

# A novel toolbox for development of a Groundwater Health Index



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# So thirsty!

Can't wait to drink a glass of water...



Which one would **you** choose?

## Sources of bacterial detections

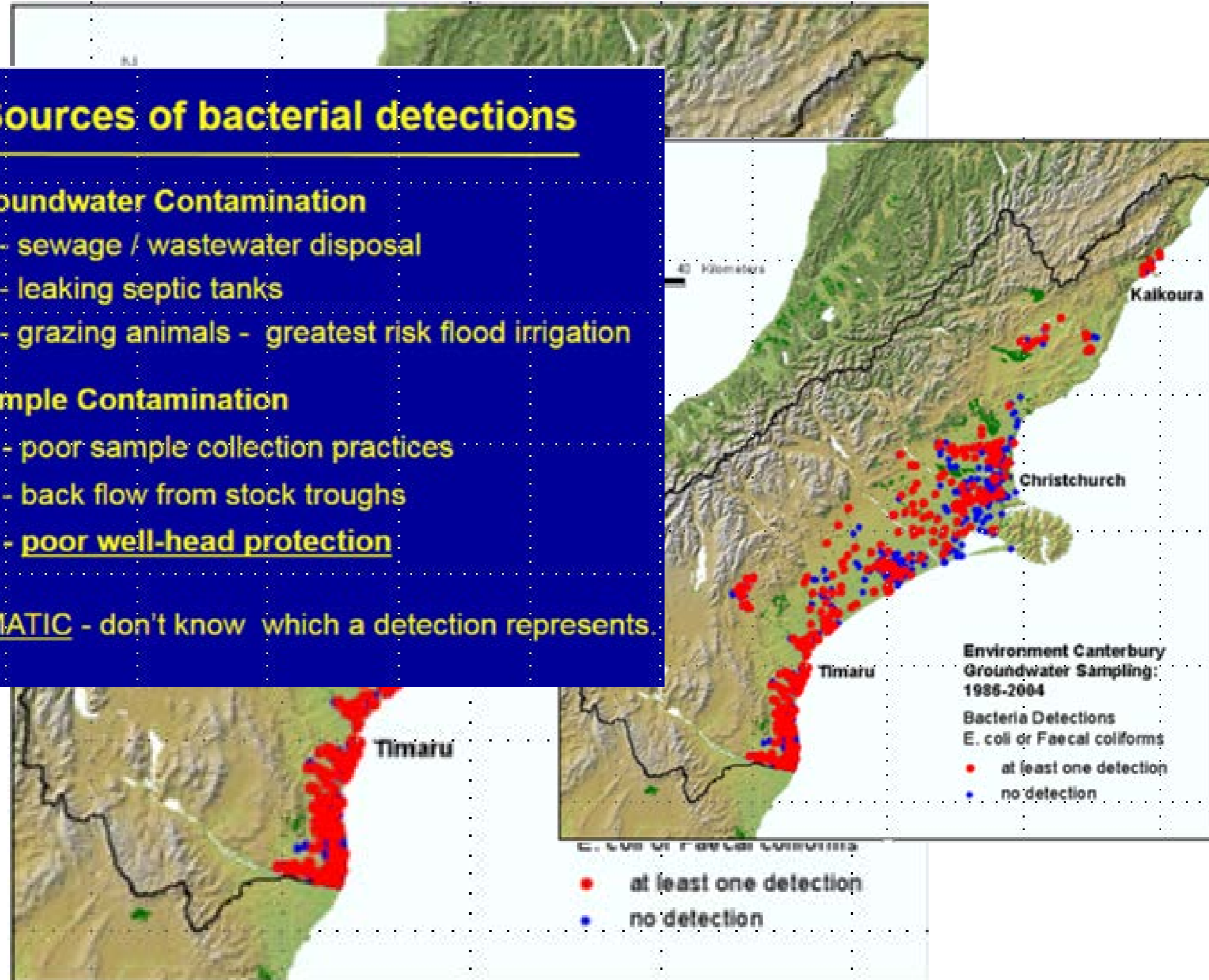
### 1. Groundwater Contamination

- sewage / wastewater disposal
- leaking septic tanks
- grazing animals - greatest risk flood irrigation

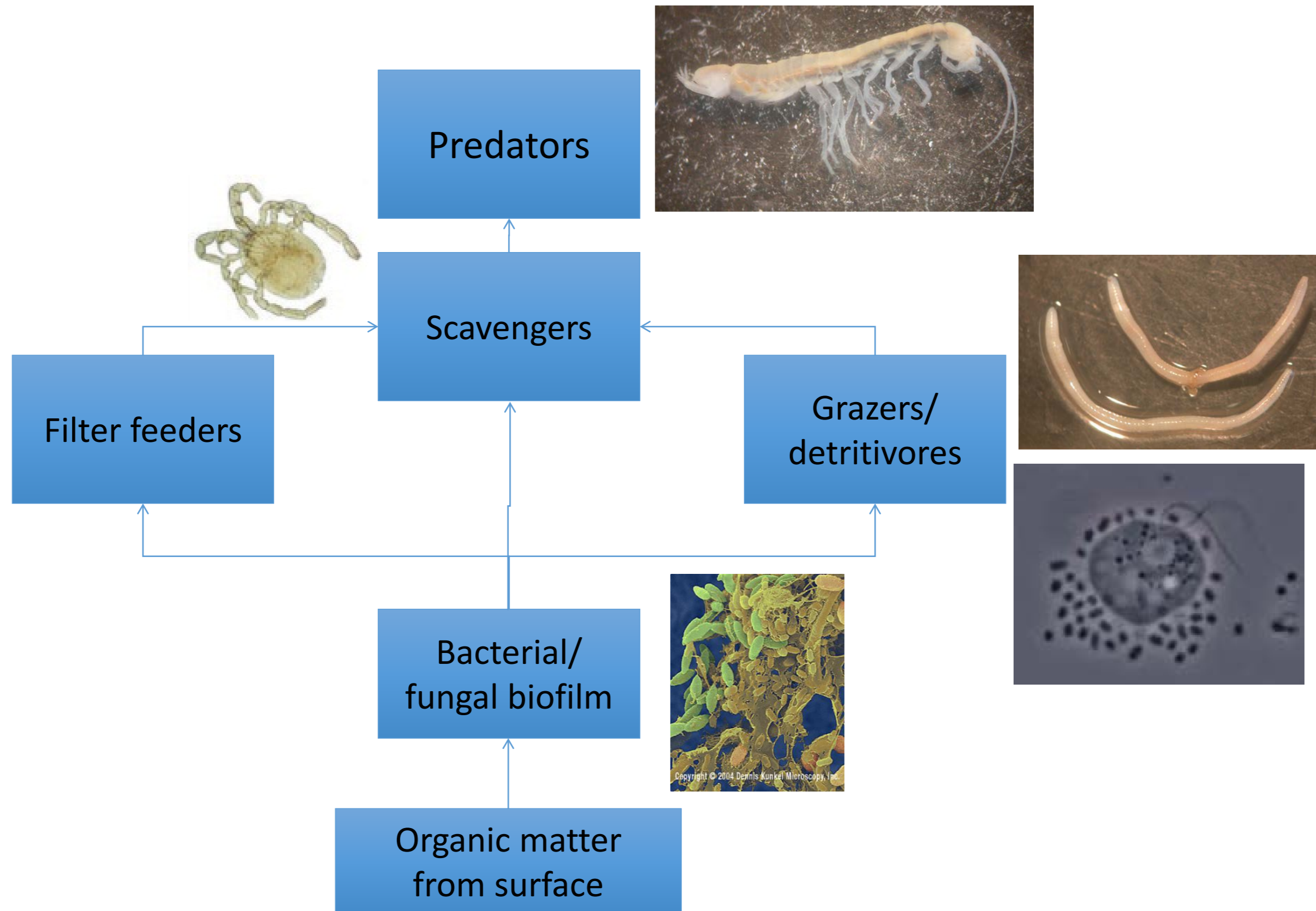
### 2. Sample Contamination

- poor sample collection practices
- back flow from stock troughs
- poor well-head protection

