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Rapport final du projet réalisé dans le cadre de l'AIP ECOSOL

(Animation : Pierre Renault)

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Rapport final – fiche administrative

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Documents joints :

Le rapport final est présenté sous la forme d'un article :

[1] Renault, P., R. Lensi, C. Chenu, C. Hénault, J. Chadœuf, S. Parry, L. Philippot, A. Clays-Josserand, R. Chaussod, K. Heurlier, et D. Chèneby. 1998. Respiration, anoxie et dénitrification des mottes de sol : Distributions spatiale et temporelle des fonctionnements microbiens en fonction de la distribution des pores et de la matière organique des mottes. *AIP ECOSOL, Rapport scientifique final.*

Nous lui avons adjoint en annexe 1 article publié, 1 article accepté, et 1 sur le point d'être soumis :

[2] Philippot L., P. Renault, J. Sierra, C. Hénault, A. Clays-Josserand., C. Chenu, R. Chaussod and R. Lensi. 1996. Dissimilatory nitrite-reductase provide a competitive advantage to Pseudomonas sp. RTC01 to colonise the center of soil aggregates. *FEMS Microbiology Ecology* 21, 175-185.

[3] Parry, S., P. Renault, C. Chenu and R. Lensi. 1998a. Denitrification in pasture and cropped soil clods as affected by the pore space structure. *Soil Biology and Biochemistry*, sous presse.

[4] Parry, S., R. Lensi, J. Chadœuf, C. Chenu, and P. Renault. 1998b. Particulate organic matter as a source of denitrification variability in pasture and cropped soil clods. *European Journal of Soil Science*, soumission prévue en octobre 1998.

Un article est encore en cours d'écriture dans le cadre de cette AIP. Il est joint à ce rapport bien que sa forme soit incomplète :

[5] Parry, S., P. Renault, C. Chenu, J. Chadœuf, O. Bastien, and R. Lensi. 1999. Correlation between Clod anoxic Fraction and Denitrification: the Effects of air-filled Pore Space and Particulate Organic Matter. *Soil Science Society of America Journal*, soumission prévue en janvier 1999.

Enfin, 3 rapports sont associés à ce travail (documents non joints) :

- le DEA de K. Heurlier ;
- la Thèse de Doctorat de S. Parry ;
- la Thèse de Doctorat de L. Philippot (pour partie).

Respiration, anoxie et dénitrification à l'échelle de la motte de sol :

Influence de la matière organique et de la structure des sols sur la distribution et le fonctionnement des micro-organismes dénitrifiants

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Mots clés : Dénitrification, respiration, anoxie, sol, motte, agrégat, structure, matière organique, micro-organisme, expérience, modèle, culture, prairie.

Respiration, anoxie et dénitrification à l'échelle de la motte de sol :

Influence de la matière organique et de la structure des sols sur la distribution et le fonctionnement des micro-organismes dénitrifiants

Résumé :

La dénitrification contribue à l'émission du sol vers l'atmosphère d'oxyde nitreux, gaz à effet de serre impliqué dans la chimie de l'ozone au niveau de la haute troposphère et de la basse stratosphère. Les contributions respectives de la dénitrification et de la nitrification à ces émissions sont encore mal appréhendées et un travail considérable reste à réaliser pour mieux comprendre et décrire ces processus. L'ensemble des travaux présentés dans ce rapport a porté sur l'influence de la structure du sol et de la matière organique sur la dénitrification, à l'échelle de la motte. Il s'agissait d'une part d'analyser le déterminisme de la dénitrification potentielle au regard des populations microbiennes, de la matière organique et de l'aération du sol Il s'agissait d'autre part d'étudier l'expression de cette activité potentielle en fonction de la structure et de la répartition des activités microbiennes entre les matières organiques particulaires et le reste du sol. L'essentiel des travaux a été réalisé sur des couples "prairie – rotation céréalière" dont les deux éléments étaient issus de la même unité pédologique. Nous avons ainsi montré que l'activité dénitrifiante des mottes est la somme d'une contribution des C-POM (i.e. la matière organique particulaire accompagnée de sa gangue de sol), *a priori* non corrélée à la fraction anoxique de la motte, et d'une contribution diffuse de la matrice (i.e. le reste de la motte) dans son domaine anoxique. Bien que cette dernière contribution ait été jusqu'à présent la seule prise en compte dans les modèles mécanistes, elle ne suffit à décrire le fonctionnement dénitrifiant des mottes que lorsque l'activité dénitrifiante potentielle des C-POM est faible ou lorsque les activités respiratoires et la structure du sol ne permettent pas à cette activité de s'exprimer. La présence de quelques pores libres à l'air peut modifier le niveau d'aération des mottes et réduire ainsi la fraction de la matrice en état d'anoxie et l'expression d'une activité dénitrifiante au niveau des C-POM. Nous avons proposé un modèle mécaniste stochastique qui décrit simultanément les contributions des C-POM et de la matrice à la dénitrification, pour des mottes de structure et de teneur en C-POM variable. Concernant la dénitrification potentielle et la respiration, les différences observées entre C-POM et matrice ne semblent pas s'expliquer par des différences de densités d'hétérotrophes et de dénitrifiants. L'absence de corrélation entre densités et activités microbiennes a été couramment mentionnée dans la littérature et un autre volet de nos propres travaux confirme ce fait à une autre échelle : les différences d'activités dénitrifiantes de 5 couples "prairie – culture" provenant chacun d'une même unité pédologique ne sont pas liées à des différences

de densités microbiennes. Ainsi, la respiration maximale et l'activité dénitrifiante potentielle pourraient découler pour partie du niveau de synthèse enzymatique ; elles sont généralement plus élevées sous prairie que sous culture, suggérant l'influence de la teneur en matière organique du sol. L'absence de relation entre densités et activités microbiennes est à prendre toutefois avec précaution en raison de l'imprécision de la méthode de dénombrement MPN et en raison de l'influence de la matière organique sur le taux d'extraction de micro-organismes. En utilisant une souche marquée et son mutant isogénique Nir^-, nous avons ainsi montré que la capacité à réduire NO_2^- en N_2O pouvait favoriser la colonisation du domaine anoxique, pour une souche en phase de colonisation (i.e. initialement présente à faible densité). L'ensemble de ces travaux montre l'insuffisance d'indicateurs trop globaux pour décrire les fonctionnements microbiens. Ainsi, le rapport C/N de la matière organique et sa teneur en lignine ou en composés solubles ne semblent pas expliquer l'activité potentielle dénitrifiante au niveau des C-POM ; d'autres mécanismes comme l'accessibilité des micro-organismes aux substrats seraient importants. De la même façon, le taux de saturation de l'espace poral ne suffit pas à décrire l'aération du sol ; la distribution des pores libres à l'air est plus importante.

1. Introduction

La dénitrification dans les sols contribue aux émissions de N_2O vers l'atmosphère (Hénault et Germon, 1995 ; Conrad, 1996). Ce gaz participe à l'effet de serre (Smith, 1990 ; IPCC, 1996 ; Renault *et al.*, 1997) et intervient, après transformation en NO_x, sur la chimie de l'O_3 au niveau de la haute troposphère et de la basse stratosphère (Graedel et Crutzen, 1992). Les estimations récentes font état d'émissions annuelles de N_2O vers l'atmosphère d'environ 14.7 Tg de $N\text{-}N_2O$ (IPCC, 1996), dont 9.5 Tg proviendraient des sols. La contribution des sols agricoles serait de 3.5 Tg. Toutefois, l'importance de ces différentes émissions et leurs origines restent incertaines : ainsi, les émissions à partir des sols agricoles pourraient en fait être comprises entre 1.8 et 5.3 Tg $N\text{-}N_2O$ par an (IPCC, 1996). Au niveau de ceux-ci, les parts respectives des différents processus pouvant conduire à des émissions de N_2O (i.e. dénitrification et nitrification) sont encore mal appréhendées (Hénault et Germon, 1995) et un travail considérable reste à réaliser pour mieux comprendre et décrire les processus responsables de ces émissions.

La compréhension du déterminisme des émissions de N_2O a débouché sur de nombreuses études visant à comprendre la dénitrification (Tiedje *et al.*, 1989). L'analyse de cette dernière à l'échelle microscopique se justifie par le fait que, dans de très nombreuses situations, les conditions environnementales au niveau des micro-organismes ne sont pas reflétées par la mesure de ces conditions, quand cette dernière est réalisée sur des échantillons moyens (Parkin, 1987). En particulier, les concentrations en O_2 peuvent passer des valeurs atmosphériques à des valeurs nulles sur quelques millimètres de distance au sein de mottes saturées en eau (Currie, 1961 ; Sexstone *et al.*, 1985 ; Rappoldt, 1992 ; Sierra *et al.*, 1995 ; Sierra et Renault, 1996). Plusieurs auteurs ont par ailleurs trouvé des sites anoxiques proches des pores libres à l'air, pourvu que des résidus organiques soient présents (Rappoldt, 1992) et que, éventuellement, une fine pellicule d'eau (de l'ordre de 160 µm d'épaisseur) les recouvre pour permettre l'installation de conditions anoxiques (Parkin, 1987).

A l'échelle centimétrique ou décimétrique, de nombreux modèles décrivent les processus relatifs à la dénitrification : il s'agit de processus physiques (transferts), microbiens (respiration, dénitrification proprement dite, et éventuellement dynamique des populations microbiennes), voire géochimiques (en relation avec le pH et les formes du CO_2). Ils considèrent généralement le sol comme un ensemble d'agrégats et de mottes (Leffelaar, 1979 ; Smith, 1980 ; McConnaughey et Bouldin, 1985a et b ; Arah, 1988 ; Arah et Smith, 1989 ; Leffelaar, 1988 ; Leffelaar et Wessel, 1988 ; Renault et Stengel, 1994 ; Sierra *et al.*, 1995), mais d'autres modèles de milieux poreux ont été proposés (Arah, 1988 ; Rijtema et Kroes, 1991 ; Rappoldt, 1992). Ces modèles supposent généralement que les activités microbiennes potentielles sont uniformément réparties au sein des mottes, qu'elles n'évoluent pas avec le

temps et ne dépendent pas de la distribution des substrats ou de l'ambiance géochimique du milieu (Arah et Smith, 1989). D'autres tiennent compte d'une dynamique des populations microbiennes (Leffelaar et Wessel, 1988), mais la nature proprement des modèles et leur paramétrisation sont alors arbitraires ; les confrontations avec l'expérience restent généralement frustres. De manière générale, les modèles admettent que les zones saturées ont des propriétés de transfert uniforme.

Les études expérimentales récentes ont montré la nécessité d'une prise en compte des hétérogénéités de structure et de distribution de la matière organique en décomposition. D'une part, la présence de pores libres à l'air peut considérablement modifier la distribution des distances d'un point d'une motte au pore libre à l'air le plus proche et, par voie de conséquence modifier son niveau d'oxygénation (Sexstone *et al.*, 1985 ; Rappoldt, 1992 ; Sierra *et al.*, 1995 ; Sierra et Renault, 1996). D'autre part, les fonctionnements microbiens potentiels peuvent, eux-mêmes, être affectés par la distribution des substrats. Ainsi, plusieurs études suggéraient l'influence de la concentration en O_2 sur la respiration maximale (Sierra *et al.*, 1995 ; Sierra et Renault, 1996) et sur l'importance de la capacité à réduire NO_2^- en N_2O pour coloniser un milieu anoxique (Philippot *et al.*, 1995 et 1996). A l'échelle supérieure, les corrélations observées entre dénitrification et teneur en résidus organiques frais de plantes (Aulakh *et al.*, 1984 ; de Cantazaro et Beauchamp, 1985 ; Aulakh *et al.*, 1991) ont donné lieu à la mise en évidence d'activités dénitrifiantes très élevées au niveau de ces résidus (Parkin, 1987 ; Christensen *et al.*, 1990 ; Murray *et al.*, 1995). Parkin (1987) montrait ainsi qu'un résidu frais de plante pouvait être responsable de 25 à 85% de la dénitrification. Au sein des mottes de sol se trouve un pool de matière organique particulaire (i.e. matières organiques de dimensions supérieures ou égales à 50 µm), dont le turnover est généralement d'autant plus rapide qu'elles sont grossières (Balesdent, 1996). Il s'agit *a priori* de résidus généralement plus âgés que les résidus précédemment évoqués, de par leur incorporation lente au sein de mottes. A notre connaissance, aucun travail n'avait traité de l'influence de ce pool de matière organique sur la dénitrification.

Plusieurs objectifs ont motivé les travaux présentés dans ce rapport. Ils ont en commun de porter sur l'influence de la structure et de la distribution du C organique dans les sols sur la distribution et l'activité des micro-organismes dénitrifiants. Ainsi, l'essentiel des travaux a-t-il été réalisé sur des couples de sols dont les deux éléments étaient issus de la même unité pédologique, mais étaient soumis à deux modes d'exploitation : une prairie et une culture (rotation céréalière), en raison de leurs effets contrastés sur les facteurs "structure" et "matière organique". En première approximation, le passage d'un sol cultivé à un sol sous prairie est susceptible d'accroître sa teneur en matières organiques (Tiessen and Stewart, 1983) et, par voie de conséquence, d'augmenter la quantité de substrats disponibles pour la dénitrification. D'un autre côté, cette conversion peut aussi changer la structure de l'espace poral à l'échelle de

l'agrégat ou de la motte de sol (Pagliai, 1994) et ainsi fournir des conditions moins favorables à la dénitrification.

Une partie importante de ce travail a été réalisée à l'échelle de la motte de sol et a consisté à analyser l'influence de la structure de l'espace poral et de la distribution de la matière organique au sein d'une motte sur son activité dénitrifiante. L'analyse de cette dernière passait par l'étude de la distribution des communautés microbiennes fonctionnelles (communauté consommatrice d'O_2 et communauté dénitrifiante), par l'étude de leurs activités en conditions non limitantes, et par l'évaluation de l'expression de ces activités. Distributions spatiales et évolutions temporelles des fonctionnements microbiens étant intimement liées, cette synthèse inclut deux axes de recherche

- l'influence de la structure des pores libres à l'air et de la matière organique sur la dénitrification d'une motte à une date donnée et pour un couple "prairie – culture" (cf. paragraphe 3) ;
- l'influence du niveau d'aération sur la dynamique des populations microbiennes hétérotrophes et dénitrifiantes pour le même sol sous culture (cf. paragraphe 4).

Des variations de quantité et de qualité du C organique dans les sols pouvant induire des différences de taille, de structure et d'activités de la communauté dénitrifiante (Tiedje, 1988), une autre partie du travail a été réalisée sur différents couples "prairie – culture" avec la caractérisation des activités microbiennes (dénitrification potentielle et aptitude à réduire N_2O en N_2) et le dénombrement de différentes communautés microbiennes fonctionnelles (cf. paragraphe 2). Une tentative de caractérisation de la structure de la communauté dénitrifiante a été entreprise pour deux des couples "prairie - culture".

Pour atteindre l'ensemble de ces objectifs, 5 équipes de recherches aux compétences complémentaires se sont associées sur tout ou partie des travaux. Ces derniers ont ainsi associé des compétences en microbiologie et biologie moléculaire, en physique des transferts de gaz et de solutés, en propriétés des matières organiques, en modélisation mécaniste et stochastique des processus couplés de transferts et de transformations microbiennes.

2. Variation des aptitudes microbiennes des sols à la dénitrification

2.1. Objectifs

Dans cette partie du travail, nos objectifs étaient d'analyser l'influence du mode d'occupation du sol (i.e. rotation céréalière ou prairie permanente) sur la dénitrification et

réduction de N_2O en N_2 dans des conditions où l'expression de ces activités n'est *a priori* pas affectée par l'existence de facteurs limitants autres que le C organique. Pratiquement, des mesures ont été réalisées sur des échantillons de sol remaniés placés en condition d'anoxie avec éventuellement ajout de NO_3^-. Elles ont concerné 5 couples "prairie – culture" dont les deux éléments étaient issus de la même unité pédologique. Afin d'évaluer le rôle des composantes "quantité" et "qualité" des communautés microbiennes fonctionnelles impliquées dans ces fonctionnements, des dénombrements microbiens ont été effectués sur ces sols ainsi qu'une ébauche de caractérisation de la biodiversité pour 2 des couples "prairie - culture".

2.2. Matériels et méthodes

Les sols

Des échantillons de sol ont été prélevés dans 5 situations comprenant chacune un sol cultivé depuis plus de dix années et un sol de prairie permanente, contigus et appartenant à la même unité pédologique. Les principales caractéristiques de ces sols sont données dans le Tableau 1.

Tableau 1 : principales caractéristiques des sols ($g.kg^{-1}$).

	Montot		**Pichange**		**Moloy**		**Panthier**		**St-Thibault**	
	Prairie	***Culture***	***Prairie***	***Culture***	***Prairie***	***Culture***	***Prairie***	***Culture***	***Prairie***	***Culture***
Argile	172	127	394	389	311	287	358	362	370	374
Limons	596	605	519	515	414	418	581	572	599	576
Sables	232	268	30	21	15	15	60	63	26	48
Calcaire	<1	<1	55	72	249	299	<1	3	5	2
M.O.	33	18	55	47	89	57	41	26	52	34
C org.	19	11	32	27	52	33	24	15	30	20
C/N	10.4	11.7	9.2	9.3	9.9	9.2	8.8	9.2	9.4	8.9
PH eau	6.7	7.0	7.8	7.9	8.0	8.0	6.7	6.9	7.1	7.4

Mesure des activités dénitrifiantes

Les mesures des activités dénitrifiantes ont été réalisées sur des durées de 200 h et pour des échantillons de sol, tamisés à 5 mm, placés en condition d'anoxie à 20°C, selon les 3 modalités suivantes (Hénault *et al.*, 1998) :

- mesure de la dénitrification semi-potentielle en présence de 2.5% de C_2H_2 et NO_3^- non limitant (anoxie, pas d'ajout de C organique) ;
- mesure de la dénitrification semi-potentielle en présence de 2.5% de C_2H_2 et d'un excédent de N_2O (anoxie, pas d'ajout de C organique et de NO_3^-) ;
- mesure de la réduction de N_2O en N_2 en condition de N_2O non limitant (anoxie, pas d'ajout de C organique et de NO_3^-) ;

Les concentrations en N_2O et CO_2 ont été suivies au cours du temps par chromatographie en phase gazeuse.

Dénombrement des communautés fonctionnelles aérobies, anaérobies et dénitrifiantes

Sur l'ensemble des sols précédemment décrits, des dénombrements microbiens ont été effectués par la méthode du nombre le plus probable (méthode MPN) (Cochran, 1950) sur différentes communautés fonctionnelles :

- ensemble des bactéries aérobies (facultatives et obligées) ;
- ensemble des bactéries anaérobies (facultatives et obligées) ;
- bactéries dissimilatrices de NO_3^- ;
- bactéries productrices de N_2O et/ou de N_2.

Le milieu FT a été utilisé pour dénombrer la microflore totale et le milieu D30 pour dénombrer les bactéries anaérobies totales, les bactéries respiratrices de NO_3^- et les bactéries dénitrifiantes *sensu stricto* (Chèneby *et al.*, 1998).

Caractérisation de la structure de la communauté dénitrifiante dans les sols

Une collection de souches dénitrifiantes a été constituée à partir des sols de Montot et de Panthier, selon la procédure chronologique suivante :

- (i) obtention de clones isolés à partir d'étalements de suspensions-dilutions de sol sur milieu DNT avec incubation en anoxie ;
- (ii) purification de ces clones par repiquages successifs sur milieu DNT ;
- (iii) repérage des souches respiratrices de NO_3^- à l'aide d'un test en microplaques (Chèneby, 1995) ;
- (iv) détection de la capacité des souches ainsi isolées à produire N_2 et/ou N_2O par analyse en chromatographie en phase gazeuse de l'atmosphère de cultures en tube sur milieu D30 en présence ou non de C_2H_2. La consommation en NO_3^- a été évaluée en fin d'expérience, permettant ainsi d'estimer la part de gaz produit à partir du NO_3^- consommé.

Sur les souches pour lesquelles le N_2O dégagé dépassait 7% de la consommation en NO_3^-, nous avons alors établi des profils de restriction de leur ADNr16S amplifié, après digestion de celui-ci par les enzymes Alu I, Bst UI et Rsa I (technique PCR-RFLP).

2.3. Résultats et discussion

Vitesses caractéristiques de réduction de NO_3^- et de N_2O

Les cinétiques de production et de consommation de N_2O, réalisées en fioles sur les échantillons de sol remanié, ont permis de mettre en évidence des différences de vitesses de production de N_2O entre sols sous prairie et sols cultivés (Tableau 2).

Tableau 2 : Vitesses de réduction de NO_3^- et de production associée de CO_2 sur les 48 premières heures d'incubation, et vitesses maximales de réduction de N_2O lors des tests en fioles *(entre parenthèses : erreur standard)*.

Incubations	$NO_3^- + C_2H_2$				N_2O	
	Vitesses maximales de réduction de NO_3^- en N_2O (10^{-9}mol N-NO_3 kg^{-1} sol s^{-1})		Vitesses maximales de production de CO_2 (10^{-9} mol C-CO_2 kg^{-1} sol s^{-1})		Vitesses maximales de réduction de N_2O en N_2 (10^{-9} mol N-N_2O g^{-1} sol s^{-1})	
	Prairie	Culture	Prairie	Culture	Prairie	Culture
Montot	20.40 (7.74)	15.67 (2.98)	32.41 (11.57)	13.89 (6.94)	52.88 (11.90)	24.24 (8.33)
Pichange	32.24 (4.56)	17.26 (1.98)	16.81 (2.08)	7.45 (0.37)	52.38 (7.74)	26.39 (2.78)
Moloy	84.92 (4.37)	62.10 (6.15)	32.43 (1.85)	13.70 (2.48)	126.98 (2.78)	108.13 (40.48)
Panthier	39.48 (0.40)	15.87 (4.17)	32.13 (0.30)	9.68 (4.70)	61.11 (3.57)	34.92 (7.74)
St Thibault	46.03 (4.96)	14.88 (2.38)	16.9 (2.59)	4.65 (1.32)	51.59 (5.56)	42.46 (12.10)

Pour les situations de Pichange, Panthier, Moloy et St Thibault, les vitesses initiales de production brute de N_2O sont significativement plus élevées ($p \geq 0.99$) sous prairie que sous le sol cultivé et sont accompagnées de vitesses de dégagement de CO_2 également plus élevées sous prairie. Pour le sol de Montot, les vitesses observées sous prairie et sous culture ne sont pas statistiquement différentes. Dans ces 5 couples de sol, la teneur en C organique ainsi que la production de CO_2 sont toujours supérieures sous prairie que sous culture. Globalement, on peut mettre en évidence une corrélation significative entre la teneur en C organique des sols et leur vitesse maximale de dénitrification ($r^2 = 0.80$; $n = 10$), mais pas entre la vitesse maximale de dénitrification et la production associée de CO_2.

Les vitesses de réduction nette de N_2O ont parfois présenté un temps de latence (Pichange, Panthier et St Thibault) qui pourrait être du, pour partie, à la production concomitante de N_2O

Contrairement aux vitesses de réduction de NO_3^-, les vitesses maximales de réduction du N_2O ne sont pas systématiquement plus importantes sur sols de prairie que sur sols cultivés.

Plusieurs études avaient déjà comparé les potentiels de dénitrification (i.e. le taux de dénitrification mesuré en conditions anoxiques, avec NO_3^- et C organique non limitants) de sols sous culture et sous prairie (Bijay-Singh *et al.*, 1989 ; Weier *et al.*, 1993 ; Lensi *et al.*, 1995 ; Sotomayor & Rice, 1996). Comme dans notre étude, Lensi *et al.* (1995) et Sotomayor & Rice (1996) observaient une dénitrification potentielle supérieure sous prairie. Bijay-Singh *et al.* (1989) observaient aussi la supériorité de la dénitrification semi-potentielle sous prairie (i.e. en conditions anoxiques avec ajout de NO_3^-, mais sans ajout de C organique).

Dénombrements microbiens

Pour chacun des sites étudiés, les dénombrements microbiens n'ont pas mis en évidence de différences significatives entre sol cultivé et prairie. Le nombre total de bactéries aérobies est compris entre 8.10^7 (Montot) et 8.10^8 (Panthier) pour les sols cultivés et 6.10^7 (Montot) et 15.10^8 (Moloy) pour les sols de prairie. Le nombre total de bactéries anaérobies par rapport au nombre total de bactéries aérobies est très variable à la fois avec le site étudié et le mode d'occupation. Les bactéries dissimilatrices de NO_3^- représentaient de 2 à 52 % de la flore totale anaérobie. Enfin, les bactéries dénitrifiantes représentaient de 0.5 à 3% de la flore totale anaérobie. Pour les 5 sols étudiés, le nombre de dénitrifiants est apparu faible : il était compris entre 2.10^5 et 12.10^5 pour les sols cultivés, et $0.9.10^5$ et 16.10^5 pour les sols sous prairie.

Weier et MacRae (1992) avaient dénombré les dénitrifiants sous prairie et sous culture et avait obtenu des populations plus importantes sous culture, cette tendance s'accentuant avec la profondeur. Ces auteurs ont observé que le niveau de la population dénitrifiante dépendait essentiellement de la teneur en eau au moment du prélèvement de sol. Notre étude ne corrobore pas ce résultat. Ce travail de dénombrement montre que la taille des populations, définie avec une incertitude importante liée à la méthode MPN, n'est pas affectée par le mode d'occupation des sols alors que les activités de dénitrification peuvent l'être, suggérant que l'activité de dénitrification des sols n'est pas liée à la taille de la population dénitrifiante (Lensi *et al.*, 1995).

Caractérisation de la structure de la communauté dénitrifiante dans les sols

A l'issue des étapes de purification, 116 et 109 souches pures, aérobies facultatives et réductrices de NO_3^-, ont été isolées respectivement des sols sous prairie et cultivé de Montot

et, 260 et 246 des sols de Panthier. La caractérisation physiologique des souches isolées a permis de retenir 6 souches issues du sol sous prairie de Montot, 2 du sol cultivé de Montot, 4 du sol sous prairie de Panthier et 5 du sol cultivé de Panthier, considérées comme des dénitrifiantes *sensu stricto*. Parmi ces souches, l'amplification de l'ADNr16S n'a pas été obtenue pour 1 souche du sol de culture de Panthier et 2 souches du sol de prairie de Montot. Au total, 14 souches ont donc fait l'objet d'une caractérisation moléculaire, 8 issues du site de Panthier, dont 4 provenant du sol de prairie et 4 du sol cultivé et 6 du site de Montot, dont 4 du sol de prairie et 2 du sol cultivé. Les profils obtenus sont présentés dans le tableau n°3.

Tableau 3 : Profils de restriction obtenus après digestion de l'amplifiat de l'ADNr16S.

Site	Panthier			Montot			Montot			Montot			Montot		
Souche	P125 P204 P256 P22 C134 C88 C178 C167			P16 A109 A127			P49			P60			P90		
Enzyme	*Alu I*	*Rsa I*	*Bst UI*	*Alu I*	*Rsa I*	*Bst UI*	*Alu I*	*Rsa I*	*Bst UI*	*Alu I*	*Rsa I*	*Bst UI*	*Alu I*	*Rsa I*	*Bst UI*
									551	848					401
		467			465				467	459	467		452		
	444		295	440		302		456				305		452	303
	433	419	240	433	414	241		410			414	245		417	
Taille		362	211		362	213					363	219	410	363	216
des			187				316	219				193			189
Bandes		148	168		153	170		156			156	172	239	156	
en	211		146	214	122	149	218				126	152	219	126	150
Paires		101	124			128	206					131			131
de	186		91	192		91						92			91
Bases															
													170		
	124			126						127					
	76			80											
	42			46											
Total	1516	1497	1507	1531	1516	1534	740	1241	1018	1434	1526	1509	1490	1514	1481

L'ensemble des résultats obtenus permet de regrouper les 8 souches de Panthier et suggère leur appartenance à la même espèce. Les souches de Montot présentent des profils différents de celles de Panthier et plus variables. Les deux souches issues du sol cultivé de Montot présentent un profil similaire que l'on retrouve aussi pour une souche issue du sol de prairie de ce site. Les autres souches du sol de prairie ont des profils différents. Néanmoins on retrouve parfois des analogies entre les profils pour une enzyme particulière, tant Alu I que Rsa I et Bst UI. L'interprétation de ces résultats en terme de biodiversité reste limitée du fait du faible nombre de souches qui ont été isolées.

2.4. Conclusions et perspectives

Les résultats obtenus au cours de cette partie de travail confirment l'existence de dénitrifications semi-potentielles, en anaérobiose totale et en condition de NO_3^- non limitant, généralement plus importantes sous prairie que sous culture, en relation avec la teneur en C organique des sols (Bijay-Singh *et al.*, 1989 ; Weier *et al.*, 1993 ; Lensi *et al.*, 1995 ;

Sotomayor et Rice, 1996). Seul le sol de Montot a présenté des activités dénitrifiantes similaires sous prairie et sous culture en condition d'anoxie totale. Ce comportement est similaire à celui observé pour le sol de Citeaux (cf. paragraphe 3). Or ces deux sols ont en commun d'appartenir à l'unité pédologique *Lgll* (réf. de la carte pédologique de Dijon). A ce jour, nous considérons que ces vitesses de réduction des NO_3^- et de N_2O pourraient être considérées comme des caractéristiques biologiques des sols. Nous avons en effet vérifié que ces vitesses se reproduisaient dans le temps sur un autre sol (Chèneby, Thèse en cours). Le travail de dénombrement montre que la taille des populations, définie avec une incertitude importante liée à la méthode MPN, n'est pas affectée par le mode d'occupation des sols, ce qui corrobore le fait que l'activité de dénitrification des sols n'est pas liée à la taille de la population dénitrifiante (Lensi *et al.*, 1995). Dans les différentes situations, le nombre de dénitrifiants est apparu assez faible.

Ce travail a privilégié l'étude des fonctionnements dénitrifiants pour des conditions environnementales *a priori* optimales, à l'exception du C organique. Se pose alors le problème de l'expression de ces activités et de sa dépendance à l'ambiance géochimique au niveau des micro-organismes pour des sols non remaniés. Ainsi, Bijay-Singh *et al.* (1989) qui observaient aussi la supériorité de la dénitrification semi-potentielle sous prairie (i.e. en conditions anoxiques avec ajout de NO_3^-, mais sans ajout de C organique) trouvaient que la dénitrification réelle mesurée *in situ* ne devenait supérieure sous prairie à celle sous maïs qu'à des potentiels hydriques supérieurs à –5.5 kPa.

3. Rôles de la structure des mottes et de la matière organique particulaire sur la dénitrification à l'échelle de la motte

3.1. Objectifs

Dans cette partie du travail, nos objectifs étaient d'estimer la contribution relative des substrats organiques à la dénitrification de mottes de sol et l'influence de la structure de l'espace poral libre à l'air sur ce même processus. Ces deux variables étant fortement affectées par le mode d'occupation du sol (Pagliai, 1994 ; Balesdent, 1996 ; Besnard *et al.*, 1996), nous avons comparé les activités dénitrifiantes pour un même sol sous culture (i.e. une rotation céréalière) et sous prairie. Au sein des mottes, deux compartiments ont été distingués : les matières organiques particulaires (POM) accompagnées de leur gangue de sol (l'ensemble étant de taille supérieure à 200 µm) et le reste du sol, respectivement appelés C-POM (pour coated particulate organic matter) et matrice dans la suite de ce papier. La dénitrification

potentielle et la production de CO_2 ont été mesurées sur chacun des deux compartiments. Sur la base de ces expériences, un modèle mécaniste et stochastique a été construit pour comparer les distributions expérimentales de dénitrification par motte de sol et en condition d'aérobiose, à des distributions simulées. Pour chacun des 2 modes d'occupation du sol, le niveau d'aération a enfin été appréhendé par l'obtention de cartes 2D de concentrations en O_2 sur des mottes ayant des niveaux de dénitrification variés.

3.2. Matériels et méthodes expérimentales

Le sol et son conditionnement

Des mottes ont été échantillonnées dans la couche 10-25 cm d'un gleyic luvisol (classification FAO) a proximité de l'abbaye de Citeaux (21, Côte d'Or). Ce sol avait été mis en culture il y a 50 ans, mais une fraction de celui-ci est retourné en prairie depuis 25 ans. Les principales caractéristiques de l'horizon 10-25 cm du sol sont données dans le Tableau 4. Pour les 2 modes d'occupation du sol, des mottes ont été tamisées entre 2 et 2.5 cm à l'humidité de prélèvement, séchées à l'air pendant 1 semaine, et stockées à 2°C jusqu'à leur utilisation.

