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# Nitrogen turnover in a tidal flat sediment: assimilation and dissimilation by bacteria and benthic microalgae

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**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## Abstract

In a short-term (24 h)  $^{15}\text{N}$ -labeling experiment, we investigated reactive nitrogen cycling in a tidal flat sediment, focusing on the relative importance of assimilatory versus dissimilatory processes and the role of benthic microalgae therein.  $^{15}\text{N}$ -labeled ammonium and nitrate were added separately to homogenized sediment, and  $^{15}\text{N}$  was subsequently traced into sediment and dissolved inorganic nitrogen (DIN) pools. Integration of results in a N-cycle model allowed us to quantify rates for the major assimilatory and dissimilatory processes in the sediment.

Overall, results indicate that the balance between assimilation and dissimilation in this tidal mudflat was mainly dependent on the nitrogen source. Nitrate was utilized almost exclusively dissimilatory via denitrification, whereas ammonium was rapidly assimilated, with about a quarter of this assimilation due to benthic microalgae (BMA). Benthic microalgae significantly affect assimilation of ammonium, because in the absence of BMA activity the sediments turns from a net ammonium sink to a net source.

Nitrification rates were initially very high, but declined rapidly suggesting that nitrification rates are low in undisturbed sediments, and that in a dynamic environment like tidal flats, intense and fast nitrification/denitrification of ammonium is common. The driving mechanisms for assimilation or dissimilation accordingly appear to be ruled to a large extent by external physical forcing, with the entire system being capable of rapid shifts following environmental changes.

## 1 Introduction

Nitrogen is a key element in aquatic ecosystems and human-induced elevated inputs of nitrogen are a widespread problem that often results in reduced water quality in coastal regions. The ability of an ecosystem to deal with these increased inputs depends on its ability to remove the excess nitrogen. In coastal ecosystems, the sediment is an important component for uptake, transformation, and removal of reactive nitrogen. In

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



such shallow regions, the sediment acts as an important filter for carbon and nitrogen, removing a large share of riverine nitrate via denitrification (Heip et al., 1995; Deek et al., 2011). Overall, sediments hold a key role in nitrogen cycling, as both oxic and anoxic processes can occur simultaneously and on very small spatial scales.

5 When nitrogen is released from organic matter in the form of ammonium it can enter a cascade of dissimilatory reactions (primarily nitrification and denitrification) in which the nitrogen compounds are converted to yield energy and which eventually leads to permanent loss of N from the sediment as  $N_2$  (e.g. Dalsgaard et al., 2005; Laursen and Seitzinger, 2002). Contrasting these dissimilatory reactions, ammonium and nitrate can also be assimilated by the benthic microbial community, supporting their nitrogen demand for growth (Blackburn, 1979; Blackburn and Henriksen, 1983). These assimilatory processes can retain anthropogenic nitrogen in coastal sediments so that sediments act as a temporary buffer for aquatic nutrient concentrations (Dähnke et al., 2010; van Beusekom et al., 1999).

15 Therefore, a key feature of the sediment with respect to the removal of excess nitrogen is the balance between assimilatory and dissimilatory pathways. Processing pathways of reactive nitrogen in sediments are dependent on various environmental parameters, such as bioturbation (Holstein and Wirtz, 2009) and organic matter availability (Cornwell et al., 1999). Yet, the balance of nitrogen assimilation versus dissimilation in coastal regions has often been merely studied in the water column (e.g. Lipschultz et al., 1986; Andersson et al., 2006; Ward, 2005). In this study, we aimed for a combined assessment of the sedimentary processes, using both experimental and modeling approaches, to investigate this balance in tidal sediments from the eutrophic Scheldt Estuary.

25 An important factor regulating N-cycling in coastal regions is the presence of benthic microalgae (BMA). Interactions of BMA with the heterotrophic community are complex. In nitrogen-rich environments, BMA can increase nutrient fluxes to the sediment (Piehler et al., 2010) and to the benthic heterotrophic community (An and Joye, 2001). Competition for ammonium between BMA and the heterotrophic community can limit

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



nitrification rates (Risgaard-Petersen, 2003; Risgaard-Petersen et al., 2004) under N-starved conditions, so that light exposure can lead to reduced denitrification (Porubsky et al., 2009). BMA can also stimulate the bacterial community via exudation of dissolved organic compounds (Middelboe et al., 1998) and influence denitrification by shifting the oxic/anoxic boundary layer due to oxygen production (Risgaard-Petersen et al., 2005).

Stable isotope ( $^{15}\text{N}$ ) tracer studies have proven to be a valuable tool for quantitatively unraveling the complex benthic N-cycle, shedding light on N-transformations in various environments, such as an intertidal freshwater marsh (Gribsholt et al., 2005, 2006), estuaries (Holmes et al., 2000; Tobias et al., 2003), and marine sediments (Risgaard-Petersen et al., 2005; Thamdrup and Dalsgaard, 2002). In this study, our principal goal was to investigate the balance between assimilatory and dissimilatory processes in the benthic N-cycle and the role of BMA therein.

We performed a labeling study where  $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_3^-$  were added separately to tidal mudflat sediment and the  $^{15}\text{N}$  was traced into various particulate and dissolved N-pools. Integration of results by means of an N-cycle model allowed us to quantify rates for all major N-cycling processes and thereby assess the overall balance between assimilatory and dissimilatory processes in this sediment. To get an estimate of the role of BMA activity on individual processes and the sedimentary N-cycle light versus dark incubations.

## 2 Materials and methods

### 2.1 Sediment sampling

In early October 2008, surface sediment was collected from an intertidal mudflat (Biezelingse Ham) in the brackish/marine section of the Scheldt Estuary (The Netherlands) (Fig. 1). Surface sediment was sampled at low tide by carefully scraping off the oxic top layer of the sediment. 40 l of site water were taken from nearby intertidal drains. In situ temperature of the water was  $16^\circ\text{C}$ , and the salinity was 21.3. Sediment and site

water were returned to the lab within 1 h, where the sediment was sieved (mesh size 1 mm) to remove larger fauna. The sediment was left to settle overnight at 17 °C before the slurries were prepared.

