

# MOLECULAR APPROACHES TO IDENTIFY BENIGN DENITRIFICATION IN SHALLOW GROUNDWATERS

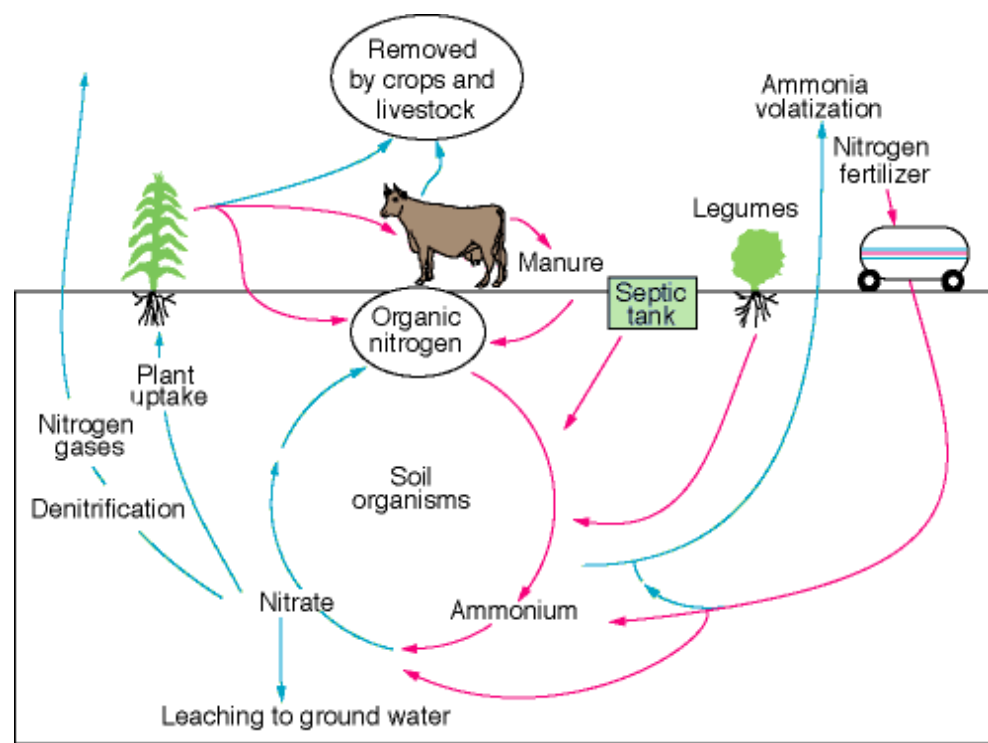
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# Intensification of agricultural activities

## Nitrate discharge in waterways

## Water pollution, GHG emissions



# SUBSURFACE DENITRIFICATION



BENIGN DENITRIFICATION

# OBJECTIVE

To identify environmental and hydrogeological conditions leading to benign subsurface denitrification in agricultural catchments

# APPROACH

# SITE SELECTION

Six Contrasting Site- Reducing and non-reducing

Palmerston North

Santoft

Dannivirke

Woodville

Pahiatua – two sites

# Monthly Groundwater Sampling

- Six Sites- Manawatu and Rangitikei Catchment
- Three Piezometers Each
- Three Replicates



# Analytical Measurements

- Redox Parameters- In field and standard laboratory practice
- Dissolved gases ( $N_2O$  and  $N_2$ ) – Denitrification Dynamics Gas Chromatograph
- Denitrifier genes abundances- qPCR techniques