Tableau 4 : Principales caractéristiques du sol de Citeaux

Mode d'occupation		Prairie	Culture
Argile (g kg^{-1})		184	145
Limon (g kg^{-1})		523	541
Sable (g kg^{-1})		293	314
C organique total (g kg^{-1})		12.1	9.0
N organique total (g kg^{-1})		1.22	0.9
Matière organique particulaire			
0.05 – 0.2 mm	C (g kg^{-1})	0.92	0.47
	C/N	11.8	13.1
0.2 – 2.0 mm	C (g kg^{-1})	0.22	0.19
	C/N	21.5	16.5

Juste avant le début des mesures réalisées sur mottes intactes (production de CO_2, dénitrification et cartographie de O_2 dans un plan), ces dernières ont été réhumectées par une solution de KNO_3 (4 g.l^{-1}) à 20°C sur des tables de succion. Pour prévenir l'apparition de fissures, la succion a été fixée à 100 cm d'eau (1 jour), puis à 50 cm (1 jour), et enfin à 10 cm (5 jours). Cette dernière valeur correspond à un potentiel de succion de –1 kPa permettant de saturer les pores de diamètre inférieur à 300 µm.

Le même procédé de réhumectation a été utilisé pour caractériser individuellement l'activité dénitrifiante potentielle sur 9 et 9 mottes des sols sous prairie et sous culture, avec utilisation ultérieure d'une aliquote d'environ 2 g de chaque motte.

Une procédure de séparation des C-POM et de la matrice a été mise au point de façon à préserver le "film" bactérien qui pourrait exister autour des matières organiques particulaires (Parry *et al.*, 1998b). La caractérisation des activités microbiennes (production de CO_2 en condition aérobie, et activité dénitrifiante potentielle) et les dénombrements microbiens (nombres d'hétérotrophes et pourcentages de dénitrifiants) spécifiques aux C-POM et à la matrice ont été réalisés dans la foulée, soit 24 h après le début de l'imbibition. Ce procédé de séparation a par ailleurs été utilisé pour caractériser la distribution des teneurs individuelles en C-POM de 100 et 100 mottes des sols sous prairie et sous culture.

Sans réhumectation, 20 et 20 mottes des sols sous prairie et sous culture nous ont permis d'obtenir des lames minces de plans passant approximativement par leur centre de gravité.

Mesure de la production de CO_2 et des activités dénitrifiantes réelles et potentielles

Après 7 jours de réhumectation, la production de CO_2 et la dénitrification ont été caractérisées sur 100 et 100 mottes des sols sous prairie et sous culture, en condition aérobie et en présence de C_2H_2. Pour ce faire, des suivis cinétiques des concentrations gazeuses dans les enceintes contenant les échantillons ont été réalisés par dosages en chromatographie en phase gazeuse.

Pour les mêmes durées de réhumectation, l'activité dénitrifiante potentielle (i.e. en condition d'anaérobiose, avec ajout d'acide succinique comme source de C et de NO_3^-) a été mesurée sur des aliquotes d'environ 2 g prises séparément sur 9 et 9 mottes des sols sous culture et sous prairie.

Après séparation des C-POM et de la matrice, la production de CO_2 et l'activité dénitrifiante potentielle ont été mesurées sur des aliquotes d'environ 0.2 g de C-POM et de 1 g de matrice pour chacun des 2 sols, dans les mêmes conditions que précédemment, excepté la durée de réhumectation alors réduite à 1 jour.

Dénombrements microbiens

Pour les C-POM et la matrice, les nombres totaux d'hétérotrophes et de dénitrifiants ont été estimés par la méthode MPN déjà décrite dans le paragraphe 2.2..

Caractérisation des matières organiques

Pour chacun des deux modes d'occupation des sols, plusieurs caractéristiques de la matière organique ont été mesurées. Il s'agit d'une part de son rapport C/N. Il s'agit d'autre part de l'importance de différents compartiments de la matière organique dont la matière organique soluble estimée selon le protocole de Adams (1980), et des matières organiques particulaires extraites et subdivisées en plusieurs classes granulométriques (0.2 - 2 mm, 0.05 - 0.2 mm et <0.05 mm) selon le protocole de Balesdent *et al.* (1991). Il s'agit enfin de la distribution des teneurs des mottes en C-POM réalisées sur 100 et 100 mottes des sols respectivement sous prairie et sous culture. Des observations en microscopie optique et en microscopie électronique nous ont permis de d'appréhender l'origine des résidus organiques contenus dans ce compartiment et leur couverture ou non par une gangue de sol.

Caractérisation de la structure des mottes

L'espace poral d'une motte peut être subdivisé en un espace poral textural spécifique de la composition minérale et organique du sol (Stengel, 1979) dont les pores sont généralement de dimensions inférieures à quelques µm, et un espace structural généralement plus grossier et dépendant de facteurs biologiques, climatiques et anthropiques. Pour estimer l'importance des pores structuraux susceptibles de rester partiellement libres à l'air, nous avons mesuré la densité de solide à l'aide de pycnomètres, la densité texturale par la méthode au pétrole (Monnier *et al.*, 1973), et la densité apparente des mottes prises individuellement par la même méthode et par la méthode à la cire (Fiès et Zimmer, 1982).

Pour la même porosité structurale libre à l'air, l'aération en un point d'une motte dépend en première approche de sa distance au pore libre à l'air le plus proche (Rappoldt, 1990). Afin d'obtenir les distributions en fréquence de ces distances, 20 et 20 lames minces des mottes de sol sous prairie et sous culture ont été préparées selon le protocole de Bruand *et al.* (1996) puis traitées par analyse d'image. Ces derniers traitements comprenaient la simulation de la saturation des pores de diamètre inférieur à 300 µm et l'analyse proprement dite de la distribution des distances au pore le plus proche.

En assimilant les mottes à des ensembles d'agrégats sphériques, nous avons estimé la distribution en fréquence de ces agrégats en utilisant la procédure de Rappoldt (1992) nous permettant de passer d'observations 2D à des milieux 3D.

Distribution d'O_2 au sein de mottes et relation avec la dénitrification

Afin de tester à l'échelle microscopique les relations entre distribution des concentrations en O_2 d'une part, et distributions des pores libres à l'air et des matières organiques particulaires d'autre part, nous avons réalisé des mesures locales de concentration en O_2 à l'aide de microélectrodes. Pratiquement et pour chaque motte, 8 profils de concentration en O_2 ont ainsi été obtenus dans un même plan (angle de 45° entre chaque profil). Ces plans ont alors été signalés et des lames minces ont été obtenues à leur niveau après inclusion dans de la résine, contenant de la fluorescéine. Les pores structuraux ont été visualisés par fluorescence sous éclairage UV. Des cartographies des matières organiques particulaires >200µm ont été obtenues manuellement et par observation des lames à la loupe pour 1 et 1 mottes des sols respectivement sous prairie et sous culture.

3.3. Modélisation du fonctionnement dénitrifiant des mottes et évaluation de l'effet de la matière organique particulaire sur l'aération des mottes

Un modèle stochastique a été développé pour décrire les contributions respectives de la matrice et des C-POM à la dénitrification des mottes du sol sous prairie et du sol sous culture (Parry *et al.*, 1998b). Ce modèle suppose que la dénitrification est la somme des deux contributions supposées indépendantes :

$$D_{clod} = D_{matrix} + D_{C-POM} \qquad [1]$$

où D_{clod}, D_{matrix} et $D_{C\text{-}POM}$ sont les taux de dénitrification au niveau des mottes, de la matrice et des matières organiques particulaires avec leur gangue de sol (mol N_2O kg^{-1} s^{-1}).

Les mottes ont été assimilées à un ensemble d'agrégats. Le nombre d'agrégats n_{agr} est aléatoire et décrit par une distribution de probabilité $q(n_{agr})$. De la même façon, les rayons des agrégats r_i ($1 \leq i \leq n_{agr}$) étaient aléatoires, indépendants entre eux et décrits par une fonction de densité de probabilité a(r). Cette dernière a été estimée à partir des distributions moyennes de distances entre un point d'une motte et le pore libre à l'air le plus proche. La distribution probabiliste des valeurs de n_{agr} a été estimée à partir de la distribution des poids de motte.
La dénitrification matricielle D_i (mol $N_2O.kg^{-1}.s^{-1}$) de chaque agrégat i a alors été supposée proportionnelle au volume anoxique de la motte et à la dénitrification potentielle matricielle :

$$D_i = V_{anox} \times \rho_{agr} \times PDA_{matrix} \qquad [2]$$

où V_{anox} et le volume anoxique de l'agrégat (m^3), ρ_{agr} est la masse volumique de l'agrégat ($kg.m^{-3}$), et PDA_{matrix} l'activité dénitrifiante potentielle de la matrice (mol $N_2O.kg^{-1}.s^{-1}$). L'activité potentielle était celle mesurée comme décrit précédemment, après séparation de la

matrice des matières organiques particulaires. Le volume anoxique était calculé pour chaque agrégat par la procédure de Currie (1961).

La dénitrification au niveau de la matière organique particulaire $D_{C\text{-}POM}$ a été supposée proportionnelle à la teneur en C-POM de la motte :

$$D_{C-POM} = \frac{P_{C-POM} \times PDA_{C-POM}}{P_{clod}} \qquad [3]$$

où $P_{C\text{-}POM}$ est la quantité de C-POM par motte (kg), $PDA_{C\text{-}POM}$ est l'activité dénitrifiante potentielle au niveau des C-POM (mol $N_2O.kg^{-1}.s^{-1}$), et P_{clod} est le poids de la motte (kg). $PDA_{C\text{-}POM}$ a été mesuré comme décrit précédemment, et $P_{C\text{-}POM}$ a été obtenu par tirage avec remise de la teneur en matière organique particulaire dans la distribution expérimentale.

En marge de ce modèle et pour étudier simultanément (i) l'expression réelle de l'activité dénitrifiante au niveau de la matière organique particulaire et (ii) l'effet de ces mêmes matières organiques sur l'aération des mottes, un modèle de perturbation locale de la concentration en O_2 au voisinage des matières organiques à été proposé (Parry *et al.*, 1998c). En fonction de la position radiale de ces matières organiques au sein des agrégats constitutifs des mottes, ce modèle nous a amenés à évaluer :

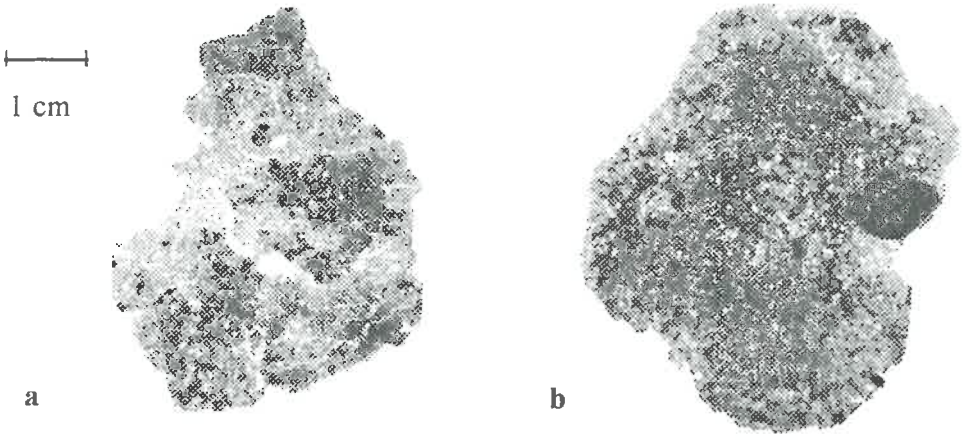
- la concentration en O_2 à la surface des C-POM et par voie de conséquence l'existence ou non d'un métabolisme anaérobie en leur sein (dont la dénitrification) ;
- le volume périphérique de sol autour des C-POM dont l'aération était perturbée par la présence des C-POM : pratiquement, ce volume a été défini par l'existence d'un abaissement de concentration en O_2 du à la présence des C-POM supérieur à 2% (valeur absolue) par rapport à ce que serait cette concentration en absence de C-POM.

3.4. Résultats et discussion

Les dénitrifications moyennes mesurées pour des mottes de sol intactes placées en condition d'aérobiose sont de $0.15\ 10^{-11}$ et de $2.1\ 10^{-11}$ mol $N_2O.kg^{-1}$ sol.s^{-1} pour les sols respectivement sous prairie et sous culture. Les différences de plus d'un ordre de grandeur, ne s'expliquent pas par les activités dénitrifiantes potentielles qui sont similaires pour les deux types de sols. Le rapport "dénitrification en condition aérobie / activité dénitrifiante potentielle" peut être considéré comme l'expression de cette dernière. Il est égal à 0.14 et 2.1% pour les mottes des sols respectivement sous prairie et sous culture.

L'analyse de la structure des mottes a clairement mis en évidence des différences entre les 2 populations de mottes avec, dans le cas des mottes du sol sous prairie, la présence d'un

réseau de macropores, qui bien que très faible d'un point de vue quantitatif (3.1%), réduit considérablement la distance des points d'une motte au pore libre à l'air le plus proche (Photos 1a et 1b).



Photos 1a et 1b : Exemples de sections fines obtenues à partir de mottes de sol sous prairie (a) et sous culture (b).

Après saturation des pores de diamètre inférieur à 300 µm, la distribution des distances au pore libre à l'air le plus proche montre que les mottes du sol sous culture ont une structure proche de celle des mottes Δ (Manichon, 1987) alors que les pores libres à l'air rendent exceptionnelles les distances supérieures à 6 mm dans le cas du sol sous prairie (Figure 1).
Ces distributions suffisent à expliquer des fractions anoxiques très différentes entre mottes des sols sous prairie et sous culture. Elles contribuent, indirectement, aux différences d'activités dénitrifiantes moyennes. Elles ne peuvent toutefois pas expliquer les distributions de d'activités entre mottes et, plus spécifiquement, les fortes valeurs observées pour les mottes du sol sous culture.

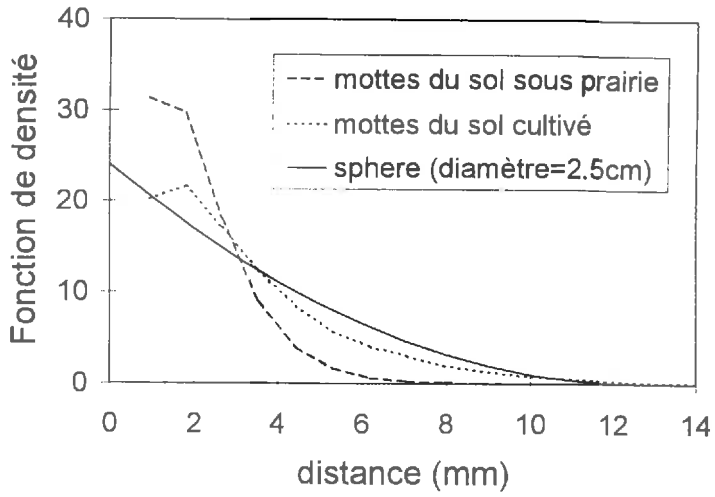


Figure 1 : distribution moyenne des distances entre points d'une motte et pore libre à l'air le plus proche, après saturation des pores de diamètre inférieur à 300 µm.

La production de CO_2 et la dénitrification potentielle sont plus importantes au niveau des C-POM qu'au niveau de la matrice (Tableau 5), ces écarts étant toutefois affectés par le mode d'occupation du sol (i.e. prairie ou rotation céréalière). Les valeurs élevées de respiration des C-POM favorisent l'installation de conditions anoxiques à leur niveau. Ces valeurs de respiration nous ont permis de définir très grossièrement une épaisseur minimale de sol les couvrant pour qu'elles puissent être en condition anoxique. En supposant que les C-POM soient distribuées de manière aléatoire au sein des mottes, 10 et 60% des matières organiques particulaires seraient alors en condition anoxique dans les sols respectivement sous prairie et sous culture. Le potentiel dénitrifiant des C-POM, plus élevé sous culture, accentue encore l'importance des C-POM dans ce sol.

Tableau 5 : Production de CO_2 et activité dénitrifiante potentielle des C-POM et de la matrice

	Prairie		**Culture**	
	C-POM	***Matrice***	***C-POM***	***Matrice***
Production de CO_2 en aérobiose (10^{-9} mol $CO_2.kg^{-1}$ sol.s^{-1})	92.9	15.4	306	9.7
Activité dénitrifiante potentielle (10^{-11} mol $N_2O.kg^{-1}$ sol.s^{-1})	103	135	945	17.8

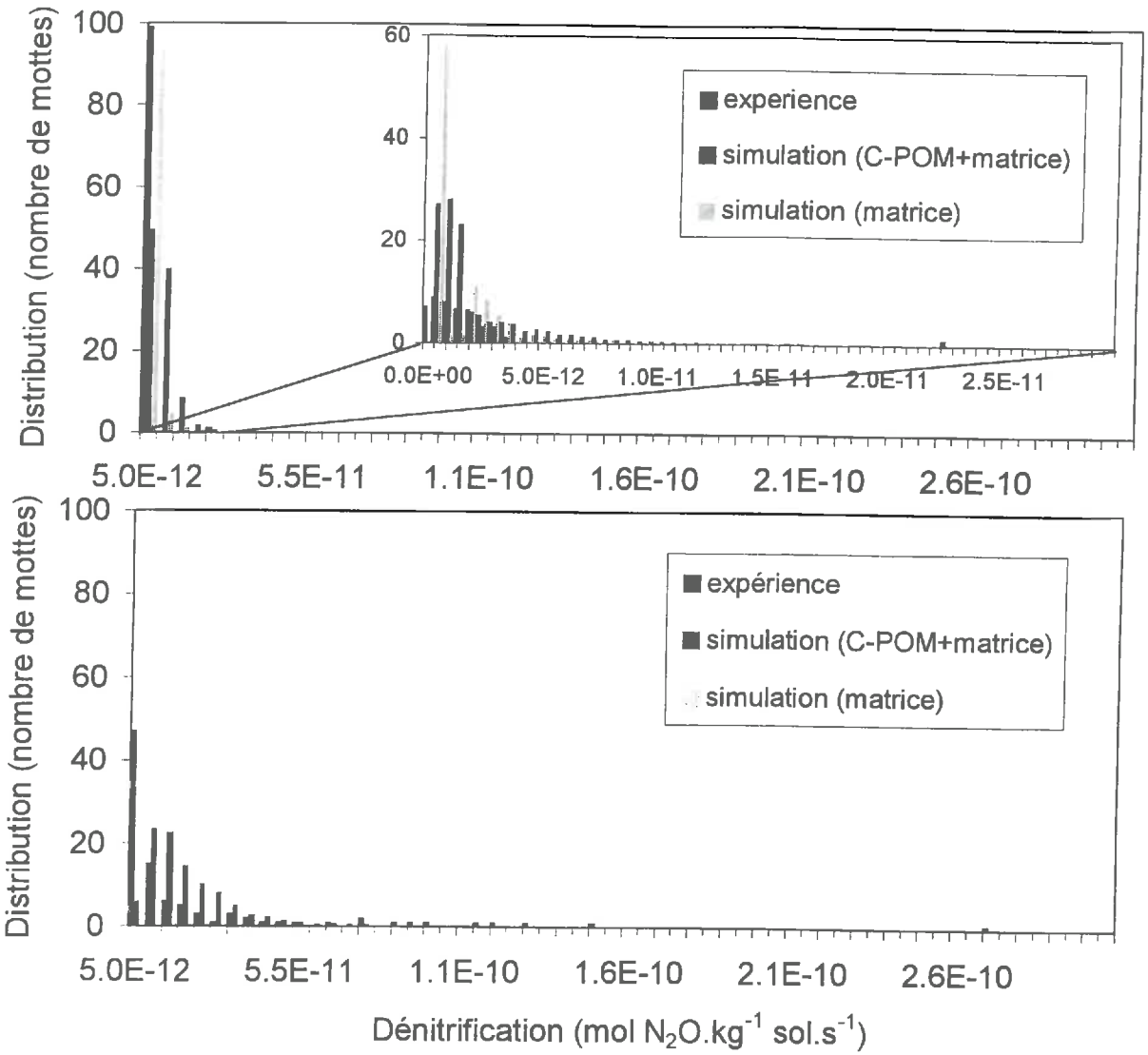
Les activités microbiennes, généralement élevées au niveau des C-POM, ne s'expliquent pas directement par les dénombrements d'hétérotrophes et de dénitrifiants. Cette absence de corrélation assez souvent rencontrée dans la littérature (Martin *et al.*, 1988; Lensi *et al.*, 1995) pourrait provenir simultanément de la difficulté à extraire les micro-organismes des POM, des incertitudes liées à la technique MPN, et à l'existence d'autres composantes impliquées dans la définition d'une activité dénitrifiante potentielle (niveau de synthèse enzymatique).

La prise en compte simultanée des activités respiratoires et dénitrifiantes, respectivement au niveau de la matière organique particulaire et au niveau du reste du sol (la matrice) nous a permis de paramétrer un modèle stochastique simulant le fonctionnement de ces 2 populations de mottes en tenant compte de la quantité variable de C-POM entre mottes et de la structure variable des mottes.

Nous avons ainsi confirmé le rôle variable joué par les C-POM, au niveau desquelles l'expression de l'activité dénitrifiante dépend simultanément des activités microbiennes et de la structure du sol :

- sous prairie, la prise en compte de l'activité dénitrifiante au niveau de la matrice suffit à expliquer la variabilité expérimentale de la dénitrification entre mottes, alors que l'ajout d'une activité sur l'ensemble des MOP aboutit à un étalement trop important des activités (Fig. 2a) ;

- sous culture, il est nécessaire de tenir compte des activités dénitrifiantes au niveau de ces deux compartiments (matrice et C-POM) pour simuler la variabilité des dénitrifications mesurées entre mottes (Fig. 2b).



Figures 2a et2b : Distributions expérimentales et simulées des activités dénitrifiantes pour des mottes des sols sous prairie (a) et sous culture (b)

L'analyse fine de la distribution de l'O_2 au sein des mottes et réalisée à l'aide de microélectrodes à O_2 semble confirmer ces conclusions : il existe une corrélation entre les niveaux d'aération observés pour les mottes sous prairie et leur activité dénitrifiante, alors que cette corrélation n'existe plus dans le cas des mottes du sol sous culture (Parry *et al.*, 1998c). L'existence ou l'absence de corrélation entre aération des mottes et activité dénitrifiante peut s'expliquer de la manière suivante :

- pour les mottes du sol sous prairie, les C-POM ne contribuent que de façon marginale à la dénitrification ; cette dernière apparaît principalement dans le compartiment

"matrice". On peut admettre que, pour les conditions expérimentales utilisées, la dénitrification soit alors proportionnelle à la fraction anoxique de la motte et donc corrélée à son niveau d'aération ;

- pour les mottes du sol sous culture, les C-POM expliquent une proportion importante de la dénitrification. La dénitrification ne serait alors corrélée au niveau d'aération des mottes que si la présence des C-POM modifiait significativement la fraction anoxique des mottes et leur niveau d'aération.

Or, l'utilisation d'un modèle nous a permis de montrer le rôle marginal joué par les C-POM dans l'aération des mottes (Parry *et al.*, 1998c). Pour le sol sous culture, l'inclusion d'une C-POM de 0.5 mm de rayon dans un agrégat de 1 cm constitutif d'une motte va entraîner un fonctionnement partiellement et totalement anaérobie de la C-POM pour des distances à la surface de l'agrégat respectivement supérieures à 2 mm et 2.5 mm (Fig. 3). Autour de la C-POM, il est possible de définir la POM-sphère comme étant la zone au sein de laquelle la présence de la C-POM entraîne une baisse de $[O_2]$ supérieure à 2% (valeur absolue). Le rayon de la POM-sphère n'excède jamais 0.9 mm (Fig. 3). Considérant au maximum 250 C-POM identiques réparties de manière aléatoire dans un tel agrégat pour simuler une motte à dénitrification élevée, il est alors possible de montrer que le niveau de $[O_2]$ baisse de plus de 2% sur 6% environ du volume de la motte. Ceci démontre clairement l'absence de corrélation entre dénitrification et niveau d'aération des mottes, alors que les C-POM jouent un rôle essentiel sur la dénitrification.

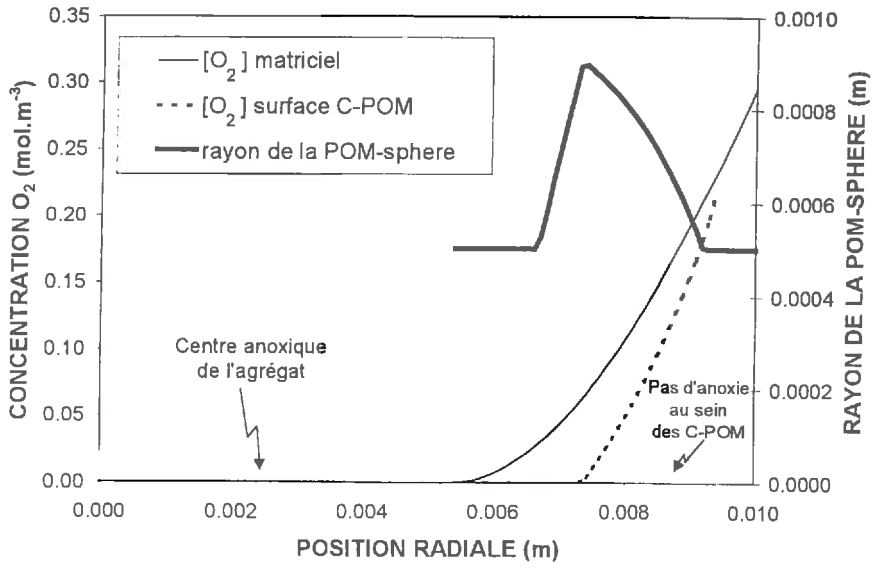


Figure 3 : Effet d'une C-POM de 1 mm de diamètre sur le niveau d'aération d'un agrégat de 1 cm de rayon constitutif d'une motte de sol sous culture en fonction de la position radiale de la C-POM au sein de l'agrégat.

La courbe "[O_2] matriciel" représente le profil de [O_2] en absence de C-POM.

2.5. Conclusion et perspectives

Nous avons montré que la présence d'un espace poral libre à l'air ne représentant pas plus de quelques pour-cent du volume de la motte pouvait modifier leur niveau d'aération et, par voie de conséquence, leur activité dénitrifiante. Plus que de son importance volumique, son effet dépend de sa répartition au sein de la motte et de la réduction des distances qu'il entraîne entre un point de la motte et le pore libre à l'air le plus proche. Ainsi, le taux de saturation des pores peut être une variable trop globale pour décrire indirectement les relations entre dénitrification et aération du sol, si cette relation n'est pas circonstanciée par la prise en compte de la structure qui dépend du contexte agro-pédo-climatique.

Nous avons par ailleurs montré que l'activité dénitrifiante des mottes de sol peut être sous-estimée si l'on admet une répartition homogène des activités respiratoire et dénitrifiante. L'activité dénitrifiante des mottes est la somme d'une contribution des C-POM, *a priori* non corrélée à la fraction anoxique de la motte, et d'une contribution diffuse de la matrice dans son domaine anoxique. Cette dernière suffit à décrire le fonctionnement dénitrifiant global des mottes lorsque l'activité dénitrifiante potentielle des C-POM est faible ou lorsque les activités respiratoires et la structure du sol ne permettent pas à cette activité dénitrifiante de s'exprimer. Les niveaux très différents d'activités respiratoire et dénitrifiante entre C-POM du sol sous prairie et C-POM du sol sous culture ne semblent pas pouvoir s'expliquer par des critères de qualité tels que le rapport C/N, ni *a priori* par leur composition (C organique soluble et taux de lignine). Il semble nécessaire de prendre en compte des problèmes d'accessibilité déjà étudiés pour la minéralisation de résidus organiques en conditions aérées.

Nous avons ainsi mis au point un modèle qui tient compte simultanément des contributions des C-POM et de la matrice, contrairement aux modèles passés qui admettaient un fonctionnement exclusivement matriciel (exception faite de la tentative de Rappoldt (1992)). De par sa simplicité de mise en œuvre, il pourrait constituer une alternative intéressante à d'autres modèles mécanistes. Il doit cependant être amélioré sur 2 points :

- la proportion des C-POM exprimant une activité dénitrifiante ;
- le comportement des mottes en condition de NO_3^- limitant.

Ce modèle pourrait servir de base d'évaluation d'autres approches simplifiées. Il pourrait aider à la conception d'une typologie des mottes de sol (au regard de la dénitrification) se référant simultanément à la structure des mottes et aux types de matières organiques incorporées dans le sol.

4. Dynamique de la distribution des communautés microbiennes hétérotrophes totales et dénitrifiantes à l'échelle de la motte : une étude sur mottes reconstituées

4.1. Objectifs

Les études qui ont eu pour objectif d'examiner les interactions entre la dénitrification et l'agrégation ont été effectuées selon 3 approches basées sur :

- (i) le fractionnement du sol en classes d'agrégats en fonction de leur taille ;
- (ii) l'analyse individuelle de petits agrégats ;
- (iii) l'observation simultanée des gradients de $[O_2]$ et de l'activité dénitrifiante effectuée sur des agrégats de grande taille ou sur des mottes de sol.

Les travaux issus des 2 premières approches montrent que des différences significatives d'activité dénitrifiante et de densité de dénitrifiants existent en fonction de la taille des agrégats (mesures effectuées sur des massifs d'agrégats de taille différente) (Beauchamp et Seech, 1990 ; Lensi *et al.*, 1995) et que, à l'intérieur d'une même classe de taille, tous les agrégats (analysés individuellement) ne sont pas colonisés (Lensi *et al.*, 1991). Ces 2 premières approches concernaient des agrégats de petite taille et n'étaient, de ce fait, pas à même de permettre l'examen des corrélations existant entre la répartition des conditions physico-chimiques et les caractéristiques de dénitrification au sein d'une même unité structurale. La troisième approche concernant des agrégats de grande taille (ou mottes) a permis ce type d'analyse et a montré :

- parfois une absence de corrélation entre l'activité dénitrifiante et le volume du centre anoxique (Sexstone *et al.*, 1985), parfois une corrélation entre ces 2 grandeurs (Parkin et Tiedje, 1984) ;
- une corrélation inverse entre les gradients radiaux de $[O_2]$ et de production de N_2O (Højberg *et al.*, 1994).

On peut constater que peu de travaux ont tenté d'établir des relations entre les situations interne ou externe et la densité des micro-organismes dénitrifiants ou la proportion relative des différentes communautés microbiennes (rapport "dénitrifiants / hétérotrophes totaux" par exemple). De même, aucune étude n'avait été faite sur le rôle de la dénitrification en tant que facteur déterminant la capacité des micro-organismes à coloniser le centre anoxique des agrégats (ou mottes) de sol.

Le travail décrit dans ce paragraphe aborde ces aspects selon une approche dynamique et tente de les relier à l'évolution de $[O_2]$. Le rôle de la dénitrification dans l'aptitude compétitive des micro-organismes a été abordé par la comparaison du comportement d'une souche dénitrifiante et d'un mutant isogénique de cette souche (Nir^-) déficient dans la capacité de synthétiser la nitrite réductase dissimilative. Nous avons réalisé une étude sur des mottes

reconstituées (donc *a priori* initialement homogènes) afin de s'affranchir de l'influence de la variabilité due aux micro-hétérogénéités.

3.2. Matériels et méthodes expérimentales

Le sol ; fabrication des mottes et incubation

Le sol de Citeaux utilisé dans cette étude a été décrit précédemment. Les mottes ont été reconstituées selon Fies et Stengel (1981) et Sierra *et al.* (1996). En bref, le sol est réhumecté puis mélangé jusqu'à obtention d'une pâte homogène qui subit, ensuite, différentes étapes de dessiccation et de façonnage jusqu'à obtention de mottes sphériques de 3 cm de diamètre supposées homogènes et similaires entre elles vis-à-vis de critères physico-chimiques et microbiens. Au cours de ce processus de fabrication, un lot de mottes a été reconstitué à partir d'une "pâte" préalablement inoculée par un mélange à parité "souche dénitrifiante - mutant Nir^{-}" (densité totale : 10^4 cell.g^{-1} sol). Les mottes reconstituées ont ensuite été réhumectées sur table de succion comme décrit précédemment. et ont atteint une teneur en eau homogène et constante de 23 à 25 g.kg^{-1}. Les mottes sont incubées à 20°C sur les tables de succion et, périodiquement une partie d'entre elles est prélevée pour subir l'un des traitements suivants :

- (i) utilisation sans perturbation ;
- (ii) mesures après écrasement et homogénéisation ; ou
- (iii) mesures spécifiques à 3 fractions concentriques (Fig. 4).

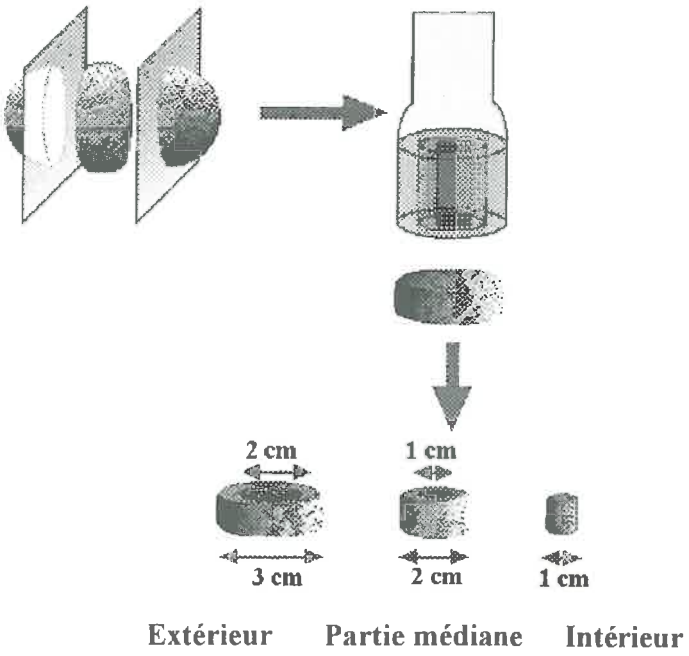


Figure 4 : protocole expérimental de séparation des mottes en fractions concentriques externe, intermédiaire et interne.

