



Università di Foggia
Scienze Agrarie, Alimenti,
Risorse Naturali e Ingegneria



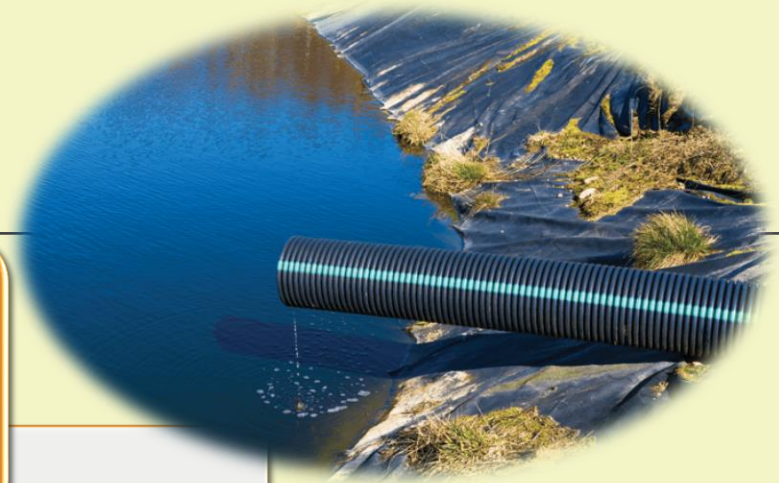
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Microbial and chemical monitoring integrated approach applied to a potential landfill leachate contamination of groundwater

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Background

Groundwater contamination by landfill leachate in agricultural and urban areas



Other routes of contamination in anthropized areas



Integrated approach to establish pollution causes



Aim of the research work

Physico-chemical and isotope



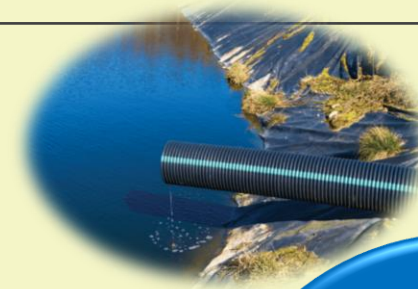
Microbial (cultivation and molecular)



