

**Comment on “Comparison of Isotope Pairing and N<sub>2</sub>:Ar Methods for Measuring Sediment Denitrification” By B. D. Eyre, S. Rysgaard, T. Dalsgaard, and P. Bondo Christensen. 2002. *Estuaries* 25:1077–1087.**

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In a recent paper in *Estuaries*, Eyre et al. (2002) concluded that the original N<sub>2</sub>:Ar technique using membrane inlet mass spectrometry (MIMS) significantly overestimates denitrification (up to 3 to 4 times, p. 1077) because of a significant oxygen effect on the mass spectrometric measurement of N<sub>2</sub>. Results obtained from identical instrumentation show that the major effect of oxygen is on the sensitivity of both N<sub>2</sub> and Ar signals and that the oxygen effect on the N<sub>2</sub>:Ar ratio is within the nominal precision of the technique when measuring replicated samples (i.e., 0.03%). Minor effects of oxygen on N<sub>2</sub> alone are documented. The discrepancies in net denitrification rates measured by the N<sub>2</sub>:Ar method and the isotope-pairing method described in Eyre et al. (2002) are not attributable to an oxygen artifact in the dissolved gas measurement procedure, but are due to unmeasured variables associated with sample collection and incubation that were not adequately controlled in the batch core experiments of that study.

Eyre et al. (2002) correctly pointed out what appears to be a systematic difference in the magnitude of sediment denitrification rates measured by the N<sub>2</sub>:Ar method and the isotope pairing method. On average, denitrification rates determined by N<sub>2</sub>:Ar measurements are higher than those determined by the isotope pairing technique, if data from all studies are considered together. They also noted that there is little geographical overlap in terms of where the two techniques have been applied. There is some uncertainty as to whether the differences in measured denitrification rates are due to differences in the biogeochemistry and physical conditions of the respective study environ-

ments or differences in the methodology. They addressed this problem directly by comparing the two methods using identical or similar sediment cores.

A principal conclusion of Eyre et al. (2002) is that a major correction of the data has to be made in order to obtain accurate N<sub>2</sub> concentrations from N<sub>2</sub>:Ar ratio measurements because of a chemical reaction between oxygen and nitrogen within the mass spectrometer. Insofar as most users of the MIMS technique have not restricted oxygen flow into their mass spectrometers, Eyre et al. (2002) imply that results from those studies are in error and that rates of denitrification calculated without the correction are consequently overestimated. We provide evidence here that the oxygen correction is substantially smaller than indicated by Eyre et al. (2002), and it is within the range of the nominal precision of the instrument.

In two independent experiments conducted by Eyre et al. (2002) in which the N<sub>2</sub>:Ar measurements were made with oxygen entering the mass spectrometer (their Fig. 1a,b), the denitrification rate determined by the N<sub>2</sub>:Ar method exceeded the rate determined by the isotope pairing method by 3–4 fold, and standardized errors were on average larger for the N<sub>2</sub>:Ar method. The difference in rates between the two methods was attributed to a putative artifact of the MIMS technique associated with the reaction of N<sub>2</sub> with O<sup>+</sup> generated in the ion source from fragmentation of O<sub>2</sub> (and H<sub>2</sub>O; Jensen et al. 1996). A reaction between N<sub>2</sub> and O<sup>+</sup> would cause a lowering of the N<sub>2</sub> (mass 28) signal as O<sub>2</sub> increased within the mass spectrometer. It was argued that the normal decrease in the O<sub>2</sub> concentration during the incubation of sediment cores would make it appear as though there was an increase in N<sub>2</sub> in the water sample (described as pseudoproduction of N<sub>2</sub> in Eyre et al. [2002]). In the absence of a true change in dissolved N<sub>2</sub>, the MIMS measurements would indicate a positive denitrification rate as long as O<sub>2</sub> decreased. A similar argument and concern can be expressed for systems in which there is net photosynthetic oxygen evolution and a time course of increasing oxygen, such as microphytobenthic systems, as Eyre et al. (2002) describe. In such systems, it would appear that nitrogen fixation was taking place to the extent of the oxygen effect on the measured N<sub>2</sub> signal.

