



Supplement of

N transformations in nitrate-rich groundwaters: combined isotope and microbial approach

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Supplementary information

N-transformations in nitrate-rich groundwaters: combined isotope and microbial approach

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Table S1: Concentrations of Nitrogen Species and Environmental Parameters in Groundwater Samples (P abbreviated for piezometer), bd – below detection, piezometers selected for incubation – in bold font.

Sample	Temperature (°C)	pH	Conductivity (µS cm ⁻¹)	Nitrate (N-NO ₃ ⁻) (mg N L ⁻¹)	Nitrite (N-NO ₂ ⁻) (mg N L ⁻¹)	Ammonium (N-NH ₄ ⁺) (mg N L ⁻¹)	DOC (dissolved organic carbon) (mgC L ⁻¹)	DON (dissolved organic nitrogen) (mgN L ⁻¹)	δ ¹⁵ N		δ ¹⁸ O	
									δ ¹⁵ N-NO ₃ ⁻	δ ¹⁵ N-NO ₂ ⁻	δ ¹⁸ O-NO ₃ ⁻	δ ¹⁸ O-NO ₂ ⁻
P-0	13.5	6.5	928	39.65	0.08	0.33	24.12	32.76	0.9	-31.0	0.5	bd
P-1	14	6.1	1000	21.05	0.284	0.224	42.06	16.76	7.7	-27.8	6.1	-7.3
P-2	13.4	7.3	1245	0.62	0.21	7.05	67.42	bd	bd	bd	bd	bd
P-3	14.1	6.7	998	39.3	0.405	0.316	27.28	57.08	4.3	-30.2	0.4	-4.2
P-4	12.8	7.3	4794	0.63	0.398	11.46	431.3	7.74	bd	bd	bd	bd
P-5	13.5	6.8	1030	18.45	0.092	0.103	176.9	6.12	9.3	bd	3.3	bd
P-6	17	7.5	1386	0.24	0.034	0.31	58.42	bd	bd	bd	bd	bd
P-7	13.2	6.9	1395	32.8	0.188	0.138	46.32	41.04	6.6	-37.0	3.2	8.5
P-9	14.4	6.7	919	0.11	<0	0.17	52.59	bd	bd	bd	bd	bd
P-10	12.2	6.8	944	0.98	0.02	1.509	24.66	bd	10.4	bd	6.1	bd
P-11	14.2	7.3	1343	0.5	0.081	2.33	bd	bd	bd	bd	bd	bd
P-14	15.5	7.1	845	0.33	0.114	14.98	bd	bd	bd	bd	bd	bd
P-15	14.7	7	512	0.44	0.146	4.397	22.28	bd	bd	bd	bd	bd
P-16	10	6.2	617	39.45	0.098	0.031	10.22	31.88	3.7	-17.3	1.6	4.4
P-17	12.7	7.1	685	0.26	0.018	0.557	51.81	bd	bd	bd	bd	bd
P-18	11.2	7.1	560	1.8	0.06	0.651	55.85	bd	8.4	bd	7.7	bd
P-19	13.9	6.6	617	0.52	0.025	0.611	9.23	bd	bd	bd	bd	bd
P-20	11.1	6.6	471	38.12	0.019	0.028	9.7	30.63	1.0	bd	-0.9	bd
P-21	10.9	7.1	574	0.43	0.087	0.565	31.49	bd	bd	bd	bd	bd
P-22	11.1	5.6	557	2.47	0.064	0.215	14.81	5.01	16.0	bd	4.8	bd
P-23	14.9	6.3	1238	89.5	0.354	0.059	21.22	91.72	5.3	-27.9	4.7	-8.9
P-L1	14.2	6.8	2581	1.26	0.226	0.817	262.4	69.28	10.3	5.4	1.9	15.7
P-L2	13.8	7	1777	0.2	0.058	17.95	68.9	25.74	bd	bd	bd	bd

Table S2: Data on GC headspace gas analyses for O₂, CO₂ and N₂O.

