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Biochemical composition of organic aggregates produced from marine macrophyte-derived dissolved organic matter

Abstract—Organic aggregates produced by bubbling dissolved organic material released from five different coastal macrophytes were analyzed for biochemical composition (carbohydrate, protein, carbon, and nitrogen content). The composition of aggregates did not reflect that of either the macrophyte or the soluble material from which they were derived and did not vary with species. These results, coupled with the fact that aggregate composition was similar to that reported for bacteria, support the hypothesis that the process of aggregation was largely driven by microbial processes. The composition of morphous detritus (recognizable fragments of decomposing macrophytes) did vary with species and was related to that of the initial macrophyte. Morphous detritus had higher C : N ratios and was lower in protein than aggregates. The composition of aggregates was also different than that reported for marine snow.

One of the fates of the dissolved organic material (DOM) released by decomposing macrophytes is aggregation into amorphous particles called organic aggregates. The mechanism of particle formation is not well understood: both biotic (incorporation of DOM into bacteria, which then form clusters, Biddanda 1985) and abiotic (direct condensation of dissolved material into particles, Kepkay and Johnson 1988) processes have been demonstrated, and both may be occurring simultaneously. We have previously shown that aggregates could be formed from the DOM released from a wide spectrum of macrophytes (Alber and Valiela 1994). We hypothesized that these aggregates are comprised of live and dead bacteria, other microbes, and extracellular material.

The purpose of the present study was to determine the biochemical composition of aggregates so that we could address several different issues. First, we compared the composition of organic aggregates with that of both the initial macrophytes and the DOM released from them during leaching in order to provide insight into the process of aggregate formation. One might expect the composition of aggregates to be influenced by that of the macrophyte from which they were derived, especially if aggregates either form abiotically or have a high proportion of DOM adsorbed directly to them. Alternately, if either the same constituents of DOM tend to precipitate or if the process is largely driven by bacteria, as we have hypothesized, aggregate composition should be similar to bacteria, regardless of the macrophyte used as the source of dissolved material.

The second purpose of the study was to distinguish between different types of macrophyte detritus. Macrophyte detritus can be classified as either morphous or amorphous (Bowen 1984). Morphous detritus consists of recognizable fragments of the original macrophyte and retains its cellular structure. It forms as dead macrophytes are fragmented into smaller pieces during decomposition. Aggregates fit the definition of amorphous detrital particles, as they lack discrete structure and form from the DOM released into the water by both live and dead macrophytes. Mann (1988) proposed that amorphous detrital pathways may be more important than morphous pathways for the flow of material from macrophytes to consumers. We therefore compared the composition of aggregates to that of the leached macrophyte, which represents the starting material for morphous detritus.

Finally, since different types of aggregates may be of different quality, we compared the composition of the organic aggregates formed in our system with values reported for marine snow, which is broadly defined as aggregates >500 μm long (Alldredge and Silver 1988).

Aggregates were formed from DOM released

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Table 1. Specifications of aggregate production. The initial wet weight (wt) of each macrophyte added to each replicate, the number of replicate jars run for each species (n), and the lengths of the leaching and bubbling periods are given below.

	Initial wt (g)	n	Leaching (d)	Bubbling (d)
Fucus	25	6	5	5
Gracilaria	50	6	4	6
Zostera	35	2	7	14
Ulva	50	6	3	5
Spartina	25	6	6	9

during leaching by three macroalgal (*Fucus vesiculosis, Gracilaria tikvahiae,* and *Ulva lactuca*) and two vascular plant (*Spartina alterniflora* and *Zostera marina*) species. Aggregate production has been described in detail elsewhere (Alber and Valiela 1994). Briefly, freeze-killed samples of five macrophytes were allowed to leach into 1,800-ml jars containing 500 ml of 0.22-μm filtered seawater for 3–7 d (Table 1). After the leaching period, the DOM-rich water was passed through a Whatman GF/F filter and then bubbled with air for 5–14 d (Table 1) to produce aggregates (aerators were fitted with in-line 0.22-μm filters). The lengths of both the leaching and bubbling periods were based on pilot studies performed to determine the number of days to reach the maximum concentration of DOM and aggregates, respectively. Aggregates were defined as any particulate organic material caught on a GF/F filter after bubbling. All experiments were run at 15°C in the dark.

