

# Short-term soil carbon dynamics of humic fractions in low-input and organic cropping systems

Timothy A. Doane, Olivier C. Devêvre, William R. Horwath\*

*Department of Land, Air, and Water Resources, University of California Davis, One Shields Avenue, Davis, CA 95616-8627, USA*

## Abstract

Observing changes in soil organic matter (SOM) is a fundamental part of defining the carbon cycle in natural and cultivated environments. However, relying on changes in the mass of soil C over short periods often produces conflicting results because of errors associated with sampling and analysis. In addition, C mass balance studies provide little interpretation of processes or turnover of specific C fractions. In the following study, we used C isotope and chemical separation of soil organic C to observe short-term soil C dynamics. With corn as the source of tracer C in two cover crop-based agricultural systems, natural abundance  $^{13}\text{C}$  measurements were used to identify changes in soil humic fractions (humic acid, fulvic acid, and humin) during two seasons under organic or low-input management treatments. All three fractions showed significant accumulation or turnover of C, with the fulvic acid fraction showing the most frequent but the smallest changes. The fulvic acid fraction showed a 5–9% turnover of C compared to 16% C turnover in the humic acid fraction. The stable soil C fraction defined as humin also exhibited an 8% turnover of C. The different humic fractions were affected at different times in the two treatments, supporting the idea that individual humic fractions may have different roles in C cycling depending on inputs and seasonal conditions. © 2003 Elsevier Science B.V. All rights reserved.

**Keywords:**  $^{13}\text{C}$  natural abundance; Cropping system; Humic fractions; Short-term; Soil carbon; Soil organic matter

## 1. Introduction

The evaluation of short-term soil C dynamics is often overlooked in favor of long-term changes because analytical techniques used to examine changes in soil C mass are often

\* Corresponding author. Tel.: +1-530-754-6029; fax: +1-530-752-1552.

E-mail address: [wrhorwath@ucdavis.edu](mailto:wrhorwath@ucdavis.edu) (W.R. Horwath).

insensitive to detecting small changes. The limitation in assessing short-term changes in soil C is that it requires researchers to estimate the effect of inputs leading to long-term changes in soil organic matter (SOM). The task of evaluating the contribution of various inputs in the cycling of carbon through SOM has been pursued effectively through isotopic approaches, which permit the separation of various sources of C. Natural abundance  $^{13}\text{C}$  techniques, in which differences in isotopic composition between  $\text{C}_3$  and  $\text{C}_4$  plants serve as a tracer of C produced in situ, have been used for an increasing variety of soil organic matter studies. This approach has formed the basis for many long-term studies in which one type of vegetation initially replaced another and was subsequently cultivated on the same site. It is regarded as a useful and convenient way to observe soil C dynamics and characterize organic matter fractions over extended periods (e.g. Cerri et al., 1985; Balesdent et al., 1987; Balesdent and Mariotti, 1996).

The supposed high limit of resolution and often small annual increments of this natural tracer have led to the belief (e.g. Balesdent and Balabane, 1992) that this approach can only be applied to soils after a number of successive cultivations of the crop used as the source of tracer, and even then only when this crop or its plant type ( $\text{C}_3$  or  $\text{C}_4$ ) has never been present on the site prior to initiating the study (Balesdent et al., 1987, 1988). Natural abundance  $^{13}\text{C}$  measurements have been put to only limited use (largely respiration studies) in an attempt to characterize changes over short periods of time (e.g. Mary et al., 1992; Cheng, 1996; Qian et al., 1997; Liang et al., 1999) and seldom under representative field conditions (e.g. Rochette and Flanagan, 1997; Rochette et al., 1999).

Here, a simple experiment was carried out to determine if changes in these pools could be observed using a short-term application of natural abundance  $^{13}\text{C}$  measurements. The study provided data on changes in size of humic fractions and the amount of new C or the turnover rate found in these fractions on a seasonal basis.

## 2. Materials and methods

### 2.1. Site and field work

The experiment was superimposed in the Sustainable Agriculture Farming Systems (SAFS) plots, located at the University of California at Davis. The SAFS project, begun in 1988, includes as its treatments two alternative (cover crop-based) management practices. The soil is classified as Reiff loam (coarse-loamy, mixed, nonacid, thermic Mollic Xerofluvents) and Yolo silt loam (fine-silty, mixed, nonacid, thermic Typic Xerorthents); both are Eutric Fluvisols under FAO classification. Most rainfall occurs during the winter months (December to March) and averages 400–500 mm annually. Daytime temperatures average 30–35 °C during the summer growing season. Additional characteristics of the SAFS site are described by Clark et al. (1998).

The two alternative systems at SAFS were used for this study: ‘organic’ plots (ORG) and ‘low-input’ (LI) plots in which corn was to be grown, replicated in each of three randomized blocks. In April 1998, one experimental microplot (9 m<sup>2</sup>) was created within each of these main plots. Soil samples, a minimum of 10 cores (0–30 cm), were taken along a line in the center area (1 m<sup>2</sup>) of each microplot. This was done just before incorporation of a winter

cover crop of vetch, an input of 900 kg C ha<sup>-1</sup> in the ORG plots and 1100 kg C ha<sup>-1</sup> in the LI plots. The ORG plots also received partially composted poultry manure following vetch, an application of 3000 kg C ha<sup>-1</sup>. Both vetch and manure were incorporated within a week prior to planting field corn. Additional N in the LI treatment was applied approximately 1 month after planting as fertilizer (90 kg urea N ha<sup>-1</sup>).

