Limnol. Oceanogr., 39(1), 1994, 37–50

Production of microbial organic aggregates from macrophyte-derived dissolved organic material

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Abstract

Organic aggregates were produced in the laboratory by bubbling dissolved organic material derived from five species of coastal macrophytes. The weight of aggregates produced after bubbling was significantly related to the amount of DOC (dissolved organic C) initially present. An average of 19% of the initial DOC was converted to aggregates, 23% remained in solution, and the remaining 58% was mineralized to CO_2. The C : N ratios of aggregates did not vary between species or runs and ranged from 4 to 12. Microscopic examination of aggregates showed that they contained large numbers of rod-shaped bacteria. The data were interpreted to suggest that aggregates are composed of live and dead bacteria, other microbes, and extracellular material. The ingestion of aggregates of this type by consumers is a potentially important route for the flow of labile organic C in marine systems.

It is estimated that 30–45% of macrophyte net production is either exuded as dissolved organic material (DOM) while the macrophytes are alive or is leached from them as they decompose (Mann 1982). The largest pulse of DOM is released from postsenescent tissues during the initial leaching phase of decomposition (Valiela et al. 1985). For example, three species of macroalgae lost between 30 and 85% of their initial wet weight as soluble substances during the first 2 weeks of decomposition, and two species of vascular plants (*Spartina alterniflora* and *Zostera marina*) lost 10–30% of their initial weight in 1 month (Buchsbaum et al. 1991; Valiela et al. 1985).

The dissolved material released as leachate is potentially nutritious as it is high in soluble proteins, carbohydrates, and lipids (Valiela et al. 1985; Buchsbaum et al. 1991). In laboratory incubations, senescent *Spartina* lost 20–25% of its C and 66% of its N as DOM after 10 d, resulting in a C : N ratio of 9 for the released DOM, whereas that of the remaining *Spartina* detritus was 17 (Marinucci 1982). Thus, the leachate was higher in N than the macrophyte, possibly indicating it had more nutritional value for consumers. DOM leached from macrophytes is also biologically labile and rapidly taken up by microbes (Benner et al. 1986; Robertson et al. 1982; Biddanda 1985).

One of the fates of DOM in seawater is aggregation into amorphous organic particles. This phenomenon first received widespread attention in the early 1960s (reviewed by Riley 1970). Since then, many investigators have demonstrated that organic particles could be produced from dissolved material (Biddanda 1985; Robertson et al. 1982; Kepkay and Johnson 1988). The mechanisms by which aggregation takes place are not well understood. Both biotic (exudation by bacteria, Robertson et al. 1982; Biddanda 1985) and abiotic (adsorption onto solid surfaces, Johnson 1976) mechanisms have been proposed for aggregate formation in the field. Both processes produce aggregates in the lab and may in fact interact. Particles formed abiotically by dissolving bubbles are quickly (3 h) colonized by microbes (Kepkay and Johnson 1988). Regardless of how they were formed, field samples of aggregates are usually colonized by bacteria and other microbes.

There is little knowledge of what fraction of released DOM does aggregate into particles or of the chemical composition of aggregates

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Acknowledgments

We thank B. Peterson, D. Caron, and two anonymous reviewers for comments on the manuscript. J. Getz, S. Englestein, and A. McKnight provided laboratory assistance; M. Hullar analyzed the DOC samples by HTCO; and Perkin-Elmer donated the CHN analyzer used in this study to the Marine Biological Laboratory. We also thank J. McDowell for her support.

This work is the result of research sponsored by the NOAA National Sea Grant College Program Office under grant NA86-AA-D-SG090 and Woods Hole Oceanographic Institution Sea Grant Projects R/A-24 and A/S-26-PD.

formed from different sources of DOM. The purposes of this study were to produce aggregates from DOM released by five species of coastal macrophytes and to quantify the conversion of dissolved C into aggregates. To accomplish these objectives, we ran two experiments. The first was designed to study in detail the time-course aggregation of DOM into particles. A brown alga, *Fucus vesiculosis*, was used to produce the DOM for this experiment. Treatments differing in initial concentration of DOM tested whether the final amount of POM (particulate organic material) produced depended on initial concentration of DOM. In the second experiment, we examined whether there were differences in amount and quality of aggregates produced from DOM released from five species of coastal macrophytes. The species were chosen to represent the major groups of macrophytes found in coastal waters. Carbon budgets were constructed in both experiments to estimate how much of the DOC (dissolved organic C) initially produced was converted into aggregates and how much remained as soluble C.

Materials and methods

Effect of the initial concentration of DOM on the formation of aggregates—Live *Fucus* was collected from a jetty in Woods Hole, Massachusetts. Whole thalli (100 g of wet wt) were frozen (−20°C) for 22 d. Freeze-killed *Fucus* was allowed to thaw and then placed in a 4-liter jar containing 2.5 liters of 0.22-μm-filtered seawater (FSW). Although some soluble material was released from the algae by the freeze/thaw process, this liquid was discarded. The jar was covered with foil and placed in the dark at 15°C in a temperature-controlled environmental chamber. The jar was gently stirred daily during the 5-d leaching period.

At the end of the leaching period, *Fucus* was removed from the jar and the leachate centrifuged for 10 min (4,500 rpm). After centrifugation, the supernatant was filtered through a Whatman GF/F filter (median pore size, 0.7 μm). The filtered leachate was placed in 500-ml mason jars and diluted with 0.22-μm FSW so that each jar contained 300 ml of material in the following concentrations: 0, 3.3, 20, 33, 66, and 100% leachate. There were 2 replicate jars for each leachate concentration. Jars were covered with foil and bubbled with air in the dark with aquarium bubblers (fitted with inline 0.22-μm bacterial filters) for 7 d. Since the leachate had passed through a GF/F filter before bubbling, any particles that appeared during the bubbling period were operationally defined as aggregates.