Samples of the initial macrophyte, leached macrophyte, DOM, and aggregates were obtained from each species as follows. Both initial and leached macrophyte samples were collected, dried to constant weight (60°C drying oven), and ground in a Wiley mill (250-μm mesh). Ground, leached macrophyte samples were considered morphous detritus, as they represented material that would be fragmented after the initial leaching phase of decomposition. Dried samples were analyzed for carbohydrate, protein, carbon, and nitrogen content. At the end of the leaching period, samples of the leached DOM were filtered through GF/F filters. Samples were stored frozen (−20°C) in scintillation vials until analysis for soluble carbohydrate and protein. Aggregates were present in each jar by the end of the bubbling period. They ranged in size from a few micrometers up to 500 μm across. These were passed through preweighed, precombusted (6 h, 550°C) GF/F filters and analyzed for carbohydrate, protein, lipid, carbon, and nitrogen content.

Carbohydrate was measured with the phenol-sulfuric acid assay (Strickland and Parsons 1972). Dried initial or leached macrophyte samples were extracted in 2 ml of 0.1 N HCl overnight at room temperature. Dried filters containing aggregates were placed into tubes with 2 ml of 0.1 N HCl and reagents were added immediately. After the reaction had cooled (>1 h), tubes with filters were centrifuged (5 min, 3,000 rpm) if necessary to clarify the mixture before reading its absorbance on the spectrophotometer. This protocol was adopted because it was found that the amount

Table 2. Composition of initial macrophytes. Percent carbohydrate, protein, carbon, and nitrogen were measured in initial macrophyte samples from each species. All numbers shown with standard errors; n is the number of replicate jars sampled; g is the grouping of species for each measurement determined by Tukey's multiple comparison of means, with significance assumed at the 95% C.L. ($P < 0.05$). The results of one-way ANOVA for each measurement are presented at the bottom of the table.

	% Carbo.	n	g	% Protein	n	g	% C	n	g	% N	n	g
Fucus	24.2±0.5	2	1	11.3±0.2	2	2	33.4±1.8	5	1,2	1.6±0.1	5	1
Gracilaria	25.1±4.5	2	1	5.8±0.7	2	1	28.7±0.7	5	1	2.4±0.1	5	3
Zostera	48.5±6.8	2	1,2	5.7±0.2	2	1	38.1±0.9	2	2,3	2.4±0.1	2	3
Ulva	48.0±1.9	2	1,2	5.1±0.1	2	1	33.7±0.7	4	1,2	2.2±0.1	4	2,3
Spartina	54.4±4.9	4	2	12.8±0.3	2	2	39.9±2.1	6	3	1.7±0.2	6	1,2
ANOVA results												
df	4,7			4,5			4,17			4,17		
F	8.0			90.7			14.6			6.9		
P	<0.01			<0.001			<0.001			<0.01		

Table 3. As Table 2, but for composition of leached macrophytes.

	% Carbo.	*n*	*g*	% Protein	*n*	*g*	% C	*n*	*g*	% N	*n*	*g*
Fucus	24.4±2.4	3	1	9.0±1.1	2	1	35.4±0.4	3	2	1.4±0.1	3	1
Gracilaria	28.3±0.3	3	1	5.2±0.4	2	1	27.8±0.6	6	1	2.2±0.1	6	2,3
Zostera	37.7±0.5	2	1,2	9.1±0.1	2	1	34.1±0.4	2	2	1.8±0.1	2	1,2
Ulva	55.1±1.9	3	1,2	5.1±0.1	2	1	36.6±0.4	3	2	2.4±0.1	3	3
Spartina	57.7±1.6	3	1,2	11.8±1.4	2	2	41.4±1.7	3	3	1.2±0.1	3	1
ANOVA results												
df	4,9			4,5			4,12			4,12		
F	87.5			12.5			43.2			20.8		
P	<0.001			<0.01			<0.001			<0.001		

of carbohydrate measured in an unfiltered aggregate suspension was equal to the amount recovered from filters prepared in this manner plus the amount measured as soluble carbohydrate in the filtrate. Extracting overnight in HCl at room temperature or at 100°C in a sealed ampoule did not improve carbohydrate recovery from filters, and filtering or centrifuging the extract before adding reagents reduced recovery by up to 45%. Leached DOM samples were analyzed without extraction.

