Updated 19.12.2023

$\delta^{13}C$ and $\delta^{15}N$ analysis of organic samples

for example, plant, fungal and animal tissues; fats, oils, plastics, fabric

We perform carbon and nitrogen isotope analyses on a wide range of mostly organic, natural materials. The most common sample types analyzed are plant and animal tissues. We also analyze fats and oil samples. See further below for an analytical description. If you're interested in the analysis of soils, sediments or other mineral rich matrices, see the dedicated information sheet for that analysis type.

General considerations

The necessary sample amount depends on the element's content in the material (dry weight).

Especially N contents in plants and fungi can display significant variability varying up to an order (or two) of magnitude depending on taxon, plant part analyzed and growing conditions. For example, C4 grasses tend to have generally lower N contents than C3 plant leaves, while their inflorescence tends to have higher contents than leaves, stalks or roots. If you're submitting these sample types for analysis**, we ask you to provide your *best guess estimate* of the typical nitrogen/carbon weight-% that is representative of your samples**. Please, search for typical mean values for your specific sample type, and circumstances from scientific literature if you have no measurement data for your own sample set. The **minimum acceptable contents of C and N is <u>0.2 Wt-%</u>.** In some cases we can measure samples down to 0.1 Wt-% at an additional charge, but the feasibility is reviewed on a case by case basis.

Examples of common C and N Wt-% values, and the matching necessary sample weights are compiled in the table below. The sample masses listed are calculated for the lowest Wt-% value in the given typical range, i.e. lower sample masses apply if your material has higher contents of the element. The value in parentheses () is the minimum applicable sample mass for still acceptable analysis quality for the lowest Wt-% value to the left, but these should not be applied unless the availability of sample material is a problem. **To find out the exact required weighing amounts for your samples, contact us at** stableisotopes@helsinki.fi **with the expected C and/or N weight-% values for your samples.**

	C wt-%	**sample mass for C, µg**	**N wt-%**	**sample mass for N, µg**
Hair, horn, hooves, nails (keratin)	45-46	170 (35)	14-16	350 (180)
Invertebrate chitin	42-43	170 (35)	5-7	950 (470)
Bulk animal (soft) tissue	43-44	170 (35)	9-12	460 (260)
Collagen, proteins	40-45	190 (40)	13-16	350 (180)
Fungi	40-45	190 (40)	3-10	1500 (780)
Leaves (C3 plants)	40-45	190 (40)	1-5	4600 (2400)
Roots	40-45	190 (40)	0.5-1	9000 (4700)
Wood (whole)	40-50	190 (40)	0.05-1.5	max. 15000
C4 grasses leaves and stalks	40-45	190 (40)	0.5-2	9000 (4700)
Seeds, grains, flour	40-45	190 (40)	1-2	4600 (2400)
Oils, fats ('wet' weight)	~80	95 (20)	-	-
Plastic (according to type)	50-85	150 (30)	-	-

Updated 19.12.2023

The isotope values of both carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) can be obtained simultaneously ("dual measurement") from the same sample for materials with a low carbon-to-nitrogen ratio**; C/N ≤ 16.5**. For example, most animal tissues have a sufficiently low C/N ratio for this type of analysis. For simultaneous analyses, the approximate sample weight listed above for N applies. For high C/N ratio materials, such as most plant tissues, two separate subsamples of the same material must be weighed and analyzed to obtain both $\delta^{13}C$ and $\delta^{15}N$ values. Check the expected C and N contents and C/N ratio of your sample material to see whether they are eligible for a dual measurement.

Constraining natural variability

Natural materials always contain a certain degree of isotopic variability on top of which come possible effects of sample preparation, and we **recommend analyzing more than one replicate per sample** to get a realistic estimate of the uncertainty for the isotope value. Note that this is a different thing than analytical uncertainty quoted in the analytical report, which only measures the precision of the instrument. If analyzing the whole set of samples in duplicate/triplicate is not feasible, we recommend choosing a representative set of samples, e.g. 10-30% of total sample number, to be analyzed in duplicate or triplicate. However, as prices are charged per analyzed replicate, the decision of replicate IRMS measurements is left up to the customer's discretion.

Sample preparation

Properly dried and homogenized sample powders, or suitable small pieces of the dried sample, are weighed and enclosed into tin (Sn) capsules of appropriate volume (see Table below). Capsule size is chosen to enable proper closing and folding of the cup without material leaking or protruding outside. Fats and oils are analyzed as is, without desiccation.

If you're weighing and closing your samples yourself, please see tips for encapsulating samples below, and note that the Wt-% results are dependent on the accuracy of your weighing. You should use a microbalance giving you 3 decimal digits below 1 mg, i.e. 0.001 mg / 1 µg precision.

