Nitrogen Removal in Fresh- and Saline-water Using Hydrogen-driven Denitrification

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Presentation Proceeding

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   II. Impacts
   III. New Zealand
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2. Methodology
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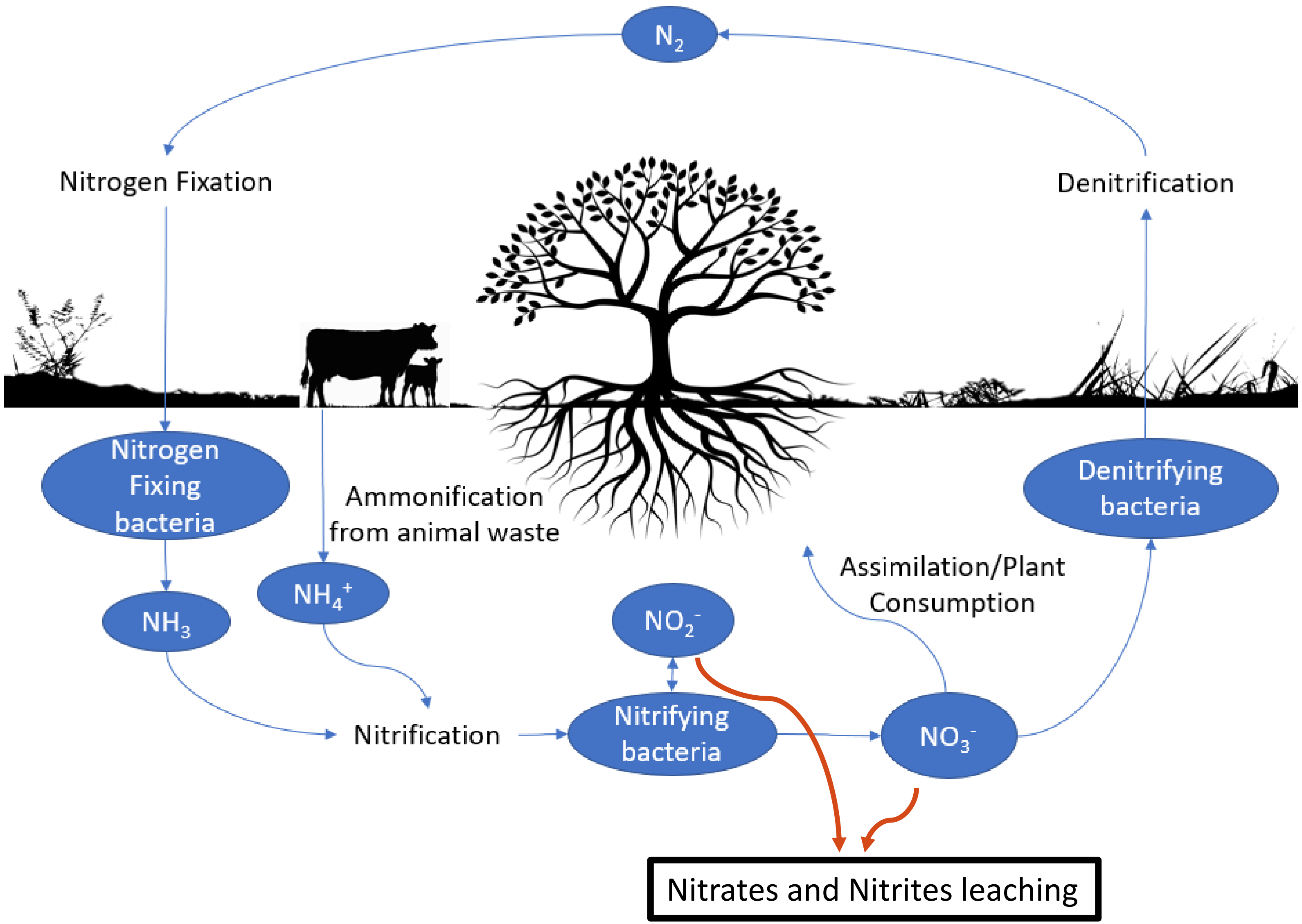
The Nitrogen Cycle

- Nitrogen is an essential element for plants and animals
- Haber-Bosch process resulted in excess nitrites/nitrates
- Nitrogen leaching into surface waters and groundwaters



Impacts

- Toxicity to humans (Cancer)
- Blue baby syndrome
- Eutrophication
- Economical Impact
- NO_3-N recommendation is 10 mg-N/L[1]

Newstalk ZB

Water New Zealand says Canterbury's nitrate levels need close
NZCity - 23/07/2019
Water New Zealand technical manager, Noel Roberts, says rural **Canterbur**
dangerously high **nitrate** levels - and people there should get ...

Freshwater scientist says Canterbury's water isn't safe to drink
Newstalk ZB - 23/07/2019

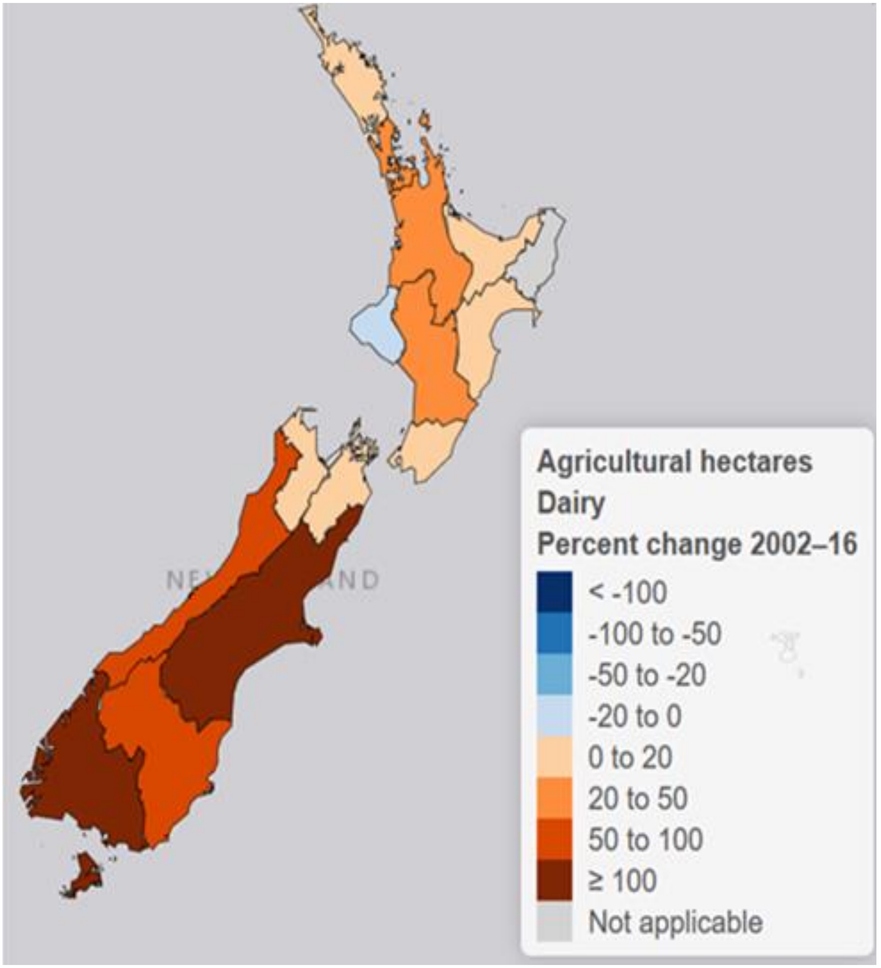
Nitrates in US drinking water may cost US$8 billion a year
Stuff.co.nz - 25/07/2019
Cancers potentially linked to high **nitrate** levels in **drinking water** coul
the United States as much as US$8 billion (NZ$12b) every ...