The lipid concentration of aggregates was determined gravimetrically with an extraction procedure adapted from Sasaki and Capuzzo (1984). The weight of the dried lipid was measured to the nearest 0.1 μg with a Cahn (model 29) electrobalance. Filters used in this analysis were prerinsed with chloroform : methanol (1 : 2), which reduced the value of the blanks by 60%. All glassware was also solvent rinsed.

Samples were analyzed for protein with the Bradford assay (Biorad). Dried samples were extracted for protein analysis following the method of Rausch (1981). Dried, ground initial or leached macrophyte samples (20–30 mg) were first extracted in 3 ml of 0.5 N NaOH for 20 min at 80°C and then re-extracted at 100°C. The second extraction increased protein recovery by 10–20%. Aggregate samples on filters were extracted following the same procedure, using pellets that were first extracted for lipids and then dried overnight at 60°C. There was no difference in protein recovered from lipid-extracted and unextracted aggregates. Leached DOM samples were run without extraction.

The CHN content of samples of the initial macrophyte, leached macrophyte, and aggregates was analyzed with a Perkin-Elmer model 240C elemental analyzer, using acetanilide as a standard. Ash-free dry weight (AFDW) of aggregates was determined by combusting preweighed filters containing aggregates in a muffle furnace at 450°C for 12 h. Dried samples of the initial and leached macrophyte were also combusted in this manner.

The five species of macrophyte differed in initial carbohydrate, protein, carbon, and nitrogen content (ANOVA, Table 2). Post hoc analyses with Tukey's multiple comparison test showed different groupings of species for each constituent (Table 2). Leached macrophytes also varied significantly among species in carbohydrate, protein, carbon, and nitrogen content (Table 3). The percentage of ash in the initial macrophytes (mean = 18, SD = 5.6) was always less than that of the leached material (mean = 28.5, SD = 8.8), suggesting that ash was not readily released during leaching (Table 4).

Since leached macrophyte samples were derived from the initial macrophyte, these two sets of data were not independent and could not be compared with parametric statistics. A nonparametric test (Wilcoxon's signed-ranks test, Sokal and Rohlf 1981) found no signifi-

Table 4. Percent ash of initial macrophyte, morphous detritus, and aggregate samples derived from five species of macrophyte. All numbers are shown with standard errors. Two replicates were analyzed in all cases except for the aggregate sample derived from *Ulva*, where *n* = 3.

	Initial macrophyte	Morphous detritus	Aggregate
Fucus	21.8±4.0	27.1±2.1	23.2±3.3
Gracilaria	21.6±0.8	41.6±2.9	−1.7±3.6
Zostera	15.1±1.5	31.0±1.1	43.1±11.5
Ulva	22.0±1.9	24.9±1.1	11.1±15.6
Spartina	9.5±0.1	17.8±1.4	38.6±9.2

Table 5. Leached DOM composition. Soluble carbohydrate and protein measured at the end of the leaching period. Data were log-transformed before ANOVAs were performed to meet the assumptions of normality.

Macrophyte source of DOM	Carbo. (μg ml^{-1})	n	g	Protein (μg ml^{-1})	n	g
Fucus	82.5±5.6	2	3	166.5±53.1	2	3
Gracilaria	111.9±7.1	2	3	9.1±2.7	2	2
Zostera	47.6±3.2	2	2	0.5±0.0	2	1
Ulva	282.6±27.6	3	4	4.0±1.0	3	2
Spartina	28.3±0.5	3	1	15.7*	1	
ANOVA results						
df	4,7			3,15		
F	163.2			76.5		
P	<0.001			<0.001		

* n = 1, not included in the ANOVA.

cant differences in carbohydrate, protein, carbon, and nitrogen content between treatments.

The amount of soluble carbohydrate and protein in DOM leached from macrophytes also differed significantly between species (Table 5). The very high concentration of protein in the *Fucus* leachate may be the result of an interaction between phenolics and protein. *Fucus* contains water-soluble polyphenols that are rapidly lost during leaching. These compounds form complexes with proteins and make them less available for microbial use (Tenore and Rice 1980). While proteins released from other macrophytes during leaching were being used by microbes, material released from *Fucus* may have been less available.

The concentration of soluble carbohydrate was not significantly correlated with initial macrophyte carbohydrate or carbon content nor with the change in either of these during leaching. Likewise, the concentration of soluble protein was not correlated with the initial concentration of macrophyte protein or nitrogen nor with the change during leaching. This lack of correlation is probably because if the leachate was used by microbes as it was produced, the soluble material remaining in the jars after 4–7 d of leaching would no longer reflect the composition of the initial macrophyte.

