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Ronald F. Follett
USDA-ARS, ronald.follett@ars.usda.gov

Eldor A. Paul
Colorado State University, eldor@nrel.colostate.edu

Elizabeth G. Pruessner
USDA-ARS, elizabeth.pruessner@ars.usda.gov

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SOIL CARBON DYNAMICS DURING A LONG-TERM INCUBATION STUDY INVOLVING ^{13}C AND ^{14}C MEASUREMENTS

Ronald F. Follett[1], Eldor A. Paul[2], and Elizabeth G. Pruessner[1]

Soil organic matter is the earth's largest terrestrial reservoir of carbon (C). Thus, it serves as a major control on atmospheric carbon dioxide (CO_2) levels. To better understand these controls, decreases in soil organic C (SOC), soil microbial biomass (SMB) C, and the role of SMB as a source of mineralizable C were measured during a long-term incubation (853 days) without added substrate. The 2 soils used were a Weld loam (fine montmorillonitic, mesic, Aridic Paleustoll) from near Akron, Colorado, and a Duroc loam (fine silty, mixed mesic Pachic Haplustoll) from near Sidney, Nebraska. The Akron soil was uniformly cropped to small grain crop-fallow rotations until 1989 when wheat (*Triticum aestivum* L.) in conventional (stubble mulch) till-fallow, reduced till-fallow, and no-till fallow treatments were adopted. On additional rotation plots, continuous corn (*Zea mays* L.) or no-till corn, fallow, wheat, and no-till corn in a 4-year rotation were grown. The Sidney soil was broken from native sod in 1970 and planted to wheat-fallow with no-till, plow-tillage, and sod-plot treatments. Moist soil samples were collected and refrigerated until plant material removal by sieving and picking. The SOC and SMB-C decreased during incubation and rates of loss measured. The results from this study allow insights into contributions of SMB and changes in soil isotope C ratios not previously available.

Soil microbial biomass C contributed an average of 31% of the evolved CO_2-C across all treatments between day 10 and day 79 of incubation and an average of about 20% during the more extended times between later measurements thereafter. Until day 160, evolution of $^{13}CO_2$ during incubation indicated that evolved C came from plant residues and was soil derived thereafter, including from the native grassland SOC. Where corn was grown, evolution of evolved C is hypothesized to have had a less negative $^{13}CO_2$ isotope signature from days 630 to 720 of the incubation because of the delayed microbial breakdown of the cob materials. After 853 days of incubation and across all plots, the SOC remaining averaged 67% and was similar to the amount of observed hydrolysis residue C. Acid hydrolysis and ^{14}C dating were also used to characterize the resistant SOC fraction and showed increased ^{14}C age with hydrolysis but not with long-term incubation. (Soil Science 2007;172:189–208)

Key words: Soil organic carbon, soil microbial biomass, long-term incubation, carbon isotopes, tillage treatments, acid hydrolysis, carbon dioxide.

[1]Soil-Plant-Nutrient Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Suite 100, Building D, 2105 Centre Avenue, Fort Collins, CO 80526. Dr. Follett is corresponding author. E-mail: ronald.follett@ars.usda.gov

[2]National Resource Ecology Laboratory, Colorado State University, Room B240, Fort Collins, CO.

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SOIL organic matter (SOM) contains the largest terrestrial reservoir of carbon (C) in the biological global C cycle. As such, it plays a major role in the control of carbon dioxide (CO_2) levels in the atmosphere. Knowledge of soil C dynamics is necessary for the interpretation of data related to ecosystem functioning,

soil fertility, and global change. It is, therefore, important to determine the controls on decomposition and the stability of soil C in different landscapes and under various managements. This is best done through knowledge of the controls that affect the pool sizes and turnover rates of soil organic C (SOC) fractions. Carbon dating using the ^{14}C naturally occurring isotope has shown SOC with overall mean residence times (MRTs) that range through years, centuries, and even millennia within the surface horizons of most soils (Stout et al., 1981). Deeper depths have been found to have much greater MRTs (Trombore, 1993; Follett et al., 2004). Cultivation often decreases the SOC by up to 50% and results in MRTs that are 1200 to 1400 years greater than those of native sites (Paul et al., 2001).

Tracer-derived turnover data must be interpreted relative to the length of the tracer exposure and the isotope of C used. For example, when a 30-year C↔C plant switch on previously forested and/or mixed cropping was replaced by corn to provide a ^{13}C tracer signal, the MRTs were much shorter than when ^{14}C dating of the same soil samples was used. The two methods of determining MRTs from different soils and management regimes were, however, highly correlated. This led Paul et al. (2003) to support the hypothesis that SOC is a related range of somewhat similar materials that are continually being formed and decomposed although at times very slowly. Fractionations together with tracers supply a great deal of information on the MRT of individual SOC fractions. They also make it possible to follow labeled plant and microbial products. Jenkinson (1971), in a review of 100 articles that examined the use of tracers (primarily ^{14}C), stated, "In general, fractionations based on acid hydrolysis are more successful in revealing the presence of biologically stable material than any other method so far tried." The use of acid hydrolysis in 6 *M* HCl to estimate a chemically resistant fraction together with incubation has more recently provided analytically derived pools and fluxes that are well correlated with tracer information (Paul et al., 2006).