## 2.2 Sediment incubations

5 For the slurry preparation, overlying water was decanted, and 50 ml of the remaining sediment were aliquoted into 250 ml glass bottles during continuous stirring. Site water was added to the bottles to a total volume of approximately 200 ml. Half the bottles were amended with 0.6  $\mu\text{mol } ^{15}\text{NH}_4^+$  (added as  $^{15}(\text{NH}_4)_2\text{SO}_4$  (Sigma, 98 at %  $^{15}\text{N}$ ), the other half was labeled with 0.6  $\mu\text{mol Na}^{15}\text{NO}_3$  (Sigma, 98 at %  $^{15}\text{N}$ ). To ensure identical starting conditions and nutrient concentrations in the different  $^{15}\text{N}$ -treatments, the same amounts of unlabeled  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were added to the  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  treatments, respectively. After nutrient and label additions, bottles were shaken to ensure homogeneous distribution of the added substrates. Final labeling percentage in the sediment slurries was 4.7 at %  $^{15}\text{N}$  for the  $^{15}\text{N}$ -nitrate additions and 4.5 at %  $^{15}\text{N}$  for the  $^{15}\text{N}$ -ammonium additions, meaning that both compounds were added at tracer level. Incubation bottles were kept at 17 °C with open caps to enable free gas exchange. To capture a maximal range of algal activity, parallel batches of slurry bottles were incubated in dark and light. Incubations were performed for 0.1, 0.5, 1, 3, 6 and 24 h. At the respective incubation time points, two replicate bottles from each treatment were homogenized to ensure equal nutrient and label distribution, followed by freezing in liquid  $\text{N}_2$  to stop microbiological activity. The bottles were then kept frozen at -18 °C until further processing. Natural, unlabeled sediment and water were sampled for analysis of natural abundance of  $^{15}\text{N}$  in the various pools analyzed for  $^{15}\text{N}$  content (see below).

## 2.3 Extraction procedures

25 Upon thawing, samples were split into a solid fraction (sediment) and a water fraction (mixture of pore water and overlying water), the total slurry volume was homogenized

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



and then centrifuged (1500 g, 15'). The supernatant was decanted, filtered (GF-D, combusted at 450 °C for 4 h), and frozen for later analysis of concentrations of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  and isotope composition of  $\text{NH}_4^+$  and  $\text{NO}_X^-$  (nitrate + nitrite). Concentrations of ammonium, nitrite and nitrate were measured with standard colorimetric techniques (Grasshoff and Anderson, 1999) in an automated flow-through system.

To distinguish the  $^{15}\text{N}$  in organic matter from ammonium that was bound to sediment particles, a subsample of sediment was transferred to a 2 M KCl solution, shaken vigorously and then centrifuged (1500 g, 15') (Rich et al., 2008). The supernatant was removed, and the pellet was washed 3 times with MilliQ water before the sediment was freeze dried for later isotope analysis.

$^{15}\text{N}$  incorporated into the KCl extracted sediment is assumed to represent  $^{15}\text{N}$  assimilation into organic matter while  $^{15}\text{N}$  in the untreated bulk sediment also includes KCl-extractable  $^{15}\text{N}$  (including sediment-bound  $^{15}\text{NH}_4^+$ ). The latter will be presented as the difference between  $^{15}\text{N}$  in untreated versus KCl extracted sediment.

## 2.4 $^{15}\text{N}$ analyses

### 2.4.1 Nitrate and nitrite

$^{15}\text{N}$  enrichment of nitrite and nitrate were measured by the Indophenol extraction method based on Preston et al. (1998). In a first step, the sample nitrate was reduced to nitrite using cadmium granules. To ensure sufficient reduction of nitrate, 5 ml of sample, containing 0.2 g of cadmium were buffered with an ammonium chloride/hydroxide buffer to a pH of 8.5 (Jones, 1984). Samples were then left to react for 3 h at room temperature on a horizontal shaker at 130 RPM. This step was followed by isotope analysis of nitrate and nitrite as described in detail by Preston et al. (1998). In brief, nitrite was converted to the colour complex Sudan-I by diazotization with naphthyl-I and annilinsulfate. The colour complex was concentrated on a C-18 extraction column, eluted with ethylacetate and derivatized for GC analysis using MTBSTFA (N-(t-butylidimethylsilyl)-N-methyltrifluoroacetamide). Derivatized samples were analyzed

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



by gas chromatography-mass spectrometry (GC-MS) on a Thermo Finnigan Voyager quadrupole mass spectrometer using selected ion monitoring of  $m/z$  305.2 and 306.2. The ratio of these two masses was used to determine the at %  $^{15}\text{N}$  of the samples by comparison with standards of different  $^{15}\text{N}$  enrichment levels (0.3 to 10 at %  $^{15}\text{N}$ ).

#### 2.4.2 Ammonium

$^{15}\text{N}$  enrichment of ammonium was measured by the ammonia diffusion method (Holmes et al., 1998; Sigman et al., 1997). Before use, glass ware used was soaked in 1M HCl over night and rinsed thoroughly with MilliQ water, the chemicals and filters were combusted at 450 °C for 4 h.

Different sample volumes, corresponding to 15  $\mu\text{g}$  of  $\text{NH}_4^+$ -N were aliquoted in 100 ml screw cap bottles (Schott Duran), followed by addition of NaCl solution ( $50\text{ g l}^{-1}$ ) to achieve a final salinity of approx.  $35\text{ g l}^{-1}$  and a final volume of 90 ml. After addition of an ammonia trap (consisting of an acidified GF/D filter sealed between two Teflon membranes) and 0.27 g of MgO per bottle, the bottles were immediately closed tightly and incubated at room temperature on a shaker table (60 rpm) for 14 days. Thereafter, filter packs were dried and filters were placed in tin capsules and analyzed for their  $^{15}\text{N}/^{14}\text{N}$  ratios on an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS, Thermo Delta V).

#### 2.4.3 Sediment samples

$^{15}\text{N}$  enrichment of freeze-dried samples of untreated bulk sediment and of KCl-extracted sediment was analyzed on an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS, Thermo Delta V).

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## 2.4.4 Calculations

Stable isotope results will be presented as concentrations excess  $^{15}\text{N}$  per bottle, which were calculated as: concentration excess  $^{15}\text{N} = [(\text{at}\% \text{ } ^{15}\text{N}_{\text{sample}} - \text{at}\% \text{ } ^{15}\text{N}_{\text{control}})/100] \times [\text{concentration N in sample}]$ . At%  $^{15}\text{N}$  was calculated from  $\delta^{15}\text{N}$  as:  $\text{at}\% \text{ } ^{15}\text{N} = [100 \times R_{\text{standard}} \times (\delta^{15}\text{N}_{\text{sample}}/1000) + 1]/[1 + R_{\text{standard}} \times (\delta^{15}\text{N}_{\text{sample}}/1000) + 1]$  and  $\delta^{15}\text{N}$  was calculated as:  $\delta^{15}\text{N}(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ .  $R = ^{15}\text{N}/^{14}\text{N}$  and  $R_{\text{standard}} = 0.003677$ . To correct for ambient  $^{15}\text{N}$ , unlabeled sediment or water were used as controls.

For an easier comparison of  $^{15}\text{N}$  in particulate and dissolved pools, excess label is expressed as total nmol  $^{15}\text{N}$  per bottle.

