Reference: *Biol. Bull.* **162:** 53–69. (February, 1982)

CARBON AND NITROGEN FLUXES DURING DECOMPOSITION OF *SPARTINA ALTERNIFLORA* IN A FLOW-THROUGH PERCOLATOR[1]

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ABSTRACT

The carbon and nitrogen in *Spartina alterniflora* litter were monitored for 4 months during decomposition at 20°C in a flow-through percolator that simulates an aerobic, moist marsh. Both the evolution of CO_2 and the loss of carbon from the litter followed exponential decay kinetics (0.5% day^{-1} and 1.0% day^{-1}). At first both total organic carbon and total organic nitrogen were lost primarily in dissolved form at high rates, but this leaching ceased rapidly. The NH_4 added to the inflow was incorporated into the litter at about 0.25 mg $N \cdot g\ litter^{-1} \cdot day^{-1}$ initially, but the rate declined eventually to about 0.08 mg $N \cdot g\ litter^{-1} \cdot day^{-1}$ after 40 days. Nitrogen enrichment of the litter occurred in two phases with peaks on days 40 and 100. Nitrification started at day 30 and was the main consumer of NH_4. The final litter nitrogen concentration was 60% of the initial.

Microbially produced organic matter, calculated from incorporated NH_4, increased during decomposition to a constant value of 250 mg $\cdot$ g dry weight $^{-1}$ in the system; about 25% of the total dry weight of the litter. The efficiency of conversion of *Spartina* biomass to microbial organic matter was also biphasic and had maxima of 50% at the start and 70% at day 80 with an intermediate value of 20%. The continuous recovery of organic matter and CO_2 exported from the percolator allowed the microbial activity to be separated into different periods dominated by bacteria in the first 40 days and then by fungi for the remaining 80 days.

INTRODUCTION

In temperate zone saltmarshes, ninety-five percent of the annual aerial production (Mann, 1972) and a substantial amount (30–100%) of the below-ground production (Howarth and Teal, 1979; Hackney and de la Cruz, 1980) of *Spartina alterniflora* undergoes decomposition before it reaches higher trophic levels (for reviews, see Marinucci, in press; de la Cruz, 1973). The energy of production in a saltmarsh may be transferred to higher trophic levels either by consumption of the decomposed litter by marsh animals (Tenore and Hansen, 1980), or through reduction and subsequent re-oxidation of sulfate by chemosynthetic bacteria (Peterson *et al.*, 1980).

Of the several ways to study overall decomposition *in situ*, litter bags are used most often. With this technique one can follow net changes in chemical and microbiological composition of the litter, but can not separate the gross chemical

Received 22 October 1981; accepted 25 November 1981.

[1] Paper of the Journal Series, New Jersey Experiment Station, Cook College, Rutgers University, New Brunswick, New Jersey. This work was performed as a part of NJAES Project No. 01501, supported by the New Jersey Agricultural Experiment Station. Additional support for completion of the project came from the National Science Foundation Grant DEB-79-5127.

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exchanges between water and litter or air and litter. Furthermore litter bag data are highly variable because of poor control of such things as oxygen tension, humidity, temperature, and nutrient availability in field experiments. Another *in situ* method used flow-through chambers to study the effect of water quality on *Spartina* decomposition (Lee *et al.*, in press). In these chambers the microbial and meiofaunal populations were altered from their natural state to measure the relative impact on decomposition of these two decomposer communities. The system does require extensive field maintenance and still has some of the drawbacks of integration of a number of environmental variables.

Another way to study decomposition is to simulate the natural environment in the laboratory (Burkholder and Bornside, 1957; Gosselink and Kirby, 1974; Haines and Hansen, 1979). In such experiments, *Spartina* litter decomposes in water in shaken flasks. These flask experiments, however, do not recreate the highly moist but aerobic conditions found in the litter layer of the marsh, and they allow buildup of metabolic products which can inhibit decomposition. In practice, flask studies have been run for only short periods; they have had a maximum duration of 40 days and most did not exceed 2 weeks.

The environment of a marsh surface can also be simulated in a percolator. This device rapidly recirculates water through a column of soil or particles suspended above a water reservoir (Lees, 1949; Temple, 1951); however, the water in the percolator is not renewed, and eventually microbial processes become inhibited because of metabolite buildup.

In this study I modified a percolator design to simulate the environment of decomposition found in the low marsh. In this new design, the water is continually replenished and metabolic products do not build up. In addition the apparatus allowed adequate control over the various environmental components that affect aerobic decomposition with the result that the variability in the data was greatly reduced. Furthermore, I could account for all major exchanges of carbon and nitrogen between water and litter or air and litter. Other advantages of this percolator are a large sample capacity of 100–125 cm^3 (6–10 g dry weight of *Spartina* litter), an incubation of the litter in a non-submerged but moist environment, and separately controlled percolation and water flow-through rates. More pragmatic features of this design are easy disassembly, chemical inertness due to the all-glass construction, and a relatively low cost. Finally, with this design I could follow decomposition for many months.

Results of *in situ* decomposition studies show that carbon and weight are lost exponentially from litter. Nitrogen, however, is first rapidly lost through leaching from litter in the marsh, then re-incorporated from the environment, and finally lost from the litter at a slow exponential rate (Odum *et al.*, 1973). During this latter stage, meiofauna increase the mineralization of *Spartina* litter to CO_2 (Lee *et al.*, in press). Because of the steady loss of carbon and increases in nitrogen during decomposition, there is a gradual decrease in the carbon:nitrogen ratio. The nitrogen increases are usually attributed to microbial activity in the detritus but little is known of the populations or processes involved. Microscopic examination of *Spartina* litter showed that bacterial biomass is relatively small in aged detritus (Odum *et al.*, 1979) while the types and concentrations of sterols suggest that fungal biomass may predominate in this litter (Lee *et al.*, 1980). Other biochemical measurements on leaf litter in a Florida estuary suggest that the initial bacterial colonization of litter was followed by fungal growth (Morrison *et al.*, 1977). Additional evidence for the importance of fungi comes from the high amounts of non-protein nitrogen in detritus; it is as high as 30% of the total litter nitrogen by dry

weight (de la Cruz and Poe, 1975, Odum *et al.*, 1979). Between 25 and 50% of this non-protein fraction of nitrogen is likely chitin, which can originate from fungal cell walls (Odum *et al.*, 1979). However, this apparent fungal dominance in the leaf litter was reduced when the litter was grazed by small crustaceans (Morrison and White, 1980).

Nitrogen incorporation from the surrounding media into *Spartina* litter had also been noted in laboratory experiments (Gosselink and Kirby, 1974) and the presence of dissolved inorganic nitrogen in the media increased decomposition rates (Haines and Hansen, 1979). Other workers show that in the laboratory the presence of meiofauna increased CO_2 evolution in the latter stages of the process (Lee *et al.*, 1976); this corroborates the results found in the field (Lee *et al.*, in press). Bacterial and overall microbial growth efficiencies, calculated from both the nitrogen and the ATP content of litter, were between 20 and 60% (weight of calculated microbial biomass produced per dry weight of plant litter decomposed) (Gosselink and Kirby, 1974; Burkholder and Bornside, 1957; Fallon and Pfaender, 1976; and Haines and Hansen, 1979). The efficiency of fungal growth, however, was as high as 82% on a substrate of *Spartina* leachate (Fallon and Pfaender, 1976). These efficiencies of bacterial growth are within values (40–60%) observed for both pure and mixed growth of bacteria on glucose in minimal medium (Payne, 1970). Fungal growth efficiencies on optimal medium (65%) are higher than bacterial values (Payne, 1970), but did not approach the high value reported by Fallon and Pfaender (1976).