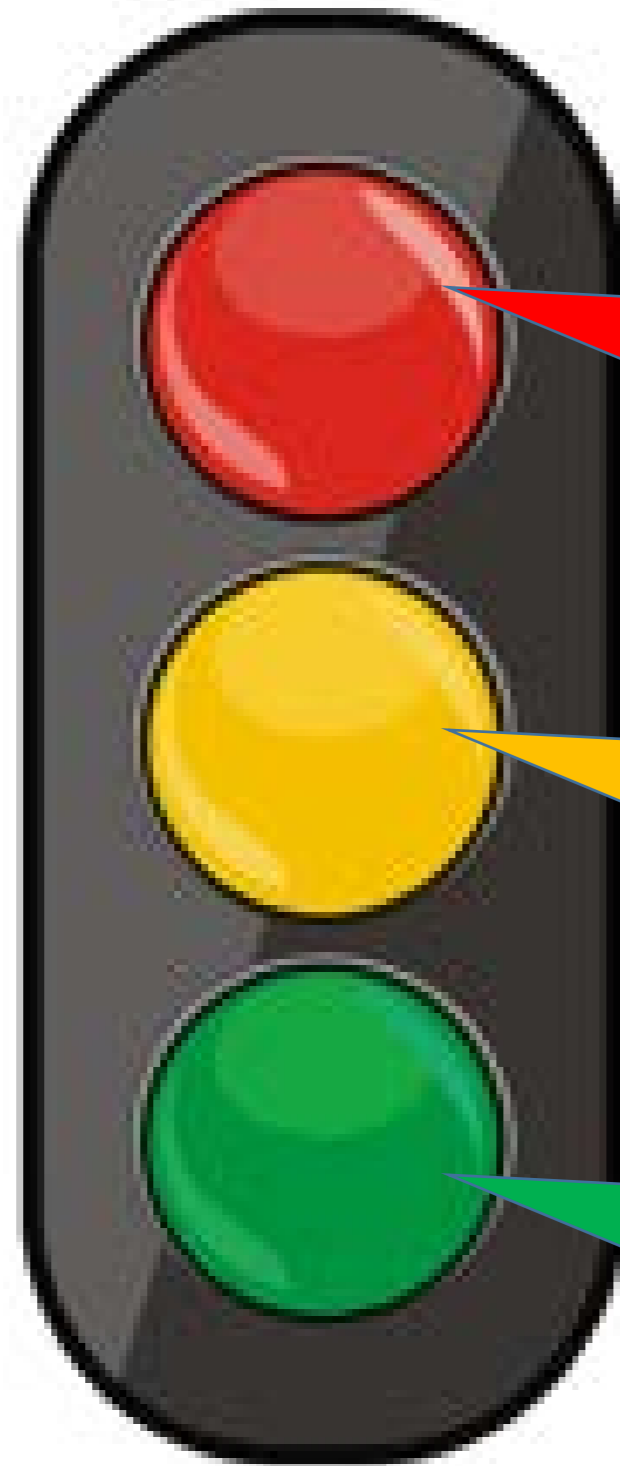
PROBLEMATIC - don't know which a detection represents.



# What lurks beneath your feet? Friend or foe??



# The Groundwater Health Index: Toolbox approach

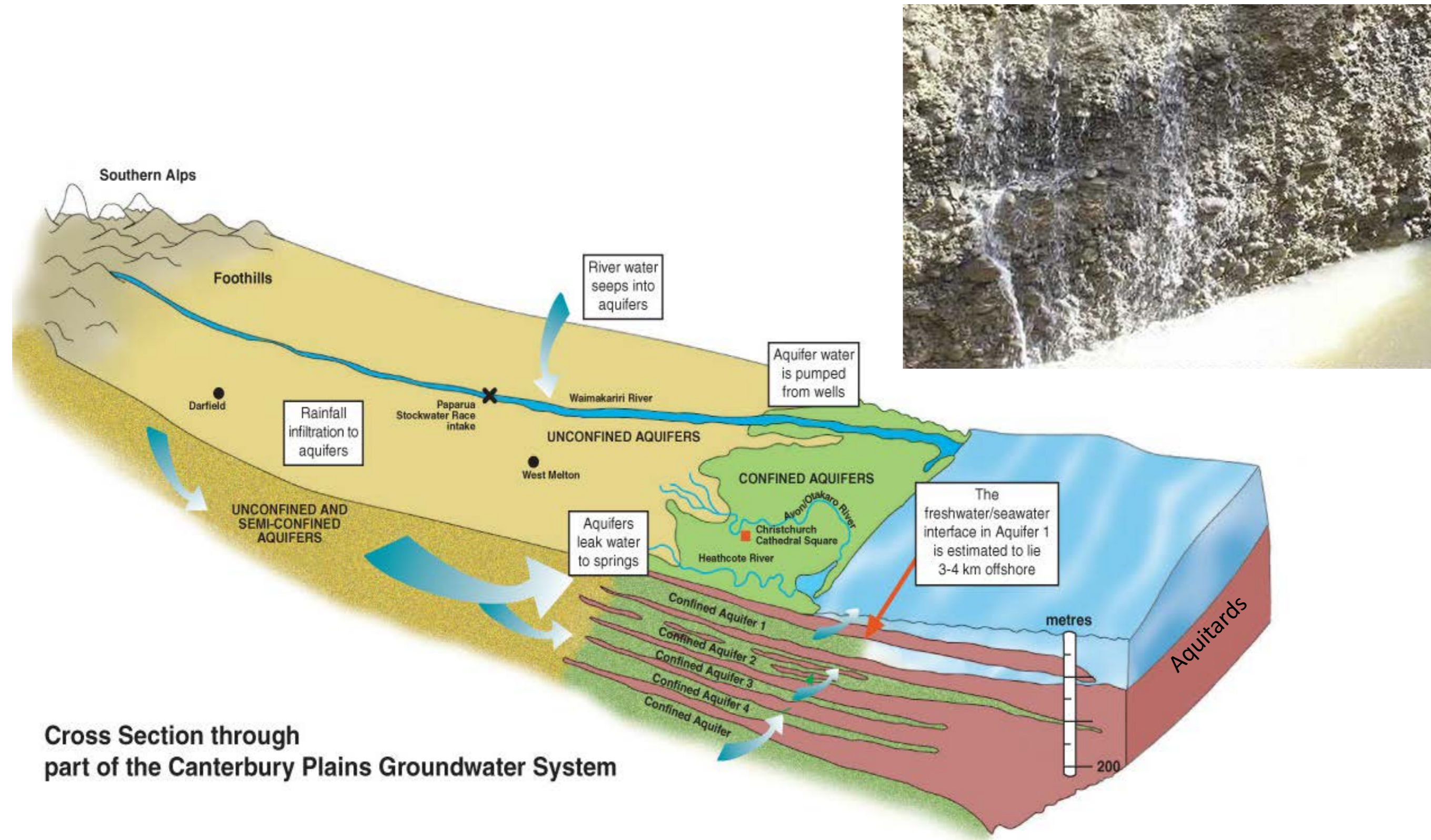


Communities can no longer protect drinking water from contamination

Communities are stressed and moving towards the tipping point ie no protection of drinking water

Communities are functioning well and not affected by contamination or no significant contamination is present

# Canterbury Aquifers micro & macro-faunal communities

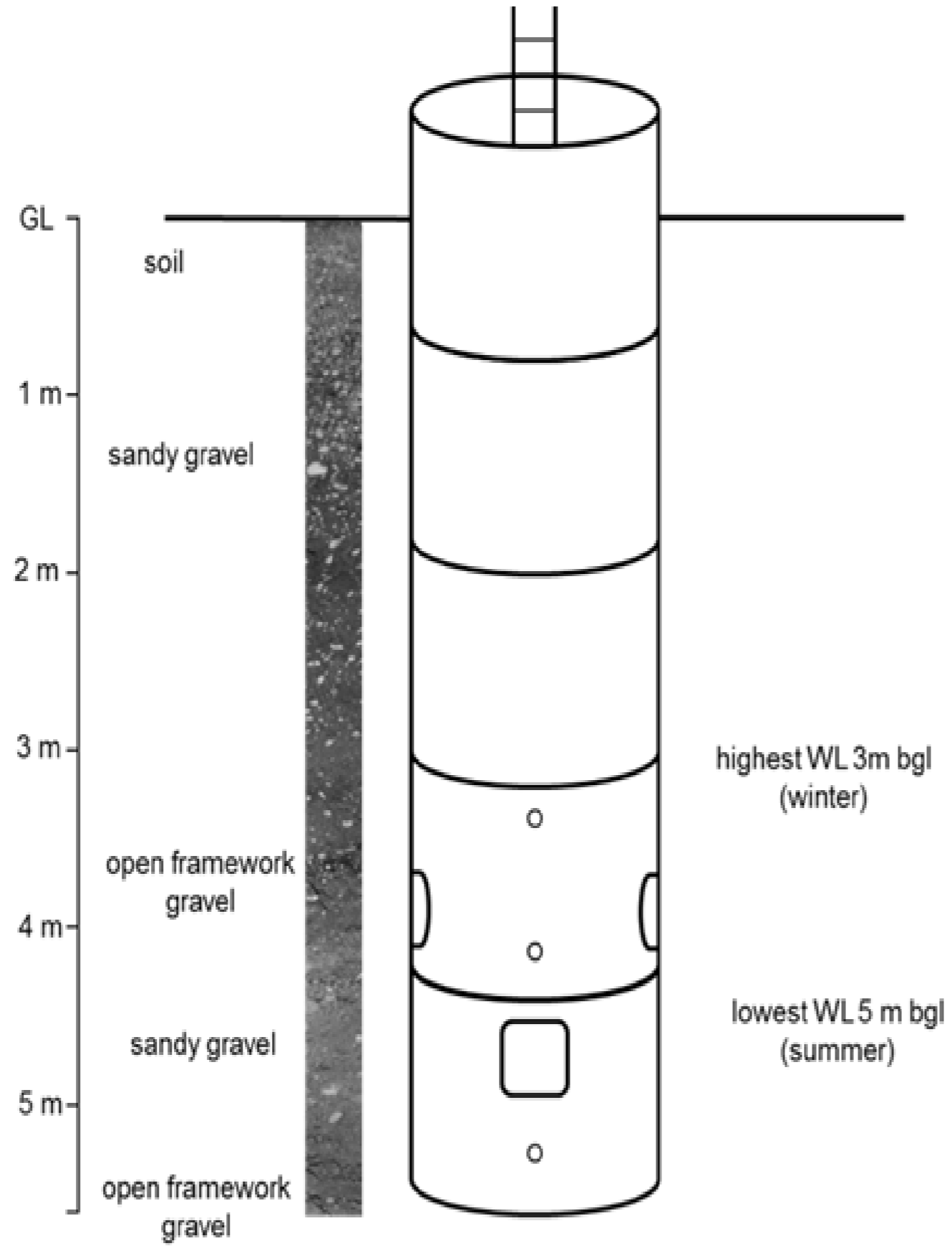


# Sampling techniques

1. Develop sampling methods to recovery sufficient DNA to obtain high quality NGS libraries for each aquifer environment.
2. Subsurface difficult to access and sample, previous pumping of GW under represents MC's
3. Investigated the following sampling methods:
  - i. ZIS sampler (best – access to sediments)
  - ii. in-situ biofilm bags (whole GW ecosystem)
  - iii. down well sonication (future research)



