Appendix A: Equivalence of Eqs. (13) and (15)

Below, we prove that the Eq. (13) is equal to Eq. (15). First of all, Eq. (15) can be rewritten as the following equation which represents an individual datum point instead of a slope from pooled data (Trimmer and Nicholls, 2009).

\[
ra = \frac{2 - 2 \cdot \frac{qN_2}{qN_2O}}{2 - \frac{qN_2}{qN_2O}}.
\]  

(A1)

On the other hand, Eq. (13) is

\[
d = \frac{A_{14}}{D_{14-N_2} + A_{14}}.
\]  

(A2)

By substituting \(D'_{14}\) and \(A_{14}\), respectively, with Eq. (5) and Eq. (6), we can express \(ra\) as

\[
ra = \frac{P_{29} - 2 \cdot r_{14-N_2}O \cdot P_{30}}{P_{29} + P_{30} \cdot (1 - r_{14-N_2}O)}.
\]  

(A3)

Since \(P_{29}/P_{30}\) is equal to \(2 \cdot r_{14-N_2}O\), the \(ra\) can be expressed in terms of \(r_{14}\) after the numerator and the denominator are divided by \(P_{30}\), which is

\[
ra = \frac{2 \cdot r_{14-N_2} - 2 \cdot r_{14-N_2}O}{2 \cdot r_{14-N_2} - r_{14-N_2}O + 1}.
\]  

(A4)

Substituting \(r_{14}\) with \(q\) using Eq. (14) produces Eq. (A1).

Appendix B: Discussions of Assumptions 5 and 6

Assumption 5 assumes that \(NO_3^-\) reduction is the only source of \(NO_2^-\) in anoxic sediment layer. That is, supplies from other potential sources, such as \(NO_2^-\) from...
ammonia oxidation or downward diffusion from overlying water, are insignificant. Under this assumption, the fraction of $^{15}$N in nitrite will be equal to that of nitrate. This assumption is indispensable for all versions of IPT; however, it is difficult to test specifically via IPT itself (see below). Several studies specifically focusing on NO$_2^-$ production showed that NO$_2^-$ in anoxic sediment mainly results from NO$_3^-$ reduction (De Beer, 2000; Meyer et al., 2005; Stief et al., 2002), which supports this assumption. Although it is untestable via IPT itself, some phenomena caused by the violation of the assumption can be recognized through slurry incubation.

Conditions of high anammox activity and significant NO$_2^-$ supply from non-labelled sources to anammox will result in inconsistent outcomes between incubations of intact core and slurry sediment. For example, significant anammox activity can be revealed in slurry incubation after adding $^{15}$NH$_4^+$; meanwhile, a positive correlation between values of $D_{14}$-classic and $^{15}$NO$_3^-$ concentrations should be obtained from the intact core experiment if all NO$_2^-$ comes from labelled sources (e.g. Fig. 7c). On the contrary, if NO$_2^-$ is largely supplied from non-labelled sources a constant value of $D_{14}$-classic will be obtained in the $^{15}$NO$_3^-$ concentration series experiment because N$_2$ produced from anammox will be supported by non-labelled NO$_2^-$. Note that the violation of Assumption 6 below might result in the same inconsistency.

In general, nitrification which uses NH$_4^+$ as the substrate will not be affected by the addition of $^{15}$NO$_3^-$ (Assumption 6). However, an indirect effect might occur in the NO$_3^-$ addition experiment since high $^{15}$NO$_3^-$ concentrations may stimulate benthic microalgae (BMA) and/or anammox activity to deplete NH$_4^+$ thus limiting nitrification. Considering an environment without anammox, reduced nitrification might happen once BMA production is stimulated by the addition of $^{15}$NO$_3^-$. Such
enhanced BMA may decrease coupled nitrification-denitrification ($P_{14n}$). Apparently, the underestimation of $P_{14n}$ causes an underestimate of $D_{14\text{-classic}}$ as the increase of $^{15}\text{NO}_3^-$ concentrations. However, if the growth of BMA doesn’t result in reduction of nitrification, $D_{14\text{-classic}}$ is expected to be independent of $^{15}\text{NO}_3^-$ additions, thus, a negative correlation between values of $D_{14\text{-classic}}$ and $^{15}\text{NO}_3^-$ concentrations should be obtained from intact core incubated in the light condition, theoretically. By comparing $D_{14\text{-classic}}$ responses between the light and dark incubations, the violation of Assumption 6 due to BMA growth can be proved and distinguished with the violation of Assumption 5.

Besides BMA, anammox is another process that might cause nitrification underestimate. Similar to the effect of BMA, this, in turn, diminishes the $\text{NO}_3^-$ supply resulting in an underestimation of $P_{14n}$ and subsequently $D_{14\text{-classic}}$. Possibly, higher $^{15}\text{NO}_3^-$ additions will cause larger degree of underestimation in $D_{14\text{-classic}}$. In contrast, if this is the case anammox must be traceable. In other words, the $^{29}\text{N}_2$ produced from anammox will cause the overestimation of $D_{14\text{-classic}}$. This overestimation of $D_{14\text{-classic}}$ is also grows with increased additions of $^{15}\text{NO}_3^-$. If both anammox and BMA co-exist, the underestimation of $D_{14\text{-classic}}$ caused by diminishing nitrification is compensated by stimulating anammox in different $^{15}\text{NO}_3^-$ treatments. Such compensation blocks a good positive correlation between $D_{14\text{-classic}}$ and the concentration spike of $^{15}\text{NO}_3^-$; more seriously, the positive correlation may even turn into negative correlation. Coupled with significant anammox activity observed in slurry incubation by adding $\text{NH}_4^+$, phenomena observed here thus resembles that caused by the violation of Assumptions 5. In addition, the degree of compensation might respond differently in light and dark incubation, the difference can be used to reveal the competition of BMA and nitrifier, and check the violation of Assumption 6.
Reference of Appendix


Acknowledgements

Special thanks to Mark Trimmer and the anonymous reviewer for constructive comments. This research was supported by the National Science Council, Taiwan (NSC 100-2621-M-001-003-MY3) and the National Natural Science Foundation of China (NSFC 41176059 and B07034).