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Abstract

We tested the singular and interactive effects of resource availability (light) and community composition (food chain length and herbivore species richness) on eelgrass (*Zostera marina*) ecosystem properties and functioning with an experimental mesocosm system. Food chain length was manipulated through the presence or absence of blue crab (*Callinectes sapidus*) predators, whereas grazer species richness varied across three levels (zero, two, or four crustacean species). We found important and interacting effects of bottom-up and top-down forcings on sediment organic matter (SOM) composition. Light increased eelgrass and algal biomass and sediment organic carbon and nitrogen content. Increasing grazer diversity generally decreased algal biomass and ecosystem production but interacted with food chain length (i.e., presence of predatory crabs) and light. Predators generally increased algal biomass and ecosystem production through a trophic cascade, which was stronger at high grazer diversity and under ambient light. SOM composition, determined with fatty acid (FA) biomarkers, was sensitive to all manipulated variables. Increasing grazer species richness often decreased the contributions of FAs derived from plant and algal sources, whereas increasing light had the opposite effect. Food chain length was generally a less important determinant of SOM composition than light, although predators did increase FAs representative of heterotrophic bacteria. Overall, resource availability and epibenthic community composition strongly influenced organic matter cycling, SOM composition, and the bacterial community in seagrass-bed sediments.

Coastal ecosystems are often affected by multiple disturbances that alter both resource availability and community composition simultaneously. In the Chesapeake Bay, for example, seagrass beds are affected by commercial harvesting of the blue crab, *Callinectes sapidus* (Stephan et al. 2000), and by suspended sediment and nutrient loading that can lead to reduced light availability (Kemp et al. 2004). Changes in abundance of important predators, such as the striped bass or the blue crab, may precipitate changes in the biomass of lower trophic levels (Hairston et al. 1960; Strong 1992; Pace et al. 1999). These shifting trophic interactions, along with reduced light availability, can affect primary producer abundance and productivity (Heck et al. 2000; Hughes et al. 2004; Borer et al. 2006) and, in turn, sediment organic matter (SOM) content (Canuel et al. 2007). Consequently, cascading changes in animal and plant biomass may alter the rates and pathways by which organic matter (OM) is cycled in an ecosystem (Schindler et al. 1997; Dangles and Malmqvist 2004).

Predicting how changing trophic structure affects OM cycling is complicated by the fact that predators induce shifts not only in prey biomass but also in prey community structure. In seagrass systems, for example, grazing invertebrates can consume epiphytic algae, macroalgae, benthic microalgae, and vascular plants (Valentine and Duffy 2006). Thus, shifts in grazer community composition may affect the abundance of different primary producers. Because seagrasses, macroalgae, and epiphytes differ in their biochemical composition and proportion of structural components, the food preferences of grazing invertebrates may, affect the quantity and lability of organic carbon delivered to the sediments and thus, the quantity and quality of sediment organic carbon (Canuel et al. 2007). Such compositional changes need not be dramatic to affect ecosystem properties: small shifts in grazer richness and species composition can significantly affect plant and algal biomass and influence total sediment organic carbon (e.g., Duffy et al. 2003; Canuel et al. 2007).

Because sediment microbial communities are important mediators of carbon and other elemental cycles in coastal environments (Boschker et al. 1999; Holmer et al. 2001, 2004), changes in aboveground trophic structure and diversity that alter OM delivery to seagrass sediments may have important consequences for carbon cycling and storage. In terrestrial soils, by analogy, microbial community composition and activity are sensitive to changes in aboveground community structure (Setälä et al. 1998; Wardle et al. 2005). Although there are fewer studies from

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marine habitats, microbial activity in sediments is strongly related to OM deposition (Canuel and Martens 1993; Boschker and Cappenberg 1998; Boschker et al. 2000). The potential cascade from consumer control of aboveground production to delivery and accumulation of belowground OM may thus be important to carbon remineralization, recycling, and sequestration in the sediments.

Effective conservation and management of seagrass ecosystems requires a clear understanding of relationships between community ecology and biogeochemical cycling. A variety of studies have investigated coastal eutrophication (Cloern 2001; Duarte 2002 and references therein), trophic interactions in seagrass beds (Heck and Valentine 2006 and references therein; Valentine and Duffy 2006), and interactions between nutrient enrichment and food-web ecology (McClelland and Valiela 1998; Deegan et al. 2002; Tewfik et al. 2005). Others have examined sediment nutrient and bacterial processes in seagrass beds (Holmer et al. 2001, 2004). Yet few studies have examined the relationships and feedbacks between aboveground ecology and belowground geochemical cycling. Geochemical tools provide a way to detect and quantify such linkages between community structure and OM cycling. Specifically, lipid biomarkers are compounds reliably produced by a specific group of organisms that are sufficiently resistant to degradation to be preserved in sediments (Killops and Killops 1993). Diagnostic biomarkers often have site-specific methyl groups, double bonds, or cyclic side chains useful in tracing the sources of OM (Killops and Killops 1993). Bacteria, for example, synthesize iso- and anteiso-branched fatty acids (BrFAs), while microalgae contain highly unsaturated long-chain fatty acids (or alkanoic acids) (Volkman et al. 1998). One class of lipids, the FAs, is particularly useful, because they have high source fidelity and exhibit a range of chemical reactivity (Canuel et al. 1995; Canuel and Martens 1996). Additionally, a subclass of the FAs, the phospholipid-linked fatty acids (PLFAs), are good indicators of recently viable cells because they are mainly derived from membrane lipids, which are rapidly hydrolyzed after cell death (White et al. 1979; Killops and Killops 1993). By quantifying both the total FAs and the PLFAs, it is possible to compare OM contributions from detrital and viable or recently viable sources. Thus, lipid biomarkers, and FAs, in particular, provide a quantifiable link between the aboveground community and sediment geochemistry.

To assess the effects of changing community structure on carbon fate and storage in seagrass beds, we conducted an experimental manipulation of bottom-up forcing (light availability), community composition (grazer diversity), and food chain length (predator presence) and measured their interacting effects on ecosystem productivity, SOM quality, and sediment microbial activity. Specifically, we built on previous studies examining top-down effects on the aboveground community (Duffy et al. 2003) and on SOM (Canuel et al. 2007) to test several hypotheses. First, higher diversity of epibenthic grazers will reduce algal biomass but increase seagrass and benthic algal biomass, leading to changes in the composition and quality of algal material incorporated in sediments. Second, predators will increase accumulation of algal biomass through a trophic cascade, thereby increasing SOM quantity, quality, and sediment microbial activity. Finally, high light availability will increase biomass accumulation of aboveground and benthic algae, SOM lability, and sediment microbial activity.

Methods

Experimental design—We conducted a mesocosm experiment to examine the main and interactive effects of grazer species richness, food chain length, and light intensity on ecosystem properties, including production, algal biomass accumulation, and SOM content and composition. We established three grazer richness treatments containing no grazer species, random combinations of two grazer species, or four grazer species. Grazers were chosen from a pool of six species, including three amphipod crustacean species (*Ampithoe longimana*, *Gammarus mucronatus*, and *Caprella penantis*), two isopods (*Idotea baltica* and *Erichsonella attenuata*), and a gastropod (*Bittium varium*). These invertebrate grazers are common in the York River estuary during the spring and summer (Duffy et al. 2001, 2003). Food chain length was manipulated by exposing a parallel set of grazer treatments to a generalist predator common in the Chesapeake Bay, the blue crab, *C. sapidus*. Light intensity was manipulated by covering half of the tanks with shade cloths (69% attenuation). There were a total of 12 treatments, each replicated five times. Because of extinctions and contaminations, however, six replicates were removed from the final analyses. Consequently, 54 replicates were used in statistical analyses; the zero-grazer treatments had five replicates in ambient light and four replicates in low light, the two grazer treatments had four replicates, and the four grazer treatments had five replicates.

