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# Molecular characterization of total organic matter and carbohydrates in peat samples from a cypress swamp by pyrolysis-mass spectrometry and wet-chemical methods

M.E.C. MOERS[1,3], M. BAAS[1], J.J. BOON[2] & J.W. DE LEEUW[1]
[1]*Delft University of Technology, Faculty of Chemical Technology and Materials Science, Organic Geochemistry Unit, de Vries van Heystplantsoen 2, 2628 RZ Delft, The Netherlands;* [2]*FOM Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands;* [3]*Present address: NRG, Drummond Bldg, The University, Newcastle upon Tyne NE 7RU, UK*

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**Abstract.** The organic matter present in coarse- and fine-grained sample preparations of a peat core from Minnie's Lake, Okefenokee Swamp (Georgia, USA) was analysed on a molecular level by gas chromatography-mass spectrometry of alditol acetates obtained from polysaccharides after acid hydrolysis and derivatization and by flash pyrolysis-mass spectrometry.

We found that the coarse-grained fraction is dominated by neutral monosaccharides derived from vascular plants whilst the fine-grained fraction is dominated by neutral monosaccharides derived from microorganisms. Relatively high contributions of xylose discriminate the *Taxodium* derived peat from the underlying peats derived from *Carex* and *Nymphaea* which show relatively low contributions of this sugar. Factor-discriminant analysis of the Py-MS data indicates that most carbohydrate degradation is restricted to the upper peat levels and that guaiacyl and syringyl type lignins are better preserved than carbohydrates. Resinous organic matter – presumably derived from *Taxodium* – is selectively preserved in the fine-grained fraction. Deviant monosaccharide concentrations and Py-MS results observed for the top sample of the *Carex* peat were explained by oxygenation of the peat, a concomitantly high microbial activity and subsequent preservation of the organic material due to the action of phytotoxins derived from *Taxodium*.

## Introduction

The function of an organic compound and its recalcitrance or lability are a reflection of its molecular structure and composition. Therefore it is important to characterize organic compounds on a molecular level. Some macromolecular compounds are recalcitrant because of their natural molecular composition, molecular configuration or degree of crystallinity in living organisms. In many cases recalcitrance appears to be a consequence of inaccessibility to enzymatic decomposition. In plants recalcitrance may be a means of protection against invasion from outside or as a protection against environmental stress. Examples are lignification, cutinization and suberinization of leaves, stems and roots. Compounds that have a protective function in living organisms are often recalcitrant after death of the organism as well. On the other hand, compounds with a metabolic function (e.g. glycogen, starch) are often destroyed during

senescence or very shortly after death of the organism (e.g. Ittekkot et al. 1982; Klok et al. 1984b).

Natural compounds that are inherently labile may become recalcitrant through association with recalcitrant compounds in living organisms. For instance, carbohydrates become more recalcitrant when they are protected by lignin in lignocellulosic complexes, when they are incorporated in the insoluble, non-hydrolyzable polymethylenic biopolymer cutan that is present in cuticulae (e.g. Nip et al. 1985a, b, 1987; Tegelaar et al. 1989a, 1990), when they are encapsulated in a mineral matrix (e.g. Liebezeit et al. 1983).

Microbially initiated (stereo) chemical transformations may also render a compound recalcitrant. For example cholesterol is transformed into 5$\beta$(H)-cholestanol (coprostanol) in the intestinal tract of mammals (e.g. Eyssen et al. 1973); coprostanol is less degradable than its precursor and it is used as a tracer for (pollution by) sewage. In sediments, organic compounds may also become more recalcitrant as a result of abiotic transformations such as photochemical reactions (e.g. light-catalyzed oxidative cross-linking of poly-unsaturated lipids in the water column (Harvey et al. 1983), sulphur incorporation and cross-linking (e.g. Sinninghe Damsté et al. 1986, 1987), condensation reactions (e.g. the Maillard reaction (Enders & Theis 1938; Stevenson 1969; Stevenson & Butler 1969; Nissenbaum & Kaplan 1972)), and hydrogenation and aromatization due to increased temperatures and pressures during diagenesis (e.g. Mackenzie et al. 1982, 1988; Peakman & Maxwell 1988; de Leeuw et al. 1989).

Compounds may also show recalcitrant behavior simply because there are no more decomposing organisms present. For example, even rather labile organic compounds from microorganisms living in deeper sediments may survive when there are no microorganisms to degrade their remains. In addition, anthropogenic compounds like plastics, paints, resins and PCBs behave recalcitrantly.

Organic compounds present in an ecosystem are mostly degraded either due to autolysis and senescence, or grazing and predation, or the activity of bacteria and fungi. In this way new compounds are formed which in turn are also prone to degradation. The result is that the more labile components participate in cycles of synthesis-degradation-resynthesis as part of the food chain. A small proportion of the organic material, generally less than 1%, consisting of the more recalcitrant organic components accumulates in sediments and participates in cycles associated with the formation of kerogen, petroleum and coals (e.g. Tissot & Welte 1984; Tegelaar et al. 1989b).

An established method to obtain information about organic material in complex mixtures on a molecular level is by means of mass spectrometry (MS) in combination with gas chromatography (GC)(Burlingame & Schnoes 1969). With this method it is possible to obtain structural information on (sub)-nanogram amounts of organic matter. This approach can be combined with pyrolysis techniques as a powerful tool in the analysis of high molecular weight material (Douglas & Larter 1982). High molecular weight compounds are thermally depolymerized by pyrolysis (Py) techniques. The resulting fragments

can either be directly analysed by mass spectrometry (Py-MS) or can first be separated by gas chromatography and then analysed by mass spectrometry (Py-GC-MS)(Boon, 1989). In either case qualitative information on the general character of the organic material is obtained. This method is used for characterization of complex organic material such as bacteria, fungi, living plants and whole sediments or selected organs (e.g. rootlets, seeds) thereof.

**Pyrolysis-(gas chromatography)-mass spectrometry**

The first step of analysis by pyrolysis-(gas chromatography)-mass spectrometry (Py-(GC)-MS) is rapid heating of the organic matter to several hundred degrees Celsius. The organic matter disintegrates into small fragments that are transported either to a mass spectrometer in the case of analysis by Py-MS or to a gas chromatograph and afterwards to a mass spectrometer in case of Py-GC-MS. A schematic representation of these techniques is given in Fig. 1. In both cases data acquisition and processing are computerized. Compound identification is based on the fact that different biopolymers yield different, partly characteristic, fragments during pyrolysis, and each of these fragments yields its own specific mass spectrum.

Rapid heating of the organic matter is achieved by applying 25 to 50 $\mu$g to a ferromagnetic wire and placing this wire in a high frequency magnetic field. The final temperature reached depends on the Curie-temperature of the wire material and consequently on its composition. In practice, temperatures of 358, 510, 610 and 770 °C are used. In Py-MS, the fragments formed during pyrolysis reach the mass spectrometer within seconds. There they are bombarded by low voltage

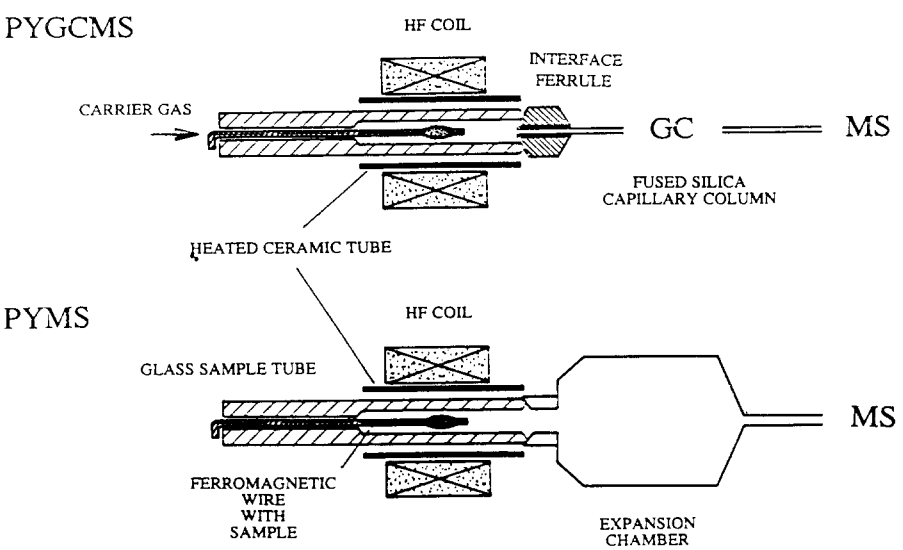



*Fig. 1.* A schematic representation of Py-MS and Py-GC-MS techniques.

electrons (16 eV) which results in the formation of a molecular ion and fragment ions. The ions formed are separated by mass using electric and magnetic fields. The overall result (the final mass spectrum) is a time integrated (over 10 sec) distribution pattern of all ion masses and the corresponding intensities observed.

