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Carbonate removal by acid fumigation for measuring the $\delta^{13}\text{C}$ of soil organic carbon

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Ramnarine, R., Voroney, R. P., Wagner-Riddle, C. and Dunfield, K. E. 2011. **Carbonate removal by acid fumigation for measuring the $\delta^{13}\text{C}$ of soil organic carbon.** *Can. J. Soil Sci.* **91**: 247–250. Complete removal of carbonates from calcareous soil samples is critical for accurate measurement of the quantity and isotopic signature ($\delta^{13}\text{C}$) of soil organic carbon (SOC). Carbonates confound SOC and $\delta^{13}\text{C}$ measurements because they have $\delta^{13}\text{C}$ values ranging from -10‰ to $+2\text{‰}$, whereas those of soil organic carbon range from -27‰ to -13‰ , depending on the source of plant residues. Commonly used methods for removing carbonates involve treatment with acid followed by repeated water washings; however, these methods are time consuming, labour-intensive and lead to losses of acid- and water-soluble organic carbon. Fumigation of soil samples with HCl was evaluated as an alternative method, and the time required for complete carbonate removal was determined in this study. Moistened soil samples, taken from 0- to 10-cm and 30- to 50-cm depths, were exposed to HCl vapours for periods of 0, 6, 12, 24, 48, 72, and 96 h, followed by measurements of total C and $\delta^{13}\text{C}$ using coupled elemental analyzer-isotope ratio mass spectrometry. The minimum time required to remove all carbonates was ca. 30 h and 56 h for surface and subsurface soils containing 0.80 and 1.94% inorganic C, respectively. Therefore, the fumigation period required is dependent on the total carbonate content of the sample and the nature of the carbonate (pedogenic vs lithogenic). In our study, the rate of removal of inorganic carbon was 0.08–0.10 mg h^{-1} for soil samples sizes with 2.4 to 5.8 mg of carbonate-C, a rate similar to previous studies on acid fumigation. A “correction factor” was used to account for a change in sample mass due to fumigation and is necessary for accurate determination of SOC concentration using our proposed methodology.

Key words: Soil organic carbon, $\delta^{13}\text{C}$, acid fumigation and carbonate removal

Ramnarine, R., Voroney, R. P., Wagner-Riddle, C. et Dunfield, K. E. 2011. **Extraction des carbonates par fumigation acide pour doser la teneur en $\delta^{13}\text{C}$ du carbone organique du sol.** *Can. J. Soil Sci.* **91**: 247–250. Tous les carbonates doivent être extraits des échantillons de sol calcaire si l'on veut mesurer précisément la concentration et la signature isotopique ($\delta^{13}\text{C}$) du carbone organique du sol (COS). En effet, les carbonates confondent la quantification du COS et du $\delta^{13}\text{C}$ parce que leur teneur en $\delta^{13}\text{C}$ varie de -10‰ à $+2\text{‰}$, alors que celle du COS fluctue de -27‰ à -13‰ , selon l'origine des résidus végétaux. Les méthodes couramment employées pour extraire les carbonates supposent un traitement à l'acide suivi de plusieurs lavages à l'eau; cependant, ces méthodes exigent du temps, sont laborieuses et entraînent la perte du carbone organique soluble dans l'eau et dans l'acide. Les auteurs ont évalué la fumigation des échantillons de sol avec du HCl comme alternative, et déterminé le temps nécessaire pour éliminer complètement les carbonates. Des échantillons de sol prélevés à une profondeur de 0–10 cm et de 30–50 cm ont été exposés aux vapeurs de HCl pendant 0, 6, 12, 24, 48, 72 et 96 h, après quoi la concentration de C totale et de $\delta^{13}\text{C}$ a été établie par spectrométrie de masse du rapport isotopique couplée à un analyseur d'éléments. Il faut au moins environ 30 h et 56 h pour retirer tous les carbonates du sol de surface et des couches inférieures contenant respectivement 0,80% et 1,94% de C inorganique. Par conséquent, la période de fumigation requise dépend de la teneur totale en carbonates de l'échantillon et de la nature de ces derniers (pédogènes c. lithogènes). Dans cette étude, le carbone minéral a été éliminé à la vitesse de 0,08–0,10 mg par heure pour les échantillons de sol contenant de 2,4 à 5,8 mg de C-carbonate; ce taux est semblable à celui rapporté lors d'études antérieures sur la fumigation acide. Les auteurs ont recouru à un « facteur de correction » pour tenir compte du changement de masse de l'échantillon après la fumigation. Pareille opération est nécessaire si l'on veut établir exactement la concentration de COS au moyen de la méthode proposée.

