Origin and fate of organic matter
in sandy soils along a primary vegetation succession

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Stellingen

1   Humificatie kan pas goed begrepen worden als zowel de locale geschiedenis als de moleculaire samenstelling van de vegetatie bekend is.

(dit proefschrift)

2   Het, tot nu toe, grotendeels of zelfs geheel over het hoofd zien van alifatische componenten in het meeste onderzoek aan bodem organische stof geeft aan dat deze onderzoekstak nog in de kinderschoenen staat.

(dit proefschrift)

3   De aan lignine toegeschreven resistentie tegen microbiële afbraak is aan hevige devaluatie onderhevig.

(dit proefschrift)

4   Chemische stabiliteit van biopolymeren als zodanig is geen maat voor hun resistentie in bodems.

(dit proefschrift)

5   Schnitzer's omschrijving van 'humic substances' is pure speculatie, en bovendien nietszeggend.

'Humic substances, which constitute 70-80% of organic matter in mineral soils, are dark-coloured partly aromatic, acidic, hydrophilic, molecularly flexible polyelectrolyte materials.'

(M. Schnitzer (1986) Binding of humic substances by soil mineral colloids. In: P.M. Huang and M. Schnitzer (Editors), *Interactions of soil minerals with natural organics and microbes.* SSSA Special publication 17, Madison, Wisc., pp. 77-101)

6   Zonder chemie géén bodemkunde, zonder bodemkunde wél chemie.

7   Veel standaard karakterisatie methoden gebruikt om organische stof kwaliteit te bepalen (Klason-lignin, 'polyphenols' etc.) zijn, chemisch gezien, misleidend.

(C.A. Palm and A.P. Rowland (1997) A minimum dataset for characterization of plant quality for decomposition. In: G. Cadish and K.E. Giller (Editors), *Driven by Nature; Plant Litter Quality and Decomposition.* CAB International, Wallingford, pp. 379-392)

8   De opbrengst van de Postcode Loterij zal grotendeels te niet gedaan worden wanneer het door haar huis-aan-huis verspreide reclamemateriaal zodanig verwerkt wordt dat het voldoet aan hun doelstelling om tot 'een betere en schonere wereld' te komen.

9   Het belang van burocratie voor de werkgelegenheid mag niet worden onderschat.

10 Het is typisch Nederlands om negatief geachte eigenschappen van Nederlanders als 'typische Nederlands' te typeren.

11 Dat een overgrote meerderheid van de voetballiefhebbers zich fan noemt van Ajax, Feyenoord of PSV geeft aan dat deze clubs relatief weinig echte (club-) supporters bezitten.

12 Vrouwelijke intuïtie is een *contradictio in terminis.*

    (J.H. Donner, Vrouwen en schaken. In: *De Nederlander en andere korte verhalen*)

13 'Uitzonderingen bevestigen de regel' is een uitzondering op de regel dat een spreekwoord of gezegde een kernachtige waarheid bevat, aangezien uitzonderingen de regels juist niet bevestigen.

14 Humor is pas echte humor als deze slechts door een beperkt aantal mensen gewaardeerd kan worden.

Stellingen behorende bij het proefschrift 'Origin and fate of organic matter in sandy soils along a primary vegetation succession'.

Klaas Nierop, 16 maart 1999.

Voorwoord

Na vier jaar komt er een einde aan mijn promotie-onderzoek. Zo beginnen de meeste voor- en nawoorden, wat geen wonder is gezien de evenzolange financiering van AIO- en OIO-projecten. Het resultaat van die periode ligt voor u en ik hoop dat velen dit van begin tot einde zullen lezen. Tenslotte heb ik dan niet voor niets al die letters, cijfers, symbolen en figuren ingetikt en geconstrueerd. Gelukkig heb ik voor wat de inhoud betreft veel hulp gehad van anderen, wat de prettige bijkomstigheid met zich meebrengt dat ik me niet alleen verantwoordelijk voel voor de eventuele onzin die er in staat. Hierbij wil ik een aantal mensen bedanken.

Allereerst buurman en Buurman. Buurman Nico van Breemen omdat hij het aandurft om als promotor op te treden en voor het (steeds sneller) doorlezen en corrigeren van manuscripten. Zo veranderden met een paar eenvoudige veranderingen krampachtige zinnen in poëtisch hoogstaande teksten (het fameuze 'Breemenization'-proces). Een enkele keer gebeurde, gelukkig maar, ook het omgekeerde, resulterende in klinklare onzin ('de-Breemenization'?). Die veranderingen heb ik dan ook meestal niet uitgevoerd, dus het netto resultaat moet leesbaar zijn. Peter Buurman wil ik bedanken voor de dagelijkse begeleiding en de grote vrijheid in het doen en laten van het onderzoek. Daarnaast heeft hij de eerste versies van de manuscripten weten door te worstelen wat door het chemische karakter vast niet altijd mee zal zijn gevallen. Als laatste wil ik hem bedanken voor het feit dat ik altijd bij hem binnen kon vallen en hij dan meteen een luisterend oor had ondanks zijn 263 andere functies en bezigheden.

Van groot belang waren ook de mensen van het lab. Met name Barend van Lagen met zijn hulp tijdens het onderzoek en het uitvoeren van de vele NMR metingen en suiker analyses. Frans Lettink, Neel Nakken en Eef Velthorst voor hun bijdragen en het gedogen van mijn aanwezigheid in hun lab.

Als je de enige in Wageningen bent die dit type onderzoek doet, is kennis nodig van buiten Wageningen. Jan de Leeuw van het NIOZ op Texel is daarbij van grote betekenis geweest. In de eerste plaats tijdens de beginfase toen ik enkele malen bij het NIOZ mocht pyrolyseren wat tegelijkertijd een 'cursus' pyrolyse betekende. Later, toen we in Wageningen zelf de beschikking hadden over Py-GC/MS apparatuur, kon ik met vragen altijd bij hem terecht en gezien de flinke brok aan pyrolsye data in dit boekje is veel direct of indirect te danken aan de eerste monsters gemeten op Texel. In de laatste fase van het onderzoek heeft Pim van Bergen van de Organische Geochemie groep van de Universiteit Utrecht een belangrijke rol gespeeld en stond hij aan de wieg van Hoofdstuk 8.

During his Erasmus period in Wageningen I enjoyed very much the cooperation with Eladio Parga Ures from the University of Santiago de Compostella. The set-up of the CuO oxidation method was quite a nightmare, resulting into complete abandonment of that method, but I never forget your astonished face discovering: "Klaas, I was thinking..."

Een chemietje tussen de bodemologen en geofielen is een raar gezicht. Voor jullie, incrowd van het Laboratorium voor Bodemkunde en Geologie, zal ik daarom (?) wel een vreemd kereltje zijn geweest. Maar weest gerust, ik vind jullie niet veel normaler. Ondanks de grote cultuur- en wetenschapsverschillen, heb ik uiteindelijk goed kunnen aarden in deze

Contents

Chapter 1

General introduction

INTRODUCTION

More than 99.9% of all carbon in the earth's crust is stored in sedimentary rocks (Berner, 1989). About 20% of this (15 000 000 Gt) is organic and mainly in the form of kerogen (Hedges and Oades, 1997). Only around 1600 Gt C is present as soil organic matter, and still smaller amounts present in living vegetation (610 Gt) and the atmosphere (750 Gt) (Schimel, 1995). Although negligible as a reservoir, soil organic matter plays a major role in the global carbon cycle. The annual net primary terrestrial production is about 60 Gt and equals the amount of litterfall and the annual exchange of C between the earth's surface and the atmosphere (Hedges and Oades, 1997). Most of the (dead) plant, animal and microbial material decomposes into CO_2 and recirculates within a few years to the atmosphere. A minor part is assimilated in microbial tissues or converted into soil organic matter, with a longer residence time.

OBJECTIVES

Organic matter plays an important role in many physical, chemical and biological processes in soils through its water holding capacity, cation exchange capacity, its ability to complex with metal ions and as a nutrient source for plants. Vegetation influences soil development among others via its litter and roots. In its turn, soil properties strongly influence vegetation composition and net primary productivity. Notwithstanding the close relationships between soil and vegetation and the important role soil organic carbon play in this interaction, relatively little is known about the chemical nature of soil organic matter and its relationship to the extant vegetation. The objective of this thesis is to extend the insight in these interactions.

Studies dealing with soil organic matter are generally concentrated on well-developed soils with a long climate and vegetative history. In such soils, causal relations between vegetation and organic matter can hardly be established, whereas temporal and vertical aspects are difficult to unravel. A relatively simple soil-vegetation system may provide a better insight in organic matter build-up and effects of vegetation on the chemical composition of soil organic matter. The area of the Hulshorster Sands and the Leuvenum Forest is an example of such a simple system: a sandy soil with a well-known vegetative history.

In the Leuvenum Forest, in the early stages of vegetation succession, the composition of organic matter depends directly on its origin, the plant species. Extensive research has been carried out on the vegetation succession and the development of humus profiles (Fanta, 1986; Prach, 1989; Emmer, 1995a).

PRIMARY SUCCESSIONS

The species composition of most forests in the Netherlands is strongly influenced by management. Therefore, relations between vegetation and humus profile development are diffuse. A primary vegetation succession is undisturbed, and has developed on a substrate,

which has not been influenced by former soil development. One example is the succession on wind-blown sands of the Hulshorster Sand and Leuvenum Forest.

The vegetation succession of this area has been studied in detail (Fanta, 1986; Prach, 1989, see also Figure 1). The succession starts with *Corynephorus canescens* (L.) Beauv. (grey hair-grass). Also algae may be the colonizers, although their role is not fully understood (Prach, 1989). In time, *Corynephorus* is joined by *Spergula morrisonii* (L.) Boreau (pearlwort spurrey) and *Festuca ovina* L. (sheep's fescue). Adjacent to these, the moss *Polytrichum piliferum* Hedw. is generally found on relatively moist places facilitated by algae. The mosses are partly succeeded by *Calluna vulgaris* (L.) Hull. (heather). The first pines (*Pinus sylvestris* L.) appear in the grass sites, but eventually all species are succeeded by these trees, which ultimately form a semi-closed canopy. Under the pines, the former ground vegetation is first replaced by lichens. These are followed by *Deschampsia flexuosa* (L.) Trin. (wavy hair-grass), which is a very dominant species in the pine forest. *Deschampsia* is accompanied by the mosses *Dicranum scoparium* Hedw. and *Hypnum cupressiforme* Hedw.. Later on, these mosses are followed by *Pleurozium schreberi* Hedw., which may even outcompete *Deschampsia* in later stages. *Pleurozium* improves the circumstances for the establishment of *Empetrum nigrum* L. (crowberry), *Vaccinium myrtillus* L. (blueberry) and *Vaccinium vitus-ideae* L. (cowberry). Meanwhile trees like *Quercus robur* L. (common oak), *Betula pendula* (L.) Roth (silver birch) respectively *pubescens* (L.) Ehrh. (downy birch) and *Fagus sylvatica* L. (beech) establish. Finally, the forest becomes completely dominated by beech.
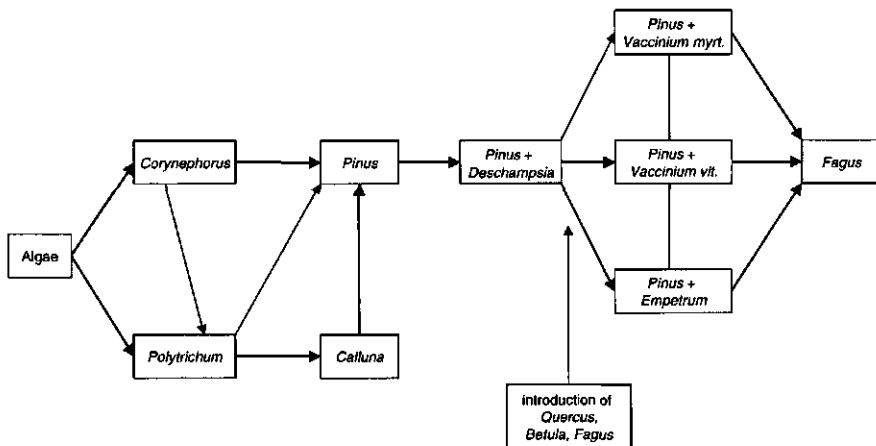

Figure 1. Simplified scheme of the vegetation sequence in the Hulshorster Sand and Leuvenum Forest.

Under the first grass vegetation there is hardly any soil formation, even though the large root system of *Corynephorus*, which dies annually, supplies organic matter into the soil. Under *Calluna* and the young pines an ectorganic layer appears, which becomes thicker with vegetation development. Also an Ah horizon is formed as a result of abundant roots in this layer. Eventually, when a pine forest has established, an ectorganic humus layer develops, with three main horizons, namely an L (litter), an F (fragmentation) and an H (humification) horizon. These three horizons reflect increasing decomposition of plant litter from top (L) to bottom (H). After about one century of forest development, the amount of organic matter in each of these horizons under pine has reached an almost constant value: a dynamic equilibrium between litter input and organic matter decay is reached (Emmer, 1996). Differences in humus profiles under pine with or without coverage of grasses or shrubs are relatively small, but the undergrowth can modify the humus form development of the organic horizons: blueberry, and especially crowberry, promote the accumulation of organic matter in the ectorganic layer more so than grasses (Emmer, 1995a). Macromorphological investigations showed that the F layer under crowberry and blueberry was mainly composed of remnants of this vegetation, while the lowest part of this horizon contained predominantly decayed residues of *Deschampsia* (Emmer, 1995a). This indicates that crowberry and blueberry indeed stimulate organic matter accumulation and do not preferably settle at humus-rich locations. Yet, the humus profile does play a crucial role in the establishment of plants. *Deschampsia* roots mainly in the F horizon. After development of an H horizon, crowberry and blueberry can establish, and will eventually outcompete *Deschampsia*. These shrubs have roots in the F as well as in the H horizon, while rhizomes of blueberry are mainly found in the H horizon (Emmer, 1996).

PODZOLIZATION

With increasing soil formation, pH decreases, eventually resulting into a decrease of biological activity and a lowering of the decomposition rate. Appreciable accumulation of organic matter and initial podzolization features are the consequences. The first traces of podzolization, such as a mini E horizon, are found after about 100 years. There are indications that the vegetation influences podzolization. Crowberry, for instance, promotes, besides organic matter accumulation, also podzolization (Emmer, 1994).

Podzols are characterized by the existence of a bleached eluvial E horizon, which overlies an illuvial B horizon resulting of accumulated organic matter in combination with iron and/or aluminium derived from overlying horizons (Petersen, 1976; De Coninck, 1980; Buol *et al.*, 1989). This type of soil is the third in terms of surface area (14.8%) and has an organic carbon pool of 0.37 Gt (Andreux, 1996). Climatic (zonal) podzols are found in the tundras and on acid parent rocks in mountain areas, degradation (intrazonal) podzols appear in Europe's temperate areas, and tropical podzols exist in South America, Africa, Asia and Oceania (Andreux, 1996).

Several mechanisms of podzolization have been proposed. In the classical *fulvate theory* (e.g. Petersen, 1976) fulvic acids release metal ions from primary and secondary minerals and move as soluble chelates through the E and Bhs horizons. When a critical Al or Fe to fulvic

acid ratio is reached the complex precipitates, causing the formation of the Bs horizon, resulting into precipitation. Farmer's *proto-imogolite theory* includes a combination of soluble Al and Si in the E horizon to give a positively charged Al/Si colloid, which elutes to the Bs horizon where it is precipitated as pH increases on negatively charged sites at the mineral surfaces (Farmer *et al.*, 1980, 1982; Anderson *et al.*, 1982). After that fulvic acids move through the E horizon and precipitates on the allophane. The *low molecular weight (LMW) organic acid theory* suggests that strong, small acids transport the Al and Fe to the subsoil and that precipitation of Al and Fe is caused by breakdown of the carrier (Lundström *et al.*, 1995).

The role of the organic matter playing in the podzolization process is far from clear. The vegetation influences the state and rate of podzolization (Hanrion *et al.*, 1975; Buurman, 1984; Emmer, 1994). Recently, the hypothesis has been put forward that exudation of LMW acids by ectomycorrhizal fungi play an important role in the formation of boreal podzols (van Breemen *et al*, 1998). Podzol B organic matter has been extensively studied, but until recently this research was severely limited by the available analytical instruments and techniques. Organic matter in the B horizons is dominated by fulvic and related acids from other mineral horizons that were purified of polysaccharides (Schnitzer, 1970; Malcolm, 1990). Especially organic C from the Bh horizon was found to be dominantly aromatic (Burges *et al.*, 1964; Buurman and Zech, 1994). However, also highly aliphatic fulvic acids were found in e.g. a Scottish podzol (Anderson and Russell, 1976). Information about non-extractable organic matter in podzol B horizons is scarce. Theng *et al.* (1992) observed that this inert humus is highly aliphatic and attached to clay as external and internal complexes. It was suggested that the aliphatic substances were derived from the upper organic horizons and transported downwards by water (Schulten *et al.* 1996). Buurman (unpublished data) noted that the organic matter in hydromorphic podzols is more aliphatic than in well-drained ones.

Detailed molecular information about the organic matter composition in podzol B horizons is rare. Using pyrolysis-FIMS characteristics of polysaccharides, alkylaromatics, lipids, lignin monomers and dimers were observed in Canadian podzols (Sorge *et al.*, 1994). Recently Wilcken *et al.* (1997) found that the spodic B horizons in northern Germany consisted mainly of alkyl C and O-alkyl C (^{13}C-NMR), independent of age of podzolization. Py-FIMS showed that weak podzolization was characterized mainly by signals from polysaccharides, phenols, lignin monomers, fatty acids and plant waxes in the B horizon, while Haplic Podzols were dominated by signals from lignin dimers, long-chain lipids and sterols (Wilcken *et al.*, 1997).

SOIL ORGANIC MATTER

Soil organic matter comprises a mixture of above- and below-ground plant, animal and microbial residues and so-called 'humic compounds', which are usually described as microbially and chemically resynthesized molecules. Complete separation of humic and non-humic substances is non-existent, because organic matter is present in a continuum of different stages of humification. Despite of that, several organic matter fractionations have been developed in order to study decomposition and humification processes. Common fractionations include the classical humus fractionation, density and particle size separations

and selective chemical treatments (extraction of lipids, hydrolysis of proteins, polysaccharides, cutin, suberin). The classical chemical approach, and still most widely applied, is based on the solubility of humus in water at varying pH's. *Humin* is insoluble in base (pH 13), the remaining soluble part is subjected to pH 2 and divided into a fraction insoluble in acid (*humic acid*) and a fraction soluble in acid (*fulvic acid*). Based on these operationally defined fractions, humic substances are classified according to the IHSS and as such used in 'humic substances' studies. Due to this limited definition, humus studies are mainly concentrated on the NaOH soluble fractions thereby excluding plant debris, which is primarily found in the humin fraction, and consequently their role in soil, whereas in 'soil organic matter' research all organic substances are included.

Perhaps because of this artificial boundary between the study of 'humic substances' and of 'soil organic matter', humification processes are far from clear. Many pathways of humus formation have been proposed. In general, two contrasting models can be distinguished: the biopolymer degradation model ('organic matter view') and the abiotic condensation theory ('humic substances view'). The *biopolymer degradation* type includes the lignin-protein theory from Wacksman (1930's), in which lignin is considered as the core of humic substances. In Hatcher and Spiker's (1988) modified degradation version, refractory biopolymers are preserved selectively and form the basic elements of humus. Besides lignin, also other polymers such as aliphatic macromolecules participate. In this theory, humin is the most recalcitrant fraction, which evolves through the humic acid fraction to the fulvic acid fraction with increasing humification. The *abiotic condensation* model, including the polyphenol theory, postulates that all plant polymers depolymerize into their monomeric units, followed by repolymerization of (mainly) phenols and quinones into humic substances (Hedges, 1988; Stevenson, 1994). In terms of classical humus fractions, the humification degree is the opposite of the polymer degradation model: humus is formed from fulvic acids via the humic acids into the 'hard core' humus, humin.

In favour of the latter theory is the fast polymerization of quinones under environmental conditions (Hedges, 1988). Against this theory, is the short life-time of small biomolecules in the presence of microbes. A strong point for the biopolymer degradation model is the discovery of the non-hydrolyzable aliphatic polymers present in plant cuticles (Nip *et al.*, 1986a, b; Tegelaar *et al.*, 1993) and barks (Tegelaar *et al.*, 1995). They appear to be diagenetically and microbially resistant and are selectively preserved with hardly or no alteration (Nip *et al.*, 1986a, b; Tegelaar, 1989b). Furthermore, the general trend in which aliphatic compounds residually accumulate with respect to e.g. lignin and polysaccharides implies a selective preservation mechanism rather than polymerization by condensation reactions. However, most likely both processes occur simultaneously (Hatcher and Spiker, 1988). A model of Kögel-Knabner (1992) based on recent discoveries and developments includes a combination of direct transformation, microbial resynthesis and selective preservation processes.

The main problem of organic matter research until the early '80s was lack of appropriate fractionation and analytical techniques. Most studies were based only on extractable humus (humic and fulvic acids) and investigated with some gross physical and chemical methods such as UV (E4/E6 ratio), titrations (functional groups), elemental analysis and IR (Schnitzer and Khan, 1972; Flaig *et al.*, 1975). Recently developed fractionations based on physical

separations and/or sequential chemical treatments together with advanced analytical methods including solid state Nuclear Magnetic Resonance (NMR), pyrolysis-MS and -GC(/MS), and chemical degradation methods (CuO oxidation, basic and acidic hydrolysis etc.) has lead to new insights in the composition and formation of organic matter. For example, ^{13}C NMR and pyrolysis techniques have shown that aromaticity in organic matter has been overestimated in the past, while the role of aliphatics appears to be much more important than recognized previously (Hatcher *et al.*, 1981a, b; Kögel-Knabner *et al.*, 1988; Kögel-Knabner and Hatcher, 1989; Tegelaar *et al.*, 1989a; Kögel-Knabner *et al.*, 1992a, b; Hempfling *et al.*, 1987; Hempfling and Schulten, 1989; Krosshavn *et al.*, 1992a, b; Amaltifano *et al.*, 1995; Zech *et al.*, 1987; Nip *et al.*, 1986a, b; Beyer, 1996; Baldock *et al.*, 1997).

PARENT MATERIALS OF SOIL ORGANIC MATTER

In addition to its classical morphological feature, plants species have particular chemical characteristics. From a chemical point of view, biodiversity is reflected by unique combinations of certain biopolymers and smaller molecules. Because fresh plant material is the main source of organic matter input, a brief review of most important biomacromolecules is in order. Knowledge of the initial composition is necessary to understand the changes at the molecular level of organic matter. Additionally, some specific compounds, biomarkers, may serve as molecular tracers of the influence of particular species. The main components of plants are polysaccharides, proteins, (poly)phenols, lignin, lipids, hydrolyzable (cutin, suberin) and non-hydrolyzable aliphatic polymers (cutan, suberan).

Polysaccharides

About 70-75% of the dry weight of plants originates from polysaccharides (Higuchi, 1997). Polysaccharides function either as storage material, thereby serving as carbon and energy supplier, or as structural component (de Leeuw and Largeau, 1993). Around 50% of wood consist of cellulose, which is also the main constituent in other plant tissue types. Alkali-soluble polysaccharides, a mixture of hetero- and homo-polysaccharides, are defined as 'hemicellulose' (Cheshire, 1979). Both cellulose and hemicellulose are structural carbohydrates. Other well-known polysaccharides such as starch serve as energy supply for the plant. Animals and microorganisms also contain specific polysaccharides. In soil organic matter, polysaccharides from many sources occur together.

Glucose is the most abundant building block of polysaccharides. It is present in e.g. cellulose, starch and glycogen. Other important monosaccharides are mannose, galactose, arabinose, xylose, ribose, fucose, rhamnose and fructose. Apart from these neutral sugars, sugar alcohols, sugar acids and amino sugars occur in nature too, but in much lower amounts.

All important monosaccharides may be present in plants as well as in microorganisms (Cheshire, 1979). Several studies established that high amounts of galactose, mannose, ribose, fucose and rhamnose were due to microbes, whereas relatively high abundances of xylose and arabinose were indicative for a plant origin (Cheshire, 1979; Murayama, 1984; Oades, 1984;

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Moers *et al.*, 1990). Oades (1984) suggested that a ratio of mannose+galactose (hexoses or C_6) over arabinose+xylose (pentoses or C_6) > 2 is indicative of microbial sugars, whereas a ratio of < 0.5 points to an important direct contribution of plant material. In addition, Murayama (1984) used the ratio of rhamnose+fucose/arabinose+xylose to discriminate between microbial sugars ('high' ratio) and plant-derived sugars ('low' ratio). Furthermore, Cowie and Hedges (1984) reported high amounts of xylose and low concentrations of mannose in angiosperms, while gymnosperms exhibited the opposite trend.

Proteins

Proteins, polymers composed of α-amino acids, generally are build from a set of 20 amino acids. Because proteins have various functions and appearances in nature, such as enzymes, transporters and regulators, they occur in many different shapes. Similar to polysaccharides, they are easily depolymerized. Because of their relatively low concentrations in plants, proteins are not considered an important source of organic matter. On the other hand, the polyphenol theory postulates that nitrogen compounds condense with phenolic or quinonic groups yielding 'humic substances' consisting of high molecular weight, amorphous, partly aromatic components (Kelly and Stevenson, 1997).

(Poly)phenols

A suite of phenolic compounds, of low and high molecular weight, can be present in plants. Low molecular weight phenols include a.o. catechol, stilbenes, quinones and flavonoids (Harborne, 1997). Polyphenols comprise mainly lignin, hydrolyzable tannins and condensed tannins. Mosses lack lignin, but do contain tannins. Hydrolyzable tannins consist of a polyhydroxy core esterified with a large number of (mainly) gallic acid (3,4,5-trihydroxybenzoic acid) units. Condensed tannins are composed of polyhydroxy flavan-3-ol units (de Leeuw and Largeau, 1993). Phenolic constituents fulfill a variety of roles in living plants (Harborne, 1997). Some of them are responsible for colour thereby protecting the plant against ultraviolet radiation. Especially the tannins have antiherbivory and antimicrobial properties through interactions with proteins (de Leeuw and Largeau, 1993; Harborne, 1997). Furthermore, tannins may have a strengthening function in plants devoid of lignin, such as mosses (Wilson *et al.*, 1989). It is speculated that tannins have a relatively resistance against decomposition, and thus may have an important contribution in organic matter formation (Wilson and Hatcher, 1988; de Leeuw and Largeau, 1993).

Lignin

After carbohydrates, lignin is the most abundant component in plants. It makes up 20-30% of the total dry matter in wood (Higuchi, 1997), and about 10-15% in other tissues. It occurs in the cell walls of vascular plants, but not in mosses, algae, and microorganisms (Higuchi,

1997) and serves as a biogeochemical tracer for vascular plants in terrestrial and marine environments. Lignin is the result of a combination of three monomers, coniferyl alcohol, synapyl alcohol and coumaryl alcohol. It occurs exclusively in cell walls, where it has a structural and protective function, providing rigidity to plants and trees. Gymnosperm lignin almost exclusively consists of polymerized coniferyl alcohol moieties, while angiosperms have both coniferyl alcohol and synapyl alcohol units in approximately equal amounts, whereas grass-lignin is composed of all three monomers. Due to the biosynthesis of lignin by coupling of oxidation-induced radicals, several stable carbon-carbon and ether bonds are present, giving lignin a heterogeneous, complex nature. Therefore, it is almost impossible to isolate the pure polymer (e.g. van der Hage, 1995).

Lipids

Lipids are defined as those compounds that are soluble in apolar organic solvents (Solomons, 1984). In contrast to other compounds or polymers, there is no structural uniformity within this group of compounds, although they all have a highly aliphatic character. Lipids may be composed of alkanes, alcohols, ketones, fatty acids, terpenoids and sterols, solely or in combinations, as in waxes, triglycerides and phospholipids. Waxes protect plants against drying out, triglycerides may serve as storage material, and phospholipids are a main constituent in cell membranes. Lipids are generally easily decomposable, and are therefore not considered to be a main organic matter component (de Leeuw and Largeau, 1993).

Aliphatic biopolyesters (cutin, suberin)

Two major types of biopolyesters can be present in plants. Cutin, present in the plant cuticle, serves as the barrier between the aerial parts of higher plants, where no secondary thickening occurs, and their environment (Kolattukudy, 1980). Suberin serves as a barrier against unfavourable external influences in roots and in physically damaged surfaces. Cutin is composed of ω-hydroxy C_{16} and C_{18} fatty acids with epoxy and hydroxy mid-chain funcionalities (Kolattukudy, 1980; Kolattukudy and Espelie, 1985; de Leeuw and Largeau, 1993). These monomeric units are also present in suberin, accompanied by ω-hydroxy fatty acids, α,ω-dicarboxylic acids, fatty acids and 1-alcohols with chain lengths of $\geq C_{20}$ (Kolattukudy, 1980; Holloway, 1983). Phenolic acids, such as *p*-coumaric acid and ferulic acid, may be attached to both polymers.

Non-hydrolyzable aliphatic polymers (cutan, suberan)

Quite recently, aliphatic biopolymers have been identified as important constituents of algae, bacteria, plant cuticles, barks and sporopollenin (McKinney *et al.*, 1996, and references cited therein). The chemical composition of these polymers is poorly understood. Cutan and

suberan produce homologous series of *n*-alkenes, *n*-alk-1-enes and *n*-α,ω-alkadienes upon pyrolysis (Nip *et al.*, 1986a, b; Tegelaar *et al.*, 1995). TMAH thermochemolysis yields mainly a homologous series of fatty acid methyl esters and methyl ethers and esters of phenols (McKinney *et al.*, 1996). A proposed tentative structure consists of a core of two phloroglucinol units linked together, which are connected to very long fatty acids through ester bonds (McKinney *et al.*, 1996). Although their concentrations in fresh plant tissues are low, cutan and suberan become more important with increasing humification due to their very high selective preservation potential (Tegelaar, 1989b; de Leeuw and Largeau, 1993; Saiz-Jimenez, 1996).

ANALYTICAL METHODOLOGY

^{13}C NMR

About 50% of all isotopes have a nucleus with a magnetic moment. In a strong magnetic field such nuclei will orient with respect to that field. When simultaneously irradiated by electromagnetic energy, the nuclei may adsorb energy through the process known as magnetic resonance. The energy required to resonate is different for each isotope. In principle each single, for example ^{13}C, isotope will have equal resonance energy. However, the atoms connected to a particular ^{13}C determine the electron density around that ^{13}C, thus the amount of resonance energy associated with that particular atom. NMR makes use of such small differences in resonance energy to analyse the different types of functional groups in a given sample.

Because only about 1% of all C atoms is a ^{13}C isotope, ^{13}C NMR is intrinsically insensitive and requires a lot of (expensive) instrument time to produce a spectrum. To overcome this problem, a method called Cross Polarization Magic Angle Spinning (CPMAS) is used to speed up the measurements. The main disadvantage of this trick is that spectra are only semi-quantitative. Despite that, NMR spectra provide a good overview of the abundance of different C types.

Chemical degradation

To obtain specific information about the composition of a given type of compound, the polymeric molecules have to be depolymerized into the monomers that can be analyzed and possibly quantified. In particular, easily hydrolyzable, chemically labile polymers such as carbohydrates, proteins, cutin and suberin can be analyzed this way.

In several chapters of this thesis, polysaccharides have been analyzed to gather information about the origin, occurrence and fate of the polysaccharide fraction. Hydrolysis under acidic conditions is a relatively simple chemical reaction. Problems, however, are the different degrees of stability of the glycoside bonds and their rates of hydrolysis. A sufficiently long reaction time or very strong acidic conditions promote complete hydrolysis. Too drastic conditions, however, may also cause partial degradation of the released monosaccharides, and

consequently bias the polysaccharide picture. According to Amelung *et al.* (1996) hydrolysis of polysaccharides with 4 *M* of trifluoroacetic acid is recommended in favour to other procedures because it has the highest yields of non-cellulosic monosaccharides, whereas cellulose is hardly affected by this agent.

After hydrolysis, the sugars have to be derivatized prior to analysis by gas chromatography. One reason is the high polarity of the sugar monomers and the consequent bad separation on the GC column. A second reason is the different appearances of the monomers in aquatic (reaction) environments, also known as mutarotation. A given C_6 or C_5 monosaccharide has five principally different structures: the open chain form, two furanose and two pyranose structures (both include an α- and β-anomer), and hence also five compounds representing only one specific sugar upon analysis. Together with the fact that monosaccharides with equal molecular mass (the hexose or pentose families) have almost equal retention times, separation and identification of the various sugar moieties is complicated, if not impossible. To reduce the number of structures, the monosaccharides are oximized resulting into two isomers. The equilibrium between those two isomers is considered to be constant, implying that only one monomer, or peak in case of chromatography, is necessary to detect, identify and quantify the sugar (Andrews, 1989). Furthermore, the polar hydroxyl groups are silylated to obtain less polar compounds, thereby enhancing resolution and separation upon gas chromatography.

Pyrolysis

Complex, heterogeneous high molecular weight compounds are rarely analysed at the molecular scale in their polymeric form. They are usually investigated after splitting the macromolecules into small pieces of up to a few hundred Daltons, which can be subsequently analysed. For example, polysaccharides and proteins can be gently hydrolysed into monosaccharides and amino acids, respectively, and further analysed by a GC, as mentioned in the previous section. However, polymers, such as lignin or 'humic substances' resist such gentle depolymerization steps, and cannot be analysed likewise.

Pyrolysis offers an alternative to chemical depolymerization. Upon pyrolysis, a given sample is rapidly heated in an inert environment, and due to the large amount of energy received, any macromolecules present will be fragmented.

Principally, two pyrolysis techniques can be distinguished. The first is temperature-resolved, in which a sample is heated at a certain rate to a final temperature. The pyrolysis products are evaporated and immediately brought into a MS so that no material is lost. A disadvantage is the low resolution: different compounds with equal mass fragment(s) cannot be separated. The second method involves the use of metal wire with a given Curie point temperature. The sample is 'immediately' heated to the Curie temperature and maintained there for several seconds. The pyrolysis products are led into and separated on a GC(/MS). Contrary to temperature-resolved pyrolysis, all compounds are separated and can be identified. However, polar and oligomeric compounds may condense in the glass liner of the GC, and never reach the column. Flash pyrolysis of polymers does not give the monomers solely, but produces a large number of other products due to numerous cleavages,

dehydrations and rearrangements. For example, pure cellulose, consisting of glucose units connected through only one bond type (β-1→4) gives upon pyrolysis 96 GC amenable products, such as furans, pyrans, anhydrosugars, carbonyl compounds, acids and methyl esters (Pouwels *et al.*, 1989).

To improve the amount of GC amenable compounds, *in situ* methylation using tetramethylammonium hydroxide (TMAH) has been applied. Because the mechanisms are not only thermolytic, but also chemolytic, the procedure is specified as thermochemolysis. Other compounds rather than strictly methylated pyrolysis fragments are produced (de Leeuw and Baas, 1993). E.g. pyrolysis of the aliphatic biopolymer cutan gives alkadiene/alkene/alkane triplets, whereas methyl esters of fatty acids are generated using TMAH (McKinney *et al.*, 1996).

SCOPE AND OUTLINE OF THIS THESIS

As mentioned earlier, organic matter research on the wind-blown sands of the Leuvenum Forest has several advantages:

- the full vegetation sequence is known
- it is a primary succession, without antecedent soil formation
- the forest ecosystem is relatively simple, with few species
- organic matter build-up can be monitored from the bare sand to the 200 years old forest through the presence of intermediate stages
- initial podzolization stages may provide better insights into the mechanism of podzolization

The following items are focal points to increase the knowledge on the molecular level of organic matter behaviour as a function of vegetation (succession):

- characterizing the chemical composition of the plant tissues of the present vegetation
- elucidating the effects of vegetation on chemical composition of organic matter
- investigating the contribution of roots to soil organic matter
- figuring out what happens to water soluble organic matter in the soil
- evaluating the temporal and vertical transformations of organic matter

Chapters 2 and 3 describe the organic matter development during the first stages of vegetation succession at the wind-blown sand area of the Hulshorster Sand. Relations between vegetative above- and below-ground input and the organic matter at and in the soil are considered. Furthermore, a comparison is made between the composition of water soluble organic matter and the bulk organic matter to trace possible relationships. In Chapter 4, H horizons of three forest profiles with different vegetation and stages of podzolization have been fractionated and characterized to find differences in composition associated with the covering species. With support of pollen analysis, the characteristics are discussed in terms of humification and vegetative influences. Chapter 5 considers the influence of vegetation on the chemical composition of the ectorganic layers, and shows the importance of root litter as

organic matter source. Chapter 6 treats the water soluble organic matter from L, F and H horizons as illuviated matter and is related to the accumulated organic matter in B horizons and humus fibers. Chapter 7 deals with the insoluble organic matter fractions in B horizons of five profiles with different vegetative coverings. To elucidate the origin of this insoluble material, the samples are further fractionated and analysed. In Chapter 8, the contribution of root material to soil organic matter and the possible origin of aliphatic compounds in particular is proven by comparing the chemical composition of roots with that of the mineral soil. Finally, Chapter 9 synthesizes all previous chapters and comprises the total organic matter development along the vegetation succession at the Hulshorster Sand and the Leuvenum Forest.

Chapter 2

Organic matter composition
of plant tissues and soil
under young vegetation on drift sand

Klaas G.J. Nierop, Barend van Lagen and Peter Buurman

ABSTRACT

Differences in organic matter composition in consecutive soil profiles (under algae, grass, moss, heather and pine) from a primary vegetation succession (central Netherlands) were investigated by CPMAS ^{13}C NMR, chemical degradation (acid hydrolysis of polysaccharides) and pyrolysis-GC/MS in relation to the vegetation. All mineral soil horizons had lower contents of polysaccharides than the ectorganic layer and/or the fresh plant material as suggested by the diminishing O-alkyl peaks by NMR. The monosaccharide distribution pointed to an increase of microbial sugars in the soil, accompanied by a significant contribution of carbohydrates from mosses in the first stages. Fresh litter and ectorganic layers under heather and pine had similar contents of aromatics, but the contribution of lignin (guaiacols and syringols) was lower in the ectorganic horizons. The degree of oxidation, as determined by the acid/aldehyde ratio, increased with progressive humification. All Ah horizons contained less aromatics, more oxidized lignin remnants and higher amounts of aliphatic compounds than the litter. One or two macromolecules, that produced n-alkenes/n-alkanes and branched alkenes/alkanes upon pyrolysis and were derived from mosses, were mainly responsible for the accumulation of aliphatics in soil, although other aliphatic polymers, such as suberin, might also play an important role.

INTRODUCTION

Soil organic matter plays an important role in biological processes and influences soil properties. Decomposition and humification is determined by many factors, such as temperature, moisture, oxygen, litter quality and nutrient status of the soil (Stevenson, 1994). Although the importance of vegetation and its organic matter for soil development processes is generally recognized, detailed information about the (organic) compounds responsible for those processes is scarce.

Understanding of organic matter development is usually hindered by the complexity of (most) soil systems, caused by e.g. bioturbation, human disturbance and complex or unknown history. Therefore, causal relations between vegetation and organic matter formation are difficult to unravel. Primary vegetation successions provide a good opportunity to study interactions between vegetation, organic matter and soil formation, because antecedent soil development is lacking. The Hulshorster Sand and Leuvenum Forest (central Netherlands) is a wind-blown sand area, which is now covered by such a primary vegetation succession. These poor sandy soils have unfavorable decomposition conditions, leading to appreciable accumulation of organic matter in the surface layer, and, eventually, podzolization. The primary vegetation succession of the area has been studied extensively (Fanta, 1986; Prach, 1989) and using this vegetation sequence, it may be possible to follow the organic matter build-up in relation to vegetation and podzolization. Figure 1 shows the simplified vegetation sequence of the Hulshorster Sand and the Leuvenum Forest. The natural succession starts with algae (profile 0), followed by grass (profile 1) and mosses (profile 2). Mosses are locally succeeded by heather (profile 3), but ultimately all vegetation are replaced by pine trees

(profile 4). Initial podzolization appears under *Pinus + Deschampsia*, and continues in the next stages.
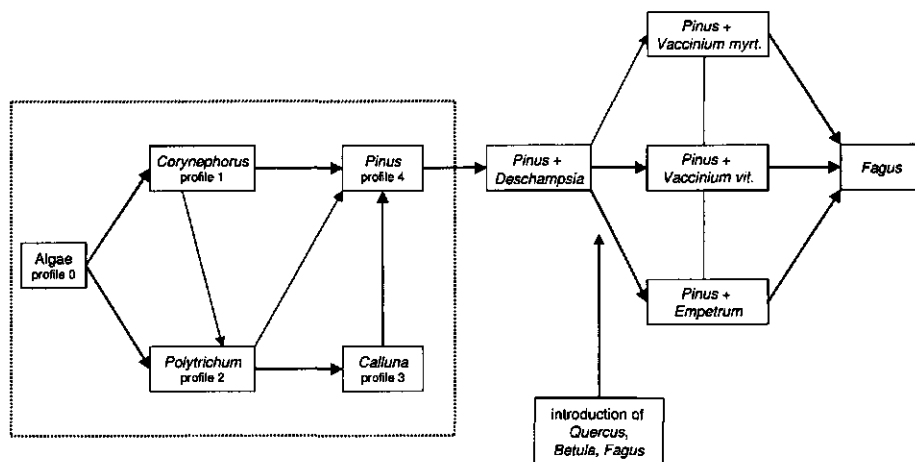
Figure 1. Vegetation sequence of the Leuvenum Forest. The profiles in the dashed box are subject of this study.

In this paper, an inventory is made of the organic matter at the molecular level in the pre-podzol stages (profiles 0-4) in order to relate the composition to the vegetation succession. Besides above-ground litter, also the below-ground litter (roots) is analyzed to trace the origin of the organic matter in the soil. Complementary chemical analyses, which are used for this study, include Cross Polarization-Magic Angle Spinning (CPMAS) ^{13}C NMR, monosaccharide determination after acid hydrolysis, and pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS).

MATERIALS AND METHODS

Vegetation, soil profiles

The following soil profiles represent the most important vegetation in the first stages of vegetation development on wind-blown sand. Profile 0 consists only of a thin mat of algae (0.5 cm). Profile 1, under sparse *Corynephorus canescens* (L.) Beauv. (grass), has an Ah horizon (2 cm) with a few fine roots and abundant moss leaves and algae remnants, which abruptly overlies a C horizon. Profile 2, under *Polytrichum piliferum* Hedw. (moss), has an Ah1 horizon (0.5 cm) with moss rhizoids, and an Ah2 horizon (2-3 cm) without rhizoids. These two horizons were sampled as one layer. Profile 3, covered by *Calluna vulgaris* (L.) Hull with some *Polytrichum*,

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has an F layer (1 cm) of which the upper part consists of *Calluna* litter and the lower part of *Polytrichum* and other moss remnants, overlying an Ah horizon (6 cm) with abundant fine roots. Profile 4, under a solitary *Pinus sylvestris* L., has an L (2 cm) and an F (4 cm) layer, both dominated by pine needles and twigs, and an Ah horizon (10 cm) with abundant fine roots and a few remnants of needles, mosses and grasses. Table 1 summarizes the C and N contents, the C/N ratios and pH values for all profiles.

Table 1. C and N mass fractions, C/N mass ratios and pH values of profiles 0-4.

