Preliminary study on the nutritional value of *Caulerpa taxifolia* and associated sediments based on elemental composition

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EXECUTIVE SUMMARY

Caulerpa taxifolia is an invasive green alga that has colonised large areas in the Mediterranean Sea, with smaller invasions on the west coast of the United States, as well as the east coast of Australia and South Australia. Despite the increasing spread of its distribution, little information is available to understand changes to food resources resulting from the replacement of seagrasses native to these regions with *C. taxifolia*. In this work, we investigated the elemental composition of tissues of *C. taxifolia* and the seagrass *Zostera* sp. (mostly *Zostera muelleri*), and of sediments under both species, in order to explore the links between elemental patterns and stoichiometric imbalances at the base of the food chain.

Caulerpa taxifolia tissues had higher nitrogen and lower phosphorus content than *Zostera* sp., resulting in lower C:N and higher N:P ratios. The higher nitrogen content in *C. taxifolia* tissues promotes fast microbial decomposition and low accumulation of organic matter in the sediments, which switch from a net sink to a net source of nutrients to the water column. In contrast, the hard tissues of seagrasses contain significant amounts of structural organic matter, leading to higher sequestration in the sediments and an increase in both organic C:N and N:P ratios. As a consequence, detritus from *Zostera* sp. beds releases energy more slowly and steadily into the food web, providing a source of nutrients for secondary production that can be transported to neighbouring environments.

1. INTRODUCTION

Caulerpa taxifolia is an invasive green alga that has colonised large areas in the Mediterranean Sea, with smaller invasions on the west coast of the United States, as well as the east coast of Australia and South Australia. Differences in sediment chemistry and morphology were observed between beds of this invasive alga and the native seagrass *Zostera* sp. in the Port River-Barker Inlet system near the city of Adelaide, South Australia (Fernandes and Deveney 2008). *Zostera* sp. beds trapped more silts and retained more water than *C. taxifolia* beds. Organic matter in the latter was quickly remineralized by microbial activity, leading to anaerobic conditions and the release of ammonium and sulfides in sediment porewaters. In contrast, sediments covered by *Zostera* sp. had lower bacterial sulphate reducing activity and acted as a long-term sink for organic carbon and nitrogen. These changes in sediment characteristics are likely to have an impact on the role of vegetated sediments on water column nutrient dynamics, and as a habitat for animal life (e.g. through changes in grain size, oxygen availability and toxicity) or as a source of energy to support secondary production and connectivity (e.g. through changes in the nutritional value of detritus).

The study by Fernandes and Deveney (2008) also suggested that detritus from *Zostera* sp. beds are more refractory, releasing energy steadily into the food web and providing connectivity with neighbouring environments. Some evidence of these assumptions came from low N:P molar ratios in sediment porewaters (2-5), indicative of low nitrogen turnover, and higher organic C:N ratios in the sediments as a consequence of the sequestration of plant structural carbon. In contrast, detritus from *C. taxifolia* beds was rapidly absorbed and broken down through microbial activity, with higher N:P ratios in sediment porewaters (~16), and lower accumulation of carbon in the sediments evidenced by virtually unchanged organic C:N ratios between vegetated and unvegetated sediments. No data were available, however, to understand how plant cover affected phosphorus dynamics. In this work, we address this gap by determining phosphorus content in sediments under *C. taxifolia* and *Zostera* sp. beds (mostly *Zostera muelleri*), and carbon, nitrogen and phosphorus content in *C. taxifolia* and *Zostera* sp. tissues. These data are used as a starting point to explore the links between elemental patterns and stoichiometric imbalances at the base of the food chain in an invaded environment, in order to identify the major changes occurring in sediment and plant nutritional value when substrate cover changes from naturally occurring seagrasses to *C. taxifolia*.