Mots clés: Carbone organique du sol, $\delta^{13}\text{C}$, fumigation acide, extraction des carbonates

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Abbreviation: SOC, soil organic carbon

Total carbon in calcareous soils consists of both organic carbon and inorganic carbon. In studies of soil organic carbon dynamics, it is essential to remove carbonates for accurate measurement of organic carbon concentrations and $\delta^{13}\text{C}$ values. One of the distinguishing features of organic and inorganic C is their $\delta^{13}\text{C}$ value. Soil organic matter is mainly derived from plant tissues that have a specific isotopic signature arising from differences in their photosynthetic carbon assimilation pathways. C_3 and C_4 plants have average $\delta^{13}\text{C}$ values of -27‰ (range of -20‰ to -35‰) and -13‰ (range of -9‰ to -17‰), respectively (Smith and Epstein 1971). Soils developed under C_3 or C_4 vegetation contain soil organic carbon (SOC) with a $\delta^{13}\text{C}$ signature reflecting the source of their plant inputs. However, primary or lithogenic carbonates (marine limestone) have $\delta^{13}\text{C}$ values of -2‰ to $+2\text{‰}$, while secondary or pedogenic carbonates (formed from dissolution and precipitation of lithogenic carbonates) have lower $\delta^{13}\text{C}$ values (range of -10‰ to 0‰) due to the contributions of CO_2 from plant and soil microbial respiration and from the influence of soil and climatic factors (Boutton 1991). Thus, measurement of $\delta^{13}\text{C}$ in soil organic matter can be confounded by the presence of carbonates.

All carbonates must be removed for the determination of the $\delta^{13}\text{C}$ of organic matter in a soil sample (Midwood and Boutton 1998). Most common methods for removing carbonates use inorganic acids, each of which has its own limitations (Midwood and Boutton 1998; Collins et al. 1999; Fernandes and Krull 2008). The treatment of soil with inorganic acids followed by repeated washings is time consuming, labour-intensive and can lead to losses of acid- and water-soluble organic carbon. Fernandes and Krull (2008) reported that treatment with 1 M HCl followed by water washing led to the preferential preservation of ^{13}C -depleted SOM components. These limitations are critical when soils contain low organic carbon concentrations and where many samples are analyzed on a regular basis.

The technique of acid fumigation has previously been used to remove carbonates without the loss of water-soluble carbon (Hedges and Stern 1984; Harris et al. 2001). The method involves exposing moistened soil samples to vapours from concentrated acids, with hydrochloric acid (HCl) being the most common. Previous studies have shown that acid fumigation does not alter the $\delta^{13}\text{C}$ values of the SOC and therefore the fumigation time for all inorganic carbon removal can be accurately determined (Midwood and Boutton 1998; Harris et al. (2001). Harris et al. (2001) reported that the time required to remove carbonates from 30-mg soil subsamples was 6 to 8 h when acid fumigation was carried out directly in Ag-foil capsules. Preliminary experiments conducted in our laboratory using the method of Harris et al. (2001) revealed some issues with the acid fumigation method, such as sample loss and contamination. Recent research has also suggested that an optimal exposure time to HCl vapors be

determined for accurate determination of SOC and $\delta^{13}\text{C}$ (Komada et al. 2008).

