Microbial Processes in Constructed Tidal Wetlands for Removal of Nitrogen from Urban Wastewaters

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Goals for Urban Wastewater Treatment

- Removal of BOD
- Removal of nutrients, particularly nitrogen
- Disinfection to kill pathogens

Typically done in large, central wastewater treatment plants, then water is discharged to the environment

Water could be reused for secondary purposes!

Furman University

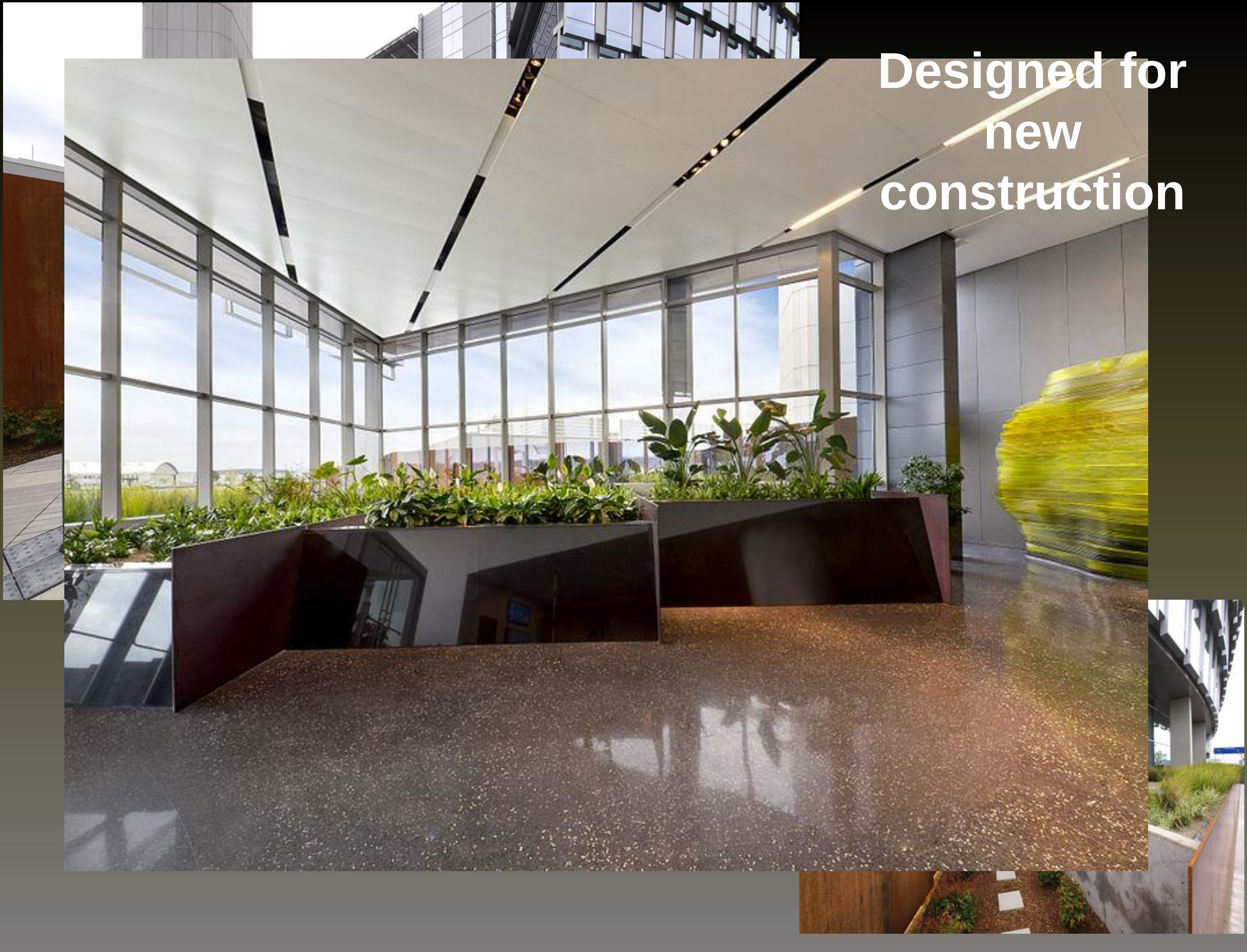
Old Trail School

Living Machine®

Guilford Co. (NC) School

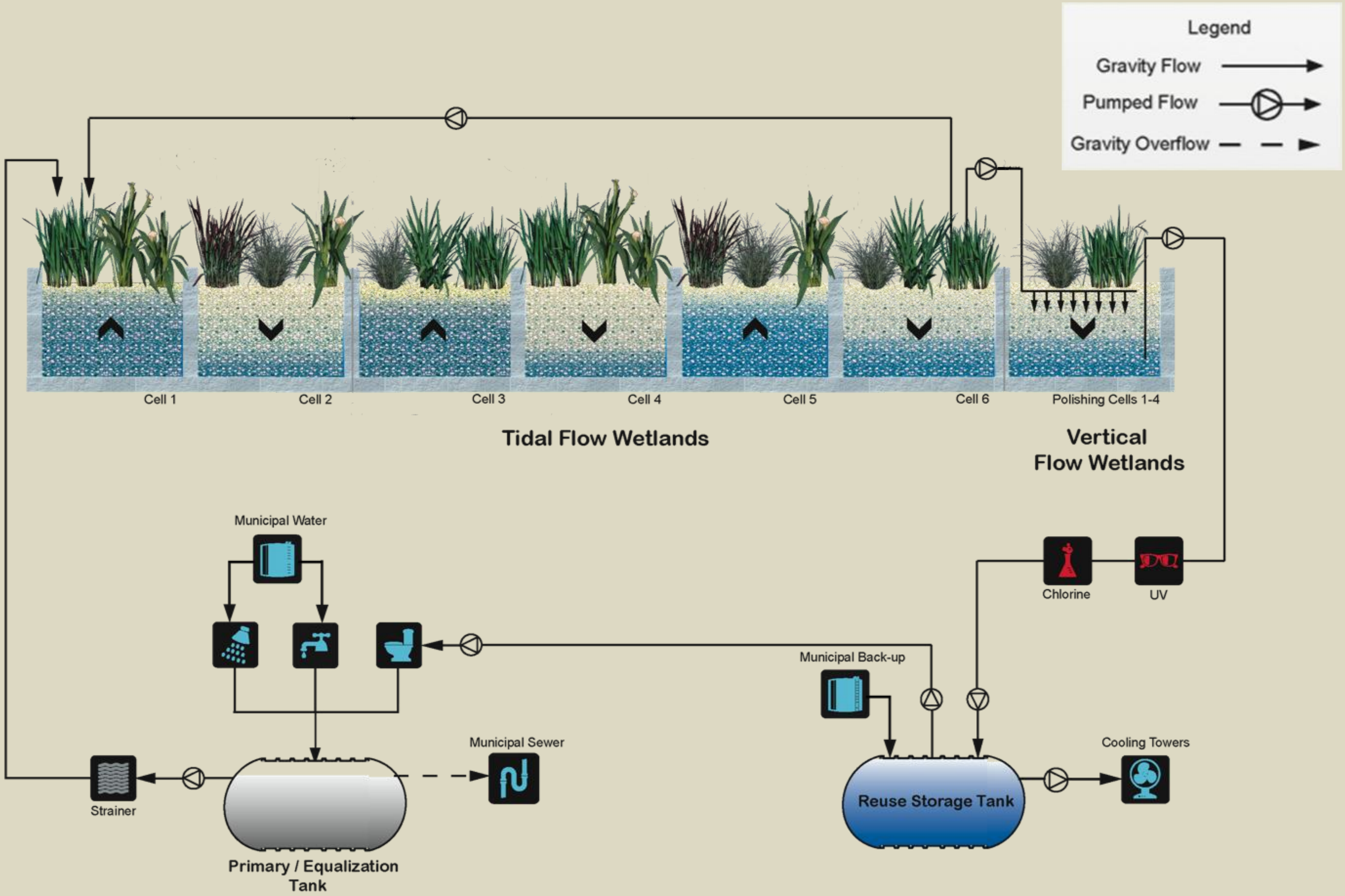
Esalen Institute

Designed for new construction

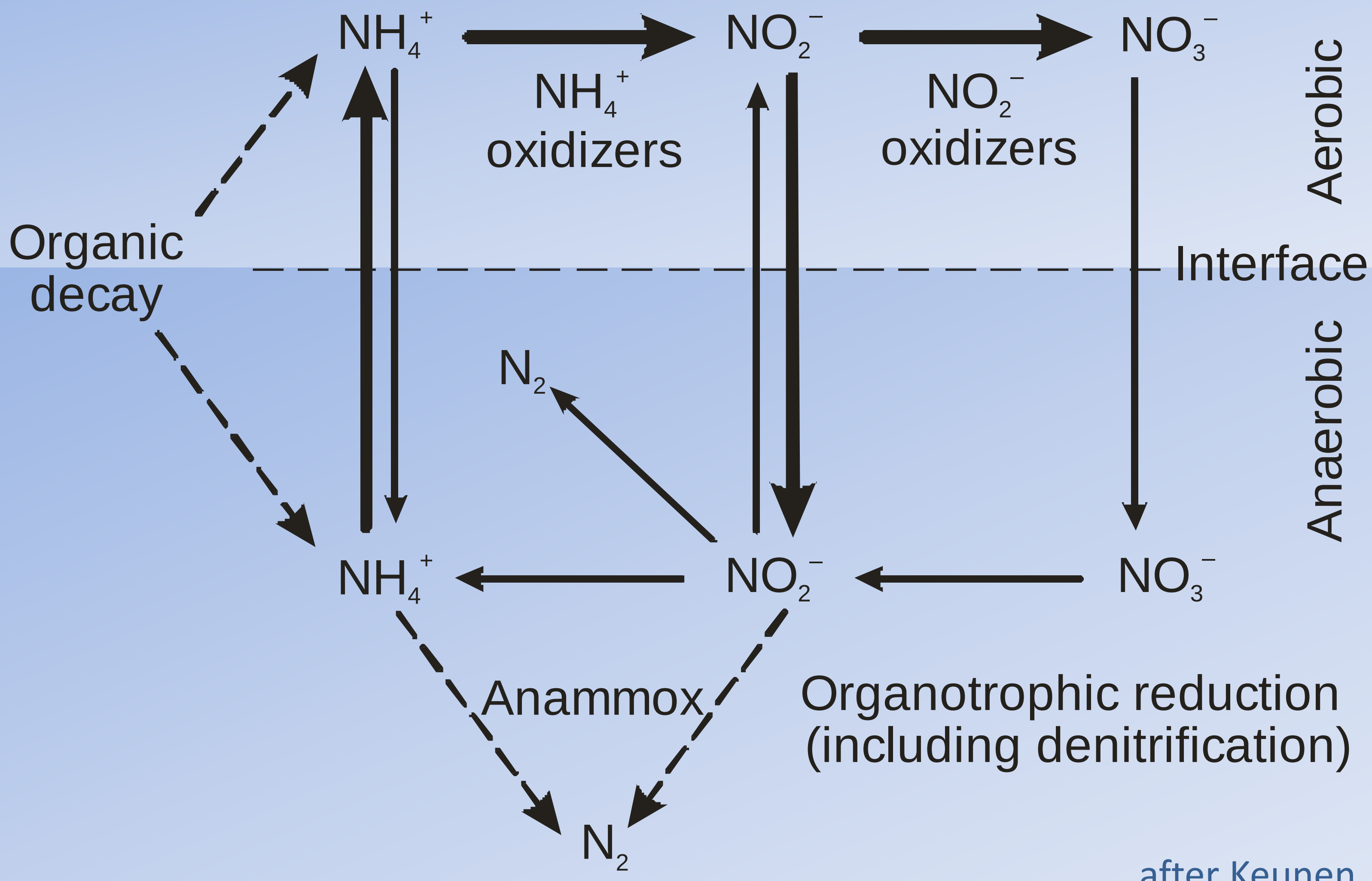


Living Machine:

Capacity = 5,000 gallons day^{-1}



Nitrogen processes important in wastewater renovation



after Keunen, 2008

Tidal reactors remove nitrogen and reduce BOD efficiently and inexpensively

Not sure what processes are responsible for N removal

Not sure how the microbes are distributed spatially within a reactor

How to optimize operation so as to exploit the microbes to greatest capacity

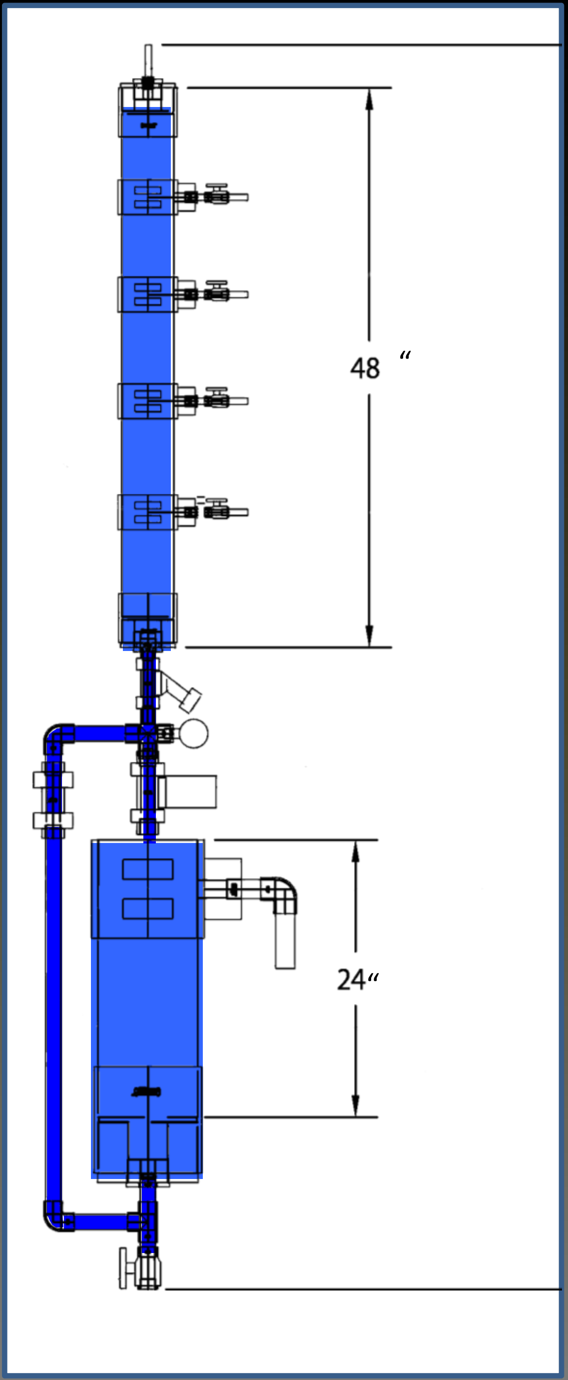
Smaller Columns

3 g LESA

	High N	Low N
NH_4^+-N (g)	1.74	0.41
COD (g O_2)	1.5	1.5

	High N	Low N
Urea (g)	3.7	0.9
Whey (g)	1.3	1.3

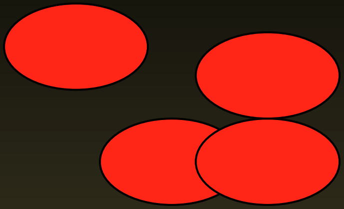
Hydraulic operation of tidal columns



Inundation Frequency (cycles day^{-1})
24
16
8
4
24

Nitrogen Cycling in Wastewater Treatment

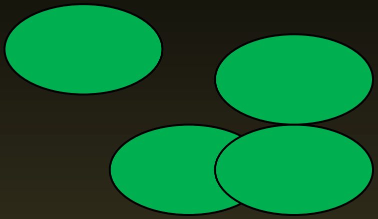
Ammonium Oxidizing Bacteria (AOB)



$NH_4^+ \rightarrow NO_2^-$

$\Delta G = -64.7$ kcal (mol NH_4^+)$^{-1}$

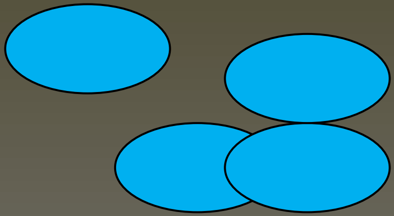
Nitrite Oxidizing Bacteria (NOB)



$NO_2^- \rightarrow NO_3^-$

$\Delta G = -18.5$ kcal (mol NO_2^-)$^{-1}$

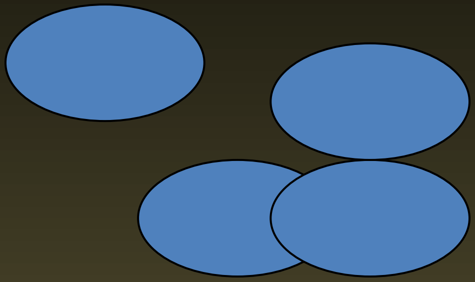
Anaerobic Ammonium Oxidizing Bacteria (AMX)



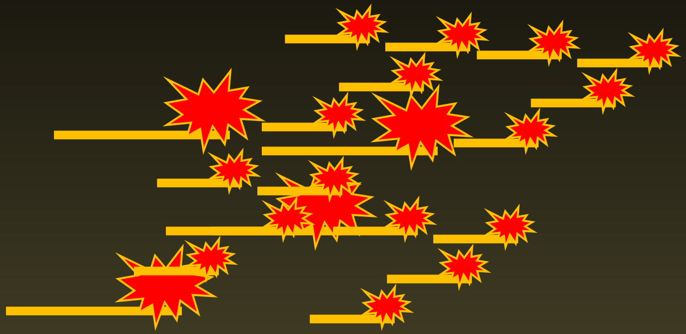
$NH_4^+ + NO_2^- \rightarrow N_2$

$\Delta G = -85.5$ kcal (mol NH_4^+)$^{-1}$

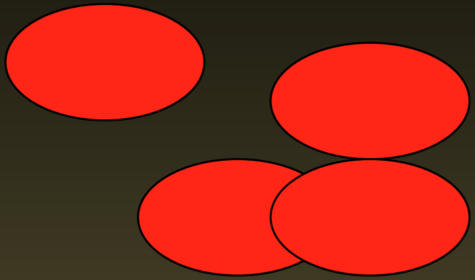
Fluorescent *In Situ* Hybridization (FISH)