In October 1998, grain was harvested in all plots and soil samples were again taken in each microplot. Corn residue was then chopped and incorporated, and a cover crop of oats, vetch, and peas was planted in all plots. The amount of corn residue returned averaged 4200 kg C ha<sup>-1</sup> in both the ORG and LI plots. The final soil samples were taken in May 1999 before complete maturity of the cover crop.

## 2.2. Analysis of humic fractions

All soil samples were sieved (2 mm), picked free of recognizable plant residues, and air-dried after collection. Chemical fractionation for isolation of classical humic fractions, based on differences in solubility in alkaline and acid solutions, closely followed International Humic Substance Society (IHSS) recommended extraction procedure (Hayes, 1985; Swift, 1996). A preliminary rinse with 0.1 N HCl was used to remove any carbonates, as well as light material and debris, such as pieces of undecomposed plant material and rootlets (Stevenson, 1965; Kononova, 1966; Anderson et al., 1974; Martel and Paul, 1974; Vose, 1980). This material was poured off with the rinse following centrifugation. After this pretreatment, soils were shaken with 0.4 N NaOH under N<sub>2</sub> overnight, centrifuged, and the extract filtered through glass wool to ensure removal of any remaining debris. The humic acid fraction was precipitated by acidifying to pH < 2, while the fulvic acid fraction remained in solution. Extractions were repeated and combined until no more organic matter (color) was present in the extractant. The fulvic and humic acid fractions were then separated by centrifugation. The remaining organic matter not directly extractable with alkali is referred to as humin. Fractions were freeze-dried and ground for analysis of C content and  $\delta^{13}\text{C}$  by dry combustion GC-IRMS (20-20/ANCA-NT, Europa, Crewe, UK). Before averaging replicates (plots) in each treatment, three analyses were performed on each sample to enhance precision and to confirm accuracy.

## 2.3. Calculations

To calculate  $f$ , the percent of C derived from the recent C<sub>4</sub> input (corn) in any soil fraction and after each interval, the same approximation used in long-term studies (e.g. Balesdent et al., 1987) was appropriate:

$$\delta \approx (\delta_i - \delta_o)f + \delta_o$$

where  $\delta_i$  is the isotopic composition ( $\delta^{13}\text{C}$ ) of corn ( $-12.5\text{‰}$ ), and  $\delta_o$  and  $\delta$  are the values, respectively, for this fraction before and after the interval. Since each interval is defined by what occurred between its beginning and end dates, the effects of residue or other additions before the start of the interval do not need to be considered.

Since cover crops were present at all times in the LI plots, corn was not the only possible external contribution to soil  $\delta^{13}\text{C}$  values or C dynamics during this experiment.

Yet, there is no possibility to accurately correct for this in the above equation. Cover crops in this study ( $\delta^{13}\text{C} < -27\text{‰}$ ) are considerably depleted in  $^{13}\text{C}$  relative to corn; all values for  $f$  and quantities derived from  $f$ , therefore, represent and should necessarily be interpreted as minimum estimates.

In the ORG plots, application of composted manure may be partly responsible for any observed increases in soil  $\delta^{13}\text{C}$ , since with a value of  $-21\text{‰}$ , it is mildly enriched in  $^{13}\text{C}$  compared to humic fractions.

The mass,  $A$ , of corn-derived C accumulated in any fraction after a given interval was calculated as the total mass of C in this fraction at the end of this interval ( $C_t$ ) multiplied by  $f$  for this interval:

$$A = C_t f.$$

The percent,  $B$ , of accumulated (new) C derived from the corn present during an interval could then be calculated as the mass of corn C added ( $A$ ) divided by the overall increase in mass of C in this fraction during the interval:

$$B = 100 * A / (C_t - C_o)$$

where  $C_o$  is the C content of the fraction at the start of the interval. This calculation was only carried out if there was a significant increase in C during this interval ( $C_t > C_o$ ).

Table 1  
Average measured carbon content of organic matter fractions in corn plots at each sampling date