Our study examined the procedure of removing carbonates by HCl fumigation from soil samples, taken at two different depths so as to span a range in soil inorganic C content. It differs from previous work on acid fumigation by using glass vials for acid treatment, a larger subsample to allow for replicates and the use of tin-foil capsules instead of silver-foil capsules. The overall goal of our study was to provide a solution and alternative to the current acid fumigation approach. The objectives of our study were (i) to determine if this alternative HCl fumigation method can be successfully used for soils with both high and low carbonate contents, and (ii) to determine the fumigation time required to remove all of the carbonates.

Site and Soil Characteristics

Soil samples were obtained from a research plot at the Elora Research Station (lat. $43^\circ 38' 29''\text{N}$, long. $80^\circ 24' 47''\text{W}$, elevation 376 m), southern Ontario, Canada (about 20 km northwest of the University of Guelph). The soil is a silt loam derived from calcareous till and classified as a Gray Brown Luvisol. Samples were taken with a hydraulic soil core sampler (38 mm i.d.) to a depth of 50 cm, and divided into 0- to 10-cm, 10- to 20-cm, 20- to 30-cm, and 30- to 50-cm depth increments. Only the 0- to 10-cm and the 30- to 50-cm depths were used in this study to capture the range in carbonate content found in soil samples in the profile.

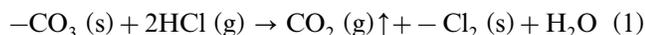
Laboratory Analysis

Triplicate composite soil samples from the 0- to 10-cm and the 30- to 50-cm depths (total of 36 samples) were fumigated with 12 M HCl for varying time periods (0, 6, 12, 24, 48, 72, 96 h) to determine the length of time required for removal of carbonates. Soil samples were homogenized, passed through a 2-mm sieve, oven-dried (105°C for 24 h), and ball-milled to a fine powder. A 300-mg subsample of soil was weighed into a 20-mL glass scintillation vial and moistened using 150 μL of deionized water. This is a modification of the procedure by Harris et al. (2001), who placed 30-mg soil subsamples directly in silver-foil capsules arranged in microtiter plates. Vials with soil were placed into a Pyrex glass vacuum desiccator (7.5 L) fitted with a porcelain plate, together with a beaker containing 100 mL of 12 M HCl. The desiccator was vacuum-sealed (80 kPa) using a water-jet vacuum system and samples were exposed to HCl vapour for the designated time periods. After the prescribed treatment period the beaker containing acid was removed. The desiccator was resealed and the samples were subjected to repeated vacuum evacuation for 1–1.5 h to remove all HCl vapour. This step is critical to preventing degradation of the tin capsules in future storage/transit and corrosion of the mass spectrometer during $\delta^{13}\text{C}$ measurement.

The samples were dried at 60°C for 16 h, removed from the oven and allowed to cool to room temperature in a desiccator cabinet. The samples were then manually disrupted (in the vial using a glass rod) returning it to a fine powder. Soil samples containing about 420 μg of carbon (based on preliminary testing) were placed in 8×5 mm tin capsules (Elemental Microanalysis Ltd, Okehampton, UK), carefully sealed (to prevent leakage), compressed (to remove trapped CO_2) and arranged in a 96-well microtiter plate. Samples were analyzed for $\delta^{13}\text{C}$ using a Tracermass[®] Isotope Ratio Mass Spectrometer (Europa Scientific, Crewe, UK) at the Stable Isotope Laboratory, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Use of a Correction Factor

The chemical reaction that occurs during HCl fumigation of carbonate is:



Since the formula mass of the $-\text{Cl}_2$ formed (70.91 g mol^{-1}) is greater than that of the $-\text{CO}_3$ (60.01 g mol^{-1}) eliminated, the organic C remaining becomes diluted within the acidified soil sample that weighs more than the original soil sample. This change in mass needs to be included for reporting the organic carbon content on a pre-treated soil mass basis. To correct for this mass change, the mass of the sample is recorded before and after the fumigation process so as to obtain a correction factor. The correction factor is used to calculate the actual mass of the acid fumigated sample used for SOC and $\delta^{13}\text{C}$ analysis. This correction factor does not apply when soil samples are treated directly in silver-foil capsules because the quantity of organic carbon measured is referenced to the original pre-acidified soil weight.