The microbial biomass in soils comprises 1 to 8% of the C of most mineral soils (Smith and Paul, 1990), with most soils falling within a fairly narrow range of 2 to 5%. The SMB-C pool is often considered of similar size to the active SOC fraction and shows a high correlation with total SOC (Jenkinson and Ladd, 1981). The aforementioned relationship raises the question of whether the biomass is a SOC pool or instead a catalyst for conversion of plant C into other SOC pools. In a series of articles that began in 1958, Simonart and Mayaudon (1958) reported the results of up to 12 years of incubation of ^{14}C plant residues and microbial constituents to establish the kinetics of decomposition and the movement of the ^{14}C into SOC fraction (Paul and van Veen, 1978). The incubation of added bacterial cells showed an average decomposition of 30% during the first week, slowing down to 60% losses after 16 weeks (Martin et al., 1974). They concluded that the products of microbial decomposition of polysaccharides tended to be concentrated in the hydrolyzable fraction, especially the amino acids. More resistant microbial components such as melanic fungal cell walls were found to be particularly resistant to decomposition (Hurst and Wagner, 1969). The early literature on tracer use to measure soil organic matter dynamics was summarized by Jenkinson (1971) and Paul and Van Veen (1978).

The extensive use of natural abundance ^{13}C to measure the turnover of soil organic matter was reviewed by Balesdent and Marriotti (1996). Other ^{13}C measurements have been used to measure the distribution of SOC within chemical (Boutton, 1996) and physical fractions (Jastrow et al., 1996) as well as for determining the effect of management (Collins et al., 1999). The use of the ^{13}C signal, available in the growth of wheat *Triticum sativum* (C3 photosynthetic pathway) on mixed (C3 plus C4) prairie soil on the Sanborn plots in Missouri, showed that 100 years of wheat had replaced 50% of the original prairie soil with that derived from wheat residues (Balesdent et al., 1988). Similarly in Colorado, Follett et al. (1997) observed that only 46% of the original prairie SOC remained in the 0- to 15-cm depth under a wheat-fallow system after 84 years with 24% of the remaining SOC derived from wheat. They also measured the ^{13}C isotope ratios of two great-plains soils where wheat, with an isotope signature of approximately −26‰, was grown on soils in Sidney, Nebraska, and Akron, Colorado, with a ^{13}C signature of approximately −16‰. They determined that 5.4% of the agricultural crop residues from the long-term Akron site remained after 84 years of cropping. About 10.5% of the residues remained at Sidney after 20 years of cropping. Paul et al. (1997) ^{14}C dated the soils from the same sites and found a

1200-year increase in MRT with depth. The nonhydrolyzable C that accounted for 23 to 70% of the SOC was, on average, 1500 years older in MRT relative to the total SOC.

The use of hydrolysis-incubation together with tracers has been found to be useful in establishing the kinetics and characteristics of SOC turnover (Collins et al., 2000; Mariam et al., 2000; Fortuna et al., 2003). This study reports on a long-term incubation of the soils from the different management practices of the Akron and Sidney sites previously studied by Follett et al. (1997) and Paul et al. (1997). Other studies on these soils have established the role of aggregates, particulate organic matter, and the light fraction in SOC dynamics (Cambardella and Elliott, 1992, 1993; Six et al., 1999, 2000). The measurement of microbial biomass during incubation made it possible to determine the role of the biomass as a source of mineralizable C. The measurement of the ^{13}C of the evolved CO_2 during this 853-day incubation made it possible to determine the source of the evolved C relative to more recent residues or from the soil SOC derived from the original native grassland. Acid hydrolysis and ^{14}C dating before and after the extended incubation characterized the more resistant SOC components. This combination of methodology has also allowed insights into the contribution of biomass and changes in isotope ratios in soil that were not previously available in the scientific literature. Our hypothesis for this study is that SMB serves to catalyze the conversion of plant C into other SOC pools and for the continual breakdown and recycling of the remaining soil C into increasingly resistant forms. The objective of this long-term study was to determine controls on decomposition and the stability of soil C under various managements that affect pool sizes and turnover rates of SOC fractions.

MATERIALS AND METHODS

Field Sampling

This study was conducted using soils collected from the Central Great Plains Research Center near Akron, Colorado, and from the High Plains Agricultural Laboratory located 8.3 km north of Sidney, Nebraska. The Akron site is on a Weld loam, a fine montmorillonitic, mesic, Aridic Paleustoll (30% sand, 40% silt, and 30% clay), with <1% of slope. Although experimental farming practices at the Akron location began in 1907, the field area sampled for this study was farmed from 1928 to 1954. During that time, half of the plots sampled for this study (plots 205, 207, and 208) were in various cultural studies and rotations, and the other half (plots 306, 307, and 308) was farmed to study methods of fallow (Brandon and Mathews, 1944). Either barley (*Hordeum vulgare* L.) or wheat (*Triticum aestivum* L.) was cropped on all of the plots. In 1955, the entire area was cropped to grain sorghum that would have supplied a C4 type signal for that one year. From 1956 to 1966, the entire area was uniformly cropped to winter wheat in a crop-fallow rotation (Halvorson et al., 1997). In 1967, a study was initiated to evaluate the use of herbicides and tillage for weed control during fallow (Smika, 1990; Halvorson et al., 1997). Conventional (stubble mulch) till-fallow, reduced till-fallow, and no-till fallow treatments were adopted in 1989 (Halvorson et al., 1997), with rotation plots that included either the growing of continuous corn (*Zea mays* L.) (plots 208 and 306) or 2 years of corn in a 4-year rotation of corn, fallow, wheat, corn (plot 308). When spoken of collectively, these plots are referred to as the Akron various-rotation (AVR) plots. The remaining plots (numbers 207, 205, and 307) were farmed as wheat-fallow in combination and no-tillage (plot 207), stubble mulch (plot 205), or plow tillage (plot 307) and collectively will be referred to as the Akron wheat-fallow (AWF) plots. Harvesting of the grain was by combine with a chopper attachment to uniformly distribute the straw to the soil surface of the plots. For harvesting the AVR plots, the knives were removed from the straw chopper, but the cobs and stalks of the corn plants were flailed through the combine to reduce their size and help distribute them as they were returned to the soil surface.