| Soil fraction              | April 1998      | October 1998<br>(mg C g <sup>-1</sup> soil) |           | May 1999  |
|----------------------------|-----------------|---------------------------------------------|-----------|-----------|
| <i>Low-input treatment</i> |                 |                                             |           |           |
|                            | <i>0–15 cm</i>  |                                             |           |           |
| Humic acid                 | 3.3 (0.3)       | 3.1 (0.1)                                   | $P=0.015$ | 3.7 (0.2) |
| Fulvic acid                | 2.0 (0.1)       | 2.1 (0.1)                                   |           | 2.0 (0.1) |
| Humin                      | 5.5 (0.7)       | 6.3 (0.1)                                   | $P=0.10$  | 6.8 (0.1) |
|                            | <i>15–30 cm</i> |                                             |           |           |
| Humic acid                 | 2.3 (0.1)       | 2.6 (0.1)                                   |           | 2.6 (0.2) |
| Fulvic acid                | 1.3 (0.1)       | 1.4 (0.1)                                   |           | 1.3 (0.1) |
| Humin                      | 3.7 (0.3)       | 4.4 (0.2)                                   |           | 4.7 (0.4) |
| <i>Organic treatment</i>   |                 |                                             |           |           |
|                            | <i>0–15 cm</i>  |                                             |           |           |
| Humic acid                 | 2.9 (0.3)       | 2.9 (0.5)                                   |           | 3.8 (0.2) |
| Fulvic acid                | 1.8 (0.1)       | 2.2 (0.1)                                   | $P=0.042$ | 2.1 (0.1) |
| Humin                      | 6.3 (0.2)       | 6.8 (0.4)                                   |           | 6.9 (0.2) |
|                            | <i>15–30 cm</i> |                                             |           |           |
| Humic acid                 | 2.3 (0.3)       | 2.4 (0.3)                                   |           | 2.4 (0.2) |
| Fulvic acid                | 1.4 (0.1)       | 1.4 (0.1)                                   |           | 1.3 (0.1) |
| Humin                      | 3.8 (0.1)       | 4.0 (0.4)                                   |           | 4.2 (0.2) |

Standard errors are given in parentheses. The number between each pair of measurements indicates the probability level ( $P$ ) at which a significant difference was indicated (paired  $t$ -test), reported for all  $P < 0.10$ .

Table 2

Average measured isotopic composition of organic matter fractions in corn plots at each sampling date

| Soil fraction              | April 1998      |           | October 1998,<br>$\delta^{13}\text{C}$ (‰) |           | May 1999       |
|----------------------------|-----------------|-----------|--------------------------------------------|-----------|----------------|
| <i>Low-input treatment</i> |                 |           |                                            |           |                |
|                            | <i>0–15 cm</i>  |           |                                            |           |                |
| Humic acid                 | – 26.52 (0.19)  | $P=0.069$ | – 27.59 (0.32)                             | $P=0.004$ | – 25.22 (0.17) |
| Fulvic acid                | – 23.89 (0.12)  |           | – 23.86 (0.10)                             | $P=0.007$ | – 23.14 (0.07) |
| Humin                      | – 22.57 (0.12)  |           | – 22.78 (0.28)                             |           | – 22.41 (0.14) |
|                            | <i>15–30 cm</i> |           |                                            |           |                |
| Humic acid                 | – 26.20 (0.27)  |           | – 26.13 (0.41)                             |           | – 27.06 (0.32) |
| Fulvic acid                | – 24.69 (0.33)  |           | – 24.46 (0.10)                             | $P=0.017$ | – 23.46 (0.16) |
| Humin                      | – 21.32 (0.11)  | $P=0.001$ | – 21.91 (0.11)                             |           | – 21.68 (0.08) |
| <i>Organic treatment</i>   |                 |           |                                            |           |                |
|                            | <i>0–15 cm</i>  |           |                                            |           |                |
| Humic acid                 | – 26.27 (0.58)  |           | – 26.12 (0.15)                             |           | – 26.49 (0.21) |
| Fulvic acid                | – 23.92 (0.10)  |           | – 23.66 (0.11)                             | $P=0.050$ | – 22.67 (0.12) |
| Humin                      | – 22.54 (0.12)  |           | – 22.77 (0.05)                             | $P=0.027$ | – 21.95 (0.12) |
|                            | <i>15–30 cm</i> |           |                                            |           |                |
| Humic acid                 | – 26.14 (0.41)  |           | – 26.67 (0.76)                             |           | – 26.41 (0.45) |
| Fulvic acid                | – 24.30 (0.30)  |           | – 24.34 (0.09)                             | $P=0.042$ | – 23.74 (0.11) |
| Humin                      | – 21.48 (0.06)  |           | – 21.83 (0.29)                             |           | – 21.40 (0.20) |

Standard errors are given in parentheses. The number between each pair of measurements indicates the probability level ( $P$ ) at which a significant difference was indicated (paired  $t$ -test), reported for all  $P < 0.10$ .

## 2.4. Identification of significant effects

For any given fraction at any given soil depth, comparisons were made across time. The beginning and end of each interval were compared using a paired  $t$ -test so that probability levels ( $P$ ) could be reported for all  $P < 0.10$  (Tables 1 and 2). The significance of each effect may therefore be interpreted individually. Values for  $f$  and related quantities were calculated for all changes for which  $P < 0.10$ .

## 3. Results and discussion

The amount and timing of available nutrients for crop uptake in these systems differs significantly; however, the yields in both systems are comparable (Clark et al., 1999a,b). This suggests that the cycling of nutrients from organic matter fractions behave differently among these systems. The cycling of nutrients is linked to the size and turnover rate of soil organic fractions. However, the relationship between nutrient availability and humic fractions is poorly understood. Part of the problem lies in trying to relate long-term data to short-term nutrient availability.

