Data management: how to process the output

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Stable Isotope Ecology Short Course

We have numbers from the IRMS, what now?

- Calibration of the data: assigning isotope delta values of the measurements relative to the zero-points of the appropriate delta scale
- Using Reference Materials (RM)
  - Primary RM
  - Secondary RM
  - Tertiary RM
  - Laboratory or in-house RM

Primary reference materials

- Defines the position of the zero-point
- Exact isotope-delta values without uncertainty

Table 1. Internationally agreed zero-points of the light element isotope ratio δ scales, their primary RM(s) and (currently available) highest metrological realisations.

Ratio	Zero-point material	primary RM(s)	Highest metrological realisation(s)	
			Name	δ value (‰)[a,b]
$^{2}H/^{1}H$	VSMOW	VSMOW	VSMOW2[c]	0.00 ± 0.3
		SLAP	SLAP2[c]	−427.5 ± 0.3
$^{13}C/^{12}C$	VPDB	NBS 19	IAEA-603[d]	+2.46 ± 0.01
$^{15}N/^{14}N$	Atmospheric nitrogen	*NA* [e]	IAEA-N-1[f]	+0.43 ± 0.04
		USGS32	USGS32	+180
$^{17}O/^{16}O$	VSMOW	VSMOW	VSMOW2[b]	0.00 ± 0.03
		SLAP	SLAP2[b]	−29.697 ± 0.05
$^{18}O/^{16}O$	VSMOW	VSMOW	VSMOW2[b]	0.00 ± 0.02
		SLAP	SLAP2[b]	−55.5 ± 0.02
	VPDB	NBS 19	IAEA-603[d]	−2.37 ± 0.04
	Atmospheric oxygen	*NA*	*NA*	*NA*
$^{34}S/^{32}S$	VCDT	IAEA-S-1	IAEA-S-1	−0.3

(FIRMS, 2004)

Secondary reference materials

- Assigned δ values are agreed upon and adopted internationally
- Some uncertainty associated with the values
- Gets reviewed over time: check latest value and provide the used value when reporting

Tertiary reference materials

- Calibrated using secondary RMs
- Some are available through universities or commercial organisations

Data handling

1. Initial data evaluation
2. Isobaric interferences
3. Scale calibration/normalisation
4. Other corrections

Over-corrections can introduce as many problems as it appears to solve, so be critical about which corrections are needed.

Initial data evaluation

- Usually done by IRMS instrument software to provide you with raw isotope-delta values
  - Integration of the peak area
  - Correction for isobaric interferences
  - Conversion to raw isotope delta values

Isobaric interferences

- ^{17}O correction for C isotope ratios of CO_2
- H_3^+ correction for H isotope ratios of H_2
- ^{13}C correction for O isotope ratios of CO and CO_2
- m/Z 28 interferences for isotopic ratios determined on CO
- O isotope corrections for S isotope ratios of SO_2

Scale calibration/normalisation

- Performed in external spreadsheets
- Based on reference material
- Two-point linear normalisation = linear shift normalisation = stretch-shift correction

Other corrections

- Blank correction
- Drift correction
- Linearity (peak size) correction
- Memory correction
- Correction for derivatisation
- Correction for extrinsic hydrogen

How does the output look like?

- Open the file: Thermo_EA_180711_Naomi_Saipan2.xlsx
- Let’s look at the different columns