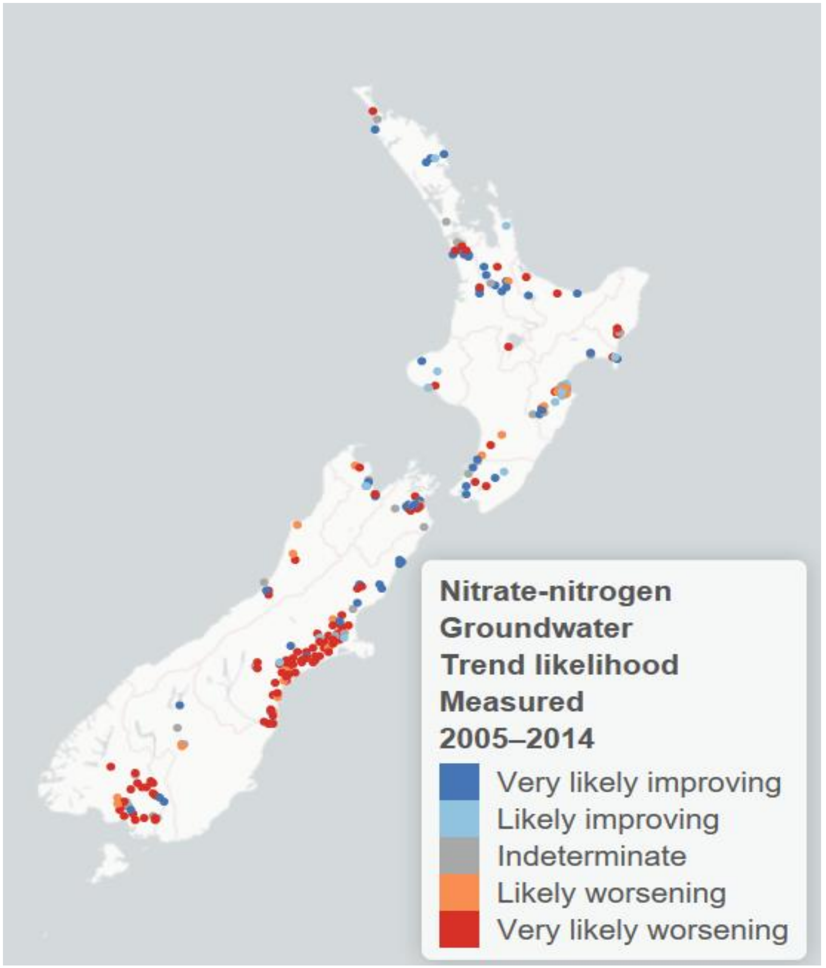
New Zealand

- NZ's Dairy Industry = $17 Billion (NZD)[2]
- 25% Increase in the use of nitrogen-based fertilizers since 2002[3]

New Zealand Groundwater

- 200 monitored groundwater sources
- 29% increase in nitrogen leaching[3]





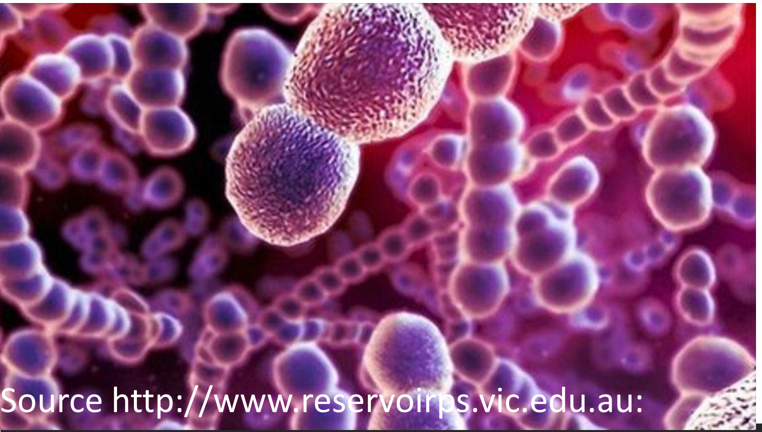
Source: (Ministry for the Environment and Stats NZ, 2019)[4]

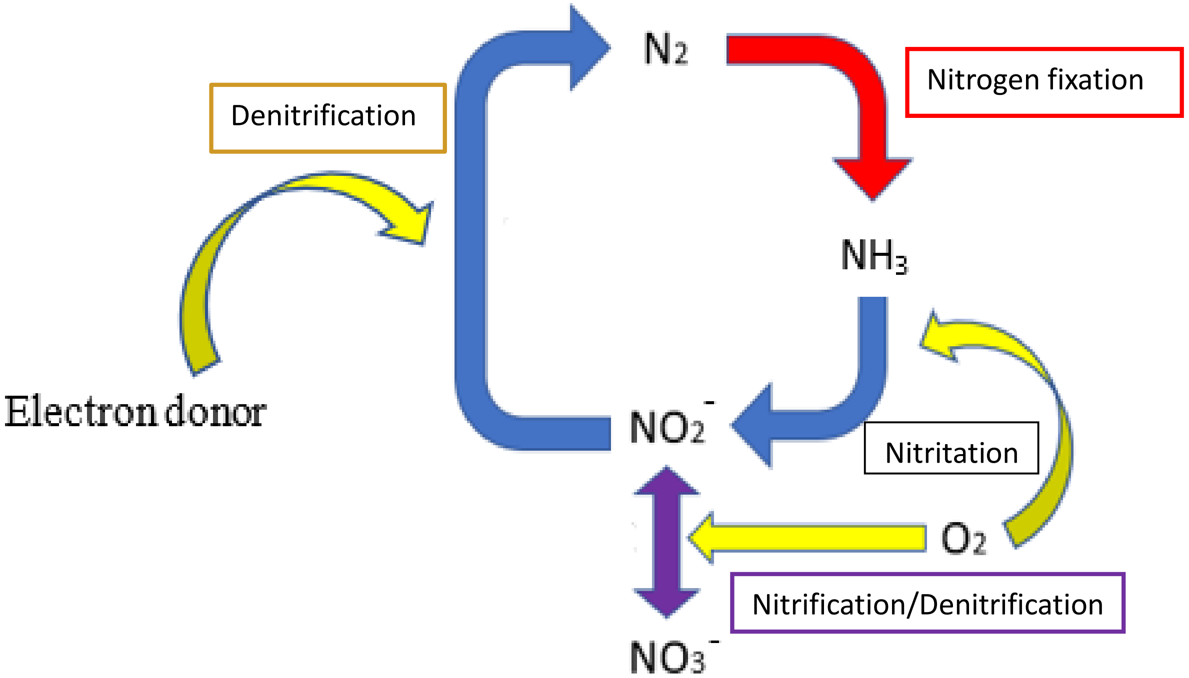
Biological Nitrogen Removal

Source http://www.reservoirps.vic.edu.au:

Hydrogenotrophic Denitrification

- Autotrophic Denitrification
- $2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O$
- WWTP use heterotrophic denitrification
- Works well in organic carbon limited zones





