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REPORT

Distribution of dissolved organic carbon and nitrogen in a coral reef

Y. Tanaka · T. Miyajima · A. Watanabe · K. Nadaoka · T. Yamamoto · H. Ogawa

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Abstract Dissolved organic matter (DOM) concentrations in a fringing coral reef were measured for both carbon and nitrogen with the analytical technique of high-temperature catalytic oxidation. Because of high precision of the analytical system, not only the concentrations of dissolved organic carbon and nitrogen (DOC and DON, respectively) but the C:N ratio was also determined from the distribution of DOC and DON concentrations. The observed concentrations of DOC and DON ranged 57–76 and 3.8–5.6 μmol l^{-1}, respectively. The C:N ratios of the DOM that was produced on the reef flat were very similar between seagrass- and coral-dominated areas; the C:N ratio was 10 on average. The C:N ratio of DOM was significantly higher than that of particulate organic matter (POM) that was produced on the reef flat. Production rates of DOC were measured on the reef flat during stagnant periods and accounted for 3–7% of the net primary production, depending on the sampling site. The production rate of DON was estimated to be 10–30% of the net uptake of dissolved inorganic N in the reef community. Considering that the DOM and POM concentrations were not correlated with each other, a major source of the reef-derived DOM may be the benthic community and not POM such as phytoplankton. It was concluded that a widely distributed benthic community in the coral reef released C-rich DOM to the overlying seawater, conserving N in the community.

Keywords DOM · POM · Coral reef · C:N ratio · Primary production

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Y. Tanaka (✉) · A. Watanabe · K. Nadaoka · T. Yamamoto
Graduate School of Information Science and Engineering, Tokyo Institute of Technology, Ookayama 2-12-1, Meguro-ku, Tokyo 152-8552, Japan
e-mail: tanaka.yask@gmail.com

T. Miyajima · H. Ogawa
Marine Biogeochemistry Laboratory, Atmosphere and Ocean Research Institute, The University of Tokyo, Kashiwanoha 5-1-5, Kashiwa, Chiba 277-8564, Japan

Introduction

Coral reefs have developed in tropical and subtropical areas, which are surrounded by oligotrophic seawater. The concentrations of inorganic nutrients in coral reef waters are typically 0.1–1.0 μmol l^{-1} for dissolved inorganic nitrogen (N) and 0.05–0.30 μmol l^{-1} for dissolved inorganic phosphorus (P) (Atkinson and Falter 2003). In spite of the low nutrient environment, gross primary production of organic carbon (C) per unit surface area of coral reefs is the highest among marine ecosystems (Gattuso et al. 1998; Atkinson and Falter 2003). A prevalent hypothesis to explain this paradoxical ecosystem is that the relationship between heterotrophs and autotrophs is physically close, and thus, dissolved inorganic N and P (DIN and DIP, respectively) are rapidly recycled within the coral reef community. A more recent hypothesis is that low-quality (i.e., high C:N or C:P ratios) dissolved and particulate organic matter (DOM and POM, respectively) are continuously produced and released by the biota, in order to conserve N and P (Atkinson and Falter 2003). A major obstacle in understanding coral reef biogeochemistry has been that the fluctuation of DOM, POM, and inorganic nutrient concentrations is small and often not well documented.

DOM and POM produced in a coral reef function as energy sources for microorganisms where the energy is

transferred to higher trophic levels via the microbial food web (Ferrier-Pagès and Furla 2001; Rochelle-Newall et al. 2008). Because the availability of N and P strongly controls the biological metabolism in oligotrophic coral reef waters, it is necessary to measure the quality of produced DOM and POM (i.e., C:N:P ratios) to understand the biogeochemical cycle of coral reef ecosystems. Since DOM is a much larger pool than POM in coral reefs (Hata et al. 2002; Atkinson and Falter 2003; Miyajima et al. 2007a), DOM should have a more important contribution to the planktonic food web. Considering that DIN is generally very low in coral reefs, DON might be the most important N source for microorganisms. However, there have been very few data sets on both DOC and DON concentrations in coral reefs (Miyajima et al. 2007a). Thus, the C:N ratio of the bulk DOM produced in coral reefs has not been precisely evaluated.

Until recently, precise routine measurements of DOM in oligotrophic seawater were lacking (Sharp 2002; Ogawa and Tanoue 2003). In the 1990s, seawater DOM concentration was determined via simultaneous measurement of DOC and DON (actually, total dissolved N; TDN) using high-temperature catalytic oxidation (HTCO) (Hansell et al. 1993; Kähler et al. 1997). Though the analysis of DON was relatively imprecise, Ogawa et al. (1999) and Sharp et al. (2004) improved the simultaneous measurement system and obtained much higher precision for DON. Although these improved measurements of DOC and DON have been applied for many marine ecosystems (summarized by Carlson 2002), coral reefs still lack in reliable DOM data, especially for DON. If the HTCO technique is applied to coral reef waters, it may be possible to detect the dynamics of DOC and DON concentrations and to obtain the C:N ratios with small error of analysis.

