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High-throughput method for simultaneous quantification of N, C and S stable isotopes and contents in organics and soils

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RATIONALE: Information about the sulfur stable isotope composition ($\delta^{34}S$ value) of organic materials and sediments, in addition to their nitrogen ($\delta^{15}N$ value) and carbon ($\delta^{13}C$ value) stable isotope compositions, can provide insights into mechanisms and processes in different areas of biological and geological research. The quantification of $\delta^{34}S$ values has traditionally required an additional and often more difficult analytical procedure than NC dual analysis. Here, we report on the development of a high-throughput method that simultaneously measures the elemental and isotopic compositions of N, C and S in a single sample, and over a wide range of sample sizes and C/N and C/S ratios.
METHODS: We tested a commercially available CHNOS elemental analyzer in line with an isotope ratio mass spectrometer for the simultaneous quantification of N, C and S stable isotope ratios and contents, and modified the elemental analyzer in order to overcome the interference of ^{18}O in $\delta^{34}S$ values, to minimize any water condensation that could also influence S memory, and to achieve the complete reduction of nitrogen oxides to N_2 gas for accurate measurement of $\delta^{15}N$ values. A selection of organic materials and soils was analyzed with a ratio of 1:1.4 standards to unknowns per run.
RESULTS: The modifications allowed high quality measurements for N, C and S isotope ratios simultaneously (1 SD of ±0.13‰ for $\delta^{15}N$ value, ±0.12‰ for $\delta^{13}C$ value, and ±0.4‰ for $\delta^{34}S$ value), with high throughput (>75 unknowns per run) and over a wide range of element amount per capsule (25 to 500 μg N, 200–4000 μg C, and 8–120 μg S).
CONCLUSIONS: This method is suitable for widespread use and can significantly enhance the application of $\delta^{34}S$ measurements in a broad range of soils and organic samples in ecological and biogeochemical research. Copyright © 2016 John Wiley & Sons, Ltd.

Information about the natural abundance of stable isotopes is now regularly integrated into a wide range of studies. Isotope data can be used to indicate, record, trace and interpret fundamental processes, interactions and changes within biological, ecological, climatological and geological systems.[1–3] Many investigations, particularly in the biological sciences, have focused on quantifying variation in carbon (C) and nitrogen (N) stable isotope ratios. This is because a great deal is understood about what leads to their variation and because the dual stable isotope analysis of these two elements using gas-source continuous flow isotope ratio mass spectrometry (CF-IRMS) is simple and cost-effective.

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However, the aforementioned studies might all benefit by including details of sulfur (S) stable isotope ratios to glean additional information about the biological and inorganic reactions occurring in environmental systems.[4–7] For example, the inclusion of S as a third element during multi-isotope analysis would facilitate a deeper understanding of the elemental cycles of N, C and S and their interactions,[8] the movement and transformations of organic matter in ecosystems and food webs,[9,10] the exchanges of organic matter and nutrients between ecosystems and habitats,[11] and investigations on animal movements.[12]

Unfortunately, the measurement of $\delta^{34}S$ values by CF-IRMS is more problematic than that of C and N stable isotope ratios. The S content is usually much lower than that of C and N in biological or geological samples, and sulfur dioxide (SO_2) produced from sample combustion tends to adhere to surfaces within the system, slowly desorbing throughout the run and producing a memory effect.[13] Concurrent measurement of NCS isotope ratios has been difficult to perform and is not yet practised widely due to several technological constraints:

(a) a traditional bulk measurement of S isotopes uses a combined combustion/reduction tube that limits space for reduction and limits throughput; (b) nitrogen gas (N_2), carbon dioxide (CO_2) and SO_2 derived from sample combustion have been difficult to separate using one gas chromatography (GC) column; (c) ash buildup during analysis causes deterioration of SO_2 peak shape; (d) possible SO_2 memory effect in the NCS configuration, and (e) ^{18}O interference in $\delta^{34}S$ measurement.[13–15]

An additional challenge for the simultaneous NCS determination, in particular for soils, is the ratio of these elements in a sample. A large sample size (30–130 mg) is usually required to reach a high enough concentration for S analysis, but samples of this size often overwhelm the IRMS system with too much CO_2 or N_2 gases produced by combustion.

It is common to analyze each organic or soil sample twice, once for C and N isotopes, and once for S isotopes. This approach severely restricts the number of samples that can be analyzed, as it is time-consuming and costly. In many cases it renders it impractical to adequately determine and interpret the field variation of all three isotopes. For example, very few studies have looked at isotope profiles of all three elements in soils,[16,17] limiting our ability to explain the patterns of element cycling in environmental systems.

In recent years, systems allowing for the simultaneous quantification of NCS isotope ratios have become operational in a few laboratories.[13,18–20] However, most of the published NCS analytical methods have relatively low throughput (<50 samples plus standards per day), or have not addressed the issue of interferences, such as nitrogen oxides (NO_x) in $\delta^{15}N$ measurements or of ^{18}O in $\delta^{34}S$ measurements, or have not been tested over a wide range of sample NCS contents and sample C/N and C/S ratios.

A high-throughput method for the simultaneous assessment of NCS isotopes is clearly necessary to increase our knowledge of S dynamics and NCS interactions. In order for the method to be of value, it must be able to accurately analyze a comprehensive range of organics and soil types and sample sizes, while also keeping costs low and sample run time relatively short. With these requirements in mind, we report here on the development of a new high-throughput method that simultaneously quantifies the elemental and isotopic compositions of NCS in a single sample and across a wide sample size range, and that requires daily instrument maintenance slightly more complex than NC analysis alone, but not as difficult as that required for most S methods alone. Based on our tests, the proposed high-throughput method is robust enough for the routine simultaneous determination of NCS isotopes in a range of organics and common soil types.

EXPERIMENTAL

Instrument configuration and modifications for NCS analysis

We tested and modified a new commercially available CHNOS elemental analyzer (vario ISOTOPE cube, Elementar, Hanau, Germany) interfaced in line with a gas isotope ratio mass spectrometer (IsoPrime 100, Isoprime Ltd, Cheadle, UK). The mass spectrometer is fitted with a 100-V head amplifier to provide a wide measuring range and universal triple gas detectors for isotope measurement of N_2, CO_2 and SO_2. Computer control of the mass spectrometer allows rapid switching between gases with specific tunings and magnet settings for each gas to allow measurement of all three gases in each sample (Supplementary Table S1, Supporting Information). The stability and linearity of the working gases were tested periodically and were found to be within the acceptable limits given by the manufacturer of the mass spectrometer.

