Bacteria in a North Carolina Salt Marsh: Standing Crop and Importance In the Decomposition of *Spartina alterniflora*

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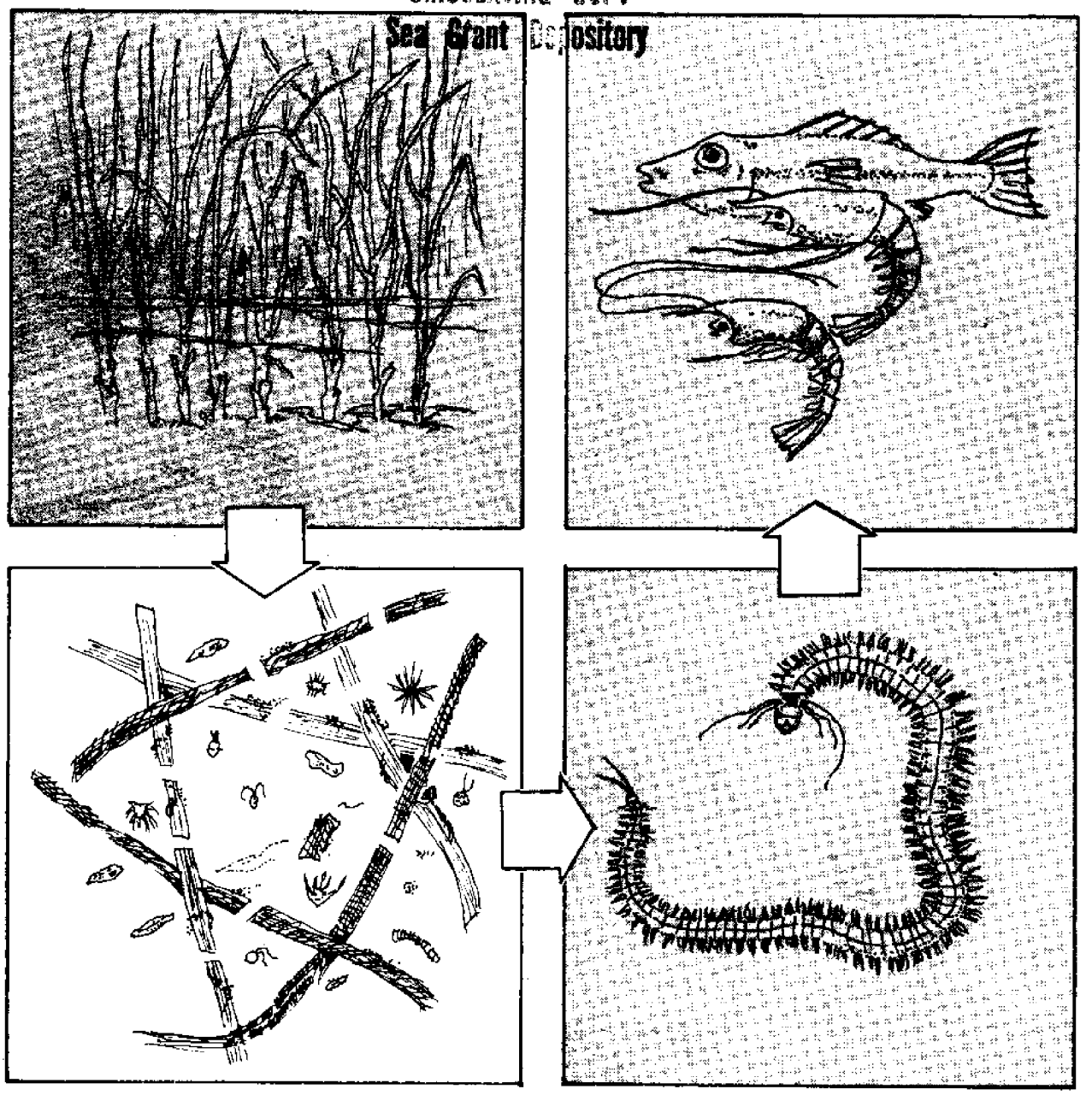

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BACTERIA IN A NORTH CAROLINA SALT MARSH: STANDING CROP AND IMPORTANCE IN THE DECOMPOSITION OF SPARTINA ALTERNIFLORA

By

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ABSTRACT

Bacteria in a North Carolina Salt Marsh: Standing Crop and Importance in the Decomposition of Spartina alterniflora.

The number of bacteria in sediments of a North Carolina salt marsh was determined by direct counts with epifluorescent illumination and acridine orange stain. Cell numbers decreased from 8.36 - 10.9 x 10^9 cells cm^{-3} of sediment at the surface of the marsh to 2.19 - 2.59 x 10^9 cells cm^{-3} of sediment at a depth of 20 cm. No significant difference was found among four stations located on a transect which crossed the marsh and spanned subtidal to intertidal sediments.

The number and size of bacteria at four depths in the marsh were monitored by direct counts for thirteen months. The number of cells reached a maximum of 13.51 x 10^9 cells cm^{-3} at the sediment surface in October, corresponding to the period of Spartina alterniflora die-back. Cell numbers were lowest and most consistent throughout the year at the 20 cm depth of sediment. Cell volumes averaged 0.2 μm^3 at the marsh surface and decreased with depth. Mean yearly estimates for standing crop indicated that bacteria contribute about 36% of the microbial carbon (as estimated by ATP) in surface sediments by only 0.6% of total organic carbon and about 1.5% of total nitrogen. Bacteria are the major constituent of total microbial carbon at depth in the sediment. Mean standing crop of bacteria to a depth of 20 cm of sediment was about 14 g carbon m^{-2}.

Nylon litterbags of 3 mm mesh containing Spartina alterniflora were placed on the surface of the marsh in the fall and sampled for about

200 days. About 0.3% of AFDW was lost from the litterbags per day. The initial bacterial population on the Spartina plants was 1.53×10^9 cells (g dry weight)$^{-1}$ of 1.76×10^9 cells (g AFDW)$^{-1}$. In 184 days the number of bacteria on the detrital complex in the litterbags increased to 29.8×10^9 cells (g dry weight)$^{-1}$ or 68.8×10^9 cells (g AFDW)$^{-1}$. Nitrogen content of the litter increased from about 1.35 to 1.8% of AFDW, carbon content decreased from about 50 to 35% of AFDW, and C/N ratio decreased from an initial value of 35 to a final value of 20. The contribution of bacterial carbon and nitrogen to the total carbon and nitrogen pools of the litter increased over time, but after 184 days was still only about 0.5% of total carbon and about 3.3% of total nitrogen. Thus, on a weight basis bacteria do not comprise a large fraction of total Spartina litter over the first 200 days of its decomposition.

The mineralization of ^{14}C-labelled Spartina alterniflora detritus was monitored at 1 to 5 day intervals over an incubation period of up to 53 days in microcosms consisting of 10 cm^3 of marsh sediments and several milliliters of seawater. In all treatments the rate of mineralization was initially rapid (up to 8% day^{-1}) but decreased over the incubation period. The rate of mineralization over 29 days was higher in microcosms incubated at 30°C (1.37% day^{-1}) than in microcosms incubated at 20°C (1.09% day^{-1}) or 10°C (0.82% day^{-1}). Spartina leachate was mineralized at a faster rate during the early part of the incubation than either whole plant material or leached plant material, but after 53 days more of the leached Spartina detritus had been mineralized than either of the other fractions. Experimental microcosms had higher rates of mineralization than undisturbed sediment cores. Higher rates of mineralization were observed in flasks with 8 mg NO_3-nitrogen addition than in flasks with 2 or 4 mg additions.

Estimates of turnover time of the material in the microcosms from an empirical model were in the range of 200 to 300 days for most treatments.

TABLE OF CONTENTS

SECTION I

DIRECT COUNTS OF BACTERIA IN THE SEDIMENTS OF A NORTH CAROLINA SALT MARSH

Introduction

Although the ecology of estuarine bacteria has received increased attention in recent years, few studies have utilized direct techniques to assess bacterial populations. The majority of studies have utilized selective media to focus on specific physiological types of bacteria or to relate estimates of viable bacteria to physical and chemical conditions (e.g. Stevenson and Colwell 1973). Exceptions are the studies of the number of bacteria on the surface of pebbles by Batoosingh and Anthony (1971) and in intertidal sediments by Dale (1974), who utilized direct count techniques similar to those currently used to examine planktonic bacteria (Daley and Hobbie 1975, Hobbie and Rublee 1975, Ferguson and Rublee 1976, Hobbie et al. 1977).

Bacteria within salt marshes are assumed to play major roles, as for example in the breakdown and conversion of dead macrophytic tissues into utilizeable forms (i.e. bacterial biomass). Although most above-ground primary production of salt marsh macrophytes enters the estuarine detrital system (Teal 1962, Reimold et al. 1975), there is significant belowgroumd production (Stroud 1976) which probably is decomposed within the marsh. Thus saprophages may be a key component of the energy flow in marsh systems. This study deals with the enumeration of bacteria in marsh sediments as a first step in quantitatively assessing the role of bacteria in the ecology of salt marshes.

Study Area and Methods

The study area was a Spartina alterniflora marsh approximately 3 km from Beaufort Inlet, and within the Newport River Estuary of North Carolina. Spartina marshes in this area have an average above ground standing crop of 545 g dry wt m^{-2} and an annual above ground production of 650 g dry wt m^{-2} (Williams and Murdoch 1969). Salinity of the overlying water ranges from about 22-36 o/oo, and temperature of the surface sediments ranges from about 0°C to 35°C. Sediments below 1 cm are generally anaerobic and have more moderate temperatures, varying from surface temperatures by as much as 11°C for depths of 20 cm.

A transect with four stations was established across the marsh (Fig. 1). Station 1 was a subtidal station in the embayment bordering the marsh and had no macrophyte vegetation. Station 2, located approximately 12 m from the low tide line, was within the tallest Spartina in this marsh. Station 4, located near the high tide line, and Station 3, midway between Stations 2 and 4, were within areas of short Spartina.

Triplicate cores were taken with a 4.45 cm internal diameter piston corer at each station on July 19, 1976. Each core was sectioned to sample 0-2, 4-6, 9-11, and 19-21 cm; the corresponding sections from the three cores were combined for sediment analysis. Particle size analysis was according to Morgans (1956), in which sediment, oven-dried for 48 hr at 90°C, was passed through sieves according to the Wentworth Scale. The sediment grades were: granules (greater than 2.0000 mm), very coarse sand (1.0000 - 2.0000 mm), coarse sand (0.50000 - 1.0000 mm), medium sand (0.2500 - 0.5000 mm), fine sand (0.1250 - 0.2500 mm), very fine sand (0.0625 - 0.1250 mm), and silt and clay (less than 0.0625 mm). The

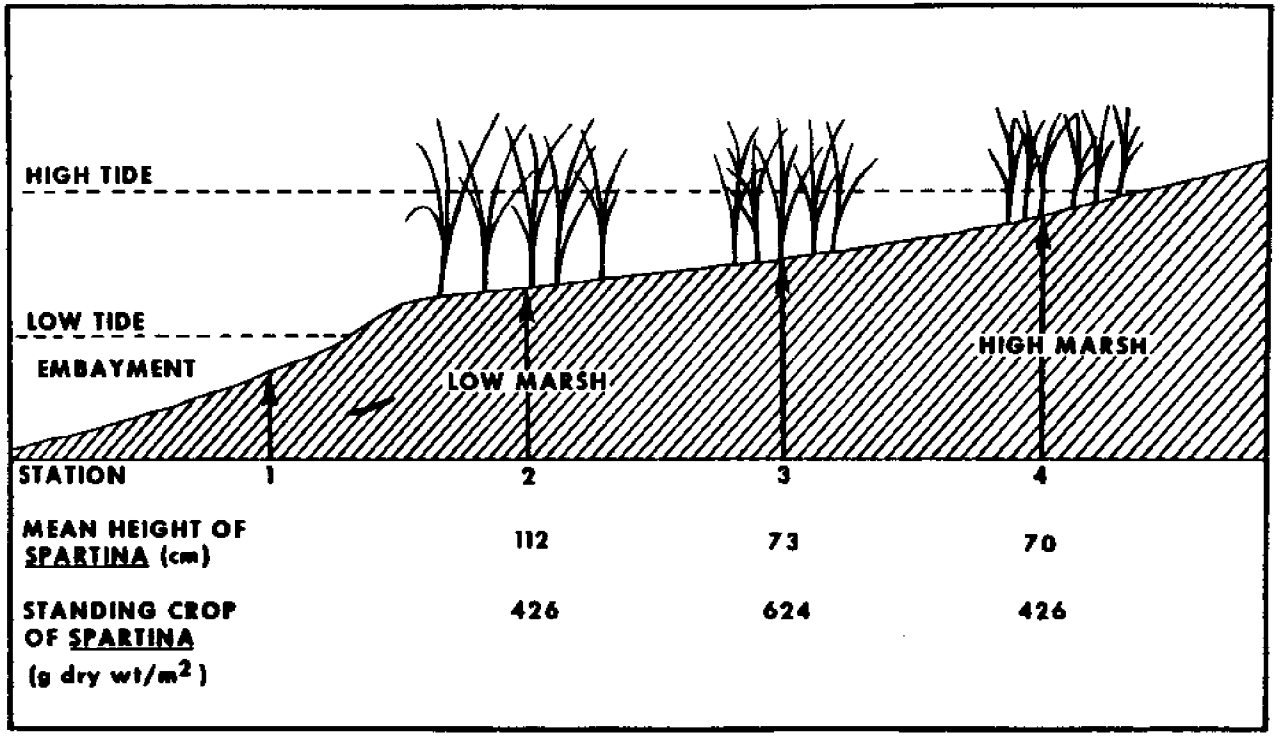

Fig. 1. Transect across a North Carolina salt marsh showing relative locations of sampling stations and the standing crop and mean height of Spartina plants.

weights of the fractions were plotted cumulatively according to Phi notation. Median grain size, Md$\emptyset$, and Phi quartile deviation, QD$\emptyset$, were calculated for each sediment sample.

Replicate portions of each sample also were analyzed for organic carbon by the Walkley-Black wet oxidation method (Morgans 1956) after sieving through a 1 mm mesh screen to remove most root material. None of the corrections suggested by Morgans were applied to the values obtained by this method. Additionally, an index of interstitial space and an estimate of surface area were calculated. The formula for interstitial space, Spatial index = $1 \times (Md\emptyset)^{-1} \times [\log(1\% + \%\ \text{silt and clay})]^{-1}$, is from Dornseif (1976). The estimate of surface area assumed a density of dry sediment material of 2.65 gm cm^{-3} (Grim 1962, Buchanan and Kain 1971) and a median particle size for each of the seven sieve fractions (equal to a sphere with a diameter one-half the mesh opening).

Replicate cores were taken for bacteria counts on 23 August 1976 for Stations 3 and 4, and two days later for Stations 1 and 2. Cores were placed on ice in the dark and taken to the laboratory where a 0.1 cm^3 sediment sample was taken by filling small aluminum planchets (Hewlett Packard # 5080-5045) with sediment from each core at depths of 0, 5, 10, and 20 cm. Each sample was placed in a vial containing 20 ml of a filtered (0.2 μm Nuclepore filters) solution of Formalin in seawater (5% v/v) and all vials were stored in a refrigerator and counted within 48 hours. Before counting, each sample was diluted further with 100 ml filtered seawater and blended for 60 seconds at high speed in a Waring Blendor. A 0.5 ml subsample of the homogenate was removed and added to 1 ml of a prefiltered solution of acridine orange stain (0.01%). After

a 60 second incubation the mixture was filtered onto a 0.2 μm Nuclepore filter which had been previously stained with Irgalan Black (Hobbie et al. 1977). The filter then was examined under epifluorescent illumination at a magnification of 1250 x. A minimum of ten fields or 200 cells were counted on each filter and the mean number of cells per field converted to number of cells cm^{-3} of sediment. This method will miss cells on the underside of particles. A correction factor of 1.15 was used to account for this error. The correction factor was determined on these samples by observing the area of the field covered by particles, the density of cells on the exposed surface of particles, and by assuming that the distribution of cells was similar on the observed and hidden surfaces of the particle. This procedure indicated that the counting method was 87% (S.E.±2%) efficient at counting bacterial cells in these sediments. Coefficient of variation of bacteria counts was 18.7%.

