Supplementary Material

1 Description of the model

A zero-dimensional model describing the main diagenetic reactions affecting dissolved and particulate nitrogen species in the sediment was constructed. The state variables, two derived species and their units are presented in Table 1, a summary of the biochemical reactions is given in Table 2, the model equations can be found in Table 3 and the parameters with their units are listed in Table 4.

The biochemical equations were formulated as in the diagenetic model OMEXDIA (Soetaert et al., 1996). The rates are proportional to the concentration of the dissolved or particulate species taking part to the reaction; rate limiting terms are expressed by a Monod (hyperbolic) function; inhibition terms are represented by a reciprocal hyperbolic function.

The model distinguishes between light and heavy nitrogen isotopes, denoted as ^{14}N and ^{15}N respectively. It was assumed that isotope fractionation was insignificant, and the same values of reaction rates, limitation and inhibition constants were used in the biogeochemical reactions involving the two isotopic species.

OMEXDIA describes two fractions of organic matter which differ in degradability and C/N ratio; a fast decaying fraction (see Table 1) with Redfield stoichiometry and a slow decaying fraction with a higher C/N ratio of 7.5 (Soetaert et al., 1996). Due to the short time of the experiment, the less labile fraction was not dynamically described here; rather a constant degradation was assumed. The fast decaying fraction is dynamically modeled using a first order degradation rate. The model includes three different mineralization pathways: oxic mineralization (Tab 3.a, Eqs. 2 and 3), consuming oxygen (see Tab 2 and Tab 3), anoxic mineralization (Tab 3.a, Eqs. 4 and 5), consuming oxidants other than oxygen and nitrate and denitrification (Tab 3.a, Eqs. 6 and 7) using nitrate as the terminal electron acceptor.

As in OMEXDIA, all the organic matter oxidation pathways proceed at the same degradation rate but unlike the original model formulation, the ammonium produced via oxic mineralization is not directly oxidized to nitrate, so that competition between nitrification (Table 2 and Table 3.a, Eq.1) and ammonium uptake (Table 3.a, Eq. 8) can occur (Hochard et al., 2010).

Four different simulations were run to reproduce the different scenarios of the labeling study: under dark and under light conditions, and for homogenized sediment spiked with, in the same concentrations, $^{15}N\text{-}NH_4$ in combination with $^{14}N\text{-}NO_x$ (NO_3+NO_2), and $^{15}N\text{-}NO_x$ together with $^{14}N\text{-}NH_4$ (see Materials and methods section for a complete description of the experimental set up).

As bacterial and algal biomass is not explicitly modeled, it is assumed that nitrogen uptake (Table 3a, Eqs. 8 and 10) is a function of the substrate concentration only. In order to clarify the role of microphytobenthos , dark and light conditions were simulated using different expressions for the microbial uptake rate. The initial value of nitrogen uptake rate was assumed to be the same for both dark and light simulations but while it remains constant in the light, it tapers off toward the end of the dark simulation (Table 3.a, Eq. 9).

Uptake of ^{15}N containing compounds produces labeled microbial biomass but, given the short duration of the experiment, it is assumed that mortality of this biomass is negligible, thus there is no production of ^{15}N-detritus. For this reason only the dead organic matter present at the beginning of the experiment is mineralized, with production of $^{14}N\text{-}NH_4$ only.

$^{15}N\text{-}NO_x$ storage into diatoms cell, which selectively removes $^{15}N\text{-}NO_x$ from the dissolved pool, is described by a simple first order reaction proportional to the difference between the internal stored pool and the dissolved $^{15}N\text{-}NO_x$ (Table 3.a, Eq.11).

Ammonium adsorption to sediment particles (Table 3.a, Eq.12), and ammonium desorption (Table 3.a, Eq.13), is assumed to affect only the new added $^{15}N\text{-}NH_4$, i.e. the adsorption-desorption of unlabeled NH_4 is at equilibrium.

Anammox, the anaerobic formation of N_2 from NH_4 and NO_3, is described by a first order reaction proportional to dissolved NH_4 concentration, limited by nitrate and inhibited by oxygen (Table 3.a, Eq. 14). Because of the presence of 15/14N ammonium and nitrate potentially interacting with each other, anammox can lead to the formation of three species of molecular nitrogen with a different isotopic composition: $^{28}N_2$,$^{29}N_2$ and $^{30}N_2$. Although only the production of $^{29}N_2$ was measured, every possible anammox reaction that can potentially take place is included in the model.