The original CHNOS elemental analyzer pneumatic configuration in NCS mode and the modifications added are shown in Fig. 1. This system uses separate combustion and reduction tubes and is based on a patented 'purge and trap' technology for gas separation. Our main modifications to the CHNOS elemental analyzer were aimed: (a) to minimize ^{18}O interference and memory effect for accurate $\delta^{34}S$ measurement by adding a quartz tube as a buffering reactor[13] using the third heater provided with the original configuration, and placing an additional drying tube immediately after the quartz tube, and (b) to achieve complete reduction of NO_x to N_2 gas for accurate $\delta^{15}N$ measurement by adding a second reduction tube in an external heater. We also substituted the original water trap connected to the original (1st) reduction tube with a custom-designed trap positioned directly on top of the tube and divided into two sections for easy removal and daily change of the drying agent. The cost of the modifications applied to the CHNOS elemental analyzer was approximately US$1000, mostly for the external reduction furnace.

Method of NCS measurement with modified elemental analyzer configuration

The NCS analysis was performed using: (1) a helium (He) carrier gas flow rate of 220 mL/min; (2) a 1150 °C combustion tube containing 50 mm of tungsten trioxide (WO_3) at its center with an ash finger placed on top of it; (3) a 880 °C reduction tube packed with around 100 g of pure copper (Cu) in the center; (4) a 900 °C buffering quartz tube filled with quartz chips and 20 mm cupric oxide (CuO) in the center; (5) several water traps; (6) a second 650 °C Cu reduction tube; and (7) CO_2 and SO_2 traps (Fig. 1).

During the analysis, samples wrapped in tin capsules and placed in a 120-space autosampler were sequentially dropped into the combustion tube by a ball valve mechanism. Oxygen (O_2), which is added at variable flow rate and dosing time depending on sample type and weight (Supplementary Table S1, Supporting Information), ignites the tin, and the organic compounds in the samples are oxidized to NO_x, N_2, CO_2, SO_2, sulfur trioxide (SO_3), and H_2O. These gas molecules move through a heated (130 °C) quartz bridge to the reduction tube kept at 880 °C, where the excess O_2 is reduced to copper oxide (Cu_2O) and SO_3 is reduced to SO_2. Excess water is also removed by the water trap filled with magnesium perchlorate ($Mg(ClO_4)_2$) placed on top of the reduction tube.

The gas stream containing NO_x, N_2, CO_2, and SO_2 then flows through a 900 °C quartz tube that minimizes interferences from ^{18}O,[13,14] and then to a phosphorus pentoxide (P_2O_5) water trap to remove any final traces of water. Subsequently, SO_2 is specifically trapped in a 100 °C trap with a proprietary absorber while NO_x, N_2 and CO_2 are allowed to pass through the trap. The NO_x are completely reduced to N_2 in the 2nd reduction tube (450 mm × 6 mm diameter) filled with Cu and

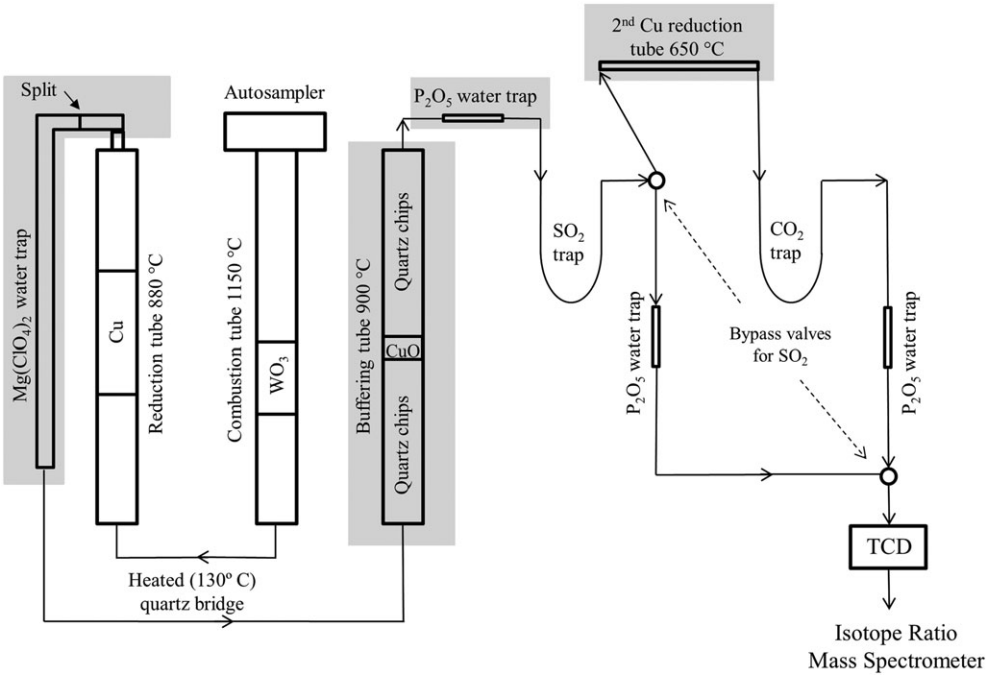

Figure 1. Schematic of the CHNOS elemental analyzer for the simultaneous elemental separation and quantification of nitrogen, carbon and sulfur contents in a single sample. Modifications to the original configuration are shown in gray.

kept at 650 °C. CO_2 is then trapped on a CO_2 trap with a specific absorber held at 38 °C. N_2 passes to a thermal conductivity detector (TCD) where the N content is measured, then through the capillary split to the mass spectrometer for N isotope measurement. As the CO_2 trap is heated to 130 °C, CO_2 elutes as a sharp peak through the TCD for C content measurement, and it is then transferred to the mass spectrometer for C isotope measurement. Bypass valves are then activated and, as the SO_2 trap is heated to 240 °C, SO_2 bypasses the CO_2 trap and elutes as a sharp peak through the TCD for S content determination. SO_2 is then transferred to the mass spectrometer for S isotopic measurement. Detailed information about the CHNOS elemental analyzer and the IRMS settings are reported in Supplementary Table S1 (Supporting Information).

The samples were analyzed within a wide range of element amount per capsule (25–500 µg N, 200–4000 µg C, and 4–120 µg S), and of C/N and C/S ratios. Each sample analysis of N, C and S elemental composition and isotope ratio determinations takes 10 min, allowing the analysis of at least 75 unknown samples per day plus standards.

Although the combustion tube is capable of analyzing over 1000 samples before being changed, it became apparent that as the number of samples analyzed increased, the memory effect for SO_2 slightly increased. Therefore, we used the combustion tube for 3 days of NCS analysis in total (around 400 capsules). Subsequently, the same tube was used in routine NC mode of analysis, or cleaned and reused in the NCS mode of analysis. The cleaning procedure involved immersing the bottom 10 cm of the tube (once emptied) and the quartz bridge between the combustion and reduction tube in a cleaning solvent (1% Micro cleaning solution, Electron Microscopy Sciences, Hatfield, PA, USA) inside an ultrasonic bath overnight.

