学位論文

Impacts of Nutrient Enrichment on Carbon and Nitrogen Cycling through Reef-building Coral Colonies

富栄養化が造礁サンゴ群体を通した炭素・窒素循環に与える影響

東京大学大学院

新領域創成科学研究科　自然環境学専攻

田　中　　泰　章

2008 年 3 月

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General Introduction

1-1. Importance and environmental problems of coral reefs

Coral reefs are the biggest and most spectacular structures made by living organisms. The distinctive scene composed of various coral species and colorful fishes has long attracted people in the world. Although they look permanent and indestructible, what we see is only superficial and as fragile as any on earth.

The total area of coral reefs exceeds 600,000 km^2 (United Nations Environment Programme and World Conservation Union 1988). The area of near-surface reefs, which is the most ecologically diverse, productive and economically important reefs, was estimated to be 255,000 km^2 (Spalding and Grenfell 1997). Although coral reefs cover <0.2% of the total area of oceans, they are home to more than a quarter of all known marine fish species (McAllister 1995). About 4000 species of fish and 800 species of reef-building corals have been described to date (Paulay 1997). Coral reef ecosystems have been called as the rainforests of the marine world: highly productive and rich in species.

Coral reefs have provided many important assets to local communities, serving as a source of seafood, generating income from tourism, buffering coastal cities and settlements from wave action and storm damage. Almost half a billion people live within 100 km of a coral reef, benefiting from the reef roles of the production and protection (Bryant et al. 1998). Moreover, in recent years,

reef-associated species have come under the spotlight as a new target for scientists to search for useful antibiotic chemicals (Birkeland 1997). Corals are already being used for bone grafts, and chemicals found within several species appear useful for treating viruses. A recent study found that the costs of destroying just 1 km of reef range from about $137,000 to almost $1.2 million over 25 years, when fishery, tourism, and protection values alone are considered (Cesar 1996).

Despite the importance, coral reefs are recently threatened by human activities around the world. Increasing coastal development is one of the major threats to reefs. Dredging of harbors and shipping channels result in the complete destruction of habitats for reef organisms. Airports and other construction projects are also continually built on reef communities. Construction materials such as sand and limestone, which are made into cement, are mined from coral reefs in many areas.

Not only the direct effects of those destruction, but indirect effects also spoil reef ecosystems. Sewage releases containing excess nutrients induce algal bloom and decline the water quality. Hot-water discharges from power plants raise water temperature around and seriously reduce diversity of species within the habitat (Brown 1997). Sediment, pesticides, and other pollution from inland, can also damage coral reefs when transported by rivers into coastal waters (Fabricius 2005).

Overexploitation of reef species, including giant clams, sea cucumbers, sharks, lobsters, groupers, snappers, is also a problem for the majority of the world's reefs. Overfishing results in shifts in fish size, abundance, and species composition within reef communities. Removal of key herbivore and predator species may ultimately affect large-scale ecosystem changes (Roberts 1995).

Global climate change is a long-term threat to reefs (Hughes et al. 2003). Current models predict that increasing atmospheric carbon dioxide (CO_2) released by human activities will elevate sea surface temperatures (SST) in many places. The elevation of SST will cause sea levels to rise, and result in greater frequency and intensity of storms, which may directly destruct coral reef structures. The increase in SST and/or its variability could also produce an increasing frequency of coral bleaching, resulting in sublethal responses that might include reduced growth or reproductive potential of corals and increased partial mortality (Brown 1997). Whether reef organisms can adapt to the increasing sea surface temperature is not well understood. Destruction of the stratospheric ozone layer by chlorofluorocarbons, and consequent increase in ultraviolet radiation levels also act as an additional stress on coral reefs (Hoogenboom et al. 2006).

All these anthropogenic impacts have the potential to threaten coastal coral reef ecosystems. According to the report of World Resources Institute (Bryant et al. 1998), 36% of all reefs were classified as threatened by overexploitation, 30% by coastal development, 22% by inland pollution and erosion, and 12% by marine pollution. When these threats are combined, 58% of the world's reefs are at risk defined as medium and high risk.

1-2. Carbon and nitrogen cycling in coral reefs and the contribution of reef-building corals

Coral reef ecosystems are originally characterized by low nutrients and high

productivity. Despite the low nutrient concentration in the water column, the rate of gross photosynthetic carbon (C) fixation per unit projected area of coral reefs was estimated to be 500–4000 g C m^{-2} per year, which was comparable to the most productive terrestrial ecosystem, tropical rainforests (1000–3500 g C m^{-2} per year) (Whittaker 1974). Photosynthesis proceeds as follows and makes organic carbon and oxygen:

$$CO_2 + H_2O \rightarrow CH_2O + O_2 \qquad (1\text{-}1)$$

The opposite reaction of Eq. 1-1 indicates consumption of organic C by respiration. Inorganic nitrogen (N) species, i.e., nitrate (NO_3^-), ammonium (NH_4^+), and nitrite (NO_2^-), are simultaneously incorporated by the primary producer. The N uptake rate is dependent on the organism and the nutrient concentration. For example, the ratio of NO_3^- incorporation to organic C production in the coral colony *Acropora pulchra* would be >60 under in-situ NO_3^- level (Tanaka et al. 2006a). The ratio is much higher than the C:N ratio of the coral tissue (Tanaka et al. 2007), implying that available inorganic N is insufficient for the coral colony.

The apparently paradoxical phenomenon, i.e., low nutrients and high productivity in coral reefs, has been explained by tight recycling of nutrients in the reef community: close physical relationships between heterotrophs and autotrophs (e.g., symbiosis) create an ecosystem where nutrients are either retained within the biota or recycled within the community. A more recent view to explain the paradox, however, is that coral reefs produce mostly low quality organic C and inorganic C, requiring relatively little input of nutrients (Atkinson and Falter 2003). Atkinson and

Falter (2003) reviewed the data of metabolic rates of entire reef ecosystems, and the average ratio of photosynthesis to respiration ratio was 1.05, suggesting that photosynthetically produced organic C in coral reefs is mostly consumed for the respiration and thus excess production could be nearly zero.

The other important C flux characteristic of coral reefs is calcification, i.e., the deposition of carbonate calcium ($CaCO_3$).

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O \qquad (1\text{-}2)$$

Coral reefs have the greatest diversity of calcifying organisms such as hermatypic (reef-building) corals, calcareous algae, foraminiferans, and molluscs. When these organisms calcify, organic N is also incorporated into the skeleton mainly as proteins (Young 1971, Constantz and Weiner 1988, Allemand 1998). Because calcification has the potential to release CO_2 (Eq. 1-2), net CO_2 fluxes in the reef water depends on the balance between photosynthesis and calcification. Considering carbonate equilibrium in the seawater, when the ratio of net organic to inorganic C production (R_{OI}) exceeds approximately 0.6, partial pressure of CO_2 in seawater decreases and the atmospheric CO_2 is potentially absorbed into reef water (Ware et al. 1992, Frankignoulle et al. 1994).

The balance between photosynthesis and calcification depends on several reef conditions such as coverage of each organism performing calcification and photosynthesis, nutrient condition, and light intensity (depth). In addition, water residence time in the reef also affects the ratio R_{OI}, because photosynthetically produced organic matter is gradually mineralized as time passes. In general, most

reef flats have been reported to be sources of CO_2 to the atmosphere on a 24-h basis, due to low net fixation by photosynthesis and rather large release of CO_2 by calcification (Gattuso et al. 1993, Kawahata et al. 1997, Suzuki and Kawahata 2003). On the other hand, it has also been observed that reef communities which exhibit a larger community excess production and/or a lower community calcification were sinks for atmospheric CO_2 (Kayanne et al. 1995, Gattuso et al. 1997). On a global scale, net $CaCO_3$ production in reefs have been roughly estimated at 111×10^{12} g C per year (Kinsey and Hopley 1991), which is >5 times more than that of net organic C (20×10^{12} g C per year; Crossland et al. 1991).

One of the most major contributors to the reef C dynamics is hermatypic corals. The coral colony is composed of host corals and their intracellular algae (zooxanthellae). Host corals belong to mostly the order of Scleractinia and perform calcification. Zooxanthellae are mainly placed in the dinoflagellate genus *Symbiodinium* and perform photosynthesis. Net production rates of organic matter by coral colonies have been reported to be within the range of ca. 10–20 μmol C cm^{-2} d^{-1} per unit surface area of the massive or branched corals (e.g., Muscatine et al. 1984, Tanaka et al. 2007). Large parts of the photosynthetic products (ca. >80%) are translocated from the algae to the host coral and are used for the host metabolism (Muscatine et al. 1984, Edmunds and Davies 1986, Tanaka et al. 2006a). Because corals are morphologically complex, projected (horizontal) area probably extends ca. 3 times of the skeletal surface area of corals (Dahl 1973). Thus, simply calculating the production rate per year, the net organic production normalized to projected area is equivalent to 1300–2600 g C m^{-2} yr^{-1}. The value is comparable to the gross photosynthetic rate for a whole reef community (see above), showing that

photosynthesis by zooxanthellae accounts for considerable parts of the whole reef production. The rate of coral calcification is same order of magnitude as the photosynthesis (reviewed by Gattuso et al. 1999). It has been suggested that both reactions of photosynthesis and calcification are closely related and potentially enhance each other, which were explained by several mechanisms (Goreau 1959, McConnaughey and Whelan 1997, Suzuki et al. 1995, Furla et al. 1998, 2000).

From the viewpoint of organic matter production by reef-building corals, the other notable process is the release of organic matter from the corals. Hermatypic corals release both of organic C and N to the ambient seawater and the released organic matter has been proposed to support various reef organisms from bacteria to fishes as energy sources (Benson and Muscatine 1974, Ducklow and Mitchell 1979a). The release rate of organic C has been reported to be sometimes equivalent to large parts of the photosynthetically fixed C (6%–40%; Crossland et al. 1980, Muscatine et al. 1984, Crossland 1987). To specifically analyze net production of organic matter by reef-building corals, the organic matter release from corals and subsequent fates of the released organic matter in the water column are important processes. Because inorganic N and phosphorus (P) are generally depleted in coral reef waters, the release of organic matter containing N and P from coral colonies might be an important nutrient source for the other heterotrophic organisms.

1-3. The utilization of inorganic nitrogen and the maintenance of symbiotic association for reef-building corals

Coral reefs are generally surrounded by oligotrophic seawater. Typical concentrations of NO_3^- and NH_4^+ are the range of 0.05–0.5 μmol L^{-1}, while NO_2^- is usually below 0.1 μmol L^{-1} (Atkison and Falter 2003). Reef-building corals can take up these inorganic nutrients and synthesize organic N for the biomass growth.

NH_4^+ uptake has been relatively well documented (Muller-Parker et al. 1994, Koop et al. 2001), compared to NO_3^-. It is mostly accepted that both fractions of host corals and zooxanthellae possess anabolic enzymes for NH_4^+, i.e., glutamine synthetase (GS) and NADPH-dependent glutamate dehydrogenase (NADPH-GDH) (Catmull et al. 1987, Yellowlees et al. 1994), although it is still not clear whether both fractions independently assimilate NH_4^+ or not (Roberts et al. 1999, Lipschultz and Cook 2002).

On the other hands, NO_3^- assimilation by coral–algal symbiotic systems has been less investigated than NH_4^+, though it is sometimes a major form of exogenous N input to coral reefs (Umezawa et al. 2002). The essential enzymes for NO_3^- assimilation, NO_3^- and NO_2^- reductases, were detected once in zooxanthellae (Crossland and Barnes, 1977), and recently, the appearance of NO_3^--derived N in zooxanthellae using ^{15}N-tracer technique (Grover et al., 2003) indicated that NO_3^- is actually incorporated into zooxanthellae. Coral colonies take up these inorganic nutrients at the rate dependent on the concentration (Grover et al. 2002, 2003, Tanaka et al. 2006a).

However, coral colonies are known to be successful in nutrient-poor seawaters, because they are closed system with regard to inorganic N (Falkowski et al. 1993). NH_4^+ excreted by the animal coral is assimilated by the symbiotic algae and translocated back to the animal as organic N (Roberts et al. 1999). The

population density of zooxanthellae is controlled by systematic N limitation of the algal growth. This nutrient limitation makes the algae to produce excess photosynthetic products, and host factors promote the leakage of the photosynthates from the algae to the host tissue (Dubinsky and Jokiel 1994, Grant et al. 1998). The growth of zooxanthellae is far from balanced, and the imbalance simultaneously ensures a supply of photosynthetically produced organic C for the host metabolism. Thus, the maintenance of a balanced coral symbiotic association appears to require low ambient nutrient concentrations (Falkowski et al. 1993).

1-4. Increasing eutrophication of coral reefs and the purpose of this study

Eutrophication is defined as excessive nutrients in water ecosystems, usually caused by runoff of nutrients (animal waste, fertilizers, and sewage) from the land (Fabricius 2005). The nutrients include inorganic N and P, e.g., NH_4^+, NO_3^-, NO_2^-, and phosphate (PO_4^{3-}), which causes dense growth of plants. Increasing nutrient input to coral reefs is perceived as one of the principal deleterious problems for many reefs in the world (Walker and Ormond 1982, Tomascik and Sander 1985, Fabricius 2005).

In the west coast of the Caribbean island of Barbados, nutrient concentrations along the coast substantially increased by sewage discharge around 1980, which caused considerable changes in community structure on the reefs: reduced coral species diversity, high abundances of macrophytic and filamentous

algae, and a reduction in skeletal growth rates in the massive coral *Montastrea annularis* (Tomascik and Sander 1985).

In the Great Barrier Reef (GBR), Australia, there has also been concern for some time about increasing nutrient loading (Bell 1991), which based on: (i) rapid increases in the number of tourists visiting the GBR and associated development of resorts on the reef, (ii) increasing urbanization along the Queensland coast during the 1980s-1990s, (iii) continuing intensive agricultural development and (iv) loss of wetlands (Koop et al. 2001). It has been claimed that much of the GBR is already in eutrophic condition (Bell and Elmetri 1995), while other work identified nutrient pollution problems as confined to the inshore GBR and not yet affecting the offshore reefs (Brodie et al. 1997).

In most reefs where eutrophication was considered to be the major cause of the reef degradation, however, it is actually difficult to identify which components are directly responsible for the reef degradation: the complex of the inputs to coastal areas including nutrients, sediment, and heavy metals from industrial and domestic sources and runoff from land has simultaneously occurred. Tomascik and Sander (1985) monitored coral growth rates and many environmental variables such as temperature, light intensity, and water movement, at several locations of Barbados, which were under different degrees of environmental stress caused by increased eutrophication. Though both concentrations of inorganic N (<0.5 μmol L^{-1}) and P (<0.01 μmol L^{-1}) were negatively correlated with the coral growth rate, they concluded that the concentration of suspended particulate matter was the best indicator of deteriorating coral growth. As coastal development seriously proceeds, it needs to specifically investigate how each reef organism responds to various shifts in

water quality.

The purpose of this study is to clarify how C and N cycling via reef-building coral colonies are affected by increasing nutrient levels. As described above, the coral colonies perform both photosynthesis and calcification with the incorporation of nutrients, and significantly influence biogeochemical cycling in coral reefs. Three phases in which corals particularly contribute were focused on in this study: (1) the balance between photosynthesis and calcification, (2) organic matter release from coral colonies, and (3) bacterial decomposition of the released organic matter.

Previous studies on nutrient impacts on the coral colonies have focused on NH_4^+, rather than NO_3^-, because it has been widely accepted that coral colonies can assimilate NH_4^+ with the anabolic enzymes (Catmull et al. 1987, Yellowlees et al. 1994). However, it has been recently observed with ^{15}N-tracer technique that N derived from NO_3^- was actually incorporated into both of the host and algal fractions (Grover et al. 2003, Tanaka et al. 2006a). N generally flows into coastal ecosystems by river, groundwater discharge and atmospheric deposition (Morris 1991, Miyajima et al. 2007). As the study site described below, NO_3^- is sometimes the most major form of exogenous N input to coral reefs by groundwater discharge (Umezawa et al. 2002). It should be further investigated how reef-building corals respond to increasing NO_3^- loading.

1-5. Study site description

Coral colonies used in this study were collected at Shiraho Reef in Ishigaki

Island, Japan, and the incubation experiments were conducted in the laboratory of Seikai National Fisheries Research Institute near Urasoko Bay in the island (Fig. 1-1). The island (24° 21′–31′N, 124° 4′–16′E) is located in the southwest of the Ryukyu Islands and surrounded by 0.5–2.0 km wide fringing reefs. The climate of the island is subtropical, with the annual mean temperature, relative humidity and precipitation being 24.3°C, 78% and 2000 mm, respectively (Ishigaki Island Meteorological Observatory). The mean surface seawater temperature is from 21.0°C at minimum in February to 29.5°C at maximal in August (Seikai National Fisheries Research Institute). There are two rainy seasons, monsoon (March to May) and typhoon (July to September). The Shiraho Reef is located at the southeast in this island. The reef water is isolated from the outer ocean during the low tide for maximal 5–6 h due to the emerged reef crest, which is ca. 850 m offshore from the coast. The depth of the lagoon is mostly <3 m, and the lagoon water is almost completely replaced with outer reef water during a single high tide period.

It has been reported that around the coastal area of the Shiraho Reef groundwater perennially contains inorganic N (Umezawa et al. 2002). Umezawa et al. (2002) estimated N input from several sources to the Shiraho Reef and compared the input with Kabira Reef, which is also in Ishigaki Island but less affected by human activity. While N input by groundwater discharge accounted for only about 5% to the total N input in Kabira Reef, it accounted for about 35% in Shiraho Reef, indicating that the ecosystem of Shirho Reef is strongly affected by anthropogenic N. It was also shown that NO_3^- was the most major species of dissolved inorganic N (DIN) in the groundwater (>99%) and that NO_3^- concentration in seawater near the shoreline of Shiraho Reef was actually elevated by the groundwater (3.3–230 μmol L^{-1} NO_3^-;

Umezawa et al. 2002).

Not only NO_3^-, but PO_4^{3-} also flows into the coastal area of Ishigaki Island through river and groundwater discharge (Kawahata et al. 2000, Abe 2007). Abe (2007) monitored PO_4^{3-} in Urasoko Bay (Fig. 1-1), and observed the concentration of 0.016–0.31 μmol L^{-1}. The PO_4^{3-} concentrations were negatively correlated with the water salinity, indicating that the observed PO_4^{3-} had terrestrial sources. Agricultural activity might be a source of the PO_4^{3-} from phosphate fertilizer.

Groundwater- and river water-loaded seawater flows along the shoreline in Shiraho Reef, and it is eventually flushed out from the channels of the reef crest. Due to the rapid dilution of the lagoon water with the offshore water, the majority of the lagoon in Shiraho Reef had only slightly higher NO_3^- (0.2–1.5 μmol L^{-1}), NH_4^+ (0.2–1.0 μmol L^{-1}), and PO_4^{3-} (0.02–0.08 μmol L^{-1}) than those in the offshore waters (NO_3^-: <0.2 μmol L^{-1}, NH_4^+: <0.2 μmol L^{-1}, PO_4^{3-}: <0.02 μmol L^{-1}; Miyajima et al. 2007). However, it would be sure that more N input into the reef is a serious concern in the future.

The vegetation in the lagoon of Shiraho Reef is typical belt-like zones: seagrass beds (mainly *Thalassia hemprichii* and *Cymodocea rotundata*) occur up to 300 m offshore; large patches of live corals (mainly *Montipora digitata*, *Acropora pulchra*, *Pocillopora damicornis*, and *Porites cylindrica*) are abundant at 400–700 m offshore; the seaward area of the reef crest (700–800 m offshore) is covered with dense brown algae from spring to autumn (mainly *Sargassum* spp. and *Padina* spp.) (Miyajima et al. 2001, Iryu et al. 1991). The remaining area of the lagoon (300–400 m) is low on macrophytes or corals and is composed of typical carbonate sand. Coral colonies for the present study were collected near the reef crest (ca. 700 m offshore).

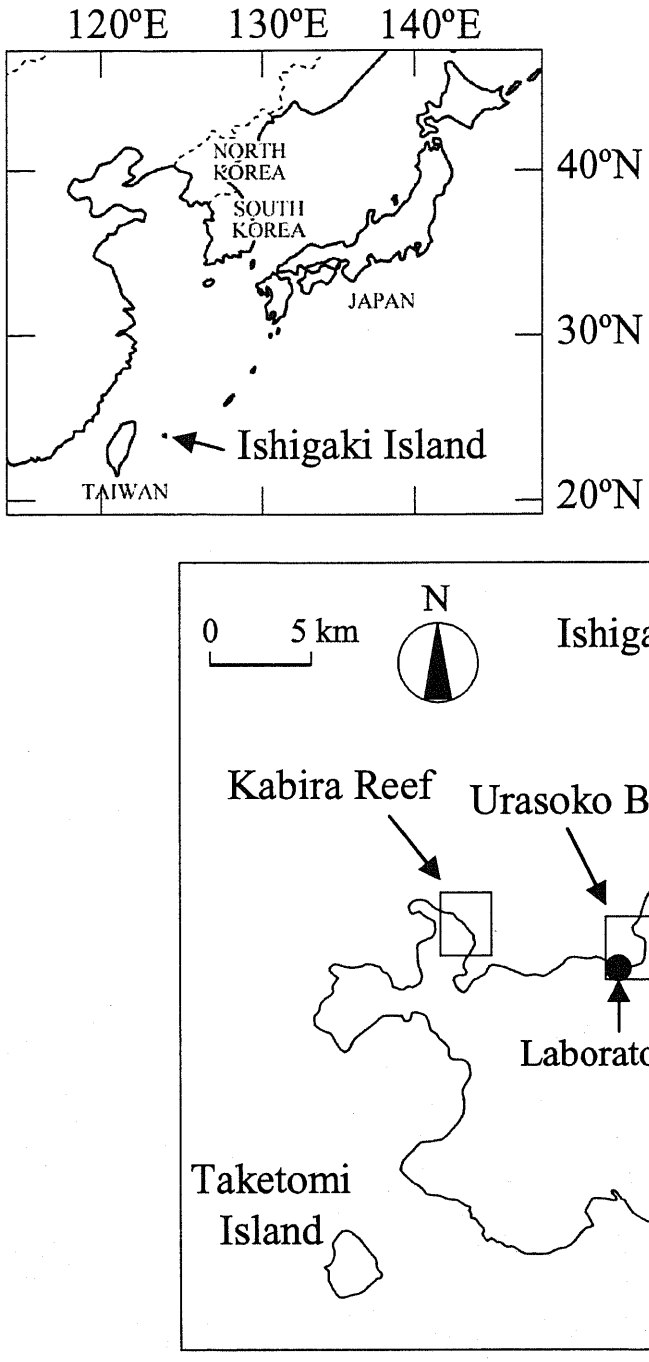

Fig. 1-1 The map of Ishigaki Island and the location of Shiraho Reef, Kabira Reef, and Urasoko Bay.

Chapter 2

The Balance between Photosynthesis and Calcification

2-1. Introduction

Hermatypic corals play a significant role for carbon (C) dynamics in tropical and subtropical coastal area due to the activity of calcification by the animal coral and photosynthesis by its symbiotic algae (zooxanthellae). These C fixation processes strongly influence carbonate chemistry in reef waters, and thus CO_2 exchange with atmosphere. Because photosynthesis absorbs CO_2 (Eq. 1-1) and conversely calcification has the potential to release CO_2 (Eq. 1-2), whether atmospheric CO_2 is absorbed or released from coral reefs depends on the proportion of photosynthesis to calcification.

It has long been considered that coral calcification and algal photosynthesis are strongly coupled with each other. Calcification rates have been observed to be higher in light than in dark (e.g., Kawaguti and Sakumoto 1948), suggesting the stimulation of calcification by algal photosynthesis. The opposite interaction, i.e., the stimulation of photosynthesis by calcification, has also been suggested in several studies (McConnaughey 1991, McConnaughey and Whelan 1997), though Gattuso et al. (2000) argued that CO_2 released by calcification was not a significant source for the algal photosynthesis. Suzuki et al. (1995) simulated a model of coexisting effects of photosynthesis and calcification, and showed that both reactions potentially enhance each other.

Nutrient loading and its subsequent impact is one of the important issues concerning conservation and protection of coral reefs (Walker and Ormond 1982, Tomascik and Sander 1985). For hermatypic coral colonies, dissolved inorganic nutrients potentially enhance zooxanthellate photosynthetic activity (Dubinsky et al. 1990, Marubini and Davies 1996, Ferrier-Pagès et al. 2000a). Both increases in algal population density per unit surface area of the coral and Chl *a* density per algal cell have been observed in previous studies because of nutrient enrichment (Hoegh-Guldberg and Smith 1989, Muller-Parker et al. 1994, Marubini and Davies 1996). Coral and algal biomass was also reported to increase with nutrients (Muller-Parker et al. 1994).

Many studies have shown negative effects of nutrient enrichment on bulk coral growth (Stambler et al. 1991, Marubini and Davies 1996, Marubini and Atkinson 1999, Ferrier-Pagès et al. 2000a) from observations of skeletal length or weight during a relatively long term (>30 d), while some others suggested positive effects (Atkinson et al. 1995, Steven and Broadbent 1997). Because skeletal density is not invariable and thus its extension rate could not always reflect calcium carbonate ($CaCO_3$) precipitation rates (Dodge and Brass 1984), it is necessary to directly measure calcification rates in order to estimate actual C fixation amount as $CaCO_3$. Moreover, to measure calcification rates for corals having different photosynthetic activity will clarify the relationship between photosynthesis and calcification, which is still a matter for discussion (Gattuso et al. 1999)

Because most of the previous studies set nutrient concentration at considerably high levels (e.g., NO_3^-: 10–50 μmol L^{-1}) compared to in situ levels (usually, <1 μmol L^{-1}), the present study regarded moderate nutrient loading (NO_3^-:

<5 μmol L^{-1}, PO_4^{3-}: <0.3 μmol L^{-1}), which can sometimes be seen at coastal area affected by river input and groundwater discharge (see below). The purpose of this study is to investigate the nutrient effects on the balance between organic tissue production and calcification. The nutrient enrichment clearly resulted in the increase in coral and algal biomass and Chl *a* density per unit surface area of the coral. Using these coral branches, net C fixation rates into organic tissue and carbonate skeleton were measured in normal nutrient-depleted seawater with ^{13}C labeling technique. The technique required only a few days to determine the rates and clarified how each C fixation rate was affected by the level of algal Chl *a* density.

2-2. Material and methods

2-2-1. Coral collection and pre-incubation under nutrient enrichment

The experiment was performed using zooxanthellate corals *Acropora pulchra*, which were collected on the reef flat of Shiraho Reef in Ishigaki Island (24° 21′–31′ N, 124° 4′–16′ E), Japan in Jul 2005. Coral branches of ca. 6 cm were collected ca. 500 m offshore, where the depth is ca. 0.5 m at low tide. Within 1 h after the collection, corals were transferred to an outdoor aquarium (inner volume: 90 L) in Seikai National Fisheries Research Institute (Fig. 3-1a). Seawater containing nutrients (NO_3^-: 2.1 μmol L^{-1}, NO_2^-: 0.06 μmol L^{-1}, NH_4^+: 0.03 μmol L^{-1}, PO_4^{3-}: 0.09 μmol L^{-1}, on average) was continuously supplied in the aquarium. Each coral was suspended using nylon line. NO_3^- and PO_4^{3-} were additionally supplied once a day to

get a final concentration of 5 μmol L^{-1} and 0.3 μmol L^{-1}, respectively. After the addition of NO_3^- and PO_4^{3-}, the aquarium was kept closed for a few hours to prevent rapid dilution of the nutrients, during which the seawater was agitated with magnetic stirrers. Samples for nutrient concentrations were taken in 10 ml acrylic tubes before and after the extra NO_3^- and PO_4^{3-} addition, and stored at -20°C until analysis.

Corals were collected from the reef in three separate timing to make the difference in coral nutrient conditions: One group (n = 8) was sampled 10 d before the following experiment of ^{13}C incorporation, another (n = 9) was 5 d before, and the last group (n = 12) was just sampled 15 h before and not affected by the extra NO_3^- and PO_4^{3-} addition.

2-2-2. ^{13}C incorporation experiment

After the nutrient enriched treatment, three groups of the collected corals were incubated for 4 d with ^{13}C-labeled dissolved inorganic C (DIC). Seawater used during the period contained low nutrients (NO_3^-: 0.11 μmol L^{-1}, NO_2^-: 0.03 μmol L^{-1}, NH_4^+: 0.05 μmol L^{-1}, PO_4^{3-}: 0.03 μmol L^{-1}, average of the 4 d) to minimize changes in coral and algal biomass and to measure photosynthetic and calcification rates under the same nutrient condition for all the corals. The seawater was gently stirred with magnetic stirrers, and exchanged twice a day (10:00 and 17:00 h). $NaH^{13}CO_3$ (purity 99%; Cambridge Isotope Laboratories, Inc.) was added after each water exchange to get 0.1 mmol L^{-1} excess ^{13}C (ca. 5 atom% ^{13}C). Temperature of the water was controlled by flowing seawater outside the aquarium (28.9°C on average during

the ^{13}C incorporation). Corals of each group were randomly arranged in the aquarium and systematically moved everyday to minimize position effects such as light intensity and water velocity.

Samples for nutrient concentrations, DIC concentrations, and DIC isotope ratios were taken before and after the seawater exchange. Samples for the DIC concentration and its isotopic ratio were taken in 15 mL and 30 mL of a glass vial, respectively. After fixed with saturated $HgCl_2$ to final concentrations of 0.03 and 0.07% (v/v), respectively, they were sealed with a PTFE (polytetrafluoroethylene)-coated butyl rubber septum and an aluminum seal.

After the ^{13}C incorporation for 4 d, each incubated coral was divided into two tips. One of them was put in 15 mL of methanol to extract whole Chl *a* of the tip. Another tip was processed according to Tanaka et al. (2006a) to separate coral and zooxanthellae: the coral tip was placed in a small glass vial containing 8.0 mL of GF/F-filtered seawater, and the vial was sonicated with an ultrasonic cleaner (composite frequency of 24 and 31 kHz, 110 W, inner volume 3.4 L) for 10 min, which resulted in peeling of organic tissue from the skeleton (Piniak and Lipschultz, 2004). It was confirmed by microscopic enumeration that >98% of algal cells remained intact after this 10 min sonication. Tissue suspension was centrifuged at 750 × g for 5 min to separate zooxanthellae (pellet) from animal tissue (supernatant). The supernatant tissue suspension was stored at -20°C. The precipitated algal pellet was washed and centrifuged twice with GF/F-filtered seawater to minimize contamination by animal tissue, and stored at -20°C until analysis. Because the tissue could not completely be separated from the skeleton with the procedure, Chl *a* was also extracted from the obtained algal tissue to determine at what ratio the tissue was

collected from the skeleton.

2-2-3. Chemical analysis and calculations

Dissolved inorganic nitrogen (N) (DIN: NO_3^-, NO_2^-, NH_4^+) and phosphorus (DIP: PO_4^{3-}) were quantified by using a nutrient analyzer AACS-III (BRAN+LUEBBE; Detection limit: <0.01 μmol L^{-1}). DIC concentration was measured with a Shimadzu TOC 5000 instrument, and its isotopic ratio was determined with GC-IRMS (gas chromatography-isotope ratio mass spectrometer; Agilent Technolgy GC-6890 + Finnigan DELTA plus XP; Miyajima et al. 1995). GC was equipped with a capillary column J&W GS-GASPRO (30 m length, 320 μm inner diameter), and oven temperature was kept at 70°C.