Outdoor mesocosm experiments were conducted for 6 weeks during the summer of 2003 in an array of 113-liter, translucent fiberglass tanks that were continuously supplied with flowing estuarine water from the York River, Virginia (Duffy et al. 2003). Water passed first through a sand filter and then through a 150-μm mesh. This eliminated larger invertebrates and minimized invasion by nontarget animals while permitting passage of invertebrate larvae and algal spores, which often colonized the tanks. Water was supplied through ''dump buckets,'' which regularly spilled the filtered water into the tanks, providing both turbulence and aeration. Tanks were stocked with clean sand to a depth of 10 cm; the percent total organic carbon (%TOC) was below detection. Low OM content sand was used as a substrate to reduce initial heterogeneity between the tanks and to increase our ability to detect newly deposited SOM (Canuel et al. 2007). Seventy-five preweighed eelgrass (*Zostera marina*) shoots, cleaned of grazers and epiphytes, were planted in the sand in each tank. This eelgrass density is within the range found in the York River estuary system (Orth and Moore 1986). One week after the grass was planted, invertebrate grazers were added to each grazer mesocosm (45 each for two-species treatments, 15 of each for four-species treatments); these densities were near the low end of those found in the York

River. Blue crabs (*C. sapidus*) were added 2 d after the grazers had acclimated. The 6-week experimental incubation time was chosen to minimize the risks of invasion by nontarget grazer species and of complete consumption of the eelgrass, which increase at longer time intervals. This time period permits major changes in animal (one to two grazer generations) and plant community development and in surface sediment characteristics (*see* Duffy et al. 2003, 2005; Canuel et al. 2007). Despite limitations, this experimental infrastructure simulates several aspects of the biotic and abiotic field situation well (Duffy et al. 2001). Results of the experiment for aboveground biomass and composition of seagrass and the associated community are reported elsewhere (Duffy et al. unpubl. data). Here, we focus on patterns of SOM accumulation and composition.

Gross ecosystem production—As an estimate of whole-ecosystem metabolism, we measured gross ecosystem production (GEP; mmol L^{-1} O_2 d^{-1} m^{-2}) 1 week before the experiment was terminated. Because of time constraints and instrument availability, these measurements were conducted only in ambient light treatments. Clear plastic wrap was placed on the water's surface of each tank to minimize oxygen exchange with the atmosphere, and the water supply was shut off. Dissolved oxygen (DO) measurements were taken three to four times during each of two 4-h incubations (10:00–14:00 h and 22:00–02:00 h) with a YSI Data Sonde to capture net daytime production and total respiration, respectively (assuming that little-to-no production occurs at night). Tank water was stirred prior to each reading to disrupt any temperature or DO stratification that may have formed while maintaining a closed system. If DO fell to hypoxic levels (2 mg L^{-1}), measurements ceased on that tank, and the plastic cover was removed. We calculated the slope of changes in DO concentration versus the time elapsed and divided this by the area of the tank to obtain flux in O_2 mmol L^{-1} d^{-1} m^{-2}. Hourly light and dark rates were scaled to 14 h of daylight and 10 h of darkness to estimate net daily summertime GEP.

Bulk SOM—At the end of the experiment, three sediment cores (2.6 cm in diameter) were collected from each mesocosm, and the upper 1 cm from each core was removed. Subsamples from each core were combined into a composite sample in a precombusted (450°C) jar. The sediment sample was homogenized, and aliquots were removed to precombusted glass scintillation vials for analyses of benthic chlorophyll a (Chl *a*; a measure of microalgal biomass) and sediment TOC and total nitrogen (TN). All samples were stored at −20°C until analysis. Samples of benthic Chl *a* were analyzed within 6 weeks of collection according to Neubauer et al. (2000). Concentrations of TOC and TN were analyzed by standard methods with a Fisons CHN analyzer (model EA1108) after removing inorganic carbon (Hedges and Stern 1984); acetanilide was used as the standard.

Lipid biomarker analyses—Lipid biomarker compounds were analyzed by a modified Bligh and Dyer (1959) method (Canuel and Martens 1993; Canuel et al. 2007). Briefly, sediment samples were extracted with methylene chloride : methanol (2 : 1, v : v) with an accelerated solvent extraction system (Dionex ASE 200). Following extraction, the samples were partitioned, and the organic phase was removed. Hexane was added to the aqueous phase, and the samples were partitioned a second time, after which the hexane layer was added to the original organic phase. The combined organic phases sat over anhydrous Na_2SO_4 overnight to remove traces of water and were concentrated to 1 mL (Zymark Turbo Vap 500). The total lipid extracts were separated into nonpolar (F1/2) and polar (F3) fractions by eluting solvents of increasing polarity through silica gel columns (Guckert et al. 1985). F1/2 (neutral and glycolipids) and F3 (phospholipids) were each saponified by procedures described in Canuel et al. (2007). Following saponification, the residue was extracted under basic (saponified-neutral; SAP-N) and acidic pH (saponified-acids; SAP-A). The SAP-A fractions were methylated with BF_3-CH_3OH and purified by silica gel chromatography. Just before gas chromatographic (GC) injection, samples were evaporated to dryness under N_2, and a small volume of hexane (30 μL for the polar fraction and 100 μL for the nonpolar) was added. The FAs (as methyl esters) were analyzed by gas chromatography following previously published procedures (Canuel et al. 2007 and references therein). Peaks were quantified relative to an internal standard, methyl heneicosanoate, added just prior to GC analysis. Peak identities were verified by reference standards and by combined gas chromatography–mass spectrometry (GC-MS) with a Hewlett-Packard 6890 GC interfaced with a mass selective detector operated in electron impact mode. FAs are designated A:BωC, where A is the total number of carbon atoms, B is the number of double bonds, and C is the position of the first double bond from the aliphatic "ω" end of the molecule. The prefixes "i" and "a" refer to *iso* and *anteiso* methyl BrFAs (*see* Canuel et al. 1995 and references therein). Results for two classes of FAs are presented: PLFAs, which represent viable or recently viable biomass, and total FAs, which represent neutral, glycolipids, and phospholipids and include the sum of the viable and detrital contributions.

Statistical analyses—The experiment was analyzed as a fully factorial three-way analysis of variance, with grazer treatment (df = 2), food chain length (i.e., predator presence or absence, df = 1), and light availability (df = 1) as fixed variables, by SAS version 9.0 for Windows. Analyses of FA data were conducted on percent abundance. GEP data were subjected to a two-way analysis of variance, because data were only available for ambient light treatments. From the analyses of variance, we calculated the magnitude of main and interactive effects (ω^2, percentage of the variance explained). Because of contaminations and extinctions, two control and four 2-species mesocosms were removed from all statistical analyses; results presented here use the type III sum of squares (SS) from the analysis of variance model. Included in the statistical analyses were five replicates in ambient light and four in low light of the zero-grazer treatments, four

Table 1. Tests of significance, estimated magnitudes of effect (ω^2), and contrasts of grazer richness versus presence for light availability, food chain length, grazer species richness, and their interactions on ecosystem production, plant biomass, sediment total nitrogen, sediment organic carbon, and sediment fatty acid abundance. When an interaction between grazers and light or predators was significant, the data set was divided according to the interaction (i.e., low light vs. ambient light or no predators vs. predators), and an analysis of variance was performed again. AFDW, ash-free dry weight. For interactive effects: G, grazers; C, crab predators; L, light. For contrast analyses, P indicates where grazer presence affected the response variable, and R refers to a richness effect. Significant p values are in bold.