Profile	Vegetation	Horizon	C (%)	N (%)	C/N	pH (H$_2$O)	pH (KCl)
0	Algae	A	1.04	0.07	14.4	4.8	4.2
1	Grass	A	0.43	0.04	10.0	5.0	4.3
		C	0.07	0.01	-	4.5	4.5
2	Moss	A	0.39	0.03	13.0	4.2	3.8
		C	0.07	0.01	-	4.4	4.6
3	Heather	F	33.80	1.29	26.2	nd	nd
		A	1.18	0.06	19.7	4.2	3.7
		C	0.07	0.01	-	4.6	4.7
4	Pine	L	52.13	1.55	33.6	nd	nd
		F	26.26	1.00	26.3	nd	nd
		A	2.17	0.12	18.1	4.0	3.2
		C	0.10	0.01	-	4.1	4.6

nd = not determined

Sample pretreatment

- Bulk samples
Fresh plant materials (leaves, needles, flowers, woody stems, roots) and organic horizons (L, F) were analysed after freeze-drying and ball milling. After freeze-drying and sieving (2 mm), the Ah horizons were shaken for 5 minutes in a 0.5 *M* NaOH solution (sample:solution ratio 1:2 (w/w)). After decantation of the solution including soluble and insoluble organic matter, the residue (sand) was washed with water until all organic matter was separated from the sand (colour of water almost clear). The combined 'solutions', containing both soluble and insoluble organic matter, was shaken with HCl/HF (ratio 1:3) at pH 1-2 for 24 h., dialysed and freeze dried. Less than 5% of the initial organic matter was lost during dialysis. The residual sand contained virtually no organic C according to C analysis.

- Selective enrichment of aliphatic biopolymers
To obtain specific information about the aliphatic biopolymers, sequential enrichment steps were performed to yield a chemically resistant residue (Tegelaar *et al.*, 1995; van Heemst *et al.*, 1996). Freeze-dried and ball-milled fresh tissues of mosses (*Polytrichum* and *Bryum* sp.) were extracted for 48 h. with CH_2Cl_2/methanol (1:1) in a Soxhlet apparatus to remove extractable lipids. After drying, the residue was saponified with 1% KOH in methanol for 2 h. under reflux. The obtained residue was acidified with HCl, centrifuged, washed with water (twice), water/methanol, methanol (twice) and CH_2Cl_2 (3 times) and dried. To remove polysaccharides, the sample was then subjected to acid hydrolysis by standing in concentrated sulphuric acid for 2 h., followed by dilution to 1 M with water and subsequent refluxing for 5 h. After centrifugation, the residue was washed with water until pH 6-7 and finally freeze-dried.

Chemical analysis

- C, N and pH
Carbon and nitrogen were measured with an Interscience Elemental Analyser EA1108; pH (H_2O and 1 M KCl) in a sample:solution ratio of 1:5.

- ^{13}C NMR
Cross Polarization-Magic Angle Spinning (CPMAS) was used for the NMR measurements, and performed on a Bruker AMX 400 operating at 100.628 MHz. The used parameters were: a spinning rate of 4.5 KHz; an acquisition time of 0.033 s; a pulse time of 4 μs; a contact time of 0.8 ms and a line broadening of 50 Hz.

- Monosaccharides
Determination of the monosaccharide composition of the non-cellulosic polysaccharides was performed by the method of Amelung *et al.* (1996). Duplicates of 10-100 mg, depending on the C content, were hydrolyzed with 4 M trifluoroacetic acid (TFA) at 105°C for 4 h. The hydrolysate was purified using XAD-7 and Dowex 50 W X8. Monosaccharides were oximized with *O*-methylhydroxylammonium hydrochloride in *N*-methylpyrrolidone (NMP) at 75°C for 1 h. and further derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 75°C for 5 min (Andrews, 1989). Separation of the reaction products was performed on a CE gas chromatograph with a J&W DB-5 column (30 m, film thickness 1.00 μm, i.d. 0.25 mm) connected to a Fisons MD 800 mass spectrometer (mass range *m/z* 50-450, cycle time 0.50 s). After 4 min at 160°C, the GC temperature was raised at a rate of 8°C/min to 180°C (2 min), after which it was further raised by 1°C/min to 195°C (2 min), and finally increased to 255°C at 10°C/min and maintained there for 5 min. Quantification was carried out using *myo*-inositol as internal tracer and *O*-methylglucose as internal standard. The standard error did not exceed 10% for individual sugars, except for sugars in ver low concentrations (less than 0.5 mg/g C), which may have errors up to 50%.

- Pyrolysis-GC/MS

Pyrolysis was carried out on a Horizon Intruments Curie-Point pyrolyser. Samples were heated for 5 s at 610°C. The pyrolysis unit was connected to a CE gas chromatograph and the products were separated on a fused silica column (Chrompack, 25 m, 0.25 mm i.d.) coated with CP-Sil 5 (film thickness 0.40 μm), with helium as carrier gas. The initial oven temperature was 40°C. After that it was heated at a rate of 7°C/min to 320°C and held for 20 min. The end of the GC column was coupled to a Fisons MD 800 mass spectrometer (mass range m/z 45-650, cycle time 1 s). Pyrolysis-methylation was performed by adding one or two drops of a solution of tetramethylammonium hydroxide in water (25%) onto the sample, followed by drying for 1-2 minutes, and immediate pyrolysis.

RESULTS

^{13}C NMR

Peak assignments of Cross Polarization-Magic Angle Spinning (CPMAS) ^{13}C NMR spectra are performed with data from literature (e.g. Hatcher, 1987; Kögel *et al.*, 1988; Kögel-Knabner, 1992; Kögel-Knabner, 1997). Chemical shifts around 62, 72 and 103 ppm, characteristic for polysaccharides, dominate the spectra of all profiles, but are particularly strong in samples from algae (Figure 2) and from profiles 1 (not shown) and 2 (Figure 3).

In the alkyl C range (0-50 ppm), the signal at 21 ppm is associated to methyl carbons, whereas resonances at 29 and 32 ppm are typical for methylene carbons. The major source of these carbons are lipids and aliphatic biopolymers (Tegelaar *et al.*, 1989a; Kögel-Knabner *et al.*, 1992a, b). In the spectra of the Ah horizons under grass and moss, the intensity of alkyl C is higher with respect to the fresh plant material. The algae have virtually no aromatic moieties (see Figure 2). Small but distinct peaks are observed in the aromatic region of the other spectra. In profile 1 (not shown), the grasses have signals at 119, 128 and 148 ppm, and together with the shoulder at 56 ppm (methoxy), they are lignin-derived carbons. In mosses the aromatic carbons (e.g. 117 and 143 ppm) are derived from tannins. In the aromatic part of profiles 3 and 4, the ectorganic horizons under pine have one broad signal at 148 ppm, whereas under heather two peaks can be distinguished at 144 and 153 ppm. This difference is due to the composition of lignin: lignin of pine is mainly composed of coniferyl alcohol, while heather lignin also contains synapyl alcohol. In addition, tannins may also resonate in the aromatic region. In the Ah horizons of profiles 1 and 2, the aromatic signals almost disappear. A similar trend is observed in profiles 3 and 4. Whereas the spectra of the roots have considerable signals in the aromatic region, the Ah horizons have much lower amounts of aryl carbons (see for profile 3, Figure 4). The high signal at 128 ppm is a spinning side band from the carbonyl peak at 172 ppm, just like the one at 217 ppm. In the spectrum of the moss leaves, a clear signal at 206 ppm is observed, and may be due to amides or ketones.
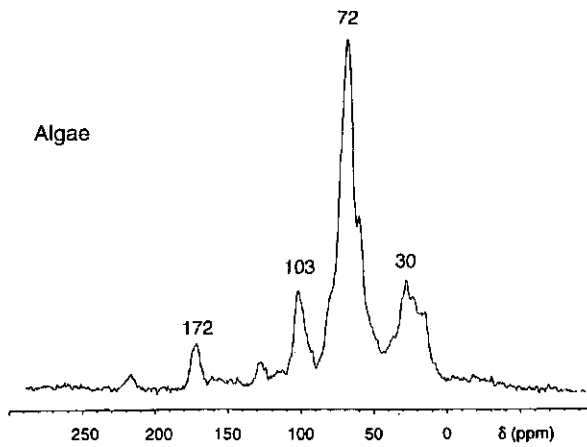
Figure 2. ^{13}C-NMR spectrum of algae (profile 0).
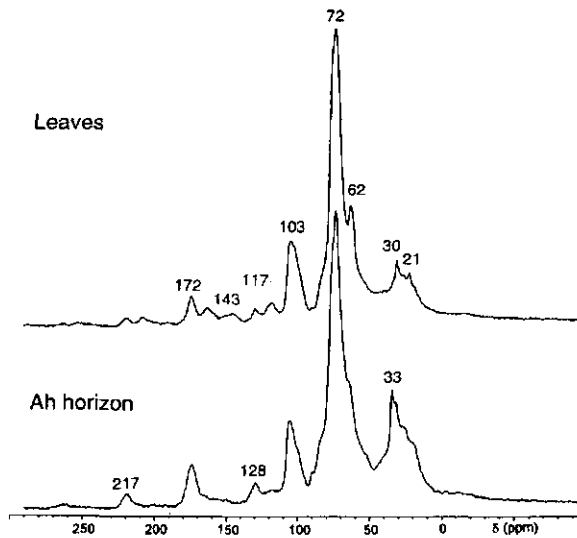

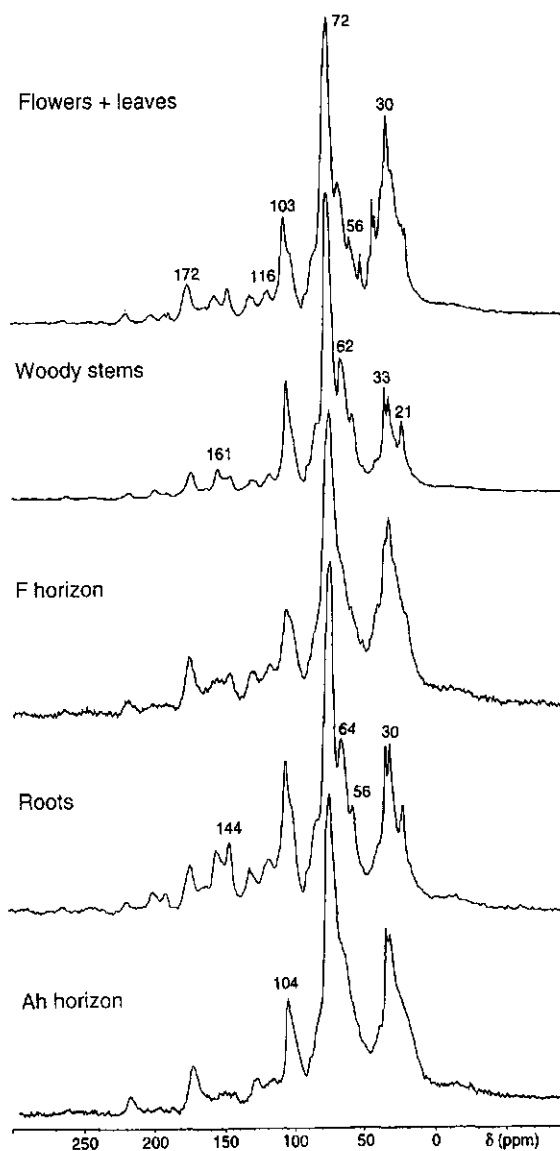**Figure 3.** ^{13}C-NMR spectrum of moss vegetation and Ah horizon under moss (profile 2).

Figure 4. ^{13}C-NMR spectrum of flowers + leaves, woody stems of *Calluna*, F horizon, *Calluna* roots and the Ah horizon (profile 3).

Table 2. Composition of non-cellulose polysaccharides of litter input and horizons.

Sample	xylose	arabinose	ribose	rhamnose	fucose	fructose	mannose	galactose	glucose	total	C6/C5	Deoxy/C5
Profile 0	Algae											
Mat	126.1	110.9	1.5	48.8	51.9	14.1	22.1	237.2	44.7	655.7	1.09	0.42
Profile 1	Grass											
Leaves	307.0	82.2	1.9	7.1	4.3	3.3	5.2	38.6	37.8	485.4	0.11	0.03
Roots	290.7	104.2	2.5	12.1	11.3	3.4	24.2	93.4	57.0	598.8	0.30	0.06
Ah	100.8	74.8	2.8	42.5	46.1	2.6	44.4	155.1	44.0	513.1	1.14	0.50
Profile 2	Moss											
Leaves	22.8	32.1	1.0	7.3	4.6	4.8	55.4	89.9	23.6	241.5	2.65	0.22
Ah	35.5	40.8	1.0	17.2	10.3	2.4	37.5	101.8	41.6	288.2	1.82	0.36
Profile 3	Heather											
Flo+lea	81.5	54.8	1.5	7.3	1.5	13.4	2.4	47.0	36.3	244.3	0.36	0.06
Wood	201.0	49.2	2.2	7.7	1.8	12.1	6.9	46.7	59.7	385.1	0.22	0.04
F	40.7	41.1	2.0	11.2	7.3	0.2	21.4	71.4	36.7	230.0	1.13	0.23
Roots	142.3	35.2	1.1	11.5	4.8	0.5	17.2	48.7	36.8	298.0	0.37	0.09
Ah	33.6	28.4	1.8	16.7	8.0	0.9	21.3	64.2	35.8	208.9	1.38	0.40
Profile 4	Pine											
Needles	23.6	56.0	0.0	7.1	4.4	9.0	41.0	49.6	65.9	256.5	1.14	0.14
L	19.3	24.2	0.5	6.2	2.9	0.7	29.5	39.5	31.8	154.6	1.59	0.21
F	18.2	18.4	0.3	5.5	3.0	0.6	19.5	29.4	24.4	119.3	1.33	0.23
Roots	42.0	44.0	0.1	7.7	5.1	1.0	10.8	38.6	35.9	185.1	0.57	0.15
Ah	29.4	25.1	0.3	9.2	6.7	1.3	8.9	32.9	25.4	139.0	0.76	0.29

Amounts (mean, n=2) in mg compound/g C. Ratio C_6/C_5 = (mannose + galactose)/(xylose + arabinose). Ratio Deoxy/C_5 = (rhamnose + fucose)/(xylose + arabinose). Flo+lea = flowers + leaves.

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Monosaccharides

Table 2 presents the most important monosaccharides released from fresh plant materials and the different soil horizons by hydrolysis with 4 M TFA. Hydrolysis with this agent liberates non-cellulosic sugars from soil and has a negligible effect on cellulose (Amelung *et al.*, 1996). The ratios C_6/C_5 (mannose + galactose/xylose + arabinose) and deoxy/C_5 (rhamnose + fucose/xylose + arabinose) are used to discriminate between polysaccharides predominantly derived from plants (low ratios, $C_6/C_5 < 0.5$) and microbial input (high ratios, $C_6/C_5 > 2$) (Oades, 1984; Murayama, 1984; Guggenberger *et al.*, 1994).

The algal mat shows high amounts of the deoxysugars rhamnose and fucose, which are mainly synthesized by microbes (Moers *et al.*, 1990). Under grass, the distribution of the monosaccharides in roots and soil differs substantially. The sugars in the soil have relatively high C_6/C_5 and deoxy/C_5 ratios, suggesting an important microbial contribution.

The monosaccharide distribution in flowers + leaves of heather resembles that of the stem wood, except for a large contribution of xylose, prominently present in wood. *Calluna* roots exhibit a somewhat different polysaccharide composition. The F as well as the Ah horizon is much more abundant in rhamnose, fucose, mannose and galactose; both sugar ratios increase in comparison with the fresh plant materials.

Pine needles contain more sugars hydrolysable with 4 M TFA than pine roots. Especially hexoses are more abundant in the needles, giving a twice as high C_6/C_5 ratio. The F horizon has a composition similar to that of the L horizon, except for lower concentrations of C_6 sugars in the first. In comparison with the fresh needles, a decrease of all monosaccharides is found in the L and F horizons. The main difference between roots and the Ah horizon is the lower quantity of pentoses in the latter.

Pyrolysis-GC/MS

Figures 5-7 show the Py-GC traces of the algae (profile 0), roots from and Ah horizon under *Corynephorus* (profile 1) and leaves, branches, roots and F and Ah horizons of *Calluna* (profile 3). Identified peaks are listed in Table 3.

- Polysaccharides
Typical pyrolysis products of polysaccharides are acids (peaks **1** and **3**), carbonyl compounds (peaks **4, 11, 20** and **21**), furans and reduced furans (peaks **2, 6, 9, 10, 12, 15, 16, 17, 32** and **37**), pyrans (peaks **18, 24, 28, 34** and **43**) and anhydrosugars (peaks **22, 27, 35, 36, 41, 53, 57** and **61**) (Pouwels *et al.*, 1987, 1989; Pouwels and Boon, 1990). Of these, peaks **18, 36** and **41** were found to be characteristic for pentoses, **22** and **24** for deoxysugars and peaks **27, 28, 35, 43, 53, 57** and **61** for hexoses (Pouwels *et al.*, 1989; van Smeerdijk and Boon, 1987). Products attributed to sugars dominate the pyrolysate of the algae (Figure 5). Particularly abundant are peaks **9, 12, 17, 18, 22, 35, 36, 57** and **61**, suggesting that a variety of different polysaccharides is present. All these compounds have also been found in pyrolysates of marine algae (van Heemst *et al.*, 1996). In pyrolysates of the Ah horizons under grass (Figure 6), under moss, and of moss tissue (both not shown), the same compounds are present in a
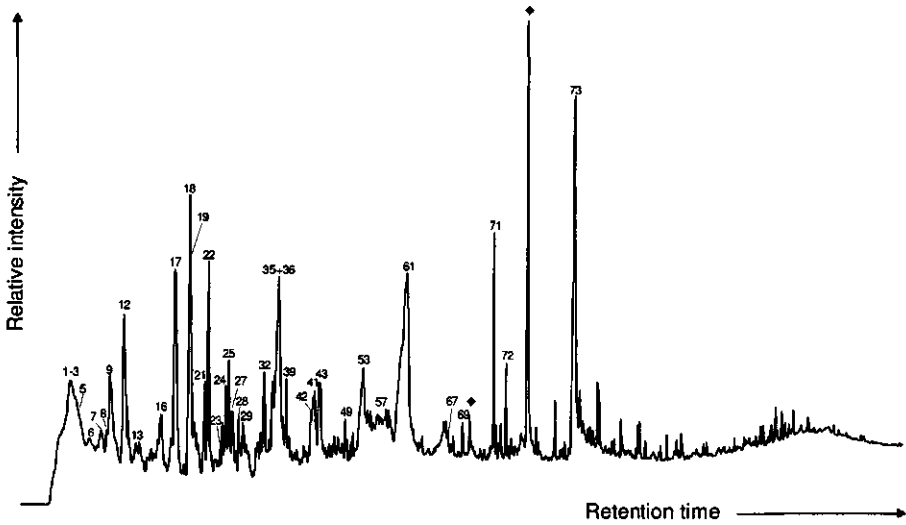
Figure 5. Pyrolysis-GC trace of algae (profile 0).

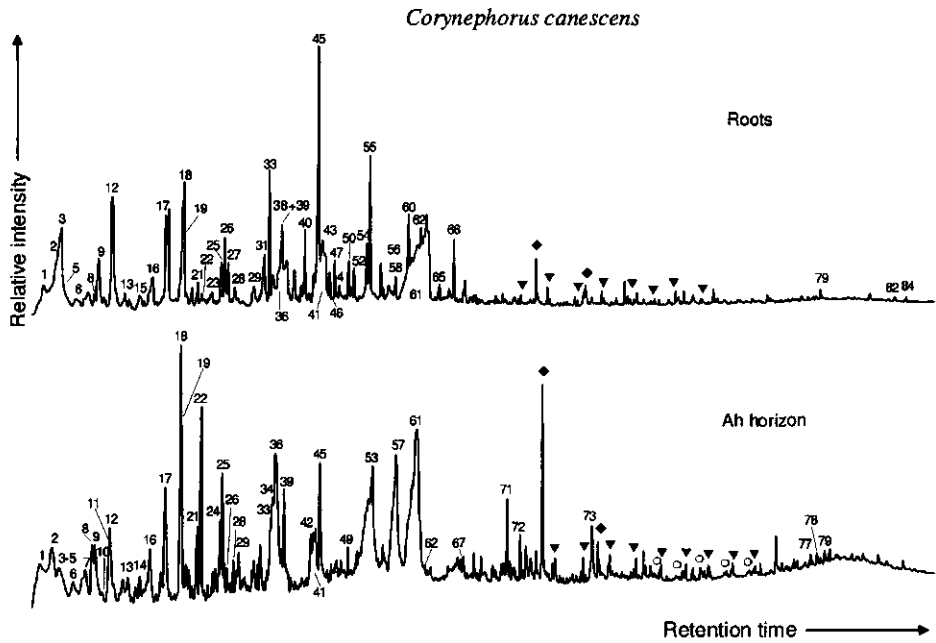*Corynephorus canescens*

Figure 6. Pyrolysis-GC traces of profile 1, roots of *Corynephorus* (a) and Ah horizon (b).
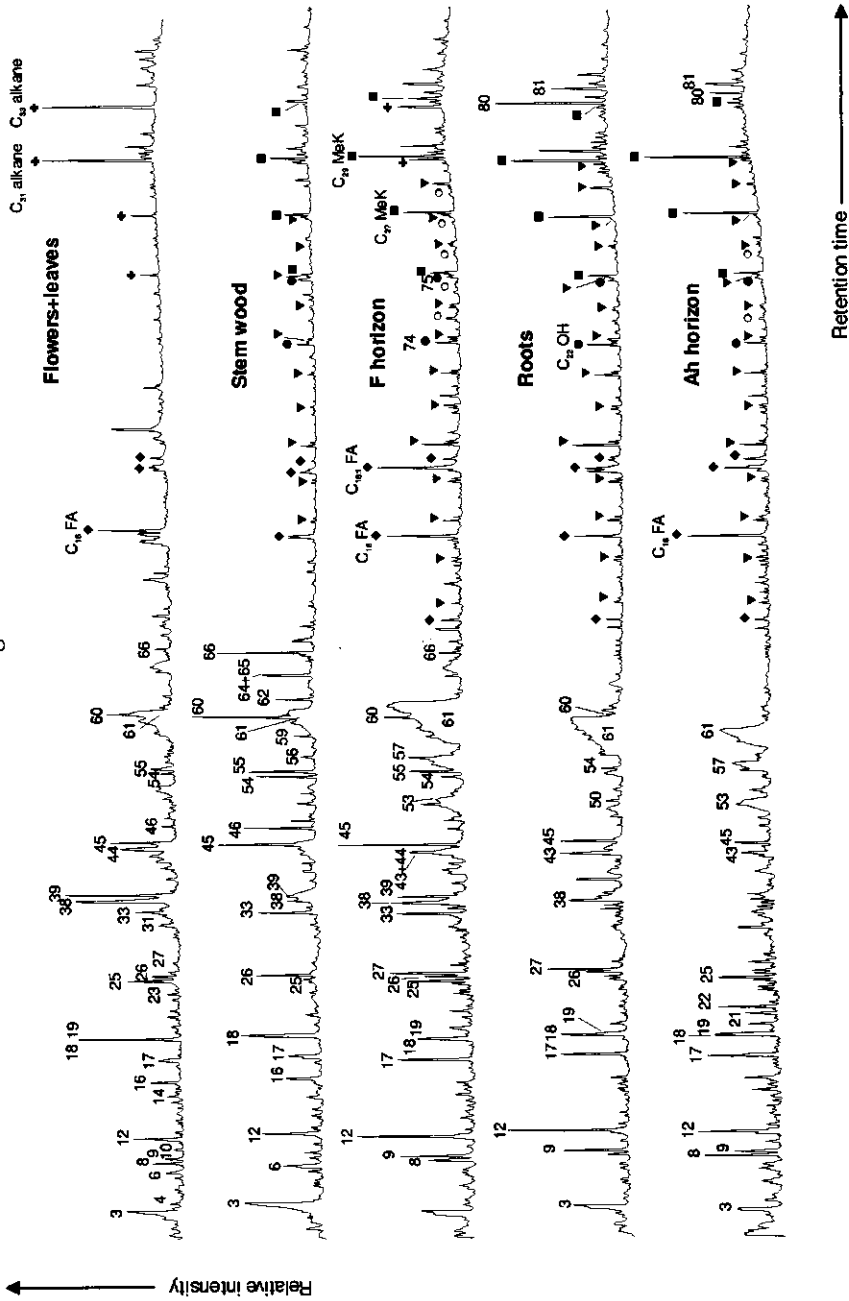
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Figure 7. Pyrolysis-GC traces of profile 3, flowers + leaves, stem wood, F horizon, roots and Ah horizon of or under *Calluna*.

Table 3. List of main pyrolysis products.

No.	Compound	Source	M$^+$
1	Formic acid	Ps	46
2	2-Methylfuran	Ps	82
3	Acetic acid	Ps	60
4	2-Butenal	Ps	70
5	Benzene	Pp	78
6	2,5-Dimethylfuran	Ps	96
7	Pyrrole	Pr	67
8	Toluene	Pp	92
9	(2H)-Furan-3-one	Ps	84
10	3-Furaldehyde	Ps	96
11	2,4-Pentadienal	Ps	82
12	2-Furaldehyde	Ps	96
13	C$_2$-Benzene	Pp	106
14	Vinylbenzene (styrene)	Pp	104
15	2-Acetylfuran	Ps	110
16	2,3-Dihydro-5-methylfuran-2-one	Ps	98
17	5-Methyl-2-furaldehyde	Ps	110
18	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	Ps	114
19	Phenol	Pp/Lg/Pr	94
20	2,3-Dimethylcyclopent-2-en-1-one	Ps	110
21	2-Hydroxy-3-methyl-2-cyclopenten-1-one	Ps	112
22	Dianhydrorhamnose	Ps	128
23	2-Methylphenol	Pp/Lg/Pr	108
24	4-Hydroxy-5-methyl-5,6-dihydro-(2H)-pyran-2-one	Ps	128
25	3/4-Methylphenol	Pp/Lg/Pr	108
26	Guaiacol	Lg	124
27	Levoglucosenone	Ps	126
28	2-Methyl-3-hydroxy-(4H)-pyran-4-one	Ps	126
29	Benzyl cyanide	Pr?	117
30	Dimethylphenols	Pp/Lg	122
31	Ethylphenols	Pp/Lg	122
32	5-Hydroxymethyl-2-tetrahydrofuraldehyde-3-one	Ps	144
33	4-Methylguaiacol	Lg	138
34	3,5-Dihydroxy-2-methyl-(4H)-pyran-4-one	Ps	142
35	1,4:3,6-dianhydro-α-D-glucopyranose	Ps	144
36	Anhydropentose ?	Ps	132
37	5-Hydroxymethyl-2-furaldehyde	Ps	126
38	Dihydroxybenzene (catechol)	Pp/Lg	110
39	4-Vinylphenol	Pp/Lg	120
40	4-Ethylguaiacol	Lg	152
41	Anhydropentose	Ps	132
42	Indole	Pr	117
43	1,4-Dideoxy-D-*glycero*-hex-1-enpyranos-3-ulose	Ps	144
44	4-Methylcatechol	Pp	124

45	4-Vinylguaiacol	Lg	150
46	Syringol	Lg	154
47	4-(1-Propenyl)guaiacol (eugenol)	Lg	164
48	4-Propylguaiacol	Lg	166
49	Methylindole		131
50	4-Formylguaiacol (vanillin)	Lg	152
51	(4?)-Ethylcatechol	Pp	138
52	*cis* 4-(2-Propenyl)guaiacol	Lg	164
53	Anhydrohexose	Ps	162
54	4-Methylsyringol	Lg	168
55	*trans* 4-(2-Propenyl)guaiacol	Lg	164
56	4-Acetylguaiacol	Lg	166
57	Anhydrohexose (mannose ?)	Ps	162
58	4-(Propan-2-one)guaiacol	Lg	180
59	4-Ethylsyringol	Lg	182
60	4-Vinylsyringol	Lg	180
61	Anhydroglucosan (levoglucosan)	Ps	162
62	4-(1-Propenyl)syringol	Lg	194
63	4-Propylsyringol	Lg	196
64	*cis* 4-(2-Propenyl)syringol	Lg	196
65	4-Formylsyringol	Lg	182
66	*trans* 4-(2-Propenyl)syringol	Lg	194
67	Biphenol	Pp	186
68	4-Acetylsyringol	Lg	196
69	1-Pristene		266
70	4-(Propan-2-one)syringol	Lg	210
71	Phytadiene		278
72	Phytadiene		278
73	Oleic acid ($C_{18:1}$ FA)	Lp	254
74	Docosanol (C_{22} OH)	Lp	326
75	Tetracosanol (C_{24} OH)	Lp	354
76	Stigmast-2-ene	Lp	398
77	Stigmasta-2,5-diene	Lp	396
78	Stigmastatriene	Lp	394
79	Stigmasta-3,5-diene	Lp	396
80	Friedoolean-2-ene	Lp	410
81	Friedoolean-14-en-3-one (taraxerone)	Lp	424
82	Friedoolean-14-en-3β-ol (taraxerol)	Lp	426
83	Stigmasta-3,5-dien-7-one	Lp	410
84	Stigma-4-en-3-one	Lp	412
▼	*n*-Alkenes and *n*-alkanes	Lp/Abp	
O	Branched methyl-alkenes and -alkanes	Lp/Abp	
◆	Fatty acids	Lp	
●	Alcohols	Lp	
■	2-Methylketones	Lp	
✚	*n*-Alkanes	Lp	

Legend: Ps: polysaccharides, Pp: polyphenols, Pr: proteins, Lg: lignin, Lp: lipids, Abp: aliphatic biopolymer.

slightly different distribution. Grass roots themselves have a completely different polysaccharide composition than the organic matter in the Ah horizon (Figure 6), thereby confirming the results of chemical degradation (acid hydrolysis of polysaccharides). Under *Calluna* (Figure 7), the relative abundance of polysaccharide pyrolysis products is low in the above-ground plant tissues, and higher in the roots. In pyrolysates of both the F and Ah horizons, sugars seem more intense, and much richer in anhydrosugars attributed to galactose and mannose than those of the fresh plant material. Similar trends are recognized under pine, but less pronounced (not shown).

- Aromatics

Peaks **5, 8, 13, 19, 23, 25** and **39** are the major aromatic compounds identified in the pyrolysate of the algae (Figure 5). Of these, phenol **(19)** and 2- **(23)**, 3- and 4-methylphenols **(25)** have the most intense signals. They may derive from polyphenolic macromolecules, which have also been found in marine algae (van Heemst *et al.*, 1996). However, a protein origin may not be excluded (van Heemst *et al.*, 1998). The same alkylbenzenes and alkylphenols are also the main aromatics present in the pyrolysate of *Polytrichum* tissue. In addition to alkylphenolic compounds, both grass leaves and roots produce methoxyphenols upon pyrolysis. Yet, hardly any methoxyphenols are observed in the pyrolysate of the Ah horizon under grass (Figure 6). Similar low abundances of methoxyphenols are present in the pyrolysates of the Ah horizon under moss.

In the pyrolysate of *Calluna* flowers + leaves (Figure 7), important signals are found of phenol **(19)** and methylphenols **(23, 25)**, and especially of catechol **(38)**, 4-methylcatechol **(44)** and, to a lesser extent, ethylcatechol **(51)**. Pyrolysis of stem wood gives these compounds in low abundance. The wood pyrogram is dominated by guaiacols **(26, 33, 40, 45, 47, 48, 50, 52, 55, 56** and **58)** and syringols **(46, 54, 59, 60, 62-66, 68** and **70)**, typical pyrolysis products of angiosperm lignin (van der Heijden *et al.*, 1994). Compared with the stems, flowers + leaves have syringols in much lower abundance than their guaiacolic counterparts. The F horizon is a mixture of flowers + leaves, wood and mosses. This is reflected in the abundances of phenols, catechols, guaiacols and syringols, being a mixture of those several inputs. The lignin composition of *Calluna* roots differs substantially from that of the above ground parts, in having a low content of syringols. Similarly to flowers + leaves, phenols and catechols are important pyrolysis products of roots. The distribution of aromatics in the pyrolysate of the Ah horizon resembles that of the roots, although phenol and methylphenols are higher and the methylcatechols are lower in abundance with respect to the guaiacols. Pine lignin is almost exclusively composed of coniferyl alcohol units, and produces merely guaiacols upon pyrolysis, with very small traces of syringol and 4-vinylsyringol. The relative abundance of all methoxyphenols as determined by pyrolysis is lower in roots than in needles. Consequently, this corresponds with a lower concentration of methoxyphenols in the pyrolysate of the Ah horizon than in those of the L and F horizons.

To find any trends in composition or alteration of the phenolic and lignin compounds, calculations were carried out using the peak areas of phenols plus catechols (P), guaiacols (G) and syringols (S) to determine the ratios of G/P, (G+S)/P and S/G. In addition, pyrolysis in the presence of TMAH was used to determine the acid/aldehyde ratios (Ac/Al) of each type of phenol (Hatcher *et al.*, 1995). The results are presented in Table 4.

Table 4. Ratios of phenols, guaiacols, syringols and of their acids and aldehydes (Ac/Al) of fresh plant tissues and soil horizons.

Profile	Horizon	Tissue/sample	G/P	(G+S)/P	S/G	Ac/Al G	Ac/Al S
Profile 1		roots	3.54	4.43	0.25	0.67	0.51
Grass	Ah	total	0.45	0.54	0.19	1.68	2.86
Profile 2		leaves	0.00	0.00	nd	1.60	nd
Moss	Ah	total	0.13	0.15	0.13	2.19	1.12
Profile 3		flowers + leaves	0.29	0.41	0.41	2.04	1.93
Heather		stem wood	1.97	4.37	1.22	1.31	0.26
	F	total	0.67	0.93	0.39	3.67	1.59
		roots	0.47	0.55	0.16	2.64	1.35
	Ah	total	0.40	0.46	0.10	6.04	2.92
Profile 4		needles	1.11	1.11	0.00	0.94	nd
Pine	L	total	1.34	1.34	0.00	2.44	nd
	F	total	1.21	1.22	0.01	2.90	nd
		roots	0.82	0.82	0.00	1.15	nd
	Ah	total	0.61	0.62	0.02	3.82	nd

Abbreviations: P: phenols+catechols, G: guaiacols, S: syringols, Ac: acid, Al: aldehyde and nd: not determined. Values are mean (n=2), relative standard deviation of ratios G/P, (G+S)/P and S/G: 4-7%, and of Ac/Al ratios: 3-5%.

Profiles 1 and 3 show the usual lignin degradation trend with decreasing S/G ratios. The high (G+S)/P ratios of *Calluna* wood and grass roots give a rough indication of the relatively high abundance of lignin in relation to non-lignin (poly)phenols. In contrast, *Calluna* flowers + leaves and roots seem to have the highest amounts of (poly)phenols. The Ac/Al ratio of guaiacols increases from fresh plant tissue to humus and with depth in all profiles. In profiles 3 and 4, the increase of that ratio in the Ah horizons with respect to the roots is stronger than that of the F horizon in comparison with the above-ground litter. As far as could be determined, increasing Ac/Al ratios for syringols are found in all profiles. The presence of guaiacol and syringol acids in pyrolysates of fresh material is probably due to cleavage and oxidation of lignin by TMAH (Hatcher and Minard, 1995). In moss the aldehydes and acids are probably derived from (poly)phenols, which may produce 3,4-dimethoxybenzaldehyde and 3,4-dimethoxybenzoic acid methyl ester after pyrolysis-methylation. Such may also happen in other samples, e.g. the high Ac/Al ratios of flowers + leaves and roots of *Calluna* in comparison with the stem wood is accompanied by higher amounts of phenols and catechols.
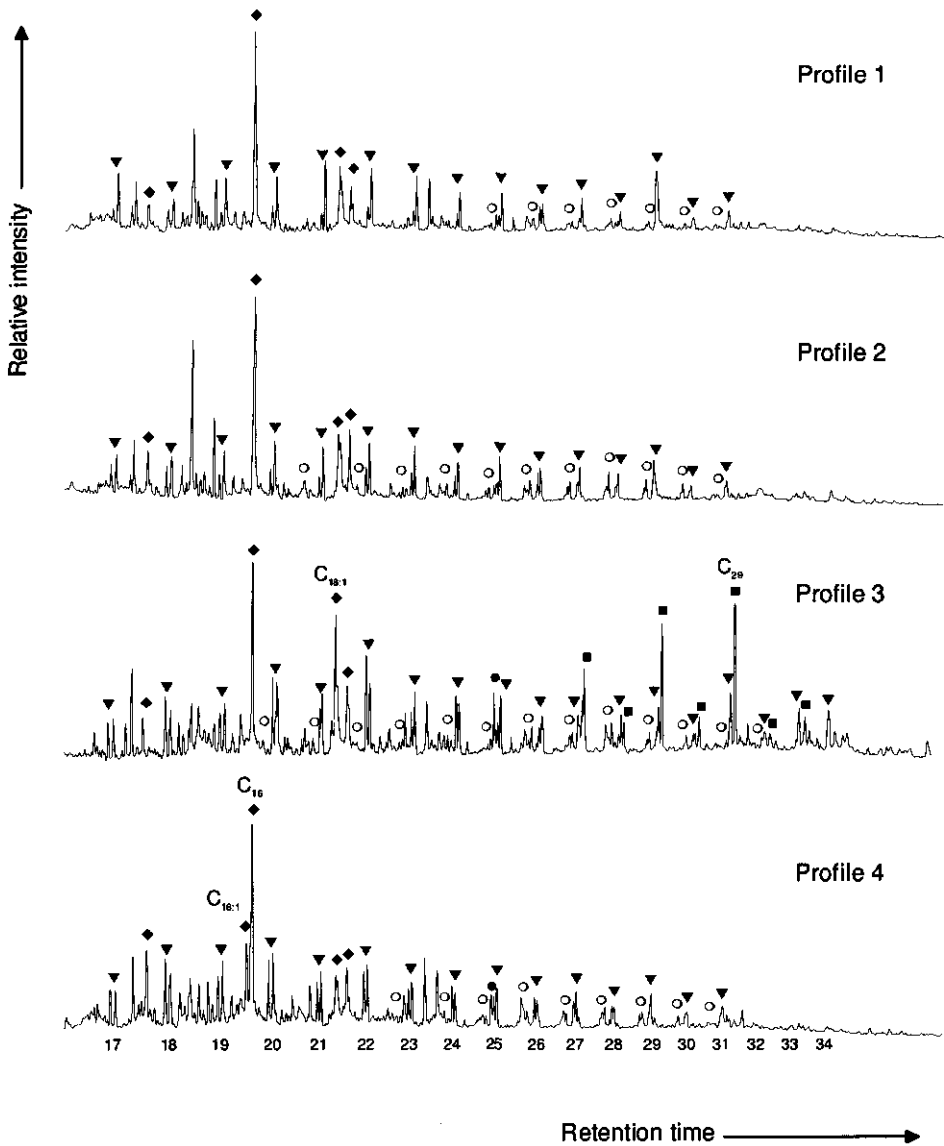
Figure 8. Partial mass chromatogram (*m/z* 55+57+59+60) showing the aliphatic 'fraction' of the Ah horizons of profile 1-4. For symbol legend, see Table 3. The numbers indicate the carbon chain lengths of the straight-chain alkenes/alkanes.

- Aliphatics

As shown by NMR, alkyl C accumulates in the Ah horizons. Figure 8 shows the partial summed mass chromatograms (m/z 55+57+59+60) of the Ah horizons of profiles 1-4. Several series of different aliphatic compounds are identified, including fatty acids (mainly C_{14}, $C_{16:1}$, C_{16}, $C_{18:1}$ and C_{18}), n-alkene/alkane pairs (C_7-C_{34}) and branched alkene/alkane pairs (C_{20}-C_{32}). All these compounds are also observed in pyrolysates of the F layers under heather and pine. The straight-chain alkenes/alkanes are a common phenomenon in pyrolysates of plant tissues (Nip *et al.*, 1986a, b; Tegelaar *et al.*, 1991, 1995) and of soil samples (Augris *et al.*, 1998; Kögel-Knabner *et al.*, 1992a, b). In contrast, branched alkene/alkane pairs have never been observed.

Pyrolysis of the lowest part of the F layer of profile 3, dominated by moss with a few heather litter, revealed a higher abundance of the branched aliphatics than in the upper, heather dominated, part of the F horizon (not shown). In moss plants themselves, these aliphatics were hardly detectable. However, after chemical treatment of the mosses *Polytrichum* and *Bryum*, including Soxhlet extraction with CH_2Cl_2/MeOH, saponification in basic methanol and hydrolysis with concentrated H_2SO_4, straight-chain as well as branched alkenes/alkanes could be observed clearly in pyrolysates (Figure 9).
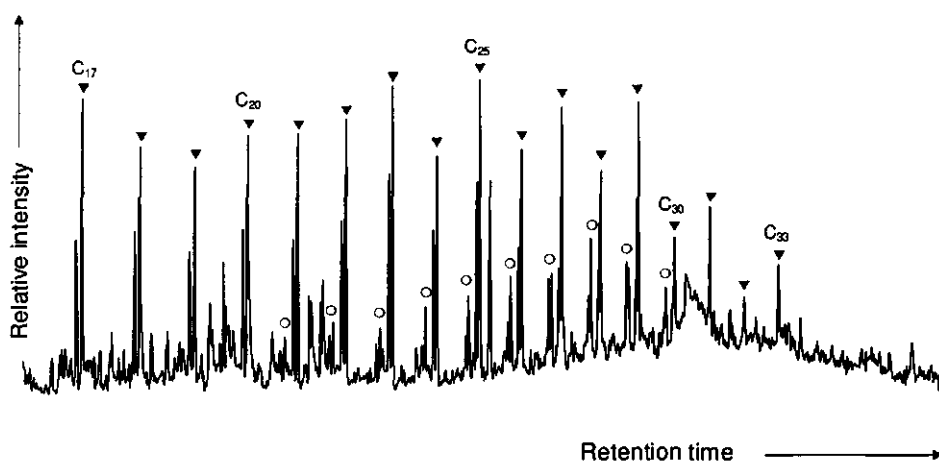
Figure 9. Partial pyrolysis-GC trace of *Polytrichum* after extraction with CH_2Cl_2/methanol, saponification and acid hydrolysis. For symbol legend, see Table 3.

The distribution of both homologous series is very similar to those found in pyrolysates of all Ah horizons and in the ectorganic layers under *Calluna* and pine. This implies that mosses have an important, persistent and chemically recognizable contribution to the organic matter in these Ah horizons.

Apart from fatty acids, high amounts of alkanes are observed in the pyrolysate of *Calluna* flowers + leaves, mainly C_{31} and C_{33} with smaller contributions of C_{27} and C_{29}. The aliphatic fraction of the stem wood is composed of fatty acids (C_{16} and C_{18}), 2-methylketones (dominated by odd-numbered carbon chain lengths of C_{25}-C_{31}), C_{22} and C_{24} alcohols and small traces of alkene/alkane pairs (C_{17}-C_{33} are visible, but most probably lower chain lengths are also present). Of the latter series, the C_{22} and C_{24} alkenes are relatively abundant.

This is due to esterified alcohol moieties, which produce their alkene analogues upon pyrolysis (van Smeerdijk and Boon, 1989). Except for the alkene/alkane pairs, all these aliphatics are likely to originate from, or adsorbed on, the biopolyester suberin present in the outer bark of the *Calluna* stems (van Smeerdijk and Boon, 1989). The alkenes/alkanes point to the non-hydrolyzable aliphatic biopolymer suberan (Tegelaar *et al.*, 1995).

Under pine, typical conifer lipids, such as abietic acid and pimaric acid, are identified in the pyrolysates. As in results from Dijkstra *et al.* (1998), they decrease with depth.

DISCUSSION

The algal mat is predominantly composed of polysaccharides as revealed by ^{13}C NMR, sugar analysis and pyrolysis. Aromatics are virtually absent in the NMR spectrum, whereas a small contribution of aliphatics is noticed. According to Oades (1984), the ratio of C_6/C_5 should be >2 for microbial polysaccharides, but this is not true for the algae in this study (1.09).

