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> Interactive Comment

Interactive comment on "Seasonal variations in concentration and composition of dissolved organic carbon in Tokyo Bay" *by* A. Kubo et al.

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We thank Referee #1 for providing helpful comments and suggestions to improve and clarify the manuscript. The suggestions were carefully considered and implemented in the text. Our replies to individual comments are detailed below:

Replies to comments from anonymous referee #1

Comments: Generally the quality of the English writing needs improvement; I recommend getting a native English speaker to read the manuscript before you submit a revised version

Author response: We had the manuscript polished by a native English speaker.





Abstract: Comment: Line 1-6: Be more specific and concise.

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

Comment: Line 6-10: Please join these two sentences.

Author response: According to the comment, we joined these two sentences in the revised manuscript.

Comment: Line 13: Use "concentration" instead of "abundance", and specify what causes the higher levels during autumn and winter.

Author response: According to the comment, we changed to "concentration" in the revised manuscript and added a sentence regarding the reason of seasonal variation in the revised manuscript.

Introduction: Comment: Page 10204 Line 23-25: Combine these sentences.

Author response: According to the comment, we combined these sentences in the revised manuscript.

Comment: Page 10205 Line 3: Is this "open ocean" or "coastal waters"?

Author response: This is "open ocean", we rearranged the sentence in the revised manuscript.

Comment: Line 17: Use "measured" instead of "observed"

Author response: According to the comment, we changed to "measure" in the revised manuscript.

Material and methods: Comment: Page 10206 Line 6: How long time after collection did you start the filtration?

Author response: We started the filtration within 2 hours of sample collection. We added this issue in the revised manuscript.

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Comment: Line 10-16: Was a microbial community added? Or did you assume that microbes passing the GF/F filter did the job? Did you filter your DOC samples? Were initial inorganic nutrient concentrations measured either in the field or your incubations? Were nutrients likely limiting the microbes?

Author response: 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. DOC and the degradation experiment samples were filtered through GF/F filters. Nutrient concentrations in freshwater and Tokyo Bay sites were high throughout the year (Table S1 and S2). During summer, the phosphorus concentration generally decreased and the nitrogen/phosphorus ratio was higher than the Redfield ratio of 16 (Redfield et al., 1963), suggesting that phosphorus acts as a limiting factor of primary production at the bay. We did not add nutrients for the degradation experiment because we assumed nutrients were not limiting the microbial growth. A degradation experiment with phosphate (KH2PO4, 2 umol L-1) was conducted to ensure that phosphorus was not a limiting factor in July 2012, at which time the concentration of phosphate was lowest in the year (0.1 umol L-1; Table S1 and S2). The results of the degradation experiment with added phosphorus were not significantly different from those of the degradation experiment without added phosphorus (y=1.1x-8.2, R2=0.97, p<0.05).

Comment: Line 16-18: Why did you store the samples differently?

Author response: Sea water DOC samples were usually frozen without adding HCl or HgCl2. However, there is a possibility that freezing glass vials with freshwater is cracked. More often, freshwater DOC samples were preserved with HCl or HgCl2 in a refrigerator. A comparison with these methods showed no significantly difference (Chen and Wangersky, 1996).

Comment: Line 18-20: How did you ensure the consistency of your DOC measurements? Were DOC reference samples used?

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Author response: We used potassium hydrogen phthalate as a standard for the measurement of DOC. The sentence added in the revised manuscript.

Comment: Line 21: Rephrase to "RDOC was here defined as : : :.".

Author response: According to the comment, we changed to "RDOC was here defined as" in the revised manuscript.

Comment: Page 10207 Line 1: I guess BDOC was calculated as the difference between initial and final DOC concentration, and RDOC was defined as the end concentrations, right?

Author response: It is right, and we modified the sentence in the revised manuscript as follows: "where DOC(t) is the amount of DOC remaining at time t (day), k is the degradation rate constant (day-1), and RDOC is the remaining DOC pool after 150 days of incubation. BDOC is the bioavailable DOC (umol L-1) at the beginning of incubation and practically equals to subtraction of RDOC from initial DOC. Using BDOC and RDOC concentrations, k can be estimated by fitting the observed DOC(t) values to equation (1) using Matlab 2012a."

Results and discussion: Comment: This section needs to be more precise as currently various ideas/results are discussed more than once.

Author response: We rearranged this section in order to omit a repetition.

Comment: Page 10208 Line 5-10: These concentrations are very low, is this due to e.g. a large input of groundwater? How did you verify that these low concentrations were correct?

