Dear Dr. Silvio Pantoja

We thank you for your helpful comments to improve and clarify the manuscript. All suggestions were carefully considered and implemented in the revised text. Our replies to individual comments are detailed below.

Major issues:

1. There are a few studies that have determined rate constants or resident times of bioactive/reactive DOC. How do your rates compare? This is very important because there are always issues with long incubation times, bottle effect, selection of specific microbial communities with time, etc.

Author response: We conducted degradation experiment using glass bottles, and with no microbial community inoculum following Ogura (1975). He conducted degradation experiment with short incubation time (about 50 days) relative to our experiment (150 days). However, BDOC can be consumed by bacteria over short periods (days to weeks; Lønborg and Álvarez-Salgado, 2012) and therefore, the remaining DOC pool after 150 days of incubation, used in equation (1), is not significantly different from RDOC concentration at 50 days. Actually, degradation rate constants calculated using data from the first 50 days (k_{50}) and those using all data from 150 days of experiment (k_{150}) are not significantly different ($k_{50}=0.90 \times k_{150}+0.016$, R²=0.86, p<0.01). Therefore, we assume that our degradation rate constants are comparable to those reported by Ogura (1975). The average degradation rate constants of other coastal waters of 0.066±0.065 d⁻¹ (n=127) in Lønborg and Álvarez-Salgado (2012) was derived from different experiments using different bottles or microbial communities but incubation time longer than 40 days. Therefore, we assume that our degradation rate constants are also comparable to this data (Lønborg and Álvarez-Salgado, 2012).

In the revised manuscript, we have added the following sentences:

(L 405-) "Ogura (1975) conducted degradation experiment with short incubation time (about 50 days) relative to our experiment (150 days). However, BDOC can be consumed by bacteria over short periods (days to weeks; Lønborg and Álvarez-Salgado, 2012) and therefore, the remaining DOC pool after 150 days of incubation, used in equation (1), is not significantly different from RDOC concentration at 50 days. Actually, degradation rate constants calculated using data from the first 50 days (k_{50}) and those using all data from 150 days of experiment (k_{150}) are not significantly different ($k_{50}=0.90 \times k_{150}+0.016$, $R^2=0.86$, p<0.01). Therefore, we assume that our degradation rate constants are comparable to those reported by Ogura (1975)."

2. Since most of the ocean is oxygenated, and most likely your bottles went anoxic, way before 25 days, Could you say that RDOC concentrations, or rate constants are valid only for DOC undergoing suboxic and anoxic microbial reactions as opposed to the most frequent aerobic degradation?

Author response: The 100 mL headspace in each glass bottle contains about 800 μ mol oxygen. The highest initial DOC concentration in this study was 430 μ mol L⁻¹ (Table 1). If we assume that one mole of oxygen is consumed when one mole of organic carbon is mineralized into CO₂, oxygen in headspace should have provided sufficient oxygen supply for heterotrophic decomposition by bacteria. This is now mentioned in the text (L 94-).

3. Error propagation. In your regression analyses, if the coefficient of determination R2 is 0.71 it means that 71% is explained and 29% is error. Will propagation of this error would impact your estimate of % RDOC and therefore your conclusion that "Tokyo Bay exported mostly RDOC to the open ocean". In other words, Would you be able to distinguish terrestrial and ocean origins when you include error bars?

Author response: We have included error estimates in equations (3) - (6) and Table 5. Also the following sentences have been added to the text:

(L 344-) "For each multiple linear regression equation (equation 3-6), the two sided 95% confidence bounds of each coefficient and intercept were estimated. For the concentrations of RDOC originating from phytoplankton, terrestrial, and open oceanic waters, we estimated the upper and lower bounds by changing an equation within its error range."

From these results, we concluded that terrestrial RDOC was significantly higher than phytoplankton RDOC. Hence, Tokyo Bay exports mostly terrestrial RDOC to the open ocean.

Minor issues:

4. Line 38 The word "Conversely" does not fit here. Better use something like "In coastal waters, DOC consists of …"

Author response: The sentence has been changed to read: "In coastal waters, DOC consists of..."

