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Decomposition dynamics of six salt marsh halophytes as determined by cupric oxide oxidation and direct temperature-resolved mass spectrometry

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Abstract

This paper presents the results of a comparative study on the aerobic decomposition of six salt marsh plant species over a period of 2 yr. In addition to ash-free dry weight (AFDW) determination and elemental analysis (C and N), two analytic methods have been applied to obtain insight into the decomposition dynamics of lignin in the various plant tissues. The analytic methods are (1) cupric oxide (CuO) oxidation followed by gas chromatography–mass spectrometry (GC-MS) and (2) direct temperature-resolved mass spectrometry (DT-MS).

AFDW losses could generally be well described by double exponential relations with time. Carbon-to-nitrogen ratios increased during the initial stages of decomposition and decreased again afterward. For five of the six plant species, a correlation between initial lignin content and AFDW loss was observed. Decay dynamics of lignin denoted a rapid relative increase during the first weeks of field exposure, followed by stabilizing contents over the next 2 yr. CuO oxidation data indicate the establishment of a stable "lignin endmember" within 1–2 months. DT-MS data, on the contrary, showed continuous modification of the lignin polymer throughout the duration of the experiment.

Evidence was found for the incorporation of (presumably) microbial N-acetylglucosamine in the complex residue produced upon decomposition. Combination of CuO oxidation and DT-MS data suggested that lignin degradation products became attached to the original macromolecular material and could still be identified as lignin-derived material. The data support a humification mechanism via condensation of small degradation products instead of the selective preservation of certain biomacromolecules (like lignin).

Lignin, a structural component of the cell walls of vascular plants and the second most abundant naturally occurring polymer in the biosphere (after cellulose), has a high preservation potential (Hedges and Mann 1979*a*; Swift et al. 1979; Kirk and Shimida 1985). Its decomposition is often regarded as the rate-limiting step in the biospheric cycle of carbon (Colberg 1988). Notwithstanding its relatively high recalcitrance, lignin does not behave conservatively during decay of plant litter. Indeed, numerous reports exist on lignin degradation (with partial or complete mineralization) by both aerobic and anaerobic decomposers. White-rot fungi are the best known representatives of the first group (Amer and Drew 1980; Chen and Chang 1985; Higuchi 1985*a,b*; Kirk and Shimida 1985; Harvey et al. 1987; Hedges et al. 1988*a*; Lewis and Yamamoto 1990; Goñi et al. 1993; Gamble et al. 1994), whereas the second group is represented by bacteria (Benner et al. 1984*a,b*, 1991; Young and Frazer 1987). Owing to modification of the macromolecular lignin structure during decay, the lignin moiety of fresh plant material may be an unsuitable tracer of vascular plant remnants in the environment.

Here we present the results of a litterbag study on the decay dynamics of six different salt marsh plant species during 2 yr of aerobic decomposition. The main objectives for this study were (1) to find out whether the lignin moiety of halophytic herbaceous plants shows high resistance to bio-

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degradation relative to bulk organic matter and (2) if so, to establish a chemically defined endmember of biodegraded lignin suitable to serve as a tracer for halophytic material exported from salt marshes into the marine environment.

Throughout the decomposition period, remaining ash-free dry weights (AFDWs) have been determined, as well as organic carbon and nitrogen contents. Two different analytic methods were applied to monitor chemical modification of the decomposing litter. The first method, oxidation of lignin with cupric oxide (CuO) under alkaline conditions, yields distinct classes of monomeric and dimeric products that can be quantitatively analyzed by means of gas chromatography (Hedges and Mann 1979*a*; Hedges and Ertel 1982; Goñi and Hedges 1992). Ratios between those different product classes are used as indicative parameters for the diagenetic stage of the material. CuO oxidation has often been applied to study the dynamics of lignin decay (Haddad and Martens 1987; Hedges et al. 1988*a*; Benner et al. 1991; Haddad et al. 1992; Goñi et al. 1993; Opsahl and Benner 1993, 1995; Louchouarn et al. 1998) and to characterize and quantify the inputs of terrigenous matter to diverse coastal and deep-sea marine environments (Hedges and Mann 1979*b*; Moran et al. 1991*a,b*; Goñi and Hedges 1992; Gough et al. 1993; Prahl et al. 1994; Louchouarn et al. 1998). The second method used is direct temperature-resolved mass spectrometry (DT-MS), in which polymeric material is thermally dissociated into fragments that are subsequently analyzed by means of mass spectrometry (Boon 1992). DT-MS has often been used for the analysis of lignin and lignified (fossil) plant material (Genuit et al. 1987; Saiz-Jimenez et al. 1987; Stout et al. 1988; Boon 1989; Scheijen and Boon 1989; Stout et al. 1989; Faix et al. 1990; Pouwels and Boon 1990; Ralph and Hatfield 1991; Van der Hage et al. 1993; Van der Heijden and Boon 1994).

Combination of these two methods provides the means to overcome some of the limitations of each individual method (Van Bergen et al. 1994). Oxidation with CuO is, for instance, more suitable for quantitative lignin determination, whereas structural details of the polymer can better be obtained with DT-MS (Van der Hage et al. 1993). In a separate paper on the degradation of *Spartina anglica* litter, it has been shown that purification of this material is required to obtain valuable analytic data on the lignin fraction (Klap et al. 1998). The purification method applied was a twofold enzymatic digestion (protease followed by polysaccharidase), yielding a residue called milled wood enzyme lignin (MWEL). All DT-MS results presented here concern samples that have undergone this purification procedure. CuO oxidation was conducted on material that was ball milled but otherwise unpurified, because it has been reported that major matrix-related effects do not occur on the yield of lignin-derived compounds upon CuO oxidation (Young and Frazer 1987; Goñi and Hedges 1995).

Experiment

Litterbag experiment—Aboveground parts of the six halophytic herbaceous plant species *S. anglica, Festuca rubra, Aster tripolium, Limonium vulgare, Artemisia maritima,* and *Suaeda maritima* were harvested in the first week of September 1992 in the Sint Annaland salt marsh (51°36′N, 4°07′E). This marsh is situated in the northeastern part of the Oosterschelde (The Netherlands), an arm of the North Sea. By the time of harvesting, the plants were in their earliest stage of senescence (first yellowing of the leaves). Different types of tissues were selected for each of the three major plant groups used in this study: for the grasses (*S. anglica* and *F. rubra*), the complete aboveground parts were selected; the nonwoody herbs (*A. tripolium* and *L. vulgare*) were exclusively represented by their leaf material; and the shrub-like herbs (*A. maritima* and *S. maritima*) were represented by small twigs, from which thicker branches were removed. *A. maritima* and *S. maritima* already carried seeds with diameters exceeding the mesh size of the bags. All plant parts (except those from *F. rubra*) were cut into small pieces ~8 cm long and dried with tissue paper; ~25 g of this material was put into nylon litterbags of 12 × 12 cm (1-mm mesh size) without any further treatment. The tissue was just paper dried before it was left in the field to avoid unnatural changes caused by extensive drying. The bags were sewn shut, weighed, and brought to the marsh the following day. They were attached with thin lines to long horizontal sticks within the canopy of the vegetation, at a distance of ~15 cm above the marsh surface. The location was near the boundary between marsh and intertidal flat. The bags were submerged for short times (<1 h) during spring tide periods. Samples were collected 6, 16, 31, 56, 196, 259, 386, and 766 d after the start of the experiment.

At the start of the experiment, the weight ratio between fresh and freeze-dried material was determined for five independent samples of each species. This ratio was used to calculate the dry weights at $t = 0$ of the samples that were left in the field. This indirect calculation of the starting weight was a consequence of our choice to fill the litterbags with undried starting material. Subsequently, the ash contents of three subsamples of each of these freeze-dried samples were determined by combustion in a muffle furnace at 570°C for 3 h. The AFDWs of the samples at $t = 0$ were thus based on a total of 15 analyses.

At each collection date, three bags per species were taken from the field. Macromaterial adhering to the bags, for instance macroalgae, was rarely noticed, but in the few occurring cases the material was removed. The bag contents were freeze dried, without previous rinsing, and weighed. The dry material was ground, and the ash content was determined in triplicate for each litterbag. The low amounts of remaining *A. tripolium* material after some weeks did not allow the determination of the ash contents in triplicate and after 1 yr not even in singular. Instead, the material was saved for chemical analyses. After gravimetric analysis, material from three bags was pooled and organic carbon and nitrogen contents were determined with a Carlo Erba NA 1500 C/N analyzer. The remaining material was powdered for 3 h (at 5°C) in a vibratory ball mill (Retsch MM2) and used for CuO and DT-MS analysis or for lignin preparation with subsequent DT-MS analysis.

Milled wood enzyme lignin preparation—MWEL preparation was conducted according to the procedure described

elsewhere (Klap et al. 1998). Briefly, 200 mg of the ball-milled material was percolated at room temperature with ethanol : benzene (2 : 1) and acetone : water (9 : 1) and extensively washed with Milli-Q water. This extractive-free material was incubated for 6 h with 0.07% (w/w) protease (Pronase E from *Streptomyces griseus,* EC 3.4.24.4, 4 U mg^{-1}, Merck) in a 50 mM Tris solution at pH 7.4 and washed with Milli-Q water afterward. Then it was incubated for 12 h with 0.5% (w/w) polysaccharidase (Cellulase Onozuka R-10 from *Trichoderma viride,* EC 3.2.1.4, >1.0 U mg^{-1}, Merck) in a 50 mM ammonium acetate solution at pH 4.5 and again extensively washed with Milli-Q water afterward. Both incubations were carried out in the dark at 37°C under continuous rotation of the test tubes. Finally, all samples were freeze dried to produce a residue ready for analysis.

Cupric oxide oxidation—Lignin-derived CuO oxidation products (LOP) were determined by the method developed by Hedges and coworkers (Hedges and Ertel 1982; Goñi and Hedges 1992), with slight modifications (Louchouarn et al. 1998). Briefly, 25–50 mg of grounded material was hydrolyzed for 3 h in a nitrogen-purged 2 M NaOH solution in the presence of CuO (~1 g) and $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (~100 mg) within a stainless steel minibomb. Four such reaction chambers were loaded, under an inert atmosphere (N_2), into a larger bomb (Parr) that contained additional base. This container was heated in an insulated heating sleeve, the heating rate and final temperature of which were controlled by a CN76000-series temperature controller (Omega Engineering). Heating from room temperature to a final internal temperature of 162°C was performed in 30 min; this final temperature (±2°C) was maintained for 2.5 h. Ethyl vanillin (3-ethoxy-4-hydroxybenzaldehyde; Aldrich Chemical) was employed as a recovery standard and was added directly (~20 μg) to the minibombs upon cooling. After oxidation, samples were acidified with HCl (6N) to pH 1, extracted with freshly distilled diethyl ether, concentrated by rotoevaporation, and dried under N_2. Dried extracts were then stored frozen until analysis.