Samples for DOC analysis were removed daily from each mason jar during the leaching period. Twenty-milliliter samples were filtered through precombusted (>6 h at 550°C) GF/F filters and the filtrate was stored frozen in scintillation vials until analysis. DOC in the filtrate was measured with acid persulfate oxidation (Menzel and Vaccaro 1964). Samples were acidified (pH < 2) and pipetted into precombusted ampoules containing 50 mg of $K_2S_2O_8$. Ampoules were bubbled vigorously (20 min) with pure (CO_2-free) oxygen during sealing to minimize entry of CO_2 from the air (MacDowell et al. 1987) and then autoclaved for 1 h at 121°C. CO_2 in the digested samples was measured on a gas chromatograph (Shimadzu, Poropak Q column, TCD detector) with a syringe-stripping technique (MacDowell et al. 1987). Dextrose was used as a standard.

Twenty-milliliter samples of water were removed from each jar on days 0, 1, 2, 3, 4, and 7 of bubbling, and both dissolved and particulate materials were measured. Dissolved samples were analyzed for DOC as described above. Particulate samples were collected by gently filtering (Hg. 10–15 mm) water through precombusted (6 h at 550°C), prerinsed (DI water), preweighed (Mettler AE163 5 place balance) GF/F filters; rinsing the filtered samples with two 1-ml aliquots of DI water; and drying them to constant weight in a drying oven at 60°C. Filters were stored dried and later analyzed for percent C and N (Perkin-Elmer model 240C elemental analyzer, acetanilide used as a standard). Due to the variability of salt retention on blank filters, final particulate weights below 0.8 mg (0.8 mg/20 ml sample = 40 mg liter^{-1}) were not distinguishable from background.

Aggregate production from different species of macrophytes—Aggregates were produced from five coastal macrophytes in a manner similar to that described above. Five initial trials were carried out using a brown (*F. vesiculosis*), a red (*Gracilaria tikvahiae*), and a green (*Ulva lactuca*) alga, and two vascular plants (*S. alterniflora* and *Z. marina*). Tw additional trials, one with *Fucus* and one with *Gracilaria*, were also performed to examine variability within a species.

All macrophytes were collected from nearshore environments in Falmouth, Massachusetts, and freeze-killed in 25–50-g (wet wt) subsamples. Upon thawing, samples were leached into 500-ml FSW in 4-liter jars. Leaching and bubbling protocols were as described above for the first experiment, except that the leachate was never diluted with FSW before bubbling. Initial wet weight of the macrophytes, the number of replicate jars, and the leaching and bubbling periods for each trial are listed in Table 1.

Samples of the initial and leached macrophyte material were both dried in a 60°C oven. Dried samples were ground in a Wiley mill (250-μm screen). These samples were stored desiccated and later analyzed for percent C and N (Perkin-Elmer model 240C elemental analyzer).

Samples were taken during both the leaching and bubbling periods, filtered through precombusted GF/F filters, and measured for DOC as described above for the first experiment. POM was also sampled during the bubbling period, as described above, and analyzed for dry weight and percent C and N.

Because of uncertainty regarding the accuracy of measuring DOC with wet persulfate oxidation (e.g. Sharp et al. 1993), a batch of 32 samples was also analyzed with an Ionics high-temperature combustion DOC analyzer. There was good correlation between the two methods ($r = 0.93$), and the slope of the regression line was 1.07 (unpubl. data).

Budget calculations—Calculations were performed on the data obtained during the bubbling period to determine what portion of the DOC present at the beginning of bubbling was still present as DOC and what portion had aggregated into POC (particulate organic C). The portion that was lost was calculated by difference.

The cumulative percentage of DOC converted to POC or respired was calculated as

$$\%\ \mathrm{DOC\ lost} = [(\mathrm{DOC}_0 - \mathrm{DOC}_t)/\mathrm{DOC}_0] \times 100,$$

and net conversion for POC production was calculated as

$$\mathrm{net\ conversion} = [(\mathrm{POC}_t)/(\mathrm{DOC}_0 - \mathrm{DOC}_t)] \times 100.$$

0 and t are concentrations on days 0 and t of the bubbling period. These calculations were performed for all treatments where the difference between initial and final DOC concentrations was >15 mg C liter^{-1} to ensure that the calculations were done only in cases where the difference between final and initial was larger than the error term. All comparisons except the 3% leachate treatment in the first experiment and the *Spartina* treatment in the second experiment met this constraint.

Table 1. Specifications for leaching and bubbling of DOM to produce aggregates from different species of producers.

	Trial	Jars (*n*)	Initial macrophyte wet wt (g)	Leaching period (d)	Bubbling period (d)
Fucus	1	6	25	5	5
	2	3	100	5	5
Gracilaria	1	6	50	4	6
	2	7	100	4	6
Ulva	1	6	50	3	5
Zostera	1	2	35	7	4
Spartina	1	6	25	6	9

Results

Effect of initial concentration of DOM on the formation of aggregates—Leaching of DOC from *Fucus* led to increases of soluble material, and the water in the jars turned a deep yellow. At the end of the 5-d leaching period, the DOC concentration was ~450 mg C liter^{-1}. Leachate was mixed with FSW to make treatments that had DOC concentrations ranging from 20 to 450 mg C liter^{-1}; the FSW control had a concentration of 3 mg C liter^{-1} (Table 2).

Bubbling of the treatments led to decreased DOC concentrations in all cases and final concentrations of DOC reflected initial concentrations of leachate (Fig. 1). There was a significant relationship between the amount of DOC present initially and the amount remaining after 7 d of bubbling (Fig. 1B).

Microscopic examination of samples during the bubbling period showed large numbers of free-swimming rod-shaped bacteria after the first day of bubbling; these bacteria clumped

Table 2. Initial concentration of DOC (±SD) measured in 300-ml jars with different amounts of *Fucus*-derived leachate added. There were 2 replicate jars at each concentration.

