GISERA W17 Environmental monitoring and microbial degradation of onshore shale gas activity chemicals and fluids

Knowledge Transfer Session | Presenter: Dr David Midgley | 31/08/21

Nai Tran-Dinh, Carla Mariani, Richard Schinteie, Kaydy L. Pinetown, Se Gong, Tania J. Vergara, Paul Greenfield and David J. Midgley

Australia's National Science Agency

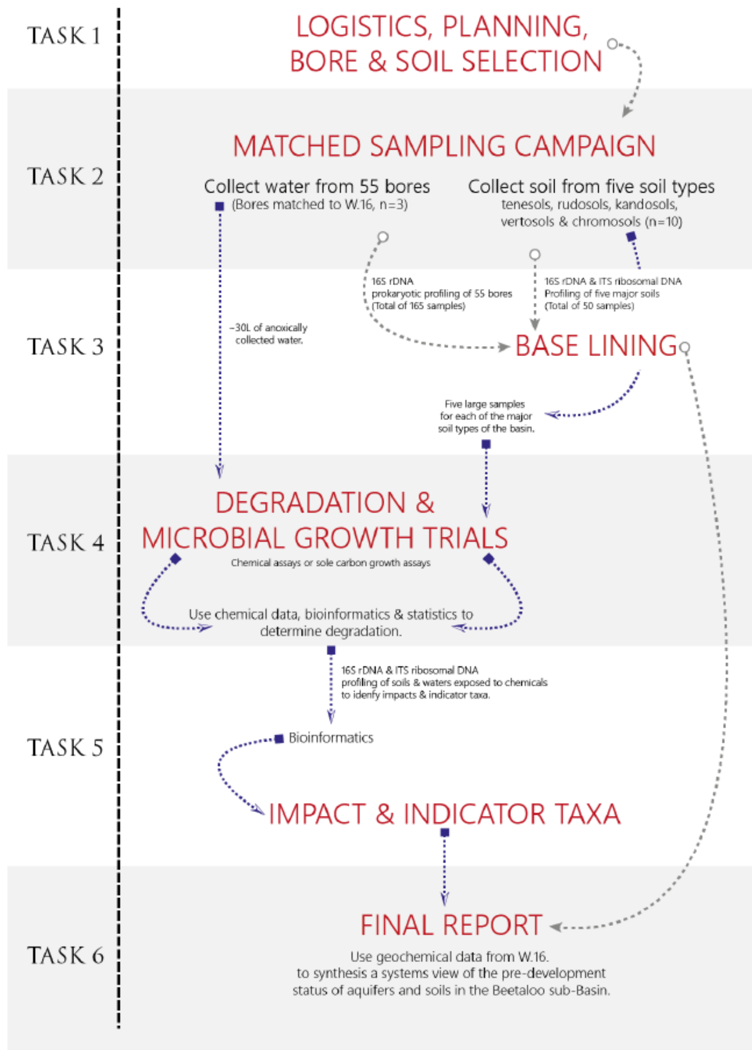
Objectives

1. Establish microbial community baselines in aquifer waters and soil samples of sites proximal to prospective unconventional gas activities in the Northern Territory.
2. Understand the microbial degradation of a range of chemicals likely to be used in unconventional gas activities, in both the five major soil types of the region and in relevant aquifer environments.

Objectives

- Spills and leaks are rare. Multiple hurdles exist to prevent their occurrence.
- Microbial biodegradation offers an addition defence against environmental harm
- Understanding organisms that respond to chemicals provides additional indicators of leaks/spills.
- This study aimed to study soils and aquifers of NT Australia, to understand chemical
  - Biodegradation
  - Biodegrading organisms
  - Effects on microbial communities.

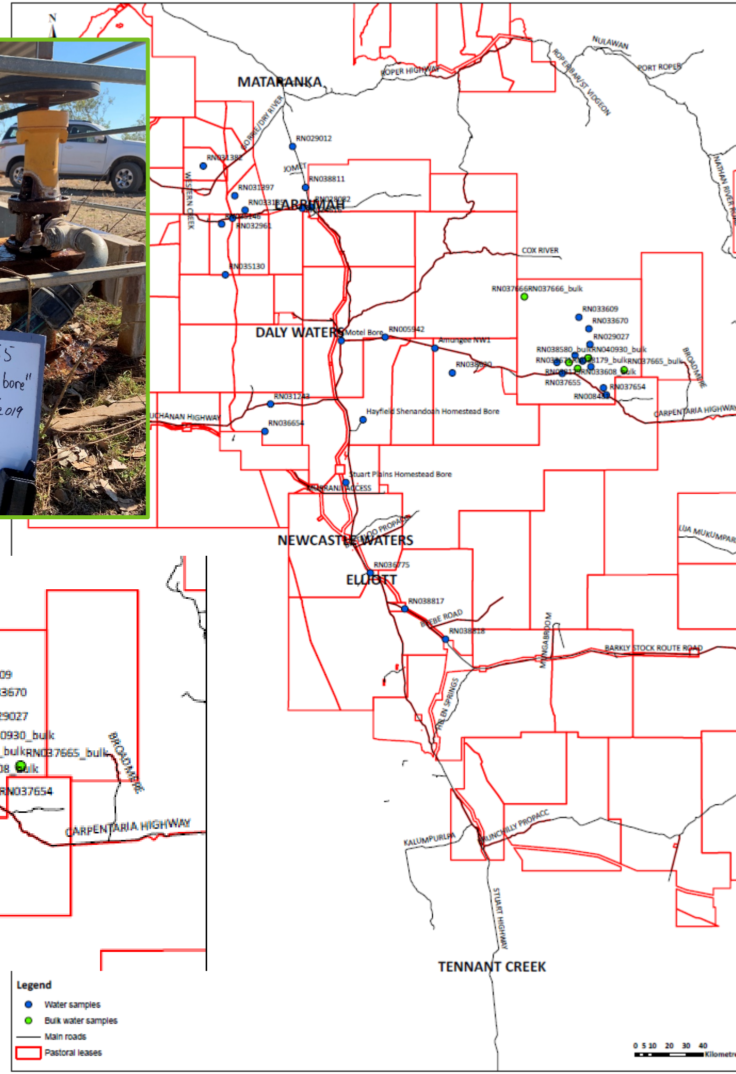
Chemicals	Additive role in onshore gas activities
2-aminoethanol	Viscosity management/ drilling additive
2-butoxyethanol	Surfactant
2-ethylhexanol	Surfactant
benzisothiazolinone	Biocide
bronopol	Biocide
c12 alcohol ethoxylate	Surfactant
diesel	Fuel
diethylene glycol ethyl ether	Solvent
d-limonene	Surfactant
eicosane	Surfactant
Hydrotreated light petroleum distillate	Fuel
ethylene glycol	Viscosity management
glutaraldehyde	Biocide
glyoxal	Viscosity management/ crosslinker
hexahydro-1,3,5-tris(2-hydroxyethyl)-sym-triazine	Biocide
isopropanol	Surfactant
methanol	Surfactant
methylchloroisothiazolinone	Biocide
methylisothiazolinone	Biocide
naphthalene	Corrosion inhibitor
o-cresol	Biocide
polyacrylamide	Friction reducer
polyoxypropylene diamine	Pipework/Epoxy resins/Hardener
pristane	Surfactant
propylene glycol	Viscosity management
triethanolamine	Viscosity management

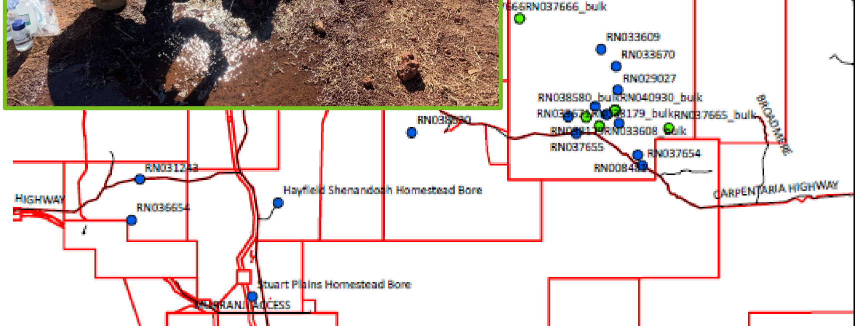


TASK 1
LOGISTICS, PLANNING, BORE & SOIL SELECTION
TASK 2
MATCHED SAMPLING CAMPAIGN
Collect water from 55 bores
(Bores matched to W.16, n=3)
Collect soil from five soil types
tenesols, rudosols, kandosols, vertosols & chromosols (n=10)
TASK 3
~30L of anoxically collected water.
16S rDNA prokaryotic profiling of 55 bores (Total of 165 samples)
16S rDNA & ITS ribosomal DNA Profiling of five major soils (Total of 50 samples)
BASE LINING
Five large samples for each of the major soil types of the basin.
TASK 4
DEGRADATION & MICROBIAL GROWTH TRIALS
Chemical assays or sole carbon growth assays
Use chemical data, bioinformatics & statistics to determine degradation.
TASK 5
16S rDNA & ITS ribosomal DNA profiling of soils & waters exposed to chemicals to idenfy impacts & indicator taxa.
Bioinformatics
IMPACT & INDICATOR TAXA
TASK 6
FINAL REPORT
Use geochemical data from W.16. to synthesis a systems view of the pre-development status of aquifers and soils in the Beetaloo sub-Basin.