## 2.5 Modeling of process rates

To unravel the complex fluxes of nitrogen in the sediment incubations, the experimental data were incorporated into a zero-dimensional N-cycle model. A schematic of the relevant reactions in the model is shown in Fig. 2, the reaction kinetics and a detailed description of the model can be found in the Supplement.

In a 0-D model, processes can occur simultaneously, and there is no vertical resolution, which resembles the conditions in the homogenized slurries, where oxic and anoxic processes will occur simultaneously despite varying diffusion gradients. The relevant biogeochemical reactions that were taken into account in the final version of the model were incorporation of nitrate and ammonium into biomass, mineralization of organic matter (including heterotrophic denitrification), bulk  $\text{N}_2$  production, nitrification, dissimilatory nitrate reduction to ammonium (DNRA), storage of nitrate in diatom cells and physical ad- and desorption processes of ammonium to sediment particles (see Fig. 2). Furthermore, the model was based on the following assumptions:

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





**Nitrogen turnover in  
a tidal flat sediment**

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



1. There is no stable isotope fractionation during biological processing over time.
2. Due to the short incubation time, there is no recycling of  $^{15}\text{N}$ -labeled organic matter to the ammonium pool. Overall mineralization of  $^{14}\text{N}$  organic matter depends on two pools of decaying detritus, one rapidly decomposing fraction and one that decomposes slowly. The former pool represents easily accessible organic matter.

The same process formulation, using the same parameter values were used to describe the four different experimental conditions ( $\text{NO}_3/\text{NH}_4$  addition versus dark/light). To improve the model fit, a process reacting differentially on the two isotope species of  $\text{NO}_x$  needed to be included, in this case preferentially reacting on heavy isotopes. While such preferential turnover of heavy isotopes is not expected for the majority of biological pathways – a basic assumption of labeling studies – any process driven by diffusion (including ion-exchange between pore-water, sediment and biota) will preferentially sequester heavy isotopes upon addition of a labeled substrate until isotopic equilibrium is achieved. Hence, removal rates for the heavier isotope are expected to be fast at the beginning of the experiment, until equilibrium is reached.

The model reflects the different conditions in light and dark incubations by toggling on and off MPB assimilation. The slurries were prepared in light and thus treated equally in the beginning, so initial rates should be identical for dark and light incubations, assuming changes will occur with or some time after the change of light conditions. Light conditions should primarily affect nutrient uptake by BMA, effects on heterotrophic uptake should be less pronounced. We furthermore assume that dissimilatory reaction rates will remain constant in the dark.

## 3 Results

### 3.1 Dissolved inorganic nitrogen concentrations

The total concentration of dissolved inorganic nitrogen (DIN) decreased in both light and dark incubations (Fig. 3). This decrease was most pronounced in the light incubations, where total DIN had decreased by 51 % after one day. An initial drop in ammonium concentrations coincided with increasing nitrite concentration. Barring a DIN maximum after 6 h, concentrations of all 3 inorganic nitrogen species dropped to final values of 27, 0.8 and 12  $\mu\text{M}$  for ammonium, nitrite and nitrate, respectively.

Up to three hours after the start of the experiment, the dark nutrient concentrations were roughly comparable to those measured in light. After this time, the most pronounced difference was a steady increase in ammonium concentrations from 35  $\mu\text{M}$  after 1 h to 53  $\mu\text{M}$  after 24 h in the dark. Overall, the development of the nitrite and nitrate concentration was comparable in light and dark incubations over the course of the entire incubation.

### 3.2 $^{15}\text{N}$ in $\text{NO}_x^-$ and $\text{NH}_4^+$

The label distribution in light or dark incubations was comparable for up to 3 h. Absolute values of measured  $^{15}\text{N}$ -excess in the various pools and model fits are shown in Fig. 4. The relative percentage of the label distribution in different pools during the incubation time is outlined in Fig. 5.

#### 3.2.1 $^{15}\text{N}$ -nitrate additions

In the  $^{15}\text{NO}_3^-$  incubations, the majority of added label was not recovered in the dissolved pool, and we only found a slight  $^{15}\text{N}$  increase in ammonium and organic matter. For the sediment compartment, the increase corresponded in only a small  $\Delta\delta^{15}\text{N}$  of  $\sim 3\text{‰}$ , and is thus subject to some uncertainty. Nevertheless, standard deviations for

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



replicate  $\delta^{15}\text{N}$  measurements were usually better than 0.3‰, and indicate that the data are reasonably robust. Over the course of the entire experiment, a large part of the added label (i.e. the sum of all  $^{15}\text{N}$  recovered after 0.1 h) disappeared. Only a small fraction ( $\sim 8.0\%$  after 24 h) of  $^{15}\text{N}$  was found in ammonium or organic matter, and the main transfer of label to these pools occurred within the first hours of the incubation, when  $^{15}\text{N}$  in KCl-extracted sediment (representing assimilation into organic matter), peaked, and  $^{15}\text{N}$  in ammonium rose to 7.3 and 7.4  $\mu\text{mol}$  per incubation bottle in light and dark, respectively (Fig. 4, Fig. 5). Overall, the comparison of light vs. dark incubations showed only little differences in nitrate incorporation into organic matter or reduction of  $^{15}\text{NO}_x$  to ammonium, while the  $^{15}\text{NO}_x$  pool decreased slightly faster in light than in dark incubations (Fig. 5). Still, the final amount of missing  $^{15}\text{NO}_3^-$  in the budget was roughly comparable, 77% and 72% in dark and light, respectively. The label distributions for these pools show good agreement with the model results, verifying the underlying assumptions (Fig. 4).

### 3.2.2 $^{15}\text{N}$ -ammonium additions

The  $^{15}\text{N}$  in the ammonium additions was mostly recovered in the sediment over the course of the experiment, only minor amounts of label were recovered in oxidized nitrogen components. In both light and dark,  $^{15}\text{NH}_4^+$  was rapidly removed from the dissolved pool, with only 21% and less than 4% remaining in the free ammonium pool after 24 h of dark and light incubations, respectively (Figs. 4 and 5). Initially, a considerable fraction of the label was transferred to the  $\text{NO}_x$  pool, with a peak of  $\sim 65\text{ nmol}$  excess  $^{15}\text{N}$  after 0.5 h (Fig. 4b). In both the light and dark incubations, the portion of labeled  $\text{NO}_x$  then decreased, dwindling at the detection limit after 24 h.

A large fraction of labeled ammonium rapidly appeared in the KCl-extractable pool representing particle bound ammonium (Figs. 4 and 5). After an initial peak a few minutes after slurry preparation (Fig. 4b), the  $^{15}\text{N}$  excess in this pool remained rather stable at around 20% of the total label addition. In the non-extractable nitrogen pool

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



(assumed to be a good approximation of  $^{15}\text{N}$  assimilated into organic matter) excess  $^{15}\text{N}$  increased steadily, containing 56 % and 34 % after 24 h in light and dark, respectively.