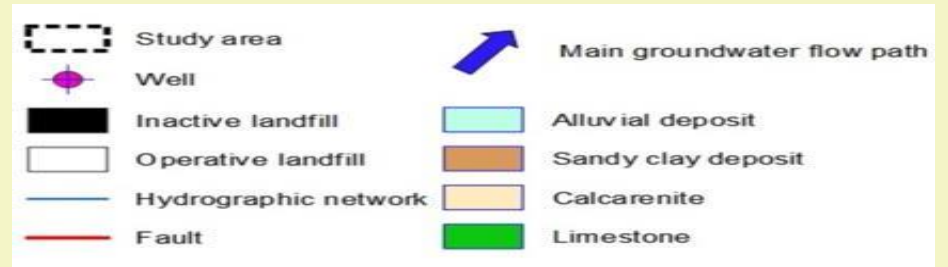
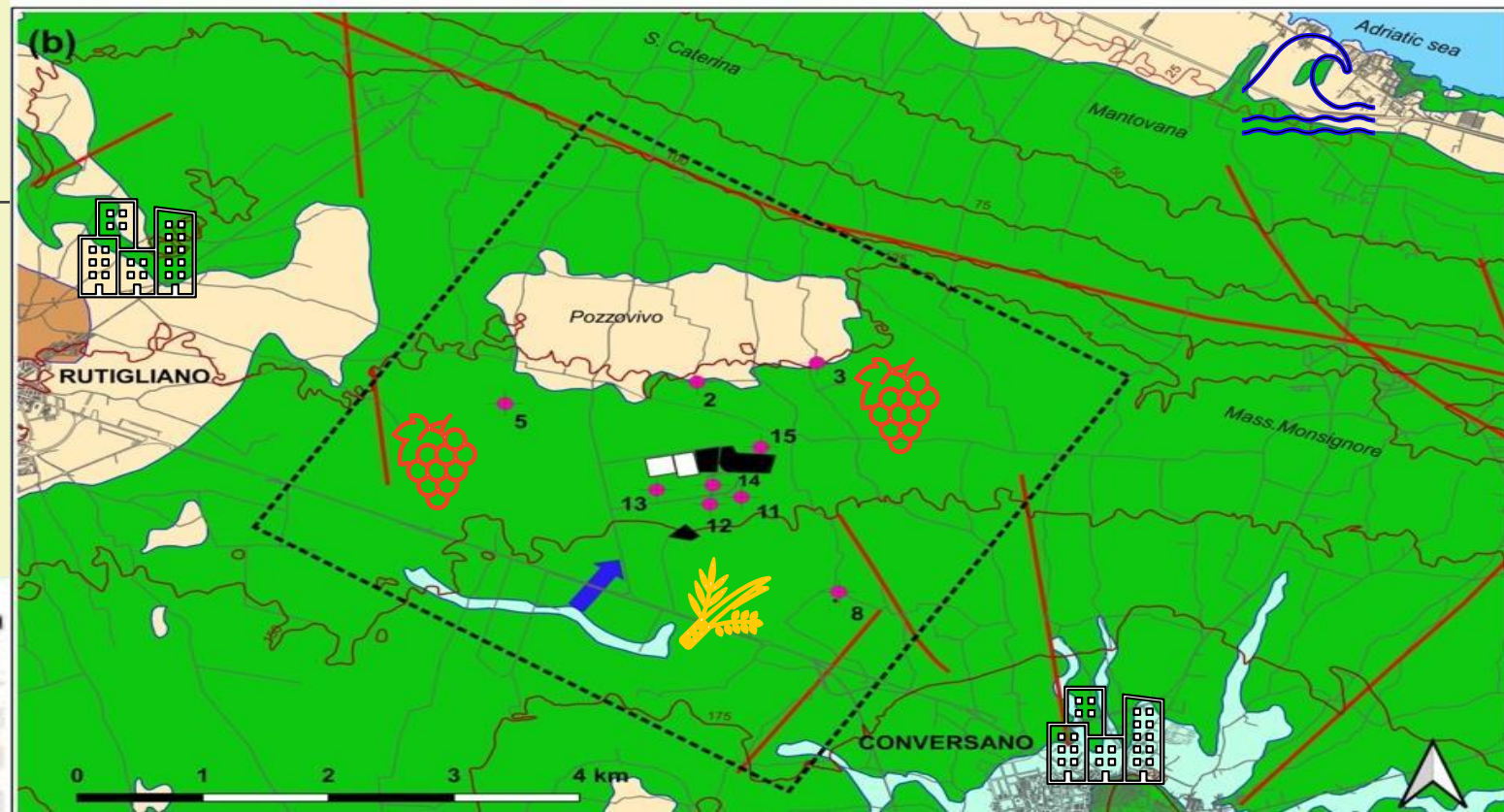
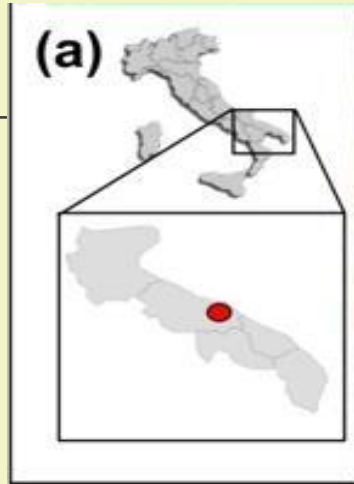
Characterize groundwater environment



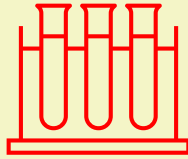
Confirm pollution source hypothesis



Landfill district – sampling site



methods



Physical and
Chemical
analyses



Isotopic
analyses

C,H, N-NO₃



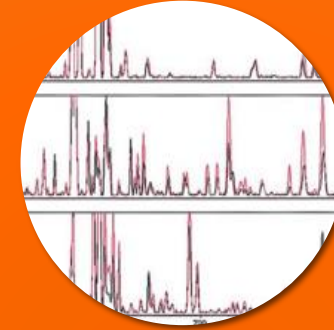
Microbial
Plate count

TMC
E.coli



Microbial PCR

Fecal
Nitrogen cycle



Microbial
fingerprint
(PCR-ARISA)

