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SOIL CARBONATE DECOMPOSITION BY ACID HAS LITTLE EFFECT ON $\delta^{13}C$ OF ORGANIC MATTER

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Summary—The $\delta^{13}C$ of soil organic C is often utilized to document vegetation changes and to estimate rates of soil organic C turnover. Soil carbonate C is isotopically distinct from organic C, and must be removed by acidification prior to isotopic analysis of soil organic matter. We determined the effects of acid concentration (0.1, 0.5, 1, or 6 M HCl) and duration of treatment (1, 2, 3, or 8 d) on soil $\delta^{13}C$ and the concentration of C and N in a carbonate-free entisol and a calcareous vertisol both obtained from Texas. $\delta^{13}C$ values of organic C in the entisol were reduced significantly by treatment with 6 M HCl for more than 1 d; however, all other combinations of acid strength and treatment duration had no effect on $\delta^{13}C$ of organic C in that soil. In the calcareous soil, $\delta^{13}C$ of organic C was largely unaffected by acid concentration or duration of treatment; however, soils treated with 0.1 M HCl required 3 d to fully remove all carbonate and achieve $\delta^{13}C$ values comparable to those in other treatment combinations. Treatment of calcareous soils with 0.5 M HCl for 24 h appears sufficient to remove all carbonate C prior to isotopic analysis, and this treatment has no discernible effect on the $\delta^{13}C$ of the soil organic matter.

INTRODUCTION

The natural stable carbon isotopic composition of soil organic matter is a function of the relative contribution of C_3 vs C_4 plants to the soil organic C pool. The isotopic difference between C_3 ($\delta^{13}C \approx -27‰$) and C_4 ($\delta^{13}C \approx -13‰$) plants has been utilized to document changes in plant community composition (Dzurec *et al.*, 1985; McPherson *et al.*, 1993; Boutton *et al.*, 1993) and to estimate rates of soil organic matter turnover (Balesdent *et al.*, 1987; Andreux *et al.*, 1990; Wolf *et al.*, 1994; Jastrow *et al.*, 1996). However, the presence of inorganic C in soil may confound the measurement and interpretation of $\delta^{13}C$ values of soil organic matter. Lithogenic carbonates are generally derived from marine limestone parent material and have $\delta^{13}C$ values near 0‰ (West *et al.*, 1988), while pedogenic carbonates have $\delta^{13}C$ values that are generally 14–16‰ greater than those of the associated soil organic matter (Cerling and Quade, 1993). Because of the large isotopic differences between organic and inorganic soil C, the presence of inorganic C can strongly influence $\delta^{13}C$ measurements of soil organic C.

To ensure that $\delta^{13}C$ measurements of soil organic C are not influenced by inorganic C, soil samples must be treated with acid to volatilize C associated with calcite and/or dolomite to CO_2 prior to isotopic analysis. This treatment may induce loss of acid-soluble organic C, and has the potential to alter the concentration and isotopic composition of organic C in the treated soils. Acid treatment of calcareous soils and sediments is practiced routinely, and several studies have documented a reduction in organic C concentration following treatment (Roberts *et al.*, 1973; Froelich, 1980; Yamamuro and Kayanne, 1995). Despite the fact that loss of acid-soluble organic C creates opportunity for isotope fractionation, there have been no studies on the effect of this practice on the $\delta^{13}C$ of soil organic C. Our purpose was to determine the effects of acid strength and duration of treatment on soil $\delta^{13}C$ values. Soil C and N concentrations were also measured to monitor the loss of $CaCO_3$ and to evaluate any other losses of these elements during acid treatment.

MATERIALS AND METHODS

Soils

Two soils were used: a carbonate-free forest entisol (Pophers, Aeric Fluvaquent, fine-silty, siliceous, acid, thermic) obtained from Houston County, Texas and a calcareous vertisol (Houston Black Clay, Udic Pellustert, fine, montmorillonitic, thermic) obtained from Dallas County, Texas. The entisol was included because the $\delta^{13}C$ of its organic

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Table 1. Physical and chemical characteristics of soils. Analyses were performed by the soil characterization laboratory, Department of Soil and Crop Sciences, Texas A and M University using procedures described by Soil Survey Staff (1984)