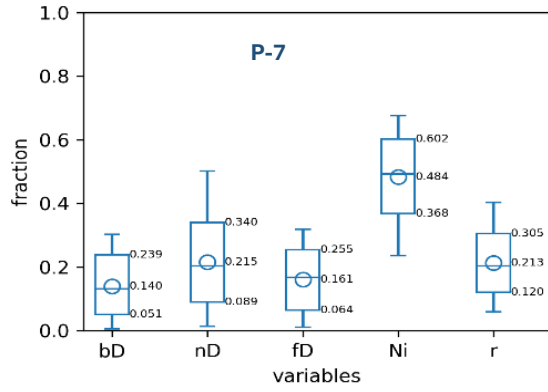
The average values and standards deviations of 4 repeated incubation flasks are shown. The respective: dissolved O₂ concentration, N₂O production and CO₂ production were calculated taking into account the gas constant for gases dissolution in water for the incubation temperature of 16 C. For sterile samples the average data for the samplings before glucose addition (1 and 3) and after glucose addition (4+6).

piezometer	sampling	day	O ₂ [%]		Dissolved O ₂ [mg L ⁻¹]	CO ₂ [ppm]		N ₂ O [ppb]		N ₂ O µg/L/d	CO ₂ mg/L/d
			average	stdev		average	St dev	average	st dev		
P7	1	2	8.5	1.5	3.5	865	225	918	189	0.66	1.82
P16	1	2	7.6	1.2	3.2	761	184	524	311	0.38	1.60
P20	1	2	7.3	1.6	3.0	391	48	258	179	0.18	0.82
P23	1	2	7.9	0.7	3.3	575	50	365	178	0.26	1.21
P7	3	7	4.3	2.3	1.8	1755	145	3316	3378	0.82	2.88
P16	3	7	3.2	1.4	1.3	1129	234	540	561	0.13	1.85
P20	3	7	3.8	1.4	1.6	337	156	527	547	0.13	0.55
P23	3	7	4.4	1.2	1.8	762	129	654	257	0.16	1.25
<i>flushing + glucose addition</i>											
P7	4	9	4.2	1.5	1.7	1340	294	393	246	0.28	2.82
P16	4	9	4.4	1.3	1.8	781	329	1290	1157	0.92	1.65
P20	4	9	2.6	1.1	1.1	426	399	133	85	0.10	0.90
P23	4	9	2.4	0.9	1.0	326	283	497	374	0.36	0.69
P7	6	14	3.9	2.2	1.6	1195	1545	5993	5657	1.49	1.96
P16	6	14	2.9	1.4	1.2	1823	1369	46578	91114	11.58	2.99
P20	6	14	2.4	1.2	1.0	1908	475	4459	7284	1.11	3.13
P23	6	14	4.1	2.0	1.7	1807	774	2052	2050	0.51	2.96
<i>Sterile samples</i>											
P7	1+3	4	4.4		3.0	1928		70		0.03	3.43
P16	1+3	4	8.1		4.0	2160		224		0.09	3.84
P20	1+3	4	5.5		2.6	632		0		0.00	1.12
P23	1+3	4	6.4		3.3	1205		0		0.00	2.14
<i>flushing + glucose addition</i>											
P7	4+6	4	3.5		2.6	1381		218		0.08	2.46
P16	4+6	4	3.5		1.7	1299		1654		0.64	2.31
P20	4+6	4	2.3		1.1	387		106		0.04	0.69
P23	4+6	4	2.6		0.8	1044		517		0.20	1.86

Table S3: qPCR primer pairs and programs for targeted genes

Target gene	Primer	Primer concentration (μM)	Program	
bacterial 16S rRNA	Bact517F	0.3	95°C 30s; 60°C 45s; 72°C 45s	x 40 cycles
	Bact1028R			
archaeal 16S rRNA	Arc519F	0.3	95°C 15 s; 56°C 30 s; 72°C 30 s	x 45 cycles
	Arch910R			
<i>nirK</i>	nirK876	0.4	95°C 15 s, 58°C 30 s; 72°C 30s, 80°C 30 s	x 45 cycles
	nirK1040			
<i>nirS</i>	nirSCd3af	0.4	95°C 15 s, 55°C 30 s; 72°C 30s, 80°C 30 s	x 45 cycles
	nirSR3cd			
<i>nosZI</i>	nosZ2F	0.4	95°C 15 s, 60°C 30 s, 72°C 30 s, 80°C 30s	x 45 cycles
	nosZ2R			
<i>nosZII</i>	nosZIIF	1	95°C 30 s, 54°C 45 s, 72°C 45 s, 80°C 45 s	x 45 cycles
	nosZIIR			
bacterial <i>amoA</i>	amoA-1F	0.4	95°C 30 s, 57°C 45 s, 72°C 45 s	x 45 cycles
	amoA-2R			
archaeal <i>amoA</i>	CrenamoA 23F	0.4	95°C 30 s, 55°C 45 s, 72°C 45 s	x 45 cycles
	CrenamoA 616R			
comammox <i>amoA</i>	comamoA AF	0.6	95 °C 15 s, 55 °C 30 s, 72 °C 30 s	x 40 cycles
	comamoA SR			
<i>nrfA</i>	nrfAF2awMOD	0.6	95 °C 15 s, 56 °C 30 s, 72 °C 30 s	x 45 cycles
	nrfAR1MOD			
<i>nifHA</i>	Ueda19F	0.4	95 °C 30 s, 53 °C 45 s, 72 °C 45 s	x 45 cycles
	Ueda407R			