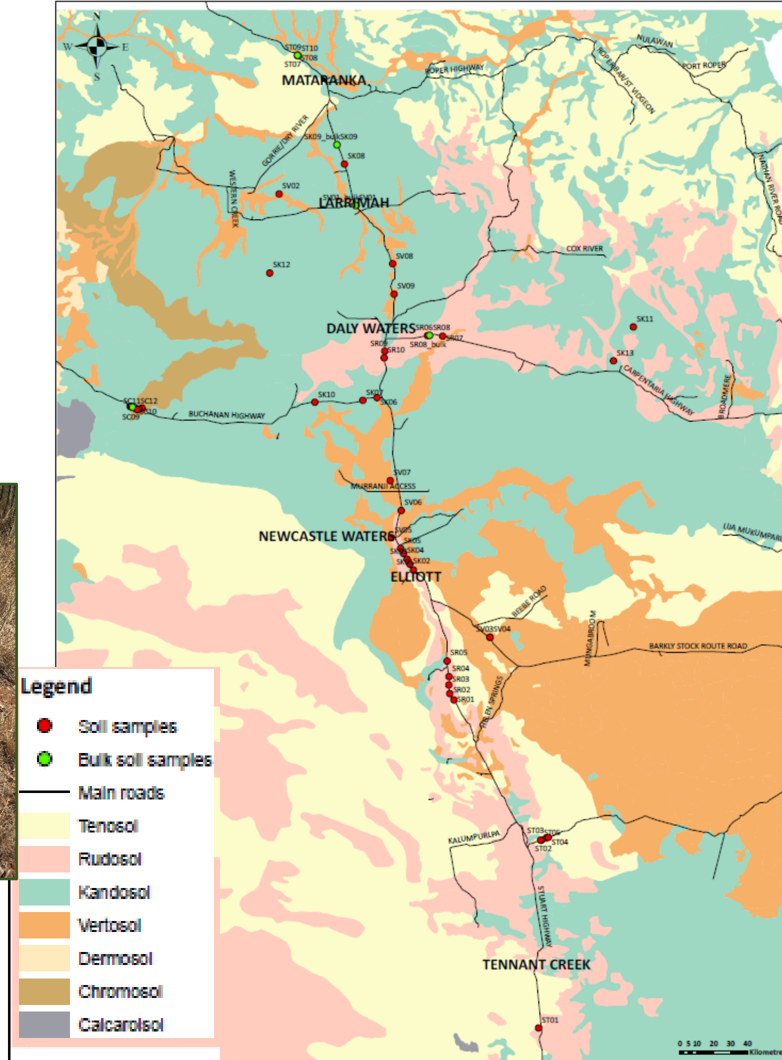
CSIRO

Aquifer sampling





Soil sampling



Methods


TENOSOL
14.792916
132.915328
5/8/2019
2:20pm
RN028082
Vertosol
8/8/19
9:30am
RN005942
8/8/19
5:30pm
Rudosol

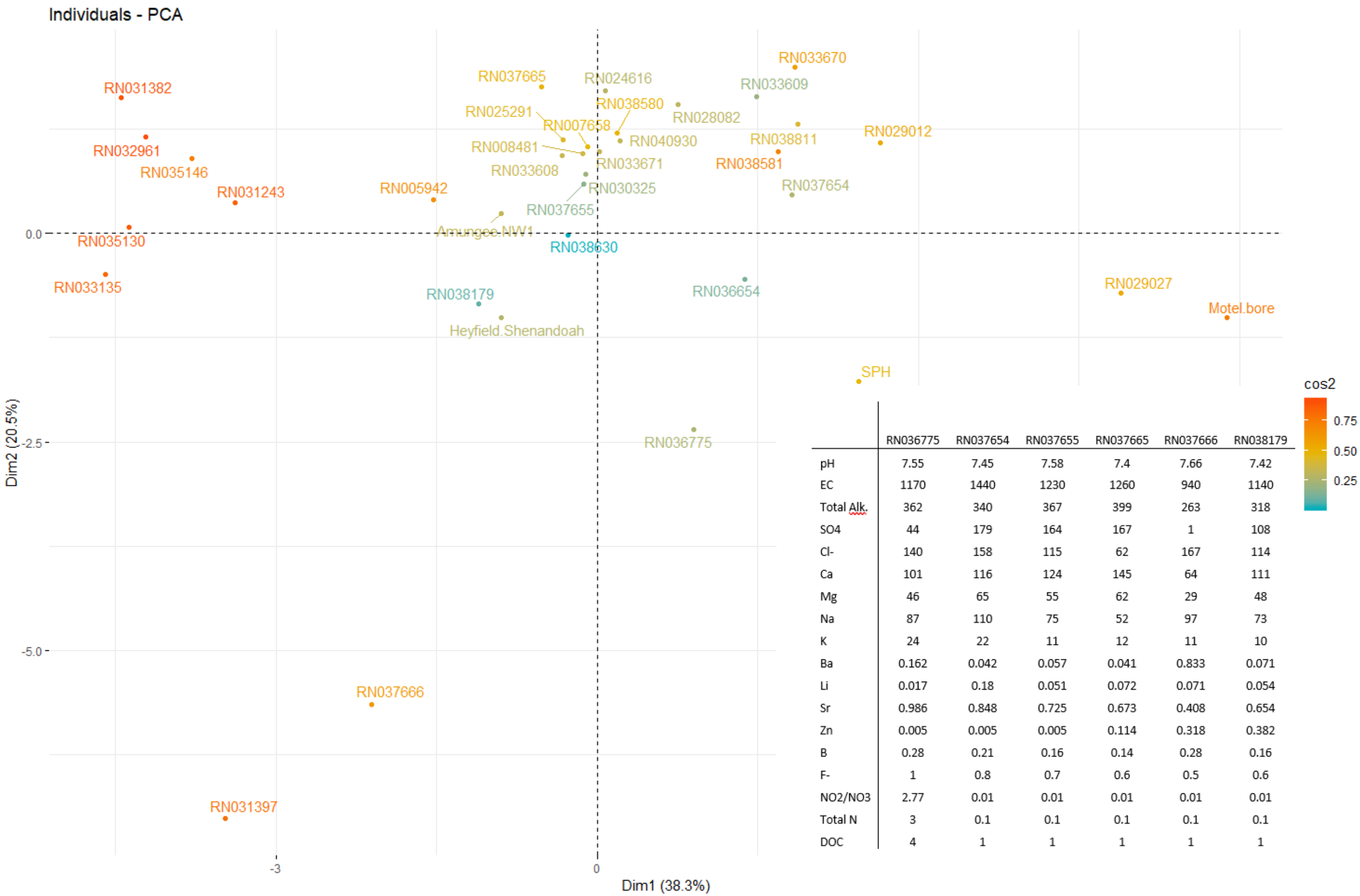
Microcosm Experiments

Results:
Baseline data

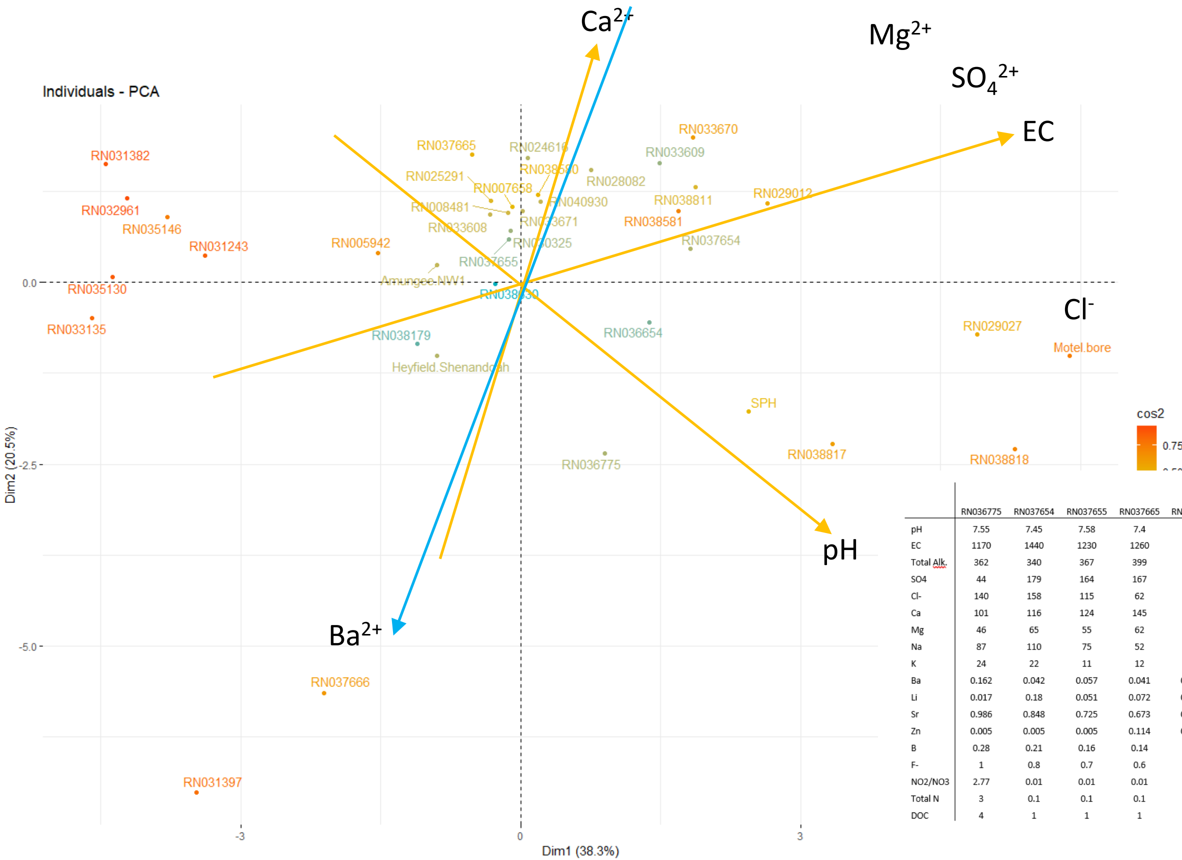
Water chemistry measurements obtained from 38 wells

In broad terms, samples were neutral to moderately alkaline. They varied in their EC (ranging from 650 to just over 2000 mgL^{-1})

	Mean	Std	Min	Max
pH	7.5	0.18	7.21	8.03
EC	1291.0	344.19	650	2140
Total Alk.	368.4	43.08	263	453
SO4	137.7	88.49	1	347
Cl-	137.9	82.58	7	378
Ca	120.8	22.79	44	161
Mg	51.6	13.94	8	72
Na	99.7	64.70	9	302
K	14.6	8.57	1	35
Ba	0.1	0.15	0.018	0.833
Li	0.1	0.04	0.01	0.24
Sr	0.6	0.32	0.074	1.47
Zn	0.0	0.08	0.005	0.382
B	0.2	0.14	0.05	0.79
F-	0.6	0.31	0.2	1.7
NO2/NO3	0.5	0.84	0.01	2.77
Total N	0.6	0.87	0.1	3
DOC	1.9	1.47	1	7



