DNA/RNA-targeted molecular analysis of bacterial communities

Lysis, DNA/RNA extraction →

DNA/RNA purification →

PCR/RT-PCR amplification of 16S rRNA or functional genes

Cloning → Sequencing → Identification

Sequencing → Phylogenetic analysis

Fingerprinting: DGGE, TGGE, RFLP, t-RFLP, SSCP

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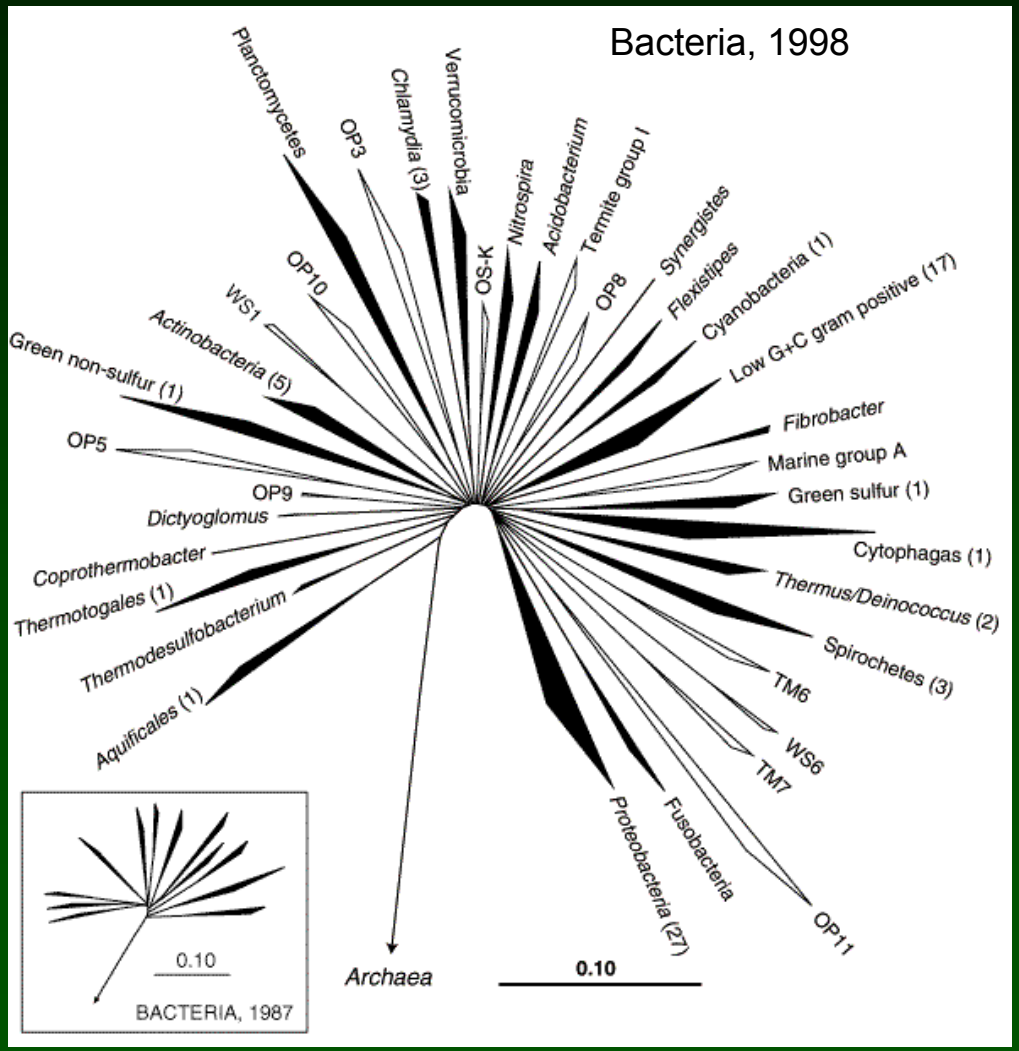
The universal SSU rRNA tree



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Hugenholtz *et al*. 1998 *J Bacteriol* 180, 4765–4774.

