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Decomposition of macroalgae, vascular plants and sediment detritus in seawater: Use of stepwise thermogravimetry

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Abstract. The applicability of a recently presented method (Stepwise Thermogravimetry, STG) to characterize biogenic organic matter (Kristensen 1990) was tested in comparative decomposition experiments. The initial microbial decay of pre-dried, fresh detritus from 6 different plant materials (2 macroalgae, 2 seagrasses, and 2 tree leaves) was examined for 70 days in aerobic seawater slurries. In addition, slurries of sediment detritus of low reactivity, representing the late stage of plant decay, were allowed to decompose aerobically and anaerobically for 200 days. Macroalgae lost 40–44% carbon over 70 days, whereas seagrasses lost 29–33% and tree leaves lost 0–8%. After a 3–5 days leaching phase, the temporal pattern of POC and PON loss from the plant detritus was exponential with higher rates for the former resulting in a 5–28% reduced C:N ratio. The Rp index decreased (<20%) during the initial leaching phase followed by a 30–40% increase to the end. Initial Rp was directly proportional to decay rate. POC loss in the anaerobic sediment slurry was 10% over the 200 day period (the aerobic was hampered by low pH). Preferential loss of PON caused a 30% increase in C:N ratio. The Rp index of sediment detritus also increased with 30%. Although the present laboratory experiments not fully simulate the natural environment, the Rp-C:N relationship obtained from the two slurry experiments can illustrate the general pattern of plant decay from fresh to refractory (humic) detritus. During initial aerobic decay, rapid leaching and microbial growth causes a decrease in both Rp and C:N ('Rapid growth', phase 1). When all labile substrates have been consumed and the slower decay is controlled by enzymatic attack on particles with an associated production of humic compounds and accumulation of nitrogen rich bacterial cell remains, Rp increases and C:N decreases ('Slow growth', phase 2). Later, when condensed humic compounds have accumulated, decay ceases. Most carbon is now bound in forms which are of low availability to bacteria and a preferential mineralization of nitrogen occurs; both Rp and C:N increases ('Condenzation', phase 3).

Introduction

The chemical composition and nitrogen content of plant detritus are major factors determining rates of microbial decay (e.g. Godshalk & Wetzel 1978c; Rice & Hanson 1984; Lancelot & Billen 1985; Buchsbaum et al. 1991; Enriquez et al. 1993). The most common biochemical compounds studied in relation to the diagenesis of plant materials in water and sediment are carbohydrates, proteins and lignins (Rice 1982; Valiela et al. 1984; Benner & Hodson 1985; Hamilton & Hedges 1988; Harrison 1989). Although these compounds are important nutrients for decomposers during early diagenesis, they also act as precursors of refractory humic geopolymers formed late in the

decomposition sequence (Rice & Hanson 1984). Much is known about the decay dynamics of each polymer group found in many source materials, but the knowledge on overall transformation rates of various compounds and nitrogen into humified and complex geopolymers during different decay phases is rather limited (Rice & Hanson 1984; Wilson et al. 1986).

The Rp index obtained by Stepwise Thermogravimetry (the ratio between weight loss after combustion in the interval 280 to 520 °C and the total loss on ignition at 520 °C) as proposed by Kristensen (1990), offers an experimentally simple, but valuable tool to describe overall compositional changes in decaying detritus. Rp values around 0.2 are typical for plant materials rich in structural carbohydrates (e.g. cellulose), whereas Rp values around 0.5–0.6 represent organic matter rich in protein and/or aromatic, refractory 'humic' compounds (Kristensen 1990). However, the Rp index seems to be most powerful when used in combination with other parameters, such as oxygen uptake, elemental C and N composition, biochemical composition, bacterial density and activity (e.g. ATP). Thus, Kristensen (1990) found a highly significant negative logarithmic relationship between Rp and C:N of a number (13) of fresh biogenic materials ranging from eggwhite to tree leaves. The combination of Rp and C:N ratio of organic matter provides a particularly promising approach in the study of chemical changes occurring in organic substrates during decomposition. Recent studies have shown that the Rp index provides information on the nature and compositional changes of decaying organic matter (Kristensen et al. 1991, 1992).

The purpose of this study was to describe the decay pattern of a variety of plant materials, starting from the leaching phase and early aerobic microbial attack on the dissolved organic matter to the slow anaerobic decay of the humified remains after prolonged decay. The decay of pre-dried, fresh plant materials was examined for 70 days in seawater slurries by measuring O_2 uptake, POC and PON, DOC and DON, protein content, and Rp. Old and humified sediment detritus, representing the late decomposition stage of plant material, was allowed to decompose for 200 days with frequent samplings for POC, PON, NH_4^+ and Rp analysis. Although the experimental conditions not fully simulated the natural environment, the results provide valuable comparative information on general principles of plant decay.

Materials and methods

Origin of substrates

Six different plant species (experiment 1) and one type of marine sediment detritus (experiment 2) were used in the present decomposition assays. The plant materials were chosen to represent a variety of sources (2 species of macroalgae, 2 species of seagrasses and leaves from 2 species of trees) and to cover a wide spectrum of biochemical composition and relative degrad-

ability. Macroalgae contain alginates (e.g. *Fucus vesiculosus*) and pectin (e.g. *Ulva lactuca*) as the main structural polysaccharide with cellulose microfibrils arranged in an amorphous matrix (Round 1973). These carbohydrates usually account for ≈ 50% of the cell dry wt. The intracellular pools of storage products (e.g. mannitol and starch) and protein may each account for up to 20% of the cell dry wt (Round 1973; Kristensen 1990). The content of phenolics (e.g. lignin) is usually low, although *F. vesiculosus* can contain up to 5% (Buchsbaum et al. 1991). The major structural polysaccharide (ca. 75% of dry wt.) of aquatic, vascular plants like *Zostera marina* and *Ruppia maritima* is cellulose (Hamilton & Hedges 1988; Harrison 1989). The lignin content in these submerged plants usually accounts for less than 10% of dry wt. Protein content is usually 10–15% (Kristensen 1990). Terrestrial (e.g. *Fagus silvatica*) and mangrove (e.g. *Rhizophora apiculata*) plants are rich in lignocellulose, a macromolecular complex of the structural polysaccharides, cellulose and hemicellulose, and the aromatic heteropolymer lignin. Lignocellulose comprises up to 70% leaf detritus (Benner & Hodson 1985), and the polysaccharide component accounts for 50–70% of lignocellulose (Benner et al. 1984; Benner & Hodson 1985). Only 5–10% of dry leaf wt. is protein (Kristensen 1990).

The two macroalgae, *Ulva lactuca* and *Fucus vesiculosus*, and two seagrasses, *Zostera marina* and *Ruppia maritima* were collected during fall in the intertidal zone of the estuary Odense Fjord, Denmark. Beech (*Fagus silvatica*) leaves were collected from the ground just after leaf fall in a small forest on the Island of Fyn, Denmark. Live, green leaves of the mangrove *Rhizophora apiculata* were picked during January in the mangrove forest, Ao Nam Bor, on Phuket Island, Thailand. All plant materials were dried at 105 °C for 12 h immediately after arrival to the laboratory. After cooling in a desiccator, the dried materials were ground in a mortar. Only particles smaller than 500 µm were used in the experiments.

The upper ca. 10 cm of an organic rich estuarine sediment was sampled at ca. 5 m water depth in the estuary Kolding Fjord, Denmark. The sediment was sieved through a 1.5 mm mesh immediately after sampling and stored cold (5 °C) for one week before the start of experiment.

Plant decomposition (experiment 1)

The dried and ground plant materials were suspended into 0.45 µm filtered 20 ‰ seawater to provide a 1:20 wt/wt slurry. A volume of 200 ml (10 g dry material) of each slurry was transferred in duplicates to 500-ml Erlenmeyer flasks. All flasks were continuously stirred and aerated. The air stream was pre-moistened in a 40 cm column of distilled water before entering the slurries. Immediately before the start of incubation, all slurries were inoculated with extract of surface sediment from Odense Fjord. Five ml taken from a mixture of 5 g wet surface sediment and 50 ml seawater, after settling of the heavy particles, was added to each experimental batch. The cultures were then

incubated in darkness at 21 °C for 70 days. At regular intervals (day (0), 1, 3, 7, 15, 21, 30, 39, 49, 59, 70), 10 ml samples were taken and filtered through 0.45 µm filters. The residues were analysed for stepwise thermogravimetry (STG), particulate organic carbon (POC) and nitrogen (PON). The residue of *Fucus vesiculosus, Ruppia maritima* and *Fagus silvatica* was, in addition, analysed for total protein content. The filtrate was analysed for pH, dissolved organic carbon (DOC) and nitrogen (DON) (unfortunately, the DOC and DON samples from *F. vesiculosus, R. maritima* and *R. apiculata* were lost!).

