Response to reviewers #1 and #2 **August 29, 2013**

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Anonymous Referee #1

This manuscript compares bioavailable DOC and DON between 3 estuaries and discusses the effects of DOM quality on bacterial growth efficiency (BGE), which is further linked to the estimation of CO_2 emissions from estuaries. Differences in bioavailable DOM between estuaries were observed and attributed to the contrasting land use. CDOM, fluorescent DOM, and DOM molecular weight were used as DOM quality parameters and were concluded to be the major drivers of BGE. Daily bacterial CO_2-emissions from estuaries were estimated and discussed. Overall, this manuscript presents interesting data and can be of interest for the readers of Biogeosciences. Generally the scientific significance, scientific quality and presentation quality are fair to good. There are some points needing reworking. I would recommend publication if the following comments are addressed in the revised manuscript.

General comments:

1). The introduction gives a review on DOM. But it is too long and the significance of the study is missing from the introduction. It will be better to present a more concise introduction that is developed to reveal the necessary for conducting this study. The revision will also help reduce the number of references, which are too many from my point of view.

> **We have now shortened Introduction from ~1100 words to ~950 words, and also the amount of references have been reduced. Significance of the study is now elaborated in the last paragraph of the Introduction.**

2). The discussion about the effects of land use on DOM degradation needs to be strengthened. For instance, lines 5-21 on page 9837, which actually should be in the result sections, are too descriptive and the discussion is insufficient. Why significant differences in bioavailable DOM are observed between estuaries? What role does the land use play in leading to the differences and how?

> **We think that lines 5-21 from page 9837 wrap up concisely the key results regarding DOM degradation and the variation between catchments, and provide a good way to start the Discussion in section 4.2. But we have now linked this better with other studies, and the discussion regarding catchment land use and the differences in BDOM is in that way strengthened. More precisely, we have added following:**
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> **We have added a more detailed description about the influence of agricultural DIN inputs to DOC and DON leaching (increases DON, not DOC; McDowell et al. 2004, cited in text).**
>
> **We have linked the lower bioavailability of DOC from forests compared to agricultural lands to previous findings (Boyer & Groffman 1996, cited in text).**
>
> **Effect of DOM C:N ratio to bioavailability has now been linked to previous studies (Sun et al. 1997; Opsahl & Benner 1997, cited in text).**

3). What are the concentrations of total dissolved nitrogen (TDN)? Oxidation efficiency for TDN are reported to be >90%, which still leaves up to 10% for errors. Concentrations of bioavailable DON (BDON) are only a few μmol/L and have relatively large deviations. It is possible that the differences in BDON between estuaries were largely

due to the errors of TDN measurement.

There is an error in section 2.3: the oxidation efficiency >90% does not refer to oxidation efficiency of total nitrogen, but to oxidation efficiency of nitrite to nitrate. This efficiency is not of importance regarding our study, since our NO_2^- values were on average 0.18 ±0.13 µmol l^{-1}.

Oxidation of total nitrogen is typically very efficient, only some cyclic nitrogen compounds may be left unoxidized (Grasshoff et al. 1999, cited in text). A study which assessed the total nitrogen oxidation efficiency of various water types (waste water, freshwater and various seawaters) concluded that coefficient of variation is low, ±8.3 % (Dahl 1974, Vatten, 30, 180). In addition, the method we are using (Koroleff 1977, cited in text) is the typical method used universally in studies regarding total nitrogen and dissolved organic nitrogen. Also, our experiment units showed systematic degradation of DON during incubations, which would be unlikely if it were due to analysis bias. From all this we are confident that the DON values and the degradation amounts calculated from those are credible.

4). Is the incubation time the same in every experiment unit? If not, the concentrations of bioavailable DOC and DON between experiment units are not directly comparable.

Incubation times vary (from 12 to 18 days), but this does not affect the %BDOC and %BDON, nor the daily degradation rates, since those calculations are insensitive to the experiment duration. However, when presenting total BDOC and BDON (in µmol l^{-1}), the experiment duration has to be taken into account. We have now normalized the BDOC and BDON values in Table 3 to 14 days, which enables the direct comparison between experiment units.

5). BGE is shown to be significantly different between estuaries and is later shown to be affected by DOM qualities by using the whole dataset from 3 estuaries (section 4.4). But still it is not clear how the distinct BGE value is explained by DOM quality in each estuary. For instance, how can the DOM quality in KY estuary cause the lowest BGE value in that region?

