

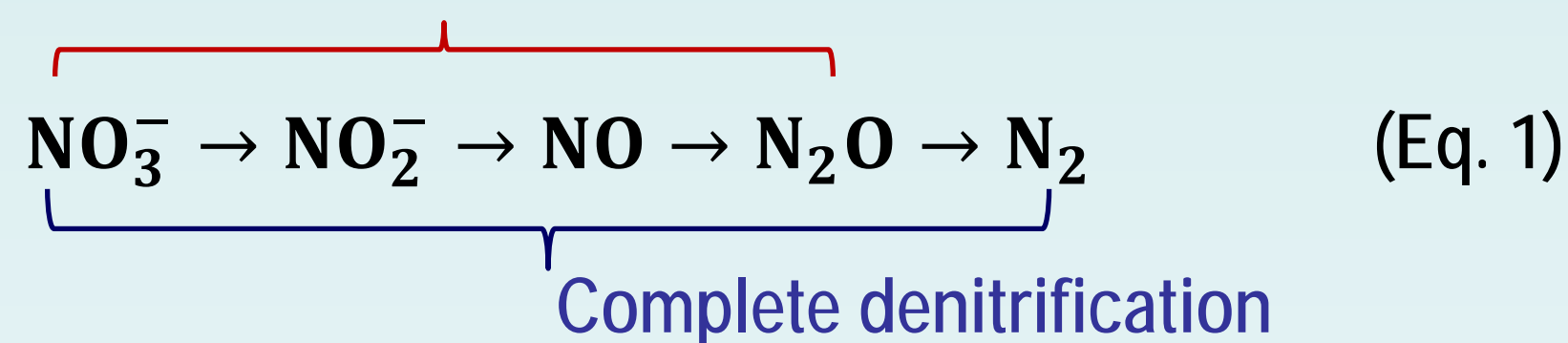
Benign denitrification in shallow groundwaters

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Denitrification in shallow groundwater – an ecosystem service or pollution swap?

Denitrification in groundwaters has been identified as a key nitrate (NO₃⁻) attenuation process, where leached NO₃⁻ can be microbially reduced to dinitrogen (N₂ - a harmless gas), offering an ecosystem service in terms of water quality protection. However, a partial denitrification can release nitrous oxide (N₂O - a greenhouse gas), resulting in a pollution swap protecting water quality but adding to global warming potential.

Partial denitrification



The main limiting factors of subsurface denitrification are the presence of suitable environment, lack of dissolved oxygen, denitrifying microbes, NO₃⁻ and electron donors, a C source (Korom, 1992; Rivett *et al.*, 2008). In many cases NO₃⁻ inputs exceed subsurface attenuation capacity and NO₃⁻ accumulates. There is yet limited information available about dynamics - **partial or complete** - of subsurface denitrification across sensitive agricultural catchments in New Zealand and elsewhere.

Objectives

- Assess biogeochemical transformation of nitrate in shallow groundwater at 6 pastoral farms across the Manawatu and Rangitikei River catchments;
- Quantify the process and products of shallow groundwater denitrification through in-field and laboratory measurements; and
- Assess effects of different hydro-geo-chemical factors on subsurface denitrification in shallow groundwaters.

Observations and Experiments

We collected monthly observations (August 2017 – September, 2018) of groundwater chemistry, redox parameters, dissolved gases and denitrifying genes in shallow groundwaters at the study sites (Table 1).

- Analysis of relevant anions, NO₃-N, SO₄²⁻, and Br⁻ was conducted using a Dionex™ Aquion™ IC (ThermoFisher Scientific), at the Soil and Water Laboratory, Massey University.
- Analysis of relevant cations, Fe²⁺, Mn²⁺ and Al³⁺ was conducted using a 4200 MP-AES (Agilent Technologies), at the Soil and Water Laboratory, Massey University.
- Dissolved N₂O was analyzed by a Shimadzu 2010-Plus gas chromatograph system (Shimadzu Corp, Kyoto, Japan) with an electron capture detector (ECD), flame-ionization detector (FID) and thermal conductivity detector (TCD) (McMillan *et al.*, 2014) at the Environmental Chemistry Laboratory, Manaaki Whenua Landcare Research.
- Dissolved N₂ and Ar were measured using the Membrane Inlet Mass Spectrometry (MIMS) at the National Institute of Water and Atmospheric Research (NIWA) in Wellington. Excess N₂ was quantified as follows (Weymann *et al.*, 2008).
- Denitrifying genes, qPCR was performed using the LightCycler, Massey University (Jha *et al.*, 2018).

Table 1: Description of the study sites across Manawatu and Rangitikei River catchments, located in the lower part of North Island, New Zealand.