A. First Samples



B. Later Samples

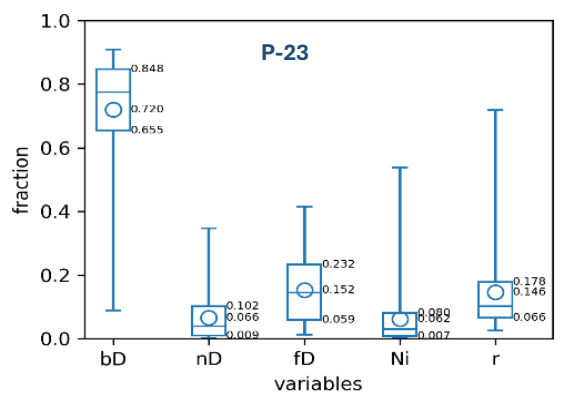
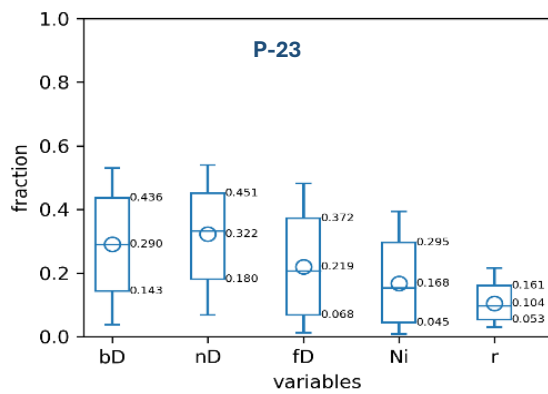
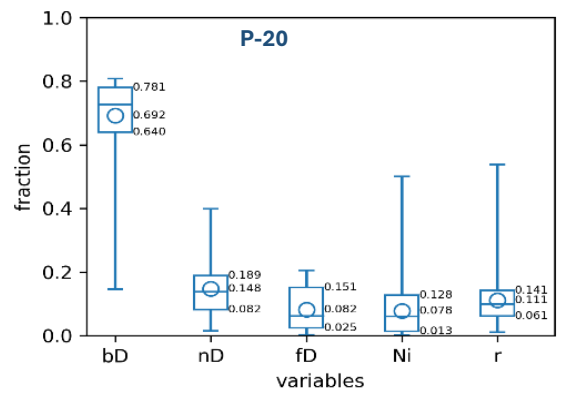
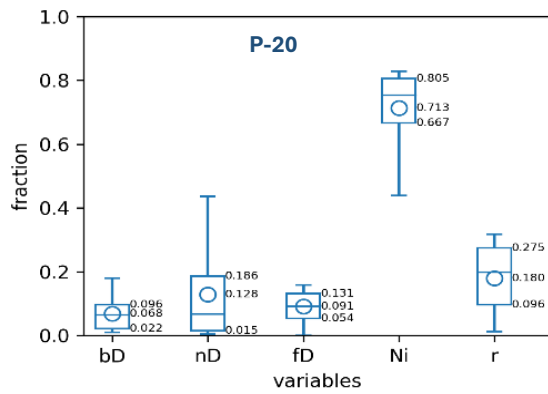
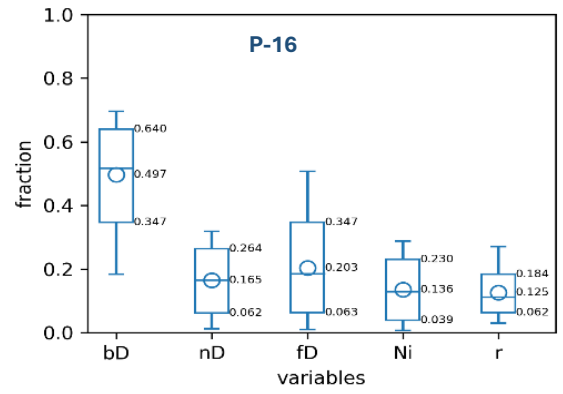
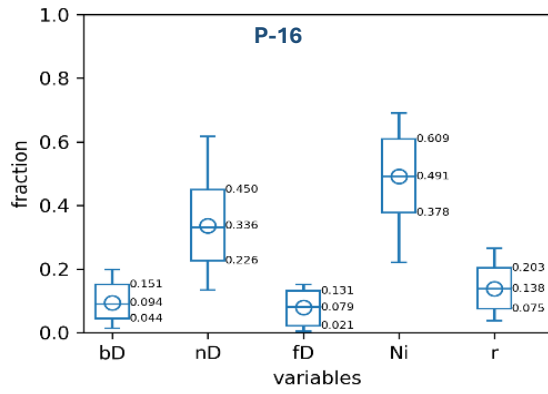
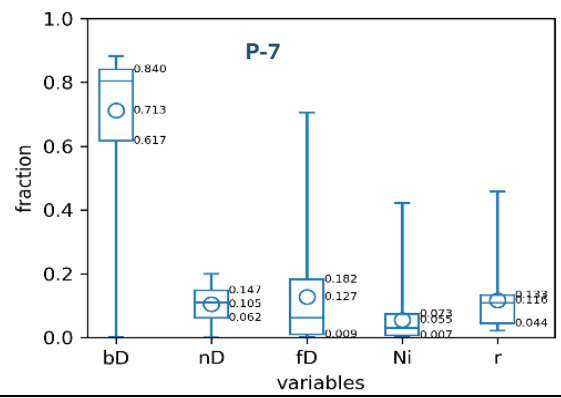