The present study was carried out to develop and test the use of flow-through percolators for the study of the chemical and biological processes and controls during *Spartina* decomposition. In one test I compared the time course of decomposition of *Spartina alterniflora* in litter bags in a natural salt marsh with decomposition of the same litter in percolation chambers. In another test, I compared decomposition in seven percolators and computed standard deviations of measurements of weight, carbon, and nitrogen in the litter, the amount of carbon and nitrogen exported from the percolators in the water, and the respiration as calculated from loss of CO_2 in the air stream. Finally, a budget of carbon and nitrogen entering and leaving the percolators over a 4 month period was constructed both to test the accuracy of measurements and to investigate the relative importance of various processes.

Materials and Methods

Laboratory experiment and apparatus

The litter used in each flow-through percolator was 6 g dry weight (5.1 g ash free dry weight) of air dried, mechanically shredded (commercial garden shredder) *Spartina alterniflora* leaves and stalks (approximately 10–20 × 1–2 mm after shredding) harvested in August from green plants from an undisturbed saltmarsh (Island Beach, N. J.). A gram of dry weight of litter was 13% ash and the ash free dry weight contained 48% C and 1.28% N.

In the percolators (Fig. 1), the litter sample was held in the column (A) above the water level by glass nubs and a small amount of coarse glass wool. An air lift pump with a flow of 100 ml/min (D) rapidly recycled water from the reservoir (B) to C and then A. The water level was maintained below the litter by the position of the air lift junction, which is just below the bottom of the litter sample. Input water was added (E) to the percolator with an Ismatic® peristaltic pump (flow rates between 0.32 and 0.42 ml/min). Residence time of the 200 ml of water in

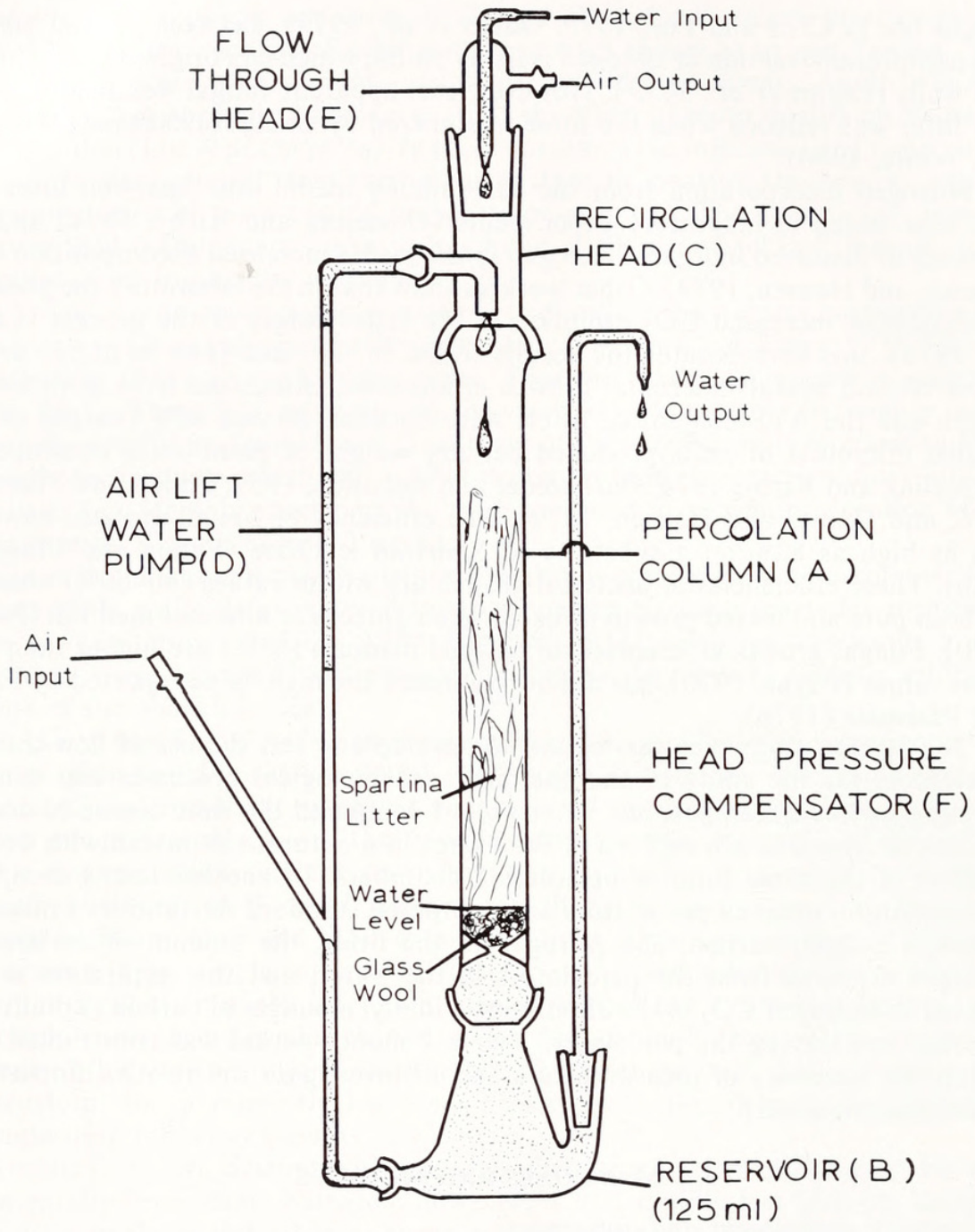

FIGURE 1. Flow-through litter percolator. Unit is approximately to scale with overall length of 58 cm (Construction details in Marinucci, 1981, or from author; modified units commercially available from Belco Glass Co., Vineland, NJ, Cat Nos. 9105-S0009 and 9105-S0011). Assembly of unit requires two #12 and two #35 ball and socket clamps (Arthur H. Thomas Co., Philadelphia). Joints should be either coated with a small amount of silicone grease or lined with teflon (Ace Glass Co., Vineland, NJ) to prevent small leaks.

the system was 6 to 8 hrs. The water leaves the system through the head pressure compensator (F) which was high enough to balance the head pressure generated by bubbling air through a total of 30 cm of KOH solution in the two CO_2 traps connected in series to the air output (E) (Fig. 2). Each trap was a 25 × 250 mm test tube with 50 ml of 0.2 *N* KOH. Influent air was bubbled through an alkali solution (15% KOH) and then distilled water to remove CO_2 and replace moisture in the air stream. The input was artificial sea water (Utility Marine Mix, Chemical

