Dissolved Organic Carbon Cycling and the Roles of the Microbial Community in the Coexistence of Corals and Seagrasses in Bise, Okinawa, Japan

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ABSTRACT

The coexistence of seagrasses and corals at Bise, Okinawa, Japan, was investigated in terms of nutrient dynamics, dissolved organic carbon (DOC), and microbial abundance. Four incubation conditions (seawater, seagrass, coral, and seagrass with coral [sg+cr]) were examined using in situ incubation methods. $^{13}$C was used as a tracer to evaluate primary production in the water column and carbon fixation in seagrass and coral tissues. Primary production was higher in the water column in seagrass incubations, resulting in increased dissolved oxygen (DO) concentrations in the surrounding water. In this treatment, DO was also significantly positively correlated with DOC concentration. Seagrass produced higher amounts of DOC in sg+cr compared to coral incubations (1.2 $\pm$ 8.8 $\mu$M) by generating 21.6 $\pm$ 19.1 $\mu$M into the water column. DOC concentrations in the seawater were positively correlated with bacterial abundance. Bacteria are important in the recycling of carbon in the system of coexisting coral and seagrasses due to their use of DOC exuded from both seagrasses and coral, which, in turn, enhances the assimilation of carbon by corals. Therefore, the production of carbon in coral tissues increased when coral was incubated together with seagrass. Similarly, nutrient uptake was highest in the sg+cr incubations. In this environment, where seagrass and coral coexist, corals can utilize DOC exuded by seagrass to increase carbon assimilation via microbial activity, while seagrass can assimilate nutrients and microbially degraded DOC from coral metabolism in the environment.

Key words: Coexistence area, dissolved organic carbon (DOC), microbial activity, nutrient

1. Introduction

Seagrass beds are systems adjacent to coral reefs in coastal zones (Hemminga et al., 1994; Unsworth et al., 2012). Seagrasses interact with corals in several important ways. For example, seagrass areas receive and stabilize sediment from the reef, and can also function as deposition sites of sediments, trapping sediment runoff from the land before it reaches coral-reef areas (Rasheed et al., 2006; Sheppard et al., 2009). Therefore, sedimentation rates are higher in seagrass beds than in coral reefs (Stapel et al., 1996; Hemminga and Duarte, 2000). In addition, the concentrations of nutrients that accumulate in seagrass sediments are also higher than in coral sediments (Maheswari et al., 2011). Seagrasses have the capacity to take up nutrients from both the water column and sediment, whereas corals can take up nutrients only from the water column (Stapel et al., 1996; Sheppard et al., 2009). Coral itself can release certain amounts of organic matter as particulate organic carbon (POC), dissolved organic carbon (DOC), particulate organic nitrogen (PON), and dissolved
organic nitrogen (DON) to the surrounding water, mainly as mucus. Seagrasses can also release large amounts of organic matter via exudation or leaching and mineralization of seagrass photosynthate, which can act as an important source of inorganic nutrient in the water column in coastal ecosystems (Ziegler et al., 2004). Clearly, seagrasses and corals each exhibit their own biogeochemical characteristics, and both can provide organic matter to support organisms within the ecosystem.

Dissolved organic matter (DOM) plays important chemical and biological roles in the marine environment by controlling nutrient dynamics and fueling the microbial loop (Ogawa and Tanoue, 2003). The largest pool of DOM in the ocean exists as dissolved organic carbon (DOC) (Shinomura et al., 2005). Seagrasses can release DOC through exudation during photosynthesis (Ziegler and Benner, 1999). However, the release of DOC by seagrass leaves accounts for less than 5% of the carbon fixed during photosynthesis (Ziegler and Benner, 1999). The seagrass community may also contribute to the DOC pool via exudation by epiphytes, excretion and sloppy feeding by consumers, and diffusive release from sediments (Moncreiff et al., 1992; Barn and Duarte, 2009). On the other hand, 6–40% of DOC released as mucus from corals consists of carbon that is photosynthetically fixed by symbiotic algae (zooxanthellae) (Ferrier-Pages et al., 1998; Tanaka et al., 2008). Increases in DOM in the surrounding water lead to increases in the activity and growth of microbial communities, which, in turn, play important roles in the remineralization of organic to inorganic compounds and the transfer of energy to other organisms (Shnit-Orland and Kushmaro, 2009; Haas et al., 2011; Tanaka et al., 2011). In addition, high microbial abundance in the surrounding water can be used as a food source by coral through grazing (Coffroth, 1990).