Recommended maintenance routines included: daily changing of the ash finger, the Cu in the 880 °C reduction tube and the used $Mg(ClO_4)_2$ in the trap placed on top of the same reduction tube. In addition, the quartz chips and the CuO inside the buffering quartz tube were changed after every 2000–3000 analyses, the drying agent in the water traps was replaced after every 600–800 analyses, and the Cu in the 2nd reduction tube was changed only when the mass 30 signals in N_2 measurements increased (after approximately every 1000–2000 analyses).

Data correction

The stable isotope abundances are presented in δ notation as deviations from the standard references (atmospheric nitrogen (AIR), Vienna PeeDee Belemnite (V-PDB) and Vienna Canyon Diablo Troilite (VCDT) for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values, respectively) in parts per thousand (‰) according to the following equation:

$\delta X = \left(R_{sample}/R_{standard}\right) - 1$ where X represents ^{15}N, ^{13}C or ^{34}S and R the ratio of the heavy and light isotope (e.g., $^{15}N/^{14}N$) in the sample and in the standard, respectively.

Post-analysis corrections of the measured delta values (raw delta values) were based on representative organic materials with different N, C and S contents and isotope compositions which included bovine liver (SRM 1577b), available from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), and with known elemental composition, and two working in-house standards – fish and algae. These materials were previously calibrated versus the $\delta^{15}N$ and $\delta^{13}C$ values of IAEA (International Atomic Energy Agency, Vienna, Austria) standards in our laboratory, and versus the $\delta^{34}S$ values of silver sulfide standards, IAEA S-1 and IAEA S-2, in this study.

A typical NCS run during normal operation included: (a) SRM 1577b at 3.8–4.2 mg (every 12 samples); (b) SRM 1577b (n = 10) at variable weights interspersed with the unknown

samples; and (c) fish and algae in-house standards (n = 10 each) at variable weights interspersed with the unknown samples.

The post-analysis processing procedure of the raw isotope values involved these steps in the following order: (i) correction of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ raw values for drift in time based on SRM 1577b at 3.8–4.2 mg; (ii) memory correction of drift-corrected $\delta^{34}S$ values; (iii) blank correction of drift-corrected $\delta^{15}N$ values based on SRM 1577b at variable weights; (iv) blank correction of drift- and memory-corrected $\delta^{34}S$ values based on fish and algae in-house standards at variable weights; and (v) conversion to the international stable isotope reference scale (normalization). Unless otherwise indicated, the results presented in the tables and figures refer to post-analysis processed data.

The SRM 1577b standard randomly placed within each sample list at variable weights was not used for S data correction, and therefore functioned as a quality control (QC) for ^{34}S measurement. Similarly, fish and algae in-house standards randomly placed within each sample list at variable weights were not used for ^{15}N and ^{13}C data correction, and functioned as the QCs for ^{15}N and ^{13}C isotopic measurements.

The presence of an S memory effect, or carry-over of residual amounts of S from one sample to the other, was tested and corrected for as described below. No memory effect was detected for N and C isotopic measurements.

The N and S blank corrections for unknown samples were performed by mass balance using the subtraction method,[21] where the sample blank corrected delta value is obtained by subtraction of the blank as:

$$\delta_S = [(n_T)\delta_T - (n_B)\delta_B]/(n_T - \delta_B) \quad (1)$$

where δ_S is the sample blank-corrected δ value (‰), n_T is the measured amount of total element in the sample (μg), δ_T is the sample measured value (‰), n_B is the estimated amount of element in the blank (μg), and δ_B is the estimated blank δ value (‰). This approach requires an estimation of the amount and isotope data of the blank.

For the N blank estimation, we followed the semi-indirect method,[22] where n_B is estimated from direct measurement, while the δ_B value is calculated using regression parameters (intercept and slope) of a linear regression of the delta value versus the inverse of the observed sample size of multiple measurement results for one reference material. The blank correction based on measurements of SRM 1577b, at variable weights and interspersed with the unknown samples, was effective in yielding accurate $\delta^{15}N$ values even for small nitrogen samples of fish and algae in-house standards.

The S blank estimation proved to be more challenging and required a hybrid approach between indirect[21] and semi-indirect[22] methods that is fully described in the Results and Discussion section. No blank correction for C was necessary.

Evaluation of instrument performance in NCS configuration

We characterized the system with respect to (1) ^{18}O interferences in $\delta^{34}S$ measurement, (2) S memory in $\delta^{34}S$ measurement, (3) optimization of $\delta^{15}N$ measurement, and (4) accuracy and precision of NCS isotopic measurements of organics and soils.

^{18}O interferences in $\delta^{34}S$ measurement

In order to evaluate the magnitude of the interference of ^{18}O in $\delta^{34}S$ analysis,[13,14] we analyzed the same amount of barium sulfate material (~15 μg S in capsule) with varying amounts of glucose (Alfa Aesar, Ward Hill, MA, USA) added to create mixtures with C/S ratios between 10 and 300 (n = 41) and without glucose (C/S = 0, total n = 12). Measurements were performed using three different configurations of the CHNOS elemental analyzer: (1) configuration with P_2O_5 as the drying agent inside the water trap positioned on top of the 1st reduction tube and without the buffering quartz tube in line (n = 17 mixture, n = 4 pure); (2) configuration with a different drying agent (by substituting P_2O_5 with $Mg(ClO_4)_2$ but still without the buffering quartz tube in line) (n = 16 mixture, n = 4 pure); and (3) modified configuration (Fig. 1) with the buffering tube in line and using $Mg(ClO_4)_2$ as the drying agent (n = 20 mixture, n = 4 pure).

Memory in $\delta^{34}S$ measurement

Sample-to-sample S memory in NCS mode was investigated by analyzing IAEA silver sulfide and barium sulfate standards alternated with a series of commercially available barium sulfate materials using the modified CHNOS elemental analyzer configuration (Fig. 1).

Optimization of $\delta^{15}N$ measurement

Preliminary NCS analysis of SRM 1577b using the modified CHNOS elemental analyzer configuration but without the 2nd Cu tube in line produced erratic $\delta^{15}N$ values compared with the true values. The isotopic N chromatograms had abnormally high mass 30 signals, suggesting that the reduction of NO_x to N_2 gas was not complete. Thus, we designed a specific comparison by analyzing SRM 1577b at different weights, and therefore different N contents (30–400 μg N), with (n = 36) and without (n = 36) an external 2nd reduction tube both with and without the addition of the same amount (5 mg) of sucrose (Difco Laboratories Inc., Detroit, MI, USA) added in order to create mixtures with different C/N ratios (4.7–80).

Accuracy and precision of NCS isotopic measurements in organics and soils

The reliability of the data obtained with the modified CHNOS elemental analyzer configuration (Fig. 1) was initially tested on a series of internationally certified standards for $\delta^{15}N$ values (ammonium sulfate IAEA N-1, potassium nitrate IAEA NO-3 and caffeine IAEA 600), $\delta^{13}C$ values (sucrose IAEA CH-6, graphite USGS24 and caffeine IAEA 600) and $\delta^{34}S$ values (silver sulfides IAEA S-1 and IAEA S-2, and barium sulfates IAEA SO-5 and NBS 127). These standard materials were analyzed in three different batches.

