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Katell Quénéa

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Présentée par

Katell QUENEA

Pour obtenir le grade de
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# ETUDE STRUCTURALE ET DYNAMIQUE DES FRACTIONS LIPIDIQUES ET ORGANIQUES REFRACTAIRES DE SOLS D'UNE CHRONOSEQUENCE FORET/MAÏS (CESTAS, SUD OUEST DE LA FRANCE)

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# INTRODUCTION GENERALE

Depuis la mise en évidence de l'augmentation du $CO_2$ atmosphérique et son implication dans l'effet de serre, la connaissance du cycle global du carbone est plus que jamais nécessaire. En effet, elle permet une meilleure modélisation du cycle du carbone et ainsi de prévoir les variations du $CO_2$ atmosphérique au cours du temps. Alors que l'évolution des principaux stocks de carbone (ceux compris dans les sédiments, les combustibles fossiles et les océans) est principalement soumise à des processus physico-chimiques, la majorité de la matière organique contenue dans la biosphère (et localisée dans les sols) est soumise à d'importants facteurs biologiques. De ce fait, c'est un compartiment bien plus réactif aux variations naturelles et anthropiques. Cependant, la matière organique est très hétérogène, aussi afin de prédire son évolution dans les sols, il est indispensable d'approfondir l'étude de sa composition mais aussi de sa dynamique. Une chronoséquence impliquant une succession de plantes de métabolismes différents ($C_3$/$C_4$) constitue un site privilégié pour suivre la dynamique de cette matière organique grâce au marquage isotopique naturel du $^{13}C$.

La dynamique de la matière organique des sols dépend aussi bien de sa composition, que des facteurs extérieurs. En effet, des facteurs environnementaux et anthropiques peuvent jouer un rôle important. Ainsi, la mise en culture d'un sol conduit à une perte importante du carbone organique total de ce sol. De plus, des pools cinétiques présentant des taux de renouvellement très différents ont été identifiés dans la matière organique des sols. Un de ces pools, présentant un temps de renouvellement de l'ordre du millénaire, est considéré comme stable. La caractérisation et la quantification de ce pool peu sensible aux influences extérieures s'avèrent désormais essentielles. La stabilité de la matière organique peut être due à une protection physique mais aussi à la composition chimique intrinsèque de celle-ci. En effet, dans des sédiments, des macromolécules extrêmement résistantes à la biodégradation, isolées par des hydrolyses drastiques et présentant une structure spécifique, ont été mises en évidence. Ces résultats ont permis d'envisager un lien entre la résistance chimique et la résistance à la biodégradation. Des molécules de structure comparable ont aussi été identifiées dans des parois d'algues, des parties de plantes (cuticule et racine) et des sols. Il paraît donc intéressant d'isoler la matière organique chimiquement réfractaire des sols, de la caractériser et de vérifier sa stabilité vis-à-vis d'une perturbation environnementale.

D'autre part, les lipides des sols, bien que relativement peu abondants, sont des constituants considérés comme relativement résistants. Ils jouent un rôle important dans les caractéristiques du sol (stabilité, capacité de rétention de l'eau) et interagissent avec de nombreux composés xénobiotiques. De plus, de par leur relativement bonne spécificité, les lipides peuvent servir de biomarqueurs et fournissent donc des informations sur leur diagenèse dans les sols. L'examen de ces composés peut ainsi fournir de nombreuses informations, tant sur leur origine que sur leur devenir (ainsi que celui des composés xénobiotiques avec lesquels ils sont associés) dans les sols.

Dans ce contexte, nous avons étudié la fraction chimiquement réfractaire et la fraction lipidique d'une chronoséquence forêt/maïs d'un podzol. Les caractéristiques de ce sol (sol sableux et acide), impliquant une faible protection physique de la matière organique ainsi qu'une faible activité microbienne, permettent d'envisager la préservation de la matière organique dans des conditions particulières. De plus, la succession d'une plante en $C_4$ à une plante en $C_3$ permet d'accéder à la dynamique de la matière organique et permet d'estimer l'influence de la mise en culture aussi bien sur la matière organique totale, que sur les lipides et sur la matière organique réfractaire.

Les objectifs de ce travail étaient la caractérisation de la fraction lipidique et de la fraction réfractaire aux hydrolyses de la matière organique d'un sol forestier des Landes et d'une parcelle cultivée depuis plusieurs années, ainsi que l'estimation de la dynamique de ces fractions suite à la mise en culture du site. Ainsi ce travail s'articule en trois parties :

- La partie A présente le cadre général de l'étude par des rappels bibliographiques sur la matière organique des sols : sa composition, son devenir dans le sol, ses principaux rôles et notamment son implication dans le cycle global du carbone.
- La partie B concerne le sol forestier et est subdivisée en trois chapitres. Le premier chapitre porte sur les lipides extraits de trois fractions granulométriques. Dans le second, nous avons caractérisé la matière organique réfractaire à des hydrolyses successives par des méthodes spectroscopiques (IRTF, RMN $^{13}C$ à l'état solide) et pyrolytiques (pyrolyse au point de Curie et pyrolyse en présence d'un agent alkylant). Dans le troisième chapitre, la structure de cette matière organique réfractaire a été examinée par des dégradations chimiques puis observée en microscopie électronique à transmission haute résolution. La combinaison des ces différentes méthodes d'analyse, rendue nécessaire par la grande inertie chimique de la matière organique réfractaire, permet d'obtenir de précieux renseignements sur sa structure.

- La partie C considère l'influence de la mise en culture du sol et la dynamique de la matière organique. Dans un premier chapitre, nous avons déterminé la composition et la distribution des lipides du sol sous culture puis nous les avons comparées à celles du sol forestier. Puis la dynamique de la matière organique, des lipides et des alcanes a été estimée. L'impact de la mise en culture sur la matière organique réfractaire a été estimé dans le deuxième chapitre. Dans le dernier chapitre, nous avons isolé et quantifié la matière organique des différentes fractions granulométriques des deux sols ainsi que les carbonisats qui avaient été préalablement identifiés dans la matière organique.

## VALORISATION DES RESULTATS DU TRAVAIL

Les résultats obtenus lors de ce travail ont conduit à la rédaction de plusieurs articles parus ou à paraître dans des revues internationales. Ainsi les différents chapitres sont basés sur les articles suivants :

- Partie B, chapitre 1 : *« Variation in lipid abundance and composition among different particle-size fractions of a forest soil »,* Organic Geochemistry, sous presse
- Partie B, chapitre 2 : *« Abundance, spectroscopic and pyrolytic studies of the macromolecular refractory fraction in a sandy forest soil (Landes de Gascogne, France) »,* Organic Geochemistry, accepté
- Partie B, chapitre 3 : *« Structural study of the macromolecular refractory fraction in a sandy acid forest soil (Landes de Gascogne, France) by chemical degradations»,* Organic Geochemistry, soumis
- Partie C, chapitre 1 : *« Molecular and isotopic study of lipids in particle-size fractions of a sandy cultivated soil (Cestas cultivation sequence, southwest France) : sources, degradation, comparison with the Cestas forest soil »,* pour soumission à Soil Bioloby and Biochemistry
- Partie C, chapitre 2 : article en préparation.

Les parties « résultats et discussions » de chaque article ont été incluses dans les chapitres correspondants.

# PARTIE A :

# CADRE GENERAL DE L'ETUDE

# I. LE SOL

## 1.1 Définition

Le sol est la partie supérieure de la croûte terrestre. Il est l'interface entre l'atmosphère, l'hydrosphère, la lithosphère et les organismes vivants. Il est composé d'une fraction minérale et d'une fraction organique : la pédogenèse résulte de l'altération d'un substrat minéral par des phénomènes physiques, chimiques et biologiques (figure 1). Au cours de cette transformation, le substrat minéral est colonisé par des organismes qui composent peu à peu la matière organique. Cette matière organique s'associera ainsi au minéral pour former entre autres des complexes organo-minéraux.

Le sol a de nombreuses fonctions. Il est un milieu biologique dans/et sur lequel se développent des êtres vivants. Ce développement va dépendre de la qualité de ce sol ou fertilité (quantité de carbone, d'azote, capacité d'échange cationique, etc…). Il est aussi un acteur déterminant du cycle de l'eau (stockage et régulation) et de la qualité de cette eau (source de pollution, capacité de rétention des polluants mais aussi biodégradation de ceux-ci). Mais le sol joue aussi un rôle prédominant dans les cycles biogéochimiques.

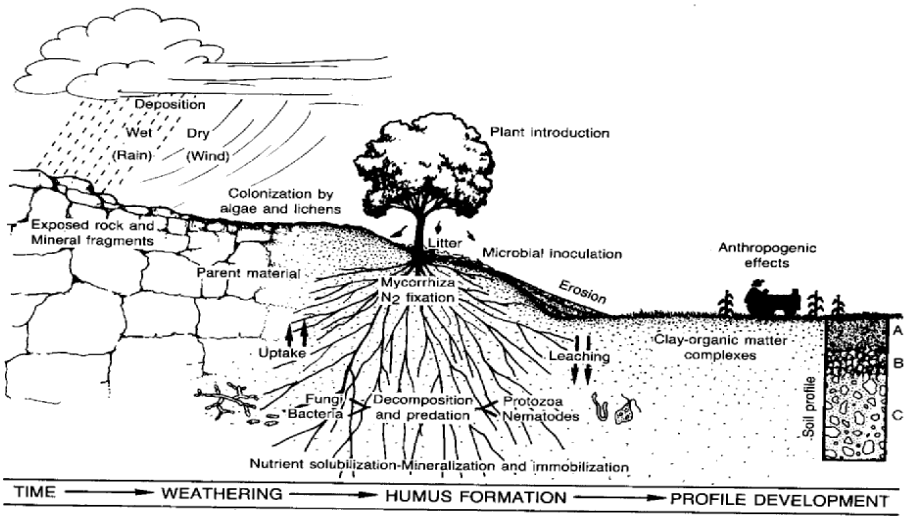



Figure 1 : Relation entre organismes, matière organique et roche mère dans le développement du sol (d'après Paul and Clark, 1996)

## 1.2 Fraction minérale

Les minéraux constituent, en général, de 95 à 99% du sol. La composition minérale dépend de la nature de la roche-mère. La nature des minéraux peut être extrêmement diverse. Ces éléments minéraux peuvent avoir différentes tailles granulométriques :

- Sable (Ø=2000 à 50µm)
- Limon (Ø=50 à 2µm)
- Argile granulométrique (Ø <2µm)

La définition de l' « argile granulométrique » ne reposant que sur la taille des particules, celle-ci ne comprend pas uniquement de l'argile minéralogique mais aussi tous les minéraux de diamètre inférieur à 2µm.

Selon les proportions de ces trois fractions granulométriques, la texture du sol peut être qualifiée de sableuse à argileuse. La capacité du sol à remplir ses fonctions dépend de la nature de la roche-mère et de la texture.

## 1.3 Fraction organique

La fraction organique est constituée à plus de 80% de matière organique (MO) morte (tissus végétaux, résidus d'organismes). La MO morte subit de nombreuses transformations dans le sol : elle est fragmentée, altérée chimiquement mais aussi biologiquement car, au cours de la minéralisation, elle sert de source d'énergie pour les organismes vivants du sol. Certaines molécules non minéralisées vont subir une humification, c'est-à-dire une réorganisation en molécules plus ou moins complexes qui constituent les substances humiques.

Dans la fraction organique, on trouve aussi des organismes vivants : des bactéries, les actinomycètes, les champignons mais aussi des racines ou encore la faune (protozoaires, nématodes, certains insectes, vers de terre). Bactéries et champignons sont les principaux responsables de la minéralisation des MO mais ils participent aussi à l'humification notamment par l'excrétion d'enzymes dans le sol ainsi qu'à la formation des complexes organo-minéraux.

# II. LA MATIERE ORGANIQUE DU SOL

## 2.1 Définition

Par le terme « MO des sols (MOS) » on entend l'ensemble des composés organiques qui sont issus de résidus d'organismes à différents stades de décomposition, synthétisés par les organismes vivants ou qui sont des produits de dégradation. Les quantités de MOS et de carbone organique sont corrélées. Il est arbitrairement admis que la MOS est le double du carbone organique dans un sol non cultivé et que dans un sol cultivé, elle est égale à 1.73 fois la teneur en carbone organique (Duchaufour, 2001). La majorité du carbone organique du sol provient des plantes vasculaires (racines et feuillages) (Oades, 1993; Kögel-Knabner, 2002).

## 2.2 Analyses de la MOS

La MO des sols a été abondamment étudiée mais du fait de son hétérogénéité et de sa complexité, sa structure n'est toujours pas entièrement élucidée. Pour ces mêmes raisons, et du fait que chaque méthode peut induire un biais dans les résultats (tels que la production de réactions secondaires ou la sur(sous)estimation de l'abondance de certains composés), Kögel-Knabner (2000) a souligné l'importance de combiner les différentes méthodes analytiques. En effet, les techniques analytiques utilisées pour étudier la MOS sont très diverses. Afin de caractériser la MOS, les méthodes les plus fréquemment employées ont été les techniques spectroscopiques (résonance magnétique nucléaire (RMN) $^{13}C$, l'infra rouge à transformée de Fourier (IRTF)), les couplages chromatographie gazeuse-spectrométrie de masse (CG-SM) après dégradation thermiques (pyrolyses) ou chimiques (oxydations).

La RMN du carbone et de l'azote à l'état solide des sols ou les fractions du sol, et l'IRTF sont des méthodes non destructives qui permettent d'obtenir des informations structurales sur la MOS (Preston et al., 1987; Kögel-Knabner, 1997). Baldock et al. (1997) ont aussi utilisé la RMN $^{13}C$ afin d'estimer le degré de dégradation de la MOS d'un même sol

en utilisant un rapport C-alkyle/O-alkyle. En effet, au cours de la dégradation, la proportion de groupements C-alkyles augmente par rapport aux groupements O-alkyles.

La pyrolyse couplée à la CG-SM permet d'avoir des indications sur la composition moléculaire de macromolécules que ce soit sur les substances humiques (Saiz-Jimenez and De Leeuw, 1985, 1987; Saiz-Jimenez, 1994; Fabbri et al., 1996), la MO totale (Hempfling et Schulten, 1990; Leinweber et Schulten, 1995), la fraction lignine (Saiz-Jimenez et De Leeuw, 1986). Cette méthode pyrolytique est aussi fréquemment associée à une méthylation in situ par ajout d'un agent méthylant (le plus fréquemment utilisé étant l'hydroxyde de tetramethylammonium (TMAH)) qui permet notamment la méthylation des acides et la coupure de liaisons éthers et esters de la macromolécule (Fabbri et al., 1996; Del Rio et Hatcher, 1998; Challinor, 2001). Cette chemopyrolyse a permis de révéler la présence de macromolécules telles que la cutine et la subérine dans des sols (Nierop et Verstraten, 2003) et souligne la contribution de la lignine (Clifford et al., 1995; Hatcher et al., 1995).

L'utilisation d'oxydants plus ou moins agressifs, ou de réactifs chimiques agissant spécifiquement sur certaines liaisons, ainsi que l'identification des produits de dégradation par CG-SM, ont aussi permis de mieux caractériser la structure de la MOS. Les réactifs employés sont très nombreux (Griffith et Schnitzer, 1989) : le permanganate de potassium (Almendros et Gonzalez-vila, 1987; Almendros et al., 1989), le persulfate de sodium (Martin et al., 1981; Cuypers et al., 2002), le perborate de sodium (Almendros et Sanz, 1992), le tetroxide de ruthénium (Gonzalez-vila et al., 1994), l'oxyde de cuivre (Hatcher et al., 1995; Sanger et al., 1997), etc...

D'autres méthodes d'études ont aussi été employées telles que la microscopie électronique (Boulmier et al., 1982) afin d'examiner la morphologie, le marquage isotopique naturel ($^{14}C$ ou $^{13}C$) (Balesdent et Balabane, 1992; Guillet, 1994; Monreal et al., 1997) pour suivre la cinétique de la MOS.

## 2.3 Composition de la MOS

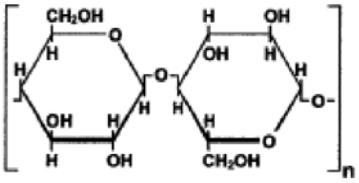
Au sein de la MOS, on distingue généralement les substances non-humiques et les substances humiques. Les substances non-humiques sont des composés aux caractéristiques chimiques reconnaissables dont la majorité provient des résidus de constituants organiques végétaux ou animaux ou de molécules excrétées par ces organismes dans le milieu. Ce sont les constituants de ces résidus organiques qui forment la MO du sol. Ainsi, on peut distinguer:

des carbohydrates dont principalement la cellulose et l'hemicellulose qui sont les constituants les plus abondants des plantes, la lignine, les composants azotés, les lipides, les tannins, la cutine, la subérine, etc...Des résidus de carbonisation de végétaux ou carbonisats ont été également observés dans les sols. Ces principaux constituants seront brièvement présentés.

## 2.3.1. Les substances non-humiques

### 2.3.1.1 Carbohydrates

La cellulose est le constituant principal des parois cellulaires des plantes. Elle est composée d'unités de glucose liées par des liaisons glucosidiques β-(1-4) (fig 2). L'hemicellulose est un polymère hautement ramifié constitué d'hexoses et de pentoses et parfois d'acide uronique**.** Les arbres à feuilles caduques contiennent ¾ de pentose et ¼ d'hexose. Ce rapport est inversé dans les conifères.




Cellulose

Figure 2 : Unité de base de la cellulose avec liaison β-(1-4)

### 2.3.1.2 Les composés azotés

Les protéines sont les composés azotés les plus abondants. Ce sont des polymères constitués par la formation de liaisons peptidiques entre les 20 monomères, les acides aminés.

### 2.3.1.3 Les tannins

Les tannins constituent un mécanisme de défense des plantes contre les pathogènes, les herbivores et des conditions environnementales hostiles. On distingue dans les tannins : les tannins hydrolysables et les tannins non-hydrolysables ou condensés (proanthocyanidine et phlorotannins). Les phlorotannins sont basés sur des unités de phloroglucinol liées par des liaisons éthers (Fig 3a). Les tannins condensés, polymères de proanthocyanidine, sont des

polyphénols d'unités polyhydroxy-flavan-3-ols liés par des liaisons C-C, C-4, C-8 (Fig 3b). Les tannins hydrolysables ont deux unités de base, un sucre estérifié soit par l'acide gallique (gallotannins) soit par l'acide ellagique (ellagitannins) (Fig 3c).

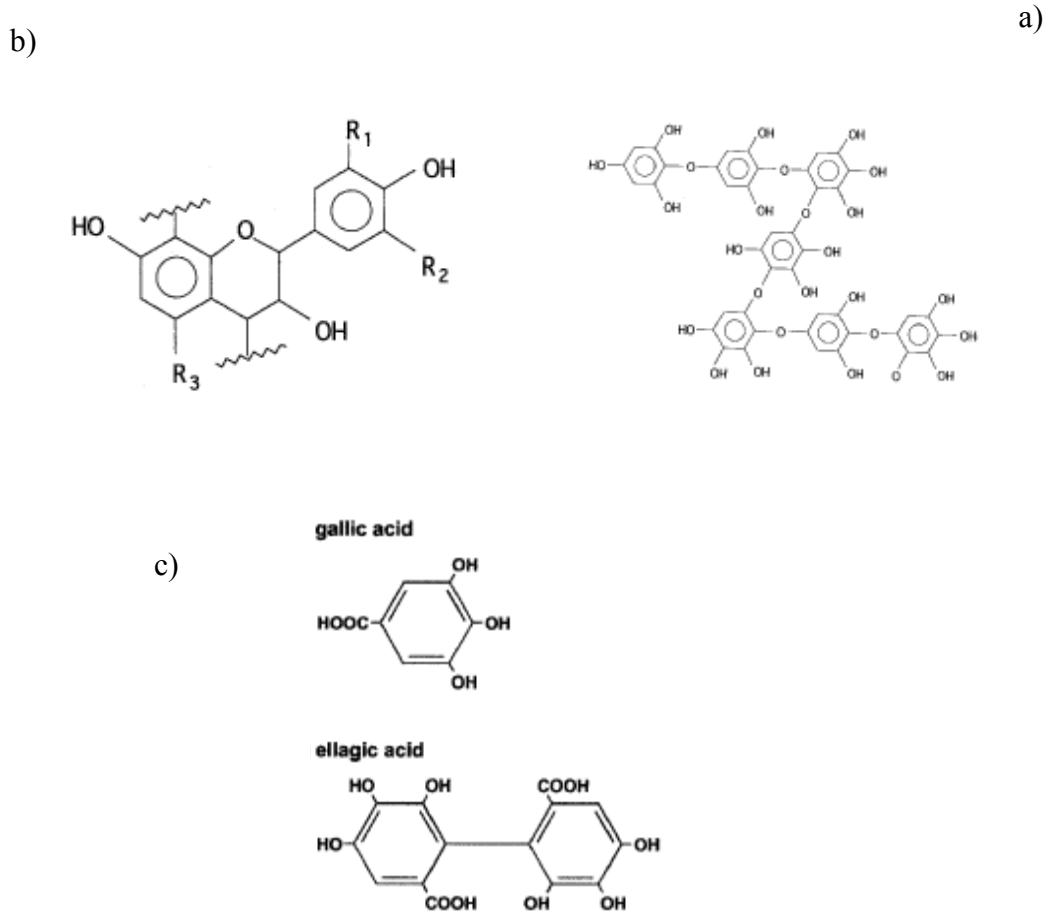



Figure 3 : unités de base de tannins a) phlorotannins, b) proanthocyanidine, c) tannins hydrolysables

2.3.1.4 Les lipides

Les lipides sont les composés solubles dans les solvants organiques (Dinel et Schnitzer, 1990). Ce terme regroupe des composés très divers qui comprennent des glycérides, des cires, des phospholipides, des stéroïdes, des terpénoïdes et des caroténoïdes (Stevenson, 1966; Morrison, 1969) regroupant ainsi de nombreuses fonctions chimiques tels que les alcanes, les alcènes, des acides gras, des alcools, les diacides, les stérols, etc…(Fig 4).

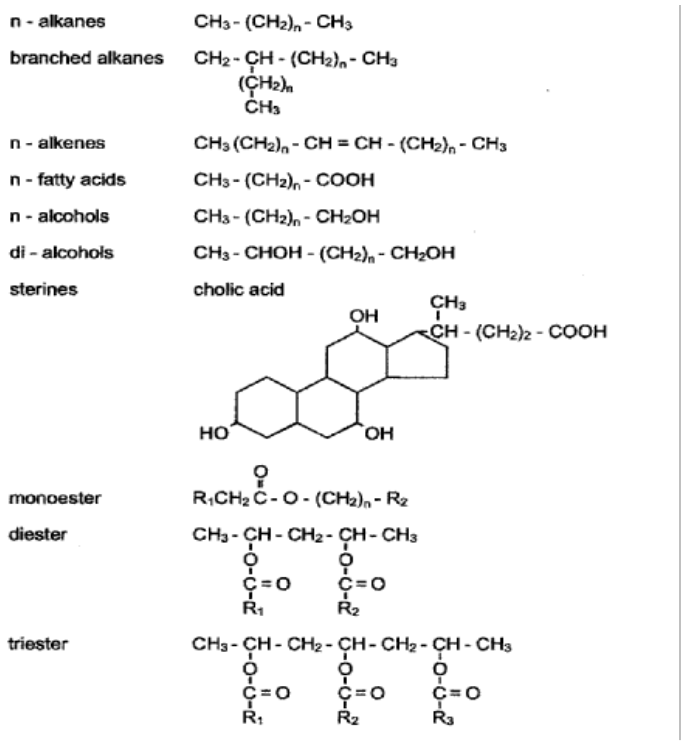



Figure 4 : Composants majeurs des lipides de plantes et de microorganismes d'après Dinel et Schnitzer (1990)

Les lipides peuvent avoir des conséquences sur les propriétés physiques du sol (effet positif sur l'agrégation du sol et sa stabilité mais effet négatif sur la rétention de l'eau) d'autant plus que les lipides sont considérés comme relativement résistants à la biodégradation.

On distingue dans les lipides ceux libres, directement extraits par des solvants organiques et les lipides liés. Les lipides peuvent être liés dans le sol soit par des liaisons esters, et sont alors obtenus par hydrolyse basique, soit par des liaisons amides ou éthers, ils sont alors extraits par hydrolyse acide.

#### 2.3.1.5 La cutine et la subérine

La cutine et la subérine sont des polyesters qui, associés aux cires, forment une enveloppe protectrice des plantes. Elles forment ainsi une barrière imperméable entre la plante et son environnement grâce à laquelle la diffusion des gaz peut être contrôlée. Ces

macromolécules sont constituées de monomères, en majorité des acides et des hydroxy acides, liés par des liaisons esters (Kolattukudy, 1980; Matzke et Riederer, 1991). Un modèle pour ces polymères a été proposé par Kolattukudy (1980) (Fig 5).

- La cutine constitue avec les cires la cuticule qui protège les parties aériennes des plantes supérieures (Fig 6). Elle est composée de deux familles d'acides carboxyliques, d'hydroxy et d'epoxy acides, une famille en $C_{16}$ et une famille en $C_{18}$. Le composé en $C_{16}$ majoritaire est un dihydroxy acide en $C_{16}$ et ceux en $C_{18}$ sont l'acide, l'ω-hydroxy acide, ω-hydroxy-9,10-epoxy acide, le 9,10,18-trihydroxy acide et leurs analogues insaturés.
- La subérine est localisée dans les parties souterraines des plantes ainsi que dans l'écorce et les tissus cicatriciels. Les principaux monomères de la subérine sont des acides (de $C_{20}$ à $C_{32}$), des n-alcools (de $C_{20}$ à $C_{32}$), des ω-hydroxy acides (de $C_{16}$ à $C_{24}$) et des α,ω-diacides (de $C_{16}$ à $C_{24}$). De plus, la subérine contient des acides phénoliques tels que l'acide coumarique et ferulique (Kolattukudy, 1980; Bernards et Lewis, 1998).

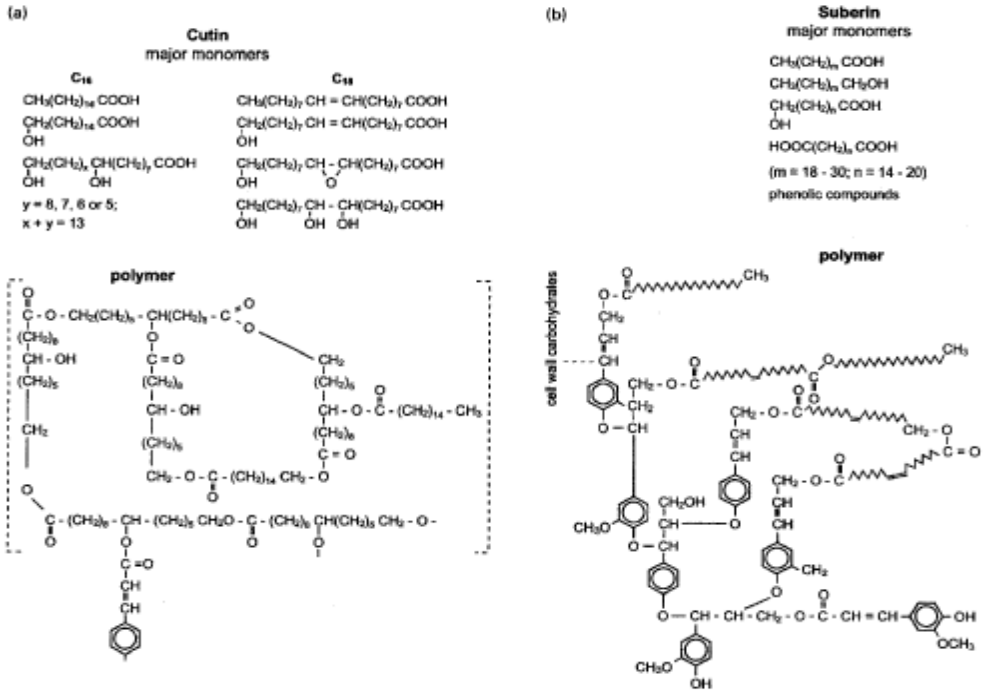



Figure 5 : Monomères et structure de a) la cutine et de b) la subérine d'après Kolattukudy (1980)

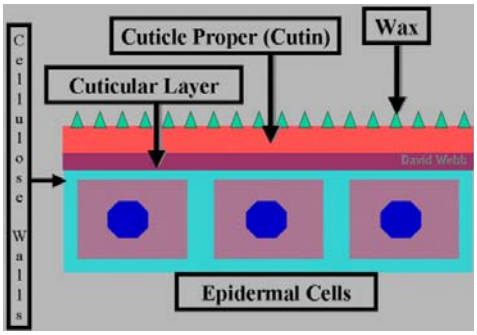



Figure 6 : Représentation schématique de cuticule

2.3.1.6 Lignine

La lignine est une macromolécule de haut poids moléculaire qui compose une partie de la paroi des plantes ligneuses, des fougères et de certaines mousses et permet leur rigidité. La structure de la lignine est polyaromatique, avec trois types d'unités de base qui comprennent un noyau aromatique et une chaîne latérale en C3. Les trois principaux précurseurs de la lignine sont les alcools synapyl, coniferyl et p-coumaryl (Fig 7).

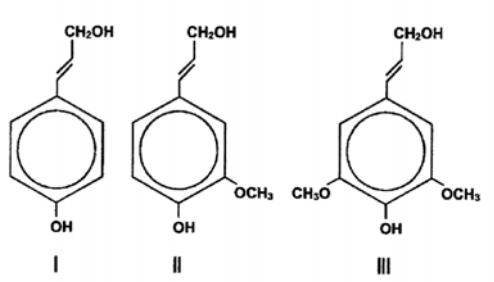



Figure 7 : Précurseurs de la lignine I) alcool coumaryl, II) alcool coniferyl, III) alcool synapyl

Ils sont liés par des liaisons arylglycerol-β-arylether (β-O-4) ou biphenyl (5-5) ou phenylcoumaran (β-5) (fig 8). La lignine des gymnospermes, angiospermes et herbacées est classée selon les proportions de ces trois précurseurs. La lignine des gymnospermes est composée presque exclusivement de monomères propane guaiacyl derivés de l'alcool coniféryl, la lignine des angiospermes contient autant de monomères derivés propane guaiacyl que de monomères propane syringyl dérivé de l'alcool syringique (Hedges et Mann, 1979). Dans les herbacées, les trois monomères sont en proportions égales. Le rapport

acides/aldehydes des dérivés des unités vanillyl et syringyl obtenus après oxydation à l'oxyde cuivrique ou par pyrolyse en présence de TMAH est également souvent utilisé comme indication du degré de dégradation de la lignine (Hedges et al., 1988): ce rapport diminue au cours de la dégradation.

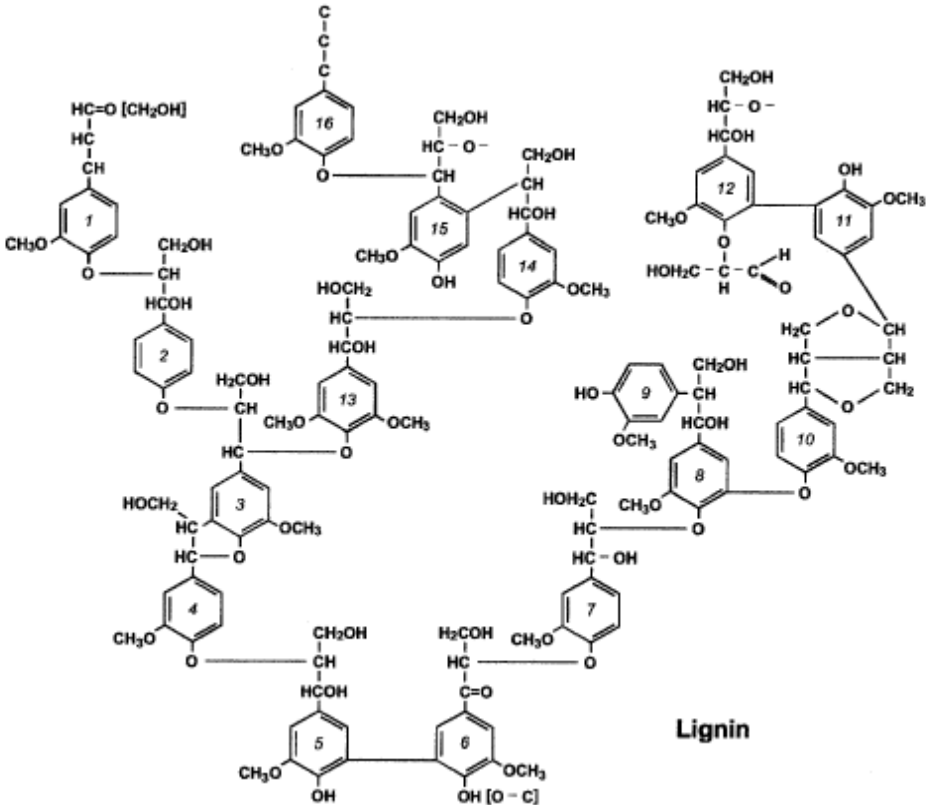



Figure 8 : Modèle de structure de la lignine

2.3.1.7 Les carbonisats

La définition des carbonisats ou noirs de carbone proposée par Goldberg (1985) est la suivante : « Le noir de carbone est une forme impure d'un élément produit par la combustion incomplète de combustibles fossiles ou de biomasse. Elle contient plus de 60% de carbone avec comme éléments accessoires majeurs de l'hydrogène, de l'oxygène, de l'azote et du soufre ». Les carbonisats sont produits par tous les processus de combustion incomplète. Ils forment un continuum allant de plantes partiellement carbonisées, à la suie et aux particules de graphite en passant par le charbon (Seiler et Crutzen, 1980).

Les trois principales sources de carbonisats sont la combustion d'huile, de charbon et de biomasse. Dès 1979, Griffin (1979) a observé une différence dans les caractéristiques

morphologiques (forme, taille) pouvant permettre de déterminer l'origine de ces carbonisats. D'après cet auteur, les particules issues de la combustion d'huile ont une structure convolutée en couche alors que les particules provenant de combustion de bois ou de charbon végétal ont une structure plus homogène avec parfois des structures reconnaissables de végétaux.

Actuellement, on distingue généralement différents types de carbonisats selon leur structure morphologique. En effet, les petites particules sphériques (diamètre entre 50 et 10 nm) avec une microstructure en « oignon » (fig 9) sont classiquement formées par combustion de matériaux peu oxygénés comme les cires de plantes ou les combustibles fossiles alors que les unités structurales de base polyaromatiques faiblement orientées pourraient provenir de l'aromatisation de précurseurs ligno-cellulosique par un processus partiel de carbonisation (Oberlin et al., 1980). On peut supposer que ces deux types de noir de carbone ont des propriétés physiques et chimiques différentes.

Figure 9 : Structure schématique de carbonisats issus de suie (« structure en oignon »)

Les carbonisats sont formés par l'empilement de couches d'unités de base polyaromatiques qui présentent de grandes différences dans l'étendue et le niveau d'organisation de la structure. Ce sont des composés polyaromatiques fortement condensés présentant un rapport H/C faible. Ceci a conduit Kuhlbusch (1995) à définir les carbonisats comme le résidu après un traitement à 340°C dans de l'oxygène pur dont le rapport H/C est inférieur à 0.2.

### 2.3.2. Les substances humiques

Les substances humiques sont le résultat de l'humification. Celle-ci résulte soit de la néoformation de composés humiques des composés organiques du sol, soit de l'héritage et de la transformation des molécules du sol. Généralement on distingue dans ces substances humiques : les acides humiques (AH), les acides fulviques (AF) et l'humine. Ceux-ci sont extraits du sol grâce à leur différence de solubilité en milieu basique et acide. En effet, seuls les acides humiques et fulviques sont solubles en milieu basique, l'humine peut ainsi être

isolée aisément. En milieu acide, les acides humiques précipitent contrairement aux acides fulviques (schéma 1). Cette définition est strictement opérationnelle et n'a pas de réalité biologique.

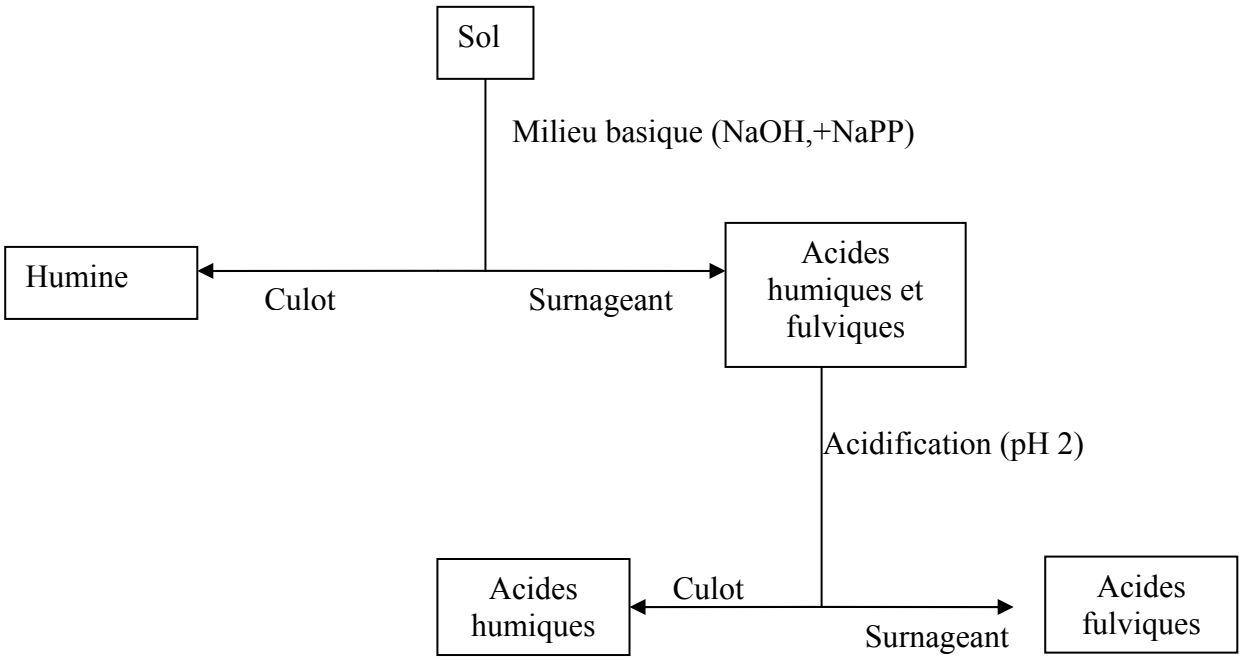



Schéma 1 : Protocole d'isolement des substances humiques

Il est généralement considéré que les composés humiques ont des structures analogues et sont formés de noyaux aromatiques oxydés, essentiellement des acides benzène carboxyliques et des acides phénoliques, liés par des chaînes aliphatiques et des groupements acides et alcools. Le degré de polymérisation varie au sein des composés humiques, il est plus élevé dans l'humine. Les acides fulviques ont le poids moléculaire le plus faible (1000 à 30000 Da), alors que les acides humiques ont un poids moléculaire compris entre 10000 et 100000 Da. Ces acides comportent de nombreux groupes fonctionnels carboxyliques, phénols, alcools. Il est couramment admis que les acides fulviques possèdent davantage de groupements carboxyliques et phénols (Schulten et Schnitzer, 1997).

Schnitzer et Schulten (1992) ont démontré par Py-FIMS que les principaux composants des acides humiques étaient les carbohydrates, phénols, monomères et dimères de lignine, alcanes, alcènes, acides gras et n-alkyl esters. Une étude par Py-CG-SM a révélé que les acides humiques produisent par pyrolyse essentiellement des alkylbenzenes (Schulten et Schnitzer, 1992) auxquels s'ajoutent n-alcanes, n-alcènes, phénols, methylphénols, alkyl et

methoxyphenols, furans, indènes, pyrroles et pyridine et donc que les AH sont principalement formés de noyaux aromatiques liés à des chaînes aliphatiques.

Puis, en combinant les résultats obtenus par ces deux méthodes, Schulten et Schnitzer (1997) ont proposé un modèle de structure en trois dimensions des acides humiques basé sur un réseau formé de noyaux aromatiques (benzène, phénols, methoxyphénols, furans, pyrroles et pyridine) et de chaînes aliphatiques (alcanes, alcènes, acides, esters) comportant ainsi de nombreuses fonctions acides, alcools, esters. Ce modèle d'acides humiques est basé sur un squelette carboné ménageant des vides qui peuvent piéger ou lier d'autres composants organiques tels que des sucres, des protéines, des lipides ou des éléments inorganiques (Fig 10). Le modèle proposé montre l'importance de la MOS pour l'interaction avec des molécules aussi bien hydrophiles, au niveau des fonctions oxygénées, qu'hydrophobes, au niveau des composants aromatiques et aliphatiques. Ainsi de nombreux auteurs ont mis en évidence le piégeage de composés : encapsulation de carbohydrates (Schulten et Schnitzer, 1997), lipides (Schnitzer et Neyroud, 1975; Grasset et Amblès, 1998; Grasset et al., 2002), de protéines (Zang et al., 2000) et de polluants (Richnow et al., 1997).

D'après Schulten et Schnitzer (1997), l'humine et les AH sont les même substances mais l'humine n'est pas extraite en milieu basique du fait de sa complexation par les argiles et les oxydes.

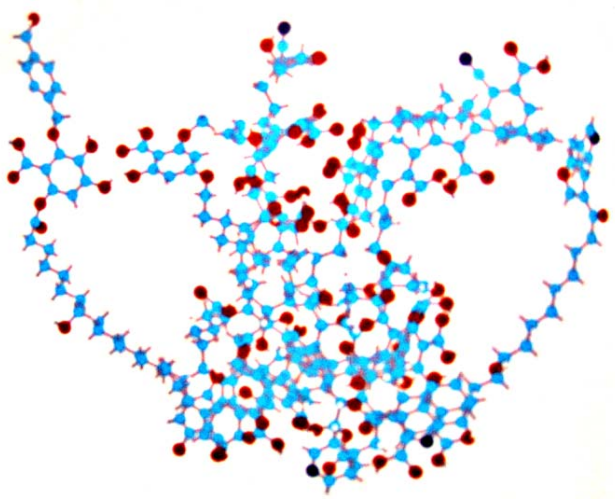


Figure 10 : Modèle d'acides humiques selon Schulten et Schnitzer (1997)

Cependant, une autre théorie a été émise par Piccolo et al. (1999). Ils évoquent la possibilité, pour les substances humiques, d'être constituées d'unités de petites tailles formant des agrégats de grande taille en solution aqueuse, maintenus ensemble par des liaisons hydrophobes et hydrogènes et présentant des propriétés similaires à celle des macromolécules.

## 2.4 Evolution de la MO dans le sol

Le devenir de la MO dans le sol peut être très différent. Une fois incorporée au sol, cette MO peut suivre trois voies : la minéralisation, l'humification ou la stabilisation.

### 2.4.1 Minéralisation

La minéralisation est l'assimilation par les organismes du sol de la matière organique comme source d'énergie et comme élément pour leur métabolisme au cours de laquelle la MO est transformée en composés minéraux ($CO_2$, $N_2$, etc…). La vitesse de minéralisation est très fortement dépendante de la nature de la MOS d'une part et des facteurs environnementaux (Davidson et al., 1995) tels que l'aération du sol (fonction pour partie de la quantité d'eau dans le sol), la teneur en nutriments, de la température, du pH, de l'accessibilité aux organismes de la MO, la teneur en eau d'autre part. Ainsi les interactions du sol avec la matrice minérale et la formation de complexes organo-minéraux sont très importantes. La nature de la MO elle-même joue un rôle car tous les composants de la MO ne sont pas dégradés de manière identique, différents organismes peuvent intervenir. La lignine par exemple n'est dégradée totalement que par certains champignons appelés « pourriture blanche ». La biodégradabilité des composants de la MO diffère : alors que les carbohydrates et les composés azotés sont minéralisés rapidement (80% de la cellulose est dégradée en un an (Sorensen, 1987)**,** les composés humiques, la lignine, les cires, les lipides, la cutine, la subérine, cutane, subérane, les carbonisats et les tannins condensés semblent présenter une résistance intrinsèque plus ou moins grande liée à leur structure chimique et qui réduit leur biodégradabilité.

### 2.4.2 Humification

L'humification résulte soit de la néoformation de composés humiques par insolubilisation des composés organiques du sol, soit de l'héritage et de la transformation des molécules du sol.

Le mécanisme de formation des substances humiques dans les sédiments est vu comme un mécanisme de dégradation-recondensation. De manière similaire, le processus de formation des substances humiques dans les sols décrit par Andreux et Munier-Lamy (1994) comporte trois mécanismes : -Les mécanismes lytiques (lyse de parois cellulaires, protéolyse et ligninilolyse, etc…) qui conduisent à la décomposition de macromolécules en molécules de poids moléculaire plus faible

- Brunissement et mélanisation par interactions chimiques et biochimiques des oligomères peptidiques, glucidiques ou phénoliques comme notamment la condensation de sucres et d'acides aminés conduisant à la formation de mélanoïdines (Maillard, 1917) et la polymerisation spontanée des semi-quinones issues de l'oxydation des composés dihydroxylés de la lignine et de pigments cellulaires qui constituent la principale voie d'incorporation de l'azote.

- Polycondensation des précurseurs ainsi formés par création de liaisons C-C, C-O ou C-N.

D'autre part, certains auteurs ont avancé la possibilité de l'incorporation de molécules plus résistantes à la biodégradation à ces composés néoformés. Ainsi Nip et al. (1986) et De Leeuw et Largeau (1993) ont mis en évidence la présence de macromolécules résistantes telles que les cutanes ou les subéranes conduisant à la formation de longues chaînes polyméthyléniques.

Oades (1988) a résumé les principaux facteurs contrôlant le principe d'humification : la quantité de litière apportée au sol, la proportion des différentes parties de plante et leur distribution aérienne ou souterraine, la proportion relative des différents tissus de plante et leur composition chimique.

### 2.4.3 Stabilisation

La MO peut être stabilisée dans le sol du fait de sa composition chimique comme nous l'avons vu précédemment, mais aussi par les conditions environnementales (pH, teneur en eau, teneur en $O_2$) ou par les interactions avec les minéraux ou les oxydes du sol qui les rendent inaccessibles aux organismes minéralisateurs. La MO peut se trouver localisée dans des agrégats du sol ou adsorbée sur les minéraux. La structure physique du sol est ainsi à l'origine de l'occlusion et donc de la protection de la MOS. Celle-ci se trouve protégée contre la biodégradation et préservée (Gregorich et al., 1989; Skjemstad et al., 1993; Sollins et al., 1996; Baldock et Skjemstad, 2000; Eusterhues et al., 2003). La MOS peut se complexer avec les minéraux, mais aussi avec les oxydes de fer ou d'aluminium du sol. Ceux-ci peuvent permettre une protection par complexation et donc une stabilisation de la matière organique directement par interaction (Oades, 1988; Kaiser et Guggenberger, 2000). En effet, les molécules de MO de faible poids moléculaire peuvent interagir avec l'argile qui présente une importante surface spécifique et qui porte une charge sur laquelle elles s'adsorbent (Figure 11). Ces molécules peuvent aussi se glisser entre les feuillets constitutifs de l'argile ou dans les micropores formés par les agrégats d'argiles (Theng et al., 1992; Kaiser et Guggenberger, 2003). Ces protections physiques de la MOS (occlusion et complexation) la rendent inaccessible aux micro-organismes et permettent une stabilisation de la MOS. Les interactions MOS-argile ont été largement étudiées par Christensen (1996), Wattel-Koekkoek et al. (2001). Dalal et Mayer (1986) ont par ailleurs démontré une stabilité de la MOS liée à la teneur en argile du sol. Finalement, Sollins et al. (1996) résument la stabilité comme étant l'effet intégré de la nature récalcitrante à la biodégradation, des interactions et de l'accessibilité. En effet, un autre facteur entrant en jeu dans la stabilisation de la MOS dans le sol est la composition de cette MOS (Derenne et Largeau, 2001; Gleixner et al., 2001). Certaines molécules sont plus récalcitrantes, c'est-à-dire que ces composés organiques présentent des caractéristiques moléculaires qui diminuent leur biodégradabilité par les microorganismes et les enzymes du sol. Certains auteurs ont ainsi expliqué la préservation de molécules labiles par encapsulation dans le réseau macromoléculaire de MO résistante (Zang et al., 2000).

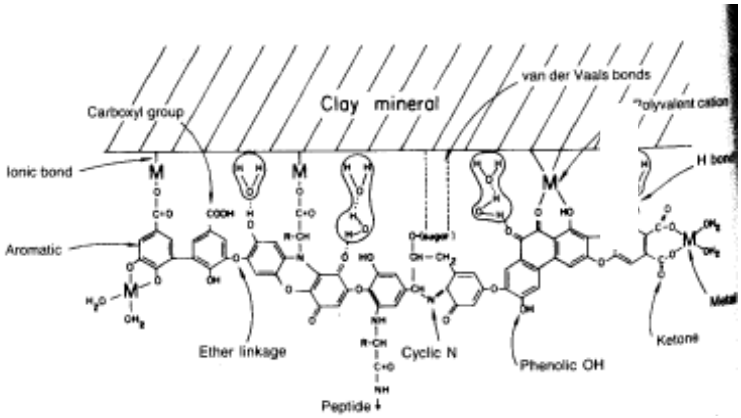



Figure 11 : Diagramme schématique du complexe argile-humate (Paul et Clark, 1996)

## III. ROLE DE LA MOS

La MOS contribue à la formation d'un environnement favorable pour les organismes se développant dans le sol. Elle est une source de nutriments, elle possède une capacité de rétention de l'eau qui permet de réguler le système hydrique du sol (Rawls et al., 2003), elle agit comme un tampon contre les variations de pH, elle contribue à la structure physique du sol (Oades, 1988) au travers de la formation de complexes organo-minéraux et à la stabilité de cette structure contre l'érosion en liant les particules de sols entre elles. Le maintien de cette structure favorise la circulation de gaz et d'eau dans le sol.

### 3.1 Nutrition

Bien que minoritaire dans le sol, la fraction organique joue un rôle très important. Tout d'abord, l'incorporation de MO fraîche dans le sol puis sa minéralisation fournissent les nutriments nécessaires à la nutrition des plantes et des organismes du sol. Elle sert aussi de ligand aux ions métalliques et évite ainsi leur lessivage hors d'atteinte du système racinaire.

### 3.2 Interaction avec les polluants

Les interactions MO-polluants sont un sujet très vaste, chaque contaminant ayant ses propres caractéristiques et donc des comportements différents vis-à-vis de la MOS.

Le pouvoir complexant de la MOS ainsi que sa nature hydrophobe sont à l'origine de la sorption des produits xénobiotiques (pesticides, HAPs, PCBs) (Kohl et Rice, 1998; Nam et Kim, 2002; Capriel et al., 1985) et des éléments traces métalliques dans les sols (Schnitzer et al., 1980 ; Ducaroir et al., 1995). Richnow et al. (1997) ont même démontré que les HAPs pouvaient établir des liaisons covalentes (éthers, esters, C-C) avec les substances humiques, probablement par un processus de couplage catalysé par une enzyme oxydative. Weber et al. (1991) se sont également particulièrement intéressé à ces phénomènes de sorption des contaminants dans les sols. Il a par la suite élaboré un modèle en tenant spécifiquement compte (Weber et al., 2001).

Ces interactions jouent un rôle dans la mobilité, la toxicité, la biodisponibilité des polluants dans le sol et ainsi que dans les estimations de transferts entre les compartiments (sols, eaux, biomasse). Ainsi, Lichtfouse et al. (1997) ont mis en évidence la prolongation de leur durée de vie par la sorption de contaminants dans un sol.

## 3.3 Structure du sol

La structure du sol est définie comme étant l'arrangement de particules et de pores associés dans le sol. Le principal facteur de la formation de cette structure est le phénomène de dessication-humectation. Par ailleurs, la MOS interagit avec les minéraux du sol et notamment avec l'argile pour former des liaisons, la MOS a un effet de stabilisation structurale d'autant plus important que le sol est sableux. Les liaisons entre les argiles (chargées négativement) et les fonctions acides et/ou les noyaux aromatiques s'établissent par l'intermédiaire des cations du sol (Oades, 1988) pour former les complexes organo-minéraux ou microagrégats (<250µm) présentant une grande stabilité physique, comme vu précédemment figure 11. A une plus grande échelle, la biomasse du sol joue aussi un rôle dans la structure de celui-ci, en particulier sur sa stabilisation. En effet, les plaquettes d'argiles et la MOS forment des microagrégats qui s'accumulent, ils sont maintenus ensemble par les polysaccharides des mucilages de plantes ou de bactéries pour former des agrégats eux-mêmes associés en macroagrégats (>250µm) grâce à l'action enveloppante des hyphes de champignons ou des racines (concept de hiérarchie des agrégats) (fig 12) (Gleixner et al., 2001). Cette accumulation de microagrégats autour de bactéries ou d'hyphes a été observée en microscopie électronique à balayage (Oades, 1988). Golchin et al. (1994) a proposé un concept de renouvellement de la MOS et de structure du sol faisant intervenir les micro-

organismes dans la formation et la stabilisation d'agrégats (fig 13) (Balesdent et al., 2000). Les organismes du sol sont aussi des acteurs de la formation de la structure que ce soit la faune par l'intermédiaire de ses boulettes fécales, les racines par leur effet asséchant du sol, leurs sécrétions, ou la formation de pore lors de leur développement (Oades, 1993).

