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Decomposition and microbial dynamics for standing, naturally positioned leaves of the salt-marsh grass *Spartina alterniflora*

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Abstract

Decomposition of leaves of smooth cordgrass (*Spartina alterniflora* Loisel.) was monitored for two cohorts of leaves from September 1984 to May 1985 (autumn and winter-spring) at Sapelo Island (31°23′ N; 81°17′ W). The leaves were tagged in place at the ligule, rather than cut and placed in litterbags. Dead leaves were not abscised from shoots. Loss of organic mass from the attached leaves was at least 60 to 68% of the original values. Fungal mass, as measured by an enzyme-linked immunosorbent assay, formed >98% of the microbial standing crops in two of three autumn samples, and in all samples for the colder, drier, winter-spring cohort. Fungal mass was probably mostly in the form of the mycelium and pseudothecia of an ascomycete, *Phaeosphaeria typharum* (Desm.) Holm. Fungal dominance of microbial standing crops declined when autumn leaves bent downward and acquired a large sediment content (ash = 35% of dry matter); the bacterial crop then rose to 7% of the total microbial crop. Microphotoautotrophic mass was always measurable, but was never more than 2% of the microbial crop. Carbon-dioxide fixation was much lower than carbon-dioxide release, and a substantial portion of the fixation may have been anaplerotic fungal fixation. Three- to 8 wk net fungal productivity (average per day) was much greater (16 to 26 times) than measured instantaneous bacterial productivity (extrapolated to per-day values) early in each decay period. Fungal productivity was negative late in the decay period for autumn leaves, and was approximately equal to bacterial productivity late for winter-spring leaves. Net nitrogen immobilization was observed only late in the decay period for autumn leaves, implying that nearly all dead-leaf nitrogen was scavenged into fungal mass after the first sampling interval. Flux estimates for dead-leaf carbon indicated a flow of 11–15% of the original to fungal mass, 2% to bacterial mass, 15–21% to carbon dioxide, 10–12% to dissolved leachate, and 34–36% to small particles; 32–39% remained attached as shreds at the end of the study periods. Salt-marsh periwinkles (*Littorina irrorata* Say) appeared to be the major shredders of dead leaves and conveyors of leaf-particulate material to the marsh sediment, at least in those parts of the marsh where the snails are densely concentrated (usually areas of short- and intermediate-height cordgrass shoots).

Introduction

Cutting of shoot material is commonly involved at the outset of a study of decomposition of aboveground material of the smooth cordgrass *Spartina alterniflora* (Christian 1984). Severed shoots are placed in litterbags on the marsh surface to begin their decay. This results in marked changes in the physical/chemical environmental conditions (moisture, light, nitrogen access, oxygen access, shredder access) for decomposer microbes and invertebrates (Swift et al. 1979), and is contraindicated in attempts to simulate natural decomposition since (a) leaves of *S. alterniflora* are not naturally abscised (Sutherland and Eastwood 1916), and (2) assemblage of microorganisms is adapted to the natural, standing-dead system (Newell et al. 1985). In a direct comparison of decay phenomena of natural, standing leaves of *S. alterniflora* and leaves cut and placed in litterbags, the cut leaves exhibited sharply different patterns of microbial dynamics (Newell and Fallon 1989). In order to determine how cordgrass decomposition would proceed under natural circumstances, we followed Hardisky (1980) and Hicks (1983) by using plastic tags to mark senescent leaves in situ.

Materials and methods

Bright orange, plastic electrical-cable ties (Pan-Ty PLT1M-M3, Panduit Corporation) were used to tag leaves within a 10 × 1 m plot of intermediate-height (tallest shoots ca. 1 m) *Spartina alterniflora* Loisel., 100 m north of the edge of Doboy Sound on Sapelo Island, Georgia (31°23′ N; 81°17′ W:

Table 1. Basic environmental data for tagging site at Sapelo Island, Georgia, USA. *T* and S: measured in creekwater adjacent to tagging site between 09.00 and 11.00 hrs; Rainfall: approximate duration of rainfall between sampling dates and (quantity of rainfall); High tides: number of diurnal high tides which rose to or above average height above the sediment of tagged-leaf ligules; Leaf wetness: average hours per diel period in which leaves near ground were recorded as wet (dew or rain) with an electronic sensor at Hastings, Florida, a 25 km inland site 180 km SSW of tagging site; because site was inland, estimation of dew persistence may be conservative for the marsh. nd: no data

Date	*T*	S (‰)	Rainfall	High tides	Leaf wetness
5 Sep. 1984[a]	26 °C	22	–	–	–
24 Sep. 1984	24 °C	31	23 h (5.5 cm)	27	13.5
17 Oct. 1984	22 °C	30	23 h (17.1 cm)	35	nd
27 Nov. 1984	15 °C	27	71 h (12.6 cm)	62	nd
19 Dec. 1984[a]	14 °C	26	–	–	–
30 Jan. 1985	10 °C	32	15 h (2.9 cm)	14	11.6
14 Mar. 1985	15 °C	25	39 h (11.4 cm)	16	10.7
15 May 1985	25 °C	28	31 h (20.2 cm)	26	10.3

[a] Initial sampling date in series of four

Table 2. *Spartina alterniflora*. Data for mature green leaves of smooth cordgrass. No data were obtained for algal volume, CO_2 exchange, or bacterial productivity. ELISA: enzyme-linked immunosorbent assay

Variable	Mean ±1 SD
Leaf area[a]	26 ±4 cm^2
Organic mass[a]	281 ±43 mg
Ash	11 ±0%
Carbon	45 ±1%
Nitrogen	1 ±0.1%
Bacterial vol[a]	0.1 $\pm 0.0 \times 10^{-2}$ mm^3
Fungal vol[a]	$<10 \times 10^{-2}$ mm^3
ELISA-fungal mass	<3 mg organic g^{-1} organic
Ergosterol	<0.1 μg g^{-1} organic

[a] These values given per standard sample (36 cm length of leaf)

Pomeroy and Wiegert 1981). Basic physical/chemical data for the site are given in Table 1. Tags were looped onto leaves at the ligule, just tightly enough to hold without deforming the leaf. Leaves were tagged at the yellow-green, senescent stage, defined here as leaves with at least 25% but less than 100% yellow coloration. Tags (ca. 500) were placed on leaves on two dates (5 September and 19 December, 1984). The mean height of the tagged-leaf ligules above the marsh sediment was 15 cm (September) and 30 cm (December). The difference in height resulted from the fact that leaves of *S. alterniflora* die in succession from the sediment up during the growing season (Hardisky 1980).