	RN036775	RN037654	RN037655	RN037665	RN037666	RN038179
pH	7.55	7.45	7.58	7.4	7.66	7.42
EC	1170	1440	1230	1260	940	1140
Total Alk.	362	340	367	399	263	318
SO4	44	179	164	167	1	108
Cl-	140	158	115	62	167	114
Ca	101	116	124	145	64	111
Mg	46	65	55	62	29	48
Na	87	110	75	52	97	73
K	24	22	11	12	11	10
Ba	0.162	0.042	0.057	0.041	0.833	0.071
Li	0.017	0.18	0.051	0.072	0.071	0.054
Sr	0.986	0.848	0.725	0.673	0.408	0.654
Zn	0.005	0.005	0.005	0.114	0.318	0.382
B	0.28	0.21	0.16	0.14	0.28	0.16
F-	1	0.8	0.7	0.6	0.5	0.6
NO2/NO3	2.77	0.01	0.01	0.01	0.01	0.01
Total N	3	0.1	0.1	0.1	0.1	0.1
DOC	4	1	1	1	1	1



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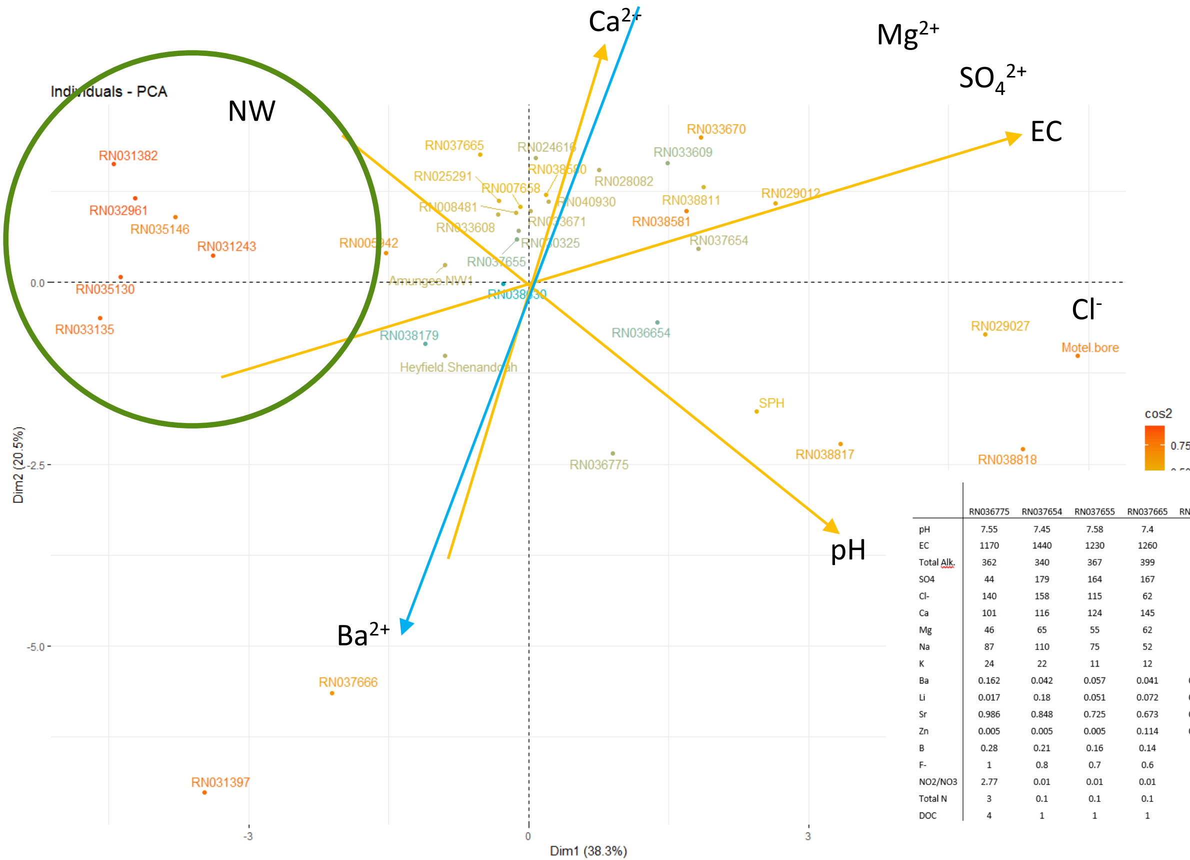
B	0.28	0.21	0.16	0.14	0.28	0.16
F-	1	0.8	0.7	0.6	0.5	0.6
NO2/NO3	2.77	0.01	0.01	0.01	0.01	0.01
Total N	3	0.1	0.1	0.1	0.1	0.1
DOC	4	1	1	1	1	1



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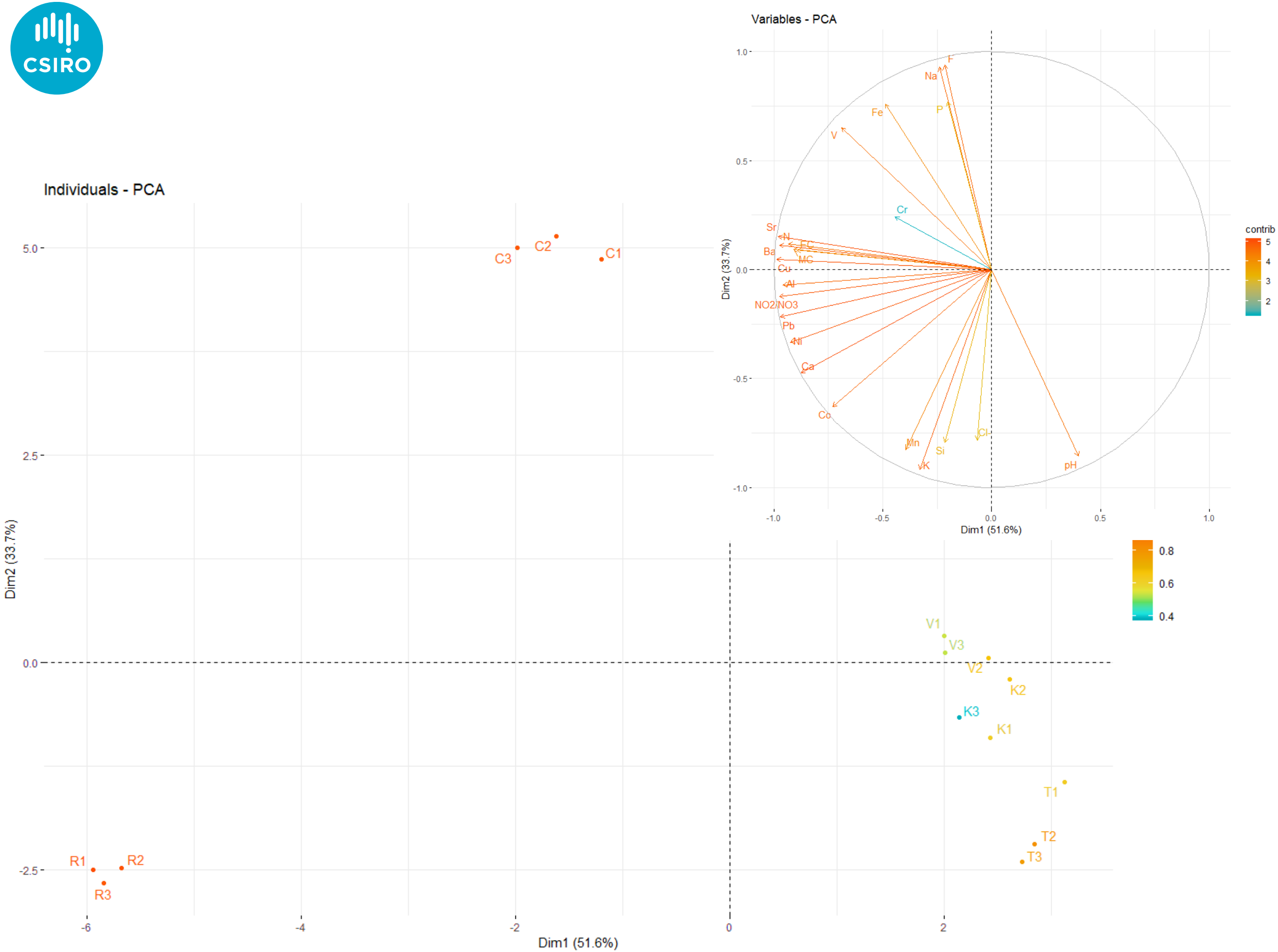
Sr	0.986	0.848	0.725	0.673	0.408	0.654
Zn	0.005	0.005	0.005	0.114	0.318	0.382
B	0.28	0.21	0.16	0.14	0.28	0.16
F-	1	0.8	0.7	0.6	0.5	0.6
NO2/NO3	2.77	0.01	0.01	0.01	0.01	0.01
Total N	3	0.1	0.1	0.1	0.1	0.1
DOC	4	1	1	1	1	1

Soil chemistry measurements obtained from the bulk samples of chromosol, kandosol, rudosol, tenosol & vertosol.

In broad terms, soils varied from acidic (pH 5) to neutral (pH 7), the main drivers of variation are in metal (Al, Fe) content (tensol much lower than other soils).