Results and Discussion

The highest and lowest number of bacteria were found at subtidal Station 1, 10.90×10^9 cells cm^{-3} at the sediment surface and 2.19×10^9 cells cm^{-3} at a depth of 20 cm. Although there appeared to be trends in the numbers along the transect (Table 1), an analysis of variance indicated that there was no significant difference between stations. Thus, the absence of macrophytes at the subtidal station did not appear to influence the distribution of bacteria that I found. The correlation of cell numbers with depth (Fig. 2) was highly significant ($r = -0.88$, $p < 0.0001$).

The use of acridine orange direct counts with 0.2 μm Nuclepore filters revealed bacteria populations similar to those seen by scanning

Table 1. Sediment properties and number of bacteria from the sediments of a North Carolina salt marsh.

Station	Depth	MdØ	QdØ	% Silt and Clay	% Organic carbon	Index of Space	Surface Area (cm^2/cm^3)	Sediment weight[1]	Bacteria (10^9 cells/cm^3)
1	0	2.35	0.80	11.17	1.34	0.392	200	0.909	10.90 ± 1.20
	5	2.60	0.80	15.56	1.71	0.316	230	1.236	5.68 ± 0.84
	10	2.60	0.65	8.75	1.58	0.389	246	1.167	3.11 ± 0.66
	20	2.73	0.45	6.64	0.80	0.415	300	1.390	2.19 ± 0.43
2	0	2.55	0.90	17.00	4.10	0.312	112	0.621	9.91 ± 0.86
	5	2.60	0.60	13.77	3.37	0.329	163	0.948	5.06 ± 1.07
	10	2.67	0.58	12.33	3.21	0.334	188	1.056	2.45 ± 0.38
	20	2.76	0.28	8.16	1.27	0.377	234	1.042	2.28 ± 0.39
3	0	2.50	1.03	15.92	3.86	0.326	119	0.509	8.52 ± 0.00
	5	2.65	0.55	9.71	1.59	0.366	165	0.791	7.12 ± 0.89
	10	2.67	0.28	7.20	1.62	0.410	166	0.790	3.91 ± 0.91
	20	2.80	0.31	4.66	1.30	0.474	235	1.145	2.59 ± 0.83
4	0	0.06	1.33	5.53	10.62	20.450	26	0.245	8.36 ± 0.26
	5	1.50	1.53	3.37	0.57	1.040	81	0.662	6.50 ± 0.16
	10	2.60	0.78	5.82	0.19	0.461	281	1.493	4.40 ± 0.08
	20	2.75	0.95	18.67	1.37	0.281	262	0.969	2.20 ± 0.36

1/ g dry wt/cm^3 of wet sediment.

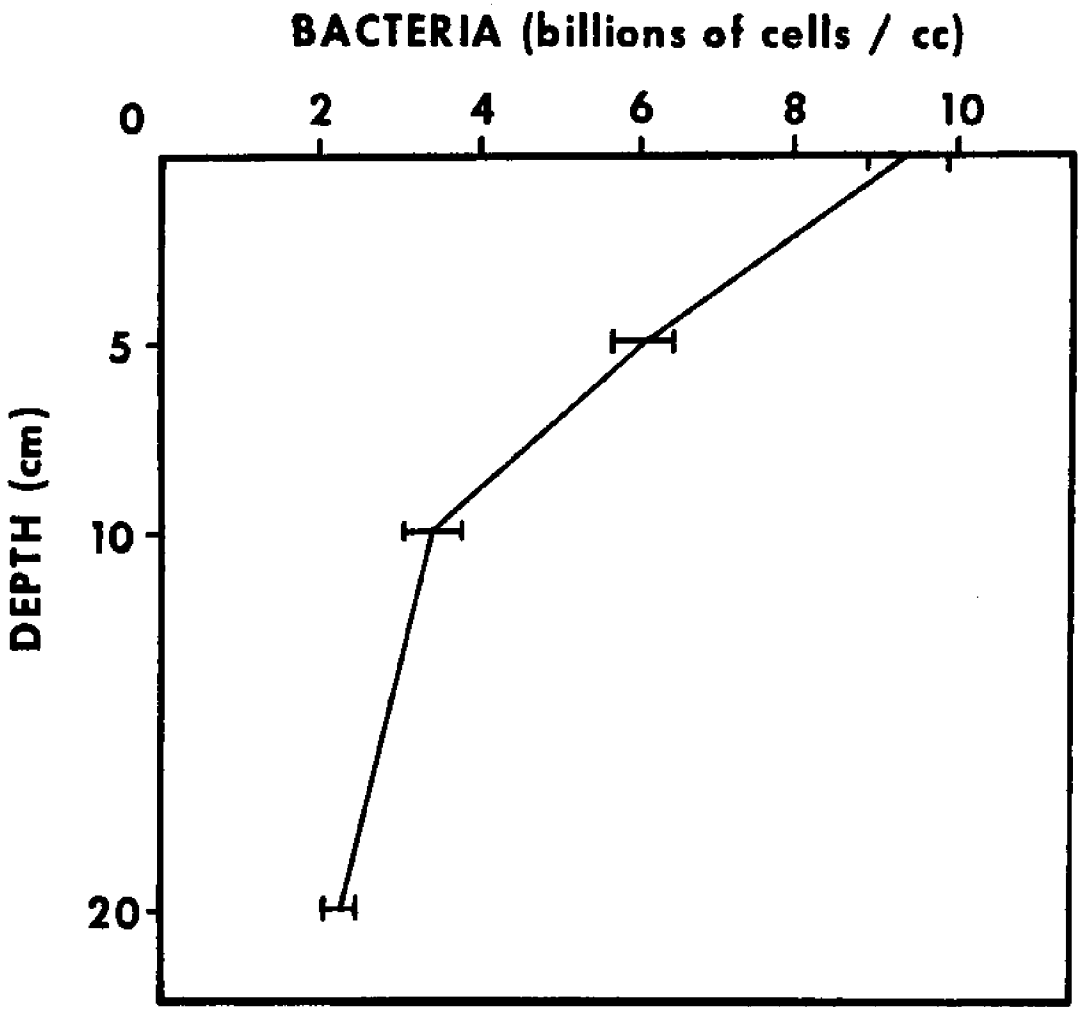

Fig. 2. Mean number of bacteria in marsh sediments of four transect stations. Horizontal bars represent ± one standard error.

electron microscopy (Bowden 1977) and up to four orders of magnitude greater than viable count methods (Francisco et al. 1973, Batoosingh and Anthony 1971). Comparison of our results with other studies will be restricted, therefore, to those which have used acridine orange stain and epifluorescent illumination to examine sediment bacteria. For example, Dale (1974) found the number of bacteria in intertidal sediments of Nova Scotia to range from 0.32 - 9.97 x 10^9 cells (g dry wt)$^{-1}$ of sediment at the sediment surface and 0.14 - 4.68 x 10^9 cells (g dry wt)$^{-1}$ at a 10 cm depth. A greater range in numbers, 0.10 - 55.0 x 10^9 cells (g dry wt)$^{-1}$ of sediment has been found in the highly organic and flocculent surface sediments of a freshwater tundra pond (Hobbie and Rublee 1975). My numbers, when converted to a dry weight basis, yield values ranging from 1.58 - 34.12 x 10^9 cells, somewhat higher than those of Dale (1974) but within the range reported by Hobbie and Rublee (1975). Any differences are probably the result of differences in location, salinity, and the fact that we used 0.2 µm pore size Nuclepore filters which retain more bacteria than the 0.45 µm pore size filters used in other studies (Hobbie et al. 1977). Finally, bacterial counts in subtidal sediments of the Newport River estuary demonstrate numbers of bacteria similar to those I report (C. Shelton, North Carolina State University, pers. comm.).

Most stations had similar sediment properties (Table 1). With the exception of the surface and 5 cm depth of Station 4, all samples were characterized by well-sorted, medium to fine sand, with similar values for surface area and interstitial space. Organic carbon was low in the

subtidal sediment (around 1%), and somewhat higher at Stations 2 and 3 (up to 4%). The surface and 5 cm depth of Station 4 had larger particles, less surface area, more interstitial space, and the highest value of organic carbon (over 10%). These differences, which were probably due to tidal action, did not appear to affect bacterial distribution.

Pearson correlation coefficients (Snedecor and Cochran 1971) were determined for bacteria numbers and sediment properties. The single strong correlation was a negative correlation of bacteria with depth. Other correlations were less important since first, they were not large, and second, they were strongly intercorrelated with depth. This is not surprising since the range of values of our sediment properties was narrow. Other studies which have demonstrated relationships of bacteria with sediment properties (e.g. Zobell 1946, Dale 1974) have been based on broader ranges of data. My values complement the positive relationship of bacteria to organic carbon reported by Dale (1974), but at similar values for mean grain size, my numbers of bacteria are one to two orders of magnitude higher.

The strong positive relationship of bacteria to surface area has been noted by many investigators (e.g. Zobell 1946, Hargrave 1972). Reported density of bacteria ranges from one cell per 30 μm^2 on the surface of decomposing turtle grass (Fenchel 1970) to one cell per 411 μm^2 on the surface of marine sediment particles (Dale 1974). I found an average density of one cell per 5.31 μm^2 (range of 0.31 - 13.73 μm^2) on the surface of marsh sediment. This value is probably high since our methods yield a minimum estimate of surface area. My values do, however, compare favorably with those of Zvyaginsev (1972), who found a density as

high as one bacterial cell per 4 μm^2 in terrestrial soils. This relationship requires further investigation, in particular with regard to the effect of bacterial density on decomposition in aquatic environments.

I have demonstrated that within the sediments of this marsh in August there was a standing crop of about 10^{10} bacteria cm^{-3} in surface sediments and macrophyte vegetation did not appear to influence bacterial distribution. Whether the presence of macrophytes has an effect on distribution at other times or an effect on bacterial activity remains to be seen.

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SECTION II

CONTRIBUTION OF BACTERIA TO THE SALT MARSH MICROBIAL COMMUNITY

Introduction

The role of microorganisms in natural systems includes decomposition, nutrient regeneration and cycling, and production of particulate matter. Such roles are of particular importance in highly productive systems where direct grazing of primary production by herbivores is not important, such as estuarine salt marshes. In these systems detrital food webs are well developed and have been the focus of many studies (e.g. Burkholder and Bomside 1957, Odum and de la Cruz 1967, Morrison et al. 1977). One theory of how marsh macrophyte production is utilized is that the consumers assimilate the microflora on detrital particles rather than assimilation of the particle itself. Assessment of the validity of this theory is difficult due to lack of definitive techniques.

Bacteria are a key component of the detrital microbial community because of their apparently rapid colonization and high metabolic activity in association with detrital material (Fenchel 1970, Hargrave 1972, Fallon and Pfaender 1976, Morrison et al. 1977, SECTION III). Few studies have looked at the bacteria directly, however, and most of our information on standing crop is from indirect estimates of the total microbial biomass from measurement of ATP (e.g. Christian et al. 1975) or constituents of procaryotic cell walls such as muramic acid (Moriarty 1975, 1977, Morrison et al. 1977). Both methods require poorly defined conversion factors and may include nonbacterial sources of the measured substrate.

This paper reports the results of a thirteen month study of the standing crop of bacteria in a salt marsh and their contribution to the total standing crop of microbiota.

Study Area

The Newport River estuary is a broad (31 km^2), shallow (mean depth at low tide about 1 m), coastal plain estuary of the southeastern United States. It has been described by Wolfe (1975), who points out that the primary production of the whole system results mainly from phytoplankton, macrophytes, and benthic algae. The importance of Spartina alterniflora to the system as a whole may be less than 10% in terms of net primary production, but within the extensive marshes annual aboveground production averages 650 g dry wt m^{-2} (Williams and Murdoch 1969). The specific study site was a 100 m^2 plot in a Spartina alterniflora marsh located approximately 3 km from Beaufort Inlet and within the estuary.

Characteristics of this marsh include a salinity range of the water over the marsh from about 22 - 36 o/oo, dependent upon rainfall, river flow, and stage of the tide (amplitude approximately 1 m). Temperature of the surface sediments are similar to air temperature throughout the year and range from 0 - 35°C. Subsurface sediments had more moderate temperature ranges and they are generally anaerobic within several millimeters of the surface. The composition of the sediments across the marsh have been described as fine sand with 5 - 17% silt and clay at the surface (SECTION I). The organic carbon content decreased with depth of sediment (Table 1). Analysis of surface sediments with a CHN analyzer yielded total nitrogen values of about 0.33% of dry weight (SECTION III).

Table 1. Sediment characteristics of the study site.

Depth (cm)	% Silt and Clay	% Organic Carbon[1]	Wet Weight of Sediment (g cm^{-3})
0-2	17.0	4.1	0.62
4-6	13.8	3.4	0.95
9-11	12.3	3.2	1.06
19-21	8.2	1.3	1.04

1/ Determined by wet oxidation.

Materials and Methods

Salt marsh sediments were sampled monthly from May 1975 until June 1976. Samples were analyzed for bacteria, for ATP in all months except May 1975, and for chlorophyll *a* in all samples after July 1975. Generally on each sampling date four samples were taken to a depth of at least 25 cm by a hand-held piston corer with removable sleeve (internal diameter of 4.45 cm). Cores were taken at or near low tide. Two cores were used for ATP and chlorophyll *a* analysis and the remaining two for bacterial enumeration.

The two cores for ATP and chlorophyll *a* were subsampled in the field immediately after collection. At four depths (surface, 5, 10, and 20 cm) the core was sectioned and subsamples of 0.2 cm^3 were taken by filling two aluminum planchets (each 0.1 cm^3) with sediment. Replicates were taken at each depth from each core for both ATP and chlorophyll *a* determinations. Subsamples for ATP were placed in small plastic vials with several pieces of dry ice for rapid freezing. Vials were then stored on dry ice. The subsamples for chlorophyll *a* were placed in plastic vials with several drops of a $MgCO_3$ slurry and approximately 5 ml of acetone and shaken vigorously. Vials were then stored on dry ice, moved to the laboratory and placed in a freezer until analysis.

ATP analysis followed the method of Ferguson and Murdoch (1975). Five ml boiling Tris (hydroxymethylaminomethane) buffer, pH 7.75, was added to each subsample which was then placed in a boiling water bath for five minutes, followed by storage at -20°C until assay. The amount of ATP was determined by the luciferin-luciferase method and calibrated with standards run the same day. The efficiency of the extraction of ATP

from sediment was tested by addition of known amounts of a lyophilized bacterial culture to replicate sediment samples. Recovery was 39.9 ± 5.7%, so all values were multiplied by a correction factor of 2.5. ATP values were converted to estimates of total microbial carbon by multiplying by a factor of 250 (Hamilton and Holm-Hansen 1967, Holm-Hansen 1973).

The amount of chlorophyll _a_ in sediment samples was determined fluorometrically following the procedure described by Ferguson and Murdoch (1975), except that the samples were extracted for 1 to 3 weeks at $-20^{\circ}C$ in the acetone mixture rather than by mechanical means. These methods give essentially equivalent results (R. Ferguson, pers. comm.) although probably neither method yields a complete extraction of chlorophyll (Daley et al. 1973). Algal standing crop was estimated by multiplying the concentration of chlorophyll _a_ by 30 (Ferguson and Murdoch 1975).

The cores for bacterial enumeration were returned to the laboratory on ice, within the removable sleeve of the coring device. In the laboratory the cores were removed from the sleeves and subsamples were taken from the same depths as ATP and chlorophyll samples. Generally, one core was sampled in duplicate and a single sample was taken from the other. Occasionally more replicates were taken from the same core, or more cores were sampled to assure that variation within and between cores was adequately estimated. Each subsample consisted of 0.1 cm^3 of wet sediment which was placed in a plastic vial containing a solution of 19 ml seawater and 1 ml Formalin. Vials were then capped, shaken to disperse the sample and stored in a refrigerator at about $4^{\circ}C$ until counting,

usually within three days of collection. All preserving solution was filtered on the day of addition to samples through a 0.2 μm pore size Nuclepore filter. A 5% Formalin solution (v/v) preserved all bacteria in the sample for at least 7 days.