Post processing – Raw output

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Row	Analysis	Identifier 1	Identifier 2	Date	Time	Peak Nr	Rt	Amount	Amt%	Ampl 28	BGD 28	Area 28	Ampl 29	BGD 29	Area 29	Area All	d 15N/14N
4	1958	blank		10/15/18	10:36:35	2	89.5		0	132	80.1	2.21	98	60.9	0.016	2.226	-6.129
5	1959	iACET	E1	10/15/18	10:50:21	2	89.5	0.343	11.29592	1880	79.5	25.448	1404	68.8	0.19	25.638	0.142
6	1960	S1	C1	10/15/18	11:06:46	2	317.1	0.339								141.342	
7	1961	S1	A11	10/15/18	11:26:03	2	316.8	0.376								141.717	
8	1962	A1T	G10	10/15/18	11:40:12	2	91.1		0	1766	80.4	24.873	1327	80.6	0.187	25.06	6.228
9	1963	A1B	G11	10/15/18	11:53:58	2	90.9		0	1114	82.9	15.934	835	89.3	0.119	16.053	2.248
10	1964	A2-1	G12	10/15/18	12:07:44	2	90.7		0	1216	92.6	16.865	914	93.1	0.127	16.992	5.95
11	1965	A2-2	H1	10/15/18	12:21:31	2	90.7		0	1381	91.9	19.033	1038	88.3	0.143	19.176	7.843
12	1966	A3T	H2	10/15/18	12:35:17	2	90.7		0	1452	92.0	20.398	1092	85.7	0.153	20.551	7.744

Two identifiers possible

Time stamp

Peak number and timing

Mass and %N

Amplitude Background Area Per mass

Amplitude Background Area Per mass

Area d15N

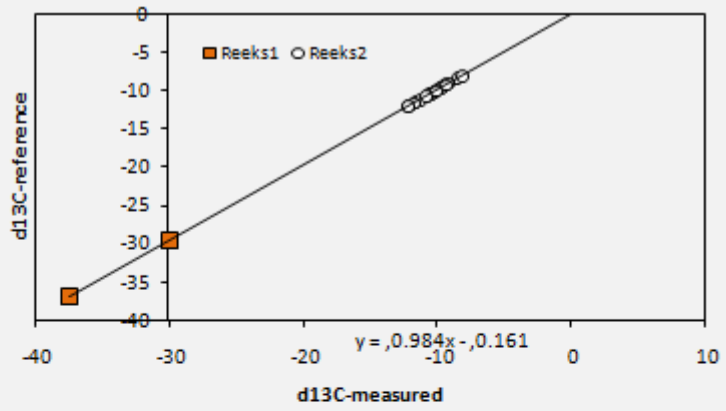
Summary of the standards at the bottom (for easy correction)

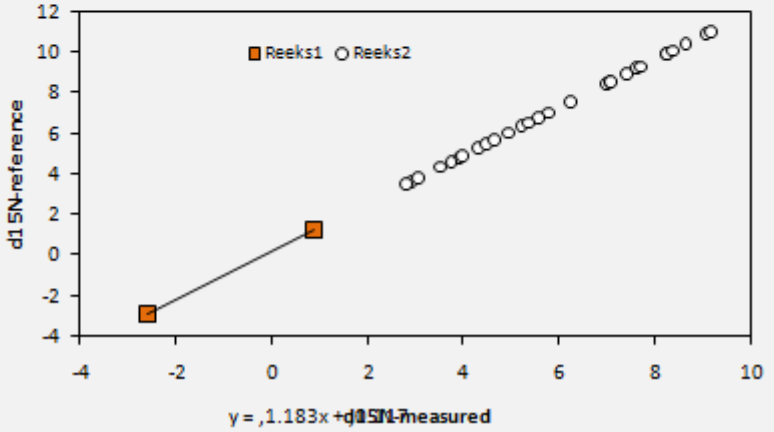
Post processing – scale calibration

Fill in data on standards in green area
Corrections based on linear regression

References used		$d^{13}C$ corrections iACET	urea	Ref 3	$d^{15}N$ corrections iACET	urea	Ref 3
Measure	1	-29.712	-37.366		0.958	-2.59	
	2	-29.863	-37.498		0.806	-2.742	
	3	-29.957	-37.768		1.011	-2.78	
	4	-29.861	-37.617		0.792	-2.473	
	5	-29.948	-37.637		0.742	-2.639	
	6	-29.907	-37.279		0.973	-2.268	
	7	-29.712	-37.186		1.066	-2.375	
	8	-29.81	-37.313		0.838	-2.595	
	9						
	10						
	11						
	12						
	13						
	14						
	15						
	16						
	17						
Average		-29.85	-37.46	#DEEL/0!	0.90	-2.56	#DEEL/0!
Stdev		0.10	0.20	#DEEL/0!	0.12	0.18	#DEEL/0!
Reference values		-29.53	-37.02	0.00	1.18	-2.91	0.00
Dd		0.32	0.44	#DEEL/0!	0.28	-0.35	#DEEL/0!