	Vertisol	Entisol
Horizon	A	A
Depth (cm)	13–36	0–13
pH	7.8	4.3
Total carbon (g kg^{-1})	52.0	50.7
Organic carbon (g kg^{-1})	15.6	50.7
$CaCO_3$ equivalent (g kg^{-1})	304.0	0.0
Texture		
Sand (%)	6.2	1.0
Silt (%)	41.1	60.4
Clay (%)	52.7	38.6
Cation exchange capacity (cmol kg^{-1})		
Ca	79.7	0.3
Mg	1.1	2.1
Na	0.4	1.1
K	0.4	0.1
Total	81.7	3.6

C could be measured without acid treatment, and these control values could be used to statistically evaluate the effects of acid treatment on that same soil. Physical and chemical characteristics of these soils are presented in Table 1. Both soils were dried at 60°C and ground (2 mm). An aliquot of the calcareous soil was ground further (425 μm) to determine the influence of grinding on the acid treatment procedure.

Acid treatment

Four acid concentrations were used (0.1, 0.5, 1 and 6 M HCl) in factorial combination with four treatment durations (1, 2, 3 and 8 d). Triplicate 10 g aliquots of each soil (calcareous vertisol ground to 2 mm, calcareous vertisol ground to 425 μm, and acidic entisol) were treated with 300 ml of acid at 25°C. For the 2, 3 and 8 d treatments, acid was poured off daily and replenished with new acid. The soil-acid mixtures were stirred three times over each 24 h period. After the appropriate treatment duration, the acid was poured off, replaced with distilled water, and stirred. The distilled water was replaced every 12 h until the soil became dispersed and remained suspended. If acidic salts are not washed out of the soil after acid treatment, the quartz tubes used to combust organic C to CO_2 (see below) are likely to crack at high temperature. Soil suspensions were then dried at 60°C, and the soil was ground (425 μm). Soil loss was calculated from the difference in soil dry weight before and after acid treatment. For control and comparative purposes, samples of both entisol and vertisol were obtained but not treated with acid, these "untreated soils" were dried at 60°C and ground (425 μm).

Isotopic and elemental analysis

$\delta^{13}C$ of organic C ($\delta^{13}C_{OC}$) and total C ($\delta^{13}C_{TC}$) in the treated soils was determined on CO_2 produced by combustion of soil at 850°C in the presence of CuO and Cu in sealed quartz tubes (Boutton, 1991). The $\delta^{13}C_{TC}$ and total C content of the untreated soils was determined using a TracerMAT, continuous flow — isotope ratio mass spectrometer (Finnigan MAT). The elemental analyzer used by this instrument combusts the samples at a temperature close to 2000°C and was used here to ensure complete dissociation of the carbonate content of the calcareous soil. The $\delta^{13}C$ of the carbonate C ($\delta^{13}C_{IC}$) in the untreated calcareous soil was determined on CO_2 produced by reacting this soil with 100% H_3PO_4 under vacuum at 25°C (Boutton, 1991). CO_2 from both the sealed quartz bombs and carbonate reactions was purified cryogenically and its isotopic composition determined on a VG-903 (VG Isogas, Middlewich) dual inlet, triple collector isotope ratio mass spectrometer. All results are reported relative to the international V-PDB standard by calibration through NBS-19 (Hut, 1987). Overall precision (machine error plus sample preparation error) was ±0.15‰.

Total C and N concentrations of both the acid treated and untreated soils were determined by automated Dumas combustion using a Carlo Erba NA-1500 elemental analyzer (Thermo Instruments, Pella and Colombo, 1973). Repeated measurements (n = 5) of a soil standard (Leco 502-062, Leco, St. Joseph, MI) yielded a precision (±1 S.D.) of 150 mg kg^{-1} for C, and 20 mg kg^{-1} for N.

Statistical analyses

Dunnett's test (SAS/STAT, SAS Institute, Cary, NC) was used to test for differences in $\delta^{13}C$ and elemental composition between controls and specific treatments. Analysis of variance was used to determine the effects between treatments of acid concentration, duration of treatment, and their interaction on $\delta^{13}C$ and elemental composition of soils (Genstat 5, Numerical Algorithms Group, Oxford).

RESULTS

Isotopic and elemental composition of acidic entisol

The $\delta^{13}C_{OC}$ of the entisol was not significantly different from the $\delta^{13}C_{OC}$ of the untreated control soil following treatment with 0.1 M, 0.5 M or 1 M HCl regardless of duration (Fig. 1). Soils treated with 6 M HCl for longer than 24 h had $\delta^{13}C_{OC}$ values significantly lower ($P < 0.05$) than that of the control soil. Significant reductions in organic C relative to the control occurred in half of the treatment combinations, and were particularly pronounced in the soil treated with 6 M HCl (Fig. 1). Soil total N content was significantly ($P < 0.05$) reduced in nearly all treatment combinations, with the greatest losses occurring in soil treated with 6 M HCl. The C-to-N ratio of the soils treated with 6 M HCl for longer than 24 h were significantly ($P < 0.05$) greater than that of the control soil.

