# Ecological Perspective on New Connectivity between Nitrogen and Carbon Cycle

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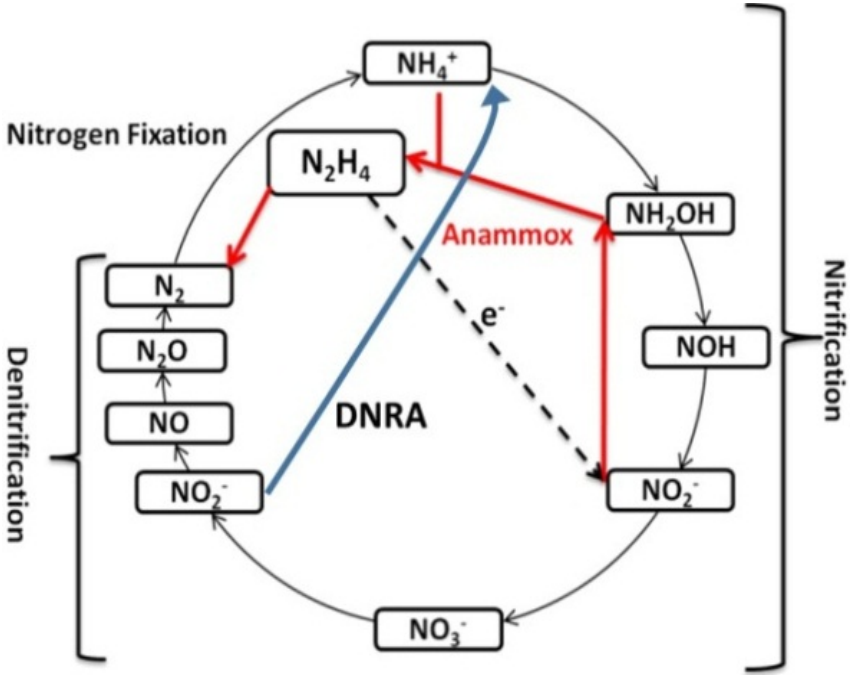
# Introduction: Nitrogen Cycle &Nitrogen Contamination

**Nitrogen contamination** is one of the 14 grand challenges prioritized by the National Academy of Engineering.

The **innovative** and effective **N transformations** through **prokaryotic** mediated pathways have been well received.

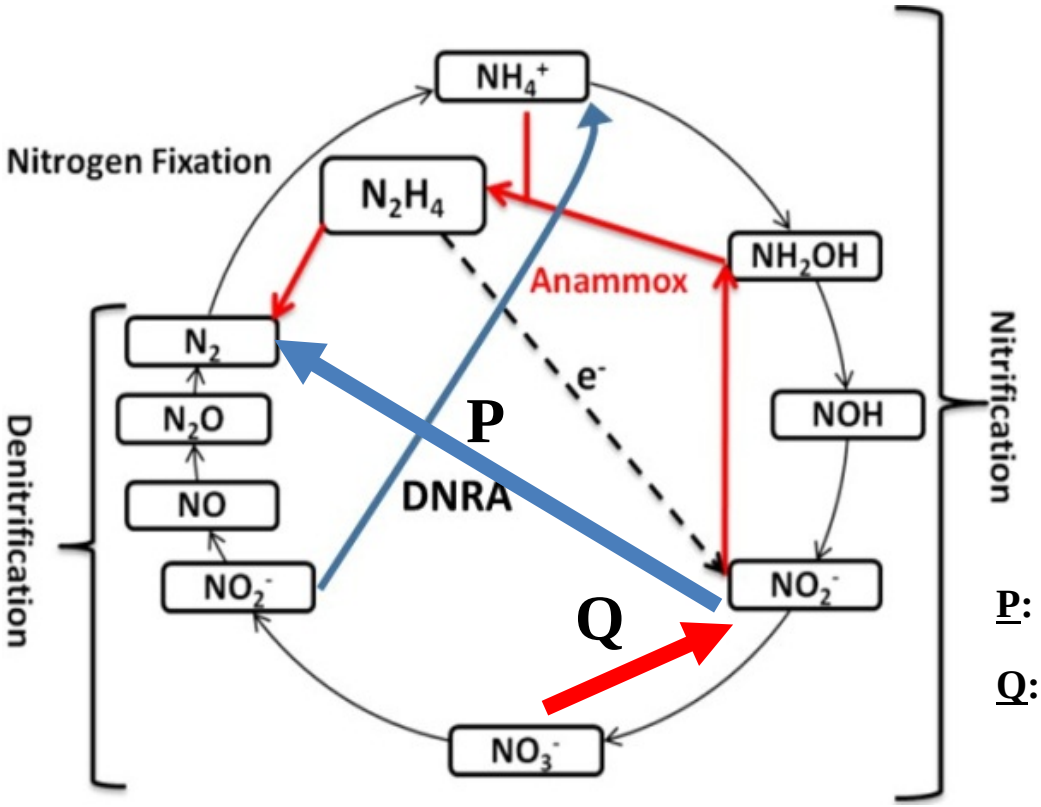
**Example: Ananerobic ammonia oxidation** was included in the overall N cycle after its inception in mid 1990's.




**Denitrification** is an important component of the overall nitrogen cycle.

- **Heterotrophic process** →organic carbon
- **Autotrophic process** →hydrogen and reduced sulfur compounds.