In Py-GC-MS, the fragments formed by pyrolysis are first separated by capillary-column gas chromatography, so that (ideally) they arrive separately at the mass spectrometer. There they are bombarded with electrons of 70 or 80 eV. Typically, one mass spectrum is recorded per second, so that mass spectra can be obtained of virtually all the pyrolysis products. These spectra can be compared with mass spectra in (computer) libraries and with spectra of reference compounds and reference mixtures and so the pyrolysis products can be identified.

Thus, Py-MS yields one mass spectrum of all pyrolysis products together, while Py-GC-MS yields mass spectra of all pyrolysis products separately. An advantage of Py-MS is that analysis time is much shorter than for Py-GC-MS. A disadvantage is that it is very difficult or impossible to recognize individual components/precursors in the data contained in the pyrolysis mass spectrum. Data processing with statistical methods like factor and discriminant analysis is almost mandatory when one wants to obtain more information from Py-MS than just a fingerprint.

**Case study: a cypress swamp**

The major aims of this investigation were firstly to determine how the degradation of peat is reflected in its carbohydrate abundance and in its lignin and carbohydrate pattern and secondly to establish how the carbohydrates in peat can be attributed to their precursors. To this end coarse- (+20 mesh) and fine-grained (−80 mesh) peat samples from several depths within swamp sediments were analysed by pyrolysis-mass spectrometry to evaluate qualitatively the nature of organic material in the peats, and by wet chemical methods to determine the different kinds of saccharide monomers present and their concentrations. Thus we hoped to gain clues as to the sources of the organic matter and possible changes which may have occurred during peatification.

Minnie's Lake, a cypress swamp in the Okefenokee Swamp in southern Georgia (USA), was selected for the purposes of this study because a peat core existed which represents different environments of peat deposition with several different vascular plants present. Another advantage is that the upper three samples from this core are mainly composed of the same vascular plant species, i.e. *Taxodium*, and comparisons of the carbohydrate and lignin abundances at the various depths may give insight into the effects of degradation of this species.

The Okefenokee swamp/marsh system consists of swamp forests dominated by *Taxodium distichum* (swamp or bald cypress), of glades or island fringes inhabited by emergent aquatic plants like *Carex* (sedge), *Panicum* (maidencane)

and *Woodwardia* (chainfern), and of open water environments dominated by *Nymphaea* (waterlilly)(Cohen 1973a; Spackman et al. 1981). The swamp is underlain by a Pleistocene marine terrace, which in most cases is a relatively pure sand. Peat accumulation in the Okefenokee started about 6500 y. B.P. in a fresh water environment (Cohen 1973b).

The basal peat at Minnie's Lake has been dated at 4000 y. B.P. (Casagrande et al. 1985) and a total of 3.5–3.7 m of peat has since accumulated (Cohen 1973b). The present vegetation at Minnie's Lake consists of a dense *Taxodium* swamp (Cohen 1973b; Spackman et al. 1981). Components observed microscopically by Ryan (1985) in the same peat core as used in this study were: 0–10 cm: partially disrupted *Taxodium* twigs, leaves and rootlets. 71–81 cm: fragments of cortical tissue from *Taxodium* roots and rootlets, though few recognizable organs. 112–122 cm: similar to previous level, but mainly cell fragments. 152–163 cm: tissues from leaves, roots and rhizomes of *Carex*. 183–193 cm: cortical tissue from rhizomes and roots of *Carex*. 244–254 cm: mixture of *Nymphaea* and *Carex* peat, and some fusinite. It appears from these observations that the Minnie's Lake area was previously an open marsh which has since been overgrown by swamp vegetation (Cohen 1973b). A description of the microscopic observations appears in Cohen & Spackman (1977).

## Experimental

Peat samples from six different depths within the core (see Table 1) were used for the analyses. The core was obtained by using a modified, hand-operated, piston-coring device as described in detail by Cohen & Spackman (1972). The core was taken in 7.5 cm diameter aluminium pipes. Sections of 122 cm were sealed in the field using plastic caps and plastic tape. After return to the laboratory, sections destined for chemical study were frozen and stored in a deep-freeze unit. During previous investigations by Rhoads (1985) and by Ryan (1985), each sample had been separated into coarse-grained (+20 and +80 mesh) fractions consisting of vascular plant tissues and tissue fragments, and into a fine-grained (−80 mesh) fraction consisting of amorphous matter using the fractionation procedures of Given et al. (1984) or Rhoads (1985). The −80 mesh fraction had been treated with 0.1 M HCl to remove carbonates and all peat fractions had been Soxhlet extracted with benzene/ethanol 2/1 v/v for 48 h by Rhoads (1985) and freeze dried. This extraction removed ca. 12 wt% of the dry material. The organic carbon content of the peat fractions is ca. 63 wt% with a carbon/nitrogen weight ratio of ca. 21 (Rhoads 1985).

### *Analysis of alditol acetates*

For carbohydrate determinations +20 mesh (coarse-grained) and −80 mesh (fine-grained) samples were analysed, half in duplicate. From each fractionated, freeze-dried and pulverized peat sample, ca. 100 mg was pretreated with 5 ml

*Table 1.* Monosaccharide yields in mg per gram dry peat fraction or per gram dry organ/tissue.

| Peat | −80 mesh (fine) | | | | | |
|---|---|---|---|---|---|---|
| Depth (cm) | 0 −10 | 71 −81 | 112 −122 | 152 −163 | 183 −193 | 244 −254 |
| Rhamnose | 3.7 | 1.3 | 1.7 | 3.1 | 2.1 | 3.3 |
| Fucose | 0.8 | 0.4 | 0.4 | 0.7 | 0.6 | 1.6 |
| Ribose | 0.5 | 0.2 | 0.2 | 0.4 | 0.2 | 0.3 |
| Arabinose | 2.3 | 0.6 | 0.6 | 1.9 | 0.7 | 1.1 |
| Xylose | 6.0 | 2.4 | 2.1 | 7.2 | 3.0 | 3.3 |
| Mannose | 8.0 | 3.1 | 2.9 | 7.6 | 3.7 | 4.8 |
| Galactose | 7.0 | 2.3 | 2.4 | 6.7 | 2.9 | 5.1 |
| Glucose | 36.4 | 23.6 | 21.3 | 43.2 | 30.7 | 19.3 |
| Min. comp. | 5.9 | 3.1 | 3.2 | 7.0 | 3.5 | 6.3 |
| Total | *70.6* | *37.0* | *34.8* | *77.8* | *47.4* | *45.1* |
| COHC/TOC* | 4.5 | 2.3 | 2.2 | 4.9 | 3.0 | 2.9 |

| Peat | +20 mesh (coarse) | | | | | |
|---|---|---|---|---|---|---|
| Depth (cm) | 0 −10 | 71 −81 | 112 −122 | 152 −163 | 183 −193 | 244 −254 |
| Rhamnose | 4.2 | 0.9 | 1.2 | 0.8 | 1.4 | 2.0 |
| Fucose | 0.6 | 0.3 | 0.2 | 0.2 | 0.3 | 0.9 |
| Ribose | 0.6 | 0.4 | 0.4 | 0.4 | 0.3 | 0.4 |
| Arabinose | 3.8 | 2.0 | 1.7 | 0.9 | 0.9 | 1.2 |
| Xylose | 25.9 | 17.5 | 12.9 | 7.5 | 8.1 | 7.6 |
| Mannose | 16.9 | 4.8 | 6.1 | 3.5 | 4.5 | 5.2 |
| Galactose | 12.2 | 4.5 | 3.9 | 2.7 | 3.3 | 5.3 |
| Glucose | 119.8 | 91.5 | 75.9 | 79.3 | 77.3 | 67.7 |
| Min. comp. | 1.7 | 2.1 | 1.3 | 1.1 | 1.6 | 2.2 |
| Total | *185.7* | *124.0* | *103.6* | *96.4* | *97.7* | *92.5* |
| COHC/TOC* | 11.8 | 7.8 | 6.6 | 6.1 | 6.2 | 5.9 |