# ZIS sampler

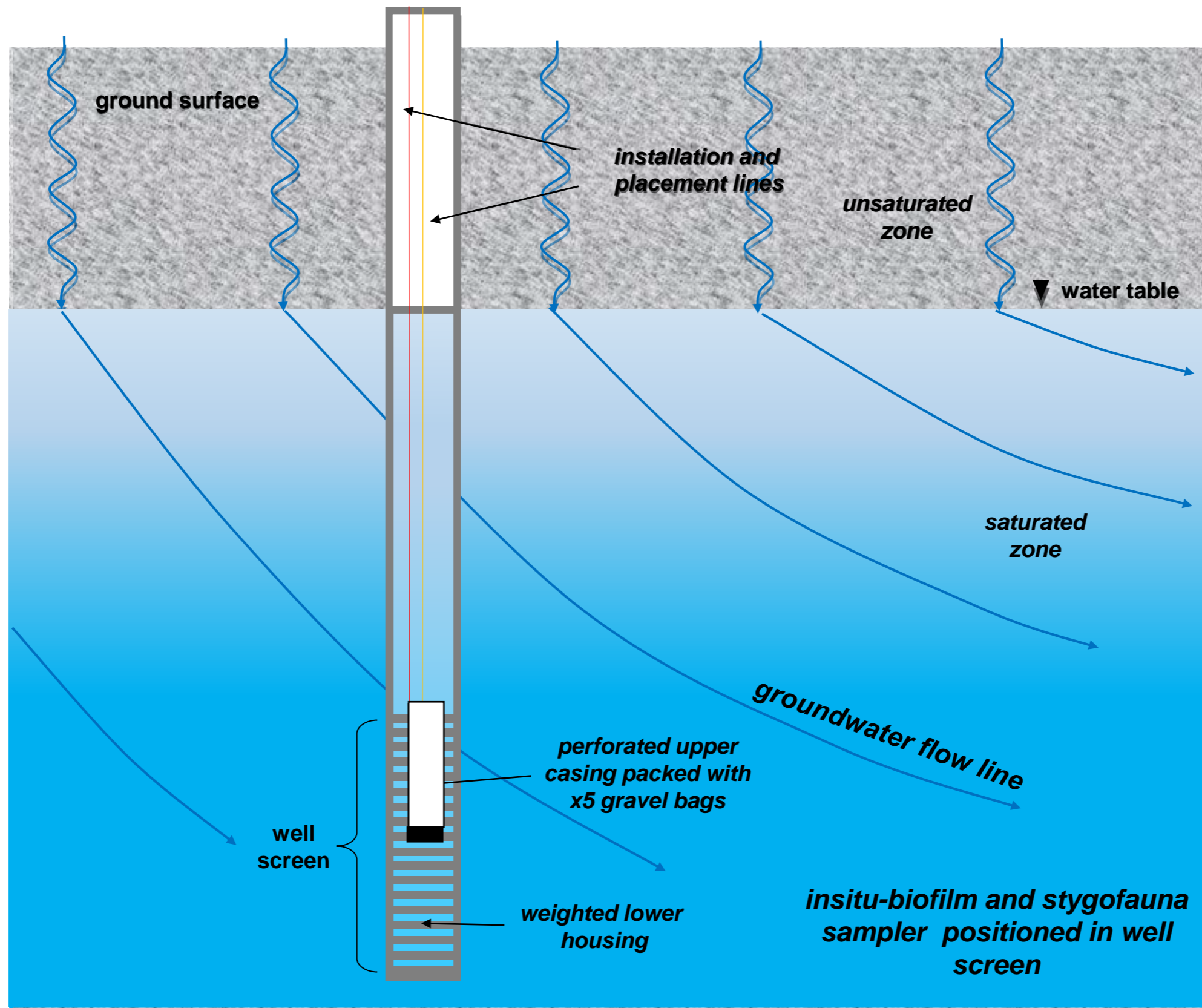




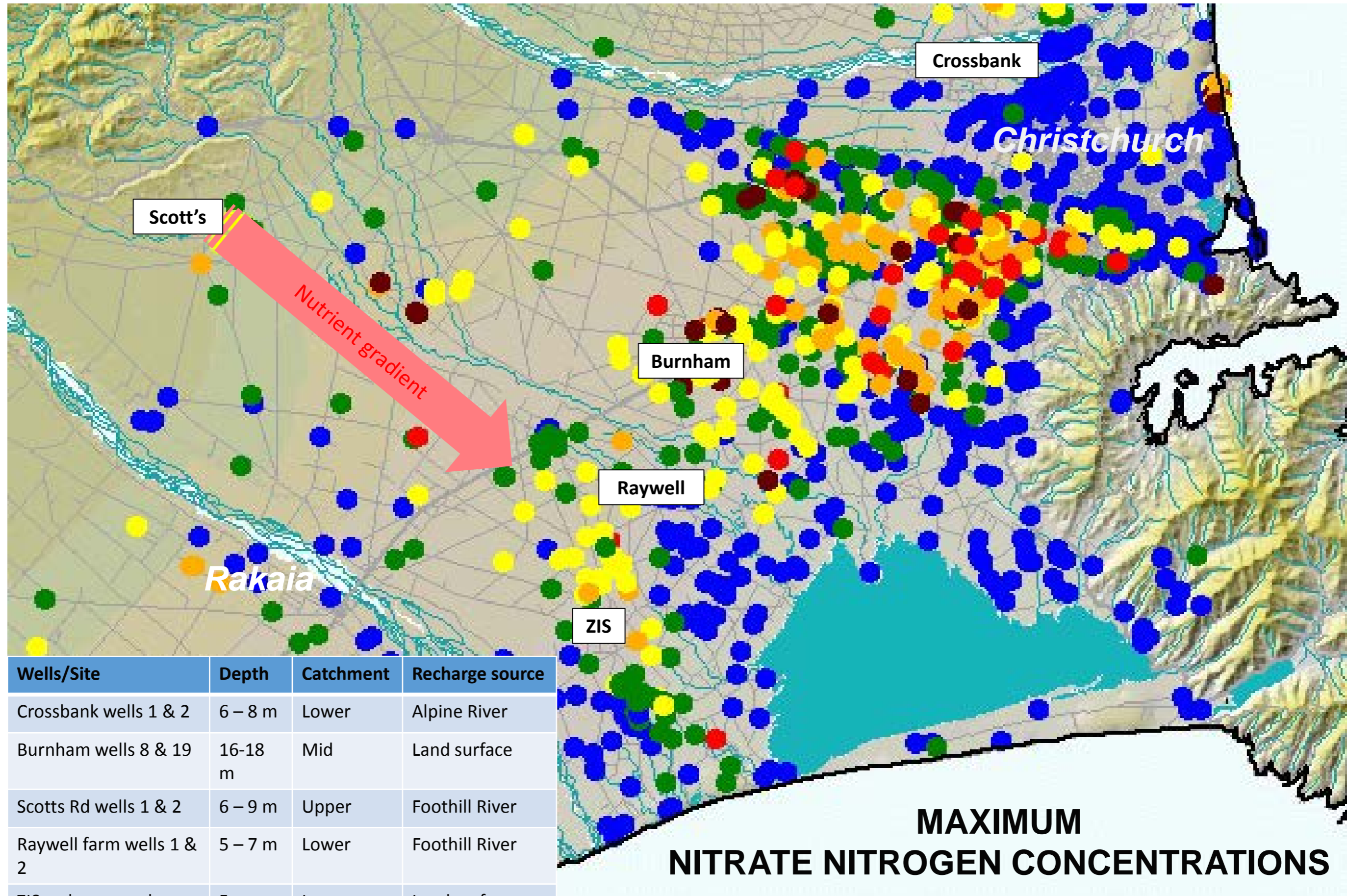
# *In-situ* biofilm bag sampler



# *In-situ* sampler installed in well



# Field sites

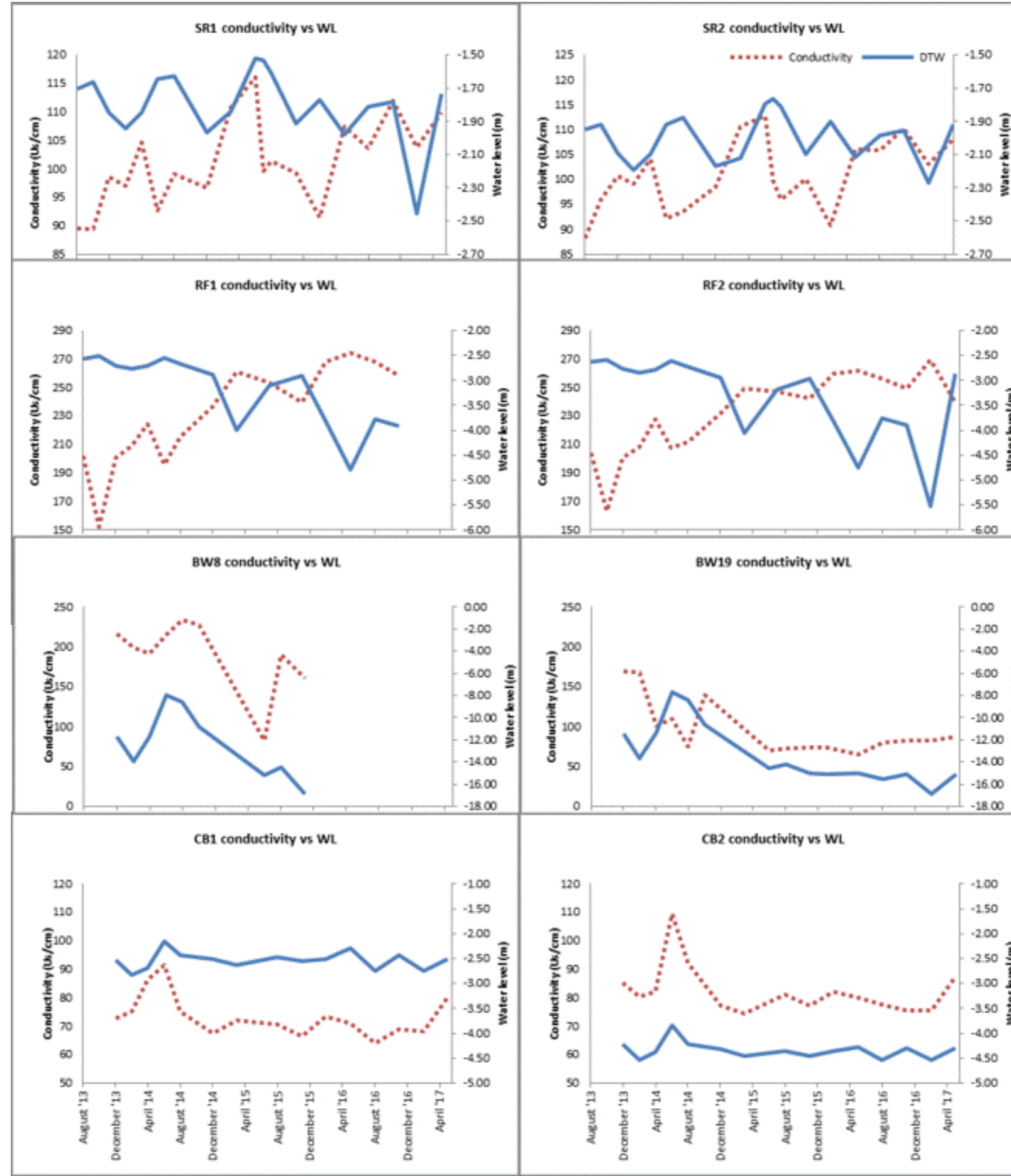


# Sampling

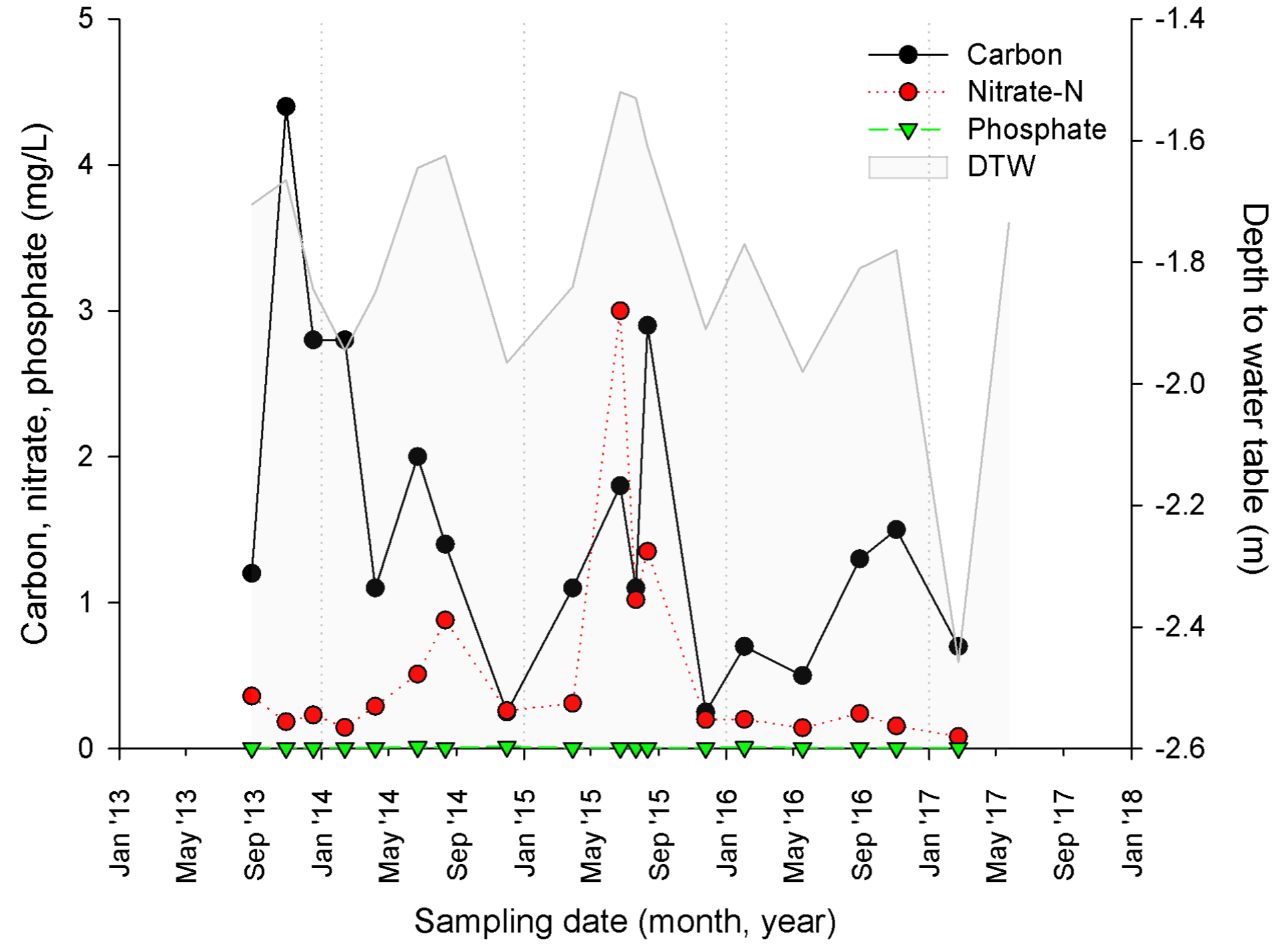
- Quarterly sampling:
  - Groundwater levels
  - Water chemistry
  - Field parameters
- Water chemistry for 22 determinands, including major ions, metals and nutrients
- Groundwater, biofilm bags and Stygofauna
  - Summer and Winter
  - Lab analysis and water chemistry
- Seasonal data on the microbial communities and their subsurface environment



# GW Chemistry Results



# Scotts Road, well SR1



## Nitrate and dissolved oxygen (DO) readings per year at Canterbury sites

July-July	Site	Well ID	Mean Nitrate-N g/m3	Min Nitrate-N g/m3	Max Nitrate-N g/m3	Mean DO mg/L	Min DO mg/L	Max DO mg/L
<b>2013-2014</b>	Scotts Road	SR1	0.29	0.15	0.51	4.25	3.60	4.900
	Scotts Road	SR2	0.36	0.13	0.76	3.45	1.70	5.900
	Raywell Farm	RF1	5.17	3.40	5.90	4.33	2.60	5.900