The Sidney site is on a Duroc loam, a fine silty, mixed mesic Pachic Haplustoll (40% sand, 35% silt, and 25% clay), with <1% slope. The plot area was broken from native sod in 1970 and planted to wheat-fallow. Three replications of no-till (Sidney no-till, hereafter referred to as SNT) and plow-tillage (Sidney plow, hereafter referred to as SP) treatments were sampled. In addition, at the Sidney site, a replicated sod-plot treatment was sampled and will hereafter be referred to as the Sidney sod (SSD) treatment. The sod plots were randomized within the cultivated plots as part of the original layout of the research area (Fenster and Peterson, 1979), but never cultivated. Grass species present in the sod plots included native wheat grasses

(*Agropyon* sp. L.), which are cool season C3 plants. Random "grab" samples of the aboveground biomass (clipped at about one cm height) were collected to measure $\delta^{13}C$ and use in a mixing equation (Kelly et al., 1993) to estimate relative amount of aboveground biomass from C3 vs. C4 plant tissue. Originally, the estimated $\delta^{13}C$ values used in the mixing equation for C3 (−26‰) and C4 (−12‰) grasses resulted in our estimating that the grab samples contained an average of 98% C3 vegetation and the surrounding native pasture area contained 70% C4 vegetation (Follett et al., 1997). Since then, Follett et al. (2004) has reported the mean $\delta^{13}C$ for 70 C3 grasses and 20 C4 grasses collected throughout the Great Plains and Western Corn Belt to be −26.8% and −12.9‰, respectively. Thus, recalculation of the fraction of C3 plants in the grab samples from the SSD plots is 92.5% C3 vegetation and that of the surrounding native prairie is 76.4% C4 vegetation.

All soil samples were collected in April of 1993. At Akron, soil samples were collected from an approximate 30 × 30-cm area excavated to a 10-cm depth by flat-bladed shovel. At the Sidney site, the 0- to 10-cm depth increment was sampled with a hydraulic coring system using a 3.8-cm-diameter tube. The collected samples were refrigerated until processed. Processing included removing plant material by sieving the moist samples through a number 10 sieve (2-mm opening), with remaining >0.2 mm removed by handpicking. Moisture determination was obtained from dried subsamples (55 °C) also used to analyze for total C, total nitrogen (N), and for $\delta^{13}C$.

Laboratory Analyses

Microbial Biomass

Moist soil samples equivalent of 50 g of oven-dried soil were weighed into glass snap-cap vials and brought to −0.05 MPa water content with distilled water. Duplicate samples were prepared and placed in separate glass containers (1.89 L) that were made air tight with a rubber ring and screw-type lid. Periodically, the vials were weighed, and distilled water was added to return the soils to −0.05 MPa water content. Each container had an alkali trap (1 *M* NaOH) placed in it to determine CO_2 evolution. Samples were incubated in the dark at constant temperature of 30 °C. However, as a result of a malfunction, the incubator temperature spiked to between 33 and 36.5 °C for a period of <2 weeks between day 500 and day 520. At day 545, the incubator again developed a 1 to 3 °C elevated temperature that returned to 30 °C by 553. Biomass C calculations were made after 10, 79, 161, 322, and 842 days of incubation by the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976; Voroney and Paul, 1984) and for biomass C calculation using Eq. (1).

$$\text{Biomass C} = \text{Cf}/0.41 \qquad (1)$$

where Cf is the CO_2-C evolved during 10 days of incubation for the chloroform-fumigated soil.

Carbon dioxide in the NaOH traps was determined by titration of excess base with standard HCl in the presence of $BaCl_2$ at days 1, 2, 4, 10, 21, 39, 64, 79, 90, 122, 160, 171, 202, 265, 322, 378, 442, 522, 553, 634, 720, 748, 842, and 853. As a result of the incubator malfunction described previously, elevated levels of CO_2 evolution were observed in the NaOH traps on days 522 and 553.