Gas chromatography—Gas chromatographic analysis of silylated oxidation products was carried out with a Hewlett-Packard 5890 GC fitted with a fused capillary column (DB-1, 30 m by 0.25 mm i.d.; J & W Scientific) attached to a flame ionization detector (FID). The injector and detector were maintained at 300°C, and split injections (~1/30 to 1/40) were performed with helium as carrier gas. The oven temperature was programmed to rise from 100°C to 270°C at a rate of 4°C min^{-1} and was kept isothermal for 15–20 min. Retention time and quantity of all eight major monomeric LOP studied here (V, the sum of monomeric guaiacyl structures; S, the sum of syringyl structures; and C, the sum of p-hydroxycinnamic acids; *see below*) were determined from their FID responses. Identification of all lignin-derived monomers and dimers was performed by GC-MS, and LOP response factors were assigned equal to the recovery standard, ethyl vanillin (Goñi and Hedges 1992; Opsahl and Benner 1995).

Direct temperature-resolved mass spectrometry—A double focusing (B/E geometry) JEOL SX-102 mass spectrometer was used for the analysis of ~5 μg of material. Drops of suspended sample material were deposited on a Pt/Rh wire and dried under vacuum. During the analysis period of 90 s, the mass spectrometer scanned a mass range between m/z 20 and m/z 1,000 each second at a resolution of 3,000. Source temperature was 180°C. Filament current for pyrolysis was programmed from 0 to 1.5 A in 90 s, inducing final temperatures of 800°C. Both electron impact (EI, 16 eV) and chemical ionization (CI, NH_3, 200 eV) were employed. Source pressure for EI was 10^{-4} Pa, whereas for CI a pressure of 20 Pa (NH_3) was maintained. Data acquisition was performed on a JEOL MP-7000 system. The set of integrated spectra of the total ion current (TIC) intervals between 30 and 60 s was used for factor analysis by the FOMpyrMAP program, a modified version of the Arthur multivariate analysis program (Infometrix) (Hoogerbrugge et al. 1983; Windig et al. 1983). Samples were analyzed in singular except for *A. maritima* under EI conditions, on which triplicate analysis was performed, thus allowing discriminant analysis.

Nomenclature—Some differences in terminology exist between the two fields of lignin research, which are united in this project. In this paper the terminology of the pyrolysis field will be applied, so the terms ''guaiacyl'' and ''p-hydroxycinnamic acid'' are used rather than ''vanillyl'' and ''cinnamyl phenol.''

Results and discussion

Gravimetric analysis—AFDWs of the different plant species are shown in Fig. 1. As outlined in the Experiment section, the initial dry weights of material left in the field were not directly measured but were calculated on the basis of measured fresh weight : dry weight ratios (fivefold) at $t = 0$. As a consequence, the weight percentages at $t = 0$ bear a standard deviation around 100%. Decomposition rates for litter can usually be well described by a double exponential equation, suggesting that litter can roughly be regarded as being composed of two fractions with distinct decomposition rates (Wieder and Lang 1982). It is evident from Fig. 1 that in four out of six cases, the double exponential model curve fits nicely indeed to the weight-loss data. The calculated decomposition rates and the relative sizes of the two fractions, as well as the squared correlation coefficients of the fittings, are shown in Table 1.

S. anglica and *A. maritima* show similar decomposition curves, defined by equally large fractions of labile and refractory material. For both species, ~35% of the initial weights remain after 2 yr of field decay. A very good fit is achieved for *F. rubra,* characterized by relatively small differences in decomposition rates between the labile and refractory fraction. The fact that no AFDWs were determined for this species after 200 d of exposure has undoubtedly improved the fit of the curve ($r^2 = 0.98$). Actually, reliable determination of the size and the decomposition rate of the refractory fraction requires data points at prolonged exposure times. Therefore, for *F. rubra,* the calculated values for

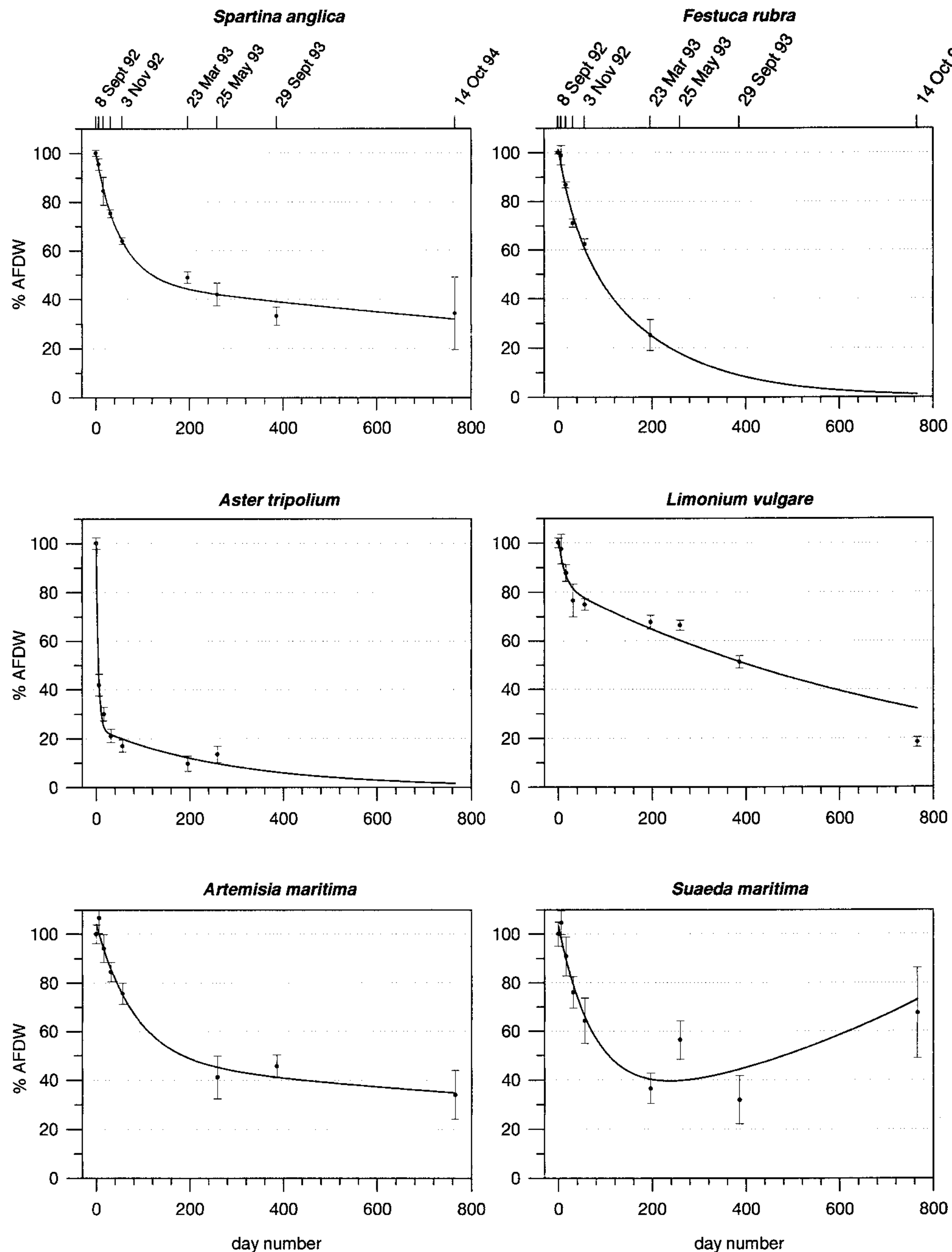

Fig. 1. Ash-free dry weight losses for the six plant species over a period of 766 d. The standard deviations at $t = 0$ are due to the experimental set up (*see text*). The curves represent the best fitting double exponential models ($X = Ae^{-k_1 dt} + (1 - A)e^{-k_2 dt}$). Values for A, k_1, and k_2 are given in Table 1.

the respective fractions and their decomposition rates should be considered as indicative only. The weight loss of *A. tripolium* material occurs so rapidly that after 1 yr not enough material was left for ash content determination. The remarkable shift in weight loss after ~20 d suggests a very labile fraction comprising ~75% of the organic material of *A. tripolium.* The uncertainty about the fraction sizes and decomposition rates as mentioned above for *F. rubra* is smaller for *A. tripolium,* although there are also no data after prolonged exposure times available for this species. The clear kink in the curve indicates that modeling the weight loss of this species requires at least a double exponential function. The good fit suggests that the material consists of two fractions, one of which is extremely labile.

A peculiar decomposition curve is calculated for *S. maritima,* indicating a shift from weight loss to weight increase after 237 d. The outcome of the model calculation does not indicate two fractions of different stabilities, but rather one

Table 1. Calculated parameters for achieving the best fit of a double exponential function ($X = Ae^{-k_1dt} + (1 - A)\, e^{-k_2dt}$) to the measured weight-loss values. The accompanying curves are plotted in Fig. 1.

Species	k_1 (10^{-4} d^{-1})	A (Organic matter)	k_2 (10^{-4} d^{-1})	1-A (Organic matter)	r^2
Spartina	194	0.521	5.3	0.479	0.96
Festuca	260	0.276	54.9	0.739	0.98
Aster	2280	0.756	35.2	0.242	0.99
Limonium	749	0.186	12.4	0.829	0.93
Artemisia	122	0.566	4.1	0.476	0.96
Suaeda	127	0.773	−13.5	0.260	0.86

decomposing fraction (A) and a produced fraction (1-A). This does not seem to be a realistic situation, although a slight mass increase might occur because of colonization by decomposers. The magnitude of the apparent increase, however, makes colonization an unlikely primary cause in this case. More probably the heterogeneous composition of the litterbag contents (characterized by relatively high contents of seeds and woody parts with deviating decomposition rates) has incidentally led to the peculiar weight-change pattern.

The other species for which the fit is not satisfactory is *L. vulgare,* which shows unexpected high weight losses for the samples collected after 386 and 766 d. The 766-d sample is based on one bag only, because the other bags were lost or torn by that time (the standard deviation thus equals that of $t = 0$). The unexpected high weight loss during the later stages of the experiment is explained by the fact that *L. vulgare* leaves lose their robust structure and become brittle after some time. Particles smaller than the mesh size of the litterbag are lost, which wrongly suggests chemical rather than physical decomposition. This is an intrinsic disadvantage of the litterbag method and leads to an overestimation of the chemical decomposition rate. In general, leaf tissue is more susceptible to comminution than other tissues. For the set of plant tissues used in this study, the process only seems to be of concern for the leaves of *L. vulgare.*

Elemental analysis—The organic carbon and nitrogen contents of both the untreated and MWEL samples are given in Table 2. The carbon values do not show a clear correlation with exposure time, but the nitrogen contents tend to increase with increasing exposure times, which is in accordance with literature data (Buth and Voesenek 1987; Hemminga et al. 1988; Benner et al. 1991). It is noteworthy that, with the exception of *L. vulgare,* nitrogen contents are far more influenced by the enzymatic purification than carbon contents. In Fig. 2, the atomic C : N ratios for the untreated and purified material are shown. It can be seen that the ranges for the two types of samples differ considerably. The enzymatic treatment (including a protease digestion) clearly removes relatively more nitrogen than carbon, yielding higher C : N ratios than for the untreated samples. Especially for the MWEL samples of the two grass species, the C : N ratios reach high values of more than 100. Even higher values have been reported for *Spartina alterniflora* tissue that had been extracted with neutral detergent (Buchsbaum et al. 1991). In general, C : N patterns for salt marsh macrophytes are characterized by increasing values during the leaching stage, which steadily decrease (to below the initial value) during the later stages of decomposition (Buth and De Wolf 1985; Benner et al. 1991; Buchsbaum et al. 1991). In this study, the C : N patterns of the MWEL preparations show better resemblance to this general pattern than the untreated material. An exception to this rule is *L. vulgare,* the C : N ratio of the MWEL of which never reaches a lower value than at $t = 0$.

