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Characterization and chemodynamics of plant constituents during maturation, senescence and humus genesis in spruce ecosystems

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Abstract. Spruce needles of different age, litter materials and soil samples from the L-, O- and A-horizons of a mor profile were analysed by temperature-programmed pyrolysis (Py) in combination with field ionization mass spectrometry (FIMS). The integrated Py-FI mass spectra give characteristic fingerprints of the biomaterials investigated. The application of principal component analysis to the mass spectral data results in a clear discrimination and classification of the samples reflecting the chemical modifications and transformations of organic matter by biochemical and biogeochemical processes. The chemical compositions are determined by processes such as enrichment and/or translocation of plant constituents (e.g. carbohydrates, lignin, lipids, suberin, and aliphatic polymers) during maturation and senescence of needles; amendment of new components; decomposition; selective preservation and humification processes in the soil environment.

During needle maturation, major chemical changes include the decrease of carbohydrate content, condensation of lignin, and crosslinking of waxes. Senescent needles are characterized by lower contents of carbohydrates and lower yields of monomeric pyrolysis products from lignin. The contribution of different litter materials to the humus layer can be estimated by differences in chemical composition. During litter decomposition and humification on the forest floor, carbohydrate content decreases rapidly. The lignin content remains almost constant but some subunits are continuously oxidized. Wax material accumulates until the mechnical disintegration of the needle occurs. In the O-horizons polymeric aliphatic materials are enriched in humified plant remains. A constant increase of aryl-alkyl esters from suberin in the O-horizons is due to both root input and selective preservation. In general, mainly aliphatic polymers and aryl-alkyl esters accumulate during the genesis of mor profiles under conifers.

Introduction

In forest ecosystems, scientists have been concerned about plant growth and nutrition (e.g. Thomas & Stoddart 1980; Horner et al. 1987) and humus genesis (e.g. Blume 1965; Ogner 1985; Kögel-Knabner et al. 1988). From a geoecological point of view, it would be desirable to connect these processes in a way that the natural maturation and senescence of leaves, the quality of plant litter and the genesis of humus are studied together to ascertain the coherence between the biochemical processes above and the biogeochemical processes below ground.

In conifer ecosystems needles persist for several years before they contribute to litter. The accumulation on the forest floor of senescent needles and other litter materials often results in the genesis of mor profiles. This type of forest humus is an indicator that some retardation occurs in the transfer of energy and the cycling of nutrients. Both litter quality and site conditions may contribute to this accumulation.

As plant litter constitutes the main material from which soil organic matter is derived, the availability of nutrients to trees is highly dependent on the quality and the rate of decomposition of these primary resources. The mean composition of the annual little input in European forests is estimated as 48—80% leaves and needles, 10—31% twigs and branches, 9—14% bark, 7—50% root litter, and 1—17% fruits (von Buch 1962; Babel 1975; Mangenot 1980; Vogt et al. 1986). This demonstrates the dominant role of foliage in the annual litter input.

For the characterization of complex organic materials, it is desirable to have a general analytical technique that gives a rapid and comprehensive overview of the chemical composition of biomaterials. Analytical pyrolysis methods and in particular pyrolysis (Py)-mass spectrometry (MS) have proved to be generally applicable in organic geochemistry (Barker & Wang 1988) and environmental research (Simmleit & Schulten 1989a). Under optimized pyrolysis conditions, thermal degradation products reflect the original structure of polymeric material (Garozzo & Montaudo 1985). The recorded mass signals supply a characteristic fingerprint that is particularly suited for pattern recognition studies (Meuzelaar et al. 1984).

In this study temperature-programmed pyrolysis in combination with time-resolved field ionization (FI)MS (Schulten et al. 1987; 1989) was used to analyze spruce needles, litter, and horizons from a mor profile.

The objectives of the project were

- to characterize and classify samples representing different compartments of a forest ecosystem by principal component analysis of their mass spectral fingerprints;

- to describe individual compounds and compound classes of spruce needles during maturation and senescence, and of various litter materials and humus horizons at different stages of humification on a molecular basis;
- to compare the chemical alterations during senescence and humification, and
- to gain insight about the origin and chemical nature of the organic material that is accumulated on the forest floor in spruce stands.

Materials and methods

Materials

Plant, litter and humus materials were taken from two mountainous sites in the Naturpark Fichtelgebirge (900 m a.m.s.l.) and the Taunus Mountains (800 m a.m.s.l.), both in Germany. The site conditions are very similar and described in detail by Simmleit (1988) for the Taunus location and by Hempfling (1988) for the Fichtelgebirge location. Except for the samples of young spruce needles, all samples were taken in late autumn. Materials sampled included leaves of *Vaccinium myrtillus* and *Deschampsia flexuosa*, spruce stemwood, spruce bark, spruce twigs, spruce cones, spruce root bio- and necromass with a diameter < 2 mm, freshly fallen spruce needles and spruce needles of different age classes. A list of all litter samples is given in Table 1. Needle samples (about 10 g fresh material) were taken from sixteen ca. 90-year old Norway Spruce (*Picea abies*) trees and combined to produce composite samples of each age class.

The humus samples were taken from a mor profile (L- and O-horizons). Samples were taken on a volumetric basis. For each horizon pooled samples (about 750 g fresh material) were produced from profiles situated in an area of about 500 m^2. The soil is classified as Lithic Udorthent developed from weathered granite. The morphological differentiation of the humus profile was carried out according to Bochter (1984) and is illustrated in Table 2. A comprehensive morphological and chemical description of the humus samples is given by Kögel-Knabner et al. (1988). From selected soil samples it was possible to separate mycelia of soil fungi. Before Py-FIMS, needles, litter and soil samples were dried and milled (< 0.2 mm).

Table 1. Description of litter samples analysed.

DFL	Leaves of *Deschampsia flexuosa*
FM	Mycelia of soil fungi
FSN	Freshly fallen spruce needles
SB	Fresh spruce bark
SBD	Biodegraded spruce bark
SC	Spruce cone
SN1A	One year old spruce needles
SN2A	Two years old spruce needles
SN3A	Three years old spruce needles
SN4A	Four years old spruce needles
SN5A	Five years old spruce needles
SN2M	Two months old spruce needles
SN5M	Five months old spruce needles
SN7M	Seven months old spruce needles
SNS	Some week old needles from spruce seedlings
SR	Spruce root bio- and necromass, ø < 2 mm
ST	Spruce twigs
SW	Fresh spruce stemwood
SWD	Biodegraded spruce stemwood
VML	Leaves of *Vaccinium myrtillus*

Table 2. Horizons of the mor profile investigated (Kögel-Knabner et al. 1988).

Horizon	Depth (cm)	C_{org} (%)	C/N	$pH(H_2O)$
Ln	0.5	47	34	3.7
Lv	1	46	35	3.6
Ld	1	45	26	3.8
Odf	2	43	25	4.2
Odhf	1	43	27	3.6
Odfh1	3	38	30	4.1
Odfh2	1	45	34	3.3
Odh	3	45	32	3.3
AehOh	1	24	31	3.5

Analytical method

Temperature-programmed pyrolysis is carried out in the high vacuum (10^{-3} Pa) of a combined EI/FI/FD-ion source. Approximately 100 μg of homogenous sample was heated in a quartz crucible at a heating rate of 1 °C/s from 50 to 500 °C using a novel sliding rod (Schulten et al. 1987). The ion source was maintained at a temperature of 200 °C and a potential of +8 kV. Magnetic scans were recorded in 10 °C intervals in the mass range m/z 70 to m/z 1000 with a Finnigan MAT 731 mass spectrometer. Between the repetitive scans, the FI emitter was flash-heated to about 1500 °C in order to avoid condensation. All electrically recorded spectra were integrated by using the Finnigan MAT SS200 data system to give a summed spectrum of the sample. Prior to chemometric evaluation, all summed spectra were normalized to 1,000,000 counts.

Chemometric evaluation

All chemometric calculations were carried out with the ARTHUR statistical software package (Harper et al. 1977) on a pdp 11/34-computer. The mass spectra were transformed into ARTHUR compatible data, using the ARTHURSSX program. The averaged mass spectra of each sample were divided into three groups and one test set. The first group comprised all spruce needle spectra (SN-samples), the second all L-horizon spectra and the third all O-horizon spectra. The test set contained Py-FI mass spectra of parent and degraded biomaterials that may be important for humus genesis (DFL, FM, FSN, SB, SBD, SC, SR, ST, SW, SWD, VML). Mean Fisher weights among the three groups were calculated for all mass variables recorded and only the 70 FI nominal mass signals with high Fisher weights were used for principal component analysis. These mass signals are very consistent within one group but differ among the three groups of samples. The use of principal component analysis for the evaluation of Py-FI mass spectral data sets has been described recently (Simmleit & Schulten 1989b). The principal component score plots are used to classify the samples. The most discriminating mass signals are concluded from loading plots.

Interpretation of mass spectra

Recent studies of spruce needles and humus samples by Curie-point Py-GC/NS and Py-FIMS in the low and high resolution mode (Simmleit 1988; Hempfling 1988) have shown that temperature-programmed Py-FIMS produces almost exclusively molecular ions of thermal degradation products. Using the elemental composition achieved by high resolution MS and thermal degradation products identified from Py-GC/MS analyses, it is possible to assign the structures of distinct thermal degradation products in a mass range up to m/z 500 (Simmleit & Schulten 1989c; Hempfling & Schulten 1990a). This allows the selection of series of nominal FI signals that are typical for specific compound classes. The compound classes suitable for the classification of environmental compartments are of special interest because they are the basis for the description of biochemical and biogeochemical processes in spruce ecosystems.

The mean coefficient of variation for intensities of individual mass signals from 4 replicates was < 10 % (Schulten et al. 1987; Hempfling et al. 1988). For the summed intensities of 5 mass signals, this results in a relative variation of the summed intensities < 22 %. Hence, the summed relative intensities of selected mass signal series are presented as percentage of total ion intensity (% TII), which provides a semiquantitative measure of the chemical composition of the samples investigated (Kögel et al. 1987; Hempfling 1988). Their variation indicates

- chemical changes during maturation, senescence, and humus genesis,
- differences in the chemical composition of litter material, and
- a semiquantitative measure of the chemodynamics of plant constituents in plant litter and humus.