Nitrification

Decomposing organic matter

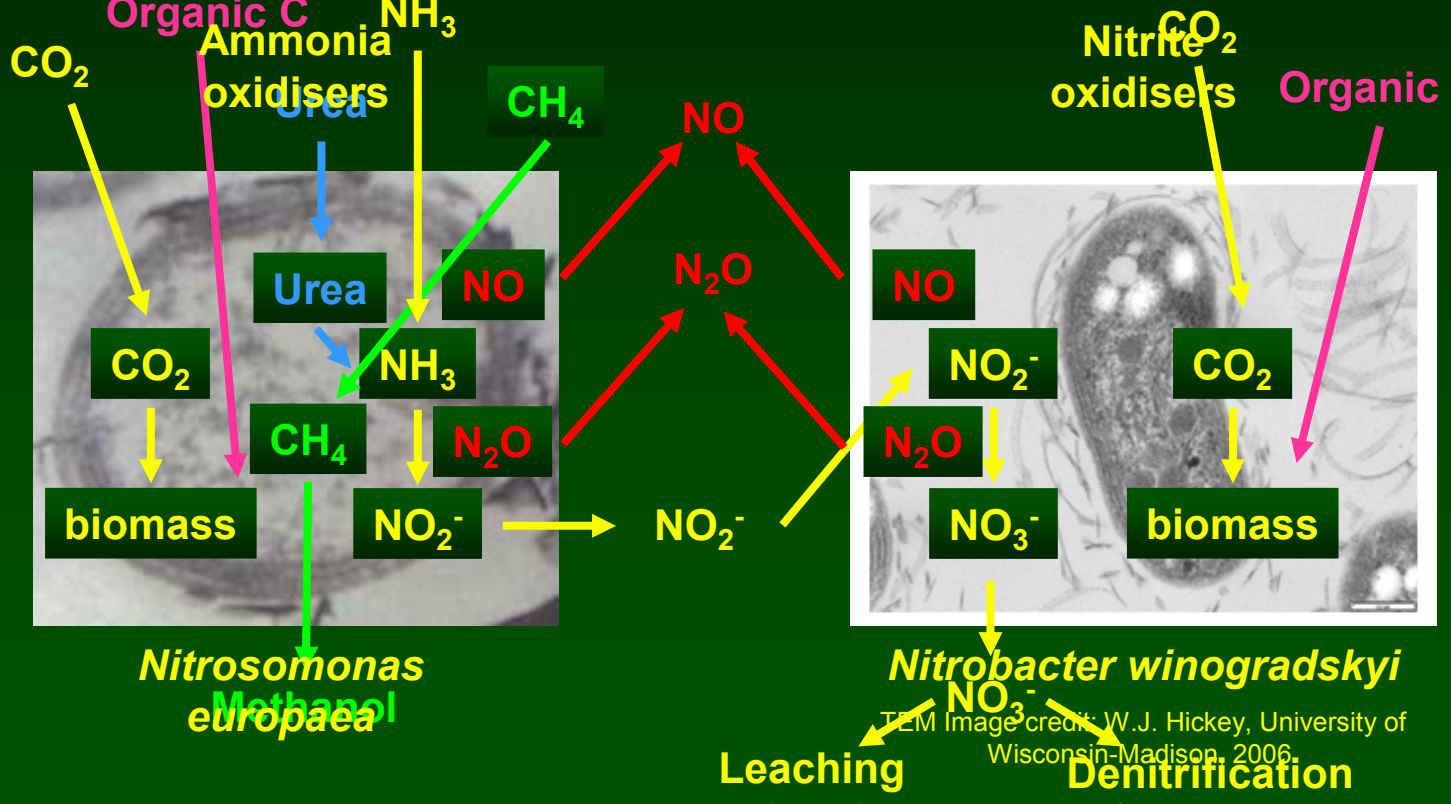
Animal excreta

Fertiliser N

N flux

1.5 x 10^{15} g soil N y^{-1}

6 x 10^{15} g marine N y^{-1}



TEM Image credit: W.J. Hickey, University of Wisconsin-Madison, 2006.

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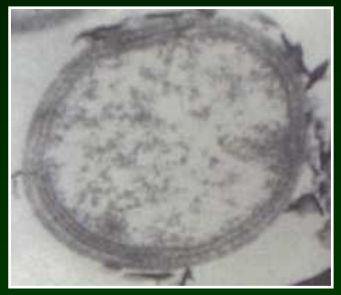
Ammonia oxidiser taxonomy

Nitrosomonas



Betaproteobacteria

Nitrosospira

Nitrosolobus

Nitrosovibrio

Nitrosococcus

Gammaproteobacteria

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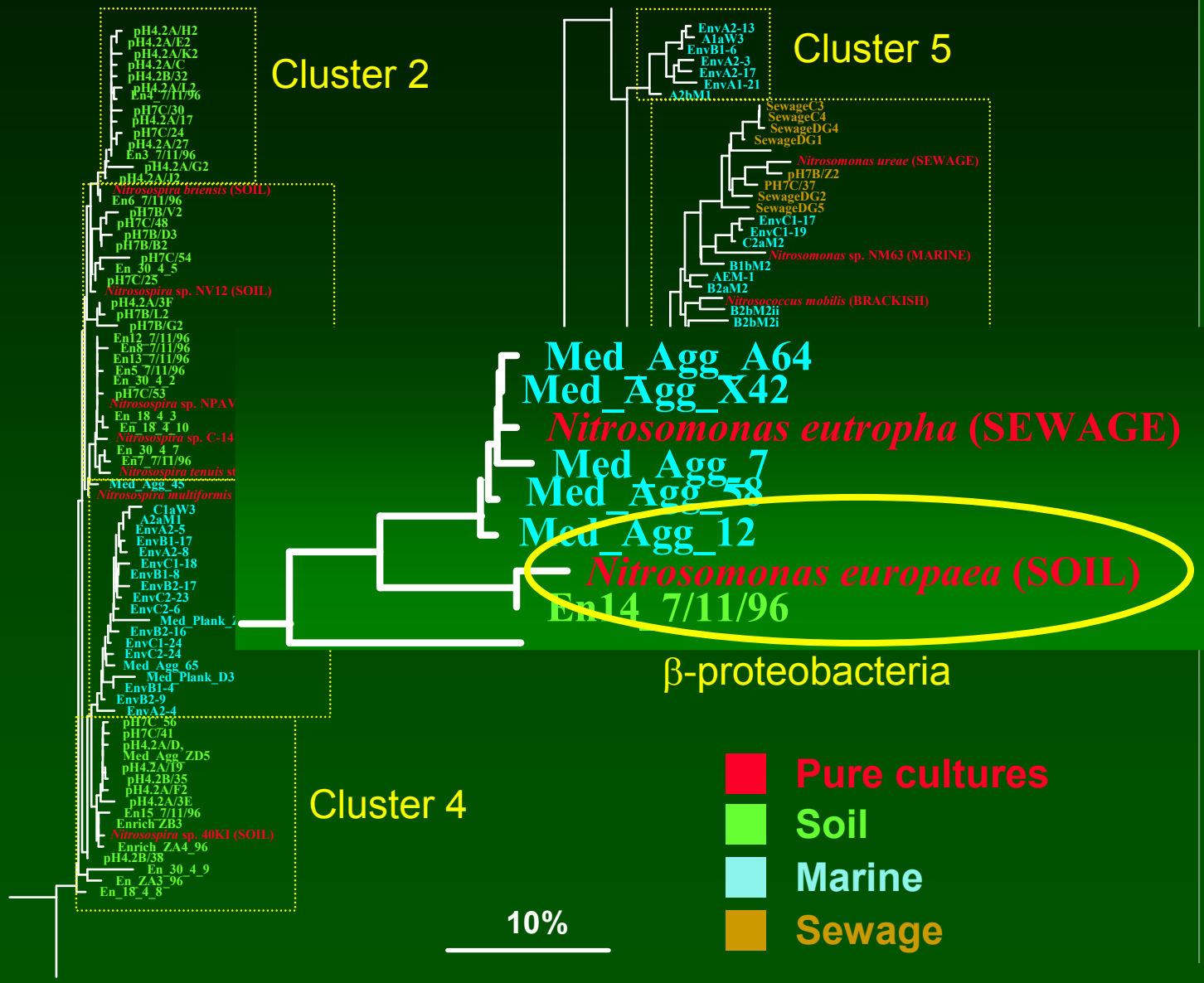
Natural sequence diversity



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Stephen *et al.*, 1996 AEM 62, 4147-54.