Oxidation products—In addition to the carbon and nitrogen contents, the measured concentrations of lignin oxidation products (LOP) and calculated parameters are listed in Table 2. p-Hydroxyphenyl structures, which are produced

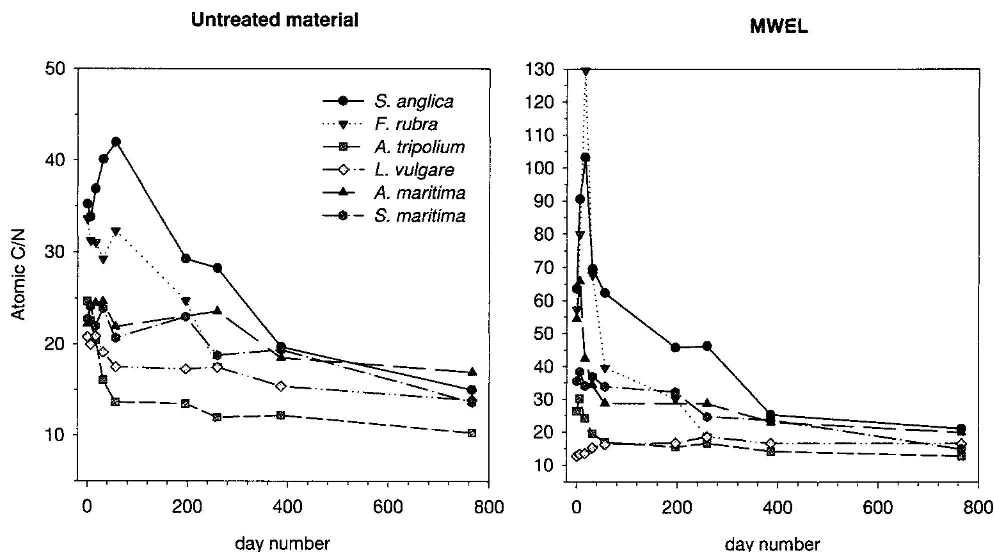

Fig. 2. Atomic C : N ratios for unpurified and MWEL samples.

upon alkaline oxidation of lignin (Sarkanen and Ludwig 1971; Hedges and Mann 1979*a*), are not included in this list because nonlignin sources such as marine plankton and especially bacteria have been shown to be significant sources of these compounds (Hedges et al. 1988*b*; Goñi and Hedges 1995). Moreover, GC-MS analyses of these compounds indicate that coelution with other compounds occurs, which hampers reliable quantification (Haddad and Martens 1987).

In Fig. 3, the lignin dynamics for the different plant species are expressed by $\lambda6$, the normalized sum (mg per 100 mg OC) of the main three guaiacyl and three syringyl structures produced upon oxidation. This sum-parameter has been proposed as a more reliable indicator of total lignin in nonwoody and herbaceous plant tissues than the traditional $\lambda8$, in which the p-hydroxycinnamic acids are included (Opsahl and Benner 1995). Those p-hydroxycinnamic acids (p-coumaric and ferulic acid) are considered to act as linkages between lignin and hemicellulose in nonwoody and particularly in gramineous tissues (Hedges and Mann 1979*a*; Hartley and Haverkamp 1984; Aiken et al. 1985; Jung and Ralph 1990) and therefore are not an integral part of the lignin polymer. Hence, $\lambda6$ is used to represent lignin in this set of grassy and herbaceous tissues. Figure 3 indicates that the six plant species can be roughly divided into three groups: lignin-rich *S. anglica, A. maritima,* and *S. maritima;* lignin-poor *A. tripolium* and *L. vulgare;* and *F. rubra,* which is characterized by an intermediate lignin content. A considerable increase in $\lambda6$ values is found for all species during the first month of the experiment, covering the leaching stage. After this period, most $\lambda6$ values vary somewhat around a constant value until the end of the experiment.

The previous broad classification is confirmed by the values of additional lignin-derived monomers and dimers (Table 2). Ratios between additional monomers and major monomers (Mono : $\lambda6$; Table 2) remain rather constant throughout the degradation period at approximately 0.10 for all six species, suggesting equal stabilities for both groups of monomers. The CuO oxidation procedure cleaves ether bonds in the lignin macromolecule efficiently, whereas many of the carbon–carbon bonds remain intact under these conditions (Chang and Allan 1971). Hence, this method yields a suite of lignin-derived dimers that retain their original carbon–carbon linkages. Furthermore, it is believed that a higher diagenetic stability of carbon–carbon bonds relative to ether bonds and/or active cross linking during degradation induces increasing yields of dimeric moieties relative to monomeric ones upon lignin decay (Goñi and Hedges 1992). To test for enrichment in lignin dimers relative to lignin monomers, their ratios (Dim : $\lambda6$; Table 2) are monitored. No increase in this ratio is found for any of the plant species, suggesting that carbon–carbon and ether bonds are cleaved at similar rates throughout the degradation process, an observation consistent with results obtained for *S. alterniflora* (Opsahl and Benner 1995). For all fresh tissues analyzed, the summed contribution of these additional monomers and dimers to the total pool of LOP is moderate at ~25% (w/w) of $\lambda6$.

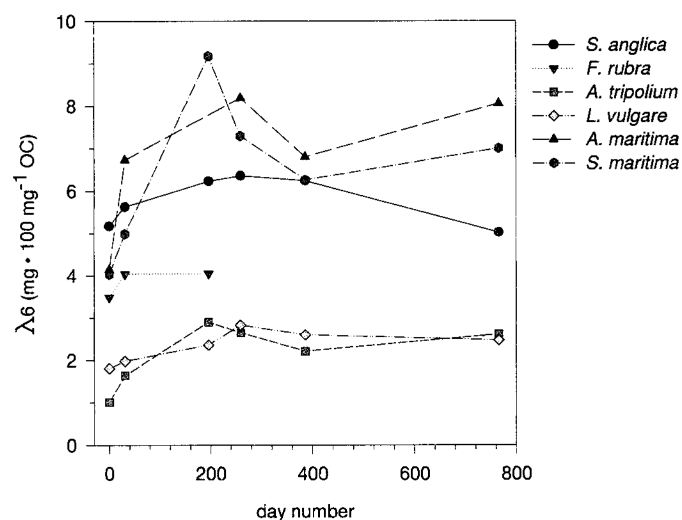

Fig. 3. Lignin dynamics during decomposition expressed as $\lambda6$ (summations of 3 guaiacyl and 3 syringyl oxidation products per 100 mg organic carbon) against time. Other lignin parameters are given in Table 2.

The normalized lignin contents do not provide a clear view on the remaining quantities in the litterbags during the decomposition experiment. In Fig. 4, the remaining percentages of the summed lignin oxidation products ($\lambda6$ + monomeric + dimeric products = ΛLig) are presented together with the remaining percentages of dry weight (DW), organic carbon (OC), and total nitrogen (TN). For most species, the loss of lignin during the initial stage of the field exposure is retarded compared to DW, OC, and TN. In some cases the amount of remaining lignin even exceeds the initial amounts, leading to percentages above 100. Especially for *A. maritima* and *S. maritima,* the immediate increase to values above 100% probably indicates an underestimation of the lignin content at t = 0. This might be related to the initial presence of compounds other than lignin that suppress the yield of lignin oxidation products. If these compounds are leached very rapidly, they will not influence yields of lignin oxidation products after t = 0. This explanation, however, is not in line with the findings of Young and Frazer (1987), who reported minimal influence of the matrix on CuO oxidation product yields. After the initial leaching phase, the loss of lignin does not grossly deviate from DW, OC, and TN, so that lignin then basically behaves like bulk organic matter.

During the experiment, the ratio between p-hydroxycinnamic acids and guaiacyl structures (C : V) decreases conspicuously for all plant species except for *F. rubra* (Table 2), a trend that is consistent with the diagenetic evolution of this ratio reported in previous studies (Hedges and Weliky 1989; Benner et al. 1990, 1991; Haddad et al. 1992; Opsahl and Benner 1993, 1995). An interesting point to note is that this ratio stabilizes rather quickly (1–2 months) after the start of the degradation process. This phenomenon can be explained by the rapid loss of ester-bound p-hydroxycinnamic acids. It seems that after loss of these structures from the periphery of the lignin macromolecule, a C : V endmember is produced that resembles more closely the more cross-linked internal part of the lignin macromolecule. Indeed, previous degradation studies (Opsahl and Benner 1993, 1995) have shown that correcting the yields of both p-hydroxycinnamic and guaiacyl CuO reaction products by subtracting

Table 2. Carbon and nitrogen contents (based on dry weights) of untreated and purified material from litterbags as well as lignin oxidation products (yields expressed in milligrams per 100 mg of organic carbon) and parameters of untreated material. Abbreviations: V, the sum of monomeric guaiacyl structures; S, the sum of syringyl structures; C, p-hydroxycinnamic acids; Mono, additional monomers; Dim, dimers; $\lambda 6$, the sum of V and S; $\lambda 8$, the sum of V, S, and C; ΛLig, C, the sum of all truly lignin-derived oxidation products (sum of $\lambda 6$, Mono, and Dim); Mono : $\lambda 6$, the ratio of additional monomers over the sum of guaiacyl and syringyl phenols; Dim : $\lambda 6$, the ratio of lignin-derived dimers over the sum of guaiacyl and syringyl phenols; C : V and S : V represent, respectively, the ratios of p-hydroxycinnamic acid and syringyl structures over guaiacyl structures; (Ad:Al)v and (Ad:Al)s represent, respectively, the ratios of acid over aldehyde moieties in the guaiacyl and syringyl families.