Results

I. Chemical characterization of biomaterials

Spruce needles

Figure 1 shows integrated Py-FI mass spectra of some week-old (a) and four-year-old (b) spruce needles. These Py-FI mass spectra show characteristic mass signals of the major organic components of the needles such as polysaccharides, lignin and lipids and some thermostable plant constituents. Typical Py-FI mass signals of cellulose, hemicellulose and lignin are listed in Table 3 (Simmleit & Schulten 1989c; Hempfling and Schulten 1990a). These signals show high abundances in the Py-FI mass spectra of Fig. 1. Comparison of Figs 1a and 1b indicate that the young needles from spruce seedlings have relatively higher polysaccharide and lower lignin contents than the four-year-old needles. It has been shown previously that the Py-FI mass spectra of spruce needles older than two months are in general similar, indicating that the development of the basic structures of a spruce needle is finished within the first two months of its life-time (Horner et al. 1987; Simmleit 1988).

Other abundant FI signals of spruce needles, at m/z 157, 297 and 406, are due to fragment ions of 10-nonacosanol, a typical constituent of the coniferous epicuticular wax (Schulten et al. 1986). The FI signals in the mass range above m/z 550 are due to molecular ions of plant lipids such as wax esters and estolides. Thermostable plant constituents (Niemann

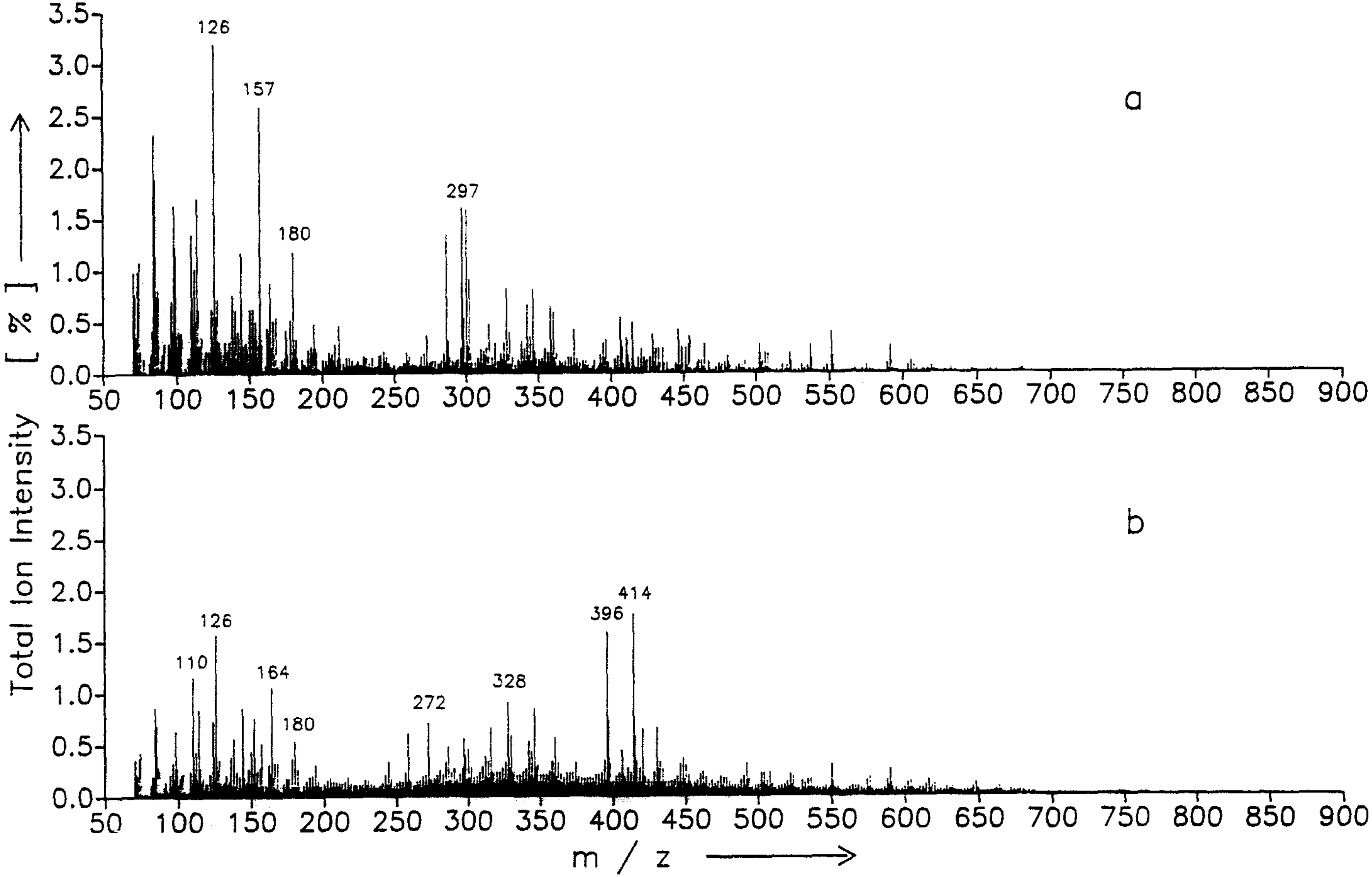

Fig. 1. Integrated Py-FI mass spectra of a few week old needles from spruce seedlings (*a*) and four year old spruce needles from 90-year old *Picea abies* trees (*b*).

Table 3. Assignment of selected, characteristic FI signals recorded after the thermal degradation of spruce needles.

Carbohydrates

m/z 72, 74, 75, 84, 85, 86, 96, 98, 110, 112, 114, 126, 128, 132, 144, 162
with m/z 114 and 132 as indicator signals for pentose units
with m/z 126, 144 and 162 as indicator signals for hexose units

Monomeric lignin units

m/z 124, 138, 140, 150, 152, 164, 166, 168, 178, 180, 182, 194, 208, 210

Dimeric lignin units

m/z 246, 260, 270, 272, 274, 284, 288, 296, 298, 300, 302, 312, 316, 326, 328, 330, 342, 344, 346, 354, 356, 358, 360, 372, 376, 386

Stilbene units

m/z 212, 226, 228, 238, 240, 242, 244, 246, 258, 260

1979; Berg et al. 1980) produce molecular FI ions at m/z 300, 302, 304 (diterpene acids), m/z 396 (ergosterol), m/z 414 (β-sitosterol) and m/z 430 (α-tocopherol). The presence of cutin is indicated by the abundant FI signals at m/z 286, 316 and 446 which are characteristic thermal degradation products of spruce cuticles (Simmleit & Schulten 1989a).

Litter materials

The integrated Py-FI mass spectrum of a spruce twig is shown in Fig. 2a. The inner woody part of twigs consists mostly of cellulose, hemicellulose and lignin. Hence, most of the FI signals seen in Fig. 2 are of the same chemical origin as the FI signals listed in Table 3. The most abundant FI signals occur in a homologuous series at m/z 432, 446, 460, 474, 488, 502, 516, 530 and 544, which can be assigned to aromatic esters released as typical thermal degradation products of suberin (Hempfling et al. 1988). However it is known that the FI signal at m/z 446 also arises from the thermal degradation of both cutin and suberin, biopolymers of different chemical structure. This emphasizes the need for a careful selection of compound specific key signals. Suberin is one of the major constituents of bark, the outer surface of twigs (Fig. 2b). Again, the FI signal at m/z 414 indicates the presence of β-sitosterol.

The Py-FI mass spectrum of a spruce cone (not shown) is very similar to the spectrum of young spruce needles in Fig. 1a. However, the cone spectrum shows less abundant fragment ions at m/z 157, 297 and 406

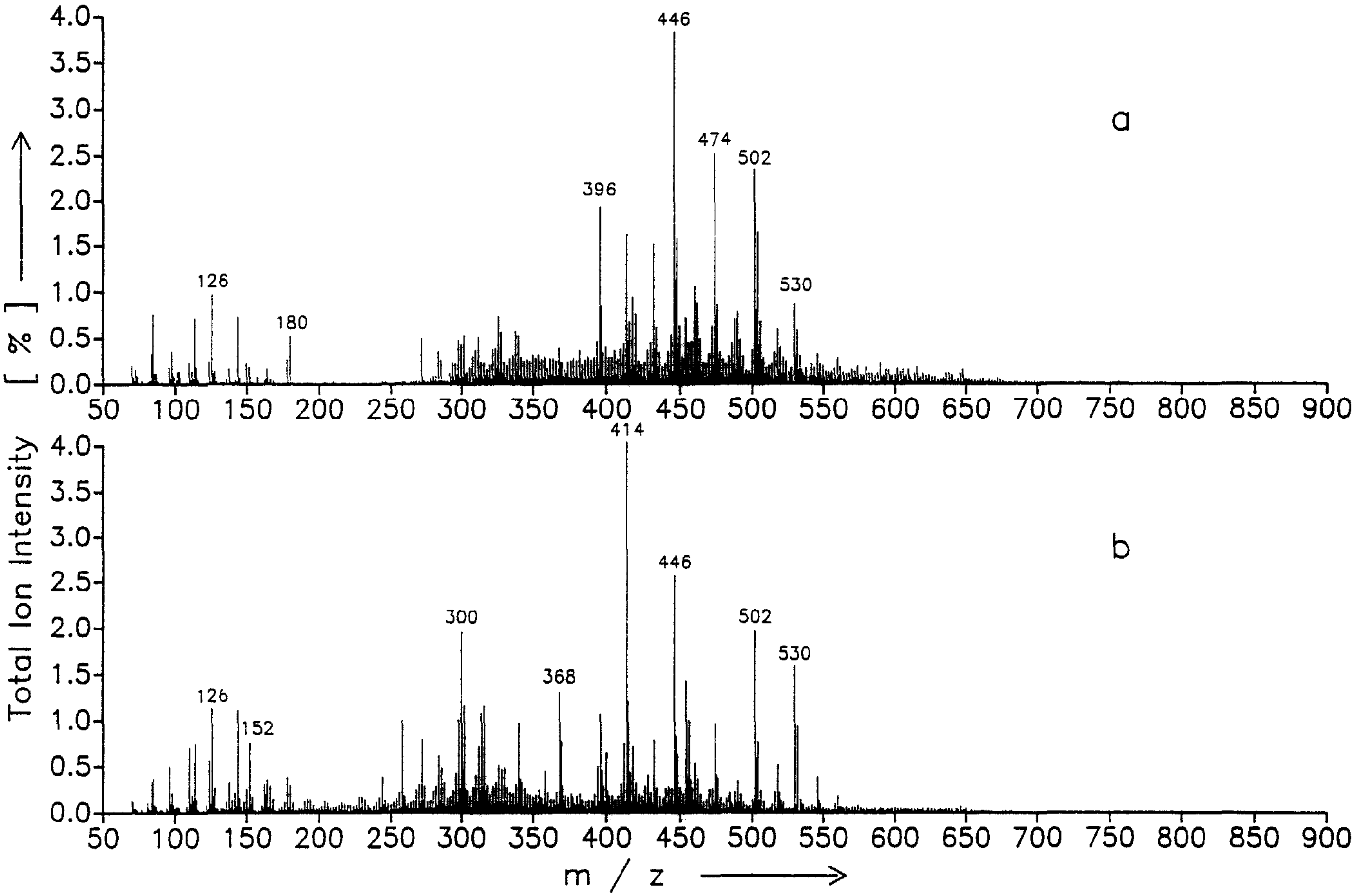

Fig. 2. Integrated Py-FI mass spectra of spruce twigs (*a*) and spruce bark (*b*).

indicating the lack of spruce wax. In comparison to older spruce needles (Fig. 1b) and spruce branches (Fig. 2a), the cone contains a higher portion of polysaccharides, and shows a smaller degree of lignification. Ergosterol and β-sitosterol are observed, and the FI signals of volatile, thermostable plant constituents at m/z 298 and 300 are abundant. These signals may be due to flavonoid compounds or diterpene acids.