Coral and algal tissue suspension was infiltrated into a pre-combusted glassfiber filter (Whatman GF/D: pore size 2.7 μm, filter diameter 10 mm). The filters were dried on a hot plate at 80°C and treated with a vapor of 12 mol L^{-1} HCl for 12 h at room temperature to remove inorganic C. After evaporating extra HCl on the hot plate under vacuum, the filters were dried again in an oven at 50°C for a few hours. Amounts and isotope enrichments of C and N on the filter were measured by combination of CHN analyzer (Fisons; NA-1500) and IRMS connected via Conflo-III interface (Thermo-electron Co. Ltd.). The sum of the coral and algal parts was used to calculate net C fixation rates by photosynthesis.

To quantify the calcified ^{13}C, one part of the incubated coral, from which Chl *a* was already extracted with methanol, was dried in an oven at 50°C and crushed

into a fine powder with a grinder (Osaka Chemical Co.; WB-1). About 20 mg of the powder and 24 mL of distilled water were sealed in a glass vial (30 mL) with a butyl rubber septum and aluminum seal. One milliliter of 1 mol L^{-1} HCl was then injected with a polypropylene syringe to dissolve $CaCO_3$ into CO_2 and get CO_2 gas into the headspace of the vial. ^{13}C enrichment of CO_2 in the headspace was measured by GC-IRMS in the same way as DIC isotopic ratios (Miyajima et al. 1995).

Bulk organic C in coral and algal tissue per unit surface area (T; μmol cm^{-2}) was determined from partially separated tissue C (T_p; μmol) and the ratio (R) of Chl *a* extracted from the obtained algal tissue to the total Chl *a* of the branch:

$$T = T_p R^{-1} A^{-1} \qquad (2\text{-}1)$$

where A (cm^{-2}) is a skeletal surface area determined by aluminum foil method (Marsh, 1970). It was supposed in this calculation (Eq. 2-1) that the ratio of Chl *a* amount to the bulk organic tissue was not different between partially separated tissue and the remaining tissue on the skeleton, thus, it might involve some error (see '3-3-1'). In this Chapter, it was assumed that the error occurs at same extent for all the incubated coral branches.

Skeletal weight (S; g) of each coral tip, from which organic tissue was partially separated, was calculated as follows:

$$S = W - TA(1 - R) \times 12.01 \times 10^{-6} \qquad (2\text{-}2)$$

where W (g) is a coral tip weight still containing a part of organic tissue. Because the

remaining tissue on the skeleton was not composed of only C but also of other organic elements such as N and oxygen, S must be overestimated in Eq. 2-2. However, the weight of the remaining tissue C (i.e., $TA\ (1 - R) \times 12.01 \times 10^{-6}$) was minor, 5% on average, compared to the whole skeletal weight (W). Thus, S largely represented the carbonate weight. Newly incorporated C (C_{new}; μmol) in organic tissue and carbonate skeleton during ^{13}C labeling period was calculated as follows:

$$C_{new} = (\text{excess } ^{13}C \text{ amount of the sample}) (\text{average APE DI}^{13}C \text{ in seawater} \times 0.01)^{-1} \quad (2\text{-}3)$$

$$\text{Excess } ^{13}C \text{ amount of the sample} = (\text{bulk organic C}) \times (\text{APE } ^{13}C \text{ of the sample} \times 0.01) \quad (2\text{-}4)$$

$$\text{APE } ^{13}C \text{ of the sample} = (\text{atom\% of the sample}) - (1.108\% \text{ as a natural ratio}) \quad (2\text{-}5)$$

where APE means atom% excess. Net photosynthesis and calcification rates were normalized by dividing C_{new} by the labeling period of 4 d and surface area of the coral tips.

2-3. Results

2-3-1. Chl *a* density and tissue biomass

The nutrient condition in this study (NO_3^-: <5 μmol L^{-1}, PO_4^{3-}: <0.3 μmol L^{-1}) increased Chl *a* density per unit surface area of the coral by a factor of 2.0 for 5 d and 2.6 for 10 d on average (Table 2-1). The lower increase rate from day 5 to 10 indicates that Chl *a* content per unit area gradually approached saturation state.

Both coral and zooxanthellate biomass also increased with the nutrient enrichment days. Algal C and N biomass increased 2.0 and 1.7 fold for 10 d, respectively (Table 2-1). Thus, Chl *a* : C and Chl *a* : N ratios of the zooxanthellae increased 1.3 and 1.5 fold during the period, respectively.

Host C and N biomass increased 1.6 and 1.7 fold for 10 d, respectively (Table 2-1). Biomass ratios of the zooxanthellae to the host coral were not significantly affected for both C and N even after 10 d (Student's *t*-test, $p = 0.1$ and 0.2, respectively).

2-3-2. Photosynthesis and calcification rates

During the ^{13}C incorporation experiment, DIC concentration of newly exchanged seawater was 1.70 ± 0.02 mmol L^{-1} (mean ± SD), and it decreased to 1.48 ± 0.02 mmol L^{-1} at 17:00 h and to 1.61 ± 0.03 mmol L^{-1} at 10:00 h. Relatively low DIC concentration of the used seawater compared to usual ocean could be because it was taken from the inner reef at evening, when DIC concentration decreased due to photosynthesis and calcification during the daytime (Hata et al. 2002).

The rate of net organic C production by the zooxanthellae increased 2.1 fold for the corals of the 5 d nutrient enrichment and 2.8 fold for those of the 10 d (Table

2-2). The increase rate was similar to that of Chl *a* density per unit surface area, meaning that the net production per Chl *a* (assimilation number) was almost constant (average: 4.0 μmol C (μg Chl *a*)$^{-1}$ d^{-1}). There was a clear correlation between the Chl *a* density and the net photosynthetic rate ($r^2 = 0.75$, $p < 0.0001$; Fig. 2-1).

The calcification rate also showed a positive correlation with the Chl *a* (Fig. 2-1), but the increase ratio (1.3 fold for the 10-d-enriched corals; Table 2-2) was significantly lower than that of photosynthesis ($p < 0.001$), and the correlation coefficient was low ($r^2 = 0.18$; Fig. 2-1). The relationship between photosynthesis and calcification also showed a significantly positive correlation ($p = 0.01$; Fig. 2-2).

The C fixation ratio of the net photosynthesis to the calcification was 0.76 ± 0.06 for the nutrient-untreated corals (Table 2-2 and Fig. 2-3). On the other hand, the structural ratio of tissue organic C to skeletal inorganic C was 0.31 ± 0.03 for the same corals (Table 2-2 and Fig. 2-3). Supposing that the coral colony grows at a constant ratio of organic tissue C to skeletal C, the required net production of organic C was calculated to be 4.1 μmol C cm^{-2} d^{-1} ($10.1 \times 0.31 \times 0.76^{-1}$). Thus, extra organic C was calculated to be 6.0 μmol C cm^{-2} d^{-1}. For the 10 d nutrient-treated corals, the C fixation ratio was 1.73 ± 0.19 and the structural ratio was 0.75 ± 0.06 (Table 2-2 and Fig. 2-3). The extra organic C was calculated to be 16 μmol C cm^{-2} d^{-1} for these corals (Table 2-2).

2-4. Discussion

2-4-1. NO_3^- availability and the effect on coral and algal biomass

Algal C and N biomass increased with the nutrient enriched days (Table 2-1), and the ratio of Chl *a* density to the algal biomass also increased during the first 5 d. These results suggest the increase in algal population density per unit area and/or the increase in Chl *a* content per algal cell. Both of them can be considered, because similar observations have been found in previous studies with enrichment of NH_4^+ (e.g., Hoegh-Guldberg and Smith 1989, Stambler et al. 1991, Muller-Parker et al. 1994) and NO_3^- (Marubini and Davies 1996). Hoegh-Guldberg and Smith (1989) observed with *Stylophora pistillata* and *Seriatopora hystrix* that the mean cell diameter of the zooxanthellae was unaffected by elevated NH_4^+ (10–40 μmol L^{-1} for 3 weeks). Snidvongs and Kinzie (1994) also reported that C contents per zooxanthellate cell of *Pocillopora damicornis* were not influenced by 15 μmol L^{-1} NH_4^+ enrichment for 8 weeks. These observations suggest that nutrient enrichment increases the population density of zooxanthellae, but the cell size is almost kept constant.

Not only the abundance of zooxanthellae, but host coral biomass also increased with the nutrient treatment, keeping an relatively constant algal : coral biomass ratio (Table 2-1). These implies that host tissue growth is stimulated by NO_3^- (Tanaka et al. 2006a), as well as NH_4^+ (Muller-Parker et al. 1994). Because enzymes for NO_3^- assimilation, i.e., NO_3^- and NO_2^- reductases, have never been found in host corals, the enhanced host tissue growth could be due to translocation of N-rich compounds from the zooxanthellae (Tanaka et al. 2006a). Grover et al. (2003) assessed the uptake rates of NO_3^- by *Stylophora pistillata* using the ^{15}N technique, and found that the ^{15}N enrichment of the algal fraction was up to 12 times greater

than that of the host, which suggests that the zooxanthellae are the primary site of NO_3^- incorporation.

Yet, considering that NO_3^- has to cross at least two animal membranes to reach the zooxanthellae, Grover et al. (2003) implied that the host has a role in NO_3^- assimilation by the zooxanthellae. Some other works also suggested that the host is primarily involved in the N uptake (Miller and Yellowlees 1989, Wang and Douglas 1998). Miller and Yellowlees (1989) proposed two possibilities of the host role that (1) NO_3^- transport across the animal membrane is mediated by a non-specific carrier whose physiological function is the transport of another species and (2) the host synthesizes a specific NO_3^- carrier for the sole benefit of the zooxanthellae. On the other hand, they also implied that the ability of coral colonies to utilize ambient NO_3^- may depend on the environmental nutrient status: In corals which reside in relatively high-nutrient environments, NO_3^--uptake system should be repressed due to readily available reduced N such as NH_4^+ and amino acids. Considering that coral colonies used in the present study actively took up NO_3^- in their tissue, they might be originally in nutrient limited environment. Translocation of organic N from zooxanthellae to the host may also depend on the host nutrient condition.

2-4-2. The interaction between algal photosynthesis and coral calcification

The ^{13}C incorporation experiment revealed that the net photosynthetic rate increased nearly in direct proportion to the Chl *a* density per unit area (Fig. 2-1 and Table 2-2). Though some studies have suggested DIC limitation of zooxanthellate

photosynthesis due to diffusional boundary layer (Lesser et al. 1994), our results suggested that the algal photosynthesis was not limited by DIC in the seawater at least within the range of the Chl *a* observed in this experiment (Burris et al. 1983). Whether the DIC limitation effect occurs or not probably depends on water velocity, which affects boundary layer thickness, and on used coral species, which could have different Chl *a* density and metabolic rates.

The increase rate of calcification was less than half of that of the net photosynthesis (Fig. 2-1 and Table 2-2). It has long been thought that photosynthesis stimulates calcification from the observation that calcification rates are higher in light than in dark (e.g., Kawaguti and Sakumoto 1948), though the interactions between the two reactions are still matters of continuous controversy. Gattuso et al. (1999) reviewed the literature and found that the average ratio of light : dark calcification rates was 3.0, indicating that photosynthesis considerably affects calcification rates. One of the well-known mechanisms to explain higher calcification rates in light (light-enhanced calcification) begins with photosynthetic CO_2 uptake by zooxanthellae (Eq. 2-3), which increases pH in seawater by the shift in carbonate equilibrium (Eq. 2-4). This pH shift increases carbonate ion concentration (Eq. 2-5) and consequently stimulates $CaCO_3$ precipitation according to Eq. 2-6 (Goreau 1959).

$$CO_2 + H_2O \rightarrow CH_2O + O_2 \quad (2\text{-}3)$$

$$HCO_3^- \rightarrow CO_2 + OH^- \quad (2\text{-}4)$$

$$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O \quad (2\text{-}5)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (2\text{-}6)$$

The hypothesis of McConnaughey and Whelan (1997) brought up the importance of H^+ produced by calcification (Eq. 2-7), and they suggested that calcification may stimulate photosynthesis (Eq. 2-7 → 2-4 → 2-3)

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+ \qquad (2\text{-}7)$$

However, this model was recently contradicted by Gattuso et al. (2000), who demonstrated that light-enhanced calcification can be inhibited without influencing photosynthesis. Another most recent hypothesis for the light-enhanced calcification is based on the titration of H^+ produced by calcification (Eq. 2-7) with the OH^- produced by photosynthesis (Furla et al., 1998, 2000).

In those previous studies, however, nutrient effects have never been regarded. Nutrient incorporation generally enhances algal photosynthetic activity, and accordingly gross and net primary production (Dubinsky et al. 1990, Marubini and Davies 1996, Ferrier-Pagès et al. 2000a) as also found in the present study. Here, attention was paid to how calcification was affected by the increased photosynthetic DIC uptake due to the nutrient incorporation. As a result, the relationship between net photosynthesis and calcification showed statistically significant correlation (p = 0.01; Fig. 2-2), but the slope of 0.16 suggests that the calcification was not considerably stimulated by the enhanced photosynthetic activity. In other words, the light-enhanced calcification was almost at a maximum level, and other factors such as organic matrix synthesis (see below) limit the calcification. Even moderate nutrient loading for 10 d in the present study disrupted the balance between

photosynthesis and calcification, implying that the skeletal extension could not keep up with the tissue growth. The ratio of photosynthesis to calcification (*P*:*C* in Table 2-2) did not almost change after Day 5 and got close to the value of 1.7. This indicated that the balance between photosynthesis and calcification was nearly in a steady state.

2-4-3. Factors in stimulating coral calcification

Though the increase rate of calcification was significantly lower than that of net photosynthesis, this study is one of the few reports which suggested a positive effect of nutrients on coral calcification (Meyer and Schultz 1985, Atkinson et al. 1995, Steven and Broadbent 1997). Some possibilities can be suggested for the increased calcification with the nutrient-enhanced photosynthesis. First, the light-enhanced calcification was directly raised by the increased algal photosynthesis. Algal photosynthesis enhanced by nutrient incorporation would produce more OH^- (Eqs. 2-3 and 2-4) and increase CO_3^{2-} (Eq. 2-5), taking into account the hypothesis of Goreau (1959). Otherwise, from the perspective of the titration hypothesis, enhanced photosynthesis would alkalize coral coelenteric space more strongly (Eqs. 2-3 and 2-4) and may facilitate consumption of H^+ produced by the $CaCO_3$ precipitation (Eq. 2-7), which results in enhancement of the calcification (Furla et al. 1998).

Second, the nutrient enrichment enhanced synthesis of an organic matrix, which is a prerequisite step for the formation and growth of coral skeleton. It has been reported that coral skeleton contains organic matters such as

mucopolysaccharides (Goreau 1959), proteins (Allemand et al. 1998), calcium-binding phospholipids (Isa and Okazaki 1987), and glycoproteins (Constantz and Weiner, 1988). Allemand et al. (1998) demonstrated that inhibition of protein synthesis reduced Ca deposition rate simultaneously, suggesting that organic matrix biosynthesis, rather than calcium deposition, may be a limiting factor controlling the coral skeletogenesis. In the present study, the nutrient enrichment could have increased amino acid synthesis by the zooxanthellae. This higher amount of amino acids would have been translocated to the host, and consequently, host biomass was enlarged with the increased supply of organic matter with low C:N ratios (Table 2-1). Though C:N ratio of the host did not significantly change, a protein pool for synthesis of the organic matrix might have increased with the nutrient pretreatment. Several authors have shown that photosynthetic products of zooxanthellae may be used as precursors for the organic matrix (e.g., Young et al. 1971).

Third possibility is that increased supply of metabolic CO_2 in the calicoblastic cell enhanced the calcification rate. Furla et al. (2000) showed that metabolic CO_2 would be the main source (70%–75%) of total C for calcification. It has also been shown that respiration rates can be increased by higher nutrient conditions and subsequent coral biomass increase (Marubini and Davies 1996, Ferrier-Pagès et al. 2000a). Respiration rates might also have increased in the present study due to the biomass increase (Table 2-1). The nutrient input in this study might have stimulated calcification through increased tissue biomass and supply of metabolic CO_2 (Furla et al. 2000). All of the three possibilities might have had a complex effect and stimulated calcification.

2-4-4. Negative effects of nutrients on calcification

Unlike the present results, many others have conversely reported negative effects of nutrient enrichment on calcification (Stambler et al. 1991, Marubini and Davies 1996, Ferrier-Pagès et al. 2000a). One well-known hypothesis to explain the decreased calcification is that photosynthesis and calcification may compete for the same DIC pool (Stambler et al. 1991). Photosynthetic activity stimulated by nutrient enrichment may draw down the internal DIC pool for calcification. Langdon and Atkinson (2005) measured photosynthesis and calcification rates in low nutrient seawater after exposing corals to very high nutrient conditions (NH_4^+: 109 μmol L^{-1}, PO_4^{3-}: 13 μmol L^{-1}) for 4 h. They observed that the increase in photosynthesis was almost mole for mole with the decline in calcification, suggesting that under saturation of the internal nutrient pool, the two C fixation processes compete for the same DIC pool.

However, at least under the condition of the present study, both of photosynthesis and calcification more or less increased, suggesting that the internal DIC pool was not in the upper limit, supposing that photosynthesis and calcification use DIC in the same pool. Whether or not the increase in photosynthesis induces the decline in calcification might depend on several conditions such as nutrient levels, exposure period to the nutrients, and subsequent change in Chl *a* density. In most observations which reported significant growth rate reduction (Stambler et al. 1991, Marubini and Thake 1999, Ferrier-Pagès et al. 2000a), DIN concentration was set at >15 μmol L^{-1}, except for Marubini and Davies (1996). This very high level of DIN

compared to in situ concentrations could cause saturation of the internal DIC pools (Langdon and Atkinson 2005) and subsequent competition for the limited DIC supply, which might have reduced skeletal growth rates.

In the study of Marubini and Davies (1996), a significant decrease in calcification rates of *Porites porites* and *Montastrea annularis* was observed by 30 to 60%, even using a relatively low range of NO_3^- concentrations (1, 5 and 20 μmol L^{-1}). They also adopted the hypothesis that calcification rates were decreased due to the competition with the photosynthesis for the same DIC pool, although the hypothesis did not fit the case of the corals under 1 μmol L^{-1} NO_3^- condition: the calcification rate was reduced without any change in photosynthesis. The different result in calcification from the present study might be derived from nutrient availability during measurement of calcification: While Marubini and Davies (1996) measured the calcification rate under the each NO_3^- concentration, calcification in the present study was measured under the nutrient-depleted condition for the all corals in order to focus on the direct effect of photosynthetic DIC uptake rates on the calcification.

With nutrients, zooxanthellae use more of their photosynthetic products for their own growth, and consequently translocation of the algal photosynthates to the host could be decreased (Dubinsky et al. 1990). In Marubini and Davies (1996), the calcification rate under relatively low NO_3^- concentrations such as 1 μmol L^{-1} might have been reduced by the decreased translocation of organic matter (Stambler et al. 1991), a part of which may be used for synthesis of organic matrix as mentioned above (Allemand et al. 1998). In the present study, the reduction of translocation during measurement of photosynthesis and calcification could have not occurred because nutrients were depleted during the period. Therefore, calcification rates

might be decreased by nutrient enrichment according to the processes of not only DIC competition between photosynthesis and calcification but also reduced translocation of photosynthetic products to the host. This is supported by why the DIC competition hypothesis did not fit the case of the 1 μmol L^{-1} NO_3^- condition in Marubini and Davies (1996).

Some authors also observed negative effects of nutrient enrichment on coral growth from in situ investigation (Walker and Ormond 1982, Tomascik and Sander 1985). Tomascik and Sander (1985) monitored environmental variables and coral growth rates at several locations, which were under different degrees of environmental stress caused by increased eutrophication. Though both concentrations of inorganic N (<0.5 μmol L^{-1}) and P (<0.01 μmol L^{-1}) were negatively correlated with the growth rate, they concluded that the nutrients had little or no direct causal effect on growth themselves and that concentration of suspended particulate matter was the best univariate estimator of coral growth rates. It might be difficult to evaluate nutrient effects alone, because many other environmental factors, such as temperature, light intensity, and water movement, simultaneously have impacts on corals in actual reefs.

2-4-5. Production of extra organic matter

The measured C fixation ratio of the net photosynthesis to the calcification was 0.76 for the corals untreated with nutrients (Table 2-2). This corresponds well to the average ratio 0.77 of previous studies (reviewed by Gattuso et al. 1999). On the

other hand, structural ratios of coral plus algal C biomass to skeletal C for each coral branch were 0.31 on average, which were significantly (60%) lower than the measured fixation ratio (Table 2-2). Supposing that the structural ratio would keep constant under the incubation condition, the extra organic C (6.0 μmol C cm^{-2} d^{-1}) should be gradually consumed for respiration. Most of newly produced respiratory substrates must be lost from the tissue within 24 h after synthesized, but some of them could be gradually consumed over a longer period (Tanaka et al. 2006a). Another possibility of the extra organic production is organic matter release to the ambient seawater. It has been well known that corals release some parts of photosynthetically fixed C as dissolved and particulate organic matter (Crossland 1987, Schlichter and Liebezeit 1991, Ferrier-Pagès et al. 1998).

For the nutrient enriched corals for 10 d, the C fixation ratio increased 2.3 fold (Table 2-2; $P{:}C$ = 1.73 ± 0.19), and the structural ratio was also raised comparably (2.4 fold). Assuming that the structural ratio was already in the steady state, the extra organic C production mentioned above was equivalent to 16 μmol C cm^{-2} d^{-1}. In this case, even considering that the enlarged tissue biomass (Table 2-1) leads to the respiration increase roughly in proportion to the biomass, the extra primary production and subsequent organic matter release might become greater with nutrient enrichment. Whether or not organic C release from coral colonies is stimulated by nutrient enrichment remains to be resolved (Ferrier-Pagès et al. 1998, Tanaka et al. 2006b).

Given longer time, the structural ratio might approach the observed C fixation ratio even for the corals having high Chl *a* density. In this case, the estimated extra organic C (16 μmol C cm^{-2} d^{-1}) could be more or less overestimated. However,

a question arising here is what range of tissue : skeleton ratios are appropriate or possible for the coral growth. If photosynthetic products were stored in the tissue at the observed fixation ratio, tissue : skeleton ratios would gradually increase under the high Chl *a* density, and at some future point, the carbonate skeleton might brim over with the excessively produced organic matter.

2-5. Conclusions

In summary, the nutrient level in this study (<5 μmol L^{-1} NO_3^- and <0.3 μmol L^{-1} PO_4^{3-}) increased zooxanthellate Chl *a* density per unit surface area, and photosynthetic C fixation rates were enhanced in proportion to the Chl *a*. Though most of the previous studies set nutrient concentration at considerably high levels (e.g., NO_3^-: 10–50 mmol L^{-1}), this study has shown that even moderate nutrient loading, which is already seen at coastal area of the study site, can alter coral physiological states. Tissue biomass of both the host coral and the zooxanthellae was consequently doubled for 10 d of the nutrient enrichment. In contrast, calcification was only left behind with respect to C fixation rates. Unless carbonate skeletal density was changed, the skeletal extension might not keep up with the tissue growth.

Though the increase ratio of the calcification was less than that of the net photosynthesis, the present results suggest that calcification does not always decrease when the photosynthetic rate is increased by nutrient enrichment. Both hypotheses, i.e., the DIC competition and the reduction of organic matter translocation from zooxanthellae to the host, might be involved in decreased calcification found in the

previous studies.

The more algal Chl *a* density increased, the more produced organic C would be stored for the tissue growth and/or excreted as organic matter into the ambient seawater. In the former case, it should be investigated how this imbalance between the two C fixation rates affects longer-term coral growth. It may cause excess and even pathological tissue hypertrophy and finally lead to competition for the internal DIC pool between photosynthesis and calcification as implied in other previous studies (Stambler et al. 1991, Marubini and Davies 1996). In the latter case, the excreted organic matter could be utilized by microorganisms, and therefore change organic C flux from the coral colony to detritus food webs in the reef water. Several studies have focused on this phenomenon (Ferrier-Pagès et al. 2000b, Wild et al. 2004a). Because zooxanthellae in hermatypic corals are one of the major primary producers in coral reefs, net CO_2 fixation of a whole reef would be affected by at what rate the released organic matter is mineralized to CO_2.

Table 2-1 Chl *a* density and biomass of the host coral and zooxanthellae affected by the nutrient enrichment for each scheduled period. Data are shown as mean ± standard error (SE); $n = 12, 9, 8$ for 0, 5, 10 d enrichment, respectively.

Enriched period (d)	Chl *a* (μg cm^{-2})	Coral (μmol cm^{-2})			Zooxanthella (μmol cm^{-2})			Zooxanthella:Coral (%)	
		C	N	C:N	C	N	C:N	C	N
0	2.6±0.2	343±29	46±4	7.4±0.1	26±2	3.5±0.4	7.5±0.3	7.5±1.0	7.6±1.1
5	5.2±0.1	495±40	63±6	7.9±0.1	38±3	4.4±0.4	8.8±0.3	7.7±0.9	7.0±0.9
10	6.9±0.3	588±56	72±7	8.1±0.1	52±5	6.0±0.6	8.7±0.2	8.8±1.2	8.3±1.2

Table 2-2 Photosynthetic C fixation rate (*P*) (μmol C cm^{-2} d^{-1}) and calcification rate (*C*) (μmol C cm^{-2} d^{-1}) calculated from ^{13}C-tracer accumulation. Tissue C:skeletal C is the structural ratio of tissue organic C to skeletal C forming $CaCO_3$ of each coral branch. Extra organic C (μmol C cm^{-2} d^{-1}) was calculated from the difference between the fixation ratio (*P*:*C*) and the structural ratio. Data are given as mean ± SE; $n = 12, 9, 8$ for 0, 5, 10 d enrichment, respectively.

Enriched period (d)	Chl *a* (μg cm^{-2})	*P*	*C*	*P* : *C*	Tissue C : skeletal C	Extra organic C
0	2.6±0.2	10.1±0.9	13.3±0.6	0.76±0.06	0.31±0.03	6.0±1.1
5	5.2±0.1	21.0±1.7	12.8±1.1	1.69±0.14	0.49±0.06	15±3
10	6.9±0.3	28.3±2.2	16.8±0.7	1.73±0.19	0.75±0.06	16±4

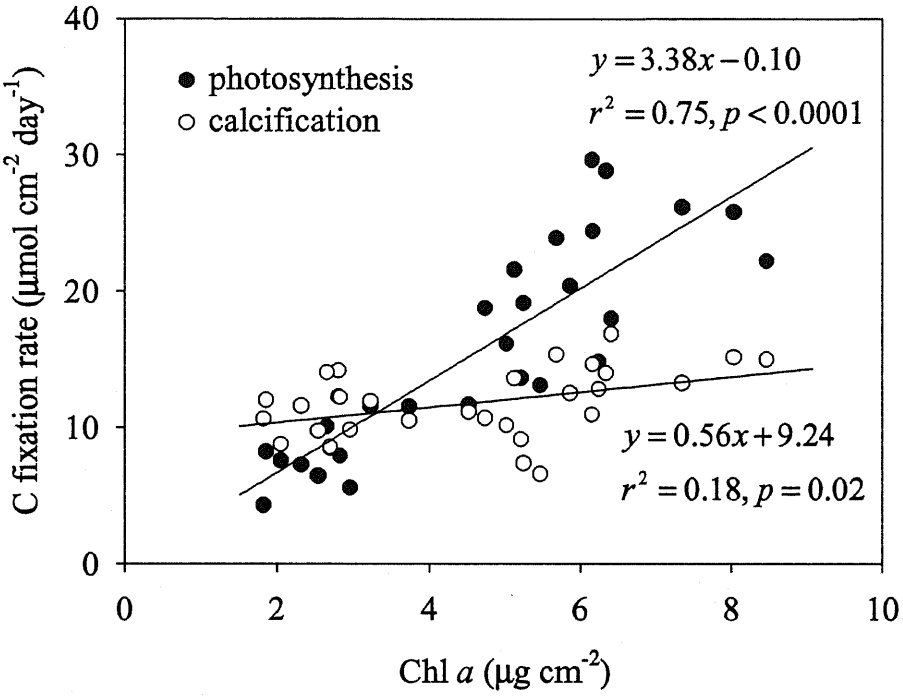

Fig. 2-1 Relationship between Chl *a* (μg cm^{-2}) density and C fixation rates (μmol cm^{-2} d^{-1}) of photosynthesis and calcification per unit surface area of the corals.

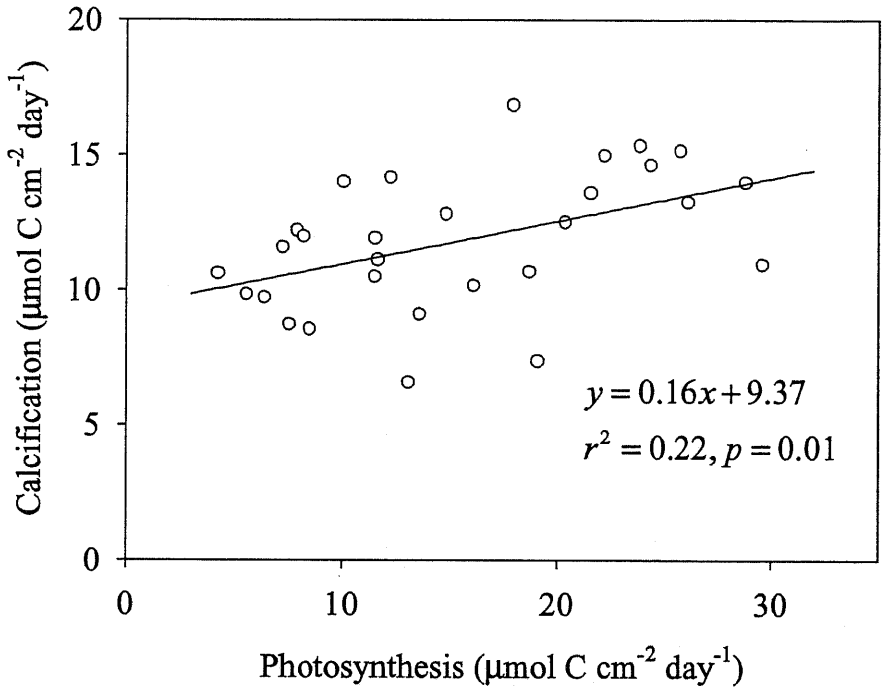

Fig. 2-2 Relationship between photosynthesis and calcification of the corals incubated under the nutrient-enriched condition.