Micro-organismes

La souche dénitrifiante RTC01 de *Pseudomonas* sp. (naturellement résistante à la rifampicine et contenant une NO_2^--réductase à cuivre) et son mutant isogénique déficient dans la capacité de synthétiser la NO_2^--réductase dissimilative (Nir^- résistant à la rifampicine et à la kanamycine grâce à l'insertion du groupement Tn5 dans le gène de structure de la nitrite réductase) ont été utilisés. La souche Nir^- a été obtenue par Ye *et al.* (1992) et validée pour ce type d'étude par Philippot *et al.* (1995).

Profils de $[O_2]$

La technique d'évaluation de la distribution de $[O_2]$ par insertion de micro-électrodes dans la motte de sol a été décrite précédemment. Pour chaque motte testée, 2 profils ont été effectués : l'un à partir du pôle en contact avec la table de succion et l'autre à partir du pôle opposé.

Caractérisations microbiologiques

Les nombres de micro-organismes hétérotrophes totaux, dénitrifiants, et capables de réduire NO_3^- en NO_2^- ont été estimés par la technique MPN en plaques de microtitration. Cette technique a été décrite précédemment. La souche sauvage de *Pseudomonas* et le mutant Nir^- ont été dénombrés par étalement sur boîte de Pétri et distingués entre eux par résistance différentielle aux antibiotiques.
La biomasse microbienne totale a été estimée par une technique de fumigation-extraction (Chaussod *et al.*, 1988) adaptée au traitement de petits échantillons de sol. La biomasse microbienne "active" a été mesurée par la technique INT (Norton et Firestone, 1991).

Production de CO_2 et activité dénitrifiante

La production de CO_2 et l'activité dénitrifiante ont été mesurées sur des mottes intactes, maintenues en conditions aérobies. La dénitrification potentielle a été mesurée sur des aliquotes de sol des compartiments externes, intermédiaires et interne après séparation des mottes en fractions concentriques.

4.3. Résultats et discussion

Evolution des profils de $[O_2]$, et des distributions des souches inoculées et des micro-organismes indigènes

La fiabilité des mesures de profils de $[O_2]$ est démontrée par le fait que les 2 profils opposés réalisés sur chaque motte sont remarquablement similaires. Cette similarité suggère également que les protocoles de préparation et de réhumectation permettent d'obtenir des mottes très homogènes. La Figure 5 montre l'évolution du volume aérobie (calculé d'après ces profils) d'une part pour les mottes entières et, d'autre part, pour les zones internes, intermédiaire et externe.

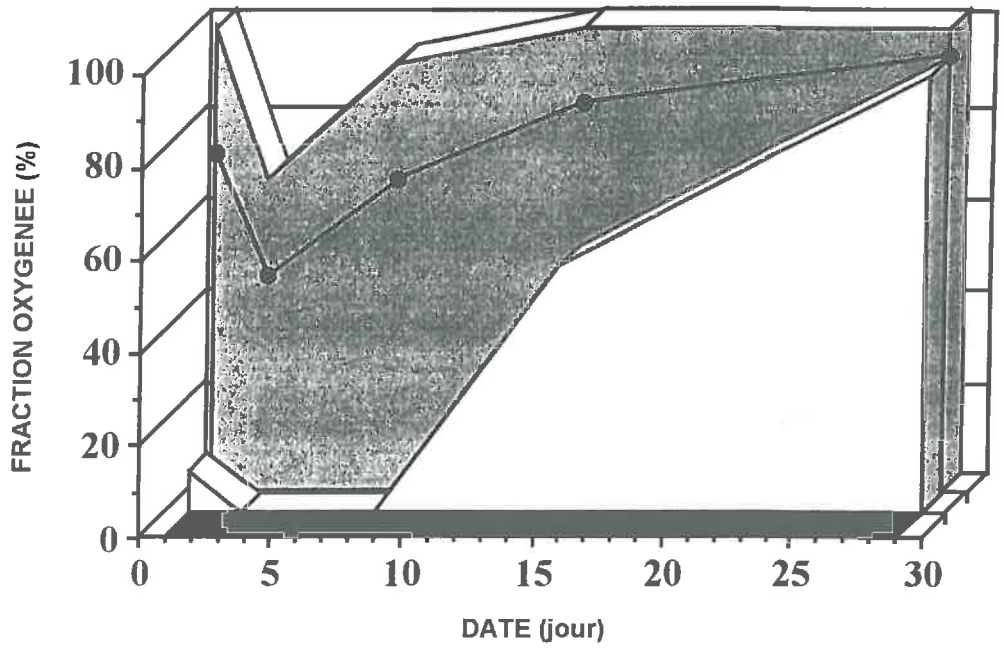


Figure 5 : Evolution du pourcentage d'aérobiose dans la motte entière (—●—) et dans les zones interne (▭) intermédiaire (▭) et externe (▬) de la motte.

Au niveau de la motte entière, on observe :

- une phase initiale de décroissance du volume aérobie due à l'activité métabolique intense qui suit généralement la réhumectation du sol (et qui confirmée par l'augmentation correspondante de la production de CO_2). Pendant cette phase, les zones intermédiaire et interne sont totalement anoxiques ;
- une phase d'augmentation du volume aérobie correspondant à la mise en place de conditions aérobies dans la zone intermédiaire. Cette phase correspond probablement à

l'épuisement progressif des composés donneurs d'électrons dans le sol comme le suggère la décroissance simultanée de la production de CO_2.

Dans les premiers jours suivant l'inoculation, on observe un accroissement significatif du nombre de *Pseudomonas* tant en ce qui concerne la souche sauvage que la souche mutante. Cette situation permet de suivre l'évolution du rapport "Nir^- / inoculum total" pour des micro-organismes en phase de colonisation. Dans les 3 compartiments de la motte de sol, l'évolution de ce rapport montre un désavantage de la souche mutée par rapport à la souche sauvage d'autant plus important que l'on s'adresse à des compartiments plus internes (Fig. 6). Ce résultat suggère que la capacité de synthétiser la Nir^--réductase dissimilative peut conférer aux micro-organismes un avantage compétitif pour la colonisation des zones internes des mottes. On peut noter que la différentiation entre les compartiments se met en place très rapidement après le début de l'incubation et se maintient globalement constant jusqu'à la fin de l'expérience. Ceci est en accord avec les résultats de Philippot *et al.* (1995) et suggère que l'essentiel des différences de comportement entre souches sauvage et mutante intervient durant la phase de multiplication cellulaire.

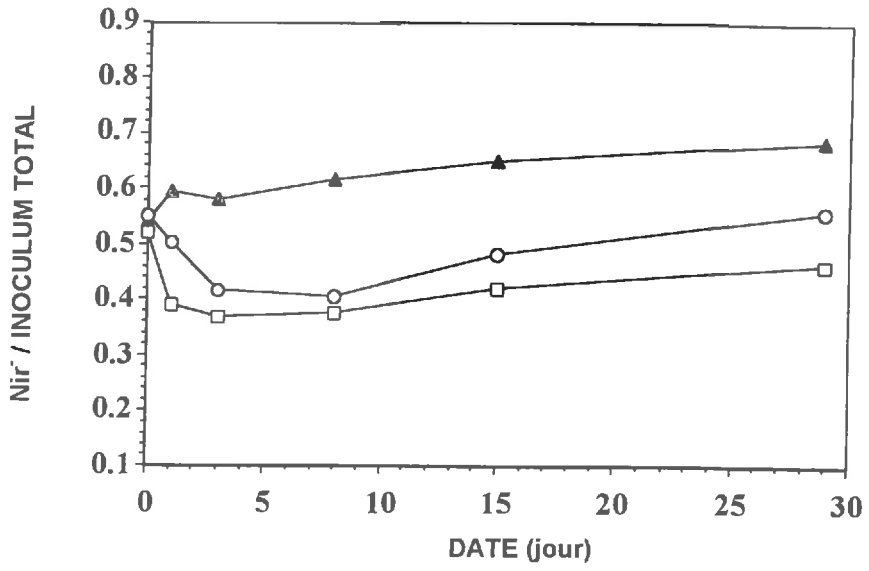


Figure 6 : Evolution du rapport "Nir^- / inoculum total" de la souche *Pseudomonas* RTC01 dans les compartiments externe (▲), intermédiaire (O) et interne (□) des mottes de sol.
Les différences entre les compartiments sont significatives à $P< 0.001$, excepté entre les compartiments internes et intermédiaires aux jours 3 et 8.

L'analyse conjointe de l'évolution des volumes aérobies (Fig. 5) et du rapport "Nir^- / inoculum total" (Fig. 6) montre que l'aération est un déterminant puissant de l'avantage observé pour la souche sauvage dans les 2 compartiments les plus internes de la motte. Cette assertion est renforcée par le fait que le passage progressif de l'anoxie vers l'aérobiose du compartiment

intermédiaire correspond à une différentiation progressive du rapport "Nir⁻ / inoculum total" dans ce même compartiment par rapport au compartiment interne. La légère augmentation de ce rapport dans le compartiment externe (qui présente des conditions aérobies de manière permanente au cours de l'expérience) est attribuée à un effet intrinsèque du transposon Tn5 indépendamment de sa localisation dans le génome (Biel et Hartl, 1983 ; Marshall *et al.*, 1988 ; Blot *et al.*, 1991). Cet effet, par ailleurs déjà été observé pour le même couple microbien dans des conditions expérimentales similaires à celles de la présente étude (Philippot *et al.*, 1995), est certes indésirable mais n'est cependant pas à même d'interférer significativement avec nos interprétations.

Compte tenu de ces résultats, on pourrait attendre une fréquence de micro-organismes dénitrifiants indigènes (ou, de manière plus générale, capables de dissimiler l'azote) plus importante dans le compartiment interne de la motte par rapport au compartiment externe. Or le Tableau 6 montre que la proportion de dénitrifiants et de NO_3^--réducteurs rapportée à la microflore hétérotrophe indigène totale est similaire entre ces 2 compartiments et demeure constante jusqu'à la fin de l'expérience.

Tableau 6 : Evolution de la microflore hétérotrophe totale et de la proportion de dénitrifiants ***(entre parenthèses : erreur standard, - : non mesuré).***

	Compartiment	Date (jour)				
		1	3	8	15	29
Hétérotrophes (10^7 cfu.g^{-1} sol)	Interne	5.0 (0.2)	-	5.7 (1.3)	6.1 (0.5)	-
	Intermédiaire	-	-	-	-	-
	Externe	4.4 (0.4)	-	5.3 (0.2)	7.2 (0.7)	-
% dénitrifiants	Interne	6	-	6	7	-
	Intermédiaire	-	-	-	-	-
	Externe	7	-	9	8	-

Différentes hypothèses peuvent être avancées pour expliquer cette contradiction apparente entre les résultats obtenus à l'échelle de la population inoculée et ceux obtenus de la communauté indigène. On peut se référer à l'article fourni en annexe (Philippot *et al.*, 1996) pour une description détaillée de ces hypothèses. En résumé, les raisons principales sont probablement liées au fait que, pendant l'expérience, la souche de *Pseudomonas* inoculée est en phase de multiplication cellulaire alors que les micro-organismes hétérotrophes indigènes présentent une densité constante (Tableau 6). Dans ces conditions, 1 mois d'incubation est probablement un temps beaucoup trop court pour que se mette en place une restructuration perceptible des communautés fonctionnelles indigènes. Ce temps apparaît d'ailleurs également insuffisant pour générer des différentiations observables de l'état physiologique des micro-organismes indigènes. En effet, les mesures d'INT comme les mesures de dénitrification potentielle ne sont pas significativement différentes entre les 3 compartiments de la motte.

4.4. Conclusions et perspectives

Les conditions générales de ce travail expérimental, au niveau de la motte entière, peuvent se résumer comme suit :

- la concentration en C organique assimilable a probablement diminué progressivement pendant la durée de l'expérience en entraînant une amélioration progressive de l'aération de la motte ;
- pendant une partie importante de l'expérience, la souche de *Pseudomonas* inoculée a été en phase active de colonisation alors que la microflore indigène était en état de stagnation démographique ;

Ces conditions ont permis de mettre en évidence ce qui constitue le résultat principal de cet aspect du travail : l'aptitude à synthétiser la NO_2^--réductase dissimilative peut conférer à une souche microbienne un avantage pour coloniser les zones internes des mottes de sol.

5. Conclusion générale

L'ensemble des travaux présentés dans cette synthèse a porté sur l'étude, à l'échelle de la motte de sol, des relations entre dénitrification d'une part, et structure du sol et matière organique d'autre part. Ces deux derniers facteurs (structure et matière organique) étant affectées à l'échelle de la motte par le mode d'occupation du sol, l'essentiel des travaux a été réalisé sur des couples de sols dont les deux éléments étaient issus de la même unité pédologique, mais étaient soumis à deux modes d'exploitation : une prairie et une rotation céréalière.

Nous avons ainsi montré que l'activité dénitrifiante des mottes est la somme d'une contribution des C-POM (i.e. la matière organique particulaire accompagnée de sa gangue de sol) *a priori* non corrélée à la fraction anoxique de la motte, et d'une contribution diffuse de la matrice (i.e. le reste de la motte) dans son domaine anoxique. Cette dernière suffit à décrire le fonctionnement dénitrifiant global des mottes lorsque l'activité dénitrifiante potentielle des C-POM est faible ou lorsque les activités respiratoires et la structure du sol ne permettent pas à cette activité dénitrifiante de s'exprimer. La présence de quelques pores libres à l'air peut modifier le niveau d'aération des mottes et réduire ainsi le domaine matriciel en état d'anoxie et l'activité dénitrifiante au niveau des C-POM. Concernant la dénitrification potentielle et la respiration, les différences observées entre C-POM et matrice ne semblent pas trouver leur origine dans les densités de microbiennes d'hétérotrophes et de dénitrifiants. L'absence de corrélation entre densité et activités microbiennes a été couramment mentionnée dans la

littérature et un autre volet de nos propres travaux confirme ce fait à une autre échelle : les différences d'activités dénitrifiantes entre modes d'occupation du sol pour 5 couples "prairie – culture" provenant chacun d'une même unité pédologique ne sont pas corrélées aux densités microbiennes. Ainsi, la respiration maximale et l'activité dénitrifiante potentielle pourraient résulter pour partie du niveau de synthèse enzymatique. Ce résultat est à toutefois à prendre avec précaution en raison de l'incertitude de la méthode de dénombrement MPN proprement dite et du taux d'extraction de micro-organismes qui est affecté par la teneur en matière organique du sol. Nous avons ainsi montré en utilisant une souche marquée et son mutant isogénique Nir$^-$ que la capacité à réduire NO_2^- en N_2O pouvait être un avantage pour coloniser un domaine anoxique dans le cas d'une souche en phase de colonisation (i.e. initialement présente à faible densité).

L'ensemble de ces travaux renvoie à plusieurs questions concernant la prise en compte de la structure du sol et de la matière organique :

- plus que de l'importance volumique de l'espace poral libre à l'air, son effet dépend de la distribution des pores dans l'espace et l'on peut s'interroger sur la pertinence de variables globales telles que le taux de saturation de l'espace poral pour décrire son effet sur la dénitrification sans prendre en compte simultanément le contexte agro-pédo-climatique qui peut jouer sur la structure du milieu ;
- les niveaux très différents d'activités respiratoire et dénitrifiante entre C-POM du sol sous prairie et C-POM du sol sous culture ne semblent pas pouvoir s'expliquer par des critères de qualité tels que le rapport C/N, ni *a priori* par leur composition (C organique soluble et taux de lignine). Il semble nécessaire de prendre en compte des problèmes d'accessibilité déjà étudiés pour la minéralisation de résidus organiques en conditions aérées.

Nous avons mis au point un modèle qui tient compte des contributions des C-POM et de la matrice, contrairement aux modèles passés qui décrivaient un fonctionnement exclusivement matriciel (exception faite de la tentative de Rappoldt (1992)). De par sa simplicité de mise en œuvre, il pourrait constituer une alternative intéressante à d'autres modèles mécanistes. Il doit cependant être amélioré sur 3 points :

- la proportion des C-POM exprimant une activité dénitrifiante ;
- le comportement des mottes en condition de NO_3^- limitant ;
- l'effet de la teneur en eau sur la distribution simulée des tailles d'agrégats constitutifs des mottes et sur l'anoxie au niveau des C-POM.

Couplé à un modèle de transferts verticaux de masse et de chaleur dans le sol et à un modèle décrivant les autres composantes des cycles de C et de N, il pourrait servir d'interpolateur dans l'espace et dans le temps de mesures des flux de N_2O à l'interface sol-atmosphère pour aboutir à des estimations régionales des émissions de N_2O.

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DOCUMENTS ANNEXES

1. **Philippot L., P. Renault, J. Sierra, C. Hénault, A. Clays-Josserand., C. Chenu, R. Chaussod and R. Lensi. 1996.** Dissimilatory nitrite-reductase provide a competitive advantage to Pseudomonas sp. RTC01 to colonise the center of soil aggregates. *FEMS Microbiology Ecology* 21, 175-185.

2. **Parry, S., P. Renault, C. Chenu and R. Lensi. 1998a.** Denitrification in pasture and cropped soil clods as affected by the pore space structure. *Soil Biology and Biochemistry*, sous presse.

3. **Parry, S., P. Renault, J. Chadœuf, C. Chenu, and R. Lensi. 1998b.** Particulate organic matter as a source of denitrification variability in pasture and cropped soil clods. *European Journal of Soil Science*, soumis le 12 novembre 1998.

4. **Parry, S., P. Renault, C. Chenu, J. Chadœuf, O. Bastien, and R. Lensi. 1999.** Correlation between Clod anoxic Fraction and Denitrification: the Effects of air-filled Pore Space and Particulate Organic Matter. *Soil Science Society of America Journal*, soumission prévue en janvier 1999. (Document inachevé)

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Dissimilatory nitrite-reductase provides a competitive advantage to *Pseudomonas* sp. RTC01 to colonise the centre of soil aggregates

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Abstract

The effect of soil aggregation on denitrification has been studied in different ways: artificial or natural aggregates individually analysed or comparison of denitrifying activity of different size classes of aggregates. However, until now, no work has been conducted to evaluate the role of denitrification in the microbial colonisation of soil aggregates. Over a one-month period, we examined on remoulded nonsterile soil aggregates the survival of inoculated wild-type *Pseudomonas* strain and of its corresponding isogenic Tn*5* mutant (Nir^-) lacking the ability to synthesize the dissimilative nitrite-reductase. Simultaneously, the evolution of the O_2 repartition inside the soil aggregates was assessed by the use of microelectrodes. The inner and outer portions were roughly anoxic and oxic, respectively, during the entire experiment while the intermediate portion showed fluctuating aeration conditions. The values of the Nir^- to wild-type + Nir^- ratio were found in the following order: inner < intermediate < outer portion, demonstrating that the nitrite-reductase may provide a competitive advantage to the *Pseudomonas* strain to colonise the centre of soil aggregates. However, a clear differentiation between inner and outer aggregates portions was not observed with the indigenous microflora (denitrifiers-to-total heterotrophs, biomass or physiological abilities).

Keywords: Denitrification; Nitrite-reductase; Aggregate; *Pseudomonas* sp.; Oxygen profile

1. Introduction

The expression of microbial functions in soil closely depends on the distribution of the microorganisms in the soil structure. Selective distribution of microorganisms according to their internal or external position in the soil aggregates was previously reported [1,2]. As examples, outer fractions were dominated by Gram-positive bacteria whereas inner fractions were comprised mainly of Gram-negative ones [1], and the inner or outer position also affects ammonium oxidizers-to-nitrite oxidizers ratio [2].

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One of the main factors differentiating the inner from the outer part of saturated soil aggregates is the O_2 concentration. As a first approximation, this parameter depends on the O_2 maximal consumption-to-O_2 diffusion coefficient ratio. In some conditions (e.g. large aggregates saturated with water and exhibiting a high maximal O_2 consumption rate), the centre of the aggregates may be anoxic [3–6]. The anoxic fraction of the aggregate then depends on several factors including its shape and dimensions, O_2 diffusion and maximal O_2 consumption.

Several authors have attempted to relate denitrification, a highly O_2-dependent microbial function, with soil aggregation. Until now, this relationship has been studied according to three experimental approaches based on either soil fractionation, analysis of individual small aggregates, or simultaneous establishment of physico-chemical profiles and denitrification within large aggregates [4,7–10]. Thus, soil aggregates of different sizes exhibited contrasting denitrifying activities and denitrifier densities [7,8], whereas the analysis of individual soil aggregates within the same size class suggested that only some of them are colonised by denitrifiers [9]. However, these studies were only able to correlate the distribution of denitrification poorly with the physico-chemical conditions prevailing in the soil aggregates. Such relationships performed by simultaneous measurements of oxygen profiles and denitrification rates demonstrated a lack of correlation between denitrification and size of anoxic centre [4] and inverse correlation between the O_2 partial pressure and N_2O production through a gradient from the surface to the centre of soil aggregate [10]. None of these works were concerned by the significance of denitrification as a determinant of aggregate colonisation. The ability to use electron acceptors other than O_2, such as NO_3^- or NO_2^-, can be supposed to help microorganisms to colonise the centre of aggregates.

In a previous study [11], using isogenic mutants, we demonstrated that the inability to reduce NO_2^- substantially decreased the competitive abilities of the studied *Pseudomonas* strains to colonise rhizosphere and anoxic soil.

By using the same approach (i.e. isogenic mutants of *Pseudomonas*), our objective was to compare the capacity of a strain lacking NO_2^- reductase and of the corresponding wild-type to survive in the different concentric zones of artificial aggregates. Simultaneously, the dynamics of oxygen transfer from the surface to the centre of the aggregates was monitored using O_2-microsensors previously used on soil aggregates by Sexstone et al. [4], Zausig et al. [12], Højberg et al. [10] and Sierra et al. [6]. In order to evaluate the ecological significance of our results, the evolution of some biological features of the indigenous microflora of the studied soil (such as denitrifiers-to-nondenitrifiers ratio, respiratory and denitrifying activities and microbial biomass) was followed.

2. Materials and methods

2.1. Soil

The 0–30 cm horizon of a Gleyic luvisol (FAO classification) was used for this study. It was sampled in the region of Dijon (Burgundy, France), air-dried and sieved to less than 2 mm. The properties of the soil were as follows: clay, 198 g kg^{-1}; silt, 511 g kg^{-1}; sand, 291 g kg^{-1}; pH_{water}, 7.3; organic C, 10.0 g kg^{-1}; total N 1.3 g kg^{-1}.

2.2. Organisms

Pseudomonas sp. RTC01 (wild-type), containing the copper nitrite-reductase, is a natural rifampicin-resistant clone of G-179 denitrifying strain which was isolated from a pampa agricultural soil [13]. Strain RTC22 lacking the ability to reduce nitrite (Nir^- mutant: resistant to rifampicin and to kanamycin) has been obtained by Ye et al. [14] by Tn*5* insertion into the nitrite-reductase structural gene of RTC01.

Strains were kindly supplied by J.M. Tiedje (Michigan State University, USA).

2.3. Preparation and incubation of aggregates

In order to obtain homogeneous aggregates with regard to structure, initial organic matter, indigenous microflora and inoculated bacteria distributions, remoulded soil aggregates were prepared according to Fies and Stengel [15] and Sierra et al. [6]. For the

noninoculated aggregates, the soil was wetted (320 g kg^{-1} water), thoroughly mixed (in order to obtain a plastic paste). This paste was then put in parallelipedic moulds of 28 cm length, 4 cm depth and 4 cm high whose bottom was replaced with a thin synthetic nonbiodegradable textile. Each mould was placed at room temperature on a 3 cm layer of dry sieved soil allowing an absorption of water from the bottom of the paste. After a 7-day period of gentle drying, each soil slab was cut in cubes of approximately 3.5 cm side length. The drying process was then continued until 5–7% of water content. Spherical aggregates (3 cm diam.) were obtained from the dry soil cubes by the use of an electric potato peeler.

For the inoculated aggregates, cultures of the wild-type and Nir^- mutant were collected by centrifugation at $5500 \times g$ for 15 min and the cells resuspended in sterile water. The procedure of aggregate preparation was the same as that for the noninoculated aggregates but the paste was initially prepared with a lower water content. A final water content of 320 g kg^{-1} was obtained by adding a suspension of a wild-type/Nir^- mutant mix (about 1:1) to the plastic paste. The bacterial density of the inoculum was calculated from previous assays in order to obtain about 10^4 cells g^{-1} of dry soil at day 0 of the experiment (Table 1).

Spherical inoculated and noninoculated aggregates were then rewetted on suction tables with a 20 cm potential water (day −1, Table 1). This procedure allowed a low rewetting process avoiding the risk of aggregates cracking. The water used to rewet the aggregates was supplemented with KNO_3 (4.2 g l^{-1}) ensuring a continuous NO_3^- diffusion through the whole aggregates during the entire experiment. The rewetting process was done at 4°C to prevent the creation of microbial gradients during this step. After 1 day, a constant water content comprised between 23 and 25 g kg^{-1} was uniformly obtained in all the aggregates. Then the suction tables were placed at 20°C (day 0, Table 1) until the end of the experiments. The schedule of measurements of the various parameters is given in Table 1.

At set times (Table 1), several aggregates were sampled and submitted to one of the three following treatments according to the measurement performed: (i) undisturbed, (ii) crushed and (iii) divided into three concentric portions (outer, intermediate and inner). The separation procedure of the aggregates into portions was performed with a pastry cutter according to Fig. 1.

NO_3^- and NO_2^- measurements were performed on each aggregate portion by ionic chromatography to verify throughout the experiment that: (i) NO_3^-–N concentration was never less than 100 μg NO_3^-–N g^{-1} dry soil; and (ii) nitrite accumulation did not occur.

2.4. Oxygen profiles

Oxygen distributions inside the aggregates were determined using O_2-microelectrodes according to

Table 1
Schedule of the experimental procedure and measurements

	Time (days)						
	−1	0	1	3	8	15	29
Beginning of saturation of soil aggregates	+						
Change of incubation temperature (4–20°C)		+					
Enumeration of inoculated *Pseudomonas* strains		+	+	+	+	+	+
Enumeration of total heterotrophic microorganisms plus evaluation of the percent of NO_3^- reducers and of identifiers			+	+	+	+	+
Microbial biomass			+	+	+	+	+
INT			+	+	+	+	+
Oxygen profiles			+ (1)	+ (2)	+ (3)	+ (3)	+ (3)
Denitrifying activity			+	+	+	+	+
Global CO_2 production			+	+	+	+	+

All measurements have been performed on three aggregates except for oxygen profile measurements (in this case values in parentheses indicate the number of tested aggregates).

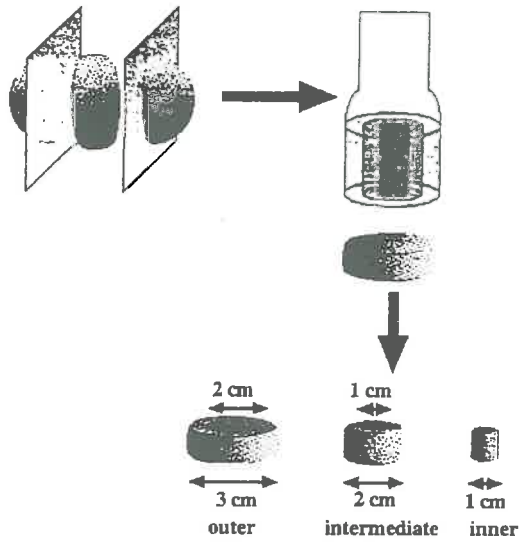

Fig. 1. Separation procedure of the aggregates into concentric portions using a pastry cutter.

Revsbech and Ward [16]. This method was fully described in Revsbech and Jorgensen [17] and improved by Revsbech [18]. The tips of the electrodes (between 40 and 60 μm in this study) were small enough to avoid O_2 diffusion perturbations and the electrode O_2-consumptions were small enough to prevent any signal disturbance. At set times (Table 1), O_2-profiles were obtained on up to three aggregates when possible (the number of treated aggregates at each time is given in Table 1). For the O_2-profile measurements, the aggregates were placed on a stand. The electrode was first calibrated at 21% O_2 and at 0% O_2. It was then inserted into the aggregate using a micromanipulator and an arm-mounted stereo-microscope. O_2-profiles were obtained between the surface of the aggregate and 1.4 cm depth with a step of 100 or 200 μm. For each aggregate, a profile was done from the surface in contact with the suction table (profile L) and another was done by inserting the electrode from the opposite aggregate surface (profile H).

2.5. Enumeration of bacteria

At set times (Table 1), each portion of three inoculated and three noninoculated aggregates was suspended in 100 ml of sterile water with a Waring blender. For the determination of total and denitrifying indigenous bacteria, appropriate dilutions of the soil were spread onto tryptic soy agar diluted 1/10 (Difco Laboratories, Detroit, MI). Counts were performed after one week incubation at 28°C to determine the number of total bacteria. To determine the denitrifiers-to-nondenitrifiers ratio, 100 cfu randomly chosen from each portion were picked into microtiter plates containing tryptic soy broth 1/10 supplemented with 1 mM KNO_3. The microtiter plates were then incubated in anaerobiosis in Gaspak Pouch system (Becton Dickinson, Cockeysville, MD) for 1 week at 20°C. After incubation, NO_2^- and NO_3^- were tested with Griess-Ilosway's reagent and Morgan's reagent respectively. Bacteria were considered as: (i) denitrifiers if neither NO_2^- nor NO_3^- was detected in the medium; and (ii) nitrate-reducers if only NO_2^- was detected [19].

To count inoculated *Pseudomonas*, soil dilutions were spread onto King's B agar (KB, Difco Laboratories, Detroit, MI) supplemented with rifampicin (50 μg ml^{-1}) for enumeration of total inoculant (wild-type plus Nir$^-$ mutant), and rifampicin (50 μg ml^{-1}) plus kanamycin (50 μg ml^{-1}) for enumeration of Nir$^-$ mutants. Cycloheximide (200 mg ml^{-1}) was added to KB agar to prevent fungal growth. Bacteria were counted between 40 and 400 cfu after 2 days incubation at 28°C. Background counts of the studied soil on KB with 50 μg of rifampicin ml^{-1} indicated a naturally rifampicin-resistant population lower than 10^2 cfu g^{-1} dry soil.

2.6. Microbial biomass

At set times (Table 1), microbial biomass measurements were performed on the different portions of three aggregates by fumigation-extraction [20] with special adaptations for handling small soil samples. Portions of 1–2 g of soil were placed into 20-ml vials and fumigated for 16 h with chloroform vapours. These fumigated samples, and corresponding unfumigated samples, were extracted by K_2SO_4 0.05 N (soil/solution: 1/5). The soil suspensions were pelleted by centrifugation in disposable plastic vials and soluble organic carbon was measured in the supernatant by persulfate-UV oxidation [21]. The microbial extractable carbon (EC), expressed as μgC

g^{-1} soil, is given by the difference between fumigated and unfumigated samples. The microbial biomass ($C - Biom. = EC/K_{ec}$) can be calculated by using a K_{ec} factor of 0.38.

2.7. INT measurements

The vital redox dye 2 [*p*-iodophenyl]-3-[*p*-nitrophenyl]-5-phenyl tetrazolium chloride (INT) directly competes with O_2 as an artificial electron acceptor [22]. The reducing power generated by the electron transport system of microorganisms converts INT into soluble INT-formazan crystals, which accumulate in metabolically active cells and may be quantified. The procedure used in the present study was developed by Norton and Firestone [23]. Soil samples (0.5–1 g dry weight) were placed in sterile 20-ml vials, and INT solution was added (0.4 ml of 0.2% aqueous solution). Incubations were conducted at 25°C for 4 h. Samples were then extracted with 8 ml of *N-N*-dimethylformamide by vortexing for 1 min and filtering through Whatman filter paper. The concentration of INT-formazan was determined from the A_{485} and standard curves of INT-formazan in dimethylformamide. A dimethylformamide extract of soil without added INT was used as a blank. There was no detectable INT reducing activity in autoclaved soil samples.

2.8. Denitrification activity

At set times (Table 1), potential and actual denitrifying activity were measured. For potential denitrification, three aggregates were cut into three portions as described above, and each sub-sample was crushed. The entire inner portion (about 1.5–2 g) and 2 g of each of the other portions were placed in 38-ml penicillin flasks containing 2 ml of a succinate-nitrate solution (10 mM succinate, 40 mM KNO_3^-). The soil slurries were made anoxic by alternately evacuating the flasks and flushing with N_2 three times. Then, 3 ml of N_2 were replaced by C_2H_2. For actual denitrification, three undisturbed aggregates were placed into 150-ml flasks without any supply and were maintained in oxic conditions. Ten millilitres of the ambient gas were replaced by C_2H_2.