Figure 3 shows a comparison of measured and model-predicted pool sizes of ammonium and nitrate/nitrite. Generally, the changes in total pool size of DIN (i.e. predominantly the unlabeled fraction) are predicted well by the model, with ammonium concentrations dropping initially and then rising in the dark incubations, whereas they dropped in the light incubations (Fig. 3). For total  $\text{NO}_x$  concentration (nitrite + nitrate), the model predicts an initial rise in concentration, followed by a decrease towards the end of the incubation, with no significant differences in light and dark incubations, which is in accordance with the data. This suggests that the model assumptions are correct and provide valuable estimates not only of DIN pool sizes, but also of process rates of assimilatory and dissimilatory turnover.

For both nitrogen species, ammonium and nitrate, data were generally well described by the model (Figs. 3 and 4). The only exception is the prediction of the  $^{15}\text{NH}_4^+$  distribution in the  $^{15}\text{N}$ -ammonium incubations: the model overestimates particle bound N in  $^{15}\text{NH}_4^+$  incubations while underestimating it in  $^{15}\text{NO}_3^-$  additions (Fig. 4).

### 3.3 Process rates – model results

Figure 6 gives an overview of the model-derived process rates for the light and dark incubations. Model results for  $^{15}\text{N}$  ammonium and nitrate additions are comparable and are thus not discussed separately. Due to the addition of unlabeled substrates in the different incubations, total initial nutrient concentrations are identical, thus the same should apply to process rates. Generally, there is a pronounced difference in process rates between the first hours of the experiment versus those for the 6–24 h period, which often reduce to but a fraction of the initial rate (Fig. 6).

In both light and dark incubations, assimilation of ammonium clearly out ruled assimilatory nitrate uptake. Initial ammonium uptake rates were as high as

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



0.8  $\mu\text{mol bottle}^{-1} \text{h}^{-1}$ , whereas nitrate uptake occurred at a fraction of this rate,  $\sim 0.02 \mu\text{mol bottle}^{-1} \text{h}^{-1}$  (Fig. 6). Overall uptake rates for nitrate and ammonium were higher in light than in dark incubations. Initial rates of light and dark uptake were comparable, but there was a marked decrease in process rates. Ammonium uptake slowed down to about 75 % of the initial rate, whereas  $\text{NO}_x$  uptake decreased gradually, approaching zero after 24 h (Fig. 6).

Regarding dissimilative processing, the model reveals only few differences between light and dark incubations. In both setups, nitrification is most pronounced at the beginning of the incubation, with initial rates of more than  $5 \mu\text{mol bottle}^{-1} \text{h}^{-1}$ , that decrease to lower, but substantial, turnover rates of  $0.16 \mu\text{mol h}^{-1}$  over the course of the experiment. It was not possible to make a reliable distinction between  $\text{N}_2$  production by denitrification versus anammox. Therefore these two processes are presented as their sum, but gross  $\text{N}_2$  production is remarkable, with maximal rates approaching  $1 \mu\text{mol bottle}^{-1} \text{h}^{-1}$ , leveling off to  $\sim 0.25 \mu\text{mol h}^{-1}$  (Fig. 6). DNRA, however, was quantitatively less important, with rates  $> 0.1 \mu\text{mol h}^{-1}$  only within the first few hours of the incubation.

## 4 Discussion

The evaluation of experimental data provides a good overview of the relative importance of various turnover mechanisms in the slurries, and the comparison of these estimates with the model-derived process rates brings up interesting questions on the controlling mechanisms. In what follows, we will first discuss the process rates that are derived from model and experimental data, and then go into detail regarding the influence of benthic microalgae. We will also specify the added value and insights regarding process rates that we gained from the modeling approach.

### Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## 4.1 The fate of ammonium

In contrast to nitrate processing (see above), the major share of ammonium is turned over via assimilatory pathways, whereas nitrification seems to play a subordinate role. Illumination almost doubled ammonium uptake, suggesting a large share of BMA uptake on total sedimentary ammonium uptake. Overall, assimilatory processes clearly prevail for ammonium turnover, 56 and 34 % of the added label were recovered in the organic matter fraction in light and dark, respectively. In comparison, nitrification appears to be only relevant in the initial phase of the experiment. This is consistent with findings by Blackburn and Henriksen (1983), who investigated nitrogen fluxes in Danish sediments and found that largest fractions of ammonium were either assimilated or bound in the sediments, whereas nitrification was of subordinate role.

However, in the initial incubation phase, we found a clear transfer of label to the  $\text{NO}_x^-$  pool (Fig. 4b) and accordingly high initial nitrification rates of  $\sim 5 \text{ nmol bottle}^{-1} \text{ h}^{-1}$  (Fig. 6). After this, no further label appeared in the  $\text{NO}_x^-$  pool, and nitrification rates dwindled to a steady state of  $0.17 \mu\text{mol bottle}^{-1} \text{ h}^{-1}$ . This decrease was swift and most likely linked to oxygen limitation. During slurry preparation, oxygen was mixed into the sediment, and all preformed biogeochemical gradients were disrupted. Nitrification could thus proceed at a high rate until oxygen was depleted, after which it became limited by oxygen diffusion into the sediment.

Another candidate sink for ammonium is the anammox reaction. We did not measure the production of labeled  $\text{N}_2$  in the slurries and can thus not distinguish between denitrification and anammox, but it seems unlikely that anammox will play a significant role in a shallow, tidal, i.e. dynamic, environment like the tidal flat, because anammox bacteria are slow-growing and thus usually favored by stable environmental conditions (Jensen et al., 2009). Denitrification, the alternative source for dinitrogen, appears to play a more important role, removing large amounts of nitrate in the incubation assay.

**BGD**

9, 6987–7019, 2012

### Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## 4.2 The fate of nitrate

Regardless of the presence or absence of light, there was little assimilation of nitrate into organic matter, and light conditions had only little influence on net nitrate uptake (Figs. 6 and 7). Rates for assimilation of nitrate were negligible in comparison to those for ammonium or to  $N_2$ -production and had no significant effect on nutrient concentrations or label appearance in organic material. Overall, it appears that nitrate was almost exclusively subject to dissimilatory turnover (Fig. 6).

Dissimilatory processing of nitrate (i.e. denitrification or DNRA) decreased rapidly within the first hours of the incubation. Nitrate turnover was mostly coupled to a transport into the “missing label” pool (Fig. 5), which is consistent with the high rates of  $N_2$  production (Fig. 6). Rates of  $N_2$  production decreased during the experiment and were apparently controlled by the availability of easily degradable organic matter. Denitrification depends on the availability of readily available electron acceptors (Pfenning and McMahon, 1997) as they are present in the labile detritus fraction. The concentration of this labile fraction was high at the beginning of the experiment, fuelling high denitrification rates and decreased during the experiment. A further kinetic control on denitrification is exerted by the diffusion of nitrate into the denitrification zone: as the nitrate concentration in the homogenized sediment decreases, less and less nitrate will find its way into the active denitrifying zone in the sediment, with rates consequently leveling off towards the end of the incubation.

