Development of a Methodology for Analyzing Organic Carbon and δ^{13}C in Soil and Sediment Samples through EA-IRMS

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This study presents a method for analyzing carbon content and carbon isotope ratio (δ^{13}C) of soil and sediment samples. Quality control (QC) samples were used to evaluate analytical precision and accuracy. The standard deviations and biases of δ^{13}C (‰) and carbon content were calculated for each QC sample. The results showed that the measurements for B2153 Low Organic Content Soil IRMS Standard and IVA Peat Soil had the largest and smallest standard deviation values for δ^{13}C (‰), respectively. The bias of δ^{13}C (‰) also varied significantly among the samples, with the measurements for B2151 exhibiting the highest bias and those for IAEA-CH3 exhibiting the smallest. Regarding carbon content, the standard deviation values for the samples ranged from 0.07% to 0.20%. The B2151 High Organic Content Sediment IRMS Standard sample showed the smallest bias, while the largest bias belonged to sample IVA Peat Soil. In addition, the process of removing carbonates from soil and sediment samples using a 0.5M HCl solution was shown to be stable and dependable. The δ^{13}C of total organic carbon (TOC) values of the analyzed samples ranged from -28.56 ± 0.01 to -23.35 ± 0.11 (‰). Sample soil mix had the highest concentration of TOC, resulting in a depleted δ^{13}C value that was much lower than the remaining samples. Lastly, a strong correlation between the δ^{13}C of Total C values in the sediment samples and TOC/Total C ratio was observed.

Keywords: *δ^{13}C, organic carbon, carbonate removal*

1. Introduction

Soil and sediment are important components of the Earth's ecosystem, and understanding their composition is crucial for many environmental and agricultural applications. One important aspect of soil and sediment analysis is the determination of the organic carbon content, which is used to evaluate the quality and fertility of soils, and to assess carbon sequestration potential. Organic carbon (OC) is a key component of the carbon cycle as it represents a large pool of carbon in the Earth's system. OC can function as a sink or source of carbon depending on the environmental conditions, and its fate can have significant implications for the global carbon cycle [1–2]. OC and δ^{13}C analysis are powerful tools for

understanding the origin and fate of carbon in soil and sedimentary environments. One widely used method for analyzing carbon content and isotopic composition is elemental analysis-isotope ratio mass spectrometry (EA-IRMS) [3–4].

EA-IRMS allows for simultaneous determination of carbon content and isotopic composition in the samples. The $\delta^{13}C$ value, the ratio of ^{13}C to ^{12}C expressed as a deviation from the Vienna Pee Dee Belemnite (VPDB) standard, is commonly used to evaluate the isotopic composition of OC in the samples [5]. The ratio of stable isotopes $\delta^{13}C$ is commonly used as a proxy for the source of organic matter, the degree of degradation, and the environmental conditions during sediment formation. A higher $\delta^{13}C$ ratio indicates a higher contribution of terrestrial plant material or anoxic conditions, while a lower ratio indicates a higher contribution of marine organic matter or oxide conditions [6–7].

Carbonate minerals, however, can interfere with the accurate measurement of organic carbon content, particularly when using isotopic analysis techniques such as EA-IRMS. Carbonates contain carbon that can be easily converted into CO_2 during analysis, leading to erroneous results for the organic carbon content and isotope ratios. Therefore, it is necessary to treat the sediment samples with acid to remove the carbonates and retain only the organic carbon for analysis.

To address this issue, researchers have developed methods to remove carbonates from soil and sediment samples prior to analysis. These methods typically involve the use of strong acids, such as hydrochloric acid (HCl) or acetic acid (CH_3COOH), to dissolve the carbonates while leaving the organic matter intact [8].

This study aims to contribute to this effort by developing a procedure for carbonate removal from soil and sediment samples using HCl. The procedure will be optimized to ensure efficient removal of carbonates while minimizing damage to the organic matter and will be validated using a range of soil and sediment samples from different environments. The effectiveness of the procedure will be assessed by comparing the results obtained before and after carbonate removal, and by evaluating the accuracy and precision of the organic carbon content and isotope ratios obtained using EA-IRMS.