Treatment (% leachate)	Amount of leachate added (ml)	DOC concn (mg C liter^{-1})
0	0	3.4±0
3.3	10	20.3±1.1
16.7	50	89.9±1.2
33	100	168.1±2.5
66	200	297.2±5.1
100	300	448.3*

* Estimated based on regression of above data: DOC concn (mg C liter^{-1}) = 1.47 × amount of leachate added (ml) + 7.35.

together after 1–2 d (Fig. 2A). Aggregates were observed after 2–4 d (Fig. 2B). These were not recognizable as bacterial clumps under the light microscope. However, DAPI, a DNA stain used to visualize bacteria (Porter and Feig 1980), revealed large numbers of bacteria in aggregates (Fig. 2C). Aggregates were also stained with Alcian Blue (Fig. 2D)—a stain that is positive in the presence of acidic polysaccharides such as uronic acid, commonly present in bacterial exudates (Decho 1990). A diverse population of microheterotrophs (ciliates and flagellates) was observed feeding at the edges of aggregates.

Aggregates were visible in the jars as dark-brown amorphous particles. Although many were free-floating, a large number were also attached to surfaces. Aggregates ranged from a few μm up to 1 cm in diameter but were usually <500 μm. It was not feasible to obtain a complete size-frequency distribution because aggregates broke up when they were sampled and clumped when they were left standing.

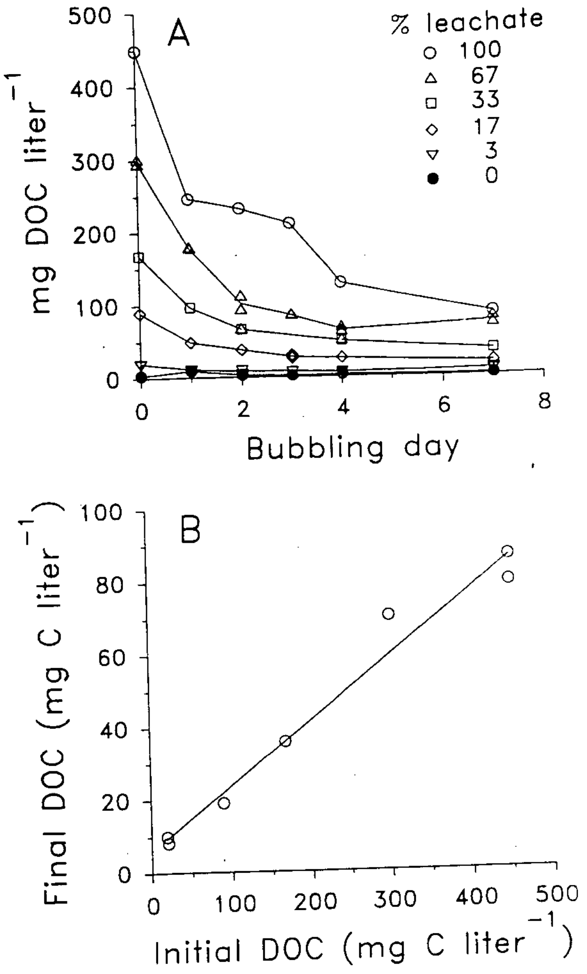

Fig. 1. A. Concentration of DOC during bubbling in treatments with different percentages of *Fucus*-derived leachate. Individual points represent replicate jars. B. Final vs. initial DOC concentration for each treatment. Line equation is: Final DOC concn = 0.18 × initial DOC concn + 5.9, $r^2 = 0.98$, $P < 0.001$.

Aggregates (measured as POC) were produced in all bubbled treatments. DOC in the control (0% leachate) remained constant (3.7 mg C liter^{-1}, SD = 2.9) and did not form measurable aggregates. Concentrations of both POC and PON (particulate organic N) after 7 d of bubbling were significantly ($P < 0.001$) related to the amount of leachate initially present (Fig. 3). These relationships suggest that DOM was indeed the source of POM.

The C and N content of aggregates did not vary systematically among treatments or over