# Introduction: Recent Developments In N Cycle: Methane Coupled Denitrification



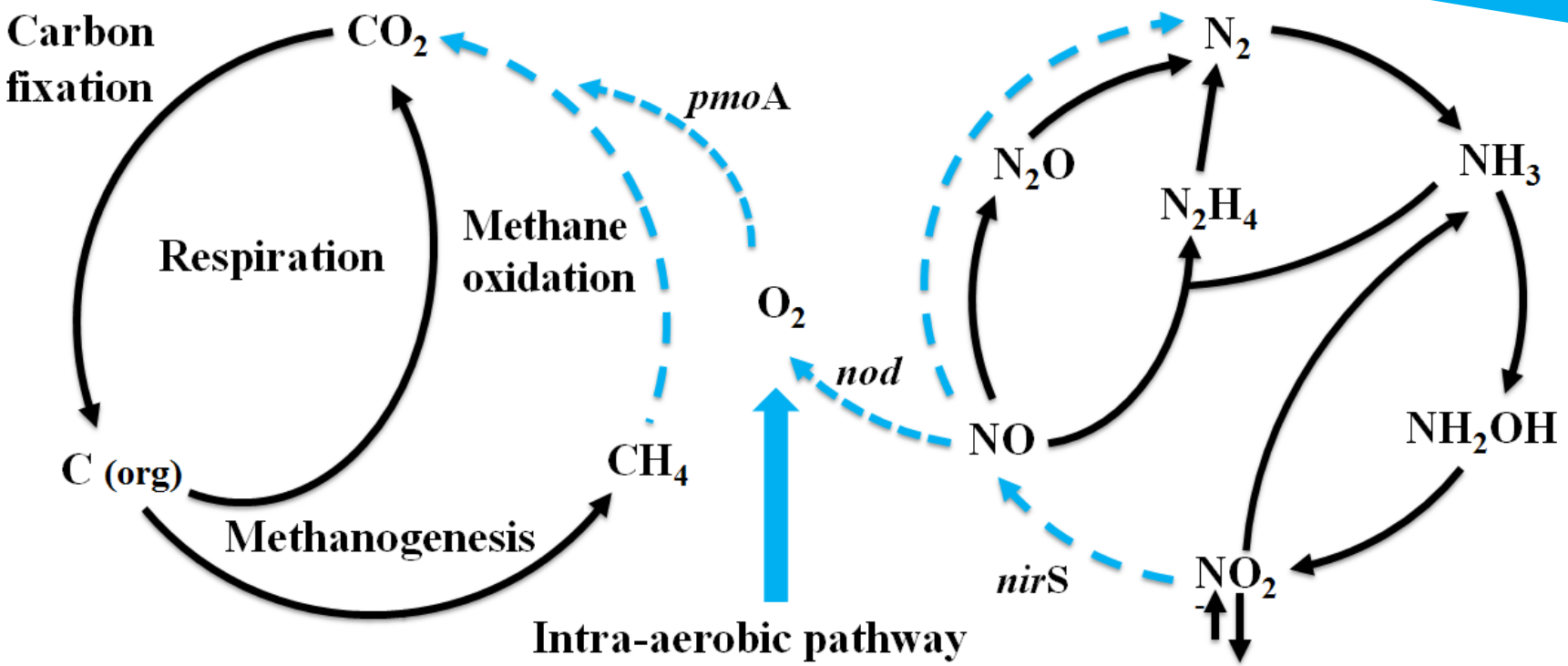

Adapted from Galloway et al., 2008

**Denitrification with anoxic methane oxidation (DAMO) is a very recent development**

**P:** $3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O$

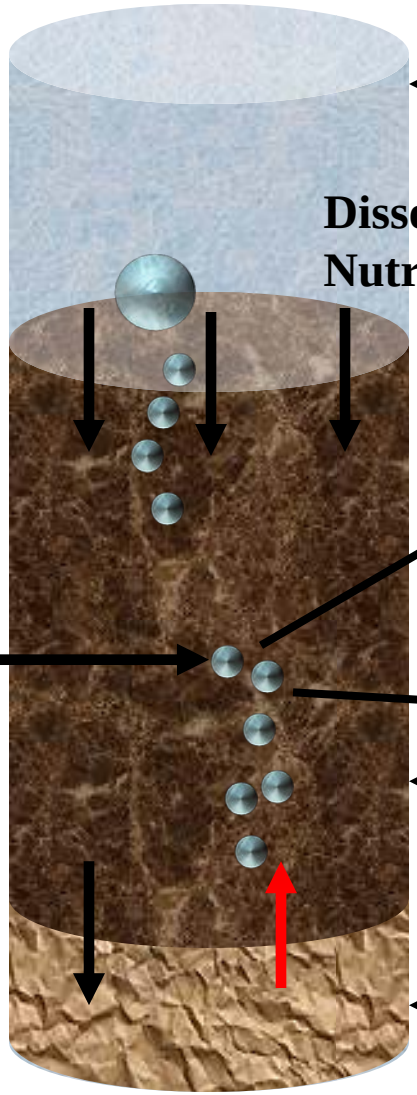
**Q:** $CH_4 + 4NO_3^- \rightarrow CO_2 + 4NO_2 + 2H_2O$