Other potential dissimilatory processes like DNRA may also be involved in nitrate turnover. For example, Kamp et al. (2011) found that diatom cells switch to DNRA in the dark, processing nitrate that was previously stored in the cells. For our experiment, though, the model approach revealed that initial DNRA were reasonably high ( $\sim 0.1 \mu\text{mol bottle}^{-1} \text{h}^{-1}$ ), but these rates decreased over the course of the experiment and resulted in little net production of labeled ammonium. Contrastingly, Porubsky et al. (2009) investigated tidal flats in South Carolina and Georgia and found that DNRA was significant and in direct competition with denitrification in sediments. They argue

**BGD**

9, 6987–7019, 2012

### Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



that DNRA appears to be stimulated and favored over denitrification at temperatures above 20 °C. While we used a moderately high temperature of 17 °C, it seems plausible that bacterial communities are adapted to lower temperatures at our study site. This apparently resulted in little quantitative importance of DNRA in our experiment in comparison to the competing process, denitrification, regardless of light conditions or BMA activity.

### 4.3 The balance between assimilatory versus dissimilatory processes

The primary goal of this study was to evaluate the balance between assimilative and dissimilative nitrogen cycling processes in a tidal mudflat sediment exposed to different light levels. By comparing illuminated slurries and those kept in the dark, we attempted to separate the bacterial from the microalgal contribution to uptake and turnover.

A limitation to this approach is that a certain portion of algal N uptake can continue under dark conditions. Evrard et al. (2008), for example, found that dark nitrate uptake was decoupled from carbon assimilation and approached 75 % of the light uptake. For ammonium, dark uptake rates can also approach 20 % of those found in light (Thornton et al., 1999). However, the lack of light clearly reduced algal uptake and provides us with a minimum estimate of their impact in the sediment.

The most important result from our study is that ammonium is predominantly assimilated in the sediment regardless of the light conditions (see Sect. 4.2). Nitrification of ammonium only plays a significant role at the beginning of the experiment, before re-establishment of biogeochemical gradients in the sediments. In contrast, assimilation is of subordinate role for nitrate or nitrite, only 5 and 7 % of the added  $^{15}\text{NO}_3^-$  enter the organic matter pool in light and dark, respectively. Dissimilatory processing of nitrate clearly prevails over the course of the incubation; a grand total of ~ 75% cannot be recovered after 24 h and is eventually denitrified.

But what are the implications for a natural system? Today, the main input of reactive nitrogen to the Scheldt estuary is in the form of nitrate, ammonium comprises <10%

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





of the total reactive nitrogen load (e.g. Soetaert et al., 2006). Our results indicate that nitrate will mainly be removed via denitrification in the estuary.

While net nitrate retention in the Scheldt estuary has decreased, our results indicate that perturbations in the sediment, like we induced by shaking the slurries have a very high impact on the N-cycle. The natural equivalent of this manual shaking is bioturbation or -irrigation and wave- or tide-induced mixing of sediment. Particularly in the outer tidal flat system, the estuary is heavily influenced by tidal forcing (Vanderborght et al., 2007) and waves (Callaghan et al., 2010). Our results suggest that these processes can significantly increase rates of both denitrification and nitrification for a short time period. After re-establishment of stable biogeochemical gradients, other factors, like the occurrence of benthic microalgae, apparently gain in importance. These interactions of physical processing and biological turnover rates should be kept in mind as natural process rates can be altered in disturbed systems due to human interactions not only in the Scheldt estuary, but also in other shallow coastal regions.

#### 4.4 The role of benthic microalgae

The nutrient and label distribution points towards some marked differences between light and dark incubations, providing clear evidence that the activity of benthic microalgae had an impact on sedimentary N-cycling in tidal sediments from the Scheldt estuary.

The most striking difference between light and dark incubations was the change in assimilation rates. Initially, ammonium uptake into organic matter was comparable in light and dark (Fig. 6), but the dark assimilation rate dropped rapidly to ~ 75% of that in light (Fig. 6). Thus, BMA seem to have been responsible for at least a quarter of the overall uptake rate in the light. Interestingly, this was sufficient to convert the sediments from being a net ammonium source (dark incubations, cf. Figs. 3 and 6) into being a net sink for reactive nitrogen.

Besides this obvious effect on ammonium assimilation, this shows that BMA compete with the bacterial community – including nitrifiers – for ammonium. This can lead

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



to reduced nitrification and a lower flux of nitrate from nitrification to denitrification. Coupled nitrification/denitrification often represents a significant share of total denitrification and is reduced when assimilation by MPB is increased. Thornton et al. (2007) explored sedimentary nutrient fluxes in the Colne estuary and found that denitrification was governed by light conditions, with significantly higher rates during dark phases. We did not see such inhibition in our experiment; it is apparently overcome by other factors favouring denitrification. A candidate mechanism is the exudation of organic matter during illumination (Cook et al., 2009): denitrifiers rely not only on nitrate but also on organic substrates, and accordingly, easily accessible sources of organic matter can potentially enhance rates of denitrification.

The combined effect of the observed higher assimilation of ammonium and continuous nitrification/denitrification in the light resulted in a much stronger sequestration of  $\text{NH}_4^+$  in the experiment (cf. Fig. 3) in light. While the sediments still were net total DIN sinks due to strong denitrification in both light and dark, they shifted from being a net sink for ammonium under illuminated conditions to being a net ammonium source in the dark. Such net efflux of ammonium can be due to DNRA in some environments (Fulweiler et al., 2008; Gardner et al., 2006), but the role of DNRA appears to be limited in intertidal Scheldt estuary sediments (see above). We presume that in this case, the shift to net sedimentary ammonium production in the dark is due to dwindling BMA ammonium uptake when light is limiting, not to increasing DNRA.

#### 4.5 Closing the budget – where does the nitrogen go?

One common feature of  $^{15}\text{N}$ -nitrate and  $^{15}\text{N}$ -ammonium addition is the fast disappearance of  $^{15}\text{N}$  from both incubation experiments. For the nitrate incubations, this can be explained by the rapid initiation of denitrification in the anoxic parts of the homogenized sediment. While oxygen was introduced in significant amounts during slurry preparation, the oxygen consumption rate was high enough to ensure fast development of anoxic conditions in subsurface parts of the sediment, allowing denitrification to take

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



place, as illustrated by the  $N_2$ -production rates of  $\sim 1.5 \mu\text{mol bottle}^{-1} \text{h}^{-1}$  at the beginning of the incubation (Fig. 7).

