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STABLE ISOTOPE FACILITY
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$\delta^{13}C$ and $\delta^{15}N$ of Natural Abundance Solid Samples by EA-IRMS

Methodology

Solid $\delta^{13}C$ and $\delta^{15}N$ samples are analyzed on one of seven EA-IRMS systems. All systems utilize the same chemistry and are fully equivalent. Result files specify the analytical system in the Mass Spec column that corresponds to an entry in the table below.

Tissue samples are combusted at 950°C in a reactor packed with chromium oxide and silvered copper oxide; complex matrices such as soils, sediments, filters are combusted at 1080°C. Oxygen is dosed with sample introduction to ensure complete combustion. Following combustion, residual oxygen and nitrogen oxides are removed by passing the combustion products over reduced copper at 650°C. Water is trapped by magnesium perchlorate and phosphorous pentoxide. CO_2 and N_2 are separated by a GC column in the Sercon EAs and by an adsorption trap in the Elementar EAs. After separation, an aliquot of the analyte gases is carried to the IRMS for measurement.

Mass Spec	System Description
Cube (Q)	Elementar vario EL cube elemental analyzer interfaced to an Elementar VisION IRMS (Elementar Analysensysteme GmbH, Langenselbold, Germany)
Dave (D)	Elementar vario MICRO cube elemental analyzer interfaced to an Elementar VisION IRMS (Elementar Analysensysteme GmbH, Langenselbold, Germany)
Hydra (H)	Elementar vario MICRO cube elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany) interfaced to a Sercon Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom)
MuadDib (M)	Carlo Erba NC2500 elemental analyzer (Thermo Fisher Scientific, formerly CE Instruments/Carlo Erba) interfaced to a Sercon 20-22 IRMS (Sercon Ltd., Cheshire, United Kingdom)
Nook (N)	Elementar vario MICRO cube elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany) interfaced to a Sercon Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom)
Petra (P)	Sercon Europa ANCA-GSL elemental analyzer interfaced to a Sercon Europa 20-20 IRMS (Sercon Ltd., Cheshire, United Kingdom)
Terra 2.0 (A)	Elementar vario MICRO cube elemental analyzer interfaced to a Elementar VisION IRMS (Elementar Analysensysteme GmbH, Langenselbold, Germany)

Calibration and Reporting of Stable Isotope Ratios

Quality control and assurance materials have been calibrated against international reference materials, including: IAEA-600, USGS40, USGS41, USGS42, USGS43, USGS61, USGS64, and USGS65. All are directly traceable to the primary isotopic reference material for each element (i.e., VPDB for $\delta^{13}C$ and Air for $\delta^{15}N$).

Calibration procedures for solids are applied identically across reference and sample materials. First, a pure CO_2 or N_2 reference gas is used to calculate provisional isotopic values of the sample peaks. Next, isotopic values are adjusted for changes in linearity and instrumental drift using in-house reference materials, Nylon Powder and Glutathione. Finally, measurements are scale-normalized to the primary reference materials using in-house reference materials, Glutamic Acid and Enriched Alanine or Caffeine. Elemental totals are calculated based on IRMS peak area using a calibration curve of linearity reference material Glutathione.

Final quality assessment is based on the accuracy and precision of the unbiased quality control materials.

> Quality assurance reference materials: Nylon, Glutathione, Glutamic Acid, Caffeine, Enriched Alanine, Chitin (being retired), Alfalfa Flour (being retired)
> Quality control reference materials: Amaranth Flour, Scallop

Measurement Uncertainty

Mean measurement error (σ) and accuracy, as determined by replicate measurements of the quality control and assurance materials, must fall below expected measurement error (± 0.2 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$) across well-sized, natural abundance plant and animal samples. Accuracy and precision of the co-measured calibrated quality control and assurance materials are provided with data reports.

Sample materials are inherently variable in composition. Precision for complex matrix samples, such as filters, soils/sediments, salt extracts, and enriched materials may vary. Results for replicate matrix-matched samples should be used to assess analytical precision.

In general, the analytical range is 200–2000 µg C and 20–200 µg N, but additional references are frequently added to cover larger samples. Data outside the range of the references are flagged in the results; those data should be interpreted cautiously.

Limit of quantification (LOQ), based on peak area, is 100 µg C for $\delta^{13}C$ and 20 µg N for $\delta^{15}N$.

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Approved By

Emily Ngo Schick

Glossary

ºC	degrees Celsius
‰	per mil; 1 ‰ is equivalent to 0.001 or 1 mUr
^{13}C	stable isotope of carbon; mass number of 13
^{15}N	stable isotope of nitrogen; mass number of 15
δ	delta notation for isotopic composition; in per mil (“‰”) or mUr; 1 ‰ equals 1 mUr
σ	standard deviation
Air	atmospheric N_2; primary reference for measurements of nitrogen isotopes
Alfalfa Flour	alfalfa flour reference material
Amaranth Flour	amaranth flour reference material
C	carbon
Caffeine	caffeine reference material
Chitin	chitin reference material
CO_2	carbon dioxide
EA	elemental analyzer
Enriched Alanine	alanine reference material enriched in ^{15}N
GC	gas chromatograph
Glutathione	glutathione reference material
Glutamic Acid	glutamic acid reference material
IAEA	International Atomic Energy Agency
IAEA-600	caffeine prepared by W. Brand and R. Werner, Max-Planck-Institute for Biogeochemistry, Jena, Germany
IRMS	isotope-ratio mass spectrometry
LOQ	limit of quantification; minimum signal for analyte to meet required signal-to-noise ratio
mUr	milliurey; 1 mUr is equivalent to 0.001 or 1 ‰
N	nitrogen
NIST	National Institute of Standards and Technology
Nylon	nylon reference material
QA	quality assurance; overall laboratory measures to ensure measurement quality
QC	quality control; activities and procedures used to evaluate quality requirements
RSIL	USGS Reston Stable Isotope Laboratory, Reston, Virginia
Scallop	scallop reference material
USGS	United States Geological Survey
USGS40	L-glutamic acid prepared by H. Qi et al., RSIL, USGS
USGS41	L-glutamic acid enriched in ^{13}C & ^{15}N prepared by H. Qi et al., RSIL, USGS
USGS42	human hair (Tibetan) prepared by H. Qi et al., RSIL, USGS
USGS43	human hair (Indian) prepared by H. Qi et al., RSIL, USGS
USGS61	caffeine prepared by H. Qi, RSIL, USGS, and A. Schimmelmann, Indiana University, Bloomington, Indiana
USGS64	glycine prepared by H. Qi, RSIL, USGS, and A. Schimmelmann, Indiana University, Bloomington, Indiana
USGS65	glycine prepared by H. Qi, RSIL, USGS, and A. Schimmelmann, Indiana University, Bloomington, Indiana
VPDB	Vienna PeeDee Belemnite; primary reference for measurements of carbon isotopes

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