

We would like to thank Mark Trimmer for the constructive comments and suggestions. Please find our detailed responses to all comments below.

The paper is presented as a technical note and whereas as I think the technical advance presented which enables the simultaneous cryo-focusing and quantification of N₂ and N₂O from the same sample is good, the claims of a new ¹⁵N isotope pairing technique are somewhat overstated. For example, the abstract and introduction describe the various processes within the N cycle that may produce both N₂ and N₂O and how to disentangle the contribution from each pathway is indeed challenging. Whereas Nielsen's original isotope pairing technique (IPT 1992) was perfectly suited to measuring denitrification, it came undone with the advent of anammox; though if anammox is absent it is still absolutely ok. The logic behind the IPT was then reworked by Risgaard-Petersen et al (2003) to include the key parameter r₁₄ and they proposed two techniques for its quantification – one direct (V in intact sediment cores) and one indirect (ra% from parallel homogenous sediments). Later, in collaboration with Trimmer et al (2006), they proposed the combined N₂ and N₂O technique for direct quantification of r₁₄ and thereby the simultaneous quantification of anammox and denitrification in intact sediment cores. At around the same time, Master et al (2005) proposed a purely theoretical framework for the combined quantification of N₂O and N₂ in a pure denitrification assay. This latter framework did not include N₂ from anammox, which, as at that time the occurrence of anammox was being reported in more and more marine and estuarine sediments, the new framework of Master was, in effect, already out of date when published – though the rationale behind wanting to quantify both N₂ and N₂O was, and still is, fully justified.

We agree that the claim of “new ¹⁵N isotope pairing technique” was overstated. We appreciate your positive evaluation of our work on the simultaneous quantification of N₂ and N₂O. The statements about the novelty were toned down in our manuscript and the title has been changed to “modified ¹⁵N isotope pairing technique”. All relevant statements in the manuscript have been rephrased.

The authors here claim advances on both of these new formulations as 1: the reworking by Risgaard-Petersen/Trimmer overlooked N₂O production and 2: Master did not include anammox. While the shortfall in Master et al (2005) is true, as described above, Trimmer et al (2006) did indeed show the production of N₂O over time in their time series incubations (Fig 3b ^{45,46}N₂O nmol m⁻² against time). The point to appreciate is that the focus of the 2006 paper was to simply focus on N₂ production by both anammox and denitrification and NOT the fractional split in N₂ to

N₂O in the denitrification pathway but it was quantified. Admittedly, we take two samples from the head-space of the helium equilibrated gas tight vial - one for cryo-focusing of ¹⁵N-N₂O and one for quantification of ¹⁵N-N₂. If this can be done in a single step, then yes, that is an advance, but I don't think that in itself qualifies as a new ¹⁵N isotope pairing technique – but what the authors do propose is a simple reformulation to include both N₂O and N₂ (equation 10 page 6871).

Thanks for reviewer's sharp comments. In the Abstract, the statement related to Trimmer et al. (2006) work has been rewritten. It is now "*Recently, the classical isotope pairing technique (IPT), the most common ¹⁵N-nitrate enrichment method to quantify denitrification, has been modified by different researchers to 1) discriminate relative contribution of N₂ production by denitrification from anammox or to 2) provide more accurate denitrification rate by considering both N₂O and N₂ productions. The former one focused on N₂ production and was suitable in the environments of low ratio of N₂O to N₂ production. The latter one neglected anammox.*"

In the Introduction, we also rewrote the sentences (p. 6864. L. 3-6), "*Based on the IPT_{classic}, Risgaard-Petersen, et al. (2003) and Trimmer, et al. (2006) proposed IPT_{ana} enabling the estimation of anammox (yellow and blue plates in Fig. 1). The above methods were mainly focused on N₂ production by denitrification (IPT_{classic}) or by both denitrification and anammox (IPT_{ana}). Although the ¹⁵N-N₂O production was quantified in Trimmer et al. (2006) to derive the ratio between ¹⁴NO₃⁻ and ¹⁵NO₃⁻ but the ¹⁵N-N₂O production was not involved in their estimation of denitrification due to its insignificance (see section 3.1).*"

The whole manuscript has been rechecked, especially section 3.1, to make sure all descriptions related to different versions of IPT were accurately described.