There were no significant differences in the biochemical composition (carbohydrate, protein, lipid, carbon, or nitrogen) of the aggregates formed from the DOM released from the five macrophyte species (1-way ANOVAs, $P > 0.05$, Table 6). Protein, nitrogen, carbohydrate, and carbon contents of aggregates were not correlated with either the amounts present in the initial or leached macrophytes, the soluble material, or with the changes in any of these materials during leaching or bubbling. Therefore, none of these constituents could be used to predict aggregate composition.

These results suggest that aggregate composition is not related, at least in terms of gross biochemical constituents, to that of either the

Table 6. Composition of aggregates produced from the DOM released from five macrophyte species. Data presented as in Table 3, with the addition of percent lipid. Tukey's test for species groupings was not performed on these data, because none of the ANOVAs yielded significant differences between species.

	% Carbo.	n	% Protein	n	% Lipid	n	% C	n	% N	n
Fucus	18.0±5.3	3	18.6±1.6	3	3.6±0.3	3	65.3±20.7	2	10.4±2.6	2
Gracilaria	13.4±1.3	4	26.9±4.3	5	9.0±1.5	5	39.5±5.6	2	9.4±1.1	2
Zostera	7.4±0.3	2	18.5±1.1	3	5.3±0.6	3	20.4±6.4	2	4.4±1.3	2
Ulva	7.9±2.3	2	15.6±3.9	3	10.0±3.4	2	30.6±2.6	2	6.4±0.2	2
Spartina	5.7±1.9	3	11.9±2.5	2	5.0±2.4	3	20.5±5.7	2	2.7±0.1	2
ANOVA results										
df	4,9		4,11		4,10		4,5		3,4	
F	3.3		2.3		2.0		3.2		3.1	
P	n.s.		n.s.		n.s.		n.s.		n.s.	

macrophyte from which the DOM was derived or the DOM itself. The data therefore shed light on the first objective of this study: they suggest that the process of aggregation in this system is not merely an abiotic condensation of all the DOM. Rather, the data are consistent with the interpretation that the process is biological, wherein DOM that varies in composition is transformed by bacteria into aggregates that do not. Moreover, the composition of aggregates was similar to that of bacteria. C : N ratios of marine bacteria range from 3 to 9 (Lee and Fuhrman 1987; Nagata 1986) and those of the aggregates range from 4 to 7.

The second objective of this study was to contrast the composition of morphous detritus (leached macrophytes) with that of amorphous detritus (aggregates). As can be seen very clearly in terms of either C : N or carbohydrate : protein ratios (Fig. 1), the composition of aggregates differed from that of morphous detritus. Both C : N and carbohydrate : protein ratios of morphous detritus samples were similar to those observed in initial macrophytes, whereas those of aggregates were not. Both C : N and carbohydrate : protein ratios of morphous detritus were greater than those of aggregates. Carbohydrate : protein ratios were >1 in morphous detritus because the samples were always higher in carbohydrate than in protein, whereas those of aggregates were <1.

The fact that aggregates have lower C : N values and are higher in protein than morphous detritus might explain why amorphous aggregates are more readily incorporated by consumers than morphous detritus. In marine systems, several species of bivalves (*Mytilus edulis, Geukensia demissa, Argopecten irradians*) incorporated more nitrogen when fed organic aggregates than when fed morphous detritus (Alber 1992), and D'Avanzo et al. (1991) found the same was true for a fish (*Cyprinodon variegatus*). In freshwater, Bowen (1984) showed that amorphous aggregates contained less refractory organic material and were more digestible than morphous particles. The digestive enzyme pancreatin hydrolyzed an average of 1.8% (AFDW) of morphous particles, as compared to 11% of amorphous material. In addition, tadpoles fed aggregates survived longer than either starved controls or those fed morphous material (Bowen 1984; Ahlgren and Bowen 1991).