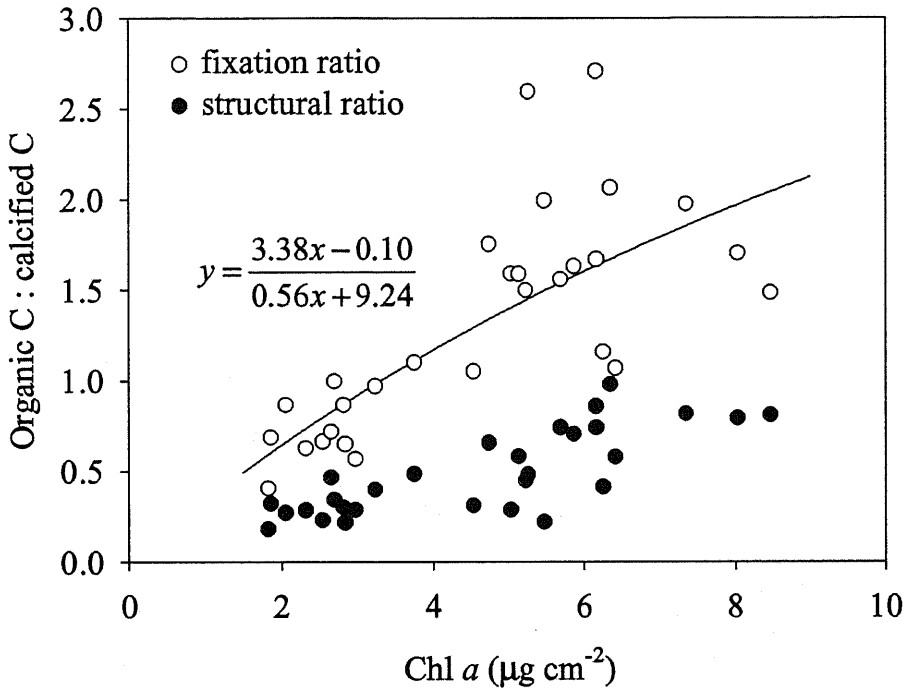

Fig. 2-3 The difference in organic C : skeletal C ratios between the measured C fixation ratios and the structural ratios of each coral branch, depending on the Chl *a* density of zooxanthellae (μg cm^{-2}). The fitting curve for the fixation ratio was obtained from the linear regressions in Fig. 2-1.

Chapter 3

The Release of DOM and POM

3-1. Introduction

It is well known that hermatypic corals release mucoid organic matter (mucus) to the ambient seawater. The mucus is massively released under stressful conditions such as aerial exposure (Daumas et al. 1982, Wild et al. 2004a, b), cold treatment (Meikle 1988, Coffroth 1990), and sedimentation (Mitchell and Chet 1975). It has been proposed that the mucus has many protective functions against desiccation (Krupp 1984, Daumas and Thomassin 1977), pathogens (Cooney et al. 2002, Ritchie 2006), UVR damage (Drollet et al. 1993), smothering by sediment (Schumacher 1977). These functions were reviewed by Brown and Bythell (2005).

Not only such stressful conditions, but it is also known that corals usually release organic matter as a result of the daily metabolism (Crossland et al. 1987, Ferrier-Pagès et al. 1998, Tanaka et al. 2006b). Davies (1984) first proposed that mucus release may function primarily as an excretory pathway for excess organic C produced by symbiont photosynthesis. The release of mucus may therefore be a direct consequence of nitrogen (N) limitation and the low quality (high C:N ratios) food provided to the host coral, due to high levels of the symbiont photosynthesis and rates of translocation to the host (78%–97%; Muscatine et al. 1984, Edmunds and Davies 1986, Tanaka et al. 2006a)

The organic matter released from corals contains various biological compounds such as carbohydrates, lipids, and proteins (Ducklow and Mitchell 1979b, Meikle 1988, Coffroth 1990), and therefore, has been considered to support a large variety of organisms from bacteria to fishes in the coral reef as energy sources (Benson and Muscatine 1974, Ducklow and Mitchell 1979a, Ferrier-Pagès et al. 2000b, Wild et al. 2004a). The release rates of organic carbon (C) under normal metabolism have been reported to be 6%–40% of the net photosynthetically fixed C (Crossland et al. 1980, Muscatine et al. 1984, Crossland 1987, Ferrier-Pagès et al. 1998). This indicates that the organic matter release from coral colonies could be greatly fueled by photosynthesis of the endosymbiotic algae (zooxanthellae), because corals with the symbiotic algae obtain >90% of the required organic C from the algal photosynthesis (Bythell 1988).

Nowadays, anthropogenic eutrophication is increasingly becoming an issue in many coral reefs around the world. However, the effect of nutrient enrichment on organic matter release from coral colonies has been scarcely investigated (Stimson and Kinzie 1991, Ferrier-Pagès et al. 1998, Tanaka et al. 2006b). Because reef water is generally depleted with inorganic nutrients, the growth of coral colony is limited by nutrients (Cook and D'Elia 1987). As a result, an increased nutrient concentration has been reported to enhance the photosynthesis of zooxanthellae in previous studies (e.g., Hoegh-Guldberg and Smith 1989, Muller-Parker et al. 1994, Marubini and Davies 1996, Tanaka et al. 2007). Moreover, effects of the nutrient enrichment have also been observed in coral calcification, and many studies have suggested that

excess nutrient incorporation by zooxanthellae reduces the host calcification rate (Stambler et al. 1991, Marubini and Davies 1996, Ferrier-Pagès et al. 2000a). The most predominant hypothesis to explain this reduction is a competition for an internal pool of dissolved inorganic C (DIC) between algal photosynthesis and coral calcification (Stambler et al. 1991): photosynthetic activity enhanced by inorganic nutrients may draw down the internal DIC pool for calcification.

In Chapter 2 of the present study, it has been shown that inorganic nutrient enrichment leads to imbalanced coral growth between organic tissue and carbonate skeleton. This result suggested that organic matter release from the corals might be enhanced to reduce excess organic matter in the tissue. The purpose of this chapter was to investigate the effect of increasing nutrient levels on the release of dissolved and particulate organic matter (DOM and POM, respectively) from reef-building corals. Stimson and Kinzie (1991) reported that release rates of zooxanthellae per unit surface area of the coral *Pocillopora damicornis* was increased by the incubation under N-enriched condition. Not only zooxanthellae but other organic matter released from corals might be influenced by nutrient enrichment. In the present study, POM and DOM were simultaneously observed and the contributions of bacteria and released zooxanthellae to the bulk POM were also evaluated to investigate the composition of the released POM.

3-2. Material and methods

3-2-1. Coral collection and pre-incubation

The experiment was performed using the zooxanthellate coral branches of *Acropora pulchra*, which were collected on the reef flat of Shiraho Reef in Ishigaki Island, Japan in August 2006. Coral branches of ca. 6 cm were collected 500 m offshore from the coast, where the depth was ca. 0.5 m at low tide.

Within 1 h after the collection, the corals were transferred to the prepared outdoor aquarium (AQ1, inner volume: 90 L) in Seikai National Fisheries Research Institute (Fig. 3-1a). Seawater was supplied to the aquarium with the rate of 3 L min^{-1}, and the temperature was controlled at 28.7 ± 0.7°C (mean ± SD), which was automatically measured every 10 min (COMPACT-CT; Alec Electronics Co. Ltd.). Each coral branch was suspended in the aquarium using nylon line. Seawater in the aquarium was kept in a nutrient-enriched condition, where the stock solution of KNO_3 (28 mmol N L^{-1}) and NaH_2PO_4 (1.4 mmol P L^{-1}) was continuously pumped with a peristaltic pump. Consequently, nutrient concentrations in the aquarium AQ1 were 4.8 ± 0.6 μmol L^{-1} NO_3^-, 0.03 ± 0.01 μmol L^{-1} NO_2^-, 0.05 ± 0.02 μmol L^{-1} NH_4^+, 0.28 ± 0.11 μmol L^{-1} PO_4^{3-} (mean ± SD, n = 19). The pre-incubation of the collected corals in the nutrient-enriched aquarium started 5, 7, 10, 12 and 14 d before the following bottle experiment on organic matter release. Two corals at each time point (total 10 corals) were pre-incubated in AQ1.

To prepare corals close to the in situ physiological state, more six coral

branches were collected 1 d (four corals) and 3 d (two corals) before the following bottle experiment, and pre-incubated in a different aquarium (AQ2), where extra addition of KNO_3 and NaH_2PO_4 was not conducted to reduce physiological change of the corals (Fig. 3-1a). Consequently, AQ2 had lower nutrients during the pre-incubation (1.8 ± 0.3 μmol L^{-1} NO_3^-, 0.03 ± 0.01 μmol L^{-1} NO_2^-, 0.04 ± 0.02 μmol L^{-1} NH_4^+, 0.08 ± 0.02 μmol L^{-1} PO_4^{3-}; mean ± SD, n = 20). The pre-incubation in AQ1 and AQ2 made the collected corals different in physiological status, depending on the pre-incubated period of 1−14 d.

3-2-2. Bottle experiment

To measure organic matter release rates, the pre-incubated coral branches were incubated for 5 h in glass bottles containing 700 mL seawater (Fig. 3-1b), which was filtered with Whatman GF/F filters (pore size 0.7 μm, filter diameter 47 mm) in advance. The bottle experiment was conducted under both light and dark conditions, because it has been reported that the release rate of DOC from corals was dependent on the light intensity (Crossland 1987). The light experiments were conducted in the laboratory using halogen lamps (LL) and outdoors under natural sunlight (LO). Both experiments of LL and LO were performed at 10:00−15:00 h. The average irradiance during the 5 h was 400 and 230 μmol photons m^{-2} s^{-1} in LL and LO, respectively. The dark experiment (DL) was conducted in the laboratory at

night (22:00–03:00 h). The pre-incubated corals for 1–14 d (total 16 corals) were used for one of the LL, LO and DL experiment: the corals pre-incubated for 3, 7 and 12 d were used in LL, 1, 5 and 10 d in LO, 1 and 14 d in DL. The incubation was conducted with duplicate bottles, and one coral branch was suspended in each bottle using nylon line. One control bottle without a coral was also prepared for each light condition.

The incubation seawater was obtained at the outer reef, and had very low nutrients (0.02 μmol L^{-1} NO_3^-, 0.03 μmol L^{-1} NO_2^-, 0.07 μmol L^{-1} NH_4^+, 0.03 μmol L^{-1} PO_4^{3-}) and usual DIC (2.0 mmol L^{-1}). At the beginning of the bottle experiments, KNO_3 and NaH_2PO_4 were added for the corals pre-incubated in AQ1 to get the final concentrations of 5 and 0.25 μmol L^{-1}, respectively. For the corals pre-incubated in AQ2, KNO_3 and NaH_2PO_4 were not added to the seawater to create an oligotrophic condition. The seawater in the bottles was gently stirred with a magnetic stirrer, and the temperature was controlled at 28.8 ± 0.0°C (mean ± SD) by flowing seawater outside the bottles.

After the bottle incubation of 5 h, the coral branches were taken out from the seawater, and subsamples for the abundance of bacteria and algae were collected in 10-mL acrylic tubes. They were immediately fixed with pre-filtered formalin (final concentration 2%, v/v). The remaining incubated seawater was filtered with pre-combusted Whatman GF/F filters, and the filtrate was sealed into pre-combusted glass ampoules of 20 mL and stored at -20°C until analysis for inorganic nutrients and DOM. The GF/F filters were also stored at -20°C to analyze POM and Chl *a*.

Each of the incubated coral branches was divided into several parts, and three of them were put in polypropylene tubes containing 5 mL of methanol (MeOH) to extract Chl *a*. The other parts were stored at -20°C to determine the tissue biomass and surface area.

3-2-3. Chemical analysis and calculations

Dissolved inorganic N (DIN: NO_3^-, NO_2^-, NH_4^+) and PO_4^{3-} concentrations were quantified with a nutrient analyzer AACS-III (BRAN+LUEBBE). The concentration of DOC and total dissolved N (TDN) was measured by the high temperature catalytic oxidation method (HTCO) using TOC-5000 (Shimadzu) and ECL-880 US (Yanaco) (Ogawa et al. 1999). DON concentrations were calculated by subtraction of DIN from TDN.

The GF/F filter containing POM was divided into two parts. One of them was immersed in N, N-dimetylformamide (DMF) in polypropylene tubes to extract Chl *a*, and another was used for quantifying bulk POM. The filter for POM quantification was dried in an oven at 50°C for 3 h, and then treated with the vapor of 12 N HCl for 12 h to remove inorganic C. After evaporating extra HCl on a hot plate (80°C) under vacuum, the filters were dried again in the oven at 50°C. Quantity of POM on the filter was determined with a CHN elemental analyzer (Fisons; NA-1500).

Chl *a* concentration in the DMF and MeOH extracts was measured with a fluorometer (Turner 10-AU; Holm-Hansen et al. 1965). The abundance of bacteria and algae fixed by formalin was counted with an epifluorescence microscopy after staining with 4',6-diamidino-2-phynylindole (DAPI) as previously described (Porter and Feig 1980). At least 400 and 200 cells were counted for bacteria and algae, respectively. Because most of the algal subsamples did not contain enough algae to count 200 cells, only limited data were shown in the present study.

The coral branches used for LL and LO experiments were immersed in 0.1 N NaOH solution of 20 mL (50°C for 2 h) to determine the tissue biomass (Muller-Parker et al. 1994). With this procedure, organic tissue was dissolved in the solution and the skeleton was whitened. A part of the tissue suspension (180 μL) was infiltrated into a pre-combusted Whatman GF/D filter (pore size 2.7 μm, filter diameter 10 mm), taking care not to overflow the filter. The quantity of organic matter in the filters was measured with a CHN analyzer (Fisons; NA-1500). The tissue analysis was conducted with duplicate filters from each coral branch.

DOM and POM release rates from the corals (R: mol cm^{-2} h^{-1}) were calculated from the difference in organic matter concentration (mol L^{-1}) at the end of the bottle incubation between coral incubated (C) and control seawater (C_c).

$$R = (C - C_c) \times V \times S^{-1} \times t^{-1} \qquad (3\text{-}1)$$

where V, S and t are seawater volume (L), coral surface area (cm^2) and the

incubation period of 5 h, respectively. Surface area of the corals was determined by the aluminum foil method (Marsh 1970). Chl *a* release rates from the corals (g Chl *a* cm^{-2} h^{-1}) were also calculated in the same way as *R*, using Chl *a* concentration (g L^{-1}) in the incubated seawater.

Linear regression was conducted with Sigma Plot 8.02 (SPSS Inc.) and significant changes were shown with solid lines in the figures. Difference of average values between two groups was evaluated by Student's *t*-test. The change in C:N ratios of the released POM and DOM was assessed by the Spearman's rank correlation.

3-3. Results

3-3-1. Chl *a* density and tissue biomass

Chl *a* density per unit surface area of the corals increased with the duration of the nutrient-enriched pre-incubation (hereafter, nutrient-enriched period). The Chl *a* density was initially 4.0 ± 0.3 μg cm^{-2} (mean ± SD, $n = 4$). The density increased during the first 10 d and then kept almost constant until Day 14 (Fig. 3-2). The average Chl *a* density during 10–14 d was 8.8 ± 0.7 μg cm^{-2} (mean ± SD, $n = 6$).

C and N biomass of the coral colony (coral + zooxanthellae) significantly increased with the nutrient-enriched period for both C and N ($p < 0.01$; Fig. 3-3a).

From the regression lines, the C and N biomass at Day 1 was calculated to be 160 and 20 μmol cm^{-2}, respectively, and they increased to 190 and 28 μmol cm^{-2} at Day 12 (Fig. 3-3a, Table 3-1). Though the obtained biomass was greater than those in Table 2-1, this could be caused by the error of calculation method in Chapter 2 (see '2-2-3'). During the period of the pre-incubation, C:N ratio of the colony significantly decreased from 8.2 to 6.6 (average of duplicate samples) ($p < 0.05$; Fig. 3-3b). The correlation coefficients (± SE) of the increasing C and N biomass indicated that net tissue growth rates were 2.7 (± 0.8) and 0.67 (± 0.16) μmol cm^{-2} d^{-1} for C and N, respectively (Fig. 3-3a). Thus, the C:N ratio of the increased biomass during the nutrient enrichment was calculated to be 4.0 ± 1.5.

3-3-2. Release of bulk POM

During the 5-h bottle experiment, POC accumulated in the incubated seawater with the range of 4.4–36 μmol L^{-1} in the light conditions (LL and LO), and 2.1–9.2 μmol L^{-1} in dark (DL). The POC release rates per unit surface area of the corals ranged 16–179 nmol cm^{-2} h^{-1} (Fig. 3-4a). Positive correlation was found between the POC release rates and the nutrient-enriched period in the LL condition ($p < 0.05$; Fig. 3-4a).

PON accumulated with the range of 0.3–3.9 μmol L^{-1} in LL and LO, and 0.5–1.2 μmol L^{-1} in DL. The PON release rates per unit coral surface area were

1.4–20 nmol cm^{-2} h^{-1} (Fig. 3-4b). In the both conditions of LL and LO, positive correlation was found between the PON release rates and the nutrient-enriched period ($p < 0.05$; Fig. 3-4b).

The C:N ratio of the released POM well accorded with each other between duplicate incubation (Fig. 3-4c). The C:N ratios in LO significantly decreased with the nutrient-enriched period ($p < 0.05$), while the relationship was not barely significant in LL ($p = 0.06$). The C:N ratios in LL and LO appeared to become constant at ca. 10 after 1 week of the nutrient enrichment. In DL, the C:N ratios were lower than any values observed in LL and LO (Fig. 3-4c), and the average C:N ratio was 5.8.

3-3-3. Release of zooxanthellae

Chl *a* concentration in the incubated seawater was 0.02–0.6 μg L^{-1} after the 5-h bottle incubation. The Chl *a* release rates per unit surface area of the corals were <0.5 ng cm^{-2} h^{-1} for the original corals, and the pre-incubation for 12 d under the nutrient enrichment increased the Chl *a* release rate up to 2.5 ng cm^{-2} h^{-1} (Fig. 3-5a). Significant correlation between the Chl *a* release rates and the nutrient-enriched period was found in LL ($p < 0.05$). When Chl *a* was released most (2.5 ng cm^{-2} h^{-1}), the release rate of algal cells, which was determined by direct cell count was 1060 ± 70 cells cm^{-2} h^{-1} (mean ± SE). There was also found significant correlation between

the Chl *a* release rates and the bulk release rates of POC ($p < 0.01$; Fig. 3-5b).

3-3-4. Release of bulk DOM

DOC accumulated in the incubated seawater with the range of 2.6–10 μmol L^{-1} in LL and LO, and 0.2–2.3 μmol L^{-1} in DL. Significant correlation between the DOC release rates and the nutrient-enriched period was not found for the all light conditions (Fig. 3-6a). The average DOC release rates in LL, LO and DL were 36 ± 10, 22 ± 5 and 6.6 ± 5.1 nmol cm^{-2} h^{-1} (mean ± SD), respectively (Table 3-2). The light intensity significantly affected the average DOC release rates: the release rates in LL and LO were significantly higher than that in DL ($p < 0.001$ and $p < 0.01$, respectively), and there was also found significant difference between LL and LO ($p < 0.05$). The release rates of bulk DOC in LL and LO were significantly correlated with the Chl *a* release rates as in the case of POC ($p < 0.05$; Fig. 3-5b).

DON accumulated in the incubated seawater with the range of 0.2–1.4 μmol L^{-1} in LL and LO, and 0.2–0.6 μmol L^{-1} in DL. Significant correlation between the DON release rates and the nutrient-enriched period was found only in LO ($p < 0.05$; Fig. 3-6b). The average DON release rates in LL and DL were 5.3 ± 1.0 and 2.1 ± 0.7 nmol cm^{-2} h^{-1} (mean ± SD), which were significantly different ($p < 0.001$; Table 3-2). Significant difference in the average DON release rate was also found between LL and LO ($p < 0.001$)

The C:N ratio of the released DOM involved large error of analysis for the corals pre-incubated for 1 d (Fig. 3-6c). This was caused by small increase of DON concentration (0.2–0.3 μmol L^{-1}) compared to the analytical precision (± 0.1 μmol L^{-1}). Excluding the data at Day 1, significant changes were not observed by the nutrient treatment for both LL and LO. The average C:N ratios during the period of 3–12 d was 7.5 ± 1.6 (mean ± SD, n = 10) in the light conditions.

3-3-5. Bacterial abundance

The bacterial abundance at the beginning of the bottle experiment was 4.9 ± 0.2 × 10^5 cells mL^{-1} (mean ± SE), and changed to 3.4–5.7 × 10^5 cells mL^{-1} at the end of the 5-h coral incubation. In LL and LO, the bacterial abundance did not significantly change in seven of twelve incubation (Fig. 3-7), and significantly decreased in the four incubation (no data for the rest one). In DL, bacterial abundance increased in all the incubation bottles, and three of them were statistically significant (Fig. 3-7). The increased bacterial abundance in DL was 5.0 × 10^4 cells mL^{-1} on average during the 5-h bottle incubation.

3-4. Discussion

3-4-1. Chl *a* density and coral plus algal biomass

The pre-incubation of the coral *Acropora pulchra* in the nutrient enrichment of the present study has clearly increased Chl *a* density per unit surface area of the corals (Fig. 3-2). The maximum Chl *a* density in this experiment (ca. 9 μg cm^{-2}) was similar to that in Chapter 2 (Fig. 2-1). The maximum density might be controlled by regulatory mechanisms in the symbiotic colony (Muscatine and Pool 1979). The increase in Chl *a* in reef-building corals has also been observed in previous studies as a result of nutrient enrichment (Muller-Parker et al. 1994, Marubini and Davies 1996, Tanaka et al. 2007). The increase in Chl *a* density due to nutrient enrichment indicates that nutrient availability limited the algal growth when the corals inhabited in natural reef water (Cook and D'Elia 1987). In this study, newly accumulated organic matter in the coral colonies had the C:N ratio of 4.0 ± 1.5 (Fig. 3-3a), and the C:N ratio of the whole biomass of the colonies significantly decreased from 8 to 7 (Fig. 3-3b). These results show that the coral colonies were conservative for the N acquired during the pre-incubation and stored organic matter of the lower C:N ratio than that of the original tissue. This response also suggests that inorganic nutrients were insufficient for the coral colonies in the natural reef water.

3-4-2. Bacterial abundance and evaluation of the release rates of organic matter

Using the corals pre-incubated for different periods under the nutrient enrichment, the release rates of DOM and POM from the corals were measured in this study. The release rates were calculated from finally accumulated amount of the organic matter after the 5-h bottle incubation, therefore, can be considered net release rates. Because a part of DOM can be rapidly incorporated by bacteria, the observed release rates of DOM might be more or less underestimated compared to the gross release rates. However, the abundance of free-living bacteria did not significantly change in the most incubation under the light conditions of LL and LO (Fig. 3-7), and in four of them the abundance significantly decreased. The decrease might be caused by coral feeding on the bacteria (Houlbrèque et al. 2004). Contrarily, the bacterial abundance in DL increased by 5.0×10^4 cells ml^{-1} on average after the 5-h incubation. This probably resulted from bacterial proliferation fueled by the DOM released from the corals (Vacelet and Thomassin 1991, Ferrier-Pagès et al. 2000b). These two processes, i.e., coral feeding on bacteria and bacterial growth enhanced by DOM released from corals, could simultaneously occur in coral incubation in a closed system. The bacterial abundance observed in the present study would represent apparent changes affected by the two processes. It was also suggested that bacterial community near surface of the coral *Galaxea Fascicularis* had an important role for the removal of the DOM released from the coral (Ferrier-Pagès et al. 1998). These bacterial activities might induce underestimation of the release rate of organic matter, especially DOM.

However, supposing that the bacterial contribution to the consumption of

organic matter was equal for all the coral incubation, it would be reasonable to discuss the effect of nutrient enrichment on the organic matter release. Moreover, NH_4^+ production due to bacterial mineralization of organic matter was <0.2 nmol cm^{-2} h^{-1}, which was calculated from NH_4^+ accumulation in the incubation seawater (data not shown). The NH_4^+ production rate was far lower than the DON and PON release rates (Figs. 3-4b, 3-6b), suggesting that bacterial consumption of the released organic matter was very minor compared to the whole release. The observed release rates in this study were comparable to some previously reported values: 150 nmol C cm^{-2} h^{-1} as TOC at daytime from *Acropora palmata* (Means and Sigleo 1986), 1.8 and 2.3 μmol C cm^{-2} d^{-1} as mucus plus DOC-lipid from *A. variabilis* and *Stylophora pistillata*, respectively (Crossland 1987), 58 and 83 nmol C cm^{-2} h^{-1} as POC at daytime from *A. millepora* and *A. aspera*, respectively (Wild et al. 2005a). These data support that the release rates obtained in the present study were not considerably underestimated due to the bacterial consumption.

3-4-3. Enhancement of POM release rates and the contribution of symbiotic algae

The present study has shown that the release rate of PON significantly increased due to the nutrient enrichment in LL and LO ($p < 0.05$; Fig. 3-4b), and the release of POC was also significantly enhanced in LL ($p < 0.05$; Fig. 3-4a). One of the components of the POM released from hermatypic corals can be symbiotic algae

(Hoegh-Guldberg et al. 1987, Stimson and Kinzie 1991). In this study, Chl *a* concentration increased in most of the incubation seawater (Fig. 3-5a), and direct count of algal cells confirmed that algae were actually released at the rate of 1100 cells cm^{-2} h^{-1} at maximum. Using the Chl *a* release rate in the same incubation bottle (2.5 ng cm^{-2} h^{-1}; Fig. 3-5a), Chl *a* content per algal cell was calculated to be 2.3 pg, which was within the range of previously reported values for zooxanthellae (ca. 1–4 pg; Hoegh-Guldberg and Smith 1989, Marubini and Davies 1996, Koop et al. 2001), implying that the observed Chl *a* could be derived from the released zooxanthellae. Similar release rate of zooxanthellae has been observed by Stimson and Kinzie (1991), who incubated *Pocillopora damicornis* under inorganic N enrichment (17 μmol L^{-1} NH_4^+) for 3–4 months. The maximum algal release rate from the corals was ca. 1000 cells cm^{-2} h^{-1}, which was recorded around 13:00 h.

The release rates of Chl *a* were initially 0–0.3 ng cm^{-2} h^{-1} (Fig. 3-5a), which accounted for <0.01% h^{-1} of the total standing stock of Chl *a* in the coral colony (Fig. 3-2). The release ratio to the standing stock was comparable with the previously reported range of <0.1% d^{-1} (Hoegh-Guldberg et al. 1987). With the nutrient treatment, the chl *a* release rate increased up to >2 ng cm^{-2} h^{-1} (Fig. 3-5a), while the increase rate of Chl *a* density in the colony was 2.5 times at most (Fig. 3-2). Thus, the ratio of released Chl *a* to the standing stock could have increased with the nutrient treatment, suggesting a response of the corals to reduce algal population in the coral colony. Inorganic nutrients could first be incorporated by symbiotic algae (Muller-Parker et al. 1994, Grover et al. 2003, Tanaka et al. 2006) and the algal

biomass would increase compared to the host (Muscatine et al. 1989, Marubini and Davies 1996). The priority of algae to utilize inorganic nutrients could have stimulated algal growth relative to the host and, subsequently, induced release of excess algae from the symbiotic colony.

In Chapter 2, *A. pulchra* was incubated under the similar nutrient condition to the present experiment for 10 d, and observed that the C : Chl *a* ratio of zooxanthellae decreased from 120 to 90 (g g^{-1}). Using the values, the observed Chl *a* release rates in the present study (<2.5 ng cm^{-2} h^{-1}; Fig. 3-5a) corresponded to <18 nmol C cm^{-2} h^{-1}. The release rates were much lower than the bulk POC release rates (Fig. 3-4a), indicating that the released bulk POC in LL and LO largely (ca. 90 %; Fig. 3-8) consisted of not algae but other organic matter.

Bacteria could also be contained in the bulk POM, but the final bacterial abundance in the incubated seawater was 3.4-5.7 × 10^5 cells mL^{-1}. Assuming that each bacterial cell contains 30 fg C (Fukuda et al. 1998), the bacterial C in the seawater was calculated to be 0.9–1.4 μmol L^{-1}, which accounted for <13% (average 8%) of the accumulated bulk POC. Because all of the bacteria could not be captured with GF/F filters, the value of average 8% is the maximum estimation. Therefore, it has been suggested that >80% of the released POM consisted of not algae and bacteria but presumably non-living organic matter derived from the host such as mucus and tissue fragments (Fig. 3-8).

It is noteworthy that the Chl *a* release rates in light were significantly correlated with both release rates of the bulk POC and DOC ($p < 0.01$ and 0.05,

respectively; Fig. 3-5b). The significant correlation was also found for the PON and DON ($p < 0.01$ and 0.05, respectively; figure not shown). These results implied that the release of zooxanthellae from the host simultaneously involved other POM and DOM release. Release of mucus or other tissue components might be necessary for the algal release to the ambient seawater. Chl *a* was also contained in the mucus released from the coral *Acropora* spp. in a normal submerged incubation (Wild et al. 2005a), suggesting that zooxanthellae and mucus were simultaneously released. It remains to be resolved that the release of zooxanthellae was due to active host expulsion of excess algal cells, or due to migration of zooxanthellae from the limited space in the host tissue (Hoegh-Guldberg et al. 1987, Stimson and Kinzie 1991).

The release rate of POC in LL was initially 53 nmol cm^{-2} h^{-1} at Day 3 and increased to 140 nmol cm^{-2} h^{-1} at Day 12, both of which were estimated from the regression line (Fig. 3-4a). In DL, the POC release rate was 17 and 35 nmol cm^{-2} h^{-1} at Day 1 and 14, respectively, which were calculated as the average values of the duplicate incubation (Fig. 3-4a). Supposing that each release rate continued for 12 h in the light and dark condition, the daily release rate of POC was calculated to be 840 nmol cm^{-2} d^{-1} for the initial corals and 2100 nmol cm^{-2} d^{-1} for the corals pre-incubated under the nutrient enrichment (Table 3-1). Similarly, the release rate of PON increased from 4.9 to 16 nmol cm^{-2} h^{-1} in LL, and from 3.7 to 5.0 nmol cm^{-2} h^{-1} in DL (Fig. 3-4b). The daily release rate of PON increased from 100 to 250 nmol cm^{-2} d^{-1}. Thus, both release rates of POC and PON increased approximately 2.5-fold (Table 3-1). Though the POM release in dark was not significantly enhanced in the

present study, the daily release rates of POC and PON could increase 2.3- and 2.4-fold, respectively (Table 3-1), in the supposition that the POM release in dark was same as the initial corals. On the other hand, C and N biomass of the coral colonies increased 1.2- and 1.4-fold, respectively (Table 3-1). These indicate that the ratio of POM release to the biomass increased with the nutrient enrichment, which might be the response of the corals to reduce excess organic matter in the coral colony. It has been observed that inorganic nutrient enrichment increases algal photosynthesis and contrarily reduce coral calcification (Marubini and Davies 1996, Ferrier-Pagès et al. 2000a, Langdon and Atkinson 2005). The present results have suggested that the imbalanced growth between soft tissue and carbonate skeleton could induce POM release from the coral colony to reduce excess organic matter in the tissue.

3-4-4. Release rates of DOM and the C:N ratios

DOM release rates were not significantly enhanced by the nutrient enrichment, except for DON release in LO (Figs. 3-6a, b), but were significantly enhanced by high light intensity (Table 3-2). The light enhancement of organic matter (mucus and DOC-lipid) release was also reported by Crossland (1987) using *Acropora variabilis* and *Stylophora pistillata*. Schlichter and Liebezeit (1991) observed that longer light pretreatment resulted in higher release rates of dissolved

free amino acids from the coral *Heteroxenia fuscescens*. These observations suggest that DOM release from coral colonies was strongly influenced by the algal photosynthetic rate.