	Light			Crab predators			Grazers			Interactions				Error		
Response	MS	p	ω^2	MS	p	ω^2	MS	p	ω^2		MS	p	ω^2	MS	ω^2	Contrast
Gross ecosystem production	NA			23.38	**<0.001**	0.29	5.42	**0.014**	0.12	GxC	8.33	**0.002**	0.19	1.03	0.40	PR
No predators							13.26	**0.001**	0.39					0.59	0.61	PR
Predators							0.49	0.661	0.00					1.14	1.03	
Z. marina (AFDW)	80.95	**0.027**	0.06	28.50	0.180	0.01	37.11	0.101	0.04	GxC	61.94	**0.025**	0.09	15.31	0.61	P
No predators	78.71	**0.005**	0.17				67.22	**0.002**	0.28					7.87	0.57	PR
Predators	14.84	0.428	0.00				31.82	0.269	0.03					22.75	1.04	
Total algae (log AFDW + 0.001)	6.79	**0.005**	0.04	35.46	**<0.001**	0.25	16.69	**<0.001**	0.23	GxC	12.52	**<0.001**		0.76	0.33	PR
No predators	0.65	0.473	0.00				56.83	**<0.001**	0.77					1.22	0.25	PR
Predators	1.46	**0.019**	0.15				0.35	0.230	0.03					0.23	0.87	
Benthic Chl *a* ($\mu g\ cm^{-2}$)	352.73	**0.023**	0.06	100.12	0.217	0.00	408.49	**0.004**	0.01					63.60	0.78	P
%TOC	0.06	**0.004**	0.12	0.01	0.669	0.00	0.01	0.398	0.00					0.01	0.90	
%TN	0.01	**<0.001**	0.18	0.00	0.955	0.00	0.00	0.294	0.00					0.00	0.89	
Total fatty acids (TFA)*																
TFA : OC ($\mu g\ mg^{-1}_{TOC}$)	193.25	**0.016**	0.08	1.18	0.846	0.00	32.46	0.359	0.00					30.87	0.89	
%SCFA (C_{12}+C_{14}) of TFA	95.18	**<0.001**	0.30	20.06	**0.014**	0.06	15.70	**0.010**	0.08					3.02	0.59	P
%$C_{16:0}$ of TFA	3.43	0.851	0.00	61.69	0.427	0.00	206.40	0.129	0.04					95.84	1.00	
%$C_{18:0}$ of TFA	0.44	0.826	0.00	9.25	0.319	0.00	1.01	0.895	0.00							
%C_{24} of TFA	13.64	**0.003**	0.11	0.00	0.983	0.00	1.85	0.277	0.01	GxL	6.98	**0.011**	0.10	1.40	0.72	
										GxC	6.58	**0.014**	0.09			
Low light				0.15	0.636	0.00	0.88	0.290	0.02	GxC	2.37	**0.047**	0.15	0.67	0.86	
Ambient light				0.14	0.799	0.00	8.48	**0.030**	0.16					2.06	0.76	P
No predators	4.97	**0.045**	0.09				0.75	0.516	0.00					1.09	0.80	
Predators	8.95	**0.032**	0.10				7.67	**0.023**	0.16					1.70	0.67	P
%PUFA of TFA	0.20	0.927	0.00	104.82	**0.042**	0.05	9.18	0.682	0.00	GxL	100.43	**0.021**	0.10	23.77	0.89	
										LxC	100.01	**0.047**	0.05			
Low light				197.48	**0.002**	0.27	23.42	0.259	0.02					16.21	0.73	
Ambient light				0.03	0.976	0.00	91.26	0.072	0.13					30.63	0.97	P
%($C_{18:2}$+$C_{18:3}$) of TFA	44.80	0.061	0.04	4.75	0.534	0.00	43.86	**0.035**	0.07	GxL	67.44	**0.007**	0.12	12.10	0.77	P
Low light				5.33	0.398	0.00	2.34	0.725	0.00					7.16	1.09	
Ambient light				30.67	0.188	0.02	116.77	**0.004**	0.28					16.59	0.69	P
%BrFA (iso-, anteiso-C_{19}) of TFA	47.83	**0.023**	0.06	57.81	**0.013**	0.07	46.15	**0.008**	0.11	GxL	47.37	**0.007**	0.11	8.57	0.72	R
Low light				23.75	**0.005**	0.24	3.86	0.215	0.03					2.32	0.78	
Ambient light				34.81	0.132	0.04	89.57	**0.007**	0.26					14.25	0.74	R
Phospholipid-linked fatty acids (PLFA)*																
PLFA : OC ($\mu g\ mg^{-1}_{TOC}$)	0.35	0.399	0.00	0.39	0.375	0.00	0.45	0.407	0.00					0.49	1.04	
%SCFA (C_{12}+C_{14}) of PLFA	1.39	0.587	0.00	7.11	0.223	0.01	10.19	0.125	0.03	GxCxL	21.20	**0.016**	0.10	4.66	0.85	
%$C_{16:0}$ of PLFA	38.88	0.536	0.00	36.72	0.548	0.00	246.27	0.097	0.04	GxCxL	469.76	**0.014**	0.10	99.90	0.84	
%$C_{18:0}$ of PLFA	148.45	0.188	0.01	162.97	0.168	0.02	105.10	0.291	0.01					82.74	1.00	
%$C_{24:0}$ of PLFA	9.98	**<0.001**	0.17	0.44	0.425	0.00	0.46	0.516	0.00					0.68	0.76	
%PUFA of PLFA	19.39	0.221	0.01	37.30	0.092	0.03	56.42	**0.017**	0.10					12.57	0.83	P

Table 1. Continued.

Response	Light			Crab predators			Grazers			Interactions			Error		
	MS	*p*	ω^2	MS	*p*	ω^2	MS	*p*	ω^2		MS	*p*	ω^2	MS	ω^2	Contrast
%($C_{18:2}$+$C_{18:3}$) of PLFA	587.83	**0.005**	0.08	223.76	0.074	0.02	377.71	**0.007**	0.09	LxC	303.22	**0.039**	0.03	66.52	0.57	P
										GxL	499.44	**0.002**	0.12			
										GxCxL	253.48	**0.030**	0.05			
Low light				2.90	0.319	0.00	4.34	0.235	0.03	GxC	10.14	**0.045**	0.15	2.79	0.82	
Ambient light				544.12	**0.048**	0.07	940.86	**0.003**	0.25	GxC	463.56	**0.04**	0.11	124.46	0.58	P
%BrFA (iso-, anteiso-C_{13}–C_{19}) of PLFA	60.24	**0.006**	0.08	1.68	0.629	0.00	60.19	**<0.001**	0.15	GxL	51.29	**0.002**	0.12	66.52	0.60	R
										GxCxL	27.38	**0.029**	0.06			
Low light				5.29	0.394	0.00	18.22	0.098	0.10					6.97	0.88	P
Ambient light				18.09	0.126	0.03	91.99	**<0.001**	0.40					7.15	0.51	R

* Biomarker response variables are expressed either as percentage of total fatty acids or as percentage of phospholipid-linked fatty acids. Data were analyzed by three-factor model III analysis of variance. $p < 0.05$ is in bold. P and R indicate whether grazer presence or richness effects, respectively, were significant, as determined through contrast analysis.

replicates of the two grazer treatments, and five replicates of the four grazer treatments. There were two criteria for elimination: (1) grazer contamination totaled more than 500 mg of ash-free dry matter and (2) failure of two grazer species (*Caprella* and *Bittium*) to establish necessitated elimination of mesocosms where this pair of species was initially stocked. To separate effects of grazer presence versus grazer species richness, we conducted a priori contrasts that partitioned the grazer SS from the analysis of variance into two orthogonal components (*see* Duffy et al. 2005). The first contrast compared the two- and four-species treatments against the zero-species treatment (species presence contrast), and the second compared the two- versus four-species treatments (species richness contrast).

To aid in interpreting the FA data, we performed multiple regression analyses modeling the FA groups as a function of biomass of the major primary producers, eelgrass, total algae, and benthic Chl *a*. The partial r^2 was calculated by dividing the type III SS for each response variable by the total SS. The analyses were performed on %TOC, individual FAs, and groups of FAs normalized to the sum of all FAs (%total FA or %PLFA). Additionally, we conducted principal components analysis (PCA; by Minitab 14) to better elucidate relationships between manipulated and response variables. We only performed PCA on SOM variables, as these responded to primary producer abundance determined by grazers and crab predators. PCA loadings describe the relationships between the SOM response variables and the dominant principal components. PCA scores illustrate relationships between the observations and the dominant principal components. PCA loadings were also regressed against the major primary producer groups (*Z. marina*, total algal biomass, and benthic Chl *a*) to help interpret the nondimensional results.