Because the data set was not detailed enough for the model to discriminate between the contribution of anammox and denitrification to N_2 production, these two processes were lumped together.

Dissimilatory nitrate reduction to ammonium, an anaerobic process inhibited by oxygen, is modeled as a first order reaction proportional to the concentration of nitrate (Table 3.a, Eq. 15)

Oxygen is consumed by nitrification and oxic degradation of organic matter and is replenished by diffusion at the sediment-water interface (Table 3.a, Eq. 16).

The concentrations of the dissolved species measured at the beginning of the study were used as initial values for the state variables.

The model was implemented in the computing environment R (R Development Core Team, 2011), and solved with the ode function from R-package deSolve (Soetaert et al., 2010).

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2 Sensitivity analysis, parameters fitting and identifiability

The R package FME (Soetaert and Petzoldt, 2009) was used to calibrate the model. The package contains functions that, in a stepwise procedure allow fitting the model to data and assessing the parameter uncertainty.

As a first step, model parameters were manually tuned until a good fit to the data was achieved. From this set of parameters, first the ones to which the model is most sensitive to and which are identifiable (i.e. can be uniquely estimated) were selected. The model was then fitted to the data by finetuning the identifiable parameters, using the pseudo-random search algorithm of Price (Price, 1977; Soetaert and Herman, 2009). Unidentifiable parameters (i.e. linearly correlated in a way that an equal change in different directions has no effect on the model output) were kept at the initial value assigned (Table 4).

Finally the parameter uncertainty was evaluated by a Markov Chain Monte Carlo (MCMC) which generates a sample of the parameter probability density function (PDF, Soetaert and Petzoldt 2009). The parameter PDF was then used to estimate the uncertainty on the model variables. This was achieved running the model several times with a randomly chosen set of parameters; the mean ± standard deviation, and the minimum and the maximum value of model variables at each time point are then represented as envelopes around the mean trajectory (Soetaert and Petzoldt, 2009).

References:

Hochard, S., Pinazo, C., Grenz, C., Evans, J., and Pringault, O.: Impact of microphytobenthos on the sediment biogeochemical cycles: A modeling approach. Ecological Modelling, 221, 1687-1701, 2010.

Price, W.L.: A controlled random search procedure for global optimisation. The Computer Journal, 20, 367-370, 1977

Soetaert, K., and Herman, P. M. J.: A practical guide to ecological modelling: using R as a simulation platform. Springer Verlag, New York, 2009.

Soetaert, K., Herman, P.M.J., and Middelburg, J. J.: A model of early diagenetic processes from the shelf to abyssal depths. Geochimica Et Cosmochimica Acta 60, 1019-1040, 1996.

Soetaert, K., and Petzoldt T.: FME: A Flexible Modelling Environment for inverse modelling, sensitivity, identifiability, monte carlo analysis. R package version 1, 2009.

Soetaert, K., Petzoldt, T., and Setzer, R.: Solving differential equations in R: Package deSolve. Journal of Statistical Software 33, 1-25, 2010

Table 1a. State variables included in the model

NAME	UNITS	DESCRIPTION
^{14}NOx	µmol /slurry	Concentration of ^{14}N - nitrate+ nitrite
$^{14}NH_4$	µmol /slurry	Concentration of ^{14}N – ammonium
$^{14}NDETf$	µmol /slurry	Concentration of ^{14}N - fast decaying dead organic matter
^{15}NOM	µmol /slurry	Concentration of ^{15}N - organic nitrogen
^{15}NOx	µmol /slurry	Concentration of ^{15}N - nitrate + nitrite
$^{15}NH_4$	µmol /slurry	Concentration of ^{15}N - ammonium
$^{15}NH_{4a}$	µmol /slurry	Concentration of ^{15}N - adsorbed ammonium
$^{15}NOxs$	µmol /slurry	Concentration of ^{15}N - internal stored nitrate
N_2	µmol /slurry	Concentration of ^{29}N - dinitrogen
O_2	µmol /slurry	Concentration of oxygen