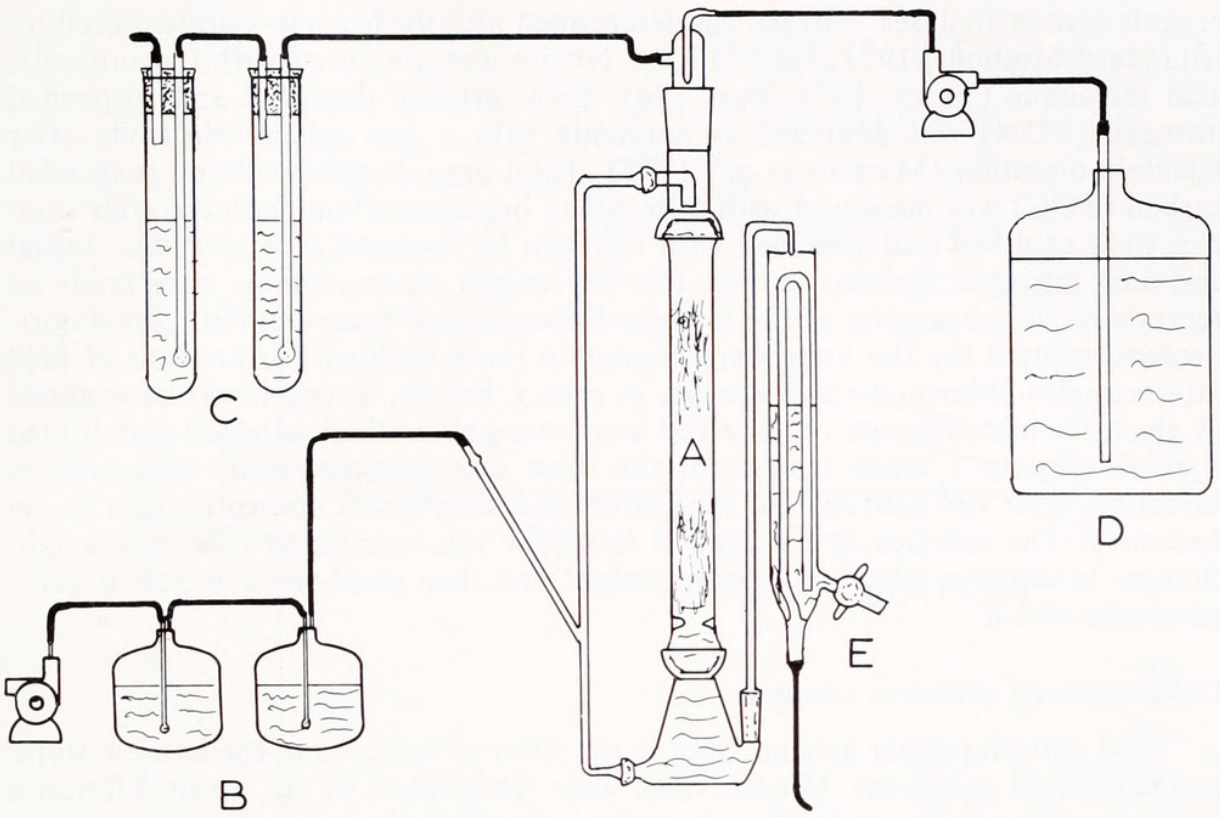

FIGURE 2. Diagram of the system showing the percolator (A), the influent air scrubbers (B), the effluent scrubbers (C), the reservoir (D), and the self-emptying collection device (E).

Co., Paterson, N. J.), diluted to 22‰ salinity and containing 9 mg $N \cdot liter^{-1}$ as $(NH_4)_2SO_4$. All percolators were operated at 20°C in an incubator and were kept in total darkness to prevent algal growth. One percolator was poisoned with 0.1% sodium azide in the inflow water to test for abiotic changes in the litter.

Comparative in situ *study*

To compare the percolator experiments with *in situ* experiments, I also measured decomposition in a New Jersey salt marsh with the same litter as used in the percolators (for details see Marinucci and Bartha, in press). The initial chemical composition of this litter was the same as that in the percolator experiments. In these experiments, which began in early to mid-summer, litter was placed in both high and low marsh locations in 18 × 30 cm litter bags with 2 mm mesh. Periodically, a bag was removed from the marsh and the contents analyzed for total dry weight, ash free dry weight, total carbon, and total nitrogen.

Chemical analysis of nitrogen and carbon components

Evolved CO_2 was trapped and measured by titration of the residual alkalinity after precipitation of carbonate with excess $BaCl_2$ (5 ml of 1 *M* solution). The chemical equivalents of CO_2 as carbonate in each trap was equal to ½ the equivalents of base neutralized. Ammonia was analyzed immediately after sampling in both inflow and outflow waters with an ammonia gas sensing electrode and standard addition techniques (Orion Research Inc., 1975; Gilbert and Clay, 1973; Srna *et al.*, 1973). Samples of influent and outflow water were periodically collected and frozen in polyethylene bottles for nitrate, nitrite, total organic nitrogen, and total

organic carbon analyses. Nitrate was determined with the brucine-sulfate procedure (Standard Methods, 1971, Part 213 C). Nitrite was measured with the sulfanilic acid technique (Taras, 1971, Part 134). Total organic dissolved and suspended nitrogen (TON) was analyzed as ammonia with a gas sensing electrode after Kjeldahl digestion (Mertens *et al.*, 1975). Total organic dissolved and suspended carbon (TOC) was measured with a Beckman organic carbon analyzer after samples were acidified and scrubbed with nitrogen for removal of carbonates. Initial and final nitrogen, carbon, and ash-free dry weight measurements were made on representative subsamples of the fresh and decomposed litter from all percolators. Procedures used for the litter were similar to those outlined for analysis of field litter samples (Marinucci and Bartha, in press). Briefly, nitrogen was determined by electrode measurement of dissolved ammonia gas in alkali-adjusted and diluted Kjeldahl digests. Carbon content of the litter was gravimetrically measured as carbonate after wet oxidation of the carbon and subsequent absorption of CO_2 on Ascarite®. The ash-free dry weight of the litter was calculated from net weight changes in samples dried to constant weight and then combusted at 550°C for a minimum of 2 h.

Calculation of chemical changes

Total material either accumulated in the litter or removed in the outflow water was calculated as follows. Uptake rates were the product of the mean difference in concentration between input and output water and the mean flow rate for the time interval. The total material was the summation of the products of the uptake rates and the time interval between the samples.

Values for days in which no samples were taken were calculated from linear interpolation of the adjacent data. The carbon and nitrogen contents of the litter, as well as incorporated nitrogen, were then calculated for each day of the experiment with equations 1, 2, and 3:

$$\text{Total litter carbon} = \text{Initial carbon} - \text{Carbon dioxide} - \text{TOC} \qquad (1)$$

$$\text{Total litter nitrogen} = \text{Initial nitrogen} + \text{Ammonia utilized} - \text{TON} - \text{Nitrate} - \text{Nitrite} \qquad (2)$$

$$\text{Ammonia incorporated into litter} = \text{Ammonia utilized} - \text{Nitrate} - \text{Nitrite} \qquad (3)$$

Data shown in this paper are the mean results of seven identical percolator experiments run at two different times.

