Responses to reviewers' comments

We thank the anonymous reviewers for the comments on our manuscript. The comments have been fully considered and responded as below.

Anonymous Referee #1

Referee #1 -1. General comments: The paper addresses the consumption of DOC by bacterioplankton in the western Pacific gyre in 20L microcosm experiments. Several treatments were established with glucose or algal exudates with and without nitrate and phosphate. The authors found that nitrate and phosphate additions led to a higher consumption of DOC (glucose-treatment) than without N+P additions. This conclusion has been reached before in similar experiments (see Malfunction of the microbial loop-Thingstad, L&O and numerous other papers). Hence, the finding presented here is not new.

Response: We did not find the same conclusion that enrichment of inorganic nutrients lead to a higher DOC consumption and less DOC stored in the water in the mentioned paper (Thingstad *et al.*, 1997 about malfunctioning microbial loop).

In another Thingstad *et al*., paper (1998) about P limitation of heterotrophic bacteria and phytoplankton in northwest Mediterranean, their incubation volume was only 250ml or 500ml and incubation time was only 3.5 days (ours: 20 liters, and 14 days). A more important difference is that they did not measure DOC.

Nevertheless, we thank the reviewer to mention the Thingstad *et al.* paper which is an important contribution regarding the understanding of bottom-up and top-down control of microbial community (including phytoplankton, bacteria and predators) and its effects on degradable DOC accumulation in surface water. They developed a comprehensive model to investigate the balance between DOC production and consumption. Their finding suggested that DOC consumption was influenced by both bacteria-phytoplankton competition for nutrients and predatory pressure on bacteria. They concluded that the accumulation was due to "malfunction of the microbial loop".

We will cited this paper and discuss the points in the revised version of our manuscript.

Referee #1 -2 As shown by Goldman, J. C., D. A. Caron, and M. R. Dennett (1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnol. Oceanogr. 32:1239-1252) the utilization of C depends on the availability of N (and P) as shown also by Thingstad and others. The present paper is not building on these papers nor citing these findings. Hence the paper critically lacks novelty. Also, the English needs major revision.

Response: Yes, there are quite some experimental studies addressing the effects of elemental ratios of C,N,P. Goldman *et al.*, cultured natural assemblages of marine bacteria with NH_4^+, PO_4^-, amino acids and glucose (but not NO_3^- -N), they also studied the rate of NH_4^+ regeneration. They concluded that the carbon gross growth

efficiency (GGE) generally was independent of the sources of C and N, but increased with decreasing C:N ratio of the substrate.
In addition to the reviewer mentioned papers, we also checked other similar studies, and found that addition of inorganic N and/or P has been shown to stimulate bacterial biomass and production, but DOC concentrations were usually not monitored except for a few studies. Even in the cases where DOC concentrations were measured, their purposes were to determine whether nutrient or organic carbon was a limiting factor for bacterial growth, rather than carbon left over in the environment (please see the relevant papers attached). In addition, our study site was located in the Western Pacific gyre at the warm pool where similar experiment had never been done. Since some studies suggested that one underlying mechanism for accumulation of labile DOC was low temperature constrained bacterial growth (Zweifel, 1999; Peter A. Raymond et al., 2000; Laura. Hoikkala et al., 2009). Our study in the warmest oceanic area is therefore necessary, and our results did show differences from previous studies (please also refer to our response to the first comment of the second reviewer).
Finally, we will cite the mentioned papers and other relevant works in the revised version of our manuscript. And language will be polished by a native English speaker.

Referee #1-3. on p 10, line 17 and following: deep ocean RDOC may become bioavailable when enriched with (inorganic) nutrients. This is not true since in the deep ocean there is plenty of inorganic nutrients but nevertheless, the DOC remains recalcitrant.
Response: We did not say "deep ocean RDOC may become bioavailable when enriched with (inorganic) nutrients".
What we said was that "situational RDOC which means it can hold refractory under certain conditions but may become bioavailable when the conditions change (such as nutrient enriched)", which refers to the situation of upper layer (where the study took place) rather than the deep ocean.
By the way, we do know the situation in the deep sea and have already stated the possible mechanisms/hypothesis why deep-sea water can hold ~40uM DOC in the presence of abundant hungry microbes. Please refer to the IME seminar minutes (page 4-5, 16 May 16, 2011) as well as the IMBER IMBIZOIII synthesis paper (the same issue) where the points have been distributed and discussed among the 21 co-authors for almost one year.

Anonymous Referee #2

Referee #2 -1. The objective of the present study was to investigate the effect of inorganic nutrients and different organic carbon sources on the degradation of the resident DOC pool. The authors tested this idea in 20-L mesocosm experiments through the addition of inorganic nutrients, glucose, and the SPE-extracted fraction of a diatom exudate to 3 μm filtered seawater collected at 75m in the western Pacific Ocean. The authors followed basic microbial parameters and concentrations of inorganic nutrients and dissolved organic carbon (DOC) over about 1 week. The

major finding of the present study is that the combined addition of glucose+N+P had the most pronounced effect on microbial activity and DOC consumption. The addition of N+P, and of SPE-extracted DOM also yielded higher rates than in the control treatment, while rates in the glucose-amended treatment were not different from the control treatment. The authors conclude from their study that inorganic nutrient limitation can profoundly affect DOC dynamics, and thus potential storage of DOC in the deep ocean. This finding and conclusion is not new. A large body of literature exists on this issue, in many oceanic regimes.

