Biogeosciences Discuss., 11, 2973–2991, 2014
www.biogeosciences-discuss.net/11/2973/2014/
doi:10.5194/bgd-11-2973-2014

Biogeosciences Discussions
Open Access

An experimental study on the effects of nutrient enrichment on organic carbon storage in western Pacific oligotrophic gyre

J. Liu[1,2], **N. Jiao**[1,2], **and K. Tang**[1,2]

[1]State Key Laboratory of Marine Environmental Sciences, Xiamen University, Xiamen 361005, China
[2]Institute of Marine Microbes and Ecospheres, Xiamen University, Xiamen 361005, China

Received: 31 January 2014 – Accepted: 4 February 2014 – Published: 21 February 2014

Correspondence to: N. Jiao (jiao@xmu.edu.cn)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Carbon sequestration in the ocean is of great concern with respect to the mitigation of global warming. How to hold the fixed organic carbon in the presence of tremendous heterotrophic microorganisms in marine environments is the central issue. We have previously hypothesized that excessive nutrients would ultimately decrease the storage of organic carbon in marine environments. To test it out, a series of in situ nutrient enrichment incubation experiments were conducted at a site (17.59° N, 127.00° E) within the Western Pacific oligotrophic gyre. Five treatments were employed: glucose or algal exudation organic material (EOM) and nitrate and phosphate were added alone or in combination to approximate final concentrations of 10 μmol C kg^{-1}, 1 μmol N kg^{-1} and 0.11 μmol P kg^{-1} respectively. The results showed that the dissolved organic carbon (DOC) consumption rates and bacterial community specific growth rates were enhanced by inorganic nutrients enrichment treatments during the initial 48 h incubation. At the end of 14 days incubation, about 1/3 (average 3.29 μmol C kg^{-1}) more organic carbon was respired from the glucose enriched incubation with addition of inorganic nutrients compared to that without addition of inorganic nutrients. In the case no essential nutrients were available, even glucose could not be efficiently used by bacteria and thus remained in the environment. These results suggest that repletion of inorganic nutrients has negative impacts on carbon preservation, presumably due to elevated nutrient-stimulated bacterial metabolism and respiration, which is meaningful for potential coastal water management and worth for further studies.

1 Introduction

Dissolved organic carbon (DOC) in the ocean, as one of the largest active carbon reservoirs on the earth, is comparable to the entire atmospheric CO_2 reservoir (~ 750 Gt; Hedges, 1992; Ogawa and Tanoue, 2003) and plays an important role in global carbon cycling and climate change. Due to the critical role of the ocean in global carbon

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cycling, a great deal of research efforts have been put on the processes and mechanisms involved in DOC dynamics, including production, consumption, and long-term storage of DOC etc. Recalcitrant dissolved organic carbon (RDOC), which comprises the largest portion of the bulk DOC reservoir in the ocean, can persist for thousands of years in the water column (Blitz, 1992). Therefore, how RDOC is produced and stored is the key for understanding DOC dynamics and has been one of the hot topics among biogeochemists for more than a decade (Søndergaard et al., 2000; Kragh and Søndergaard, 2004, 2009; Eichinger et al., 2009). Recently, a new conceptual framework, the microbial carbon pump (MCP), was proposed to address the processes and mechanisms involved in the sources of RDOC (Jiao et al., 2010). In the MCP framework, microorganisms that are primarily responsible for the decomposition and remineralization of organic matter, in an ironic twist, are considered to play a critical role in bio-sequestration of organic carbon. The rationale lies in at least two aspects: the microbial production of RDOC compounds and constrains of microbial consumption of DOC. While the former is well understood, the later remain unraveled due to lack of systematic studies. One of the critical issues is whether or not nitrogen (N) and phosphorus (P) nutrients enrichment is positive or negative for carbon preservation in the marine environment (Zweifel et al., 1993; Carlson et al., 2002; Gasol et al., 2009).