Blank data		Area C	d13C	Area N	d15N
Blank	1	0	0	0	0
Blank	2				
Blank	3				
Blank	4				
Blank	5				
Blank	6				
Avg		0.000	0.00	0.000	0.00
Stdev		#DEEL/0!	#DEEL/0!	#DEEL/0!	#DEEL/0!





Linear regression:
(corrected=slope*measured+intercept)

d13C
slope: 0.984
intercept -0.161

d15N
slope: 1.183
intercept 0.117

Post processing – scale calibration and blank correction

										Approach: first calibrate measured delta values, then perform blank-correction						
			Mass samp	Area C	d13C	Area N	d15N	Area S	d34S	Calibrated	Calibrated	Calibrated	Final	Final	Final	
	SampleID	SampleID	mg	measured	measured	measured	measured	measured	measured	d13C	d15N	d34S	d13C	d15N	d34S	
67	5A		5.09	731.193	-12.007	38.085	9.09			-11.98	10.87	-2.37	-11.98	10.87	#DEEL/0!	
68	6A		4.938	602.885	-10.594	39.497	4.348			-10.59	5.26	-2.37	-10.59	5.26	#DEEL/0!	
69	6B		4.761	565.078	-11.664	35.128	4.982			-11.64	6.01	-2.37	-11.64	6.01	#DEEL/0!	
70	6C		5.077	616.923	-12.243	32.078	5.81			-12.21	6.99	-2.37	-12.21	6.99	#DEEL/0!	
71	6D		5.038	588.435	-11.334	35.055	5.246			-11.31	6.33	-2.37	-11.31	6.33	#DEEL/0!	
72	6E		4.99	602.43	-11.732	52.314	8.278			-11.71	9.91	-2.37	-11.71	9.91	#DEEL/0!	
73	8A		5.101	574.575	-9.552	32.577	7.008			-9.56	8.41	-2.37	-9.56	8.41	#DEEL/0!	
74	8B		4.998	636.24	-10.536	32.072	8.676			-10.53	10.38	-2.37	-10.53	10.38	#DEEL/0!	
75	8C		4.937	584.619	-9.906	35.323	7.441			9.91	8.92	2.37	9.91	8.92	#DEEL/0!	

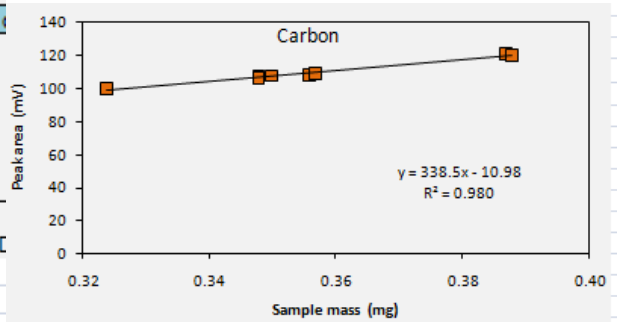
$$\delta_{\text{blk corr}} = \frac{\delta_{\text{meas}} \times Area_{\text{meas}} - \delta_{\text{blk}} \times Area_{\text{blk}}}{Area_{\text{meas}} - Area_{\text{blk}}}$$

