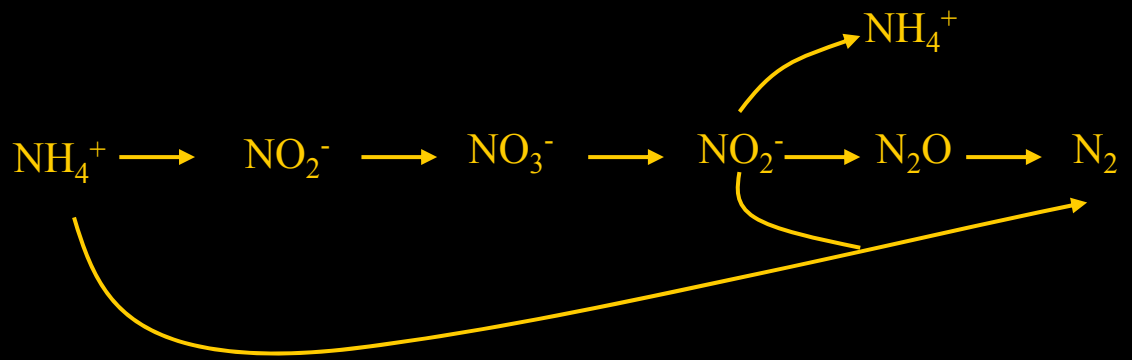
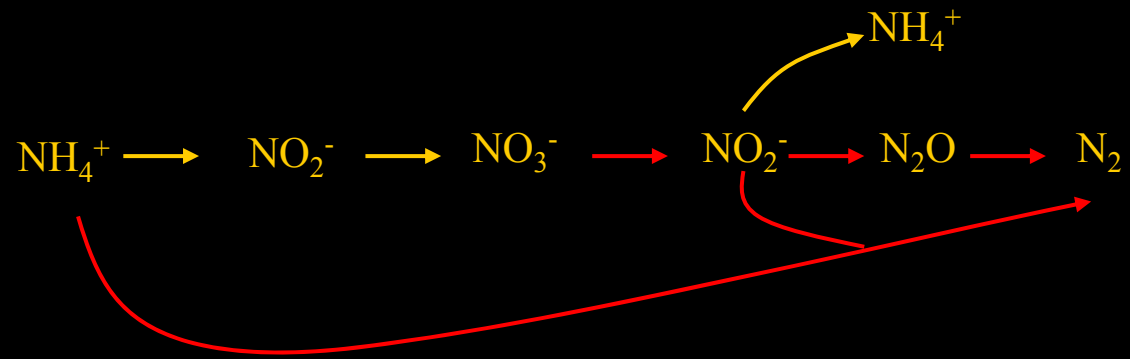


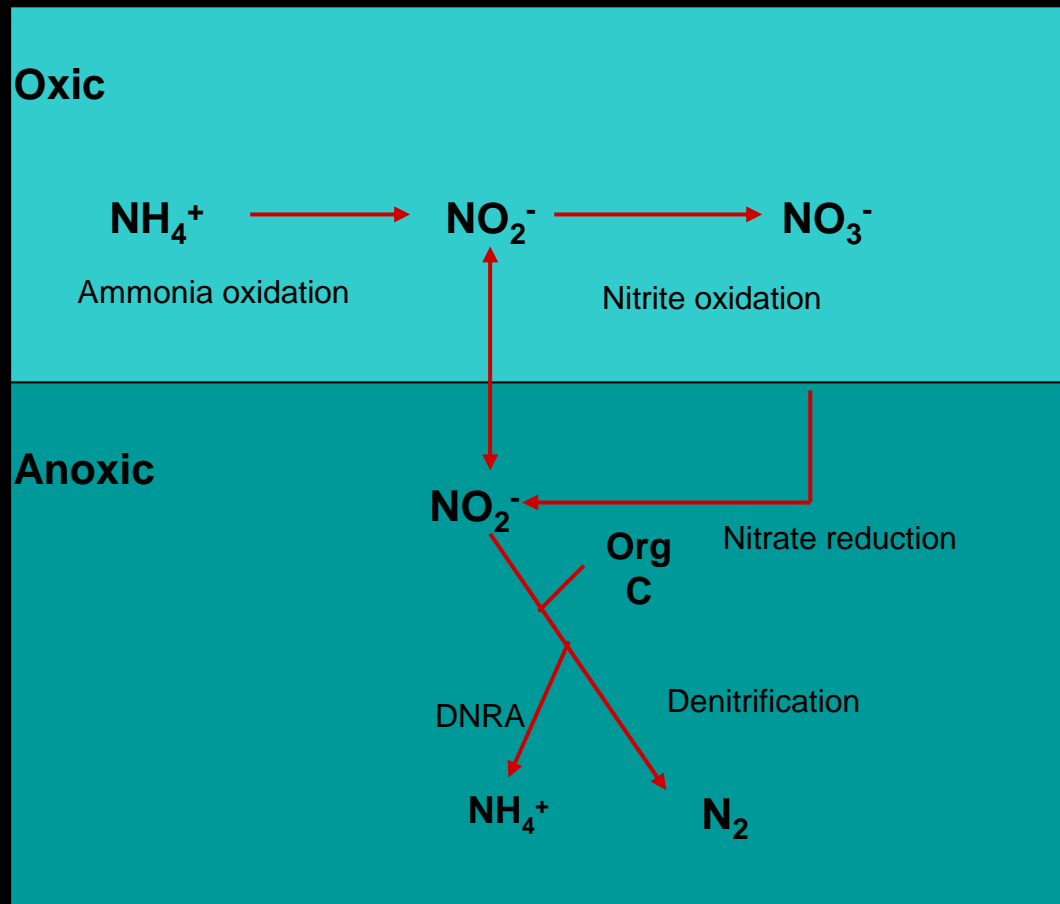
Alternative pathways for removal of nitrogen from the sea

Nils Risgaard-Petersen, National Environmental Research Institute, Denmark





The classical benthic nitrogen cycle



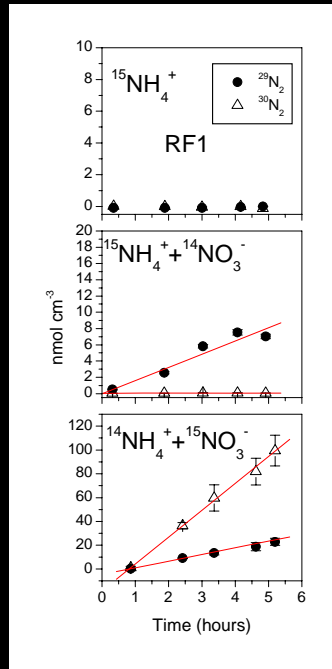
The Anammox process:



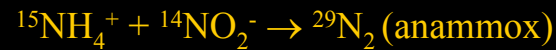
Historic:

- **Process suggested :Hamm & Thomason 1941**
- **NH₄⁺ deficit in oxygen minimum zones ⇒ anaerobic oxidation of NH₄⁺: Richards 1965**
- **Disappearance of NH₄⁺ from fluidized bed reactor: Mulder 1995**
- **Reaction identified in wastewater sludged: Van de Graff 1997**
- **Bacteria responsible for the reaction identified: Schmid 2000**
- **First direct evidence for existence of the process in marine sediments: Thamdrup and Dalsgaard 2002.**

Experimental evidence for anammox: Incubations of sediment slurries with ^{15}N -isotopes