In the present study, the coexistence of seagrass and coral was investigated at Bise in northwest Okinawa, Japan. Despite the different characteristics of seagrass and coral, the seagrass Thalassia hemprichii and the coral Montipora digitata were found coexisting in the same habitat at the study site. This situation is rarely observed in other coastal regions. Coral colonies grow and survive in the seagrass meadow, which covers a large portion of the coastal area. The stability of the seagrass bed is enhanced as the roots grow and adapt in such a way that they form a web around the coral colonies (Inoue et al., 2008; Ninomiya et al., 2008). To understand this coexistence better, the exchange and recycling of DOC and nutrients were examined together with bacterial abundance using in situ incubation methods in three sub-environments: seagrass meadow, coral reef, and seagrass with coral.

2. Methodology

2.1 Study site

This study was conducted in the coastal area of Bise, Okinawa, Japan (26°42’ N, 127°52’ E). The area is characterized by an intertidal zone, seagrass meadow, and coral reef with a shallow surrounding platform (2 m at high tide, 0.6 m at low tide). The distribution of seagrass in Bise is generally restricted by substrate type and water flow in this environment. The substrate contains rich dead coral rubber, and the seagrasses have adapted to growing their roots in this substrate (Inoue et al., 2008; Ninomiya et al., 2008; Takagi et al., 2008). In addition, the seagrass Thalassia hemprichii contributes to a stable meadow with the branching coral Montipora digitata. For the purposes of this study, four sub-environments were delineated within this environment: seawater (control), corals (A), seagrasses (B), and seagrasses coexisting with corals (C) (Fig. 1). Sample collection was conducted at 18:00, after sunset, on 14 May 2013, to limit photosynthesis prior to incubation. Seawater, seagrass (Thalassia hemprichii), and coral (Montipora digitata) were collected from Bise (Fig. 1) for the incubation experiment. Initial concentrations of DOC, nutrients (ammonium, nitrite, nitrate, and phosphate), and the abundances of bacteria and cyanobacteria in the seawater were sampled as initial values.

![Fig. 1: Three different sites on Bise reef, Okinawa, Japan; A: coral ecosystem; B: seagrass ecosystem, and C: area of seagrass and coral coexistence (sg+cr).](image-url)
2.2 Incubation experiment

To evaluate primary production, organic carbon cycling, nutrient dynamics, and the role of microbes in the four sub-environments (seawater, seagrass, corals, and seagrass with coral), each incubation condition was prepared in triplicate in 1,000-ml polycarbonate plastic bottles (Niskin bottles): seawater (control), seagrass, coral, and seagrass with coral (sg+cr). Three seagrass plants and three coral branches of similar sizes and weights were carefully selected for each incubation condition. The average length of the seagrasses was ~25 cm including the root and the average weight was 2 g, including the rhizome. For coral, the average size of branches was ~5 cm long, and the average weight was ~4 g. Seagrasses and coral were collected at selected sites, together with surrounding water to be used for incubations. In addition, 2 ml of a $^{13}$C tracer solution (1 g NaH$^{13}$CO$_3$ in 100 ml) were added to each bottle for measurements of primary production rates during daytime and nighttime (Casareto et al., 2009). The bottles were then filled to volume (1,000 ml) with seawater from Bise, and seagrass and weights were carefully selected for each incubation condition. The bottles used for measurements of nutrients and DOC were washed and rinsed with 5% HCl and Milli-Q water before use. Incubations in the bottles were carried out for 24 h in situ at Bise under natural light and temperature conditions. Two batches of nighttime (12 h) incubations were prepared (total of $n=24$). Temperature and light were monitored over the duration of the experiment using in situ sensors (MDS-MkV/T and MDS-MkV/L, Alec Electronics; Japan). At the start time (06:00), the first batch of triplicate samples was removed at 18:00. The remaining bottles were sampled at 06:00 the next morning (24-h incubations). During the experiment, the temperature ranged from ~23–25°C, and the highest light intensity was 1238.4 µmol m$^{-2}$ s$^{-1}$ at noon. The incubation bottles were transported to the laboratory, was used as a reference material. DOC concentrations were determined by filtering 50-ml seawater samples from the incubation bottles using a glass syringe connected directly to a filter holder containing a glass-fiber filter (Whatman GF/F, Toyo Roshi Kaisha, Ltd). The filters were pre-combusted at 500°C for 4 h. The filtered seawater was kept in glass vials (Shinomura et al., 2005). DOC concentrations were determined using a high-temperature catalytic combustion (HTC) method using a Shimadzu TOC-5000A analyzer (Sumika Analysis Service Co., Australia) (Suzuki et al., 1992). The average analytical error of DOC measurements using this instrument is 0.95%. The system was standardized daily before operation with a four-point calibration curve using an EDTA–2Na solution in Milli-Q water. A total blank was determined from the intercept and slope of the calibration curve. To evaluate primary production, organic carbon cycling, nutrient dynamics, and the role of microbes in the four sub-environments (seawater, seagrass, corals, and seagrass with coral), each incubation condition was prepared in triplicate in 1,000-ml polycarbonate plastic bottles (Niskin bottles): seawater (control), seagrass, coral, and seagrass with coral (sg+cr). Three seagrass plants and three coral branches of similar sizes and weights were carefully selected for each incubation condition. The average length of the seagrasses was ~25 cm including the root and the average weight was 2 g, including the rhizome. For coral, the average size of branches was ~5 cm long, and the average weight was ~4 g. Seagrasses and coral were collected at selected sites, together with surrounding water to be used for incubations. 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A total blank was determined from the intercept and slope of the calibration curve. Deep Sargasso Sea water, provided by the Bermuda Biological Station (BBS, USA) as part of an international certified reference material program for DOC measurements by Hansell Laboratory, was used as a reference material. DOC concentrations were determined by subtracting the intercept and dividing by the slope of the calibration curve (Shinomura et al., 2005).