| Nymphaea organs and tissues: | Leaf | Root | Rhizome | Periderm |
|---|---|---|---|---|
| Rhamnose | 3.0 | 2.2 | 3.7 | 4.4 |
| Fucose | 1.1 | 1.2 | 1.3 | 2.0 |
| Ribose | 0.7 | 0.8 | 1.5 | 1.1 |
| Arabinose | 3.9 | 3.9 | 6.9 | 9.3 |
| Xylose | 9.3 | 14.0 | 8.1 | 15.8 |
| Mannose | 3.4 | 4.4 | 8.8 | 8.0 |
| Galactose | 13.4 | 14.5 | 12.3 | 13.7 |
| Glucose | 54.8 | 121.4 | 251.7 | 224.9 |
| Min. comp. | 0.0 | 0.0 | 0.0 | 0.0 |
| Total | *89.6* | *162.4* | *294.3* | *279.2* |

*: gram carbohydrate carbon (COHC) per 100 g total organic carbon (TOC).

12 M $H_2SO_4$ for 2 h at room temperature, after which the acid was diluted to 1 M and the sample hydrolysed at 100 °C for 4.5 h. In addition various organs and tissues (leaves, roots, rhizomes, and the periderm of a rhizome) of waterlilly (*Nymphaea alba*) were analysed for neutral monosaccharides as well. They were freeze-dried, pulverized and subjected to the same acid treatment as described above. In all cases myo-inositol was added as an internal standard. The monosaccharides released by hydrolysis were reduced to alditols by $NaBH_4$ (room temperature, 16 h) and then acetylated to alditol acetates by acetic anhydride/pyridine (100 °C, 3 h). The reaction mixtures were analysed by GC on a Carlo Erba 4160 gas chromatograph and by GC-MS on a Hewlett-Packard 5890 gas chromatograph coupled to a VG 70-250SE mass spectrometer. In both cases a CPsil88 fused silica capillary column (l = 25 m, i.d. = 0.32 mm, $d_f$ = 0.12 $\mu$m; Chrompack, Middelburg, The Netherlands) was used for gas chromatographic separations. The samples were injected at 50 °C (GC-MS) or 70 °C (GC). The temperature was rapidly raised to 150 °C and then programmed at 3 °C/min to 230 °C and maintained at this final temperature for 40 min. The mass spectrometer was operated in the electron impact mode at 70 eV, with a source temperature of 250 °C.

Mass spectral data and relative retention times (Stoffel & Hanfland 1973; Schwarzmann & Jeanloz 1974; Jansson et al. 1976; Radziejewsky-Lebrecht et al. 1979; Wong et al. 1980; Klok et al. 1982) were used to identify the alditol acetates, except in the cases of some heptoses and amino sugars where only mass spectral data were available.

Quantification of the compounds analysed was achieved by integration of the GC peak areas with the aid of a Maxima Chromatography Workstation (Dynamic Solutions Corporation, Ventura, CA, USA) and comparing the responses with the response of myo-inositol hexaacetate (the internal standard). The flame ionization detector responses of all alditol acetates were assumed to be equal on a weight basis. In a few cases where compounds coeluted, quantification was achieved by integration of the peak areas of selected characteristic ion fragments from GC-MS analyses. Duplicate analyses showed that the error in the determinations of the major sugars is 10% or less and that the error in the determinations of the minor sugars ranges from ca. 50% for the compounds present in less than 0.02 mg/g to 10% for the others. The definitions of 'major' and 'minor' sugars are explained below.

*Analysis by pyrolysis-mass spectrometry*

Aliquots of peat samples suspended in water were applied to ferromagnetic wires and dried in vacuo. Curie point pyrolysis mass spectra were obtained with the FOMautoPYMS system described previously by Meuzelaar et al. (1982) and Boon et al. (1984). This is a PyMS system with a fully automated sample exchange system using a low voltage EI quadrupole MS, built at the FOM Institute for Atomic and Molecular Physics, Amsterdam, The Netherlands. The conditions were as follows: Curie temperature, 610 °C; heating time, 0.1 sec;

total pyrolysis time, 0.8 sec; heated pyrolysis chamber, ca. 180 °C; temperature of the expansion chamber, 210 °C; ionization by electron impact, 16 eV; mass range, m/z 25–240; scan speed, 10 scan/sec; total number of averaged spectra, 200. The samples were analysed in triplicate.

*Multivariate techniques*

Comparison of the Py-MS spectra was carried out by a factor and discriminant analysis procedure using a modified ARTHUR computer program package, adapted to accept Py-MS data (Hoogerbrugge et al. 1983; Boon et al. 1984). Factor analysis is a data reduction technique which reduces the (large number of) original variables (the individual masses and their intensities in the original pyrolysis-mass spectra) to a minimum number of new variables (the factors) that are linear combinations of the original variables. The aim of the analysis is the comparison of large series of mass spectra for the extraction of the most significant differences. Each spectrum is compared with the overall average spectrum of the file and resulting positive and negative differences are factor-analysed. In this way groups of similar spectra can be detected. The linear combinations of the original variables determined by factor analysis are based on the largest variance in the data set. This variance has a part which is due to real differences between the samples ('signal') and a part that is based on differences not representative of the samples ('noise'). Discriminant analysis calculates linear combinations of the variables with maximum between-group variance (signal) to within-group variance (noise) ratios. If, as in the present case, samples are analysed in triplicate, each triplicate analysis can be considered as one group and the variance within each group (the noise) will be minimized. The variance between the triplicates (the signal) will be maximized. The new variables which describe the discrimination are called discriminant functions. The discriminant functions are used to calculate the contributions (loadings) of the original mass variables to the discriminant function spectra. These spectra show the mass peaks which best describe the differences between the samples. Positive and negative discriminant spectra (D+ and D−) have mass peaks which have higher and lower values, respectively, than the zero point spectrum. The analysis output is also presented as plots which show the scores of the samples on the discriminant functions (i.e. the contribution of a discriminant function to each sample) versus sample depth.

Factor analysis was also applied to the results from the 33 minor sugar analyses (see below). The relations between the 'old' variables (the individual minor sugars) are expressed in a table of factor loadings, expressing the contribution of each 'old' variable to the 'new' variables (the factors). The output is also presented as plots which show the scores of the samples on the factors (i.e. to which extent the various factors are reflected in the samples) versus sample depth.

## Results and discussion

*Analysis of carbohydrates as alditol acetates*

Hydrolysis of the peat samples yielded a great variety of neutral monosaccharides

- Glucose, galactose, mannose, xylose, arabinose, ribose, fucose and rhamnose (see Table 1). This group will be called '*major*' sugars and comprises 98 to 99 wt% of the total neutral monosaccharides in the coarse-grained fraction and 86 to 93 wt% in the fine-grained fraction.
- Partially methylated saccharides, amino sugars, heptoses, allose, altrose, tetroses and glycerol, which are collectively called '*minor*' sugars in this paper (Tables 1 and 2).