Species	Days	%OC (Un-treated)	%N (Un-treated)	%OC (MW-EL)	%N (MW-EL)	V	S	$\lambda 6$	C	$\lambda 8$	Mono	Dim	ΛLig	Mono-$\lambda 6$	Dim/$\lambda 6$	C/V	S/V	$(Ad/Al)_v$	$(Ad/Al)_s$
Spartina	0	41.34	1.37	45.43	0.83	3.03	2.14	5.17	1.77	6.93	0.38	0.72	6.26	0.07	0.14	0.58	0.71	0.42	0.73
	6	39.41	1.36	44.76	0.58														
	16	38.23	1.21	49.94	0.56	3.32	2.45	5.77	1.96	7.73	0.52	0.88	7.17	0.09	0.15	0.59	0.74	0.33	0.74
	31	40.90	1.19	51.06	0.86	3.17	2.46	5.63	1.47	7.10	0.57	0.78	6.98	0.10	0.14	0.46	0.77	0.25	0.61
	56	41.37	1.15	46.24	0.87														
	196	39.63	1.58	41.91	1.07	3.41	2.81	6.23	1.46	7.68	0.70	1.68	8.60	0.11	0.27	0.43	0.82	0.23	0.62
	259	39.01	1.61	41.75	1.05	3.56	2.80	6.36	1.75	8.11	0.55	1.11	8.02	0.09	0.18	0.49	0.78	0.26	0.67
	386	40.69	2.41	38.44	1.77	3.39	2.85	6.24	1.41	7.64	0.68	0.94	7.86	0.11	0.15	0.42	0.84	0.25	0.70
	766	34.55	2.69	29.74	1.65	2.61	2.41	5.02	1.03	6.06	0.64	0.84	6.50	0.13	0.17	0.40	0.92	0.30	0.78
Festuca	0	43.5	1.51	44.54	0.91	2.15	1.34	3.49	0.86	4.35	0.29	0.75	4.52	0.08	0.21	0.40	0.62	0.42	0.42
	6	41.23	1.54	47.01	0.69	2.28	1.36	3.64	1.69	5.32	0.30	0.41	4.35	0.08	0.11	0.74	0.60	0.34	0.58
	16	40.69	1.53	46.16	0.42	2.55	1.72	4.27	1.98	6.25	0.41	0.54	5.22	0.10	0.13	0.78	0.67	0.38	0.70
	31	41.16	1.64	43.85	0.76	2.41	1.63	4.04	1.80	5.84	0.43	0.54	5.01	0.11	0.13	0.75	0.68	0.29	0.59
	56	39.05	1.41	41.68	1.23	1.95	1.36	3.31	1.01	4.32	0.33	0.53	4.17	0.10	0.16	0.52	0.70	0.24	0.45
	196	38.16	1.8	37.74	1.46	2.31	1.75	4.05	1.05	5.10	0.46	0.39	4.89	0.11	0.10	0.46	0.76	0.23	0.40
	259	34.4	2.29	32.33	2.02														
Aster	0	33.45	1.58	26.1	1.15	0.63	0.39	1.01	0.22	1.23			1.01	0.00	0.00	0.35	0.61	0.47	0.52
	6	31.67	1.64	38.88	1.5	0.79	0.48	1.27	0.25	1.52	0.12	0.19	1.58	0.10	0.15	0.32	0.61	0.64	0.68
	16	34.74	1.98	47.9	2.31	0.99	0.79	1.79	0.26	2.05	0.17	0.09	2.05	0.10	0.05	0.26	0.80	0.39	0.37
	31	40.54	2.95	45.54	2.72	1.01	0.63	1.64	0.16	1.81	0.18	0.14	1.96	0.11	0.08	0.16	0.62	0.31	0.39
	56	40.46	3.46	41.79	2.87	1.43	0.83	2.26	0.25	2.51	0.23	0.14	2.63	0.10	0.06	0.17	0.58	0.38	0.45
	196	38.87	3.37	40.15	3.02	1.96	0.94	2.90	0.27	3.17	0.47	0.46	3.83	0.16	0.16	0.14	0.48	0.34	0.41
	259	38.48	3.76	38	2.66	1.71	0.94	2.65	0.32	2.97	0.28	0.24	3.17	0.11	0.09	0.19	0.55	0.34	0.42
	386	37.55	3.6	34.16	2.78	1.51	0.70	2.21	0.18	2.39	0.28	0.17	2.67	0.13	0.08	0.12	0.47	0.31	0.37
	766	32.95	3.75	25.65	2.34	1.72	0.89	2.61	0.27	2.88	0.31	0.21	3.13	0.12	0.08	0.16	0.52	0.41	0.55
Limonium	0	39.4	2.21	36.08	3.3	1.01	0.80	1.81	1.03	2.85	0.16	0.21	2.18	0.09	0.12	1.02	0.80	0.64	0.57
	6	38.86	2.27	47.9	4.18	0.79	0.65	1.44	0.56	2.00	0.15	0.12	1.70	0.10	0.08	0.71	0.83	0.49	0.41
	16	39.85	2.23	50.11	4.31	1.20	1.05	2.25	1.04	3.29	0.25	0.32	2.82	0.11	0.14	0.86	0.87	0.47	0.46
	31	44.08	2.69	49.71	3.8	1.05	0.93	1.98	0.76	2.74	0.24	0.20	2.43	0.12	0.10	0.72	0.88	0.40	0.41
	56	44.67	2.98	45.91	3.29	1.33	1.17	2.50	0.95	3.45	0.27	0.24	3.01	0.11	0.09	0.71	0.88	0.37	0.43
	196	42.31	2.86	46.88	3.27	1.30	1.06	2.36	0.76	3.12	0.29	0.22	2.86	0.12	0.09	0.58	0.81	0.36	0.37
	259	42.05	2.81	42.35	2.65	1.50	1.33	2.83	1.12	3.95	0.30	0.48	3.61	0.11	0.17	0.74	0.88	0.40	0.44
	386	42.79	3.25	38.69	2.7	1.37	1.23	2.60	0.81	3.42	0.30	0.22	3.12	0.12	0.08	0.59	0.90	0.35	0.37
	766	35.96	3.03	38.48	2.71	1.40	1.08	2.47	1.14	3.61	0.33	0.44	3.24	0.13	0.18	0.82	0.77	0.54	0.60

Table 2. Continued.

Species	Days	%OC (Un-treated)	%N (Un-treated)	%OC (MW-EL)	%N (MW-EL)	V	S	λ6	C	λ8	Mono	Dim	ΛLig	Mono-λ6	Dim/λ6	C/V	S/V	(Ad/Al)$_v$	(Ad/Al)$_s$
Artemisia	0	42.3	2.22	53.93	1.16	2.05	2.10	4.14	0.56	4.70	0.36	0.46	4.96	0.09	0.11	0.27	1.03	0.35	0.43
	6	40.53	1.95	51.49	0.91	2.59	2.78	5.37	0.54	5.91	0.46	0.60	6.43	0.08	0.11	0.21	1.07	0.35	0.47
	16	41.33	1.97	52.75	1.45	3.01	3.38	6.39	0.41	6.80	0.54	0.70	7.62	0.08	0.11	0.14	1.12	0.33	0.41
	31	44.59	2.11	46.87	1.59	3.10	3.63	6.73	0.34	7.07	0.53	1.21	8.47	0.08	0.18	0.11	1.17	0.28	0.35
	56	42.81	2.28	48.5	1.96	2.94	3.40	6.34	0.35	6.69	0.49	1.05	7.88	0.08	0.17	0.12	1.16	0.24	0.30
	259	45.07	2.23	42.49	1.73	3.81	4.38	8.19	0.27	8.47	0.71	1.08	9.98	0.09	0.13	0.07	1.15	0.29	0.34
	386	42	2.65	42.52	2.15	3.38	3.42	6.80	0.31	7.11	0.65	0.87	8.32	0.10	0.13	0.09	1.01	0.29	0.35
	766	38.28	2.64	37.5	2.2	4.18	3.87	8.05	0.25	8.31	0.81	1.11	9.97	0.10	0.14	0.06	0.93	0.29	0.35
Suaeda	0	31.37	1.61	26.02	0.86	2.33	1.70	4.03	1.73	5.75	0.22	0.35	4.60	0.05	0.09	0.74	0.73	0.38	0.34
	6	30.62	1.48	43.26	1.32	2.33	2.47	4.80	1.44	6.24	0.29	0.31	5.40	0.06	0.07	0.62	1.06	0.31	0.29
	16	31.28	1.66	39.81	1.36	2.87	2.74	5.61	1.90	7.50	0.37	0.37	6.34	0.07	0.07	0.66	0.96	0.37	0.33
	31	35.42	1.73	40.49	1.28	2.19	2.80	4.99	0.73	5.72	0.30	0.38	5.68	0.06	0.08	0.33	1.28	0.29	0.23
	56	38.44	2.17	38.49	1.33	2.14	4.20	6.34	0.41	6.75	0.40	0.45	7.20	0.06	0.07	0.19	1.96	0.29	0.26
	196	39.21	1.99	40.87	1.48	2.65	6.53	9.17	0.30	9.47	0.58	0.56	10.30	0.06	0.06	0.11	2.46	0.25	0.25
	259	38.79	2.41	33.64	1.59	2.49	4.80	7.29	0.40	7.69	0.72	0.95	8.96	0.10	0.13	0.16	1.93	0.28	0.26
	386	38.88	2.34	34.6	1.7	2.22	4.04	6.26	0.34	6.60	0.48	0.57	7.31	0.08	0.09	0.15	1.82	0.25	0.24
	766	34	2.91	31.43	2.45	2.53	4.47	7.00	0.35	7.35	0.58	0.67	8.25	0.08	0.10	0.14	1.76	0.28	0.32

the alkali-extracted amounts of esterified acids produces close to constant C : V ratios in all plant tissues studied. In line with this explanation, it is observed that another common parameter, the ratio between syringyl and guaiacyl structures (S : V), is relatively invariant upon degradation (Table 2). Only *S. maritima* shows a clear increase during the first 200 d, whereas *S. anglica* and *F. rubra* show small, more or less consistent S : V changes throughout the whole duration of the experiment. The remaining three species show no trend in their S : V ratios, indicating comparable stabilities for guaiacyl and syringyl structures under those conditions.

Elevated acid over aldehyde ratios (Ad : Al) have been used as indicators for oxidative degradation of lignin (Hedges et al. 1988*a*; Goñi et al. 1993; Nelson et al. 1995). Diagenetic alteration of the side chains of lignin has always been associated with oxic decomposition processes; however, recent evidence has shown that these ratios can increase substantially during long-term anoxic (subaqueous) degradation of nonwoody plant material (Opsahl and Benner 1995). Thus, elevated Ad : Al ratios should be attributed to both oxic and long-term anoxic degradation. These ratios in the six plants studied are presented in Table 2. Contrary to the expected increases in acid over aldehyde ratios, the (Ad : Al)$_v$ ratios (vanillic acid over vanillin) decrease for all six plant species during the first year of degradation. In the second year, the ratio increases slightly for *S. anglica, A. tripolium,* and *L. vulgare.* Less evident trends can be extracted from the (Ad : Al)$_s$ ratios (syringic acid over syringaldehyde). Nonetheless, some evidence can be found for a general decrease during the first month followed by stabilization in the subsequent year and a slight increase during the last year of degradation. The observed reduction of Ad : Al ratios in the early stage of degradation might be explained by loss of ester-bound acidic components from the halophyte tissues, as has been observed previously for seagrasses (Opsahl and Benner 1993). Subsequent increase of Ad : Al ratios would then indicate common diagenetic alteration of lignin.