QC assessment was based on the isotopic results of multiple analyses of SRM 1577b (n = 94) as the QC of the corrected $\delta^{34}S$ data, and of the fish (n = 93) and the algae (n = 92) in-house standards as the QCs for the corrected $\delta^{15}N$ and $\delta^{13}C$ data treated as unknown samples and analyzed in multiple batches (n = 8–10). The measured isotopic values were then compared with published or laboratory calibrated isotopic values.

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Evaluating the accuracy and precision of our method for soils was more problematic, as there is no certified standard for $\delta^{34}S$ values in soils and there are very few published data on soil $\delta^{34}S$ values. As a result, we chose to test one sediment material (High Organic Sediment Standard OAS Cat No. B2151, Certificate No. 162517) and one soil material (Low Organic Content Soil Standard OAS Cat No. B2153, Certificate No. 114524) from Elemental Microanalysis Ltd (Okehampton, UK) (EM) that have certified NCS elemental composition values and certified values for $\delta^{15}N$ and $\delta^{13}C$, but only approximate $\delta^{34}S$ values. These two materials were separately compared at two different sample sizes in order to cover a range of N, C and S contents.

In order to test soil materials that cover the typical range of NCS contents and isotope values in soils, we extended the above-mentioned test to include two other soil types, Icacos (Caribbean National Forest, Puerto Rico) and Yolo (Yolo County, CA, USA), from the National Cooperative Soil Survey, USA.[23] Icacos soils are very deep, poorly drained and acidic soils on perennial river flood plains that support tropical rain forests. Yolo soils formed in fine-loamy alluvium derived from sedimentary formation have neutral pH and are used for intensive row, field and orchard crops. These soils were run in triplicate at different weights (Icacos at 40, 60, and 80 mg, for a total of 9 samples; Yolo at 50, 70, 100, 120, and 140 mg, for a total of 15 samples).

Since the measurements were fully replicated, one-way analysis of variance (ANOVA) (Systat 10.8, Systat Software, Inc., San Jose, CA, USA) was performed to compare soil sample size separately for each soil type.

RESULTS AND DISCUSSION

Using 'purge and trap' chromatography in the vario ISOTOPE cube, N_2, CO_2 and SO_2 gases can be effectively separated with complete baseline resolution in 10 min, as shown in the representative isotopic chromatogram during the simultaneous NCS analysis of 4 mg of SRM 1577b (Fig. 2). In addition, with the use of wide trapping columns, there was no deterioration in the SO_2 peak shape during the daily run even after 120 capsules, as illustrated in the insert in Fig. 2. This allows the number of analyses performed per day to be maximized and the number of standards versus unknown analyses to be minimized compared with other NCS analytical methods.[13] In addition, the common procedure for minimizing asymmetrical S peaks by adding relatively toxic catalysts, such as vanadium oxide or niobium oxide, to all samples was not necessary using our NCS configuration (on average, we found that the differences in the $\delta^{34}S$ values of different types of organic samples between using a catalyst and not using a catalyst were <0.2 ‰ (n = 30).

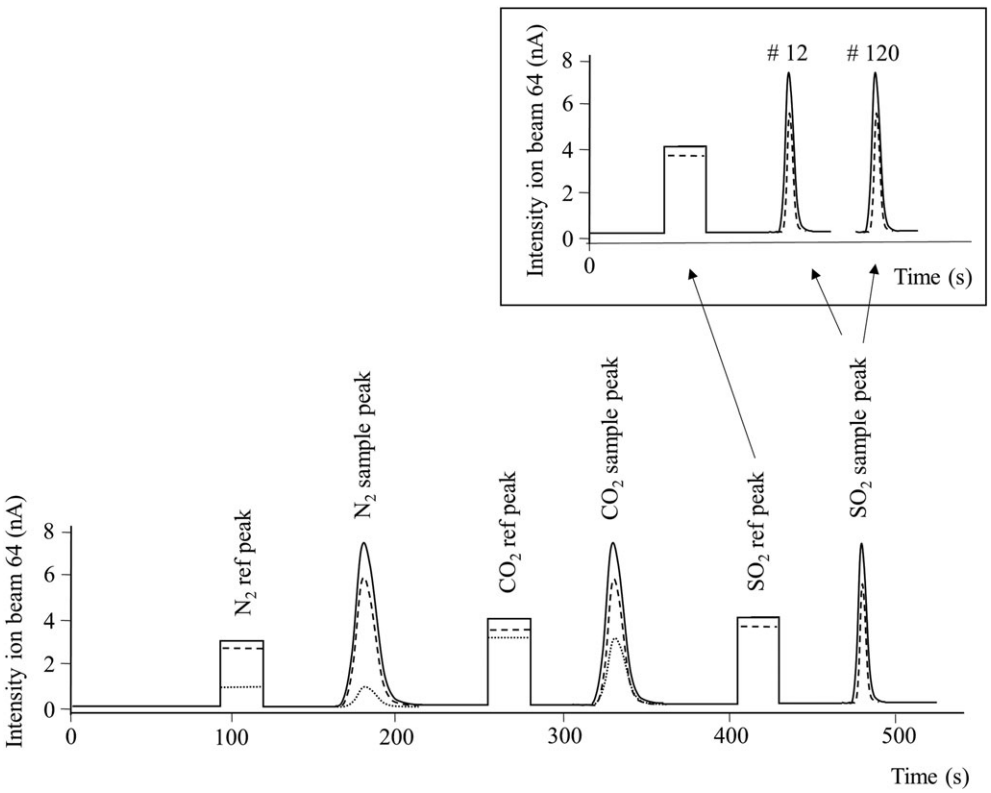

Figure 2. Example of isotopic chromatogram measured with the IRMS system during simultaneous NCS analysis of 4 mg SRM 1577b bovine liver. The insert shows the SO_2 peak shape of 4 mg SRM 1577b bovine liver at the beginning and at the end of the 120 sample queue on the same day. The lines represent: masses 28, 44 and 64 (straight), masses 29, 45 and 66 (dashed), and masses 30 and 46 (dotted) for N_2 , CO_2 and SO_2, respectively.

^{18}O interferences in $\delta^{34}S$ measurement

Previous studies have shown that the oxygen isotope variations of SO_2 produced by online automated preparation systems affect the measured sulfur isotopic compositions, particularly of organic materials.[13–15] Since the S isotopic composition is measured by IRMS as the ratio of masses 66/64 of SO_2, the presence of ^{18}O in the SO_2 molecule ($^{32}S^{16}O^{18}O$) can contribute to mass 66. The combustion of organic material generates water that constitutes an additional ^{18}O source for the produced SO_2, possibly via oxygen exchange reactions between quartz, SO_3 and SO_2 in the combustion reactor.[14] On the contrary, very little or no water is produced from the combustion of inorganic S compounds. Post-analysis corrections for oxygen isotope contributions rely on the assumption that the oxygen content and isotope composition of the SO_2 generated from samples and from reference materials are identical. Unfortunately, there are currently no organic materials with certified $\delta^{34}S$ values with which to test these assumptions.