Results

Primary producer biomass and GEP—In general, primary producer biomass was enhanced by light and predator presence and decreased by grazers. Aboveground, light increased the biomass of both *Z. marina* and algae (Table 1; Fig. 1A,B). Cascading predator effects resulted in grazers reducing primary producer biomass only in the absence of predators (grazer × predator interaction, Table 1). For example, grazer presence and richness decreased *Z. marina* biomass in the absence of predators ($p = 0.002$, $\omega^2 = 0.28$). Further, total algal biomass was reduced by grazer presence and richness but increased when predators were present. In the sediments, benthic Chl *a* was increased by ambient light ($p = 0.023$, $\omega^2 = 0.06$, Fig. 1C), decreased by grazer presence ($p = 0.004$, $\omega^2 = 0.01$), and unaffected by crab predators.

GEP in the ambient light mesocosms was influenced by the interaction of predators and grazers ($p = 0.002$, $\omega^2 = 0.19$, Table 1; Fig. 2). Overall, blue crab predators increased GEP ($p < 0.001$, $\omega^2 = 0.29$) but only in the presence of grazers, reflecting a trophic cascade. Increasing grazer species richness reduced GEP but only in the

absence of predators ($p = 0.001$, $\omega^2 = 0.39$). Thus, grazer presence, richness, and predator presence are all important, interacting determinants of GEP (Table 1).

Bulk SOM—During the 6-week experiment, measurable levels of TOC and TN accumulated in surface sediments (Table 1; Fig. 3). Sediment %TOC and %TN content were higher in ambient light than in shaded treatments ($p = 0.004$, $\omega^2 = 0.12$ and $p < 0.001$, $\omega^2 = 0.18$, respectively). Neither grazers nor predators significantly affected %TOC or %TN. Thus, bottom-up forcing had a stronger effect on TOC and TN accumulation than top-down processes.

Total FAs—While bulk indicators of SOM were sensitive only to light availability, FA composition was strongly influenced by both bottom-up and top-down forcing. On average, total FA abundance normalized to TOC ($\mu g\ mg^{-1}$) was significantly reduced by light but was unaffected by predators and grazers (Fig. 4A). For further analysis, both the total FAs and PLFAs were categorized into subclasses on the basis of chain length, number of double bonds, and carbon branching patterns representing different sources of OM (Fig. 5).

Total FA composition was dominated (29–47% total FA) by even-numbered saturated compounds ($C_{12:0}$–$C_{18:0}$), representing algal and bacterial sources. The relative abundance of short-chain FAs (SCFAs; $\%(C_{12:0} + C_{14:0})$) was highest in ambient light in the presence of predators (Table 1; Fig. 5A). Grazer presence, however, decreased SCFAs. SCFAs were also positively related to benthic Chl *a* (Table 2). The contributions of $\%C_{16:0}$ and $\%C_{18:0}$ FAs were unaffected by any of the treatments and were unrelated to either eelgrass or benthic Chl *a* abundance (Table 1). The long-chain FA $C_{24:0}$, composing 3–16% of the total FAs, was increased by ambient light on average (Table 1; Fig. 5B) and decreased by grazers but more so in ambient light and predator treatments. $C_{24:0}$ was also positively related to benthic Chl *a* (Table 2). Overall, light increased FAs that were positively associated with benthic microalgae (Chl *a*), whereas grazers, the dominant top-down control, generally had the opposite effect.

Relative abundance of polyunsaturated FAs ($C_{18:4}$, $C_{20:4}$, $C_{20:5}$, $C_{22:5}$, and $C_{22:6}$; grouped as polyunsaturated FAs [PUFAs]) was reduced by predators but only in shaded treatments, reflecting an interaction between predators and light (Table 1; Fig. 5C). %PUFA abundance was not related to either eelgrass biomass or benthic Chl *a* (Table 2). Linoleic ($C_{18:2\omega6}$) and linolenic ($C_{18:3\omega3}$) acids were decreased when grazers were present ($p = 0.035$, $\omega^2 = 0.07$; Table 1; Fig. 5D) but only in ambient light treatments (grazer × light interaction, $p = 0.007$, $\omega^2 = 0.12$). *Z. marina* was positively related to $\%(C_{18:2\omega6} + \%C_{18:3\omega3})$ (Table 2). Overall, top-down controls were important determinants of PUFA abundance, with predators decreasing %PUFA and grazers decreasing linoleic and linolenic acids.

BrFAs (iso- and anteiso-$C_{13:0}$, $C_{15:0}$, $C_{17:0}$, and $C_{19:0}$), representative of sediment heterotrophic bacteria, were sensitive to all three manipulated variables (Fig. 5E). Light

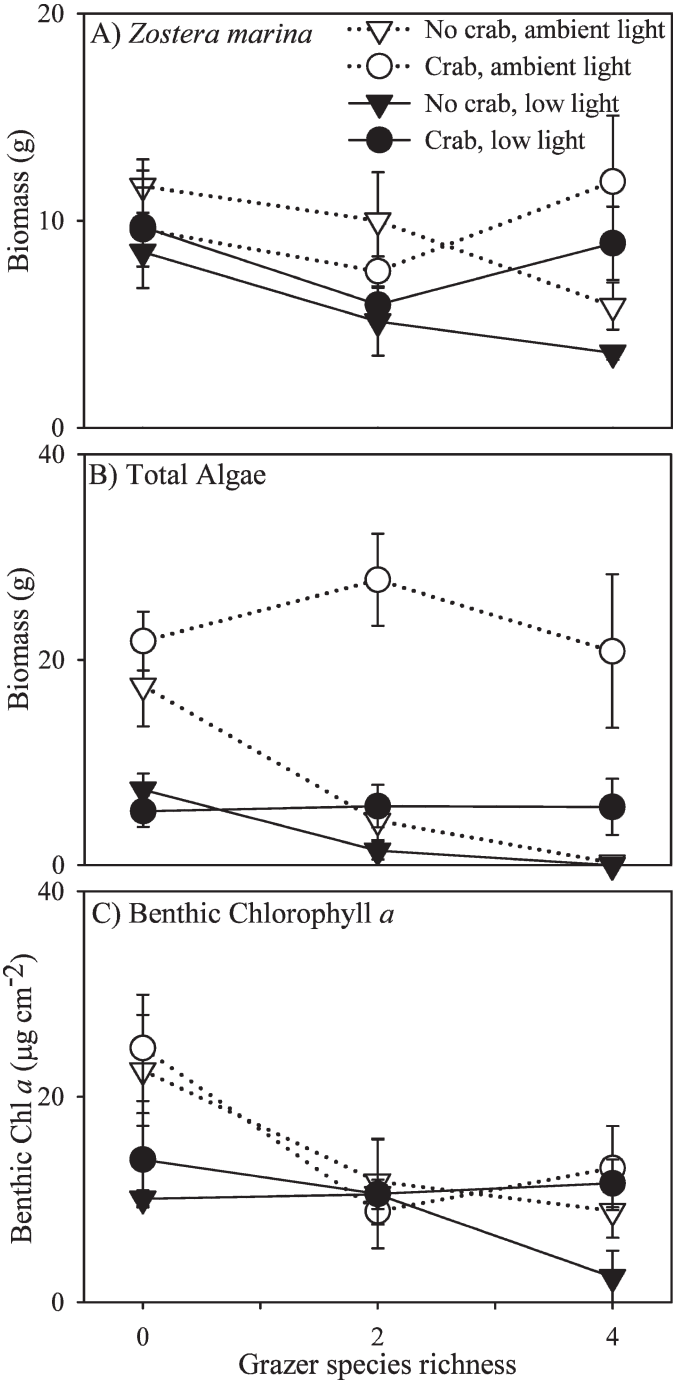

Fig. 1. Effects of grazers, predators, and light availability on aboveground primary producers (*Z. marina* and total algae) and benthic Chl *a*. Light, generally, increased primary producer biomass. Grazers decreased *Z. marina* biomass (in the absence of predators) and total algal biomass. Predators decreased both *Z. marina* and total algal biomass, but the magnitude of this effect varied with grazer richness, resulting in grazer × predator interactive effects. Error bars represent standard error. There were four replicates of each zero-grazer treatment in low light and five in ambient light: four replicates of each two grazer treatment and five replicates of each four grazer treatment. Statistical results are reported in Table 1.