Besides coniferous trees, herbaceous plants as parent materials also contribute to humus genesis. As a typical example leaves of *Vaccinium myrtillus* have been chosen; the corresponding Py-FI mass spectrum is presented in Fig. 3. This indicates the dominances of cellulose, hemicellulose and β-sitosterol, with little intensity of signals derived from lignin. Other abundant signals are due to a homologuous series of alkyl fatty acids or esters at m/z 396 (C_{26}), m/z 410 (C_{27}), m/z 424 (C_{28}), m/z 438 (C_{29}), m/z 452 (C_{30}), and m/z 466 (C_{31}). Another homologuous alkyl series can be seen at m/z 444, 472, 500, 528, 556, 584 and m/z 612. At present the chemical origin of this series and of the abundant FI signal at m/z 590 is unknown. However, a similar series of signals has been found for thermal degradation products derived from the cuticle of *Agave americana* (Tegelaar et al. 1989). In this study it has been shown that the elemental composition of compounds of the series is $C_n H_{2n-12} 0_4$.

Below the forest floor, soil fungi, soil fauna and root bio- and necromass as parent materials also contribute to humus genesis. As an example Fig. 4 shows the Py-FI mass spectrum of spruce fine roots. This spectrum indicates that fine roots are composed mainly of cellulose, hemicelluslose and lignin subunits. In comparison to four-year-old needles, bark, and *Vaccinium myrtillus* leaves, the FI signals of sterol compounds play only a minor role and all FI signals derived from suberin in fine roots show relative intensities less than 1 %. These aromatic ester signals are centered around the FI signal at m/z 474 in a homologuous series and have been already discussed in relation to the bark of the twigs (see above). The base peak at m/z 454 is due to molecular ions of epoxy-D:A-friedooleanandione a typical suberin lipid (Hempfling & Schulten 1990a). Comparison with the Py-FI mass spectrum of bark in Fig. 2b shows that this compound is much more abundant in roots than in bark.

Humus horizons

In a spruce ecosystem the upper litter layer is composed largely of conifer needles and twigs. Therefore, the Py-FI mass spectrum of the Ln-horizon (not shown) is similar to that of matured spruce needles (Fig. 1b), but shows enhanced signal intensities of the aromatic ester peaks derived from thermal degradation products of bark suberin. As suberin is a stable biopolymer, which is not subjected to immediate biodegradation, its

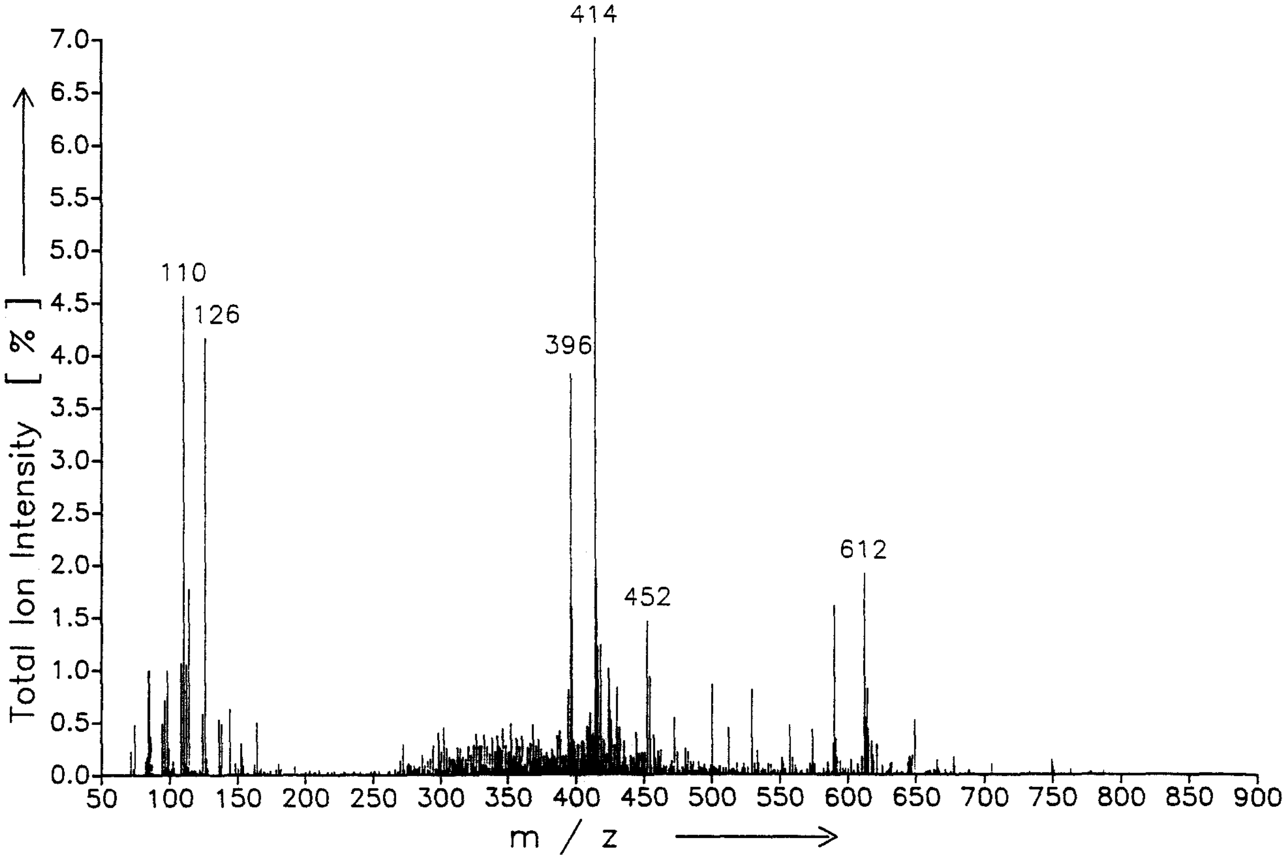

Fig. 3. Integrated Py-FI mass spectrum of *Vaccinium myrtillus* leaves.

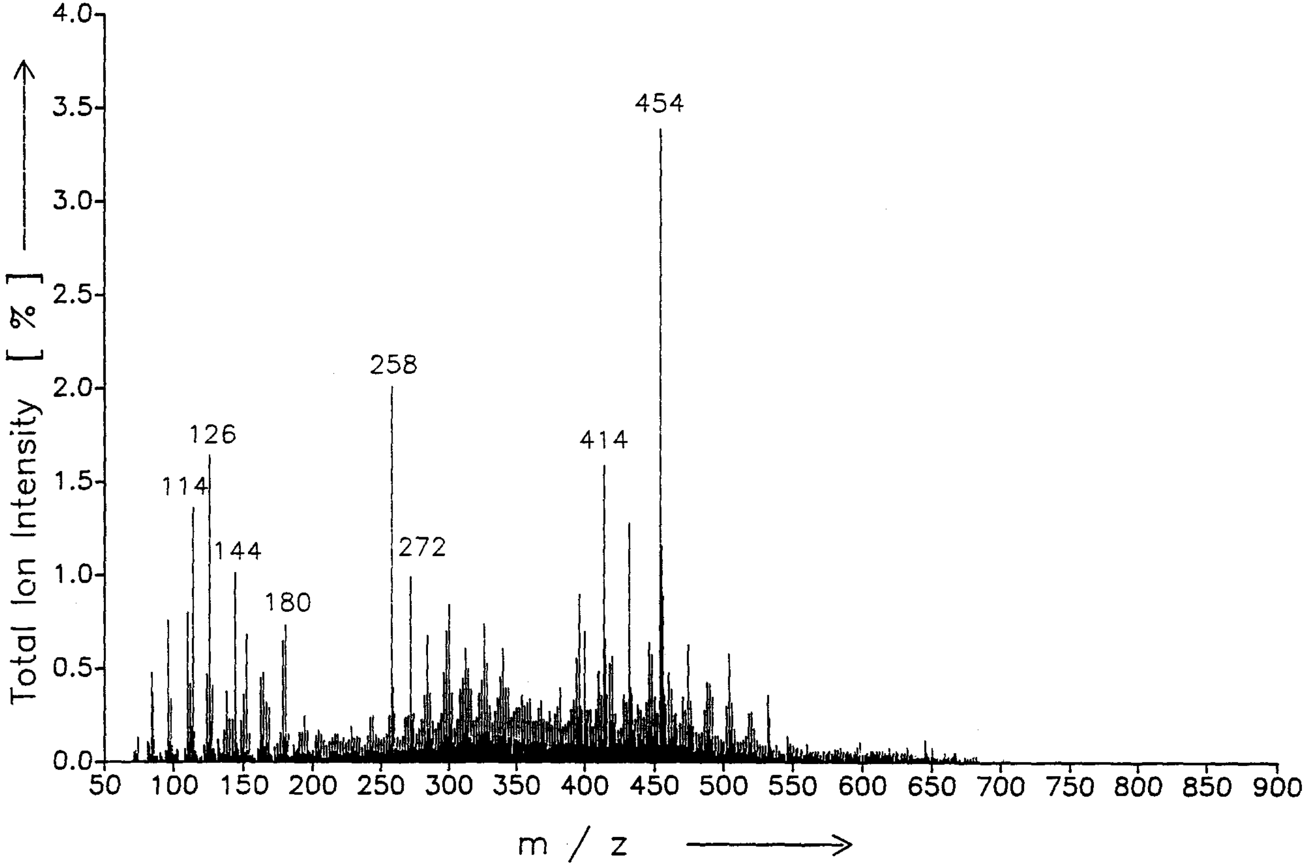

Fig. 4. Integrated Py-FI mass spectrum of spruce root bio- and necromass.

content is relatively enriched during the first stages of humus genesis in the litter layer. Hence, the homologuous series of aromatic ester signals at m/z 418, 446, 474, 502, 530 and 558 dominates the Py-FI spectrum of the Lv-horizon shown in Fig. 5a. Most of the other FI signals are due to thermal degradation products of cellulose, hemicellulose and softwood lignin (Tab. 3). Other homologuous series of prominent FI signals are due to C_{18}—C_{24} alkanoic acids at m/z 284, 298, 312, 326, 340, 354 and 368 and C_{28}—C_{40} alkenes at m/z 392, 420, 448, 476, 504, 532 and 560 (Kotra & Hatcher 1988). The alkanoic acids are derived from plant materials such as wax esters, whereas the alkene compounds could be derived by water loss from alcohols. In FI it is known that aliphatic alcohols preferentially form $[M\text{-}H_20]^+$ ions (Schulten et al. 1986).