Reports of the occurrence of the major sugars in natural samples are common. For example, recent sediments contain major sugars which show inputs from marine and terrestrial sources (e.g. Myklestad 1974; Cowie & Hedges 1984; Klok et al. 1984a, b; Michaelis et al. 1986; Tanoue & Handa 1987; Hamilton & Hedges 1988; Moers et al. 1990a). Microorganisms, i.e. (cyano)bacteria and algae, are thought to be the source of the minor sugars (Given 1972; Given & Dickinson 1975; Casagrande & Park 1978; Klok et al. 1984a, b; Casagrande et al. 1985; Moers et al. 1989, 1990a, b). Notably lipopolysaccharides in the outer cell membranes of gram-netative bacteria and cyanobacteria are known for their great variety of partially methylated monosaccharides (e.g. Laskin & Lechevalier 1982; Aspinall 1983; Kennedy & White 1983; Brade et al. 1988; Ratledge & Wilkinson 1988). Algae are also reported to contain partially methylated monosaccharides but not in such great varieties (e.g. Percival & McDowell 1967, 1981; Stewart 1974). Vascular plants are reported to contain minute amounts of a few of the monomethylated neutral monosaccharides and amino sugars (Lowe 1978; Cheshire 1979; Aspinall 1983). However, analysis of a variety of vascular plant organs and tissues (a.o. of sawgrass, cotton grass, bog myrtle, red mangrove and beech) using the method as described in the experimental has never revealed the presence of any of the partially methylated monosaccharides or amino sugars in the hydrolysates (Moers 1989; Moers et al. 1989, 1990b; Tegelaar, pers. comm.; Moers & Baas, unpublished results). To verify these results for the present case, various organs and tissues of *Nymphaea* (waterlily) were analysed. The absence of partially methylated monosaccharides and amino sugars in the hydrolysates of the *Nymphaea* organs and tissues (Table 1) indicates that the minor sugars identified in the peat core samples in the present case are probably also not derived from vascular plants, but more likely from microorganisms, i.e. (cyano)bacteria and algae.

The coarse-grained samples yield higher amounts of neutral monosaccharides than the fine-grained samples, irrespective of depth and composition of the peat (Table 1). The saccharide concentrations in the coarse-grained samples initially

*Table 2.* Minor sugars and loadings on the first four factors obtained from factor analysis.

| Variance preserved factors | 30% F1 | 25% F2 | 15% F3 | 10% F4 |
|---|---|---|---|---|
| 1. glycerol | −0.30 | *−0.82* | +0.42 | +0.12 |
| 2. erythrose | −0.38 | *−0.84* | −0.19 | −0.17 |
| 3. threose | −0.45 | *−0.54* | −0.39 | −0.04 |
| 4. allose | −0.30 | +0.07 | *−0.53* | −0.25 |
| 5. altrose | +0.03 | −0.11 | −0.39 | *−0.73* |
| 6. a heptose | +0.38 | *−0.62* | −0.00 | −0.04 |
| 7. glucoheptose | *+0.78* | −0.30 | −0.31 | +0.13 |
| 8. a heptose | *+0.76* | +0.10 | +0.43 | −0.20 |
| 9. an amino sugar | *+0.62* | +0.47 | −0.00 | −0.27 |
| 10. glucosamine | *+0.72* | −0.35 | +0.47 | −0.11 |
| 11. an amino sugar | *+0.79* | +0.40 | +0.22 | −0.08 |
| 12. 2-O-methyl-rhamnose | *−0.60* | −0.26 | −0.45 | +0.34 |
| 13. 2-O-methyl-fucose | *−0.64* | −0.28 | −0.39 | +0.09 |
| 14. 4-O-methyl-rhamnose | *−0.79* | *+0.52* | −0.13 | −0.22 |
| 15. 4-O-methyl-fucose | +0.06 | +0.12 | *−0.89* | +0.23 |
| 16. 3-O-methyl-rhamnose | *+0.61* | +0.43 | *−0.50* | −0.25 |
| 17. 3-O-methyl-fucose | +0.29 | +0.24 | *−0.81* | +0.06 |
| 18. 2/4-O-methyl1ribose* | *−0.79* | +0.35 | +0.14 | +0.02 |
| 19. 2-O-methyl-arabinose | −0.23 | *−0.52* | *−0.65* | −0.38 |
| 20. 4-O-methyl-arabinose | +0.10 | *−0.58* | *−0.62* | +0.20 |
| 21. 2/4-O-methyl-xylose* | *−0.82* | +0.26 | +0.41 | +0.05 |
| 22. 3-O-methyl-arabinose | −0.09 | *+0.86* | −0.30 | −0.10 |
| 23. 3-O-methyl-xylose | −0.21 | *+0.84* | −0.17 | −0.13 |
| 24. 6-O-methyl-mannose | *+0.72* | +0.31 | −0.21 | +0.47 |
| 25. 6-O-methyl-galactose | *+0.75* | +0.29 | −0.09 | *+0.55* |
| 26. 6-O-methyl-glucose | +0.31 | −0.28 | +0.02 | *+0.81* |
| 27. 2/5-O-methyl-mannose* | *−0.73* | +0.31 | +0.49 | +0.02 |
| 28. 2/5-O-methyl-galactose* | *−0.98* | −0.00 | +0.12 | −0.06 |
| 29. 2-O-methyl-glucose | *−0.58* | −0.32 | +0.08 | +0.18 |
| 30. 3/4-O-methyl-mannose* | −0.20 | *+0.79* | +0.04 | +0.30 |
| 31. 3/4-O-methyl-galactose* | −0.08 | *+0.79* | −0.13 | −0.11 |
| 32. 3-O-methyl-glucose | −0.26 | *+0.69* | −0.02 | +0.14 |
| 33. 4-O-methyl-glucose | −0.39 | *+0.75* | −0.23 | +0.39 |

*: Alditol acetates not separated on CPsil88.

decrease with increasing depth followed by stabilization to ca. 95 mg/g. The fine-grained samples also show an initial decrease in total monosaccharide concentration, followed by an unexpected irregular increase in the samples from 152–163 cm (see Table 1).

Figure 2 shows the sum of all individual monosaccharide yields (the total monosaccharide yield in mg monosaccharides per g peat fraction) plotted against the relative yields of individual monosaccharides in wt%. This Fig. 2 shows the relationship between the relative monosaccharide contributions, the grain size and the total monosaccharide yields: the minor sugars together with rhamnose, fucose, ribose, mannose and galactose tend to be relatively depleted in the coarse-grained fraction and enriched in the fine-grained fraction. Glucose,