When a CHNOS elemental analyzer configuration similar to the original design is used (e.g., with P_2O_5 as drying agent inside the water trap positioned on top of the 1st reduction tube and without the buffering quartz tube in line), the amount of C and therefore of oxygen produced by water formation during combustion increases, resulting in the raw $\delta^{34}S$ values of barium sulfate/glucose mixtures changing by up to 2‰ compared with the raw $\delta^{34}S$ values of pure barium sulfate (Fig. 3, open circles; see also previous research[13–15]). Substituting the drying agent with $Mg(ClO_4)_2$ improved the reliability, but the variability across C/S ratios was still higher than the acceptable standard deviation (mean difference from pure barium sulfate of 0.42 ± 0.29 ‰) (Fig. 3, closed circles).

After testing various configurations for ^{18}O interferences in $\delta^{34}S$ measurement, we adopted a published method for minimizing the oxygen effect by buffering the isotopic composition of SO_2 gas produced from organic samples via isotopic exchange reaction between SO_2 and quartz (SiO_2). Specifically, buffering occurred in the presence of a trace amount of water in a large uniform reservoir of oxygen in the form of quartz chips that were contained in a heated furnace.[14] After this quartz buffering tube was added,[14] the $\delta^{34}S$ values of barium sulfate/glucose mixtures did not change as the C/S ratio increased and were similar to the values of pure barium sulfate (Fig. 3, open triangles). These comparisons illustrate that the quartz buffering tube added to the NCS configuration (Fig. 1) effectively buffers the oxygen in SO_2, provided that magnesium perchlorate is used as the drying chemical. In our experience, reliable buffering of the oxygen requires the use of $Mg(ClO_4)_2$ and not P_2O_5 as the drying agent, probably because the trace amount of water allowed to pass by the $Mg(ClO_4)_2$ is necessary for oxygen buffering to occur.

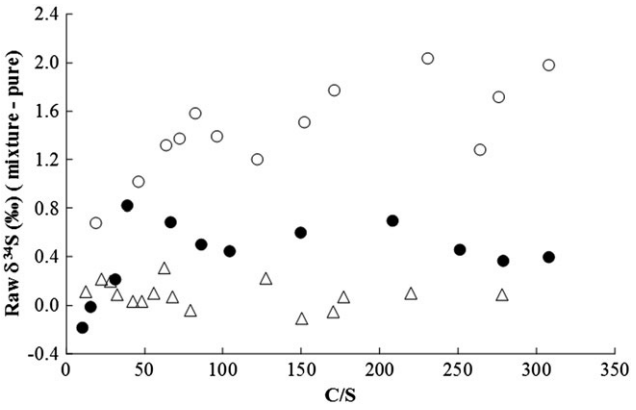

Figure 3. ^{18}O interferences in $\delta^{34}S$ measurement. Barium sulfate molar C/S ratios versus the difference between the raw $\delta^{34}S$ values (‰) measured for barium sulfate/glucose mixture (C/S = 10–300, total n = 41) and those measured for pure barium sulfate (C/S = 0, total n = 12) using the following CHNOS elemental analyzer configurations: (a) with P_2O_5 as the drying agent inside the water trap positioned on top of the 1st reduction tube and without the buffering quartz tube in line) (n = 17 mixture) (open circles); (b) with different drying agent (by substituting P_2O_5 with $Mg(ClO_4)_2$ and without the buffering quartz tube in line) (n = 16 mixture) (closed circles); and (c) modified configuration (Fig. 1) with the buffering tube in line and Mg $(ClO_4)_2$ as the drying agent (n = 20 mixture) (open triangles).

Memory in $\delta^{34}S$ measurement

Since SO_2 can persist in the analytical system, the quantification of the contamination from the previous sample is an important aspect to consider. Our initial carry-over test showed that the sample isotope value was influenced, on average, by <2% from the isotope value of the precursor sample based on the difference between the raw non-memory corrected $\delta^{34}S$ values and the raw memory corrected $\delta^{34}S$ values of several IAEA standards and commercially available barium sulfate materials with different isotopic compositions and analyzed at the same amount of S (~25 μg S per capsule) (Fig. 4).

However, during regular laboratory operation, samples within a wide range of sulfur amounts (4–120 μg S per capsule) were analyzed. Therefore, the S memory was calculated not as a fixed amount of exchange based on the delta value, but based on the percentage of the peak area of the previous sample that carries over into the next sample peak area. The following equation was applied:

$$\delta_{S\ mem\ corr} = [((A_S)(\delta_S))-((\%A_{PS})(\delta_{PS}))]/(A_S-\%A_{PS}) \quad (2)$$

where $\delta_{S\ mem\ corr}$ is the sample memory corrected δ value (‰), A_S is the sample peak area (nA), δ_S is the sample δ value (‰), A_{PS} is the peak area (nA) of the previous sample, and

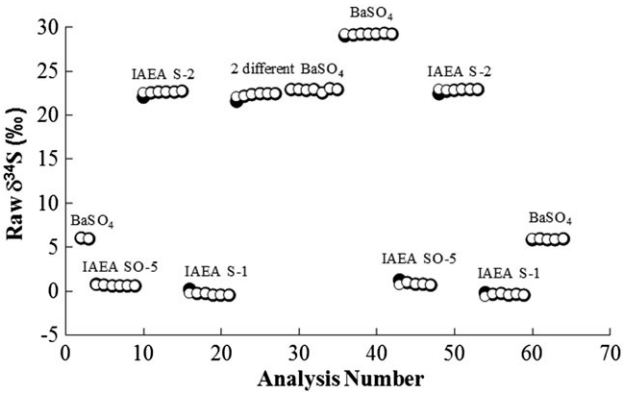

Figure 4. Memory in $\delta^{34}S$ measurement. Raw $\delta^{34}S$ values (‰) of several IAEA standards and commercially available barium sulfate materials with different isotopic compositions analyzed in NCS mode in sequence as original data (closed symbols) and after carryover correction (open symbols) (total n = 63).

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δ_{PS} is the previous sample δ value (‰). For each run, we adopted the peak area percentage value that minimized the drift correction based on the actual $\delta^{34}S$ value of SRM 1577b (i.e., calibrated against IAEA S1 and IAEA S2). The carry-over varied among batches but was found always to be between 0.5 and 1.5%, in agreement with previously published carry-over effects for $\delta^{34}S$ measurements of organic materials.[13]

Adding a drying tube directly on top of the reduction tube (a modification from the original configuration of the CHNOS elemental analyzer, Fig. 1) helped to minimize the potential condensation of water and the dissolution of SO_2 in the water film, which could potentially result in memory effects for SO_2 isotopic analysis.

