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Supplementary material

Dialysis is superior to anion exchange for removal of dissolved inorganic nitrogen from freshwater samples prior to dissolved organic nitrogen determination

Daniel Graeber,[A,D] *Björn Gücker*,[B] *Elke Zwirnmann*,[C] *Brian Kronvang*,[A] *Christoph Weih*[C] *and Jörg Gelbrecht*[C]

[A]Department of Bioscience, Aarhus University, Vejlsøvej 25, DK-8600 Silkeborg, Denmark.

[B]Department of Biosystems Engineering, Federal University of São João del-Rei, Campus Tancredo Neves, 36301-160 São João del-Rei, Minas Gerais, Brazil.

[C]Central Chemical Laboratory, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, D-12587 Berlin, Germany.

[D]Corresponding author. Email: dgr@dmu.dk

Accuracy of total dissolved nitrogen and dissolved organic carbon measurements

In order to test the accuracy of the total dissolved nitrogen (TDN) measurement by high-temperature catalytic oxidation, recovery was checked with imidazole (>99 % purity, Merck, Darmstadt, Germany), urea (>98% purity, Merck), glycine (>99.7 % purity, Merck), nicotic acid (>99 % purity, Merck) and L-tyrosine (100 % purity, Sigma Chemical CO, St Louis, MO), each with a DON concentration of 4 mg N L^{-1}.

The recovery rates of the TDN measurements were 93–108% depending on the compound (Table S1). For the same standard compounds, the DOC recovery rates were 95–108%.

Table S1. Recovery rates of total dissolved nitrogen and dissolved organic carbon for dissolved organic standard substances

Standard deviations are based on six measurement replicates

	Total dissolved nitrogen			Dissolved organic carbon		
	True concentration (mg L^{-1})	Recovery rate		True concentration (mg L^{-1})	Recovery rate	
Substance		Mean	s.d.		Mean	s.d.
Imidazole	4.0	93	(±2)	6.0	97	(±5)
Urea	4.0	108	(±2)	2.0	108	(±4)
Glycine	4.0	98	(±4)	20.6	100	(±1)
Nicotic acid	4.0	106	(±2)	8.0	99	(±1)
L-tyrosine	4.0	100	(±3)	36.0	95	(±1)

Ammonium measurements of L-tyrosine

For pure L-tyrosine, a considerable part (10 %) of the calculated DON concentration was measured as NH_4^+. The reason for this could either be that some of the L-tyrosine was mineralised to NH_4^+ during storage or that a small percentage of the amine groups of this amino acid are measured as NH_4^+. If a part of the L-tyrosine would have been mineralised to NH_4^+, then this should show the same behaviour as the NH_4^+ of other samples during DP. However, for L-tyrosine, the recovery rates of NH_4^+ were respectively 89, 125.7 and 50.8 % after 24, 48 and 72 h This is in contrast to agricultural ditch 1 and the wetland outflow, the only other samples which exhibited measurable NH_4^+ concentrations. For these, the recovery rates were respectively <34, <24 and <11 % after 24, 48 and 72 h. Thus, low removal of NH_4^+ observed for L-tyrosine was not observed for the other two samples, which is best explained by the fact that a part of the L-tyrosine itself is measured as NH_4^+. This notion is supported by a study, in which, with the same method of NH_4^+ determination as used in our study, the authors found significantly higher NH_4^+ concentrations as a result of amino acid interference in NH_4^+ determinations.[1] For L-tyrosine, we therefore decided to assign all measured NH_4^+ to DON for both AEP and DP. It is clear to us that this may result in an overestimation of L-tyrosine; however, if we would not have done that, we surely would have underestimated the L-tyrosine concentrations.

Different settings of dialysis pre-treatment (DP)

In two trials in addition to the one described in the study, we used a slightly different DP setting in order to test DP under alternative conditions. We tested if DP would still function with less buffer and at a different temperature. Instead of 20 mL in 12–14-cm dialysis tubes, we used 40 mL in 63-cm dialysis tubes. For each sample, we put all dialysis tubes to be measured at the two (48, 72 h) dialysis times for the agricultural ditches or to be measured at the four dialysis times for the wetland outflow (15 or 16, 24, 48, 72 h) in one vessel. For each dialysis time, we took only a single dialysis tube out of the vessel and we used smaller vessels (2.5 L). Thus, a buffer volume to sample volume ratio of 13:1–63:1 was given. This is in contrast to the recommendations of the dialysis tube producer, as generally a buffer volume to sample volume ratio of 100 is recommended (Spectrum Europe B.V., Breda, the Netherlands, see http://www.spectrumlabs.com/dialysis/FAQ.html, accessed 5 December 2012). However, if we could prove that the DP also works for smaller ratio, then the buffer volume needed could be reduced and this would increase the applicability of DP. Finally, the DP was conducted in a refrigerator at 4 °C and not at room temperature as in the first trial, because temperature could affect the pore size of the dialysis tubes and could therefore result in less removal of DON while still removing DIN.