We use BGE as an integrative proxy for DOM quality, i.e. it reveals the functional response of the bacterial community to the substrate (DOM) in case. Currently, with our data we can present linkages between DOM quality and BGE (Figure 8), and between estuaries and BGE. We found, that there is correlation between selected DOM quality properties ("humic-like" DOM) and BGE, but still leaves room for variation to other, unknown factors (which can be interpreted from the R^2 values in Figure 8). From this it follows, that even though we can explain variation of BGE by variation in DOM quality to some extent, linking particular DOM quality properties to BGE in an estuary is a step further that needs additional research to be answered.

6). As stated in the 2nd paragraph on page 9842, DOM properties that affect BGE are linked to humic substances (line 15), which are resistant to biodegradation (line 20). Given that, those DOM quality indicators (molecular weight, spectral slope, florescence properties) are then very insensitive to biotransformation. So how can they be used to explain the variation in BGE? I feel the conclusion about the drivers of BGE (page 9820, lines 17-20) is somewhat overstated.

We think that the DOM properties that have been linked to BGE in this study (Figure 8), can be viewed as different aspects of the same phenomenon. In Figure 8 and the

respective Discussion, we state that "these properties of DOM that most affect BGE can be seen to represent different facets of the same general underlying denominator, as they all can be linked to aromaticity and humic-type characteristics". This negative correlation between BGE and molecular weight, $SUVA_{254}$ and humic-like fluorescence peak means that the more pronounced the humic-like signal in bulk DOM is, the lower the BGE. This is actually very well in line with previous findings that consider the humic-like material most resistant to biodegradation (Amon and Benner 1996; Moran and Hodson 1990; Boyd and Osburn, 2004; Berggren et al., 2009; Guillemette and del Giorgio, 2012; Shimotori et al., 2012; Hulatt et al. 2013, cited in text).

Specific comments:

Introduction

Page 9822 Line 3: Give the definition for "BDOM".

Definition given.

Methods

Page 9826 Line 28: The full name of "SEC" should be given when it first appears here.

Full name given.

Page 9827 Lines 15-25: How is $SUVA_{254}$ calculated?

$SUVA_{254}$ calculation procedure described.

Line 23: You mention "the quality of the CDOM", please specify what "quality" means here.

Here we refer to the qualitative properties of CDOM, instead of just quantitative (like CDOM absorption at a certain wavelength). The reference given (Asmala et al. 2012), provides additional information about this subject. We rephrased the sentence.

Results

Page 9831 Lines 10-13: Please explain how the "source" and "sink" of DOM are assessed using the two end members.

We consider the estuaries to act as marginal filters (Lisitsyn 1995, reference added to manuscript), which changes the quantity and quality of DOM in coastal areas. In order to be able to assess the functioning of this marginal filter, there has to be distinct end-members, which are chosen operationally. This is now clarified in the manuscript.

Line 20, 23: What do "DOM quality parameters" refer to?

We refer to optical and SEC values, which are now explained in that sentence.

Page 9832 Lines 1-5: I suggest placing this paragraph in the discussion section.

Paragraph moved to discussion (4.1)

Line 8: I think you meant "significant differences" rather than "differences".

True, word added.

Page 9833 Lines 5-6: BDOC in Kiiminkijoki is 64.1 µmol L-1, which is not the lowest (Table 3)

According to general comment #4, we have now normalized the BDOC and BDON amounts to 14 days, which allows direct comparison of the experiment units. After this change, the sentence is correct.

Page 9835 Line 16: "absorption" should be "absorbance".

Corrected.

Line 18: The word "drivers" should be used very carefully. Those quality parameters influence BGE but I don't think they are the drivers of BGE. Please rephrase the sentence.

Sentence rephrased.

Discussion

Page 9836 Lines 14: The use of "seawater" can be misleading here, as you only investigate low salinity waters, not real seawater.

Wording changed.

Line 17: I suggest replacing "importance" by "influence" or "effect".

Wording changed.

Page 9837 Line 4: Is "seasonality" discussed in the section 4.2?

Seasonality is discussed briefly with the special case of Kyrönjoki in figure 4. Indeed, with only this short section, including the word seasonality in the section title might be too much, so we have removed it.