The purpose of this study was (1) to precisely measure the concentrations of DOC and DON in a coral reef using a HTCO technique and to determine C:N ratios of the DOM and (2) to evaluate the difference in the C:N ratio between DOM and POM that were produced in a coral reef. At extreme low tide, the seawater overlying the coral reef of the study site was separated from the outer ocean because of the emerging reef crest (stagnant period). Using this period, the production rate of DOC was also quantified on the reef flat and was compared with the corresponding primary production rate.

Materials and methods

Study site

This study was performed in August and September 2009 at Shiraho fringing reef (24°21–22′N, 124°15′E) in Ishigaki Island, Japan. The reef has a shallow flat lagoon (reef flat), with a depth of 0.5–4.0 m, surrounded by the reef crest and the shoreline: the crest is developed roughly parallel to the shoreline at a distance of ca. 850 m (Fig. 1). The reef flat exchanges seawater with the outer ocean during the high tide (flooding) period. Water turnover time on the reef flat during the flooding period has been estimated to be ca. 3 h (H. Yamano personal observation). At spring tide, the seawater on the reef flat is isolated from the outer ocean by the emerged reef crest for 1–6 h (depending on the tide range), and the water current on the reef flat is stagnant (stagnant period).

The biota on the reef flat shows broadly belt-like zonation (Miyajima et al. 2007a, b; Nakamura and Nakamori 2009): Seagrass and benthic algae occurred up to 200 m from the shoreline, and large colonies of hermatypic corals abundantly inhabited the zone between 400 and 700 m from the shoreline. Dominant coral species were *Acropora* spp., *Porites* spp., and *Montipora* spp. A gap zone between the seagrass bed and the coral community was mainly covered with pristine carbonate sand with sparse patches of living corals (Nakamura and Nakamori 2009).

Fig. 1 The location of sampling stations at Shiraho fringing reef (Image: IKONOS^{TM} image taken on 26 Aug 2003)

Water sampling

Water sampling was mainly conducted at four stations (Sta) in the coral reef: two of which were located 100–150 m from the shoreline, where the bottom substrate was mainly dominated by seagrass and benthic microalgae (Sta. SG1 and SG2; Fig. 1). The other two sampling stations were located 400–500 m from the shoreline, where the bottom was mainly covered by branching and massive corals (Sta. CR1 and CR2; Fig. 1). At these four stations, seawater was sampled on the boat every hour for 24 h as scheduled in Table 1. In order to compare between lagoonal and oceanic seawater, oceanic seawater was also sampled at Sta. B (Fig. 1) once on 24th August and then again on 11th September (Table 1).

Seawater samples were immediately filtered on pre-combusted (450°C for 3 h) Whatman GF/F filters (pore size: 0.7 μm, filter diameter: 25 mm) to obtain DOM, using a hand-operated glass syringe (100 ml). The filtrate was placed in pre-combusted (550°C for 5 h) glass ampoules (20 ml) for DOC, DON, and inorganic nutrients analysis. The remaining filtrate was placed in acid-washed polypropylene bottles for DOP analysis. All filtrates were stored at −20°C until the analyses. For POM samples, a 10-l seawater sample was filtered with pre-combusted GF/F filters (47 mm diameter) and the filters were stored at −20°C until analysis. A 300-ml seawater sample for chlorophyll *a* (Chl *a*) was filtered on a GF/F filter (25 mm diameter), and the filters were placed in *N*, *N*-dimethylformamide (DMF) to extract Chl *a*. The solvent for Chl *a* analysis was stored at −20°C until analysis. During the survey periods, seawater temperature was measured with a sensor (Compact-CT; JFE Advantec). Water pressure was also monitored at the reef bottom of each sampling station using a logger (HOBO; Onset) in order to calculate the fluctuation of the water level. The salinity was determined with a Portasal salinometer (8410A; Guildline Instruments).

To measure the rates of DOC production and net primary production on the reef flat, additional seawater sampling was conducted at two stations on the reef flat during daytime and nighttime (Sta. S1 and S2; Fig. 1, Table 1). There were clearly two high tide and two low tide periods in the day. The seawater level at Sta. B was below the mean level at ca. 10:30–15:30 h on 3rd and 4th September and at ca. 2:00–7:00 h on 10th and 11th September. The daytime and nighttime sampling was conducted when the seawater level at Sta. B was below the mean level and thus the water current on the reef flat was stagnant. At these stations, the bottom was sparsely covered with branching and massive corals. Because the seawater on the reef flat was minimally exchanged with the outer ocean during these periods, the change in the measured chemical parameters of the lagoonal seawater was influenced only by the inner-reef biogeochemical cycle. The seawater was sampled every 30 min and was processed for DOC analysis as above. To measure dissolved inorganic C (DIC) and total alkalinity (TA), 300 ml of seawater was collected in borosilicate glass bottles, which were immediately fixed with saturated mercuric chloride ($HgCl_2$) to a final concentration of 0.03% (v/v).