As another example of a humus sample, Fig. 5b shows the Py-FI mass spectrum of the AehOh-horizon which represents an advanced stage of humification (100% macromorphological fine substance). This horizon is a mixture of mineral soil and humified parent materials, with an organic carbon content less than half that of the L-horizons (Tab. 2). In general, this spectrum shows the same FI signal patterns as the Py-FI spectrum of the L-horizons. However, the relative intensities of the mass signal at m/z 446 and the alkene signals are significantly less. The same is true for β-sitosterol at m/z 414. In the low mass range, shifts can be seen in comparison to the spectrum of the Lv-horizon (Fig. 5a). First, there is an increase of the FI signal at m/z 98 attributed to molecular ions of furfuryl alcohol, methylfuranone ($C_5H_6O_2$), methylpentenone ($C_6H_{10}O$) and heptene (C_7H_{14}), thermal degradation products which cannot be related to a specific compound class. Thus, the increase of pyrolysis products at m/z 98 relative to the increase of hexose signals at m/z 126 and 144, may be either due to an enrichment of microbially modified or synthesized carbohydrates (Hempfling et al. 1988), or to non-carbohydrate structures. Second, the increase of the FI signals at m/z 168 ($C_8H_8O_4$) which represents vanillic acid and the drastic decrease of the FI signals at m/z 180 ($C_{10}H_{12}O_3$), due to coniferyl alcohol (Evans et al. 1986), indicates an advanced degree of lignin oxidation in the AehOh-horizon.

II. Classification of precursor and humus samples

Principal component analysis of the mass spectral data suggests that the dimensionality of the data set can be reduced to the first two principal components that comprise 82.7 % of the variance. As shown in Fig. 6, a first classification of the sample set can be achieved by plotting the scores of the first two principal components. For comparison other litter materials (test set) are also illustrated. The first principal component clearly

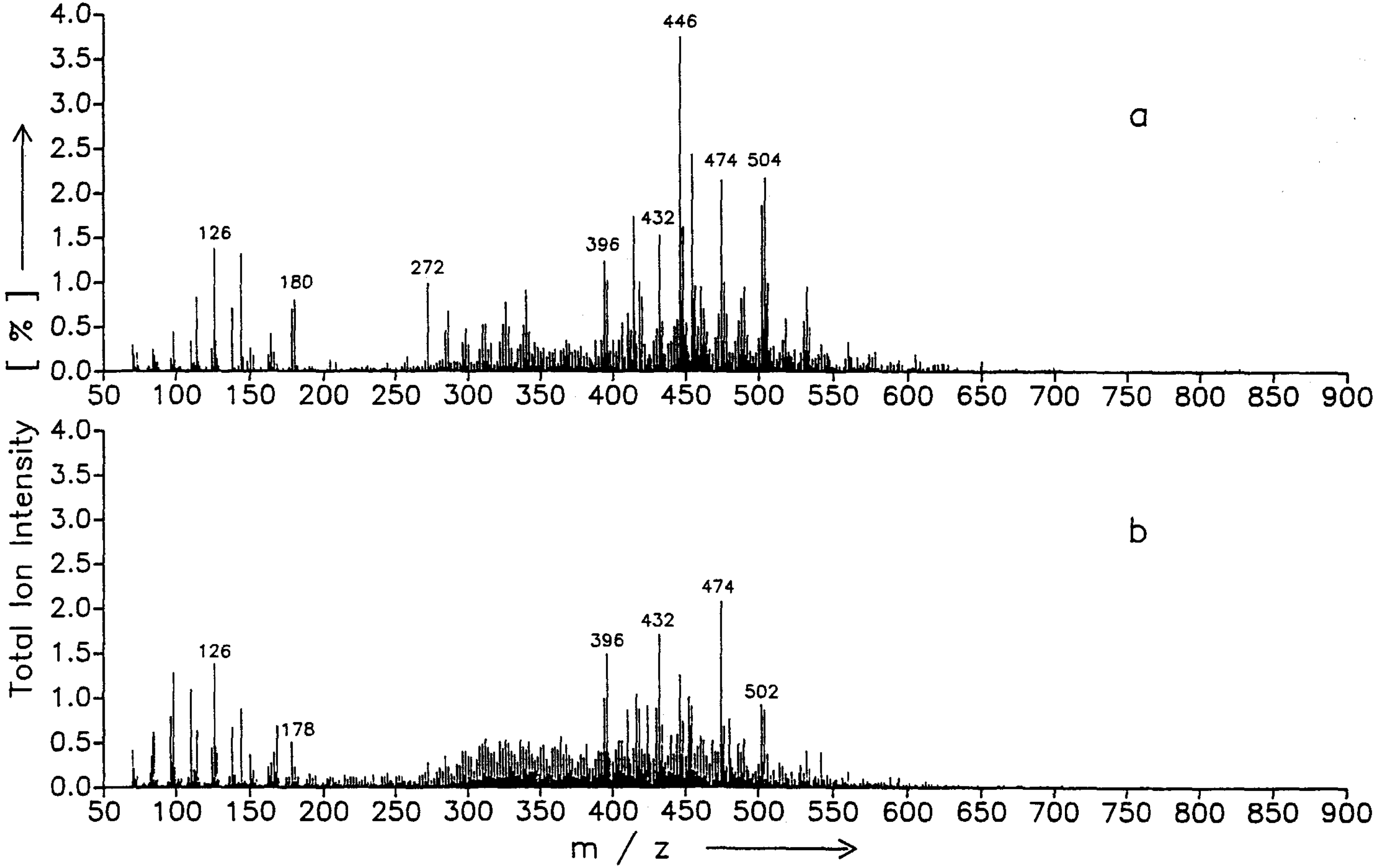

Fig. 5. Integrated Py-FI mass spectra of humus samples taken from the Lv-horizon (*a*) and the AehOh-horizon (*b*).

separates the spruce needles from the soil horizons whereas the second principal component represents the soil formation discriminating the L- and the O-horizon.

First principal component

The main portion of the variance, i.e. the major differences in chemical composition within a conifer ecosystem, which can be detected by Py-FI mass spectral fingerprints, is due the chemical differences between spruce needles with negative scores and mor humus horizons with positive scores. Increasing scores of the first principal component (Fig. 6) indicate the maturation of the spruce needles on the tree, the senescence of spruce needles (FSN) and the first decomposition of spruce needle litter (LN,

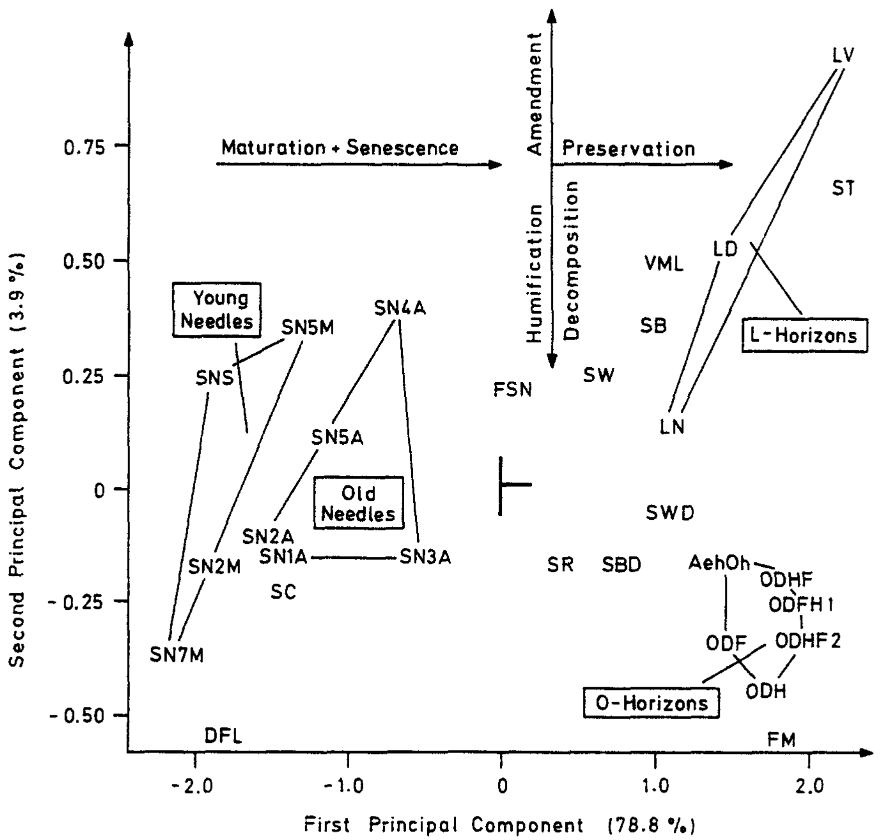

Fig. 6. Score plot of the first versus the second principal component calculated from a Py-FI mass spectral data set of nine spruce needle and nine humus samples from a mor profile. These samples have been divided into three groups consisting of spruce needles, L-horizons and O-horizons, respectively. Samples of other precursor material also are shown (test set). All sample names are explained in Tables 1 and 2.

LV). Spruce needle decomposition starts with loss of easily degradable constituents causing enrichment of other constituents by selective preservation. For example, the macromorphological differentiation of horizons shows the enrichment of small pieces of twigs in the Lv-horizon. Similarily, biodegradation of spruce wood starts with the decomposition of cellulose, causing a selective enrichment of lignin and an increase of the scores from SW to SWD in the principal component analysis. The opposite process leading to lower positive scores represents diminution of the preserved components. Obviously, this process prevails during the development of the humus profile between the Lv- and the Ld-horizon or between the Odhf2-, Odh- and AehOh-horizons.