Mature green (September only; Table 2) and yellow-green leaves were collected at the time of tagging, and tagged leaves were collected at 3 to 8 wk intervals thereafter. Leaves were cut at the ligule, rinsed in seawater from a settling tank (2 min agitation in each of three changes), and three standard 3 cm-long pieces were cut from each leaf. The standard pieces were: basal, 5 cm distal to ligule; tip, 15 cm distal to tip; and mid, the approximate center of the leaf. For each variable measured (except where otherwise indicated), four leaves (12 standard pieces) were pooled to serve as individual replicate samples, and there were four replicate samples per time of collection.

Two sets of four replicate samples were stored in buffered, seawater-formaldehyde solution at 4 °C in darkness, one set for subsequent acridine-orange direct counting/sizing (AODC) of bacteria and epifluorescence direct-microscopic, conservative determination of fungal-hyphal volume (Newell et al. 1985), and one set for direct-microscopic determination of epiphytic microphotoautotroph volume (Fallon et al. 1985). One set of four replicates was frozen to −70 °C in Whirl-Pak bags for a subsequent enzyme-linked immunosorbent assay (ELISA; alkaline-phosphatase-linked rabbit antibodies) (Fallon and Newell 1989; based on Johnson et al. 1982) of the total (live plus dead) mass of *Phaeosphaeria typharum* (Desm.) Holm, the predominant fungal secondary producer in leaves of *Spartina alterniflora* (Newell and Fallon 1983).

The content of living (membrane-containing) fungal mass within leaves was estimated via analysis of ergosterol content (Newell et al. 1986). Large samples of leaves (50 per sampling time) were rinsed, lyophilized, ground and pooled for analysis by high-pressure liquid chromatography (HPLC: Miller et al. 1983).

Leaf pieces for determination of rates of bacterial cell production were soaked for 1 h in settled seawater from the marsh in Whirl-Pak bags. Then the rate of incorporation of [methyl-^{3}H] thymidine incorporation into DNA was measured at field temperature (Table 1) (Fallon and Newell 1986; 4 μ*M* added thymidine in autoclaved seawater, 1.5 h incubation). The factor for conversion from moles thymidine incorporated to bacterial cells produced (4×10^{18} cells mol^{-1}) was determined as the mean from three experiments performed in the fashion of Kirchman et al. (1982), except that bacterial assemblages (0.8 μm filtrates, 60 cm Hg vacuum filtration) were grown on autoclave-sterilized dead *Spartina alterniflora* leaves (Fallon and Newell 1986). Thymidine incorporation was measured for conversion-factor experiments in the same way as for field samples, by removing leaves to autoclaved seawater. Conversion of bacterial cell production to bacterial organic-mass production was made using measured mean cell volumes and an average factor (570 fg μm^{-3}) for bacterial organic density from the literature (Newell et al. 1987).

Rates of carbon-dioxide release were measured for four replicate samples by infrared gas analysis (Newell et al. 1985). Just prior to analysis, leaf samples were photocopied for later determination of total leaf area. Leaf pieces were soaked in settled seawater for 15 min, drained, and incubated in stoppered serum vials at 100% relative humidity in darkness for 1 h at field temperature (Table 1). The vials were then flushed for 10 s with standard compressed air, restoppered, and incubated for another hour, after which gas samples were withdrawn and immediately analyzed. The first incubation in darkness permitted initial CO_2 bursts and

remnant light-stimulated CO_2 fixation to be excluded from measurement of net CO_2 exchange (Legendre et al. 1983, Newell et al. 1985). Following withdrawal of gas samples, the leaf pieces were dried to constant weight at 100 °C. The weights were recorded and the leaf pieces were each cut in half. One half of each replicate set was weighed, ashed (450 °C, 4 h) and reweighed for determination of organic content. The other half of each replicate set was ground for analysis of carbon and nitrogen content (Perkin-Elmer Model 240 CHN analyzer; Newell et al. 1984). Acetanilide used as a standard gave correct values for citrus leaves from the U.S. National Bureau of Standards.

Carbon-dioxide fixation into standing leaves was measured as $^{14}CO_2$ incorporation into organic matter by a method derived from that of Darley et al. (1976). Six leaf pieces (each 6 cm in length) were used. These pieces were derived from two leaves. Basal, middle, and tip sections were cut from each leaf. Sections were placed into 250 ml plastic cell-culture bottles in the natural attitude (i.e., abaxial side down). These bottles had been modified by drilling holes in the sides and placing two 4 mm diam glass rods across the major axis width of the bottle, approximately 1 and 8 cm from the bottom, respectively. The rods were sealed in place with silicone adhesive. These glass rods held the leaf pieces in place, out of contact with the sides of the bottle.

Five light, five dark (aluminum foil covers), and four killed-control (autoclaved, 15 min at 121 °C) replicates were connected in series to a manifold and peristaltic pump which circulated air at about 300 ml min^{-1} through all replicates. A heat-exchanging coil, cooled by tap water, was included in the loop in order to ensure that temperature remained near ambient. The total system volume was about 4 liters. Bottles and leaves were held at approximately 45° to horizontal. With samples held in the dark, air was circulated for 1 h before starting the experiment. Incubations were performed outdoors, at the edge of the marsh.

At the end of the equilibration period, 100 μCi of gaseous $^{14}CO_2$ was added to the system. After 15 min, the cover was removed, exposing the bottles to sunlight. A 1 ml sample of air was taken for total CO_2 and gaseous radioactivity. Temperature and light readings were recorded periodically throughout the experiment. Samples for total CO_2 and radioactivity were collected hourly. A neutral-density, gray, fiberglass screen was used to keep the light intensity $\leq 1\,300$ $\mu E\ m^{-2}\ s^{-1}$. Preliminary experiments had shown that photoinhibition occurred above this light intensity. The incubation was ended after 3 h by adding 10 ml of ethanol to each of the replicates.

Samples were processed by adding 30 ml of distilled water to the contents of each sample bottle. The suspension was homogenized in a Waring blender for 3 min. A 1 ml sample of the suspension was filtered onto a Gelman A/E glass-fiber filter. Solids collected on the filter were exposed to fumes of concentrated HCl overnight to remove inorganic CO_2. The filtrate was acidified with 85% phosphoric acid and sparged for 1 h to remove inorganic CO_2. One ml of methyl benzethonium hydroxide was added to each retentate sample. These were held overnight to partially digest the leaf material. Radioactivity in the processed samples and gas samples was measured by liquid scintillation counting with quench correction. Light and dark values were corrected for killed-control values.

