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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

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Interactive Comment

Reply to anonymous referee # 2

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on behalf of all authors of the manuscript

7 August 2012

In this reply to anonymous referee # 2 (referred to as Reviewer 2 throughout the reply) we show the referee's main comment - that the number of natural samples is too low to substantiate our conclusions - is not justified since the working principle of the new direct DON measurement technique applies in similar way to all freshwater samples. Moreover, we will address all other comments and show that our main conclusions are valid.

Reply to comments on low number of natural samples The low number of natural samples are the main argument of Reviewer 1:

1: *"...the conclusions appear too far-reaching to me and are insufficiently supported by the limited number of natural samples tested in this study."*

2: *"Errors of the standard approach Did I understand correctly, that you use only ONE natural sample (wetland outflow) and two standard compounds (L-tyrosine and imidazole) to test the recovery rates in dependence of the DIN:TDN ratio (which is a central part of this study)? In view of the far-reaching conclusions (P 7038 "With this novel*

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technique, the scientific community will be able to gather more information on DON concentrations especially for anthropogenically disturbed systems such as freshwaters in agricultural and urban areas") the limited sample size appears insufficient to justify the conclusions and therefore lacks of more natural samples from different freshwater systems and ecosystem compartments (throughfall, forest floor and soil solutions). What about the involvement of blanks to check for a background signal?"

- It is not true that we only used one natural sample to compare the new technique to the standard method of DON determination as is implied in the comment. We used three natural samples with three different treatments for one of these samples. Thereby and by testing model substances, we demonstrate the validity of the working principle of the direct measurement of DON by size-exclusion chromatography (SEC): DON is separated from DIN by molecular size and can be measured directly by a UV detector. There is no reason that this working principle should not apply to other freshwater samples, since DON molecules are always larger than DIN molecules. However, for urea, the DON molecule with the smallest molecular weight, the direct determination of DON by SEC does not work with our setting. We already clearly mentioned this in the discussion (page 7037, lines 6 - 11) and also give a citation describing how this problem can be solved with a slightly altered SEC setting (Huber S. et al. J. Water Supply Res. T., 60, 159–166, 2011).
- The errors of the standard method of indirect DON determination have been thouroughly tested and demonstrated in two previous studies: Lee, W. and Westerhoff, P. Environ. Sci. Technol., 39, 879–884, 2005 and Vandenbruwane, J. et al. Sci. Total Environ., 386, 103–113, 2007. Thus, we think that we do not need to show these problems in detail for several ecosystem compartments for the third time. However, we show the relationship between the error of DON determination and the DIN:TDN ratio for 99 natural samples from different sites within a screening . Additionally, recovery rates were calculated for 3 samples at different

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DIN:TDN ratios (these could not be calculated for the screening samples, see Specific Comment 5 for reasons). All of this was done to exemplify the problems of the indirect DON determination by the standard approach at high DIN:TDN ratios. We will add a sentence in the revision of the Methods section to make our intention clearer.

- The mentioned statement on page 7038 refers to anthropogenically disturbed freshwaters in urban and agricultural areas. In the revision, we will tone down the conclusion and will clearly mention that we mean surface freshwaters and that SEC should be tested in further systems. For throughfall, forest floor and soil solutions, the problems of the standard method have also been demonstrated by Vandenbruwane et al. (Sci. Total Environ., 386, 103–113, 2007). We agree that SEC could and should be tested in these as well as in several other ecological compartments of soils, freshwaters as well as in marine waters. However, this clearly exceeds the scope of our study.

- We have measured blanks of all measurements and found no effect on the sample measurements. Data and statements on this will be included in the revised version of the manuscript. Please see detailled comments on this in the Reply to Anonymous Referee # 1.

Specific comments

1. *"Specific comments Introduction - In general, I miss the Kjeldahl approach (DON + NH4-N) as one traditional method already reducing the number of N species. Can you please comment on its advantages and disadvantages?"*

   As suggested, we will comment on the Kjelldahl approach in the introduction of the revised manuscript. This approach does not perform well at high nitrate concentrations, as was already shown in a US EPA report from 1977 (Schlueter, A.:

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Nitrate interference in Total Kjeldahl nitrogen (TKN, DON + NH4+) determinations and its removal by anion exchange resins, technical report, Environmental Monitoring Support Laboratory, Cincinnati, USA, 1977). In the abstract, the author states that: "TKN losses greater that 90% were observed in solutions containing a nitrate-nitrogen concentrations ten times greater than the TKN level". In other words: At a DIN:TDN ratio of 0.9 an underestimation of 90% of the TKN occurred. If all TKN is DON then this will result in an underestimation of the DON concentration by 90%. Schlueter moreover mentions that only removal of nitrate can prevent such underestimations and proposes anion-exchange (which we mentioned in the text: page 7025, line 16).