Measured C content and  $\delta^{13}\text{C}$  values for each sampling date are listed in Tables 1 and 2. The humic acid and humin pools in the LI plots accumulated C during the winter season, while the ORG plots accumulated C in the summer, only in the fulvic acid pool. Incorporation of new corn-derived C into all three fractions was observed during the winter

season. Table 3 gives the quantities calculated for the winter interval. These observations show the immediate effects of the C inputs present during this experiment upon the different fractions analyzed.

### 3.1. Humic acid fraction

Only the LI treatment showed changes in the humic acid fraction. Since  $\delta^{13}\text{C}$  decreased significantly from April to October 1999 (Table 2), the effect of non- $\text{C}_4$  C (i.e. vetch) dominated over that of the corn roots. This effect may be appreciated since it was strong enough to affect the isotopic composition of this fraction, i.e. a significant amount of vetch C became part of this fraction. The acquired C represented turnover of the humic acid pool (there was no increase in C during the interval).

From October 1998 to May 1999, again in the LI treatment, the humic acid pool from 0 to 15 cm increased in size by  $0.62 \text{ mg C g}^{-1} \text{ soil}$  (Table 1). Using a bulk density of  $1.3 \text{ g cm}^{-3}$  at SAFS, this increase is equivalent to  $1200 \text{ kg C ha}^{-1}$ . This is notable in that C derived from corn decomposing during this time accounted for essentially all of the new C accumulated in the humic acid. This is approximately 28% of the C incorporated as corn residue. Such noticeable short-term dynamics during the above two intervals would be obscured if the period from April 1998 to May 1999 had been considered as the minimum time required for adequate observations. In this experiment, such a ‘minimum time’ was actually composed of two very different shorter periods, the net effect of which was insignificant (i.e.  $\delta_{\text{April 1998}} \approx \delta_{\text{May 1999}}$  but  $\delta_{\text{April 1998}} \neq \delta_{\text{October 1998}}$  and  $\delta_{\text{October 1998}} \neq \delta_{\text{May 1999}}$ ).

### 3.2. Fulvic acid fraction

In both the LI and ORG systems, and at both depths measured, some of the C in the fulvic acid fraction was replaced by a similar amount of new corn C from October 1998 to May 1999. This new corn-derived C in the LI and ORG plots, respectively, was  $0.13$  and  $0.19 \text{ mg C g}^{-1} \text{ soil}$  (Table 3), equivalent to  $250$  and  $370 \text{ kg C ha}^{-1}$ , or 6% and 9% of the

Table 3

Minimum estimates of changes in each organic matter fraction in corn plots during one interval (no changes were observed in the preceding season), described by  $f$  (percent of C derived from recent corn crop),  $A$  (mass of new corn-derived C), and  $B$  (percent of accumulated C that is corn C)

| System                          | Depth (cm) | Soil fraction | $f$ (%) | $A$ (mg C g <sup>-1</sup> soil) | $B$ (%) |
|---------------------------------|------------|---------------|---------|---------------------------------|---------|
| <i>October 1998 to May 1999</i> |            |               |         |                                 |         |
| Low input                       | 0–15       | humic acid    | 16      | 0.58                            | 97      |
|                                 |            | fulvic acid   | 6.3     | 0.13                            |         |
| Organic                         | 15–30      | fulvic acid   | 8.4     | 0.11                            |         |
|                                 | 0–15       | fulvic acid   | 8.9     | 0.19                            |         |
|                                 |            | humic acid    | 8.0     | 0.55                            |         |
|                                 | 15–30      | fulvic acid   | 5.1     | 0.07                            |         |

Values not given cannot be calculated.

C incorporated as corn residue. Turnover of this pool was associated only with the input of corn residue (in the presence of a growing cover crop), while it actually increased in size during the previous interval (April to October 1998) in the ORG plots. This response is perhaps attributable to the large amount of C applied to these plots in April, mostly as manure, although corn residue in October ( $4200 \text{ kg C ha}^{-1}$ ), similar in size to the input of manure plus vetch in April ( $3900 \text{ kg C ha}^{-1}$ ), did not cause this fraction to grow as did the manure plus vetch. This may be due to the season of application or to the greater presence of nutrients in the manure and vetch. Other differences in the chemical properties of these additions may have also influenced their metabolism and form of incorporation into fulvic acids.

In the ORG plots, manure, with a  $\delta^{13}\text{C}$  of  $-21\text{‰}$ , must be considered along with corn as a potential source of enrichment whenever increases in soil  $\delta^{13}\text{C}$  are observed. In practice, however, much greater humification of mildly enriched manure than highly enriched corn would be required to cause a similar increase in soil  $\delta^{13}\text{C}$ , especially after October, when only relatively recalcitrant components of manure would be left. In three other summer seasons measured at the SAFS site where manure was applied to either corn or tomato crops, none showed an increase in total soil  $\delta^{13}\text{C}$  (data not shown). Table 2 shows increases in  $\delta^{13}\text{C}$  only after October. This does not eliminate the uncertainty with respect to manure or corn as the cause of any increase in  $\delta^{13}\text{C}$  from October to May, it just allows such changes to be more confidently attributed to corn<sup>1</sup>.