Table 1b. Model derived species

NAME	UNITS	DESCRIPTION
NOx	µmol /slurry	Concentration of $^{14}N\text{-}NO_x + {}^{15}N\text{-}NO_x$
NH_4	µmol /slurry	Concentration of $^{14}N\text{-}NH_4 + {}^{15}N\text{-}NH_4$

Table 2. Chemical reactions included in the model

Nitrification: $NH_3 + 2O_2 \rightarrow NO_3^- + H_2O + H^+$

Oxic mineralization: $(CH_2O)_\gamma(NH_3) + \gamma O_2 \rightarrow NH_3 + \gamma CO_2 + \gamma H_2O$

Denitrification: $(CH_2O)_\gamma(NH_3) + 0.8\gamma NO_3^- + 0.8\gamma H^+ \rightarrow NH_3 + \gamma CO_2 + 0.4\gamma N_2 + 1.4\gamma H_2O$

Anoxic mineralization: $(CH_2O)_\gamma(NH_3) + An\ oxidant \rightarrow NH_3 + \gamma CO_2 + \gamma ODU + \gamma H_2O$

Anammox: $NO_2^- + NH_4^+ \rightarrow N_2 + H_2O$

DNRA: $NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$

Table 3.a Model equations

$$Nit = R_{nit} * \frac{(O_2)}{(O_2) + K_{oxni}} * [NH_4] \quad (1)$$

$$NDETfmin = R_{denf} * \frac{[O_2]}{(O_2) + K_{oxmin}} * NDET_f \quad (2)$$

$$NDETsmin = pdenf_s * R_{denf} * \frac{[O_2]}{(O_2) + K_{oxmin}} \quad (3)$$

$$NDETfamin = R_{denf} * \frac{K_{oxde}}{(O_2) + K_{oxde}} * \frac{K_{nitrde}}{[NO_x] + K_{nitrde}} * NDET_f \quad (4)$$

$$NDETsamin = pdenf_s * R_{denf} * \frac{K_{oxde}}{(O_2) + K_{oxde}} * \frac{K_{nitrde}}{[NO_x] + K_{nitrde}} \quad (5)$$

$$NDETfden = R_{denf} * \frac{K_{oxde}}{(O_2) + K_{oxde}} * \frac{[NO_x]}{[NO_x] + K_{nitrde}} * NDET_f \quad (6)$$

$$NDETsden = pdenf_s * R_{denf} * \frac{K_{oxde}}{(O_2) + K_{oxde}} * \frac{[NO_x]}{[NO_x] + K_{nitrde}} \quad (7)$$

(for i= light, dark)

$$Ammup = Uptake_i * \frac{[NH_4]}{[NH_4] + K_{amm}} \quad (8)$$

$$Uptake_{light}^{up} = N_{up}; \qquad Uptake_{dark} = (N_{up} - N_{updk}) * \frac{0.1}{0.1 + t} + N_{updk} \quad (9)$$

$$Nitup = Uptake_i * \frac{[NO_x]}{[NO_x] + K_{nitr}} * \frac{K_{ammin}}{[NH_4] + K_{ammin}} \quad (10)$$

$$NO_{xstor} = Rstor * (NO_x - NO_{xs}) \quad (11)$$

$$NH_{4ads} = Rads * NH_4 \quad (12)$$

$$NH_{4des} = Rdes * NH_{4a} \quad (13)$$

$$Anmox = R_{anamox} * \frac{K_{oxax}}{(O_2) + K_{oxax}} * \frac{(NO_x)}{(NO_x) + K_{nitr}} * [NH_4] \quad (14)$$

$$DNRA = R_{dnra} * \frac{K_{oxde}}{(O_2) + K_{oxde}} * [NO_x] \quad (15)$$

$$OXrear = RO_2 * ([O_{2sat}] - [O_2]) \quad (16)$$

Table 3.b Rate of change of the state variables

$$\frac{dNO_x}{dt} = Nit * \frac{(NH_4)}{[NH_4]} - (Nitup + DNRA + 0.8*(NDETfden*CN_{rf} + NDETsden*CN_{rs}) + Anmox) * \frac{[NO_x]}{[NO_x]}$$