The procedure for counting the bacteria is a modification of that used for planktonic samples (Daley and Hobbie 1975, Hobbie et al. 1977). Briefly, the samples are placed in a Waring Blendor with 100 ml filtered seawater and blended at high speed for 60 seconds. Next, a 0.5 ml subsample of the dilution is incubated with 1 ml of a filtered 0.01% solution of acridine orange in seawater for 60 seconds. The sample is then drawn onto a black membrane filter by vacuum filtration at 0.5 atmospheres. Finally, the filter is placed on a slide with immersion oil, covered with a No. 1 coverslip, and viewed with oil immersion and epifluorescent illumination at 1250 x. The number of cells in at least 20 randomly chosen fields were noted and the total number of cells cm^{-3} of sediment calculated using conversion factors for magnification, dilution, per cent of filter observed, and efficiency of counting (see SECTION I). Blanks were prepared at the time of sampling in a manner similar to preparation of sample but without sediment addition, treated like samples for counting, and subtracted from the sample counts.

In addition to counting the number of cells in a sample, I determined the average cell size for most samples. For this purpose random fields on the filter were chosen and a minumum of 100 cells per sample were observed and placed within one of five categories:

1) cocci less than 0.5 μm in diameter,

2) cocci between 0.5 and 1.0 μm in diameter,

3) cocci greater than 1.0 μm in diameter,

4) rods approximately 0.5 m x 1.0 μm,

5) cells which did not fit in any of the first four categories and whose dimensions were noted individually.

Most cells fit in categories 1, 2, or 4. The average cell size was then determined by assuming the mean volume for each of the first four categories was 0.330, 0.221, 0.524, and 0.246 μm^3, respectively, and by calculating the volumes for individuals in category 5. Biomass estimations were made following the procedure given in Ferguson and Rublee (1976) who assumed a density of cell of 1.07 g cm^{-3} and used values for dry weight (27% of wet weight) and carbon (34.4% of dry weight) based on analyses of cultures of estuarine bacteria.

During the period of this investigation the counting procedure was changed in the following manner: Counts before February 1976 were made using 0.2 μm pore size Sartorius filters (cellulose acetate). Afterwards counts were made on 0.2 μm pore size Nuclepore filters (polycarbonate membranes) which have a more discrete pore, but must be dyed black (Hobbie et al. 1977, Bowden 1977). Nuclepore counts were 24% higher than those on Sartorius for replicate samples. All counts are corrected to the higher value. Additionally, Wayfos, a wetting agent, was used to rinse hydrophobic batches of Nuclepore filters during the early months of this study. Its use was discontinued when hydrophobic filters were no longer detected (Hobbie et al. 1977). When Wayfos or dyes were used blanks were also treated appropriately to assess any contamination of samples from those sources.

Results

The number of bacteria was highest in the fall at the surface of the salt marsh sediments (Fig. 1A). The number ranged from 13.50×10^9 cells cm^{-3} of sediment at the surface during October to 1.00×10^9 cells cm^{-3} of sediment at a depth of 20 cm in August. Generally, the number of cells in the surface sediments was about 10^{10} cells cm^{-3}. In all months the number of bacteria at 5 cm was about 60% of the surface value (Table 2) and exhibited a similar seasonal variation. At sediment depths of 10 and 20 cm the numbers of bacteria averaged 35 and 22% of the surface value (Table 2), but displayed relatively little seasonal variation. An analysis of variance of all bacterial counts indicated that depth, month and depth x month interaction terms were all highly significant (Table 3). Coefficient of variation for all counts was 29%.

The average cell volume of bacteria also decreased with depth and was highest during the summer (Fig. 1B). The range of values for cell volumes was 0.260 μm^3 $cell^{-1}$ at the marsh surface in July 1975 to a low value of 0.108 μm^3 $cell^{-1}$ at 20 cm in March. At all depths the largest cell volumes were noted in summer (June, July, and August) and the smallest in spring (March and April). An analysis of variance performed on the estimates of cell volume showed the same factors (depth, month, and depth x month interaction) were significant sources of variation for cell volume as they were for cell numbers.

The estimated standing crop of bacteria (Fig. 1C) followed the same trends as cell numbers and volumes. The highest values for bacterial standing crop were found in late summer and fall and reached a maximum of 221 μg bacterial carbon cm^{-3} of sediment at the surface in October.

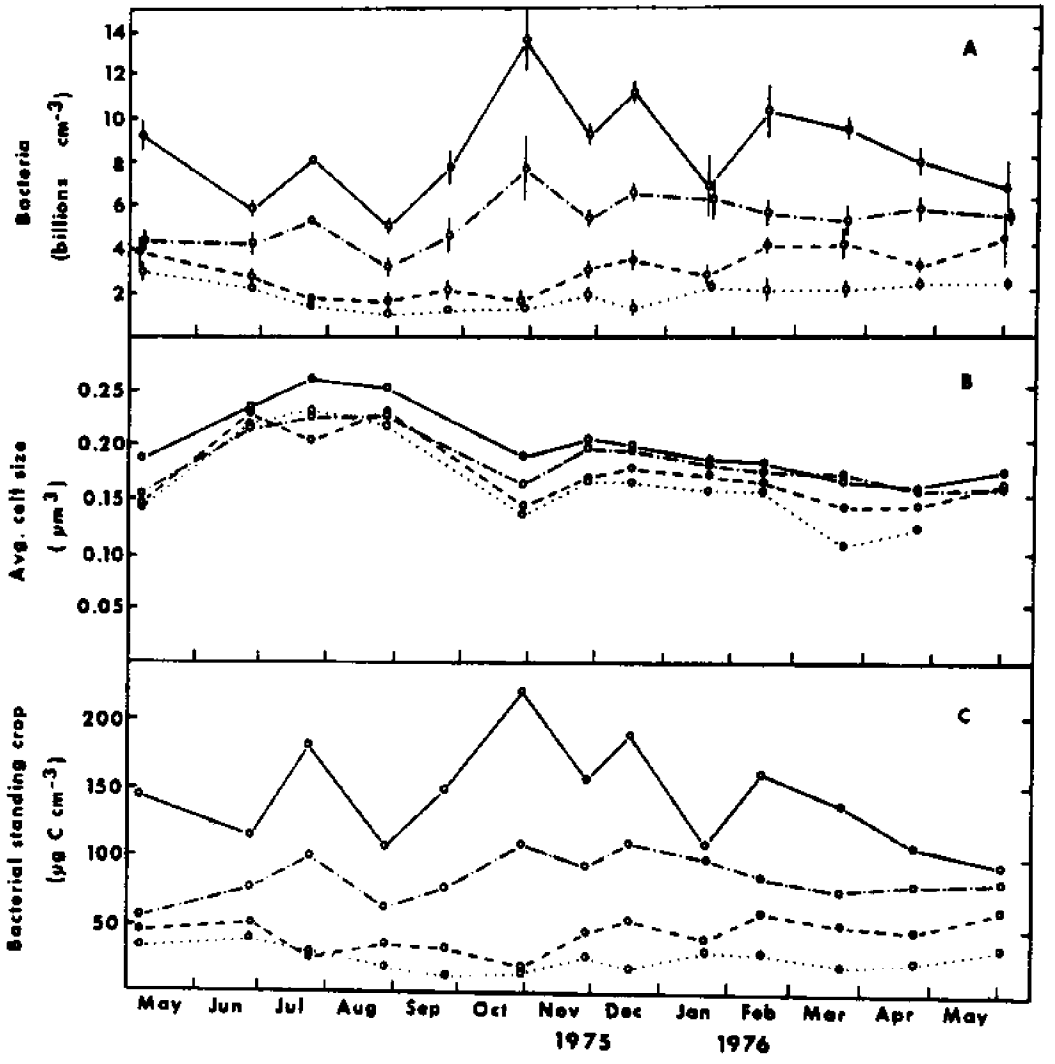

Fig. 1. Bacterial characteristics of a North Carolina salt marsh. A. Number of bacteria. B. Average bacterial cell size. C. Bacterial standing crop. In all figures solid line represents samples from surface sediments (0-1 cm), dash-dot line represents 5-6 cm depth, dashed line represents 10-11 cm depth, and dotted line represents 20-21 cm depth.

Table 2. Mean yearly values (± 1 SE) for some microbial characteristics of a North Carolina salt marsh.

Depth (cm)	Microbial ATP (μg ATP cm^{-3})	Bacteria		Benthic Algae[1]
		Number (10^9 cells cm^{-3})	Volume (μm^3)	Chlorophyll a (μg chlor a cm^{-3})
0-1	1.64 ± 0.22	8.54 ± 0.63	0.20	3.00 ± 0.45
5-6	0.25 ± 0.06	5.36 ± 0.31	0.19	0.77 ± 0.22
10-11	0.07 ± 0.01	3.01 ± 0.29	0.18	0.10 ± 0.02
20-21	0.04 ± 0.01	1.89 ± 0.16	0.18	0.07 ± 0.01

1/ Values calculated for only 10 months.

Table 3. Analysis of variance of bacterial counts.

Source of variation	DF	MS	F-ratio	Significance level
Month (M)	12	10.580	4.63	0.0001
Depth (D)	3	380.57	166.40	0.0001
M x D	36	6.11	2.67	0.0001
Error	101	2.29		

Minimum values occurred in early summer (93 μg bacterial carbon cm^{-3} of sediment). Standing crop decreased with depth; the average value at 20 cm was only 17% of the surface value.

ATP and chlorophyll *a* also decreased with depth of sediment and exhibited high values during the fall months (Fig. 2). ATP attained a maximum value of 3.13 μg ATP cm^{-3} of sediment at the surface of the marsh in October. In all months the ATP content of the sediments decreased significantly with depth. The peak chlorophyll *a* value was found on the marsh surface in June (5.2 μg chlor *a* cm^{-3} of sediment), and values greater than 3.0 μg cm^{-3} of sediment were found during all but the coldest months. Generally, the amount of chlorophyll decreased rapidly with depth.

Discussion

The number of bacteria found in this marsh is similar to that found in other sediment studies. For example, studies in the Newport River estuary have demonstrated numbers of bacteria in subtidal sediments (C. Shelton, unpublished data) and in a transect across a salt marsh (SECTION I) that are similar those found here. Dale (1974) reported numbers of bacteria in intertidal sediments of Nova Scotia to range from 0.32 - 9.97 x 10^9 cells (g dry wt)$^{-1}$ of sediment at the sediment surface. Hobbie and Rublee (1975) found 1.0 - 55.0 x 10^9 cells (g dry wt)$^{-1}$ of sediment in the highly organic surface sediments of Alaskan tundra ponds. Both of these studies utilized the same acridine orange direct counting method as used in this study except that 0.45 m pore size filters were used. On a dry weight basis the numbers found in the present study range from 8.11 to 21.97 x 10^9 cells g^{-1}.

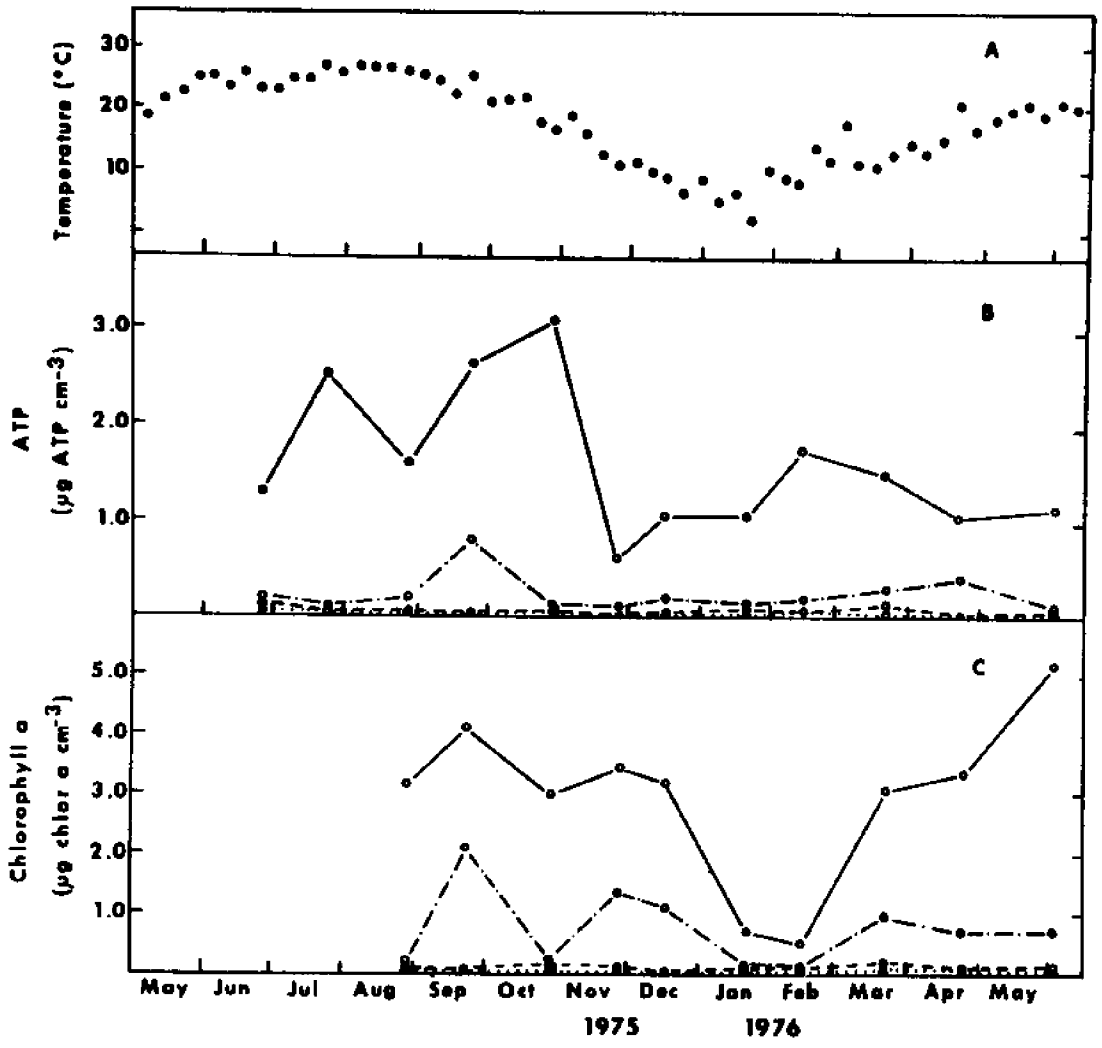

Fig. 2. A. Mean weekly air temperature. B. Concentration of ATP in surface (solid line), 5 cm (dot-dash line), 10 cm (dashed line) and 20 cm (dotted line) sediments of a North Carolina salt marsh. C. Concentration of chlorophyll a in surface, 5, 10, and 20 cm sediments.

The analysis of variance of bacterial numbers (Table 3) indicated strong depth and seasonal components as well as a significant interaction term. The decrease in the number of bacteria with depth has been noted by many investigators (e.g. Zobell 1946) and need not be discussed further except to note that the seasonal trends at the surface and 5 cm depths of sediment are similar and much more variable than the trends at 10 and 20 cm depths (Fig. 1). The variance in the upper sediments suggests a seasonal pattern related to nutrient input, while the relatively stable number of bacteria at depth suggests less strong seasonal influences. The input of particulate and dissolved carbon from macrophytes has been well documented (Odum and de la Cruz 1967, Reimold et al. 1975) and its utilization the subject of many studies (Burkholder and Bornside 1957, Adams and Angelovic 1970, Gosselink and Kirby 1974, SECTION IV). The high number of bacteria found in the marsh sediments in the fall are undoubtably a reflection of nutrient input (both dissolved and particulate) following the die-back of Spartina alterniflora in the fall.

Bacterial volume also varies significantly with depth and season. The maximum average cell volume was observed in the summer, 0.26 μm^3 at the marsh surface, and the minimum volume, 0.09 μm^3, was found at the 20 cm depth of sediment in spring. There was a significant correlation of cell volume with temperature ($r = 0.62$, $p > 0.95$). The average cell volume in the surface sediments was 0.20 μm^3. This value may be compared with those found for nearshore ocean waters of 0.09 μm^3 (Ferguson and Rublee 1975) and estuarine waters of 0.047 μm^3 (Bowden 1977).