2. METHODS

2.1. Sampling

Sediments were collected from two *Zostera* sp. and two *C. taxifolia* beds in the Port River-Barker Inlet system in April-May 2008 (Figure 1) (Fernandes and Deveney 2008). Water depth at low tide was typically <2 m. One transect was surveyed in each bed, with 4 sites per transect: (1) at least 1 m inside the bed, (2) 10 cm inside and (3) 10 cm outside the edge, and (4) unvegetated sediments at least 1 m away from the bed edge. Five replicate cores were collected at each site using 67 mm (internal diameter) PVC tubes capped with rubber bungs. These cores were transported on ice to a temporary laboratory set up in a warehouse at the North Arm of the Port River (Figure 1). In the laboratory, the overlying water in the tube was carefully discarded to minimise surface disturbance, the top 1 cm was sliced for analysis, and any fragments of roots and other plant material were removed. Samples were transferred into pre-combusted scintillation vials and frozen at -20ºC.

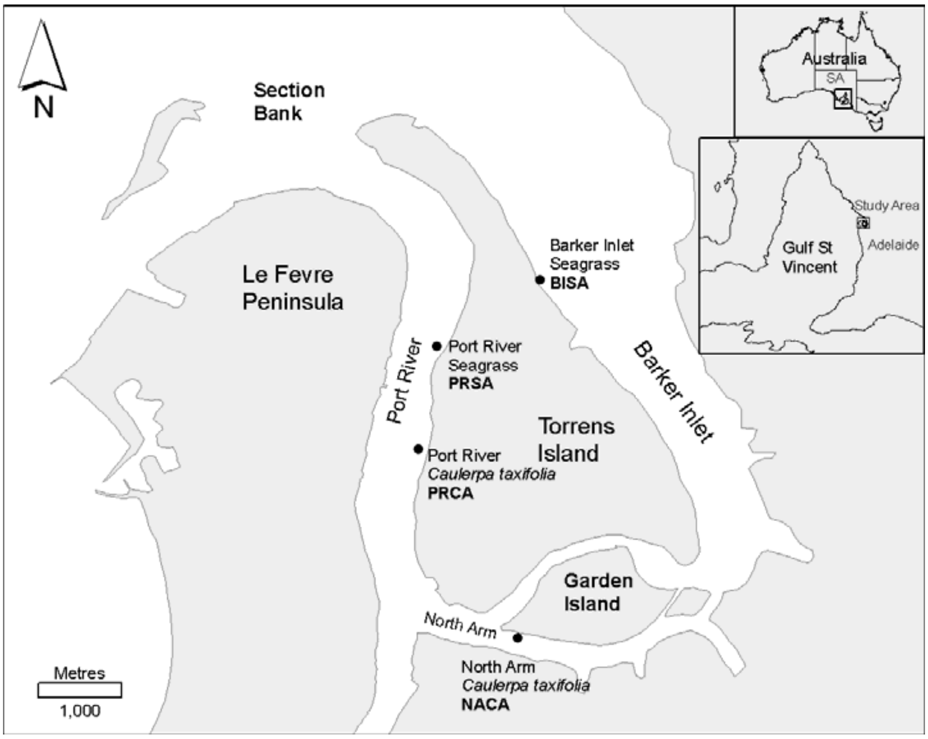

Figure 1. Sampling sites in the Port River-Barker Inlet system.

Caulerpa taxifolia and *Zostera* sp. samples were collected in December 2008 from the same 4 sites (Figure 1). At each site, six replicate samples were collected, consisting of a minimum of six *C. taxifolia* plants, or six clumps of above and below ground *Zostera* sp. tissues. Depending on the size of the meadow, replicates were taken a minimum of 2 m apart. Samples were transported on ice and stored frozen at -20 ^{o}C.

2.2. Analytical

Sediment samples were freeze-dried, sieved to 500 μm to remove large shell fragments, and homogenized with a ball mill grinder. Phosphorus content was analysed by ICP-AES after digestion with aqua regia (1:3 HNO_3/HCl mixture) (Standards Australia 1997).