Site Code	DF	SC	ARM	SR	BUR	CAM
Area of sampling site	Palmerston North	Santoft	Woodville	Pahiatua	Pahiatua	Dannevirke
Piezometer N ^o , Depth (m, bgl)	1 5.5 2 7.5 3 8.7	1 6.0 2 3.0 3 5.2	1 5.0 2 6.0 3 7.5	1 4.5 2 5.5 3 6.5	1 3.6 2 4.3 3 6.1	1 4.5 2 - 3 7.5
Land use	Dairy	Dairy	Beef/ Sheep	Dairy	Dairy	Dairy
Soil type*	Manawatu fine sandy loam	Foxton brown sand-Pukepuke black sand Himatangi sand	Kairange silt loam and clay loam	Kopua stony silt loam	Kopua stony silt loam	Takapau silt loam
Rock type*	Alluvium	Sand flat	Alluvium	Loess over gravel	Loess over gravel	Loess over gravel

$$\text{Excess N}_2, X_{\text{ExcessN}_2} = X_{\text{N}_2\text{T}} - X_{\text{N}_2\text{EA}} - X_{\text{N}_2\text{EQ}}$$

Where, X is the molar concentration of each parameter, N₂T is the total dissolved N₂ in the groundwater sample; N₂ EA is the N₂ from excess air; and N₂ EQ is the dissolved N₂ in equilibrium with the atmospheric concentration.

$$\text{N}_2 \text{ from excess air}, X_{\text{N}_2\text{EA}} = (X_{\text{ArT}} - X_{\text{ArEQ}}) * \left(\frac{X_{\text{N}_2\text{atm}}}{X_{\text{Aratm}}} \right)$$

Where, N₂ atm is the atmospheric mole fraction of N₂; Ar atm at the atmospheric mole fraction of Argon (Ar); Ar T is the total dissolved Ar in the groundwater sample; and Ar EQ is the dissolved Ar in equilibrium with the atmospheric concentration.

Results and Discussion

Table 2: Groundwater redox status and process based on average monthly groundwater values (August 2017 - September 2018) at the study sites (Table 1). Groundwater redox status and process were defined based on McMahon and Chapelle (2008) criteria/threshold.

Parameter	DO	NO ₃ -N	Mn ²⁺	Fe ²⁺	SO ₄ ²⁻	Redox status	Redox process	
Threshold value, mg·L ⁻¹	0.5	0.5	0.05	0.1	0.5			
Site	Piezometer	mg·L ⁻¹						
DF	1	1.76	0.59	0.10	1.62	4.20	Mixed(oxic-anoxic)	O2-Fe(III)/SO4
	2	0.28	0.04	0.16	2.99	2.55	Anoxic	Fe(III)/SO4
	3	0.15	0.11	0.15	2.68	2.20	Anoxic	Fe(III)/SO4
SC	1	0.54	0.58	0.71	5.30	4.74	Mixed(oxic-anoxic)	O2-Fe(III)/SO4
	2	0.96	4.46	0.41	0.52	10.29	Mixed(oxic-anoxic)	O2-Fe(III)/SO4
	3	0.25	0.20	0.19	3.53	15.45	Anoxic	Fe(III)/SO4
ARM	1	0.20	0.02	0.11	3.62	4.33	Anoxic	Fe(III)/SO4
	2	0.20	0.02	0.16	3.49	1.19	Anoxic	Fe(III)/SO4
	3	0.14	0.03	0.15	1.13	2.04	Anoxic	Fe(III)/SO4
CAM	1	4.79	5.41	0.13	0.12	8.84	Mixed(oxic-anoxic)	O2-Fe(III)/SO4
	3	0.33	0.21	0.82	0.22	2.88	Anoxic	Fe(III)/SO4
SR	1	7.95	3.48	0.02	0.03	4.58	Oxic	O2
	2	7.64	3.57	0.02	0.07	4.53	Oxic	O2
	3	7.55	3.84	0.02	0.07	5.04	Oxic	O2
BUR	1	3.53	6.61	0.03	0.14	5.67	Mixed(oxic-anoxic)	O2-Fe(III)/SO4
	2	5.01	4.45	0.03	0.37	5.50	Mixed(oxic-anoxic)	O2-Fe(III)/SO4
	3	3.55	3.69	0.03	0.37	4.31	Mixed(oxic-anoxic)	O2-Fe(III)/SO4

Groundwater redox status and processes (Table 2):