2.4 Dissolved organic carbon (DOC) measurements

DOC concentrations were determined by filtering 50-ml seawater samples from the incubation bottles using a glass syringe connected directly to a filter holder containing a glass-fiber filter (Whatman GF/F, Toyo Roshi Kaisha, Ltd). The filters were pre-combusted at 500°C for 4 h. The filtered seawater was kept in glass vials (Shinomura et al., 2005). DOC concentrations were determined using a high-temperature catalytic combustion (HTC) method using a Shimadzu TOC-5000A analyzer (Sumika Analysis Service Co., Australia) (Suzuki et al., 1992). The average analytical error of DOC measurements using this instrument is 0.95%. The system was standardized daily before operation with a four-point calibration curve using an EDTA–2Na solution in Milli-Q water. A total blank was determined from the intercept and slope of the calibration curve. Deep Sargasso Sea water, provided by the Bermuda Biological Station (BBS, USA) as part of an international certified reference material program for DOC measurements by Hansell Laboratory, was used as a reference material. DOC concentrations were determined by subtracting the intercept and dividing by the slope of the calibration curve (Shinomura et al., 2005).

2.5 Abundances of bacteria and cyanobacteria

Bacteria and cyanobacteria were filtered onto black, 0.2-µm polycarbonate filters (Millipore) by filtering 5- to 10-ml aliquots stained with 4’, 6-diamidino-2-phenylindole (DAPI) (Porter and Feig, 1980). Bacteria were counted under ultraviolet excitation, and cyanobacteria were counted under blue excitation using an epifluorescence microscope (Nikon; Eclipse/E600, Japan).

2.6 Primary production measurements

For primary production measurements, 500 ml of seawater from each incubation bottle were filtered through GF/F filters (pre-combusted at 550°C for 4 h). To measure carbon production in coral, tissue was removed from the coral nubbins by to Hansen and Koroleff (2007). Nitrate was determined by subtracting the values of nitrite from the values of nitrate + nitrite. The detection limit was estimated as 3 σ, where σ is the standard deviation of the replicated ($n=5$) analyses for a 3.5% NaCl (blank) solution. The reproducibility (precision) of the nutrient analyses was ± 0.2% for NO$_3^-$, ± 0.5% for NO$_2^-$, ± 1.2% for NH$_4^+$, and ± 0.8% for PO$_4^{3-}$. 