2. Material and methods

2.1. Sample description

Determining the carbon content and isotopic ratios in soil and sediment samples is an important task for geologists and environmental scientists. The EA-IRMS method is a well-established technique that allows for the accurate measurement of both the amount of total organic carbon (TOC) and the isotope ratio $\delta^{13}C$ in various soil and sediment samples. We first established a standardized method for analyzing the samples by using standard reference material (SRM) samples. To ensure the accuracy and precision of the analysis, a set of carbon content and carbon isotope ratio standards are used for quality control (QC). Afterwards, we evaluated several sediment samples that contained varying levels of TOC and TIC (total inorganic carbon) and used the established method to determine the TOC and $\delta^{13}C$ isotope ratio. The information on samples was shown in Table 1.

Table 1. Information of samples

Type	Sample	Matrix	$\delta^{13}C_{VPDB}$, (‰)	C (%)
SRM	IVA Ure	Ure	-36.54±0.06	-
	IAEA-603	Calcite	+2.46±0.01	-
	IVA Sulphanilamide	Sulphanilamide	-	41.81±0.21
QC	IVA Peat Soil	Soil	-	15.95±0.03
	IAEA-CH-3	Cellulose	-24.72±0.04	-
	Elemental Microanalysis B2151	High Organic Content Sediment IRMS Standard	-28.85±0.10	7.45±0.14
	Elemental Microanalysis B2153	Low Organic Content Soil IRMS Standard	-22.88±0.40	1.86±0.14
Sample	LS	Lake Sediment		
	MS	Marine Sediment		
	NIST 2709 mix with CaCO$_3$	Soil		
	Soil mix with CaCO$_3$	Soil		

2.2. Carbonate removal

The carbon content of a sedimentary sample was analyzed in this study. The sample consisted of both organic and inorganic carbon, and to determine the $\delta^{13}C_{TOC}$, a weak acid solution (dilute HCl) was used to remove the carbonate component.

To remove the carbonate, a portion of the air-dried sample (0.5g) was placed in a round-bottom centrifuge tube and a weak acid solution (0.5M HCl) was added. The tube was gently shaken to ensure the acid covered the entire sample and then placed in an ultrasonic bath for 60 minutes to break up any remaining carbonate particles. The tube was then centrifuged to separate the remaining sample residue from the liquid, and the process was repeated twice to ensure complete removal of the carbonate.

The remaining sample residue was washed six times with deionized water to remove any remaining acid or carbonate particles. The liquid was then pipetted out, and the sample was dried in an oven at 60°C. Finally, the sample was ground to a fine powder (100 to 200 μm) using an agate mortar before being analyzed with the EA-IRMS.

To conduct this study, the sample was treated twice more with HCl solution using the same procedure used to remove carbonate. The aim was to assess how effective the carbonate removal process was, as well as to see how using HCl solution affected the TOC content and $\delta^{13}C$ value of the sample being analyzed.

2.3. Analysis

To analyze stable isotope ratios of carbon in sediment samples, the system EA-IRMS (model: Flash IRMS), made by Thermo Scientific in Germany, was used.

To begin the analysis, each sample is weighed at 0.5 – 3 mg and placed into a small tin capsule 8 mm × 5 mm (height × diameter). The sample is then loaded into the MAS 200R autosampler and fed into the EA's combustion chamber.

In the combustion chamber, the sample is ignited with O_2 gas in a quartz tube at 1020°C. The resulting product is a mixture of gases, which includes CO_2 and H_2O. These gases are transported by a high-purity helium gas (99.999% purity) at a rate of 110 mL/min to

the reduction chamber. Then, residual O_2 is removed. The H_2O component formed during the combustion process is removed and retained by the water trap column, which contains magnesium perchlorate. The only gases that remain is CO_2, which continue to be transported into the gas chromatographic column.