Each day, more than one hundred million tons of CO_2 is fixed through phytoplankton photosynthesis in the ocean (Behrenfeld, O'Malley et al., 2006). It is generally considered that enhancement of inorganic nutrients could result in enhancement of primary production (Falkowski et al., 1998), subsequently leading to enhanced DOC release in seawater (Biddanda and Benner, 1997; Hansell and Carlson, 1998). It is also reported in the field that nutrient supplementation in late spring could stimulate phytoplankton blooms, and then increase the DOC concentration in northwestern Sargasso Sea (Carlson et al., 1994). However, usually only less than 15 % of total primary production, in the form of particulate organic carbon, could be exported out of the euphotic zone and only 0.1 % of total primary production could reach the seafloor for long term carbon storage (Houghton, Ding et al., 2001). Obviously, the amount of carbon storage in the

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ocean interior is quite different from the amount fixed in the euphotic zone. Enhancement of nutrients may not necessarily lead to enhancement of carbon storage. In fact, high nutrient input waters like the Pearl River estuary can be sources rather than sinks of CO_2 (Yuan et al., 2010). Therefore, it was proposed that the mobilization of DOC by microbial respiration, as a result of enhanced nutrient input in coastal waters, may have negative impacts on carbon sequestration and storage (Jiao et al., 2010b). To test this point, a set of in situ experiments was conducted in oligotrophic seawater with supplemented inorganic nutrients (N and P) and organic carbon (glucose or EOM), alone or in combination, in ten microcosms. In the oligotrophic seawater, the low level of nutrients was comparatively constant, for securing that the response of nutrients enrichment on carbon storage was adequately sharp to be detected clearly. This is an advantage over the coastal water where the chemical composition and source of DOC were so myriad that they could profoundly influence its bioavailability (Coble et al., 1990; Cabaniss and Shuman, 1987). The objective of this study was to test the hypothesis that excess inorganic nutrient enrichment (N and P) would decrease storage of organic carbon in the ocean.

2 Materials and methods

2.1 Experimental design and sampling

Seawater was collected from a depth of 75 m in the western Pacific Ocean (17.59° N, 127.00° E), using a rosette sampler with conductivity–temperature–depth (CTD) on 27 November 2012. The experiment was carried out with ten microcosms (polycarbonate bottles, 20 L) which were pre-acid washed and sample water rinsed. The filter system was pre-cleaned with copious ultra-pure water and seawater in turn to minimize carbon contamination. Each microcosm was filled with 20 L seawater pre-filtered through 3 µm filter. Treatments were amended with organic carbon sources and inorganic nutrient as described in Table 1. Each treatment was conducted in replicates. The microcosms

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were incubated at 28 ± 0.5 °C in darkness. Cultures were sampled at hour 0, 3, 6, 12, 24, 36, 48, 96, 168 and 336, and water samples were stored at −20 °C until analysis.

2.2 Algal culture and dissolved organic material extraction

Axenic culture of *Phaeodactylum triconutum* was incubated in f/2 medium with artificial seawater under a photon flux of 112 µE m^{-2} s^{-1} and light/dark cycle (10 h/14 h), with a temperature of 20 ± 0.5 °C. To separate the medium from algal cells, the culture was filtered through 3 µm filter when stationary growth phase was reached. The algal exudation organic material (EOM) was extracted from the medium using solid phase extraction (SPE) cartridges (PPL, 1 g, Agilent, Bond Elut) according to Dittmar et al. (2008). The algal filtrate was acidified with HCl (final pH = 2) prior to passing through the cartridges. Ultrapure water (also acidified to pH = 2 with HCl) was then used to remove excess salt from the cartridges. EOM was eluted with methanol into pre-combusted glass vials (40 mL) after the sorbent was dried with nitrogen gas. EOM was stored at −20 °C until the evaporation of methanol by nitrogen gas had been achieved. Before being transferred to the microcosms, EOM was re-dissolved within 10 mL ultra-pure water.

2.3 Total dissolved organic carbon (TOC) analysis

Total dissolved organic matter was measured by high temperature combustion method using Shimadzu TOC-V CPH TOC analyzer. To avoid potential organic carbon contamination, filtration procedure was not applied in sampling. Consequently, bacterial biomass carbon was not ruled out and only contributed for less than 1 % of TOC (Carlson and Ducklow, 1996). The samples were collected in glass vials (40 mL) with glass pipets. All the glass apparatus, employed in sampling, were pre-acid cleaned and combusted (500 °C, 6 h). Water samples were then acidified to pH = 2 with H_3PO_4 and stored at −20 °C until analysis. TOC measurement was according to Julie Callahan

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et al. (2004). Culture samples were taken at hour 0, 3, 6, 12, 24, 36, 48, 96, 168 and 336.