Bacterial
diversity



Groundwater main features

On-site measurements

Sampling well	Elevation m (asl)	Depth m	EC mS/cm	T (°C)	pH	DO mg/L	Eh (mV)	TDS (mg/L)
2	130	260	1.477	16.68	7.58	5.45	345	<u>1081</u>
3	125	277	1.019	16.60	7.54	5.83	291	595
5	133	289	0.888	16.56	7.51	5.59	345	501
8	162	452	0.869	16.83	7.46	4.76	346	527
11	146,7	198	0.952	16.45	7.34	5.61	135	812
12	144,35	250	1.047	16.49	7.40	4.47	145	561
13	142,02	349	1.640	16.97	7.32	7.54	121	<u>1249</u>
14	141,5	250	<u>3.170</u>	17.90	7.14	<u>1.39</u>	142	<u>1651</u>
15	141,02	250	1.155	17.03	7.30	8.03	108	855

(all data are averaged)

Main cations and anions concentration in sampled groundwater and leachate

HPLC



Sampling well	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	B ⁻	F ⁻	Br	Cl ⁻	SO ₄ ²⁻	NH ₄ ⁺	NO ₃ ⁻	NO ₂ ⁻	HCO ₃ ⁻
2	5.3	116.2	99.9	58	0.03	<0.1	0.5	179.6	48.8	<0.1	36.8	<0.1	520
3	2.8	48.7	121.4	28.1	0.02	<0.1	0.2	92.2	13.6	<0.1	56.0	<0.1	410
5	2.2	27.7	90.9	46	0.03	<0.1	<0.1	55.3	11.1	<0.1	33.7	<0.1	440
8	2.4	27.3	86.3	48.3	0.02	<0.1	0.4	49.2	13.2	<0.1	20.9	<0.1	455
11	2.1	27.3	112.8	49.2	0.02	<0.1	<0.1	52.8	16.9	<0.1	40.9	<0.1	510
12	2.7	44.8	109.7	53.8	0.02	<0.1	<0.1	76.8	18.0	<0.1	33.7	<0.1	540
13	5.2	131.8	120.3	65.8	0.05	<0.1	1.1	182.6	33.8	<0.1	32.9	<0.1	675
14	16.1	438.9	137.3	107.2	0.14	<0.1	1.6	389.7	105.0	<0.1	17.2	<0.1	1325
15	3.5	33.6	126.4	56.3	0.02	<0.1	<0.1	132.5	6.5	0.30	38.8	<0.1	495
Leachate	1883.1	2134.6	65.6	53.6	1.43	2.1	2,4	2464.4	53.3	2156,7	27.5	<0.1	5035

(all data are averaged and expressed in mg/L).

Microbial indicators of potential faecal contamination

determined by viable cell count and PCR

Microbial target	Sample									
	11	12	13	14	15	2	5	3	8	Leachate
Mesophilic count (30 °C - UFC/100 mL)	300	100	3	500	<u>3200</u>	70	50	160	230	<u>5x10⁷</u>
E. coli (37 °C-UFC/100mL)	ND	ND	ND	<u>3</u>	ND	ND	ND	ND	ND	ND
Bacteroides /Prevotella	-	+	-	+	+	-	-	+	-	-
Bifidobacterium spp.	-	-	-	-	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-	-	-	-
Enterococcus faecium	-	-	-	-	-	-	-	-	-	-
Enterococcus spp.	-	-	-	-	-	-	-	-	-	-

Data averaged, + when at least one sample was found positive; ND = not detected;

PCR targeting nitrigen cycle microorganisms

Microbial target	Sample									
	11	12	13	14	15	2	5	3	8	Leachate
Nitrobacter spp.	-	+	-	+	-	-	-	+	-	+
Nitrospira spp.	-	+	-	+	-	-	-	-	-	-
AOB	-	-	-	-	-	-	-	-	-	+
AOA	-	+	+	+	+	-	-	-	-	-
NosZ Denitro	-	+	-	+	-	-	-	-	-	+
NirK Denitro	-	+	-	-	-	-	-	-	-	+