Response: Yes, there are quite some similar studies as we summarized in the attached table. However, there are also quite some differences between our work and previous studies: Such as,

1) Most of the concerns addressed in the previous studies are the effects of enhancement of nutrients on bacterial growth rate, bacterial production and BGE (bacterial growth efficiency); These incubations were carried out in relatively small volumes (as microbes don't need much space to grow comfortably) for relatively short time periods (as microbial generation time is very short); And ambient DOC is usually not monitored (as they focus on biological responses rather than environmental effects). In contrast, our concern is the effects of enhancement of nutrients on DOC remained in the environment, therefore we used large volume (20 liter) and conducted the incubation for two weeks; and we followed the DOC dynamics in the whole incubation time course.

2) As for those studies concerning the effects of nutrients on microbial mediated DOC dynamics (see attached table), conclusions from different authors are often controversial: some suggested that inorganic nutrient amendments had effects on bacterial growth/production or DOC uptake (Zweifel et al., 1993;Rivkin and Anderson, 1997;Cotner et al., 1997;Thingstad et al., 1998;Caron et al., 2000;Sala et al., 2002;Pinhassi et al., 2006); while others did not conclude the same (Cherrier et al., 1996;Carlson and Ducklow, 1996;Kirchman and Rich, 1997; Rivkin and Anderson, 1997;Carlson et al., 2002;Pinhassi et al., 2006). Even compared to a most similar study by Carlson et al., 2002, our study is still distinct at least in the following aspects: (a) experimental design -- Carbon *et al*., used NH_4^+-N as the nitrogen source in their incubation while we used NO_3^--N to simulate the scenario of upwelling injection to the oligotrophic surface water or the situation of river input to coastal oceans, which made a difference beyond availability of nitrogen source (the reason previous studies did not use NO_3^--N could be that it is just recently recognized that many heterotrophic bacteria have the *nasA* gene and thus are able to take up NO_3^--N (Kirchman D. L., 2000; Cai & Jiao *et al*., 2008)); (b) In terms of results -- Although both studies observed the highest responses in bacterial abundance and DOC utilization to "glucose+N+P" enrichment. There are essential differences behind: the microbial responses were mainly stimulated by nutrients (rather than glucose) enrichment in our case, but by glucose (rather than nutrients) in Carlson *et al*.'s case.

3) Our study site was in the Western pacific warm pool where similar experiment had never been done. Such unique and typical environment (warmest oceanic water in the

world) makes a difference and necessity for our study, and our results should be of interest to the community.

Referee #2 -2. The overall conclusion drawn by the authors that "nutrient repletion has negative effects on carbon preservation which is meaningful for coastal water management" is quite far-fetched. This link does not really make sense.

Response: Thanks for the comments, and sorry for the confusion. We agree that the first half and latter half of this sentence is not closely connected, it jumped too much from the former to the latter. What we wanted to say were 1) that a purpose of the present study was to test out the hypothesis by Jiao *et al.*, (2010) that microbial carbon sequestration in eutrophic coastal waters would be enhanced by reducing terrestrial nutrient input; 2) that it is difficult to test the hypothesis in coastal waters because nutrients are replete there and no way to demonstrate the effects of reducing nutrient inputs. In contrast, it is easy to make it in oligotrophic waters, and the results of present study provided the evidence.

We will revise this part in the new version.

Referee #2 -3. Also, in the conclusion, the authors argue that their experiment has shown a "reduction of carbon storage", which I do not agree with. Even if the DOC was not consumed in the time frame of their experiment, it could be degraded under changing environmental conditions, or at longer time scales before being stored in the strict sense.

Response: Agreed. The comments "Even if the DOC was not consumed in the time frame of their experiment, it could be degraded under changing environmental conditions, or at longer time scales before being stored in the strict sense.", is absolutely right. In fact, the three authors here are also involved as coauthors in the synthesis paper of this special issue where most RDOC is defined as "$RDOC_{context}$" which means such DOC that can not be consumed here could be re-used somewhere else when conditions permit.

We will constrain the implication here in a more strict sense in the revised version.

Referee #2 -4. Finally, the title of the MS is not appropriate.

Response: We tend to change the key word in the title "organic carbon storage" to "organic carbon recalcitrance" or alike.

Referee #2 -5. Specific comments: p. 2975: Line 13-15: The authors state that the production of RDOC is well understood. I do not agree with this statement, as a very limited number of studies exist on this topic.

Response: Agreed. And the statement is revised to "the mechanisms of production of RDOC are still poorly understood".

Referee #2 -6. p. 2976, line 9-13: The first sentence describes the inorganic nutrient concentration, while the follow-up sentence is about DOC: What is the connection between them?

Response: The message we wanted to deliver here is "why we conducted the experiment in oligotrophic seawater rather than coastal water". The low background levels of nutrients as well as DOC in the oligotrophic water secured the visibility of the effects of nutrient and/or carbon enrichments.
We will revise the statements to make the points clear.

Referee #2 -7. p. 2977, line 7-9: Why was the algal exudate concentrated on a SPeE cartridge and not added as a whole to the mesocosms?
Response: The main purpose of the experiment was to test the effect of nutrient enrichment on organic carbon uptake / storage. Therefore, it is absolutely necessary to avoid nutrient contaminations. That is why the SPE cartridge, with a desalting process, was applied to concentrate the algal exudate.

Referee #2 -8. Table 1: It appears this Table provides the added concentrations to the mesocosms? It would be important to provide also the final concentrations of DOC and inorganic nutrients to appreciate the amount of the respective nutrients added. Why are all the concentrations only provided approximatively?
Response: Valid comments. We will provide the initial real concentrations which were measured immediately after the addition of the nutrients.

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