model simulating microbial biomass-C and cumulative CO_2 at all other rates of C added.

2.7 Modelling aggregate stability

Our hypothesis was that for a given soil type, aggregate stability should be a constant function of microbial agents of aggregate stability such as microbial biomass, fungal abundance, extracellular polysaccharides and general microbial activity represented by evolved CO_2. Hence, we first analyzed the quantitative relationships between these

* In C. Neel. 1996. *Modèlisation couplée du transfert et des transformations de l'azote: paramétrisation et évaluation d'un modèle en sol nu.* Ph.D., Université Pierre et Marie Curie, Paris.

Table II-1. Specifications of symbols, parameters, units, values and percentages of pool considered in CANTIS model.

Symbol	Parameter	Unit	Value	% of pool considered
k_1	Decomposition rate of rapidly decomposable material (RDM)	day^{-1}	0.25*	27
k_2	Decomposition rate of hemi-celluloses (HCE)	day^{-1}	0.04†	31
k_3	Decomposition rate of celluloses (CEL)	day^{-1}	0.01†	38
k_4	Decomposition rate of lignin (LIG)	day^{-1}	0.0022*	4
k_S	Decomposition rate of soluble compounds (SOL)	day^{-1}	1.49*	
k_Z	Decomposition rate of zymogenous µbiomass (ZYB)	day^{-1}	0.323†	
k_A	Decomposition rate of autochthonous µbiomass (AUB)	day^{-1}	0.011†	
k_H	Decomposition rate of humified organic matter (HOM)	day^{-1}	$8.65\ 10^{-5}$†	
Y_S	C assimilation yield of the soluble pool by ZYB	$g\ g^{-1}$	0.62*	
Y_Z	C assimilation yield by ZYB	$g\ g^{-1}$	0.62*	
Y_A	C assimilation yield by AUB	$g\ g^{-1}$	0.62*	
Y_H	C assimilation yield of the humified pool by AUB	$g\ g^{-1}$	0.62*	
h_Z	Humification coefficient by ZYB		0.6†	
h_A	Humification coefficient by AUB		0.01†	
h_L	Humification coefficient of lignin		1.0	
V_{max}	Maximal nitrification rate	$mg\ N\ kg^{-1}\ day^{-1}$	5.0*	
K_N	Affinity constant of NH_4^+ for nitrifying bacteria	$mg\ N\ kg^{-1}$	3.5*	
β	Langmuir coefficient for immobilization		0.045*	
α_Z	Proportion of direct assimilation of N by ZYB		1.0*	
α_A	Proportion of direct assimilation of N by AUB		0*	
K_{MZ}	Michaëlis-Menten constant for decomposition of SOL	$mg\ kg^{-1}$	0*	
K_{MA}	Michaëlis-Menten constant for decomposition of HOM	$mg\ kg^{-1}$	0*	

* (Garnier et al., 2003)

† estimated with the incubation experiment

k_2 et k_3 were adjusted on data from 5g C-straw kg^{-1} soil incubation.

variables and MWDs over time for the different doses of straw by regression analysis. Then, for the slow wetting test only, we performed multiple regression analysis to express the MDW as a function of initial MWD of the control sample and of biological variables. Among possible linear regressions able to predict MWD to slow wetting, we selected the better one based on variables that could be generated by the decomposition model, CANTIS (CO_2 evolved, cumulative CO_2, microbial biomass C). We finally simulated the evolution of MWD at different rates of straw addition coupling CANTIS and the multiple regressions and compared it to experimental data.

2.8 Statistical analysis

Soil data is given as arithmetic means and standard errors. Single and multiple regressions were obtained using SigmaStat ver 3.11 (Systat Software, Inc.). When analysis of variance was performed the means were separated by the Tukey test.

We statistically evaluated the multiple linear regression model with determination coefficients (R^2) and model's efficiency coefficient (Ef) from Smith et al. (1997).

3 Results

3.1 Microbial activity, biomass and by-products

The respiration rates were strongly affected by the addition of C over all the incubation time. Samples showed an increase in respiration rate (Fig. II-2), which was directly related to the amount of straw added. In samples with no addition of C respiration varied very little during incubation. Instead, treatments with added C showed a peak of respiration rate at < 48 h of incubation. For the 2.5 and 5 g C kg^{-1} soil added, the peaks of respiration rate were at < 24 h of incubation, showing a delay of ~ 24 h in the maximum values of respiration rate increasing C addition. At 48 h of incubation the highest dose of C added had a respiration rate ~ 400 times higher than the control and, at the end of incubation (day 253) it was only ~10 times higher. As a result, cumulative C respiration also increased with increasing amount of straw added (Fig. II-3 a). Significant linear correlations ($P < 0.001$) were found between C of straw added and cumulative C

respiration for all measured incubation dates. The correlation (R^2) varied from 0.929 to 1 from day 2 to 253 of incubation respectively. Thus, the proportions of cumulative C respiration to the added C were very similar among rates of added C over the incubation time (Fig. II-3 b) and ca. 50 % at the end of the incubation. The straw decomposition was proportional to the amount of maize straw added.

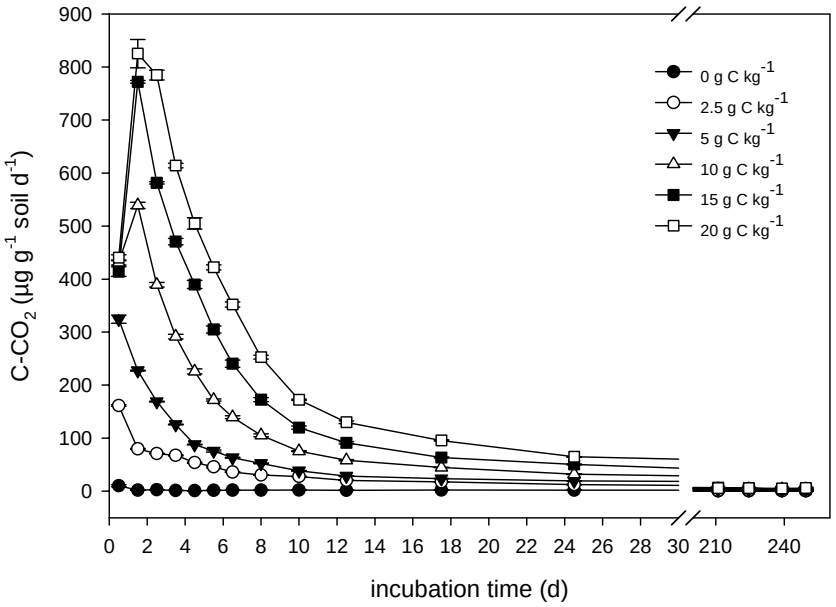

Fig. II-2. Soil respiration rate during 253 days of incubations after the addition of 0 to 20 g C kg^{-1} soil. Error bars represent the standard error of the means.

The addition of straw strongly increased ($P < 0.01$) the microbial biomass-C at all sampling dates (Fig. II-4). It was a net peak at day 3 of incubation for the highest amounts of straw added. With the addition of 2.5 g C kg^{-1} soil the peak was less evident and no significant difference ($P < 0.01$) was found between day 3 and 7 of incubation. No significant peak was found in the control treatment and the microbial biomass diminished slightly after 28 days of incubation. At day 3, 20 g of C added kg^{-1} soil induced ~ 12 times more the microbial biomass-C than in the control. At the end of incubation this ratio was only of 2.3.

Table II-2. Incubation dates of maximum values for microbial biomass-C, ergosterol content, respiration rate and hot-water extractable carbohydrate-C for each rate of addition of maize straw.

Added C (g C kg^{-1} soil)	Microbial biomass-C	Ergosterol	Respiration rate	HWEC
	days			
2.5	3-7	14	1	ND
5	3	14	1	3
10	3	14	2	3
15	3	28	2	ND
20	3	14	2	3

ND: not determined

Table II-3. Ergosterol to microbial biomass-C ratio (%) at two incubation dates after the addition of different rates of maize straw added (from 0 to 20 g C kg^{-1} soil).

Incubation time	Added C (g C kg^{-1} soil)					
	0	2.5	5	10	15	20
28 d	0.44±0.03	1.02±0.01	2.04±0.06	3.26±0.16	3.92±0.26	3.74±0.14
135 d	0.40±0.01	0.78±0.06	1.16±0.05	2.03±0.08	1.65±0.06	1.64±0.13

Mean values ± standard errors of the means.

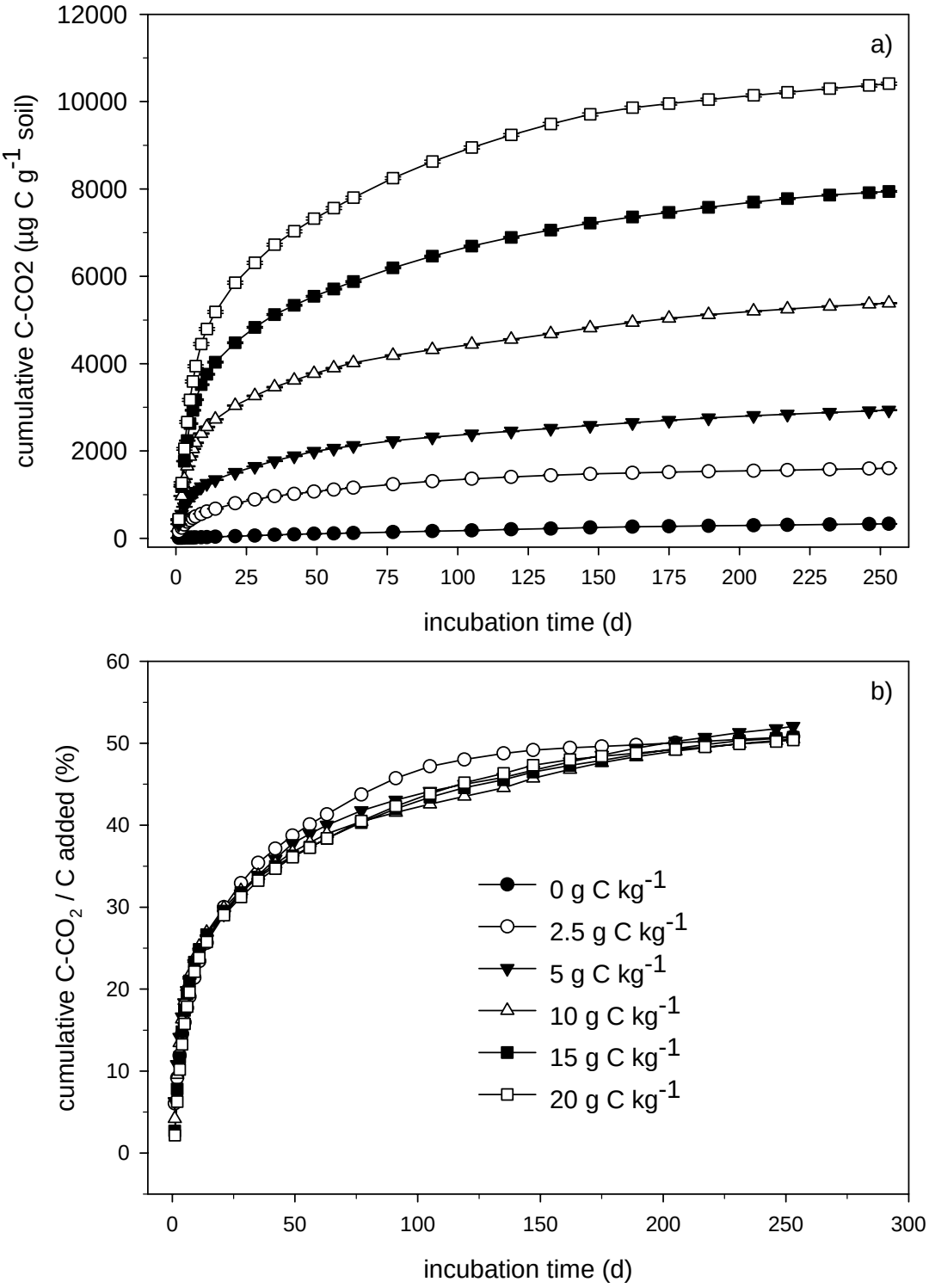

Fig. II-3. a) Cumulative soil respiration evolution and b) straw cumulative mineralization evolution (% C addition) after the addition of 0 to 20 g C kg^{-1} soil. In a) error bars represent the standard error of the means (mostly hidden by symbols).

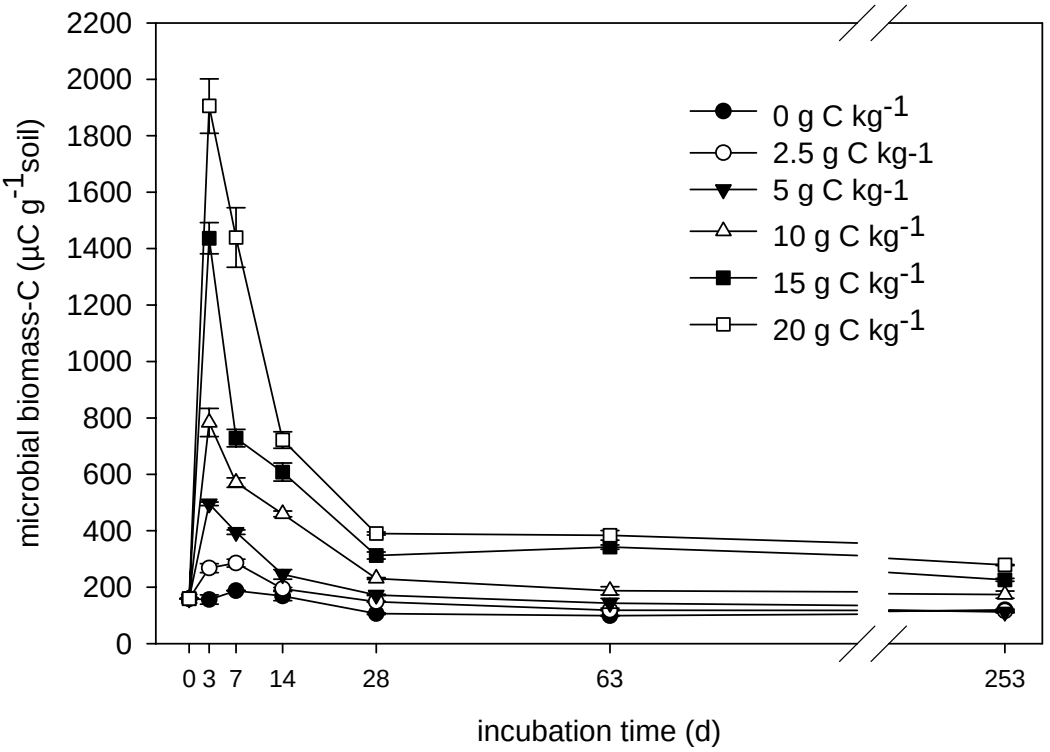

Fig. II-4. Microbial biomass carbon evolution after different rates of addition of maize straw to soil. Error bars represent the standard error of the means.

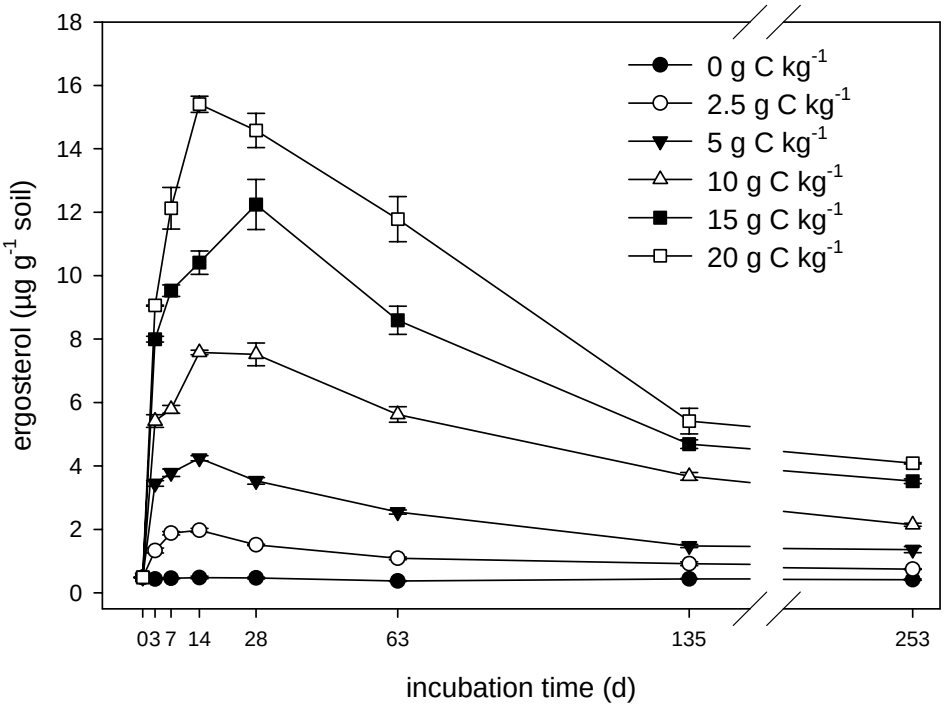

Fig. II-5. Ergosterol content evolution after the addition of maize straw at different rates. Error bars represent the standard error of the means.

Fungi content (estimated by ergosterol) also increased linearly ($R^2 = 0.736$) with added C at all incubation dates (Figs. II-5 and II-6 b). The treatment with no addition of straw did not vary over the entire incubation with a mean value of 0.44 µg ergosterol g^{-1} soil. Maximum values of ergosterol were found at 14-28 days of incubation, thus later than microbial biomass-C (Table II-2). Maximum increases of ergosterol were 32 times at day 14th and ~10 times at the end of incubation. Relative increases of ergosterol were more persistent than that of microbial biomass-C as is showed in Fig. II-6 (a and b).

The addition of maize straw significantly ($P < 0.01$) and persistently modified the composition of the microflora, the relative percentage of fungi being increased with rates of addition (Table II-3).

The production of exudates by microorganisms also reflected their stimulated activity. Adding maize straw increased HWEC 5.5 times on day 3 and 2.6 times on day 253 for the highest rate of addition (Fig. II-7). The amount of HWEC varied slightly with no addition of straw, averaging 168 µg g^{-1} soil. The peaks of HWEC production with the addition of straw were observed at day 3.

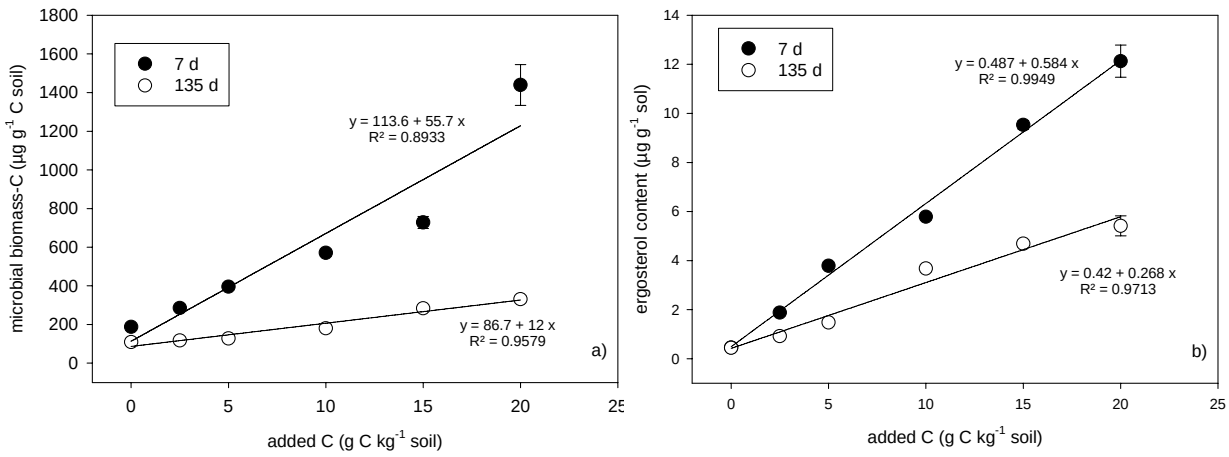

Fig. II-6. Microbial biomass carbon (a) and ergosterol content (b) as a function of different maize straw rates of addition the 7th and 135th days of incubation. Error bars represent the standard error of the means.

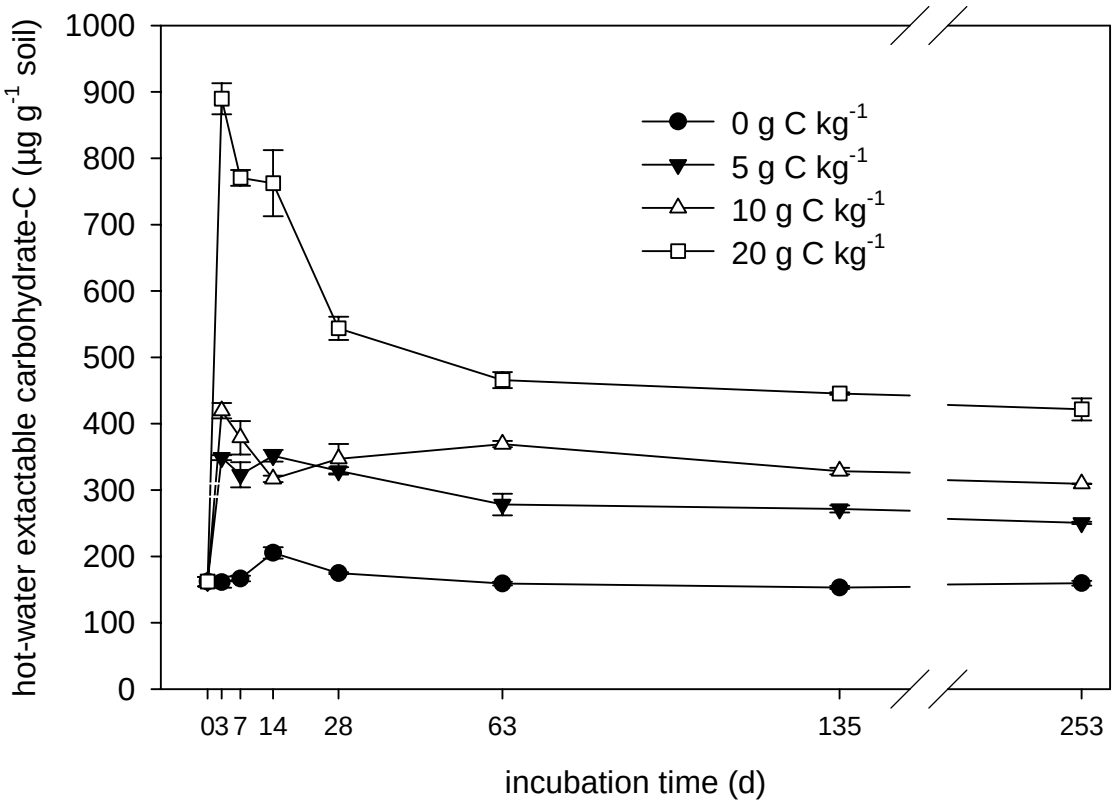

Fig. II-7. Hot-water extractable carbohydrate-C evolution during incubation after maize straw addition. Error bars represent the standard error of the means.

3.2 Aggregate stability

The control soil sample showed a very small MWD at the start of incubation, describing its instability. We found MWDs of 0.44 mm after the slow wetting test, 0.23 mm after the fast wetting test and 0.65 mm after the stirring following the prewetting test (Fig. II-8). The MWDs of the control samples remained almost constant over the incubation.

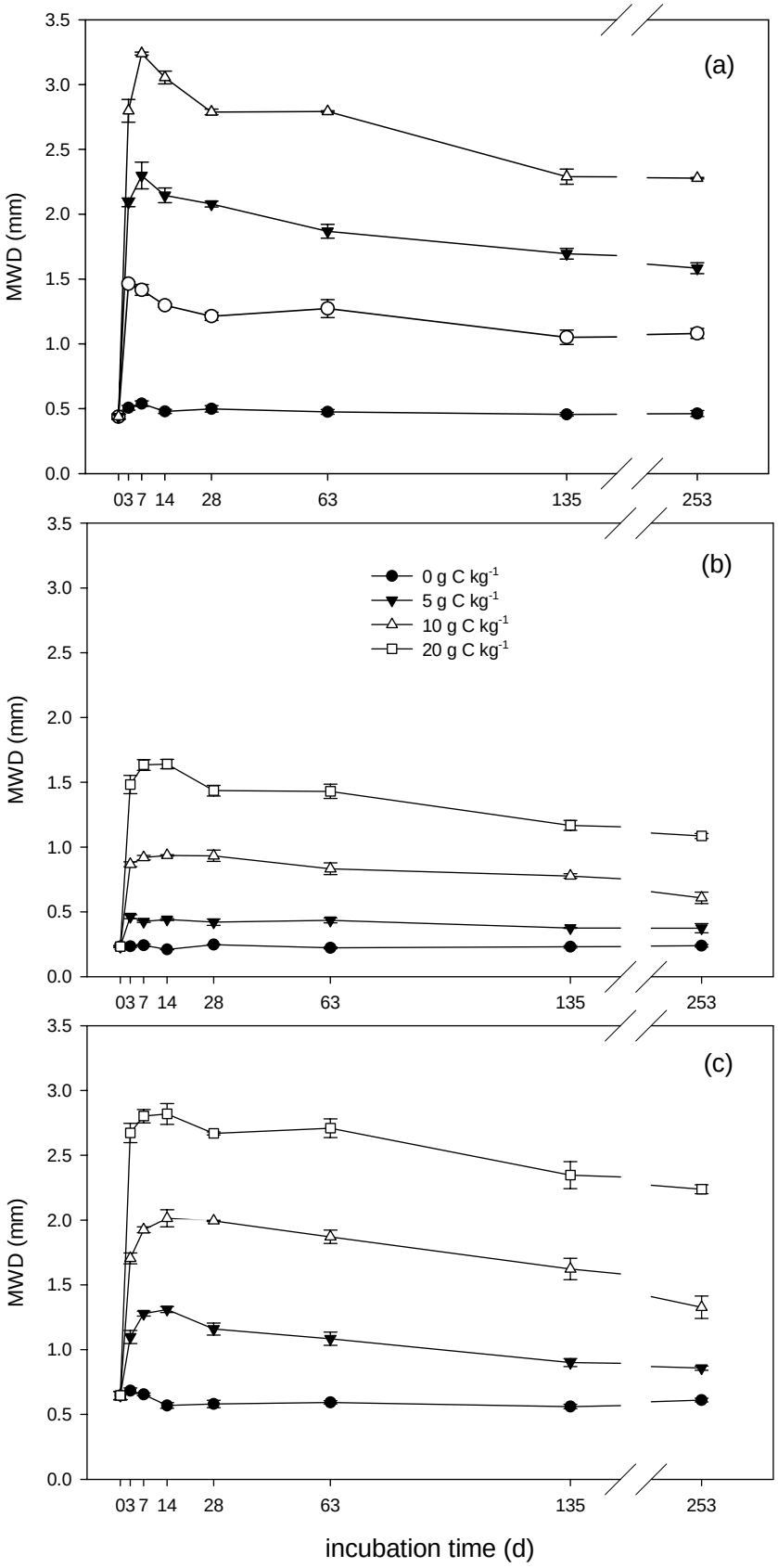

Fig. II-8. Aggregate stability evolution during incubation after maize straw additions for (a) slow wetting test, (b) fast wetting test and (c) stirring after prewetting test. Error bars represent the standard error of the means.

Table II-4. a) R^2, slopes and intercepts of linear regressions between MWDs and rates of C addition at days 7 and 135 of incubation; b) average for all dates for slow wetting test vs residual C**

a)	R^2		Slopes		Intercepts	
Days of incubation	7	135	7	135	7	135
Slow wetting	0.965	0.955	0.134±0.008*	0.091±0.006*	0.702±0.092*	0.575±0.072*
Fast wetting	0.981	0.969	0.072±0.003*	0.049±0.003*	0.173±0.036*	0.211±0.032*
Stirring	0.987	0.961	0.107±0.004*	0.092±0.006*	0.729±0.045*	0.552±0.067*
b)						
Slow wetting	0.93		0.151		- 0.65	

*Values are presented with standard errors and $P < 0.001$.

** Residual $C_{ti} = C_{t0}$ initial + C_{t0} added – C_{ti} cumulative respiration

Table II-5. CANTIS efficiency coefficient (E_f) and R^2 simulating cumulative CO_2 and microbial biomass-C for the rates of C addition used.

Rates of added C	Cumulative CO_2		Microbial biomass-C	
(g C kg^{-1} soil)	E_f	R^2	E_f	R^2
All	0.9959	0.9963	0.9388	0.945
2.5	0.9943	0.9970	0.7445	0.9104
5	0.9873	0.9926	0.9235	0.9964
10	0.9833	0.9906	0.7612	0.9665
15	0.9895	0.9915	0.8219	0.8763
20	0.9940	0.9943	0.9835	0.9905

The addition of straw had a large and significant ($P < 0.001$) impact on aggregate stability at all doses of C applied, dates of incubation and tests of aggregate stability measured. The dynamics of the three aggregate stability tests was similar and characterized by an early peak at 7-14 days of incubation and a relative plateau from day 135 to 253. The smallest values of MWD were obtained at the fast wetting test (from 0.2 to 1.6 mm) and the largest with the slow wetting test (from 0.47 to 3.24 mm) because of the different intensities and nature of stresses applied. The aggregate stability was the variable that showed the most permanent effect of the addition of straw. As an example, in the slow wetting test the highest dose of C added increased the MWD 6 times in relation to the control at day 7, whereas at day 135 it was 5 times. At each date, aggregate stability was proportional to initially added C but slopes decreased with time (Fig II-9 a, b and c). The slopes of regressions between added C and aggregate stability diminished from slow wetting > stirring > fast wetting tests and from day 7 to 135 of incubation (Table II-4, a).

We plotted the relationship between residual C (control soil C + added maize C – evolved C-CO_2) and the slow wetting test (Fig. II-9 d) and we found that, after 7 days of incubation, aggregate stability was proportional to residual C in the system whatever time and rate of addition considered (Table II-4 b).

3.3 Relationships between aggregate stability and microbial variables

Correlations between all biological variables and the results of three tests of aggregate stability including all rates of residues addition at all sampling dates indicated that each variable was significantly related to aggregate stability (Table II-6). The microbial biomass-C and the respiration rate had the lower correlation coefficients, likely because they peaked and decreased earlier than aggregate stability (Table II-2). On the contrary ergosterol, HWEC, and even cumulative respiration described better the aggregate stability because of their importance after 28 days of incubation. Finally, added C and residual C exhibited the best correlation coefficients with MWDs (Table II-4).

To test whether aggregating agents would be different at different dates of the incubation or for different doses of C, we performed the same analysis by date (Table II-7) and by dose (Table II-8). It showed that each biological variable was significantly correlated with aggregate stability with fairly similar correlation coefficients (r), at all

incubation times and at the different doses, not allowing us to determine a hierarchy among variables with either date or dose.

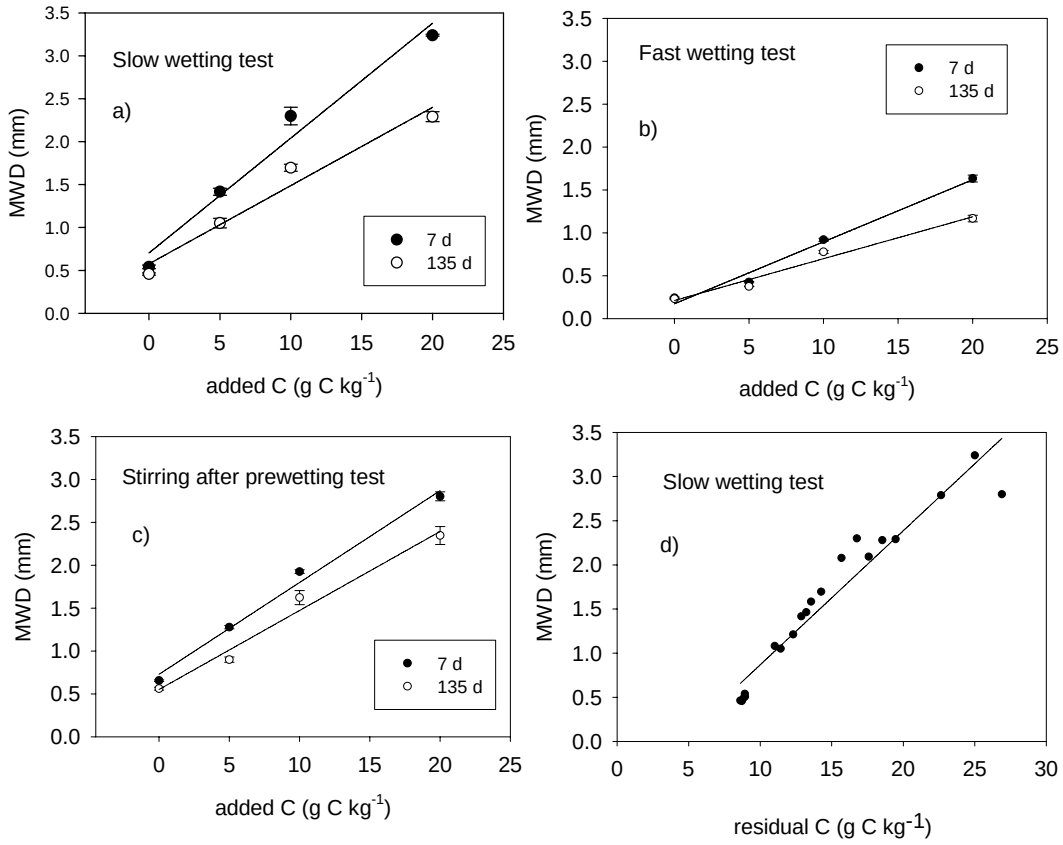

Fig. II-9. Mean weight diameters for (a) slow wetting test, (b) fast wetting test and (c) stirring after prewetting test for four different residue additions at 7 and 135 days of incubation and (d) slow wetting test vs. soil residual C at 3, 7, 28, 135 and 253 days of incubation ($R^2 = 0.93$). Error bars represent the standard error of the means.