	Raywell Farm	RF2	5.43	3.80	6.50	3.75	3.00	4.600
	Burnham	B8	4.90	4.30	5.30	7.35	6.80	7.700
	Burnham	B19	2.83	1.30	4.10	8.25	7.90	8.600
	Crossbank	CB1	0.29	0.15	0.47	6.33	4.70	8.300
	Crossbank	CB2	0.32	0.12	0.77	8.35	7.00	9.300
<b>2014-2015</b>	Scotts Road	SR1	1.11	0.26	3.00	6.65	4.70	8.600
	Scotts Road	SR2	0.87	0.23	2.60	5.38	2.60	8.100
	Raywell Farm	RF1	7.20	6.70	7.90	5.20	3.40	8.100
	Raywell Farm	RF2	7.30	7.10	7.40	5.43	4.50	7.100
	Burnham	B8	4.55	0.75	6.50	7.20	5.10	9.600
	Burnham	B19	1.30	0.15	3.00	9.33	7.70	10.300
	Crossbank	CB1	0.13	0.08	0.20	6.77	6.00	8.000
	Crossbank	CB2	0.14	0.07	0.23	7.90	6.60	8.700
<b>2015-2016</b>	Scotts Road	SR1	0.69	0.20	1.35	5.98	2.50	9.500
	Scotts Road	SR2	0.50	0.18	0.90	5.93	1.10	9.300
	Raywell Farm	RF1	7.10	6.50	7.80	4.50	4.00	5.100
	Raywell Farm	RF2	7.30	6.90	7.90	3.60	2.60	5.000
	Burnham	B8	5.40	3.90	6.90	8.40	8.30	8.500
	Burnham	B19	0.24	0.17	0.30	10.18	8.30	0.000
	Crossbank	CB1	0.16	0.10	0.19	6.23	4.90	7.600
	Crossbank	CB2	0.18	0.13	0.27	8.05	6.50	9.700