Results and Discussion

Comparison of percolator decomposition with in situ *litter bag results*

Decomposition of litter was similar in the percolator and in bags from the high and low marsh (Fig. 3). Data points (n = 3) from these litter bag experiments had a standard deviation of around ±5–8% while percolator data points had an average deviation of around ±1–2%. Decomposition rate constants were calculated for these experiments by regression of the natural logarithm of the total litter carbon against time (Table I). The percolator data correlated best with this exponential function and falls between the decay rates for litter in the high and low marsh. Carbon loss rates of 0.86 to 2.31% · day^{-1}, which were determined by Lee *et al.* (in press) from $^{14}CO_2$ evolution from radiolabeled *Spartina* in a field microcosm in the low marsh also bracket the percolator decay rate.

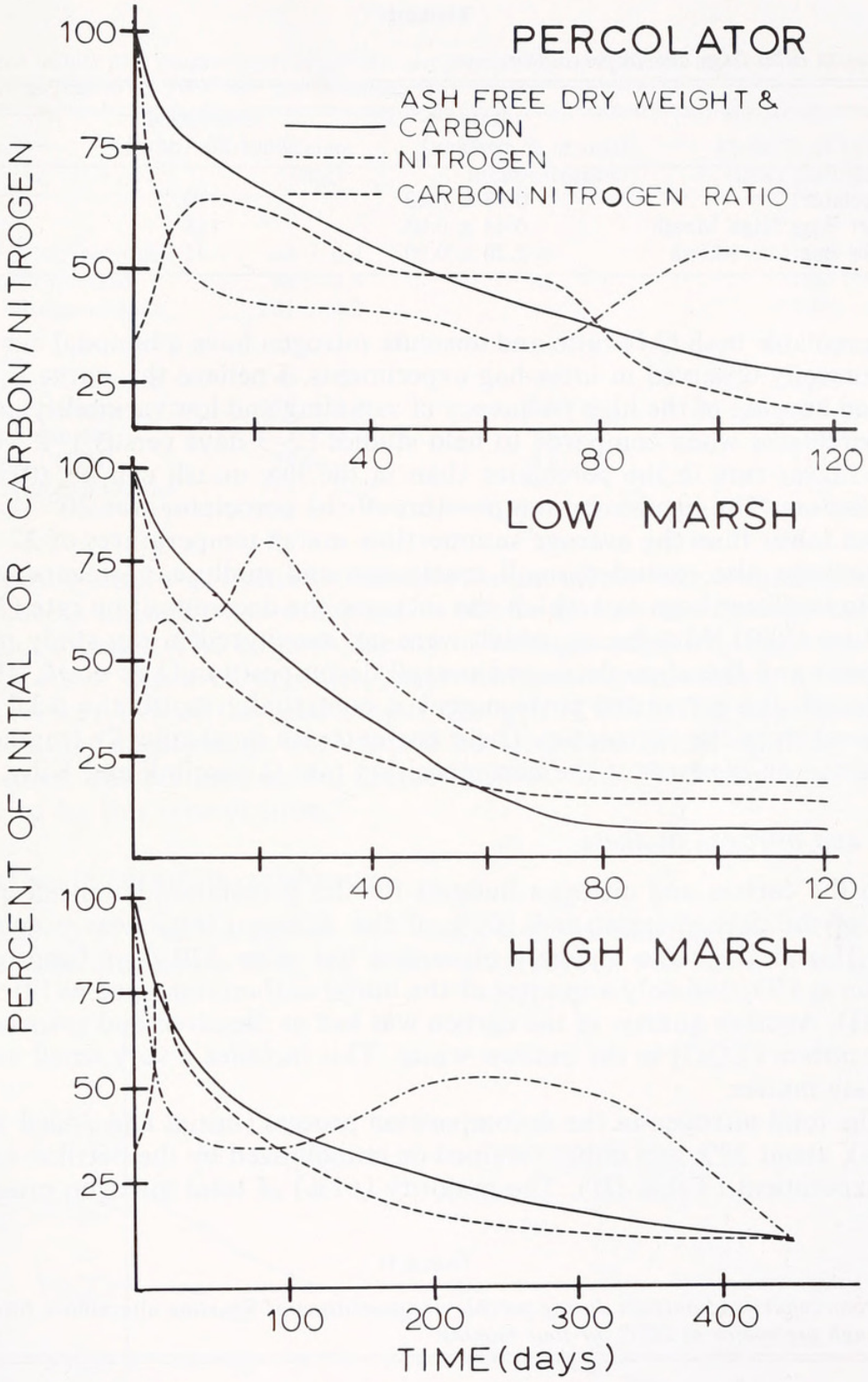

FIGURE 3. Decomposition of *Spartina alterniflora* litter in percolators and in litter bags in the high and low salt-marsh. Carbon and nitrogen quantities are expressed as percent of the initial amount while the C/N ratios are actual values. Field data were redrawn from Marinucci and Bartha (in press).

Nitrogen changes were much more complex in percolator-decomposed litter than in the litter bag study. However, there was a general pattern of net nitrogen accumulation in the litter after an initial rapid loss (see also Mann, 1972; Odum *et al.*, 1973). Overall, the C:N ratios increase sharply from the initial level of 38 but all eventually decline to the relatively nitrogen-rich level of 18 to 20. However,

TABLE I

Decay rates in litter bags and in percolator.

	Rate (% per day)	Calculated half-life (days)	R^2
Percolator	0.98 ± 0.03	70	0.99
Litter Bags High Marsh	0.44 ± 0.05	158	0.80
Litter Bag Low Marsh	2.20 ± 0.20	31	0.81

in the percolator both C:N ratio and absolute nitrogen have a bimodal curve which is not normally observed in litter bag experiments. I believe this curve is real and was found because of the high frequency of sampling and low variability in samples in the percolator when compared to field studies (2–3 days versus 1–2 weeks).

The slower rate in the percolator than in the low marsh can be attributed to several factors. The incubation temperature of the percolator was 20° C, which is somewhat lower than the average summertime marsh temperatures of 22 to 28°C. The percolator also excluded small crustacean and molluscan grazers which are present in the litter bags and which can increase the decomposition rate (Morrison and White, 1980). Meiofauna, which were not monitored in my study may have been absent and therefore decreased overall decomposition (Lee *et al.*, 1976). Finally, though the percolator environment is continually moist, the tidal currents were absent from the percolator. These currents can mechanically fragment litter and result in an increase of the decomposition rate (Gosselink and Kiby, 1974).

Carbon and nitrogen budgets

Both the carbon and nitrogen budgets for the percolators balanced quite well as 99% of the carbon input and 107% of the nitrogen input was accounted for (Tables II and III). The majority of carbon lost after 120 days (approximately 50%) was as CO_2 and only a quarter of the initial carbon remained as litter carbon (Table II). Another quarter of the carbon was lost as dissolved and suspended total organic carbon (TOC) in the outflow water. This includes a very small amount of particulate matter.