As the effects of the different microbial determinants on aggregate stability which act simultaneously are independent of doses of residues applied, and the correlation coefficients by incubation date are better than those by added C rate, the aggregate stability could be well predicted as a function of a biological agent at different incubation dates. Based on these results, we expressed aggregate stability to slow wetting test as a function of the MWD of the control sample at time 0, and of biological variables. When aggregate stability was simulated from our experimental data, the best subset of variables to predict

MWDslow with a multiple linear regression model was microbial biomass-C, ergosterol and cumulative CO_2.

$$MWD_{slow} = 0{,}450 + (0.000703 * \text{microbial biomass-C}) + (0.000120 * \text{cumulative } CO_2) + (0.109 * \text{ergosterol}). \quad (3)$$

Considering 5, 10 and 20 g C kg^{-1} added the correlation coefficient (r) was 0.977. However, taking just microbial biomass-C and cumulative CO_2 the coefficient only diminished to 0.923 (r).

Based on these results, we selected along the different variables those that CANTIS could generate and we expressed aggregate stability to slow wetting test as a function of the MWD of the control sample at time 0, microbial biomass-C and cumulative CO_2:

$$MWD_{slow\ ti} = MWD_{slow\ t0} + (\gamma_1 + (\gamma_2\ \Delta\text{microbial biomassC}_{\ ti}) + (\gamma_3\ cumCO_{2\ ti})) \quad (4)$$

where MWDslow ti is the mean weight diameter to slow wetting at the time ti after an addition of residue, MWDslow t0 is the mean weight diameter to slow wetting at time 0 (at the addition of residue), Δmicrobial biomassC (µg C g^{-1} soil) and cumCO_2 (µg C g^{-1} soil) are the increase of microbial biomass carbon and the cumulative CO_2 respired. γ_1, γ_2 and γ_3 were derived based on biological variables and aggregate stability experimental data at all dates and all doses. The coefficients from model (4) were γ_1: 0.2783; γ_2: 0.00197 and γ_3: 0.0001489. Coefficient γ_1 was added to better fit the simulated values to the observed ones in all the kinetics of aggregate stability and for all doses of added C considered. The multiple linear regression model adjusted quite well the MWDslow data ($R^2 = 0.90$), all independent variables appear to contribute to predicting aggregate stability ($P < 0.05$). The goodness of fit was significant for each independent variable ($P < 0.001$).

3.4 Simulation of straw decomposition with CANTIS

After calibrating CANTIS at 0 and 5 g C kg^{-1} maize added, we simulated CO_2 evolution and microbial biomass-C with CANTIS for all other rates of C added (Fig. II-

10). Simulations agreed very well with our experimental data as evaluated by the model's efficiency coefficient (Ef) (Smith et al., 1997) and the determination coefficient (R^2) (Table II-5). CANTIS simulated well not only the rate of C input that we used to optimise some parameters (5 g C kg^{-1}) but all doses tested. Cumulative CO_2 was slightly better predicted from CANTIS than microbial biomass-C (coefficients were always > 0.9 for cum CO_2). The model tended however to overestimate the initial peak of microbial biomass-C at low rates of C input (Fig. II-10).

3.5 Aggregate stability model

We used CANTIS model outputs and the established equation (4) to predict aggregate stability to slow wetting, over time after adding 5, 10 and 20 g C-straw kg^{-1} soil (Fig. II-11).

This mechanistic 2-parameter model described the complete dynamics of aggregate stability after residue incorporation (the initial and fast increase and its latter decrease) in the short term. The model's efficiency coefficient (Ef) predicting MWDslow for three rates of addition of maize was 0.70, which indicates that the model simulated satisfactorily the experiment. When Ef was calculated independently for each rate of C input, we found 0.71, 0.53 and 0.39 for 5, 10 and 20 g C kg^{-1} soil added, showing that the largest the rate of C added to soil the poorest the prediction of MWD. The model could simulate rather well the initial peak and the final phase of MWD evolution (from 135 to 253 days) at 5 and 10 g C kg^{-1} added. However, the decrease that occurs immediately after the peak was not well predicted by the model. With the highest rate of C added, the model only estimated reasonably well the values from 135 to 253 days.

Table II-6. Correlation coefficients (r) between biological variables and aggregate stability (MWD). All dates and all C inputs.

	Microbial biomass-C	Ergosterol	HWEC	Respiration rate	Cumulative CO_2	C added	Residual C
Slow wetting	0.669	0.919	0.893	0.525	0.794	0.959	0.969
Fast wetting	0.684	0.929	0.905	0.528	0.815	0.967	0.977
Stirring	0.637	0.921	0.858	0.487	0.790	0.971	0.967

all significant ($P < 0.01$)

Table II-7. Correlation coefficients between aggregate stability and biological variables by date.

Test	Incubation time (days)	HWEC	Ergosterol	µbiomass-C	Respiration rate	Cumulative CO_2
Slow wetting	3	0.921	0.988	0.915	0.959	0.994
	7	0.967	0.980	0.942	0.971	0.996
	14	0.867	0.981	0.972	0.971	0.988
	28	0.936	0.975	0.959	0.971	0.981
	63	0.989	0.950	0.925	0.980	0.989
	135	0.982	0.969	0.888	0.959	0.974
	253	0.992	0.984	0.874	0.910	0.988
Fast wetting	3	0.971	0.977	0.976	0.995	0.971
	7	0.975	0.977	0.978	0.991	0.988
	14	0.913	0.993	0.992	0.992	0.993
	28	0.932	0.977	0.973	0.972	0.983
	63	0.953	0.969	0.961	0.994	0.991
	135	0.961	0.991	0.954	0.980	0.979
	253	0.977	0.990	0.958	0.866	0.989
stirring after prewetting	3	0.968	0.980	0.970	0.993	0.977
	7	0.969	0.980	0.947	0.980	0.999
	14	0.854	0.976	0.966	0.970	0.986
	28	0.931	0.978	0.964	0.973	0.983
	63	0.971	0.966	0.938	0.987	0.990
	135	0.960	0.964	0.917	0.976	0.979
	253	0.972	0.989	0.961	0.826	0.990

all significant ($P < 0.001$)

Table II-8. Correlation coefficients between aggregate stability tests and biological variables by added C rate.

Test	Added C (g C kg^{-1} soil)	HWEC	Ergosterol	µbiomass-C	Respiration rate	Cumulative CO_2
slow	5	0.9014	0.8168	0.5584	0.5673	0.2549
wetting	10	0.8772	0.8756	0.5249	0.3972	0.4321
	20	0.8207	0.8343	0.5102	0.3794	0.3939
fast	5	0.8501	0.7812	0.4605	0.4904	0.2879
wetting	10	0.859	0.9012	0.443	0.3177	0.4584
	20	0.8479	0.8568	0.5335	0.405	0.3423
stirring	5	0.852	0.9406	0.4869	0.3627	0.026
after	10	0.7864	0.924	0.3389	0.2	0.4806
prewetting	20	0.7737	0.8066	0.454	0.3489	0.4644

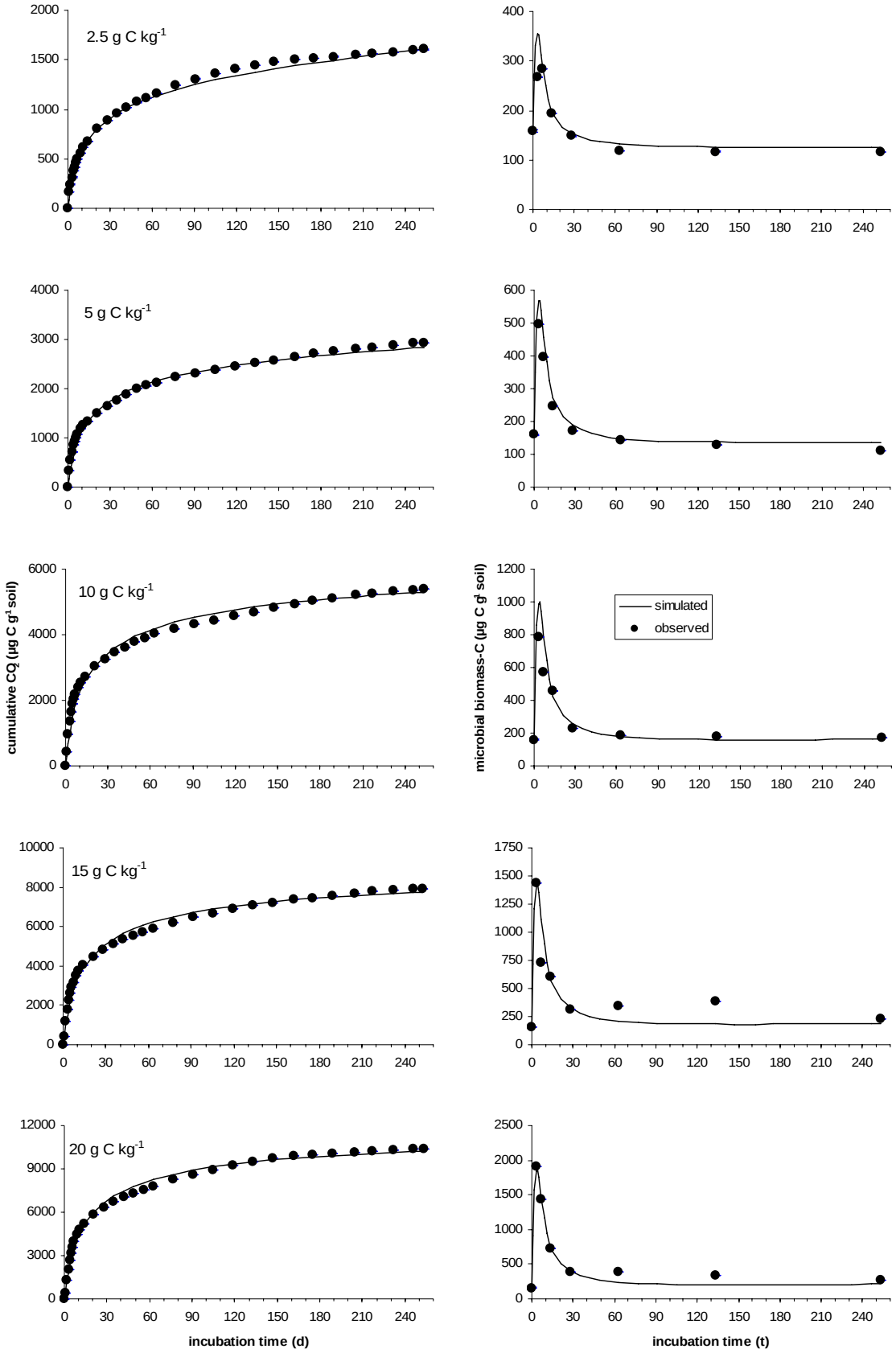

Fig. II-10. Observed and CANTIS simulated values of cumulative CO_2 and microbial biomass-C with 5 rates of C added. Note the different y-scales.

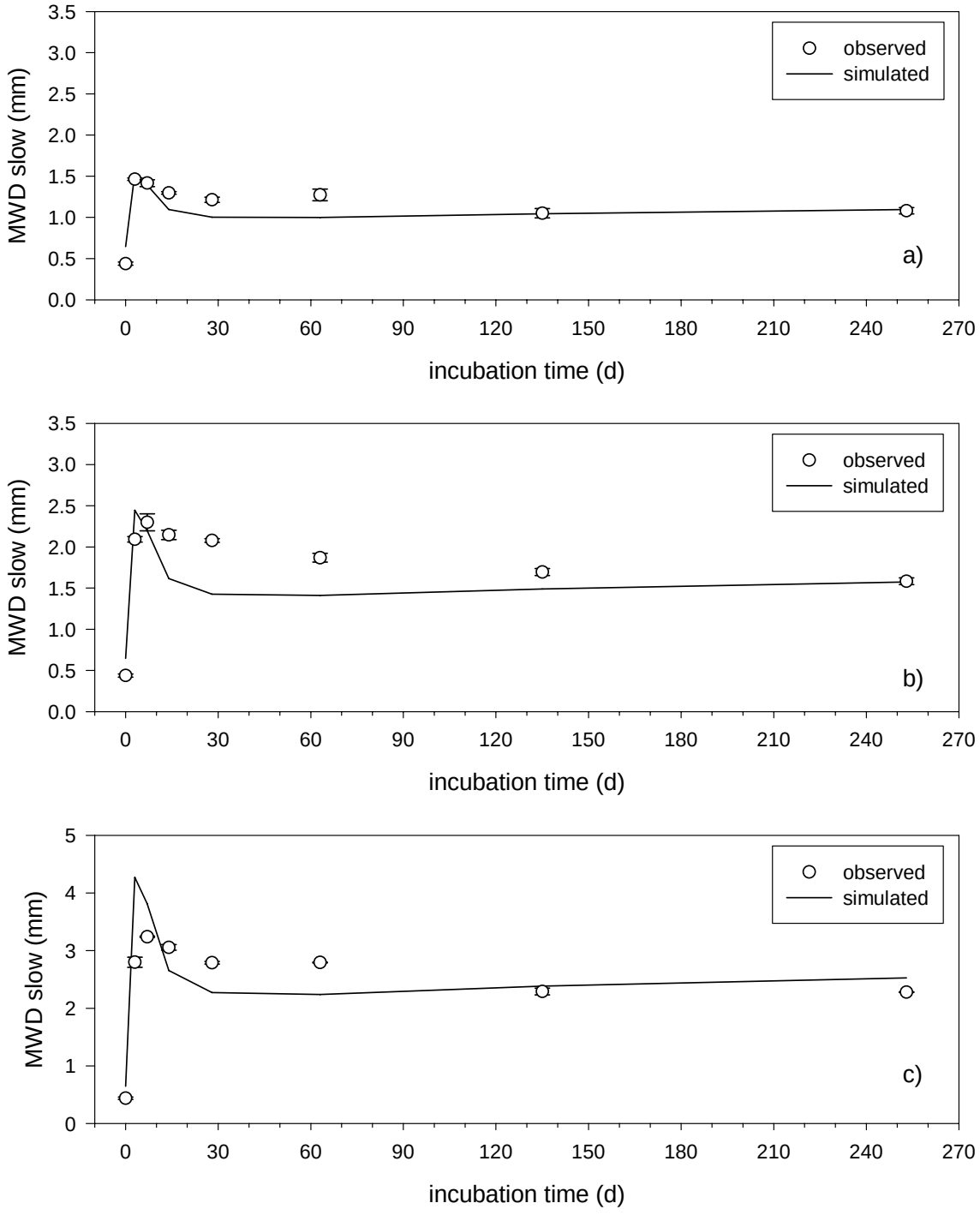

Fig. II-11. Observed and simulated aggregate stability evolution after the addition of a) 5; b) 10 and c) 20 g C kg^{-1} soil for the slow wetting test. Fitted values were obtained with an additive linear model from microbial biomass carbon and cumulative CO_2. Standard errors of means are represented with bars in observed values. Note the different y-axis scale in c).

4 Discussion

4.1 Impact of C inputs on microorganisms

It is well recognized that the quality and quantity of available substrates are major factors influencing soil microbial biomass and activity. The manipulation of native microorganisms via different C-to-N ratio inputs to alter soil physical processes was done in field and laboratory experiments (Roberson et al., 1995; Preston et al., 1999). Meanwhile, quantitative relationships between the rate of addition of substrates to soil and the corresponding response in terms of microbial abundance, activities or by-products production are scarce.

In our experiment, all microbial variables measured were strongly affected by the addition of residues over all the incubation time and they increased linearly with C inputs. Respiration rates were rapidly affected (< 48 h) and they correlated well with the C inputs during the duration of incubation. However, a more integrative variable as the cumulative respiration was better correlated with the doses of residue at the end of incubation ($R^2 = 1$ at day 253) than respiration rates. Microbial biomass-C also increased linearly with the rate of substrate entry to soil (Fig. II-6 a) similarly to its respiration rate.

HWEC is frequently used as a surrogate for extracellular polysaccharides (Haynes & Francis, 1993). The production of microbial extracellular polysaccharides was correlated with the addition of C to soil ($R^2 = 0.758$). At the beginning of incubation the peaks of microbial biomass-C and HWEC were concomitants (Table II-2) suggesting that both bacteria and fungi produced extracellular polysaccharides. However, the decline in HWEC concentrations was less rapid than that of microbial biomass C, suggesting a higher residence time of the polysaccharides (Fig. II-7). The ratio of HWEC to microbial biomass-C was of 0.5 at their peak at 3 days and it stabilized at 1.2 to 2.3 thereafter.

We quantified fungi, via the ergosterol biomarker because these organisms are often better related to soil aggregation than bacteria or total microbial biomass (Beare *et al.*, 1997; Abiven *et al.*, 2006). Fungi also increased proportionally to the C added (Fig. II-6 b) but later in the incubation than the other biological variables (Table II-2). Apparently, bacteria to fungi is a common succession of microorganisms during residue decomposition

because of the decreasing proportion of easily assimilated substrate that occurs during decomposition (Griffiths et al., 1999).

Ergosterol content better discriminated the substrate-loading rate at the end of incubation (253 d) than did the microbial biomass-C: ergosterol content was 10 times that of the control for the highest C application rate, instead of 2.4 times for the microbial biomass-C. The proportion of fungi to total microbial biomass significantly increased ($P < 0.01$) at high substrate loading rates (Table II-3) showing a change in the community structure. However, the real significance of this enhanced proportion (from 0.4 to 3.7 % at 28 d) is difficult to evaluate since ergosterol is a biomarker of fungi difficult to convert to fungal biomass, because ergosterol abundance depends on fungal species and physiological state (Bermingham et al., 1995). Fungal enrichment at high C loading rates was also described by Griffith et al. (1999).

In our experiment, the activity and growth of the soil microbial biomass was limited by the availability of substrate, i.e. carbon. All biological variables responded linearly to the addition of straw and no plateau was attained. Hence, C inputs remained the limiting factor, which can be related to the optimal incubation conditions, i.e. T°, available water and O_2, provided by our incubation set-up. As a result, the microbes adapted their activity and size to colonize and decompose the same proportion of residue added (Fig. II-3 b), confirming that entry of substrate into the soil is a key factor which governs the size and activity of the microbial biomass and so its capacity to decompose an available substrate.

4.2 Relation of aggregate stability to C inputs

It is generally accepted that there are three phases in the life cycle of an aggregate, i.e., formation from non-aggregated material, stabilisation and destruction (Tisdall & Oades, 1982). These processes are difficult to separate and they likely occur simultaneously (Oades, 1993). Working with “natural” pre-existing 3-5 mm aggregates we focused on their stabilization by added organic matter, and we evaluated the capacity of aggregates to overcome external stresses in terms of a final measured aggregate diameter. The proof that we are only assessing aggregate stabilisation is that the aggregate stability of control samples (no addition of C) did not change during the incubation. Samples were not air-dried and T° and water potential were kept constant during the 8-month incubation.

Thus, the control treatment showed a steady state with a very low aggregate stability that is common in a silty soil with low organic matter content (Le Bissonnais & Arrouays, 1997).

Our experimental set up has advantages not commonly found in the literature. First, we aimed to describe the whole kinetics of aggregate stability after the addition of an organic source, so we incubated aggregates for 253 days whereas few papers performed similarly long incubations. Second, we applied a wide range of C additions, from 0 to 20 g C kg^{-1} soil. If we consider 10 cm depth residue incorporation in a cultivated soil with a bulk density of 1.3 Mg m^{-3}, the C additions correspond to a range from 0 to 26 Mg C ha^{-1} and the highest rate corresponds to ~ 4-5 times a current agronomic rate of maize residue input. Nevertheless, several circumstances may lead to a localized high concentration of C in cultivated soils. For example, when a residue is incorporated by tillage its distribution is very heterogeneous leading to local zones with a high concentration of substrates (hot spots). Direct drilling leaves organic residues on the soil surface creating very high C contents at the mulch-soil interface. In horticulture, high C inputs are not uncommon. Besides, our goal in using a wide range of C inputs was to evaluate the potential response of soil aggregate stability to infer quantitative relationships. The carbon contents and levels of aggregate stability attained with the higher dose of straw addition at the end of incubation were similar to C contents and MWD of silty forest soils cultivated for a few years (Chenu et al., 2000) or of temporary prairies on silty soils (M. Hedde, personal communication). Finally, we assessed the aggregate stability with three tests that cover different intensities and kinds of stresses that allow us to approximately distinguish different mechanisms of aggregate breakdown (Le Bissonnais, 1996).

We observed a transient increase in aggregate stability followed by a decrease in MWD as in other works where plant residues had been added to soil (Monnier, 1965; Angers et al., 1997; Kiem & Kandeler, 1997; Martens, 2000).

We found that the aggregate stability increased linearly with C inputs in the whole incubation period measured. Even with very high amounts of residue, we found no maximum threshold of aggregate stability. Most published relationships between aggregate stability and OM added to soil are established in long term field experiments with more stabilised organic matter, comparing different tillage or cropping systems. A positive and linear relation is frequently found (N'Dayegamiye & Angers, 1990; Angers et al., 1997; Roldan et al., 2003). However, the relationship between organic matter and aggregation is soil type dependent and it can vary widely within the same soil, depending in part of when

the OM was added, what type of OM it was, and when the aggregates were subsequently taken for measurements (Loveland & Webb, 2003). Thus, some authors (N'Dayegamiye & Angers, 1993; Spaccini et al., 2004) also reported the absence of any significant relationship between soil C and aggregate stability.

In the short term, several authors showed that aggregate stability increased with C input under laboratory conditions (Hadas et al., 1994; Martens, 2000). A linear and positive relationship was showed in controlled conditions by Roldan et al. (1994) but only two doses of C were added with a maximum of 10 g C kg^{-1} soil. De Gryze et al. (2005) and Denef et al. (2002) also described a linear relationship, but they focused on the process of aggregate formation and thus used crushed soil in their experiments. In our experiment, we found no direct effect of the straw on aggregate stability on day 0, hence the effect of different doses of added straw on aggregate stability is due to the action of microbial decomposers and their by-products.

The added C increased the aggregate stability irrespective of the test considered (Fig. II-10), with similar patterns over the incubation time for the three tests. This means that the addition of organic matter and the resulting abundance and activity of microorganisms acted on the three breakdown mechanisms: slaking, microcraking and mechanical breakdown (see 4.1.) The differences of MWDs among stability tests reflected the different intensities of stresses applied.

4.3 Biological variables determining aggregate stability

When fresh organic matter is added to soil, stimulated bacterial and fungal populations enhance directly the aggregate stability by three ways (i) direct enmeshment of aggregates and particles by fungal hyphae (Degens, 1997), (ii) production of extracellular polysaccharides by bacteria and fungi which glue mineral particles (Chenu, 1995), and (iii) production of hydrophobic substances (Capriel et al., 1990).

In our experiment all measured biological variables exhibited similar time patterns with that of aggregate stability. Thus straw additions led to the production of aggregating agents in proportion to its rate of decomposition. We observed some variations of polysaccharide to biomass ratio and ergosterol contents but these changes were limited to the beginning of decomposition. Therefore it is reasonable that the residual C (1-decomposition) had a link with aggregate stability as show it in Fig. II-9d. Residual C

takes into account the action of microbial activity, supporting the idea that several mechanisms influenced the aggregate stability dynamics but always linked with the activity of microorganisms.

Biological variables had similar correlation coefficients among added C rates, confirming the linearity of the relation between the biological variables and the aggregate stability. This behaviour was observed in all aggregate stability tests measured. As each test attempt to separate different breakdown mechanisms we can conclude that in the case of our silty soil and for the range and type of maize residue added, the same biological agents of aggregate stability acted at the same time on them, increasing the aggregate cohesion, and diminishing differential swelling and slaking.

4.4 Predicting aggregate stability with time

To date few quantitative models have addressed the dynamics of soil structure. Even fewer take explicitly into account the relationships between soil organic matter – soil microorganisms and soil structure. However, such models are truly needed to predict changes in soil structure associated with erodibility, specific cultivation practices, or with changes in crops or cultivation systems. Furthermore, since soil structure protects organic matter from decomposition, an incorporation of data on and prediction of aggregate dynamics are needed to improve predictive models of soil organic matter dynamics or soil C sequestration.

One quantitative attempt in modelling the aggregate formation and the addition of organic matter was performed by De Gryze et al. (2005). In a 21 days experiment they tested 4 mathematical models. Two models were purely deterministic (linear and sigmoidal model), whereas the other two were mechanistic in that they related the rates of formation and destruction of aggregates with the abundance of macroaggregates /non-aggregated soil. They found that the four models performed equally well. As models were performed in soils with different textures, they concluded that mechanisms by which fungi or bacteria influence aggregate formation are texture dependent. However, predictive models should be able to take into account both the aggregation or stabilization process and the following decrease in aggregate stability, i.e. integrate longer times.

In our model, we used a variable associated with living microorganisms to predict aggregate stability very early after the C input (MBC) and the cumulative CO_2 as an

integrative variable more associated to the decrease in aggregate stability. Taking the cumulative CO_2 production in time to predict the slowly decrease in aggregate stability after the initial peak seems not to be logical, since by definition cumulative CO_2 never decreases. However, after 4 months of incubation the increase in cumulative CO_2 is counteracted by the diminution of microbial biomass-C. As a result the linear model predicts reasonably well the final phase of aggregate stability dynamics.

One important advantage in using variables as microbial biomass-C or cumulative CO_2 is that models that simulate C transformations in soil after a C input can predict them. Moreover, they can be predicted from different OM qualities and quantities added to soil and from different temperatures and soil water contents. Thus, CANTIS was tested simulating microbial biomass-C and cumulative CO_2 from different rates of C inputs and then aggregate stability was predicted from our linear model. Most C transformation models in soil can also predict the humified organic matter but we preferred to use CO_2 rather than a humified pool since the later can not be directly measured, so the model could not be tested.

The concept behind the aggregate stability model is that in similar soil environmental conditions (water potential, porosity, temperature and nutrient availability) the aggregate stability depends linearly on the relative abundance of aggregating agents. This abundance is controlled by the amount of C added to soil. The concept of linearity was used by Perfect and Kay (1990) defining the relative stability of soil structure as $Ri/R0 = \alpha\ (Ci/C0) + \beta$, where Ci and C0 are the aggregating agents and α and β are constants. They applied this model to five different cultivation systems over 15 years and to several soil structure attributes, but did not derive predictions due to the high number of missing variables (including the nature and abundance of the aggregating agents). However, the relation between abundance of aggregating agents and aggregate stability may change in different soil environmental conditions, e.g. nitrogen availability, texture, etc (Bossuyt et al., 2001). Moreover, this relation could be time dependent. Therefore, the same amount of microbial biomass-C or respiration stimulated by different organic quality additions could lead to a different aggregate stability dynamics by the effect of, for instance, a change in the microbial community structure or distribution.

5 Summary and conclusions

Our experimental set-up allowed us to show that in optimum controlled conditions when C is added to soil as a plant residue the response in microbial activity and its by-products is linear. This linearity was observed in a wide range of C input. Microbial activity and by-products were the main determinants of aggregate stability in the short-term and indicators of living microorganisms were responsible determining aggregate stability very early after the C input. Aggregate stability to slaking, microcracking and mechanical breakdown (as measured by three aggregate stability tests) responded similarly to stimulated microorganisms. A hierarchy among biological variables determining aggregate stability could not be established since they reacted fairly concomitantly to the addition of straw.

In constant conditions, a mechanistic and linear model based on biological variables simulated reasonably well the whole dynamics of aggregate stability over several months. This allowed coupling this linear model with a C transformations model (CANTIS) that simulate biological variables in wider conditions than our experimental set-up.

Chapitre 3

L`hydrophobie du sol: une comparaison des méthodes

Measuring subcritical water repellency: an appraisal of current methods [1]

Résumé

L'hydrophobie sub-critique est une propriété physique élémentaire d`importance majeure dans la stabilité de la structure. Cependant, une caractérisation correcte de cette propriété est difficile pour des raisons méthodologiques. Différentes méthodes existent, mais n'ont pas été comparées entre elles, s'appuient sur des formalismes physiques distincts, et sont techniquement différentes. Notre objectif a donc été de comparer la trois principales méthodes de mesure de l'hydrophobie des sols.

Nous avons utilisé des échantillons de sol limoneux provenant d'une expérience d'incubation destinée à analyser les impacts de la matière organique sur la stabilité des agrégats. Les échantillons couvraient une grande gamme de teneur en matière organique, d'abondance des microorganismes, de stabilité des agrégats et à priori d'hydrophobie. Sur des macroagrégats calibrés de 3 à 5 mm nous avons comparé trois méthodes : l'ascension capillaire (CRM), l'index de repellence à l`eau (R) et le temps de pénétration d'une goutte d`eau (WDPT).

Les résultats des méthodes montrent une concordance générale. En se basant sur les concepts de Philip (1957) et de Tillman et al. (1989) nous avons développé les relations théoriques qui existent entre WDPT, R et CRM qui s'avèrent cohérentes avec les résultats expérimentaux. Les trois méthodes ne déterminent pas la même propriété. Ainsi, le CRM et R estiment l'hydrophobie d'un sol alors que le WDPT estime la vitesse d'humectation qui dépend à la fois de l'hydrophobie et du spectre poral des agrégats. Ce travail nous a permis des recommandations quant au choix des méthodes en se basant sur les aspects mécanistiques comme pratiques

Mots-clés : *Hydrophobie, taux de réhumectation, rpellence `l'eau, temps de pénetration d'une goutte d'eau, méthode d'ascension capillaire, index de répellence.*

Abstract

Subcritical water repellency is a major property underlying soil structural stability. However, a good characterization of this property is not simple since a general methodological framework is missing in this area. Selecting the appropriate method to soil repellency is not ovbious because (i) these methods have been used in separate studies and not compared to date, (ii) they rely on different theoretical backgrounds and (iii) they are technically different. Therefore, the objective of this paper was to compare three methods which allow measurements of sub-critical water repellency.

[1] Ce chapitre est constitué d'un article en préparation pour Geoderma — D. Cosentino, P. Hallett, J.C. Michel, and C. Chenu. Measuring subcritical water repellency: an appraisal of current methods.

We used silty soil samples issued from an incubation experiment designed to assess the impact of organic matter additions on aggregate stability. The samples provided a wide range of organic matter contents, microbial abundances, aggregate stability and a priori water repellency. In macroaggregates (3-5 mm) we compared the capillary rise method (CRM), the water-repellency index (R) and the water drop penetration time (WDPT).

The results of three methods were in general agreement. Based in the concepts of Philip (1957) and Tillman et al. (1989) we developed theoretical relationships between WDPT, R index and CRM that are coherent with experimental data. The methods do not determine the same soil property. CRM and R index estimate soil hydrophobicity whereas WDPT estimates the rate of wetting which depends on both hydrophobicity and porosity of aggregates. Based on our results we propose criteria to select the appropriate method based on mechanistic and practical aspects.

Keywords: *Hydrophobicity, rate of wetting, water repellence, water drop penetration time, capillary rise method, repellency index.*

1 Introduction

Hydrophobic compounds produced by the microbial degradation of organic residues can influence soil wettability (Tillman et al., 1989; Wallis & Horne, 1992; Hallett et al., 2001a). This has important consequences on soil properties. It affects hydraulic transport of water and solutes in soils, enhances surface runoff and decreases aggregate breakdown by rapid wetting. When aggregates are rapidly wetted, air may become trapped, causing the aggregates to slake; soil colloids may swell, which generates internal stresses and microcracks. A reduction in the wettability of aggregate pore walls reduces the rate of wetting and subsequently the extent of slaking (Concaret, 1967). Subcritical water repellency is thus a major property underlying soil structural stability. Organic matter has a large influence on water repellency since hydrophobic compounds may decrease wettability, and hydrophilic and hydrophobic compounds may stabilise soil pores against external stresses (Eynard et al., 2004). Subcritical water repellency is enhanced in the short term by microbial activity (Hallett & Young, 1999; White et al., 2000; Feeney et al., 2004).

There are several methods to measure the resistance of soil to be wetted by water and two approaches are generally considered: (i) the measurement of liquid-solid contact angles and (ii) quantifying the affinity of the soil to liquids by infiltration. The direct and optical measurement of contact angles is a specific measure of water repellency. It is physically correct since wettability has been defined by laws that describe the interactions among the surface tensions between solid-liquid, solid-vapour and liquid-vapour interfaces

(Young, 1805). Contact angles have to be measured on homogeneous isotropic planar surfaces, otherwise the influence of roughness and porous system on the measurement is significant (Jouany et al., 1992). Thus, the method is somehow restricted to isolated soil components (mineral and organic fractions) with modified arrangement, i.e. pressed or oriented deposits of the materials. An "apparent" contact angle can be assessed by measuring the rate at which liquids wet soil by capillarity, e.g. the capillary rise method (CRM) on powders or soil material. This method is restricted to advancing contact angles < 90 ° (Woche et al., 2005).

The second approach is based on measuring infiltration or sorptivity. The water drop penetration time (WDPT) is a widespread and familiar method that evaluates the time that a water drop takes to enter completely into the soil. In fact, the WDPT test is essentially a measure of the infiltration rate modified by the persistence of the hydrophobicity, since hydrophobicity is a time dependent property (Doerr, 1998). It is an easy test that allows classifying soil hydrophobicity. WDPT and the "molarity of an ethanol droplet" have become the two most commonly used methods in the last two decades (Doerr, 1998). The lately (also called "critical surface tension") is well suited to field investigations on hydrophobic soils. King (1981) proposed another technique which uses a miniature ring infiltrometer for field measurements and expresses the results as infiltration rates. A relatively new technique is the use of a microinfiltrometer to measure the sorptivity of water in individual aggregates, from which a water-repellency index (R) can be calculated (Hallett & Young, 1999). The R index is directly proportional to the reduction in water sorptivity caused by hydrophobicity of pore walls (Feeney et al., 2004).

Other approaches based in visualization techniques such as computed tomographs are useful for non-destructive analysis of flow patterns (DeBano, 2000), but very expensive apparatus are necessary.

The different methods available have been used to show that soil repellency is related to soil organic matter contents (Bisdom et al., 1993), that the addition of organic matter affects soil repellency, generally by increasing it (Cosentino *et al.*, 2006a), and that microorganisms could induce subcritical water repellency (Tillman et al., 1989; Smits et al., 2003). Selecting the appropriate method to characterise the changes in soil repellency due to microbial activities is not evident because (i) these methods have been used in separate studies and not compared to date, (ii) they rely on different theoretical backgrounds and (iii) they are technically slightly different. Hence a general

methodological framework is missing in this area. Therefore, the objective of this paper was to compare three methods presented above, which allow measurements of sub-critical water repellency.