The nutrient enrichment in the present study has increased Chl *a* density per unit surface area of the coral (Fig. 3-2). This could be ascribed to the increase in Chl *a* content per algal cell and the increase in algal density per unit surface area (Muller-Parker et al. 1994, Marubini and Davies 1996). Consequently, algal photosynthetic rate per surface area would have also increased under the nutrient enrichment (Hoegh-Guldberg and Smith 1989, Marubini and Davies 1996, Ferrier-Pagès et al. 2000a). Supposing that both of the algal density and photosynthetic rate increased in this study, the relatively constant release rates of DOM per surface area (Figs. 3-6a, b) mean that the contribution of each algal cell (or Chl *a*) to the whole DOM release decreased. Assuming that DOM release from the coral colony was only influenced by the algal photosynthetic rate, the decrease in DOM release per algal cell might be caused by increased nutrient availability: when inorganic nutrients limit the growth of zooxanthellae, the algae could not use the photosynthetic products efficiently and excess organic C would be released from the algae. On the other hand, when inorganic nutrients are sufficient, zooxanthellae could efficiently utilize the newly-synthesized organic C with acquired N and P, and DOM release per algal cell would decrease (Dubinsky and Jokiel 1994, Dubinsky and Berman-Frank 2001). These physiological changes are also known as one of the mechanisms of DOM release from phytoplankton (Carlson 2002). The changes in

the two phases, i.e., increase in the symbiotic algal density but decrease in DOM release per algal cell, might have resulted in apparently constant release rates of DOM per unit surface area, when affected by nutrient enrichment.

With inorganic nutrients, zooxanthellae probably synthesized organic matter of lower C:N ratios than that of nutrient-depleted condition, and a part of the organic matter might be released to the ambient seawater passing through the host coral tissue. However, C:N ratios of the DOM released from the coral colony were not significantly affected by the nutrient enrichment at least within the time scale of the present study (Fig. 3-6c). This suggested that the coral tissue had a pool of DOM to be released, and the pool was relatively large compared to the flux of organic matter from the symbiotic algae. It has been measured with ^{13}C tracer that DOM released from the colony *A. pulchra* and *Porites cylindrica* contained newly-synthesized DOM, which was photosynthetically produced by zooxanthellae within the last a few days, by only 7%–9% (Tanaka et al., in press). This implied that most DOM released from the coral colonies was not composed of new photosynthetic products but was derived from stored large DOM pool in the coral tissue. Thus, the chemical composition (C:N ratio) of the released DOM might have reflected the composition of not fresh photosynthetic products but previously synthesized DOM pool. The average C:N ratios of DOM in the present study were 7.5 ± 1.6 (mean ± SD, n = 10) in LL and LO during the period of 3–12 d (Fig. 3-6c). The values were slightly lower than those of simultaneously released POM (Fig. 3-4c), indicating that DOM contained nitrogenous compounds at higher proportions than POM. The obtained

C:N ratios of DOM and POM were within the range of previously reported values for coral-derived organic matter: 7.2 for mucus from *Fungia scutaria* (Krupp 1984), 6.9–14 for fluid mucus and 4.8–5.9 for mucus sheet from *Porites* spp. (Coffroth 1990), 2.5–13 for DOM from *Galaxea fascicularis* (Ferrier-Pagès et al. 1998). It might take some longer time that the C:N ratio of released DOM is affected by nutrient enrichment.

3-4-5. The ratio of organic matter release to the biomass production

POC and DOC release rates after 12 d of the nutrient enrichment were estimated to be 2100 and 510 nmol cm^{-2} d^{-1} (Tables 3-1 and 3-2). Similarly, PON and DON release rates were 250 and 89 nmol cm^{-2} d^{-1} (Tables 3-1 and 3-2). On the other hand, the growth rates of C and N biomass were estimated to be 2.7 and 0.67 μmol cm^{-2} d^{-1}, respectively (Fig. 3-3a). Supposing that the growth rates of the biomass were constant during the pre-incubation period, the release of total organic matter (POM + DOM) after the nutrient treatment was equivalent to 50% and 34% of the net organic production (biomass production + POM and DOM release) for C and N, respectively. The lower value of N than C suggests that the growth of coral and zooxanthellae was limited by N and thus conservative for acquired N (Tanaka et al. 2006a). The value for the release ratio of organic C to net C fixation can be compared to the previous studies: 40% as mucus (Crossland et al. 1980), 20% as

mucus and DOC-lipid (Crossland 1987), 14% as DOC (Ferrier-Pagès et al. 1998). The higher value in the present study might be partially ascribed to the inclusion of POM, but corresponded to the highest range of the previous reports. Nutrient enrichment might have increased the ratio of organic matter release to the net organic production.

3-5. Future problems and conclusions

In this study, coral observation under the nutrient enrichment was performed for 2 weeks, and Chl *a* density per unit surface area of the corals clearly increased during the period. However, the density of zooxanthellae must have an upper limit from the perspective of space limitation in the host tissue. Relatively constant range of the Chl *a* density during Day 10–14 might imply the upper limit (Fig. 3-2). This suggests that production rates of organic C by algal photosynthesis per surface area could also have an upper limit. Because NO_3^- must be first incorporated and synthesized into organic matter by zooxanthellae (Grover et al. 2003), NO_3^- uptake rates would also have an upper limit. It can be guessed that the release rate of POM from the coral colony do not continue to increase forever but have a maximum value. Longer-term observation will elucidate steady metabolic rates under a nutrient-enriched condition.

In summary, the present study has shown that nutrient enrichment enhanced

POM release rates at daytime from the coral colony, while the effect was not clear for DOM. Algal biomass, which was released from the host coral, explained only ca. 10% of the POM, suggesting that not only symbiotic algae but other POM such as coral mucus and tissue fragments were also simultaneously released as a result of the nutrient enrichment. The release rate of bulk POM increased relative to the growth rate of the whole biomass, suggesting that the organic matter excessively accumulating in the colony was released to the ambient seawater as POM. This study has suggested that the imbalanced growth between algal photosynthesis and coral calcification due to nutrient enrichment could induce POM release, at least within the time scale of the present study.

Table 3-1 Effects of the nutrient enrichment in the present study on the C and N biomass (nmol cm^{-2}) of the coral colonies and the release rates of POM (nmol cm^{-2} h^{-1}). The daily POM release rates (nmol cm^{-2} d^{-1}) were calculated on the supposition that the release rates in light (LL) and dark (DL) continued for 12 h each. Because the release rates in dark (DL) were not significantly changed in the present study, the estimation in the unaffected case, i.e., the same rate as the initial, was also shown in parentheses.

		Initial	After 12 d nutrient enrichment	Increase ratio
Biomass C		160×10^3	190×10^3	1.2
Biomass N		20×10^3	28×10^3	1.4
POC release	Light (LL)	53	140	2.6
	Dark (DL)	17	35 (17)	2.1 (1.0)
	Daily	840	2100 (1900)	2.5 (2.3)
PON release	Light (LL)	4.9	16	3.4
	Dark (DL)	3.7	5.0 (3.7)	1.4 (1.0)
	Daily	100	250 (240)	2.5 (2.4)

Table 3-2 Average release rates of DOM (nmol cm^{-2} h^{-1}) with SD of the incubations (n = 6 for LL and LO, n = 4 for DL). Significant differences between the light conditions are indicated with *** ($p < 0.001$), ** ($p < 0.01$), or * ($p < 0.05$).

	Light conditions	Release rates (SD)		
DOC	LL	36 (10)	*	***
	LO	22 (5)	**	
	DL	6.6 (5.1)		
DON	LL	5.3 (1.0)	***	***
	LO	2.2 (0.8)		
	DL	2.1 (0.7)		

Fig. 3-1 (a) The pre-incubation of corals in the outdoor aquarium. (b) The bottle experiment to measure organic matter release from corals.

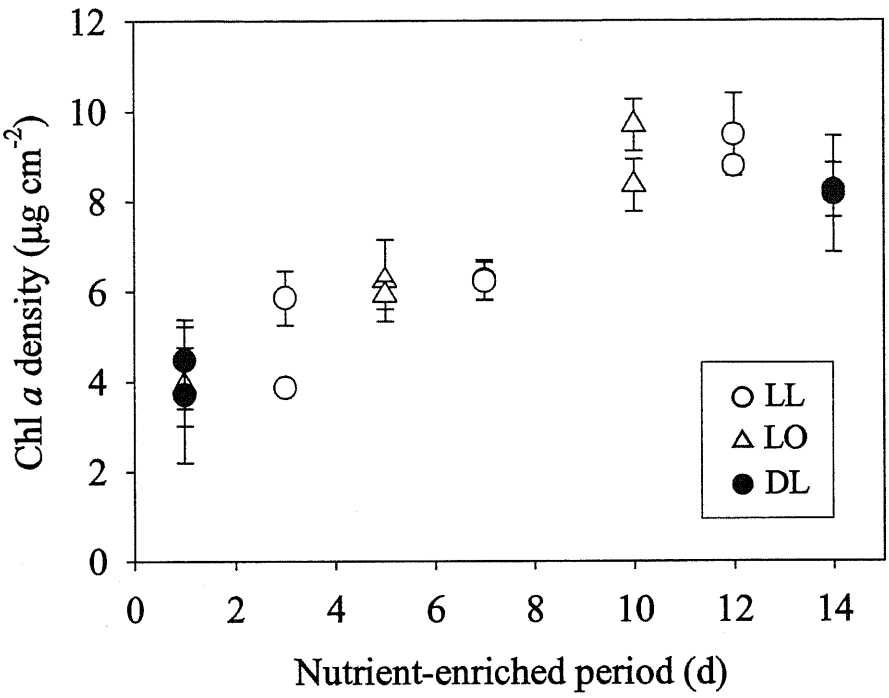

Fig. 3-2 Chl *a* density (μg cm^{-2}) per unit surface area of the corals incubated in the nutrient-enriched condition for different period of 1−14 d. Symbols explain which experiments of LL, LO, and DL each coral was used for. Error bars indicate standard deviation (SD) of triplicate subsamples collected from one coral branch.

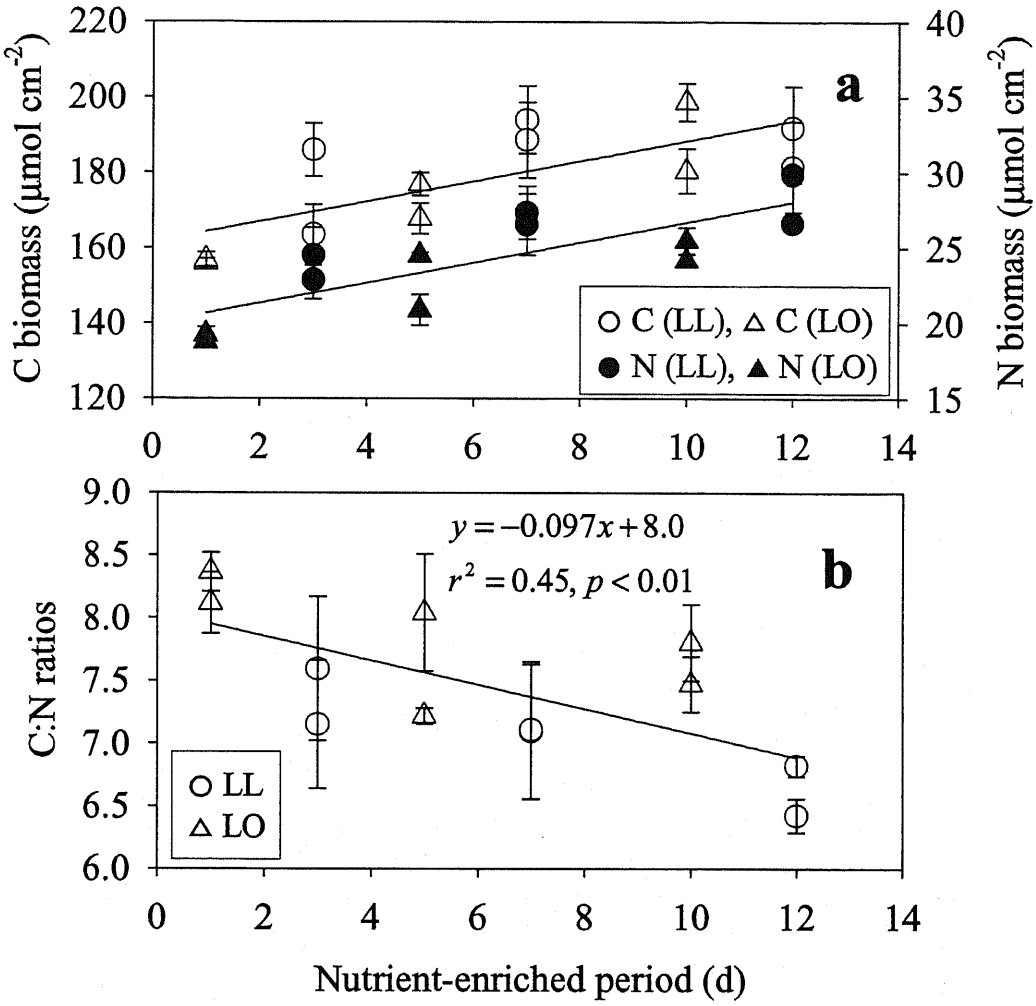

Fig. 3-3 C and N biomass (a) and its C:N ratio (b) of the corals incubated in the nutrient-enriched condition for different periods. The solid lines indicate significant change with the enriched period ($p < 0.01$). C biomass: y = 2.7x + 160, N biomass: y = 0.67x + 20.

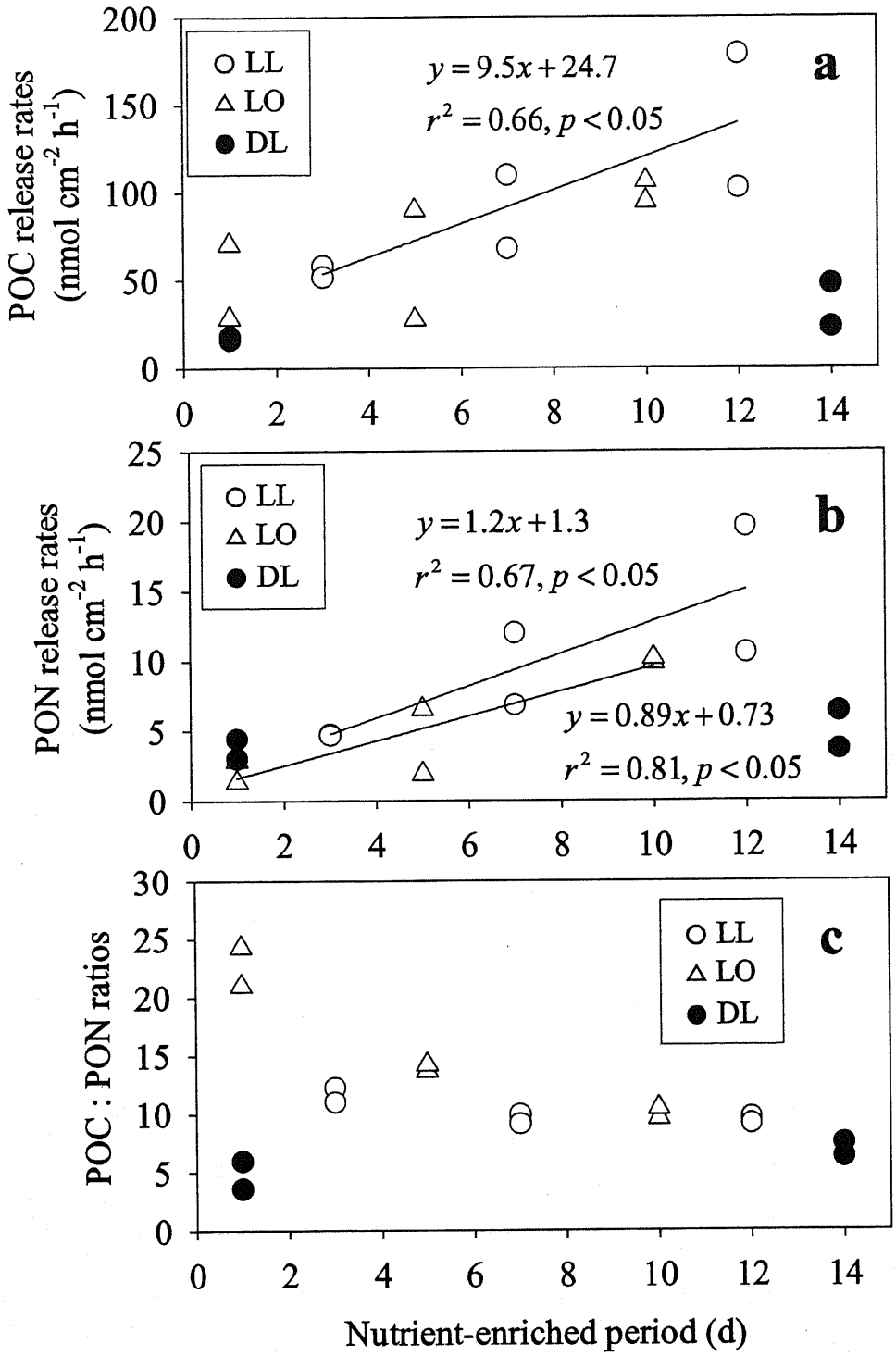

Fig. 3-4 The release rates of POC (a) and PON (b) per unit surface area of the corals, and the POC:PON ratios (c). The solid lines for LL in POC, and LL and LO in PON indicate significant increase in the release rates ($p < 0.05$).

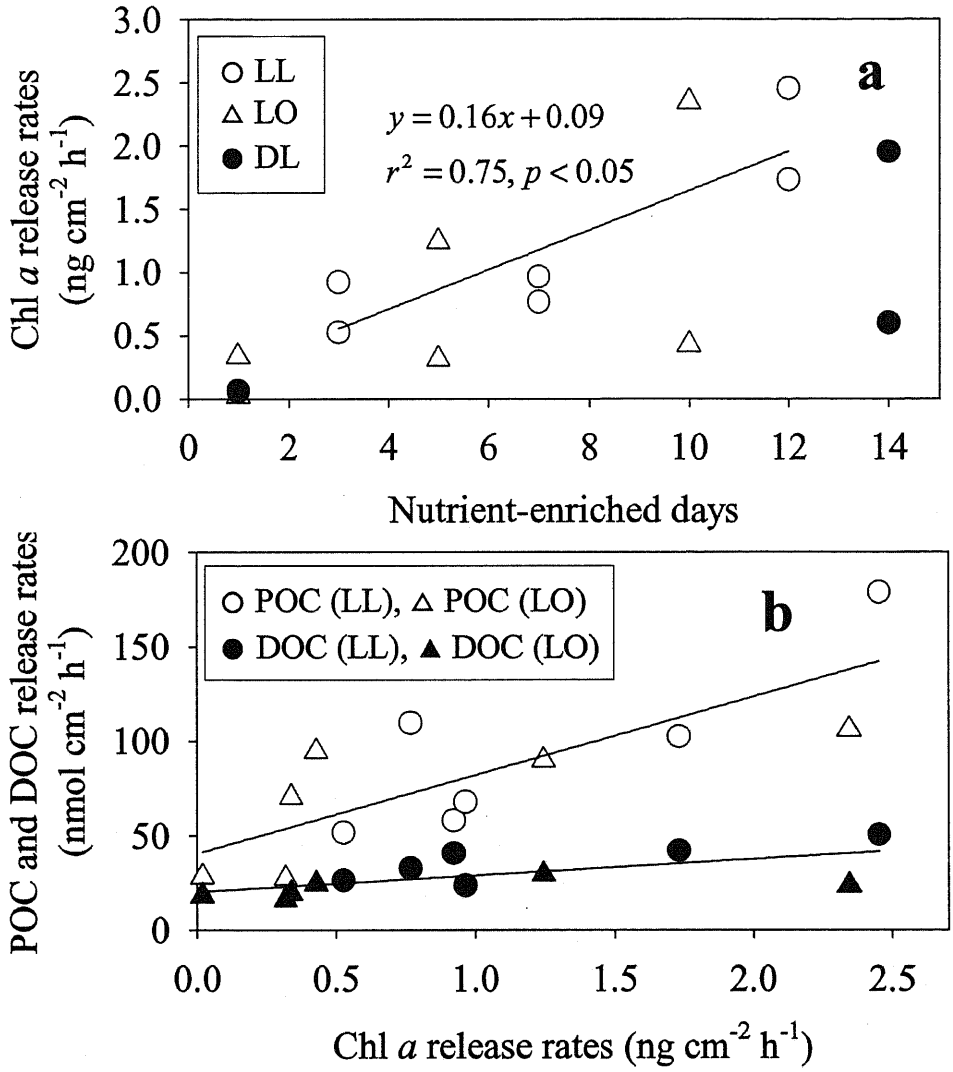

Fig. 3-5 (a) The relationship between Chl *a* release rates and nutrient-enriched periods in the light conditions of LL, LO, and DL. The solid line indicates significant increase in the release rate for LL ($p < 0.05$). (b) The relationship between the release rates of Chl *a* and organic C (POC and DOC). Both release rates of POC and DOC were significantly correlated with the Chl *a* release rates. POC: $y = 41x + 40$ ($p < 0.01$), DOC: $y = 8.7x + 20$ ($p < 0.05$).

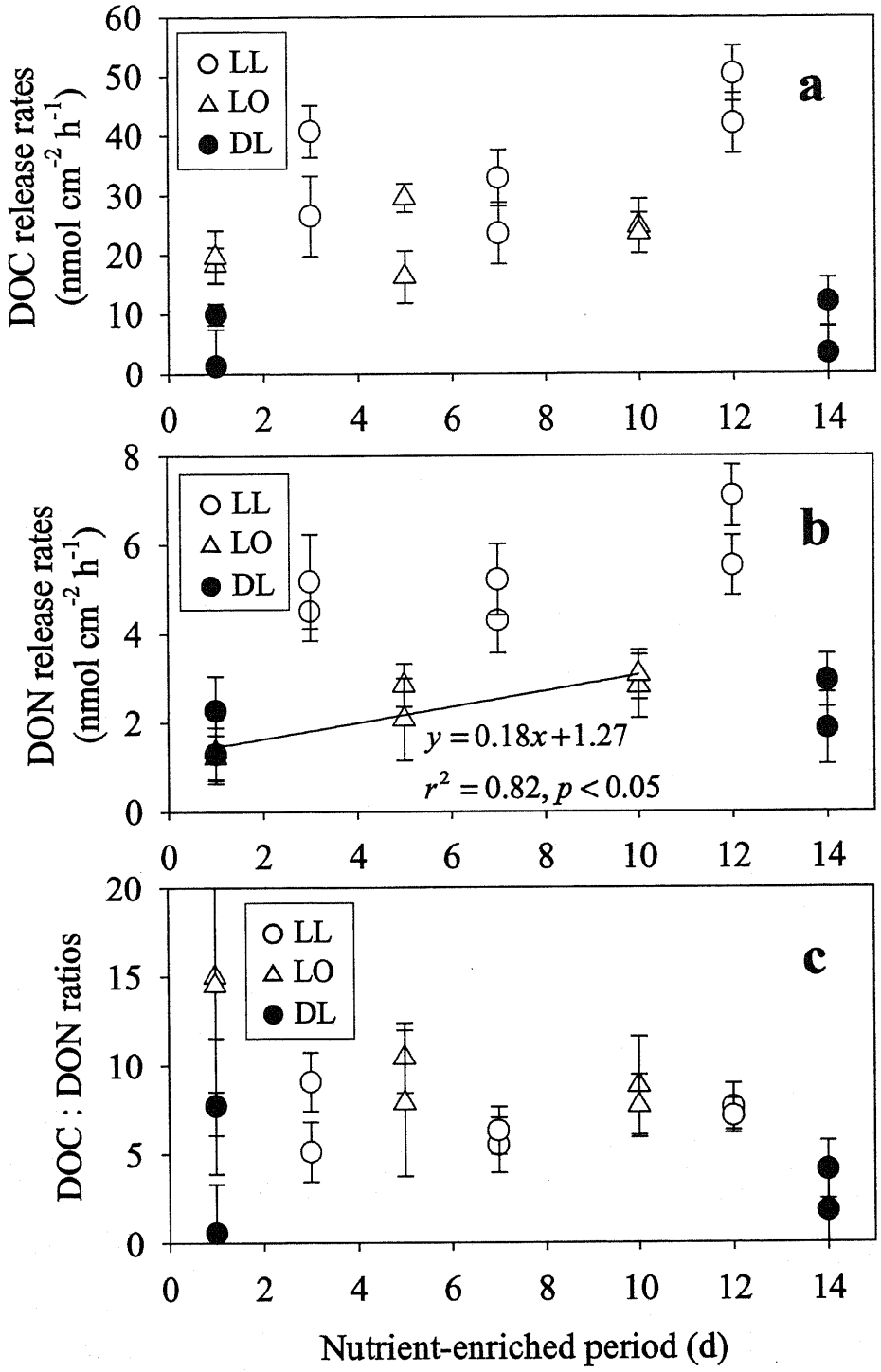

Fig. 3-6 The release rates of DOC (a) and DON (b) per unit surface area of the corals, and the DOC:DON ratios (c). The solid line for LO in DON indicate significant increase in the release rate ($p < 0.05$).

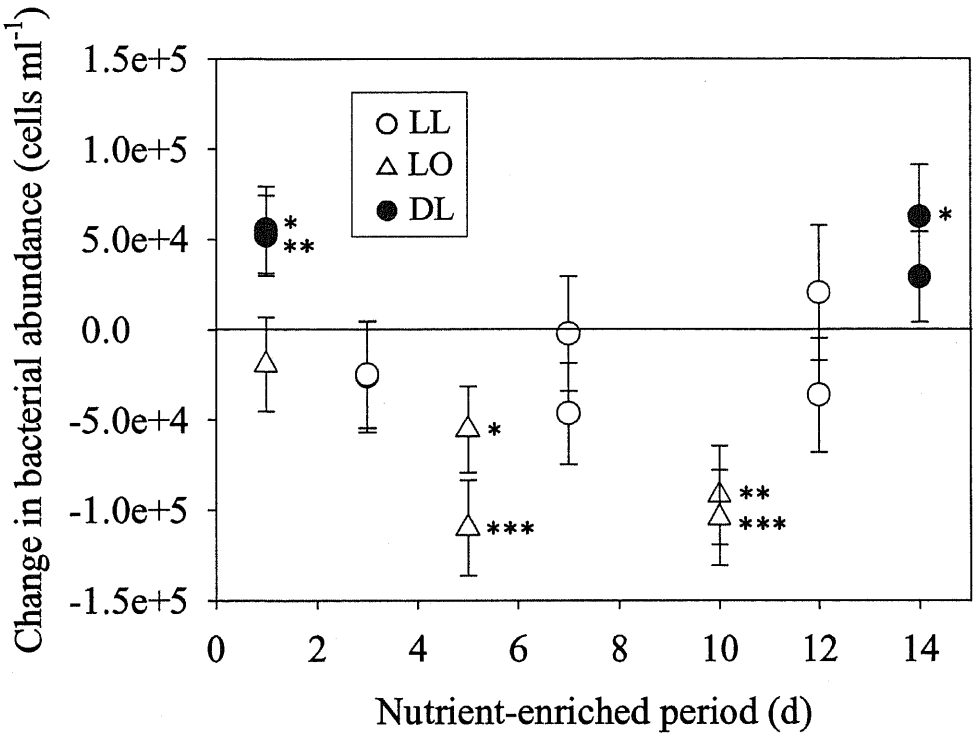

Fig. 3-7 The change in bacterial abundance in seawater after the 5-h bottle incubation, compared to the initial abundance. Significant changes are indicated with *** ($p < 0.001$), ** ($p < 0.01$), and * ($p < 0.05$).

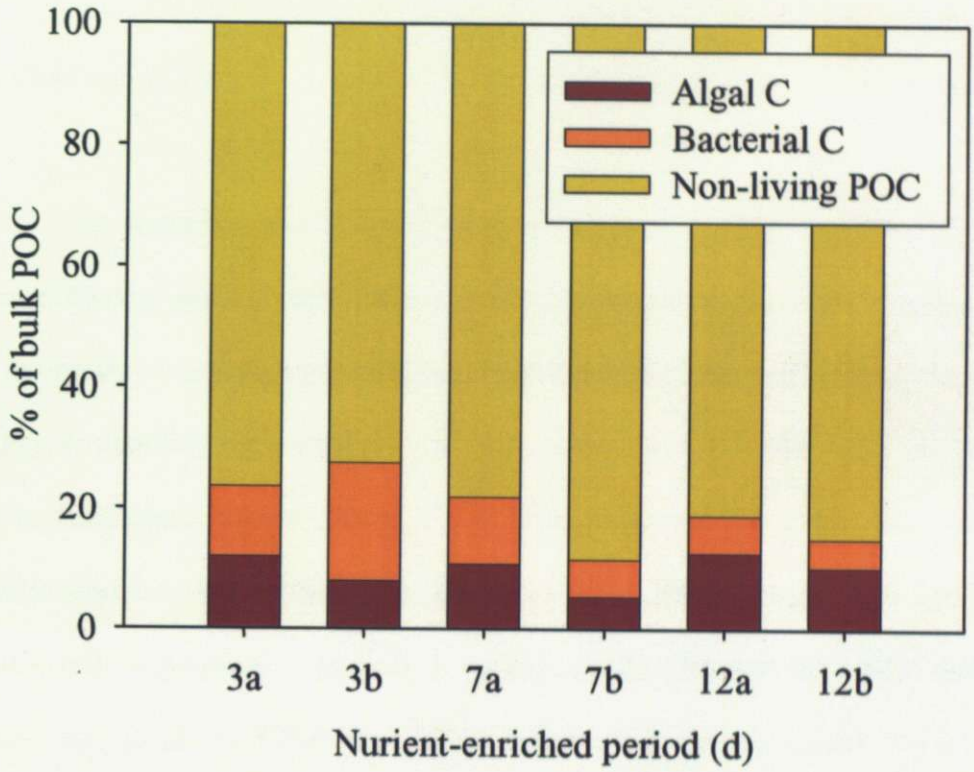

Fig. 3-8 The composition of POC released from the corals in light (LL). 'a' and 'b' mean duplicate bottle incubation for each nutrient-enriched period. Algal C and bacterial C were estimated from Chl *a* concentration and bacterial abundance in the incubated seawater, respectively. As the conversion factor from Chl *a* to algal C, 120 (g C (g Chl *a*) $^{-1}$) was used for the corals at Day 3, and 90 (g C (g Chl *a*) $^{-1}$) for Day 7 and 12 (Tanaka et al. 2007). The factor of 30 fg C cell^{-1} was used to calculate bacterial C (Fukuda et al. 1998). Non-living POC is the materials that algae and bacteria could not explain.

Chapter 4

Bacterial Degradability of the Released Organic Matter

4-1. Introduction

The organic matter released from reef-building corals has been well known as 'coral mucus,' and attracted the reef scientists for a long time. It has been reported that the mucus contains energy-rich lipid compounds (Benson and Muscatine 1974) and has a relatively high nitrogen (N) content (Coles and Strathmann 1973), while most reef detritus is N-poor (Hickel 1974). This suggested that coral mucus may be a valuable source of nutrients and energy for bacteria (Ferrier-Pagès et al. 2000b) and detritus and suspension feeders such as reef fishes (*Spratelloides delicatulus* and *Chromis* sp.; Johannes 1967). The mucus is famous as visible organic materials, but invisible dissolved fraction has also taken attention in more recent years (Crossland 1987, Ferrier-Pagès et al. 1998). Ferrier-Pagès et al. (2000b) showed that DOM (<14000–20000 Da) released from some hermatypic corals significantly enhanced pico- and nanoplankton growth. From these observations, organic matter released from corals has been supposed to be rapidly decomposed and mineralized into CO_2 in the reef water (Ferrier-Pagès et al. 1998, Wild et al. 2004a, b).