Of the total nitrogen in the decomposition process (initial and added ammonia nitrogen), about 39% was either retained or immobilized by the detritus at the end of the experiment (Table III). The majority (61%) of total nitrogen processed by

TABLE II

Total carbon inputs and outputs during aerobic decomposition of Spartina alterniflora *litter in a flow-through percolator at 20°C for four months.*

	Weight (mg)	Mean % of total and initial
Input:		
Initial Litter Carbon	2462 ± 15	—
Output:		
Final Litter Carbon	655 ± 49	26
Carbon Dioxide Evolved	1095 ± 32	45
Carbon (TOC)	694 ± 37	28
Total Carbon Output	2445 ± 77	—

TABLE III

Total nitrogen inputs and outputs during aerobic decomposition of Spartina alterniflora *litter in a flow-through percolator at 20°C for four months.*

	Weight (mg)	Mean % of total input nitrogen	Mean % of initial litter nitrogen
Input:			
Initial Litter Nitrogen	63 ± 0.4	39	100
Ammonia Utilized	98 ± 4.5	61	156
Total Nitrogen Input	161 ± 4.2	—	256
Output:			
Final Litter Nitrogen	43 ± 2.8	25	68
Nitrate Produced	52 ± 5.2	29	82
Nitrite Produced	2 ± 0.2	1	2
Nitrogen (TON)	74 ± 7.8	43	117
Total Nitrogen Output	173 ± 10.9	—	275

the decomposing litter was from external sources (ammonia-N). A significant quantity of ammonia-nitrogen was converted to nitrite and nitrate through microbial nitrification processes. The largest quantity of nitrogen exported, however, was in the form of either dissolved organics, suspended micro-particulates, or microbial biomass; this was 43% of the total output nitrogen (TON). The large fraction of the nitrogen that appeared as TON could be attributed to the leaching of non-senescent grass which I used as my source of litter. The TOC may likewise have been affected by this type of litter.

Gross changes in chemical components

About 20 to 25% of the carbon was leached as dissolved carbon in the initial stages of the decomposition (Fig. 4). Afterwards, carbon was lost at a steady rate

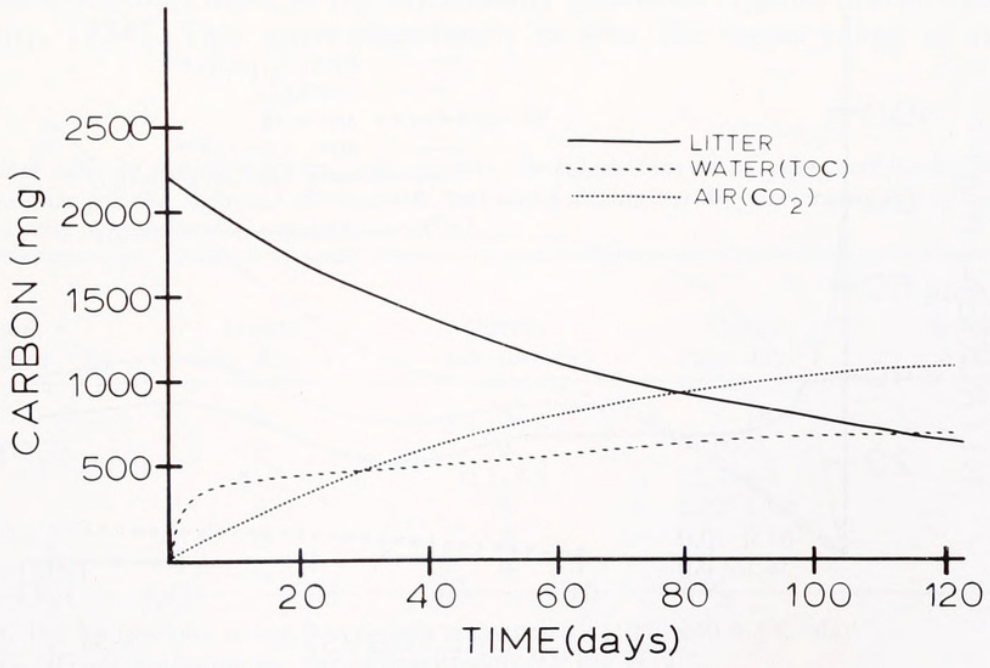

FIGURE 4. Cumulative amounts of carbon gained or lost by *Spartina alterniflora* litter during aerobic decomposition in a percolator at 20°C.

of about 2.5 mg C · day^{-1}. The CO_2 evolution was initially less rapid than the total organic carbon (TOC) losses but was much more constant during the experiment. The rate of CO_2 evolution did, however, decrease as litter was consumed.

The rate of CO_2 evolution from each percolator decreased over time, and the total evolved approached a value of 1250 mg C (Fig. 4). This value and the 700 mg TOC substracted from the initial carbon (2450 mg) leaves 500 mg of undecomposable carbon. This is probably the lignin fraction which remains fairly intact after cellulose mineralization (see Swift *et al.*, 1979, Chapter 2). This amount of recalcitrant carbon, about 20% of the initial carbon, is the same as the lignin found in fresh *Spartina alterniflora* by MacCubbin and Hodson (1980).

Nitrogen losses were also rapid at the start of decomposition with 66% of the initial nitrogen lost as TON in the first 10 days (Fig. 5). The TON was continually lost for the remainder of the experiment, and the TON loss rate decreased to a constant value of 0.5 mg N · day^{-1} after day 30. Cumulative TON lost during decomposition surpassed by a small quantity the initial nitrogen of the *Spartina* litter. In litter poisoned with sodium azide (data not shown), there was a TON total loss of only 50%, 9/10ths of which was lost in 20 days. This indicates that a lot of the TON loss from the litter was microbially mediated.

The ammonia utilization curve shows net nitrogen incorporation from the water into the decomposing litter (Fig. 5). This incorporation was initially rapid and constant (0.25 mg N · g litter^{-1} · day^{-1} as NH_4), but became slow and variable after 40 days (approximately 0.08 mg N · g litter^{-1} · day^{-1}). Then ammonia oxidation to nitrate and nitrite became important. This process of nitrification was first detected after day 30 of the experiment, and significant quantities of products were measured after day 40. The slow development of nitrification in the percolation system may have been the result of the relatively slow generation time of the nitrifiers of 20–40 hrs (Focht and Verstraete, 1977). Nitrification may also have been suppressed by the high dissolved organic matter in the percolators before day 30; this is significantly reduced about the same time as commencement of nitrification (data not shown).