We used soil samples issued from an incubation experiment designed to assess the impact of organic matter additions on aggregate stability (Cosentino et al. in prep, Chap. 2). The samples provided a wide range of organic matter contents, microbial abundances and aggregate stability. We decided to use macroaggregates (3-5 mm) to be in line with the measurements of aggregate stability tests and we used the capillary rise method (Michel et al., 2001), the water-repellency index (Hallett & Young, 1999) and the water drop penetration time (Letey, 1991).

2 Materials and methods

2.1 Samples

The soil used was a cultivated silt loam Luvisol sampled at the experimental site of the Institut National de la Recherche Agronomique – INRA – at Versailles, France. It had 167 g kg^{-1} clay, 562 g kg^{-1} silt and 271 g kg^{-1} sand, with a total carbon content of 9.0 g kg^{-1}, Ct/Nt: 9.3 and pH (H2O) of 7.0. Soil was sampled with a spade from 0-20 cm depth (Ap horizon from six points). In the laboratory, larger clods present in the sample were gently crumbled by hand at field moisture along their natural fissures and sieved to obtain aggregates between 3.15 and 5 mm. Great care was taken to avoid damaging the natural aggregates. After sieving, coarse organic matter (free roots and plant debris) were removed with tweezers.

Maize straw (*Zea mays L.*) from stems and leaves dried at 40°C and ground < 500 µm was added at 0, 2.5, 5, 10, 15 and 20 g C kg^{-1} soil to the aggregates. The aggregates were then incubated at 20°C ± 0.5 °C at a water potential (Ψ) of -10 kPa which corresponds to 0.20 – 0.29 g g^{-1} water content, for 135 days. At days 3, 7, 14, 28, 63, and 135 of incubation three replicate jars per concentration of C added (i.e. three replicates per sample) were destructively sampled. Three additional jars without the addition of C were used to assess the initial state of the soil (day 0 of incubation). Samples were dried during at least 72 h at 40 °C in a ventilated oven. To assess aggregate stability and hydrophobicity

we carefully re-sieved the samples by hand into aggregates from 3.15 to 5 mm (hereinafter referred to 3-5 mm). The mean proportion of aggregates obtained (3-5 mm/total incubated mass) was 89 % with a standard deviation of 3.9 %.

2.2 Aggregate porosity

On samples incubated for 7 and 135 days, we measured the aggregate volume by immersing samples in kerosene and using Archimedes's principle according to Monnier et al., (1973). The water content was measured on the same samples and aggregate porosity was calculated from the apparent volume and water content assuming a density of solid of 2.65 g. cm^{-3}.

2.3 Water drop penetration time (WDPT)

We used the method from Letey (1991), modified by Chenu et al. (2000), where we assessed the time taken by a 3 µl deionized water drop to enter completely into an individual aggregate, i.e. the aggregate surface where the water drop enters becomes totally mat. The weight of the water drop is ~5% of mean aggregate weight (57 mg). Twenty-two aggregates per replication were used (i.e. 66 per sample). All measurements were done at 20 °C with an ambient relative humidity around 40 %, under a stereomicroscope using a CR-700-200 Hamilton CO. micro-syringe.

WDPT was measured at the following incubation days: 0, 7, 28, and 135.

2.4 Capillary rise method (CRM)

To measure the hydrophobicity by the capillary rise method, we used the same procedure and apparatus detailed in Michel et al. (2001). Briefly, we determined the capillary rise with two liquids (deionised water and n-hexane) by using the Krüss Processor Tensiometer K12 ®. The aggregate capillary rise is done in a glass tube with a porous glass base. The tube was fixed to a microbalance (readability = 0.1 mg), then placed automatically in contact with the liquids to measure the increase in weight in the soil sample in relation to time controlled by a computer program (K121®) which monitors the

tensiometer. The tube was filled by 3-5 mm aggregates (1.1 g; 2 cm height) and packed manually (3 series of 10 soft strikes). The error from variations in compacting the sample could cause variations in porosity and permeability, especially for crushed material and powders where the distinction between internal and external porosity is less evident than for natural large macroaggregates. Also the wetting process could affect the geometry of pores especially in unstable materials. The objective in compacting the samples was to enhance and standardize the contact among aggregates but not to change their internal poral geometry, which was achieved by working with dry aggregates. We also visually verified that no aggregate volume change occurred during the wetting process inside the tube. Capillary wetting the sample significantly diminished the extent of slaking in our soil (Cosentino *et al.*, 2006a).

The contact angles were obtained from the Washburn equation (1921)

$$h^2 = \frac{r\,\gamma_L \cos\theta}{2\eta}\,t \qquad (1)$$

where h is the height of the rising liquid front (cm), t is the time (s), η and γL are, respectively, the viscosity (mPa s) and the surface tension of the liquid (mJ m^{-2}), r represents the radius of the capillary (cm), and θ is the contact angle between solid and liquid.

The Washburn equation (1) defines the flow of a liquid through a capillary, which, for a bundle of capillaries and replacing the height of the wetting front by the increase in weight and simplified, becomes:

$$\cos\theta = \frac{m^2\,\eta}{t\,\rho^2\,\gamma_L\,c} \qquad (2)$$

in which

$$c = 0.5\,\pi^2(\tau\,r)^5 n^2 \qquad (3)$$

where θ is the contact angle between solid and liquid, m, η, ρ and γL are the mass (g), the viscosity (mPa s), the density (g cm^{-3}) and the surface tension (mJ m^{-2}) of the absorbed liquid, respectively. The time is t (s), and c (cm^5) is an empirical constant related to the porosity and tortuosity of capillaries, which depends on particle size and degree of packing. In c formula (3), τ is a constant to approximate the tortuosity of the capillaries, r and n are the mean radius (in cm) of a bundle of capillaries and their number, respectively.

A liquid with a contact angle = 0 (n-hexane) was used to estimate c in Washburn's equation (2). Knowing c, the apparent contact angle using deionised water could be calculated with the same equation.

Three to five curves (m vs t) of water and n-hexane were assessed per replication at the incubation days of 0, 7 and 135, performed each on a different set of aggregates.

2.5 Water-repellency index (R)

We determined the sorptivity of individual aggregates to deionized water and ethanol (95% v/v) with a microinfiltrometer device developed by Hallett & Young (1999). Sorptivity is soil water infiltration at early stages.

Liquids were supplied to the aggregates through a tip with a 140 µm radius from a source at constant hydraulic head (Ψ = 0 cm). A stereo microscope was used to verify the good contact with the soil aggregate. A microbalance (readability = 0.1 mg) was logged to register the liquid uptake with time from which the sorptivity values were calculated as follow:

$$S = \sqrt{\frac{Q\,f}{4\,r\,b}} \qquad (4)$$

where S is sorptivity (mm $s^{-0.5}$), Q is the liquid flow (mm3 s^{-1}), f is the air-porosity (mm3 mm^{-3}), r is the radius of the infiltrometer tip (mm) and b is a constant that depends on the soil-water diffusivity function. We used the same value of b = 0.55 for both liquids (White & Sully, 1987).

Finally, the water-repellency index (R) can be calculated from:

$$R = d\left(\frac{S_{ethanol}}{S_{water}}\right) \qquad (5)$$

The constant d accounts differences in the surface tension and viscosity between liquids. In the case of ethanol and water d = 1.95. An R = 1 indicates a soil that is not influenced by repellency (Tillman et al., 1989).

Water and ethanol sorptivity were measured on different aggregates to obtain R index. Ten values of R per sample were established at 0, 7, 28 and 135 days of incubation.

3 Results and discussion

3.1 Developed hydrophobicity

The samples exhibited a wide range of repellency levels measured using the WDPT, capillary rise and water repellency index approaches (Table III-1). As WDPT is often not normally distributed, medians are shown to diminish the weight of extreme values. WDPT classified the aggregates as completely wettable to strongly hydrophobic (Bisdom et al., 1993). However, the water repellency index indicated that none of the soils were perfectly wettable, thus exhibiting low to sub-critical levels of water repellency (Tillman et al., 1989). CRM classified the samples from 0 to 89°, 90° being the upper limit of the method. The range of variations is similar to that described for soils with contrasted type and management for WDPT (Chenu et al., 2000), R index (Hallett et al., 2001a) and CRM (Poulenard et al., 2004; Goebel et al., 2005).

Table III-1. Global descriptive statistics for the population of samples used for water drop penetration time (WDPT), repellency index (R) and the capillary rise method (CRM).

	WDPT	R index	CRM
Number of measurements per sample	66 (22 per replication)	30 (10 per replication)	9-15 (3-5 per replication)
Minimum mean sample value	1.4 s	2.55	0°
Maximum mean sample value	103.4 s	14.56	89°
Mean*	23.01	6.59	57.1°
Coefficient of variation (%)*	109.2	52.9	60.9

* taking into account all samples values.

3.2 Relationships between results obtained by the different methods.

Even though the expression of results is conceptually different (time, apparent contact angles or a relative index) the same physical principle is used to determine hydrophobicity. The three methods are based on the quantification of the time taken by liquids to enter soil. In the three methods water enters aggregates from a very small area relative to the external aggregates surface area. In the CRM method water enters soil from a zero to -2 cm pressure head (height of the column). To obtain the R index, instead, it enters soil with a 0 cm pressure head. In the WDPT method water enters with a zero or very slightly positive head (weight of a 3 µl drop). Hence, in the three methods it is the capillary pressure which basically drives the liquid into the soil. When only the early stages of capillary rise or infiltration (sorptivity) are considered, the hydrostatic pressure can be neglected (Woche et al., 2005). The flow of water entering the porous system by capillarity is described by the Washburn equation (1). It depends on (i) the number, radii and tortuosity of the capillaries, i.e. pore system characteristics, (ii) the contact angle between the pore walls and the liquid, (iii) the characteristics of the liquid (viscosity, surface tension) and (iv) the height of the wetting front.

The WDPT method uses only water; hence, it is basically a measure of the rate of wetting and cannot distinguish between changes in the poral system and changes in

hydrophobicity. CRM and R methods use non-polar liquids to assess independently the effect of the poral geometry and hydrophobicity on the rate of wetting. CRM uses n-hexane and R uses ethanol.

Philip (1957) introduced the concept of intrinsic sorptivity that is measured with liquids which do not react with the medium and is "essentially an expression of the geometry of the medium":

Then $Sw = Si \cos\theta$ (6)

where *Sw* is water sorptivity, *Si* is the intrinsic sorptivity and *θ,* the apparent contact angle.

This concept was taken by Tillman et al. (1989) to build the water-repellency index (R) as defined in (5) using ethanol to determine Si. In a similar way, CRM indirectly determines the apparent contact angle measuring an "intrinsic capillary rise" to calculate a constant "c" representing the geometry of pores in the formula of Washburn (1). Therefore, using aggregates in the CRM the determination of the constant "c" accounts for the contact points among aggregates and for the internal geometry of pores of all aggregates placed into the small tube where the capillary rise takes place.

3.2.1 WDPT and R

WDPT is basically a measure of the water infiltration rate so it should be related to water sorptivity (Sw) using equation (4). From (4) and grouping 4, r and b in a new constant *a*, we can rewrite it as follows:

$$Sw = \frac{\sqrt{Q\,f}}{a} \qquad (7)$$

and as by definition

$$Q = \frac{1}{WDPT} \qquad (8) \text{ so,}$$

$$Sw = \frac{\sqrt{f}}{a\sqrt{WDPT}} \qquad (9)$$

In a system where air-filled porosity (f) remains constant, equation (9) can by simplified as:

$$Sw = \frac{1}{a\sqrt{WDPT}} \qquad (10)$$

The observed relationships between experimental values of Sw and WDPT and the equation 10 fitted to the observed values are presented in Fig. III-1. Several considerations can be drawn in comparing observed and theoretical relationships. Porosity was not constant in the experiment (see 3.3). However, its impact is minor since the difference between the square root of the max and min porosity value is 0.042. Another error in this relationship is that water transport in soil takes time to reach a steady state and with WDPT this can not be checked (Hallett et al., 2001b). Also WDPT is influenced by surface roughness. Even thought the R^2 (0.64) was satisfactory.

From equations (5) and (9) we can link R index with WDPT as follows:

$$\frac{d\ Si}{R} = \frac{\sqrt{f}}{a\ \sqrt{WDPT}} \qquad (11)$$

regrouping and simplifying and considering air-filled porosity as a constant and so intrinsic sorptivity (Si):

$$R = c\ \sqrt{WDPT} \qquad (12)$$

where c is a fitting parameter.

The same errors must be considered in the relationship between R and WDPT than in Sw and WDPT. From observed values we deduce that c = 1.69 and R^2 = 0.8 (Fig. III-2a).

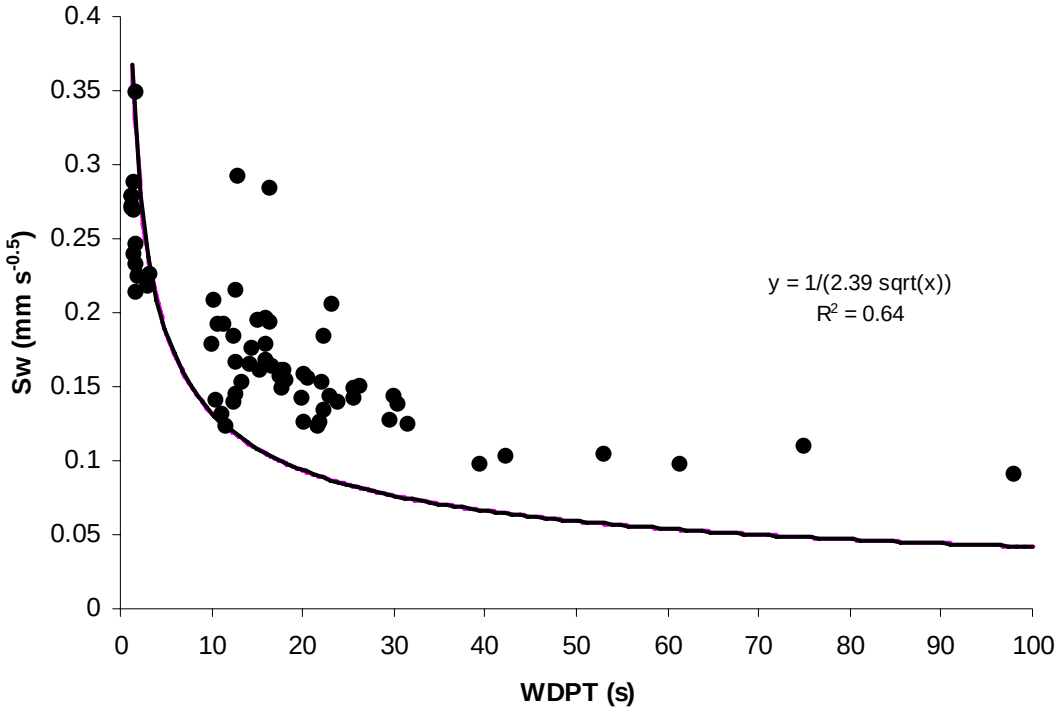

Fig. III-1. The relationship between water sorptivity (Sw) and water drop penetration time. Dots: observed values, line: theoretical relationship with constant air-filled porosity.

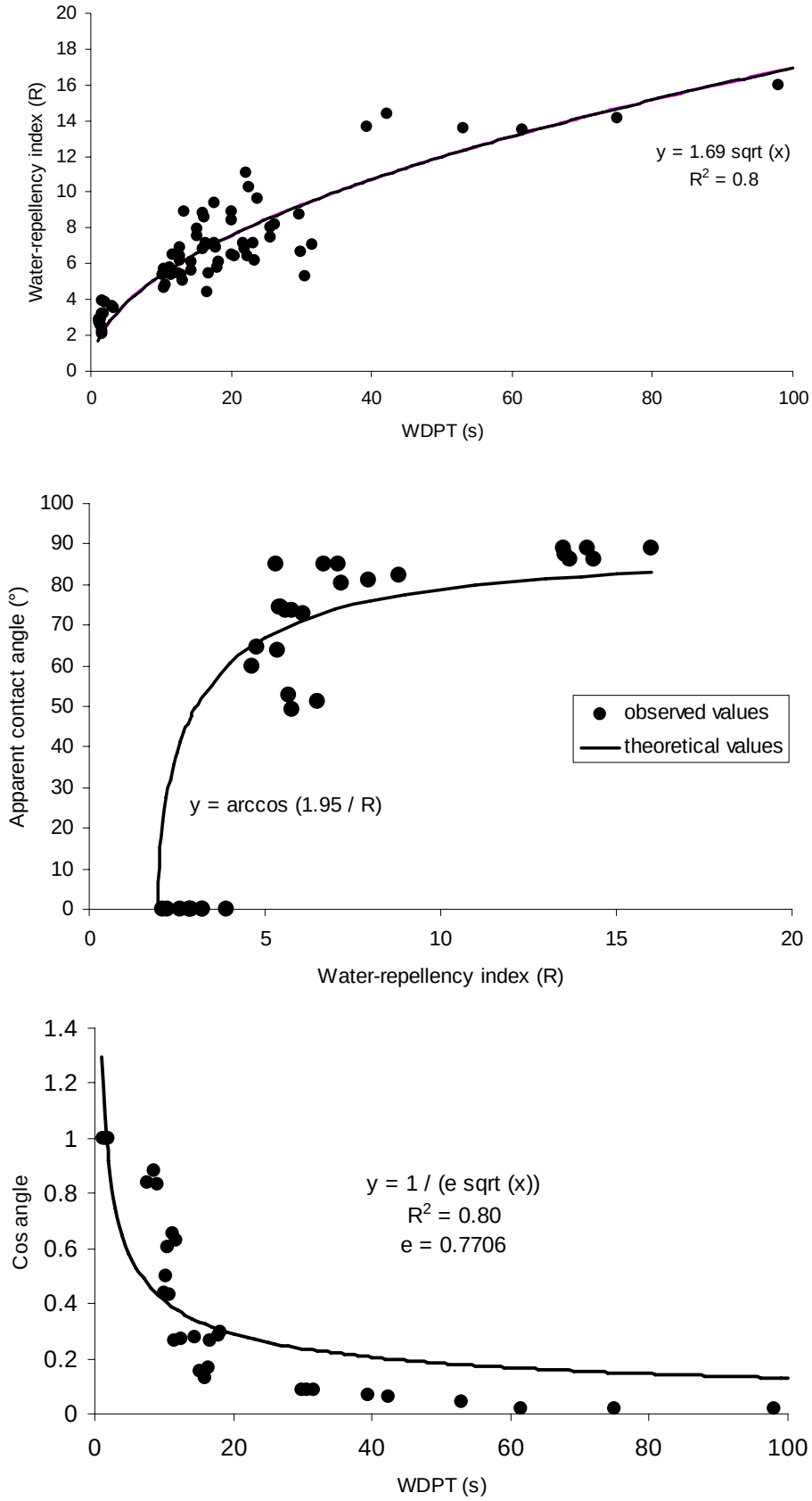

Fig. III-2. The relationships between a: the water-repellency index (R) and the water drop penetration time test (WDPT); b: the apparent contact angle and R; and c: cosine of the apparent contact angle and WDPT

3.2.2 R and the soil-water apparent contact angle

From the equations (6) (Philip, 1957) and (5) (Tillman et al., 1989) the theoretical relationship between the soil-water apparent contact angle and R index is expressed by the following formula:

$$\cos\theta = \frac{d}{R} \qquad (13)$$ and so the apparent contact angle is:

$$\theta = \arccos\left(\frac{d}{R}\right) \qquad (14)$$ or

$$\theta = \arccos\left(\frac{1.95}{R}\right) \qquad (15)$$ using ethanol and water to obtain R index.

In Fig. III-2b is represented the observed relationship between the experimental value for R index and apparent contact angles as measured by the CRM and the theoretical ones according to equation (15). The R^2 between observed and theoretical values was 0.78 confirming the applicability of the relation (15). However, for low values of R, the experimental relationship between these variables was not that expected from the theory. In particular, we found contact angles of 0 for samples exhibiting R ~ 3 where we would have expected apparent contact angles ~ 50°. A possible explanation is that the shape of the equation (15) determines that small experimental errors in measuring low values of R lead to a very different calculated values of apparent contact angles. Arc cosine function is valid from +1 to -1, meaning that theoretically it is not physically possible to measure R index values smaller than 1.95 (Angle = 0). Another source of difference between methods is that CRM takes into account the geometry of pores from a group of aggregates meanwhile R index is determined in a single aggregate. This difference may be important at rapid capillary rise where contact angles are small.

3.2.3 WDPT and the apparent contact angle

From equations (12) and (13) we can obtain the relationship between the apparent contact angle and WDPT as follows:

$$\cos\theta = \frac{1}{e\sqrt{WDPT}} \quad (16)$$

where e is a fitting parameter.

Observed relationship between measured CRM and WDPT and the theoretical ones from equation (16) are represented in Fig. III-2c. With e = 0.7706. Observed and fitted values correlate reasonable well (R2 = 0.80).

We have to be cautious relating the cosine of apparent contact angles and WDPT since many assumptions are done: (i) soil is not highly water repellent, (ii) water repellency does not change with time (i.e. as the soil hydrates), (iii) the WDPT test in early time detects mainly the impedance to flow by repellency whereas at longer times it detects the rate of hydration of organic compounds. So it is related to water flow for low values, (iv) the WDPT is a point value and influenced by surface roughness and (v) we consider a simple system where porosity does not change with hydrophobicity.

Table III-2. Effects of organic matter additions on poral system

Sample	Date (day)	Aggregate density (Mg m^{-3})	Aggregate porosity (% apparent volume)	Ethanol sorptivity	c*
Control (no straw addition)	0	1.78±0.008	32.8	0.413±0.011	$8.7\ 10^{-6}$
	7	1.79±0.009	32.6	0.346±0.044	$8.7\ 10^{-6}$
	135	1.78±0.009	32.8	0.413±0.031	$7.98\ 10^{-6}$
Straw addition (20 g C kg^{-1})	7	1.65±0.008	37.8	0.737±0.064	$2.53\ 10^{-5}$
	135	1.65±0.011	37.8	0.723±0.077	$1.69\ 10^{-5}$

*c parameter from Washburn's formula (2)

3.3 Porosity

In our experiment, the sorptivity of soil aggregates to ethanol, (i.e. intrinsic sorptivity) was rapidly enhanced after the addition of maize straw and it remained larger than in the reference soil until the end of incubation at 135 d (Table III-2). This increase in intrinsic sorptivity is also supported by measurements of aggregate density. At day 7 it diminished from 1.79 Mg m^{-3} in the control to 1.65 Mg m^{-3} with the addition of 20 g C kg^{-1}, corresponding to a total aggregate porosity from 32.6 % to 37.8 %. As the solid density of aggregates were not measured, we assumed a general value of 2.65 Mg m^{-3}, the impact of taking into account the straw maize particle density in the calculation of total porosity to each added C treatment is minor (reduction in total aggregate porosity < 2.3 % for the highest dose of C). Hence, in our data set, not only the surface properties of the solids were modified, but also the porosity. Very limited quantitative data is available regarding the impacts of soil organic matter or soil microorganisms on total porosity, pore size distribution or pore connectivity. The hydraulic conductivity can be decreased by pore clogging with cells (Vandevivere & Baveye, 1992) or colloidal organic matter, or by trapped air that cause a partial or complete occlusion of the pore system (Hafida *et al.*, 2005). However, plant residues additions can form additional porosity or change the spatial size distribution particularly in the vicinity of straw residues (De Gryze et al., 2006b).

The addition of straw had simultaneously two opposite effects on the rate of wetting, increasing it by enhanced porosity and reducing it by the impact on hydrophobic substances (Fig. III-3). Nevertheless, in our conditions, the impact of hydrophobicity on the rate of wetting was much higher than the change in the poral system since WDPT clearly increased several times with the doses of added C (and also the water sorptivity). Our results are in line with those of Hallett et al. (2001b) that working with field soil samples from a range of cultivation practices also showed that subcritical water repellency had a more dominant effect on reduced water sorptivity than that of the pore structure. Not taking into account change in the poral system and measuring WDPT can lead to a wrong estimation tems of hydrophobicity. This could also explain differences between WDPT and R-values in the higher doses of added C (see 3.2). Heterogeneity in individual aggregates can not be discarded.

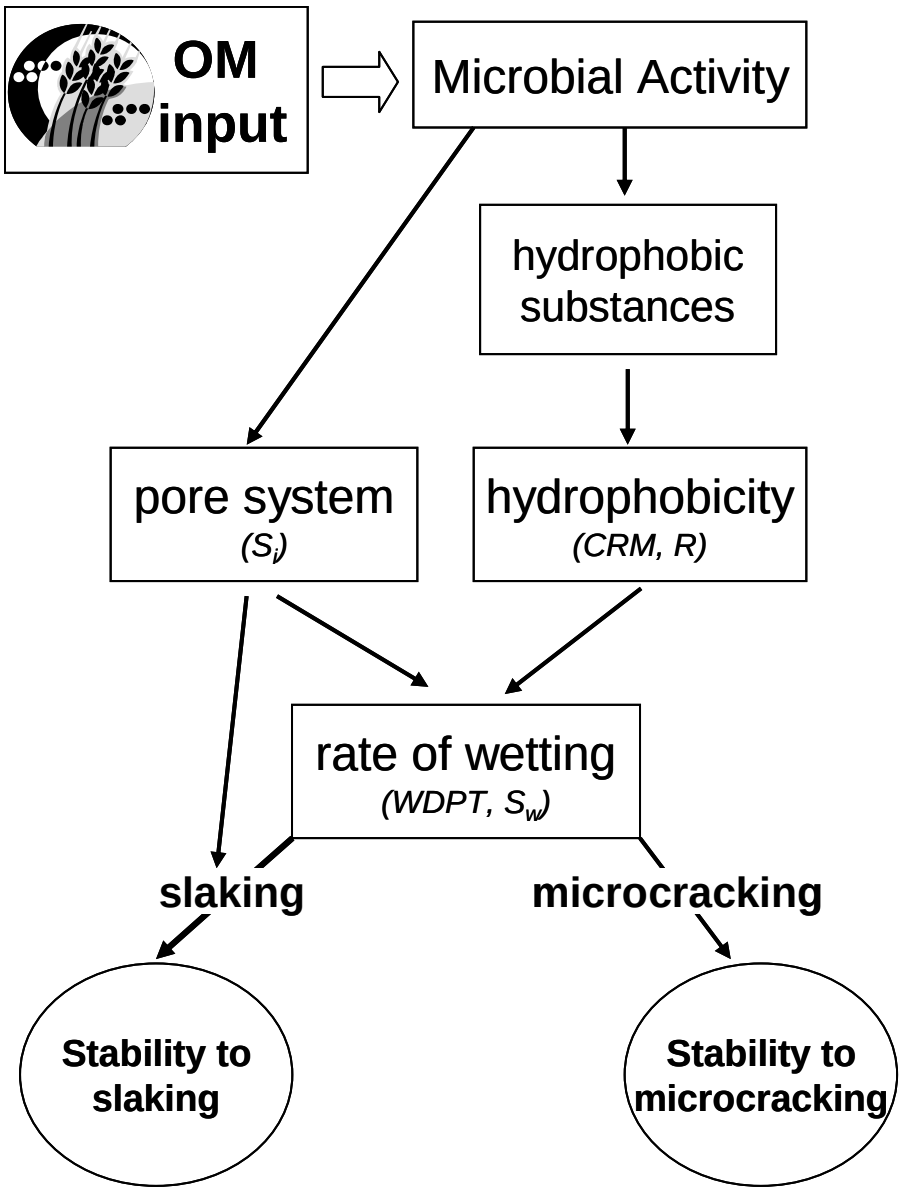

Fig. III-3. Impact of added organic matter on the aggregate stability through the influence on the rate of wetting. In italics methods to measure each property: *Si* and *Sw*: intrinsic and water sorptivity; *CRM, R* and *WDPT* are the capillary rise method, the water-repellency index and the water-drop penetration time, respectively

3.4 Practical aspects

In Table III-3 we compare our practical experience in using three methods to determine hydrophobicity or the rate of wetting. The sample used for measurements are not the same: individual aggregates in the case of WDPT and R, and a loosely packed column of aggregates for CRM. Hence, rates of wetting are not strictly comparable since in the case of CRM the porosity defined by the arrangement of aggregates is involved. Goebel et al. (2005) notes that the difficulty in achieving the same packing density between samples is the main source of error of the capillary rise method. This source of error does not exist in the R method since single aggregates are measured. However, the variability in hydrophobicity among aggregates is higher than for a group of aggregates so several replicates must be processed.

Table III-3. Practical aspects for three techniques of hydrophobicity or rate of wetting: the water-drop penetration time (WDPT), the water-repellency index (R) and the capillary rise method (CRM).

	WDPT	R Index	CRM
Weight of soil sample used	3.76 g	3.42 g	21.8 g
Samples used	Individual aggregates	Individual aggregates	Cores of slightly packed aggregates
Relative time employed	1	5.6	8
Equipment	Micro-syringe Stereo microscope Chronometer	Microbalance Stereo microscope Computer – data register	Special microbalance Tensiometer Computer – data register

The total weight of soil sample used was calculated in the base of doing 22 measurements of WDPT per replicate and 3 replicates per treatment (corresponding to 3 independent incubated jars). In the case of R, the weight was calculated using 20 independent aggregates per replicate with 3 replicates per treatment. Finally, the weight of soil used to CRM was calculated considering 1.1 g of aggregates per curve (ethanol or water), doing 6 curves per replication with 3 replications per treatment.

The relative time employed refers to the same set of samples assessed. We preferred to express a relative time of a set of samples that include all the range of developed hydrophobicity because of the obvious time dependency of hydrophobic measurements.

Finally, WDPT and R index use simple laboratory equipment, while CRM needs a relatively sophisticated apparatus.

If we compare the variability of hydrophobicity methods in terms of coefficients of variation the WDPT >> CRM > R index (in Table III-1, the extreme outliers in the calculation of the coefficients of variation for WDPT were discarded). The high variability of WDPT is caused by the influence of extreme values, likely due to the fact that in WDPT method the initial infiltration of water is not in the steady state and is highly influenced by the heterogeneous distribution of hydrophobicity at the aggregates surface. Moreover, the values are not normally distributed and the standard deviation increases with the doses of C added. Bi-modal distributions were also found (Scott, 2000). Two examples are illustrated in Fig. III-4. Taken the variability of medians among replicates, instead of means to avoid the influence of extreme values (Doerr, 1998; Mataix-Solera & Doerr, 2004; Cosentino *et al.*, 2006a) we calculated a coefficient of variation < 10 % aligned with R index and CRM methods.

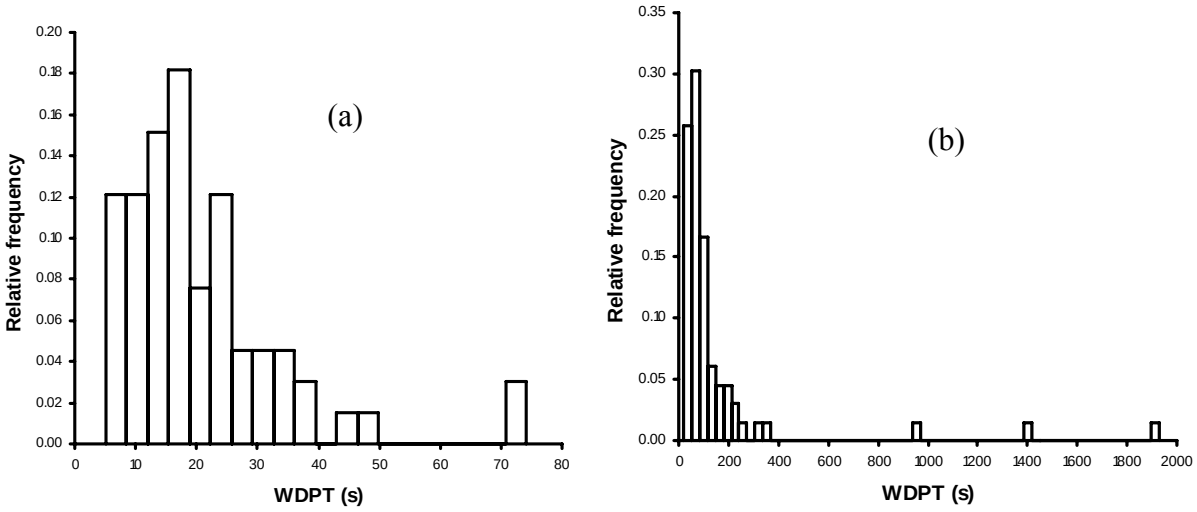

Fig. III-4. Frequency distribution of water-drop penetration time (WDPT) at the day 7 of incubations: (a) 5 g C kg^{-1} added, n = 66 with 20 intervals and (b) 20 g C kg^{-1} added, n = 66 with 60 intervals.

4 Conclusions

Three independent methods: WDPT, R and CRM showed the same tendency in characterizing the water-repellency. They use the same physical principle; however they measure different soil properties that are related. WDPT determines the rate of wetting to water and the R index and CRM the sub-critical hydrophobicity.

Using a silty soil sample set characterized by a wide range of samples from non repellents to subcritical water repellents, the results of three methods were in general agreement. Based in the concepts of Philip (1957) and Tillman et al. (1989) we developed theoretical relationships between WDPT, R index and CRM that are coherent with reality even when several considerations should be done (e.g. changes in porosity) confirming their competence to the soil we used.

We suggest using WDPT for field survey applications since it is simple, cheap and discriminates well the sub-critical rate of wetting. However, it is not appropriate from a mechanistic point of view since it does not separate between changes in the poral system and changes in hydrophobicity.

R index and CRM are strong, sensitive and repetitive techniques that may be used to separate soils with different sub-critical hydrophobicity independently of their geometry of pores. CRM and R techniques have some similarities: both use non-polar liquids to cancel the effect of porosity. Using water as one of the liquids, both are restricted to advancing contact angles < 90°. Meanwhile, using different liquids, CRM can measure the solid surface free energy (Owens & Wendt, 1969) and can thereby calculate the contact angles > 90°, extending its applicability. The fact of measuring a group of aggregates or powders seems to be the causes of the highest repeatability. However, CRM is very time consuming and the more expensive technique tested. The use of dangerous non polar liquids (e.g. n-hexane) is not negligible. The R index seems to be the easiest and cheapest technique to measure sub-critical hydrophobicity.