It is interesting to note that the average cell volume determined by direct observation in this study is only about 20% of the volume of the

average E. coli cell as reported by Luria (1960), a value that has often been used in calculation of bacterial biomass of natural populations (e.g. Dale 1974). There is compensation for this error, however, since improved methodology in counting bacteria (Hobbie et al. 1977, Bowden 1977) indicates that previous methods have underestimated the number of bacteria by about the same factor. The point serves to illustrate one of the problems of direct counting of bacteria; accurate estimation of cell sizes is difficult because such counts are made near the limits of microscopic resolution. In fact, comparison of acridine orange direct counts (the method used in this study) and scanning electron micrograph preparations suggest that the direct count method over estimates the size of the bacteria (Bowden and Rublee, unpublished data).

The estimates of microbial biomass reflect the strong influence of cell numbers and size estimates in their calculation. The seasonal pattern is less pronounced, but indicates high standing crop in both summer and fall. An integrated value of the average standing crop of bacteria over the top 20 cm of sediment is approximately 14 g C m^{-2} or about 40 g dry weight m^{-2}. Hobbie and Rublee (1975) reported a value of approximately 9 g C m^{-2} for the bacterial standing crop in the sediments of Alaskan tundra ponds. Dale (1974) found 5.5 - 21.5 g dry weight m^{-2} in subtidal sediments, and Zhukova and Fedosov (1963) reported 17 - 56 g dry weight m^{-2} in shallow muds of the Azov Sea. It is surprising that these values agree so well in light of the differences in techniques and conversion factors used in calculating the standing crop.

The determinations of ATP and chlorophyll a are also in agreement with literature values. Similar amounts of ATP have been found in

subtidal estuarine sediments in North Carolina and in salt marsh sediments of South Carolina and Georgia (Table 4). Additionally, Christian et al. (1975) have cited studies from temperate lakes, Alaskan tundra, and sandy coral reefs which also report similar quantities of ATP in sediments. Chlorophyll *a* values are also in the range reported in the literature for estuarine sediments (Ferguson and Murdoch 1975), but are somewhat lower than those reported for some marsh sediments (Estrada et al. 1974).

Mean values for bacterial and sediment characteristics suggest that bacteria contribute significantly to total microbial biomass (Table 5). At the sediment surface bacterial standing crop accounts for 36% of the total microbial standing crop as determined by the ATP method. The percentage contribution of bacteria at depths below the surface are all greater than 100% and suggest that cell sizes were overestimated by a significant factor or that the conversion factor of 250 for ATP is inappropriate. It is likely that cell sizes were overestimated, as mentioned previously, but in a natural system where growth of microbes may not be optimum and in fact large portions of the populations may be non-viable (Postgate 1973, Zavarzina 1955, as cited in Kusnetsov 1970) it is also likely that the conversion factor is inaccurate. Ausmus (1973) found a factor of 500 for bacteria in one study and this would bring the values of ATP-biomass at depth more in line with those of the estimate from the direct counts. A conversion of the mean chlorophyll *a* value, 3.0 μg chlor *a* cm^{-3}, to algal biomass by a conversion factor of 30, indicates that the benthic algae contribute about 22% of the total standing crop at the surface. This is likely an underestimate because of the method used to

Table 4. ATP content of some marsh and estuarine sediments.

Location	Depth (cm)	ATP ($\mu g\ cm^{-3}$)	Reference
Spartina marsh, N.C.	0-1	0.61 - 3.13	This study
	5-6	0.11 - 0.81	
	10-11	0.02 - 0.16	
	20-21	0.01 - 0.12	
Spartina marsh, S.C.	0-1	1.72 - 13.63	Simons (1976)
Spartina marsh, Ga.	0-1	0.37 - 3.61	Christian et al. (1975)
	5-10	0.14 - 0.80	
	10-15	0.02 - 0.35	
	15-25	0.01 - 0.33	
Tidal creek, S.C.	0-1	0.90 - 3.21	Simons (1976)
Subtidal sediments, N.C.			
sand biocenosis	0	1.34 - 2.45	Ferguson and Murdoch (1975)
	5	0.34 - 0.67	
	15	0.08	
sulfuretum	0	0.27 - 1.30	
	5	0.02 - 0.21	
Subtidal sediments, N.C.			
sand biocenosis	0	0.75 - 1.27	Shelton (personal communication)
	5	0.30 - 0.51	
sulfuretum	0	0.55 - 1.27	
	5	0.08 - 0.23	

Table 5. Contribution of microbial and bacterial standing crop to total sediment organic carbon and nitrogen in a North Carolina salt marsh.

Depth (cm)	Microbial Carbon[1] (mg C cm^{-3})	Bacterial Carbon[2] (mg C cm^{-3})	Sediment Carbon[3] (mg C cm^{-3})
0-1	0.408	0.147	25.40
5-6	0.063	0.088	31.90
10-11	0.018	0.047	33.80
20-21	0.010	0.014	13.20

Depth (cm)	Microbial Nitrogen[4] (mg N cm^{-3})	Bacterial Nitrogen[5] (mg N cm^{-3})	Sediment Nitrogen[6] (mg N cm^{-3})
0-1	0.069	0.042	2.19

1/ ATP x 250.

2/ Estimated from direct counts.

3/ Determined by wet oxidation (SECTION I).

4/ ATP x 42 (Christian et al. 1975).

5/ Bacterial carbon/3.54 (R. Ferguson, unpublished data).

6/ Determined by CHN analyzer.

determine chlorophyll a and because the missing chlorophyll data is from summer months which would probably raise the mean value. In any case, the standing crop of algae below the surface appears to be small and not an important contributor to microbial standing crop at depth. The remaining carbon at the surface is presumably composed of an array of organisms including protozoans and small metazoans.

The contribution of total microbes and bacteria to sediment carbon and nitrogen pools is not large (Table 5). In both this study and one in a Georgia salt marsh (Christian et al. 1975) the contribution of the total microbiota (from ATP) was not more than 2% of the sediment organic carbon value and just over 3% of the sediment nitrogen. Similarly, the contribution of bacteria as estimated in this study is even less important (about 0.6% of sediment organic carbon and less than 2% of the nitrogen at the sediment surface). These values compare well with those reported by Ernst (1960) and Ferguson and Murdoch (1975) where contributions of microbes to sediment organic carbon were in the range of 0.05 - 2.98%. These values are all significantly lower than those reported by King and White (1977) in a Florida bay and Moriarty (1975, 1977) for a sand seagrass bed of very low organic carbon content.

The apparent small contribution of the bacteria and total microbial population to organic carbon and nitrogen content of sediments may not give a true picture of their importance, however, since standing crop measures are often poor indicators of metabolic activity. This is particularly important with respect to microorganisms due to their potentially high rates of metabolism. Thus, their true contribution to the ecological community in terms of recycling nutrients can only be

assessed accurately with measures of activity in addition to standing crop values.

Estimates of the generation time of natural populations of bacteria cover a wide range of values. Brock (1967) reported generation times of the epiphyte Leucothrix mucor to be about 11 hours in Long Island Sound. Doubling times of 10 hours to over 280 hours have been reported in lacustrine sediments by Gambaryan (1976) and a range of 9 to 20 days has been cited for various studies in soils (Gray 1976). Recently, values from about 17 to 150 hours have been determined using ATP and lipid-phosphate assays on decomposing oak and pine vegetation in a Florida estuary (Morrison et al. 1977). A similar value (about six days) has been determined for the apparent doubling time of bacteria colonizing fresh Spartina detritus (SECTION III). Wiebe and Bancroft have suggested that a realized doubling time is on the order of 50 - 60 days. My calculations agree that doubling time is probably slow, but the apparently stable standing crop suggests that the term turnover time may be more appropriate than doubling time and, in a manner analogous to a chemostat, the loss rate of bacterial biomass (via death, predation, etc.) may be of the same order of magnitude as the growth rate.

Certainly the potential for bacterial production in salt marshes is high and of interest from the standpoint of mineral and nutrient cycling and as a food resource. This study has confirmed that bacteria constitute a significant portion of the microbial biomass. With the addition of new techniques, such as assay of bacterial lipids (Morrison et al. 1977, King and White 1977), muramic acid (Morrison et al. 1977, Moriarty 1975, 1977), and specific staining techniques (Mayfield 1976), assessment of the

microbial community may become a routine procedure. When combined with activity measurements such as tritium isotope techniques now in use, and the assays for metabolic activity recently developed (King et al. 1977, Morrison et al. 1977) our understanding of the importance of the microbial community in both grazing and detrital ecosystems should be much improved.

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SECTION III

COLONIZATION OF *SPARTINA ALTERNIFLORA* LITTER BY BACTERIA

Introduction

The importance of vascular plant detritus to estuarine and coastal food chains has been recognized by many investigators. Within estuarine systems there are several generally accepted characteristics of such detrital food chains, including (1) significant detrital input by extensive monospecific stands of marsh vegetation (Reimold et al. 1975); (2) rapid leaching of labile components of the fresh detritus followed by slower utilization of the structural material by colonizing microflora (Odum et al. 1973); and (3) grazing of the detrital-microbial complex by consumers (Newell 1965, Mann 1972, Wetzel 1975). While these concepts are now incorporated into our understanding of estuarine ecology, the dynamics of the detrital process are not totally known, particularly with respect to the significance of the microbial community.

A first step in any study to determine the cycling of material must include assessments of the standing pools of such material. Since microorganisms are such a key component in our concept of detrital food chains, this study was conducted to determine the bacterial biomass associated with decomposing *Spartina alterniflora*. The study combined the litterbag technique with the recently developed acridine orange direct count method (AODC) for direct enumeration of bacteria (Hobbie et al. 1977, SECTION II).

Decreases in weight and carbon content of decomposing detrital material such as *Spartina alterniflora* are accompanied by increases in nitrogen content, presumably a result of microbial colonization. Weight loss of both emergent and submergent macrophytes has been studied with litterbags *in situ* as well as in laboratory microcosm (Burkholder and

Bornside 1957, de la Cruz 1965, Ustach 1969, Gosselink and Kirby 1974). In situ rates of loss of litter from litterbags are around 1% day^{-1} for Spartina. Laboratory rates of litter decomposition may be much faster due to preparation of the material (drying, grinding, addition of nutrients) but they demonstrate the effects of environmental factors in the process. Studies assessing qualitative changes of decomposing macrophyte litter have shown that the concentration of carbon decreases as detrital material ages but that the concentration of nitrogen increases (Teal 1962, Heald 1969, Odum et al. 1973, Gosselink and Kirby 1974, de la Cruz et al. 1975). This nitrogen increase is presumed due to the colonization of the detritus by microbes which have a lower carbon to nitrogen ratio than the plant material. Thus, the aged detrital-microbial complex with its decreased carbon to nitrogen ratio is likely a more nutritious food source than the original material (Newell 1965, Odum et al. 1973).

Studies of the microbial community associated with plant detritus suggest that significant populations of bacteria and fungi do act as the primary decomposers. The presence of fungi on both live and decomposing Spartina alterniflora plants has been noted and their importance in decomposition suggested by a number of investigators (Gessner et al. 1972, Gosselink and Kirby 1974, May 1974, Meyers 1974). The importance of bacteria to the microbial community has not been clearly defined. Significant numbers of bacteria at concentrations up to six orders of magnitude higher than the surrounding medium were found on Spartina by plate count methods in Louisiana (Alexander 1972). Scanning electron micrographs of Spartina litter did not reveal large populations of bacteria in a study by May (1974), however, and he suggested that fungi were important as

degraders of detritus along with macrofauna. In laboratory experiments Gosselink and Kirby (1974) reported significant conversion of Spartina litter to microbial biomass which appeared to be primarily bacteria and secondarily fungi during microscopic examination of the samples. They were not able to quantify these populations, however, The single study that has quantified the microbial community on detritus by direct observation has been that of Fenchel (1970) on detrital material derived from Thalassia testudinum, a submerged macrophyte. On detrital particles of undetermined age he noted 3×10^9 bacteria, 5×10^7 flagellates, 5×10^4 ciliates, and 2×10^7 diatoms (g dry weight)$^{-1}$ of the detrital material. He also noted the presence of, but did not quantify, fungi, nematodes, and amphipods. Two studies which have used non-microsopic methods to examine the metabolism of detrital material have suggested that bacteria colonize detrital material rapidly, followed by slower fungal colonization (Morrison et al. 1977) and that bacteria are important in mineralization of the detrital material, whereas fungi are more efficient at conversion of plant litter into microbial tissue (Fallon and Pfaender 1976).

Recent studies have served to reemphasize that salt marshes are important areas of conversion of plant material into microbial and finally animal tissue. For example, Cammen (1975) has noted that there appears to be a relatively short turnover time for oganic carbon in some barrier island salt marshes. In light of recent studies which have emphasized the magnitude of belowground production of Spartina (Valiela et al. 1976, Stroud 1976), the amount of carbon cycled by the detrital pathway within salt marshes is likely of high significance to estuarine species. Additional evidence has suggested that the export of detrital material

from marshes via the water column may not be as important as previously believed (Haines 1976). If such export cannot be demonstrated via sediment movement or storm surges, the amount of detrital material decomposed within the marsh may indeed be considerable.

Materials and Methods

Two sets of litterbags were filled with Spartina alterniflora plant material and were placed in the field near the same area from which the plants were harvested. Each bag was made of nylon mesh net (3 mm mesh opening), measured approximately 10 x 25 cm, and was closed by means of a drawstring at the open end. The procedure for filling the bags consisted of harvesting the aboveground portion of small Spartina plants (3 - 25 cm in height), washing them carefully in the laboratory to remove an accumulated sediment material, removing brown or yellowed leaves, and air drying the plant until no moisture was evident on the surface of the material, and finally, placing 20 to 40 g wet weight of the plant material in the weighed litterbags. The material was returned to the field in the litterbags within two days after harvesting. The first set of litterbags consisted of material harvested on October 22 - 25, 1976 and the second set was harvested on 8 - 10 November, 1976. Each set was placed in the area of the marsh with the tallest Spartina plants (low marsh) in rows perpendicular to the water line at low tide. Individual bags were tied to lines attached to stakes so that the bag lay partially on the sediment surface and partially suspended in air during low tide. All bags were completely submerged at high tide. Both sets of bags were sampled initially and at intervals thereafter for ash free dry weight (AFDW), number of bacteria, carbon and nitrogen content.

Samples for weight loss were taken in triplicate. Material was removed from each bag and placed in tared, ashed beakers. No attempt was made to wash the sediment from any samples in order not to disturb any microbial community which may have been attached to the litter material. Samples were dried at 90°C for 48 hours, weighed, ashed at 500°C for at least 24 hours, and reweighed. AFDW and % ash were determined from these measurements.

Samples for bacterial colonization generally consisted of duplicate subsamples from replicate litterbags, and were counted by a modified AODC method (Hobbie et al. 1977, SECTION II). Each bag was opened and small whole plants or portions of larger plants were cut into 1 - 2 cm segments and placed in a Waring Blendor^{R} with 100 ml prefiltered (through 0.2 µm pore size filters) seawater. The sample was blended at high speed for 60 seconds and then a 0.5 ml subsample of the dilution removed. The subsample was incubated for 60 seconds in 1 ml of a prefiltered 0.01% acridine orange seawater solution, after which it was drawn onto a 0.2 µm Nuclepore^{R} filter by gentle vacuum (less than 0.5 atmospheres) until all water was removed. The filter was then carefully placed on a drop of immersion oil on a glass slide, covered with immersion oil, followed by a No. 1 glass coverslip, and an additional drop of oil. The slide was then viewed under epifluorescent illumination at a magnification of 1250 x. A minimum of twenty cells per field or 200 cells per sample were counted in random fields to assure a confidence level of approximately 10% around the mean number of cells per field. The efficiency of this counting method was assumed to be the same as for counting sediment bacteria, about 87%, and all counts were corrected for this efficiency (SECTION I, SECTION II).

The solution remaining in the blendor was filtered onto pre-ashed and pre-weighed glass fiber filters for determination of dry weight and AFDW of the sample. Filters were dried at 90°C for 24 hours, weighed, ashed at 500°C for 24 hours, and finally reweighed. The number of bacteria (g dry weight)$^{-1}$ or (g AFDW)$^{-1}$ was then determined from the mean number of cells field^{-1} and appropriate conversion factors for dilution of the sample, magnification of the sample, and percentage ash in the filters.