Patterns for the development of DOC and NO_3^- concentrations during DP were slightly deviating between the trial shown in the study and second or third trial (Fig. S1). In contrast, the patterns were very similar between second and third trial itself. In both trials, DP resulted in recovery rates of 80–85% of the original DOC concentration after 72 h (Fig. S1a). However, after 24 h, DOC recovery rates were 94 % for the wetland outflow and 90 % of the initial NO_3^- + NO_2^- concentration had already been removed. For the agricultural streams, the recovery rate of DOC was 82–94 % after 48 h and 87–96% of the initial NO_3^- + NO_2^- concentration had been removed (Fig. S1b). Therefore, loss of DOC was apparent for the DP; however, it was small for a dialysis time of 24–48 h and at the same time NO_3^- + NO_2^- was removed with high efficiency.

From these additional trials, DP also works with a reduced sample volume to buffer volume ratio and in the refrigerator at 4 °C. Recovery rates of DOC were partly higher than for the trial of the study which was conducted at room temperature.

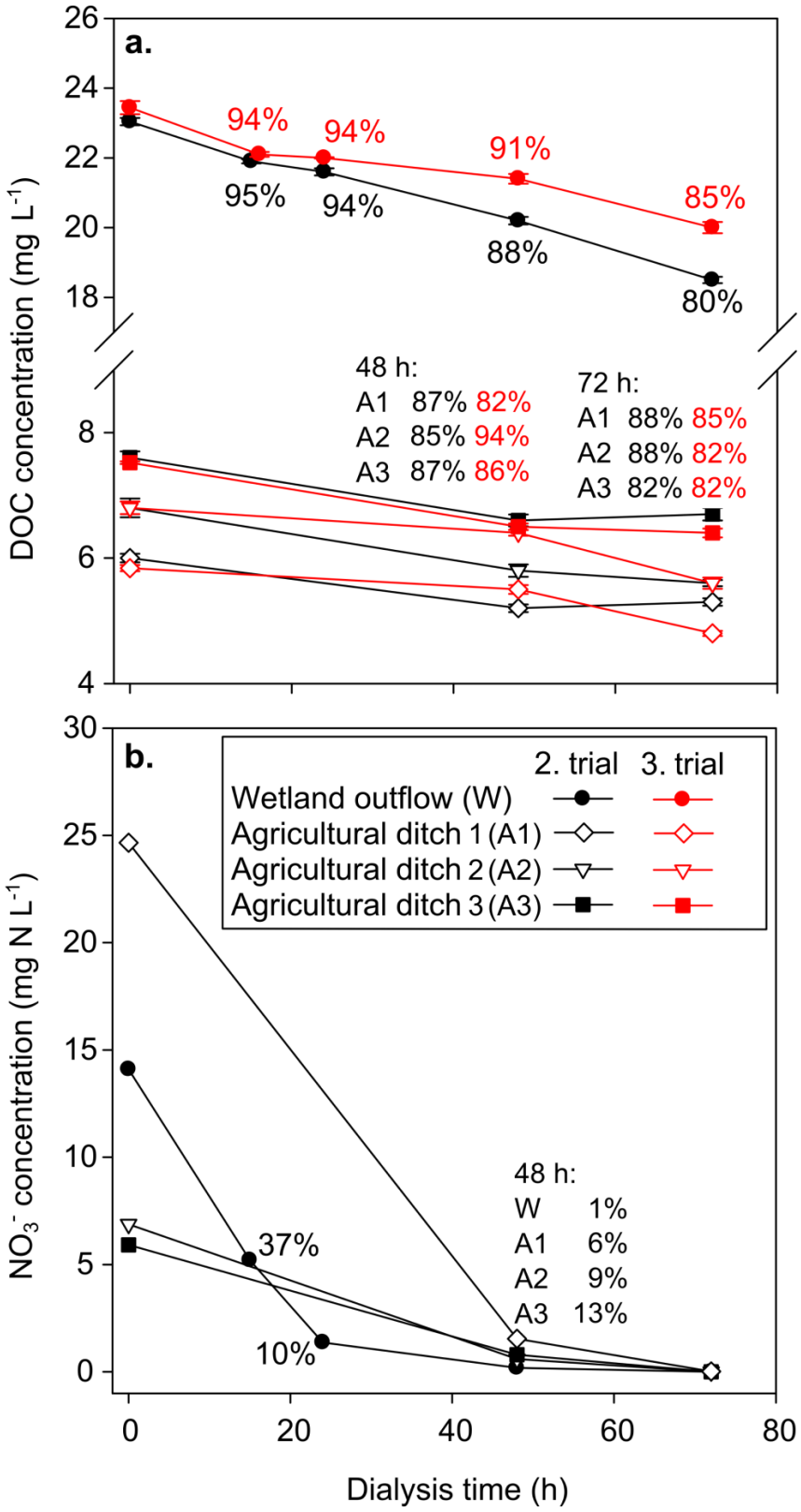

Fig. S1. Concentrations and recovery rates of DOC (a) and NO_3^- (b) after different times of dialysis pre-treatment (DP) for the second and third trial. In (a), mean DOC concentrations (±1 s.d.) of the measurement replicates (n = 5) are given. In (b), mean NO_3^- + NO_2^- (n = 2 measurement replicates) concentrations are only given for the second trial. One standard deviation of the measurement replicates are denoted by error bars and some of the error bars are very small because of the small measurement error.

