

An isotopically light nitrogen reservoir in the ocean: evidence from ferromanganese crusts

E.E. Stüeken, M. Bau

Supplementary Information

The Supplementary Information includes:

- Analytical methods
- Figure S-1
- Table S-1
- Supplementary Information References

Analytical methods

The samples were analysed in bulk and after acid treatment, using facilities in the St Andrews Isotope Geochemistry Laboratories (StAIG) (e.g., Stüeken *et al.*, 2020). For the acid treatment, approximately 0.5-1 g of rock powder were weighed into pre-combusted glass centrifuge tubes (500 °C for 2 hours) and mixed with 10 ml of 2N HCl (reagent grade). The tubes were capped loosely to allow escape of CO₂ gas. The acid was left to react overnight at 70 °C in a closed oven and decanted the next day after centrifugation (700 rpm for 15 minutes). The residues were washed three times with 18.2 MΩ·cm⁻¹ and then left to dry in the oven for two days before transfer into pre-combusted scintillation vials. For determination of carbon and nitrogen abundances and isotopic ratios in acid-treated residues and in bulk rocks, the powders were weighed into tin capsules and analysed by flash combustion in an elemental analyser (EA Isolink) coupled to an isotope-ratio mass spectrometer (MAT253) via a ConFlo IV (all Thermo Fisher). The EA was operated in dual-reactor mode, where the first reactor was packed with Cr₂O₃ and silvered cobaltic cobaltous oxide at 1020 °C for complete combustion and sulphur removal, respectively, while the second reactor was packed with Cu wire at 650 °C for conversion of nitrogen oxides into N₂ gas. A magnesium perchlorate column at room temperature was used to remove water from the gas stream. The gas chromatograph column of the EA was ramped from 35 °C

to 240 °C during each analysis to optimize elution of CO₂. The standards USGS-40 and USGS-41 were used to calibrate the data to standard delta notation (δ [‰] = $[R_{\text{sample}}/R_{\text{standard}} - 1] \times 1000$), where $R = {}^{13}\text{C}/{}^{12}\text{C}$ for $\delta^{13}\text{C}$ and $R = {}^{15}\text{N}/{}^{14}\text{N}$ for $\delta^{15}\text{N}$. Analytical quality was monitored with USGS-62 (measured $\delta^{13}\text{C} = -14.58 \pm 0.18$ ‰, $\delta^{15}\text{N} = +20.16 \pm 0.33$ ‰, 1SD), which gave good agreement with expected values ($\delta^{13}\text{C} = -14.79 \pm 0.04$ ‰, $\delta^{15}\text{N} = +20.17 \pm 0.06$ ‰). Replicate analyses on samples yielded a precision of 0.4 ‰ for $\delta^{15}\text{N}$ and 0.5 ‰ for $\delta^{13}\text{C}$. In addition, we analysed three international iron-oxide reference materials and obtained good reproducibility for those as well (Table S-1). The decarbonated residues were too small to perform nitrogen isotope analyses, but they could be used to measure $\delta^{13}\text{C}_{\text{org}}$. To our knowledge, these reference materials have not previously been analysed for nitrogen isotopes, prohibiting us from comparing our results to the published literature. But hopefully, our work will stimulate more work on these materials in the future.

We note that the analytical uncertainty for USGS-62 in our measurements is slightly larger than that of the expected value reported by Schimmelmann *et al.* (2016). In their study, this standard was analysed by nine different laboratories, and the reported standard deviation ranges from 0.02 to 0.34 ‰. Hence our measured standard deviation falls within the range of other laboratories. The fact that it is larger than elsewhere does not impact the interpretation of our data as the total range in $\delta^{15}\text{N}$ values of the samples is more than one order of magnitude greater than the analytical uncertainty.

To ensure that the measured values are not impacted by the low N-quantities and hence small peak sizes in our samples, we looked at the measured $\delta^{15}\text{N}$ values of a series of USGS-41 standards, which were used for calibrating N abundances in the samples (Figure S-1). We find good reproducibility down to peak areas (total integrated peak for both isotopes) of at least 4 Vs, which was the smallest area assessed here. In our sample set, almost all peaks were above 7 Vs and many above 10 Vs, meaning that we have no reason to suspect poor accuracy due to low N abundances. Only two samples produced smaller peaks of 2.5 and 2.8 Vs (SO33-52GTV-1a and SO33-52GTV-2), which explain the slightly larger standard deviations for those, but even those slightly larger uncertainties are still small enough to distinguish those samples from others in the data set.