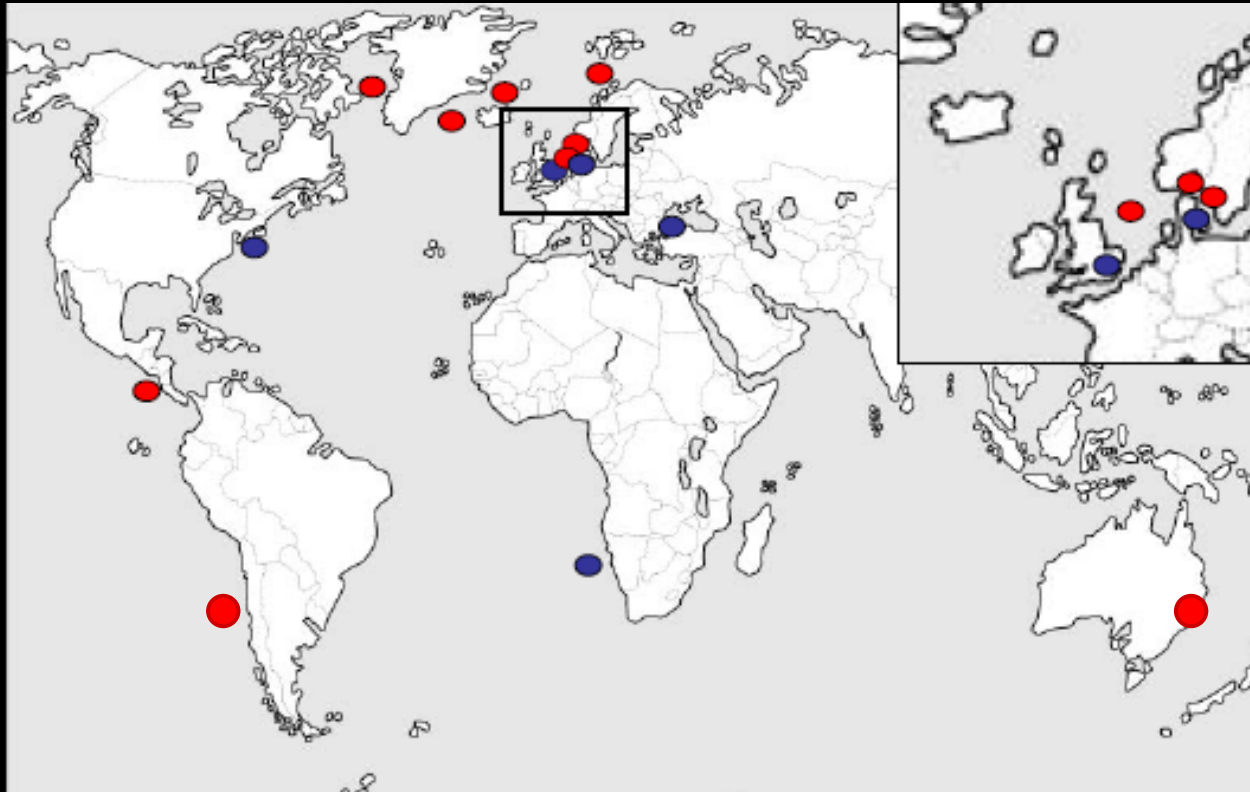


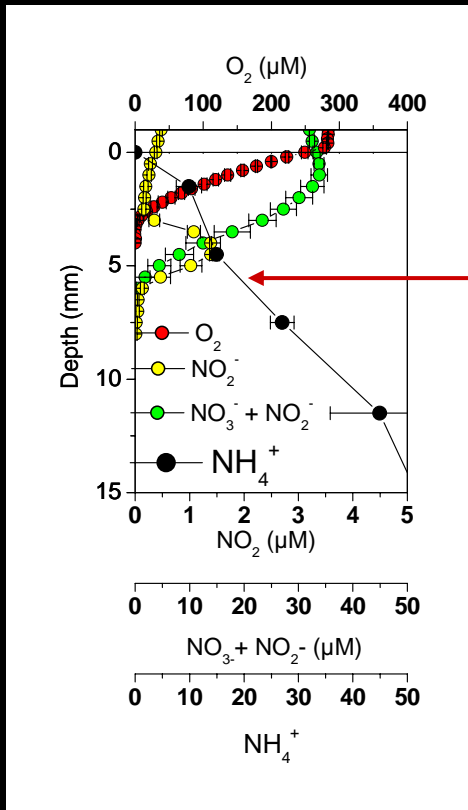
Nothing happens!



Data from Randers Fjord, DK

Anammox is ubiquitous in the marine environment

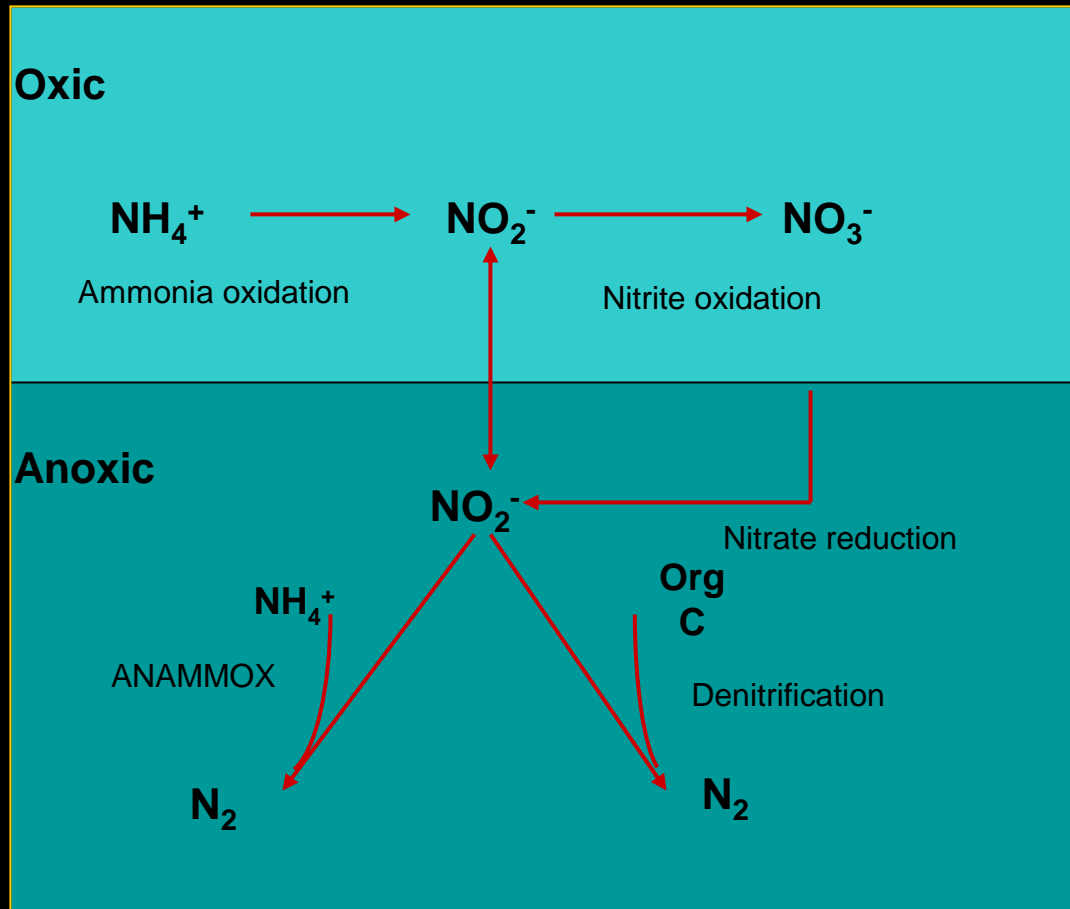




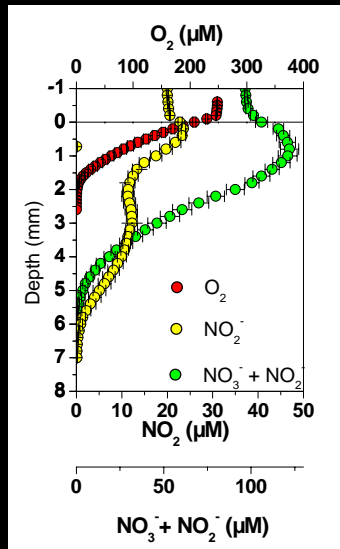
In sediments Anammox occurs below the oxic/anoxic interface

Randers Fjord

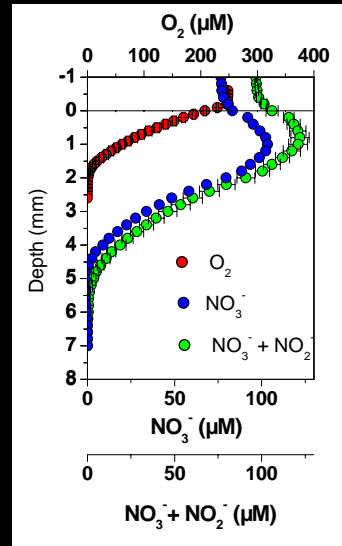
Which processes are responsible for the supply of NO_2^- to the ANAMMOX reaction?