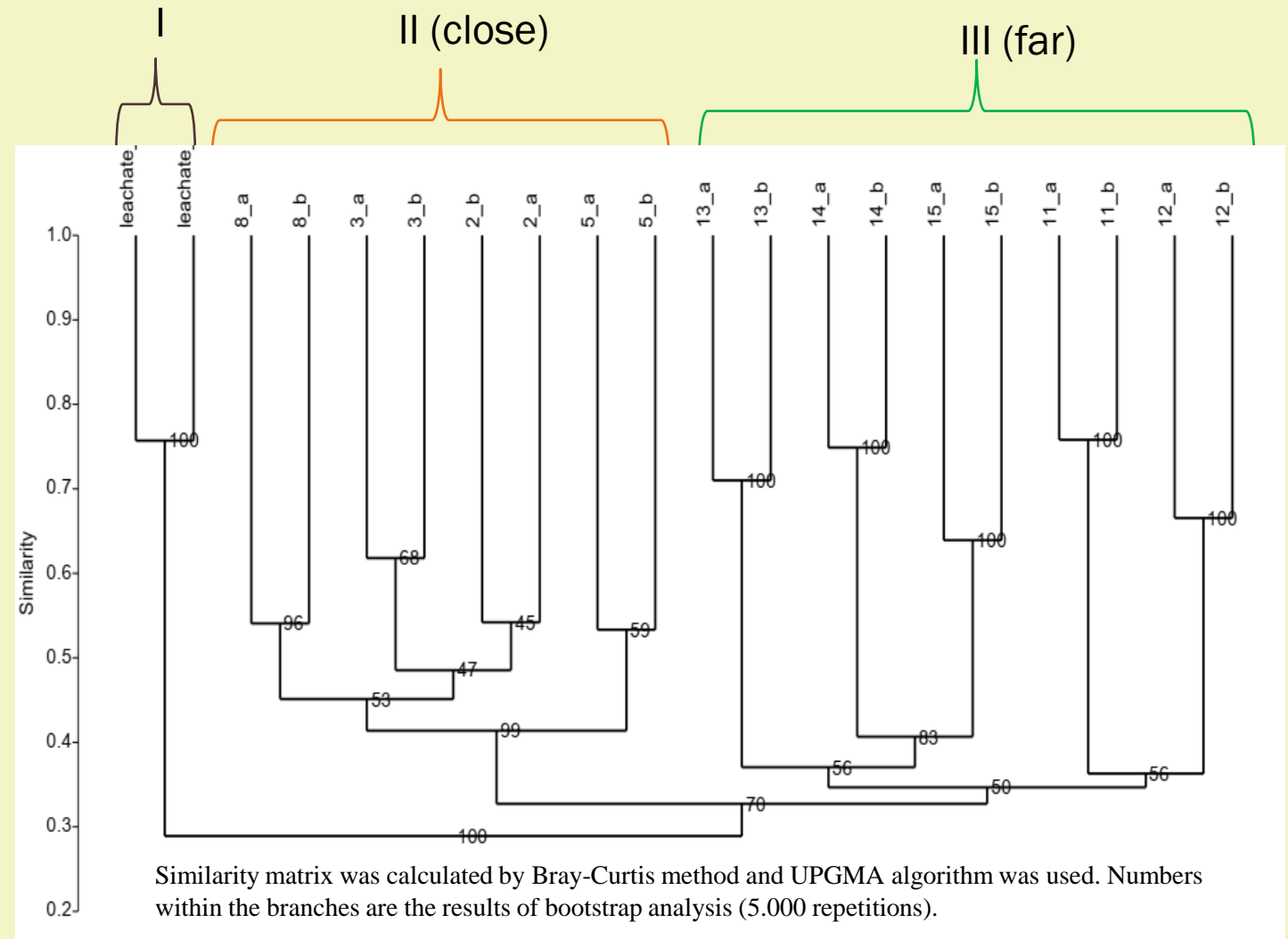
AOB = ammonia oxidizing BACTERIA

AOA = ammonia oxidizing ARCHAEA