# Introduction: Connectivity Between C & N Cycle : Methane oxidation coupled to $NO_2$




**P:** $3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O$

# DAMO Process in Sediments



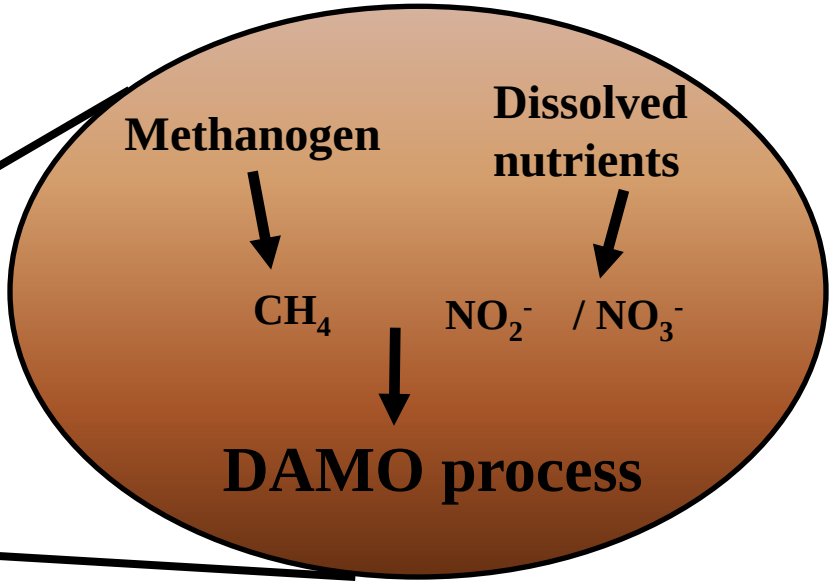
Water Column

Dissolved Nutrients

Methane produced

Sediments

Bed Rocks

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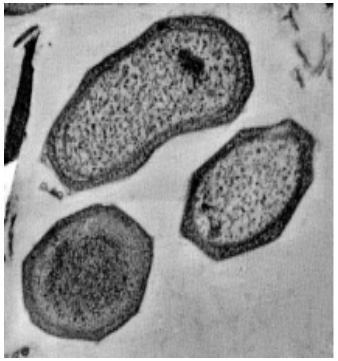
# Introduction: Denitrifying Anaerobic Methane Oxidizing (DAMO) Prokaryotes

## Bacteria ($NO_2^-$)

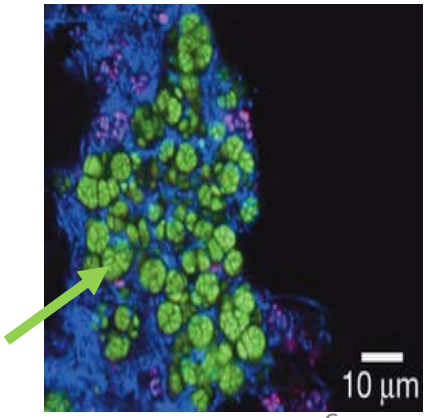
- *Candidatus* ' Methylomirabilis oxyfera only known bacteria to show DAMO activity.
- NC 10 phylum
- NC10 phylum bacteria are ubiquitous.
- Slow growth (1-2 weeks)
- Genome size of 2.75 (Mb)
- Gram negative
- Size of 0.8-1.1µm
- Mesophilic



Wu *et al*., 2012

## Archaea ($NO_3^-$)

- *Candidatus* ' Methanoperedens nitroreducens
- ANME-2d lineage
- Enriched from freshwater sediments and wastewater sludge.
- Genome size of 3.2 (Mb)
- Size of 1-3µm
- Mesophilic




Haroon *et al*., 2013

# Research Objectives: So what is next?

## Objectives in this study:

- to confirm the presence of DAMO activity in ecosystem (Jordan river, UT)
- to enrich the prokaryotes involved in DAMO process from the sediment (Jordan river, UT).
- to compare microbial diversity in the enriched DAMO reactors and the Jordan river

# Methods and Results: Rate of DAMO process In Jordan River

**20grams** of sediments
(a) 0-5cms
(b) 5-10cms
(c) 10-20cms

**$NO_2$** medium Headspace **$CH_4$**

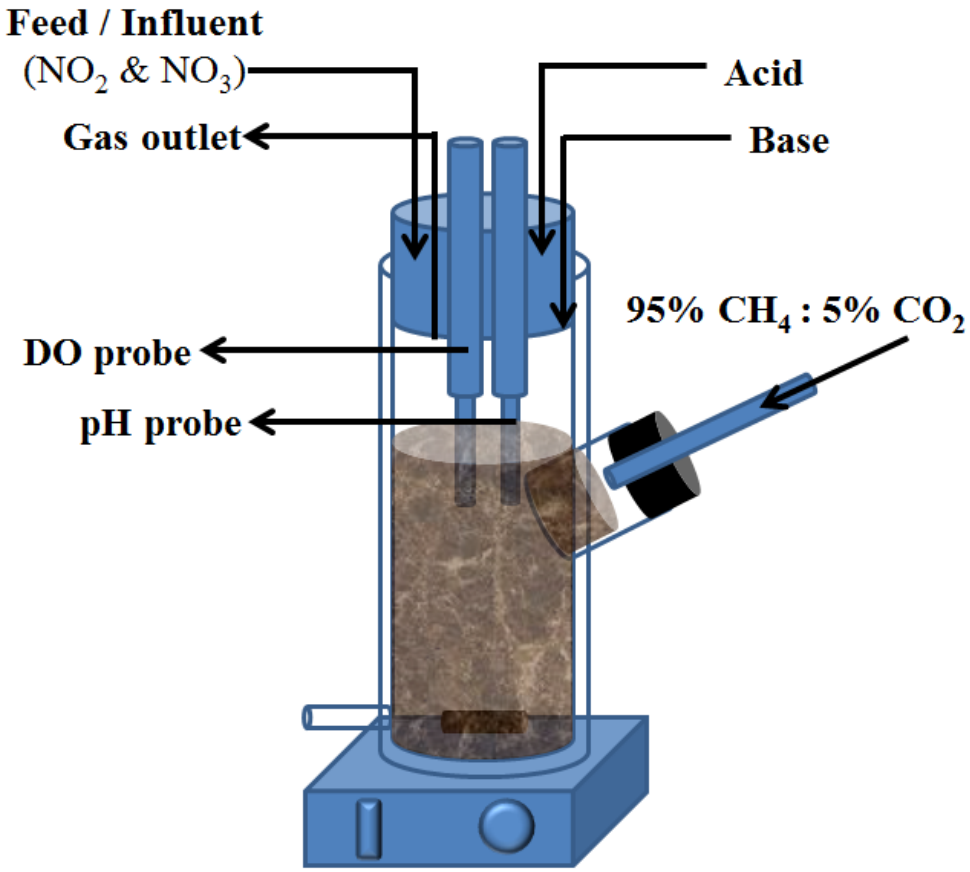
DAMO coupled with **$NO_2$**

**$NO_3$** medium Headspace **$CH_4$**

DAMO coupled with **$NO_3$**

| **$CH_4$ (Headspace)** | **0-5cms Depth (µM/L.day)** | **5-10cms Depth (µM/L.day)** | **10-20cms Depth (µM/L.day)** |
|---|---|---|---|
| DAMO-**$NO_2$** | **0.77** | **0.6** | **0.78** |
| DAMO-**$NO_3$** | **0.07** | **0.06** | **0.037** |