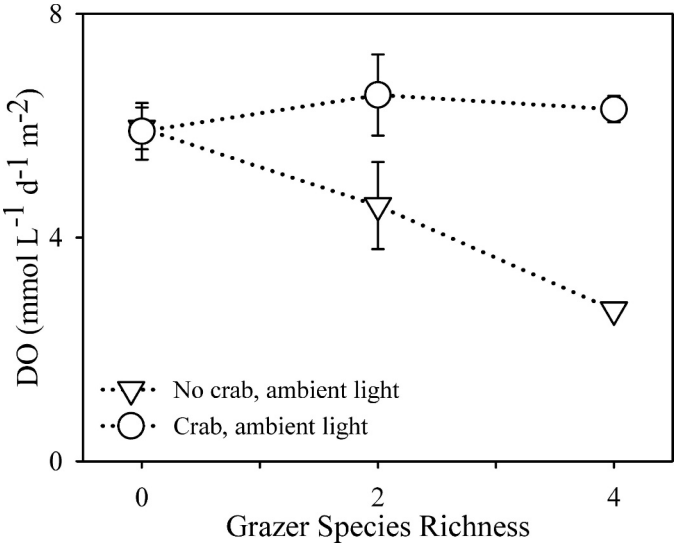

Fig. 2. Effects of grazers and predatory crabs on summer gross ecosystem production, measured as dissolved oxygen (DO) flux. Predators (crabs) mediated a negative grazer effect on gross ecosystem production through a trophic mechanism. The magnitude of the predator effect increases with grazer richness. Data are only from ambient light treatments. Error bars represent standard error. Statistical results are reported in Table 1.

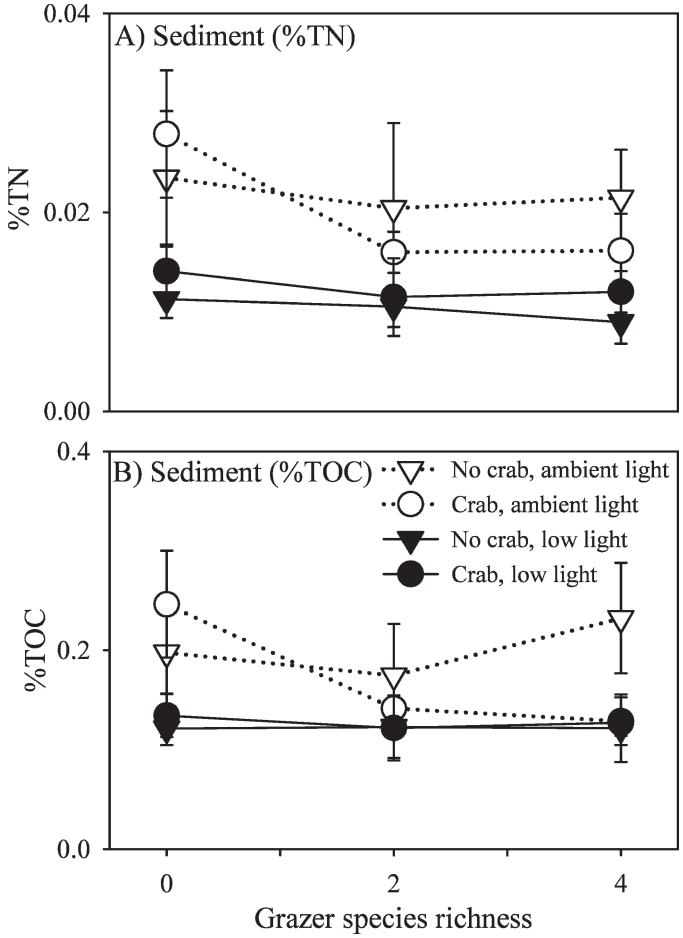

Fig. 3. Effects of light, grazers, and predators on sediment nitrogen and carbon. (A) Light increased sediment total nitrogen (%TN) and (B) total organic carbon content (%TOC). Neither grazer richness nor food chain length affected %TN or %TOC. Error bars represent standard error. Statistical results are reported in Table 1.

generally decreased the relative abundance of BrFAs, though this effect was driven mainly by the two-grazer species treatment and translated into a grazer × light interaction effect (Table 1). Relative abundance of BrFAs was consistently higher in predator treatments ($p = 0.013$, $\omega^2 = 0.07$). BrFAs were positively related to benthic Chl *a* (Table 2). These results suggest that sediment heterotrophic bacteria are sensitive to both bottom-up and top-down controls.

PCA provided a summary of these changes in SOM with manipulation of light and epibenthic community composition. Principal components 1 (PC1) and 2 (PC2) explained 31.7% and 25.9% of the variance in total FA composition, respectively (Fig. 6A,B). %TOC, $\%C_{24:0}$, and $\%(C_{12:0} + C_{14:0})$ had the most positive loadings on PC1 (Table 3) and also responded positively to ambient light (Figs. 3, 5). The association between PC1 and light is also supported by the positive relationship between benthic Chl *a* and PC1 loadings ($r^2 = 0.33$; $p < 0.001$). In contrast, PC2 separated SOM variables according to crab predator or grazer effects. Variables with negative PC2 loadings (%PUFA and %BrFA) were affected by crab predators, albeit in opposite directions, whereas those with positive PC2 loadings ($\%(C_{12:0} + C_{14:0})$, $\%(C_{18:2} + C_{18:3})$, and $\%C_{24:0}$ (ambient light only)) were decreased by grazers (Table 3). Bottom-up forcing interacted with top-down forcing of SOM composition, as PC scores were influenced by crab predators and grazers differently, depending on light availability (Fig. 6A,B). In ambient light (Fig. 6A), grazer-free treatments had positive PC1 and PC2 scores, whereas the two- and four-grazer treatments were near zero or negative on PC2. In contrast, under low light (Fig. 6B), treatments with crabs had more positive PC2 scores, whereas no-crab treatments were negative. Under both light regimes, the pattern is most evident for the zero- and four-grazer treatments (Fig. 6A,B). Thus, PCA results suggested that the dominant top-down control (grazers vs. crab predators) influenced total FA composition differently with light availability.

Phospholipid-linked FAs—Like total FAs, PLFAs (μg PLFA mg^{-1} TOC; Fig. 4B), indicative of viable or recently viable OM sources, were sensitive to top-down and bottom-up influences. None of the manipulated treatments affected total PLFAs, $\%(C_{12:0} + C_{14:0})$, $\%C_{16:0}$, or $\%C_{18:0}$ PLFAs (Table 1). The relative abundance of $C_{24:0}$ PLFAs and linoleic and linolenic PLFAs ($\%C_{18:2\omega6}$ and $\%C_{18:3\omega3}$) was higher under ambient light but only in the absence of grazers, which reduced linoleic and linolenic acid contributions (Fig. 5G,I). Predators increased linoleic and linolenic ($C_{18:2\omega6}$ and $C_{18:3\omega3}$) PLFAs only under ambient light; this translated into a predator × light interaction (Table 1). BrFAs were lower in ambient light treatments ($p = 0.006$, $\omega^2 = 0.08$). In addition to main effects, there were a variety