The terms "water repellence", "wettability", "rate of wetting", "rate of water sorption" and "hydrophobicity" are currently use in soil scientific bibliography as a synonyms. However, we suggest that the word "hydrophobicity" would be reserved specifically to the property of the surface of soil solids. Thus, measurements of solid-

water-air contact angles or the ones that can cancel the effect of the poral system refer to "hydrophobicity". Thereby, a highly hydrophobic soil will have a low rate of wetting but the contrary will not be necessarily true.

Chapitre 4

Les variables physiques élémentaires qui déterminent la stabilité de la structure

Physical properties that control aggregate stability after the addition of maize straw to soil [1]

Résumé

La stabilité des agrégats a été proposée comme un indicateur de qualité de sol grâce à son importance dans la dégradation physique et sa sensibilité à la teneur en matière organique.

L'instabilité des agrégats vis à vis de l'eau est causée par la désagrégation mécanique due à l`énergie cinétique des gouttes d'eau, par le gonflement - retrait des argiles et des matières organiques qui entraînent une microfissuration et lors de l'humectation rapide par le piégeage d'air dans les pores (éclatement). Ainsi, plusieurs propriétés physiques contribuent à la stabilité des agrégats, qui est une propriété intégrative : la cohésion interparticulaire, l'hydrophobie et le système poral. Leur contribution relative est un sujet de débat.

Nous avons déjà analysé l'impact de l'apport de matière organique sur l'abondance et l'activité des microorganismes et ses conséquences sur l'évolution de la stabilité des agrégats (Chapitre 2). Le but de ce travail a été d'analyser et quantifier la contribution de plusieurs propriétés physiques du sol, i.e. l`hydrophobie, le système poral et la cohésion, à l'amélioration de la stabilité de la structure après un apport de matière organique.

Pour cela, nous avons suivi une expérience où nous avons apporté différentes doses de résidus de maïs aux agrégats millimétriques d'une sol limoneux cultivé pour avoir une large gamme de teneurs de MO et d'activité des microorganismes.

Après l'apport de MO, le système poral a amélioré la conduction de l'eau, la cohésion a augmenté et les agrégats ont devenus plus hydrophobes. Tous les mécanismes d'action possibles de la matière organique contre l`action disruptive de l'eau ont donc été mis en évidence. La mesure de la stabilité de la structure, propriété intégratrice apparaît donc pertinente pour évaluer les effets des matières organiques sur la stabilité des agrégats.

***Mots-clés** : stabilité structurale, mécanismes de désagrégation, cohésion, hydrophobie, addition de matières organiques*

[1] Ce chapitre est constitué d'un article en préparation pour *Soil Science Society of America Journal* — D. Cosentino, C. Chenu, P. Hallet, J-C. Michel, D. Tessier & P. Défossez. Physical properties that control aggregate stability after the addition of maize straw to soil

Abstract

Aggregate stability was proposed as a soil quality indicator because of its relevance to physical degradation and sensibility to organic matter, that most of the time are linked.

Aggregate disruption by water is caused by a mechanical breakdown due to the kinetic energy of rain drops, the swelling of clay and organic domains causing microcracking and, with rapid wetting, by the entrapment of air in aggregate pores causing slaking. Hence, several physical properties contribute to aggregate stability: interparticle cohesion, hydrophobicity and the characteristics of the poral system. Their relative contribution to aggregate stability is under debate.

As an integrative soil property, aggregate stability should not be evaluated with a single test but by assessing the different elementary breakdown mechanisms that control aggregate stability, or directly by measuring the elementary physical properties that determine it.

In a previous study we analyzed the impact of added OM on the abundance and activity of microorganisms in relation to aggregate stability (chapter 2). The purpose of the present study was to investigate and quantify the contribution of several soil physical properties, i.e., hydrophobicity, poral system and cohesion, in the amelioration of aggregate stability when adding organic matter to soil. For this we monitored an incubation experiment with different doses of added straw in order to have samples exhibiting a wide range of OM contents and microbial activity.

Adding fresh OM strongly modified the physical properties of a silty soil, rather poor in OM and unstable. The pore network was modified being more conducive to liquids, pore walls were rendered more hydrophobic and wet cohesion was increased. All possible mechanisms of action of OM against the disruptive action of water were thus affected. Hence, it seems meaningful to still consider aggregate stability as an integrative property when analysing the effect of OM additions to soil aggregate stability.

Keywords*: aggregate stability, breakdown mechanisms, cohesion, hydrophobicity, organic matter addition*

1 Introduction

Soil structure and its stability have been suggested as a key factors in the functioning of soil (Bronick & Lal, 2005) because of their implication in environmental quality (e.g. C sequestration and water quality) and supporting life. Aggregate stability was therefore identified as a quality indicator (Shukla et al., 2004) because of its relevance to physical soil degradation and sensibility to organic matter (OM), that most of the time are linked. Resistance of soil to erosion depends largely upon the stability of the aggregates (Le Bissonnais, 1988). Aggregate stability is a soil property that result from intrinsic soil characteristics (especially texture and organic matter) as well as from external factors such as climate and agricultural management (Amézketa, 1999).

Aggregate disruption by water is caused by a mechanical breakdown due to the kinetic energy of rain drops, the swelling of clay and organic domains causing microcracking and, with rapid wetting, and by the entrapment of air in aggregate pores causing slaking. Hence, several properties contribute to aggregate stability: interparticle cohesion, hydrophobicity and characteristics of the poral system. However, their relative contribution to aggregate stability is under debate. According to Caron et al. (1996) the increased rate of water entry is the major mechanism for decreased aggregate stability whereas aggregate cohesion and swelling would be less important processes (Zaher *et al.*, 2005). Conversely, De Gryze et al. (2006a) concluded that water repellency was not a dominant process influencing aggregate stability in a pasture soil.

Another problem that contributes to a certain degree of confusion is that there is no unique way to measure aggregate stability and, in addition, the term is not completely objective and express a qualitative concept that can only be evaluated using direct or indirect measurements procedures (Díaz-Zorita et al., 2002). Dozens of methods for measuring aggregate stability have been developed and the lack of a satisfactory standard methodology is a problem in this field (Le Bissonnais, 1996). As an integrative soil property, aggregate stability cannot be evaluated just with a single test but is better evaluated by assessing the resistance of soil to the different elementary breakdown mechanisms that control aggregate stability, or directly by measuring the elementary physical properties that determine it.

Silty soils are fragile because of their texture and they have a high susceptibility to crusting and erosion. OM is the main agent of their aggregate stability (Le Bissonnais & Arrouays, 1997). In the short-term, aggregate stability often exhibits large seasonal variability controlled mainly by climate and organic matter incorporations through their action on soil moisture and stimulation of microbial activity (Cosentino *et al.*, 2006a).

Many studies have shown that adding fresh organic matter to soil stimulated microbial activity and increased aggregate stability transiently (Monnier, 1965; Tisdall & Oades, 1982; Metzger et al., 1987). Polysaccharide secretion by soil microorganisms was observed (Haynes & Beare, 1997) and assumed to increase cohesion as demonstrated in model systems (Chen & Guérif, 1991). Hyphal entanglement of soil particles was observed (Molope & Page, 1986). Hydrophobicity is also enhanced by microbial activity, presumably by exuding hydrophobic substances (Capriel et al., 1990; Hallett & Young, 1999), and the pore network can be modified by occlusion of pores (Caron et al., 1996) or

by changes in connectivity or tortuosity (Preston et al., 1999). However mechanistic studies regarding OM-mediated aggregation, generally considered only one of the potential roles of OM.

Knowledge of the short-term effects of organic matter additions on aggregate stability becomes essential when considering soil conservation strategies. In a previous study we analyzed the impact of added OM on the abundance and activity of microorganisms in relation to aggregate stability (chapter 2). The purpose of the present study was to investigate and quantify the contribution of several soil physical properties, i.e., hydrophobicity, poral system and cohesion, in the amelioration of aggregate stability when adding organic matter to soil. For this we monitored an incubation experiment with different doses of added straw in order to have samples exhibiting a wide range of OM contents, quality and microbial activity (Cosentino, chapter 2). We measured the effect of OM additions on hydrophobicity, cohesion and porosity and we discussed the data in relation to the observed aggregate stability changes and changes in biological variables.

2 Materials and methods

2.1 Experimental area, site description and sampling procedure

The soil used in this experiment was a silt loam Luvisol sampled at the experimental site of the Institut National de la Recherche Agronomique – INRA – (48°48’29”N, 2°04’58”E), at Versailles, France. The climate is temperate with an annual rainfall of 639 mm yr^{-1} (1928-2003) and 10.5°C annual mean temperature. The texture was of 167 g kg^{-1} clay, 562 g kg^{-1} silt and 271 g kg^{-1} sand, with a total carbon content of 9.0 g kg^{-1}, Ct/Nt: 9.3 and pH (H_2O) of 7.0. It had been cultivated for more than 50 years with conventional tillage (mouldboard plow at 0-30 cm) with a rotation based on wheat (*Triticum aestivum* L.), colza (*Brassica napus* L.) and pea (*Pisum sativum* L.).

We sampled the soil from six points of the parcel at 0-20 cm depth (Ap horizon) with shovels to keep the natural structure of the soil as much as possible. The soil was sampled at a water content of 0.19 g H_2O g^{-1} soil. At the laboratory, the larger clods were gently crumbled by hand at field moisture along their natural fissures and sieved to obtain an adequate amount of aggregates between 3.15 (here referred to 3 mm) and 5 mm. Great

care was taken to avoid damaging the natural aggregates. After sieving, coarse organic matter (free roots and plant debris) was removed with tweezers and the soil was then stored in the dark in plastic boxes at 4°C for one month (0.16g H_2O g^{-1} soil). To avoid any compaction the aggregates were disposed in a 2 cm layer. The aggregates were incubated at 20°C for twelve days before incubation to minimize the variations in microbial activity due to changes in temperature conditions.

2.2 Incubations procedures and experimental treatments

We incubated soil aggregates (3-5 mm) in optimal conditions for biodegradation in terms of N and O_2 availability. The aggregates were incubated at 20 °C ± 0.5 °C for 8 months with different concentrations of added maize (Zea mays L.) straw: 0, 2.5, 5, 10, 15 and 20 g C kg^{-1} soil. Additionally, the treatments with 0, 5 and 20 g C added kg^{-1} were incubated for 343 days.

The equivalent of 160 g dry sol (105°C, 24h) was placed in glass jars and we added 0, 0.93, 1.86, 3.72, 5.58 and 7.45 g of straw to get the concentrations described above. The samples with added maize straw were cautiously mixed and immediately sprayed with a solution of NO_3NH_4 to adjust simultaneously the soil+straw sample C/N ratio to 10 and its water potential (Ψ) to -10 kPa. Thus, the corresponding water contents to the different added straw concentrations were: 0.20, 0.21, 0.23, 0.25, 0.27 and 0.29 (g/g). The samples with no straw addition (controls) were treated in the same way, but with deionized water. All treatments were kept at field capacity (Ψ = -10 kPa) during all the incubation. A beaker without direct contact to soil containing 20 ml of deionized water was also added to the jars to minimize desiccation. At the end of the incubation the jars had lost < 1 % (g/g) of initial water content. In treatments (concentrations of added straw) designated to be sampled at the 8^{th} month, a second beaker with NaOH was also placed in the jars to trap and measure the CO_2 produced during incubation by respiration of microorganisms.

2.3 Soil analyses

At days 0, 3, 7, 14, 28, 63, 135, 253 and 343 of incubation three replications per concentration of C added were sacrificed (only 3 concentrations at 343 days). At each sampling date the soil was dried during at least 72 h at 40 °C in a ventilated oven and then

we carefully re-separated the samples into aggregates from 3 to 5 mm. Almost no energy was applied and only attached aggregates > 5 mm were separated by hand. Thus, we obtained a mean size proportion of aggregates from 3-5 mm respect to the entire sample (3-5 mm/< 5 mm) of 89 % with a standard deviation of 3.9 % for all treatments and incubation dates. Physical properties were measured on these aggregates. Not all soil analyses were performed in all rates of C input or all incubation dates. As some of them are very time consuming we focused in the first 4 months of incubations for infiltration determinations. Compression tests were performed at 343 days of incubation.

2.3.1 Biological variables

At each incubation date we determined immediately in a moist subsample the microbial biomass-C by fumigation-extraction according to Vance et al. (1987).

Another subsample was taken and frozen to measure within the month the ergosterol content with a technique modified from Djajakirana et al., (1996) and Gong et al., (2001) developed by Annabi (2005). Ergosterol was used as a biomarker of living fungi. A third aliquot was taken to estimate the hot-water extractable carbohydrate-C (HWEC) (Puget et al., 1999). Details on the methods can be found in Cosentino et al. 2006 (in prep, chapitre 2).

2.3.2 Water drop penetration time (WDPT)

We used the method from Letey (1969), modified by Chenu et al. (2000), where we assessed the time taken by a 3 µl deionized water drop to enter completely into an individual aggregate (i.e. the aggregate surface where the water drop enters become totally mat). WDPT is essentially a measure of the rate of wetting. The weight of the water drop is ~5% of mean aggregate weight (57 mg). Twenty-two aggregates per replication were used (i.e. 66 per sample). For some samples, measurements were performed on aggregates that had been cut in two along their diameter, and we recorded the WDPT of the outside surface of the aggregate and the WDPT of the inner volume of the aggregate. All measurements were done at 20 °C with an ambient relative humidity around 40 %, under a stereomicroscope using a CR-700-200 Hamilton CO. micro-syringe. WDPT was measured at the following incubation days: 0, 7, 28, and 135.

2.3.3 Water-repellency index (R)

We determined the sorptivity of individual aggregates to deionized water and ethanol (95% v/v) with a microinfiltrometer device developed by Hallett & Young (1999). Sorptivity is the soil infiltration at early stages.

Liquids were supplied to the aggregates trough a tip with a 140-µm radius from a source at constant hydraulic head (Ψ = 0 cm). A stereomicroscope was used to verify good contact with the soil aggregate. A microbalance (readability = 0.1 mg) was logged to register the liquid uptake with time from which the sorptivity values were calculated as follows:

$$S = \sqrt{\frac{Q\ f}{4\ r\ b}} \qquad (1)$$

where S is sorptivity (mm $s^{-0.5}$), Q is the liquid flow ($mm^3\ s^{-1}$), f is the air-filled porosity ($mm^3\ mm^{-3}$), r is the radius of the infiltrometer tip (mm) and b is a constant that depends on the soil-water diffusivity function. We used the same value of b = 0.55 for both liquids (White & Sully, 1987).

Finally, the water-repellency index (R) can be calculated from:

$$R = 1.95\left(\frac{S_{ethanol}}{S_{water}}\right) \qquad (2)$$

The infiltration of ethanol is not affected by hydrophobicity and thus allows measuring the "intrinsic sorptivity" which is essentially an expression of the geometry of the medium. Therefore, R index is directly proportional to the reduction in water sorptivity caused by hydrophobicity (Feeney et al., 2004). An R<1.95 corresponds to a non-repellent soil (Tillman et al., 1989).

Ten water and ten ethanol sorptivity curves per replication of individual aggregates were measured (i.e. 10 R values per replication and 30 per sample) at 0, 7, 28 and 135 days of incubation.

2.3.4 Aggregate porosity

On samples incubated for 0, 7 and 135 days, we measured the aggregate apparent volume by immersing samples in kerosene and measured Archimedes's force according to Monnier et al., (1973). The water content was measured on the same samples and total porosity was calculated from the apparent volume and water content assuming a density of solid of 2.65 g. cm^{-3}.

2.3.5 Tensile strength and uniaxial compression (oedometer test)

The tensile strength of soil aggregates was measured with the "crush test" (Guérif, 1988). Single soil aggregates 3-5 mm in diameter dried at 40°C for 72 h were crushed using a simple compression press at a constant rate of strain of 0.433 mm s^{-1}. The apparatus is described in detail by Guérif (1988). Tensile strength (T in 100 kPa) can be calculated from the force (F in N) required to fail the aggregate as:

$$T = \frac{0.576\,F}{d^2 * 1000} \qquad (3)$$

where d (mm) is the mean of 3-D diameter of soil aggregates.

Tensile strength was measured on the samples from 7 days of incubation with 0 and 20 g C kg^{-1} of straw added. Twenty individual aggregates per replicate (i.e. 60 per sample) were used. Contrastingly to the method proposed by Guérif (1988), we did not "round" artificially the aggregates, thus we preserved their external surfaces, where the straw was added and microbial activity was enhanced.

The uniaxial compression was performed with an oedometer. The oedometer apparatus applies a one-dimensional load to a confined soil sample and the volume change is registered by measuring the vertical displacement of the rigid top porous plate used to apply the load (Fig. IV-1). The single-oedometer test consists of loading the sample incrementally to a specific state of vertical stress and allowing the sample to come to equilibrium under the applied pressure. The stresses applied ranging from 0 to 1500 kPa on the top of a 50 mm diameter cylinder with 19 mm of maximum height. In our experiment, the stresses included 29 stages (between 2 and 1500 kPa) of 5 minutes each (to reach equilibrium). The apparatus also allows injecting water from the top of the cell at a

constant rate and pressure. Drainage of water is assured through a porous plate at the bottom of the cell (Fig. IV-1). The internal deformation of porous plates and the elasticity of the membrane were taken into account by making a test on uncompressible Plexiglas cylinder.

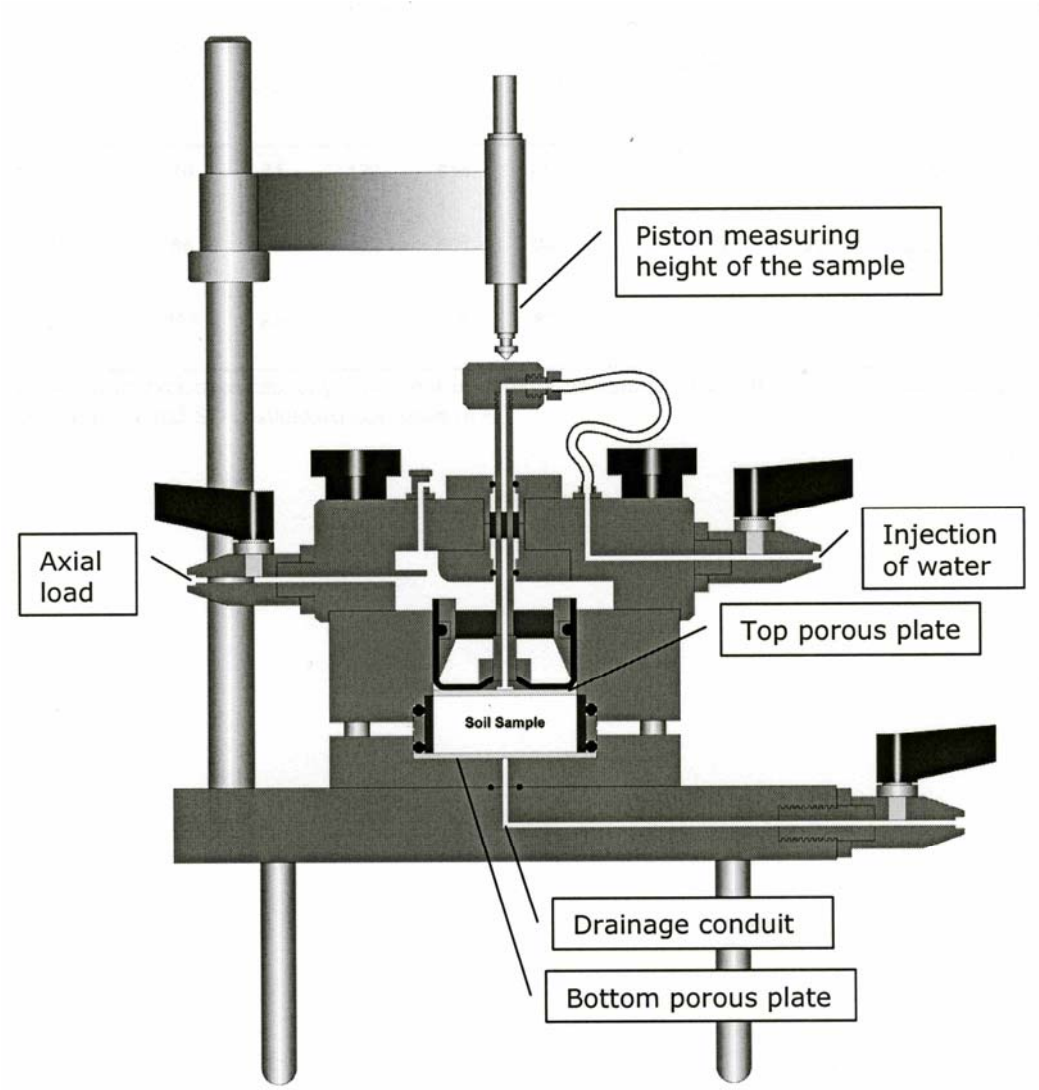

Fig. IV-1. Oedometer schema.

Aggregates (3-5 mm) were poured into a 50 mm diameter ring using a small funnel fixed 5 cm above the middle of the ring located on a porous plate. The ring was overfilled and the surface was carefully levelled off with tweezers. The assemblage was then disposed in the oedometer and the top cap gently positioned.

At day 343 of incubation, samples with three concentrations of added maize straw (0, 5 and 20 g C kg^{-1} soil) were taken to measure uniaxial compression. Three different curves were obtained by flooding the samples at different stages: (i) where the samples were flooded at the start of the test (0.1 kPa) (i.e. wet curve); (ii) at 100 kPa and (iii) at

1500 kPa (dry curve). Three replications per curve were performed. The corresponding axial pressure was maintained during the injection of water which rated 25 mm^3 min^{-1} during 12 h. Water continued to be injected at a constant pressure assuring the wetting of the sample till the end of the test. This is also called the double-oedometer test (Assallay et al., 1996). The collapse or abrupt changes in the bulk density that occur when a soil is loaded and flooded is often called hydroconsolidation in soil mechanics.

2.3.6 Aggregate stability

We measured the soil aggregate stability according to Le Bissonnais (1996). The method is performed on 3 – 5 mm aggregates. It involves three pre-treatments with different subsamples before sieving in alcohol at 50 µm and dry sieving of the resulting fraction (> 50 µm).

Each pre-treatment attempts to separate different breakdown mechanisms: in the slow-wetting test, 6 g of aggregates are capillary rewetted with water on a tension table at a potential of – 0.3 kPa for > 60 minutes; in the fast-wetting test, 6 g of aggregates are immersed in deionized water for 10 min and in the stirring after prewetting test, the aggregates are saturated in ethanol for 30 min, then manually agitated in deionized water in an Erlenmeyer end over end for 20 times. Dry-sieving was performed by hand with a nest of six sieves (2,000, 1,000, 500, 200, 100 and 50 µm) and the mean weight diameter (MWD) or aggregate size distribution can be calculated as the sum of the mass fraction remaining on each sieve after sieving, multiplied by the mean aperture of the adjacent sieves.

3 Results

3.1 Water uptake and hydrophobicity

The addition of maize residues caused a rapid and significant decrease ($P < 0.01$) in soil water sorptivity (Fig. IV-2 b). Accordingly, the WDPT significantly ($P < 0.01$) and transiently increased with the addition of maize straw (Fig. IV-3), corresponding to slightly to strongly hydrophobic soil in the classification of Bisdom et al. (1993). The effect of added straw stabilized after 30 days, but remained significant up to 135 days of incubation.

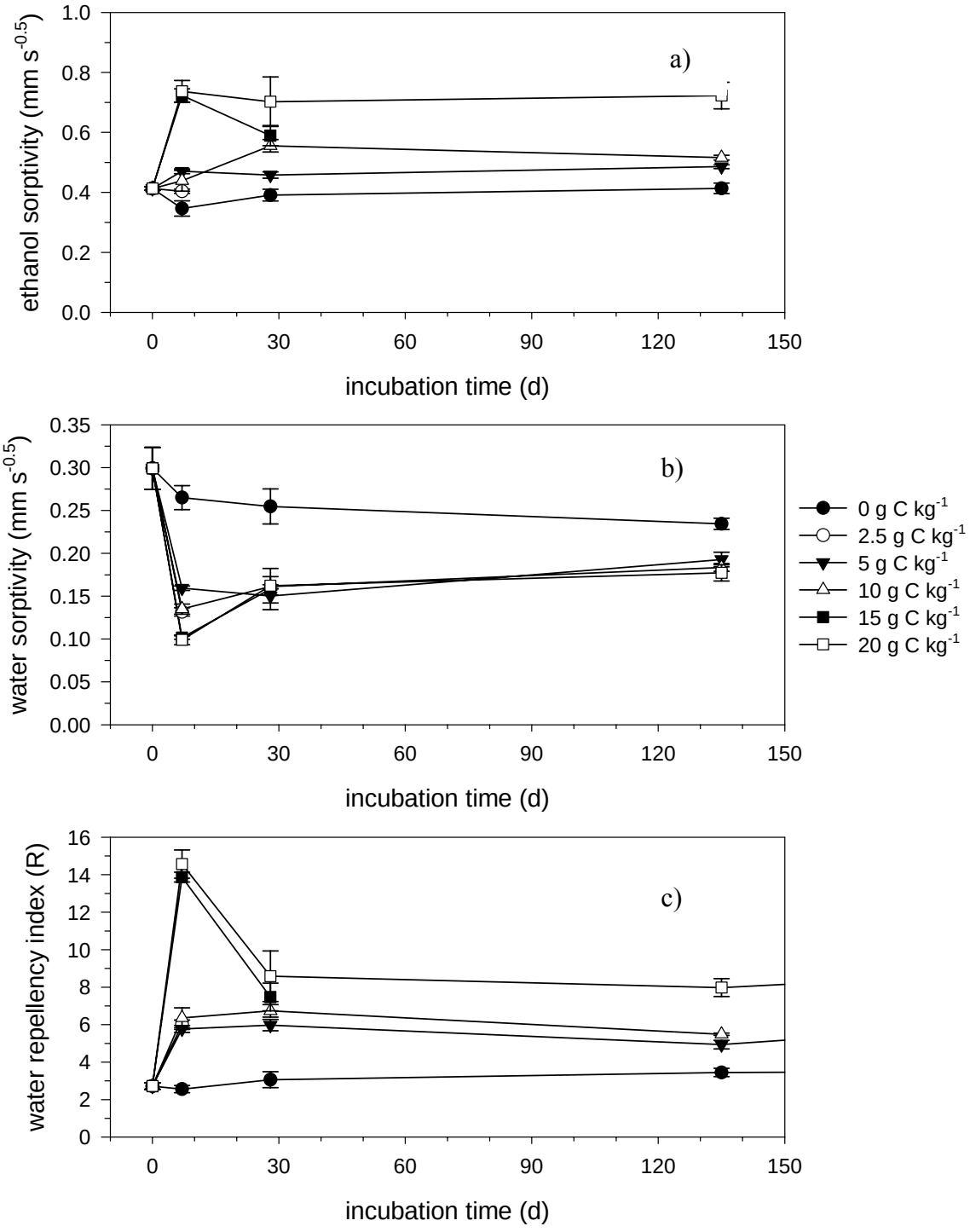

Fig. IV-2. a) Evolution of ethanol sorptivity, b) water sorptivity and c) water repellency index during incubation of 3-5 mm soil aggregates with 6 rates of added maize straw. Standard errors of the mean are indicated.

Only small variations during incubation were measured in control samples for both variables. WDPT was larger on the outer surface of aggregates incubated with straw than on internal surfaces, whereas there was no significant difference for control aggregates. Data not shown.

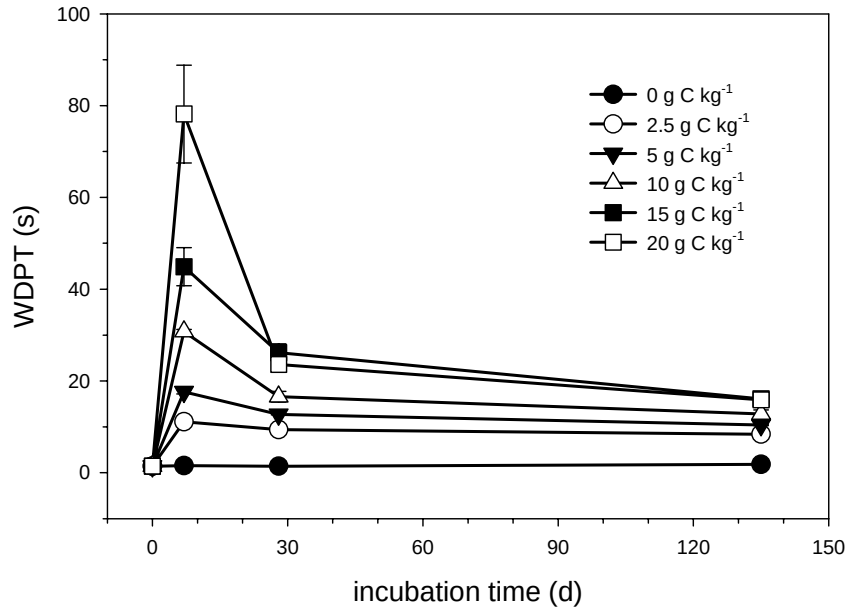

Fig. IV-3. Water drop penetration time evolution during incubation of 3-5 mm soil aggregates with 6 rates of added maize straw. Standard errors of the medians are indicated.

After 7 days of incubation, adding 20 g C kg^{-1} to soil increased the repellency index 5.7 times compared to the control (Fig. IV-2c). This difference decreased at 28-day and did not vary until 135 days, where it was 2.3 times higher than the control. The kinetics of WDPT during incubation was similar to that of R index showing a more pronounced peak at 7 days (51 times higher with the highest dose of straw added than with the control) and stabilization from 28 to 135 days of incubation.

R index and WDPT increased linearly (R^2 = 0.89 and 0.96 at day 7) with added C at 7 and 135 days of incubation (Fig. IV-4 a and b), however the slopes of linear regression lines at day 135 were smaller than that of 7 days for both variables (Fig. IV-4).

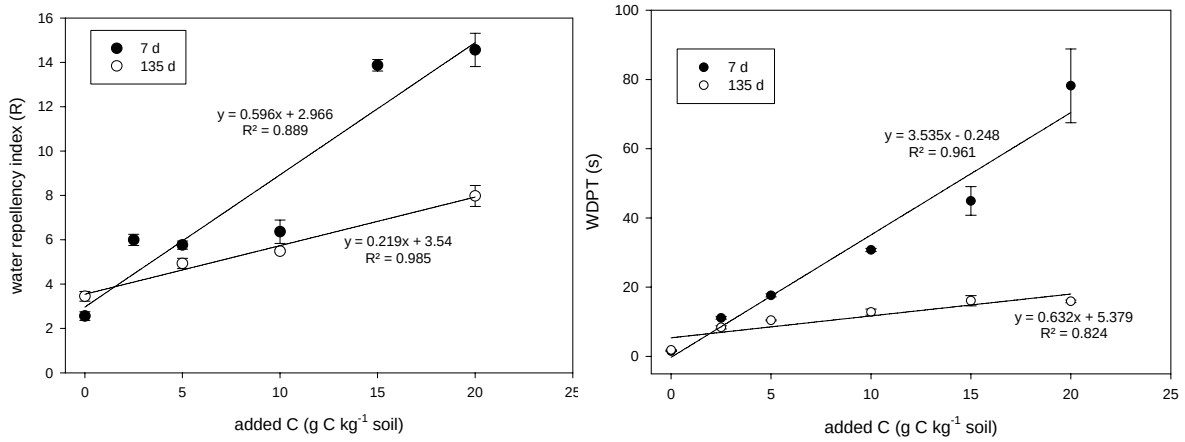

Fig. IV-4. a) Water repellency index and b) water drop penetration time as a function of maize straw addition the 7th and 135th days of incubation. Error bars represent the standard error of the means and medians respectively.

3.2 Pore system

We evaluated the change in pore system of aggregates due to the addition of maize straw from both the kinetics of ethanol sorptivity and aggregate density. Ethanol sorptivity was significantly and rapidly increased due to maize addition ($P < 0.001$ at day 7) (Fig. IV-2 a) and remained almost constant from day 7 to day 135 at all doses of C applied (no significant differences among incubation dates for 0, 5 and 20 g C kg^{-1} added – $P < 0.01$). Adding straw increased ethanol sorptivity by 112 % at day 7, to 74 % at the end of incubation with 20 g C kg^{-1} added with respect to the control treatment.

The aggregate porosity also increased with C added in a similar way to ethanol sorptivity (Fig. IV-5). All doses of C added increased aggregate porosity significantly ($P < 0.01$) at day 7 with respect to control and remained relatively constant to day 135. The aggregate porosity increased linearly with the amount of C added ($R^2 = 0.96$ and 0.97 for day 7 and 135 respectively) and the slopes of linear regressions between aggregate porosity and C added at day 7 and 135 were not significantly different.

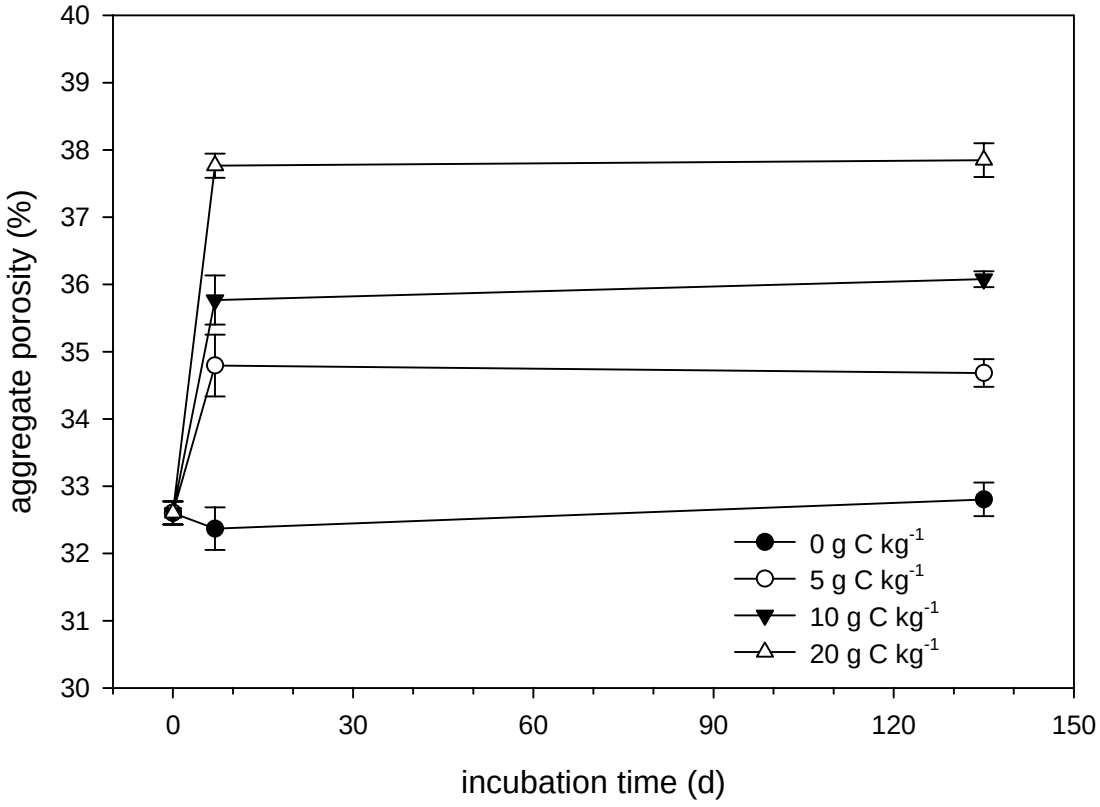

Fig. IV-5. Aggregate porosity over incubation time for 0, 5, 10 and 20 g C kg^{-1} soil of straw maize added.