Our measurements of instantaneous rates of primarily saprotrophic microbial activity (bacterial growth rate, total CO_2 release) were for submerged or water-saturated leaves, whereas leaves in the marsh undergo irregular drying/wetting cycles. We used the data in Table 1 to calculate the approximate percentage of time per diel period that leaves were wet during the standing-decay intervals. The leaf-wetness data in Table 1 are consistent with measurements of rainfall and periods of 90 to 100% relative humidity in the marshes of Sapelo in autumn-spring 1987–1988 (T. Arsuffi and L. Scott personal communication), and with measurements of summer dew persistence in a temperate meadow at ground level [$\bar{x} \cong 10$ h (diel period)$^{-1}$: see Dowding 1986]. Assuming that half of the leaf-contacting tides (Table 1) did not coincide with dew or rain, and resulted in 2 h of wetness, dew plus coincident tidal submergence would account for some 80% of total wetting (rain, 9%; non-coincident tides, 10%) for the autumn leaves, and autumn leaves would be wet 62% of the time; corresponding values for winter-spring leaves: dew, 91%; rain, 5%; tides, 3%; percent time wet, 47%. We averaged the rate values for carbon release as CO_2 and microbial values (latter from Table 5, omitting periods of net loss for fungal mass), and multiplied by the duration of wetness to obtain estimates for flow of carbon from dead leaves to microbial mass, carbon dioxide, and particulate/leachate/translocation output. Note that algal contributions were ignored. (The data in Table 4 indicate that this is justifiable.) We calculated leachate values using data from Gallagher and Pfeiffer (1977) for rate of carbon leaching from standing-dead *Spartina alterniflora*, and our estimates for duration of rain and tidal wetting.

Results

The first (autumn) cohort of tagged *Spartina alterniflora* leaves lost both organic mass and leaf area from standard samples in a smooth fashion (exponential decay constants: organic mass, $k = -0.011$ d^{-1}; leaf area, -0.006 d^{-1}; $r^2 = 0.93$ to 0.95), resulting in losses of 60% of organic mass and 37% of leaf area by 83 d after tagging of leaves (Fig. 1 a). The loss of measurable leaf area was a combination of loss of material and collapse of leaf mesophyll between longitudinal vascular bundles. By 83 d, this loss of mesophyll had resulted in the reduction of leaves into tangles of long shreds (Fig. 2). In spite of this extensive degradation of leaves, 92% of the autumn tagged leaves were recovered in standing position, although many leaves had bent down and were touching the sediment.

Although all leaves had turned brown by 42 d after tagging, the second (winter-spring) cohort of leaves lost little mass or leaf area until after mid-March (Fig. 1 a). This cohort experienced colder and drier conditions (Table 1). If the losses over the whole sampling period (December–May) are

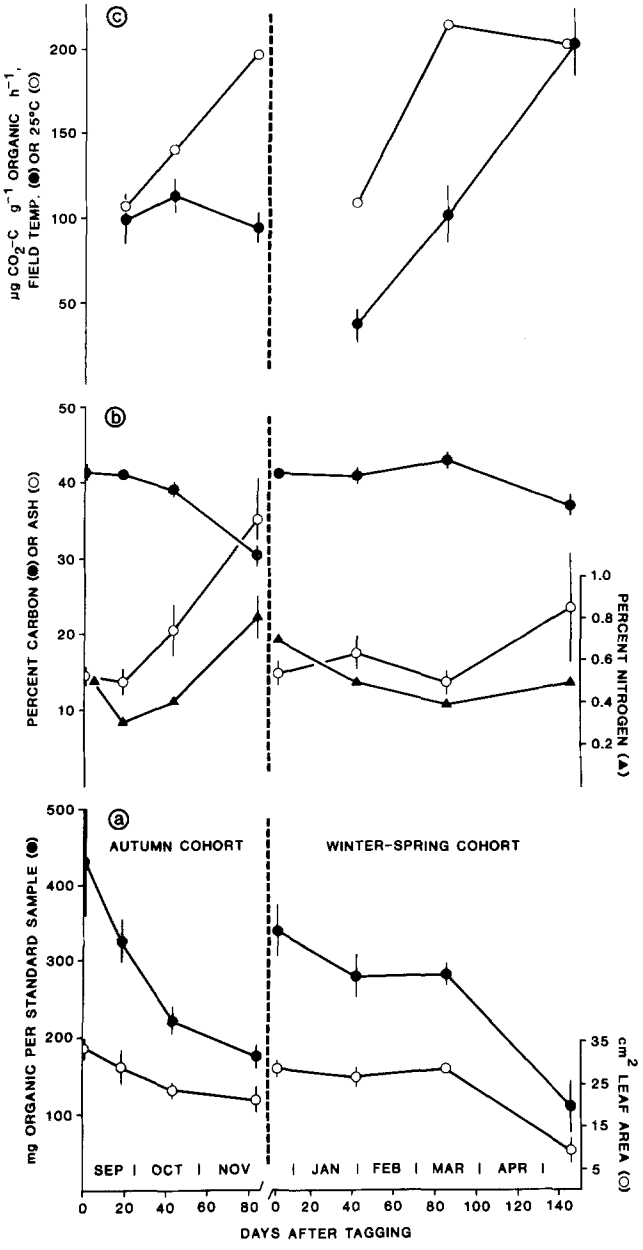

Fig. 1. *Spartina alterniflora.* Dynamics of organic mass, ash, carbon, and nitrogen for tagged leaves decaying in place from September 1984 to May 1985. (a) Organic mass and leaf area per standard sample (see "Materials and methods" for description of standard sample); (b) percentages of dry mass for carbon, nitrogen and ash; (c) rate of release of carbon dioxide from water-saturated leaves in air, at field temperature and at 25°C. Bars indicate one standard deviation

treated as exponential, then decay constants are only marginally different from those of the first cohort of leaves (organic mass, $k = -0.007\ d^{-1}$; leaf area, $k = -0.007\ d^{-1}$) but r^2 is lower (0.69 to 0.79). Percentage losses from original values at 147 d were organic mass 68%, and leaf area 66%; most of the loss occurred between 85 and 147 d (Fig. 1a). Ninety-seven percent of the tagged leaves were recovered in standing position during sampling of the second cohort. Although leaves were shredded during decay as for the first cohort, the higher (30 cm vs 15 cm) average height of the leaves on the shoots resulted in leaves less commonly bending down to touch the sediment; rather, they usually lay tangled across other shoots above the sediment.