Lastly, the acid treatment of the samples mobilized carbonate, as well as phosphate and some metal oxides. The total organic carbon content of the residue (TOC_{residue}) was corrected for this mass loss to obtain the total organic carbon content of the bulk rock (TOC_{bulk}). To determine the abundance of total inorganic carbon (TIC), we subtracted the calculated TOC_{bulk} concentration from the measured total carbon content of untreated sample (TC). The isotopic composition of inorganic carbon ($\delta^{13}\text{C}_{\text{carb}}$) was calculated by mass balance, using the abundances and measured values of $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{bulk}}$.



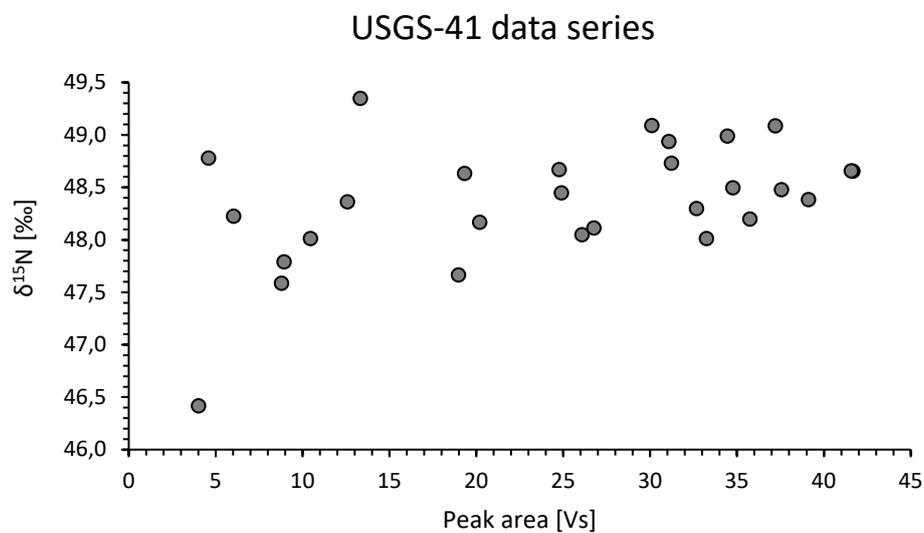


Figure S-1 Series of USGS-41 standards analysed with different masses to test for systematic isotopic bias in small samples. No such bias was found down to peak sizes of at least 4Vs.

Supplementary Table

Table S-1 Analytical results. TN = total nitrogen, TC = total carbon, TOC = total organic carbon, TIC = total inorganic carbon, SD = standard deviation, RE = relative error, HCl loss = fraction of material lost during HCl treatment.