For all the treatments, gas samples of 0.5 ml were withdrawn 3 h, 4 h, 5 h and 6 h after the introduction of C_2H_2 and were immediately analysed for N_2O with an electron capture detector – gas chromatograph Varian 3400 Cx. The kinetics obtained were linear allowing the calculation of soil denitrification rates.

2.9. Carbon dioxide production

At set times (Table 1), actual CO_2 production was measured on undisturbed aggregates. The aggregates were placed in 150-ml flasks previously partially filled with approximately 110 ml of paraffin in order to reduce the gas volume of the flasks and maintained in aerated conditions. Gas samples of 0.3 ml were withdrawn immediately after closing the flasks and after 10 h incubation and analysed for CO_2 with a thermal conductivity detector gas chromatograph HP 5890. The CO_2 production was estimated from the difference between CO_2 concentration at 10 and 0 h.

2.10. Statistical treatment of data

An analysis of variance was performed on the data to test differences between portions. Comparisons of the Nir^--to-total inoculant ratios of *Pseudomonas* was performed by using an arcsin $\sqrt{ratio}$ transformation before the ANOVA test.

3. Results

3.1. Evolution of O_2 partial pressure in the soil aggregates

Fig. 2 shows three examples of the oxygen profiles obtained by insertion of the O_2-microelectrodes at two diametrically opposite points of the aggregates (profile L and profile H). The comparison of the two profiles performed on the same aggregate generally shows significant but very small differences. The percentages of aerobiosis in the whole aggregate and in the three portions were calculated from these oxygen profiles (Fig. 3). After an initial decrease from 71 to 45%, the percentage of aerobiosis in the whole aggregate increased to 94% at day 29. The inner portion remained strictly anoxic throughout the experiment while the outer portion

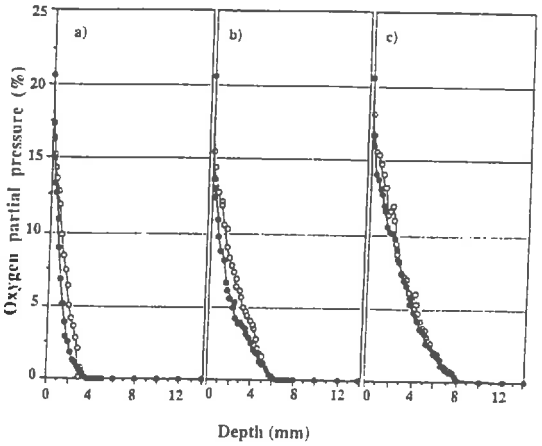

Fig. 2. Examples of oxygen profiles obtained at day 3 (a), 8 (b) and 15 (c). (●) profile L; (○) profile H.

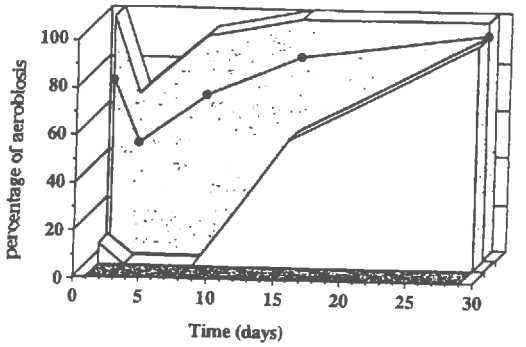

Fig. 3. Evolution of the percentage of aerobiosis in the whole aggregate (●) and in the outer (medium-shaded area), intermediate (light-shaded area) and inner (dark-shaded area) portions of soil aggregates.

remained roughly oxic. The intermediate portion was roughly anaerobic until day 8, then the percentage of aerobiosis increased to 92% at day 29.

3.2. Survival of the inoculated strains

Table 2 shows the population dynamics of the RTC01 (wild-type) strain in the different portions of the aggregates. As the logarithmic scale used to express bacterial population dynamics did not allow clear distinction between the evolution of the wild-type and Nir$^-$ populations, only the wild-type results are presented. When all the points of the population dynamics were taken into account, no significant differences could be recorded between the kinetics in the three portions of the soil aggregates. However, a significant increase in bacterial populations occurred in each portion with maximal densities at day 3 in the outer portion (about 8×10^4 cfu g^{-1} dry soil), at day 8 in the intermediate portion (about 2×10^5 cfu g^{-1} dry soil) and at day 15 in the inner portion (about 6×10^4 cfu g^{-1} dry soil).

Fig. 4 shows that the Nir$^-$-to-total inoculant (wild-type plus Nir$^-$ mutant) ratio, initially at 0.5 for all the portions (day 1), significantly differed between the portions after one day of incubation. The ratio observed in the inner portion decreased to 0.37 after 1 day and then slightly increased until 0.46 at day 29 while the ratio of the outer portion constantly increased to reach 0.68 at time 29. Thus the Nir$^-$-to-total inoculant ratio was significantly lower ($P < 0.001$) in the inner portion than in the outer portion from day 1 to day 29.

The kinetics of the ratio in the intermediate portion, situated between the two other portions during the whole experiment, could be divided into two phases. From day 0 to day 8, the ratio decreased to

Table 2
Evolution of the number of the wild-type *Pseudomonas* RTC01 (cfu g^{-1} dry soil) in the different compartments of the soil aggregates

Time (days)	Number of cfu of wild-type *Pseudomonas* RTC01 (10^4 cfu g^{-1} dry soil)		
	Outer compartment	Intermediate compartment	Inner compartment
0	0.98 (0.75)	1.09 (0.49)	1.01 (0.77)
1	4.71 (4.19)	9.02 (5.36)	1.19 (0.97)
3	7.7 (0.20)	4.96 (3.76)	2.88 (2.70)
8	4.6 (0.25)	15.7 (1.45)	3.17 (1.70)
15	4.7 (0.19)	5.3 (0.25)	6.18 (4.87)
29	1.3 (0.86)	2.67 (1.37)	0.89 (0.02)

Values in parentheses are S.D

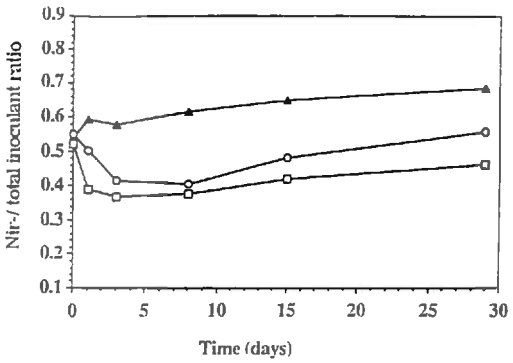

Fig. 4. Evolution of the Nir^--to-total inoculant ratios of *Pseudomonas* RTC01 in the outer (▲), intermediate (○) and inner (□) portions of soil aggregates. Differences between compartments are significant at $P < 0.001$ except between the inner and intermediate portion at days 3 and 8 (no statistical differences).

reach values close to those observed in the inner portion (no statistical differences at days 3 and 8 between intermediate and inner portions). From day 8 to the end of the experiment, the ratio increased more rapidly in the intermediate than in the inner portion becoming significantly different ($P < 0.001$) at days 15 and 29.

3.3. Evolution of microbial biomass, total heterotrophic microflora and proportion of denitrifiers

Table 3 shows that the total number of heterotrophic organisms (measured only in the inner and outer portions at days 1, 8 and 15; see Table 1) remained roughly constant ($P < 0.05$) at $5–7 \times 10^7$ cells g^{-1} dry soil without significant differences between the two portions. When analysed on the whole kinetics, the proportion of denitrifiers as well as the proportion of nitrate-reducers did not significantly differ between the two portions. The INT reduction remained roughly constant at 30 $\mu g\ g^{-1}$ dry soil during the experiment in the different portions. The variability of the INT reduction between aggregates and dates was not significantly dependent on the position of sampling in the soil aggregates. The microbial biomass slightly decreased from 100–110 $\mu gC\ g^{-1}$ soil at day 1 to 92–95 $\mu gC\ g^{-1}$ soil at day 29 without significant differences between the three portions.

Table 3
Evolution of total heterotrophic microflora and proportion of denitrifiers plus nitrate-reducers, microbial biomass, INT and potential denitrifying activity in the three portions

	Compartment	Time (days)				
		1	3	8	15	29
Total heterotrophs ($\times 10^7$ cfu g^{-1} dry soil)	Inner	5.0 (0.2)	–	5.7 (1.3)	6.1 (0.5)	–
	Intermediate	–	–	–	–	–
	Outer	4.4 (0.4)	–	5.3 (0.2)	7.2 (0.7)	–
% of denitrifiers	Inner	6	–	6	7	–
	Intermediate	–	–	–	–	–
	Outer	7	–	9	8	–
% Nitrate-reducers	Inner	29	–	30	33	–
	Intermediate	–	–	–	–	–
	Outer	35	–	35	23	–
Microbial biomass $\mu g\ C\ g^{-1}$ soil	Inner	103.3 (1.9)	121.8 (19.6)	103.3 (3.4)	99.6 (3.1)	94.7 (4.5)
	Intermediate	109.8 (0.9)	110.3 (6.7)	107.3 (2.5)	99.9 (3.5)	92.7 (2.7)
	Outer	116.6 (3.5)	106.4 (4.3)	108.6 (3.8)	95.0 (4.7)	92.5 (4.8)
INT $\mu g\ g^{-1}$ soil	Inner	33.7 (11.3)	32.7 (13.3)	36.4 (1.87)	34.7 (4.79)	44.0 (14.9)
	Intermediate	29.0 (1.65)	18.7 (2.81)	30.5 (2.49)	29.2 (4.67)	29.4 (8.11)
	Outer	28.8 (6.68)	26.6 (4.96)	29.7 (7.68)	26.9 (3.77)	29.8 (11.6)
Potential denitrification $\mu mol\ N_2O\text{-}Nm^{-1}\ s^{-1}$	Inner	1.47 (0.48)	0.77 (0.21)	2.01 (0.42)	2.41 (1.21)	2.33 (0.53)
	Intermediate	3.33 (0.44)	2.66 (0.67)	2.35 (0.43)	3.46 (0.89)	3.17 (0.84)
	Outer	3.04 (0.17)	3.44 (0.58)	2.47 (0.86)	2.45 (0.10)	1.99 (0.89)

Values in parentheses are S.D.
–, not measured.

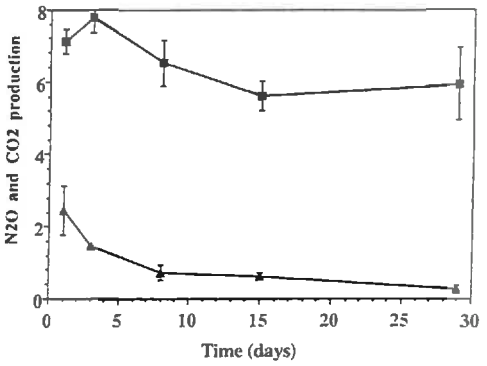

Fig. 5. Evolution of the production of N_2O (▲) in μmol N_2O-N m^{-3} s^{-1} and CO_2 (■) in μmol m^{-3} s^{-1} in the whole aggregate. Error bars denote the standard deviation.

3.4. Evolution of respiratory activities

Fig. 5 shows that, after an initial increase from 6.7–7.7 μmol m^{-3} s^{-1} at day 3, the actual CO_2 showed a decline to about 5.9 μmol m^{-3} s^{-1} at day 29. Similar decline was observed for the actual denitrification from 2.45 μmol N_2O–N m^{-3} s^{-1} at day 1, to 0.27 μmol N_2O–N m^{-3} s^{-1} at day 29. The relationship between the evolution of the actual denitrification and the evolution of the percentage of aerobiosis in the whole aggregate (Fig. 3) was linear ($r = 0.84$) between day 3 and day 29 (day 1 was not taken into account because the O_2 profiles were measured on only one aggregate). The results of potential denitrification measurements for the three portions are presented in Table 3. The N_2O production remained roughly constant between 1–3 μmol N_2O–N m^{-3} s^{-1} from day 1 to day 29 in all the portions without any significant evolution during the experiment and any significant difference between the three portions.

4. Discussion

During this work, the experiments were conducted at two levels: (i) inoculated microorganisms to evaluate the role of denitrification (and more precisely nitrite-reduction) in the colonisation of aggregates; and (ii) indigenous soil microflora (density, biomass, activity) in order to assess the evolution of the microbial characteristics in the different portions of the soil aggregates or in the whole aggregate. The use of artificial aggregates was a prerequisite to compare the evolution of the repartition of inoculated microbial strains. Indeed, a homogenous initial repartition of the inoculated strain in the different portions was needed for this comparison. This homogeneity could only be obtained by inoculating the soil before the formation of the aggregates. Using microelectrodes, oxygen profiles were performed on the soil aggregates in order to be able to correlate the distribution of organisms, biomass, and activities with the evolution of the anoxic volume in the soil aggregates.

4.1. Evolution of the oxygen concentration in the soil aggregates

The microelectrodes used to establish the O_2 profiles were already described by Sexstone et al. [4], Zausig et al. [12] and Sierra et al. [6]. The accuracy of the O_2 measurements was validated by the obtention of the same profiles when inserting the electrode inside the aggregate and when putting the electrode out of the aggregate. The similar L and H O_2 profiles indicated that the preparation and the rewetting processes resulted in homogenous aggregates. Such homogeneity was not found for natural aggregates as shown by the fact that, in this case, anaerobic zones are often asymmetric and do not always occur at the aggregate centre [4]. According to our goal, this symmetrical repartition of physico-chemical parameters in the aggregate was a prerequisite to obtain valid results. The initial decrease of the aerobic volume of the whole aggregate (Fig. 3) was probably due to an intense metabolic activity following soil rewetting and rewarming as suggested by the concomitant initial increase in CO_2 production (Fig. 5). This resulted in allowing complete anoxic conditions to take place in the inner and intermediate portions of the aggregates (Fig. 3). The subsequent and progressive increase in the oxic volume of the whole aggregate until the end of the experiment could be attributed to the progressive depletion of electron donors as suggested by the corresponding decrease in CO_2 production (Fig. 5). The stronger consequence was a drastic change in the aeration status of the intermediate portion where oxic conditions progressively took place (Fig. 3).

4.2. Relationships between inoculated Pseudomonas and oxygen profiles in the soil aggregates

No significant difference appeared between the population dynamics of the *Pseudomonas* strain in the three portions and especially between the oxic outer and the anoxic inner portions (Table 2). A previous work [11], performed on a silt-loam soil of the region of Lyon, showed that the RTC01 strain exhibited a better survival in anoxic than in oxic soil. The use of another soil type in the present study (due to technical reasons related to insertion of O_2-microelectrodes in the aggregate) could explain these two apparently contrasting results. Indeed, the fact that a denitrifying strain exhibits a higher competitive ability under oxic or anoxic conditions not only depends on isolate but also on soil type [24]. Moreover, the experimental conditions were not similar between the two experiments: imposed aeration conditions to individualised bulk soil samples in the work of Philippot et al. [11], naturally occurring aeration status through a continuous gradient of O_2 consumption-diffusion ratio in this work.

The level of inoculation of RTC01 strains has been chosen in order to obtain a low bacterial density at the beginning of the experiment (10^4 cells g^{-1} of dry soil; day 0). In such conditions, a significant bacterial growth occurred in the three portions (Table 2). This situation allowed us to compare the evolution of the Nir^--to-total inoculant ratio during a colonisation process.

The use of Tn*5* mutants may be questionable due to a possible 'self effect' of the Tn*5* transposon irrespective to its location in the genome [25–28]. This could be the reason why the Nir^--to-total inoculant ratio increased in the oxic outer portion (where dissimilative nitrite-reductase was not expected to express its activity) (Fig. 4). However, as explained in a previous study [11], our experiments being based on comparison between different portions, the Tn*5* effect can be taken into account in our interpretation.

The comparison of the evolution of the Nir^--to-total inoculant ratio in the three aggregate portions showed that the dissimilative nitrite-reductase could provide to the studied strain a competitive advantage for colonisation of the centre of aggregates. It is interesting to note that this advantage can be observed after only one day of incubation and remained globally constant during the entire experiment in agreement with the results obtained previously by Philippot et al. [11] working on the same strains inoculated in soil under different conditions. This suggested that the differences mainly took place during the step of cell multiplication.

Several studies have dealt with the influence of aggregation on soil denitrifying activity or denitrifiers [3–10], but the present study is the first report showing that denitrification may help organisms to reach higher proportions in the centre of soil structures. Moreover, the simultaneous observation of the evolution of the Nir^--to-total inoculant ratio (Fig. 4) and of the percentage of aerobiosis (Fig. 3) in the three portions allows us to hypothesise that the distribution of oxygen (due to an increasing consumption-to-diffusion ratio from the surface to the centre of the aggregates) was a strong determinant of the advantage of the nitrite dissimilative strain in the inner portion. This hypothesis is strengthened by the fact that the dynamics of the Nir^--to-total inoculant ratio in the intermediate portion appear closely related to the evolution of the oxygen concentration in this portion. Indeed, at days 3 and 8, the simultaneous complete anaerobiosis of the intermediate and inner portions corresponded to the same Nir^--to-total inoculant ratio in these two portions while at days 15 and 29, the progressive occurrence of oxic conditions in the intermediate portion resulted in significant differentiation of the corresponding ratio from the ratio of the inner portion (Figs. 3 and 4).

4.3. Evolution and repartition of indigenous microorganisms and microbial activities in soil aggregates

The competitive advantage of the denitrifying inoculated strain for colonisation of the centre of aggregates suggested that the proportion of denitrifiers must be higher at the centre than at the surface of the aggregate. Surprisingly, the denitrifiers-to-total heterotrophs and nitrate reducers-to-total heterotrophs ratios were similar in the two studied portions (inner and outer) and constant during the entire experiment (Table 3). Three hypothesis can be proposed to explain why no significant differences of the indigenous microflora could be observed between the inner and outer portions while the use of a denitrifying

population, considered as representative of the denitrifying community, clearly showed that such differentiation may exist: (i) in the studied soil, the equal competitive ability in oxic and anoxic conditions of the inoculated *Pseudomonas* could not be representative of the competitive ability of the indigenous denitrifying community. Indeed, Murray et al. [24] demonstrated that some denitrifiers are better competitors under anoxic conditions while others better compete under oxic conditions. If the majority of denitrifiers of our soil belonged to the latter class, the selective advantage given by the ability to denitrify could be hidden; (ii) the physiological and/or competitive status of the inoculated and the indigenous microflora were different in that the former was, at least at the beginning of the incubation, in a growth phase (during which differentiation occurred) while the latter remained roughly at the same density as demonstrated by the stability of the number of heterotrophs as well as of the total microbial biomass (Table 2). Moreover it is possible that the inoculated strain was submitted to a stronger competition than the previously adapted indigenous microflora resulting in a higher sensitivity to the physico-chemical fluctuations; and (iii) the enumeration of inoculated cells is more precise than the enumeration of indigenous microorganisms.

As for the denitrifiers or nitrate-reducers-to-non-dissimilators ratios, the new physico-chemical conditions generated by the formation of the aggregates appeared not selective enough to induce significant differences in the repartition of the total microflora (as suggested by the measurements of microbial biomass and total heterotrophic organisms; Table 3). These conditions also seemed not sufficient to induce significant differentiation in the physiological potentialities of the indigenous organisms. Indeed neither INT measurements used as an index of active microbial cells [23] nor potential denitrification (both being basically potential measurements) were significantly different between the 3 portions (Table 3). In fact, the absence of significant differentiation in the indigenous microbial characteristics might be due to too light physico-chemical gradients or too short period of incubation. Indeed, after a modification of the environmental conditions, the microbial parameters are influenced in the following order: firstly, expression of enzymes; secondly, ability of organisms to express their denitrification genes; and thirdly, number of organisms. In our work, the expression of the real activities (CO_2 production and denitrification) decreased during the experiment, likely due to the depletion of available organic carbon, and for denitrification, to the increase in aeration (linear relationship between the percentage of aerobiosis in the whole aggregate and actual denitrification rate).

The use of isogenic mutants was the only way to assess the role of denitrifying enzymes in the colonising ability of microorganisms. The main result of our work was to demonstrate that the dissimilative nitrite reductase conferred to the inoculated *Pseudomonas* a competitive advantage to colonise the centre of soil aggregates. However, even though the ability to denitrify may improve the survival of each indigenous denitrifying population in the centre of soil aggregates, the differences in other metabolic capacities between the different denitrifying populations appeared able to hide this advantage. Moreover, considering the variety of the mechanisms of microbial selection in natural environments, the use of artificial aggregates (required to study the behaviour of inoculated microbial populations) must only be considered as a preliminary step to assess the influence of soil aggregation on indigenous organisms. Thus, even if the understanding of the determinism of the denitrifiers distribution may be strongly improved by the use of experimental models of their microhabitats, studies on natural stable aggregates are needed to produce more realistic investigations.

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Denitrification in pasture and cropped soil clods as affected by pore space structure

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Abstract

To assess the influence of the pore space structure and organic matter on denitrification, a comparative study was performed on clods in a soil under cropped and pasture managements. For each management, the potential denitrification rate was estimated. Denitrification under oxic conditions was also measured on 100 clods, which were saturated with KNO_3 solution (4 g l^{-1}). Size and density fractions of the soil were separated, and the C and N contents of their particulate organic matter were determined. Clod porosities were measured and the distributions of distances of any point within the clod to the nearest air-filled pore were estimated on 20 thin sections for each soil management. Potential denitrification rates were similar (105×10^{-11} and 98×10^{-11} mol N_2O kg^{-1} dry soil s^{-1} for pasture and cropped soil, respectively). The mean denitrification rate under oxic conditions was only equal to 0.14% of the potential denitrification rate for pasture, whereas it was about 2.1% for cropped soil. The total and soluble organic C content was significantly higher in pasture than in cropped soil clods. The quantity or the quality of organic matter fractions did not explain the difference in denitrification activities. Even if macroporosity represented a small fraction of the total porosity in both soils, the differences in macropore distribution induced by soil management practices led to significantly different maximal distances between any clod point and the nearest air-filled pore (8 and 14 mm for pasture and cropped soils, respectively). Consequently, we demonstrated that the pore space structure appears to be the major factor explaining the difference in mean denitrification rates between pasture and cropped soil clods, while the distribution of particulate organic matter is suspected to be involved in the differences in denitrifying activity distribution between the clods of the two soils.

1. Introduction

Denitrification is a soil microbial process leading to the production of nitrous oxide (N_2O) (Hénault and Germon, 1995; Conrad, 1996). This gas participates in the terrestrial greenhouse effect (Smith, 1990; IPCC, 1995) and affects the chemistry of ozone (O_3) in the upper troposphere and lower stratosphere (Graedel and Crutzen, 1992).

Efforts at understanding the determinism of N_2O emissions in soils have resulted in several studies on factors affecting denitrification (Tiedje et al., 1989). The microscale approach to study denitrification is motivated by the fact that, in many cases, the conditions experienced by soil organisms at the microscale are not reflected by measurements of these conditions made on bulk soil samples (Parkin, 1987). For example, O_2 concentrations may decrease from values nearly equal to the atmospheric concentration to zero values within a few millimeters in soil clods (Currie, 1961; Sexstone et al., 1985; C. Rappoldt, unpub. Ph.D. Thesis, Wageningen Agricultural University, 1992; Sierra et al., 1995). For readily-decomposable organic matter particles, a thin layer of covering water (i.e. thickness of about 160 μm) may be sufficient for anaerobiosis to occur (Parkin, 1987). Rappoldt (1992) found anoxic sites close to air-filled pores, provided that organic residues were present. Therefore, to understand the determinants for soil denitrification, a description of the microbial processes with regard to

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the substrate gradients at these scales is needed. Experimental studies have pointed out the necessity of taking into account such micro-heterogeneities (Sexstone et al., 1985; Rappoldt, loc. cit.; Sierra and Renault, 1996).

As the pore space structure and organic matter (quantity and quality) are primarily affected by land use (Pagliai, 1994; Balesdent, 1996; Besnard et al., 1996), comparative studies on denitrification in soils under very different management practices (e.g. in pasture and cropped soils) may be a good mean of assessing the effect of the soil structure and organic matter on denitrification. Some comparisons have dealt with the potential of denitrification, i.e. the rate of denitrification measured under anoxic conditions in soil samples, supplemented with NO_3^- and organic C (Bijay-Singh et al., 1989; Weier et al., 1993; Lensi et al., 1995; Sotomayor and Rice, 1996). Lensi et al. (1995) and Sotomayor and Rice (1996) used glucose as organic C supply andfound that potential denitrification was significantly higher in pasture than in cropped soil. Bijay-Singh et al. (1989) measured denitrification under anoxic conditions, but with only an additional supply of NO_3^-. They found higher denitrification in pasture than in cropped soil. In the field, Bijay-Singh et al. (1989) found a higher actual denitrification in cropped soil than in pasture, despite similar NO_3^- contents. They explained their results as the consequence of the easier water drainage in the pasture soil, due to the higher porosity of this soil. Complementary measurements after the application of various amounts of water agreed with this analysis: denitrification in pasture soil became higher than denitrification in cropped soil only at water suctions greater than −5.5 kPa (Bijay-Singh et al., 1989). All these studies show the importance of considering both actual and potential denitrification when assessing the role of organic matter and structure (microheterogeneitites) on denitrification.

Several investigators have attempted to identify the influence of different organic substrates on denitrification. Denitrification was shown to be highly correlated with soluble organic matter (Burford and Bremner, 1975; de Cantazaro and Beauchamp, 1985; Bijay-Singh et al., 1988), and easily mineralizable C (Burford and Bremner, 1975; Bijay-Singh et al., 1988). In addition, several investigations have shown that denitrification intensity was positively related to the distribution of fresh plant residues in the soil profile (Aulakh et al., 1984; de Cantazaro and Beauchamp, 1985; Aulakh et al., 1991; Parkin, 1987; Christensen et al., 1990). Particulate organic matter (POM) is identified as a pool of labile organic matter and the coarser the residues the more rapid their turnover in the soil (Balesdent, 1996).

In a first approximation, converting cropped soil to pasture may increase the organic matter content of the soil (Tiessen and Stewart, 1983) and, as a consequence, increase the amount of substrates available for denitrification. However, conversion from cropped soil to pasture may also change the pore space structure at the scale of soil aggregates and clods (Pagliai, 1994), and thus provide conditions less favourable to denitrification.

Our purpose was to assess the relative contribution of organic substrates and the pore space structure on denitrification, at the clod scale. As these two variables are primarily affected by land use, we compared the same soil cropped or under permanent pasture. In order to study the effects of organic matter and the pore space structure, denitrification was measured in intact soil clods incubated under oxic conditions without organic substrate supply, but with non-limiting NO_3^-. This "actual" denitrification was compared to the "potential" one (i.e. crushed clods, anoxic conditions, non-limiting C and NO_3^- substrates). The influence of soil management practices on "actual" and "potential" denitrification was analysed by referring to the effect of soil use on the total and labile organic matter content (measured on size-class soil fractions) and the soil structure (assessed by both bulk density and image analysis) in the two soils.

2. Materials and methods

2.1. The soil and clod incubation

Clods were sampled in the 10–25 cm layer of a gleyic luvisol (FAO classification), located near the Citeaux Abbey (Burgundy, France), 47°09′ N and 5°05′ E. This soil has been cropped for 50 y. One area of this soil is still cropped (maize) and the other area has been in pasture for 25 y. Clods from each of these two areas were sieved between 2 and 2.5 cm at the field moisture content, air-dried for 1 week and stored at 2°C until the beginning of the experiment. The properties of the soil sampled were as follows: clay, 184 and 145 g kg^{-1}; silt, 523 and 541 g kg^{-1}; sand, 293 and 314 g kg^{-1}; pH water, 6.8 and 6.8; organic C, 12.1 and 9.0 g kg^{-1}; total N, 1.22 and 0.9 g kg^{-1}; for pasture and cropped soil, respectively.

Just before the experiment, each clod was weighed at residual moisture (4.4 and 2.1% for pasture and cropped soil, respectively). Clods were then rewetted with a solution of KNO_3 (4 g l^{-1}) at 20°C on suction tables for 24 h at 100 cm water suction, and then for 24 h at a suction of 50 cm, and for the 5 following days at a suction of 10 cm. This procedure ensured a slow rewetting process which prevented the occurrence of additional clod cracks.

To check the uniform distribution of NO_3^- within the clod, tests were made. Four clods from each of the two plots were divided into three portions after the rewetting procedure (lower, intermediate and upper, the lower portion being in contact with the suction table). Nitrate concentrations were measured on each portion by capillary electrophoresis (Waters Capillary Ion Analyzer). In addition, three clods from the pasture and cropped soils were divided into two portions (internal and external) by peeling and also analyzed for NO_3^- concentrations. Relative deviation of NO_3^- concentrations did not exceed 11% between the different regions.

2.2. Denitrification measurements

After the rewetting procedure, potential denitrification rates were measured on 9 pasture clods and 9 cropped soil clods. For each clod (previously crushed and thoroughly mixed) 2 g (oven dried basis) subsamples were placed into 38 ml penicillin flasks, which had been previously filled with 2 ml of a solution containing 10 mM succinic acid and 40 mM KNO_3. Anoxic conditions were created by flushing with N_2 for 5 min. Three ml of N_2 were then replaced by C_2H_2 to block the N_2O reductase and 1 ml of N_2 was replaced by Kr to detect possible leakage. Then 0.3 ml gas samples were withdrawn with a syringe 180, 270 and 360 min after the introduction of C_2H_2 and were immediately analyzed for N_2O using a gas chromatograph equipped with an electron capture detector (HP 5890 Series II). The kinetics of N_2O production were linear in all experiments (the mean of the correlation coefficients was 0.98—SD = 0.03) which allowed to express the potential of denitrification proportional by the values of the slopes of these kinetics.

Denitrification of intact soil clods, rewetted by the above described procedure, was also measured under oxic conditions. In order to assess the variability of denitrification between clods, individual measurements were performed on 100 pasture and 100 cropped soil clods. Each clod was placed into a 150 ml plasma flask (care was taken to avoid any modification of the clod structure). Seven and 1 ml of gas were replaced by C_2H_2 and Kr, respectively. Gas samples of 0.3 ml were withdrawn with a syringe 18, 20, 22 and 24 h after the addition of C_2H_2 and analyzed for N_2O concentrations as described above. The kinetics obtained were linear which allowed us to calculate the soil denitrification rates (the mean of the correlation coefficients was 0.94 (SD = 0.12)). To estimate clod water contents of these two soil managements, wet weights were determined after their rewetting by the procedure described above. The clods were then dried for 24 h at 105°C and weighed again.

2.3. Organic matter

For each soil management, the organic matter content was determined on 5 size soil fractions. To obtain these fractions, 10–12 clods were sieved (< 2 mm) and pooled before analysis. Size and density fractions of the soil were separated according to Balesdent et al. (1991), i.e., 50 g of soil was dispersed in water with agate beads for 16 h, then sieved into 0.2–2 mm, 0.05–0.2 and < 0.05 mm fractions. In the three coarser size fractions, particulate organic matter was separated from dense minerals by flotation-panning (Feller, 1979). The < 0.05 mm fractions were dispersed with ultrasonics and separated into clay (< 0.002 mm), fine silt (0.002–0.02 mm) and coarse silt fractions (0.02–0.05 mm) by sedimentation. Fractions were oven dried and their C and N contents were determined with a Carlo Erba element Analyzer NA 2500. All fractionations and extractions were performed in triplicate.

To quantify water soluble C, 50 g of soil were dispersed in deionized water for 1 h according to Adams (1980) and the solution was recovered by centrifugation and filtration. Soluble carbon was measured with a Dorhman DC 190 analyzer.

2.4. Bulk density measurements and morphological characterization of the air-filled pore space

The pore space of the clod may be divided into a pore space specific to the soil composition, i.e., a textural pore space (Stengel, 1979), and a pore space which depends on climatic, anthropic and biological effects, i.e., a structural pore space. Generally, structural pores have larger dimensions than textural pores. Solid density was measured using water pycnometers. To estimate the total porosity of the clods, bulk densities were measured on 20 pasture and 20 cropped soil clods, using the kerosene method proposed by Monnier et al. (1973). The results were confirmed by measurement with the wax method (Fiès and Zimmer, 1982). To estimate the textural porosity of the soil clods, similar measurements were performed on five sets of 2–3 mm aggregates for each soil management. Indeed, the volume of structural pores may be neglected in small aggregates (Monnier et al., 1973).

For the same porosity, the aeration status of a given point depends on its distance to the nearest air-filled pore (Rappoldt, 1990). In order to obtain distributions of these distances for individual clods, 20 thin sections of both pasture and cropped soil clods were prepared and analyzed by morphological operations. Thin sections were obtained for dry clods, which were included in resin with a fluorescent dye (Bruand et al., 1996). Care was taken to systematically center the section on the center of the clods.