In this study, bacteria were considered as particulate organic carbon (POC) in the microcosm culture. POC concentration was estimated from bacterial abundance and bacterial carbon conversion factor (CCF), which was assumed to be 20 fg C cell^{-1} based on the natural planktonic assemblage (Lee and Fuhrman, 1987). POC concentration could therefore be calculated by the following equation:

$$\text{POC concentration} = \text{Bacterial abundance} \times \text{CCF} \quad (1)$$

Dissolved organic carbon (DOC) was defined as total dissolved organic carbon (TOC) which did not include POC (bacterial biomass carbon). Therefore, DOC concentration was calculated as:

$$\text{DOC concentration} = \text{TOC concentration} - \text{POC concentration} \quad (2)$$

In theory, the organic carbon decrease in the incubation system was the overall consequence of bacterial biomass carbon (POC) increase and bacterial respiration consumption which is comparable to observed net TOC reduction (Carlson et al., 1999). Therefore, bacterial respiration rate was estimated as:

$$\text{Bacterial respiration rate} = \Delta\text{TOC concentration}/\Delta t \quad (3)$$

2.4 Bacterial abundance (BA) analysis and specific growth rate

The culture was sampled at hour 0, 3, 6, 12, 24, 36, 48, 96, 120, 144, 168 and 336 respectively. Samples were fixed with glutaraldehyde to final concentration 1 % (Vaulot et al., 1989) and frozen in liquid nitrogen prior to storage at −80 °C. Bacteria were stained with SYBR green I (Marie et al., 1997) before flow cytometer analysis (Becton–Dickinson), and the autotrophs were run separately without being stained (Jiao et al., 2005).

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Specific growth rate was measured in 0–48 h and 120–168 h and calculated as follows:

$$\text{Specific growth rate} = \ln(\Delta BA)/\Delta t \quad (4)$$

Where, ΔBA represents the observed net change in bacterial abundance.

2.5 Analysis of dissolved inorganic nutrient concentration

The concentrations of dissolved inorganic nutrients in the samples were measured by Technicon AA3 Auto-Analyzer (Bran-Lube, GmbH). The copper-cadmium reduction method was employed to determine the dissolved inorganic nitrogen concentrations (DIN, nitrate and nitrite) of each sample. The spectrophotometric method was employed to determine dissolved inorganic phosphorus concentration (DIP) (Knap et al., 1996). The detection limits for DIN and DIP were 0.1 $\mu mol\,kg^{-1}$ and 0.08 $\mu mol\,kg^{-1}$ respectively.

3 Results and discussion

3.1 Dynamics of bacterial abundance and growth rate

In general, bacterial abundance (BA) in all treatments showed similar growth pattern transitioning through lag, logarithmic and stationary phases but with different levels of the maximum abundance (Fig. 1). Compared to the control, the most pronounced differences were observed in Glu +N + P treatment, followed by EOM and N + P treatments. In contrast, the Glu treatment did not make much difference from the control. The specific growth rates during 0–48 h of all the treatments, except for Glu treatment, were significantly higher than that of the control (ANOVA test, $p < 0.05$) (Table 2). The results from the Glu treatment that no/slight bacterial response to enrichment of glucose, a labile DOC source, seemed to be unreasonable apparently, but such situations

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do exist in oligotrophic oceans as reported in the Sargasso Sea (Carlson et al., 1996). These results actually suggest a case of nutrient rather than carbon limiting for the microbial community. In our study, the bacterial specific growth rate in N + P treatments was significantly higher than that in the control (ANOVA test, $p < 0.05$) (Table 2), and the same result was obtained for BA at 48 h (Fig. 1), indicating that inorganic nutrient addition could stimulate bacterial growth in logarithmic phase. This is consistent with the results by Carlson et al. (2002) from the Sargasso Sea (2 days, 0.08 cell L^{-1} d^{-1} and 0.06 cell L^{-1} d^{-1}). When Glu met with N + P, it made the most robust difference, where BA reached 1.25×10^6 cells ml^{-1} at 48 h, which was the highest value among all the treatments, with specific growth rates of 1.8 to 2.5 fold that of N + P treatments during the 0–48 h incubation (Fig. 1 and Table 2). These results demonstrated that even such labile DOC as glucose could not be used by microbes and left over in the environment if no essential nutrients are available. On one hand, it means that bacteria growth not only requires organic carbon but also requires inorganic nutrients; It could be possible for a labile DOC molecule to become semi-labile or instantly refractory to bacteria if nutrients are not available. Then an argument could be raised here: are inorganic nutrients essential for bacteria? The results of our EOM treatment incubations (without supplementary inorganic nutrients) showed that, BA and specific growth rate were significantly higher than the control and even other treatments (except for Glu +N + P treatment) during the 0–48 h period. Since EOM must contain nitrogen, phosphorus and other elements, it is to say, elemental balance is a key for bacterial growth and abundance. In the case labile organic matters that contain diverse elements are not enough, inorganic nutrients could be substituted to meet the bacterial elemental demand for growth in the oligotrophic ocean.