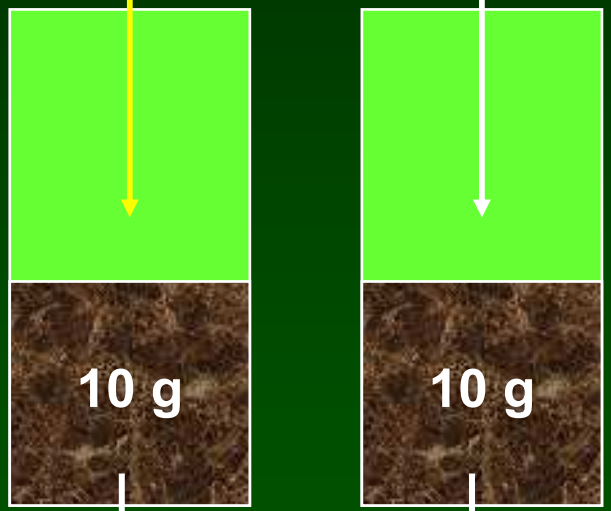
Influence of sheep urine on ammonia oxidisers

- $KHCO_3$
- KCl
- K_2SO_4
- Glycine
- Urea

1 ml synthetic sheep urine

1 ml distilled water

1 mg N g^{-1} soil



pH Ammonia Nitrate Urea DGGE

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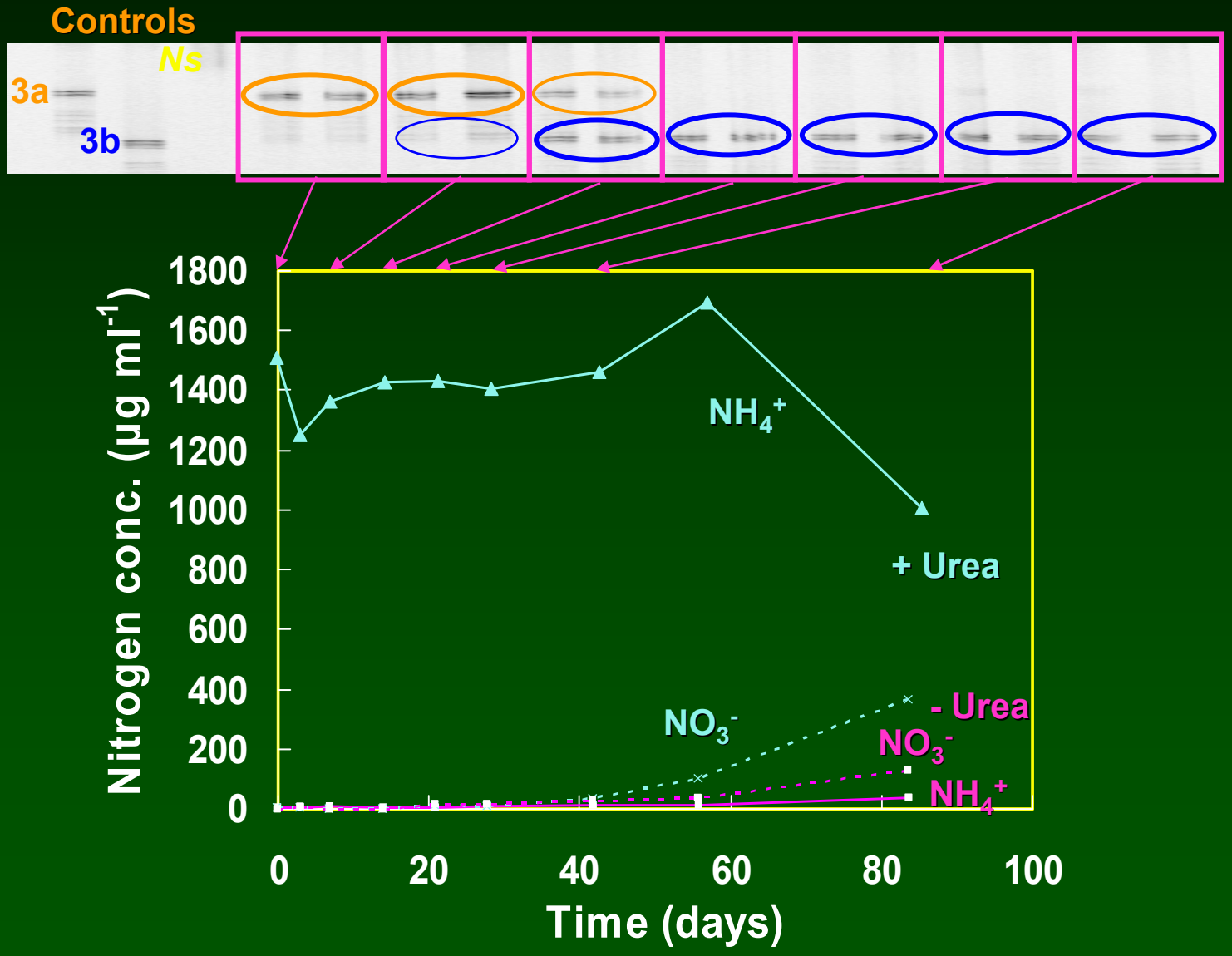
Webster *et al.*, 2005 *Env Micro* 7, 676-684.