Hydrogenotrophic Denitrification

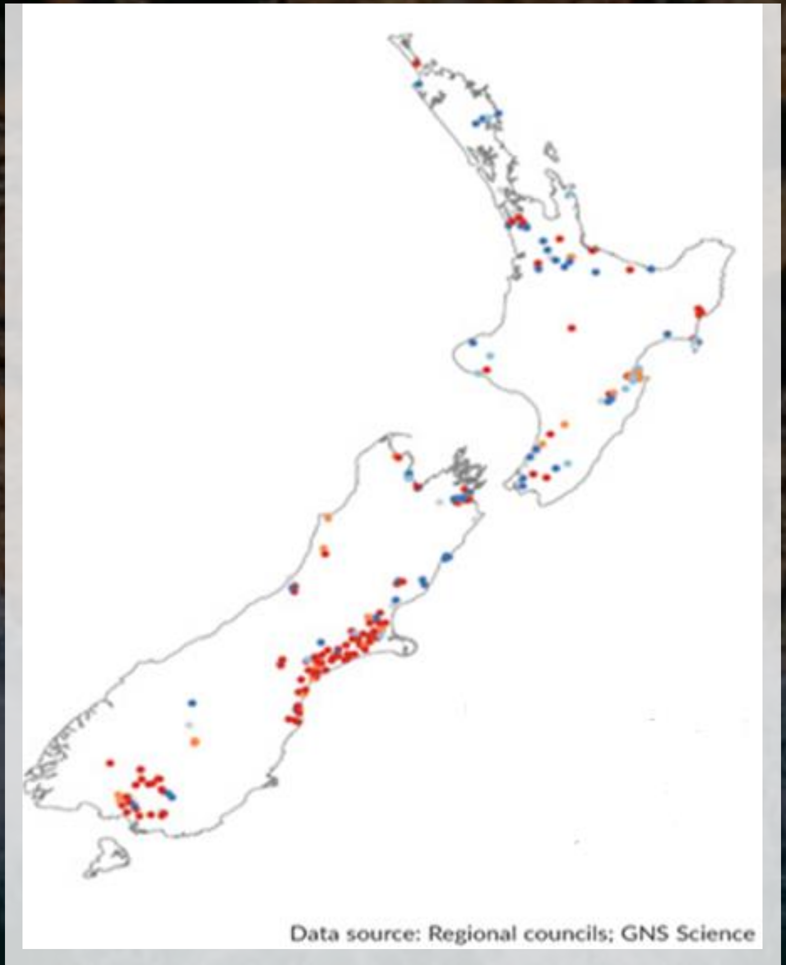
Substrate	Cost $/kg substrate	Consumption kg substrate/kg $N\text{-}NO_3^-$	Cost of denitrification $/kg $N\text{-}NO_3^-$	Nitrate Removal rate kg-N $m^{-3}d^{-1}$
Methanol	0.92	2.08-3.98	1.8-3.6	1-27
Acetic Acid	2.21	2	4.42	-
Acetate	1.67	2.7	4.37	0.6-1
Ethanol	1.10	3.5	3.85	0.4-1.2
Cotton	0.53	2.8	1.48	0.36
Sulphur	0.1	2.5	0.25	0.05
Hydrogen	**2.2-3.1**	**0.43**	**0.95-1.3**	**0.5-2.4**

Source: Park and Yoo, (2009) [5]

Cons
Low Solubility
Explosive Risk

Seawater Intrusion

- 150/200 situated near the coast
- Ion exchange - brine treatment (4%-26%)



Source: (Ministry for the Environment and Stats NZ, 2019)[4]

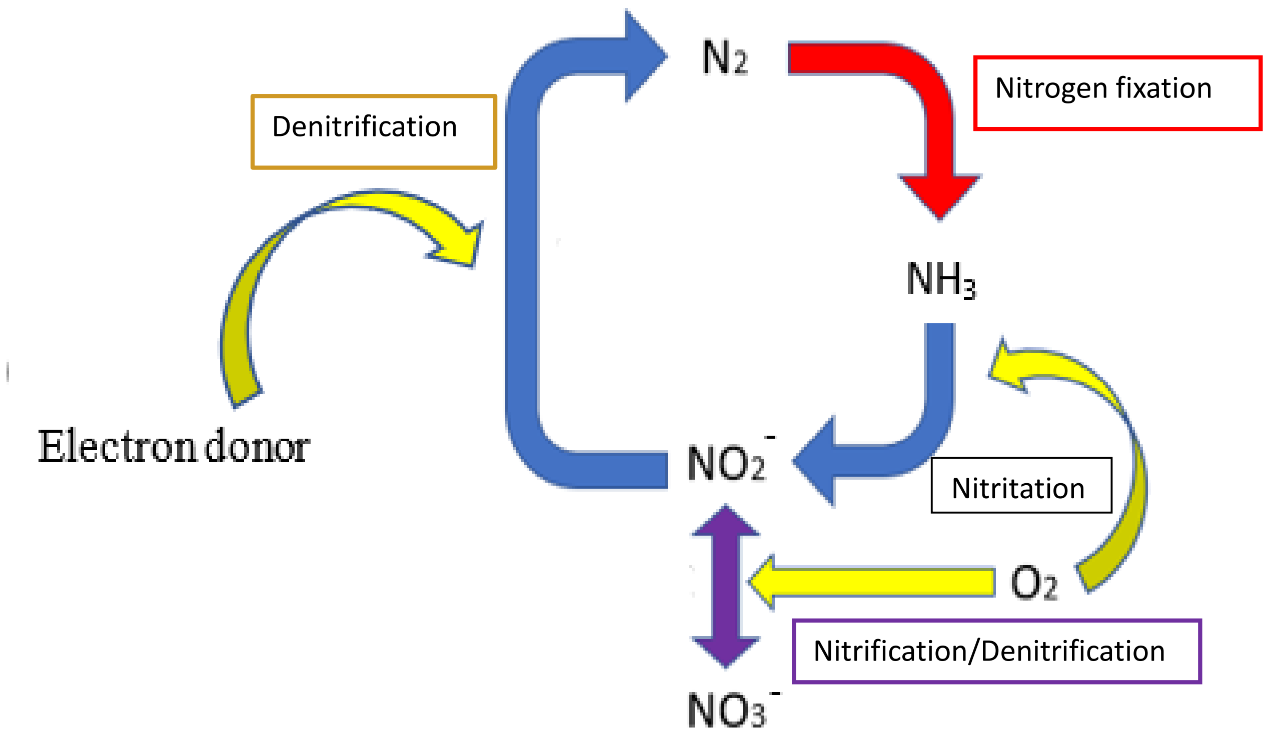
Objectives

1. Enriching hydrogenotrophic denitrifiers using **indigenous seed samples**
2. Investigating nitrate removal efficiencies of the enrichment cultures.
3. Understanding the functional redundancy of hydrogen-utilising microbes within the enriched hydrogenotrophic denitrifying cultures.

❖ **Both Saline and non-Saline environments**

Methodology

Saline Reactors	Non-Saline Reactors
• Batch Reactors • 100 mL working volume • Vacuumed and replenished/pressurised with H_2 gas. • Incubated at 30°C • Seed from a local beach • Salinity 4%	• Batch Reactors • 100 mL working volume • Vacuumed and replenished/pressurised with H_2 gas. • Incubated at 30°C • Seed from a local wastewater treatment plant



Methodology

Kinetic study for set-up conditions

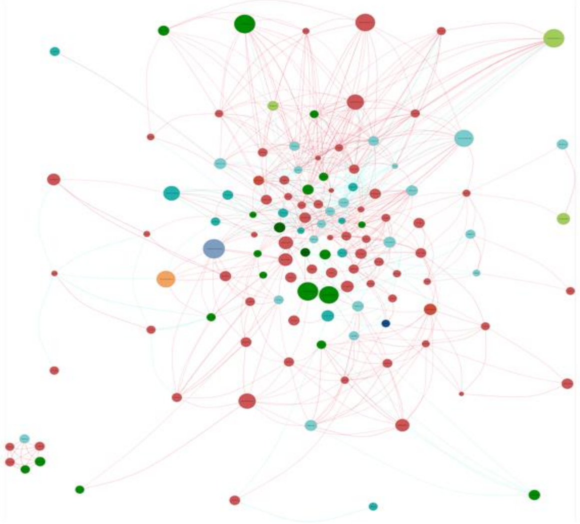
1. Non-saline Nitrate Reducers
2. Non-Saline Nitrite Reducers
3. Saline Nitrate Reducers
4. Saline Nitrite Reducers

Nitrate reducing bacteria	**True Denitrifying Bacteria**
Nitrate → Nitrite → Nitrogen	Nitrite → Nitrogen
• Replenished with Nitrate	Replenished with Nitrite