$^{13}C/^{12}C$ Isotope Ratio of the Evolved CO_2-C

The technique used to measure the $^{13}C/^{12}C$ isotope ratio of CO_2-C is described by Harris et al. (1997) wherein the excess base in the NaOH traps was titratrated with standard HCl in the presence of $SrCl_2$. The resulting $SrCO_3$ precipitate had the supernate removed, and the $SrCO_3$ precipitate was transferred into a centrifuge tube using cold previously boiled water as part of the rinsing procedure to remove excess Cl^- ion from the sample. The precipitate was rinsed with cold boiled water and collected by centrifugation for 5 minutes at 939 × g with the centrifuge cooled to −1 °C before centrifugation. After removal of the supernate the sample was allowed to sit approximately 5 more minutes to "soften," then the cold boiled water was re-added and the procedure repeated. The rinsing procedure was repeated three times. After rinsing, the $SrCO_3$ precipitate was analyzed for its $^{13}C/^{12}C$ isotope ratio by isotope-ratio mass spectrometry as described subsequently. The $^{13}C/^{12}C$ isotope ratio analyses on the evolved CO_2-C were determined after soil incubation times of 10, 20, 65, 79, 160, 320, 553, 634, 720, 842, and 853 days.

Total Soil C and N

No soil inorganic C remained in these samples. Measurement of SOC, total soil N, and the $^{13}C/^{12}C$ isotope ratio were determined after incubation of the soils for 0, 172, 333, and

TABLE 1

Soil organic C (%) measured in incubated soils at various times†

Plot	Day 0	Day 172	Day 333	Day 853
Akron, Colorado				
Various-rotation plots				
AVR 208	1.01	0.87	0.76	0.61
AVR 306	1.18	0.97	0.79	0.69
AVR 308	0.87	0.81	0.65	0.55
Mean	1.02	0.88	0.74	0.62
S.D.	0.16	0.08	0.07	0.07
Wheat-fallow plots				
AWF 207	0.91	0.88	0.75	0.64
AWF 205	1.09	0.97	0.81	0.70
AWF 307	0.74	0.70	0.58	0.50
Mean	0.91	0.85	0.71	0.61
S.D.	0.17	0.14	0.12	0.10
Sidney, Nebraska				
No-tillage plots				
SNT I	1.99	1.87	1.61	1.48
SNT II	2.21	2.07	1.77	1.58
SNT III	2.08	1.97	1.71	1.50
Mean	2.09	1.97	1.70	1.52
S.D.	0.11	0.10	0.08	0.06
Plowed plots				
SP I	1.44	1.29	1.12	0.98
SP II	1.43	1.28	1.09	0.80
SP III	1.14	1.17	1.01	0.92
Mean	1.34	1.25	1.08	0.90
S.D.	0.17	0.06	0.06	0.09
Sod plots				
SSD I	2.99	2.68	2.33	1.98
SSD II	3.39	2.83	2.42	2.08
SSD III	2.70	2.57	2.15	1.93
Mean	3.03	2.69	2.30	1.99
S.D.	0.35	0.13	0.14	0.08

†Soils were from the 0- to 10-cm depth increment from all field plots at both Akron, Colorado, and Sidney, Nebraska. Data points were the average of analyses of four samples for days 0 and 853 and two samples for days 172 and 333.

853 days. Analyses for total SOC, total N, and the $^{13}C/^{12}C$ isotope ratio were accomplished using a Carlo Erba C/N analyzer (Haake Buchler Instruments, Saddle Brook, New Jersey)[3] interfaced to a Tracer mass isotope-ratio mass spectrometer (Europa Scientific Ltd., Crewe, England). The $^{13}C/^{12}C$ isotope ratio was used to calculate $\delta^{13}C$, which has per mill units as shown in Eq. (2). By convention, $\delta^{13}C$ values are expressed relative to a calcium carbonate standard known as PDB, from the Cretaceous Pee Dee formation in South Carolina (Boutton, 1991). Sign of $\delta^{13}C$ indicates whether a sample has higher or lower $^{13}C/^{12}C$ isotope ratio than PDB.

$$\delta^{13}C\ (‰) = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{reference}}{(^{13}C/^{12}C)_{reference}} * 1000 \quad (2)$$

Carbon Dating

The SOC from Akron research plot numbers 205, 208, 306, and 307 was dated for ^{14}C age at day 0 and after day 853 of the incubation. The ^{14}C age was measured by combustion of delimed soil in quartz tubes at 900 °C in presence of copper oxide, silver foil, and copper turnings to convert SOC to CO_2 (Coleman and Fry, 1991). Carbon yield was measured manometrically from the CO_2 combustion product.

Trade and company names are given for the reader's benefit and do not imply endorsement or preferential treatment of any product by the USDA.