The organic matter of profile 1, under grass, is dominated by O-alkyl carbon (mainly polysaccharides) as determined by NMR. Lignin is hardly present in the Ah horizon: no characteristic lignin aromatic signals are assigned in the NMR spectrum, and very low amounts of lignin-derived products are observed in the pyrolysate. The overall distribution of monosaccharides as well as both sugar ratios (C_6/C_5 and deoxy/C_5) of the Ah horizon under grass are comparable to those of the algae, suggesting that the organic matter is mainly derived from algae. This is compatible with the vegetation sequence, because the grass vegetation is very sparse and establishes itself in the algal mat. The algal input is accompanied by that of mosses, as indicated by the presence of branched and unbranched alkenes/alkanes in the pyrolysates. The total Py-GC-trace of Ah horizon under grass more closely resembles the pyrogram of the Ah horizon under moss than that of the algae. The moss organic matter originated probably from elsewhere and was transported by wind together with the sand, because mosses follow grasses instead of preceding them. Anyway, the Ah horizon of profile 1 has a low input of the current plant material.

The Ah horizon under moss has an increase of alkyl C and a simultaneous decrease of O-alkyl C with respect to fresh plants as determined by NMR. Pyrolysis results suggest that the increase of aliphatic C is mainly due to hitherto unknown branched and unbranched aliphatic biopolymer(s). Up to now, it is not clear whether there is one aliphatic polymer, which produces branched as well as unbranched alkene/alkane pairs upon pyrolysis, or two separate ones, a branched and an unbranched one. Whatever the molecular structure may be, those aliphatics exhibit, already in these young profiles, a high selective preservation potential. The aromatic peaks in the NMR spectrum of moss tissue, at 117, 128, 143 and 161 ppm, are not from lignin, because this polymer is not present in mosses (no methoxyphenols are detected

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by pyrolysis), and is further substantiated by the absence of the shoulder at 56 ppm. These aromatic carbons will result therefore from tannins or other phenol-containing compounds (Wilson et al., 1989). Also the relatively broad signal around 103 ppm suggests the presence of tannins, in addition to a contribution of anomeric polysaccharide carbons (Wilson and Hatcher, 1988). The small traces of lignin in the Ah horizon are most probably derived from earlier grasses. In the Ah horizon of profile 2, a similar monosaccharide distribution with respect to fresh moss material is observed, although the C_6/C_5 ratio is lower and the deoxy/C_5 ratio is higher. These trends may be due to remnants of algae, from an earlier succession stage. Fresh *Polytrichum* tissue has a high C_6/C_5 ratio (2.65), implying that a high C_6/C_5 ratio is not always due to microbial polysaccharides.

The F horizon under *Calluna* (profile 3) is composed of (partly) humified flowers, leaves, woody branches and mosses. The pyrolysate of this horizon reflects this combination, such as: a high amount of $C_{18:1}$ fatty acid, indicative for mosses; 2-methylketones from to *Calluna* bark; and C_{31} and C_{33} alkanes, derived from the flowers + leaves of *Calluna*. Both sugar ratios are higher in the F horizon in comparison with the input materials. Part of this will be due to microbial sugars, but the main reason will be most likely the presence of moss litter in F horizon. Lignin, however, can be used to determine the degree of humification: the S/G ratio is lower in the F horizon than in the fresh tissue types and also the Ac/Al ratios of guaiacol and syringol are higher in the ectorganic layer, indicative of considerable degradation.

Calluna roots appear to have a much lower syringyl content in the above-ground plant tissues. Figure 7 shows that the relative intensities of guaiacols in comparison with, e.g., levoglucosan and the aliphatic compounds, are lowest for roots and highest for wood (stems), which gives an indication of the lignin concentration in the several *Calluna* parts. This seems at variance with the lignin contents in *Calluna* wood (33.4%) than in flowers + leaves (40.3%) and roots (40.6%) as described by van Vuuren (1992). However, these determinations were based on 'Klason lignin', the residue after extraction of lipids by ethanol and polysaccharides by concentrated sulphuric acid. Such residues do not contain lignin only, but also polyphenols and aliphatic biopolymers. As shown in Figure 5, the pyrolysates of *Calluna* flowers + leaves and roots contain significant amounts of phenols and catechols, indicative for a considerable (poly)phenol content (Galletti and Reeves, 1992). Also aliphatic compounds are more abundant in these pyrolysates than in that of wood. Both raise the 'lignin' concentrations. Besides this, wood contains in general (much) higher amounts of lignin than non-woody tissues (Higuchi, 1997). NMR showed that aromatic signals decrease significantly from root to soil, implying a low input, or a fast decay of lignin and tannin in soil. Furthermore, the larger differences in Ac/Al ratios between Ah horizon and roots compared to the differences of these ratios between above-ground litter and F horizon suggest a faster decay of lignin in the soil than on the soil.

The lipid fraction of *Calluna* roots has a composition similar to that of *Calluna* stem wood. This suggests that roots will have a comparable suberin/suberan composition. The great similarity of the Py-GC traces of the roots and Ah horizon reflects the important input of roots. But also moss remnants are present as indicated by the branched alkenes/alkanes.

Going from needles to L horizon (profile 4), the increases of the alkyl/O-alkyl ratio (NMR), the C_6/C_5 and deoxy/C_5 ratios (monosaccharides), and the Ac/Al ratio of G all imply

(rapid) decay of fresh needles. From L to F horizon, however, the alkyl/O-alkyl ratio and the C_6/C_5 ratio decrease, whereas the deoxy/C_5 and Ac/Al ratios increases slightly. Apparently, decomposition is not fast, or no discrimination takes place among the participating compounds. In the F and Ah horizons the accumulation of unbranched alkenes/alkanes points to, again, moss litter. Because practically no moss remnants are present in the Ah horizon, mosses appear to be strongly decayed, leaving the highly resistant aliphatics in the soil.

CONCLUSIONS

All profiles show progressive decay phenomena from plant materials to soil horizons, although such trends are not clear between L and F horizons under pine. Most evident trends are increases in alkyl C contents with humification accompanied with a decrease of O-alkyl C as determined with ^{13}C NMR. Also Py-GC/MS reveals the increasing contribution of aliphatics in soil. The trends are caused by decomposition of polysaccharides, the most labile biopolymer(s), and residual accumulation of very resistant aliphatic polymers, mainly derived from mosses. Aromaticity decreases towards the Ah horizons. Within the aromatic fraction, lignin is degraded and non-methoxyphenolics are accumulated. In relation to the extant vegetation, the organic matter in the profiles is dominated by the covering plants, except that under grass, which organic matter is clearly affected by algae and mosses. Besides recognizable moss influence, the organic matter of the Ah horizons under heather and pine have a significant root input, and above-ground litter contribution is of negligible importance.

Additionally, the results also show that parameters, such as C_6/C_5 and deoxy/C_5 for sugars and, to a lesser extent, the Ac/Al ratios for aromatics, commonly used to describe the organic matter composition and humification may be influenced strongly by input of earlier vegetation. Knowledge of vegetation history is therefore indispensable when trying to interpret such parameters.

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Chapter 3

Composition of soil organic matter and its water soluble fraction under young vegetation on drift sand, central Netherlands

Klaas G.J. Nierop and Peter Buurman

ABSTRACT

Podzols tend to form on drift sands in the Netherlands given sufficient time. Differences in organic matter composition related to vegetation succession were investigated on a soil chronosequence on drift sand that evolves eventually into a micro-podzol. We examined the relation between the water soluble organic matter (WSOM) and the total organic matter composition in the Ah horizons of the first vegetation stages, under grass (*Corynephorus canescens*), moss (*Polytrichum piliferum*), heather (*Calluna vulgaris*) and pine (*Pinus sylvestris*), as determined by Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) and analysis of polysaccharides after acid hydrolysis. Polysaccharides dominated the organic matter in the Ah horizons under grass and moss. Lignin and aliphatic compounds were minor compounds in these profiles, but were more important under heather and pine. The Py-GC traces of the WSOM under grass and moss contained abundant products derived from polysaccharides. The water soluble fraction was enriched in phenolic and aliphatic compounds in comparison with their contents in pyrolysates of the total organic matter. In the WSOM fractions under heather and pine, phenols dominated the pyrolysates, whereas lignin-derived methoxyphenols were rare. Remarkably large amounts of alkenes and alkanes were present in the pyrolysates of these water soluble fractions, most likely derived from aliphatic biopolymers. Their presence in water might be explained through the formation of colloids, which were stabilized by micelle-like arrangements.

INTRODUCTION

Podzols are characterized by an illuvial horizon, as a result of accumulation of organic matter in combination with aluminium and/or iron eluted from overlying horizons. A bleached eluvial horizon may overlie the illuvial horizon. A number of theories have been proposed to explain podzolization in which the role of organic matter is emphasized. The vegetative cover and the organic matter it produces plays an important role in podzolization and little is known about the specific effect of different organic compounds. However, the organic matter analyses usually applied have not gone beyond separation of humic and fulvic acids. Furthermore, through lack of advanced analytical instruments, only some general characteristics of organic matter involved in podzolization have been noticed. Large differences in the nature of the organic matter in the illuvial B horizons have been found, varying from highly aromatic to predominantly aliphatic, depending on environmental conditions and vegetation (Andreux, 1996).

Only few scientists have investigated the molecular nature of podzol organic matter. Sorge *et al.* (1994) observed characteristics of polysaccharides, alkylaromatics, lipids, lignin monomers and dimers, using pyrolysis-Field Ionization Mass Spectrometry (FIMS). Hempfling and Schulten (1990) also detected these compounds in dissolved organic matter in forest humus profiles. Recently, Wilcken *et al.* (1997) elucidated that alkyl and O-alkyl C predominated the organic matter of podzol B horizons, consisting of lipid-derived structures, alkylaromatics and lignins, while carbohydrate accumulation was site specific. Amblès *et al.* (1983) and Colina-Tejada *et al.* (1996) observed dissolved lipids in percolating water and rainwater leachates under

pine. Because water soluble compounds are considered as the main source of organic matter in the B horizons of podzols, lipids can be expected in forest podzol B horizons.

Most of the research on organic matter has been done on old, well-developed podzols, with unknown or complex vegetation history, so that a specific relation between organic matter composition and vegetation cannot be traced. To study such a relation, we analysed the water soluble organic matter (WSOM) in a young soil (no more than 200 years old) on drift sand in the early stages of podzolization. Two factors determine the chemical composition of these water soluble fractions: the presence of the particular compounds in the original Ah horizons and their solubility in water. Therefore, we also characterized the total organic matter composition of the Ah horizons.

MATERIAL AND METHODS

Vegetation, soil profiles

The study sites are in the Hulshorster Sands and the Leuvenum Forest (central Netherlands). The area was originally an inland dune field. About 200 years ago a pioneer vegetation established itself. Nowadays, the older vegetative parts of the area carry a beech (*Fagus sylvatica* L.) forest, while some parts are still bare, and all other intermediate vegetation stages are locally present. The vegetation succession is well-documented (Fanta, 1986; Prach, 1989) and a simplified scheme of the vegetation sequence is given in Figure 1.
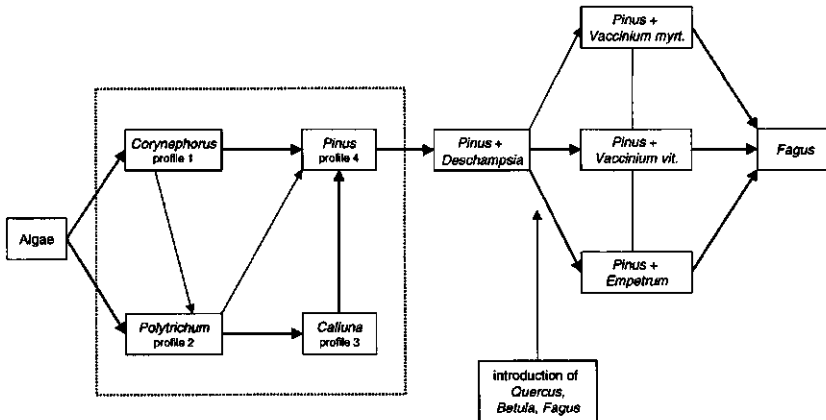

Figure 1. Simplified scheme of vegetation succession on the Hulshorster Sands/Leuvenum Forest area, modified after Prach (1989). The profiles indicated in the dotted box are investigated in this paper.

The dunes are first colonized by algae, which form algal mats in moist spots. A sparse vegetation of the grass *Corynephorus canescens* (L.) Beauv succeeds algae and the moss *Polytrichum piliferum* Hedw. growing at slightly moister sites. Heather (*Calluna vulgaris* (L.) Hull) locally follows *Polytrichum*. Solitary pine trees (*Pinus sylvestris* L.) establish themselves in the preceding vegetation. Under these pines, needles eventually drown a sparse growth of grasses. When a pine forest is established (spontaneously or by planting), the grass *Deschampsia flexuosa* (L.) Trin. first dominates the undergrowth, accompanied by the moss *Pleurozium schreberi* Hedw. This stage is followed by Ericaceae, accompanied by the spontaneous appearance of other trees: *Betula pubescens* L. (birch), *Quercus robur* L. (oak) and *Fagus sylvatica* L. (beech). Eventually, the beech takes over completely, and all undergrowth disappears. Traces of podzolization appear under *Pinus + Deschampsia*, and the podzol morphology is clear under *Empetrum* and the subsequent vegetation stages.

The following profiles are used in this study:

Profile 1 (under *Corynephorus*): Two cm of weakly developed Ah horizon abruptly overlying a C horizon.

Profile 2 (under *Polytrichum*): Half a cm of Ah1 horizon with *Polytrichum* rhizoids overlying an Ah2 horizon without rhizoids; sampled as one horizon.

Profile 3 (under *Calluna*): One cm of F layer, the upper part of which consists of *Calluna* litter and the lower part of *Polytrichum* litter, overlying an Ah horizon of 6 cm with abundant fine roots.

Profile 4 (under *Pinus*): Two cm of L layer on 4 cm of F layer, both dominated by pine needles and twigs. The F layer overlies a 10 cm Ah horizon with abundant fine roots).

In the early vegetation stages, there is little biological activity in the mineral horizons, and negligible mixing of above-ground litter with the mineral matrix. This implies that organic matter in B horizons may be (partly) derived from illuviated water soluble organic matter (WSOM).

For this reason, special attention is paid to the water soluble and the total organic matter from the Ah horizons of the first stages of profile development (grass through to solitary pines, see Figure 1). The profiles have incipient Ah and organic horizons without signs of podzolization. Yet these pre-podzol profiles may influence later soil development, because they produce (water soluble) organic matter that may be involved in podzolization and identified in the Bh horizons in the later stages of soil formation (pine + *Deschampsia* through beech, see Figure 1).

MATERIAL AND METHODS

Sample pretreatment

- Bulk samples

Soil samples were taken from Ah horizons in March 1996 and frozen until further treatments. There was too little organic C in the original samples to obtain reliable chromatograms and mass spectra upon pyrolysis. Therefore the samples were enriched by removing the sand. Each sample was shaken with 0.5 M NaOH for two hours under nitrogen, and then allowed to stand for 1 minute. The sand immediately sank to the bottom, while the solution with dissolved and

particulate organic matter remained in suspension at the top. The supernatant was poured into an Erlenmeyer, and the procedure was repeated until the solution was colourless, and all the organic matter was isolated. The addition of NaOH was necessary to isolate all the organic matter, which was impossible solely with water. The supernatants were combined, acidified to pH 2-3 with HCl/HF (1:3), shaken for 24 hours, dialyzed against demineralized water and finally freeze-dried. This was done three times for each Ah horizon, after which the organic matter obtained was thoroughly mixed before use.

- Water soluble organic matter (WSOM)
Water soluble organic matter can be obtained by several methods (Herbert and Bertsch, 1995; Zsolnay, 1996), which can be divided into two main approaches: those collected in the field (called dissolved organic matter (DOM)) and the others extracted from soil samples in the laboratory (called water soluble organic matter (WSOM)). Initially, both methods were used in order to compare the organic matter composition. However, due to insufficient yields of field-collected DOM, only laboratory-obtained WSOM was used for analysis. Soil samples from the Ah horizons were shaken for 24 hours with distilled water at a solid:solvent ratio of 1:3 by weight. After centrifuging (2200 g) for one hour, the solutions were filtered through a 0.45-μm filter. The final filtrate was freeze-dried. For each site, this was done four times. The yields were 1-2% based on organic matter content of the original soil samples.

- Extractions
Low molecular weight (LMW) and high molecular weight (HMW) compounds were separated by extraction of 100 ml of a solution of WSOM, as obtained above, with 100 ml of (a) dichloromethane (DCM), (b) diethyl ether, (c) DCM followed by diethylether, or (d) diethyl ether followed by DCM. All extractions were repeated once. The remaining WSOM in the water fractions was freeze-dried and subsequently pyrolyzed.

Chemical analysis

- C, N and pH
Carbon and nitrogen were measured in duplicate with an Interscience Elemental Analyser EA1108, pH (H_2O and 1 M KCl) in a sample:solution ratio of 1:5. The relative error for carbon was 0.3%, for nitrogen 0.5%.

- Pyrolysis-GC/MS
Pyrolysis was carried out on a Horizon Intruments Curie-Point pyrolyser. Samples were heated for 5 s at 610°C. The pyrolysis unit was connected to a CE gas chromatograph and the products were separated by a fused silica column (Chrompack, 25 m, 0.25 mm i.d.) coated with CP-Sil 5 (film thickness 0.40 μm). Helium was used as carrier gas. The initial oven temperature was 40°C and maintained for 1 minute. Then it was raised at a rate of 7°Cmin[-1] to 320°C and maintained at that temperature for 20 min. The end of the GC column was coupled to a Fisons MD 800 mass spectrometer (mass range *m/z* 45-650, ionization energy 70 eV, cycle time 1 s). Pyrolysis in the presence of tetramethylammonium hydroxide (TMAH)

was performed by adding a droplet of a 25% solution of TMAH in water to the sample, after which the sample was dried for 1-2 minutes, and immediately pyrolyzed.

- Monosaccharides
Hydrolysis of polysaccharides was performed by the method described by Amelung *et al.* (1996) and is described in Chapter 2. Briefly, the non-cellulosic polysaccharides in soil samples and WSOM were hydrolyzed by trifluoroacetic acid. After purification, the monosaccharides were derivatized by oximation and sylilation, and analysed by GC. The soil samples were analysed in quadruplicate, and the WSOM samples in duplicate.

RESULTS AND DISCUSSION

By decanting to concentrate the organic matter, less than 5% of the OM originally present was lost during dialysis. The C/N ratios of these treated samples were at most 1 unit larger than those of the untreated bulk samples (see Table 1). The residues (sand) contained < 0.005 % organic carbon.

Table 1. C and N mass fractions, C/N mass ratios, and pH values of soil, total OM and WSOM.

Profile	Vegetation	Sample	C (%)	N (%)	C/N	pH (H_2O)	pH (KCl)
1	Grass	Soil	0.4	0.04	10.0	5.0	4.3
		Total OM	45.7	4.3	10.6		
		WSOM	32.8	3.7	8.8		
2	Moss	Soil	0.4	0.03	11.8	4.2	3.8
		Total OM	47.1	3.8	12.5		
		WSOM	35.2	3.1	11.5		
3	Heather	Soil	1.2	0.06	19.8	4.2	3.7
		Total OM	41.0	2.0	20.4		
		WSOM	21.8	2.6	8.3		
4	Pine	Soil	2.2	0.12	18.1	4.0	3.2
		Total OM	43.3	2.3	19.0		
		WSOM	20.9	2.1	9.8		

Of course, the concentration treatment changes the organic matter to some extent. The main chemical effect of NaOH will be hydrolysis of esters, whereas HCl/HF can create new esters. However, these effects appeared to be negligible as determined by pyrolysis for samples of L, F and H horizons before and after the procedure. Therefore, we assume that differences in the composition of organic matter from Ah horizons caused by this procedure are also small and not significant.

Table 1 lists the C and N contents and their ratios, and pH values of the four profiles. The C contents in the Ah horizons increases as vegetation develops. The C/N ratios of profiles 1 and 2 differ from those of profiles 3 and 4. Under grass and moss this ratio (10-12) is fairly low and indicates either highly humified material, and/or important contribution of microbial material. The C/N ratios of the WSOM are less than in of the Ah horizons from which WSOM was extracted, and the largest differences were in profiles 3 and 4. A decrease of both pH values is observed on going from profile 1 to profiles 2 and 3 and to profile 4.

Figures 2-5 show the GC-traces of the pyrolysates of the total organic matter (above) and the WSOM (below) of the Ah horizons. The numbers and symbols corresponding to the peaks and their assignments are summarized in Table 2.

Polysaccharides

In the pyrolysates of all total organic matter samples many furans (**2, 6, 9, 10, 12, 15, 16** and **17**), pyrans (**18, 23, 26, 31** and **38**), a dianhydrodeoxyhexose (**21**), two anhydropentoses (**32, 36**), three anhydrohexoses (**46, 50, 54**), and other compounds such as formic acid (**1**), acetic acid (**3**), 2-butenal (**4**), 2,4-pentadienal (**11**) have been identified. All these compounds have been found after pyrolysis of polysaccharides (Pouwels *et al.*, 1987; 1989; Pouwels and Boon, 1990; van Smeerdijk and Boon, 1987). Many of these compounds, especially those with lower molecular weights, cannot be related to a specific sugar, but some of the larger fragments are more specific. Compounds **18, 32** and **36** are identified as typical markers of pentose-containing polysaccharides (Pouwels *et al.*, 1987), compounds **21** and **23** are related to rhamnose (van Smeerdijk and Boon, 1987), whereas the pyrans **26** and **38**, and the anhydrosugars **46, 50** and **54** are attributed as indicators of hexoses (Pouwels *et al.*, 1987, 1989). In the WSOM of all Ah horizons, the same polysaccharide-derived pyrolysis products are identified as in the total organic matter. A shift from anhydrosugars, which dominate the polysaccharide-derived pyrolysis products of the total organic matter, to furans, predominantly present in pyrolysates of the WSOM, is evident. Because pyrolysis is not the most sensitive method for sugar analysis, monosaccharides released by trifluoroacetic acid (TFA) were also determined. Quantitative information obtained after acid hydrolysis of polysaccharides in soil and WSOM samples is presented in Table 3.

Table 3 shows that profiles 1 and 4 have similar concentrations of non-cellulosic sugars in the Ah horizons and WSOM, whereas the concentration of those sugars is greater in the WSOM fractions of profiles 2 and 3 with respect to the bulk soil samples. Pyrolysis as well as carbohydrate analysis show that polysaccharides strongly dominate the organic matter in the Ah horizon under grass, and that their importance decreases from grass via moss and heather to pine, under which they are relatively unimportant.

Although specific monosaccharides cannot be attributed as markers for either plant or microbial species (Cheshire, 1979), pentoses (C_5) are important constituents of plants, whereas deoxyhexoses (deoxy) and hexoses (C_6) are considered as being synthesized mainly by microbes (Cheshire, 1979; Murayama, 1984; Oades, 1984; Moers *et al.*, 1990).

Figure 2. Py-GC traces of total OM (a) and WSOM (b) of Ah horizon under *Corynephorus canescens* (profile 1).

Figure 3. Py-GC traces of total OM (a) and WSOM (b) of Ah horizon under *Polytrichum piliferum* (profile 2).
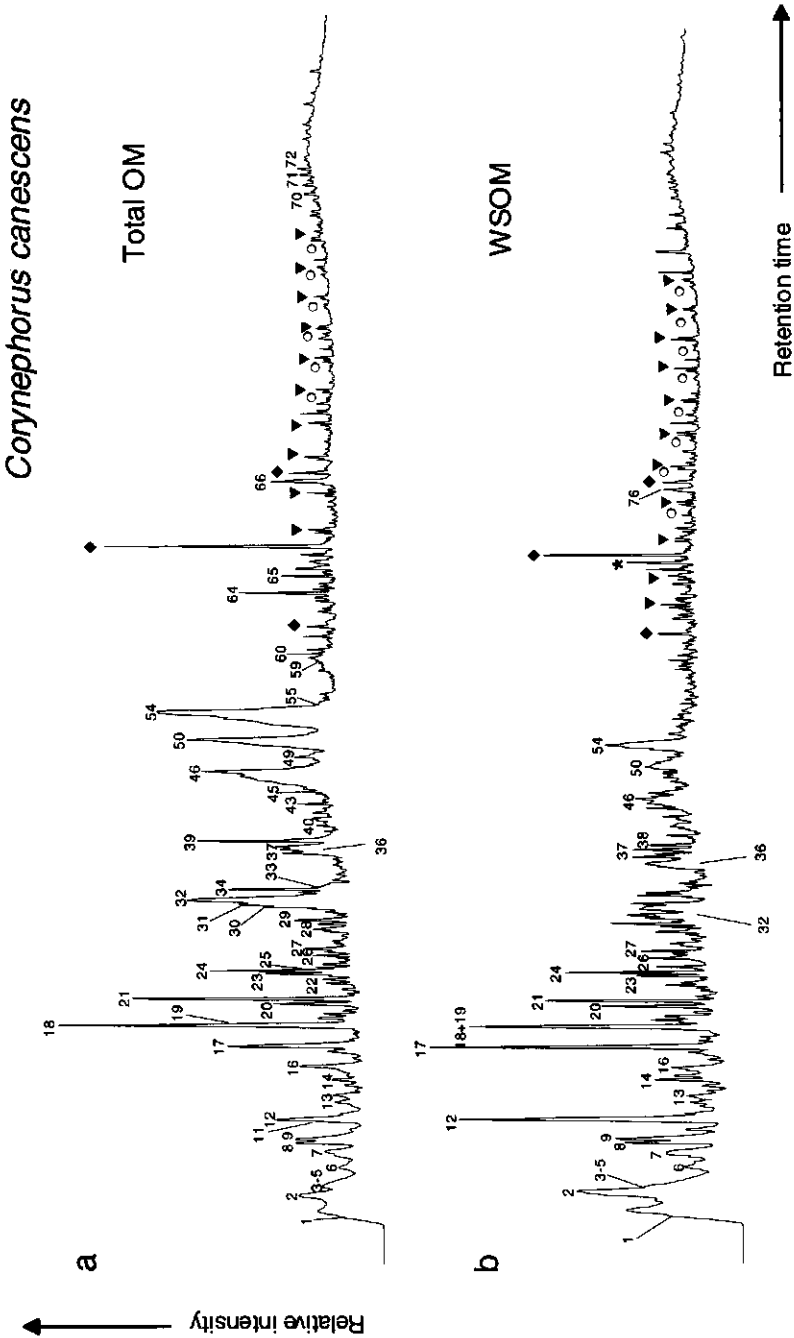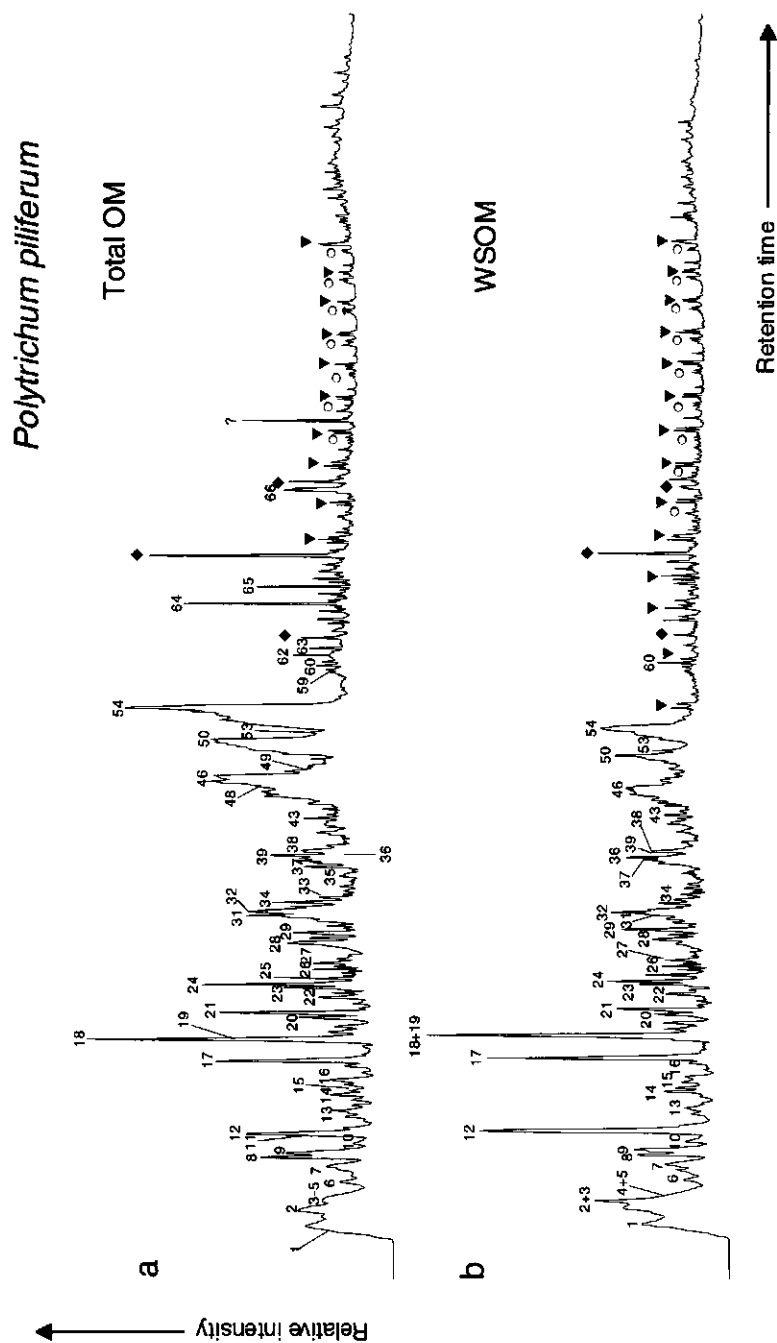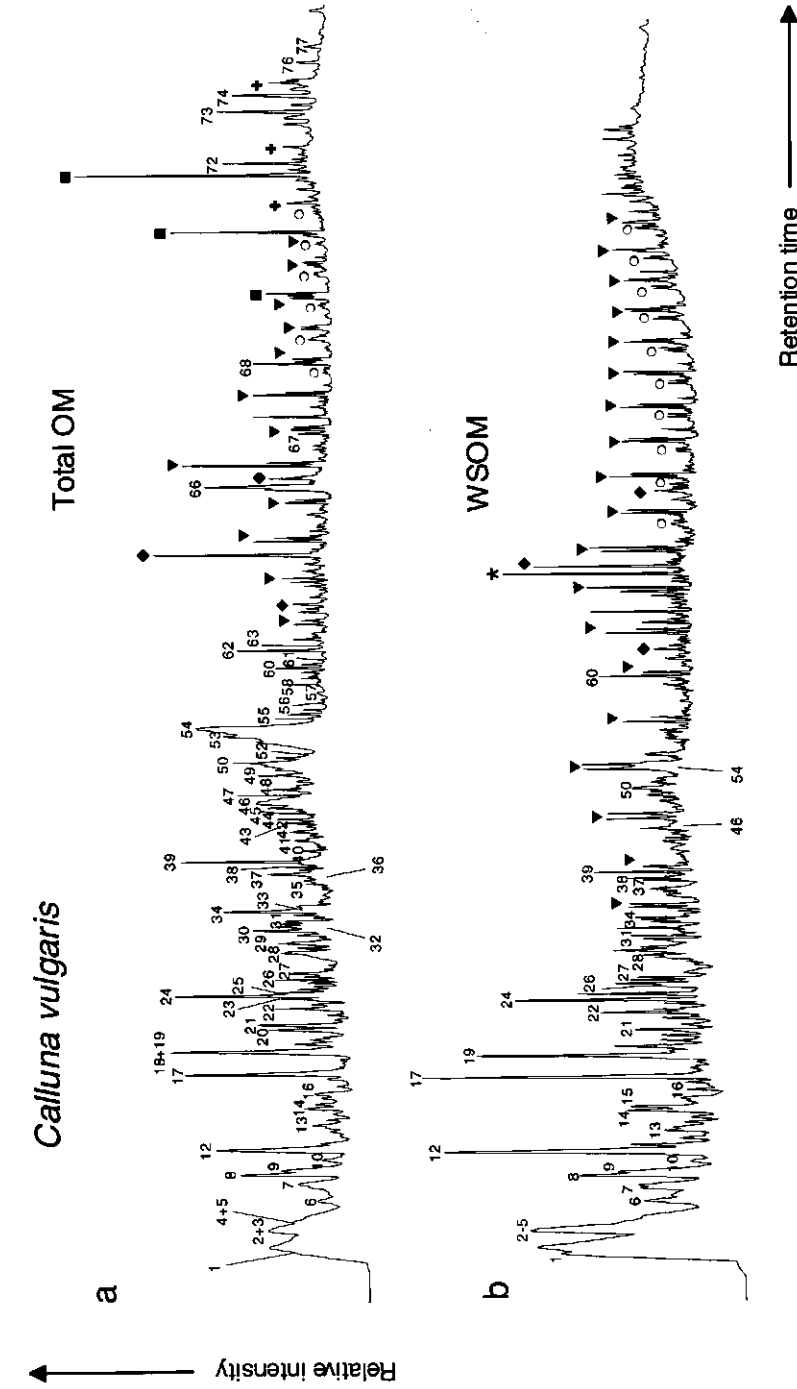
Figure 4. Py-GC traces of total OM (a) and WSOM (b) of Ah horizon under *Calluna vulgaris* (profile 3).
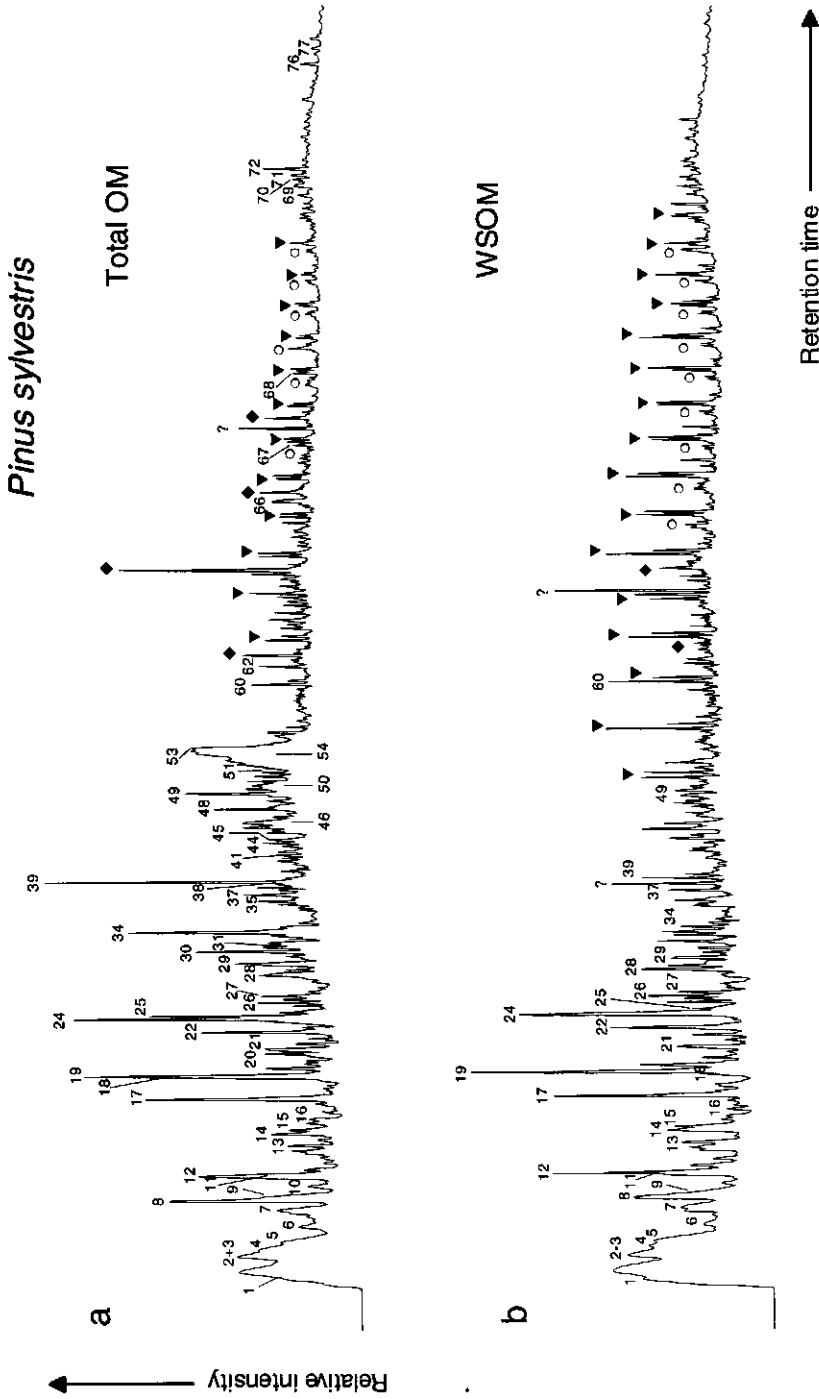
Figure 5. Py-GC traces of total OM (a) and WSOM (b) of Ah horizon under *Pinus sylvestris* (profile 4).

Table 2. List of main pyrolysis products.

No.	Compound	Source	M$^+$
1	Formic acid	Ps	46
2	2-Methylfuran	Ps	82
3	Acetic acid	Ps	60
4	2-Butenal	Ps	70
5	Benzene	Pp	78
6	2,5-Dimethylfuran	Ps	96
7	Pyrrole	Pr	67
8	Toluene	Pp/Pr	92
9	(2H)-Furan-3-one	Ps	84
10	3-Furaldehyde	Ps	96
11	2,4-Pentadienal	Ps	82
12	2-Furaldehyde	Ps	96
13	C$_2$-Benzene	Pp	106
14	Vinylbenzene (styrene)	Pp/Pr	104
15	2-Acetylfuran	Ps	110
16	2,3-Dihydro-5-methylfuran-2-one	Ps	98
17	5-Methyl-2-furaldehyde	Ps	110
18	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	Ps	114
19	Phenol	Pp/Lg/Pr	94
20	2-Hydroxy-3-methyl-2-cyclopenten-1-one	Ps	112
21	Dianhydrorhamnose	Ps	128
22	2-Methylphenol	Pp/Lg/Pr	108
23	4-Hydroxy-5-methyl-5,6-dihydro-(2H)-pyran-2-one	Ps	128
24	3/4-Methylphenol	Pp/Lg/Pr	108
25	Guaiacol	Lg	124
26	2-Methyl-3-hydroxy-(4H)-pyran-4-one	Ps	126
27	Benzyl cyanide	Pr	117
28	Dimethylphenol	Pp/Lg	122
29	Ethylphenol	Pp/Lg	122
30	4-Methylguaiacol	Lg	138
31	3,5-Dihydroxy-2-methyl-(4H)-pyran-4-one	Ps	142
32	Anhydropentose	Ps	132
33	Dihydroxybenzene	Pp/Lg	110
34	4-Vinylphenol	Pp/Lg	120
35	4-Ethylguaiacol	Lg	152
36	Anhydropentose (xylose?)	Ps	132
37	Indole	Pr	117
38	1,4-Dideoxy-D-glycero-hex-1-enpyranos-3-ulose	Ps	144
39	4-Vinylguaiacol	Lg	150
40	Syringol	Lg	154
41	4-(1-Propenyl)guaiacol (eugenol)	Lg	164
42	4-Propylguaiacol	Lg	166
43	Methylindole	Pr	131
44	4-Formylguaiacol (vanillin)	Lg	152

45	*cis* 4-(2-Propenyl)guaiacol	Lg	164
46	Anhydrohexose (galactose?)	Ps	162
47	4-Methylsyringol	Lg	168
48	*trans* 4-(2-Propenyl)guaiacol	Lg	164
49	4-Acetylguaiacol	Lg	166
50	Anhydrohexose (mannose?)	Ps	162
51	4-(Propan-2-one)guaiacol	Lg	180
52	4-Ethylsyringol	Lg	182
53	4-Vinylsyringol	Lg	180
54	Anhydroglucosan (levoglucosan)	Ps	162
55	4-(1-Propenyl)syringol	Lg	194
56	4-Propylsyringol	Lg	196
57	*cis* 4-(2-Propenyl)syringol	Lg	196
58	4-Formylsyringol	Lg	182
59	*trans* 4-(2-Propenyl)syringol	Lg	194
60	Biphenol*	Pp	186
61	4-Acetylsyringol	Lg	196
62	1-Pristene		266
63	4-(Propan-2-one)syringol	Lg	210
64	Phytadiene		278
65	Phytadiene		278
66	Oleic acid ($C_{18:1}$ FA)	Lp	254
67	Eicosanol (C_{20} OH)	Lp	298
68	Docosanol (C_{22} OH)	Lp	326
69	Stigmast-2-ene	Lp	398
70	Stigmast-2,5-diene	Lp	396
71	Stigmastatriene	Lp	394
72	Stigmast-3,5-diene	Lp	396
73	Friedoolean-2-ene	Lp	410
74	Friedoolean-14-en-3-one (taraxerone)	Lp	424
75	Friedoolean-14-en-3β-ol (taraxerol)	Lp	426
76	Stigmasta-3,5-dien-7-one	Lp	410
77	Stigmast-4-en-3-one	Lp	412
▼	*n*-Alkene and *n*-alkane (doublet)	Lp/Abp	
O	Branched methyl-alkene and -alkane (doublet)	Lp/Abp?	
◆	Fatty acid	Lp	
■	2-Methylketone	Lp	
✚	2,4-Diketone	Lp	

Abbreviations: Ps: polysaccharides, Pp: polyphenols, Pr: proteins, Lg: lignin, Lp: lipids, Abp: aliphatic biopolymer. * the compound tentatively identified as biphenol appeared to be diketodipyrrole (see e.g. Chapter 8).

Table 3. Non-cellulosic polysaccharide composition of the total OM and WSOM of the Ah horizons after TFA hydrolysis.

Profile	Sample	Total (mg /g C)		C_6/C_5*		Deoxy/C_5*	
1	Total	513	(17)	1.14	(0.01)	0.50	(0.02)
	WSOM	570	(44)	1.20	(0.14)	0.56	(0.03)
2	Total	288	(13)	1.82	(0.06)	0.36	(0.01)
	WSOM	577	(58)	1.09	(0.05)	0.29	(0.04)
3	Total	209	(7)	1.38	(0.06)	0.40	(0.02)
	WSOM	689	(15)	0.35	(0.02)	0.11	(0.01)
4	Total	139	(13)	0.76	(0.07)	0.28	(0.03)
	WSOM	107	(12)	1.44	(0.01)	0.70	(0.06)

*C_6/C_5 = (mannose + galactose)/(xylose + arabinose); deoxy/C_5 = (fucose + rhamnose)/(xylose + arabinose). The standard error is given in parenthesis.

A C_6/C_5 (mannose + galactose)/(xylose + arabinose) ratio < 0.5 is supposed to be indicative for polysaccharides mainly derived from plants, whereas a high ratio (>2) may suggest that microbial sugars dominate the carbohydrates (Oades, 1984). Furthermore, the deoxy/C_5 (fucose + rhamnose)/(xylose + arabinose) ratio indicates the origin of the polysaccharides (Murayama, 1984). High values of C_6/C_5 and of deoxy/C_5 imply that the microbial contribution is fairly important under moss and in WSOM under pine (Table 3). These ratios do not agree well between soil and WSOM fractions: both ratios are higher in WSOM than in the bulk in profile 4 (pine), whereas the reverse is true in profiles 2 and 3. Mosses, which have high sugar ratios in their fresh plant material already, could cause these contrasting trends (e.g. *Polytrichum* has a C_6/C_5 ratio of 2.65). Mosses dominate profile 2, and are an important constituent in the litter on profile 3. This may also explain the different trends observed for the sugar contents of soil and WSOM of profiles 1 and 4 on the one hand, and profiles 2 and 3 on the other hand.