L'existence de cette structure a un effet majeur sur la dynamique de la MOS (circulation des gaz et de l'eau). Elle induit la formation de microenvironnements à l'intérieur même du sol. Dans ces agrégats la diffusion des gaz et donc l'aération peuvent être réduites. De plus, ces agrégats fournissent une protection physique à la MO qui s'y trouve (cf 2.3.3).

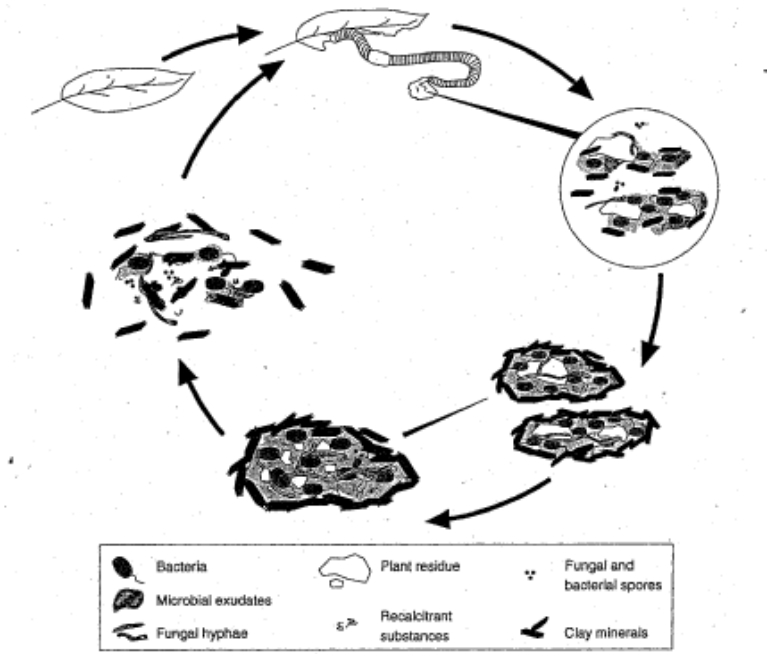



Figure 12 : Incorporation de la MOS dans les agrégats et leur évolution (Gleixner et al., 2001)

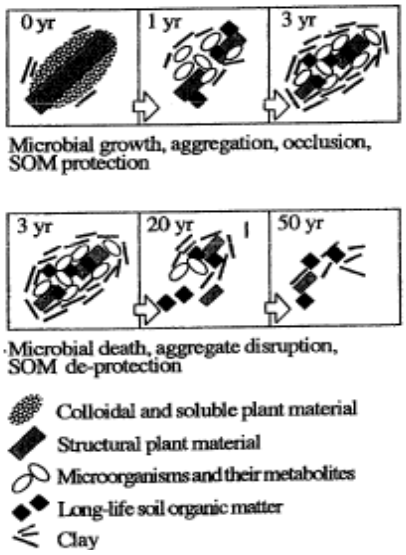



Figure 13 : Représentation schématique du devenir de la MOS et des agrégats (Golchin et al., 1994)

Les interactions organo-minérales se sont aussi révélées utiles pour l'étude des sols. Du fait de la grande hétérogénéité de la MOS, de nombreux auteurs ont tenté de mettre en évidence des pools de matière organique présentant une plus grande homogénéité dans leur composition et donc dans leur dynamique. Les méthodes les plus efficaces, qui sont depuis couramment appliquées, sont les fractionnements granulométriques et densimétriques (Christensen, 1987; Oades et al., 1987; Christensen, 1992; Golchin et al., 1995; Kaiser et al., 2002). Lors du fractionnement densimétrique, les matières organiques de faible densité principalement constituées de matière organique fraîche sont séparées de la MO plus humifiée (Golchin et al., 1994; Neufeldt et al., 2002). Lors du fractionnement granulométrique, il a été mis en évidence des concentrations en carbone organique inversement proportionnelles à la taille des particules de la fraction (Anderson et al., 1981; Christensen, 1987; Schulten et al., 1993). De plus, Buyanovsky et al. (1994) ont étudié la dynamique de dégradation dans différentes fractions granulométriques du sol et ont démontré que le temps de résidence de la MO est d'autant plus important que les fractions dont elle est issue sont fines. Ce résultat a par la suite été maintes fois confirmé par des méthodes très diverses (datation au $^{14}C$ (Paul et al., 1997), marquage isotopique naturel (Balesdent et al., 1988; Balesdent, 1996; Monreal et al., 1997; Balesdent et al., 1998; Roscoe et al., 2001). Oades et al. (1987), Guggenberger et al. (1995) et Chen et Chiu (2003) ont caractérisé la MO de fractions granulométriques par RMN $^{13}C$ à l'état solide et ont révélé l'augmentation de la contribution des groupes alkyles et aromatiques dans les fractions plus fines du sol et la diminution des groupes O-alkyles. Selon ces auteurs, ces observations sont le résultat de la dégradation des composés labiles et de l'accumulation de résidus résistants. Ainsi, comme l'avait déjà remarqué Baldock et al. (1997), la MOS localisée dans les fractions fines présente un degré de dégradation plus important que celle se trouvant dans les fractions grossières et en conséquent une vitesse de dégradation plus faible. Guggenberger et al. (1994) ont étudié la composition et la distribution de carbohydrate non-cellulosiques et de lignine dans différentes fractions du sol et ont eux aussi démontré une plus grande dégradation de la lignine dans les fractions fines.

La détermination de pools plus homogènes de MO par des méthodes physiques a donc simultanément révélé l'existence de pools cinétiques de MOS dont les taux de renouvellement sont différents. Du fait des échanges gazeux entre le sol et l'atmosphère, les diverses dynamiques de minéralisation de ces pools vont avoir une influence variable sur le cycle global du carbone.

## 3.4 La MOS dans le cycle global du carbone

### 3.4.1 Le cycle global du carbone

Le cycle global du carbone s'exerce à la fois dans l'atmosphère, la biosphère, les océans et la lithosphère. Le principal stock de carbone se trouve dans la lithosphère où il représente $30x10^6$Pg de carbone (1Pg=$10^{15}$g) auxquels s'ajoutent 10000 Pg de combustibles fossiles. Les océans constituent le deuxième réservoir avec 39000 Pg. La biosphère (2000 Pg) et l'atmosphère (760 Pg) sont des réservoirs bien plus modestes. Le carbone atmosphérique est pourtant actuellement au centre des discussions. L'augmentation du taux de $CO_2$ atmosphérique (un des gaz à effets de serre) observée depuis plus d'un siècle est due aux activités anthropiques (combustion fossile, déforestation, etc…) et elle conduit à une augmentation du $CO_2$ atmosphérique de 1.6 ppm/an (Amundson, 2001)**.** Cet accroissement du taux de $CO_2$ est responsable du réchauffement climatique actuel et a des répercussions importantes sur le milieu. Pour appréhender le cycle global du carbone et prévoir ses évolutions, il est nécessaire de mieux le connaître. Or, alors que dans les divers compartiments les mécanismes sont essentiellement physico-chimiques, dans la biosphère, les facteurs biologiques sont majoritaires ; c'est donc le compartiment le plus difficile à modéliser et le plus réactif aux variations aussi bien naturelles, qu'anthropiques.

La biosphère regroupe la végétation (500 Pg de carbone) et les sols (1500 Pg dans le premier mètre (Eswaran et al., 1993)). Depuis quelques années, l'attention s'est portée plus spécifiquement sur le sol (Eswaran et al., 1993; Bruce et al., 1999; Amundson, 2001). Non seulement car il constitue le réservoir le plus important (2 fois le stock de $CO_2$ atmosphérique et 3 fois celui de la végétation) mais aussi car la dynamique du carbone organique du sol est encore mal connue. Les émissions annuelles de carbone du sol sont estimées à 60 Pg.an$^{-1}$ soit 10 fois les émissions par combustions d'origine fossile. De plus, les sols sont susceptibles de constituer le puits manquant du cycle du carbone (Houghton, 1995). L'existence de ce puits de carbone manquant dans la biosphère terrestre, plus probablement localisé dans l'hémisphère nord, a été mis en évidence par le déséquilibre entre l'estimation du carbone stocké dans les océans et l'atmosphère et l'émission de $CO_2$ calculée dans le même temps (Tans et al., 1990). Pour que le bilan soit équilibré, Siegenthaler et Sarmiento (1993) ont calculé qu'il doit exister un puits de carbone accumulant 1,9 Pg C.an$^{-1}$ :

Emissions (7,3 Gt C.an$^{-1}$)= Combustion d'énergie fossile (5,4 Gt C.an$^{-1}$)
+ déforestation (1,9 Gt C.an$^{-1}$ )

Accumulation 5,4 Gt C.an$^{-1}$= atmosphère 3,4 Gt C.an$^{-1}$+ océans 2 Gt C.an$^{-1}$.

Puit estimé=Emissions- accumulation estimée= 1.9 Pg.C.an$^{-1}$

Comme le stock constitué par la végétation n'a pas pu être mis en évidence, ce puits est probablement le sol (Siegenthaler et Sarmiento, 1993). Cependant le carbone organique du sol est un pool de carbone très sensible aussi bien aux variations des paramètres climatiques qui influent sur le taux de minéralisation, mais aussi à l'influence anthropique notamment par la mise à nu du sol ou la mise en culture. En conséquence, tout changement dans la taille du réservoir de carbone du sol peut potentiellement altérer la concentration de $CO_2$ atmosphérique et le climat terrestre global.

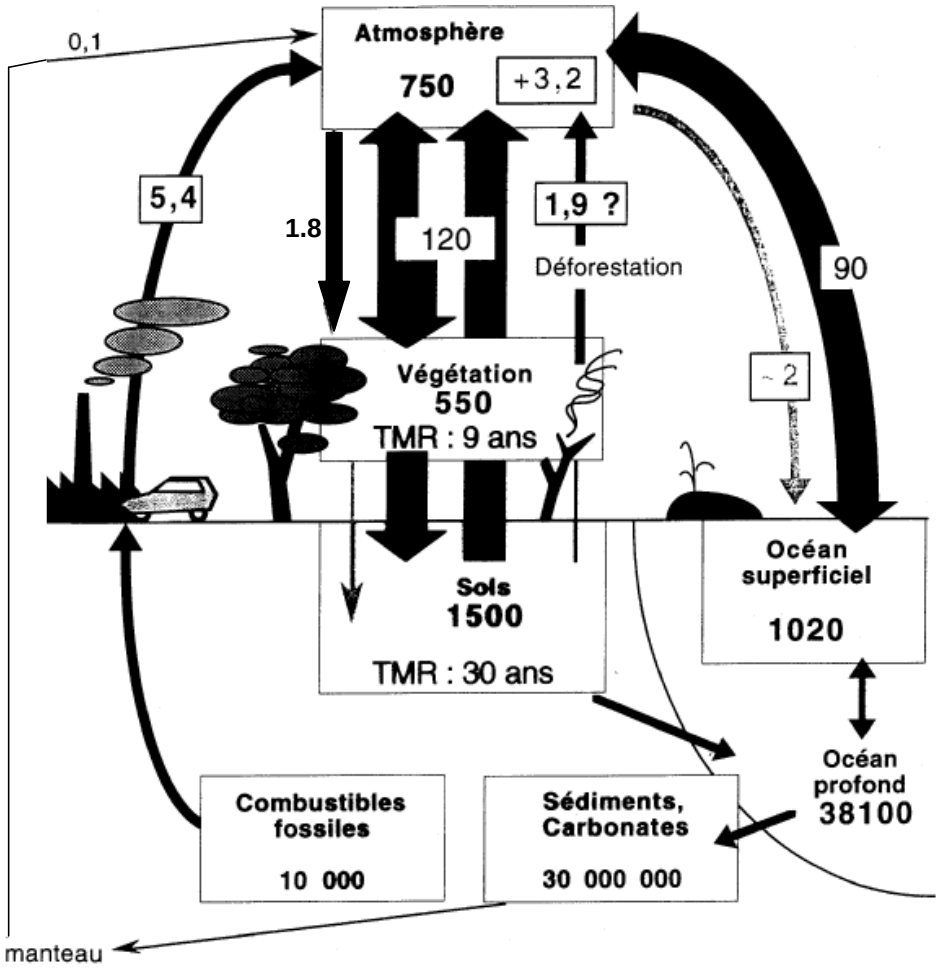



Figure 14 : Cycle global du carbone, stock et flux annuels (milliards de tonnes)

### 3.4.2 Effet de la mise en culture sur le stock de carbone du sol

Le stockage du carbone par le sol est contrôlé par deux processus fondamentaux : l'apport par la production primaire des végétaux et la décomposition. De là, la gestion culturale du sol par l'homme a de nombreuses conséquences. Les perturbations générées par l'homme sur le sol sont multiples mais les études s'accordent à montrer un déclin de la teneur en carbone organique suite à sa mise en culture. Son impact sur le stock de carbone organique diffère selon le climat de la région et la texture du sol : les pertes sont plus importantes sous un climat humide (Islam et Weil, 2000), pour un sol pauvre en carbone (Dale, 1992) et dans un sol sableux (Guggenberger et al., 1994; Neufeldt et al., 2002). Cette perte est très importante les premières années, puis la teneur en carbone se stabilise pour atteindre un nouvel équilibre en 30 à 50 ans (Balesdent et al., 1988; Arrouays et Pelissier, 1994).Toutefois, la mise en culture conduit à une perte moyenne de carbone de 30% (Murty et al., 2002).

Cette diminution du carbone organique total est liée à un apport en matière organique issue de la végétation à la fois de nature différente et le plus souvent en quantité moindre ainsi qu'à une augmentation de la décomposition de la MOS. Cette augmentation est la conséquence de plusieurs facteurs. Tout d'abord, le labour détruit les macroagrégats du sol, la MOS auparavant protégée est alors accessible aux microorganismes et est minéralisée (Balesdent et al., 2000). Le milieu est aussi modifié : le sol reçoit davantage d'énergie solaire et se réchauffe, l'aération est améliorée, le pH peut changer (chaulage), le labour apporte de la MO fraîche en profondeur et l'activité bactérienne est ainsi stimulée. De la même façon, l'utilisation d'engrais permet aux bactéries de proliférer. De plus, la mise à nu du sol le rend plus enclin à l'érosion.

Les études cinétiques de dégradation de la MOS ont introduit la notion de pools de MOS. La mise en culture affecte tous les pools à un taux dépendant de la durée de vie intrinsèque de chaque pool (Oades, 1995; Balesdent et al., 1998).

### 3.4.3 Pools cinétiques de la MOS

Le taux de dégradation de la MOS est, comme nous l'avons vu précédemment, influencé par de nombreux facteurs. Le niveau de dégradation de la MOS diffère aussi selon les fractions granulométriques considérées. La MOS des fractions grossières est plus récente que celle des fractions fines. De plus, la composition de la MOS dans ces fractions varie.

C'est pourquoi des pools de MOS présentant des cinétiques de dégradation différentes ont été envisagés afin de modéliser le devenir de la MOS.

Les modèles considèrent un nombre de pools cinétiques variable selon les auteurs (Smith et al., 1997). Les outils qui ont permis de mettre en évidence ces différents pools sont la datation au $^{14}C$ et le marquage isotopique naturel de la MO.

Actuellement, un consensus a été trouvé et la MOS peut être approximée par trois pools conceptuels de différents temps de résidence (Parton et al., 1993; Balesdent, 1996) :

-un pool actif, avec un temps de résidence d'environ une année, constitué de MO fraîche

-un pool intermédiaire, avec un temps de résidence de 10 ans à un siècle

-un pool passif, avec un temps de résidence supérieur à 1000 ans, dans lequel on trouve la MOS protégée physiquement ou chimiquement de la dégradation.

#### 3.4.3.1 Le marquage isotopique naturel de la MO

Le dioxyde de carbone atmosphérique est formé à 98.9% de $^{12}C$ mais il contient aussi une faible proportion de $^{13}C$ (1.1%). Au cours de la photosynthèse, les plantes assimilent le $CO_2$ atmosphérique pour synthétiser leur propre matière. Or, selon leur métabolisme, les plantes peuvent présenter une proportion $^{13}C$ /$^{12}C$ différente (Bender, 1971; O'Leary, 1981). Cette différence est due d'une part à la résistance variable de diffusion par les stomates (le $^{12}CO_2$ diffuse plus rapidement que le $^{13}CO_2$) et d'autre part aux métabolismes photosynthétiques des plantes. On distingue trois groupes selon leur métabolisme : les plantes en $C_4$ (en majorité des plantes provenant de pays « chauds »), les plantes en $C_3$ (en majorité originaires de la zone tempérée) et les plantes CAM (Crassulacean acid metabolism, surtout issues de déserts). Au cours de la photosynthèse, les plantes en $C_3$ synthétisent leurs sucres en suivant le cycle de Calvin dont la première molécule synthétisée est une molécule en $C_3$ alors que les plantes en $C_4$ forment leur sucre au cours du cycle de Hatch-Slack, la première molécule est en $C_4$. Ces deux cycles ne font pas intervenir les mêmes enzymes et celles-ci n'ont pas la même affinité pour le $CO_2$. Dans les deux cas, les organismes utiliseront de préférence le $^{12}CO_2$, il y a donc discrimination entre le $^{13}C$ et le $^{12}C$. Les plantes utilisant préférentiellement le $^{12}C$, elles sont donc appauvries en $^{13}C$. Cependant les plantes en $C_4$, du fait de leur métabolisme, utilisent davantage de $^{13}C$ que celles en $C_3$ et seront moins appauvries en $^{13}C$. Ainsi il se produit un marquage isotopique naturel de la végétation que

l'on peut suivre avec le $\delta^{13}C$. La valeur du $\delta^{13}C$ est exprimée par rapport à un standard (PDB) et ce calcule de la façon suivante :

$$\delta^{13}C‰= [(^{13}C/^{12}C)_{échantillon}/(^{13}C/^{12}C)_{référence}-1]x1000)$$

Les plantes en $C_3$ ont un $\delta^{13}C$ de -27.6 à -30.5‰ alors que les plantes en $C_4$ ont un $\delta^{13}C$ de -10 à -12‰ (Bender, 1971; O'Leary, 1981; Collister et al., 1994). Les plantes CAM présente un $\delta^{13}C$ entre -13 et -27‰.

La MOS provenant essentiellement de la végétation, le rapport isotopique de celle-ci est quasiment identique à celle de la végétation se développant sur le sol. Grâce à ce marquage isotopique naturel, la dynamique de la MOS dans le sol, mais aussi dans les différentes fractions granulométriques a pu être estimée par de nombreux auteurs tels que Cerri et al. (1985); Balesdent et al. (1987); Skjemstad et al. (1990); Feller et al. (1991); Gregorich et al., (1994); Golchin et al. (1995); Huggings et al. (1998); Roscoe et al. (2001), lors de la succession de plantes $C_3/C_4$ et en particulier lors de l'étude de chronoséquences. Une chronoséquence consiste en une série de sols ayant le même matériel source, la même topographie et ayant subi le même climat et dont seul un processus varie entre les sols (amendement, lessivage, changement de gestion du sol, changement de culture, etc…). L'étude de chronoséquence représente un grand intérêt dans la compréhension des mécanismes influençant le sol aussi bien sa structure que sa teneur en carbone par exemple (Mariotti, 1991; Boutton, 1996).

La proportion (*f*) de carbone du sol provenant d'un type de végétation par rapport à une autre végétation peut être ainsi calculée grâce au $\delta^{13}C$ (Balesdent and Mariotti, 1996):

$$f= (\delta_{SOM}-\delta_{végétation\ originelle})/(\delta_{nouvelle\ végétation}-\delta_{végétation\ originelle})$$

Dans cette équation, $\delta_{SOM}$ représente la valeur du $\delta^{13}C$ de la MOS, $\delta_{végétation\ originelle}$ est la valeur du $\delta^{13}C$ de la végétation de départ, et $\delta_{nouvelle\ végétation}$, la valeur du $\delta^{13}C$ de la végétation nouvellement incorporée au sol. Lors de l'utilisation de cet « outil », il faut toutefois rester vigilant et tenir compte de la variation du $\delta^{13}C$ au cours d'autres processus tels que l'humification (enrichissement de 0,5 à 1,5‰ (Balesdent and Mariotti, 1996) dû aux fractionnements isotopiques intervenant lors de la respiration microbienne et de la dégradation des MOS) et l'appauvrissement du $\delta^{13}C$ du $CO_2$ atmosphérique par injection dans

l'atmosphère du $CO_2$ provenant de combustibles fossiles appauvris en $^{13}C$. Malgré tout, ces variations sont nettement plus faibles que la différence due à la composition isotopique des plantes en $C_3$ et $C_4$.

Un nouveau champ d'étude s'attache à présent à déterminer le taux de renouvellement des molécules constitutives de la MOS. Il est basé sur la composition isotopique de composés individuels (Collister et al., 1994; Lichtfouse et al., 1994; Lichtfouse et al., 1995; Lichtfouse et Budzinski, 1995; O'Malley et al., 1997; Van Bergen et al., 1998; Zegouagh et al., 1998; Cayet and Lichtfouse, 2001; Gleixner et al., 2002; Chikaraishi et Naraoka, 2003).

#### 3.4.3.2 Datation $^{14}C$

Le rapport atmosphérique $^{14}CO_2/^{12}CO_2$ est relativement constant ($1.2x10^{-12}$). Comme le $^{14}C$ est un élément instable, sa radioactivité diminue avec le temps (demi-vie=5730 ans). Les organismes photosynthétiques utilisent ce $^{14}CO_2$ pour former leur matière carbonée et présentent ainsi une radioactivité. Comme celle-ci décroît dès la mort du végétal, à partir de la radioactivité de la MOS, on peut donc calculer l'âge de cette MOS.

Le renouvellement de la MOS peut être calculé à partir de l'âge apparent de la MOS :

$$Age = (-1/\lambda_{14C})x\ [ln(k/(k+ \lambda_{14C})]$$

$$Temps\ de\ renouvellement=C/I=1/k$$

Où $\lambda^{14}C$ est la constante de décroissance radioactive du $^{14}C$ ($\lambda$=1/8270 par an), k le taux de décomposition, I le taux d'apport et C la teneur en carbone (Trumbore et Druffel, 1995).

L'utilisation du $^{14}C$ a aussi permis l'estimation de taux de renouvellement de la MO dans un sol total et dans différentes fractions (Paul et al., 1997). Cette méthode s'est avérée efficace dans l'étude de l'effet de la mise en culture d'un sol (Hsieh, 1992; Harrison et al., 1993), de la stabilisation de la MOS par la fraction minérale du sol (Monreal et al., 1997). La MOS se trouvant en profondeur dans un sol est plus ancienne et donc plus stable (Schwartz et al., 1992; Paul et al., 2001; Rumpel et al., 2002), elle est appauvrie en $^{14}C$.

# IV. MO REFRACTAIRE

Le compartiment de MO constituant le pool le plus stable soulève de nombreuses questions. Tout d'abord, son isolement est difficile. Ainsi, certains isolent la matière organique réfractaire (MOR) par hydrolyse acide (Leavitt et al., 1996; Paul et al., 1997; Paul et al., 2001), hydrolyses acides et basiques (Zegouagh et al., 1999; Poirier et al., 2002; 2003), photooxidation (Skjemstad et al., 1993), densimétrie (Golchin et al., 1995), fractionnement granulométrique (Christensen, 1996), oxydation (Theng et al., 1992), etc… Cependant, alors que les connaissances concernant les mécanismes de protection physique ont beaucoup progressées, la composition de la MOS présentant une résistance à la biodégradation liée à sa structure chimique intrinsèque reste encore à élucider.

Comme nous l'avons vu précédemment, l'étude de la dynamique de la matière organique des sols et des fractions granulométriques du sol, associée aux résultats obtenus tant au niveau structural (spectroscopie IR, RMN $^{13}C$) que moléculaire (CG-SM, pyrolyse couplée au CG-SM, CG-FIMS, etc…) indique que les divers constituants chimiques de la MOS ont des temps de résidence variés. Les composés qui ont une durée de vie longue, liée à leur structure chimique intrinsèque, sont des éléments fondamentaux dans le cycle du carbone car ils constituent un puits de carbone stable qui ne sera pas (ou peu) influencé par les facteurs anthropiques. Ces molécules présentent une grande inertie chimique aussi il est indispensable de combiner les méthodes d'analyses afin d'obtenir des informations.

Dans les sols, on a pu distinguer en particulier d'importantes familles de macromolécules résistantes (revues par Derenne et Largeau, 2001) dont les carbonisats (Poirier et al., 2002) et des structures aliphatiques (Baldock et al., 1997). De plus, la résistance chimique peut être acquise comme lors de la formation de mélanoïdines.

## 4.1 Les carbonisats

Les carbonisats sont retrouvés dans les sols à une échelle de temps géologique. Thinon (1978) a noté leur présence dans des profils de sols dont les âges moyens étaient supérieurs à 2000 ans et des carbonisats sont retrouvés dans des sédiments anciens (Gustafsson et Gschwend, 1998; Schmidt et Noack, 2000). Cette résistance est souvent attribuée à leur structure (Cf 2.2.1.6). Ils sont ainsi des traceurs de feux à l'échelle géologique, et leur étude a des utilités multiples (telles que la reconstitution de climats (Smith et al., 1973; Griffin et

Goldberg, 1975) ou de biomes (Bird et Cali, 1998) à l'échelle terrestre). Le carbone végétal transformé en carbonisats lors de la combustion est soustrait au cycle du carbone à court terme pour un cycle à long terme (Seiler et Crutzen, 1980). Le stock de carbonisat constitue donc un puits de $CO_2$ à long terme et selon Kuhlbusch et Crutzen (1995, 1996), il pourrait constituer une partie du puits de carbone manquant dans le cycle global du carbone. Les carbonisats représentent un pool de carbone stable utilisé dans les modèles de matières organiques des sols (Crutzen et Andreae, 1990; Oades, 1995).

Dans certains sols, les résidus de carbonisation représentent une part importante du carbone du sol (45% du carbone organique d'un chernozem allemand soit 8 g/kg (Schmidt et al., 2001), 35% de la matière organique du sol Terra Preta au Brésil (Glaser et al., 2000).

## 4.2 Les unités aliphatiques

Des structures aliphatiques extrêmement résistantes ont été mises en évidence dans les couches externes d'organismes. Ces structures sont issues aussi bien d'algues (algaenanes), que de plantes supérieures (cutanes, subéranes), que de champignons, de graines ou de pollens (De Leeuw et Largeau, 1993).

Ces macromolécules sont résistantes à des hydrolyses acides et basiques drastiques. Cette résistance est liée à leur structure chimique. Les caractères communs à ces structures sont leur très grande aliphaticité et l'abondance de liaisons intra moléculaires. Du fait de leur grande résistance aux dégradations, ces molécules sont fréquemment retrouvées dans les sols (Kögel-Knabner et al., 1992; Van Bergen et al., 1997; Augris et al., 1998; Nierop, 1998; Van Bergen et al., 1998). Dans les sols, ces macromolécules aliphatiques correspondent aux cutanes et subéranes. En effet, elles sont identifiées dans la cuticule (cutane) et dans les couches péridermiques (liège et racine) de plantes supérieures (Nip et al., 1986; Tegelaar et al., 1995). Elles sont aisément mises en évidence lors de pyrolyse au point de Curie couplée à une CG-SM car leur motif est alors très caractéristique : la pyrolyse de ces composés conduit à la production d'une série de n-alcanes/n-alc-1-ènes ainsi que d'alcadiènes ne présentant ni prédominance paire ni impaire. Certaines chaînes aliphatiques dans la MOS peuvent aussi provenir de l'incorporation de lipides dans les structures macromoléculaires.

# V. OBJECTIFS DE L'ETUDE

Depuis le protocole de Kyoto, l'augmentation du $CO_2$ atmosphérique est devenu une préoccupation majeure. Il est devenu essentiel de mieux connaître le cycle global du carbone afin d'estimer l'impact anthropique sur ce cycle. Afin de modéliser ce cycle et ainsi de pouvoir prévoir les évolutions du taux de $CO_2$ atmosphérique, il est important de parfaitement connaître tous les composants intervenant dans le cycle. Or comme nous l'avons vu, la biosphère continentale constitue le compartiment le plus sensible car elle représente un stock important de carbone sur lequel les hommes ont une grande influence. En effet, la déforestation, la mise en culture de sols vierges sont des sources de carbone importantes. Du fait de l'expansion démographique, de plus en plus de surfaces sont déforestées et mises en culture, il est donc nécessaire de connaître la MOS et son taux de renouvellement pour estimer la réponse à ces facteurs. La biodégradation de la MOS conduit à la production de $CO_2$. Il est donc primordial de pouvoir caractériser et estimer les pools dynamiques de la MOS, en particulier le pool stable qui constitue un stock de carbone à une grande échelle temporelle sur lequel l'homme a peu d'impact (Falloon et al., 1998). De nombreuses études se sont attachées à la protection physique de la MOS mais de nombreuses interrogations concernant la protection chimique intrinsèque de molécules subsistent. Des molécules subsistant dans les sédiments à une grande échelle de temps se sont avérées, de la même façon, résistantes aux hydrolyses (Nip et al., 1986; Tegelaar et al., 1989). L'hypothèse d'une corrélation entre résistance chimique et résistance à la biodégradation a été émise.

Dans cette étude, nous avons décidé de nous intéresser à l'étude structurale et dynamique des fractions lipidiques, de la MOR et des carbonisats d'un podzol. Plusieurs auteurs ont noté le caractère aliphatique de fractions de sol résistantes à la dégradation. De plus, des études spécifiques des lipides du sol laissent à penser que ces composés seraient relativement résistants à la biodégradation. Ainsi, l'étude des lipides, de la matière organique réfractaire aux attaques acides et basiques ainsi que celle des carbonisats a pour objectif d'apporter de nouvelles informations sur la stabilité de la MOS.

Les podzols représentent une part importante des sols terrestres. Ils ont la caractéristique d'être des sols sableux acides. Ainsi ils présentent un taux de dégradation assez faible (Motavalli et al., 1995; Nierop et Verstraten, 2003), et sont plus sensibles à la mise en culture (Guggenberger et al., 1994; Neufeldt et al., 2002). Il nous a donc paru intéressant de considérer ce type de sol dans notre étude. De plus, nous avons choisi d'étudier

un site localisé dans le sud ouest de la France qui présente plusieurs avantages supplémentaires : il s'agit d'une chronoséquence forêt/maïs. Ainsi, nous avons pu nous intéresser à l'effet de la mise en culture au niveau moléculaire, mais aussi estimer le renouvellement de la MOS grâce au marquage isotopique naturel car la forêt est constituée de pins maritimes (métabolisme photosynthétique en $C_3$) alors que le maïs est une plante en $C_4$.

# PARTIE B :

# LE SOL FORESTIER

Le site d'étude est un sol forestier localisé dans le sud ouest de la France, à proximité de Cestas. L'exploitation forestière a été développée sur ce site depuis environ 40 ans. L'espèce dominante est donc un pin (*Pinus pinaster)* dont la production a été optimisée par l'apport d'engrais inorganique. Jolivet et al. ayant étudié la teneur en carbone organique des sols de Gascogne, l'historique de ce site mais surtout sa pédologie sont bien connus (Jolivet, 2000).

Ce sol est un podzol de texture sableuse (plus de 90% de sable) et son pH est faible (4.3). Les podzols sont principalement présents sous des climats frais et humides, souvent sous couverts forestiers, où les conditions favorisent le développement d'une couche de surface organique (mor). Ce type de sol se forme par podzolisation, c'est-à-dire lors d'un processus par lequel des composés organiques solubles acides (incluant acides humiques et fulviques) migrent en profondeur entraînant par la même occasion l'aluminium et le fer (revue par Lunström et al., 2000). Les acides organiques sont produits par lessivage des plantes, décomposition de la litière par les microorganismes et par les exudats. Lors de leur passage sur les matériaux sableux, ils solubilisent par complexation les ions $Al^{3+}$ et $Fe^{3+}$ compris dans les feuillets cristallins et ils contribuent ainsi au lessivage des minéraux. Les ions $Al^{3+}$ et $Fe^{3+}$ sont alors entraînés sous forme de complexes et transportés en profondeur. Les éléments organiques et minéraux précipitent alors et se polymérisent dans l'horizon inférieur (l'horizon B).

Les caractéristiques de ce sol en font un site intéressant pour l'étude de la matière organique des sols et en particulier de sa préservation. En effet, comme nous l'avons vu dans la partie A, la MOS interagit préférentiellement avec les argiles pour former des complexes argilo-humiques. Aussi, dans les sols sableux tels que les podzols, l'agrégation est faible. Ceci pourrait laisser à penser que la MOS est peu protégée. Cependant, certains auteurs ont noté une minéralisation plus faible dans les sols acides (Oades, 1988; Motavalli et al., 1995; Van Bergen et al., 1997; 1998; Nierop et al., 2003). Ce phénomène peut être lié à plusieurs facteurs : alors que généralement les bactéries prédominent dans les sols, dans les sols acides les communautés fongiques sont largement développées (Dinel et Schnitzer, 1990) et les bactéries ont un rôle plus restreint (Neale et al., 1997). De plus, la concentration en $Al^{3+}$ augmente, or c'est un cation toxique pour les microorganismes. Du fait de cette apparente et relative préservation dans les sols acides, nous avons donc décidé d'étudier les lipides ainsi que la matière organique réfractaire (MOR) de ce podzol des Landes.

# I LIPIDES: VARIATION DE L'ABONDANCE RELATIVE ET DE LA COMPOSITION DES LIPIDES DE DIFFERENTES FRACTIONS GRANULOMETRIQUES

Des études préalables ont mis en évidence une augmentation du caractère aliphatique du sol et des fractions granulométriques au cours de l'humification (Preston et al., 1987; Hempfling et al., 1991; Kögel-Knabner et al., 1992a; Baldock et al., 1997; Nierop et al., 2001; Chen et Chiu, 2003). Aussi certains auteurs ont considéré que ces composés aliphatiques pouvaient constituer une source de carbone stable (Almendros et Dorado, 1999).

Les lipides du sol représentent de 4 à 8% du carbone organique mais des pourcentages bien plus élevés ont été trouvés (20% dans un podzol de l'ouest de la France (Jambu et al., 1978) et 42% par Preston et al. (1987) dans des tourbes). Comparés aux protéines et aux sucres, les lipides du sol sont considérés comme relativement résistant à la biodégradation, ils peuvent persister dans les sols. Les lipides ayant des propriétés hydrophobes, ils jouent un rôle important dans les caractéristiques du sol telles que leur stabilité ou leur capacité de rétention en eau (Jambu et al., 1978; 1983; Dinel et Schnitzer, 1990). Un autre intérêt des lipides concerne leur utilité en tant que biomarqueurs. Pour cette raison, les lipides ont été largement étudié dans les sédiments et ils ont fourni de nombreuses informations sur les sources de la MO mais aussi sur sa diagenèse (Cranwell, 1981; Matsumo et al., 1987; Grimalt et Albaigés, 1987; Goosens et al., 1989; Rieley et al., 1991; Saliot et al., 1991; Haddad et al., 1992; Meyers et Ishiwatari, 1993; Shi et al., 2001), sa dégradation ou ses mécanismes de préservation. Les études de la MO des sols concernant spécifiquement les lipides sont plus restreintes. Nous pouvons néanmoins citer les travaux de Amblès et al., (1989b; 1989a; 1991 ), Jambu et al. (1991, 1993), Amblès et al. (1994), Jaffé et al. (1996), Van Bergen et al. (1997), Bull et al. (2000), dont les résultats ont démontré l'importance de l'étude des lipides du sol.

De plus, d'après Stevenson (1966), le taux de lipides s'accroît avec l'acidité du sol. Jambu et al. (1978), Moucawi et al. (1981), Dinel et Schnitzer (1990) ainsi que Bull et al. (2000) ont noté l'accumulation des lipides avec l'augmentation de l'acidité (mais aussi en cas d'anaérobiose ou de sécheresse). De façon plus précise, selon Amblès et al. (1994), les acides gras s'accumulent dans un podzol sous forêt. Ainsi, les lipides d'un podzol peuvent constituer une part non négligeable de carbone organique relativement stable.

Au vu des renseignements fournis sur la dégradation des lipides d'une part, et sachant que le niveau de dégradation de la MOS dépend de la fraction granulométrique considérée d'autre part (Cf partie A 3.3), l'investigation de la composition lipidique de fractions granulométriques pourrait fournir de nouvelles informations sur l'évolution des lipides dans le sol. Après isolement des lipides, nous nous sommes donc attachés à la quantification et à la composition chimique des lipides de trois fractions granulométriques du sol : la fraction sableuse (2000-50µm), la fraction limoneuse (50-2µm) et la fraction argileuse (<2µm). L'analyse des lipides isolés a été effectuée par chromatographie gazeuse couplée à un spectromètre de masse.

## 1.1. Results and discussion

### 1.1.1. Mass balance

In agreement with the sandy nature of the Cestas soil, particle-size fractionation showed that the sand fraction accounts ca. 90wt% of this whole soil (Table 1.1). However, this fraction is very poor in organic matter (1.4wt %) whereas the silt and clay ones are largely composed of organic matter (ca. 48 and ca. 67wt% respectively). Such an OM enrichment in the fine fraction of soil is classically observed (Christensen, 1987; Gregorich et al., 1988, 1989; Feller et al., 1991; Hassink et al., 1993; Andreux and Munier-Lamy, 1994; Buyanovsky et al., 1994; Christensen, 1996; Schmidt et al., 1999).

| | Relative abundance of the size fractions (wt% of total soil) (a) | OM abundance in each size fraction (wt% of the total fraction) | Lipid abundance in each size fraction (wt% of the total fraction) | Lipid abundance in each size fraction (wt% of the total OM in the fraction) | Absolute amount of lipids (mg) from 1kg of soil |
|---|---|---|---|---|---|
| Sand | 90.2 | 1.4 | 0.1 | 7.1 | 900 |
| Silt | 6.7 | 48.4 | 3.4 | 7 | 2280 |
| clay | 2.3 | 67.4 | 15.8 | 23.4 | 3635 |

Table 1.1: Quantitative features from particle-size fractionation and lipid extraction

Lipid abundance in each size fraction, as wt% of the total mass of this fraction, exhibits large differences and ranges from a very low value of 0.1% for the sand fraction up to ca. 16% for the clay fraction. These lipids accounts for 7 to ca.23wt% of bulk organic matter depending on the fraction. When the absolute amounts of lipids are considered, a substantially lower value is noticed for the sand fraction compared to the silt and clay ones. Lipid enrichment thus occurs in the finest fractions, especially in the clay one.

### 1.1.2. Molecular composition

So as to determine whether differences in lipid content between the three particle-size fractions are associated with differences in molecular composition, each lipid extract was analysed by GC/MS. The total ion current (TIC) traces are complex but similarities are observed between those of the two fine fractions in contrast with that of the sand fraction (Fig.1.1). Amides, carboxylic acids, methylesters of fatty acids, and alkanes are the main series observed in the three fractions although with different relative abundances and distributions. Their distribution will be described and their origin discussed in the following along with that of the ketones which are only present in the two fine fractions.

So as to get further insight in the hydrocarbon composition, the less polar fraction was separated from the total lipid extract by TLC. Methylation and silylation were also performed to improve the detection of the polar lipids. Finally, high molecular weight lipids which are not GC-amenable and thus cannot be directly analysed were investigated through saponification of the total lipids and further GC/MS analyses.

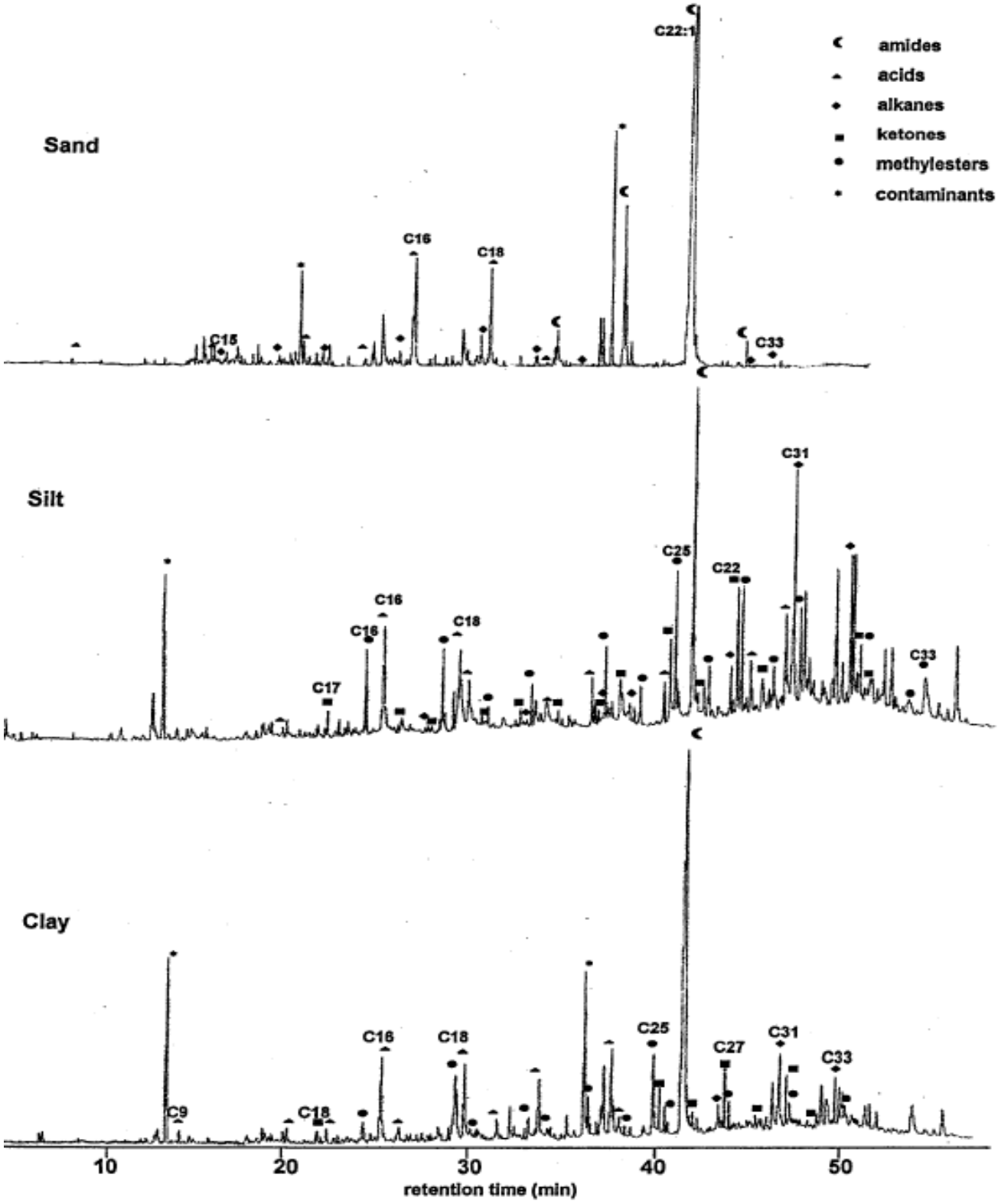



Fig 1.1: Total ion current (TIC) trace of the total lipid extracts from the three particle-size fractions

#### 1.1.2.1. Free amides.

The TIC traces of the lipids from the three fractions are dominated by a $C_{22:1}$ amide which, in the case of the sand fraction, occurs as the major constituent of a series of even carbon numbered unsaturated amides ranging from $C_{18:1}$ to $C_{24:1}$. The origin of these amides is not clear but, as discussed below, they are likely to be contaminants. Indeed, as far as we are aware, long chain amides have not been reported as higher plant lipids. A $C_{22:1}$ acid, named erucic occurs in the lipids of some higher plant seeds such as Cruciferae (Padley et al., 1992) but such plants are not growing in the sampling site. Moreover, there is no relationship between the distributions of the acids and amides which precludes a common origin. In addition, erucylamide was found as a water contaminant originating from poly(ethylene) (Impact analytical, www.impactanalytical.com)**.** In consequence, the amides observed in the lipids of the Cestas soil should originate from contamination by such products. However, when the relative abundance of erucylamide with respect to the total lipids is considered, it shows a maximum in the sand fraction where it accounts for 15% of the GC-amenable products (according to GC integration). However, as reported below, in the study of the saponification products, non GC-amenable compounds represent a rather large proportion of the lipids. As a result, erucylamide should not affect significantly the lipid content reported in table 1.1.

#### 1.1.2.2. Free carboxylic acids.

Carboxylic acids were detected in the lipids from the three fractions. In the two fine fractions, these acids occur along with a series of fatty acid methylesters. It is well documented that methyl esters of fatty acids can be formed during rock extraction with methanol-containing solvents, since methylation is catalysed by various minerals, especially the clayey ones. Accordingly, the methylesters observed in the present case probably originate from such a process and they reflect the methylation of a part of the fatty acids during extraction with dichloromethane-methanol mixture (see annexes 3.1). These esters shall therefore be taken into account for determining the distribution of the fatty acids in the extracts. Moreover, long chain fatty acids with more than $C_{22}$ carbon atoms are hardly detected under usual GC conditions. To overcome these two difficulties, complete

methylation of the acids was therefore performed prior to further GC/MS analysis. The distributions thus obtained for the fatty acids are shown in Figure 1.2.

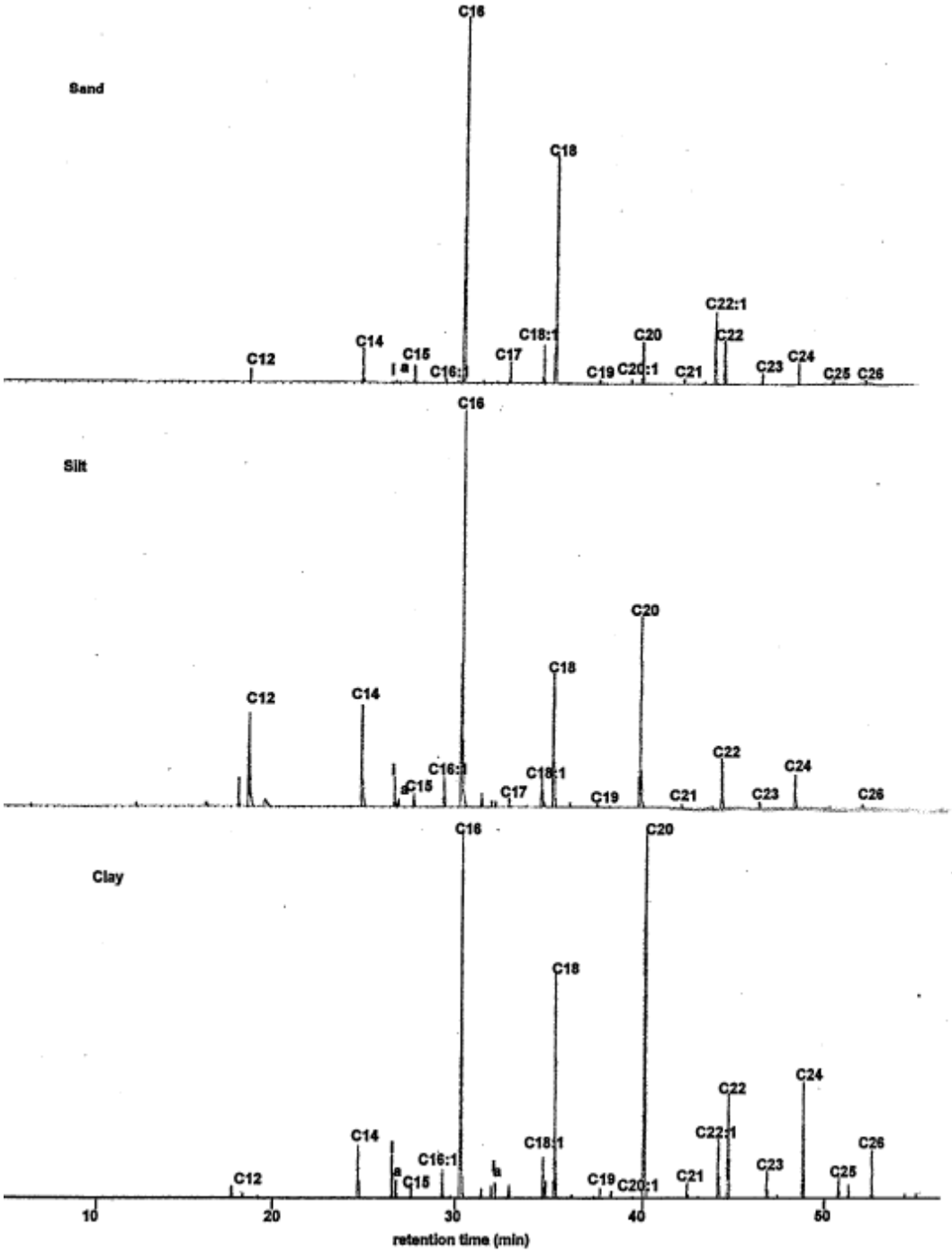



Fig 1.2: Ion chromatogram (*m/z* 74) showing the distribution of the free carboxylic acids as their methylesters in the total lipid extracts of the three particle-size fractions (a: anteiso-, i: iso-).

In the three particle-size fractions, the main free fatty acid is the saturated $C_{16}$ one along with the $C_{18}$ one. In addition, large amounts of the $C_{20}$ saturated acid are observed for the silt and clay fractions. Moreover, the distributions of the saturated fatty acids in the lipid extracts show a relative increase of the $C_{20+}$ compounds with respect to the $C_{16}$ one from the sand fraction to the silt and clay ones. These long chain even-carbon-numbered fatty acids are typical biomarkers of higher plants (Kolattukudy, 1980; Wannigama et al., 1981). The increase in the $C_{20+}/C_{20-}$ ratio from the coarse fraction to the fine ones thus reflects differential contributions of higher plant acids within the free lipids of the particle-size fractions with a maximum relative contribution for the clay fraction.

These saturated fatty acids are characterized by a strong even-over-odd carbon number predominance ($CPI_{12-24} < 0.1$ in sand, silt and clay). This indicates a moderate level of alteration since fatty acids with odd carbon number are considered to be mostly derived from even-carbon-numbered compounds.

Short chain ($C_{15}$, $C_{17}$) fatty acids are detected in the three fractions in low amount along with their *iso*- and *anteiso*-isomers. These acids are generally considered as bacterial markers (Boon et al., 1975; Meyers and Ishiwatari, 1993) and their low abundance points to a low contribution of bacterial acids in these free lipids of the size fraction of the Cestas soil.

Some unsaturated fatty acids were also observed (Fig. 1.2). Although the $C_{16:1}$ and $C_{18:1}$ acids are present in the three extracts, differences are observed between the different particle-size fractions. Indeed, no additional unsaturated acid is detected in the silt whereas $C_{20:1}$ and $C_{22:1}$ acid occur in the sand and clay fractions along with a $C_{18:2}$ acid in the latter fraction. The occurrence of unsaturated fatty acids in the three extracts is therefore consistent with the low to moderate level of lipid degradation inferred from the distribution of the saturated acids as shown in marine sediments by Haddad et al. (1992). The presence of a diunsaturated compound in the case of the clay reflects a low level of degradation for this fine-grained fraction. This is in agreement with the lower turnover rate generally observed for the OM in the finest particle-size fractions. The occurrence of several unsaturated fatty acids observed for this sand fraction, in spite of its coarse size should reflect the contribution of relatively fresh material. Indeed, poorly humified components are known to be preferentiallly associated with coarse fraction (Oades et al., 1987; Feller et al., 1991; Buyanovsky et al., 1994; Balesdent et al., 1998).

$\alpha,\omega$-$C_8$ and $C_9$ diacids are detected in low amounts in the silt and clay lipids. These diacids are commonly observed in soils where they are considered as the result of the

oxidative degradation of unsaturated lipids such as the ubiquitous oleic acid (Grasset and Amblès, 1998a).