3.2 Dynamics of inorganic nutrients

Dissolved inorganic nutrients (N and P) concentrations in non-nutrient enrichment treatments (Glu, EOM and control treatments) were all below detection limits (Fig. 2a and b). For the nutrients (N and P) enriched treatments, the variation of nutrients con-

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centrations during the incubation time course were different between Glu + N + P and N + P treatments. In the Glu + N + P treatment, after a lag phase (0–12 h), dissolved inorganic nitrogen (DIN) decreased rapidly from 1.45 to 0.77 $\mu mol\,N\,kg^{-1}$ during the 12–48 h period, and then maintained a low level around 0.66 $\mu mol\,N\,kg^{-1}$ during the 48–336 h period (Fig. 2a). In contrast, DIN in the N + P treatment showed a prolonged gradual reduction over the 0–336 h incubation time course, decreasing from 1.54 to 1.13 $\mu mol\,N\,kg^{-1}$ in total (Fig. 2a). Moreover, in the Glu + N + P treatment, dissolved inorganic phosphate (DIP) was rapidly consumed during the first six hours (0.11 to 0.09 $\mu mol\,P\,kg^{-1}$) and reduced down to below detection limit within 12 h (Fig. 2b). In the N + P treatment however, DIP decreased slowly from 0.11 to 0.09 $\mu mol\,P\,kg^{-1}$, during 0–72 h, and then suddenly dropped down to below detect limit at the 96 h (Fig. 2b). The difference in nutrient uptake patterns between Glu + N + P and N + P treatments suggest that inorganic nutrients uptake could be significantly enhanced by labile organic carbon (e.g. glucose) enrichment.

3.3 Dynamics of TOC and DOC concentrations

TOC concentrations in all treatments and the control were monitored during the entire incubation time course (336 h) to check the differences in consumption of organic carbon between the treatments. Generally, TOC concentrations decreased rapidly in the initial 48 h and then became relatively stable during the later hours (48–336 h) at different levels in different treatments (Fig. 3). The fraction of DOC consumed in the first 48 h accounted for 8 % to 13 % of the initial bulk DOC varying with different treatments (Table 2). Glu + N + P treatment showed the highest DOC consumption rate (0.232 $\mu mol\,C\,kg^{-1}\,h^{-1}$) among all the treatments, most likely due to the combined effects of nutrient and organic carbon enrichment. DOC consumption rates in the N + P treatment ranked the second highest (0.170 $\mu mol\,C\,kg^{-1}\,h^{-1}$), indicating that nutrient enrichment did stimulate uptake of organic carbon as suggested before (Jiao et al., 2011). The DOC consumption rate in the EOM treatment (0.163 $\mu mol\,C\,kg^{-1}\,h^{-1}$) was similar to the N + P treatment. But the mechanism behind the DOC utilization could

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be quite different. EOM provided not only organic carbon but also other elements including N and P that bacteria demand. In contrast if only organic carbon is supplied, like the case of Glu treatment, bacteria may not be able to use much carbon ($0.141\,\mu mol\,C\,kg^{-1}\,h^{-1}$, similar to the control, $0.134\,\mu mol\,C\,kg^{-1}\,h^{-1}$) due to elemental limitation by N and P (Table 2). A number of studies have shown that algal excretion is a complex mixture of organic material, comprised largely of polysaccharides, other small nitrogenous compounds, lipids, vitamins, etc. (Goldman et al., 1992; Myklestad et al., 1989; Mague et al., 1980). Therefore EOM could be more efficient than glucose alone for bacterial growth in oligotrophic waters. It is noteworthy that while DOC consumption rate in EOM and N + P treatments are similar, bacterial respiration rates in the two treatments are quite different (Table 2), which is likely due to that more energy is needed for synthesis of biomass (protein etc.) with inorganic nutrients. Among all the treatments, the highest bacterial respiration rates were observed in Glu + N + P treatment, contrasting to the lowest in the Glu treatment which was actually the same as the control. Taken together DOC consumption and bacterial respiration, it is clear that inorganic nutrient could stimulate bacterial respiration and lead to more carbon consumption in the same organic carbon availability scenario.