We also observed that anoxic sediments have the tendency to show quite asymmetric SO_2 peaks, with possible confounding effects on subsequent samples. We recommend performing a specific test to quantify the memory effect when analyzing these types of soil samples for $\delta^{34}S$ values.

Optimization of S blank correction

The blank contribution to the isotopic composition of a sample represents a systematic error and a blank correction is usually performed,[24] particularly to improve the data for small samples. In this study, we first tested if a blank correction was necessary by evaluating the relationship between the drift- and memory- corrected $\delta^{34}S$ values of SRM 1577b, fish and algae in-house standards and the size (µg S per capsule) of the sample. Even with optimization of the S peak integration, we found that the SRM 1577b drift- and memory-corrected $\delta^{34}S$ values were relatively constant with size, while the drift- and memory- corrected $\delta^{34}S$ values of the fish standard and of the algae standard became progressively lower and higher, respectively, below a sample size of 15 µg S per capsule (Fig. 5(a)). It is important to mention that this effect is only noticeable if a range of standards with different $\delta^{34}S$ values over a range of sizes is measured. When an analytical blank is present, the isotopic ratio actually determined during the mass spectrometric measurement is that of the sample plus the blank, and the discrepancy between the observed and true values is usually higher for small samples.

As mentioned in the Experimental section, in order to apply a mass balance approach and subtract the blank effect from the sample delta (Eqn. (1)), knowledge of the amount and isotopic value of the blank is necessary. The S blank is usually so small that its size and delta value cannot be measured precisely. Therefore, we applied published blank estimation procedures.[21,22] Initially, we assessed these blank parameters using the indirect method,[21] where the blank size and isotope value are calculated from the intersection of the linear regression solutions of the delta value with the inverse of the observed sample size of multiple measurements for two reference materials with different isotopic composition. Our initial evaluation using fish and algae in-house standards measurements from different batches (n = 4) resulted in estimated blank sizes of <1 µg S and blank $\delta^{34}S$ values of 5 ± 2 ‰, depending on the batch (Fig. 5(b), example of the results obtained using same measurement data as in Fig. 5(a)).

However, when these blank values were subtracted from the fish and algae $\delta^{34}S$ values using Eqn. (1), the blank

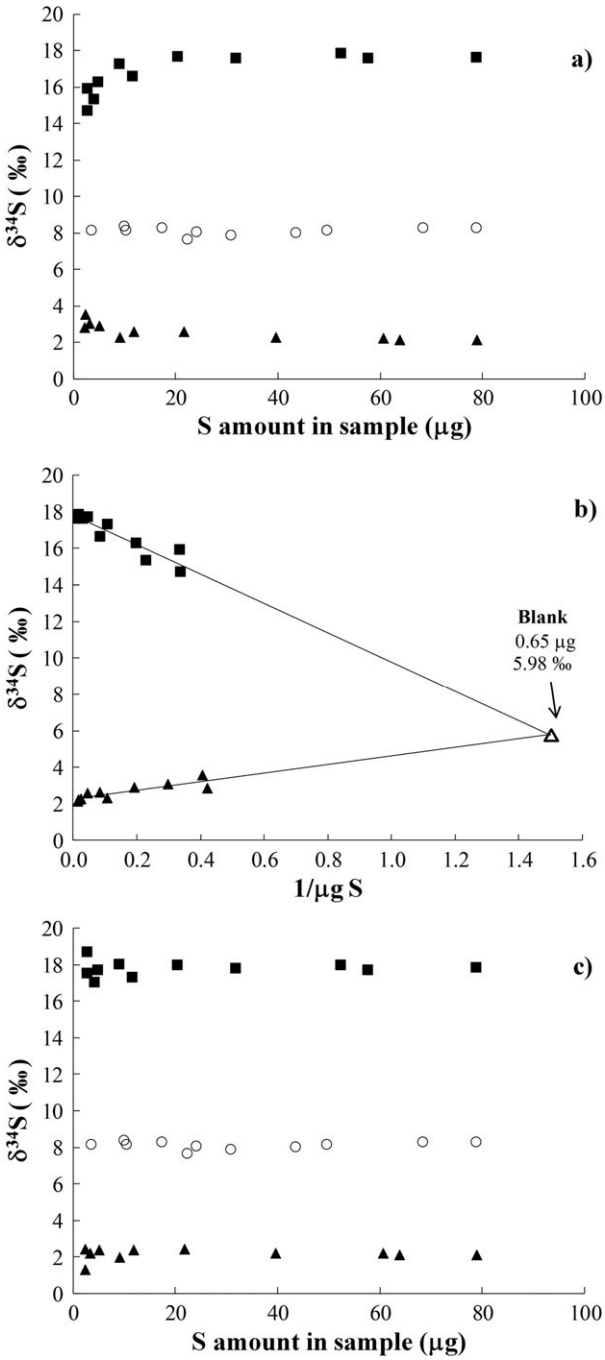

Figure 5. Example of optimization of S blank correction using data from a typical batch of analysis: (a) relationships between the $\delta^{34}S$ values (‰) (drift- and memory-corrected) and the S amount in sample (µg) of SRM 1577b bovine liver (open circles, n = 10), fish (closed squares, n = 10) and algae (closed triangles, n = 10) in-house standards; (b) linear regressions (black lines) of $\delta^{34}S$ values (‰) (as (a)) versus 1/sample mass (µg S) of fish (closed squares) and algae (closed triangles) in-house standards for estimation of isotopic composition and S mass of blank following the indirect method;[21] and (c) relationships between the $\delta^{34}S$ values (‰) (drift-, memory- and blank- corrected) and the S amount in sample (µg) of SRM 1577b bovine liver (open circles, n = 10), fish (closed squares, n = 10) and algae (closed triangles, n = 10) in-house standards after estimation of isotopic composition and S mass of blank following the modified semi-direct method.[22]

corrected values for small samples (<15 μg S in capsule) were not accurate, exhibiting higher than acceptable standard deviation compared with the true values. The best blank correction was reached by applying a modification of the semi-indirect method,[22] previously used to estimate the N blank effect. Briefly, for each batch, the blank isotopic composition was derived from the intercept of the linear regression of the $\delta^{34}S$ value with the inverse of the observed S sample size for multiple measurements of one standard (fish) as described for the N blank correction in the Experimental section. However, the S blank size was not obtained by direct measurement as in the original semi-indirect method,[22] but it was slightly adjusted from the initial estimates based on the indirect method (Fig. 5(b)), so that the $\delta^{34}S$ values of the series of variable size algae in-house standard were also corrected accurately (Fig. 5(c)).

The sets of fish and algae in-house standards at variable weights were analyzed in each batch and the blank parameters then calculated. Using this hybrid approach, the calculated size of the blank varied from 0.5 to 1.5 μg S, and its isotopic composition varied from 2.70 to 12.05 ‰ over several batches.