Some minor aromatic acids were identified in the lipids from the silt and the clay fractions: hydroxy- and dihydroxybenzoic acids in both case, methoxybenzoic acid, hydroxymethoxybenzoic acid and cinnamic acid from the silt, benzoic acid from the clay. Benzoic acid is a rather ubiquitous compound in higher plant remains and the other aforementioned aromatic acids are considered as depolymerisation products of lignin (Young and Frazer, 1987). Lignin is a major polymeric constituent of conifer shoots and needles which chemical structure is based on phenyl propanoid units (Sarkanen and Ludwig, 1971; Kögel-Knabner et al., 1994) and the aforementioned aromatic acids were shown to occur in the lipid extract of shoots of a fossil conifer infected by fungi (Nguyen Tu et al., 2000). The presence of such acids in the lipid extracts clearly reflects the contribution of lignin degradation products to the silt and clay fractions.

#### 1.1.2.3 Free hydrocarbons.

In order to analyse the hydrocarbons of the lipid extracts, the less polar compounds were separated by thin layer chromatography. The chromatograms of these compounds (Fig. 1.3) reveal a much wider diversity of alkanes than that directly visible on the chromatograms of the total extracts.

In the sand fraction, the alkanes constitute a highly complex mixture which distribution pattern shows alkane clusters from $C_{14}$ to $C_{33}$. These clusters exhibit an odd-over-even carbon number predominance from $C_{17}$, especially for the long chain $C_{25}$-$C_{33}$ alkanes. The odd-carbon-numbered long chain *n*-alkanes are typical constituents of higher plant waxes (Tulloch, 1976; Rieley et al., 1991) whereas the shorter compounds are usually considered to reflect a bacterial origin (Han and Calvin, 1969). Such a bacterial contribution is confirmed by the occurrence of many branched alkanes among which *iso*- and *anteiso*-isomers.

When compared with the sand fraction, the distribution of the alkanes from the silt fraction is characterized by a much simpler pattern. The hydrocarbons mainly correspond to $C_{15}$-$C_{33}$ *n*-alkanes dominated by odd-carbon-numbered compounds ($CPI_{23\text{-}31}$=3.9), with a maximum at $C_{31}$. This type of distribution has already been observed in several soils (Morrison, 1969; Amblès et al., 1989a; Jambu et al., 1991; Jaffé et al., 1996; Bull et al., 1998;

Baas et al., 2000; Bull et al., 2000) and sediments (Cranwell, 1981) and reflect a higher plant input.

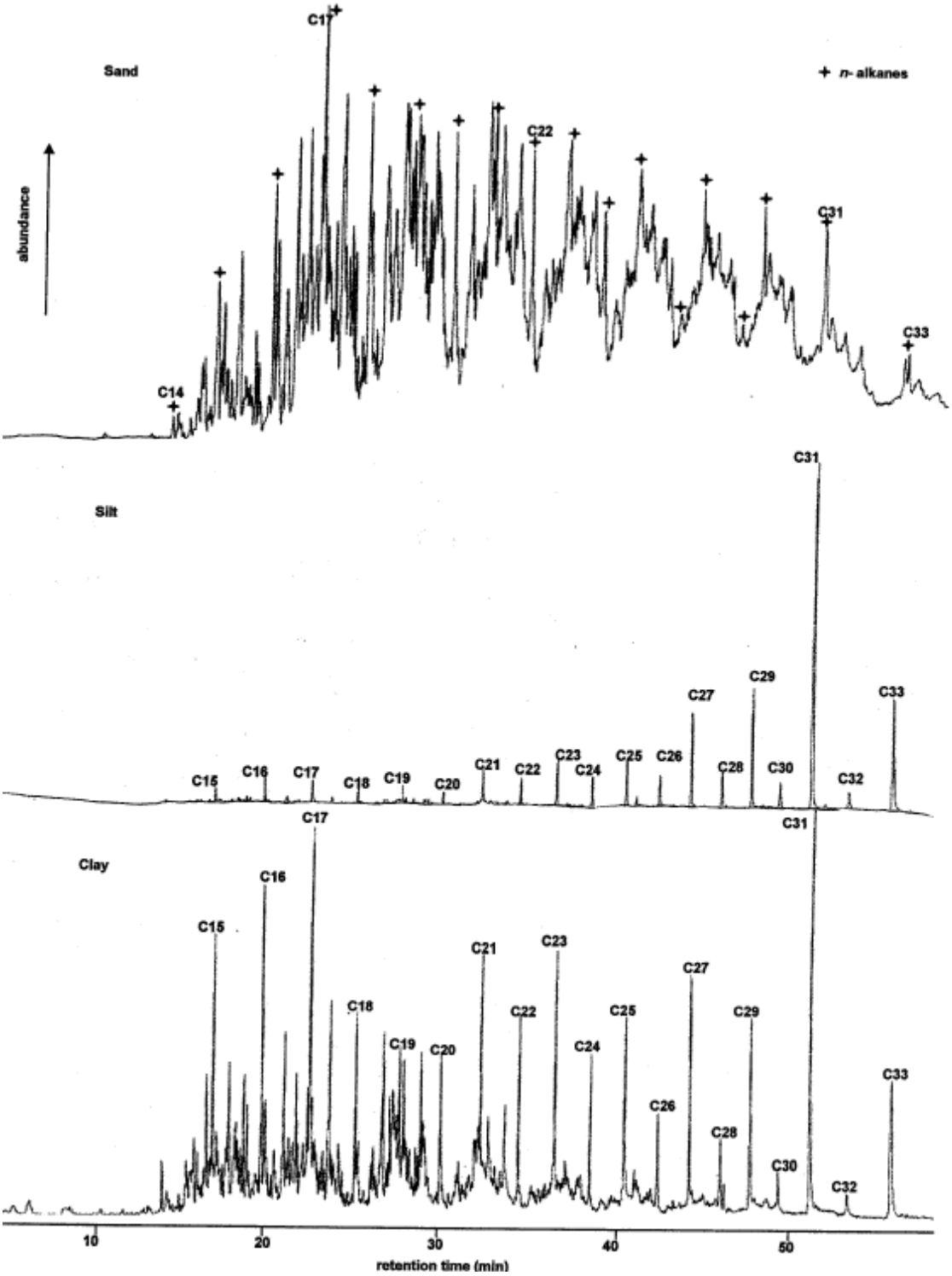



Fig 1.3: Ion chromatogram (*m/z* 57) showing the distribution of the alkanes isolated by TLC from the total lipid extracts of the three particle-size fractions. Alkanes from the sand fraction exhibit complex clusters of isomers.

In the clay fraction, a bimodal distribution is observed with long chain $C_{20+}$ compounds dominated by odd-carbon-numbered *n*-alkanes maximizing at $C_{31}$ and clusters of shorter ($C_{20-}$) alkanes corresponding to complex mixtures of branched compounds along with the *n*-isomers. These clusters are similar to those observed in the chromatogram of the hydrocarbons from the sand fraction. The high relative abundance of branched alkanes in addition to the occurrence of short chain linear alkanes point to a bacterial origin for these $C_{20-}$ clusters whereas the long chain *n*-alkanes are derived from higher plants. When the silt and clay fractions are compared, the main difference is the absence of the aforementioned clusters in the silt hydrocarbons. Short chain compounds are known to be more sensitive to biodegradation than long chain homologues (Moucawi et al., 1981). As a result, the distribution of alkanes in the clay likely indicates a better preservation of these hydrocarbons of bacterial origin in clay with respect to silt.

#### 1.1.2.4. Free ketones.

*n*-Alkan-2-ones ranging from $C_{17}$ to $C_{31}$ in the silt (and $C_{33}$ in the clay), with a strong odd-over-even carbon number predominance ($CPI_{22-30}$ =3.5 in silt and clay) and a maximum at $C_{27}$ are detected in the two fine fractions (Fig. 1.4). As reported by Rieley et al. (1991), such *n*-alkan-2-ones have not been detected in lipids from higher plants. However, a similar series of ketones was previously observed by Morrison et al.(1969) and by Jaffé et al. (1996) in soil lipids. In the latter case, the ketones exhibited the same distribution as the *n*-alkanes and were thus considered as formed by bacterial sub-terminal oxidation of the *n*-alkanes as commonly assumed in sediments (Jaffé et al., 1996) and in soils (Amblès et al., 1989a). Another possible mechanism which was put forward to account for *n*-alkan-2-one formation is $\beta$-oxidation of fatty acids followed by decarboxylation (Amblès et al., 1989a). In the latter case, the distribution of the ketones should correspond to that of the fatty acids, minus one carbon.

In the present study, the distribution of the *n*-alkan-2-ones, i.e. mainly long chain compounds with a strong odd-over-even carbon number predominance points to a higher plant origin. However, the ketone series maximize at $C_{27}$ (Fig. 1.4) whereas the *n*-alkanes are characterized by a maximum at $C_{31}$ in the extracts of the silt and clay fractions (Fig. 1.3) so that a direct formation by sub-terminal oxidation of the *n*-alkanes cannot be considered.

Similarly, no relationship can be observed between the distribution of the fatty acids (minus one carbon) (Fig. 1.2) and that of the ketones, thus ruling out a formation by fatty acid $\beta$-oxidation and decarboxylation. These ketones can be considered as humification products but mechanism responsible for their formation is not yet elucidated. A similar observation has been reported by Volkman et al. (1981) in the study of a coastal marine sediment. Whatever this mechanism for ketone formation is, it seems to be catalysed by the minerals in the soil since the *n*-alkan-2-ones are only detected in the silt and clay fractions and are lacking in the sand one in spite of a careful search by selective ion detection. Such a catalytic oxidation has been recently put forward by Faure et al. (2003).

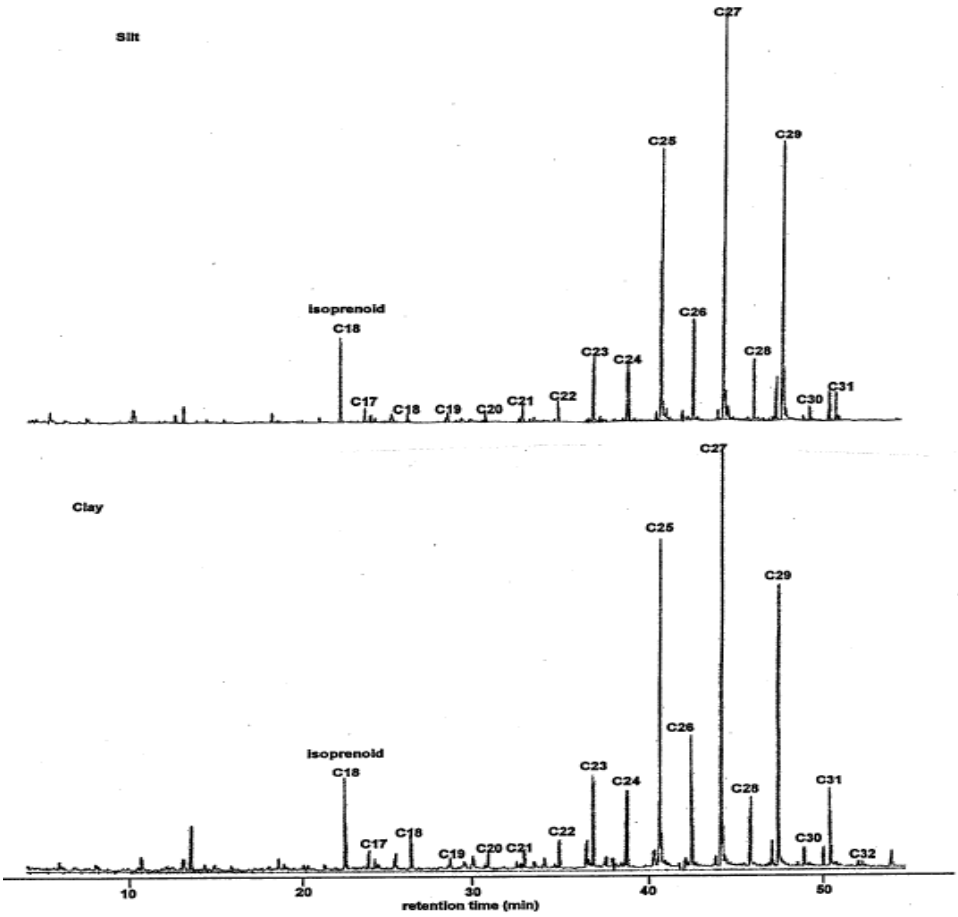



Fig 1.4: Ion chromatogram (*m/z* 58) showing the distribution of the *n*-alkane-2-ones in the total lipid extracts of the silt and clay fractions.

In addition to the *n*-alkan-2-ones, the $C_{18}$ regular isoprenoid ketone (6,10,14 trimethylpentadecan-2-one) is identified in substantial amounts in the lipids from the silt and clay fractions. This ketone is commonly observed in sediment extracts where it is considered as resulting from the oxidation of the phytyl chain of chlorophyll (Ikan et al., 1975; Brooks et

al., 1978). It must be noted that no phytol could be identified in these extracts whereas it was reported to occur in large abundance in some rain forest soils (Jaffé et al., 1996).

1.1.2.5. Free alkanols.

Silylation of the total lipids reveals a series of primary *n*-alkanols in the three fractions. Again, sharp differences are observed between the sand fraction, on the one hand, and the silt and clay ones on the other hand. Indeed, in the sand, only short chain *n*-alkan-1-ols ($C_{18}$ and $C_{20}$) are observed, whereas in the two fine fractions, a series of even carbon numbered *n*-alkan-1-ols ($C_{18}$-$C_{30}$ in the silt, $C_{20}$-$C_{28}$ in the clay) with a maximum at $C_{22}$ is present (Fig. 1.5). Long chain *n*-alkan-1-ols ($C_{20+}$) with even carbon numbers have been reported in higher plant leaf waxes (Kolattukudy, 1980; Rieley et al., 1991). Such compounds are commonly observed in soil extracts (Amblès et al., 1989a; Rieley et al., 1991; Jaffé et al., 1996; Van Bergen et al., 1997) sometimes along with shorter homologues. The latter are usually considered to be of microbial origin. It must be noted that large amounts of $C_{18}$ and $C_{20}$ alkanols have been identified in spore waxes (Albro, 1976). The distribution of the *n*-alkan-1-ols thus points to a predominant microbial contribution in the sand fraction, in agreement with hydrocarbon distribution. In contrast, *n*-alkan-1-ols in the fine fractions of the Cestas soil are mainly of higher plant origin.

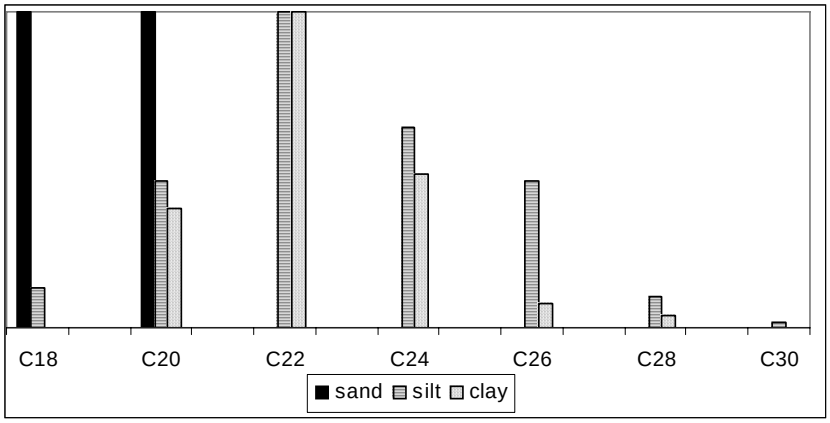



Fig 1.5 : Free alkanol distribution in the lipid extracts of the three particle-size fraction (for each fraction the distribution is normalized to the maximum)

1.1.2.6. Lipid saponification.

High molecular weight compounds and/or highly polar lipids are not GC-amenable and thus cannot be directly analysed. Information on the structure of some of the units

building up such constituents can however be obtained through lipid saponification, e.g. Amblès et al. (1991).

The saponified extracts obtained from the three particle-size fractions were methylated and silylated prior to GC/MS analyses. Complex chromatograms dominated by series of acids, diacids and hydroxy acids (as methyl esters and trimethylsilyl ether derivatives) were thus observed as shown for the clay fraction (Fig. 1.6). The same series were obtained for the three particle-size fractions. The large amount of GC-amenable lipids released upon saponification compared to the free lipids is reflected by the relative abundance of the erucylamide (occurring as free compound) before and after saponification (Fig. 1.1 and 1.6).

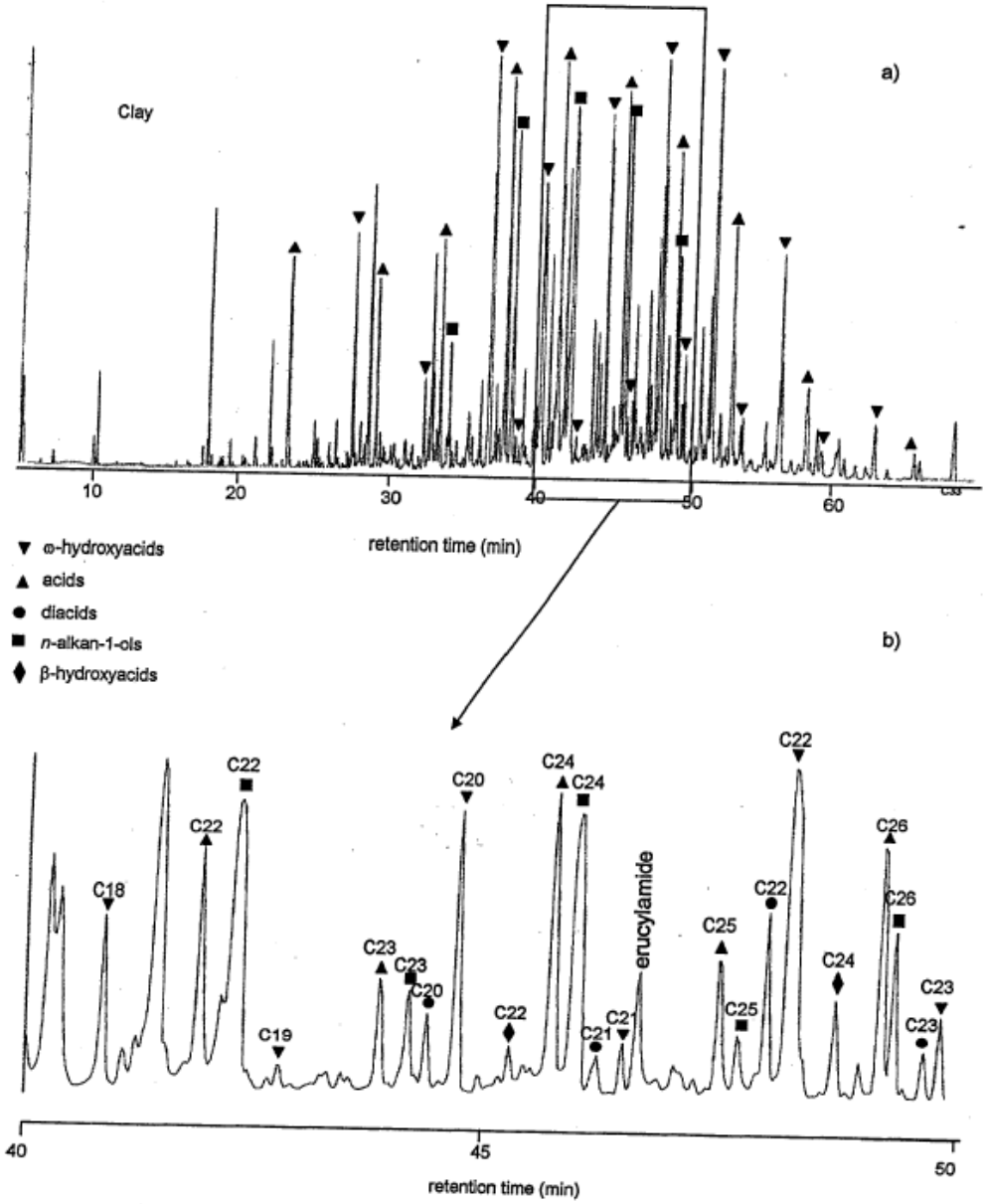



Fig 1.6: (a)TIC trace of the saponified lipids from the extract of the clay fraction; (b) Expanded area showing the various series.

*Carboxylic acids*. After saponification, carboxylic acids ranging from $C_{12}$ to $C_{30}$ with an even-over-odd carbon number predominance ($CPI_{16-28}$ from 0.2 to 0.4) are obtained, as shown by the ion chromatogram at m/z 74 of the methylated acids (Fig. 1.7). These acids, observed after saponification, can originate either from the cleavage of ester linkages in high molecular weight and/or highly polar lipids or they can correspond to free acids. The contribution of linked esters can be evidenced by the comparison of the distribution of the acids in the free and saponified lipids (Figs. 1.2 and 1.7). Moreover, two types of high molecular weight lipids can be considered, either lipids directly inherited from the biomass (such as wax esters or cutin-derived products) or lipids formed upon humification by condensation of more or less altered smaller molecules.

When compared to the free acids, the acids observed after saponification comprise a higher relative contribution of long chain homologues ($C_{20^{+}}$), with a predominance of the *n*-isomers. These long chain fatty acids thus largely originate from the cleavage of ester bonds in high molecular weight and/or polar lipids. Wax esters and suberin are known to comprise ester-linked long chain fatty acid moieties with an even carbon number and thus can account for the increase in these long chain compounds after saponification, especially in the sand fraction. Evidence for such a suberin contribution in a temperate forest soil was previously reported by Riederer et al. (1993) based on chemical depolymerization. Cutin is a biopolyester dominated by $C_{16}$ and $C_{18}$ monomers; since these acids are ubiquitous, it is difficult to infer suberin (cutin)-derived products contribution from the distributions of the fatty acids before and after saponification. However, as discussed later on, cutin input is evidenced by the occurrence of typical hydroxy acids in the saponified lipids. As a result, a part of the $C_{16}$ and $C_{18}$ acids observed after saponification should originate from cutin. When comparing the long chain fatty acids from the free and saponified lipids, a higher contribution of odd-carbon-numbered acids is observed in the latter. As previously mentioned, these acids reflect alteration of the initial distribution. The occurrence of substantial amount of these odd-carbon-numbered acid moieties in the non GC-amenable lipids thus suggests that a part of the latter have been formed upon humification and that the incorporated acid moieties had undergone some alteration prior to incorporation.

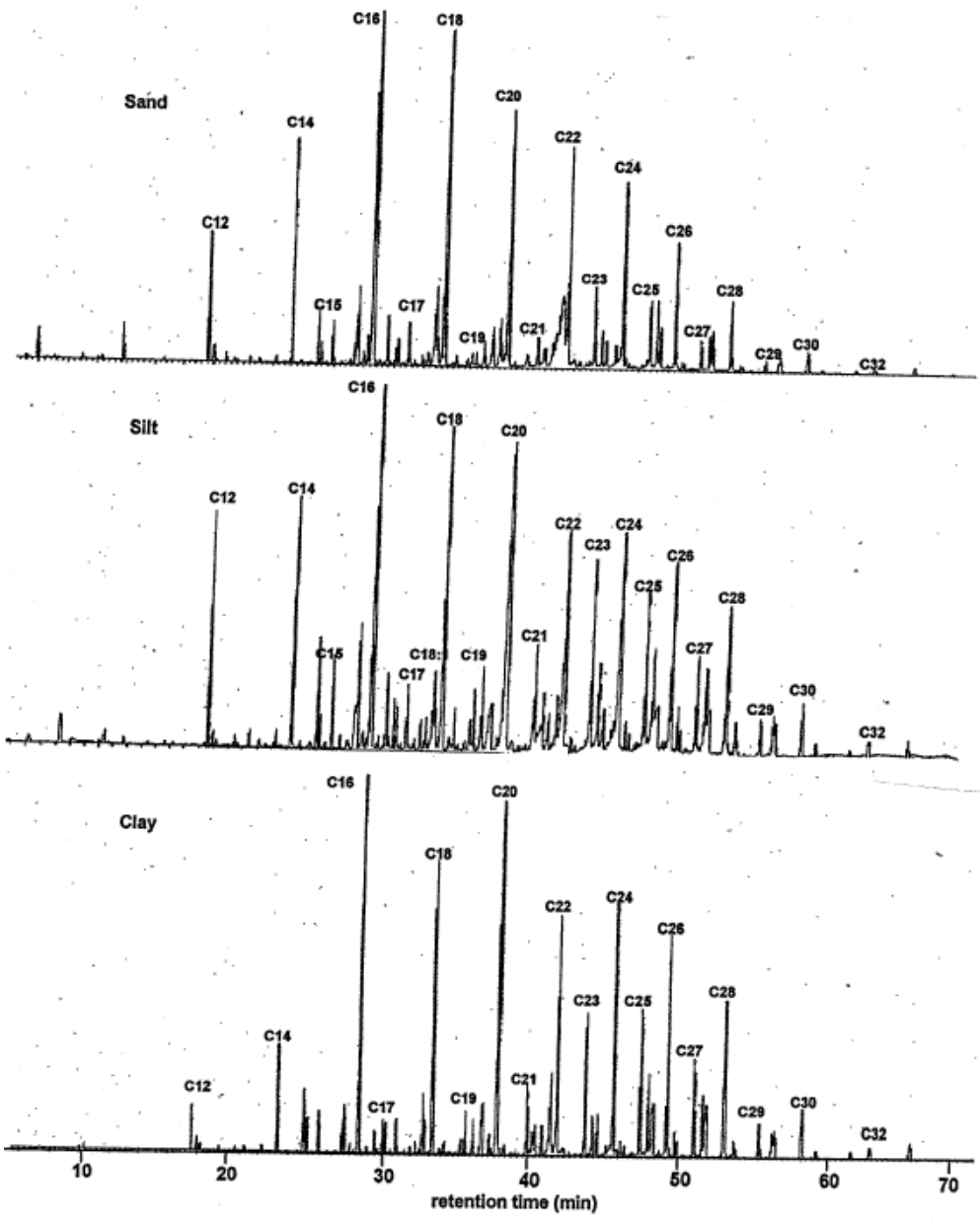



Fig 1.7: Ion chromatogram (*m/z* 74) showing the distribution of the carboxylic acids as their methylesters in the saponified lipids from the three fractions after saponification

*Hydroxy acids*. Hydroxy acids dominate the TIC trace of the saponified lipids (Fig. 1.8) whereas none of these compounds were detected in the free lipids. These hydroxy acids thus exclusively correspond to ester-linked moieties in high molecular weight and/or polar lipids released by basic treatment. $\omega$– and $\beta$-hydroxy acids are identified in the three particle-

size fractions, the former being predominant. The $\omega$–hydroxy acids range from $C_{12}$ to $C_{28}$, show a substantial even-over-odd carbon number predominance for the silt and clay fractions ($CPI_{20-26}$ of 0.54 and 0.32 respectively, and only even-carbon-numbered compounds are present for the sand fraction). These acids are characterized by a high contribution of $C_{12}$, $C_{16}$, $C_{20}$, $C_{22}$ and $C_{24}$ homologues (Fig. 1.8). Similar distributions were previously reported for $\omega$-hydroxy acids released by saponification of the lipid extract of an acidic andosol (Naafs and Van Bergen, 2002), transesterification or pyrolyses of macromolecular lipids from soils (Riederer et al., 1993; Gobé et al., 2000) or by thermochemolysis of a forest soil (Nierop, 1998). It must be noted that saponification of sediments also afforded such compounds (Goosens et al., 1989; Wakeham, 1999).

Several origins can be considered for these $\omega$-hydroxy acids. Cutins and suberins are polyesters which major monomers comprise even-carbon-numbered $\omega$-hydroxy acids with chain length of $C_{16}$ and $C_{18}$ for cutins and from $C_{16}$ to $C_{26}$ for suberins (Eglinton and Hunneman, 1968; Eglinton et al., 1968; Kolattukudy, 1980; Holloway, 1983; Matzke and Riederer, 1991; Santos-Bento et al., 2001). $C_{16}$ and $C_{18}$ dihydroxy acids along with a $C_{18}$ unsaturated hydroxy acid are also typical constituents of cutins. In addition, polyesters of $C_{12}$-$C_{18}$ even ω-hydroxy acids are common constituents of gymnosperm waxes (Tulloch, 1976) and homologues with up to 30 carbons have also been reported as constituents of conifer waxes (Murray and Schoenfeld, 1955). The occurrence of a series of $\omega$-hydroxy acids in the saponified lipids thus probably reflects contribution of the aforementioned polyesters and especially of suberins. Indeed, a similar distribution was reported for the $\omega$-hydroxy acids from suberin in periderm depolymerizates (Matzke and Riederer, 1991). This strongly suggests a marked input of root- and bark-derived products in the saponified lipids from the three particle-size fractions. Such a root contribution in SOM was also recently demonstrated by pyrolysis of a forest soil (Nierop, 1998). In addition, cutin contribution is supported by the presence of a $C_{18:1}$ $\omega$-hydroxyacid in the saponified lipids from the three particle-size fractions and of $C_{16}$ and $C_{18}$ $\omega$-dihydroxy acids in the silt and clay fractions. Along with these direct biological sources, it cannot be excluded however that a part of these $\omega$-hydroxy acids originates from microbial hydroxylation of fatty acids. Indeed, $\omega$-hydroxy acids are considered as intermediates in the formation of $\alpha,\omega$-diacids. However, no direct relationship can be established between the distribution of the fatty acids (Fig. 1.2) and that of the $\omega$-

hydroxy acids, thus showing that at most a minor fraction of the $\omega$-hydroxy acids should originate from this pathway.

$C_{24}$ and $C_{26}$ $\beta$-hydroxy acids were identified, as their methyl esters trimethylsilyl ether derivatives in the saponified lipids from the sand fraction. These compounds show a wider distribution for the silt and clay fractions ($C_{12}$-$C_{29}$) with a pronounced even-over-odd predominance. Short chain ($C_{12}$-$C_{20}$) $\beta$-hydroxy acids occur as constituents of structural biopolymers of many microorganisms including bacteria, yeasts, fungi and protozoa (Ratledge and Wilkinson, 1988). Based on their distribution, the shortest $\beta$-hydroxy acids of the silt and clay fractions may originate from such biopolymers. However, hydroxy acids are also known to be intermediates in oxidative degradation of fatty acids, the $\beta$-oxidation pathway being more important than the $\alpha$-one, hence a generally higher abundance of $\beta$-hydroxy acids over the $\alpha$-isomers. The occurrence of long chain fatty acids in the silt and clay fractions supports such an origin for the $\beta$-hydroxy acids. It must be noted that no $\alpha$-hydroxy acids could be detected in these saponified lipids from any fraction. In the fine fractions, a double origin (microbial structural biopolymers and fatty acid oxidation) can be considered for the shortest $\beta$-hydroxy acids, whereas the longest compounds likely result from the latter process. The restricted range of the $\beta$-hydroxy acids (only $C_{24}$ and $C_{26}$) in the saponified lipids of the sand fraction makes their origin less clear.

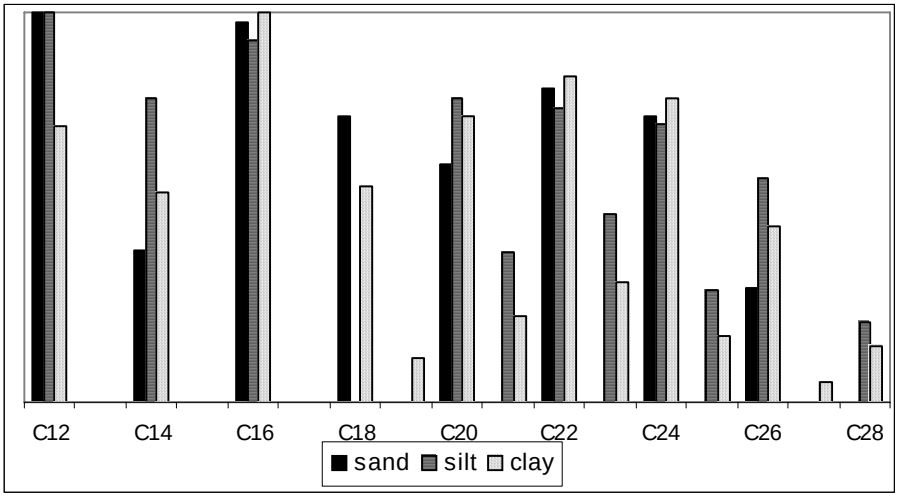



Fig 1.8 : $\omega$-hydroxy acid distribution of the saponified lipids from the three particle-size fractions

*Diacids.* Whereas only a few diacids ($C_8$ and $C_9$) were present in the free lipids, a series of *α,ω*-diacids was observed in the saponified lipids of each particle-size fraction. This series ranges from $C_{16}$ for the sand fraction ($C_{11}$ for the silt and $C_{14}$ for the clay) to $C_{29}$ with even-over-odd carbon number predominance ($CPI_{22-26}$=0.22 in the sand, $CPI_{20-28}$=0.45 in the silt and $CPI_{20-28}$=0.31 in the clay) and a maximum at $C_{22.}$ Diacids with a similar distribution were also identified in the saponified lipids from an anmoor soil (Amblès et al., 1991), pyrolysates of macromolecular lipids from an acidic anmoor soil (Gobé et al., 2000) and in trace amounts after saponification of an acidic andosol (Naafs and Van Bergen, 2002).

Two main origins are generally considered for these compounds:

- $C_{16}$ to $C_{22}$ α,ω-diacids are major components of suberins (Eglinton and Hunneman, 1968; Eglinton et al., 1968; Kolattukudy, 1980; Holloway, 1983; Matzke and Riederer, 1991; Santos-Bento et al., 2001) and their occurrence in soil lipids would thus reflects input from roots and bark.

- *α,ω*-diacids are formed by microbial oxidation of fatty acids, with the intermediate formation of *ω*-hydroxy acids (Eglinton and Hunneman, 1968; Hita et al., 1996).

The distribution observed for the *ω*-hydroxy acids points to a predominant contribution of suberin and thus supports a suberin origin for the *α,ω*-diacids. However, based on similarities in the distributions of the *ω*-hydroxy acids and the *α,ω*-diacids, the formation of the latter by microbial oxidation cannot be excluded.

*n-alkan-1-ols.* In the saponified lipids from the three fractions, a series of primary *n*-alcohols with an even-over-odd carbon number predominance ($CPI_{23-26}$=0.5) is detected as trimethylsilyl ethers, ranging from $C_{16}$ to $C_{28}$ (Fig. 1.9). Such even-carbon-numbered alkanols with a similar range were identified in the saponified lipids from two acidic soils (Amblès et al., 1991), upon saponification of an acidic andosol (Naafs and Van Bergen, 2002) and pyrolyses of macromolecular lipids from an acidic anmoor soil (Gobé et al., 2000).

The distribution of the alkanols in the saponified lipids being markedly different from that observed for the free lipids (Fig. 1.5), these alcohols appear to mainly correspond to moieties occurring in high molecular weight, non GC-amenable molecules. Long chain alkanols are typical constituents of higher plant wax esters and $C_{20}$-$C_{32}$ compounds with predominance of the $C_{22}$ and $C_{24}$ homologues occur in suberins (Eglinton and Hunneman, 1968; Eglinton et al., 1968; Matzke and Riederer, 1991; Nierop, 1998; Santos-Bento et al., 2001). It is thus likely that most of the alkanols observed in the saponified lipids are directly

derived from such biomolecules. However, incorporation of microbially altered compounds into high molecular weight lipids should also be considered. The oxidation of *n*-alkanes can however be ruled out since it would lead to odd-carbon-numbered alkanols as stressed by (Amblès et al., 1989a). In contrast, microbial reduction of long chain fatty acids may have played a role in alkanol formation as suggested by Jambu et al. (1993).

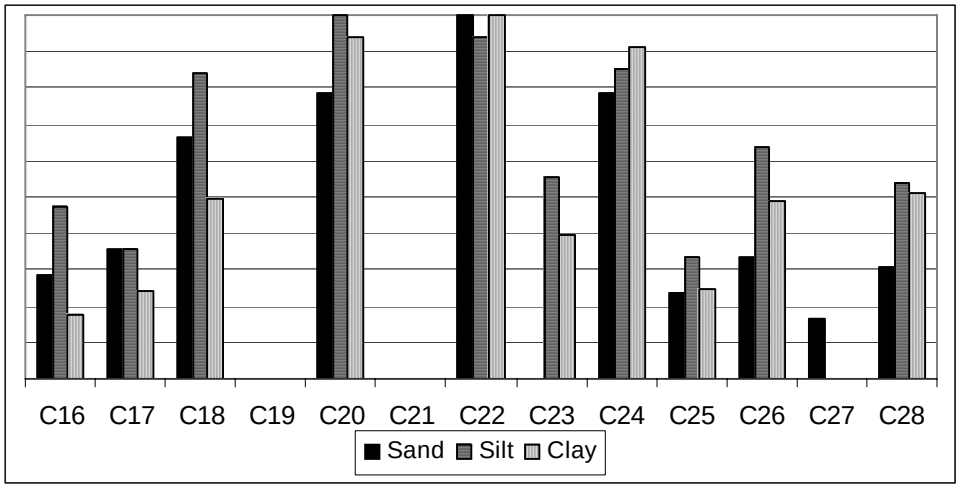



Fig 1.9 : *n*-alkan-1-ol distribution in the saponified lipids from the three particle-size fractions

*Dehydroabietic acid.* Dehydroabietic acid is detected, after saponification in the lipids of the silt and clay fractions, this acid is widely used as conifer biomarker (Simoneit, 1986). Indeed, it originates from the oxidation of dehydroabietan which occurs as a natural component of resins. The occurrence of this acid in the saponified lipids reveals that it has been incorporated in high molecular weight and/or polar lipids upon humification. It must be noted that this specific marker of the vegetation is not detected in the sand.

Whereas the free lipids show remarkable differences in composition between the three particle-size fractions, the saponified lipids are rather similar. Indeed, fatty acids, hydroxy acids, diacids and alkanols almost exhibit the same distributions in the saponified lipids whatever the considered fraction. As discussed above, these compounds mainly originate from cutin, suberin and wax esters. Due to their high intrinsic resistance, these biopolymers and high molecular weight lipids remain largely unaltered even when not protected within a fine-grained mineral matrix. The survival of cutin and suberin was also evidenced through lipid analysis in lacustrine sediments (Eglinton et al., 1968). In contrast, low molecular weight

compounds recovered as free lipids are preferentially preserved in the fine fractions, especially the clay one.

## 1.2. Conclusion

Particle-size fractionation provided new insight in both the lipid sources and their preservation mechanisms. The main results of this study are the following:

- Lipids are preferentially located in the fine fractions, especially in the clay one
- Lipid sources show differences between the 3 particle-size fractions, with predominant bacterial contribution in the sand fraction and higher plant input dominating the fine fractions as shown by fatty acid, alkane and *n*-alkan-1-ol distributions. In addition, lignin degradation products (aromatic acids) are observed in the silt and clay fractions. The free lipids of the latter two fractions also contain *n*-alkan-2-ones, probably originating from oxidation reactions catalysed by the mineral phase.
- A differential degradation of free lipids depending on the fraction size is revealed with a low level of degradation in the sand fraction globally due to major input of fresh OM, substantial degradation in the silt fraction and a preferential preservation in the clay fraction as revealed for example by the persistence of short alkanes and unsaturated acids. This is likely due to adsorption or physical protection provided by the fine minerals as commonly considered for soils and sediments. Thus, Meyers and Ishiwatari (1993) have assigned the high quantity of alkanes and acids observed in the lipids of clay-rich samples of Recent sediment to the sorption capacity of clay, and Schulten and Schnitzer (1990) reported a large retention capacity of clay in various soils.
- An intrinsic resistance to biodegradation of biopolymers such as cutin, suberin and wax esters was indicated by the lack of differential degradation observed for the cutin-bound moieties in these compounds depending on fraction-size, in contrast with free lipids. These bound moieties, released by saponification of these three extracts, include fatty acids, $\omega$-hydroxy acids, $\beta$-hydroxy acids, $\alpha,\omega$-diacids and *n*-alkan-1-ols. Dehydroabietic acid, a biomarker of conifer resin was also detected from the silt and clay fractions.

- Incorporation of resistant molecules and some microbial degradation products to high molecular weight structure during humification was also reflected by lipid analyses.

## II MATIERE ORGANIQUE REFRACTAIRE

L'étude de la MO fossile a permis de mettre en évidence, à côté du mécanisme classique par dégradation-recondensation, un nouveau mécanisme de préservation dit de préservation séléctive (Tegelaar, 1989a). Ce mécanisme est basé sur le fait que certaines macromolécules organiques sont sélectivement préservées durant les processus de sédimentation. Tegelaar et al. (1989a) ont rapporté que la préservation sélective a joué un rôle majeur dans la formation des kérogènes. Ainsi, des structures ayant survécu à des échelles de temps géologiques présentent une structure chimique bien particulière constituée de chaînes polyméthyléniques liées par des liaisons éthers. Ces structures, des algaenans, ont pu être isolées par des hydrolyses drastiques à partir de biomasse algale. Par la suite d'autres structures aliphatiques hautement résistantes ont été mise en évidence par des méthodes d'isolement similaires dans les cuticules de plantes (Nip et al., 1986; McKinney et al., 1996) et de tissus subérisés (Tegelaar et al., 1995). Ces molécules présentent une grande similitude de structure, ce qui a permis d'envisager une corrélation entre structure et stabilité. Ainsi l'hypothèse d'un lien entre résistance chimique et résistance à la biodégradation a été émise. Il a été envisagé que certaines macromolécules naturelles pouvaient persister dans l'environnement (De Leeuw et Largeau, 1993). Derenne et Largeau (2001) ont ainsi discuté les degrés de résistance de six familles de macromolécules considérées comme réfractaires aussi bien aux hydrolyses qu'à la biodégradation.

En raison de son caractère insoluble, l'humine a été considérée comme la fraction des substances humiques susceptibles d'être chimiquement réfractaire. L'humine, qui peut constituer plus de 80% des MOS, est par ailleurs souvent considérée comme stable (Almendros et Sanz, 1992). Cette stabilité est importante, non seulement car ce carbone organique n'est que très peu sensible aux perturbations de l'environnement (facteurs anthropiques ou climatiques) et est donc stocké durablement dans les sols, mais les substances liées à cette MO (par adsorption ou formation de complexes), comme les contaminants (Cf partie A)**,** seront par la même occasion immobilisés durablement. Le risque de transfert dans la chaîne trophique est alors écarté.

L'estimation quantitative de cette MO stable est essentielle à la mise en place de modèle du cycle du carbone. De plus, du fait des possibilités d'interaction de la MO stable avec les polluants, l'amélioration de la compréhension des mécanismes d'interaction mais

aussi de la dynamique de la MO stable est nécessaire. Une meilleure connaissance de la composition et de la structure chimique de cette MO stable paraît dès lors indispensable. La principale difficulté est d'isoler cette fraction stable. Il a alors été suggéré que, comme dans la MO sédimentaire, la MO réfractaire de sols résistante à des traitements drastiques était stable. Diverses méthodes ont alors été appliquées pour isoler une fraction réfractaire de la MOS. Une fraction des substances humiques isolée de diverses façons s'est révélée être très ancienne (Leavitt et al., 1996; Paul et al., 1997; Poirier et al., 2000; Paul et al., 2001; Poirier et al., 2002; 2003). La caractérisation de cette MOR est délicate en raison de la complexité et de l'inertie chimique de cette fraction. Il est donc essentiel de mettre en œuvre une combinaison de méthodes analytiques.

Dans ces chapitres, nous avons examiné la structure et la composition chimique de la MOR du sol forestier. Dans une première partie, nous avons recherché des informations structurales globales, grâce aux méthodes spectroscopiques (IRTF, RMN $^{13}C$ à l'état solide), puis nous avons analysé sa composition moléculaire par pyrolyse au point de Curie couplée à une chromatographie gazeuse et spectrométrie de masse. Dans une seconde partie, nous avons employé une succession de dégradations chimiques en utilisant plusieurs oxydants.

## 2.1 Caractéristiques spectroscopiques et pyrolytiques de la fraction macromoléculaire réfractaire d'un sol forestier

Les analyses spectroscopiques et pyrolytiques sont largement répandues dans l'étude des macromolécules naturelles. Elles ont été appliquées aussi bien à l'étude de MO sédimentaire telle que le kérogène (e.g. Largeau, 1986; Deniau et al., 2001), que dans l'étude des substances humiques : acides humiques, acides fulviques, humine (e.g. Saiz-Jimenez et De Leeuw (1987), Schulten and Schnitzer (1992), Martin et al. (1995), Fabbri et al. (1996), Grasset et Amblès (1998c), Chefetz et al. (2002)). Elles ont permis de fournir de nombreux renseignements aussi bien sur leur composition chimique que sur leur structure. Schulten et Schnitzer (997) en ont d'ailleurs déduit un modèle théorique de structure de la MOS et du sol.

Des études précédentes sur la MOR de la fraction granulométriques inférieure à 50µm d'un sol des Landes et d'un sol ferralitique du Congo ont révélé un important degré d'humification (Poirier et al., 2000; 2002).

Nous avons à présent porté notre attention sur la MOR du sol total. Celle-ci a été isolée (Cf protocole 3.2.2), quantifiée et sa structure chimique a été examinée en combinant les résultats d'analyse élémentaire, d'analyses spectroscopiques (IRTF et RMN $^{13}C$ à l'état solide) et d'analyses pyrolytiques (conventionnelle et en présence de TMAH).

### 2.1.1 Results and discussion

#### 2.1.1.1. Refractory organic matter abundance and elemental composition

Efficient demineralization was achieved by the HF/HCl treatment, as indicated by the low content of residual ash of only 4wt% retained in the isolated material. Analysis of the refractory organic matter (ROM) showed a TOC content of 58.7wt% and 1.59 g of ROM (corrected for ash) were obtained from 100 g of sieved soil. From the above values and the TOC content of the soil (2.6 wt%), it appeared that the carbon of the isolated ROM represents 34% of the total initial carbon. The non-hydrolysable insoluble macromolecular fraction thus accounts for a substantial part of the whole organic matter of the soil. Nitrogen is

preferentially eliminated during ROM isolation, due to hydrolysis of N-containing components such as proteinaceous materials. This preferential removal is reflected by the markedly higher C/N atomic ratio of the isolated ROM: 61 against 30 for the OM of the sieved soil. Elemental analysis showed an H/C atomic ratio of only 0.9 for the isolated material. This relatively low value ruled out a major contribution of aliphatic moieties, such as alkyl chains, in the macromolecular structure of the ROM from the Cestas soil.

### 2.1.1.2. ROM spectroscopic features

The FTIR spectrum (Fig.2.1.1) exhibits relatively weak bands at 2970, 2925 and 2850 $cm^{-1}$ corresponding to alkyl groups (symmetric and asymmetric C-H stretching in $CH_2$ and $CH_3$), in agreement with the relatively low aliphaticity inferred from elemental analysis data. The spectrum is dominated by a broad band centred at 3400 $cm^{-1}$ (OH groups), absorptions at 1710 $cm^{-1}$ (C=O functions), 1600 $cm^{-1}$ (predominantly aromatic C=C bonds) and several bands in the 1050-1260 $cm^{-1}$ range (ethers and/or alcohols C-O bonds).

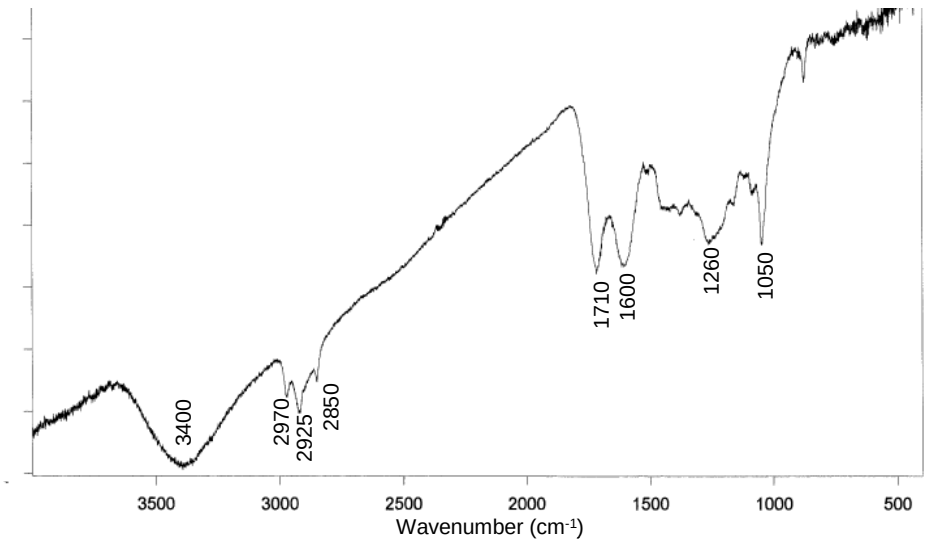



Fig 2.1.1: Fourier transform infrared spectrum of the Cestas ROM.

The solid-state $^{13}C$ NMR spectrum (Fig. 2.1.2) is dominated by an aliphatic peak in the 10-40 ppm range with a maximum at 30 ppm corresponding to methylenic carbon atoms in aliphatic chains. This feature may seem at variance with the lack of predominance for such aliphatic structures showed by elemental and FTIR data. However, it is well documented that the relative abundance of the aliphatic moieties can be largely overestimated, by CP/MAS

solid state $^{13}C$ NMR spectroscopy, in macromolecular material that also contain condensed units such as poly aromatic and highly cross-linked structures. Indeed, carbon atoms in the latter structures cannot be “seen” by this spectroscopic method, when they are too far from protons, since they cannot be reached by spin diffusion. Such a bias was observed for various types of natural materials and for mixtures of model compounds (e.g. Hatcher et al. (1981), Wilson et al. (1987), Oades et al. (1987), Preston et al. (1997), Poirier et al. (2000)). In contrast to this lack (or incomplete) detection of the carbon atoms in condensed units, the carbon atom in aliphatic chains are very efficiently detected so that their relative abundance is largely overestimated.

In addition to the signal at 30 ppm, the ROM spectrum exhibits several relatively intense peaks. The peaks at 72 ppm (oxygen-linked carbon atoms in pyran units) and 104 ppm (anomeric carbon atoms in glycosidic linkages) reflect the presence of polysaccharides like cellulose (e.g. Pastorova et al. (1994), Preston (1996)). The peak around 85 ppm is attributed to aliphatic carbon atoms in C-O bonds and they might be also related to polysaccharides. The pronounced shoulder at 150 ppm (oxygen-linked aromatic carbon atoms) and the peak at 56 ppm (methoxyl groups) reflect the presence of lignin (e.g. Hatcher (1987); Preston (1996)). Condensed tannins may also contribute to the 150 ppm shoulder. The main $^{13}C$ resonances of such compounds are a peak around 105 ppm (non-protonated aromatic carbon atoms $\alpha$ to oxygen-linked aromatic carbon atoms) and a double peak at 145-155 ppm (phenolic carbon atoms) (Wilson and Hatcher, 1988; Preston et al., 1997). These peaks interfere with resonances from polysaccharides and lignin, respectively, so that clear-cut evidence for condensed tannin occurrence in the ROM can hardly be derived from NMR data. However, the broadness of the shoulder around 150 ppm points to some tannin contribution to the Cestas ROM. The broad resonance in the 110-140 ppm range can be attributed to olefinic and/or aromatic carbons; it chiefly corresponds to the latter based on the FTIR data and includes the aromatic carbon atoms of lignin and condensed tannins not substituted by oxygen. The broad peak centred at 170 ppm is attributed to carbon atoms in C=O bonds of esters and/or acids.