In the  $^{15}\text{N}$ -ammonium incubations, approximately 25 % of the added label could not be recovered over the course of the incubation. However, this pool is a well-known phenomenon in labeling studies (Laws, 1984), and is often attributed to the formation of labeled DON (Bronk and Glibert, 1994; Collos, 1992). The size of this DON pool appears to be rather variable, in estuarine and coastal environments, DON comprises 13 (Berman and Bronk, 2003) to 25 % of the dissolved nitrogen pool (Schlarbaum et al., 2010), which is in accordance with the missing N-pool size in our slurry incubation. The stimulation of algal activity in light can enhance DON production in the slurries, leading to an overall increase in DON and a larger fraction of missing  $^{15}\text{N}$  in light vs. dark (Cook et al., 2009).

Alternatively, some loss of  $^{15}\text{NH}_4^+$  may have been due to rapid and tightly coupled nitrification/denitrification, which can account for 60–100 % of the total denitrification in coastal systems (Laursen and Seitzinger, 2002) From our data, we can not decide which of these two pathways, DON production or coupled nitrification/denitrification likely is responsible for the missing  $^{15}\text{N}$  in the ammonium incubations, but both processes may also act simultaneously, contributing to the “missing N” pool in the ammonium incubations. However, the faster disappearance of  $^{15}\text{N}$ -labeled nitrate in light speaks in favor of the hypothesis of enhanced denitrification rates.

#### 4.6 Selective removal of $^{15}\text{N}$ ?

The model was essential in unraveling our  $^{15}\text{N}$  results and translating these to process rates for the complex benthic N-cycle. In some cases, the initial model assumptions and the experimental data were not entirely consistent and modification of the original conceptual model was required in order for the model to be able to reproduce the experimental results. The most profound difference was that the original model was unable to reproduce the initial decrease in the labeling percentage of nitrate. This offset

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



between data and model could be overcome by inclusion of a process that selectively removed  $^{15}\text{NO}_3^-$  from the total dissolved  $\text{NO}_3^-$  pool (see Sect. 2). We can only speculate on what process accounted for this selective removal, but one probable mechanism is intracellular nitrate storage by diatoms. Diatoms can represent a substantial part of the BMA in the sediment (Sundback et al., 2011) and dominate the BMA community in our study area (Veuger and van Oevelen, 2011). They are known to store nitrate internally in up to millimolar concentrations (Dortch et al., 1984; Pettersson and Sahlsten, 1990; Flynn et al., 2002), thus playing an important role in marine nutrient cycling. It has long been hypothesized that this uptake of nitrate by diatom cells is at least partly regulated via diffusion (Lomas and Glibert, 1999; Serra et al., 1978). Consequently,  $^{15}\text{N}$  will initially diffuse into the cells until equilibrium with  $^{15}\text{N}$  in the environment is achieved. This can explain the rapid decrease in labeled  $^{15}\text{NO}_3^-$  in the dissolved pool in our experiment (Fig. 4a). This pool of internally stored nitrate is not captured in the pool of  $^{15}\text{N}$  in organic matter because the latter involves particulate organic material (i.e. biomass) while intracellular dissolved  $\text{NO}_3^-$  was likely removed by the KCl extraction.

Interestingly, a similar phenomenon can be seen for the removal of  $^{15}\text{NH}_4^+$ . There appears to be a similar process acting preferentially on the heavier ammonium isotope, but it is not easy to infer which process might be responsible for this behavior. Some authors (Lomas and Glibert, 2000; Baek et al., 2008) observed internal storage of excess ammonium in flagellates, but it is not clear whether these mechanisms play a role in our experiment, where sedimentary turnover governs process rates. Furthermore, there is no proof of diffusion-driven uptake of ammonium by these organisms, so the mechanistic basis of this ammonium uptake remains somewhat speculative.

Barring uncertainty about the precise processes involved for ammonium, these selective uptake processes are only revealed by the modeling approach used in this study in addition to the experimental data. This shows that such a combined approach provides valuable additional information on the processes under study – in this case the presence and relevance of diatoms in coastal nitrogen cycling. Consequently, even though our data do not allow the precise identification and quantification of the two

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



mechanisms that are responsible for the selective removal of labeled ammonium or nitrate, our results clearly demonstrate their relevance for interpretation of the stable isotope tracer results and quantification of resulting process rates. This highlights the added value of the combined approach of modeling and experimental data, which allowed us not only to determine process rates for our setup, but also revealed these – unexpected – selective removal mechanisms that we would have been unable to detect using the experimental data alone. Yet, such selective processes have a significant impact on the pool size of labeled compounds, which are the basis for most calculations of process rates. Accordingly, disregarding these processes can obviously lead to erroneous process rate estimations.

## 5 Conclusions

Our results indicate that the balance between assimilation and dissimilation in the investigated sediment differed for ammonium and nitrate. Ammonium was primarily assimilated, while nitrate was utilized almost exclusively in dissimilatory processes like denitrification. There is only little evidence of dissimilatory processes like nitrification beyond the initial phase of the experiment and benthic microalgae apparently did not stimulate dissimilatory pathways in our sediment. Assimilation of ammonium by BMA is substantial to the extent that, if it ceases, sediments in our experiment switched from being a net sink for ammonium to being a net source.

One significant result is the behavior of the slurry at the start of the incubation, which showed highly elevated nitrification rates. This means that, even though nitrification rates on the long term are low, such sediments have a high potential for nitrification which is expressed if they are physically perturbed. In the field, such perturbations are expected by fauna or currents, suggesting that assimilation or dissimilation can be driven to a large extent by external physical or biotic forcing, with the entire system being capable of rapid shifts following environmental changes.

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Our combined experimental and modeling approach highlights the importance of pathways that selectively remove labeled ammonium, as well as nitrate. We can merely speculate on the precise mechanisms involved, but there is further need to study the relevance of these processes in natural environments.

5 **Supplementary material related to this article is available online at:**  
**[http://www.biogeosciences-discuss.net/9/6987/2012/  
bgd-9-6987-2012-supplement.pdf](http://www.biogeosciences-discuss.net/9/6987/2012/bgd-9-6987-2012-supplement.pdf)**

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- 25

**BGD**

9, 6987–7019, 2012

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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**Nitrogen turnover in  
a tidal flat sediment**

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Gardner, W. S., McCarthy, M. J., An, S., Sobolev, D., Sell, K. S., and Brock, D.: Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries, *Limnol. Oceanogr.*, 51, 558–568, 2006.

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**Nitrogen turnover in a tidal flat sediment**

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Nitrogen turnover in  
a tidal flat sediment**

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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**Nitrogen turnover in a tidal flat sediment**K. Dähnke et al.