Optimization of $\delta^{15}N$ measurement

We found that the addition of a second Cu reduction tube at 650 °C was necessary to obtain reliable $\delta^{15}N$ data in the NCS mode of analysis (Fig. 1). Without the 2nd reduction tube in line the mass 30 signal detected by IRMS was off scale, probably resulting from the presence of NO because of incomplete reduction of NO_x as no CO (i.e., another possible contaminant that could yield mass 30) was present within the system and the C percentages were accurate, indicating complete thermal decomposition of the samples.

The results from our comparisons (Fig. 6) show that the incomplete reduction of NO_x affects the 29/28 ratio used to calculate $\delta^{15}N$ values, thus resulting in erratic $\delta^{15}N$ values. The N isotopic composition of a series of SRM 1577b standards analyzed over a wide range of N amount per capsule (25–400 μg) using the modified configuration but without the 2nd reduction tube in line differed by up to 1‰ from the true value (measured 6.78 vs actual 7.78 ‰) and had large standard deviation (±0.5‰) (Fig. 6(a), open symbols). Varying the sample C/N ratios, by adding the same amount of C as glucose to the SRM 1577b standard, affected the results in a negative way even more when the samples had little N (<50 μg per capsule), and therefore C/N ratios between 40 and 70 (Fig. 6(a), closed symbols).

Instead, with the 2nd reduction tube in line (Fig. 1) the sample mass 30 signal detected by IRMS was small, consistent, and similar to that routinely detected in regular dual NC analysis, and SRM 1577b analysis gave good accuracy (measured 7.86 vs actual 7.78‰) and good precision (±0.12‰) independently from the sample N mass (Fig. 6(b), open symbols) and the C/N ratio (Fig. 6(b), closed symbols).

Accuracy and precision of NCS measurement in organics and soils

The performance of the NCS method using the modified configuration (Fig. 1) and the data correction procedure described previously was evaluated for all three elements by analyzing several international reference materials covering a wide range of N, C and S isotopic compositions (Table 1), QC materials (SRM 1577b and fish and algae in-house standards) (Table 2), and a selection of soils (Tables 3 and 4). The average differences between the measured and known values for all three elements for several available international reference materials with sample sizes between 50 and 70 μg N, 200 and 500 μg C and 15 and 80 μg S per capsule were small (0.2‰ for $\delta^{15}N$ values, 0.04‰ for $\delta^{13}C$ values, and 0.1‰ for $\delta^{34}S$ values) and very precise (max standard deviation of 0.4‰ for $\delta^{34}S$ values) (Table 1). The SRM 1577b $\delta^{34}S$ values obtained in the NCS configuration in the sample size ranges of 8–80 μg S and analyzed over 10 runs in a 6-month period were comparable with results obtained using the Parr bomb method[14] (7.77 ± 0.43‰ vs 7.5 ± 0.2‰, respectively) (Table 2). Below a sample size of 8 μg S per capsule, the $\delta^{34}S$ value precision degraded (±1‰ for samples containing 4 μg S per capsule). In addition, the N and C isotopic compositions of the fish and algae in-house standards analyzed in the same runs and in the sample size ranges between 25 and 500 μg N and 200 and 4000 μg C per capsule agreed well with reference values (Table 2).

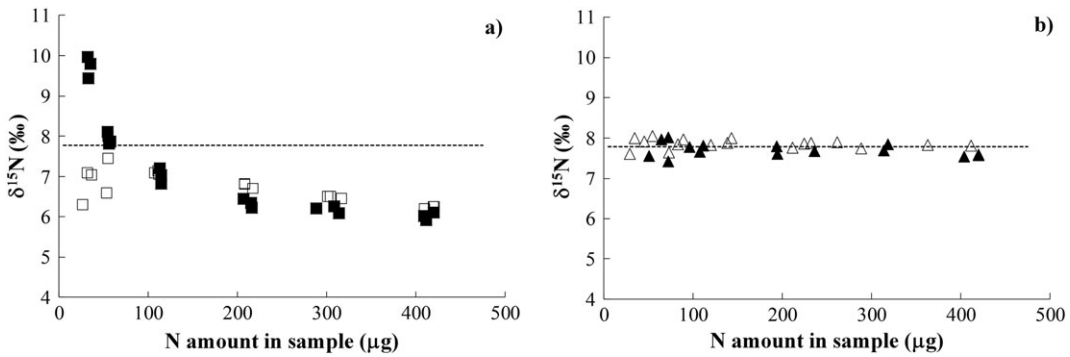

Figure 6. Optimization of $\delta^{15}N$ measurement. $\delta^{15}N$ values (‰) versus N amount in sample (μg) of SRM 1577b bovine liver at sample weight range between 0.3 to 4 mg analyzed in NCS mode with the modified configuration (Fig. 1) (a) without the second reduction Cu tube in line (squares, $n = 36$) and (b) with the second reduction Cu tube in line (triangles, $n = 36$). For each configuration, SRM 1577b was analyzed with (closed symbols) and without (open symbols) 5 mg of added glucose in order to vary the sample C/N ratio. The dashed line represents the actual δ^{15} N value (‰) of SRM 1577b.

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Table 1. Comparison of N, C and S isotope ratios, as $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values (‰), for measured vs known values of international reference materials analyzed using the modified CHNOS elemental analyzer configuration (sample sizes varied between 50 and 70 μg N, 200 and 500 μg C and 15 and 80 μg S)

Standard	Known $\delta^{15}N$ (‰)	Measured $\delta^{15}N$ (‰)	Known $\delta^{13}C$ (‰)	Measured $\delta^{13}C$ (‰)	Known $\delta^{34}S$ (‰)	Measured $\delta^{34}S$ (‰)
IAEA S-1					−0.3	−0.35 (0.32)
IAEA S-2					22.70 (0.20)	22.58 (0.40)
IAEA SO-5					0.50 (0.20)	0.56 (0.15)
NBS 127					21.17 (0.40)	21.34 (0.35)
IAEA N-1	0.40 (0.20)	0.58 (0.15)				
IAEA NO-3	4.70 (0.20)	4.85 (0.17)				
IAEA 600	1.00 (0.2)	1.18 (0.04)	−27.77 (0.04)	−27.68 (0.04)		
IAEA CH-6			−10.45 (0.03)	−10.57 (0.07)		
USGS24			−16.05 (0.04)	−16.04 (0.03)		

Values are means (n = 32 for IAEA S-1 and IAEA S-2, n = 6 for all other standards) with 1 SD in parentheses.