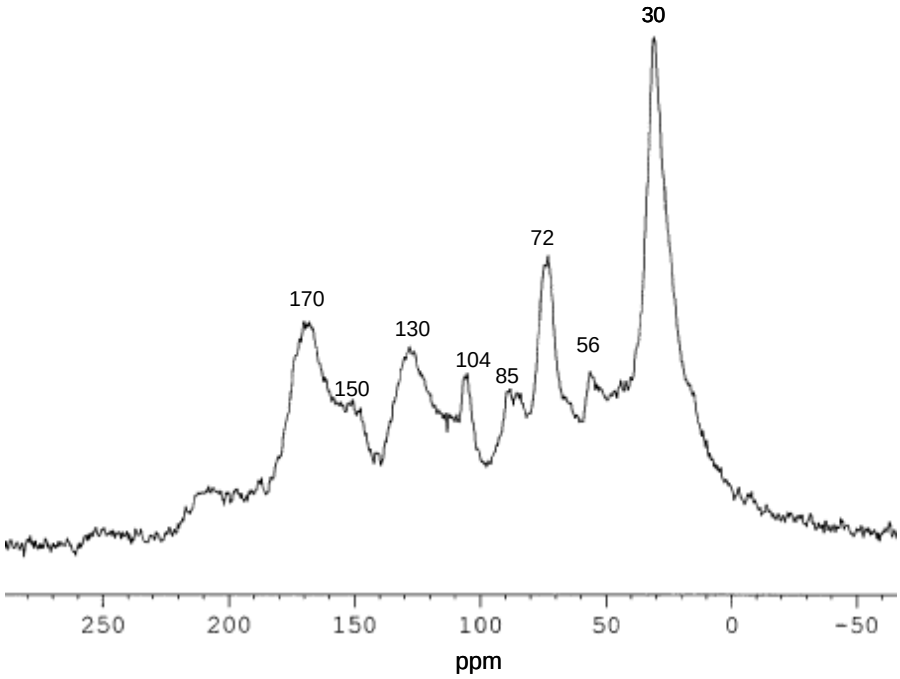



Fig 2.1.2: Solid-state 13C NMR spectrum of the Cestas ROM

Taken together, the FTIR and solid-state $^{13}C$ NMR spectra indicate the occurrence of a large variety of oxygen-containing groups. In addition, $^{13}C$ NMR reveals the presence of lignin and of polysaccharides attributed to cellulose and the NMR data also points to the presence of condensed tannins. It is well documented that most lignin fractions are not solubilized by hydrolysis (e.g. Kögel-Knabner et al., 1994; Poirier et al., 2003). Cellulose resists TFA treatments (Amelung et al., 1996) but it can be hydrolysed by heating with strong acids like HCl unless under a highly crystalline form. The survival of a cellulose fraction in the isolated ROM is therefore probably related to lignin occurrence and the resulting protection of cellulose microfibrils within the so-called ligno-cellulose complex and/or to a high degree of cristallinity. Condensed tannins are ubiquitous in higher plants and highly resistant to hydrolysis (De Leeuw and Largeau, 1993). Such aromatic compounds were previously shown to contribute to the refractory fraction of the loamy forest and crop soils examined by Poirier et al. (2000, 2003).

## 2.1.2. ROM thermal degradation

### 2.1.2.1 Conventional pyrolysis (CuPy-GC/MS)

The total ion current (TIC) trace obtained by CuPy-GC/MS is dominated by levoglucosenone (peak 11) (Fig 2.1.3 and Table 2.1.1). Several other typical pyrolysis products of polysaccharides (e.g. Pouwels et al., 1989; Pastorova et al., 1994), i.e. anhydrosugars and furanic compounds, are also observed in large relative amounts: 2-furancarboxaldehyde (3), methylfurancarboxaldehyde (5), dihydrobenzofuran (17) and levoglucosan (23). The lack of significant amounts of levulinic acid indicated that the cellulose fraction was not significantly altered upon the acid treatments since the latter compound is the major pyrolysis product of acid-altered saccharides (Almendros et al., 1997). This lack of alteration of cellulose in the isolated ROM is likely related to protection by lignin.

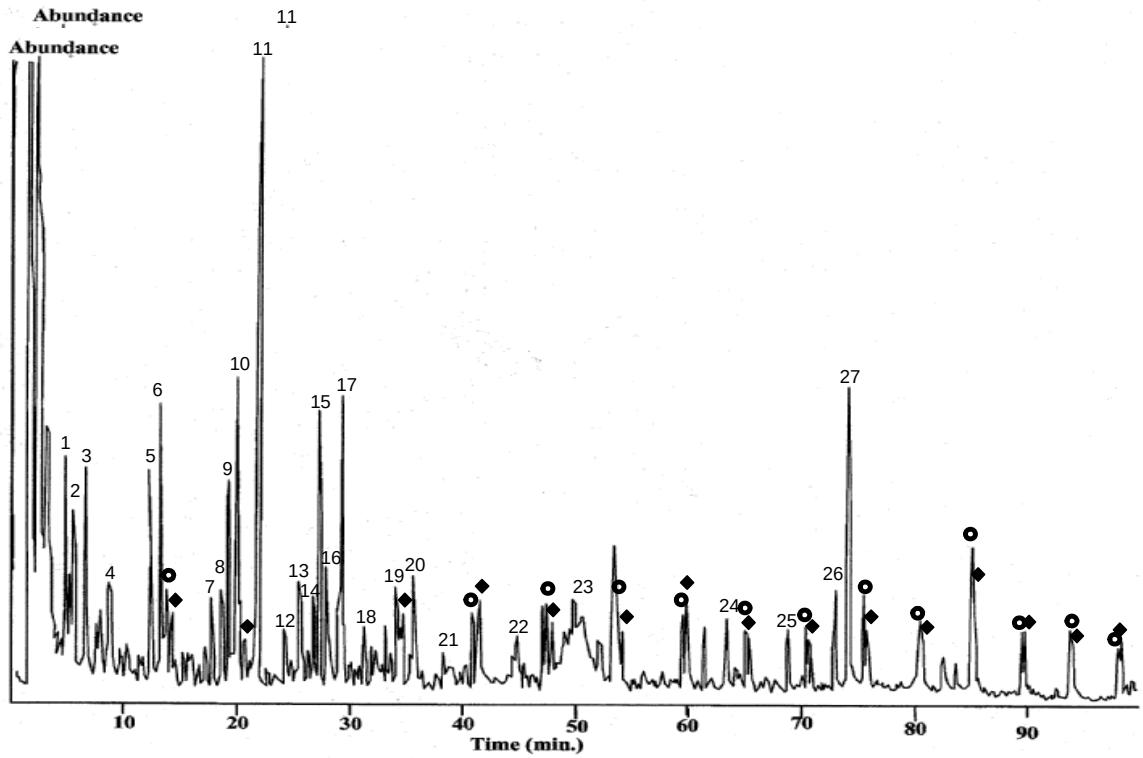



Fig 2.1.3: Total ion current trace of the 650°C Curie point pyrolysate of the Cestas ROM. See Table 2.1.1 for the identification of the numbered peaks; n-alkanes (◆), *n*-alk-1-enes (⭘).

| Peak number | Compound | Origin |
|---|---|---|
| 1 | ethylbenzene | |
| 2 | dimethylbenzene | |
| 3 | 2-furancarboxaldehyde | Ps |
| 4 | propylbenzene | |
| 5 | methylfurancarboxaldehyde | Ps |
| 6 | phenol | (L) |
| 7 | 2-methylphenol | (L) |
| 8 | acetophenone | L |
| 9 | 3-methylphenol | (L) |
| 10 | 2-methoxyphenol | L |
| 11 | levoglucosenone | Ps |
| 12 | 2-4-dimethylphenol | (L) |
| 13 | 3-ethylphenol | (L) |
| 14 | naphthalene | |
| 15 | 4-methyl-2-methoxyphenol | L |
| 16 | 1,2-benzenediol | (L) |
| 17 | dihydrobenzofuran | Ps |
| 18 | 4-methyl-1,2-benzenediol | (L) |
| 19 | 4-ethyl-2-methoxyphenol | L |
| 20 | 4-ethenyl-2-methoxyphenol | L |
| 21 | 2,6-dimethoxyphenol | L |
| 22 | 4-propenyl-2-methoxyphenol | L |
| 23 | levoglucosan | Ps |
| 24 | $C_{14}$ acid | |
| 25 | $C_{15}$ acid | |
| 26 | $C_{16:1}$ acid | |
| 27 | $C_{16}$ acid | |
| ○ | *n*-alk-1-enes | |
| ◆ | *n*-alkanes | |

Table 2.1.1: Products formed upon 650°C Curie point pyrolysis of the Cestas ROM and their origin (L: Lignin, Ps: Polysaccharide, (L): can also partly originate from other sources, like polysaccharides and tannins)

A number of phenolic products (phenol, alkylphenols, methoxyphenols and benzenediols) are also observed in the ROM pyrolysate, including several compounds with high relative abundances (Fig 2.1.3 and Table 2.1.1). 2-Methoxyphenol (10, one of the main

peaks of the trace), 2,6-dimethoxyphenol (21) and $C_1$- to $C_3$-substituted methoxyphenols (15, 19, 20 and 22) are typical products of lignin, the mono- and dimethoxylated compounds being derived from guaiacyl and syringyl units, respectively (e.g. Saiz-Jimenez and De Leeuw, 1986, Hatcher and Clifford, 1997). Acetophenone (8) is also a typical product in lignin pyrolysates.

Phenol (6) and $C_1$- and $C_2$-alkylphenols (7, 9, 12 and 13) are ubiquitous pyrolysis products that can be generated from several groups of biomacromolecules, including lignin, polysaccharides and tannins (Saiz-Jimenez and De Leeuw, 1986; Almendros et al., 1997; van Heemst et al., 1999). Some of these compounds in the ROM pyrolysate are likely related to lignin. The occurrence of these phenols suggests that a part of the lignin moieties in the ROM underwent alteration since demethoxylation is a major process during the diagenetic alteration of lignin (e.g. Hatcher, 1988). The presence of some altered lignin moieties is also supported by the occurrence in the pyrolysis products of 1,2-benzenediol (16) and of 4-methyl-1,2-benzenediol (18) that can originate from demethylated and/or demethoxylated units in degraded lignin (Gonzalez-vila et al., 1996); however, these two compounds could also originate from condensed tannins (Galletti and Reeves, 1992). The high relative abundance of the methoxyphenols and of the other phenolic compounds in the pyrolysate supports a substantial contribution of lignin to the ROM, including both unaltered and altered lignin. Some condensed tannins also probably contribute to this refractory fraction.

Weak peaks corresponding to *n*-alkane/*n*-alk-1-ene doublets were detected in the pyrolysate (Fig. 2.1.3). The mass chromatogram of *m/z* 57 shows a homologous series of doublets ranging from $C_9$ to $C_{32}$ with a maximum in the $C_{10}$-$C_{23}$ range (Fig. 2.1.4). No significant odd- or even-carbon-number predominance was observed in the latter range whereas the longer homologues, from $C_{23}$, are predominantly odd-carbon-numbered. *n*-Alkane/*n*-alk-1-ene doublets commonly occur in the pyrolysates of natural macromolecular organic materials (e.g. Largeau et al., 1986) where they chiefly originate from the homolytic cleavage of C-C bonds in polymethylenic chains. It thus appears that some polymethylenic chains, including long chains up to $C_{32}$ contribute to the macromolecular structure of the ROM. The occurrence of such chains was previously observed upon pyrolysis of various fractions of soil organic matter ranging from complex lipids (Gobé et al., 2000) to the insoluble non-hydrolysable fraction (Augris et al., 1998). Different possible sources were considered for these chains : highly aliphatic, non-hydrolysable, biomacromolecules of higher plants, i.e. cutan or suberan (Tegelaar et al., 1989b; 1995;

Van Bergen et al., 1997; Augris et al., 1998; Nierop, 1998), or of micro-organisms (Lichtfouse et al., 1995) and lipids condensed in resistant macromolecular structures (Almendros et Sanz, 1992; Almendros et al., 1996). The distribution of the *n*-alkane/*n*-alk-1-ene doublets generated from the ROM is characterised by an extended carbon number range and a wide maximum with similar intensities except for the longest homologues. Similar features were observed both for cutans and suberans (Tegelaar et al., 1989b; 1995). However, the distribution of the longest homologues, predominantly odd-carbon-numbered for the ROM, recalls in that range neither the distribution reported for cutans (rather regular decrease in intensity with increasing carbon number) nor that observed for suberans (sharp decrease in intensity from $C_{20}$). These similarities and differences suggest that the doublets probably originate both from cutans and/or suberans and from long chain lipids of higher plants. The latter lipids were condensed in the macromolecular structure and they would largely contribute to the longest doublets.

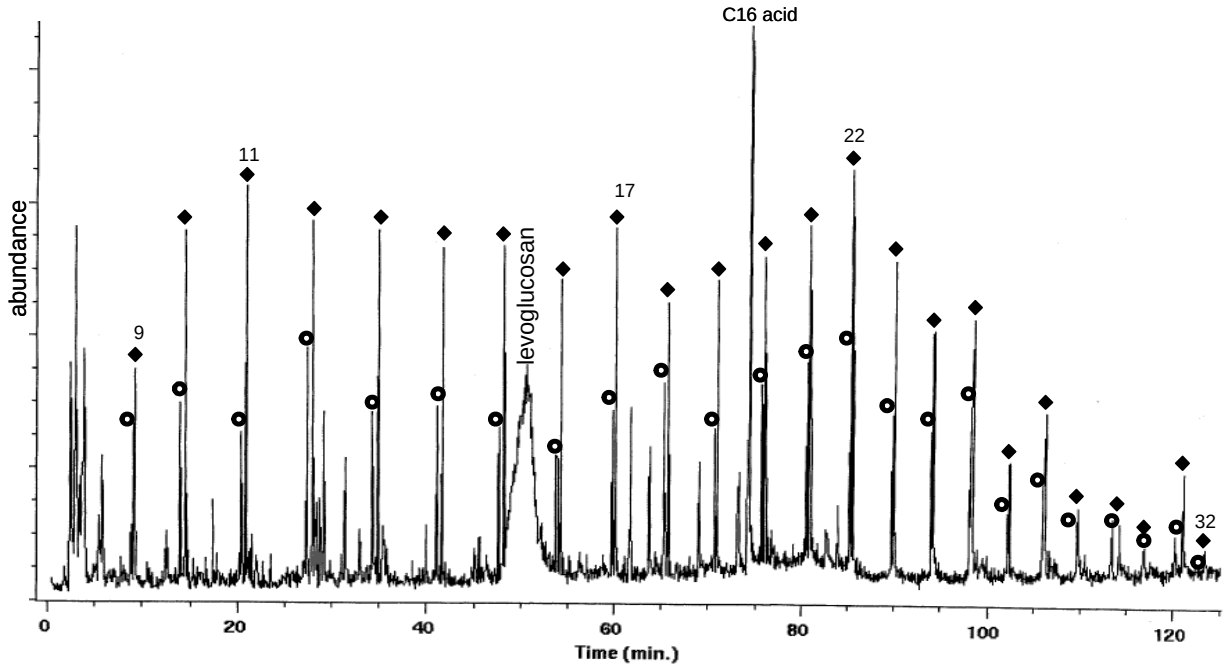



Fig 2.1.4: Ion chromatogram (*m/z* 57) showing the distribution of the *n*-alk-1-enes (◎) and *n*-alkanes (◆) in the 650°C Curie point pyrolysate of the Cestas ROM (some other products, exhibiting ions at *m/z* 57, like levoglucosan and palmitic acid methyl ester, as also observed on the trace).

Whatever their origin, polymethylenic chains only afford a minor contribution to the refractory material of the Cestas soil. Indeed, the *n*-alkane/*n*-alk-1-ene doublets only occur in

low relative amounts in the pyrolysate. Furthermore, the effective contribution of the aliphatic chains is still lower than suggested by the relative abundance of the doublets, because pyrolysis tends to overestimate such aliphatic moieties compared with other moieties like polysaccharidic and (to a lesser extent) lignin (Poirier et al., 2000). This bias is due to the markedly higher yield of GC-amenable volatile pyrolysis products generated from the aliphatic moieties. The $C_2$- and $C_3$-alkylbenzenes (1, 2 and 4) may originate from cyclisation/aromatization of aliphatic chains as commonly observed in pyrolysates (e.g. Largeau et al., 1986). Relatively large amounts of palmitic acid (27) occur in the pyrolysate along with minor amounts of $C_{14}$, $C_{15}$ and $C_{16:1}$ fatty acids (24-26). These acids originate from the thermal cleavage of esters that were sterically protected in macromolecular structures of the ROM and, hence, not hydrolysed by the extensive acid treatments applied for ROM isolation.

#### 2.1.2.2. Thermochemolysis (THM-GC/MS)

Thermally assisted hydrolysis and methylation (THM) in the presence of TMAH has been largely applied, in the recent years, to a wide range of macromolecular materials, including soil organic matter and the refractory fraction of recent sediments (e.g. Schulten et Sorge, 1995; Nierop, 1998; Garcette-Lepecq et al., 2001). Compared to conventional pyrolyses, THM affords a more efficient cleavage of macromolecular structures, especially those with oxygen-containing linkages like esters or ethers. Furthermore, the carboxylic acid groups and the hydroxy groups are methylated in situ, thus making GC-amenable a number of polar pyrolysis products. In addition, secondary reactions like decarboxylation and dehydration are limited (e.g. Challinor, 1994; Clifford et al., 1995; del Río et al., 1996).

The TIC trace obtained by THM (TMAH)-GC/MS of the Cestas ROM shows a large number of components (Fig. 2.1.5a and table 2.1.2). The first part of the chromatogram (Fig 2.1.5b) chiefly shows the methylated counterparts of the phenolic compounds, derived from lignin, that were observed in the conventional pyrolysate. These methylated products correspond to most of the identified methoxy- and dimethoxybenzenes (9, 11, 20, 23, 24, 30 and 35). Such compounds are typical products generated upon THM of lignin through cleavage of the $\beta$-O-4 linkages by TMAH (Hatcher et Minard, 1995). The occurrence of methoxyphenols (17, 25, 27, 28, and 34) and of phenol and alkylphenols (8, 14 to 16, 19, 21 and 22) indicates that methylation was incomplete. This was previously observed in THM

experiments, for example with standard lignin (Gauthier et al., 2003) and attributed to insufficient contact between the reagent and the examined solid materials. This problem should be especially important with non-hydrolysable macromolecular materials like the ROM studied here. Indeed, this refractory material contains potentially labile linkages, such as ethers, not affected by acid attacks during the isolation treatment due to efficient steric protection. TMAH access to these linkages is also probably difficult so that the corresponding products are partly generated through purely thermal reactions. In addition to phenolic compounds, THM of lignin results in the production of methyl esters of aromatic carboxylic acids, including various methoxy-substituted products, not formed upon conventional pyrolyses (Clifford et al., 1995; Martin et al., 1995). Several of these compounds were generated from the ROM (26, 36, 37, 38, 40, 42-45) with 42 and 44 dominating the first part of the trace. 3,4-Dimethoxyacetophenone (41) and 3,4-dimethoxybenzaldehyde (39) are also methylated THM products related to lignin.

Previous studies indicated that products related to carbohydrates are generally lacking in TMAH thermochemolysates (Clifford et al., 1995). In agreement with these observations, none of the numerous polysaccharide-derived compounds that abundantly occurred in the conventional pyrolysate were generated upon THM of the ROM

The second part of the trace shows the most abundant peaks (Fig. 2.1.5a). These compounds chiefly correspond to the methylated counterparts, as methyl esters and methyl ethers, of normal carboxylic acids: alkanoic acids, alkenoic acids, ω-hydroxy alkanoic acids and α,ω-alkanedioic acids (Fig 2.1.5a). These acids, except for palmitic acid and minor amounts of $C_{14}$ $C_{15}$ and $C_{16:1}$ fatty acids, were not observed in the conventional pyrolysate. In order to indicate the lengths of the corresponding moieties, as they occur in the ROM, the carbon numbers reported below, for these four homologous series, do not take into account the methyl groups that were introduced upon THM. These groups (one or two) were added through esterification and etherification of the carboxylic and hydroxy functions, respectively. The series of alkanoic acid ranges from $C_6$ to $C_{30}$, and exhibits a trimodal distribution (maximum at $C_{16}$ and submaxima at $C_8$ and $C_{26}$). No clear-cut odd- or even-carbon-number predominance is observed in the $C_6$-$C_{13}$ range whereas the even-carbon-numbered compounds predominate for the higher homologues. The monounsaturated alkenoic acids correspond to a minor series only ranging from $C_6$ to $C_{16}$. This series shows a maximum at $C_8$ and does not exhibit any clear carbon number predominance so that its distribution is similar to the distribution of the alkanoic acids in the $C_6$-$C_{13}$ range. An abundant series of even-carbon-

numbered ω-hydroxy alkanoic acids is observed in the $C_{16}$-$C_{28}$ range with a maximum at $C_{22}$ and this compound is one of the major peaks on the second part of the trace. The α,ω-alkanedioic acids correspond to a minor series of even-carbon-numbered compounds ranging from $C_{18}$ to $C_{24}$. α,ω-Diacids can be formed by microbial oxidation of fatty acids, with the intermediate formation of *ω*-hydroxy acids (Eglinton and Hunneman, 1968; Hita et al., 1996). However marked differences in the distribution of the *n*-alkanoic acids, including the presence of both even- and odd-carbon-numbered homologues, compared to the distributions of the former two series, exclusively comprising even-carbon-numbered compounds, rules out such an origin.

In fact, these two series reveal the presence of suberin in the ROM. Indeed, ω-hydroxy alkanoic acids and α,ω-alkanedioic acids are typical products of suberin when submitted to THM (Gonzalez-vila et al., 1996; Del Rio et Hatcher, 1998). Suberins are polyesters, forming a protective barrier on the underground surfaces of plants and also occurring in bark. These insoluble biomacromolecules are mostly composed of interesterified ω-hydroxy alkanoic acids and α,ω-alkanedioic acids (e.g. Eglinton et al., 1968; Kolattukudy et al., 1976, 1980; Holloway, 1983). The resulting aliphatic domains in suberins are also covalently linked, by ester linkages, to aromatic domains composed of aromatic carboxylic acids like ferulic acid and *p*-coumaric acid. Upon conventional pyrolyses, suberins do not generate significant amounts of specific GC-amenable pyrolysis products (Gonzalez-Vila et al., 1996). On the contrary, THM results in efficient ester cleavage releasing the typical monomers of suberins (Gonzalez-Vila et al., 1996; Del Rio and Hatcher, 1998; Santos-Bento et al., 2001). The distributions observed here from the ROM, for the ω-hydroxy alkanoic acids and the α,ω-alkanedioic acids, are similar to those reported upon THM of standard suberins in the above studies. Suberin contribution to the ROM is also supported by the formation of *p*-coumaric acid and ferulic acid (44 and 45 in Fig. 2.1.5a) originating from the aromatic domains of suberin. The long chain *n*-alkanoic acids up to $C_{30}$ may also partly originate from suberin since such acids also contribute to suberin structure (e.g. Kolattukudy, 1976). This suberin fraction accounts for the broad peak centred at 170 ppm (C=O in esters) observed in the solid state $^{13}C$ NMR spectrum of the ROM (Fig. 2.1.2). A major contribution of suberin to the alkyl components of soil organic matter was inferred from solid state $^{13}C$ NMR data (Kögel-Knabner et al., 1992b). In addition, the presence of suberin was recently evidenced through THM in soil samples (Nierop, 1998; 2001) and an important contribution of root-derived components, especially of suberin, was reported by the former author for an acid sandy soil.

However, all these previous studies were performed on the bulk of the organic matter of the soil, only the lipid fraction being removed, and not on the insoluble non-hydrolysable fraction as in the present case.

Cutins, the insoluble polyesters forming a protective layer on the aerial surfaces of plants, like leaves and twigs, exhibit the same type of structures as the aliphatic domains of suberins and are also well detected by THM (De Leeuw and Baas, 1993). However, suberins and cutins are differentiated by the chain length of their monomeric units and the abundance of mid-chain hydroxy or keto functions (e.g. Kolattukudy, 1976, 1980; Holloway, 1982; Matzke and Riederer, 1991): long chains (> $C_{20}$) are dominant for suberins while shorter chains occur in cutins and much less mid-chain oxygenated groups occur for suberins compared to cutins. The distributions of the ω-hydroxy alkanoic acids and α,ω-alkanedioic acids generated from the Cestas ROM and the lack of significant amounts of mid-chain substituted products thus demonstrate that the polyester contribution in this refractory fraction corresponds to suberin rather than cutin. Such distributions, characterised by the exclusive occurrence of even-carbon-numbered homologues, also indicate that the suberin fraction of the ROM is not (or only weakly) altered. The occurrence of suberin rather than cutin within the Cestas soil is in accordance with field observations indicating that the bulk OM derived from aerial parts of the plants are remaining on the acid soil surface due to low biological activity.

Suberin, like cutin, being a polyester based on a network of interesterified monomeric units, is potentially a hydrolysable biopolymer. Thus, no biopolyesters were retained in the refractory components isolated from maize leaves by using successive TFA and HCl treatments (Poirier et al., 2003). However, some studies aimed at releasing the monomeric units, including alkaline or acid hydrolyses, only resulted in partial depolymerization of suberin and an insoluble residue was retained (e.g. Holloway, 1973). The present observations indicate that the suberin fraction of the Cestas soil (or at least a part) survived the extensive treatments applied for ROM isolation. The occurrence of this non-hydrolysable fraction should reflect either intrinsic resistance to hydrolysis and/or physical protection by some resistant components in the heterogeneous matrix of the ROM. Some increase in the relative contribution of suberin-related components to soil organic matter was previously observed with age (Rumpel et al., 2002) and also upon long-term soil acidification (Nierop et al., 2003). It was considered, by Kögel-Knabner et al. (1992b), that increased cross-linking of suberin would occur in soil. This may result in increasing resistance to biodegradation and also in chemical hydrolyses as observed in the Cestas ROM.

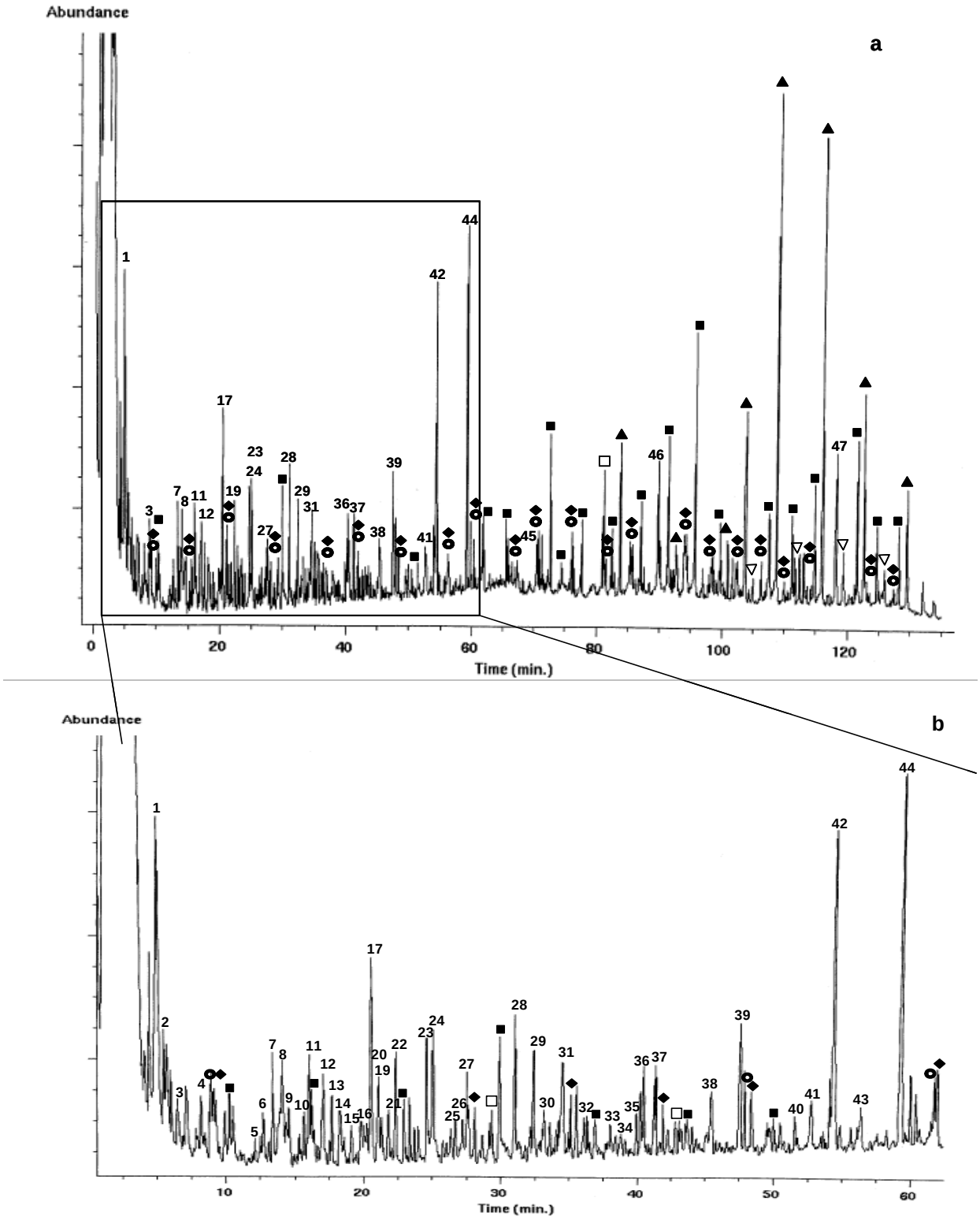



Fig 2.1.5: (a) Total ion current trace of the 650°C Curie point pyrolysate of the Cestas ROM in presence of TMAH, (b) Expanded area of the beginning of the trace. See Table 2 for the identification of the numbered peaks; n-alkanes (◆), n-alk-1-enes (◎).

| Peak number | Compound |
|---|---|
| 1 | toluene |
| 2 | ethylbenzene |
| 3 | 1,4 dimethylbenzene |
| 4 | 1,2 dimethylbenzene |
| 5 | propylbenzene |
| 6 | 1-ethyl-4-methylbenzene |
| 7 | unidentified |
| 8 | phenol |
| 9 | 1-methoxy-2-methylbenzene |
| 11 | 1-methoxy-4-methylbenzene |
| 12 | butanedioic acid |
| 13 | 1,2-ethanediol1-(2-furanyl) |
| 14 | 2-methylphenol |
| 15 | 4-methylphenol |
| 16 | 3-methylphenol |
| 17 | 4-methoxyphenol |
| 19 | dimethylphenol |
| 20 | 1-ethyl-4-methoxybenzene |
| 21 | 2,4,6-trimethylphenol |
| 22 | ethyl,methylphenol |
| 23 | 1,2-dimethoxybenzene |
| 24 | ethenyl-3-methoxybenzene |
| 25 | methyl-1,4-dimethoxyphenol |
| 26 | 3-methylbenzoic acid |
| 27 | 2-methoxy-4-methylphenol |
| 28 | 5-methoxy-2,3-dimethylphenol |
| 29 | 2-hydroxy-3-methyl benzaldehyde |
| 30 | 4-ethyl-2-methoxybenzene |
| 31 | trimethoxybenzene |
| 32 | 2-methylnaphthalene |
| 33 | 4-methyl-3-methoxy benzoic acid |
| 34 | 2,6-dimethoxyphenol |
| 35 | 1,2-dimethoxy-4-ethenylbenzene |
| 36 | 3-methoxybenzoic acid |
| 37 | 4-methoxybenzoic acid |
| 38 | 4-hydroxy-3-methoxybenzoic acid |
| 39 | 3,4-dimethoxybenzaldehyde |
| 40 | 4-methoxybenzenepropanoic acid |
| 41 | 3,4-dimethoxyacetophenone |
| 42 | 3,4-dimethoxybenzoic acid |
| 43 | 3,4-dimethoxy benzeneacetic acid |
| 44 | 3-(4-methoxyphenyl-2-)propenoic acid (p-coumaric acid) |
| 45 | 3-(3,4-dimethoxyphenyl)-2-propenoic acid (ferulic acid) |
| 46 | unidentified |
| 47 | unidentified |
| ◆ | *n*-alk-1-enes |
| ⊙ | *n*-alkanes |
| ■ | acids |
| □ | unsaturated acids |
| ▲ | ω-hydroxyacids |
| ▽ | diacids |

Table 2.1.2: Products formed upon 650°C Curie point thermochemolysis of the Cestas ROM

*n*-Alkane/*n*-alk-1-ene doublets were observed for both conventional and THM experiments and they exhibited similar distributions. However, the relative abundance of these doublets is still lower in the latter case, thus reflecting the higher efficiency of THM in terms of production of GC-amenable products. The distribution of the doublets rules out tight relationships with suberin or cutin. On the contrary, as discussed in the section on conventional pyrolysis, this distribution is consistent with an origin from cutan and/or suberan for a large part of the doublets. However, the above observations, indicating a substantial contribution of suberin to the ROM while no cutin contribution occurred, favour a suberan source rather than a cutan one for these doublets.

Contrary to the predominantly even-carbon-numbered *n*-alkanoic acids in the $C_{14}$-$C_{30}$ range, the shorter $C_6$-$C_{13}$ *n*-alkanoic and *n*-alkenoic acids are unlikely to be directly related to unaltered (or weakly altered) lipids or biopolymers. Such acids probably correspond to oxidative degradation products from lipids as observed by Amblès et al. (1994) for extracts from a forest podzol. The resulting short chain products were subsequently protected against further alteration. This protection resulted either from incorporation in the macromolecular components of the ROM through covalent linkages by non-hydrolysable ester bonds or from entrapment in the three-dimensional macromolecular structure of the ROM, as observed for various low molecular weight products in humin (Grasset et al., 2002).The lack of branched alkanoic acids in the thermochemolysate suggests that no substantial amounts of bacterial components contributed to the ROM, since such acids especially the iso and anteiso ones are typical bacterial markers (e.g. Perry et al., 1979; Boon et al., 1975; Meyers and Ishiwatari, 1993; Garcette-Lepecq et al., 2001). Such a weak bacterial input is consistent with the low bacterial growth typically observed in acid soils (e.g. Motavalli et al., 1995).

No nitrogen-containing products were detected either in the conventional pyrolysate or in the thermochemolysate. On the contrary, previous studies showed that these two methods can afford various nitrogenous compounds, including pyrroles, indoles, pyridines and nitriles, from the refractory fraction isolated form recent sediments and the whole organic matter of soils (e.g. van Bergen et al., 1998; Garcette-Lepecq et al., 2000, 2001; Nierop et al., 2001). Upon THM of the refractory OM of recent sediments, a number of other nitrogen-containing compounds (piperidines, pyrimidines and triazines) and methyl derivatives of amino acids were also observed in large relative abundance (Garcette-Lepecq et al., 2000). These nitrogen-containing products are thought to be derived from proteinaceous materials, especially from melanoidin-type macromolecules formed by random condensation of monomers and other alteration products of proteins and polysaccharides (Maillard, 1917). It was noted, however,

that melanoidins are not well detected by pyrolysis, especially when they exhibit a high degree of cross-linking (Poirier et al., 2000). A poor detection is also encountered for such melanoidins when using solid state $^{13}C$ NMR (Poirier et al., 2000). On the contrary, melanoidins exhibit intense FTIR absorptions around 1630 and 1710 $cm^{-1}$ but such absorptions are far from being specific. Based on the low nitrogen content and high C/N atomic ratio of the ROM and with the results of thermal degradation it appears that protein-derived materials do not afford a large contribution to the ROM. However, some contribution of highly cross-linked melanoidins cannot be discarded.

### 2.1.2 Conclusions

The main conclusions derived from bulk analysis, spectroscopic (FTIR and solid state $^{13}C$ NMR) and pyrolytic (conventional and TMAH thermochemolysis) studies of the insoluble and non-hydrolysable material isolated from an acid sandy forest soil are summarised below:

- This refractory fraction (ROM) accounts for a substantial part, ca. one third of the whole organic matter of the soil.
- Elemental analysis and spectroscopic features ruled out a major contribution of alkyl chains, and hence a highly aliphatic nature for this ROM, while a variety of oxygen-containing groups occurs along with aromatic units.
- Unaltered and weakly altered (demethoxylated) lignin occurs in the ROM and also, probably, some condensed tannins.
- Polysaccharidic material, attributed to cellulose, survived in the isolated ROM, in spite of the drastic acid treatments applied, and its chemical structure remained largely unaltered, probably due to efficient protection within ligno-cellulose complex and/or high crystallinity.
- Suberin affords a substantial contribution and specific products originating from its aliphatic and aromatic domains were observed upon THM, thus reflecting a substantial input of root-derived material to the ROM. The survival in the ROM of this biopolymer, although it is based on interesterified monomeric units, indicates a high steric protection within the macromolecular matrix of the ROM and/or the occurrence of a suberin fraction exhibiting a high intrinsic resistance to acid hydrolysis.

- Suberan only occurs as a minor component in the ROM, in spite of its non-hydrolysable nature, thus indicating a low input of this intrinsically refractory biomacromolecular material to the OM in the Cestas soil.
- Fatty acids, with an extended carbon number range, are present in the ROM. They are either linked to the macromolecular structure, through sterically protected and thus non-hydrolysable ester bonds, or trapped within this structure. Some of these acids, the short chain ones, correspond to oxidative degradation products of lipids, while the other directly originate from biomolecules, including suberin and higher plant waxes.
- The ROM exhibits a low nitrogen content. Furthermore, no N-containing products were detected either upon conventional pyrolysis or upon THM. Accordingly, protein-derived moieties do not afford a significant contribution to the ROM. However, the occurrence of some highly cross-linked melanoidins, hardly detectable via thermal degradation or spectroscopic observations, cannot be discarded.
- The lack of branched compounds in the acids released from the ROM through pyrolyses points to the lack of a significant contribution of bacterial components to these acid moieties.
- Different constituents of the ROM were evidenced by conventional pyrolysis and by THM. Thus, the occurrence of cellulose was well revealed by the former method but not by the latter, whereas a reverse situation was found for the suberin fraction.

## 2.2 Dégradations chimiques de la fraction macromoléculaire réfractaire d'un sol forestier

De nombreuses informations sur la structure et la composition de macromolécules complexes ont été apportées par l'utilisation d'oxydants. Ainsi, de précieux renseignements ont été obtenus tant sur les acides humiques (Martin et al., 1981; Almendros et al., 1987; 1989; Gonzalez-vila et al., 1994), que les acides fulviques (Martin and Gonzalez-vila, 1982), que l'humine (Almendros et Gonzalez-vila, 1987; Almendros et al., 1991; Gonzalez-vila et al., 1994) ou encore des kérogènes (Boucher et al., 1989; Rullkötter et Michaelis, 1989; Bajc et al., 2001; Kribii et al., 2001). Les réactifs utilisés sont très divers et dépendent des objectifs considérés : soit la détermination de la structure globale ou la mise en évidence de liaisons spécifiques.

L'analyse des produits de dégradation chimique de la matière organique réfractaire (MOR) suite à l'utilisation d'oxydants plus ou moins forts doit permettre de confirmer et/ou de compléter les observations obtenues par pyrolyse et spectrométrie. Nous avons donc analysé les produits de plusieurs oxydations (oxydation au persulfate de sodium, au permanganate de potassium, au perborate de sodium et au tetroxyde de ruthenium) de la MOR du sol forestier de Cestas. Des produits de combustion incomplète (carbonisats), difficiles à mettre en évidence par les méthodes spectroscopiques et pyrolytiques, peuvent fournir par dégradations oxydatives, des composés caractéristiques (Glaser, 1998). Leur contribution à la MOR a déjà été révélée par METHR (Poirier, 2000) aussi cette technique sera également utilisée pour examiner la MOR du sol sous forêt de Cestas.

### 2.2.1. Results and discussion

#### 2.2.1.1. Sodium persulfate degradation of the refractory organic matter (ROM)

This mild oxidant was previously used for studying the most accessible and easily released components of soil organic matter, including those associated with high molecular weight structures through physical interactions (like van der Waals forces and hydrogen

bonds) or through easily cleaved covalent bonds. This method (see annexes 4.1) has been chiefly applied so far to humic and fulvic acids (Martin et al., 1981; Martin and Gonzalez-vila, 1983), it was also used for examining the whole humin, from various soil samples (Almendros and Gonzalez-Vila, 1987). This reagent was recently applied for a comparative study on the “amorphous” and “condensed” fractions of whole organic matter in soil (Cuypers et al., 2002).

Persulfate oxidation resulted in a weak degradation and only yielded a low amount of degraded products, in agreement with both the use of a mild reagent and the refractory nature of the ROM, so that the bulk of this insoluble material was retained after the treatment. As shown by measurements with an internal reference, the reaction products recovered after extraction and analysed by GC accounted for ca. 0.03 wt% of the initial ROM. These persulfate oxidation products simply consist in a series of normal alkanoic acid (Fig. 2.2.1). These fatty acids range from $C_{12}$ to $C_{32}$, with a maximum at $C_{16}$, and they mostly correspond to even-carbon-numbered compounds. In spite of the drastic acid treatments applied for isolating the ROM, a few aliphatic moieties still can be released by mild oxidation. Based on their distribution, these aliphatic moieties should mostly originate from higher plant lipids. No compounds related to aromatic moieties were obtained from the ROM. On the contrary, sodium persulfate treatment of whole humin from different soils (Almendros and Gonzalez-Vila, 1987) afforded, in addition to predominant fatty acids, substantial amounts of phenolic and benzenecarboxylic acids. However, the extent humin oxidation was not quantified. These aromatic acids included methoxy-substituted products that were thought to originate from lignin or lignin-derived components. The lack of aromatic acids in the compounds released from the Cestas ROM indicates that the lignin retained in this material is not affected by mild oxidation and that the susceptible lignin fractions, possibly occurring in the organic matter of the Cestas soil, were eliminated upon ROM isolation.

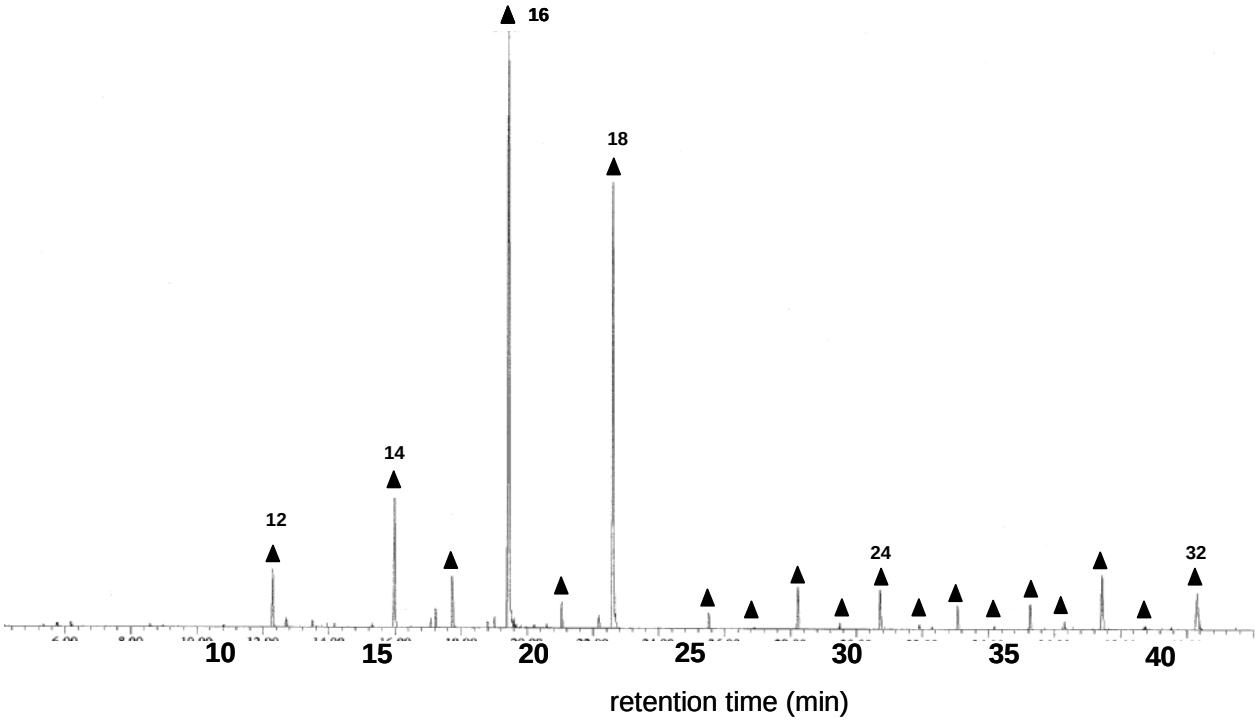



Fig 2.2.1: TIC trace of the Na persulfate oxidation products, (▲) alkanoic acid

2.2.1.2. Alkaline permanganate degradation of ROM

Alkaline permanganate oxidation has been largely applied for structural studies on fossil macromolecular organic matter from sedimentary rocks and the different types of kerogens were thus examined (e.g. Vitorovic et al., 1984; Bajc et al., 2001). This strong oxidant was also used for studies on humins (Almendros and Gonzalez-Vila, 1987) and humic acids (Almendros et al., 1989).

The solid material left after the persulfate treatment of the Cestas ROM was further oxidised by alkaline permanganate. This second treatment resulted in a much higher degradation. Indeed, the ROM was almost entirely degraded and only a negligible amount of residual insoluble material was recovered. Nevertheless, the reaction products obtained after extraction and analysed by GC accounted for only ca. 1.5 wt% of the ROM. A large difference was thus observed between the extent of degradation and the amount of the analysed products. This should reflect the occurrence of extensive secondary degradation that resulted in the formation of low molecular weight water soluble oxidation products like oxalic acid, and eventually in $CO_2$ formation, as commonly observed in kerogen studies (e.g. Vitorovic et al., 1984). The formation of soluble but non GC-amenable oligomers of the macromolecular structure of the ROM was also probably implicated.

The GC-amenable reaction products corresponded to a predominant series of normal $C_4$-$C_{28}$ α,ω-alkanedioic acids, with a maximum at $C_9$, a weak submaximum at $C_{16}$ and a low relative abundance of the $C_{16+}$ homologues (Fig. 2.2.2). No clear-cut odd- or even-carbon-number predominance is observed in this series, except for a weak but significant even predominance for the long chain compounds in the $C_{18}$-$C_{28}$ range. This major series of diacids, not formed upon the persulfate treatment, should originate either from $(CH_2)_n$ chains bearing functional groups (or branching points) at the place of the acid groups or from the corresponding diesters occurring as bridges in the macromolecular structure of the ROM. The distribution of the diacids, with a pronounced dominance of the short chains, rules out any direct relationship with suberins for the bulk of these compounds. However, the occurrence of predominantly even-carbon-numbered homologues for the long chain diacids, especially the $C_{22}$ and $C_{24}$ ones, and the submaximum at $C_{16}$ point to a suberin origin for these minor products. In suberins, ω-hydroxyalkanoic acids are major building blocks along with α,ω-alkanedioic acids and they exhibit the same distribution as the latter. The lack of ω-hydroxyalkanoic acids in the products obtained from the ROM probably reflects secondary oxidation of these compounds into α,ω-diacids. The main diacids, i.e. the short chain ones around $C_9$, likely reflect the occurrence, in the ROM, of moieties originating from the condensation of unsaturated lipids. Indeed, ω8, ω9 and ω10 double bonds commonly occur in biolipids and the oxidative cross-linking of such unsaturated lipids would contribute to the formation of resistant geomacromolecules, as previously considered for the formation of some humin moieties (Almendros et al., 1991; Almendros and Sanz, 1992; Gonzalez-vila et al., 1994).

In addition to the predominant diacids, a minor series of normal alkanoic acids was detected ($C_{12}$-$C_{31}$ range, maxima at $C_{16}$ and $C_{18}$, dominated by even-carbon-numbered compounds). The latter series was also observed, with a similar distribution reflecting a higher plant origin from the persulfate treatment. The lack of branched acids, contrary to observations on alkaline permanganate oxidation of kerogens (e.g. Bajc et al., 2001), indicates the lack of significant input of bacterial lipidic components, in agreement with previous pyrolytic studies of this ROM (part B 2.1).

No aromatic acids were detected although lignins can be oxidised by alkaline permanganate (Griffith and Schnitzer, 1976) and also the aromatic domains of suberin. However the resulting phenolic acids can be secondarily oxidised, by alkaline permanganate, into quinones that undergo further extensive degradation through ring opening. Similarly, the

phenolic acids formed from the condensed tannins, possibly present in the ROM, would be eliminated through secondary oxidation by $KMnO_4$. On the contrary, the lack of benzenecarboxylic acids cannot be explained by secondary degradation. Indeed, such compounds can resist permanganate oxidation (Almendros et al., 1989) and variable amounts of benzenecarboxylic acids were obtained through alkaline permanganate oxidation of some humins (Almendros and Gonzalez-Vila, 1987). Accordingly, contrary to these humins, no moieties capable of generating benzenecarboxylic acids, upon $KMnO_4$ treatment, occurred in the ROM.

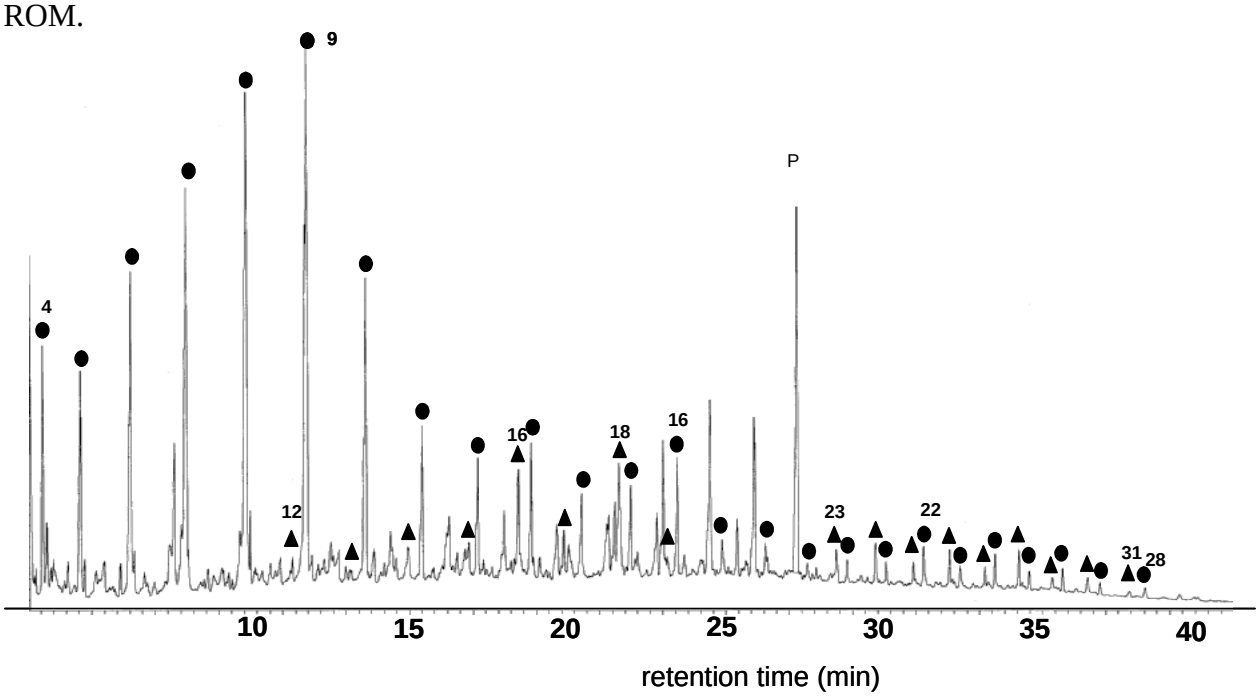



Fig 2.2.2: TIC trace of the $KMnO_4$ oxidation products, (▲) alkanoic acid, (●) diacids

2.2.1.3. Sodium perborate degradation of ROM

This oxidation was performed under the same conditions (see annexes 4.2) as the persulfate treatment, but sodium perborate, although considered as a rather mild oxidant, is a markedly stronger reagent than sodium persulfate (Almendros et al., 1987). The low amount of residual material recovered after this oxidation indicated that the bulk (ca. 75 wt%) of the ROM was degraded into soluble compounds. However, the reaction products obtained by extraction and analysed by GC only represented 0.8 wt% of the ROM. The causes of such a large difference are the same as considered above for the permanganate treatment, including the formation of soluble but non-GC amenable oligomers. Indeed, a large formation of such oligomers was observed following treatment by sodium perborate of humic acids (Almendros

et al., 1987) and humins (Almendros et al., 1991; Almendros and Sanz, 1992). In the latter study, a degradation into soluble compounds of ca. 80 wt% was obtained but the extracted, GC-amenable reaction products accounted for only ca. 4 wt% of the different humins examined.

The recovered reaction products are dominated by a series of normal alkanoic acids ranging from $C_{12}$ to $C_{32}$, with a maximum at $C_{22}$ and a submaximum at $C_{16}$ (Fig. 2.2.3). The long chain $C_{20+}$ acids are the main components of the series and the even-carbon-number compounds predominate along the whole carbon number range. A series of normal α,ω-alkanedioic acids, exhibiting a substantial abundance, was also observed in the $C_{18}$-$C_{28}$ range. This series shows a sharp maximum at $C_{24}$ and also a rather large relative abundance for the $C_{22}$ homologue. The distribution of these diacids indicates that they are derived from suberin moieties. The lack of ω-hydroxyalkanoic acids, although they are the major monomers of suberins, should reflect secondary oxidation into α,ω-diacids, as already discussed for the permanganate treatment. Normal saturated alkanoic acids also contribute to suberin formation. However, the abundance of the series of alkanoic acids that dominates the reaction products (Fig. 2.2.3) shows that only a weak part of these compounds originates from monomeric units of suberin. Indeed, acids are much less abundant than alkanedioic acids among suberin monomers whereas the reverse is observed here. A large part of these alkanoic acids therefore likely originate from ROM moieties that were formed through the incorporation of higher plant lipids, especially long chain lipids of waxes, to macromolecular structures by esterification.