The second principal component

This component separates the L- (positive scores) from the O-horizons (negative scores). All materials that contribute to above-ground litter input (FSN, SW, SB, VML, ST) also show positive scores. By contrast, all materials that represent degraded biomaterials or below-ground litter input (SWD, SBD, SR, FM) have negative scores. Amendment of fresh plant constituents is indicated by the increase of scores. For example, the accumulation of twigs in the Lv-horizon causes an enrichment of suberin and lignin. This can be also interpreted as the addition of lignin and suberin to the spruce needle system in relation to the score level of FSN and LN. During the first phase of decomposition, twigs (ST) are degraded more slowly than freshly fallen spruce needles (FSN, LN), resulting in the Lv-horizon. Then, bark decomposition prevails, leading to the Ld-litter horizon and a decrease of the scores. The decrease of scores indicates humification processes that are similar to those occuring during the degradation of wood (SW vs. SWD) and bark (SB vs. SBD). The chemical transformation of the Ld-litter to the Odf-humus is characterized by a balanced wood, bark and needle degradation. The addition of root material (SR) leads to increased scores for the Odhf-horizon. In the lower Odfh1-, Odfh2- and Odh-horizons humification processes dominate the chemodynamics leading to lower scores of the second principal component. The increased scores of the AehOh sample can be explained by precipitation of water-soluble humus materials mainly from the L-horizons.

III. Description of compound classes

Figure 7 shows the loading plot of the principal component analysis. The exact loadings of the mass signals which comprise 82.7 % of the variance are not shown but rather the sector in which they are located. The approximate values of the corresponding loadings are indicated by the

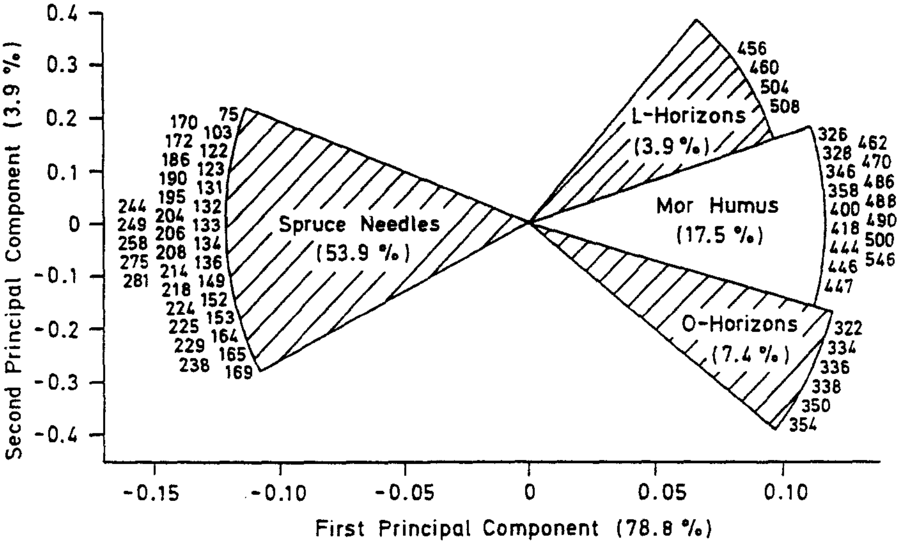

Fig. 7. Loading plot of the first versus the second principal component (compare to score plot in Fig. 6). Only the sectors are shown in which certain FI mass signals are situated. The hatched sectors show the direction of the groups of samples in the corresponding score plot. According to the sum of individual loadings, the proportion of variance of each sector is indicated.

radius of each sector. The sectors have been defined by the directions of samples groups in the classification pattern in Fig. 6.

The mass signals which are related to the sectors of "spruce needles" and "mor humus" are responsible for the distinction between plant materials and humus samples (first principal component). Signals with negative loadings are more abundant in the Py-FI mass spectra of the spruce needles whereas the signals with positive loadings are more abundant in the spectra of the soil samples. Thus, the Py-FI mass spectra of plant materials show higher intensities of FI signals in the mass range m/z 75-281 which are due to lignin monomers and dimers and N-containing plant materials. The levels of these compounds are lower in the soil samples. The humus samples are characterized by higher signal intensities of oligolignols and suberin constituents. Apart from the principal differences between plant materials and humus, the litter horizons are also characterized by high intensities of FI mass signals at m/z 456, 460, 504 (trilignols) and m/z 508. The latter signal could be due to an aliphatic monoester, a typical wax constituent. In contrast, the O-horizons are differentiated from the L-horizons by alkene-derived FI signals at m/z 322, 336 and 350 indicating an increase of aliphatics during humification.

IV. Chemodynamics

The most important classes of coumpounds suitable for the differentiation of compartments of the forest ecosystem by principal component analysis of Py-FI mass spectra proved to be lignin, lipids, suberin, and aliphatic polymers (see Section III). Surprisingly FI signals that relate to carbohydrates were not suitable. This may be due to either minor changes of carbohydrate content within the system, or to high variability within the selected compartments, exceeding the differences between the compartments. A pyrolysis-mass spectrometric study of paper pulp from several sagebrush (*Artemisia*) species by Windig et al. (1984) did not show a hexose pattern in the factor analysis. This was interpreted as a result of the normalization and data analysis procedures used that filter the constant part of the spectra out and focus mainly on differences between the samples. Although carbohydrate signals were not suitable for the chemometric differentiation, the description of chemodynamics of carbohydrates was regarded as relevant, because they represent major buildings blocks of biomaterials.

In addition, signal series of compound classes suitable for discrimination were supplemented with further typical mass signals that showed similar characteristics in principal component analyses under slightly modified conditions. This was to avoid misinterpretation in the chemodynamic description caused by different chemical sources for one mass signal.

Lignin

Typical mass signals for monomeric pyrolysis products from softwood lignin are m/z 138 (guaiacylmethane), 150 (guaiacylethene), 152 (guaiacylethane), 164 (guaiacylpropene), and 180 (coniferyl alcohol). The summed intensities (% TII) of these FI signals as calcuated from the mass spectra of the investigated materials are shown in Fig. 8. During maturation of spruce needles (Fig. 8A), the chemical composition of lignin changes so that the yields of monomeric pyrolysis products decrease from the first to the second month, increase from the second to the twelth month and decrease from one to five year old needles, and during senescence. For litter materials (Fig. 8B), intensities of these mass signals are comparatively very high for cones, high for fresh and degreaded wood and for degraded bark, and medium for fresh bark, twigs and roots. In the humus layer (Fig. 8C) the lignin macromolecules are characterized by decreasing yields of monomeric pyrolysis products from Ln to Ld. The O horizons are characterized by fairly constant values which are increased in the Odhf, Odfh2, and AehOh horizons.

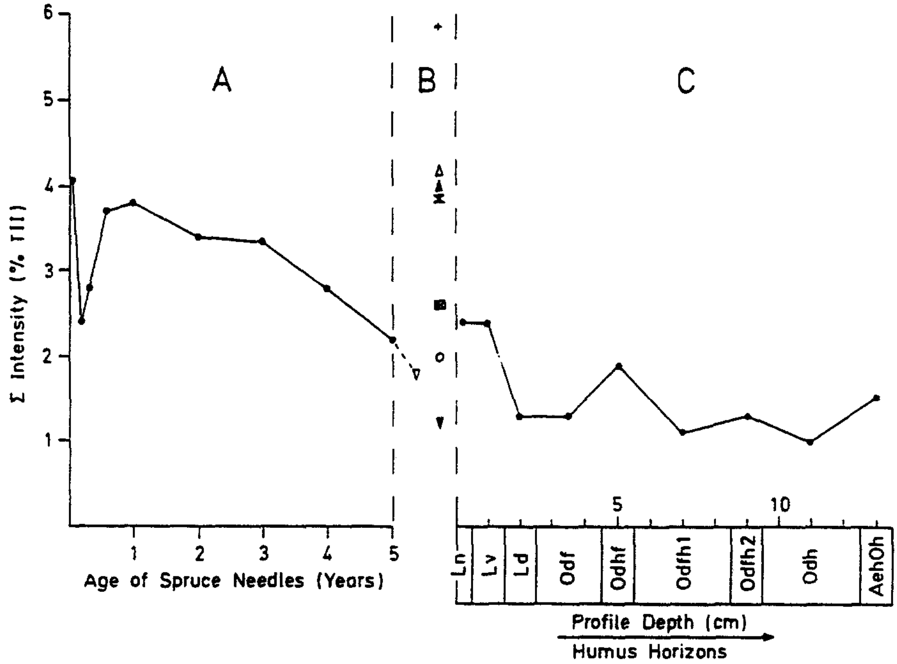

Fig. 8. Summed intensities of characteristic FI signals from needles (*A*), litter materials (*B*), and humus horizons (*C*) of spruce ecosystems that relate to monomeric pyrolysis products from lignin (m/z 138, 150, 152, 164, 180). In Fig. 8A chemical changes during needle maturation and senescence are described; Fig. 8B compares individual litter material such as freshly fallen spruce needles (▽), twigs (▼), cones (+), wood (△), degraded wood (▲), bark (○), degraded bark (⧗), and roots (■); litter decomposition and humification with increasing profile depth is described in Fig. 8C.

Recently, possible structures for pyrolysis products of condensed, dimeric lignin subuits have been proposed (Simmleit and Schulten 1989; Hempfling & Schulten 1990a). Modifications of the lignin macromolecules during maturation and senescence of spruce needles are of minor importance for dimeric subunits, as the yields of related pyrolysis products remain rather constant (not shown). The various litter materials yield very high amounts of dimeric lignin subunits from fresh and degraded wood, medium amounts from roots, fresh and degraded bark, and cones, and low amounts from twigs during pyrolysis.