2.3 Nutrient measurements

For nutrient measurements, samples were collected in 125-ml polyethylene bottles and were preserved at ~20°C for analysis of dissolved inorganic nutrients (ammonium [NH$_4^+$], nitrite [NO$_2^-$], nitrate [NO$_3^-$], and phosphate [PO$_4^{3-}$]). Nutrient concentrations were determined using an auto analyzer (TRAACS 2000, BL Tek, K.K., Germany) in the laboratory according
blasting with 35% NaCl water using an air-jet and homogenizing the blastate before filtration with GF/F. For seagrass, leaves and roots were cut and their weight was measured before placing them on GF/F filters. The filters were kept frozen at -20°C until analysis. POC and 13C isotopes on POC filters (samples) were measured using a mass spectrometer (DELTA plus Advantage, Thermo Finnigan, USA) equipped with an elemental analyzer EA1110 (Casareto et al., 2009). The primary production rate was determined using the 13C tracer technique according to Hama et al. (1993). The analytical precision (SD) of POC and 13C measurements was <±3%. Prior to analysis, POC filters were acidified using HCl fumes to remove inorganic carbon from the filters and then dried again to remove water from the filter papers.

2.7 Statistical analysis

Correlations between variables were determined using Pearson’s correlation tests. Two-way ANOVA tests were performed to determine differences among experimental conditions at different times. Post hoc Tukey’s tests were used to assess pairwise differences when ANOVAs revealed statistically significant effects. The software MINITAB ver. 14 was used for all statistical analyses.

3. Results

3.1 Nutrient dynamics

The initial concentrations of ammonium, nitrate, and phosphate were 0.6 ± 0.3, 11.8 ± 0.3, and 0.35 ± 0.01 µM (n = 3), respectively. The concentration of ammonium in controls (seawater) increased from the initial value, whereas nitrate and phosphate concentrations decreased from initial values. Higher uptake of phosphate (0.09 ± 0.04 µM) and much lower uptake of nitrate (0.6 ± 0.2 µM) were observed in incubations of coral compared to seagrass incubations (Table 1). However, the highest uptakes of nitrate and phosphate compared to seawater controls occurred in the sg+cr incubations.

### Table 1: Concentrations (µM; means ± standard deviation) of ammonium (NH₄⁺), nitrate (NO₃⁻), and phosphate (PO₄³⁻) under initial conditions and after 24 h. Net daily changes (Δ) in NH₄⁺, NO₃⁻, and PO₄³⁻ compared to controls (seawater).

<table>
<thead>
<tr>
<th>Condition</th>
<th>NO₃⁻</th>
<th>NH₄⁺</th>
<th>PO₄³⁻</th>
<th>Δ NO₃⁻</th>
<th>Δ NH₄⁺</th>
<th>Δ PO₄³⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>11.8 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.35 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Seawater</td>
<td>8.9 ± 0.1</td>
<td>0.9 ± 0.6</td>
<td>0.19 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Seagrass</td>
<td>4.9 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.18 ± 0.0</td>
<td>-4.0 ± 0.1</td>
<td>-0.3 ± 0.2</td>
<td>-0.01 ± 0.0</td>
</tr>
<tr>
<td>Coral</td>
<td>8.3 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.10 ± 0.0</td>
<td>-0.6 ± 0.2</td>
<td>-0.5 ± 0.1</td>
<td>-0.09 ± 0.0</td>
</tr>
<tr>
<td>Sg+Cr</td>
<td>4.1 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.07 ± 0.0</td>
<td>-4.7 ± 0.1</td>
<td>-0.4 ± 0.1</td>
<td>-0.12 ± 0.0</td>
</tr>
</tbody>
</table>

3.2 DOC and DO concentrations

The initial concentration of DOC was 83.9 ± 0.7 µM (Table 2). After 24 h, the DOC concentration increased in the seagrass incubation (105 ± 19.1 µM, n = 3), and the values were higher than those in all other incubation treatments. The coral incubations did not exhibit significant changes in DOC compared to seawater controls. However, when seagrass and coral were incubated together, DOC concentrations increased by 9.3 ± 5.6 µM (n = 3) compared to controls. During the daytime, seagrass and coral together released more DOC than at night (Table 3). During the nighttime, both the coral and sg+cr incubations exhibited net negative values of DOC (–3.9 ± 1.8 and –27.4 ± 1.1 µM, respectively). However, this trend was not reflected in the seagrass incubations, which exhibited net positive DOC concentration values at night (2.2 ± 2.6 µM, n = 3; Table 3). Similar trends were observed for DO under all incubation conditions, and DO was positively correlated with DOC (r = 0.42, n = 24; Fig. 2). The highest concentration of DO occurred in the incubations of seagrass (10.3 ± 0.5 mg l⁻¹, n = 3), which differed significantly from the control, whereas DO in coral incubations (8.7 ± 1.2 mg l⁻¹, n = 3) did not. In the sg+cr incubations, DO (1.9 ± 0.5 mg l⁻¹, n = 3) differed significantly from controls (p < 0.01); these values were lower than in seagrass incubations but higher than in coral incubations (0.3 ± 1.2 mg l⁻¹, n = 3).