# Laboratory analyses

## Biomass

- Heterotrophic plate counts (HPC)
- Protein concentration

## Enzyme activity

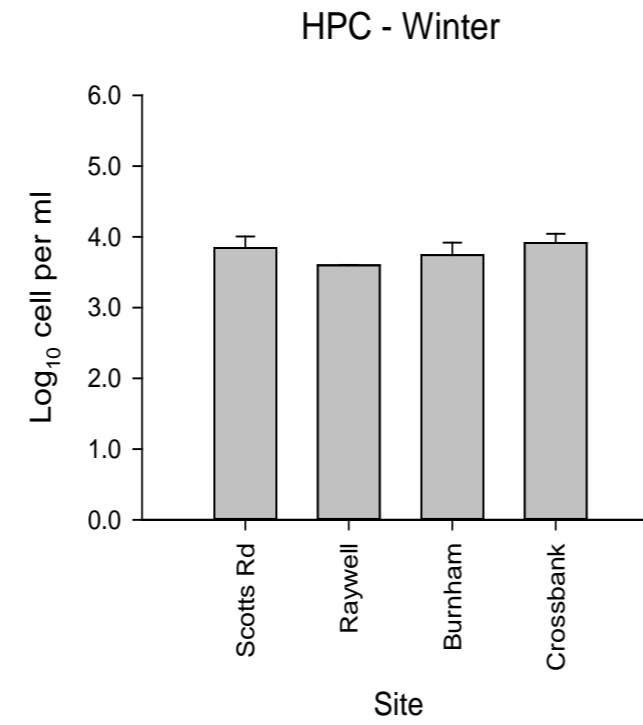
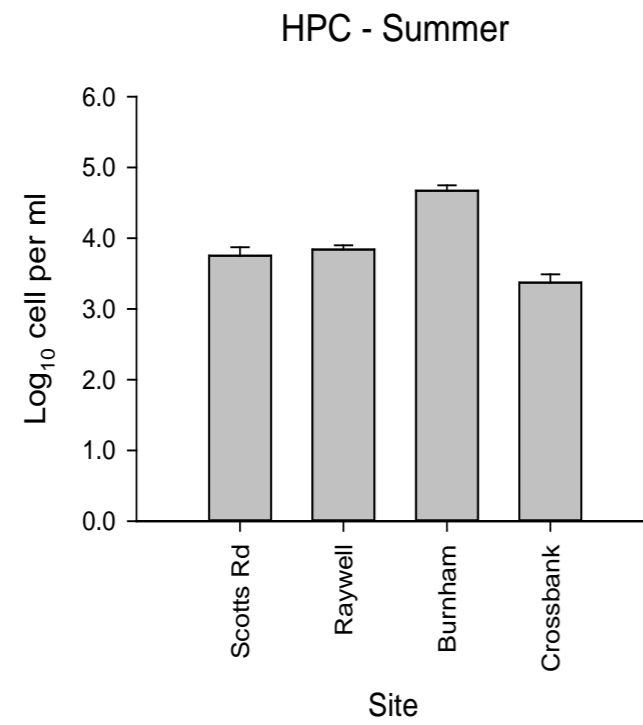
- Carbon, nitrogen and phosphorous acquisition

## Targeted metagenomics

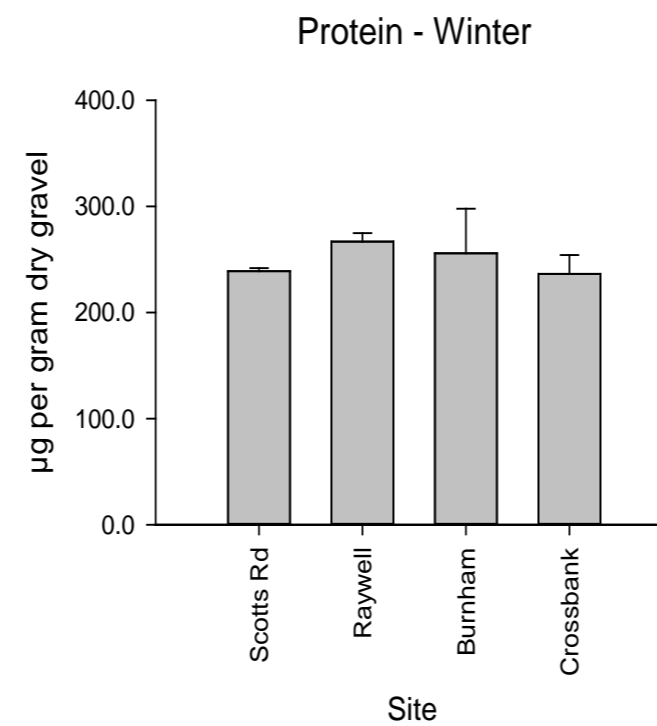
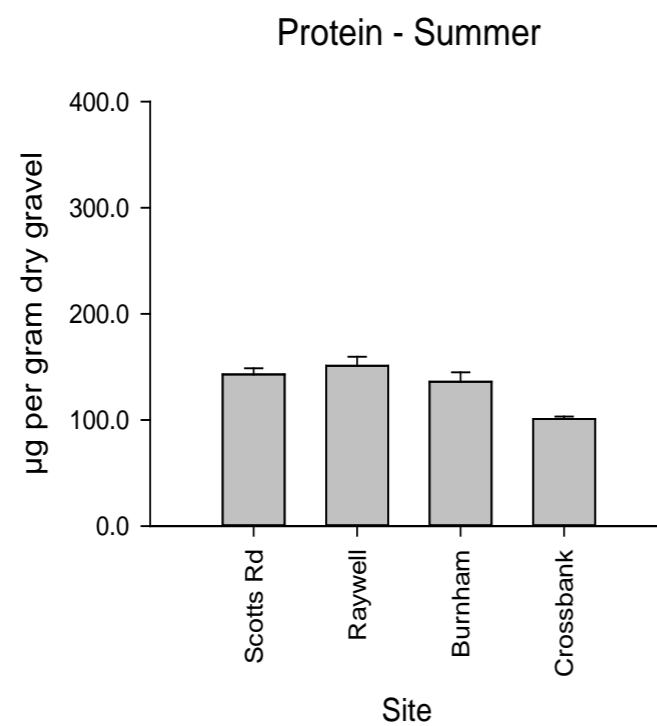
- DNA was extracted from all samples and sent for next generation sequencing using the Illumina platform
- Used 16S, 18S rDNA genes and ITS amplicons to target Prokaryotes, Eukaryotes and Fungi.
- Analysis performed using QIIME



