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Biogeosciences Discussions

Interactive comment on "Technical Note: Comparison between a direct and the standard, indirect method for dissolved organic nitrogen determination in freshwater environments with high dissolved inorganic nitrogen concentrations" *by* D. Graeber et al.

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Interactive Discussion

Discussion Paper

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Interactive Comment

Reply to anonymous referee # 1

Daniel Graeber and Björn Gücker
on behalf of all authors of the manuscript

24 July 2012

In this reply to the comments of Anonymous Referee #1 (referred to as Reviewer 1 throughout the reply), we address the reviewer's allegation that we have largely ignored his/her original review, mainly a request to report blank measurements. Furthermore, we address all other comments by Reviewer 1 and show that many of his/her previous concerns were not addressed in a prior revision of the manuscript, because we did not regard them important or justified. We also detail the changes that we will make to the manuscript in response to the reviewer's comments.

The comments of Reviewer 1 are given in italics to separate them from our reply.

Reply to comments on missing blanks

- The missing blanks are the main argument of Reviewer 1:
  1: *"The most substantive issue, and for me it is a deal breaker, is the complete lack of discussion of blanks as I note below."*

  2: *"What were the blanks of the standard and SEC method? This can have a huge impact on the calculation of final concentrations and percent recoveries. I*

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brought this up in an earlier review. The fact that it is still left unaddressed suggests that the authors don't have blank estimates they can present, the blanks are something they don't want to show, or they didn't appreciate the time a overworked reviewer took to try to help them improve their paper."

3: *13. Page 7035, top of paragraph – The issue of nitrogen remaining in the column comes up again. The authors assume the retention must be low because of the high recovery rates but as a reader I can't have much confidence in the recoveries when I have no information on the blanks. I myself have had beautiful recoveries ruined by an ugly blanks on a number of occasions. All may be well with the data provided but the can't know for sure.*

- It is true that we did not present blank measurements in the manuscript and that Reviewer 1 had asked for blank measurements in his/her previous review. However, in the previous review round (review for L&O: Methods, attached to this reply), Reviewer 1 was the only of three reviewers asking for blanks. Accordingly, we and probably also the other two reviewers of L&O: Methods regarded the proper consideration of sample blanks a standard procedure that goes without saying. As the reviewer insists on this methodological detail, we report the blank measurements here and in the supplement of the revised manuscript, but we strongly disapprove the reviewer's accusation that we either want to hide these measurements or did not even perform them. Blank measurements are standard procedure in our laboratories.

- High-temperature catalytic oxidation-total dissolved nitrogen (HTCO-TDN): During the experiment we used direct blank measurements of MilliQ water to measure blanks several times. We found a mean blank concentration of 0.048 $mgNL^{-1}$($\pm$ 0.030 1SD, n = 8). Moreover, we used three different concentrations of nitrate and ammonium (0.5, 4 & 8 $mgNL^{-1}$) to calculate the blank indirectly as intercept with the y axis and found a blank concentration of 0.087 for nitrate

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and 0.055 $mgNL^{-1}$for ammonium. Both, direct and indirect determinations of the blank are thus in a similar range.

- Size-exclusion chromatography (SEC): During the experiment we performed blank measurements with MilliQ water (n=5). For these, we only found baseline noise but no measurable nitrogen peak. We will add a figure of the blank chromatograms to the manuscript supplement (see Fig. 1 in the supplement of this reply for the blank chromatograms). The mean SD of the noise for the blanks was 0.03 arbitrary units, with a minimum of 0.021 and a maximum of 0.05. Expressed as a concentration, this SD was 0.00085 $mgNL^{-1}$, with a minimum of 0.00057 $mgNL^{-1}$ and a maximum of 0.0014 $mgNL^{-1}$. Therefore, using the mean SD of the noise as blank value, it is $<$ 0.001 $mgNL^{-1}$ which equals $<$ 1 μgNL^{-1} and a determination limit of 3 μgNL^{-1}. This sensitivity is less than the one reported by Huber et al. (2011, Water Research 45 (2): 879-885), who found a determination limit of 1 μgNL^{-1} for urea with the same instrument. Thus, SEC can be considered as highly sensitive and absolutely sufficient for freshwaters. Normally, we assume a detection limit of 0.05 $mgNL^{-1}$ for this method, as we want to make sure that the samples have a high enough signal:noise ratio to be measured with high accuracy by SEC.