We have reproduced the N<sub>2</sub> versus O<sub>2</sub> relationship (Fig. 1) shown in their Figs. 2 and 3 using a MIMS configuration and Balzers QMG 422 mass spectrometer (with the exception of the in-line furnace) that was the original design for the MIMS used by Eyre et al. (2002). In our experiment, we varied the O<sub>2</sub> concentration by slowly injecting a solution of sodium dithionite by syringe pump into

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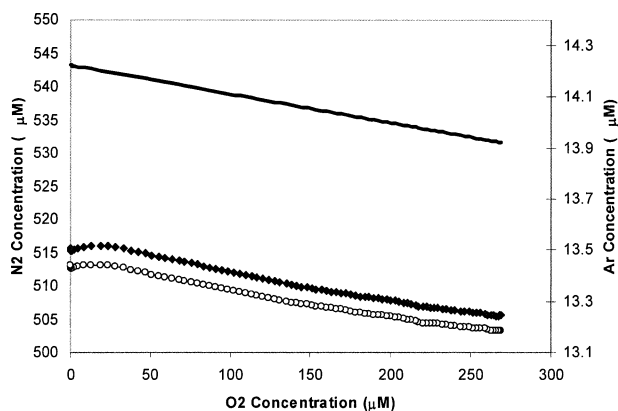


Fig. 1. Relationship between  $N_2$  (mass 28; closed diamonds) and Ar (mass 40; open circles) with respect to  $O_2$  (mass 32). Oxygen was varied by injecting a solution of sodium dithionite. The line is the regression equation provided by Eyre et al. (2002) for a comparable experiment. The offset between the Eyre et al. (2002) data and our experiment is related to the different temperature and salinity of the tested water. Ar concentration axis is scaled to the same relative change as the  $N_2$  concentration axis.

pH-buffered water at a rate sufficient to remove all of the dissolved  $O_2$  in c. 0.5–1 h. The MIMS sampled this water continuously while logging measurements. This type of experiment was repeated no fewer than 15 times while investigating the effects of instrumentation parameters on the results. Our data should be comparable to the technique used by Eyre et al. (2002) in which the signals were recorded during the furnace warm-up period (but see note added to final response statement). During this period there was a progressive decrease in the amount of oxygen that reached the mass spectrometer. Both techniques are significantly better in precision to the discrete vial method originally used to test for an effect (Kana et al. 1994). In addition to  $N_2$ , we show the relationship between Ar (mass 40) and  $O_2$  (Fig. 1). Inspection of Fig. 1 shows an obvious effect of  $O_2$  on Ar that is nearly proportional to the effect on  $N_2$ . This suggests the possibility that  $ArO^+$  ions are being formed, a phenomenon that has been observed in inductively couple plasma mass spectrometry (ICPMS) methods that involve Ar plasmas. Measurements at mass 56 indicated only trace amounts of  $ArO^+$  in our experiments (c. 0.03% of the mass 40 signal).

The relationship between the  $N_2$ :Ar ratios and  $O_2$  is shown in Fig. 2. It is clear that the relatively large changes in  $N_2$  and Ar with  $O_2$  are very close to proportional, insofar as the maximum difference ( $\Delta$ ) in the ratio was 0.13%, whereas the  $\Delta N_2$  was 2%. This range overestimates the practical range of oxygen concentrations found in experimental samples, since it is common practice to limit sediment core incubation conditions to 50–100%

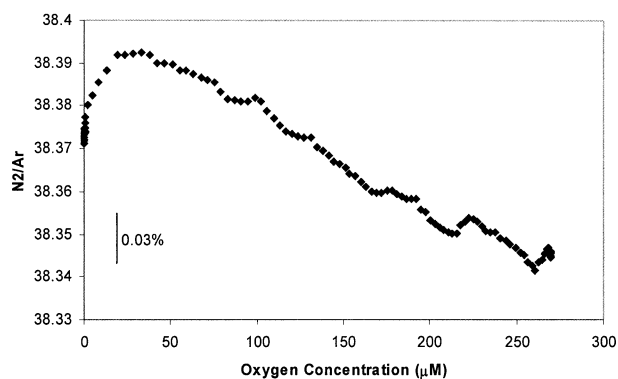


Fig. 2. Relationship between the  $N_2$ :Ar ratio and  $O_2$  concentration. Data from Fig. 1.