Nitrification after addition of urea



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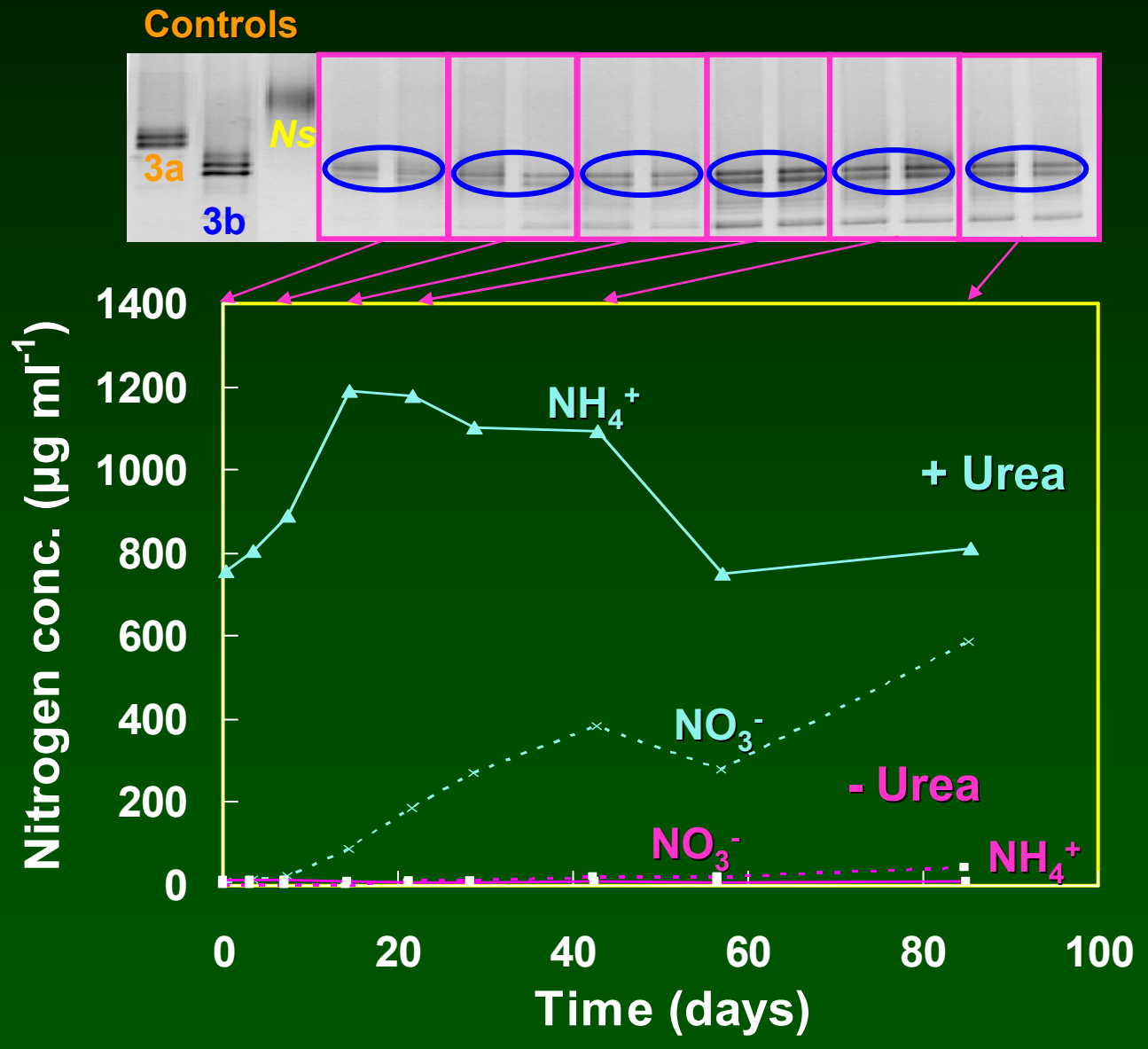
Webster *et al.*, 2005 *Env Micro* 7, 676-684.

Nitrification after addition of urea



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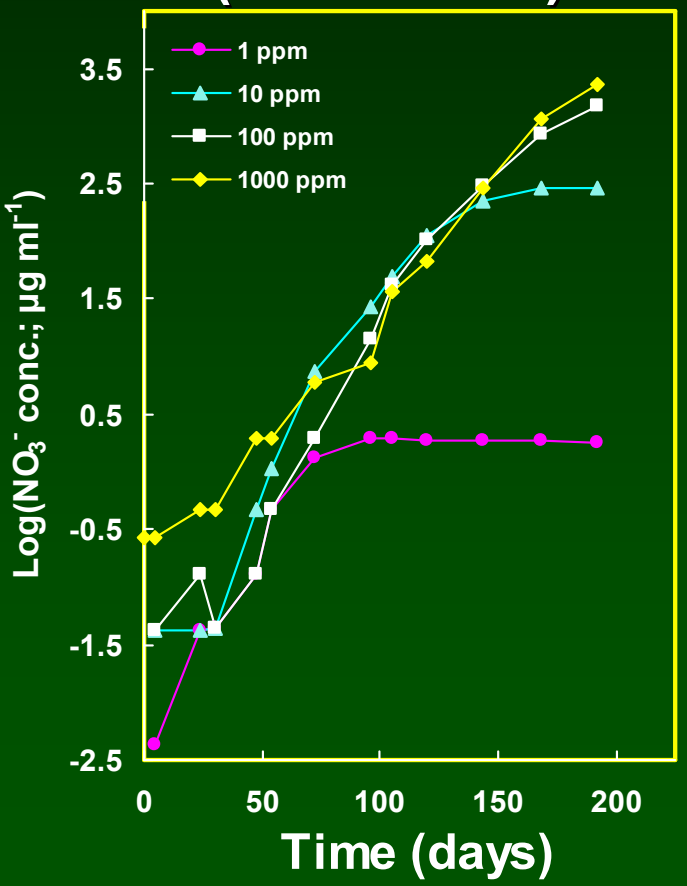
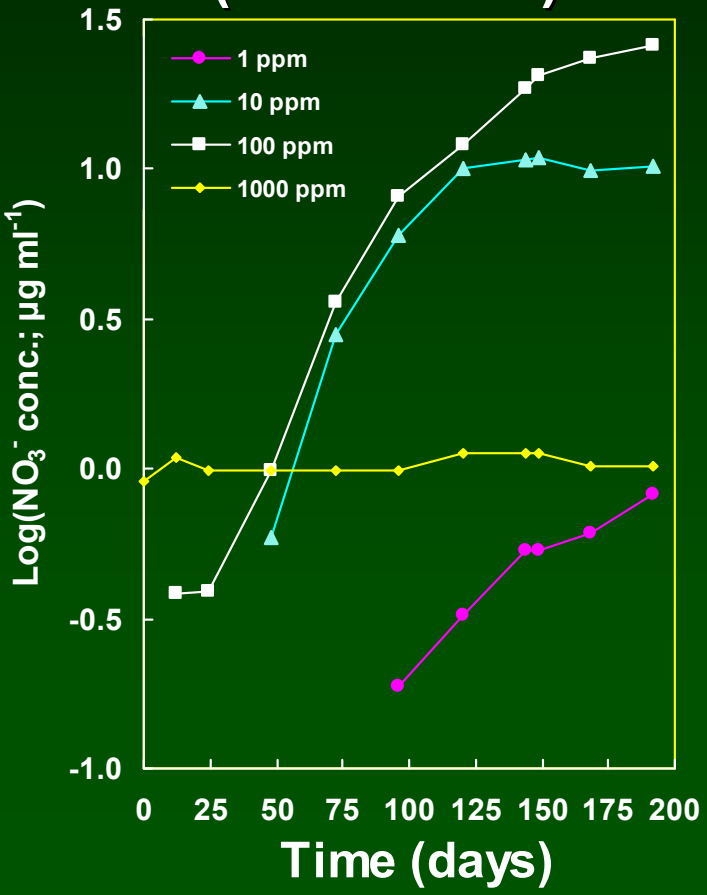
Webster *et al.*, 2005 *Env Micro* 7, 676-684.