Estimates of bacterial carbon and nitrogen were obtained with the use of appropriate conversion factors. In the case of bacterial carbon the procedure followed was that of Ferguson and Rublee (1975) except that the average cell volume was assumed to be 0.2 μm^3, the value of the average volume of bacterial cells found in the surface sediments of this marsh (SECTION II). The bacterial nitrogen was determined in a similar manner by using a value for bacterial nitrogen equivalent to 28.3% of bacterial carbon (R.L. Ferguson, unpublished data).

Samples remaining from the bags used in the counting procedure were saved for carbon and nitrogen analysis. The material was dried at 90°C for at least 24 hours and then ground in a Wiley Mill^{R} and stored in a desiccator. Subsamples of about 1 g dried litter were prepared for analysis by addition of several ml of 10% HCl solution followed by several minutes of incubation to remove carbonate material, and then freeze-drying to remove water. Triplicate aliquots were then analyzed in an F & M Model 185 CHN analyzer against an acetanilide standard. The remainder of the material was then weighed, ashed, and reweighed so that results could be reported on an AFDW basis.

Results and Discussion

An analysis of variance indicated that the loss of AFDW from the two sets of litterbags was significantly different ($p > 0.99$), although by 175 days the differences appeared minimal (Fig. 1). As a further comparison, a regression analysis was performed on the data after a log transformation. Analyses of this type are commonly used for "decay" functions (Poole 1974) and yield a constant, k, which is the instantaneous rate of decay. In this instance the resultant equation is:

$$\% \text{ AFDW remaining} = a \cdot e^{-k(\text{days in field})}$$

where a and k are constants. These regression coefficients were only slightly different for the two litterbag sets: $a = 94.6$ and $k = 0.0033$ for set 1, $a = 102.5$ and $k = 0.0031$ for set 2, and the correlation coefficients were greater than 0.95 in each case. Thus, the loss rate of material from the litterbags was about 0.3% of AFDW day^{-1}, and corresponds to a loss of 50% of the AFDW in about 230 days.

The pattern of weight loss from the litterbags, as well as the rates, is similar to that found in other studies. Rapid initial weight loss followed by slower rates have been demonstrated in studies by Burkholder and Bornside (1957), de la Cruz (1965), and Odum et al. (1973). The rates of loss are also similar to those found by de la Cruz (1965) who placed Spartina material in various parts of a marsh. He found that 41.5% of the dry weight of the Spartina litter remained after 300 days. Extrapolation of the equations for AFDW in this study yields a similar value. Such similarities suggest that the loss of material from litterbags in this study is not significantly different from that found in

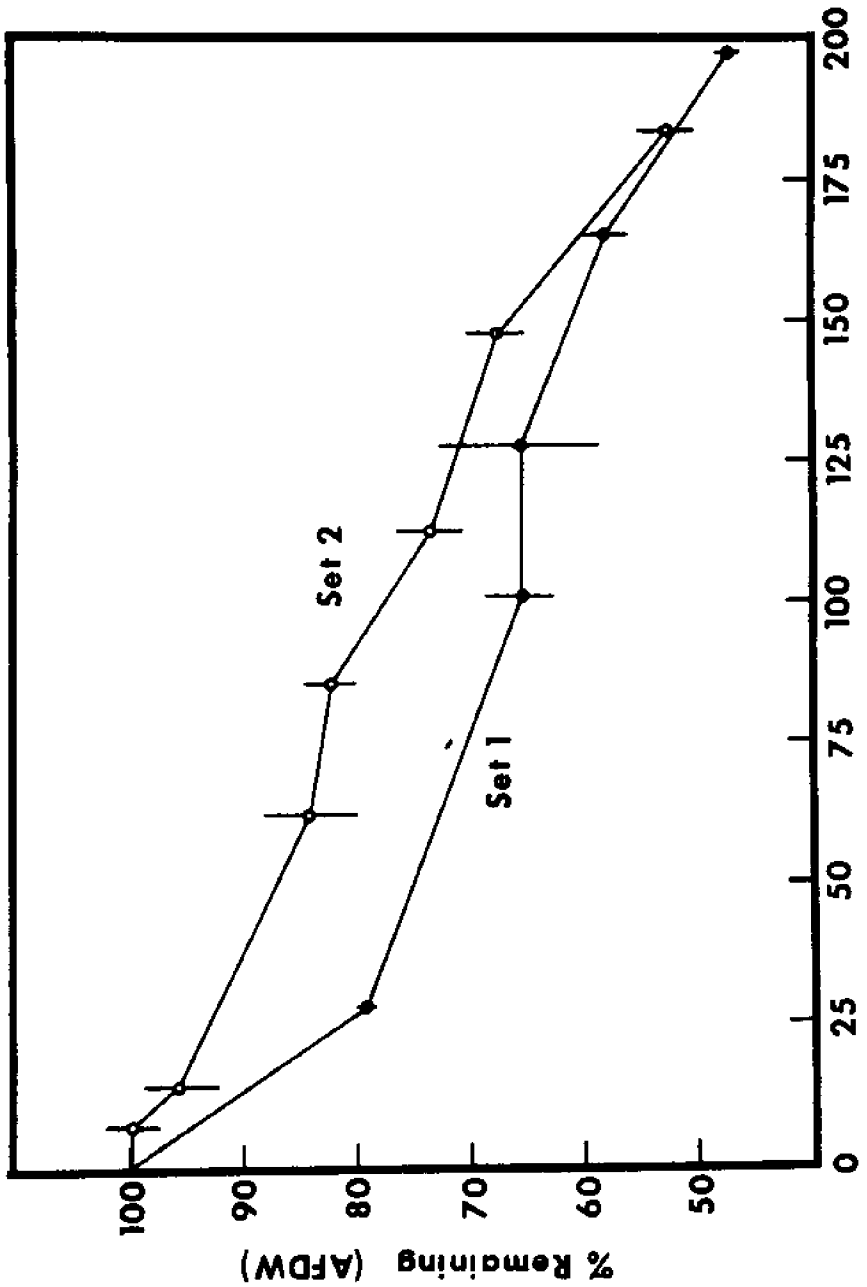

Fig. 1. Weight loss from litterbags in a North Carolina salt marsh.

other studies and that the results reported here for bacterial colonization may be generalized to other Spartina marshes.

The number of bacteria found on the litter material increased by more than an order of magnitude over the observation period (Fig. 2). Initial samples exhibited 1.53×10^9 bacteria (g dry weight)$^{-1}$ or 1.76×10^9 bacteria (g AFDW)$^{-1}$ of detritus, concentrations less than that found in surface sediments of the marsh (SECTION I, SECTION II). The number of bacteria on the litter increased over the 184-day observation period to 29.8×10^9 bacteria (g dry weight)$^{-1}$ or nearly 68.8×10^9 bacteria (g AFDW)$^{-1}$ of litter material. Although the coefficient of variation for these counts was high, around 50%, linear regression equations using the number of days in the field as the single independent variable were highly significant ($p > 0.99$) and had high correlation coefficients ($r = 0.83$ for dry weight and $r = 0.86$ for AFDW). The equations were:

$$\text{No. of bacteria (g dry weight)}^{-1} = 0.135 \times 10^9 \, (t) + 3.78 \times 10^9$$

$$\text{No. of bacteria (g AFDW)}^{-1} = 0.283 \times 10^9 \, (t) + 1.83 \times 10^9$$

where t = number of days in the field. These two equations represent the extreme values for the number of bacteria found on litter material in this study. That is, the equation based on dry weight includes a significant contribution from the weight of accumulated sediment and the number of bacteria is therefore lower than would be on the detrital material alone on a dry weight basis. Conversely, the AFDW basis includes bacteria associated with the sediment material and relegates them to the detritus. Thus, the actual number of bacteria probably lies somewhere between these determinations. An attempt to correct for this was made by using the

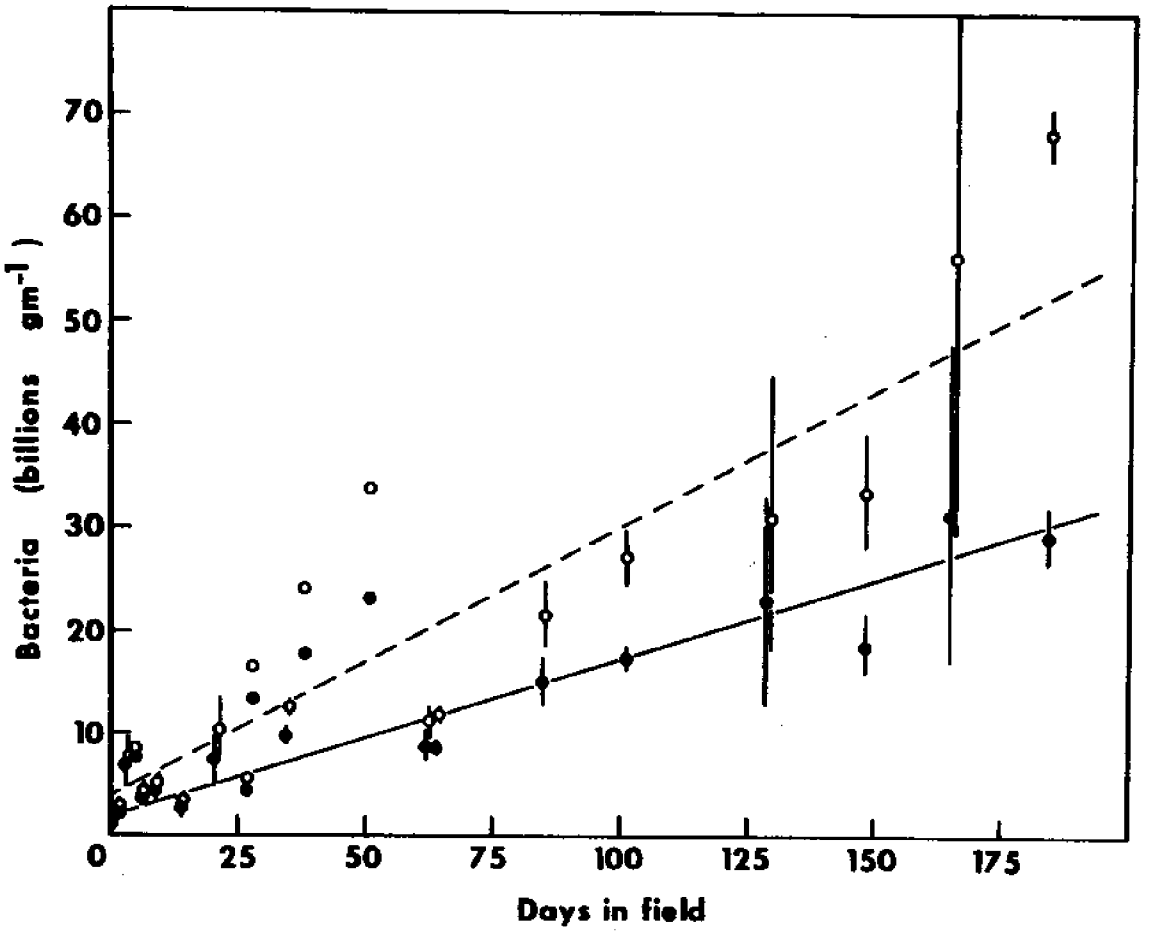

Fig. 2. Colonization of Spartina alterniflora litter by bacteria. Dashed line represents number of bacteria on an AFDW basis and solid line represents number of bacteria on a dry weight basis. Vertical lines represent ± 1 SE of mean at points where multiple samples of litter were analyzed.

assumption that all the ash material in the sample was sediment derived and that the bacteria in the sediment maintain a population of 8.54×10^9 cells cm^{-3} of sediment (the mean yearly value found for surface sediments of this marsh, SECTION II). Under these assumptions and with appropriate conversion (for sediment density and % sediment in each sample) the maximum apparent contribution of sediment bacteria is no more than 30% of the total number on an AFDW basis. Even this conservative estimate then indicates that the number of bacteria on the litter at the end of the field exposure period is a significant increase and cannot be attributed to sediment accumulation on the samples.

The values found for the number of bacteria on the Spartina litter are generally higher than those found in other in situ studies. For example, Alexander (1972) using plate count techniques found several orders of magnitude fewer bacteria than this study, and May (1974) using scanning electron microscopy techniques simply noted a paucity of bacteria. These numbers are similar, during the early days of field exposure, to those found by Fenchel (1970, 1977) on decomposing Thalassia and hay. During the latter stages they exceed the values found by Fenchel. Fenchel also noted strong positive correlations between the number of bacteria, activity (as oxygen uptake), and surface area of the detritus. Although no quantitative data on surface area were taken during this study, the relationship of number of bacteria to surface area was qualitatively evident since there was a positive correlation of bacteria with length of time in the field (Table 1) and since the litter material appeared increasingly "fissured" and shredded during the course of the field exposure.

Table 1. Correlation coefficients of various characteristics of litter material. All values are significant at $p > 0.99$.

	Days in field	Bacteria	Carbon	Nitrogen	C/N Ratio	% Remaining (AFDW)
Days in field	1.000					
Bacteria	0.904	1.000				
Carbon	-0.728	-0.715	1.000			
Nitrogen	0.572	0.598	-0.616	1.000		
C/N Ratio	-0.690	-0.694	0.823	-0.946	1.000	
% Remaining (AFDW)	-0.963	-0.937	0.830	-0.794	0.839	1.000

A qualitative observation from the direct counts suggests that the majority of the bacterial population may have been metabolically active during the first 100 days of litter decomposition. This observation is based on the fact that at low concentrations acridine orange fluoresces red when bound to RNA and green when bound to DNA (Rigler 1966). Hobbie et al. (1977) noted that in active, growing cultures acridine-orange stained bacteria generally appeared red, presumably due to relatively high levels of RNA in the active cells, while in senescent cultures the cells appeared green. Thus, the color of fluorescence may be related to the level of activity of the cells. During the course of this study the majority of cells were red during the early stages of field exposure of the litter (less than 100 days) while green fluorescence of bacteria became increasingly predominant thereafter. The bacterial population as a whole may still have been active during the latter stages of this study, but due to increasing dilution of the sample and successively smaller portions of the population observed microscopically, no determination of this type could be made. Additionally, since the effects of concentration, pH, and ionic strength of the solutions used in staining are not well known, it is difficult to interpret any such information. Certainly this is an area for further research which may provide an assay for activity of natural bacterial populations.

Analyses of carbon and nitrogen content of litterbag material indicated relatively stable concentrations of these elements during the first 100 to 125 days in the field, but changing concentrations after that. In the case of carbon this value was initially about 50% of the AFDW of the litter and decreased to a value near 35% by 198 days (Fig. 3).