# Biomass

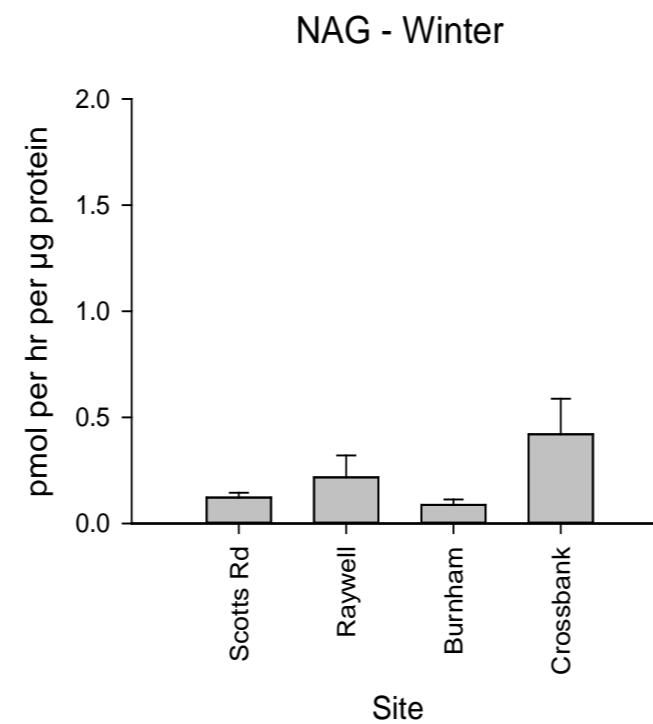
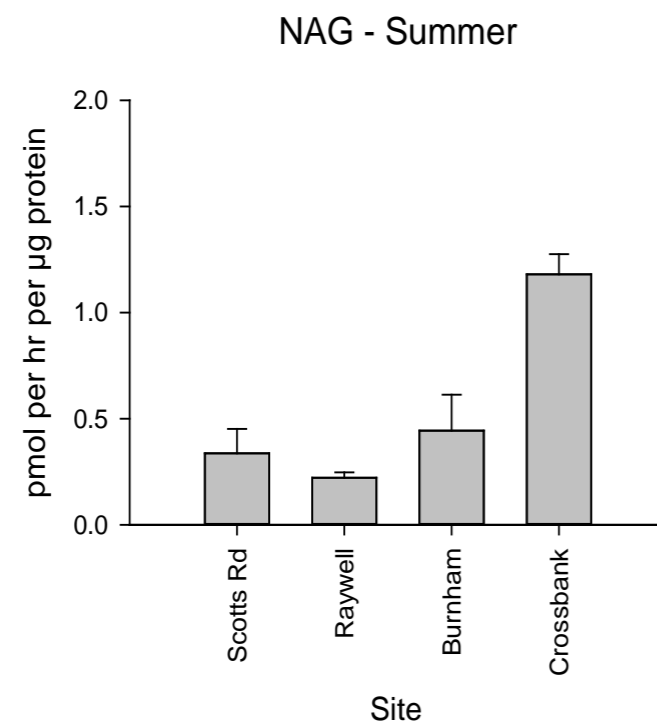
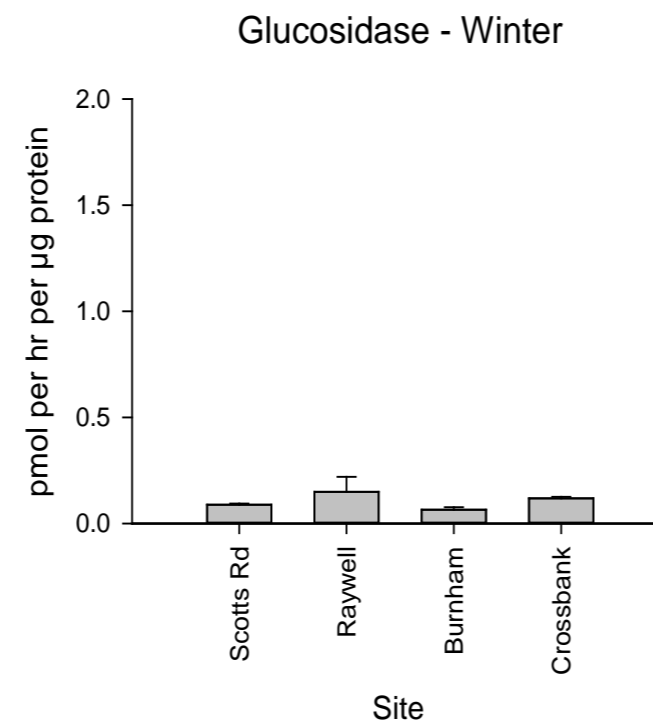
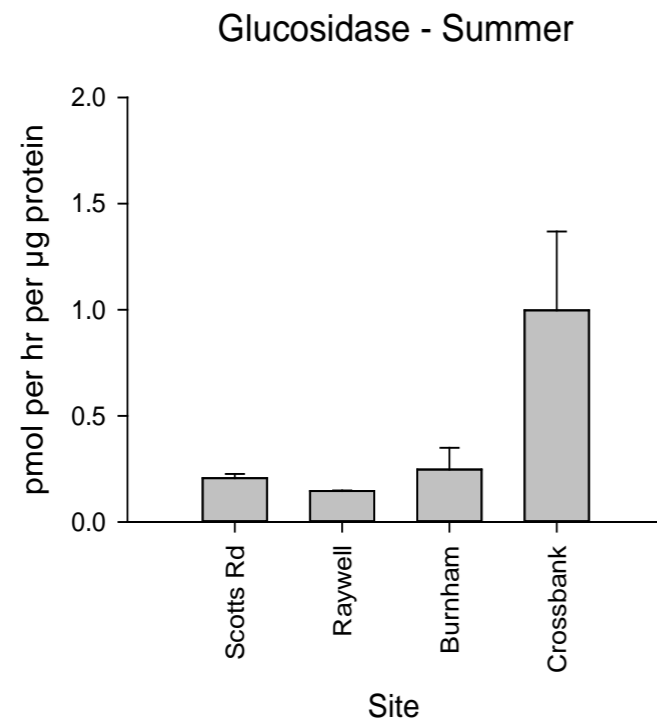


- **HPC** - culture of microorganisms that use organic carbon sources to grow

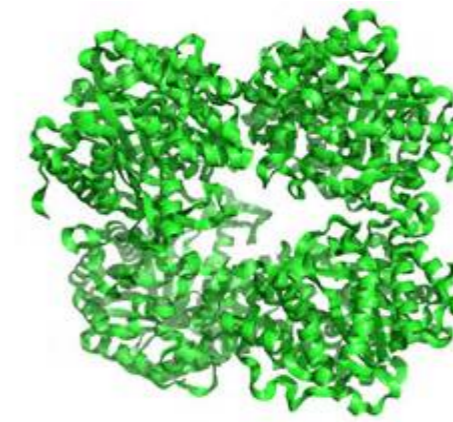


- **Protein assay** - determines total protein concentration within a sample

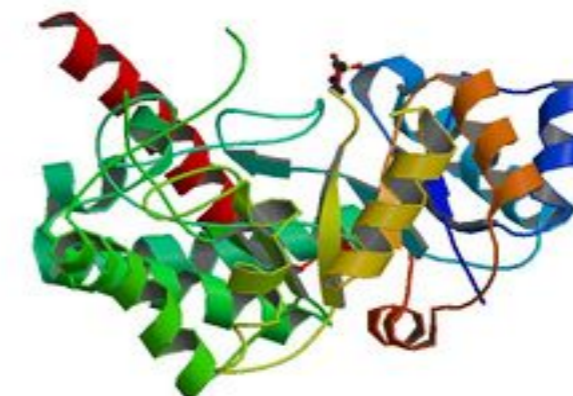
# Carbon acquisition



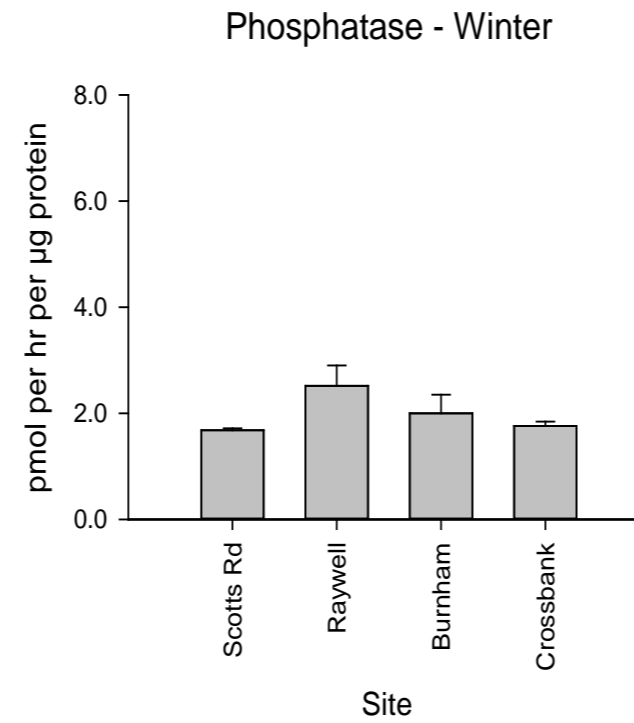
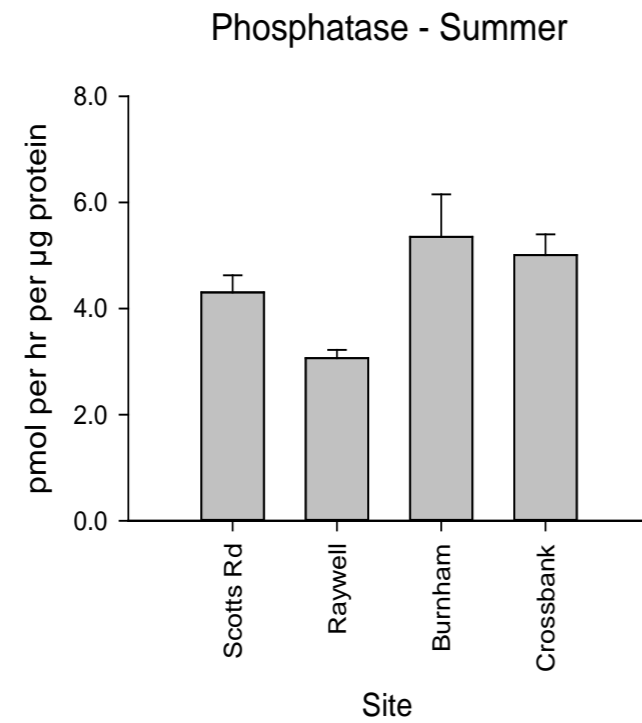
- **Glucosidase** - breakdown of complex carbohydrates