Aromatic compounds

Under pine (profile 4, Figure 5a) important signals come from typical gymnosperm lignin pyrolysis products (**25, 30, 35, 39, 41, 42, 44, 45, 48, 49** and **51**). In the Ah horizon of heather (profile 3, Figure 4a), an angiosperm, all syringolic counterparts (**40, 47, 52, 53, 55-59, 61** and **63**) of the guaiacols are present as well. In the grass and moss profiles (1 and 2, Figures 2a and 3a) only small amounts of lignin-derived products are present in the pyrolysates. In profile 1 this may be because most of the organic matter originates from algae and mosses, which are devoid of lignin. Another possibility may be rapid decay of lignin. The only methoxyphenols detected in the pyrolysates of all WSOM fractions are guaiacol (**25**) and 4-

vinylguaiacol (**39**). A shift towards guaiacol units with smaller side-chains has been found with increasing humification (Dijkstra *et al.*, 1998).

With Py-GC/MS in the presence of TMAH, the carboxylic acids and phenolic hydroxyl groups are methylated, preventing the acids to decarboxylate (Challinor, 1989). Using this method on the WSOM fractions reveals the methylated version of vanillic acid as by far the most abundant pyrolysis product with an aromatic core, whereas only a small peak of methylated guaiacol is detected. This means that during conventional pyrolysis guaiacol results mainly from decarboxylated vanillic acid. Thus, lignin is present only in WSOM in a very oxidized state. This is in agreement with Guggenberger and Zech (1994), who suggested that lignin is only soluble in water when it is highly oxidized.

Another series of dominant signals in the GC-traces of all sites are from non-methoxyaromatics such as alkylbenzenes (**5, 8, 13** and **14**), alkylphenols (**19, 22, 24, 28, 29, 34**), dihydroxybenzene (**33**) and biphenol (**60**). These can originate from lignin (Saiz-Jimenez and de Leeuw, 1986; van der Hage *et al.*, 1993) and/or from other polyphenols (Harborne, 1997; van der Heijden, 1994). The (non-methoxy) phenols are important compounds in the pyrolysates of the WSOM fractions, in particular phenol (**19**) and methylphenols (**22, 24**). These compound are most probably derived from the water soluble polyphenols that are present in plants (Harborne, 1997).

Aliphatic compounds

Several homologous series of aliphatic compounds are observed in the pyrolysates of the total organic matter. Series of fatty acids, in the range of C_{12}-C_{24}, methylketones (C_{19}-C_{31}) and 2,4-diketones (C_{25}-C_{31}), and some alcohols (**67** and **68**), terpenoids (**73, 74** and **75**) and steroids (**76** and **77**) have been identified. All these compounds are pyrolysis or evaporation products of lipids and of the biopolyesters cutin and suberin (van Smeerdijk and Boon, 1987). The presence of suberin is especially pronounced under heather (profile 3).
Palmitic acid (C_{16}) is prominently present in pyrolysates of all WSOM fractions, as well as in all total organic matter samples. Other fatty acids observed are C_{14}, C_{18} and traces of C_{20} and C_{22}. All other aliphatic compounds, such as the long-chain alcohols and ketones, are hardly present in the pyrolysates of WSOM.

Other hydrocarbons identified in all profiles are homologous series of *n*-alkenes and *n*-alkanes (C_7-C_{33}) and of branched alkenes and alkanes (C_{20}-C_{31}). Straight-chain alkenes and alkanes are considered to be derived from non-hydrolyzable aliphatic macromolecules (Tegelaar *et al.*, 1989, 1995). Methyl-branched alkenes and alkanes are rarely found in pyrolysates of samples containing aliphatic biopolymers, but have recently been observed in fresh moss tissues (Chapter 2) and always in combination with the unbranched alkenes and alkanes. Therefore, it seems that most of the alkenes and alkanes in the pyrolysates of Ah horizons are derived from mosses.

A characteristic of aliphatic compounds is that they are sparingly soluble in water. Hence, the presence of homologous series of *n*-alkenes and *n*-alkanes and branched alkenes and alkanes in pyrolysates of the WSOM fractions is very remarkable. To find out whether the alkenes and alkanes were derived from low molecular weight (LMW) or high molecular

weight (HMW) components, the water soluble fractions of profiles 3 (heather) and 4 (pine) were extracted by dichloromethane (DCM) and diethylether. LMW compounds are removed by these extractions, while HMW compounds are not. Extraction of WSOM with DCM resulted in a precipitate between the DCM and water layers.
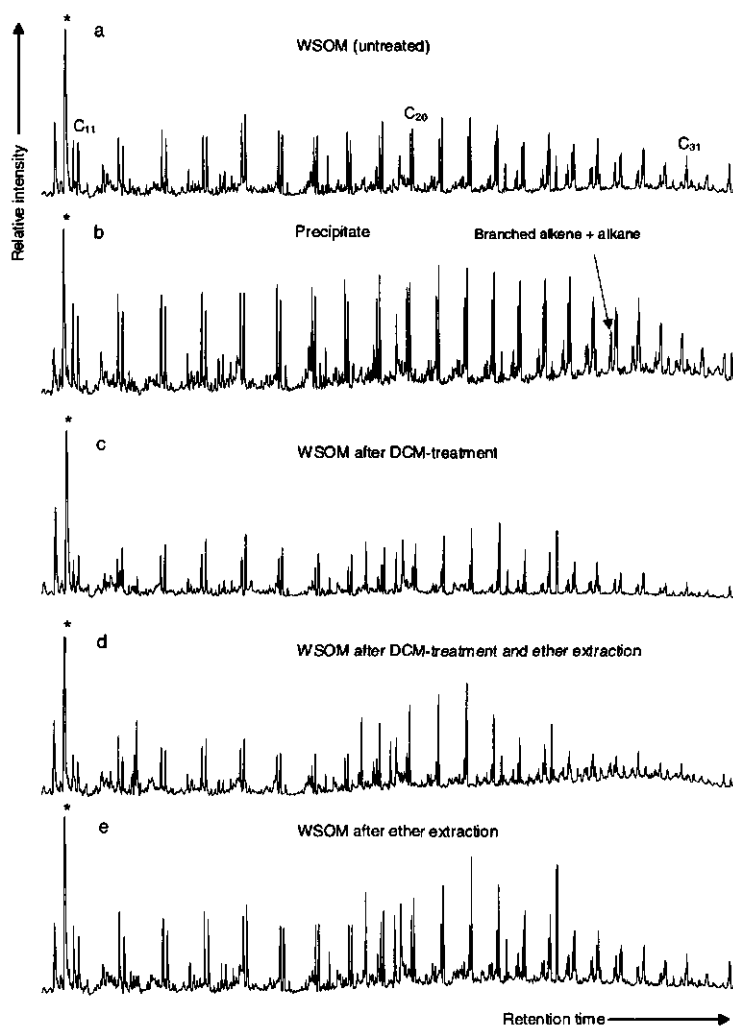

Figure 6. Alkene and alkane distribution (*m/z* 55+57) relative to 3- and 4-methylphenol peak (*) (*m/z* 108) in pyrolysates of WSOM of the Ah horizon under *Calluna vulgaris* (a), the precipitate (b), and the residual water soluble fractions after DCM-treatment (c), after DCM-treatment and ether extraction (d) and extraction with ether (e).

This phenomenon was observed before upon the characterization of the cuticles of *Symplocos paniculata* (Tegelaar *et al.*, 1993) and the outer bark tissues of some angiosperms (Tegelaar *et al.*, 1995). Pyrolysis of the precipitate revealed the presence of almost exclusively cutan, a non-hydrolyzable aliphatic biopolymer in plant cuticles (Tegelaar *et al.*, 1993), and suberan, a macromolecule analogue of cutan present in suberized plant tissues (Tegelaar *et al.*, 1995). In our samples, pyrolysis shows that the precipitate is enriched in alkene and alkane pairs in comparison with other compounds, such as methylphenols, which are also present in the precipitate (Figures 6a and c). For clarity, the mass chromatograms of the alkenes and alkanes (*m/z* 55+57) and of 3- and 4-methylphenol (*m/z* 108, used as 'internal standard') are used to show the changes in abundances. The residual organic matter in the WSOM fraction showed the opposite trend to that observed for the precipitate: the peaks of the alkenes and alkanes are much lower (Figure 6b). Extractions with ether, before or after the 'DCM-treatment', did not induce much difference (Figures 6d and e). Thus, DCM effects the behaviour of the biopolymer that gives rise to alkenes and alkanes upon pyrolysis, while ether does not. Therefore, we conclude that the alkenes and alkanes are not likely to be LMW compounds, but pyrolysis products of aliphatic biopolymers. The formation of a precipitate between DCM and water, and the absence of such a phenomenon between water and ether, may be characteristic for the aliphatic biopolymers.

Because of their poor solubility in water, the aliphatic biopolymers are probably present in water as colloidal components in micelle-like structures (Piccolo *et al.*, 1996a, b), or associated with hydrophilic compounds in 'pseudomicelles' (Engebretson and von Wandruszka, 1994). Aliphatic biopolymers have rarely been found in Py-FIMS spectra of dissolved organic matter fractions in forest soils (Hempfling and Schulten, 1990; Guggenberger and Zech, 1994) and podzol B horizons (Sorge *et al.*, 1994; Wilcken *et al.*, 1997). A reason for this may be the insensitivity of Py-FIMS in detecting these aliphatic polymers (Tegelaar *et al.*, 1989), in contrast with Py-GC/MS that we used.

The compounds found in the water soluble fractions are, in principle, the first contributors leading to podzolization. The dominant compounds in pyrolysates of WSOM under heather and pine, phenols and aliphatics, were found to be major constituents in podzol B horizons (Sorge *et al.*, 1994; Wilcken *et al.*, 1997). Nevertheless, no indications of podzolization are found in the Ah horizons of profiles 1-4. One reason may be that little WSOM is produced. Only a few percent of the total organic matter, which is also a few percent of the bulk, can be extracted with water. So these potential agents of podzolization make up only about 0.01% of the total soil, and a major part of it may be decomposed before it can cause podzolization.

CONCLUSIONS

In this study, a comparison is made between soil organic matter from Ah horizons and their WSOM fractions under young vegetation on wind-blown sand. This young vegetation is the first stage of a chronosequence, in which the soil eventually develops into a podzol. In the pre-podzol stages investigated here, the first organic matter progenitors may already be present, which could be recovered in later (podzol) stages.

Table 4 presents the relative water solubilities of the most important groups of compounds obtained by pyrolysis, as relative 'shifts' from total to WSOM fractions. Polysaccharide-derived furans, (alkyl) phenols and alkenes and alkanes dominate the pyrolysates of the WSOM fractions of all profiles. Products derived from polysaccharides are more abundant in pyrolysates of WSOM under grass and moss than in those of WSOM under heather and pine. The soil organic matter under heather and pine (profiles 3 and 4) exhibits a clear influence of the covering vegetation, and therefore differs substantially from each other.

Table 4. Relative 'shifts' in abundances of pyrolysis products in pyrolysates of WSOM with respect to the total organic matter.

	Furans	Pyrans	Anhydro-sugars	Phenols	Guaiacols and syringols	Fatty acids	Alkenes and alkanes	Alcohols, ketones, terpenoids and steroids
Profile 1	+	–	–	=	–	–	=	–
Profile 2	+	=	–	=	–	–	=	–
Profile 3	+	–	–	+	–	–	+	–
Profile 4	=	–	–	+	–	–	=	–

Explanation: +: abundance higher in WSOM than in total organic matter, =: little or no differences, and –: abundance lower in WSOM than in total organic matter.

In sharp contrast, the composition of the WSOM fractions upon pyrolysis appears to be largely independent of the vegetation, showing a great uniformity. However, the composition of the polysaccharides in the WSOM, as determined after TFA hydrolysis, differs completely between heather and pine.

With increasing development of the vegetation, lignin, (poly)phenols and aliphatic compounds exceed the polysaccharides in the Ah horizons. In WSOM, phenol-containing and aliphatic components become more important as the vegetation develops. Although visible signs of podzolization are still lacking, the compounds identified in the WSOM fractions have been found as major constituents in B horizons of well-developed podzols. This implies that the organic matter in the pre-podzol stages may indeed play a role in the initialization of podzolization.

Chapter 4

Effect of vegetation on chemical composition of H horizons in incipient podzols as characterized by ^{13}C NMR and pyrolysis-GC/MS

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ABSTRACT

In a primary vegetation sequence on wind blown sands in the Netherlands, podzolization becomes evident under pine forest with various undergrowth and is more expressed under the subsequent beech forest. Recognizable podzol-B horizons appear together with H (humified O) horizons. In order to investigate whether the vegetation influences H horizon composition and the water-soluble fraction of this horizon, H horizons of three subsequent succession stages (*Pinus/Empetrum - Fagus/Vaccinium - Fagus* without undergrowth) were sampled, fractionated, and analysed with ^{13}C CPMAS-NMR, pyrolysis-GC/MS, and by pollen analysis. Water soluble and NaOH soluble fractions of the three H horizons were very similar, suggesting that the humified material in the H horizon was formed exclusively from pine litter and that beech litter, yearly added to the soil surface in large amounts, did not influence the composition of the H horizon. That input of beech litter into the H horizon under beech was small, was supported by very low amounts of *Fagus* pollen in all H layers. Humin fractions of the three H horizons were remarkably different, which was mainly due to varying amounts of beech root litter. The similarity of the water soluble organic matter fractions suggested that a direct relation between vegetation and organic matter mobilized by humification in the H horizon only exists under pine forest. Hence, increased podzolization under beech was probably not due to changing organic matter input, but to the factor time.

INTRODUCTION

Soil organic matter is composed of a variety of compounds of plant, animal and microbial origin (Schnitzer and Kahn, 1972; Stevenson, 1994). Degradation and transformation of these compounds, together with input of fresh litter and biological homogenization, makes soil organic matter a very complex system. Furthermore, the understanding of humification is complicated in most cases by antecedent soil development and complex or unknown vegetation history. Although interactions between vegetation and soil have been recognized, - e.g. pine and Ericaceae accelerate podzolization (Petersen, 1976; Emmer, 1995a), - there is only little information about such relationships. Therefore, primary vegetation successions, which have no antecedent soil development, provide a good opportunity to study vegetation-soil organic matter relationships.

The primary vegetation succession at the Leuvenum Forest (central Netherlands) has been studied extensively and is well-documented (Fanta, 1986; Prach, 1989). Originally, the area consisted of sand dunes. About 200 years ago, reforestation and gradual colonization began. Nowadays, the area is largely covered by vegetation, and all vegetation succession stages are still present. The vegetation starts with algae, which are successively replaced by *Corynephorus canescens* (L.) Beauv., *Polytrichum piliferum* Hedw. and widely spaced *Pinus sylvestris* L. As the pine vegetation becomes denser, the undergrowth is dominated first by *Deschampsia flexuosa* (L.) Trin., which is later succeeded by Ericaceae. The undergrowth transition from *Deschampsia* to Ericaceae is found under pine trees of similar age. Meanwhile, deciduous trees appear, such as *Quercus robur* L., *Betula pubescens* L. and *Fagus sylvatica* L.. Finally, the forest is completely dominated by *Fagus* without undergrowth. In this sequence, podzolization

starts under pine trees with *Deschampsia* and is clearly developed under pine with Ericaceae and in the later stages under beech.

Under pine with *Deschampsia*, the first traces of an H horizon are found. This H horizon becomes thicker in the next stages. It appears to play a crucial role in the competition between grasses and Ericaceae, in favour of the latter (Emmer, 1995a). Furthermore, the simultaneous appearance and development of the H horizon and podzolization suggests a relation between these two.

To investigate whether any vegetation-podzolization relation is affected by the chemical composition of the H horizon, the organic matter of the H horizon was fractionated and subsequently characterized. Usually, soil organic matter is fractionated into a fulvic acid, a humic acid and a humin fraction. However, the most important source of podzol B organic matter from the upper organic horizons is water soluble organic matter. Therefore, the water soluble organic matter was extracted from the H horizons, which were subsequently extracted with 0.5 M NaOH to separate the NaOH soluble and insoluble substances.

The purpose of the present study is to characterize the organic matter, with ^{13}C NMR and flash pyrolysis-GC/MS, to get a better insight in the chemical composition of the obtained fractions from three sites with increasing vegetation succession and podzolization degree. Additionally, pollen analysis of the H horizon was performed to identify presence or absence of biological mixing. In acid media, such as podzol H and B horizons, pollen grains are preserved over time spans of centuries to millennia. If an H horizon that was formed under pine is 'fossilized' under the following beech vegetation, i.e., no biological mixing has taken place, the pollen content will only reflect the pine forest with its local undergrowth. In this case, the H horizon has only changed through decay and through addition of soluble organic matter from overlying litter. If, on the other hand, biological mixing under beech strongly affects the previously formed H horizon, the pollen spectrum will reflect the change in vegetation, and the organic matter of the H horizon will be a mixture of inputs from both pine and beech forest.

MATERIALS AND METHODS

Vegetation, soil profiles

The profiles chosen for this study are from three sites with comparable soil profiles (Cambic Arenosols, according to FAO (1988)), but with different vegetation.

Profile 1 has pine trees (*Pinus sylvestris*, about 100 years) with dominant undergrowth of crowberry (*Empetrum nigrum*). Profile 2 consists of beech (*Fagus sylvatica*) vegetation with remnants of *Vaccinium myrtillus*, and pine trees in the surroundings. Profile 3 has only beech trees.

All profiles have a thin L horizon, a considerable F horizon and a 1-2 cm thick H horizon overlying a bleached E and an illuvial Bh horizon. Biological homogenization in the whole mineral soil is virtually zero. The collected H horizons of profiles 1, 2 and 3 will be mentioned as sample 1, 2 and 3, respectively. Because the vegetation sequence is also a time sequence, the H horizon becomes considerably older from profile 1 to 3.

Fractionation

- Water soluble organic matter:
After removal of roots, field-moist samples were shaken with distilled water (sample:solvent ratio 1:4) for 16-20 hours. The solution was centrifuged, and the residue was shaken for 4 hours with distilled water (1:2). After centrifugation, both solutions were combined, filtered over a 0.45 µm filter, and freeze-dried. The yields were 0.1-0.5% (dry weight).

- NaOH soluble organic matter:
The residue after water extraction was shaken with 0.5 *M* NaOH (sample:solution ratio 1:20) under nitrogen for 24 hours. The solution was centrifuged, and the residue was shaken with distilled water for 2 hours. After centrifugation the combined solutions were filtered over a 0.45 µm filter, acidified with conc. HCl to pH=1, dialysed against distilled water, and freeze-dried. The yields were 45-55% (dry weight).

- Humin (NaOH insoluble organic matter):
The residues after extraction with NaOH were washed several times with distilled water until pH 7 and freeze-dried. The yields were 45-55% (dry weight). (Aliquots of the residues after removal of the NaOH-solution which were subsequently acidified, dialysed and freeze-dried gave similar results upon pyrolysis as the residues which were only washed with water. This means that the time-consuming acidification and dialysis steps were not necessary).

Chemical analysis

- C, N and pH
Carbon and nitrogen are measured with an Interscience Elemental Analyser EA1108, pH (H_2O and 1 *M* KCl) in a sample:solution ratio of 1:5.

- ^{13}C NMR spectroscopy
Cross Polarization-Magic Angle Spinning (CPMAS) was used for the NMR measurements, and performed on a Bruker AMX 400 operating at 100.628 MHz. The used parameters were: a spinning rate of 4.5 KHz; an acquisition time of 0.033 s; a pulse time of 4 µs; a contact time of 0.8 ms and a line broadening of 50 Hz.

- Pyrolysis-gas chromatography and pyrolysis-gas chromatography/mass spectrometry
Samples were pressed onto flattened ferromagnetic wires with a Curie temperature of 610°C. Pyrolysis was carried out by inductive heating of the wires to their Curie temperature, held for 10 s using a high frequency generator (Fischer 9425) and a FOM-4LX pyrolysis unit. The pyrolysis products were separated by a HP 5890 series II gas chromatograph equipped with a fused silica column (25 m, 0.32 mm i.d.) coated with CP-Sil 5 (film thickness 0.45 µm). Helium was used as carrier gas. The initial oven temperature was 0°C and kept for 5 minutes after injection. Then it was raised with a rate of 3°C /min to 320°C and held for 20 min. The end of

the column was coupled to a FID detector (Py-GC) or, the EI ion source of a VG Autospec Ultima mass spectrometer (mass range: *m/z* 45-800; cycle time 2 s; ionization energy 70 eV) (Py-GC/MS).

Pollen analysis

Preparation of samples for pollen analysis were performed according to Fægri and Iversen (1964). Samples from the H horizons were boiled for a few minutes in a 10% KOH solution in water. The solutions were sieved over 300 µm and centrifuged, after which the residues were washed with water. After centrifugation, the obtained residues were boiled in a solution of concentrated HF/HCl, and the residues after centrifugation were washed twice with water. The final residues were stained with basic fuchsine in glycerine. When enough pollen grains were present, 200 tree pollen were counted.

RESULTS AND DISCUSSION

General

Table 1 shows that the carbon contents of the fractions are independent of the vegetation, while the nitrogen concentrations increase strongly from humin through the NaOH soluble to the water soluble fractions, and also from sample 1 to 2 and 3 for all fractions.

Table 1. C and N mass fractions, atomic C/N ratios and ash contents of the three fractions of samples 1, 2 and 3.

Sample/fraction	C (%)	N (%)	C/N	Ash (%)
1 (pine + *Empetrum*)				
Water	45.3	1.92	27.5	7.4
NaOH	50.4	1.55	37.9	4.9
Humin	49.1	1.00	57.3	11.7
2 (beech + *Vaccinium* remnants				
Water	44.6	2.14	24.3	7.0
NaOH	51.7	1.95	30.9	5.4
Humin	49.0	1.33	43.0	12.9
3 (beech)				
Water	44.4	2.44	21.2	9.3
NaOH	51.6	2.39	25.2	4.8
Humin	49.5	1.60	36.1	6.0

Consequently, the C/N ratios decrease accordingly, with the strongest trend for the humin fractions. This suggests a prolonged decomposition of C with respect to N from the youngest sample, 1, to the oldest, 3.

^{13}C NMR

The NMR spectra of the different water soluble fractions closely resemble each other. Similarly, the spectra of NaOH soluble fractions are comparable, and so are the spectra of the humin fractions.
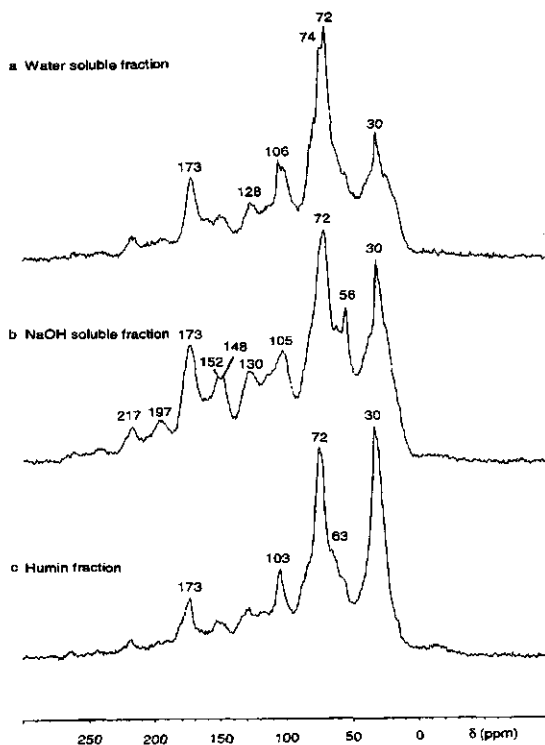

Figure 1. CPMAS ^{13}C NMR spectra of water soluble (a) NaOH soluble (b) and humin fractions (c) of sample 2 (beech + blueberry remnants).

Assignments of the peaks are based on data reported by Nimz *et al.* (1981), Hatcher (1987), Kögel *et al.* (1988), Wilson and Hatcher (1988), and Preston (1992). The chemical shift at 217 ppm is due to a spinning sideband of the carbonyl carbon peak (173 ppm). Also part of the signal at 128 ppm is caused by sideband spinning.

Peaks in the O-alkyl carbon range dominate the spectra of the water soluble fractions (Figure 1a). Signals at around 72, 74, 80 and 83 ppm are attributed to C_2, C_3, C_4, and C_5 (in case of hexose) of the sugar unit. The shoulder at 63 ppm and the peak at 103 ppm are derived from C_5 (pentose) or C_6 (hexose) and the anomeric C_1, respectively. Spectra of the NaOH soluble (Figure 1b) and humin fractions (Figure 1c) also have these peaks derived from polysaccharides, but relatively less abundant compared to the water soluble fractions.

In the water soluble fraction an important signal is observed at 106 ppm. Besides lignin, the aromatic fraction of soil organic matter can also contain tannins. Considerable amounts of tannins are soluble in water (Harborne, 1997) and the signal at 106 ppm may be derived from tannins (Wilson and Hatcher, 1988).

In the NaOH soluble fractions the aromatic peaks (115, 130, 148 and 152 ppm) are more abundant than in the other fractions. The shoulder at 115 ppm is due to unsubstituted aromatic carbons *ortho* or *para* to O-substituted carbons. C-substituted aromatic carbons appear at 130 ppm. The signal at around 148 ppm is assigned to O-substituted (OH and OMe groups) aromatic carbons of guaiacol, while the resonance at 152 ppm may be derived by aromatic carbons connected to methoxy groups in syringol units. Also the signals of aldehydes and ketones (197 ppm), carboxylic acids and amides (173 ppm) and methoxy groups (56 ppm) are more intense in the NaOH soluble fractions. The signals in the aromatic region together with the methoxy group are unambiguously due to lignin, mainly composed of coniferyl alcohol (guaiacyl-lignin).

The humin fraction has the most intense alkyl carbon signal at 30 ppm and is attributed to methylene groups. Most of these will be derived from lipids and aliphatic polymers, like the (hydrolyzable) polyesters cutin and suberin, and the non-hydrolyzable cutan and suberan (Nip *et al*, 1986a; Tegelaar *et al*, 1989b, 1995). The aliphatic signals have the lowest intensities in the water soluble fractions. The chemical shift at 26 ppm in the NMR spectrum of this fraction is probably due to methyl groups.

These results are in line with findings of Fründ and Lüdemann (1991), who studied a range of soils. These authors found, however, an increase of polysaccharides in the NaOH soluble fractions in comparison with the humin fractions.

Using NMR, integrated peaks or areas are directly related to the amounts of the measured carbon types. However, experiments with CPMAS are not appropriate for absolute quantification because a range of factors change the relative intensities of peaks or areas in different regions of the spectrum (Kinchesh *et al.* 1995a, b). Because similar types of samples do not differ much in types of carbon, relative changes in quantities of those carbon types can be obtained using the integrals. The spectra are divided in the commonly used groups of carbons: alkyl C (0-50 ppm), O-alkyl C (50-110 ppm), aromatic C (110-160 ppm) and carbonyl C (160-220 ppm). The integrated areas and the ratios of alkyl/O-alkyl, carbonyl/aromatic and carbonyl/alkyl are presented in Table 2.

Table 2. Integrated areas and ratios of different carbon types measured by ^{13}C-NMR.

Sample/ fraction	Carbonyl	Aromatic	O-alkyl	Alkyl	Alkyl/ O-alkyl	Carbonyl/ aromatic	Carbonyl/ alkyl
1 (pine + *Empetrum*)							
Water	16	16	43	24	0.56	0.98	0.66
NaOH	17	19	38	26	0.69	0.90	0.65
Humin	9	12	45	33	0.74	0.73	0.27
2 (beech + *Vaccinium* remnants							
Water	14	16	48	22	0.47	0.91	0.65
NaOH	18	19	40	23	0.58	0.93	0.77
Humin	12	14	43	31	0.71	0.84	0.39
3 (beech)							
Water	16	16	46	22	0.49	1.01	0.71
NaOH	17	18	40	25	0.67	0.92	0.68
Humin	12	14	40	34	0.85	0.84	0.35

In all samples and fractions, the O-alkyl C resonances have the highest integrals. The integrated areas in the alkyl C range increase from the water soluble fractions through the NaOH soluble fractions to the humin fractions, and the contribution of aromatic C and carbonylic C are the lowest for the humin fractions. The alkyl/O-alkyl ratio is increasing from water soluble through NaOH soluble to humin fraction. The ratio between carbonyl C and aromatic C diminishes from water soluble trough NaOH soluble to humin, although in case of sample 2 this ratio is somewhat higher for the NaOH soluble fraction in comparison with that of the water soluble organic matter. This suggests a higher solubility for aromatic moieties containing oxygen-rich functional groups. The carbonyl/alkyl ratio is much lower for the humin fractions than for the water soluble and NaOH soluble fractions. A reason for this is most probably the presence of long-chain aliphatic components in the humin fractions, whereas both soluble fractions have considerably lower amounts of aliphatics.

Pyrolysis-GC and pyrolysis-GC/MS

The pyrolysis-GC traces of the water soluble fractions are very similar. The same is valid for the NaOH soluble fractions. In contrast, the pyrolysates of the humin fractions differ substantially, particularly in the aliphatic part.

- Polysaccharides
A series polysaccharide-derived products is noticed in pyrolysates of all fractions (see Figure 2 for sample 2). The relative abundance of these products increases from the humin fractions through the NaOH soluble fractions to the water soluble fractions. The low concentrations of polysaccharides in pyrolysates of the humin fractions contrasts the significant amount of
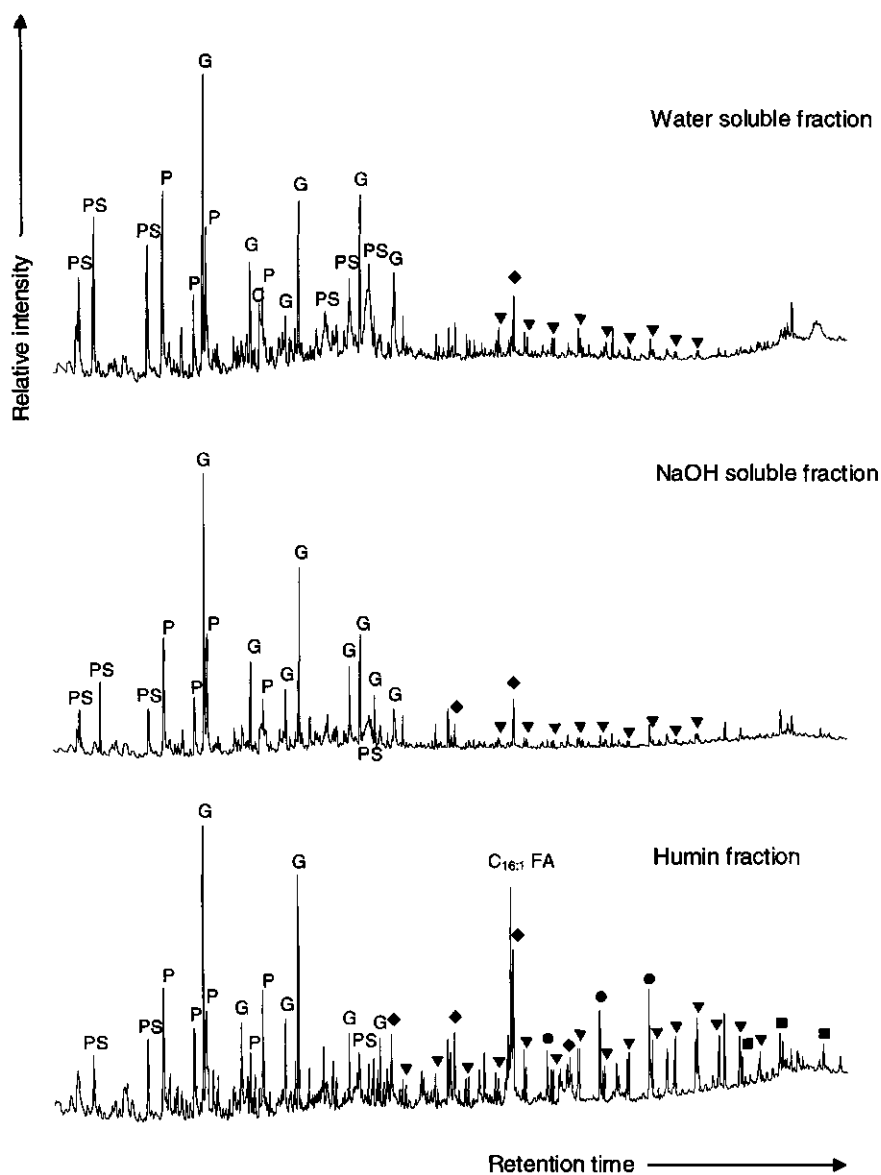
Figure 2. Partial Py-GC traces of pyrolysates of water soluble, NaOH soluble and humin fractions of sample 2 (beech + blueberry remnants). (PS: polysaccharide; P: (alkyl)phenol; G: guaiacol; ■: methylketone; ◆: (saturated) fatty acid; ▼: alkene/alkane; ●: alcohol).
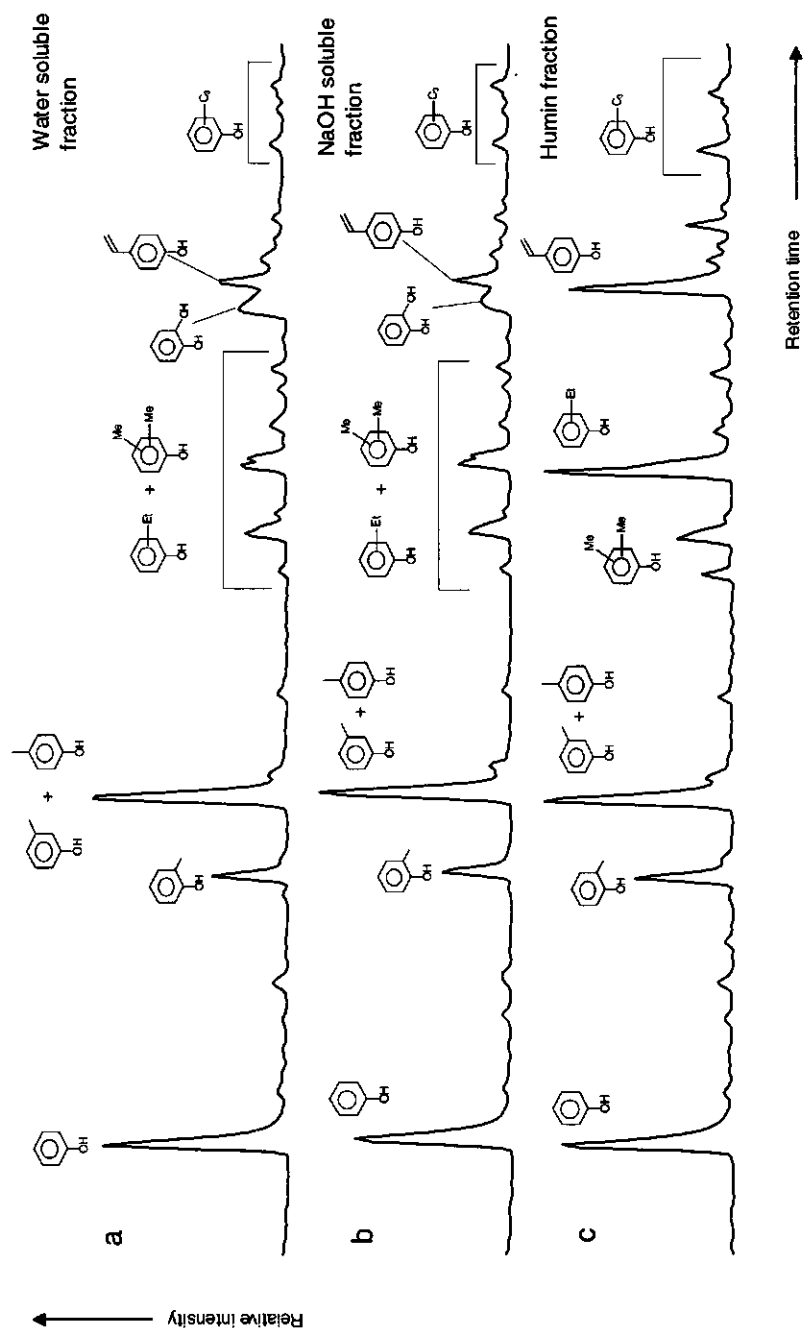
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Figure 3. Partial mass chromatogram of alkylphenols (*m/z* 94+107+108+110+120+121+122+136) of the water soluble (a), NaOH soluble (b) and humin (c) fractions of sample 2 (beech + blueberry remnants).

polysaccharide pyrolysis products in such fractions as found by Saiz-Jimenez and de Leeuw (1986b), whereas Fabbri *et al.* (1996) noticed a higher abundance of polysaccharides in humin fractions than in the humic acid fraction. The differences may be related to the different types of soils investigated.

- Phenols

Phenol and the three isomers of methylphenol (*ortho, meta* and *para*) are important compounds in the pyrolysates of the water soluble fractions. Ethyl- and dimethylphenols, catechol (1,2 dihydroxybenzene) and 4-vinylphenol are encountered in much lower amounts, while C_3-substituted phenols are rare. These phenolic compounds are observed also in the pyrolysates of the NaOH soluble fractions. Their distribution resembles that of the water soluble fractions (see mass chromatograms in Figure 3), although catechol and 4-vinylphenol are somewhat lower in intensity. The humin fraction shows a different distribution of alkylphenols. C_2- and C_3-phenols are very abundant in these fractions, especially 4-ethylphenol and 4-vinylphenol are prominently present. No traces of catechol are observed.

(Alkyl)phenols may have several origins, such as lignin (e.g. Saiz-Jimenez and de Leeuw, 1986a; van der Hage, 1995) and tannins (e.g. van der Heijden, 1994; Tegelaar *et al.*, 1995). 4-Vinylphenol is also found as the decarboxylated product from esterified coumaric acid in cuticles (Tegelaar *et al.*, 1989a). Very recently, van Heemst *et al.* (1998) found that alkylphenols are also produced upon pyrolysis after subsequently basic and acid hydrolysis of samples containing proteins and polysaccharides. The distribution of phenols was similar to those shown in Figure 3. However, also pyrolysates of dissolved organic matter, not chemically treated, had comparable phenolic compositions (van Heemst *et al.*, 1998). Up to now, it is not possible to discriminate whether differences in the phenolic distribution of the fractions after NaOH treatment and subsequent acidification reflect the natural situation, or an artificial one.

- Methoxyphenols

A series of typical pyrolysis products of gymnosperm lignin is found in the GC-traces of the pyrolysates of all fractions (see mass chromatograms in Figure 4). In the water soluble fraction the most prominent peaks represent guaiacol (**1**), 4-methylguaiacol (**2**), 4-vinylguaiacol (**4**), 4-acetylguaiacol (**11**), 4-propan-2-oneguaiacol (**12**), vanillic acid (**14**) and 4-propan-1-oneguaiacol (**15**). The latter three compounds are especially abundant. Minor compounds are 4-ethylguaiacol (**3**), eugenol (**6**), 4-propylguaiacol (**7**), vanillin (**8**), *cis* (**9**) and *trans* isoeugenol (**10**). Small traces of syringol (**5**) are observed.

The pyrolysates of the NaOH soluble fractions contain more 4-ethylguaiacol, 4-vinylguaiacol, eugenol, *cis* and *trans* isoeugenol and 4-propan-2-oneguaiacol than those of the water soluble fractions, but less vanillin and especially 4-acetylguaiacol and vanillic acid plus 4-propan-1-oneguaiacol.

In the pyrolysates of the humin fractions, all guaiacol units with oxidized side-chain are lower in intensity than in the NaOH soluble fractions, whereas the syringyl compounds (**5** and **13**) are more pronounced. In relation to (alkyl)phenols, the methoxyphenols are more abundant in the pyrolysates of NaOH soluble fractions and even more in the pyrolysates of the humin fractions than in those of the water soluble fractions.
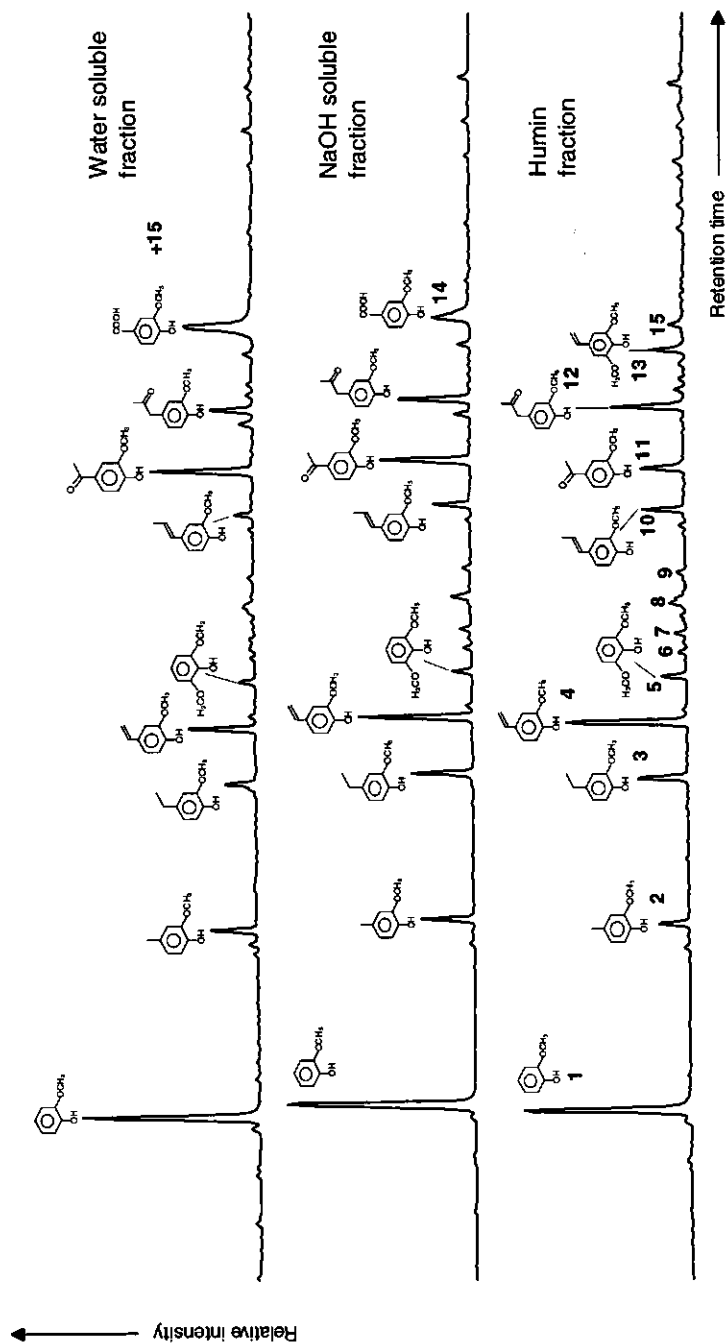
Figure 4. Partial mass chromatogram of methoxyphenols of the water soluble, NaOH soluble and humin fractions of sample 2 (beech + blueberry remnants) based on the base masses of the observed methoxyphenols.

Biodegradation of lignin is most rapidly performed by Basidiomycetes (Kirk and Farell, 1987), leading to oxidation and cleavage of the propanoid units, mainly at C_α and C_β (Saiz-Jimenez and de Leeuw, 1984). The observation of 4-acetylguaiacol, vanillic acid and 4-propan-1-oneguaiacol as relatively prominent pyrolysis products in all water soluble fractions suggests that the humification degree is the highest in these fractions. Furthermore, compounds like 4-vinylguaiacol and *trans* isoeugenol as important products in the pyrolysates of the humin fractions point to slightly alterated lignin (Saiz-Jimenez and de Leeuw, 1986b) and is in line with results of Ertel and Hedges (1984). Compared to guaiacyl-lignin, the contribution of syringyl-lignin is very small. Their abundance increases from sample 1 to samples 2 and 3. Because plant litter is mainly found in the humin fraction, and the syringols are more abundant in the humin fractions, differences in presence of syringyl-lignin in the H horizons are probably due to undecomposed plant litter (Beyer, 1996). Because biological mixing is virtually absent, roots are probably the most important source of fresh litter input (Emmer, 1995a). So even under beech forest, the H horizon almost exclusively contains gymnosperm lignin. The relative abundance of aromatics in the fractions as determined by NMR coincides with the relative intensities of (methoxy)phenols in the pyrolysates: it increases from humin fractions through water soluble fractions to NaOH soluble fractions.