Another 1–2 ml samples from each batch were taken at regular intervals (day 1, 2, 3, 4, 7, 10, 15, 21, 30, 49, 59, 70) for oxygen uptake measurements at 21 °C. The samples, which were diluted to 10 ml with 0.45 µm filtered seawater, were transferred to a continuously stirred 10-ml respiration chamber equipped with a precalibrated oxygen electrode (Radiometer, Denmark). The electrode reading was recorded continuously for 1–2 h after sealing the chamber. Oxygen uptake was determined from the initial linear phase of oxygen removal and corrected for dilution. Seawater controls showed no measureable O_2 uptake within 3 h.

Detritus decomposition (experiment 2)

Wet estuarine sediment was suspended in 0.45 µm filtered seawater on a 1:1 wt/wt basis. Since the original sediment contained 78% water, the amount of dry matter in the slurry was only 11%, of which ca. 17% was organic matter. A slurry volume of 400 ml was transferred to each of 4 continuously stirred 500-ml Erlenmeyer flasks. Two of the flasks were incubated with aeration as mentioned above for the plant materials (aerobic). The remaining 2 flasks were sealed and kept permanently anoxic during the incubation period (anaerobic). Twice a week the anaerobic slurries were opened and flushed with N_2 for 45 min to remove excess gaseous metabolites (CO_2 and H_2S) and each added ca. 1 mmol SO_4^{--} to maintain maximum sulfate reduction. All sediment batches were incubated for 188 days in darkness at 24 °C. At frequent intervals (day 0, 7, 14, 28, 42, 63, 83, 106, 132, 161, 188), 10 ml samples were taken and filtered through 0.45 µm filters. The residue was analysed for STG, POC, and PON and the filtrate was analysed for pH and NH_4^+.

Analysis

The stepwise thermogravimetric procedure of Kristensen (1990) was applied to characterize the overall chemical composition of the decaying plant and sediment materials. Briefly, samples of 0.5 g were pre-dried at 130 °C for 6 h. After cooling in a desiccator, the sample weight was determined with a precision of 0.1 mg. Subsequently, the samples were combusted at precisely 280 °C for 6.0 h in a computer controlled Heraeus MR 170 muffle furnace. After cooling in a desiccator and re-weighing, the samples were returned to the muffle furnace and combusted at 520 °C for 6.0 h. After cooling in a

desiccator the final ash weight was determined. The weight loss in the temperature range 280–520 °C (PII) was related to the total loss-on-ignition (LOI) in the range 130–520 °C (PI + PII) to provide the Rp index according to, Rp = PII/(PI + PII).

Samples for POC, PON were analyzed on a Hewlett-Packard 185B CHN-analyzer by the method of Kristensen & Andersen (1987). Water samples for DOC and DON analysis were acidified with HCl to pH 2 in order to remove inorganic carbon from the solution. About 18 h later, the samples were dried at 100 °C for 18 h. After cooling in a desiccator the dried material was analyzed for carbon and nitrogen on the CHN-analyzer. This procedure is likely to underestimate the dissolved organic pool due to evaporation of low-molecular, volatile compounds. However, the error was probably of minor importance here since only a few percent of naturally occurring dissolved organic matter usually is considered to be volatile (Sansone & Martens 1982; Sugimura & Suzuki 1988).

The protein content of *Fucus vesiculosus, Ruppia maritima* and *Fagus silvatica* was determined by the micro-biuret method (Rausch 1981; Meyer & Walther 1988). Freeze-dried and ground samples (0.2 g) were suspended in 100 ml distilled water. Subsamples of 2 ml were extracted in 4 ml 0.5 M NaOH at 100 °C for 20 min. After cooling to room temperature the solution was shaken and centrifuged (3000 rpm for 10 min). Two ml samples of the supernatant were added 1 ml 0.21% $CuSO_4$ solution. The color reaction of dissolved protein with $CuSO_4$ (cuprate-complex) was measured spectrophotometrically at 310 nm.

Dissolved NH_4^+ was determined by the standard autoanalyzer method of Solorzano (1969).

Results

Experiment 1

Carbon and nitrogen. After an initial leaching phase of 3–5 days, the (aerobic) decay rate of POC in the examined plant materials (except *Fagus silvatica*) decreased exponentially through time (Figs. 1A & 2A) and could be adequately described by first-order decay kinetics: dG/dt = kG, where G is the concentration of organic matter (mol l^{-1}), k is first-order decay constant. The most rapid decay was found for the macroalgae *Ulva lactuca* nd *Fucus vesiculosus* which over 70 days lost 40 and 44%, respectively, of the initial POC (decay constants, k_C of 1.6 and 2.3 yr^{-1}, Table 1). The seagrasses *Zostera marina* and *Ruppia maritima* lost 29 and 33% (k_C of 1.0 and 0.8 yr^{-1}, Table 1), whereas leaf material from the trees *Fagus silvatica* and *Rhizophora apiculata* was most resistant to decay with 0 and 8% loss of POC during the 70 day period (k_C of 0 and 0.5 yr^{-1}, Table 1). Between 60 and 90% of the

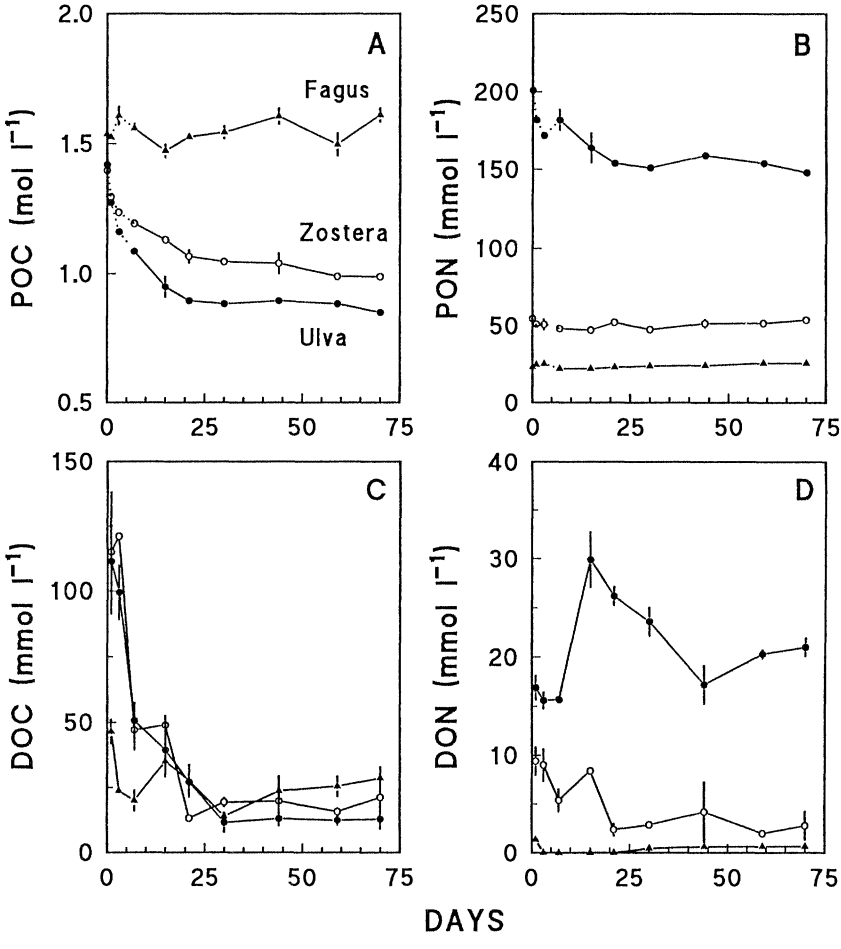

Fig. 1. Ulva lactuca, Zostra marina and *Fagus silvatica.* Temporal changes of (A) POC, (B) PON, (C) DOC and (D) DON content in batch cultures over 70 days. Error bars indicate range of 2 cultures. Dotted lines in (A) and (B) represent the leaching phase (not included in estimates of first order decay constants).

total POC loss of all materials occurred within the first 20 days. Leaching of DOC appeared most conspicuous for the labile materials; almost 10% of the initially added *U. lactuca* and *Z. marina* carbon was recovered as DOC at day 1 in contrast to 3% for *F. silvatica* (Fig. 1C). For *U. lactuca* and *Z. marina* the concentration of DOC decreased rapidly from day 3 to 30 and remained constant throughout the remaining experimental period. DOC accounted for only 1.5–2.1% of the total carbon remaining at day 70.

