Evaluation on nitrogen isotopes analysis in high-C/N-ratio plants using elemental analyzer/isotope ratio mass spectrometry postprint

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Abstract

Elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) has been widely applied to analyze the 15N/14N isotope composition ($ $15N) of plants and soils, but the $ $15N results may be inaccurate due to incomplete combustion of the high-C/N-ratio plant samples by EA. Therefore, it is necessary to develop a method to solve the problem of imperfect combustion. In this study, we used two methods: 1) adding copper oxide powder to the samples, and 2) increasing the O2 flow (from 100 mL min-1 to 200 mL min-1) for the auto sampler inlet purge line of the EA. The $ $15N values of the plant samples became more positive and tended to be stable after complete combustion. Also, the required blank samples for each plant sample decreased with increasing amount of the added CuO powder. However, at 200 mL min-1 of the oxygen flow in the EA, complete combustion could not be achieved without adding copper oxide, but this was done with decreased amount of CuO powder. Therefore, mixing cupric oxide into the high-C/N-ratio samples was an efficient, simple and convenient way to solve the problem of imperfect combustion in the EA.

Full Text

Preamble

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Evaluation on nitrogen isotopes analysis in high-C/N-ratio plants using elemental analyzer/isotope ratio mass spectrometry

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Elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) has been widely applied to analyze the $^{15}N/^{14}N$ isotope composition ($\delta^{15}N$) of plants and soils, but the $\delta^{15}N$ results may be inaccurate due to incomplete combustion of high-C/N-ratio plant samples by EA. Therefore, it is necessary to develop a method to solve the problem of imperfect combustion. In this study, we used two methods: (1) adding copper oxide powder to the samples, and (2) increasing the O_2 flow (from 100 mL min^{-1} to 200 mL min^{-1}) for the auto sampler inlet purge line of the EA. The $\delta^{15}N$ values of the plant samples became more positive and tended to be stable after complete combustion. Additionally, the required blank samples for each plant sample decreased with increasing amount of added CuO powder. However, at 200 mL min^{-1} of oxygen flow in the EA, complete combustion could not be achieved without adding copper oxide, though this could be accomplished with decreased amounts of CuO powder. Therefore, mixing cupric oxide into high-C/N-ratio samples represents an efficient, simple, and convenient way to solve the problem of imperfect combustion in the EA.

Keywords: Nitrogen isotopes, Cupric oxide, O_2 flow of EA/IRMS, High-C/N-ratio plants
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Introduction

Continuous-flow elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) has been extensively used to analyze nitrogen isotopes in plant and soil samples [1–6] and to study the global nitrogen cycle [7–11]. EA analysis is based on flash combustion, where the nitrogen content in a sample is converted to N_2 and NO_x in the oxidation furnace through instantaneous and complete oxidation [12]. However, Wang et al. [13] found that natural plant samples with high C/N ratios could not be completely combusted, resulting in nitrogen isotopic compositions that were less than expected. Therefore, methods must be developed to ensure complete combustion of these plant samples.

To ensure sufficient nitrogen ($\geq$ 100 µg) is introduced into the mass spectrometer, larger sample amounts are required for nitrogen isotopic analysis of high-C/N-ratio plants. However, these samples are tightly loaded in Ag (or Sn) foil capsules to avoid air contamination, and the plant powder in the packet core may not contact oxygen adequately, leading to incomplete oxidation and nitrogen isotope fractionation [14]. Vanadium pentoxide (V_2O_5) has been added to samples to enhance combustion in sulfur stable isotope measurements by EA/IRMS [15–17], but this method proved ineffective for high C/N ratio samples because the background intensity of the m/z 28 ion increased with V_2O_5

content, and the plant-V_2O_5 mixture could not be completely reacted [13]. In this paper, copper oxide powder is used as a combustion-supporting reagent to find a simple and effective way to obtain accurate isotope results for high-C/N-ratio plant samples analyzed by EA/IRMS.

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Experimental

A. Materials

Copper oxide was ground into powder with particle size < 110 µg after sintering at 650 °C for two hours. The high-C/N-ratio plant samples, designated Plant 1 (P1) and Plant 2 (P2), were collected from Xinjiang, China. The plant samples were soaked in deionized water for 24 hours (with the deionized water renewed every 8 hours) to remove salts from the sample surface, then dried at 40 °C in an oven for 48 hours. The dried samples were ground into powder with particle size < 74 µm. Carbon and nitrogen contents and C/N ratios were determined using an elemental analyzer. The carbon content was 44.14% for P1 samples and 44.77% for P2 samples, while the nitrogen content was 1.37% for P1 and 0.75% for P2, yielding C/N ratios of 32.22 and 59.69 for P1 and P2, respectively.

Plant sample powders were weighed on a fresh sheet of paper and mixed with CuO powder at various mass ratios. The prepared samples were loaded into Ag and Sn capsules (5 mm × 9 mm), which were tightly crimped to avoid trapping air that would perturb combustion.