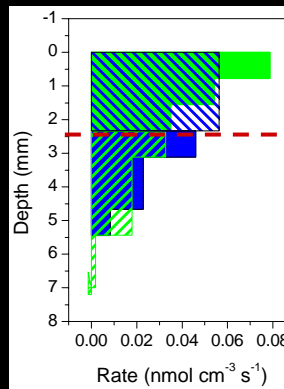
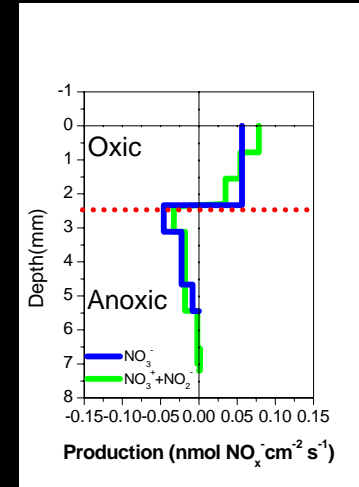
Inference of process rates from microprofiles



calculation of Nitrate profile

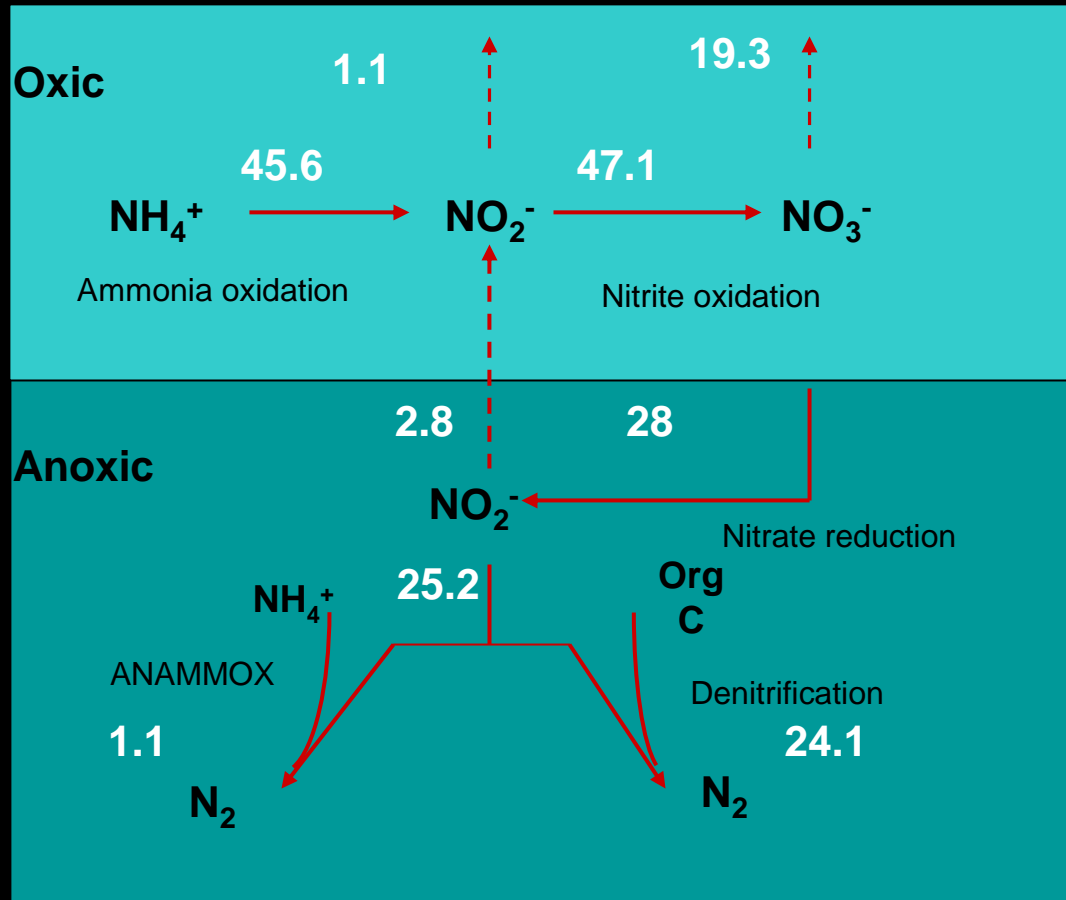


modeling

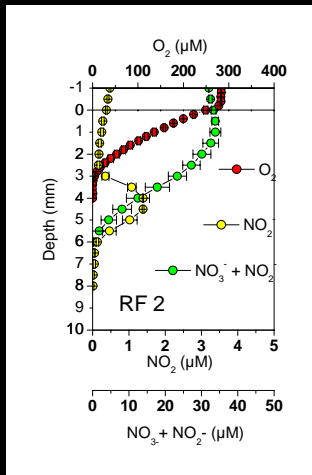


- Ammonia oxidation
- ▨ Nitrite reduction
- ▨ Nitrite oxidation
- Nitrate reduction

The principal Nitrite source for anammox is Anaerobic Nitrate Reduction
Example from Logan River Australia. Units: $\text{nmol cm}^{-2} \text{ h}^{-1}$

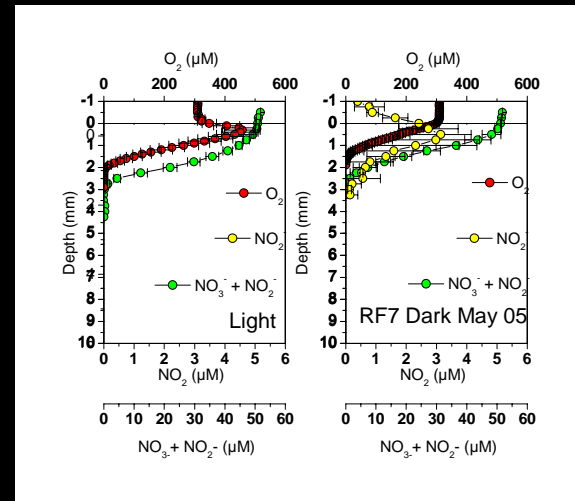


Anammox Station



Deep profiles of NO_x
 NO₂ present in anoxic zone most of the year
 Relative low Denitrification capacity
 Relative low O₂ consumption
 Relative low C-mineralization