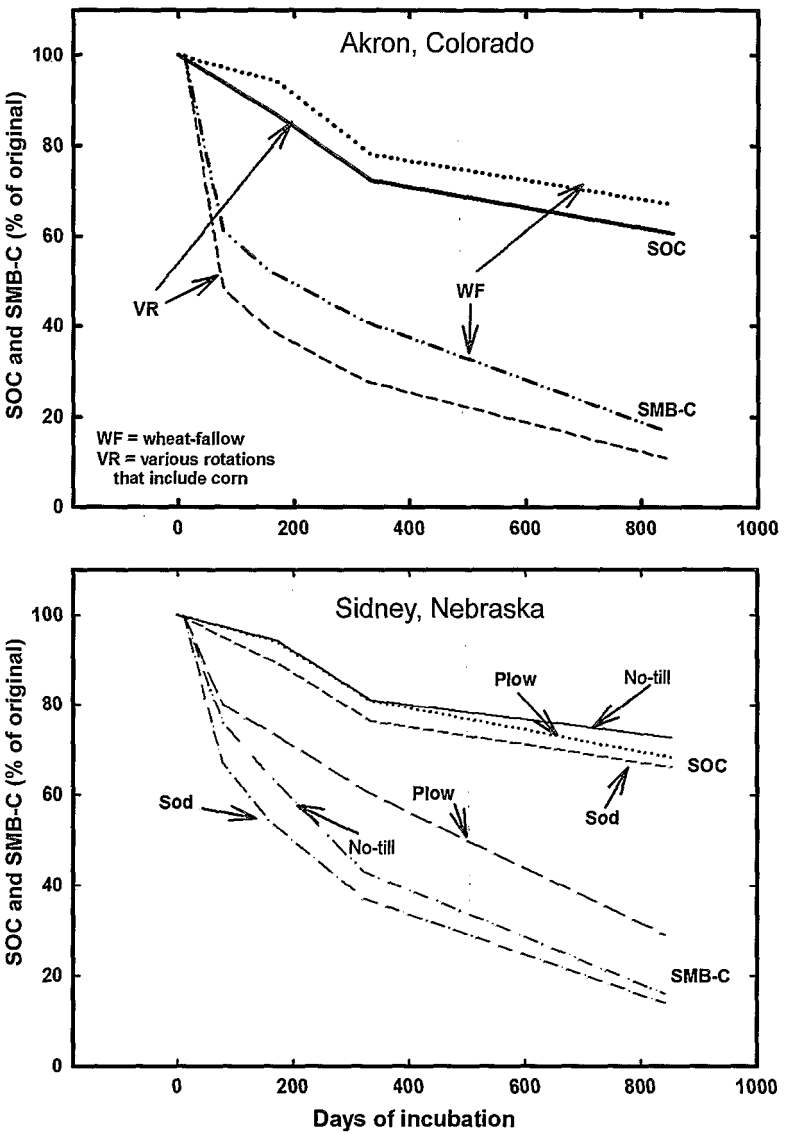

Fig. 1. The effect of incubation time on the percentage of original SOC at days 0, 172, 333, and 853, and soil microbial biomass C (SMB-C) remaining after 10, 79, 161, 322, and 842 days for wheat-fallow (WF) and various-rotation (VR) treatments at Akron, Colorado, and on wheat-fallow plow and wheat-fallow no-till and sod-plot treatments at Sidney, Nebraska.

After reducing the CO_2 to CO on hot zinc and then to graphite on hot iron, soil C age was determined from ^{14}C activity on a tandem accelerator mass spectrometer at the NSF-Arizona Accelerator Facility. A $\delta^{13}C$ correction for isotope fractionation (Goh, 1991) of accelerator measurements of ^{14}C activity was used to arrive at the percentage of modern C and then converted to ^{14}C years BP, with AD 1950 as 100% modern C and 0 year BP.

The residue of acid hydrolysis was determined on selected plots as an estimate of the resistant C pool. This was accomplished by refluxing 1 g of soil in 6 *M* HCl for 18 h. Refluxed samples were washed three times with deionized water to remove excess Cl^- ion, dried at 55 °C, and ground to pass a 180-μm screen. Carbon dating was used to determine MRT (Paul et al., 1997).

RESULTS AND DISCUSSION

Soil Organic C

The SOC for the AVR treatment plots at the Akron site ranged from 1.18 to 0.87% at day 0, with decreases to 0.69 to 0.55% after 853 days

TABLE 2

Soil microbial biomass C (μg C g soil^{-1}) in incubated soils at various times†

Plot	Day 10	Day 79	Day 161	Day 322	Day 842
Akron, Colorado					
Various-rotations plots					
AVR 208	365	194	175	127	38
AVR 306	622	243	174	120	54
AVR 308	412	213	170	124	55
Mean	466	217	173	124	49
S.D.	137	25	3	3	10
Wheat-fallow plots					
AWF 207	261	149	119	102	36
AWF 205	377	210	194	106	49
AWF 307	199	139	118	114	46
Mean	279	166	143	107	43
S.D.	90	38	43	6	7
Sidney, Nebraska					
No-tillage plots					
SNT I	799	587	516	411	160
SNT II	878	689	556	354	132
SNT III	792	600	499	300	109
Mean	823	625	524	355	134
S.D.	48	56	29	56	26
Plowed plots					
SP I	404	319	291	243	137
SP II	385	306	288	238	90
SP III	339	273	254	209	99
Mean	376	299	278	230	109
S.D.	34	23	21	19	25
Sod plots					
SSD I	1538	996	797	515	207
SSD II	1626	1100	882	607	195
SSD III	1582	1082	883	645	280
Mean	1582	1059	854	589	227
S.D.	44	56	49	67	46

†Soils were from the 0- to 10-cm depth increment from all field plots at both Akron, Colorado, and Sidney, Nebraska. Data points were the average of analyses of four samples for days 10 and 842 and two samples each for days 79, 161, and 322.

of incubation (Table 1). For the AWF treatment plots, the SOC ranged from 1.09 to 0.74% at day 0 with decreases to 0.50 to 0.70% at 853 days. The corresponding decreases in average SOC from day 0 to day 853 for the SNT, SP, and SSD treatments were from 2.09 to 1.52%, 1.34 to 0.90%, and 2.70 to 1.93%, respectively. At the Akron site (Fig. 1), the soils from treatments with less total SOC at the beginning (day 0) of the incubation (wheat-fallow) retained a larger fraction of their original SOC than did the treatment with a larger amount of SOC (various-rotation plot) at the beginning (67 vs. 61%). This is likely explained by a greater loss of active and slow SOC pools relative to the amount of the resistant SOC pool remaining where the fields from which the soils were collected had historically received more cultivation.