2. *"Materials and methods - P 7029, L 19 Why does the number of replicates differ between 5- 11? Why not using a uniform, standardized number of replicates?"*

   The different number of replicates does not have any effect on our conclusions, which are based on Monte Carlo (MC) simulation, since the MC simulation is based on standard deviations that are independent of the number of replicates. The reasons for the differences in sample sizes were partially simple misunderstandings between coauthors in the laboratory. Moreover, in the HTCO measurements additional measurement replicates were performed by the equipment, when the standard deviation was above a threshold value. We could simply not report these values, but we decided to include all available replicates in order to improve the data quality.

3. *"- Section 2.4 Samples and treatments Where do "all natural samples used" come from? Could you please provide a map of the sampling spots and more information when the sampling was performed and which range of TDN concentrations they cover? Why was HTCO-TDN measured with three measurement replicates and NO3 + NO2, and NH4+ with only two?"*

   We do not think that the origin of the samples does affect the relationship between

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DON and DIN:TDN ratio since this is a sample-independent measurement error problem. However, we will provide character, GPS coordinates, concentrations (TDN, nitrate and ammonium) and a map of the sampling sites in the appendix of the revised manuscript. The reason for the different measurement replicates was that HTCO usually has a higher error than nitrate or ammonium measurements, which made it necessary to measure one more replicate. This does not have an effect on MC simulations as stated above (reply to specific comment 2).

4. *"Section 2.4.1 Errors of the standard approach..."*

   We responded to this comment in detail at the beginning of this document.

5. *P 7031 L 1-2 " "No recovery rates were calculated for the samples of the screening due to unknown DON concentrations." I do not understand why you left out the screening samples (n = 99! which would be great), due to the fact that the DON concentrations are unknown. The same is true for the one natural sample (wetland outflow) you used. I cannot follow that line of argumentation."*

   There was no way to determine the true DON concentration for the screening, only the measured (which we show). The reason for this was that the partly high DIN:TDN ratio of the screening samples resulted in an interference of DIN in the indirect determination of DON. In contrast, the wetland outflow exhibited a relatively low DIN:TDN ratio of 0.4, a DIN:TDN ratio for which DON can be determined indirectly with high certainty (Lee, W. and Westerhoff, P. Environ. Sci. Technol., 39, 879–884, 2005 and Vandenbruwane, J. et al. Sci. Total Environ., 386, 103–113, 2007). Subsequently, we added nitrate to increase the DIN:TDN ratio of the wetland outflow in order to investigate the effect of this on recovery rate (section 2.4.1 of the manuscript). In the revised version, we will add a sentence stating that: "No recovery rates were calculated for the samples of the screening due to unknown DON concentrations. DON could not be determined due to the interference of DIN in the indirect determination of DON at the partly

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high DIN:TDN ratios."

6. *"- Section 2.4.2 Comparison of standard approach and size-exclusion chromatography. This section is hard to follow and to understand. For comparing SEC and the standard approach, you use four standard compounds (L-tyrosine, imidazole, nicotinic acid and glycine) and one natural NOM sample (natural organic matter extracted from a pond) to test the recovery rates at different DIN:TDN ratios. Additionally, you applied both methods to water samples from an agricultural stream and from an agricultural tile, but again using different measurement replicates for the two methods. Could you please explain why?"*

   The reason for the different numbers of replicates is stated above (Reply to Specific comment 2). As explained previously, we decided not to ignore additional replicates measured by the equipment. As already mentioned, this does not have any effect on the MC simulations or standard deviations reported in the figures.

7. *"Results In general, it is hard to follow the results and the different experimental approaches. It might be helpful for the reader to give an overview table presenting the methods, levels of DIN:TDN, number of measurement replicates and the different samples used. I am a little bit surprised by the results presented in Fig. 4. How can the DON concentration be that different after the addition of inorganic N, while the measurement errors of the pure NOM sample were low? The authors stated that this was due to an overestimation by the HTCO-TDN measurement. This appears erratic to me. I also wonder why different dilution levels were applied for the measurements. Please, comment on this."*

   We will provide a table with replicates, DIN:TDN ratios and samples used in the revised manuscript. The high overestimation was also mentioned by anonymous reviewer # 1. Please see our reply to his comment on Figure 4:

   Comment of anonymous reviewer # 1: "12. Figure 4 – I am completely baffled by how the standard method could be off by this much at the concentrations

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being measured. Were samples diluted? Could this be a calculation problem somewhere? Something seems very wrong to me."

Our reply: "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."

Since both reviewers mentioned this, we will add a paragraph to the discussion of the revised manuscript similar to the cited reply.

8. *Conclusions As already mentioned, the conclusions appear too far-reaching to me and are insufficiently supported by the limited number of natural samples tested in this study. Also some methodological approaches are irritating, especially the differing numbers of measurement replicates for the two methods and the lack of blank measurements. The paper needs mayor revisions to be considered for publication.*

   As mentioned before, we will clarify that our conclusions apply to freshwaters in agricultural areas. In the replies above, we also explained the differing numbers of replicates and clarified that these did not affect our results and conclusions. Finally, we have already addressed the blank issue and reported blank data in our reply to anonymous reviewer # 1, in which we demonstrated that there were no effects of high blanks measurements on our results.

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