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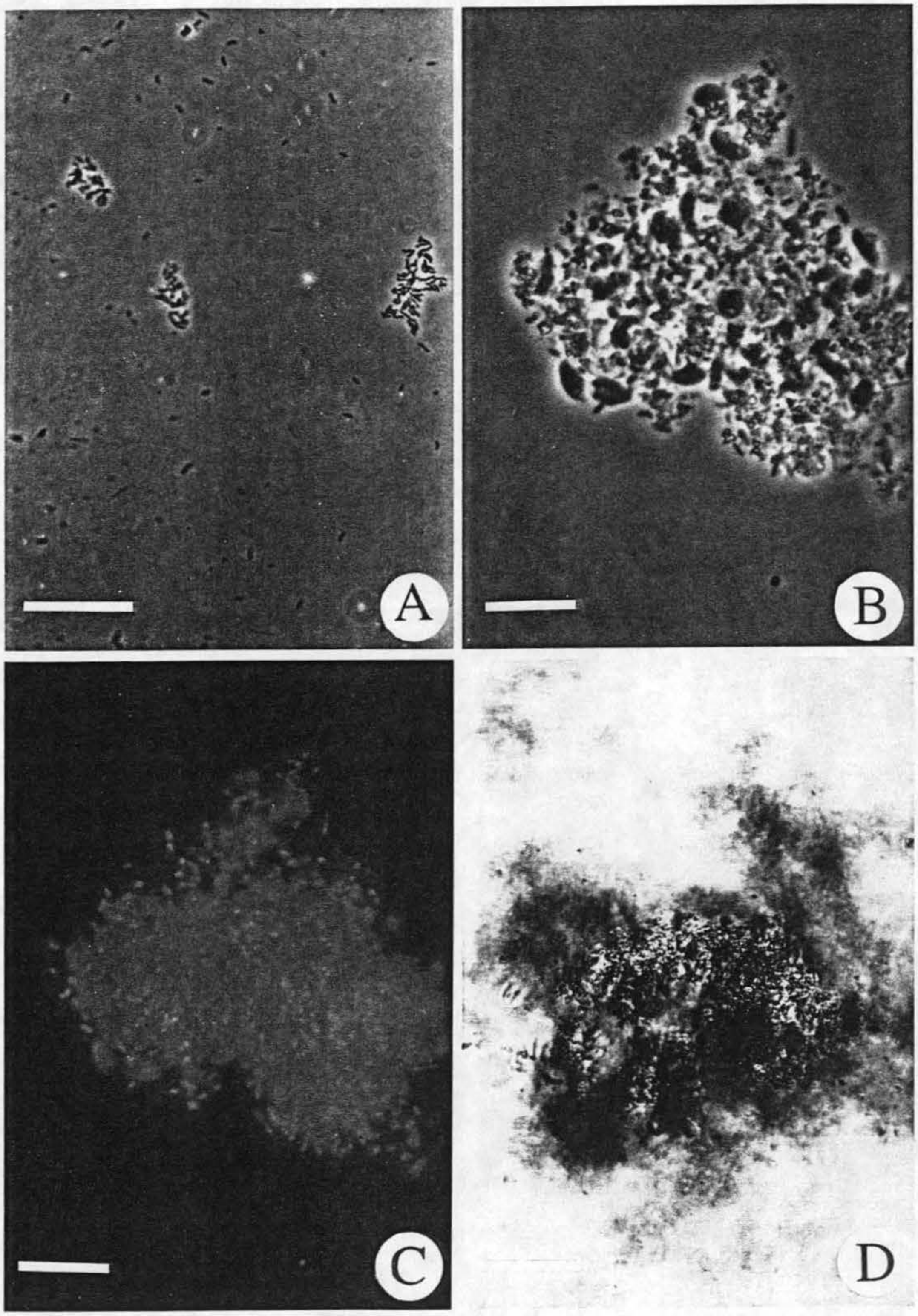
Fig. 2. A. Phase-contrast photograph of water sample as it appeared on day 2 of the bubbling period. Note clumping of rod-shaped bacteria. B, C, D. Photographs of aggregates sampled after 4 d of bubbling *Fucus*-derived leachate. B. An organic aggregate viewed with phase contrast. Note the presence of microheterotrophic protozoa. C. The same aggregate as in panel B, photographed under epifluorescence with DAPI stain. D. Phase-contrast photograph of an aggregate stained with Alcian Blue. (Scale bar: 10 μm in A, B, and C; 25 μm in D.)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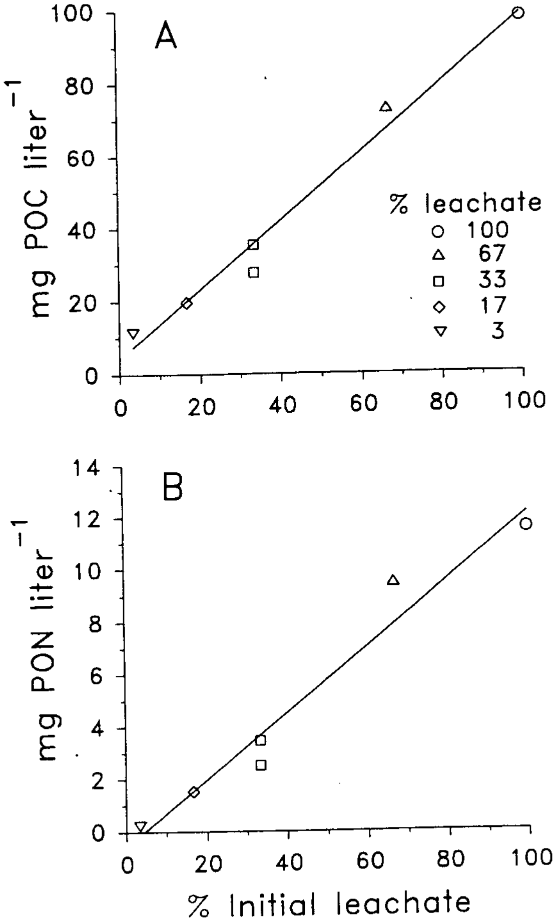

Fig. 3. Final weight of POC and PON vs. the percent of leachate initially present in treatments containing different amounts of *Fucus*-derived leachate. Line equations are: POC concn = 0.94 × % leachate + 43, $r^2 = 0.98$, $P < 0.001$; PON concn = 0.13 × % leachate − 0.54, $r^2 = 0.96$, $P < 0.001$.

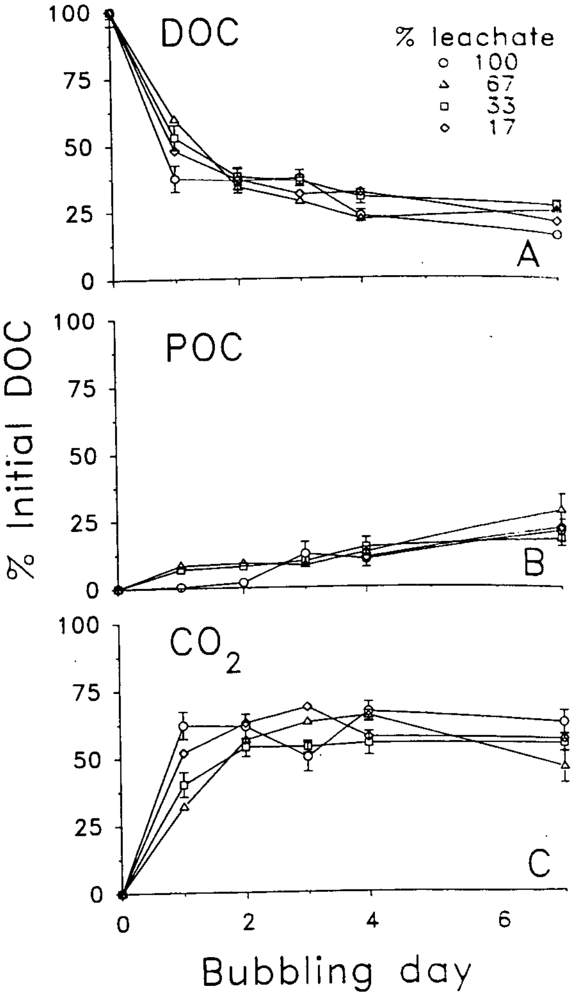

Fig. 4. Pattern of DOC, POC, and the difference expressed as a percent of initial DOC concentration in treatments containing differing amounts of *Fucus*-derived leachate.

time (Table 3) despite differences in the weight of aggregates produced (Fig. 3). Aggregates averaged 24.3% C (SD = 5.9) and 3.1% N (SD = 1.4), giving them an average C : N ratio (by atom) of 8.3 (SD = 1.6).