3.3 Cohesion

The aggregate dry tensile strength did not vary between the control and added straw treatment at day 7. However, the tensile strength tended to increase with the addition of C, from a mean of 291 to 322 x 100 kPa for 0 and 20 g kg^{-1} added C respectively. Coefficient of variations were similar with ~26 %.

The uniaxial compression test showed that deformation due to axial load was significantly different between dry and wet curves independently of C addition rate. However, after 343 days of incubation the control tended to be more compressive and more sensitive to hydrocollapse (Fig. IV-6). The Δbulk density at 100 kPa diminished with increasing C but the Δbulk density of maximum and minimum values for dry and wet curves were not significantly different (Table IV-1).

Initial and final bulk density of aggregates beds significantly diminished with straw addition in ~8 % (i.e. a void ratio increasing from 1.36 to 1.57) (Table IV-1). The applied pressure at which the bulk density started to increase when soil is saturated was 10 kPa, irrespective of C addition.

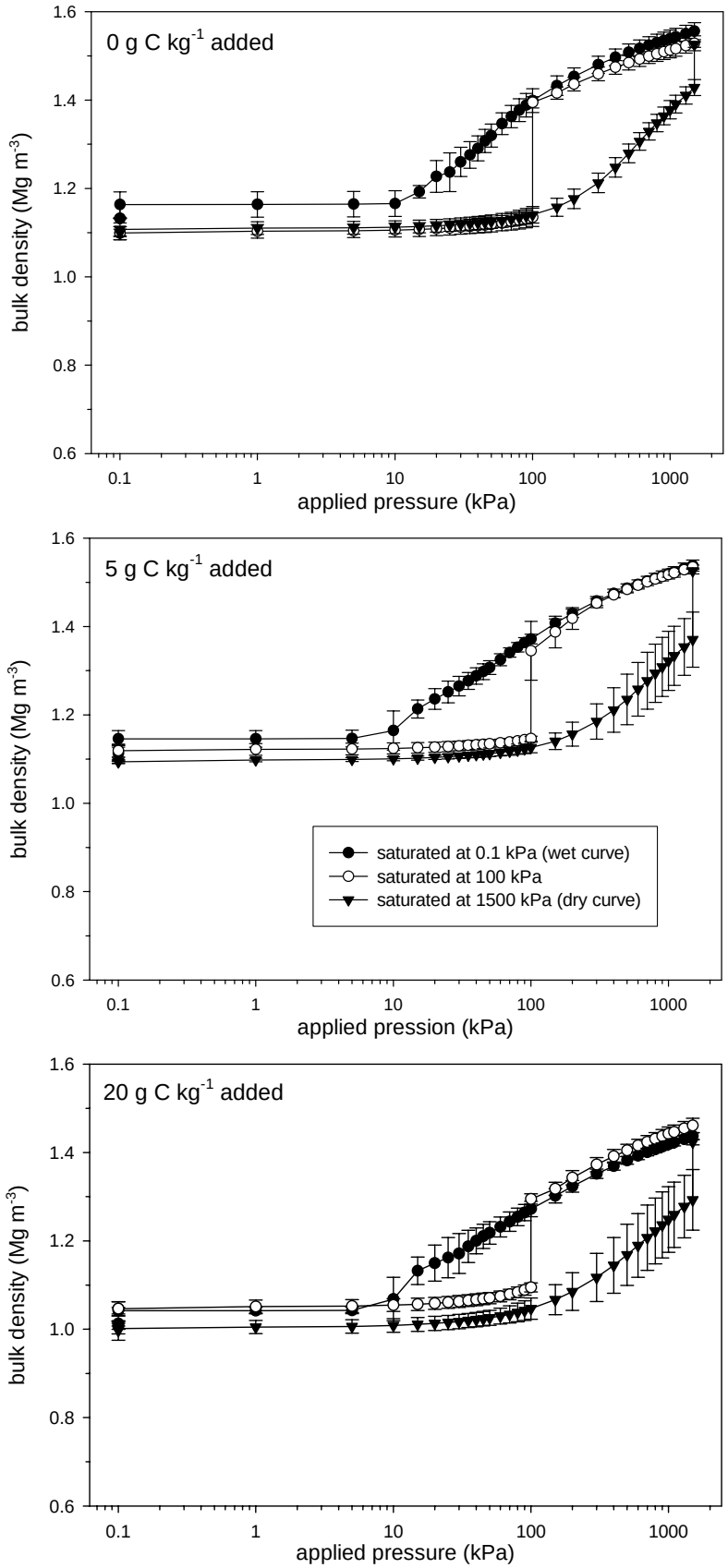

Fig. IV-6. Compression curves (oedometer test) with water injections at 0.1, 100 and 1500 kPa for three maize straw additions: a) 0, b) 5 and c) 20 g C kg^{-1} soil.

Table IV-1. Compression curve parameters from the oedometer test of 3 rates of added C after 343 days of incubation.

Parameters		Rates of C added (g C kg^{-1} soil)		
		0	5	20
Bulk density at 0.1 kPa (mean of 3 curves)	$Mg\ m^{-3}$	1.12a	1.12a	1.03b
Maximum bulk density (mean of 3curves)		1.537a	1.533a	1.440b
Δbulk density (max-min of dry curve)		0.316a	0.267a	0.284a
Δbulk density (max-min of wet curve)		0.39a	0.41a	0.39a
Δbulk density at 100 kPa (after and before saturation of 100 kPa curve)		0.26a	0.2b	0.2b
Inflexion point of wet curve		at 10 kPa	at 10 kPa	at 10 kPa

Values followed by a different lowercase letter among rates of C added are significantly different according to Tukey's test ($P < 0.05$).

3.4 Aggregate stability

We previously reported the results of the aggregate stability tests during the incubation experiments, as MWDs and found that adding straw significantly increased aggregate stability for the three aggregate stability tests and with the same dynamics, aggregates being more stable at day 7 than at day 135. Here, we expressed the results as size distributions after the tests (Figs. IV-7, IV-8 and IV-9) and found that the stirring after prewetting test and the slow wetting test showed a similar aggregate size distribution; i.e. increased proportion of aggregates > 2 mm with the addition of C, and decreased other size classes (Figs. IV-8 and IV-9). In the fast wetting test, the organic matter also increased the proportion of aggregates > 2 mm and diminished the abundance of size classes of 0.05-0.2 mm and < 0.05 mm. There was not a clear effect of OM addition on the proportion of 0.2-2 mm aggregates after the fast wetting test, presumably because of the counteracting of simultaneous gain and loss of aggregates (Fig. IV-7).

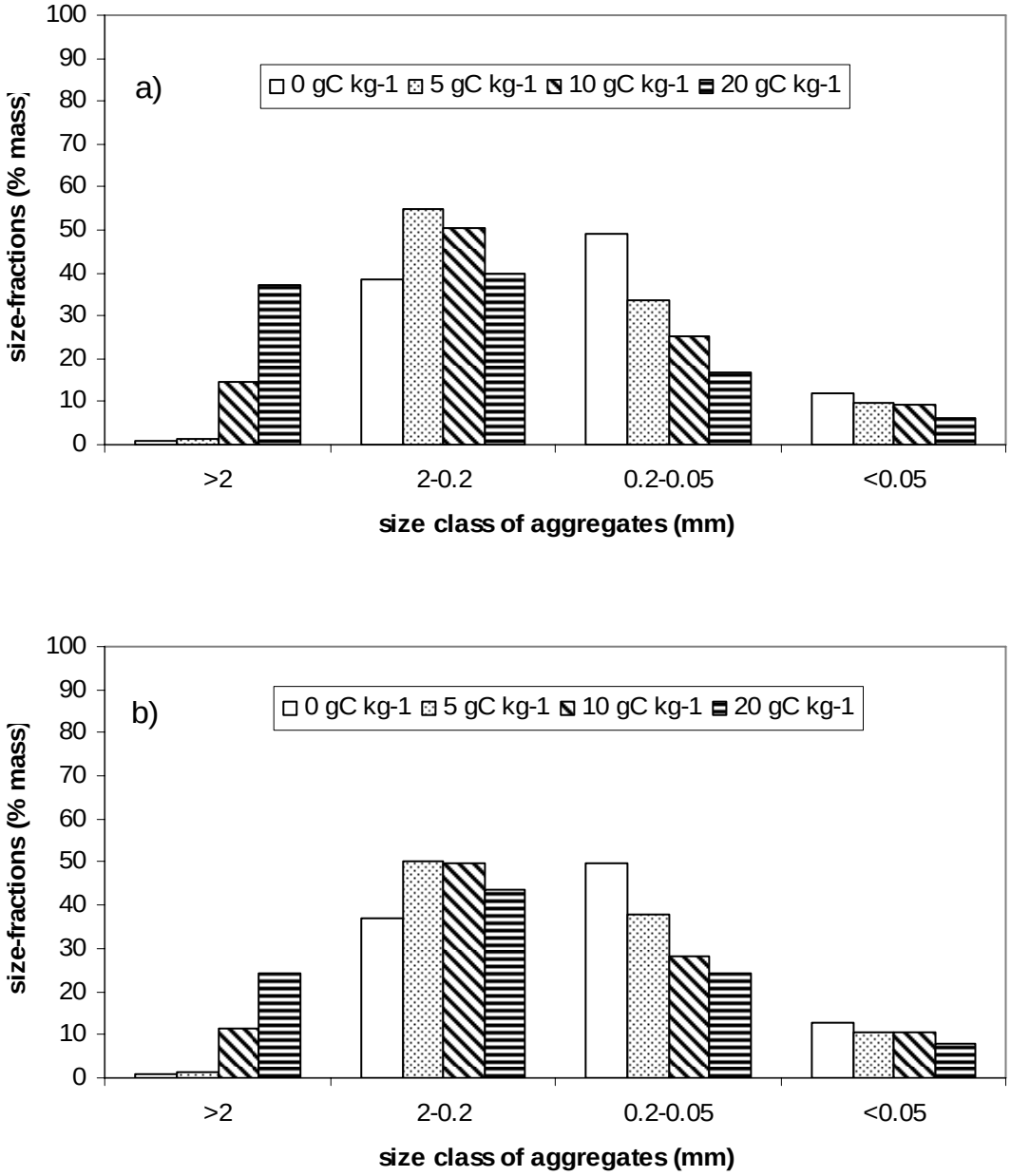

Fig. IV-7. Aggregate size distribution after the fast wetting test for four maize straw additions after a) 7th and b) 135th days of incubation.

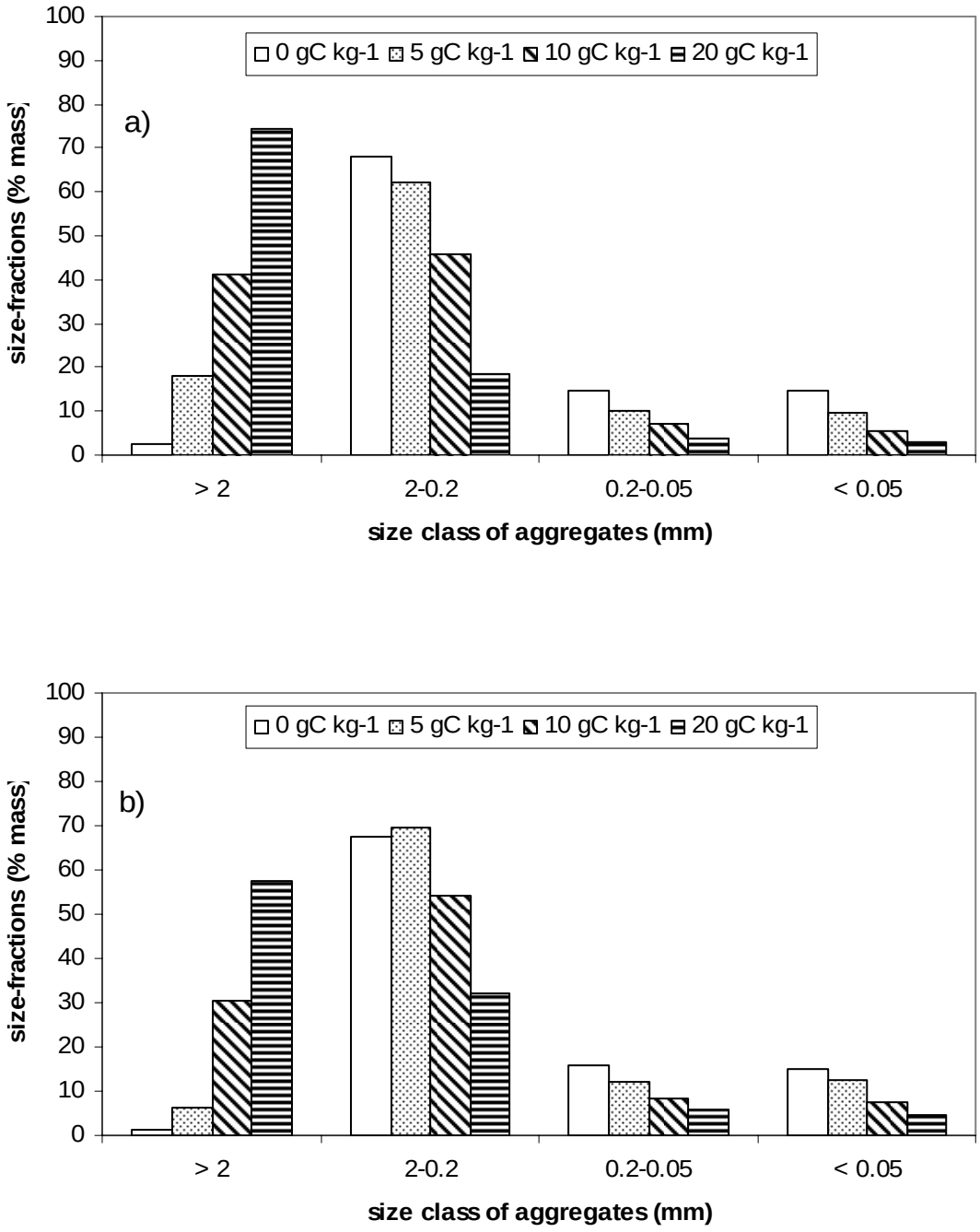

Fig. IV-8. Aggregate size distribution after the stirring after prewetting test for four maize straw additions after a) 7th and b) 135th days of incubation.

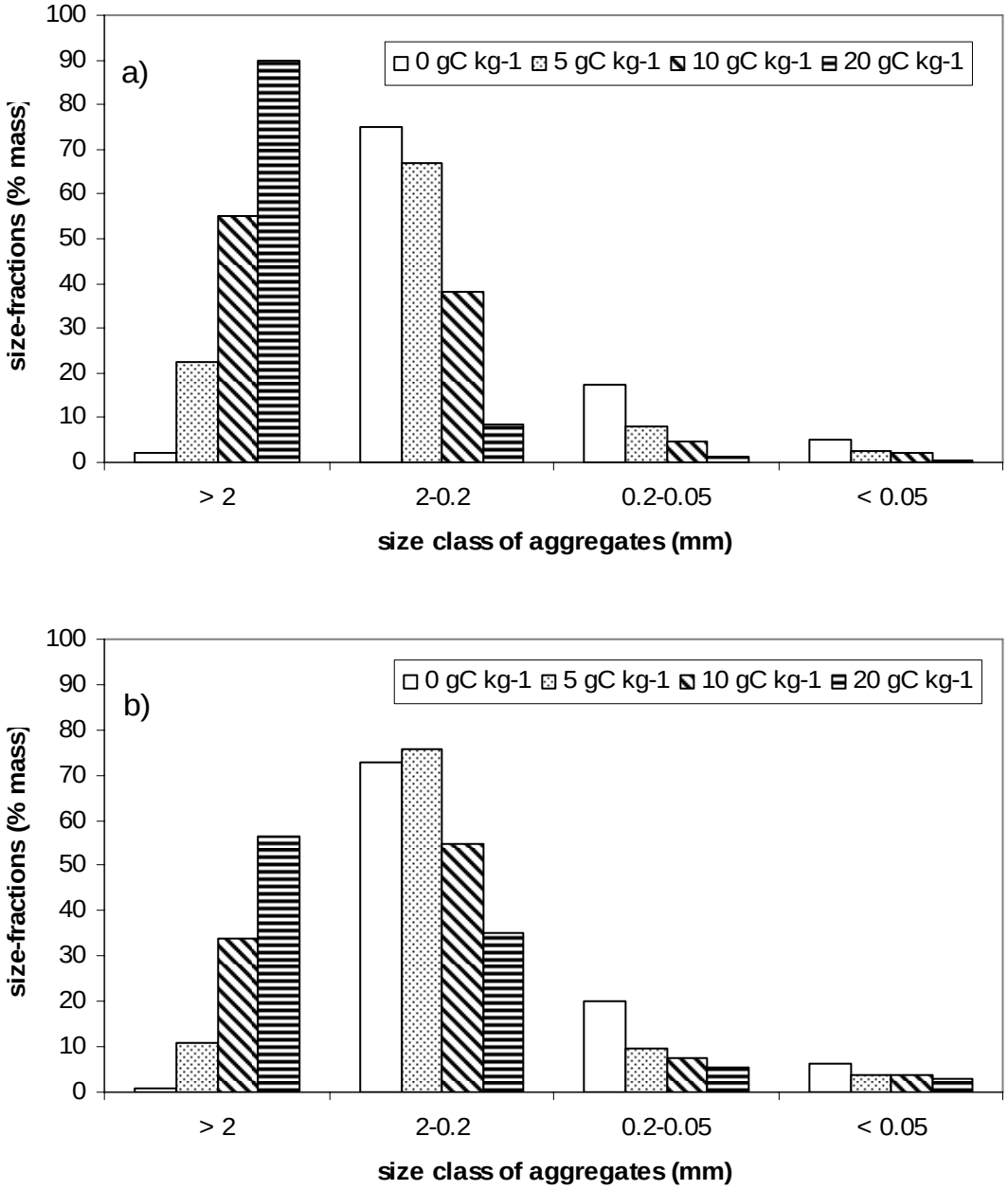

Fig. IV-9. Aggregate size distribution after the slow wetting test for four maize straw additions after a) 7th and b) 135th days of incubation.

3.5 Biological variables

All biological variables measured were significantly and linearly correlated ($P < 0.01$) with the WDPT and repellency indexes (Table IV-2). Variables that peaked early in the incubation (< 7 days) and stabilized at 28 days were better correlated than the others (in chapter 2), as found for microbial biomass-C, HWEC and respiration rate. These properties therefore had better correlation coefficients than ergosterol content.

Table IV-2. Correlation coefficients (r) among WDPT, R index and biological and C related variables

	Microbial biomass-C	Ergosterol	HWEC	Respiration rate
WDPT	0.923	0.726	0.893	0.916
R index	0.810	0.744	0.930	0.828
Added C	-	0.842	0.892	-

HWEC: hot-water extractable carbohydrate-C; WDPT: water drop penetration time; R index: water-repellency index. All values were significant at least at $P < 0.001$.

4 Discussion

We performed a simple experiment in which we incorporated fresh residues in a range of rates to natural soil aggregates to measure at the same time the physical properties that determine aggregate stability and the aggregate stability itself. The latter was assessed with three tests that relatively emphasize different breakdown mechanisms (slaking, microcraking and mechanical breakdown) controlled by the physical properties, basically the rate of wetting and cohesion.

4.1 Porosity

Using two different ways to characterize the pore system (aggregate porosity – Fig. IV-6 and ethanol sorptivity – Fig. IV-2a) we found that the decomposition of plant residues added to soil increased the total porosity, and also its capacity to infiltrate liquids. This

effect did not vary from the early stage of incubation until at least 4 months for any rate of residue addition; hence it was probably due to the abundant microorganisms present at this stage of incubation and remained thereafter, although the microbial populations decreased after day 7. We assume that this additional porosity was not due to the straw particles themselves because they were initially located outside of the aggregates.

The decomposition of added organic matter was previously shown to cause an increase in total porosity (Pagliai & Vittori Antisari, 1993; Zhang, 1994); and recent studies based on Xray-CT tomography found that porosity was increased in the vicinity of the added plant residues (De Gryze et al., 2005; Feeney et al., 2006). The addition of easily utilizable organic substrates modifies the soil cracking pattern, in terms of connectivity, density and heterogeneity at such different scales from µm (Dorioz *et al.*, 1993) to cm (Preston *et al.*, 1999). This mechanism is microbially-mediated since cracks are generated by dry-wetting events where physical discontinuities exist, these being either due to the microbial bodies themselves or to contrasted shrink-swell capacities of polysaccharide-rich zones compared to rather mineral zones (Dorioz et al., 1993). Dry-wet events are not a necessary condition since fungi may create < 30 µm pores as was suggested by Emerson & McGarry (2003) by leaving voids behind their dead hyphae.

Contrastingly, bacteria were shown to decrease the hydraulic conductivity in sand and soil columns because they clogged pores with their biomass and extracellular polysaccharides (Vandevivere & Baveye, 1992) (Wu et al., 1997). This did not occur in our samples, where the poral system became more capable to infiltrate liquids, if neglecting hydrophobicity.

4.2 Hydrophobicity

In a previous study, we compared three methods commonly used to measure water repellency :WDPT, Repellency Index and the Capillary Rise Method (Chapitre 3). We found a good correlation of their results, consistent with the theoretical relationships linking them. We emphasized that the soil rate of wetting or water uptake is controlled both by hydrophobicity, which is a surface property of solids, and by the quality and volume of pore network. Here, we observed that another consequence of adding residues to soil aggregates was a marked increase of their hydrophobicity over all the incubation time, but particularly at day 7 (Fig. IV-2c). As described above, the addition of maize straw also

induced a more "open" and conductive poral system, property that has an opposite action on the rate of wetting compared to hydrophobicity. However, the final result was a highly reduced rate of wetting with water, as show by the water sorptivity (Fig. IV-2 b) and the WDPT (Fig. IV-3). WDPT cannot separate the effect of hydrophobicity from that of a change in the pore system. Increased WDPT by organic matter was also found by Chenu et al., (2000) and Cosentino et al., (2006a). The repellency index, using two liquids allows to determine hydrophobicity independently of porosity. Hallett & Young (1999) and Hallett et al., (2001a) also found increased soil hydrophobicity when adding organic substrates to soil and thereby stimulating microbial growth and activity.

It is generally accepted that organic compounds derived from living or decomposing plants or microorganisms may cause hydrophobicity (Doerr et al., 2000). Vegetation with a high amount of resins, waxes or aromatic oils, may induce severe water repellency, impeding water infiltration and causing overflow erosion (Wallis & Horne, 1992); whereas subcritical water repellency is widespread in cultivated soils. In our experiment, the developed hydrophobicity was due to microorganisms since the added maize straw was partially hydrophilic. We measured the contact angle of water on the maize straw with the capillary rise method (Michel et al., 2001) and we obtained a mean angle of 83°. Patterns of hydrophobicity and WDPT dynamics were similar to that of microbial variables (Chap. 2). Indeed, respiration rate and microbial biomass-C were highly correlated with WDPT and R index (Table IV-2). Hallett & Young (1999) suggested that fungi are the dominant microbial group causing water repellency. However, Feeney et al. (2005) found a poor relationship between fungal biomass and water repellency. In our work the peak of hydrophobicity was earlier than that of ergosterol and thus seems to be more related with general microbial activity, rather than only to the presence of living hyphae.

It is still not clear which compounds are hydrophobic. Lipids may be responsible for microbially induced soil repellency, as suggested by (Capriel et al., 1990). Bacteria as well as yeasts exude lipids. Melanins are dark pigmented polymers which are present in the fungal wall or exuded in the media. Melanins are hydrophobic and resistant to biodegradation (Martin *et al.*, 1959; Martin & Haider, 1979).

Fungi commonly exude a specific class of molecules in the medium, hydrophobins. When secreted in the media hydrophobins self-assemble to form highly insoluble amphiphilic membranes. Several mycorrhizae exude an insoluble class of proteins,

glomalin, well correlated with aggregate stability (Wright & Upadhyaya, 1998; Piotrowski et al., 2004). Glomalin was suggested to stabilize aggregates by rendering them more hydrophobic (Wright & Upadhyaya, 1998).

A specific feature of the amended aggregates was that the increased hydrophobicity was restricted to the outer surfaces of the aggregates. At time 0 we mixed the ground straw with preexisting aggregates. Straw was thus located on the surface of the aggregates. Studies on the spatial extent of the detritusphere (Gaillard et al., 1999) suggest that in our experiment the microbial development was very concentrated on the aggregate surfaces of superficial layer. Indeed, abundant hyphal filaments were observed on the aggregate outer surfaces. The changes in physical properties of the aggregates by microorganisms were thus very localized. However, they modified the overall stability of the aggregate to disruption by water.

Goebel et al. (2005) ascribed the reduced rate of entry of water in OM-rich aggregates to hydrophobicity; contrastingly Zaher et al., (2005) and Caron et al. (1996) attributed it to pore clogging by OM. In our case, we found no evidence of pore clogging with enhanced microbial activity, but hydrophobicity was increased.

4.3 Cohesion

Interparticle cohesion opposes the increase in internal pressure generated by fast wetting (Concaret, 1967). Soil cohesion is not easy to measure directly, because it depends on texture, poral system, organic matter, water content, structural form and furthermore, on the spatial scale being considered. Here, we investigated aggregate cohesion in three ways:

(i) we measured the tensile strength of individual dry aggregates. The tensile strength of soil aggregates depends on soil cohesion and correlates well, in general, with soil resistance to water, compaction or machinery (Guerif, 1988). However, its determination is restricted to brittle aggregates with elastic deformation until rupture (Rogowsky et al., 1968), condition that is only achieved at or near air-dry states.

(ii) we performed uni-axial compression tests on wet beds of aggregates; their cohesion was measured at two scales: intra aggregate and interagregate, e.g. the contact between aggregates; and

(iii) we submitted the aggregates to mechanical energy provided by agitation in water, after having rewetted the aggregates in ethanol and replaced it with water (stirring

after prewetting test). Here interparticle cohesion is involved at the intra aggregate scale, but neither the result of the stress nor the stress applied can be expressed in physically meaningful units.

We found that tensile strength and resistance to compression were unaffected by the addition of organic matter, even at the peak of aggregate stability. Contrastingly resistance to mechanical agitation was increased by decomposing organic matter. We expected to measure increased cohesion with the three methods. Indeed, two biological mechanisms may increase cohesion, bonding by extracellular polysaccharides and fungi (Chenu & Guérif, 1991; Degens et al., 1996), and we found that the addition of straw stimulated both hot water extractable polysaccharides, which are surrogates for extracellular polysaccharides (Haynes & Francis, 1993) and ergosterol (Chapter, 2, Table II-2).

One hypothesis for the absence of increased cohesion during the incubation with straw is that biological bonds are weak and cannot be detected in the dry state. However, adsorbed extracellular polysaccharides were shown to increase the tensile strenght of dry clay aggregates (Chenu & Guérif, 1991). Furthermore, the oedometry experiment was done in the wet state and exhibited no increase in cohesion. Then, another hypothesis is that the observed increased in porosity (confirmed by bulk density measurements in the oedometer experiments) counter-balanced the increased bonding between mineral particles, as suggested by (Zhang, 1994). Like in our experiment, several authors found no relation between organic matter addition and tensile strength (Dexter et al., 1984), or a slight one (Guerif, 1988); while others found a positive correlation (Rogowsky et al., 1968). The quality of organic matter an its degree of decomposition may affect the relationship (Zhang, 1994). We suggest that in our experiment interparticle cohesion was increased but that it did not increase the aggregate cohesion due to increased porosity.

A third hypothesis regards the spatial distribution of the binding agents. As underlined previously, microorganisms and their binding agents were probably located on the superficial layer of soil aggregates. A circlet of well-bound soil particles around the aggregates might protect it from collisions with aggregates but not from a crushing test.

From the initially added C and the cumulative respiration of treatments and control we can calculate the C added still not decomposed at any time of incubation as: C from straw not decomposed = C added$_{t0}$ – (cum respirationC_{ti} – cum respiration control$_{ti}$). Priming effect is neglected. Thus, 5 and 20 g C kg^{-1} initially added becomes 2.12 and 9.24

g C kg^{-1} at 343 days of incubation. When total residual organic C is calculated ($C_{res\ ti} = C_{t0}$ + $C\ added_{t0}$ – $cum\ respiration_{ti}$) the control, 5 and 20 g C kg^{-1} at 343 days of incubation are equivalent to 8.5; 10.6 and 17.7 g C kg^{-1} soil. This calculation corroborates that, at the end of incubation, the difference in C between treatments is still very important (25 and 100 % more residual C than control in 5 and 20 g C kg^{-1} initially added respectively). However, the relative increase in bulk density (i.e. Δbulk density) due to compaction applied was similar in wet and dry curves to 3 rates of C added. Only the hydrocollapse at 100 kPa was slightly but significantly ($P < 0.05$) greater in the control treatment than with 5 or 10 g C kg^{-1} initially added (Table IV-2). The most apparent impact of the addition of straw in compression curves was that the absolute values of bulk density diminished when added C increased (see bulk densities at 0.1 kPa and maximum bulk densities in Table IV-2). This behaviour agrees to the increased aggregates porosity and increased ethanol sorptivity with added C showed in Figs IV-6 and IV-2a and also to the similarity in aggregate tensile strength performed with the crash test. In other words, the two approaches in measuring cohesion for an individual or a bed of aggregates used suggest that the addition of C increased the volume of pores but did not modify interparticle strength. According to Dexter (1988) uniaxial compression of beds of aggregates express the ability of an aggregate bed to support a load in terms of tensile strength of the individual aggregates.

4.4 Contribution of the different aggregate physical properties to the extent of slaking, mechanical breakdown and differential swelling

As we demonstrated, the microbial activity decomposing the added straw enhanced the porosity, hydrophobicity and wet cohesion of soil aggregates and diminished the rate of wetting.

4.4.1 Slaking

One of the most destructive breakdown mechanisms in silty soils is slaking. Concaret (1967) showed that increased internal pressure when soil is fast wetted depends on hydrophobicity and the possibility of the entrapped air to escape. Thus, slaking depends on the rate of wetting of the aggregates, the ability of air to escape and hence pore

diameters, connectivity and tortuosity (Zaher et al., 2005) and interparticle cohesion, that can, or not, withstand the forces exerted by entrapped air. Microcracking depends on the swelling capacities of the material, the rate of wetting and interparticle cohesion. Hence, the resistance of soil aggregate to the two tests, fast wetting involving mainly slaking and slow wetting involving microcraking and some slaking should increase, with a decrease in the rate of wetting of the aggregates. Indeed, aggregate stability increases with WDPT and the repellency index (Fig. IV-10). Furthermore, the extent of swelling is expected to be decreased when hydrophobicity increases, which should promote a positive correlation between WDPT, R, and MDW to slow wetting. We used the same silty soil with different doses of C so, texture or clay mineralogy did not vary among treatments.

Although WDPT, R and MWD to fast and slow wetting were fairly well linearly correlated ($R^2 \sim 0.6$; $R^2 \sim 0.8$), we distinguished three zones. When sample hydrophobicity was low, i.e. WDPT < 5 s or R<4, MWD to fast or slow wetting was constant. When sample hydrophobicity was intermediate (5s ≤ WDPT≤ 40s or 4≤ R ≤11,5) MDW increased with the hydrophobicity of the sample. For higher values of WDPT and R, MWD did not increase with hydrophobicity. The threshold estimated independently for WDPT and R are consistent considering the theoretical relationships we established in chapter 3.

$$R = c\sqrt{WDPT}$$

where c is a fitting parameter that had a numerical value of 1.69.

If we take for example threshold of 4 and 11,5 for R, it gives threshold of 5.6 s and 46.3 s for WDPT.

These thresholds would mean that in our samples, the pore walls have to become hydrophobic enough for the resulting reduction in the rate of water to change the resistance of aggregates to wetting. Similarly, above an R of 11.5 or WDPT of 46.3 s a further reduction in the rate of wetting does not change aggregate stability, presumably because entrapped air has ample time to escape.