The rapid mineralization of the organic matter has also been suggested from another perspective: Gattuso et al. (1996) measured community metabolism of two reef flat at Moorea (French Polynesia) using a Lagrangian flow respirometry technique, and showed that the ratio of gross primary production (*P*) to respiration

(*R*), i.e., *P*:*R* ratio, was 1.1–1.2. Atkinson and Falter (2003) reviewed the data of metabolic rates of entire reef ecosystems, and the average *P*:*R* ratio was 1.05. These data suggest that photosynthetically produced organic matter in coral reefs is consumed for the respiration and thus net primary production could be near zero (Kinsey 1985).

However, contrarily, excess production of organic matter has been suggested in some previous studies. Organic carbon (C) fluxes were directly measured in the barrier reef of Palau (Hata et al. 1998) and in Shiraho coral reef, Japan (Hata et al. 2002), and it has been shown that a part of the organic matter produced in the reefs was exported to the offshore water. Miyajima et al. (2007) investigated the hydrodynamics of the Shiraho Reef and also showed that both organic C and N are exported from the lagoon to the outer ocean. As a different technique, Kayanne et al. (1995) measured the partial pressure of CO_2 and total alkalinity in reef waters at Shiraho Reef and also suggested net production organic matter. Kayanne et al. (2005) stated that the organic production was attributable to the coral community on the reef flat by comparing the metabolic rate between before and after bleaching event of corals. These observations suggest that a part of the organic matter released from corals and other benthic community is not rapidly utilized by bacteria but remained as organic compounds for a while.

The idea of excess production in coral communities could be supported by the suggestion that coral mucus is a material of low nutritional value for reef organisms. Krupp (1984) argued that though low C:N ratios of coral mucus were previously reported (e.g., 4.1–7.7; Coles and Strathman 1973), it could resulted from colonization by microorganisms and adsorption of organic sediments. Coffroth

(1990) concluded that aged mucus was not an important nutrient source compared to fresh liquid mucus because of the higher ash and lower organic contents of the aged mucus. Coffroth (1990) attributed low C:N ratios of the aged mucus to a loss of C rather than an increase in N. Moreover, Vacelet and Thomassin (1991) observed that neither bacteria nor eukaryotes completely degraded the coral mucus over 21 days. These observations propose that some of the organic matter released from corals are not good food source for bacteria, and thus, are not rapidly mineralized into CO_2 in the reef ecosystem.

Therefore, it is still not well understood at what % of coral mucus is actually utilized by reef organisms and contrarily what % is not mineralized and remained as organic forms. The purpose of this chapter is to quantitatively investigate bacterial degradability of the organic matter released from coral colonies. The organic matter easily mineralized within several days, though the period depends on the water residence time of the reef, could influence the organic to inorganic fluxes in the reef ecosystem. The remaining organic matter could be exported to the outer ocean and not affect the reef ecosystem directly. In the present study, the released organic matter was first fractionated into dissolved and particulate organic matter (DOM and POM, respectively), and the degradability of both size fractions was observed under dark during the first several days and subsequent longer periods.

During the decomposition experiment, inorganic nutrients of NO_3^- and PO_4^{3-} were added into some of the incubation bottles from the perspective of eutrophication of coral reefs as discussed in Chapter 2 and 3. If nutrient enrichment stimulated decomposition rates of the organic matter, the availability of inorganic nutrients by bacteria could influence organic to inorganic fluxes in the coral reef.

4-2. Material and methods

4-2-1. Experimental designs

A. Gently released DOM in submerged condition

In Aug 2006, the zooxanthellate coral branches of *Acropora pulchra* were collected on the reef flat of Shiraho Reef in Ishigaki Island. The size of the coral branches was ca. 6 cm, which were collected 500 m offshore from the coast. The collected corals were transferred to one of the two outdoor aquariums (inner volume: 90 L each) in Seikai National Fisheries Research Institute. Seawater was supplied into each aquarium with the rate of 3 L min^{-1}. The supplied seawater had nutrient concentrations of 1.8 ± 0.2 μmol L^{-1} NO_3^-, 0.03 ± 0.01 μmol L^{-1} NO_2^-, 0.04 ± 0.02 μmol L^{-1} NH_4^+, 0.08 ± 0.02 μmol L^{-1} PO_4^{3-} (means ± SD, n = 54). One of the aquariums was additionally supplied with KNO_3 and NaH_2PO_4 with a peristaltic pump, and consequently, nutrient concentrations in the aquarium were kept at 10.9 ± 0.4 μmol L^{-1} NO_3^-, 0.03 ± 0.01 μmol L^{-1} NO_2^-, 0.06 ± 0.05 μmol L^{-1} NH_4^+, 0.53 ± 0.02 μmol L^{-1} PO_4^{3-} (means ± SD, n = 44). The seawater temperature in the aquariums was controlled at 28.7 ± 0.7°C (mean ± SD), which was recorded every 10 min with a data logger (COMPACT-CT; Alec Electronics Co. Ltd.). Each coral branch was suspended in the aquariums using nylon line and acclimated to the aquarium conditions for 28 d (Table 4-1).

To obtain organic matter released from the corals, the acclimated coral branches were incubated for 5 h in glass bottles. Each bottle contained artificial seawater (Table 4-2) of 700 mL. Two coral branches from each aquarium, i.e., low and high nutrient conditions, were collected (total 4 corals) and each coral branch was suspended in the glass bottle (total 4 bottles) using nylon line. For the corals (DLN1, DLN2; see the footnotes in Table 4-1) acclimated in the low nutrient aquarium, the seawater in the bottles were nutrient-depleted (0.01 ± 0.00 μmol L^{-1} NO_3^-, 0.01 ± 0.00 μmol L^{-1} NO_2^-, 0.02 ± 0.00 μmol L^{-1} NH_4^+, 0.03 ± 0.00 μmol L^{-1} PO_4^{3-}; means ± range of two bottles). For the other two corals (DHN1, DHN2; see the footnotes in Table 4-1) acclimated in the high nutrient aquarium, the nutrients of KNO_3 and H_3PO_4 were artificially added into the glass bottles, and the final nutrient concentrations were 10.2 ± 0.0 μmol L^{-1} NO_3^-, 0.02 ± 0.01 μmol L^{-1} NO_2^-, 0.03 ± 0.00 μmol L^{-1} NH_4^+, 0.49 ± 0.01 μmol L^{-1} PO_4^{3-} (means ± range of two bottles). The bottle incubations were conducted under light (10:00–15:00 h) using halogen lamps in the laboratory. The average irradiance during the 5 h was 400 μmol photons m^{-2} s^{-1}. Seawater in the bottles was gently stirred with a magnetic stirrer, and the temperature was controlled at 28.8 ± 0.0°C (mean ± SD) by flowing seawater outside the bottles.

After the bottle incubations for 5 h, the coral branches were taken out of the bottles and stored at -20 °C to determine the coral surface area by aluminum foil method (Marsh 1970). The incubation seawater was well agitated and then filtered to remove POM with pre-combusted Whatman GF/F filters (pore size 0.7 μm, filter diameter 47 mm). From each filtrate, subsamples for the bacterial abundance were collected in 10-mL acrylic tubes and fixed with pre-filtered formalin (final concentration 2%, v/v). The remaining filtrate containing DOM was divided into

pre-combusted glass ampoules of 20 mL. The filtrate of 15 mL was put in each glass ampoule (total 18 ampoules), and extra KNO_3 and H_3PO_4 were added into the ampoules for DHN1 and DHN2 by 5 and 0.25 μmol L^{-1}, respectively, to make the difference in nutrient conditions during the decomposition process. Headspace of the glass ampoule contained natural air of ca. 10 mL, which was enough for aerobic bacterial decomposition of organic matter by 90 μmol C, assuming that one mole of oxygen was consumed when one mole of organic C was mineralized. The glass ampoules were immediately sealed and then stored at 20°C under dark to follow the bacterial decomposition of DOM. Subsamples of the incubation seawater were taken at 0, 2, 7, 30, 120 and 365 d of the decomposition experiments. To stop bacterial decomposition, three glass ampoules were frozen at -20°C every sampling time.

B. Gently released POM in submerged condition

In Aug 2006, the coral branches of *Acropora pulchra* were collected as described in the above section "A. Gently released DOM", and transferred to the low nutrient outdoor aquarium (1.8 ± 0.2 μmol L^{-1} NO_3^-, 0.03 ± 0.01 μmol L^{-1} NO_2^-, 0.04 ± 0.02 μmol L^{-1} NH_4^+, 0.08 ± 0.02 μmol L^{-1} PO_4^{3-}; means ± SD, n = 54). The other conditions (light, temperature, water residence time) of the aquarium were same as explained in the above section. The size of the coral branches was ca. 4 cm in diameter. Six coral branches were acclimated to the aquarium for 19 d, and the other six corals were collected one day before the following experiment on organic matter release (Table 4-1).

To obtain POM released from the corals, four polycarbonate bottles (inner volume 8 L each) were prepared for the coral incubations. Two of the bottles (PHN1, PHN2; see the footnotes in Table 4-1) were used for the six corals acclimated to the aquarium for 19 d (three corals per bottle), and the other two bottles (PLN1, PLN2; see the footnotes in Table 4-1) were used for the corals collected one day before (three corals per bottle). Incubation seawater was collected out of the reef, and filtered to remove POM with pre-combusted Whatman GF/F filters before the coral incubations. The seawater had the nutrient concentration of 0.04 ± 0.02 μmol L^{-1} NO_3^-, 0.01 ± 0.00 μmol L^{-1} NO_2^-, 0.02 ± 0.01 μmol L^{-1} NH_4^+, 0.01 ± 0.00 μmol L^{-1} PO_4^{3-} (means ± range of the two bottles). KNO_3 and H_3PO_4 were added into PHN1 and PHN2 to get a similar nutrient condition to the pre-incubation, and the final nutrient concentrations were 2.0 ± 0.0 μmol L^{-1} NO_3^-, 0.00 ± 0.00 μmol L^{-1} NO_2^-, 0.05 ± 0.03 μmol L^{-1} NH_4^+, 0.11 ± 0.00 μmol L^{-1} PO_4^{3-} (means ± range of the two bottles). The coral incubations were conducted outdoor at daytime (10:00–15:00). The average irradiance measured with a logger-type iluminometer (MDS-MKV/L; Alec Electronics Co. Ltd.) was 860 μmol photons m^{-2} s^{-1}. The average temperature was 30°C during the 5 h incubation.

After the bottle incubations for 5 h, the coral branches were taken out of the bottles and stored at -20°C to determine the coral surface area by the aluminum foil method (Marsh 1970). The incubation seawater was well agitated and subsamples for the bacterial abundance were collected in 10-mL acrylic tubes, which were fixed with pre-filtered formalin (final concentration 2%, v/v). To measure POM concentrations in the incubation seawater, a part of the seawater (700–1000 mL) was collected and filtered with pre-combusted Whatman GF/F filters. The filters were

immediately stored at -20°C until analysis. Subsamples for bacterial abundance were also taken from the filtrates to estimate bacterial contribution to the bulk POM by subtraction. The remaining incubation seawater was stored at 20°C under dark to observe the bacterial decomposition of POM. A portion of the incubation seawater was collected at 0, 2, 7, 30, and 120 d of the decomposition processes, and subsamples for POM, bacteria and inorganic nutrients were taken in the same way as described above.

C. Massively released DOM by air exposure

In Jun 2007, coral branches of *A. pulchra* and *Porites cylindrica* were collected as described in the above section "A. Gently released DOM." After the collection, the corals were immediately put in two glass bottles per species (abbreviated as DAC1 and DAC2 for *A. pulchra*, DPO1 and DPO2 for *P. cylindrica*; see the footnotes in Table 4-3) containing artificial seawater of 300 mL each (Table 4-2). Coral surface area was 110–150 cm^{-2} per bottle. The nutrient concentrations in the incubation seawater were 0.06 μmol L^{-1} NO_3^-, 0.05 μmol L^{-1} NO_2^-, 1.1 μmol L^{-1} NH_4^+, 0.16 μmol L^{-1} PO_4^{3-}. Within 1 h after the collection, the glass bottles containing the corals were carried to the laboratory with gently being shaken at the rate of one swing per second (amplitude 10 cm). With this procedure, the corals were repeatedly exposed to the air and consequently released visible mucus into the ambient incubation seawater.

In the laboratory, the corals were taken out of the bottle and stored at -20 °C,

and after a while the coral surface area was determined by the aluminum-foil method (Marsh 1970). Additional artificial seawater of 400 mL was pored into the incubation seawater, and 5 mL of in situ seawater, which was collected the day before, was also added into the each bottle. The in situ seawater was added to inoculate free-living bacteria in the natural reef water. The incubation seawater (total ca. 700 mL) was filtered with pre-combusted Whatman GF/F filters to remove POM, using a hand-operated vacuum pump. The filtrate was stored at 20°C under dark to follow bacterial decomposition of DOM in the seawater. Subsamples for total organic matter, inorganic nutrients, and bacterial abundance were taken at 0, 2, 7, 30, 90 d of the decomposition processes. For the total organic matter and nutrients, a part of the incubation seawater was sealed in 20-mL glass ampoules and stored at -20°C until analysis. For the bacterial abundance, the incubation seawater was collected in 10-mL acrylic tubes and fixed with pre-filtered formalin (final concentration 2%, v/v).

4-2-2. Chemical analysis and calculations

The concentrations of dissolved inorganic nitrogen (DIN: NO_3^-, NO_2^-, NH_4^+) and phosphorus (DIP: PO_4^{3-}), DOC and DON were quantified using the same instruments as Chapter 3. The abundance of bacteria in the incubation seawater was also counted in the same ways as Chapter 3.

Because amino acids are one of the major organic matter released from corals (Ducklow and Mitchell 1979b, Meikle et al. 1988), the concentrations of

hydrolysable amino acids were measured in this Chapter. The concentrations were quantified by HPLC (high-performance liquid chromatography) with fluorometric detection, after derivatization of primary amines with 2-mercaptoethanol and *o*-phthaldialdehyde (Mopper and Dawson 1986). Total hydrolysable amino acids (THAA) were measured after vapor-phase acid hydrolysis (Tsugita et al. 1987). In the beginning of the decomposition experiment of DLN, DHN, DAC, and DPO, THAA was all composed of dissolved hydrolysable amino acids (DHAA) because POM was removed in advance. Amino acids were abbreviated as follows: asparagine + aspartate (Asx), glutamine + glutamate (Glx), arginine (Arg), histidine (His), lysine (Lys), serine (Ser), glycine (Gly), threonine (Thr), tyrosine (Tyr), alanine (Ala), methionine (Met), phenylalanine (Phe), valine (Val), isoleucine (Ile), leucine (Leu). These amino acids were categorized in four families: acidic (Asx, Glx), basic (Arg, His, Lys), neutral (Ser, Gly, Thr, Tyr), and hydrophobic (Ala, Met, Phe, Val, Ile, Leu). Because the acid hydrolysis procedure alters asparagine and glutamine to aspartate and glutamate, respectively, the amount of N contained in THAA was calculated in the both cases that Asx and Glx are all derived from aspartate and glutamate or asparagine and glutamine.

The GF/F filter containing POM was dried in an oven at 50°C for 3 hours, and then treated with a vapor of 12 N HCl for 12 h to remove inorganic C. After evaporating extra HCl on a hot plate (80°C) under vacuum, the filters were dried again for 2 h in the oven at 50°C. Quantity of POM on the filter was determined by CHN analyzer (Fisons; NA-1500).

The release rate of DOM and POM from the corals (R: μmol cm^{-2} h^{-1}) was calculated from the organic matter (C: μmol L^{-1}) newly accumulated in the seawater

during the 5-h bottle incubation.

$$R = C\,V\,S^{-1}\,t^{-1} \qquad (4\text{-}1)$$

where V, S and t are seawater volume, coral surface area and the incubation period of 5 h, respectively. Because artificial seawater used for the experiments contained some DOC (4.8–7.3 μmol L^{-1}) in the beginning of the incubation, the initial DOC concentration was subtracted from all the observed concentrations of DOC at each sampling time, supposing that the initial DOC concentration did not change during the whole experimental period. The concentration of initial DON was ignored because the value was 0.0–0.1 μmol L^{-1}, which was within the range of analytical error of DON. The remaining percentage (r: %) of the organic matter during the decomposition experiment was calculated as follows:

$$r = C_s\,C^{-1} \times 100 \qquad (4\text{-}2)$$

C_s (μmol L^{-1}) is the concentration of organic matter at a sampling time.

4-3. Results

4-3-1. Release rates of organic matter

In the incubations of DLN and DHN, DOM accumulated in the seawater at

the range of 9.3–16 μmol C L^{-1} and 1.0–1.8 μmol N L^{-1}. The release rates per unit surface area of the corals were calculated to be 25–48 nmol C cm^{-2} h^{-1} and 2.8–5.4 nmol N cm^{-2} h^{-1} (Table 4-3). These release rates were not affected by the nutrient conditions during the pre-incubation, which was same as the results in Chapter 3. The C:N ratio of the released DOM in DLN and DHN was 9.5 ± 0.7 (mean ± SD). DHAA accumulated in the seawater at the range of 320–660 nmol L^{-1}. Supposing that all of the detected Asx and Glx were derived from aspartate and glutamate, N contained in the DHAA (DHAA-N) accounted for 32–41% (average 36%) of the bulk DON (Table 4-4). Conversely, if the detected Asx and Glx were all derived from asparagine and glutamine, DHAA-N would account for 53%–64% (average 59%) of the bulk DON (Table 4-4). The contribution of DFAA to DHAA was 5.8%–10% (mole/mole).

In the incubations of PLN and PHN, POM accumulated at the range of 4.6–11 μmol C L^{-1} and 0.4–1.2 μmol N L^{-1}. The release rates per unit surface area of the corals were influenced by the pre-incubation period: 116–121 nmol C cm^{-2} h^{-1} and 11 nmol N cm^{-2} h^{-1} for PLN, and 180–186 nmol C cm^{-2} h^{-1} and 20–21 nmol N cm^{-2} h^{-1} for PHN (Table 4-3).

Under the stressful incubations of DAC and DPO, the corals released about 10 times more DOM than those in DLN and DHN (Table 4-3). DOM accumulated in the seawater at the range of 80–97 μmol C L^{-1} and 5.7–13 μmol N L^{-1}. The release rates per unit coral surface area were 400–440 nmol C cm^{-2} h^{-1} and 32–35 nmol N cm^{-2} h^{-1} for *A. pulchra*, and 510–650 nmol C cm^{-2} h^{-1} and 66–89 nmol N cm^{-2} h^{-1} for *P. cylindrica*. DHAA accumulated in the seawater at the range of 1.8-3.1 μmol L^{-1}. DHAA-N accounted for 40%–86% and 17%–28% of the bulk DON for *A. pulchra*

and *P. cylindrica*, respectively (Table 4-5), considering both cases that Asx and Glx were all derived from aspartate and glutamate or asparagine and glutamine. The contribution of DFAA to DHAA was 9.7%–17%.

4-3-2. Mineralization of DOM in the incubations of DLN and DHN

The concentrations of NO_3^- and PO_4^{3-} in DLN were <0.05 μmol L^{-1} at the beginning of the decomposition experiment, and almost constant during the whole experimental period (Figs. 4-1a & 4-1d). In DHN, the concentrations of NO_3^- and PO_4^{3-} were 8–9 μmol L^{-1} and 0.3–0.9 μmol L^{-1} at the beginning of the experiment, respectively, and also constant during the experiment (Figs. 4-1a & 4-1d). Thus, the nutrient conditions were clearly different between DLN and DHN. NH_4^+ concentrations were initially 0.1–0.9 μmol L^{-1} and increased by 0.5–1.1 μmol L^{-1} during the decomposition period (Fig. 4-1c). NO_2^- concentrations were almost same as the initial level in three of the four incubations (Fig. 4-1b).

The concentration of organic matter in DLN and DHN drastically decreased for both C and N during the first 1 week of the decomposition experiment (Figs. 4-2a & 4-3a). The decrease rates were not affected by the nutrient conditions of DLN and DHN (Table 4-6). The average remaining % after 1 week was 67% and 76% for C and N, respectively (Table 4-6). After 1 week, the decrease rates became far slower and the concentration remained almost constant for the next 1 year (Figs. 4-2a & 4-3a). The average remaining % after 1 year was 63% and 70% for C and N, respectively (Table 4-6), irrespective of the nutrient conditions.

During the decomposition, the contribution of THAA-N to the bulk TON decreased to 21%–50% after 7 d and to 14%–26% after 120 d (Table 4-4). Within the first 7 d, Glx % drastically decreased, and conversely, some other amino acids significantly increased (Fig. 4-4). The *p*-values indicating the significant changes were <0.001 for Asx, Glx, Gly, Ala, and Leu, <0.01 for Arg, Val, Phe, and Orn, <0.05 for Lys and Ile. After 7 d, the compositional changes were not found for all the amino acids.

4-3-3. Degradation of POM in the incubations of PLN and PHN

The NO_3^- concentrations in PLN were <0.1 μmol L^{-1} throughout the experiment, and the concentrations in PHN were 1.1–1.4 μmol L^{-1} (Fig. 4-5a). The PO_4^{3-} concentrations in PLN were <0.05 μmol L^{-1}, and the concentrations in PHN were >0.5 μmol L^{-1} (Fig. 4-5d). Thus, the nutrient conditions were clearly different between PLN and PHN. NH_4^+ concentrations were initially <0.1 μmol L^{-1} and increased for 3 months by 1.3–1.8 μmol L^{-1} for three of the four incubations (Fig. 4-5c). NO_2^- concentrations tended to increase after NH_4^+ concentrations decreased (Fig. 4-5b).

The POM concentration in PLN and PHN considerably decreased for both C and N during the first 1 week of the decomposition experiment (Figs. 4-6a & 4-7a). The concentrations did not drastically change after 1 week, and a constant amount of POM remained in the seawater after 3 months, irrespective of the nutrient conditions. Because produced amount of POM was different between PLN and PHN, the

remaining % of POM also differed: 64% of POC and 38% of PON still remained after 3 months for the PLN incubations, and 24% of POC and 16% of PON remained for PHN. (Table 4-7).

4-3-4. Mineralization of DOM in the incubations of DAC and DPO

NH_4^+ concentrations were 0.8–0.9 μmol L^{-1} at the beginning of the decomposition experiment, and increased by 4.2–5.3 μmol L^{-1} for the first 1 week (Fig. 4-8c). The NH_4^+ concentrations reached at maximum after 30 d of the decomposition processes, and then decreased <0.1 μmol L^{-1} at 90 d. Alternatively, NO_2^- increased by 5.6–7.8 μmol L^{-1} from Day 30 to Day 90 (Fig. 4-8b), and NO_3^- also increased by 0.3–0.5 μmol L^{-1} (Fig. 4-8a). Total DIN and PO_4^{3-} gradually increased by 5.2–7.4 μmol L^{-1} and 0.08–0.17 μmol L^{-1}, respectively, during the whole decomposition period (Figs. 4-8d & 4-8e).

The concentration of organic matter in DAC and DPO drastically decreased for both C and N during the first 1 week of the decomposition experiment (Figs. 4-9a & 4-10a). The average remaining % of C and N after 1 week was 56% and 59% for *A. pulchra*, and 63% and 58% for *P. cylindrica*, respectively (Table 4-8). C:N ratios of the DOM mineralized during the first 1 week were 13 and 6.6 on average for *A. pulchra* and *P. cylindrica*, respectively (Table 4-8).

After 1 week, the decrease rates of organic matter became slower and the concentration gradually decreased until Day 90 (Figs. 4-9a & 4-10a). The average remaining % after 90 d was 33% and 27% for C and N, respectively, for *A. pulchra*,

and 40% and 26% for *P. cylindrica* (Table 4-8). C:N ratios of the organic matter decomposed during the period were 8.6 and 5.2 on average for *A. pulchra* and *P. cylindrica*, respectively (Table 4-8).

During the decomposition, the compositional change of THAA was found for some amino acids as in the case of DLN and DHN incubations: Glx % drastically decreased within the first 7 d, and conversely, the contribution of neutral and hydrophobic amino acids increased (Figs. 4-11a & 4-11b). The ratios of THAA-N to the bulk TON were not considerably changed during the decomposition period (Table 4-5).

4-4. Discussion

4-4-1. Comparison in bacterial degradability of the released organic matter between labile and refractory fractions

Decomposition of the organic matter released from corals seemed to proceed in two steps: a rapid decrease during the first several days, and following slow decrease after that time. This indicates that the organic matter released from the corals was composed of two different fractions in bacterial degradability. In this study, the organic matter easily decomposed within 1 week was defined as labile organic matter (L-OM), and the organic matter slowly decomposed after 1 week was defined as refractory organic matter (R-OM).

Assuming that each fraction of the organic matter was decomposed as a

first-order reaction, the concentration of total organic matter (C) after a decomposition period t (day) would be represented as the following equation.

$$C = A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t) \qquad (4\text{-}3)$$

where k_1 and k_2 ($k_1 > k_2$) are first order decay constants of L-OM and R-OM, respectively, and A_1 and A_2 are initial concentrations of the each fraction. During the initial stage of the decomposition (<1 week), the concentration of R-OM could not be changed considerably, thus, the second part of the equation ($A_2 \exp(-k_2 t)$) can be regarded as constant during the period. Supposing that the constant value was the lowest concentration during the decomposition period (mostly, at the last sampling time), the equation can be altered to,

$$C - C_c = A_1 \exp(-k_1 t) \qquad (4\text{-}4)$$

where C_c is the lowest concentration. Using the concentrations of organic matter at 0 d and 7 d (C_0 and C_7, respectively), the decay constant k_1 is calculated as,

$$-k_1 (7\text{ d} - 0\text{ d}) = \exp(C_7 - C_c) - \exp(C_0 - C_c) \qquad (4\text{-}5).$$

Considering that most of the L-OM was decomposed within 1 week, the first part of Eq. 4-3, i.e. $A_1 \exp(-k_1 t)$, can be neglected after 1 week, and the decay constant k_2 was calculated using the concentration of organic matter at 7 d and the last sampling time as

$$-k_2 (7\ \mathrm{d} - t_f) = \exp (C_f - C_c) - \exp (C_7 - C_c) \qquad (4\text{-}6)$$

where t_f and C_f is the sampling time (day) and the concentration of organic matter at the last time.

The calculated decay constants for L-OM and R-OM showed that the degradability were completely different between L-OM and R-OM by more than one order of magnitude (Table 4-9). L-OM could have the turnover time of 3.2–9.1 d (average 6.2 d) for the mineralization of DOC and DON, and 5.6–9.1 d (average 7.4 d) for the decomposition of PON. Contrarily, R-OM had the turnover time of >100 d in the most incubations.

A similar trend of the decomposition processes in two steps, i.e., a rapid decomposition during the first a few days and following slow decrease after that time, has also been observed for DOC sampled at natural marine ecosystems: Sagami Bay and Tokyo Bay, Japan (Ogura 1975), and the North Atlantic Ocean during a phytoplankton bloom (Kirchman et al. 1991). The origin of the rapidly decomposed DOM in the two previous studies was both attributed to phytoplankton, which is generally considered to be one of the major sources of newly-produced marine DOM (Nagata 2000). It has not been resolved whether DOM released from coral colonies are directly derived from the symbiotic algae or controlled by the host corals (Crossland 1987, Ferrier-Pagès et al. 1998). However, light enhancement of DOC release observed in Chapter 3 and by Crossland (1987) implied that the algal photosynthetic activity strongly influences DOM release from the colony. Assuming that a part of the photosynthetic products is released from the algae as discussed in

Chapter 3, chemical characteristics of the organic matter released from the coral colony might be very similar to those released from typical phytoplankton. However, Tanaka et al. (in press) has observed that newly-synthesized organic matter by zooxanthellae accounted for 7%–9% of the bulk released DOC, suggesting that >90% of the organic matter released from corals could be derived from a stored DOC pool (see Chapter 3). It remains to be resolved exactly what chemical components the released DOM has.

POM released from corals could be composed of mainly non-living organic materials such as coral mucus and host tissue fragments, rather than living organisms such as zooxanthellae and bacteria (Fig. 3-8). The decay constants of the released PON were similar to the values for DOM (Table 4-9), suggesting that non-living organic components in the POM had similar bacterial degradability to the released DOM, although transfer of POM to DOM fraction by fragmentation was not considered here. Considering the observation that coral mucus of molecular weight larger than 6000–8000 was not completely degraded by bacteria and eukaryotes even after 21 d (Vacelet and Thomassin 1991, see below), easily decomposed POM might be not mucus but a part of the host tissue, which was simultaneously released with zooxanthellae as discussed in Chapter 3. Under light condition, the released algae would more or less grow with nutrient incorporation, thus, the bulk POM concentration might not decrease as observed in the present study.

4-4-2. Effects of nutrient enrichment on the organic matter decomposition

POM production was stimulated by the pre-treatment in the nutrient-enriched condition (Table 4-3), as shown in Chapter 3. The increasing POM by nutrient enrichment could be composed of zooxanthellae released from the host and other non-living organic materials such as coral mucus and host tissue fragments. It was also shown in Chapter 3 that the release of zooxanthellae and bulk POM was significantly correlated with each other (Fig. 3-5b), suggesting that release of zooxanthellae simultaneously involved the other POM release. The present decomposition experiment on POM has shown that inorganic nutrient enrichment stimulates POM production from the corals, but the increased fraction might be susceptible to bacterial decomposition. It has been reported that some parts of coral-derived organic matter enhance bacterial growth (Ferrier-Pagès et al. 2000b, Wild et al. 2004a, b). Therefore, inorganic nutrients might stimulate the fluxes of organic matter from coral colonies to bacteria and other heterotrophic communities in coral reefs.

The residual concentrations of POM were not different after 120 d among the incubations of PLN and PHN (Figs. 4-6a & 4-7a), implying that the corals released undegradable POM at a constant rate, which was not dependent on the pre-incubation under the nutrient enrichment. The POM fraction included bacterial cells, but the abundance after 120 d was 1.3×10^5 cells ml^{-1} at most. Using the conversion factor of 15 fg C per bacterial cell (Fukuda et al. 1998), the bacterial biomass was calculated to be 0.16 μmol C L^{-1} at most, which was <10% of the remaining POC at Day 120 (Fig. 4-6a). This estimation confirmed that the remaining POM was not bacteria but non-living organic compounds such as coral mucus (Vacelet and Thomassin 1991). Coffroth (1990) indicated that mucus sheet (aged

mucus) released from *Porites* spp. had higher ash and lower organic contents than mucus fluid (fresh mucus) and therefore its nutritional value could be low for bacteria. Krupp (1984) also argued that because pure mucus might provide corals with some protection against desiccation, it was not a particularly rich food source for reef heterotrophs. Corals might release such nutritionally low POM to the ambient seawater at a constant rate.