Experimental Design and Statistical Analyses

The experiment was arranged as a two-way factorial with three replicates; depth (0–10 and 30–50 cm) and treatment time (0, 6, 12, 24, 48, 72, 96 h) being the two factors. Both treatment time and depth were analyzed as fixed effects with the response variable being $\delta^{13}\text{C}$. All data were analyzed using SAS version 9.1 (SAS Institute, Inc., Cary, NC). A segmented linear regression (quadratic model with plateau) was carried out to determine the minimum treatment time for all carbonates to be removed. Least square means and standard errors were computed for $\delta^{13}\text{C}$ using the Proc GLM procedure in SAS.

Results

Changes in the $\delta^{13}\text{C}$ of soil samples over time from the 0- to 10-cm and 30- to 50-cm depths indicate the removal of carbonates by HCl fumigation treatment (Fig. 1). Untreated samples from the 0- to 10-cm depth had a mean $\delta^{13}\text{C}$ of -17.02‰ , which decreased with treatment time reaching a steady state at -22.68‰ (Fig. 1. A). The time required for complete removal of

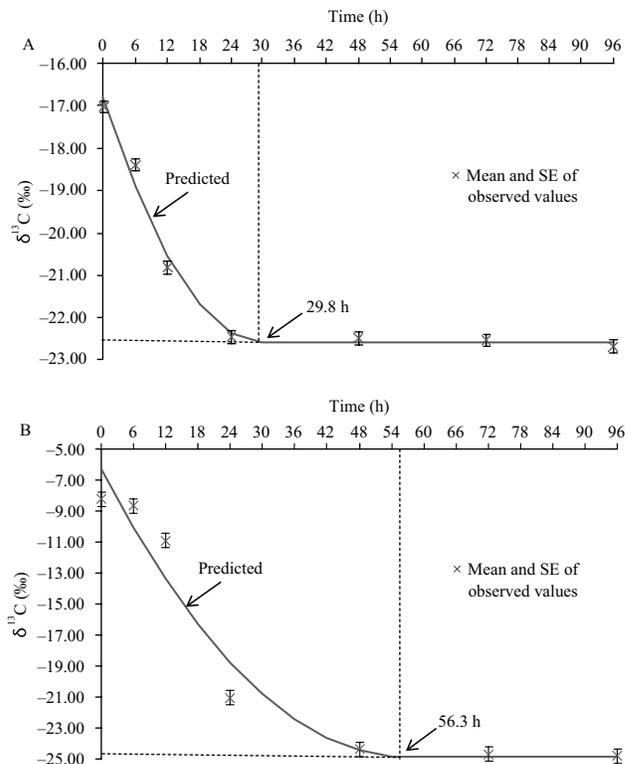


Fig. 1. Relationship between $\delta^{13}\text{C}$ value and treatment time by 12 M HCl fumigation from soil sampled from the 0- to 10-cm (A) and 30- to 50-cm (B) depths. The standard error (SE) of observed values was 0.15‰ and 0.47‰ for the 0- to 10-cm and 30- to 50-cm depths, respectively.

carbonates from the 0- to 10-cm depth samples was 30 h. Samples from the 30- to 50-cm depth had initial $\delta^{13}\text{C}$ values of -8.22‰ , which decreased to -24.81‰ after 56 h of acid fumigation treatment (Fig. 1 B). The higher $\delta^{13}\text{C}$ values of untreated samples were caused by the presence of carbonates, and, as acid fumigation time progressed, $\delta^{13}\text{C}$ values became more negative and levelled off when all carbonates were removed.