Caulerpa taxifolia and *Zostera* sp. samples were thawed and rinsed thoroughly with fresh water to remove salt and sediments. For each replicate, the fronds or leaves were cut and cleaned of epiphytes using the blunt edge of a scalpel. Samples were freeze-dried and ground to a fine powder with a ball mill grinder. The amount of *Zostera* sp. collected was insufficient for all analyses, so replicates 1, 2 and 3 were combined to produce one sample, and replicates 4, 5 and 6 were combined to produce a second sample. The same was done for the analyses of phosphorus in *C. taxifolia* tissues. Carbon and nitrogen contents were determined in a LECO TruSpec elemental analyser. Phosphorus content was analysed by ICP-AES after digestion with nitric acid, hydrogen peroxide, and hydrochloric acid (US Environmental Protection Agency 1991).

2.3. Statistics

Results were analysed with the software package STATISTICA (StatSoft, Tulsa, OK). Analysis of variance (ANOVA) was used to identify statistical differences (α = 0.05) between substrate cover (*C. taxifolia* vs *Zostera* sp.) and distance from the bed edge, both of which were treated as fixed factors. Variables were transformed when there was a need to improve normality and heterogeneity of variances. Tukey post-hoc tests were performed when significant differences were detected.

3. RESULTS

3.1. Plant tissues

Although the data show some variability in the carbon content of *Zostera* sp. leaves, this was indistinguishable from the carbon content of *C. taxifolia* fronds (Figure 2a). Nitrogen content was on average 40% higher in *C. taxifolia* fronds, whereas phosphorus content was slightly higher in *Zostera* sp. leaves (Figure 2b,c). These differences are reflected in the lower C:N and higher N:P ratios of *C. taxifolia* fronds (Figure 3). The significance of the differences observed between species is highlighted in Table 1.