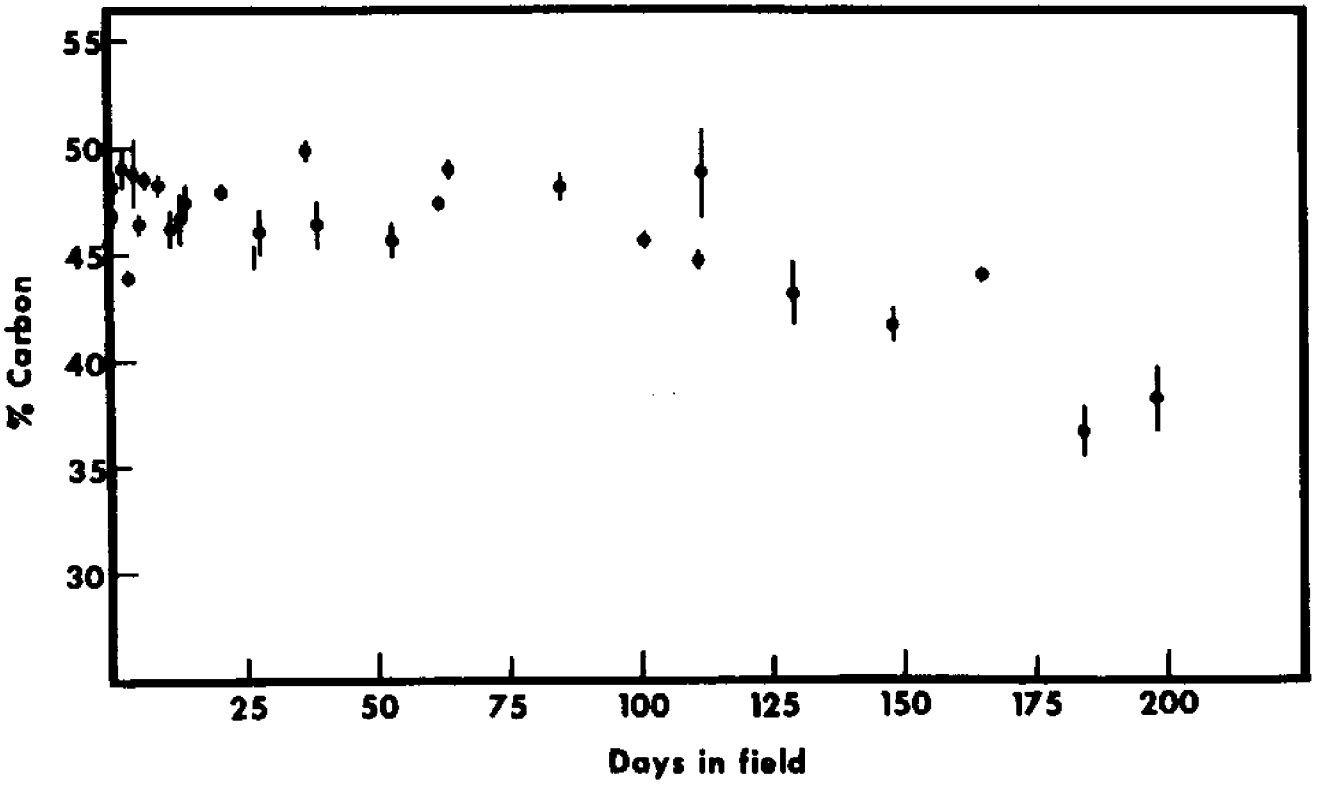

Fig. 3. Percentage of carbon in Spartina alterniflora litterbag material during field exposure. Vertical lines represent ± 1 SE of mean of triplicate subsamples.

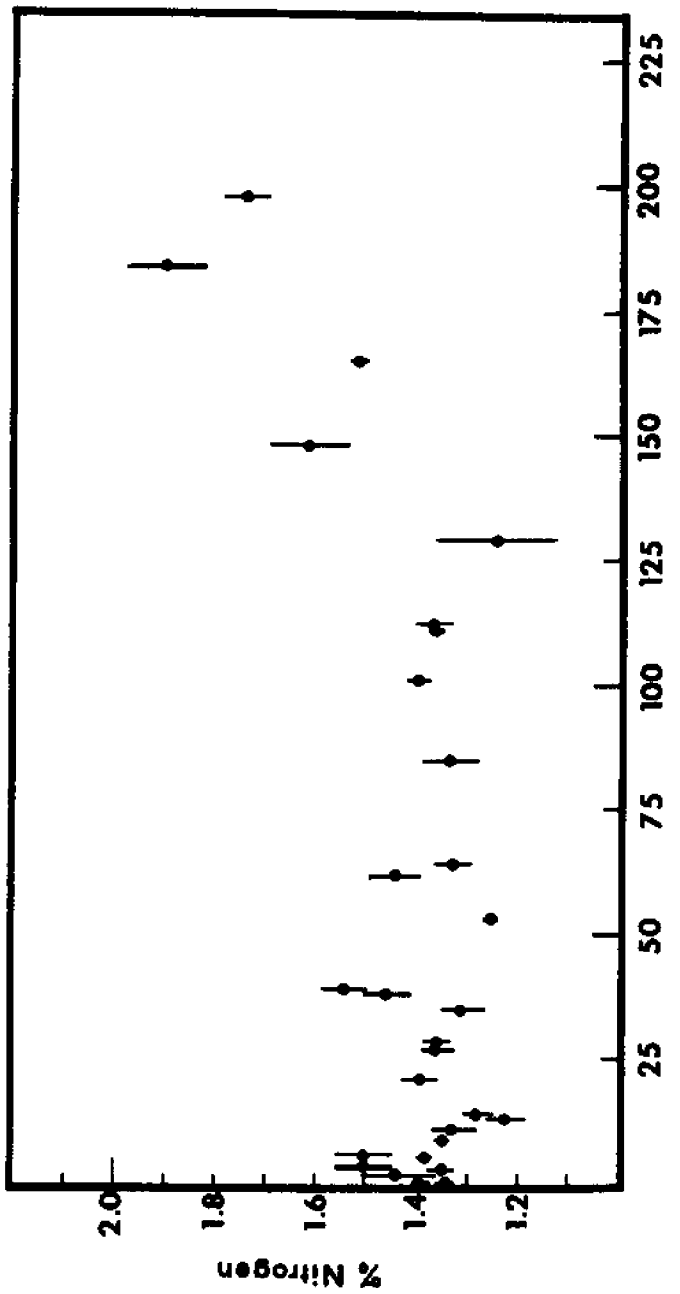

Fig. 4. Percentage of nitrogen in Spartina alterniflora litterbag material during field exposure. Vertical lines represent ± 1 SE of mean of triplicate subsamples.

In contrast, nitrogen increased from its original value of about 1.3 - 1.4% of AFDW to values of about 1.7 - 1.9% of AFDW (Fig. 4). C/N ratio decreased during the same period from an initial value of about 35 to a final value of 20. The initial period of field exposure (0 - 125 days) when concentrations of carbon and nitrogen in the litter do not appear to change may be explained by the cold temperatures during this period when reduced levels of biological activity would be expected. Overall, correlations of carbon, nitrogen, and C/N ratio with days in the field were significant at the $p > 0.99$ level (Table 1).

The contribution of bacteria to the carbon and nitrogen pools of the litter material was small. Bacteria constituted less than 0.01% of the initial total carbon pool, and this percentage increased to only 0.5% after 184 days (Table 2). Similarly, the bacterial contribution to the nitrogen pool was only about 0.07% of the initial total nitrogen and about 3.3% at the end of the field exposure period (Table 2). The implication clearly is that bacteria are not contributing very much to the standing pools of carbon and nitrogen in the detrital complex.

The overall results of this study agree well with a similar study which examined bacteria on litter by indirect methods (Morrison et al. 1977). In that study pine and oak litter was incubated in a Florida Bay and biomass estimates for the bacteria were 20 - 170 mg dry weight of bacteria by an assay of ATP content of the litter. While the values found in this study are not directly comparable since this study was on Spartina and was begun in the fall, there is reasonable agreement. I found 1.2 mg dry weight of bacteria (g dry weight detrital complex)$^{-1}$, a value not too different from the ATP-based estimate of Morrison et al. (1977).

Table 2. Carbon and nitrogen pools of bacteria associated with Spartina litter.

Day	Bacterial carbon		Bacterial nitrogen	
	as ug C g^{-1} dry weight of litter	as % of C in litter	as ug N g^{-1} dry weight of litter	as % of N in litter
0	26	0.01	8	0.07
14	48	0.01	14	0.14
28	229	0.06	64	0.61
53	397	0.10	112	1.17
85	261	0.08	74	0.85
112	290	0.09	82	0.95
129	397	0.14	113	1.47
165	562	0.22	159	1.99
184	508	0.51	144	3.29

Additionally, the maximum activity values demonstrated in the Florida study indicated a slow growth rate for the bacteria, between 0.01 and 0.11% per 2 hours by their various assays. Slow growth rates are also suggested in this study where the number of bacteria was only 19 to 39 times greater after 184 days.

In summary, this study and others have indicated that there is a large bacterial population on detrital material in the field. The standing crop of the bacteria, however, appears to be a minor contribution to the total carbon or nitrogen pools of the litter material. Thus, if increases in nitrogen of decomposing macrophyte material are indeed attributable to the microbial community, it must be increases in organisms other than the bacteria, such as fungi, algae, and protozoans.

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SECTION IV

DECOMPOSITION OF SPARTINA ALTERNIFLORA IN LABORATORY MICROCOSMS

Introduction

Detrital pathways are important in coastal ecosystems but our understanding of such energy transformations is incomplete. High rates of primary and secondary production within coastal ecosystems and the scarcity of organisms that feed directly upon and assimilate the primary production has often been noted. Studies have suggested that an indirect pathway, that is, primary producer → saprophage → consumer, may be the key to energy transfer in these systems. Recent studies (e.g., Wetzel 1975, Cammen, in prep.) tend to confirm the importance of the detrital complex in secondary production, but suggest that such a view is a simplistic representation of the natural system. Thus, the process of decomposition and subsequent utilization of macrophyte detritus is currently an area of intense study.

Previous studies of macrophyte decomposition have characterized the pattern of decomposition and pointed out some factors which may control the process. Both in situ litterbag and laboratory studies have demonstrated that decomposition of detrital material is initially rapid followed by a slower rate over the long term (Burkholder and Bornside 1957, de la Cruz 1965, Saunders 1972, Odum et al. 1973, SECTION III). The initial rapid phase is presumably due to leaching of soluble material and rapid utilization by microbes and the slower phase reflects utilization of the more refractory structural components of the detritus (Olah 1972, Odum et al. 1973). Some of the factors affecting decomposition

include the quality of the detritus (including particle size, age, carbon to nitrogen ratio, and amount of structural carbohydrates), quantity and types of decomposers, presence of higher trophic levels (predators on saprophages), and environmental conditions (Ustach 1969, Odum et al. 1973, Gosselink and Kirby 1974, Parnas 1975, 1976, Fenchel 1977a, 1977b). Decomposition then is the sum of biological and physical processes affecting the detritus (Goksyr 1975).

This study attempted to assess the effects of some environmental factors on the decomposition of the salt marsh cordgrass, Spartina alterniflora, in laboratory microcosms consisting of marsh sediment and a small layer of overlying water, but with organisms larger than 1 millimeter excluded. The basis for such a study includes recognition of the importance of the sediment microbial community as well as the detritus in the decomposition process. The study was designed to consider the effects of temperature, "location" of the detrital material, fraction of the plant material, and of small nitrogen additions on the decomposition rates within these microcosms. An additional feature was the use of Spartina material that had been labelled with $^{14}CO_2$ by growth of the plant in a $^{14}CO_2$ atmosphere for 60 days.

Previous studies of decomposition utilizing labelled plant material have aided our understanding of the role of specific organisms and plant fractions in the breakdown process. For example, at the average rate of mineralization (as per cent of available label) over 7 days in a seawater microcosm was about 3.6% day^{-1} for both whole Spartina detritus and a Spartina leachate, as compared to 0.23% day^{-1} for the leached plant fraction (Fallon and Pfaender 1977). In the same study, antibiotics were added to some microcosms in an attempt to selectively inhibit metabolism

of fungi or bacteria. Although complete inhibition of microbial metabolism is not possible with antibiotics, those experiments suggested that fungi had higher growth efficiencies than bacteria and consequently that bacteria were important in rapid short-term mineralization of labile components while the fungi were more important in conversion of detritus to microbial carbon. Similar results were found in laboratory chemostats which included sediment and water as well as combinations of microflora, meiofauna, and a consumer, Capitella capitata (Lee et al. 1976). The rates of mineralization of radioactively labelled eelgrass, Zostera marina, ranged from about 1.5 to 2.0% of original label day^{-1} over the remaining three weeks of the experiment. Mineralization was most rapid in the systems containing microflora and meiofauna. The half-life of the Zostera detritus was estimated to be about 330 days. In both of these studies the general decomposition pattern of rapid initial mineralization followed by slower rates was observed.

Materials and Methods

Labelled Spartina alterniflora

The Spartina alterniflora used in this experiment consisted of small plants that were transplanted from a North Carolina salt marsh and grown in buckets within an incubation chamber in a $^{14}CO_2$ atmosphere (see description by Ferguson and Williams 1974). After a 60-day incubation the plant material was harvested, freeze-dried, separated into components (leaf, stem, or root), and ground so that it would pass through a 0.35 mm mesh. All sample material was sterilized by gas to prevent chemical or biological changes of the material during the preparation procedures.

Subsamples of the Spartina leaves, stems, and roots were combined in proportions of about 2:1:7 (dry weight), which approximated the proportion of each component at the time of harvest. Some of this material (about 0.5 g) was then placed in a scintillation vial with 10 ml of 0.2 μm prefiltered seawater and allowed to leach for 4 days at 4°C with occasional agitation. The mixture was then filtered through a glass fiber filter, the plant residue freeze-dried, and the leachate saved. The fractions were analyzed for radioactivity by digestion in NCS^R, a tissue solubilizer, followed by liquid scintillation counting in Aquasol^R with benzoyl peroxide added to reduce chemical quench. Subsamples of the leachate were injected directly into the counting solution. Activity of the plant fractions by these methods was:

whole plant	24.3 μCi g^{-1}
leached fraction	17.0 μCi g^{-1}
leachate	0.1 μCi ml^{-1}

Finally, all material was stored at 4°C to inhibit chemical or biological change of the material.

Microcosms

The microcosms were 50-ml flasks with 10 cm^3 of marsh surface sediment and 2 ml of overlying water. After the top 2 cm of sediment was collected from a Spartina marsh, it was passed through a 1 mm mesh sieve to remove large shell fragments and macrofauna. Replicate 10 cm^3 portions of the sediment were then added to each flask and allowed to settle evenly on the bottom of the flask. Seawater which had been passed through a 0.2 μm membrane filter was then gently layered onto the mud surface of each flask. The flasks were stored in the dark at the experimental temperature

for 24 hours before the experiment. During this period the sediment in the flasks developed an oxidized surface layer of about 1 mm thickness.

Five sediment cores were also taken from the marsh surface at the same time the sediment was collected and were treated as additional experimental microcosms. These cores were collected in short section of Plexiglass tubing with a diameter (4.45 cm) similar to that of the flasks at the mud surface. Next, the bottom of the core tubes were sealed with rubber stoppers; the top of the core tube had been previously covered with Plexiglass containing a small hole. Filtered seawater was then layered on the cores to about the same level as in the flasks and cores were stored in the same manner as the flasks. Several millimeters of oxidized surface layer was always present in the sediment cores.

Experimental procedure

After the 24 hr equilibration period each flask received either 10 mg of whole Spartina material, 10 mg of leached Spartina material, or 1 ml of Spartina leachate. Core tubes received 10 mg of whole plant material. Blanks (killed controls) consisted of replicates in which 3 ml of 0.2 N H_2SO_4 was added. All flasks and cores were sealed with rubber serum stoppers with a plastic cup attached. The plastic cup was positioned in the air space above the sediment and water and contained a folded piece of chromatography paper.

Thirteen treatments tested differences in mineralization of the organic matter due to environmental or physical factors or to differences in the plant fractions. Each treatment included three live replicate flasks or cores and one or two blanks. Three treatments consisted of incubation of a set of flasks at temperatures of 10, 20, or 30°C. The

flasks incubated at 20°C were also considered as controls for all experiments. The effect of surface versus subsurface incubation was tested by mixing the labelled material into the sediment. Mixing disrupted the surface oxidized layer, but it was soon reestablished. Incubation of labelled material with sediment in the core tubes assessed the effect of disturbance of the sediment. A test for differences in plant fraction consisted of the previously mentioned control (whole plant fraction), a treatment where leached material was added, and a treatment where the leachate was added. Two experiments, consisting of three treatments each, were used to look at nitrogen additions. Three of these treatments were additions of 2, 4, or 8 mg of nitrogen as NH_4Cl and three consisted of additions of the same levels of nitrogen as KNO_3. These levels provided inorganic nitrogen in quantities which significantly exceeded those of the organic nitrogen in the detritus.

The microcosms were incubated for 29 days, except that the control and plant fraction treatments were incubated for 53 days. At 1 to 6 day intervals (the shorter intervals were used during the first 15 days of the incubation) the amount of mineralized label in the microcosms was determined by the method of Hobbie and Crawford (1969). Briefly, this involved injection of phenethylamine through the septa of the rubber stopper and onto the chromatography paper in each flask or core. After 8 hr the rubber stopper was removed, the chromatography paper placed in a scintillation cocktail for determination of $^{14}CO_2$, a new piece of chromatography paper placed in the cup, and the stopper replaced.