# RESULTS

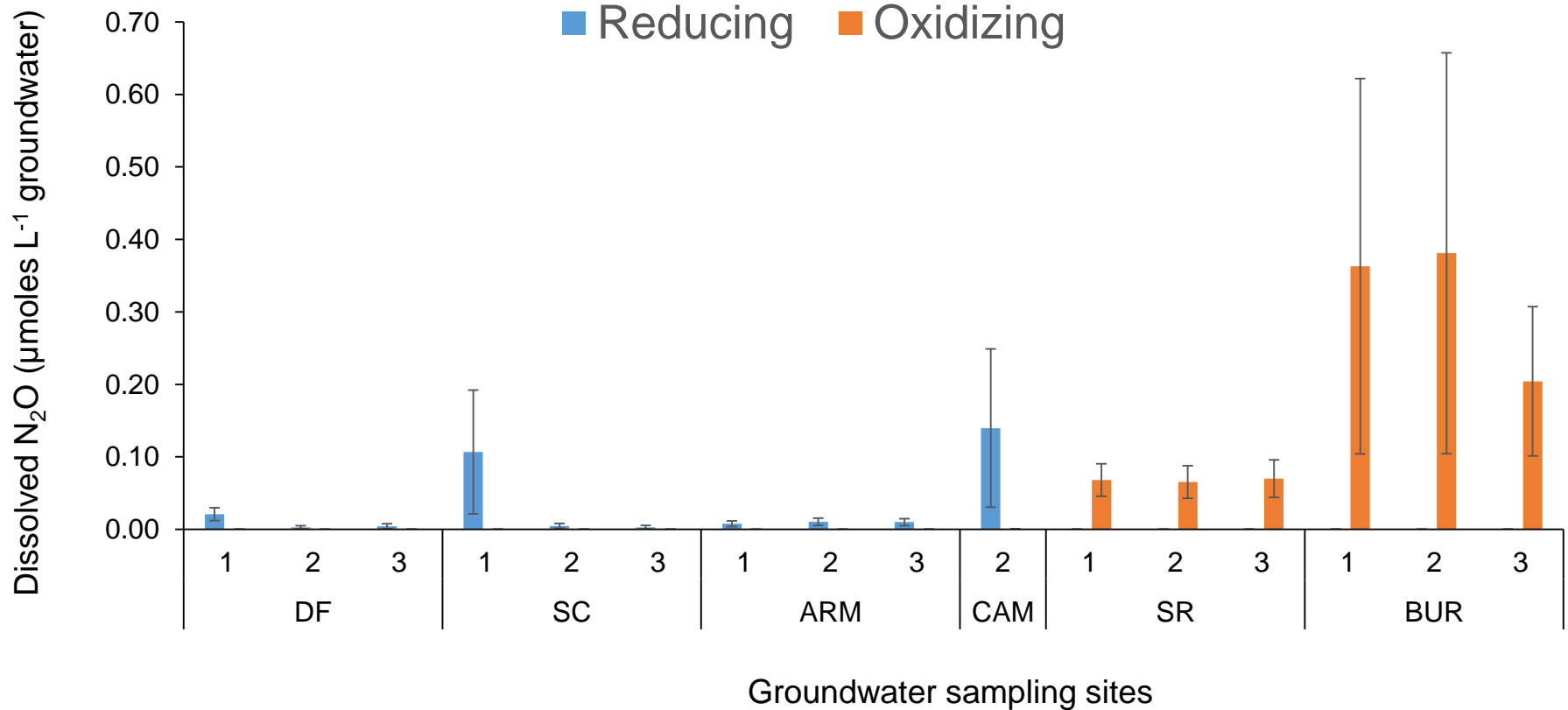
# REDOX PARAMETERS

# Redox parameters in groundwater samples collected from Manawatu and Rangitikei catchments from Aug 2017 to Sep 2018

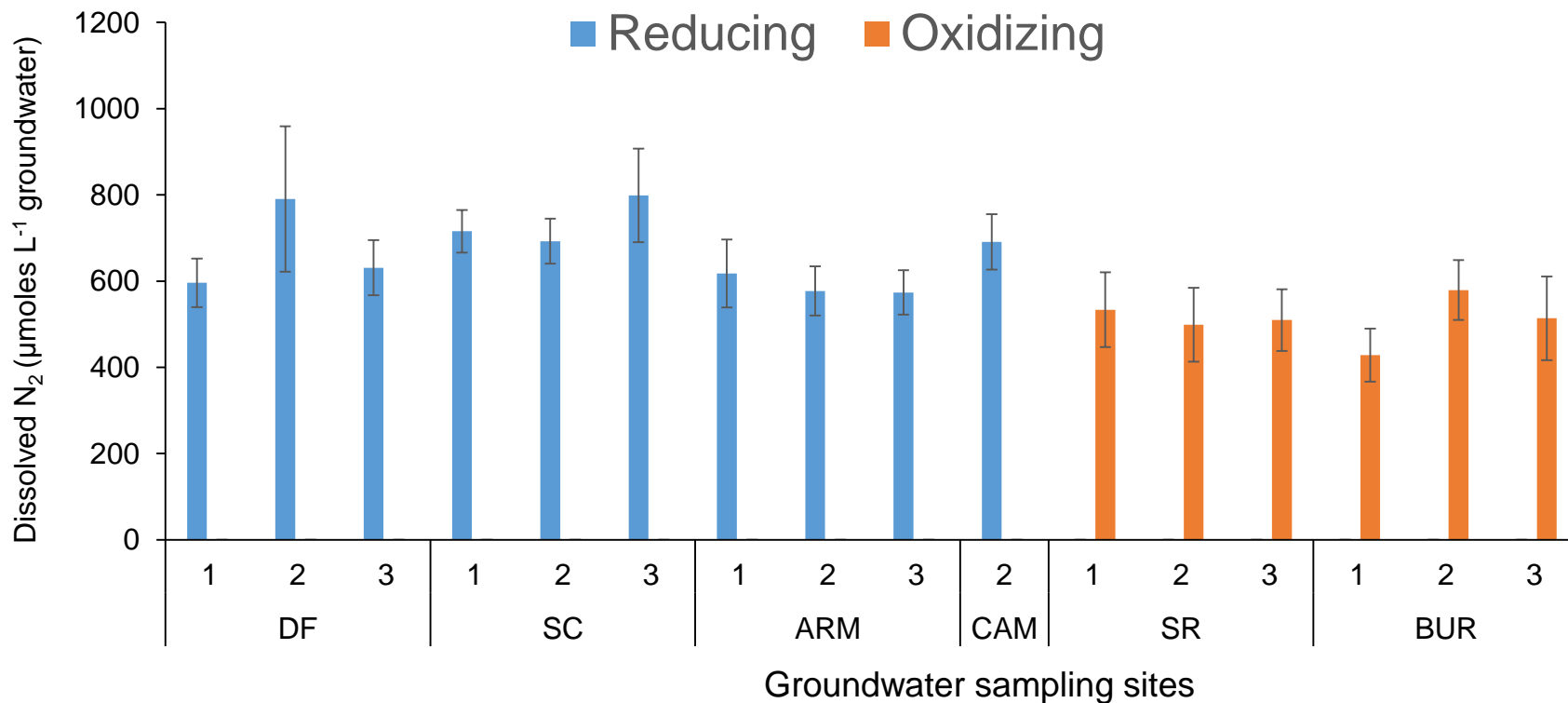
Redox Parameter	Range (min-max)	
	Anoxic	Oxic
Dissolved Oxygen ( $O_2$ ) ( $mg\ L^{-1}$ )	0.13 to 1.31	3.36 to 7.81
Nitrate ( $NO_3^-$ -N) ( $mg\ L^{-1}$ )	0.02 to 1.50	3.17 to 6.93
Iron ( $Fe^{2+}$ ) ( $mg\ L^{-1}$ )	0.23 to 5.62	0.02 to 0.13
Manganese ( $Mn^{2+}$ ) ( $mg\ L^{-1}$ )	0.03 to 0.52	0.02 to 0.10
Sulfate ( $SO_4^{2-}$ ) ( $mg\ L^{-1}$ )	0.32 to 18.38	4.32 to 11.60
Dissolved organic carbon ( $mg\ L^{-1}$ )	0.00 to 27.06	0.05 to 3.37

# DISSOLVED GASES

# Dissolved nitrous oxide in groundwater samples collected from Manawatu and Rangitikei catchments from Aug 2017 to Sep 2018