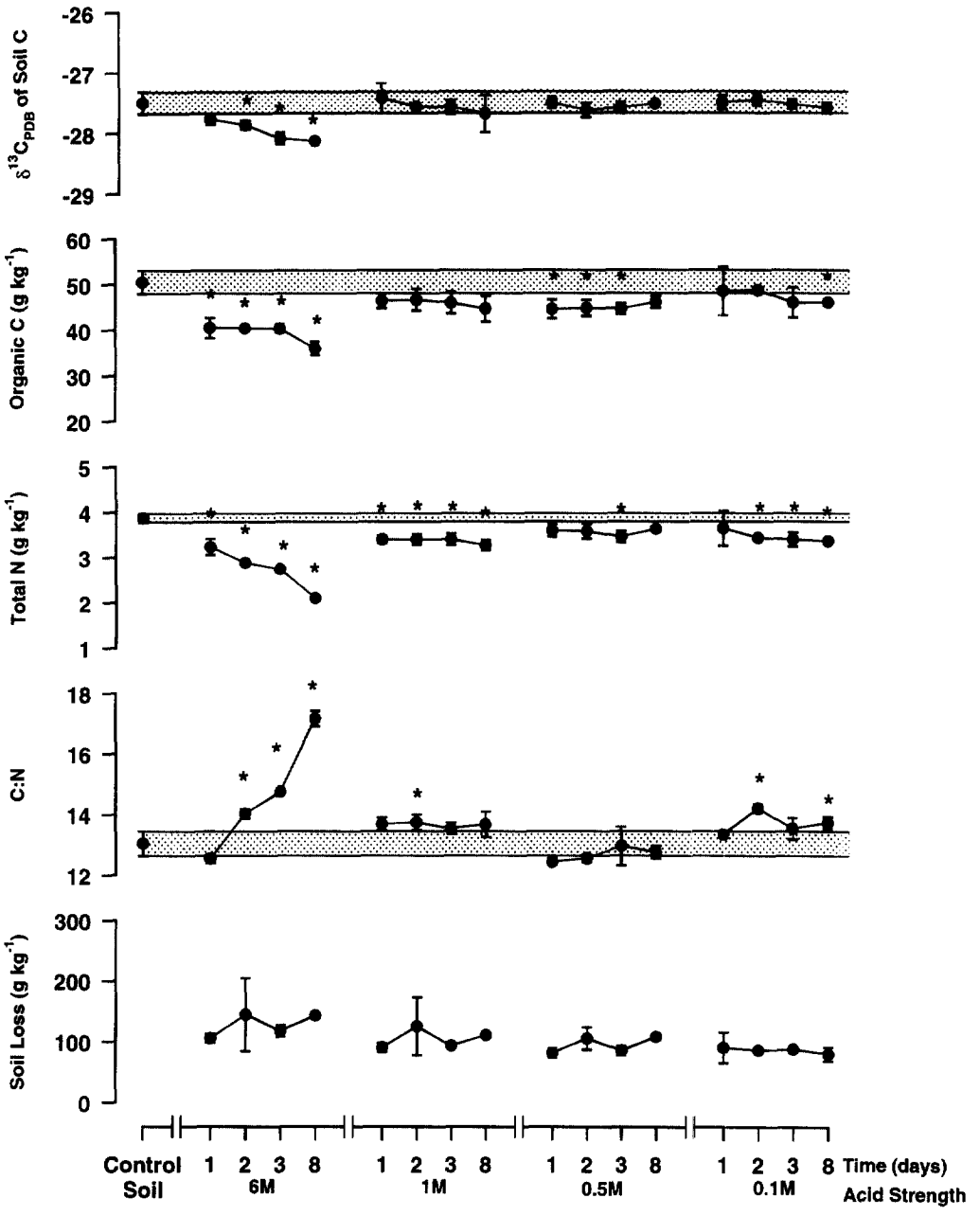

Fig. 1. $\delta^{13}C_{OC}$, organic C, total N, C-to-N ratio, and soil loss of the acidic entisol in response to acid treatment. Error bars represent ± 1 S.D. of mean ($n = 3$). The shaded areas correspond to ± 1 S.D. from the control soil mean ($n = 6$); the asterisks indicate treatments which are significantly different ($P < 0.05$) from the control soil, as determined by Dunnett's test.