Page 9838 Lines 11-12: Why increasing agricultural land would increase BDON, but not BDOC?

This is likely due to the continuous addition of inorganic nitrogen to the agricultural soils in the catchment area, which have been shown to increase DON leaching, but not DOC (McDowell et al. 2004). This is now added to manuscript.

Page 9839 Line 21: Previous studies have observed the production of CDOM by microbes (e.g., Nelson et al. Mar. Chem. 89, 273-287, 2004) and can be used here to underline the statement.

Agreed, reference added.

Lines 22-28: What is the main point of this paragraph? The higher riverine SUVA254 value in summer could be simply due to larger inputs of plant-derived DOM at that time and is not a robust diagenetic indicator of DOM. In the last sentence of this paragraph (page 9840, line 3), I would suggest to replace "utilized" by "altered" as it is not clear whether CDOM is utilized or removed during the incubation, as stated in the first sentence (page 9839, lines 22-24).

The main point of the paragraph is to underline the fact that the microbial utilization (or alteration) of DOM does not happen uniformly throughout the CDOM spectrum. We link this non-uniform degradation to selective (preferential) degradation of different DOM compounds. From this follows, that differences in both UV slope and

$SUVA_{254}$ mean differences in the bulk DOM quality. It is true that the seasonal variation of $SUVA_{254}$ may also be the result of differences in plant-derived matter amounts, but previous studies have indeed linked increasing $SUVA_{254}$ to bacterial metabolism (Berggren et al. 2009, cited in text), which means that both these drivers can cause $SUVA_{254}$ to change in the summer. This has now been added to text.

Word "utilized" replaced.

Page 9840 Lines 23-25: Any references?

Reference added (Kirchman 2003).

Line 26: The title for section 4.4 should be revised. This section discusses the effects of water sources (river vs. sea end-members), seasonality, substrate concentrations and DOM quality on BGE, but not how BGE is used as a proxy for DOM quality.

Section title has now been changed according to suggestion.

Page 9841 Line 1: Please specify what kind of DOM quality leads to "faster degradation of DOM and higher BGE".

The influence of DOM quality to BGE is presented in Figure 8, and in section 4.4 (paragraphs 4 and 5). We have now added a reference to page 9841, line 1, which immediately directs the reader to Figure 8.

Lines 5-6: Why those transformative forces (e.g., photo-oxidation, microbial activity) occurring in the sea end-member do not cause DOM to be more refractory?

Our view is that the mentioned transformative forces in the sea end-member are dynamic processes, but resulting as a net increase of DOM reactivity. This is a complex interaction, where forces making DOM more and less refractory occur simultaneously, but on the bulk level the DOM becomes less refractory. This has now been clarified in text.

Lines 10-11: Is the effect of seasonality on BGE described in the result section?

Yes, we state in section 3.2.2 that season did not have an effect on BGE. For this reason, the BGE seasonality is not covered in more detail.

Page 9842 Line 2: "Fig. 7" should be "Fig. 8".

Corrected.

Line 25: Replace "proxies" by "parameters".

Replaced.

Table 2 Remove the unit of the third $SUVA_{254}$.

Unit removed.

Figure 1 The map has insufficient labels. Estuary information (e.g., abbreviation, land use, etc.) can be included.

Information to figure added.

Figure 6 In the figure legend, "$P < 0.001$." should be "$P < 0.001$"

Corrected.

Anonymous Referee #2

Received and published: 9 August 2013

General comments

In this manuscript, the authors determined several quantitative and qualitative parameters of DOM in addition to the bacterial growth parameters during incubation experiments using rivereine and seawaters of Baltic Sea estuaries with the different land-use characteristics. From these experiments, the authors found that DOM qualities that might be controlled by the watershed's characteristics affect the DOM degradability and bacterial growth efficiency. The results described in this manuscript, especially combination of chemical and biological parameters during the microbial degradation experiments, are novel and very interesting. Thus, I believe this manuscript would be great interest of readers in Biogeosicences. Even that said, there are a couple of unclear issues in the present manuscript.