DT-MS analysis—The DT-MS results concern enzymatically purified lignin residues (MWEL). It was shown for *S. anglica* that untreated tissues yield less specific DT-MS spectra than their MWEL counterparts (Klap et al. 1998). This phenomenon was confirmed for the other five plant species. Therefore, only results on purified samples are presented. The specificity of the technique was also modified using different ionization methods, i.e., electron impact (EI) and chemical ionization (CI). EI is the more universal ionization technique with a broad analytic window, whereas CI has a narrower window with high ionizing efficiency for polysaccharides (Pouwels 1989). However, CI has also been successfully applied for the ionization of dehydrated monolignols (Van der Hage et al. 1995). More information on the technique is given elsewhere (Boon 1992; Klap 1997).

In general, DT-MS generates complex spectra, since no molecular separation is conducted prior to analysis. Moreover, usually the spectra of a defined time (~temperature) window are integrated to provide one spectrum for each sample. In order to highlight compositional variation between individual samples, multivariate factor analysis or dis-

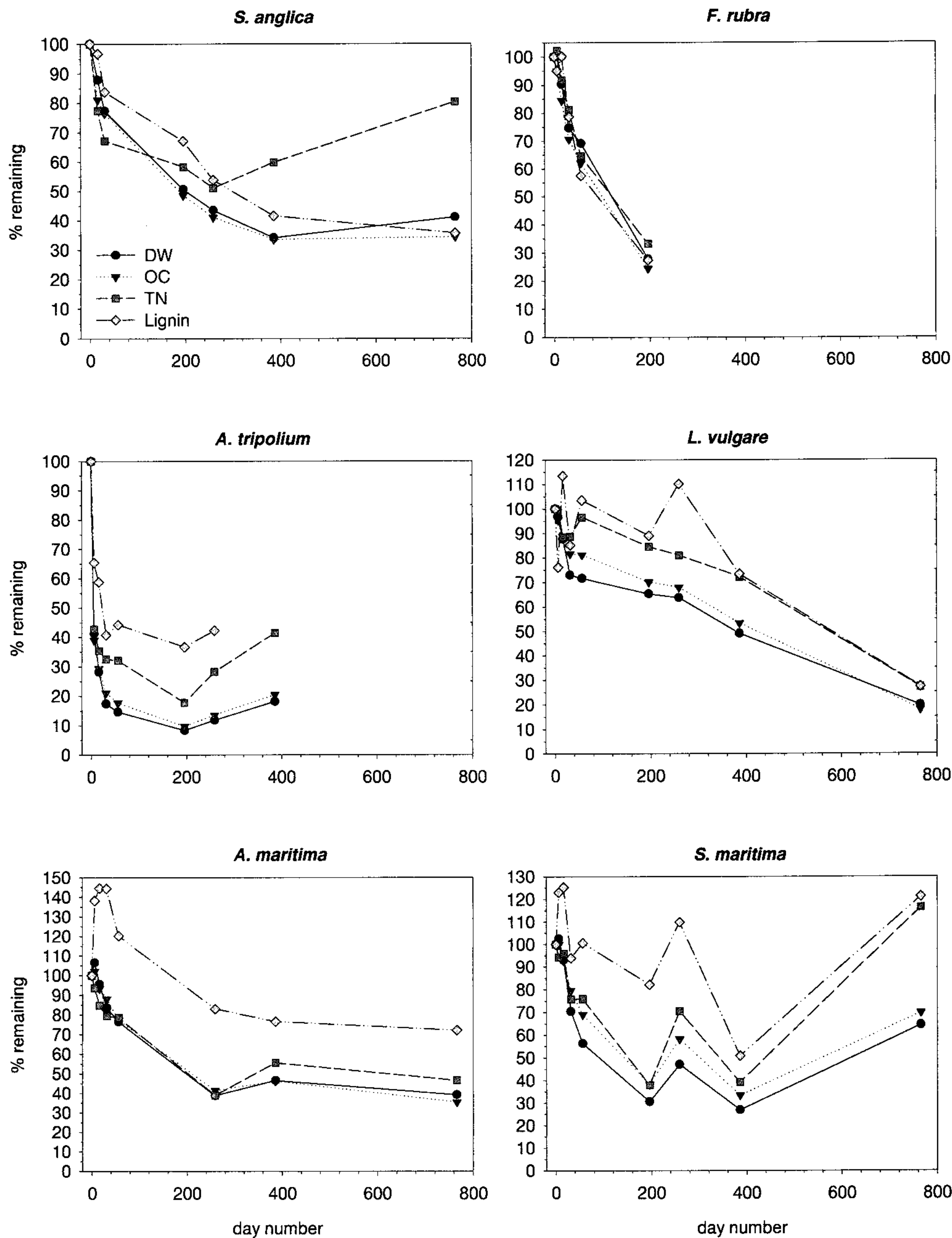

Fig. 4. Remaining quantities of dry weight, organic carbon, total nitrogen, and lignin (Λlig) as a function of time, normalized to initial amounts.

criminant analysis was carried out on sets of these integrated spectra. The essence of this numeric analysis method involves calculation of the weighted average of all spectra in the set and subsequent comparison of individual spectra with this average spectrum. Differences are expressed as factor scores or (when analyzed in multiplicate) discriminant scores. Details on the procedure can be found elsewhere (Hoogerbrugge et al. 1983; Windig et al. 1983; Boon et al. 1984; Tas 1991; Klap 1997).

Figure 5 shows two average spectra, in this case the results obtained after EI (Fig. 5a) and CI (Fig. 5b) ionization of *A. maritima.* Both spectra show many lignocellulose characteristics (*see symbol designations*), although in different ratios. The CI results (Fig. 5b) confirm the high sensitivity of sugars (Pouwels and Boon 1990) and dehydrated coniferyl and sinapyl alcohol (Van der Hage et al. 1995) for this ionization method. In Figs. 6b, 7b, the scores of individual spectra in the first discriminant or factor function are plotted respectively. The zero lines in these plots represent the average spectra.

Samples were analyzed in triplicate in the EI mode; hence, discriminant analysis was performed (Fig. 6). The first discriminant function accounts for 82% of the characteristic variance (=29% of the total variance in the set). The scores

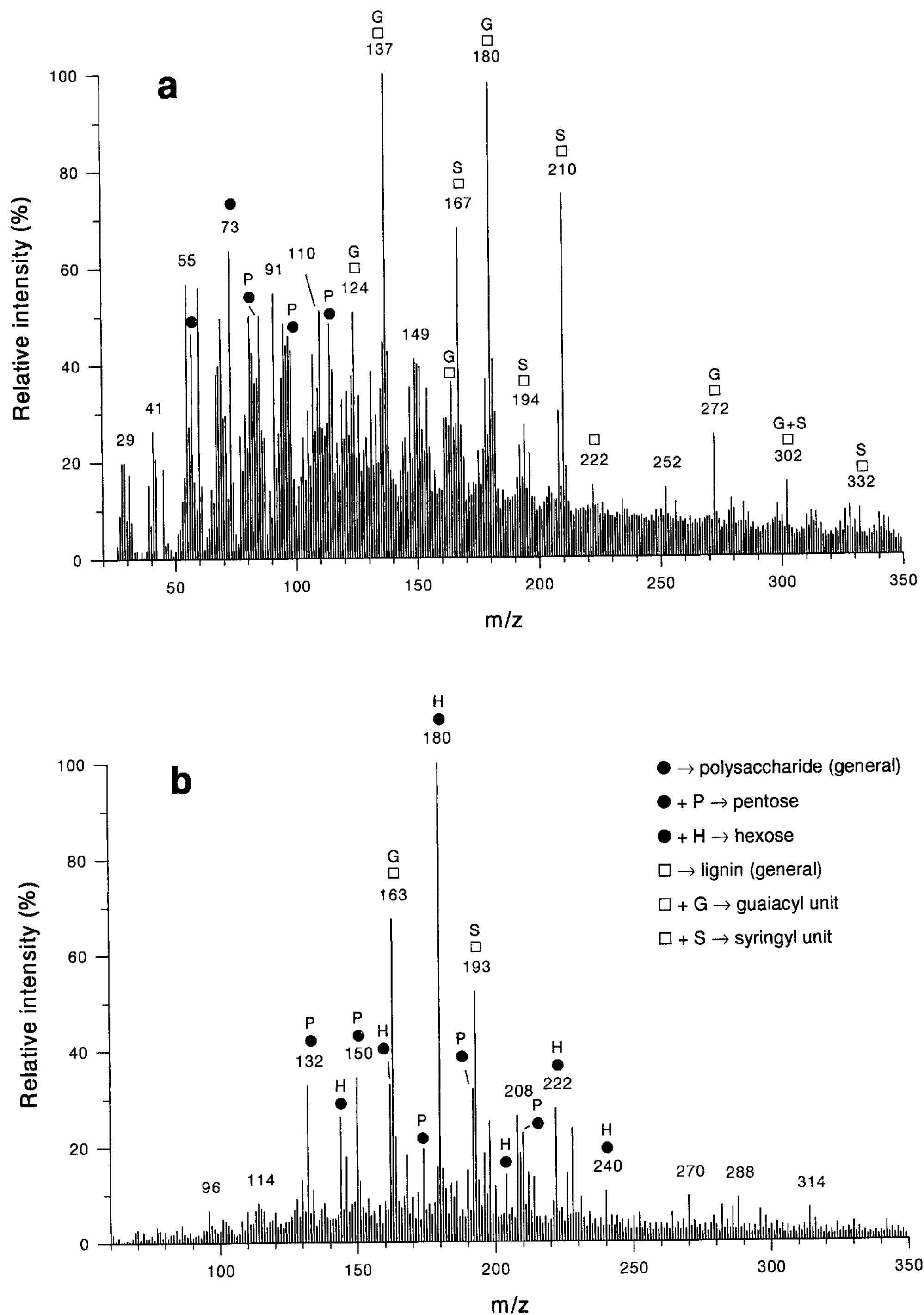

Fig. 5. Average DT-MS spectra (EI and CI, [a] and [b], respectively) of all purified *A. maritima* litterbag samples. These spectra represent the zero lines in Figs. 6b and 7b.

in Fig. 6b illustrate the mass spectral differences between the individual spectra with respect to the average spectrum. The negative DF_1 axis expresses compositional modifications related to the leaching process, whereas the positive part of the DF_1 space is representative for the decomposition process. It must be realized that the discriminant spectra of Fig. 6 are calculated distributions that do not closely resemble the individual spectra with the highest and lowest scores; they only indicate which sets of masses covary relative to the average spectrum. The spectrum of day 766, for instance, still contains the two main peaks of the lower spectrum, m/z 210 and 180, but their intensities are less dominant than in the spectrum of day 16.