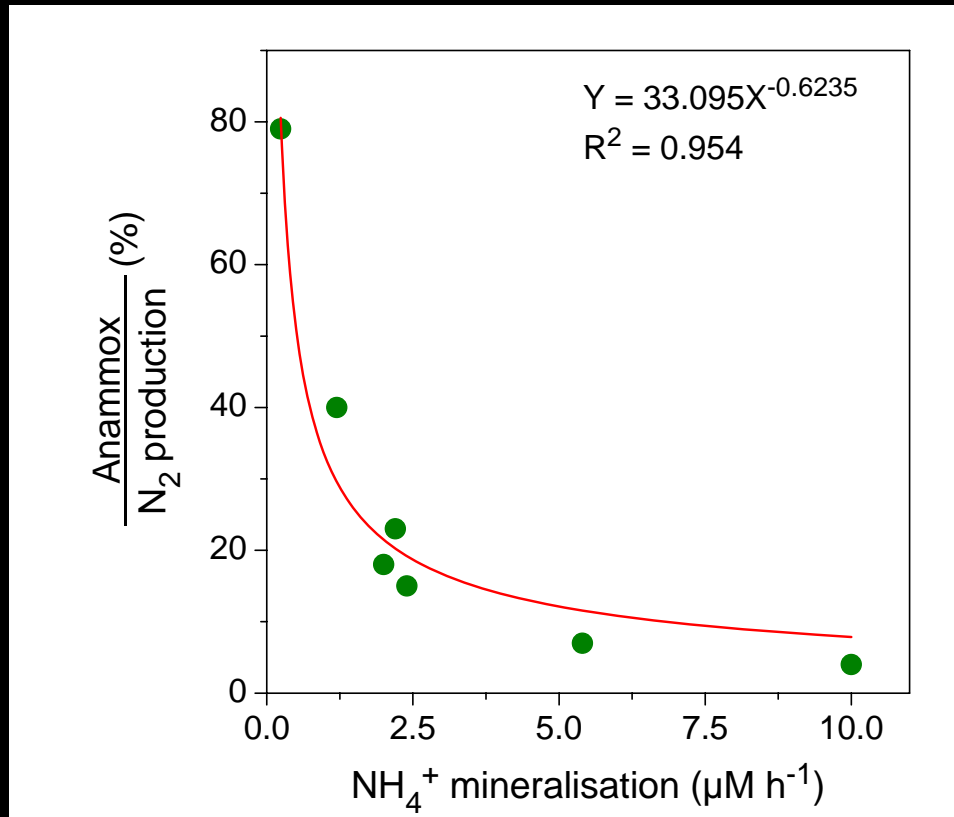
“NO anammox” station



Shallow profiles of NO_x
 Relative High Denitrification capacity
 Relative High O₂ consumption
 Relative High C-mineralization

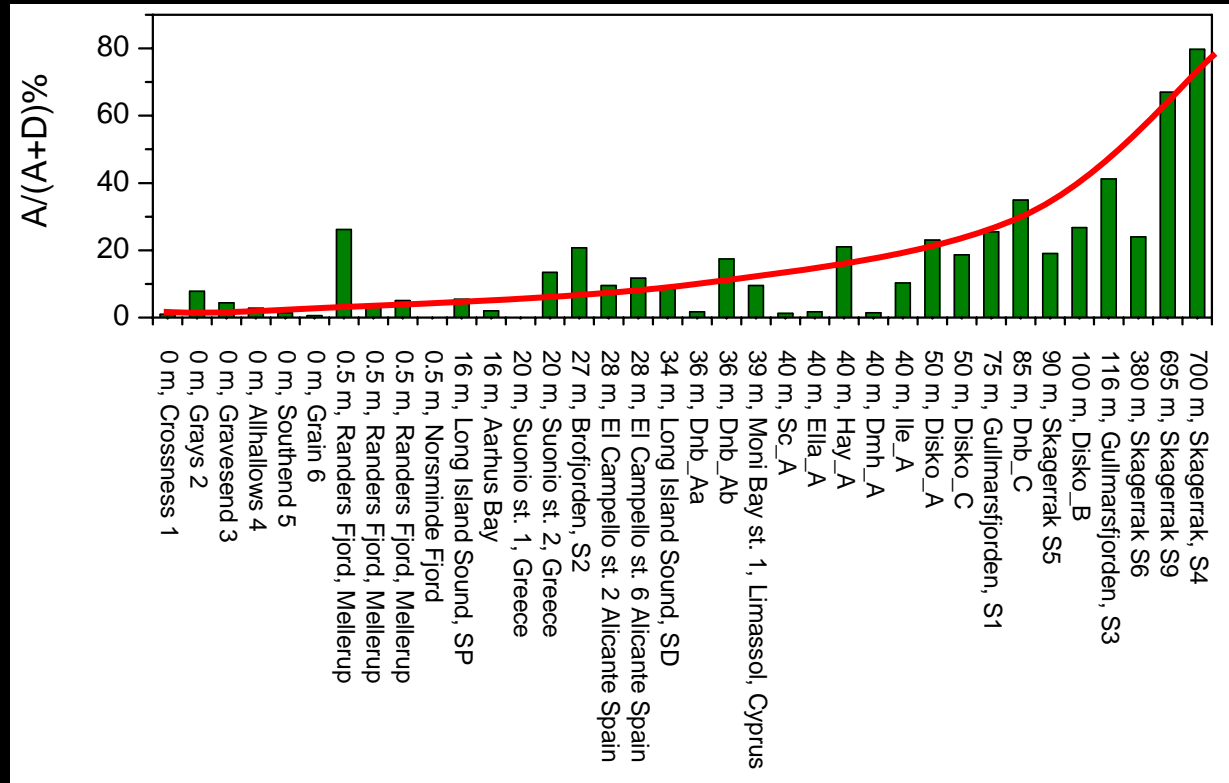
Ho: High Org C load favours heterotrophic denitrification on the expense of Anammox.

The contribution of anammox to benthic N_2 production decreases with increasing sediment reactivity, because heterotrophic denitrifiers do better in sediments with high organic loadings.



Data from Engström et al. 2005

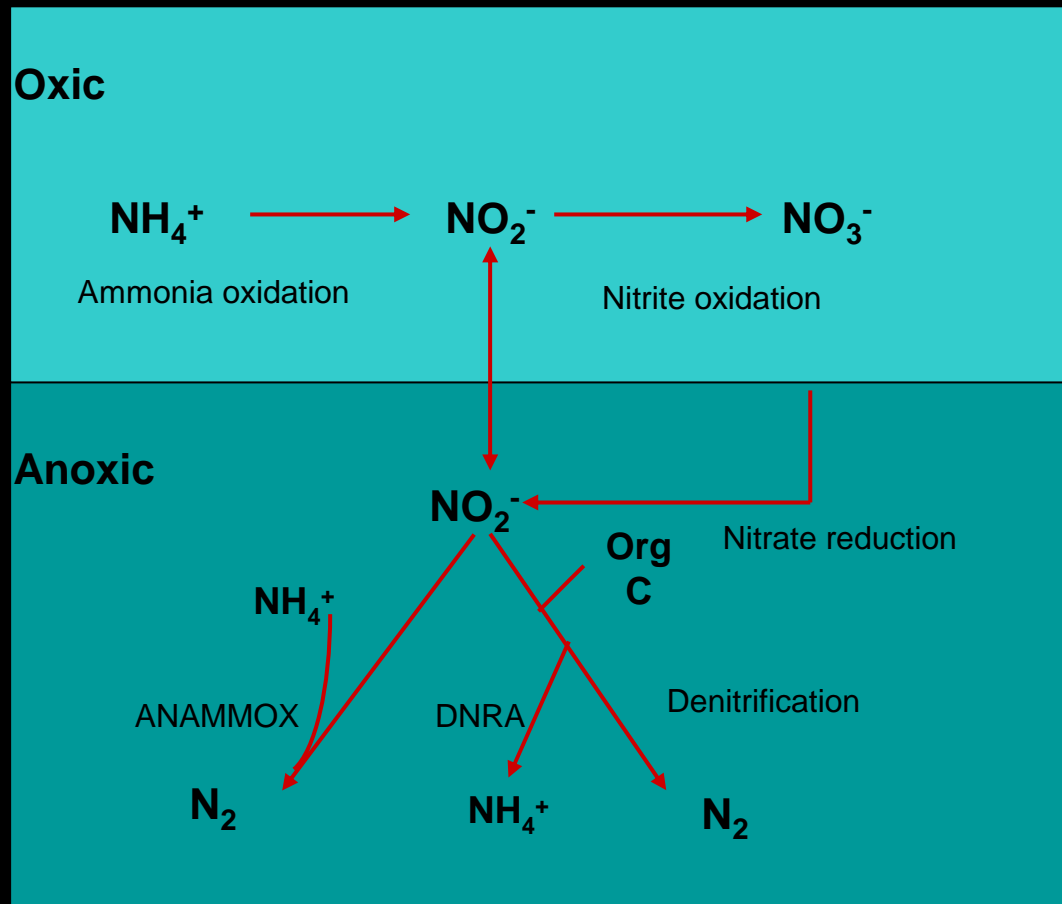
The contribution of anammox to benthic N removal increases with water depth