	TN [µg/g]	RE [%]	$\delta^{15}\text{N}_{\text{bulk}}$ [‰]	SD [‰]	TC [wt. %]	RE [%]	$\delta^{13}\text{C}_{\text{bulk}}$ [‰]	SD [‰]	HCl loss [wt. %]	TOC _{resid.} [wt. %]	RE [%]	$\delta^{13}\text{C}_{\text{org}}$ [‰]	SD [‰]	TOC _{bulk} [µg/g]	TIC [µg/g]	$\delta^{13}\text{C}_{\text{carb}}$ [‰]
Hydrogenetic crusts:																
SO66-500	67	2.1	-6.52	0.49	0.39	1.5	-22.78		97.40	2.87		-27.74		744	3122	-21.60
SO66-503	81	0.8	-7.87	0.13	0.37	2.2	-21.91		97.42	3.45		-27.93		890	2803	-20.00
SO66-506	46	0.8	-4.89	0.05	0.37	2.4	-21.50		98.08	4.86		-27.74		933	2769	-19.40
SO66-507	49	3.4	-9.48	0.69	0.44	1.8	-22.08		98.98							
SO66-513	57	2.5	-12.07	0.34	0.14	2.1	-13.76	0.61	96.12	0.23		-28.60		89	1348	-12.78
SO66-518	62	0.9	1.47	0.05	0.46	4.6	-23.03		99.96	9.15		-27.89		36	4585	-23.00
SO66-522	51	2.6	1.08	0.13	0.22	1.4	-16.04	0.20	98.12	0.56		-28.33		106	2084	-15.42
SO66-524	39	1.8	3.04	0.41	0.31	1.6	-17.40	0.24	98.25	1.31		-28.04		230	2880	-16.54
SO66-526	66	1.7	1.68	0.04	0.17	2.1	-17.92	0.96	100.00							
SO66-531	64	0.1	2.98	0.24	0.29	0.1	-13.41	0.00	89.79	0.27		-26.53		280	2621	-12.01
SO66-545	43	3.2	-3.58	0.95	0.34	3.2	-21.44	1.06	98.76							
Hydrogenetic nodules:																
Chinese Nodule-1a	214	2.6	10.04	0.19	0.18	2.8	-18.00	0.40	59.25	0.07		-23.08		304	1446	-16.93
Chinese Nodule-1b	228	3.2	10.11	0.39	0.17	1.8	-19.11	0.01	56.67	0.07	3.5	-22.89	0.34	290	1452	-18.29
Chinese Nodule-2	173	1.6	12.21	0.07	0.10	5.3	-14.07	0.81	49.21	0.04		-19.43		179	810	-12.88
SO33-52GTV-1a	30	5.0	4.30	1.61	0.16	5.0	-11.89	0.82	68.15	0.04		-22.91		121	1492	-11.26
SO33-52GTV-2	31	3.4	4.51	0.94	0.17	4.7	-13.80	0.88	85.21	0.04		-23.95		53	1647	-13.47
Diagenetic nodules:																
Discol-KD1313	151	7.3	5.14	0.98	0.08	2.6	-19.41	0.26	41.20	0.03		-21.07		192	573	-18.85
SO106-159KG	116	2.4	3.66	0.27	0.28	3.0	-25.84	0.44	31.84	0.13		-25.41		913	1910	-26.04
SO106-159KG-125-180um	118	2.3	2.93	0.60	0.11	4.7	-23.50	0.81	32.10	0.07		-24.89		496	571	-18.20
SO106-163KG-1	164	2.2	6.35	0.38	0.17	0.9	-15.83	1.37	34.58	0.06	6.7	-23.34	0.10	397	1307	-14.26
SO106-163KG-1a	163	0.7	6.44	0.36	0.14	3.0	-15.76	0.29	44.98	0.03		-20.84		166	608	-14.55
SO106-163KG-2	83	0.5	6.13	0.08	0.11	5.4	-14.27	0.77	46.16	0.08		-24.69		436	706	-8.34
SO79-62KD-1a	155	4.6	6.79	0.90	0.11	1.1	-18.51	0.38	37.18	0.03	1.6	-20.36	0.02	162	904	-18.18
SO79-62KD-2	82	5.2	6.98	0.30	0.05	2.2	-18.84	0.45	39.74	0.02	2.6	-20.38	0.28	101	370	-18.49



	TN [μg/g]	RE [%]	$\delta^{15}\text{N}_{\text{bulk}}$ [‰]	SD [‰]	TC [wt. %]	RE [%]	$\delta^{13}\text{C}_{\text{bulk}}$ [‰]	SD [‰]	HCl loss [wt. %]	TOC _{resid.} [wt. %]	RE [%]	$\delta^{13}\text{C}_{\text{org}}$ [‰]	SD [‰]	TOC _{bulk} [μg/g]	TIC [μg/g]	$\delta^{13}\text{C}_{\text{carb}}$ [‰]
<i>Certified Reference materials (FeMn nodules):</i>																
CRM-JMn-1 (mix hyd+dia)	125	2.4	6.78	0.02	0.12	9.8	-13.82	0.25								
CRM-Nod-A1 (hydrogenetic)	103	9.8	4.33	0.13	0.20	0.1	-7.99	0.16								
CRM-Nod-P1 (mix hyd+dia)	154	1.4	9.13	0.12	0.20	4.9	-7.03	0.50								



Supplementary Information References

- Schimmelmann A., Qi H., Coplen T.B., Brand W.A., Fong J., Meier-Augenstein W., Kemp H.F., Toman B., Ackermann A., Assonov S., Aerts-Bijma A.T. (2016) Organic reference materials for hydrogen, carbon, and nitrogen stable isotope-ratio measurements: caffeine, n-alkanes, fatty acid methyl esters, glycines, L-valines, polyethylenes, and oils. *Analytical Chemistry* 88, 4294-4302. <https://doi.org/10.1021/acs.analchem.5b04392>
- Stüeken E.E., de Castro M., Krotz L., Brodie C., Iammarino M., Giuzzi G. (2020) Optimized switch-over between CHNS abundance and CNS isotope ratio analyses by Elemental Analyzer-Isotope Ratio Mass Spectrometry: Application to six geological reference materials. *Rapid Communications in Mass Spectrometry*, 34(18):e8821. <https://doi:10.1002/rcm.8821>