The amount of C in the DOC, POC, or CO$_2$ (calculated by difference) pools on each day of the bubbling period was expressed as a percentage of the initial DOC (Fig. 4). There were similar proportions in all treatments despite large differences in initial DOC (Table 2). The percentage remaining as DOC decreased dramatically during the first day of bubbling and then dropped off more slowly (Fig. 4A). By the end of day 1, <50% of the initial DOC remained, and by day 4 the concentration was <35% of the initial concentration of DOC in all treatments.

The percentage of initial DOC present as POC increased significantly over time (Fig. 4B, $y = 3.5x + 0.5$, $r^2 = 0.85$, $P < 0.001$). Between 17 and 25% of the initial DOC was converted to POC by the end of bubbling.

The final component of the DOC budget is the amount of material no longer measured as either DOC or POC. This difference is DOC that has presumably been mineralized to CO$_2$ by microbes. It is possible, however, that some of the DOC lost from the budget was concentrated on the sides of the jars. DOC lost in this manner would result in an overestimate of CO_2. If DOC concentrated on filters when the samples were being processed, it would affect the distinction between dissolved and particulate C but not the CO_2 term. There was a very steep increase in the amount of DOC lost during the first day of bubbling (Fig. 4C); after that, the increase slowed.

Aggregate production from different species of macrophytes—Initial C : N ratios of the five species of macrophytes ranged from 13 to 28 (Table 4). The C : N of both *Fucus* and *Spartina* increased during leaching but did not change in the other species. The concentrations of DOC, measured at the end of the leaching period, ranged from 48 to 834 mg C liter^{-1} (Table 4).

During bubbling, the DOC concentration decreased rapidly during the first 1–2 d and then remained constant (Fig. 5A). This pattern was similar to the pattern observed during the first experiment (Fig. 1A). By day 4 or 5, an average of 73% of the initial DOC was removed, and the DOC decrease per day after day 4 was always <5% (Table 5).

As in the first experiment, the final concentration of DOC at the end of the bubbling period was significantly related to initial concentration (Fig. 5B). The slope of the relationship for both experiments together is similar to that found in the first experiment alone (0.23 vs. 0.18), suggesting that, regardless of species of producer and over a very wide range of initial DOC, the percent of the initial DOC that remained as soluble material at the end of bubbling was constant. This constancy is remarkable since the actual concentration of DOC, the length of the bubbling period, and the chemical composition of the leachate produced from the different macrophytes (Alber 1992) varied among trials. The most likely explanation may be that there is a refractory fraction of DOC that is not available to bacteria.

Table 4. C : N ratios of initial and leached macrophytes (±SD) and concentration of DOC (mg C liter^{-1}) released from them during leaching in each experimental trial.

	Trial	Initial C:N	Leached C:N	DOC
Fucus	1	21.2±1.2	25.8±2.1	395±73
	2	23.7±0.2	25.2±1.5	834±58
Gracilaria	1	12.9±0.9	12.6±1.8	283±12
	2	13.8±0.3	14.2±0.1	185±34
Ulva	1	16.3±0.3	15.5±1.6	427±5
Zostera	1	18.2±4.4	18.3±2.8	293±45
Spartina	1	28.0±5.9	34.3±2.8	48±5

Aggregates were formed from all species of algae during the bubbling period. Microscopically, aggregates were similar to those observed during the first experiment and followed a similar pattern of initial clustering of rod-shaped bacteria followed by the appearance of microheterotrophic protozoa. The final concentration of POM ranged widely, from 80 to 330 mg dry wt ml^{-1} (Table 6).

Initial DOC concentration was a significant predictor of final aggregate POC both within and across species (Table 7). Although variability is introduced when the relationship obtained from the first experiment is extended to include the additional trials and species used in the second experiment (Table 7), the overall relationship ($y = 0.16x + 12.7$, $P < 0.01$) may be useful for predicting the final amount of POC that can be produced by bubbling macrophyte DOC.

Despite differences in the species of producer or the amount of aggregated DOC, the C : N of aggregates did not vary appreciably

Table 3. %C, N, and C : N (by atoms) of aggregates produced during bubbling treatments with different initial percentages of *Fucus*-derived leachate.

Bubbling day	Treatment											
	17%			33%			67%			100%		
	%C	%N	C:N	%C	%N	C:N	%C	%N	C:N	%C	%N	C:N
1				27.9	6.4	4.4	42.3	9.5	4.5			
2				30.5	3.3	9.3	21.8	2.5	8.7	25.6	4.2	6.2
3				31.5	3.2	9.8	23.3	2.3	10.0	34.8	7.1	4.9
4	8.1	0.9	8.8	22.1	2.4	9.1	16.8	2.3	7.5	17.9	2.2	8.2
7	17.5	1.3	12.7	28.8	2.7	10.6	19.2	2.5	7.7	19.0	2.4	7.9

Table 5. Cumulative percent of DOC released during 7 d of bubbling, calculated as $[(DOC_0 - DOC_t)/DOC_0] \times 100$, where 0 and t are DOC concentrations on days 0 and t of the bubbling period. Data are presented for treatments from experiment 1, which had different amounts of *Fucus*-derived leachate, and experiment 2, where 100% leachate derived from several different macrophyte species was bubbled.

		Bubbling day						
	Trial	1	2	3	4	5	6	7
Exp. 1 (% leachate)								
17					71			79
33		42	61	71	71			79
67		40	66	71	78			75
100		45	48	53	71			81
Exp. 2								
Fucus	1		69		78	77		
	2				74	76		
Graciliaria	1			60		70	76	
	2					68	71	
Ulva	1	73		77	81			
Zostera	1					79		

(Table 6). The average for aggregates in the second experiment was 31% C and 6.4% N. As might be expected from the range of final POM measurements, final concentrations of both POC and PON also ranged widely, from 14 to 136 mg C $liter^{-1}$ and 2 to 31 mg N $liter^{-1}$ (Table 6).