Harris et al. (2001) reported that the time required for removing all carbonates from a 30-mg soil sample containing 0.72 mg inorganic carbon was 6 h. This apparent discrepancy is explained by the total inorganic carbon present in the sample, which is a function of the inorganic carbon content of the soil and quantity of sample fumigated. In our study the sample size was 300 mg and the inorganic carbon content was 0.80 and 1.94%, for the upper and lower depths, respectively. The total inorganic carbon in the sample was 2.4 mg for the upper depth and 5.8 mg for the lower depth. Therefore, the rate of inorganic carbon removal was 0.10 mg h^{-1} and 0.08 mg h^{-1} for the upper and lower depths, respectively, in this study, which was similar to that (0.12 mg h^{-1}) in the study reported by Harris et al. (2001). The lower rates of inorganic carbon removal in this study may be due to a slower diffusion time of the

HCl vapours because of a larger sample size used, the size and shape of the soil sample container, and/or the presence of dolomite [$\text{CaMg}(\text{CO}_3)_2$] and metal oxides in the soil samples.

The acid fumigation technique describes the treatment of soil samples in silver-foil capsules with 12 M HCl (Harris et al. 2001). Issues with this method involve samples with high calcium carbonate content that can effervesce intensely and overflow causing loss of sample and contamination of other capsules in the microtiter plate. After acid treatment, silver-foil capsules can also become brittle, leading to sample loss during folding/compression of the capsules. The above observations were also reported in a recent study by Walthert et al. (2010) who used "stepwise-acidification" as a modification of the acid fumigation method. Placing treated samples and capsules into a second capsule prior to folding [as suggested by Harris et al. (2001) and Walthert et al. (2010)] is more difficult, time-consuming and increases cost. Double encapsulating the sample is also problematic for the mass spectrometer because more oxygen is required for combustion and results in frequent replacement of copper wires used to trap excess oxygen.

This experiment was conducted with ball-milled soil samples, which is advantageous to treating <2 mm soil samples that will still require grinding before mass spectrometer analysis. This 300-mg sample size allows for replicated analyses, but can be decreased to 100 mg with the use of a smaller glass bottle if desired. The acid-fumigated samples increased in mass by 1.02% for the upper depth and 1.06% for the lower depth when compared with the original soil samples. After correction, organic carbon values averaged 1.89 and 0.57% for the upper and lower depths, respectively. The use of a "correction factor" is a deviation from Harris et al. (2001), who used the original pre-acidified soil weight as the reference for SOC determination since acid fumigation was carried out directly in the capsules with soil to be analyzed. The "correction factor" is necessary when transferring the acid-fumigated sample from one vessel (vials) to the capsules. This study focused on refining the acid fumigation technique using a single soil (Gray Brown Luvisol) but it is anticipated that the results will be similar for other soils. Previous studies have incorporated soil types with varying levels of organic and inorganic carbon (Harris et al. 2001; Walthert et al. 2010).

Conclusion

The unique and valuable aspect of the proposed method is the use of glass vials for acidification of larger soil samples and the subsequent transfer to less costly tin-foil capsules rather than silver-foil capsules. Fumigation with 12 M HCl can be successfully used to remove carbonates from surface and subsoil samples prior to the determina-

tion of $\delta^{13}\text{C}$ of SOC. The length of treatment time, as depicted by the stabilization of $\delta^{13}\text{C}$ values, is dependent on the total carbonate content of the soil sample.

In this study, the time required for complete carbonate removal was ca. 30 and 56 h for soils containing 0.80 and 1.94% inorganic C, respectively. The removal rate of inorganic C was 0.08–0.10 mg h^{-1} for samples ranging from 2.4 to 5.8 mg of carbonate C. This experiment has generated successful results as an alternative to traditional carbonate removal techniques and may provide solutions to researchers who have tried adopting the acid fumigation technique.

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