The change in PON through time also showed a post-leaching exponential decrease for *U. lactuca, F. vesiculosus* and *R. maritima* (Fig. 1B & 2B). The rates were, however, lower than found for POC, but 80–90% of the total loss

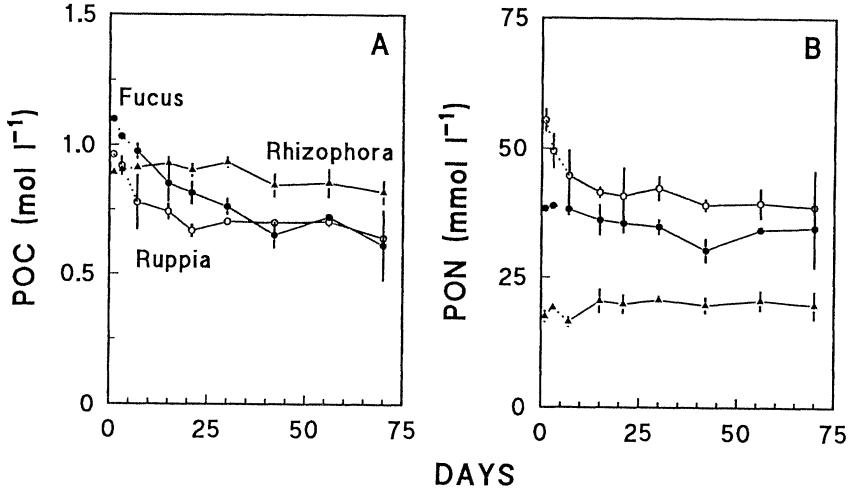

Fig. 2. Fucus vesiculosus, Ruppia maritima and *Rhizophora apiculata.* Temporal changes of (A) POC and (B) PON content in batch cultures over 70 days. Error bars indicate range of 2 cultures. Dotted lines represent the leaching phase (not included in estimates of first order decay constants).

Table 1. Average first order decay constants, k = –dG/Gdt (yr^{-1}) for POC (k_C) and PON (k_N) in the 2 experiments. The initial leaching phase in experiment 1 and organic enrichment phase in experiment 2 are not included in the estimates. Initial C:N ratio and Rp index are given for the various materials.

	k_C	k_N	C:N	Rp
Anaerobic sediment	0.24	0.81	11	0.420
Ulva lactuca	1.64	0.97	7	0.520
Zostera marina	0.99	≈0	26	0.388
Fagus silvatica	≈0	≈0	66	0.248
Fucus vesiculosus	2.33	0.80	30	0.359
Ruppia maritima	0.75	0.70	18	0.406
Rhizophora apiculata	0.50	≈0	44	0.290

occurred within 20 days. *U. lactuca* and *R. maritima* lost 26 and 30%, respectively, of the initial PON during the course of the experiment (decay constants, k_N of 1.0 and 0.7 yr^{-1}, Table 1), *F. vesiculosus* lost 10% (k_N of 0.8 yr^{-1}), and *Z. marina, F. silvatica* and *R. apiculata* lost 0–1% ($k_N \approx 0$). The DON concentration pattern was erratic, but showed a decreasing trend through time (Fig. 1D). For *U. lactuca* DON accounted for 8% of the initially added nitrogen at day 1. The figures for *Z. marina* and *F. silvatica* were 17 and 6%, respectively. In the *U. lactuca* batch culture DON increased to 18% at day 15 and again gradually decreased to 14% at day 70. For *Z. marina* and *F. silvatica* the final DON concentration was 5 and 2%, respectively, of the nitrogen remaining at day 70.

The initial protein content of *F. vesiculosus* was, in contrast to PON, 2–3 times higher than that of *R. maritima* and *F. silvatica*. The former two materials showed a rapid initial decrease (Fig. 3, only followed for 20 days). Both plant materials lost about half of the protein content in 20 days; about 5 and 2 times higher rate than for PON. The protein content in *F. silvatica* decreased to half within the first 3 days, but gradually increased again to the initial level at day 20.

The fast decay of carbon relative to nitrogen resulted in a decreasing C:N ratio of the residues throughout the experiment (not shown). The decrease was most pronounced for the materials with largest difference between decay constants of carbon (k_C) and nitrogen (k_N), i.e. 28% for *Z. marina* and *F. vesiculosus* and 18% for *U. lactuca*. The C:N ratio was most insensitive to decay in *F. silvatica* (5% decrease), *R. maritima* (7%) and *R. apiculata* (10%).

The pH remained stable between 6 and 8 in all batches throughout the experiment.

Oxygen uptake. The uptake of O_2 by the batch cultures reflected the rapidly decreasing decay rate with time and the relative difference in reactivity between the various plant materials (Fig. 4). For all materials except *Z. marina* and *F. silvatica* there were a few days lag phase before O_2 uptake peaked. The most active period occurred within the first 25 days. After this, the O_2 uptake rates remained low, but with almost the same relative differences

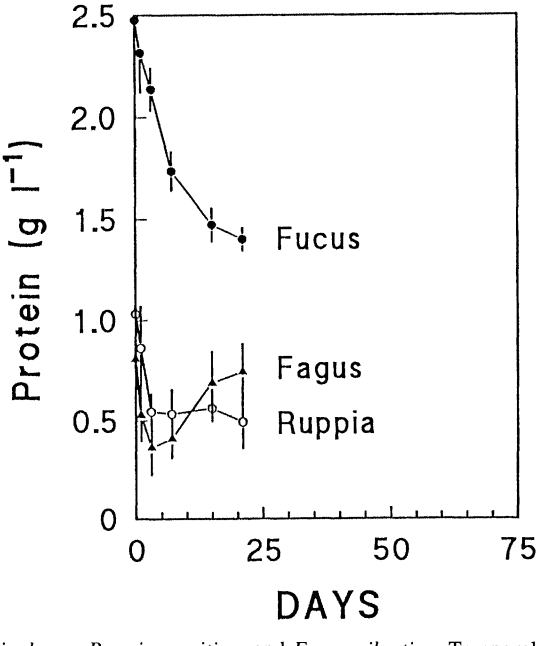

Fig. 3. Fucus vesiculosus, Ruppia maritima and *Fagus silvatica*. Temporal changes of protein content in batch cultures over 21 days. Error bars indicate ± S.D. of 5 determinations on pooled samples from 2 cultures.

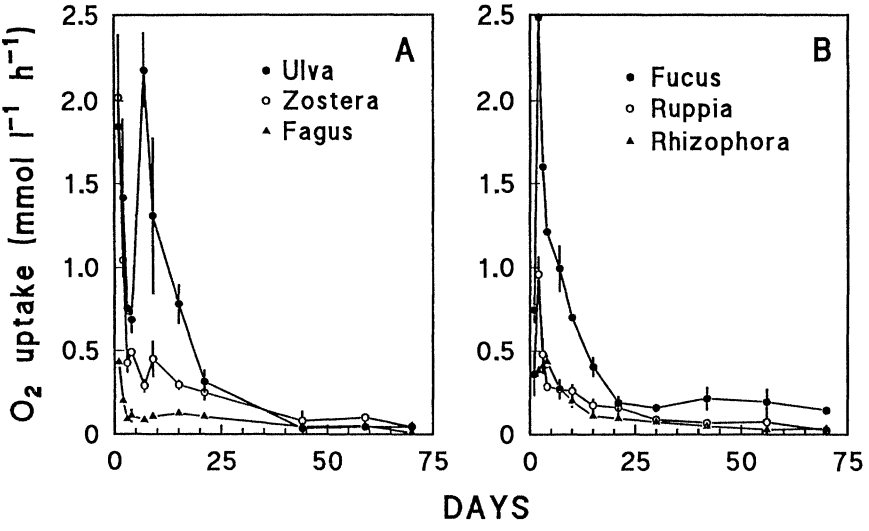

Fig. 4. Temporal changes of oxygen uptake in batch cultures over 70 days. (A) *Ulva lactuca, Zostera marina* and *Fagus silvatica*; (B) *Fucus vesiculosus, Ruppia maritima* and *Rhizophora apiculata*. Error bars indicate range of 2 cultures.

between the various materials. The 70 day integrated O_2 uptake for the most reactive materials corresponded well (within 30%) with the loss of POC in the cultures (Table 2). For the least reactive, *F. silvatica* and *R. apiculata*, there were an excessive O_2 uptake compared to the very low measured POC loss.

STG. During initial leaching and decay, the Rp index generally decreased (<20%) followed by a more or less rapid increased throughout the rest of the experiment (Fig. 5). Materials with the most reactive nitrogen pool, *U. lactuca, F. vesiculosus* and *R. maritima* showed the steepest increase in Rp with maximum values at day 25 or later, which were 38, 47 and 36%, respectively, higher than the minimum recorded within the first 10 days. The low degradable materials, *F. silvatica* and *R. apiculata*, showed only minor variations in Rp throughout the experiment. These latter materials exhibited the lowest Rp's, 0.20–0.25 and 0.28–0.30, respectively. The reactive materials all had Rp's around 0.35 or higher.

Table 2. Total net POC and PON change in plant batch cultures (exp. 1). The integrated oxygen uptake is presented for comparison. Values are in mmol l^{-1} for a 70 day period.