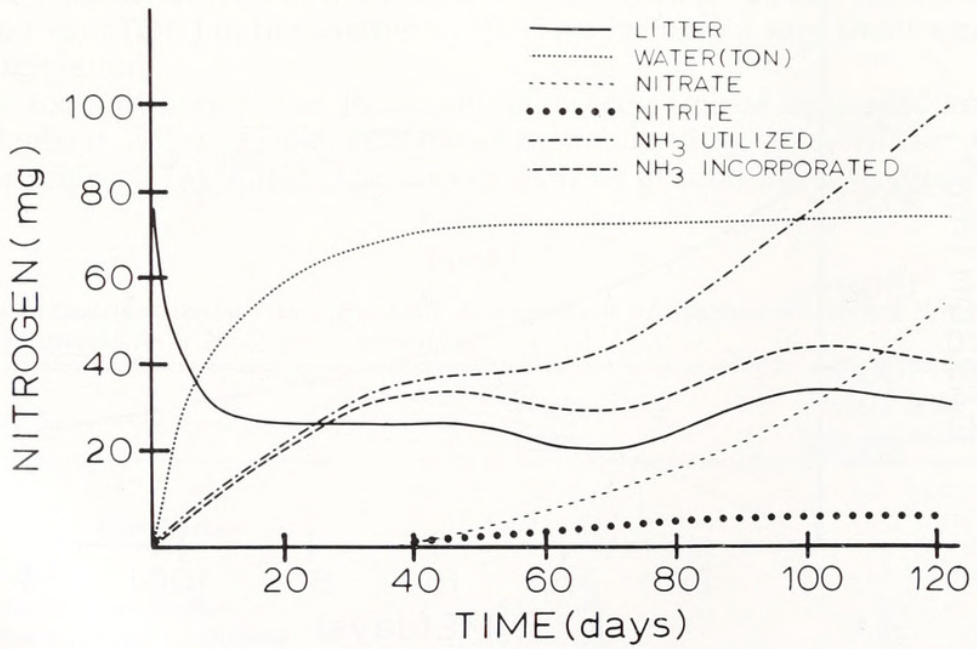

FIGURE 5. Cumulative amounts of nitrogen gained or lost by *Spartina alterniflora* litter during aerobic decomposition in a percolator at 20°C.

The high amounts of TOC and TON produced during the first few days of the study probably result from the type of litter used in this study. This grass was actively growing; therefore, much of the nutrients were still in the stems and leaves. Scenescent grass, which is the major litter input in nature, would have lost most of these nutrients to the roots for storage.

The soluble organic matter lost after the initial rapid leaching of litter at the beginning of the experiment (TOC and TON after day 30) had a mean C/N value of 5. Pure protein and micro-organisms can also have this low C/N value. However, since the outflow water was nearly devoid of culturable microorganisms (J. C. Hunter, personal communication), this organic material was probably either soluble protein, amino acids or polypeptides.

The percolator which was poisoned as a control for abiotic litter decay did not evolve any CO_2, nitrate, or nitrite (data not shown) and did not accumulate any nitrogen. About 20–25% of the carbon as TOC and 45–50% of the nitrogen as TON was leached abiotically during the first few days of the study, but this loss then ceased and the litter did not decompose further.

Representative values of rates and concentrations are given in Table IV to illustrate the levels found.

Contribution of microbes to the litter

Since a large percentage of the initial nitrogen is rapidly leached from the litter in the early stages of decomposition, I assumed that the incorporation of ammonia nitrogen into the litter is an indicator of the magnitude of microbially produced organic matter. This assumption was supported by the fact that ammonia was accumulated in litter at the same time as TON leached from the litter (see Fig. 5). However, it is not known whether this incorporated nitrogen remains in microbial biomass (bacteria, hyphae) or becomes a part of microbial slimes or fragments of cell walls and hyphae. For the purpose of this paper I assume that the composition of the microbially produced material is the same as the composition of microbes. Accordingly, the incorporated ammonia nitrogen was divided by 0.13% to calculate the total mass of the microbially produced organic matter (Gosselink and Kirby, 1974). This conversion factor is near the upper range of values of

TABLE IV

Approximate rates of inputs, uptakes, and outputs; concentrations of outputs; and variation of cumulative data for the chemical components measured during aerobic decomposition of Spartina alterniflora *in a percolator for 4 months at 20°C.*

Chemical component	Inputs ($mg \cdot day^{-1}$)	Uptake ($mg \cdot day^{-1}$)	Output ($mg \cdot day^{-1}$)	Median output concentrations ($mg \cdot l^{-1}$)
CO_2-C	0	0	5–18	—
Carbon (TOC)	0	0	0–7[1]	6.30
NH_4-N	4–5[2]	0.3–2.3	2.7–4.7	5.40
NO_3-N	0	0	0.02–1.20[3]	1.00
NO_2-N	0	0	0.01–0.10[3]	0.09
Nitrogen (TON)	0	0	0.02–1.4[4]	1.26

[1] TOC lost by leaching in the first 5 days was approximately 250 mg $C \cdot day^{-1}$.
[2] Input NH_4-N concentration was approximately 9.0 mg $N \cdot l^{-1}$.
[3] Nitrification products were first detected at day 30.
[4] TON lost by leaching was approximately 10 mg $N \cdot day^{-1}$.