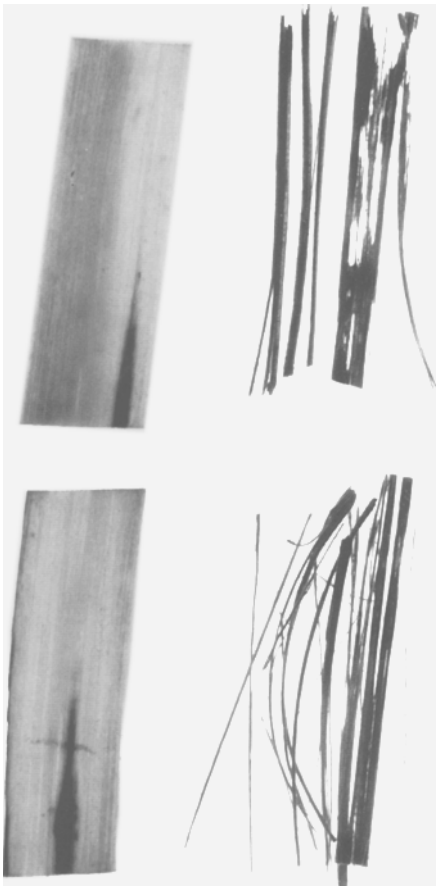

Fig. 2. *Spartina alterniflora.* 3 cm-long pieces of leaves collected as portions of standard-sample replicates from tagged leaves. Pieces on left were cut from yellow-green senescent leaves at time of tagging (Time zero), those on right from leaves at final sampling. Upper set: autumn cohort; lower set: winter-spring cohort

After the autumn leaves had begun to lose integrity and bend downwards, the fine clay sediment of the marsh surface infiltrated them thoroughly. This is reflected by a sharp rise in ash content after September (Fig. 1b). To a lesser extent, this was also true for winter-spring leaves. The percentage carbon of remaining leaf material varied inversely with ash content (time-zero values excluded), as expected ($r = -0.99$, $p < 0.001$), but the nitrogen content varied positively with ash content ($r = +0.93$, $p < 0.01$) (Fig. 1b), counter to expectation if the clay were simply diluting organic nitrogen content. Both cohorts of leaves showed net mineralization of nitrogen per standard sample for the period between tagging and final sampling (−12% of the original value for autumn; −75% for winter-spring). Only one sampling interval exhibited net nitrogen immobilization (October–November, +1.1 mg N per standard sample = 100%) (calculated using data from Fig. 1a, b).

Rates of carbon mineralization as carbon-dioxide release at field temperature were level at about 100 µg CO_2-C g^{-1}

Table 3. *Spartina alterniflora*. Mean rates of carbon-dioxide fixation (μg C g^{-1} organic matter h^{-1}) measured using $^{14}CO_2$ for standing senescent and dead leaves, with temperature and light ranges for each determination; autoclaved-control values were subtracted from the values presented. L–D: light minus dark

Date	Bottles[b]		Light-stimulated (L–D)	T (°C)	Light (μE m^{-2} s^{-1})
	Light	Dark			
5 Sep. 1984[a]	435.8	5.8	430.0	25–29	350–1 050
24 Sep. 1984	33.2	1.7	31.6	31–37	500– 750
17 Oct. 1984	18.4	2.8	15.6	32–36	450– 700
27 Nov. 1984	30.6	lost	lost	20–24	300– 710
20 Dec. 1984[a]	85.4	3.9	81.5	12–17	140–1 050
1 Feb. 1985	1.2	0.3	0.9	17–23	270– 750
15 Mar. 1985	1.2	1.2	0.0	28–31	350– 760
17 May 1985[c]	14.7	6.5	8.2	26–31	180–1 120

[a] Initial sampling date in series of four each of two cohorts of tagged leaves; 5 September and 20 December samples were of yellow-green, senescent leaves; remaining samples were of dead leaves

[b] Average standard deviation equaled 54 and 25% of mean for light and dark values, respectively

[c] Data for this date based on estimated ^{14}C-specific activity, since total-CO_2 samples were lost

organic matter h^{-1} for water-saturated autumn leaves, but showed a steady increase to near 200 μg CO_2–C h^{-1} when normalized to 25 °C (using a Q_{10} of 2.1: Newell et al. 1985) (Fig. 1c). Temperature-normalized rates of CO_2 release from water-saturated winter-spring leaves also were about 100 μg CO_2–C g^{-1} organic matter h^{-1} at the first sampling, and also rose to about 200 μg CO_2–C h^{-1} by about 80 d, apparently remaining at this level through 147 d (Fig. 1c). The calculated time-averaged rates of *total* carbon loss per gram organic matter, for the two periods of sharpest mass loss (September, March–May), were 312 and 290 μg C h^{-1}, 3.2 and 1.4 times larger than the instantaneous CO_2–C output rates measured at the ends of those periods.

Excluding data for yellow-green leaves, light-stimulated carbon-dioxide fixation rates ranged from 0.0 to 31.6 μg CO_2–C g^{-1} organic matter h^{-1} (Table 3). Values were greater in the autumn cohort than in the winter-spring cohort (Table 3). Because leaves were still yellow-green at the first harvesting for each cohort, the relatively high fixation values on 5 September and 20 December most probably represent *Spartina alterniflora* leaf activity. Excluding these dates, dark carbon-dioxide fixation ranged from 5 to 99% of total fixation (Table 3). This percentage was substantially greater in the winter-spring cohort than in the autumn cohort.

The biovolumes of microbes at the surfaces of (as opposed to within) leaves were strongly dominated by bacteria (exluding cyanobacteria) at all samplings except those of yellow-green leaves (Time zero) (Fig. 3a), when algal volume was near to or greater than that of bacteria. Microphotoautotroph volume was dominated by the green alga *Pseudendoclonium submarinum* Wille (Fallon et al. 1985). Green algal volume rose steadily with time on autumn leaves to