	Ulva	*Zostera*	*Fagus*	*Fucus*	*Ruppia*	*Rhizophora*
ΔPOC	–567	–406	0	–480	–320	7
ΔPON	–53.0	–0.8	0	–3.7	–16.8	2.2
O_2 upt	622	347	125	589	223	165

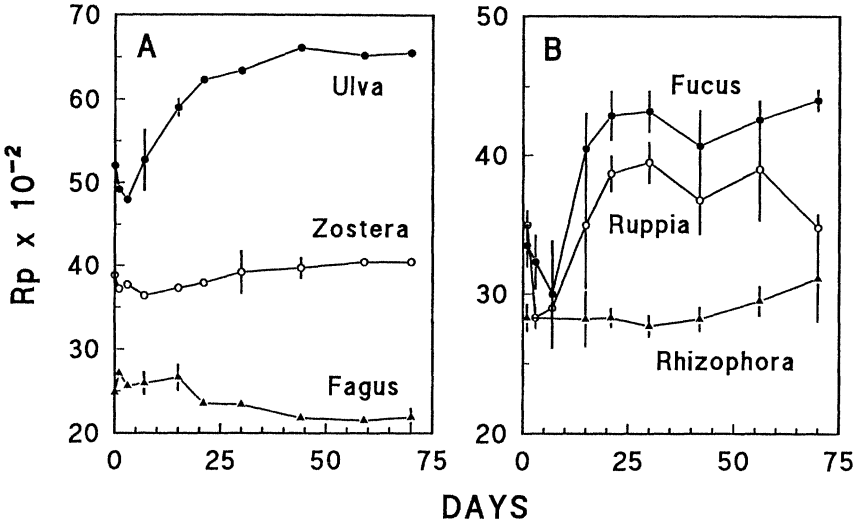

Fig. 5. Temporal changes of Rp index in batch cultures over 70 days. (A) *Ulva lactuca, Zostera marina* and *Fagus silvatica*; (B) *Fucus vesiculosus, Ruppia maritima* and *Rhizophora apiculata*. Error bars indicate range of 2 cultures.

The changes in Rp and C:N through time provide a two-dimensional characteristic of changes in quality of the organic matter during aerobic decay (Fig. 6). All examined materials (except *F. silvatica*) showed the same pattern. During the first 5–10 days both Rp and C:N decreased rapidly. Subsequently, Rp increased while C:N continued to decrease, but at a gradually slower rate. Rp was always higher and C:N lower after 70 days of decay than found at day 0. An intercomparison of the Rp-C:N relationship for all materials showed a significant relationship (power function) both at day 0 ($r^2 = 0.954$) and day 70 ($r^2 = 0.934$, Fig. 7), but with a 30% steeper slope at the end.

Experiment 2

Carbon and nitrogen. The decay of sediment detritus was more erratic than for the pure plant materials (Fig. 8). The aerobic batches showed a 20% increase in POC during the first 15 days (Fig. 8A). The associated rapid decrease in pH was probably due to sulfide oxidation (Fig. 8C). Between day 28 and 42, where POC decreased rapidly again, pH reached a minimum of 2.5–3.0. The lack of any subsequent POC change in the aerobic batch may be due to inhibition of microbial activity by low pH. The gradual increase in pH to about 6 from day 42 to 190 indicated that the capacity for sulfide oxidation was exhausted. In the anaerobic batch, POC also increased during the first 15 days, but only with about 7% (Table 3). During the rest of the experiment POC in this batch gradually decreased, reaching 90% of the initial value at day 190. The POC loss observed after day 15 can be described by a

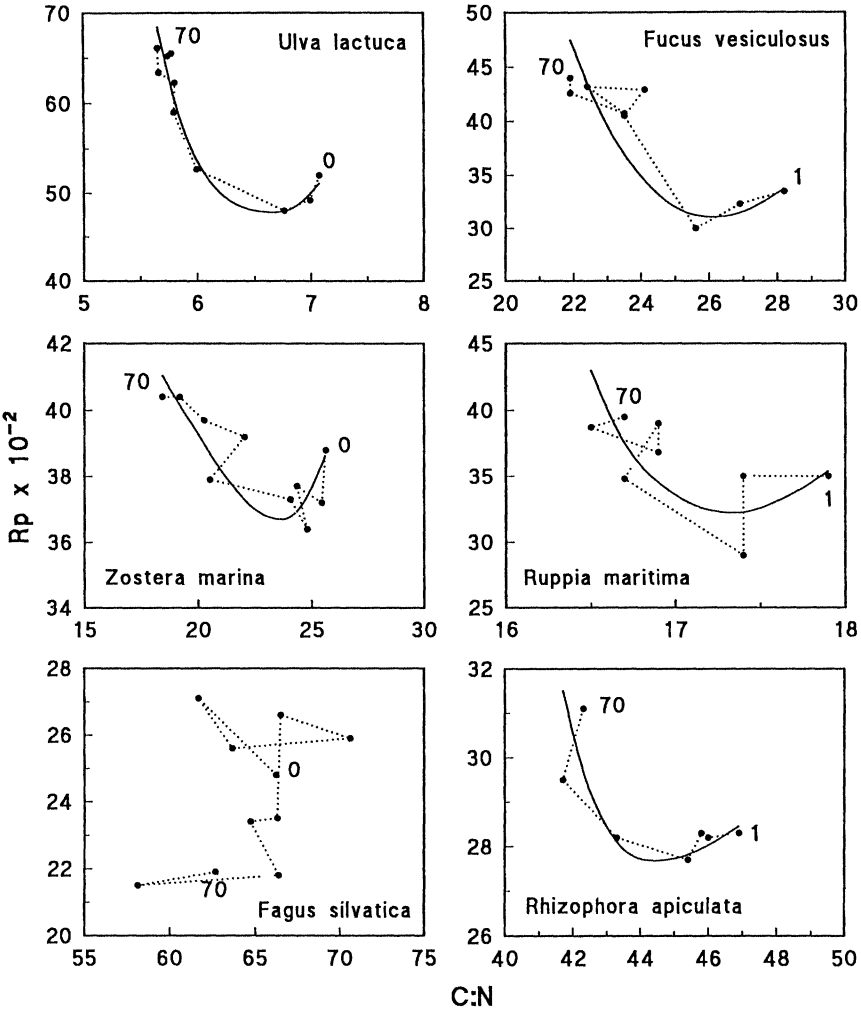

Fig. 6. Temporal relationships between the Rp index and C:N ratio of 6 different plant materials being decomposed aerobically in batch cultures. Dotted lines indicate the sequence of time points from the first (0 or 1) to the last sampling day (70). Smooth curves are drawn by eye.

first order rate constant, k_C, of 0.24 yr^{-1}. No major changes in pH were observed during the anaerobic incubation.

PON in the aerobic batch showed a pattern similar to POC, but with an initial increase of about 30% which peaked at day 28 (Fig. 8B). The C:N ratio of the material gained was 7.5 (Table 3). After a rapid decrease from day 28 to day 63, PON remained constant until day 132 followed by a 9% decrease until day 190. The C:N ratio of the material lost was 3.7. The initial PON increase in the anaerobic batch was only 6% (day 14) providing a C:N ratio of the gained material of 13. During the rest of the anaerobic incubation PON

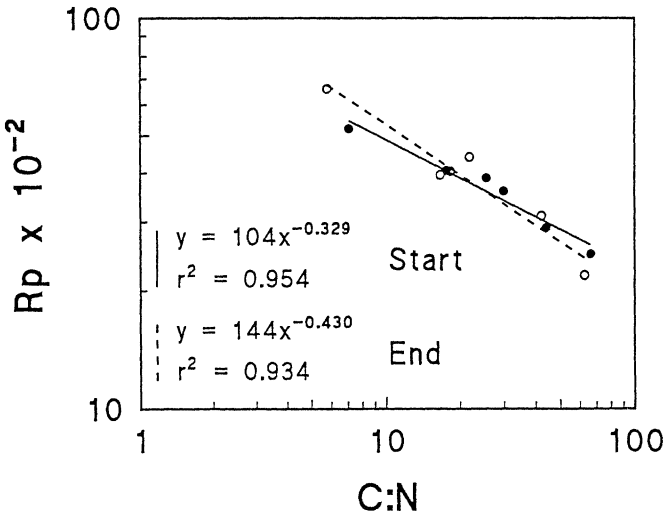

Fig. 7. The relationship between Rp index and C:N for the 6 plant materials examined, before (Start) and after (End) the 70 day decomposition period in batch cultures. Equations obtained by least squares linear regression are given.

decreased with a rate which was equivalent to a rate constant (k_N) of 0.81 yr^{-1}, reaching about 70% of the initial value at day 190. The C:N ratio of the material lost was 4.9.

The concentration of NH_4^+ in the aerobic batch was closely associated to pH (Fig. 8D). No NH_4^+ was detected when pH was below 3, and the increase in pH after day 42 was accompanied by a gradual increase in NH_4^+. The anaerobic batch, in contrast, showed a constant increase in NH_4^+ throughout the experiment at a rate (8.2 μM d^{-1}) similar to that found in the aerobic batch after day 42 (8.0 μM d^{-1}). The production of dissolved NH_4^+ in the two culture types only accounted for 7–10% of the measured loss of PON. The remainder is probably adsorbed to particles or transformed into DON.

The gradually increasing C:N ratio in both culture types after day 28 indicated that a preferential removal of N had occurred (Fig. 9A). The increase was largest in the active anaerobic (30%) compared to the aerobic batch (20%) resulting in final C:N ratios of 14.9 for the former and 13.7 for the latter. The decreasing C:N ratio observed within the first 28 days reflected the larger initial increase in POC relative to PON.