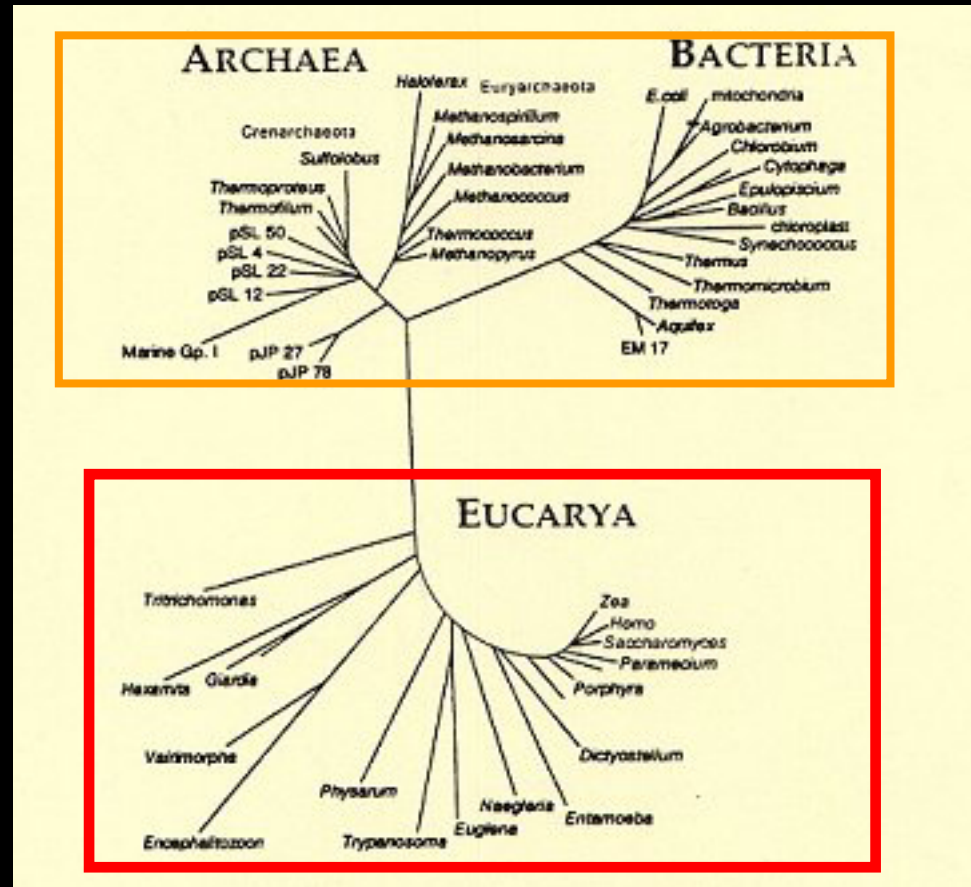
Dalsgaard et al 2005

The average depth of the ocean is 3800 m.
Anammox might be the dominant process for removal of N

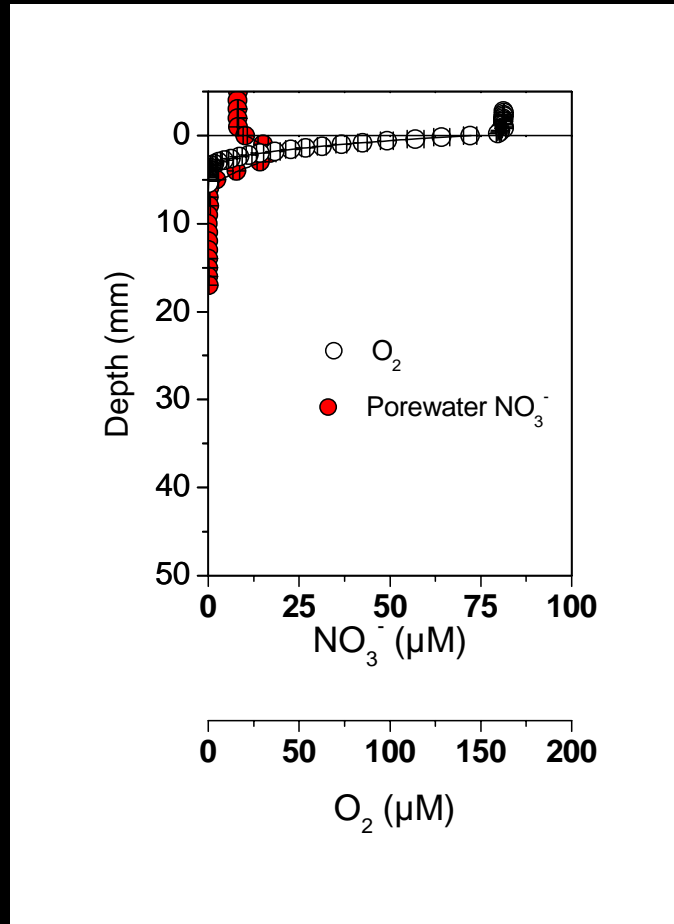
The updated benthic nitrogen cycle



According to the present conceptual view removal of fixed N
the oceans is mediated by prokaryotes
But is it true?