Author response: Headstream water sources in Japan are mostly surface runoff from neighboring watersheds and ground water input through the mineral soil horizon before entering surface water (Nakamura et al., 2011). The precipitation is characterized by very low DOC concentrations (Avery et al., 2003). Ground water inputs through the mineral soil horizon typically have low DOC concentrations because mineral soils have

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the ability to adsorb a significant amount of DOC (Aitkenhead et al., 2003). Such low concentrations of DOC in headstream waters have commonly been reported in Japan (e.g. Maki et al., 2010), as well as in other countries (e.g. Yamashita et al., 2002). According to the comment, we added the reasons of low DOC concentrations in the revised manuscript.

Comment: Line 24: Please explain what a "secondary treatment" is?

Author response: According to the comment, we added explanation of secondary treatment in the revised manuscript.

Comment: Page 10209 Line 23: If you consider the variation in your rates and the standard deviation of your average, this value is not statistical different from the average reported for coastal waters in the cited paper.

Author response: According to the comment, we revised this sentence in the revised manuscript. We modified the sentence as follows: "The annual average degradation constant normalized to 15° C at the lower Arakawa River station was 0.031 ± 0.005 , which was similar to other coastal waters (0.066 ± 0.065 ; Lønborg and Álvarez-Salgado, 2012)."

Comment: Line 29: The methods used by Ogura (1975) were different from the ones you used (e.g. He used GF/C filters and a wet oxidation to measure DOC) this should be mentioned and the likely implications of these differences for the comparison with your results needs to be discussed.

Author response: According to the comment, we added the differences of method in the revised manuscript. We also combined with results and discussion about comparison of Ogura (1975) in revised manuscript (see first paragraph of section 3.3 in revised manuscript).

Comment: Page 10210 Line 5: The rates reported by Ogura (1975) (measured at 20_C) are generally lower than the rates you report in table 4 (at 20_C), following I

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don't agree with this statement.

Author response: We argue in this sentence about freshwaters not Tokyo Bay waters, so please see "Table 2" not "Table 4". We restructured table caption in the revised manuscript (see second paragraph of section 3.3 in revised manuscript).

Comment: Line 13-24: This section should be rewritten and shortened, currently it is very difficult to read.

Author response: According to the comment, we restructured the section in the revised manuscript. In addition, we moved results and discussion about comparison of Ogura (1975) to section 3.3 in revised manuscript. We modified the sentence as follows: "Seasonal variations in temperature, salinity, chl a, POC, and DOC at the three stations in Tokyo Bay are presented in Figure 3. High values of temperature, chl a, POC, and DOC were observed during spring and summer, while low values were observed during autumn and winter. Salinity was higher during autumn and winter than spring and summer. DOC concentrations ranged from 81 to 182, 76 to 153, and 60 to 108 umol L-1 at stations F3, F6, and 06, respectively (Figure 4). The concentrations of DOC were generally lower than these at the lower Arakawa River station."

Comment: Page 10211 Line 9: Previously you mentioned that the DOC variations were controlled by BDOC, so how does this fit with your previous statement?

Author response: Freshwater DOC was mainly controlled by BDOC. In contrast, DOC in Tokyo Bay waters was controlled by both RDOC and BDOC due to freshwater input and biological production.

Comment: Line 12-13: What does this suggest? Please explain.

Author response: According to the comment, we restructured the section in the revised manuscript. We modified the sentence as follows: "RDOC concentrations in Tokyo Bay were negatively correlated with salinity and positively correlated with chl a (Table 4). In the bay, salinity was lower in spring and summer than in autumn and winter (Fig-

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ure 3) because of high freshwater input during spring and summer. The freshwater RDOC concentration was higher than that of Tokyo Bay water; therefore, a negative relationship between RDOC and salinity was observed. RDOC is also produced directly by phytoplankton (Kragh and Søndergaard, 2009). Hence, the positive relationship between RDOC and chl a observed in this study likely reflected RDOC produced by phytoplankton."

Comment: Page 10212 Line 2-3: This has already been mentioned previously, so there is no reason to repeat the same idea.

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

Comment: Line 4 to page 10213 line22: The multiple linear regression approach is very nice, but I am not convinced by the validity of the individual functions proposed in this section. The assumptions made are very rough (E.g. I don't believe our assumption of 0 ug L-1 chl a in the open ocean), so I suggest that this part is shortened and only the multiple linear regression approach is reported. Also why is the same type of relationship not shown for BDOC?