It is interesting to note that this fraction is considerably more enriched in  $^{13}\text{C}$  than the humic acids; the humin is even more so. It has been stated that the similarity in isotopic composition between the fulvic acid and plants, as well as the comparative depletion in  $^{13}\text{C}$  of humic acids (believed to occur during biological processing), indicate the greater dynamic state of the fulvic acid fraction and its place as an active precursor to both humic acid and humin pools (Nissenbaum and Schallinger, 1974). Paul and van Veen (1978), on the other hand, describe similarity in  $^{13}\text{C}$  content among humic fractions and conclude that “no gradual transformation of organic compounds from one class to another occurs, i.e. fulvic acids are not converted to humic acids”. Goh et al. (1976), observing similar proportions of humic fractions in young and old soils, concluded that “the relative proportions of the different fractions are a feature of humus formation from the earliest stages of decomposition and are not the result of subsequent transformation of one fraction into another”. Observations like the above, however, consider SOM genesis in general (a very long-term effect), and cannot be used towards any detailed interpretation in the context of our study and site, especially in the confounding presence of both  $\text{C}_3$  and  $\text{C}_4$  plants. Table 1 shows that the relative proportions of humic fractions at our site did change over the course of the experiment. Any continuum among humic fractions would be hard to observe, however, since SOM is continuously formed and decomposed.

<sup>1</sup> Assuming all of the manure C was still labile in October, then  $f$  for ORG from October to May in the fulvic acid fraction =  $(23.66 - 22.67) / (23.66 - 21) = 37\%$ .  $A = (0.37)(2.1) = 0.78 \text{ mg C g}^{-1} \text{ soil}$  or  $1550 \text{ kg C ha}^{-1}$ . Application of manure in April was  $3000 \text{ kg C ha}^{-1}$ , so half of this would have had to be converted into fulvic acid C after October if manure was solely responsible for the increase in  $\delta^{13}\text{C}$  of this fraction. Performing the analogous calculation for humin, values of  $f$  and  $A$  are, respectively, 46% and  $6350 \text{ kg C ha}^{-1}$ .

### 3.3. Humin fraction

The non-alkali extractable organic matter showed significant changes during this study. From October 1998 to May 1999 in the ORG treatment, C derived from corn residue decomposing during this period replaced at least 8% of the C in this pool (Table 3). Most of the corn C acquired by the soil during this interval appeared in this relatively large pool.

In contrast to the ORG treatment, the LI plots during this time showed a tendency to accumulate C in humin ( $P=0.10$ ). Although the value of  $\delta^{13}\text{C}$  for this fraction increased, this difference was not significant and implies that non- $\text{C}_4$  C (i.e. root exudates or turnover) of the growing cover crop was responsible for a large part of this observed increase in humin C.

The humin in the 15–30 cm layer in the LI treatment showed a significant increase in non- $\text{C}_4$  (i.e. vetch) C, acquired through turnover, during the first interval. Since the  $\delta^{13}\text{C}$  values of vetch and humin are fairly well separated, the  $\delta^{13}\text{C}$  value of vetch may be used to estimate  $f$  for this observation (7%). It is possible that corn roots primarily affected the humic and fulvic acid fractions, although no differences great enough to be significant were noted in these pools. Different characteristics of these inputs (C/N ratio, relative amounts of protein, polysaccharides, etc.), as well as external conditions such as moisture and temperature, might have influenced their breakdown and incorporation into different fractions at different times.

It is also unknown, given this short period, whether the new C in humin in the above observations is really ‘humified’ (extensively chemically altered) or simply other forms of C such as unextractable microbial residues or recently formed simple organic compounds stabilized quickly with the soil minerals. Devèvre and Horwath (2001) report a treatment effect on short-term recovery of tracer  $^{15}\text{N}$  in non-extractable organic matter, suggesting that there are in fact active components of such a pool, a pool which is effectively the residue left after alkali extraction. Shields and Paul (1973) found at least half of a  $^{14}\text{C}$ -labeled plant residue addition in humin after 2–3 years. This large fraction of SOM must not be too quickly assumed to “contribute only a small portion to the annual cycling of soil C”, as stated by Collins et al. (1997).

### 3.4. Possible artifacts of the extraction technique

Of the large body of both recent and earlier literature in which humic fractionation has been used or discussed, only minimal consideration has been given to possible discrepancies with the technique and its interpretation (e.g. Anderson et al., 1974). Published protocols may (e.g. Kononova et al., 1966) but often do not (e.g. Schnitzer, 1982) mention sources of artifacts such as the presence of plant debris during extraction. It is known that plant residue contains humic (alkali-soluble) material, which further accumulates as decomposition progresses (e.g. Shindo and Kuwatsuka, 1977; Swift and Posner, 1977). Following the initial acid wash, however, there would be little fresh corn residue or its labile fraction left in the soil. Using density flotation and alkali extraction of corn residue, we determined that the amount of corn residue and its contribution to humic fractions was insignificant in our soil (data not presented). Ensuring the absence of undecomposed debris is important so as not to include it in the subsequent alkali extraction, and confound

the results by including material extracted from undecomposed organic matter as part of soil humic fractions. This is especially important when considering the potential significance of small changes in  $\delta^{13}\text{C}$ . It is possible that particulate debris in various stages of decay would not be removed by the initial wash or even the subsequent alkali washes, if this debris were well trapped within aggregates. As stated in [Anderson et al. \(1974\)](#), a portion of this ‘hidden’ material would be soluble in alkali and potentially contribute to humic and/or fulvic acid fractions. As these authors state for their work, however, this contribution is likely trivial. In a similar context, the only way that corn (tracer) C would be erroneously found in humin would be if pieces of residue were analyzed as part of this fraction. In our study, most, if not all, of the corn residue would have been removed during the HCl and NaOH extractions.