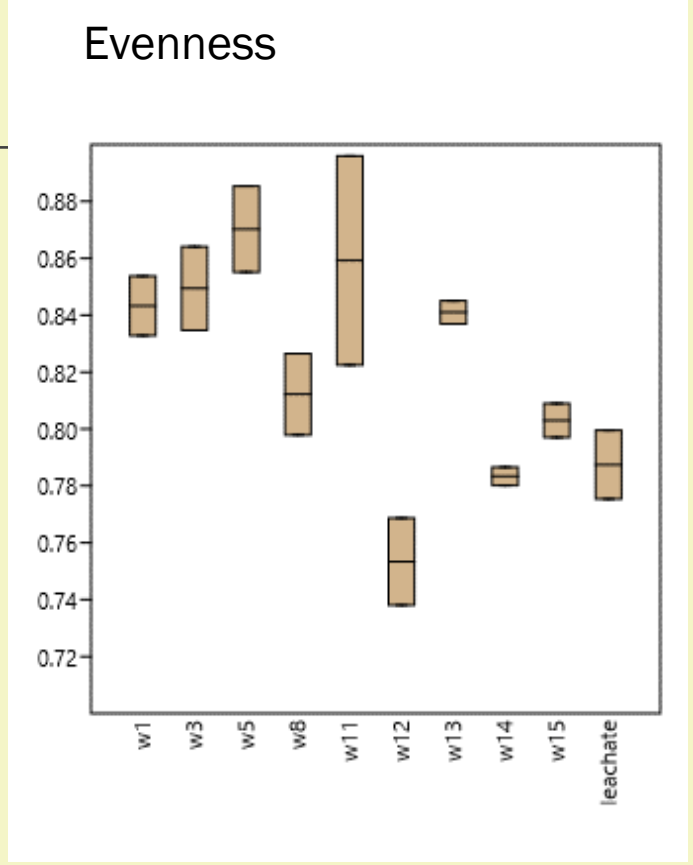
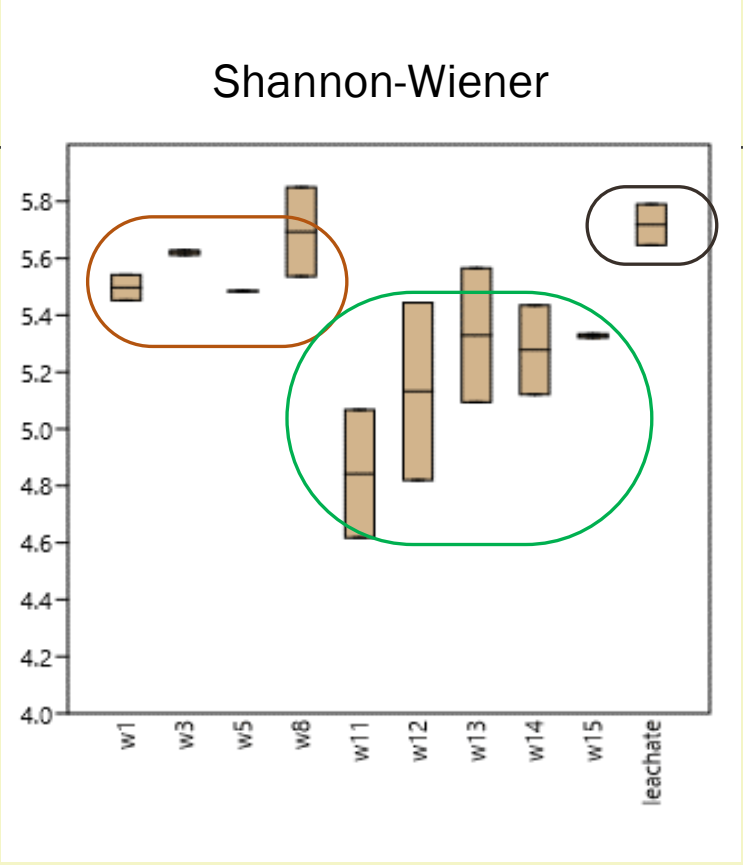
NosZ-NirK = bacterial oxydoreductases