What is interesting, however, is the very high ratio of N₂O to N₂ in the denitrification pathway in these sediments. Most studies report some 2% but here the authors record 66% of denitrification gases as N₂O –yet there is very little consideration of what other studies have shown for estuarine sediments or indeed (beyond the sulphide story) what may actually cause this. If, indeed, 66% of the ¹⁵N gases are accumulating as N₂O in the denitrification pathway, then the formulation that includes this would obviously be necessary but it seems quite out of the ordinary.

Besides our study, some other studies also reported such high N₂O yields in eutrophic estuary sediments. In our revised manuscript, we added new references and more discussions. The new paragraph is now "*Although many studies reported N₂O*

yield ($N_2O/(N_2+N_2O)$) via denitrification were less than 2%, there are still some studies recorded high N_2O yield in estuarine sediments. For example, Dong et al. (2002) observed N_2O yield from 0 to ~9% with one exceptionally high (~50%) in the Colne estuary. They concluded that NO_2^- is favored for denitrifier to form N_2O and may be a critical factor regulating the formation of N_2O . In other British rivers and estuaries, wide range of N_2O yield (0 to 100%) had also been found (García-Ruiz et al., 1998a; García-Ruiz et al., 1998b). The causes reasonable for such high N_2O yield were not well explored; however, our high yield might not be out of the ordinary.”

Finally, the direct quantification of the production of $^{44}N_2O$ by IRMS should enable any additional production or excess production above and beyond that traced with ^{15}N to be assigned to nitrification but I think it might be helpful to simply term this as N_{44} as a logical contrast to that ascribed to denitrification as D_{44} , rather than P'_{44} (which would just be the total?) as the authors currently have it.

The term P'_{44} has been change to N_{44} as suggested.

In relation to this point, the title and the abstract may lead the reader to believe that all of the N_2O and N_2 coming from denitrification, anammox and nitrification can be accounted for in one measurement and formulation but the quantification of N_2O from nitrification (i.e. equations 16 and 17) is not included in the overall estimate of N_2 and N_2O as $P_{14-anaN_2O}$ (equation 10)?

Yes, reviewer is right. The estimation of N_2O from nitrification is not a part of $P_{14-anaN_2O}$. We have clarified it in Abstract. It is now “Based on the six m/z peaks of the two gases, the ^{15}N -nitrate traceable processes including N_2 and N_2O from denitrification and N_2 from anammox were estimated through our modified IPT. In addition, N_2O produced via nitrification could also be estimated by using the production rate of unlabelled gas species, $^{44}N_2O$.”

However, we think the title is fine. It would not misleading readers that N_2O from nitrification is a part of IPT.

Also, if it is possible to measure the production of $^{44}N_2O$ i.e. without a tracer, then, with cryo-focusing, is it not also possible to measure $^{28}N_2$ directly and check that against the ^{15}N -traced estimates in $P_{14-anaN_2O}$?

Unfortunately, direct measurement the production of $^{28}N_2$ is difficult using cryo-focusing IRMS due to the high background of N_2 . However, it can be measured

by using membrane inlet mass spectrometry. A good example was An et al. (2001) which can simultaneously quantify denitrification and nitrogen fixation using IPT. We had added more illustrations in Section 4.4 pointing out difficulties and possible ways to explore entangled processes, such as BMA and N-fixation.

References:

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- García-Ruiz, R., Pattinson, S. N., and Whitton, B. A.: Denitrification and nitrous oxide production in sediments of the Wiske, a lowland eutrophic river, *The Science of the Total Environment*, 210, 307-320, 1998a.
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