(or narrower) oxygen saturation. The standardized error of the  $N_2$ :Ar ratio within this region of the dataset is 0.02%, which is at the limit of precision of measurement of discrete samples (Kana et al. 1994). With the definable relationship shown in Fig. 2, it is possible to derive a correction factor for the  $N_2$ :Ar ratio to improve the accuracy beyond the 0.02% level if desired. As described below, this error is at the same magnitude or smaller in comparison to potential errors associated with sediment and sample handling.

The likely cause of the major oxygen effect on  $N_2$  and Ar has to do with the effect of  $O_2$  on the ionization energy of the mass spectrometer source. The process of ionizing gases for detection by a mass filter typically results in a fraction of gas molecules being fragmented (e.g.,  $N_2$  is broken into 2  $N^+$  ions) or laden with a double charge (e.g.,  $N_2^{++}$ ). Both of these ions are detected at (apparent) mass 14. Increased ionization energy will lead to an increase in apparent mass 14 ( $N^+$  and  $N_2^{++}$ ) relative to mass 28 ( $N_2^+$ ). In our dithionite experiments, the mass 14:mass 28 ratio increased with  $O_2$  (data not shown) indicating that the presence of  $O_2$  increases the ionization energy. A change in ionization energy in the mass spectrometer source should also influence the measurement of other molecules or atoms, including Ar.

Eyre et al. (2002) accepted the relationship between mass 30 ( $NO^+$ ) and  $O_2$  (their Fig. 2) as evidence of the role of the  $N_2 + O^+$  reaction, and they scaled their chart to make it appear at first glance as though the extent of the increase in NO is equivalent to the decrease in  $N_2$  (see their Fig. 2). Although Eyre et al. (2002) did not report the ion currents for mass 28 ( $N_2$ ), for our instrument, the ratio of mass 30 ion current to mass 28 ion current was approximately  $1.2 \times 10^{-3}$ , or roughly 0.1%. It is possible that the 0.13% change in the  $N_2$ :Ar ratio reported here (Fig. 2), was due to an

oxygen effect on the nitrogen signal, though this tentative conclusion warrants further study.

Regardless of the mechanism, the empirical result shown in Fig. 2 justifies the direct measurement of the mass 28:mass 40 ratio in the presence of oxygen as a measure of the N<sub>2</sub>:Ar ratio in water as it is within the general precision of the instrument. We conclude that the specific manner of correction used and advocated by Eyre et al. (2002), i.e., using the “relation obtained between the O<sub>2</sub> and N<sub>2</sub> signal” to correct N<sub>2</sub>:Ar ratios “back to the O<sub>2</sub> concentration at the start of the incubation” (p. 1079), is invalid and results in erroneous data. The fact that the oxygen-corrected data from that study resulted in denitrification rates that were closer to the results from the isotope pairing method is not appropriate justification for performing the oxygen correction as proffered by Eyre et al. (2002, p. 1081). The suggestion in Eyre et al. that there may be instrument-specific differences in the oxygen correction is not supported given the fact that our respective experiments were conducted on essentially identical instruments. Rather, the difference has to do with the way in which the data were analyzed.