If the above artifacts were still significant in this study, indiscriminate ‘contamination’ should be found in both treatments (LI and ORG plots), since both received an input of corn residue. In fact, while a notable change was observed for the humic acid pool in the LI treatment, this pool showed no change in the ORG treatment. The humin pool, on the other hand, was affected in the ORG but not in the LI system.

### 3.5. General observations

The LI plots, which contained less total C, were slightly more dynamic than the ORG plots, with six significant observations (C accumulation or turnover) compared to four for the ORG plots. Interestingly, the only instance of C accumulation in the ORG system was seen in the fulvic acid fraction, the smallest of the fractions, while in the LI plots C accumulation occurred in the larger humic acid and humin pools. Unlike the experiments mentioned by [Paul \(1970\)](#), the fulvic acid, humic acid, and humin fractions during the course of this short experiment did not show a uniform distribution of tracer C.

There have been several proposed routes of SOM formation, such as those in which fulvic acids are precursors to humic acids and humin ([Kononova, 1966](#); [Nissenbaum and Schallinger, 1974](#)), or in which more complex, larger materials are formed first and then decomposed, i.e. humic acids broken down into fulvic acids ([Schnitzer, 1978](#)). An alternative view holds that organic compounds are not transformed from any one class into another ([Paul and van Veen, 1978](#)). Our data set is small, and we can not suggest any movement (or lack of movement) of C between fractions. Estimates of turnover only indicate the change—the amount of C replaced—in the same analytically defined pool from one date to another. During our experiment, these pools were dynamic, but, as [Stevenson \(1994\)](#) appropriately writes, “at this time, interrelationships between humic and fulvic acids and other natural products are not clear”. [Balesdent and Mariotti \(1996\)](#), by observing similar kinetics of enrichment in new (corn-derived) C in all humic fractions over time, concluded that “a scheme that would link humin, HA, and FA as sequential stages in the decomposition process would be false if applied to these separates in the soil”. Even the non-extractable humin, often described as a resistant, old, or terminal pool, acquired new C in both treatments. What might be ‘passive’ pools as a whole are perhaps better defined as heterogeneous pools, of which a certain part can be actively involved in short-term cycles ([Devêvre and Horváth, 2001](#); also suggested by [Balesdent et al., 1987](#) for certain clay size fractions). Collective (whole fraction) estimates of mean residence or

turnover times, on the order of hundreds of years for fulvic acids and thousands of years for humic acids and humins (e.g. Paul and van Veen, 1978), overlook the heterogeneous nature of these fractions, both in age and in biochemical incorporation and removal of C. In a simple study, Mayaudon and Simonart (1959) found that the C of most plant constituents could quickly become part of every humic fraction to quite a large extent.

Stevenson (1965) mentioned, at the time, that the usefulness of chemical fractionation was evidenced by the fact that the majority of studies conducted on soil humus involved such fractionation. As other authors have stated (Jenkinson, 1971; Paul and McGill, 1977; Paul, 1984), we recognize that humic fractions still lack a true practical significance outside of that defined by the extraction procedure, i.e. no one extraction scheme, including classical humic fractionation, has been demonstrated to yield biologically or temporally (with respect to turnover) meaningful fractions. The results of this study imply that individual humic fractions, however, may in fact have different roles in C cycling under different conditions. This in turn may be related to the turnover and availability of nutrients associated with these fractions. For example, the fulvic acid fraction seemed most sensitive to change, even at the lower levels of SOM and inputs present at the 15–30-cm depth. Each fraction may be affected differently by different inputs and seasonal conditions, although we lack sufficient data to be able to describe trends in or patterns of C movement particular to each fraction.

Stevenson and Elliott (1989) emphasized that techniques used to assess SOM dynamics, if they are to relate to the ability of the soil to supply nutrients, should involve fractions or pools having biological significance. In this experiment, changes in certain pools were associated with specific conditions such as the amount and nature of inputs, differences in soil type and activity, and/or certain seasonal variables. Many such conditions can be directly related to consequent changes in the soil microbial community, which governs residue decomposition and consequent organic matter formation. The changes we observed in certain pools were therefore likely associated with unique biological situations, supporting to some extent the separation of SOM into these classical humic fractions. This method provides a basis for evaluating the sustainability of alternative agricultural practices from the perspective of SOM dynamics and nutrient cycling. More research is needed to determine the role of these C fractions in both short-term and long-term soil fertility.