*n*-Alkanes in the $C_{27}$ to $C_{31}$ range with a maximum at $C_{31}$, dominated by odd-carbon-numbered compounds, were also identified but they only occur in minor amounts. In fact these hydrocarbons, not clearly observed on the total ion current trace, were identified by selective ion detection of *m/z* 57. A *n*-alkane series, with a similar distribution, was observed in the lipid fraction extracted from the Cestas soil (see part B.1). These compounds should correspond, in the perborate oxidation products, to alkanes trapped in the macromolecular structure of the ROM, as previously observed for alkanes trapped in humins (Almendros and Gonzalez-Vila, 1987; Almendros and Sanz, 1992; Grasset and Amblès, 1998b) and kerogens (e.g. Kribii et al., 2001; Deniau et al., 2004).

Similarly to alkaline permanganate treatment, no aromatic acids were detected among the perborate reaction products, except for a low amount of a benzenedicarboxylic acid. The lack of phenolic acids derived from lignin, tannins and the aromatic domains of suberin can

again be explained by secondary oxidative degradation via quinone formation and ring opening. On the contrary, perborate does not affect benzenecarboxylic acids once formed and a substantial contribution of such acids was observed in sodium perborate oxidation products of humins (Almendros and Sanz, 1992). These observations therefore confirm, in agreement with the $KMnO_4$ experiment, the lack in the Cestas ROM of moieties capable to directly generate benzenecarboxylic acids upon treatment with such oxidants. A benzenedicarboxylic acid was detected, but only in low amounts, in the ROM reaction products (Fig. 2.2.3). The lack of some bacterial biomarkers such as β-hydroxyalkanoic acids and branched acids, previously detected upon perborate treatment of various humins (Almendros and Sanz, 1992), indicates the lack of a significant bacterial contribution to the perborate-released moieties for the Cestas ROM.

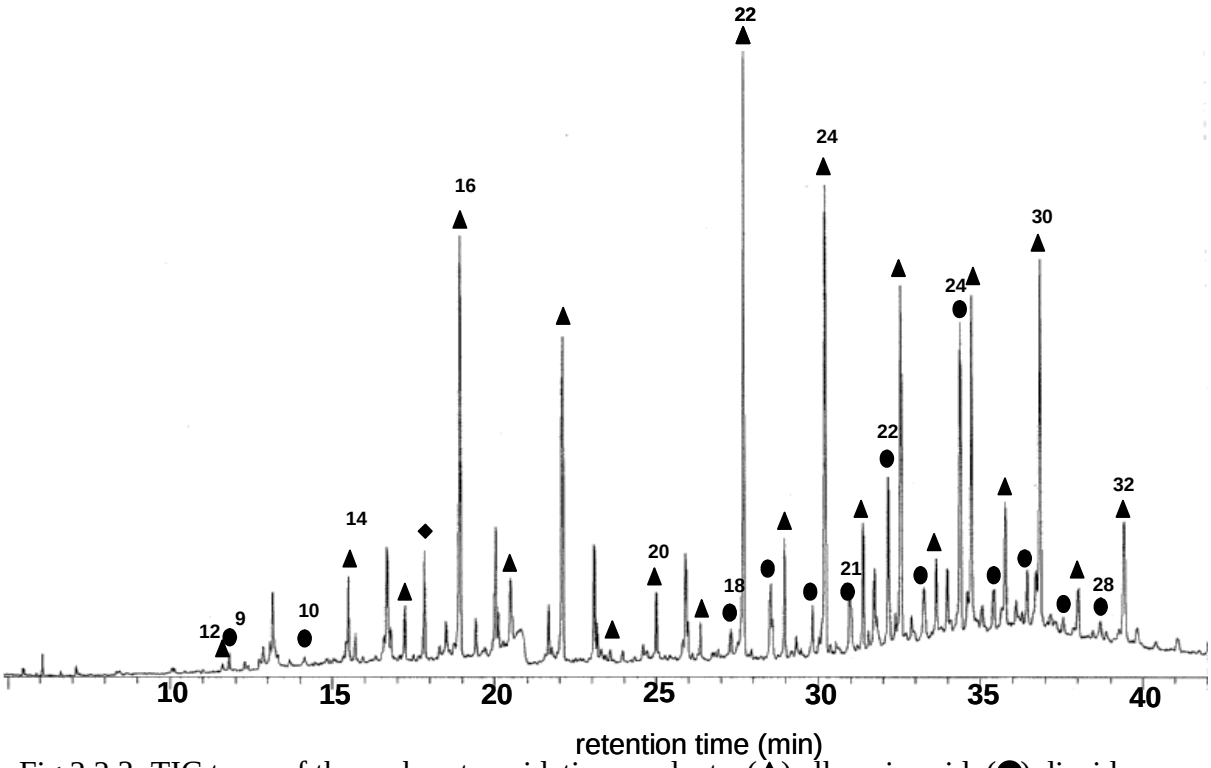



Fig 2.2.3: TIC trace of the perborate oxidation products, (▲) alkanoic acid, (●) diacid, (◆) benzene dicarboxylic acid

2.2.1.4. Ruthenium tetroxide degradation of ROM and HRTEM observations

This reagent preferentially oxidises aromatic carbons but it also cleaves several types of functional groups (e.g. Rullkötter and Michaelis, 1989). Ruthenium tetroxide releases the alkyl substituents of aromatic rings, affording alkanoic acids with one carbon atom more than

the substituent. The carboxyl carbon atom in these acids originates from the junction of the alkyl substituent to the ring. Non-substituted aromatic carbon atoms are oxidised to $CO_2$, so that isolated aromatic rings are entirely degraded. On the contrary, polyaromatic structures and polysubstituted aromatic rings can be transformed into benzenepolycarboxylic acids. Ruthenium tetroxide also oxidises esters, vicinal ethers, primary alcohols and olefinic double bonds for producing carboxylic acids (e.g. Schouten et al., 1998). $RuO_4$ oxidation has been so far mostly applied to kerogens (e.g. Boucher et al., 1989; Kribii et al., 2001) and non-hydrolysable aliphatic biopolymers, algaenans and cutans (e.g. Schouten et al., 1998; Blokker et al., 2000). Only one previous paper, dealing with humic acids and whole humins, was concerned with $RuO_4$ oxidation of soil organic matter (Gonzalez-Vila et al., 1994).

The ruthenium tetroxide treatment resulted in a large degradation (ca. 80 wt%) of the ROM. However, the extracted reaction products, analysed by GC, only represented ca. 1wt% of the starting material. $RuO_4$ oxidation commonly affords low yields, of a few wt%, of GC-amenable degradation products when applied to complex macromolecular natural materials (Blokker et al., 1998, 2000; Schouten et al., 1997, 1998; Kribii et al., 2001). Nevertheless, this method is thought to afford representative information on the main components of such materials (Blokker et al., 1998, 2000; Schouten et al., 1998). Indeed, $RuO_4$ released a somewhat larger variety of reaction products from the Cestas ROM compared to the other oxidants. These products comprise two main series of normal α,ω-alkanedioic acids and benzenepolycarboxylic acids (Fig. 2.2.4). The diacids range from $C_4$ to $C_{27}$ with a maximum at $C_7$-$C_9$, a weak submaximum at $C_{16}$, a low contribution of long chain homologues and no clear-cut odd- or even-carbon-number predominance. The benzenepolycarboxylic acids correspond to di-, tri-, tetra-, penta- and hexa-substituted compounds and several isomers are observed for the tri- and tetracarboxylic acids. The minor series of normal alkanoic acids ranges from $C_9$ to $C_{30}$, it exhibits a maximum at $C_{16}$ and submaxima at $C_{26}$ and $C_{28}$. In this series, the long chain $C_{22}$ to $C_{28}$ homologues are relatively abundant and the even-carbon-numbered compounds predominate in the $C_{14}$ to $C_{30}$ range while no odd or even dominance occurs for the short chain $C_9$-$C_{13}$ acids. The distribution and the abundance of the α,ω-alkanedioic acid series are similar to those observed for the alkaline permanganate treatment. Similarities are also found for the alkanoic acids that represent, both in the $RuO_4$ and permanganate reaction products, a minor series of predominantly even-carbon-numbered compounds with an extended range and a maximum at $C_{16}$. However, the submaxima for the long chain homologues was not observed in the case of the permanganate treatment while such a feature was noted upon perborate oxidation The origin of these two series of acids was

discussed in the preceedings sections on permanganate and perborate. For the $RuO_4$ experiment, the short chain diacids may also originate from alkyl bridges between aromatic rings in the ROM, affording diacids with two carbon atoms more than these alkyl chains. However, the similar distribution, compared to the permanganate experiment, favours structures formed by oxidative cross-linking of unsaturated lipids as the source of the diacids rather than alkyl bridges between aromatic units.

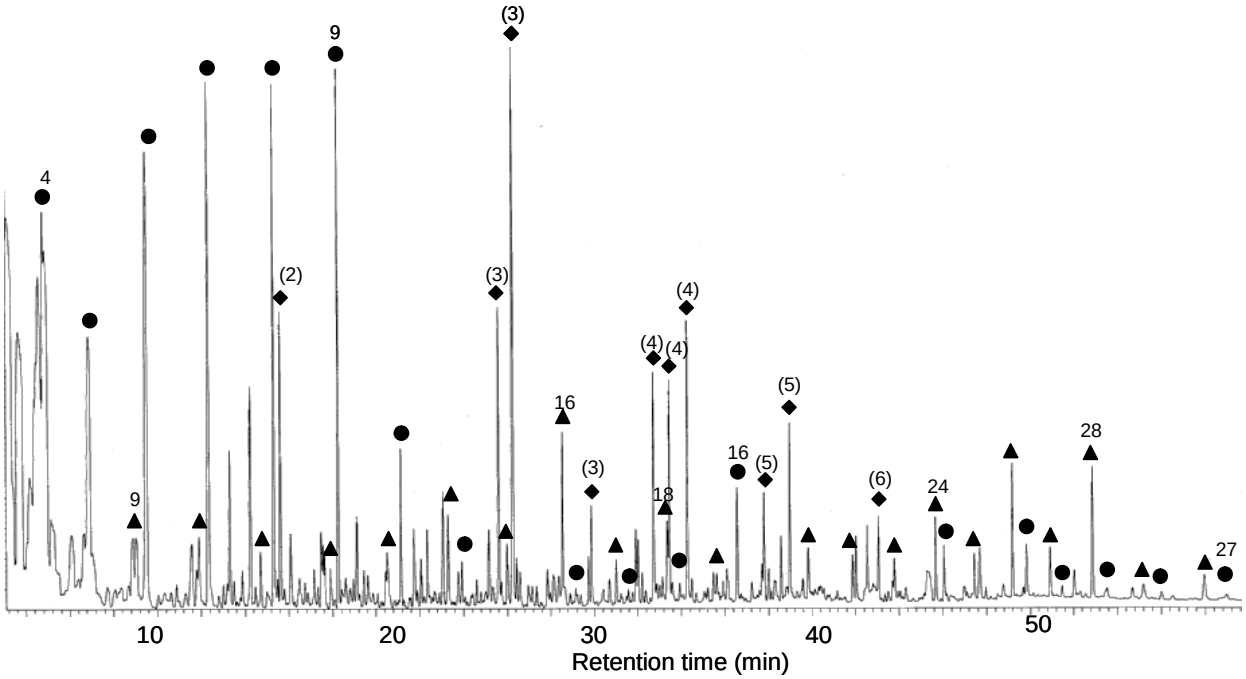



Fig 2.2.4: TIC trace of the ruthenium tetroxide oxidation products, (▲) alkanoic acid, (●)diacid, (◆) benzene polycarboxylic acid (number of carboxylic fonctions)

The lack of iso- and anteiso-acids, contrary to observations on the $RuO_4$ oxidation products of some humic acids (Gonzalez-Vila et al., 1994), indicates the lack of a significant bacterial contribution to the $RuO_4$-sensitive alkyl moieties in the ROM.

Benzenepolycarboxylic acids are the second predominant series in the $RuO_4$ reaction products of the ROM. On the contrary, such compounds were not formed with the other reagents, except for a low amount of a benzenedicarboxylic acid, with perborate. Benzenepolycarboxylic acids are typically formed upon $RuO_4$ oxidation of condensed polyaromatic structures or of polysubstituted aromatic rings. The ruthenium tetroxide treatment thus indicates that such structures substantially contribute to the ROM. A part of these aromatic structures probably correspond to polysubstituted aromatic rings in condensed tannins. Indeed, solid state $^{13}C$ NMR data (part B 2.1.1.2) pointed to the occurrence of condensed tannins in the ROM and such compounds exhibit a high resistance to hydrolysis

(De Leeuw and Largeau, 1993). Nevertheless, condensed tannins can only account for the formation of the di-, tri- and tetracarboxylic acids and an additional source has to be considered for the penta- and hexacarboxylic homologues. This other source may correspond to condensed polyaromatic units in black carbon, i.e. in incomplete combustion products from vegetation fires and fossil fuel burning. Black carbons are also resistant to drastic hydrolyses (Poirier et al., 2000, 2002) and they can generate penta- and hexacarboxylic acids upon $RuO_4$ oxidation (Glaser et al., 1998). However, this type of condensed polyaromatic materials is poorly (or even not) detected by solid state $^{13}C$ NMR and pyrolyses (Poirier et al., 2000). Black carbons exhibit a strong IR absorption around 1600 $cm^{-1}$ but this band is not specific at all. On the contrary, direct evidence for the presence of black carbon can be obtained through HRTEM (Rouzaud and Oberlin, 1990).

HRTEM observations, using the lattice fringe mode, confirmed the presence of black carbon in the Cestas ROM. This mode allows for the direct observation of the polyaromatic basic structural units of black carbon and thus provides information on its origin. Mostly poorly organized black carbons (Fig 2.2.5), with scarce contribution of onion-shaped black carbons (Fig 2.2.6), were observed. Such features indicate that the black carbon particles probably originate from the partial carbonisation of ligno-cellulosic materials (charcoal), probably related to fire events. This contribution might be important in relation with organic matter stabilisation in the soil, due to the relative inertness of charcoal compared to non-combusted vegetal biomass (e.g. Kuhlbusch and Crutzen, 1995), and with the fate of organic pollutants, due to very high sorption capacity of charcoal compared to bulk organic matter in soil (e.g. Bucheli and Gustafsson, 2000).

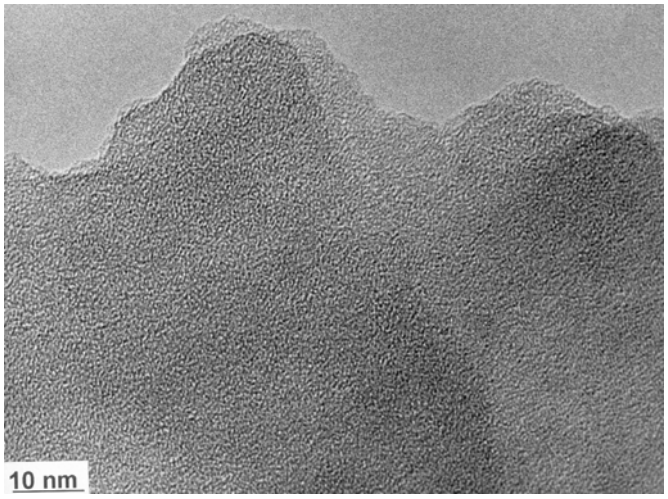



Fig 2.2.5: Lattice fringe HRTEM observation of the ROM, poorly organized black carbon

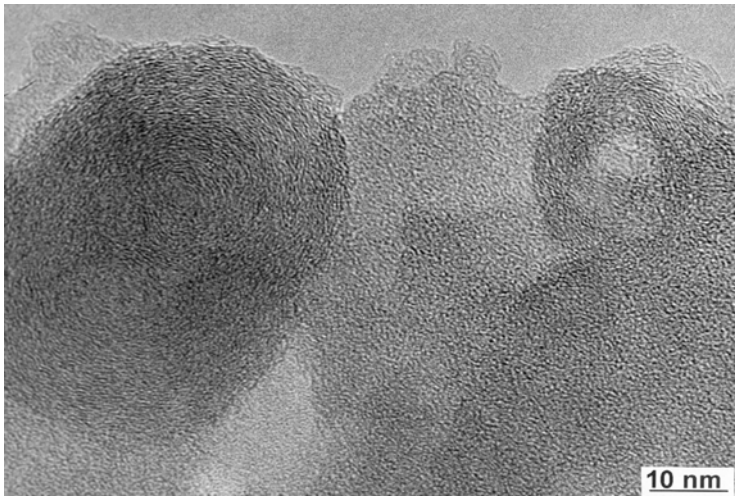



Fig 2.2.6: Lattice fringe HRTEM observation of the ROM, onion shaped black carbon

### 2.2.2. Comparison of the oxidative treatments

Large quantitative and qualitative differences were observed between the four treatments applied to the ROM (Table 2.2.1, fig.2.2.7 and fig 2.2.8). The bulk of the initial material was retained after the persulfate treatment since only the most accessible and easily released moieties are freed by this mild oxidant. On the contrary, extensive degradation (from ca. 75 to almost 100 wt%) was obtained with the other three oxidants. However, even in these cases, the organic solvent-extracted and GC-amenable reaction products only accounted for a low percentage, 0.8 to 1.5wt%, of the ROM. These low yields should reflect the formation of $CO_2$, and of low molecular weight compounds through extensive secondary degradation reactions and also the formation of extractable but not GC-amenable oligomers of the macromolecular structure, as observed in previous studies on kerogens and humins. Low yields of analysed products were also commonly obtained in these studies through oxidation by alkaline permanganate, sodium perborate and ruthenium tetroxide.

| | n-alkanes | n-acids | α,ω-diacids | benzene carboxylic acids |
|---|---|---|---|---|
| Na Persulfate | | $C_{12}$-$C_{32}$ ($C_{16}$) | | |
| K permanganate | | $C_{12}$-$C_{31}$ ($C_{18}$) | $C_4$-$C_{28}$ ($C_9$) | |
| Na perborate | $C_{17}$-$C_{31}$ ($C_{31}$) | $C_{12}$-$C_{32}$ ($C_{22}$) | $C_{18}$-$C_{28}$ ($C_{24}$) | dicarboxylic acids |
| Ruthenium tetroxide | | $C_9$-$C_{30}$ ($C_{16}$) | $C_4$-$C_{27}$ ($C_9$) | di-to hexa-carboxylic acids |

Table 2.2.1: Distribution of the oxidation products obtained with the different reagents

The sodium persulfate treatment only afforded a single series of normal alkanoic acids whereas both such acids and a series of α,ω-alkanedioic acids were obtained through alkaline permanganate and sodium perborate treatments. The largest diversity of reaction products was obtained with $RuO_4$, the latter two series occurring along with a number of benzenepolycarboxylic acids with high relative abundance.

The sodium perborate treatment and (to a lesser extent) the alkaline permanganate treatment showed the presence of suberin, reflected by the production of long chain α,ω-alkanedioic acids. These two reagents also evidenced the contribution of lipids to the macromolecular structure, reflected by the production of short chain α,ω-alkanedioic acids and of alkanoic acids. However, conspicuous differences were observed both in the relative

abundance of these two series and in the distribution of the diacids between the alkaline permanganate and sodium perborate treatments. Firstly, the alkanedioic acids predominate over the alkanoic acids for the former whereas the reverse is observed for the latter and, secondly, these diacids mostly correspond to $C_4$-$C_{11}$ short chain compounds instead of the exclusive occurrence of long chain $C_{18}$-$C_{28}$ homologues in the case of the perborate treatment. Permanganate being a stronger oxidant than perborate, these differences likely reflect a higher production of short chain diacids with the former. These short chain compounds, not released by perborate, would largely dilute the long chain mono- and diacids formed in both experiments. As previously discussed, the short chain diacids probably originate from geomacromolecules formed by the oxidative cross-linking of predominantly ω8, ω9 and ω10 unsaturated lipids. These macromolecular components would therefore exhibit a higher degree of cross-linking, related to the production of short chain acids, when compared to the ROM components releasing the long chain acids. Hence the former would be more resistant to oxidation and therefore only cleaved by permanganate. Sodium perborate showed the occurrence of low amounts of *n*-alkanes, originating from higher plant waxes, trapped in the macromolecular structure of the ROM. The lack of *n*-alkanes in the permanganate reaction products probably reflects secondary oxidation into acids by this stronger oxidant.

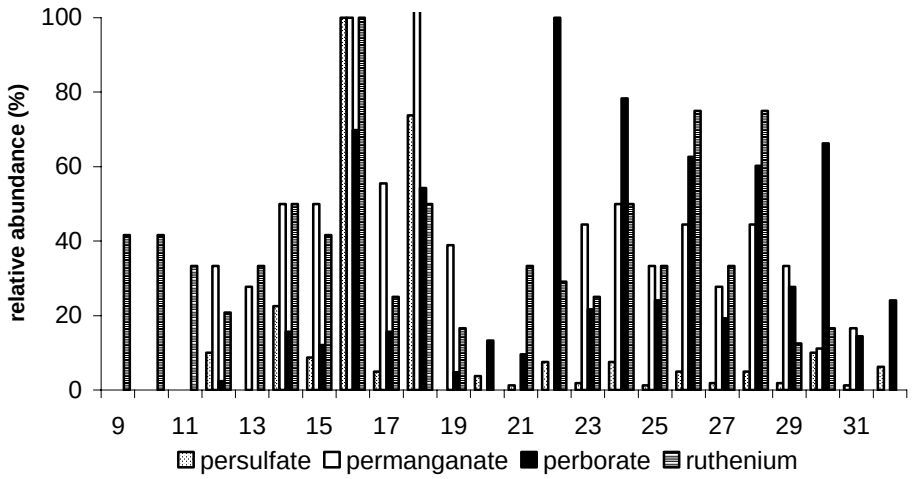



Fig 2.2.7 : Distribution and relative abundance of alkanoic acids

In addition to suberin and lipid contributions, the $RuO_4$ treatment revealed the presence of aromatic moieties, reflected by the formation of benzenepolycarboxylic acids. These aromatic structures correspond to condensed tannins and to black carbon. Such aromatic contributions were not reflected at all by the permanganate and perborate oxidations. In the previous study concerned with the Cestas ROM, the occurrence of condensed tannins was

only suggested by the NMR data and the presence of black carbon could not be revealed by the spectroscopic and pyrolytic methods applied.

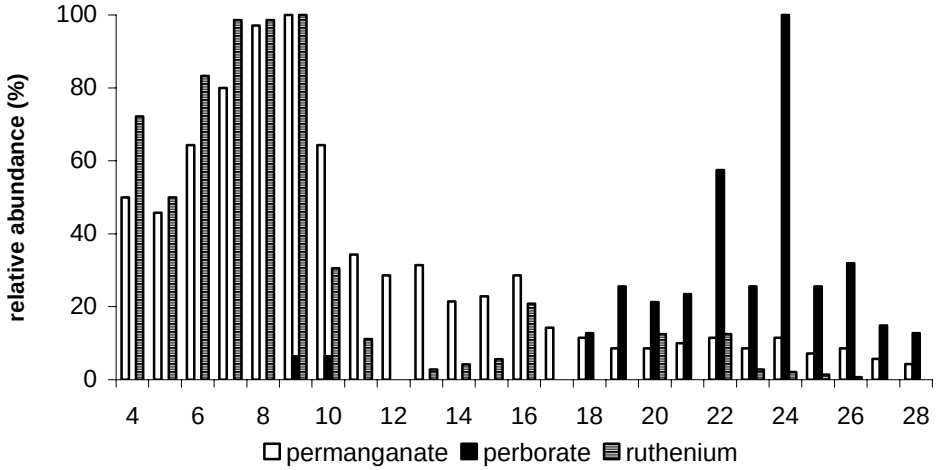



Fig 2.2.8: Distribution and relative abundance of diacids

## 2.2.3 Conclusions

The main conclusions derived from chemical degradation by various oxidants (sodium persulfate, alkaline permanganate, sodium perborate and ruthenium tetroxide) of the insoluble and non-hydrolysable material isolated from a sandy acid forest soil, and from HRTEM observations, are summarised below:

- Conspicuous differences in the level of degradation of this refractory organic material (ROM) and in the nature and relative abundance of the GC-amenable reaction products were observed between these four treatments.
- The persulfate treatment showed that the ROM contains a few moieties that can be released by mild oxidation, in spite of the drastic acid attacks applied for ROM isolation. These easily accessible and released moieties mostly originate from higher plant lipids.
- The sodium perborate, the alkaline permanganate and the $RuO_4$ treatments showed the presence of suberin. These reagents also evidenced the contribution of lipids to the macromolecular structure of the ROM. Such lipid-derived moieties corresponded to unaltered long chain lipids from higher plant waxes and to oxidatively cross-linked unsaturated lipids.
- The occurrence of condensed tannins, previously suggested by the solid state $^{13}C$ NMR features of the ROM (part B 2.1), was ascertained by the $RuO_4$ treatment.

- This latter treatment also revealed the presence of black carbon, confirmed by HRTEM observations. In addition, HRTEM showed that black carbon in the ROM corresponds to poorly organized charcoal particles probably related to fire events.
- Low amounts of long chain n-alkanes are trapped in the macromolecular structure of the ROM, as shown by the perborate treatment.
- No bacterial markers (branched acids and β-hydroxyalkanoic acids) were formed from the ROM in any of the applied oxidation treatments, contrary to observations on the oxidation of whole humins from various soils. This feature confirms the lack of significant contribution of bacteria-derived lipids to the Cestas ROM, previously suggested by pyrolysis experiments.
- The different treatments revealed different components of the ROM depending on the strength of the oxidant and the type of linkages affected. Thus, the occurrence of suberin was better indicated by the perborate treatment compared to alkaline permanganate and $RuO_4$ oxidations. On the contrary, the contribution of moieties originating from the oxidative cross-linking of unsaturated acids was well evidenced by alkaline permanganate and $RuO_4$ oxidations while no indices of such a contribution was observed in the perborate reaction products. The presence of condensed tannins and black carbon was only shown by the $RuO_4$ treatment.

## Conclusion partielle de la partie B

**Deux grands types de constituants, les lipides et la fraction organique réfractaire ont été étudiés dans le sol forestier de Cestas.**

**L'analyse des lipides des fractions granulométriques (sable, limon, argile) a montré que ces composés constituent une part importante de la MOS. Ceux-ci sont répartis de façons inégales entre les fractions granulométriques et leur concentration atteint 23% de la MOS dans l'argile. La prise en considération des différentes fractions a permis d'apporter des indications précieuses sur les différences de sources majoritaires de lipides selon la fraction considérée. De plus, nous avons observé un degré d'altération des lipides variables dans les trois fractions granulométriques et notamment la présence de composés préservés dans l'argile, ce qui implique une protection physique des lipides par interaction avec l'argile. Cependant, les macromolécules telles que les cires, la cutine et la subérine semblent présenter une résistance intrinsèque à la dégradation, indépendamment de leur environnement minéral.**

**Les hydrolyses acides et basiques ont permis l'isolement de la MO réfractaire. Elle représente un tiers de la MOS. Les méthodes spectroscopiques associées aux méthodes pyrolytiques ont montré que cette MOR est principalement constituée de lignine peu altérée et de subérine. Ces résultats indiquent une préservation de ces molécules au sein de la structure macromoléculaire de la MOR et/ou une résistance intrinsèque notamment de la subérine. Les dégradations chimiques, et notamment l'utilisation conjointe d'oxydants de force variable, ont fourni des indications sur la composition, la structure et l'organisation de cette MOR. Ainsi, il apparaît que, malgré le protocole d'isolement, des acides faiblement liés subsistent dans la MOR. Des alcanes sont piégés et ainsi stabilisés dans la MOR. Des lipides et de la subérine contribuent à cette fraction réfractaire dont la structure implique de nombreux ponts diesters ainsi que de nombreuses liaisons inter- et intramoléculaires. L'utilisation du tetroxide de ruthénium a révélé la présence de carbonisats ce qui n'était pas apparu par les méthodes spectroscopiques ou pyrolytiques. Cette contribution est confirmée par l'observation en microscopie électronique à transmission haute résolution.**

# PARTIE C :

# INFLUENCE DE LA MISE EN CULTURE

Comme nous l'avons vu dans la partie A, de nombreux auteurs ont souligné l'impact du changement d'utilisation du sol sur la MOS. La mise en culture a de nombreuses répercussions sur le sol (Cf partie A). Elle implique généralement la fertilisation et le labour du sol. Ces deux facteurs conduisent à une augmentation du taux de minéralisation par les microorganismes (Amblès et al., 1989; Jambu et al., 1991; Dinel et al., 1998). Balesdent et al. (1998) ont noté un taux de renouvellement après mise en culture 8 fois supérieur à celui observé antérieurement. D'autre part, Van Bergen et al. (1997; 1998) ont aussi souligné l'impact de la mise en culture sur la composition de la MOS.

Une parcelle adjacente au sol forestier étudié dans la partie B 1 a été convertie à la culture de maïs il y a 22 ans. Le passage d'une couverture végétale dominée par des plantes au métabolisme en $C_3$ à une couverture de plantes en $C_4$, rend possible l'étude de la dynamique de la MOS, notamment l'incorporation de la MO issue du maïs. Grâce à cette chronoséquence, nous avons pu estimer l'impact de la mise en culture non seulement sur la quantité de carbone dans le sol et les différentes fractions granulométriques, mais surtout nous avons quantifié et caractérisé les lipides du sol et de ces fractions ainsi que la MOR après la mise en culture.

# I. ETUDE MOLECULAIRE ET ISOTOPIQUE DE LIPIDES DANS TROIS FRACTIONS GRANULOMETRIQUES D'UN SOL CULTIVE : SOURCES, DEGRADATION, COMPARAISON AVEC LE SOL FORESTIER

L'analyse des lipides de différentes fractions granulométriques du sol forestier (partie B 1) a montré la localisation préférentielle des lipides dans les fractions fines. De la même façon, nous avons observé que selon la fraction considérée, les lipides sont majoritairement soit issus des plantes supérieures, soit des microorganismes. D'autre part, les lipides liés ont révélé une importante proportion de lipides provenant de cires mais aussi de la cutine et de la subérine. Ainsi, ces macromolécules semblent présenter une résistance intrinsèque à la biodégradation.

De plus, plusieurs auteurs ayant souligné le caractère résistant des lipides du sol, il nous a paru intéressant de nous interroger sur la possibilité d'un impact de la mise en culture sur la fraction lipidique du sol et notamment sur les macromolécules considérées comme plus résistantes (i.e cutine et subérine). Les principaux objectifs de ce travail étaient de déterminer si des différences notables sont observées dans les lipides des différentes fractions granulométriques, de comparer ces résultats à ceux préalablement obtenus dans le sol sous forêt et finalement d'examiner la contribution des composants issus de la forêt et du maïs. Dans ce but, les lipides extraits ont été analysés par CG-SM puis des mesures isotopiques ont été réalisées.

## 1.1 Results and discussion

### 1.1.1 Bulk features of the particle-size fractions

A very high recovery was obtained following particle-size fractionation and, together, the three fractions represent around 98wt% of the cultivated soil. In agreement with the sandy nature of the Cestas soil, the sand-sized fraction largely dominates (903 g were recovered

from 1 kg of whole soil) over the silt- and clay-sized fractions. The clay fraction is the least abundant (25 g against 55 g for the silt). However, the sand fraction exhibits a very low content in organic carbon of only 4.4 g $C_{org}.kg^{-1}$of fraction, whereas contents above 200 g $C_{org}.kg^{-1}$ are found for the silt and clay fractions (Table 1.1). In fact, calculation of $C_{org}$ distribution in the cropped soil, using the latter values and the distribution of the particle-size fractions, showed that most of the organic carbon is located in the silt fraction followed by the clay and sand fractions (Table 1.1). The organic carbon enrichment ratios $E_{Corg}$ (g $C_{org}.kg^{-1}$ of size fraction/g $C_{org}.kg^{-1}$ of whole soil) were also calculated, according to Guggenberger et al. (1994). The large differences in the ratios thus obtained (Table 1.1) illustrate the commonly observed organic matter enrichment in the finest particle-size fractions along with depletion in the coarse one (e.g. Zhang et al., 1988; Guggenberger et al., 1994). The very low ratio exhibited by the sand fraction (ca. 0.2) against ca. 10 for the other two fractions reflects an especially high depletion in organic matter for the sand in the Cestas cultivated soil.

| | Particle-size fraction distribution ($g.kg^{-1}$ of soil) | $C_{org}$ content in fractions (g $C_{org}.kg^{-1}$ of fraction) | $C_{org}$ distribution in soil (g $C_{org}.kg^{-1}$ of whole soil) [b] | $E_{Corg}$ (g $C_{org}.kg^{-1}$ of fraction/g $C_{org}.kg^{-1}$ of soil) [c] |
|---|---|---|---|---|
| Sand | 903 (902) | 4.4 (7.1) | 3.97 (6.40) | 0.19 (0.23) |
| Silt | 55 (67) | 226.5 (242) | 12.52 (16.31) | 10 (8) |
| Clay | 25 (23) | 244.7 (337) | 6.19 (7.65) | 10.8 (11) |

Table 1.1**:** Bulk features of the Cestas cultivated soil after 22 years of maize cropping: distribution of the particle-size fractions, organic carbon ($C_{org}$) content of the fractions, $C_{org}$ distribution in whole soil and $C_{org}$ enrichment factor ($E_{Corg}$) of the fractions [a] The bracketed numbers correspond to the values for the forest soil, calculated from the results reported in part B.1, [b] Calculated from $C_{org}$ contents and fraction distributions,[c] $C_{org}$ of Cestas soils determined from soil fraction measurement ($g.kg^{-1}$): 22.7 (cultivated), 30.4 (forest)

Comparison with the Cestas forest soil showed the same trends between the particle-size fractions for all the above features (Table 1.1) and close $E_{Corg}$ values were obtained for the different fractions for both soils. The substantially lower $C_{org}$ content of the cultivated soil (ca. 23 $g.kg^{-1}$ instead of ca. 30 $g.kg^{-1}$ for the forest soil) is reflected, for all the fractions, by lower values for $C_{org}$ contents and distributions (Table 1.1). This decrease is especially marked for

the sand-sized fraction, in agreement with previous studies (Guggenberger et al., 1995; Jolivet et al., 2003) showing that sand-associated SOM is more sensitive to cultivation.

The content of lipidic carbon (g $C_{lip}$.kg$^{-1}$ of fraction) exhibits large differences, with a value below 1 for the sand-sized fraction and values above 10 and 20 for the silt- and clay-sized fractions, respectively (Table 1.2). However, these differences are relatively less pronounced when compared to total organic carbon ($C_{org}$ values in Table 1.1). In fact, calculation of $C_{lip}$ distribution showed similar values for the three fractions, around 0.6 g $C_{lip}$.kg$^{-1}$ of whole soil (Table 1.2). When the abundance of the lipidic C relative to total organic carbon is considered, the largest percentage is observed for the sand-sized fraction and the lowest one for the silt fraction. The lipidic carbon enrichment ratios $E_{Clip}$ show a wide range reflecting, as in the case of $C_{org}$, depletion for the coarse fraction and enrichment for the fine ones. However, comparison between $E_{Clip}$ and $E_{Corg}$ reveals a relatively less pronounced depletion for the lipids compared to the total organic carbon in the sand-sized fraction (Tables 1.1 and 1.2). For the clay-sized fraction, similar ratios are obtained. In contrast, a substantially lower enrichment (ca. two times) is noted for lipids in the silt fraction compared to the total organic carbon.

| | $C_{lip}$ content in fractions [b] (g $C_{lip}$.kg$^{-1}$ of fraction) | $C_{lip}$ distribution in soil (g $C_{lip}$.kg$^{-1}$ of whole soil) [c] | $C_{lip}$ abundance [d] (% of Corg) | $E_{Clip}$ (g $C_{lip}$.kg$^{-1}$ of fraction/ g $C_{lip}$.kg$^{-1}$ of whole soil) [e] |
|---|---|---|---|---|
| Sand | 0.61 (0.76) | 0.55 (0.69) | 14 (11) | 0.32 (0.17) |
| Silt | 11.1(19.4) | 0.61 (1.30) | 4.9 (8) | 5.7 (4.4) |
| Clay | 23.1 (96.6) | 0.58 (2.22) | 9.4 (28.5) | 12 (21) |

Table 1.2: Bulk features of the lipidic carbon ($C_{lip}$) of the Cestas cultivated soil after 22 years of maize cropping: content of the fractions, distribution in whole soil, abundance relative to $C_{org}$ and enrichment factor ($E_{Clip}$) of the fractions [a] The bracketed numbers correspond to the values for the forest soil, calculated from the results reported part B.1,[b] Calculated from the mass of lipids obtained from the considered fraction (g lipids.kg$^{-1}$ of fraction) and the carbon content (%) of these lipids determined by elemental analysis,[c] Calculated from $C_{lip}$ contents and from fraction distributions (Table 1),[d] Calculated from $C_{lip}$ contents and from $C_{org}$ contents (Table1),[e] $C_{lip}$ of Cestas soils: 1.74 g kg$^{-1}$ (cultivated), 4. 21 g kg$^{-1}$ (forest)

Comparison with the results from the forest soil showed a similar trend for $C_{lip}$ content with, however, a markedly higher content (ca. four times) for the clay-sized fraction of the forest soil. The enrichment factors also exhibit the same trend, i.e higher values as the fraction becomes finer, but the resulting difference between the sand and clay fractions is much less

pronounced for the cultivated soil. Different trends were observed for Clip distributions for the two soils.

These bulk features determined on particle-size fractions therefore show some marked differences between the cultivated and the forest soil and, also, between the lipidic carbon and the total organic carbon in the same soil.

### 1.1.2 Lipid composition of the particle-size fractions

Analysis by GC/MS of the lipid extracts from the three particle-size fractions showed the occurrence of several series of components, dominated by fatty acids (analysed as methyl esters, see annexes 3.1.3.2). Relatively abundant series of alkanes and ketones were also observed. Alkanols were analysed (as trimethylsilyl ethers) after silylation of aliquots of the extracts. Aliquots were also used for separating the apolar components by TLC, in order to improve the identification of the various series of alkanes by GC/MS. Lipid extracts generally comprise large amounts of components that are not GC-amenable due to a relatively high molecular weight and/or polarity. Some of these components contain acid or alcohol moieties occurring as esters. Such moieties, contrary to the “free” acids and alcohols, thus escape detection through direct GC/MS analysis. For obtaining information on these “bound” moieties, aliquots of the lipid extracts were saponified, so as to release the corresponding acids and alcohols through cleavage of the ester linkages, before additional GC/MS analysis. The saponified lipids show important series of fatty acids (analysed as methyl esters), hydroxy acids (analysed as trimethylsilyl ethers and methyl esters), diacids (analysed as methyl diesters) and alkanols (analysed as trimethylsilyl ethers). Saponification results in a large release of these ester-bound compounds so that the alkanes and ketones are highly diluted. In fact, the latter, that can only occur as free components, now appear as minor series while they were relatively abundant before saponification.

The information obtained on these different types of lipids are presented and discussed below in relation with their origin and differences between the three size fractions.

1.1.2.1 Free fatty acids.

The distribution of the fatty acid methyl esters was determined by selective detection of their specific ion (*m/z* 74) and the corresponding ion chromatograms are shown in Figure 1.1 a-c. Normal saturated fatty acids are predominant for the three fractions and they exhibit a maximum at $C_{16}$ (palmitic acid). These acids range from $C_{12}$ to $C_{30}$ and a marked dominance of the even-carbon-numbered homologues is always observed. However important differences in the distribution of this series are noted between the silt-sized fraction, on the one hand and the sand and clay fractions on the other hand. Indeed, the former exhibits a large contribution of $C_{22}$ to $C_{28}$ compounds (ca. 48% of the total series) whereas these long chain acids only show a weak relative abundance in the case of the sand- and clay-sized fractions (ca. 10% of the total series). Such acids are commonly found in extracts of whole soils (e.g. Jambu et al., 1985; Amblès et al., 1994, 1998; Jaffé et al., 1996; Van Bergen et al., 1997; 1998, Bull et al., 1998; Naafs et al., 2004). Even-carbon-numbered long chain *n*-saturated fatty acids are considered as typical of higher plants where they occur in epicuticular waxes (Kolattukudy et al., 1976; Wannigama et al., 1981). The odd-carbon-numbered homologues observed in substantial amounts for the three fractions probably originate form partial microbial reworking of these long chain even acids, through $\alpha$-oxidation and subsequent chain shortening. The differences in distribution between the three fractions of the Cestas maize soil therefore point to a relatively large contribution of acids derived from higher plant waxes in the free fatty acids of the silt fraction, in contrast with the weak contribution for the sand and clay ones. Palmitic acid is ubiquitous and generally abundant in a number of living organisms, including higher plants and bacteria (Padley et al., 1992) and in soil extracts (e.g. Amblès et al., 1998; Bull *et al.*, 1998). Accordingly, its abundant presence in the extracts does not afford any specific information. The $C_{16:1}$ and $C_{18:1}$ monounsaturated fatty acids only occur in low amounts and they are, also, not highly specific in terms of sources. On the contrary, the branched acids are thought to be indicative of bacterial contribution to SOM (e.g. Jambu et al., 1985; Jaffé *et al.*, 1996; van Bergen *et al.*, 1998; Zelles, 1999). These bacterial acids chiefly correspond to *iso* and *anteiso* compounds and they generally exhibit an odd carbon number with maxima at $C_{15}$ or $C_{17}$. Therefore the occurrence, in the three extracts, of $C_{15}$ and $C_{17}$ *iso* and *anteiso* branched compounds (Figure 1.1) shall reflect the contribution of bacterial acids. This bacterial contribution is markedly larger in the free fatty acids of the

clay-sized fraction where the $C_{15}$ iso and anteiso acids are abundant and the former almost as intense as palmitic acid while these acids are only minor components for the other two fractions, especially the silt-sized one.

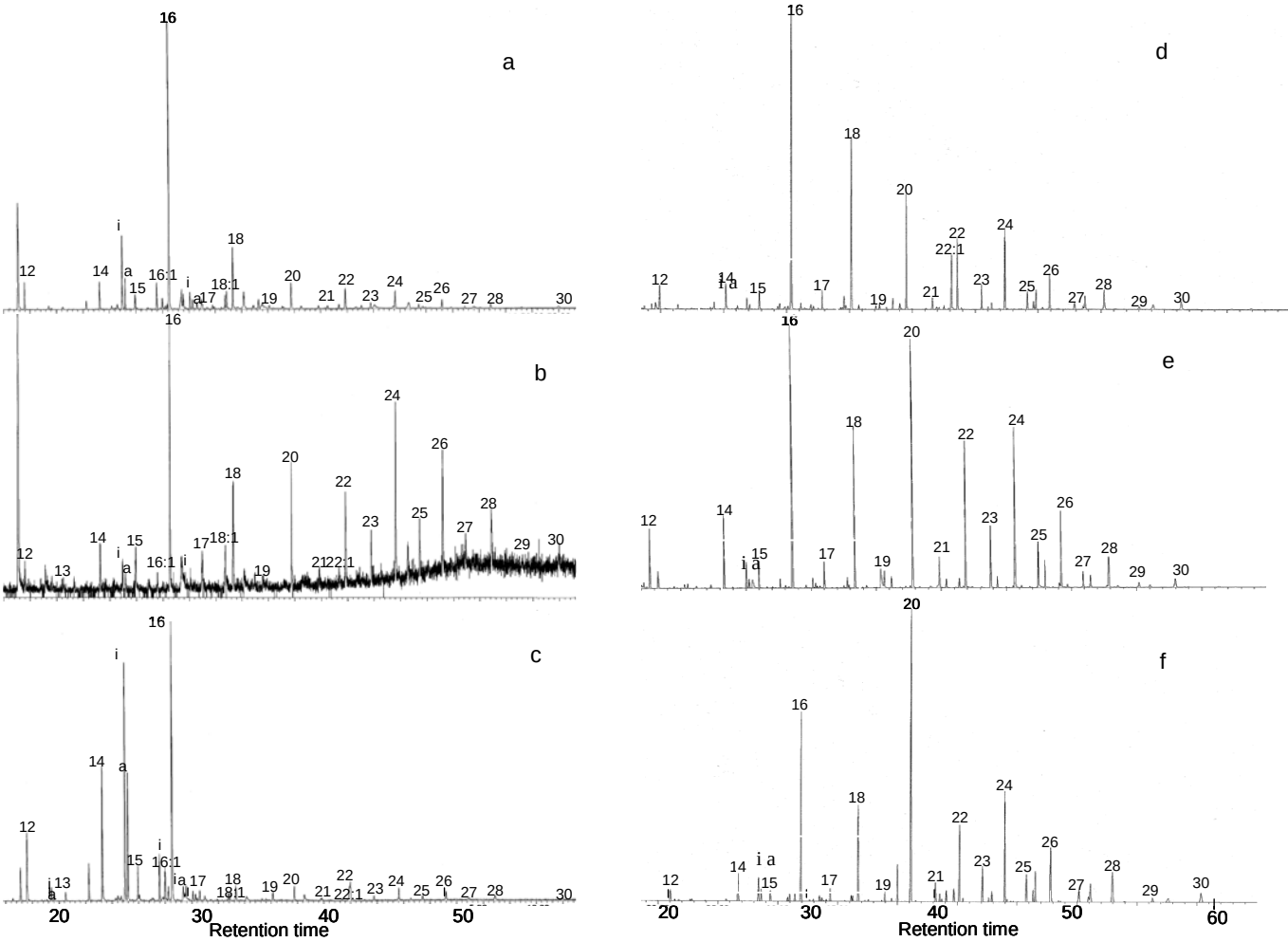



Fig 1 a-f: Ion chromatograms (*m/z* 74) showing the distribution of the free carboxylic acids as their methylesters in the total lipid extracts (a-c) and in the saponified lipid extracts (d-f) of the three particle-size fractions (a: anteiso-, i: iso-).

### 1.1.2.2 Bound fatty acids.

The fatty acids obtained after saponification of the lipid extracts contain both the "free" acids that occurred, as such, in the extracts and the acids released by the cleavage of ester linkages. The distributions of the saponified fatty acids are reported in Figure 1.1d-f. Comparison with the distribution of the "free" acids (Figure 1.1a-c) showed important differences, especially for the sand- and clay-sized fractions. These two fractions are still dominated by predominantly even-carbon-numbered *n*-saturated fatty acids and the presence of branched acids and of *n*-monounsaturated fatty acids is also noted. However, the saponified

acids exhibit, in these two fractions, larger relative contributions in the $C_{18}$ to $C_{30}$ range (e.g. 60% of the total series for the sand fraction against 10% in the case of the free acids). These variations reflect a large release of acid moieties through saponification. Such a release was especially important in the case of the clay fraction since (i) palmitic acid that dominated the free acids is now largely outweighed by the $C_{20}$ acid and (ii) the $C_{15}$ *iso* and *anteiso* acids that exhibited a high relative abundance now appear as minor components (Figure 1.1f). The increase in the relative abundance of the $C_{18}$ to $C_{30}$ *n*-saturated fatty is substantial, although somewhat less pronounced, in the case of the silt-sized fraction. These observations indicate that the *n*-saturated fatty acids released in large amounts by saponification of the lipid extracts chiefly correspond to long-chain, predominantly even-carbon-numbered, compounds. According to such a distribution, these acid moieties probably originated from ester-containing components of higher plants like wax esters and suberin-derived components. The latter would correspond either to suberin that underwent some alteration and was partly depolymerised so that it became soluble or to soluble precursor that would be biosynthesized by the plant. The presence of substantial amounts of odd-carbon-numbered long chain fatty acids indicates, as for the free acids, that a part of these acids originates from microbial reworking of higher plant components.

#### 1.1.2.3 Bound hydroxy acids.

The saponified lipids show an important series of ω-hydroxy acids, while such components were not detected before saponification. This series is predominant in the clay-sized fraction. These hydroxy acids that exclusively occur as ester-bound moieties in the non GC-amenable lipids of the three fractions chiefly correspond to even-carbon-numbered compounds, ranging from $C_{12}$ to $C_{26}$ (or $C_{28}$) and showing a maximum at $C_{22}$ (Figure 1.2). No large differences are noted in the distribution of the ω-hydroxy acids between the three fractions. Such hydroxy acids, with similar distributions, were previously observed in extracts of whole soils (van Bergen *et al.*, 1998; Naafs *et al.*, 2004) and after saponification of the lipid extracts of acidic soils (Naafs and Van Bergen, 2002 and partie B, 1). Transesterification of high molecular weight lipids from soils also afforded a similar series of ω-hydroxy acids (Riederer et al., 1993). Even-carbon-numbered ω-hydroxy acids are typical components of the higher plant polyesters, cutins and suberins and such hydroxy acids are among the main building blocks of these biopolymers. However, they exhibit different carbon number ranges

for cutins and suberins: only $C_{16}$ and $C_{18}$ in the former case whereas a large range of even-carbon-numbered compounds, from $C_{16}$ to $C_{26}$, is typically observed for suberins (Kolattukudy, 1980). The presence of the even ω-hydroxy acids, observed in the saponified lipids, thus probably reflects the contribution of components derived from higher plant polyesters, especially of partly depolymerised suberin-derived components. The occurrence of low amounts of odd-carbon-numbered homologues indicates that some bacterial reworking probably took place for these components, through α-oxidation and chain shortening. The above results thus point to a marked input of root-derived components for the lipids of the three fractions. A large contribution of root material was previously observed, to total SOM, through pyrolysis of forest soils (Nierop, 1998; Naafs and Van Bergen, 2002). Such a feature was also noted by Balesdent (1996) for the total OM of cultivated soils and interpreted as possibly reflecting a lower biodegradability of root components compared to aboveground material.

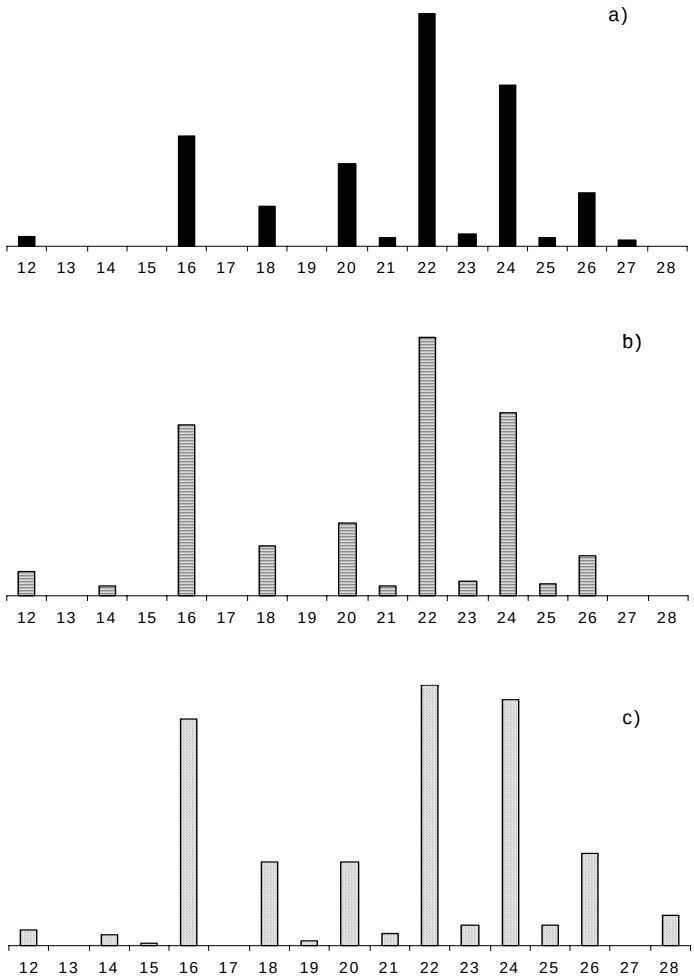



Fig 1.2: Distribution (%) of the ω-hydroxy acid obtained after saponification of the total lipid extract of the three particle-size fractions. a) sand fraction, b) silt fraction, c) clay fraction.

A part of the $C_{16}$ and $C_{18}$ ω-hydroxy acids may also originate from partly depolymerised cutin. Indeed, the contribution of cutin-derived compounds is supported by (i) the presence in the three saponified fractions of a typical building block of cutin, a $C_{18:1}$ ω-hydroxy acid and (ii) the presence of the $C_{16}$ 10,16-dihydroxy acid, also a typical building block of cutin (Del Rio and Hatcher, 1998). The ω-hydroxy acids may also partly originate from microbial oxidation of fatty acids on the terminal carbon and be intermediates in the formation of α,ω-diacids (Haug and Sever, 1971; Naafs *et al.*, 2004). However, the lack of a clear-cut relationship with the distribution of the saponified fatty acids (Figure 1.1d-f) indicates that such a process did not play an important role in the present case.