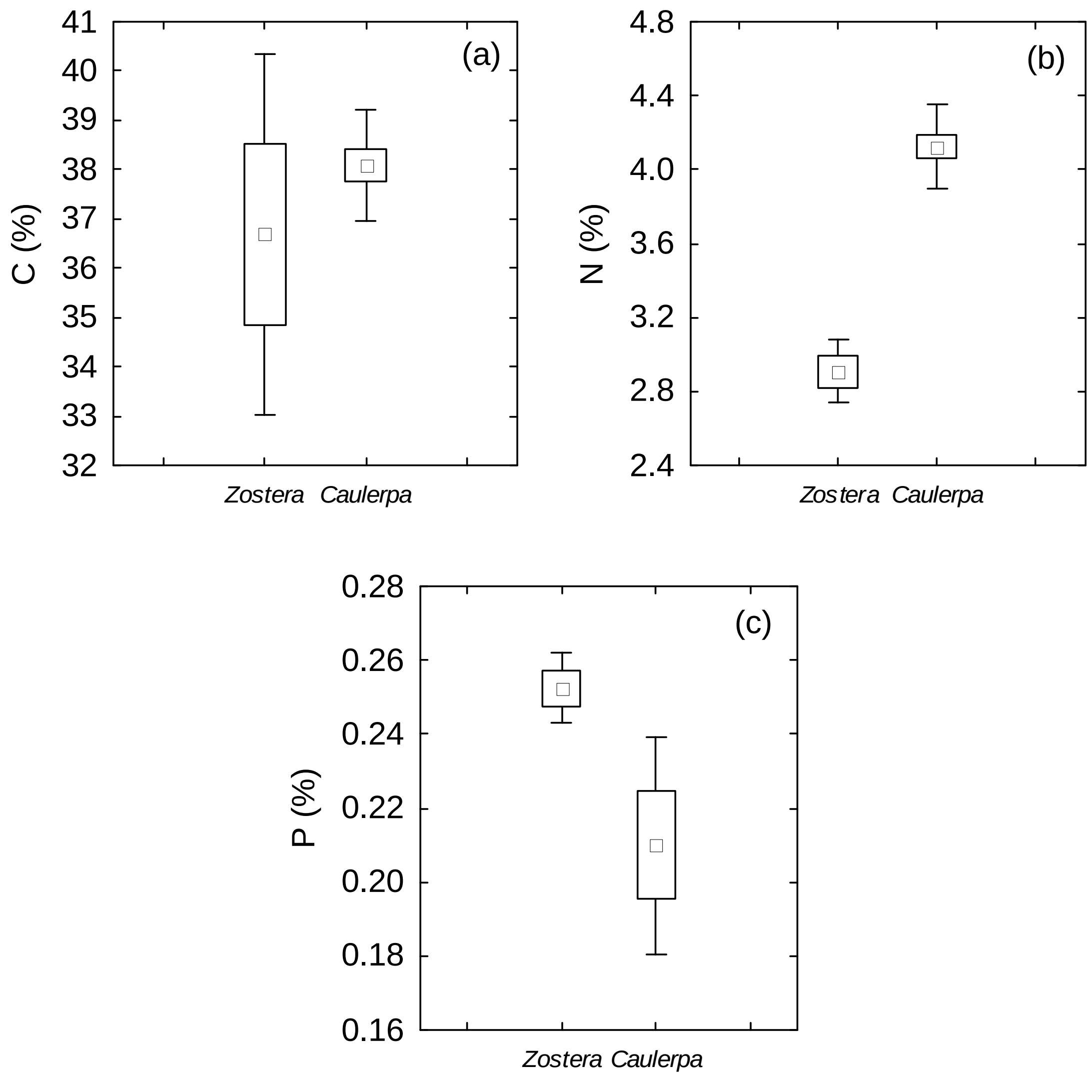

Figure 2. Carbon (a), nitrogen (b), and phosphorus content (c) in *Zostera* sp. leaves when compared to *C. taxifolia* fronds. Error bars indicate the standard deviation, and error boxes the standard error.

Table 1. ANOVA results for tissue nutrient content and elemental molar ratios as a function of species (*C. taxifolia vs Zostera* sp.).

Variable	Source	df	MS	F	p
Carbon (%)	Species	1	5.860	1.526	0.237
	Error	14	3.840		
Nitrogen (%)	Species	1	4.445	92.652	**<0.001**
	Error	14	0.048		
Phosphorus (%)	Species	1	0.004	7.539	**0.033**
	Error	6	0.001		
C:N ratio	Species	1	46.667	73.126	**<0.001**
	Error	14	0.638		
N:P ratio	Species	1	676.165	74.697	**<0.001**
	Error	6	9.052		