B. Sample Analysis Using EA/ConFlo III/IRMS

Determination of $^{15}N/^{14}N$ isotope ratios was performed with a FLASH 1112 Elemental Analyzer (CE Instruments, Rodano, Italy) equipped with an AS200 auto sampler interfaced to a DeltaPLUS isotope ratio mass spectrometer (ThermoQuest Finnigan, Bremen, Germany) via a ConFlo III interface (ThermoQuest Finnigan, Germany). The elemental analyzer consisted of an oxidation furnace filled with chromium(III) oxide and silvered cobalt oxide for combustion and a reduction furnace filled with copper to reduce nitric oxide compounds. A CO_2 adsorption trap (to avoid CO_2 interference) and a water trap (to protect the GC column from humidity) were connected between the reduction reactor and the GC column. The mixed gas flowing from the furnace was separated via the chromatographic column.

The following EA conditions were used: oxidation reactor temperature 1000 °C, reduction reactor temperature 650 °C, and GC column temperature 40 °C [18]. High-purity helium was used as a carrier gas at a flow rate of 85 mL min^{-1}. All $^{15}N/^{14}N$ isotope ratios were expressed in conventional δ notation in per mil versus air, defined as:

$$\delta^{15}\mathrm{N}(‰)(\mathrm{air}) = \left[\frac{(^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{sample}}}{(^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{standard}}} - 1\right] \times 1000$$

A tank of high-purity nitrogen with known δ^{15}N value was used as the working reference gas. The δ^{15}N standards of IAEA-NO_3 and a soil standard SN-2 were measured daily to monitor analytical accuracy. The standard deviation for duplicate analyses was less than 0.3‰.

C. Improvement of Oxidizing Conditions

To supply sufficient oxygen for complete conversion of high-C/N-ratio plant samples by flash combustion in the EA, two methods were tested. The first method involved adding CuO to the samples, while the second method directly increased the oxygen injection of the EA from 100 mL min^{-1} to 200 mL min^{-1}. Because excess O_2 would shorten the lifetime of the reduction reactor [19], we did not further increase the oxygen flow.

Results and Discussion

A. Checks on CuO and Capsules

To determine whether copper oxide would induce contamination, we monitored the blank of the CuO reagent. Different amounts of CuO powder were used to determine N signal intensities of the blank. Additionally, CuO powder was mixed with the SN-2 standard and the δ^{15}N data were compared with that of SN-2 (Table 1). The results showed that the N intensity of the CuO blank was zero and the δ^{15}N results of SN-2 were not affected by the added CuO. Furthermore, when CuO and the CuO/SN-2 mixture were loaded in both Ag and Sn capsules, the capsules contributed no nitrogen contamination.

To examine whether Ag capsules prevent complete combustion, δ^{15}N values for samples wrapped in Ag capsules and Sn capsules were compared. As shown in Table 2 , no difference was observed between plant samples loaded into Ag capsules versus those in Sn capsules.

TABLE 1. Analysis of the N blank of CuO powder in Sn and Ag capsules, and nitrogen isotope results of the SN-2 standard and its mixture with CuO powder (mean values ± standard deviation)

Sample	Capsule	CuO added	Ion intensity of m/z 29 (mV)	δ^{15}N (‰ vs. air)

Note: No.1–4, CuO in Sn or Ag capsules; No.5–8, CuO/SN-2 mixture in Sn or Ag capsules; No.9, SN-2 standard.

TABLE 2. Comparison of δ^{15}N data between samples in Ag and Sn capsules and the intra-assay precision assessed by replicate analysis in a single day and several days (mean values ± standard deviation)

Sample	δ^{15}N (‰ vs. air) by Ag capsules	δ^{15}N (‰ vs. air) by Sn capsules
P1/CuO = 1/5	8.00$±0.090(n = 6)^b$	8.58$±0.108(n = 3)^a$
P2/CuO = 1/3	5.36$±0.099(n = 2)^a$	5.41$±0.046(n = 2)^a$
P2/CuO = 1/5	11.69$±0.081(n = 2)^a$	11.80$±0.035(n = 3)^a$
SN-2/CuO = 1/4	8.46$±0.016(n = 2)\|8.45±0.058(n = 3)^a$	
P1/CuO = 1/1	—	8.46$±0.058(n = 3)^a$

[a] Replicate analysis in a single day.
[b] Replicate analysis on different days.

B. Precision of EA-IRMS δ^{15}N Analysis

The precision of the EA/IRMS system must be guaranteed to study δ^{15}N values. Replicate analyses were performed in which plant samples mixed with different amounts of CuO were analyzed either in a single day or across several days. The results demonstrated high precision, with standard deviations less than 0.2‰ (Table 2).