Chemical analyses and calculations

The concentrations of DOC and TDN in seawater were measured by the HTCO technique using the combination of total organic C analyzer (TOC-5000; Shimadzu) and NOx analyzer (ECL-880 US; Yanaco) (Ogawa et al. 1999). Each seawater sample was injected into the analytical system 3–5 times, and standard deviation (SD) of the analysis was within the range of 0.0–1.3 μmol l^{-1} (average

Table 1 Summary of the survey periods at each sampling station, and the mean values ± SD of water temperature (Temp.), salinity, chlorophyll *a* (Chl *a*) concentrations, and dissolved inorganic N (DIN) and P (DIP) concentrations during each survey period

Sta.	Survey period	Temp. (°C)	Salinity (psu)	Chl *a* (μg l^{-1})	DIN (μmol l^{-1})	DIP (μmol l^{-1})
B	24/Aug 11:00 h	29.6	33.9	0.33	0.83	0.06
SG1	29/Aug 9:00 h–30/Aug 8:00 h	31.6 ± 1.1	33.2 ± 0.4	0.43 ± 0.17	1.3 ± 0.7	0.03 ± 0.01
SG2	30/Aug 10:00 h–31/Aug 10:00 h	31.0 ± 1.4	33.4 ± 0.2	0.36 ± 0.14	0.42 ± 0.31	0.03 ± 0.01
S1	3/Sep 10:30–13:30 h	30.0 ± 0.3	33.7 ± 0.0	0.18 ± 0.03	0.35 ± 0.03	0.03 ± 0.01
S2	4/Sep 11:00 h–13:00 h	31.0 ± 0.2	33.9 ± 0.0	0.20 ± 0.02	0.40 ± 0.03	0.04 ± 0.00
CR1	6/Sep 8:00 h–7/Sep 8:00 h	29.5 ± 0.7	34.0 ± 0.0	0.28 ± 0.07	0.54 ± 0.23	0.06 ± 0.01
CR2	7/Sep 10:00 h–8/Sep 10:00 h	29.2 ± 0.4	33.9 ± 0.0	0.24 ± 0.09	0.38 ± 0.11	0.04 ± 0.01
S1	10/Sep 2:30–5:30 h	28.8 ± 0.1	33.6 ± 0.0	0.21 ± 0.02	0.87 ± 0.12	0.05 ± 0.01
S2	11/Sep 2:30–5:30 h	29.8 ± 0.1	34.0 ± 0.0	0.18 ± 0.01	0.55 ± 0.10	0.03 ± 0.00
B	12/Sep 13:00 h	30.5	34.0	0.11	0.79	0.05

0.5 μmol l^{-1}) for DOC and 0.00–0.11 μmol l^{-1} (average 0.05 μmol l^{-1}) for TDN. DIN (NO_3^-, NO_2^-, NH_4^+) and phosphate (PO_4^{3-}) concentrations were quantified with a nutrient analyzer (AACS-III; BRAN + LUEBBE). DON concentrations were calculated by subtraction of DIN from TDN. Because the analytical error for DIN concentrations was <0.02 μmol l^{-1}(depending on the nutrient species), which was determined by the analysis of standard solutions, each seawater sample was measured once. Thus, SD of DON was calculated to be equal to SD of TDN (i.e., ±0.05 μmol l^{-1} on average). DOP concentration in seawater was quantified by the high-temperature dry-combustion method described by Suzumura (2008).

The GF/F filters on which POM was collected were dried in an oven at 50°C and were treated with vapor of 12 N HCl for 12 h at a room temperature to remove inorganic C. Excess HCl was then removed completely from the filters by placing the filters in a vacuum desiccator with NaOH pellets for at least 1 week. Particulate organic C and N (POC and PON, respectively) on the filters were quantified with a CHN analyzer (FLASH-EA; Thermo-Fisher Inc.). Chl *a* content in DMF extract was analyzed using a fluorometer (10AU; Turner Designs) (Suzuki and Ishimaru 1990).

To calculate photosynthetic organic production, DIC and TA concentrations in seawater were determined by a continuous-flow-type analyzer (Watanabe et al. 2004, 2006). Both DIC and TA were calibrated using at least three different batches of certified reference materials, which were distributed by Scripps Institution of Oceanography, USA (Watanabe et al. 2004, 2006). The precisions of DIC and TA were 2 and 1.5 μmol kg^{-1}, respectively.