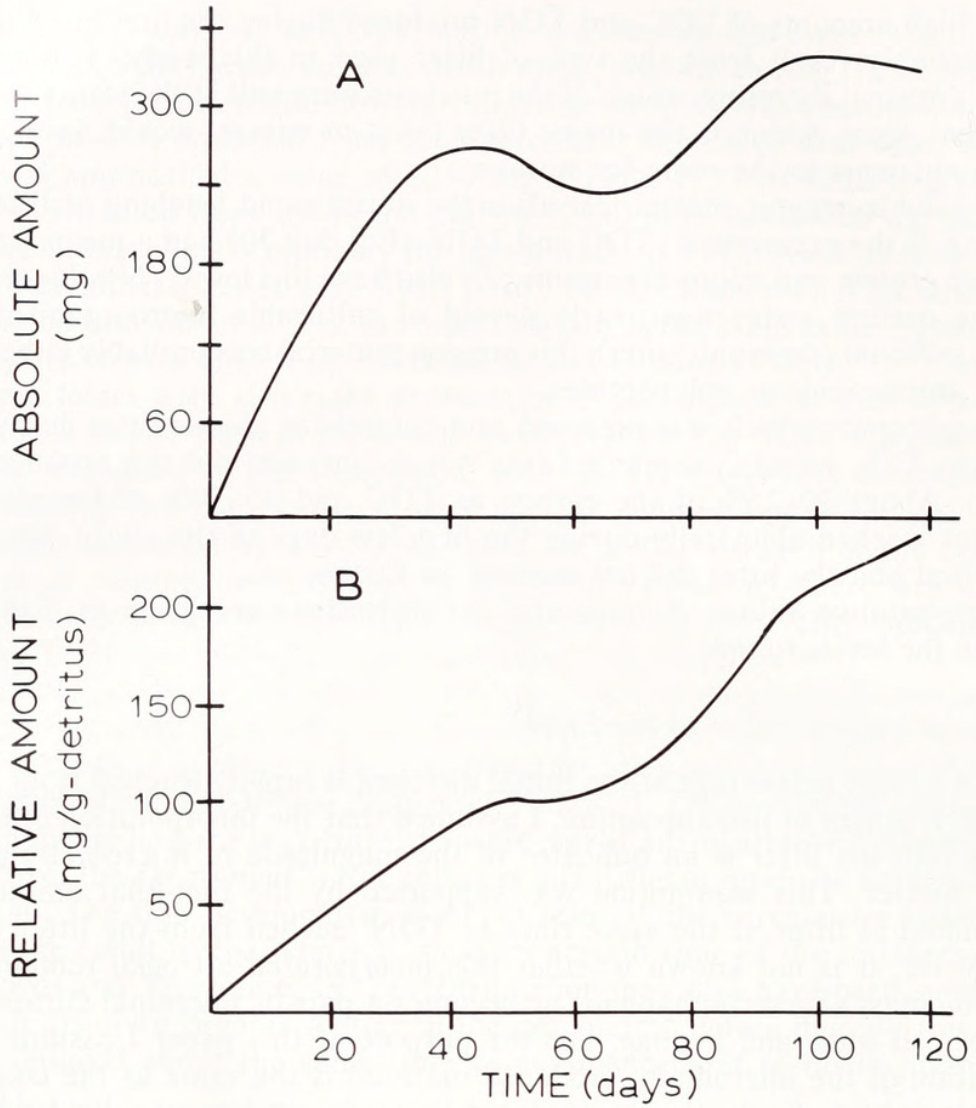

FIGURE 6. Absolute (A) and relative (B) amounts of microbially produced organic matter in decomposing *Spartina alterniflora* litter in a percolator at 20°C.

microbial nitrogen content (microbes are 5.2 to 13.6% N) and thus makes the most conservative estimate of microbial produced organic matter (Lipinsky and Litchfield, 1970). Absolute amounts of the microbially produced organic matter increased rapidly in the first few days of decomposition and then became relatively constant, though there was a slight biomodal shape to the curve (Fig. 6). The amounts per gram of litter, on the other hand, increased in a nearly linear fashion throughout the experiment. The calculated microbial production in the resultant litter was 250 $mg \cdot g^{-1}$ or 25% by dry weight of detritus at the end of the experiment.

The bimodal shape of both the C/N curve (Fig. 1) and the absolute amount curve (Fig. 6A) indicates a two-phased microbial enrichment of the litter which is also seen in decomposition data for other types of macrophyte litter in aquatic systems (Hunter, 1976; Andersen, 1978; Seki and Yokohama, 1978; Blackburn and Petr, 1979; Kruczynski *et al.*, 1979) and in terrestrial systems (Howard and Howard, 1974). However, this two-phased pattern was not very pronounced in these cited works, and therefore was not discussed by these authors.

The initial nitrogen enrichment during decomposition in the first 40 days was probably the result of rapid colonization by heterotrophic microbiota which ex-

ploited the more easily decomposable fractions of the litter. The second step of nitrogen build-up in the remaining 80 days could indicate a shift of the microbiota in response to a depletion of the more readily utilizable substrates. This in turn causes a buildup of a population which attacks the less available bio-polymers (see Swift *et al.*, 1979, Section 4.5). In *Spartina* litter, Lee *et al.* (1980) found a similar pattern for fungal biomass calculated from the ergosterol content but only a one step increase of bacterial biomass in the same litter (direct counts). Since the more recalcitrant lignocellulosic components of the litter are degraded primarily by fungi, it is likely that a shift from primarily cellulolytic fungal population to lignolytic types was responsible for the two step enrichment. Extrapolation of lignin mineralization data gathered *in vitro* strongly indicates that most (approx. 70%) of the lignin fraction of *Spartina alterniflora* is not decomposed by the microbial community present during the initial stages of decomposition (MacCubbin and Hodson, 1980). Further decomposition of this litter requires a subsequent increase in the population of a lignolytic microbiota.

The second step of nitrogen enrichment of the litter correlated with the increase in nitrate and nitrite in the outflow waters (Fig. 5) so it is possible that nitrifying bacteria contributed to the organic matter. To test this, the maximum contribution of chemoautotrophic microbial biomass to the litter was calculated from the amount of ammonia oxidized to nitrite and then to nitrate. This value was derived from the nitrate production data and from published conversion factors (Focht and Verstraete, 1977) and is about 10^5 cells · mg NO_3-N^{-1}. I assumed some diameters and densities for small rod-shaped nitrifying bacteria (Ferguson and Rublee, 1976), and then calculated a mean dry weight for the nitrifier cell of 3.05×10^{-14}g. With these two factors and the total nitrate production at the end of the study (Table III), only a maximum of 0.2 mg of nitrifier biomass could be produced in this experiment. If the maximum rate of nitrate production (Table IV) as an indicator of active nitrifier biomass (10 g NH_3-N was oxidized to $NO_3 \cdot day^{-1} \cdot g\ cell^{-1}$), it can be calculated that only about 0.12 mg of nitrifiers were active at the end of

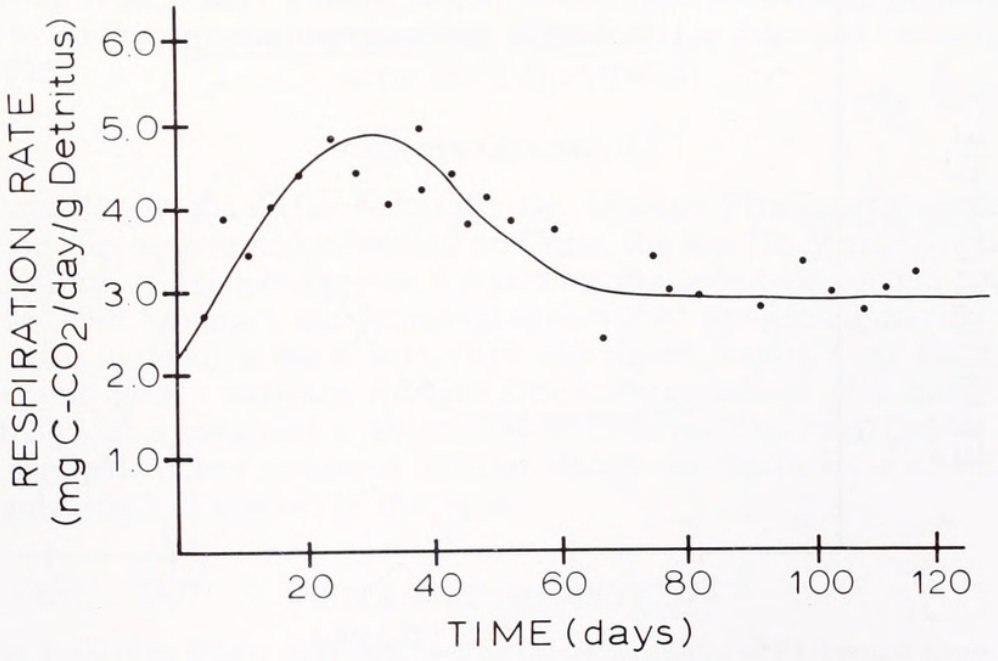

FIGURE 7. The CO_2-C evolution rate from *Spartina alterniflora* litter which was decomposed in percolator at 20°C. The data were corrected for litter weight. Total mass of litter was calculated from litter carbon changes (0.48 g C · g $AFDW^{-1}$).

the experiment. Both of these values are insignificant when compared to 320 mg of microbially produced organic matter in the litter at the end of the experiment.