$$\frac{dNH_4}{dt} = NDETfmin + NDETfamin + NDETfden + NDETsmin + NDETsamin + NDETsden + DNRA*\frac{(NO_x)}{[NO_x]} - (Nit + Ammup + Anmox)*\frac{[NH_4]}{[NH_4]}$$

$$\frac{dNDETf}{dt} = -NDETfmin - NDETfamin - NDETfden$$

$$\frac{dNOM}{dt} = Ammup * \frac{[NH_4]}{[NH_4]} + Nitup*\frac{(NO_x)}{[NO_x]}$$

$$\frac{dNO_x}{dt} = Nit * \frac{(NH_4)}{[NH_4]} - NOxstor - (Nitup + DNRA + 0.8*(NDETfden*CN_{rf} + NDETsden*CN_{rs}) + Anmox) * \frac{[NO_x]}{[NO_x]}$$

$$\frac{dNH_4}{dt} = DNRA*\frac{(NO_x)}{[NO_x]} - (Nit + Ammup + Anmox) * \frac{[NH_4]}{[NH_4]} - NH_{4ads} + NH_{4des}$$

$$\frac{dNH_{4a}}{dt} = NH_{4ads} - NH_{4des}$$

$$\frac{dNO_{xs}}{dt} = NOxstor$$

$$\frac{dN_2}{dt} = Anmox * \frac{(NH_4)}{[NH_4]} * \frac{(NO_x)}{[NO_x]} + Anmox * \frac{(NH_4)}{[NH_4]} * \frac{(NO_x)}{[NO_x]} + 0.8*\frac{(NO_x)}{[NO_x]} * (NDETfden*CN_{rf} + NDETsden*CN_{rs})$$

$$\frac{dO_2}{dt} = Oxreae - Nit*2 - NDETfmin*CN_{rf} - NDETsmin*CN_{rs}$$

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Name	Value	Description	Unit	References
CNrf	6.6	C:N ratio for fast decay detritus	dimensionless	[Soetaert et al, 1996a]
CNrs	7.5	C:N ratio for slow decay detritus	dimensionless	[Soetaert et al, 1996a]
RO_2	0.001	O_2 reaeration coefficient	day^{-1}	See text
Rnit	0. 8	Nitrification rate	day^{-1}	Fitted
Rdenf	1	Mineralization rate fast decay detritus	day^{-1}	See text
pdenf_s	1.22	Production rate slow versus fast decay detritus	dimensionless	Fitted
Nup	5.26	DIN uptake in light	$\mu mol\ L^{-1}\ day^{-1}$	See text
Nupdk	3.83	DIN uptake in the dark	$\mu mol\ L^{-1}\ day^{-1}$	Fitted
Koxni	5	Monod constant for O2 limitation in nitrification	$\mu mol\ L^{-1}$	[Soetaert et al, 1996a]
Koxde	10	Monod ct for O2 inhibition of denitrification	$\mu mol\ L^{-1}$	[Soetaert et al, 1996a]
Koxax	12.8	Monod ct for O2 inhibition of anammox	$\mu mol\ L^{-1}$	Fitted
Koxmin	33.7	Monod ct for O2 limitation oxic mineralization	$\mu mol\ L^{-1}$	Fitted
Kamm	7.7	Ammonium half saturation constant	$\mu mol\ L^{-1}$	Fitted
Knitr	36.4	Nitrate half saturation constant	$\mu mol\ L^{-1}$	Fitted
Knitrde	$33.7*10^2$	Monod ct for NO3 inhibition of anaerobic mineralization and limitation in denitrification	$\mu mol\ L^{-1}$	Fitted
Kammin	1.87	Monod ct for NH4 inhibition of NO3 uptake	$\mu mol\ L^{-1}$	Fitted
Rdnra	0.012	DNRA rate	day^{-1}	Fitted
Ranamox	0.149	Anammox rate	day^{-1}	Fitted
Rads	0.5	Ammonium adsorption coefficient	day^{-1}	See text
Rdes	0.2	Ammonium desorption coefficient	day^{-1}	See text
Rstor	0.35	Selective removal of 15Nitrate	day^{-1}	Fitted

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