5. Map of Tokyo Bay needs to be improved. Land is not shown here (remember that not everyone is familiar with the area). Show the open ocean and the entire bay as well as rivers and the populated area.

Author response: Figure 1 has been revised accordingly.

6. It is not clear in the Method section if experiments were carried out on board or in the lab, and how many hours passed after sampling.

Author response: We have modified the sentences in the revised manuscript as follows:

L69-

Freshwater samples were collected using a bucket, transferred into HCl acid-washed 1-L polyethylene bottles and kept in the dark until being processed in the laboratory. The bucket and sample bottles were rinsed three times with sample water before being filled. Within 2 h of after sample collection, the freshwater samples were carried back to the laboratory. DOC and the degradation experiment samples were filtered immediately after arrival in the laboratory through GF/F filters (nominal pore size; 0.7 μ m) that had been precombusted at 450°C for 3 h.

L79-

Within 1 h after sample collection, DOC and the degradation experiment samples were filtered through precombusted GF/F filters on board. Then, samples were kept in the dark and carried back to the laboratory within 4 h.

7. Lines 78-80. "We assumed that GF/F filters allow the passage of a significant fraction of free-living bacteria into DOC samples (e.g. Bauer and Bianchi, 2011); therefore, we did not add the microbial community." How good is this assumption considering you are selecting the smaller fraction of the microbial population? Would it affect your results?

Author response: Tranvik and Höfle (1987) investigated the interactions between bacterial assemblages and DOC consumption using batch cultures and found that the

DOC bioavailability was independent of the inoculum. Tanaka et al. (2011) also showed that mineralizatioin rate of the BDOC fraction in coral reef was not different between natural waters and waters filtrated by GF/F, although the initial bacterial abundance in the incubated waters filtrated by GF/F was about 30-50% of bacteria abundance in natural waters. Based on these insights, we assume that the assumption does not significantly affect our results.

In the revised manuscript, this sentence has been changed to read:

(L 81-) "We assumed that GF/F filters allow the passage of a significant fraction of free-living bacteria into DOC samples (e.g. Bauer and Bianchi, 2011). In addition, Tranvik and Höfle (1987) investigated the interactions between bacterial assemblages and DOC consumption using batch cultures and found that the DOC bioavailability was independent of the inoculum. Tanaka et al. (2011) also showed that mineralizatioin rate of the BDOC fraction in coral reef was not different from natural waters and waters filtrated by GF/F, nevertheless the initial bacterial abundance in the incubated waters filtrated by GF/F was about 30-50% of bacteria abundance in natural waters. Therefore, we did not add the microbial community."

8. L 84. Blank for "samples were dispensed into glass vials that had been pre-washed with HCl."; Were they combusted? Show blank measurements for ambient and experiment analyses.

Author response: We used pre-combusted glass vials at 450°C for 3 h. DOC blank including pure water (Milli-Q water, Millipore Corp., Bedford, MA, USA), instrument blank, and any carbon derived from vial was about 3 μ mol L⁻¹ in total. This value is comparable to values reported in the other studies using high temperature catalytic oxidation method (Sharp et al., 2002). The blank is now mentioned in the text (L 107).

9. L 86. Add the word "sample" between "the" and "volume"

Author response: The word "sample" has been added.

10. L. 91-96. This paragraph includes results so it should be summarized and moved to the Results + Discussion section

11. L. 96-101. Result as well

Author response: These paragraphs have been moved to the Results and Discussion (section 3.1. in revised manuscript).

12. L 152-154 could be combined in one sentence for easy reading.

Author response: These sentences have been modified to read: "The average RDOC concentration of 25 μ mol L⁻¹ was the lowest among our freshwater and Tokyo Bay sites, and its contribution to the total DOC was 67%."

13. L 161, 180. Replace good with significant

Author response: "good" has been replaced with "significant".