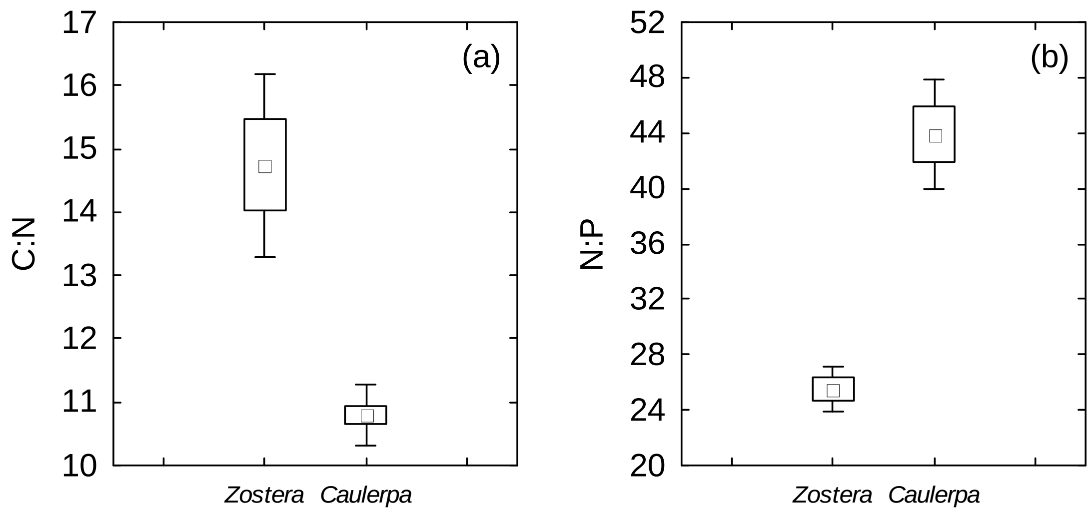

Figure 3. C:N (a), and N:P (b) molar ratios in *Zostera* sp. leaves when compared to *C. taxifolia* fronds. Error bars indicate the standard deviation, and error boxes the standard error.

3.2. Sediments

The differences in organic carbon and nutrient content of sediments between type of vegetative cover (*C. taxifolia* vs *Zostera* sp.) and distance from the bed edge are highlighted in Table 2. Organic carbon, nitrogen and phosphorus contents were higher in sediments colonized by both *C. taxifolia* and *Zostera* sp. (Figure 4), but the difference between unvegetated and vegetated sediments was only significant for the *Zostera* sp. beds ($p<0.05$, Tukey post-hoc tests). The edge effect (i.e. difference between 10 cm inside and 10 cm outside the bed) was also only important for organic carbon and nitrogen in sediments from seagrass beds ($p<0.05$, Tukey post-hoc tests). Elemental ratios were virtually unchanged between sediments colonized by *C. taxifolia* and unvegetated sediments (Figure 5). In contrast, sediments colonized by *Zostera* sp. had higher organic C:N and N:P ratios ($p<0.05$, Tukey post-hoc tests), with a significant edge effect for C:N ratios ($p<0.05$, Tukey post-hoc tests).

Table 2. ANOVA results for sediment elemental composition and molar ratios as a function of substrate cover and distance from bed edge.

Variable	Source	df	MS	F	p
Organic C (%)	Cover	1	0.123	23.009	**<0.001**
	Distance	3	0.155	29.061	**<0.001**
	Cover x Distance	3	0.045	8.429	**<0.001**
	Error	72	0.005		
Nitrogen (%)	Cover	1	0.004	14.255	**<0.001**
	Distance	3	0.005	16.772	**<0.001**
	Cover x Distance	3	0.001	4.328	**0.007**
	Error	72	0.000		
Phosphorus (%)	Cover	1	0.001	5.724	**0.019**
	Distance	3	0.001	13.729	**<0.001**
	Cover x Distance	3	0.001	0.859	0.466
	Error	69	0.001		
Sediment C:N (molar ratio)	Cover	1	0.025	5.290	**0.024**
	Distance	3	0.031	6.730	**<0.001**
	Cover x Distance	3	0.011	2.460	0.070
	Error	72	0.005		
Sediment N:P (molar ratio)	Cover	1	285.753	67.025	**<0.001**
	Distance	3	8.545	2.004	0.121
	Cover x Distance	3	4.669	1.095	0.357
	Error	69	4.263		