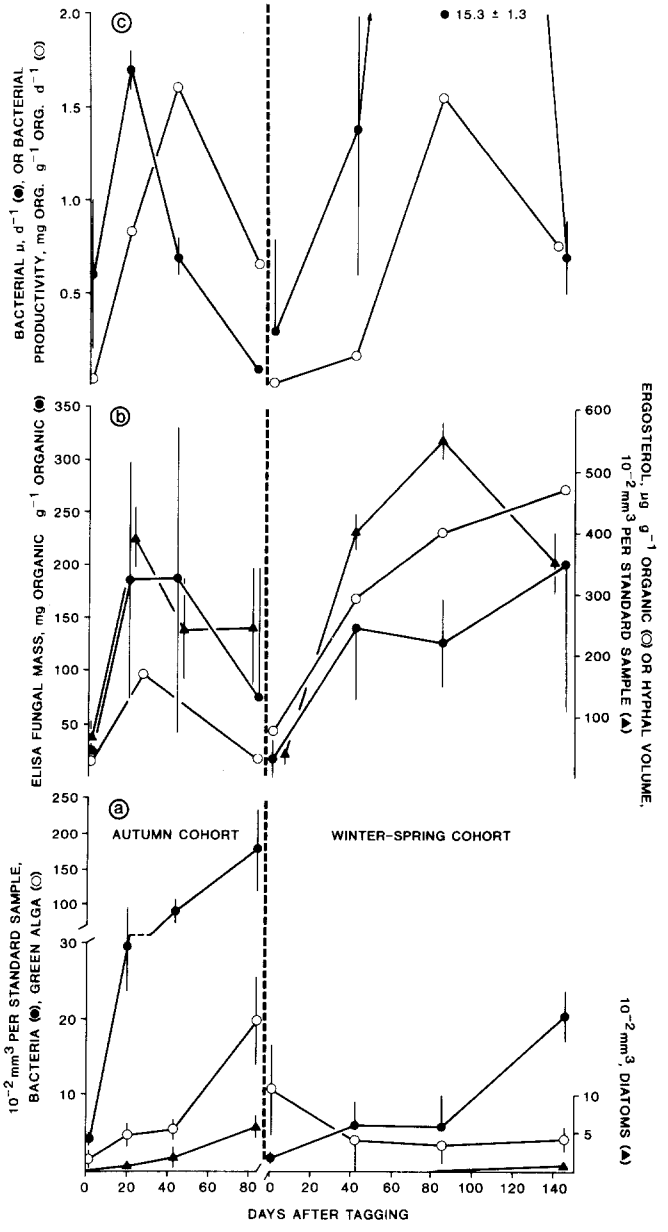

Fig. 3. *Spartina alterniflora*. Microbial dynamics for tagged leaves decaying in place (see also Table 4). (a) Concentration of surface-oriented microbes (microphotoautotrophs, bacteria) per standard sample; (b) concentration of fungal biovolume per standard sample, ergosterol (an index of living fungal mass) per gram organic mass of leaf, and probable total fungal mass per gram organic mass of leaf, as measured via enzyme-linked immunosorbent assay (ELISA); (c) rate of bacterial production and bacterial specific growth rate (μ) on submerged leaves, calculated after measurement of rate of incorporation of ^3H-thymidine into DNA. Bars indicate one standard deviation (except ergosterol, for which values are means of triplicate runs of large pooled samples)

about 0.2 mm^3 per standard sample (ca. 5×10^{-3} mm^3 cm^{-2} leaf) at 83 d after tagging, but stayed near 0.04 mm^3 per standard sample on the winter-spring leaves (Fig. 3a). Diatoms rose to about 0.06 mm^3 per standard sample (ca. 1×10^{-3} mm^3 cm^{-2} leaf) at 83 d for the first cohort, but were nearly absent for the second cohort of leaves. Other forms of phylloplane microphotoautotrophs (Fallon et al.

1985) were absent or present at levels less than 0.1×10^{-3} mm^3 cm^{-2} leaf (except spheroidal cyanobacteria, 83 d autumn leaves, 0.6×10^{-3} mm^3 cm^{-2} leaf). Bacterial biovolumes rose sharply as autumn leaves decayed, to about 40×10^{-3} mm^3 cm^{-2} leaf (1.76 mm^3 per standard sample), but were not much greater than algal volumes on winter-spring leaves (maximum of ca. 10×10^{-3} mm^3 cm^{-2} leaf) (Fig. 3a).

Direct microscopy of intact leaf surfaces indicated that nearly all fungal hyphae in homogenates had originated from *within* dead leaves. Hyphal volumes for fungi were much greater (1 to 3 orders of magnitude) than bacterial volumes for all samples except the October and November samples of autumn leaves, when equality between the two was approached (Fig. 3a, b). The patterns of change in hyphal volume were similar to those in the fungal content of leaves as measured via ergosterol and immunosorbent assay (ELISA), (Fig. 3b). Both autumn and winter-spring leaves showed steep increases in fungal content for the first sampling interval ($\bar{x} = 706\%$ increase, average for all three methods). After the first sampling interval, autumn leaves exhibited a declining trend in fungal content (Fig. 3b), falling by an average of 71% from the ergosterol and ELISA peaks. Winter-spring leaves showed increases in fungal content to the final sampling at 147 d (Fig. 3b; note that hyphal volume is given per standard sample). In contrast to the findings for algal and bacterial volumes, the fungal contents of leaves were as high or higher for the colder, drier winter-spring study period than for the autumn period, with the exception of ELISA values for January–March.

Table 4 compares estimates of microbial organic masses per gram organic matter of senescent and decaying leaves. Eukaryotic microphotoautotroph mass was least, reaching 1 mg g^{-1} only once, on Day 83 in autumn leaves. Bacterial mass was >1 mg g^{-1} on all but two samples of decaying leaves, but was lower than even the minimum estimate of fungal mass, with only one exception, on Day 83 in autumn leaves. The living fungal mass (ergosterol estimate) was 3 to 8 times greater than direct microscopically-estimated fungal mass, except at the end of the autumn study period. Immunologically estimated fungal mass was consistently by far the greatest microbial mass estimate, reaching 19 to 20% of organic mass early in the autumn leaves and late in the winter-spring leaves (but note in Fig. 3b that standard deviations for the immunological estimates were quite large when the estimates were large).

Cross-sections of decaying autumn leaves taken at the time of peak ELISA-fungal mass revealed that longitudinal rows of pseudothecia (ascomata, sexual reproductive organs) of *Phaeosphaeria typharum* had formed within the former mesophyll chlorenchyma of the leaves. (Longitudinally running bands of chlorenchyma alternate with vascular bundles in leaves of *Spartina alterniflora*: Sutherland and Eastwood 1916, Anderson 1974). The pseudothecia, which are about 50 to 100 μm wide (cf. Kohlmeyer and Kohlmeyer 1979, Newell and Hicks 1982) were located just inside the abaxial epidermis, and were visible under the dissecting microscope as rows of dark brown-black dots between the lighter-colored vascular-bundle zones. Mature pseudothecia were open to the exterior of the leaf via a small ostiole (ca. 25 μm diam: Kohlmeyer and Kohlmeyer 1979). Beneath the rows of pseudothecia, melanic fungal hyphae were intermixed with the remains of the mesophyll chlorenchyma. Hyphae were present within the highly lignified vascular cells, but penetration of vascular tissue appeared to be less extensive than that of the mesophyll chlorenchyma.