While NO_3^- and PO_4^{3-} concentrations were rich in DHN incubations, both concentrations were depleted in DLN incubations (Figs. 4-1a & 4-1d). The difference in the nutrient conditions, however, did not affect DOM mineralization. NH_4^+ concentrations were 0.5–1.5 μmol L^{-1} (Fig. 4-1c), but not consumed as well. These results indicate that bacterial utilization of the organic matter did not require inorganic nutrients. Bacteria need N and P as well as C to synthesize new cells, where not only organic N and P but inorganic nutrients such as NH_4^+, NO_3^- and PO_4^{3-} are also utilized (Kirchman 2000). The corals in this study released organic matter with the C:N ratios of 7.3–14 (Table 4-3), suggesting that it contained organic compounds of low C:N ratios such as proteins and nucleic acids. Organic N and P released from the corals could have proliferated the bacterial growth, rather than inorganic nutrients. In other words, the depletion of inorganic nutrients did not limit the bacterial further degradation of organic matter, but the organic matter itself was recalcitrant to bacterial decomposition. The levels of inorganic nutrients would not determine bacterial degradability of DOM released from the corals, at least in normal submerged condition.

4-4-3. Comparison of the released DOM between normal and stressful conditions

The organic matter released from corals under stressful conditions such as air exposure and water jet has been obtained in many previous studies (reviewed by Brown and Bythell 2005). Under the stressful condition in the present study, the coral colonies produced about 10 times more DOM than those of the normal incubations of DLN and DHN (Table 4-3). Similar enhancement was reported by Wild et al. (2005a), who observed that *Acropora millepora* exposed to air increased the POM release rate 12-fold, compared to the submerged condition. The enhanced release rates can be considered as a potential of the coral to release organic matter to the ambient seawater.

Remaining % of the released organic matter was lower in DAC and DPO than that released in the incubations of DLN and DHN (Tables 4-6 & 4-8), suggesting that the corals released easily-decomposed DOM at higher % under the stressful condition (DAC and DPO). It has been reported that many kinds of organic matter such as carbohydrates, proteins, and lipids (Meikle et al. 1988, Coffroth 1990, Wild et al. 2005a) are contained in coral-derived organic matter, which could be gradually utilized by bacteria. The enhancement of organic matter release due to stressful environment would result in an increase in the easily-decomposed organic matter, and the ratio of undegradable organic matter might decrease in relative. Therefore, it has been implied in the present study that stressful conditions induce massive release of organic matter from coral colonies, and consequently, stimulate the fluxe of organic matter from coral colonies to bacteria and subsequent heterotrophic communities.

After 1 week, the remaining % of the organic matter released in the submerged condition of DLN and DHN did not change considerably for 1 year (Table 4-6). On the other hand, the organic matter released under the stressful condition (DAC and DPO) continued to be mineralized for 3 months and the remaining % decreased by 20%–30% during the period (Table 4-8). These result means that the organic matter produced in the DAC and DPO incubations still contained degradable fraction after 1 week more than that in DLN and DHN. This was supported by the data of THAA % of the bulk TON: while the THAA % decreased to 14%–26% in the DLN and DHN incubations, THAA still accounted for 53%–73% in the DAC incubations, suggesting higher % of hydrolysable proteins in DAC. Though the THAA % for DPO was lower than that for DAC (Table 4-5) during the whole decomposition period, this could be due to higher % of glucosamine and galactosamine (Meikle et al. 1988, Wild et al. 2005a) as discussed above.

4-4-4. The possibilities for long-term preservation of the organic matter

In previous studies, the organic matter released from hermatypic corals has been considered to be good energy sources for bacteria (Benson and Muscatine 1974, Ducklow and Mitchell 1979a, Wild et al. 2004a, 2005a), and therefore, to be rapidly mineralized into CO_2. However, these studies have focused on only bacterial growth or regeneration of inorganic nutrients in a short time period of a few hours, and not investigated quantitatively by longer term experiments. In the present study, the

concentration of organic matter released from the corals was followed during the period of >3 months, and showed that some parts of the organic matter was not rapidly mineralized but remained even after 1 year.

Some possibilities could be suggested for the undegradability of the coral-derived organic matter. First, the organic matter originally had resistance against bacterial degradation. Using coral mucus with molecular weight larger than 6000–8000, it was observed that the mucus web was not completely degraded by bacteria and eukaryotes even after 21 d (Vacelet and Thomassin 1991). They explained that mucus excretion from coral is a defensive reaction against physical and chemical stresses, thus mucus is a poor, or even inhibiting medium for the bacterial degraders isolated from the mucus itself. Similar results were also reported by Pascal and Vacelet (1981). Krupp (1984) and Coffroth (1990) also suggested that coral mucus is a material of low nutritional value for reef organisms. More recently, Ritchie (2006) observed that mucus from *Acropora palmata* had antibiotic properties that were likely to play a role in ordering only beneficial microbial communities on the coral surface. Antibacterial substance was also found from coral mucus of *Symphyllia gigantea* (Chen et al. 2007) and from tissue extraction of some soft corals (Kelman et al. 2006). The present results suggest that corals or coral-associated bacteria routinely produce organic matter resistant to bacterial degradation.

Second possibility for the undegradability is that the organic matter was once utilized by bacteria and converted into different organic forms, which were recalcitrant to further bacterial decomposition. Ogawa et al. (2001) reported that when marine bacteria utilized labile compounds (glucose, glutamate), they produced refractory DOM (R-DOM) that persisted for more than a year. The result suggested

that microbial processes alter the molecular structure of DOM, making it resistant to further degradation. Considering that 5%–7% of original substrate C (glucose, glutamate) were altered to R-DOC in Ogawa et al. (2001), the R-DOM produced by bacteria could account for only a few % of the remaining organic matter in this study (Figs. 4-2b & 4-3b), supposing that bacteria produce R-DOM with the similar ratio to the utilized organic matter. Therefore, the preservation of the organic matter in the present experiment could not be explained by only bacterial activity. Some parts of the remaining organic matter could originally have resistance against bacterial decomposition.

Third possibility is a technical problem that the community of bacteria used in DLN and DHN might be very specific to the coral surface area. In the experiments of DLN and DHN, the corals were incubated in artificial seawater and the bacteria in in-situ reef water were not inoculated. Though a few milliliter of natural seawater attached to the coral surface was dropped in the incubation bottles with the coral transfer, bacterial community in the attached seawater might be specific to near-surface seawater of the coral (Ritchie 2006). This idea was also suggested by the change of nutrient concentrations during the decomposition processes: as organic matter was mineralized, NH_4^+ concentrations first increased for all the incubations (Figs. 1c, 5c, 8c). In the experiment of PLN, PHN, DAC and DPO, the NH_4^+ was then converted to NO_2^- and NO_3^- (Figs. 5c & 8c) probably due to the activity of nitrifying bacteria. On the other hand, the nitrification was not observed in DLN and DHN experiments, suggesting that nitrifying bacteria did not live near the coral surface. These observations imply that not only the community of nitrifying bacteria but the community of other bacteria might be different between natural seawater and

near-coral seawater. Limited community of bacteria might have reduced degradability of the organic matter in DLN and DHN.

4-4-5. Amino acids and the other organic components in decomposed organic matter

In freshly produced DOM, Glx accounted for the highest % (30%–60%) of DHAA in all the incubations, irrespective of the coral species (Figs. 4-4, 4-11a, b). Glx in DFAA accounted for 2%–4% of the Glx in DHAA (data not shown), indicating that most of the released Glx was derived from combined amino acids such as proteins. Daumas and Thomassin (1977) also reported high % of glutamic acid (48%) in the organic matter released from *Fungia scutaria*. Means and Sigleo (1986) observed that Glx was one of the major amino acids in coral mucus, though the contribution (10%) was less than the present study. Fitzgerald and Szmant (1997) measured amino acid composition in the tissue of five scleractinian corals, and reported that Glx accounted for the highest % (12%–14%). These previous studies and the present results suggest that Glx is a major amino acid in both of coral tissue and organic matter released from the colony.

Within 1 week of the decomposition experiment, the Glx concentration and its % drastically decreased in all the incubations (Figs. 4-4, 4-11a, b). This suggested that a part of the released DHAA was selectively consumed by bacteria and it contained Glx at high % compared to the other DHAA. Glx is a primary amino acid which is first synthesized when corals incorporate inorganic N, and can be used as a precursor of the other amino acids. The rapidly decomposed Glx during the first 1

week might not have been locked into proteins of high molecular weight, but be a part of smaller combined amino acids in order to be utilized for other amino acids. Further investigation would be needed to clarify how the Glx was positioned in the released DHAA.

Newly produced DON in the DAC and DPO incubations (t = 0 d; Table 4-5) contained DHAA-N with the ratio of 40%–86% and 17%–28% for *A. pulchra* and *P. cylindrica*, respectively. This suggests that amino acids were one of the major DON released from the corals but other organic N was also simultaneously contained. The ratio of such unknown organic matter (OM_u) seemed to be higher for *P. cylindrica* than *A. pulchra*. The ratios of decreased THAA-N (ΔTHAA-N) to decreased bulk TON (ΔTON) during the first 1 week (42%–100% for *A. pulchra* and 19%–43% for *P. cylindrica*; Table 4-5) were within the similar range to those of the newly produced DOM, suggesting that both of amino acids and OM_u were simultaneously decomposed at a constant ratio. The C:N ratios of the decreased organic matter during the period were 13 and 6.6 for *A. pulchra* and *P. cylindrica*, respectively (Table 4-8), while the C:N ratio of amino acids must be about 5 on average. Therefore, the OM_u could have C:N ratios of greater than 13 for *A. pulchra* and nearly 6.6 for *P. cylindrica*. Assuming that ΔTHAA:ΔTON during the first 1 week was 50% and 20% for *A. pulchra* and *P. cylindrica*, respectively, C:N ratios of the OM_u were calculated to be 21 ($5 \times 0.5 + x \times 0.5 = 13$), and 7.0 ($5 \times 0.2 + x \times 0.8 = 6.6$). The OM_u might have been mainly composed of carbohydrates and lipids for *A. pulchra*, and acetyl glucosamine and acetyl galactosamine (C:N = 8 for both) for *P. cylindrica*. N-acetyl glucosamine and N-acetyl galactosamine have been both found from coral mucus in the previous studies (Ducklow and Mitchell 1979b, Meikle et al.

1988)

4-5. Summary and future problems

The organic matter released from the corals had two different fractions in bacterial degradability. Easily-mineralized fraction (L-OM) was decomposed within the first several days (turnover time 3.2–9.1 day for both DOM and POM), and the decomposition rate became far slower after that time. The production of L-OM was enhanced under both of the nutrient-enriched condition and stressful condition for POM and DOM, respectively. The enhancement would stimulate the flux of organic matter from the coral colonies to bacteria and other heterotrophic communities. Inorganic nutrient enrichment did not affect preservation of the released organic matter, indicating that the mineralization by bacteria was not limited by inorganic N and P availability. The present results have also suggested that a part of the coral-derived organic matter could be very recalcitrant to bacterial decomposition, and thus, be exported to the outer ocean. It remains to be resolved what makes the organic materials persistent to bacterial degradation.

Table 4-1 Summary of the pre-incubation conditions (NO_3^-, PO_4^{3-}, and the period) for the experiments of organic matter release.

Incubation names	NO_3^- (μmol L^{-1})	PO_4^{3-} (μmol L^{-1})	Period (day)
DLN1	1.8	0.08	28
DLN2	1.8	0.08	28
DHN1	11	0.53	28
DHN2	11	0.53	28
PLN1	1.8	0.08	1
PLN2	1.8	0.08	1
PHN1	1.8	0.08	19
PHN2	1.8	0.08	19

DLN: The decomposition target was DOM released from the coral less affected by the nutrient enrichment.
DHN: The decomposition target was DOM released from the coral more affected by the nutrient enrichment.
PLN: The decomposition target was POM released from the coral less affected by the nutrient enrichment.
PHN: The decomposition target was POM released from the coral more affected by the nutrient enrichment.

Table 4-2 The concentrations of ion species in the artificial seawater used for the decomposition incubations.

ions	(g kg^{-1})
Cl^-	19.4
Na^+	10.8
SO_4^{2-}	2.64
Mg^{2+}	1.29
Ca^{2+}	0.41
K^+	0.38
HCO_3^-	0.15
Br^-	0.07
BO_3^{3-}	0.03
Sr^{2+}	0.01

Table 4-3 Release rates of organic matter (nmol cm^{-2} h^{-1}) per unit surface area of the corals, and the C:N ratios. Standard deviations of analysis are shown in parenthesis.

Incubation names	Targets	C	N	C:N
DLN1	DOM	36 (1)	3.4 (0.0)	10.4 (0.2)
DLN2	DOM	36 (1)	3.8 (0.1)	9.5 (0.4)
DHN1	DOM	48 (1)	5.4 (0.2)	8.9 (0.4)
DHN2	DOM	25 (2)	2.8 (0.3)	9.0 (1.2)
PLN1	POM	116	11	11
PLN2	POM	121	11	11
PHN1	POM	186	21	8.9
PHN2	POM	180	20	8.9
DAC1	DOM	444 (2)	32 (1)	14.0 (0.4)
DAC2	DOM	399 (8)	35 (1)	11.4 (0.3)
DPO1	DOM	514 (4)	66 (0)	7.8 (0.1)
DPO2	DOM	650 (7)	89 (1)	7.3 (0.1)

DAC: The decomposition target was DOM released from *Acropora pulchra*.
DPO: The decomposition target was DOM released from *Porites cylindrica*.

Table 4-4 The concentrations of THAA (nmol L^{-1}) in the incubations of DLN and DHN. The ranges for the contribution of THAA-N to the bulk TON (THAA-N:TON) indicate the both cases that Asx and Glx in THAA are all aspartate and glutamate (low %) or asparagine and glutamine (high %). THAA and TON at t = 0 d equal DHAA and DON, respectively.

	Days	THAA (nmol L^{-1})	Remaining (%)	THAA-N:TON
DLN1	0	435	100	32-53
	7	209	48	24-31
	120	153	35	18-23
DLN2	0	323	100	32-53
	7	239	74	30-50
	120	121	37	20-26
DHN1	0	661	100	40-64
	7	239	36	21-28
	120	181	27	15-20
DHN2	0	380	100	41-64
	7	166	44	27-34
	120	82	22	14-17

Table 4-5 The concentration of THAA (nmol L^{-1}) and its contribution to the bulk TON concentration (THAA-N:TON, %) in the incubations of DAC and DPO. THAA and TON at t = 0 d equal DHAA and DON, respectively. ΔTHAA:ΔTON (%) is the ratio of decreased THAA-N to the decreased bulk TON during the period of 0-7 d and 7-90 d. The ranges for THAA-N:TON and ΔTHAA:ΔTON indicate the both cases that Asx and Glx in THAA are all aspartate and glutamate (low %) or asparagine and glutamine (high %).

	Days	THAA (nmol L^{-1})	Remaining (%)	THAA-N:TON	ΔTHAA:ΔTON
DAC1	0	3140	100	60-86	
	7	1230	39	44-56	78-100
	90	702	22	53-65	36-57
DAC2	0	2710	100	40-56	
	7	1710	63	39-46	42-80
	90	1110	41	61-73	18-31
DPO1	0	1830	100	18-28	
	7	956	52	17-21	20-43
	90	733	40	35-43	6-13
DPO2	0	2130	100	17-27	
	7	1030	48	15-18	19-42
	90	666	31	21-24	10-16

Table 4-6 The concentrations and remaining % of the organic matter during the incubations of DLN and DHN. TOC and TON at t = 0 d equal DOC and DON, respectively. The data of DLN and DHN are given as the mean ± SD of analysis. The average values of DLN and DHN are shown as mean ± SD of the four incubations.

		TOC		TON	
	Days	Concentration ($\mu mol\ L^{-1}$)	Remaining (%)	Concentration ($\mu mol\ L^{-1}$)	Remaining (%)
DLN1	0	15.1±0.3	100	1.5	100
	7	9.1±0.5	60±4	1.0	72±1
	365	10.1±0.6	66±4	0.9	60±3
DLN2	0	10.2±0.3	100	1.1	100
	7	7.5±0.9	73±9	0.8	79±5
	365	6.2±0.5	61±5	0.7	68±7
DHN1	0	16.0±0.4	100	1.8	100
	7	10.1±1.4	63±9	1.3	75±9
	365	8.6±0.4	54±3	1.2	70±3
DHN2	0	9.3±0.8	100	1.0	100
	7	6.7±0.7	72±10	0.8	77±14
	365	6.4±1.1	70±14	0.8	84±15
Average	7		67±6		76±3
	365		63±7		70±10

Table 4-7 The concentrations and remaining % of POM in the incubations of PLN and PHN. The average values are given as the mean ± |mean-replicate| of duplicate incubations for PLN and PHN.

	Days	POC		PON	
		Concentration ($\mu mol\ L^{-1}$)	Remaining (%)	Concentration ($\mu mol\ L^{-1}$)	Remaining (%)
PLN1	0	5.5	100	0.5	100
	7	3.2	59	0.3	61
	120	4.0	73	0.1	26
PLN2	0	4.6	100	0.4	100
	7	1.9	41	0.3	73
	120	2.5	55	0.2	50
Average	7		50±9		67±6
	120		64±11		38±12
PHN1	0	8.7	100	1.0	100
	7	4.6	53	0.4	39
	120	1.9	22	0.1	14
PHN2	0	11	100	1.2	100
	7	2.9	27	0.5	42
	120	2.9	26	0.2	17
Average	7		40±13		40±2
	120		24±2		16±2

Table 4-8 The concentrations and remaining % of organic matter during the incubations of DAC and DPO. TOC and TON at t = 0 d equal DOC and DON, respectively. C:N of ΔOM indicates the C:N ratio of the decomposed organic matter during the period. The data of DAC and DPO are given as the mean ± SD of analysis. The average values of DAC and DPO are shown as mean ± SD of the four incubations.

		TOC		TON		
	Days	Concentration ($\mu mol\ L^{-1}$)	Remaining (%)	Concentration ($\mu mol\ L^{-1}$)	Remaining (%)	C:N of ΔOM
DAC1	0	80±0	100	6.0±0.2	100	
	7	47±0	59±1	3.5±0.0	58±2	13±1
	90	26±1	32±1	1.3±0.0	22±1	10±0
DAC1	0	84±2	100	7.6±0.2	100	
	7	45±1	53±2	4.6±0.1	60±2	13±1
	90	29±2	35±3	2.4±0.1	32±1	7.4±1.3
DPO1	0	85±1	100	11±0	100	
	7	52±1	62±1	6.3±0.0	57±0	6.8±0.2
	90	31±1	37±1	2.6±0.1	24±1	5.8±0.3
DPO2	0	97±1	100	14±0	100	
	7	61±0	63±1	8.0±0.0	59±1	6.4±0.2
	90	42±1	44±1	3.8±0.1	28±1	4.5±0.2
Average	7		59±4		59±1	
	90		37±5		27±4	

Table 4-9 First order decay constants (day^{-1}) of labile and refractory organic matter released from the corals. 'nd' means that no data was obtained because of the calculation method in the present study.

	Labile (0-7 d)		Refractory (>7 d)	
	C (d^{-1})	N (d^{-1})	C (d^{-1})	N (d^{-1})
DLN1	0.31	0.18	-0.0003	0.0005
DLN2	0.16	0.15	0.0005	0.0004
DHN1	0.22	0.25	0.0005	0.0002
DHN2	0.21	0.25	-0.0001	-0.0003
DAC1	0.14	0.11	0.007	0.012
DAC2	0.18	0.13	0.005	0.008
DPO1	0.13	0.12	0.006	0.011
DPO2	0.15	0.12	0.004	0.009
PLN1	nd	0.11	-0.002	0.008
PLN2	nd	0.11	-0.003	0.003
PHN1	0.14	0.18	0.008	0.009
PHN2	0.66	0.17	0.0002	0.008

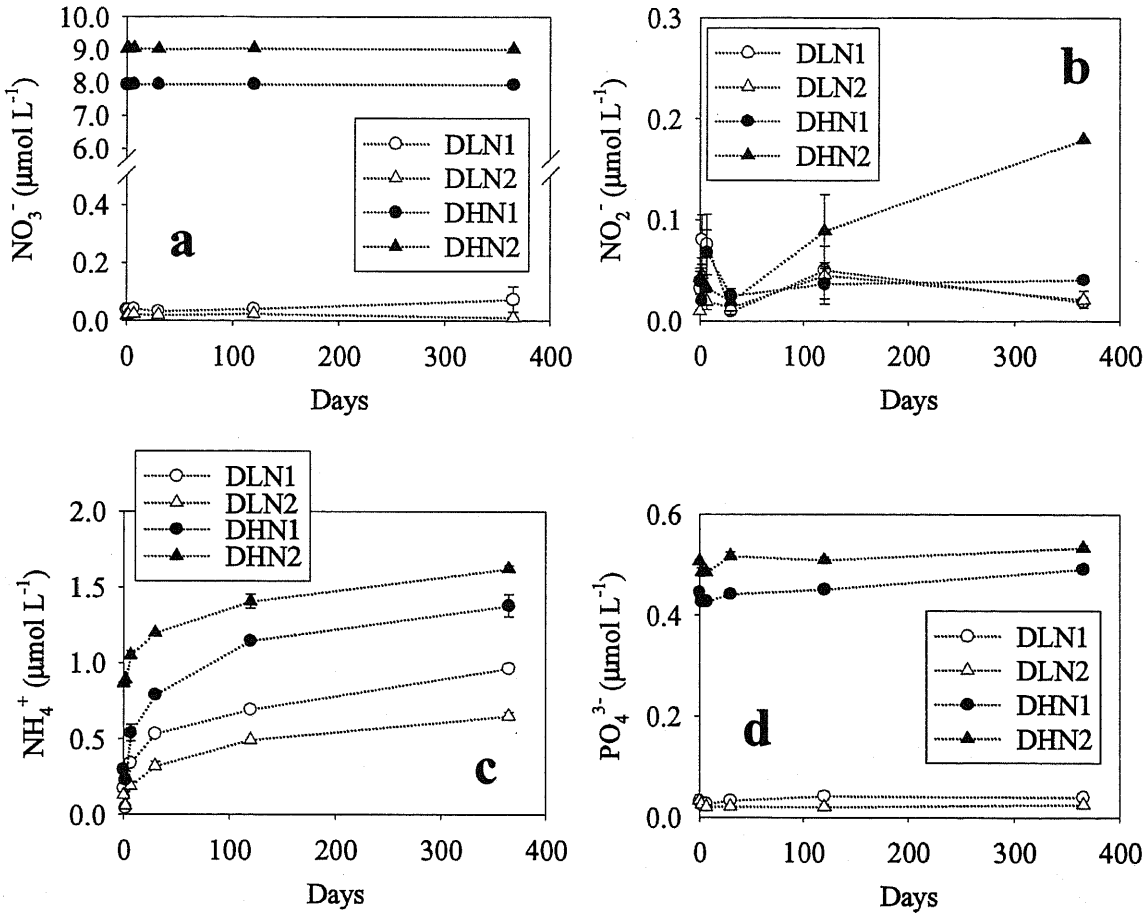

Fig. 4-1 The nutrient concentrations of NO_3^- (a), NO_2^-(b), NH_4^+ (c), PO_4^{3-} (d) during the decomposition experiment of DLN and DHN.

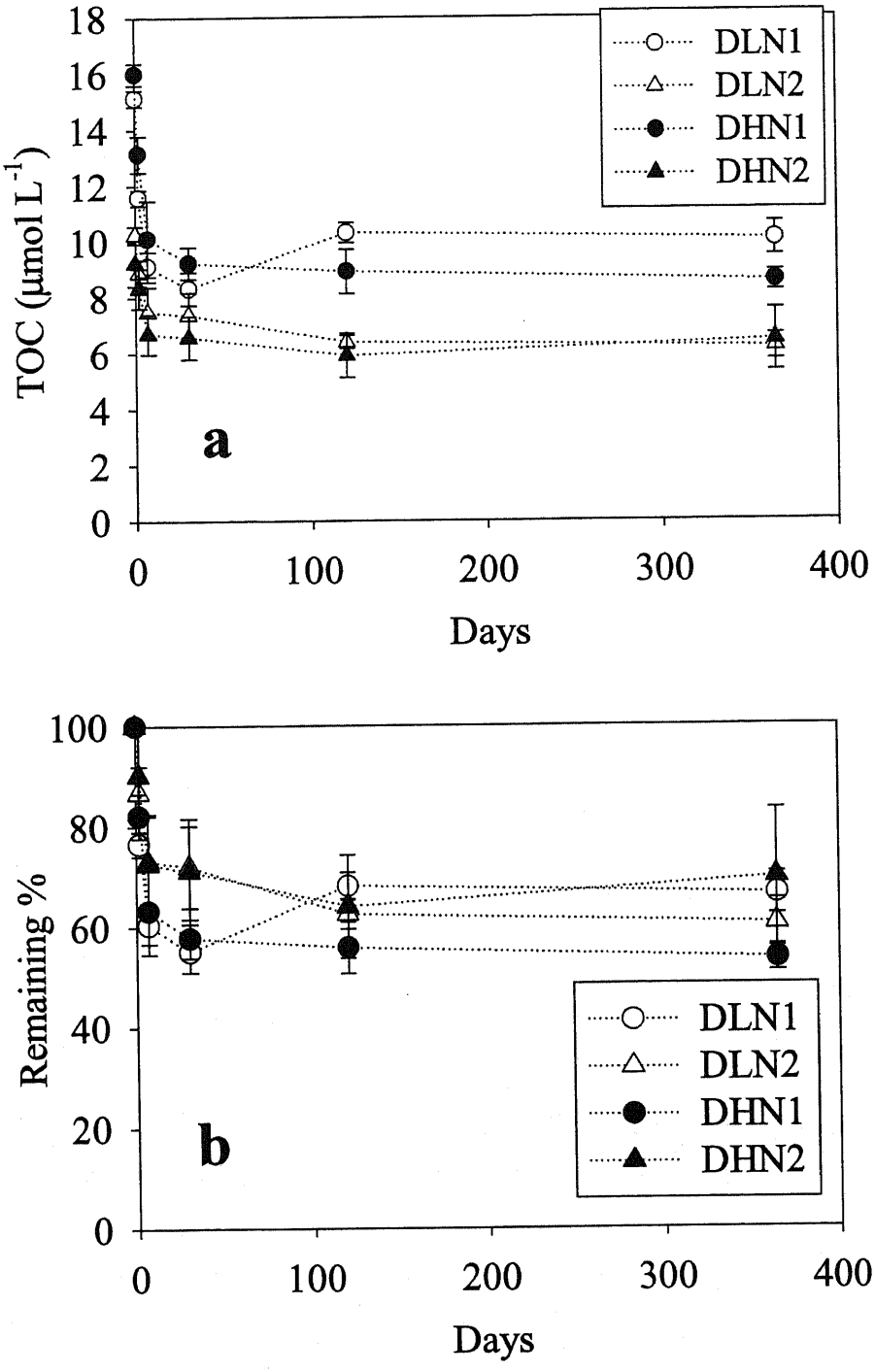

Fig. 4-2 The concentrations (a) and remaining % (b) of total organic C (TOC) in the decomposition experiments of DLN and DHN. The initial organic matter (t = 0) contained only DOC. The data are given as the mean ± |mean-replicate| of duplicate ampoule incubations.

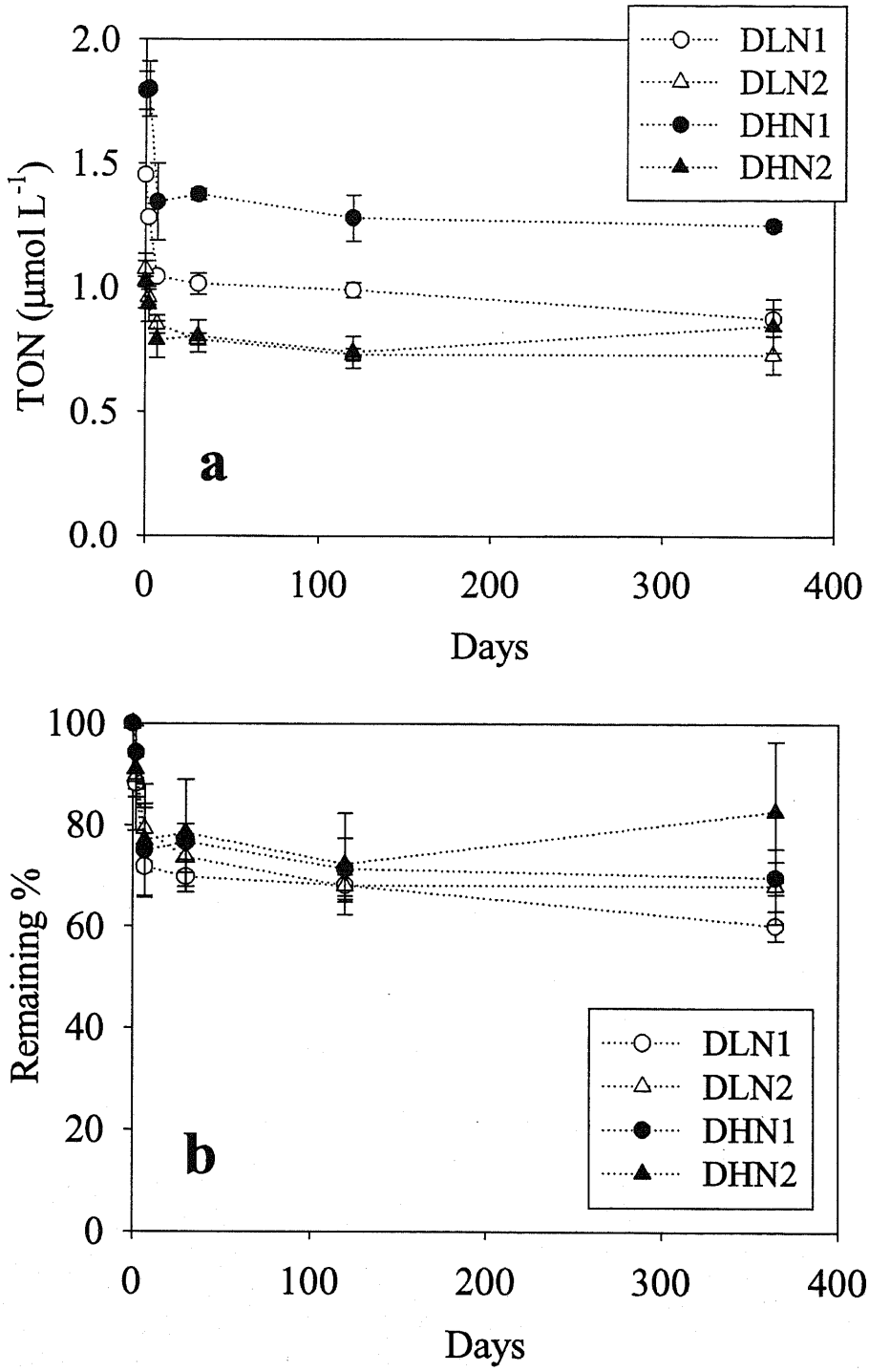

Fig. 4-3 The concentrations (a) and remaining % (b) of total organic N (TON) in the decomposition experiments of DLN and DHN. The initial organic matter (t = 0) contained only DON. The data are given as the mean ± |mean-replicate| of duplicate ampoule incubations.