- Lipids
Aliphatic pyrolysis compounds, mainly fatty acids, alkenes and alkanes, are present in the pyrolysates of the water soluble fractions in low abundances. In the pyrolysates of the NaOH soluble fractions the contribution of aliphatic pyrolysis products is even lower than in the water soluble fractions, while the composition is very similar. Differences between samples are very small. The fatty acids in the NaOH fractions may be released from (poly)esters during extraction with NaOH, resulting in an enhanced solubility.

Notwithstanding the great similarity of water soluble and NaOH soluble products, major differences are found among the humin fractions of the three investigated samples (see mass chromatograms, Figure 5). As shown in Figure 2, the aliphatic compounds are present in much higher quantities in the humin fractions, in accordance with the NMR data.

Fatty acids (FA's) are observed in the range C_4-C_{22}, and are dominated by those with an even number of carbons. In samples 1 and 2 C_{16} fatty acid is dominating, while in sample 3 this fatty acid has similar concentrations as the other fatty acids. Unsaturated fatty acids, one $C_{16:1}$ FA and at least four $C_{16:2}$ FA's, are decreasing from sample 1 through 2 to 3. Simultaneously, a similar increase is found with respect to long-chain alcohols (C_{22} and C_{24}).

All these compounds are pyrolysis products from (extractable) lipids, like waxes and triglycerides, and from monomers released during hydrolysis (NaOH treatment) of the polyesters cutin and suberin (Tegelaar *et al.*, 1991, 1995). Cutin is composed of hydroxy and epoxy fatty acids with predominant chain lengths of C_{16} and C_{18} and forms the extracellular membrane between the aerial parts of plants and their environment (Kolattukudy, 1977, 1980). Relatively high amounts of phenolics, ω-hydroxy fatty acids, dicarboxylic acids and long-chain alcohols (>C_{18}) distinguishes suberin from cutin (Kolattukudy, 1980). The relatively high abundance of C_{18}, C_{20} and C_{22} fatty acids in relation to C_{16} fatty acid in the pyrolysate of the humin fraction of sample 3 points to a higher concentration of suberin in the original H horizon. The C_{22} and C_{24} alcohols are evaporation products rather than pyrolysis products. After
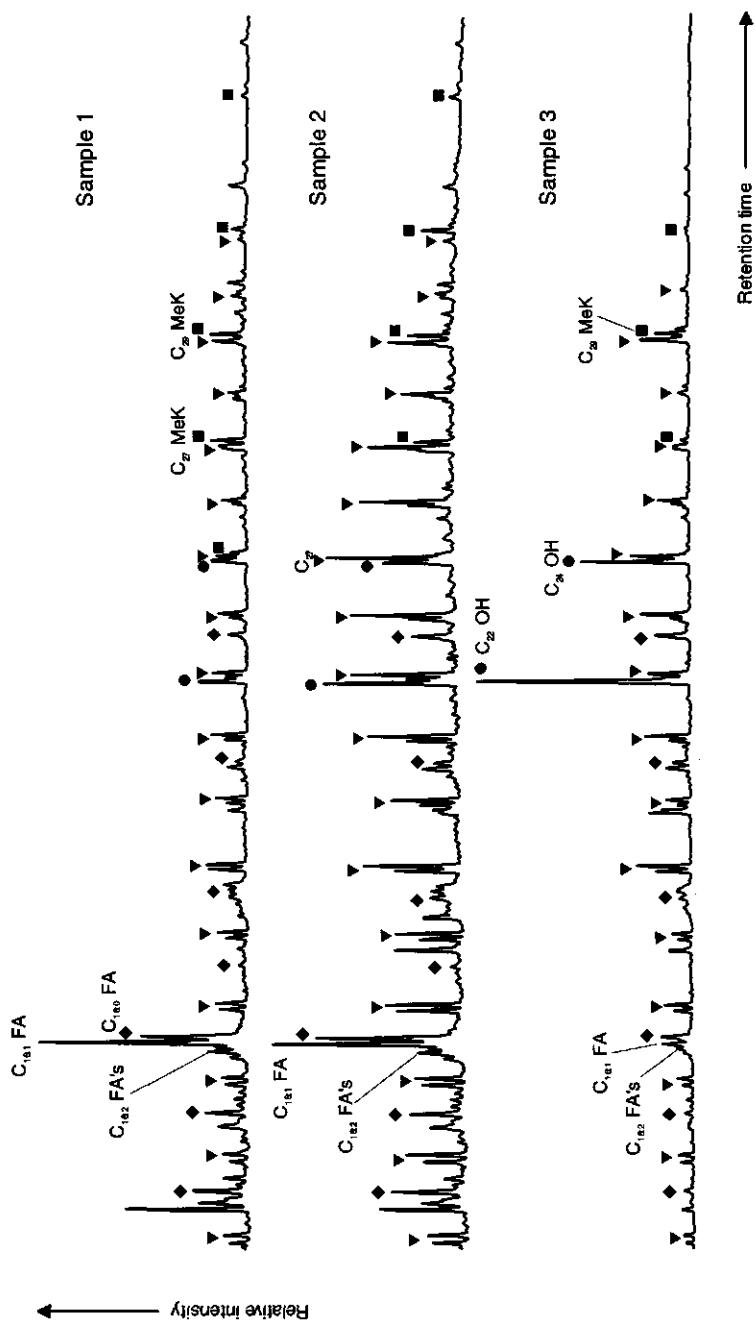
Figure 5. Partial summed mass *(m/z 55+57+59)* Py-GC traces of aliphatic components in the humin fractions of samples 1 (pine + crowberry), 2 (beech + blueberry remnants) and 3 (beech) (♦: (saturated) fatty acid; ▼: alkene/alkane doublet; ●: alcohol; ■: methylketone).

extractions with methanol/dichloromethane those alcohols are hardly present in the pyrolysates. Pyrolysis of the untreated samples of H horizons of profiles 1, 2 and 3 revealed intense signals of C_{22} and C_{24} alkenes and minor amounts of the corresponding alcohols (Chapter 5). The relative amounts of these alkenes and alcohols were the lowest in sample 1 and the highest in sample 3, a similar trend as found for the alcohols in the humin fractions. The alkenes are derived from esterified alcohol moieties (van Smeerdijk and Boon, 1987; de Leeuw and Baas, 1993). This means that most of the alcohols in the humin fractions were originally present as esters. These are probably hydrolysed during the extractions with NaOH, and since no increase is found for the C_{22} and C_{24} alkenes, the hydrolysis is almost completely. Suberin of beech contains both C_{22} and C_{24} alcohols (Holloway, 1983; Matzke and Riederer, 1991), and the increase of these alcohols coincides with the increase of beech root input from sample 1 to sample 3. Figure 5 also shows a homologous series of alkenes/alkanes. They are abundant in the pyrolysates of humin fractions, though they also appear in the pyrolysates of the water soluble and NaOH soluble fractions, in much smaller amounts (see Figure 2). They indicate the presence of aliphatic polymers, such as cutan (Nip *et al.*, 1986), or suberan (Tegelaar *et al.*, 1995). However, also salts of fatty acids produce alkenes and alkanes upon pyrolysis (Hartgers *et al.*, 1995). This last phenomenon may be important in the pyrolysis of the humin fractions, because these fractions are only washed with water after extraction with NaOH, and are not acidified, so that saponified fatty acids can be anticipated in the final residue. However, upon pyrolysis the non-acidified samples appeared to be very similar acidified samples, implying that the alkenes and alkanes are indeed from aliphatic biopolymers

Methylketones are observed in the pyrolysates of the humin fractions ranging from C_{19} to C_{33}, with an odd over even predominance. A symmetrical distribution is observed, with the top at C_{27} and C_{29} in sample 1, and at C_{29} for sample 2, whereas for sample 3 only C_{29} is highly abundant. After extractions, these ketones are removed, implying that they are evaporated during pyrolysis. The origin of the ketones is most probably the suberin matrix as found for roots in *Calluna* (van Smeerdijk and Boon, 1987), and which may also be true for *Empetrum*.

Pollen analysis

Pollen analysis was applied to establish whether the H horizons could really have formed under pine forest and be preserved without biological mixing under beech. The results concerning pollen analysis of the H horizons are presented in Table 3, which is divided into two parts. The first part contains the data about tree pollen (AP), and the second part deals with the non-tree species.

The *Pinus* pollen dominate in all H horizons. Under crowberry (sample 1) only a small percentage of Ericaceae pollen is noticed. Similarly, in samples 2 and 3 under beech, very low amounts of *Fagus* pollen are counted, but relatively high amounts of pollen from Ericaceae and Gramineae. Therefore, the absence of beech pollen in H horizons under beech indicates the absence of biological mixing. This, together with the strong chemical resemblance of the H horizons under beech with those under pine, and the obvious chemical difference with beech litter, indicates that the H horizon that is now found under beech was already formed before the

beech vegetation was established and can be considered as 'fossilized', notwithstanding the huge annual addition of beech leaf litter.

Pollen decay in leaf mold is similar for *Betula, Fagus, Pinus* and *Quercus* (Havinga, 1984). Thus, the relative low amounts of beech pollen in the H horizons of soils under beech canopy are probably not due to selective preservation of the other pollen types. This means that the H horizons are formed under pine forest and reflect the vegetation at the time of formation. Mixing of leaf mold of subsequent vegetation with the H horizon that formed under pine forest does not occur.

Table 3. Pollen analysis (in duplicate) of samples 1, 2 and 3.

Pollen type	Sample 1		Sample 2		Sample 3	
	% with respect to arboreal pollen (AP)					
Trees						
Alnus	0	4	2	1	5	0
Betula	9	12	9	12	22	8
Carpinus	0	0	0	1	1	0
Cornus	0	0	1	0	0	0
Corylus	4	1	0	1	2	2
Fagus	0	2	1	3	3	5
Pinus	82	76	84	80	59	79
Quercus	5	5	2	2	8	6
Ulmus	0	0	1	0	0	0
Others						
Cerealia	127	4	1	3	5	6
Chenopodiaceae	0	2	0	0	1	0
Compositae	0	0	0	1	1	0
Cyperaceae	0	4	0	1	0	1
Ericaceae	9	5	3	4	28	2
Filices	0	0	0	0	1	0
Gramineae	23	26	17	13	18	15
Unidentified	0	4	6	1	8	3
Sum AP grains	22	200	201	100	191	196

CONCLUSIONS

Organic matter in the H horizon of the three investigated stages, under pine and crowberry (1), beech with remnants of blueberry (2) and without undergrowth (3) appeared to be mainly derived from pine litter: pyrolysis showed an almost exclusively gymnosperm character of lignin and pollen analysis supported the evidence. Only the humin fractions show a relation with the current vegetation. The composition of the aliphatic part of the humin fractions differ substantially: from sample 1 to 3 the abundance and distributions of unsaturated C_{16} fatty acids, methylketones and C_{22} and C_{24} alcohols in pyrolysates differ. The differences can be related to suberin, and its presence may be associated to the input of beech root litter.

The water soluble as well as the NaOH soluble fractions are similar for all samples and seem to be dependent of the previous (pine) vegetation. These fractions, and especially the water soluble fractions, are important sources for podzol B horizon organic matter. Therefore a direct relationship between the present vegetation and organic matter composition in the H horizon and the underlying Bh horizon is only expected under pine vegetation. The large amounts of beech leaf litter that are yearly deposited on the soil surface do not influence the composition of the H horizon or of the water soluble fraction. Most probably such a relation may be found in the upper (L and F) horizons.

Chapter 5

Organic matter composition in ectorganic horizons as affected by vegetation in a primary succession on drift sand

ABSTRACT

The origin and nature of organic matter in the ectorganic layers from four profiles from different stages of a primary vegetation succession on drift sand has been used to evaluate the impact of the vegetation on organic matter formation. With depth, and thus increasing humification degree, the contribution of polysaccharides decreased, the contents of lignin and tannins remained approximately constant and aliphatic compounds accumulated relatively, partly due to cutan/suberan and partly to cutin and suberin. The bulk of the ectorganic horizons in the pine forest seemed hardly affected by the understorey. The aliphatic fraction, however, clearly showed the input of the undergrowth by its cutin and suberin composition. Using the distribution of the cutin and suberin building blocks, an inventory was possible of above- and below ground litter as organic matter sources. Especially the F1 and F2 horizons had distinct input of undergrowth-derived organic matter. In the beech forest, the L and F horizons were mainly composed of above-ground beech litter, whereas the H horizons appeared to be largely derived from pine litter of the previous vegetation stages. Beech roots, however, most clearly contributed to the organic matter in the H horizons as observed for beech-suberin, which contents increased from younger to older beech stands. Lignin was not useful as biomarker in the investigated profiles because of its relative fast decomposition, and cutin and suberin appeared to be better tracers of the organic matter origin.

INTRODUCTION

Organic matter development on drift sand has been studied in the past as a function of primary vegetation succession (Fanta, 1986; Prach, 1989), with emphasis on humus forms and soil development (Emmer, 1995a) in the Leuvenum Forest (central Netherlands). During the succession, the initial vegetation of grasses, mosses and herbs makes way for Scots pine and beech forest. The dominant forest undergrowth is wavy hair-grass (*Deschampsia flexuosa* (L.) Trin. but the formation of an H horizon appears to be crucial for *Empetrum nigrum* L. and *Vaccinium myrtillus* L. to establish and outcompete *Deschampsia* (Emmer, 1995b). When the pine forest is about 100 years old, organic matter input and decomposition reach an equilibrium (Emmer and Sevink, 1994). Pines dominate the development of the ectorganic layer, but the shrubs have a modifying effect. For instance, *Empetrum* and to some extent also *Vaccinium*, positively affect accumulation of organic matter in the ectorganic layer (Miles, 1985; Emmer, 1994). The L and F1 horizons have significantly higher decomposition rates than the underlying F2 and H horizons (Emmer, 1995a). The undergrowth roots predominantly in the ectorganic layers, suggesting that a decay continuum with depth is not occurring, although the increasing fragmentation from L to H horizons would indicate such continuum (Emmer, 1995a).

Little is known about the organic matter formation at the molecular level in the research area of the Hulshorster Sand and Leuvenum Forest. Organic chemical characterization methods have been proven to be a useful tool to increase the knowledge of origin and occurrence of organic matter, thus providing additional information concerning development

of soil organic matter. To investigate if and to what extent above- and below-ground understorey litter contributes to the ectorganic horizons and thus verifying the hypothesis about decay continuum or not, the organic layers of four profiles from different stages of a primary vegetation succession are analyzed for its organic chemical composition using a combinational approach including ^{13}C NMR spectroscopy, chemical degradation (acid hydrolysis of polysaccharides), pyrolysis and thermochemolysis.

MATERIALS AND METHODS

Vegetation, profiles

Profiles 1 and 2 are under *Pinus sylvestris* L. with *Deschampsia flexuosa* and *Empetrum nigrum*, respectively, as dominant understorey vegetation. Profile 3 is under beech trees (*Fagus sylvatica* L.), with some *V. myrtillus*, and profile 4 is under beech without undergrowth (see also Figure 1). Descriptions of the profiles are summarized in Table 1. Prior to analysis, the samples were air-dried and milled. Prior to pyrolysis and thermochemolysis, samples were extracted with methanol/dichloromethane (1:1) in a Soxhlet apparatus to remove lipids.
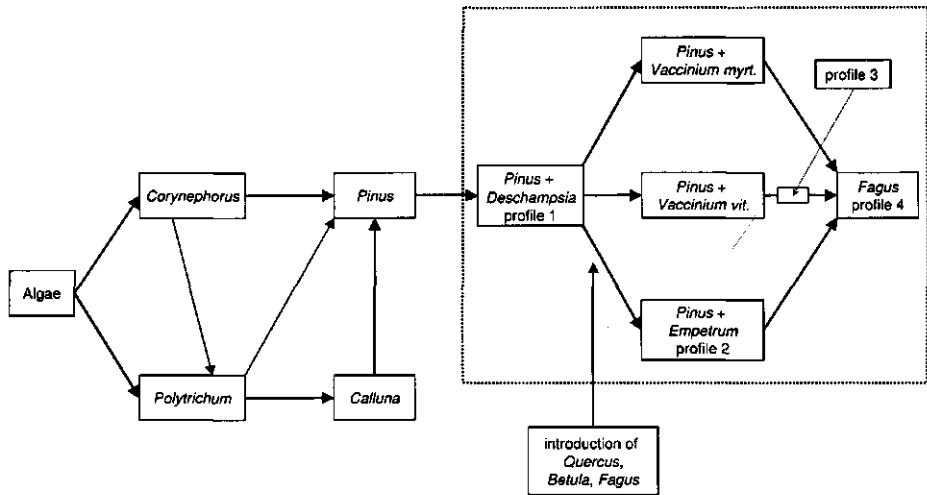

Figure 1. Simplified scheme of vegetation succession at Hulshorster Sand and Leuvenum Forest. Profiles 1-4 are indicated in the dashed box.

Table 1. Profile descriptions, total carbon and nitrogen, C/N ratios and pH in soil samples of profiles 1-4.

Profile	Horizon	Thickness (cm)	Description	C(%)	N(%)	C/N	pH(H₂O)	pH(KCl)
1	L	0.5	Pine needles, bark and some grass foliage	48.7	2.0	24.5		
	F1	5	Grass roots and partly fragmented grass foliage and needles	47.5	2.0	24.1		
	F2	3	Abundant grass roots and fragmented grass foliage and pine needles	43.1	1.6	26.4		
	H	0.5	Completely humified brown/black material and a few grass and pine roots	35.1	1.3	26.6		
	AE	1	Few roots	0.7	0.04	19.4	3.8	3.4
	BC	3	Very few roots	0.3	0.02	20.2	3.9	3.8
2	L	0-1	Pine needles and *Empetrum* parts	50.6	2.2	22.8		
	F1	6	Fragmented pine and *Empetrum* parts and abundant *Empetrum* roots	52.0	2.0	26.0		
	F2	5	Fragmented material and abundant *Empetrum* roots	52.7	1.8	29.3		
	H	3	Black, with humified material and a few roots and a sharp transition to:	45.5	1.3	34.2	3.5	2.5
	E	2	Grey-white with few roots	0.4	0.02	20.2	3.9	3.4
	B	6	Very few roots	0.4	0.02	20.4	3.7	3.5
3	L	3	Dominated by beech leaves and a few pine needles and oak leaves	41.8	1.7	24.6		
	F	3	Fragmented parts of the same tissues as in the L and abundant fine beech roots	47.9	2.1	22.4		
	H	2	Black with some beech roots in humified material and a very sharp transition to:	32.7	1.1	30.6	3.5	2.5
	E	4	Grey with some roots	0.4	0.02	19.5	3.8	3.4
	B	6	Yellow-brown Bh with a few roots	0.4	0.02	20.3	3.8	3.7
4	L	4	Beech leaves	42.1	1.9	22.1		
	F	4	Fragmented beech parts and a root mat at the bottom	46.2	2.0	23.5		
	H	3	Black with few roots	33.5	1.4	24.1	3.7	2.6
	E	8	Grey with few roots	0.7	0.04	18.4	3.8	3.4
	B	11	Yellow-brown with very few roots	0.8	0.04	22.2	3.7	3.4

Chemical analysis

-C, N and pH
Carbon and nitrogen were measured in duplicate with an Interscience Elemental Analyser EA1108, pH (H_2O and 1 *M* KCl) in a sample:solvent ratio of 1:5. The relative standard deviation for carbon was 0.3%, for nitrogen 0.5%. The results are summarized in Table 1.

-^{13}C NMR
Cross Polarization-Magic Angle Spinning (CPMAS)-NMR measurements were performed on a Bruker AMX 300 operating at a frequency of 75.48 MHz, with an acquisition time of 0.033 s; a pulse time of 4 µs; a contact time of 0.8 ms; spinning rate 3.375 KHz and a line broadening of 50 Hz.

-Monosaccharides
The monosaccharide composition of the non-cellulosic polysaccharides was analysed according to Amelung *et al.* (1996). Duplicates of 10-100 mg, depending on the C content, were hydrolyzed with 4 *M* trifluoroacetic acid (TFA) at 105°C for 4 h. The hydrolysate was purified using XAD-7 and Dowex 50 W X8. Monosaccharides were oximized with *O*-methylhydroxylammonium hydrochloride in *N*-methylpyrrolidone (NMP) at 75°C for 1 hr. and further derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 75°C for 5 min (Andrews, 1989). The reaction products were separated on a Interscience GC8000 with a J&W DB-5 column (30 m, film thickness 1.00 µm, i.d. 0.25 mm) connected to a Fisons MD 800 mass spectrometer (mass range *m/z* 50-450, cycle time 0.50 *s*). The GC temperature started at 160°C for 4 min, next was raised at a rate of 8°C/min to 180°C. After 2 min. it was further raised by 1°C/min to 195°C (2 min), and finally increased to 255°C at 10°C/min and maintained there for 5 min. Quantification was carried out using *myo*-inositol as internal tracer and *O*-methylglucose as internal standard. The standard error did not exceed 10% for individual sugars, except at concentrations below 0.5 mg/g C, where errors reach 50%. The errors of the ratios (C_6/C_5 and deoxy/C_5) are below 6%.

-Pyrolysis-GC/MS and thermochemolysis
Pyrolysis was carried out on a Horizon Instruments Curie-Point pyrolyser. Samples were heated for 5 s at 610°C. The pyrolysis unit was connected to a CE gas chromatograph and the products were separated by a fused silica column (Chrompack, 25 m, 0.25 mm i.d.) coated with CP-Sil 5 (film thickness 0.40 µm). Helium was used as carrier gas. The oven was initially kept at 40°C for 1 minute, next it was heated at a rate of 7°C/min to 320°C and maintained at that temperature for 20 min. The end of the GC column was coupled to a Fisons MD 800 mass spectrometer (mass range *m/z* 45-650, ionization energy 70 eV, cycle time 1 s). Thermochemolysis (J.W. de Leeuw, pers. com.) was performed by adding a droplet of a 25% solution of tetramethylammonium hydroxide (TMAH) in water to the sample, after which the sample was slightly dried, and immediately pyrolyzed using the same the GC program as with pyrolysis.

RESULTS

13C NMR

The spectra of L, F1, F2 and H horizons of profile 1 (Figure 2) are dominated by peaks in the O-alkyl region (62, 72 and 104 ppm) and indicate the presence of carbohydrates (e.g. Kögel-Knabner, 1992, 1997). In the L and F1 horizons alkyl peaks (around 30-33 ppm) have more or less the same intensity but are distinctly lower than the O-alkyl peaks. Alkyl peak intensity increases with depth to values similar to those of the O-alkyls in the H horizon.
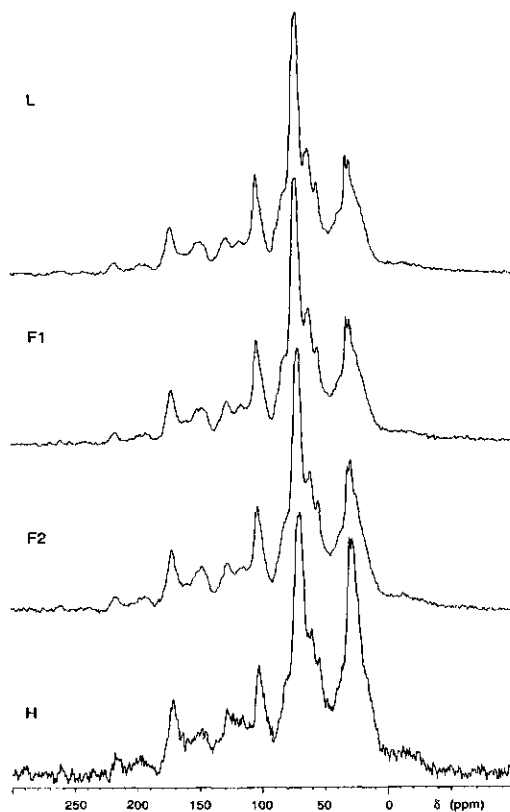
Figure 2. ^{13}C NMR spectra of profile 1 (under pine and *Deschampsia*).

The aromatic and carbonylic peaks hardly change in all spectra, which is substantiated by the integrated values of the different carbon types as presented in Table 2.

Table 2. Integrated values and alkyl/O-alkyl ratio of carbon types as determined by ^{13}C-NMR.

Sample		Carbonyl	Aromatic	O-alkyl	Alkyl	Alkyl/O-alkyl
Profile 1	L	6	12	56	26	0.47
Pine + *Deschampsia*	F1	6	12	55	26	0.48
	F2	8	12	51	29	0.57
	H	6	13	45	35	0.77
Profile 2	L	6	12	51	31	0.61
Pine + *Empetrum*	F1	8	14	50	29	0.57
	F2	8	13	49	30	0.61
	H	7	14	44	35	0.80
Profile 3	L	8	10	60	22	0.37
Beech	F	9	14	51	26	0.51
	H	10	14	43	33	0.77
Profile 4	L	9	11	57	23	0.40
Beech	F	9	14	53	24	0.45
	H	11	14	44	31	0.70

Carbonyl = 220-160 ppm; aromatic = 160-110 ppm; O-alkyl = 110-50 ppm and alkyl = 50-0 ppm.

The alkyl/O-alkyl ratio is supposed to indicate the degree of decomposition (Baldock *et al.*, 1997). For profile 1 these values suggest comparable degrees of decomposition in the L and F1, and extents of decomposition in the F2 and H horizons. For profile 2, the spectra of L, F1 and F2 horizons closely resemble each other, whereas the H horizon is much richer in alkyl C. Under beech (see Figure 3) the profiles differ from profiles 1 and 2 in that alkyl carbons regularly increase with respect to the other carbon types from L through F to H horizon, whereas the aromatic signals remain approximately constant. Accordingly, the alkyl/O-alkyl ratio increases from L via F to H in profiles 3 and 4.
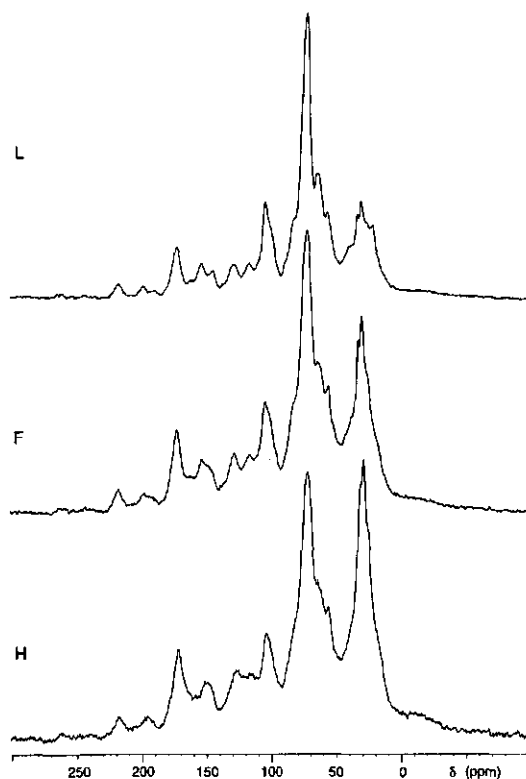
Figure 3. ^{13}C NMR spectra of profile 4 (under beech).

Polysaccharides

Table 3 presents the data dealing with the occurrence and origin of carbohydrates in the ectorganic layers. The L and F1 horizons of profile 1 and 2 have similar amounts of monosaccharides, which decrease with depth in the F2 and H layers. The lower ratios in profile 1 compared with the respective layers in profile 2 is mainly due to higher amounts of xylose, probably derived from the *Deschampsia* litter. The contents of non-cellulosic sugars are much higher in the L horizon (profiles 3 and 4) than profiles1 and 2. Their contents decrease with depth into the F and H horizon. High ratios of C_6/C_5 (galactose+mannose/arabinose+xylose) and deoxy/C_5 ratio (fucose+rhamnose/

arabinose+xylose) point to an important microbial influence, whereas 'low' ratios mark a predominant plant origin (Oades, 1984; Murayama, 1984). In all profiles both ratios hardly differ between L and F horizons, but are much higher in the H horizons.

Table 3. Total amounts of non-cellulosic sugars in mg/gram C, C_6/C_5* and deoxy/C_5* ratios of ectorganic layers.

Sample	Total	C_6/C_5	Deoxy/C_5
Profile 1			
L	343	0.36	0.07
F1	337	0.30	0.06
F2	173	0.44	0.10
H	141	0.95	0.20
Profile 2			
L	195	0.60	0.14
F1	161	0.80	0.17
F2	236	0.67	0.17
H	78	1.13	0.24
Profile 3			
L	619	0.21	0.09
F	264	0.39	0.13
H	97	1.07	0.25
Profile 4			
L	605	0.23	0.08
F	244	0.39	0.12
H	111	0.78	0.23

*C_6/C_5 ratio: (galactose+mannose/arabinose+xylose) and
deoxy/C_5 ratio: (fucose+rhamnose/arabinose+xylose).

Pyrolysis

- Vegetation
The pyrolysates of pine needles and fresh leaves of *Deschampsia*, *Empetrum* and *Fagus* are dominated by alkylphenols and methoxyphenols (guaiacols and syringols), typical pyrolysis products of lignin (Figure 4 and Table 4 for peak identifications). In agreement with usually observed for grass-lignin, 4-vinylguaiacol is by far the dominant lignin-derived pyrolysis product (Saiz-Jimenez and de Leeuw, 1986; van Bergen *et al.*, 1997; Huang *et al.*, 1998).
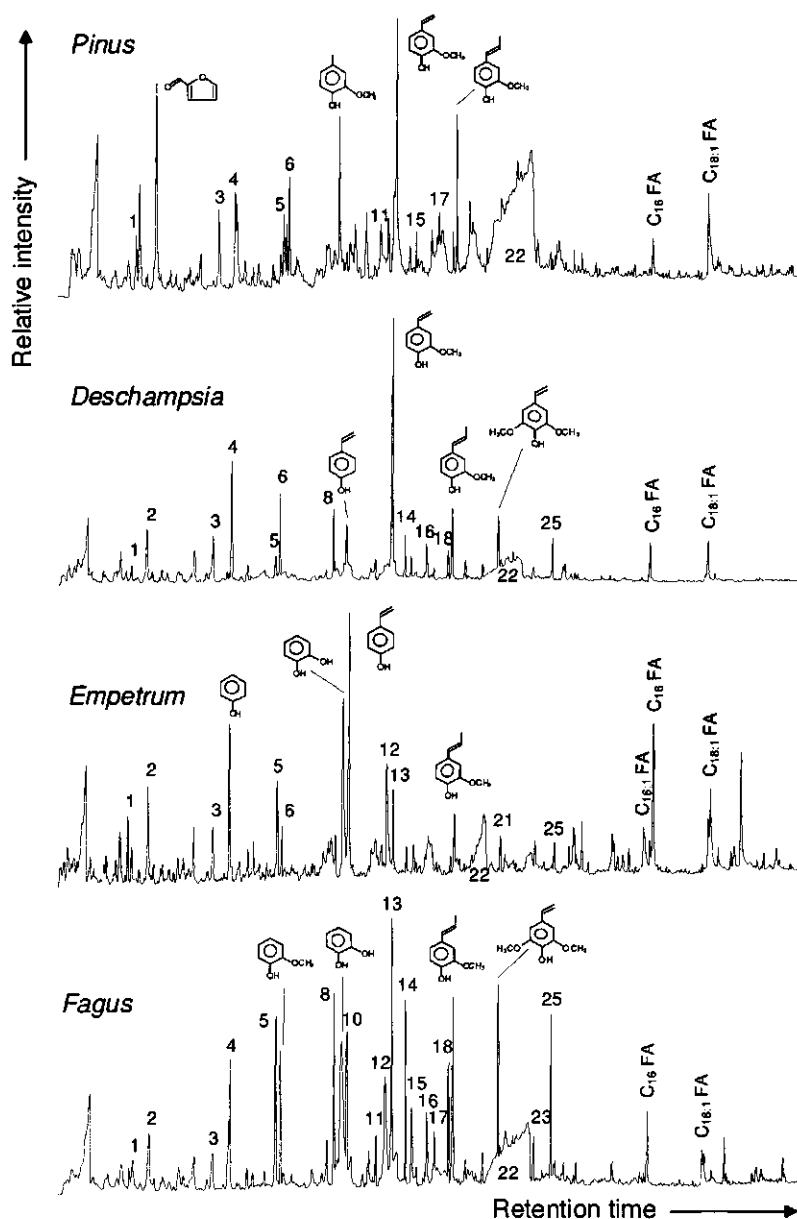
Figure 4. Pyrolysis-GC traces of leaves of *Deschampsia*, *Empetrum* and *Fagus*. Numbers correspond to compounds listed in Table 4.

Table 4. Main pyrolysis products.

No.	Compound	Origin
1	Toluene	Pr/Pp
2	2-Furaldehyde	Ps
3	5-Methyl-2-furaldehyde	Ps
4	Phenol	Pp/Pr/Lg
5	Methylphenol	Pp/Pr/Lg
6	Guaiacol	Lg
7	Levoglucosenone	Ps
8	4-Methylguaiacol	Lg
9	Catechol	Pp
10	4-Vinylphenol	Lg/Pp
11	4-Ethylguaiacol	Lg
12	Methylcatechol	Pp
13	4-Vinylguaiacol	Lg
14	Syringol	Lg
15	4-(Prop-1-enyl)guaiacol (eugenol)	Lg
16	Vanillin	Lg
17	*cis* 4-(2-Propenyl)guaiacol	Lg
18	4-Methylsyringol	Lg
19	*trans* 4-(2-Propenyl)guaiacol	Lg
20	4-Acetylguaiacol	Lg
21	4-Vinylsyringol	Ps
22	Levoglucosan	Ps
23	4-(Prop-1-enyl)-syringol	Lg
24	*cis* 4-(2-Propenyl)syringol	Lg
25	*trans* 4-(2-Propenyl)syringol	Lg
26	4-Acetylsyringol	Lg
27	Diketodipyrrole	Pr/Pep
28	Phytadiene	

Abbreviations and symbols: Pr: protein, Pp: (poly)phenol, Ps: polysaccharide, Lg: lignin, Pep: (poly)peptide.

Pyrolysates of leaves of *Empetrum* and *Fagus* have abundant signals of 4-vinylphenol, catechol and methylcatechol, which likely originate from tannins (Galletti and Reeves, 1992).

Other important pyrolysis products in all pyrolysates are derived from polysaccharides (e.g. Pouwels and Boon, 1990) and fatty acids ($C_{16:1}$, C_{16} and $C_{18:1}$). Pine roots appear to have a much higher content of tannins than the needles as suggested by the intense signals of phenols and catechol. Apart from a lower abundance of 4-vinylguaiacol and almost complete

lack of lipids, the pyrolysates of *Deschampsia* roots (not shown) are very similar to those of the leaves.In contrast to *Deschampsia*, roots of *Empetrum* and *Fagus* differ substantially from the respective above-ground tissues. The pyrolysates of roots of *Empetrum* and *Fagus* have almost no syringyl-lignin, a high abundance of 4-vinylguaiacol and hardly any 4-vinylphenol.

- Profiles
The pyrolysates of the ectorganic layers of profile 1 and 2 (see Figure 5 for profile 1) are dominated by pyrolysis products of polysaccharides (2-furaldehyde, 5-methyl-2-furaldehyde, levoglucosenone, anhydrosugars), tannins (phenol, methylphenols, catechol), lignin (guaiacol, 4-methylguaiacol, 4-vinylguaiacol, vanillin, *trans* isoeugenol, 4-acetylguaiacol). Syringols are hardly present in all pyrolysates of profiles 1 and 2. The distribution of compounds in the pyrolysates of L and F1 horizons is very similar to each other, and resembles that of pine needles (see also Figure 4), and the transition from F1 via F2 to H is marked by a relative increase of levoglucosenone, vanillin and 4-acetylguaiacol, and decreases of 4-vinylphenol, catechol, methylcatechol, eugenol, *cis* and *trans* isoeugenol.

In both profiles under beech, the pyrolysates are dominated by syringols and guaiacols, as shown for profile 4 in Figure 6. Also phenol, methylphenol, catechol, levoglucosan, C_{16} and $C_{18:1}$ fatty acids, and a series of *n*-alkenes/alkanes are distinct pyrolysis products in the L horizon.The latter alkene and alkane pairs indicate the presence of macromolecules like cutan (e.g. Nip *et al.*, 1986). From L through F to H horizon, the concentration of syringols decreases. Along the same sequence, catechol and the three propenylguaiacol isomers decrease, a trend coinciding with increases of 4-acetylguaiacol, diketodipyrrole, $C_{18:1}$ fatty acid, and the alkenes and alkanes. Especially pronounced are the increases in C_{22} and C_{24} alkenes and C_{22} alcohol. These compounds are present in considerable amounts in pyrolysates of profile 3, but are particularly prominent in those of profile 4.

Thermochemolysis

- Vegetation
Thermochemolysis yields mainly two sets of products. The first set includes aromatic compounds, such as methylated versions of guaiacol, catechol, three isomers of trihydroxybenzene, vanillin, vanillic acid, syringaldehyde, syringic acid, coumaric acid, ferulic acid and two isomers of 4-(2-hydroxyethylene)guaiacol. These compounds are mainly derived from lignin (Clifford *et al.*, 1995; Hatcher and Minard, 1996) and tannins (van der Heijden, 1994). The second set contains several series of methyl esters and ethers of fatty acids, (mono, di and tri)hydroxy fatty acids, dioic acids and alcohols, which are all derived from cutin (de Leeuw and Baas, 1993) or suberin (Nierop, 1998).

Table 5 shows the most important cutin- and suberin-derived products upon thermochemolysis in needles, leaves and roots from the vegetation. Although it is not clear whether, and to what extent, *p*-coumaric and ferulic acid are connected to cutin and suberin, they are included in this table.
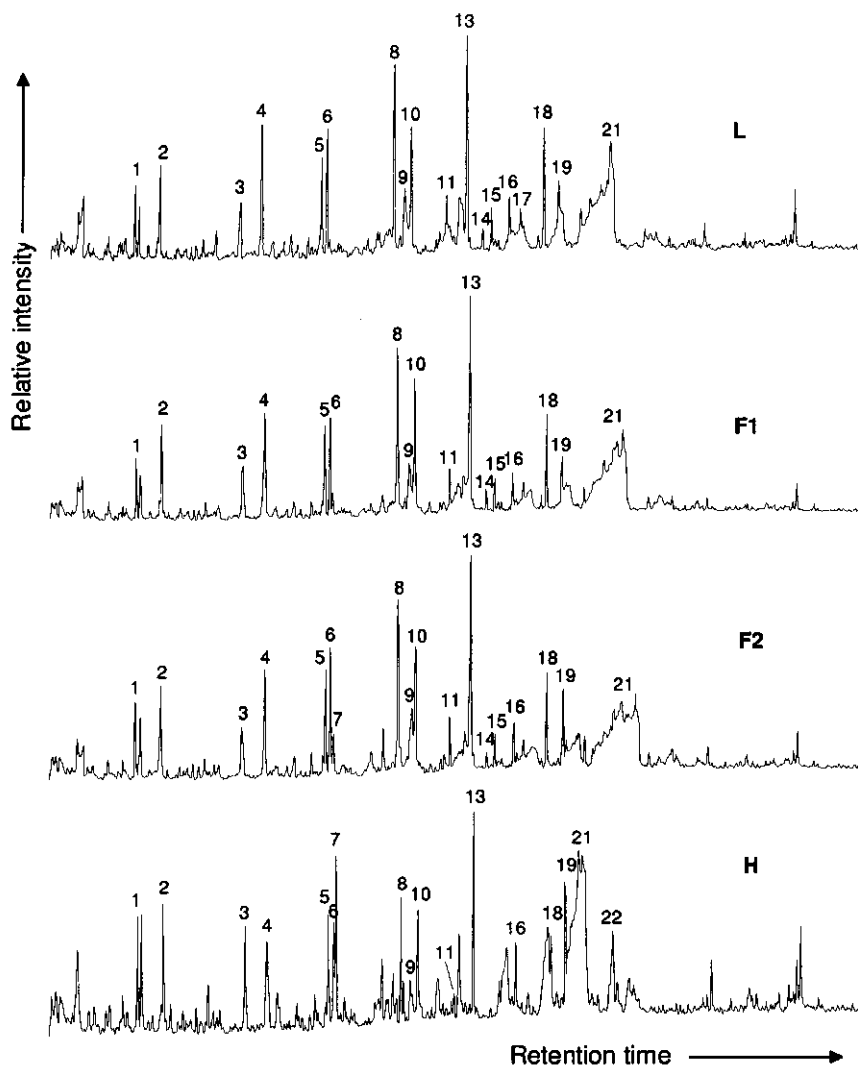
Figure 5. Pyrolysis-GC traces of profile 1 (*Pinus + Deschampsia*). Numbers correspond to compounds listed in Table 4.
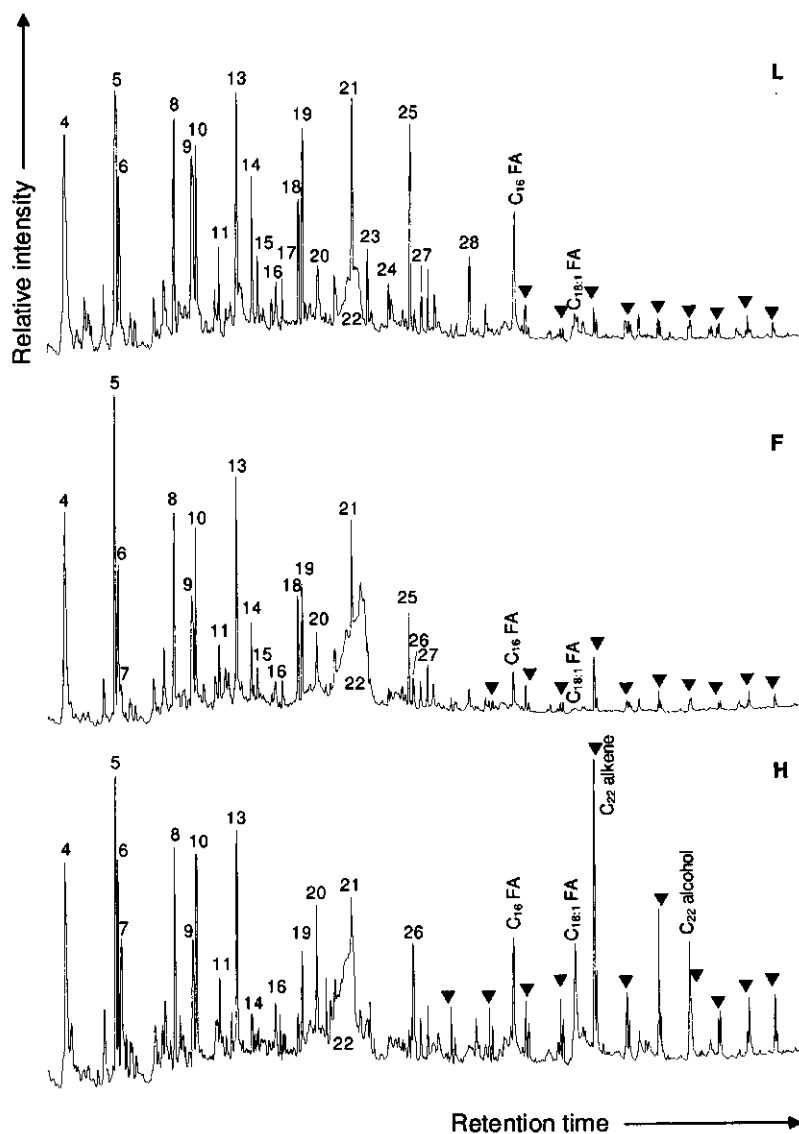
93

Figure 6. Pyrolysis-GC traces of profile 4 (*Fagus*). Numbers correspond to compounds listed in Table 4. FA = fatty acid; (e.g.) $C_{18:1}$ indicates number of C atoms and, if necessary, the number of double bonds; ▼ = alkene/alkane pair.