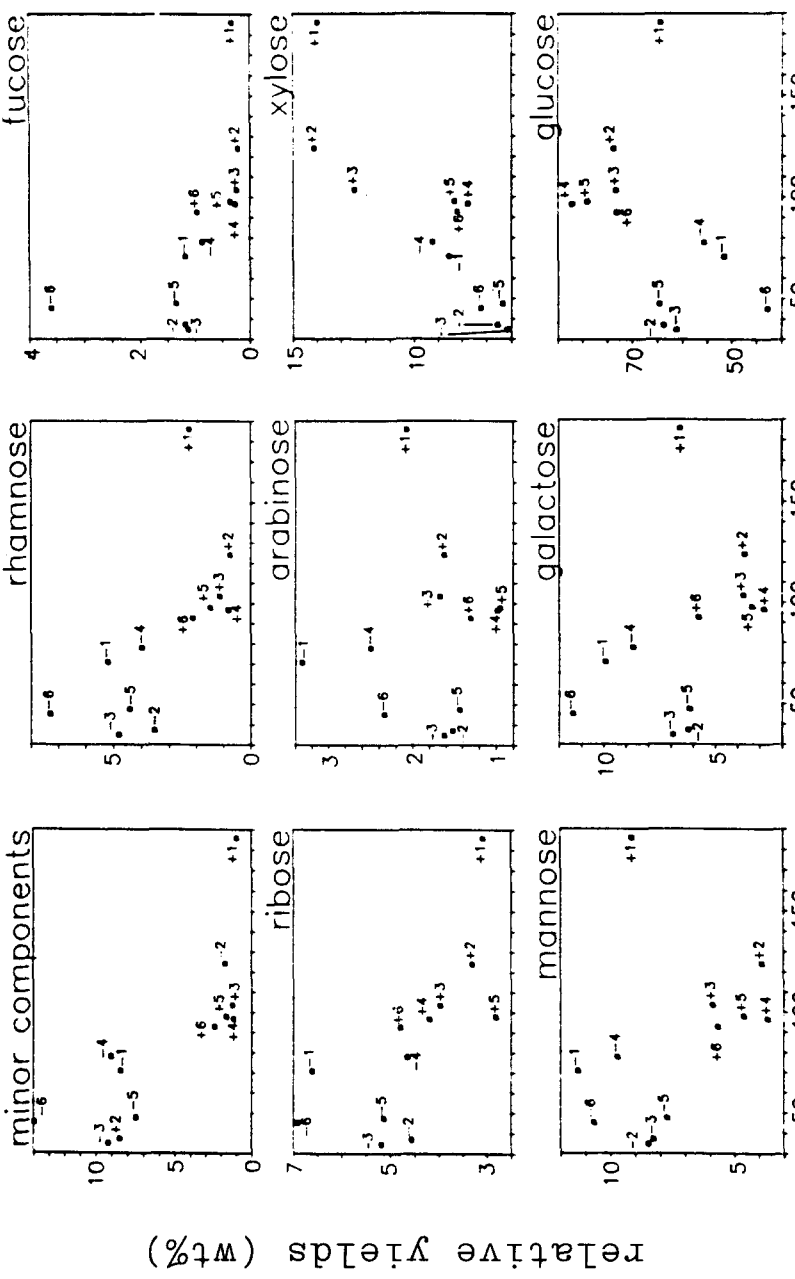



*Fig. 2.* (Individual) relative monosaccharide yields (in wt% of the total monosaccharide yield) versus the total monosaccharide yield (in mg/g). The numbers next to the dots within the figures indicate the depth of the peat sample: 1 = 0–10 cm, 2 = 71–81 cm, 3 = 112–122 cm, 4 = 152–163 cm, 5 = 183–193 cm, 6 = 244–254 cm, '+' and '–' indicate coarse-grained (+20 mesh) and fine-grained (–80 mesh) samples, respectively.

on the other hand, tends to be enriched in the coarse-grained fraction. Comparison with the results of handpicked vascular plant organs and tissues confirms these trends (compare Table 1, Moers et al. 1989, 1990b): vascular plant organs and tissues generally yield higher amounts of total neutral monosaccharides than peat samples, no minor sugars and their relative monosaccharide distributions show rather high contributions of glucose and xylose and/or arabinose and rather low contributions of rhamnose, fucose, ribose and mannose. The case of galactose is not so clear cut: fine-grained peat samples show higher relative contributions of galactose than coarse-grained samples (Fig. 2, Moers et al. 1989, 1990b) and the relative contributions of mannose and galactose are highly covariant which suggests a similar source. However, 'fresh' vascular plant organs and tissues may also yield reasonable amounts of galactose (up to 11 wt%, Moers & Baas, unpublished results). It is speculated that the galactose in 'fresh' vascular plant organs and tissues could be derived from easily degradable polymers that will not contribute (much) to the sediment. Summarizing, we suggest that relatively high contributions of minor sugars, rhamnose, fucose, ribose, mannose and galactose in the peat samples indicate a significant input of microbial organic matter, whereas relatively high contributions of xylose and glucose indicate a predominant vascular plant input. This interpretation is corroborated by the total monosaccharide yields of the two peat fractions which are higher for the coarse-grained and lower for the fine-grained samples (Table 1), because microbial activity likely results in a reduced saccharide yield, not only because vascular plant tissues themselves yield more carbohydrates than bacteria per g of tissue (Cowie & Hedges 1984), but also because the microbes consume and respire the polysaccharides present originally.

Carbohydrate compositions are different for different vascular plant species (e.g. Kirk 1973; Cowie & Hedges 1984), so that the neutral monosaccharide compositions of the coarse-grained samples may reflect their vascular plant precursors. Only xylose seems to give clues in this respect: The coarse-grained samples of the *Taxodium* peat show higher xylose contributions than the coarse-grained samples from the *Carex/Nymphaea* peat (see Fig. 2), namely 13.5 $\pm$ 0.8 and 8.1 $\pm$ 0.2 wt%, respectively. However, it is hard to prove whether this higher xylose contribution is really due to the presence of remains of *Taxodium* organs and tissues in the peat, because incorporation of vascular plant material in peats is accompanied by various selective degradative processes which cause plant organs and tissues in peat to differ in colour, structure and composition from the living equivalents (Cohen & Spackman 1980; Hedges et al. 1985). Moreover, the different types of cell walls (e.g. primary and secondary cell walls) contain contributions of different polysaccharides and the different tissues (e.g. xylem, pith, cortex) are comprised of different types of cell walls (Sjöström 1981; Aspinall 1983; Bacic et al. 1988). The analysis of the various *Nymphaea* organs and tissues may serve as an illustration: the rhizome and the periderm of the rhizome yield more glucose per g tissue than the leaf and root and the relative contributions of xylose and galactose are higher in the case of the leaf (10.4 and

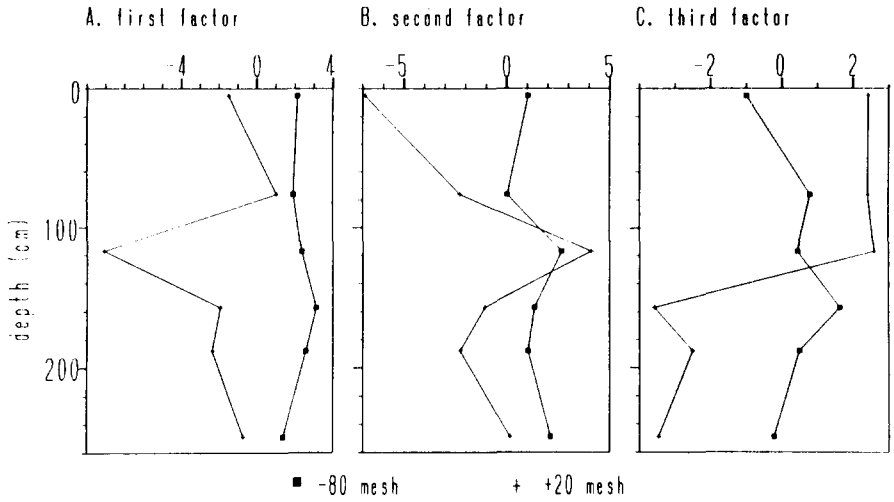



*Fig. 3.* Depth profiles of the scores of the samples on the first three factors that describe the variance in the contributions of the minor sugars. The identifies of the sugars that load on these factors are listed in Table 2.

14.9 wt%, resp.) and root (8.6 and 9.0 wt%, resp.) than in the case of the rhizome (2.8 and 4.2 wt%, resp.) and periderm (5.7 and 4.9 wt%, resp.). So, it would be hard to predict the composition of a *Nymphaea* peat on the basis of the analysis of 'fresh' plant organs and tissues that are known to contribute to the peat. An extra complication in the case of the *Taxodium* peat is that Py-MS results indicate an angiosperm admixture to the peat (see below).

Factor analysis of the minor sugars was carried out in order to investigate possible relationships between the contributions of certain minor sugars and sample grain size, sample depth, microbial communities etc. This technique permits the group of components that are covariant. Table 2 lists the loadings of the minor sugars on the first four factors, i.e. the degree to which the individual minor sugars (the old variables) contribute to the new variables (the factors). The factors describe 30, 25, 15 and 10% of the total variance respectively.

Analyses of minor sugars in cell walls and membranes of several photosynthetic prokaryotes by Weckesser et al. (1979) and Schmidt et al. (1980a, b) have shown that the composition of the polysaccharide moiety of lipopolysaccharides is species specific. However, interpretation of minor sugar contributions to peats in terms of differences in microbial populations is hampered by a general lack of knowledge about systematic occurrences of partially methylated sugars, heptoses, amino sugars etc in microorganisms. Consequently, it is at the moment not possible to use the variations in the minor sugar distributions for the identification of microbial populations.

Figure 3 shows depth profiles of the scores of the samples on the first three factors, i.e. the degree to which the new variables (the factors) are reflected in

the samples. The fine-grained samples do not show much differentiation in their contributions of minor sugars with depth. This indicates rather similar microbial populations in these samples, irrespective of depth. The coarse-grained samples show much more variation, most likely due to the presence of different microbial populations in the various coarse-grained samples. No relationship exists between the distributions of minor sugars in the coarse- and fine-grained samples. This might point to a corresponding lack of relationship between the microbial populations in the two fractions.

The coarse-grained samples tend to score higher on the negative side of the first factor (F1 −) than the fine-grained samples, apparently showing more characteristics of 2-O-methyl-sugars while the fine-grained samples show more characteristics of amino sugars, heptoses, and 6-O-methyl-mannose and 6-O-methyl-galactose (see Fig. 3 and Table 2). It is speculated that this first factor differentiates between bacterial sugars on the positive side (F1 +) and sugars from algae (and maybe fungi) on the negative side (F1 −), because especially the lipopolysaccharides of gram-negative bacteria and cyanobacteria are known to contain substantial amounts of heptoses and amino sugars (Carr & Whitton, 1982; Laskin & Lechevalier 1982; Aspinall 1983; Brade et al. 1988). Part of the amino sugars, of course, must also be derived from peptidoglycans in bacterial cell walls.