STG. The Rp index showed a temporal pattern similar to that of the C:N ratio, except for the first 42 days in the anaerobic batch (Fig. 9B). For the entire period, the increase in Rp was larger in the anaerobic batch (29%) than in the aerobic batch (19%). Within the first 30 days, the aerobic batch exhibited a steep decrease in Rp which corresponded to the decrease in C:N ratio during the same time period. In the anaerobic batch, on the other

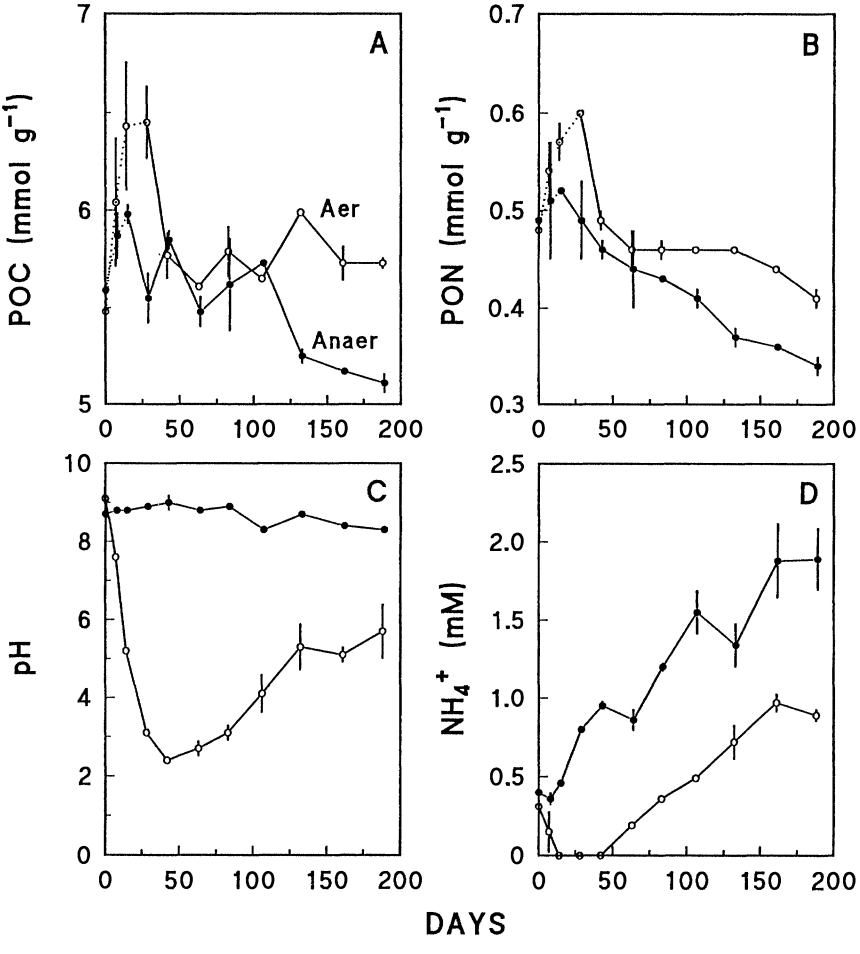

Fig. 8. Aerobic (Aer) and anaerobic (Anaer) sediment. Temporal changes of (A) POC, (B) PON, (C) pH and (D) NH_4^+ in batch cultures over 190 days. Error bars indicate ± S.D. of 4 cultures. Dotted lines in (A) and (B) represent the organic enrichment phase (not included in estimates of first order decay constants).

hand, Rp first started to decrease at day 28 after a rapid initial increase. This batch resumed the increasing trend after day 63. The anaerobic batch generally exhibited higher Rp values than the aerobic (final Rp of 0.54 and 0.51, respectively).

The Rp-C:N relationship of the sediment cultures showed a trend which was almost opposite to that of the plant materials in experiment 1 (Fig. 10). Except for some noise in the start due to initial variations in POC and PON content, the Rp-C:N relationship for both culture types showed almost similar linear patterns. Both Rp and C:N was generally lower in the aerobic compared to the anaerobic batch.

Table 3. Total net POC, PON and C:N change in aerobic and anaerobic sediment batch cultures (exp. 2). The budget is presented as total, with the initial gain (positive) and subsequent loss (negative) periods specified. Values are in μmol g dw^{-1}.

	GAIN	LOSS	TOTAL
Aerobic			
	day 0–28	day 28–188	day 0-188
ΔPOC	970	–720	250
ΔPON	130	–193	–63
C:N	7.5	3.7	–
Anaerobic			
	day 0–14	day 14–188	day 0-188
ΔPOC	390	–867	–477
ΔPON	30	–178	–132
C:N	13.0	4.9	3.6

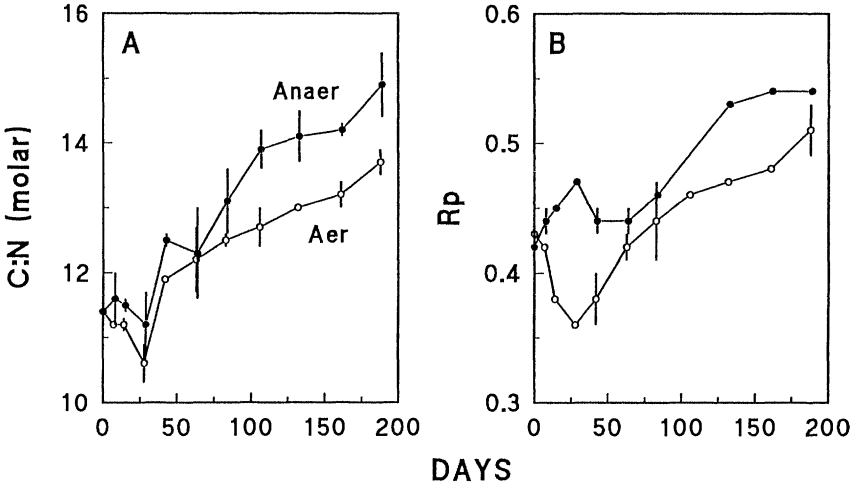

Fig. 9. Aerobic (Aer) and anaerobic (Anaer) sediment. Temporal changes of (A) C:N ratio (molar) and (B) Rp index in batch cultures over 190 days. Error bars indicate ± S.D. of 4 cultures.

Discussion

Decay of fresh materials

Although the present laboratory experiment not fully simulated the natural environment (i.e. pre-drying and slurry incubation of plant detritus), the initial decay rates of the selected plant species clearly reflect their chemical composition, origin and taxonomy. Aerobic decay was generally most rapid for

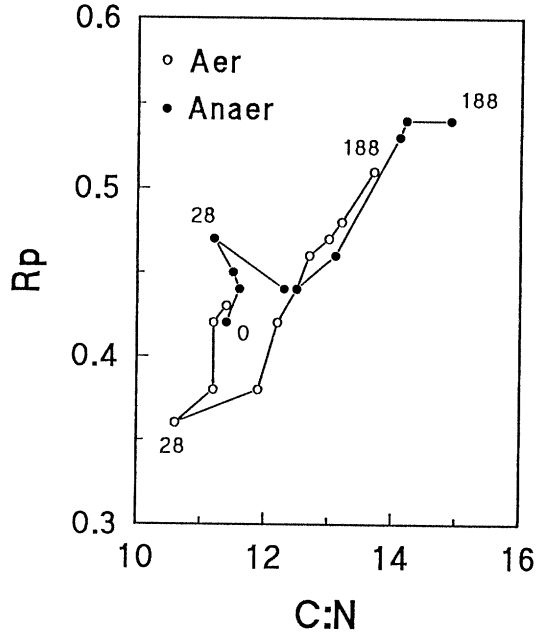

Fig. 10. Temporal relationships between the Rp index and C:N ratio of sediment detritus being decomposed under aerobic (Aer) and anaerobic (Anaer) conditions in batch cultures. Numbers shown represent the first (0), an intermediate (28) and the last sampling day (188).

macroalgae (i.e. *Ulva lactuca* and *Fucus vesiculosus*), intermediate for seagrasses (i.e. *Zostera marina* and *Ruppia maritima*) and slowest for tree leaves (i.e. *Fagus silvatica* and *Rhizophora apiculata*). Previous studies on aquatic and terrestrial plants have shown a similar pattern, indicating that decay of plant detritus is dependent on the size and lability of carbohydrate, phenolic (e.g. lignin) and organic nitrogen pools (Rice & Tenore 1981; Rice 1982; Valiela et al. 1984; Twilley et al. 1986; Buchsbaum et al. 1991; Enriquez et al. 1993).