Table 5. Most abundant cutin- and suberin-derived thermochemolysis products in above- and below-ground tissues.

Vegetation	FA	OH	diFA	diOH	triOH	OL	Aromatic acids
Cutin							
Pinus	C_{16}	C_{12}^*, C_{14}^*, C_{16}^*		C_{16}^*			
Deschampsia	C_{16}, $C_{18:1}$	C_{16}		C_{16}	C_{18}^*		ferulic acid*
Empetrum	C_{16}^*, $C_{18:1}$	C_{16}^*, $C_{18:1}^*$		C_{16}^*			ferulic acid
Fagus	C_{16}^*, $C_{18:1}^*$, C_{20}, C_{22}^*, C_{24}, C_{26}, C_{28}	C_{16}		C_{16}			
Suberin							
Pinus	$C_{18:1}^*$, C_{20}^*, C_{22}^*, C_{24}, C_{26}	C_{16}, $C_{18:1}^*$, C_{20}, C_{22}	C_{20}, C_{22}, C_{24}				ferulic acid*
Deschampsia	C_{20}	C_{16}^*, $C_{18:1}$, C_{20}, C_{22}, C_{24}	C_{20}, C_{22}, C_{24}				coumaric acid, ferulic acid*
Empetrum	$C_{18:1}$, C_{20}^*, C_{22}, C_{24}, C_{26}, C_{28}	$C_{18:1}^*$, C_{20}, C_{22}	C_{20}, C_{22}, C_{24}	C_{16}			
Fagus	$C_{18:1}$	C_{16}^*, $C_{18:1}^*$, C_{20}, C_{22}	C_{16}^*, $C_{18:1}$, C_{20}, C_{22}	C_{16}	C_{18}^*	C_{22}^*, C_{24}	ferulic acid

Explanation: FA: fatty acid, OH: ω-hydroxy fatty acid, diFA: α,ω-dioic acid, diOH: 9/10,16-dihydroxy fatty acids, triOH: 9,10,18-trihydroxy fatty acid, OL: alcohol. $C_{n:1}$ indicates carbon chain length, and where appropriate, the number of double bonds. * mentioned building blocks are the dominating compounds released upon thermochemolysis.
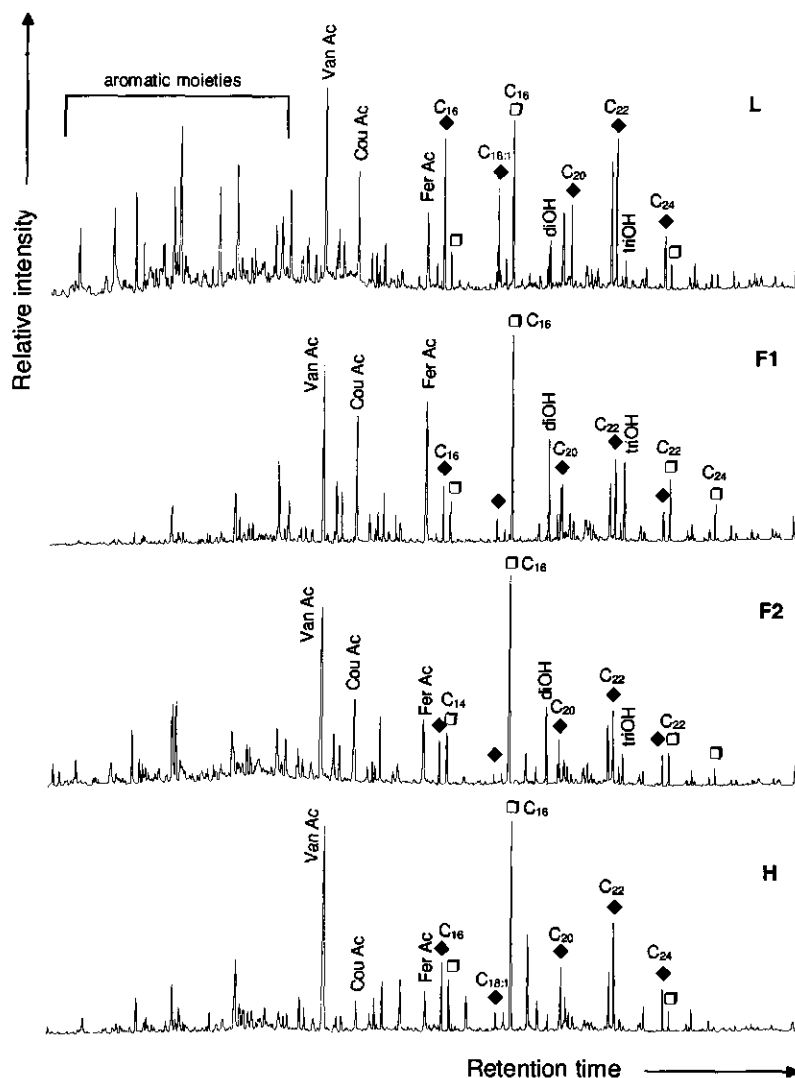
Figure 7. Thermochemolysis traces of profile 1. Van Ac = vanillic acid; Cou Ac: *p*-coumaric acid; Fer Ac: ferulic acid; $C_{18:1}$ indicates number of C atoms and, if necessary, the number of double bonds; ◆: fatty acid; ◻: ω-hydroxy fatty acid; diOH: 9/10,16-dihydroxyhexadecanoic acids; triOH: 9,10,18-trihydroxyoctadecanoic acid.

Needle and leaf cuticles contain the biopolymer cutin which predominantly consists of ω-hydroxy C_{16} and C_{18} fatty acids, mostly with a mid-chain double bond or functional groups such as hydroxyl or epoxy (Kolattukudy, 1980). Additionally, straight-chain fatty acids and small contributions of *p*-coumaric and ferulic acid may be present. Pine needles appear to have mainly C_{16} monomers, but also contain considerable amounts of hydroxy C_{12} and C_{14} fatty acids. These hydroxy fatty acids have also been measured in cutin of *Picea abies* (Matzke and Riederer, 1991). *Deschampsia* and *Empetrum* cutin mainly have the C_{16} and C_{18} monomers, whereas *Fagus* cutin has a prominent contribution of fatty acids with chain lengths greater than C_{18}, in addition to the C_{16} and C_{18} building blocks.

Suberin is the cutin-analogue in roots. Apart from the building blocks present in cutin, suberin may have important contributions of fatty acids, ω-hydroxy fatty acids, alcohols with chain lengths $\geq C_{20}$ and alkanedioic acids (C_{16} - C_{22}) (Kolattukudy, 1980). Suberin of *Pinus* has high concentrations of unsaturated C_{18} constituents, namely oleic acid and 18-hydroxy fatty acid. In contrast to the high amounts of di- and trihydroxy fatty acids in the leaves of *Deschampsia*, these building blocks are hardly present in their roots. *Empetrum* roots resemble *Pinus* in the composition of suberin, but the distribution differs. The thermochemolysate of beech roots has prominent signals from C_{22} and C_{24} alcohols, which are very characteristic for *Fagus* suberin (Holloway, 1983; Matzke and Riederer, 1991), and from 9,10,18-trihydroxyoctadecanoic acid and 9,10-epoxy-18-hydroxyoctadecanoic acid.

- Profiles
Figure 7 shows that in the L horizon of profile 1, coumaric acid, ferulic acid, $C_{18:1}$ fatty acid, C_{16} hydroxy fatty acid, 9/10,16-dihydroxyhexadecanoic acids, C_{16}-C_{24} fatty acids are the most abundant cutin- and suberin-derived building blocks. Compared to the L horizon, the F1 has a much lower concentration of $C_{18:1}$ fatty acid, but a much higher abundance of hydroxy C_{16} fatty acid, 9/10,16-dihydroxyhexadecanoic acids, and 9,10,18-trihydroxyoctadecanoic acid. With depth, coumaric and ferulic acid decreases in the F2 and even more in the H horizon. In the H horizon di- and trihydroxy fatty acids also decrease, while C_{20} and C_{22} fatty acids increase.

Fatty acids (C_{16}-C_{28}) and hydroxy fatty acids (C_{16}, $C_{18:1}$) dominate the thermochemolysate of the L horizon in profile 2. Towards the F2 horizon, the fatty acids, in particular C_{22}, increase relative to the hydroxy fatty acids with depth. In the H horizon, the aliphatic composition resembles that of the pine needles.

In profiles 3 and 4, aliphatic compounds derived from beech leaves dominate the thermochemolysate of the L. In the F horizon, di- and trihydroxy fatty acids are predominant whereas traces of the C_{22} and C_{24} alcohols are observed, especially in profile 4. The H horizon differs completely from the L and F horizon, in having high contributions of 16-hydroxy fatty acid, C_{16} dioic acid, 9/10,16-dihydroxyhexadecanoic acids, $C_{18:1}$ fatty acid, 9,10,18-trihydroxy fatty acid, C_{22} alcohol, C_{22} fatty acid, C_{22} hydroxy fatty acid and C_{24} alcohol. The contribution of the alcohols is much higher in the thermochemolysate of profile 4 than in that of profile 3, suggesting a much higher contribution of beech suberin in profile 4.

In general, vanillic acid clearly increases with depth in comparison with vanillin, suggesting a progressive oxidation of lignin (Ertel and Hedges, 1985; Hedges *et al.*, 1988; Clifford and Hatcher, 1995; Hatcher *et al.*, 1995). This confirms the trend suggested by the results of conventional pyrolysis of oxidized lignin increasing with respect to non-oxidized lignin fragments from L to H horizon.

DISCUSSION

The great similarity of NMR spectra, sugar composition and pyrolysis data between L and F1 horizons in profiles 1 and 2 strongly suggests that the initial litter decomposes slowly. The organic chemical data reveal important changes between F1 and F2 and especially towards H horizon implying a pronounced humification. This is in strong contrast with interpretations about decomposition based on morphological observations as well as experimental data on the decomposition of organic matter in the Leuvenum Forest (Emmer, 1995a). Emmer (1995a) observed significantly higher decomposition rates in the L and F1 than in the underlying F2 and H horizons in pine stands with understorey of *Deschampsia*. The apparent discrepancy between decomposition and chemical data may be explained as follows: the F1 is extensively rooted by the undergrowing species, thus supplying fresh litter to this layer. Because the organic chemical data averages all organic matter - fresh and decomposed -, the decomposed, partly humified material in the F1 is diluted with undecomposed 'fresh' roots of the undergrowth.

The lignin composition in the F1 horizon in profile 1 closely resembles that of the L, especially in comparison with the F2 and H horizons, by having a very low syringyl content and lacking a dominant of 4-vinylguaiacol peak typical of pyrolysates of the *Deschampsia* litter. This suggests preferential decay of *Deschampsia* over pine litter, leaving pine litter more or less untouched. *Deschampsia* decomposes faster than *Calluna* (van Vuuren, 1992) and probably also more rapidly than *Empetrum* and *Pinus* (Emmer, 1994; Dijkstra *et al.*, 1998).

In profiles 3 and 4, beech leaves dominate the organic matter in L and F horizons. The H horizon, however, is devoid of syringyl lignin, confirming earlier results that above-ground input into these horizons is almost exclusively derived from pine litter (Nierop *et al.*, 1998).

Lignin

Most of the soil science literature, as well as models describing litter decomposition emphasizes lignin as the recalcitrant fraction, which in particular determines the decomposition rate in the later phases of the decay process (e.g. Parton *et al*, 1987, Cadisch and Giller, 1997). A two-phase decomposition pathway with lignin as the recalcitrant fraction was suggested by Emmer (1995a) for the ectorganic layer under pine and undergrowth, and supported by an increase of lignin (determined according to Klason lignin) with depth.

However, the NMR spectra gave no support for a residual enrichment of lignin with depth in the ectorganic layer in any of the investigated profiles. With increasing depth, O-alkyl carbon decreases, aromatic carbon remains approximately constant, and alkyl carbon increases. The discrepancy between the results of Emmer (1995a) and this study must be ascribed to the fact that Klason 'lignin' - the part of organic matter after removal of ethanol soluble substances (lipids) and polysaccharides - contains tannins and aliphatic biopolymers (cutin and suberin, cutan and suberan) in addition to lignin. Especially the aliphatic biopolymers accumulate as decomposition proceeds. Zech *et al.* (1987) observed a considerable amount of aliphatic carbon in NMR spectra of Klason lignin of spruce litter, and the aliphatic fraction increased compared with the aromatic carbons in spectra of Klason lignin of decomposed litter (after 1106 days).

Pyrolysis and thermochemolysis give evidence for a progressive alteration of the lignin macromolecules from L to H horizon, although differences between L and F1 in profiles 1 and 2 are minimal. The general composition of lignin in the first two profiles suggests that lignin is mainly derived from pine. This may be due in part to chemical reasons, e.g. demethoxylation or selective degradation of syringyl-lignin leaving mainly guaiacyl-lignin (van der Heijden *et al.*, 1994). On the other hand the predominance of coniferous lignin may have a physical/biological cause: as easily decomposable vegetation tissues are removed the more recalcitrant pine litter accumulates residually. In profiles 3 and 4, the L and F layers are dominated by beech litter remnants and so is the organic matter composition, but the H horizon is mainly derived from pine litter. Thus, with depth a decrease of syringyl-lignin is noticed, but mainly due to different amounts of contributing vegetation litters than due to a selective degradation of syringyl-lignin (Nierop *et al.*, 1998).

The progressive oxidation of lignin with depth in all profiles, as observed by pyrolysis and thermochemolysis, is a common feature (e.g. Kögel, 1986; Kögel *et al.*, 1988; Kögel-Knabner *et al.*, 1991; Guggenberger and Zech, 1994) and implies (fungal) degradation of lignin (Saiz-Jimenez and de Leeuw, 1984; Goñi *et al.*, 1993; Hatcher *et al.*, 1995).

Cutin and suberin: impact of roots

Because bark contributes very little to the organic matter, I assume that all suberin is derived from roots. Furthermore, decomposition rates of cutin and suberin are presumed to be more or less similar. Although Riederer *et al.* (1993) suggested that suberin may be degrading somewhat slower than cutin, until now no real evidence for a difference in decomposition rates has been found. Riederer *et al.* (1993) suggested that cutin uniformly decomposes, i.e. all monomers have similar decomposition rates. However, this was only analysed for the five main constituents of beech cutin, all di-, tri- and tetrafunctionalized monomers. Nierop (1998) observed a non-uniform decomposition of beech suberin with a relative increase of the trihydroxyoctadecanoic acid with respect to some mono- and difunctionalized monomers. Nevertheless these differences in distribution of the constituents between fresh and humified suberin were not too striking, and made it possible to indicate the importance of the several cutins and suberins in soil.

Although grass roots are frequently present in the F1 and F2 horizons of profile 1, they appear to have no effect on the chemical composition as reflected by pyrolysis, i.e. the typical grass-lignin signature is absent. However, thermochemolysis reveals the presence of grass leaf cutin and suberin by characteristic compounds such as 9,10,18-trihydroxyoctadecanoic acid and ferulic acid. The very intense peak of ferulic acid can also be derived from pine needles which input is suggested by the presence of C_{12} and C_{14} hydroxy fatty acids, and typical pine cutin monomers as C_{16} hydroxy fatty acid and 9/10,16-dihydroxyhexadecanoic acids. From F1 through F2 to the H horizon, the grass cutin and suberin biomarkers decrease, whereas pine suberin (roots) become more important, as indicated by C_{20} and C_{22} fatty acids and pine cutin. The presence of suberin from pine in the L horizon will be mainly derived from bark (exception of the assumption), which is present in relatively high amounts (see also Table 1).

Under *Empetrum*, the aliphatic part is dominated by cutin monomers, such as 16-hydroxy fatty acid, and 9/10,16-dihydroxyhexadecanoic acids. The C_{20} and C_{22} fatty acids point to suberin. From L to F2, the C_{20} and C_{22} fatty acids increase relatively, implying an increasing root input. Whether this is due to pine roots or those of *Empetrum* is not clear due to their similar suberin composition. The H horizon differs completely, and has a close resemblance to pine cutin, thereby suggesting a low root contribution.

L and F layers of both beech profiles are dominated by cutin derived from beech leaves. Suberin from beech is brought to light in the F horizons, but much more pronounced in the H horizons, especially in profile 4. Also cutin and suberin from pine is present in the H horizons, but is exceeded by suberin of beech. Thus, beech roots have an increasing impact on the organic matter of the H horizons, but, yet, beech leaves do not.

CONCLUSIONS

Progressive decomposition and humification in all profiles is reflected by residual enrichment of alkyl C relative to aromatic C and O-alkyl C, by increasing C_6/C_5 and deoxy/C_5 sugar ratios, and increases in oxidized lignin fragments. Although there is a significant contribution of root litter in the F and H horizons, and thus supply of fresh litter, root input at depth does not greatly disturb the 'decay continuum' pattern sketched above.

Influence of *Deschampsia* and *Empetrum* (profiles 1 and 2, respectively) seems to be mainly restricted to cutin and suberin, and especially in F1 and F2 horizons, whereas other biopolymers derived from these species are decomposed extensively. In the H horizon, the contribution of suberin of the understorey is small, especially under *Empetrum*.

The transition from pine to beech forest is reflected by the changing organic matter composition at different depth: in profiles 3 and 4 the organic matter composition of the L and F horizons is dominated by beech litter, whereas the H horizons are mainly composed of pine litter, although beech root litter input increasingly contributes to the composition of H horizons, from profile 3 to 4. Cutin and suberin appear to be more stable in the organic horizons than lignin, and therefore the two aliphatic biopolyesters are more useful as biomarkers than lignin.

Chapter 6

Water soluble organic matter in incipient podzols: accumulation in B horizons or in fibers?

Klaas G.J. Nierop and Peter Buurman

ABSTRACT

To study the fate of the water soluble organic matter (WSOM) in incipient podzols in sandy soils, a comparison was made of the composition of the WSOM from L, F and H horizons with the bulk of the Bh horizons and fibers of three profiles. WSOM appeared to have significant contributions of ligno-cellulose and proteins, but these biopolymers were hardly present in the Bh horizons. The fibers, however, showed a great resemblance with the WSOM composition thereby suggesting that in these soils most of the WSOM was transported through the B horizon and accumulated hardly changed in thin bands when the water movement stopped. This implied that in the early steps of podzolization, accumulation of organic matter in the B horizon was not likely caused by water soluble material.

INTRODUCTION

Podzols are among the most studied soils. They are the dominant soil types in the boreal forest zone, where they occur on a wide range of parent materials. Outside the boreal zone, they are restricted to poor sandy soils and wet climates. They consist of a thick, dark ectorganic surface layer, overlying a bleached eluvial E horizon, followed by a dark illuvial B horizon that is enriched in organic matter, Al and Fe in comparison to the E and C horizons. Part of the organic matter is not precipitated in the B horizon and moves through the B horizon to deeper horizons. This organic matter may precipitate when the waterfront stops moving, forming thin, dark fibers. Such fibers are very common in sandy, well-drained podzols.

Several mechanisms for the podzolization process have been proposed. All of them, the classical fulvate theory (e.g. Petersen, 1976), the proto-imogolite theory (Farmer *et al.*, 1980; Anderson *et al.*, 1982) and the low molecular weight (LMW) organic acids theory (Lundström *et al.*, 1995) explain precipitation of organic matter-metal complexes in the podzol B horizon, but none addresses the fate of the organic matter precipitated in fibers.

Although organic matter of podzol B horizons has been investigated intensively in the past, only some general characteristics were noticed, there is little consensus about its composition, and descriptions vary from 'highly aromatic' to 'predominantly aliphatic' (see for a review Andreux, 1996). However, on the detailed molecular level, only a few studies of podzol B organic matter have been reported. Using Pyrolysis-Field Ionization Mass Spectrometry (Py-FIMS), Sorge *et al.* (1994) analyzed the total soil and particle-size fractions of a Canadian podzol B horizon. In the total soil as well as in the silt and clay fractions, organic matter was dominated by signals from lipids, alkylaromatics, carbohydrates, and intact and altered lignins, whereas in the sand fraction mainly carbohydrates, phenols/lignin monomers and dimers were identified. Spodic B horizons from northern Germany consisted mainly of alkyl C and O-alkyl C (^{13}C-NMR), independent of podzolization age (Wilcken *et al.*, 1997). By Py-FIMS, Wilcken *et al.* (1997) elucidated that incipient podzol B horizons were characterized by signals predominantly from polysaccharides, phenols, lignin monomers, fatty acids and plant waxes, whilst well-developed Haplic podzol B horizons were dominated by signals from lignin dimers, long-chain lipids and sterols. In general, all these

compounds in young and old podzols were also detected in dissolved organic matter in percolating water from podzol profiles (Hempfling and Schulten, 1990).

Virtually nothing is known about the organic matter in fibers. ^{13}C signatures of bulk organic matter in well-developed podzols in central Netherlands appear to indicate a difference between B horizon and fibers (Buurman *et al.*, 1998). Does the material in these fibers (below the B horizon) differ from that of the B horizons? Were fibers formed from dissolved organic matter that passed through the B horizon? These questions were studied as part of a research project dealing with organic matter and soil development in relation to the vegetation succession at the wind-blown sand area of the Leuvenum Forest. In the course of this succession, the soil develops towards a podzol. Because all succession stages from bare sand to beech forest with podzolic features are currently present, the initial steps of podzolization can be monitored. In this paper, we try to unravel the relationships between the WSOM of organic layers, organic matter in B horizons and fibers of three profiles, in subsequent development stages: which compounds are transported, accumulated or leached away? We compare the organic chemical composition of the organic matter as characterized by combining ^{13}C-NMR and pyrolysis-GC/MS techniques.

MATERIALS AND METHODS

Sites, profiles

The studied soil profiles are from three sites with comparable soil profiles, but with progressive podzolization and under different vegetation. Although the soils show distinct podzolization features, they are classified as Cambic Arenosols (FAO).

Profiles 1 and 2 have pine trees (*Pinus sylvestris* L., about 100 years) with dominant undergrowth of wavy hair-grass (*Deschampsia flexuosa* (L.) Beauv.) and crowberry (*Empetrum nigrum* L.), respectively. Profile 3 has only beech (*Fagus sylvatica* L.) trees. Biological homogenization in the mineral soil is virtually zero. For detailed information, including layer thicknesses, C, N, C/N ratios and pH, see Table 1.

Sample pretreatment

- Water soluble organic matter (WSOM)
Field-moist L, F and H horizons were shaken with distilled water (sample solution ratio 1:4 (w/w)) for 24 h. After centrifugation, the solution was decanted, and the residue was shaken for 2 h. with water and subsequently centrifuged. The combined solutions were filtered over a 0.45 μm filter and freeze-dried.

-B horizons and fibers
Samples of B horizons and fibers were freeze-dried and sieved over a 2 mm sieve. For NMR and pyrolysis, the organic matter was concentrated. Samples were shaken with 0.5 *M* NaOH (sample solution ratio 1:2) for 5 minutes. After decanting of the 'solution', including all insoluble (organic) material, the residue (sand) was washed several times with water until the

Table 1. C, N, C/N ratios, pH values and Fe and Al contents of soil horizons and WSOM.

Profile	Horizon	Thickness (cm)	Soil C(%)	N(%)	C/N	pH(H$_2$O)	pH(KCl)	Al(%)	Fe(%)	WSOM C(%)	N(%)	C/N
1	L	0.5	48.7	2.0	25					38.2	4.6	8
	F1	5	47.5	2.0	24					50.0	4.1	12
	F2	3	43.1	1.6	26					43.3	2.6	17
	H	0.5	35.1	1.3	27							
	AE	1	0.69	0.04	19	3.8	3.4	0.01	0.01			
	BC	3	0.27	0.02	20	3.9	3.8	0.01	0.03			
	C		0.18	0.01		4.2	4.5	0.04	0.01			
	fiber		0.63	0.05	13							
2	L	0-1	50.6	2.2	23					37.1	4.7	8
	F1	6	52.0	2.0	26					47.5	4.1	12
	F2	5	52.7	1.8	29					44.6	2.6	17
	H	3	45.5	1.3	34	3.5	2.5	0.15	0.16			
	E	2	0.38	0.02	20	3.9	3.4	0.01	0.01			
	B	6	0.41	0.02	20	3.7	3.5	0.01	0.03			
	C		0.23	0.01		4.2	4.5	0.05	0.01			
	fiber		0.74	0.07	11							
3	L	4	42.1	1.9	22					45.7	5.0	9
	F	4	46.2	2.0	24					42.3	3.6	12
	H	3	33.5	1.4	24	3.7	2.6	0.09	0.09	44.3	2.4	18
	E	8	0.70	0.04	18	3.8	3.4	0.01	0.01			
	B	11	0.82	0.04	22	3.7	3.4	0.02	0.07			
	C		0.22	0.01		4.3	4.7	0.07	0.02			
	fiber		1.01	0.08	12							

supernatant was clear. The combined 'solutions' were acidified with HCl/HF (1:3), shaken for 24 hours, dialysed and freeze-dried. (For detailed information, see Nierop and Buurman, 1998).

Chemical analysis

- C, N, pH, Al and Fe
Carbon and nitrogen were measured with an Interscience Elemental Analyser EA1108, pH (H_2O and 1 M KCl) in a sample:solution ratio of 1:5. Amorphous Al and Fe contents were determined as acid-oxalate extractable metals (Buurman *et al.*, 1996).

- ^{13}C NMR
Cross Polarization-Magic Angle Spinning (CPMAS)-NMR measurements were performed on a Bruker AMX 300 operating at a frequency of 75.48 MHz, with an acquisition time of 0.033 s; a pulse time of 4 μs; a contact time of 0.8 ms; spinning rate 3.375 kHz and a line broadening of 50 Hz.

- Pyrolysis-gas chromatography/mass spectrometry and thermochemolysis
Samples were pressed onto flattened ferromagnetic wires with a Curie temperature of 610°C. Pyrolysis was carried out by inductive heating of the wires to their Curie temperature, held there for 5 s using a Horizon Intruments Curie-Point pyrolyser. The pyrolysis unit was connected to a Carlo Erba gas chromatograph and the products were separated by a fused silica column (Chrompack, 25 m, 0.25 mm i.d.) coated with CP-Sil 5 (film thickness 0.40 μm). Helium was used as carrier gas. The initial oven temperature was 40°C, followed by heating at a rate of 7°C/min to 320°C and held for 20 min. The end of the GC column was coupled to a Fisons MD 800 mass spectrometer (mass range *m/z* 45-650, cycle time 1 s). Pyrolysis-methylation (thermochemolysis) was performed by adding one or two drops of a solution of tetramethylammonium hydroxide (TMAH) in water (25%) on the sample, which was briefly dried and pyrolyzed immediately afterwards (J.W. de Leeuw, pers. com.).

RESULTS

^{13}C NMR

All ^{13}C NMR spectra of WSOM from the L, F and H horizons of all profiles are very similar. The spectra of WSOM from profile 3 (Figure 1a-c) show resonances at 25 and 33 ppm, indicative of methyl and methylene carbons, respectively. Furthermore, high signals are present in the O-alkyl range, centered around 72 ppm. Together with the chemical shift at 105 ppm, these signals are ascribed to polysaccharides (Kögel *et al.*, 1988; Kögel-Knabner, 1997). The peak at around 172 ppm, derived from carboxylic acids or esters, has two spinning side bands, at 128 and 216 ppm. Only weak signals are found of aromatic carbons, at 153 and 'under' the side band, which resonates at 128 ppm. The B horizons (cf. Figure 1d) give much
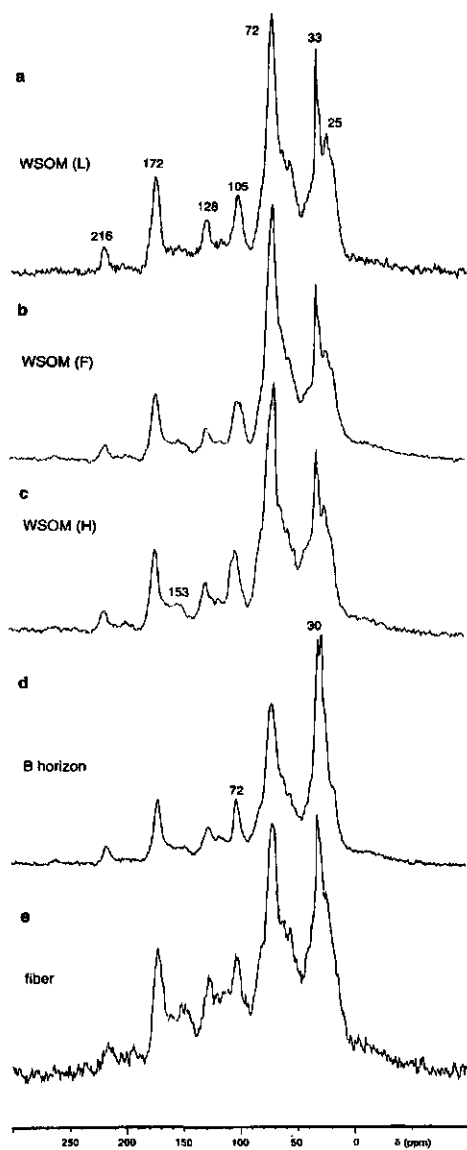
Figure 1. ^{13}C NMR spectra of water soluble organic matter (WSOM) from L (a), F (b) and H (c) horizons and total organic matter in the B horizon (d) and fiber (e) of profile 3.
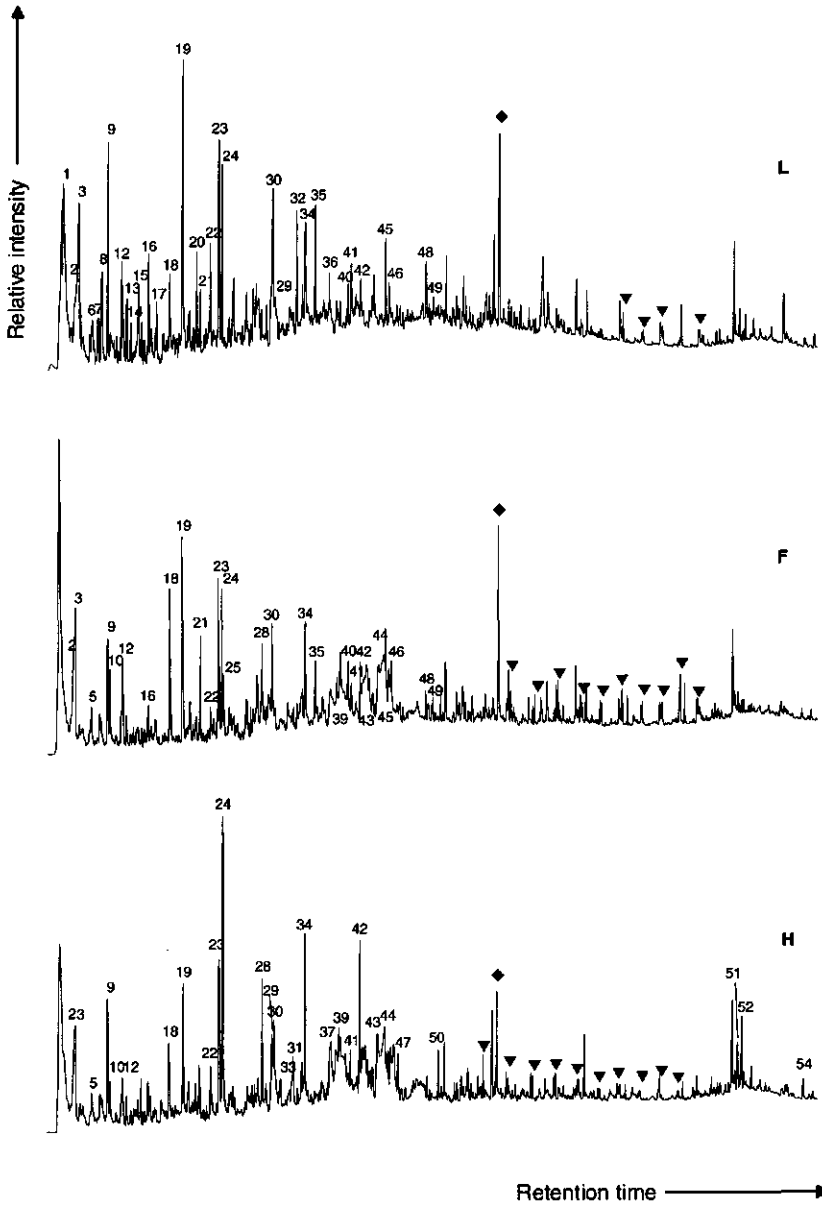
Figure 2. Total ion current chromatograms of pyrolysates of water soluble organic matter (WSOM) from L, F and H horizons of profile 3.

higher signals of alkyl C (0-50 ppm), and lower intensities of the other carbon types than those of the WSOM of L, F and H horizons. In the spectra of the fiber (cf. Figure 1e), alkyl C and O-alkyl C predominate and aromatic C is relatively abundant.

Pyrolysis-GC/MS and thermochemolysis

- WSOM
Pyrolysis results of the WSOM fractions of profiles 1, 2 and 3 are rather similar for each horizon (L, F, and H). Polysaccharide-derived products identified in the pyrolysates of WSOM obtained from L, F and H horizons are furans (**1, 2, 5, 10-12, 17, 18**) and anhydrosugars (**25, 39, 43, 44**). From L to F, (2H)-furan-3-one (**10**), 2-furaldehyde (**12**), 5-methyl-2-furaldehyde (**18**), dianhydrorhamnose (**21**) and the anhydrohexoses (**39, 43, 44**) increase relatively, while the other compounds show the opposite trend or hardly any change (Figure 2 for profile 3). Table 2 lists identified peaks and symbols. Other prominent compounds in the pyrolysates of WSOM are lignin-derived 2-methoxyphenols (guaiacols **24, 28, 31, 34, 37, 41, 42** and **46**) and 2,6-dimethoxyphenols (syringols **35, 40, 45, 48** and **49**). The syringols are only present in WSOM of profile 3, and not in profiles 1 and 2. After *in situ* methylation using TMAH, also known as thermochemolysis, the far most dominating product is the methylated version of vanillic acid, whereas methylated syringic acid is also relatively important.

Nitrogen-compounds, encountered in the pyrolysates of the water soluble fractions, are (alkyl)pyrroles (peaks **6, 8, 13** and **14**), pyridine (**7**) and (alkyl)indoles (**32, 36**). Their abundance decreases from L to F and H horizon. Together with (part of) toluene, styrene, phenol and methylphenols, all N-compounds may be derived largely from proteins. Indoles are thought to be pyrolysis products from proteins including tryptophan (Tsuge and Matsubara, 1985). Pyrroles have been identified as pyrolysis products of proline and hydroxyproline, whereas pyridine is related to alanine, amino sugars or even to amino acid-polysaccharide complexes (Stankiewicz *et al.*, 1996; van Bergen *et al.*, 1998; Bland *et al.*, 1998, and references cited therein). In addition, phenol and methylphenol, toluene and styrene have been identified as degradation products of the amino acids tyrosine and phenylalanine, respectively (Tsuge and Matsubara, 1985).

The aliphatic fraction is of minor importance in WSOM. The aliphatics are dominated by a series of fatty acids and terpenoids, and small traces of alkenes and alkanes. Upon thermochemolysis, the most prominent compounds are methyl esters of fatty acids (C_{14}-C_{28}), followed by methyl esters of dehydroabietic acid and a diterpenoic acid.

- B horizons
The pyrolysates of all B horizons (Figure 3) are dominated by acetic acid (**3**), toluene (**9**), 2-furaldehyde (**12**), styrene (**16**), 5-methyl-2-furaldehyde (**18**), phenol (**19**), methylphenols (**22, 23**), guaiacol (**24**) and 4-vinylphenol (**30**). Other important pyrolysis products are pyridine (**7**), pyrrole (**8**), ethylphenols (**27**), indole (**32**), 4-vinylguaiacol (**34**), vanillin (**37**), levoglucosan (**44**), 4-acetylguaiacol (**42**), vanillic acid (**46**), diketodipyrrole (DKDP), unsaturated fatty acids (C_{16} and C_{18}) and homologous series of 1-alkenes and alkanes (C_7-
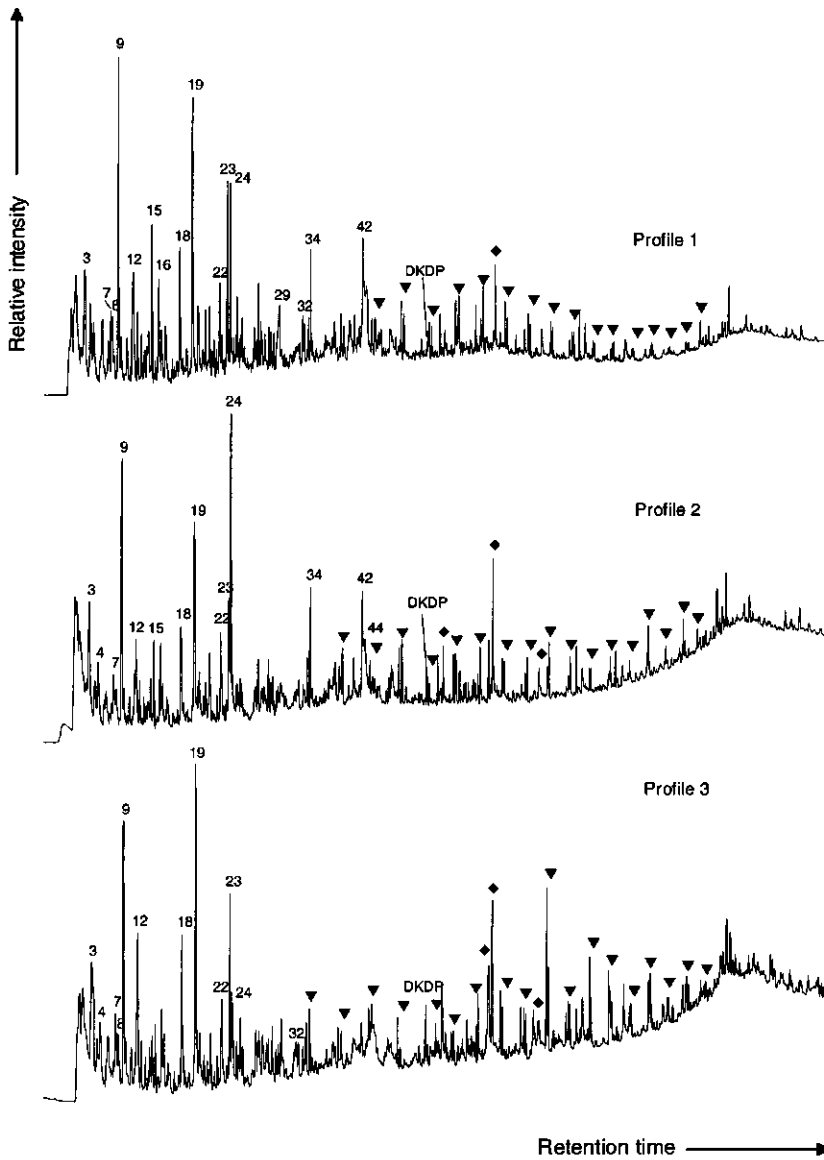
Figure 3. Total ion current chromatograms of pyrolysates of organic matter in B horizons of profiles 1-3.

Table 2. List of main pyrolysis products.

No.	Compound	Source	M⁺
1	Furan	Ps	68
2	2-*Methylfuran*	Ps	82
3	Acetic acid	Ps	60
4	Benzene	Pp	78
5	2,5-Dimethylfuran	Ps	96
6	*N*-methylpyrrole	Pr	81
7	Pyridine	Pr	79
8	Pyrrole	Pr	67
9	Toluene	Pp/Pr	92
10	(2H)-furan-3-one	Ps	84
11	3-Furaldehyde	Ps	96
12	2-Furaldehyde	Ps	96
13	2-Methylpyrrole	Pr	81
14	3-Methylpyrrole	Pr	81
15	C₂-benzene	Pp/Pr	106
16	Vinylbenzene (styrene)	Pp/Pr	104
17	2,3-Dihydro-5-methylfuran-2-one	Ps	98
18	5-Methyl-2-furaldehyde	Ps	110
19	Phenol	Pp/Lg/Pr	94
20	2-Hydroxy-3-methyl-2-cyclopenten-1-one	Ps	112
21	Dianhydrorhamnose	Ps	128
22	2-Methylphenol	Pp/Lg/Pr	108
23	3/4-Methylphenol	Pp/Lg/Pr	108
24	Guaiacol	Lg	124
25	Levoglucosenone	Ps	126
26	Dimethylphenols	Pp/Lg	122
27	Ethylphenols	Pp/Lg	122
28	4-Methylguaiacol	Lg	138
29	1,2-Dihydroxybenzene (catechol)	Pp/Lg	110
30	4-Vinylphenol	Pp/Lg	120
31	4-Ethylguaiacol	Lg	152
32	Indole	Pr	117
33	4-Methylcatechol	Pp	124
34	4-Vinylguaiacol	Lg	150
35	Syringol	Lg	154
36	Methylindole	Pr	131
37	4-Formylguaiacol (vanillin)	Lg	152
38	Dimethylnaphtalene		156
39	Anhydrohexose	Ps	162
40	4-Methylsyringol	Lg	168
41	*trans* 4-(2-Propenyl)guaiacol	Lg	164
42	4-Acetylguaiacol	Lg	166
43	Anhydrohexose	Ps	162
44	Anhydroglucosan (levoglucosan)	Ps	162
45	4-Vinylsyringol	Lg	180
46	4-Carboxyguaiacol (vanillic acid)	Lg	168
47	Trimethylnaphtalene		170
48	*trans* 4-(2-Propenyl)syringol	Lg	194
49	4-Acetylsyringol	Lg	196
50	1-Pristene		266
51	*Stigmastatriene*	Lp	394
52	Stigmasta-3,5-diene	Lp	396
53	Friedoolean-2-ene	Lp	410
54	Stigmasta-3,5-dien-7-one	Lp	410
▼	*n*-Alkene and *n*-alkane (doublet)	Lp/Abp	
◆	Fatty acid	Lp	
●	Alcohol	Lp	
DKDP	Diketodipyrrole		

Abbreviations: Ps: polysaccharides, Pp: polyphenols, Pr: proteins, Lg: lignin, Lp: lipids, Abp: aliphatic biopolymer.
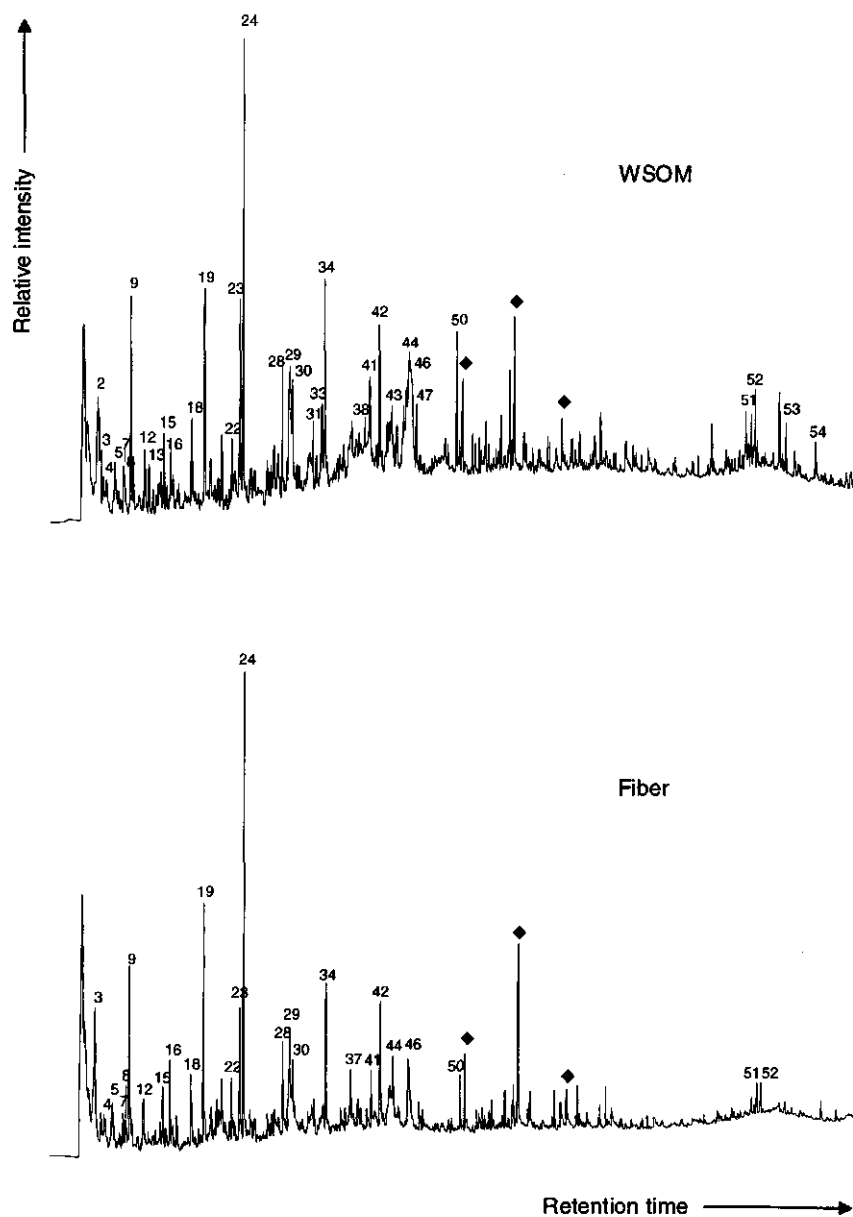
Figure 4. Total ion current chromatograms of pyrolysates of water soluble organic matter (WSOM) from H horizon and of total organic matter in fiber from profile 2.