Pictures of the anion-exchange columns

A yellowish-brownish stain could clearly be seen in the columns after pre-treatment of natural samples (Fig. S1b, c, d) in comparison to a standard compound (Fig. S1a).

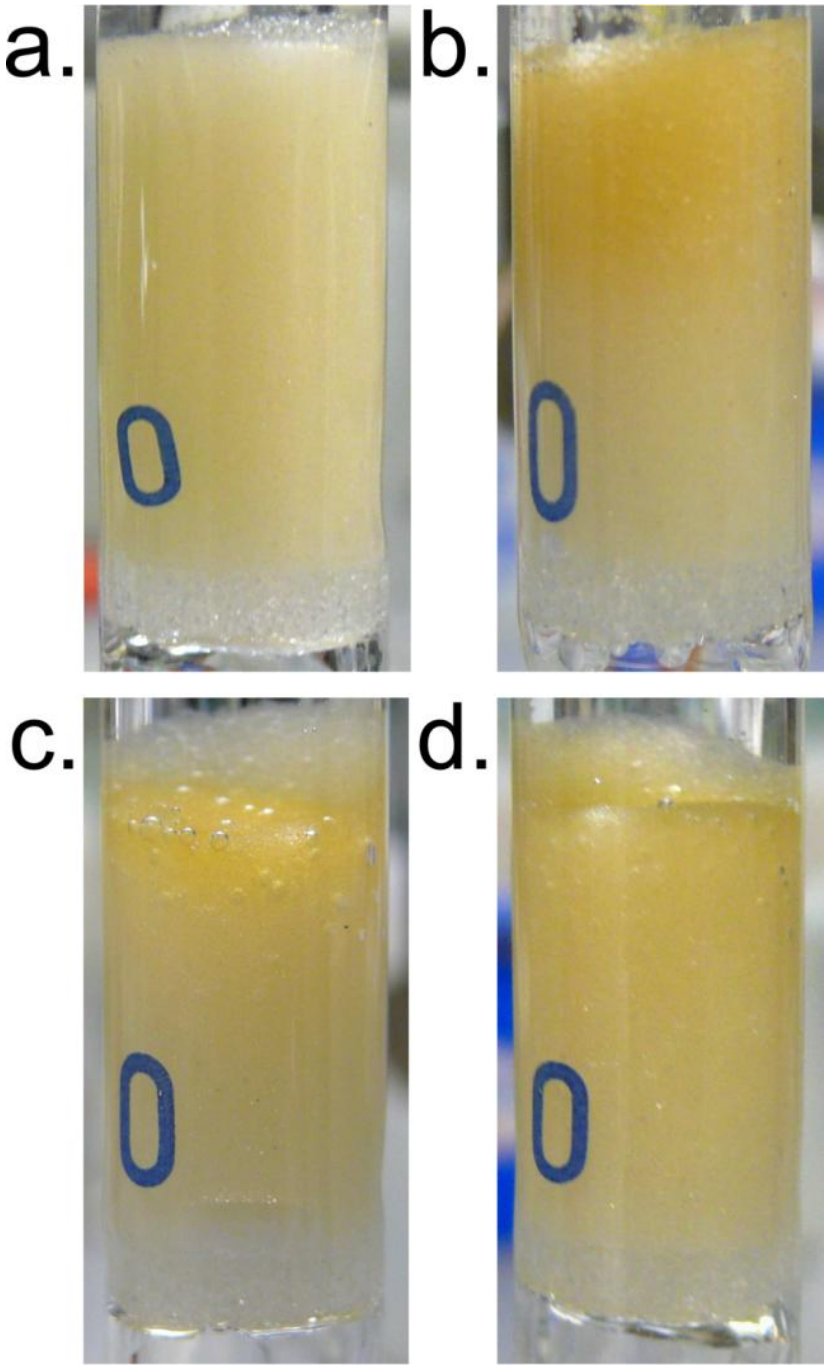

Fig. S2. Examples of chromatographic columns after anion-exchange pre-treatment (AEP) of L-tyrosine (a) and samples from a wetland outflow (b), agricultural ditch 1 (c) and waste water (d).

References

[1] D. L. Burton, D. A. Gower, P. M. Rutherford, W. B. McGill, Amino acid interference with ammonium determination in soil extracts using the automated indophenol method. *Commun Soil Sci Plan* **1989**, *20*, 555. doi:10.1080/00103628909368100