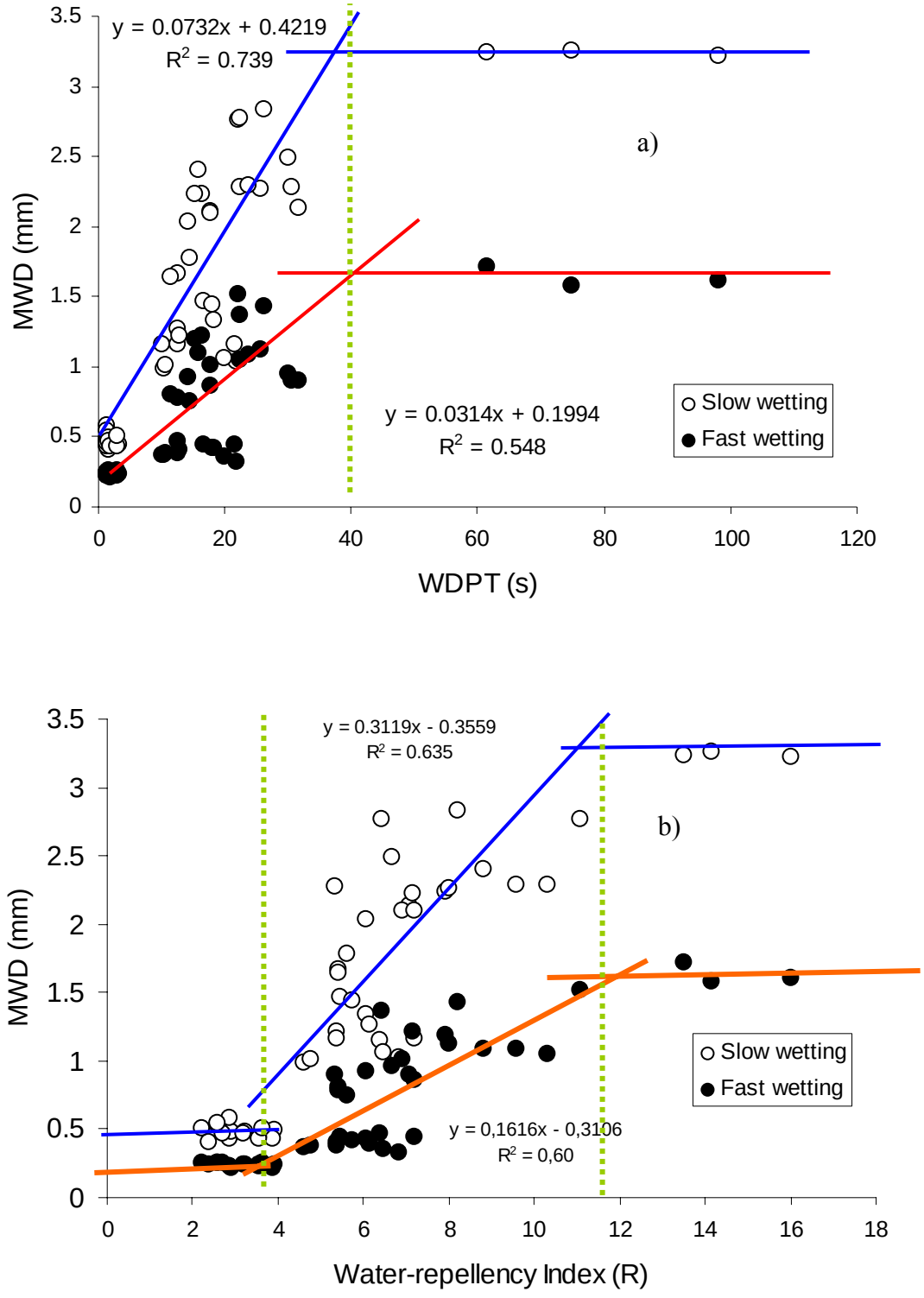

Fig. IV-10. Mean weight diameter (MWD) after the slow and fast wetting tests vs a) the water drop penetration time (WDPT) and b) the water-repellency Index (R). Dotted lines were fitted by hand.

Even though there are several articles that correlate aggregate stability with hydrophobicity (Chenu et al., 2000; Hallett et al., 2001b; Mataix-Solera & Doerr, 2004),

the concomitant increase of aggregation and water repellency was not always found. De Gryze et al. (2006a) observed that aggregate stability always increased after the addition of wheat straw to soil, but not repellency. Thus, they concluded that in some soils water repellence did not influence aggregate stability and that other stabilisation mechanisms dominated. In fact, articles showing enhanced hydrophobicity, after addition of hydrophobic substances, increasing microbial activity or fire, always showed increased aggregate stability (Capriel, 1997; Piccolo & Mbagwu, 1999; Chenu et al., 2000; Eynard et al., 2004; Mataix-Solera & Doerr, 2004; Woche et al., 2005), demonstrating a causal effect of hydrophobicity on aggregate stability.

4.4.2 Mechanical breakdown

The concomitant impact of C input on the rate of wetting and cohesion is showed by the relationship between WDPT and MWD after the stirring plus prewetting test (MWDsti) (Fig. IV-11). Although the increase in WDPT and MWD after the stirring test was simultaneous over all the incubation times (Fig. IV-10), their relationship is most likely not causal since microbial polysaccharides are generally hydrophilic substances (Chenu, 1993). Fungi can bond particles by exudate polysaccharides and, at the same time, produce hydrophobic substances when they colonize and enmesh soil aggregates. The slopes of MWDsti vs WDPT curves changed over time (Fig. IV-11) showing that the mechanism of increasing wet cohesion is independent of increased hydrophobicity and aggregate stability is the final result of several mechanisms with different kinetics over time.

Concaret (1967) proposed a model to describe aggregate stability:

$$s = C - Pi \qquad (4)$$

where s is the aggregate stability; C is the wet cohesion and *Pi* is the internal pressure.

The differential contribution of physical properties that govern structural stability could not be separated because after the addition of C, wet cohesion, hydrophobicity and the pore system were simultaneously enhanced. The conceptual model (4) seems to be validated and aggregate stability could be theoretically described as being a weighed function of several basic soil properties: repellency or the rate of water entry, cohesion, etc.

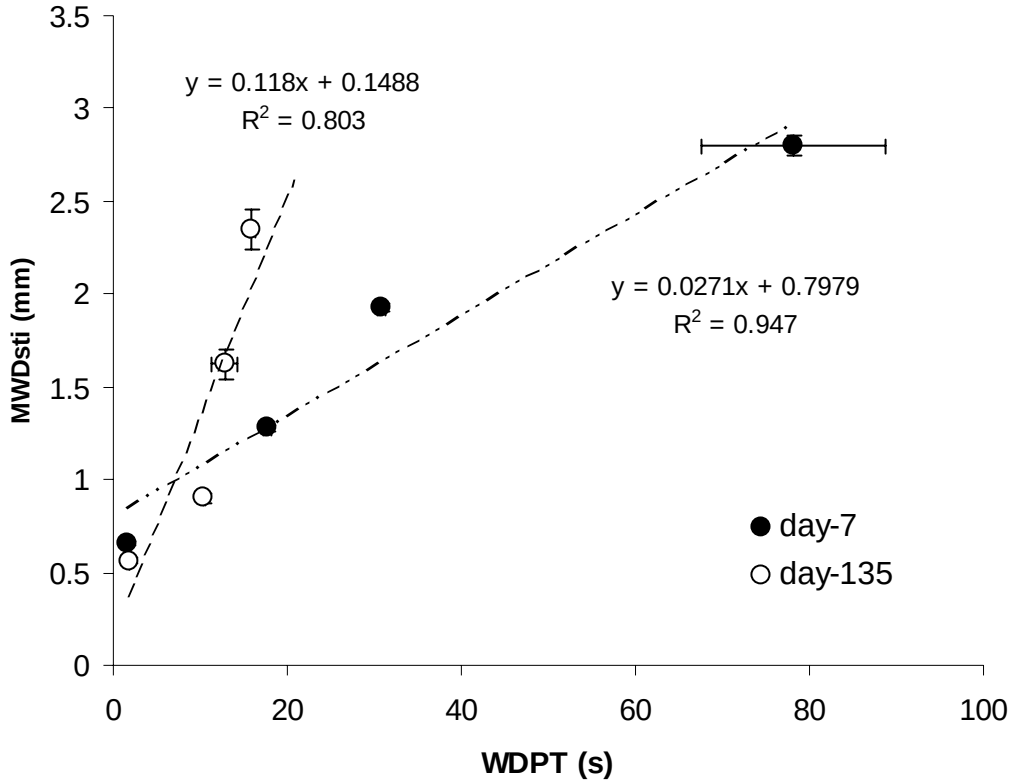

Fig. IV-11. Relationship between the mean weight diameter after the stirring after prewetting test and the water drop penetration time at day 7 and 135 of incubation. Bars represent the standard errors of the means.

4.4.3 Differential swelling

The last main breakdown mechanism measured was the breakdown by differential swelling (or microcracking). It was evaluated by the slow wetting test. Again, the addition of organic matter strongly increased the proportion of aggregates > 2 mm at the expense of < 2 mm aggregates. Twenty g of C kg^{-1} added increased 43 times the proportion of > 2 mm aggregates in relation to the control treatment (Fig. IV-9). According to Le Bissonnais (1996), the breakdown by microcracking depends on the same properties as slaking (including rate of wetting and cohesion), but the distinction is done because slaking decreases with higher clay content, whereas breakdown by differential swelling increases with it. Thus, the physical processes are different. However, the distinction is only apparent when comparing soils with different clay content or swelling potential.

The relationships among aggregate stability tests are showed in Fig. IV-12. Relative mean weight diameter (MWD/MWDmax) of fast and slow wetting test are linearly and

highly correlated with the stirring after prewetting test (both $R^2 = 0.97$). As stated previously, each test emphasizes different breakdown mechanisms that are controlled by different physical properties, which were all modified by the incorporation of organic matter. Fig. IV-11 clearly revealed that the addition of exogenous organic matter to a silty soil diminished the effect of all breakdown mechanisms at the same time independently of the dose of C added or the incubation time. Also, the relative impact of each breakdown mechanism to soil aggregates was the same, again irrespective of added C rate or incubation time. Thus, the stability to slaking (fast wetting test) was always ~50 % than stability to stirring (Fig. IV-12). Moreover, it is very surprising that the stability to slow wetting was most the same to stability to stirring (note the proximity of lines 1:1 and slow to stirring correlation in Fig. IV-12) even though the tests apply different kinds and intensities of stresses to soil aggregates.

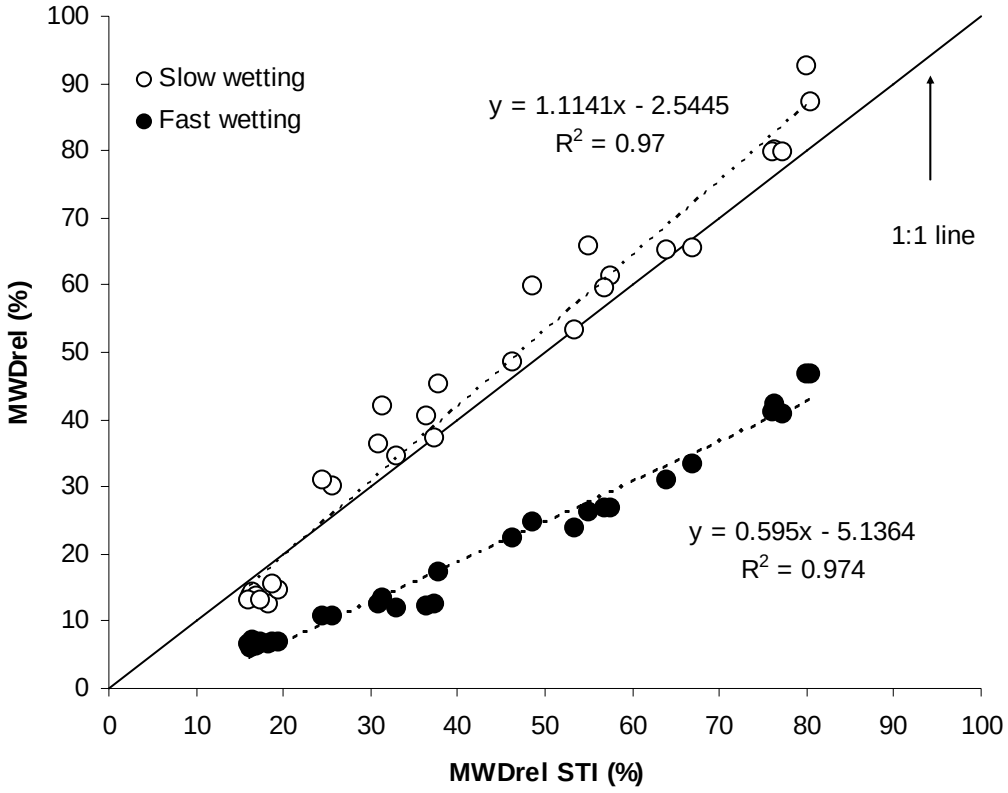

Fig. IV-12. Relative mean weight diameters (MWDrel: MWD after each test / MWDmaximal * 100) relationships between stirring after prewetting test and slow and fast wetting tests including all doses of C added and all incubation dates. MWD maximal = 3.5 mm.

Here, we hypothesize that since the effects of mechanical breakdown (stirring test) and breakdown by microcraking (slow wetting test) on aggregate stability were the same, both breakdown mechanisms depended on the same soil property, i.e. wet cohesion. It is important to take into account that we incubated aggregates with the same texture and without dry-wetting cycles. Thus, only the increased wet cohesion by microbial bonding and enmeshment controlled at the same time the stability to mechanical breakdown and to microcracking. The effect of different rates of wetting that can also influence microcracking was eliminated by comparing soils with the same texture and by the test itself (slow wetting at – 3 kPa during at least 1 hour).

5 Conclusions

- Adding fresh OM strongly modified the physical properties of a silty soil, rather poor in OM and unstable. The pore network was modified being more conducive to liquids, pore walls were rendered more hydrophobic and wet cohesion was increased.
- The relative impact of the different aggregate breakdown mechanisms was constant irrespective of added C rate or incubation time. The relative contribution of the stability to slaking and to mechanical breakdown for the energy applied was determined. We hypothesized that when C is added to soil the effect of differential swelling on the aggregate stability was negligible.
- All possible mechanisms of action of OM against the disruptive action of water were affected. Hence, it seems meaningful to still consider aggregate stability as an integrative property considering the effect of OM additions to soil aggregate stability.
- Here, we added one type of OM, maize straw, which had no direct effect on aggregate stability per itself. Whether different qualities of added organic matter can play differently on soil rate of wetting, hydrophobicity, porosity and interparticle cohesion, either by their direct effect, or by promoting the growth of specific microbial populations with specific by-products remains to be determined.

Chapitre 5

Impact des cycles de réhumectation et dessiccation sur la dynamique de la stabilité de la structure

Aggregate stability and microbial community dynamics under drying-wetting cycles in a silt loam soil [1]

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Résumé

La stabilité des agrégats a fréquemment une grande variabilité saisonnière et interannuelle quelque soit le type d'apport au sol. Cette variabilité est fréquemment plus grande que la celle entre différents types de sols ou systèmes de culture. Des variations de la teneur en eau et la stimulation saisonnière de l'activité microbienne sont les causes les plus citées. Le but de ce travail a été d`évaluer les effets des cycles de réhumectation et dessiccation sur la stabilité des agrégats et sur les principaux agents microbiens d'un point de vue mécaniste. Des agrégats calibrés de 3 à 5 mm d`un sol limoneux ont été incubés à 20ºC pendant 63 jours avec les traitements suivants et ses combinaisons : (i) avec ou sans apport de paille de maïs et (ii) avec ou sans exposition à quatre cycles de réhumectation et dessiccation. On a estimé l`activité microbiologique par la mesure de la respiration du sol et les agents microbiens de la stabilité de la structure par les polysaccharides extractibles à l`eau chaude, le carbone de la biomasse microbienne, et la teneur en ergostérol. On a mesuré le temps de pénétration d`une goutte d`eau pour estimer l`hydrophobicité et la stabilité de la structure par la méthode de Le Bissonnais (1996) pour distinguer trois mécanismes de désagrégation : l`éclatement, la microfissuration et la désagrégation mécanique. L`apport de paille de maïs a stimulé l`activité microbienne et a augmenté la résistance des agrégats aux trois tests de stabilité de la structure augmentant la cohésion interparticulaire et l`hydrophobie des agrégats. Tous les agents microbiens de la stabilité de la structure estimés ont répondu positivement à l`apport de matière organique et ont corrélés avec la stabilité des agrégats. La biomasse fongique est mieux corrélée avec la stabilité des agrégats que la biomasse microbienne totale, ce qui montre le rôle clé des champignons par leur contribution triple : enrobage physique, production de polysaccharide extracellulaires et de substances hydrophobes. Les cycles de réhumectation et dessiccation ont eu un impact moindre sur la stabilité des agrégats que l`apport de paille de maïs, mais ses effets ont été plus prononcés quand l`activité microbienne a été stimulée montrant une interaction positive.

***Mots-clés** : Stabilité structurale, cycles de dessiccation-réhumectation, La méthode de Le Bissonnais, temps de pénétration d'une goutte d'eau,Ergosterol; polysaccharides extractibles à l'erau chaude.*

[1] Ce chapitre est constitué d'un article publié dans *Soil Biology & Biochemistry* — Cosentino, D., Chenu C. and Le Bissonnais, Y. 2006. Aggregate stability and microbial community dynamics under drying-wetting cycles in a silt loam soil. 38, 2053-2062.

Abstract

Aggregate stability often exhibits a large inter-annual and seasonal variability which occurs regardless of residue treatments and is often larger than the differences between soils or cropping systems. Variations in soil moisture and seasonal stimulation of microbial activity are frequently cited as the major causes. The goal of this paper was to evaluate the effects of drying-rewetting cycles on aggregate stability and on its main microbially-mediated agents from a mechanistic point of view. The 3 – 5 mm aggregates of a silty soil were incubated at 20°C for 63 days with the following treatments and their combinations: (i) with or without straw input and (ii) with or without exposure to four dry-wet cycles. Microbial activity was followed by measuring the soil respiration. We estimated the microbial agents of aggregate stability measuring hot-water extractable carbohydrate-C, microbial biomass carbon and ergosterol content. We measured the water drop penetration time to estimate the hydrophobicity and aggregate stability according to Le Bissonnais (1996) to distinguish three breakdown mechanisms: slaking, mechanical breakdown and microcracking. The addition of straw stimulated microbial activity and increased the resistance to the three tests of aggregate stability, enhancing the internal cohesion and hydrophobicity of aggregates. All the estimated microbial agents of aggregate stability responded positively to the addition of organic matter and were highly correlated with aggregate stability. Fungal biomass correlated better with aggregate stability than total microbial biomass did, showing the prominent role of fungi by its triple contribution: physical entanglement, production of extracellular polysaccharides and of hydrophobic substances. Dry-wet cycles had less impact on aggregate stability than the addition of straw, but their effects were more pronounced when microbial activity was stimulated demonstrating a positive interaction.

Keywords*:Aggregate stability; Dry-wet cycles; Le Bissonnais method; Water drop penetration time; Ergosterol; Hot-water extractable carbohydrate-C*

1 Introduction

Aggregate stability often exhibits large inter-annual and seasonal variability. Aggregate stability is usually lowest during winter and increases in spring. Such variations occur regardless of residue treatments and are often larger than the differences between soils or cropping systems (Perfect et al., 1990; Angers et al., 1999).. Two factors are mainly controlling these fluctuations: climate and organic matter incorporations. Climate can directly influence aggregate stability through its action on soil moisture (Perfect et al., 1990) and indirectly through seasonal stimulation of microbial activity.

Soil moisture affects aggregate stability in several ways. First, the soil water content at the moment of the test impacts slaking. The extent of slaking decreases as the initial moisture content increases until saturation is reached (Panabokke & Quirk, 1957).

This effect is particularly evident in soils with low contents of organic matter (Haynes, 2000). Given the impact of sample physical conditions, such as aggregate size and soil moisture content before applying the test (Amézketa, 1999), it is obvious that the lack of a satisfactory standard methodology is a problem in this field (Le Bissonnais, 1996). The methods are, as well, often poorly described (Yang & Wander, 1998). The soil water content at the time of sampling thus impacts aggregate stability when it is measured on field moisture samples (Perfect et al., 1990). Furthermore, even though aggregate stability is measured on air-dried samples, the antecedent water content has been shown to affect aggregate stability (Caron et al., 1992). The effect of moisture on aggregate characteristics cannot be generalized and, alone, have no consistent effect on soil aggregate size and stability (Yang & Wander, 1998). Instead, soil moisture interacts with other events to influence aggregation.

It is not easy to link the influence of dry-wet cycles on macroaggregation as they can affect it directly through physical or chemical process (Utomo & Dexter, 1982) and/or indirectly through their action on microbial activity (Denef et al., 2001). Effects of drying on soil structure are still unclear, since both increases and decreases in water stable aggregation have been observed following drying (Denef et al., 2001). The contradictory results found in the literature can be explained by different initial physical conditions of the aggregates, organic matter contents, intensities and durations of drying and rewetting phases and aggregate stability methods. The term ‘initial physical conditions’ refers particularly to soil water content, aggregate size and degree of disturbance (that is to say, analyzing the process of aggregate formation or the process of aggregate stabilization). Finally, the cohesion of macroaggregates can be modified by ageing or thixotropy. As suggested by Suwardji and Eberbach (1998), inter-annual and seasonal variability in aggregate stability may result from seasonal wetting and drying interacting with the accumulation of plant and microbial debris associated with the growing crop.

The incorporation of organic matter to soil may also largely contribute to intra-annual variations in aggregate stability. It is well established that the addition of OM to soil increases aggregate stability within a few weeks due to the stimulation of microbial decomposers. Microorganisms increase the stability of aggregates in several ways. Fungi act mainly by mechanical enmeshment of soil particles (Degens et al., 1996), bacteria and fungi may exude extracellular polysaccharides which bond the particles and increase interparticle cohesion (Chenu & Guérif, 1991). Microorganisms have also been observed

to increase the repellency of soil aggregates, presumably by exuding hydrophobic substances (Capriel *et al.*, 1990; Hallett & Young, 1999). This may stabilize the aggregates by decreasing their rate of wetting.

Notwithstanding the complexity of factors that govern the short-term variations in aggregate stability in relation to soil moisture and OM additions, not too much effort has been given to understand these variations from an aggregate breakdown mechanistic point of view. Predictions of long-term changes in aggregate stability require a good record of the variability encountered within a single year, involving both a statistical description (Caron et al., 1992) and a mechanistic understanding of the factors governing aggregate stability. Such mechanistic approach is facilitated by the use of a method, based on three tests combining two liquids (ethanol and deionized water), that achieves the distinction of three basics breakdown mechanisms: slaking, mechanical breakdown and microcracking (Le Bissonnais, 1996).

The general objective of our study was to better understand short-term variations in aggregate stability for a temperate silty loam cultivated soil in order to improve its prediction. The goal of this paper is thus to evaluate the net effects of dry-wet cycles on aggregate stability and on its main microbially mediated agents. We hypothesized that there was an interaction between dry-wet cycles and microbial agents of aggregation. To evaluate this position, we carried out an experiment over 63 days under controlled conditions in the laboratory where we managed dry – wet cycles and microbial activity separately. We investigated the changes in aggregate stability in terms of slaking, mechanical breakdown and microcracking.

2 Materials and methods

2.1 Soil and sampling

We collected a surface soil (0-20 cm) from the “La Cage” plots of the experimental site of the Institut National de la Recherche Agronomique – INRA – (48°48’29”N, 2°04’58”E), in Versailles, France. The climate is temperate with an annual rainfall of 639 mm yr^{-1} (1928-2003) and 10.5°C annual mean temperature.

The soil was a silt loam Luvisol with a texture of 167 g kg^{-1} clay, 562 g kg^{-1} silt and 271 g kg^{-1} sand, with a total carbon content of 9.2 g kg^{-1}, C_t/N_t: 10.5 and pH(H_2O) of 7.0. The plot had been cultivated for more than 50 years with conventional tillage (mouldboard plow at 0-30cm) with a rotation based on wheat (Triticum aestivum L.), colza (Brassica napus L.) and pea (Pisum sativum L.).

In September 2003, one week after the seeding of colza, we carefully sampled the soil at a water content of 0.18 g H_2O g^{-1} soil. This work was carried out manually, using a shovel, to avoid disturbing the natural structure of the soil as much as possible. The larger clods were gently crumbled by hand at field moisture along their natural fissures and sieved to obtain an adequate amount of aggregates between 3.15 and 5 mm (hereinafter referred to as 3-5 mm aggregates). Great care was taken to avoid damaging the natural aggregates. After sieving, coarse organic matter (free roots and plant debris) were removed with tweezers and the soil was then stored in the dark at 4°C for two months (~0.15g H_2O g^{-1} soil).

The aggregates were stocked in a plastic box at 20°C for ten days before incubation to minimize the variations in microbial activity due to changes in temperature conditions (Kiem & Kandeler, 1997).

2.2 Incubation and experimental treatments

Sixty cores with 3 – 5 mm aggregates were incubated at 20°C ± 0.5°C for 63 days with the following treatments and their combinations: (i) without (-ST) or with (+ST) straw input (4 g C kg^{-1} soil) and (ii) without (W) or with (DW) exposure to four dry-wet cycles, characterized by a fast air drying and a slow rewetting to field capacity. Treatment -ST W was the control treatment; it was kept at field capacity (0.195 g g^{-1}, Ψ -10 kPa) and with no straw input. At field capacity the porosity was 42.8 % filled by water. The straw was from maize (Zea mays L.) stems and leaves ground between 200 and 500 µm (414.5 g C kg^{-1}; C/N: 54.7).

We placed the equivalent of 80 g dry sol (105°C, 24h) in plastic cylinders (diameter = 7.9 cm, height = 2.4 cm) closed at the bottom by a nylon cloth (20 µm mesh) suspended in sealed 1 l glass jars with 40 ml of deionized water at the base to minimize desiccation. The samples were carefully packed to obtain a bulk density of 1.2 Mg m^{-3}. A beaker

containing NaOH was also placed in the jars to trap and measure the CO_2 produced during incubation.

The samples with added maize straw were cautiously mixed and immediately sprayed with a solution of NO_3NH_4 to adjust the sample C/N ratio to 10 and its water content to field capacity. The added N suppresses the limiting effect of N during the decomposition of crop residues (Recous et al., 1995). The samples with no straw addition were treated in the same way, but with deionized water.

Four dry-wet cycles were started from day 7 of incubation and then every ~ 15 days (i.e. days 7, 21, 36 and 49). In each cycle (72 h) the samples were removed from the jars and put under a fan at room temperature (20 °C) for ~10 h until reaching the air-dry moisture content (0.012 g g^{-1}). Afterwards, the samples were slowly capillary-wetted to –3.1 kPa over a period of 48 h and to –10 kPa over a period of 14 h. The rewetting process was carried out on tension tables to minimize slaking. All samples were taken right before the start of each dry-wet cycle.

2.3 Measurements

To estimate soil respiration the evolved C-CO_2 trapped in NaOH was measured at days 2, 4, 7, 14, 21, 36, 49 and 63 in the treatments kept at field capacity. In those subjected to dry-wet cycles, the respiration was not measured during the cycles themselves (days 7-9, 21-23, 36-38 and 49-51).

At days 0, 7, 21, 36, 49 and 63 three replications per treatment were used to determine immediately in a moist subsample the microbial biomass carbon (MBC) by fumigation extraction according to Vance et al. (1987). We froze another subsample to measure within the week the ergosterol content (ERG) modified from Djajakirana et al. (1996) and Gong et al. (2001) ergosterol being a biomarker of fungi. The rest of the soil was dried at 40° C during 48 h and an aliquot was taken to estimate the hot-water extractable carbohydrate-C (HWEC) (Puget et al., 1999). Then we carefully re-separated the soil into aggregates from 3.15 (here referred to as 3 mm) to 5 mm. From these aggregates we assessed aggregate wettability by measuring the water drop penetration time (WDPT) as performed by Chenu et al. (2000) and aggregate stability (Le Bissonnais, 1996). Subsamples were also taken to measure and express all results at 105°C 24 h standard moisture content.

To estimate MBC, the difference between soil carbon (C) extracted with 0.03 M K_2SO_4 from chloroform-fumigated and unfumigated soil samples (20 g) were measured with a TOC-5050A Shimadzu elemental analyzer (Kc = 0.45). The ergosterol content was estimated with a Waters 2695 HPLC using 3.5 g of moist soil extracted with 120 ml ethanol agitated with glass beads for 30 min. HWEC were obtained from the extract of 1 g of soil suspended in 20 ml of hot water (80 °C) for 24 h, the carbohydrate content of the extract was analyzed by the H_2SO_4/phenol method (Dubois et al., 1956).

2.4 Aggregate stability

We measured the soil aggregate stability according to Le Bissonnais (1996) to distinguish three breakdown mechanisms: slaking, mechanical breakdown and microcracking. Briefly, the test is performed on 3 – 5 mm aggregates, dried at 40 °C for 48 h. It involves three pre-treatments with different subsamples before sieving in alcohol at 50 µm and dry sieving of the resulting fraction (> 50 µm) : (i) Slow-wetting, where the aggregates are capillary rewetted with water on a tension table at a potential of – 0.3 kPa for > 60 minutes; (ii) fast-wetting, where 5 g of aggregates are immersed in deionized water for 10 min; and (iii) stirring after prewetting, where the aggregates are saturated in ethanol for 30 min, then agitated in deionized water in an Erlenmeyer end over end 20 times. Dry-sieving was performed by hand with a nest of six sieves (2,000, 1,000, 500, 200, 100 and 50 µm) and the mean weight diameter (MWD) was calculated as the sum of the mass fraction remaining on each sieve after sieving, multiplied by the mean aperture of the adjacent sieves.

2.5 Statistical analyses

We used analysis of variance (ANOVA, 4x6x3 factorial design) at 6 sampling dates with three replications to determine the effects of straw and dry-wet cycles on MBC, ERG, WDPT, HWEC and structural stability. When effects were significant at a level of 0.05, means were tested with the Tukey test. For WDPT, we show the medians of 20 measures per repetition analyzed by a non-parametric test (Kruskal-Wallis One-Way AOV). Data are presented as mean or median values with standard errors. Pearson's correlations were made among variables.

3 Results

3.1 Biological variables

The respiration rates in +ST treatments were significantly ($P < 0.05$) higher than the -ST ones throughout the entire incubation except for the last period (49 to 63 d) (Fig. V-1). In the -ST samples the total microbial activity varied slightly during incubation whereas the +ST treatments showed a peak of the respiration rate at day 3 and its stabilization from day 28 at < 20 µg $C\text{-}CO_2$ g^{-1} soil per day. Only the first dry-wet cycle significantly increased the respiration rates, and it was more important in the +ST treatment.

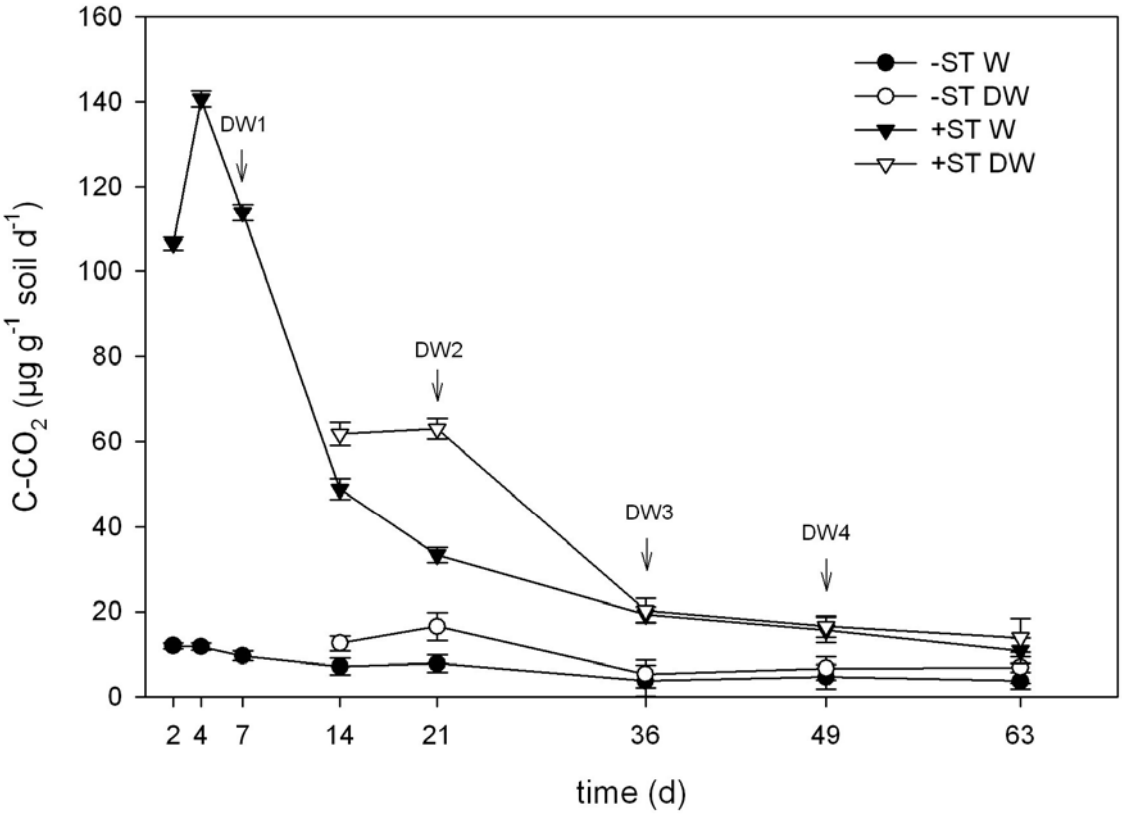

Fig. V-1. Soil respiration during incubation. No added straw (-ST), added straw (+ST), dry-wet cycles (DW) and continuously wet (W) treatments. Error bars represent the standard error of the means. DW events are indicated with arrows.

The addition of straw strongly increased MBC at all sampling dates (Fig. V-2), with a more persistent effect than on respiration rates. Incubations without straw affected MBC only slightly, showing a cyclical pattern over the incubation period. DW cycles affected MBC negatively up to the end of the experiment while maintaining a similar difference between +ST W and +ST DW treatments averaging 45 µg C g^{-1} soil in the last three DW cycles. The effect of straw addition was even stronger on the ergosterol content, with a peak at day 7, and a stabilization from day 49 (Fig. V-3). It was the biological variable that showed the largest difference between the +ST and the -ST treatments, about ten times at day 7 and nearly six times at the end of the experiment. However, almost no effects of dry-wet cycles were observed on ergosterol content over the 63 days of the trial.