#### 4. Conclusions

This study demonstrates that long periods of time and ‘clean’ experimental plots (i.e. one C input) and history were not necessary to at least semi-quantitatively observe changes in soil humic fractions at our site using  $^{13}\text{C}$  natural abundance measurements. This approach is adaptable to a variety of experimental settings and may be controlled to isolate certain effects, such as that of active roots. The presence of non-tracer sources of C in our study, while possibly masking certain subtle effects of corn, was not a handicap in that several observations could be made with respect to these other inputs.

As summarized by Collins et al. (1997), the current theory of humus formation is based on a step by step process involving decomposition of plant material to simple compounds,

assimilation and repeated cycling of C through the microbial biomass, and simultaneous joining of microbially synthesized and altered plant-derived compounds to form large polymers. Our results show that soil humic fractions are dynamic at our site, responding quickly and notably to changes in external inputs and conditions. These temporary variations are likely continuous, responding to each new change in external conditions such as inputs and climate.

While interrelationships between humic fractions remain unclear, these fractions appear to have different roles in C cycling. This study lacks sufficient data to be able to describe trends, but nonetheless demonstrates the dynamic nature of these SOM pools. Using data such as that presented here, the conditions under which changes in each fraction occur can be identified and used to investigate the composition and function of these fractions. Understanding the short-term as well as the long-term role of these fractions and the pools of soil C that they represent will be of value in developing and evaluating alternative agricultural practices that will rely on soil organic matter to supply available nutrients.

## 5. Uncited references

He et al., 1988  
Kelley and Stevenson, 1995  
Martin and Haider, 1980  
Olk et al., 1996

## Acknowledgements

We would like to thank Andrea D. De Lisle and Elisabeth L. Palmer for their valuable technical assistance. Carbon-13 measurements were performed by Dr. David Harris at the Stable Isotope Facility at UC Davis. This research was funded by the United States Department of Agriculture (USDA-NRI, grant no. 9604453) and by the Kearney Research Foundation for Soil Science (University of California).

## References

- Anderson, D.W., Paul, E.A., St. Arnaud, R.J., 1974. Extraction and characterization of humus with reference to clay-associated humus. *Can. J. Soil Sci.* 54, 317–323.
- Balesdent, J., Balabane, M., 1992. Maize root-derived soil organic carbon estimated by natural  $^{13}\text{C}$  abundance. *Soil Biol. Biochem.* 24, 97–101.
- Balesdent, J., Mariotti, A., 1996. Measurement of soil organic matter turnover using  $^{13}\text{C}$  natural abundance. In: Boutton, T.W., Yamasaki, S. (Eds.), *Mass Spectrometry of Soils*. Marcel Dekker, New York, pp. 83–111.
- Balesdent, J., Mariotti, A., Guillet, B., 1987. Natural  $^{13}\text{C}$  abundance as a tracer for soil organic matter dynamics studies. *Soil Biol. Biochem.* 19, 25–30.
- Balesdent, J., Wagner, G.H., Mariotti, A., 1988. Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. *Soil Sci. Soc. Am. J.* 52, 118–124.
- Cerri, C., Feller, C., Balesdent, J., Victoria, R., Plenecassagne, A., 1985. Application du traçage isotopique naturel en  $^{13}\text{C}$  à l'étude de la dynamique de la matière organique dans les sols. *C. R. Acad. Sci., Ser. II* 9, 423–428.