Metagenomics Analysis

DNA extraction

MiSeq- Illumina Sequencing



Bioinformatics – Obtaining Binned Genomes

1. Phylogenetic tree (16S rRNA)
2. Genomic Pathways (KEGG)

Results

Nitrate → Nitrite → Nitrogen

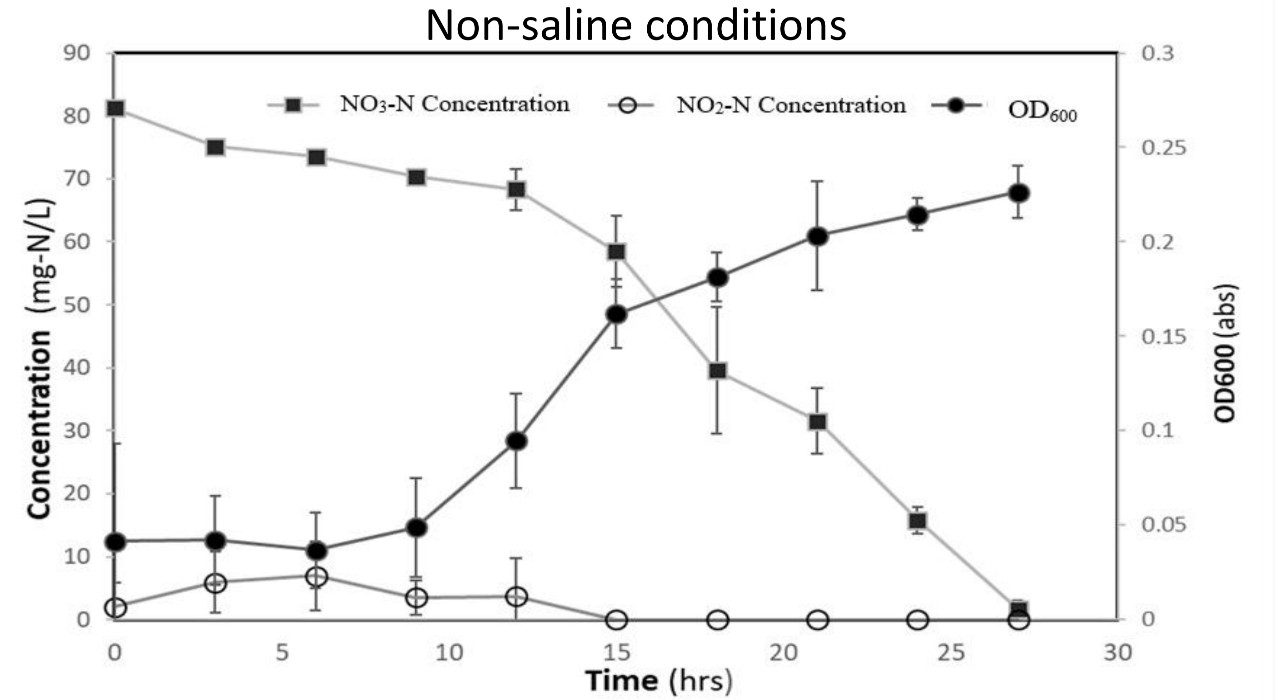
Nitrate removal

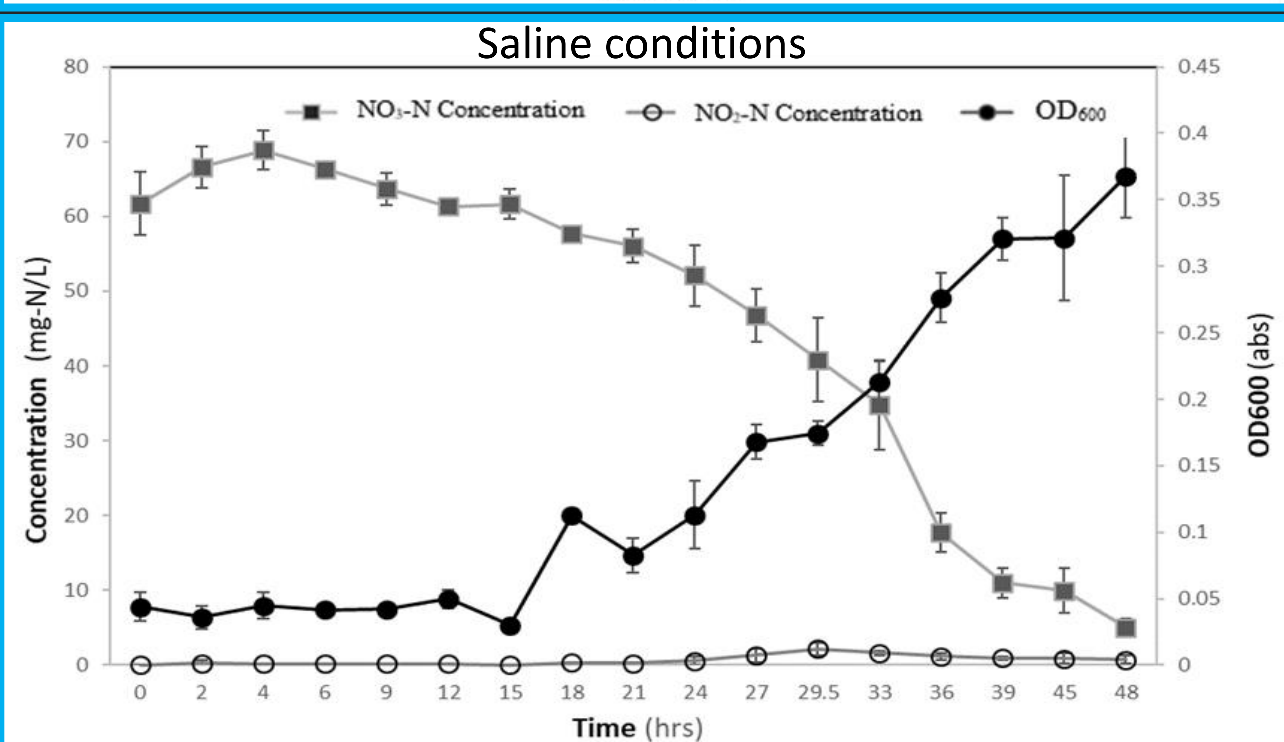
Non-Saline Results

- 98% removal of NO_3-N over a 26-hour period. With an average removal rate of 70 mg NO_3^--N $L^{-1} \cdot d^{-1}$, (peak 195±2 mg NO_3^--N $L^{-1} \cdot d^{-1}$)
- A lag phase of 10 hours, and a doubling time of 4 hrs

Saline Results

- 92% nitrate removal of NO_3-N over a 48-hour period. With an average removal rate of 28 mg NO_3^--N $L^{-1} \cdot d^{-1}$ (peak 135±2 mg NO_3^--N $L^{-1} \cdot d^{-1}$)
- Lag phase of 15 hours and a peak doubling time of 5 hours





