

We would like to thank Reviewer #1 for very useful suggestions, which obviously promote the scientific level of our manuscript. Please find our point-by-point responses to all comments below.

Only one validation experiment in the dark was done. Most of the environments where this technique will be applied, including the site where the authors did their validation, would contain benthic microalgae (BMA). As such, I would like to have seen a light and dark validation to make the technique more broadly applicable (see also comment on assumptions: BMA below).

Light and dark incubation comparison has been studied to explore the enhancement of nitrification-denitrification coupling during daytime (Dong et al., 2000); however, this is not the scope of our method study.

Theoretically, our method is still applicable when light drives benthic algae to stimulate oxygen production and subsequently nitrification rate and the consequent denitrification. Nevertheless, we add this part in Section 4.4. We also believe that under light condition nitrification will be inhibited to some extent; thus, this comparison will be worthy to carry out in the future to explore the relative importance of individual processes over diurnal cycle in some shallow water environments.

More importantly, a N_2O yield of 66% is exceptionally high, and the estimate N_2O production rates didn't match the directly measured N_2O flux. Although the authors did discuss both these issues, they highlight that the technique requires more validation.

The respond is the same as our reply to the third question by Trimmer.

Although we did only one validation experiment, the small standard deviations of replicate incubations indicated that our experimental case was a reliable example to demonstrate IPT_{anaN_2O} . We chose to make a conservative conclusion because we know that our technique have to be tested further under various environments to ensure its applicability in the future.

It would also have been nice to have done a validation on subtidal sediment and compared to directly measured N_2 fluxes across the sediment water interface. The authors would then have validation of both the N_2O and N_2 fluxes. Due to the inclusion of N_2O perhaps the two techniques would compare better than previous comparisons between ITP and $N_2:Ar$ that found IPT underestimated denitrification (e.g. Ferguson 2007. MEPS 350, 19; Gihring 2010. L&O 55, 740).

This suggestion is well taken; unfortunately, we did not measure N_2 flux during the sampling. We will follow this suggestion in our next study combining with the effect of BMA.

The whole manuscript needs to be edited for expression and grammar. I haven't made these corrections.

The revised manuscript will be edited by native English speaker.

p. 6863. L. 19. See Dong 2006. L&O. 51, 545. Who did dual measurements of N_2 and N_2O .

We made an unclear statement. What we wanted to emphasize is that there is no simultaneous quantification of N_2 and N_2O for "the same vial". We have rewritten the sentence to clarify it. According to reviewer's reminder, we included those studies that had done dual measurements of N_2 and N_2O into References including Dong et al. (2006), Minjeaud et al. (2008), Trimmer and Nicholls (2009) and Trimmer et al. (2006).

p. 6864. L. 5. Need to make it clear that they did not take N_2O into account for denitrification, as some did measurement N_2O .

We have corrected it. It has been rewritten as "*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 only focused on N_2 production by denitrification ($IPT_{classic}$) or both denitrification and anammox (IPT_{ana}). Although the $^{15}N-N_2O$ production was quantified in Trimmer et al. (2006) to derive the ratio between $^{14}NO_3^-$ and $^{15}NO_3^-$ but the N_2O production was not involved in their estimation of denitrification due to its insignificance (see section 3.1).*"

p. 6874. It's not clear how many times the time series was sampled- start and end point only?

Yes, we sampled at start and end point only. It indeed was mentioned in p. 6874 from Line 26 to p. 6875. Line 2. The linear response of $^{15}N-N_2$ and $^{15}N-N_2O$ over

incubation time was reconfirmed in the time series experiment. If we had increased sampling frequency, the extra incubation of sediment cores might not have been finished within the incubation period of 3 hours.

p. 6876. L. 25 . see Dong 2006. L&O. 51, 545

To our knowledge, only two studies, Dong, et al. (2006) and Minjeaud et al. (2008), applied IPT_{N₂O}. We don't refer to Dong et al. (2006) here as an example because they did not have data/experiments of constant response of N₂O yield with various ¹⁵NO₃⁻ additions which is important in our discussions.

p. 6880. Section 4.4. Should also consider production of N₂O from co-denitrification see Spott 2011. Soil Biology and Biogeochemistry 43, 1995.