Although fungal mass dominated the microbial standing crop within the dead-leaf system, the nitrogen content of the decaying leaves (Fig. 1b) did not vary in the same direction as the fungal standing crop. Excluding Time-zero values, the correlation coefficient for %N versus ELISA-fungal mass was $r = -0.74$ ($0.1 > p > 0.05$). In contrast, the nitrogen content of decaying leaves varied in the same direction as bacterial standing crop ($r = +0.81$, $p = 0.05$), a relationship nearly as strong as that for ash content versus nitrogen (see above).

Changes in the instantaneous rate of bacterial production (based on thymidine incorporation on submerged leaves) were similar in pattern for the two cohorts of leaves (Fig. 3c). In each case there was a rise from <0.5 mg bacte-

Table 4. *Spartina alterniflora.* Estimated microbial organic masses (to nearest whole mg) g^{-1} organic matter of leaf substrate for the two cohorts of senescent (Day 0) and decaying tagged leaves. EP: eukaryotic photoautotrophs (green alga + diatoms); B: bacteria (except cyanobacteria); HV: fungal mass based on conservatively measured hyphal volume; FE: fungal mass based on ergosterol measurements; FI: fungal mass based on immunosorbent assay

Microbial compartment	Autumn cohort				Winter-spring cohort			
	Day 0	Day 19	Day 43	Day 83	Day 0	Day 42	Day 85	Day 147
EP[a]	0	0	0	1	0	0	0	0
B[a]	0	1	2	6	0	0	0	1
HV[a]	1	7	6	6	1	8	10	18
FE[b]	3	18	no data	3	8	31	42	50
FI	22	186	188	77	19	141	128	203

[a] For uniformity in calculations, one value was used to convert biovolumes to organic mass (570 fg μm^{-3}, Newell et al. 1987)

[b] Factor for conversion from ergosterol content to fungal organic mass = 105.8 mg mg^{-1}, based on ergosterol content of *Phaeosphaeria typharum* in leaf extract (Newell et al. 1987)

Table 5. *Spartina alterniflora*. Saprotrophic productivities (mg organic mass standard sample^{-1} d^{-1}) for standing-dead leaves, calculated for first and last sampling intervals for each set of tagged leaves. Fungal productivities are calculated as net changes in standing crop (based on enzyme-linked immunosorbent assay) divided by number of days; bacterial productivities are mean instantaneous rates (based on ^{3}H-thymidine incorporation into DNA for submerged leaves)

Microbial type and sampling intervals	Autumn	Winter-spring
Fungal		
first	2.68	0.79
last	−0.71	0.22
Bacterial		
first	0.17	0.03
last	0.23	0.23

rial organic matter g^{-1} leaf organic matter d^{-1} to about 1.5 mg g^{-1} d^{-1}, and then a drop at the final sampling to near 0.7 mg g^{-1} d^{-1}. Bacterial productivity per cell (cf. Fig. 3a and 3c) declined sharply at the final samplings, especially for autumn leaves. Calculated bacterial doubling time ($0.693 \div \mu$) for autumn leaves increased from 1 to 7 d between the October and November samplings.

Minimum estimates of rates of fungal production (net, $\text{time}_n - \text{time}_{n-1}$ divided by number of days, each cohort) were compared with calculated bacterial productivity for the same time intervals, taking ELISA estimates of fungal mass to represent total (live + dead) fungal mass (Table 5). Bacterial productivity was calculated as the mean rate for two consecutive sampling times (ignoring the fact that bacterial productivities are only valid for water-saturated or submerged leaves). The calculated net organic fungal productivities were 16 to 26 times greater than the gross bacterial organic productivities for the first sampling intervals of each leaf cohort, but over the final intervals for each cohort, gross bacterial productivity was greater or equal to net fungal productivity.

Discussion

Gallagher and Pfeiffer (1977) discovered that there was a metabolically active "dead-plant community" on/in submerged, standing-dead *Spartina alterniflora* shoots. Newell et al. (1985) found that other types of wetting episodes (rain, dew, high humidity) permitted the microbial assemblages of standing-dead cordgrass shoots to initiate rapid carbon mineralization. Thus, it should not be surprising that standing-dead leaves undergo substantial decomposition while still attached to the stem (minimum losses from original organic mass of 60 to 68%). That >90% of tagged leaves were recovered attached to shoots during our study periods demonstrates that the natural process of flow of cordgrass-leaf carbon to the atmospheric, water-column, and sediment compartments of the marsh ecosystem does not involve separation and movement of whole leaves. This is consistent with the facts that very little fallen litter is found free on the *S. alterniflora* marsh surface in Georgia (<1% of total aboveground dead material at the time of peak dead-shoot standing crop for intermediate-height cordgrass in January 1988: T. Arsuffi and L. Scott, personal communication) and that very little flux of large-particulate dead-shoot material passes through the marsh water column (Dame 1982, Chalmers et al. 1985).

Grasses with annual shoots and perennial rhizomes, such as smooth cordgrass, generally do not abscise their leaves (Kozlowski 1973), so the processes of translocation of nitrogen and other nutrients to rhizomes and the early stages of microbial decomposition of leaves can overlap. Translocation (and leaching) should result in reduction of leaf nitrogen content, and saprotrophic microbial production would be expected to have an opposing effect. Hopkinson and Schubauer (1984) found that 54% of cordgrass-shoot nitrogen was translocated to rhizomes prior to death of shoots, resulting in a mean nitrogen percentage for standing-dead material of 0.4%. We found a similar decrease in nitrogen content of dying tagged leaves during the first sampling intervals (Fig. 1b). This interval (Time_0–Time_1) was also one of rapid increase in fungal content of tagged leaves, indicating that nitrogen may be in severe short supply (see Paustian and Schnürer 1987a). The maximum nitrogen content of fungal mass (ELISA) at the first sampling for autumn and winter-spring leaves could have been 1.8 to 4.3%, similar to the range measured for the mycelia of *Spartina alterniflora* fungi (1.9 to 6.9%: Newell and Statzell-Tallman 1982) growing on *S. alterniflora*. If our values for ELISA-fungal mass are correct, then either the fungus (*Phaeosphaeria typharum*) growing within the tagged leaves rapidly immobilized virtually all the leaf nitrogen not translocated to rhizomes, or, if not all the leaf nitrogen was mycelial, then the fungus was producing low-nitrogen mycelium. This may tie in with the fact that there is evidence to suggest that *P. typharum* produces less glucosamine per unit mycelial mass when growing within leaves of *S. alterniflora* than when growing in liquid leaf extract (Newell et al. 1987).