	Mean	Std	Min	Max
pH	6.52	0.60	5.10	7.00
EC	37.80	15.42	18.00	72.00
Moisture	1.29	0.42	1.00	2.40
Si	827.47	215.55	505.00	1110.00
Cl	160.00	50.71	100.00	200.00
Ca	12.00	4.14	10.00	20.00
Na	12.00	4.14	10.00	20.00
K	25.33	10.60	10.00	40.00
Al	5322.00	2320.49	2490.00	10300.00
Ba	64.67	51.81	20.00	150.00
Cr	51.60	22.80	8.00	82.00
Co	6.20	5.21	2.00	16.00
Cu	7.60	3.56	5.00	14.00
Fe	30686.00	16280.77	5940.00	57000.00
Pb	8.93	4.89	5.00	18.00
Mn	265.00	200.44	20.00	553.00
Ni	3.13	1.55	2.00	6.00
Sr	9.67	9.75	2.00	25.00
Va	79.53	39.25	16.00	134.00
U	0.69	0.48	0.10	1.40
F	68.00	52.40	40.00	180.00
NO2/NO3	8.33	7.09	1.80	21.10
Total N	364.00	224.17	120.00	720.00
Total P	182.67	107.56	96.00	409.00



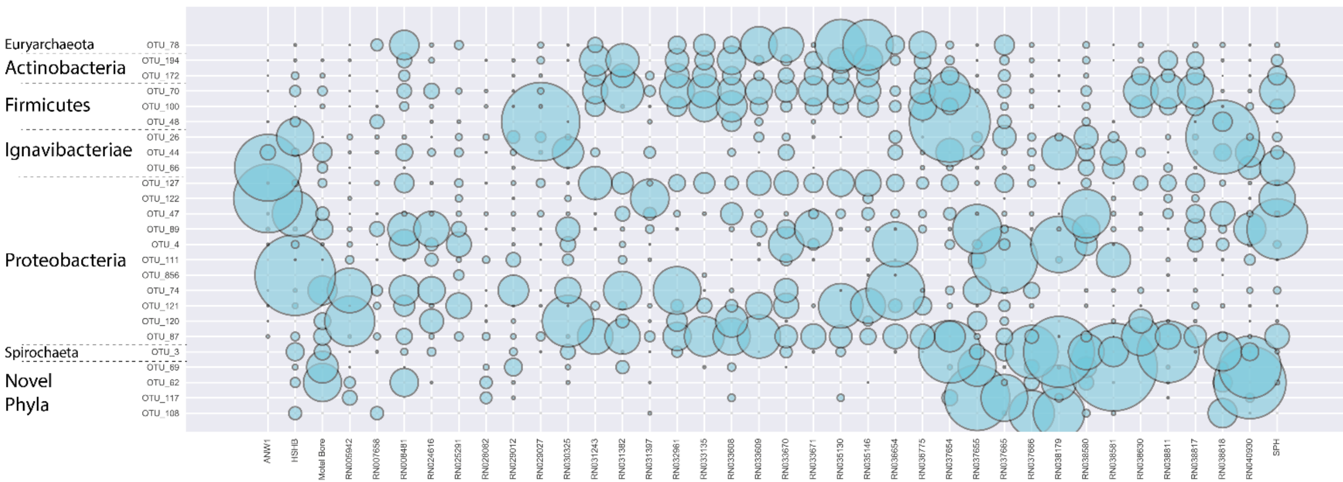
Variables - PCA
Individuals - PCA
Dim1 (51.6%)
Dim2 (33.7%)
contrib
F
Na
P
Fe
V
Cr
Sr
N
Ba
EC
MC
Cu
Al
NO2/NO3
Pb
Ni
Ca
Co
Mn
K
Si
Cl
pH
C1
C2
C3
R1
R2
R3
V1
V2
V3
K1
K2
K3
T1
T2
T3

Most comprehensive survey of aquifer microbiomes for the NT to date.

1467 OTUs detected across the 38 aquifer samples
- **31 different phyla detected**
- **334 OTUs from unknown phyla**

DNA yields poor. Low cell number in aquifers.

Diversity
319 ± 15.9 OTUs per aquifer sample
Max: 503 OTUs (RN029027)
Min: 157 OTUs (RN031243)



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Summary stats of the soil microbiomes

1251 OTUs detected across the soil samples

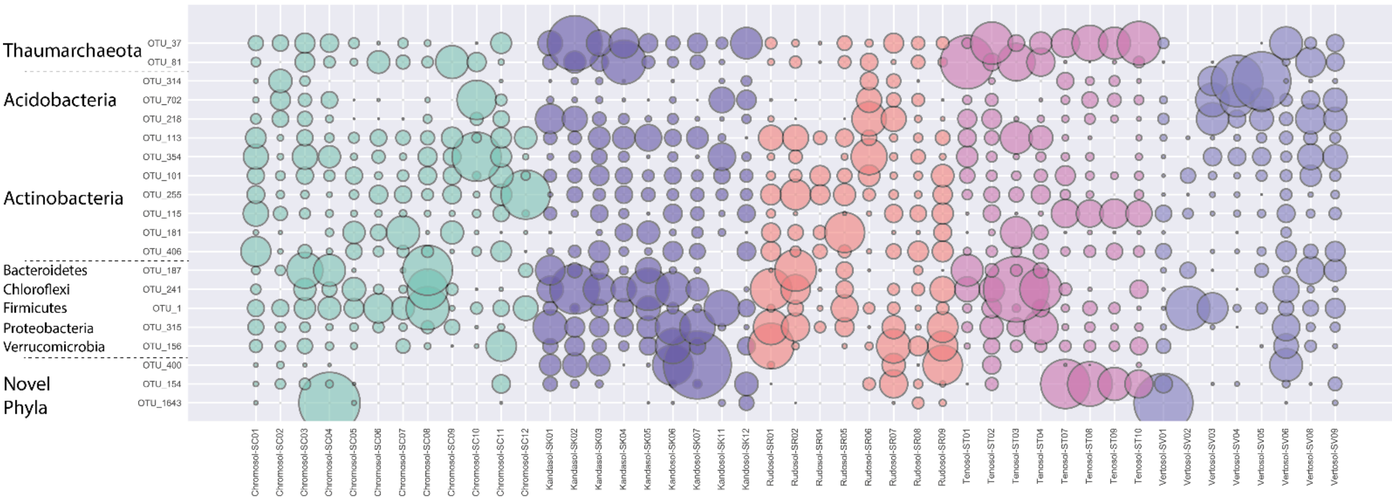
- **22 different phyla detected**
- **191 OTUs from unknown phyla**

Diversity

477 ± 20.9 OTUs per soil sample

Max: 713 OTUs (Vertosol-SV06)

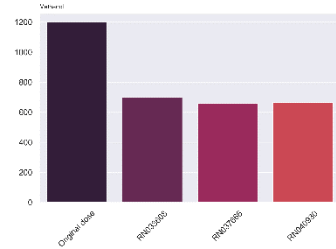
Min: 79 OTUs (Vertosol-SV02)



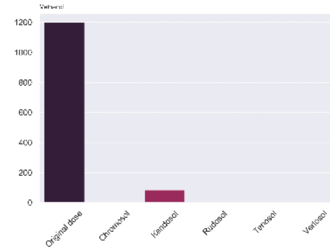
Effect of chemicals of potential concern (COPC)

Aquifers have slower rates of degradation than soils for tested chemicals.

Methanol



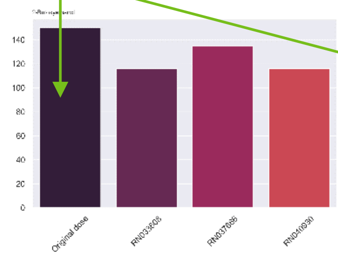
3 months



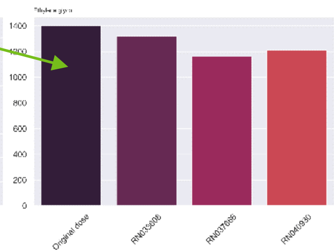
1 month

Original dose

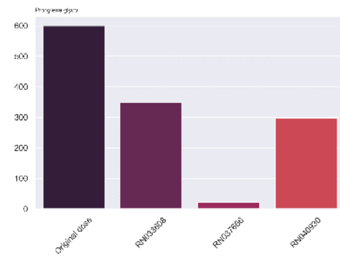
Butoxyethanol



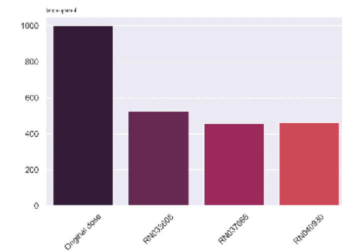
Ethylene glycol

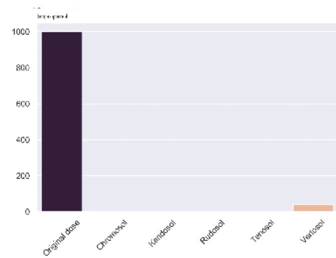


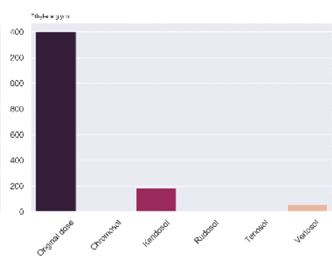
Propylene glycol

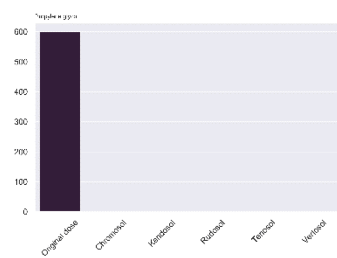


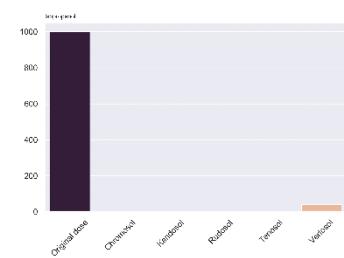
Isopropanol





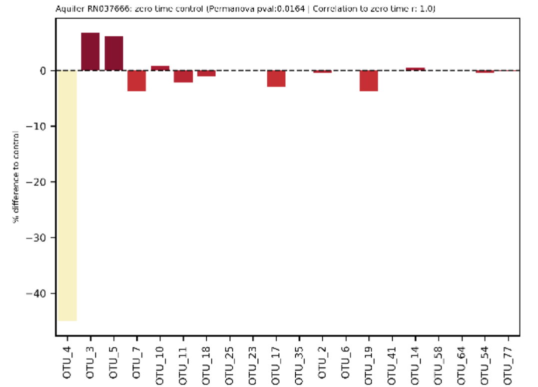






Aquifers - COPC

	RN033608		RN037666		RN040930	
	pval	r	pval	r	pval	r
2-aminoethanol	0.0021	0.902	0.0083	0.856	0.0145	0.772
C12 ethoxylated alcohol	ND	0.054	0.0025	0.796	0.0022	0.596
2-butoxyethanol	0.0287	-0.102	0.0398	0.635	0.0224	0.608
bronopol	0.0167	0.849	0.008	0.808	0.002	0.77
benzisothiazolinone	0.0018	-0.796	0.0017	0.762	0.0035	0.664
diethylene glycol ethyl ether	ND	0.265	0.0006	0.841	0.0007	0.576
d-limonene	0.0037	-0.63	0.0085	0.902	0.0057	0.328
diesel	0.0075	-0.705	0.0021	0.807	0.0027	0.388
eicosane (linear-C20)	0.0063	-0.351	0.0098	0.852	0.0027	0.331
ethylene glycol	0.0085	0.127	0.0008	0.836	0.0039	0.487
2-ethylhexanol	0.0126	-0.265	ND	0.696	0.0022	0.58
glutaraldehyde	0.0202	0.763	0.001	0.905	0.0057	0.641
glyoxal	0.0077	0.894	0.0069	0.96	0.0108	0.604
hydrotreated light petroleum distillate	ND	0.089	ND	-0.139	0.0018	0.354
hexahydro-1,3,5-tris(2-hydroxyethyl)-sym-triazine	0.0052	0.829	0.0097	0.892	0.0026	0.74
isopropanol	ND	-0.376	0.0275	0.885	0.0024	0.616
methylchloroisothiazolinone	ND	-0.196	0.004	0.858	0.0136	0.377
methylisothiazolinone	0.0071	0.716	0.0076	0.777	0.0037	0.654
methanol	ND	0.65	0.0081	0.732	0.0048	0.564
naphthalene	0.0194	0.293	0.0066	0.874	0.0022	0.61
2-methylphenol (o-cresol)	0.0081	0.273	0.0011	0.875	0.0049	0.591
polyacrylamide	ND	0.124	0.0017	0.944	0.0008	0.369
polyoxypropylenediamine	ND	0.66	0.0078	0.932	0.0007	0.279
propylene glycol	ND	0.799	0.0186	0.868	0.009	0.494
pristane (C15)	0.0353	0.514	0.0091	0.788	0.0023	0.466
triethanolamine	0.0088	0.619	0.0032	0.806	0.0023	0.562