- Oxic site (SR 1, 2 and 3) showed NO₃-N accumulation > 3 mg·L⁻¹.
- Mixed (oxic-anoxic) sites (DF 1 and 3; SC 1 and 2; CAM 1; and BUR 1, 2 and 3) showed O₂-Fe³⁺/SO₄²⁻ as the main redox process. The monthly average NO₃-N ranged between 0.58 to 6.91 mg·L⁻¹.
- Anoxic sites (DF 2 and 3; SC 3; ARM 1, 2, and 3; CAM 3) (DO < 0.5 mg·L⁻¹) showed Fe³⁺/SO₄²⁻ as the main redox process. Very low NO₃-N (< 0.3 mg·L⁻¹) suggests NO₃-N reduction occurring in the subsurface environment.
- Presence of Fe²⁺ (> 0.1 mg·L⁻¹) and/or Mn²⁺ (> 0.05 mg·L⁻¹) suggest reduction processes occurring at sites DF, SC, CAM and BUR sites.

Conclusions

- Subsurface denitrification in shallow groundwaters is spatially variable.
- A **complete** subsurface denitrification appears as predominant process in the mixed and anoxic groundwaters (see the sites mixed DF1, and SC 1 & 2; and the anoxic sites DF 2 & 3, ARM 1, 2 & 3, CAM3).
- A **partial** subsurface denitrification process appears as predominant process in the oxic groundwater (see the oxic sites SR 1, 2 & 3, and BUR 1, 2 & 3).
- A combined measurement of groundwater redox species, dissolved (N₂ and N₂O) gases, and denitrifier genes provide a full insights into dynamics of denitrification in groundwaters

