

634 **Appendix**

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

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

$$639 \quad ra = \frac{2 - 2 \cdot \frac{qN_2}{qN_2O}}{2 - \frac{qN_2}{qN_2O}}. \quad (A1)$$

640 On the other hand, Eq. (13) is

$$641 \quad ra = \frac{A_{14}}{D'_{14-N_2} + A_{14}}. \quad (A2)$$

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

$$644 \quad ra = \frac{P_{29} - 2 \cdot r_{14-N_2O} \cdot P_{30}}{P_{29} + P_{30} \cdot (1 - r_{14-N_2O})}. \quad (A3)$$

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

$$647 \quad ra = \frac{2 \cdot r_{14-N_2} - 2 \cdot r_{14-N_2O}}{2 \cdot r_{14-N_2} - r_{14-N_2O} + 1}. \quad (A4)$$

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

649 **Appendix B: Discussions of Assumptions 5 and 6**

650 Assumption 5 assumes that  $NO_3^-$  reduction is the only source of  $NO_2^-$  in anoxic  
651 sediment layer. That is, supplies from other potential sources, such as  $NO_2^-$  from

652 ammonia oxidation or downward diffusion from overlying water, are insignificant.  
653 Under this assumption, the fraction of  $^{15}\text{N}$  in nitrite will be equal to that of nitrate.  
654 This assumption is indispensable for all versions of IPT; however, it is difficult to test  
655 specifically via IPT itself (see below). Several studies specifically focusing on  $\text{NO}_2^-$   
656 production showed that  $\text{NO}_2^-$  in anoxic sediment mainly results from  $\text{NO}_3^-$  reduction  
657 (De Beer, 2000; Meyer et al., 2005; Stief et al., 2002), which supports this assumption.  
658 Although it is untestable via IPT itself, some phenomena caused by the violation of  
659 the assumption can be recognized through slurry incubation.

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

671 In general, nitrification which uses  $\text{NH}_4^+$  as the substrate will not be affected by the  
672 addition of  $^{15}\text{NO}_3^-$  (Assumption 6). However, an indirect effect might occur in the  
673  $\text{NO}_3^-$  addition experiment since high  $^{15}\text{NO}_3^-$  concentrations may stimulate benthic  
674 microalgae (BMA) and/or anammox activity to deplete  $\text{NH}_4^+$  thus limiting  
675 nitrification. Considering an environment without anammox, reduced nitrification  
676 might happen once BMA production is stimulated by the addition of  $^{15}\text{NO}_3^-$ . Such

677 enhanced BMA may decrease coupled nitrification-denitrification ( $P_{14n}$ ). Apparently,  
678 the underestimation of  $P_{14n}$  causes an underestimate of  $D_{14\text{-classic}}$  as the increase of  
679  $^{15}\text{NO}_3^-$  concentrations. However, if the growth of BMA doesn't result in reduction of  
680 nitrification,  $D_{14\text{-classic}}$  is expected to be independent of  $^{15}\text{NO}_3^-$  additions, thus, a  
681 negative correlation between values of  $D_{14\text{-classic}}$  and  $^{15}\text{NO}_3^-$  concentrations should be  
682 obtained from intact core incubated in the light condition, theoretically. By comparing  
683  $D_{14\text{-classic}}$  responses between the light and dark incubations, the violation of  
684 Assumption 6 due to BMA growth can be proved and distinguished with the violation  
685 of Assumption 5.

686 Besides BMA, anammox is another process that might cause nitrification  
687 underestimate. Similar to the effect of BMA, this, in turn, diminishes the  $\text{NO}_3^-$  supply  
688 resulting in an underestimation of  $P_{14n}$  and subsequently  $D_{14\text{-classic}}$ . Possibly, higher  
689  $^{15}\text{NO}_3^-$  additions will cause larger degree of underestimation in  $D_{14\text{-classic}}$ . In contrast,  
690 if this is the case anammox must be traceable. In other words, the  $^{29}\text{N}_2$  produced from  
691 anammox will cause the overestimation of  $D_{14\text{-classic}}$ . This overestimation of  $D_{14\text{-classic}}$   
692 is also grows with increased additions of  $^{15}\text{NO}_3^-$ . If both anammox and BMA co-exist,  
693 the underestimation of  $D_{14\text{-classic}}$  caused by diminishing nitrification is compensated by  
694 stimulating anammox in different  $^{15}\text{NO}_3^-$  treatments. Such compensation blocks a  
695 good positive correlation between  $D_{14\text{-classic}}$  and the concentration spike of  $^{15}\text{NO}_3^-$ ;  
696 more seriously, the positive correlation may even turn into negative correlation.  
697 Coupled with significant anammox activity observed in slurry incubation by adding  
698  $\text{NH}_4^+$ , phenomena observed here thus resembles that caused by the violation of  
699 Assumptions 5. In addition, the degree of compensation might respond differently in  
700 light and dark incubation, the difference can be used to reveal the competition of  
701 BMA and nitrifier, and check the violation of Assumption 6.

702 **Reference of Appendix**

703 De Beer, D.: Potentiometric microsensors for *in situ* measurements in aquatic  
704 environments, in: In situ monitoring of aquatic systems: chemical analysis and  
705 speciation., edited by: Buffle, J., and Horvai, G., Wiley, 161-194, 2000.

706 Meyer, R. L., Risgaard-Petersen, N., and Allen, D. E.: Correlation between anammox  
707 activity and microscale distribution of nitrite in a subtropical mangrove  
708 sediment, Appl. Environ. Microb., 71, 6142-6149, 2005.

709 Stief, P., Beer, D., and Neumann, D.: Small-scale distribution of interstitial nitrite in  
710 freshwater sediment microcosms: the role of nitrate and oxygen availability, and  
711 sediment permeability, Microb. Ecol., 43, 367-377, 2002.

712 Trimmer, M., and Nicholls, J. C.: Production of nitrogen gas via anammox and  
713 denitrification in intact sediment cores along a continental shelf to slope transect  
714 in the North Atlantic, Limnol. Oceanogr., 54, 577-589, 2009.

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