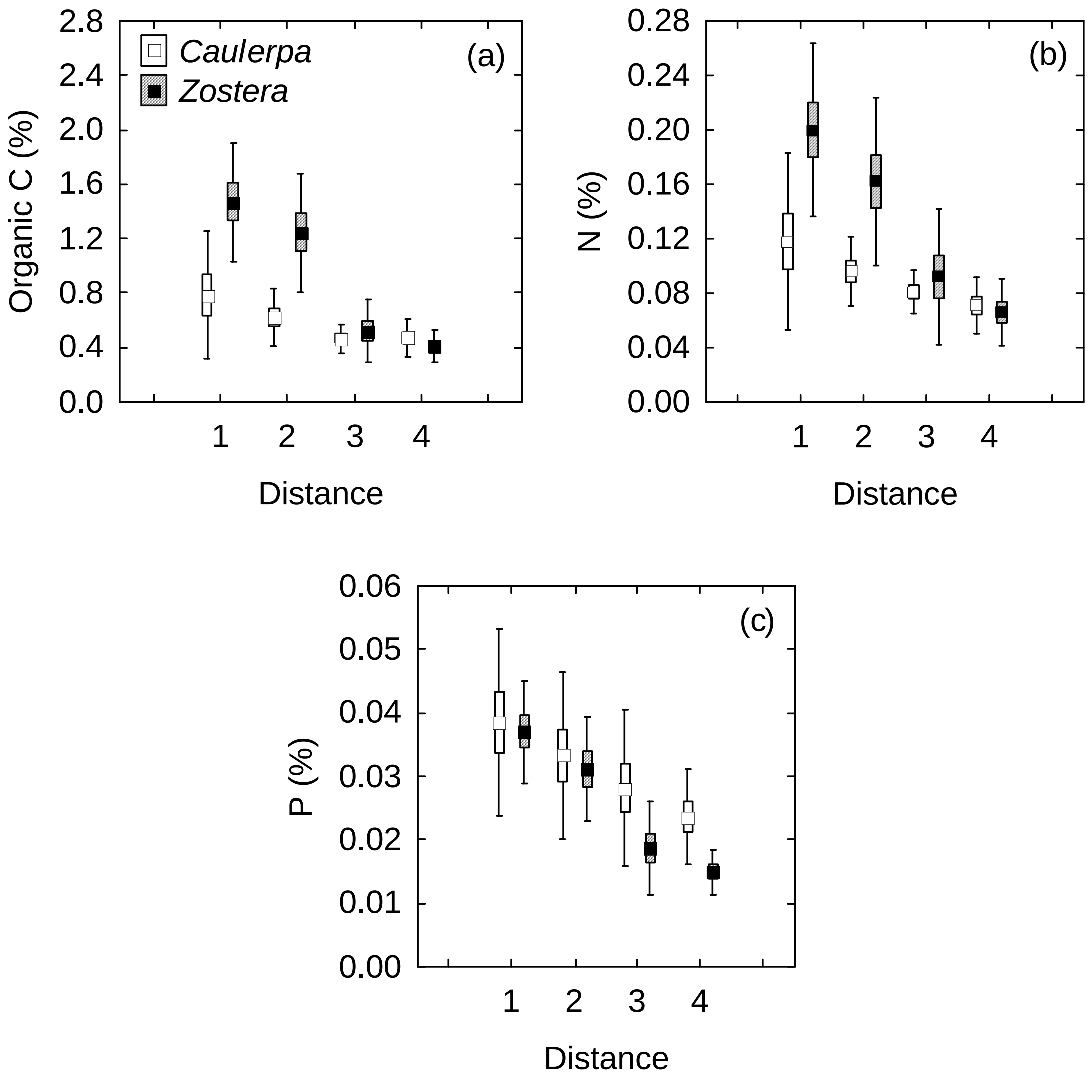

Figure 4. Sediment organic carbon (a), nitrogen (b), and phosphorus content (c) for both *C. taxifolia* and *Zostera* sp. beds according to distance from the edge: (1) at least 1 m inside, (2) 10 cm inside, (3) 10 cm outside, and (4) at least 1 m outside. Error bars indicate the standard deviation, and error boxes the standard error. Organic carbon and nitrogen values are from Fernandes and Deveney (2008).