Carbon budgets—As the initial concentration of DOC decreased during bubbling, some of it was converted to aggregate C and some was lost. Averaged over all trials and species, 19% of the initial DOC was converted to aggregates, 23% remained in solution as DOC, and 58% was presumably mineralized to CO_2 by the end of the bubbling period (Table 8). The amount of initial DOC converted to aggregates in these budgets ranged from a low of 9% in *Zostera* to a high of 34% in one *Fucus* trial. When expressed as a percentage of the decrease in initial DOC, net conversion of DOC to aggregates at the end of bubbling ranged from 10 to 45% (Table 8).

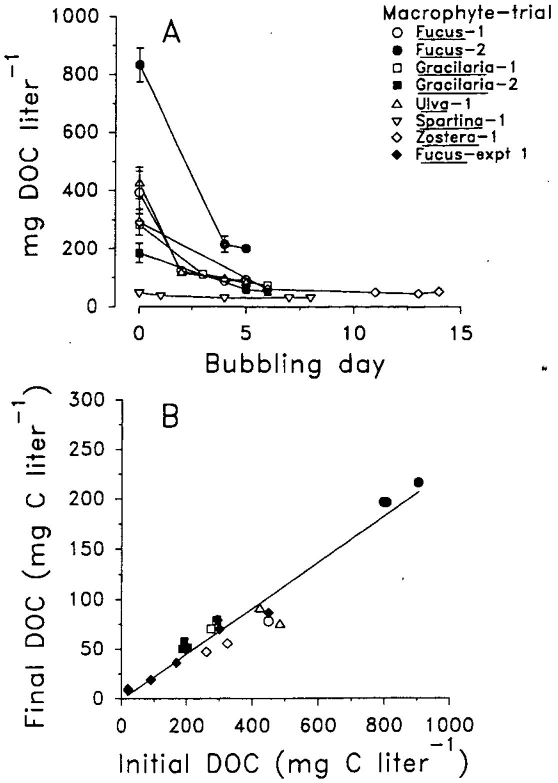

Fig. 5. A. DOC concentration during bubbling in experiment 2, where leachate was derived from different macrophytes. Individual points represent replicates. B. Final vs. initial DOC concentration for treatments from both experiments. Line equation is: Final DOC concn = 0.23 × initial DOC concn − 1.14, $r^2 = 0.94$, $P < 0.001$.

Table 6. POM, POC, and PON (mean ± SD) of aggregates produced from macrophyte-derived leachate, as well as %C, %N, and C:N.

Leachate source	Trial	POM (μg ml⁻¹)	POC (μg C ml⁻¹)	PON (μg N ml⁻¹)	%C	%N	C:N
Fucus	1	333±71	136.0±11.0	22.1±0.4	44.6	7.8	6.6±0.6
	2	304±117	131.0	31.0	26.4±5.9	6.0±1.7	4.4±0.3
Gracilaria	1	132±26	60.8±9.8	14.5±1.8	39.5±7.9	9.4±1.6	4.2±0.2
	2	105±32			34.3	7.8	4.4
Ulva	1	204±300	53.2	11.9	31.4±2.6	6.6±0	4.7±0.4
Zostera	1	120±42	25.2±0.7	5.4±0.1	19.8±9.0	4.3±1.7	4.6±0.1
Spartina	1	81±9	13.9±7.6	2.2±0.9	16.5	2.7±0	6.1±0.1

Table 7. Regression relationships between initial DOC concentration and final POC concentration. Relationships are shown for all data, and for data grouped by experiment, for data from aggregates derived from *Fucus* leachate only, and for aggregates derived from species other than *Fucus*. (Not significant—ns.)

Data used	n	Slope	Intercept	P	r^2
All data	15	0.16	12.7	<0.01	0.57
Exp. 1	5	0.21	4.6	<0.001	0.98
Exp. 2	10	0.15	14.7	<0.05	0.48
Fucus only	8	0.15	31.3	<0.05	0.59
Other species	7	0.13	3.9	ns	0.55

Discussion

Organic aggregates were produced by bubbling the leachate released by all five species of coastal macrophyte used. The concentrations of DOC in these experiments were much higher than those observed in coastal waters, which are typically <5 mg C liter^{-1} (e.g. Sharp et al. 1993). The data presented here, therefore, should be extrapolated to the field with caution. Nevertheless, this study does enable us to examine the effect of a range of initial DOC concentrations on the process of aggregate formation. Despite the very wide range of initial concentrations observed here, all treatments behaved similarly. The amount of POC produced depended on the amount of DOC in the leachate, as evidenced by the significant relationships between initial DOC and final POC concentration (Table 7). On average, 19% of the leachate was converted to POC, 23% remained as DOC, and 58% was lost (Table 8). The C : N ratio of aggregates produced from the DOC released by the different species ranged from 4 to 12 (Tables 3, 6).

The results of these experiments are in keeping with a series of notions about aggregates: first, that they consist largely of bacterial clusters; second, that they stay together once formed; and third, that they are surrounded by extracellular material. We will examine each of these suggestions in turn.

Aggregates consist of bacterial clusters—The experiments reported here were not designed to determine the mechanism of aggregate formation. We did, however, observe large numbers of rod-shaped bacteria that clumped during the bubbling process (Fig. 2). Aggregates stained heavily with DAPI and looked similar to the biologically produced aggregates described by Biddanda (1985, 1986) and others (Paerl 1978; Robertson et al. 1982). Biddanda (1985) found that microbial aggregates formed when he bubbled DOC released from macrophytes, but they did not form in treatments where antibiotics were added. Paerl (1974, 1978) also reported bacteria-particle aggregates that did not form in sterile controls.

Table 8. Carbon budgets on the final day of bubbling. Budgets were calculated for treatments from experiment 1, which had different amounts of *Fucus*-derived leachate, and experiment 2, where 100% leachate derived from several different macrophyte species was bubbled. Data are presented for the final concentrations of carbon in the DOC and POC pools, and the amount of carbon in each pool as a percent of the initial DOC measured in the leachate. Net conversion of DOC to POC on the final day of bubbling, calculated as $[POC_t/(DOC_0 - DOC_t)] \times 100$, where 0 and t are concentrations measured on days 0 and t, is also presented.