### Table 2: Concentrations (means ± standard deviation) of DOC (mg l⁻¹), dissolved organic carbon (DOC; µM) under initial conditions and after 24 h under seawater, seagrass, coral, and seagrass with coral (sg+cr) incubation conditions. Net daily changes (Δ) in DO (mg l⁻¹) and DOC (µM) compared to controls (seawater).

<table>
<thead>
<tr>
<th>Condition</th>
<th>DO</th>
<th>DOC</th>
<th>Δ DO</th>
<th>Δ DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>7.4 ± 0.1</td>
<td>83.9 ± 0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Seawater</td>
<td>8.4 ± 0.2</td>
<td>83.3 ± 5.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Seagrass</td>
<td>10.3 ± 0.5*</td>
<td>105.0 ± 19.1</td>
<td>1.9 ± 0.5</td>
<td>21.6 ± 19.1</td>
</tr>
<tr>
<td>Coral</td>
<td>8.7 ± 1.2</td>
<td>84.6 ± 8.8</td>
<td>0.3 ± 1.2</td>
<td>1.2 ± 8.8</td>
</tr>
<tr>
<td>Sg+Cr</td>
<td>9.6 ± 0.9*</td>
<td>92.3 ± 5.6</td>
<td>1.2 ± 0.9</td>
<td>9.3 ± 5.6</td>
</tr>
</tbody>
</table>

Two-way ANOVAs were performed and post hoc Tukey’s tests were then applied to the data to determine significant differences among incubation conditions. * Indicates a significant difference (p < 0.01) compared to controls (seawater).

### Table 3: Net DOC (µM; means ± standard deviation) for daytime (12 h), nighttime (12 h) and 24-h periods. Negative values indicate consumption of DOC.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Average net DOC in daytime</th>
<th>Average net DOC in nighttime</th>
<th>Net daily DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seagrass</td>
<td>19.4 ± 9.9</td>
<td>2.2 ± 2.6</td>
<td>21.6 ± 19.1</td>
</tr>
<tr>
<td>Coral</td>
<td>5.1 ± 1.4</td>
<td>-3.9 ± 1.8</td>
<td>1.2 ± 8.8</td>
</tr>
<tr>
<td>Sg+Cr</td>
<td>36.7 ± 5.4</td>
<td>-27.4 ± 1.1</td>
<td>9.3 ± 5.6</td>
</tr>
</tbody>
</table>
3.3 Abundances of bacteria and cyanobacteria

The abundances of bacteria and cyanobacteria in the surrounding water of the sg+cr incubations differed significantly from controls \((p < 0.01)\) and coral incubations \((p < 0.01)\). Coral incubations contained the lowest abundance of bacteria \((2.5 \pm 0.5 \times 10^6 \text{ cells ml}^{-1})\), followed by seagrass incubations \((3.3 \pm 0.7 \times 10^6 \text{ cells ml}^{-1})\); however, the abundance of cyanobacteria in coral incubations \((12.7 \pm 5.5 \times 10^3 \text{ cells ml}^{-1})\) was higher than in seagrass incubations \((6.7 \pm 2.9 \times 10^3 \text{ cells ml}^{-1})\); Table 4). Bacterial abundance was positively correlated with DOC concentrations during the experiment \((r = 0.39; \text{Fig. 3}); \text{i.e.}, high abundances of bacteria occurred when concentrations of DOC were high in the water column.

### Table 4: Abundance of bacteria and cyanobacteria \(\text{(cell ml}^{-1})\); means ± standard deviation under initial conditions and after 24 h.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Bacterial abundance ((\times 10^9 \text{ cell ml}^{-1}))</th>
<th>Cyanobacterial abundance ((\times 10^3 \text{ cell ml}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>1.2 ± 1.1</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>Seawater</td>
<td>1.9 ± 0.4</td>
<td>9.6 ± 1.4</td>
</tr>
<tr>
<td>Seagrass</td>
<td>3.3 ± 0.7</td>
<td>6.7 ± 2.9</td>
</tr>
<tr>
<td>Coral</td>
<td>2.5 ± 0.5</td>
<td>12.7 ± 5.5</td>
</tr>
<tr>
<td>Sg+Cr</td>
<td>3.4 ± 0.5*</td>
<td>18.9 ± 0.7</td>
</tr>
</tbody>
</table>

Two-way ANOVAs and post hoc Tukey’s tests were used to determine significant differences between incubation conditions compared to controls (seawater). * Indicates a significant difference at \(p < 0.01\).