The standing-crop mass of procaryotic microbes is not believed to contain much of the nitrogen of vascular plant litter. As others have pointed out (Rublee et al. 1978, Lee et al. 1980), bacterial nitrogen (calculated here as 10% of bacterial mass) could account for less than 5% of the total dead-cordgrass-leaf nitrogen (cf. the maximum for our tagged leaves of 4.8% of total nitrogen). However, in these and other litterbag studies of decomposition of *Spartina alterniflora* (Valiela et al. 1985, Twilley et al. 1986), nitrogen concentrations of material in the bags rose steadily over several months and/or did not show the natural declines found for leaves which were not artificially separated from shoots (Hopkinson and Schubauer 1984). (This suggests that the nitrogen dynamics of litterbagged leaves were a partial result of mechanical disruption of translocation: Newell and Fallon 1989.) For the tagged, attached leaves, changes in nitrogen content were positively correlated with changes in ash (probably mostly sediment) content of the leaves ($r^2 = 0.86$), suggesting that sediment microbes were

the primary immobilizers of nitrogen. The surface 2 cm of *S. alterniflora* marsh sediment contains 50% of the total nitrogen-fixing activity down to 22 cm depth (Whiting and Morris 1986), and at least part of the surface sediment nitrogen-fixation is light-stimulated (Carpenter et al. 1978, Whiting and Morris 1986). Also, we found measurable concentrations of spheroidal cyanobacteria (0.6×10^{-3} mm^3 cm^{-2} leaf) on tagged leaves when sediment (ash) content was highest. Since all the litterbag experiments were conducted on the sediment surface, it may be that increases in nitrogen content of decaying *S. alterniflora,* although not due to an increase in bacterial cell mass, are brought about by sediment nitrogen-fixing microbes in combination with humification processes (Wilson et al. 1986).

In the one litterbag experiment with smooth cordgrass in which fungal mass was estimated, via ergosterol measurement (Lee et al. 1980), ergosterol concentrations in the cordgrass leaves were considerably lower than those we found for tagged leaves. This is partially due to the fact that Lee et al. measured only free sterols (no alcoholic-base hydrolysis: see Newell et al. 1988a), but our preliminary, unpublished measurements indicate that free ergosterol makes up about 75% of total ergosterol in standing-dead leaves of *Spartina alterniflora.* The highest concentration that Lee et al. observed in their litterbagged leaves was 51 μg g^{-1} dry mass, 54 d after cutting leaves. We observed peak values of ergosterol concentration for tagged leaves of 132 (autumn) and 359 (winter-spring) μg g^{-1} dry mass. Several samples of standing-dead cordgrass leaves of unknown age collected in autumn-winter. 1987–1988, gave an average total ergosterol content of 392 μg g^{-1} dry mass (Newell et al. 1988a). (Our lower values for autumn tagged leaves may have resulted from losses during preservation by lyophilization: Newell et al. 1988a.) Leaves cut and placed in litterbags had ergosterol contents 5 to 9 times lower than tagged leaves (Newell and Fallon 1989). Unless environmental factors associated with latitude were dominant [Lee et al. 1980, 42°N; Sapelo (present study), 31°N], it is probable that cutting and placing the leaves in sediment contact (constant high moisture, early availability of sediment nitrogen sources, lower oxygen availability, high bacterial inocula) led to low levels of fungal standing crop (Newell 1984, Griffin 1985).

We measured fungal content of tagged leaves in three ways: (a) conservatively, by direct epifluorescence microscopy (only clearly tubular, large hyphal pieces at 400×, no corrections for extraction inefficiency); (b) by measuring an index of living mass (ergosterol, Newell et al. 1987); (c) by measuring all soluble antigenic mass of *Phaeosphaeria typharum,* the predominant ascomycete of dead cordgrass leaves (Newell and Fallon 1983, Fallon and Newell 1989). Our direct-microscopic values for fungal volume are substantially lower ($\geq \times 18$) than the hyphal biovolume (uncorrected for extraction inefficiency) obtained earlier for cordgrass (Newell and Hicks 1982). Newell and Hicks used higher magnification (1 000×, rather than 500× as incorrectly reported). This may have been the principal reason for their larger average value (see Bååth and Söderström 1980), especially since they did not take the conservative approach which we adopted for the present work. When measuring hyphal diameters at 1 000× in the current work, it was clear that many hyphal bits had not been taken into account during hyphal-length estimation at 400×. Hyphal masking by non-fungal material may interact with indistinctness of hyphal fragments in causing low fungal-volume estimates (Newell and Hicks 1982, Elmholt and Kjøller 1987). Another potential factor is that Newell and Hicks (1982) worked with dead leaves from the lower 50 cm of creekbank shoots (ca. 2 m tall), as opposed to the intermediate-height shoots (ca. 1 m tall), of the present study.

Since ergosterol is probably primarily an index molecule for living (as opposed to total, live plus empty mycelial walls) fungal mass (see Margalith 1986, Newell et al. 1987, West and Grant 1987), basing fungal mass estimates upon the ergosterol assay would be expected to yield lower values than estimates based on total soluble fungal antigens. The percentage (ergosterol estimate) ÷ (immunoassay estimate) × 100, may be an approximation of the percentage of fungal mass that is living (Newell et al. 1986). If so, the percentage of living fungal mass would fall from 10 to 4% for autumn leaves between September and November, and vary between 22 and 33% for winter-spring leaves. The winter-spring values are similar to percentages (16 to 31%) estimated using direct microscopy of percent fluorescein-diacetate-active hyphae for early stages of decay of barley straw (Wessén and Berg 1986), but much higher than percentages usually found for hyphae in soil (e.g. <6%: Paustian and Schnürer 1987b). Our lower values for autumn leaves may have resulted from ergosterol losses during preservation (Newell et al. 1988a).

Our method for approximately measuring total fungal mass (ELISA) gave mean values with large uncertainties. One source of this uncertainty is the marked variability of antigenic responsiveness of the mycelium of *Phaeosphaeria typharum* as its mycelium ages: 70 d-old mycelium had range/mean values which were 14 times higher than those of 3 d mycelium (Fallon and Newell 1989), although the means were within 21% of each other. Also the high end of the range of mean values for three strains of *P. typharum* of the same mycelial age was 1.6 times the low end of the range. All these error estimates are for mycelium without sexual structures (pseudothecia). Pseudothecia are regularly produced in the field, and they may well be antigenically different from mycelium. Patchiness of strain clones, of mycelial age, and of fungal morphogenetic status which are likely in natural substrates (see e.g. Rayner et al. 1987 and references therein) will probably intractably block the obtaining of precise estimates of mycelial mass by ELISA in the dead-cordgrass system. Perhaps the best means of challenging the accuracy of our imprecise ELISA estimates of fungal mass would be to apply a mycological method analogous to the tritiated-thymidine method of measuring bacterial productivity (Newell et al. 1988b). Fungal specific-growth rates could be used to test whether peak ELISA-fungal masses are likely to be achievable.