C. δ^{15}N Linearity Range of the IRMS System

The ion intensity of N_2 from plant samples increases with the degree of combustion completeness. It is essential that the range of ion intensity variation falls within the δ^{15}N linearity range of the IRMS system. This linearity range was tested by investigating the relationship between ion intensity and δ^{15}N values [20]. First, the pressure controller of the reference gas N_2 in the Finnigan ConFlo III interface was adjusted. Eleven pulses of various N_2 amounts were measured to achieve a diverse range of ion intensities without changing EA conditions. The measured δ^{15}N values remained stable across an ion intensity range of 500–6600 mV (Fig. 1 Figure 1: see original paper). Next, different amounts of the SN-2 standard were loaded in Sn or Ag cups to examine the linearity of the EA/IRMS system. In the N_2 ion intensity range of 750–4300 mV, the measured δ^{15}N value showed linear correlation with ion intensity, but in the range of 1500–4000 mV, the δ^{15}N value did not vary significantly (Fig. 1(b)). These results demonstrate that the increase in δ^{15}N values as a function of increasing plant sample size was not caused by any non-linearity offset of the mass spectrometer.

FIGURE:1 Linearity of the mass spectrometer for nitrogen isotope analysis. (a) δ^{15}N of N_2 standard working gas at N_2 ion intensities of 500–6600 mV; (b) δ^{15}N of SN-2 at N_2 ion intensities of 750–4300 mV.

D. δ^{15}N Values with CuO Addition and O_2 Increase

It is well known that isotope fractionation occurs if samples are not fully oxidized using EA/IRMS [14, 17]. The ^{14}N isotope can participate in reactions more easily than ^{15}N, resulting in more negative δ^{15}N values when samples are only partially converted.

In this study, the nitrogen isotopes of the two high-C/N-ratio plant samples showed similar trends in m/z 29 ion intensity. When the oxygen loop in the EA was set to 100 mL min^{-1} or 200 mL min^{-1}, the nitrogen isotopes of both samples were more negative without CuO powder, indicating incomplete combustion. As shown in Fig. 2 [Figure 2: see original paper], adding CuO powder to the samples at 100 mL min^{-1} O_2 flow increased isotopic compositions until reaching CuO/plant ratios of 5/1 for P1 and 3/1 for P2. However, at 200 mL min^{-1} O_2 flow, isotope results tended to stabilize when the CuO/plant mass ratio exceeded 3/1 for P1 and 1/1 for P2. The nitrogen isotopes for P1 and P2 showed the same variation trend under different experimental conditions. As mentioned above, this stable behavior indicates complete combustion of the plant samples, which can be achieved by adding CuO powder or increasing O_2 flow in the EA.

FIGURE:2 The δ^{15}N values of samples P1 (a) and P2 (b) at different mass ratios of CuO/plant-sample.

E. Blank Samples After Each Sample Analysis

To verify complete combustion of plant samples, blank samples were measured after each plant sample analysis until no nitrogen peak appeared. As shown in Fig. 3 Figure 3: see original paper, the ion intensity of the first blank sample was 35–60 mV at O_2 flows of both 100 and 200 mL min^{-1}, indicating that nitrogen in the two plant samples was not completely converted. At 100 mL min^{-1} O_2 flow, four or five blank samples were required to eliminate the m/z 29 peak without adding CuO (Fig. 3(a)), whereas this was achieved with just two blank samples at 200 mL min^{-1}. Thus, increasing O_2 flow to 200 mL min^{-1} promotes sample combustion to some extent, but complete conversion cannot be achieved by simply increasing O_2 flow.

As shown in Figs. 3(b) and 3(c), the number of blank samples decreased with increasing amounts of added CuO. In the 100 mL min^{-1} scenario, the number of blank samples required was reduced from three to two when the copper oxide/plant sample mass ratio increased from 1/1 to 3/1. With a 200 mL min^{-1} flow, high-C/N-ratio plant samples were completely combusted when the copper oxide/plant sample mass ratio exceeded 1/1. This indicates that copper oxide is an efficient reagent for promoting combustion in nitrogen isotope analysis of high-C/N-ratio samples using EA/IRMS.

FIGURE:3 Nitrogen signal intensity from blank samples analyzed after plant sample analysis at different mass ratios of CuO/plant-sample.

Conclusion

Adding copper oxide powder to high-C/N-ratio plant samples ensures complete oxidation of nitrogen contents by flash combustion in EA/IRMS, enabling accurate determination of nitrogen isotope values. While increasing oxygen flow in the EA improves nitrogen oxidation of high-C/N-ratio plant samples, complete combustion cannot be achieved by this measure alone. Additionally, it is necessary to analyze blank samples after each partially combusted sample to avoid interference from memory effects of residual nitrogen from the previous sample. Therefore, adding CuO powder and increasing EA O_2 flow can greatly reduce the time spent on blank samples when high-C/N-ratio plant samples are completely combusted by flash combustion. In nitrogen isotope analysis with EA/IRMS, mixing copper oxide into samples is an easy and effective way to solve the problem of incomplete combustion of high C/N ratio plant samples. The true nitrogen compositions can be obtained and analysis efficiency can be improved by adding copper oxide and increasing O_2 flow.

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Note: Figure translations are in progress. See original paper for figures.