Bacterial community structure

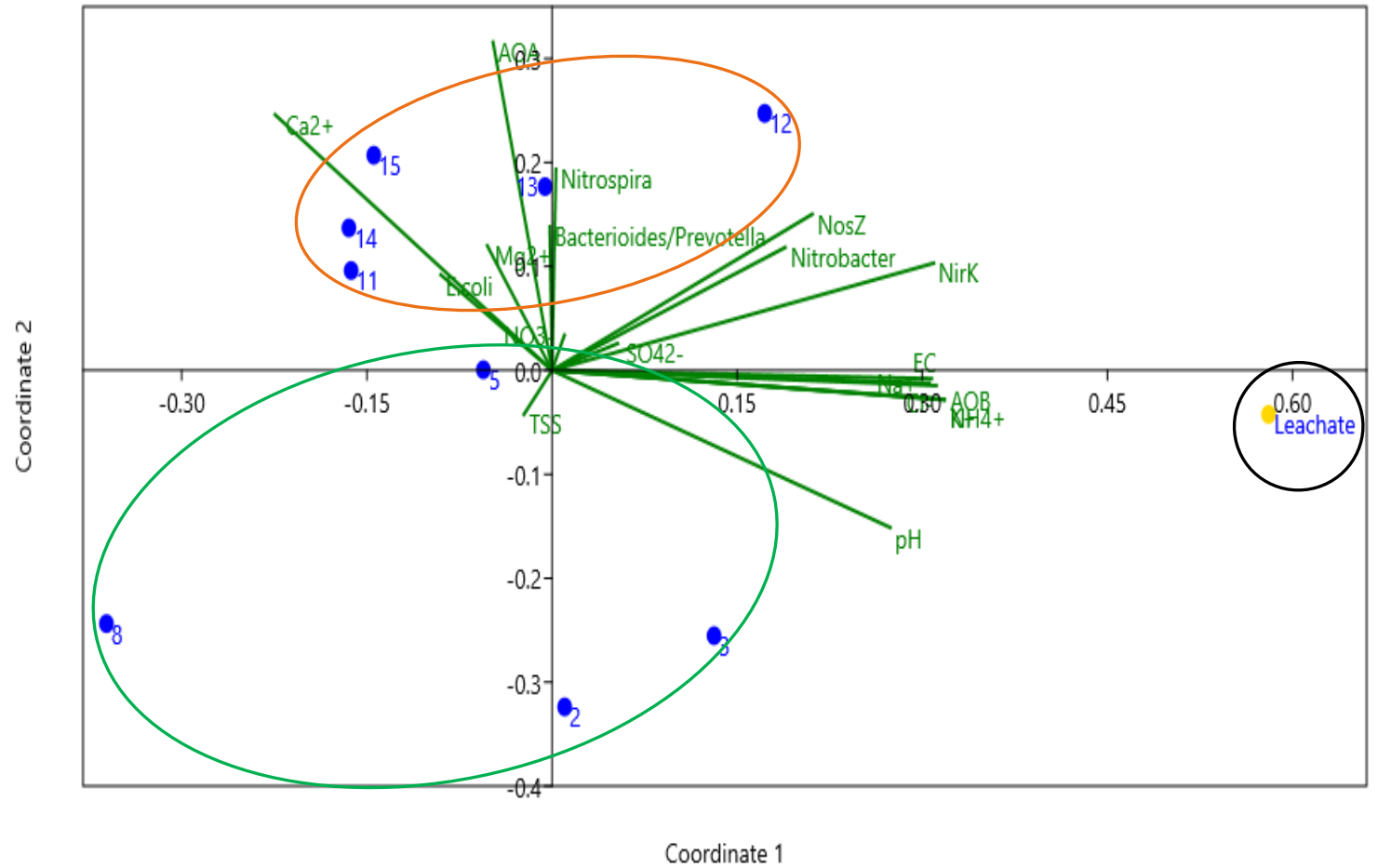
Cluster analysis realized on the bacterial community fingerprint determined by PCR-ARISA on the groundwaters and leachate



Diversity indexes based on the ARISA profiles of analyzed samples.