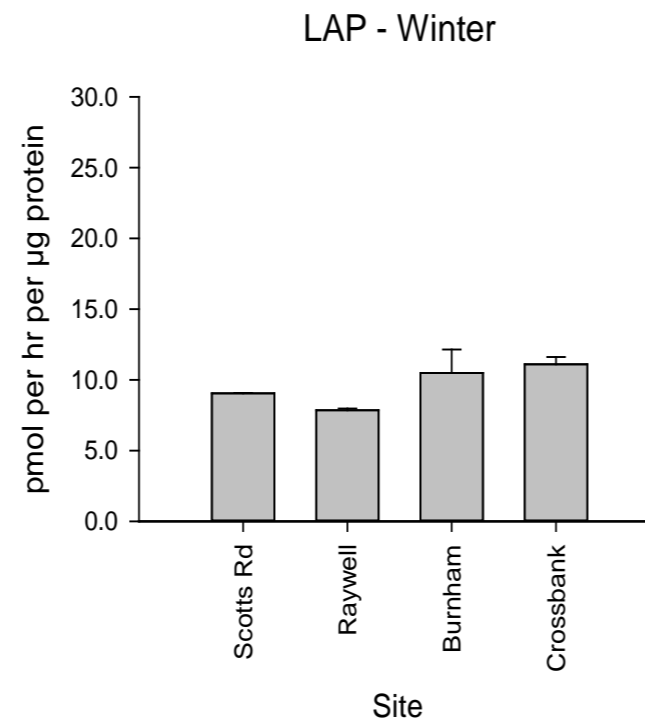
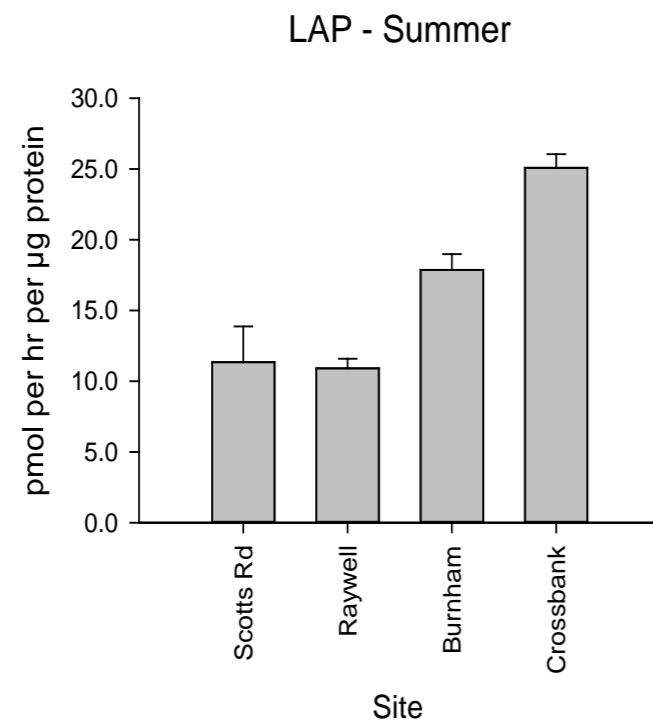
- **Glucosaminidase (NAG)** - degrades chitin from higher organisms



# Phosphorous and nitrogen acquisition



- **Phosphatase** - Phosphorus mineralization



- **Leucine peptidase (Lap)** - degrades proteins into amino acids



# Metagenomics results

- 10-100 thousand reads per sample
- High diversity seen in all samples 300->600 different species
- Predominantly Proteobacteria (~25-70%)
- Groundwater higher 50-70%  
Biofilm more variable 25-50%



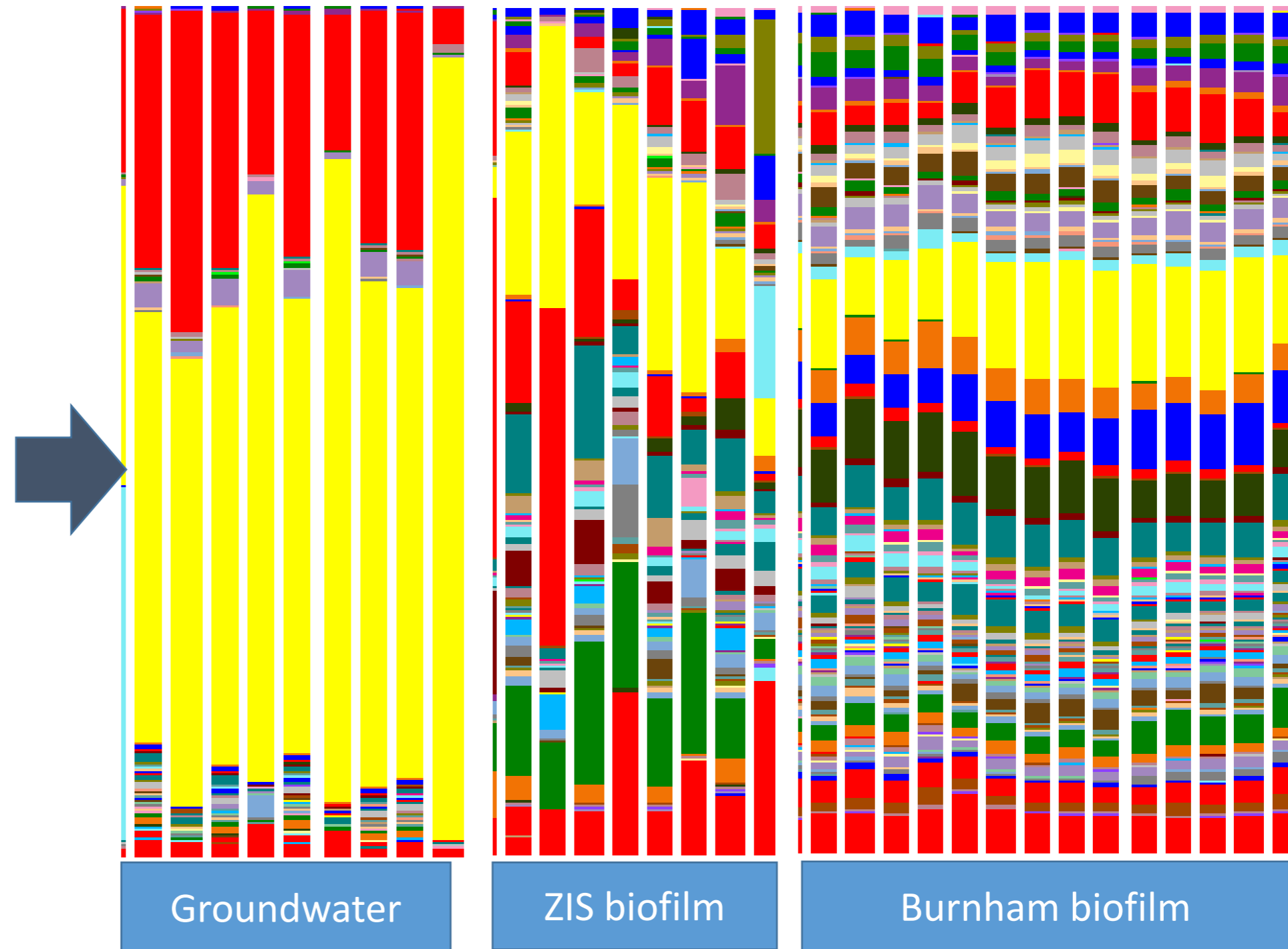
# Site comparisons

At all sites:

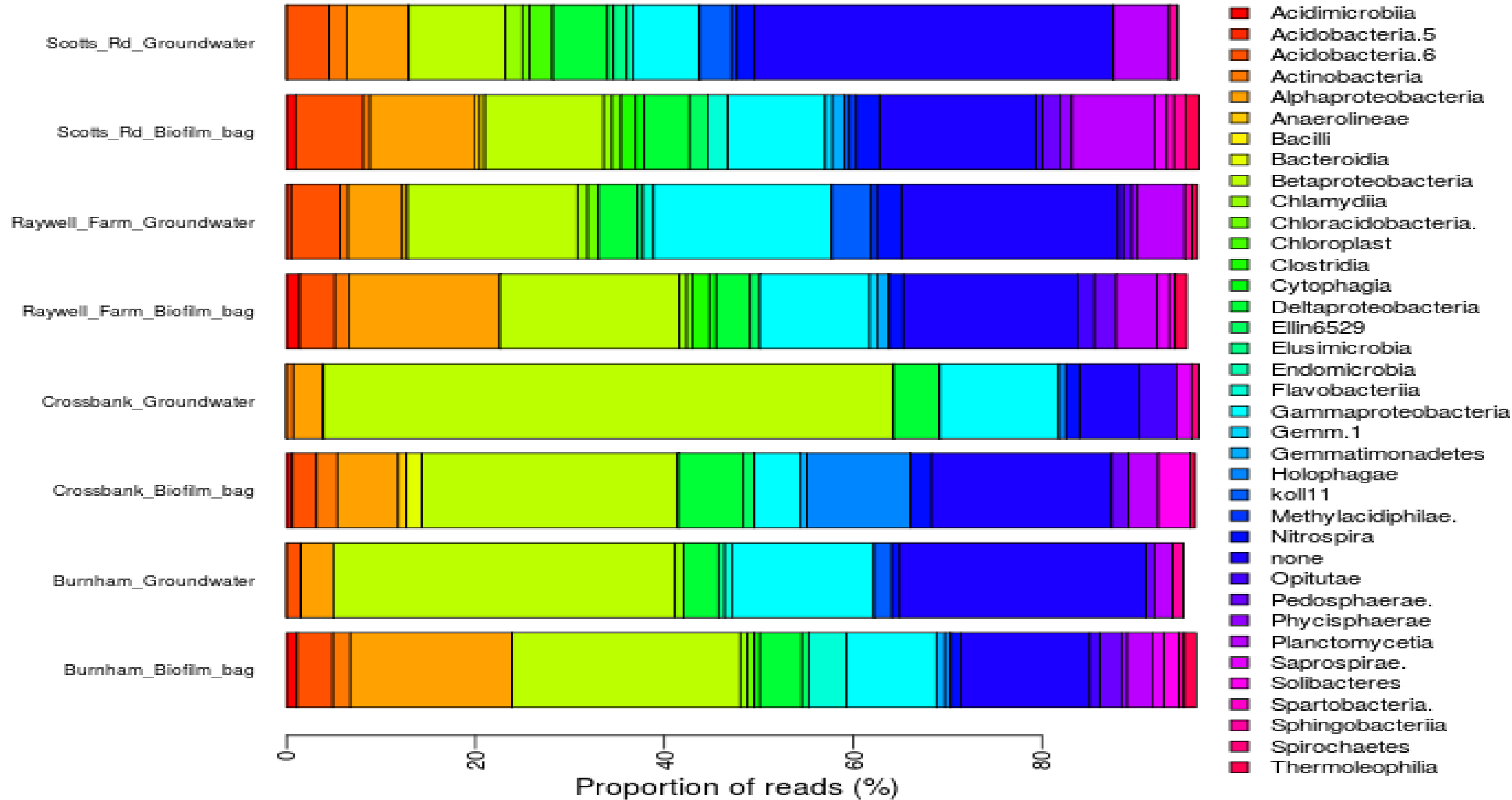
- GW high abundance Beta Proteobacteria (**yellow**)

Biofilms

- Wide range species
- Some differences between sites – Good!