However, the C-to-N ratio was largely unchanged in soils subject to all other treatment combinations (Fig. 1). Average soil loss across all treatment combinations was 104 ± 26.8 g kg^{-1} (mean $\pm$ SD), or approximately 10% of the initial soil weight (Fig. 1).

Isotopic and elemental composition of calcareous vertisol

No significant differences were observed between the soils ground to 2 mm or 425 μm in terms of $\delta^{13}C$, organic C, total N or the amount of soil lost following acid treatment. As a consequence, the two data sets were combined and the overall means were used to test for differences between the treatments (Fig. 2).

Analysis of variance revealed significant differences in $\delta^{13}C$ values due to acid concentration and treatment duration (Fig. 2). These differences were attributed to soil treated with 0.1 M HCl for 1 or 2 d. These treatments failed to remove all carbonate C, resulting in significantly higher $\delta^{13}C$ values. $\delta^{13}C$ values of soils in all other treatment combinations

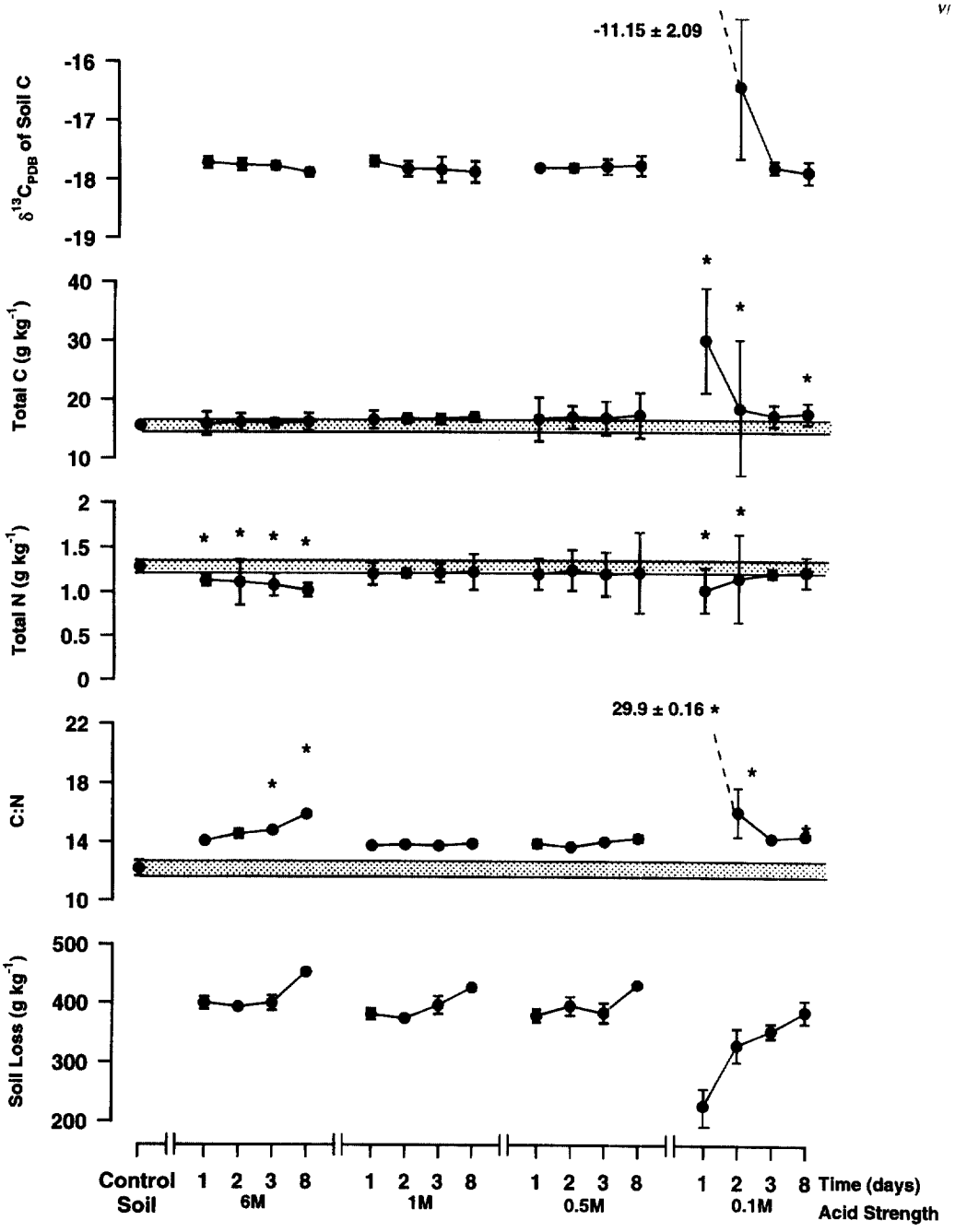

Fig. 2. $\delta^{13}C_{TC}$, total C, total N, C-to-N ratio, and soil loss of the calcareous vertisol in response to acid treatment. Error bars represent ±1 S.D. of the pooled mean (n = 6) of soil ground to 2 mm or 425 μm. Note that both the total C and total N of the control are expressed on a carbonate-free basis. The shaded areas correspond to ±1 S.D. from the control soil mean (n = 12); the asterisks indicate treatments which are significantly different ($P < 0.05$) from the control soil, as determined by Dunnett's test.

were not significantly different from each other, and averaged −17.8 ± 0.13‰ (n = 72) (Fig. 2).

Since we could not directly measure the actual $\delta^{13}C$ of the organic matter without acid treatment to remove carbonate C, it was not possible to compare this overall mean value with a true value. However, we estimated the organic matter $\delta^{13}C$ using a mass balance approach. According to mass balance:

$$\delta^{13}C_{TC} = (\delta^{13}C_{IC}) \cdot (f_{IC}) + (\delta^{13}C_{OC}) \cdot (f_{OC}) \quad (1)$$