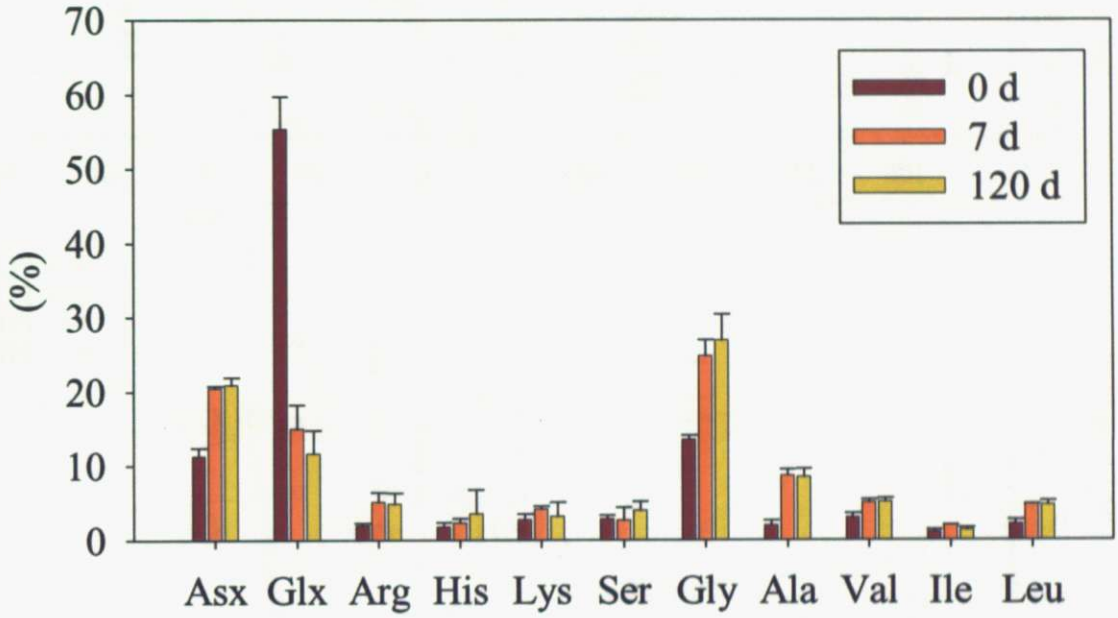

Fig. 4-4 Amino acid composition during the decomposition of organic matter in the incubations of DLN and DHN (mean ± SD of the four incubations).

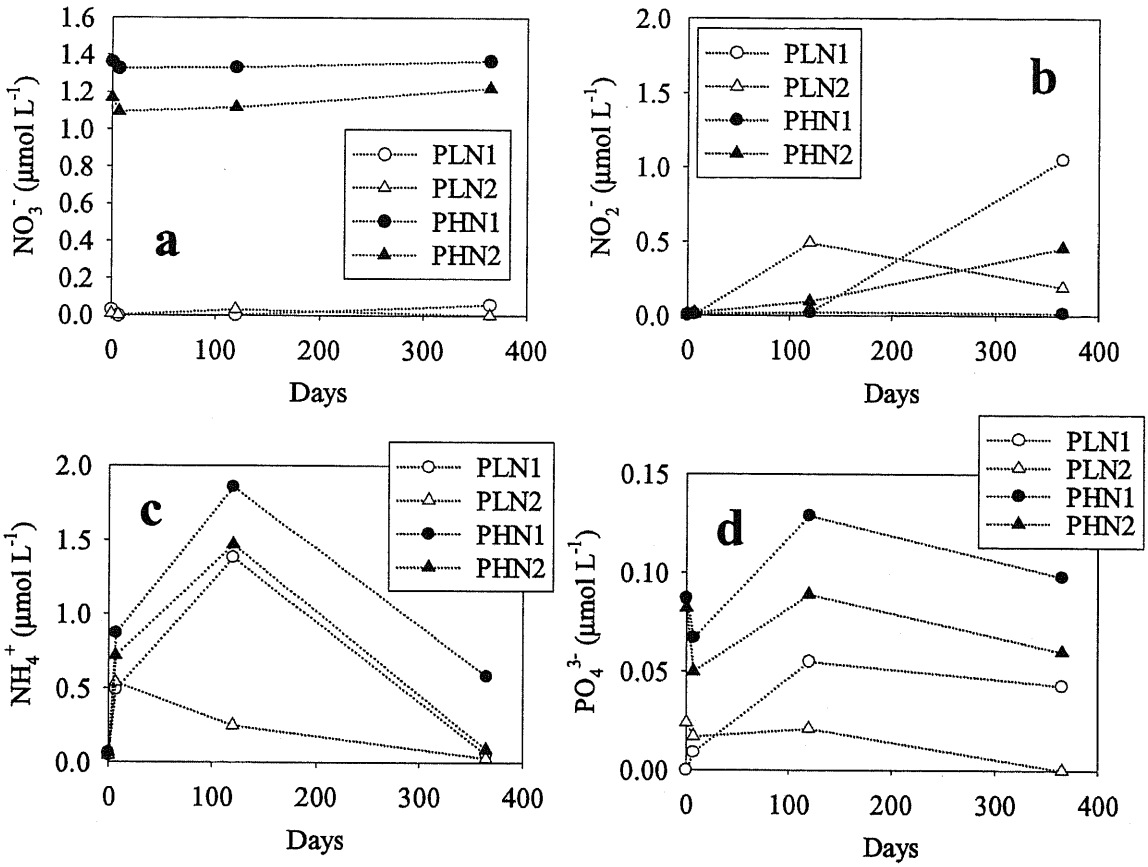

Fig. 4-5 The nutrient concentrations of NO_3^- (a), NO_2^-(b), NH_4^+ (c), PO_4^{3-} (d) during the decomposition experiment of DLN and DHN.

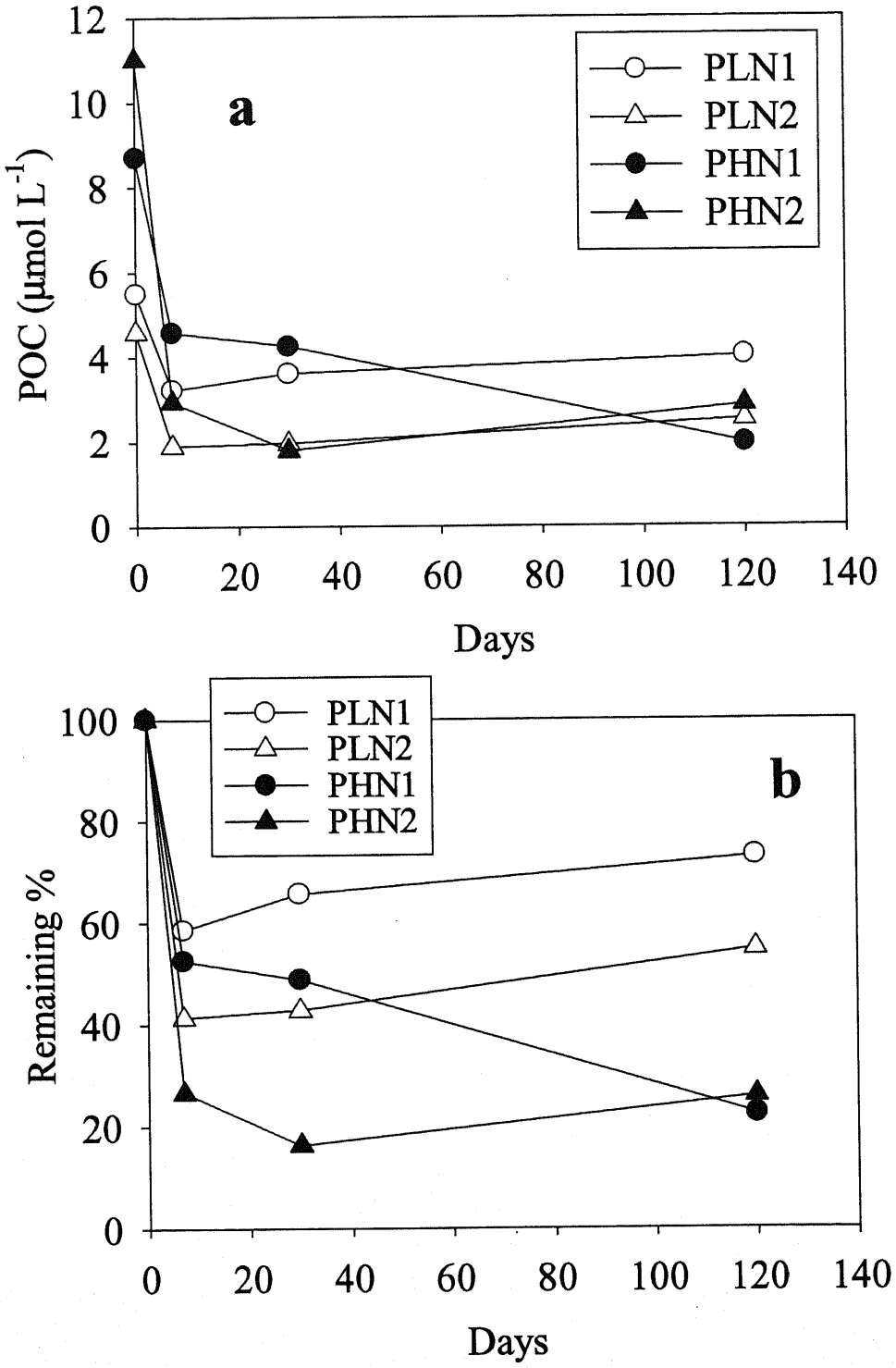

Fig. 4-6 The concentrations (a) and remaining % (b) of particulate organic C (POC) in the decomposition experiments of PLN and PHN.

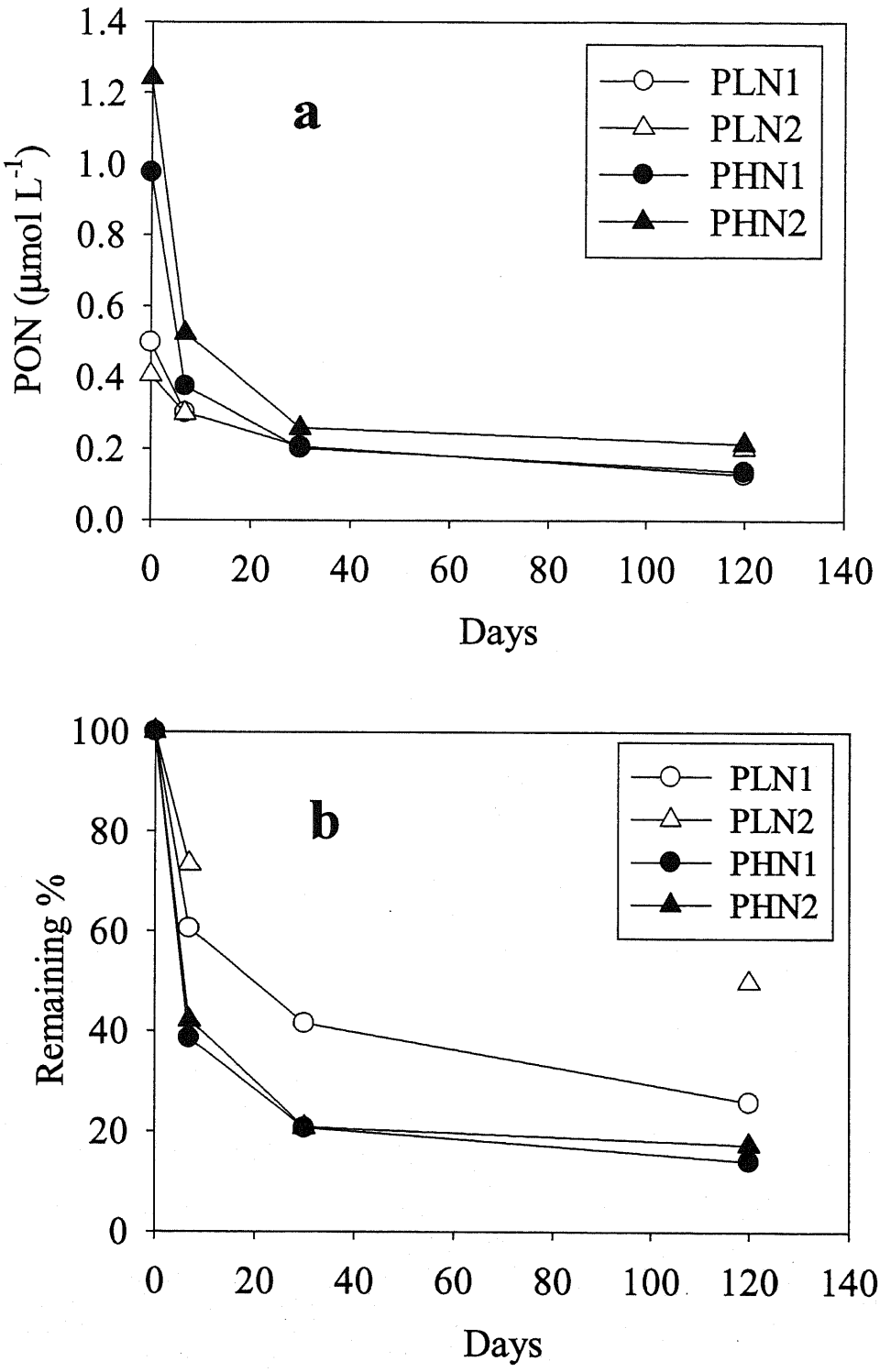

Fig. 4-7 The concentrations (a) and remaining % (b) of particulate organic N (PON) in the decomposition experiments of PLN and PHN.

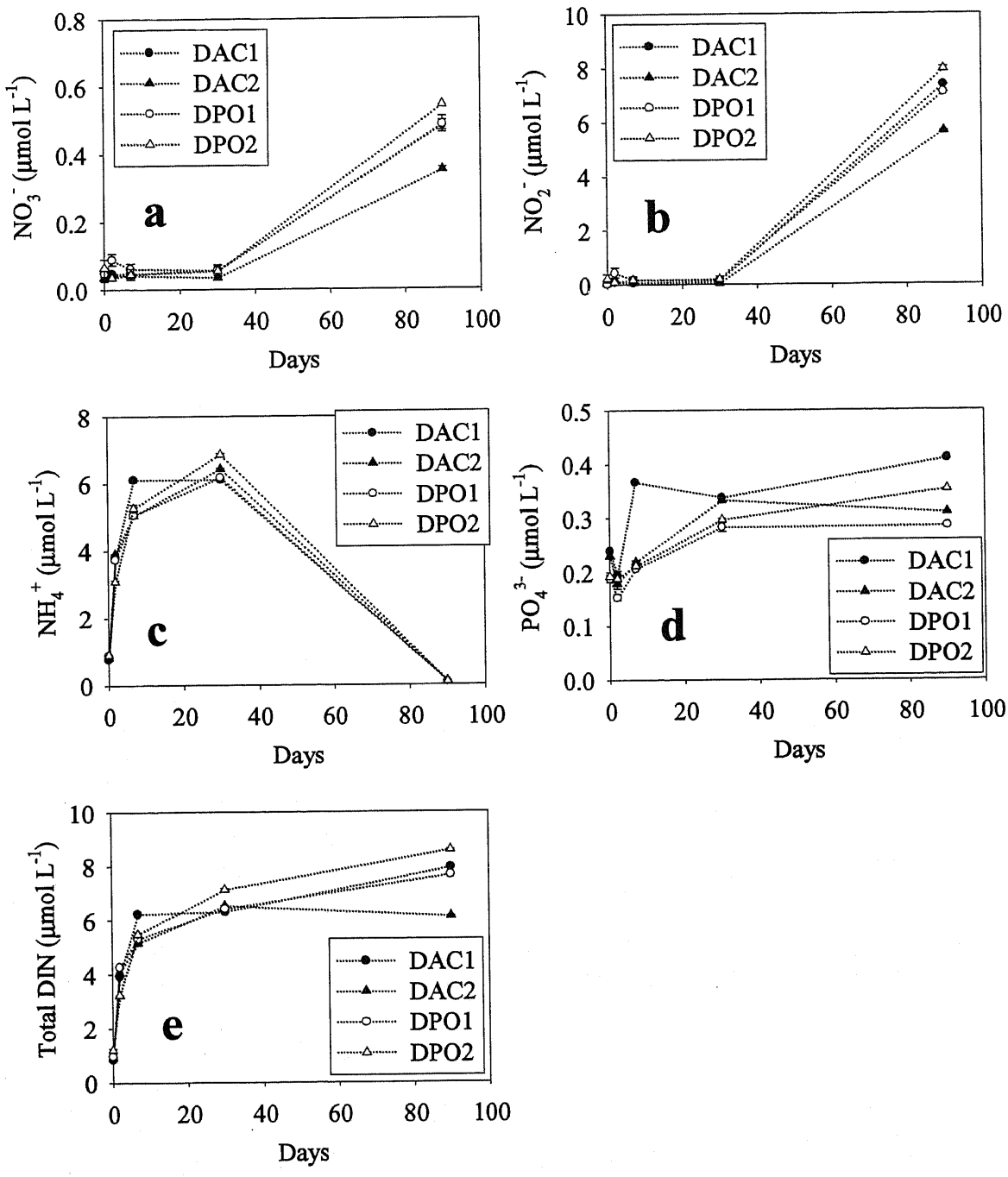

Fig. 4-8 The nutrient concentrations of NO_3^- (a), NO_2^-(b), NH_4^+ (c), PO_4^{3-} (d), and total DIN (NO_3^- + NO_2^- + NH_4^+) (e) during the decomposition experiment of DAC and DPO.

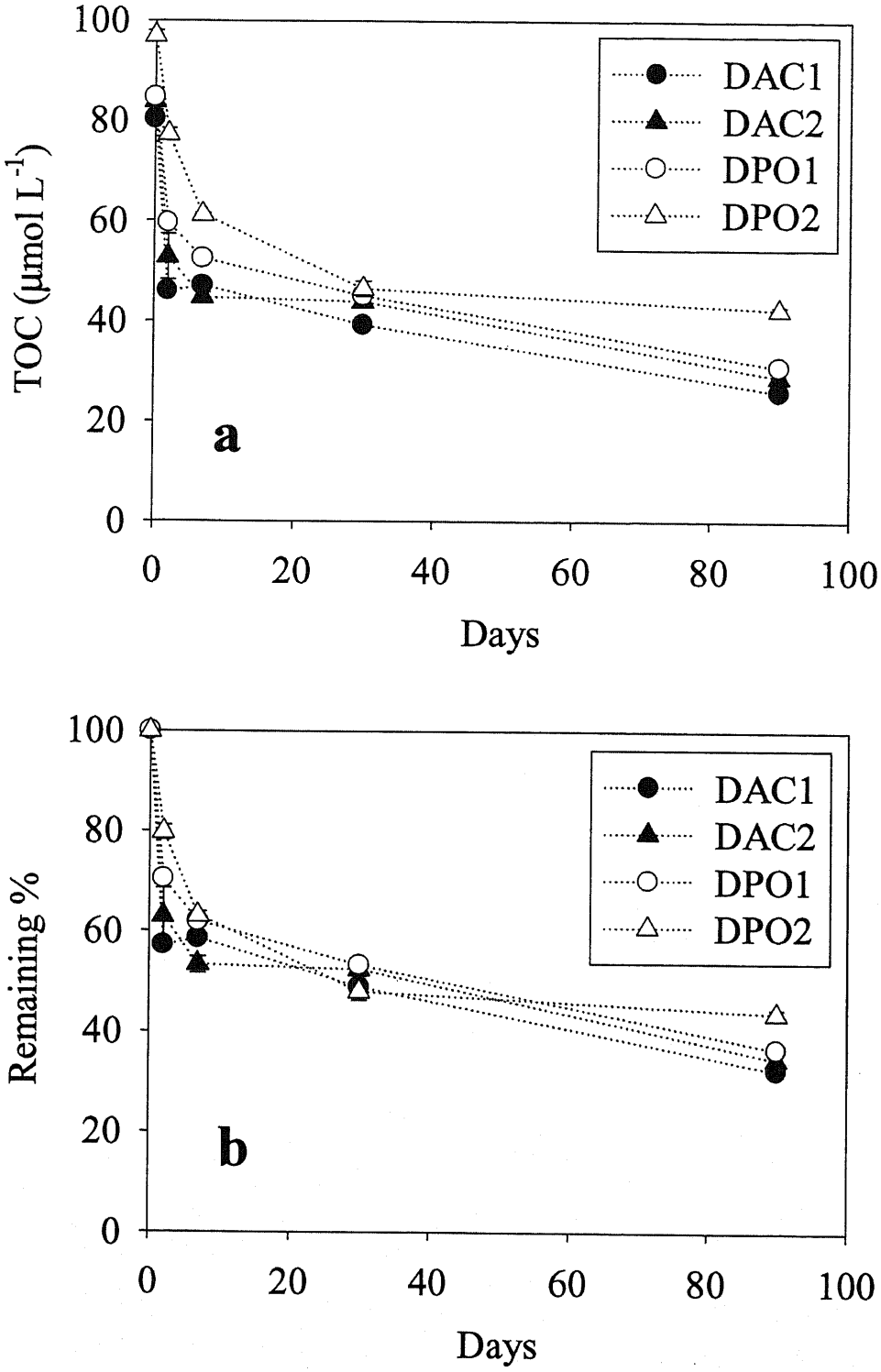

Fig. 4-9 The concentrations (a) and remaining % (b) of total organic C (TOC) in the decomposition experiments of DAC and DPO. The initial organic matter (t = 0) contained only DOC. The data are given as the mean ± |mean-replicate| of duplicate ampoule incubations.

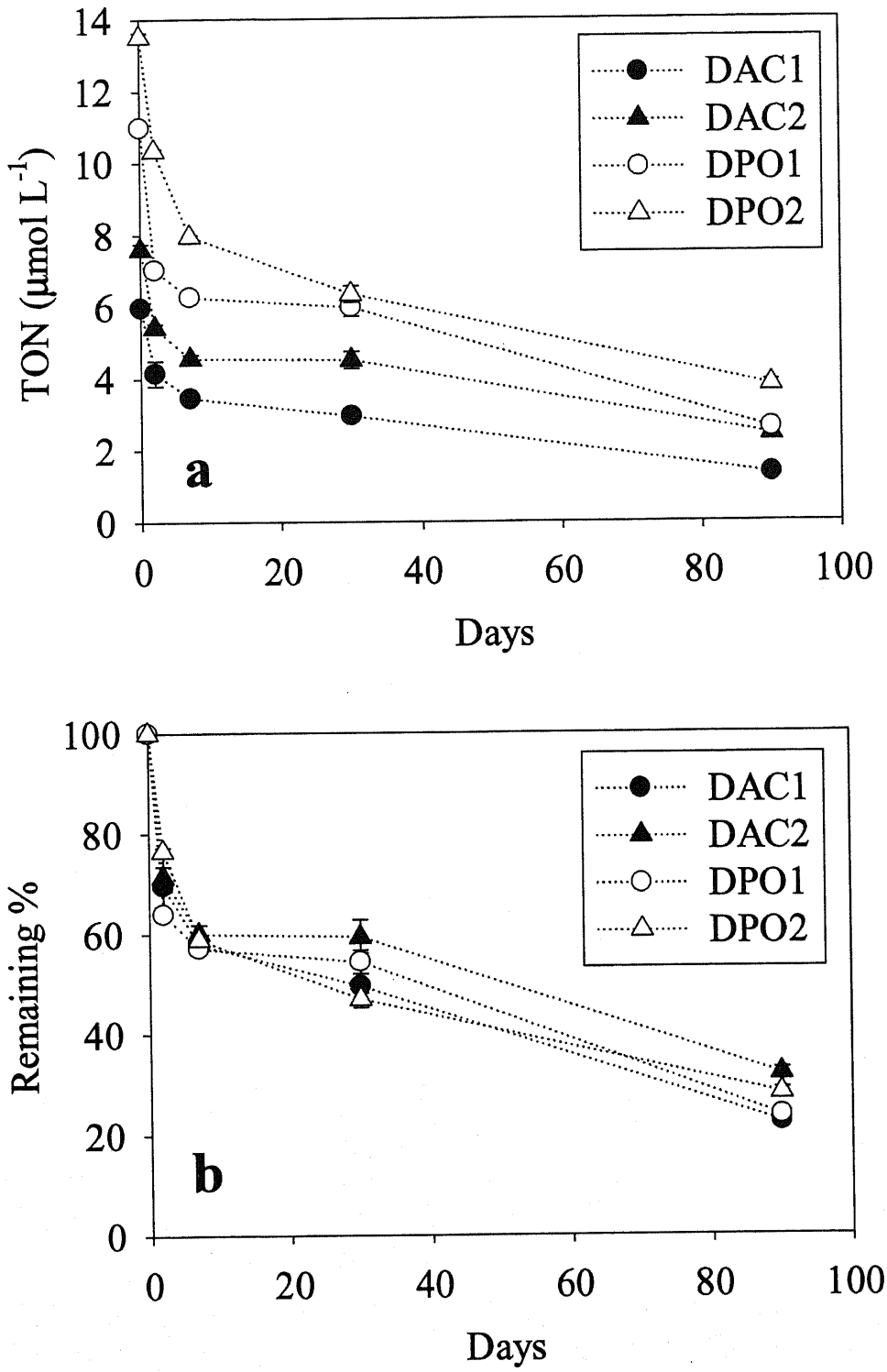

Fig. 4-10 The concentrations (a) and remaining % (b) of total organic N (TON) in the decomposition experiments of DAC and DPO. The initial organic matter (t = 0) contained only DON. The data are given as the mean ± |mean-replicate| of duplicate ampoule incubations.

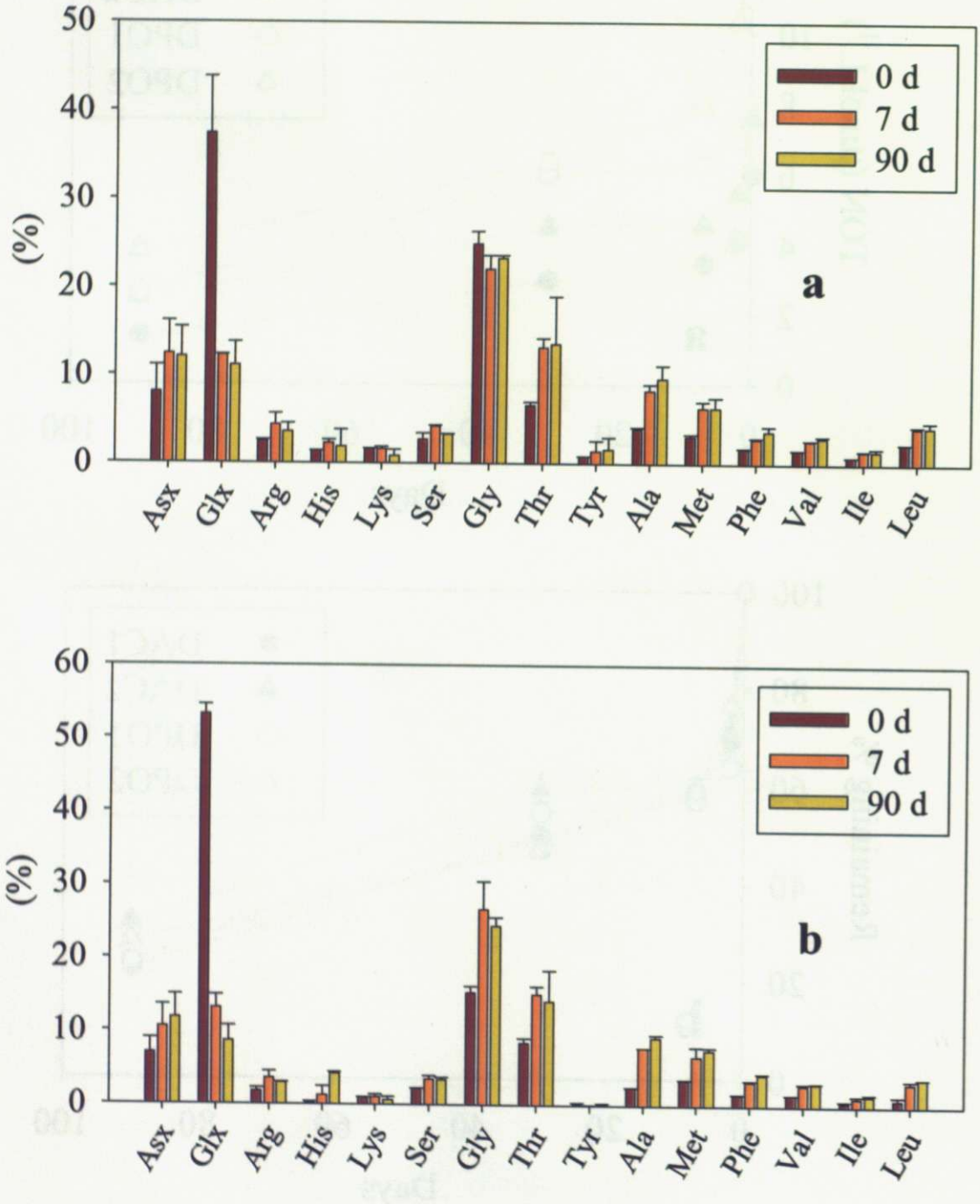

Fig. 4-11 Amino acid composition during the decomposition of organic matter released in the incubations of DAC (a) and DPO (b). The data are given as the mean ± |mean-replicate| of duplicate bottle incubations.

Chapter 5

General Discussion

5-1. Integration of the data obtained in the present study on carbon and nitrogen cycling through coral colonies

Carbon (C) and nitrogen (N) fluxes obtained in the present study were summarized in Figs. 5-1 and 5-2. When NO_3^- and PO_4^{3-} concentrations increased, it was actively taken up by the corals. It has been confirmed that the uptake rate of NO_3^- was dependent on its concentration (Tanaka et al. 2006a). When NO_3^- concentration increased from 0.5 to 5 μmol L^{-1}, the uptake rate would be enhanced from 230 to 980 nmol cm^{-2} d^{-1} (Fig. 5-2). The increased nutrient incorporation significantly enhanced photosynthetic activity of the symbiotic algae and the photosynthetic C fixation rate measured by ^{13}C-tracer technique increased from 10 to 28 μmol C cm^{-2} d^{-1} during the nutrient-enriched period of 10 d in Chapter 2 (Table 2-2). The growth rate of coral plus algal biomass, i.e., net production, was calculated in Chapter 3 to be 2.7 and 0.67 μmol cm^{-2} d^{-1} for C and N, respectively (Fig. 3-3a).

Bythell (1988) estimated annual C and N budgets via *Acropora palmata*, and observed that the tissue C growth including gamete production accounted for 33% of the respiratory C consumption. Using the budget estimation, the respiration rate of the coral in Chapter 3 was calculated to be 8.2 (= 2.7/0.33) μmol C cm^{-2} d^{-1}. Thus, the ratio of gross primary production (P_g) to respiration (R) was estimated to be 1.7 (= (2.7+2.1+0.56+8.2)/8.2) for the corals under the nutrient enrichment in this

study. These metabolic rates shown in Figs. 5-1 and 5-2 were normalized to unit surface area of the coral branches. The rates could become greater when normalized to projected area of coral reefs because coral branches are morphologically complex. Dahl (1973) calculated a surface index (SI: the ratio of actual surface area to that of a plane with similar boundaries) for several types of corals, and the SI value for branched corals (*Acropora palmata*) was 3. The present metabolic rates normalized to projected area using the SI were shown in Table 5-1.