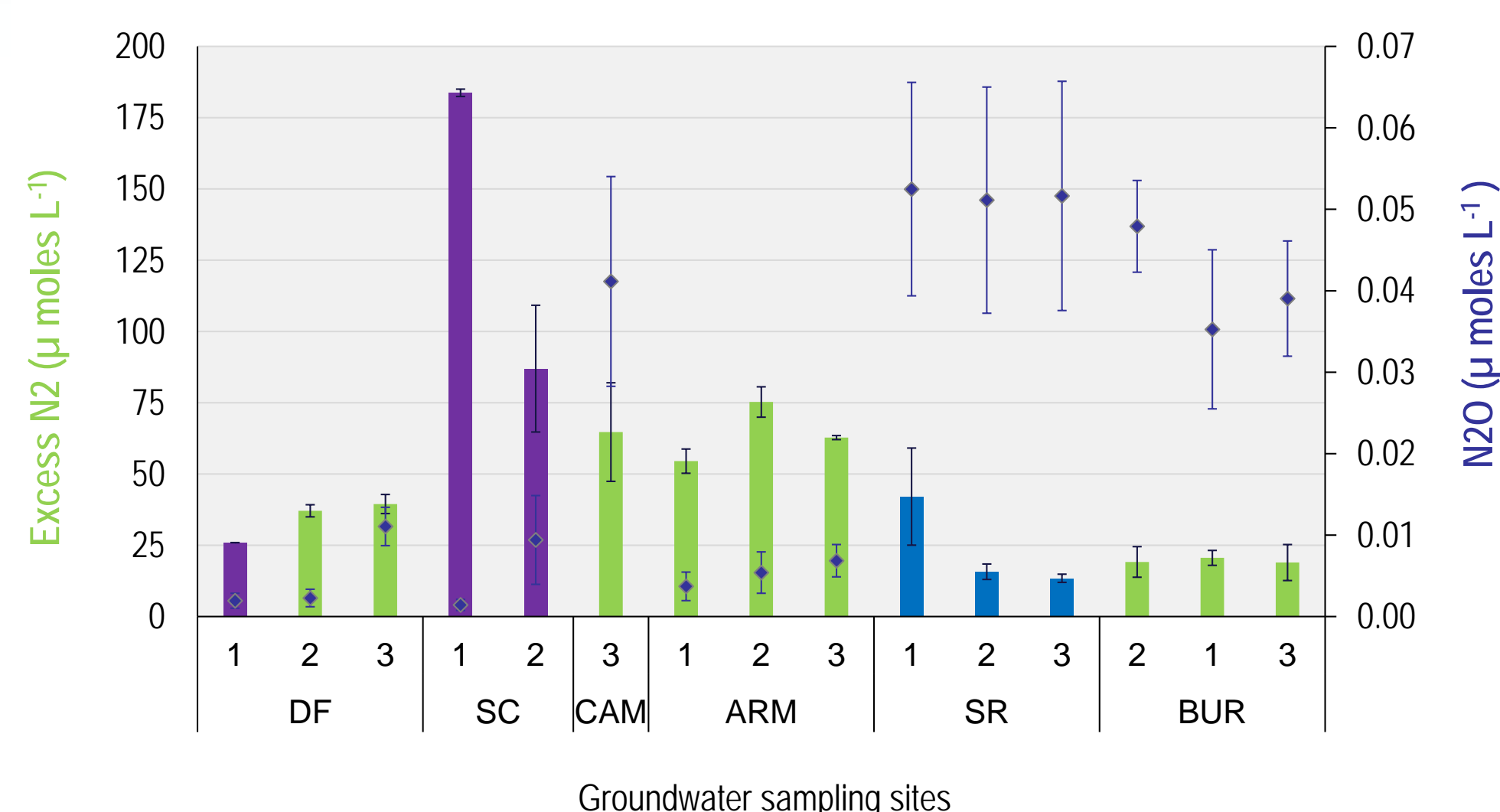


Figure 1: Monthly average of Excess N₂ and N₂O (June, August and September 2018) (n=3 ± SEM) measured at the study sites (Table 1) (Gonzalez *et al.*, 2019).

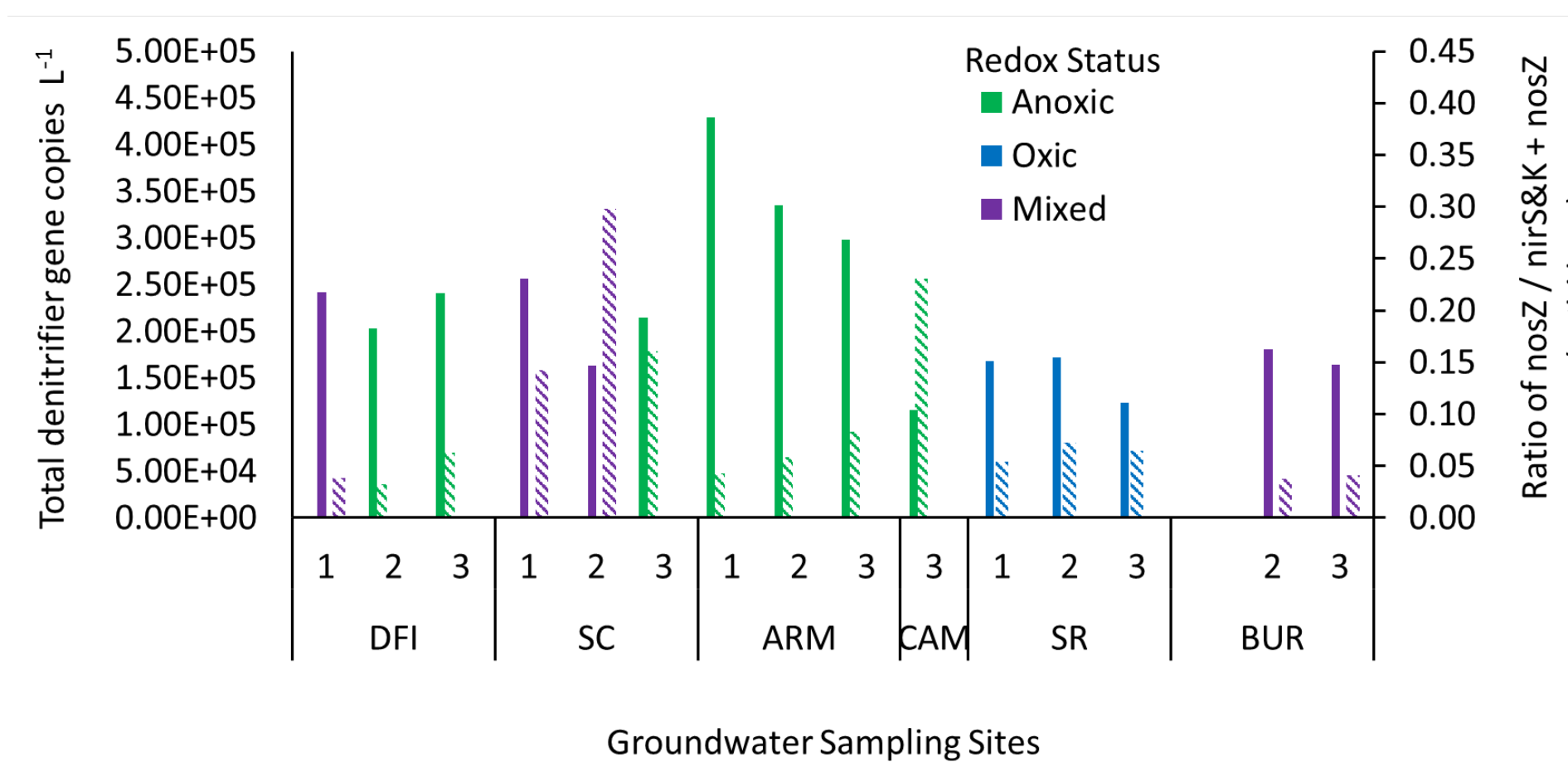


Figure 2: Monthly average of denitrifier gene copies measured at the study sites (Table 1).