Table 2. Accuracy and precision for N, C and S isotope ratios, as $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values (‰), and contents (%, w/w) of SRM 1577b (bovine liver material) and fish and algae in-house standards analyzed in separate runs using the modified CHNOS elemental analyzer configuration (sample size varied between 25 and 500 μg N, 200 and 4000 μg C, and 8 and 80 μg S)

Reference Material	n	$\delta^{15}N$ (‰)	% N (w/w)	$\delta^{13}C$ (‰)	% C (w/w)	$\delta^{34}S$ (‰)	% S (w/w)
SRM 1577b	94		10.52 (0.27)		50.46 (0.09)	7.77 (0.43)	0.80 (0.43)
Reference values*			10.60 (0.10)		50.15 (0.08)	7.50[14]	0.75 (0.03)
Fish	95	16.26 (0.20)	11.77 (0.50)	−17.64 (0.12)	46.17 (0.50)		
Reference values		16.36		−17.63			
Algae	92	10.96 (0.20)	10.33 (0.60)	−32.09 (0.14)	46.70 (0.65)		
Reference values		11.20		−32.01			

Values are means with 1 SD in parentheses.
*C and N contents (%, w/w) were obtained by independent EA analysis; S percentage is certified by NIST.

Table 3. Accuracy and precision for N, C and S isotope ratios, as $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values (‰), and contents (%, w/w) of Elemental Microanalysis Ltd. soil (Low Organic Content Soil Standard OAS Cat No. B2153, Certificate No. 114524) and sediment (High Organic Sediment Standard OAS Cat No. B2151, Certificate No. 162517) reference materials analyzed at several weight ranges up to 140 mg using the modified CHNOS elemental analyzer configuration

Reference Material	Sample weight (mg)	n	$\delta^{15}N$ (‰)	% N (w/w)	$\delta^{13}C$ (‰)	% C (w/w)	$\delta^{34}S$ (‰)	% S (w/w)
EM soil (low organic content)								
	70	3	6.91 (0.04)	0.14 (0.01)	−27.50 (0.08)	1.55 (0.01)	4.71 (0.08)	0.02 (0.01)
	140	3	6.87 (0.07)	0.14 (0.01)	−27.32 (0.07)	1.54 (0.01)	4.56 (0.08)	0.02 (0.01)
Certified values			6.70 (0.15)	0.13 (0.02)	−27.46 (0.11)	1.52 (0.02)	4.94* (1.40)	0.02 (0.01)
EM sediment (high organic content)								
	5	3	5.05 (0.06)	0.69 (0.01)	−26.23 (0.07)	9.37 (0.06)	4.37 (0.48)	0.80 (0.13)
	10	3	4.61 (0.05)	0.67 (0.01)	−26.39 (0.04)	9.36 (0.03)	4.26 (0.15)	0.73 (0.01)
Certified values			4.42 (0.29)	0.62 (0.02)	−26.27 (0.15)	9.15 (0.12)	4.20*	0.69 (0.04)

Values are means with 1 SD in parentheses. No statistically significant differences with significance threshold set at 0.05 (one-way ANOVA test) were found between sample size for each reference material.
*Defined as 'approximate values' by Elemental Microanalysis Ltd.

The precision for soils was tested by analyzing the EM soil and sediment materials. Table 3 shows the isotope values (certified and non-certified) for each reference material, as well as the results from our testing. Our values show good accuracy and very low standard deviation from one another, and they fall within the range reported by Elemental

Table 4. N, C and S isotope ratios, as $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values (‰), and contents (%, w/w) of Icacos and Yolo soil series analyzed at a range of weights up to 140 mg using the modified CHNOS elemental analyzer configuration

Soil type	Sample weight (mg)	$\delta^{15}N$ (‰)	% N (w/w)	$\delta^{13}C$ (‰)	% C (w/w)	$\delta^{34}S$ (‰)	% S (w/w)
Icacos							
	40	3.45 (0.05)	0.29 (0.01)	−28.25 (0.02)	5.04 (0.07)	15.44 (0.12)	0.06 (0.01)
	60	3.47 (0.04)	0.29 (0.01)	−28.26 (0.03)	5.11 (0.02)	15.33 (0.12)	0.06 (0.01)
	80	3.46 (0.04)	0.27 (0.01)	−28.06 (0.04)	5.01 (0.03)	15.94 (0.01)	0.05 (0.01)
Yolo							
	50	4.45 (0.07)	0.11 (0.01)	−25.44 (0.03)	0.88 (0.01)	−3.44 (0.15)	0.02 (0.01)
	70	4.44 (0.03)	0.11 (0.01)	−25.40 (0.02)	0.87 (0.01)	−3.58 (0.20)	0.02 (0.01)
	100	4.52 (0.02)	0.11 (0.01)	−25.43 (0.03)	0.98 (0.01)	−3.67 (0.22)	0.01 (0.01)
	120	4.50 (0.09)	0.11 (0.01)	−25.43 (0.04)	0.86 (0.02)	−3.69 (0.47)	0.01 (0.01)
	140	4.50 (0.05)	0.10 (0.01)	−25.51 (0.03)	0.84 (0.01)	−3.53 (0.15)	0.01 (0.01)

Values are means (n = 3) with 1 SD in parentheses. No statistically significant differences with the significance threshold set at .05 (one-way ANOVA test) were found among sample size for each soil type.

Microanalysis Ltd. The $\delta^{15}N$ and $\delta^{13}C$ values of the Icacos and Yolo soils had a maximum standard deviation of 0.1‰, and the $\delta^{34}S$ values had a maximum standard deviation of 0.3‰ (Table 4). As expected, the results for the $\delta^{34}S$ values were more variable than those for the $\delta^{15}N$ and $\delta^{13}C$ values. However, all these values are well within acceptable error ranges. In agreement with the data obtained for the certified EM materials, sample size does not appear to have any impact on the isotopic composition of the Icacos and Yolo soils. These data (Tables 3 and 4) show that our method is able to accurately analyze the N, C and S isotopic composition of different soil types.

CONCLUSIONS

Recent technical advancements such as the 'purge and trap' technology in elemental analyzers and the adoption of wide dynamic range in IRMS measurement have shown potential in advancing the simultaneous measurements of the concentrations and isotopic ratios of N, C and S in a single sample.[18] Here, we made modifications to the CHNOS elemental analyzer (vario ISOTOPE cube) to maximize sample throughout, minimize the interference of ^{18}O on $\delta^{34}S$ measurements, and achieve accurate $\delta^{15}N$ values. The described NCS setup has the capacity to analyze over 130 total capsules per day, and was tested on organic compounds and on different types of soils, and over a wide range of sample sizes, and C/N and C/S ratios. The precision of the N and C isotopic measurements is comparable with the one usually attained in NC mode alone. The precision for S isotopic measurement in the range 8–120 µg S in capsule is ±0.4‰. The only significant additional maintenance compared with routine dual NC measurement is the changing of the first Cu reduction tube and the magnesium perchlorate water trap daily. Soils with masses up to at least 140 mg can be analyzed with good accuracy and precision.

This method is suitable for widespread use, and can significantly enhance the application of $\delta^{34}S$ measurements in both soils and a broad range of organic samples in biological, ecological and biogeochemical research. Future efforts should include additional testing to improve precision at low S content (<8 µg S) and for difficult soils such as anoxic sediments. The main unresolved issue is the lack of available certified international standards of biological nature for the simultaneous determination of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values.

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