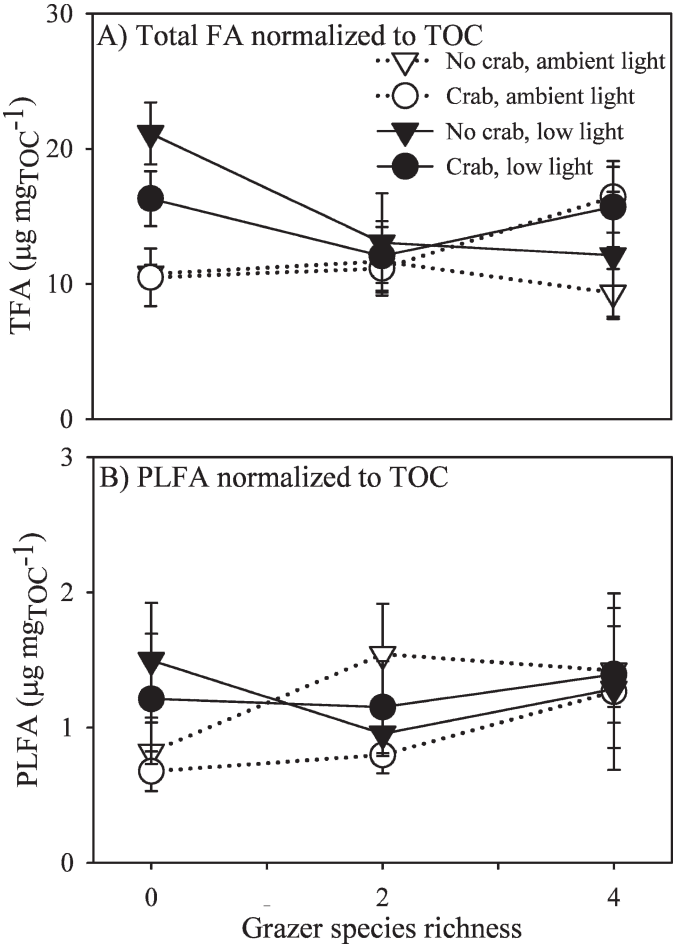

Fig. 4. (A) Abundance of total fatty acids (total FAs) and (B) phospholipid-linked fatty acids (PLFAs) normalized to total sediment organic carbon content (μg mg$_{TOC}^{-1}$). Light decreased total FA (μg mg$_{TOC}^{-1}$ (A)) but had no effect on PLFA (μg mg$_{TOC}^{-1}$ (B)). Error bars represent standard error. Statistical results are reported in Table 1.

of interactive effects on PLFA composition and abundance (Table 1). Overall, the PLFA results echo those for total FAs, showing that community structure and light availability alter SOM deposition and probably sediment microbial response.

PC1 and PC2 explained 25.3% and 19.5% of the variance, respectively, in PLFA composition (Fig. 6C,D). Similar to the results for total FAs, PC1 separated PLFA variables according to light availability. %TOC, %$C_{24:0}$, which were increased by light (Figs. 3, 5) %($C_{18:2}$ + $C_{18:3}$), which was increased by light and correlated with benthic Chl *a* (Fig. 5; Table 2), had more positive PC1 loadings (Table 3). PC1 was also positively related to total algal biomass, which increased in ambient light ($r^2 = 0.10$; $p = 0.018$). In ambient light, PC2 separated response treatments by grazer presence (near zero) and absence (more negative) (Fig. 6C). The association of PC2 with grazers is supported by the negative relationship between PC2 and benthic Chl *a* ($r^2 = 0.10$; $p = 0.017$). In shaded treatments, neither PC1 nor PC2 clearly separated grazer and crab treatments (Fig. 6D).

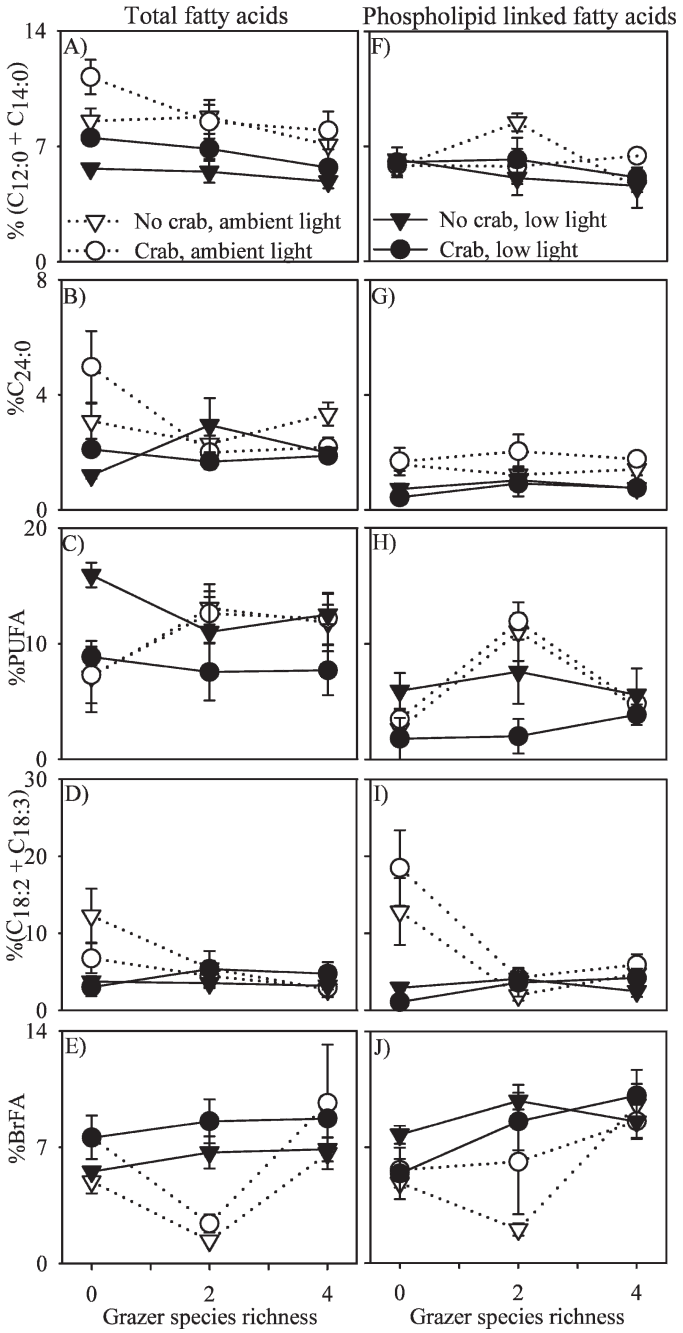

Fig. 5. (A–J) Effects of light, grazers, and predators on total fatty acids (total FAs) and phospholipid-linked fatty acids (PLFAs) subclasses. Light, predators, and grazers had strong singular and interactive effects on total FAs and PLFAs. The polyunsaturated fatty acid (%PUFA) subclass, representing fresh algal material, is composed of $C_{18:4}$, $C_{20:4}$, $C_{20:5}$, $C_{22:5}$, and $C_{22:6}$. The branched fatty acid (%BrFA) subclass, representing heterotrophic bacteria, includes iso- and anteiso-$C_{13:0}$, $C_{15:0}$, $C_{17:0}$, and $C_{19:0}$. Error bars represent standard error. *See text for biomarker sources and Table 1 for statistical results.*

Discussion

A realistic assessment of ecosystem functioning under changing conditions requires simultaneous consideration of

Table 2. Regression analyses of *Z. marina* biomass (ash-free dry weight, g) and benthic Chl *a* (μg cm^{-2}) against the major fatty acid groups. Significant relationships ($p < 0.05$) are noted in bold. TFA, total fatty acids; PLFA, phospholipid-linked fatty acids; i,a, iso-, anteiso-.