		Final concn (mg C liter^{-1} ± SD)		As % of initial DOC			
	Trial	DOC	POC	DOC	POC	CO_2	Net conversion
Exp. 1 (% leachate)							
17		19±0	20±1	21±1	22±1	57	28
33		36±1	30±4	21±1	18±3	61	23
67		75±6	85±18	25±2	29±6	46	38
100		86±2	94±3	19	21	60	26
Exp. 2							
Fucus	1	93±14	136±90	23±6	34	43	45
	2	203±11	131±31	24±2	16±4	60	21
Gracilaria	1	74±4	52±10	26±2	18±4	55	25
	2	53±4	36±11	29±6	20±7	52	27
Ulva	1	82±8	64	19±3	12	69	18
Zostera	1	51±6	24±9	18±3	9	73	10
Average				23±4	19±8	58	10

There have been many observations of bacterial aggregates in natural waters. They have been described in kelp beds (Linley and Field 1982), lakes (Paerl 1975), and the euphotic zone (Seki 1971). Schoenberg and Maccubin (1985) described aggregates of bacteria as the dominant form of attached bacteria in both marsh and lake water. Gordon (1970) described "flakes" that stained heavily for protein and looked like the aggregates produced in our study.

Several other lines of evidence also suggest that the aggregates produced in this system consisted largely of clumped bacteria. First, the C : N ratio of the aggregates was similar to reported values for bacteria (4.5–6.7, Bratbak 1985). Furthermore, the C : N ratio was constant (and low) for aggregates produced from different species, whereas that of the starting macrophytes was always higher and varied between species (Table 4). The C : N makeup of the aggregates was therefore independent of the DOM from which they were formed, which is consistent with a biological source of aggregation, since one might expect an abiotic condensation mechanism of aggregation to produce particles that reflect the C : N of the source material.

Finally, the time-course of DOC decrease and POC formation can be explained in terms of microbial processes. The initial rapid decrease in DOC observed in all experiments (Figs. 1A, 5A) is probably due to rapid growth of free bacteria which metabolized the DOC, converting some of it to bacterial biomass and some to CO_2. Ogura (1975) found that the kinetics of microbial oxidation of DOC in seawater could be described in two phases. During the first phase, up to 40% of the initial DOC is quickly removed in <7 d (much of it as low molecular weight material); after that DOC use decreases considerably.

Aggregates stay together once they have formed—Once aggregates were formed, DOC loss slowed to $<5\%$ per day (Table 5). This slow loss suggests that once bacteria formed into aggregates they were no longer actively metabolizing DOC. A decrease in DOC metabolism is consistent with the observation that bacterial aggregation in cultures occurs in late log phase when growth rate slows: cells in aggregates may not respire, metabolize, or grow as much as dispersed cells (Calleja 1984). The slowed DOC loss also suggests that the microheterotrophic protozoa observed in the jars did not have a large impact on the system, since neither the DOC nor POC concentrations changed.

A similar pattern of POC production was observed by Robertson et al. (1982) who reported that $>90\%$ of the bacteria in their system was in aggregates after 5 d of bubbling. Both the absolute number of bacteria and the percentage that were aggregated remained relatively constant during the next 9 d despite the sustained presence of flagellates and ciliates after day 5. The number of bacteria Biddanda (1985) observed in aggregates formed from bubbling DOM also remained relatively constant after 2 d, although flagellates were not observed until day 8.

Our observation that bacterial aggregates remained intact over the course of these experiments (4–9 d) is in contrast to the suggestions of Biddanda and Pomeroy (1988) that bacterial-particle aggregates produced from bacteria and degrading phytoplankton are part of a process of microbial succession. They showed that bacteria grew, colonized phytoplankton, formed aggregates, were grazed by protozoa, and disaggregated. The entire process took 8–16 d. Particle disaggregation did not occur in this study. It may be that there were not enough protozoa present in the jars to have an effect on the aggregates, or the experiments may not have been run for long enough or stirred vigorously enough to effect disaggregation. We have, however, produced aggregates that remained intact even after 2 months of bubbling (M. Alber pers. obs.). It is possible that aggregates formed as bacterial clusters are harder to break down than the phytoplankton-containing bacterial-particle aggregates produced by Biddanda and Pomeroy (1988).

Aggregates contain extracellular material—The hypothesized "stability" of the aggregates in this study may have been due to the presence of inactive bacteria and extracellular material. This explanation is also consistent with the observations that the DOC concentration decreased slowly after aggregates were formed, the weight of the aggregates did not decrease over time, and the C : N ratio of the aggregates always resembled that of bacteria and protozoa.

Paerl (1974) showed that dissolved organic compounds are rapidly incorporated into slimes and capsular materials and that a small live fraction of bacteria can produce up to 20 times its C weight in 1 week (Paerl 1978). He also observed that dead bacteria and extracellular material remained within clusters and did not decompose rapidly (Paerl 1978). Robinson et al. (1982) grew bacteria in *Laminaria* leachate and measured 3 times more C in these cells by CHN analysis than they calculated with ATP measurements. They attributed the high carbon value to the presence of large quantities of extracellular material that would not be accounted for in the ATP technique.

The presence of extracellular material has also been reported in other environments. Investigators working on flocs formed in activated sewage sludge have found that only 10–15% of the mass is composed of viable cells and that large quantities of polysaccharides and protein and up to 40% of the nucleic acids can be found extracellularly (Harris and Mitchell 1973). Parsons and Dugan (1971) estimated that extracellular material represented 40% of the total solids of a bacteria grown in pure culture.

The aggregates stained with Alcian Blue (Fig. 2D), and this fact also supports the notion that they have extracellular material associated with them, because the stain is positive in the presence of bacterial exudation products. Autoradiographs of aggregates and naturally occurring particles show that bacterial extracellular fibrils are frequently present either between adjacent bacteria or between bacteria and nonliving solids (e.g. Biddanda 1986). These mucopolysaccharides, mucilaginous capsules, slimes, and fibrillar excretions have been proposed as the glue that holds aggregates together (Hobbie and Lee 1980). Robertson et al. (1982, p. 282) described their clusters as "densely packed bacterial cells embedded in an amorphous matrix which fluoresced" when stained with acridine orange.