# Methods and Results: Reactor Configuration: DAMO reactor



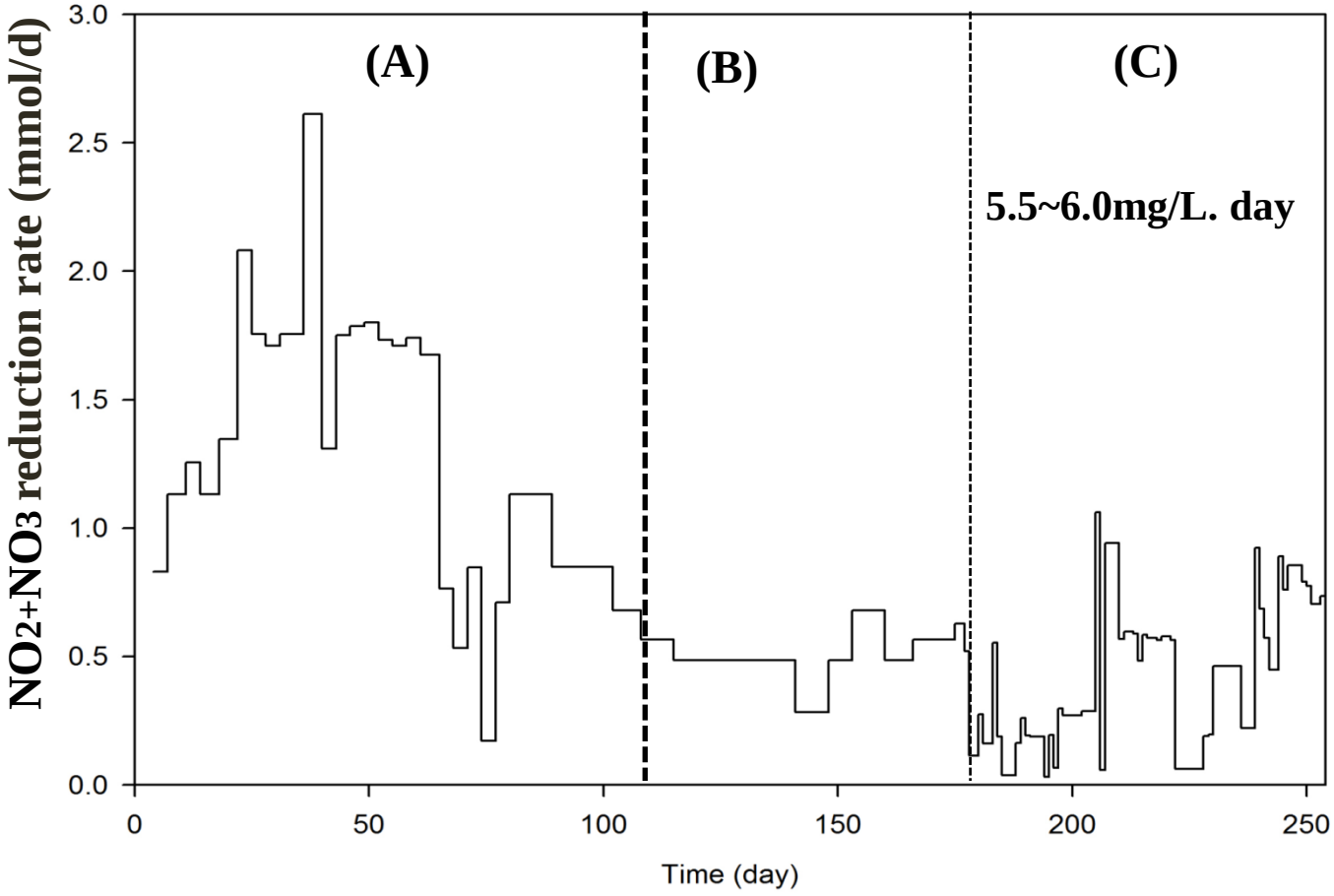

## Lab scale DAMO reactor

**Semi-continuously fed sequencing batch reactor (FBR)**

- 1.9L reactor volume
- 6 day cycle includes 400ml of feeding* ($NO_2$ & $NO_3$) (2.78ml/h)
- HRT of 29 days
- SRT of 100 days
- Operating at 35$^{O}$ C
- Purged with 95% $CH_4$ : 5% $CO_2$ (8ml/min)
- pH 7.5±0.2
- DO maintained below detection level (Anoxic)

*Feed composition based on Ettwig *et al.*, 2009

# Methods and Results: Reactor Performance




**(A) Reactor start-up**

**(B)Denitrification became negligible.**

**(C)Monitoring frequency changed from 7 days to daily.**

# Methods and Results: Methane Oxidation Rates

**$NO_2$** medium Headspace **$CH_4$** → DAMO coupled with **$NO_2$**

**$NO_3$** medium Headspace **$CH_4$** → DAMO coupled with **$NO_3$**

**$NO_2$/ $NO_3$** medium Headspace **$N_2$** → Denitrification

$CH_4$ mass balance in serum bottle (duplicate)