The coarse-grained samples from the *Taxodium* peat show the largest variation in their scores on the first and second factor as can be seen from Fig. 3A and 3B. The third factor clearly distinguishes the coarse-grained *Taxodium* samples from the coarse-grained *Carex/Nymphaea* samples. The latter group is characterized by relatively high contributions of 3- and 4-O-methyl-fucose and of 2- and 4-O-methyl-arabinose. Again it is not clear whether and how this can be interpreted in terms of differences in microbial populations associated with different plant species.

*Comparison of sugar data with literature data*

Cowie & Hedges (1984) used xylose and mannose contributions (on a glucose free basis) to distinguish angiosperms from gymnosperms: They report xylose contributions to be relatively high in angiosperms and low in gymnosperms, while the opposite is true for mannose. They also state that nonwoody tissue shows higher arabinose + galactose contributions (on a glucose free basis) than woody tissue. Application of these parameters in the present investigation would strongly indicate a non-woody angiosperm source of the organic matter (i.e. relatively high xylose values, low mannose values and high arabinose + galactose values and no trends with depth in any of the cases). This is in contradiction with the results from microscopic determinations (Cohen 1973a; Spackman et al. 1981; Ryan 1985), which have shown that the upper three coarse-grained samples are derived from woody and nonwoody gymnosperm (roots/rhizomes and leaves of *Taxodium*), while the lower three samples

are predominantly derived from nonwoody angiosperms (roots/rhizomes of *Carex* and *Nymphaea*).

A second contradiction between our data and literature data concerns the specificity of vascular plant versus microbial sugars: Hamilton & Hedges (1988) report, among other things, results of carbohydrate analyses in very recent sediments from a coastal marine environment. They were able to distinguish a vascular plant input (indicated by high contributions of glucose, lyxose, mannose and xylose), and input from marine algae and presumably bacteria (indicated by high contributions of arabinose, galactose, rhamnose and fucose). For the present case we suggest instead that the origin of high contributions of mannose is from microorganisms. Several freshwater algae are known to contain appreciable amounts of structural mannans, in contrast to marine algae (Stewart 1974; Aspinall 1983). Maybe this could explain why Hamilton & Hedges consider mannose as derived from vascular plants.

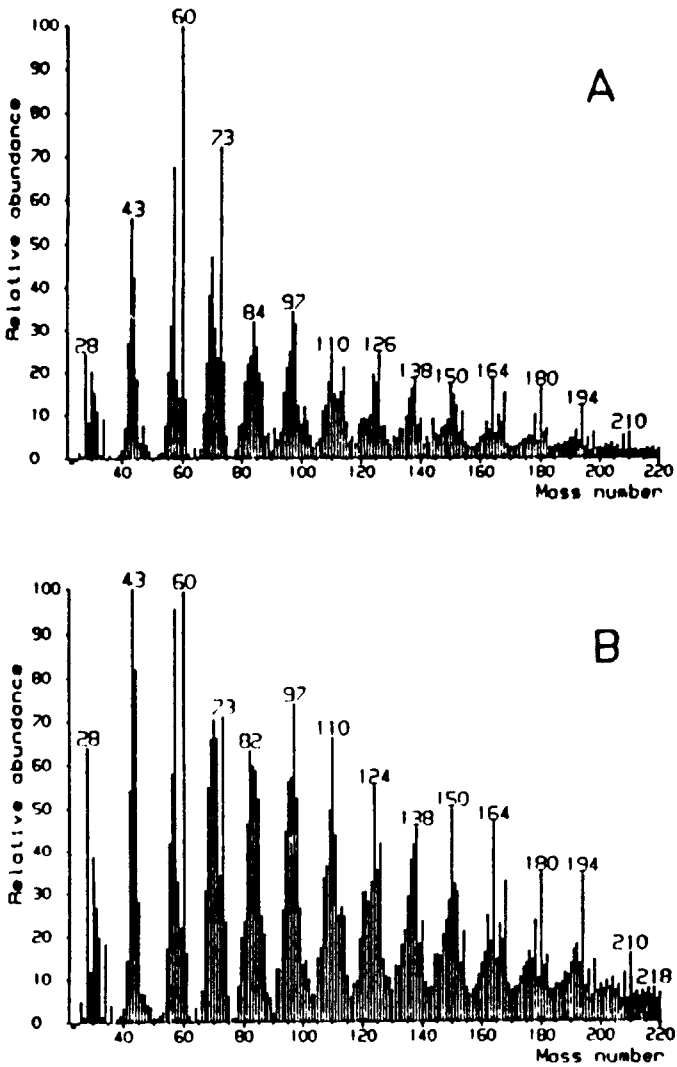
From further comparisons of several studies in which comparable hydrolysis methods were used (e.g. Ittekkot et al. 1982; Degens & Mopper 1976; Cowie & Hedges 1984; Hedges et al. 1985; Tanoue & Handa 1987; Hamilton & Hedges 1988; Moers et al. 1989, 1990b), it appears that the environment of sedimentation and the microbial processes taking place in the watercolumn and/or sediment leave complicated imprints with respect to sugar concentrations. This makes it impossible to formulate universally applicable rules concerning the origin of sugar monomers in sediments on the basis of individual monosaccharide contributions. Liebezeit (1987) reached similar conclusions concerning attempts to use individual sugars as source indicators from investigations of carbohydrates in the water column.

*Py-MS mapping*

Figure 4 shows representative pyrolysis mass spectra of fine-grained peat samples. The mass peaks in these spectra are tentatively attributed to various sources on the basis of literature data obtained for pure biopolymers and sedimentary organic matter: The presence of guaiacyl-syringyl lignin is deduced from mass peaks at m/z 124, 138, 150, 152, 154, 164, 166, 178, 180, 182, 194, 196, 208, 210 (Genuit et al. 1987; Saiz-Jimenez et al. 1987). The peaks at m/z 94, 107, 108, 110, 122 are indicative of modification of the lignin, because increasing decomposition of the organic material and modification of lignin is attended by demethoxylation and demethylation of the phenol derivatives and consequently by shifts to lower m/z values in the Py-MS spectra (Stout et al. 1988). Polysaccharides are indicated by several series of mass peaks (e.g. van der Kaaden et al. 1984; Genuit & Boon 1985; Helleur et al. 1985; Pouwels et al. 1987). The peaks at m/z 85, 86, 114 are attributable to xylose and arabinose; m/z 128 represents a characteristic pyrolysis product of rhamnose. Hexoses are indicated by peaks at m/z 126, 144; hexosan and pentosan polymers also produce anhydrosugars which are evident in the spectra by fragment ions at m/z 57, 60, 73 (e.g. Helleur 1987). Mass fragments at m/z 34, 48, 56, 64, 69, 80, 81, 83, 92, 97, 100, 117 are

interpreted as being derived from proteins (Meuzelaar et al. 1982; Brock et al. 1985).

Markers for polysaccharides and lignin constitute the major signal in the pyrolysis mass spectra of the peat samples as discussed above (Fig. 4). Apart from polysaccharide and lignin contributions mass peaks at m/z 42, 43, 56, 57, 70, 71, 82, 84, 85, 97, 98 may point to the presence of aliphatic hydrocarbons. The fine-grained samples from 0–10 and 152–162 cm and the coarse-grained one from 0–10 cm mainly show saccharide characteristics. The pyrolysis mass spectra of the samples from 0–10 cm resemble spectra of *Taxodium* peats from different locations (Stout et al. 1988; J.J. Boon, unpublished data). The pyrolysis



mass spectra of the peat samples from 183–193 and 244–254 cm resemble those recorded for a *Mariscus/Nymphaea* peat from another location (Moers et al. 1989).