We have added co-denitrification into consideration in our revised manuscript. We also referred to Spott et al. (2011) showing that if N₂O from co-denitrification predominates, the ratio of qN₂ to qN₂O would be >1. By contrast, anammox-effective samples will reveal ratios of <1.

p. 6881. L.3. Welsh 2001. Marine Biology 139, 1029. found N₂O production from DNRA in the field.

In this sentence, we stated that no "direct" field evidence showing DNRA is a significant N₂O source in sediment. In Welsh et al. (2001), the parallel measurements of denitrification by two techniques suggested that denitrification rate measured by IPT_{classic} (N₂ only) can't explain the excess N₂O production in acetylene-block technique under light incubations. They concluded that those excess N₂O should be attributed by DNRA. However, this evidence by Welsh et al. (2001) was thought to be indirect. One of the possibilities was ignored in their paper, the excess N₂O could be ¹⁵N-N₂O from denitrification which was the fraction they didn't quantify in their IPT_{classic} experiments. Nevertheless, we added this paper into References.

p. 6885. Assumption 6. You need to consider the effect of the ¹⁵NO₃⁻ addition on the stimulation of BMA production, which may reduce nitrification, due to completion for ¹⁴NO₃, particularly if this technique is to be used in the light, although BMA can still consume NO₃ in the dark.

Assumption 6 is about nitrification. But this question seems to be related to

substrate competition between BMA and denitrifier. Thus we don't quite follow this comment since nitrification uses NH_3 as substrate, which should not be affected by the addition of $^{15}\text{NO}_3^-$.

Assumptions. What is the effect of N-fixation on this technique? What if the added $^{15}\text{NO}_3^-$ stimulated heterotrophs, sulphate reducers which can fix N?

In brief, if N-fixation coexists with denitrification, the rate of $^{15}\text{N-N}_2$ will be underestimated (net production) due to the consumption of N_2 via synchronous N-fixation. An et al. (2001) has proposed a modified IPT_{classic} to quantify both processes at the same time. On the other hand, since N-fixation does not produce or consume N_2O , no effect on the calculations is related to N_2O production. We have added above sentences into the manuscript.

The following paragraphs are our answer to the reviewer's question, but it is too complicated to put into our paper.

The effect of N-fixation on IPT_{classic} and IPT_{ana} can be resolved. If the N-fixation coexists with denitrification, the net N_2 production (n) should be the net of the gross N_2 production (d) and consumption (f , N-fixation), $d = n + f$. Here, n actually refers to the sum of measurable parameters, P_{28} , P_{29} and P_{30} . For example, An et al. (2001) had proposed a modified IPT_{classic}, which applied MIMS analysis to discern denitrification and N-fixation. In their method, they assumed N-fixer is BMA which uses N_2 mixtures from ambient water and sedimentary denitrification (see Eq. 10, 11, and 12 in An et al., 2001). If anammox was also involved (IPT_{ana}), it would not be difficult to further modify their formulas to quantify N-fixation, denitrification and anammox separately. This issue has great potential to be another paper. Our IPT_{anaN₂O} is similar to IPT_{ana} since N_2O is not involved. However our cryo-focusing IRMS cannot measure $^{28}\text{N}_2$ precisely due to high background $^{28}\text{N}_2$, thus, unable to produce reliable parameter, P_{28} , the critical parameter in the method proposed by An et al. (2001).

In the case of $^{15}\text{NO}_3^-$ addition to stimulate heterotrophs and/or sulphate reducers to fix N_2 , it is reasonable to assume these N-fixer uptakes freshly produced N_2 from denitrification and/or anammox. In other words, the ^{14}N and ^{15}N ratio calculated from gaseous N_2 products ($r_{14-\text{N}_2}$ or $r_{14-\text{N}_2\text{O}}$) should be equal to that from PO^{15}N . Since the N-fixers can be stimulated by $^{15}\text{NO}_3^-$ addition, which is similar to the response of denitrification. We think the linear relationship of $D_{15-\text{N}_2}$ and $^{15}\text{NO}_3^-$ spike should be the net result driven by those two processes which are difficult to separate. Therefore, the quantification of N-fixation and gross denitrification will be a challenge. Finally, we will reveal explicitly that limitations are there and a lot of work to be done in the future though we improved the IPT method one step forward.

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