Photographs taken under U.V. radiation were scanned (1 pixel = 35 μm × 35 μm) and analyzed with VISILOG software commercialized by NOESIS. At first, the saturation of the pore having an equivalent dia of less than 300 μm (corresponding to an experimental water suction of 10 cm) was simulated by a closing operation (Serra, 1982). Successive erosion operations were then performed to measure the distance between a point in the soil matrix and its nearest air-filled pore (Serra, 1982). By measuring the surface area, which disappeared at each erosion process, distributions of distances of points within the clods to the nearest air-filled pore were obtained for each clod. Cumulative distribution functions were then evaluated for each of the 40 clods and used to estimate mean cumulative distribution functions F_p and F_c for pasture and cropped soil clods, respectively.

2.5. Statistical analyses

Statistical tests were performed to check whether the denitrification rates were significantly different between pasture and cropped soils. For potential denitrification, we used a Student–Fisher test. As the same high number of measurements for each of the two soil treatments was carried out, pair tests were performed for denitrification in oxic conditions, using the differences of randomly-associated denitrification rates of pasture and cropped soil clods (Dagnelie, 1975). χ^2-tests allowed to check whether the actual distribution of clod denitrification in oxic conditions could be described as normal, lognormal or exponential distributions (Dagnelie, 1975). In addition, we used Student–Fisher tests to check whether the total porosities, the total and soluble C content and the C-to-N ratio were significantly different between the two soil managements.

To check whether the distributions of distances, between any clod point and the nearest air-filled pore, were significantly different between pasture and cropped soils, a Monte Carlo test was performed (Barnard, 1963; Diggle, 1983). The maximum absolute value of the differences between the cumulative mean distributions F_p and F_c was obtained. Assuming an independence between the observations of clods (i.e., no statistical difference between pasture and cropped soil clods), similar maximum differences could be obtained if the 40 clods were randomly distributed in two sets of 20 clods. By successively performing 19 such random distributions, 19 maximum absolute differences were obtained. The probability that the maximum difference between pasture and cropped soils is over all these 19 values is equal to 0.05, assuming an independence between clod observations.

3. Results

3.1. Denitrification activities

Similar potential denitrification rates ($P < 0.05$) were obtained for pasture and cropped soil, 105×10^{-11} (SD = 60×10^{-11}) and 98×10^{-11} (SD = 78×10^{-11}) mol N_2O kg^{-1} dry soil s^{-1}, respectively. In contrast, the mean denitrification measured on undisturbed soil clods under oxic conditions was 13 times higher in the cropped than in the pasture soil. Indeed, the mean rate and the standard deviation of pasture soil clods were equal to 0.15×10^{-11} and 0.22×10^{-11} mol N_2O kg^{-1} dry soil s^{-1}, respectively. The mean rate and the standard deviation of cropped soil clods were equal to 2.1×10^{-11} mol N_2O kg^{-1} dry soil s^{-1} and 3.8×10^{-11} mol N_2O kg^{-1} dry soil s^{-1}, respectively. Fig. 1a shows that, when incubated under oxic conditions, denitrification in pasture soil clods exhibits a symmetrical distribution. The distribution was approximated either by a lognormal or by a normal probability density function. The mean and standard deviation of the corresponding Ln-distribution were −28 and 1.1, respectively. For cropped soil clods, denitrification rates presented a higher variability and a skewed frequency distribution (Fig. 1b). It appeared that, while most of the denitrifying rates were low, some samples exhibited extremely high rates (Fig. 1a). From a statistical point of view, only the lognormal distribution can be accepted. The mean and standard

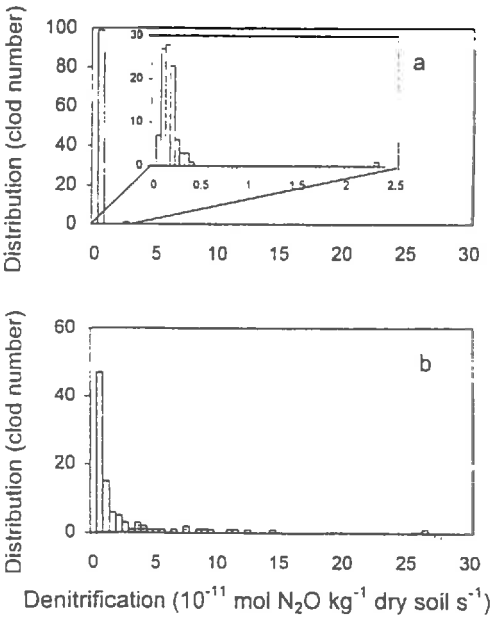

Fig. 1. Distribution of the denitrification rates of intact soil clods (2–2.5 cm dia) under oxic conditions (atmospheric O_2 concentration) with KNO_3^- (4 g l^{-1}) for (a) 100 pasture soil clods and (b) 100 cropped soil clods.

Table 1
Organic C and C-to-N ratio for POM fractions from pasture and cropped soils. Numbers in parentheses are the standard deviations. Fraction POM > 2 mm was not analyzed because not enough material was recovered. POM = particulate organic matter

POM fractions	Permanent pasture		Cropped soil	
	C content mg g^{-1} soil	C-to-N	C content mg g^{-1} soil	C-to-N
0.05–0.2 mm	0.92 (0.08)	11.8 (0.8)	0.47 (0.04)	13.1 (0.1)
0.2–2 mm	0.22 (0.06)	21.5 (0.7)	0.19 (0.05)	16.5 (0.4)
Unfractionated clods	11.5 (0.1)	10.7 (0.1)	8.3 (0.02)	10.1 (0.0)

deviation of the Ln-distribution were −25 and 1.2, respectively.

3.2. Organic matter content

The total organic C contents of the pasture and cropped soil clods were 11.5 and 8.3 mg C g^{-1} soil, respectively (Table 1). Particulate organic matter (> 0.05 mm) represented 1.02 and 0.5% of the pasture and cropped soil mass, respectively. This mass corresponded to 9.9% of the organic C content of the pasture soil and 8.0% of the cropped soil. Their C-to-N ratios were 13.7 and 14.1, respectively.

Soluble organic C contents were 157 μg C g^{-1} and 114 μg C g^{-1} for pasture and cropped soil, respectively. However, soluble organic C represented a higher fraction of total organic C in cropped soil than in pasture, i.e., 1.25 and 0.72%, respectively.

3.3. Pore space characteristics

From solid and bulk density values (summarized in Table 2), we estimated the total porosities to be significantly different for the two soil managements ($P < 0.05$). In addition, the structural porosity of pasture clods nearly doubled in comparison to cropped soil. At the 10 cm water suction (pores having diameters lower than 300 μm are water saturated), the saturation rate of macropores was 28 and 30% in pasture and cropped soil clods, respectively (Table 2). Because of the initial structural porosity values (Table 2), the air-filled porosity remained twice as high in pasture as in cropped soil clods. The structure of the pore space seemed to be different between pasture and cropped soil clods (Fig. 2a and b). It was clear in the different sections (e.g., Fig. 2a and b), that pasture clods presented an extensive network of cracks, which were not present in cropped soil clods. This network of cracks reduced the distances of points within the clods to the nearest air-filled pores (Fig. 3). In pasture clods, distances never exceeded 8 mm whereas distances in cropped soil clods could reach 14 mm. The Monte Carlo test showed that distributions of distance of points to the nearest air-filled pore were significantly different in pasture and cropped soil clods ($P < 0.05$). In addition, Fig. 3 shows the distributions of distances to the nearest air-filled pore for a homogeneous aggregate of 2.5 cm dia (i.e., an aggregate without air-filled macropores). This distribution of distances was similar to the mean distribution for cropped soil clods. This implies that the air-filled macropores within cropped soil clods had little effect upon the distribution of distances to the nearest air-filled pore and, as a consequence, did not significantly affect the aeration status of these clods.

Table 2
Physical characteristics of the studied soil under contrasting management. Numbers in parentheses are the standard error of the mean

	Permanent pasture	Cropped soil
Bulk density of clods (kg m^{-3})	1646 (43)	1755 (44)
Textural bulk density (kg m^{-3})	1700 (20)	1790 (16)
Solid density (kg m^{-3})	2661 (5)	2679 (15)
Total porosity (m^3 m^{-3})	0.382	0.345
Structural porosity (m^3 m^{-3})	0.031	0.019
Saturation rate of macropores (%)	28	30

4. Discussion

Denitrification rates have generally been found to be affected by land management practices (Bijay-Singh et al., 1989; Sotomayor and Rice, 1996). Differences in denitrification rates between pasture and cropped soils may be due to the structure of the pore space or the organic matter contents (Parkin, 1987) affecting the microbial distributions and activities (Lensi et al., 1995). Therefore, comparisons between pasture and cropped soils could be a good way to investigate the effects of spatial heterogeneities in the structural pore space and organic matter. The main results obtained in our study were that land use did not affect the potential denitrification rate, but strongly influenced denitrification measured on intact soil clods under oxic conditions without organic substrate supply, but with

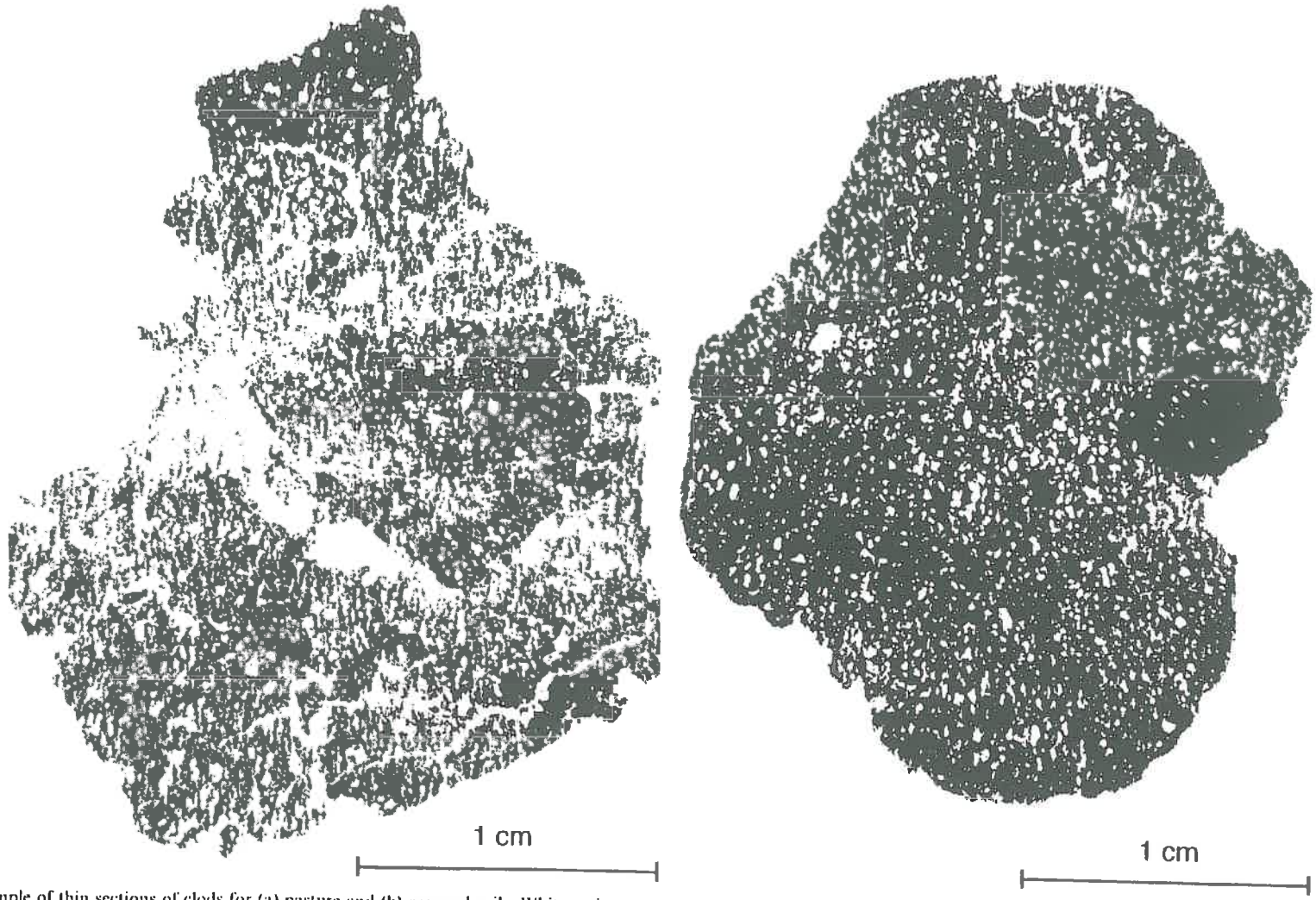

Fig. 2. Example of thin sections of clods for (a) pasture and (b) cropped soils. White regions are either for macropores or quartz particles, whereas grey and black are other solid particles, micropores and particulate organic matter.

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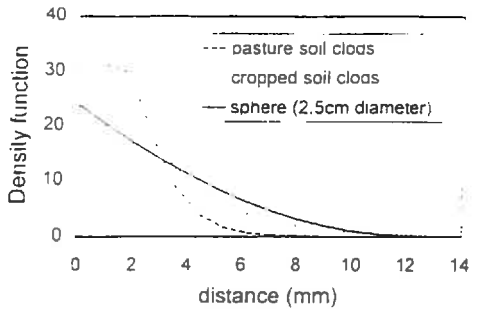

Fig. 3. Mean distributions of distances (density function) of points to the nearest air-filled pore for pasture and cropped soil clods. The theoretical distribution of distances was also reported for a homogeneous spherical clod of 2.5 cm dia.

non-limiting NO_3^-. It is thus possible to define the rate of denitrifying expression as the "potential denitrification rate–to–actual denitrification rate" ratio.

The obtained potential denitrification rates were of the same order of magnitude as those of Lensi et al. (1995) and Sotomayor and Rice (1996). Nevertheless, in our study the land use did not influence this potential denitrification rate, suggesting that the denitrifier microbial status was similar for pasture and cropped soils. These different behaviours could result from the fact that these studies have been performed on three different soils or from differences in the experimental procedures, including the initial drying and subsequent wetting of the clods specific to our study. In contrast, for intact soil clods under oxic conditions, denitrification rates in cropped soil clods were about 13 times higher than in pasture. The mean denitrification rates correspond to the expression of 0.14 and 2.1% of the potential denitrification rates for pasture and cropped soil clods, respectively. Moreover, the distribution pattern was also different between the two soil managements (Fig. 1).

With regard to organic matter, we found that total and soluble organic C contents were significantly higher in pasture than in cropped soil clods (Table 1) ($P < 0.05$), in agreement with the results of Tiessen and Stewart (1983). The quantitative and qualitative distributions of organic matter in soil fractions (Table 1) were similar for the two soil managements and in agreement with other data (Tiessen and Stewart, 1983; Cambardella and Elliot, 1992; Balesdent, 1996). We found significant differences in the organic matter content and the C-to-N ratio only in the coarse organic fractions from the two soil managements ($P < 0.05$). These fractions are generally easily decomposed (Turchenek and Oades, 1979). Therefore, total C, labile organic matter and soluble C were higher in pasture than in cropped soil. These three factors should favour denitrification in pasture. This strongly suggests that, in our case, others factors than the organic matter (in its quantitative and qualitative components) affected denitrification, such as for instance the soil structure which can greatly affect the soil aeration status. However, it can not be excluded that organic matter was directly involved in the distribution patterns of denitrification rates measured on intact soil clods under oxic conditions.

With regard to the soil structure, there was no significant difference in total porosity between pasture and cropped soil clods (Table 2). Nevertheless, the structural porosity in pasture clods were 1.5 times higher than in cropped soil clods, and the simulated air-filled pores were more numerous in pasture clods than in cropped soil clods at a 10 cm water potential (Table 2). As illustrated in Fig. 2a and b, pasture and cropped soil clods had different structures. According to the classification of H. Manichon (unpub. Ph.D. Thesis, INAPG, 1982), cropped soil clods can be regarded as Δclods, i.e., clods having a bulk density equal to the textural one without visually observable structural pores. Pasture clods can be regarded as Γclods, i.e., clods including numerous structural pores. Although representing a very small volume fraction of the clods, the structural pore space may be seen as a partially air-filled network of pores, which may greatly modify the aeration status of the clods. The mean distribution of distances showed maximum distances of 8 and 14 mm for pasture and cropped soil clods, respectively. Some estimates of the critical radius, i.e., the minimum radius of a spherical and saturated clod having an anoxic region, were proposed by Greenwood and Berry (1962), Zausig et al. (1993) and Sierra et al. (1995), among others. The range of proposed values lies between 3 and 7 mm, approximately. Assuming a nearly equal critical radius for pasture and cropped soil clods, the proportion of clod sites which were at distances to the nearest air-filled pore larger than this radius, was higher in cropped soil than in pasture clods. For example, if we choose a critical radius of 7 mm, this proportion represented 10 and 1% of the clod volume for cropped soil and pasture, respectively. If the critical radius was only 3 mm, the difference between pasture and cropped soil clods would be much smaller: 20 and 8% of the clod surface for cropped soil and pasture, respectively. These estimates depend on the critical radius which in turn may differ between pasture and cropped soil clods: the critical radius depends on O_2 consumption rate and O_2 diffusion (Currie, 1961). Moreover, the use of a "distance to nearest air-filled pore" criterion does not take into account the fact that anaerobiosis can appear near a crack if labile organic matter was present (Rappoldt, 1990).

For intact soil clods under oxic conditions, we observed a skewed distribution of denitrification rates. Because nearly all the cropped soil clods were without visually observable structural pores (Fig. 2a and b),

they should have similar anoxic fractions and denitrification rates, as long as organic matter (content and quality) and microbial activity do not vary between clods. Under these conditions (i.e., no differences in microbial activity, organic matter content and quality between clods), the presence of air-filled cracks would improve clod aeration and, as a consequence, decrease the anoxic fraction and the denitrification rate. As a consequence, the soil structure cannot explain the skewed distribution of denitrification, especially the high rates observed for some cropped soil clods. We may hypothesise that the different distributions of denitrification rates in the two soils were due to the particulate organic C being heterogeneously distributed between clods: the 20 thin sections of cropped clods included either no or some visible particulate organic matter, suggesting a variability in the particulate organic matter distribution between clods.

In this study, we showed that land use did not significantly affect the microbial denitrifier status (as demonstrated by similar potential denitrification rates) of this soil, but greatly influenced the mean denitrification rate of intact soil clods under oxic conditions (the mean value being 13 times higher in cropped soil than in pasture). As total C, labile organic matter and soluble C content were higher in the soil under permanent pasture, the observed differences in denitrification rates between the two soils could not be explained by these variables. The soil management practices also influenced the structure of the air-filled pore space which, in turn, influenced the distribution of distances of clod sites to their nearest air-filled pore. As a consequence, the anoxic fraction and its distribution were probably affected. It is important to highlight that global indicators, such as porosity values, do not accurately describe the aeration status of the soil and that image analysis can be considered as an essential tool for improving this description. However, the determinism of the skewed distribution of denitrification rates in the cropped soil clods cannot be explained by the structure of air-filled pore space, and is probably closely associated to the distribution of particulate organic matter.

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Particulate organic matter as a source of denitrification variability in pasture and cropped soil clods

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Summary

In order to assess the effect of Particulate Organic Matter (POM) on denitrification, we studied soils under pasture and crop management. For each treatment, clods were fractionated into two compartments: Coated Particulate Organic Matter, C-POM (POM over 200 µm and adherent soil) and the matrix (the rest of the soil). Potential denitrification, CO_2 production and number of heterotrophic and denitrifying bacteria were estimated in C-POM, matrix and bulk soil samples. The quantity and the quality of C-POM were assessed individually in 100 pasture and 100 cropped soil clods. Potential denitrification rates were neither significantly different between bulk samples from pasture and cropped soils, nor between C-POM and matrix from the pasture soil clods. Conversely, potential denitrification was 70 times higher in the C-POM than in the matrix from the cropped soil. CO_2 production in C-POM was 9 and 33

times higher than in the matrix from pasture and cropped soils, respectively. Microbial activities were not correlated to the number of bacteria, which was similar in C-POM and matrix from pasture and cropped soils. C-POM presented skewed frequency distributions for the two soil management. These observations were the basis for a stochastic mechanistic model of denitrification, taking into account C-POM and matrix contributions. Denitrification rates in computerised representation of pasture and cropped soil clods had approximately the same variability as the experimental data from Parry *et al.* (1998), considering both (i) the matrix and C-POM contributions in the cropped soil and (ii) only the matrix contribution in pasture soil.

1. Introduction

Denitrification is one of the major microbial processes that leads to the production of nitrous oxide (N_2O) (Hénault & Germon, 1995; Conrad, 1996). Consequently, efforts to understand the determinism of N_2O emissions in soils, have often led to studies on the factors that affect denitrification at different scales (Tiedje *et al.,* 1989). The microscale approach of the study on denitrification is motivated because the conditions experienced by soil organisms at microscale are often not satisfactorily reflected in the assessements of these conditions when performed on bulk soil samples (Parkin, 1987). More precisely, O_2 concentrations could decrease from atmospheric concentration to zero values within a few millimetres in soil clods (Sexstone *et al.,* 1985; Rappoldt, 1992; Sierra *et al.,* 1995). Authors found examples of anoxic sites close to air-filled pores when organic residues were present (Rappoldt, 1992) with a thin layer of covering water (i.e. thickness of about 160 μm) sufficient for anaerobiosis to occur (Parkin, 1987). Mechanistic models of denitrification do not take into account these micro-heterogeneities, i.e. the authors generally considered the soil as an assembly of homogeneous aggregates in which they model transport and microbial processes (Arah & Smith, 1990; Renault & Sierra, 1994). In particular, all these models neglected air-filled pores within the clods and assumed homogeneous microbial activities. As a result, high microbial activities in the vicinity of organic residues were not taken into account.

In the last few decades, the effect of organic matter on denitrification has predominantly been studied considering the different forms of carbon or the different carbonaceous molecules occuring in the soil. For example: (i) the denitrifying process was shown to be strongly correlated with soluble organic carbon (Burford & Bremner, 1975; de Cantazaro & Beauchamp, 1985; Bijay-Singh *et al.,* 1988) and easily mineralisable carbon (Burford & bremner, 1975; Bijay-Singh *et al.,* 1988); (ii) the relationship between

denitrification and organic carbon was closer with first total organic carbon, then hot water-soluble carbon, water-soluble carbon and microbial biomass carbon (Germon *et al.*, 1983); (iii) Paul *et al.* (1989) demonstrated that denitrifying capacity increased with organic molecules in this order : sucrose, glucose, acetate, propionate and butyrate. Recent experiments have underlined the effect of micro-heterogeneities caused by the presence of fresh plant residues which might significantly affect denitrification on a larger scale (Aulakh *et al.*, 1984; 1991; de Cantazaro & Beauchamp, 1985; Parkin, 1987; Christensen *et al.*, 1990; Murray *et al.*, 1995). For example, a single fresh plant residue could be responsible for 25 to 85% of the denitrification in decimetric soil cores (Parkin, 1987). Particulate Organic Matter (POM) is a labile organic matter pool (Cambardella & Elliott, 1992; Balesdent, 1996), which may play a major role in the patchy functioning of the soil at a microenvironment scale. This POM, slowly incorporated in the soil clods, was *a priori* more decomposed than fresh plant residues (Balesdent, 1996). Its effects on denitrification are still unknown. However, Parry *et al.* (1998) have suggested that POM distribution might be responsible for denitrification variability between soil clods, using a comparative approach between different cultural practices applied to the same soil (permanent pasture or continuous cultivation).

In the present work, our objective was to confirm and specify this hypothesis, i.e. the contribution of POM to denitrification variability. Based on the same comparative approach as used by Parry *et al.* (1998), this study was conducted in two stages. First, a representative set of clods from pasture and cropped soil were fractionated into two compartments: particulate organic matter with soil coating over 200 µm (C-POM), and the remaining soil called the `matrix`. Potential denitrification (measured in anoxic conditions, non limiting C and NO_3^- substrates), CO_2 production and the number of total heterotrophic and denitrifying bacteria were measured in each compartment. The quality of the C-POM in each soil

treatment was also compared by microscopic observation. Second, the quantity and other aspects of the quality (C – to – N ratio) of the C-POM were estimated in 100 pasture and 100 cropped soil clods. On this basis, a stochastic model was constructed to compare the denitrification rates in the computerised representation of pasture and cropped soil clods within C-POM and the experimental data from Parry *et al.* (1998).

2. Materials and methods

Soil clod sampling

Clods were sampled in the 10 - 25 cm layer of gleyic luvisol (FAO classification), located near the Citeaux Abbey (Burgundy, France), 47°09' N and 5°05' E. This soil has been cropped for 50 years. One part of this soil is still cropped (maize), whereas the other part returned to pasture 25 years ago. Clods from these two parts were sieved between 2 and 2.5 cm at the field moisture content, air-dried for one week and stored at 2°C until the experiment. The properties of the clods sampled were: clay, 184 and 145 g kg^{-1}; silt, 523 and 541 g kg^{-1}; sand, 293 and 314 g kg^{-1}; pH water, 6.8 and 6.8; organic C, 12.1 and 9.0 g kg^{-1}; total N, 1.22 and 0.9 g kg^{-1}; for pasture and cropped soil, respectively.

Physical fractionation of soil clods

A mass of 0.8 g of coated particulate organic matter (C-POM) and 3 g of the remaining soil clods (matrix) were obtained from each soil management as described below. Sub-samples of about 30 g of dry clods (4 - 5 individual clods) were carefully dispersed in 150 ml of distilled water by horizontal agitation in a 1000 ml bottle at 120 rpm for 15 min in order to preserve the integrity of the C-POM coating and its micro-organisms. The soil suspension was

then sieved at 200 µm by gentle washing with distilled water to separate a fraction > 200 µm from a fraction < 200 µm. Coarse organic debris (> 200µm) were collected with tweezers. The remaining fraction > 200 µm was divided by flotation in 100 ml of distilled water into a dense fraction and a light fraction. This stage was repeated twice to collect all the C-POM that could be trapped in mineral fractions. All C-POM collected with tweezers and light fractions were pooled. Several sub-samples were fractionated until the total quantity of the collected C-POM reached 0.8 g. All fractions < 200 µm and dense fractions were pooled and homogenised to constitute the matrix. Two aliquots of the matrix (50 g of soil in 500 ml of distilled water) were centrifuged at 8000 rpm for 15 min at 20°C and the pellets were pooled. The supernatant was transparent. In each soil management, the physical fractionation was performed in triplicate.

The same fractionation procedure was applied to 100 individual soil clods from pasture and cropped soils to estimate the distribution of C-POM in clods for the two treatments.

We also used the procedure described by Balesdent *et al.* (1991) to separate the POM over 200 µm from the rest of soil.

In order to compare the C-POM and matrix microbial characteristics to those of the unfractionated soil clods, bulk soil samples were obtained by submerging similar amounts to those used above in distilled water in a 1000 ml bottle and carefully dispersing them by horizontal agitation at 120 rpm for 15 min. After homogenisation, two aliquots (50 g of soil in 500 ml of distilled water) were centrifuged at 8000 rpm during 15 min at 20°C and pellets were pooled. The supernatant was transparent. In each soil management, this treatment was performed in triplicate.

Characterisation of C-POM, matrix and bulk soil samples

The C and N contents of the POM, C-POM, matrix and bulk soil samples obtained using the above procedures were determined with a Carlo Erba element Analyser NA 2500. Each sample was oven dried, crushed and forced through a 200 μm sieve.

Sub-samples of air-dried C-POM fractions from pasture and cropped soils were observed with binoculars (LEICA MZ8). They were also mounted on metal stubs, coated with gold and studied under a Philips Scanning Electron Microscope (100SM).

The relative proportions of organic matter and mineral particles present in the C-POM were estimated by considering the organic C content of the C-POM and the organic C content of the POM with a dimension over 200 μm.

Denitrification measurements

Potential denitrification rates were assessed in the aliquots of the C-POM, matrix and bulk soil samples. In each soil, sub-samples of either 0.2 g of the C-POM or 1 g of the matrix and bulk soil samples were put into 10 ml tubes previously filled with 2 ml of a solution containing 10 mM of succinic acid and 40 mM KNO_3. The atmosphere in each tube was evacuated and replaced by a 90:10 He:C_2H_2 mixture. Gas samples of 0.2 ml were withdrawn with a syringe 180, 240 and 300 min after the introduction of C_2H_2 and immediately analysed for N_2O by a gas chromatograph equipped with an electron capture detector (Varian, 3400CX).

Denitrification in intact soil clods (rewetted by the procedure described in this article) has been previously measured under oxic conditions in the 100 pasture and 100 cropped soil clods individually (Parry *et al.*, 1998). Each clod was put into a 150 ml plasma flask. Seven and 1 ml of gas were replaced by C_2H_2 and Kr, respectively. Gas samples of 0.3 ml were

withdrawn with a syringe 18, 20, 22 and 24 h after adding C_2H_2 and analysed for N_2O concentrations using a gas chromatograph equipped with an electron capture detector.

All the kinetics of N_2O production were linear, which allowed us to express the N_2O productions proportional to the kinetics slope values.

CO_2 production measurements

CO_2 production rates in oxic conditions were taken from the aliquots of C-POM, matrix and bulk soil samples as an indicator of the global heterotrophic activity level. In each soil, sub-samples of 0.2 g for C-POM and 1 g for matrix and bulk soil samples were put into 10 ml tubes. Tubes were tightly sealed and 0.4 ml gas samples were withdrawn with a syringe 180, 240 and 300 min after closing the tubes to be immediately analysed for CO_2 using a gas chromatograph equipped with a catharometer (Girdel, 30). We assumed that, due to the short incubation time, the decrease in O_2 partial pressure in the tubes was not sufficient to significantly affect the rate of CO_2 production. This assumption was strengthened by the fact that the kinetics of CO_2 production was linear. Such linearity made it possible to express CO_2 production as proportional to the kinetics slope values.

Enumeration by MPN of the total heterotrophs and denitrifying bacteria

In each soil management and compartment, an aliquot was homogenised in 9 ml of NaCl (0.8%) in a Waring blender for 2 min followed by serial 10-fold dilutions in the same solution. Dilutions were aliquoted in 8 × 12 wells microtiter plates (eight replicates) in Difco nutrient broth supplied with cycloheximide (0.2 g l^{-1}). For denitrifying bacteria (Staley & Griffin. 1981), the medium was modified with KNO_3 (5 mM), and the plates were incubated at 28°C for one week in anoxic conditions. NO_2^- and NO_3^- were tested with Griess-Ilosway's

and Morgan's reagents, respectively. The presence of denitrifiers was considered positive when neither NO_2^- nor NO_3^- was detected in the medium after incubation. The most probable number (MPN) of heterotrophic or denitrifying micro-organisms was then determined according to Cochran (1950).

Statistical analyses

Student-Fisher tests were used to check whether the potential denitrification, CO_2 production rates and the number of heterotrophic and denitrifying bacteria were significantly different between C-POM, matrix and bulk soil samples, and between the two soil management (Dagnélie, 1975).

Descriptive model of denitrification

We developed a stochastic model to describe the effect of C-POM on the distribution of denitrifying activities in pasture and cropped soil clods. This model was based on the assumption that denitrification in soil clods was the result of two independent factors:

$$D_{clod} = D_{matrix} + D_{C-POM}$$

where D_{clod}, D_{matrix} and $D_{C\text{-}POM}$ are the denitrification rates of the clod, the matrix and C-POM respectively (mol N_2O kg^{-1} s^{-1}).

Clods were represented by an assembly of spherical aggregates. The number n_{agr} of aggregates was random and described by a probability density function $q(n_{agr})$. Similarly, aggregate radii r_i ($1 \leq i \leq n_{agr}$) were random, independent, with a common probability density function a(r). Aggregate radii density was estimated following Rappoldt (1992) by minimising the distance between theoretical probability density function of distances from one point to the nearest air-filled pore ($P_a(d)$) and its empirical estimation performed on plane cross

sections in the same clods $\left(\hat{P}(x)\right)$ (Parry *et al.*, 1998).

$$\hat{a}(.) = \arg\min_{a} \int_{0}^{\infty} \left(P_a(x) - \hat{P}(x)\right)^2 dx \quad [1]$$

where $\hat{a}(.)$ is the estimated function *a* over the whole range of r values.

The probability distribution $q(n_{agr})$ was then estimated by minimising the distance between:

- (i) the empirical Laplace transform of clod masses $\hat{F}(s)$:

$$\hat{F}(s) = \frac{1}{n_{clod}} \sum_{i=1}^{n_{clod}} e^{-sM_i} \quad [2]$$

where M_i is the mass of clod i (kg) and n_{clod} the number of clods;

- and (ii) the theoretical Laplace transform $F_p(s)$ under distribution $p(n_{agr})$ and $\hat{a}(.)$:

$$F_p(s) = \sum_{n_{agr}=1}^{\infty} g(s)^{n_{agr}} p(n_{agr}) \quad [3]$$

$$\text{with } g(s) = \int_{0}^{\infty} e^{-s\frac{4\pi}{3}r^3 d} \hat{a}(r)dr$$

where d is the textural bulk density (kg m^{-3}).