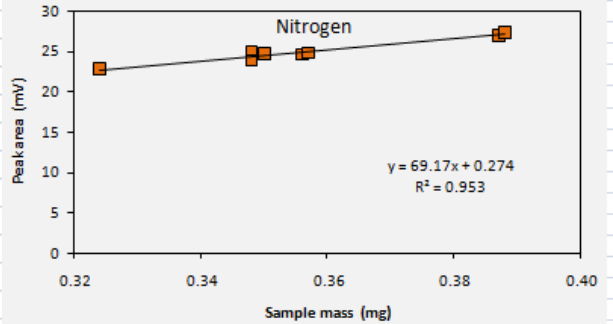
Post processing - %C and %N

Blank data		Area C	d13C	Area N	d15N	Area S
Blank	1	0	0	0	0	0
Blank	2					
Blank	3					
Blank	4					
Blank	5					
Blank	6					
Avg		0.000	0.00	0.000	0.00	0.000
Stdev		#DEEL/0!	#DEEL/0!	#DEEL/0!	#DEEL/0!	#DEEL/0!

Standard		mg	Area C	Area N	Area C-cor	Area N-cor	mg C	mg N	K-C	K-N
iACET	18	0.387	121.383	26.957	121	27	0.27512	0.04009	441.2029	672.3584
iACET	1	0.348	107.301	24.844	107	25	0.24739	0.03605	433.7266	689.1004
iACET	2	0.356	107.894	24.606	108	25	0.25308	0.03688	426.323	667.1619
iACET	3	0.357	108.867	24.791	109	25	0.25379	0.03699	428.9627	670.2951
iACET	4	0.324	99.781	22.789	100	23	0.23033	0.03357	433.2059	678.923
iACET	5	0.388	120.237	27.351	120	27	0.27583	0.04020	435.9111	680.4273
iACET	6	0.348	106.352	23.846	106	24	0.24739	0.03605	429.8906	661.4188
iACET	7	0.35	107.867	24.722	108	25	0.24882	0.03626	433.5229	681.7981

	K-C	K-N
avg	432.8432	675.1854
stdev	5	9
%err	1.06	1.33
Slope	476	668
interc	-11	0





Post processing - %C and %N

										Approach uses regression line between element amounts and blank-corrected areas						
SampleID	SampleID	Mass samp mg	Area C measured	d13C measured	Area N measured	d15N measured	Area C Corrected	Area N Corrected	Area S Corrected	mg C	mg N	mg S	%C	%N	%S	C/N
5A		5.09	731.193	-12.007	38.085	9.09	731.193	38.085	0.000	1.558484	0.056624	-6.6E-19	30.62	1.11	0.00	27.5
6A		4.938	602.885	-10.594	39.497	4.348	602.885	39.497	0.000	1.289053	0.058739	-6.6E-19	26.10	1.19	0.00	21.9
6B		4.761	565.078	-11.664	35.128	4.982	565.078	35.128	0.000	1.209662	0.052196	-6.6E-19	25.41	1.10	0.00	23.2
6C		5.077	616.923	-12.243	32.078	5.81	616.923	32.078	0.000	1.318531	0.047629	-6.6E-19	25.97	0.94	0.00	27.7
6D		5.038	588.435	-11.334	35.055	5.246	588.435	35.055	0.000	1.258709	0.052087	-6.6E-19	24.98	1.03	0.00	24.2
6E		4.99	602.43	-11.732	52.314	8.278	602.430	52.314	0.000	1.288097	0.077934	-6.6E-19	25.81	1.56	0.00	16.5
8A		5.101	574.575	-9.552	32.577	7.008	574.575	32.577	0.000	1.229605	0.048376	-6.6E-19	24.11	0.95	0.00	25.4
8B		4.998	636.24	-10.536	32.072	8.676	636.240	32.072	0.000	1.359094	0.04762	-6.6E-19	27.19	0.95	0.00	28.5
8C		4.937	584.619	-9.906	35.323	7.441	584.619	35.323	0.000	1.250696	0.052488	-6.6E-19	25.33	1.06	0.00	23.8
8D		4.874	568.194	-10.192	28.214	4.511	568.194	28.214	0.000	1.216206	0.041842	-6.6E-19	24.95	0.86	0.00	29.1
8E		5.051	435.277	-11.73	25.286	3.94	435.277	25.286	0.000	0.937096	0.037457	-6.6E-19	18.55	0.74	0.00	25.0
11A		4.92	661.002	-8.478	41.929	7.075	661.002	41.929	0.000	1.411091	0.062381	-6.6E-19	28.68	1.27	0.00	22.6
11B		4.843	502.052	-10.522	25.42	7.659	502.052	25.420	0.000	1.077315	0.037658	-6.6E-19	22.24	0.78	0.00	28.6
11C		4.906	617.098	-10.237	37.742	6.272	617.098	37.742	0.000	1.318898	0.056111	-6.6E-19	26.88	1.14	0.00	23.5
11D		5.106	619.783	-10.411	29.915	5.588	619.783	29.915	0.000	1.324536	0.044389	-6.6E-19	25.94	0.87	0.00	29.8