The respiration rate of the litter increased continually from about 2 mg C · day^{-1} · g detritus^{-1} at the start of the experiment to about 5 mg C · day^{-1} · g detritus^{-1} at day 25 (Fig. 7); this agrees with the rapid increase in the microbially produced organic matter (Fig. 6). Afterwards, respiration decreased to a relatively steady level of 3.5 mg C · day^{-1} · g detritus^{-1} for the duration of the experiment. Such a decrease in respiration rate without a simultaneous decrease in the relative amounts of microbially produced organic matter strongly supported the hypothesis that the majority of this organic matter is not active microbial biomass (Lee *et al.*, 1980). The same pattern was also seen in O_2 respiration in field decomposed *Spartina alterniflora* (Lee *et al.*, 1980). They attributed the pattern to environmental temperature changes, but because a similar pattern was found in the percolators at a constant temperature, their conclusion may not be correct.

Instantaneous conversion efficiencies of plant biomass to microbially produced organic matter (Fig. 8) were calculated from NH_3-N litter uptake data (Fig. 5) and CO_2-C evolution data (Fig. 4). The curve accentuates the bimodal nitrogen enrichment of the litter, and also suggests a two-phase microbial colonization of the litter by microbiota. Peak conversion efficiencies averaged around 60–70%, though individual points were as high as 90%. Though these extremely high values might be artifacts of the iterative nature of the efficiency calculation or of the value of nitrogen content of the microbiota, the trends in microbial growth dynamics are definitely present. Furthermore, the mean peak efficiencies are reasonable and consistent with those found in pure culture experiments for both bacteria (40 to 60%) and fungi (65%) (Payne, 1970). The initial microbial conversion efficiency could be associated primarily with bacterial growth as suggested by Morrison and White (1977). However, from the results of Lee *et al.* (1980), this initial efficiency was more likely associated with both bacterial and fungal growth on the litter. The

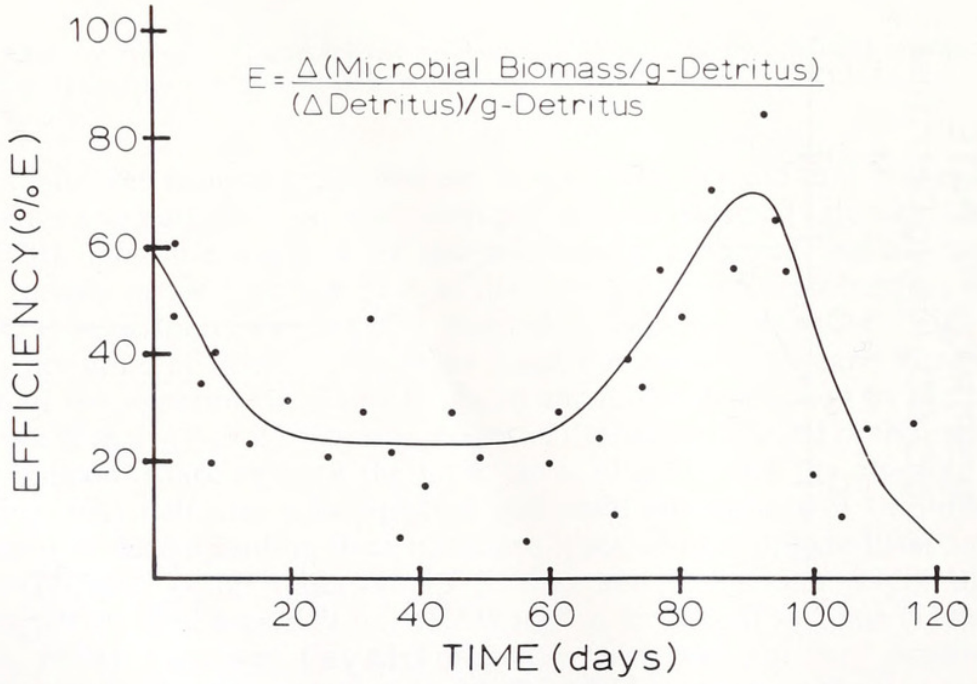

FIGURE 8. Instantaneous microbial conversion efficiencies of *Spartina alterniflora* litter biomass to microbially produced organic matter calculated from NH_4-N incorporation into litter. Plant biomass consumption was calculated from evolved CO_2-C (detritus is 48% C · AFDW^{-1}).

peak efficiency of the secondary colonization is somewhat higher than the first and reflects the slightly higher optimal fungal growth efficiencies as compared to bacterial efficiencies. These results further indicate that fungi are the major decomposers in the latter stages of decomposition. However, Fallon and Pfaender (1976) reported 82 and 54% conversion efficiency values for fungal and bacterial communities, respectively, grown on *Spartina alterniflora* leachate. Their peak fungal conversion efficiency is somewhat higher than other reported values; nevertheless, their values are very close to the peak efficiencies measured in my study and suggest that initial decomposition is mediated by bacteria. The low conversion efficiencies of my experiment (the flat sections of Fig. 6), which are between 20 and 30%, are similar to conversion efficiencies for mixed fungal and bacterial populations (Fallon and Pfaender, 1976).

Microbial succession and biomass contribution during *Spartina alterniflora* litter decomposition is still an area of much controversy (for review, see Lee, 1980). These percolator observations indicate that fungi contribute a larger biomass to detritus than bacteria and this agrees with actual measurements of fungal biomass by S. Y. Newell (personal communication). One reason for this may be that the bacterial cell walls are much less recalcitrant than fungal hyphal components so that bacteria disappear after death while fungi remain.

The conclusion of this study is that the decomposition process can be successfully studied in well-controlled laboratory flow-through percolators. Not only was there good control over variables in these percolators but there was also low variability and complete recovery of chemical components. As a result, there was better resolution of changes in litter over time and it became apparent that nitrogen, and presumably microbially produced organic matter, increased in two steps. This could be caused by bacterial and fungal succession. One way to investigate the succession would be with antibiotics (Fallon and Pfaender, 1976). Biomass and successional information can also be obtained by concurrent direct measurements of bacteria (Daley and Hobbie, 1975) and fungi (Berg and Söderström, 1979) and with indirect biochemical measurements which relate to microbial biomass (Paul and Voroney, 1980; White *et al.*, 1980). Finally, the impact of meiofauna on this system can be checked by adding or removing these organisms from the litter and innoculum (Lee *et al.*, 1976).

ACKNOWLEDGMENTS

I would like to thank the following: Dr. Michael Pirnik for suggesting the percolation approach to decomposition problems; the late Dr. Walter J. Nickerson of the Institute of Microbiology at Rutgers for the prototype column and airlift design; Mr. Bill Shipman, the former glassblower at Rutgers University, for his aid and skills in creating the flow-through unit shown here; George Doza for use of his organic carbon analyser; Richard Driese for assistance with sampling; Dr. Jerry Melillo for a review of a preliminary manuscript; Dr. John Hobbie for discussion, suggestions, and reviews of this manuscript; and finally Dr. Richard Bartha for the guidance and support of this work.

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