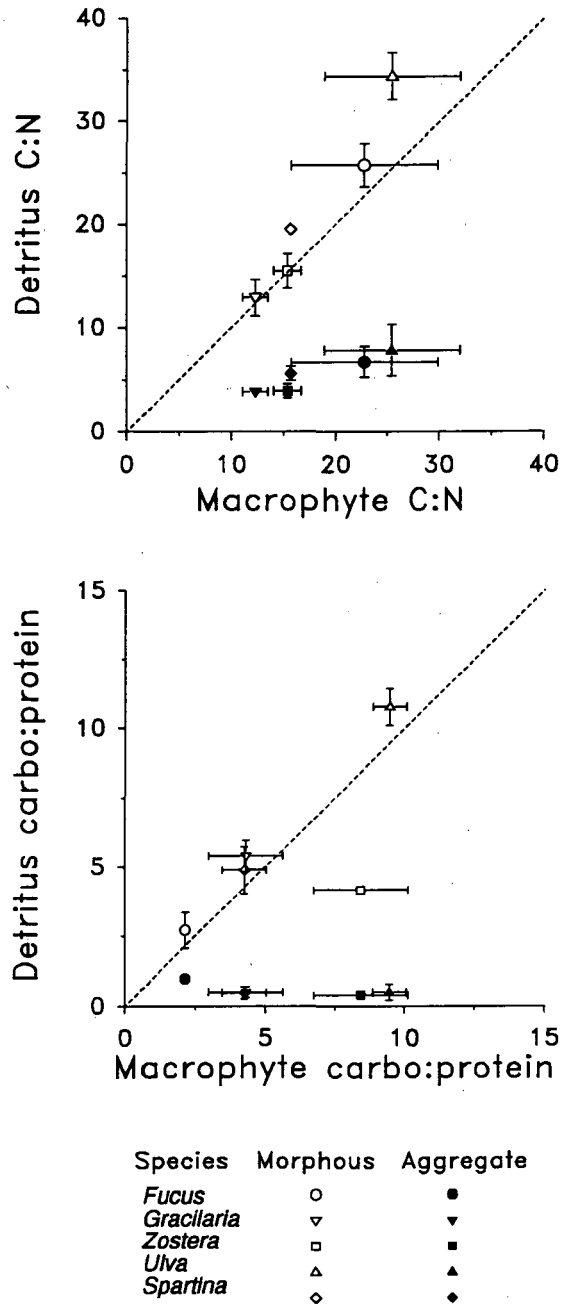

Fig. 1. C : N and carbohydrate : protein ratios of both morphous detritus (open symbols) and aggregates (closed symbols) plotted against those of initial macrophytes. Bars represent standard errors. Dashed lines (1 : 1 line, where $x = y$) are included for reference.

We have taken the composition of leached macrophytes as a representation of morphous detritus, but aggregates were formed after a week of microbial activity, whereas morphous detritus samples were not similarly incubated. It could be argued that upon further decomposition leached material would be colonized by microbes and its composition could change. However, morphous detritus takes much longer than a week to decompose, and there is evidence that a week of bacterial action does not fundamentally change its composition. Nitrogen content of the same five species used in the present study varied between 1 and 2.5% after a week of decomposition (Buchsbaum et al. 1991). These values are in the same range as the nitrogen content reported here for leached macrophytes (Table 3) and much less than those measured in aggregates (Table 6). The point remains that soluble material leached from macrophytes can be quickly converted into aggregates that are potentially higher quality food than morphous detritus.

The final finding of this study is that the composition of the aggregates produced in this system was distinct from reported values for marine snow particles. Reported C : N values of marine snow collected in surface waters generally range between 7 and 10 (compiled by Alldredge and Silver 1988), whereas the aggregates produced here had C : N ratios that ranged from 4 to 7. Marine snow aggregates collected from nearshore waters in California ranged from 47 to 80% ash (Alldredge 1979), whereas the aggregates produced here did not contain inorganic enclosures and ranged from 0 to 43% ash (Table 4). Finally, Alldredge and Cox (1982) reported average carbohydrate : protein ratios of 12 (SE = 5.3, $n = 5$) for marine snow particles formed from discarded appendicularian houses, whereas carbohydrate : protein ratios of the aggregates produced here averaged 0.57 (SE = 0.1, $n = 5$).

Aggregate composition does not vary with species and does not reflect the composition of either the macrophyte or the DOM from which it was derived. Instead, aggregates are similar in quality to bacteria. Their larger size (1–500 μm), however, makes them more accessible to larger consumers that are less efficient at (or incapable of) capturing individual bacteria. Aggregates are likely to be better quality food than either morphous detritus or marine snow. The aggregation pathway of detritus utilization may therefore represent a potentially important route for the flow of carbon and energy from primary producers to consumers.

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