Similar results to those at the Akron site were observed at the Sidney site. The average SOC in the no-till and plow treatments, respectively, decreased from 2.1 and 1.3% SOC at day 0 to 1.5 and 0.9% SOC at day 853 of the incubation. The corresponding values for the percentage of SOC in the sod treatment were a decrease of 3.0 to 2.0%. The no-till, plow, and sod treatments retained 73, 68, and 66% of their SOC after 853 days of incubation (Fig. 1). The sod treatment retained the smallest fraction of its SOC, which would be consistent with the buildup and then more rapid loss of the active and slow SOC fractions under the sod treatment than under the cropped treatments.

TABLE 3
Contribution of soil microbial biomass C to CO_2-C evolved during the 842-day incubation†

Day	Percentage of CO_2-C derived from the microbial biomass				
	Akron corn	Akron wheat	SNT	SP	SSD
10–79	41.84 (7.43)	32.46 (5.81)	23.31 (4.39)	26.60 (1.44)	32.61 (4.60)
80–161	24.84 (4.73)	19.46 (0.19)	18.34 (0.82)	16.52 (1.15)	25.13 (2.71)
162–322	22.17 (4.91)	16.58 (3.81)	20.53 (1.28)	15.69 (0.82)	23.68 (2.67)
323–842	21.02 (3.33)	16.38 (1.55)	20.07 (1.18)	15.74 (0.95)	18.17 (0.91)

†Data points were the average of the analyses of four samples. Value in parentheses is S.D.

The relative retention of SOC after incubation for 853 days provides insight about the time required for breakdown and release as CO_2-C from the less resistant soil C fractions. The amount of SOC present at the beginning of the incubation was indicative of a larger pool of the less resistant fractions that were available to be broken down and recycled, thus resulting in lower percentages of the original SOC remaining after 853 days of incubation.

Soil Microbial Biomass C

Soil microbial biomass C, measured at day 10, was 3 to 5% of the total SOC in plots from both the Akron and Sidney sites (Table 2). These values compare well to values reported for cultivated soils by Collins et al. (2000), who report SMB-C as 2 to 3% in the surface 20 cm of prairie sites and 3 to 6% in cultivated forest soils. For the AVR and AWF plots at the Akron site, the average SMB at day 10 was 470 and 280 μg C g soil^{-1}, respectively. By day 842, the values for these corresponding treatments had decreased to 50 and 40 μg SMB C g soil^{-1}, thus having retained only 11 and 16%, respectively, of SMB-C that was originally present on day 10 (Fig. 1). At the Sidney site (Table 2), the amounts of SMB-C on day 10 were higher where less soil disturbance had occurred as shown by the SSD treatment containing an average of 1580 μg SMB-C g soil^{-1} and lesser amounts were observed under the SNT and SP treatments with 820 and 380 μg SMB C g soil^{-1}, respectively. At the Sidney site, the fraction of the SMB-C remaining after 842 days was only 16, 29, and 14% for the SNT, SP, and SSD treatments, respectively (Fig. 1). The higher percentage of retention for the AWF and SP treatments likely reflects the lower beginning amounts of SMB in these treatments that had resulted from type of tillage and/or lower residue C inputs they received in the years before our collection of samples from them.

In this experiment, at steady abiotic conditions and no external substrate inputs, the survival of the microbiota depended on maintenance of the population and its feeding on an increasingly recalcitrant substrate. Measurement of both microbial biomass and CO_2-C at various stages of the incubation made it possible to determine the net contribution of the biomass to mineralization of C (Table 3). The decrease in biomass accounted for 32 to 42% of the CO_2 accumulation from days 10 to 79 of incubation of the Akron soil. This rate dropped to 16 to 21% of the CO_2 accounted for during the last 500 days of the incubation, at which time only about 10% of the original amount of SMB-C remained (Fig. 1). The initial contribution was somewhat lower in the Sidney soils but also was still substantial in the latter parts of the experiment (Table 3 and Fig. 1). One must ask, was the lower biomass responsible for the lower asymptotic decomposition rates of the SOC that were observed in the latter part of this experiment, i.e., was the decomposition activity limited by enzymatic capacity rather than by substrate? This question was partially answered by a change in the incubation temperature between days 500 and 550 (from a refrigeration malfunction) that caused an increase of 3 to 6.5 °C in incubation temperature. Figure 2 (plotted from day 21) shows that the microorganism responded with an average 50% increase in the daily rate of CO_2 respiration at day 522. After the readjustment of the incubation temperature, the rate of CO_2 respiration dropped back at subsequent measurement times. Such an active response so late in the incubation was observed when the population averaged only 27 and 37% of the original SMB-C for the Akron and Sidney plots, respectively (Fig. 1). The issue is whether by day 522 the substrate should have been more recalcitrant, but the aforementioned observations lead to questions concerning the SOC at this time and whether