In terms of carbon storage/sequestration in the ocean, the less organic carbon could be used by microbes, the more organic carbon would be left over in the environment. Compared to Glu treatment, more than $3.29\,\mu mol\,C\,kg^{-1}$ TOC on the average was consumed in the Glu + N + P treatment after 336 h incubation. This suggested that inorganic nutrients enrichment was not beneficial for organic carbon preservation as proposed by Jiao et al. (2011); and that the bio-availability and lability of one organic carbon compound can be situation-specific. Therefore, the term RDOC can be refined as deep ocean RDOC which has been generally referred by chemists, and situational RDOC which means it can hold refractory under certain conditions but may become bioavailable when the conditions change (such as nutrient enriched). Such situations actually exist in the oceans. For example, the highest DOC concentration among the world

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oceanic waters located in the South Ocean Gyre (Hansell et al., 2009) where nutrients are limiting, and stratifications are strong.

4 Conclusions

It is generally known that enrichment of inorganic nutrients would increase carbon fixation, but it is not necessarily true for carbon preservation in the environment. Our in situ incubation experiments in the western Pacific gyre showed that nitrate and phosphate addition stimulated organic carbon consumption and bacterial respiration and ultimately resulted in reduction of organic carbon storage. On the other hand, if nitrogen and phosphorus are limiting (oligotrophic case), even if in the presence of labile pure carbon DOC molecules like glucose, bacteria may not grow efficiently. In contrast, natural labile organic matters like EOM, containing multiple elements, would be easily used by bacteria for growth in more proportion relative to respiration. These recognitions are useful for interpretations of some paradoxes such as why turbid estuarine waters with rich inorganic nutrients are often sources rather than sinks of CO_2, and thus can be referred for coastal water managements regarding to ecological health and carbon sequestration. Further studies are desired to explore the thresholds of nutrient concentrations or ratios that are most favorable for shifting the carbon cycle equilibrium towards organic carbon storage in marine environments.

References

Behrenfeld, M. J., O'Malley, R. T., Siegel, D. A., McClain, C. R., Sarmiento, J. L., Feldman, G. C., Milligan, A. J., Falkowski, P. G., Letelier, R. M., and Boss, E. S.: Climate-driven trends in contemporary ocean productivity, Nature, 444, 752–755, 2006.

Biddanda, B. and Benner, R.: Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton, Limnol. Oceanogr., 42, 506–518, 1997.