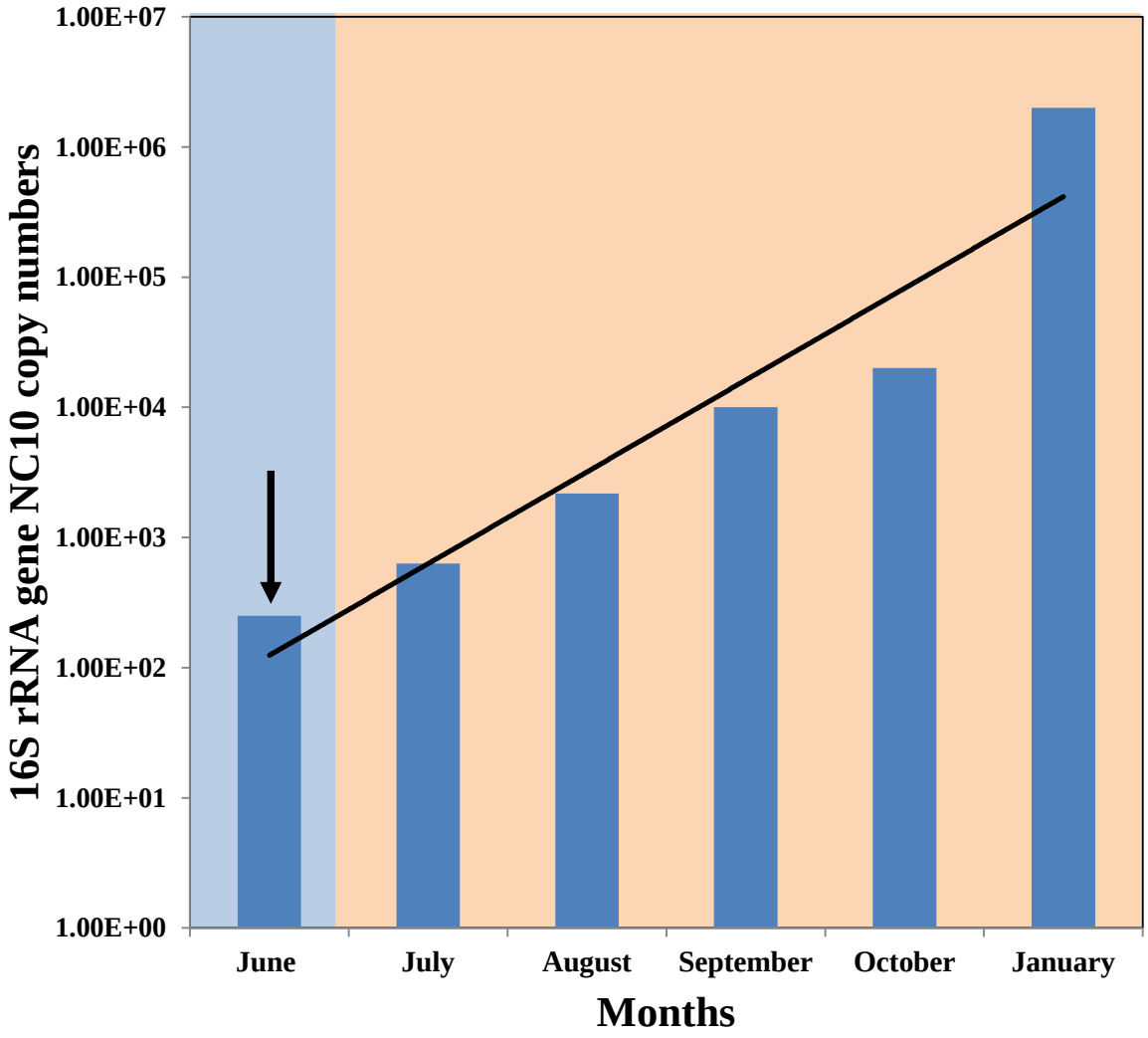
**Average 80-85% = ($CH_4$ lost from reactor/ $CH_4$ expected based on stoichiometry) * 100**

| **$CH_4$ (Headspace)** | **March (mM/day)** | **December (mM/ day)** |
|---|---|---|
| DAMO-**$NO_2$** | 0.021 | 0.19 |
| DAMO-**$NO_3$** | 0.005 | 0.007 |

| **$N_2$ (Headspace)** | **March (mM/day)** | **August (mM/day)** |
|---|---|---|
| Denitrification -**$NO_2$** | N.D. | N.D. |
| Denitrification -**$NO_3$** | N.D. | N.D. |