The discriminant spectra are interpreted by comparison

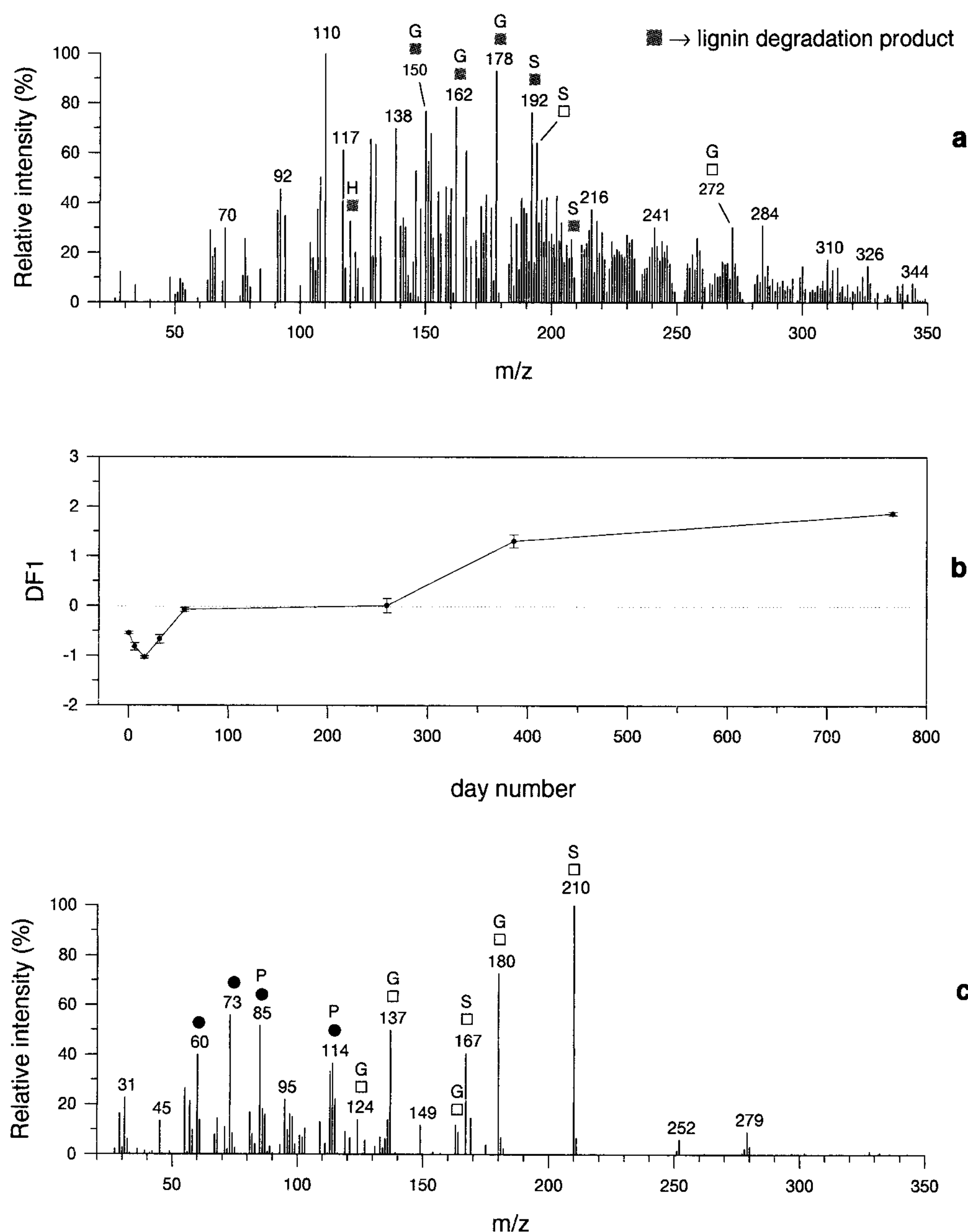

Fig. 6. DT-MS (EI) results after discriminant analysis of purified *A. maritima* litterbag set. Score plot (b) and calculated positive (a) and negative (c) discriminant spectra. Samples were analyzed in triplicate; error bars denote standard deviations of the means. Variance described is 82% of characteristic and 29% of total variance. Symbols as in Fig. 5.

with peak patterns of pure compounds, polymers, and plant material or by comparison with Py-GC-MS data obtained independently (Pouwels 1989; Van der Hage et al. 1993; Klap et al. 1998). Interpretation of the $DF_1(-)$ spectrum reveals a strong lignocellulose character. Interpretation of the $DF_1(+)$ spectrum is much more complicated, because it contains many peaks with rather high intensities but without a recognizable signature of bio-organic material. Considering that this fraction is produced upon decomposition of natural material, it can probably also be referred to as "humic substances." Assigning structures to individual masses in such full spectra is problematic, but the simultaneous presence of the ions m/z 208, 192, 178, 162, 150, and 120 (shaded squares) in this spectrum strongly suggests the presence of degraded lignin. The score pattern and the general features of the discriminant spectra and the average spectrum are very comparable to the results of *S. anglica* (Klap et al. 1998). Taken together, these DT-MS (EI) data describe a set of samples with a high average content of lignocellulose (Fig. 5). This lignocellulose character is enhanced during the

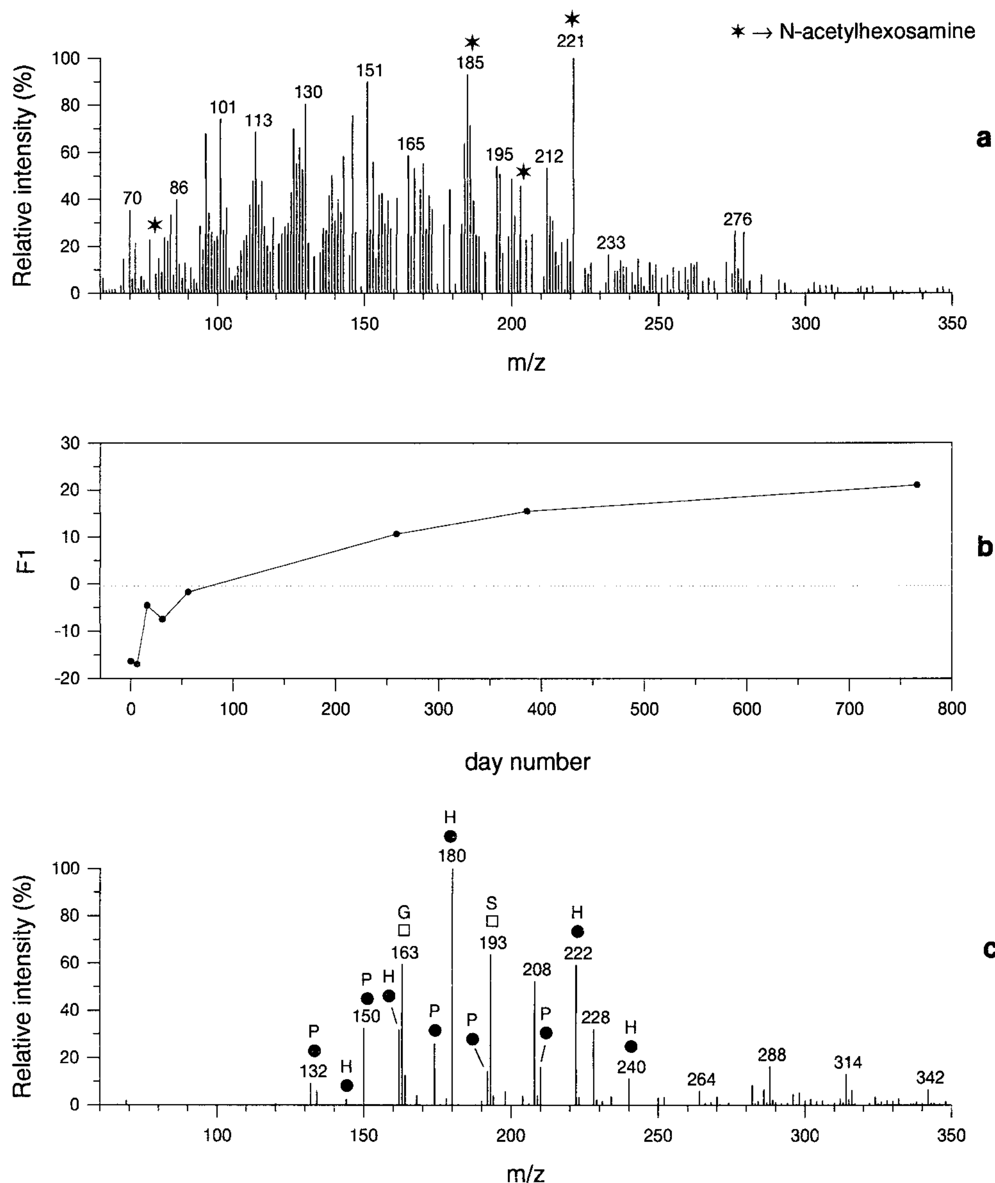

Fig. 7. DT-MS (CI) results after factor analysis of purified *A. maritima* litterbag set. Score plot (b) and spectra (a and c) of first factor function. Samples were analyzed in triplicate; error bars denote standard deviations of the means. Variance described is 52% of total variance. Symbols as in Fig. 5.

leaching stage and is gradually substituted by a combination of degraded lignin and a complex heterogeneous organic fraction during the later stages of decomposition (Fig. 6).

The CI results in Fig. 7 confirm the EI results, although the score pattern of Fig. 7b is not identical to Fig. 6b. The initial decrease over the first weeks is lacking, but the gradual shift from negative to positive scores coinciding with an increase in highly complex material at the expense of lignocellulose is similar to the EI results. Again, most of the mass peaks in the $F_1(-)$ spectrum (Fig. 7c) can be assigned to lignocellulose, whereas the complex $F_1(+)$ spectrum designates a poorly defined heterogeneous organic fraction. In this case, however, $F_1(+)$ also contains specific additional peaks indicative of N-acetylglucosamine, which appear at m/z 221, 203, 185, and 77. This aminosugar is an important constituent of bacterial cell walls (Boon et al. 1987), fungi (De Nobel et al. 1993), and the monomeric unit of chitin, the structural polysaccharide of arthropod skeletons (De Leeuw and Largeau 1993). The identity of the mentioned fragment ions was confirmed by MS-MS comparison with ions of equal nominal mass resulting after pyrolysis of pure chitin.