The differences in DOM and POM concentrations between Sta. SG1 and SG2 and between Sta. CR1 and CR2 were evaluated by two-sided Student's *t* test. The relations between C and N concentrations for both DOM and POM were assessed with simple regression analysis. The slopes (regression coefficients) of the regression lines were interpreted to be the C:N ratios of the organic matter that was produced on the reef flat. The differences of the slopes between Sta. SG1 and SG2 and between Sta. CR1 and CR2 were determined by testing the *t* value. For both the statistical analyses of DOM and POM concentrations and C:N ratios, when significant differences were not found between Sta. SG1 and SG2 and between Sta. CR1 and CR2, the data were combined for each dominating benthic community (SG or CR) and the difference between the two groups was evaluated again as shown above. The correlation between DOM and POM was investigated by Pearson's correlation coefficient test for both C and N. A difference at the 5% level was considered as significant for all the statistical tests.

For the survey of the stagnant periods, linear regression was conducted with the graphic software SigmaPlot 8.02 to determine the rate of change in DOC, DIC, and TA concentrations with time (mmol m^{-3} h^{-1}). Because the water depth was almost constant on the reef flat during the stagnant period, the rate of change in DOC, DIC, and TA concentrations was converted to the rate of change per unit horizontal surface area of the reef flat (mmol m^{-2} h^{-1}) by multiplying the rate per volume (mmol m^{-3} h^{-1}) by average water depth (m) during the stagnant period at each sampling station. The rate of net organic C fixation (net primary production, NPP: mmol m^{-2} h^{-1}) in coral reefs can generally be calculated from DIC and TA (e.g., Watanabe et al. 2006): when one mole of organic C is produced by photosynthesis, one mole of DIC decreases. When respiration occurs, the opposite reaction proceeds. Similarly, when one mole of calcium carbonate ($CaCO_3$) is produced by calcification, one mole of DIC decreases. Considering that only the process of calcification changes TA and that two moles of TA decrease with the production of one mole of $CaCO_3$, NPP can be calculated as follows:

$$\mathrm{NPP} = -(R_{\mathrm{DIC}} - R_{\mathrm{TA}} \times 0.5) \quad (1)$$

where R_{DIC} and R_{TA} are the rate of change in DIC and TA per unit horizontal surface area of the reef flat (mmol m^{-2} h^{-1}).

Results

Fluctuation of DOC and DON

The concentration of bulk DOM on the reef flat fluctuated within the range of 57–76 μmol l^{-1} for DOC, 3.8–5.6 μmol l^{-1} for DON, and 0.13–0.27 μmol l^{-1} for DOP (average ± SD given in Table 2). The fluctuations of DOC and DON concentrations tracked well with each other (Fig. 2), while DOP fluctuations did not follow DOC or DON. The concentrations of DOC, DON, and DOP were not correlated with the salinity at any sampling stations. The concentrations of DOC and DON were not significantly different between Sta. SG1 and SG2 and between Sta. CR1 and CR2 (Table 2). Combining the data for each dominating benthic community, the seawater sampled at the seagrass-dominated stations (the data set of Sta. SG1 + SG2) contained significantly more DOC and DON than that sampled at the coral-dominated stations (the data set of Sta. CR1 + CR2; $P < 0.01$). The seawater sampled out of the reef flat (Sta. B) on different days showed that the DOM concentration at Sta. B was steady and was lower than those on the reef flat for both C and N (Table 2).

The slopes of the relationship between DOC and DON concentrations were not significantly different between Sta. SG1 and SG2 and between Sta. CR1 and CR2. After combining the data for each benthic community, the observed C:N ratio was 10.4 at the seagrass-dominated

Table 2 The average concentrations ± SD of DOM (C, N, P) and POM (C, N) observed at each station during the 24-h survey scheduled in Table 1

	DOM (μmol l^{-1})			POM (μmol l^{-1})	
Sta.	DOC	DON	DOP	POC	PON
SG1	68 ± 3	5.0 ± 0.3	0.19 ± 0.02	6.2 ± 0.9	0.87 ± 0.16
SG2	69 ± 5	4.9 ± 0.4	0.22 ± 0.03	6.5 ± 1.0	0.98 ± 0.19
CR1	60 ± 2	4.1 ± 0.2	0.17 ± 0.02	4.8 ± 1.6	0.67 ± 0.25
CR2	62 ± 3	4.2 ± 0.2	0.18 ± 0.03	4.8 ± 1.4	0.66 ± 0.19
B	57 ± 1	4.0 ± 0.0	0.16 ± 0.01	3.2 ± 0.1	0.47 ± 0.03

For Sta. B, means ± range of duplicate seawater samples taken on the 2 days are shown

stations (the data set of Sta. SG1 + SG2; Fig. 3a) and was 10.3 at the coral-dominated stations (the data set of Sta. CR1 + CR2; Fig. 3b).