**Currently on going experiment for Stable isotope probing**

# Methods and Results: 16S rRNA gene (NC10)quantification




**Quantification of 16SrRNA gene specific to NC10 phylum**

**qP2 F/R primers**

**(Amplify position 1169 to 1460bps)**

**Melting curve analysis (Tm):**

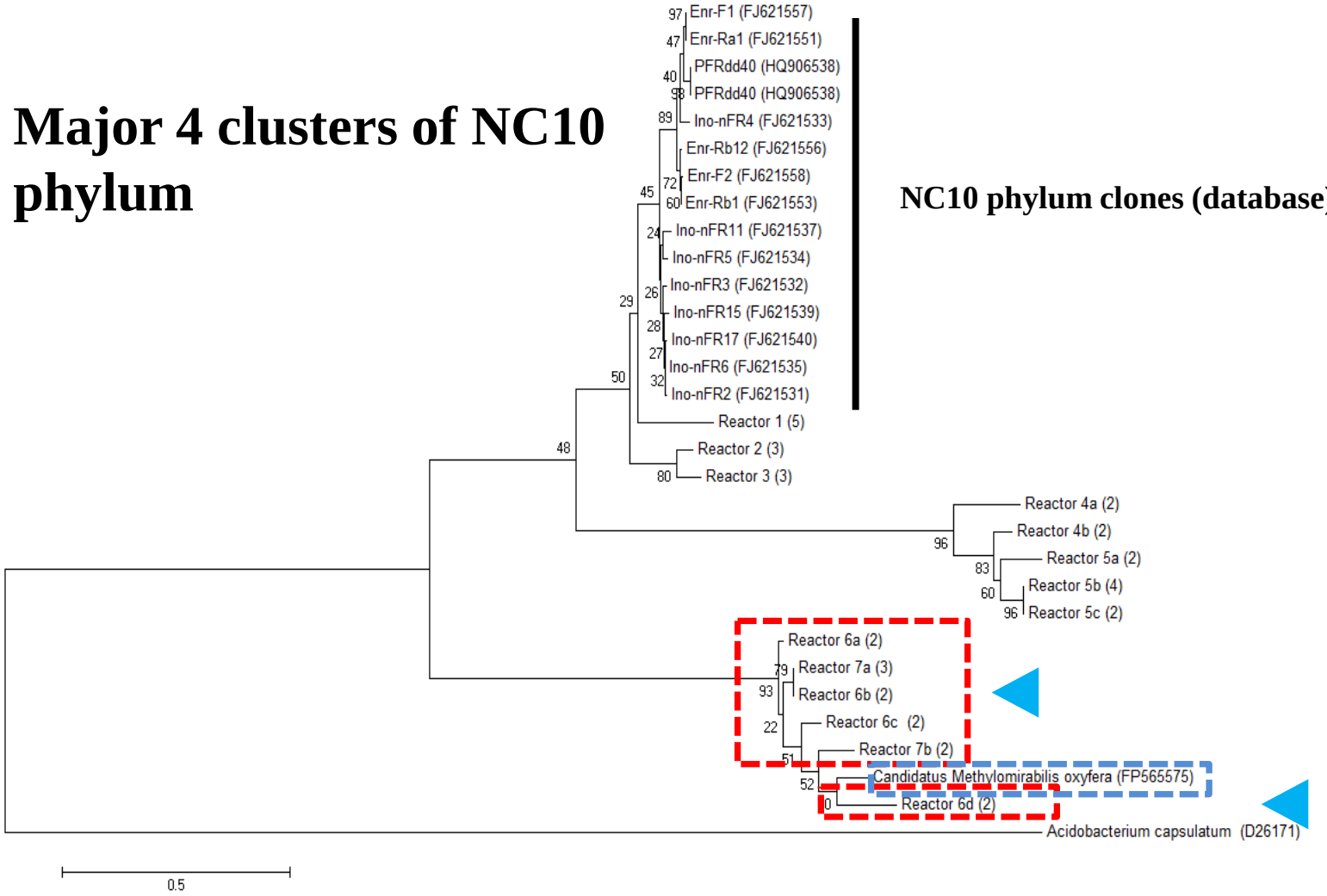
**84.1-84.2°C)**

**Significant increase in 16SrRNA gene (NC10 phylum) copy number**

**Graphical representation of Copy number in logarithmic scale (base 10)**

# Methods and Results: 16S rRNA gene (NC10 phylum clones)

**Major 4 clusters of NC10 phylum**



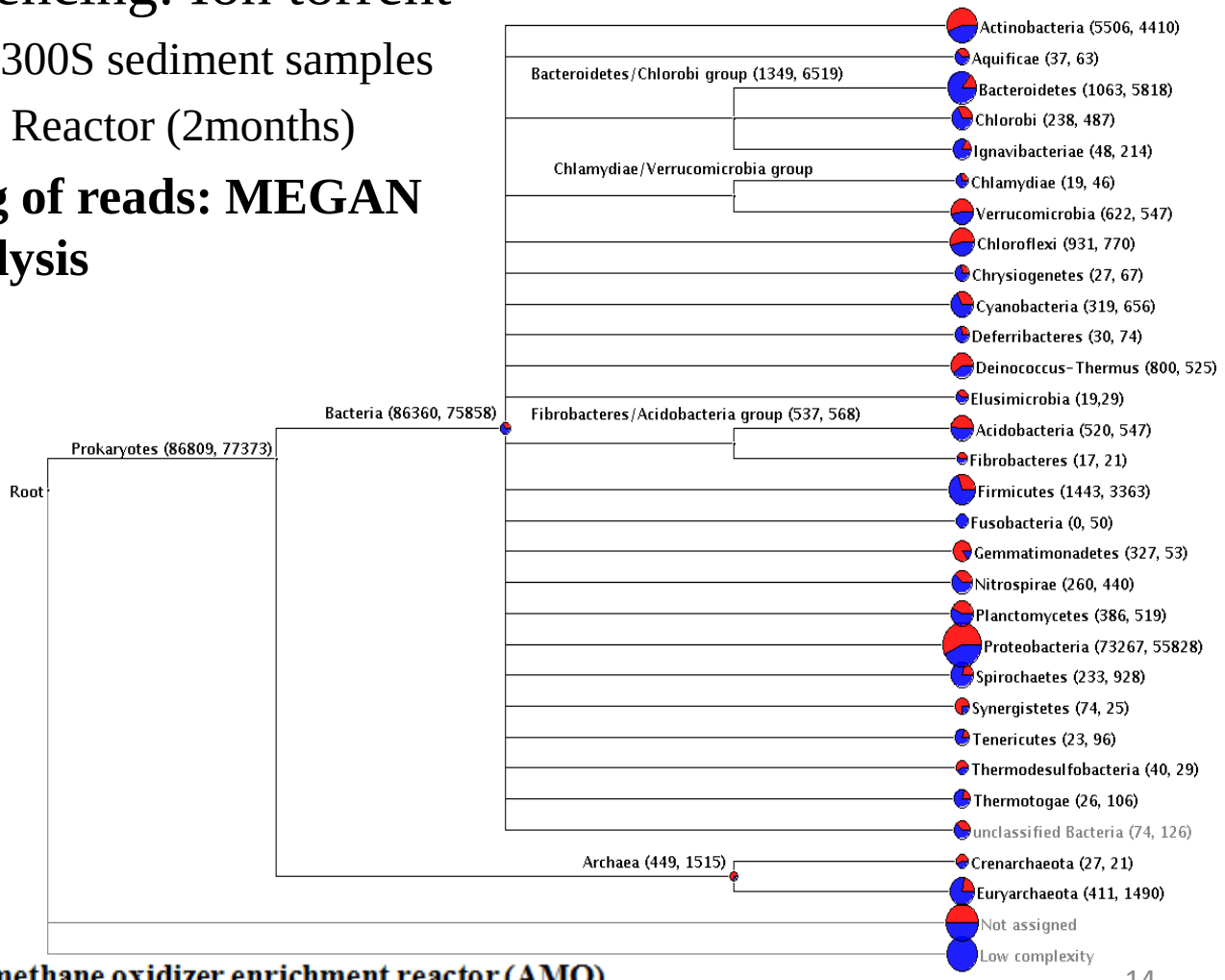

Neighbor-joining method. Boot strap support values (1,000 replicates)