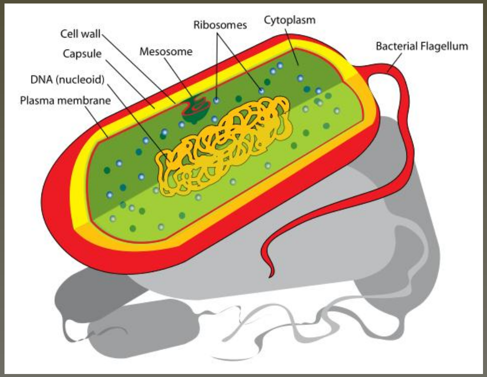
1) Bacterial cells are made permeable



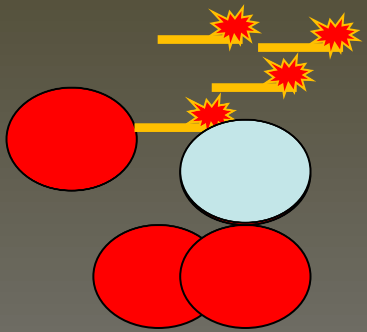
2) Labeled DNA probes are added



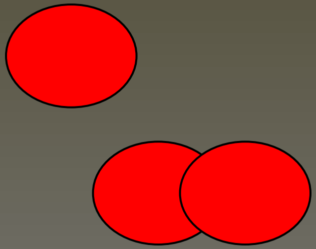
3) Probes pass through the cell wall



4) Probes bind with the 16S region of the ribosome

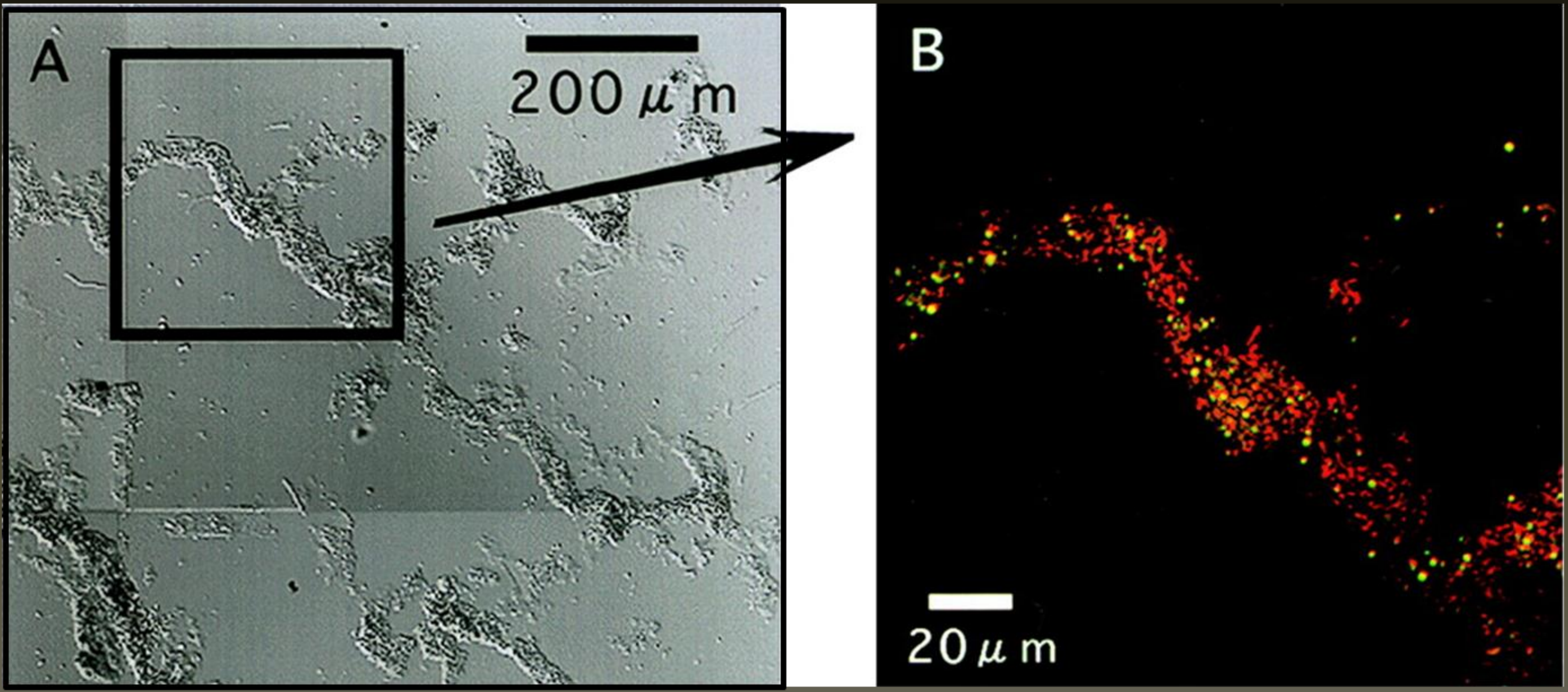


5) Unbound probe is rinsed away



6) Labeled cells are visible under fluorescence microscopy

In situ hybridization of a wastewater biofilm



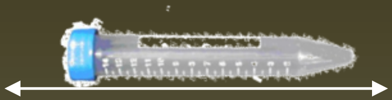
Okabe et al. Appl. Environ. Microbiol. 1999, 65:5107-5116

Applied and Environmental Microbiology

Extract Cells

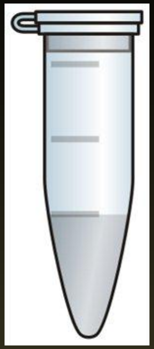
1. Combine 1 g LESA with 2 mL PBS

2. Vortex for 5 min



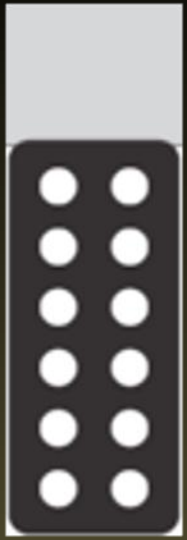
3. Transfer to micro-centrifuge tube.

Preserve Cells



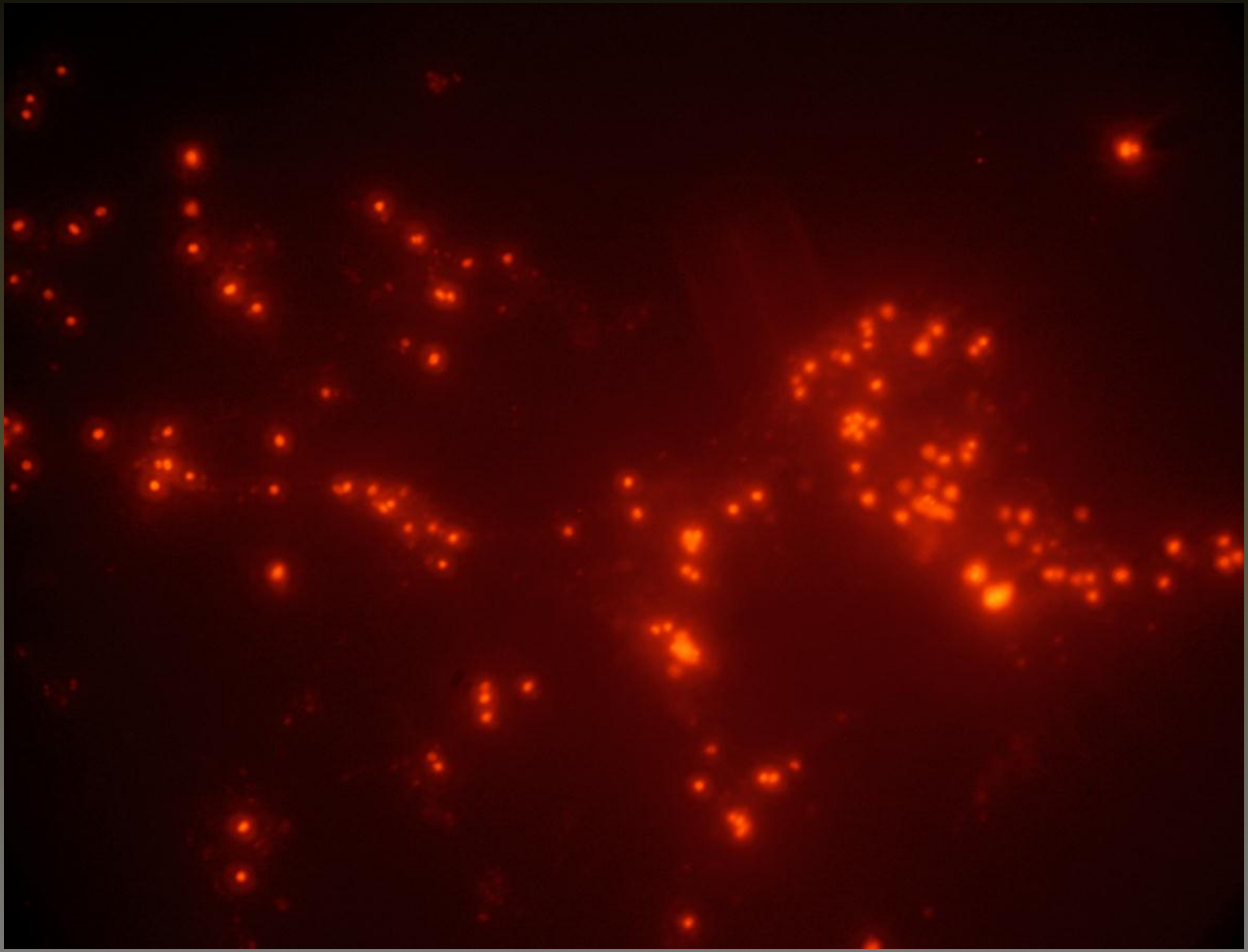
1. Preserve cells in methanol
2. Transfer to ethanol
3. Store @ -20° C