14. L 160-163. Do you think winter would have DOC concentration in that range as well?

Author response: DOC concentration at Shibaura STP in winter was within the range as well. We observed monthly DOC concentrations at Shibaura STP during 2011 and 2012. There was no significant seasonal variation throughout the year. In January and February 2012, DOC concentrations were 387 and 305 μ mol L⁻¹, respectively. Average DOC concentration was 360±48 (standard deviation) during 2011 and 2012 and this value was comparable to the DOC variation in this research. According to the comment, we added the DOC data at Shibaura STP in January and February 2012 (Table 1).

15. L204-210. Check figures. There is no DOC data in Figure 3 but in Figure 5, and Figure 4 is about incubations, etc.

Author response: Thank you for pointing this out. We added the DOC data in Figure 3 and replaced "Figure 4" with "Figure 3" in L 229 in revised manuscript.

16. L 213-215. Replace

"The results of DOC degradation experiments at Tokyo Bay are shown in Figure 4. Rapid degradation of the labile pool occurred within the first 20 days of incubation, indicating that BDOC were remineralized during the residence time of the bay water." With:

"Rapid degradation of the labile pool occurred within the first 20 days of incubation,

indicating that BDOC were remineralized during the residence time of the bay water (Figure 4)."

Author response: According to the comment, we replaced the sentence in the revised manuscript.

17. L 200, 238, 390. Add units to rate constants (d-1)

Author response: According to the comment, we added units to rate constants (d^{-1}) in the revised manuscript.

18. L 265. Replace "chl a" with "chlorophyll a"

Author response: According to the comment, we have replaced "chl a" with "chlorophyll a" in the revised manuscript.

19. L 394-395 "and the quality of DOC has become more recalcitrant owing to an increase in STP effluent.". It is not clear in the text how this conclusion was drawn. Please explain.

Author response: According to the comment, we have rearranged this paragraph in the revised manuscript (L 414-428) in the revised manuscript) as follows:

"In 1972, the average concentrations of RDOC and BDOC were 224 and 337 μ mol L⁻¹ (40% and 60% of the total DOC, respectively) in the freshwater environment of the lower Tamagawa River, which flows into Tokyo Bay (Ogura, 1975). The present RDOC and BDOC concentrations at the lower Arakawa River station (149 and 86 μ mol L⁻¹) are lower than those reported by Ogura (1975). If we assumed that the amount of freshwater discharge into the bay has increased by 24% (Okada et al., 2007), the amount of RDOC and BDOC flowing into the bay would have decreased by 17% and 68%, respectively. Ogura (1975) also estimated a degradation rate constant (k₁₅) of 0.087 d⁻¹, which is much higher than that observed in the present study (Table 2). These changes are consistent with the fact that proportion of treated wastewater to the total freshwater inflow to the bay increased from 11% to 28% from 1970 to 2000 (National Institute for Land and Infrastructure Management, 2004). Degradation of DOC at STPs before being discharged should lower BDOC flowing into the bay has decreased, and the quality of DOC

becomes more recalcitrant."

20. L 405-406. Even though I know what you mean "the lability of DOC has become more recalcitrant." DOC becomes recalcitrant, not its lability. Please re-write. Check line 420 as well.

Author response: According to the comment, we changed to "DOC becomes recalcitrant" in the revised manuscript.

21. L 422. Either "decreased, especially the BDOC fraction." or "decreased, especially BDOC." would be better

Author response: According to the comment, we replaced "decreased, especially the BDOC." with "decreased, especially the BDOC fraction." in the revised manuscript.

References

Lønborg, C., and Álvarez-Salgado, X.A.: Recycling versus export of bioavailable dissolved organic matter in the coastal ocean and efficiency of the continental shelf pump, Global Biogeochemical Cycles, 26, GB3018, doi:10.1029/2012GB004353, 2012. Ogura, N.: Further studies on decomposition of dissolved organic matter in coastal seawater, Marine Biology, 31, 101-111, 1975.

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Sharp, J.H., Carlson, C.A., Peltzer, E.P., Castle-Ward, D.M., Savidge, K.B., and Rinker, K.R.: Final dissolved organic carbon broad community intercalibration and preliminary use of DOC reference materials, Marine Chemistry, 77, 239-253, 2002.

Sincerely yours

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