Trimeric pyrolysis products of lignin are indicated by mass signals at m/z 420, 450, 476, 504, and 522. The summed intensities of these signals from the Py-FI mass spectra of the various samples are shown in Fig. 9. During the first 12 month of maturation, a significant increase of the summed intensities is visible. Yields of trimeric pyrolysis products from lignin remain constant for one to three year old needles, and then increase

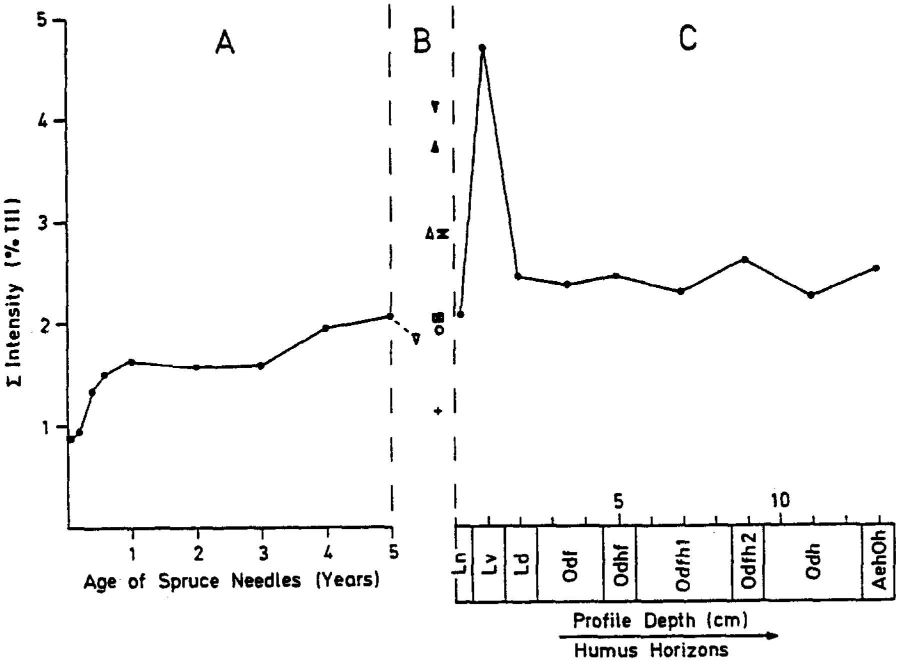

Fig. 9. Summed intensities of characteristic FI signals from needles (*A*), litter materials (*B*), and humus horizons (*C*) of spruce ecosystems that relate to trimeric pyrolysis products from lignin (m/z 420, 450, 476, 504, 522). Details for Fig. A, C are given in Figure 8. Litter materials in Fig. 9B: freshly fallen spruce needles (▽), twigs (▼), cones (+), wood (△), degraded wood (▲), bark (o), degraded bark (⧗), roots (■).

again from 3 to 5 years. For senescent material, the yield is comparable to 4 and 5 year old needles. The other litter material is characterized by comparatively high yields from twigs, fresh and degraded wood and degraded bark, medium yields from fresh bark and roots, and low yields from spruce cones. The Py-FI mass spectra of the humus horizons indicate that during litter decomposition and humification, the yield of trimeric lignin subunits increases sharply from Ln to Lv, decreases again to Ld, and then remains almost constant from Ld to AehOh.

The ratio of the intensities of signals that relate to coniferyl aldehyde (m/z 178) and to coniferyl alcohol (m/z 180) is shown in Fig. 10. This ratio indicates the degradation (oxidation) status of lignin subunits. However, this ratio should be used only for monomeric subunits because the chemodynamics of monomers differ from dimer and trimer subunits. As expected, there are no significant changes for this ratio for needles on the tree. The ratios for lignin-containing litter samples (only partly shown) are very similar to those of spruce needles. There is a slight increase from

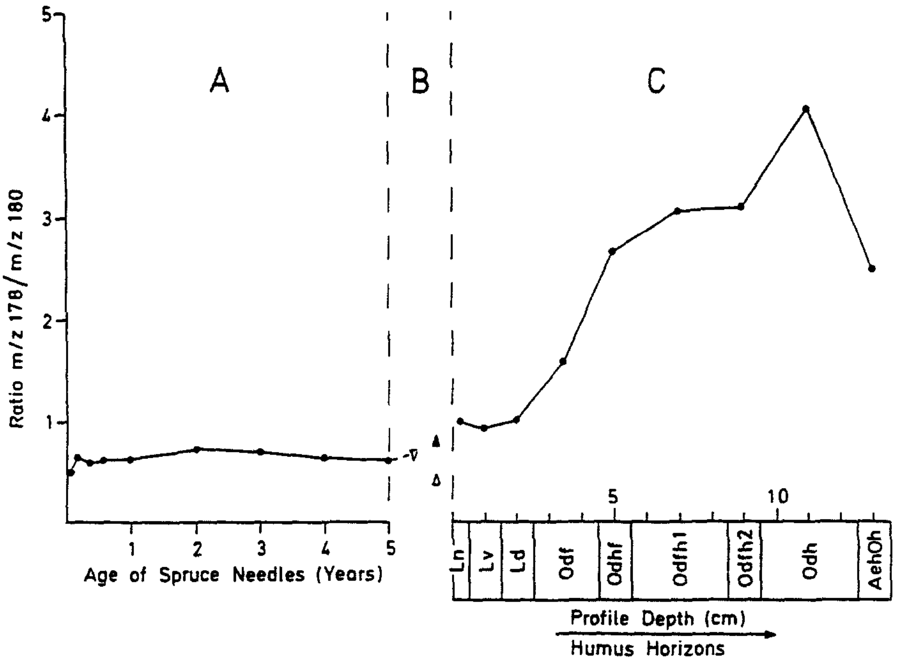

Fig. 10. Ratio of the intensities of the FI-signals m/z 178 (coniferyl aldehyde) to m/z 180 (coniferyl alcohol) for needles (*A*), litter materials (*B*), and humus horizons (*C*) of spruce ecosystems. Details for Fig. A, C are given in Fig. 8. Litter material in Fig. 10B: freshly fallen spruce needles (∇), wood (△), degraded wood (▲).

fresh wood to degraded wood. In the humus layer the ratio increases constantly from Lv to Odh, and decreases to AehOh.

Suberin

Figure 11 shows summed intensities of characteristic signals that relate to suberin constituents (m/z 474, 488, 502) for some litter material and humus horizons. These suberin subunits as well as suberin lipids and lignin subunits proved to be the major differences between spruce needles and the related mor humus horizons. Proposed structures for the suberin subunits are aryl-alkyl esters with an increasing length of the aliphatic chains (Hempfling et al. 1988). In the litter materials, very high contents of suberin are indicated for twigs and high contents for bark. The contents of degraded barks and roots are similar to those of the litter layer. As suberin is major constituent only of bark and roots, signal intensities for other litter materials such as needles are below 1 % TII. Within the humus layers, suberin content increases sharply from Ln to Lv and decreases again to Ld. From Odf to AehOh a constant increase of suberin material is observed.

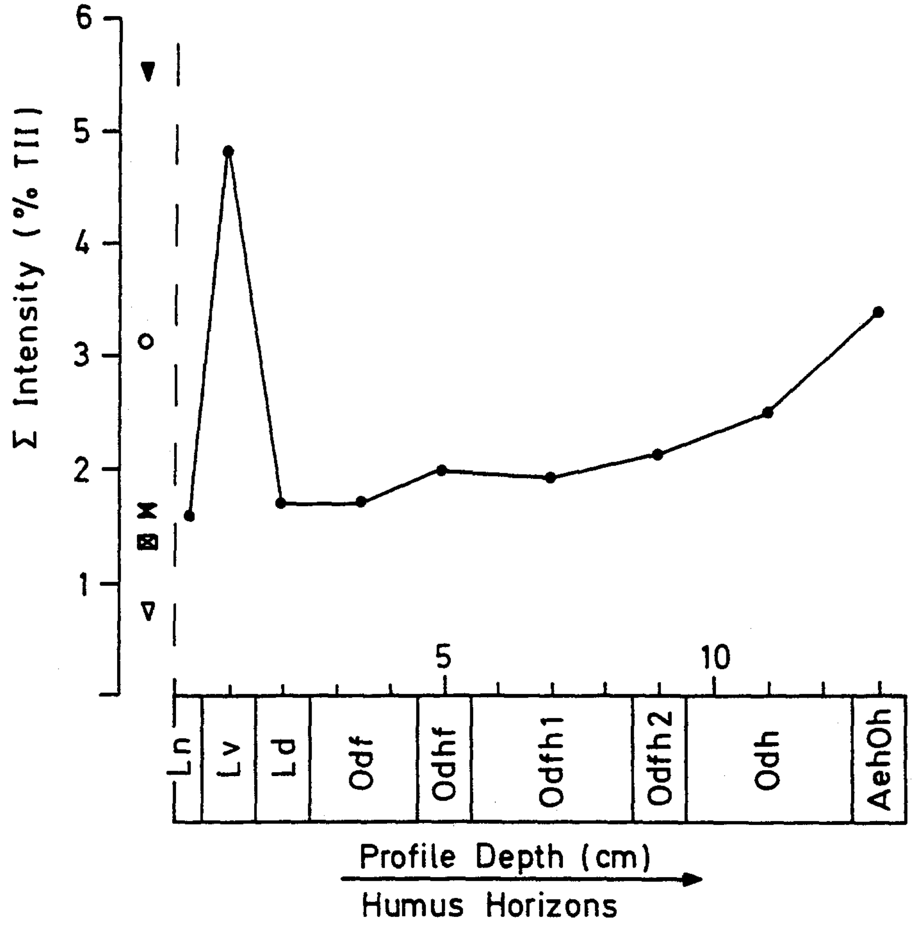

Fig. 11. Summed intensities of characteristic FI signals from litter materials and humus horizons of spruce ecosystems that relate to aryl-alkyl esters from suberin (m/z 474, 488, 502). Litter materials: twigs (▼), bark (○), degraded bark (⧗), roots (▣), and freshly fallen spruce needles (▽) for comparison.

Aliphatic constituents

The major component of the epicuticular wax layer of spruce needles is 10-nonacosanol. Yields of this lipid compound from the various samples are indicated by the summed intensities of the two typical FI fragment ions of this molecule at m/z 157 and 297 in Fig. 12. During the first 48 months of maturation, 10-nonacosanol constanly decreases. In litter materials, 10-nonacosanol is only abundant in spruce needles and cones. The humus layer is characterized by a high value in the Ld horizon, a sharply decreased value from Ld to Odf, and moderately declining values in the lower horizons.