Author response: We assumed more realistic values that chl a in the rivers were 6.0 ug L-1 (Ministry of the Environment: http://www.env.go.jp), which was the average value of surface waters in Arakawa River, and that salinity and chl a in the open ocean were 34.5 (Okada et al., 2007) and 1.0 ug L-1, respectively (Japan Meteorological Agency: http://www.jma.go.jp/jma/index.html), which were the average values of surface waters offshore from Tokyo Bay. RDOC sources estimation were essential point of our manuscript, therefore we did not delete this section. In the revised manuscript, we obtained two multiple linear regression equation. At first, we calculated that RDOC concentration at the intercept (chl a: 0 ug L-1; salinity: 0) was not fixed. Secondly, we assumed that RDOC concentration at the intercept (chl a: 0 ug L-1; salinity: 0) was fixed to be average RDOC concentration at the lower Arakawa River station (149 umol

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L-1). RDOC concentrations in Tokyo Bay could be expressed within two equations. BDOC in Tokyo Bay was not well correlated with salinity and chl a (Table 4), so multiple linear regression analysis was not applied to the BDOC data.

Comment: Page 10213 Line 10: How can a value within 3 times the standard error be reasonable?

Author response: According to the comment, we added discussion about terrestrial end-member in the revised manuscript. We modified the sentence as follows: "The end-member of terrestrial RDOC was higher than the average RDOC concentration at the lower Arakawa River station (149 umol L-1) and was similar to that of Shibaura STP (278 umol L-1). The ratio of river water to STP effluent was 1:1 (Japan Sewage Works Association, 2010; Bureau of Sewerage, 2013) and data collected at the upper Arakawa River station and Shibaura STP represent these two sources (see section 3.1.). It is possible that freshwater inputs in Tokyo Bay were more strongly influenced by STPs than headstream waters."

Comment: Line 13: I am not convinced by this statement. In order to conclude this you should e.g. construct a biogeochemical box model or use a mass balance approach. Following I suggest deleting this part of the discussion.

Author response: We used a mass balance approach data of Yanagi (1993). According to the comment, we added discussion in the revised manuscript. We modified the sentence as follows: "The influx of terrestrial TOC (POC+DOC) from the rivers to Tokyo Bay was estimated using a mass balance model (8.1×1010 gC year-1; Yanagi et al., 1993), and the DOC/TOC ratio in freshwater site was 0.62 (Kubo, unpublished data). Hence, the influx of terrestrial DOC was estimated to be 5.0×1010 gC year-1 and RDOC accounted for 66% of terrestrial DOC (see section 3.1.; 3.3×1010 gC year-1). The efflux of TOC from the surface bay to the open ocean was estimated using a mass balance model (9.4×1010 gC year-1; Yanagi et al., 1993), and the DOC/TOC ratio in the surface bay to the open ocean was estimated using a mass balance model (9.4×1010 gC year-1; Yanagi et al., 1993), and the DOC/TOC ratio in the surface bay to the open ocean was estimated using a mass balance model (9.4×1010 gC year-1; Yanagi et al., 1993), and the DOC/TOC ratio in the surface bay mouth was 0.69 (Kubo, unpublished data).

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DOC was estimated to be 6.5×1010 gC year-1 and RDOC accounted for 73% in the surface bay mouth (see section 3.2.; 4.7×1010 gC year-1). Assuming that terrestrial and phytoplankton RDOC were exported outside of the bay in the same ratio at the bay mouth (Table 5), Tokyo Bay exported mostly terrestrial RDOC to the open ocean owing to the high concentration of terrestrial RDOC and remineralization of BDOC."

Comment: Section 3.2.3., this section should be combined with your previous discussion of the Ogura (1975) data.

Author response: According the comment, we combined with previous discussion in revised manuscript (see section 3.3. in revised manuscript).

Tables/Figure Comment: Table 1: Report the standard deviations of your DOC and POC concentrations.

Author response: According to the comment, we added the standard deviations of DOC concentrations. POC samples were only one sample, so we cannot calculate the standard deviations.

Comment: Table 2 and 4: Report the standard deviations of your KDOC estimates.

Author response: According to the comment, we added the standard deviations of KDOC estimates.

Comment: Figure 3: Delete "concentrations" after salinity in the figures.

Author response: According to the comment, we omitted "concentrations" and rearranged the figures in the revised manuscript.

Comment: Figure 4: Show the standard deviations of your DOC values.

Author response: According to the comment, we added the standard deviations of DOC values.

Comment: Figure 5: Show the standard deviations of your DOC, BDOC and RDOC

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values.

Author response: According to the comment, we added the standard deviations of DOC, BDOC, and RDOC values.

Comment: Figure 6: I suggest deleting this figure as I do not trust the approach used.

Author response: We deleted this figure because we obtained two multiple linear regression equation results in revised manuscript. We added Table 5 in the revised manuscript.

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