The results for the other species are presented in abbreviated form in Figs. 8 (EI) and 9 (CI), which show the score plots with a summary of the main characteristics of the corresponding spectra. Details on the chemical characterization of *S. anglica* can be found elsewhere (Klap et al. 1998). For the other species, the EI results are first considered. The

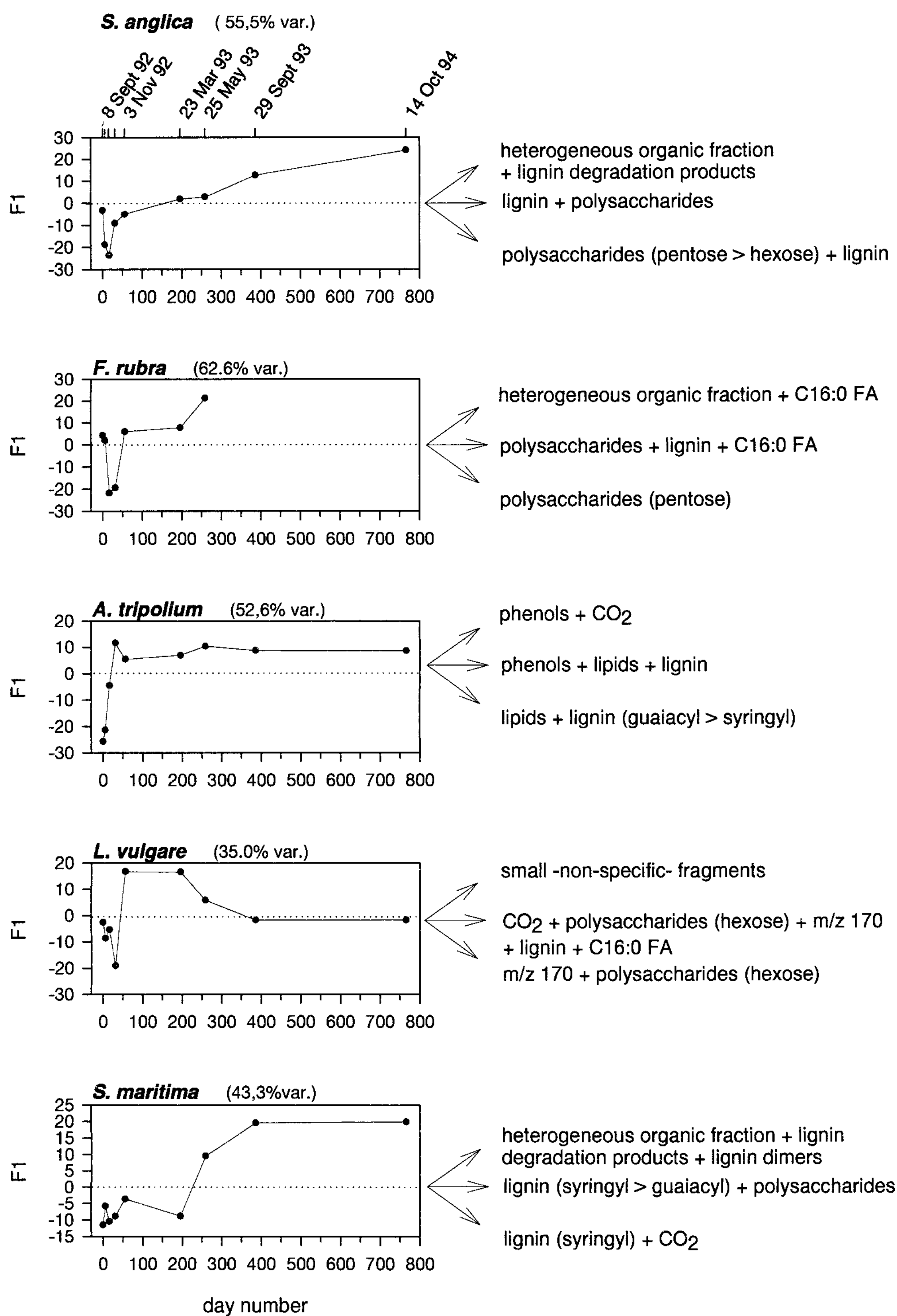

Fig. 8. EI score plots of *S. anglica, F. rubra, A. tripolium, L. vulgare,* and *S. maritima* with the amounts of variance described indicated behind the species names. The main characteristics of the accompanying spectra are expressed on the right sides of the plots. For each of these plots, the upper line gives the characteristics of the $F_1(+)$ spectrum, the middle one those of the average spectrum, and the lower one those of the $F_1(-)$ spectrum. The sequence of mentioned characteristics is the sequence of importance in the spectrum.

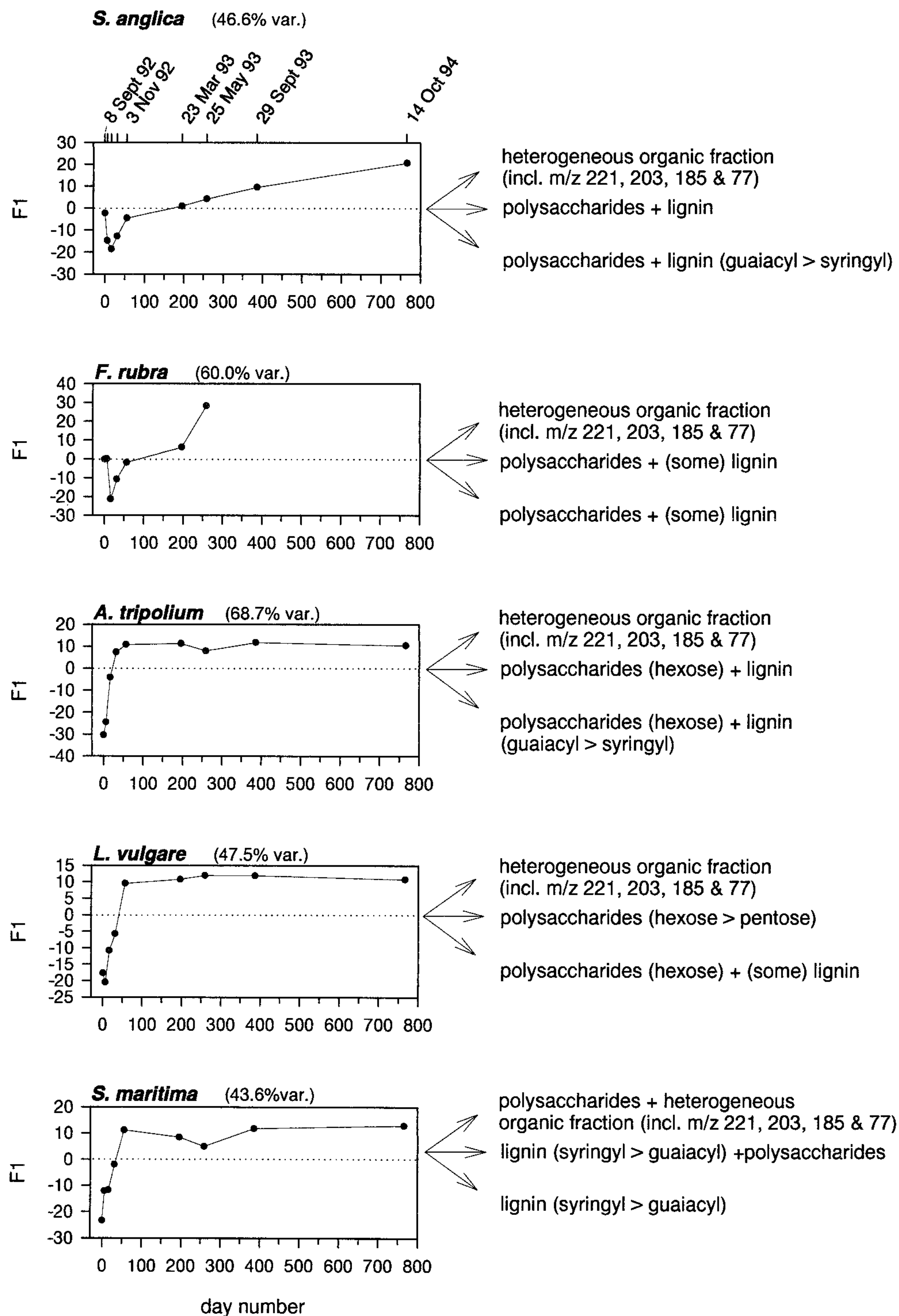

Fig. 9. CI score plots of *S. anglica, F. rubra, A. tripolium, L. vulgare,* and *S. maritima. See Fig. 8 for explanation.*

score plot for *F. rubra* shows a comparable pattern to that of *S. anglica* and *A. maritima* (Figs. 8 and 6b, respectively). For this species, the undefined heterogeneous organic fraction is negatively correlated to a pentose fraction instead of a lignocellulose fraction. The three remaining plots show different patterns. The scores for *A. tripolium* shift within 1 month from extremely negative to positive and remain stable afterward. This shift in scores, which correlates with the rapid weight loss (Fig. 1), expresses the conversion of lipid and lignin into some simple phenolic compounds. Thus, in contrast to the previous species, decomposition of most *A. tripolium* material does not gradually produce a complex mix-

ture. The scores for *L. vulgare* do not show a consistent trend during the decomposition process, and the factor spectra are not easily interpretable. It is clear, however, that lignin is not an important compound with regard to the decomposition dynamics of this species. The score plot for *S. maritima* does not reveal a shift during the first few weeks, but the general compositional shift from lignin-rich material to an uncharacterized heterogeneous organic fraction is comparable to the trends seen for *S. anglica* and *A. maritima.* The relatively high abundance of syringyl units in the lignin moiety of *S. maritima* compared to *S. anglica* and *A. maritima* is in accordance with the CuO oxidation results (Table 2). Also, the main features of the average spectra (not shown) confirm the division of the different plant tissues according to their lignin content as determined by CuO oxidation.

The CI results (Fig. 9) provide a consistent compositional shift for all species during the 2 yr of decomposition. In this period, lignin-rich (*S. maritima*) or lignocellulose-rich (all other species) material is changed into a heterogeneous organic fraction. Only *S. anglica* and *F. rubra* show a leaching pattern during the initial stages of decomposition. The previously mentioned markers of N-acetylglucosamine (m/z 221, 203, 185, and 77) are always part of the heterogeneous organic fraction. This finding correlates well with the generally observed increase in relative nitrogen contents during the decomposition process (Table 2).