#### 1.1.2.4 Bound diacids.

Diacids were not detected before saponification, except for a very low amount of $C_8$ and $C_9$ α,ω-diacids for the silt- and clay-sized fractions. On the contrary, an abundant series of α,ω-diacids was identified in the saponified lipids of the three particle-size fractions. This series shows similar distributions for the silt- and clay-sized fractions (Figure 1.3b,c). These distributions are characterised by the presence of low amounts of $C_9$ and $C_{10}$ diacids, the predominance of even-carbon-numbered compounds in the $C_{16}$ to $C_{27}$ (or $C_{29}$) range, a maximum at $C_{22}$ and a major contribution of long chain compounds from $C_{20}$. The distribution for the sand-sized fraction is substantially different due to the lack of the short chain $C_9$ and $C_{10}$ diacids, and of odd-carbon-numbered compounds (Figure 1.3a). α,ω-Diacids were previously observed, for acidic soils, through saponification or pyrolysis (Amblès et al., 1991; van Bergen *et al.*, 1998; Gobé et al., 2000; Naafs and van Bergen, 2002). Even-carbon-numbered α,ω-diacids in the $C_{16}$ to $C_{24}$ range are major building blocks of suberins (Kolattukudy, 1980; Del Rio and Hatcher, 1998). The distribution observed for the main α,ω-diacids in the saponified lipids of the three fractions thus reflects the contribution of suberin-derived components and hence of root-derived material. α,ω-Diacids can also originate from the microbial oxidation of ω-hydroxy acids (Van Bergen et al., 1998). Comparison with the distribution of the hydroxy acids suggests that such a pathway may afford some contribution to the α,ω-diacids, especially to those above $C_{24}$ in the silt and clay fractions. For the $C_9$ and $C_{10}$ diacids observed in these two fractions, exclusive formation through microbial oxidation

can be put forward. Indeed, such short-chain diacids are typical degradation products of unsaturated fatty acids and of mid-chain hydroxy acids (Haug and Sever, 1971; Amblès et al., 1991; Naafs *et al.*, 2004) and their carbon number reflects the locations of unsaturation and of mid-chain hydroxy substituents in these acids. In contrast, the occurrence of several odd-carbon-numbered homologues suggests that diacids underwent some reworking for the silt and clay fractions.

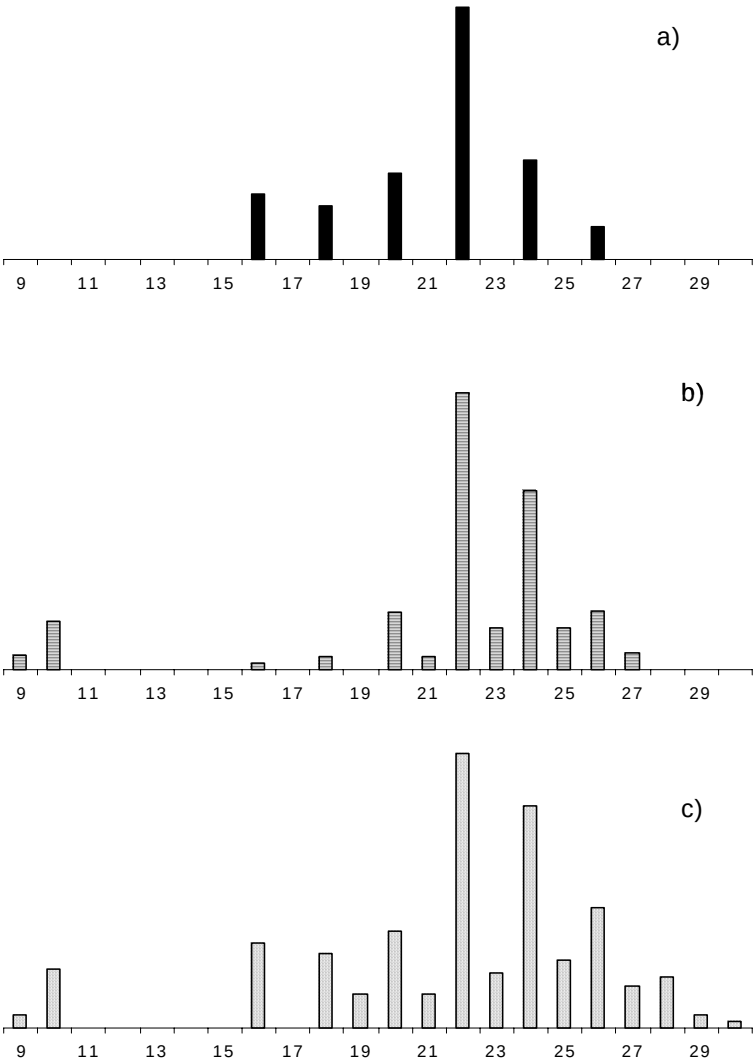
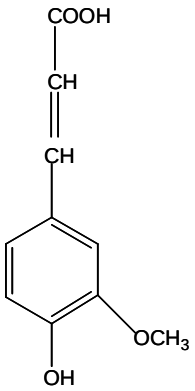



Figure 1.3: Distribution (%) of the α,ω-diacids obtained after saponification of the total lipid extract of the three particle-size fractions. a) sand fraction, b) silt fraction, c) clay fraction.

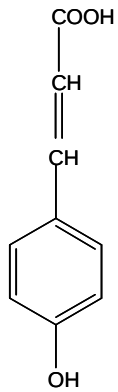
1.1.2.5 Bound aromatic acids.

Some aromatic acids were detected after saponification of the lipid extracts. They are dominated by the 3-methoxy 4-hydroxy cinnamic acid (ferulic acid), while the 4-hydroxy

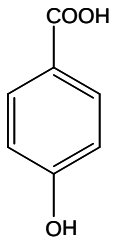
cinnamic acid (*p*-coumaric acid), the 4-hydroxy benzoic acid and the 3-methoxy 4-hydroxy benzoic acid only exhibit low relative abundances. Similar distributions were observed for these aromatic acids from the three particle-size fractions. Two assumptions can be put forward. Indeed, the ferulic and p-coumaric acid are typical compounds of lignin, so these acids can originate either from lignin or suberin. Suberins are characterized by the presence of aromatic domains, in addition to the aliphatic domains formed by interesterified ω-hydroxy acids and α,ω-diacids (Kolattukudy, 1980; Bernards and Lewis, 1998). The former domains are composed of aromatic acids like ferulic and *p*-coumaric acids. The occurrence of ferulic and *p*-coumaric acid moieties therefore probably reflects the contribution of suberin-derived components to the lipids of the three fractions. The benzoic acids could also correspond to moieties in suberin-derived components that underwent oxidative degradation of the $C_3$ alkyl chain resulting in the cleavage of the unsaturation. However, based on their structure, all these aromatic acid moieties released by saponification of the lipid extracts of the three fractions might also originate from lignin-derived components. Indeed, lignin in monocotylodenous angiosperms like maize is characterised by the presence of both *p*-coumaryl units (no $OCH_3$ substituents on the aromatic ring) and guaiacyl units (one $OCH_3$ substituent).



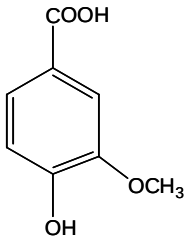

Ferulic acid
(4-hydroxy-3-methoxy cinnamic acid)




p-coumaric acid
(4-hydroxy cinnamic acid)




4-hydroxy benzoic acid




4- hydroxy- 3-methoxy- benzoic acid

1.1.2.6 Free alkanols.

Normal primary alkanols were detected in the three lipidic extracts after silylation. These *n*-alkan-1-ols are exclusively, or predominantly, even-carbon-numbered and they occur in the $C_{18}$ (or $C_{20}$) to $C_{28}$ range. The *n*-alkan-1-ols from the silt- and clay-sized fractions show similar distributions but marked differences are noted with the sand fraction (Figure 1.4a-c). The latter is characterised by the abundant presence of the $C_{18}$ alkanol (lacking for the other two fractions) and by the exclusive occurrence of even compounds. *n*-Alkan-1-ols are commonly observed in lipids extracted from whole soils (Amblès et al., 1985; Jambu et al., 1985; 1993; Schulten and Schnitzer, 1990; Jaffé et al., 1996; van Bergen *et al.*, 1997, 1998; Bull *et al.*, 1998, 2000; Naafs et al., 2004). In fact, long-chain (above $C_{20}$) even-carbon-numbered *n*-alkan-1-ols are typical components of the epicuticular waxes of higher plants with maxima generally found at $C_{22}$ and/or $C_{24}$. Accordingly, the distribution observed for the silt and clay fractions indicates a major contribution of *n*-alkan-1-ols from higher plants, including some microbially reworked products as indicated by the presence in substantial amounts of odd-carbon-numbered alkanols. Large amounts of the $C_{18}$ and $C_{20}$ *n*-alkan-1-ols have been observed in the lipids of some microbial spores (Amblès et al., 1989; Jaffé *et al.*, 1996; van Bergen *et al.*, 1997, 1998; Naafs et al., 2004). The distribution of the sand-sized fraction therefore points to a large contribution of such a source in the coarse fraction. n-Alkane oxidation may also contribute to the formation of *n*-alkan-1-ols (Amblès *et al*., 1985, 1989). However the major differences observed with the distribution of the former (see below), especially the predominance of the odd-carbon-numbered *n*-alkanes that should form odd *n*-alkanols upon oxidation, rules out a significant role for this pathway. Previous studies on the lipids of whole soils suggested that *n*-alkan-1-ols can also be formed by fatty acid reduction (Jambu *et al*., 1993; Bull *et al.*, 1998). However, comparison with the distributions of the fatty acids (Figures 1.1a-c and 1.4a-c) showed major differences indicating that such a process played, at most, a minor role in the formation of the free *n*-alkan-1-ols for the Cestas maize soil.

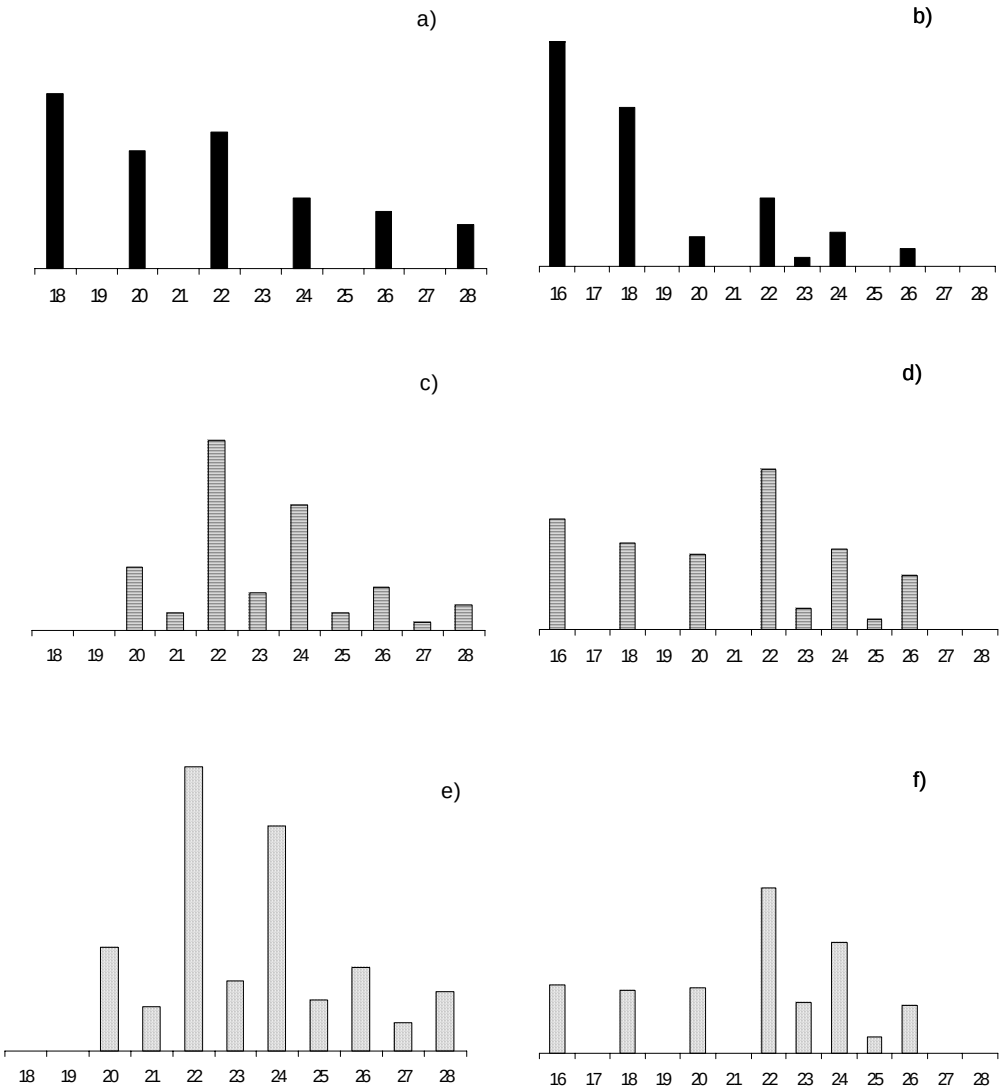



Fig 1.4: Distribution (%) of the free alkanols obtained after saponification of the total lipid extract of the three particle-size fractions. a) sand fraction, b) silt fraction, c) clay fraction and of the alkanols obtained after saponification: d) sand fraction,e) silt fraction, f) clay fraction

1.1.2.7 Bound alkanols.

Abundant *n*-alkan-1-ols occur in the saponified lipids of the three fractions. This series ranges from $C_{16}$ to $C_{26}$ and is dominated by even-carbon-numbered compounds (Figure 1.4d-f). Such alcohols, with the same type of distribution were previously observed, as for the α,ω-diacids, through study of acidic soils by saponification or pyrolysis (Amblès *et al.*, 1991 ; Gobé *et al*., 2000; Naafs and Van Bergen, 2002). Comparison with the distribution of the free alkanols (Figure 1.4a-c) showed important changes. These variations reflect a large release of

ester-linked alcohol moieties upon saponification. As a result, (i) for the sand-sized fraction, the $C_{16}$ *n*-alkan-1-ol now dominates while it was lacking in the free alcohols and the relative abundance of the $C_{18}$ homologue is largely increased, (ii) for the silt- and clay-sized fractions, substantial amounts of the $C_{16}$ and $C_{18}$ alkanols are observed after saponification and (iii) the relative abundance of the $C_{27}$ and $C_{28}$ components becomes negligible so that they are no longer detected in the saponified lipids contrary to the free ones. It thus appears that saponification resulted in a large release of the $C_{16}$ and $C_{18}$ *n*-alkan-1-ols, probably of microbial origin. However, predominantly even-carbon-numbered longer compounds ($C_{20}$ to $C_{26}$ range) were also freed in large amounts. Based on previous studies on higher plants and whole soils (van Bergen *et al.*, 1997, 1998; Naafs & van Bergen, 2002), the latter alkanols probably originate from wax esters and suberin-derived products and include microbially reworked components. Similar distributions are observed for the saponified alkanols of the silt and clay fractions (Figure1.4e,f). In contrast, the saponified alkanols of the sand fraction show a different distribution due to a higher relative abundance of the microbial $C_{16}$ and $C_{18}$ alkanols while the long chain alkanols of higher plants are relatively less abundant (Figure 1.4d). In fact, the relative abundance of the $C_{20}$ to $C_{26}$ *n*-alkanols increases when the fraction becomes finer (Figure 1.4d-f); a similar trend was also observed for the long chain bound fatty acids (Figure 1.1d-f) and the bound diacids (Figure 1.3a-c). The distributions of the bound alkanols in the three fractions indicated, as for the free alkanols, that neither *n*-alkane oxidation nor fatty acid reduction played a significant role in their formation.

#### 1.1.2.8 Alkanes.

Their distribution, as reflected by ion chromatograms of *m/z* 57, is shown in Figure 1.5. For the three extracts, the *n*-alkanes predominate and show a large carbon number range from $C_{12}$ to $C_{33}$. In addition, low intensity clusters are observed, especially in the first part of the chromatograms. These clusters correspond to complex mixtures of isomers of branched alkanes, including *iso*, *anteiso* and isoprenoid compounds. Apart from these common features, a marked difference is observed in the distribution of the *n*-alkanes between the sand- and silt-sized fractions, on the one hand, and the clay fraction, on the other hand. In fact, the former two exhibit a unimodal distribution with a maximum at $C_{14}$, no odd-or even-carbon number predominance in the $C_{12}$ to $C_{20}$ range and a weak contribution of predominantly odd-carbon-numbered $C_{21}$ to $C_{33}$ compounds (Figure 1.5a,b). On the contrary the *n*-alkanes of the clay-

sized fraction show a bimodal distribution with a maximum at $C_{23}$ and a submaximum at $C_{16}$ (Figure 1.5c). Again, no odd or even predominance is noted for the short *n*-alkanes while the long ones are predominantly odd-carbon-numbered in the $C_{23}$ to $C_{33}$ range. *n*-Alkanes are commonly observed in the lipids of whole soils where they generally represent the most abundant hydrocarbons (Stevenson, 1966; Jambu et al., 1991; Amblès *et al*., 1989; Jaffé *et al.*, 1996; van Bergen *et al.*, 1997; Marseille et al., 1999; Naafs et al., 2004).

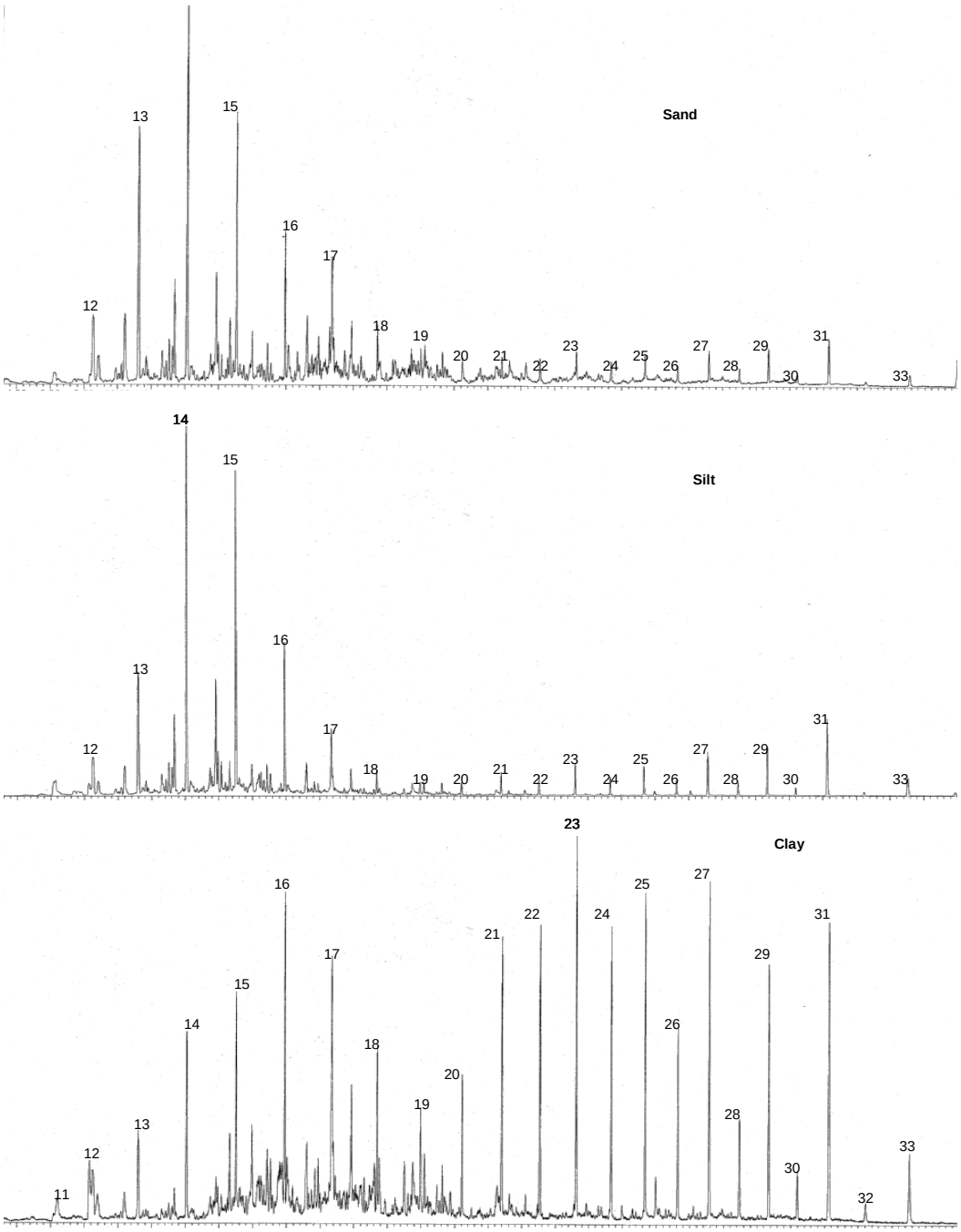



Fig 1.5: ion chromatograms (m/z 57) showing the distribution of the n-alkanes of the total lipid extract of the three particle-size fractions

For the Cestas cultivated soil, bacterial contribution is reflected by low intensity clusters of branched alkanes in the first part of the chromatograms. As previously put forward for the lipid extracts of various whole soils (e.g. Jambu et al., 1985; Amblès *et al*., 1989) bacteria should also be the source of the *n*-alkanes with no odd- or even-carbon number predominance in the $C_{12}$ to $C_{19}$ range. The latter compounds occur in the three fractions and they are dominant for the sand- and silt-sized ones. Long chain, odd-carbon-numbered $C_{23}$ to $C_{33}$ *n*-alkanes are typical components of epicuticular waxes of higher plants (Tulloch, 1976; van Bergen *et al.,* 1997). Accordingly, they are classically used as biomarkers of higher plant input. However, some recent studies indicate that such compounds can also be formed by fungi (Marseille *et al.,* 1999; see also Bull et al 2000 and Naafs et al 2004). Moreover, some fungal species were shown to produce long chain n-alkanes without predominance (Grimalt, 1988; Hu Ming et al., 1995). Accordingly, two origins can be considered for the long chain n-alkanes (abundant for the clay-sized fraction, minor for the other two ones): higher plant waxes and/or fungal lipids. The distributions observed for the three fractions thus point to a high microbial (bacterial and also possibly fungal) contribution, to the *n*-alkanes

1.1.2.9 Alkanones.

The presence of alkan-2-ones in the three extracts was revealed by selective detection of their specific ion (*m/z* 58.). The distributions thus obtained are similar for the three particle-size fractions (Figure 1.6). They are characterised by predominantly odd-carbon numbered compounds in the $C_{22}$ to $C_{31}$ range with a maximum at $C_{25}$ or $C_{27}$ and by an abundant $C_{18}$ regular isoprenoid ketone (2,6,10-trimethylpentadecan-2-one). The only substantial difference between the three fractions is the occurrence of a large amount of the $C_{13}$ regular isoprenoid ketone (2,6 -dimethylundecan-2-one) in the lipids of the sand-sized fraction while this ketone is not detected in significant amount for the other two fractions. *n*-Alkan-2-ones are generally considered as not being directly formed as primary products by living organisms (Rieley et al., 1991). Nevertheless, such compounds are commonly observed in lipids of whole soils (Amblès et al., 1989, 1993; van Bergen *et al.,* 1998; Naafs et al., 2004) where they are thought to originate either from the sub-terminal microbial oxidation of *n*-alkanes or from the β-oxidation and subsequent decarboxylation of *n*-saturated fatty acids. The implication of the former pathway is reflected by similar distributions for the *n*-alkan-2-ones and *n*-alkanes whereas, for the second pathway, the distribution for the *n*-alkan-2-ones is similar to that of

the fatty acids minus one carbon. In the present study, such relationships are not observed, either with the *n*-alkane or with the *n*-saturated fatty acid distributions. This is due to the lack of compounds below $C_{19}$ for the *n*-alkan-2-ones while such products are predominant for the acids and the alkanes (Figures 1.1 and 1.5). Moreover, when the detailed distribution of these long-chain ketones is considered, no tight relationship is noted with the corresponding long chain *n*-alkanes or with the long chain *n*-saturated fatty acids (minus one carbon). Nevertheless, the carbon number range of the *n*-alkan-2-ones and the maximum at $C_{25}$ or $C_{27}$ point to a higher plant origin.

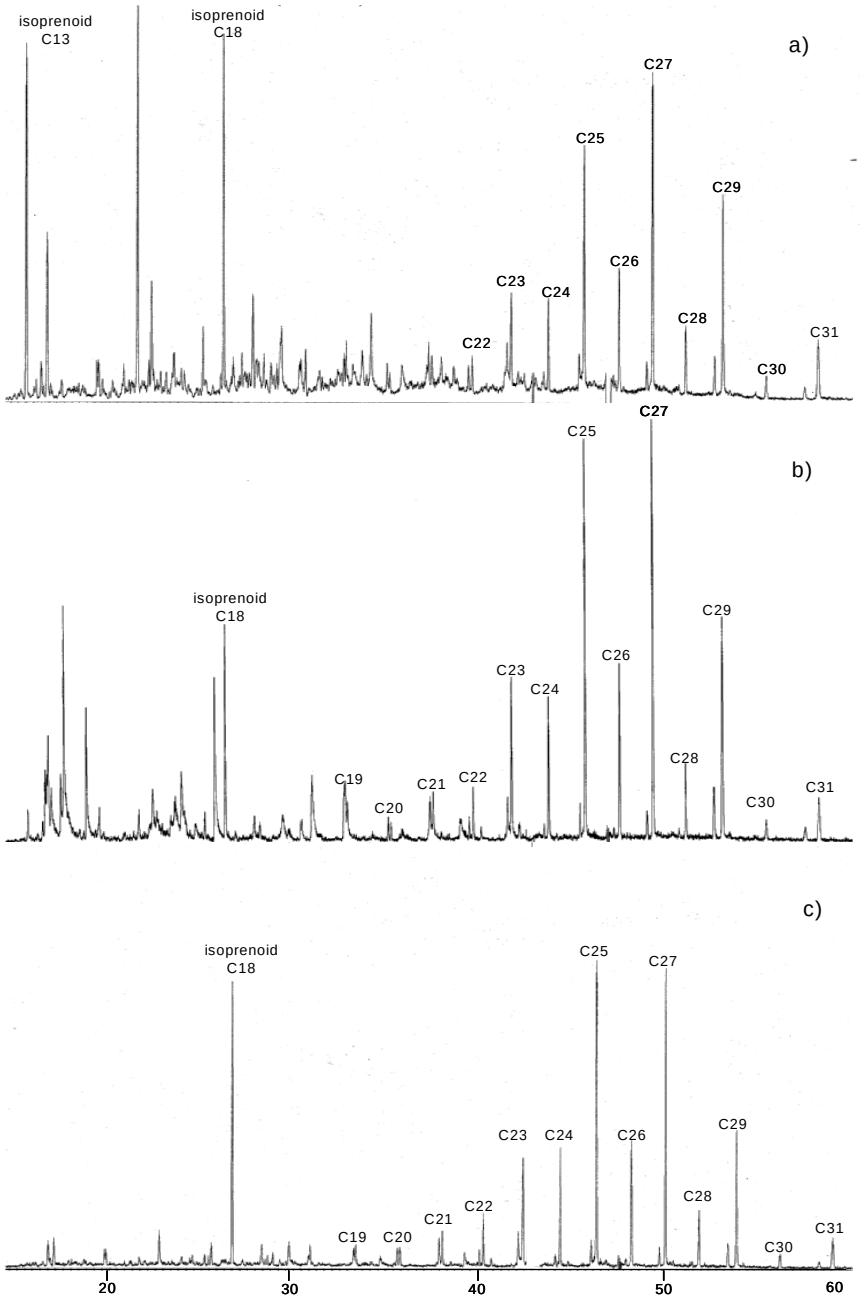



Figure 1.6: ion chromatograms (m/z 58) showing the distribution of the ketones of the total lipid extract of the three particle-size fractions a) sand, b) silt and c) clay

1.1.2.10 Lipid sources in particle-size fractions.

Analysis of the lipid extracts, before and after saponification, afforded detailed information on the sources and the relative composition of the lipids in the three fractions of the cultivated soil (Table 1.3). Several types of compounds originated from higher plants, including (i) some free fatty acids, free alkanols and possibly alkanes from waxes, (ii) the bound fatty acids and bound alkanols released from wax esters upon saponification and (iii) the suberin-, cutin- and lignin-derived components also detected through saponification, probably corresponding to partial depolymerisation products of these plant biopolymers. Most of these compounds underwent substantial microbial reworking and the alkanones and a part of the bound diacids originate from the microbial oxidation of plant components. In addition, microbial sources directly contributed to several types of lipidic components, including some free fatty acids, free and bound alkanols and alkanes. In some cases, information was obtained on the type (bacteria, fungi, fungal spores) of microorganisms implicated.

In soil, pH plays a major role in the control of microbial activity and, hence, on SOM fate. Under acid conditions fungi and Actinomycetes dominate and the microbial activity is limited (e.g. Dinel, 1990; Motavalli et al., 1995). As a result, lipid preservation is favoured in acid soils (e.g. Moucawi et al., 1981; Fustec et al., 1985; Amblès et al., 1985; Jambu et al 1978,1983; Dinel et al 1990; van Bergen et al., 1998; Bull et al., 2000). On the contrary, other fungi and bacteria predominate in alkaline soils (e.g. Amblès et al., 1989; Dinel, 1990; Bumpus 1993) and a high microbial activity takes place in such soils. The Cestas cultivated soil is weakly acid (pH of ca. 6) so that microbial activity shall be relatively intense. In fact, in strongly acidic soil, limited microbial activity is reflected by the lack of short chain ($<C_{20}$) *n*-alkanols and of branched (iso and anteiso) acids (Naafs et al., 2004). On the contrary, such components are found for the lipids of the Cestas soil, thus confirming a substantial microbial activity, also reflected by the occurrence of even carbon numbered *n*-alkane.

| | sand | silt | clay |
|---|---|---|---|
| Free FAs [a] | plant waxes* (weak)<br>bacterial (moderate) | plant waxes* (abundant)<br>bacterial (weak) | plant waxe*s (weak)<br>bacterial (abundant) |
| Bound FAs | plant (wax esters & suberin-derived)* | plant (wax esters &, suberin-derived)* | plant (wax esters & suberin-derived)* |
| Bound OH acids [b] | plant (suberin- & cutin-derived)* | plant (suberin- & cutin-derived)* | plant (suberin- & cutin-derived)* |
| Bound diacids | plant (suberin-derived)<br>no microbial oxidation products | plant (suberin-derived)*<br>microbial oxidation products [c] | plant (suberin-derived)*<br>microbial oxidation products [c] |
| Bound aromatic acids [b] | plant (suberin- & lignin-derived) | plant (suberin- & lignin-derived) | plant (suberin- & lignin-derived) |
| Free alkanols | plant waxes* (large)<br>microbial spores (large) | plant waxes* (major) | plant waxes* (major) |
| Bound alkanols | Plant* [d]<br>microbial (dominant) | Plant* [d] (dominant)<br>microbial | Plant*[d] (dominant)<br>microbial |
| Alkanes | plant waxes and/or fungal lipids (low)<br>bacterial (dominant) | plant waxes and/or fungal lipids (low)<br>bacterial (dominant) | plant waxes and/or fungal lipids (large)<br>bacterial (large) |
| Alkanones [b] | plant [e] | plant [e] | plant [e] |

Table 1.3: Sources of the different types of free and bound lipids in the three particle-size fractions of the Cestas cultivated soil*: Including microbially reworked compounds,[a] Ubiquitous palmitic acid always predominant,[b] Similar distributions were observed for the three particle-size fraction,[c] Originating from the oxidation of plant components,[d] Wax esters and suberin-derived components,[e] The precise source of the *n*-alkan-2-ones was not determined but their distribution indicates that they originate from the microbial oxidation of plant components; the $C_{18}$ isoprenoid ketone originates from the oxidation of phytyl moieties

### 1.1.3 Differences between the particle-size fractions: Comparison with the forest soil.

Similar distributions were observed for some lipid series in the three particle-size fractions of the cultivated soil, namely the bound ω-hydroxy and aromatic acids and the *n*-alkan-2-ones (Table 1.3, Figures 1.2 and 1.6). However, a number of differences, related to the distribution or the relative abundance of the considered type of compounds, were observed in the lipid composition of the three fractions (Table 1.3, Figures 1.1, 1.3-1.5). For example,

conspicuous differences occur in the distribution of the free fatty acids and of the n-alkanes depending on the particle-size fraction. It also appears that the intensity of bacterial reworking increases when the fraction becomes finer, as reflected by changes in distributions of ω-hydroxy acids, diacids and free and bound alkanols. Thus, for example, the diacids of the sand fraction exclusively correspond to even-carbon-numbered (non-reworked) homologues while increasing abundance of the odd-carbon-numbered (reworked) homologues is observed for the silt and clay fractions. Also, ω-hydroxy acid and diacid distributions may reflect a relatively large input of fresh plant debris in the sand fraction, in agreement with the classically considered transfer processes between size fractions upon humification and the relatively larger input of maize carbon in this fraction revealed by isotopic measurements (see below). As in the case of the Cestas forest soil (partie B, 1), the results obtained on this cultivated soil therefore illustrate the variety in lipid composition occurring between the size fractions and the usefulness of studying the lipids on size separates.

Comparison of lipid composition in the particle-size fractions of the cultivated soil and of the forest soils showed marked differences for all the types of lipids and all the particle-size fractions. These differences are especially pronounced in a number of cases, as illustrated for the free fatty acids in the clay-sized fractions (Figure 1.7a,b), the bound alkanols in the sand-sized fractions (Figure 1.7c,d) and the n-alkanes in the silt-sized fractions (Figure 1.7e,f). As previously observed for lignin and carbohydrate components (Guggenberger *et al.*, 1994), change in land-use is associated with conspicuous variations in SOM composition of the particle-size fractions. The present results on lipids confirm that size fractionation is an efficient tool for evaluating both SOM sources and land-use induced changes in SOM composition. As illustrated in Figure 1.7, the lipids of the size fractions of the Cestas cultivated soil generally show a markedly lower contribution of higher plants components and a higher contribution of microbial products.

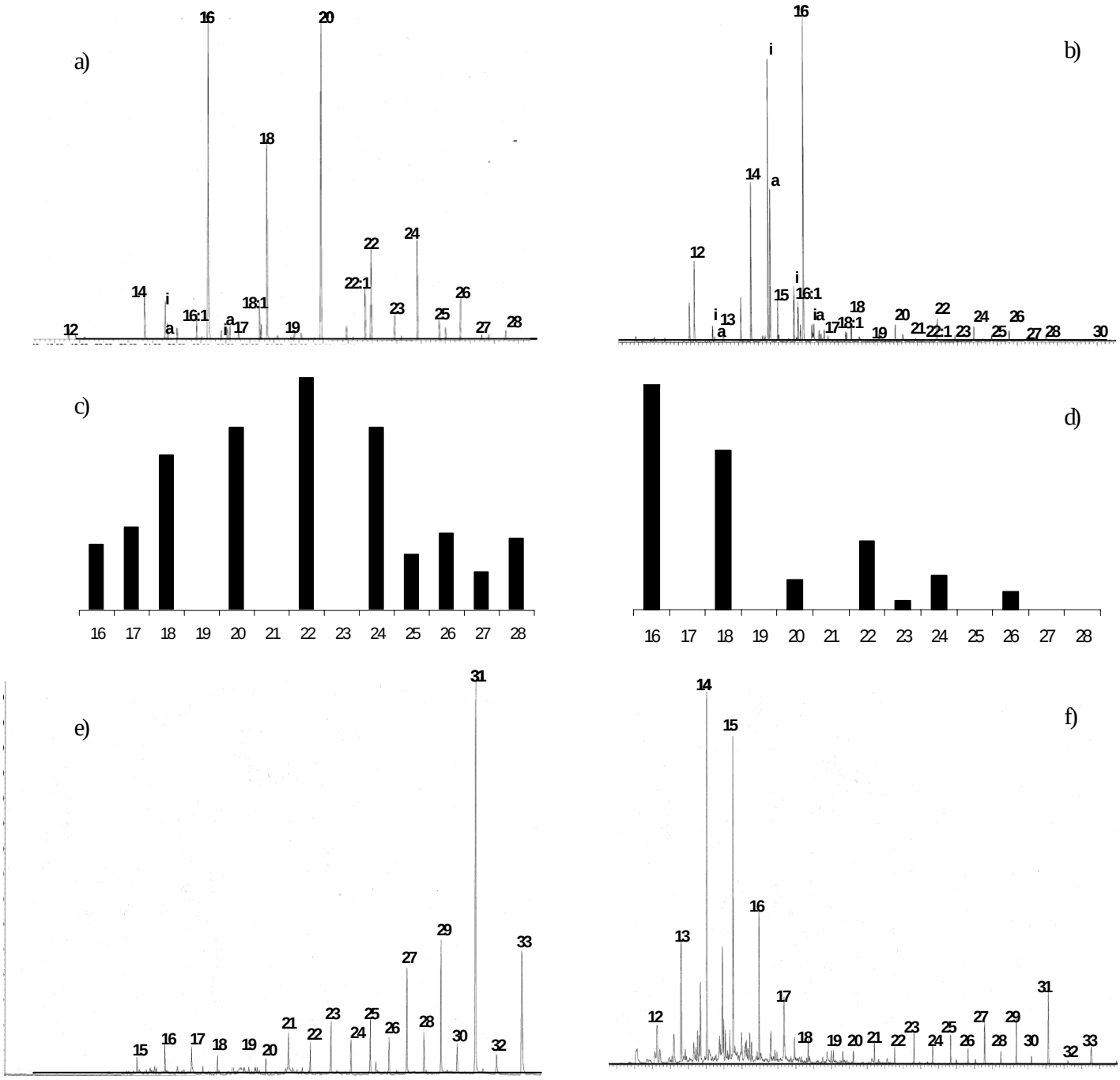



Figure 1.7: Comparison of lipid composition in the particle-size fractions of the cultivated soil and of the forest soils: a-b) ion chromatograms (m/z 74) showing the fatty acid distribution in the clay fraction a) from the forest soil, b) from the cultivated soil, c-d)alkanol distributions after saponification of the total lipid extract of the sand fraction c) in the forest soil, d) in the cultivated soil, e-f) ion chromatograms (m/z 57) showing the n-alkane distribution in the silt fraction e) from the forest soil, f) from the cultivated soil

### 1.1.4 Contribution of forest-inherited and of maize materials in the cropped soil.

The marked differences observed above in lipid composition for the particle-size fractions of the cultivated and forest soil shall reflect both large changes, after 22 years of continuous cropping, in the lipids inherited from the forest soil and input from maize components. Molecular and isotopic studies were performed to derive information on these two types of contribution with focus on hydrocarbons.

#### 1.1.4.1 *n*-Alkane distribution in maize shoots and roots. Comparison with the cropped soil

Extensive studies have been performed, on hydrocarbon composition in the aerial parts of higher plants, for a number of species including maize (Avato et al., 1990; Rieley et al., 1991; Chikaraishi et Naraoka, 2003). On the contrary, analyses on hydrocarbons from roots are very scarce (Marseille et al., 1999) and, to the best of our knowledge no such study has been reported for maize. Root material generally accounts for a large part of plant OM input to soil. In the present case the shoots were ground after harvest and left on the ground till next spring before being returned to the soil upon ploughing, so that maize OM input shall largely correspond to root material. Accordingly, hydrocarbon composition was examined for both shoot and root samples. In both cases, the *n*-alkanes are the major hydrocarbons of the lipid extracts. The *n*-alkanes from the maize shoots exhibit a wide carbon number range ($C_{17}$ to $C_{33}$) with a major contribution of predominantly odd-carbon-numbered long chain compounds and a maximum at $C_{29}$ (Figure 1.8a). This type of distribution is classically observed for the aerial parts of higher plants and has been reported for a number of species, including maize (Avato et al., 1990; Rieley et al., 1991). Remarkably, *n*-alkane distribution in maize roots is markedly different when compared to shoot distribution (Figure 1.8b). Indeed, the former distribution also shows a wide carbon number range from $C_{17}$ to $C_{33}$, but it is characterised by a low contribution of long chain compounds and a maximum at $C_{19}$. Thus, this distribution hardly exhibits the features that are generally thought to be typical of higher plant *n*-alkanes, based on studies that were concerned with numerous plants but virtually limited to shoot materials. Comparison of these distributions with those observed for the fractions of the cultivated soil indicated that the long chain compounds from maize shoot possibly contributed

to the long chain *n*-alkanes occurring, as minor hydrocarbons, in the sand- and silt-sized fractions (Figure 1.5a,b) and, as major hydrocarbons, in the clay-sized fraction (Figure 1.5c). However, some substantial differences are observed (Figures 1.8a and 1.5a-c), including a much higher relative abundance of the even-carbon-numbered long chain compounds and the lack of a maximum at $C_{29}$, for the lipids in the cropped soil contrary to maize shoots. Accordingly, alkanes from maize shoots possibly contributed, but only partly, to the long chain *n*-alkanes of the cultivated soil. For the hydrocarbons of maize roots, their distribution suggests a possible contribution to the $C_{19}$ *n*-alkane occurring in moderate relative abundance in the hydrocarbons of the clay-sized fraction (Figures 1.8b and 1.5c). These root components may also contribute to the $C_{20}$-$C_{23}$ *n*-alkanes. However, such a contribution should be low based on the rather weak intensity of the $C_{19}$ *n*-alkane in this fraction. When compared with alkanes in the forest soil (ranging from $C_{14}$ to $C_{33}$ maximizing at $C_{31}$), it appears that at least some of them are preserved in the maize soil. Together, the molecular features therefore point to a possible contribution of *n*-alkanes from maize shoots and roots to the *n*-alkanes of the particle-size fractions of the cultivated soil but this contribution was, at most, minor. Stable carbon isotopes measurements were performed to assess the relative contributions of forest-inherited carbon and of maize carbon to total organic carbon, total lipids and individual *n*-alkanes in the cultivated soil.

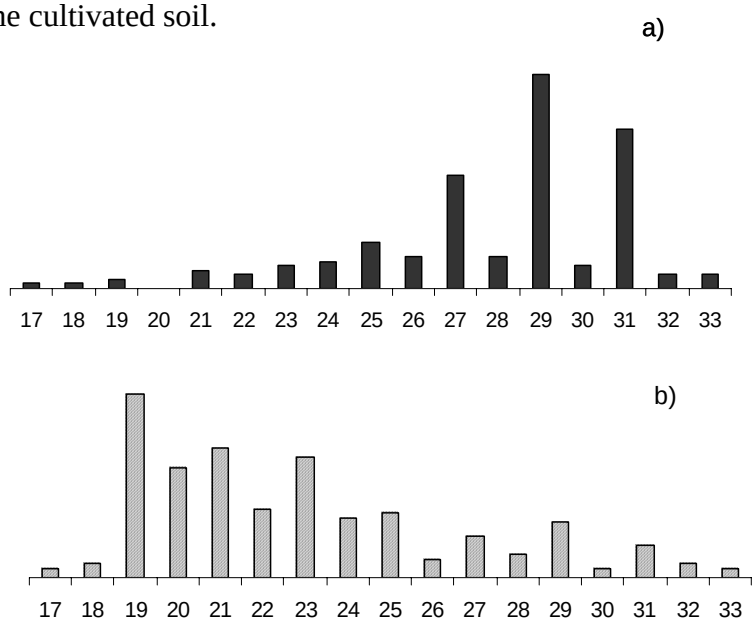



Figure 1.8 : n-alkane distribution in the total lipid extract of a) maize shoots and b)from the maize roots

1.1.4.2 Bulk isotopic measurements.

The differences in the photosynthetic pathways of $C_3$ plants (like trees in temperate forest) and $C_4$ plants (like maize) result in large differences in $^{13}C$ abundance (Bender, 1971; Smith et Epstein, 1971; O'Leary, 1981). These differences are reflected by the stable carbon isotope ratios of OM in soils with different types of vegetation. The natural isotopic labelling obtained through $C_3/C_4$ vegetation sequences thus affords information on OM dynamics in soil following change in land use (e.g. Balesdent et al., 1988; Balesdent and Mariotti, 1996; Huggings et al., 1998). In the cultivated plot of the Cestas sequence, the forest ($C_3$ plants with $\delta^{13}C$ values around –27‰ for total OM) has been replaced 22 years ago by continuous maize cropping ($C_4$ plant with a $\delta^{13}C$ value of ca. –12‰ for total OM).

| | $C_{org}$ isotopic ratios ($\delta^{13}C$ ‰) | Relative contributions (% of $C_{org}$) | | Preservation/degradation level (%) of inherited forest C [c] |
|---|---|---|---|---|
| | | Maize C [a] | Forest C [b] | |
| Whole soil | - 25.17 (- 27.77) | 17 | 83 | 62/38 |
| Sand | - 22.21 (- 27.61) | 35 | 65 | 40/60 |
| Silt | - 24.36 (-27.88) | 23 | 77 | 59/41 |
| Clay | - 25.66 (- 27.97) | 15 | 85 | 69/31 |

Table 1.4**:** Bulk isotopic data for $C_{org}$ and derived information on the cultivated soil of Cestas and the three particle-size fractions: relative contribution of carbon derived from maize and from the forest soil; level of preservation/degradation of the carbon inherited from the forest soil after 22 years of maize cropping[a] Calculated from equation (annexe 5.5) using $\delta^{13}C$ value of Cestas maize Carbon (- 12.5 ‰) and $\delta^{13}C$ value of $C_{org}$ of the forest soil and its three particle-size fractions (bracketed values),[b] Forest Carbon contribution = 100 – maize Carbon contribution,[c] Calculated from $C_{org}$ distribution in the forest and cultivated soils (whole soils and fractions, see Table 1.1) and the relative contributions of forest C

At first, the natural abundance of $^{13}C$ was measured for the total organic carbon of the cultivated and forest soils and of their particle-size fractions (Table 1.4). A weak decrease in $^{13}C$ contribution, corresponding to $\delta\ ^{13}C$ lowering of ca. 0.4‰, is observed for the forest soil as the fraction becomes finer. Previous studies on soils, where the OM can be considered to be in a steady state, also only showed weak differences, of at most 1‰, between particle-size fractions (e.g. Balesdent et al., 1987; Martin et al., 1990; Feller et al., 1991; Gregorich et al., 1994; Balesdent and Mariotti, 1996; Roscoe et al., 2001; Jolivet et al., 2003). On the contrary,

a substantial decrease (>3‰) was noted for the cultivated soil, from the sand to the clay fraction, reflecting different relative contributions of maize and forest carbon (Table 1.4). From these isotopic data, the relative amounts of maize-derived and forest-derived carbon in the total organic carbon of the cropped soil and of its fractions were calculated using equation reported annexes 5.5. It thus appeared (Table 1.4) that the contribution of “new” carbon originating from maize is moderate (only 17% for the whole soil). The contribution of maize carbon exhibits, however, fairly large variations depending on the considered fraction, from only 15% for the clay- to 35% for the sand-sized fractions. Accordingly, the contribution of maize carbon markedly decreases as the fraction becomes finer. Such a maximum contribution of “new” carbon to the coarser fraction and minimal values for the clay fraction are classically observed upon vegetation change (e.g. Cerri et al., 1985; Balesdent et al., 1988; Martin et al., 1990; Feller et al., 1991; Boutton, 1996; Jolivet et al., 2003). A substantial degradation of the carbon from the forest soil took place upon change in land use and intensive maize cropping for 22 years, as reflected by the drop in $C_{org}$ content of the whole soil (20.3 $g.kg^{-1}$ and 30.4 $g.kg^{-1}$ in the cultivated and forest soil, respectively). Nevertheless, carbon inherited from the forest soil remains predominant (at least 65%) whatever the considered fraction of the cultivated soil.

The level of preservation/degradation of the total organic carbon from the forest soil was also calculated from these isotopic data and from the bulk quantitative data reported in Table 1.1. It thus appeared that the forest carbon retained in the whole soil after 22 years of cropping represents 62% of the carbon that occurred in the initial forest soil (Table 1.4). Similar values, ca. 60 to 55%, were observed by Jolivet et al. (1997) for soils from the same area also cropped with maize for 22 years. However, this value cannot be considered, stricto sensu, as a “molecular” preservation yield (i.e. corresponding to molecules that would be directly related to components of the forest soil). In fact, this “forest” carbon (i.e. exhibiting an isotopic $C_3$ signature) occurring in the cultivated soil should be partly located in microbially reworked components of the initial soil and in components of the microbial biomass that chiefly thrived on the carbon inherited from this soil. When the particle-size fractions are considered, large differences in the level of preservation are observed and this level markedly increases from the sand- (40%) to the clay-sized (ca. 70%) fraction. The preservation yield calculated for a given fraction might be affected by organic matter transfer from (and/or to) the other fractions during cultivation. However, the observed trend is similar to that previously observed in various studies on vegetation changes (Cerri et al., 1985; Balesdent et al., 1988; Martin et al., 1990; Gregorich et al., 1994; Boutton, 1996; Jolivet et al.,

2003). As considered in the above studies, these values should mostly reflect difference in the preservation/degradation level of the carbon from the initial soil depending on fraction size. As classically observed, preservation is minimal in the sand-sized fraction and maximal in the clay-sized fraction of the Cestas cultivated soil (Christensen, 1987; Buyanovsky et al., 1994; Golchin et al., 1995).

The stable carbon isotope ratios were also determined for the lipid extracts from both soils and their particle-size fractions (Table 1.5). Contrary to classical examination on total organic carbon this is the first time, to the best of our knowledge, that this type of study is performed on lipid extracts of soil using particle-size fractionation.

| | Clip isotopic ratios ($\delta^{13}C$ ‰) | Relative contributions (% of Clip) | | Preservation/degradation level (%) of inherited forest C [c] |
|---|---|---|---|---|
| | | Maize C [a] | Forest C [b] | |
| Whole soil | - 28.5 (- 30.2) | 11 | 89 | 37/63 |
| Sand | - 27.0 (- 29.8) | 18.5 | 81.5 | 64/36 |
| Silt | - 28.5 (-29.9) | 9 | 91 | 43/57 |
| Clay | - 28.9 (- 30.25) | 9 | 91 | 24/76 |

Table 1.5**:** Bulk isotopic data for $C_{lip}$ and derived information on the lipids in the cultivated soil of Cestas and the three particle-size fractions: relative contribution of carbon derived from maize and from the forest soil; level of preservation/degradation of the carbon inherited from the forest soil after 22 years of maize cropping,[a] Calculated from equation (1) (see Experimental) using $\delta^{13}C$ values of Cestas maize lipids (- 15.05 ‰) and $\delta^{13}C$ values of $C_{lip}$ of the forest soil and its three particle-size fractions (bracketed values),[b] Forest Carbon contribution = 100 – maize Carbon contribution,[c] Calculated from $C_{lip}$ distribution in the forest and cultivated soils (whole soils and fractions, see Table 2) and the relative contributions of forest C

Lipids in living organisms and in derived materials, are substantially $^{13}C$-depleted relative to other types of components such as proteins and polysaccharides (e.g. Galimov, 1974; Deines, 1980; Monson and Hayes, 1982; Rieley et al., 1991; Collister et al., 1994). Thus, as expected, the lipids extracted from the forest and cropped whole soils and from their particle-size fractions exhibit markedly lower $\delta^{13}C$ values compared to the total organic carbon in the corresponding samples (Tables 1.4 and 1.5).

As for the total organic carbon, (i) a slight decrease in $^{13}C$ abundance in the lipidic carbon (corresponding to $\delta\ ^{13}C$ lowering of ca. 0.4‰) occurs in the forest soil from the sand-sized to the clay-sized fraction and (ii) a larger decrease is observed between these fractions for the cultivated soil reflecting different inputs of maize-derived lipidic carbon (Table 1.5).

