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Application of the isotope pairing technique in sediments where anammox and denitrification coexist

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Web Appendix 1

Relationship between N_2 gasses produced via denitrification or anammox

Denitrification—In a ^{15}N labeling experiment, the denitrification process will produce 3 isotopic N_2 species, $^{14}N^{14}N$, $^{14}N^{15}N$, and $^{15}N^{15}N$. If the ratio between ^{15}N labeled and unlabeled NO_x^- species is constant in the NO_x^- reduction zone, the produced isotopic N_2 species will be binomially distributed.

The ratio between $^{14}N^{14}N$ and $^{14}N^{15}N$ produced via denitrification, i.e., D_{28} and D_{29}, respectively, will then be as follows:

$$\frac{D_{28}}{D_{29}} = \frac{x^2}{2 \cdot x \cdot y} = \frac{x}{2 \cdot y} = \frac{r_{14}}{2} \tag{1.1}$$

where x and y are the mole fractions of $^{14}NO_x^-$ and $^{15}NO_x^-$, respectively, in the NO_x^- reduction zone—e.g., $x = {}^{14}NO_x^-/[{}^{14}NO_x^- + {}^{15}NO_x^-]$, and r_{14} is the ratio between $^{14}NO_x^-$ and $^{15}NO_x^-$ in the NO_x^- reduction zone.

D_{28} can then be expressed as a function of D_{29}:

$$D_{28} = D_{29} \cdot \frac{r_{14}}{2}. \tag{1.2}$$

Given binomial distribution of the produced N_2 species the ratio between $^{14}N^{15}N$ and $^{15}N^{15}N$ produced via denitrification, i.e., D_{29} and D_{30}, will be as follows:

$$\frac{D_{29}}{D_{30}} = \frac{2 \cdot x \cdot y}{y^2} = 2 \cdot r_{14}. \tag{1.3}$$

D_{29} and D_{30} can then be expressed as functions of each other:

$$D_{29} = 2 \cdot D_{30} \cdot r_{14}, \tag{1.4}$$

$$D_{30} = \frac{D_{29}}{2 \cdot r_{14}}. \tag{1.5}$$

From these equations it is possible to derive the equation used in the classical IPT. The classical IPT presumes that genuine N_2 production (p_{14}) is equivalent to:

$$p_{14} = 2 \cdot D_{28} + D_{29}. \tag{1.6}$$

By substitution with Eq. 1.4 and 1.5, this equation can be expressed as follows:

$$\begin{aligned} p_{14} &= r_{14} \cdot D_{29} + 2 \cdot D_{30} \cdot r_{14} \\ &= r_{14} \cdot (D_{29} + 2 \cdot D_{30}). \end{aligned} \tag{1.7}$$

According to Eq. 1.3, r_{14} can be expressed as:

$$r_{14} = \frac{D_{29}}{2 \cdot D_{30}} \tag{1.8}$$

and substituting r_{14} into Eq. 1.7 with this term gives:

$$p_{14} = \frac{D_{29}}{2 \cdot D_{30}} (D_{29} + 2 \cdot D_{30}), \tag{1.9}$$

which is the classical IPT term.

Anammox—In a ^{15}N-labeling experiment the anammox process will produce 2 isotopic N_2 species: $^{14}N^{14}N$ and $^{14}N^{15}N$. If the ratio between ^{15}N labeled and unlabeled NO_2^- species is constant in the NO_2^- reduction zone the ratio between $^{14}N^{14}N$ and $^{14}N^{15}N$ produced via anammox, i.e., A_{28} and A_{29} can be estimated from simple probability mathematics.

$$\frac{A_{28}}{A_{29}} = \frac{x}{y} = r_{14} \tag{2.1}$$

Thus, A_{28} can be expressed as a function of A_{29}:

$$A_{28} = r_{14} \cdot A_{29}. \tag{2.2}$$

Here we assume for the sake of simplicity that p, q and r_{14} are all equivalent to the terms used above (*see* assumption 2 in the *Materials and procedures* section).