The markers for lignin are for the greater part of the guaiacyl type (m/z 124, 137, 138, 150, 164, 180). Saiz-Jimenez & de Leeuw (1986) and Saiz-Jimenez et al. (1987) report the occurrence of lignin exclusively of the guaiacyl type in pyrolysates of modern and buried spruce, and generalize this phenomenon to be diagnostic for gymnosperms. This conclusion seems to be supported by the present results. Curie point pyrolysis of lignin from several angiosperms has

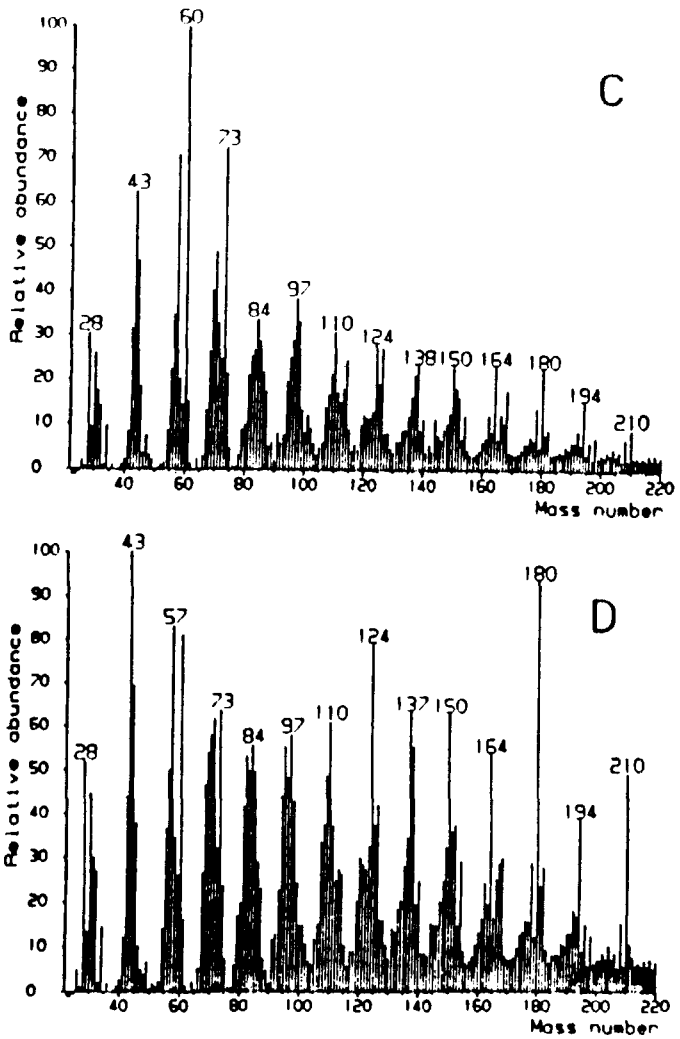



*Fig. 4.* Pyrolysis mass spectra of four fine grained samples. A: 0–10 cm. B: 112–122 cm. C: 152–163 cm. D: 244–254 cm.

revealed the presence of both syringyl and guaiacyl derivatives, whereas grasses appear to yield mainly non-methylated phenol derivatives, especially p-vinylphenol (m/z 120)(e.g. Saiz-Jimenez & de Leeuw 1986; Saiz-Jimenez et al. 1987). The presence of markers for guaiacyl/syringyl lignin in the pyrolysis mass spectra is in agreement with the microscopic observations of *Carex* and *Nymphaea* tissues in the lower three peat samples. The presence of markers for syringyl moieties (m/z 154, 168, 194, 210) in the upper three samples is thought to indicate a small admixture of angiosperm material in the peat, which is speculated to be derived from spanish moss (*Tallandsia*), an epiphytic monocotyledonous angiosperm, in the *Taxodium* trees. So the upper three samples do not represent a completely pure *Taxodium* swamp.

Modification of angiosperm lignin by defunctionalization would result in a shift from syringyl (= dimethoxy-phenol derivatives) to guaiacyl (= monomethoxy-phenol derivatives) moieties (Stout et al. 1988). This is observed indeed with increasing depth of the samples. Modification of lignin together with a change in vascular plant precursors might also explain the change with depth of the relative contributions of the guaiacyl lignin markers at m/z 110, 124, 138, 150, 164, 180; at shallower depth peaks at m/z 110, 138, 164 tend to be rather prominent, while the deepest samples show higher contributions of the peaks at m/z 124, 150, 180 (see Fig. 4).

With increasing depth in the peat the pyrolysis mass spectra of coarse- and fine-grained samples show increasing relative contributions of lignin markers and decreasing relative contributions of polysaccharide markers, with the largest changes occurring between the upper two samples. This indicates that saccharides are preferentially degraded compared to lignins (see also below). The fine-grained sample from 152–162 cm shows deviant behaviour in several aspects and will be discussed later.

Multivariate analysis yielded several discriminant functions, of which the first two are depicted in Fig. 5. The first discriminant function (D1) describes 63% of the characteristic variance (35% of the total variance) and the second discriminant function (D2) describes 22% of the characteristic variance (18% of the total variance). Figure 5 shows the sets of correlated mass peaks that differ either positively or negatively from the overall average spectrum (zero point spectrum). The positive side of the D1 spectrum (D1 +) shows contributions of polysaccharides (interpreted as being derived from both microorganisms and from vascular plants) and the negative side shows contributions of guaiacyl/syringyl lignin, which is typically derived from vascular plants. The pattern of the mass peaks in the D1 − spectrum indicates modification of the lignin (Stout et al. 1988). The positive spectrum of the second discriminant function (D2 +) represents markers for both polysaccharides and lignin, and is interpreted as indicating a more or less intact lignin-carbohydrate complex. The negative side of the D2 spectrum (D2 −) contains mass peaks that are tentatively interpreted as markers for (terpenoid) hydrocarbons (m/z 67, 69, 79, 81, 82, 93, 95, 97, 109, 111, 121, 135, 136, 191). These markers might point to the presence of resins in the peat samples.

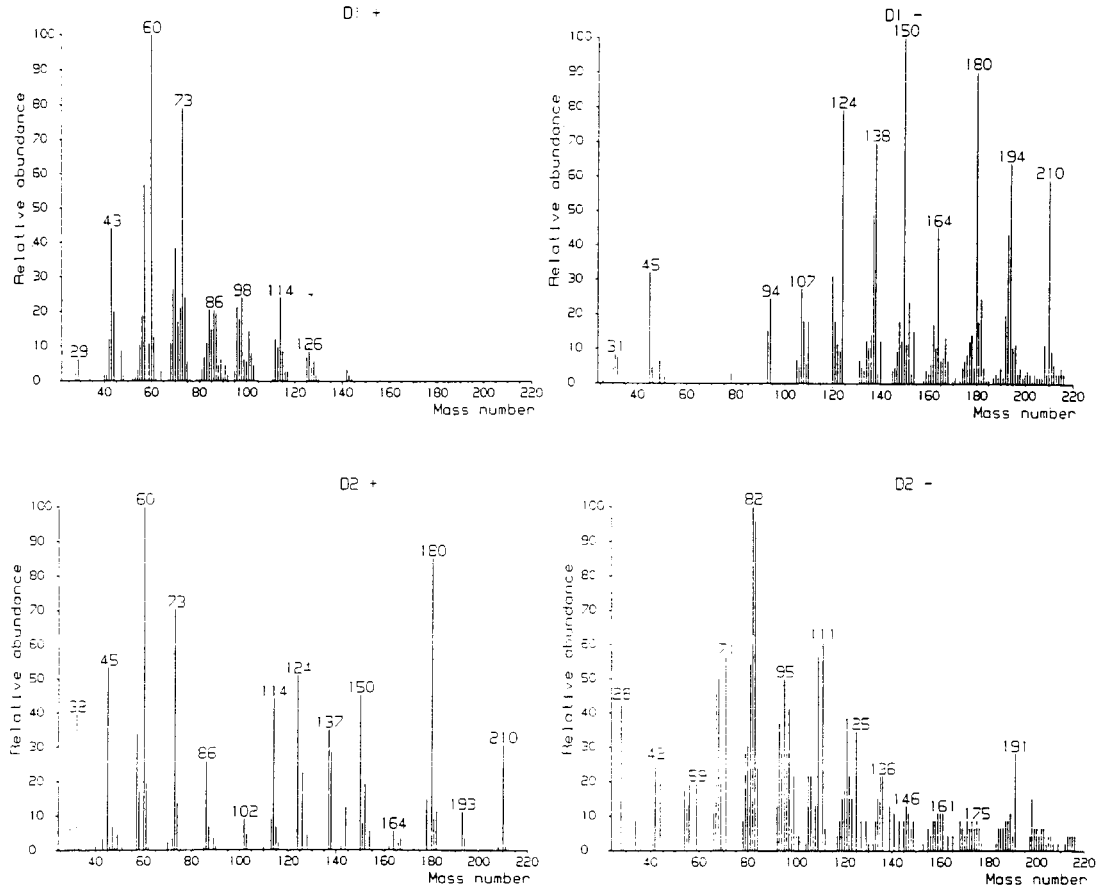



*Fig. 5.* Mass spectra of the positive and negative sides of the first (D1) and second (D2) discriminant functions that describe the variance in pyrolysis mass spectra.