However, the latter $\delta^{13}C$ decrease is substantially less pronounced (1.9‰ instead of ca. 3.4‰) for lipidic carbon compared to total carbon (Tables 1.4 and 1.5). In both cases, the relative contribution of maize carbon decreases as the fraction becomes finer and similar trends are therefore observed, between the particle-size fractions, for the lipidic carbon and the total organic carbon. However, the contribution of "new" carbon is always markedly weaker for the former. Thus the maximal input of maize carbon (in the sand-sized fraction) is of only ca. 18% for $C_{lip}$ against 35% for $C_{org}$ and the minimal inputs (in the clay-sized fraction) are of 9 and 15%, respectively. This difference in intensity for the relative contribution of the "new" carbon between the lipidic carbon and the total carbon may reflect (i) the low abundance of lipids in maize, shown by Fustec et al. (1985) and Jambu et al. (1985) for shoots and (ii) the poor relative stability of maize lipids in soil compared to TOC (Fustec et al., 1985; Moucawi et al 1981). In the latter studies, lipid extracts from maize straw were incubated in soils with different pH. A large mineralisation of these lipids was thus observed (30 to 50% after thirty days) even in acid soils. The somewhat larger contribution of maize carbon observed for the lipids in the sand-sized fraction (Table 1.5) is probably related to a larger input of relatively fresh OM in this fraction.

Preservation/degradation yields showed a substantially lower level of preservation of the carbon inherited from the forest soil for the lipidic carbon compared to the total carbon (37 against 62%) (Tables 1.4 and 1.5). This marked difference reflects a higher sensitivity to degradation upon change in land use and cropping for the lipidic components of the initial forest soil relative to total organic matter. When the particle-size fractions are considered, large variations in the preservation yield are noted. Indeed, this yield markedly decreases for the lipidic carbon from 64 to 24% between the sand- and the clay-sized fraction (Table 1.5). On the contrary, a pronounced increase was observed from 40 to 69% for the total organic carbon (Table 1.4). Reverse trends therefore occur for the lipidic compounds and the total organic matter with regard to the level of preservation of the carbon from the forest soil. Two explanations can be put forward to account for these opposite trends. Firstly, large lipid transfers may occur between the particle-size fractions upon cultivation so that comparisons between the fractions would be highly affected. Secondly, as already mentioned for total organic matter, the values calculated for the preservation/degradation of the carbon from the forest soil are unlikely to correspond to "molecular" preservation yields. Indeed, a large part of the lipids in the cultivated soil correspond to products that retained the isotopic signature of the "forest" carbon but, in fact, correspond to microbially reworked lipids and, also, to lipids formed by microorganisms (bacteria and fungi) thriving on organic components inherited

form the forest soil. This is reflected by the (i) the larger relative abundance of microbial lipids observed for the fractions of the cultivated soil compared to the forest soil and (ii) the large differences in distribution noted between both soils for most lipid series in a given fraction although lipids in the cropped soil predominantly contain forest carbon based on their isotopic signature.

#### 1.1.4.3 Isotopic measurements on individual compounds.

Stable carbon isotope ratios were measured for individual alkanes of both soils and of their particle-size fractions. It is well documented that long chain odd-carbon-numbered *n*-alkanes, usually considered as typical components of higher plant waxes, well exhibit the isotopic difference between $C_3$ and $C_4$ plants (e.g. Rieley et al., 1993; Collister et al., 1994; Chikaraishi and Naraoka, 2003). Moreover, a few previous studies showed that some direct input of *n*-alkanes from maize leaf waxes takes place in maize cropped soils (Lichtfouse et al., 1994; 1995). Accordingly, compound-specific isotope analysis (CSIA) was focused on the $C_{27}$, $C_{29}$ and $C_{31}$ *n*-alkanes of the Cestas forest and cultivated soils. These *n*-alkanes exhibit a high relative abundance in the leaf wax hydrocarbons of maize shoots and comparison of distributions, discussed in a previous section, showed that the contribution of maize components to the long chain *n*-alkanes of the cultivated soil probably originated from maize shoots rather than from roots.

The $\delta\ ^{13}C$ values obtained by CSIA for the $C_{27}$, $C_{29}$ and $C_{31}$ *n*-alkanes in the forest and cultivated soils and in maize shoots are reported in Table 1.6. The relative contribution of maize and forest carbon to these hydrocarbons in the cropped soil, calculated from these isotopic data, are also indicated. No regular trend is observed for the relative percentage of maize and forest carbon in the three *n*-alkanes in relation either with their chain length or with fraction size (Table 1.6). However, in agreement with observations on distributions discussed in a previous section, it clearly appears that the maize carbon always affords a minor contribution of at most ca. 25%. Substantially higher contributions of maize carbon to the *n*-alkanes of a cultivated soil were previously reported with values around 50% after 23 years of maize cropping (Lichtfouse et al., 1994;Cayet and Lichtfouse, 2001). However, in the latter studies, (i) maize succeeded to cropping of $C_3$ plants and not to forest as in the Cestas sequence and (ii) the examined soil was a silty loam instead of a sandy spodosol. The much

lower content of silt + clay in the Cestas soil (ca. 8% against ca. 90% for the silty loam) may largely account for the markedly lower contribution of maize carbon in the present case. In fact, the *n*-alkanes afforded by maize vegetation would be largely degraded for two reasons. Firstly, a low physical protection in the Cestas soil, characterised by a high macroporosity and aeration. Secondly, the « saturation » of the protective sites of the fine minerals by organic components from the forest soil, as considered by Jolivet et al. (2001, 2003) to account for observations on the bulk OM of this type of soil.

| *n*-alkanes | Forest | Maize shoot | Cultivated soil [b] | Relative contribution (%) | |
|---|---|---|---|---|---|
| | | | | Maize C [c] | Forest C |
| Sand | | | | | |
| $C_{27}$ | - 32.8 | -19.3 | - 29.4 | 25 | 75 |
| $C_{29}$ | - 33.2 | - 18.7 | - 30.4 | 20 | 80 |
| $C_{31}$ | - 32.5 | - 20.2 | - 30.8 | 14 | 86 |
| Silt | | | | | |
| $C_{27}$ | - 32.5 | -19.3 | - 30.8 | 13 | 87 |
| $C_{29}$ | - 32.8 | - 18.7 | - 31.0 | 12 | 88 |
| $C_{31}$ | - 32.6 | - 20.2 | - 30.8 | 14 | 86 |
| Clay | | | | | |
| $C_{27}$ | - 33.2 | -19.3 | - 30.8 | 17 | 83 |
| $C_{29}$ | - 33.6 | - 18.7 | - 30.2 | 22 | 78 |
| $C_{31}$ | - 32.3 | - 20.2 | - 30.8 | 12 | 88 |

Table 1.6**:** Individual isotopic data (δ $^{13}$C ‰) for the $C_{27}$, $C_{29}$ and $C_{31}$ *n*-alkanes from the lipid extracts of the particle-size fractions of the Cestas forest and cultivated soils and from maize shoots; calculated relative contributions of carbon derived from maize and from the forest soil in these *n*-alkanes for the cultivated soil after 22 years of maize cropping [a] CSIA measurements were performed on the hydrocarbon fractions isolated by thin layer chromatography from the lipid extracts of the particle-size fractions of both soil. [b] Close values were obtained for the even-carbon-numbered *n*-alkanes (e.g. –31.2 to –31.5‰ for the $C_{24}$, $C_{26}$, $C_{28}$ and $C_{30}$ compounds in the clay-sized fraction) compared to the odd ones. [c] Calculated according to equation reported in annexe 5.5.

The bulk (ca. 75 to 90%) of the carbon in the $C_{27}$, $C_{29}$ and $C_{31}$ *n*-alkanes occurring in the cultivated soil originates from carbon of the forest soil. However, as stressed in the discussion on distributions, a part of these odd *n*-alkanes probably corresponds to hydrocarbons synthesised by fungi thriving on components inherited from the forest soil, and thus exhibiting a “forest” isotopic signature. This is also supported by the close δ $^{13}$C values

observed both for these odd-carbon-numbered long chain *n*-alkanes and for the even-carbon-numbered ones (see Note b, Table 1.6) since the latter do not correspond to higher plant components and are thought to be of fungal origin.

## 1.2 Conclusions

The main conclusions derived from the molecular and isotopic analysis of the crude and saponified lipids in the three particle-size fractions of a cultivated sandy soil from Cestas, and from comparison with the forest soil of this cultivation sequence, are summarised below:

- The bulk quantitative features of the organic matter showed marked differences between the fractions of the cultivated and forest soils and between the fractions of each soil as well. Some pronounced differences were also observed between the features of the lipidic carbon and of the total organic carbon. Thus, for example, the enrichment ratios indicated, for the former carbon, a relatively less pronounced depletion in the sand-sized fraction and a relatively lower enrichment in the silt-sized one.

- Numerous series of lipids, including free and bound fatty acids, bound ω-hydroxy acids, bound diacids, bound aromatic acids, free and bound alkanols, alkanes and alkanones, occur in all the particle-size fractions of the cultivated soils.

- Large differences in the molecular composition of the lipids, concerned with the relative abundance and/or the distribution of these various series, were observed between the particle-size fractions of the cropped soil, as illustrated, for example, by the distribution of the free fatty acids and *n*-alkanes. Detailed information was thus obtained about the different lipid sources in these fractions, illustrating the usefulness of studying the lipids on size separates.

- Higher plant contribution corresponds not only to waxes and wax esters but, also, to partial depolymerisation products of suberin, cutin and lignin. A relatively intense microbial activity occurs in this weakly acid cultivated soil, as reflected both by the presence of various reworked and oxidised plant components and by the direct contribution of a number of lipids from bacteria, fungi and fungal spores. The intensity of the microbial reworking of the lipidic components increases as the fraction becomes finer.

- Marked differences were observed, for all types of lipids in the particle-size fractions, between the cultivated and forest soil, thus reflecting the large variations associated with change in land-use. The lipids of the fractions of the cultivated soil generally show a higher

contribution of microbial components and a lower contribution of higher plant components when compared to the fractions of the forest soil.

- Comparison with *n*-alkane distribution in maize roots and shoots indicated that such compounds do not afford a major contribution to the *n*-alkanes of the cropped soil.

- Stable carbon isotope measurements of total organic matter on the whole soils showed that the input of carbon from maize is rather moderate after 22 years of continuous cropping. Indeed, this “new” carbon accounts for only 17% of the total carbon in the cultivated soil. In spite of a substantial level of degradation of the carbon from the initial forest soil (38%) the latter thus remains predominant. Parallel measurements on the particle-size fractions showed similar general trends but also revealed marked differences between the fractions. Thus, the contribution of maize carbon increases as the fraction is coarser (up to 35% for the sand-sized fraction) and the level of degradation of the carbon from the initial forest soil also increases (up to 60% in this fraction). The carbon exhibiting a “forest” isotopic signature in the cultivated soil partly corresponds to microbially reworked components of the initial soil and to components of the microbial biomass that thrived on the organic matter inherited from this soil.

- Similar general trends with respect to the input of “new” carbon were observed for the lipidic carbon when compared to the total carbon. However, the former contribution is always weaker, with a value of only 11% (against 17%) for the whole soil and a maximum value of ca. 18% (against 35%) for the sand-sized fraction. This probably reflects the low content of lipids in maize and/or the poor stability of these lipids in soil compared to total organic matter.

- The level of degradation of the carbon from the initial forest soil is markedly higher for the lipidic carbon when compared to total carbon (63% against 38%), thus reflecting a higher sensitivity for the lipidic components upon change in land use and cropping. Large differences in this degradation level are observed between the size fractions. However, contrary to total carbon, the higher degradation (76%) is now observed for the clay-sized fraction. This reverse trend may reflect the occurrence of substantial transfers of lipids between the particle-size fractions. In addition, in agreement with molecular data, a large part of the lipids in the cultivated soil, that retained the isotopic signature of the “forest” carbon, in fact corresponds to microbially reworked lipids and, also, to lipids formed by microorganisms (bacteria and fungi) chiefly thriving on organic components inherited form the forest soil.

- Compound-specific isotope analysis on the long chain ($C_{27}$, $C_{29}$ and $C_{31}$) odd-carbon numbered *n*-alkanes showed a minor contribution (12 to 25%) of maize carbon. This low

contribution is probably related to the poor content of silt and clay in this sandy soil so that the *n*-alkanes from maize would be largely degraded. In agreement with molecular and isotopic data, a large part of the long chain *n*-alkanes with the “forest” $\delta\ ^{13}C$ signature probably corresponds to fungal components.

# II INFLUENCE DE LA CONVERSION D'UN SOL FORESTIER A LA CULTURE DE MAÏS SUR LA STRUCTURE CHIMIQUE DE LA MATIERE ORGANIQUE REFRACTAIRE

La MOR étant la fraction résistante aux hydrolyses acides et basiques, on peut supposer une corrélation entre le caractère réfractaire de cette fraction et sa résistance au dégradation et donc établir une relation directe entre la structure chimique et la biodégradabilité de la MOR. Dans ce cas, cette fraction du sol représenterait un pool stable de carbone. Etant donné que la mise en culture d'un sol conduit à une perte de carbone organique, la quantification du carbone de cette MOR pourrait permettre de vérifier la stabilité de cette fraction. De plus, les caractéristiques spectroscopiques et pyrolytiques de la MOR du sol sous forêt ont été déterminées dans la partie B, aussi l'étude de l'influence qualitative sur la MOR peut être envisagée.

Le but de cette partie est donc la caractérisation de la MOR après 22 ans de culture afin de révéler la composition moléculaire de la MOR sous maïs par combinaison de méthodes d'analyses. Puis, les MORs des deux sols seront comparées. En associant le marquage isotopique à ces analyses, la dynamique de la MOR pourra être estimée.

## 2.1 Results and discussion

### *2.1.1. Mass balance, elementary analysis*

The elemental composition and isotopic data of the soil and ROM are reported in table 2.1 for the forest soil and the maize one.

| | | | Bulk soil | | | | | ROM | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mass of soil studied (g) | Mass of refractory organic matter (g) | C% | N% | $\delta^{13}C$ (‰) | % C from maize | % of degradation of $C_{forest}$ | C% | N % | Ashes% | $\delta^{13}C$ (‰) | % C from maize | % of degradation of $C_{forest}$ |
| Forest soil | 200 | 3.18 | 2.6 | 0.09 | -27.7 | 0 | 0 | 58.67 | 1 | 4.53 | -28.2 | 0 | 0 |
| Corn soil | 200 | 1.78 | 2 | 0.11 | -25.2 | 16.4 | 35.3 | 51.52 | 1.21 | 11.97 | -25.9 | 14.6 | 57.3 |

Table 2.1: Elemental composition and isotopic data of the soil and ROM

The maize soil contains 2% of total organic carbon, i.e. 23 % less than the forest soil. A drop in carbon content is classically observed after conversion to land use. This calculated decrease leads to an underestimation of carbon loss upon cultivation since it is based on the assumption that no carbon from the maize is added to the soil, which is wrong as shown below by isotopic data. A similar range of carbon loss was previously observed by e.g. Dalal and Mayer (1986), Dale (1992), Arrouays and Pelissier (1994) and Murty et al. (2002) after conversion of forest soil to cropping. Such studies dealing with soil carbon dynamics have revealed a fast decrease in carbon content during the first 10 years of conversion, followed by a slow down to reach a new equilibrium after 30 to 50 years (Balesdent et al., 1988). Indeed, conversion of a forest soil to cropping affects the amount and composition of organic residues returning to the soil on the one hand, and the soil environment (such as temperature, moisture, density, porosity, accessibility to the organic matter) on the other hand and thus modifies the SOM turnover. Balesdent et al. (1998) have reported a turnover eight times higher after land-use conversion of a forest soil.

After treatment of 200 g of maize soil, 1.78 g of ROM was isolated, with a total organic carbon content of 51.5%. The ROM carbon thus represents 22.5% of the TOC of the cropped soil. This value is much higher than those previously reported for the ROM isolated from the forest and maize soils of Lacadée (Augris et al., 1998; Poirier et al., 2003) but it must be considered that (i) only the < 10 μm fraction, which accounted for ca. 50 % of the TOC, was used in the case of Lacadée soil and (ii) the two soils (Cestas and Lacadée) exhibit sharply different textures, sandy and loamy, repectively. When compared to the Cestas forest soil, the ROM content in the Cestas cropped soil is substantially lower (22.5% vs 34%), thus pointing to a higher rate of degradation for the ROM carbon than for the TOC upon cultivation. In the case of Cestas, it must be noted that ROM carbon and TOC showed similar degradation levels after conversion into maize (16% and 14% for the soil and ROM, respectively). Taking into account the relative amount of ROM carbon with respect to TOC on the one hand, and the TOC contents in the two soils on the other hand, it can be calculated that the ROM carbon accounts for 8.8 and 4.4 wt ‰ of the total forest and maize soils, respectively. A 50 % decrease in ROM carbon is thus observed after 22 years of maize cropping, still assuming no carbon input from the maize.

The incorporation of organic matter originating from the maize vegetation in the soil can be calculated from the $\delta^{13}C$ values in order to estimate the OM turnover. Indeed, $^{13}C/^{12}C$ ratios of plant is related to their photosynthetic pathway (Bender, 1971; O'Leary, 1981). Plants with $C_4$ photosynthetic pathway show a higher incorporation of $^{13}C$ than plants with a

$C_3$ photosynthetic cycle and thus exhibit depleted $\delta^{13}C$ values (between -27.6 and -30.5‰ in the $C_3$ pathway and -10 and -12‰ for the $C_4$ pathway according to Collister et al. (1994). As a result, the natural $^{13}C$ abundance was often used as a tracer of the soil organic matter dynamics in soils (e.g.Cerri et al., 1985; Balesdent et al., 1987, Skjemstad et al., 1990; Huggings et al., 1998) and has revealed the incorporation rate of the new vegetation (Mariotti, 1991, Mariotti and Peterschmitt, 1994; Boutton, 1996). Thanks to the change in land use in Cestas, we can use this method to assess the maize input to the SOM. In the forest soil, pine is the main source of organic matter, characterized by a $C_3$ photosynthetic pathway with a typical value of -27.7‰, whereas after conversion to maize cultivation, a maize input takes place. The fraction *f* of carbon derived from maize can be calculated according to Balesdent and Mariotti (1996):

$$f=(\delta-\delta_{ref})/(\delta_{maize}-\delta_{ref})$$

where $\delta$ stands for the measurement of $\delta^{13}C$ in the soil at a given time, $\delta_{ref}$ for the $\delta^{13}C$ of the original forest and $\delta_{maize}$ for the $\delta^{13}C$ of the maize crop. The value of -12.5‰, measured on a maize sample composed of crushed leaves and stalks by Jolivet et al. (2003), was used for $\delta_{maize}$ in this calculation and the measured $\delta^{13}C$ ($\delta_{ref}$) of the forest soil is -27.7‰. Hence, we can calculate that, in the maize soil, only 16 % of the carbon originates from the maize after 22 years of cultivation, hence a rather low maize input. A similarly low input of maize was observed in the 0-10 μm fraction of the Lacadée soil after 35 years of maize cropping. Several explanations can be put forward to account for this low incorporation. Upon maize cropping, fresh material would be extensively degraded prior to their incorporation to the soil hence a low final input. Based on a protection of the OM on mineral sites, the latter would be already saturated with forest OM and thus not available for new material coming from the maize. Since these sites mostly occur within silt and clay fractions, this second explanation must especially hold for the sandy Cestas soil. The weak percentage of maize observed in the soil can be due to the quality of the maize OM: It must be noted that angiosperm lignin (i.e. that occurring in maize) is more easily biodegradable than the gymnosperm one, such as that present in pines (Faix et al., 1985; Sanger et al., 1997). In addition, the soil environmental conditions have changed due to tillage and fertilization, and in consequence, the biodegradation rate has been probably enhanced (Balesdent et al., 2000). So, the fresh OM from the maize soil, which is not stabilized yet, has been preferentially degraded.

With the same calculation, it appears that 85.4 % of the ROM carbon originates from the forest: the ROM is slightly depressed in maize vegetation compared to the bulk soil. In addition to the aforementioned reasons to account for the low maize input in the total soil, one must assume a relatively low content in refractory material in the maize biomass. Using the same procedure as that used to isolate the ROM from the Cestas soils, fresh maize leaves afforded an insoluble residue that accounted for 6 % of the initial carbon (Poirier et al., 2003). However, as far as we are aware, no data is available on maize roots.

### *2.1.2 Solid state $^{13}C$ NMR*

The solid state $^{13}C$ NMR spectrum (Fig 3.1) is largely dominated by a peak centred at 30 ppm, corresponding to aliphatic carbons without heterosubstituents, i.e. methylene carbons in long-chain aliphatic structures such as fatty acids, cutins, and other aliphatic biopolymers. It must be noted that, as already stressed in the case of the ROM from the forest soil, this peak is likely to be overestimated by the CP-MAS sequence (Oades et al., 1987). Three additional peaks of relatively low intensity are observed in the spectrum: 75 ppm, corresponding to carbons bearing O or N atoms, a broad peak maximizing at 130 ppm due to aromatic and olefinic carbons and 170 ppm, assigned to carbons in ester and /or amide functions.

When compared with the NMR spectrum of the forest ROM, that of the cropped soil is characterized by a much lower contribution of polysaccharides and lignin. Indeed, polysaccharides are evidenced in the forest ROM by the presence of a rather intense peak at 72 ppm and of a well-resolved peak at 104 ppm (anomeric C in glycosidic bonds) (Pastorova et al., 1994) and lignin by its characteristic peaks at 150 ppm (aromatic carbons linked to oxygen) and 56 ppm (methoxy groups) along with the broad peak at 130 ppm (Hatcher et al., 1987). The occurrence of these peaks was interpreted as the survival of ligno-cellulose complex within the forest ROM (part B, II). After conversion into maize cropping, peaks of lignin are hardly visible and no evidence for cellulose can be derived from the NMR spectrum of the ROM. It thus appears that the ligno-cellulose complex has been strongly altered upon continuous maize cropping. As a result, the peak at 75 ppm in the maize ROM spectrum can be attributed to carbons linked to oxygen in altered carbohydrates but it can also reflect the contribution of other aliphatic C-O groups such as those occurring in cutins and suberins. The ester groups of these biopolymers would contribute to the 170 ppm peak. The ester groups of these biopolymers would contribute to the 170 ppm peak. No evidence for tannin contribution can be suggested by this spectrum contrary to what was observed in the forest soil.

Comparison between the two spectra thus indicates a decrease in the lignin and carbohydrate contribution and an accumulation of aliphatic moieties in the maize soil. Aliphatic units have already been reported to be relatively resistant structures, which accumulate during humification in soils (Hempfling et al., 1991; Baldock et al., 1997; Nierop et al., 2001; Chen and Chiu, 2003). These results indicate that the ROM from the maize soil has experienced a high mineralization and that the remaining components are the most refractory to biodegradation.

It must be noted that humification is usually considered to lead to the formation of melanoidins, which are poorly detected by CP/MAS NMR especially when they are highly cross-linked. Melanoidins, if present in the ROM, should exhibit a high level of cross-linking so as to survive the drastic treatments used to isolate the ROM. As a result, from the above spectrum, one cannot exclude a melanoidin contribution in the maize ROM, even in substantial amount (Gauthier et al., 2003).

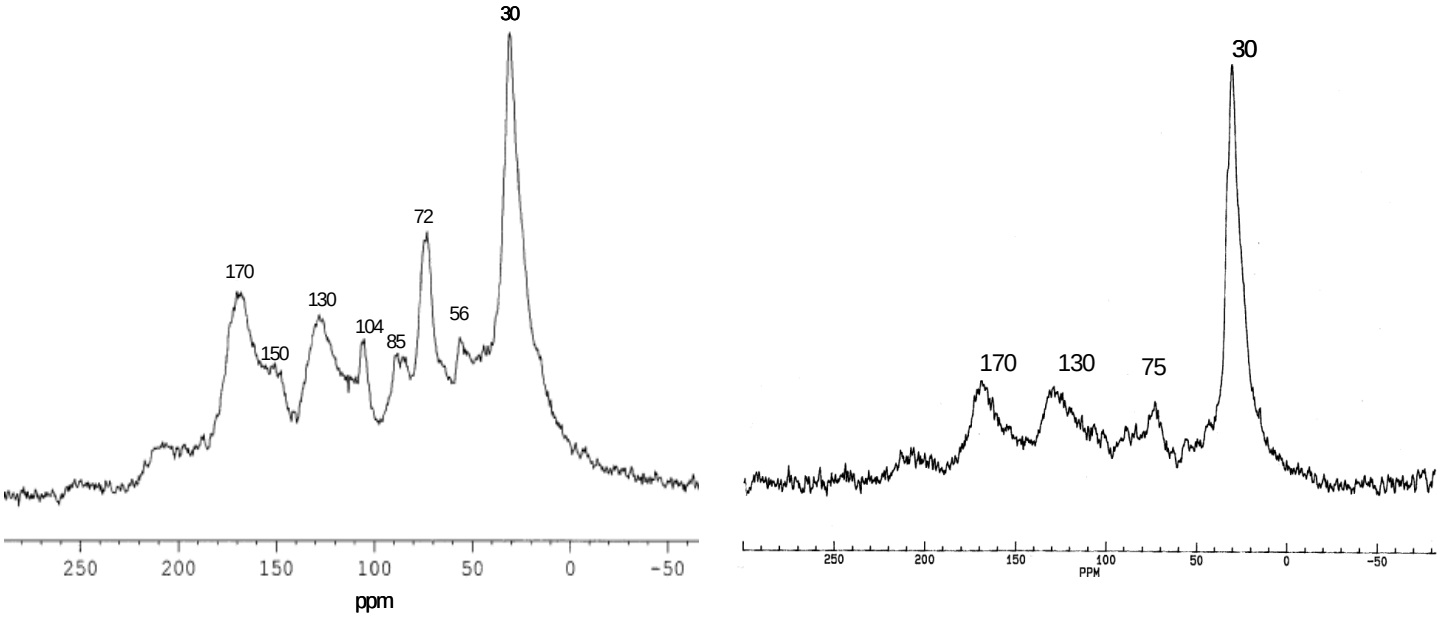



Figure 3.1: Solid-state $^{13}C$ NMR spectra of the Cestas ROM from the forest soils (a) and maize soil (b)

*2.1.3 Pyrolytic studies*

*2.1.3.1 Conventional pyrolysis (Py-GC/MS)*

The Fig 2.2 shows the total ion current (TIC) trace of the ROM from the maize soil. The pyrochromatogram is characterized by abundant phenols and methoxyphenols, alkyl benzenes and a substantial contribution of *n*-alkane/*n*-alk-1-ene doublets.

Phenol (7) and alkyl phenols (peaks 8, 10, 12, 13) (see table 2.2) are the main compounds identified (the main peak being the phenol (7)) along with methoxyphenols (11, 15, 18). Alkyl phenols are ubiquitous compounds which can result from lignin, protein and polysaccharide pyrolysis (Saiz-Jimenez and De Leeuw, 1984, 1986; Almendros et al., 1997) but methoxyphenols are typical pyrolysis products of lignin (Saiz-Jimenez and De Leeuw, 1984, 1986; Hatcher and Clifford, 1997). The relatively high abundance of these products reveals a substantial contribution of lignin to the maize ROM. Lignin degradation mostly involves demethylation (Saiz-Jimenez and De Leeuw, 1986; Filley et al., 2002) that results in the release of alkyl phenols upon pyrolysis. It can thus be anticipated that at least a part of the alkylphenols originate from altered lignin. Due to the isolation procedure for the ROM, proteins and non-cellulosic polysaccharides have been removed (Amelung et al., 1996), and these biomacromolecules cannot be considered as precursors of the alkyl phenols in the ROM pyrolysate. However, it is well-known that upon humification, these biomacromolecules undergo depolymerisation and further random recondensation to form the so-called melanoidins (Maillard, 1917). Melanoidins produce phenolic compounds upon pyrolysis along with furanic products (Pouwels et al., 1989). But since no furanic compounds are detected, this origin must be minor. Although usually not considered as a phenol precursor, suberin may also contribute to the phenolics of a pyrolysate.

Alkylbenzenes contribute significantly to the pyrolysate, especially toluene (1), 1,2-dimethylbenzene (3), 1,3 dimethyl benzene (4) and propyl benzene (5). Two origins can be considered for these compounds: cyclisation/aromatization of aliphatic chains (Largeau et al., 1986) or β-cleavage of substituted aromatic rings linked to the macromolecular structure via an alkyl chain (Hartgers et al., 1994). Due to the limited distribution of the alkylbenzenes (side chain up to C3), the first origin seems rather unlikely or at most minor, especially since long chain *n*-alkane/*n*-alk-1-ene doublets are detected in the pyrolysate, thus pointing to the occurrence in the ROM of long alkyl chains.

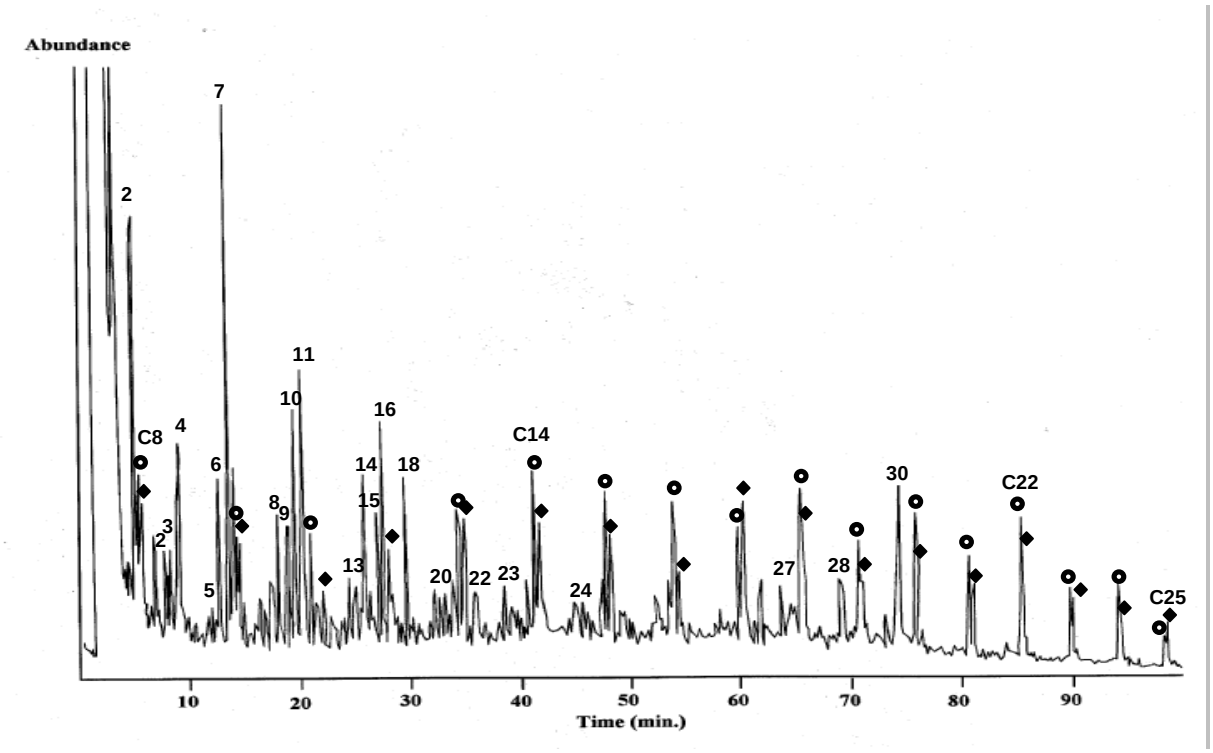



Figure 2.2: Total ion current trace of the 650°C Curie point pyrolysate of the ROM from the maize soil. See Table 2 for the identification of the numbered peaks

| Peak number | Compound |
|---|---|
| 1 | Toluene |
| 2 | Ethylbenzene |
| 3 | 1,2-dimethyl benzene |
| 4 | 1,3-dimethylbenzene |
| 5 | propylbenzene |
| 6 | 5-methyl-2-furancarboxaldehyde |
| 7 | phenol |
| 8 | 2-methylphenol |
| 9 | 1-phenyl-ethanone |
| 10 | 4-methylphenol |
| 11 | 2-methoxyphenol |
| 12 | 2,4-dimethylphenol |
| 13 | 3-ethylphenol |
| 14 | Naphthalene |
| 15 | 2-methoxy-4-methylphenol |
| 16 | 2,3-dihydrobenzofuran |
| 17 | hexylbenzene |
| 18 | 2.6-dimethoxyphenol |
| 19 | methylnaphthalene |
| 20 | octylbenzene |
| 21 | $C_{14}$ acid |
| 22 | $C_{15}$ acid |
| 23 | $C_{16}$ acid |

Table 2.2: Products formed upon 650°C Curie point pyrolysis of the ROM from the maize soil and their origin (◉) *n*-alkanes, (◆) *n*-alkenes

The *n*-alkane/*n*-alk-1-ene series ranges from $C_8$ to $C_{31}$ without even-over-odd carbon number predominance and maximizes at $C_{11}$ (Fig 2.3). Such doublets were frequently observed in pyrolysate of soil lipids (Van Bergen et al., 1997; Gobé et al., 2000), soil humin (Almendros et al., 1996; Nierop, 1998), plant (Tegelaar et al., 1995; Van Bergen et al., 1997), sediment (Zegouagh et al., 1999), kerogen-like organic matter in sediment (Garcette-Lepecq et al., 2000) and soil resistant organic residue (Augris et al., 1998; Lichtfouse et al., 1998; Poirier et al., 2000). Several origins are generally considered: thermal cleavage products from polymethylenic chain such as highly aliphatic macromolecules (cutan or suberan) (Nip et al., 1986; Tegelaar et al., 1989; Tegelaar et al., 1995; Augris et al., 1998), aliphatic biopolymer from micro organisms (Lichtfouse et al., 1995) or lipids polymerised on mineral colloids (Almendros et al., 1996). The distribution observed, without even-over-odd predominance of the doublets along with the decrease in intensity from $C_{23}$ is consistent with a substantial suberan source for the n-alkanes/n-alk-1-enes of the ROM.

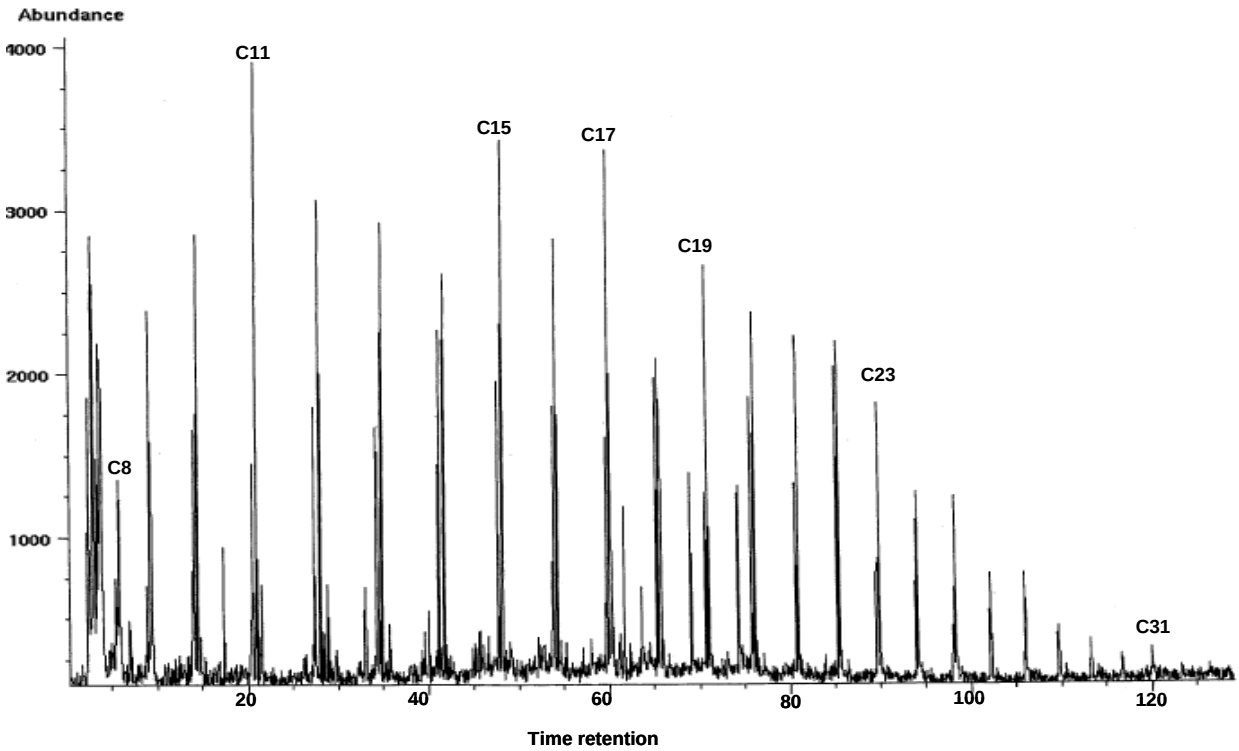



Fig 2.3: Ion chromatogram (m/z 57) showing the distribution of the n-alkanes of the 650°C Curie point pyrolysate of the ROM from the maize soil

A significant contribution of $C_{14}$, $C_{15}$ and $C_{16}$ aliphatic acids (21, 22, 23) is noted in the pyrolysate of the maize ROM. A similar abundant contribution of these acids was previously reported in the pyrolysate of a soil under a pine forest (Nierop, 1998; 2003) and these acids are considered as common products of soil under pine forest. These acids can be either released by the cleavage of ester functions sterically protected within the macrolecular network and hence not hydrolysed upon ROM isolation (Largeau et al., 1986) or trapped in the ROM structure. Such an entrapment of fatty acids has been reported in soil (e.g. in humin (Largeau et al., 1986; Grasset and Amblès, 1998a), in kerogen-like matter from a soil (Amblès et al., 1991) and in a forest soil (Naafs and Van Bergen, 2002).

When compared with the pyrolysate of the ROM from the forest soil, that of the maize ROM is characterized by a strong depletion in carbohydrate-derived compounds, especially peaks of levoglucosenon and levoglucosan which are absent on the TIC trace of the maize ROM. In addition, the relative contribution of aliphatic chains in the ROM pyrolysates seems higher after cultivation. Such an enrichment after cultivation is consistent with previous observations of a relative accumulation of alkyl components upon biodegradation (Preston et al., 1987; Kögel-Knabner et al., 1992b; Baldock et al., 1997; Nierop et al., 2001). This accumulation is usually attributed to selective preservation of aliphatic moieties, mainly originating from cutin and suberin by intense cross-linking of these aliphatic compounds in soils (Kögel-Knabner et al., 1992b). The persistence of these polymers has been reported in soils (Riederer et al., 1993; Bull et al., 2000; Nierop et al., 2003) and in sediments (Goñi and Hedges, 1990). In these preliminary studies, cutin and suberin were reported as more stable than the bulk soil (Goñi and Hedges, 1990; Riederer et al., 1993) and lignin (Van Bergen et al., 1997; Nierop, 1998; Van Bergen et al., 1998; Rumpel et al., 2002; Nierop and Verstraten, 2003). Moreover, Riederer et al. (1993) have concluded that suberin is even more stable than cutin. However, as shown in the study of the forest ROM ( part B, II), cutin and suberin contributions can be revealed upon pyrolysis provided a methylating agent, such as TMAH, would be used. We have thus performed the pyrolysis of the maize ROM in the presence of TMAH to test the contribution of these biopolymers in the maize ROM.

*2.1.3.2 Pyrolysis in the presence of TMAH (THM-GC/MS)*

When compared to conventional pyrolysis, thermochemolysis provides additional information on the ROM structure. Indeed, the presence of TMAH, an alkylating reagent, methylates the polar compounds which become more volatile and less polar (and thus GC-

amenable). Moreover, the use of TMAH avoids thermal decarboxylation that occurs in the pyrolysis chamber during conventional pyrolysis. Finally, TMAH allows the cleavage of esters and ether-bonds in macromolecules. Due to the efficiency of pyrolysis in the presence of TMAH to cleave macromolecular structures, this method has been applied to the characterization of many natural macromolecules such as lignin (Clifford et al., 1995; Hatcher et al., 1995), humic acids (Grasset and Amblès, 1998c), humin (Fabbri et al., 1996), cutin (De Leeuw and Baas, 1993), suberin (Santos-Bento et al., 2001), kerogen-like organic matter (Garcette-Lepecq et al., 2001), macromolecular lipids (Gobé et al., 2000), cutan (McKinney et al., 1996), soil organic matter (Nierop, 2001).

The TIC trace of the thermochemolysate comprises two sets of compounds, aromatics and aliphatics. The first part of the pyrochromatogram is dominated by methoxy alkylbenzenes, methoxyphenols and methoxybenzoic acids (Fig 2.4) assigned to lignin (Clifford et al., 1995; Hatcher et al., 1995; Challinor, 1996; Gauthier et al., 2003). As previously reported, lignin contribution is especially highlighted in the presence of TMAH (Clifford et al., 1995). The main peaks correspond to the 3,4-dimethoxybenzoic acid (42) and *p*-coumaric acid (44) (Table 2.3). These acids could not be detected upon conventional pyrolysis since they undergo decarboxylation under these conditions. Since such cinnamic acids are characteristic of non-woody tissues (Hedges and Mann, 1979), their occurrence in the pyrolysate might be interpreted as the reflect of the maize input. However, it must be noted that these compounds were already present in the thermochemolysate of the forest ROM, likely due to the understory vegetation. In addition, acids may also result from lignin alteration or may have been formed as secondary products upon reaction with TMAH.

Methoxyphenols and alkylphenols previously identified upon lignin conventional pyrolysis still occur in the thermochemolysate, revealing that the methylation reaction is incomplete. Similar incomplete methylation have been noticed by Saiz-Jimenez et al. (1996) in soil and soil particle-size fractions. These authors have excluded the possibility of incomplete reaction due to insufficient reagent since they had obtained the same results with previous standard methylation of lignin even in the presence of an excess of reagent (Saiz-Jimenez, 1994). The methylation could be ineffective when the compound accessibility is limited by steric protection or matrix protection. 3,4-dimethoxybenzaldehyde (39), 3,4 dimethoxyacetophenone (41), 3,4-dimethoxybenzoic acid (42) and ferulic acid (45) correspond to the main compounds identified by Hatcher et al. (1995) and Martin et al. (1995) in wood lignin chemopyrolysate.

Since maize is an angiosperm, its lignin is based on syringyl units, which would lead to trimethoxy-compounds upon TMAH pyrolysis. Only trimethoxybenzene (31) could be identified in trace amount in the TIC trace. This indicates that either the maize input to the ROM is very low or that maize markers are degraded and thus no longer recognizable. Indeed, syringyl units are known to be more sensitive to biodegradation than guaiacyl ones (Sanger et al., 1997), and it was already noted by Van Bergen et al. (1997) and Bull et al. (1998) that high molecular weight molecules did not reflect the land use conversion in their study on the Rothamsted classical experiment.

It must be noted that ferulic and coumaric acids are the aromatic monomers involved in the suberin structure. Their presence in the thermochemolysate of the maize ROM can thus also reflect a suberin input.

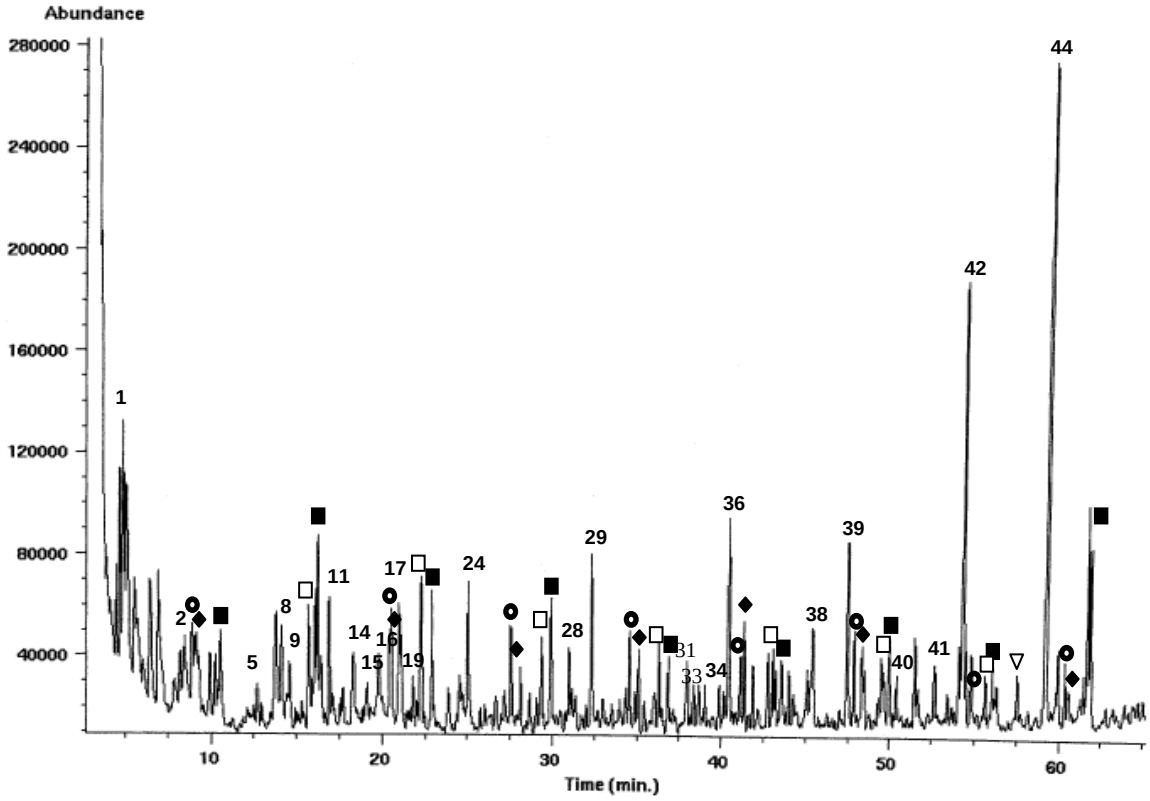



Figure 2.4: Expanded area of the beginning of the total ion current trace of the 650°C Curie point pyrolysate of the ROM from the maize soil in presence of TMAH. See Table 2.3 for the identification of the numbered peaks

The second part of the chromatogram (Fig 2.5) is dominated by aliphatic compounds, such as ω-hydroxy acids, α,ω-diacids, fatty acids and *n*-alkane/*n*-alk-1-ene doublets. The main series corresponds to even-carbon-numbered ω-hydroxy acids ranging

from $C_{16}$ to $C_{28}$, with a strong predominance of the $C_{22}$ and $C_{24}$ homologues. ω-hydroxy acids are the main constituents of cutin and suberin but they show different ranges in carbon number in the two biopolymers. Indeed, cutin is composed of fatty acids, dihydroxy acids, trihydroxy acids, epoxyacids and ω-hydroxy acids all comprising 16 or 18 carbons. The suberin monomers are mainly ω-hydroxy acids and α,ω-diacids with carbon numbers ranging from $C_{16}$ to $C_{24}$. These monomers occur along with additional fatty acids, *n*-alkanols, dihydroxy acids, trihydroxy acids and epoxy acids (Kolattukudy, 1980; Holloway, 1983; Matzke and Riederer, 1991; Riederer et al., 1993; Del Rio and Hatcher, 1998). As a result, the occurrence of long chain ω-hydroxy acids and α,ω-diacids (from $C_{18}$ to $C_{32}$) with dominant $C_{22}$ and $C_{24}$ ascertains an important suberin contribution to the ROM and hence an important root input. The presence of these characteristic markers confirms that the aforementioned p-coumaric acid (44) and ferulic acid (45) originate at least partly from suberin. Moreover, as Bernards and Lewis (1998) have reported, the contribution of hydroxy-cinnamic acid to suberized tissues, the 4-hydroxy-3-methoxybenzoic acid (38) (or hydroxy cinnamic acid) identified on the TIC trace could also be derived from such polymer.

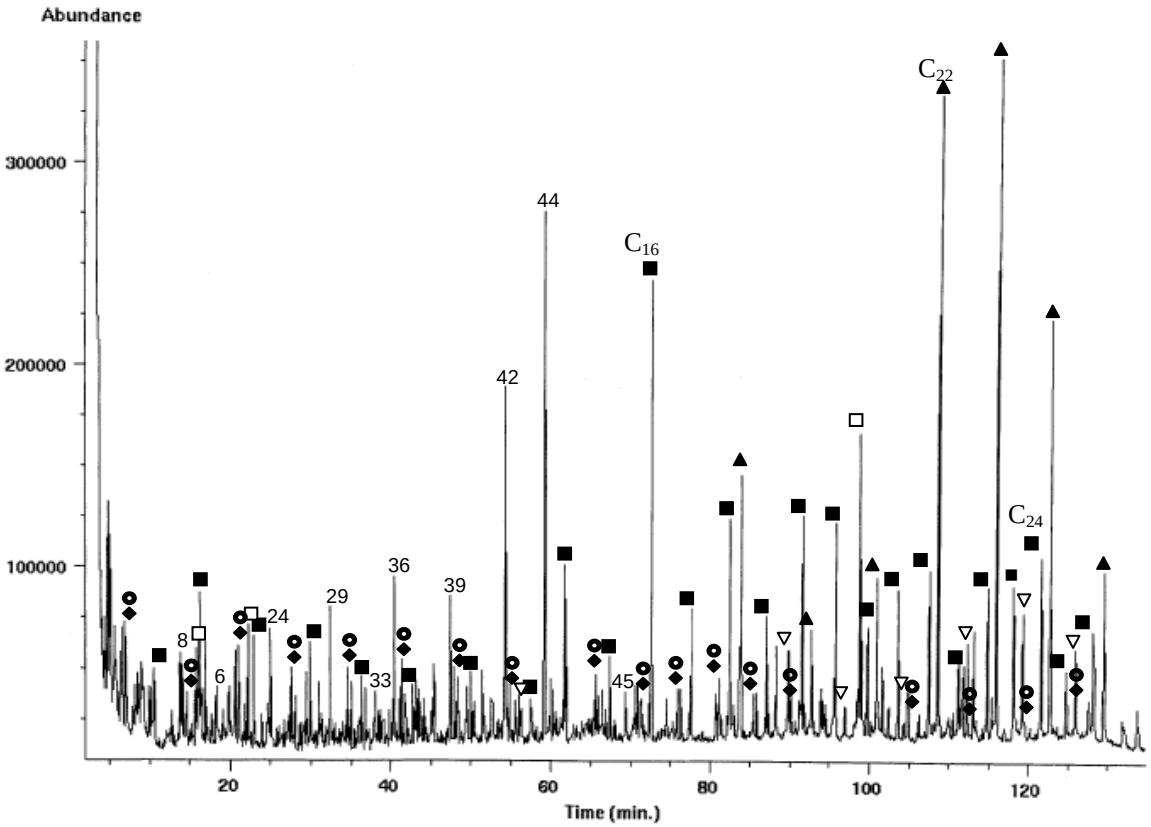



Figure 2.5: Total ion current trace of the 650°C Curie point pyrolysate of the Cestas ROM in presence of TMAH

| Peak number | Compound |
|---|---|
| 1 | toluene |
| 2 | ethylbenzene |
| 3 | 1,4 dimethylbenzene |
| 4 | 1,2 dimethylbenzene |
| 5 | propylbenzene |
| 6 | 1-ethyl-4-methylbenzene |
| 7 | unidentified |
| 8 | phenol |
| 9 | 1-methoxy-2-methylbenzene |
| 11 | 1-methoxy-4-methylbenzene |
| 12 | butanedioic acid |
| 13 | 1,2-ethanediol1-(2-furanyl) |
| 14 | 2-methylphenol |
| 15 | 4-methylphenol |
| 16 | 3-methylphenol |
| 17 | 3-methoxyphenol |
| 19 | dimethylphenol |
| 20 | 4-ethyl-1-methoxybenzene |
| 21 | 2,4,6-trimethylphenol |
| 22 | ethyl,methylphenol |
| 23 | 1,2-dimethoxybenzene |
| 24 | ethenyl-3-methoxybenzene |
| 25 | 4-methyl-1,2-dimethoxybenzenel |
| 26 | 3-methylbenzoic acid |
| 27 | 2-methoxy-4-methylphenol |
| 28 | 5-methoxy-2,3-dimethylphenol |
| 29 | 2-hydroxy-3-methyl benzaldehyde |
| 30 | 4-ethyl-2-methoxybenzene |
| 31 | trimethoxybenzene |
| 32 | 2-methylnaphthalene |
| 33 | 4-methyl-3-methoxy benzoic acid |
| 34 | 2,6-dimethoxyphenol |
| 35 | 1,2-dimethoxy-4-ethenylbenzene |
| 36 | 3-methoxybenzoic acid |
| 37 | 4-methoxybenzoic acid |
| 38 | 4-hydroxy-3-methoxybenzoic acid |
| 39 | 3,4-dimethoxybenzaldehyde |
| 40 | 4-methoxybenzenepropanoic acid |
| 41 | 3,4-dimethoxyacetophenone |
| 42 | 3,4-dimethoxybenzoic acid |
| 43 | 3,4-dimethoxy benzeneacetic acid |
| 44 | 3-(4-methoxyphenyl-2-)propenoic acid (p-coumaric acid) |
| 45 | 3-(3,4-dimethoxyphenyl)-2-propenoic acid (ferulic acid) |
| 46 | unidentified |
| ◆ | *n*-alk-1-enes |
| ⚬ | *n*-alkanes |
| ■ | acids |
| □ | unsaturated acids |
| ▲ | ω-hydroxyacids |
| ▽ | diacids |

Table 2.3: Products formed upon 650°C Curie point thermochemolysis of the ROM from the maize soil in presence of the TMAH

A wide series of alkanoic acids (from $C_6$ to $C_{32}$) is identified, as their methylesters counterpart, in the pyrochromatogram. Alkanoic acids exhibit a trimodal distribution (maximum $C_{16}$, sub-maxima $C_8$ and $C_{24}$). As previously mentioned, they can be either released by cleavage of wax ester bonds or freed thanks to the cracking of the ROM macromolecule (Grasset and Amblès, 1998a; Naafs and Van Bergen, 2002). The $C_{16}$ acid is ubiquitous and thus cannot be assigned to a specific source. Short chain acids, which do not exhibit any odd or even carbon number predominance reflect microbial input, along with the iso- and anteiso-$C_{15}$ and $C_{17}$ acids, which are generally accepted as bacterial biomarkers (Perry et al., 1979; Meyers and Ishiwatari, 1993). The main source of the long chain alkanoic acids with even carbon numbers are higher plant waxes (Tulloch, 1976) and suberin. The submaximum at $C_{24}$ strongly confirms the significant contribution of suberin to the ROM. The odd-carbon-numbered fatty acids should mainly originate from the diagenetic alteration of the biological acids. In consequence, the comparable abundance of odd- and even-carbon-numbered fatty acids indicates that fatty acids have undergone a relatively high level of degradation before incorporation in the ROM. Indeed Amblès et al. (1991) and Grasset and Amblès (1998b) have reported the preservation of acids bound to the matrix. Taken together, alkanoic acids in the ROM pyrolysate are derived from distinct sources: microbial, higher plant and degradation products.