Most of the carbohydrates in macroalgae are aliphatic, non-lignified polysaccharides of high degradability. The high Rp (0.36–0.52) observed for macroalgae also indicate a high protein content (Table 1), as Rp's of carbohydrates usually are in the range of 0.2–0.3 (Kristensen 1990). Accordingly, first order decay constants reported for macroalgal detritus are generally high, 3–18 yr^{-1} (Rice & Hanson 1984; Twilley et al. 1986), which is of similar magnitude or higher than the results obtained here for algal POC. Leaf detritus from trees, on the other hand, is usually rich in decay resistent lignocellulose and poor in protein, which is substantiated by the low Rp (0.25–0.29, Table 1) (Kristensen 1990). Reported first order decay constants of terrestrial tree leaves (e.g. *F. silvatica*) and leaves of mangrove trees (e.g. *R. apiculata*) are typically low, 0.1–0.3 yr^{-1} (Hamilton & Hedges 1988; Findlay et al. 1990), which is comparable to the present results for leaf POC. Seagrasses are

vascular plants rich in cellulose but with reduced need for supporting structural tissues due to their submersed growth form. Despite the high cellulose content, Rp's found for seagrasses are high (0.39–0.41, Table 1) compared with leaf detritus, probably due to the higher protein content (Kristensen 1990). Reported decay rates for seagrasses, typically 1.5–5.0 yr^{-1} (Harrison 1989), are somewhat higher than those obtained for seagrass POC in the present study.

The content and composition of organic nitrogen are important factors determining decay rates of plant detritus (Rice 1982; Lancelot & Billen 1985; Harrison 1989). The degradability of plant materials is generally considered directly proportional to the nitrogen content (Godshalk & Wetzel 1978b; Marinucci et al. 1983; Twilley et al. 1986; Enriquez et al. 1993). The decay of POC and PON in the present study was also most rapid for materials rich in nitrogen, i.e. those with lowest C:N ratio. The generally slower loss of PON compared to POC indicates, that through the action of aerobic microorganisms more nitrogen than carbon is retained during the early mineralization of plant materials. It is known that during initial decay of plant detritus having C:N > 10–15, the bacteria respires excess organic carbon to gain additional energy for synthesis of extra-cellular enzymes before sufficient nitrogen is obtained for biosynthesis and growth (Fenchel & Blackburn 1979; Linley & Newell 1984; Goldman et al. 1987; Tezuka 1990). Even in the present *Ulva lactuca* batch cultures with an initial organic C:N of ca. 7 and no external N-source, a decrease in C:N was observed. As C:N ratios of bacterial cells remain constant around 5 irrespective of the composition of substrates (Bratbak 1985; Goldman et al. 1987), the bacterial gross growth efficiency for carbon is lower than for nitrogen when detritus of higher C:N is being decomposed. The availability of organic nitrogen depends on the actual chemical form in which it is stored in plant cells. A significant amount of detrital nitrogen may exist as protein-carbohydrate, protein-phenol condensation products or non-protein compounds in the detritus matrix (Odum et al. 1979; Rice 1982), i.e. in a form not readily accessible for the bacteria. Total protein bound nitrogen of animal and plant tissues can be roughly estimated by dividing the protein content with 6.25 (Pirie 1955). In the present study, the initial amount of protein accounted for ca. 70% of total nitrogen in *Fucus vesiculosus*, 18% in *Ruppia maritima*, and 25% in *Fagus silvatica*. After 21 days the corresponding values were 45, 14, and 37%. For comparison, Harrison & Mann (1975a) determined that 38–90% of the nitrogen in *Zostera marina* detritus is protein bound. The remainder represent various less degradable non-protein compounds such as amino sugars associated with aromatic condensation products (Odum et al. 1979), which may be precursors of nitrogenous humic geopolymers (Sieburth & Jensen 1969).

Rapid aerobic decay of organic matter is usually associated with a high rate of O_2 uptake (Godshalk & Wetzel 1978b; Twilley et al. 1986; Peduzzi & Herndl 1991). In the present study, POC decay (k_C) and total O_2 uptake of the various plant cultures correlate well (Fig. 11A). The relationship is less

pronounced when PON decay (k_N) is considered. The very high O_2 uptake observed initially for the degradable materials (macroalgae) also corresponds to the high DOC content and very rapid loss of POC within the first few days. Initial leaching of DOC from dried and ground plant materials is usually known to account for up to 30% (10% here) of the initially added carbon (Otsuki & Hanya 1972; Harrison & Mann 1975b; Godshalk & Wetzel 1978a). The leached DOC compounds are in general highly reactive and are readily consumed by bacteria (Benner & Hodson 1985). In the present study, the initial leaching phase and subsequent rapid bacterial uptake and growth were terminated within the first 25 days. After this the decay rate was controlled by slow leaching and bacterial attack directly on the particulate substrates (Harrison 1989). The low initial O_2 uptake observed for the terrestrial leaf materials, on the other hand, is in accordance with the low DOC content and lack of measurable POC loss for these materials.

As mentioned by Kristensen (1990), a negative logarithmic relationship exists between Rp and C:N of fresh materials (Fig. 7). The initial slope found here (–0.329) for plants agrees well with that reported by Kristensen (1990) for a variety of animal, bacterial and plant materials (–0.246). Even after 70 days decomposition the relationship is still significant, although less strong, but with a 31% steeper slope. Eventually the relationship may break down due to specific decay patterns and humification pathways later in the process for each of the materials (Kristensen 1990).

The temporal pattern of the Rp index during aerobic decay was different for the various materials examined, although all showed an initial decrease followed by a gradually declining increase. Rp values of the start materials (except *F. vesiculosus*, which has an anomalous low Rp and high C:N) correlate significantly with decay constants of both POC and PON (Fig. 11B), suggesting that Rp of fresh materials can be used as an indicator of degradability. The very consistent (parabolic) relationship observed between the Rp index and the C:N ratio during initial decay of plant materials in seawater (except decay resistent materials as *F. silvatica*) suggests a general interaction, although of variable intensity, between microbes and their substrates (Fig. 6).

The decrease of both Rp and C:N found during initial aerobic decay was associated with leaching and rapid bacterial growth (phase 1, day 0 to 10). Minimum Rp was attained just after the peak in O_2 uptake and POC loss. High Rp materials such as non-structural proteins may account for a high fraction of leachates from plant materials (Otsuki & Hanya 1972; Rice & Tenore 1981; Buchsbaum et al. 1991). The amount of protein leached is generally highest during deacy of macroalgae (Buchsbaum et al. 1991). This is in accordance with the DOC:DON ratio observed initially in this study; within the first 5 days DOC:DON was ca 7 for *U. lactuca*, 13 for *Z. marina* and >50 for *F. silvatica*. During rapid bacterial growth, large ('light', low Rp) bacterial cells and quantities of extracellular mucopolysaccharides are produced (Hobbie & Lee 1980, Lee & Fuhrman 1987; van Duyl et al. 1992; Biddanda & Riemann

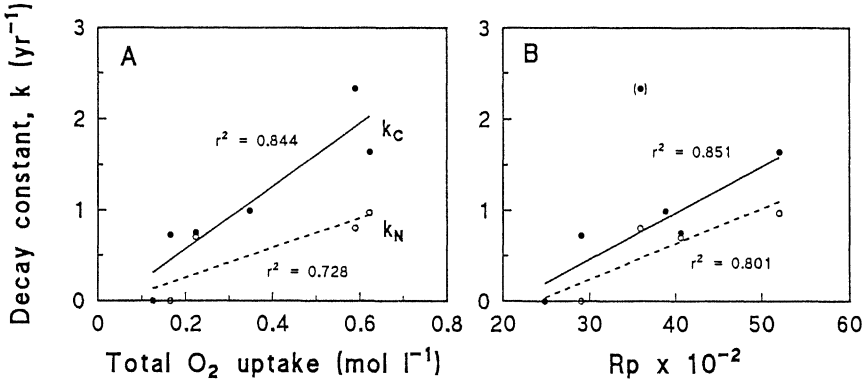

Fig. 11. The relationship between decay constants (carbon (k_C) and nitrogen (k_N)) and (A) total time integrated oxygen uptake and (B) the initial Rp index of various plant materials being decomposed for 70 days. Lines are obtained by least squares linear regression. Correlation coefficients are given. *Fucus vesiculosus* is excluded from the carbon regression analysis in (B) because of an anomalous low Rp relative to k_C.

1992). Furthermore, the leaching of proteinaceous material from the particulate substrates and subsequent rapid bacterial assimilation and growth reduces Rp and C:N; most for macroalgae and least for terrestrial trees. In a study of the decay pattern of tube material from the burrowing sea anemone, *Ceriantheopsis americanus*, Kristensen et al. (1991) found that the initial rapid decrease in both Rp and C:N was associated with a 10–30 fold increase in microbial ATP.

Later, when Rp started to increase and C:N continued to decrease (phase 2, day 10 to 35), the most degradable fractions have disappeared and bacterial growth ceased. Kristensen et al. (1991) similarly found a 50% reduction in ATP content during phase 2 of *C. americanus* tube decay. During this phase, live and dead bacterial cells may continue to enrich the entire particulate phase with nitrogen. Continued bacterial production of particulate, nitrogen-rich and condensed aromatic materials, e.g. amino-sugars in bacterial cell walls (Boon & Haverkamp 1982) drives C:N down and Rp up. The most degradable materials, *Ulva lactuca, Fucus vesiculosus* and *Ruppia maritima*, showed the most pronounced increase in Rp during phase 2, whereas tree leaves showed no or only a limited increase.