To avoid aberrant clod volumes due to the limits of this procedure, we only kept simulated clods with a volume of between $3.4\ 10^{-6}$ – to – $8.6\ 10^{-6}$ m^3 and $3.5\ 10^{-6}$ – to – $10.9\ 10^{-6}$ in pasture and cropped soils respectively. These volumes corresponded to the smallest and the largest clod volumes which were deduced from their textural bulk density (Parry *et al.*, 1998) and the experimental distribution of weights in 100 pasture and 100 cropped soil clods.

Matrix denitrification was the result of denitrification rates in the aggregates within a clod. Denitrification rate D_i of aggregate i (mol N_2O kg^{-1} s^{-1}) was expressed as:

$$D_i = V_{anox} \times \rho_{agr} \times PDA_{matrix} \quad [4]$$

where V_{anox} is the anoxic volume of the aggregate (m^3), ρ_{agr} is the bulk density of the aggregate

(kg m^{-3}), and PDA_{matrix} the potential denitrification rate of the matrix (mol N_2O kg^{-1} s^{-1}). The normalised clod denitrification rate was then expressed as:

$$D_{clod} = \sum_{i=1}^{n_{agr}} D_i \Big/ \sum_{i=1}^{n_{agr}} M_i \qquad [5]$$

Potential denitrifying activities (PDA) were measured as described in section (2.4). Anoxic volume (V_{anox}) was calculated from models combining O_2 diffusion and consumption in homogeneous spherical aggregates (Currie, 1961). Respiration rate was assumed to be equal to matrix CO_2 production. Oxygen diffusion coefficients within the matrix were equal to 1.76 and 1.43 10^{-11} $m^2.s^{-1}$ in pasture and cropped soils respectively (results not shown).

C-POM denitrification $D_{C\text{-}POM}$ (mol N_2O kg^{-1} s^{-1}) was expressed as proportional to either the C-POM content (Eq. [6a]) or the organic C content of C-POM (Eq. [6b]):

$$D_{C-POM} = \frac{P_{C-POM} \times PDA_{C-POM}}{P_{clod}} \qquad [6a]$$

$$D_{C-POM} = \frac{P_{C-POM} \times F_{C_{C-POM}} \times PDA_{C_{C-POM}}}{P_{clod}} \qquad [6b]$$

where P_{C-POM} is the quantity of C-POM in clod (kg), PDA_{C-POM} the potential denitrifying activity of the C-POM expressed as a function of the C-POM content (mol N_2O kg^{-1} C-POM s^{-1}), P_{clod} the dry mass of the clod (kg), $F_{C_{C-POM}}$ the fraction of organic C in the C-POM, $PDA_{C_{C-POM}}$ the potential denitrifying activity of the C-POM expressed as a function of organic C content in the C-POM (mol N_2O kg^{-1} C s^{-1}). When $D_{C\text{-}POM}$ was expressed as a function of the C-POM, the C-POM quantity in clod P_{C-POM} was randomly chosen from the experimental distribution of the C-POM contents (Fig. 1a), as no relationship between clod weight and C-POM content was found. When $D_{C\text{-}POM}$ was expressed as a function of the organic C content of C-POM, the product $P_{C-POM} \times F_{C_{C-POM}}$ was randomly chosen from the experimental

distribution of organic C within the C-POM (Fig. 1b), as no relationship between clod weight and organic C within the C-POM was found.

3. Results

Coated particulate organic matter (C-POM)

The mean C-POM content per clod estimated on 100 clods of each management was twice as high in pasture than in cropped soil. The mean C-POM content per clod and the standard deviation in pasture soil were equal to 76.9 and 40.9 mg dry C-POM $clod^{-1}$ respectively. The mean C-POM content per clod and the standard deviation in cropped soil were equal to 35 and 31.4 mg dry C-POM $clod^{-1}$ respectively. The mean C - to - N ratio and the standard deviation of the C-POM in pasture soil clods were equal to 15.4 and 4.4 respectively. The mean C - to - N ratio and the standard deviation in cropped soil clods were equal to 16.2 and 4.1 respectively.

Figure 1 shows that, for both soils, whereas most clods contained small amounts of C-POM, some clods had extremely high C-POM content and that C-POM content resulted in skewed frequency distributions. Observation with binoculars and scanning electron microscopy showed that: (i) the C-POM in the pasture soil was essentially composed of root fragments (Fig. 2a). These root fragments had mineral particles that adhered to their surface. The soil present in the C-POM fractions was mainly composed of small aggregates (several tens of micrometres long), trapped by rootlets or filamentous fungi (Fig. 2b), and thereby attached to the root debris. And (ii) The C-POM in the cropped soil was composed of plant debris at different stages of decomposition (i.e. fragments of stems and leaves), root fragments, spores, seeds and charcoal. The soil in C-POM fractions was essentially present as a thick (several

tens of micrometres) and continuous coating on straw debris (Fig. 2c and 2d).

The organic C-content of the POM over 200 µm was equal to 300 and 271.9 mg C g^{-1} POM in pasture and cropped soils respectively. Assuming there was no variation in these values, we thus estimated that the relative proportions of POM and mineral particles were 24% and 76% respectively, for the C-POM in pasture soil, and 16% and 84% respectively, for the C-POM in cropped soil (Table 1). The distribution of organic C content of the C-POM per clod resulted in high variability (Fig. 1b). Their means were equal to 3.06 mg $C_{C\text{-}POM}$ $clod^{-1}$ (SD: 1.4) and 2.3 mg $C_{C\text{-}POM}$ $clod^{-1}$ (SD: 2) in pasture and cropped soils respectively.

Denitrification and CO_2 production

Table 1 shows that the potential denitrification was similar in pasture and cropped bulk soil samples ($p<0.05$). In pasture soil clods, similar potential denitrification rates were found in the matrix and the C-POM ($p<0.05$). In contrast, in cropped soil clods, C-POM had a potential denitrification rate 70 times higher than the potential denitrification rate of the matrix. (Table 1). In pasture and cropped soils, potential denitrification rates were negatively correlated to the organic C-content of the C-POM: the determination coefficients were equal to 0.98** and 0.96** in pasture and cropped soils respectively (3 replicates).

As described by Parry *et al.* (1998), the mean denitrification rate (measured under oxic conditions in 100 undisturbed soil clods) was 13 times higher in the cropped than in the pasture soil clods ($2.1\ 10^{-11}$ and $0.15\ 10^{-11}$ mol N_2O kg^{-1} s^{-1} in pasture and cropped soil clods respectively). Figure 3a shows that, when incubated under oxic conditions, denitrification in pasture soil clods had a symmetrical distribution. In contrast, cropped soil clods presented denitrification rates with higher variability and skewed frequency distribution (Fig. 3b). It appeared that, whereas most denitrifying rates were low, some samples had extremely high

rates (Fig. 3b).

Similar mean CO_2 production rates ($p<0.05$) were obtained in pasture and cropped bulk soil samples (Table 1). In both soil managements, the CO_2 production rate was significantly higher in the C-POM than in the matrix.

Numbers of the total heterotrophs and denitrifying bacteria

The enumeration of the total microflora by MPN indicated that the number of total heterotrophs was not significantly different ($p<0.05$) in pasture and cropped bulk soil samples (Table 1). Similarly, the number of heterotrophic bacteria ($p<0.05$) was not significantly different in the matrix and C-POM, in both the pasture and the cropped soil clods (Table 1).

Denitrifying bacteria (MPN) represented 2.3% of the total microflora in pasture and 0.55% in cropped bulk soil samples. In fractionated pasture and cropped soil clods, the number of denitrifying bacteria was not significantly different in the matrix and C-POM ($p<0.05$) (Table 1).

Model of denitrification

The probability density functions a(r) and $q(n_{agr})$ used in the model are shown in Figures 4a and 4b.

The experimental data from Parry *et al.* (1998) were compared with the model, assuming that the denitrification rate within the C-POM was proportional to the C-POM content in clods. Taking the model (C-POM + matrix) for pasture clods gave us a histogram of the simulated denitrification rates that was different from the experimental data (Fig. 3a). The mean experimental denitrification rate was $0.15\ 10^{-11}$ mol $N_2O\ kg^{-1}\ s^{-1}$, and the mean

simulated denitrification rate was 1.2 10^{-11} mol N_2O kg^{-1} s^{-1}. However, when we used the model (matrix), the resulting histogram of the simulated denitrification rates was similar to the real data on respect to the magnitude of the mean. The mean simulated denitrification rate was 0.3 10^{-11} mol N_2O kg^{-1} s^{-1}. In contrast, when we used the model (C-POM + matrix) for cropped soil clods, the resulting histogram of the simulated denitrification rates was similar to the experimental data (Parry *et al.*, 1998) on the mean and shape (Fig. 3b). The mean experimental denitrification rate was 2.1 10^{-11} mol N_2O kg^{-1} s^{-1}, and the mean simulated denitrification rate was 3.8 10^{-11} mol N_2O kg^{-1} s^{-1}. When we consider the model (matrix) for cropped soil clods, the mean simulated denitrification rate was 0.6 10^{-11} mol N_2O kg^{-1} s^{-1}.

The experimental data from Parry *et al.* (1998) were also compared to the model, assuming that the denitrification rate within the C-POM was proportional to the organic C content of the C-POM. Simulations of the model (C-POM + matrix) had lower variability in denitrification distribution within clods (results not shown). When we consider the model (C-POM + matrix), the mean simulated denitrification rates were 0.7 10^{-11} and 2.3 10^{-11} mol N_2O kg^{-1} s^{-1} in pasture and cropped soils respectively.

4. Discussion

This study was conducted in two stages: (i) a comparison of the microbial characteristics of the C-POM and matrix fractions and the C-POM quality in a representative set of clods from the two soils, and (ii) an analysis of the quantitative and qualitative (C and N contents) distribution of C-POM performed individually in 100 clods from each soil management. After a short critical analysis of the fractionation procedure used in this study, the results will be first discussed and then associated to those of Parry *et al.* (1998) to

elaborate a stochastic model of denitrification for pasture and cropped soil, based on the distribution of the C-POM.

Critical analysis of the fractionation procedure

In this study, only C-POM greater than 200 µm were taken into account. This C-POM size class is believed to affect the soil microbial functioning more directly than the finer organic particles. Balesdent (1996) reported a positive correlation between the size of the residues and their turnover in soil. POM extraction is generally obtained by methods that involve a vigorous soil dispersion to eliminate mineral particles: agitation with glass beads or ultrasonic energy. It has been demonstrated that ultrasonic energy has germicidal effects (Scherba *et al.*, 1991). In our study, it was essential to maintain the soil as a POM coating and to preserve the integrity of micro-organisms. The low amount of energy used to disperse soil clods enabled us to maintain the POM coating, as illustrated in Figures 2c and 2d. However, this gentle procedure prevents the dispersion of dense macro-aggregates (200 - 2000 µm) due to their greater stability, especially in pasture soil (Haynes & Swift, 1990). These macro-aggregates were pooled in the matrix by our procedure, though they might have contained POM, as previously observed by Puget *et al.* (1996).

The organic C amount contained in the C-POM in pasture soil was higher than the organic C amount contained in the POM > 200 µm from Parry *et al.* (1998) (Table 1). This indicates that most POM over 200µm was included in the C-POM and the excess of organic C can be due to the presence of the POM between 50 and 200 µm. Conversely, the organic C amount contained in the C-POM in cropped soil was similar to the organic C amount contained in the POM > 200 µm. Our procedure enabled us to recover most POM > 200 µm in the C-POM fractions from pasture and cropped soils. Nevertheless, C-POM also contained

POM between 50 and 200 μm, especially in pasture soil.

Microbial characteristics of the C-POM and matrix fractions and C-POM quality in the two soils

The 'denitrifying activity of the C-POM – to – denitrifying activity of the matrix' ratios were approximately 1 and 70 in pasture and cropped soil clods, respectively. Discrepancies between these values and those which can be calculated from Table 1 are explained by the fact that the mean 'microbial activity of the C-POM - to - microbial activity of the matrix' ratios for the three replicates has been preferred to the 'mean microbial activity of the C-POM – to – mean microbial activity of the matrix' because of the dependencies between the two variables. These ratios greatly differed from the corresponding CO_2 production ratios (9 and 33 for pasture and cropped soils, respectively). In the pasture soil, contrasted 'C-POM activity - to - the matrix activity' ratios for CO_2 production and potential denitrification (9 and 1, respectively) indicated that denitrification was not stimulated in the C-POM whereas global heterotrophic activity was. On the opposite, the CO_2 production and potential denitrification ratios found in the cropped soil (33 and 70, respectively) indicated that both variables were stimulated in the C-POM and that denitrification was more favoured than global activity near organic particles.

This observation showed that the C-POM was globally more "active" in cropped soil and strongly suggested that differences in the quality of the POM and/or the C-POM local micro-environmental conditions in the two soil management resulted in a differential effect of this structural compartment on soil microbial functioning. In both soils, no significant differences in the number of micro-organisms (heterotrophs or denitrifiers) were found between the C-POM and matrix. This suggested that the variations in the POM quality and/or

the C-POM local environmental conditions probably influenced the functionality of the micro-organisms rather than their density. However, a poor correlation between microbial activities and the number of micro-organisms, although it has already been observed by other authors (Martin *et al.*, 1988; Lensi *et al.*, 1995), should still be interpreted with caution. A incomplete desorption or dispersion of the micro-organisms from the C-POM could result in an underestimation of their number by the MPN method (Richaume *et al.*, 1993). In cropped soil, the strong differences in potential denitrifying activity between the C-POM and matrix could result from (i) soluble compounds supplied by the organic particles specific to the denitrifying process and/or (ii) drastic changes in the local aeration status. This second hypothesis is more realistic, as the functionality and the density of the denitrifying bacteria are potentially affected by the oxygen level (Philippot *et al.*, 1995). In this study, the term respiration was used as CO_2 production in aerobic conditions as it was demonstrated that the respiratory quotient was generally between 0.7 and 1.3 (Bridge & Rixon, 1976) and CO_2 geochemistry may be ignored (Sierra & Renault, 1996). In cropped soil, C-POM respiration was three times higher than in pasture soil, and significantly less air-filled pores took part to the clod aeration (Parry *et al.*, 1998). According to Currie (1961), the minimum thickness of the soil surrounding the C-POM for anaerobiosis to occur at the C-POM level was estimated as a first approximation, assuming (i) uniform microbial respiration equal to that of C-POM and (ii) 21% O_2 at the coating surface. Coating thickness thresholds were then around 3 mm and 1.6 mm in pasture and cropped soils respectively. Considering the distribution of distances of a point to the nearest air-filled pore described in Parry *et al.* (1998) and assuming a random C-POM distribution within soil clods, 12 and 55% of the C-POM would then be at higher distances to the nearest air-filled pore for pasture and cropped soils respectively. Consequently, the high potential denitrifying activity of the C-POM in the cropped soil could

partially result from high respiration rates at these sites. Such respiration rates are *a priori* dependent on organic matter quality.

C-POM fractions contained few organic matter and mineral particle coatings. POM represented 24 and 16% of the C-POM weight in pasture and cropped soils respectively. These differences are likely to favour respiration in pasture C-POM. The C - to - N ratio was often used as an indicator for organic matter decomposition status. In this study, this was similar for C-POM in pasture and cropped soils (Table 1). Microscopic observation showed that the C-POM in pasture soil only contained root fragments, sometimes associated to filamentous fungi, whereas the C-POM in cropped soil also contained straw residues and various other, including seeds, spores and charcoal. Roots were composed of less lignin and more soluble organic C but decomposed slower than plant stems and leaves. These results agree with ours; i.e. the respiration rate was inversely related with the proportion of roots in the C-POM. Reasons explaining the differences in respiration between root fragments and other plant residues include accessibility and microbial adhesion. Microscopic observation confirmed that the high mineral content of the C-POM fraction (84%) in cropped soils was mainly present as a POM coating (Fig. 2d). The mineral content in pasture soil (76%) was aggregated and trapped by filamentous fungi (Fig. 2b).

Distribution of C-POM in pasture and cropped soil clods as a base for a stochastic model of denitrification

The mean denitrification rate of the 100 soil clods being thirteen times higher in cropped soil than in the pasture soil, although cropped soil contained two times less C-POM content than in pasture soil, strengthened our interpretations on the critical importance of the POM differential qualities between the two management. The experimental distributions of

the C-POM and of its organic C content in the two soils (Fig. 1a and 1b) associated with the microbiological characteristics and the results from Parry *et al.* (1998) were used to develop a stochastic model for simulating the distribution of clod denitrification rates.

Assuming that, denitrification rate within C-POM was proportional to the C-POM content in the clods, the model showed similarities to the real data from Parry *et al.* (1998). In pasture soil clods, the best adequation was obtained by considering only denitrification in the matrix fraction (Fig. 3a). The experimental mean denitrification rate was also closer to the simulated mean denitrification, considering the matrix fraction only. Conversely, we needed to take into account simultaneously denitrification in the matrix and C-POM fractions to simulate the experimental denitrification variability within cropped soil clods (Fig. 3b). Here, the experimental mean denitrification rate was closer to the simulated mean denitrification, simultaneously considering the C-POM and the matrix fractions. These results agreed with the previously trend of estimated C-POM fractions in anoxic conditions (12 and 55% in the pasture and cropped soils respectively). Assuming that 100% of the C-POM expressed its potential activities, the matrix contributed to 15% to the mean denitrification activity in an infinite set of cropped soil clods. Nevertheless, a few clods with high denitrification rates greatly reduced the matrix contribution. The relative contribution of the C-POM and matrix were then estimated individually in each soil clod. The mean relative contribution of the matrix was 83% in cropped soils.

Assuming that, denitrification rates within the C-POM was proportional to its organic C content, the contribution of the C-POM to the variability in denitrification rates within clods was reduced. In cropped soil, this was due to the low organic C content of the C-POM within clods with high C-POM contents. In pasture soil, this may result from the organic C content of the C-POM used for denitrification characterisation, which was generally higher than the

organic C content in the set of 100 clods. With regard to the negative correlation between the organic C content of the C-POM and its potential denitrification rate, the adequation of this last model is questionable. An increase in organic C content of C-POM could result from the loss of some of the C-POM coating during the fractionation procedure, leading to the decrease in the micro-organisms number around POM. Therefore, we underline the first model option, i.e. the microbial activity at the POM level was assumed to be proportional to the C-POM content.

Although simulations approximately reflected experimental data, we noticed differences here between experimental data and simulations. These differences may be due to:

i/ parameter estimations for the model, including O_2 diffusion coefficient D_{O_2}, soil clod structure characterisation (i.e. distribution of distances between clod points and their nearest air-filled pore) and microbial activity (i.e. O_2 consumption);

ii/ the basic assumptions of the model itself, including the assumed clod structure (i.e. a set of aggregates), the independence of the matrix and C-POM behaviours, and microbial activities at the C-POM level (ignored or proportional to the C-POM content in the model option used);

iii/ the low number of clods with high denitrification rates, which may prevent good estimation of the mean clod behaviour and C-POM relative contribution to denitrification.

5. Conclusions

In this study, we demonstrated that C-POM in cropped soil clods could be considered as hot spots for denitrification, in contrast to C-POM in pasture soil clods. We pointed out

that, although C-POM was constituted of residues *a priori* more decomposed than the fresh plant residues considered by Parkin (1987) and Christensen *et al.* (1990), they seemed able to remain active micro-sites. Nevertheless, the microbial activities of C-POM depended on their nature and on the soil structure, which influenced the aeration status.

Our results indicated certain limits of the previous mechanistic approaches of denitrification, including the current assumptions of spatial homogeneity in clod structure and microbial activities. We improved previous mechanistic modelling approaches of denitrification and combined them with a stochastic simulation procedure of computerised representation in pasture and cropped soil clods to consider the variability in clod structure and C-POM content. The model considers denitrification as the sum of C-POM and matrix independent contributions. This new approach enabled us to simulate the global trends in the experimental data, especially the skewed distribution of clod denitrification in the cropped soil and to assess approximately the relative contribution of POM to denitrification. A closer estimate is still needed for the proportion of microbial active POM with respect to denitrification.

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1 **Captions to figures**

2

3 Figure 1 : Distribution of C-POM (> 200 µm) content (a) and its organic C content (b) in 100
4 pasture and 100 cropped soil clods.

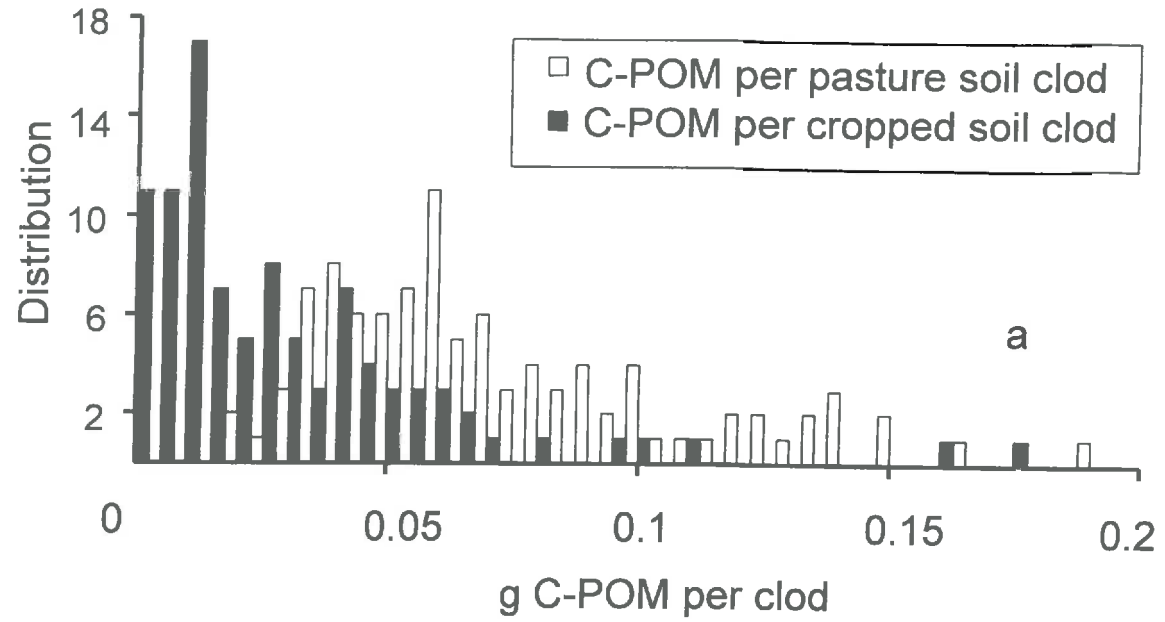
5 Figure 2 : Photographs of C-POM isolated in pasture (a and b) and cropped (c and d) soil
6 clods observed by scanning electron microscopy. The photograph (a) represents a root
7 fragment (*r*) with isolated mineral particles (*m*). The photograph (b) represents small
8 aggregates (*a*) trapped into filamentous fungi (*f*). The photograph (c) represents plant debris
9 with a soil covering (*sc*). The photograph (d) represents the continuous soil covering
10 constituted of mineral particles (*mp*).

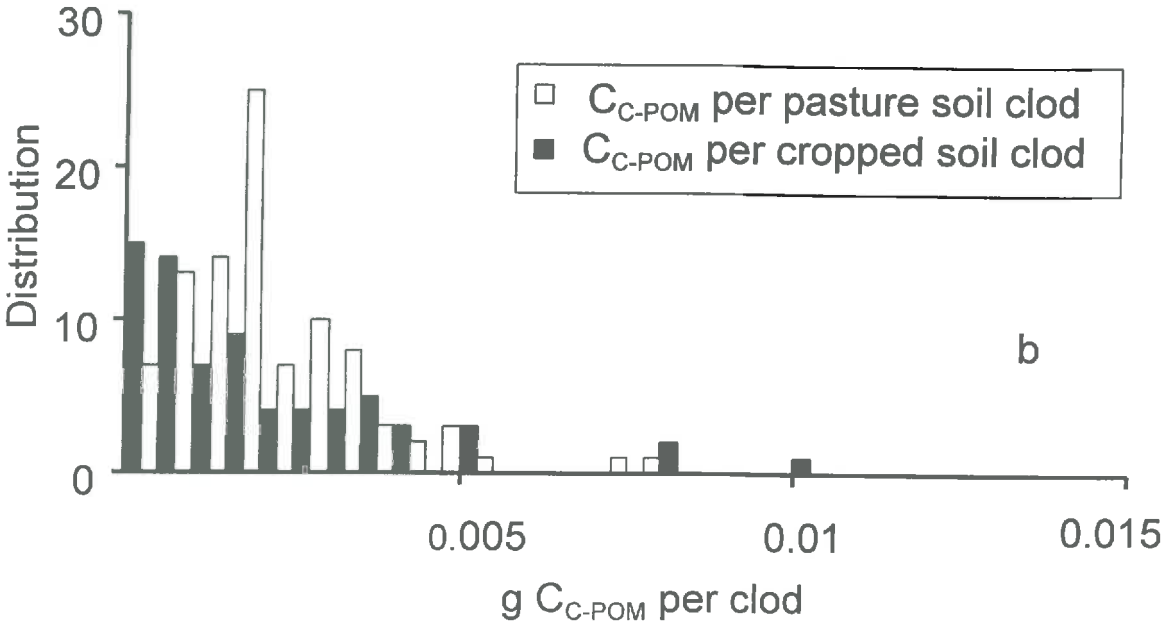
11 Figure 3 : Distributions of the experimental denitrification rates in intact soil clods (2 - 2.5 cm
12 in diameter) under oxic conditions (atmospheric O_2 concentration) with KNO_3 (4 g l^{-1}) for 100
13 pasture soil clods (a) and for 100 cropped soil clods (b) from Parry *et al.* (1998) and
14 distributions of the computer-generated denitrification rates of the model (C-POM+matrix)
15 ( ) and the model (matrix) ( ).

16 Figure 4 : Distributions of radius of aggregates in pasture and cropped soil clods a(r) (a) and
17 the distributions of the number n_{agr} of aggregates per clod in pasture and cropped soils (b).

18

19 Table 1 : Microbial activities, microbial densities and organic matter content and composition
20 in bulk soil, C-POM and matrix respectively, in pasture and cropped soil clods. Numbers in
21 parentheses are standard deviations.





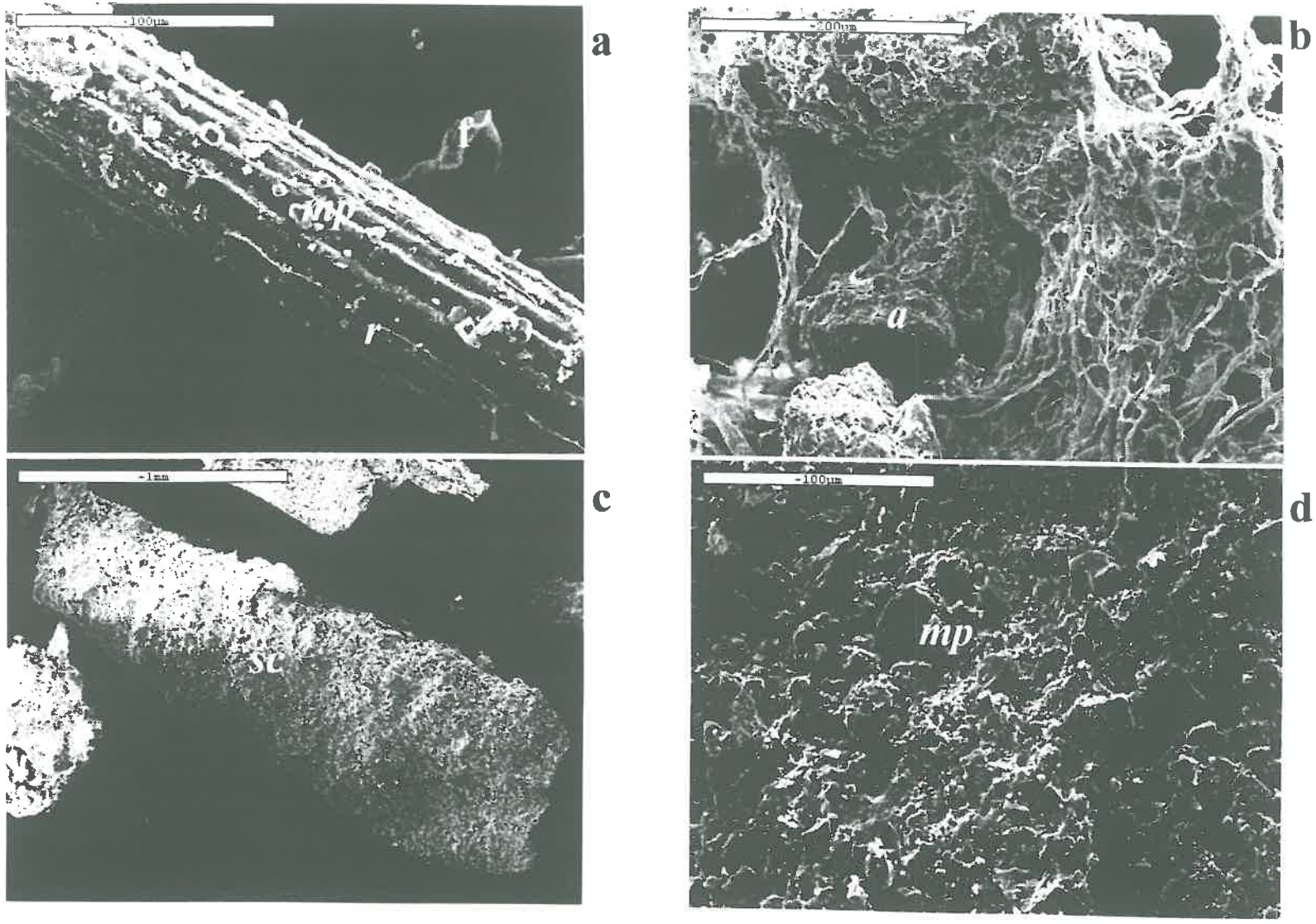
Figures 1a and 1b

Table 1 Microbial activities, microbial densities and organic matter content and composition in bulk soil, C-POM and matrix respectively, in pasture and cropped soil clods. Numbers in parentheses are standard deviations.