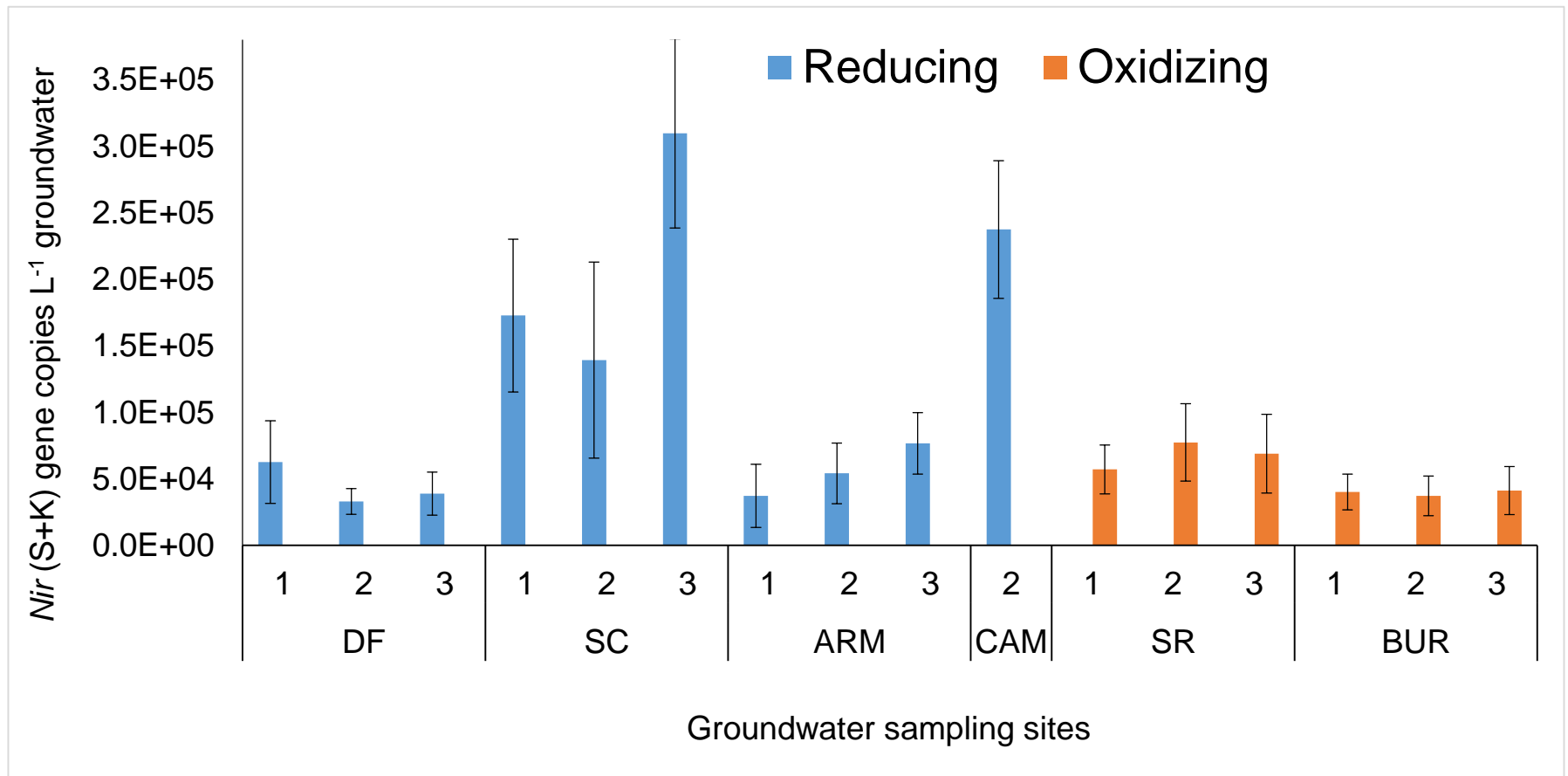


# Dissolved dinitrogen in groundwater samples collected from Manawatu and Rangitikei catchments from Aug 2017 to Sep 2018