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diesel	0.0075	-0.705	0.0021	0.807	0.0027	0.388
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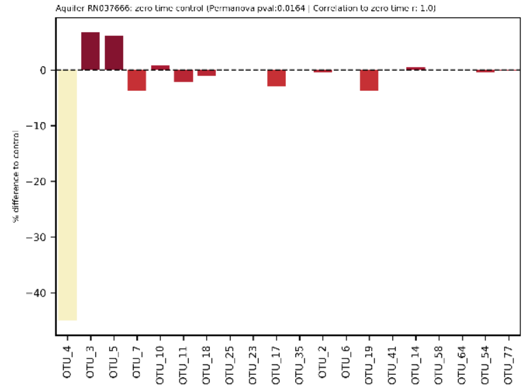
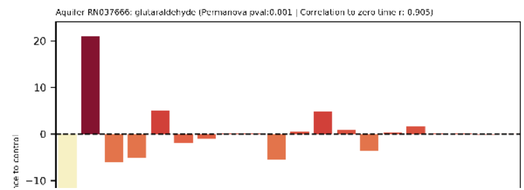
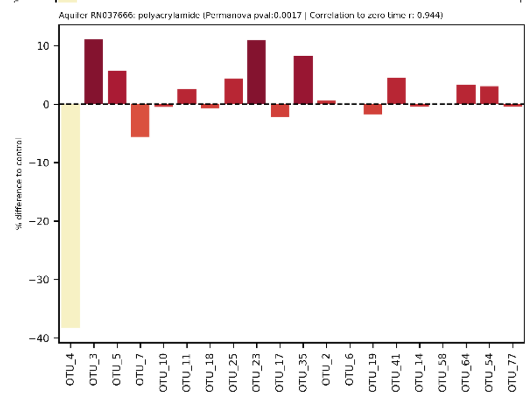
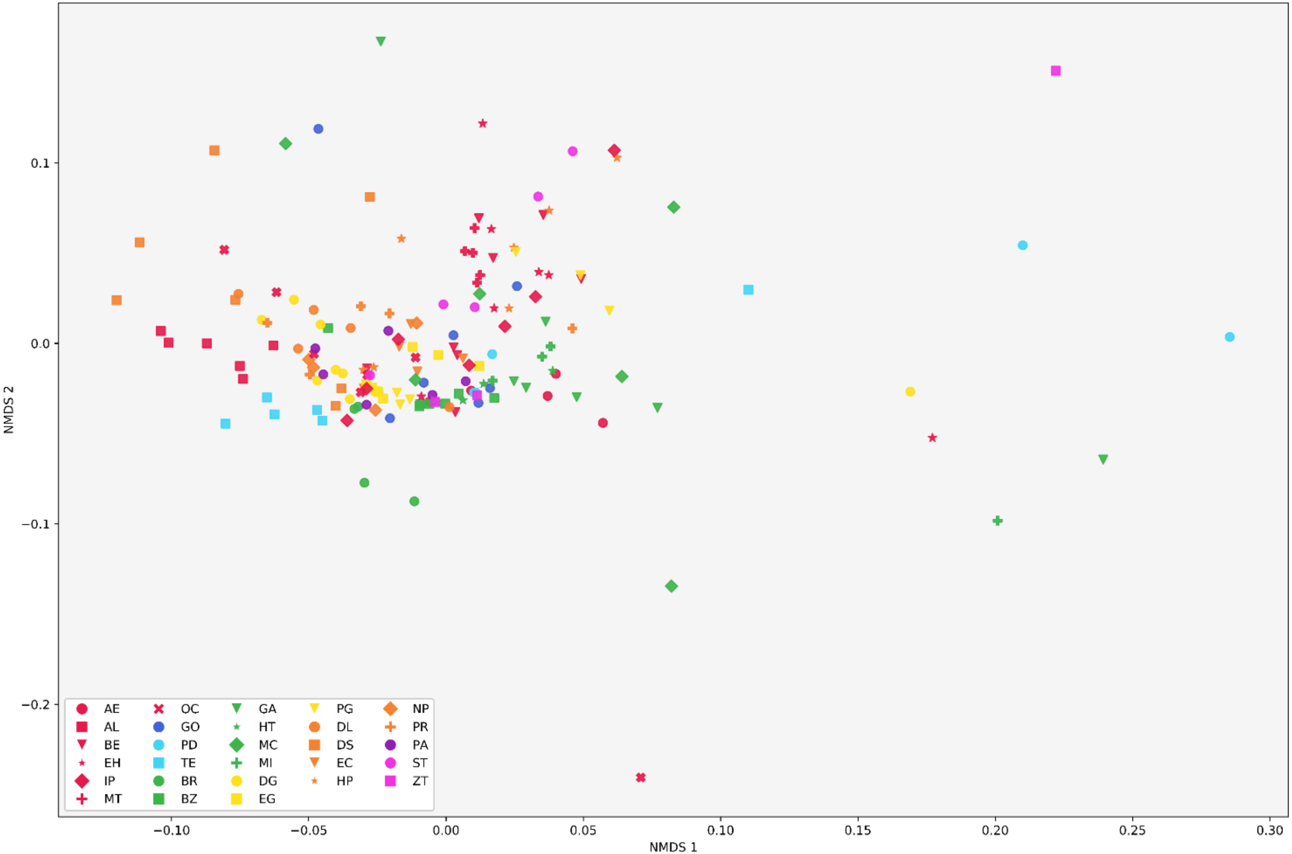
methanol	ND	0.65	0.0081	0.732	0.0048	0.564
naphthalene	0.0194	0.293	0.0066	0.874	0.0022	0.61
2-methylphenol (o-cresol)	0.0081	0.273	0.0011	0.875	0.0049	0.591
polyacrylamide	ND	0.124	0.0017	0.944	0.0008	0.369
polyoxypropylenediamine	ND	0.66	0.0078	0.932	0.0007	0.279
propylene glycol	ND	0.799	0.0186	0.868	0.009	0.494
pristane (C15)	0.0353	0.514	0.0091	0.788	0.0023	0.466
triethanolamine	0.0088	0.619	0.0032	0.806	0.0023	0.562



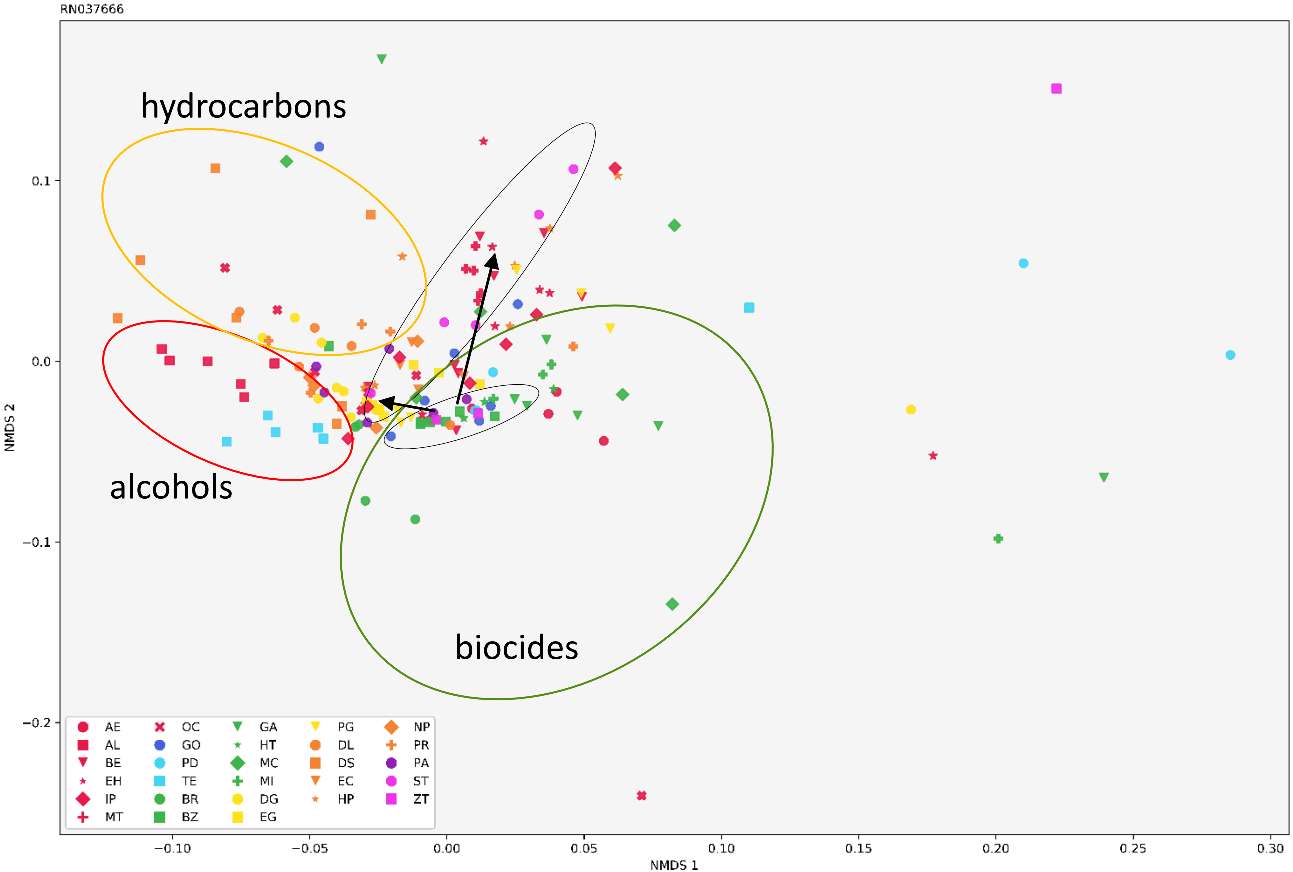




RN037666



NMDS 2
NMDS 1
0.1
0.0
−0.1
−0.2
−0.10
−0.05
0.00
0.05
0.10
0.15
0.20
0.25
0.30
AE
AL
BE
EH
IP
MT
OC
GO
PD
TE
BR
BZ
GA
HT
MC
MI
DG
EG
PG
DL
DS
EC
HP
NP
PR
PA
ST
ZT



