Technical Note: Simultaneous measurement of sedimentary N_2 and N_2O production and new ^{15}N isotope pairing technique

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Appendix

Appendix A: Equivalence of Eq. (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 individual datum point instead of slope from pooled data (Trimmer and Nicholls, 2009).

$$ra = \frac{2 - 2 \cdot \frac{qN_2}{qN_2O}}{2 - \frac{qN_2}{qN_2O}}. \tag{A1}$$

On the other hand, Eq. (13) is

$$ra = \frac{A_{14}}{D'_{14\text{-}N_2} + A_{14}}. \tag{A2}$$

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

$$ra = \frac{P_{29} - 2 \cdot r_{14\text{-}N_2O} \cdot P_{30}}{P_{29} + P_{30} \cdot (1 - r_{14\text{-}N_2O})}. \tag{A3}$$

Since $P_{29}/\ P_{30}$ equals to $2 \cdot r_{14\text{-}N_2O}$, the ra can be expressed in terms of r_{14} after the numerator and the denominator being divided by P_{30}, which is

$$ra = \frac{2 \cdot r_{14\text{-}N_2} - 2 \cdot r_{14\text{-}N_2O}}{2 \cdot r_{14\text{-}N_2} - r_{14\text{-}N_2O} + 1}. \tag{A4}$$

Substituting r_{14} with q using Eq. (14), and arranging the equation, we get Eq. (A1).

Appendix B: Discussions of Assumption 5 and 6

Assumption 5 assumes 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 nitrate. This assumption is indispensable for all versions of IPT; however, it is difficult to test specifically via IPT itself (see below). Several studies focused particularly on NO_2^- production showed that NO_2^- in anoxic sediment is mainly resulted 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 if slurry incubation is conducted.

Under condition of high anammox activity and significant NO_2^- supply from non-labelled sources to anammox, inconsistent outcomes will be obtained between incubations of intact core and slurry sediment. For example, a significant anammox activity can be revealed in slurry incubation after adding $^{15}NH_4^+$; meanwhile, a positive correlation between values of $D_{14\text{-classic}}$ and $^{15}NO_3^-$ concentrations should be obtained from 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 $D_{14\text{-classic}}$ will be obtained in $^{15}NO_3^-$ 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 happen in NO_3^- addition experiment since high $^{15}NO_3^-$ concentration may stimulate anammox activity to deplete NH_4^+ thus limiting nitrification as a consequence. The decreased nitrification therefore diminishes the NO_3^- supply resulting in an underestimation of $P_{14}n$, the genuine gases production via coupled nitrification-denitrification. The underestimation of $P_{14}n$ of course leads to underestimate of $D_{14\text{-classic}}$. Apparently, higher $^{15}NO_3^-$ additions will cause larger degree of underestimation in $D_{14\text{-classic}}$. On the other hand, if this is the case anammox must be traceable; oppositely, the $^{29}N_2$ produced from anammox will cause the overestimation of $D_{14\text{-classic}}$. This overestimation of $D_{14\text{-classic}}$ is also enlarged as increasing $^{15}NO_3^-$ additions. To summarise, the underestimation of $D_{14\text{-classic}}$ caused by diminishing nitrification will be compensated by stimulating anammox in different $^{15}NO_3^-$ treatments. Such compensation blocks a good positive correlation between $D_{14\text{-classic}}$ and the concentration of $^{15}NO_3^-$ spike. Coupled with significant anammox activity observed in slurry incubation by adding NH_4^+, phenomena observed here thus resembles that caused by the violation of Assumptions 5.

Reference of Appendix

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