Effect of ammonium conc. on growth of pure cultures

Nitrosospira briensis (Cluster 3b)



Nitrosospira NpAV (Cluster 3a)



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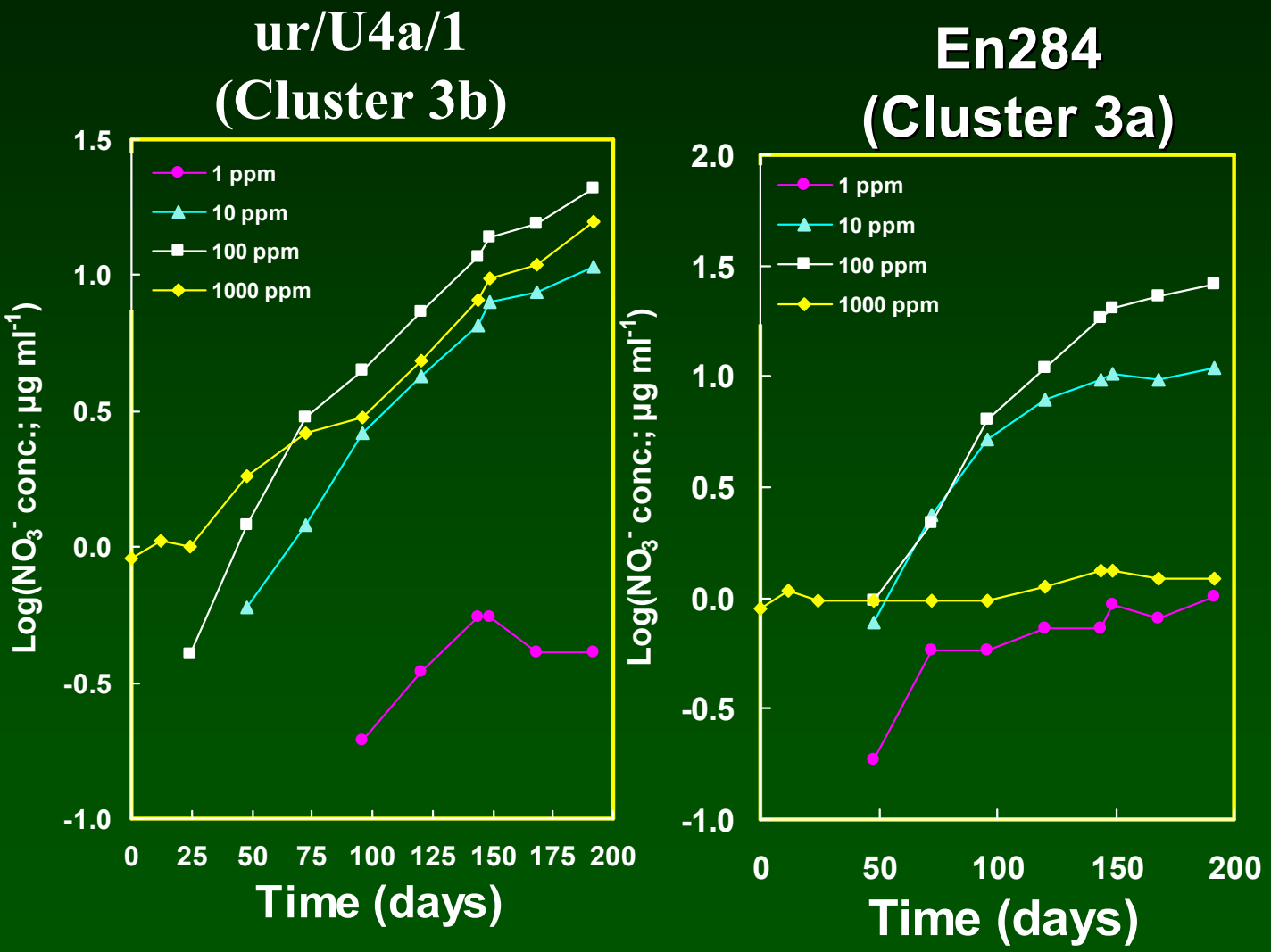
Webster *et al.*, 2004 *Env Micro* 7, 676-684.