Eyre et al. (2002) described a number of factors that can contribute to errors in the interpretation of changes in N<sub>2</sub>:Ar ratios from time-course experiments on sediment cores. We agree that a significant source of error in ascribing changes in N<sub>2</sub>:Ar ratios to denitrification and nitrogen fixation has to do with gas fluxes that are driven by solubility gradients. Eyre et al. (2002) described errors associated with temperature gradients and gas exchange between the water and plastic containers and between water and bubbles. These errors were minimized in the original technique (Kana et al. 1994, 1998) by using continuous flow-through core incubations and determining the N<sub>2</sub> flux in the steady-state condition from measurements of the inlet and outlet gas ratios. Other workers including Eyre et al. (2002) adopted the MIMS method of N<sub>2</sub>:Ar measurement for closed core incubation (batch cores) experiments in which time-course measurements are made to determine the biological fluxes. This approach has the benefit of being less time-consuming and mechanically simpler, and it allows greater replication for a given amount of effort. It has become the preferred method for those reasons, but it is subject to weaker control over the physical variables. Eyre et al. (2002) improperly attribute their batch core method to the original contributions of Kana et al. (1994, 1998), who used a continuous flow core method.

The limits of resolution for the N<sub>2</sub>:Ar method are, in our opinion, set by the ability to limit solubility-driven gas fluxes during the incubation, and

not by the measurement of the gas ratio by the MIMS. It is insightful to look at the sensitivity of the N<sub>2</sub>:Ar ratio to changes in temperature and salinity, which can both change significantly over short time frames in estuarine systems. At a reference point of 23°C and 10 PSU, the equilibrium N<sub>2</sub>:Ar ratio changes by 0.05% per PSU and 0.17% per °C. In an earlier study, we observed gradients in the N<sub>2</sub>:Ar ratio within estuarine sediment, using in situ equilibrators, of +2.8–3.4% in the top 6 cm relative to the overlying water. This delta is large in comparison to what one would expect based on temperature and salinity effects from the natural environment, and it is presumptive evidence for in situ denitrification. With such sediment, a constant (i.e., linear time-course) flux of N<sub>2</sub> relative to Ar is expected to be a good measure of net denitrification. There is less certainty when the change in N<sub>2</sub>:Ar is less than a few tenths percent, which is the magnitude of potential solubility effects. We note that Eyre et al. (2002) were able to highly resolve  $\Delta$ N<sub>2</sub>:Ar ratios of 0.05% in a time course measurement, and they obtained a linear increase of 0.12% in N<sub>2</sub> over an incubation of 10 h. At this level of analytical resolution, it is very important that solubility equilibration has taken place, because the magnitude of its potential effect is on par with the measured changes. This concern is further emphasized in Eyre et al. (2002). (We also note that in order to attain 0.01% precision on the N<sub>2</sub>:Ar ratio, it is necessary to frequently standardize the mass spectrometer as instrument drift can cause the ratio to vary by > 0.03%/h using a Balzers QMG 422 mass spectrometer.)

There is yet no acceptable technique for separating solubility effects from biological effects in sediment time-course experiments. Water blanks can account for processes occurring in the water and artifacts associated with disequilibria with the container material, and they are useful when measurements of sediment oxygen demand are made by O<sub>2</sub>:Ar measurements, which can be made simultaneously with the N<sub>2</sub>:Ar measurements (e.g., Kana et al. 1998). The development of a technique that kills all biological activity would be advantageous. Even a killed control could, in principle, exhibit an apparent linear flux over a short time-frame due to the relaxation of a biologically-induced gradient within the sediment. In such a case, the control would overestimate the solubility-driven flux.

We agree with Eyre et al. (2002) that the nature of gas measurements from closed sediment cores contains a number of uncontrolled or poorly controlled variables that can result in inaccurate determinations of denitrification. In theory, the importance of experimental error decreases as the

denitrification flux increases. We disagree with the analysis of the so-called oxygen effect and the conclusion that N<sub>2</sub>:Ar ratios must be corrected using the N<sub>2</sub> versus O<sub>2</sub> relationship. We have provided evidence that indicates that measurements of N<sub>2</sub>:Ar ratios using the mass 28:mass 40 ratios are within the precision of measured replicates. For experiments that require precision of < 0.03% or for the measurement of masses 29 and 30 in the isotope pairing technique, oxygen removal is desirable and the copper reduction method described by Eyre et al. (2002) is useful.

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