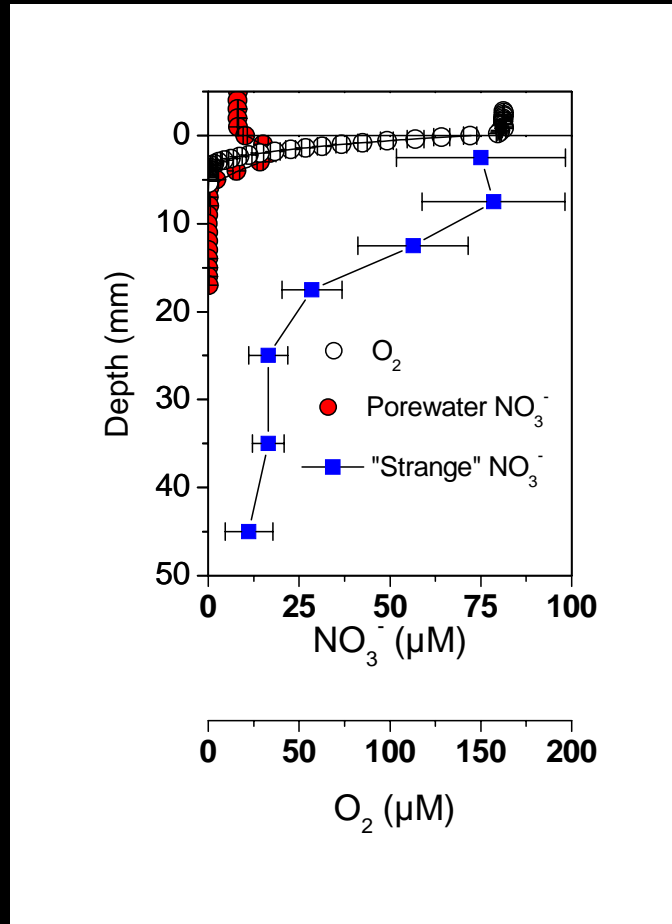


Nitrate in Gullmar Fjord (S) Sediment



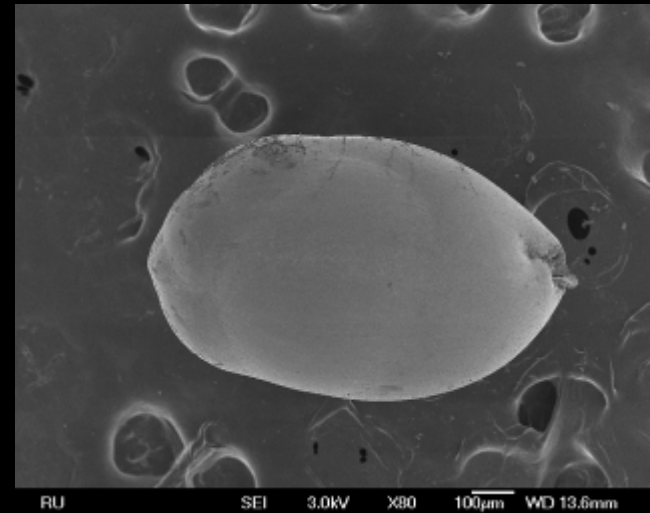
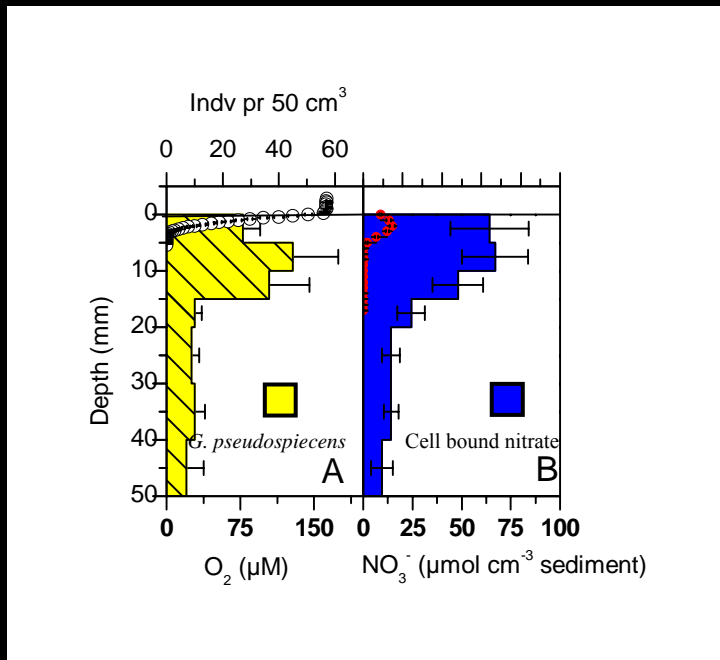
Water depth: 114 m
O₂: 25-100% sat
NH₄⁺ in water <0.05 μM
NO₃⁻ in water 9-17 μM
Temp: 6 C
Salinity 34 psu

Nitrate in Gullmar Fjord (S) Sediment



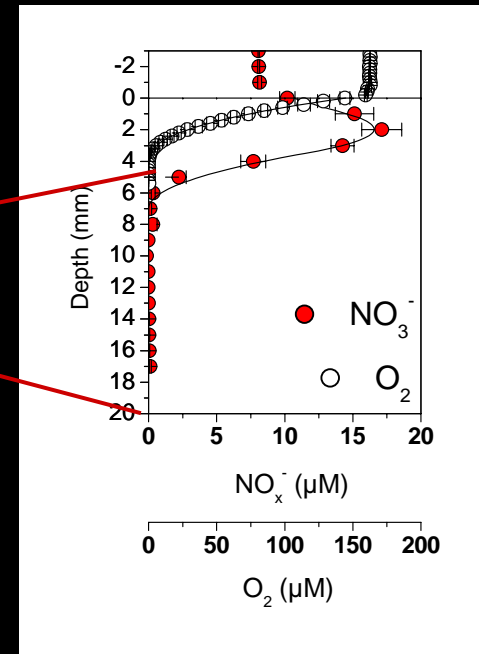
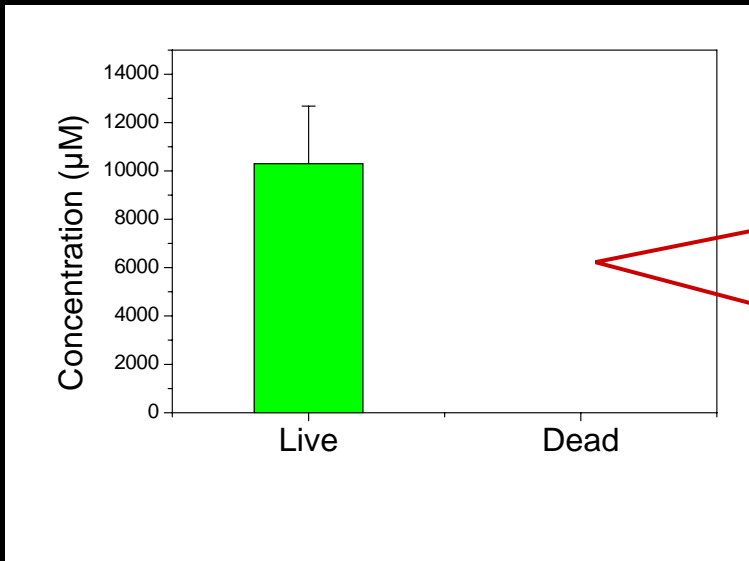
Nitrate is not only present in the porewater. There are cell bound pools in sediments

Nitrate and distribution of foraminifers (*Globobulimina pseudospiezens*)

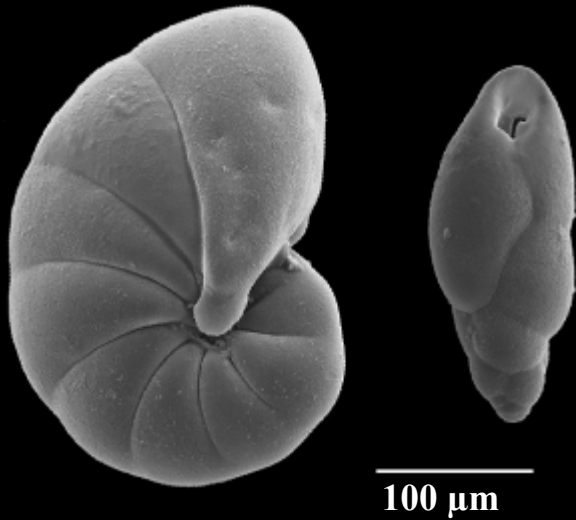


**Cell bound nitrate correlate with foraminiferal abundance
(Pearson, $r=0.95$; $p<0.001$)**

Nitrate in live and dead foraminifers (*Globobulimina pseudospiezens*)