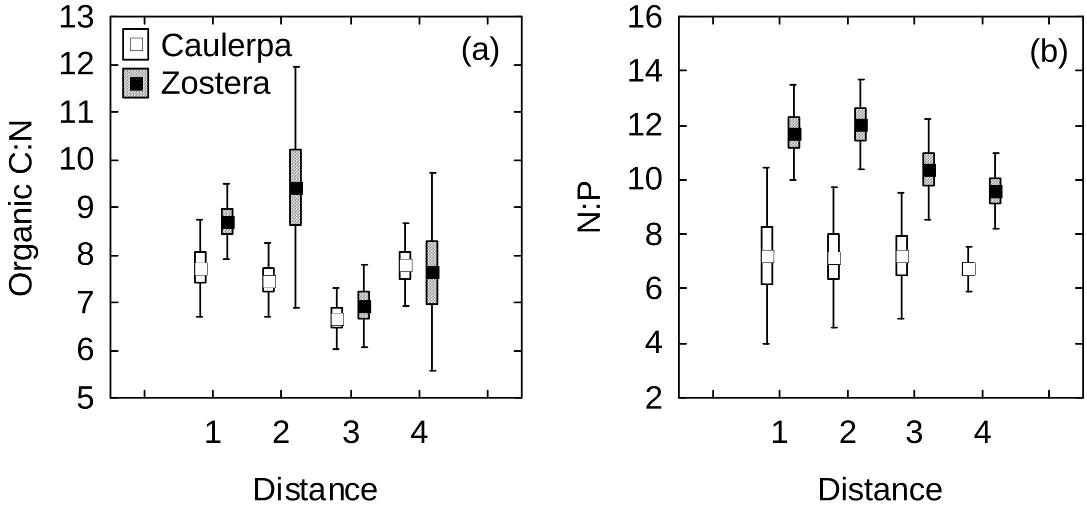

Figure 5. Sediment organic C:N (a), and N:P (b) molar ratios for both *C. taxifolia* and *Zostera* sp. beds according to distance from the edge: (1) at least 1 m inside, (2) 10 cm inside, (3) 10 cm outside, and (4) at least 1 m outside. Error bars indicate the standard deviation, and error boxes the standard error. Organic C:N ratios are from Fernandes and Deveney (2008).

4. DISCUSSION

These data confirm some of the issues raised in the first report of this series (Fernandes and Deveney 2008), particularly that differences in the nature of the detritus produced by *Zostera* sp. and *C. taxifolia* beds are reflected in changes in the composition of organic matter available as food resources to support secondary production. Because seagrasses have higher C:N ratios in comparison to *C. taxifolia*, the ability of bacteria to decompose *Zostera* sp. detritus is compromised by nitrogen deficiency (Goldman et al. 1987). This difference occurs because seagrasses are angiosperms, and invest in producing structural materials such as cellulose and lignin to a much greater degree than do algae such as *C. taxifolia*. The more refractory nature of seagrass detritus ensures that organic matter produced in *Zostera* sp. beds persists for longer in the system, with higher sequestration in the sediments. Thus, detritus from *Zostera* sp. beds releases energy more slowly and steadily into the food web, providing a source of nutrients for secondary production that can be transported to neighbouring environments. This linkage between coastal ecosystems provided by seagrasses has been observed in the gulfs of South Australia, where detritus from subtidal meadows indirectly provides nutrition to a commercial fishery species (yellowfin whiting, *Sillago schomburgkii*) feeding on an intertidal polychaete prey (Connolly et al. 2005).

In *C. taxifolia* beds, the availability of resources is transient and there is little accumulation in the sediments. *Caulerpa taxifolia* detritus is rapidly broken down by microbial activity, and is therefore unlikely to provide the same ecological services to higher trophic levels as seagrasses. The higher nitrogen content in *C. taxifolia* tissues, and low accumulation in the sediments, further indicates that *C. taxifolia* beds are likely to act as a source of nitrogen to the water column. The change of substrate cover between *C. taxifolia* and *Zostera* sp. is thus bound to alter nutrient dynamics by switching the status of sediments from a net sink to a net source of nitrogen to the water column.

5. CONCLUSIONS

The replacement of seagrasses by the invasive alga *C. taxifolia* in the Port River-Barker Inlet system has important ecological implications by modifying the nature of food resources at the base of the food web. While seagrasses provide a steady supply of detritus for secondary production both locally and in adjacent habitats, the organic matter produced by *C. taxifolia* is quickly decomposed by microbial activity and less likely to support connectivity between coastal habitats.

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