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[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[I◀](#)[▶I](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

**Nitrogen turnover in  
a tidal flat sediment**

K. Dähnke et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

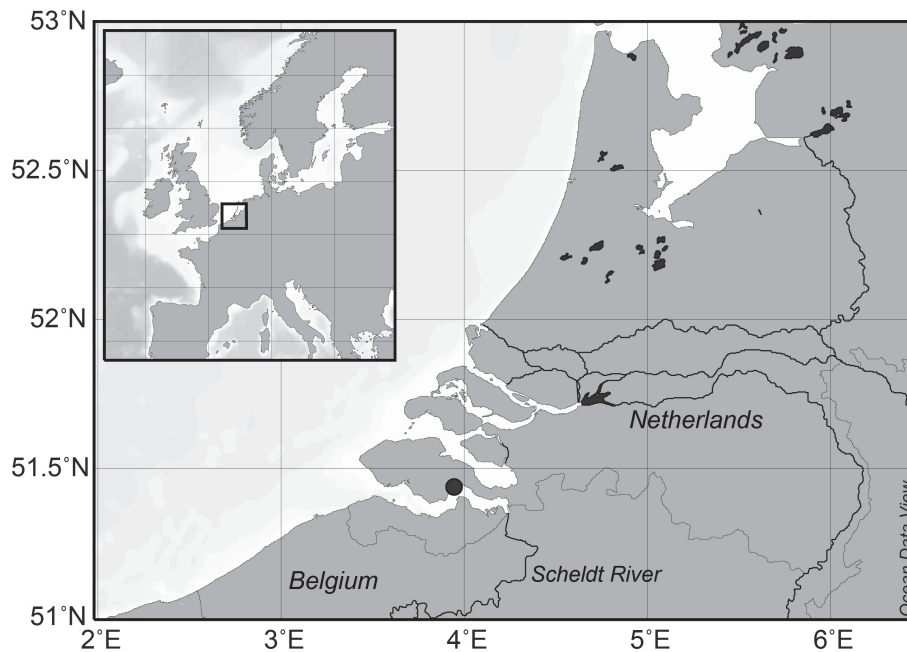
Back

Close

Full Screen / Esc

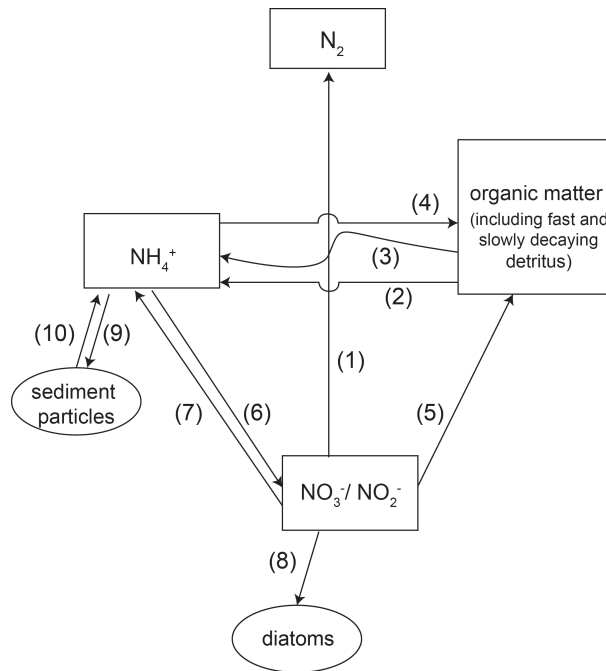
Printer-friendly Version

Interactive Discussion

**Fig. 1.** Map of the study area. Grey circle indicates sampling site.

## Nitrogen turnover in a tidal flat sediment

K. Dähnke et al.



**Fig. 2.** Conceptual diagram of N fluxes in the N-cycle model. Turnover processes: (1) Denitrification; (2) oxic and anoxic mineralization; (3) mineralization coupled to denitrification; (4) ammonium assimilation; (5) nitrate assimilation; (6) Nitrification; (7) DNRA; (8) nitrate storage in diatoms; (9) ammonium adsorption to particles, (10) desorption of ammonium from particles. Processes 8, 9 and 10 specifically apply to  $^{15}N$ -labeled pools (see text, Sect. 2.5, for details).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

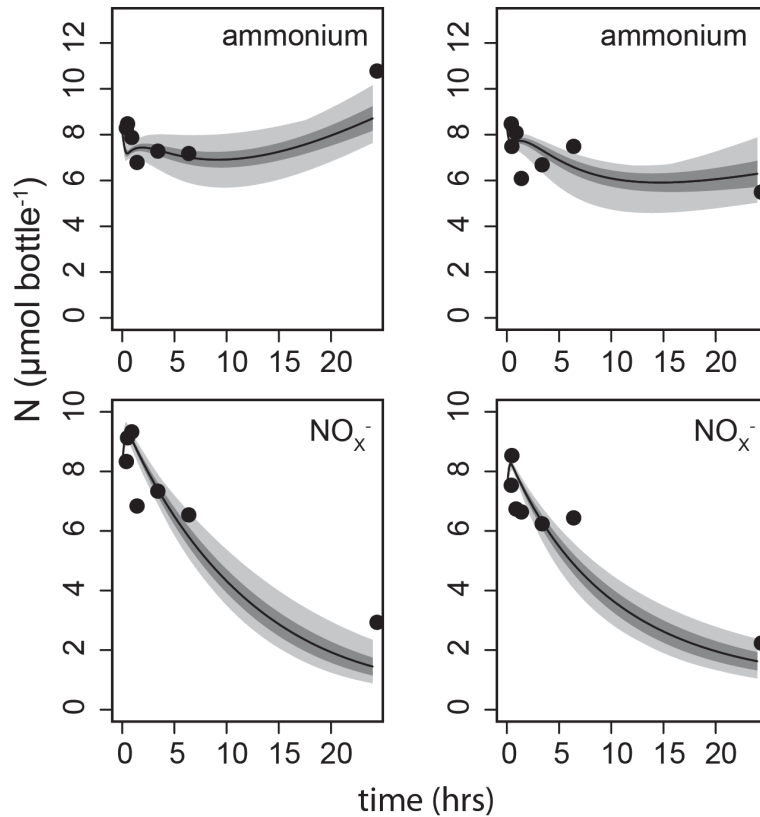
Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





**Fig. 3.** Data and model fits for concentrations of ammonium and nitrate in dark (left side) and light (right side). The dark shaded area of the modeled curve represents one standard deviation, light shading indicates minimum and maximum values.