Because calcification could not follow the tissue production rates (Chapter 2), it was expected that the imbalanced growth between carbonate skeleton and soft tissue might induce the increase in organic matter release to the ambient seawater. POC release rates actually increased twice compared to those without the nutrient enrichment (Figs. 3-4a, b), and the ratio of POC release to the biomass C also increased (Table 3-1). These suggest that photosynthetic products of zooxanthellae were gradually utilized for not tissue growth of the coral colony but POC release to the external seawater. Increased nutrient levels would enlarge algal biomass in the host coral (Hoegh-Guldberg and Smith 1989, Muller-Parker et al. 1994, Marubini and Davies 1996), and the excessive zooxanthellae could be released from the host to the ambient seawater (Stimson and Kinzie 1991). However, most of the POM was not accounted by the released algae, suggesting that other organic materials such as coral mucus and host tissue fragments were also simultaneously released with the symbiotic algae (Fig. 3-8).

The organic matter released from the corals had two fractions different in bacterial degradability. The labile fraction had the turnover time of 3.2–9.1 d, thus, it could be mineralized in the reef ecosystems before it was flushed out to the outer

ocean. On the other hand, the refractory fraction had the turnover time of >100 d, and therefore, would be exported to the outer ocean. It depends on the water residence time of the reef what % of the organic matter released from corals is exactly decomposed within the reef lagoon and what % is exported to the offshore. As for POM, the ratio would also depend on the sedimentation rates of the POM. Wild et al. (2004a) and Huettel (2006) have shown that coral mucus physically traps suspended particles from the reef water and sink to the lagoon sediments, where they are consumed by the benthic community. Nutrient enrichment enhanced POM release rates from the corals, but the increased POM was rapidly decomposed within 1 week and the remaining amount after 3 month was almost not affected by the nutrient treatment. It can be suggested that nutrient enrichment would increase the POM fluxes from corals to bacteria and subsequent heterotrophic communities in the reef system.

In previous studies, it has been considered that net primary production by corals equals to the tissue growth and that the organic matter released from corals could be all mineralized into CO_2. However, in the present study, the organic matter which remained intact for >3 months accounted for 31% of the growth rate of C biomass under the nutrient enrichment and 14% of the N biomass growth (Figs. 5-1 & 5-2). This is the first study which has demonstrated long-term remaining of organic matter released from corals.

5-2. The comparison of net primary production between coral colonies and whole reef ecosystems

C and N fluxes via hermatypic corals have been investigated in the present study from the perspective of inorganic nutrient enrichment. In this section, metabolic rates of net primary production in whole reefs were reviewed and the contribution of coral colonies was evaluated.

Reef metabolic data have been recently reviewed by Atkinson & Falter (2003). Gross community production varied from 100 to 2000 mmol C m^{-2} d^{-1}, depending on habitat (Table 5-1). Gattuso et al. (1998) also gained the similar range of the value (220–1600 mmol C m^{-2} d^{-1}; Table 5-1) in their review. Such variability could mostly be due to differences in the community structure of the sites investigated: low relief sand communities were the lowest (100–300 mmol C m^{-2} d^{-1}), and high relief communities of coral and algae showed maximal values (1000–2000 mmol C m^{-2} d^{-1}). Calcification rates were also highest in the coral and algal community. These overall metabolic rates of different reefs were relatively consistent between reefs, suggesting that metabolic rates in each community (corals, seagrasses, sediment, etc.) are independent of species composition. Community respiration also varied over the same range as gross production (Table 5-1), and the two processes were positively correlated. These indicate that much of the respiration of organic matter occurs within the community, or organisms that produce the organic C. As entire reef systems, net community production was calculated to be 0–70 mmol C m^{-2} d^{-1}, and the P_g:R ratio was 1.05, indicating a slight net autotrophy.

Comparing with these data on a reef scale, coral colonies are considered to be far autotrophic: the P_g:R ratio estimated from the present study was 1.7 for the corals incubated under nutrient enrichment. The other values for the corals not

affected by nutrient enrichment have been shown in previous studies, e.g., 2.1 for *Porites porites* (Edmunds & Davies 1986), 1.4 for *Acopora palmata* (Bythell 1988), which were also far greater than 1 (Table 5-1). The difference in estimation of the P_g:R ratio between the scales of coral colonies and whole reefs indicates that the community which excludes coral colonies and probably algal-dominated areas consumes organic matter (heterotrophy) and reduces net primary production by corals and algae.

Wild et al. (2004a, b) described that coral mucus efficiently traps organic matter from the water column and is rapidly carried to the reef sediment, which mineralizes the organic matter as a biocatalytic filter. Hansen et al. (1992) also reported that a significant fraction of the products of reef-flat primary production could be transported as particulate material and reach lagoon sediments at Davies Reef, Great Barrier Reef. Not only coral and algal-dominated communities, but seagrass beds in coral reefs might also function as a source of suspended organic matter to the water column (Miyajima et al. 1998). Organic matter released from various sources as describe above would partially settle down to the sediment and be constantly consumed by bacteria day and night. Thus, sediment might be one of the major heterotrophic communities in coral reefs (Rasheed et al. 2004, Wild et al. 2005b).

Oxygen consumption rates in reef sediments have been reported to be 50 mmol O_2 m^{-2} d^{-1} at Heron Island, Australia (Wild et al. 2004a, b), and 20 mmol O_2 m^{-2} d^{-1} at Aqaba, Red Sea (Wild et al. 2005b). The bacterial production rates in reef sediments have ranged from 1.7 to 31 mmol C m^{-2} d^{-1} with an average of 11 mmol C m^{-2} d^{-1} (Moriarty et al. 1985, Moriarty and Hansen 1990, Hansen et al. 1992).

Ammonium flux rates from sediments to the water column in coral reefs were reported to be 0.06–0.3 (Charpy-Roubaud et al. 1996, Bertuzzi et al. 1996, Rasheed et al. 2004). These data suggest that organic matter is filtered from the water column when bottom currents interact with the sediment and decomposed in the sedimentary microbial food chain. The products of the mineralization, the inorganic nutrients, are then released into the pore water and overlying water column.

However, some previous studies are negative about the idea that sediment might be a heterotrophic community. Suzumura et al. (2002) observed daily net production (6.9–24 mmol C m^{-2} d^{-1}) by micorophytobenthic communities in carbonate sediments in Shiraho Reef, Japan. Boucher et al. (1994) and Miyajima et al. (2001) showed that inorganic N had a flux from the water column to the sediment. These observations indicate that benthic communities have net primary production (i.e., autotrophy) due to the activity of benthic microalgae.

The discrepancy between those previous studies, i.e., whether reef sediments are heterotrophic or autotrophic, could be caused by physical structures of the coral reef, especially water depth in the lagoon. When water depth to the sediment is shallow, benthic algal community can receive enough light intensity and produce net organic matter by photosynthesis. On the other hand, when the benthic community does not receive enough light intensity due to the deep lagoon structure, bacterial decomposition of organic matter in the sediment would exceed the gross primary production by benthic algae. Sediment could become both autotrophic and heterotrophic, depending on physical structures of the reef.

The balance of total surface area between net autotrophic community and net heterotrophic community could affect the P_g:R ratio of whole reef ecosystems.

However, the P_g:R ratio could be more strongly influenced by water residence time of the lagoon. When the water residence time is long enough, the produced organic matter is decomposed within the lagoon and the P_g:R ratio gets closer to 1, even if P_g was initially much higher than R. Contrarily, when the water residence time is short, most of the produced organic matter are rapidly exported from the ecosystem and the P_g:R ratio becomes more than 1.

Some previous studies have shown that the P_g:R ratio in Shiraho Reef was more than 1 and organic matter was exported from the lagoon to the offshore (Kayanne et al. 1995, Hata et al. 2002, Kayanne et al. 2005). The water residence time of Shiraho Reef was estimated to be 4–8 h (H. Yamano, unpubl. data). Thus, even labile organic matter, which can be mineralized by bacteria within several days (see Chapter 4), could be exported to the offshore in such coral reefs and the P_g:R ratio of the whole ecosystem would become greater than 1.

5-3. Coral contribution in POM and DOM production to the coral reef water

In the present study, the corals released POC and DOC with the rate of 0.84 and 0.56 μmol cm^{-2} d^{-1} without nutrient enrichment, and the POC release rates increased by the nutrient enrichment to 2.1 μmol C cm^{-2} d^{-1} (Fig. 5-1). Normalized to projected area, the release rates would become three times higher (Dahl 1973) and thus, 25, 17, and 63 mmol C m^{-2} d^{-1}, respectively. These release rates obtained using *Acropora pulchra* were within the similar range to those of *Porites cylindrica* (0.59

and 0.34 μmol cm^{-2} d^{-1} for POC and DOC, respectively; Tanaka et al. in press). On the other hand, Hata et al. (2002) measured in-situ fluxes of POC and DOC at Shiraho Reef in Ishigaki Island, which is the same reef where corals were collected in the present study. The study site in Hata et al. (2002) was at the middle of the reef pavement (ca. 500 m offshore from the coast), where branched *Porites* and *Montipora*, and blue coral *Heliopora* were the dominant species of corals. In-situ fluxes of POC and DOC obtained in his study were 7 and 36 mmol m^{-2} d^{-1}, respectively. Because large variance could be involved when normalizing the surface area of coral branches to projected area, the absolute value of POM and DOM fluxes might not be able to be compared between coral incubations in a closed system of the present study and the in situ observation. However, a clear difference was found in the ratio of DOC to POC: while the DOC:POC ratio was <1 in the present study, the ratio was >5 in the in-situ observation. Some possibilities can be suggested for the difference. The ratios of DOC:POC from the corals (*A. pulchra* and *P. cylindrica*) were also <1 in the previous experiment (Tanaka et al. in press), where DOC and POC release rates were measured over 4 d, suggesting that the release ratio was relatively constant.

First, POM released from corals traps other organic materials in reef water (Rasheed et al. 2004, Wild et al. 2004a, b) and consequently, easily settles down to the sediment compared to the released DOM. Even if POM does not trap other organic matter, the POM might be trapped by complicated benthic structure of coral reefs during the course of flowing. The physical characteristics of POM might result in higher DOM:POM ratio for in-situ observations. Hata et al. (2002) sampled the seawater at the surface, and thus, POM of higher density than the reef water could

not be detected, even if actually produced from the benthic community. Moreover, they calculated the fluxes of POC and DOC using the data during the slack water period, when POM might easily sink down.

Second possibility is that reef communities except corals might release organic matter at higher DOC:POC ratios than that from coral colonies. Ziegler and Benner (1999) estimated the DOC flux in a subtropical seagrass (*Thalassia testudjnum*) -dominated lagoon (Laguna Madre, Texas) as 4–25 mmol m^{-2} d^{-1}. Tyler et al. (2001) observed that the macroalga *U. lactuca* released DON into the water during active growth and the release was higher in the light than the dark, indicating a possible association with photosynthesis. Brylinsky (1977) also reported that DOM was released from benthic macrophytes at rates of 0.5–4.4 μmol C (g dry weight)$^{-1}$ h^{-1}. These observations suggest that not only coral colony but benthic macrophytes community constantly releases DOM to the ambient reef water. It has been observed in Shiraho Reef, i.e., the study site of Hata et al. (2002) and the present study, that seagrass beds occur up to 300 m offshore from the coast (Tanaka and Kayanne 2007).

To summarize these possibilities, POM release from coral colonies might have major contribution to the bulk organic matter in reef water relative to the DOM release. DOM found in in-situ reefs might have different sources other than coral colonies. Increasing nutrient input to coral reefs would stimulate the growth of benthic macrophytes and subsequent release of DOM, while the present study has suggested that nutrient enrichment could stimulate POM release from coral communities, rather than DOM.

5-4. Multiple effects of nutrient enrichment on coral calcification

The effect of nutrient enrichment on coral calcification was first shown by Simkiss (1964) for dissolved inorganic phosphorus (DIP). It was explained that DIP acts as a crystal poison of calcification as a compound which settles on the surface of a crystal and interferes with the continued formation of the crystal lattice. Consequently, crystal growth stops and the mother liquor for calcification may become supersaturated. The effect has been experimentally observed under much higher phosphate concentrations (e.g., 2 μmol L^{-1}; Kinsey and Davies 1979, Ferrier-Pagès et al. 2000a) than in situ levels (e.g., <0.1 μmol L^{-1}; Miyajima et al. 2007). This can be defined as a direct effect of nutrient enrichment on coral calcification.

The DIP concentration in the present study was only elevated up to 0.3 μmol L^{-1} not to induce such a direct effect on calcification. The nutrient enrichment in this study resulted in the statistical increase in both of algal photosynthesis and coral calcification but the rate of increase in calcification was much lower than that of photosynthetic organic production (Fig. 2-1). Under the higher nutrient condition than the present study, it has also been suggested that calcification rates decrease as a result of increased photosynthesis (Langdon and Atkinson 2005). The decreased calcification has been explained by the competition for the same saturated DIC pool in the coral between the two C fixation reactions (Stambler et al. 1991, Marubini and Davies 1996). Marubini and Davies (1996) observed that calcification rates decreased even in the condition of 1 μmol L^{-1} NO_3^- for one month. These effects of

inorganic nutrients on coral calcification can be defined as a competitive effect (process 1 in Fig. 5-3).

It has been considered that coral calcification is stimulated by algal photosynthesis from the observation that calcification rates are higher in light than in dark (Kawaguti and Sakumoto 1948, Goreau 1959). Suzuki et al. (1995) simulated a model of coexisting effects of photosynthesis and calcification, and showed that both reactions potentially enhance each other, considering chemical equilibrium in seawater. Therefore, not only the competitive effect, but the increase in algal photosynthetic activity also has a cooperative effect on coral calcification. Whether photosynthesis stimulates or decrease calcification would depend on the level of algal photosynthetic activity, which is a consequence of the nutrient level and the exposure duration.

Not only these effects, but other indirect effects on calcification were also implied from the results of Chapter 3 and 4. In Chapter 3, increased nutrient concentrations enhanced tissue production rates of the coral colony and resulted in the increase in POM release to the ambient seawater (process 2 in Fig. 5-3). Because the increased part of POM was rapidly mineralized by bacteria as shown in Chapter 4 (Figs. 4-6a, 4-7a), inorganic nutrients could consequently stimulate the growth rate of bacteria and subsequent larger microorganisms such as heterotrophic flagellates and ciliates (Ferrier-Pages et al. 2000b, processes 3 and 4 in Fig. 5-3). The increase in heterotrophic communities would lead to the stimulation of O_2 consumption and CO_2 release due to the respiration (processes 5 and 6 in Fig. 5-3). The increase in the partial pressure of CO_2 (pCO_2) at nighttime was actually observed by in situ measurement (Gattuso et al. 1993, Kayanne et al. 1995), which was considered to be

a result from respiration by reef organisms including coral colonies. Thus, the stimulation of POM release from coral colonies might increase pCO_2 at least at nighttime (process 7 in Fig. 5-3). At daytime, the increased pCO_2 by respiration would compensate the decreased pCO_2 by photosynthesis (processes 8 & 9 in Fig. 5-3), thus, both effects should be taken into account to consider total pCO_2 in the reef water.

The increase in pCO_2 at nighttime could lower the pH according to the opposite reaction of Eq. 2-4 (Chapter 2), and the concentration of CO_3^{2-} decreases by the opposite reaction of Eq. 2-5 (Chapter 2). It has been experimentally demonstrated that the decrease in CO_3^{2-} concentration due to the increase in pCO_2 has lowered coral daily calcification (Marubini and Atkinson 1999, Langdon and Atkinson 2005). Corals are known to perform calcification in dark (dark calcification) as well as in light, though the rate in dark was about half of that in light (Al-Horani et al. 2007). Thus, the increase in POM release from coral colonies due to nutrient enrichment might decrease dark calcification of the corals (process 10 in Fig. 5-3).

Coral skeleton generally consists of two types of micro-structures, the centers of the calcification (COCs) and fasciculi. COCs are the granular shaped crystal, and fasciculi are the fish-scale-shaped bundles emerged from the COCs. In the reef coral, it is considered that COCs are formed at night with slow calcification rate (Gladfelter 1983, Cohen et al. 2001). The decrease in dark calcification due to the increase in pCO_2 at nighttime might decline the formation rate of COCs, and consequently, possibly affect calcification rates at daytime.

5-5. Conclusions and future perspectives

In this study, C and N cycling via hermatypic corals have been investigated from the perspective of inorganic nutrient enrichment. Some significant effects of the nutrients and new pathways of organic materials have been found.

(1) Nutrient enrichment increased symbiotic algal Chl *a* per unit surface area of the coral branch, and photosynthetic C fixation rates were also enhanced with the Chl *a* density. C and N biomass of the host coral and the symbiotic algae consequently increased as time passed. On the other hand, calcification could not follow the rates of algal photosynthesis and bulk tissue growth. It has been suggested that imbalanced growth of the coral colony occurs between organic tissue and carbonate skeleton at least within the time scale of 1–2 week.

(2) POC and PON release rates from the corals increased with the nutrient enrichment, relative to the coral plus algal biomass in the colony. The result implied that excessive organic matter for the colony was released to the ambient seawater as POM. The POM contained not only zooxanthellae (ca. 10 %) but other non-living materials (>80%) such as mucus and host tissue fragments. The release rates of these two fractions were significantly correlated, suggesting that the release of zooxanthellae usually involve the other POM.

(3) Despite the nutrient enrichment and subsequent increases in Chl *a* density and algal photosynthetic rate, the release rate of DOM from the symbiotic colony was

not significantly enhanced in the present study. This could be explained by the possibility that DOM release rate per algal cell (or Chl *a*) decreased due to the increased nutrient availability. C:N ratios of the bulk DOM released from the colony were also not influenced by the nutrients. Considering that the bulk DOC contained newly produced organic C by only <10%, the DOM released from the coral might have a relatively large pool in the host tissue.

(4) The organic matter released from the coral colonies had two different fractions in bacterial degradability. Easily mineralized fraction had a turnover time of 3–9 d for both DOM and POM. The POM fraction which was released as a result of inorganic nutrient enrichment was rapidly decomposed by bacteria within 1 week, suggesting that the nutrient enrichment activates heterotrophic community in the reef ecosystems.

(5) It has been shown that the corals under stressful condition (aerial exposure) released ca. 10 times more DOM than that in normal submerged condition. The massively released DOM contained labile organic matter at higher % than the normal DOM. These results implied that stressful conditions for coral colonies also make the flux of organic matter to heterotrophic community enhanced, as caused by nutrient enrichment.

(6) A part of the organic matter released from the corals was not mineralized by bacteria for >3 months. This organic matter could be exported from the coral reef ecosystem to the outer ocean and preserved for a longer period. Some

possibilities were proposed for the undegradable organic matter: (a) the organic matter was originally recalcitrant to bacterial decomposition, (b) bacteria altered the chemical structure of the initial organic matter to different forms, (c) the bacterial community around the coral surface area was very limited, i.e., other bacteria might be able to decompose the organic matter. It should be resolved in the future what made the organic matter to be remained for such a long time.

In Chapter 2 and 3, the experiment has revealed short-term (a few weeks) response of the corals to the increased nutrient enrichment. In other words, the corals could respond to nutrient loading, even if the nutrient input was temporary (e.g., typhoons, rainy seasons). However, it remains a matter of concern how long the coral colony continues to take up inorganic nutrients and to respond as the ways observed in the present study. Longer-term experiments will clarify what is the steady state for the corals under a nutrient-enriched condition.

Table 5-1 Comparison of metabolic rates between reef scales and coral community scales. P_g: gross primary production. R: respiration. P_n: net primary production. The metabolic rates were normalized to projected coral reef area (mmol C m^{-2} d^{-1}).

References	Study targets	P_g	R	P_n	P_g:R
Reef scale					
Atkinson & Falter (2003)	Entire Reef flat	640 (330-1580)	600 (290-1250)	40 (-220-310)	1.07
	Algal pavement	460 (170-580)	300 (40-560)	160 (0-130)	1.5
	High coverage	1180 (660-1920)	1280 (500-2000)	-100 (-830-250)	0.92
	Sandy areas	130 (80-230)	130 (90-200)	0 (-40-30)	1.0
	Shallow lagoon	450 (210-1080)	430 (180-790)	20 (-200-280)	1.05
	Entire Reef systems	390 (190-640)	370 (190-570)	20 (0-70)	1.05
Gattuso et al. (1998)	Coral/algal reef flats	79-584	76-538		1.07
	Algal-dominated area	30-1369	6-910		
				110	
Coral community scale					
This study	*Acropora pulchra*	410	250	160	1.7
Bythell (1988)	*Acropora palmata*				1.4
Edmunds and Davies (1986)	*Porites porites*				2.1

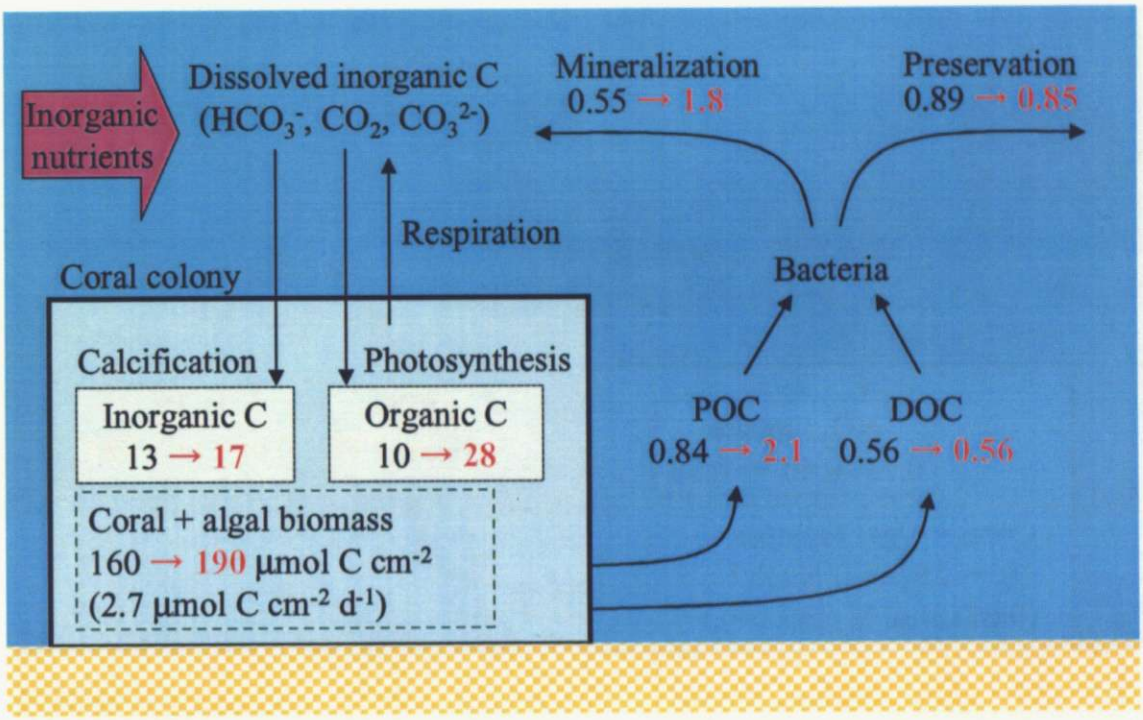

Fig. 5-1 Summary of C fluxes obtained in the present study. The data are normalized to unit surface area of the coral branch (μmol C cm^{-2} d^{-1}). The changes shown in red numbers mean the effects of nutrient enrichment within the range of the present study.

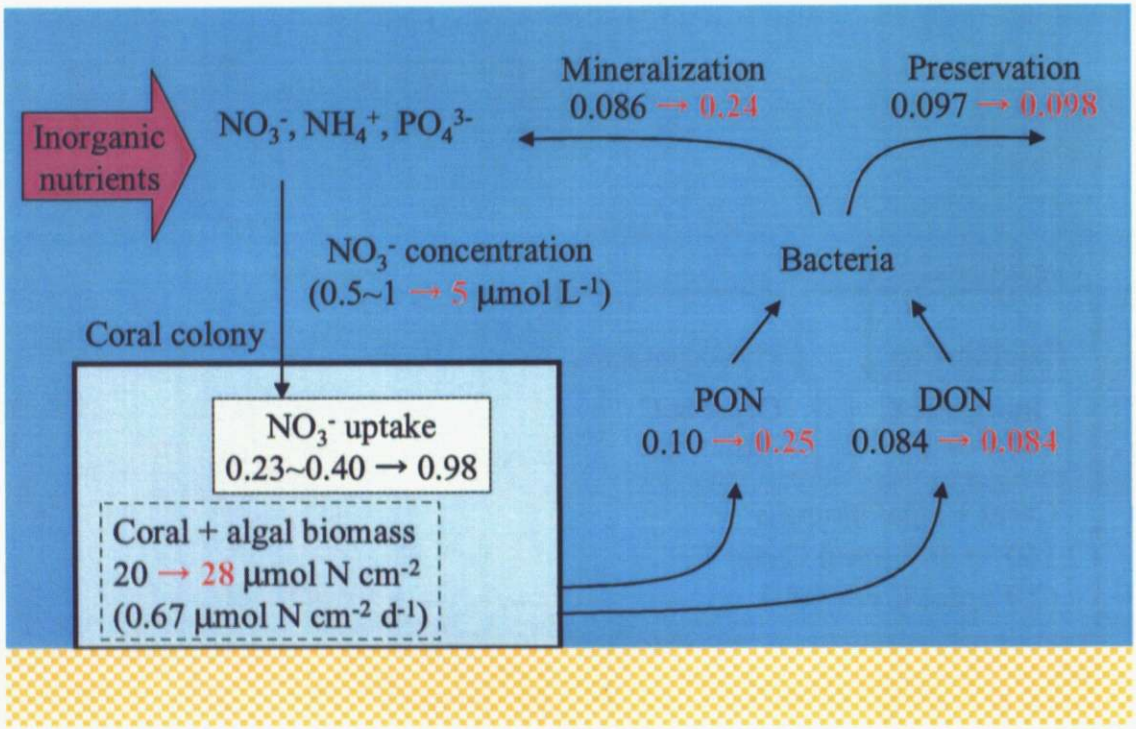

Fig. 5-2 Summary of N fluxes obtained in the present study. The data are normalized to unit surface area of the coral branch (μmol N cm^{-2} d^{-1}). The changes shown in red numbers mean the effects of nutrient enrichment within the range of the present study.

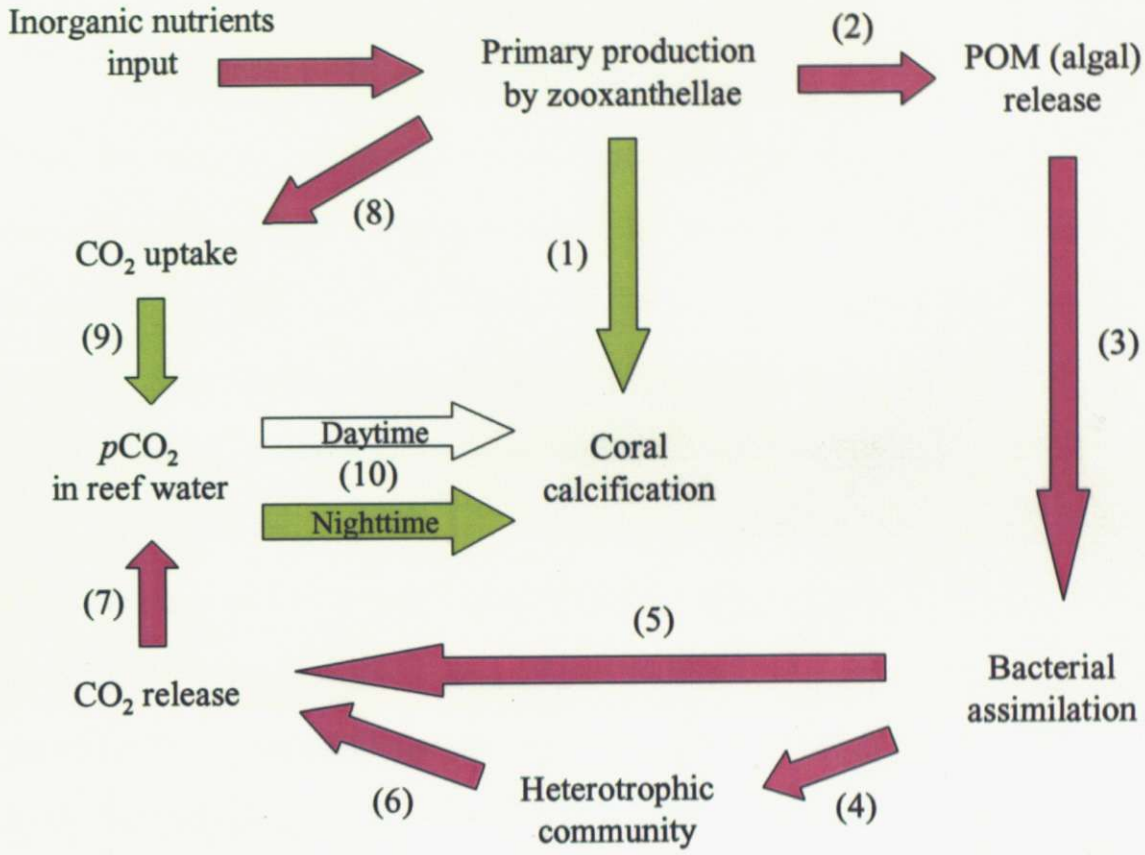

Fig. 5-3 Effects of nutrient enrichment on coral calcification and reef ecosystems. Positive effects are shown in pink arrows and negative effects are shown in green.

(1) Competitive effects of inorganic nutrients on coral calcification.

(2) Increase in POM release from coral colonies (Chapter 3).

(3) Mineralization of the released POM by bacteria (Chapter 4).

(4) Organic matter fluxes from bacteria to higher heterotrophic community.

(5) & (6) Respiratory CO_2 release.

(7) The potential to increase partial pressure of CO_2 (pCO_2)

(8) Increase in CO_2 uptake by photosynthesis.

(9) The potential to decrease pCO_2.

(10) Decrease in calcification at nighttime due to increased pCO_2. The response at daytime would be determined by the balance between processes (7) and (9).

Acknowledgements

I would like to express my gratitude to Associate Professor H. Ogawa, Ocean Research Institute (ORI), The University of Tokyo, for valuable discussion throughout this study. I would also like to my appreciation to Dr. T. Miyajima (Research Associate, ORI, The University of Tokyo), who wholly supported my study with a lot of profound advices, and to Dr. I. Koike (University of the Ryukyus), who also gave me valuable advices as a former professor in my laboratory.

In the field study at Ishigaki Island, I would like to thank Dr. T. Hayashibara and other many staff in Seikai National Fisheries Research Institute, who supported me in the field surveys and laboratory experiments for many years. I would also feel grateful to Dr. Y. Umezawa (Research Institute for Human and Nature), Dr. Y. Tanaka (ORI, The University of Tokyo), Dr. H. Fukuda (ORI, The University of Tokyo), and Y. Tsuboi (Master of environmentology, Chemicals Evaluation and Research Institute) for supporting my sampling and experiments in the field. I also thank other colleagues in my laboratory for helping my experiments.

This study was financially supported by the Sasakawa Scientific Research Grant from the Japan Science Society and by Research Fellowships of the Japan Society for the promotion of Science for Young Scientists.

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