Effect of ammonium conc. on growth of enrichments



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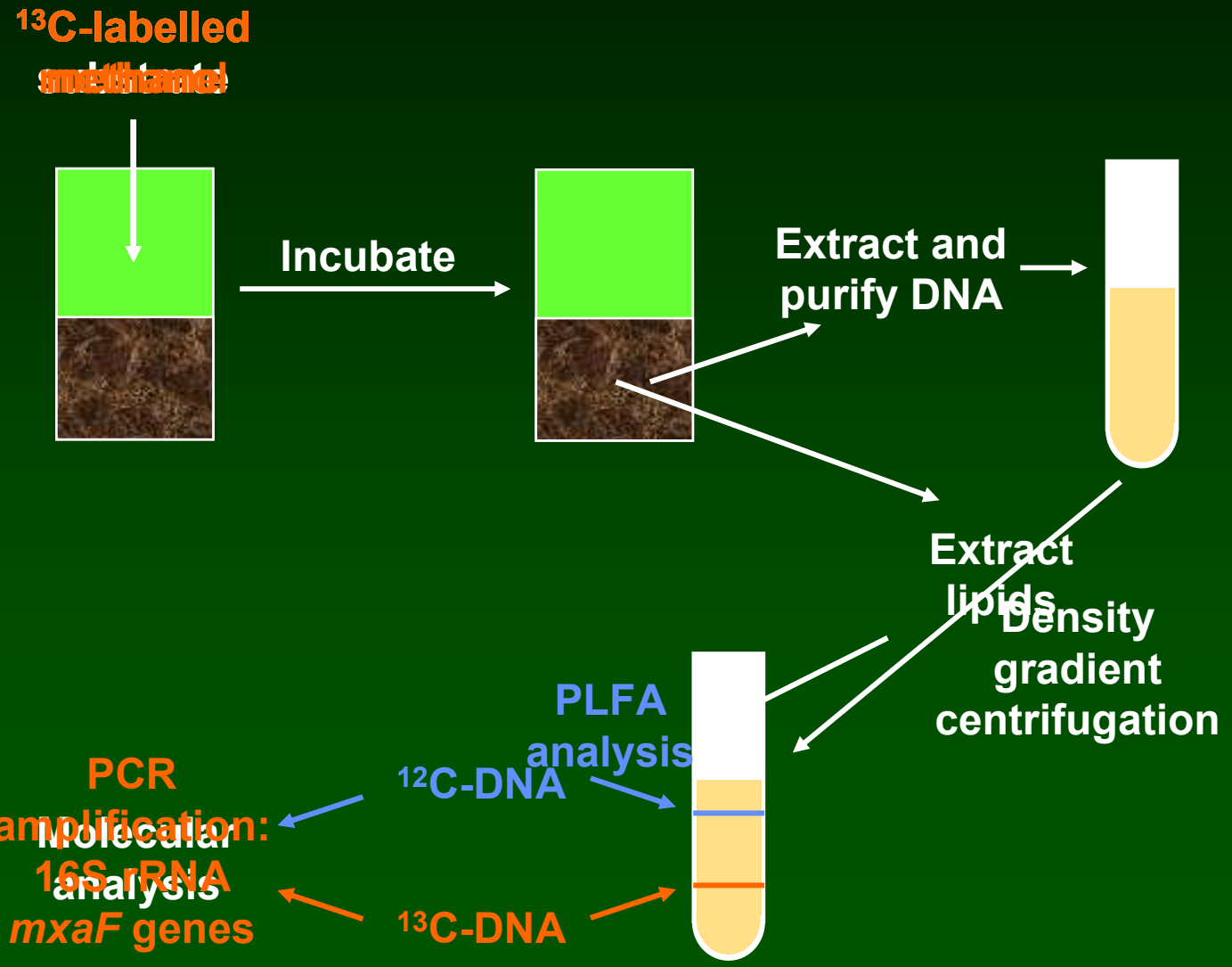
Webster *et al.*, 2005 *Env Micro* 7, 676-684.

Stable isotope probing
^{13}C-PLFA analysis



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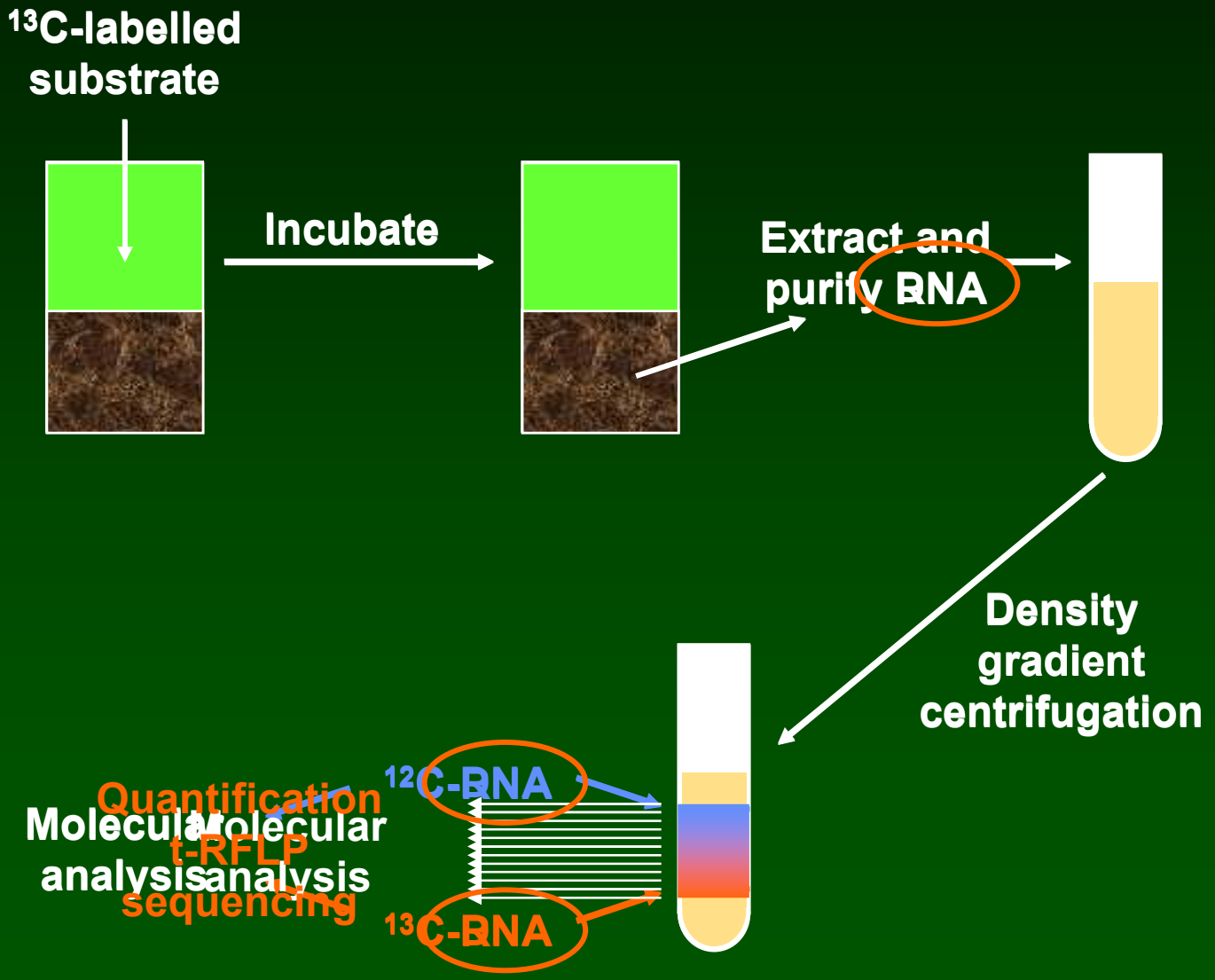
Boschker et al. 1998 *Nature* 392, 801-805.