General discussion and overview

Initial lignin content versus decomposition rate—The decomposition rates of the different plant species vary considerably. Since lignin is considered a decay-retarding substance, decomposition rates have often been correlated with initial lignin contents (Wilson et al. 1986*a,b*; Buth and Voesenek 1987; Hemminga et al. 1988; Hemminga and Buth 1991). The results after CuO oxidation and DT-MS both denote the tissues of *S. anglica, A. maritima,* and *S. maritima* as lignin rich and the tissues of *A. tripolium* and *L. vulgare* as lignin poor, and *F. rubra* occupies an intermediate position. This pattern correlates quite well with the observed weight losses (Fig. 1), except for *L. vulgare,* where a low lignin content is not correlated with rapid weight loss. This deviation becomes even more pronounced if it is realized that the decomposition rate of *L. vulgare* is probably overestimated because of the loss of brittle material from the litterbags. Obviously, another decay-retarding substance than lignin determines the decomposition rate of *L. vulgare.* As indicated in the Results section, the contribution of physical processes to the weight loss is an inherent disadvantage connected to litterbag experiments. For this sample set, this problem seems to be of concern only for the leaves of *L. vulgare.*

Lignin dynamics during decay—The results obtained with the CuO oxidation and DT-MS method partly confirm each other, but discrepancies also exist. Conformity exists on the rapid relative increase of lignin during the first weeks of the decomposition process (Figs. 3, 6–9). This increase can be explained as the leaching of soluble and easily degradable components, such as storage sugars, amino acids, etc., while structural components, including lignin and cellulose, remain in the litter. It is remarkable that the extensive extractions and rinses involved in the MWEL purification procedure apparently do not mask the leaching process. It seems that conformity does not exist on the relative lignin contents during the postleaching period. The CuO results indicate more or less stable LOP yields (normalized to OC, Fig. 3) during the second stage of decomposition for most of the studied tissues, whereas the DT-MS results indicate a gradual loss of intact lignocellulose toward the end of the experiment (Figs. 6–9).

Considering the comparative approach of this study, we should address the question of the extent to which the results obtained with the two analytic methods are comparable. Not only do the methods have their specific features, they have also been applied to different samples, i.e., untreated (only ball milled) and purified plant tissues. Before comparing the lignin dynamics in the postleaching period, these potential objections are discussed in some detail.

A rough characterization of these analytic methods might be that the combination of untreated material with CuO oxidation provides merely quantitative data, whereas DT-MS of MWEL samples yields a mostly qualitative view on the lignocellulose fraction. However, the ''quantitative'' approach of the CuO method does not mean that no qualitative indications are achieved; ratios of different compounds are used as diagenetic indicators (Hedges and Mann 1979*a*; Goñi and Hedges 1992; Haddad et al. 1992). Accordingly, the term ''qualitative'' should not be taken to be too strict for the DT-MS method. Here it indicates that relative peak intensities of lignin markers in the mass spectra of the various samples are compared. Peak intensities are proportional to detected ion numbers and represent semiquantitative data (as long as the approximate pyrolysis/ionization efficiencies of the various compounds in the sample are known). The previously mentioned disagreement between the two methods should therefore be nuanced to a lack of conformity between the amount of lignin oxidation products of whole samples and mass spectral data of thermally liberated lignocellulose fragments of purified samples.

If we consider the differences between crude and purified samples, a source of variation might be extraction with organic solvents. This is particularly the case since it has been shown that extraction of lignin by organic solvents is enhanced by ball milling (Lai and Sarkanen 1971). We have checked this effect for *S. anglica,* and it turned out indeed that MWEL obtained after 3 h of ball milling was richer in lignin than MWEL obtained after 20 h of ball milling. Ball milling thus induced enhanced lignin extraction. The risk of high depletion should not be exaggerated, however, because the ethanol-extractable lignin fraction of angiosperms (so called Brauns lignins) is generally smaller than 0.2% of the total plant material (Lai and Sarkanen 1971). Moreover, the milling time has been kept short (3 h, whereas 48 h was recommended in the original publication of Björkman 1956) in order to minimize lignin extraction. As far as intact lignin is concerned, we assume that crude and purified samples may be compared, although no data are available on whether the effect of ball milling varies as a function of decomposition period. No effect of the ball milling was found on the CuO oxidation method (comparison between unmilled and 3-h-milled material).

Quantitation of the lignin oxidation products with the CuO method does not bear large uncertainties. With regard to the DT-MS technique, the question is pertinent whether differences in pyrolysis/ionization efficiency between compound classes bias the resulting spectra in such a way that the score patterns obtained after discriminant analysis do not represent prevalent chemical changes. If, for instance, the heterogeneous organic fraction that is produced upon decomposition in the field would consist of compounds that are far more efficiently pyrolyzed and/or ionized than other compounds in the sample, this fraction might cause nonlinearity of the y-scales in Figs. 6–9. A control experiment with a series of mixtures of the two *A. maritima* MWEL samples with the most extreme scores in Fig. 6b pointed out that the discriminant scores are almost linearly related to the relative concentrations of the two samples (results not shown). It seems likely that this also counts for the other score plots (Figs. 6–9). Overall, the two analytic methods provide specific views on the lignin dynamics of the exposed plant tissues, and with this in mind the results may be compared.

At first we consider the apparent contradiction that during the postleaching period, the DT-MS results indicate a gradual conversion of intact lignocellulose into a heterogeneous organic fraction, while the relative amount of LOP as determined by CuO remains stable. It is important to realize that the DT-MS technique allows distinguishing compounds with different volatility or thermal stability. Small compounds will generally desorb at low temperatures and thus induce a shift in the thermal desorption profile of the sample. The MWEL residues did not show such a shift in the thermal desorption profile with increasing decomposition for any of the plant species in this study. Thus, the MWEL residues mainly consist of polymeric compounds throughout the experiment. The reconstructed mass spectrum that typifies the heterogeneous organic material that is produced upon decomposition includes markers of altered monolignols (Fig. 6a, dashed squares), which suggests that these chemically modified monolignols still belong to the lignin macromolecule. Unfortunately, it is not possible to make a thorough quantitative comparison between intact lignin in the residue after leaching and modified lignin in the decomposed fraction. We conclude that the DT-MS results essentially indicate the chemical modification of lignin and that it cannot be decided how the rate of this modification relates to that of bulk organic matter loss. The quantitative indication provided by CuO that lignin is not better preserved than bulk organic matter during the postleaching phase is thus not opposed by the DT-MS results, as might be concluded at first sight.

Nevertheless, there remains a quantitative peculiarity. In contradiction to the MWEL samples, which consist mainly of macromolecular material, the untreated samples still contain a fraction that can be removed by the purification procedure. One may wonder what the removable fraction of small compounds consists of. One would expect a relatively low lignin content for this fraction and thus a negative influence on the LOP yield. However, the LOP yields in the postleaching phase are stable instead of decreasing. Two reasonable explanations for this observation appear. At first the influence of the removable fraction may be compensated for by relative lignin enrichment of the remaining macromolecular fraction. Otherwise, the removable fraction may as well not be depleted in lignin (or material that produces LOP) compared to the macromolecular fraction. On the basis of the DT-MS results, which indicate that the relative lignin content of the macromolecular fraction does not increase during decomposition, we conclude that the second option is more probable. This scenario requires that the removable fraction contains small lignin decomposition products that, upon CuO oxidation, yield a comparable suit of products as the intact lignin macromolecule. This may be the case, since natural oxidation as well as CuO oxidation converts the α-carbon atom of the side chain of a phenolic lignin unit into a carbonyl group and eventually an acid group (Hedges et al. 1988*a*; Dijkstra et al. 1998). We conclude that small lignin decomposition products become attached to the tissue remains and can still be identified as LOP.

Another apparent disagreement between the results obtained with the two methods concerns a qualitative aspect. From the CuO oxidation results it appears that, for all plant species, the lignin moiety reaches an endmember within 1 month. In fact, this only relates to the rapid stabilization of the C : V ratio because other qualitative parameters (S : V and Ad : Al) do not show consistent trends during the 25 months of aerobic decomposition. The DT-MS (EI) results indicate that the intensities of several specific lignin degradation products (Fig. 6a) increase upon decay for the three lignin-rich tissues. According to these DT-MS results, a lignin endmember is not yet reached after more than 2 yr of aerobic degradation. Actually, these results are not contradictory but complementary, because the C : V ratio is better determined with the CuO method, whereas first-stage alteration of the propenolic side chain of a monolignol is more easily identified using DT-MS. Based on these results, the suitability of lignin to monitor efflux of halophytic material from salt marshes to adjacent water systems is dependent on the analytic method used. If the CuO method is used, there do not seem to be limitations with regard to the stability of the first-stage diagenetic endmember. If DT-MS is used, typical lignin characteristics are continuously lost and may eventually vanish.

Both methods reveal no clear-cut differences in degradability of guaiacyl and syringyl units. The relative stabilities of the different monomer families have been studied many times (Hedges et al. 1985; Benner et al. 1991; Haddad et al. 1992; Goñi et al. 1993; Gamble et al. 1994; Van der Heijden and Boon 1994; Opsahl and Benner 1995), but no uniform image appears from all these studies. Even in well-controlled laboratory experiments with white-rot fungi, the observations are ambiguous. The lack of uniformity, particularly under field conditions, seems logical because the stability of monolignols and oligolignols depends not only on intrinsic chemical properties, but also on (1) the biochemical and physicochemical conditions during decomposition (including the different degradation pathways employed by different decomposers), (2) their location in the lignin macromolecule (Terashima et al. 1993), and (3) interaction with other cell wall components.

An interesting observation in the DT-MS results of the three lignin-rich plant species (*S. anglica, A. maritima,* and

S. maritima) is that lignin and polysaccharide show correlated behavior during decay. The apparent comparable stabilities explain why we commonly have used the designation ''lignocellulose'' instead of ''lignin'' and ''polysaccharide'' separately. This observation is contradictory to other reports that denoted cellulose as relatively labile (Hodson et al. 1984; Opsahl and Benner 1999).

Geochemical implications from this study—Rice (1982) has exhaustively addressed the phenomenon of increasing nitrogen content with increasing decomposition time, which was also observed in this study (Fig. 2 and Table 2). His well-accepted hypothesis (Wilson et al. 1986*a*; Hemminga et al. 1988; Benner et al. 1991; Buchsbaum et al. 1991), which shares similarities with the mechanism proposed in the previous section, is that small reactive oligomeric molecules produced during the first stage of decomposition form reactive geomacromolecules. These geomacromolecules act as condensation nuclei for nitrogenous microbial exudates and become more and more refractory in the course of time. The remaining plant fraction thus becomes increasingly enriched with those nitrogen-rich geomacromolecules. Our DT-MS (CI) results indicate a gradual increase of N-acetylglucosamine for all plant species studied and thus provide strong evidence for a contribution of allochthonous nitrogen-rich material.

It is worthwhile to relate the presented results to the common viewpoints on the formation of humic substances. Two different pathways have been distinguished: (1) condensation of small degradation products, such as monopeptides and oligopeptides, monosaccharides and oligosaccharides, lipids, and phenolic substances, into a refractory heterogeneous organic polymer and (2) condensation of only slightly modified biogenic macromolecules (Mayer 1985; Hedges 1992; Ishiwatari 1992; De Leeuw and Largeau 1993). As previously outlined, we interpret the combined results of DT-MS and CuO oxidation as an indication for the occurrence of the first of the two proposed pathways. Of course different substances have different preservation potentials, so the second pathway will apply to some extent. Nevertheless, it does not seem likely that certain biogenic molecules remain intact in humus. As such, the second pathway might be regarded as an intermediate stage of the first pathway in which decomposition of the contributing substances is less prolonged.

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