1) Regarding with DOM parameters, it seemed that the authors basically used average values including every seasons and every water types for discussion (Table 3, Figs 3, 4, 6 and 8). However, there is no justification why the authors decided to use average values. In Table 2, the authors only compared the DOM parameters of "initial condition" among seasons and between river and estuary end members, but delta values (changes during incubations) were only compared among estuaries or among seasons. The use of average values is key part of data analysis for this manuscript, thus, the authors should justify why the authors decided to average every "water types".

We averaged water types, since there were no significant differences between those in the study variables. The only exception was BGE, which showed significant differences between end-members, and that data is shown in Figure 7. There was a misleading error in the caption of Table 2, indicating that data would also be shown differentiated by water types. Since there were no differences in e.g. DOC and DON degradation between water types, we think that presenting data without differences is not justified.

2) The authors collected samples several times (typically, d=0, 3, 6,10, and 14) during the incubation experiments for DOM analyses. However, it seemed that the authors used only differences in DOM parameters between initial (d=0) and end (d=14) of experiments for data analysis. Did the authors use data of d=3, 6, and 10 for data analysis, e.g., calculation of DOM degradation rate? Please clarify this issue in the revised manuscript. In addition, it is not clear which data were used for Tables and Figures. For example, were all of experimental data, i.e., d=0, 3, 6,10, and 14, used for Figure 4a? Why were number of plots different between Figure 4a and Figure 4b? Please clarify what kind of data was used for Figures 4, 6, and 8 (it seemed that the authors used all data collecting throughout the experiments, i.e., d=0, 3, 6, 10, and 14, for some figures, but used only one data, i.e., differences between d=0 and d=14 for other figures).

For DOM degradation rate calculations, we did use only end-point values (to achieve the Δ-values). We chose not to include kinetic degradation models (like first order decay or gamma models), as we find that already endpoint calculations provide enough information for our purposes in this manuscript. Our aim was essentially to elucidate the interplay between DOM quality and bacterial growth efficiency, so we

think that including degradation kinetics would be somewhat off the scope of this manuscript.

In Figures 4, 6 and 8, the amount of data points is now clarified.

3) The authors determined bacterial production using three methods, i.e., 14C-leucine, 3H-thymidine, and bacterial numbers. Did the authors use average bacterial production (and bacterial growth efficiency) determined by the three methods? Please clarify it.

Yes, we averaged the BGE values from the three different calculating methods. This is now clarified in the text.

Specific comments

Page 9822 Lines 23-25: Absorbance and fluorescence analysis can evaluate a part of DOM, i.e., CDOM, but not whole DOM.

We mean here that absorption and fluorescence characteristics of the DOM pool can tell us details about the whole DOM pool, even though they only measure directly a portion of it. This is now clarified in the sentence.

Page 9825 Lines 1-4: What is the meaning of sea samples (corresponding salinity was 6.3±0.5, 2.7±1.1, and 2.3±0.1)? It seemed river end-member samples, because the authors mentioned in the next sentence (Lines 5-9) that salinity of seawater samples were close to the open-sea salinity values.

Here we refer to open-sea salinity values of the Baltic Sea, which are of course considerably lower than those of the oceans. We have now clarified this in the text.

Page 9826 Lines 23-27: The authors mentioned that GF/F filtrate were kept at 4°C within two weeks for fluorescence and absorbance analysis. Even though the authors mentioned that nominal pore size of combusted GF/F became smaller, 4°C was the same temperature with incubation temperature of spring experiments. Is this preservation acceptable for removing bacterial activity?

The effects of storage to CDOM properties has been studied by Stedmon et al. (2000), and they found insignificant changes in CDOM during 27-d incubations. This is now mentioned in the manuscript.

Page 9827 Line 8: NO_3 should be NO_3^-.

Corrected.

Page 9830 SEC chromatogram might be affected by differences in salinity of samples loaded. Did the authors check this effect?

We tested the analysis with different buffer strengths, and found no significant effects to the SEC chromatogram. Thus we conclude that the differences in the SEC values results in actual changes in DOM size, and not from the medium.

Page 9831 Lines 10-12: How can the authors assess the differences between the "source" and the "sink" of DOM from Table 2? Please explain it.

As answered to Referee #1: We consider the estuaries to act as marginal filters (Lisitsyn 1995, reference added to manuscript), which changes the quantity and

quality of DOM in coastal areas. In order to be able to assess the functioning of this marginal filter, there has to be distinct end-members, which are chosen operationally. This is now clarified in the manuscript.