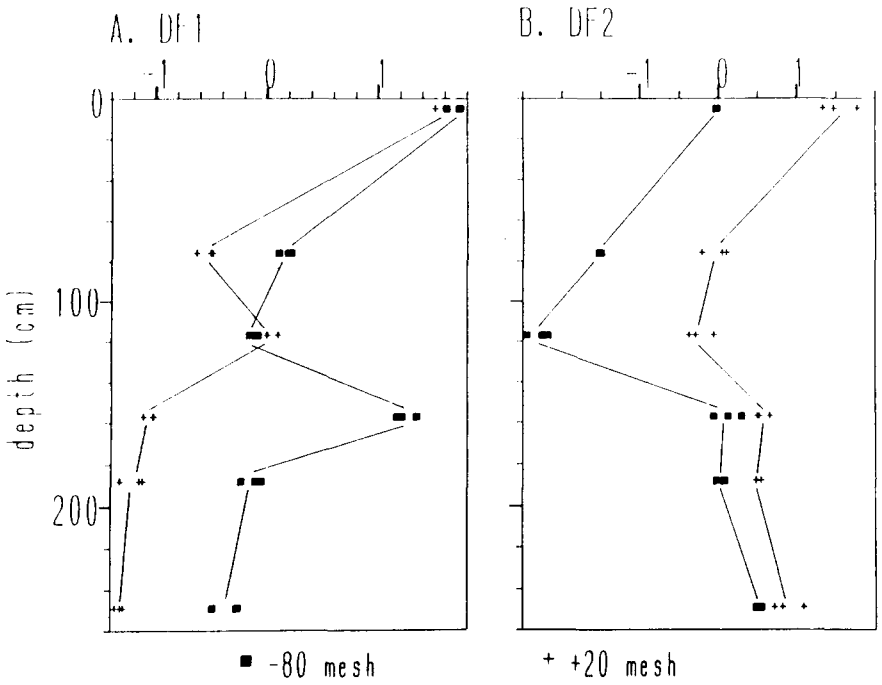



*Fig. 6.* Depth profiles of the scores of the samples on the D1 and D2 functions. D1 + is loaded with carbohydrate markers and D1 − with markers for guaiacyl/syringyl lignin. D2 + is loaded with markers for a lignin-carbohydrate complex and D2 − with markers for resinous material.

Figures 6A and 6B show depth profiles for the first two discriminant functions. Plots of the first discriminant function with depth (see Fig. 6A) show that the coarse-grained samples show more guaiacyl/syringyl characteristics and that the fine-grained samples show more carbohydrate characteristics. The coarse-grained samples are thought to contain a much greater proportion of vascular plant material than the fine-grained samples (see discussion above) which explains why the coarse-grained samples plot more on the negative side of the D1 function. This is not in contradiction with the wet-chemical carbohydrate data, because the fact that the fine-grained samples show more carbohydrate characteristics (i.e. the fine-grained samples score higher on the first discriminant function) than the coarse-grained samples does not say anything about the absolute amounts of carbohydrates present in these fractions. The general trends with depth probably reflect degradation: polysaccharides degrade more easily than lignins and a relative loss of polysaccharides from the peat is therefore accompanied by a relative preservation of lignins (Hatcher et al. 1981; Hedges et al. 1985; Wilson et al. 1987). Most saccharide degradation seems to occur in the upper peat levels which is in agreement with the results from the wet-chemical saccharide analyses (compare Table 1 and Fig. 6A). Figure 6B shows that the coarse- and fine-grained samples from the *Taxodium* peat (i.e. the

samples from the upper three levels) show increasingly more terpenoid characteristics with increasing depth. This can be explained by a relative (i.e. in comparison to the carbohydrate-lignin complex) accumulation of recalcitrant resinous material with increasing depth. Relative resistance to degradation (or selective preservation) of resins is in agreement with higher scores of the fine-grained samples on the negative side of the D2 function and with a generally more degraded character of the material in the fine-grained samples. Vascular plants like *Carex* and *Nymphaea* do not contain resins, which explains why the peat samples from the lower three levels show hardly any differentiation.

*Additional observations*

The trends with depth for total monosaccharide yields (see Table 1) from the peat samples agree well with the trends observed for the first discriminant function (see Fig. 6). The initial sharp decrease with depth in polysaccharide content in the coarse-grained fraction followed by a much slower decrease has also been observed by Rhoads (1985) in Fourier-transformed infrared (FT-IR) spectra for the same samples. This author also observed an initial decrease with depth in polysaccharide content in the fine-grained fraction followed by an unexpected high carbohydrate yield in the sample from 152–163 cm. The results from these three different analytical techniques appear to be in good agreement.

The fine-grained sample from 152–163 cm behaves differently than the other samples: it shows a high total monosaccharide yield but 'normal' relative contributions of microbial sugars (see Table 1 and Fig. 2). The pyrolysis mass spectrum of this sample (see Fig. 4) shows relatively high contributions of polysaccharide markers and low contributions of lignin markers, which is confirmed by the high score of this sample on the positive side of the first discriminant function (see Fig. 6A). Peat from this depth also contains twice as much sulphate (from 35 to 73 ppm) as at other depths, while the concentrations of other sulphur species remain low over the whole length of the core (Casagrande et al. 1977).

The discontinuity at 152–163 cm may be related to the change in environment from open marsh to *Taxodium* swamp. It is speculated that such a transition is accompanied by oxygenation of the surface peat layers at that time and subsequently higher microbial activity. This high activity could result in degradation of deeper peat layers so that the overall result would be a highly decomposed top peat layer with a large amount of fine-grained material and at the same time a large contribution from microbial sugars. *Taxodium* peat would be deposited on top of this material. *Taxodium* is rich in phytotoxins (Given & Dickinson 1975), which could protect the peat layer in question from further extensive microbial degradation.

## Conclusions

1. The coarse- and fine-grained sample preparations show contributions from vascular plants, indicated by high contributions of glucose and xylose, and from microorganisms, indicated by high contributions of partially methylated sugars, amino sugars, heptoses, tetroses and rhamnose, fucose, ribose, mannose and galactose. The contribution of saccharides from microorganisms, and consequently of microorganisms, to the fine-grained fraction is much greater than to the coarse-grained fraction. This is consistent with higher overall monosaccharide concentrations in the coarse-grained fraction.
2. In the coarse-grained samples the *Taxodium* peat can be distinguished from the *Carex/Nymphaea* peat on the basis of higher relative contributions of xylose to the former. From Py-MS data it appears that the *Taxodium* peat contains an angiosperm contribution.
3. Factor analysis of the minor sugars shows that differences exist between the microbial populations of the fine- and coarse-grained samples. The fine-grained samples show very little variation among themselves.
4. Most of the degradation of polysaccharides appears to be restricted to the upper levels of the peat.
5. Factor discriminant analysis of the Py-MS data yielded a first discriminant function that describes the relative accumulation of lignin compared to polysaccharides with increasing depth in the peat. The second discriminant function differentiates between terpenoid hydrocarbons and a lignin-carbohydrate complex. This is interpreted as selective preservation of resinous material derived from *Taxodium*, in the fine-grained samples.
6. The change in environment from open marsh to *Taxodium* swamp is thought to be reflected by deviant monosaccharide concentrations and Py-MS results of the fine-grained sample at the depth of 152–163 cm.

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