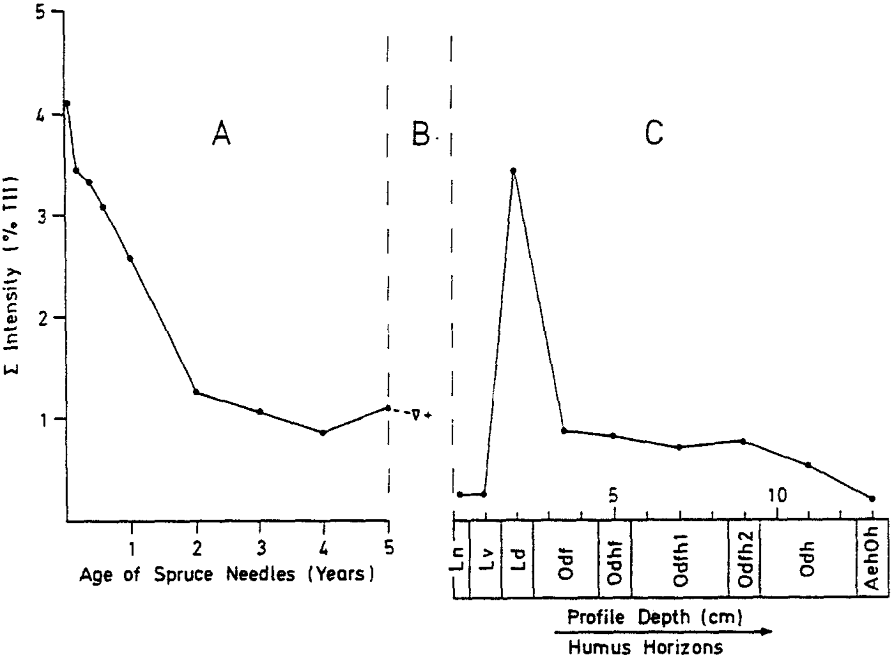

Fig. 12. Summed intensities of characteristic FI signals from needles (*A*), litter materials (*B*), and humus horizons (*C*) of spruce ecosystems that relate to mass spectrometric fragment ions of 10-nonacosanol (m/z 157, 197). Details for Fig. 12 A, C are given in Fig. 8. Litter materials in Fig. 12B, freshly fallen spruce needles (▽), cones (+).

The molecular ion of β-sitosterol at m/z 414 is suitable as an indicator for the chemodynamics of plant phytosterols. The changes in relative intensity of this signal (not shown) indicate a rapid increase of β-sitosterol in the young needles within the first 5 months, and a further increase from 7 to 60 months. During senescence the content of β-sitosterol is comparatively very high in leaves of *V. myrtillus*, high in bark, medium in twigs, roots, and cones and low in *D. flexuosa*, degraded bark, and wood. As found for 10-nonacosanol, the β-sitosterol content also increases during litter decomposition from Ln to Ld, and then decreases to AehOh.

Intense signals typical for alkenes are found at m/z 322, 336, and 350 ($C_{23}H_{46}$, $C_{24}H_{48}$, $C_{25}H_{50}$). These alkenes represent dehydration products from free alcohols or thermal degradation products from esterified alcohols and aliphatic polymers (van Smeerdijk & Boon 1987). Summed intensities of these signals from the Py-FI mass spectra (Fig. 13) are more intense in spectra of samples that represent advanced stages of needle maturation and senescence. The yield for senescent needles is comparable to that of 5 year old needles. High yields for these alkenes were obtained

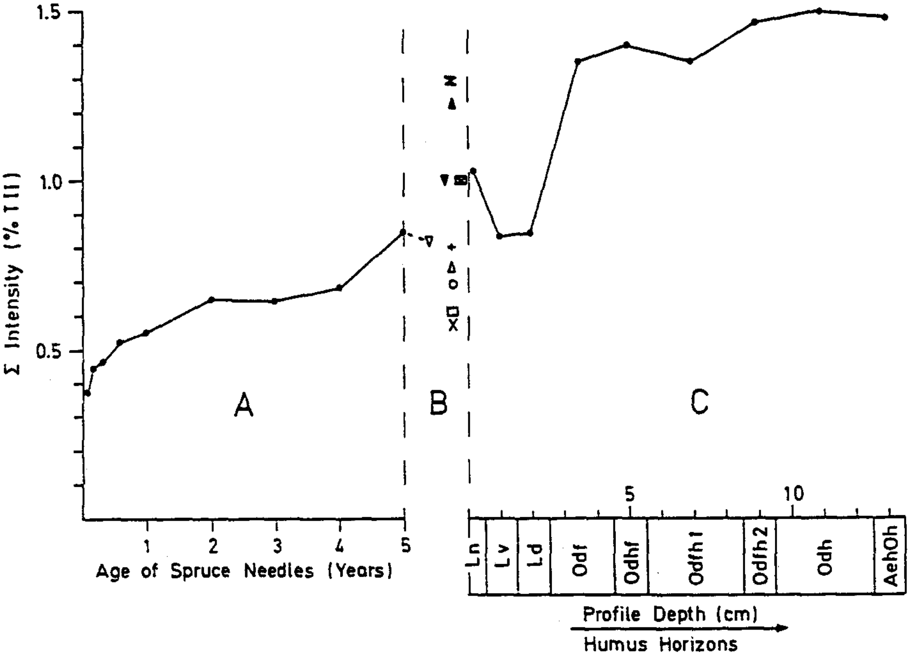

Fig. 13. Summed intensities of characteristic FI signals from needles (*A*), litter materials (*B*), and humus horizons (*C*) of spruce ecosystems that relate to alkenes (m/z 322, 336, 350). Details for Fig. 13 A, C are given in Fig. 8. Litter materials in Fig. 13B: freshly fallen spruce needles (▽), twigs (▼), cones (+), wood (△), degraded wood (▲), bark (○), degraded bark (⧗), *D. flexuosa* (□), *V. myrtillus* leaves (×), roots (▩).

from degraded bark and degraded wood, medium yields for twigs, roots, cones, and low yields from fresh wood and bark, *D. flexuosa*, and *V. myrtillus* leaves. During litter decomposition and humification, the relative pyrolysis yield of alkenes decreases from Ln to Lv, increases sharply from Ld to Odf, and then tends to increase from Odf to Odh.

Carbohydrates

For carbohydrates numerous pyrolysis products have been reported (e.g. Pouwels et al. 1989). However, for investigation of the chemodynamics of carbohydrates in forest ecosystems by Py-FIMS only selected signals in the mass range m/z 112 to 144 are suitable. The lower mass range signals with m/z values characteristic of carbohydrates can also be formed during pyrolysis of other classes of compounds (Hempfling 1988; Bracewell et al. 1989). To describe the variations of carbohydrates during maturation, senescence and humification, and their abundance in various litter materials, summed intensities of the mass signals at m/z 112, 114, 126, 128, 132, and 144 are presented in Fig. 14. These mass signals represent furanes, pyranones, levoglucosenon, anhydropentoses, and anhydrohexoses as typical pyrolysis products of carbohydrates. During the first five months of needle maturation the amount of carbohydrate content increases and then decreases up to the 5 year old needles. During senescence carbohydrate content further decreases (dashed line). In comparison to freshly fallen spruce needles and the Ln horizon, other litter materials are characterized by a very high carbohydrate content in spruce cones, high contents in *V. myrtillus* leaves and fresh wood, medium contents in roots, *D. flexuosa*, fungi mycelia, and twigs, and low contents in degraded wood. During litter decomposition and humification the carbohydrate content decreases rapidly from Ln to Odf, increases from Odf to Odh and decreases to AehOh horizon.

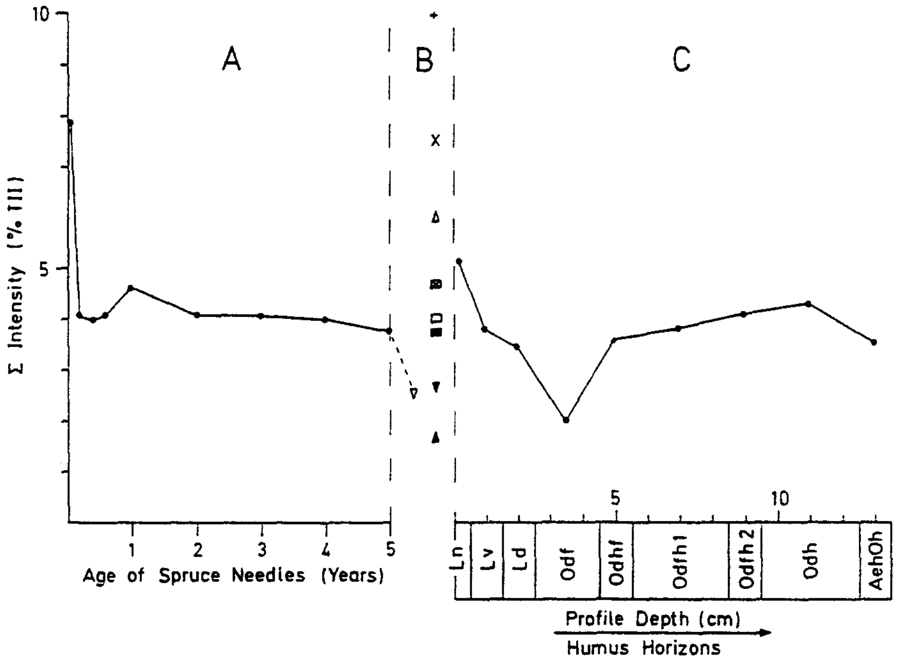

Fig. 14. Summed intensities of characteristic FI signals from needles (*A*), litter materials (*B*), and humus horizons (*C*) of spruce ecosystems that relate to carbohydrates (m/z 112, 114, 126, 128, 132, 144). Details for Fig. 14A, C are given in Fig. 8. Litter materials in Fig. 14B: freshly fallen spruce needles (▽), twigs (▼), cones (+), wood (△), degraded wood (▲), *D. Flexuosa* (□), *V. myrtillus* leaves (X), roots (▨), fungi mycelia (■),

Discussion

Maturation and senescence of spruce needles

The maturation and senescence of spruce needles is characterized by significant age-specific trends that result from enhanced or reduced synthesis of distinct plant constituents, structural rearrangements, and retranslocations of individual needle constituents. In addition, leaching may become an important factor during senescence. Major changes indicated by a decrease of carbohydrates and a reduced yield of monomeric pyrolysis products from lignin occur within the first three months. This indicates that the major processes of cell wall development have already been completed during the early stages of needle maturation. The fact that the decrease of lignin monomers corresponds with an increase of dimers and trimers within the first 5 months demonstrates that rearrangements within the lignin macromolecules are mainly condensation reactions, or that an enhanced synthesis of condensed lignin subunits is favoured in 3 to 5 month-old needles. This tendency for enhanced condensation of lignin with increasing age continues up to 5-year-old needles as shown by the relative pyrolsis yields of trimeric lignin subunits. The constant ratio of coniferyl aldehyde to coniferyl alcohol for 1 month to 5-year-old spruce needles shows that almost no lignin oxidation occurs during maturation.

