Supplementary Appendix S1 – Derivation of ρ_{reg} (nutrient uptake with regeneration and temporally-varying isotope dilution).

If we assume (as was assumed for the derivations of ρ_0 and ρ_{kan}) that nutrient uptake (ρ) is constant throughout the duration of a nutrient uptake experiment, we can define a series of differential equations defining the rate of change of the substrate (S), the particulate organic matter in the incubation (P) and the total isotope-label in the substrate (^{15}S) and particulate organic matter (^{15}P):

$$\frac{\partial S(t)}{\partial t} = (a-1)\rho \qquad \text{(A1)}$$

$$\frac{\partial P(t)}{\partial t} = (1-a)\rho \qquad \text{(A2)}$$

$$\frac{\partial\, {}^{15}S(t)}{\partial t} = a\rho \times I_P(t) - \rho \times I_S(t) \qquad \text{(A3)}$$

$$\frac{\partial\, {}^{15}P(t)}{\partial t} = \rho \times I_S(t) - a\rho \times I_P(t) \qquad \text{(A4)}$$

where a is the fraction of nutrient uptake that gets recycled in the incubation (as in Kanda et al., 1987), IP(t) is the isotope ratio of P at time t and IS(t) is the isotope ratio of S at time t. It follows that:

$$\frac{\partial I_S(t)}{\partial t} = \frac{a\rho \times I_P(t) - \rho \times I_S(t)}{S(t)} \qquad \text{(A5)}$$

$$\frac{\partial I_P(t)}{\partial t} = \frac{\rho \times I_S(t) - a\rho \times I_P(t)}{P(t)} \qquad \text{(A6)}$$

Ideally, we should solve the above system of equations to determine an unbiased estimate of ρ. Unfortunately, while the above set of differential equations has a closed form solution, the solution cannot be solved for ρ. However, we can make the simplifying assumption that substrate concentration and particulate organic matter concentration remain approximately constant during the incubation (S(t)≈S(0) and P(t)≈P(0)). I note that this approximation will be exactly true if a = 1. It should also be reasonable anytime that ρ is constant throughout the incubation. I also note that S(0) is equal to Namb + Nspk from Eq. 2. Given this assumption, and conservation of ^{15}N in the incubation, we can show that:

$$I_S(t) = \frac{I_P(0) \times P(0) + I_S(0) \times S(0) - I_P(t) \times P(0)}{S(0)} \qquad \text{(A7)}$$

Substituting A7 into A6 and rearranging gives us:

$$\frac{\partial I_P(t)}{\partial t} = \rho\left(\frac{I_P(0)\times P(0)+I_S(0)\times S(0)}{P(0)\times S(t)} - \frac{P(0)+a\times S(t)}{P(0)\times S(t)} I_P(t)\right) \qquad (A8)$$

After rearranging:

$$\int \frac{\partial I_P(t)}{\left(\frac{I_P(0)\times P(0)+I_S(0)\times S(0)}{P(0)\times S(0)} - \frac{P(0)+a\times S(0)}{P(0)\times S(0)} I_P(t)\right)} = \int \rho \partial t \qquad (A9)$$

So:

$$-ln\left(\left|\frac{P(0)+a\times S(0)}{P(0)\times S(0)} I_P(t) - \frac{I_P(0)\times P(0)+I_S(0)\times S(0)}{P(0)\times S(0)}\right|\right) = \frac{P(0)+a\times S(0)}{P(0)\times S(0)} \rho t + C \qquad (A10)$$

Where C is a constant, which, after solving at time t=0, we can show is equal to:

$$C = -ln\left(\frac{I_S(0)-a\times I_P(0)}{P(0)}\right) \qquad (A11)$$

Therefore:

$$\rho = \left(ln\left(\frac{I_S(0)-a\times I_P(0)}{P(0)}\right) - ln\left(\frac{I_P(0)\times P(0)+I_S(0)\times S(0)}{P(0)\times S(0)} - \frac{P(0)+a\times S(0)}{P(0)\times S(0)} I_P(t)\right)\right)\left(\frac{P(0)\times S(0)}{P(0)+a\times S(0)}\right)\frac{1}{t} \qquad (A12)$$

Evaluated at the end of the incubation (t = T), this defines ρ in terms of variables measured in a typical nutrient uptake experiment:

$$\rho = \left(ln\left(\frac{I_S(0)-a\times I_P(0)}{P}\right) - ln\left(\frac{I_P(0)-I_P(t)}{[N_{spk}+N_{amb}]} + \frac{I_S(0)-a\times I_P(t)}{P}\right)\right)\left(\frac{P\times[N_{spk}+N_{amb}]}{P+a\times[N_{spk}+N_{amb}]}\right)\frac{1}{T} \qquad (A13)$$

Eq. A13 should be an unbiased estimate of nutrient uptake rates if S and P remain constant throughout the incubation. This will always be true when nutrient regeneration in the bottle is complete (a=1) and the labeled nutrient is the only form of that element being utilized by organisms during the experiment. Constancy of S and P are also implied by the assumption of constant ρ throughout the incubation experiments made in the derivations of Dugdale and Goering (1967) and Kanda et al. (1987). However, it is a potentially biased estimate when $a \neq 1$ and the concentrations of nutrients change during the incubation.