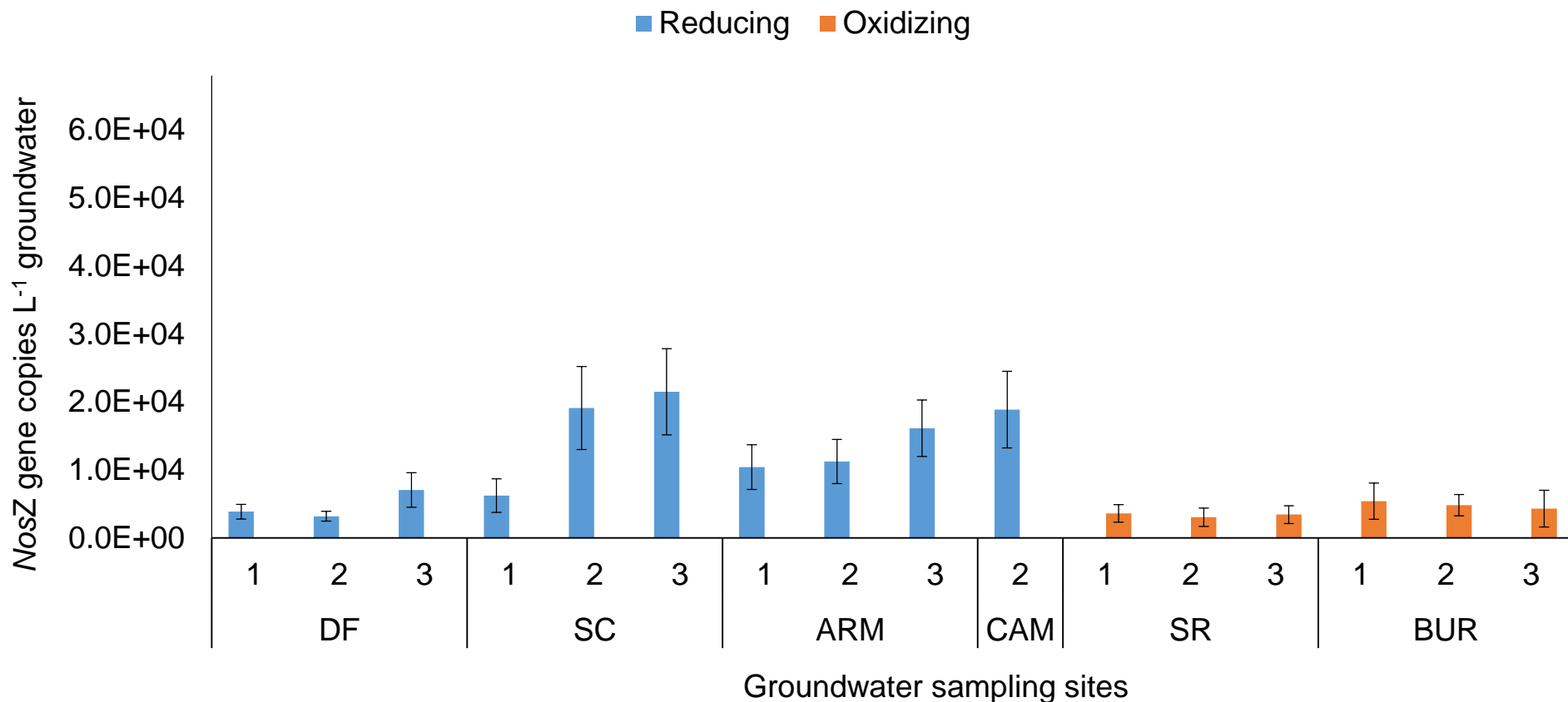
# DENITRIFIER GENE ABUNDANCE

## *Nir* (S+K) gene abundance in groundwater samples collected from Manawatu and Rangitikei catchments from Aug 2017 to Sep 2018





## Nos Z gene abundance in groundwater samples collected from Manawatu and Rangitikei catchments from Aug 2017 to Sep 2018



# Pearson's correlation coefficient ( $P < 0.05$ ) among the measured parameters

	DO	Fe <sup>2+</sup>	Mn	SO <sub>4</sub> <sup>-</sup>	NO <sub>3</sub> -N	DOC
DO	*	-0.660	-0.474	ns	0.705	ns
Fe <sup>2+</sup>	-0.660	*	ns	ns	-0.662	0.472
Mn	-0.475	ns	*	ns	ns	ns
SO <sub>4</sub> <sup>-</sup>	ns	ns	ns	*	ns	0.556
NO <sub>3</sub> -N	0.705	-0.662	ns	ns	*	ns
DOC	ns	0.472	ns	0.556	ns	*

# Pearson's correlation coefficient ( $P < 0.05$ ) among the measured parameters

	<b>N<sub>2</sub>O</b>	<b>N<sub>2</sub></b>	<b>DNA</b>	<b><i>NirS+K</i></b>	<b><i>NosZ</i></b>
<b>N<sub>2</sub>O</b>	*	ns	ns	ns	ns
<b>N<sub>2</sub></b>	ns	*	0.558	0.638	0.579
<b>DNA</b>	ns	0.558	*	0.731	0.708
<b><i>NirS+K</i></b>		0.638	0.731	*	0.441
<b><i>NosZ</i></b>	-0.778	0.585	0.752	0.753	*

# Comparison of sites based on reducing conditions

	Anoxic Sites	Oxic Sites
Nitrate Content	Low	High
Fe <sup>2+</sup>	High	Low
Nitrous Oxide	Low	High
Excess N <sub>2</sub>	Low	Marginally High
<i>NosZ</i> gene	High	Low
<i>Nir</i> gene	High	Low

# Conclusions

- Oxic sites ( $\text{DO} > 2 \text{ mg L}^{-1}$ ) shows partial subsurface denitrification of available nitrate
- Anoxic sites ( $\text{DO} < 2 \text{ mg L}^{-1}$ ) indicates relatively more benign subsurface denitrification (lack of  $\text{N}_2\text{O}$ )
- Relatively higher abundance of *nosZ* gene in the anoxic sites indicates benign subsurface denitrification
- Hydrogeological conditions and more abundant denitrifier or the presence of electron donor such as Fe leads to complete denitrification of nitrate in selected anoxic sites.

# Acknowledgements

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OUR LAND  
AND WATER

Toitū te Whenua,  
Toiora te Wai

National  
**SCIENCE**  
Challenges



**Thank You**