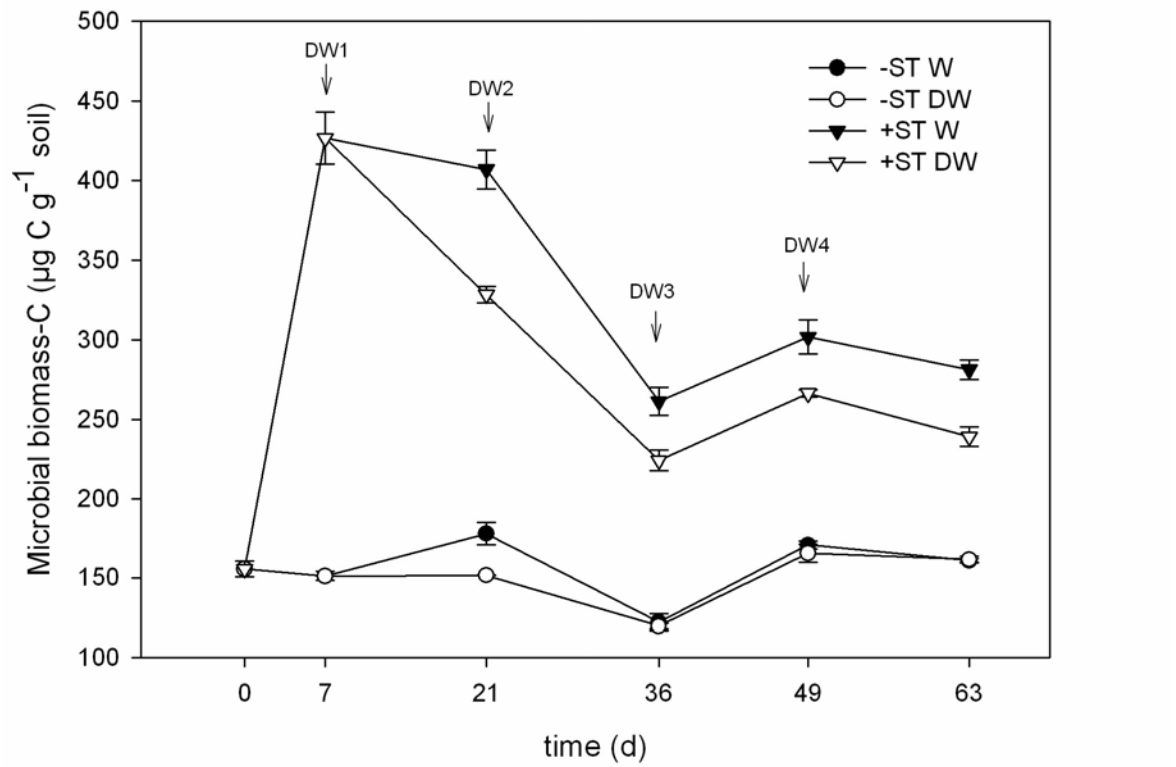

Fig. V-2. Microbial biomass carbon in control (-ST), added straw (+ST), dry-wet cycles (DW) and continuously wet (W) treatments. Error bars represent the standard error of the means (n = 3). DW events are indicated with arrows.

The amount of HWEC remained almost constant in the -ST treatments, averaging circa 180 mg C kg-1 (Fig. V-4). Adding straw increased polysaccharide contents by 57 % at day 7 to 47 % at the end of incubation, with respect to -ST treatments. In the +ST treatments, HWEC decreased significantly after the second dry wet cycle and remained low until after the fourth cycle, at which time the amounts of HWEC of both treatments became statistically the same.

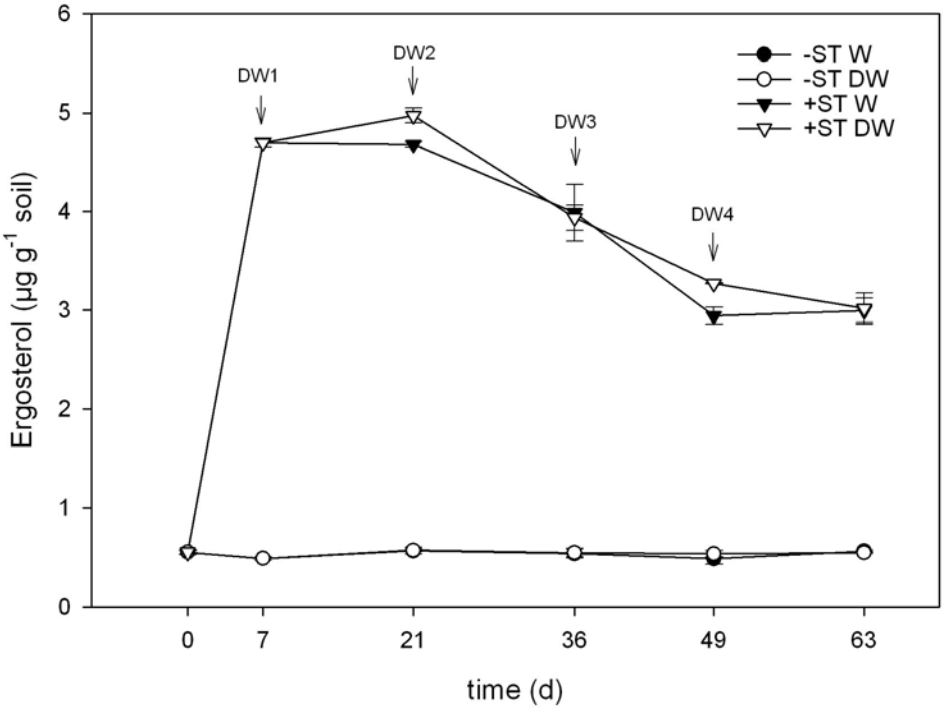

Fig. V-3. Ergosterol content in control (-ST), added straw (+ST), dry-wet cycles (DW) and continuously wet (W) treatments. Error bars represent the standard error of the means (n = 3). DW events are indicated with arrows.

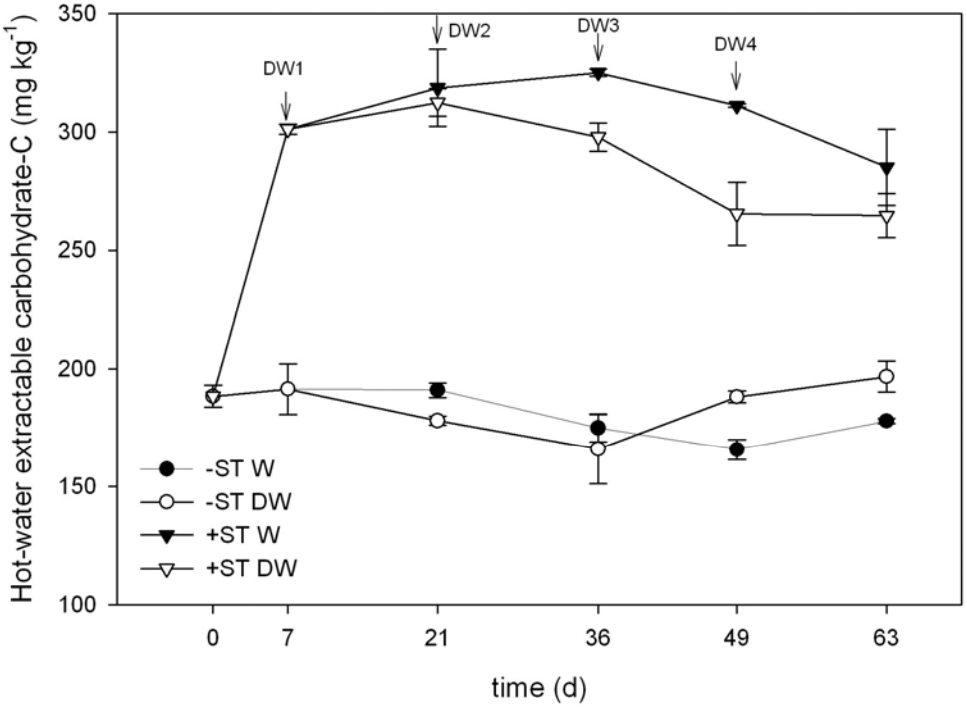

Fig. V-4. Hot-water extractable carbohydrate-C in control (-ST), added straw (+ST), dry-wet cycles (DW) and continuously wet (W) treatments. Error bars represent the standard error of the means (n = 3). DW events are indicated with arrows.

3.2 Aggregate stability and water drop penetration time

The silty soil used in this experiment exhibited very small MWDs after the tests at day 0, showing its low aggregate stability: 0.8 mm after the slow wetting test, 0.3 mm after the fast wetting test and 0.9 mm after stirring following the prewetting test (Fig. V-5). The MWD of the -ST samples varied little during incubation.

Both slow and fast wetting tests showed a similar response over the incubation time after the addition of straw but at different scales of MWD, showing the different intensities and kinds of stresses applied (Fig. V-5a, b). After the slow wetting test the MWD ranged between 0.6 and 2 mm, whereas after the fast wetting it was between 0.2 and 0.6 mm. In both cases, the addition of straw significantly increased the MWD by >110% and 60%, respectively, as an average from day 7 to day 63. The addition of straw also increased the resistance of aggregates to mechanical breakdown by about 28 % (Fig. V-5c).

The size distribution of aggregates after the tests, from which MWDs were calculated, is plotted in Figs. V-6, V-7 and V-8. When the slow wetting test was applied, the initial 3-5 mm aggregates were disrupted mostly to small macroaggregates 0.2-2 mm (69.6 % mass), large macroaggregates > 2mm (9.7 % mass), microaggregates (0.05-0.2 mm, 14.3 % mass) and to < 50 µm fraction (6.4 % mass). The fast wetting test was much more disruptive and released mostly microaggregates (41.6 % mass) and small macroaggregates (42.3 % mass) (Fig. V-6). The progressive breakdown of aggregates when subjected to increasing stresses (from slow rewetting and stirring after prewetting to fast rewetting) shows the hierarchical character of soil structure.

After the addition of straw, more macroaggregates (> 2 mm) resisted the slow wetting test than in the control –ST W treatment (Fig. V-7a, b). These were mainly formed at the expense of small macroaggregates. These large macroaggregates did not resist the fast wetting test (Fig. V-8). However, small macroaggregates were stabilized at the expense of microaggregates.

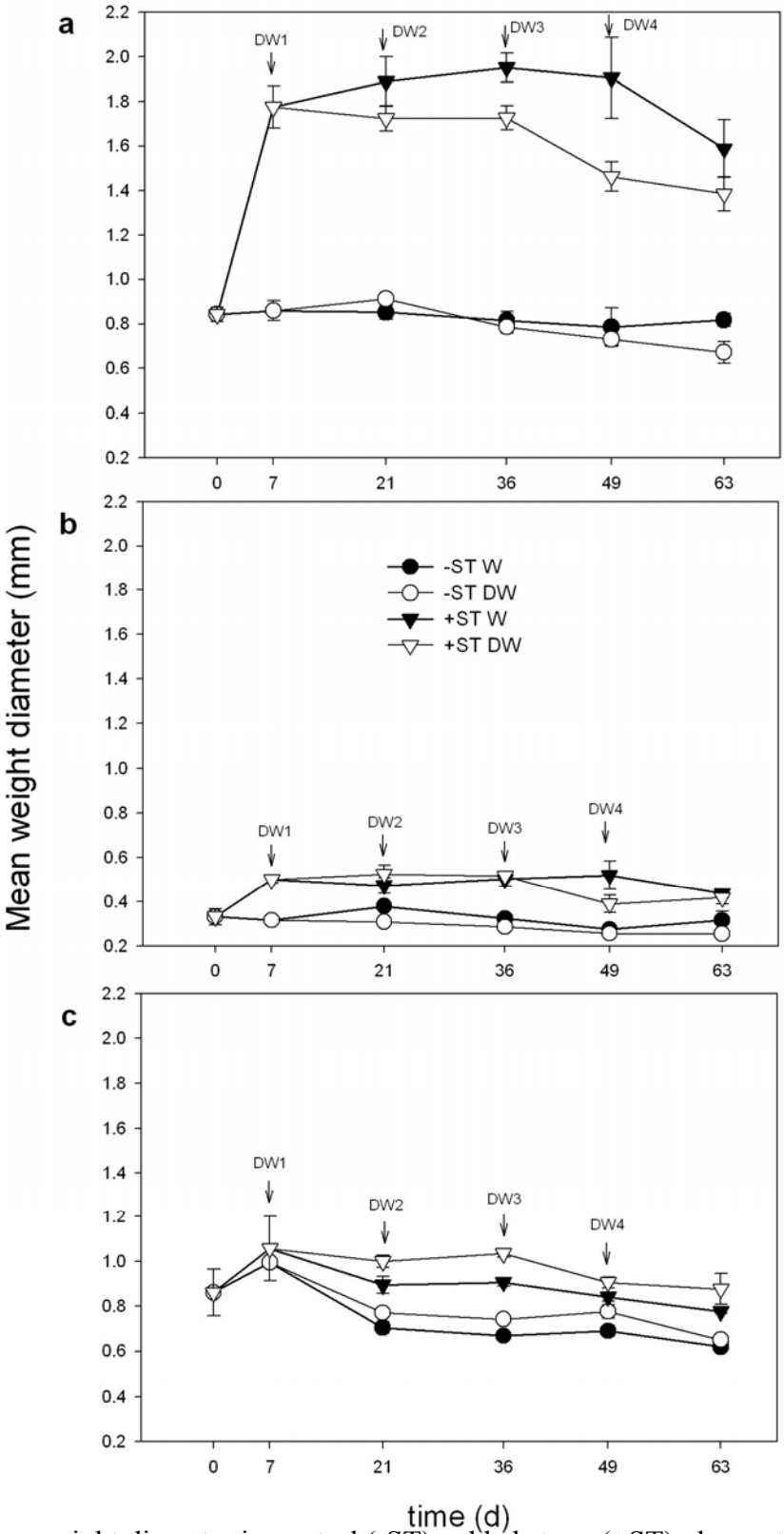

Fig. V-5. Mean weight diameter in control (-ST), added straw (+ST), dry-wet cycles (DW) and continuously wet (W) treatments for a) slow wetting test, b) fast wetting test and c) stirring after prewetting test. Error bars represent the standard error of the means (n = 3). DW events are indicated with arrows.

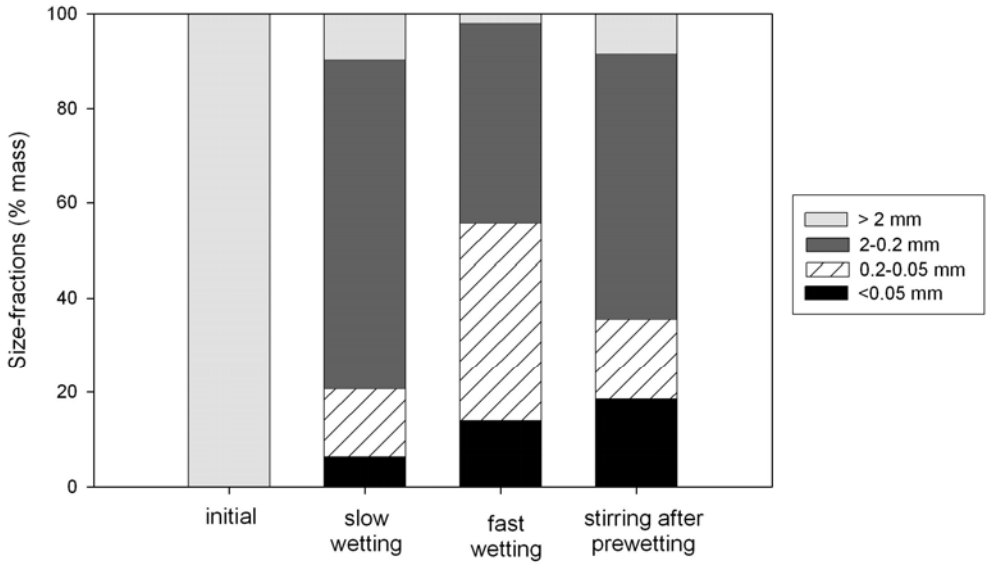

Fig. V-6. Aggregate size distribution of the control sample at time 0, initially and after the three aggregate stability tests.

In the -ST samples, the dry-wet cycles had no significant ($P < 0.05$) effect on aggregate stability (Fig. V-5). However, in the +ST samples, they decreased the MWD from the first cycle in the slow wetting test. No variations could be measured in the fast wetting test but the dry-wet cycles increased the MWD in the stirring after prewetting test because of the increase in the proportion of macroaggregates (> 0.2 mm). MWD decreased with incubation time for all treatments. Dry-wet cycles only slightly affected the size distribution (Fig. V-7b, c).

The aggregates of the silty soil were highly wettable, as shown by small WDPT (Fig. V-9). The addition of straw increased WDPT up to 10 s after one week of incubation and it then remained stable from day 7 to day 63 (Fig. V-9). The error bars of +ST treatments were higher than the control ones, showing a physical heterogeneity of the organic components that determine this variable. The WDPT was not affected by the DW cycles.

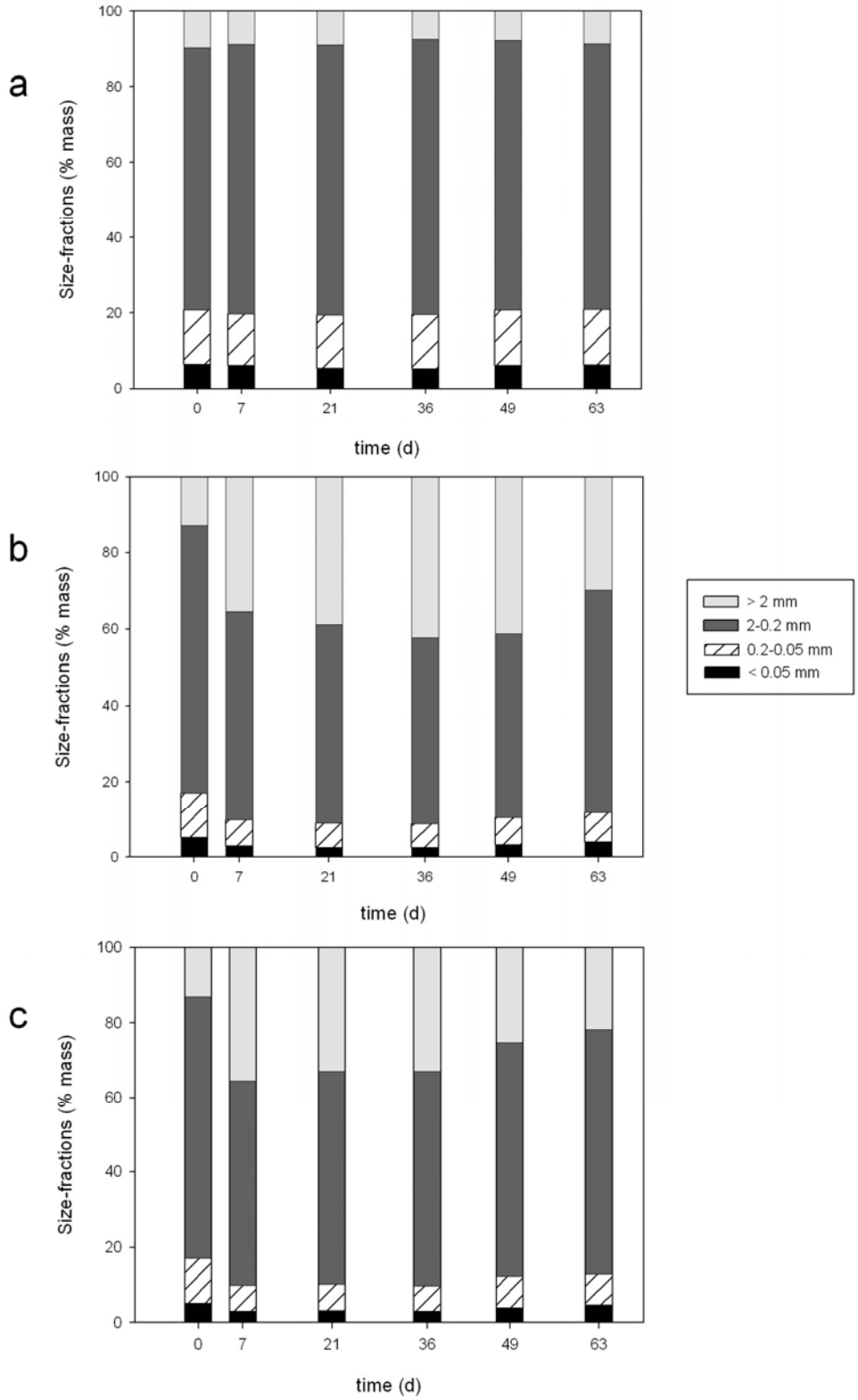

Fig. V-7. Aggregate size distribution after the slow wetting test. a: control continuously wet; b: added straw, continuously wet and c: added straw with dry-wet cycles.

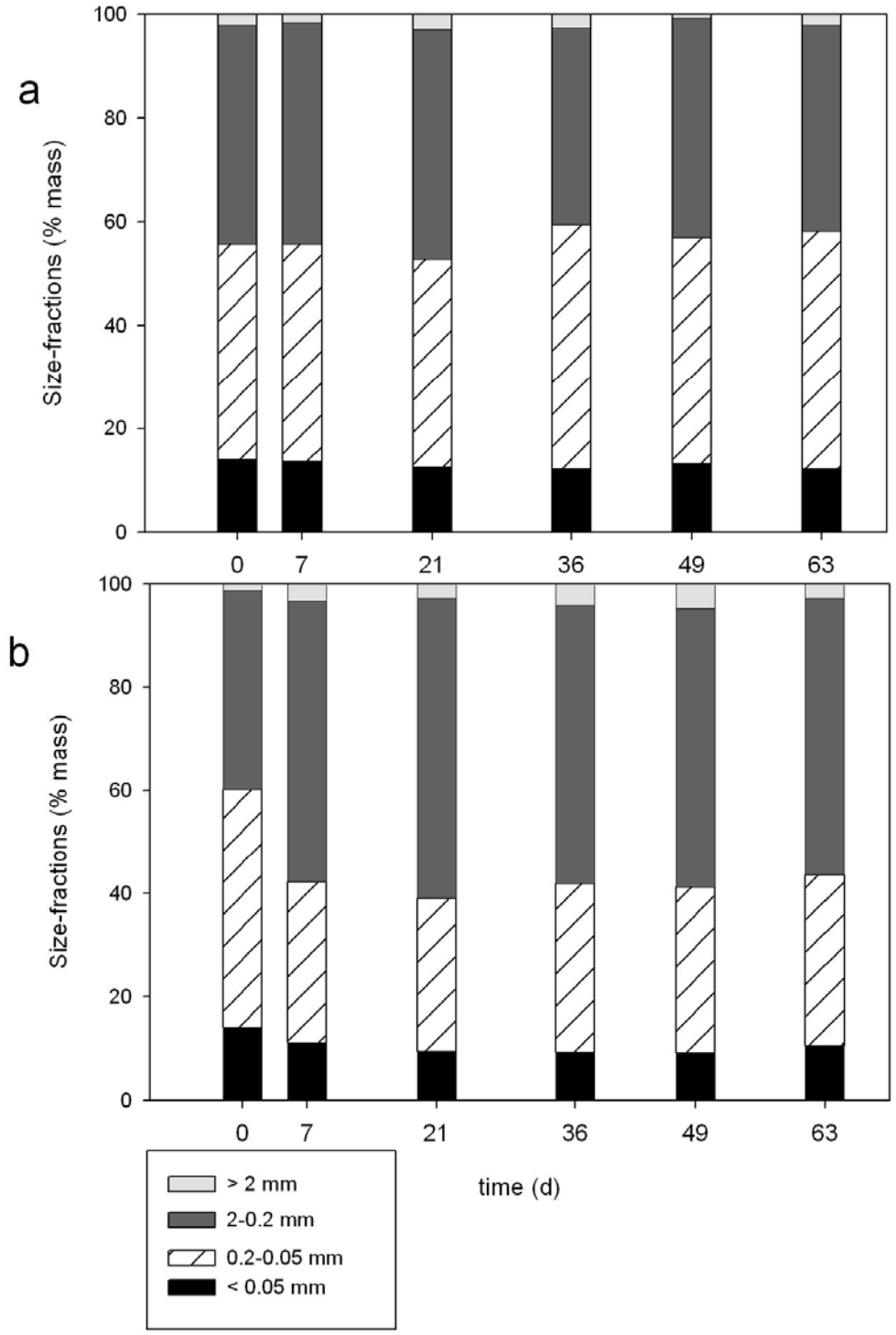

Fig. V-8. Aggregate size distribution after the fast wetting test in continuously wet treatments; a: control and b: added straw.

4 Discussion

4.1 Effect of organic matter addition on aggregate stability

Aggregate dynamics occur in three phases: formation from non-aggregated material, stabilization and breakdown (Tisdall & Oades, 1982). Here, by adding organic matter to 3-5 mm pre-existing aggregates, we focused on the stabilization of these preexisting aggregates. These had very low stability, which is typical of silty cultivated soils from northern Europe.

Many studies showed a positive, short-term effect of adding organic matter on soil aggregate formation and stabilization (Kiem & Kandeler, 1997; De Gryze *et al.*, 2005), which is due to the activity of microbial decomposers. Our results also showed a rapid increase of aggregate stability, which peaked 7 days after the beginning of the incubation. Straw had no effect at time 0. Therefore, all observed effects on aggregate stability were due to microbial activity. Furthermore, the method used here enabled us to analyze the results in mechanistic terms. Le Bissonnais (1996) reviewed the theories about the mechanisms of aggregate breakdown, highlighting three main elementary mechanisms for non dispersed soils: (i) slaking, breakdown caused by compression of entrapped air during wetting; (ii) mechanical breakdown by raindrop impact and (iii) microcracking, or breakdown by differential swelling. The method we used attempts to separate these mechanisms (Le Bissonnais, 1996). The fast wetting test emphasizes the slaking, the stirring after prewetting test isolates the wet mechanical cohesion independently of slaking and the slow wetting test expresses mainly the breakdown of the aggregates by microcracking, although some slaking also takes place. Organic matter may stabilize aggregates in two ways: by increasing the interparticle cohesion and by increasing their hydrophobicity, thus decreasing their rate of wetting (Robert & Chenu, 1992). In addition, organic matter may modify the porosity of aggregates and thus the extent of slaking.

The net, although small effect of straw addition on MWD after the stirring after pre-wetting test (Fig. V-5c) showed that microbial activity increased the internal cohesion of aggregates. WDPT increased after straw addition, which showed that microbial activity increased the hydrophobicity of aggregates, in line with results from Hallett and Young (1999). Increased stability of aggregates to fast and slow wetting tests, i.e. increased resistance to slaking and microcracking, involved increased hydrophobicity of aggregates.

Accordingly, in our experiments, the WDPT correlated well with the MWD after fast wetting test and with the MWD after slow wetting test, but it was not with the MWD after the stirring after pre-wetting test (Table V-1). Effects of microbial activity on the pore size distribution of aggregates cannot be ruled out and were not studied in this work. For example Preston et al. (1999) showed that the stimulated microbial activity produces less heterogeneous and less connected soil cracks, suggesting that polysaccharides and fungal hyphae not only contribute to aggregate stabilization through a bonding action, but also change the heterogeneity and connectivity of cracks, diminishing the negative breakdown of microcraking.

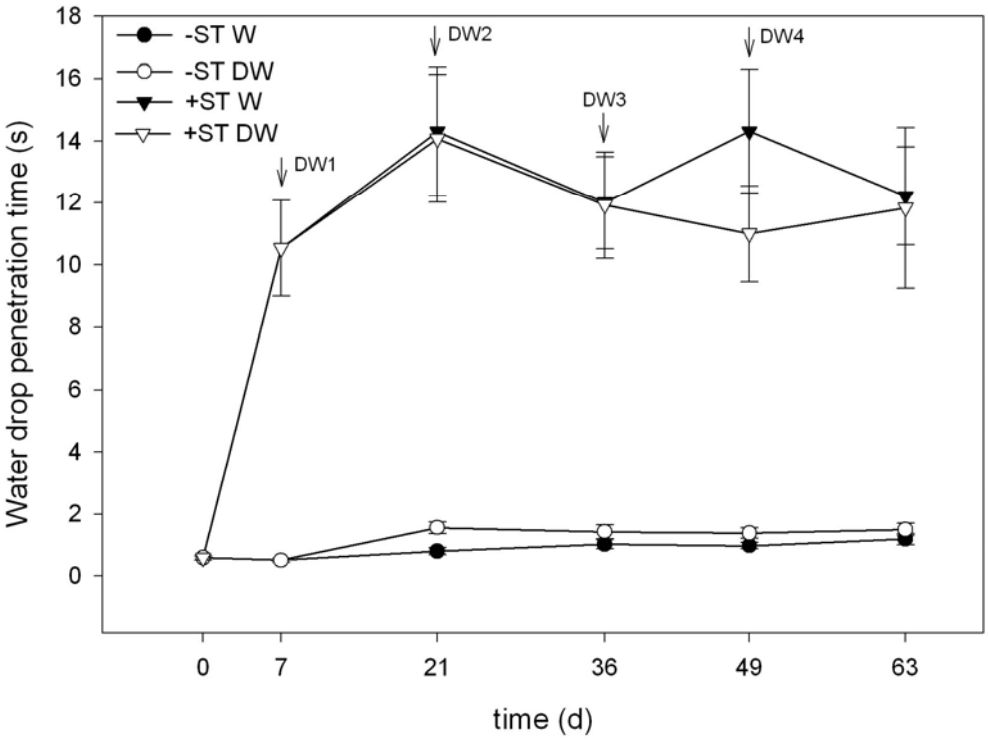

Fig. V-9. Water drop penetration time in control (-ST), added straw (+ST), dry-wet cycles (DW) and continuously wet (W) treatments. Error bars represent the standard error of the medians (N = 20). DW events are indicated with arrows.

Table V-1. Correlation coefficients (r) between measured variables.

	MBC	Respiration rate	ERG	HWEC	WDPT
Fast wetting	0.785*	0.575	0.889*	0.844*	0.887*
Stirring after prewetting	0.560	0.618	0.613	0.645*	0.467
Slow wetting	0.868*	0.603	0.950*	0.957*	0.956*
WDPT	0.836*	0.521	0.942*	0.911*	-

* significant values at $P < 0.001$ (n = 24).

MBC: microbial biomass carbon; ERG: ergosterol content; HWEC: hot-water extractable carbohydrate-C; WDPT: water drop penetration time.

4.2 Microbial agents of aggregate stability

It is well established that when fresh organic matter is added to soil, bacterial and fungal populations increase and stabilize aggregates by (i) direct enmeshment of aggregates and particles by fungal hyphae (Degens, 1997), (ii) production of extracellular polysaccharides by bacteria and fungi which glue mineral particles (Chenu, 1995), and (iii) production of hydrophobic substances (Capriel et al., 1990).

All measured microbial agents of aggregate stability (microbial polysaccharides, fungal biomass and hydrophobic compounds by measuring HWEC, ergosterol content and WDPT respectively) increased after addition of organic matter and were highly correlated with the slow and fast wetting tests (Table V-1).

We used the ergosterol content as an indicator of fungi. It was better correlated with aggregate stability than the microbial biomass. This is in agreement with several studies, based either on the use of bactericides and/or fungicides (Hu et al., 1995; Bossuyt et al., 2001), or on correlations (Chantigny et al., 1997). This prominent role of fungi compared to that of bacteria can be explained by the contribution of fungi to the three mechanisms cited above: physical entanglement, production of extracellular polysaccharides and production of hydrophobic substances. Here, high correlation coefficients between ergosterol and MWD for fast-wetting and slow-wetting aggregate stability tests were consistent with occurrence of the three roles of fungi. Furthermore, the close correlation of ergosterol with WDPT is consistent with an increased hydrophobicity due to fungi.

HWEC are a fraction relatively enriched in polysaccharides from microbial origin (Haynes & Francis, 1993) and are thus assumed to represent extracellular polysaccharides.

HWEC is a pool of C involved in changes in aggregate stability occurring over relatively short time-periods (Puget et al., 1999). In our experiments HWEC was significantly correlated with aggregate stability. The test the more closely related was the slow wetting test (Table V-1) and the time patterns of both variables are surprisingly similar (Figs. V-4 and V-5a). Thus, in agreement with Kiem and Kandeler (1997), aggregate stability depended more on the production of binding substances by microorganisms, than on microbial numbers.

4.3 Dry-wet cycles net effects

Dry-wet cycles had strong effects on microbially-derived variables over the first cycle: soil respiration was stimulated, MBC decreased, and ergosterol content slightly increased. The following cycles had no effect on respiration, no additional one on MBC, none on ergosterol and HWEC decreased. Magid et al. (1999) stated that in most papers showing a significant increase in respiration, the drying-rewetting process was accompanied by other physical changes: manual perturbation or increased temperature. We carefully manipulated the samples in such a way that no structural degradation and minimal temperature change occurred. The drying was done in only ~10 h. In addition, we rewetted the samples slowly to field capacity (-10 kPa). We assume, therefore, that previously protected organic matter was not de-protected by dry-wet cycles. Thus, as only the first dry-wet cycle significantly affected the respiration rate in the +ST treatment (Fig. V-1), we attribute it to the decomposition of the added straw which was not decomposed during drying (Magid et al., 1999) and, as microbial biomass decreased by 15-19 %, to the death of microorganisms. Subsequent dry-wet cycles did not affect respiration rates probably because the substrate became less available. Sorensen (1974) indicated that the effect of dry-wet cycles on the decomposition of organic matter decreases the longer the organic material is incubated in the soil. This suggests, according to Degens and Sparling (1995), that the dry-wet cycles have a stronger effect on mineralization of labile pools of organic matter.

Literature about the effects of dry-wet cycles on fungal and bacterial communities is not clear. Shipton (1986) reports that fungi can remain active in soils at very low water potential, contrarily to bacteria. Hattori (1988) suggested that fungi are more sensitive to drying and wetting because of their location on the outer surfaces of aggregates. West et al.

(1987) and Scheu and Parkinson (1994) found no consistent trends in the fungal and bacterial biomass after air-drying soils, indicating that the susceptibility of bacteria and fungi to drying is not generally different, and would rather depend on the bacterial and fungal communities growing in different soil materials. In our experiment, the ergosterol content was unaffected by dry-wet cycles, whereas total microbial biomass was. Hence fungi appeared to be more resistant than bacteria to dry-wet cycles, perhaps because of the short period of drying and the water potential applied ($\Psi << -1.5$ MPa).

HWEC were also affected by dry-wet cycles, especially in the +ST treatment, however, the decline in HWEC occurred later than that of microbial biomass and increased with time. Both bacteria and fungi can synthesize and exude extracellular polysaccharides and one study reported an increase in extracellular polysaccharides production by bacteria with drying (Roberson & Firestone, 1992). Here, we suggest that the decrease in HWEC with dry-wet cycles is due to bacterial death and that HWEC have a longer residence time that microbial biomass.