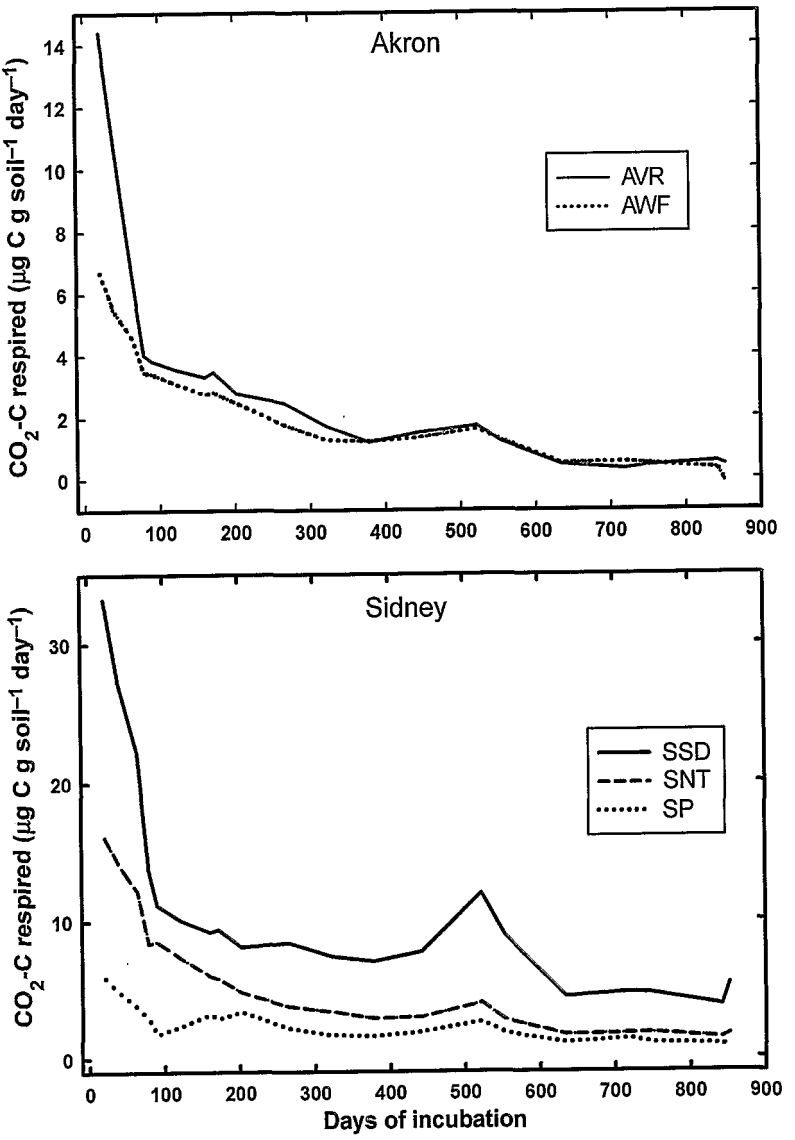

Fig. 2. The effect of days of incubation time upon the average respiration rate of CO_2-C between days 21 and 853 for wheat-fallow (WF) and various-rotation (VR) treatments at Akron, Colorado, and on wheat-fallow plow and wheat-fallow no-till and sod-plot treatments at Sidney, Nebraska. Note that day 21 was chosen as the beginning day to allow the y axis to be rescaled to better show the 500 to 550 days of temperature increase effect on rate of CO_2-C respiration.

temperature sensitivity rather than biochemical recalcitrance is the limiting factor.

Smith and Paul (1990) identified that microbial biomass in mineral soils comprises 1 to 8% of the C, with most soils falling within a fairly narrow range of 2 to 5%. Before incubation, soil from the Akron site had 3 to 4.5% of its C comprised of SMB-C, whereas at the Sidney site, the corresponding range was from 2.8 to 5.3%. By the end of the incubation, the Akron soil samples had only 0.7 to 0.8% of their soil C comprised of SMB-C, with the corresponding amounts for the Sidney sites only 1.1 to 1.4%. Therefore, the SMB served as one of the more labile pool during this incubation study. Unfortunately, data were not collected during the day 500 to 550 temperature increase, but by extrapolation, the fraction of the soil C comprised as SMB-C was 1.3 to 1.4% for the Akron samples and 1.7 to 2.1% for the Sidney samples. These decreased levels of SMB-C at the time the temperature increase occurred would seem

TABLE 4
Soil organic and hydrolysis C and ^{14}C ages measured at day 0 and 853 in selected incubated soils†

Plot	SOC (g kg^{-1})	Hydrolysis residue C (% of total)	Supernatant C (% of total)	^{14}C age	
				SOC	Hydrolysis residue C
Day 0					
Various-rotation plots					
208	10.1	—	—	1320 ± 45	—
306	11.8	—	—	650 ± 50	—
Wheat-fallow plots					
205	10.9	60.5	39.5	1015 ± 45	2658 ± 60
307	7.4	63.9	36.1	1130 ± 45	3656 ± 70
Day 853					
Various-rotations plots					
208	6.1			Modern	—
306	6.9			280 ± 50	—
Wheat-fallow plots					
205	7.0	62.4	37.6	1225 ± 50	4190 ± 80
307	5.0	62.0	38.0	1160 ± 60	5745 ± 65

†Soils were from the 0- to 10-cm depth increment from Akron, Colorado, field plots. Data points were the average of analyses of two samples.

to support that temperature may be more important than biochemical recalcitrance of the substrate for increased SOC pool availability for microbial processing.