The final phase (phase 3, day 35 to 70), where no significant short-term (within the present experiment) changes occurred in Rp and C:N, was associated with a gradually slower bacterial activity due to the beginning of the humification process, i.e. condensation and/or polymerization (Hedges 1988). The 3 phases observed here during decay of plant materials are equivalent to those suggested by Valiela et al. (1984): 'a short-lived leaching phase, during which soluble materials are lost', 'a decomposer phase, during which organisms degrade litter', and 'a refractory phase, during which there is a slow

loss of litter'. In general, the degradation path from fresh to polymerized humic material appears to be faster for labile substrates (e.g. algae) than for less reactive substrates (e.g. leaf tissue).

Decay of sediment detritus

Sediment detritus is usually a heterogeneous mixture of diagenetically formed polymeric compounds (humic substances or geopolymers) and thus less degradable than most fresh plant materials (Westrich & Berner 1984; Ertel & Hedges 1985; Hedges et al. 1988). Coastal sediments, like those in Kolding Fjord, usually receive macrophyte detritus similar to that examined in the present 'Experiment 1' (Valiela et al. 1985; Peduzzi & Herndl 1991), and therefore may illustrate the fate of detritus during phase 3 of the decomposition sequence in more detail. The (anaerobic) decay constants of POC in the present nearshore marine sediment were only 10–15% of those obtained for pure *U. lactuca* and *F. vesiculosus* cultures, whereas decay constants of PON were similar to or only slightly lower than for the algae. The decay constants for both sedimentary POC and PON obtained under anaerobic conditions in this study are similar to those reported for other coastal sediments (Westrich & Berner 1984; Kristensen & Blackburn 1987; Burdige 1991; Hansen & Blackburn 1991). In aerobic cultures, respiration processes with O_2 as the terminal electron acceptor dominate the microbial decay, whereas a consortium of hydrolysing, fermentative and sulfate reducing bacteria controls decomposition in anaerobic cultures based on seawater (Burdige 1989). The preferential degradation of PON in the present sediment cultures reflects the condensed and polymeric nature of most sedimentary POC (Krom & Sholkowitz 1977; Aller 1980; Lancelot & Billen 1985; Hedges 1988). Anaerobic microbial decay (i.e. hydrolysis and fermentation) of carbon in humified and relatively nitrogen-poor sediment detritus can be very slow, whereas nitrogen can appear in more available forms. Kristensen & Blackburn (1987) suggested that the preferential decay of PON in sediments is due to the participation of bacterial biomass in the degradative process. If the structural components of the bacterial cells, which have a high C:N ratio, tend to accumulate after the cells have died and lysed, an increase in the residual organic detritus is expected. This will result in an apparent low C:N in the organic detritus (cell plasma) being oxidized.

The rapid initial increase in POC and to a minor extent PON in both the aerobic and the anaerobic batch cultures may be attributed to an initial rapid bacterial growth. A similar increase in sediment organic content due to microbial growth have previously been reported (Kepkay et al. 1979; Kristensen et al. 1991). Handling of the sediment before the experiment may have provided reduced inorganic electron donors (e.g. HS^-) and liberated labile DOC and DON in sufficient quantities (not measured, unfortunately), which acted as substrates for autotrophic and heterotrophic bacterial growth. The dramatic decrease in POC observed for both culture types after 40–50 days indicates

microbial death and rapid decay of labile detritus (e.g. dead bacterial cells). Novitsky (1986) have shown that 30–40% of the carbon in dead bacterial cells is degraded within 3–7 days. The slow decay after day 50 in the anaerobic batch was caused by continued bacterial attack (hydrolysis, fermentation and respiration) on the more refractory, original detritus pool. In the aerobic culture, the low pH attained after 20 days restrained microbial activity. However, the gradually increasing NH_4^+ concentration after day 50 and loss of PON after day 130 indicate a pH independent recovery of heterotrophic activity. The pH effect on the aerobic culture obstructs the comparison between decay rate under oxic and anoxic conditions.

The initial variations in the POC and PON pools are also evident from the C:N and Rp data. The initial decrease in C:N (until day 25) indicates a production of low C:N organic matter (e.g. microbial biomass). The subsequent gradual increase in C:N confirms the preferential mineralization of PON in the sediment. The initial Rp patterns, on the other hand, substantiates that microbial processes, the bacterial community and the composition of produced detritus material (e.g. cells) are different in aerobic and anaerobic sediment. The initial disturbance after handling lasted for about 30 days before phase 3 actually started. Both the aerobic and the anaerobic batches showed the same phase 3 pattern with increase in both Rp and C:N, indicating that the humification (polymerization or condensation) processes occurred simultaneously under both aerobic and anaerobic conditions and that it may proceed without microbial involvement in aerobic cultures. Only the anaerobic batch appeared to reach a constant level (at least within the present time-scale) at $Rp \approx 0.54$ and $C:N \approx 15$. The partially pH inhibited aerobic batch was generally lower in both parameters, but approached the same level at the end. The final C:N of 15 is within the range proposed as the ultimate C:N of 'non-degradable' and old humic geopolymers (Lancelot & Billen 1985), indicating that the decomposition process on the time-scale examined was almost terminated.

Conclusions

The present study suggests that the Rp index can be a good predictor of composition and biodegradability of plant materials. Rp is not only directly proportional to decay rate, but also correlates with factors which have long been assumed to influence decomposition rates; e.g. nitrogen content, C:N ratio and lignin content of the detritus. The decay pathway from fresh and labile plant material to refractory humic geopolymers can be illustrated by the Rp-C:N relationship. During the initial decay of fresh plant material, rapid leaching of non-structural proteins and microbial growth primarily on DOC and DON induces a short-term decrease in Rp (Fig. 12, 'Rapid growth', phase 1). However, the simultaneous production of nitrogen rich bacterial cells and otherwise nitrogen enrichment of the detritus reduces C:N. When the initial rapid growth phase has ended and all labile DOC and DON have been consumed, the bacterial decay proceeds more slowly by enzymatic attack

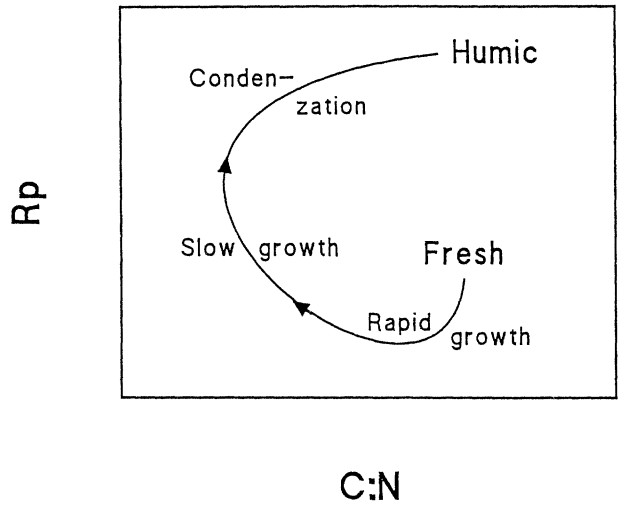

Fig. 12. Generalized Rp-C:N pattern of plant detritus during the decay sequence from fresh material to the end of humification. The 3 major decay phases are indicated; phase 1: Rapid growth, phase 2: Slow growth, phase 3: Condenzation. Scales on the Rp and C:N axis and the shape of the curve may depend on the actual source material examined.

directly on the particles (Fig. 12, 'Slow growth', phase 2). The gradual consumption of aliphatic carbohydrates, initial production of humic compounds and production of protein-rich and condensed cell materials increases Rp and decreases C:N. Later, when all labile materials are consumed, decay ceases while the production of condensed humic compounds continues (Fig. 12, 'Condenzation', phase 3). At this stage most carbon is bound in forms which are of low availability to bacteria and a preferential mineralization of nitrogen occurs; both Rp and C:N increases. Most mineralized nitrogen may origin from dead and dying bacterial cells. The first two phases in Fig. 12 represent the more or less rapid aerobic decay of plant materials before burial into the sediment. The last phase illustrates slow anaerobic decomposition and humification within the sediment. By moving along the line from 'Fresh' to 'Humic', the time horizon increases exponentially.

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References

Aller RC (1980) Diagenetic processes near the sediment-water interface of Long Island Sound. I. Decomposition and nutrient elements geochemistry (S, N, P). Adv. Geophys. 22: 237–350

Benner R & Hodson RE (1985) Microbial degradation of the leachable and lignocellulosic components of leaves and wood from *Rhizophora mangle* in a tropical mangrove swamp. Mar. Ecol. Prog. Ser. 23: 221–230.