There are two possible errors in the measurement of $^{14}CO_2$, one is incomplete absorption of CO_2 from the atmosphere in the flask, and the

other is incomplete exchange between the sediment, water, and atmosphere in the flask. A test for the first error was made by injecting a known quantity of labelled bicarbonate into nine replicate flasks and then immediately acidifying the media with 3 ml of 0.2 N H_2SO_4. After injection of phenethylamine the $^{14}CO_2$ was absorbed during an 8 hr incubation. The efficiency of absorption of CO_2 was 83.1 ± 2.5% ($\bar{X}$ ± SE). The second error was tested by an acidification of each sample on the last sampling day. The results were compared with the expected amount of mineralization based on the previous interval. No large differences were found and this second error was therefore judged to be unimportant. Thus, sample values were multiplied by a factor of 1.2 to correct for the first error.

Results

The pattern of $^{14}CO_2$ evolution from the experimental microcosms was similar in most experiments and can be illustrated by the results from the various temperature treatments (Fig. 1). Mineralization was most rapid during the first 6 to 10 days at all temperatures and decreased during the remainder of the incubation period. Rates were highest for the highest temperature (Table 1) with an overall rate of mineralization (as percent of original label added) of 0.59% day^{-1} for the 10°C incubation, 1.09% day^{-1} for the 20°C incubation, and 1.37% day^{-1} for the 30°C incubation. An analysis of variance of the data indicated that treatment differences were significant ($p > 0.99$). Replication within treatments was generally within 10% of the mean in the temperature treatments. Good replication was not always found in other treatments, however, and is

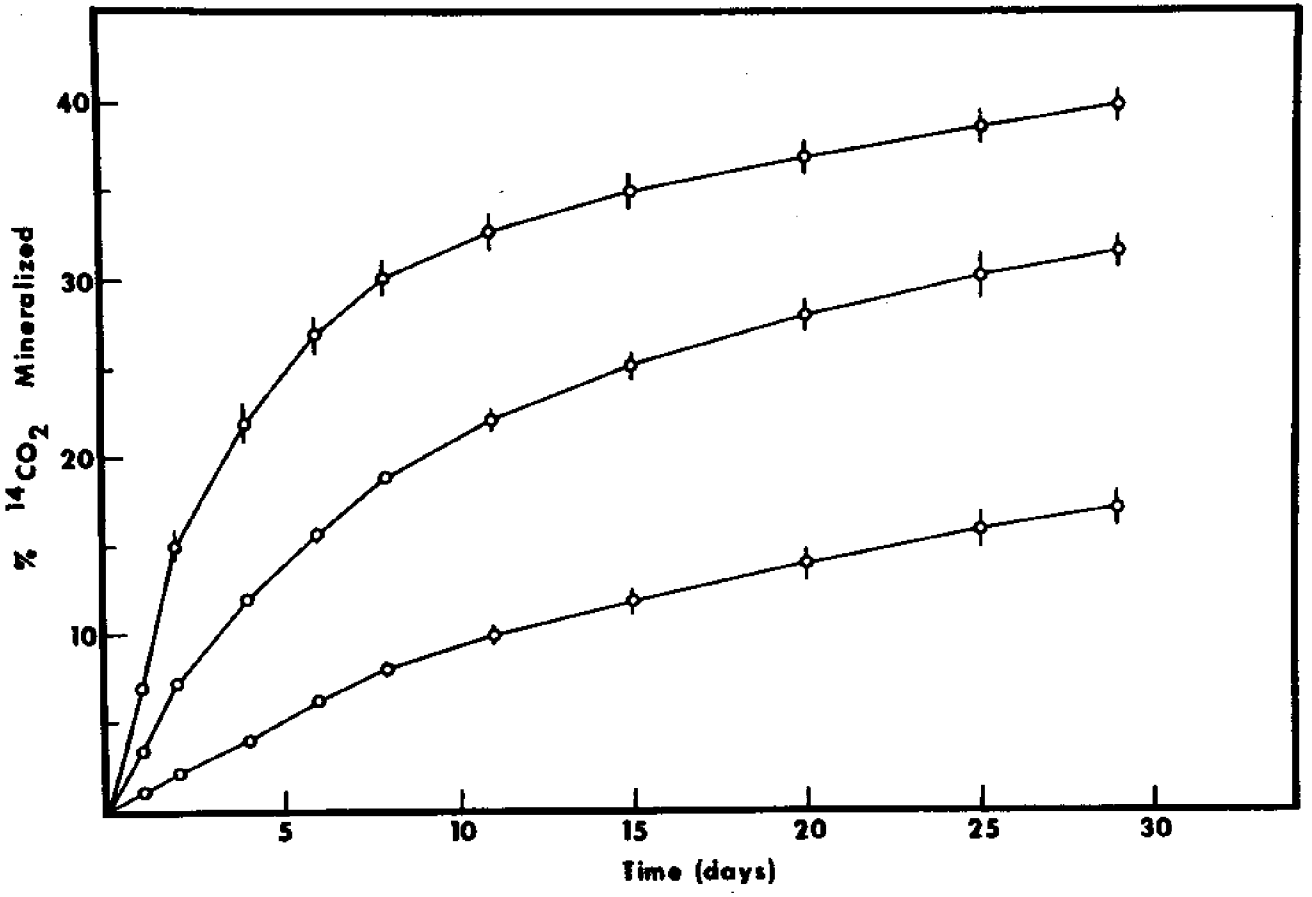

Fig. 1. Mineralization of ^{14}C-labelled Spartina alterniflora in experimental microcosms at 10°C (lower curve), 20°C (middle curve), and 30° C (upper curve). Each point represents the mean of three replicates and verticle bars represent ± 1 SE.

Table 1. Mineralization rates and coefficients of variation for experimental treatments.

Treatment	Length of Incubation (days)	Rates of mineralization[1] (as % original label day^{-1}) early phase	late phase	Coefficient of variation (%)
10°C Incubation	29	0.78	0.39	12.6
20°C Incubation - "Control"	29 (53)	1.67	0.46 (0.31)	5.2
30°C Incubation	29	2.33	0.35	5.9
Detritus mixed into flask	29	1.65	0.52	6.5
Undisturbed sediment core	29	1.30	0.32	10.3
Leached detritus fraction	52	1.91	0.44	9.8
Detrital leachate	53	1.87	0.21	42.3
NH_3 addition (2 mg)	29	1.50	0.37	31.6
NH_3 addition (4 mg)	29	1.63	0.36	34.3
NH_3 addition (8 mg)	29	1.71	0.46	20.6
NO_3 addition (2 mg)	29	1.02	0.26	26.9
NO_3 addition (4 mg)	29	1.39	0.48	7.1
NO_3 addition (8 mg)	29	1.78	0.51	16.9

1/ Early phase = 0 to 15 days, late phase = 16 to 29 days or 16 to 53 days.

reflected in the coefficient of variation for each treatment which was as high as 42% in the case of the leachate treatment (Table 1).

The location of the labelled material was not a significant source of variation ($p < 0.87$), but the experimental flasks had significantly ($p > 0.99$) higher rates of mineralization than did the sediment cores (Fig. 2, Table 1). Thus, disturbance of the sediment results in high rates that likely would not reflect in situ measures of detrital mineralization.

In the plant fraction experiment (Fig. 3), an analysis of variance indicated that the treatment effect was significant ($p > 0.95$) even though the variation within the leachate treatment was high (Table 1). A slight difference in the pattern of the mineralization was also noted in this experiment. The mineralization of the leachate was most rapid during the first 11 days of the experiment and was the highest of the three treatments. Because the mineralization rates of both this fraction and the leached material were changing rapidly relative to each other after 29 days, these treatments (and the control) were allowed to continue for an additional 24 days. At the end of the experiment, the leachate had an overall rate of mineralization lower (0.68% day^{-1}) than either the whole plant fraction (0.69% day^{-1}) or the leached material (0.85% day^{-1}). The pattern of the decomposition of the leached material was of interest also, because it appeared to have an initial lag period (the first day) followed by a rapid rate of mineralization resulting in greater overall mineralization than either whole or leachate fractions. This pattern is similar to that seen in measures of microbial growth in culture.

High variation among the replicates in the nutrient addition experiments resulted in no significant differences ($p < 0.87$) among the

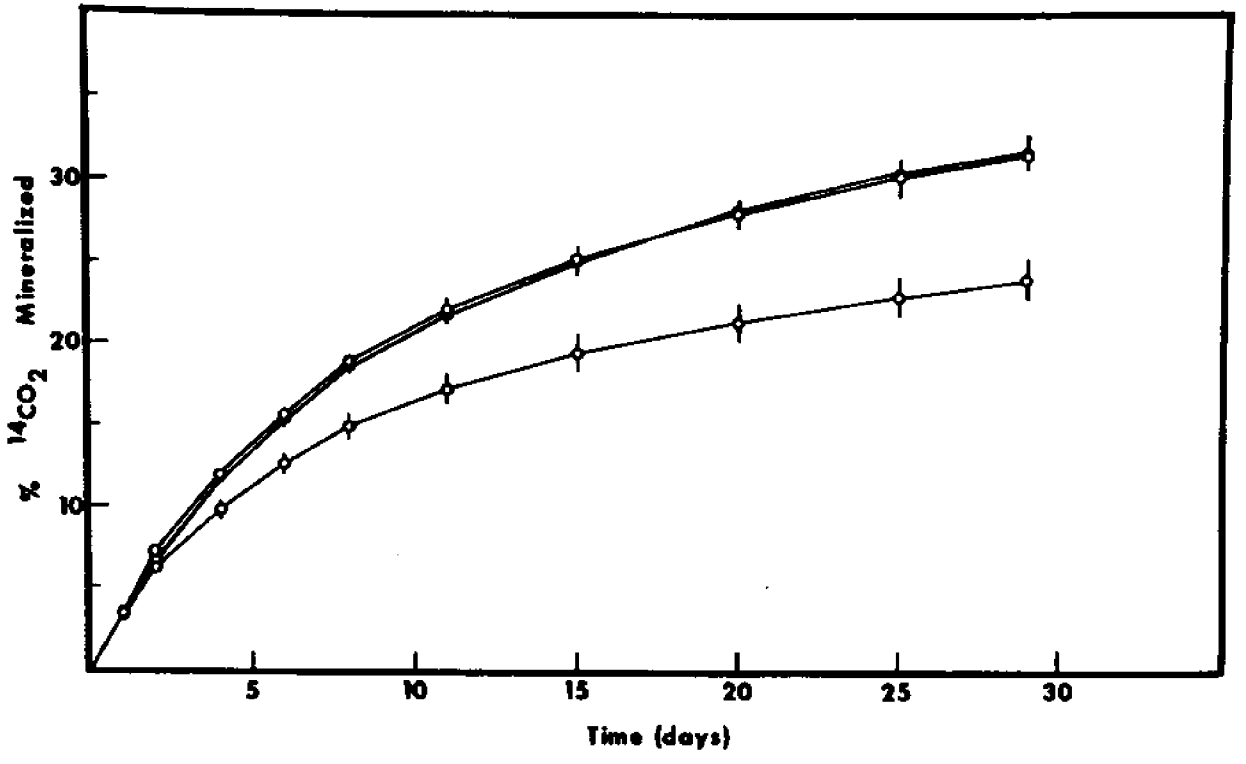

Fig. 2. Mineralization of ^{14}C-labelled Spartina alterniflora in experimental microcosms. Upper curves represent control flasks and flasks where Spartina material was mixed into sediment. Lower curve represents mineralization from undisturbed sediment cores. Vertical bars represent ± 1 SE of mean of triplicate samples.

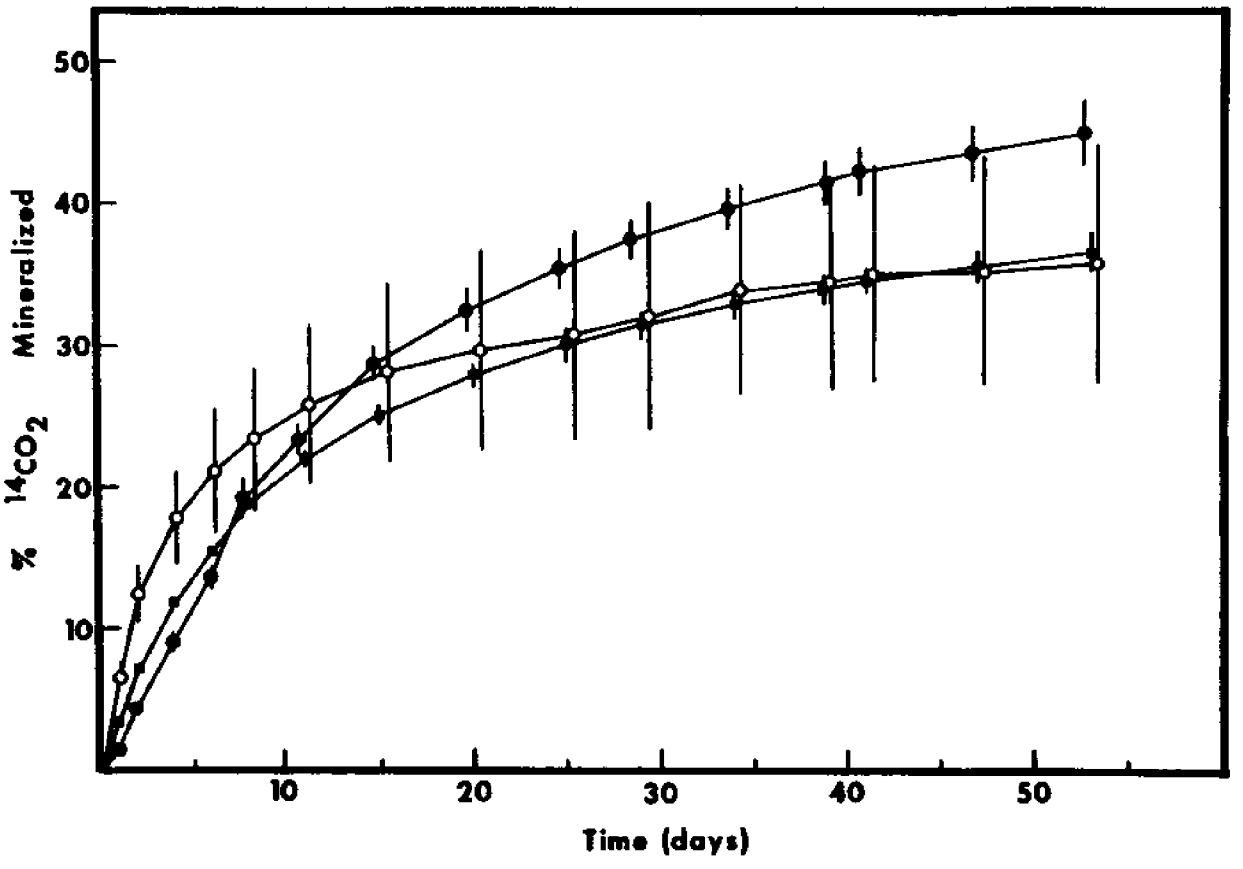

Fig. 3. Mineralization of ^{14}C-labelled Spartina alterniflora fractions in experimental microcosms. Open circles represent Spartina leachate, closed circles represent leached Spartina, and squares represent whole Spartina material. Vertical bars represent ± 1 SE of mean triplicate samples.

treatments with ammonia additions, although there were differences in the nitrate additions. In both experiments the highest level of addition resulted in the highest rates of mineralization (Figs. 4, 5). Thus, a definite stimulatory effect was found after nitrate addition ($p > 0.99$) and a possible effect after the ammonia additions.

The pattern of decomposition curves suggested that application of a non-linear model might be of aid in comparison of the results. A curvilinear model was selected on the basis of observations that a log transformation did not linearize the results, and that the curves appeared to approach an asymptote. The model chosen was that of a curve approaching a sloping asymptote (Riggs 1963) and is described by the general equation:

$$Y = a + bt - a(Y_o + e^{-kt})$$

where Y = mineralized substrate, t = time, a and b are coefficients of the asymptote (intercept and slope), and k is the coefficient of the exponent which determines how rapidly the curve approaches the asymptote. In this case, Y_o is by definition equal to zero, so that the equation becomes:

$$Y = a + bt - ae^{-kt}$$

Each treatment was then analyzed for the parameters a, b, and k by using a non-linear least squares regression procedure (Barr et al. 1976). A conservative test of the results found no significant lack of fit for this model ($p < 0.95$).