	Permanent pasture			Cropped soil		
	bulk sample	C-POM	matrix	bulk sample	C-POM	matrix
Potential denitrification						
(10^{-11} mol N_2O kg^{-1} dry material s^{-1})	130 (12.2)	103 (104)	135 (8.2)	62.1 (42.2)	945 (144)	17.8 (13.8)
(10^{-8} mol N_2O kg^{-1} C within C-POM s^{-1})	-	1.36	-	-	7.86	-
CO_2 production	12.8 (10.6)	92.9 (41.4)	15.4 (9.5)	18.8 (12.3)	306 (24)	9.74 (2.5)
(10^{-9} mol CO_2 kg^{-1} dry material s^{-1})						
C – to – N ratio	8.7 (1)	19.4 (6.2)	8.5 (0.5)	7 (1.9)	9.4 (2.8)	17.3 (0.8)
C of POM > 200 µm (mg C g^{-1} POM)	300	-	-	271.9	-	-
C of C-POM (mg C g^{-1} C-POM)	-	72	-	-	44	-
C-POM content (mg C-POM g^{-1} soil)		9.1			4	
Mineral fraction of C-POM (%)	-	76	-	-	84	-
POM fraction of C-POM (%)	-	24	-	-	16	-

Denitrifying bacteria (10^4 bact. g^{-1} dry material)	2.3 (3.5)	0.9 (0.9)	1.2 (0.8)	1.1 (0.5)	0.8 (0.5)	0.6 (0.3)
Heterotrophic bacteria (10^4 bact. g^{-1} dry material)	100 (65)	242 (202)	394 (38)	194 (85)	200 (61)	73 (30)

[a] POM = Particulate Organic Matter



Figures 2a, 2b, 2c and 2d

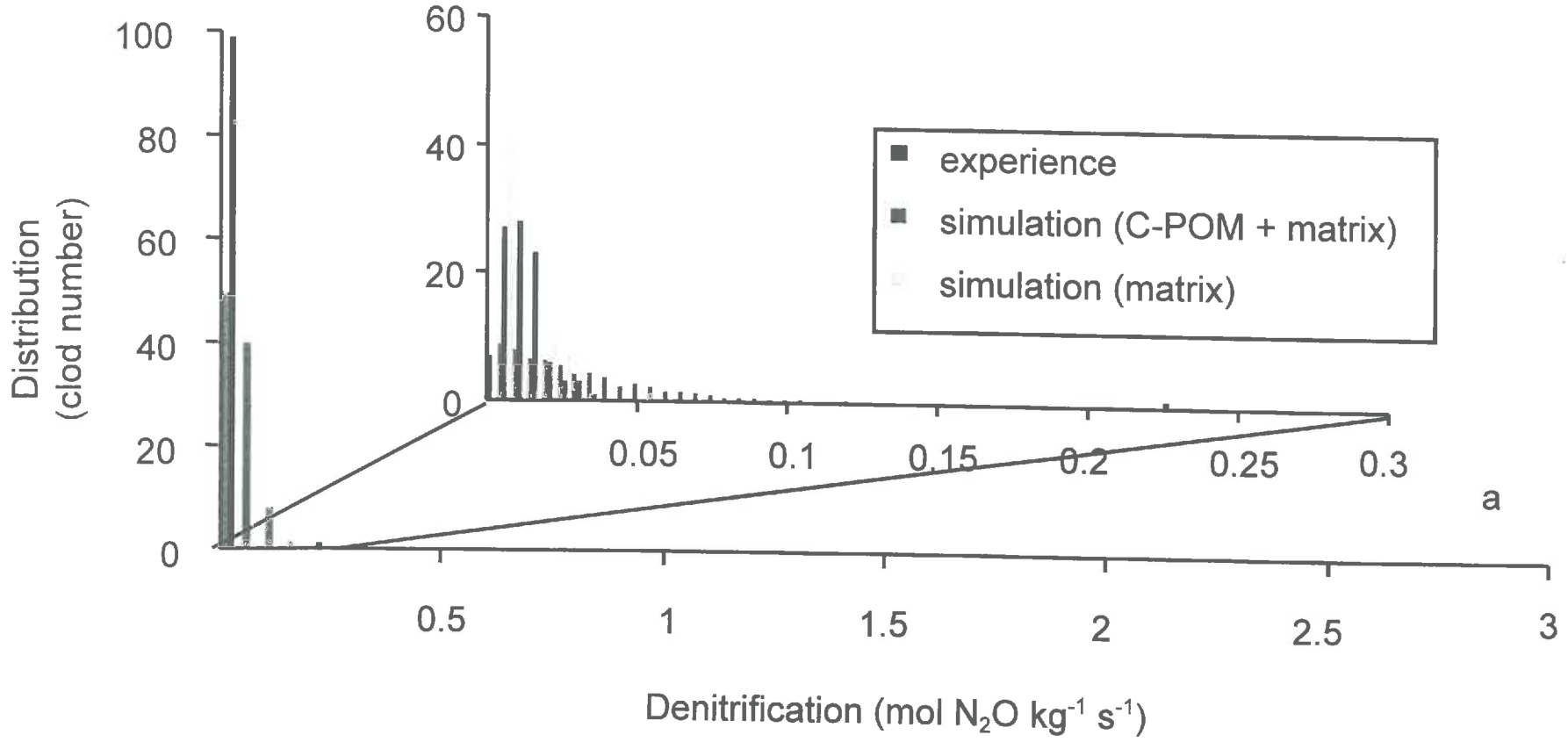

Distribution (clod number)
100
80
60
40
20
0
60
40
20
0
0.05
0.1
0.15
0.2
0.25
0.3
a
experience
simulation (C-POM + matrix)
simulation (matrix)
0.5
1
1.5
2
2.5
3
Denitrification (mol N_2O kg^{-1} s^{-1})

Figure 3a

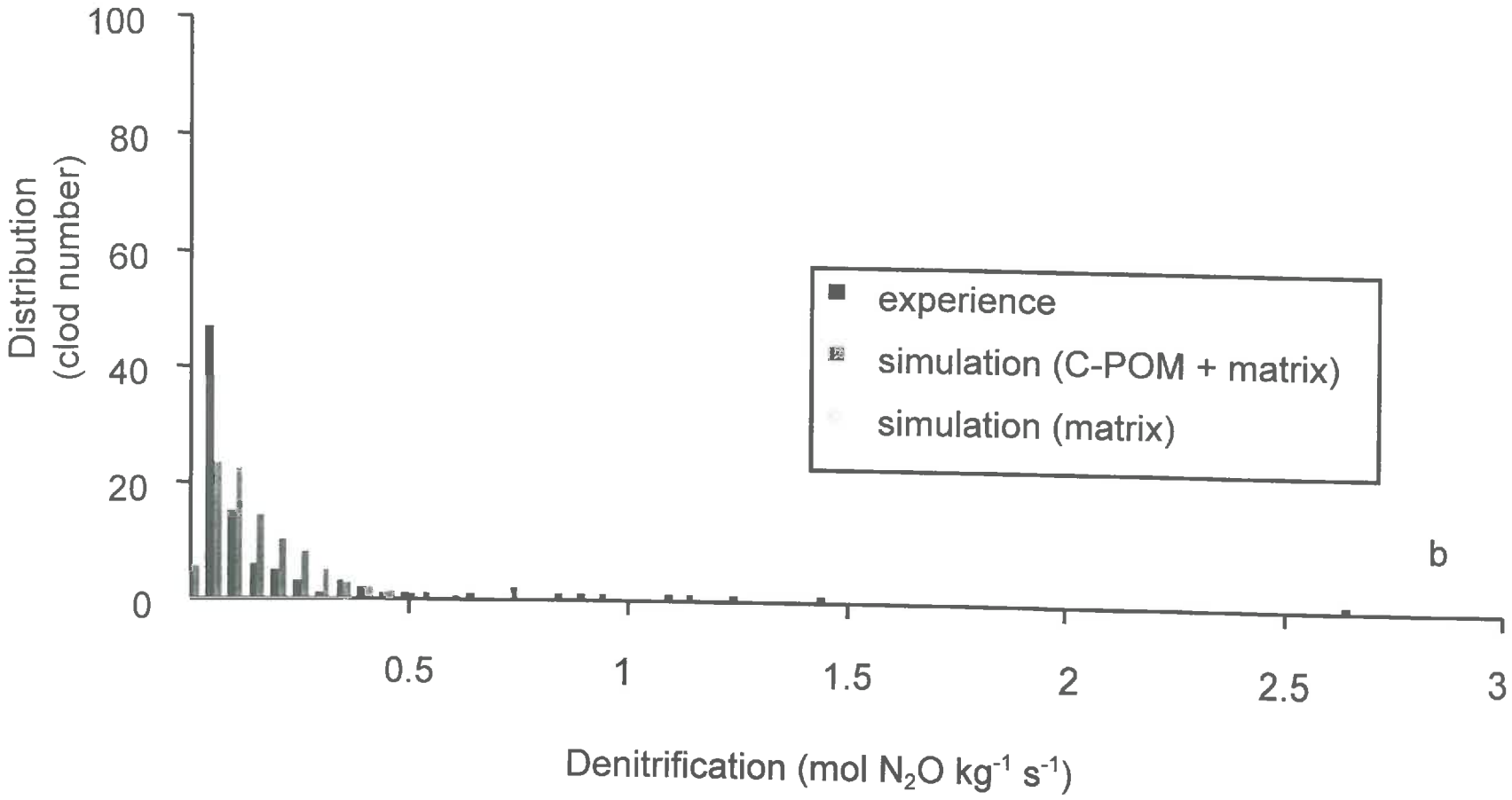
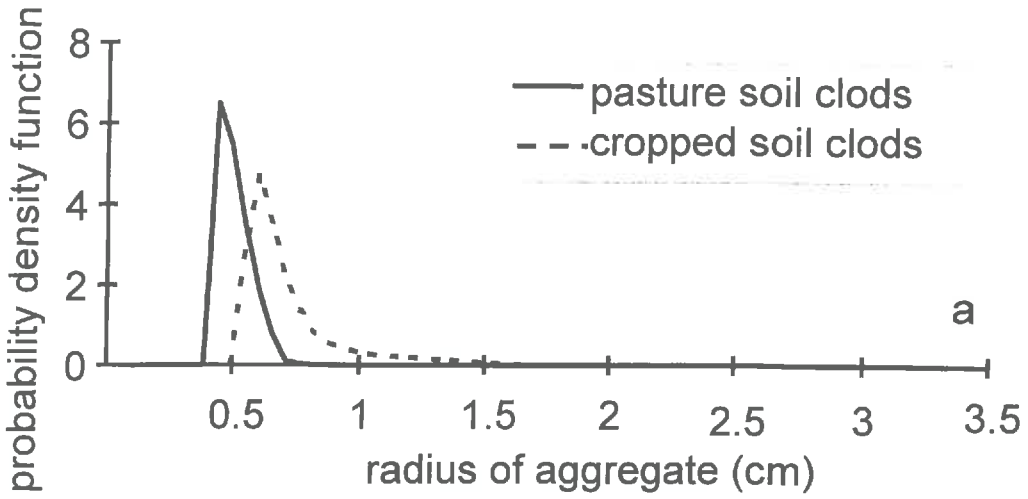
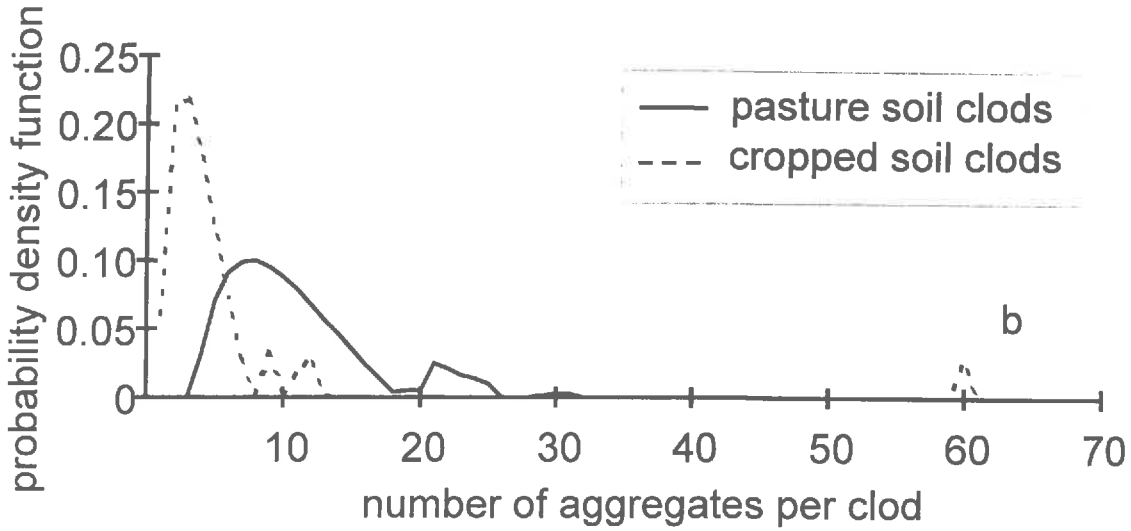

100
80
60
40
20
0
Distribution
(clod number)
experience
simulation (C-POM + matrix)
simulation (matrix)
b
0.5
1
1.5
2
2.5
3
Denitrification (mol N_2O kg^{-1} s^{-1})

Figure 3b





Figures 4a and 4b

Correlation between Clod anoxic Fraction and Denitrification: the Effects of air-filled Pore Space and Particulate Organic Matter

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Running title: Denitrification and Clod Aeration.

Correlation between Clod anoxic Fraction and Denitrification: the Effects of air-filled Pore Space and Particulate Organic Matter

ABSTRACT

To assess the effect of the air-filled pore space and particulate organic matter on clod aeration and denitrification, a comparative study was performed in a soil under pasture and cropped managements. For each management, the denitrification rate was measured on 4 intact soil clods in aerated and non limiting NO_3 conditions. Oxygen distribution was then assessed from 8 radial $[O_2]$ profiles made in a unique plane approximately centered on the clod. Thin clod sections corresponding to this plane were thereafter obtained. Distances from $[O_2]$ measurement points to the nearest air-filled pore in these sections were measured on each clod, and distances to the nearest particulate organic matter were measured on 1 clod for each soil management. An analytic model was used to evaluate the effect of particulate organic matter on $[O_2]$ distribution within the clod. Clods from pasture management generally exhibited irregular $[O_2]$ profiles due to the presence of an air-filled macropore space; only 1 $[O_2]$ profile showed anaerobiosis. On the opposite, most of the $[O_2]$ profiles made in cropped soil clods decreased 3-4 mm below the clod surface to 0 values. Denitrification rate was correlated to the clod aeration in the pasture, whereas no correlation appeared in the cropped management. This resulted from the high contribution of particulate organic matters to clod denitrification for the cropped soil and from the negligible effect of these particulate organic matters on clod aeration, as shown by model simulations.

INTRODUCTION

Denitrification leads to the production of N_2O (Hénault & Germon, 1995; Conrad, 1996), which contributes to the global warming of the Earth (IPCC, 1996) and is implied in chemical transformations of O_3 in the upper troposphere and the lower stratosphere (Graedel & Crutzen, 1992). Efforts at understanding the determinism of N_2O emissions in soils have resulted in several studies on factors affecting denitrification (Tiedje *et al.*, 1989). The microscale approach was motivated by the fact that, in many cases, the conditions experienced by soil microorganisms at this scale are not reflected by the measurements of these conditions, when performed on bulk soil samples (Parkin, 1987). Especially, O_2 concentrations may decrease from atmospheric concentration to zero value within a few millimeters in soil clods (Sexstone *et al.*, 1985; Rappoldt, 1992; Sierra *et al.*, 1995).

Mechanistic models of anoxia, denitrification and N_2O emissions generally considered the soil as a set of homogeneous clods and aggregates (Arah & Smith, 1989; Leffelaar, 1979; Rappoldt, 1992) to account for easy gas transport in the air-filled macropore space and slow transport of dissolved gases in saturated zones. To attain the same objectives, other conceptual models of soils have also been proposed: they described the soil as a medium homogeneous in structure, in which air-filled pores were randomly distributed (Arah, 1988; Rijtema & Kroes, 1991; Rappoldt, 1992). Within saturated zones of the soil, the structure was assumed to be homogeneous: gas and solute diffusion coefficients did not vary with the location. On the other hand, microbial activities were generally described with simplified formulations such as enzyme kinetics (Leffelaar, 1988; McConnaughey & Bouldin, 1985; Sierra & Renault, 1996). Spatially distributed potential activities were taken into account only in the model of Leffelaar and Wessel (1988) as a consequence of substrate gradients, which then affect growths of microbial populations (Philippot *et al.*, 1996). Whatever the model, saturated zones (e.g. soil

clods) were considered as a unique basic soil compartment, from which the behavior was described by solely one set of transport and microbial activity laws. Such conceptual models could not explain why correlation between the anoxic fraction of soil clods and their bulk denitrification rate was sometimes not observed in NO_3 non-limiting conditions (Sexstone *et al.*, 1985), whereas such correlation was indirectly pointed out in other cases (Parkin & Tiedje, 1984). Recently, Parry *et al.* (1998a and 1998b) suggested that clod denitrification rate results from additional contributions of the microbial activities specific to 2 basic compartments: the Particulate Organic Matters >200 µm (POM_{200}) and the anoxic fraction of the remaining of the clod (called "matrix" in this paper). The relative contribution of POM_{200} to clod denitrification rates depend on their specific respiratory and denitrifying activities and on the structure of the clod, which could favor or inhibit denitrification activity at POM_{200} level, as a consequence of their aeration status. These results were deduced from the comparison between experimental distribution of denitrification rates for numerous intact soil clods, and simulations from a stochastic model accounting for these two contributions. Parameters of the model were experimentally obtained. Nevertheless, no experimental evidences were directly obtained at the microscopic scale.

By using O_2-microelectrodes, O_2 distribution were mapped in 2D sections of clods, which came from the same soil submitted to different cultural practices as already used by Parry *et al.* (1998a and 1998b). To check the reliability of previously proposed hypotheses (Parry *et al.*, 1998a and 1998b), we successively (i) analyzed the correlation between the clod aeration and their denitrification rate in NO_3 non-limiting condition, and (ii) tried to explain $[O_2]$ distribution with regard to the distances to the nearest air-filled pore and nearest POM. Because it was difficult to experimentally assess the influence of POM on the clod aeration as a consequence of their few number and low weight proportion, a model of local O_2 diffusion and consumption (Renault & Stengel, 1994) was adapted to describe local $[O_2]$ depletion in

the vicinity of POM_{200}, and to evaluate their contribution to the clod anoxic fraction and their effect on clod aeration.

MATERIALS AND METHODS

Soil clods sampling

Clods were sampled in the 10 - 25 cm layer of a gleyic luvisol (FAO classification), located near the Citeaux Abbey (Burgundy, France), 47°09' N and 5°05' E. This soil has been cropped for 50 years. One part of this soil is still cropped (maize), contrary to the other part, which returned in pasture 25 years ago. Clods from each of these two parts were sieved between 2 and 2.5 cm at the field moisture content, air-dried for one week and stored at 2°C until the beginning of the experiment. The properties of the clods sampled were as follows: clay, 184 and 145 g kg^{-1}; silt, 523 and 541 g kg^{-1}; sand, 293 and 314 g kg^{-1}; pH water, 6.8 and 6.8; organic C, 12.1 and 9.0 g kg^{-1}; total N, 1.22 and 0.9 g kg^{-1}; for pasture and cropped soil, respectively.

Denitrification measurements

Before measurements of denitrification rate, clods were rewetted with a solution of KNO_3 (4 g l^{-1}) at 20°C on suction tables for 24 h at 100 cm water suction, and then 24 h at a suction of 50 cm, and for the 5 following days at a suction of 10 cm. This procedure ensured a slow rewetting process, which prevented the occurrence of additional cracks, and a uniform distribution of NO_3 within the clod (Parry *et al.*, 1998a). The same rewetting procedure was used before O_2 concentration measurements in pasture and cropped soil clods. As described in

Parry *et al.* (1998a), denitrification rate was measured on 100 pasture and 100 cropped intact soil clods under oxic conditions. Each clod was placed into a 150 ml plasma flask (care was taken to avoid any modification of clod structure). Seven and 1 ml of gas were replaced by C_2H_2 and K_r, respectively. Gas samples of 0.3 ml were withdrawn with a syringe 18, 20, 22 and 24 h after the addition of C_2H_2 and analyzed for N_2O concentrations by gas chromatography equipped with an electron capture detector (HP 5890 Series II). The linearity of kinetics allowed expressing the denitrification rates proportional to the values of the slope of these kinetics.

Oxygen profiles

In order to indirectly estimate the aeration of the clods, 8 radial O_2-profiles were performed in a 2D cross-section going through the clod center (angle between neighboring profiles: 45°) for 4 pasture and 4 cropped soil clods, respectively, by the use of O_2-microelectrodes.

Oxygen microelectrodes were constructed as proposed by Revsbech & Ward (1983). Oxygen is chemically reduced at their cathode surface. The resulting electrical current is typically comprised between 1 and 200 pA, and is proportional to $[O_2]$ at the tip of the microelectrode. Typically, their response time was about 1 s, their offset signal (i.e. at 0% O_2) was lower than 15 pA and their sensitivity was higher than 5 pA per % of $[O_2]$ change, their tip diameter was about 50 µm. This electrical current was measured using a picoammeter (Keithley 487, Cleaveland, Ohio, USA). Calibration was performed at O_2 ratio of 0% (slurry in anoxic conditions), 20.9% (air and water in equilibrium with air) and 100% of O_2 (pure O_2 and water in equilibrium with pure O_2). Offset signal was assumed as not depending on the medium. On the opposite, it could be slightly higher in air than in water. As a consequence, the simultaneous use of liquid and gaseous media for the calibration gave us the actual

calibration for the soil and enabled us to easily check a drift by recording the microelectrode signal in air between successive O_2-profiles.

After the O_2-microelectrode calibration, the clod was positioned in an experimental design (care was taken to avoid any modification of clod structure), which insured O_2-profiles to be recorded in a unique plane and enabled us to mark thereafter the position of these profiles by inserting a colored glass capillary in the hole resulting from the electrode tip perforation. The microelectrode was driven perpendicularly to the clod surface with a motor-driven micro-manipulator (Märzhäuser, Steindorf-Wetzlar, Germany), which allowed to position the electrode tip with an accuracy of 10 µm. Measurements were performed every 200 µm in the 7 mm below the clod surface, except when mechanical disturbances forbade it. The tip of the electrode and the clod surface were observed with a binocular at a ×16-40 magnification during the whole course of the experiment. All measurements took place in a laboratory at 20-21°C within a Faraday cage. After each O_2-profile, we checked the drift of the microelectrode signal in air, except when microelectrode tip broke during measurements. The time course of an experiment (initial calibration measurement and drift controls) was about 1 day per clod.

Clods were thereafter carefully conserved in wet condition, until they were included in a resin in order to characterize the distributions of POM_{200} and air-filled pores in the same cross-section.

Distributions of distances of clod sites to the nearest air-filled pore and Particulate Organic Matter (POM_{200})

In order to obtain distributions of the distances to the nearest air-filled pore and to the nearest POM_{200} for 4 pasture and 4 cropped soil clods; thin sections of both clods were prepared and analyzed by morphological operations. Thin sections were obtained for dry clods, which were

included in resin with a fluorescent dye (Bruand *et al.*, 1996). Care was taken to center the section on the plan marked by glass capillaries. In order to obtain the distribution of distances of experimental points to the nearest air-filled pore space, photographs were taken under U.V. light, scanned (1 pixel = 35 μm × 35 μm) and analyzed with VISILOG software commercialized by NOESIS. At first, the saturation of the pore having an equivalent diameter of less than 300 μm (corresponding to an experimental water suction of 10 cm) was simulated by a closing operation (Serra, 1982). By measuring the distance between an experimental point and the surface of the nearest air-filled pore, distributions of distances were obtained for each profile and each clod. In order to obtain the distribution of distances of an experimental point to the nearest POM_{200}, one thin section of a pasture and one of a cropped soil clods were observed with a binocular **(XXX)**. C-POM were then marked and analyzed with VISILOG software commercialized by NOESIS. By measuring the distance between an experimental point and the nearest C-POM, distributions of distances were obtained for each profile and each clod.

Conceptual Picture of the Clods

A stochastic model of clod (Parry *et al.*, 1998b) was developed to describe the influence of the air-filled pore space structure and POM with their soil coating (dimension > 200 μm) on the distribution of $[O_2]$ within pasture and cropped soil clods. Clods were represented by an assembly of spherical aggregates. The number n_{agr} of aggregates is random, described by a probability distribution $q(n_{agr})$. Similarly, aggregate radii r_i ($1 \leq i \leq n_{agr}$) are random, independent, with common probability density function a(r). Aggregate radii density was estimated by Parry *et al.* (1998b) as in Rappoldt (1992), by minimising the distance between theoretical probability density function $P_a(h_{2D})$ of distances h_{2D} from clod points to nearest

air-filled pores in 2D sections and its empirical estimation performed on plane cross sections of the same clods $\hat{P}(h_{2D})$ (Parry *et al.*, 1998a). The probability distribution $q(n_{agr})$ was then estimated by minimising the distance between the Laplace transform of experimental clod masses and the Laplace transform of simulated clod masses, when accounting for a(r) and $q(n_{agr})$ (Parry *et al.*, 1998b). Clod particulate organic matter > 200 µm with their coating (C-POM) was randomly chosen among the experimental distribution of C-POM contents per clod (Parry *et al.*, 1998b), as no relationship between clod weight and C-POM content was found. Their spatial distribution within clods was assumed to be random.

Estimation of POM Influence on the Aggregate Aeration Status

A model of O_2 spherical diffusion and consumption enabled to estimate the global trend of $[O_2]$ as a function of the radial position within aggregates of radii r_i (Fig. 1):

$$\frac{\partial}{\partial r}\left(r^2 D_{O_2} \frac{\partial [O_2]}{\partial r}\right) = r^2 R_{O_2-matrix} \qquad [1]$$

where r was the radial position within the aggregate (m), D_{O_2} the O_2 diffusion coefficient ($m^2.s^{-1}$), $[O_2]$ the O_2 concentration in the liquid phase at position r ($mol.m^{-3}$), and $R_{O_2-matrix}$ the O_2 consumption rate at position r in absence of C-POM ($mol.m^{-3}.s^{-1}$). Assuming that D_{O_2} did not depend on r, and $R_{O_2-matrix}$ was constant as long as O_2 was present, Eq. [1] could be solved analytically and describe $[O_2]$ as a function of r (Renault and Stengel, 1994).

In order to assess the influence of POM on the clod aeration, C-POM were positioned at various distances to the aggregate surface. Fictitious spheres centered on C-POM and having radii equal to the distance between the C-POM center and the aggregate surface were then defined (Fig. 1). To account for the variability of $[O_2]$ values at the surface of these spheres,

an intermediate $[O_2]$ concentration was defined as an equivalent uniform boundary condition (Fig. 2):

$$[O_2]_b = [O_2]_r \quad [2]$$

where $[O_2]_b$ is the equivalent $[O_2]$ at the surface of the fictitious sphere (mol.m^{-3}), and $[O_2]_r$ the O_2 concentration at radial position r within the aggregate, which may be estimated by Eq. [1], considering the aggregate without C-POM (mol.m^{-3}). In order to estimate local fluctuations of $[O_2]$, a model of O_2 spherical diffusion was applied in the fictitious sphere sub-domain without C-POM:

$$\frac{\partial}{\partial r_{fs}}\left(r_{fs}^{\,2} D_{O_2} \frac{\partial [O_2]}{\partial r_{fs}} \right) = 0 \quad [3]$$

where r_{fs} was the radial position within the fictitious sphere (m). As long as the resulting estimate of O_2 supply did not exceed the C-POM maximum O_2 consumption, the applied boundary condition at the C-POM surface was:

$$[O_2] = 0 \quad [4a]$$

Otherwise, O_2 flux at C-POM surface was assumed to counterbalance C-POM maximum O_2 consumption:

$$\left(D_{O_2} \frac{\partial [O_2]_{matrix}}{\partial r_{fs}} \right)_{r=r_{POM}} = \frac{r_{POM}}{3} R_{O_2\text{-}POM} \quad [4b]$$

where r_{POM} was the C-POM radius (m), and $R_{O_2\text{-}POM}$ the maximum O_2 consumption of C-POM (mol.m^{-3}.s^{-1}).

Although it is not strictly possible to superpose local fluctuations of $[O_2]$ as a function of the radial position within these fictitious spheres to the global trend of $[O_2]$ variations within the aggregate, the estimates of local fluctuations enabled us to assess the order of magnitude of the volume in which the $[O_2]$ was significantly modified by the O_2 consumption of POM: the POM-sphere was thus defined as a C-POM centered sphere, having a radius equal to the

distance to the C-POM center at which the local decrease of $[O_2]$ du to the C-POM was equal to 2% in air, corresponding to 0.028 $mol.m^{-3}$ in a diluted aqueous solution at 20°C. Oxygen concentration estimation at C-POM surface was used to check whether anaerobic conditions prevail within C-POM, assuming D_{O_2} within C-POM to be equal to D_{O_2} in the remaining of the aggregate (the matrix).

Simulations were performed for cropped soil clods, as it was demonstrated C-POM contributed significantly to clod denitrification rates only in this soil (Parry *et al.*, 1998b). The O_2 diffusion coefficient within the clods was then estimated from measurements by Sierra ***et al.*** (1995) on the same soil. Microbial respiration rates for the matrix medium and particulate organic matter were deduced from the CO_2 production in aerobic conditions measured on the same set of clods by Parry *et al.* (1998b), assuming the respiratory quotient was equal to 1.

Statistical Approach of the Clod Aeration

Le travail présenté dans ce paragraphe ne sera réalisé qu'à la fin de l'année 1998 ou au début de l'année 1999. La description des méthodes est incomplète. Dans la section "Results and Discussion", un titre sans contenu a été placé pour mémoire.

In order to assess whether $[O_2]$ measurements conformed to the conceptual picture of soil clods (i.e. a random set of aggregates) and the model of O_2 diffusion and consumption, $[O_2]$ values were assumed to be random with probability density functions depending on the distance h_{3D} to the nearest air-filled pore, where 3D-indicia is for the actual lowest distance in the 3-dimensional space. Using the conceptual picture of soil clod and the global model of O_2 spherical diffusion and consumption within soil aggregates, these probability distributions

$p_{h_{3D}}([O_2])$ were deduced from the probability density function a(r) for an aggregate to have a radius r:

$$p_{h_{3D}}([O_2]) = \frac{\{(r-h)^2 a(r)\}_{r/[O_2]\,at\,r-h}}{\int (r-h)^2 a(r) dh} \quad [5]$$

These theoretical probability density functions were compared to the density functions $\hat{p}_{h_{3D}}([O_2])$ deduced from the experimental probability density functions $\hat{p}_{h_{2D}}([O_2])$ of $[O_2]$ as a function of h_{2D} in the observed clod cross-sections:

$$p_{h_{3D}}(.) = F_{2D\to 3D}\left(p_{h_{2D}}(.)\right) \quad [6]$$

where (.) is for all the possible $[O_2]$ values and $F_{2D\to 3D}$ is a function converting probabilities of $[O_2]$ for distance to the nearest air-filled pore in 2D sections to probabilities of $[O_2]$ for distance to the nearest air-filled pore in the 3-dimensional space.

χ^2 - tests were used to check whether there existed statistical differences between modeling and experimental estimates of the $[O_2]$ probability density functions (Dagnélie, 1975) for fixed h_{3D} values (i.e. 1, 3 and 5 mm).

RESULTS AND DISCUSSION

Critical Analysis of the Experimental Procedure

Denitrification measurements on 100 pasture and 100 cropped soil clods were realized 18 months before O_2-profiles. During this time, regular 'denitrifying activities' tests were performed on several pasture and cropped soil clods presenting various denitrification rates. These tests showed that the relative decrease in denitrification rates was ever lower than 40%

on 12 months and more generally about 10-20%. The clod grading according to denitrification rates was preserved during this time (results not shown).

Because O_2 concentration measurements could be only realized on saturated soil clods, each clod was rewetted by the procedure described above. With regard to experiment duration for a single clod (1-day), we regularly add some water droplets on the clod surface between $[O_2]$ profiles in order to prevent the clod drying. Nevertheless, the addition of water generally led to an excess of water with regard to the water content for a 10 cm water suction. Unfortunately, it was neither possible to strictly quantify this excess nor to control it. It may decrease the aeration of the clod and smooth irregularity in $[O_2]$ profiles as a consequence of a partial saturation of pores having a diameter higher than 300 µm. On the other hand, it render difficult the actual estimate of the distance between a point and the nearest air-filled pore in 2D clod sections, because saturation corresponding to a 10 cm suction was simulated on these pore sections. Previous modeling works by Renault and Stengel (1994) showed that steady $[O_2]$ profiles can be reached in a clod initially well aerated after approximately 5 hours if no bubble of air is blocked within the clod. When air bubbles are blocked within the clods, the equilibration time may increase up to 2 days. In our study, $[O_2]$ profiles would thus indicate lower clod aeration than the actual one during denitrification measurements. Nevertheless, we may assume that this decreasing was small in the pasture soil, in which numerous macropores improved the aeration of the clod; $[O_2]$ profiles were irregular and $[O_2]$ generally remained higher than 5-10% (Fig. 3a-d)) whereas it should have decreased to zero value for saturated clods in steady state. On the opposite, only a few macropores were included in clods from the cropped soil (Parry *et al.*, 1998a). An increase of the water content could have minimized irregularities in $[O_2]$ profiles. We assumed this effect did not alter the grading of clod aeration.

In order to obtain pores and C-POM maps and O_2-profiles on a unique plan, care was taken to center the section on the plan marked by 8 glass capillaries. However, it was not always possible to find simultaneously the 8 glass-capillaries (Fig. 2a and 2b).

Denitrification measurements

Four pasture and 4 cropped soil clods have been chosen among the sets of 100 and 100 clods, which were already used by Parry *et al.* (1998a) for denitrification measurements. Pasture soil clods presented low denitrification rates, and the 4 retained clods were chosen so as to insure a distributed range of their denitrification rates, i.e. 0.06 10^{-11}, 0.15 10^{-11}, 0.18 10^{-11} and 2.2 10^{-11} mol N_2O kg^{-1} s^{-1} (Fig. 3a-3d), respectively. In the cropped soil, 4 clods presenting high denitrification rates were chosen in order to insure that C-POM greatly contributed to denitrification rates within these clods (Parry *et al.,* 1998a and 1998b). Their denitrification rates were 9.07 10^{-11}, 11 10^{-11}, 12.6 10^{-11} and 25.5 10^{-11} mol N_2O kg^{-1} s^{-1} (Fig. 3e-3h).

Oxygen profiles, air-filled pores and POM distributions

Figures 3a-3h represent the O_2-profiles obtained for 4 pasture and 4 cropped soil clods, respectively. All the profiles presented similarities: (i) a steep O_2-gradient which occurred over a small distance, and (ii) a weak variation of $[O_2]$ concentrations beyond 2 mm. On the other hand, $[O_2]$-profiles exhibited differences between soil managements, clods for each soil management and profiles within a clod. Oxygen profiles in pasture soil clods were irregulars (Fig. 3a-d), as already observed by Bakker & Bronswijk (1993) for another pasture soil. In contrast, $[O_2]$-profiles in cropped soil clods were regular, with only a few (e.g. in Fig. 3g or in Fig. 3h). For this soil, our results differed from those of Sierra & Renault (1996) who

measured more irregular [O_2]-profiles than ours, in cropped soil clods sampled from the same soil. These differences could be due to the water droplets put at the clod surface before [O_2] measurements, and/or to changes in the structure of the clods. Secondly, anoxia was reached between 3 and 4 mm below the clod surface in the clods from the cropped soil (Fig. 3e-h), whereas only one [O_2]-profile exhibited an anoxic region in pasture soil clods. These different behaviors certainly resulted from the presence or absence of an air-filled pore space within the clods. Observations of thin sections of the 4 pasture and 4 cropped soil clods (Fig. 2a-b) lead to similar pictures as those obtained by Parry *et al.* (1998a). Pasture soil clods exhibited an important network of cracks, which did not exist in cropped soil clods. The presence of macropores decreased the distances between experimental point and the nearest air-filled pore, which influenced the aeration status of the clods and, in turn, influenced their anoxic fraction (Fig. 3a and 3d), even if rarely observed at the profile locations. The few air-filled pores present in cropped soil clods seemed not to influence their aeration status. The maximum distance measured in pasture soil clod between an experimental point and an air-filled pore was 4 mm, whereas it reached 7.5 mm in the cropped soil clods (i.e. the maximum depth below the clod surface reached by the microelectrode tip, as described in Materials and Methods).