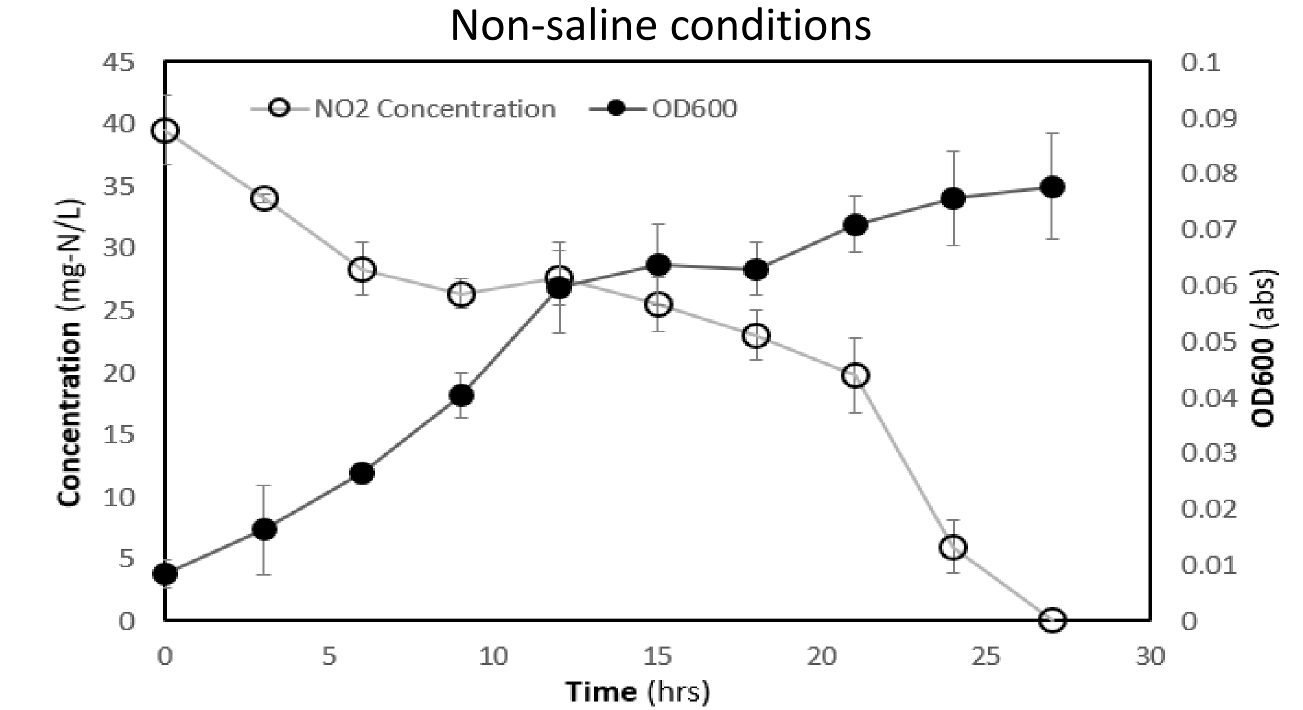
Nitrite removal

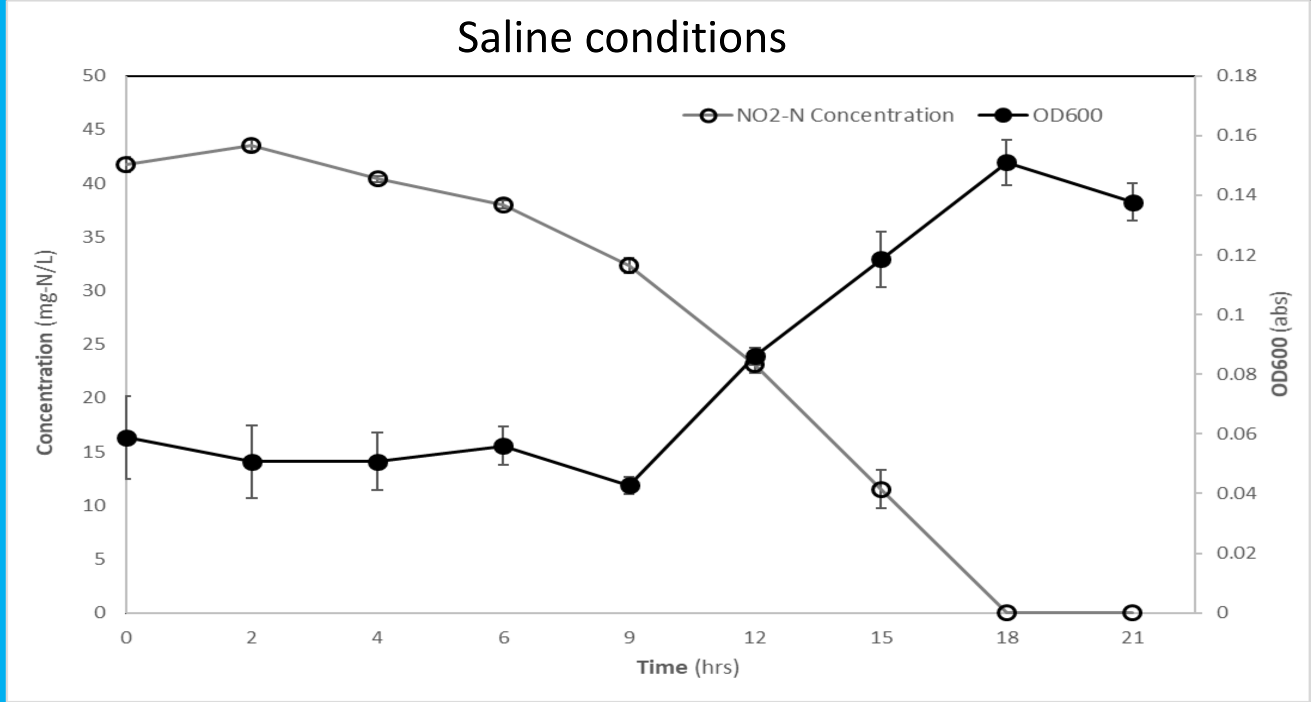
Non-Saline

- 100% removal of NO_2 -N over a 24-hour period. With an average removal rate of 34 mg NO_3^--N $L^{-1} \cdot d^{-1}$
- No lag phase with a doubling time of 4 hrs