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C_{32}). The relatively high abundance of alkenes and alkanes indicates a major contribution of aliphatic biopolymers, such as cutan (Nip *et al.*, 1986a). The other products indicate contributions of (little) amounts of lignin, proteins and polysaccharides. Diketodipyrrole may have originated from peptides including hydroxyproline (van Bergen *et al.*, 1998). The similarity between profile 1 and 2 is clear, except for a difference in abundance of lignin-derived methoxyphenols. The pyrolysate of profile 2 has a higher concentration of guaiacol, 4-vinyl- and 4-acetylguaiacol as the main lignin indicators than that of profile 1, and in particular, that of profile 3. In addition, the latter profile under beech has C_{22} and C_{24} alkenes and the corresponding alcohols as distinct pyrolysis products.

- Fibers

The pyrolysates of the (bulk) organic matter in the fibers resemble those of the WSOM, in particular of the H horizon, remarkable well, as shown in Figure 4. The dominating products identified in the pyrolysates are toluene (**9**), phenol (**19**), methylphenols (**22, 23**), guaiacol (**24**), catechol (**29**), 4-vinylguaiacol (**34**), 4-acetylguaiacol (**42**). Other prominent pyrolysis products are acetic acid (**3**), 2-furaldehyde (**12**), C_2 benzenes (**15**), styrene (**16**), 5-methyl-2-furaldehyde (**18**), 4-methylguaiacol (**28**), 4-vinylphenol (**30**), vanillin (**37**), levoglucosan (**44**), vanillic acid (**46**), *trans* 4-(2-propenyl)guaiacol (**48**) and 1-pristene (**50**). Furthermore, traces of fatty acid, alkenes and alkanes are found. In case of profile 3, no syringols are detected.

As in WSOM, the thermochemolysis data of the fibers reveal vanillic acid as dominating product. Other distinct compounds are 1,2,4- and 1,3,5-trimethoxybenzene, (methylated) vanillin and 4-acetylguaiacol, a series of methyl esters of fatty acids (C_{14}-C_{30}) and 16-hydroxyhexadecanoic acid.

DISCUSSION

In all horizons, amorphous aluminium and iron contents are (very) low, and differences between horizons may be not significant. However, an iron maximum in the B horizons and an aluminium maximum in the C horizons for the mineral soil (Table 1) are also found by Emmer (1995). No big differences in C, Al and Fe contents could be expected in these incipient podzols.

WSOM vs. total (bulk) horizons

WSOM has lower C/N ratios than the corresponding bulk horizons and may reflect the relative high water solubility of compounds containing hetero atoms, such as nitrogen and oxygen.

In the bulk horizons, the syringols decrease with respect to the guaiacols from L to F horizon and are almost completely gone in the H horizon. Very low syringyl contents in the H horizons are mainly due to the fact that this horizon is built-up of pine litter and to low mixing of beech-derived organic matter into the H horizons (Nierop *et al.*, 1998).
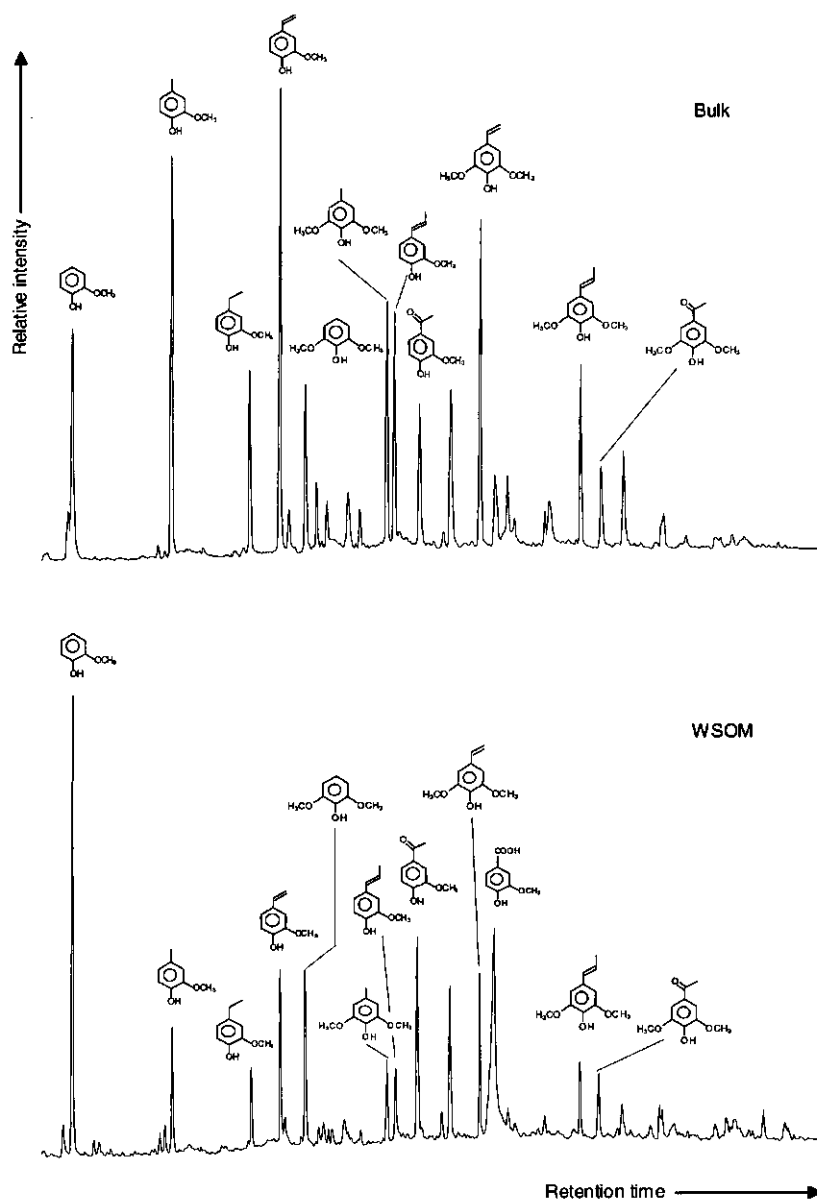
Figure 5. Partial mass chromatogram of guaiacols and syringols in pyrolysates of the F horizon and its water soluble fraction (profile 5).

Furthermore, the relative increase of oxidized guaiacol parts, such as vanillin, 4-acetylguaiacol and vanillic acid, and the simultaneous decline of *trans* 4-(2-propenyl)guaiacol with depth, indicates a progressive alteration of lignin. A similar trend can be found for syringols, from L to F horizon. Figure 5 compares the WSOM with the bulk organic matter in terms of lignin pyrolysis products: from bulk to WSOM increases of guaiacol, 4-acetylguaiacol and vanillic acid and decreases of other compounds, in particular 4-methyl-, 4-vinyl- and *trans* 4-(2-propenyl)guaiacol, within the 2-methoxyphenol family. A similar tendency is observed for the syringols in the L and F horizons of profile 3. The WSOM demonstrates a shift towards lower syringol/guaiacol ratios with respect to the bulk horizons as observed in the pyrolysis data (Figure 5). All these results suggest greater alteration of lignin in the water soluble fractions than in the bulk and in agreement with a higher solubility of increasingly oxidized lignin (Guggenberger and Zech, 1994).

High amounts of lignin-derived compounds in WSOM relative to the bulk horizons, contrast with observations from soils in the earlier vegetation succession where we found hardly any signals of lignin in WSOM extracted from bulk (Ah) horizons in which lignin was prominently present (Nierop and Buurman, 1998). A possible reason for this is that other degradation processes, or with other rates, take place in the ectorganic layers (L, F and H) than in mineral (Ah) horizons.

WSOM vs. B horizon

The NMR spectra of B horizons show a much higher content of aliphatics than in the WSOM. Furthermore, pyrolysis clearly shows that the chemical composition of WSOM differs completely from that of the B horizons: lignin-derived methoxyphenols, which dominate the pyrolysates of WSOM, are not important in the B horizons, while aliphatic compounds, such as alkenes, alkanes, fatty acids and alcohols are prominent in the pyrolysates of the B horizons, but almost absent in those of the WSOM. A reason for this lack of resemblance may be that WSOM does not accumulate in the B horizon, but passes through the B horizon and precipitates when the water movement stops, forming thin bands, the so-called fibers.

Nitrogen-containing compounds are relatively abundant in the pyrolysates of the WSOM fractions, and their abundance decreases from L to H horizons. This decline is supported by the increase of the C/N ratios with depth. In addition, these N-containing compounds are identified in the B horizons, although in relatively lower amounts. By contrast, diketodipyrrole is virtually absent in the WSOM, but relatively abundant in pyrolysates of the B horizons.

The organic matter composition of the B horizons shows a remarkable resemblance with that of soils from the Broadbalk Wilderness from the Rothamsted Classical Experiment (van Bergen *et al.*, 1997). The main differences are the lower abundances of N-compounds and higher amounts of levoglucosan in our pyrolysates. Van Bergen *et al.* (1997) observed a trend in which 'high' lignin contents came along with a low soil pH and vice versa, and stated that lignin rapidly changes diagenetically in high pH environments. However, in our case the pH is around 3.8, thus a high lignin content would be anticipated then, but, as mentioned, lignin was (very) low in abundance. Lignin is degraded predominantly by white-rot fungi, and decay

is the fastest when pH lies between 3.5 and 5.5 (Kirk and Farrell, 1987) and may therefore explain the low lignin contents observed in our B horizons.

The resemblance of the B horizons from Leuvenum Forest with the completely different soils from Rothamsted indicates that the organic matter in the B horizon does not originate from illuviation, but is humified material directly derived from plant litter. Indeed, in a parallel study, we found that an important source of organic matter in the B horizons was derived from root material (Nierop, 1998; and Chapter 7) and, apparently, is the virtually only source.

WSOM vs. fibers

The NMR spectra of WSOM suggest a higher amount of O-alkyl C and a lower amount of alkyl C and aromatic C compared to the fibers. Both pyrolysis and thermochemolysis data of WSOM remarkably resemble those of the fibers. This suggests that WSOM leaches through the soil and accumulated as such in the fibers. NMR data point to a relative accumulation of aliphatic and aromatic materials in fibers. These materials may be relatively resistant to decomposition, or, insusceptible to resolublize and subsequent transport downwards due to their hydrophobicity. It should be noted that the resemblance between WSOM and fiber in profile 3 is restricted to WSOM from the H horizon, because the fiber lacks syringols, which are important constituents of the WSOM from L and F horizons. This may suggest that WSOM from the H horizon is the main source of the organic matter in the fiber.

Prominent products in the pyrolysates of WSOM and fibers are the N-containing compounds. Their presence in fibers also points to the WSOM from the H horizon as the source material.

CONCLUSIONS

- WSOM contains more oxidized OM than the corresponding bulk horizons
- There is little evidence of appreciable accumulation of WSOM in the (incipient) B horizon as assumed by current models of podzolization
- WSOM resembles the OM in fibers suggesting they are made up of WSOM which undergoes little selective decomposition after precipitation

Chapter 7

Insoluble organic matter fractions in incipient podzol B horizons: preservation of aliphatic biopolymers from roots

Klaas G.J. Nierop and Peter Buurman

ABSTRACT

Organic matter in podzol B horizons is generally considered as the result of illuviation from upper horizons. This illuviated organic matter is thought to be largely, if not completely, extractable by sodium pyrophosphate. In incipient podzols from the Leuvenum Forest, 17-37% of the B organic matter remained insoluble after extraction with NaOH. Extraction of these insoluble fractions by sodium pyrophosphate hardly enlarged the amounts of soluble organic matter. Combining pyrolysis, thermochemolysis and SEM revealed that the insoluble organic matter consisted almost completely of root remnants. It appeared to be highly aliphatic, being composed of suberin and a 'suberan-like' polymer, whereas lignin and polysaccharides were virtually absent. This suggested that the aliphatic biopolymers were (highly) selectively preserved, not transported by illuviation and that root organic matter is an important constituent in B horizons.

INTRODUCTION

Humin is defined as the organic residue after extraction of soils and sediments with 0.5 M NaOH or other basic solutions (e.g. Schnitzer and Kahn, 1972). It may consist of fresh or slightly altered plant materials, clay-organic matter complexes, fungal melanins and highly condensed humic matter. The latter is sometimes called the 'real humin' (Stevenson, 1994). Because of lack of proper methods to separate the several components, the concepts about the origin of this material are equivocal: on one hand it is considered as the final product of the humification process in the condensation theory, whereas on the other hand, in the degradative biopolymer model, humin is the starting material (Hatcher and Spiker, 1988). The main chemical characteristics of the humin faction are less controversial: it has generally a highly aliphatic composition (Beyer, 1996).

Large portions, 50-90%, of organic matter in podzol B horizons can be extracted with NaOH (Mokma and Buurman, 1982), and thus constitute fulvic and humic acid fractions. Up to 50% remains insoluble. 0.1 M of sodium pyrophosphate ($Na_4P_2O_7$) is considered to be more effective in extracting organic matter from podzols. In general, no humin fractions are obtained, using a sample:solution ratio of 1:100 (w:w) (Mokma and Buurman, 1982). This is due to the ability of $Na_4P_2O_7$ to complex with Fe and Al, leaving the organic matter previously associated with these metals as soluble remnants.

In a study about the nature and origin of organic matter in a vegetation sequence, it appeared that the organic matter in incipient podzol B horizons was predominantly aliphatic. Furthermore, we found that about 17-37% of the organic matter was not soluble in NaOH. FTIR and DRIFT spectroscopic data of these 'humin' fractions revealed that considerable amounts of clay was present in those fractions (Buurman *et al.*, unpublished results). The organic matter in the humin fraction proved to be highly aliphatic. Also Theng *et al.* (1986) and Schulten *et al.* (1996) found that organic matter associated with clay in podzol B horizons was predominantly aliphatic. So, clay-organic matter complexes may be present in the insoluble fractions of the incipient B horizons.

To determine the origin of the organic matter, the humin fractions were subjected to further fractionation to distinguish between organic matter complexed with metals, associated with clay, or present as plant residues. Extracts and residues were analyzed by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) in the absence or presence of TMAH, while they were also studied by SEM.

MATERIALS AND METHODS

Profiles

The B horizons were sampled from sites at the Leuvenum Forest. This is a drift sand area, which is nowadays largely covered by a forest. Samples 1, 2 and 3 were collected from sites in the same Scots pine (*Pinus sylvestris* L.) stand, that differed in undergrowth: *Deschampsia flexuosa* (L.) Trin., *Vaccinium myrtillus* L. and *Empetrum nigrum* L., respectively. Under pine and *Deschampsia*, weakly developed podzols occur, whereas under the Ericaceae, podzolization is more pronounced (Emmer, 1994). When deciduous trees are introduced, they eventually tease away the pines and undergrowth. Sample 4 is under beech (*Fagus sylvatica* L.) with pines in the surroundings, and sample 5 is from a site with only beech. Both beech sites have a more developed podzol profile than in sites 1-3. All B horizons had only a few (living) roots.

Fractionation

Before use, the soil samples were air-dried and sieved (2 mm) to remove roots. Next, they were fractionated according to the scheme shown in Figure 1. About 100 g of soil was extracted with 1 L of 0.5 M NaOH for 24 h. under nitrogen. After centrifugation, the solution was decanted and the residue was shaken again with 0.5 M NaOH for 24 h. under nitrogen. The residue after centrifugation and decantation (residue 1) was acidified with HCl, washed several times until pH was neutral, and freeze-dried. Carbon contents were determined by dry combustion using an Interscience Elemental Analyzer EA1108.

Aliquots of the humins were subjected to extraction with 0.1 M of sodium pyrophosphate ($Na_4P_2O_7$), (C:solvent ratio 1:50 (w:w)). After 15 minutes of ultrasonic treatment, the samples were shaken for 24 h. After centrifugation, both solution (extract 2) and precipitate (residue 2) were acidified with HCl, dialysed, and freeze-dried.

To destroy clay, aliquots of residue 2 were treated for 24 h. with HF/HCl (3:1 v/v). After centrifugation, the solid phase was extracted by 0.5 M NaOH for 24 h. Centrifugation, acidification of the solution, and freeze-drying, yielded residue 3a. Similar procedures were carried out on aliquots of residue 2 using refluxing HF/HCl, followed by NaOH extraction, giving extract 3b and residue 3b, and refluxing in 0.5 M NaOH, resulting into extract 3c and residue 3c. These 'hot' steps were also done after ball-milling aliquots of residue 2.
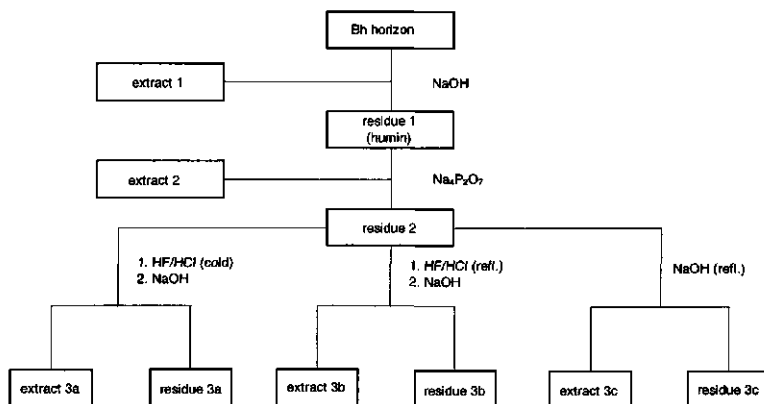
Figure 1. Fractionation scheme of organic matter in B horizons.

Pyrolysis-GC/MS and thermochemolysis

Pyrolysis was carried out on a Horizon Intruments Curie-Point pyrolyser. Samples were heated for 5 s at 610°C. The pyrolysis unit was connected to a CE gas chromatograph and the products were separated by a fused silica column (Chrompack, 25 m, 0.25 mm i.d.) coated with CP-Sil 5 (film thickness 0.40 μm). Helium was used as carrier gas. The initial oven temperature was 40°C. After pyrolysis, the oven was heated at a rate of 7°C/min to 320°C and held for 20 min. The end of the GC column was coupled to a Fisons MD 800 mass spectrometer (mass range m/z 45-650, cycle time 1 s). Pyrolysis-methylation (thermochemolysis) was performed by adding one or two drops of a solution of tetramethylammonium hydroxide (TMAH) in water (25%) onto the sample, dried for 1-2 minutes, and immediately followed by pyrolysis, using the same GC program as with conventional pyrolysis (J.W. de Leeuw, pers. com.).

SEM

Scanning Electron Microscope (SEM) analyses were performed with a Philips 535 SEM at IPO-DLO Wageningen.

RESULTS

Fractionation

Between 17 and 37% of the total organic matter present in the B horizons appears to be insoluble in 0.5 M NaOH (see Table 1). Further extraction of these humin fractions by $Na_4P_2O_7$ removes another 1-2% (samples 1-3) or 3-4% (samples 4 and 5) of the total C originally present.

Virtually no yields are obtained when treating residue 2 with HF/HCl to destroy the mineral matrix to set free the clay-bound organic matter. From this it can be concluded that the amounts of organic matter bound to clay is negligible. The final residue 3a is therefore similar to residue 2 and will not be discussed further.

When residue 2 is boiled in HF/HCl for 1 hour, the organic matter does not dissolve at all, and after adding NaOH the solution remains colourless: thus no significant amounts are soluble. Also when residue 2 is refluxed in 0.5 M NaOH for 1 hour, the organic matter is still in the residue. However, the organic matter in ball-milled aliquots of residue 2 almost completely dissolves in 0.5 M of NaOH.

Table 1. Carbon contents (mass fractions of air-dried soil) and distribution of extractable and residual C (mass fraction of total C) in B horizons of profiles 1-5.

Profile no	C	NaOH extract 1	residue 1	$Na_4P_2O_7$ extract 2	residue 2
	%	%	%	%	%
1	0.27	79	21	1	20
2	0.40	64	36	2	34
3	0.41	63	37	2	35
4	0.44	83	17	3	14
5	0.80	68	32	4	28

Humins (residue 1)

Figure 2 shows the pyrolysis-GC traces of the humin fractions of samples 1 and 5. Pyrolysis results of the humin fractions of samples 2 and 3 are similar to that of sample 1. Dominant signals in the pyrolysate of sample 1, 2 and 3 can be attributed to polysaccharides (peaks **1, 2, 4, 6, 8, 10, 13** and **16**), benzene derivatives (peaks **3, 5** and **7**), phenols (peaks **9, 11** and **14**), methoxyphenols (peaks **12** and **15**) and aliphatic compounds such as fatty acids and alkene/alkane pairs (consult Table 2 for the identified peaks). The latter are supposed to be pyrolysis products derived from highly aliphatic biopolymers, such as cutan and suberan (Nip *et al.*, 1986a, b; Tegelaar *et al.*, 1995; Augris *et al.*, 1998).

Very abundant pyrolysis products in the humin fraction of sample 5 are C_{22} and C_{24} alcohols, derived from suberin of beech (Holloway, 1983; Matzke and Riederer, 1991; Tegelaar *et al.*, 1995). These alcohols are also identified in the Py-GC trace of sample 4, but in lower amounts. Benzene and phenol derivatives (peaks **3, 5, 7, 9, 11**) and alkenes/alkanes are encountered as well in the insoluble organic matter of samples 4 and 5.
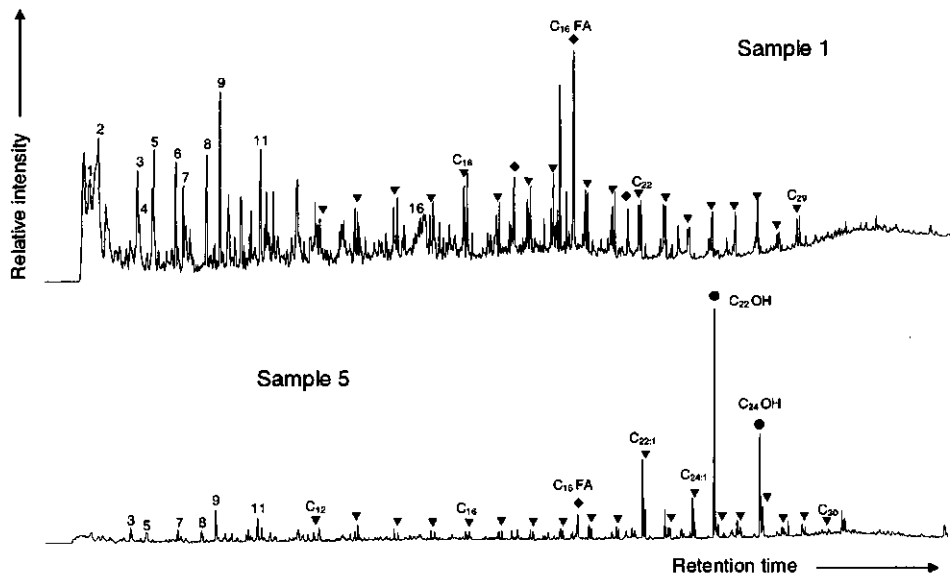

Figure 2. Pyrolysis-GC traces of humin fractions (residue 1) of samples 1 and 5. Numbers and symbols correspond to compounds listed in Table 2.
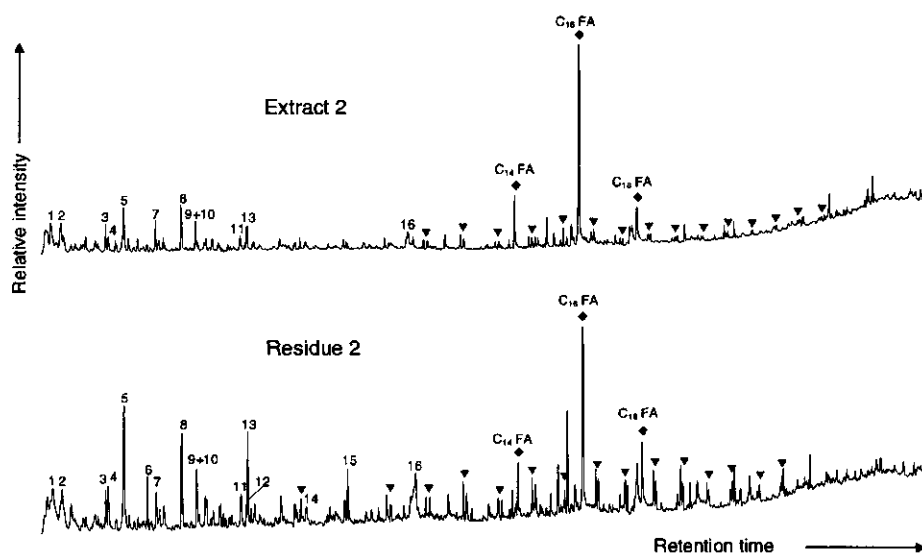
Na$_4$P$_2$O$_7$ extracts (extract 2) and residues 2

Fatty acids are enriched in the $Na_4P_2O_7$ soluble fractions (extracts 2) compared to the residues 2 obtained from samples 1, 2 and 3 (see Figure 3 for sample 1).

Similarly, samples 4 and 5 (Figure 4 for sample 5) have a relatively high concentration of fatty acids in their $Na_4P_2O_7$-extracts. In addition, higher amounts of polysaccharide-derived pyrolysis products have been found in pyrolysates of extract 2 than in those of residue 2. Apart from the C_{22} and C_{24} alkenes and the corresponding alcohols, the pyrolysate of the residue 2 contains phenols, especially 4-vinylphenol and 4-vinylguaiacol, levoglucosan and alkene/alkane pairs. Both vinylic compounds are most probably pyrolysis products from esterified *p*-coumaric acid and ferulic acid (de Leeuw and Baas, 1993), linked to e.g. polysaccharides, lignin and suberin (Kolattukudy, 1980; de Leeuw and Largeau, 1993). The alcohols (C_{22} and C_{24}) are important in extract 2, and even more so in residue 2. The presence of relatively high peaks of C_{22} and C_{24} alkenes, indicative for esters of the corresponding

Table 2. Major identified pyrolysis products.

No.	Compound	M
1	Furan	68
2	Acetic acid	60
3	Toluene	92
4	(2H)-Furan-3-one	84
5	Dimethylbenzene	106
6	2-Furaldehyde	96
7	Styrene	104
8	5-Methyl-2-furaldehyde	110
9	Phenol	94
10	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	114
11	Methylphenol	108
12	Guaiacol	124
13	Levoglucosenone	126
14	4-Vinylphenol	120
15	4-Vinylguaiacol	150
16	Levoglucosan	162
▼	n-Alkene/n-alkane doublet	
◆	Fatty acid	
●	Alcohol	

Figure 3. Pyrolysis-GC traces of $Na_4P_2O_7$-soluble (extract 2) and insoluble (residue 2) fractions of sample 1. Numbers and symbols correspond to compounds listed in Table 2.

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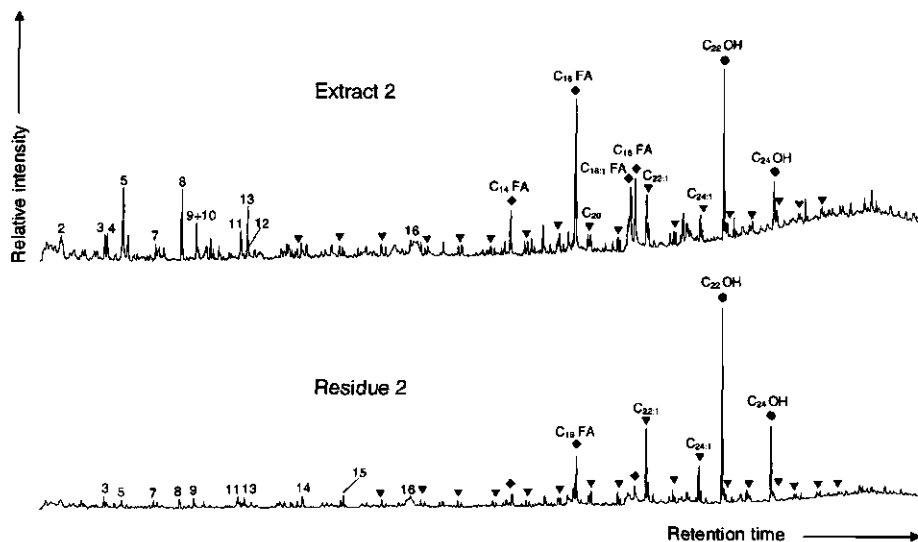**Figure 4.** Pyrolysis-GC traces of $Na_4P_2O_7$-soluble (extract 2) and insoluble (residue 2) fractions of sample 5. Numbers and symbols correspond to compounds listed in Table 2.
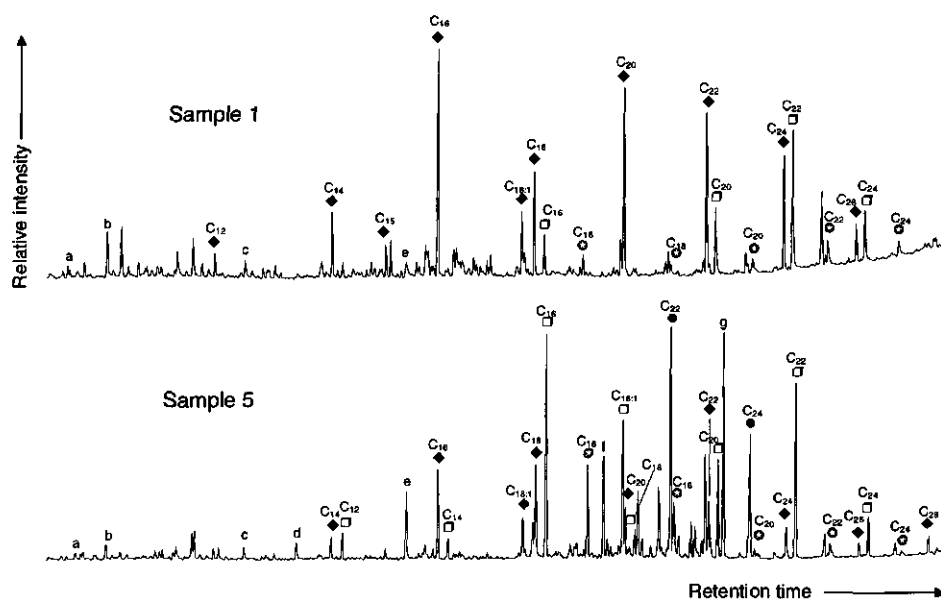

Figure 5. Thermochemolysis-GC traces of residue 2 of samples 1 and 5. For symbol legend, see Table 3.

alcohols in the pyrolysates of both the extract as the residue, suggests that three days of basic treatment (twice with NaOH and once with $Na_4P_2O_7$) hydrolyses the ester bonds far from completely.

Thermochemolysis

Pyrolysis with *in situ* methylation using tetramethylammonium hydroxide (TMAH) has been recently proven to be complementary to conventional pyrolysis in the analysis of polymers (Challinor, 1989), cutin (de Leeuw and Baas, 1993), lignin (Clifford *et al.*, 1995; Hatcher *et al.*, 1995; McKinney *et al.*, 1995), cutan (McKinney *et al.*, 1996), humic substances (del Rio *et al.*, 1994, Hatcher and Clifford, 1994) and peat (van der Heijden, 1994). Relatively polar pyrolysis products are poorly chromatographable as such, but can be separated well by GC after methylation in the presence of TMAH as methyl esters and/or methyl ethers methylated. Because the reactions are chemolytic rather than pyrolytic (de Leeuw and Baas, 1993), the procedure is more often referred to as thermochemolysis (McKinney *et al.*, 1995; Clifford *et al.*, 1995).

All thermochemolysis-GC traces of the residues 2, after NaOH and $Na_4P_2O_7$ extractions, are dominated by methyl esters and/or ethers of fatty acids (C_{12}-C_{28}), ω-hydroxy fatty acids (C_{12}-C_{24}) and α,ω-alkanedioic acids (C_{16}-C_{24}). Sample 4, and in particular sample 5 (Figure 5 and Table 3), have abundant signals of methyl ethers and/or esters of 1-alcohols (C_{22} and C_{24}), 9,16- and 10,16-dihydroxyhexadecanoic acid and 9,10,18-trihydroxyoctadecanoic acid. Also the ω-hydroxy fatty acids and α,ω-alkanedioic acids are more important in the GC traces of samples 4 and 5 than in those of samples 1-3. Only small traces of other compounds are present, confirming the results of conventional pyrolysis that the residues are predominantly aliphatic. All aliphatic compounds mentioned above can be related to the biopolyester suberin, present in the periderms that cover secondary stems, wounds and secondary roots (Kolattukudy, 1980; Matzke and Riederer, 1991; Riederer *et al.*, 1993; Tegelaar *et al.*, 1995), with the 1-alcohols (C_{22} and C_{24}) in samples 4 and 5 as marker of beech suberin in the soil. Although data about the chemical composition of suberin in roots are lacking, the ratio of the C_{22} to C_{24} alcohol of about 2 resembles that of values measured in suberin from beech bark (Holloway, 1983).

Furthermore, the relatively high abundance of 22-hydroxydocosanoic acid, hexadecanedioic acid and 9,10,18-trihydroxyoctadecanoic acid refers to suberin derived from beech (Holloway, 1983; Matzke and Riederer, 1991). The relatively high contributions in the pyrolysates of methylated versions of *p*-coumaric and ferulic acid in relation to other aromatic compounds suggest that these acids are derived from, or esterified with suberin.

Fatty acids with chain lengths < C_{20} are uncommon constituents of suberin (Kolattukudy, 1980; Holloway, 1983). Therefore, they will have originated from other compounds, most probably from triglycerides or waxes. The alkene/alkane patterns observed in the Py-GC traces of all residues are not encountered after thermochemolysis. This resembles the pyrolytic and thermochemolytic behaviour as encountered for the aliphatic biopolymer cutan from the cuticle of *Agave americana*. It gave a homologous series of alkadiene/alkene/alkane triplets upon pyrolysis, whereas, among others a series of fatty acid methyl esters (FAMEs) is

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obtained after thermochemolysis (McKinney *et al.*, 1996). Thus, the FAMEs obtained in the residues can to some extent also be derived from an aliphatic polymer similar to cutan or suberan.

In addition to the FAMEs, McKinney *et al.* (1996) identified also a number of aromatic compounds, mainly 1,3,5-trimethoxybenzene derivatives and, in lower amounts, 1,2,3-trimethoxybenzene derivatives and dimethoxybenzoic acid methyl esters. We did not observe 1,3,5-trimethoxybenzenes, whereas 1,2,3- and 1,2,4-trimethoxybenzenes were clearly present. It cannot be excluded that these trimethoxybenzenes are indeed derived from a suberan-like aliphatic biopolymer with building blocks similar to those of cutan from *Agave americana*, but structurally different.

Table 3. Major identified thermochemolysis products.

No.	Compound	M
a	1,2,3-Trimethoxybenzene	168
b	1,2,4-Trimethoxybenzene	168
c	3,4-Dimethoxybenzoic acid, methyl ester	196
d	3-(4-Methoxyphenyl)-2-propenoic acid, methyl ester	192
e	3-(3,4-Dimethoxyphenyl)-2-propenoic acid, methyl ester	222
f	9,16 and 10,16-Dimethoxyhexadecanoic acid, methyl ester	330
g	9,10,18-Trimethoxyoctadecanoic acid, methyl ester	388
◆	Fatty acid, methyl ester (FAME)	
❐	ω-Methoxy fatty acid, methyl ester	
❂	α,ω-Alkanedioic acid, dimethyl ester	
●	1-Methoxyalkane	

Scanning Electron Microscope (SEM)

SEM (not shown) was applied to assess whether the observed chemical data obtained with pyrolysis and thermochemolysis derive from recognizable plant remnants or from completely humified, i.e. unrecognizable amorphous material.

In the humin fractions, intact and partly altered plant fragments are recognized by SEM. Some of the fragments can be identified as parts from roots, but all fragments consisted predominantly of cell walls without inner cell material. $Na_4P_2O_7$ extractable organic matter (extracts 2) does not contain such remnants, but forms fibrous and flagged structures, most likely caused by freeze-drying. As the humin fractions, the residues 2 contain predominantly plant structures, albeit more degraded than in the humin fractions. Apparently extraction with $Na_4P_2O_7$ removes parts of (degraded) plant tissue.

DISCUSSION AND CONCLUSIONS

Pyrolysis, thermochemolysis and SEM results all point to a predominantly root origin of the insoluble organic matter. Emmer (1995b) determined the relative root densities of the species present at sites 1-3. Except for *Empetrum*, all species have roots in the mineral horizons. The similarity of the humin fractions, the extracts 2 and the residues 2 of the organic matter in the B horizons of the profiles 1, 2 and 3 strongly suggests that the (root) input is independent of the understorey vegetation. In profiles 4 and 5, later stages in the vegetation sequence, the influence of beech as indicated by the C_{22} and C_{24} alcohols is clearly present in the B horizons. The relative increase of mono, di and trihydroxy fatty acids and α, ω-alkanedioic acids in the pyrolysates of the two samples under beech points to an increasing concentration of suberin in the B horizon. According to SEM, the organic part of the residues is composed of intact or partly altered cell walls. About half a century ago, Kononova (1943, cited by Kononova, 1961) established the rapid degradation of medullary rays, cambium, phloem and parenchyma of the primary cortex in humified lucerne roots, leaving the periderm and xylem vessels unaffected. As determined by pyrolysis, polysaccharides and lignin are hardly present in the residues 2, suggesting that xylem is almost completely decomposed. This means that the periderm layer (suberin and a suberan-like polymer) of the cell wall are selectively preserved, giving rise to the predominant aliphatic character of the organic matter in the B horizons, and in the insoluble fractions in particular.

Considering the vegetation sequence and history of the Leuvenum Forest, pine trees and other species have abundant fine roots in the Ah horizon during the first succession stages. With time, the ectorganic layer develops and becomes the main rooting compartment. The old and dead roots originally present in the Ah horizon remain in the newly formed E and B horizons. Upon decay, microbial readily decomposable compounds, such as polysaccharides and lignin, decompose, while the less accessible aliphatics selectively preserve. Under beech, not only roots from previous species contribute to the organic matter in the B horizons, but also roots from the current trees. This is clearly manifested in samples 4 and 5, where the presence of typical beech root components, such as the already mentioned C_{22} and C_{24} alcohols, is established. Site 5 has older beech trees than site 4, but with the same amount of living roots. Hence, the relatively higher concentration of e.g. the C_{22} and C_{24} alcohols in sample 5 is most likely derived from a greater accumulation of dead beech roots.

In principle it could be possible that part of suberin in the B horizons is derived of illuviation of water soluble organic matter. However, the solubility of suberin in water is negligible (Kögel-Knabner *et al.*, 1989), which is also shown in Chapter 6 for the WSOM fractions of the investigated profiles here. In very old podzols (e.g. millions years old) the possible contribution of suberin in B horizons due to illuviation may be significant, but within the time span of two centuries in the soils of the Leuvenum Forest illuviation of suberin may be neglected.

Assuming that NaOH does not dissolve Al- and Fe-organic matter complexes, increasing amounts of $Na_4P_2O_7$ extractable C in the sequence from sample 1 to sample 5, suggest that effects of podzolization also increase in that direction. Nevertheless, metal-organic matter complexes seem to be of minor importance in these incipient podzols. $Na_4P_2O_7$ soluble fractions also contain aliphatic compounds. As seen with SEM, the residues after $Na_4P_2O_7$

extraction exhibit more degraded plant remnants, whereas pyrolysis suggests that the alcohols are to some extent present as esters in these fractions. This probably means that, in addition to metal-organic matter complexes, some polymerized parts are solubilized in 0.1 M of $Na_4P_2O_7$.

Cold treatment with HF/HCl, followed by NaOH extraction of residue 2 does not induce much effect in dissolving organic matter. This most likely means that little of the organic matter was bound to clay. Neither hot treatment with HF/HCl followed by extraction with NaOH gives a soluble fraction, nor hot NaOH only. However, when prior to treatment with hot NaOH residues 2 are ball-milled, the bulk of the organic matter becomes soluble. This can be explained by an increasing surface area of the plant fragments in the residues 2, so that the base-catalyzed hydrolysis of ester bonds of the suberin polymers takes much faster place.

In conclusion, in incipient podzol B horizons, the organic matter appears to be highly aliphatic, particularly the more recalcitrant, insoluble fractions. These fractions mainly contain outer cell wall remnants without inner cell wall material. Pyrolysis and thermochemolysis confirm this, showing that this material is practically devoid of lignin and polysaccharides, and mostly consisted of aliphatics, which can be related to a suberan-like polymer and the biopolyester suberin, present in the periderm layer of the cell wall. Periderms have a protective function in living plant roots - and apparently this function still exists -, while other plant components containing biopolymers, such as polysaccharides and lignin, are virtually absent. Hence, these root remnants seem to be a more important source of carbon sink in the incipient B horizons than the illuvial organic matter caused by podzolization.

Chapter 8

Origin of aliphatic compounds in a forest soil

ABSTRACT

The aliphatic organic matter fraction in a forest soil in the Leuvenum Forest (central Netherlands) was examined by pyrolysis-GC/MS and thermochemolysis. In addition, roots were analyzed for their aliphatic composition. Pyrolysis of soil organic matter and the roots revealed an alkene and alkane pattern, usually attributed to cutan or suberan, whereas thermochemolysis elucidated the presence of suberin. The origin of the soil organic matter could be largely ascribed to root input.