The concentrations of DOC during the stagnant periods of daytime and nighttime were in the range of 56–62 μmol l^{-1} at Sta. S1 and of 59–65 μmol l^{-1} at Sta. S2. The concentrations increased in the daytime (Fig. 4a) and decreased in the nighttime (Fig. 4b). Net fluxes of DOC at Sta. S1 and S2 were 3.9 and 1.7 mmol m^{-2} h^{-1} in the daytime and −1.9 and −0.8 mmol m^{-2} h^{-1} in the nighttime, respectively (Table 3). Both DIC and TA concentrations decreased in the daytime (Fig. 4a), while they were constant in the nighttime (Fig. 4b): net fluxes of DIC at Sta. S1 and S2 were −37 and −45 mmol m^{-2} h^{-1} in the daytime and 0.1 and 8.5 mmol m^{-2} h^{-1} in the nighttime, respectively. Net fluxes of TA at Sta. S1 and S2 were −16 and −14 mmol m^{-2} h^{-1} in the daytime and −2.4 and 0.1 mmol m^{-2} h^{-1} in the nighttime, respectively. Multiplying the daytime and nighttime flux by 12 h each, the net diel DOC flux was estimated to be 24 and 12 mmol m^{-2} d^{-1} at Sta. S1 and S2, respectively. Similarly, the net diel primary production (NPP) calculated from DIC and TA was 335 and 359 mmol m^{-2} d^{-1}, and thus, the percentage of DOC flux to NPP was 7.2 and 3.3% at Sta. S1 and S2, respectively (Table 3).

Comparison between DOM and POM

The concentration of individual sample bulk POM over the reef flat fluctuated with the ranges of 2.8–9.0 μmol l^{-1} for POC, 0.39–1.37 μmol l^{-1} for PON (average ± SD given in Table 2). The concentrations of POC and PON were not significantly different between Sta. SG1 and SG2 and between Sta. CR1 and CR2 (Table 2). Combining the data for each dominating benthic community, the seawater sampled at the seagrass-dominated stations (the data set of Sta. SG1 + SG2) contained significantly more POC and PON than that sampled at the coral-dominated stations (the data set of Sta. CR1 + CR2; $P < 0.01$).

The slopes of the relationship between POC and PON concentrations were not significantly different between Sta. SG1 and SG2 and between Sta. CR1 and CR2. Combining the data for each benthic community, the slopes showed that the C:N ratios of POM produced on the reef flat were 4.6 at the seagrass-dominated stations (Sta. SG1 + SG2; Fig. 3a) and 6.5 at the coral-dominated stations (Sta. CR1 + CR2; Fig. 3b). These C:N ratios were significantly different from each other ($P < 0.01$). The C:N ratios of DOM were significantly higher than those of POM at both seagrass-dominated and coral-dominated stations ($P < 0.01$) (Fig. 3a, b).

There was no significant correlation between DOM and POM concentrations for both C and N, except for Sta. SG1. At Sta. SG1, negative correlation was observed between

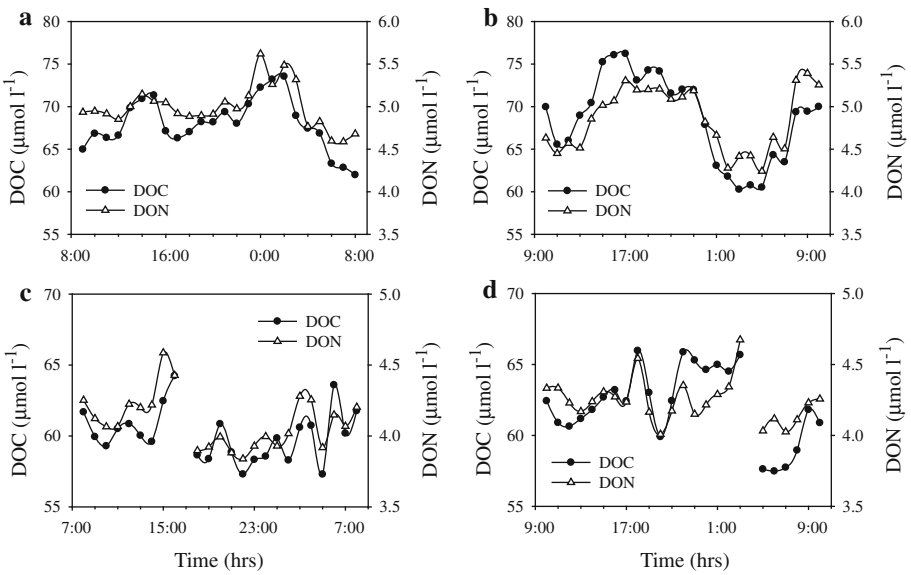

Fig. 2 Diel fluctuation of DOC (*black circle*) and DON (*white triangle*) concentrations at Sta. SG1 (**a**), SG2 (**b**), CR1 (**c**), and CR2 (**d**)

