

Interactive
Comment

***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.**

Anonymous Referee #2

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Comment on the Biogeosciences ms bgd-9-7021-2012 "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.

General comments The establishment of a novel method to directly and therefore more accurately determine dissolved organic nitrogen (DON) in solutions would be of great

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benefit to the understanding of the nitrogen cycle. This paper reports on the application of size-exclusion chromatography (SEC) to determine DON in comparison to other standard procedures. Though the authors present a number of experimental data on the application of SEC to standard compounds and natural samples covering different DIN:TDN ratios some shortcomings occurred with regard to the organization of the ms, methodological approaches, and the far-reaching conclusions.

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?

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?

- 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 H₂CO₃-TDN measured with three measurement replicates and NO₃⁻ + NO₂⁻, and NH₄⁺ with only two?

- Section 2.4.1 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 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?

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.

- 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?

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.

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.

Interactive comment on Biogeosciences Discuss., 9, 7021, 2012.

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