Several experiments report that dry-wet cycles disrupt aggregates and thus diminish the proportion of stable aggregates (Utomo & Dexter, 1982; Denef *et al.*, 2001). In most of these experiments, the wetting is sudden and slakes the aggregates. In our case, we slowly rewetted the aggregates by capillarity. We incubated 3-5 mm aggregates and most of them kept their initial size over the incubation (results not shown). However, their stability varied. The dry-wet cycles had no effect on the stability of aggregates to slaking, decreased their stability to slow rewetting and increased their stability to the stirring after pre-wetting test (Figure V-5). Microcracks are created when rewetting slowly aggregates, because of differential swelling (Le Bissonnais, 1996). Here, we propose that the differential swelling and shrinkage during dry-wet cycles created microfissures that became failure zones when the aggregates were exposed to the slow wetting. It created stable aggregates of small size and this effect of dry-wet cycles was more pronounced with straw than without, showing an interaction with microbially-mediated aggregation. This interaction was probably due to the detrimental effect of dry-wet cycles on bacteria and on extracellular polysaccharides production, which resulted in less binding agents in aggregates that are subjected to more stresses.

Dry-wet cycles had no impact on MWD after the rapid wetting test because this test is very aggressive and disrupts all aggregates to small macroaggregates (0.2-2 mm) and microaggregates (0.05 -0.2 mm).

The stirring after prewetting test assesses the ability of aggregates, prewetted with alcohol, to withstand the physical action of being hit among each other and with the Erlenmeyer's walls as a way to measure their interparticle cohesion independently of slaking. Dry-wet cycles increased the cohesion of aggregates incubated with straw, and had no such effects on reference samples. Drying probably created additional intermolecular associations between organic macromolecules, such as extracellular polysaccharides, and mineral surfaces (Haynes & Swift, 1990), and thus increased interparticle cohesion within aggregates, at the scale of small macroaggregates. In fact, the lower contents of measured HWEC after dry-wet cycles may be due either to a lower production in soil by microorganisms as suggested earlier, or to a stronger adhesion to mineral surfaces, decreasing their solubilisation by hot water.

Microorganisms decomposing straw, located at the outer surfaces of 3-5 mm aggregates were then responsible for the stabilization, within these aggregates, (i) of zones 0.2-2 mm which were stable to slaking and to mechanical breakdown, i.e. with enhanced cohesion and hydrophobicity, and (ii) of larger zones, > 2mm in diameter, which were stable to the smaller stresses of slow rewetting. This hierarchy of aggregate stabilization by microbial activity was revealed by the combination of tests. Straw located between 3-5 mm aggregates, hence stimulated microbial binding agents well within the aggregates, in agreement with results from Gaillard et al. (1999) on the detritusphere of wheat straw. Dry-wet cycles, characterized by fast drying and slow rewetting, consolidated the stabilized 0.2-2 mm zones, and created failure zones between them. We observed no effect of dry-wet cycles on microbially-mediated hydrophobicity of aggregates.

4.4 Conclusions

With an incubation experiment, we confirmed that straw addition stimulated microbial activity and showed that it stabilized aggregates both by increasing their cohesion and hydrophobicity. Microorganisms produced carbohydrates and water repellent substances which were more persistent or had more persistent effects than the microorganisms themselves. The net effects of dry-wet cycles were less important than that due to straw addition, but were more pronounced in the presence of an important microbial activity, demonstrating an interaction. Prediction of short-term variations in aggregate stability should take into account the complex interactions between drying rewetting

events, and the dynamics of microbial populations and their binding and aggregating agents. An analysis at the scale of elementary mechanisms, i.e. increasing cohesion and hydrophobicity of aggregates appears necessary, given the complex nature of aggregate stability.

5 Acknowledgements

We would like to thank Mathieu Chevalier for conducting preliminary experiments and for discussions.

Chapitre 6

Conclusions générales et Perspectives

1 Conclusions générales

L´étude bibliographique faite dans ce mémoire a mis en évidence l´importance de la structure du sol sur l´environnement, notamment sur le fonctionnement général du sol. Ainsi, la structure du sol conditionne de nombreux processus physiques et biogéochimiques dans les environnements naturels et agronomiques. La structure du sol est un facteur clé du fonctionnement d'un sol.

Malgré l`alerte de nombreux scientifiques partout dans le monde sur l`impact de l`avancée de l`érosion et de la diminution de la teneur en MO des sols connaissant leur interdépendance, il y a un manque important de connaissances mécanistes et quantitatives sur les relations MO – structure des sols. Les mécanismes impliqués sont complexes et dynamiques à court terme. La structure des sols est liée aux conditions climatiques (notamment l'eau du sol) et aux MO du sol. En plus, il n´existe pas de protocole normalisé pour la mesure de la stabilité structurale au plan international, ce qui complique les comparaisons entre les différents résultats scientifiques.

La science du sol en France a fait dernièrement d'importants efforts pour mieux comprendre les relation entre les MO, la stabilité structurale et de l`érosion au travers de projets nationaux et de thèses de doctorat. Des avancées ont étés faites sur la compréhension de la dynamique de la stabilité structurale après un apport de résidus de culture de différentes qualités (Abiven, 2004) ou de différents composts d`origine urbaine, plus ou moins évolués (Annabi, 2005), en analysant la réponse dynamique des agents microbiens responsables de la stabilisation de la structure. Notamment Abiven (2004) a présenté le premier modèle quantitatif de prédiction de la stabilité structurale à partir de la qualité biochimique initiale des MO apportées. Ses résultats expérimentaux ont confirmé le fort impact de la qualité de la MO apportée sur la dynamique de la stabilité de la structure, et confirment aussi la nécessité d`avancer sur la quantification de relations directes MO apportée → microorganismes → stabilité structurale, comme stratégie de prédiction de la stabilisation de la structure.

1.1 La démarche et le système.

Les relations entre l`évolution de stabilité de la structure et ses déterminants dans un milieu naturel sont très complexes. Le concept de stabilité structurale est déjà très global et intégrateur et dépend donc de nombreux facteurs internes (e.g. texture, teneur et nature des MOS, CEC, nature des cations etc.), mais aussi externes comme le climat, le type de couvert végétal et l`homme. Ce fait nous oblige à simplifier le système pour mieux le comprendre. Avec cette idée, nous avons fait des expérimentations basées sur des incubations de laboratoire, avec le même sol en conditions contrôlées (Tº, lumière, disponibilité de l'eau) et sans plantes. Le risque de ce type de démarche est une validité limitée des conclusions obtenues dans le contexte plus large de situations réelles.

L`objectif de ce mémoire a donc été d'analyser le déterminisme de la stabilité de la structure après des apports de MO et de quantifier ces effets, en fonction de variations de la teneur en eau du sol. Le choix a été de maintenir constantes la plupart des variables qui modifient la structure et de se focaliser sur l`impact de la quantité de MO apportée sur les agents microbiens de la structure et sur les propriétés physiques élémentaires impliquées dans la stabilisation de la structure. La modification des doses de MO apportées au sol et l`application de cycles de dessiccation – réhumectation ont donc été la démarche choisie pour essayer de mesurer chaque compartiment du modèle conceptuel proposé (Fig. VI-1) et d'analyser quantitativement les relations entre eux comme un premier pas vers la modélisation mathématique de la dynamique de la stabilité structurale à court terme.

Les deux déterminants de la stabilité de la structure étudiés dans cette thèse, la quantité de MO apportée et les cycles de dessiccation- réhumectation, sont considérés comme les plus importants dans les sols limoneux (cf Chapitre 1). Mais ils sont de natures diverses et peuvent affecter la stabilité structurale différemment. Comme la MO est la source d`énergie pour les microorganismes du sol, le contact physique MO - agrégats peut modifier la vitesse de colonisation des MO par les microorganismes et la vitesse de biodégradation. Ainsi, la granulométrie des résidus organiques apportés et la taille des agrégats du sol considéré ont été des facteurs que nous avons dû standardiser dans nos expérimentations et qui ont des valeurs qui peuvent être différentes de celles au champ. La même considération est valide pour l`intensité et la quantité de cycles de dessiccation – réhumectation appliquées à notre expérimentation. En revanche, la standardisation nous a permis d`utiliser de méthodes calibrées (stabilité structurale, Le Bissonnais, 1996) et

avancer sur la compréhension des mécanismes basiques qui modulent la structure à court terme.

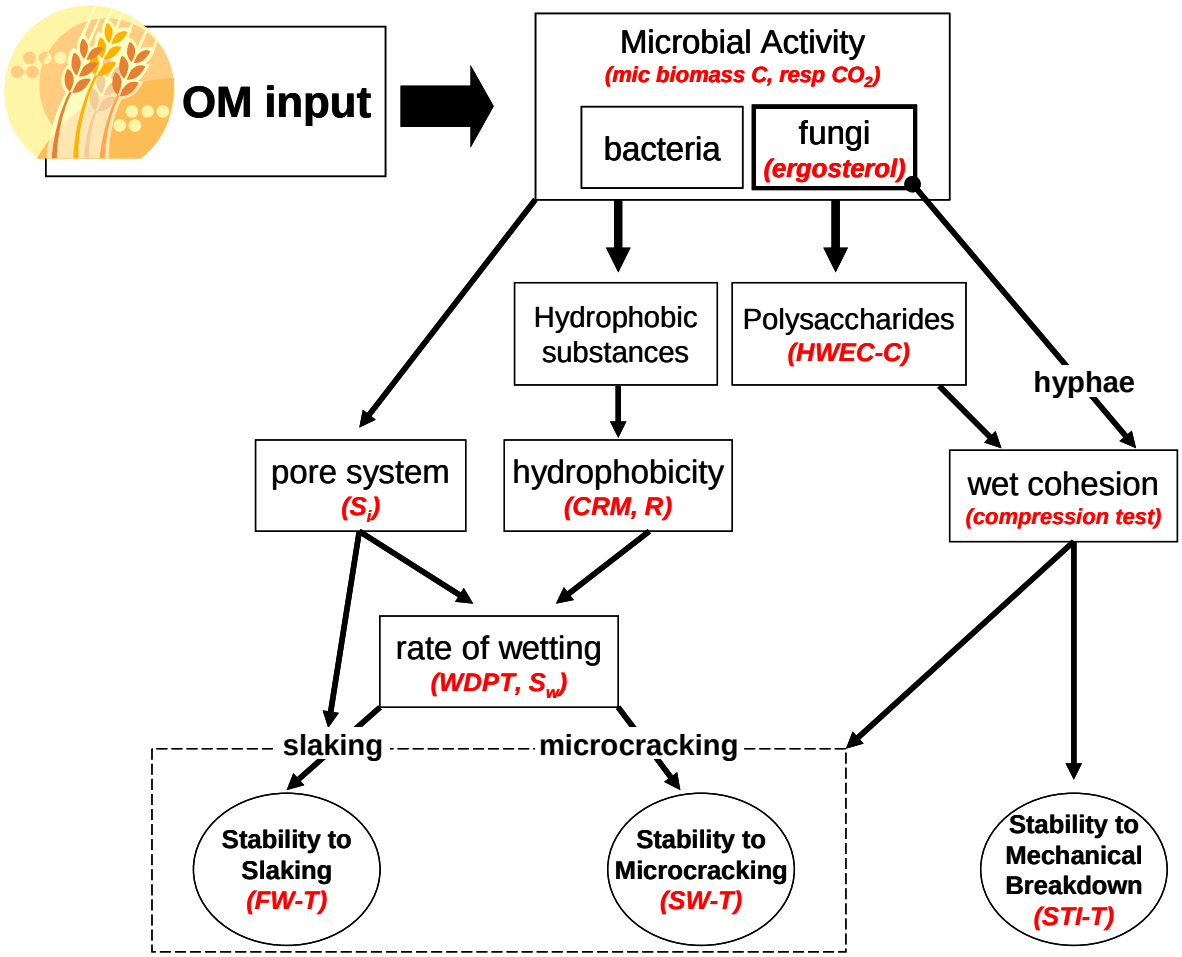

Fig. VI-1. Schéma conceptuel proposé sur l`effet de l`apport de MO sur la stabilité de la structure.

En italiques sont indiquées les méthodes utilisées pour mesurer chaque compartiment : mic biomass C (carbone de la biomasse microbienne), resp CO_2 (CO_2 respiré), ergosterol (teneur d'ergosterol), HWEC-C (polysaccharides extractibles à l'eau chaude), Si (sorptivité intrinsèque), CRM (ascension capillaire), R (index de repellence), compression test (test uniaxial de compression), WDPT (temps de pénétration d'une goutte d'eau), Sw (sorptivité à l'eau), FW-T (test d'humectation rapide), SW-T (test d'humectation lente) et STI-T (test de d'agitation).

1.2 Les compartiments microbiologiques du modèle conceptuel.

Nous avons choisi quatre déterminations liées aux variables biologiques : la respiration, le C de la biomasse microbienne, les polysaccharides extractibles à l`eau chaude et l`ergostérol. La respiration du sol et le C de la biomasse microbienne sont des mesures qui montrent l`activité et l'abondance globale des microorganismes du sol. Elles prennent en compte des processus métaboliques globalement, i.e. la dégradation catabolique dans des conditions aérobies. L`inconvénient de la mesure de la respiration est qu`en présence de substrats organiques apportés, le CO_2 produit par oxydation du composé ajouté peut seulement être estimé par différence avec un témoin sans apport. Sa mesure rigoureuse requiert un marquage des MO apportées (^{14}C ou ^{13}C). Sans marquage, le *priming effect* peut être une source d`erreur non négligeable. Néanmoins, nos résultats de respiration montrent clairement que le C disponible a été le principal facteur limitant de la croissance microbienne. Selon Garland & Mills (1991), l'utilisation du C disponible est le facteur clé qui gouverne la croissance microbienne dans les sols. Aujourd'hui l`application de techniques moléculaires peut améliorer la détermination de l`activité et de la diversité microbienne du sol. Néanmoins, la détermination de la composition de la microflore ou de la concentration de chaque métabolite ou le taux de chaque réaction de transformation sont, non seulement laborieux et coûteuxn sinon supeflus pour quantifier la décomposition de la MO. En effet, il n`a pas été observé de relation directe entre la diversité microbienne et la décomposition de la MO dans les sols (Nannipieri et al., 2003). Bien que l`activité microbiologique globale soit assez bien estimée et reliée à la décomposition de la MO, elle ne renseigne pas sur l'abondance et l`activité respective des champignons et des bactéries. Les champignons ont un rôle important sur la structure du sol notamment par l`enrobage physique des agrégats par les hyphes fongiques, la production de polysaccharides extracellulaires et la production de substances hydrophobes (cf Chapitre 1). La mesure de la concentration en ergostérol est un moyen relativement simple et économique pour estimer l'abondance globale des champignons vivants, mais cela reste une mesure relative car l`abondance de l'ergostérol dépend des espèces fongiques considérées. La comparaison entre les techniques qui mesurent le biovolume fongique (e.g. analyse d`images) et les techniques d`extraction biochimique (ergostérol) apparaît nécessaire à l'avenir dans ce domaine.

Les polysaccharides extractibles à l`eau chaude sont généralement utilisés comme estimateur des polysaccharides microbiens extracellulaires. La capacité d`en produire est largement distribuée entre groupes de microorganismes. La liaison avec la stabilisation de la structure a été bien démontrée (voir chapitre introduction). Cependant, la teneur en nutriments dans le sol (e.g. la concentration d`azote), et le groupe de microorganismes (champignons, bactéries, algues, etc.) modifient la nature des polysaccharides produits et donc, l`efficacité agglutinante de particules du sol.

Les substances hydrophobes d`origine microbienne n`ont pas été quantifiées et indentifiées ici. Nous avons mesuré leurs conséquences sur le sol : l`hydrophobie et l`impact sur la vitesse de réhumectation. Les substances qui causent l`hydrophobie dans les sols sont d`origines diverses. Les plantes, les microorganismes et même la MO des sols sont concernés. Cependant, dans nos expérimentations, le développement d`hydrophobie a été d`origine microbienne, car la MO apportée et le sol témoin étaient hydrophiles. Les champignons semblent être le groupe des microorganismes responsables de l`hydrophobie d'origine microbienne car ils peuvent produire des mélanines ou exsuder des petites protéines, les hydrophobines, à caractère hydrophobe. Plusieurs mycorhizes exsudent aussi des protéines insolubles, les glomalines, bien corrélées avec la stabilité des agrégats (Wright & Upadhyaya, 1998; Piotrowski et al., 2004), qui s'apparenteraient aux hydrophobines. Des lipides de surface ou extracellulaires peuvent aussi être responsables de l`hydrophobie induite par microorganismes (Capriel et al., 1990). Différentes espèces de bactéries et des levures peuvent en effet exsuder des lipides (Georgiou et al., 1992). La nature et le comportement des composés responsables de l'hydrophobie subcritique des sols reste cependant à identifier.

Les estimations de l`activité et l`abondance des microorganismes et de l'abondance des polysaccharides solubles à l'eau chaude nous ont permis d`établir des relations quantitatives entre l`apport de MO au sol et la réponse microbiologique générale du sol. Ainsi, dans les conditions non limitantes en N, le C semble être la ressource limitante et la réponse microbiologique du sol a été proportionnelle à l`apport carboné. La gamme d`apport de C utilisés dans notre travail comprend largement les apports de C agronomiques courants. Il ne semble pas y avoir un seuil de l`activité et de l'abondance des microorganismes vis-à-vis de la dose de matière organique apportée.

1.3 Les propriétés physiques élémentaires qui déterminent la stabilité structurale.

Le choix d`essayer de comprendre l`effet de l`apport de MO sur la dynamique de la stabilité structurale d'un point de vue mécaniste et intégrateur (Fig. VI-2), nous a conduit à « sortir » des tests de stabilité structurale, qui ne sont pas la mesure d'une propriété physique *per se*. En nous basant sur les tests de Le Bissonnais (1996) qui discriminent les principaux mécanismes de désagrégation, nous avons cherché à comprendre et quantifier la dynamique des propriétés physiques élémentaires qui régulent l'amplitude de la désagrégation du sol. Ainsi, nous avons choisi d'étudier le système poral, l'hydrophobie et la cohésion. Malheureusement, le manque de définitions claires de ces propriétés (par exemple l'hydrophobie), la complexité de leur détermination et parfois le manque de méthodes appropriés (par exemple la mesure de la cohésion du sol à l`état humide à l'échelle d'agrégats) nous ont amené à dépenser beaucoup d`efforts pour les évaluer, ceci avec des résultats positifs (hydrophobie) et négatifs (cohésion à l`état humide) vis-à-vis de la relation entre ces propriétés physiques et la stabilité de la structure.

Nous avons démontré que la stimulation des microorganismes par un apport carboné a augmentait le volume poral, l'hydrophobie et la cohésion à l'état humide des agrégats et en conséquence leur vitesse de réhumectation a diminué notablement. Cette modification a fait diminuer l'importance de tous les mécanismes de désagrégation, indépendamment de la quantité de MO apportée et du temps. Dans un sol limoneux pauvre en MO comme celui étudié, l`importance relative de chaque mécanisme de désagrégation semble être la même quelque soit la quantité de MO apportée, voire le type résidu de culture. Donc, il semble encore être très pertinent de considérer les tests de stabilité de la structure comme des tests intégratifs au lieu de seulement considérer la cohésion interparticulaire ou l`hydrobie ou la porosité.

Evidemment, il y a des situations où l'impact relatif de chaque propriété physique élémentaire sur la stabilité des agrégats peut changer. Par exemple, nous avons démontré que les cycles de dessiccation – réhumectation augmentent la stabilité du sol vis à vis de la désagrégation mécanique mais qu'ils la font diminuer vis-à-vis de la microfissuration (Fig. VI-4). Un autre exemple est celui de la disponibilité de l`azote dans le sol. Nous avons fait

une expérimentation annexe (non rapportée dans ce mémoire) similaire à celle détaillée dans les chapitres 2 avec le même type de sol, en apportant 20 g C de paille kg^{-1} de sol, mais sans ajouter d`azote. Nous avons pu constater qu'au bout d'un mois, le sol sans apport d'azote était devenu plus stable que celui ayant reçu un apport d'azote afin d'obtenir un C/N moyen de 10. Cependant, sans apport d'azote, ni l`hydrophobie, ni la biomasse microbienne, ni la biomasse fongique fongique n'étaient relativement augmentées. Il semble ici que l'importance relative de l'hydrophobie et la cohésion soient différentes. La qualité de la MO apportée et la texture du sol sont des variables qui pourraient aussi faire varier l`importance relative de chaque mécanisme de désagrégation sur la stabilité de la structure.

Dans nos expérimentations, la réponse microbiologique et la réponse générale des propriétés physiques responsables de la stabilité de la structure suite à l'apport de paille de maïs ont suivi la même dynamique temporelle. En conséquence, la linéarité de la relation « MO → microbiologie » est vraie pour la relation « réponse microbiologique → stabilité de la structure » (Fig. VI-2).

La linéarité de la relation entre l`abondance relative des agents microbiens agrégeants et la stabilité de la structure pendant toute la période d`incubation, nous a permis d'utiliser des relations mathématiques linéaires pour prévoir la dynamique de la stabilité structurale à court terme.

Fig. VI-2. Schéma de relation entre l'apport carboné et la stabilité structurale.

La linéarité des relations a permis de construire des régressions linéaires multiples entre les variables microbiologiques et la dynamique de la stabilité de la structure à court terme. Cela nous a permis de coupler le modèle CANTIS de décomposition de C à la stabilité de la structure et cela donne la possibilité de prévoir la dynamique de cette stabilité dans des conditions différentes de celles de notre expérimentation. Ainsi, CANTIS peut prévoir la dynamique de la quantité de CO_2 et de C de la biomasse microbienne qui

sera produite à partir de MO en fonction de la qualité et de la quantité de MO, de la température, de l'azote disponible etc. (Fig. VI-3) et la fonction « STAB » finalement prévoir la stabilité structurale à partir du CO_2 et du C de la biomasse microbienne obtenues par CANTIS.

Même si CANTIS réussit à modéliser la réponse microbiologique pour différentes quantités de MO apportées, des variables comme la texture, les cycles de dessiccation-réhumectation, la disponibilité des nutriments peuvent changer l`effet des agents microbiologiques sur la stabilité de la structure et aussi l`impact relatif de chacun des mécanismes de désagrégation et devraient donc être étudiés.

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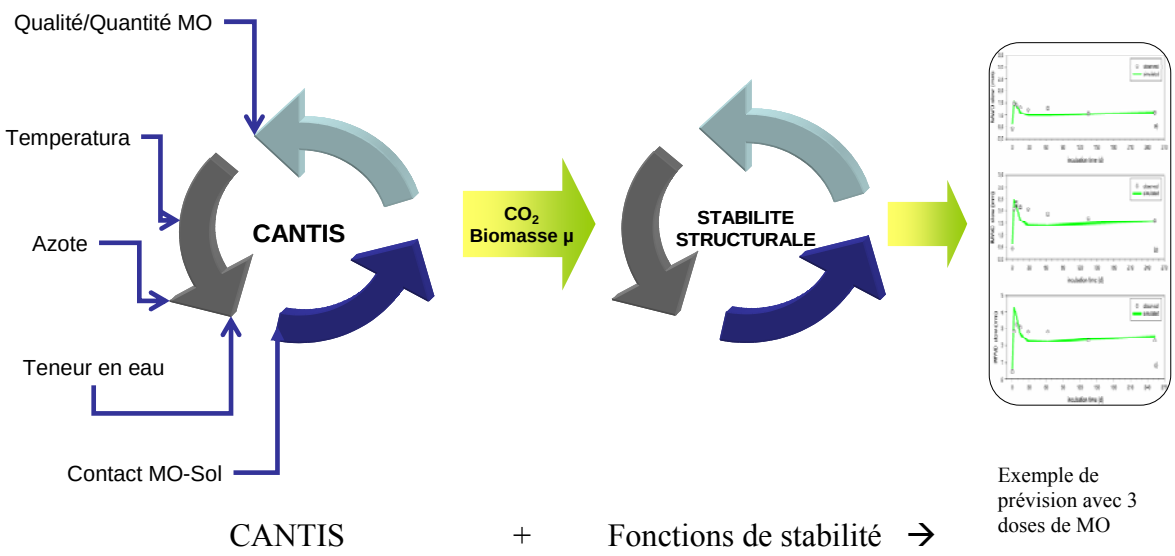

Fig. VI-3. Le couplage CANTIS – Stabilité de la structure proposé.

Au vu de nos résultats, une prévision précise des variations à court terme de la stabilité structurale doit prendre en compte les interactions complexes entre les alternances climatiques et la réponse microbiologique. Cependant, les effets des alternances d'humectation dessiccation telles que nous les avons reproduites (réhumectation non destructive) sont moins prononcés que celui de l`apport de C. Dans la recherche d`un modèle prédictif de la stabilité structurale plus général, l`effet de l`apport organique dans différent conditions sol, devient donc prioritaire.

Finalement, l`hétérogénéité spatiale des conditions de sol à l`échelle des microorganismes doit être prise en compte si nous voulons vraiment décrire et prédire le fonctionnement de sols.

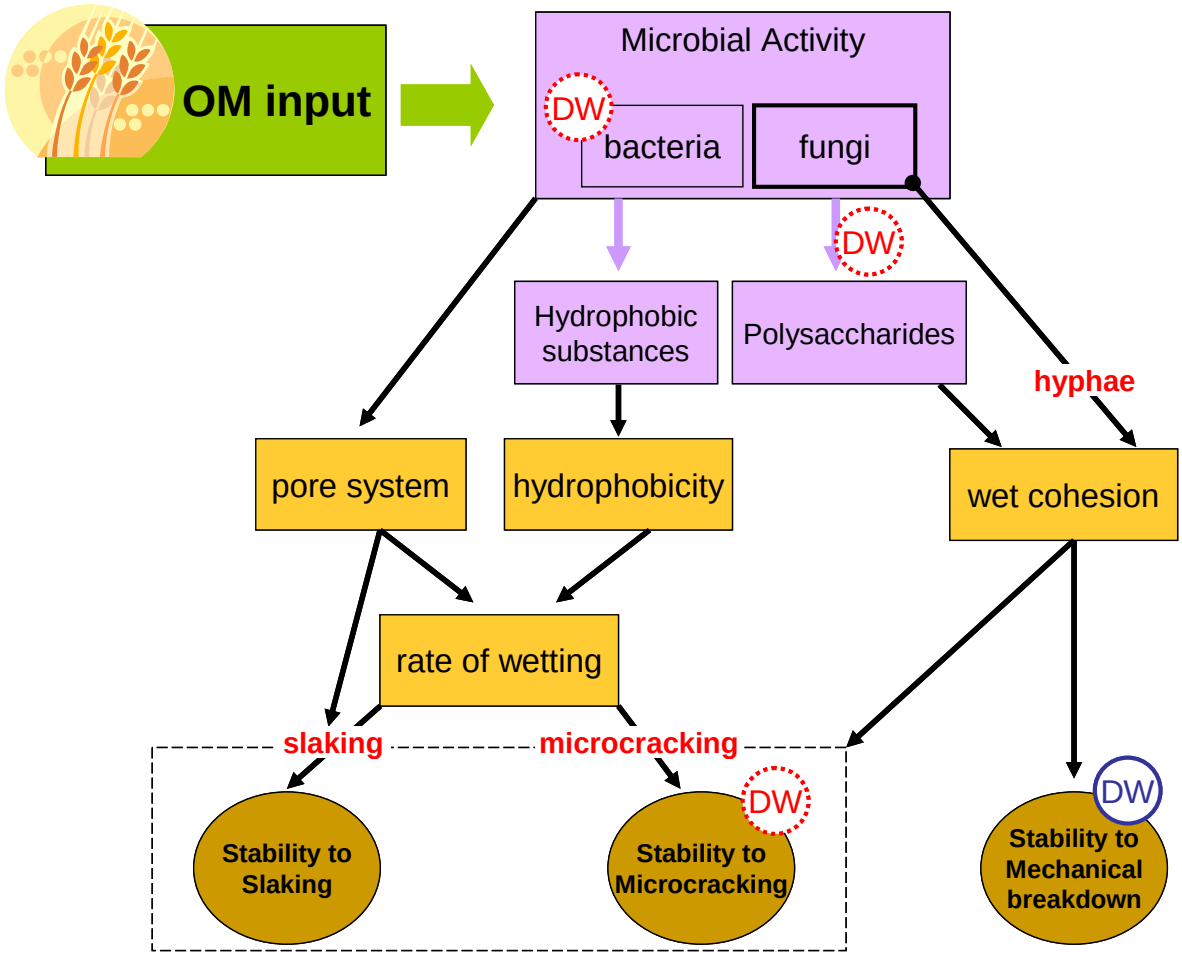

Fig. VI-4: Modèle conceptuel proposé affecté par les cycles de dessiccation et réhumectation (DW). Cercle en pointilleux : l'effet est négatif, en continu : l'effet est positif.

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ORGANIC MATTER CONTRIBUTION TO AGGREGATE STABILITY IN SILTY LOAM CULTIVATED SOILS. CARBON INPUT EFFECTS.

Abstract

Soil aggregate stability is a key property for soil functioning. However, there are still no tools to predict its temporal variations or to determine cultural practices to improve it. The subject is particularly important in regard to the effect of C inputs to fragile soils (silty cultivated soils).

The general aim of this work is to improve the knowledge in factors determining short-time aggregate stability variations caused by C inputs and soil water contents variations.

To establish quantitative relationships among aggregate microbial agents, elementary physical properties that determine aggregate stability (porosity, hydrophobicity and soil cohesion) and aggregate stability we varied the organic matter added rate (maize residue) and wetting-drying cycles in soil controlled conditions. We used three aggregate stability tests that distinguish different soil breakdown mechanisms (slaking, microcracking and mechanical breakdown) to analyze the relationships from a mechanistic point of view.

In our conditions, C inputs stimulated linearly microbial activity and by-products to all doses of C inputs used. Aggregate microbial agents measured (microbial biomass-C, ergosterol content (biomarquer of fungi), and hot water extractable polysaccharides (surrogate of extracellular polysaccharides)), had an important dynamic pattern and there were all correlated to aggregate stability. No hierarchic order among biological variables could be established. Aggregate stability to slaking, microcracking and mechanical breakdown had a similar dynamic pattern after C input. A linear semi-mechanistic model predicting aggregate stability variations based on coupling an soil C and N dynamic model (CANTIS) and multiple linear regressions linking microbial biomass-C and respired CO_2 after a C input and aggregate stability was proposed.

C input has also strongly modified the elementary physical properties that determine aggregate stability. The aggregates increased porosity, hydrophobicity and Interparticle cohesion with C input rate. Giving the impact of microbial activity in hydrophobicity, emphasis was given in determining hydrophobicity and three methods were applied and evaluated. Diversity of microbial activity effects on soil physical aggregate properties suggests that is still pertinent to consider aggregate stability as an integrative and sensitive property to organic matter input effects in soil.

Climatic conditions as wetting-drying cycles have shown to modulate the effect of C inputs on aggregate stability, particularly changing the relative impact of the organic matter in breakdown mechanisms of aggregate stability.

Key-words: aggregate stability, hydrophobicity, cohesion, aggregate microbial agents, C inputs, maize residues, microbial biomass carbon, slaking, microcracking, mechanical breakdown.

CONTRIBUTION DES MATIERES ORGANIQUES
A LA STABILITE DE LA STRUCTURE DES SOLS LIMONEUX CULTIVES.
EFFET DES APPORTS ORGANIQUES A COURT TERME.

Résumé

La stabilité de la structure des sols vis-à-vis de stress externes est une propriété clé pour le fonctionnement du sol. Cependant, il n`existe pas aujourd'hui d'outil permettant de prévoir ses variations temporelles ou de déterminer les pratiques culturales qui l`améliorent. La question est particulièrement posée en ce qui concerne l'effet d'amendements organiques sur des sols fragiles comme les sols limoneux cultivés.

L´objectif général de cette thèse est approfondir la connaissance du déterminisme de la variabilité intra-annuelle de la stabilité de la structure du sol résultat de l´apport de matières organiques et de la variation de la teneur en eau du sol.

Pour établir des relations quantitatives entre les agents microbiens impliqués dans l'agrégation, les variables physiques élémentaires qui la déterminent (porosité, hydrophobie et cohésion) et la stabilité de la structure, nous avons choisi une démarche expérimentale en laboratoire et fait varier les doses de MO apportées au sol (un résidu de culture) et appliqué des cycles de dessiccation – réhumectation. Nous avons utilisé trois tests de stabilité structurale qui mettent en évidence différents mécanismes de désagrégation (l`éclatement, la microfissuration et la désagrégation mécanique) pour analyser les relations quantitatives d'un point de vue mécaniste.

Dans nos conditions expérimentales, l'apport de MO a stimulé linéairement l'activité des microorganismes pour toute la gamme de MO apportées. Les différents agents agrégeants microbiens mesurés (biomasse microbienne, ergosterol (biomarqueur des champignons), et les sucres extraits à l'eau chaude (estimateur des polysaccharides extracellulaires)) ont une forte dynamique temporelle et sont tous significativement corrélés à la stabilité structurale, sans hiérarchie apparente. La stabilité de la structure à l'éclatement, à la microfissuration et à la désagrégation mécanique ont eu une dynamique similaire après l'apport de MO. Nous proposons un modèle semi-mécaniste d'estimation de la stabilité structurale, basé sur le couplage entre un modèle de la dynamique du C et N dans les sols (CANTIS) et une fonction statistique reliant la biomasse microbienne et la quantité de CO_2 minéralisée à partir de la MO apportée à la stabilité de la structure.

La décomposition de la MO apportée a fortement modifié les propriétés physiques responsables de la stabilité de la structure. La porosité des agrégats, leur hydrophobie et leur cohésion interparticulaire ont toutes augmenté. Etant donné l'impact de l'activité microbienne sur l'hydrophobie, nous avons comparé, trois méthodes différentes permettant son estimation. La diversité des effets de l'activité microbienne sur les propriétés physiques des agrégats du sol, suggère qu'il est généralement pertinent de mettre en œuvre des tests de stabilité de la structure, propriété sensible et intégrative des différents effets des matières organiques.

Les conditions climatiques, telles-que des alternances d'humectation dessiccation, modulent l'effet des MO apportées sur la structure du sol, en particulier en changeant l'impact relatif des MO sur chaque mécanisme de désagrégation.

Mots-clés : stabilité structurale, hydrophobie, cohésion, agents microbiens, apport de matières organiques, résidus de maïs, carbone de la biomasse microbienne, éclatement, microfissuration, désagrégation mécanique.