RN037666
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alcohols
biocides
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0.05
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AE
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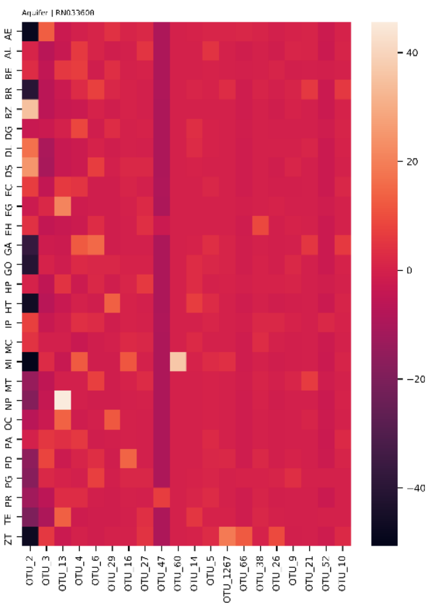
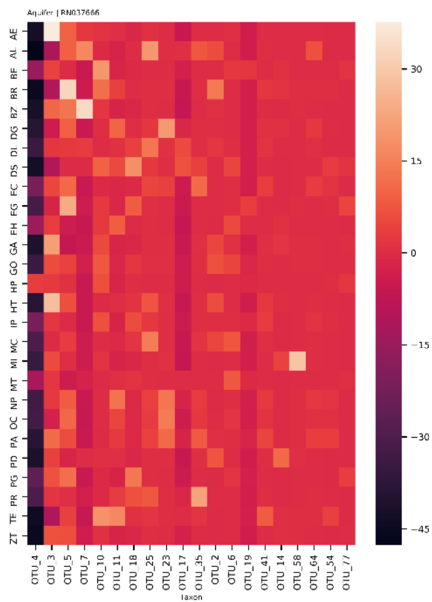
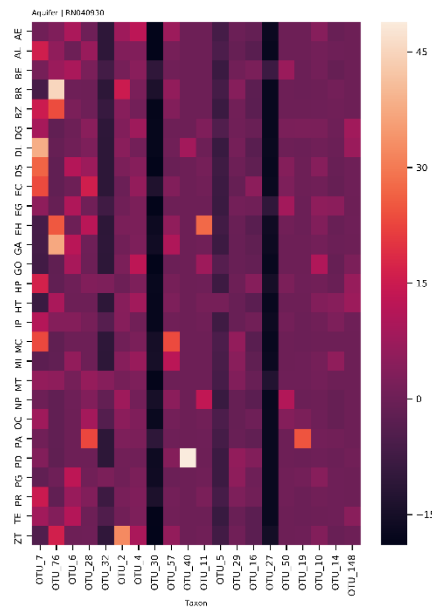
Aquifers - COPC

COPC

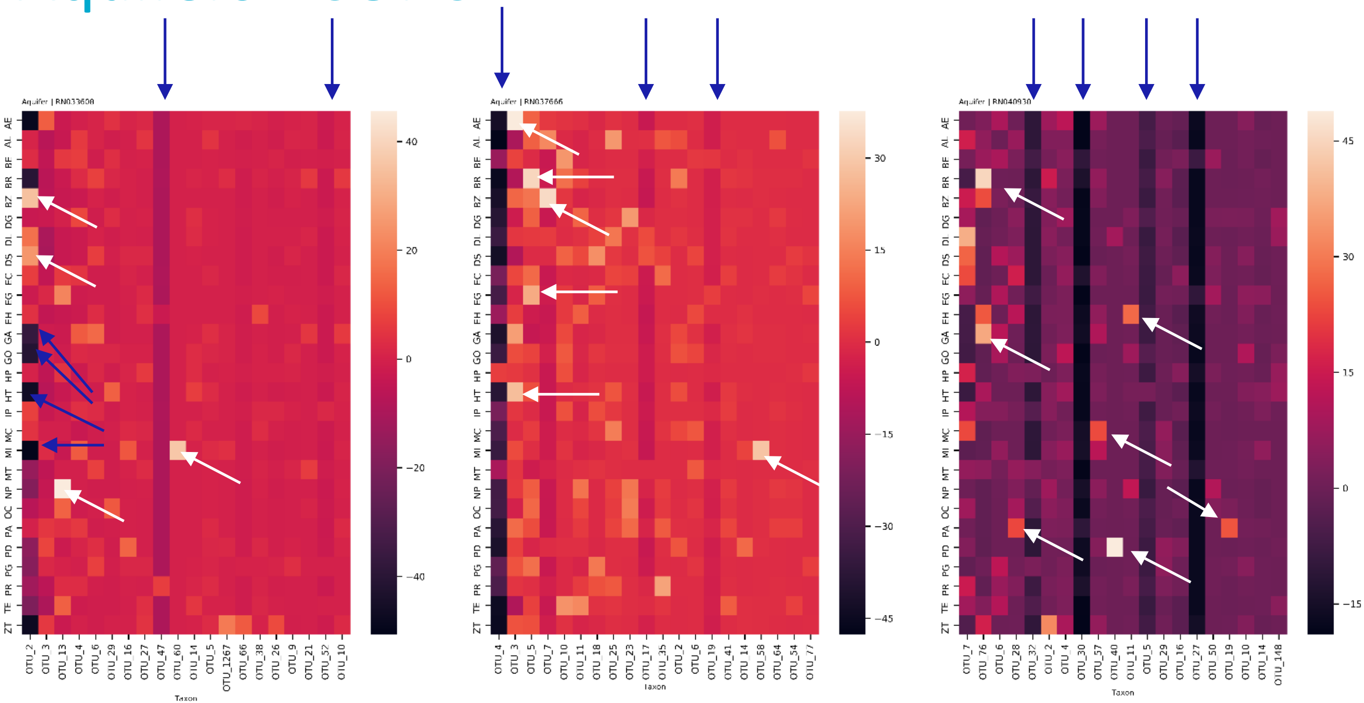






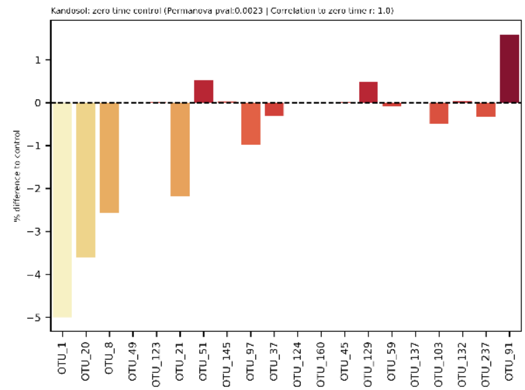
‘species’

Aquifers - COPC



Soils - COPC

	Chromosol		Kandosol		Vertosol		Tenosol	
	pval	r	pval	r	pval	r	pval	r
2-aminoethanol	0.0005	-0.316	0.0016	0.638	0.0058	0.27	0.0005	-0.005
C12 ethoxylated alcohol	0.0261	0.612	0.008	0.268	0.0082	0.305	0.0005	0.194
2-butoxyethanol	0.0002	-0.605	0.002	-0.799	0.0002	0.595		
bronopol	0.0007	0.956	0.0032	0.94	0.0004	0.952	0.0073	0.311
benzisothiazolinone	0.0013	0.949	0.0026	0.762	0.0004	0.406	0.0112	0.315
diethylene glycol lethyl ether	ND	0.733	0.0031	-0.047	ND	0.647	0.045	-0.689
d-limonene	0.048	0.585	0.0148	-0.489	0.0411	0.324		
diesel	0.0002	0.634	0.0012	-0.32	0.0006	0.388		
eicosane (linear-C20)	0.0074	0.848	ND	-0.494	0.0011	0.581	0.0046	-0.341
ethylene glycol	0.0003	0.818	0.0019	-0.575	0.0011	0.182		
2-ethylhexanol	0.0025	-0.967	0.0025	-0.199	0.0013	0.39		
glutaraldehyde	0.0003	-0.816	0.0012	0.536	0.0009	0.15	0.0015	-0.173
glyoxal	0.0003	0.605	0.0089	-0.381	0.0006	0.821	0.0009	0.431
hydrotreated light petroleum distillate	0.0191	0.43	0.0066	-0.156	0.0414	0.425	0.0285	-0.376
hexahydro-1,3,5-tris(2-hydroxyethyl)-sym-triazine	0.0008	0.948	0.0015	0.398	0.0014	0.489	0.0013	0.192
isopropanol	0.0141	0.829	0.0155	-0.602	0.0005	0.027		
methylchloroisothiazolinone	ND	0.584	0.008	0.882	0.0075	0.626	ND	0.374
methylisothiazolinone	ND	0.965	0.0071	0.841	0.0063	0.819	0.0071	0.421
methanol	0.0146	0.854	0.0027	0.268	0.0014	0.158		
naphthalene	ND	-0.836	0.0019	-0.666	0.0022	0.196		
2-methylphenol (o-cresol)	ND	-0.757	0.0025	-0.693	0.0006	0.435		
polyacrylamide	ND	-0.672	0.0131	-0.594	0.0003	0.546		
polyoxypropylenediamine	ND	-0.192	0.0017	0.346	0.0012	0.748	0.0097	-0.388
propylene glycol	0.0009	0.547	0.0025	-0.579	0.001	0.748		
pristane (C15)	0.0001	-0.088	0.0111	0.116	0.0002	0.662	0.0007	0.088
triethanolamine	0.0437	0.458	0.0081	0.706	0.0005	0.314	0.0024	-0.467