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Blitz, L.: ^{14}C activity of dissolved organic carbon fractions in the north-central Pacific and Sargasso Sea, Nature, 357, 667–670, 1992.
Cabaniss, S. E. and Shuman, M. S.: Synchronous fluorescence spectra of natural waters: tracing sources of dissolved organic matter, Mar. Chem., 21, 37–50, 1987.
Carlson, C. A. and Ducklow, H. W.: Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea, Aquat. Microb. Ecol., 10, 69–85, 1996.
Carlson, C. A., Ducklow, H. W., and Michaels, A. F.: Annual flux of dissolved organic carbon from the euphotic zone in the northwestern Sargasso Sea, Nature, 371, 405–408, 1994.
Carlson, C. A., Bates, N. R., Ducklowz, H. W., and Hansell, D. A.: Estimation of bacterial respiration and growth, Aquat. Microb. Ecol., 19, 229–244, 1999.
Carlson, C. A., Giovannoni, S. J., Hansell, D. A., Goldberg, S. J., Parsons, R., Otero, M. P., Vergin, K., and Wheeler, B. R.: Effect of nutrient amendments on bacterioplankton production, community structure, and DOC utilization in the northwestern Sargasso Sea, Aquat. Microb. Ecol., 30, 19–36, 2002.
Coble, P. G., Green, S. A., Blough, N. V., and Gagosian, R. B.: Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy, Nature, 348, 432–435, 1990.
Dittmar, T., Koch, B., Hertkorn, N., and Kattner, G.: A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater, Limnol. Oceanogr.-Meth., 6, 230–235, 2008.
Eichinger, M., Kooijman, S., Sempere, R., Lefèvre, D., Gregori, G., Charrière, B., and Poggiale, J.-C.: Consumption and release of dissolved organic carbon by marine bacteria in a pulsed-substrate environment: from experiments to modelling, Aquat. Microb. Ecol., 56, 41–54, 2009.
Falkowski, P. G., Barber, R. T., and Smetacek, V.: Biogeochemical controls and feedbacks on ocean primary production, Science, 281, 200–206, 1998.
Gasol, J. M., Vázquez-Domínguez, E., Vaqué, D., Agustí, S., and Duarte, C. M.: Bacterial activity and diffusive nutrient supply in the oligotrophic Central Atlantic Ocean, Aquat. Microb. Ecol., 56, 1–12, 2009.
Goldman, J. C., Hansell, D. A., and Dennett, M. R.: Chemical characterization of three large oceanic diatoms: potential impact on water column chemistry, Mar. Ecol.-Prog. Ser., 88, 257–270, doi:10.3354/meps088257, 1992.
Hansell, D. A. and Carlson, C. A.: Net community production of dissolved organic carbon, Global Biogeochem. Cy., 12, 443–453, 1998.

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Hansell, D. A., Carlson, C. A., Repeta, D. J., and Schlitzer, R.: Dissolved organic matter in the ocean: a controversy stimulates new insights, Oceanography, 22, 52–61, 2009.
Hedges, J. I.: Global biogeochemical cycles: progress and problems, Mar. Chem., 39, 67–93, 1992.
Houghton, J., Ding, Y., Griggs, D., Noguer, M., Van der Linden, P., Dai, X., Maskell, K., and Johnson, C.: IPCC, 2001: Climate Change 2001: The Scientific Basis, Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge, United Kingdom, New York, USA, Cambridge University Press, 2001.
Jiao, N., Yang, Y., Hong, N., Ma, Y., Harada, S., Koshikawa, H., and Watanabe, M.: Dynamics of autotrophic picoplankton and heterotrophic bacteria in the East China Sea, Cont. Shelf. Res., 25, 1265–1279, 2005.
Jiao, N., Herndl, G. J., Hansell, D. A., Benner, R., Kattner, G., Wilhelm, S. W., Kirchman, D. L., Weinbauer, M. G., Luo, T., Chen, F., and Azam, F.: Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean, Nat. Rev. Microbiol., 8, 593–599, doi:10.1038/nrmicro2386, 2010a.
Jiao, N., Tang, K., Cai, H., and Mao, Y.: Increasing the microbial carbon sink in the sea by reducing chemical fertilization on the land, Nat. Rev. Microbiol., 9, 75–75, 2010b.
Knap, A., Michaels, A., Close, A., Ducklow, H., and Dickson, A.: Protocols for the joint global ocean flux study (JGOFS) core measurements, JGOFS Report No. 19, Reprint of the IOC Manuals and Guides No. 29, UNESCO, 1996.
Kragh, T. and Søndergaard, M.: Production and bioavailability of autochthonous dissolved organic carbon: effects of mesozooplankton, Aquat. Microb. Ecol., 36, 61–72, 2004.
Kragh, T. and Søndergaard, M.: Production and decomposition of new DOC by marine plankton communities: carbohydrates, refractory components and nutrient limitation, Biogeochemistry, 96, 177–187, 2009.
Lee, S. and Fuhrman, J. A.: Relationships between biovolume and biomass of naturally derived marine bacterioplankton, Appl. Environ. Microb., 53, 1298–1303, 1987.
Mague, T., Friberg, E., Hughes, D., and Morris, I.: Extracellular release of carbon by marine phytoplankton; a physiological approach, Limnol. Oceanogr., 25, 262–279, 1980.
Marie, D., Partensky, F., Jacquet, S., and Vaulot, D.: Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I, Appl. Environ. Microb., 63, 186–193, 1997.