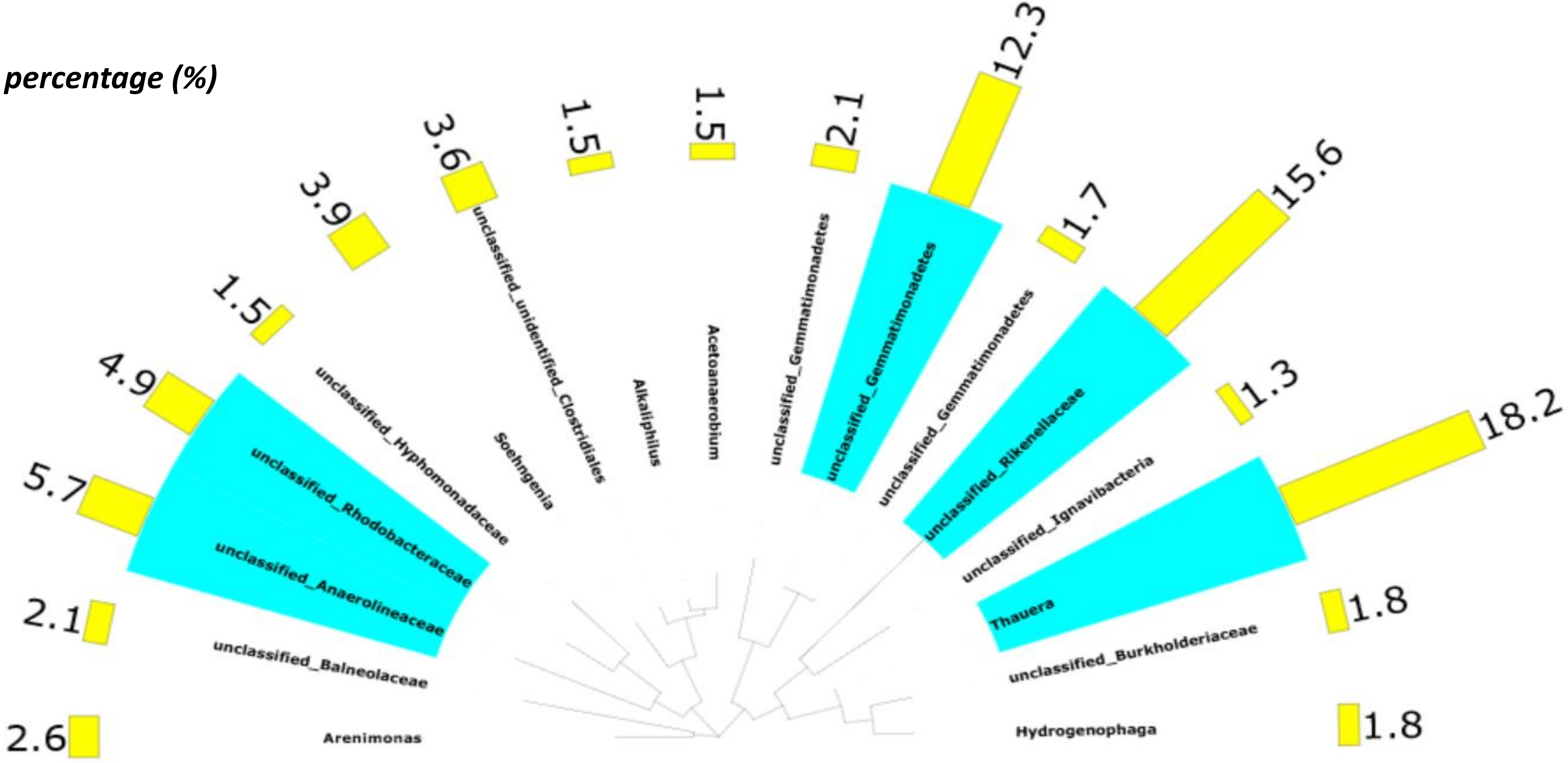
Saline

- 100% removal of NO_2 -N over an 18-hour period. With an average removal rate of 40 mg NO_3^--N $L^{-1} \cdot d^{-1}$
- 2 hr lag phase, doubling time of 3 hours





In percentage (%)



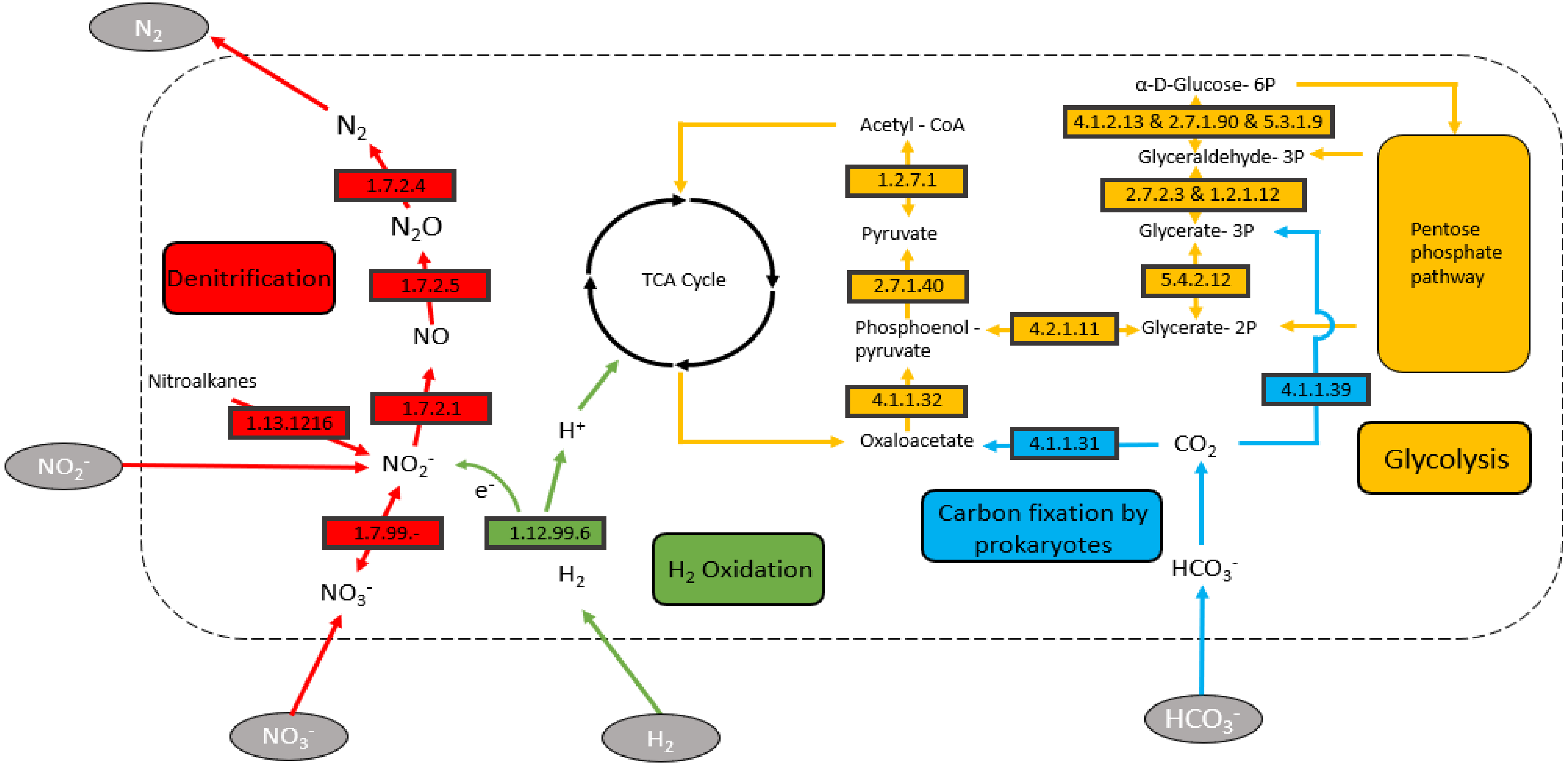
Phylogenetic Tree

- Nitrate fed, Non-Saline microbial community

Classified OTUs	Abundance (%)	Most-closely related culturable species	Known Denitrifiers
Rhodocyclaceae_Thauera	**18.2**	***Thauera mechernichensis* (~100%)**	+
Unclassified_Rikenellaceae	15.6	*Poryphyromonas pogonae* (86.2%)	-
Unclassified_Gemmatimonadetes	12.3	*Longimicrobium terrae* (84.8%)	-
Unclassified_Anaerolineaceae	5.6	*Ornatikinea apprima* (90.5%)	-
Unclassified_Rhodobacteraceae	**4.9**	***Defluviimonas pyrenivorans* (99.6%)**	+