Response	*Z. marina*			Benthic Chl *a*			Total
	Coefficient	Partial r^2*	p	Coefficient	Partial r^2*	p	Model r^2
TFA							
%SCFA ($C_{12:0}$+$C_{14:0}$) of TFA	0.09	0.03	0.171	0.12	0.22	**<0.001**	0.25
%$C_{24:0}$ of TFA	0.00	0.00	0.968	0.07	0.22	**<0.001**	0.22
%PUFA of TFA	−0.13	0.01	0.428	0.03	0.00	0.718	—
%($C_{18:2}$+$C_{18:3}$) of TFA	0.26	0.08	**0.041**	0.05	0.01	0.439	0.09
%BrFA (i,a C_{13}–C_{19}) of TFA	−0.20	0.06	0.066	0.11	0.08	**0.034**	0.14
PLFA							
%SCFA ($C_{12:0}$+$C_{14:0}$) of PLFA	0.06	0.02	0.285	0.00	0.00	0.897	—
%$C_{24:0}$ of PLFA	0.05	0.07	0.052	0.00	0.00	0.813	—
%PUFA of PLFA	−0.07	0.01	0.588	−0.05	0.01	0.449	—
%($C_{18:2}$+$C_{18:3}$) of PLFA	0.32	0.04	0.121	0.20	0.08	**0.037**	0.12
%BrFA (i,a C_{13}–C_{19}) of PLFA	−0.16	0.04	0.137	−0.03	0.00	0.509	—

* Partial r^2 values were calculated by dividing the type III SS by the total SS.

top-down and bottom-up effects (Strong 1992; Hughes et al. 2004; Borer et al. 2006). In benthic, sedimentary systems, this should include effects on biomass and composition of the aboveground primary producers and animals (Heck et al. 2000; Hughes et al. 2004; Borer et al. 2006), the belowground community (Wardle et al. 2005), and the OM composition in sediments (Holmer et al. 2004; Canuel et al. 2007). In this study, we showed experimentally that epibenthic food-web structure and resource (light) availability strongly influenced the abundance and composition of SOM. Specifically, light increased and grazers decreased most measures of primary producer biomass and SOM. Grazer effects on primary producers and SOM composition were generally stronger in ambient light treatments, showing that animal communities and resource availability together shaped properties of this seagrass ecosystem. Perhaps surprisingly, given the strong effects of predators on aboveground algal biomass in this system (Duffy et al. 2005), effects of predators (food chain length) on SOM were less pervasive than those of light availability

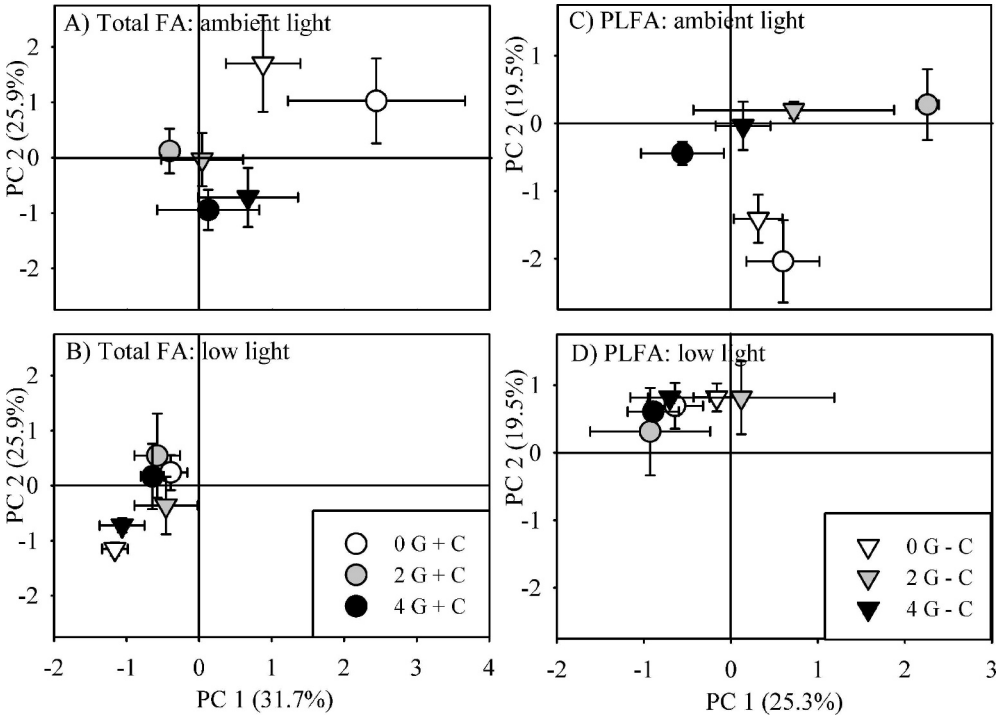

Fig. 6. Score plots from principal component analysis for total fatty acids (total FAs) and phospholipid-linked fatty acids (PLFAs) in ambient light and shaded treatments. Error bars represent standard error. G, grazers; C, crab predators.

Table 3. Loadings from principal components analysis of sediment organic matter composition and content for total fatty acids (total FA) and phospholipid-linked fatty acids (PLFA). Polyunsaturated fatty acids (PUFA) are composed of $C_{18:4}$, $C_{20:4}$, $C_{20:5}$, $C_{22:5}$, and $C_{22:6}$. i,a, iso-, anteiso-.

	Total FA		PLFA	
Variable	PC1	PC2	PC1	PC2
TOC (mg g^{-1})	0.494	−0.221	0.324	−0.148
%($C_{12:0}$+$C_{14:0}$)	0.512	0.169	0.526	0.140
%$C_{16:0}$	−0.229	0.527	−0.536	−0.168
%($C_{18:2}$+$C_{18:3}$)	0.222	0.453	0.137	−0.662
%$C_{24:0}$	0.583	0.118	0.216	−0.557
%PUFA	−0.018	−0.632	0.492	0.290
%BrFA (i,a $C_{13:0}$–$C_{19:0}$)	0.228	−0.160	−0.152	0.312

or grazers. Nevertheless, predators increased OM contributions from microbial sources generally (%SCFAs, total FAs), and from sediment heterotrophic bacteria specifically (%BrFA, total FA). This suggests that the previously demonstrated cascading effects of crab predators on primary producer biomass (Duffy et al. 2005; Canuel et al. 2007) also affect the accumulation of labile OM, eliciting a bacterial community response.

Bottom-up forcing—Many seagrass ecosystems suffer from suspended sediment and nutrient loading, both of which can reduce light availability (Duarte 2002; Kemp et al. 2004). Decreased water clarity negatively affects seagrass performance and has cascading effects on associated fauna, water quality, and sediment erosion (Orth and Moore 1983; Duarte 2002). With such wide-ranging effects, it is likely that decreased water transparency would also affect SOM accumulation and biogeochemical processes in seagrass sediments (McGlathery et al. 1998; Holmer et al. 2004). Thus, a primary goal of our study was to elucidate how light availability, alone and in concert with changing food-web structure, influences OM composition.

In our experimental system, light strongly increased aboveground plant and algal biomass (Table 1; Fig. 1), confirming that the level of shading we used limited primary production and accumulation of producer biomass. In the sediments, light increased benthic microalgal biomass and, presumably as a result, TN and TOC. These light effects translated into changing SOM composition by increasing the abundance of algal and microbial FAs (%($C_{12:0}$ + $C_{14:0}$); total FA), %$C_{24:0}$ (total FAs and PLFAs), and linoleic and linolenic acids (PLFAs) and by decreasing heterotrophic bacterial fatty acids (%BrFA; total FAs, and PLFAs) (Fig. 5). Linoleic and linolenic acids (total FA) were positively correlated with eelgrass biomass, while %($C_{12:0}$ + $C_{14:0}$) (total FAs), %$C_{24:0}$ (total FAs), and BrFAs (total FAs) were positively correlated with benthic microalgal biomass (Chl *a*). The positive relationship between benthic Chl *a* and heterotrophic bacterial FAs suggests that, in our system, microalgae served as a primary OM source for sediment bacteria. This is consistent with recent studies showing that microalgae are often a major source of SOM and drive microbial degradation processes in seagrass beds (Boschker et al. 2000; Bouillon and Boschker 2006).