**Nitrogen turnover in a tidal flat sediment**

K. Dähnke et al.

Title Page

Abstract Introduction

Conclusions References

Tables Figures

◀ ▶

◀ ▶

Back Close

Full Screen / Esc

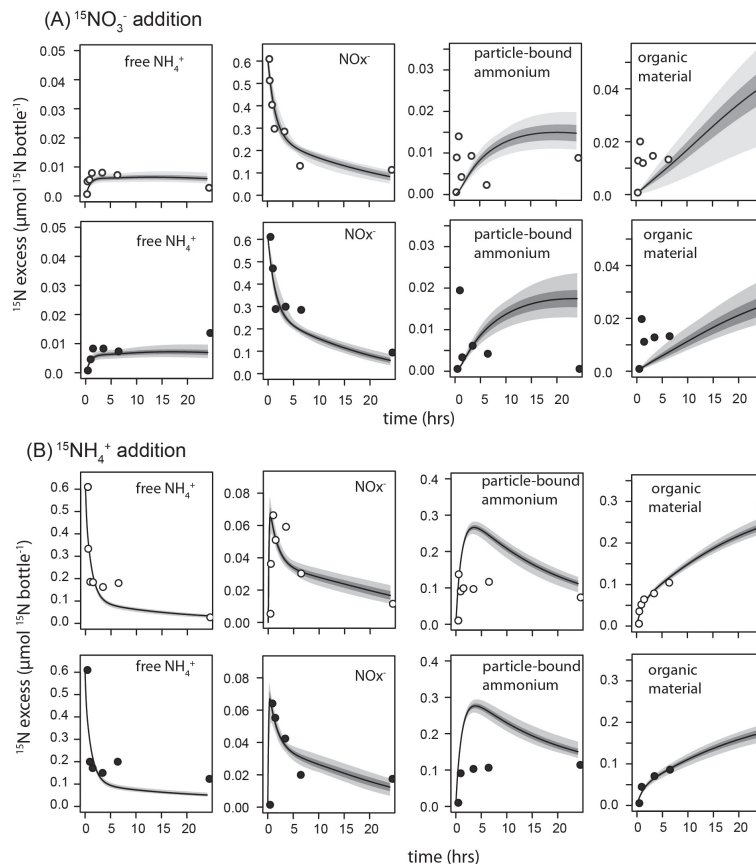
Printer-friendly Version

Interactive Discussion



Nitrogen turnover in  
a tidal flat sediment

K. Dähnke et al.



**Fig. 4.** Measured pools of  $^{15}\text{N}$  for  $^{15}\text{N}$ -nitrate additions **(A)** and  $^{15}\text{N}$ -ammonium additions **(B)** in dark (filled circles) and light (open circles). Solid lines and shading indicate model fits. Dark shaded areas represent one standard deviation, light shading indicates minimum and maximum values. Note different scales of Y-axes.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

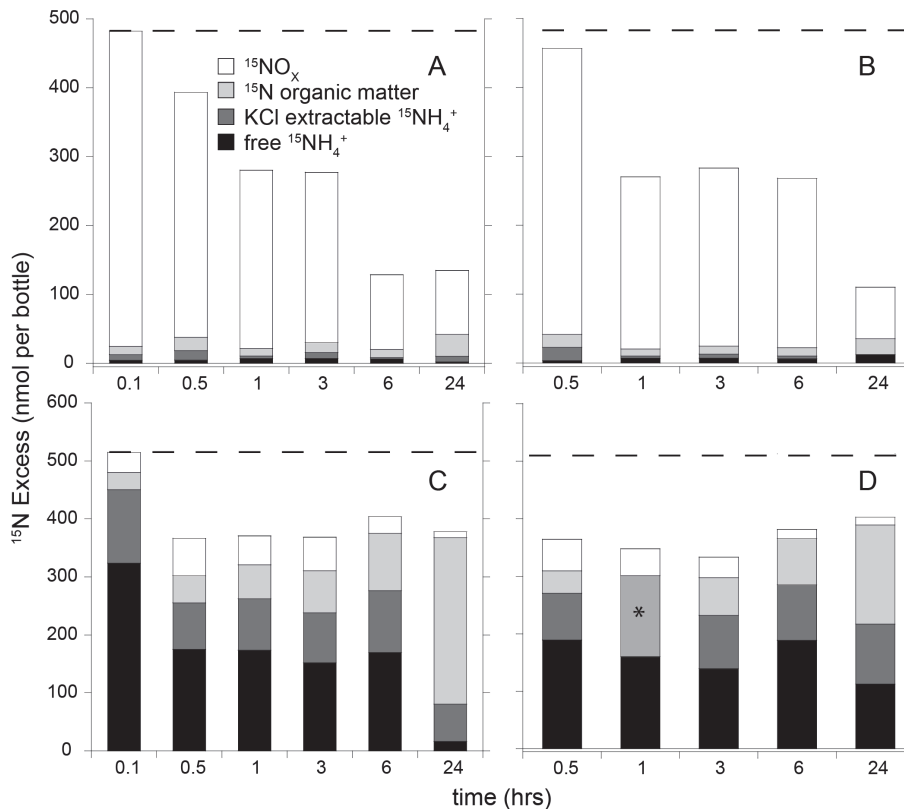
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Full Screen / Esc

Printer-friendly Version

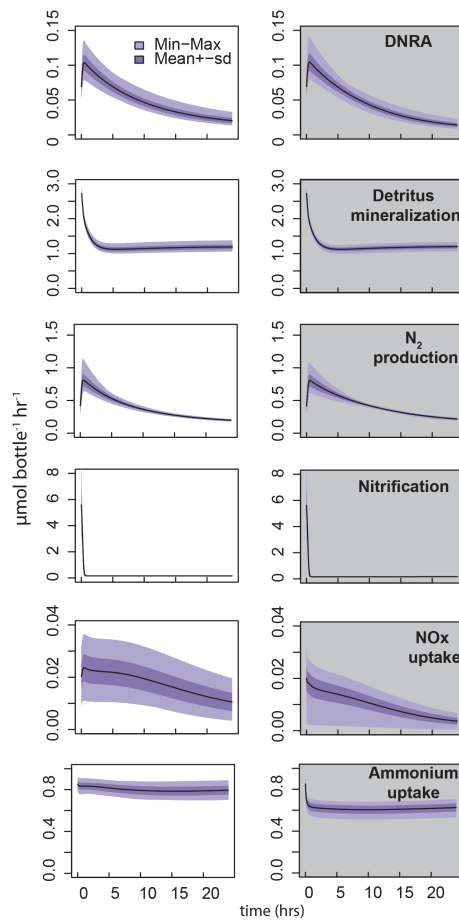
Interactive Discussion





**Fig. 5.** Relative distribution of labeled N in different pools of nitrogen. **(A)**  $^{15}\text{N}$ -nitrate addition in light and, **(B)**, dark: **(C)**  $^{15}\text{N}$ - ammonium addition in light and, **(D)**, in dark. Asterisk in plot **(C)** sediment-bound ammonium and organic matter were not determined separately. The amount of the pool “missing label” was calculated by difference in comparison to the initial label addition, indicated by the dashed line (see text for details).





**Fig. 6.** Process rates derived from model. Left: rates in light, right side: rates in dark incubations.