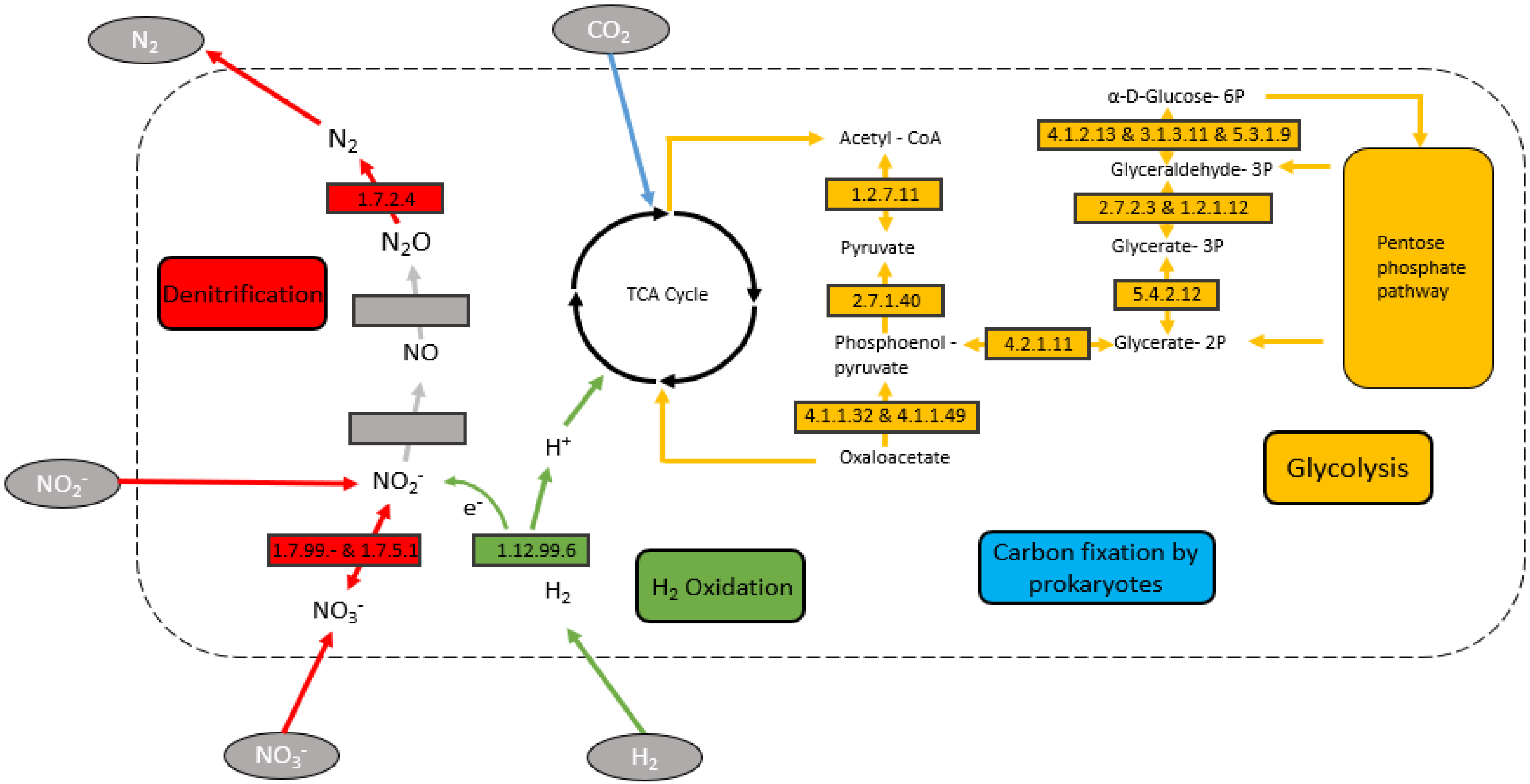
Results

- ❖ *Thauera* dominated community (56%) = 100 mg NO_3^--N $L^{-1} \cdot d^{-1}$ [6]



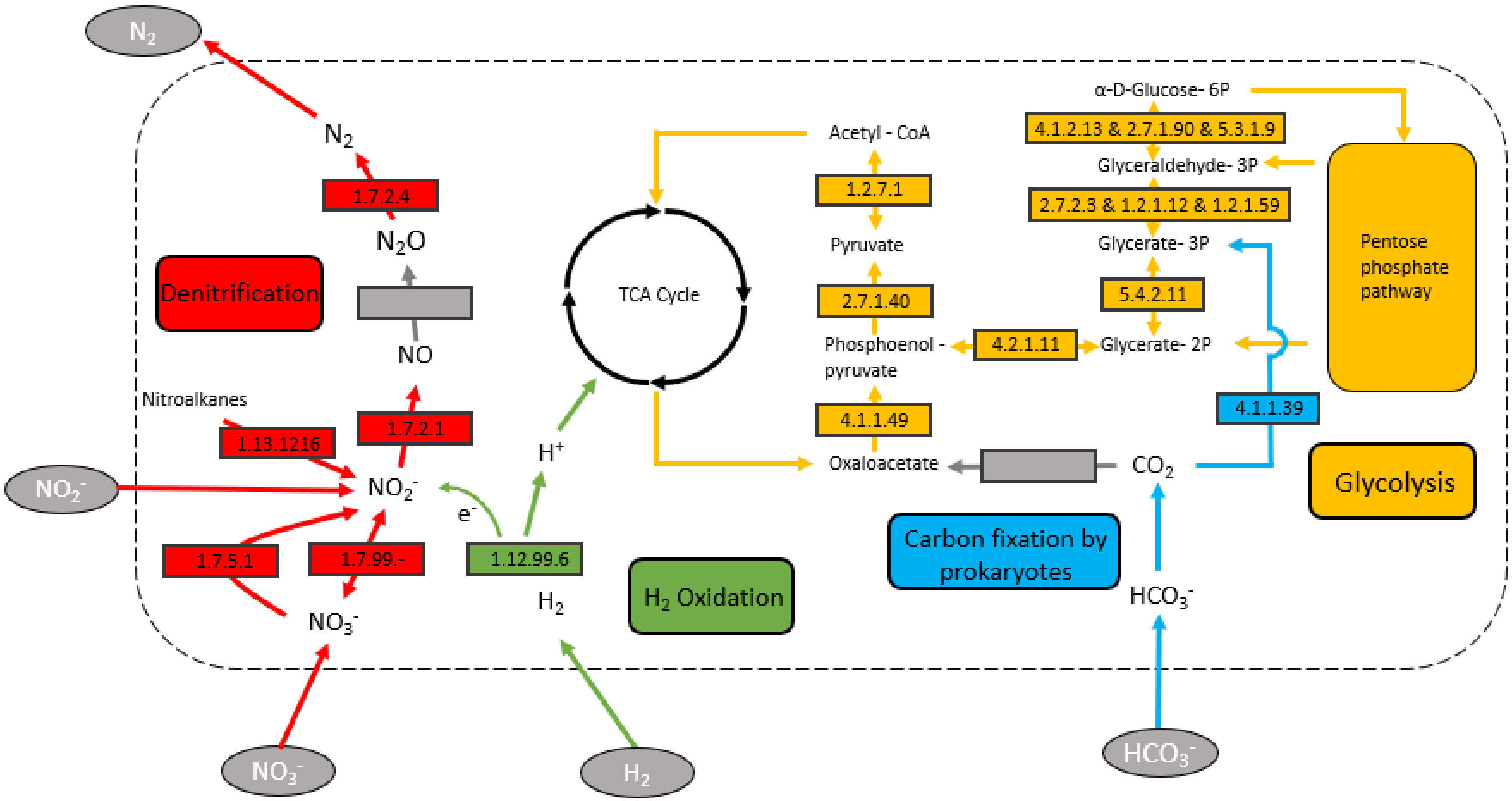
Thauera

- Capable of complete denitrification
- Autotrophic Denitrification (Pentose phosphate pathway- Calvin Benson cycle)
- Known for growth in aerobic conditions as well.



Unclassified_Rhodobacteraceae

- Incomplete denitrification pathway.