- Nitrate and Ammonium: We used a Skalar autoanalyser which performs a blank measurement every ten samples after a wash sample. This blank measurement is then automatically subtracted from the next ten measurements and not reported by the instrument.

- In a revised version of the manuscript we will report the above mentioned blank values and details on the blank measurements in the supplement information.

Specific comments

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- *1. Abstract – I think few analysts would call 2.5 hours per sample fast. Presumably samples can be staggered or run in batches. I think indicating an average number of samples that can be run in 8 hours (or some other time unit) would be more useful to the reader.*

  We have mentioned in the abstract that: "With 2.5h measurement time per sample, SEC is a moderately fast and accurate alternative to the standard approach..." According to the second specific comment of Reviewer 1 in L&O: Methods we already changed "fast" to "moderately fast". Given that the SEC measures DON, DOC and DIN for one sample in 2.5 hours, we regard this as justified. Moreover, we would now add to the text that SEC is equipped with an autosampler and thus 9 samples can be run per 24h.

- *2. Abstract – Specify that this method is for freshwater systems unless you provide evidence that it can be used in marine systems.*

  We provide this information already in the title of the BGD manuscript and in the text (page 7037, line 5 & 10; page 7039, line 1). This had already been changed in response to the reviewer's original third specific comment in L&O: Methods. However, following the reviewer's suggestion, we will repeat this information in the abstract of the revised manuscript.

- *3. Section 2.1 – Why acidify and sparge for a TDN analysis? If this if for the DOC side of things, than this should be stated.*

  Yes, this was done for DOC measurement. In the revised version of the manuscript, we will state this within the text.

- *4. Page 7028, lines 16-21 – Expand on the residual within the column. How much is retained? What is the range of effects this can produce in the final concentrations measured?*

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The effects are low as we state on page 7035, lines 1-5. Responding to the reviewer's comment, we will shift this paragraph to the respective section in the methods section. Moreover, this effect is thoroughly discussed on page 7073, lines 12-24.

- *5. Page 7030, top of page – The volumes of water used to rinse the filters seem excessive to me. Are these filters prone to contamination? If yes, why were they used when there are so many other good alternatives?*

  This intensive rinsing is a standard procedure in our laboratory. This rinsing may seem extensive, but is maintained to make perfectly sure that no impurities are affecting the measurements. To make this clear, we would now state in the text that: "This intensive rinsing was conducted to make sure that no filter impurities were affecting the measurements."

- *6. Section 2.4.1 – Only one natural sample was tested for the more extensive trials. This strikes me as very odd for a method paper. I would have loved to see several natural samples with different characteristics used. What was the rationale here?*

  The rationale was that we already tested two standard compounds with different enrichment levels of nitrate. Thus, we used and additional natural substance to test if the high recovery rates at high DIN:TDN ratios also apply for natural substances. We do not think that extensive trials for more natural samples are necessary, since we have already shown that the working principle (separating DIN and DON by molecular size, Fig. 3 of the manuscript) applies. However, please see two additional chromatograms for the agricultural ditch and the tile drain from the manuscript (Fig. 2 in the supplement of this reply) that confirm the working principle (separation of DIN from DON) also for these samples.

- *7. Section 2.4.1 – Why were model substances tested at 2 mg N/L while the natural sample was only 1.5 mg N/L? What was the rationale?*

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We do not think that a 0.5 mg concentration difference between the model substances and the natural sample makes any difference regarding the test of the underlying measurement principle.