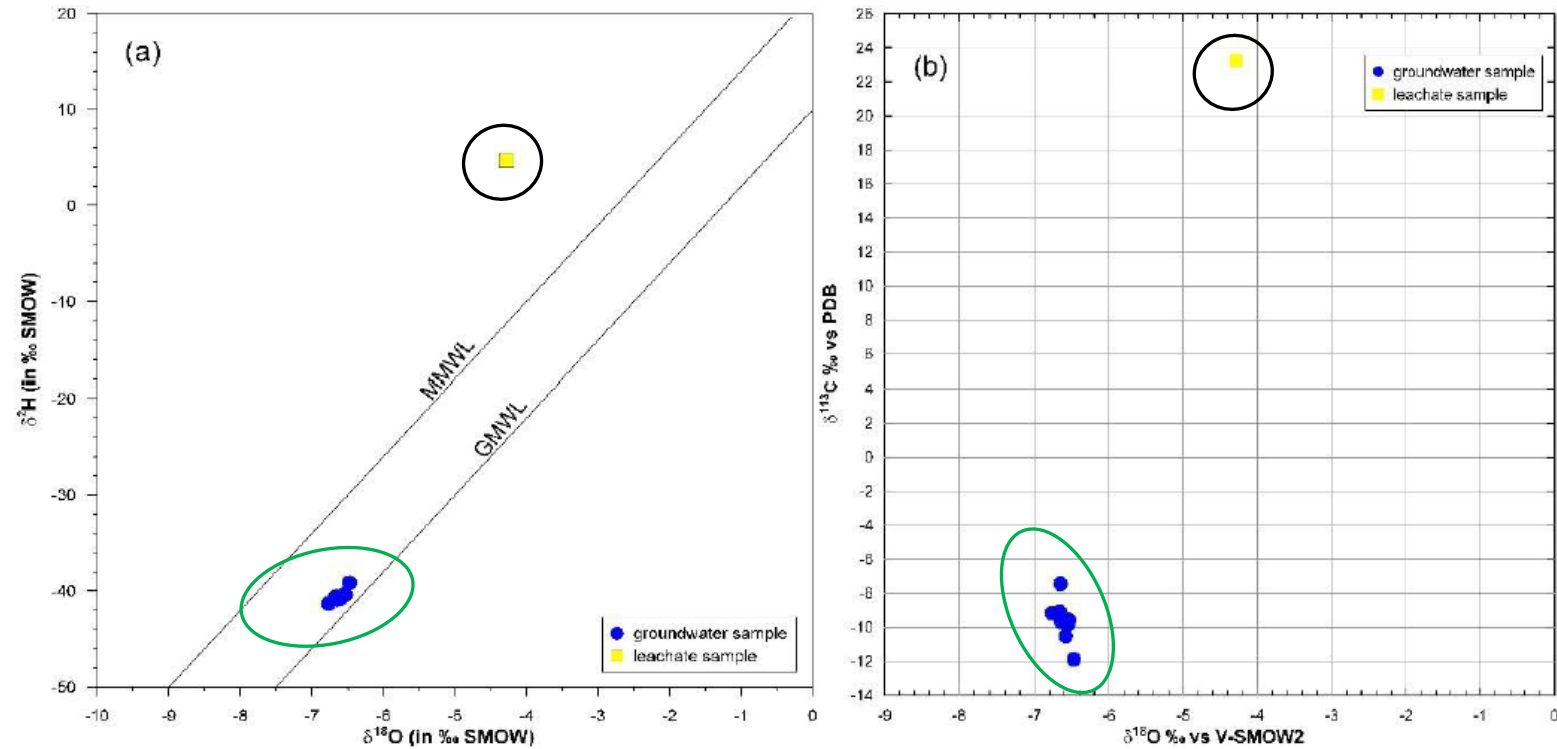


Non-metric multidimensional scaling (NMDS) ordination of the samples and main chemical parameters.