Benner R, Maccubbin AE & Hodson RE (1984) Preparation, characterization, and microbial degradation of specifically radiolabelled [^{14}C]lignocelluloses from marine and freshwater macrophytes. Appl. Environ. Microbiol. 47: 381–389

Biddanda B & Riemann F (1992) Detrital carbon and nitrogen relations, examined with degrading cellulose. P.S.Z.N.I. Mar. Ecol. 13: 271–283

Boon JJ & Haverkamp J (1982) Pyrolysis mass spectrometry of intact and decomposed leaves of *Nuphar variegatum* and *Zostera marina*, and some archeological eelgrass samples. Hydrobiol. Bull. 16: 71–82

Bratbak G (1985) Bacterial biovolume and biomass estimations. Appl. Environ. Microbiol. 49: 1488–1493

Buchsbaum R, Valiela I, Swain T, Dzierzeski M & Allen S (1991) Available and refractory nitrogen in detritus of coastal vascular plants and macrolagae. Mar. Ecol. Prog. Ser. 72: 131–143

Burdige DJ (1989) The effects of sediment slurring on microbial processes, and the role of amino acids as substrates for sulfate reduction in anoxic marine sediments. Biogeochemistry 8: 1–23

Burdige DJ (1991) The kinetics of organic matter mineralization in anoxic marine sediments. J. Mar. Res. 49: 727–761

Duyl FC van, Kop AJ, Kok A & Sandee AJJ (1992) The impact of organic matter and macrozoobenthos on bacterial and oxygen variables in marine sediment boxcosms. Neth. J. Sea Res. 29: 343–355

Enriquez S, Duarte CM & Sand-Jensen K (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94: 457–471

Ertel JR & Hedges JI (1985) Sources of sedimentary humic substances: Vascular plant debris. Geochim. Cosmochim. Acta 49: 2097–2107

Fenchel T & Blackburn TH (1979) Bacteria and Mineral Cycling. Academic Press, New York

Findlay S, Howe K & Austin HK (1990) Comparison of detritus dynamics in two tidal freshwater wetlands. Ecology 71: 288–295

Godshalk GL & Wetzel RG (1978a) Decomposition of aquatic angiosperms. I. Dissolved components. Aquat. Bot. 5: 281–300

Godshalk GL & Wetzel RG (1978b) Decomposition of aquatic angiosperms. II. Particulate components. Aquat. Bot. 5: 301–327

Godshalk GL & Wetzel RG (1978c) Decomposition of aquatic angiosperms. III. *Zostera marina* L. and a conceptual model of decomposition. Aquat. Bot. 5: 329–354

Goldman JC Caron DA & Dennett MR (1987) Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnol. Oceanogr. 32: 1239–1252

Hamilton SE & Hedges JI (1988) The comparative geochemistries of lignins and carbohydrates in an anoxic fjord. Geochim. Cosmochim. Acta 52: 129–142

Hansen LS & Blackburn TH (1991) Aerobic and anaerobic mineralization of organic material in marine sediment microcosms. Mar. Ecol. Prog. Ser. 75: 283–291

Harrison PG (1989) Detrital processing in seagrass systems: A review of factors affecting decay rates, remineralization and detritivory. Aquat. Bot. 23: 263–288

Harrison PG & Mann KH (1975a) Chemical changes during the seasonal cycle of growth and decay in eelgrass (*Zostera marina*) on the Atlantic coast of Canada. J. Fish. Res. Board Can. 32: 615–621

Harrison PG & Mann KH (1975b) Detritus formation from eelgrass (*Zostera marina* L.): The relative effects of fragmentation, leaching, and decay. Limnol. Oceanogr. 20: 924–934

Hedges JI (1988) Polymerization of humic substances in natural environments. In: Frimmel FH & Christman RF (Eds) Humic Substances and Their Role in the Environment (pp 45–58) John Wiley, Chichester

Hedges JI, Clark WA & Cowie GL (1988) Organic matter sources to the water column and surficial sediments of a marine bay. Limnol. Oceanogr. 33: 1116–1136

Hobbie JE & Lee C (1980) Microbial production of extracellular material: importance in benthic ecology. In: Tenore KR & Coull BC (Eds) Marine benthic dynamics (pp 341–346) Univ. S. Carolina Press, Columbia

Kepkay PE, Cooke RC & Novitsky JA (1979) Microbial autotrophy: A primary source of organic carbon in marine sediments. Science 204: 68–69

Kristensen E (1990) Characterization of biogenic organic matter by stepwise thermogravimetry (STG). Biogeochemistry 9: 135–159

Kristensen E & Andersen FØ (1987) Determination of organic carbon in marine sediments: A comparison of two CHN-analyzer methods. J. Exp. Mar. Biol. Ecol. 109: 15–23

Kristensen E & Blackburn TH (1987) The fate of organic carbon and nitrogen in experimental marine sediment systems: Influence of bioturbation and anoxia. J. Mar. Res. 45: 231–257

Kristensen E, Aller RC & Aller JY (1991) Oxic and anoxic decomposition of tubes from the burrowing sea anemone *Ceriantheopsis americanus*: Implications for bulk sediment carbon and nitrogen balance. J. Mar. Res. 49: 589–617

Kristensen E, Devol AH, Ahmed SI & Saleem M (1992) Preliminary study of benthic metabolism and sulfate reduction in a mangrove swamp of the Indus Delta, Pakistan. Mar. Ecol. Prog. Ser. 90: 287–297

Krom MD & Sholkovitz ER (1977) Nature and reactions of dissolved organic matter in the interstitial waters of marine sediments. Geochim. Cosmochim. Acta 41: 1565–1573

Lancelot C & Billen G (1985) Carbon-nitrogen relationships in nutrient metabolism of coastal marine ecosystems. Adv. Aquat. Microbiol. 3: 263–321

Lee S & Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl. Environ. Microbiol. 53: 1298–1303

Linley EAS & Newell RC (1984) Estimates of bacterial growth yields based on plant detritus. Bull. Mar. Sci. 35: 409–425

Marinucci AC, Hobbie JE & Helfrich JVK (1983) Effect of litter nitrogen on decomposition and microbial biomass in *Spartina alterniflora*. Microb. Ecol. 9: 27–40

Meyer E & Walther A (1988) Methods for the estimation of protein, lipid, carbohydrate and chitin levels in fresh water invertebrates. Arch. Hydrobiol. 113: 161–177

Novitsky JA (1986) Degradation of dead microbial biomass in a marine sediment. Appl. Environ. Microbiol. 52: 504–509

Odum WE, Kirk PW & Zieman JC (1979) Non-protein nitrogen compounds associated with particles of vascular plant detritus. Oikos 32: 363–367

Otsuki A & Hanya T (1972) Production of dissolved organic matter from dead green algal cells. I. Aerobic microbial decomposition. Limnol. Oceanogr. 17: 248–257

Peduzzi P & Herndl GJ (1991) Decomposition and significance of seagrass leaf litter (*Cymodocea nodosa*) for the microbial food web in coastal waters (Gulf of Trieste, Northern Adriatic Sea). Mar. Ecol. Prog. Ser. 71: 163–174

Pirie NW (1955) Proteins. In: Peach K & Tracey V (Eds) Modern methods of plant analysis. Vol 4 (pp 314–367). Springer, Heidelberg

Rausch T (1981) The estimation of micro-algal protein content and its meaning to the evaluation of algal biomass I. Comparison of methods for extracting protein. Hydrobiologia 78: 237–251

Rice DL (1982) The detritus nitrogen problem: New observation and perspectives from organic geochemistry. Mar. Ecol. Prog. Ser. 9: 153–162

Rice DL & Hanson RB (1984) A kinetin model for detritus nitrogen: Role of the associated bacteria in nitrogen accumulation. Bull. Mar. Sci. 35: 326–340

Rice DL & Tenore KR (1981) Dynamics of carbon and nitrogen during the decomposition of detritus derived from estuarine macrophytes. Estuar. Coast. Shelf Sci. 13: 681–690

Round FE (1973) The Biology of the Algae. 2nd ed. Edward Arnold Publ., London
Sansone FJ & Martens CS (1982) Volatile fatty acid cycling in organic-rich marine sediments. Geochim. Cosmochim. Acta 46: 1575–1589
Sieburth JM & Jensen A (1969) Studies on algal substances in the sea. II. The formation of Gelbstoff (humic material) by exudates of Phaeophyta. J. Exp. Mar. Biol. Ecol. 3: 275–289
Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14: 799–801
Sugimura Y & Suzuki Y (1988) A high-temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. Mar. Chem. 24: 105–131
Tezuka Y (1990) Bacterial regeneration of ammonium and phosphate as affected by the carbon:nitrogen:phosphorus ratio of organic substrates. Microb. Ecol. 19: 277–238
Twilley RR, Ejdung G, Romare P & Kemp WM (1986) A comparative study of decomposition, oxygen consumption and nutrient release for selected aquatic plants occurring in an estuarine environment. Oikos 47: 190–198
Valiela I, Wilson J, Buchsbaum R, Rietsma C, Bryant D, Foreman K & Teal J (1984) Importance of chemical composition of salt marsh litter on decay rates and feeding by detritivores. Bull. Mar. Sci. 35: 261–269
Valiela I, Teal JM, Allen SD, van Etten R, Goehringer D & Volkman S (1985) Decomposition in salt marsh ecosystems: The phases and major factors affecting disappearance of above-ground organic matter. J. Exp. Mar. Biol. Ecol. 89: 29–54
Westrich JT & Berner RA (1984) The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested. Limnol. Oceanogr. 29: 236–249
Wilson JO, Valiela I & Swain T (1986) Carbohydrate dynamics during decay of litter of *Spartina alterniflora*. Mar. Biol. 92: 277–284