Soils - COPC

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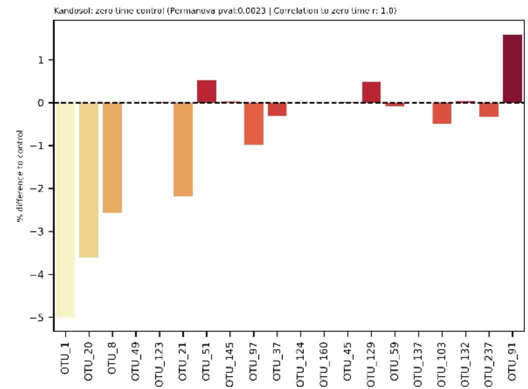

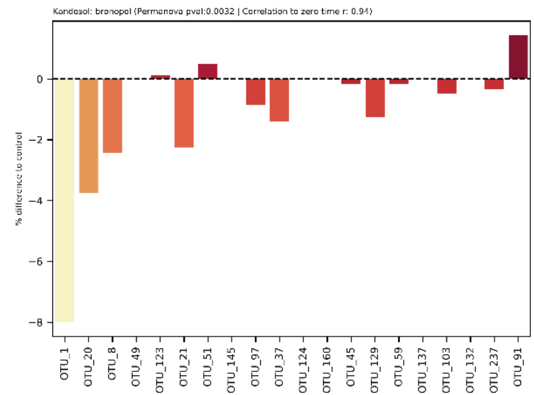

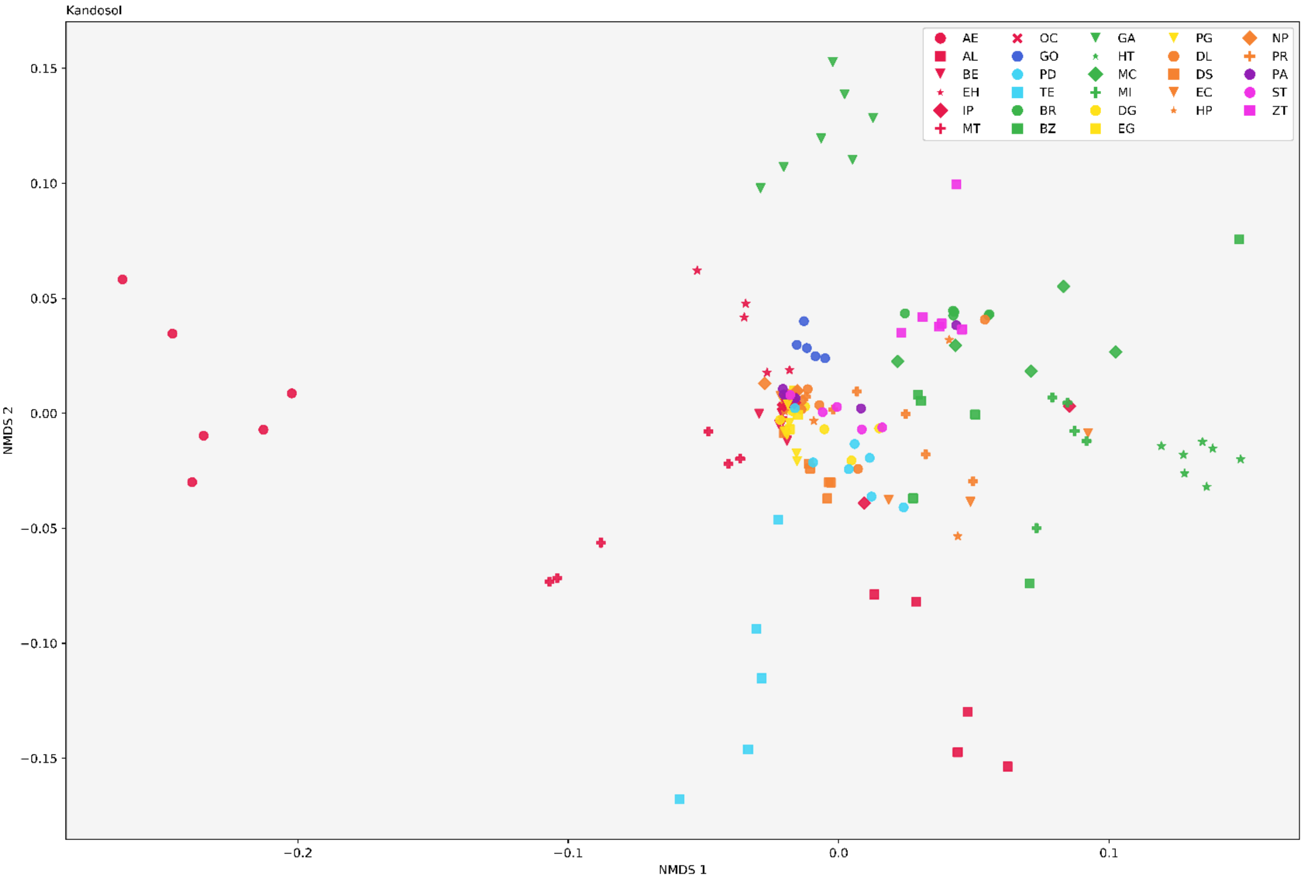

Kandosol
AE
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0.10
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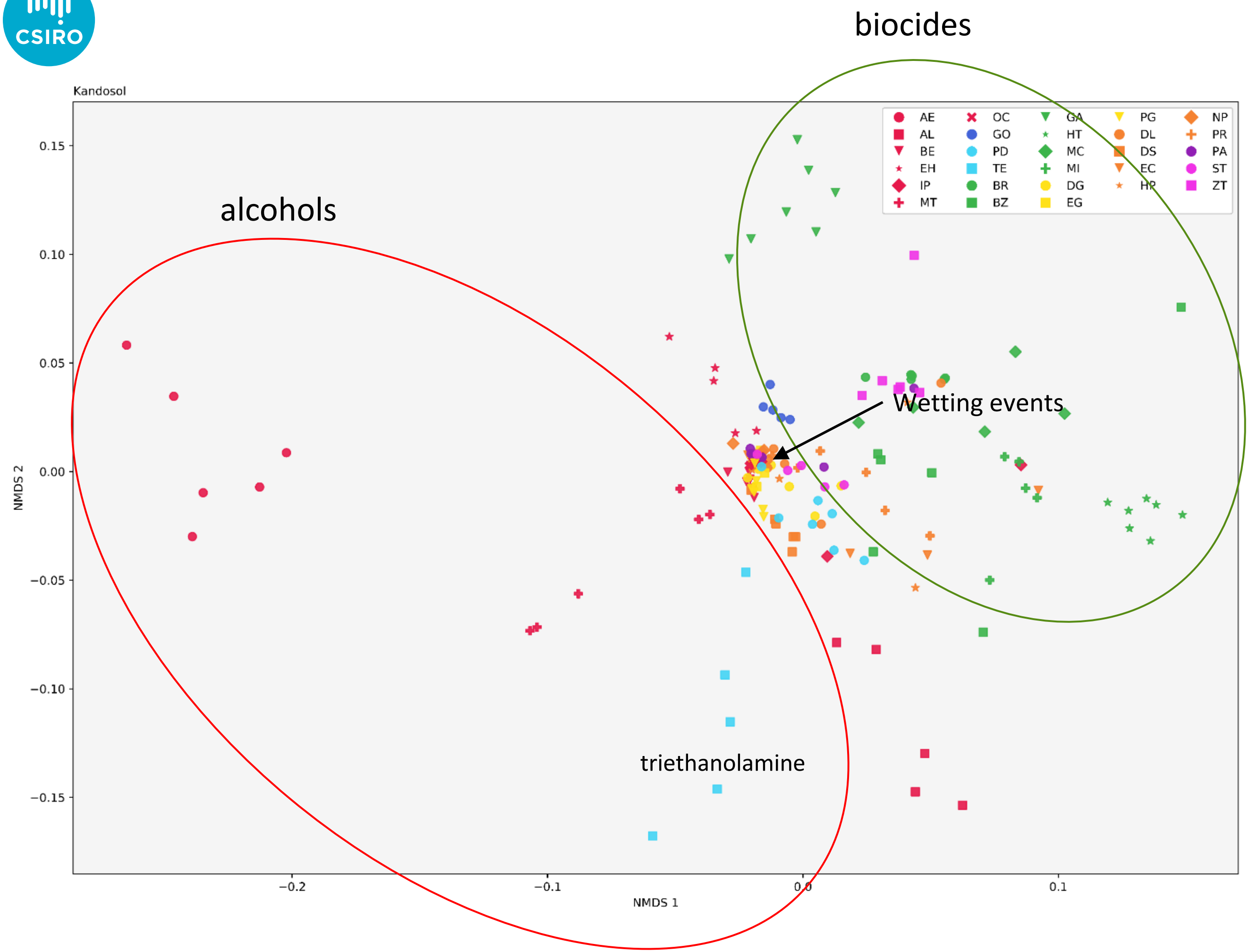
0.0
0.1
NMDS 1
NMDS 2



biocides
alcohols
Kandosol
Wetting events
triethanolamine
NMDS 1
NMDS 2
AE
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BZ
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MC
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DG
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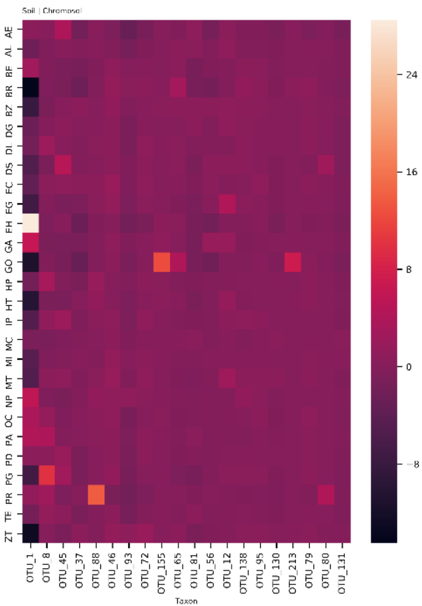
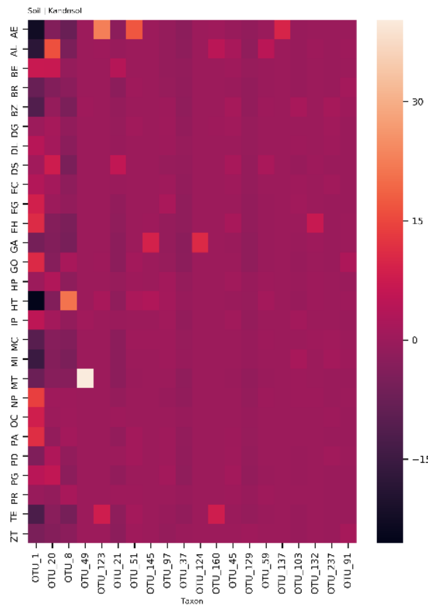
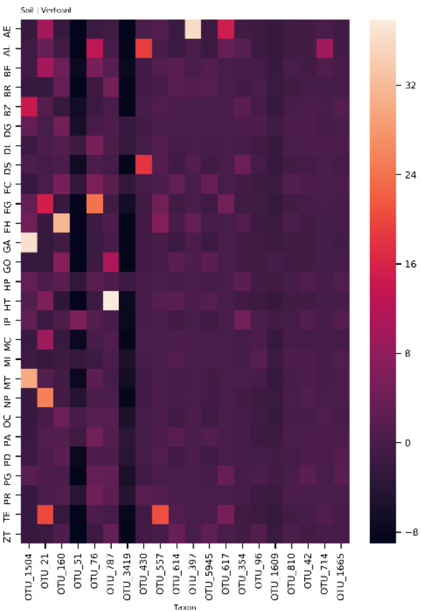
Soils - COPC