Radajewski et al. 2000 *Nature* 403, 646-649.

Stable isotope probing
RNA-SIP



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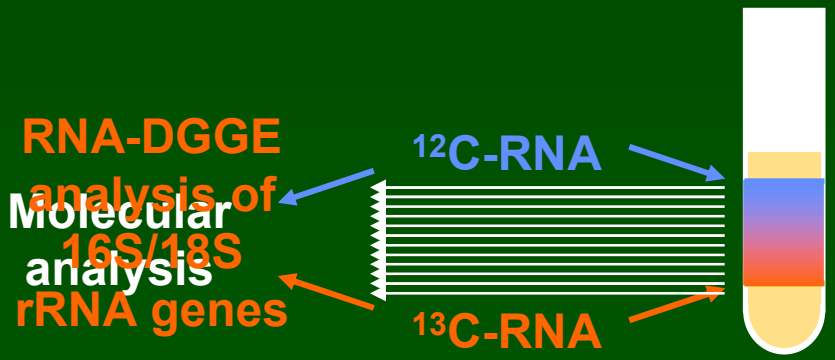
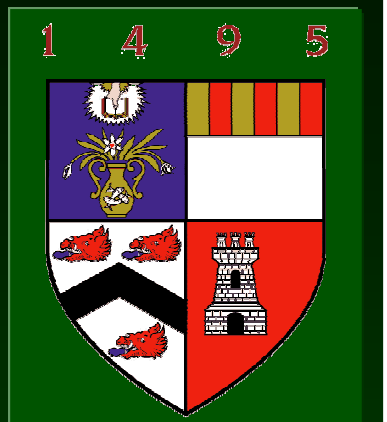
Lueders *et al.* 2003 *Env Micro* 6, 60-72
6, 73-78

Stable isotope probing in the field: RNA-SIP

Extract and purify RNA

Density gradient centrifugation





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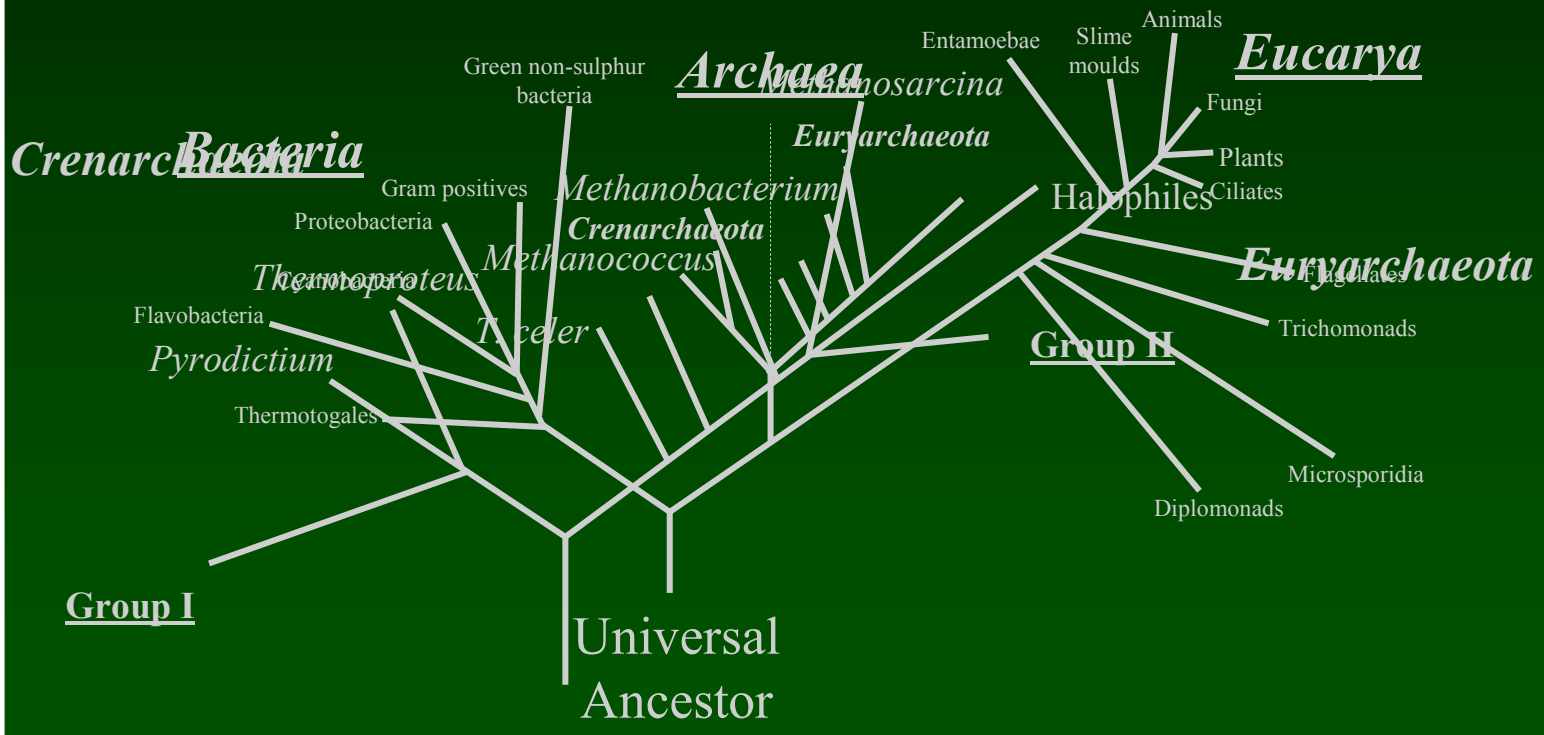
The Crenarchaeota