Adhere to Slides



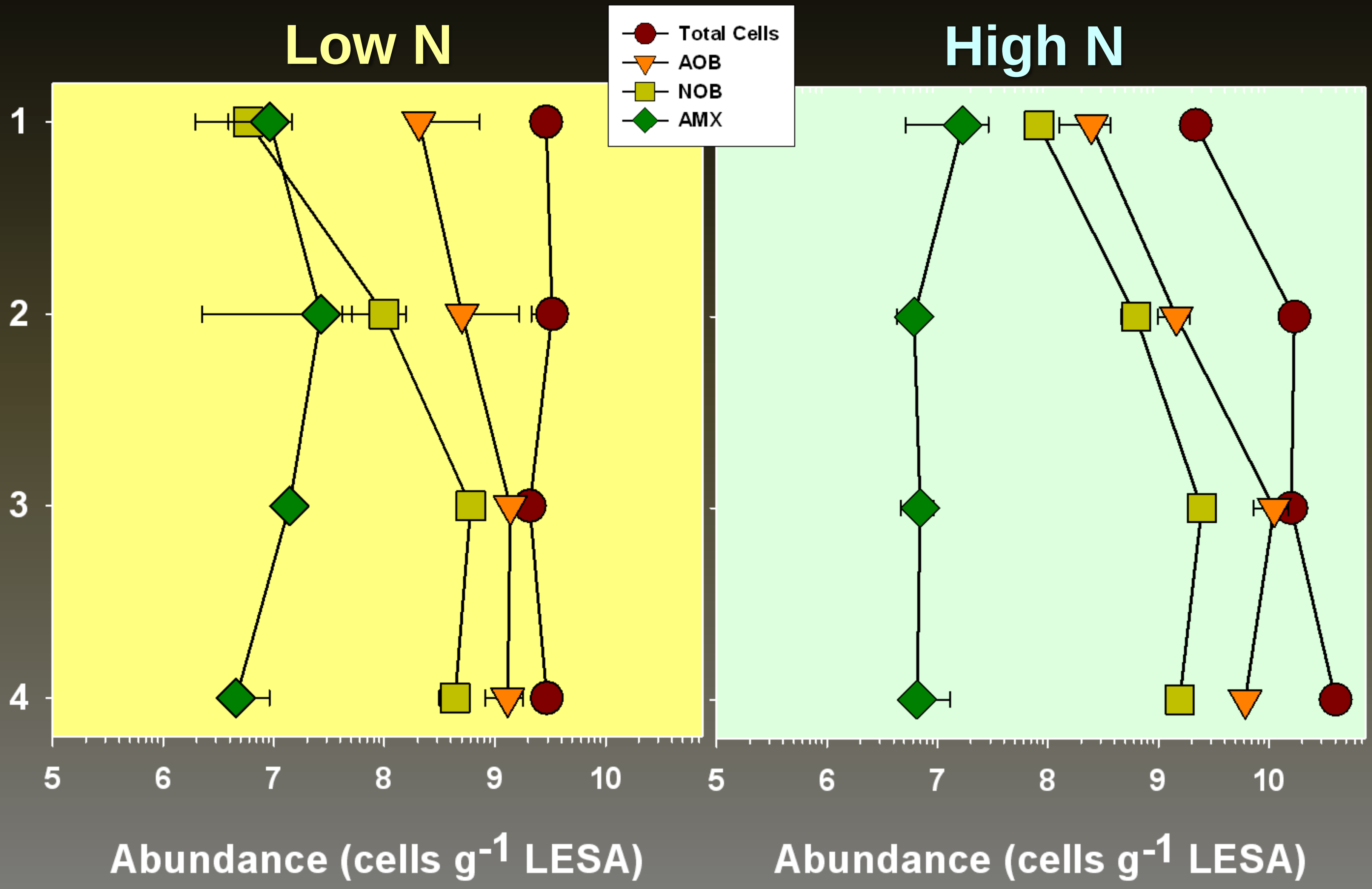
1200 samples to count!

AOB after hybridization with Cy3-labeled oligonucleotides



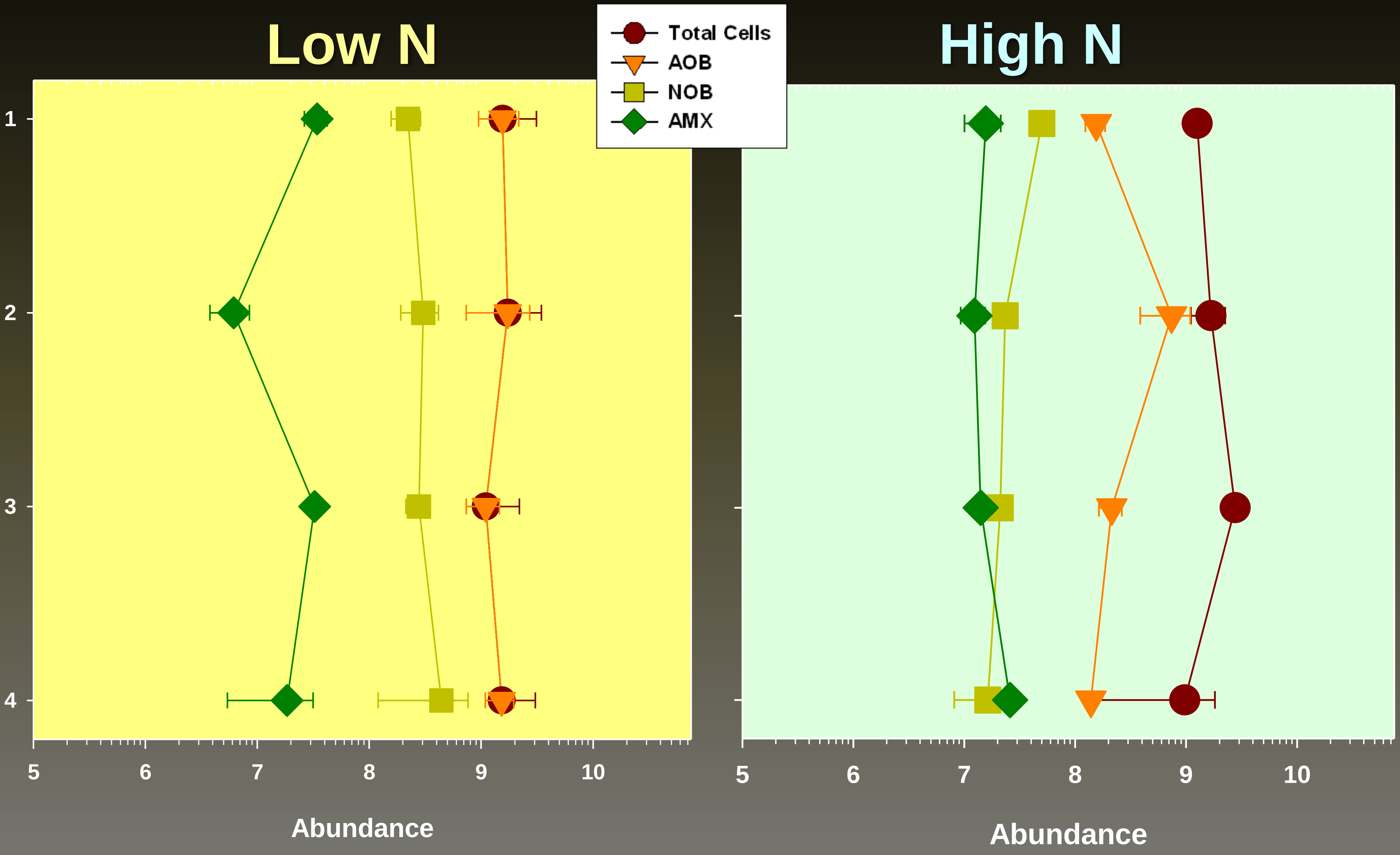
Abundance of N-cycle organisms in tidal reactors