Short chain alkanoic acids (from $C_6$ to $C_{18}$) occur as doublets along with their unsaturated counterpart. This suggests a common origin for these two series, the acids being released after cleavage of their alkyl chain rather than cleavage of ester bonds. Similarly, *n*-alkane/*n*-alk-1-ene doublets are detected from $C_8$ to $C_{31}$. The distribution of these doublets is similar to that observed upon conventional pyrolysis and thus, they have the same origin. The strong contribution of suberin in the ROM supports a suberan origin for at least a part of the doublets.

Comparison of the thermochemolysates of the forest and maize ROMs do not reveal major differences between the two samples. Based on the relative abundance of the doublets on the one hand, and on the typical ω-hydroxy-acids, on the other hand, it seems that the relative input of suberin is slightly more pronounced in the maize soil. As reported, ω-hydroxy acids accumulate in low pH condition in soil (Bull et al., 2000) either by a better preservation (Nierop et al., 2003) or/and a slower degradation rate. It must be noted that fresh suberin comprises unsaturated ω-hydroxy acids and epoxy acid, which were not detected upon pyrolysis of the ROM, suggesting that the suberin has undergone some alteration prior

to its incorporation in the ROM. Indeed, Goñi and Hedges (1990) have shown that suberin-components reactivity was correlated with the occurrence of unsaturation and to the number of hydroxyl group. This alteration holds for both samples (forest and maize) and is thus not due to cultivation.

Taken together, the conventional pyrolysis and that in the presence of TMAH reveal an important contribution of lignin, suberin and, to a less extent, of suberan to the ROM. The lack of carbohydrates in the maize ROM reflects the additional alteration undergone by the ROM upon cultivation, revealing its sensitivity to land use. It must be noted that several parameters may have increase the sensitivity of the ROM to biodegradation. These parameters are linked to the change in land use. The alteration of soil structure upon tillage may have increased the bioavailability of SOM. Another important factor which may play a role is the soil pH: the forest soil is an acidic soil (pH 4.5) whereas the maize soil pH is 6. The pH influences the microbial community and the mineralization rate (Oades, 1988; Motavalli et al., 1995; Van Bergen et al., 1997; 1998; Nierop et al., 2003). In addition, soil pH affects more the lignin chemistry than lipids (Nierop, 1998; 2001; Rumpel et al., 2002; Nierop and Verstraten, 2003).

Suberin was previously reported as an important contributor of SOM by Kögel-Knabner et al. (1992b), Gonzalez-vila et al. (1996), Nierop (1998), Van Bergen et al. (1998), Bull et al. (2000), Gobé et al. (2000) and Nierop et al. (2001), this study emphasizes this assertion by showing the survival of suberin in the ROM after 22 years of continuous cultivation.

The chemical composition of the ROM has been altered by 22 years of continuous cultivation and is thus less stable than expected. Recalcitrance to biodegradation is not directly linked to the chemical resistance of SOM. This confirms, on another type of soil, the conclusions of Poirier et al. (2002) who have highlighted the difference between chemical resistance and stability in soils.

## 2.2. Conclusions

Soil ROM isolated from the maize soil represents 22% of soil carbon. This fraction is mainly composed of lignin- and suberin-derived components and to a lesser extent of fatty acids and aliphatic moieties, at least partly, corresponding to suberans. These fatty acids originate from higher plant waxes and for a weak part from the bacterial contribution. The

occurrence of fatty acids is related to their entrapment in the matrix or to their covalent linkage to the macromolecular structure.

The conversion of the forest soil to maize cropping has many consequences:

- The total organic carbon content of the maize soil has decreased of 23%. The maize ROM accounts for 22.5% of the TOC of the soil, i.e. much lower than the forest ROM (34%). The ROM underwent a substantial degradation upon cultivation.
- The percentage of OM originating from the maize vegetation in the SOM and in the ROM are weak (16% and 14%, respectively), indicating that the contribution of maize OM to the soil is low either due to a low input of maize or to a higher degradation rate of maize OM.
- The solid state $^{13}C$ NMR indicate a higher aliphaticity of the ROM from the maize soil. The aliphaticity generally increases during humification, so the degradation level of the ROM from the maize soil is more extensive.
- Conversion into cropping thus results in an important degradation of lignin and carbohydrates, the latter being virtually absent from the maize ROM. Despite abundant lignin products observed upon pyrolysis, the molecular analysis has revealed a more important contribution of suberin and fatty acids to the ROM of the maize soil. Suberin appears to present an intrinsic resistance to degradation.

The high level of decay in the maize soil is related to the cultivation. However, the pH increase may have played a major role as pH is known to influence degradation rate and to enhance the mineralization. Although ROM exhibits a relative stability, conversion to cropping has an influence on its structure. In consequence, this fraction is less recalcitrant than expected and does not constitute the whole pool of stable carbon. Chemical recalcitrance is not equivalent to long term stability.

# III LES CARBONISATS DE 2 SOLS APPARTENANT A UNE CHRONOSEQUENCE ET DANS LES DIFFERENTES FRACTIONS GRANULOMETRIQUES

Les carbonisats sont, comme nous l'avons vu dans la partie A 2.2.1.6, des produits de combustion incomplète. Ils sont rencontrés dans de très nombreux milieux et ont été observés aussi bien dans l'atmosphère, les sédiments, les sols, les glaces ou les océans. Cette ubiquité est liée à leur capacité de transports sur de longues distances (80 % des carbonisats produits pendant un feu restent dans les résidus alors que 20% sont dispersés dans la fumée par le vent ou les rivières (Kuhlbusch et Crutzen, 1995)). De plus, une des caractéristiques des carbonisats est leur persistance dans l'environnement. Elle est liée à leur caractère résistant (Cf partie A 4.1). Par ailleurs, les carbonisats influent sur de nombreuses composantes environnementales.

## 3.1 Introduction

### 3.1.1 Rôle dans le cycle du carbone

Les carbonisats représentent une quantité non négligeable de carbone sur Terre. La quantité produite annuellement est estimée par des méthodes indirectes. De ce fait, les stocks ou les flux de carbonisats varient selon les auteurs. En 1980, la production a été évaluée à 500-1700 Tg/an avec une valeur moyenne autour de 1100 Tg/an (Seiler et Crutzen, 1980). Puis en 1990, Crutzen et Andreae (1990) ont réévalué cette valeur à 50 à 270 Tg/an. Les dernières approximations du taux de formation annuel de BC calculées par Kuhlbusch (1995) à partir du rapport carbonisat /$CO_2$ sont de 50-270Tg/an, pour la combustion de biomasse, et 12-24 Tg/an issus de combustibles fossiles. Le flux annuel dans les sédiments est de 1 à 20g.$m^2$.$an^{-1}$ (Gustafsson et Gschwend, 1998) ; ainsi, d'après Masiello et Druffel (1998), les carbonisats représentent de 4 à 22% du pool total de carbone organique dissous dans les océans. Dans les sols, les carbonisats peuvent constituer une part importante du carbone organique total. Schmidt et al. (2001b) ont observé une contribution de 45% au carbone

organique d'un chernozem et Glaser et al. (2000) a rapporté une teneur de 35% de la matière organique dans un sol brésilien. Par conséquent, les carbonisats constituent un stock de carbone important. Cependant, un problème capital quant à l'estimation du stock de carbone sous forme de carbonisats, aussi bien dans les sédiments que dans les sols, est l'absence de méthode standardisée d'isolement des carbonisats. En effet, comme les carbonisats forment un continuum allant de produits partiellement carbonisés aux carbonisats présentant une structure en « oignon ». La quantité de carbonisats varie considérablement selon le protocole d'isolement utilisé. On distingue généralement 3 catégories de méthodes de quantification des carbonisats:

- des méthodes thermiques (Cachier et al., 1989; Gustafsson et al., 2001)
- des méthodes chimiques (Wolbach et Anders, 1989; Verardo, 1997; Glaser et al., 1998; Elias et al., 2001)
- des méthodes optiques

Schmidt et al. (2001a) ont comparé les résultats obtenus par plusieurs méthodes qui différent aussi bien par les pré-traitements appliqués que par la méthode de quantification.

De plus, les carbonisats constituent un stock de carbone extrêmement stable (CF partie A 4.1), et selon Kuhlbush et Crutzen (1995), plus le rapport H/C des carbonisats est faible, plus ils sont résistants aux dégradations à la fois chimiques et biologiques. Les principaux mécanismes impliqués dans la dégradation des carbonisats dans la nature sont photo-chimiques et radicalaires d'une part et la dégradation microbienne d'autre part. Cette dernière a été mise en évidence par Potter (1908) puis par Shneour (1966) mais elle reste très lente.

### 3.1.2 Rôle dans le bilan radiatif terrestre et le climat

Les carbonisats présents dans l'atmosphère influent sur le bilan radiatif terrestre car, par leurs propriétés d'absorption des radiations mais aussi de dispersion de l'énergie solaire**,** ils ont un effet sur les radiations atmosphériques (Robinson et Roberts, 1998; Im. J.-S., 2001). La concentration des carbonisats dans l'atmosphère est estimée entre 200 et 800 $ng.m^{-3}$ dans les régions rurales de l'hémisphère nord et de 10 $ng.m^{-3}$ au-dessus des océans (Clarke et al., 1984). Ces aérosols peuvent donc jouer un rôle sur le climat. Selon Clarke et al. ( 1984), par l'accroissement de leur concentration dans l'atmosphère, l'absorption de l'énergie solaire augmenterait et provoquerait un réchauffement de la terre. Jacobson (2001) ajoute que les

carbonisats pourraient être le deuxième composant le plus important du réchauffement global, après le $CO_2$, en terme de forçage direct. D'autres auteurs, dont Crutzen et Andreae (1990) pensent qu'en servant de noyaux de condensation des nuages, les carbonisats augmentent la couverture nuageuse et modifient la répartition des pluies et l'énergie reçue au sol.

### 3.1.3 Interaction avec les polluants

Les carbonisats sont des composés très réactifs. Cette réactivité est liée à la présence de fonctions oxygénées à leur surface. En effet, si on considère une suie provenant de la combustion d'hexane comme modèle, la moitié de la surface des particules de carbone porte de nombreuses fonctions oxygénées comme des groupements alcools, phénols, carbonyls, carboxyliques, esters, éthers (Smith et Chughtai, 1995). De plus, ils présentent des pores de l'ordre de quelques dizaines de nanomètres et donc une grande surface spécifique. Ces deux facteurs sont responsables de leurs propriétés d'adsorption (Goldberg, 1985). Rockne et al. (2000) a corrélé le rapport atomique C/H et le volume ou l'aire des micropores: en considérant la surface représentée par les pores des suies, ces pores pourraient représenter un important dépôt de contaminants hydrophobes dans l'environnement.

Gustafsson et Gschwend (1997) ont mis en évidence une distribution spatiale des les hydrocarbures aromatiques polycycliques (HAP) davantage liée à des interactions avec des suies qu'avec la matière organique. La sorption étant un processus clé contrôlant le devenir des polluants organiques dans l'environnement, les carbonisats jouent donc un rôle environnemental important par leurs capacités de sorption associées à une grande stabilité. Par ce phénomène, la dégradation des polluants peut être limitée et les carbonisats peuvent influencer la biodisponibilité de composés organiques comme les HAPs (Gustafsson et Gschwend, 1997), les dérivés de pesticides, ou encore ceux proches des PCBs (Accardi-Dey et Gschwend, 2002; Bucheli et Gustafsson, 2003).

### 3.1.4 Rôle des carbonisats dans la formation de la MOS

Skjemstad et al. (1996) ont émis l'hypothèse que selon leur abondance, et de par leur structure chimique, les carbonisats puissent augmenter l'aromaticité de la matière organique

du sol et sa quantité relative de carbone inerte. Ainsi ils joueraient un rôle aussi bien dans la composition que dans le renouvellement ou la formation de matière organique du sol.

D'après Haumaier et Zech (1995) et Shindo et al. (2004), l'oxydation des matériaux les moins carbonisés des charbons, associée à une hydratation et/ou une oxydation de la suie et des matériaux davantage carbonisés font des carbonisats une source possible d'acides humiques du sol.

### 3.1.5 Les carbonisats dans le sols de Cestas

En raison de leur rôle dans le cycle du carbone, leur contribution à la MOS et leur interaction avec les polluants, il est essentiel de pouvoir quantifier les carbonisats dans le sol. Lors de l'étude de la composition de MOR à des hydrolyses acides et basiques d'un sol des Landes et d'un sol du Congo, Poirier et al. (2000; 2002) ont observé qu'une partie de cette MOR était constituée de carbonisats.

Le degré de dégradation ainsi que l'âge moyen de la MOS augmentent des fractions grossières aux fractions fines. Cette augmentation de l'âge moyen des particules fines pourrait être liée à une localisation préférentielle des carbonisats dans ces fractions.

Dans cette étude, la MOR des deux sols de Cestas (avant et après mise en culture) et de leurs différentes fractions granulométriques ont été isolées, quantifiées puis analysées par RMN $^{13}C$ à l'état solide. Puis, à partir de cette MOR, les carbonisats ont été isolés par thermooxydation. Dans les fractions granulométriques, les carbonisats ont alors été quantifiés puis observés en microscopie électronique à transmission à haute résolution.

## 3.2 Résultats et discussion

### 3.2.1 MOR des différentes fractions granulométriques

Les données quantitatives obtenues pour les MORs des différentes fractions granulométriques des deux sols sont très différentes (Tableau 3.1). Dans le sol forestier, la fraction limon est particulièrement enrichie en carbone provenant de la MOR. Dans cette

fraction 44% du carbone provient de la MOR, alors que dans les fractions sable et argile, le carbone issu de la MOR constitue respectivement 14.5% et 16.2% du carbone total de la fraction. Dans le sol sous culture de maïs, on constate que 47.5% du carbone provient de la MOR alors que dans les fractions fines, ce pourcentage est de 13%. Une grande majorité du carbone de la MOR de la fraction sableuse provient très probablement de mélanoïdines. Les mélanoïdines sont formées par la condensation d'acides aminés et de sucres (Maillard, 1917). Allard et al. (1997) ont mis en évidence la formation de ces composés en milieu acide en laboratoire et Poirier et al. (2003) ont souligné la possibilité de formation lors de l'isolement par des hydrolyses fortes de MO. Cette condensation a essentiellement lieu dans la fraction sableuse sous maïs, d'une part car la MO fraîche, plus riche en sucres et en acides aminés, se trouve dans la fraction le plus grossière (Guggenberger et al., 1994; Baldock et al., 1997), et d'autre part car la nature de la MO constitutive du maïs est plus propice à cette réaction que la MO issue des pins. La formation de mélanoïdines au cours de l'isolement de la MOR des sols totaux avait été évitée grâce à une étape supplémentaire d'hydrolyses au TFA et HCl (Cf Annexe X, protocole 1). Les sources protéiques et les sucres non-cristallisés avaient donc été préalablement soustraits. Cette étape n'a pas été effectuée lors de l'isolement de la MOR des fractions granulométriques (Cf Annexe X protocole 2) car l'objectif premier de l'étude de ces fraction est l'isolement des carbonisats par l'oxydation thermique de ces MORs et leur quantification or il a été montré que les mélanoïdines sont détruites par cette oxydation (Gustafsson et al., 2001).

| **fractions** | **Forêt** | **Maïs** |
|---|---|---|
| sable | 14,5 | 47.5 |
| limon | 44 | 13 |
| argile | 16 | 13 |

Tableau 3.1 : Pourcentage de carbone de chaque fraction granulométriques

Exception faite de la fraction sableuse du sol sous maïs, le pourcentage de carbone issu de la MOR est plus faible dans le sol sous maïs. Ce résultat confirme les observations faites lors de la comparaison de la MOR des deux sols en dans le partie C-II et qui ont conduit à considérer une dégradation plus rapide de la MO issue du maïs que celle issue des pins. La MOR apparaît encore une fois sensible à la mise en culture du sol. Cependant, le carbone provenant de la MOR a diminué dans une proportion nettement plus importante dans la fraction limoneuse.

La mesure du $\delta^{13}C$ du carbone de la MOR des différentes fractions granulométriques indique une diminution de cette valeur de la fraction grossière à l'argile (Tableau 3.2). Cet appauvrissement en $^{13}C$ dans les fractions les plus fines a été fréquemment constaté lors de l'étude de sols (Cerri et al., 1985; Balesdent et al., 1987; Mariotti, 1991). Il est dû à une dégradation différentielle des composés de la MO. L'étude de la composition isotopique du sol et de la MOR indique un appauvrissement en $^{13}C$ de la MOR par rapport au sol. De façon similaire à l'appauvrissement en $^{13}C$ au cours de l'humification de la MO, ces données montrent que l'isolement de la MOR a conduit à concentrer une MO davantage humifiée que dans les fractions fines mais aussi que le degré d'altération de la MO composant la MOR est croissant du sable à l'argile.

Dans le sol sous maïs, l'appauvrissement en $^{13}C$ par rapport au sol forestier aussi bien dans le sol que dans la MOR témoigne d'une contribution de la MO issue du maïs. Toutefois celle-ci est faible. En revanche, alors que dans le sol sous forêt l'appauvrissement entre le sol et la MOR est toujours inférieur à 0.3‰, dans le sol sous maïs celui-ci bien plus important. Ceci indique que l'humification est plus poussée, d'autant plus que, si on tient compte de l'apport du maïs, le rapport isotopique augmente. De plus, l'appauvrissement entre le sol et la MOR est croissant de l'argile vers le sable dans laquelle il atteint 2‰. La MO du maïs est plus labile que celle de la forêt. Elle est plus dégradée dans les fractions sable et limon que dans la fraction argile. La comparaison des résultats de $\delta^{13}C$ renforce l'hypothèse, avancée dans le chapitre 2 de la partie C, d'un taux de dégradation différent selon l'origine de la MOS.

| Fractions | Sol forêt | ROM forêt | Sol maïs | ROM maïs |
|---|---|---|---|---|
| Sable | -27.61 | -27.87 | -22.21 (35.7) | -24.28 (23.3) |
| Limon | -27.88 | -28.06 | -24.36 (22.9) | -26.30 (12.5) |
| Argile | -27.97 | -28.23 | -25.66 (14.9) | -26.26 (11.3) |

Tableau 3.2 : $\delta^{13}C$ (‰) des fractions granulométriques du sol et de la MOR sous forêt et sous maïs (entre parenthèse le pourcentage de MO issu du maïs en supposant que le δ13C ne varie pas entre les fractions et est de -12.5‰)

Les spectres de RMN du carbone 13 à l'état solide des fractions granulométriques des deux sols sont très similaires (Figures 3.1a et b).

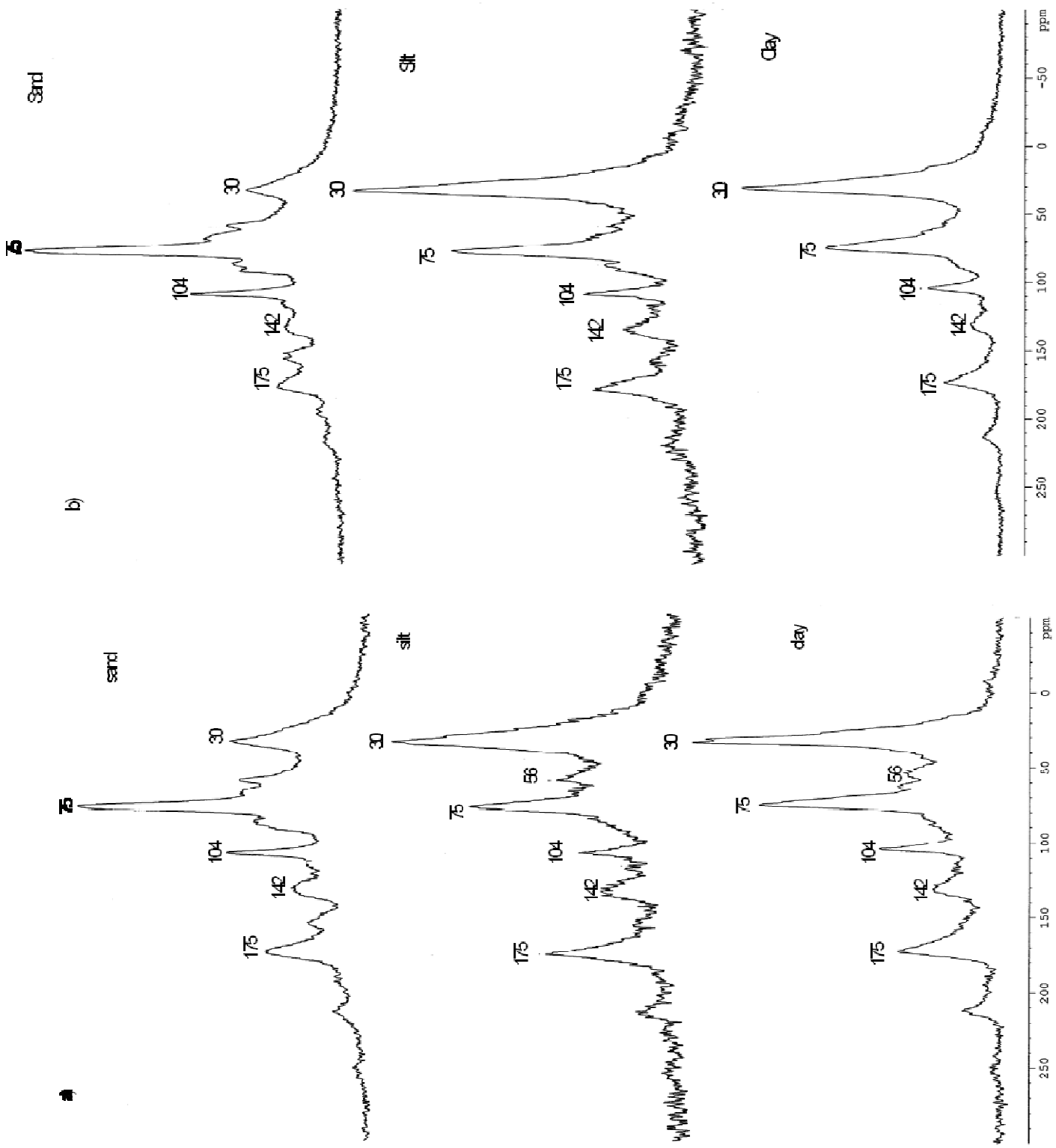



Fig 1 : a) Solid-state 13C NMR spectrum of the different particle-size fractions of the corn soil b) Solid-state 13C NMR spectrum of the different particle-size fractions of the forest soil

On constate que la MOR des fractions sableuses se distingue de celles des fractions fines. En effet, les spectres des fractions sableuses sont les seuls dominés par un pic centré à 75ppm qui, associé au pic à 105 ppm caractéristique, du carbone anomérique des

polysaccharides, indique une contribution importante des carbohydrates à la MOR. Ces pics sont accompagnés de signaux à 56 ppm (methoxy), 150 ppm (phenols) et d'un épaulement dans la région 60-90 ppm caractéristiques de la lignine. La présence d'unités carbohydrates et lignines traduit vraisemblablement une préservation de complexe ligno-cellulosique. Ces signaux diminuent fortement quand on considère les fractions fines. La MO des fractions sableuses est moins altérée que celle des fractions fines, de ce fait, une partie de cette MO fraîche, en partie protégée par la structure végétale résiduelle, subsiste dans la MOR. Cela se traduit par des spectres de RMN très différents. En contrepartie, le signal à 30 ppm révélant la présence de carbone polyméthylénique de structure à longues chaînes aliphatiques croît du sable à l'argile. Il est dominant dans les deux fractions fines**.** Kögel-Knabner et al. (1992a) a envisagé qu'un apport de cutine et de subérine puisse être à l'origine de ce pic. L'analyse préalable des lipides et de la MOR des sols a effectivement révélé une contribution importante de composés aliphatiques et notamment de subérine dans ces sols. Un pic large est observé vers 130 ppm avec la même intensité dans les trois fractions. Il correspond aux carbones aromatiques. Plusieurs composés peuvent être à l'origine de l'aromaticité de la MOR : des carbonisats, des tannins, des mélanoïdines ou le groupement aryl de la lignine. La comparaison des spectres obtenus pour la MOR des sols totaux, isolées selon le protocole 1 et ceux des fractions granulométriques (MOR isolée par le protocole 2) indique un signal dû aux carbohydrates beaucoup plus faible dans les sols totaux. Une hydrolyse des sucres ayant été réalisée pour les sols totaux, cela confirme l'efficacité de l'extraction des sucres par le TFA dans le protocole 1, et indique que dans les fractions granulométriques, des mélanoïdines ont effectivement été formées. Elles participent probablement à la bande de résonance des composés aromatiques mais aussi au signal observé à 150 ppm. Ce signal est particulièrement visible dans les fractions sableuses, ce qui tend à indiquer une contribution plus importante de mélanoïdines dans cette fraction grossière. Celle-ci contenant la MO fraîche, riche en carbohydrates, les mélanoïdines sont, en effet, davantage susceptibles de se former dans cette fraction. A 175 ppm, un signal intense traduit la présence de groupes amides et/ou esters. L'étude des MORs des sols totaux avait montré une contribution importante d'acides carboxyliques et de subérine à qui ce pic peut être attribué. La comparaison des spectres des deux sols ne révèle pas de différence marquée. Seule la contribution de lignine semble légèrement plus importante dans le sol forestier.

### 3.2.2 Carbonisats

#### 3.2.2.1 Oxydation chimio thermique

Les carbonisats des sols et des différentes fractions granulométriques ont été obtenus selon la méthode mise au point par Gustafsson et al. (2001). La MOR a été soumise à une oxydation thermique (375°C) pendant 24 heures sous flux d'oxygène. Le dosage du carbone résiduel a ainsi permis la quantification des carbonisats. Les teneurs en carbonisats obtenus sont extrêmement faibles (tableau 3.3).

| | Forêt | Maïs |
|---|---|---|
| Sol total | 0.016 | 0.008 |
| Sable | 0.014 | 0.05 |
| Limon | 0.029 | 0.009 |
| Argile | 0.012 | 0.009 |

Tableau 3.3 : Pourcentage du carbone dû aux carbonisats dans le carbone de la fraction considérée

Les carbonisats constituent une fraction négligeable du carbone contenu dans les fractions granulométriques comme dans le sol total. Cette teneur en carbonisats semble encore plus faible pour sol sous maïs. Cette différence de concentrations, qui doit être confirmée, compte tenu des faibles valeurs, peut être le résultat d'une dégradation des carbonisats lors de la mise en culture du fait d'une augmentation de l'activité bactérienne. Du fait de la stabilité des carbonisats, cette minéralisation des carbonisats est très certainement mineure. Cependant, les carbonisats peuvent aussi avoir migrer dans des horizons plus profonds. Lors de la culture de maïs l'irrigation est assez importante, le ruissellement et le lessivage ont pu mobiliser les carbonisats en profondeur.

Le terme « carbonisats » englobe des résidus de carbonisation très différents et leur résistance aux dégradations dépend de leur niveau d'organisation et, par conséquent, du rapport H/C (Kuhlbusch, 1995) car, plus celui-ci est faible, plus l'organisation est élevée. La méthode employée pour l'isolement étant très oxydante, il est probable que seul les

carbonisats les plus résistants aient subsistés. De ce fait, nous n'aurions isolé et quantifié qu'une fraction des carbonisats initialement présents.

Si les contributions en carbonisats de chaque fraction sont rapportées à un kilogramme de sol, on s'aperçoit que la majorité des carbonisats est localisée dans la fraction limoneuse (Tableau 3.4).

| | forêt | | | maïs | | |
|---|---|---|---|---|---|---|
| | Concentration en carbonistas (g/g de ROM) | Masse de MOR (g) | masse de carbonisats dans la fraction (g) | Concentration en carbonistas (g/g de ROM) | Masse de MOR (g) | masse de carbonisats dans la fraction (g) |
| sol | $0,29x10^{-3}$ | 14,2 | **$4x10^{-3}$** | $0,19x10^{-3}$ | 8,98 | **$1.6x10^{-3}$** |
| sable | $0,5x10^{-3}$ | 1,8 | **$9x10^{-4}$** | $0,20x10^{-3}$ | 5,17 | **$9x10^{-4}$** |
| limon | $0,35x10^{-3}$ | 13,84 | **$4x10^{-3}$** | $0,28x10^{-3}$ | 4,22 | **$10^{-3}$** |
| argile | $0,42x10^{-3}$ | 2,3 | **$9x10^{-4}$** | $0,39x10^{-3}$ | 15,6 | **$5x10^{-4}$** |

Tableau 3.4 : Masse de carbonisats (g) contenue dans chaque fraction (pour 1 Kg de sol)

Les résidus d'oxydation thermique ont été observés par MET à haute résolution (METHR) en réseau de franges dont le principe est résumé annexe X. Cette technique nous a permis de visualiser directement les carbonisats isolés. L'observation a révélé que la plus grande partie des carbonisats observés présentent une microtexture concentrique, très régulièrement organisée, dite en « oignon ». Cette structure est classiquement formée par combustion de matériaux peu oxygénés comme les cires de plantes ou les combustibles fossiles. De par cette structure concentrique, les carbonisats en « oignon » sont peu altérables. L'observation en METHR des MORs avait révélé la présence de tels « oignons » (partie B,III) mais la majorité des structures de type carbonisats était beaucoup plus désorganisées, c'est-à-dire que leur unités structurales de base sont faiblement orientées. Ces structures plus amorphes sont considérées comme provenant de l'aromatisation de précurseurs ligno-cellulosiques (Oberlin, 1980). Il semble donc qu'au cours de l'oxydation chimio-thermique, ces carbonisats amorphes aient été détruits et que principalement les « oignons » aient été isolés. Il est donc vraisemblable que les teneurs en carbonisats obtenues par cette méthode soient fortement sous estimées.

#### 3.2.2.2 Tri optique

Les quantités de carbonisats évaluées par oxydation chimio-thermique dans les sols étant très faibles et les observations en METHR suggérant qu'une partie des carbonisats soit détruite par cette méthode, il nous a paru intéressant de quantifier les carbonisats des sols totaux par une méthode différente. La deuxième technique employée est une méthode optique développée par le centre de Bio-Archéologie et d'écologie de Montpellier. Le sol est observé par microscopie photonique et les résidus de carbonisation sont isolés manuellement puis pesés. Par cette méthode, le pourcentage de carbonisats dans le carbone du sol mesuré sous forêt est de 4.6% du carbone et de 2.5% sous maïs. Une très grande différence est constatée entre les résultats des deux méthodes (thermique et optique). Dans la méthode optique, tous les résidus de carbonisation sont pris en compte, quel que soit l'étendue ou le degré de combustion, à condition d'être de taille suffisante pour être observé en microscopie photonique, alors que lors de l'oxydation thermique, seuls les carbonisats les plus organisés et les plus résistants ont subsisté. Ainsi la comparaison de ces deux méthodes permet d'obtenir les deux valeurs extrêmes de concentration des carbonisats : par la méthode optique cette valeur peut être surestimée, alors que la méthode thermique ne considère qu'une relativement faible partie des carbonisats.

Par ailleurs, il n'est pas exclu qu'une partie des carbonisats soit perdue au cours des traitements mis en œuvre pour isoler la MOR. Pour tester cette hypothèse, nous avons appliqué le protocole 2 à des composés de référence fournis par le « comité international de direction pour les matériaux de référence des carbonisats » : du charbon provenant de la combustion de bois et du charbon issu de la combustion d'herbe. Le protocole 2 conduit à une perte massique de 20% du charbon issu de la combustion d'herbe et de 1% du charbon issu de la combustion de bois.

Ces résultats préliminaires indiquent que selon leur origine, des carbonisats peuvent être éliminés par ce protocole. La comparaison des analyses élémentaires avant et après traitement ainsi que l'observation en HRTEM de ces charbons « standards » pourra sans doute permettre d'envisager une sensibilité liée à la composition chimique ou à la structure (faiblement ou fortement organisée) des carbonisats.

## 3.3 Conclusions

La quantification de la MOR des différentes fractions granulométriques des sols sous forêt et maïs indique une distribution hétérogène de la MOR et révèle une contribution de mélanoïdines à cette MOR. Ces résultats montre que lors de l'isolement de la MOR, l'élimination des sucres et acides aminés est nécessaire, notamment lorsque l'apport de MO peu dégradée est important (Poirier et al., 2003).

Lors de la mise en culture du sol, la MO issue du maïs contribue peu à la MOR des fractions granulométriques et des sols totaux. La MO du maïs est peu stabilisée dans les sols, probablement car son taux de dégradation est plus élevé, du fait d'une minéralisation plus importante après la mise en culture, et de la nature de la MO apportée au sol.

Les carbonisats des sols totaux et des différentes fractions granulométriques ont été isolés par oxydation thermique. Il apparaît que les carbonisats obtenus par le protocole utilisé représentent une fraction négligeable du carbone organique total. La quantification dans les différentes fractions et dans les deux sols a toutefois permis de noter une localisation préférentielle des carbonisats dans la fraction limon pour les deux sols et une proportion plus faible dans le sol sous maïs. Cette diminution dans le sol sous maïs est probablement liée à une migration en profondeur. L'observation en HRTEM des carbonisats a révélé une contribution plus importante de carbonisats présentant une structure dite « en oignon ». Ceux-ci résultent en majorité de la combustion de cires et, de part leur structure, sont des éléments très résistants aux dégradations. Bien que des carbonisats de structure moins organisée soient observables, il semble que la méthode d'isolement par oxydation thermique altère tous les constituants de la MOR excepté les carbonisats les plus résistants. Toutefois, la quantité de carbonisats étant très faible, une perte liée aux étapes successives du protocole d'isolement ne peut être exclue.

# CONCLUSION PARTIELLE DE LA PARTIE C

**Les lipides et la matière organique réfractaire du sol cultivé ont été étudiés et comparés au sol forestier afin de déterminer l'influence de la mise en culture sur ces fractions.**

**La comparaison des teneurs en carbone et en lipides des différentes fractions granulométriques a révélé des différences importantes entre les deux sols, tant sur la teneur en lipides, que sur la teneur en carbone. En effet, après mise en culture, nous avons observé une diminution de la teneur en carbone organique et en lipides dans le sol ainsi que dans les fractions granulométriques. Concernant les lipides, bien que les mêmes séries de composés soient identifiées dans les fractions granulométriques des deux sols, des différences importantes dans les distributions sont observées aussi bien entre les sols, indiquant un effet de la mise en culture, qu'entre les fractions granulométriques. Les lipides des fractions granulométriques du sol cultivé présentent une contribution de lipides issus de plantes supérieures (cire, cutine, subérine et lignine), cependant ils indiquent une contribution microbienne plus importante que dans le sol forestier.**

**D'autre part, la dynamique de la matière organique a aussi été envisagée : les taux d'incorporation du maïs dans la matière organique du sol, dans les lipides mais aussi dans les alcanes ont été calculés à partir des mesures isotopiques ($\delta^{13}C$). Ces résultats mettent en évidence une faible incorporation des composés issus du maïs (seulement 16% dans le sol) mais celle-ci diffère selon les fractions considérées : la proportion de maïs contenue dans la MOS diminue du sable à l'argile. Les mêmes tendances sont observées dans le carbone lipidique, bien que les taux d'incorporation soient plus faibles et que le taux de dégradation soit plus élevé. Ainsi, les composés lipidiques semblent plus sensibles à la conversion forêt/culture. Dans le sol cultivé, une part importante de la signature isotopique de la MO issue de la forêt correspond à de la MO bactérienne développée sur la MO de forêt et donc ayant une valeur isotopique similaire à cette MO.**

**L'étude de la matière organique réfractaire (MOR) indique une faible incorporation de MO issue du maïs à la MOR ainsi qu'une une diminution de la MOR après 22 ans de culture. Ce résultat témoigne d'une altération de la MOR liée à la mise**

**en culture du sol et indique que cette MO chimiquement réfractaire est moins stable que supposé préalablement. Cette fraction ne reflète pas le pool de carbone stable de la MOS. De ce fait la résistance chimique n'est pas strictement synonyme de résistance biologique.**

**L'isolement par traitements chimiques puis chimiothermique des carbonisats préalablement mis en évidence en HRTEM et par les dégradations chimiques (Cf partie B III) a permis leur quantification et l'établissement d'une localisation préférentielle dans la fraction granulométrique correspondant au limon. Cependant, toutes les concentrations mesurées se sont révélées extrêmement faibles (largement inférieur à 1%) et diminuent dans le sol cultivé. Par ailleurs, la quantification des carbonisats isolés par tri optique a montré une contribution plus importante des carbonisats (4.6% sous forêt et 2.5% sous maïs) ainsi qu'une influence probable de la mise en culture sur ces composés. Cette différence entre les deux modes de quantification semble indiquer une destruction des carbonisats lors du processus d'isolement par oxydation chimio-thermique.**

# CONCLUSIONS GENERALES ET PERSPECTIVES

Au cours de ce travail, nous avons examiné les fractions lipidiques et organiques réfractaires d'une chronoséquence forêt/maïs. Ces fractions ont été isolées à partir d'échantillons de sol prélevés dans le sud ouest de la France. Les sols étudiés sont des podzols présentant une structure très sableuse (90%). De ce fait, la protection physique de la matière organique des sols est faible. L'étude de la MO de ce site peut ainsi fournir des informations sur la stabilité de la MO davantage liée à sa composition chimique et aux facteurs environnementaux. La succession forêt de pins (plante en C3)/ culture de maïs (plante en C4) donne accès à des informations sur la dynamique et sur la stabilité de la matière organique du sol.

Après avoir mis en place le cadre général de l'étude (partie A), dans la partie B, nous avons tout d'abord étudié le sol forestier. Après un fractionnement granulométrique (sable >50 µm, limon 50-2 µm et argile <2 µm), les lipides ont été extraits. Ainsi nous avons quantifié les lipides des différentes fractions et mis en évidence une distribution inégale, avec un maximum de lipides dans la fraction argileuse. L'analyse par CG-SM a révélé une variation de la distribution des constituants lipidiques dans les différentes fractions et a ainsi fourni des indications sur des sources majoritaires de lipides variables selon la fraction considérée. De plus, nous avons observé différents indices démontrant une meilleure préservation dans la fraction argileuse. Une fraction des lipides a été saponifiée afin d'analyser les lipides de haut poids moléculaire présents dans le sol. Nous avons pu noté la contribution de cires et de composés issus de l'altération de la cutine et de la subérine. Contrairement aux lipides libres, ces macromolécules semblent présenter une résistance intrinsèque à la dégradation.

La matière organique réfractaire (MOR) a ensuite été isolée par une hydrolyse basique pour ne conserver que l'humine, une hydrolyse par un mélange d'acide trifluoroacétique/ acide chlorhydrique afin d'éliminer les composés protéiques et les sucres qui pourraient former des mélanoïdines lors de l'isolement, puis les minéraux ont été dissout par l'acide fluorhydrique. Les méthodes spectroscopiques (IRTF, RMN $^{13}C$ à l'état solide, associées à la pyrolyse ainsi qu'à la pyrolyse en présence d'un agent alkylant (TMAH) ont indiqué la préservation de structures de type ligno-cellulosique et de constituants de la subérine dans la MOR. La présence de ces composés dans la matière organique réfractaire indique la

possibilité d'une protection par la matrice macromoléculaire mais aussi une probable préservation de la subérine due à sa structure chimique.

L'analyse par CG-SM des monomères obtenus par des oxydants plus ou moins puissants (le persulfate (faiblement oxydant), le permanganate, le perborate et le tetroxyde de ruthenium (spécifique de certaines liaisons)) a permis d'avoir un aperçu de la structure de la matière organique réfractaire. Ces oxydations ont confirmé la contribution de subérine mais aussi de lipides provenant de plantes supérieures et de lipides insaturés réticulés au sein de la structure macromoléculaire de la MOR, ainsi que la présence de *n*-alcanes piégés dans la structure. L'oxydation au tetroxide de ruthénium a mis en évidence la contribution de carbonisats à la MOR ce qui a été ensuite été confirmé par des observations en HRTEM.. L'utilisation de divers réactifs permet d'identifier différents composants et types de liaisons selon le pouvoir oxydant. De plus, elle complète les informations obtenues par les méthodes spectroscopiques et pyrolytiques.

Dans la partie suivante, nous avons tenté d'évaluer l'influence de la mise en culture du sol. En effet, le second sol étudié a été échantillonné après 22 ans de culture. La mise en culture du sol entraîne de nombreuses perturbations : changement de la nature et de la quantité de MO qui retourne au sol, variation des facteurs environnementaux (tel que le pH), impact sur la structure du sol (qui est limité dans notre cas du fait de la texture sableuse du sol). De ce fait, la matière organique du sol subit d'importantes répercussions. La comparaison de la quantité et de la composition, aussi bien des lipides que de la MOR, des deux sols fournit de précieuses informations sur l'effet de la mise en culture mais aussi sur la stabilité des ces fractions. De plus, la transition entre l'apport de MO issue de la forêt de pins dont le métabolisme est en $C_3$ et celui de MO issue du maïs qui est une plante à métabolisme $C_4$ donne l'opportunité de suivre la dynamique de la MO.

Dans le premier chapitre de cette partie, nous avons identifié les lipides du sol cultivé puis nous avons comparé leurs distributions à celles du sol forestier. D'importantes différences de distributions des composés sont observées à la fois entre les fractions du sol cultivé, reflétant ainsi l'intérêt du fractionnement granulométrique dans cette étude, mais aussi entre les deux sols, révélant une influence de la mise en culture sur les lipides et notamment une contribution plus importante de lipides issus de microorganismes après la mise en culture. Puis, nous avons envisagé la dynamique de la MO, des lipides ainsi que des *n*-alcanes. L'apport de la MO issue du maïs après 22 ans de culture dans le sol total est plutôt faible (16%). On constate une augmentation de la contribution du maïs de la fraction argileuse vers

la fraction sableuse. Une tendance similaire est observée pour la dynamique des lipides. Le taux de dégradation calculé pour les lipides étant supérieur à celui de la MO du sol total, il apparaît que les lipides sont plus sensibles au changement de culture que la MO dans sa globalité. Du fait de la composition lipidique qui indique un apport substantiel de lipides issus des microorganismes, on peut en déduire qu'une partie du carbone ayant une signature isotopique de plante en $C_3$ est en réalité du carbone issu de la forêt métabolisé par les microorganismes.

Dans un second chapitre, la comparaison des spectres de RMN $^{13}C$ à l'état solide et des pyrolysats (conventionnel et en présence de TMAH) de la matière organique chimiquement réfractaire des deux sols permet d'obtenir des informations sur l'influence de la mise en culture sur la composition chimique de cette fraction. De plus, l'estimation de la dynamique de la MOR permet d'avoir une indication sur sa stabilité. La composition chimique des deux MOR apparaît très semblable avec une majorité des constituants provenant essentiellement composés dérivés de la lignine et de la subérine, mais aussi de composés aliphatiques (tels que les subéranes) et d'acide gras probablement piégés dans la matrice ou liés à la structure macromoléculaire. Toutefois, on constate une diminution de l'abondance des produits issus de composés ligno-cellulosiques. De plus, la quantification de la MOR indique que celle-ci a subi une dégradation relativement importante, en particulier pour les composés issus de la lignine. Les composés issus de la subérine semblent présenter une plus grande résistance intrinsèque à la dégradation. Finalement, il s'avère que la MO chimiquement réfractaire est moins récalcitrante à la biodégradation qu'on ne l'avait supposé et donc que la résistance chimique n'implique pas forcement la résistance à la biodégradation.

Les dégradations chimiques de la MOR du sol forestier et son observation en microscopie électronique à transmission à haute résolution ont révélé la présence de carbonisats dans la MOR. Ces composés sont connus pour leur grande stabilité. Nous avons donc décidé de quantifier les carbonisats des deux sols, mais aussi ceux contenus dans les fractions granulométriques. Les carbonisats sont obtenus, grâce à une oxydation chimiothermique de la MOR isolée par un protocole simplifié (l'hydrolyse à l'acide trifluoroacétique a été supprimée car elle devient inutile, l'oxydation chimiothermique détruisant les mélanoïdines artéfactuelles potentiellement formées). La quantification des carbonisats a indiqué une localisation préférentielle de ces composés dans la fraction « limon », cependant les carbonisats ne représente qu'un pourcentage extrêmement faible du carbone du sol. L'observation microscopique indique que les carbonisats observés présentent en grande majorité une structure dite « en oignon ». Cette organisation rend ces carbonisats

très stable. Cette observation associée à une quantification des carbonisats isolés de manière optique laisse supposer que le protocole d'isolement détruit une partie des carbonisats pour ne conserver que les plus stables.

Ce travail indique que la combinaison de méthodes analytiques (spectroscopiques, pyrolytiques, oxydatives et microscopiques) afin de déterminer la composition moléculaire et la structure de la MOS, associées à des mesures isotopiques peuvent fournir de précieux renseignements concernant la stabilité de la MOS. De plus, l'étude de fractions (fractions granulométriques et/ou fractions isolées chimiquement) permet de compléter et d'approfondir ces résultats. Ainsi, l'impact de la mise en culture a mis en évidence une inadéquation entre stabilité chimique et stabilité cinétique et le fractionnement granulométrique a révélé une localisation plus importante de carbonisats dans les fractions limons. Les résultats obtenus dans le chapitre 3 de la partie C mettent, une fois de plus, en évidence la difficulté d'isoler les carbonisats de sols et montre la différence de stabilité selon le type de carbonisats considéré (source, niveau d'organisation).

Plusieurs perspectives peuvent être envisagées dans ce travail :

- Concernant les lipides, il serait intéressant de renouveler cette étude sur des sols différents afin de voir si une tendance générale sur l'origine des lipides et leur évolution lors d'un changement d'occupation des sols se dessine. Dans le cas de chronoséquences, des mesures du rapport isotopique des différents constituants lipidiques fourniraient des indications sur les taux de renouvellement des différents composés.
- Les résistances chimiques et biologiques n'étant pas compatibles, il serait intéressant d'étudier la composition moléculaire de la MOS de sols sableux (pour lesquels la protection physique est faible) mais prélevée dans des horizons plus profonds, dont on sait que la MO est plus ancienne, afin de vérifier la structure et la composition chimique de cette MO biologiquement stable.
- Il serait aussi intéressant extraire et doser les oxydes d'aluminium et de fer avec lesquels la MOS peut aussi interagir et être ainsi protégé dans les sols de Cestas. Par la suite, lorsque la composition de cette MO biologiquement stable sera déterminée, on pourra essayer de l'isoler.
- La quantification et leur spéciation de polluants (pesticides ou éléments traces métalliques) spécifiquement associés à cette fraction serait intéressante et pourrait

permettre d'estimer leur biodisponibilité et leur devenir. On pourrait d'ailleurs commencer par l'étude de l'association polluants-MO ancienne (d'horizon inférieur).

- Des mesures isotopiques effectuées sur des monomères spécifiques de constituants de plantes tels que la cutine ou la subérine par couplage pyrolyseur-CG-spectromètre de masse isotopique permettraient d'obtenir des données sur la cinétique de ces composés.
- Il serait utile d'isoler les carbonisats dans les fractions granulométriques d'autres sols afin de confirmer la localisation préférentielle dans une fraction. La datation des carbonisats des différentes fractions granulométriques pourrait être envisagée. L'étude de composés standards de carbonisats pourrait fournir des précisions sur leur stabilité, selon le composé d'origine à partir duquel est formé le carbonisat, et permettre d'améliorer l'isolement des carbonisats dans les sols.

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**Résumé**

La matière organique (MO) des sols est un réservoir important de carbone sur Terre. Le stock de MO dépend de facteurs biologiques, ainsi la MO des sols constitue le compartiment du cycle global du carbone le plus sensible aux perturbations anthropiques, mais aussi climatiques. Lors de sa biodégradation, la MO représente une source de $CO_2$ atmosphérique, aussi une meilleure connaissance de sa structure et de sa dynamique est nécessaire afin de pouvoir modéliser et prévoir les variations de $CO_2$ atmosphériques liées à des modifications du milieu. Différents pools cinétiques ont été distingués au sein de la MO des sols dont les temps de renouvellement varient de la dizaine d'année au millénaire. Les pools aux temps de résidence les plus importants forment un stock de carbone stable peu soumis aux perturbations. La détermination qualitative et l'estimation quantitative de ce stock de carbone reste encore à améliorer. Au cours de ce travail, nous nous sommes intéressés à la structure et à la dynamique des lipides, qui sont souvent considérés comme relativement stables, et à une fraction chimiquement réfractaire, obtenue à la suite d'un protocole comprenant des hydrolyses acides et basiques. Cette fraction est susceptible de présenter également une résistance à la biodégradation. Pour cela, nous avons étudié deux sols sableux (podzols) des Landes appartenant à une chronoséquence : un sol forestier ayant été converti à la culture de maïs.

La détermination de la composition lipidique dans trois fractions granulométriques (sable, limon et argile), notamment par CG-SM, de ces deux sols a permis d'accéder à de nombreuses informations tant sur leur source que sur leur dynamique. Les méthodes spectroscopiques (RMN $^{13}C$, IRTF) associées à des méthodes pyrolytiques et à des oxydations chimiques ont fourni des renseignements sur la composition chimique de la matière organique réfractaire (MOR) et sa structure. La comparaison des deux sols a révélé une influence de la mise en culture du sol forestier sur les lipides ainsi que sur la MOR. De plus l'utilisation du marquage naturel par le carbone 13 a permis d'obtenir des données sur la dynamique tant de la matière organique du sol, que des lipides ou de la MOR.

**Abstract**

Soil organic matter (OM) is an important pool of carbon on Earth. The OM stock depends on biologic factors, thus soil OM constitutes the global carbon cycle compartment the more sensitive to anthropic and climatic disturbances. In the course of biodegradation, OM represents an atmospheric $CO_2$ source, hence a better knowledge of its structure and dynamic modelling is necessary and to forecast atmospheric $CO_2$ variation, linked to environmental modifications. Different kinetic pools have been distinguished in soil OM which residence times vary from years to millennia. The more important residence time pools represent a stable stock of carbon poorly submitted to disturbance. The qualitative determination and quantitative estimation of this stock of carbon still has to be improved. During this work, we have studied the structure and dynamic of lipids, often considered as relatively stable, and of a chemically refractory fraction, obtained by a protocol including acid and basic hydrolyses. This fraction can present a resistance to biodegradation. Thus, we have studied two sandy soils (podzols) belonging to a chronosequence: a forest soil converted to maize cropping.

Lipid composition determination in three particle-size fractions (sand, silt and clay) of these two soils, especially by GC-MS, makes possible to have access to substantial information on their source and dynamic. Spectroscopic methods ($^{13}C$ NMR, FTIR) combine to pyrolytic methods and chemical oxidations provide information on refractory organic matter (ROM) chemical composition and structure. Soil comparison has revealed the corn cropping influence on lipids and ROM. Moreover natural $^{13}C$ abundance makes possible to obtain data on OM, lipids and ROM dynamic.