In the gas chromatographic column, N2 is separated from CO2, and the content of C in the sample is determined by the Thermal Conductivity Detector (TCD). Finally, the He carrier CO_2 into the IRMS to determine the $\delta^{13}C$ in the sample.

The results of stable isotope ratios and uncertainties were calculated using The Kragten spreadsheet approach based on the SRM sample provided in Table 1. The QC samples were analyzed together to verify the process [5]. For the analysis of the concentration of C, a calibration curve was constructed from the Sulphanilamide sample with varying masses.

3. Results and Discussion

Table 2 presents the standard deviations of analytical values and bias for $\delta^{13}C$ (‰) and carbon content in the QC samples. The largest standard deviation value for $\delta^{13}C$ (‰) is associated with the B2153 sample (0.12 ‰), while the smallest is linked to IAEA-CH3 (0.03‰). This indicates that the measurements for B2153 are more variable compared to those of IAEA-CH3. The bias of $\delta^{13}C$ (‰) also varies significantly among the samples. The measurements for B2151 exhibit the highest bias (0.87%), while those for IAEA-CH3 exhibit the smallest bias (0.04%).

Table 2. Results analysis of QC samples

| QC	n	$\delta^{13}C_{VPDB}$ (‰)			C (%)		
		Certificate	Analysis	Bias (%)	Certificate	Analysis	Bias (%)
IVA Peat Soil	3	-	-	-	15.95±0.03	15.20±0.20	-4.70
IAEA-CH-3	4	-24.72±0.04	-24.71±0.03	0.04	-	-	-
B2151	3	-28.85±0.10	-29.10±0.09	0.87	7.45±0.14	7.32±0.07	1.74
B2153	2	-22.88±0.40	-22.82±0.12	-0.26	1.86±0.14	1.94±0.08	4.30

Regarding carbon content, the standard deviation values for the samples range from 0.07% for sample B2151 to 0.20% for sample IVA Peat Soil. Thus, the measurements for IVA Peat Soil show greater variability compared to those for B2151. The B2151 QC sample shows the smallest bias (1.74%), indicating that the measurements for this sample are closer to the true value, while the largest bias (-4.70%) belongs to sample IVA Peat Soil. This indicates that the measurements for IAV Peat Soil are further from the true value than those for other samples.

The process of removing carbonates from soil and sediment samples using a 0.5M HCl solution has been shown to be stable and dependable. This is supported by the results of a Carbonate re-removal analysis of the treated samples in Table 3, which showed that dilute HCl solution did not affect the $\delta^{13}C$ or TOC values in the analyzed samples. When comparing the results of the Carbonate re-removal samples with the results of the Carbonate removal samples, there were small fluctuations in the values within the analytical error.

Table 3. Results analysis of samples

Sample	None Carbonate removal		Carbonate removal		Carbonate re-removal	
	$\delta^{13}C_{VPDB}$	Total C	$\delta^{13}C_{VPDB}$	TOC	$\delta^{13}C_{VPDB}$	TOC
	(‰)	(%)	(‰)	(%)	(‰)	(%)
LS	-23.02±0.02	1.44±0.01	-23.35±0.11	1.29±0.01	-23.79±0.17	1.26±0.01
MS	-18.54±0.15	0.72±0.01	-23.86±0.15	0.47±0.02	-23.66±0.14	0.51±0.01
NIST 2709 mix	-15.34±0.16	2.12±0.16	-25.58±0.13	1.01±0.04	-25.56±0.10	0.90±0.10
Soil mix	-17.04±0.01	8.10±0.03	-28.56±0.01	5.18±0.08	-28.55±0.10	4.99±0.22

The $\delta^{13}C$ of TOC values of the analyzed samples ranged from -28.56 ± 0.01 to -23.35 ± 0.11 (‰). Sample Soil mix had the highest concentration of TOC (5.18 ± 0.08 %) and accounted for the majority of Total C (about 64% of Total C), resulting in a depleted $\delta^{13}C$ value that was much lower value than the remaining samples. For sample LS, the TOC content occupied a substantial portion of the sample (1.29 ± 0.01 % of TOC in 1.44 ± 0.01 % of Total C), resulting in no difference between the $\delta^{13}C$ of TOC and $\delta^{13}C$ of Total C.