24 cycles day^{-1}



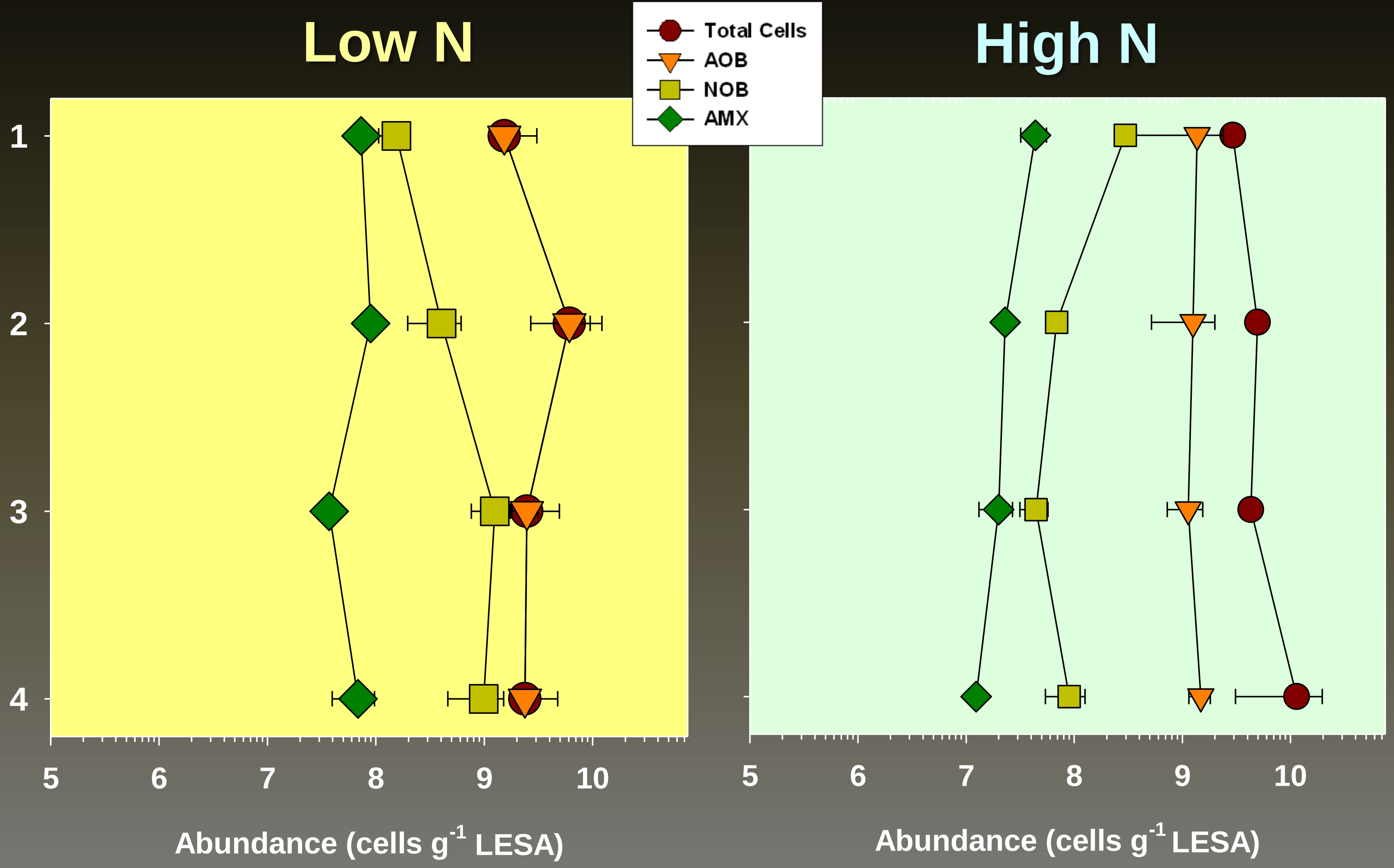
Abundance of N-cycle organisms in tidal reactors