where f_{IC} is the fraction of total C which is inor-

Table 2. Mass, mass fraction, and $\delta^{13}C$ values of carbon components of the calcareous vertisol

	Mass (g kg^{-1})	Fraction of total C	$\delta^{13}C$ (‰) (mean ± S.D.)
Total C	52.09	1.0000	−7.04 ± 0.16 (n = 5)
Organic C	15.61	0.2997	–
Inorganic C	36.48	0.7003	−2.39 ± 0.05 (n = 4)

ganic and f_{OC} is the fraction of total C which is organic. By rearrangement:

$$\delta^{13}C_{OC} = \frac{\delta^{13}C_{TC} - (\delta^{13}C_{IC}) \cdot (f_{IC})}{f_{OC}} \quad (2)$$

Utilizing the mass fractions (f_{IC} and f_{OC}) and the $\delta^{13}C$ values for the carbon components of the calcareous vertisol (Table 2), solution of equation (2) yields a $\delta^{13}C_{OC}$ of −17.9‰, nearly identical to the overall mean value of −17.8‰ obtained for the acid treated samples (Fig. 2).

Direct comparison of the elemental composition of the control and treated soils was achieved after correcting the total C and total N content of the control soil to allow for the carbonate content and mass change. The organic C content of the soil was not significantly different from that of the control except in the 0.1 M HCl group. A significantly ($P < 0.05$) higher C content was observed in the soil after treatment for 1, 2 and 8 d with 0.1 M HCl (Fig. 2). The total N content of the calcareous vertisol was not significantly different from the control after treatment with 1 M or 0.5 M HCl. A significant ($P < 0.05$) reduction in total N relative to the control soil was observed however, after treatment with 6 M HCl regardless of treatment duration, and 0.1 M HCl for 1 and 2 d (Fig. 2). The C-to-N ratio of the soil treated with 1 M and 0.5 M HCl was also not significantly different than the control. However, the C-to-N ratio was significantly ($P < 0.05$) higher after treatment with 6 M HCl for 3 and 8 d and 0.1 M HCl for 1 and 2 d (Fig. 2). Soil losses following acid treatment ranged from 220.8 to 453.8 g kg^{-1} (Fig. 2). Acid concentration ($P < 0.001$), duration of treatment ($P < 0.001$), and their interaction ($P < 0.012$) all had a significant effect on soil loss.

