**Apoplasm model code description**

The apoplasm model was built on the structure of the time-dependent box model for physiological mechanism of nitrogen (N) isotope fractionation during ammonium (NH4+) assimilation. It includes cellular apoplasm, which is the acidic space between the inner plasma membrane and the outer cell wall, into and out of which NH4+ and NH3 can diffuse freely from the cell exterior. We used this model to determine how cyclic retention of NH4+ in the apoplasm, due to the low apoplasmic pH, influences N isotope fractionation on the external NH4+. The constants and variables used in the model are described below.

**Constants**

0.00367 – initial ratio of 15N/14N for all the N pools other than external NH4+ and apoplasmic NH4+

0.003693 – initial ratio of 15N/14N for external NH4+ and apoplasmic NH4+

OH – concentration of OH- in the apoplasm at pH 6.5 or 8.0 (mol L-1)

1.76 x 10-5 – equilibrium constant for protonation of NH3

1.58 x 10-6 – concentration of OH- in the external medium at pH 8.2 (mol L-1)

1.0 x 10-7 – concentration of OH- in the cytoplasm at pH 7.0 (mol L-1)

mu\_max – maximum specific growth rate (s-1)

alpha\_GS – N isotope fractionation factor for glutamine synthetase (GS)

Mult – multiplier of the maximum specific growth rate for NH4+ uptake

PC\_NH3 – permeability coefficient for NH3 (cm s-1)

PC\_NH4 – permeability coefficient for NH4+ (cm s-1)

1.0 x 10-5 – diffusivity of NH4+ or NH3 (cm2 s-1)

3.565 – beta, a term in the constant field equation for NH4+ diffusion across the cellular membrane

exp\_beta – exponent of 3.565

vol\_apo – volume of the apoplasm with internal radius of 6.5 µm and external radius of 6.6 µm (L)

Surface area of the phytoplankton cell – 5.3 x 10-6 cm-2

Cellular N quota – 1.66 x 10-6 µmol N cell-1

Volume of the phytoplankton cell – 1.15 x 10-12 L

epsilon\_equil – NH4+-NH3 equilibrium isotope effect (‰)

602409 – initial cell density (cells L-1)

**Variables**

CellN14 – 14N in the phytoplankton nitrogen in the culture (µmol L-1)

CellN15 – 15N in the phytoplankton nitrogen in the culture (µmol L-1)

NH4\_apo14 – 14N in the apoplasmic NH4+ in the culture (µmol L-1)

NH4\_apo15 – 15N in the apoplasmic NH4+ in the culture (µmol L-1)

NH4\_in14 – 14N in the cytoplasmic NH4+ in the culture (µmol L-1)

NH4\_in15 – 15N in the cytoplasmic NH4+ in the culture (µmol L-1)

NH4\_out14 – 14N in the external NH4+ pool (µmol L-1)

NH4\_out15 – 15N in the external NH4+ pool (µmol L-1)

MMuptake – Michaelis-Menten term for NH4+ uptake with half-saturation constant of 50 nmol L-1

Cell\_N\_total – phytoplankton nitrogen in the culture (µmol L-1)

CellsL – cell density per liter of the culture media (cells L-1)

NH4cytoplasm – cytoplasmic NH4+ pool in the culture (µmol L-1)

NH3cytoplasm – cytoplasmic NH3 pool in the culture (µmol L-1)

NH4\_cytoplasm – cellular concentration of NH4+ in the cytoplasm (µmol L-1)

C\_NH4\_apo – cellular concentration of NH4+ in the apoplasm (µmol L-1)

NH3\_cytoplasm – cellular concentration of NH3 in the cytoplasm (µmol L-1)

C\_NH3\_apo – cellular concentration of NH3in the apoplasm (µmol L-1)

NH4\_out\_total – external NH4+ pool (µmol L-1)

F15NH4out – fraction of 15N in the external NH4+

F15NH4\_apo – fraction of 15N in the apoplasmic NH4+

NH3\_out – external NH3 pool (µmol L-1)

Delta\_NH3cyt – difference in NH3 concentration between the cytoplasm and apoplasm (µmol L-1)

Delta\_NH4cyt – difference in NH4+ concentration between the cytoplasm and apoplasm (µmol L-1)

DeltaC\_NH3 – difference in NH3 concentration between the external medium and apoplasm (µmol L-1)

DeltaC\_NH4 – difference in NH4+ concentration between the external medium and apoplasm (µmol L-1)

Q – a term used in determination of rates of NH4+ and NH3 diffusion between the external medium and apoplasm according to Fick’s second law of diffusion (s-1)

ENH3 – a term used in determination of the rate of NH3 efflux from the cytoplasm to apoplasm (s-1)

d15NH4cyt – N isotope composition of NH4+ in the cytoplasm (‰ vs. air)

d15NH3cyt – N isotope composition of NH3 in the cytoplasm (‰ vs. air)

R15NH3cyt – ratio of 15N in the cytoplasmic NH3

F15NH3cyt – fraction of 15N in the cytoplasmic NH3

EffluxNH314 – rate of 14N NH3 efflux from the cytoplasm to the apoplasm in the culture (µmol s-1)

ENH4 – rate of cellular NH4+ efflux from the cytoplasm to the apoplasm (µmol cell-1 s-1)

F15NH4cyt – fraction of 15N in the cytoplasmic NH4+

EffluxNH414 – rate of 14N NH4+ efflux from the cytoplasm to the apoplasm in the culture (µmol s-1)

MM\_GS – Michaelis-Menten term for GS with half-saturation constant of 10 µmol L-1

GS14 – rate of 14N NH4+ condensation with glutamate by GS in the culture (µmol s-1)

EffluxNH315 – rate of 15N NH3 efflux from the cytoplasm to the apoplasm in the culture (µmol s-1)

EffluxNH415 – rate of 15N NH4+ efflux from the cytoplasm to the apoplasm in the culture (µmol s-1)

GS15 – rate of 15N NH4+ condensation with glutamate by GS in the culture (µmol s-1)

UptakeNH414 – rate of 14N NH4+ uptake into the cytoplasm via AMTs in the culture (µmol s-1)

UptakeNH415 – rate of 15N NH4+ uptake into the cytoplasm via AMTs in the culture (µmol s-1)

d15NH4\_out – N isotope composition of NH4+ in the external pool (‰ vs. air)

d15NH3\_out – N isotope composition of NH3 in the external pool (‰ vs. air)

R15NH3\_out – ratio of 15N in the external NH3

F15NH3\_out – fraction of 15N in the external NH3

lnNH4\_out – negative natural logarithm of the external NH4+

d15NH4apo – N isotope composition of NH4+ in the apoplasmic NH4+ (‰ vs. air)

NH4diffout14 – rate of 14N NH4+ diffusion from the apoplasm to the external medium in the culture (µmol s-1)

NH4diffout15 – rate of 15N NH4+ diffusion from the apoplasm to the external medium in the culture (µmol s-1)

NH3\_diffin14 – rate of 14N NH3 diffusion from the external medium to the apoplasm in the culture (µmol s-1)

NH3\_diffin15 – rate of 15N NH3 diffusion from the external medium to the apoplasm in the culture (µmol s-1)

NH4\_diffin14 – rate of 14N NH4+ diffusion from the external medium to the apoplasm in the culture (µmol s-1)

NH4\_diffin15 – rate of 15N NH4+ diffusion from the external medium to the apoplasm in the culture (µmol s-1)