How does the output look like?

- Open the file:

  NU_EA_20190923 Naomi filter_T0.xlsx
- Let’s see how it is done here

How does the denitrifier output look like?

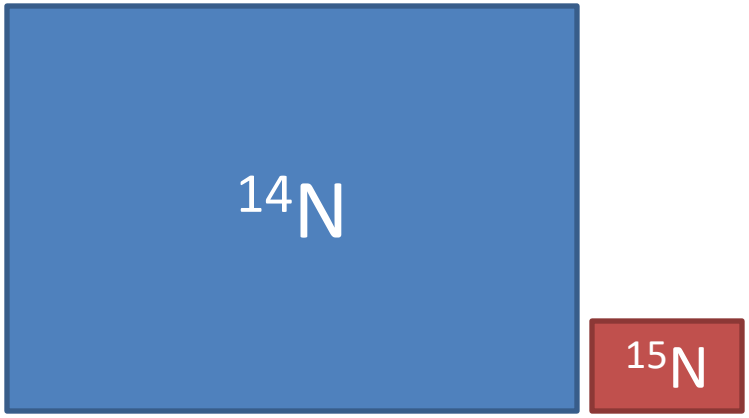
- Open the file: NO_denitrifier_template.xls

Dealing with tracers

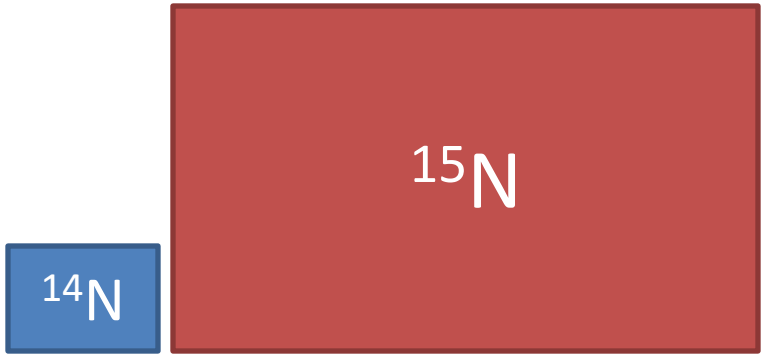
- What are tracers?
  - 99% of the heavy isotope
  - e.g. $^{15}NO_3^-$, $^{13}CO_3$, $^{15}NH_4^+$
  - To follow the elements through certain processes, such as trophic uptake or biogeochemical processes
  - Use just the amount needed to see effect

Diluting tracer samples before measurements

Normal samples:

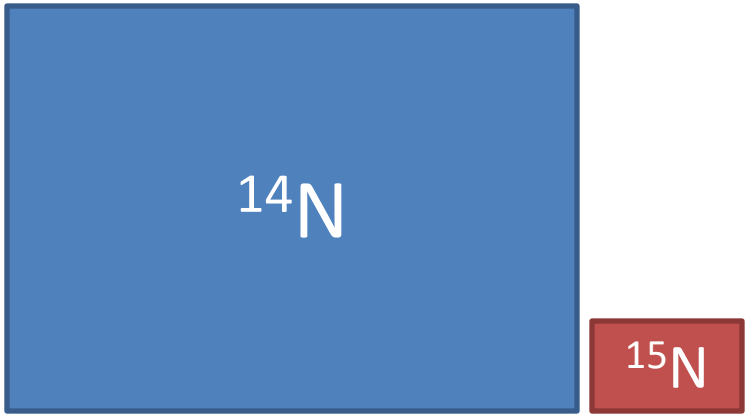


Tracer samples:

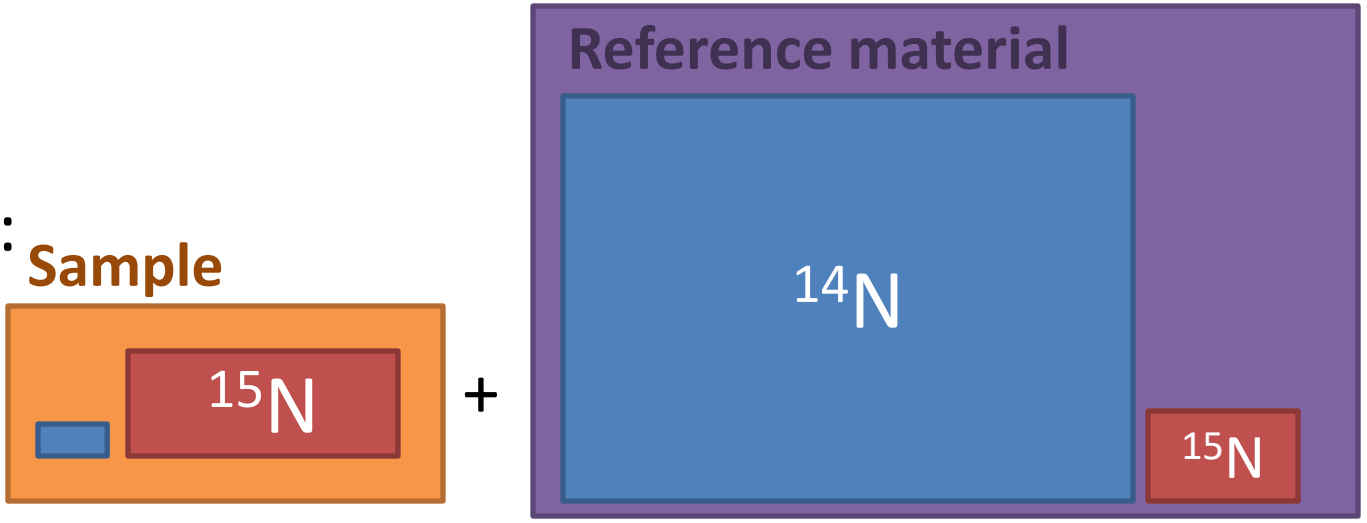


Diluting tracer samples before measurements

Normal samples:



Tracer samples:



Dilution: exercise

- Knowns:
  - Sample dry weight: 0.260 mg
  - iACET dry weight: 0.794 mg
  - δ^{15}N (after normalisation): 1424.47 ‰
  - %N: 10.2569
  - δ^{15}N (iACET): 2.588 ‰
  - %N (iACET): 10.430%

$$\delta^{15}N_{meas} * N_{meas} = \delta^{15}N_{samp} * N_{samp} + \delta^{15}N_{RM} * N_{RM}$$

Dilution: exercise

- Knowns:
  - Sample dry weight: 0.260 mg
  - iACET dry weight: 0.794 mg
  - $\delta^{15}N$ (after normalisation): 1424.47 ‰
  - %N: 10.2569
  - $\delta^{15}N$ (iACET): 2.588 ‰
  - %N (iACET): 10.430

C	D	E	F	H	J	K	L	M
					N mg			
totalDW	sampleDW	iACETDW	cor.15N	%N	total	iACET	sample	15N.final
1.054	0.260	0.794	1424.47	10.2569	10.811	8.281	2.529	**6079.88**