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Myklestad, S., Holm-Hansen, O., Vårum, K. M., and Volcani, B. E.: Rate of release of extracellular amino acids and carbohydrates from the marine diatom *Chaetoceros affinis*, J. Plankton Res., 11, 763–773, 1989.

Ogawa, H. and Tanoue, E.: Dissolved organic matter in oceanic waters, J. Oceanogr., 59, 129–147, 2003.

Søndergaard, M., Borch, N. H., and Riemann, B.: Dynamics of biodegradable DOC produced by freshwater plankton communities, Aquat. Microb. Ecol., 23, 73–83, 2000.

Vaulot, D., Courties, C., and Partensky, F.: A simple method to preserve oceanic phytoplankton for flow cytometric analyses, Cytometry, 10, 629–635, 1989.

Yuan, X., Yin, K., Harrison, P. J., Cai, W.-J., He, L., and Xu, J.: Bacterial production and respiration in subtropical Hong Kong waters: influence of the Pearl River discharge and sewage effluent, Aquatic Microb. Ecol., 58, 167–179, 2010.

Zweifel, U. L., Norrman, B., and Hagstrom, A.: Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients, Mar. Ecol.-Prog. Ser., 101, 23–23, 1993.

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Table 1. Initial treatments of organic carbon and inorganic nutrient enrichment of the incubations.

Treatments	Carbon amendment		Inorganic nutrient amendment	
	Glucose ($\mu mol\,C\,kg^{-1}$)	EOM ($\mu mol\,C\,kg^{-1}$)	Inorganic nitrogen ($\mu mol\,N\,kg^{-1}$)	Inorganic phosphate ($\mu mol\,P\,kg^{-1}$)
Glu + N + P	≈ 10	–	≈ 1	≈ 0.11
Glu	≈ 10	–	–	–
EOM	–	≈ 10	–	–
N + P	–	–	≈ 1	≈ 0.11
Control	–	–	–	–

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Table 2. The DOC consumption rate, bacterial community specific growth rate, bacterial abundance (BA) and bacterial respiration rate (BR) in the incubation experiments. DOC consumption rate was estimated from the absolute value of the slope of the linear regression on all collected data of DOC concentration during 0–48 h. Bacterial respiration rate was estimated from the observed net TOC concentration reduction in corresponding incubation time (0–48 h).

Treatments	DOC consumption rate (0–48 h)	Specific growth rate (h^{-1})		BA (336 h) (10^5 cell mL^{-1})	BR ($\mu mol\,C\,kg^{-1}\,d^{-1}$)
		0–48 h	120–168 h		
Glu +N + P	0.232 ± 0.038	0.022 ± 0.0008	0.005 ± 0.0005	12.82 ± 0.51	5.46 ± 0.19
Glu	0.141 ± 0.021	0.009 ± 0.0002	–	6.30 ± 0.06	3.45 ± 0.58
EOM	0.163 ± 0.028	0.015 ± 0.0003	0.006 ± 0.0008	8.94 ± 0.26	3.50 ± 0.88
N + P	0.170 ± 0.022	0.012 ± 0.0005	–	7.43 ± 0.17	4.03 ± 0.27
Control	0.134 ± 0.024	0.008 ± 0.0003	–	5.88 ± 0.37	3.46 ± 0.46

– Non measurement during 120–168 h due to no obvious bacterial abundance enhancement.
Data were mean ± SE (standard error).

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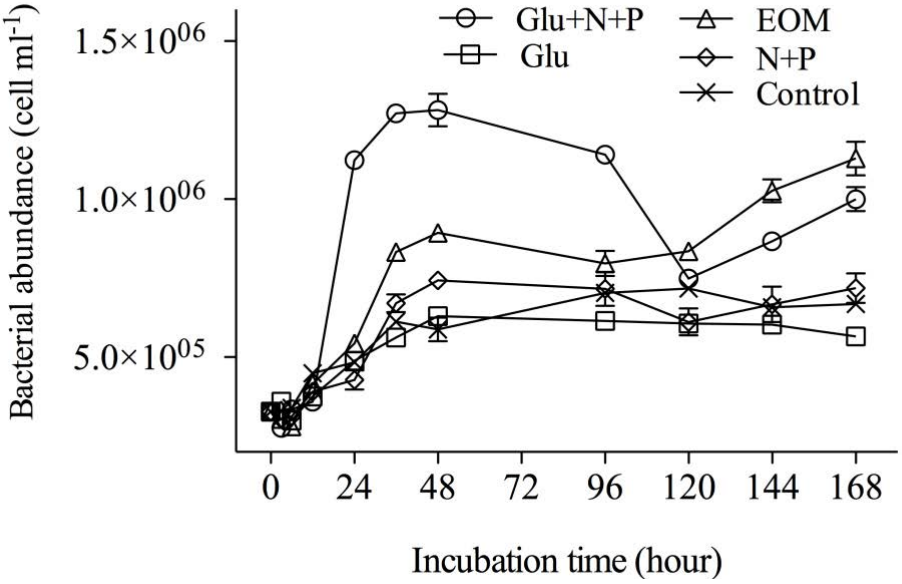