Aim at a constant weight with a precision of ± 10% throughout your sample series. For example, aiming at a mean weight of 0.350 mg, you would accept sample weights from 0.315 to 0.385 mg.

Note that for fats and oils, sample weighing for analysis must be done on site at the Laboratory of Chronology right before analysis, as they tend to leak from vials during shipment. Thus for fats and oils, 'weighing into analysis' must be ordered as an additional service, or we can arrange time for you to use the microbalance in our facilities (additional charge for external customers).

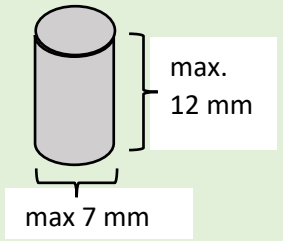
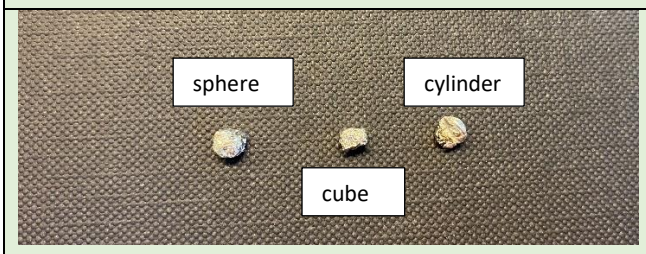
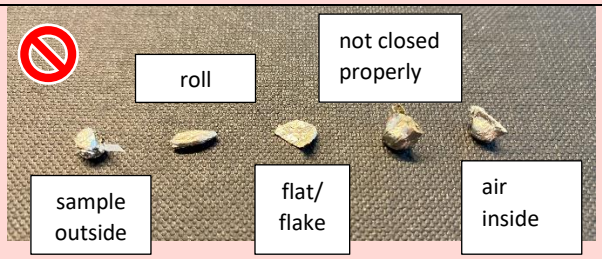
Manufacturer	Product	ID	Size (mm)	Common for sample types
IVA Analysentechnik GmbH & Co. KG	Tin capsules for solids	SA76980002	3.2 x 4	animal tissues, collagen, plants, fungi…
IVA Analysentechnik GmbH & Co. KG	Tin capsules for solids, light	SA76980702L	4 x 6	plant N, soils, sediments, food crust (arch)
IVA Analysentechnik GmbH & Co. KG	Tin capsules for solids	SA76980902	5 x 8	plant N, soils, sediments, food crust (arch)

Table: Tin cups used for C and N isotope analyses at the Laboratory of Chronology. Corresponding capsules are available from many other manufacturers.

Encapsulating samples:

Updated 19.12.2023

The aim is to have all the sample material inside, all the air squeezed out, nothing leaking out, and have a shape that is bulky (3D) enough that it will not get stuck and wedged in the narrow spaces left between the moving and stationary parts of the autosampler.

YES	NO
☺ compress, squeeze out air from sample+capsule	☹ squeeze/fold only top part of tin cup closed
☺ make bulky shape: spherical, cubical, cylinder…	☹ folded into a flat/ flake-like/ roll-like shape
☺ fold and crimp in the opening	☹ sample is leaking or protruding out of cup
 After closing and compaction, capsule must fit in a cylidrical shape of max 7 x 12 mm	NOTE THAT IF YOU'RE SAMPLES ARE SOMETHING DESCRIBED ABOVE OR PICTURED BELOW, WE WILL HAVE TO REPACK/ RESHAPE THEM AT AN ADDITIONAL CHARGE.
	

IRMS-analysis and data normalization

The isotopic composition of carbon and nitrogen is measured on a Carlo Erba NC2500 elemental analyzer coupled to a Thermo Scientific Delta V series isotope ratio mass spectrometer in continuous flow mode. Alongside samples, each analytical run contains ca. 30% reference materials that are used for calibration (i.e. normalization) of isotope values and for quality control. The isotope values of the samples and QC materials are normalized using the known isotope values of *at least two different* calibration reference materials included in the run. We use several internationally recognized secondary reference materials for this purpose: USGS-40, USGS-41, IAEA-N1, IAEA-N2, IAEA-CH3, IAEA-CH6 and IAEA-CH7. The QC materials are chosen to correspond to the analyzed sample matrix as closely as possible. Data normalization is performed using the 'LIMS for Light Stable Isotopes' software developed by Tyler Coplen of the Reston Stable Isotope Laboratory at the US Geological Survey. The long-term analytical precision is ± 0.2 for both $\delta^{13}C$ and $\delta^{15}N$ values.

In addition to the requested isotope values, the report of analysis also includes the Weight-% of the element(s) in the samples, a method description and standard deviations of the reference materials to evaluate internal precision. If the customer wishes to also receive a copy of the uncorrected, 'raw' data, this can be made available **upon prior agreement**.