COPC

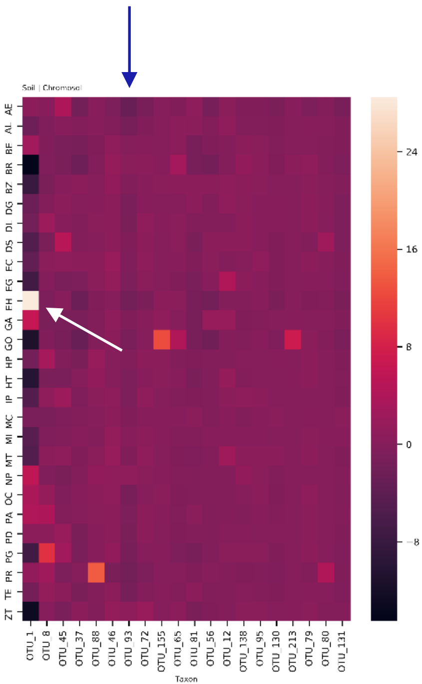




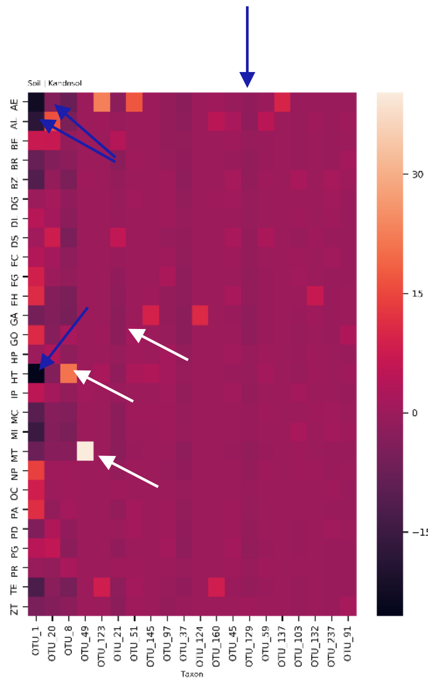


‘species’

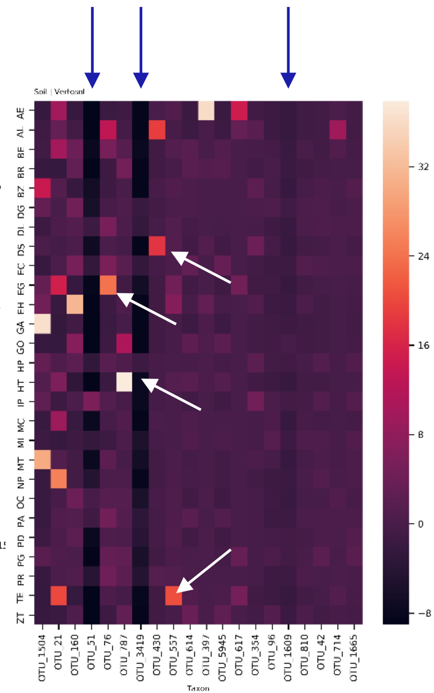
Soils - COPC



Soil | Chromosol
AE AL BF BR BZ DG DL DS FC FG FH GA GO HP HT IP MC MI MT NP OC PA PD PG PR TE ZT
OTU_1 OTU_8 OTU_45 OTU_37 OTU_88 OTU_46 OTU_93 OTU_72 OTU_155 OTU_65 OTU_81 OTU_56 OTU_12 OTU_138 OTU_95 OTU_130 OTU_213 OTU_79 OTU_80 OTU_131
Taxon
24
16
8
0
−8



Soil | Kandosol
AE AL BF BR BZ DG DL DS FC FG FH GA GO HP HT IP MC MI MT NP OC PA PD PG PR TE ZT
OTU_1 OTU_20 OTU_8 OTU_49 OTU_173 OTU_21 OTU_51 OTU_145 OTU_97 OTU_37 OTU_124 OTU_160 OTU_45 OTU_129 OTU_59 OTU_137 OTU_103 OTU_132 OTU_237 OTU_91
Taxon
30
15
0



Soil | Vertosol
AE AL BF BR BZ DG DL DS FC FG FH GA GO HP HT IP MC MI MT NP OC PA PD PG PR TE ZT
OTU_1504 OTU_21 OTU_160 OTU_51 OTU_76 OTU_787 OTU_3419 OTU_430 OTU_557 OTU_614 OTU_397 OTU_5945 OTU_617 OTU_354 OTU_96 OTU_1609 OTU_810 OTU_42 OTU_714 OTU_1665
Taxon
32
24
16
8
0
−8

Conclusions & Recommendations

Baselines

Aquifers

- Aquifer communities dominated by autotrophs
- Most extensive aquifer study to date in the Northern Territory (38 wells)
- Mean richness >300 OTUs per well.

Soils

- UV tolerant organisms detected
- Soil type did not predict microbial diversity
- Generally more diverse >475 OTUs per sample.
- Fungal diversity high and unusual (data not shown)

Chemicals of potential concern

- Microbial degradation offers an addition defence against some chemical contamination (in the unlikely event of a spill/leak)
- Biocides impact on soils & aquifers and effects are similar – maybe bacteriostatic. Various alcohols impacted soils and aquifers.

Aquifers

- Some chemicals degraded within 3 months, others did not.
- Aquifer microbiomes response was not consistent between replicates - cell numbers are low ~10^4/mL.
- Identified indicator taxa and potential catabolists.

Soils

- Those COPC tested readily degraded in soil in 1 month
- Some increasing taxa are associated with degradation of these chemicals.
- Identified numerous potential indicator taxa that could be used for biomonitoring.
- Soil wetting impacts microbial diversity markedly.

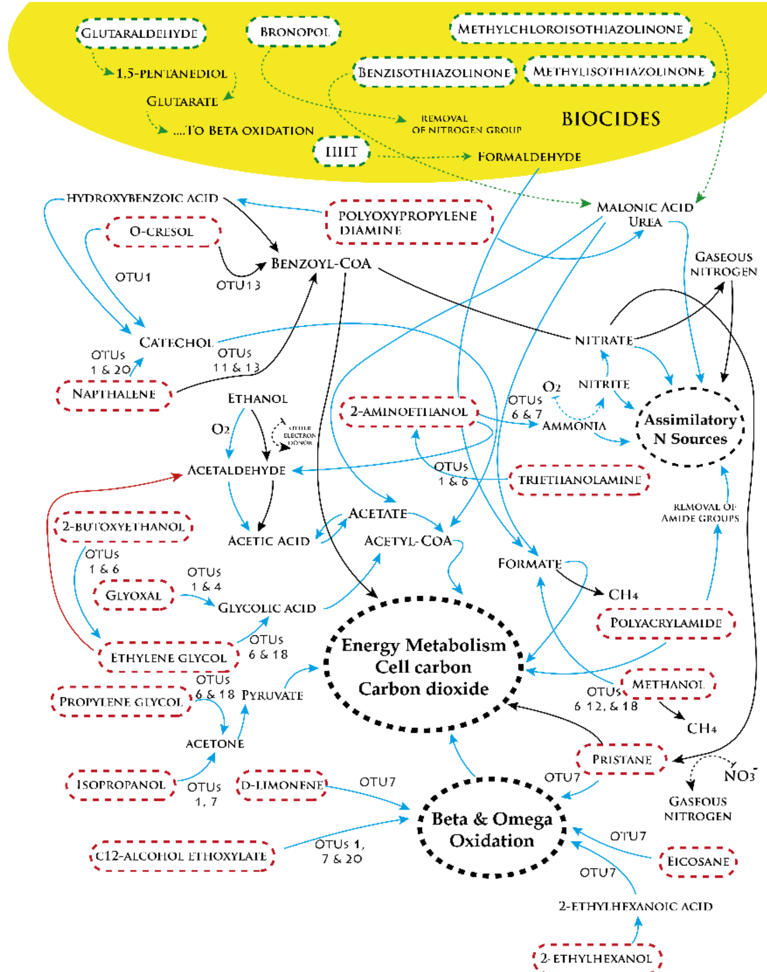
Conceptual model

Numerous OTUs were identified that have close relatives that can degrade chemicals examined.

Conceptual was developed noting these taxa.

Microbial degradation of biocides is unclear from this study.

Biocides prevent the normal shift in soils of the Beetaloo Basin region that occurs during wetting events (e.g. rain or flooding).



Recommendations

Baseline data represents an important first step, however, future surveys to determine the biodiversity and stability of these communities are advised.

Indicator taxa were identified and may be useful for biomonitoring of these aquifers. However, further baseline profiling is suggested to determine any seasonal, temporal changes.

Chemicals examined here were used at industrially relevant concentrations. It is noteworthy that chemicals were tested individually and not in combination. Combinations may change the rates of biodegradation and impacts on microbial communities.

Thank you

CSIRO ENERGY
Dr. David J. Midgley
Biogeochemistry & Microbiology Team Lead

+61 2 94137862
david.midgley@csiro.au
csiro.au/energy