INTRODUCTION

Soil organic matter (SOM) is composed of a mixture of residues of plant, microbial and/or animal origin. Mineralization and humification change the composition in general as well as at the molecular level. Originally, research focused on extractable organic matter, i.e. the fulvic and humic acid fractions. Based on these earlier studies the structure of humic substances (often defined as organic matter minus recognizable plant-derived molecules) was considered to be composed of an aromatic core formed through random condensation reactions of (poly)phenols and quinones, both mainly derived from lignin, together with N-containing compounds (Stevenson, 1994). The application of solid state ^{13}C NMR on total soil samples has changed the view on organic matter formation drastically. These data indicate that aliphatic moieties accumulate when decomposition proceeds, whereas the contents of aromatic compounds increases, decreases or remains unchanged. (For a review, see Baldock et al., 1997). Three main sources of aliphatic compounds derived from plants can be distinguished: (i) (extractable) lipids, (ii) the biopolyesters cutin and suberin, and (iii) non-hydrolyzable biopolymers, such as cutan and suberan (Tegelaar et al., 1989). The latter biomolecules are suggested to be responsible for the relative enrichment of aliphatic compounds in soil (Tegelaar et al., 1989, Augris et al., 1998), because both lipids and biopolyesters are considered to have a low preservation potential compared to cutan and suberan (de Leeuw and Largeau, 1993). The contribution of cutan, suberan or other resistant aliphatic macromolecules in soils is based on the presence of alkene/alkane doublets in pyrolysates (Tegelaar et al., 1989; Kögel-Knabner et al., 1992a, b; van Bergen et al., 1997; Augris et al., 1998).

In the present study, sandy soils from the Leuvenum Forest area (central Netherlands) have been examined with respect to their aliphatic composition. These nutrient-poor soils have a pH of about 3.8, which implies that biological homogenization is virtually absent and is furthermore reflected in well-defined profiles with thick, dark litter layers that have a sharp transition to the gray/white mineral layers (Emmer, 1995a). Due to the low mixing, roots may be the most important input of organic matter in these mineral horizons. Initial ^{13}C NMR data have revealed that the organic matter is dominated by alkyl carbon, whereas O-alkyl carbon and aromatic carbon are less abundant (Chapter 6).

The objectives of the present study are to elucidate the chemical composition of the aliphatic fraction of soil organic matter, and to what extent material from roots contributes to the organic matter in the soil. Pyrolysis-gas chromatography/mass spectrometry and

thermochemolysis (pyrolysis in the presence of TMAH) are applied to analyse soil samples and (fresh) roots.

MATERIALS AND METHODS

The soil samples were taken from a Cambic Arenosol (FAO-Unesco, 1988), with incipient podzolization, from the Leuvenum Forest (central Netherlands). The area is covered by *Fagus sylvatica* L. (beech), which succeeded *Pinus sylvestris* L. (Scots pine) (Fanta, 1986; Prach, 1989). The soil profiles consist of an ectorganic layer (10-12 cm thick) overlying a white/grayish EAh horizon (8 cm, 0.7% C) and a brown/yellow B horizon (12 cm, 0.8% C). The B horizons were used for this study. The soil samples were freed from (the few) living roots by hand-picking, subsequently dried at 50°C, and sieved (2 mm; 10 times) to remove litter material. For analyses, the samples were shaken in 0.5 *M* NaOH (1:1, w/w) for 5 minutes and allowed to stand for 1 minute. While gently shaking, the solution containing dissolved and particulate organic matter was decanted. The residual sand was washed with distilled water and this was decanted (5 times). All 'solutions', including all insoluble material, were combined, acidified to pH 2 with a solution of 0.1 *M* HCl and 0.3 *M* HF and shaken for 24 hours and finally dialysed and freeze-dried. Roots (< 1mm) (from *Calluna vulgaris* (L.) Hull, *Pinus sylvestris* L., *Deschampsia flexuosa* (L.) Trin. and *Fagus sylvatica* L.) were taken from the soil at different locations in the Leuvenum Forest and the Hulshorster Sand, dried and ground (ball-mill). Prior to pyrolysis, roots were extracted with MeOH/DCM (1:1) for 24 hours in a Soxhlet apparatus.

Samples were pyrolyzed at 610°C on a Horizon Instruments Curie-Point pyrolyser connected to a CE gas chromatograph. The products were separated on a fused silica column (Chrompack, 25 m, 0.25 mm i.d.) coated with CP-Sil 5 (film thickness 0.40 μm). After pyrolysis, the GC was heated from 40°C at a rate of 7°C/min to 320°C and maintained at that temperature for 20 min. The end of the GC column was coupled to a Fisons MD 800 mass spectrometer (mass range *m/z* 45-650, ionization energy 70 eV, cycle time 1 s). Pyrolysis in the presence of tetramethylammonium hydroxide (TMAH) was performed by adding a droplet of a 25% solution of TMAH in water to the sample, after which the sample was dried, and immediately pyrolyzed.

RESULTS AND DISCUSSION

The Py-GC trace of the soil sample is shown in Figure 1. The most abundant non-aliphatic pyrolysis products are toluene, 2-furaldehyde, 5-methyl-2-furaldehyde, phenol, 2-, 3- and 4-methylphenols, 2-methoxyphenol, levoglucosan (LG) and diketodipyrrole (DKDP). They indicate the presence of polysaccharides (e.g. Saiz-Jimenez and de Leeuw, 1986a; van Bergen *et al.*, 1997), proteins/peptides (van Bergen *et al.*, 1998), tannins (Galletti and Reeves, 1992) and (very little) lignin (e.g. Saiz-Jimenez and de Leeuw, 1986a, b; van Bergen *et al.*, 1997).
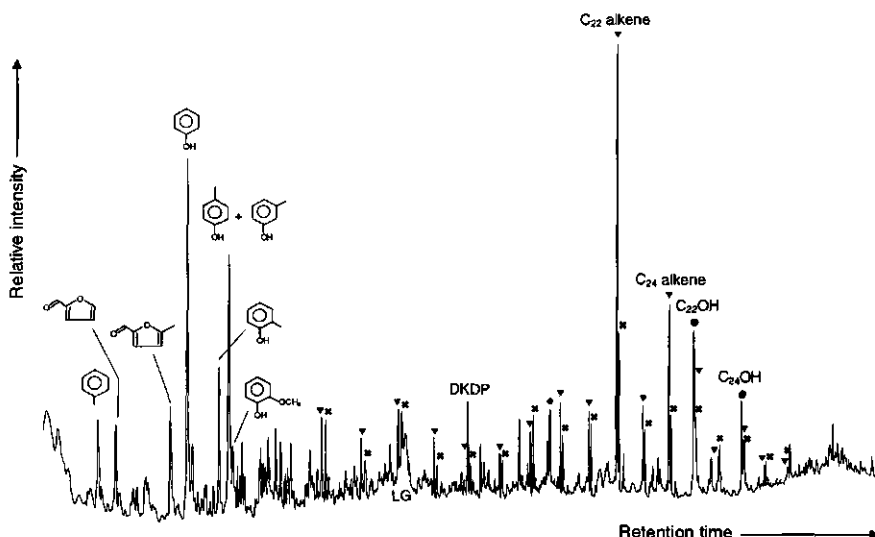
Figure 1. Py-GC trace of soil sample (under beech). Legend: LG = levoglucosan; DKDP: diketodipyrrole; ▼: *n*-alk-1-ene; ✖: *n*-alkane; ◆: fatty acid; ●: 1-alcohol.

The aliphatic fraction of the pyrolysate consists of a homologous series of alkenes and alkanes ranging from C_{11} to C_{29}, and two long-chain alcohols (C_{22} and C_{24}). The relatively high signals of the C_{22} and C_{24} alkenes are thought to be pyrolysis products from the corresponding esterified alcohols (van Smeerdijk and Boon, 1987). The pyrolysate resembles that of soils from Broadbalk Wilderness, Rothamsted (van Bergen *et al.*, 1997). The main differences were the relatively high abundance of nitrogen-containing compounds, and the absence of relatively high peaks of C_{22} and C_{24} alkenes and the C_{22} and C_{24} alcohols in the Py-GC traces of the Rothamsted soils.

The alkenes and alkanes are commonly attributed to be derived from insoluble, non-hydrolyzable aliphatic polymers, such as cutan (Nip *et al.*, 1986; Tegelaar *et al.*, 1993) and suberan (Tegelaar *et al.*, 1995). However, until now, these polymers have only been observed in above-ground plant tissues, such as cuticles and barks (de Leeuw and Largeau, 1993). It is therefore somewhat surprising to observe these compounds in the soil, because input of above-ground litter is negligible at this site due to the absence of biological mixing.

To find out whether roots contained macromolecules, which released a similar alkene/alkane distribution, fine roots from the main vegetation (*C. vulgaris, P. sylvestris, D. flexuosa* and *F. sylvatica*) in the Leuvenum Forest were pyrolyzed after extraction with MeOH/DCM. This results, in all cases, in the presence of the alkene/alkane doublets in the pyrolysates of the roots. Their relative abundance is highest in roots of *Calluna*, followed by pine and *Deschampsia*. For beech roots, the alkenes and alkanes are hardly visible due to very intensive C_{22} and C_{24} alkene peaks.

In Figure 2, the mass chromatogram, *m/z* 83 + 85, clearly reveals the alkene/alkane doublets in the pyrolysate of *Calluna* roots. The alkene/alkane (C_{10} - C_{31}) pattern is

dominated by the C_{22} and C_{24} alkenes, whereas C_{22} and C_{24} alcohols are also prominently present.
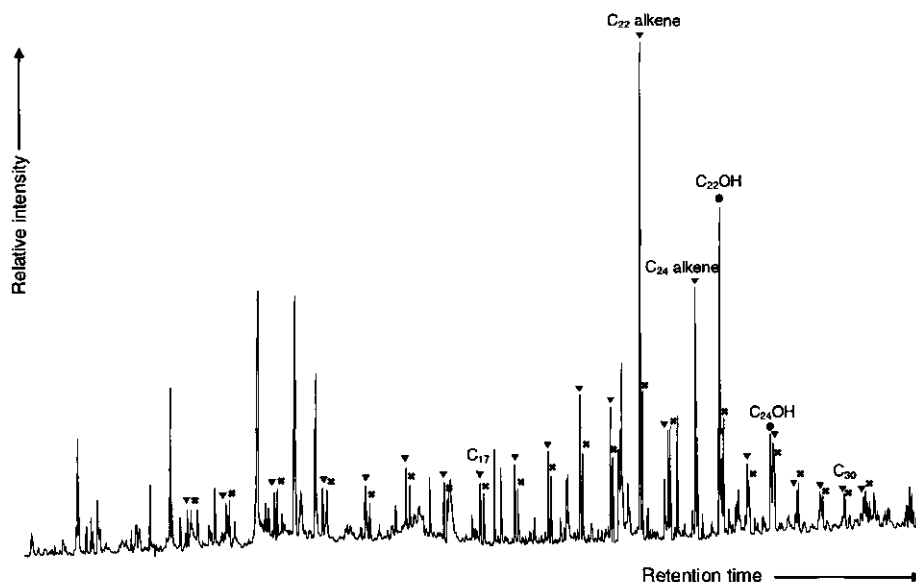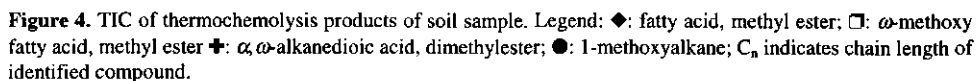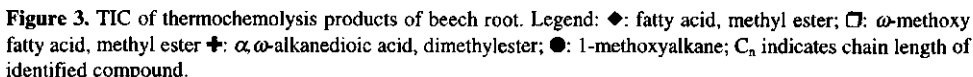

Figure 2. Summed mass chromatogram (*m/z* 83 + 85) of pyrolysate of *Calluna* root. Legend: ▼: *n*-alk-1-ene; ✖ : *n*-alkane; ◆: fatty acid; ●: 1-alcohol; C_n indicates chain lenght of identified compound.

The homologous series of alkenes and alkanes indicate that a cutan or suberan-like polymer may be present in the roots. Van Smeerdijk and Boon (1987) postulated that an aliphatic biopolymer, which gave alkenes/alkanes upon pyrolysis, was present in the cell walls of the endodermis and exodermis of the cortex of fossilized Ericaceae rootlets. The C_{22} and C_{24} alcohols and their pyrolysis products C_{22} and C_{24} alkenes in the pyrolysates of the soil samples, *Calluna* roots and *Fagus* roots suggest the presence of the polyester suberin (van Smeerdijk and Boon, 1987; Holloway, 1983; Matzke and Riederer, 1991).

Recently, pyrolysis in the presence of TMAH (actually thermochemolysis) has been successfully used to study the polyester cutin (de Leeuw and Baas, 1993). Here, soil and roots were subjected to the same procedure to determine the significance of the biopolyester suberin in SOM. Figures 3 and 4 show the TIC of the thermochemolysis of *Fagus* roots, and of the soil sample, respectively.

Both consist primarily of two sets of compounds: the first part of the chromatograms is dominated by methylated phenols (methoxybenzenes) and 2-methoxyphenols (1,2-dimethoxybenzenes), which are mainly lignin-derived products (Clifford *et al.*, 1995). The

Figure 3. TIC of thermochemolysis products of beech root. Legend: ◆: fatty acid, methyl ester; ▢: ω-methoxy fatty acid, methyl ester ✚: α, ω-alkanedioic acid, dimethylester; ●: 1-methoxyalkane; C_n indicates chain length of identified compound.

Figure 4. TIC of thermochemolysis products of soil sample. Legend: ◆: fatty acid, methyl ester; ▢: ω-methoxy fatty acid, methyl ester ✚: α, ω-alkanedioic acid, dimethylester; ●: 1-methoxyalkane; C_n indicates chain length of identified compound.

second part reveals methyl esters and/or methyl ethers of fatty acids (C_{16}-C_{30}), ω-hydroxy fatty acids (C_{16}-C_{26}), α,ω-alkanedioic acids (C_{16}-C_{24}), alcohols (C_{22} and C_{24}), 9,16- and 10,16-dihydroxyhexadecanoic acid and 9,10,18-trihydroxyoctadecanoic acid. These compounds are well-known constituents of suberin (Kolattukudy, 1980; Holloway, 1983; Matzke and Riederer, 1991), and thus these results provide unequivocal evidence for the contribution of root organic material to SOM.

The distribution of the compounds differs between *Fagus* roots and the soil sample. This may be due to the fact that *Fagus* is not the only vegetation contributing organic matter into the mineral soil, but so has pine, and maybe others too. Secondly, selective decomposition of some monomers compared to other building blocks may have taken place, the relative decrease of e.g. the 1-alcohols and $C_{18:1}$ fatty acid compared to 9,10,18-trihydroxyoctadecanoic acid may be explained in that sense. The mono functionalized hydrocarbons are at terminal positions of the polymer, and will therefore be depolymerized upon natural decomposition in favour of the trihydroxy fatty acid, which forms the core of the macromolecules.

These data clearly show that alkenes and alkanes are not restricted to pyrolysates of cuticles and periderms of above-ground plant parts, but can also be derived from roots. In addition, thermochemolysis reveal the building blocks of suberin in roots. Pyrolysis and thermochemolysis data of the soil samples reveals unambiguous evidence for the presence of suberin and a suberan-like polymer, and clearly show the importance of root material to SOM.

Chapter 9

Synthesis

In the preceding chapters, the organic chemical composition of plant tissues (leaves, needles, roots etc.), organic (L, F and H) and mineral horizons (Ah, E and Bh), water soluble, NaOH soluble and insoluble fractions in different stages of the vegetation succession (see Figure 1 in Chapter 1) has been analyzed. In this chapter I want to put together and, where possible, solve the puzzle made up of the pieces from the earlier chapters.

THE ORGANIC LAYERS IN THE COURSE OF THE SUCCESSION

In the first successional stages, when algae, grasses and mosses cover the sand, the input of litter does not exceed decomposition of organic matter, and thus no organic horizons are formed. Under heather or pine trees an ectorganic layer is developed. Heather primarily succeeds the mosses, whereas pines mainly develop on the dryer, grassy sites. Under *Calluna*, the influence of mosses is clearly present, in the ectorganic layer as well as in the endorganic layer. Especially pronounced are the aliphatic macromolecules derived from mosses (Chapter 2). Also the contributions of flowers, leaves and woody branches from the *Calluna* itself are chemically evident. Similarly, the pine soils have primarily needle-derived material in the L and F horizons. The young age of these early successional *Calluna* and *Pinus* profiles is reflected by the low humification degree: polysaccharides dominate the organic matter composition, with small contributions of lignin, tannins, cutin and suberin.

Later on in the vegetation sequence, in the pine forest, ectorganic layers of 10-15 cm develop. With depth, from L to H horizon, the typical humification trends are observed with NMR: contents of O-alkyl C decrease, aromatic C remains constant and alkyl C increases relatively. Despite the dominant undergrowth of *Deschampsia* and *Empetrum* in the older pine forest, the bulk of the organic matter composition as determined by NMR and pyrolysis seems hardly affected by the understorey. E.g. lignin-derived pyrolysis products are dominated by guaiacols, and products derived from angiosperms are hardly present, suggesting that soil organic matter is predominantly pine-derived. Thermochemolysis, however, reveals some influence of the undergrowth by the presence of cutin and suberin building blocks from grasses and dwarf shrubs. The impact of their roots is especially pronounced in the F1 and F2 horizons (Chapter 5).

In the beech forest, NMR reveals the same trend as in the pine forest: with increasing depth alkyl C increases relatively to aromatic C and O-alkyl C. The L and F horizons are dominated by beech leaves and the bulk organic matter composition is dominated by beech as well (Chapter 5). The deeper H horizons however, appear to be mainly derived from pine litter (Chapter 4); the difference between the young and old beech forest is mainly reflected by the presence of suberin derived from beech roots. Although no clear differences are observed in root density between the young and old sites, the contribution of beech suberin is much higher in the older stands (Chapter 4).

All L horizons are, of course, mainly composed of fresh plant material and consist of polysaccharides, and smaller amounts of lignin, tannins, cutins etc. With depth, towards the H horizons, the polysaccharide contribution decreases, aromatic compounds remain constant, and aliphatic compounds such as, cutin, suberin, cutan and suberan become the dominant fraction (Chapters 4 and 5).

THE MINERAL LAYERS IN THE COURSE OF THE SUCCESSION

The organic matter in the mineral soil has three possible sources: *i*) below-ground litter (roots), *ii*) above-ground litter that has been mixed in, and *iii*) illuviated water soluble organic matter. Because biological activity is very low, mixing is probably negligible.

In the first stage, when the algae establish themselves in the sand, the little organic matter in the soil is, no wonder, completely determined by these algae. The organic matter in the mineral soil consists primarily of polysaccharides and some phenolic and aliphatic compounds. In the next stage, when grasses appear, the algae still have the greatest contribution to the organic matter, although a clear influence of mosses is also present (Chapter 2). Polysaccharides still dominate the soil organic matter, lignin is hardly present, and the aliphatics are mainly derived from mosses. Under mosses, the organic matter is almost exclusively derived from the covering vegetation.

The negligible biological mixing is reflected by the fact that the organic layer is directly related to above-ground plant tissues, while, the organic matter in the Ah horizon is mainly derived from root material. Similarly, this is valid for the young pine stands, where organic matter derived from pine roots dominates the Ah horizon. In addition, aliphatics from mosses of a previous successional stage contribute to the Ah under both *Calluna* and pine. Because roots are abundant in the mineral layers, the supply of fresh organic matter is high, and the organic matter has a relatively low humification degree: polysaccharides are the most important fraction, whereas lignin and aliphatics are present in relatively small amounts. The humification degree is higher in the Ah than in the ectorganic horizons, e.g. lignin is more oxidized (Chapter 2).

In the incipient podzols, the E and B horizons under both the pine and beech forest appear to be richer in aliphatics than the ectorganic layers. The similarity in organic matter composition of E and B horizons is observed with NMR, pyrolysis and thermochemolysis (not shown in this thesis). Both appear to be strongly aliphatic, partly by a cutan or suberan-like macromolecule and partly by suberin (Chapters 7 and 8), whereas polysaccharides and lignin are hardly present.

ORIGIN, OCCURRENCE AND FATE OF POLYSACCHARIDES

Polysaccharides form the main fraction (60-70%) of initial plant litter. They form the most common food source of microbes, and are easily decomposable. Hence their relative contribution to soil organic matter decreases as decomposition c.q. humification proceeds. Microbial carbohydrates are present in small amounts but their relative concentration in the total sugar pool increases upon humification. Thus the concentration of microbial sugars provides an indication of the humification degree of the organic matter. Although all monosaccharides are present in plants as well as in microorganisms, several indicators, based on sugar monomers, have been proposed to determine the origin of polysaccharides in soil samples. One is the ratio of mannose + galactose to arabinose + xylose, another is the fucose + rhamnose to arabinose + xylose ratio. The higher these ratios, the higher the microbial sugar contribution.

The content of total and plant sugars decreases in the direction fresh litter > L > F > H, due to the preferential decomposition of these compounds. The composition of polysaccharides in the H horizons is similar in the oldest pine and in the still older beech forest, implying a sort of steady state for this horizon during these stages of development. The Ah, E and B horizons have higher relative concentrations of microbial sugars than the overlying organic layers, whereas the (main) source material (leaves, needles, roots etc.) have comparable compositions. This suggests that the carbohydrates have been more strongly transformed in the mineral layers than in the organic horizons.

ORIGIN, OCCURRENCE AND FATE OF LIGNIN

Lignin is characteristic for vascular plants; it is absent in algae and mosses, but present in grasses, shrubs and trees. Three different building blocks are available for the assemblage of the macromolecule: coumaryl alcohol, coniferyl alcohol and synapyl alcohol. Gymnosperm-lignin has virtually only coniferyl alcohol as a monomer, (dicotyledonous) angiosperms have proximately equal amounts of coniferyl and synapyl alcohol, and grasses have all three building blocks. However, this is only true for above-ground tissues: roots of both gymnosperms and angiosperms have only coniferyl alcohol in their lignin (Chapter 5). Grass roots and leaves appear to have a similar lignin composition. In a given soil (sample) the distribution of the lignin monomers provides information about the origin of the organic matter. However, degradation of lignin, e.g. demethoxylation, results into a transformation of dimethoxyphenols (syringols or synapyl alcohol) into monomethoxyphenols (guaiacols or coniferyl alcohols). Hence, the lignin signature is changed by such processes and may give a biased picture of the origin of the organic matter and of lignin in particular.

As expected, no lignin is observed in the soil under the algae. Under the first grasses, lignin is present, but only in small amounts. In the ectorganic layer of the *Calluna* profile a clear angiosperm signature of the lignin-derived organic matter is present. In the Ah horizon, hardly any syringyl components are detected, because of the minimal above-ground input and thus mainly 'gymnosperm-like' root input. Under the initial sparse pines only the characteristic gymnosperm lignin is present. In the pine forest with *Deschampsia*, *Empetrum* and *Vaccinium*, the L and to some extent the F1 have very small amounts of angiosperm lignin, but predominantly gymnosperm lignin, which is the only lignin-derived material present in the underlying organic and mineral horizons. The L and F horizons under beech have clear contributions of syringyl-lignin.

In the youngest profiles, under *Calluna* and pine, lignin is clearly oxidized with respect to the fresh lignin in tissues. In the older profiles, in the pine and beech forest, signs of lignin degradation increase with depth: oxidation, thus relative increase of carboxylic acid and ketonic groups with respect to non-oxidized side-chains, and decreasing chain length of side groups.

The lignin degradation pattern seems to be different for the ectorganic layer and for the endorganic layer. In the early stages under *Calluna* and pine, the concentration of lignin is lower in the endorganic than in the ectorganic horizons (Chapter 2), accompanied by a higher degree of oxidation in the mineral layers. In the course of the succession, such differences between organic and mineral layers increase remarkably. In the ectorganic layers of in the

pine and beech forest lignin concentrations are more or less similar to those in the fresh plant materials, whereas in the E and B horizons, lignin remnants are hardly present and only in a very oxidized state (Chapter 5 *vs.* Chapters 6, 7 and 8). This may suggest a different pathway or process of lignin degradation. 'In' the soil, the rate of lignin humification seems to be much higher than 'on' the soil, assuming that the age of organic matter is more or less similar in ect- and endorganic horizons.

ORIGIN, OCCURRENCE AND FATE OF ALIPHATIC BIOPOLYMERS

In the early stages, biopolymers derived from mosses that give homologous series of *n*-alkenes and *n*-alkanes together with a series of branched alkenes and alkanes upon pyrolysis (Chapter 2) contribute significantly to the aliphatic fraction. In later stages, moss-derived aliphatics have disappeared (at least the branched alkenes/alkanes are not detectable anymore in pyrolysates), but the series of *n*-alkenes and *n*-alkanes become more important during the succession in organic as well as in mineral layers. It is not certain from which species the cutan or suberan macromolecules are derived, but beech leaves and roots are an important source, although also other plants may add these aliphatic polymers to the soil (Chapter 8).

Another family of aliphatic biopolymers, cutin and suberin, plays a role in the selective accumulation of aliphatic compounds upon decomposition. In the ectorganic layers this is a combination of cutin and suberin mainly derived from leaves, needles and roots from both the trees and the undergrowth, whereas in the mineral soil this is mainly suberin from tree roots. In these soils cutin and suberin act as biochemical tracer for the organic matter origin.

PODZOLIZATION

In the course of the succession, the soil develops towards a micro-podzol. Pyrolysis and NMR of organic matter from different horizons in podzols may help increase the knowledge about the podzolization process, which is still a matter of debate. Current theories about the organic matter accumulation propose that soluble material move from upper layers (e.g. organic horizons) through the soil until it is precipitated by complexation with Al and Fe. This suggests that functional groups such as carboxyl and hydroxyl, capable to interact with metals, would be a characteristic for the organic matter.

The water soluble organic matter (WSOM) from ectorganic layers, as source for the podzol B organic matter, appears to have an important polysaccharide and lignin composition, whereas aliphatic compounds are hardly present. The organic matter in the B horizons, however, is highly aliphatic, derived from suberin and cutan/suberan-like polymers. Polysaccharides and lignin are very minor compounds. Thus, there is little resemblance between WSOM and B horizon organic matter. However, in the pre-podzol stages the WSOM appeared to have an important aliphatic contribution (Chapter 3). In Chapter 2 it was discovered that these aliphatic compounds are mainly derived of mosses. The distribution of alkenes and alkanes, especially the presence of branched alkenes and alkanes, in pyrolysates did not resemble that of the B horizons. Thorough analysis of the B horizon organic matter indicated that roots are the main source of organic matter, and that little or no organic matter

was metal- or clay-bound (Chapter 7). However, the composition of the organic matter in the fibers below the B horizons appeared to have a composition similar to that of the WSOM (Chapter 6)! Apparently WSOM from the ectorganic horizon passes through the B horizons and accumulates when the water stops moving, forming the organic fibers in these incipient podzols. Hence, B horizon organic matter in the initial podzolization stages are root-derived and not formed by illuviation of Al- and Fe complexed organic matter.

THE MAIN OBSERVATIONS AND CONCLUSIONS

can be summarized as follows:

- Mosses have a cutan/suberan-like aliphatic macromolecule that produces straight- and (methyl)branched-chain alkene and alkane doublets upon pyrolysis
- These aliphatic polymers appear to have a remarkable 'solubility' in water
- H horizons under different vegetation in incipient podzols are mainly derived from pine litter and only roots of covering species contribute to organic matter
- L and F horizons in the pine forest with different undergrowths have a gymnosperm-lignin composition, but the cutin and suberin composition relates directly to current vegetation and their parts (needles, leaves, roots)
- In all organic horizons the polysaccharide fraction decreases with depth, the lignin and tannin concentration remain approximately constant, and the contribution of aliphatic polymers increases relatively
- In the mineral layers, polysaccharides and aromatic compounds are both almost completely disappeared resulting into a very high content of aliphatics
- Humification in terms of composition seems to follow different pathways in ect- and endorganic layers:
  in the ectorganic horizon: polysaccharides < lignin and tannins < aliphatic polymers
  and in the endorganic horizon: polysaccharides = lignin and tannins << aliphatic polymers
- Organic matter in incipient podzol B horizons is mainly derived from roots
- Water soluble organic matter moves through the soil forming organic matter fibers below the B horizons
- Origin of alkene/alkane pairs in pyrolysates is not restricted to above-ground plant tissues, but may also be derived from roots

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Summary

Until now little is known about the role vegetation plays in the organic matter formation, particularly at the molecular level. Most ecosystems have a long history, which is unknown or too complex to find distinct relations between vegetation and the chemical composition of soil organic matter. To gain a better insight in such relationships, the relative simple soil-vegetation system of the Hulshorster Sands and Leuvenum Forest is used. The complete vegetation succession has been investigated, making it possible to monitor the organic matter development. During the succession, thus with time, the pH decreases, the ectorganic layer becomes thicker and the soil develops towards a micro-podzol.

Chapter 2 describes the organic matter composition and relation to the first stages of vegetation development in the pre-podzol phase. In the Ah horizon under grass (*Corynephorus canescens*), the organic matter has virtually no contribution of the covering vegetation. Algae, from an earlier stage, and mosses (*Polytrichum piliferum* and *Bryum* sp.), from adjacent spots, are the main suppliers. Under mosses, the organic matter is very similar to that of the original species. An aliphatic biopolymer, derived from mosses, that produces a series of methyl-branched alkene/alkane doublets (C_{20}-C_{32}) together with a homologous series of n-alkenes and n-alkanes (C_{10} up to C_{34}) upon pyrolysis, appears to be very recalcitrant in the first stages of succession. In the vegetation stages following mosses, these aliphatic macromolecules are still an important source of the aliphatic part of organic matter. Apart from the aliphatic moss contribution, the organic matter under *Calluna vulgaris* and *Pinus sylvestris* has a close relationship with the contributing plant parts: flowers, leaves and branches dominate the organic layer under *Calluna*, needles predominate the ectorganic horizon under pines, and the soil organic matter in the Ah horizons in both cases is almost completely determined by roots.

In Chapter 3 the water soluble organic matter (WSOM) of the Ah horizons is compared with the bulk horizons. The WSOM contains little lignin, also when this compound was present in significant amounts in the Ah horizons. In contrast, the moss-derived aliphatics are prominently present in the WSOM. Their presence in water is probably due to the formation of micelle-like arrangements in which the hydrophobic parts are inside a hydrophilic mantle resulting into 'soluble' aliphatics.

Chapter 4 deals with the fractionation of three H horizons under different vegetation in the forest with incipient podzolization: a water soluble fraction, a NaOH soluble fraction and the residue, the insoluble fraction (humin). The water soluble fractions are very similar to each other with regard to their chemical composition, and so are the NaOH soluble factions. The insoluble fractions differ in particular in their aliphatic part. During beech forest development, an increasing amount of suberin, mainly derived from beech roots, is observed. The bulk of the organic matter in the H horizons, however, appears to have a pine origin as concluded from the chemical data. Pollen analysis supports that beech litter did not contribute to the H horizons.

Chapter 5 addresses the analysis (NMR, composition of polysaccharides, pyrolysis and thermochemolysis) and the unraveling of the origin of the ectorganic layers in the succession

from pine to beech forest. Under pine, the bulk organic matter shows only little effect of the ground vegetation *Deschampsia* and *Empetrum* respectively. The chemical composition of the L and F1 horizons are very similar to each other, but towards the H horizons an increase of aliphatics, a decrease of polysaccharides and a more or less constant fraction of aromatic compounds is noticed. The lignin composition is dominated by guaiacol building blocks, and hardly any angiosperm-lignin is present in the ectorganic layers implying no significant contributions of the undergrowth. This contrasts with macromorphological analyses, because abundant above- and below-ground parts of *Deschampsia* and *Empetrum* are present in the organic horizons suggesting an important input of the understorey. The composition of the aliphatic fraction also shows an effect of the undergrowth. The distribution of cutin and suberin building blocks as measured upon thermochemolysis reveals the clear impact of *Deschampsia* and *Empetrum* in addition to pine, especially in the F1 and F2 horizons. The transition towards the beech forest is characterized by a more distinct influence of beech litter. From L to H horizons polysaccharide contents decrease clearly, aromatics remain constant and the amounts of aliphatic compounds increase relatively. Furthermore, with depth a clear decrease of syringyl lignin compared with guaiacyl lignin is observed. The latter is mainly due to a decrease of beech litter towards the H horizon: the L consists only of beech litter, the F is also dominated by beech litter in addition to beech roots, and the H horizon is virtually only derived from pine litter. Nevertheless, the composition of the H horizons is affected by beech through their roots, which is clearly marked by the presence of beech-suberin, and the contents of that biopolymer increases from younger to older beech stands.

In Chapter 6 the WSOM fractions from L, F, and H horizons are compared on their chemical composition with those of the bulk of B horizons and fibers of incipient podzols. The WSOM shows hardly any resemblance with the B horizons, whereas it appears to be very similar to the fibers. This strongly suggests that in the initial phases of podzolization almost all WSOM moves through the B horizons and accumulates when the water movement stops resulting into the formation of humus fibers. In Chapter 7 it is elucidated that the organic matter in the B horizons is mainly composed of root-derived material, whereas hardly any organic matter is complexed with metals or clay. The organic matter in B horizons is highly aliphatic, consisting partly of suberan-like polymers and partly of suberin, whereas lignin and polysaccharides are virtually absent. In Chapter 8, the chemical implications of the negligible organic matter sources of both illuviation as mixing of above-ground litter is described. The clear chemical evidence of root-derived organic matter could be established.

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Samenvatting

Tot nu toe is nog weinig bekend van de rol die vegetatie speelt in the vorming van bodem organische stof, met name op het moleculaire niveau. Het probleem is dat veel ecosystemen een lange geschiedenis hebben, die meestal onbekend is of veel te complex om duidelijke relaties tussen vegetatie en organische stof samenstelling te kunnen vinden. Om toch een poging te wagen is gebruik gemaakt van het relatief eenvoudige bodem-vegetatie systeem in het stuifzandgebied van het Hulshorster Zand en het Leuvenumse Bos. Hiervan is de complete vegetatie geschiedenis bekend, en is het mogelijk stap voor stap de ontwikkeling van de organische stof te volgen. Gedurende de successie daalt de pH in de bodem met als gevolg een toenemende accumulatie van organische stof op de bodem, die zelf ontwikkelt in de richting van een mini-podzol.

Hoofdstuk 2 beschrijft de organische stof samenstelling in de eerste stadia van de vegetatie successie als er nog geen podzolisatie te zien is. In de Ah horizont onder het gras *Corynephorus canescens* heeft de organische stof vrijwel geen enkele bijdrage van die vegetatie, maar is voornamelijk afkomstig van de algen die in een eerdere fase aanwezig waren. Verder is ook een bijdrage te merken van mossen zoals *Polytrichum piliferum* en *Bryum* sp., die in de nabijheid groeien. Onder diezelfde mossen is de organische stof geheel afkomstig van de dekkende plant. In de eerste stadia van de vegetatie successie blijkt een alifatisch biopolymeer afkomstig van de mossen zeer recalcitrant te zijn. Dit polymeer vertoont onder pyrolyse omstandigheden naast een homologe serie van *n*-alkenen and *n*-alkanen (C_{10} tot aan C_{34}) een tweede homologe serie van vertakte (methyl) alkenen en alkanen (C_{20}-C_{32}), die zeer karakteristiek is voor mossen. Deze alifatische verbindingen zijn onder vegetatie volgend op de mossen, met name onder *Calluna vulgaris* en *Pinus sylvestris*, nog steeds zeer nadrukkelijk aanwezig. Afgezien van deze mos-invloed heeft de organische stof in de profielen onder *Calluna* en *Pinus* een zeer duidelijke en directe relatie met de huidige vegetatie: bloemen, blaadjes en takjes bepalen de organische stof samenstelling in de ectorganische laag onder de heide, terwijl de organische horizonten onder de den vrijwel geheel door dennenaalden bepaald worden. De organische stof in minerale Ah horizonten is in beide gevallen geheel afkomstig van wortels, naast enige inbreng van mossen.

In Hoofdstuk 3 wordt de wateroplosbare organische stof (WSOM) van de Ah horizonten uit bovenstaande profielen qua chemische samenstelling vergeleken met die van de oorspronkelijke (totale) Ah horizonten. De WSOM bevat zeer weinig lignine-fragmenten, ook al was dat polymeer in behoorlijke hoeveelheden aanwezig in de Ah horizonten. Opmerkelijk was de aanwezigheid van de alifatische componenten afkomstig van mossen. Hun aanwezigheid in water is het best te verklaren door de vorming van micel-achtige ordeningen, waarbij de hydrofobe delen afgeschermd worden van het water door een hydrofiele mantel, waardoor de alifatische componenten 'wateroplosbaar' worden.

Hoofdstuk 4 beschrijft de fractionering en karakterisering van drie H horizonten uit de latere stadia van vegetatie successie, waarbij sprake is van beginnende podzolisatie. Deze horizonten zijn gefractioneerd in een wateroplosbare fractie, een NaOH oplosbare fractie en een residu, de onoplosbare fractie. De wateroplosbare fracties zijn vrijwel gelijk aan elkaar

qua organische stof samenstelling, en hetzelfde kan gezegd worden van de NaOH oplosbare fracties. Alleen tussen de verschillende onoplosbare fracties kunnen duidelijke verschillen waargenomen worden, en dan met name in het alifatische deel. Van dennenbos naar beukenbos gezien is een toename te bemerken van het suberine afkomstig van de beuk. De chemische samenstelling suggereert dat de overige organische stof van alle H horizonten vrijwel geheel afkomstig is van dennenstrooisel. Met behulp van pollen analyse blijkt inderdaad dat de H horizonten gevormd zijn toen vrijwel alleen den als vegetatie aanwezig was.

Hoofdstuk 5 behandelt de chemische analyse van de ectorganische lagen in de overgang van dennen- naar beukenbos met behulp van NMR, suiker analyse, pyrolyse en thermochemolyse. In het dennenbos met ondergroei van bochtige smele (*Deschampsia flexuosa*) en kraaiheide (*Empetrum nigrum*) lijkt het alsof de bulk van de organische stof nauwelijks beinvloed wordt door de grondvegetatie. Tussen L en F1 horizonten is vrijwel geen verschil te vinden in chemische samenstelling, maar gaande naar de H horizont is duidelijk te zien dat het gehalte aan polysacchariden daalt, de aromatische fractie min of meer constant blijft en dat er een relative aanrijking is van alifatische componenten. De lignine samenstelling in alle horizonten heeft een 'dennen-achtig' karakter, dat wil zeggen dat vrijwel alleen guaiacyl eenheden aanwezig zijn en dat syringyl bouwstenen, dominant aanwezig in *Deschampsia* en *Empetrum*, nauwelijks aanwezig zijn evenals typische gras-lignine componenten. Dit zou suggereren dat de ondergroei weinig tot niets bijdraagt aan de organische stof. Echter, macromorfologisch gezien, bevatten de ectorganische lagen duidelijk boven- en ondergrondse plantendelen afkomstig van de ondergroei. Analyse van het alifatische deel van de organische stof aan de hand van de data verkregen met thermochemolyse geeft duidelijk de invloed van de ondergroei aan, blijkens de aanwezigheid van cutine- en suberine-eenheden afkomstig van de verschillende plantendelen, zowel van den als de grondvegetatie. Met name de F1 en F2 horizonten blijken een belangrijke bijdrage van zowel boven- als ondergrondse plantendelen van *Deschampsia* respectievelijk *Empetrum* te bevatten. De overgang van dennenbos naar beukenbos wordt gekarakteriseerd door een overduidelijke bijdrage van beukenstrooisel in de chemische samenstelling van de L en F horizonten. De H horizonten zijn echter vrijwel geheel afkomstig van de vorige dennenvegatatie, zoals al in Hoofdstuk 4 was ontdekt. Het beuken-suberine, ook al opgemerkt in Hoofdstuk 4, neemt toe in de H horizonten van jonge naar oude beuken locaties. Van L naar H horizont is de bekende humificatie trend te zien: afname van de koolhydraten, gelijk blijven van het gehalte aan aromatische verbindingen en een relatieve stijging van de alifatische fractie. Verder daalt het gehalte aan syringyl lignine ten opzichte van guaiacyl lignine met toenemende diepte. Dit heeft meer te maken met de afnemende bijdrage van beukenstrooisel dan met een selectieve afbraak van het syringyl lignine. Tenslotte kan geconcludeerd worden dat cutine en suberine een uitstekende rol als biomarkers kunnen vervullen, en in ieder geval beter dan lignine.

In Hoofdstuk 6 wordt de chemische samenstelling van wateroplosbare organische stof (WSOM) van L, F en H horizonten vergeleken met die van de totale organische stof in B horizonten en van humus fibers. Het blijkt dat de WSOM geheel anders is samengesteld dan de B horizonten, maar zeer veel gelijkenis vertoont met de fibers. Dit suggereert dat in de eerste fasen van het podzolisatie proces vrijwel al het WSOM door de B horizonten loopt, en neerslaat als het water transport stopt resulterende in humus fibers. In Hoofdstuk 7 blijkt dat

de organische stof in B horizonten vrijwel geheel afkomstig is van wortels, en dat vrijwel geen organische stof gecomplexeerd wordt door metalen of klei. Daarnaast is de organische stof van een zeer hoog alifatisch gehalte, deels als cutan/suberan-achige polymeren en deels als suberine en dat suikers en lignine in slechts zeer kleine hoeveelheden terug te vinden zijn. Hieruit blijkt dat lignine in deze zure bodems veel minder resistent is dan men altijd heeft gedacht en dat het veel labieler geachte suberine veel stabieler lijkt te zijn. Als we inspoeling en biologische menging van bovengronds materiaal kunnen verwaarlozen als bronnen van organische stof in de minerale lagen, kunnen we duidelijk aantonen aan de hand van de chemische gegevens dat wortels een duidelijke rol spelen als organische stof bron (Hoofdstuk 8). Bovendien blijkt dat de aanwezigheid van alkeen/alkaan doubletten in pyrolysaten niet automatisch wijst op cutan of suberan uit bovengrondse plantendelen, maar dat wortels evenzeer een bron van dit soort alifatische biopolymeren kan dienen.

Curriculum Vitae

Klaas Gerrit Johannes Nierop werd geboren op 22 mei 1968 te Andijk. Na een seizoen (80-81) op de Groen van Prinsterer MAVO te Enkhuizen te hebben rondgelopen, werd de rest van de middelbare schooltijd doorgebracht op het Marcus College te Grootebroek en afgesloten in 1986 met het VWO diploma. In dat zelfde jaar begon hij de studie Chemische Technologie aan de Universiteit Twente te Enschede. Tijdens de doctoraalstage bij het Philips Research Laboratorium te Eindhoven verrichte hij onderzoek op het gebied van de vloeibaar kristallijne materialen en behelsde de synthese en karakterisering van cholesterische diacrylaten. Hij studeerde in 1992 af bij de vakgroep Organische Chemie op het gebied van de supramoleculaire chemie. Dit leidde o.a. tot de synthese van 12,22,40,50,82,88,93,99-Octakis(2-ethoxyethoxy)-67,73,74,75-tetraundecyl-14H,20H,26H,42H,48H,54H-4,58(epoxy-methanoxy)-1,69:3,59:61,65-trimethano-11,23:39,51-bis-(methano[1,3]benzenomethano)-9,13:15,19:21,25:37,41:43,47:49,53-hexametheno-6H,34H,67H-dibenzo[d,d'][1,3]dioxocino[4,5-f_1:8,7-f_1]bis[1,3,6,30,9,27]benzotetraoxadiazacyclodotriacontine-7,27,35,55(8H,28H,26H,56H)-tetrone. Na en tijdje minder wetenschappelijk te zijn bezig geweest, werd hij in 1994 via de Nederlandse organisatie voor Wetenschappelijk Onderzoek (NWO) aangesteld als zgn. onderzoeker in opleiding (oio) bij de vakgroep (tegenwoordig Laboratorium) Bodemkunde en Geologie aan de Landbouwuniversiteit Wageningen. Het onderzoek verricht tijdens deze periode staat beschreven in dit proefschrift.