DISCUSSION

The $\delta^{13}C_{OC}$ of the acidic entisol was not affected by 1 M, 0.5 M and 0.1 M HCl, regardless of treatment duration. A significant decline in $\delta^{13}C_{OC}$ was brought about by 6 M HCl. Using an acid weaker than 6 M HCl to decompose soil carbonates in some soils may be essential if the isotopic integrity of the organic matter is to be preserved. All of the acid treatments lowered the organic C and total N content of the soil relative to the control, especially those in the 6 M HCl treatment. Losses of acid soluble organic matter and soil N were probably responsible for these changes in the elemental composition. Similar losses have been documented following the acid treatment of marine sediments prior to elemental analysis (Froelich, 1980; Yamamuro and Kayanne, 1995). Carbohydrates, N-containing compounds, carboxyl groups, and some aliphatics and lignins are particularly acid soluble, whereas long alkyl-chain aliphatics, aromatics, and some phenolic compounds tend to be insoluble (Schulten, 1996). Lower $\delta^{13}C_{OC}$ values in the soils treated with 6 M HCl may be attributable to a relative increase in aliphatic compounds, which are known to be low in ^{13}C (Deines, 1980).

In the calcareous vertisol decomposition of carbonate was complete within 24 h using 6 M, 1 M and 0.5 M HCl. However, the $\delta^{13}C_{OC}$ and C content were significantly greater and the total N significantly lower in vertisols treated with 0.1 M HCl for 1 and 2 d, indicating that traces of carbonate remained.

Following acid treatment, the measured $\delta^{13}C_{OC}$ of the calcareous vertisol (−17.8‰), was very close to the $\delta^{13}C_{OC}$ predicted by mass balance (−17.9‰). Furthermore, the isotopic difference between soil organic matter and pedogenic carbonates formed in association with that organic matter is known to be −15‰ (Cerling *et al.*, 1989). In this soil, $\delta^{13}C_{IC}$ was −2.4‰, yielding a difference of 15.6‰ between the organic and inorganic C. In contrast to the acidic entisol, the $\delta^{13}C_{OC}$ of the vertisol was not significantly reduced relative to the other treatments by the use of 6 M HCl. The differential response of these soils to 6 M HCl may have been due to: (1) variation between soils in the relative proportions of acid-soluble vs acid insoluble compounds, and or (2) differences between soils in the degree of organic matter protection afforded by clay mineral complexes.

Grinding the calcareous vertisol further from 2 mm to 425 μm did not produce significantly different results and the degree of grinding may only be important when the soil has a high dolomite content. The calcareous vertisol used here contained 0.8% dolomite; in soils containing significantly higher amounts, grinding to 150 μm may be required to facilitate reaction with the acid (Allison and Moodie, 1965).

No comparable studies have been conducted on soils, and only a few studies of riverain and marine sediments and biota are available to provide comparative data. Bird *et al.* (1992) found that Brazilian river sediments treated with 1 M HCl overnight had $\delta^{13}C$ values that were 0.4‰ lower than untreated samples. They reported later (Bird *et al.*,

1994) that riverain sediments from Cameroon treated with 1 M HCl overnight had $\delta^{13}C$ values which were reduced by on average only 0.18% relative to the untreated samples. Naidu *et al.* (1993) reported that $\delta^{13}C$ values of sediments from the Bering Sea were not altered by treatment with 10% HCl for an unspecified duration, although data were not provided to substantiate the point. Bunn *et al.* (1995) found that treatment of shrimp and seagrass with 0.1 to 1.0 M HCl for 1 h had no significant effect on their $\delta^{13}C$ values. Collectively, these reports substantiate the results of our study, and suggest that there is little or no effect of acid treatment on $\delta^{13}C$ values of organic C.

It should be noted that several acid pretreatment techniques have been developed for the measurement of organic C in calcareous sediments using automated Dumas-type elemental analyzers (Verardo *et al.*, 1990; Cutter and Radford-Knoery, 1991; Nieuwenhuize *et al.*, 1994; Yamamuro and Kayanne, 1995). These methodologies may be particularly well suited for the measurement not only of organic C concentration, but also $\delta^{13}C_{OC}$ by continuous flow-isotope ratio mass spectrometry. In these techniques, excess acid is driven off the sample by drying rather than decanting, thereby minimizing loss of acid soluble compounds.

In conclusion, treatment with 0.5 M HCl for 24 h was the most rapid and least costly (in terms of labor and consumables) approach for the complete removal of carbonate prior to the measurement of $\delta^{13}C_{OC}$. The $\delta^{13}C_{OC}$ of both the calcareous vertisol and acidic entisol were unaffected by this treatment. Use of 0.1 M HCl for less than 3 d appears to be inadequate for the rapid removal of carbonate. Treatment with 6 M HCl may alter the $\delta^{13}C_{OC}$ in some soils.

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