# Methods and Results: High Throughput Sequencing

Next generation sequencing: Ion torrent

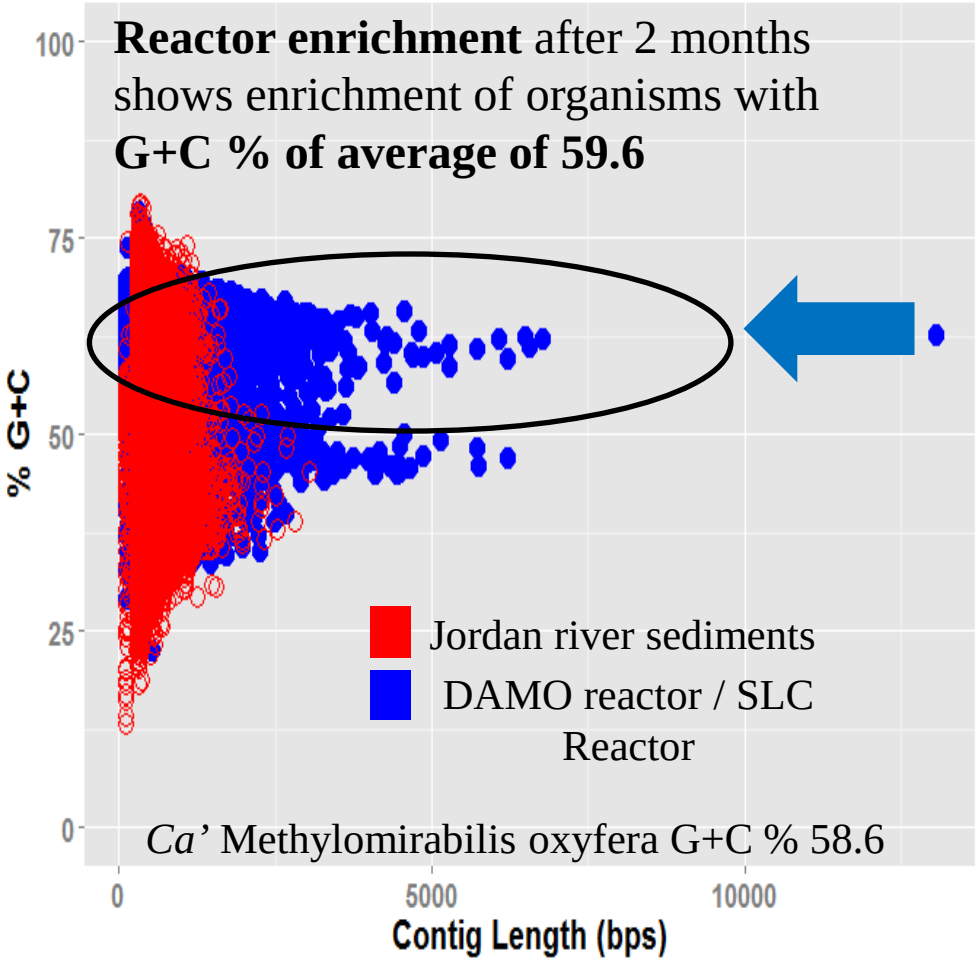
- Riverine sediments: 1300S sediment samples
- DAMO reactor / SLC Reactor (2months)