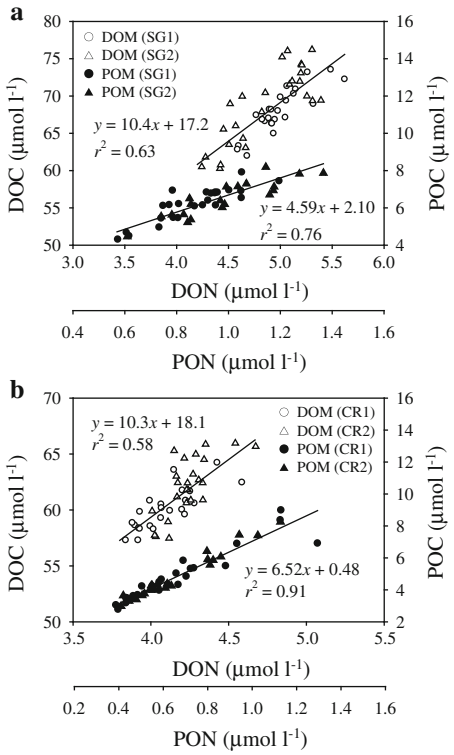

Fig. 3 The relationship between C and N concentrations for both DOM (*white*) and POM (*black*) at seagrass-dominated (**a**) and coral-dominated (**b**) stations. Regression lines were made for the combined data of DOM for Sta. SG1and SG2; POM for Sta. SG1 and SG2; and for DOM and POM for Sta. CR1 and CR2. The slopes of the regression lines indicate C:N ratios of DOM and POM produced on the reef flat

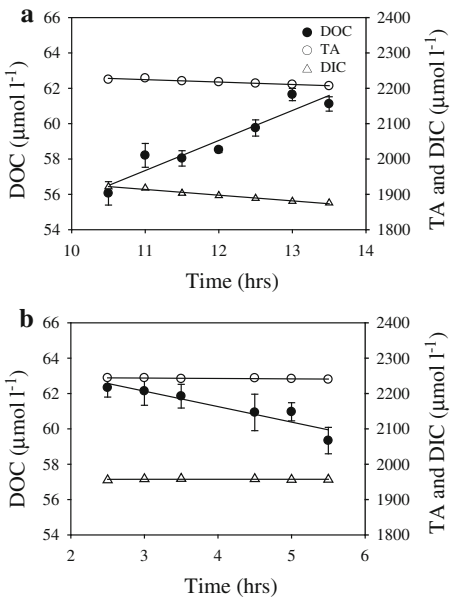

Fig. 4 The changes in DOC, TA, and DIC concentrations during the stagnant periods of daytime (**a**) and nighttime (**b**) at Sta. S1. The DOC concentrations are shown as means ± SD of analysis

Table 3 Means ± SE of net DOC flux and net primary production (NPP) were calculated from the survey during the daytime and nighttime stagnant periods at Sta. S1 and S2

	Daytime flux (mmol m^{-2} h^{-1})		Nighttime flux (mmol m^{-2} h^{-1})		Diel flux (mmol m^{-2} d^{-1})	
Sta.	DOC	NPP	DOC	NPP	DOC	NPP
S1	3.9 ± 0.6	29 ± 1	−1.9 ± 0.4	−1.3 ± 0.5	24 ± 12	335 ± 19
S2	1.7 ± 0.2	38 ± 5	−0.8 ± 0.4	−8.5 ± 1.5	12 ± 7	359 ± 73

The diel flux was estimated by multiplying the obtained daytime and night time flux by 12 h each

DOC and POC concentrations ($r^2 = 0.20$, $P < 0.05$) and between DON and PON concentrations ($r^2 = 0.19$, $P < 0.05$).

Chl *a* and nutrient concentrations

The average concentration of Chl *a* at each sampling station ranged from 0.11 to 0.43 µg l^{-1} (Table 1). Higher Chl *a* concentrations were observed at Sta. SG1 and SG2 than at the other stations. The average concentration of DIN was <0.9 µmol l^{-1} at most sampling stations, but was higher at Sta. SG1 (1.3 µmol l^{-1}). DIP concentrations were within the range of 0.03–0.06 µmol l^{-1} (Table 1).

Discussion

This is the first study that simultaneously reports the concentrations of DOC and DON on a coral reef using a HTCO technique. The measurement system used in this study had analytical errors of <1.3 µmol l^{-1} (average 0.5 µmol l^{-1}) for DOC and <0.12 µmol l^{-1} (average 0.05 µmol l^{-1}) for DON. This high precision revealed very consistent fluctuation between DOC and DON concentrations (Fig. 2), implying that the DOM produced on the reef flat had a constant C:N ratio.

