**Model code description**

To query the physiological mechanism of nitrogen (N) isotope fractionation during ammonium (NH4+) assimilation, we constructed a time-dependent finite-differencing box model that tracks discrete 14N and 15N in the different N pools during the growth of marine algae with NH4+ as the sole N source. The model entails three N reservoirs: external N (medium), intracellular reservoir, and the phytoplankton nitrogen (biomass). We prescribe a non-fractionating active NH4+ uptake by the cells via specialized NH4+ transport proteins (AMTs), that follows Michaelis-Menten kinetics with half-saturation constant of 50 nM. Inside the cell, NH4+ is condensed with glutamate by glutamine synthetase (GS) to biomass – the rate-determining and isotope-fractionating step for NH4+ assimilation. The isotope fractionation imparted internally is then communicated to the external medium largely by passive diffusion of ammonia (NH3). A full description of the physiological model and the different scenarios tested can be found in the article. Below is an outline of the various constants and variables used in the model.

**Constants**

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

0.003695 – initial ratio of 15N/14N for external NH4+

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)

1.0 x 10-9 – concentration of OH- in the vacuole at pH 5.0 (mol L-1)

µmax – maximum specific growth rate (hr-1)

VGSmax – maximum GS rate for NH4+ condensation with glutamate (hr-1)

alphaGS – N isotope fractionation factor for GS

alphaCat – N isotope fractionation factor for catabolic NH4+ production

MultLA – multiplier of the maximum specific growth rate for the low-affinity uptake

MultHA – multiplier of the maximum specific growth rate for the high-affinity uptake

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

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

BetaNH4 – a term in the constant field equation for NH4+ diffusion across the cellular membrane

expBetaNH4 – exponent of BetaNH4

BetaVac – a term in the constant field equation for NH4+ diffusion across the vacuolar membrane

expBetaVac – exponent of BetaVac

SA – surface area of the phytoplankton cell (cm-2)

SAVac – surface area of the cell vacuole (cm-2)

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

Phytoplankton cell volume – 1.15 x 10-12 L

Vacuole volume – 3.45 x 10-13 L

NH4+-NH3 equilibrium isotope effect – 15‰

**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)

Gln14 – 14N in the glutamine pool in the culture (µmol L-1)

Gln15 – 15N in the glutamine pool in the culture (µmol L-1)

NH4cyt14 – 14N in the cytoplasmic NH4+ in the culture (µmol L-1)

NH4cyt15 – 15N in the cytoplasmic NH4+ in the culture (µmol L-1)

NH4out14 – 14N in the external NH4+ pool (µmol L-1)

NH4out15 – 15N in the external NH4+ pool (µmol L-1)

Vacuole14 – 14N in the vacuolar NH4+ in the culture (µmol L-1)

Vacuole15 – 15N in the vacuolar NH4+ in the culture (µmol L-1)

MMLAU – Michaelis-Menten term for low-affinity NH4+ uptake with half-saturation constant of 30 µmol L-1

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

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

"Cells\_L-1" – cell density per liter of the culture medium (cells L-1)

CytoplasmNH4conc – cellular concentration of NH4+ in the cytoplasm (µmol L-1)

CytoplasmNH3conc – cellular concentration of NH3 in the cytoplasm (µmol L-1)

NH4concOut – external NH4+ pool (µmol L-1)

F15NH4out – fraction of 15N in the external NH4+

NH3concOut – external NH3 pool (µmol L-1)

Delta\_NH3in – difference in NH3 concentration between the cytoplasm and external medium (µmol L-1)

EffluxNH3 – rate of cellular NH3 efflux from the cytoplasm to the external medium (µmol cell-1 hr-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

VENH314 – rate of 14N NH3 efflux from the cytoplasm to the external medium in the culture (µmol hr-1)

Delta\_NH4in – difference in NH4+ concentration between the cytoplasm and external medium (µmol L-1)

EffluxNH4 – rate of cellular NH4+ efflux from the cytoplasm to the external medium (µmol cell-1 hr-1)

F15NH4cyt – fraction of 15N in the cytoplasmic NH4+

VENH414 – rate of 14N NH4+ efflux from the cytoplasm to the external medium in the culture (µmol hr-1)

VINH3Vac14 – rate of 14N NH3 influx from the cytoplasm to the vacuole in the culture (µmol hr-1)

MMGS – 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 hr-1)

GlnConc – cellular concentration of glutamine (µmol L-1)

MMGln – Michaelis-Menten term for glutamate synthase (GOGAT) with half-saturation constant of 700 µmol L-1

F15Gln – fraction of 15N in the glutamine pool

GOGAT14 – rate of assimilation of 14N glutamine into phytoplankton nitrogen by GOGAT in the culture (µmol hr-1)

VENH315 – rate of 15N NH3 efflux from the cytoplasm to the external medium in the culture (µmol hr-1)

VENH415 – rate of 15N NH4+ efflux from the cytoplasm to the external medium in the culture (µmol hr-1)

VINH3Vac15 – rate of 15N NH3 influx from the cytoplasm to the vacuole in the culture (µmol hr-1)

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

GOGAT15 – rate of assimilation of 15N glutamine into phytoplankton nitrogen by GOGAT in the culture (µmol hr-1)

VENH4Vac14 – rate of 14N NH4+ efflux from the vacuole to the cytoplasm in the culture (µmol hr-1)

VENH4Vac15 – rate of 15N NH4+ efflux from the vacuole to the cytoplasm in the culture (µmol hr-1)

VU14 – rate of 14N NH4+ uptake into the cytoplasm via AMTs in the culture (µmol hr-1)

alphaTR – sigmoidal parametrization of N isotope fractionation factor for NH4+ transport via AMTs

VU15 – rate of 15N NH4+ uptake into the cytoplasm via AMTs in the culture (µmol hr-1)

d15NCell – N isotope composition of cellular N (‰ vs. air)

d15NH4Vac – N isotope composition of NH4+ in the vacuole (‰ vs. air)

d15NH3Vac – N isotope composition of NH3 in the vacuole (‰ vs. air)

d15NH4out – N isotope composition of NH4+ in the external pool (‰ vs. air)

NH4concVac – vacuolar concentration of NH4+ (µmol L-1)

NH3concVac – vacuolar concentration of NH3 (µmol L-1)

Delta\_NH3Vac – difference in NH3 concentration between the cytoplasm and vacuole (µmol L-1)

Delta\_NH4Vac – difference in NH4+ concentration between the cytoplasm and vacuole (µmol L-1)

EffluxNH4Vac – rate of cellular NH4+ efflux from the vacuole to the cytoplasm (µmol cell-1 hr-1)

F15NH4Vac – fraction of 15N in the vacuolar NH4+

InfluxNH3Vac – rate of cellular NH3 influx from the cytoplasm to the vacuole (µmol cell-1 hr-1)