Nonionella cf. stella and *Stainforthia* sp from OMZ off Chile



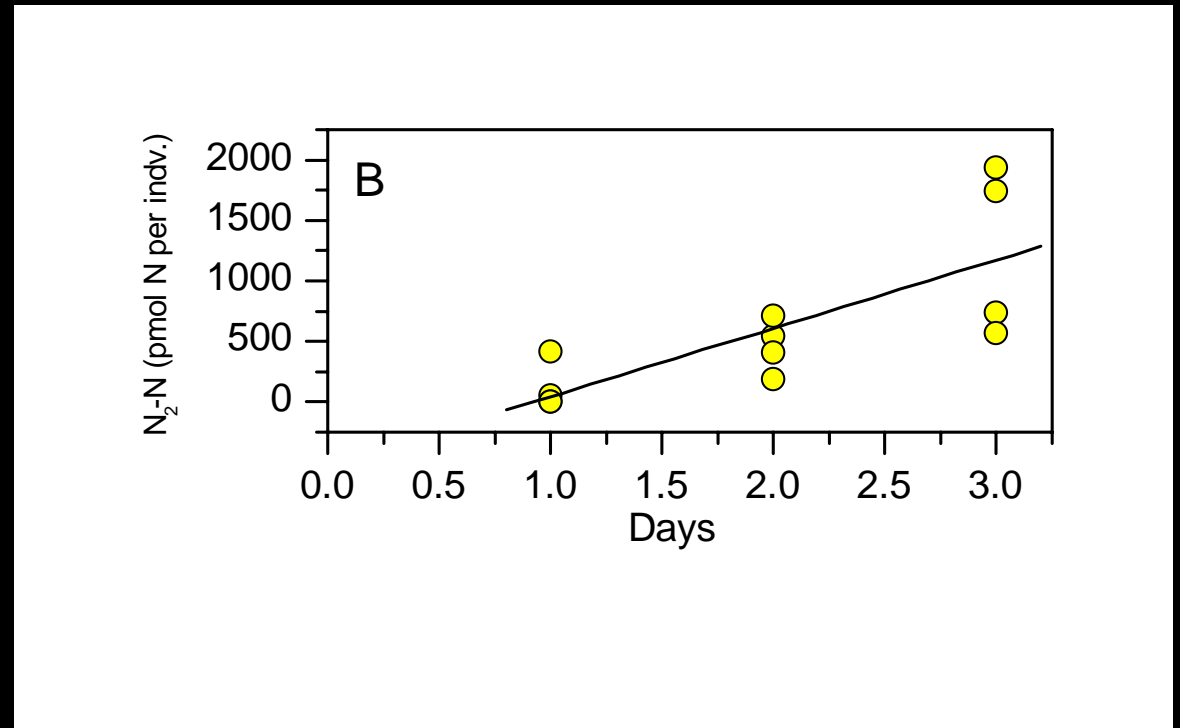
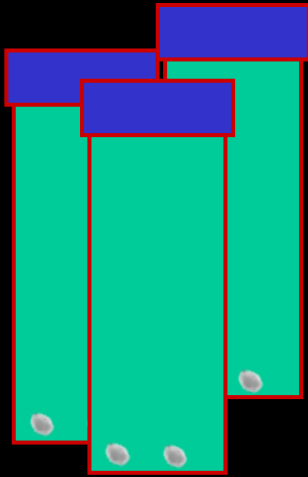
Maximum NO_3^- concentration in porewater (μM)	Species	Intracellular NO_3^- content (pmol per individual)		Intracellular NO_3^- concentration (μM)
		Mean	Range	
12	<i>Nonionella cf. stella</i>	186 (25, n=43)	8-794	35000
12	<i>Stainforthia</i> sp.	60 (9, n=26)	0- 172	180000

Why do benthic foraminifers accumulate nitrate?



$$\Delta G = -545 \text{ kJ per mol NO}_3^-$$

Denitrification by foraminifers (*Globobulimina pseudospinences*)



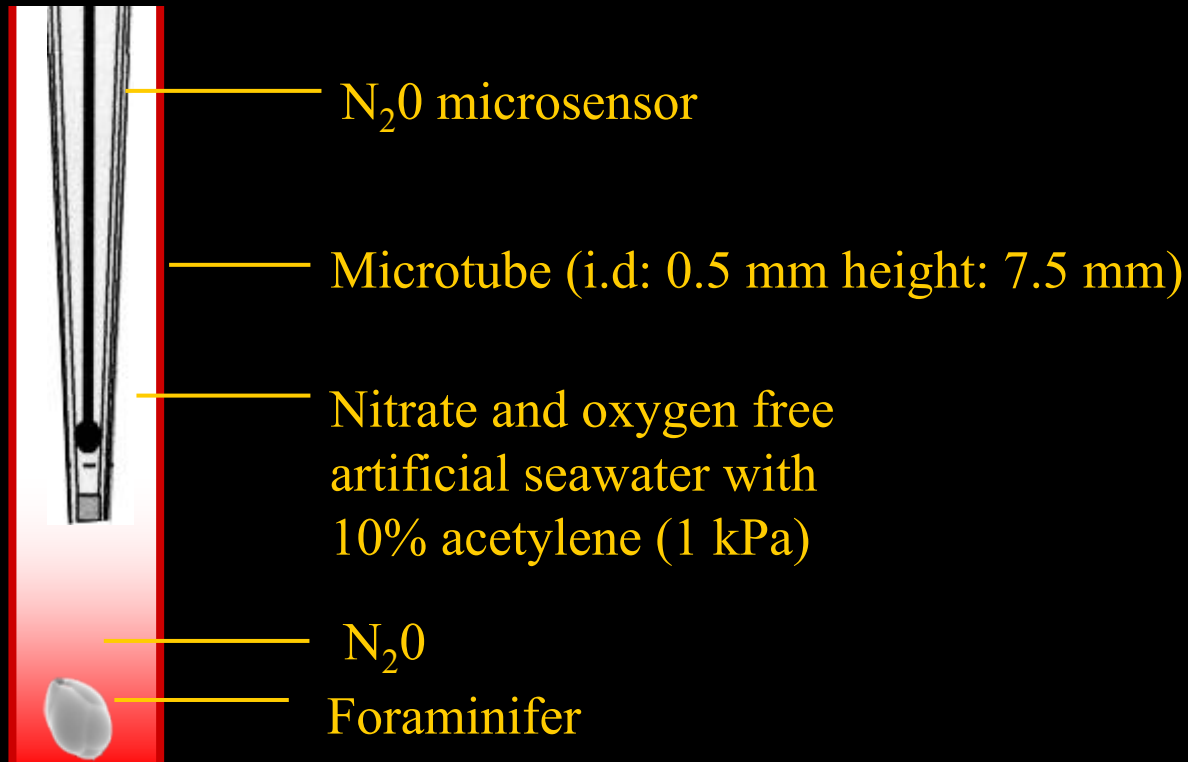
Denitrification rate = 565 ± 151 pmol N cell⁻¹ day⁻¹

Anoxic incubations of
foraminifers with a ¹⁵N-labelled
intracellular nitrate pool

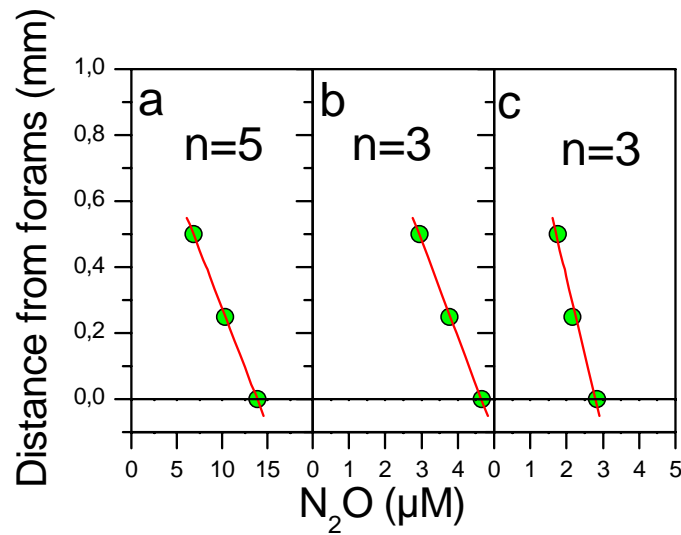
Denitrification by foraminifers
(Nonionella cf. Stella)