**Taxonomic Binning of reads: MEGAN analysis**




**Denitrifying anaerobic methane oxidizer enrichment reactor (AMO)**
**Jordan River sediments (JRM)**

# Methods and Results: Evidence Showing Enrichment of *Methylomirabilis* Sp.

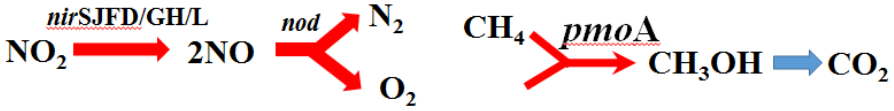



Reads from each metagenome were mapped on

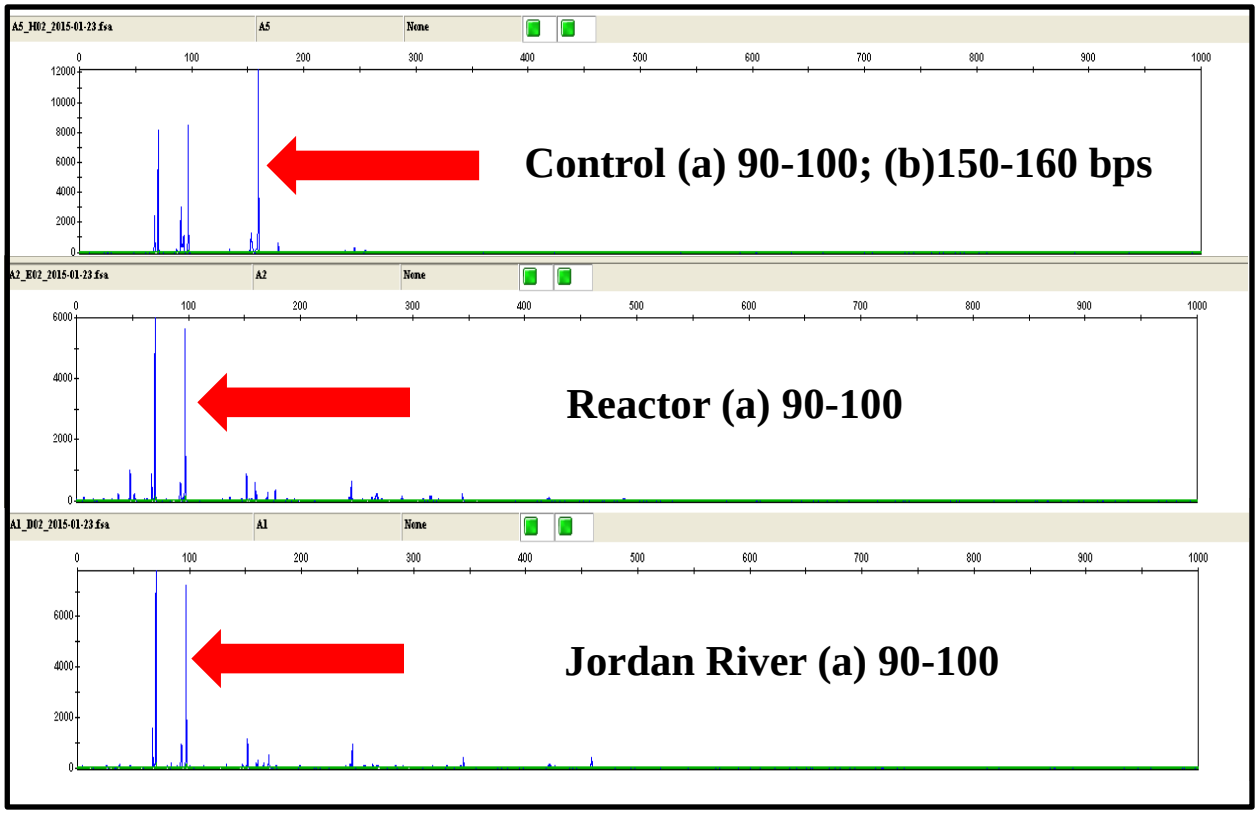
***Candidatus* Methylomirabilis oxyfera** genome.

| | % Reads mapped | Length of genome covered (bp) |
|---|---|---|
| DAMO reactor | 5.29 | 2, 581, 385 |
| Jordan River sediments | 1.39 | 1, 511, 690 |

# On-going: T-RFLP:*pmo*A gene NC10 specific




**Anaerobic methane oxidation coupled to nitrite**




**T-RF profile** of

(a) Jordan River Samples (JRM)

(b) (b) Enrichment reactor (AMO).

(c) DAMO control*

**Results**

**T-RF cuts of 90-100bps observed in all samples.**

**More detailed analysis under processing for developing a robust method for T-RFLP of *pmo*A gene specific to NC 10 phylum**

* DNA extracted from DAMO enriched reactors from Netherlands

# Conclusions

- **Presence of DAMO activity in Jordan River**
- **Enrichment of bacteria capable of anoxic methane oxidation from sediments of riverine system was achieved.**

  Based on

  - Cloning and sequencing of 16SrRNA gene (NC10 phylum)
  - Quantification of 16S rRNA gene (NC10 phylum)
  - Metagenomics: Enrichment of organism with G+C% average 59.6%


# Thank you

# Questions

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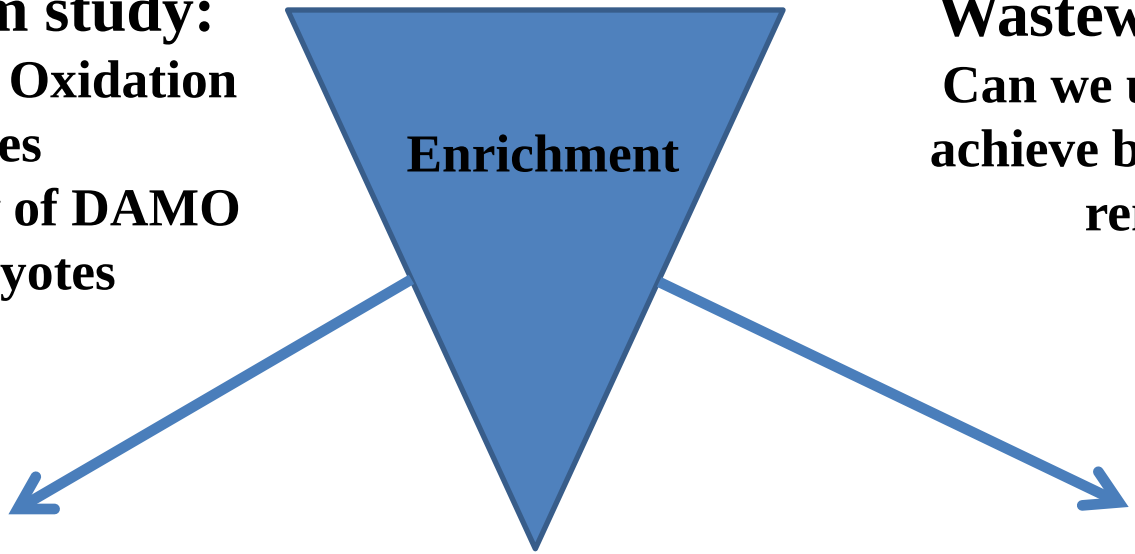
# On Going Research

**Ecosystem study:**
**(a) Methane Oxidation Rates**
**(b) Diversity of DAMO prokaryotes**

**Wastewater study:**
**Can we use DAMO to achieve better nitrogen removal?**




**Geochemistry**

**pH, Temp and DO**

**Lab-scale enrichment**
**(a) Methane Oxidation Rates.**
**(b) Quantification of NC 10 Phylum.**
**(c) Metagenomics: Whole Community Analysis.**
**(d) Gene expression based study.**