$$y = -0.051x - 0.286$$
$$R^2 = 0.963$$
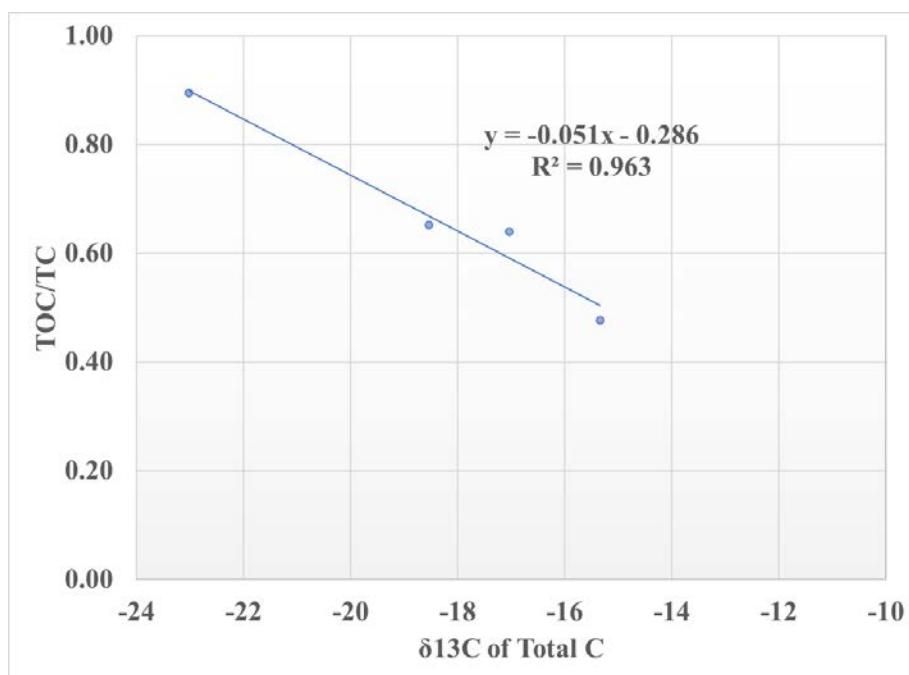
Figure 1. Relationship between $\delta^{13}C$ of Total C and TOC/Total C ratio.

The $\delta^{13}C$ of Total C values in the sediment samples strongly correlated between the ratio of TOC/TC and $\delta^{13}C$ of TOC in the sample. The linear regression equation in Figure 1 shows that when the ratio of TOC/TC in the sample is small, indicating a larger TIC content, the mutual influence of the mixture of TOC and TIC in the sample will lead to a higher $\delta^{13}C$ of Total C value than samples with a large TOC/TC ratio (which are samples containing mainly organic carbon). The strong correlation with $R^2 = 0.963$ can be used as a reference to predict the TIC present in the sample and choose a suitable method to remove carbonate in the sample.

4. Conclusion

The results of this study indicate that the use of a 0.5M HCl solution to remove carbonates from soil and sediment samples is a stable and dependable method for preparing samples for $\delta^{13}C$ and TOC analysis. The Carbonate re-removal analysis showed that the dilute HCl solution did not affect the analytical values of the samples, indicating that the method is reliable. The $\delta^{13}C$ values for the analyzed samples ranged from -28.56 ± 0.01 to -23.35 ± 0.11 (‰), with the sample with the highest TOC content showing the lowest $\delta^{13}C$ value. The standard deviation and bias values varied among the QC samples, indicating differences in measurement variability and accuracy among the samples. Overall, the results of this study provide important insights into the preparation and analysis of soil and sediment samples for $\delta^{13}C$ and TOC measurements and contribute to the development of more accurate and reliable methods for studying carbon cycling in terrestrial ecosystems.

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