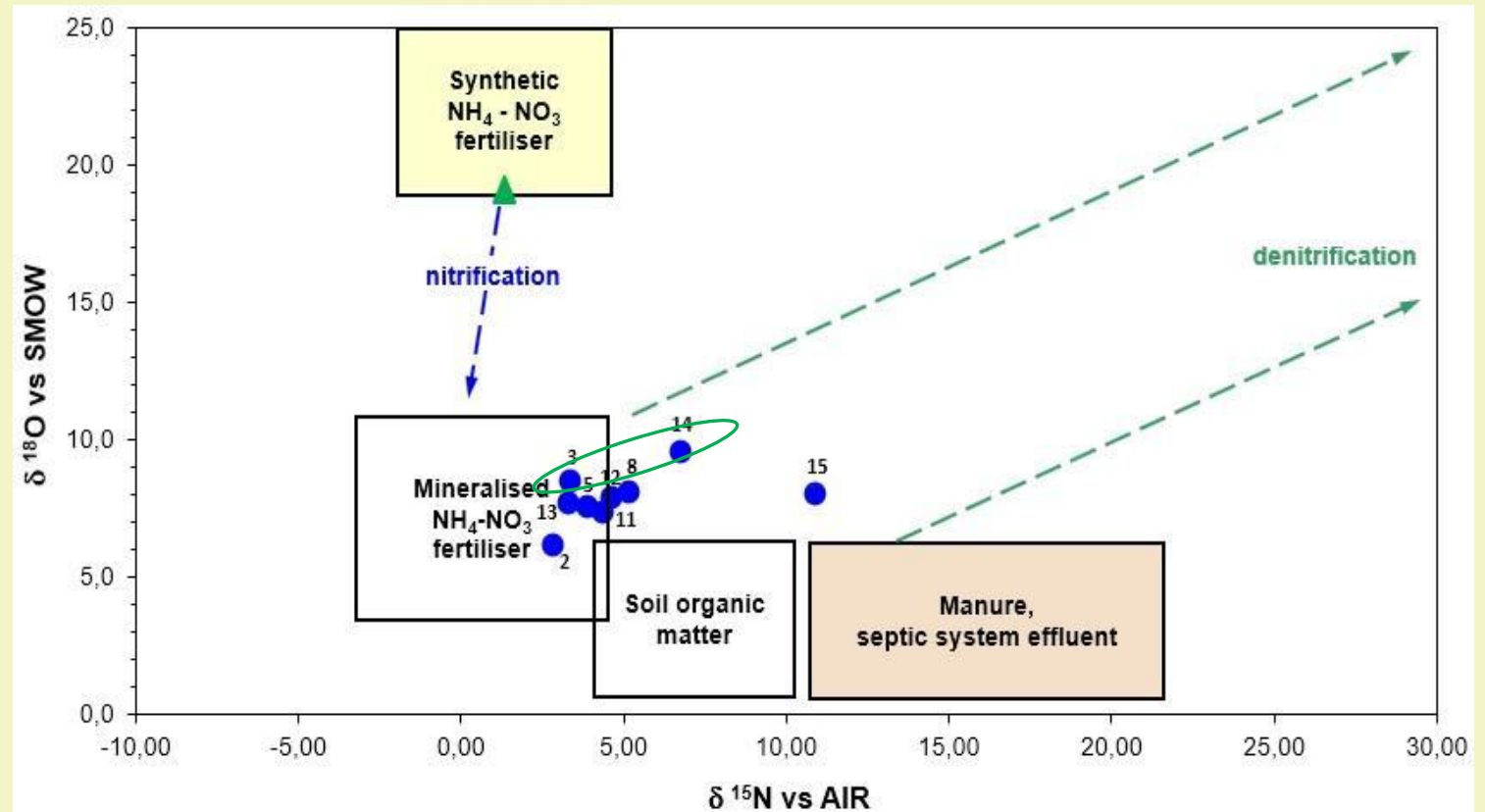


a) Binary $\delta^2\text{H}$ – $\delta^{18}\text{O}$ diagram for water samples and leachate.

(b) Binary $\delta^{13}\text{C}$ – $\delta^{18}\text{O}$ diagram.



Isotopic signature of N-NO₃



Samples with higher isotopic signature were also found positive to ammonia oxidizers and nitrifiers

Conclusions



Groundwater was not influenced by the landfill site and leachate composition

Agriculture and sea intrusion contributed to the variability in the groundwater characteristics.

Utility of integrated approach with microbial markers and isotopes (particularly for nitrogen cycle as a proxy of pollution-disturbance).

Wider monitoring period will increase the awareness of multiple source of disturbance and allow early detection of possible leachate intrusion

Thank you for the attention



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