The relationship between C and N concentrations of DOM and POM gives information about the organic matter that was produced on the reef flat and the observed DOM had a similar C:N ratio irrespective of the sampling stations (Fig. 3a, b). Because the bulk DOM concentration was not positively correlated with POM, it was not likely that the DOM was directly released from phytoplankton in the water column. Because the DOM concentration was not negatively correlated with the salinity, it was also not likely that DOM was delivered to the reef flat via river and/or groundwater discharge. The remaining possibility was that DOM was released from benthic communities on the reef flat. Rochelle-Newall et al. (2008) incubated the seawater in the lagoon of New Caledonia and showed that DOC production by phytoplankton did not fulfill bacterial C

demand in the seawater, which suggested that the bacteria utilized DOC released from the benthic community. Several DOM sources could be considered in benthic processes of coral reefs: (1) direct release by benthic organisms, e.g., coral-zooxanthellae symbiotic colonies (Ferrier-Pagès et al. 1998; Wild et al. 2008; Tanaka et al. 2008, 2009, 2010), seagrass (Kirkman and Reid 1979; Ziegler and Benner 1999), benthic algae (Haas and Wild 2010) and (2) solubilization of organic matter on sediment by bacteria (Smith et al. 1992; Urban-Rich 1999). The C:N ratios of DOM released by corals have not been constant in previous studies: 2.5–13 (Ferrier-Pagès et al. 1998), 5.4–9.5 (Tanaka et al. 2009), 21 ± 5 (Tanaka et al. 2010). This inconsistency might be caused by the difference in experimental conditions such as coral species. As for seagrass and benthic algae, there are no previous studies on C:N ratios of the released DOM. Therefore, it is difficult to specify the major DOM producer on the reef flat from C:N ratios of DOM released from each benthic community.

Considering that DOM had a similar C:N ratio in seagrass- and coral-dominated areas, the second possibility, i.e., solubilization of organic matter in the sediment by bacteria, may be the major DOM producer from the sediment to the water column, because it could occur everywhere on the reef flat. Wild et al. (2004) reported that mucoid organic matter released from corals (coral mucus) gradually sank to the sediment with the attachment of POM and was utilized by bacteria on the surface of the sediment. Due to the activity of bacterial hydrolytic ectoenzymes, a part of the settled organic matter might be continuously released in the form of DOM (Smith et al. 1992). The organic matter that could become a source of DOM might not be necessarily derived from coral mucus: Urban-Rich (1999) showed that DOC was released from zooplankton fecal pellets. Whatever the source of the accumulated organic matter on the reef sediment, the hypothesis that widely distributed sediment was a major DOM source for the lagoonal seawater due to bacterial degradation activity might explain the very similar C:N ratio at the seagrass- and coral-dominated areas in the present study site (Fig. 3a, b). Significantly higher DOM concentrations at Sta. SG1 and SG2 than Sta. CR1 and CR2 (Table 2) suggested that the seagrass-dominated benthic community retained more organic matter in the sediment (Miyajima et al. 1998) and thus acted as a larger DOM producer for the reef seawater.

The POM produced on the reef flat had a C:N ratio near to or less than the Redfield ratio (6.6; Fig. 3a, b). The observed ratios were lower than previously reported C:N ratios of POM released from corals (7.8–19; Wild et al. 2005; Tanaka et al. 2008) and benthic algae (12 on average; Haas et al. 2010), implying that most of POM observed in the present study was not released from those benthic organisms but was composed of planktonic microorganisms and fresh detritus. On the other hand, the DOM produced on the reef flat had a C:N ratio of 10, which was significantly higher than that of POM at both seagrass- and coral-dominated areas (Fig. 3a, b). Supposing that DOM was derived from solubilization of organic matter due to bacterial degradation, the released DOM would have a higher C:N ratio than the original organic matter because bacteria preferentially solubilize organic N and P and incorporate this for cell synthesis (Smith et al. 1992). The remaining C-rich DOM might have been released from the sediment to the overlying water column. Even if the major DOM producer was not heterotrophic bacteria but photosynthetic communities, it could be possible that DOM had a significantly higher C:N ratio than POM: it is well known that under sufficient light and low nutrient concentrations, phytoplankton release excess organic C as DOC to the ambient seawater (Carlson 2002). Because coral reefs are certainly situated in typically high light and low nutrient conditions, primary producers would have a metabolic property to conserve N and P and to release excess C-rich organic matter. Sakka et al. (2002) observed high percentage of extracellular DOC release (34–68%) to the total primary production by phytoplankton in a coral reef and explained the metabolic property by nutrient limitation. Though it is difficult to conclude that phytoplankton were the major source of DOM in the present study site (see above), it was also reported that the coral *Acropora pulchra* released DOM of higher C:N ratios than that of the original coral tissue (Tanaka et al. 2009). This difference in the C:N ratios could be due to the coral metabolic property to conserve N in the tissue (Tanaka et al. 2006). Though the actual DOM source was not exactly determined, the observation that DOM had a higher C:N ratio than POM corresponded to the findings that each reef organism had a property to conserve N.