3.4 Primary production

Primary production in seawater controls and in the surrounding waters of incubated organisms (seagrass, coral, and sg+cr) was measured during the experiment. Moreover, carbon fixation rates in seagrass and coral tissues were also measured under the various incubation conditions (Table 5). The highest primary production in the seawater occurred in the seagrass incubations \((15.3 \mu\text{mol C l}^{-1} \text{ day}^{-1})\), followed by the sg+cr incubations \((10.0 \mu\text{mol C l}^{-1} \text{ day}^{-1})\). However in the coral incubations, primary production in the water column was lower than in controls, indicating that coral consumed all available carbon in the water column. Carbon fixation rates in tissues were higher in seagrass tissues \((991.4 \mu\text{mol g}^{-1} \text{ wet weight day}^{-1})\), but values decreased in sg+cr \((897.6 \mu\text{mol g}^{-1} \text{ wet weight day}^{-1})\). Primary production in coral tissues was higher in sg+cr incubations \((13.6 \mu\text{mol g}^{-1} \text{ wet weight day}^{-1})\) than in coral-only incubations \((9.0 \mu\text{mol g}^{-1} \text{ wet weight day}^{-1})\).

4. Discussion and conclusions

The present study was conducted to examine the coexistence of seagrass \((\text{Thalassia hemprichii})\) and coral \((\text{Montipora digitata})\) in terms of nutrient dynamics, DOC cycling, and the role of microbes. Nutrient dynamics indicated that the uptake of ammonia and phosphate was higher in coral incubations than in seagrass incubations, whereas seagrass exhibited higher uptake of nitrate than did coral. In sg+cr incubations, higher nitrate and phosphate uptake was observed. The higher uptake of ammonia in coral may be because ammonia is the primary source of nitrogen for the coral-zooxanthellae complex, as it can be easily taken up through passive diffusion. Both host and symbiont can convert ammonia to amino acids, which is not the case for nitrate. Only zooxanthellae have the ability to convert nitrate to ammonia and subsequently assimilate it into

### Table 5: Net primary production \(\text{(NPP; µmol C l}^{-1} \text{ day}^{-1})\) and change \(\Delta\) of NPP compared to controls (seawater). Production of carbon \((\mu\text{mol C g}^{-1} \text{ wet weight day}^{-1})\) in the tissues of seagrass and coral.

<table>
<thead>
<tr>
<th>Condition</th>
<th>NPP</th>
<th>Δ NPP</th>
<th>Seagrass tissue</th>
<th>Coral tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater</td>
<td>9.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Seagrass (Sg)</td>
<td>15.3</td>
<td>6.0</td>
<td>991.4</td>
<td>-</td>
</tr>
<tr>
<td>Coral (Cr)</td>
<td>6.5</td>
<td>-2.8</td>
<td>-</td>
<td>9.0</td>
</tr>
<tr>
<td>Sg+Cr</td>
<td>10.0</td>
<td>0.7</td>
<td>897.6</td>
<td>13.6</td>
</tr>
</tbody>
</table>
amino acids (Sheppard et al., 2009). Nitrate uptake also occurs in both coral and zooxanthellae, but only zooxanthellae can convert nitrate to ammonia for synthesis of amino acids. In the case of seagrass, ammonia, nitrate, and phosphate are used as ambient sources of nitrogen and phosphorus (Hemminga and Duarte, 2000). Overall, concentrations of nitrate and phosphate were lower compared to initial concentrations in all incubations (Table 1). In addition, controls took up nitrate and phosphate compared to the values prior to incubation, indicating that the planktonic community and microbes compete for nitrate uptake. Coincident with the uptake of nitrate and phosphate, bacterial abundance also increased (Table 4).