- Cheng, W., 1996. Measurement of rhizosphere respiration and organic matter decomposition using natural  $^{13}\text{C}$ . *Plant Soil* 183, 263–268.
- Clark, M.S., Horwath, W.R., Shennan, C., Scow, K.M., 1998. Changes in soil chemical properties resulting from organic and low-input farming practices. *Agron. J.* 90, 662–671.
- Clark, M.S., Horwath, W.R., Shennan, C., Scow, K.M., Lanini, W.T., Ferris, H., 1999a. Nitrogen, weeds, and water as yield-limiting factors in conventional, low-input, and organic tomato systems. *Agric. Ecosyst. Environ.* 73, 257–270.
- Clark, M.S., Klonsky, K., Livingston, P., Temple, S., 1999b. Crop yield and economic comparisons of organic, low-input, and conventional farming systems in California's Sacramento Valley. *Am. J. Altern. Agric.* 14, 109–121.
- Collins, H.P., Paul, E.A., Paustian, K., Elliot, E.T., 1997. Characterization of soil organic carbon relative to its stability and turnover. In: Paul, E.A., Paustian, K., Elliott, E.T., Cole, C.V. (Eds.), *Soil Organic Matter in Temperate Agroecosystems: Long-term Experiments in North America*. CRC Press, Boca Raton, pp. 51–72.
- Devêvre, O.C., Horwath, W.R., 2001. Stabilization of fertilizer- $^{15}\text{N}$  into humic substances in aerobic vs. water-logged soil following straw incorporation. *Soil Sci. Soc. Am. J.* 65, 499–510.
- Goh, K.M., Rafter, T.A., Stout, J.D., Walker, T.W., 1976. The accumulation of soil organic matter and its carbon isotope content in a chronosequence of soils developed on Aeolian sand in New Zealand. *J. Soil Sci.* 27, 89–100.
- Hayes, M.H.B., 1985. Extraction of humic substances from soil. In: Aiken, G.R., et al. (Eds.), *Humic Substances in Soil, Sediment, and Water*. Wiley, New York.
- He, X.T., Stevenson, F.J., Mulvaney, R.L., Kelley, K.R., 1988. Incorporation of newly immobilized  $^{15}\text{N}$  into stable organic forms in soil. *Soil Biol. Biochem.* 20, 75–81.
- Kelley, K.R., Stevenson, F.J., 1995. Forms and nature of organic N in soil. *Fertil. Res.* 42, 1–11.
- Kononova, M.M., 1966. *Soil Organic Matter*, 2nd ed. Pergamon, New York.
- Liang, B.C., Gregorich, E.G., MacKenzie, A.F., 1999. Short-term mineralization of maize residues in soils as determined by carbon-13 natural abundance. *Plant Soil* 208, 227–232.
- Martel, Y.A., Paul, E.A., 1974. The use of radiocarbon dating of organic matter in the study of soil genesis. *Soil Sci. Soc. Am. Proc.* 38, 501–506.
- Martin, J.P., Haider, K., 1980. Microbial degradation and stabilization of  $^{14}\text{C}$ -labeled lignins, phenols, and phenolic polymers in relation to soil humus formation. In: Kirk, T.K., et al. (Eds.), *Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications*, vol. 2. CRC Press, Boca Raton.
- Mary, B., Mariotti, A., Morel, J.L., 1992. Use of  $^{13}\text{C}$  variations at natural abundance for studying the biodegradation of root mucilage, roots and glucose in soil. *Soil Biol. Biochem.* 24, 1065–1072.
- Mayaudon, J., Simonart, P., 1959. Étude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. *Plant Soil* 9, 170–175.
- Nissenbaum, A., Schallinger, K.M., 1974. The distribution of the stable carbon isotope ( $^{13}\text{C}/^{12}\text{C}$ ) in fractions of soil organic matter. *Geoderma* 11, 137–145.
- Olk, D.C., Cassman, K.G., Randall, E.W., Kinchesh, P., Sanger, L.J., Anderson, J.M., 1996. Changes in chemical properties of organic matter with intensified rice cropping in tropical lowland soil. *J. Soil Sci.* 47, 293–303.
- Paul, E.A., 1970. Plant components and soil organic matter. *Recent Adv. Phytochem.* 3, 59–104.
- Paul, E.A., van Veen, J.A., 1978. The use of tracers to determine the dynamic nature of organic matter. *Proceedings of the 11th International Congress of Soil Science*, vol. 3. Intl. Soil Sci. Soc., Edmonton.
- Qian, J.H., Doran, J.W., Walters, D.T., 1997. Maize plant contributions to root zone available carbon and microbial transformations of nitrogen. *Soil Biol. Biochem.* 29, 1451–1462.
- Rochette, P., Flanagan, L.B., 1997. Quantifying rhizosphere respiration in a corn crop under field conditions. *Soil Sci. Soc. Am. J.* 61, 466–474.
- Rochette, P., Flanagan, L.B., Gregorich, E.G., 1999. Separating soil respiration into plant and soil components using analyses of the natural abundance of carbon-13. *Soil Sci. Am. J.* 63, 1207–1213.
- Schnitzer, M., 1978. Humic substances: chemistry and reactions. In: Schnitzer, M., Khan, S.U. (Eds.), *Soil Organic Matter*. Elsevier, Amsterdam.
- Shields, J.A., Paul, E.A., 1973. Decomposition of  $^{14}\text{C}$ -labeled plant material under field conditions. *Can. J. Soil Sci.* 53, 297–306.
- Shindo, H., Kuwatsuka, S., 1977. Behavior of phenolic substances in the decaying process of plants: VII.

- Characteristics of phenolic substances in the humic acids of decayed rice straw and compost-supplied field soil. *Soil Sci. Plant Nutr.* 23, 333–340.
- Stevenson, F.J., 1965. Gross chemical fractionation of soil organic matter. In: Black, C.A., et al. (Eds.), *Methods of Soil Analysis: Part 2. Chemical and Microbiological Properties*. Amer. Soc. Agr., Madison.
- Stevenson, F.J., 1994. *Humus Chemistry: Genesis, Composition, Reactions*, 2nd ed. Wiley, New York.
- Stevenson, F.J., Elliott, E.T., 1989. Methodologies for assessing the quality and quantity of soil organic matter. In: Coleman, D.C., et al. (Eds.), *Tropical Soil Organic Matter*. Univ. of Hawaii Press.
- Swift, R.S., 1996. Organic matter characterization. In: Sparks, D.L., et al. (Eds.), *Methods of Soil Analysis: Part 3. Chemical Methods*. Soil Sci. Soc. Am. Book Ser., vol. 5. Soil Sci. Soc. Am., Madison.
- Swift, R.S., Posner, A.M., 1977. Humification of plant materials—properties of humic acid extracts. *Soil Organic Matter Studies*, IAEA/FAO, vol. 1, pp. 171–182.
- Vose, P., 1980. *Nuclear Techniques in Agronomy and Plant Biology*. Pergamon, Oxford.