# Bacteria observed in samples (Class, > 0.5% relative abundance)



# Stygofauna ID

Taxonomic ID – Graham Fenwick (NIWA) & Annette Bolton (ESR)

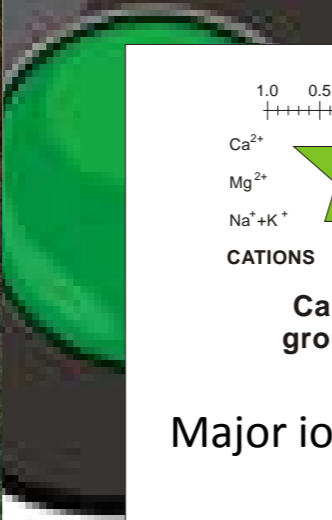
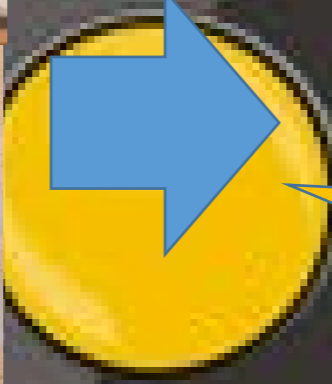
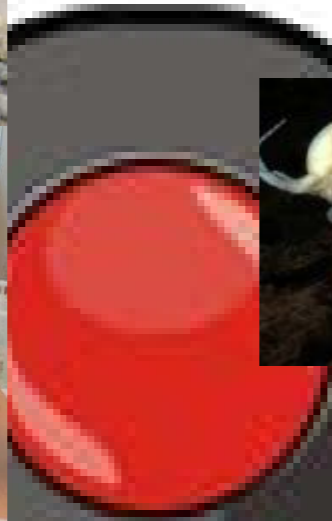
Molecular ID – targeted approach in development

Preliminary results look promising – we can identify Isopoda and Amphipoda using molecular approaches

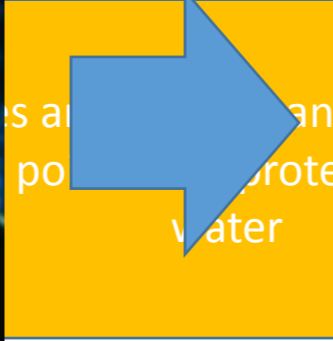
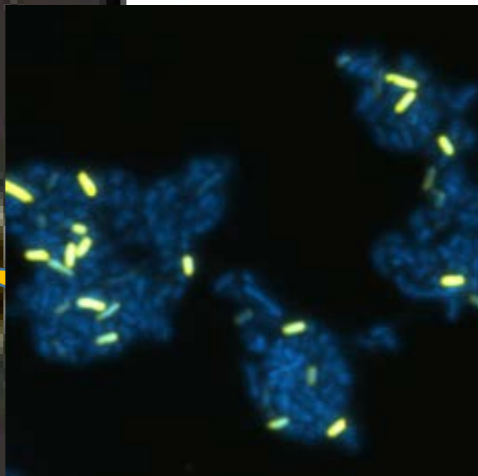
More to come!



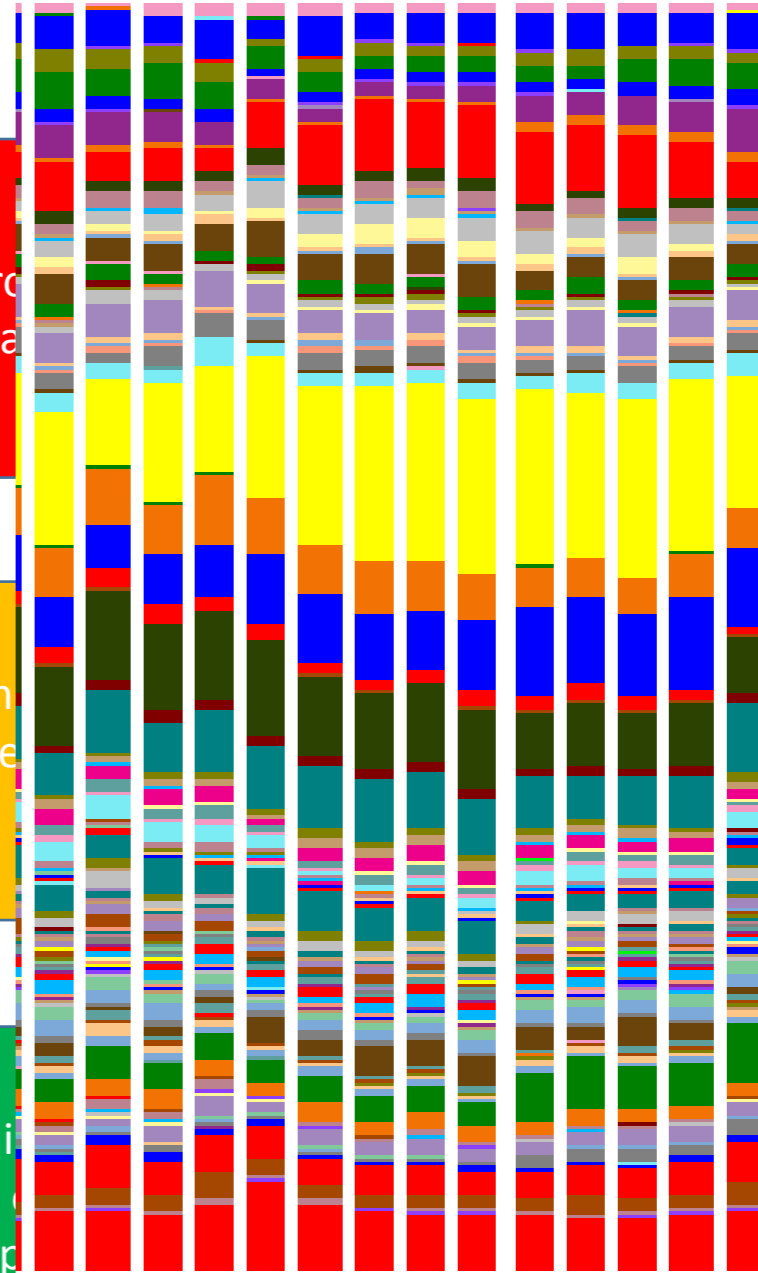
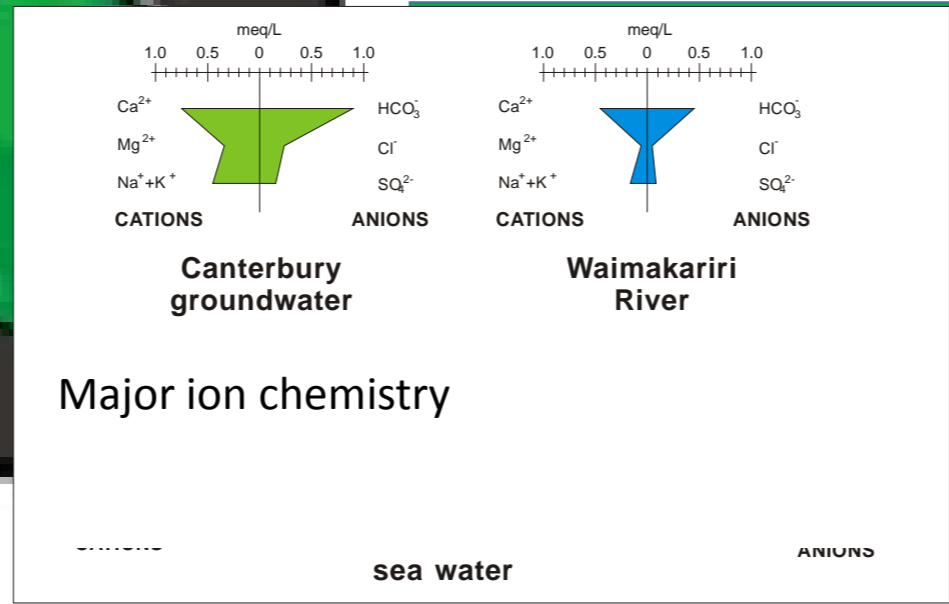
# The toolbox



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water



Burnham biofilm



# Conclusions & next steps

- Differences seen in microorganisms between sites – now comparing to GW chemistry
- Preliminary results Stygofauna ID from DNA - promising

Where next:

- Expanding approach to Archaea, fungi and Stygofauna (GW macroinvertebrates)
  - Whole community assessment
- Developing methods for activity assessment (proteomics, metabolomics)
- Ultimate goal – Groundwater health index

**Thank you for listening!**