Table 2: Correlation between the groundwater chemistry, dissolved N gases and denitrifier gene copies measured all study sites across the Manawatu and Rangitikei River catchments.

Parameters	DO	DOC	Fe ²⁺	Mn ²⁺	SO ₄ ²⁻	NO ₃ -N	N ₂ O	Excess N ₂	nirS&K	nosZ
DO	*	-0.39	-0.68	-0.51	0.05	0.70	0.79	-0.51	-0.31	-0.60
DOC		*	0.29	0.72	0.57	0.02	-0.24	0.63	0.65	0.35
Fe ²⁺			*	0.32	-0.02	-0.72	-0.69	0.76	0.13	0.49
Mn ²⁺				*	0.01	-0.37	-0.36	0.72	0.61	0.65
SO ₄ ²⁻					*	0.30	-0.09	0.14	0.69	0.27
NO ₃ -N						*	0.66	-0.51	-0.17	-0.50
N ₂ O							*	-0.55	-0.23	-0.49
Excess N ₂								*	0.46	0.76
nirS&K									*	0.73
nosZ										*

Dissolved gases (Figure 1):

- The oxic sites SR 1, 2 & 3; and the mixed sites BUR 1, 2 & 3 have less Excess N₂ (< 20 μmol·L⁻¹) and higher N₂O concentration (> 0.035 μmol·L⁻¹) → indication of **partial** subsurface denitrification.
- The mixed sites DF1 and SC 1 & 2, and the anoxic sites DF 2 & 3, and ARM 1, 2 & 3 have the highest average Excess N₂ (> 25 μmol·L⁻¹) and lower N₂O (< 0.02 μmol·L⁻¹) → indication of **complete** subsurface denitrification.
- The anoxic site CAM 3 have slightly higher N₂O concentration (0.04 μmol·L⁻¹), but also > 60 μmol·L⁻¹ of Excess N₂ → indication of favorable conditions for **complete** subsurface denitrification.

Denitrifier gene copies (Figure 2):

- The total denitrification gene copies L⁻¹ (including *nirS*, *nirK* and *nosZ*) varied from 3.61E+04 to 3.31E+05, showing no corresponds with the groundwater redox status.
- However, the ratio of *nirZ* / (*nirS&K* + *nosZ*) was measured relatively higher (>0.15) at the anoxic and mixed (oxic-anoxic) sites (except CAM3) → indication of favorable conditions for **complete** subsurface denitrification.
- The oxic sites SR 1, 2 & 3 and the mixed sites BUR 2 & 3 showed relatively smaller count of denitrification gene copies and relatively lower ratio of *nirZ* / (*nirS&K* + *nosZ*). This corresponds well with the measured less Excess N₂ and higher N₂O at the SR and BUR sites (Figure 2) → indication of **partial** subsurface denitrification.

References:

- *Collins (2015) and New Zealand Fundamental Soil Layer and Rock type from the New Zealand Land Resource Inventory based on the location of the study sites (<https://soils.landcareresearch.co.nz/soil-data/the-iris-portal/>) *Collins, S. (2015). Investigating the Transport and Fate of Nitrogen from Farms to River in the Lower Rangitikei Catchment. (Master of Science), Massey University, New Zealand.
- Korom, S. 1992. Natural denitrification in the saturated zone: A review. Water resources research, Vol. 28, N°6, Pages 1657-1668.
- McMillan, A., Phillips, R., Berben, P., Thilak, P., Jha, N., & Saggari, S. (2014). Automated N₂O/N₂ analysis- a new tool for studying denitrification dynamics and testing mitigation strategies. In L. D. Currie & C. L. Christensen (Eds.), Nutrient Management for the Farm, Catchment and Community Occasional
- Rivett, M., Buss, S., Morgan, P., Smith, J., Bement, C. 2008. Nitrate attenuation in groundwater: A review of biogeochemical controlling processes. Water Research 42 (2008) 4215 – 4232.
- Weymann, D., Well, R., Flessa, H., von der Heide, C., Deurer, M., Konrad, C., Walther, W. 2008. Groundwater N₂O emission factors of nitrate-contaminated aquifers as derived from denitrification progress and N₂O accumulation. Biogeoscience, 5, 1215-1226.