Figure S1: N_2O production pathways contribution and N_2O reduction progress based on the FRAME modelling of the experimental N_2O isotope data collected for the first analysed samples (before glucose addition, 1st and 2nd sampling, day 2 and 4) (A) and mean value of the later samples (after glucose addition, 4th and 5th sampling, day 9 and 11) (B).

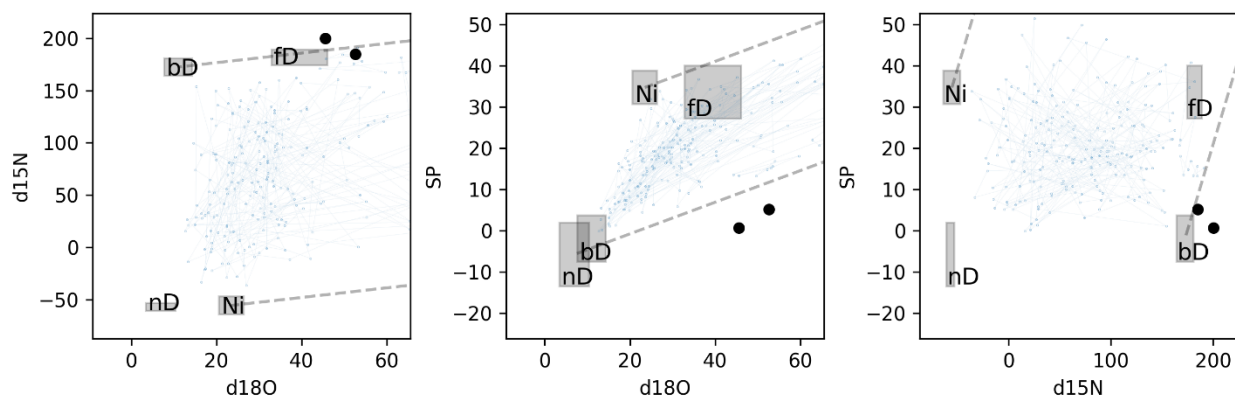


Figure S2: An example of the FRAME modelling path illustration for the last incubation samples (P-16, day 14), which do not provide modelling results.

Data availability

Original data are available in the zenodo repository files (<https://zenodo.org/records/15076761>)