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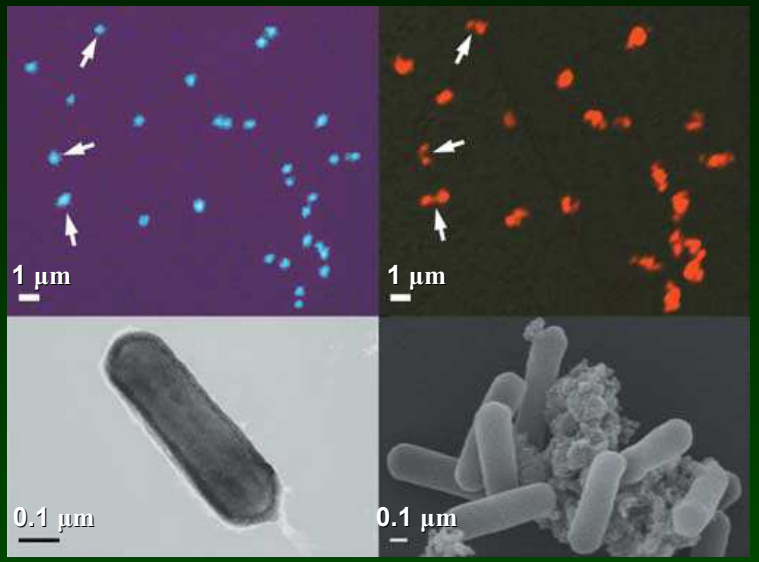
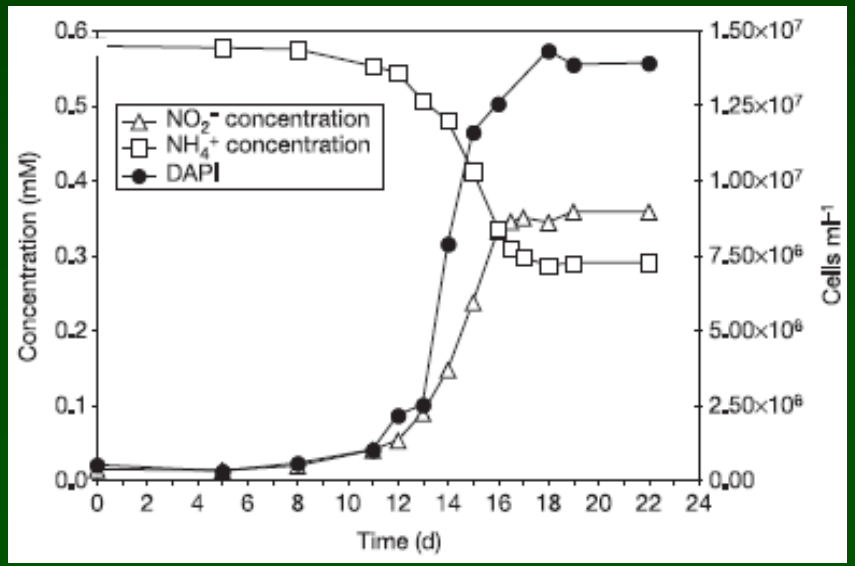
Isolation of *Nitrosopumilus maritimus*





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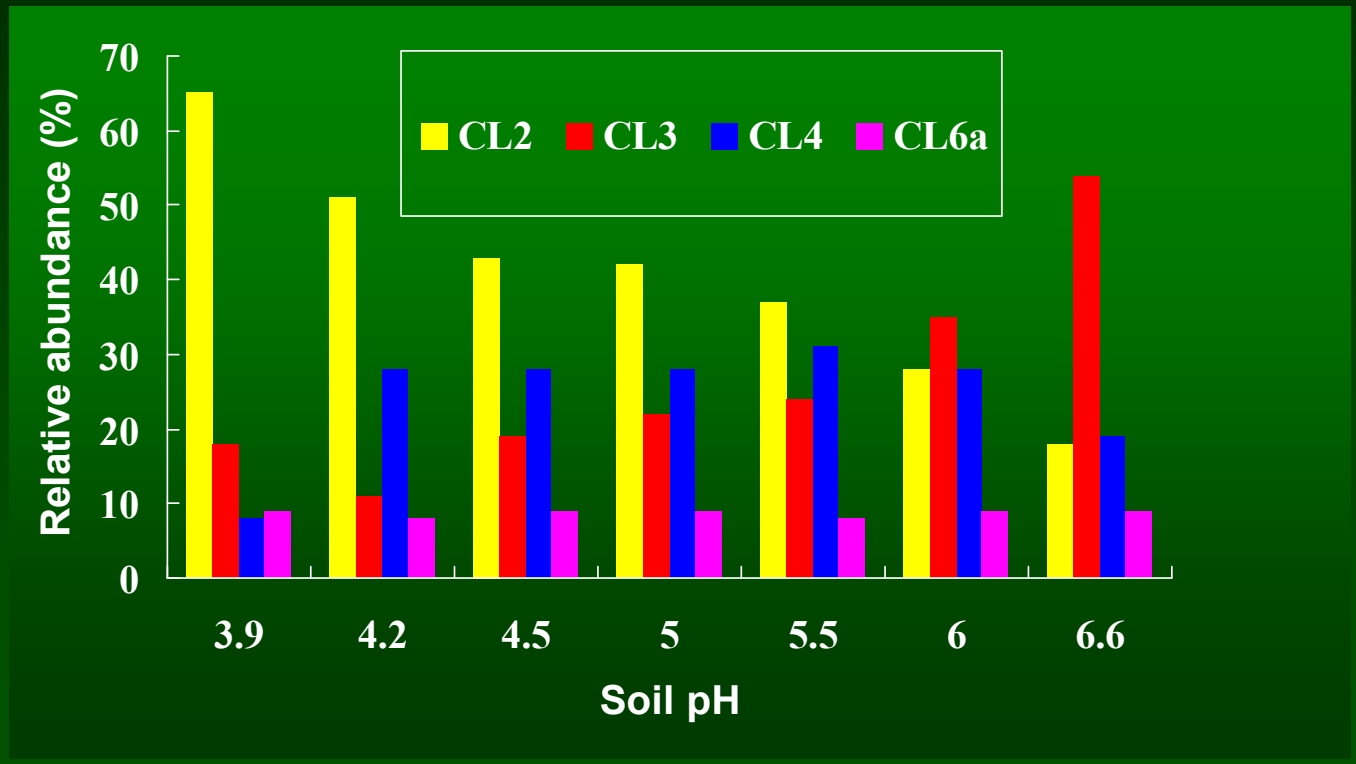
Könnneke *et al*. 2005, *Nature* 437, 543-546.

Relative abundance of bacterial ammonia oxidiser clusters in soils of different pH



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Stephen *et al.* 1998, *Appl. Env. Micro.* 64, 2958-2965