Acetylene block: $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$

Setup

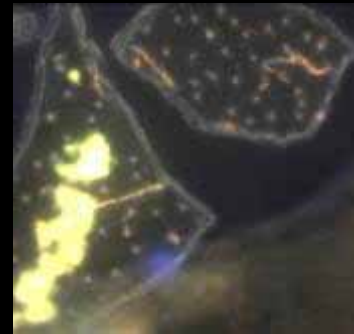
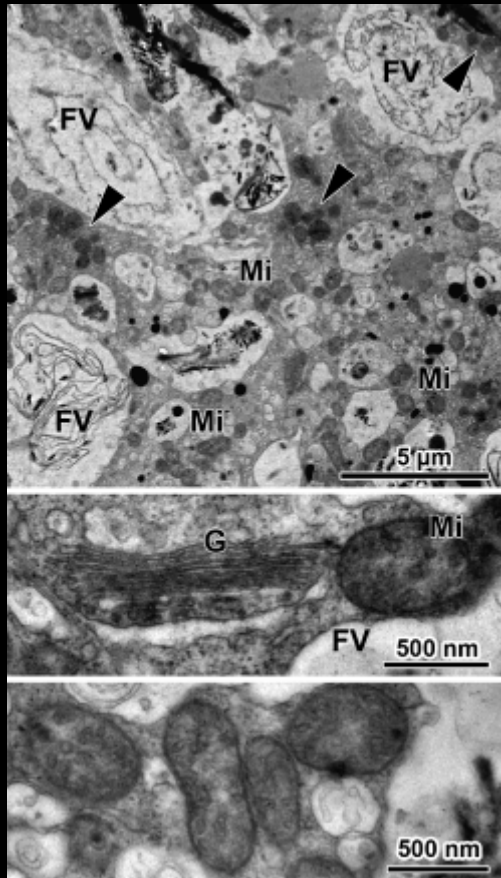


Denitrification by foraminifera (*Nonionella cf. Stella*)



Denitrification =
 $D_{N_2O} * dC/dX * \text{surface area}$
 $= 84 \pm 33 \text{ pmol N cell}^{-1} \text{ d}^{-1}$

Who is responsible for denitrification of the intracellular nitrate??



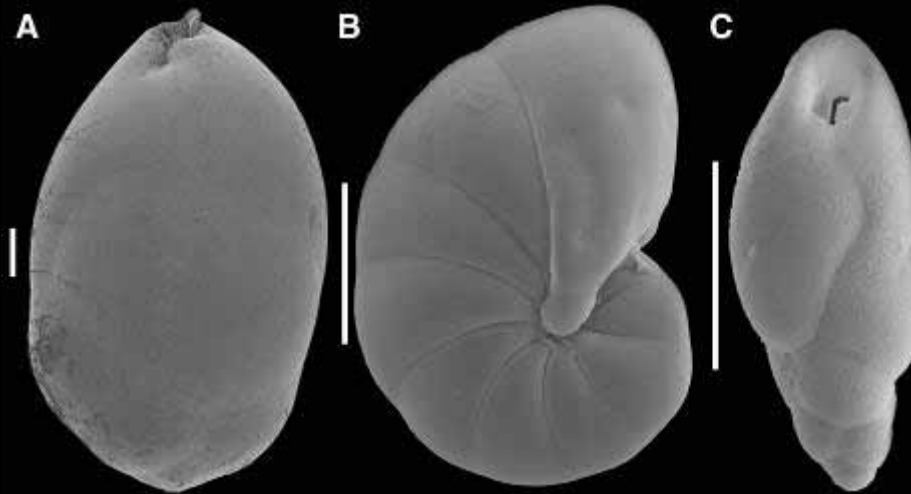
- GS2
- EUB
- DAPI
- Autofluorescence

FISH applied on *G. pseudospinencens*

FISH analysis and TEM micrographs shows that endosymbiotic bacteria are absent

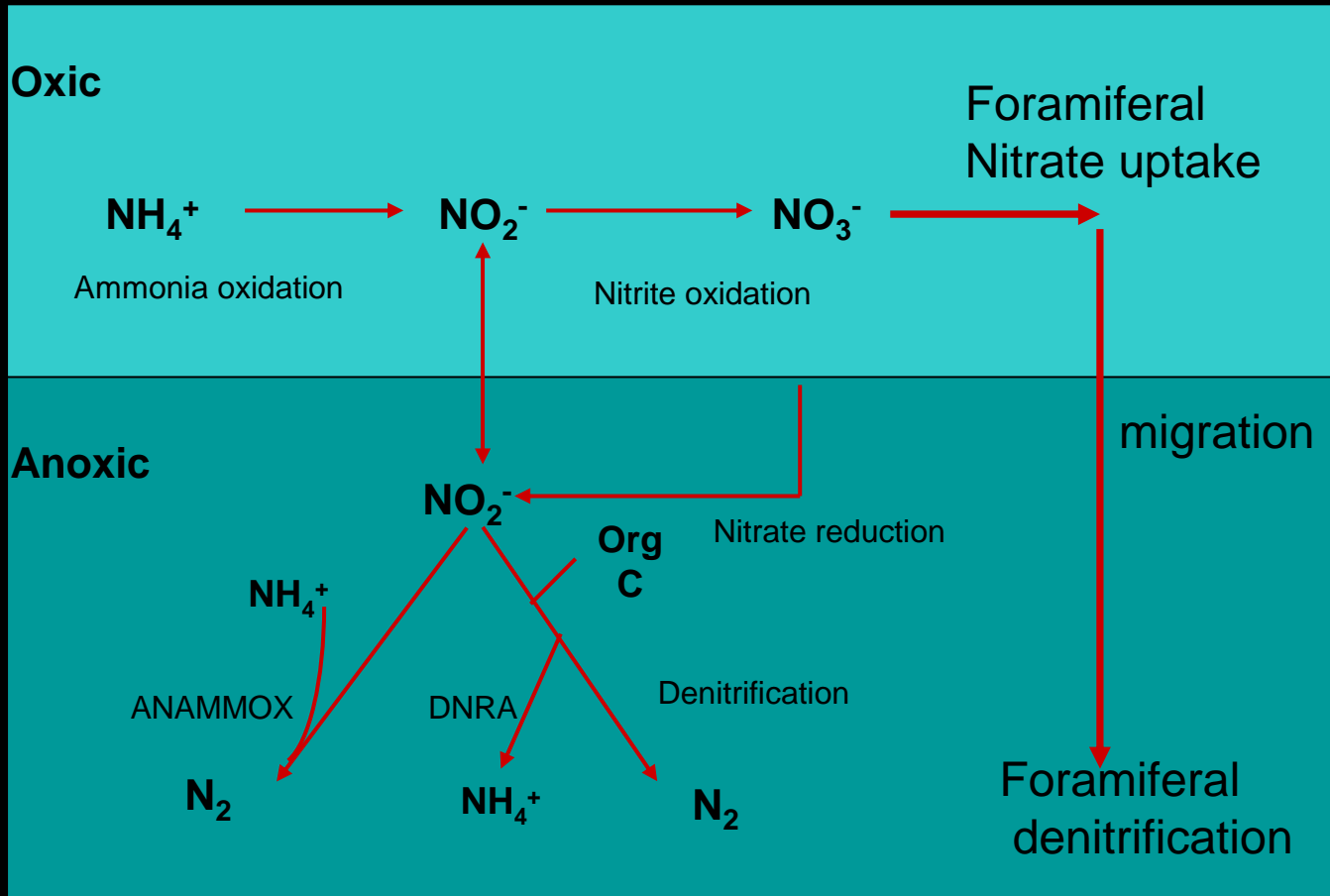
TEM micrograph of *G. pseudospinencens*

**This implies that denitrification is performed by
the foraminifers**



**Benthic foraminifers forms a hitherto unknown
pathway for removal of fixed N from the sea**

The updated benthic nitrogen cycle Version 2.0



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