- *8. Section 2.4.1 – Why was NH4+ not measured in the model substance samples? Amino acid standards can often have relatively high NH4+ concentrations depending on how the standards are prepared. If there is any sample left, I suggest checking the NH4+ concentration as it could explain some of the deviations in DON values.*

  We have not measured NH4+, because we have added none to the standard compounds. The rationale was that in most freshwaters NO3- represents the largest fraction whereas ammonium occurs in much lower concentrations. Moreover, we think it is a false assumption that amino acid standards have high NH4+ concentrations. In contrast, interference between the amino acids and the NH4+ measurement by the indophenol blue method results in measurement errors that produce high NH4+ values. This was shown by Burton et al. (1989, Communications in Soil Science and Plant Analysis, 20(5-6)) some time ago. In another study of the same batch of L-tyrosine used in this manuscript, we could show that the NH4+ measurement interference at high amino acid concentrations is indeed the source of potentially high NH4+ concentration measurements and not amino acids mineralized to NH4+ (submitted to Environmental Chemistry). However, the described potential NH4+ - amino acid measurement interference does not affect our results: In the HTCO-TDN, all N is measured independent of the N fraction (amino acids or NH4+) and in the nitrate measurement, the described potential NH4+ - amino acid measurement interference does not have any effect. Thus, deviations in measured NH4+ concentrations cannot have any effect on the DON concentrations determined as HTCO-TDN concentration minus nitrate. For SEC, this also does not have any effect, as NH4+ is separated from DON.

- *9. As I said in my earlier review, it strikes me that a summary table of the various*

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treatments and sample sizes would be useful. As written it is this seemingly endless list of things that sound the same but are not. It makes the reader work too hard.

Treatments and sample sizes are correctly reported in the methods section. Thus, we do not think that such a table is necessary. If the second reviewer also wants a summary table, we will provide it in the supplement.

- *10. Tables 1 and 2 – Here I am baffled. The point of the manuscript is to test the SEC-DON method and yet they don't report measures of error. This is standard for any study but it is particularly important when one is establishing the limitations of a new method.*

  Actually, we reported the requested measures of error in Figure 4 of the manuscript. Additionally, we can report measures of error for three of the model substances of Table 1 for which we measured in 6 replicates for a concentration of 4 (imidazole, nicotinic acid) or 8 $mgNL^{-1}$ (L-tyrosine) of the standard compound. We found a coefficient of variation (CV) of 7%, 2%, 3% for imidazole, nicotinic acid and L-tyrosine, respectively. We will report this in the text of the revised manuscript. Moreover, we will also report the SDs and CVs of the natural samples from Figure 4 in the text.

- *11. Section 4.1 – The authors state that the main reason DON concentrations are overestimated was an overestimation of HTCO-TDN. There are also recoveries far in excess of 100. Is this a blank issue? There are no data provided for blanks and no discussion of how blank issues could impact the results or whether the data presented were corrected for blanks. I'm sorry but a method paper that doesn't have a thorough description and discussion of blanks associated with the analysis is not publishable in my view.*

  As we have shown at the beginning of the reply, this is not a blank issue and as we already stated in the text this is not an issue of the calibration curve (page

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7033 lines 24-25). However, our findings of high errors at high DIN:TDN ratios are strongly supported by the literature (page 7024 line 11 - page 7025 line 4), suggesting that we are not confronted with exceptionally high errors.

- *12. Figure 4 – I am completely baffled by how the standard method could be off by this much at the concentrations being measured. Were samples diluted? Could this be a calculation problem somewhere? Something seems very wrong to me.*

  We have again checked all potential error sources thoroughly (blanks, calibration curves, dilutions) but found no error. In addition to our study two papers have reported that DON concentrations determined at DIN:TDN ratios $>$ 0.6-0.8 are not reliable (Lee, W. and Westerhoff, P. Environ. Sci. Technol., 39, 879–884, 2005 and Vandenbruwane, J. et al. Sci. Total Environ., 386, 103–113, 2007). Especially, Lee and Westerhoff showed that at DIN:TDN ratios of 0.8-0.9, DON concentrations of the samples were over- or underestimated by a factor of 0.5 to 2 (Fig. 4 of Lee and Westerhoff 2005). In figure 4 of our study, we found an overestimation by a factor of 1.9 - 2.1 for a DIN:TDN ratio 0.8 which is accordance to Lee and Westerhoff (2005). Thus, the only thing wrong here is he standard method as discussed in our paper in detail and which is the reason that we propose SEC as an alternative measurement method.

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