Because measurements of carbon-dioxide fixation were performed near mid-day, with shading to prevent photo-

inhibition, total fixation estimates are probably at the high end of the actual daily range. When integrated to an hourly basis, total carbon-dioxide fixation ranged from 1 to 33 μg C g^{-1} organic matter h^{-1} during the sampling periods following the yellow-green stage. Comparing this range to the estimated respiration rates shows that the standing-dead leaf system was a net heterotrophic system, as one would expect. Our rates are far lower than those estimated by Jones (1980) for standing-dead shoots from Sapelo Island marshes (Jones: 5 to 14 μg C cm^{-1} length of leaf h^{-1}; present data: 0.0 to 0.3 μg C cm^{-1} h^{-1}). However, considering the relatively low algal biomass and low net growth rates on the standing-dead leaves, light-stimulated carbon-fixation rates much greater than we observed would imply high (implausible?) rates of consumption of fixed algal carbon.

Algal mass was positively related to light-stimulated fixation after the yellow-green stage. Periods of minimal light-stimulated fixation by the dead-leaf systems were associated with periods of minimal algal numbers. The lower rates seen in the winter-spring cohort (30 cm above the marsh) were probably due to a less favorable microenvironment higher above the marsh surface (Fallon et al. 1985). Jones (1980) reported a negative correlation between height and maximum total carbon fixation. The very high ratios of dark to total fixation seen in the winter-spring cohort may be related to anaplerotic fixation by fungi (e.g. Martin and Canet 1986). Such high ratios are unusual for normal photosynthetic populations (e.g. Goldman and Dennett 1986). In laboratory experiments, high weight-specific rates of dark carbon-dioxide fixation have been detected for fungal species common to standing-dead leaves (own unpublished observations).

Leaf photosynthesis was active at the yellow-green phase (i.e., Day-0 samplings). Fungal growth was very rapid following this sampling in the fall. Since fungal invasion was occurring when the leaf was still actively fixing carbon dioxide, the fungus possibly used photosynthetic end products for rapid growth. This is another argument for incubation of standing-dead material in a natural state rather than in litter bags.

We present crude values for carbon flow from standing-dead leaves in Table 6 (see "Materials and methods" for details of calculations). Based on these flux estimates, if both bacterial and fungal carbon-conversion efficiencies were equal to 50%, then $\geq 70\%$ of the carbon released as CO_2 would be due to fungal respiratory activity (see Padgett et al. 1985).

Our estimates of carbon flow indicate that about 70% of the loss of organic matter from standing-dead leaves was not in the form of CO_2–C, but as particles, dissolved leachate, and material translocated to rhizomes. The magnitude of this type of loss was greater than the quantity of leaf material remaining attached at the ends of the study periods (Table 6). Calculations based on literature values indicate that ca. 25% of the non-CO_2 loss of carbon would have been as leachate during rain and tidal submergence. Thus, approximately half of the total loss of organic matter from the tagged standing-dead leaves was probably in the form of small particles and translocated substance. That much of the loss was as particles is consistent with our observation that loss of leaf area was largely due to loss of mesophyll material from between longitudinally running vascular bundles. Since much of the mass of *Phaeosphaeria typharum* visible in cross-sections of leaves was located in the former leaf mesophyll (present results, and Newell and Hicks 1982), loss of mesophyll/fungal material would help explain the declines or plateaus observed in the fungal mass estimates.

Table 6. *Spartina alterniflora*. Estimates of carbon flow (mg) from standing-dead leaves, based on data from Tables 1 and 5, average values for carbon-dioxide release at field temperature, and values for total organic-mass loss and percent carbon (Fig. 1). Values given are per standard replicate sample. Values in parentheses are percentages that each category represents of original carbon per standard sample

Compartment	Autumn leaves (83 d)	Winter-spring (147 d)
Original content	210	165
Attached shreds[a]	82 (39)	53 (32)
Total loss	128 (61)	112 (68)
To carbon dioxide[b]	31 (15)	35 (21)
Loss in non-CO_2 form	97 (46)	77 (47)
To fungal mass[c]	23 (11)	24 (15)
To bacterial mass[b]	5 (2)	4 (2)

[a] Longitudinally shredded leaf material remaining attached to shoots at end of each study period (Fig. 2)
[b] Values are based on instantaneous rate measurements and duration of activity, and thus are total estimates
[c] Values are based on net changes in standing crop, and thus are minimal estimates

Invertebrate activity at the tagged-leaf site may be involved in leaf-particle output. The density of salt-marsh periwinkles (*Littorina irrorata* Say) $\gtrsim 5$ mm shell diameter averaged 243 m^{-2} (counts from three 0.25 m^2 plots in December 1984). (For comparison, number of brown, dead leaves in December averaged 331 m^{-2}). This is not an unusually high value for periwinkles in portions of Sapelo and some other southern marshes with intermediate-height cordgrass shoots (Odum and Smalley 1959, Warren 1985), but it is higher than values reported in other North American cordgrass marshes (Stiven and Kuenzler 1979). Periwinkles were dormant on the marsh surface during periods of temperatures < 15 °C (first two sampling intervals of our winter-spring cohort), but were active grazers upon dead shoots during warmer periods, ranging high into the plant canopy. Periwinkles which were offered leaves from the field containing pseudothecia of *Phaeosphaeria typharum* rasped away the abaxial sides of the mesophyll/fungal material and produced feces containing undigested bits of plant and fungal material, as found by Alexander (1979) for standing-dead cordgrass in Louisiana. *L. irrorata* can ingest and assimilate radiolabeled fungal tissue (B. Bebout, University of North Carolina unpublished data), and Cammen et al. (1980) calculated that *L. irrorata* consumes about 10% of the net aboveground production of *Spartina alterniflora* in North Carolina. Thus, one of the ways in which dead-leaf

material is shredded and transferred to the marsh sediment is as particles in periwinkle feces. Remaining, attached lignocellulosic shreds are probably made frangible by continuing fungal and bacterial (Benner et al. 1984) activity, and by solar ultraviolet radiation (Chang et al. 1982), until they crumble to the sediment also. The fact that fungal mass was not rapidly removed from tagged leaves during winter could be explained by the infrequency of shredding activity by snails due to their low-temperature dormancy.

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