Particulate organic matter with their soil coating (>200 µm) (POM_{200}) were observed on thin sections for 1 pasture and 1cropped soil clods. They were randomly distributed within the cross-section. Unfortunately, no POM_{200} intersected an [O_2] profile. Although some of the POM_{200} were in the 1mm vicinity of [O_2] profiles, they apparently did not affect [O2] values on these profiles

Irregularities in [O_2] profiles were generally not correlated with the presence of an air-filled pore or a C-POM. This could partially result from the fact that pores and POM_{200} distribution were observed in 2D sections with no description in adjacent clod medium.

C-POM effect on clod aeration; correlation between the clod aeration and denitrification

In order to assess the influence of C-POM, the model was used to simulate $[O_2]$ distribution within large aggregates (i.e. radius=1cm) of cropped soil clods, with regard to probability density function q(r) of the radii r of the aggregates (Fig. 4a). Even without accounting for the O_2 consumption of C-POM, the model simulate anoxic conditions as soon as the distance to the aggregate surface go beyond 4-5 mm (Fig. 6), in agreement with experimental $[O_2]$ profiles for the cropped soil clods (Fig. 3e-h). Simulated $[O_2]$ (without accounting for C-POM respiration) were used as boundary conditions for the fictitious spheres of soil surrounding C-POM. The local $[O_2]$ depletion due to C-POM and the dimensions of the POM-sphere were then estimated. For example, local $[O_2]$ depletion within a fictitious sphere of 4 mm diameter containing C-POM of 0.5 mm radius was reported in Fig. 5, assuming boundary $[O_2]$ to be in equilibrium with atmospheric O_2 (Fig. 5); for this example, simulated O_2 supply exceeded O_2 consumption of C-POM when considering 0% $[O_2]$ at the C-POM surface, and more realistic simulations were then obtained by assuming that O_2 supply counterbalanced exactly the O_2 consumption of C-POM (Fig. 5). Local $[O_2]$ depletion and the POM-sphere dimensions were estimated for C-POM at various distances to the aggregate surface, these last distances defining the radius of the fictitious sphere surrounding C-POM and the corresponding uniform $[O_2]_b$ at the surface of these spheres (Fig. 6). The POM-sphere radii never exceeded 0.9 mm for 0.5 mm radius C-POM. Minimum POM-sphere radii were obtained for C-POM in the vicinity of the aggregate surface; O_2 transport concerned distances not long enough to induce a depletion of $[O_2]$ at C-POM surface higher than the previously defined $[O_2]$ threshold. Minimum POM-sphere radii were also obtained for C-POM near the aggregate anaerobic center, where $[O_2]$ was lower than 0.0283 $mol.m^{-3}$ before accounting for C-POM effect. When POM-sphere radius was maximum (i.e. 0.9 mm), the corresponding volume of

aggregate "significantly affected" by C-POM was about 3.1 mm^3. Assuming a clod having a volume of 4.2 cm^3 (r=1 cm), the POM-sphere volume corresponds to 0.5‰ of the clod volume. The maximum C-POM content of a clod (0.19 g C-POM) would correspond to 250-300 C-POM of 0.5 mm radius, and induce an $[O_2]$ perturbation in less than 18% of the clod volume. More precisely, considering that 250 C-POM are randomly distributed within the 1 cm radius clod and accounting for the relationship between the POM-sphere radius and the radial position within the aggregate, we calculated that $[O_2]$ were significantly affected by C-POM in only 6% of the aggregate volume. Therefore, we consider that C-POM of the cropped soil clods did not significantly affect their aeration status. Because most of the denitrifying activity took place within C-POM in the retained cropped soil clods (Parry et al., 1998b), the low effect of C-POM on clod aeration would explain why no correlation between denitrification and the aeration level was found for cropped soil clods. On the opposite, it was demonstrated that C-POM did not significantly contribute to the denitrification rates of pasture soil clods (Parry et al., 1998b); clod denitrification occurred mainly in the anoxic central zone of the largest aggregates, which constituted the clods. Denitrification rates were thus correlated with the aeration status of these clods.

Simulated and experimental probability density functions of $[O_2]$ as a function of the nearest air-filled pore

Le travail correspondant à ce paragraphe ne sera réalisé qu'à la fin de l'année 1998 ou au début de l'année 1999. Il est placé ici pour mémoire, afin de permettre au lecteur d'avoir une vue d'ensemble sur les objectifs sous-jacents à ce travail.

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CAPTION TO FIGURES

Figure 1: Conceptual picture of the model developed to estimate local [O_2] fluctuations caused by particulate organic matter with their soil coating.

Figures 2a-b: Clod cross sections with [O_2] profile localization and corresponding [O_2] profiles. Particulate organic matter (>200 µm) were redrawn on the section in green color:

a/ clod from pasture management;

b/ clod from cropped soil management.

Figures 3a-h: [O_2] profiles obtained on the cropped soil managements (a-d) and the pasture management (e-h).

Figures 4a-b: Statistical characteristics of the clods from pasture and cropped soil managements when representing the clods as a random number of aggregates having random radii:

a/ probability density function of of aggregate radii estimated as Rappoldt (1992);

b/ probability density function of the number of aggregates per clod.

Figure 5: Example of [O2] fluctuation in a spherical soil aggregate (radius = 4 mm) containing a centered C-POM (radius = 0.5 mm) with [O2] concentration at the aggregate surface equal to the atmospheric concentration. Two calculation procedures were used:

- (i) [O_2] was put to 0 at the C-POM surface; this led to an O_2 supply higher than the maximum C-POM O_2 consumption;

- (ii) $[O_2]$ gradient counterbalanced maximum C-POM $[O_2]$ consumption.

No O_2 consumption was taken into account in the soil to describe only $[O_2]$ fluctuation caused by the C-POM.

Figure 6: $[O_2]$ profile within an aggregate (radius = 1 cm) of the cropped soil, when accounting for microbial respiration only in the matrix (i.e. neglecting C-POM), and $[O_2]$ fluctuation due to the presence of a C-POM (radius = 0.5 mm) as a function of its radial position. $[O_2]$ fluctuation is described by (i) $[O_2]$ at the C-POM surface and (ii) by the POM-sphere radius (i.e. the radius of the sphere centered on the C-POM and in which $[O_2]$ decrease caused by the C-POM corresponded to more than % O_2 in air).

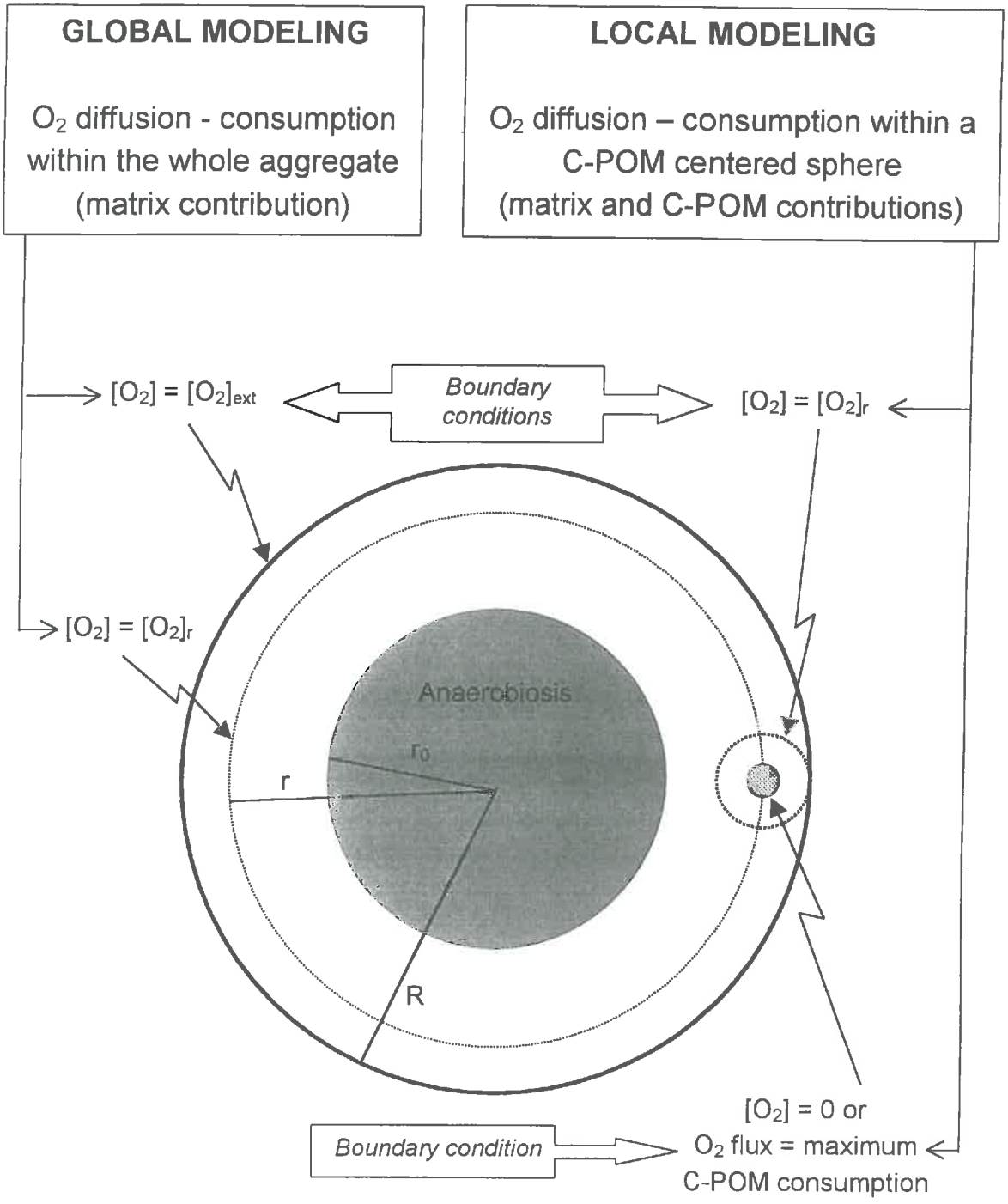

GLOBAL MODELING
O_2 diffusion - consumption within the whole aggregate (matrix contribution)
LOCAL MODELING
O_2 diffusion – consumption within a C-POM centered sphere (matrix and C-POM contributions)
$[O_2] = [O_2]_{ext}$
Boundary conditions
$[O_2] = [O_2]_r$
$[O_2] = [O_2]_r$
Anaerobiosis
r_0
r
R
$[O_2]$ = 0 or
O_2 flux = maximum C-POM consumption
Boundary condition

Figure 1

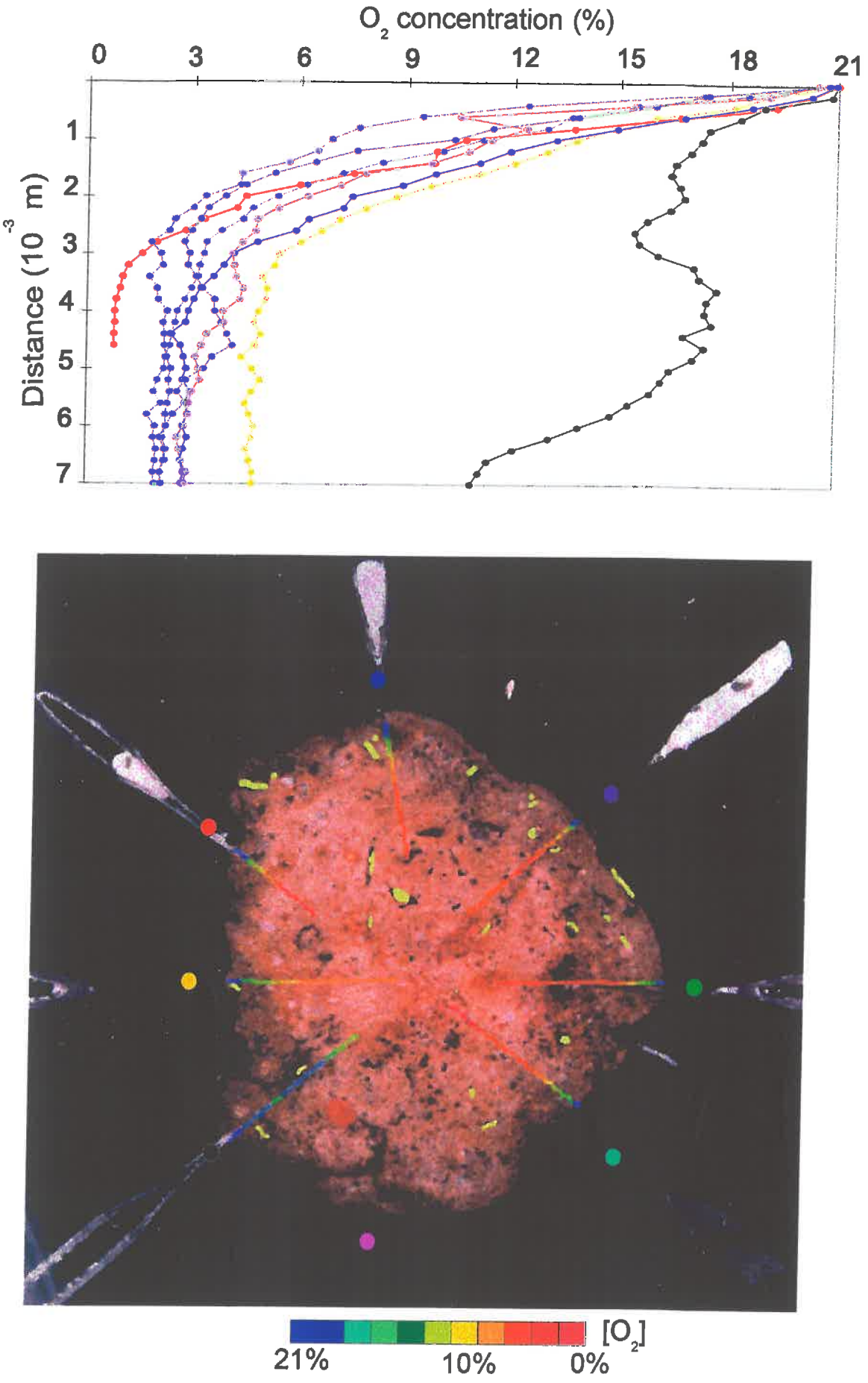

O2 concentration (%)
0
3
6
9
12
15
18
21
Distance (10 -3 m)
1
2
3
4
5
6
7
21%
10%
0%
[O2]

Figure 2a

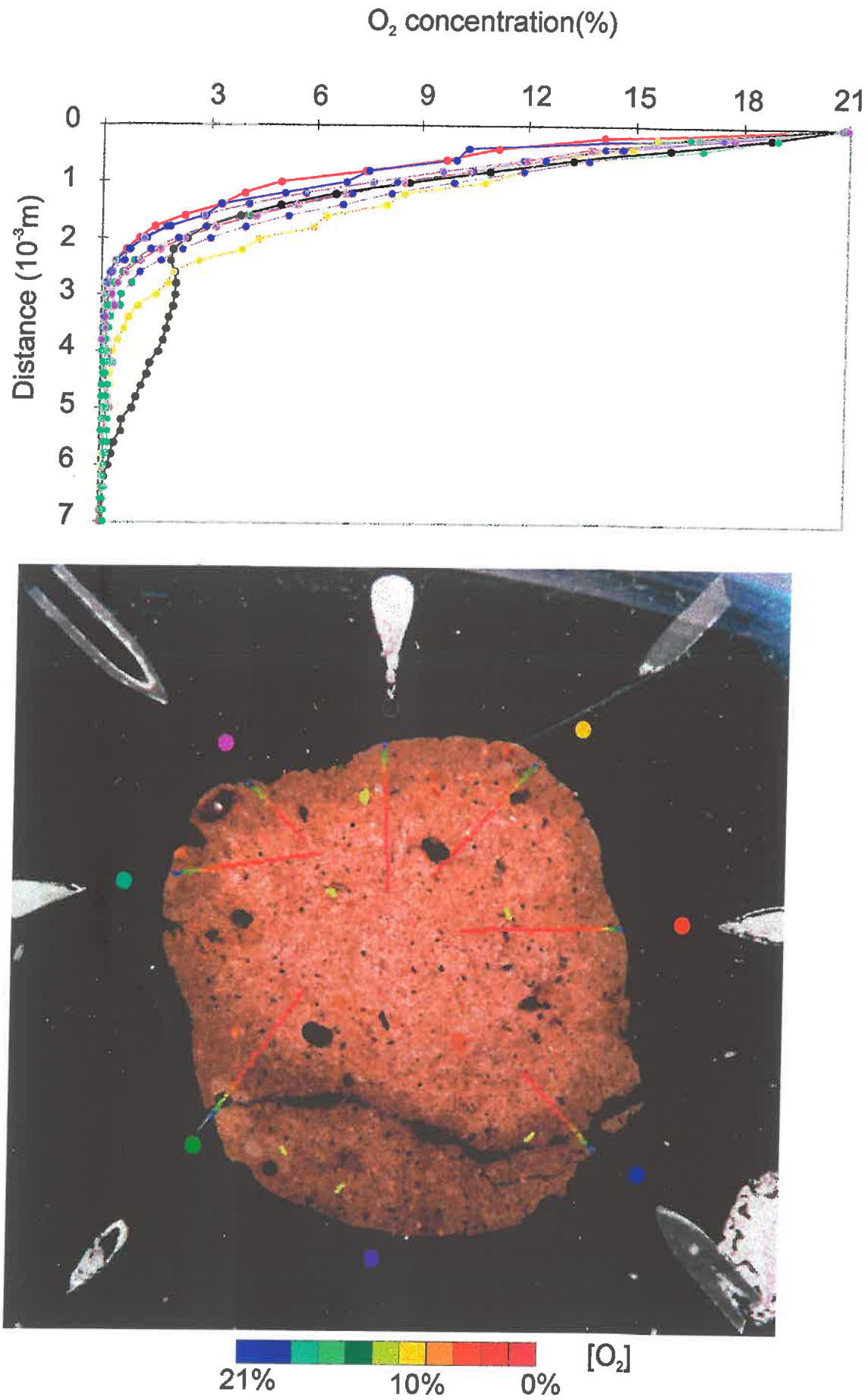
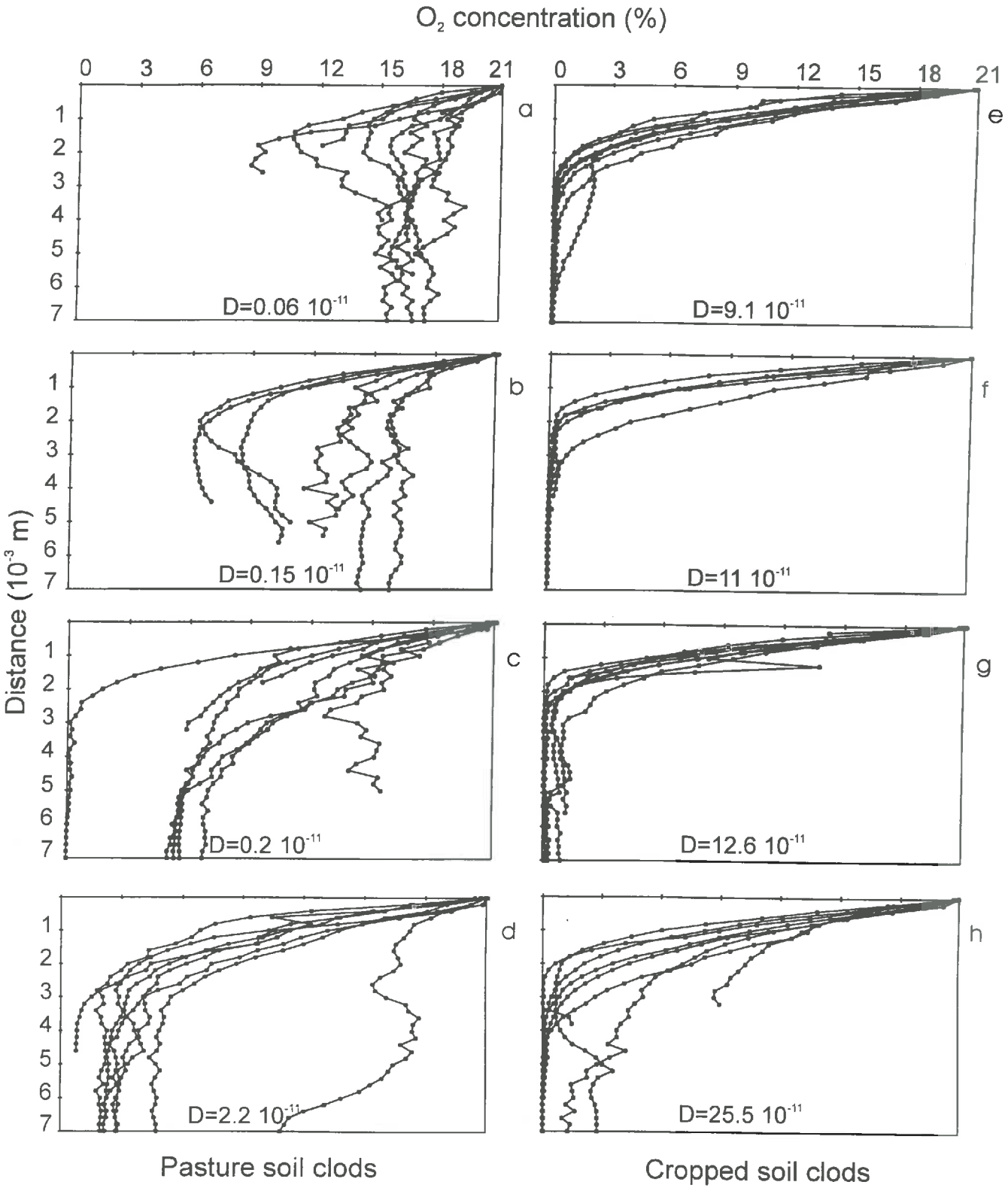
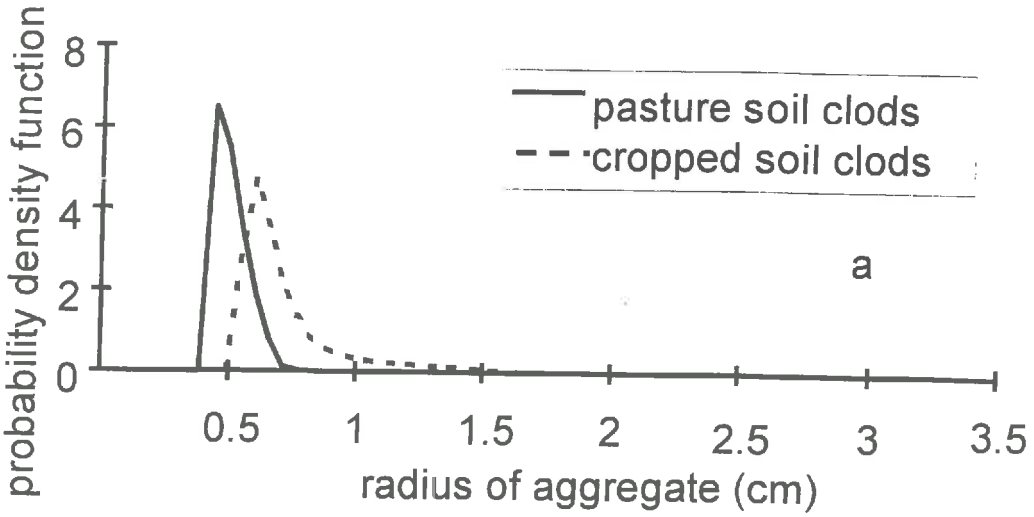
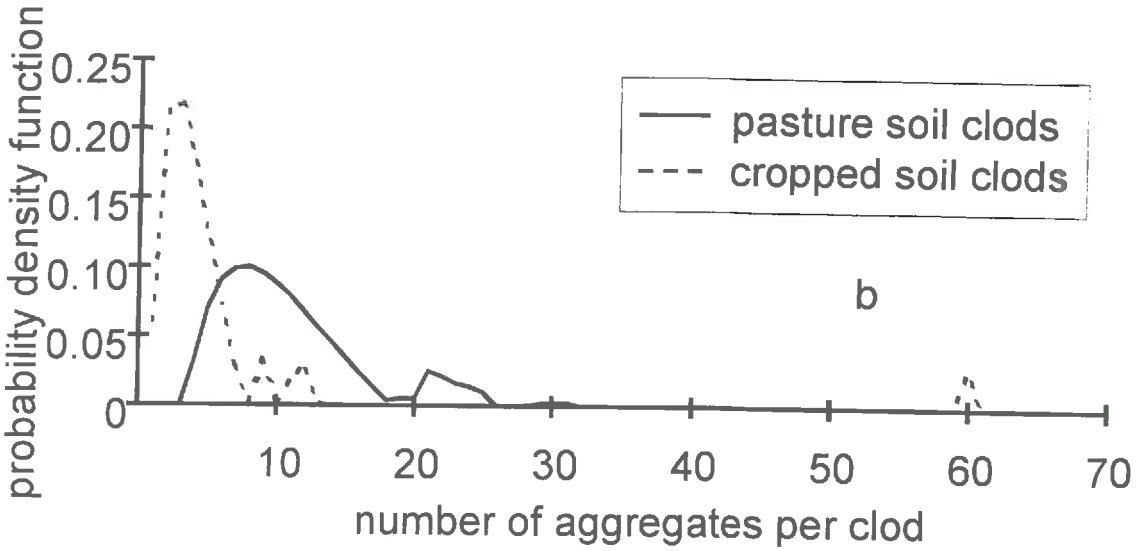

O2 concentration(%)
3
6
9
12
15
18
21
0
1
2
3
4
5
6
7
Distance (10-3m)
21%
10%
0%
[O2]

Figure 2b



Figures 3a, 3b, 3c, 3d, 3e, 3f, 3g, 3h





Figures 4a and 4b

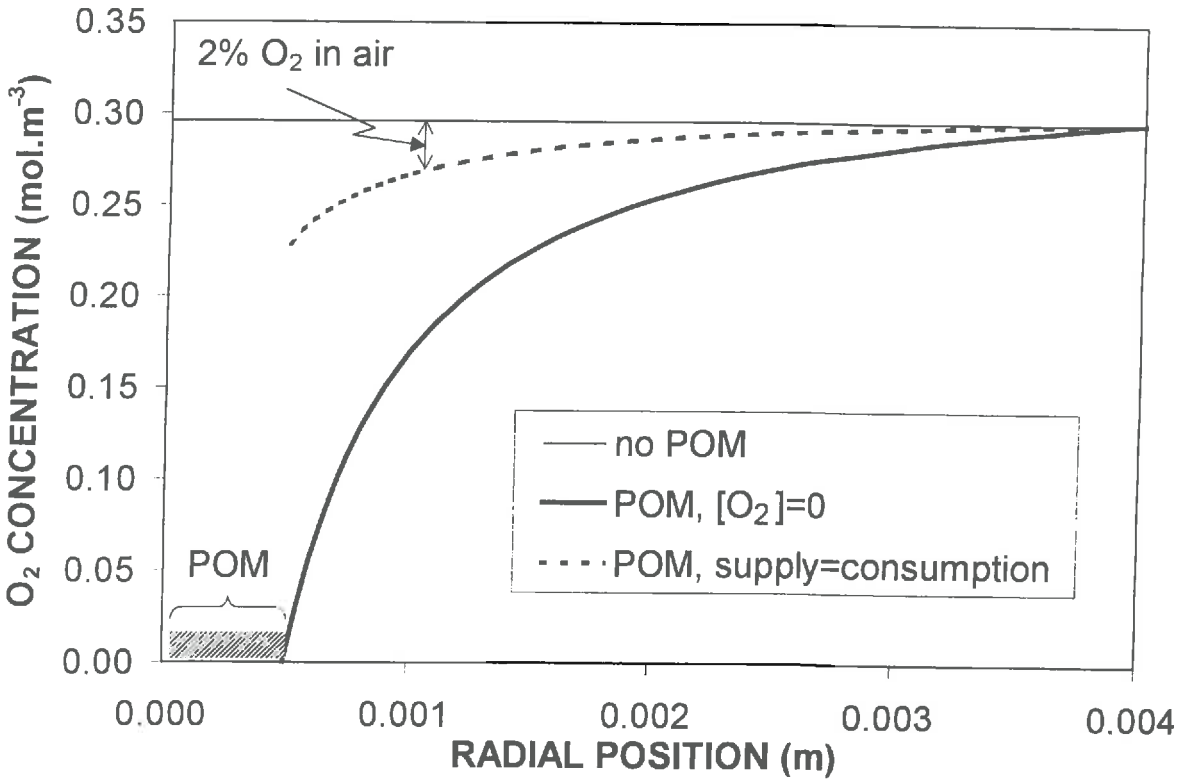

0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
2% O_2 in air
POM
no POM
POM, $[O_2]$=0
POM, supply=consumption
O_2 CONCENTRATION (mol.m^{-3})
0.000
0.001
0.002
0.003
0.004
RADIAL POSITION (m)

Figure 5

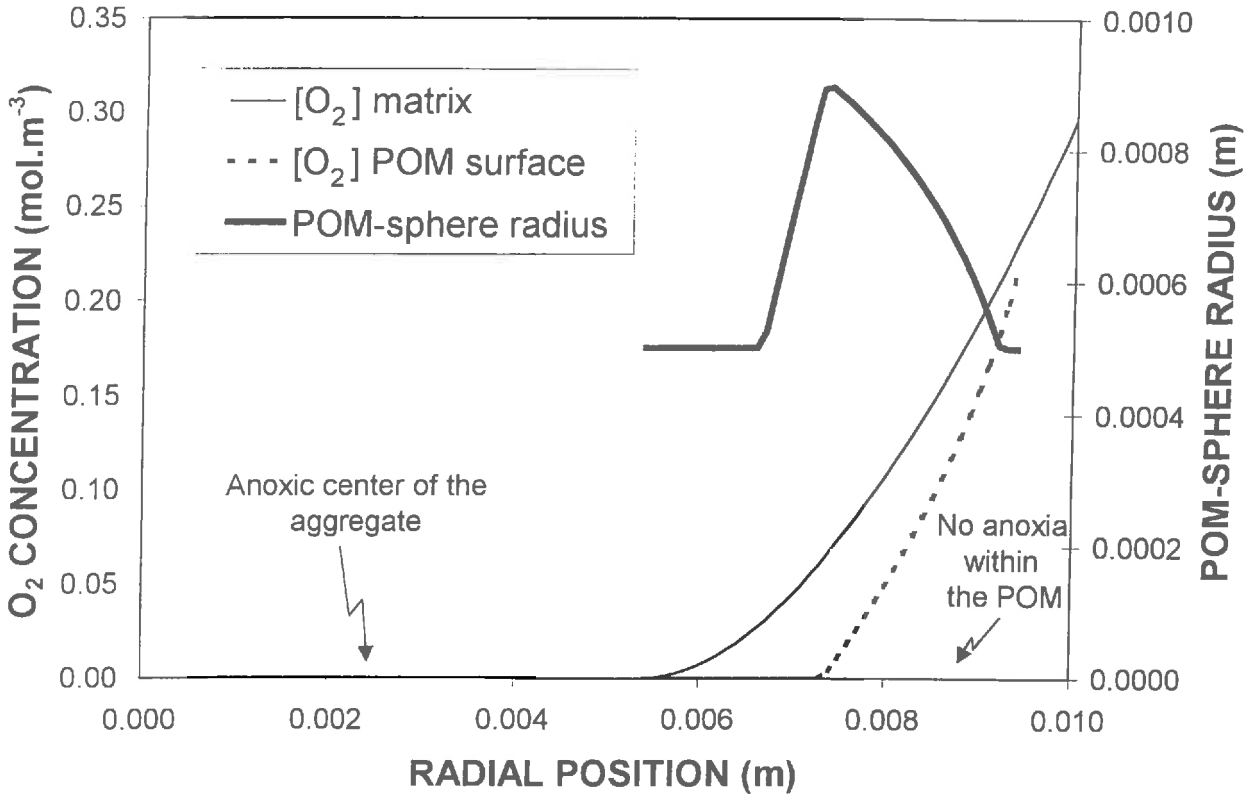

0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
O_2 CONCENTRATION (mol.m^{-3})
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
POM-SPHERE RADIUS (m)
[O_2] matrix
[O_2] POM surface
POM-sphere radius
Anoxic center of the aggregate
No anoxia within the POM
0.000
0.002
0.004
0.006
0.008
0.010
RADIAL POSITION (m)

Figure 6