Page 9832 Line 8 and elsewhere: I thinks it's better to use "delta(%) value" rather than "delta value" throughout the manuscript.

Agreed, this is now changed throughout the manuscript.

Page 9832 Lines 11-12: How DON degradation were different among water type? Please describe it in detail.

There is a mistake in that sentence; DON degradation did not vary between water types. The sentence is now corrected.

Page 9833 Figure 3: If the authors used average data of all water types for Figure 3, relationships between BDOM and lake percentage confused me. The estuarine samples occupied more than half of average data, if the authors averaged all of water types. The authors claimed that longer retention time expressed by the lake percentage affected the degradability of DOM. However, if the riverine DOM enter the estuary, it is hard to compare the residence time among estuaries.

Yes, average data of all water types is used for Figure 3. The lake percentage of the catchment is highly relevant, as it increases the processing time of DOM within the lotic system before entering the estuary (Mattsson et al. 2005, cited in text). As our river end-member sampling was very close the river mouth, the processing of DOM had already occurred in the lakes preceding the river sampling. In that sense, all study estuaries are comparable, as their riverine sampling points were similarly very close to entering the Baltic Sea. So the DOM processed in the estuaries has been processed for different periods in lakes, depending on the catchment properties.

Page 9836 Lines 8-10 and elsewhere The authors compared their results among seasons and discussed seasonality of DOM quantity/quality and biodegradability. The quantity/quality of DOM are strongly affected by hydrological conditions. For example, quantity/quality of DOM in rivers are controlled by snowmelt events or rain events like storm (e.g., Neff et al., 2006, GRL, 33, L23401; Hood et al., 2006, JGR 111, G01007). So, if the authors discuss differences in seasonality, the authors should show the discharge data for justifying their discussion.

We state that "season surprisingly did not have significant effect on DOC and DON degradation", which in our opinion justifies the lack of more thorough comparison between seasons. We think that it is better to focus on factors that do make difference, instead of those that do not. Therefore we think that the seasonality (or actually the lack of it) in the data should not be given more emphasis in the text.

Page 9836 Lines 12-16: Again, please show the results regarding with differences in DOM degradability among water types.

There were no significant differences between water types (see answer above), so we think that there is no reason to present those results.

Page 9837 Lines 21-23: It seemed that relationship between BDOC and BDON was also significant for Kajaanjoki. Please check it again.

This is true, the linear relationship between BDOC and BDON in Karjaanjoki is significant to $P = 0.034$. This is now corrected to the text.

Page 9840 Line 29-page 9841, Line 2: From Table 3 and Fig.7, it seemed that BGE did NOT covary with degradation rates of DOC and DON. For example, highest DOM degradation rates were found for Kyronjoki, but highest BGE was found for Karjaanjoki.

This is right, we meant proportional BDOC, which is now corrected in text.

Page 9841 Lines 7-9: Did the authors find any autochthonous signature in DOM quality for estuarine samples?

No, we found no indication of autochthonous DOM from the samples (e.g. slope coefficients 300-650 nm and 350-400 nm, and relationship of $a_{(CDOM254)}$:$a_{(CDOM440)}$).

Page 9843 I think the authors can evaluate C:N of bacterial biomass more preciously, if the authors used changes in inorganic nitrogen concentration during incubation experiments.

This is true, but since our focus is on the organic matter, we think that including a more thorough assessment of inorganic nutrient cycling would be outside of the scope of our manuscript. We acknowledge the role of inorganic nitrogen, and we do not want to say that all nitrogen is utilized from organic sources. So we have changed the wording on page 9843 and Table 4 to more precise form.

Table 4: How did the authors estimate refractory DOC and DON? Also, what is the meaning of the unit of these, e.g., kg C d^{-1}?

Refractory DOC and DON are the amounts not assimilated to bacterial biomass or respired, i.e. that fraction of the DOM pool that is not affected by biodegradation. This is inferred value, so it is the net result of the processes affecting DOM cycling. This is now clarified in Table 4 caption. The unit (kg C d^{-1}) enables the comparison between end-members and between study estuaries.

Figure 4b: In Kyronjoki plot, were spring samples included? If so, please mark spring samples as red triangle like Figure 4a.

Kyrönjoki spring samples marked with red triangle now in both plots in Figure 4.