Fig. 1. Variations of bacterial abundance during the incubation time course of various treatments. Error bars indicate standard errors.

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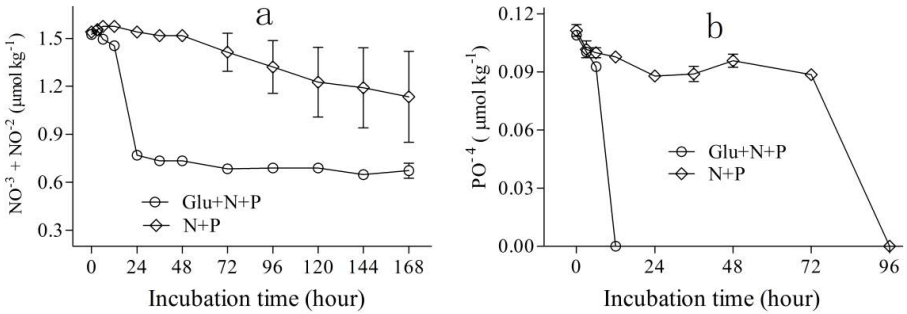

Fig. 2. Variations of dissolved inorganic nitrogen and phosphate concentrations during the incubation time course of the nutrient-enriched treatments. (Non-inorganic-nutrient-enrichment treatments were not shown due to below detected limits.) Value "0" in **(b)** means below detected limits. Error bars indicate standard errors.

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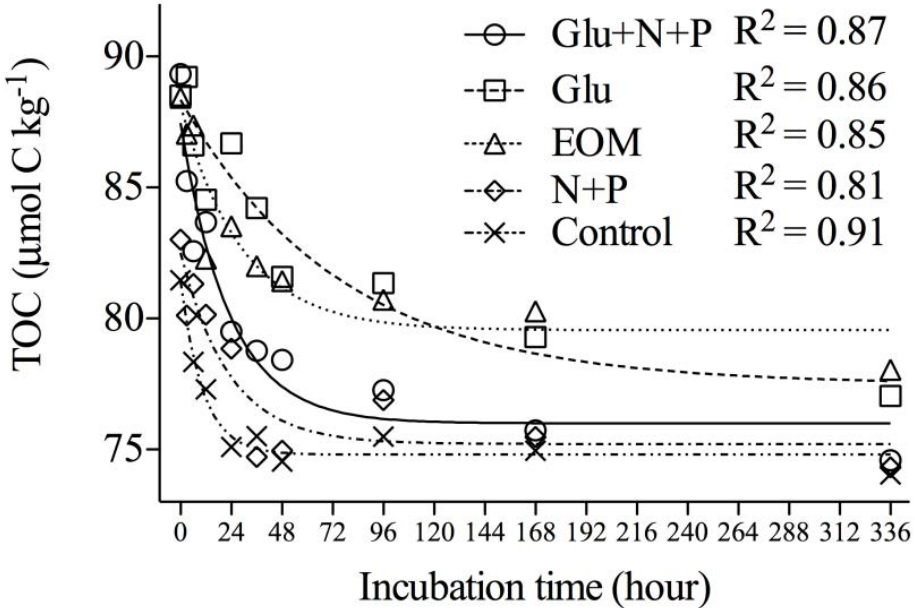

Fig. 3. Variations of TOC concentrations during the incubation time course of various treatments. The non-linear regression lines were obtained from the corresponding TOC concentration observations during the entire incubation. Symbols shown in the figure were the mean values of TOC concentration of each sampling point.

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