Further support for this suggestion comes from the data of Rice and Hanson (1984) who showed that during decomposition of macrophyte detritus accumulated N was sequestered in bacterial exudates. At any one time the bacterial biomass is low, yet the N concentration of the material reflects the cumulative number of bacteria that have been present—the bacterial history of the detritus. In a similar manner, the low C:N ratios observed here (Tables 4, 6) may be due to the presence of either dead bacteria or N-rich extracellular material such as mucopolysaccharides and glycoproteins.

Carbon conversion—There is considerable variation in reported values of conversion efficiency for macrophyte and phytoplankton-derived detritus (Table 9). From 20 to 30% of the metabolized detrital C (the amount converted to bacterial C or mineralized to CO_2) at any given time is measured as live bacterial C, whereas anywhere from 10 to 90% of the detrital C is measured as POC. In general, efficiencies based on direct measurements of POC are greater than those based on live bacterial C because the POC pool includes not only live bacterial biomass but other C as well.

In this study, 10–45% of the DOC that was removed from solution at the end of bubbling was measured as POC. These efficiencies are on the low end of values reported for conversion of detrital C to POC (Table 9). Low efficiencies may be due to the large pore size of the filter used here to measure POC compared with that used by others. It is also possible that the other reported values (based on ^{14}C) are overestimates. ^{14}C incubations can underestimate CO_2 losses if isotopic equilibrium has not been established in internal C pools: Moran and Hodson (1989) suggested this was the reason their conversion efficiency was so much higher after their 16-h incubation than after 32 h.

Implications for detrital food webs—The low C:N ratios of the aggregates produced here (range, 4–12) suggest that they are comprised largely of bacteria and are potentially nutritious food particles. Their size range (a few μm to 500 μm in diameter) may make aggregated bacteria available to larger consumers that are not as effective at capturing free-living bacteria. Moreover, if aggregates contain dead bacteria or bacterial exudates, as has been suggested here, then consumers that eat aggregates are eating not only the small live fraction of bacteria but food particles that contain a nonliving pool of material as well. This nonliving material is not usually accounted for in considerations of C flow or calculations of conversion efficiency based on bacterial production.

The aggregate trophic pathway—primary producer to released DOM to aggregate to

Table 9. Literature values for net conversion of detritus to POC or bacterial C. Estimates are grouped according to whether efficiency was calculated in terms of the total amount of POC or the amount of live bacterial C present in the sample. Values are presented along with their methodology, relevant conversion factors, and the length of time detritus was incubated.

Substrate	Time	Conversion (%)	Method* [comments]	Reference
Net POC conversion				
Present study				
Zostera leachate	11 d	10	CHN (0.7-μm GF/F)	
Ulva leachate	5 d	18		
Gracilaria leachate	6 d	25–27		
Fucus leachate	1 d	4–21		
	5–7 d	21–45		
Mangrove leachate	12 h	64–94	^{14}C (0.2-μm Nuclepore)	Benner et al. 1986
Spartina leachate (lignocellulose fraction)	16 h	62	^{14}C (0.2-μm Gelman GA8)	Moran and Hodson 1989
	32 h	27		
Spartina particles	2 d	73	^{14}C (0.45-μm Gelman)	Fallon and Pfaender 1976
	2 d	58	[microbes present]	
Whole *Spartina* particles	145 d	20	^{14}C (0.2 μm)	Moran et al. 1989
Lignocellulose only	145 d	10		
Phytoplankton exudate	1 h	68–82	^{14}C (0.2 μm)	Iturriaga and Hoppe 1977
Phytoplankton exudate	3 h	57–76	^{14}C (0.2 μm)	Bell and Sakshaug 1980
Net bacterial conversion				
Spartina particles (lignocellulose only)	4–6 d	17–37	Biovolume (0.22 g C cm^{-3})	Benner et al. 1988
	4–6 d	3–14	[microbes present]	
Spartina particles	4 d	10	Biovolume (0.11 g C cm^{-3})	Linley and Newell 1984
Thalassius leachate	2 d	4	Biovolume (0.09 g C cm^{-3})	Robertson et al. 1982
Syringia leachate	2 d	17		
Zostera particles	4 d	8	Biovolume (0.35 g C cm^{-3})	Blum and Mills 1991
Phytoplankton leachate	1 d	24–33	Cell count (20 fg C cell^{-1})	Biddanda 1988
Phytoplankton debris	1 d	22–36		
Phytoplankton debris	3 d	10	Biovolume (0.11 g C cm^{-3})	Newell et al. 1981
Phytoplankton detritus	24 h	17–27	Biovolume (0.1 g C cm^{-3})	
Laminaria mucilage	7–9 d	6–15	Biovolume (0.11 g C cm^{-3})	Lucas et al. 1981
Laminaria particles	2 d	27–43	Cell count (200 fg C cell^{-1})	Robinson et al. 1982†
Ecklonia particles	8 d	23–27	Biovolume (0.11 g C cm^{-3})	Koop et al. 1982

* Filter size for net POC conversion; conversion for net bacterial conversion.

† Conversion efficiency calculated as a percentage of CO_2 lost instead of as a percentage of CO_2 lost plus DOC converted to bacterial C.

metazoan consumer—has been proposed as a route for the assimilation of primary production by metazoa in detrital food webs (Mann 1988). We have demonstrated that aggregates can be ingested and assimilated by suspension-feeding bivalves (Alber 1992) and other metazoa (D'Avanzo et al. 1991).

Production of aggregates in this study was independent of the source of the DOM. It may be, therefore, that a fifth to a third of all the DOC released both by living and dead macrophytes and by phytoplankton can potentially be transformed into aggregates—with a conversion efficiency ranging up to 45%—and that these aggregates can in turn be used as food by consumers. The aggregation of DOC into amorphous particles is a little-recognized but potentially important route for the flow of labile organic C in marine systems.

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Submitted: 12 June 1992
Accepted: 7 June 1993
Amended: 27 July 1993