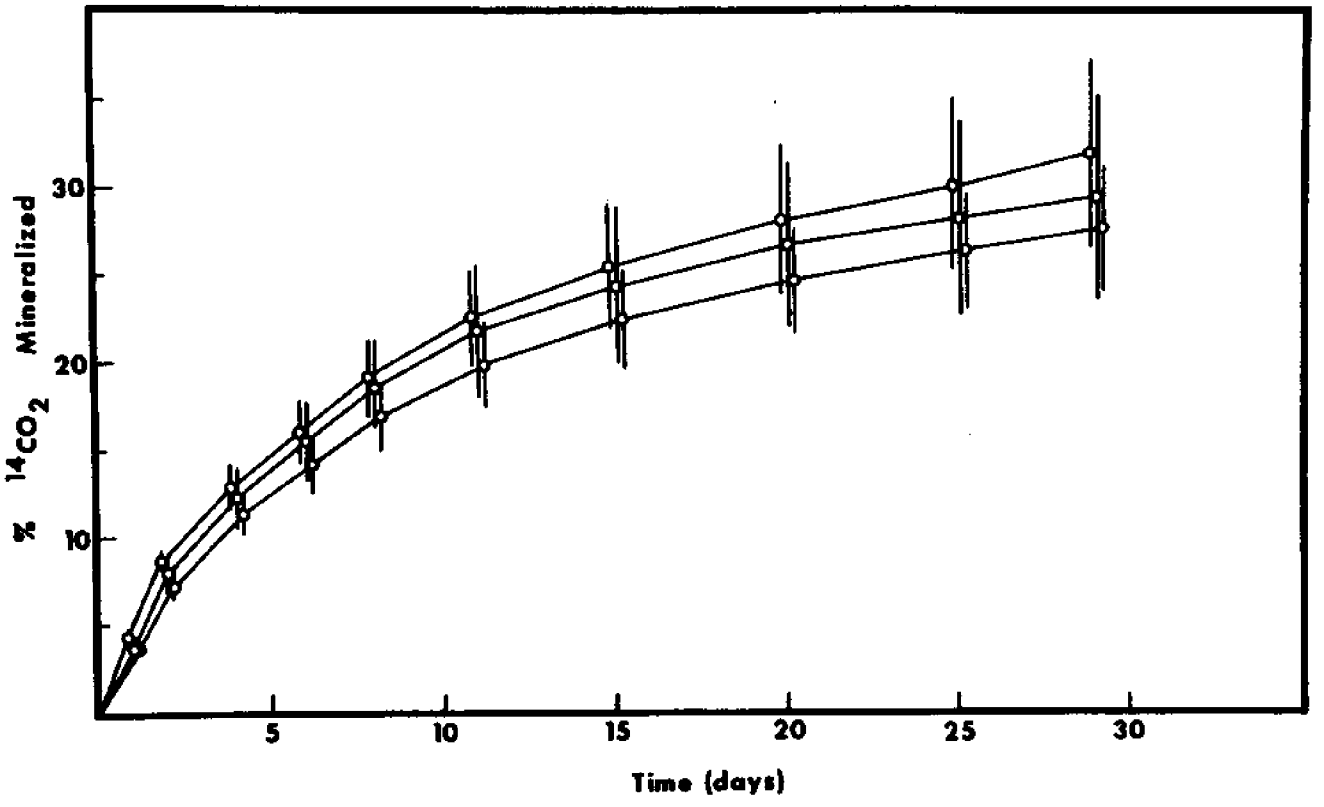

Fig. 4. Mineralization of ^{14}C-labelled Spartina alterniflora in experimental microcosms with additions of 2 mg (lower curve), 4 mg (middle curve), or 8 mg (upper curve) nitrogen as NH_4Cl. Vertical bars represent ± 1 SE of the mean of three replicates.

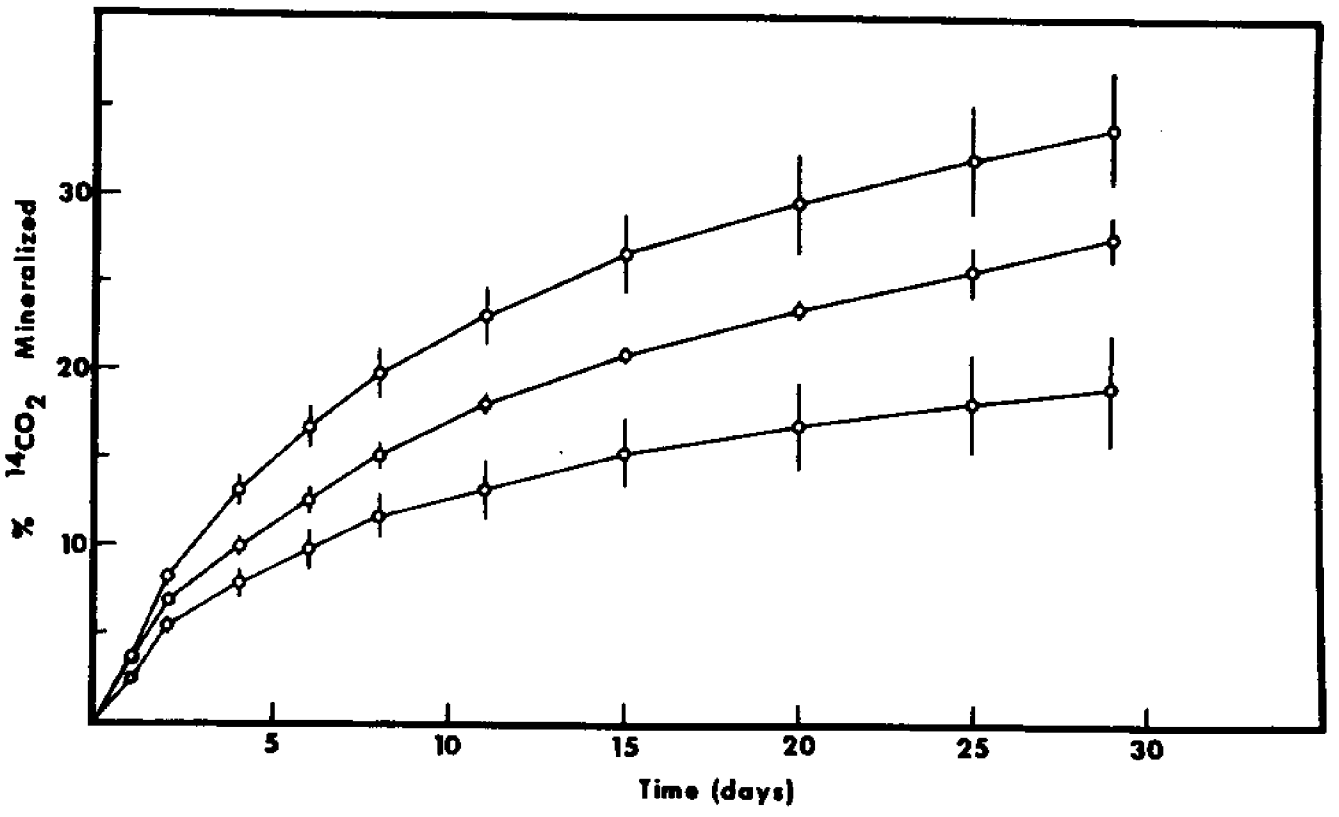

Fig. 5. Mineralization of ^{14}C-labelled Spartina alterniflora in experimental microcosms with additions of 2 mg (lower curve), 4 mg (middle curve), or 8 mg (upper curve) nitrogen as KNO_3. Vertical bars represent ± 1 SE of the mean of three replicates.

The parameters produced by the model allowed estimation of turnover times of the Spartina material and in most treatments this was 200 - 300 days (Table 2). It should be noted that the paramters a and k seem most sensitive to temperature or plant fraction differences. The most important coefficient of the model may be the slope of the asymptote, however, since it is assumed to represent the rate of community mineralization over the long term and thus allowed calculation of the turnover rate of the plant material added to each treatment. For example, in the temperature experiment the turnover times calculated for 10, 20 and 30°C are 396, 237, and 209 days, respectively (Table 2).

Discussion

Modelling approaches to the understanding of natural processes are based upon the premise that model systems reduce the complexities of the natural system, but retain the relationships under question. In practice, any modelling approach, whether biological or mathematical, cannot simultaneously be general, precise, and real (Levings 1966). Thus, controlled laboratory experiments that are highly simplified and illustrate direct cause-effect relationships may sacrifice information on community response to a particular factor, and conversely, complex systems may show indirect responses while overshadowing the direct effect. The microcosm approach is a simplification of the system intended to illustrate general ecological concepts; however, due to inherent alteration of the biological parts of the microcosm (even if they were generated from a natural sample) and due to the sometimes extreme variation found within such experimental systems, all results need to be interpreted with caution (Cooke 1977).

Table 2. Coefficients of the model $Y = a + bt - ae^{-kt}$ for the experimental treatments, and estimated turnover time based on the model coefficients.

Treatment	Coefficients			Turnover time (days)
	a	b	k	
10°C Incubation	12.16	0.222	0.079	396
20°C Incubation - "Control"[1]	20.00 (19.91)	0.368 (0.306)	0.185 (0.190)	217 (262)
30°C Incubation	30.25	0.334	0.290	208
Detritus mixed into flask	20.09	0.381	0.178	210
Undisturbed sediment core	13.75	0.229	0.196	377
Leached detritus fraction	30.13	0.297	0.094	232
Detrital leachate	24.14	0.292	0.294	260
Ammonia addition (low - 2 mg)	18.45	0.330	0.195	247
Ammonia addition (medium - 4 mg)	20.00	0.300	0.200	267
Ammonia addition (high - 8 mg)	20.41	0.394	0.204	202
Nitrate addition (low - 2 mg)	11.02	0.280	0.238	318
Nitrate addition (medium - 4 mg)	11.68	0.560	0.272	158
Nitrate addition (high - 8 mg)	21.08	0.447	0.187	177

1/ Numbers in parenthesis indicate values for 53-day incubation, other numbers are for 29-day incubation.

Within this study specific examples of the necessity for careful interpretation can be found in the nutrient addition and leached plant fraction treatment results. In both types of nutrient additions the "control" (no added nutrient) yielded rates of mineralization that were similar to the intermediate level of nitrogen addition. Since significant differences were found among the treatment levels in the nitrate addition it is likely that the control is in fact not a proper control. That is, addition of the nitrate in quantities that are higher than those found in the natural marsh (DeLaune et al. 1976) probably stimulated denitrifier microbial types so that these treatments cannot be directly compared with the control. The ammonia additions were not significantly different, even though a trend of higher mineralization was noted with increasing levels of additions. In this case the control flask may indeed be an adequate control since the ambient ammonia concentrations in marsh sediments can reach high levels (DeLaune et al. 1976). The response of the various treatments then may be an indirect community response. The best example of this is found in the leached Spartina treatment, where the mineralization rates was slow at first, then rose, and then leveled off. That type of response is highly suggestive of the typical growth curve of a bacterial culture. In other words, it is likely that the leached Spartina treatment was selective for a microbial community that could utilize the refractory components of the detritus effectively. Hence, it is important to monitor the successional pattern in the microcosms.

The pattern of mineralization of the labelled Spartina material compared favorably with the patterns found in other studies of mineralization of substrates and was complementary to patterns of material remaining after a period of decomposition. For example, two studies

(Ustach 1969, Fallon and Pfaender 1977) conducted similar experiments with Spartina material in microcosms with seawater over 7 to 9 day incubations. Ustach (1969) used short term labelling procedures but found similar rates of mineralization to those found in this study; 1.3 - 2.2% day^{-1} over the first 7 days. The result of Fallon and Pfaender (1977), who labelled their plant material in a manner similar to that used here, were also comparable, except that they had less mineralization of the leached fraction over their 9 day incubation period. The difference is probably due to a more vigorous leaching procedure (boiling) than that used here. Results from studies which used other substrates, such as Zostera marina (Lee et al. 1976) or synthetic ^{14}C-labelled lignins (Hackett et al. 1977) also showed the same pattern in spite of the fact that the Zostera material was decomposed in a chemostat and the lignins were decomposed in soils as well as lake sediments. Finally, complimentary data, that is, a measure of residue remaining, can be found in another study of Spartina decomposition (Burkholder and Bornside 1957). The microcosms in that study consisted of flasks of seawater innoculated with sediment bacteria and with Spartina. After 25 days about 70% of the material remained in the flasks as particulate residue and the pattern of particulate loss was almost exactly complimentary to that found in this study. Thus, a variety of microcosm and substrate types as well as various environmental conditions, reveal similar patterns of decomposition for plant material.

The results of this study with Spartina detritus and a microcosm including sediment demonstrated temperature, plant fraction, and nutrient dependence of the decomposition process. Increased temperature resulted

in more rapid mineralization; during the early phase of the incubation the mineralization rate doubled between 10 and 20°C and increased by a factor of 1.5 between 20 and 30°C (Table 1). It is interesting to note that the late-phase rates of mineralization did not appear to be temperature dependent. The experiments with the plant fractions indicated a more rapid initial decomposition of those portions containing highly soluble organic and long term mineralization rate that might be related to microbial succession in the microcosm. Whether the material was on top of or within the sediment did not appear to be a source of variation, but rates in the flasks were higher than those in undisturbed cores, and likely neither rate is the same as might be found in situ. Finally, nutrient additions appeared to stimulate mineralization even though they could not be directly compared with unamended controls.

The analysis of this data by the model equation presented in the results section is of considerable comparative value for this type of experiment, and may provide some insight into the decomposition process. For example, the equation is an improvement over the "stripping technique" sometimes used to analyze results of decomposition experiments. In that method successive exponential terms are stripped from the data to create equations of the form:

$$Y = a_1 e^{-k_1 t} + a_2 e^{-k_2 t} + \ldots a_i e^{-k_i t}$$

where Y = % remaining, t = time, and a_{1-i}, k_{1-i} are coefficients determined by the procedure. Generally, such equations are limited to two or three terms that adequately describe the results of the experiment, and each term may be designated as some approximation of a process occurring

during the breakdown of the material (e.g., leaching). A significant criticism of such analysees is the arbitrary nature of the selection of data points to be included in the determination of each successive exponential term. Riggs (1963) has discussed the arbitrary nature of this selection as well as the resultant problems of extrapolation of the equation for prediction. The model and procedure used in this study may avoid those problems to some extent because first, only three coefficients are used here and second, use of a non-linear least squares regression reduced investigator bias. Thus, within constraints of the regression procedure the model represents a "best fit" of the data.

The parameters of the model may also represent specific portions of the decomposition process. For example, if the assumption is correct that there is a rate of community metabolism in these systems at equilibrium, then the coefficient b, the slope of the asymptote, should be an estimate of this rate. In addition, the asymptote itself provides the terms necessary to determine a turnover time for the detrital material (keeping in mind the reservations on extrapolation expressed previously). Similarly, the coefficients a and k are the primary determinants of the shape of the curve during the early portion of the incubation period. They are sensitive to temperature and to the plant fraction (Table 2). They represent, in some manner, the leaching and mineralization during the early stages of decomposition and as such are probably closely related to the nutritional quality of the detritus and the activity of the bacterial community which seems to dominate in the early stages of decomposition (Fallon and Pfaender 1977, Morrison et al. 1977). Further studies in gnotobiotic systems and <u>in situ</u> would serve to

better evaluate the relationships of the coefficients with various decomposition factors, including those not studied here, such as the effects of macrofauna on microbial activity.

The model used here remains a statistical one, however, and should not be confused with process models such as that of Parnas (1975, 1976). Her process model accounts for carbon and nitrogen in decomposition of organic materials based on the assumption that decomposition is proportional to growth of the decomposer population. It is of interest to note that the results of this study are not incompatible with predictions based on that process model. Thus, use of model systems, mathematical and biological, should be useful in elucidating the pathways of detrital decomposition which are of great importance in coastal areas.

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