Unclassified_Gemmatimonadetes

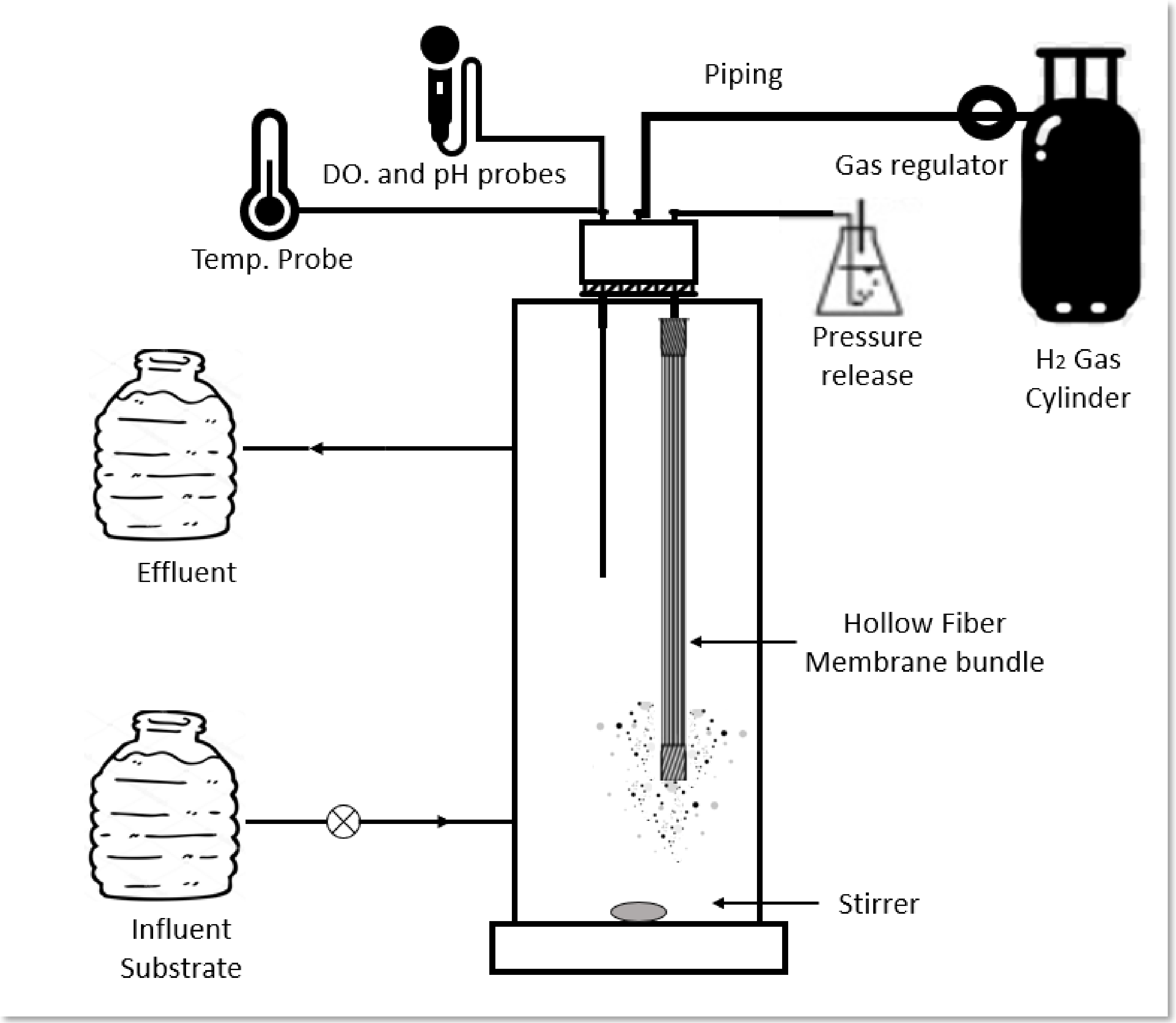
- Almost complete denitrification pathway.

Conclusion

- Water regulation of 10 mg/L NO_3-N can be achieved using indigenous strains of hydrogenotrophic denitrifiers
- Significant Nitrate removal was achieved in both fresh- and saline-water, (98% and 92% respectively)
- Salinity affects nitrate consuming bacteria, with no conceivable effect for nitrite reducing
- Abundance of *Thauera* helpful for groundwater treatment (Capable of growth in aerobic conditions)

Future Developments

- Incorporating Hollow Fiber membranes
- Point Source Treatment System



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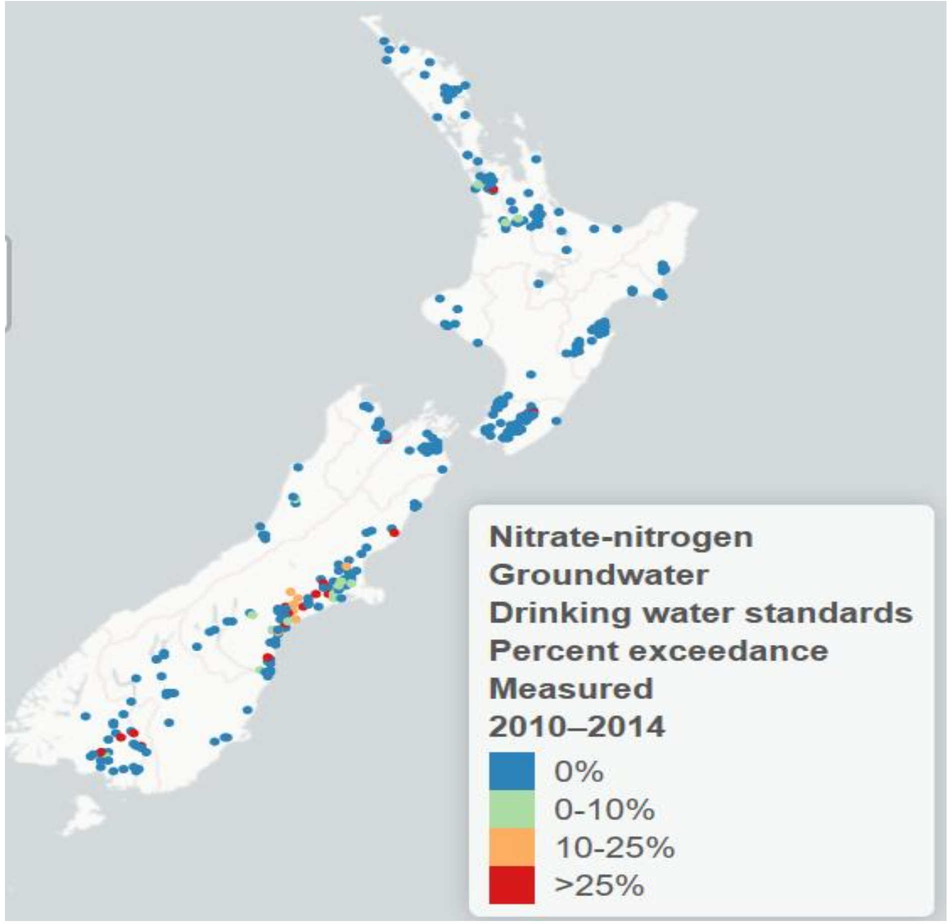
Thank you.
Any Questions?

Appendix

NZ groundwater conditions

- The Canterbury region has on average higher than 5 mg-N/L in groundwater
- 13% of the country's monitored groundwater have exceeded acceptable WHO values on at least one occasion (2010-2014)



https://www.stats.govt.nz/indicators/groundwater-quality

Physical nitrogen treatment methods

Method	Description	Advantages	Disadvantages	References
IE	NO_3^- ions are removed from the treatment stream by displacing chloride on an anion exchange resin. The resin is made to minimize adsorption of other anions/cations so NO_3^- can be removed. Subsequently, regeneration of the resin is necessary to remove the nitrate from the resin.	• High availability of nitrate selective resins Effective for removal in low to moderate nitrate concentration Can remove multiple contaminants (including arsenic, perchlorate, and chromium) Improved efficiency of low brine in recent years	• Produces concentrated waste brine. Wastewater must be treated before discharge. Difficult and expensive. May not be feasible for extremely high nitrate levels	Harter, T., & Lund, J.R., 2012
RO	Second most common nitrate treatment alternative. A semi-permeable membrane separates contaminants (predominantly those with higher valences) when water is forced through. The process will not change the compounds' molecular structures. The process has an energy demand of 3.7 kW h/m^3.	• Feasible for municipal and direct/on location use application. Can be used simultaneously for multiple contaminant removal and desalination	• NO3-is a monovalent ion, RO is not as effective. Higher costs relative to IE (pre-treatment requirements and power consumption) Produces concentrated waste brine requiring further treatment.	Song, Zhou, Li, & Mueller, 2012; Ergas & Rheinheimer, 2004.
CD	Chemical denitrification uses metals to transform nitrate to other nitrogen species.	• No waste brine produced, so no need to dispose Potential for multiple contaminant removal Recent progress has been made in improving efficiency	• The nitrate reduction reactions are inconsistent, potential for incomplete denitrification, and risk of nitrite formation Dependence on temperature and pH Lack of full scale systems.	Harter, T., & Lund, J.R., 2012
ED	Process involves ion flow across anion-exchange and cation-exchange membranes in a constant electric field. The membranes trap nitrate and other ions in a concentrated waste stream. Ion Exchange resin is used in a sheet form. Build up on the membrane is minimized by reversing the polarity several times per hour to change the ions' direction of movement.	• Multiple contaminant removal and desalination Less waste produced than RO Fewer pre-treatment requirements than RO Possible to selectively remove nitrate ions	• Pre-treatment requirements High energy demands and operating costs Operational complexity Waste disposal	Rozanska & Wisniewski, 2007; Prato & Parent, 2017