During the stagnant periods, the concentration of DOC gradually increased in the daytime (Fig. 4a) and decreased in the nighttime (Fig. 4b). The net diel production rate of DOC was estimated to be 12–24 mmol m^{-2} d^{-1} depending on the sampling site (Table 3). This means that DOC was exported from the reef flat to the surrounding outer ocean via the water exit of the reef flat (Miyajima et al. 2007b). Constantly higher DOC concentrations at other stations on the reef flat than those at Sta. B (Fig. 2) also support the net export of DOC from the reef flat. The diel DOC flux at our study site was comparable with previous reports. Hata et al. (2002) conducted research at the same site as the present study and reported similar DOC fluxes of 30–36 mmol m^{-2} d^{-1}. Ziegler and Benner (1999) estimated the DOC flux in a subtropical seagrass-dominated lagoon (Laguna Madre, Texas) as 4–25 mmol m^{-2} d^{-1}.

DON concentrations on the reef flat were also constantly higher than those at Sta. B (Fig. 2). Though it was

uncertain that the net flux of DON was imported to or exported from the reef flat at Shiraho reef (export flux 2.6 ± 6.7 mmol m^{-2} d^{-1}; Miyajima et al. 2007a), the present study suggests that the reef flat acted as a net source of DON at least during the survey period. Assuming that the production rate of DON on the reef flat was 10% of DOC (Fig. 3a, b), DON could be produced at the rate of 2.4 and 1.2 mmol m^{-2} d^{-1} at Sta. S1 and S2, respectively (Table 3). Miyajima et al. (2007b) observed the fluctuation of nutrient concentrations at Shiraho reef (near Sta. S2 of the present study site) and estimated the uptake rate coefficient for NO_3^- as 0.6–0.7 m h^{-1}. Assuming that NO_3^- and NH_4^+ had the same uptake rate coefficient, and considering that DIN concentration was 0.5–1.0 μmol l^{-1} during the present survey period (Table 1), the uptake rate of DIN could be estimated to be 7.2–17 mmol m^{-2} d^{-1}. In different coral reefs, Steven and Atkinson (2003) estimated the uptake rate of NH_4^+ as 7.8 mmol m^{-2} d^{-1} in the microatolls at One Tree reef lagoon. Atkinson et al. (2001) reported that the uptake rate of $NH_4^+ + NO_3^-$ in the reef flat of the experimental Biosphere was 0.3–9.8 mmol m^{-2} d^{-1}. DON production in coral reefs might be considerably small (10–30% at Shiraho reef) compared with DIN uptake by the reef community.

In this study, some parameters exhibited spatial variation: for example, the C:N ratio of POM was lower at seagrass-dominated area (4.6) than coral-dominated area (6.5; Fig. 3). Because seagrass beds have a function to trap organic matter in the water column and are enriched with accumulated organic detritus (Miyajima et al. 1998; Barrón et al. 2004), the seawater at Sta. SG1 and SG2 might have had a higher bacterial contribution to the bulk POM than the other reef flat area. The much lower C:N ratio at a seagrass-dominated area than the Redfield ratio (6.6) suggests that the POM was mainly composed of bacteria. Another spatial variation observed in this study was that the DOC production rate measured during the stagnant period was twice as high at Sta. S1 as at S2 (Table 3). These differences indicate that the coral reef ecosystem has large spatial heterogeneity. It could be important to observe parameters in multiple sampling stations to evaluate C and N cycling and biological metabolism of the whole reef system.

In summary, the present study measured the distribution of DOC and DON concentrations on a coral reef using a precise HTCO technique and has shown that the C:N ratio of DOM produced on the reef flat is significantly higher than that of POM. The C:N ratio of produced DOM was very similar at coral- and seagrass-dominated areas, suggesting that DOM was derived from a widely distributed source such as bacterial solubilization of organic matter on the sediment. The higher C:N ratio of DOM than POM might be caused by a metabolic property of the benthic community to conserve N and to release C-rich organic matter to the overlying seawater. Because the dynamics of DOM in coral reefs has scarcely been measured, it could be an important step to determine the major DOM source more precisely. In this regard, stable isotopes may prove to be useful. Quantification of the sink and source of DOM in coral reefs remains one of the most important and unknown problems in understanding biogeochemical cycles in coral reefs more completely.

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