Microbes also compete for the consumption of nutrients; therefore, an increase in bacterial populations also suggests the consumption of nutrients (Ye et al., 2012). The highest uptake of nutrients and the highest abundance of heterotrophic bacteria were both observed in the sg+cr incubations. In addition, the abundance of bacteria was significantly negatively correlated with the concentrations of nitrate \( (r = -0.42, p < 0.05, n = 24) \) and phosphate \( (r = -0.41, p < 0.05, n = 24) \). Cyanobacterial abundance was also significantly negatively correlated with the concentrations of nitrate \( (r = -0.56, p < 0.01, n = 24) \) and phosphate \( (r = -0.62, p < 0.01, n = 24; \) Glibert et al., 2004; Boyer et al., 2006; Michelou, 2010). Cyanobacteria photosynthesize and proliferate in the presence of light by taking up nutrients from seawater. These photosynthetic products are released into the surrounding water, which could also account for the increase in DOC concentrations during the experiment (Ye et al., 2012). Similarly, several studies have demonstrated that the release of DOC from the cyanobacteria Prochlorococcus accounts for \(~9-24\%) of total assimilated inorganic carbon, with slightly lower values for nitrate- and phosphorus-limited cultures (Bertilsson et al., 2005; Znachor and Nedoma, 2009).

During the present experiment, seagrasses made a greater contribution to the DOC pool compared to corals. In addition, this treatment also exhibited high concentrations of DO (Table 2) and high net primary production (Table 5), resulting in the release of DOC from seagrass and other organisms in the incubation bottles. The increase in DO concentration due to increased photosynthesis led to the high metabolic rate. Thus, comparatively large amounts of DOC were released to the surrounding seawater during the daytime (photosynthetic active period; Table 3). Similarly, several studies have reported increased oxygen concentrations and DOC during the day (Ziegler and Benner, 1999; Barn and Duarte, 2009; Haas et al., 2011). In addition, production of DOC was higher during the photosynthetic period compared to at night under all incubation conditions (Fig. 2 and Table 3). Furthermore, the concentrations of DOC and DO in the water column were significantly positively correlated \( (r = 0.42, p < 0.05, n = 24; \) Fig. 2), indicating that the increases in DOC concentrations were related to increases in DO due to photosynthesis. During the experiment, lower values of DO (Table 2) and primary production (Table 5) were observed in coral incubations relative to seagrass incubations, potentially due to the lower photosynthetic activity of coral symbionts compared to seagrasses, which have higher photosynthetic capacity. Because primary production was lower for coral, the exudation of DOC was restricted to improve carbon assimilation in the tissues.

The assimilation of carbon in coral tissue was higher when incubated together with seagrass than when coral was incubated alone. However, seagrass tissue exhibited lower assimilation of carbon in sg+cr incubations. These results suggest that in sg+cr treatments, higher DOC release rates from seagrasses support and benefit carbon assimilation in coral tissue, which may be due to the role of bacteria in the remineralization of organic to inorganic constituents (Hemminga and Duarte, 2000; Haas et al., 2011). During this process, inorganic carbon released through bacterial consumption of DOC may support carbon assimilation in coral (Fig. 4). In addition, DOC concentration and bacterial abundance were positively correlated in the water column \( (r = 0.39, p < 0.05, n = 24; \) Fig. 3). Therefore, the increase in bacterial abundance could be linked to DOC recycling in the incubation experiment, as bacteria can use DOC for growth and proliferation (Fig. 4). The abundance of bacteria in the water column was higher in both the seagrass and sg+cr incubations compared to the other treatments. However, despite the highest concentration of DOC being in the seagrass incubation, the abundance of bacteria in the water column was similar to that in sg+cr incubations. These microbes were likely exposed to an initial surplus of labile carbon, after which they were able to utilize the refractory carbon over a longer time scale (Zweifel et al., 1993; Haas et al., 2013). DOC from the surrounding water within the seagrass incubations may have contained greater refractory than labile carbon. Bacterial abundance in this incubation treatment was similar to that in the sg+cr treatment because high
refractory DOC in the seagrass incubation was not utilized as rapidly as was labile DOC.

Our results indicate that areas in which seagrasses and coral coexist harbor a constant supply of DOC, mainly exuded from seagrasses, which can be utilized by corals through microbial activity, resulting in increased carbon assimilation by corals. In contrast, seagrasses can assimilate nutrients and microbially degraded DOC from coral metabolism in the area of coexistence. Overall, our findings clarify several of the processes involved in the coexistence of seagrasses and corals; however, the long-term sustainability of this coexistence remains unpredictable due to the dynamic nature of the coral-reef environment.

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