Although it is generally accepted that $C_{12:0}$ + $C_{14:0}$ derives from aquatic algal and microbial sources, the origin of $C_{24:0}$ is less clear. Vascular plants are typically considered the source of long-chain FAs; however, diatoms have been reported to contribute as much as 30% of $C_{24:0}$ in some sediments (Volkman et al. 1980). Other studies have reported $C_{24:0}$ FAs in cyanobacterial mats (Edmunds and Eglinton 1984), diatoms (Viso and Marty 1993), and microalgae (Volkman et al. 1998 and references therein). These organisms are often associated with the community of organisms composing the microphytobenthos. In addition, %($C_{12:0}$ + $C_{14:0}$) (TFA; aquatic algal and microbial OM) and %$C_{24:0}$ (TFA) had similar PC1 and PC2 scores (Table 3). These FA classes responded similarly to light and food-web treatments, suggesting that they share an OM source in our system.

Overall, light availability increased the abundance of aboveground primary producers, sediment TN and TOC content, and the relative contributions of FA typically thought to derive from aquatic sources, such as algae and microbes. These results demonstrate that resource availability affects belowground OM storage and cycling in this seagrass system in addition to the more obvious accumulation of plant biomass aboveground. Consequently, changes in water quality that result in reduced light availability may alter carbon cycling and storage in seagrass ecosystem sediments.

Community structure and top-down forcing—The community structure of seagrass ecosystems is rapidly changing as a result of reduced water quality, fishing pressure, and other human influences (Duarte 2002; Orth et al. 2006). The resulting shifts in community composition at multiple trophic levels may precipitate changes in ecosystem functioning (Heck et al. 2000; Duffy 2002). For example, loss of a top predator can indirectly reduce primary producer biomass via a trophic cascade (Hairston et al. 1960; Pace et al. 1999; Shurin et al. 2002). In seagrass systems specifically, shifts in species composition at intermediate trophic levels may also alter ecosystem properties and OM accumulation (Duffy et al. 2003; Canuel et al. 2007). A goal of this experiment was to determine how simultaneous changes in food-web composition and resource availability influence ecosystem properties and functioning.

Food chain length (predator presence or absence) strongly influenced GEP, total algal biomass, and SOM composition (Table 1). This effect of crab predators was evidently mediated indirectly; as crabs inhibited or consumed grazing invertebrates, increasing algal biomass and, consequently, GEP. In the sediments, predators increased algal and microbial OM (%($C_{12:0}$ + $C_{14:0}$) total FA; Fig. 5A), presumably through the same trophic cascade mechanism. Interestingly, predators decreased the relative contribution of even-numbered PUFAs (%PUFA total FAs; Fig. 5C), which are considered proxies for "fresh" algal material (Canuel and Martens 1993). This effect was strongest in shaded treatments where primary producer

biomass was lower. Importantly, predators also increased OM contributions from sediment heterotrophic bacteria (%BrFA, total FA; Fig. 5E), suggesting that trophic cascades can extend beyond animals and plants to OM and biogeochemical cycling. Consequently, the removal of top predators may alter not only biomass and production of herbivores and plants, but also ecosystem processes mediated by sediment or soil communities (Setälä et al. 1998; Wardle et al. 2005). This has implications for seagrass ecosystems in Chesapeake Bay and elsewhere where blue crabs and predatory fishes are commercially harvested.

Overall, grazers strongly decreased ecosystem production, plant and algal biomass, and the contributions to the sediments of FA deriving from these sources (Table 1; Figs. 1, 2, 5). Aboveground, grazer presence decreased total algal and *Z. marina* biomass, resulting in reduced GEP, but only in the absence of predators, reflecting the strong trophic cascade demonstrated previously in the aboveground portion of this system (Duffy et al. 2005). Both grazer presence and richness were strong determinants of GEP, confirming that invertebrate species composition and diversity can influence ecosystem-level rate processes (Jonsson and Malmqvist 2003; Dangles and Malmqvist 2004). In the sediments, grazer presence decreased benthic microalgal biomass (Chl *a*), microbial FAs (%($C_{12:0}$ + $C_{14:0}$) total FAs), linoleic and linolenic acids (total FAs and PLFAs), and %C_{24} (ambient light and with predators, total FAs) (Fig. 5). Thus, grazing reduced the contribution of FAs characteristic of eelgrass and algae to SOM. Grazer richness influenced only heterotrophic bacterial FA abundance (%BrFA, total FA and PLFA; Fig. 5E), though this effect was mainly driven by the two-species treatment. Overall, our results indicate that the presence of grazers is more important than the number of species in determining SOM composition and quality.

Overall, food chain length and grazers strongly affected GEP, primary producer biomass, and SOM composition. Predators mediated carbon flow and accumulation between lower trophic levels while grazers altered the composition of OM delivered to the sediment. Further, our results suggest that aboveground communities may influence sediment heterotrophic bacteria. Consequently, human-induced shifts in the abundance or composition of aboveground communities can indirectly affect sediment biogeochemistry by influencing the pathways (invertebrate grazers vs. bacteria) through which OM is cycled.

Interactions between bottom-up and top-down forcings—Because seagrass habitats are perturbed by multiple stressors, developing a comprehensive understanding of ecosystem responses is imperative for conservation and restoration (Duarte 2002; Orth et al. 2006). However, most studies have investigated the effects of human stressors, such as eutrophication (*see* Cloern 2001) or changing biodiversity (*see* Duffy 2006), on seagrass systems singularly (*but see* Heck et al. 2000; Hughes et al. 2004). Thus, a major goal of this study was to investigate how interactions between decreased resource (light) availability and altered food-web structure (grazer community and predator presence) affect ecosystem properties. Interactions between the three manipulated variables had pervasive effects on the abundances of aboveground eelgrass and algal biomass and SOM composition. Although the majority of interactions were between grazers and light or predators, there were also several three-way interactions.

Overall, most interactive effects of the treatments on SOM largely stemmed from light or predators mediating grazer effects on primary producer biomass and OM. Generally, grazer effects were stronger in ambient light, while predator controls were more prevalent in shaded treatments (Table 1), suggesting that the strength of trophic cascades depends on the availability of light or other resources, as in some freshwater systems (Chase 2003). The results of the PCA analyses best summarize the interactive effects of light, grazers, and predators on SOM (Table 3; Fig. 6), suggesting that grazers can strongly determine SOM composition, that their effects are damped by predators, and that changing light intensity affects the relative strength of this trophic cascade.

Our results largely confirm our original hypotheses. Grazers decreased total algal biomass and altered SOM composition. Predator inclusion resulted in a trophic cascade whereby total algal biomass, algal and microbial OM (%($C_{12:0}$ + $C_{14:0}$) total FA), and bacterial FA abundance (%BrFA, total FA) in the sediments were increased. Ambient light increased aboveground and sediment primary producer abundance, sediment TN and organic carbon, and algal and microbial OM (%($C_{12:0}$ + $C_{14:0}$) total FA, %$C_{24:0}$ total FA and PLFA). Unlike our predictions, grazers decreased *Z. marina* biomass, benthic Chl *a*, and FAs derived from algal and microbial OM (%($C_{12:0}$ + $C_{14:0}$) total FA), while reduced light availability increased bacterial OM (%BrFA, total FA). This latter result was largely driven by the treatment with two grazer species. The complex interactive effects among resources, predators, and grazers suggest that aboveground and sediment properties are unlikely to respond in simple, predictive ways to multiple disturbances. Further, our results demonstrated that resource availability and food-web structure strongly influence ecosystem properties and that synergism between bottom-up and top-down controls may affect sediment carbon composition and storage in natural seagrass beds. This underscores the need for additional multifactorial experimental and field approaches to understanding the cycling of OM in estuarine systems. Realistic mesocosm experiments are initially helpful in identifying subtle changes in SOM and focusing research questions and methods. However, field experiments will clearly be necessary to explore how linkages between aboveground processes and SOM are related in the more complex natural environment. Combined, results from both approaches should be useful in designing more effective management strategies for the preservation of productive seagrass ecosystems.

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