Further significant chemical changes during maturation and senescence occur in the epicuticular wax layer that mainly consists of 10-nonacosanol. During maturation a relative decrease of this compound is observed, perhaps due to crosslinking of wax constituents to high-molecular weight compounds (Schulten et al. 1986). In contrast, phytosterols such as β-sitosterol are accumulated during the first 5 months of needle maturation. The decrease of β-sitosterol from 5 to 7 months indicates that β-sitosterol synthesis did not cease but was reduced relative to production of other constituents. Similar contents of β-sitosterol in 1 to 4 years old needles suggest that β-sitosterol production continued as the tissue aged, because needles increased in mass with age. The higher proportion of β-sitosterol in the 5-year-old needles probably reflects the retranslocation of other components from the tissue as indicated by the decreasing yields of pyrolysis products from carbohydrates from senescent material. In contrast to a decreasing contribution of non-polymeric wax materials (e.g. 10-nonacosanol) to needle composition during maturation and senescence, the contribution of chemical subunits yielding alkenes as pyrolysis products constantly increases. Therefore, not free alcohols but esterified alcohols and/or aliphatic polymers are proposed as major origin for these alkenes. One possibility would be the macromolecular network of cutin that is synthesized during maturation and relatively enriched during senescence.

Litter quality

The various litter materials can be distinguished chemically by contents of the organic constituents. The specific composition makes it possible to estimate the contribution of individual litter inputs to the humus layers and to evaluate their impacts on humus quality. There is, for example, a high content of aryl-alkyl esters from suberin in spruce twigs and bark, and of carbohydrates as well as of β-sitosterol in *V. myrtillus* leaves. High contents of lignin are typical for spruce cones, twigs, and wood. The epicuticular wax constituent 10-nonacosanol is only abundant in spruce needles and cones.

Plants growing on unfavorable sites are often observed to have relatively high contents of lignin in green foliage and litter. The lignin content of litter is an important indicator of litter quality, because the lignin component of plant appears to regulate litter decomposition (Horner et al. 1987). For the mor profile, a high lignin and suberin input by twigs, bark and cones slows decomposition due to the accumulation of compounds poor in energy. In earlier stages of decomposition, specific pathways of degradation in the interior of the needle lead to a rapid decrease of compounds rich in energy resulting in the enrichment of compounds poor in energy.

Humus genesis

Chemical changes that occur within humus profiles may be due to decomposition, selective preservation and polymerization reactions of individual compounds, as well as from amendments and accumulations of root material and microbial biomass. For any individual humus constituent, a constant content throughout the profile would indicate that the rates of accumulation and decomposition are similar to the rates of total carbon accumulation and decomposition. In this approach humification is considered only in relation to modification and polymerization. The importance of individual components and processes for the development of the mor profile investigated will be discussed in the following.

Based on area measurement of individual chemical shift ranges from CPMAS ^{13}C NMR spectroscopy of samples from this mor profile, Kögel-Knabner et al. (1988) observed a decrease of O-alkyl carbon and an increase of alkyl carbon and carboxyl groups as depth increases. The amount of aromatics remained constant and the general decrease of

O-alkyl carbon was interrupted by a secondary maximum in the Odh horizon. In their study the amount of organic carbon identified as plant or microbial-derived biopolymers by wet-chemical methods accounted for 65% of carbon in the litter layer and 34% of carbon in the mineral soil horizon.

The Py-FI mass spectra indicate that the carbohydrate content rapidly decreases from Ln to Odf, thus indicating that these humus constituents are preferably decomposed serving as energy sources for microorganisms. Aryl-alkyl esters from suberin and lignins are more recalcitrant against primary degradation and hence relatively enriched in the Lv horizon. The increase of wax constituents and phytosterols as indicated by increases in 10-nonacosanol and β-sitosterol from Ln to Ld is due to the typical decomposition pattern of spruce needles that starts from the interior and leads to an enrichment of hollow needles which consist mainly of epidermic and cuticular components. Spruce wax is a highly selective substrate for microbial colonisation that retards needle degradation (Günthhardt-Georg and Rossi 1987).

The content of 10-nonacosanol in partly degraded hollow needles is similar to that of very young needles ($<$ 1 year) on the tree. Because alkenes decrease from Ln to Lv, it is assumed that some alcohols and/or polymeric aliphatic material are partially decomposed.

The mechanical disintegration of the needles that occurs from the Ld to the Odf layer coincides with a rapid decrease of wax constituents and phytosterols. This may be due either to decomposition or polymerization, or both. With advanced decomposition the contents of these compounds further decrease. This decrease coincides with a significant increase of alkenes indicating that, in contrast to waxes, the degradation of esterified alcohols and/or polymeric aliphatic materials is reduced in the O layers resulting in a selective preservation of individual aliphatic subunits in humified plant remains.

The slight increase of the β-sitosterol content in the Odfh2 and Odh horizons may be due to microbial biomass that accumulates in the rhizosphere region. The decreasing trend for carbohydrates and monomer subunits from lignin is also interrupted in Oh-horizons due to the high input of root litter. Moreover, carbohydrate content increases from Odf to Odh. This corresponds with high contents of fine roots and root residues that are reported as typical morphological features of these horizons by Kögel-Knabner et al. (1988). In addition to root input, the increase of carbohydrate content may also be due to microbially synthesized carbohydrates. For hexose material, which is indicated by the signal intensities of m/z 126 and 144, there is an equilibrium of decomposition and root input from Odf to Odh. Pentose content as indicated by the intensities of m/z 114 and 132 increases from Odf to Odh (data not shown). This is mainly due to the fact that compared to needles and other litter materials, root material is characterized by a higher pentose content than hexose. Besides root input, the varying contributions of lignin dimers and trimers are also due to selective preservation of condensed building blocks during lignin decay (Hempfling et al. 1988).

The oxidative degradation of lignin is clearly demonstrated by the constantly increasing ratio of conifery aldehyde to coniferyl alcohol from Lv to Odh. The input and decay of root material also cause the increase of aryl-alkyl ester from suberin in the Odhf horizon. The constant increase of this material from Odfh1 to AehOh can be explained by both root input and selective preservation of suberin constituents. The influence of mineral soil constituents on the transport of water-soluble humus constituents from upper to lower parts of humus profile results in an increasing yield of monomeric pyrolysis products from lignin in the AehOh horizon. This is due to precipitation of partly altered, water soluble lignin subunits (Hempfling & Schulten 1990b), a fact that has been recently reported in subalpine Rendzinas by Zech et al. (1990). The low ratio of m/z 178/180 in the AehOh horizon (Fig. 10) indicates that the lignin material precipitated in the AehOh horizon is less oxidized than the lignin subunits in the corresponding Oh horizons.

Geoecological comparison of above and below ground processes

In order to ascertain similarities and dissimilarities during the maturation and senescence of foliage and during humus genesis, enrichment factors of individual compound classes and subunits were calculated. For this purpose, the values of the summed intensities of selected and characteristic mass signals and signal series related to specific compounds and compound classes for freshly fallen spruce needles were subdivided by the values calculated for spruce seedlings (factors for biochemical processes). In a similar way the values for the AehOh-horizon were subdivided by those of the Ln-horizon resulting in factors for biogeochemical processes. These values are summarized in Table 4. Values > 1 characterize the enrichment of specific compounds, whereas values < 1 result from a decreasing content of plant constituents during maturation/senescence and humus genesis.

For lignin clear differences in the chemodynamics of monomer, dimer and trimer subunits are seen. Similar increasing or decreasing trends for monomers and trimers are obtained for both the maturation/senescence of needles and during humus genesis. The factors for 10-nonacosanol and carbohydrates both indicate decreasing contents during maturation/senes-

Table 4. Enrichment factors for individual compounds and compound classes during maturation/senescence and humus genesis.

Compounds Compound classes	Enrichment factors during maturation/senescence	 humus genesis
Lignin		
monomers	0.4	0.6
dimers	1.2	0.8
trimers	2.2	1.2
10-nonacosanol	0.3	0.7
β-sitosterol	3.2	0.3
Alkenes	2.2	1.4
Carbohydrates	0.3	0.6

cence on the tree and during early stages of humus genesis on the forest floor. In contrast, an enrichment of alkenoic subunits is observed during biochemical and biogeochemical processes. The chemodynamic behaviour of β-sitosterol is characterized by an accumulation during maturation and senescence and a strong decrease during humus genesis. This may be due to the importance of sterols for the regulation of plant growth and their function as antioxidants.

In general, biochemical and biogeochemical processes modifying major plant constituents result in similar chemodynamics on the tree and on the forest floor. During needle maturation and senescence, the driving forces for biochemical processes are the conversion of storage compounds to building blocks of plant tissues during maturation, and retranslocation of compounds rich in energy during senescence. During humus genesis the driving force is the reduction of organic matter rich in energy by microorganisms using this material as an energy source.

Conclusions

The fingerprinting approach gives an overview of the chemical composition of distinct environmental compartments and of chemical transformations during biochemical and biogeochemical processes. Therefore, it is possible to differentiate and classify various biomaterials of spruce ecosystems by pyrolysis mass spectra in combination with principal component analysis. The principal components calculated on the basis of thermal degradation products of the biomaterials contain relevant chemical information. This allows to subdivide maturation, senescence and humus genesis into individual processes such as amendment, decomposition, preservation and humification.

Enrichment of distinct plant constituents during maturation and sencescence is continued in the first stages of humus genesis (selective preservation). By contrast, plant constituents, which are retranslocated from senescent plant leaves, are also preferably decomposed in the first degradation steps. Hence, it is concluded that the first principal component of the spruce ecosystem investigated represents chemical changes that are either due to genetically controlled reactions or due to energetically regulated processes.

The second principal component which represents only a small portion of the total variance is caused by biogeochemical processes that lead to a non-regulated, chaotic transformation of biomaterials in the plant/soil-system (e.g. humification).

The changes in intensity of mass signal sets characteristic of individual compounds and compound classes can be used to follow the details of chemical variations caused by individual processes, to separate several coupled processes, and to compare them. In addition, knowledge of the chemical composition of individual litter inputs permits estimation of their contribution to the various humus horizons and their impacts on humus quality.

For different compound classes, a number of similar effects of biochemical and biogeochemical processes have been demonstrated indicating the existence in these spruce ecosystems of an underlying dependence on energy transfer. This energetic regulation affects numerous different processes including biosynthesis, transformation, and retranslocation on the tree, as well as decomposition, selective preservation, and humification on the forest floor. For example the decrease of compounds rich in energy such as carbohydrates can be observed during senescence and humus genesis, but this effect is due to retranslocation on the tree and to decomposition on the forest floor.

The preferential decomposition of compounds rich in energy on the forest floor leads to the accumulation of aliphatics that yield alkenes as pyrolysis products and of suberin constituents within the mor profile. The high lignin and suberin content of spruce litter and the accumulation of highly aliphatic humified plant remains on the forest floor may be reasons for the retardation of energy transfer and nutrients cycling that occurs in mor profiles.

Acknowledgement

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