8 cycles day^{-1}

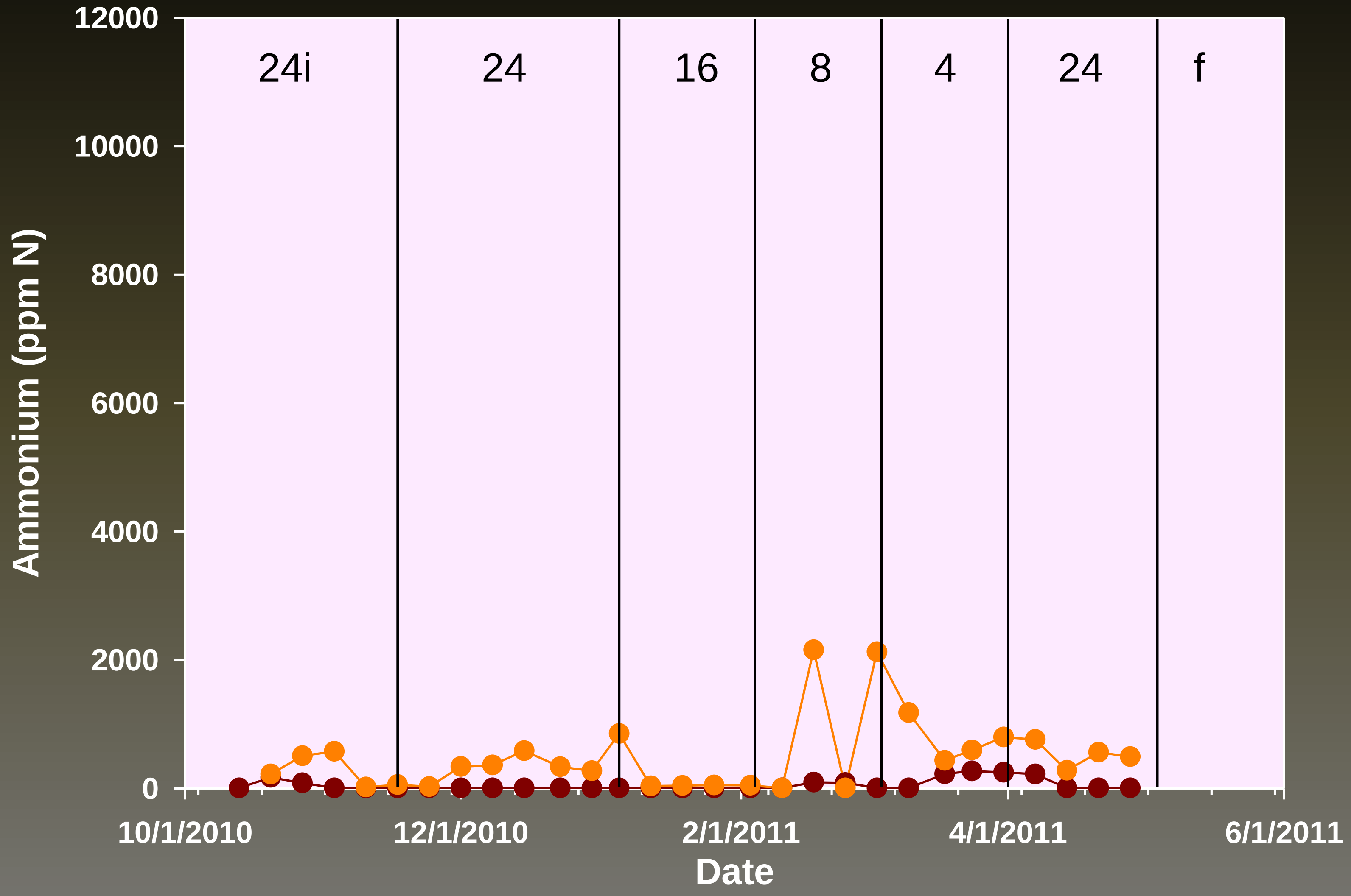


Abundance of N-cycle organisms in tidal reactors

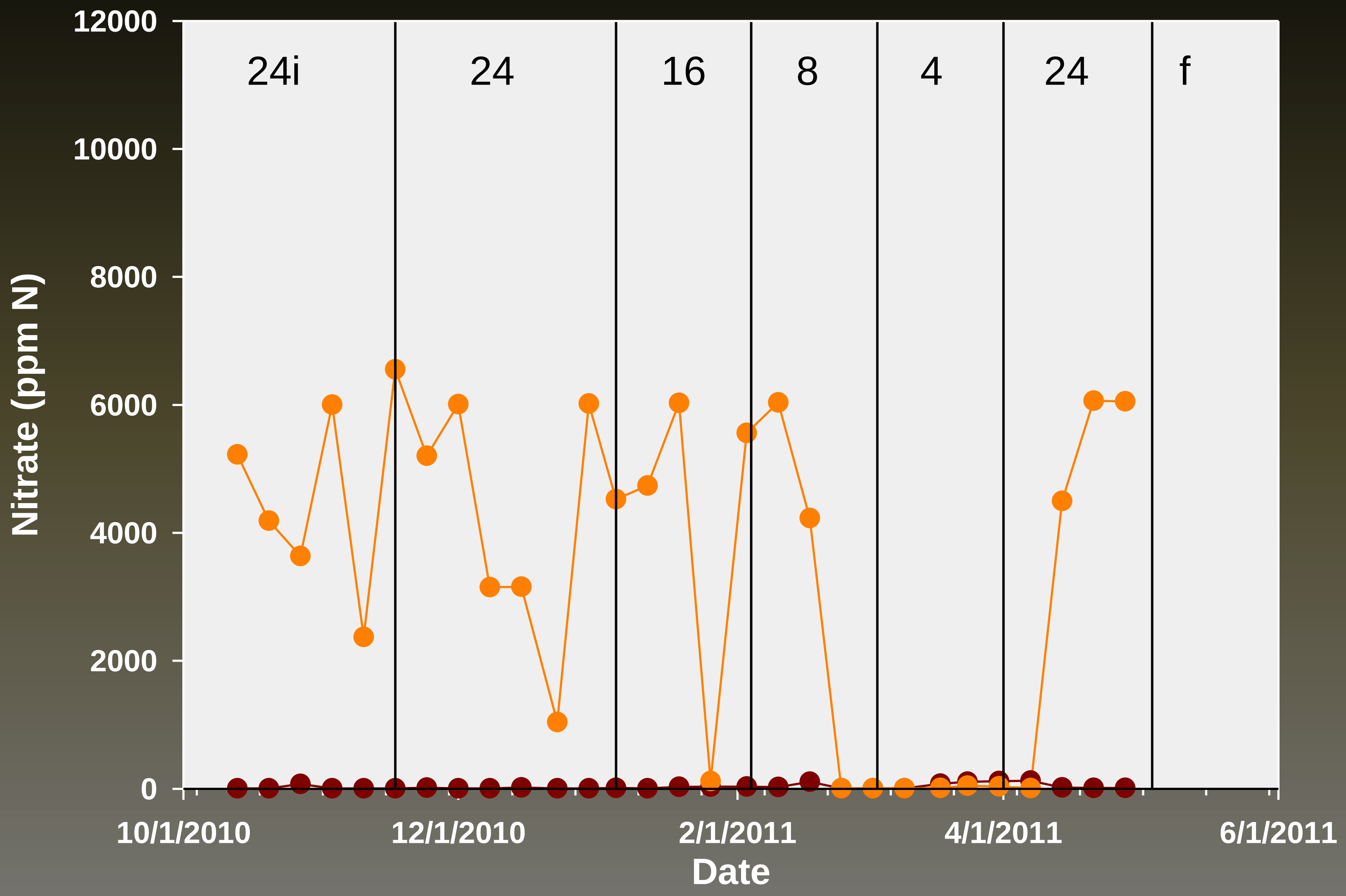
4 cycles day^{-1}



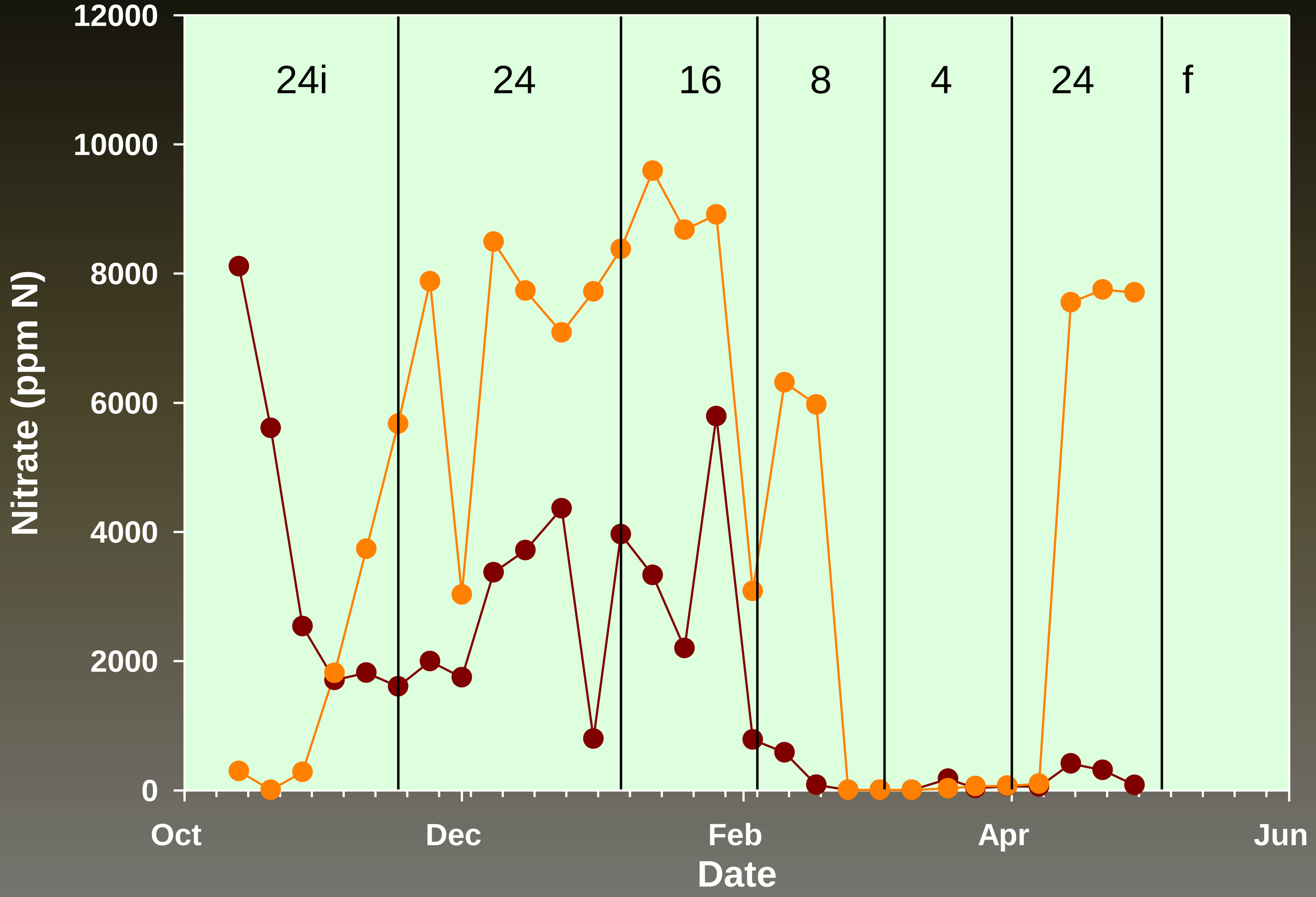
Ammonium concentration in tidal columns



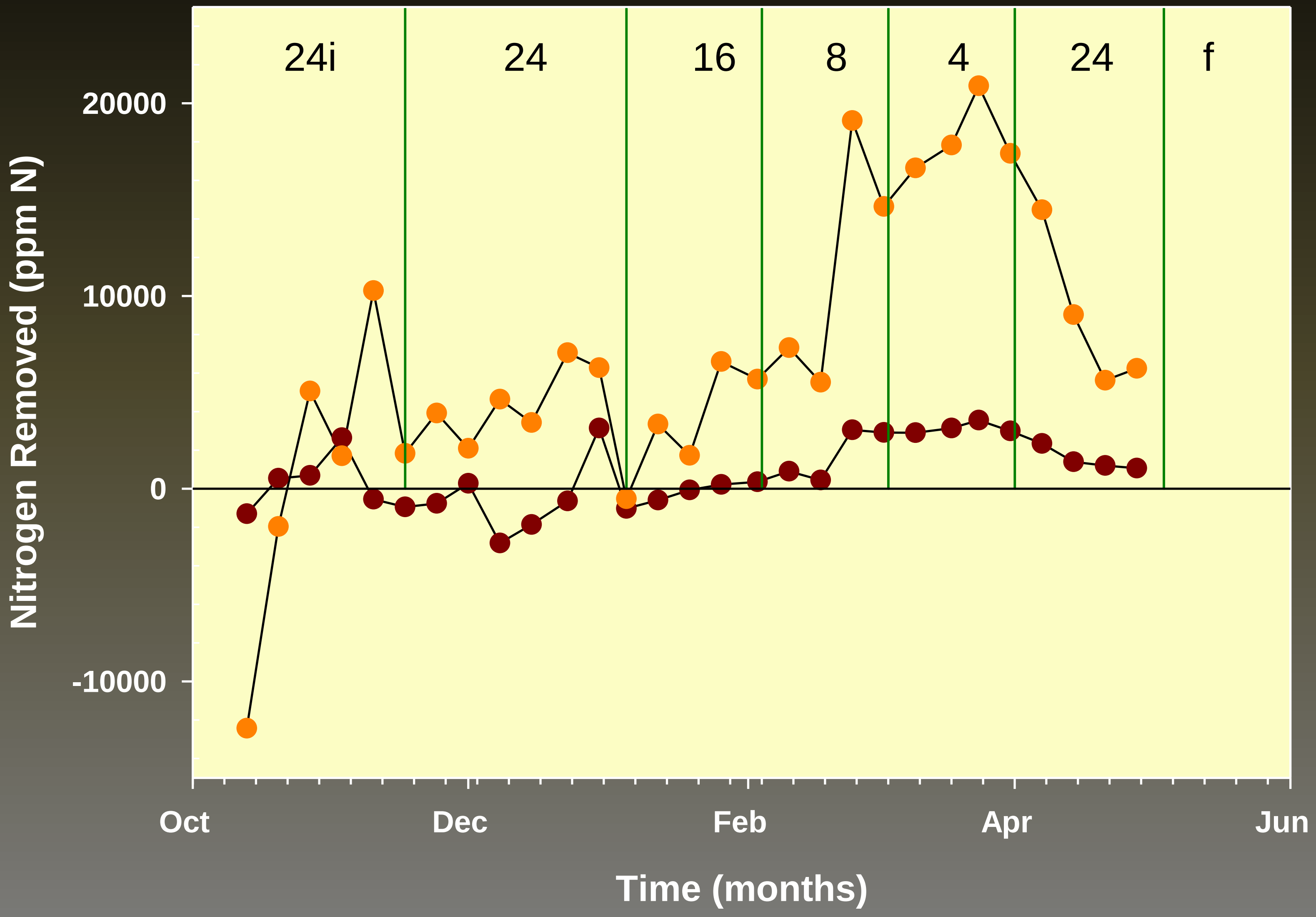
Nitrite concentrations in tidal columns



Nitrate concentrations in tidal columns



Nitrogen removal in tidal columns



Conclusions

- The dominant reaction sequence for N removal from wastewater in tidal reactors is denitrification

- Anammox capable organisms are present, but at insignificant numbers

- Under optimal conditions, AMX may become as plentiful as NOB

- The optimal cycle rate for reactors of the size used in this study is 4 cycles day^{-1}

Living Machine Demonstration: Tema, Ghana

LMECOL

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