^{14}C Ages of SOC in Selected Plots

Discussions in the preceding sections allude to decreases in the active and slow fractions during this long-term incubation study. To the degree that SMB-C represents an active fraction, the data indicate lower levels under treatments that would have been subjected to either more intensive tillage and/or less plant residues return. Carbon dating, using the naturally occurring ^{14}C isotope after acid hydrolysis, has been used to estimate the resistant fraction (Collins et al., 2000). Because measurement of naturally occurring ^{14}C is quite expensive, we obtained measurements on only selected plots as shown in Table 4. The SOC was ^{14}C dated in the Akron soil on nonincubated soil (equivalent to day 0) and after incubation for 853 days for two of the AVR plots (208 and 306, continuous corn) and two of the AWF plots (205 and 307, stubble mulch and plow tillage, respectively). The soils from 205 and 307 (AWF) were hydrolyzed, and the hydrolysis residue C was ^{14}C dated for nonincubated soil (day 0) and after incubating for 853 days. The column showing SOC (in g kg^{-1}) was shown in Table 1 but is also shown here for reader convenience. The residue of the acid hydrolysis estimates the size of the resistant C pool and amounted to 60 and 64%, respectively, for plots 205 and 307 at day 0, with corresponding values of 62 and 62% at day 853. These percentage sizes of the acid hydrolysis residue pools are quite similar for different treatments at the different incubation times. However, great changes are shown because of the great differences in the content of SOC at these different incubation times. The total amount of SOC remaining at day 853 is considerably diminished from that at day 0 (Fig. 1).

The ^{14}C ages measured for the nonhydrolyzed soil at day 0 are similar to those observed at Akron by Paul et al. (1997) on nearby plots. The MRT of the SOC for plots 208 and 306 (AVR) decreased in ^{14}C age by an average of about 850 years after 853 days of incubation, whereas the MRT of the SOC for plots 205 and 207 (AWF) increased in ^{14}C age by an average of 120 years after the 853-day incubation, an average difference of 970 years. These results may possibly be explained by the fact that there is as much microbial decomposition of the older fractions of SOC during incubation as the modern SOC. Also, there could have been recalcitrant modern C fractions in the AVR plots (208 and 306) that resisted microbial decomposition during incubation more than older SOC fractions possibly did and that were not in the AWF plots (205 and 307). Acid hydrolysis is known to have a limitation in that modern lignin fractions would appear in the nonhydrolyzable

TABLE 5

The $\delta^{13}C$ (‰) of SOC measured for incubated soils†

Plot	Day 0	Day 172	Day 333	Day 853
Akron, Colorado				
Various-rotation plots				
AVR 208	−20.75	−17.96	−18.62	−18.42
AVR 306	−23.69	−18.67	−19.14	−19.05
AVR 308	−18.44	−18.99	−18.86	−18.89
Mean	−20.96	−18.54	−18.87	−18.79
S.D.	2.63	0.53	0.26	0.26
Wheat-fallow plots				
AWF 207	−19.45	−19.54	−19.81	−19.74
AWF 205	−22.74	−20.34	−20.01	−19.65
AWF 307	−20.55	−19.30	−18.72	−18.67
Mean	−20.91	−19.73	−19.51	−19.36
S.D.	1.68	0.54	0.70	0.49
Sidney, Nebraska				
No-tillage plots				
SNT I	−19.64	−20.90	−20.41	−20.46
SNT II	−20.04	−21.00	−20.65	−20.46
SNT III	−19.61	−20.46	−20.35	−20.20
Mean	−19.76	−20.79	−20.47	−20.37
S.D.	0.24	0.29	0.16	0.15
Plowed plots				
SP I	−20.67	−19.11	−19.56	−19.34
SP II	−20.93	−19.24	−20.42	−19.52
SP III	−18.40	−19.06	−19.72	−19.29
Mean	−20.00	−19.14	−19.90	−19.38
S.D.	1.39	0.10	0.46	0.20
Sod plots				
SSD I	−20.29	−20.69	−20.61	−20.62
SSD II	−20.18	−19.62	−20.20	−19.88
SSD III	−20.08	−19.31	−19.98	−19.55
Mean	−20.18	−19.87	−20.26	−20.02
S.D.	0.11	0.72	0.32	0.55

†Soils were from the 0- to 10-cm depth increment from all field plots at both Akron, Colorado, and Sidney, Nebraska. Data points were the average of analyses of four samples for days 0 and 853 and two samples for days 172 and 333.

fraction (Paul et al., 2006). Later discussion of the ^{13}C data in this article notes the likely existence of such modern lignin from the presence of resistant corncob materials in the AVR plots.

Paul et al. (1997) had observed that hydrolysis of the Akron surface soil increased the ^{14}C age by about 1600 years. In this study, hydrolysis of the day 0 soil increased the ^{14}C ages of the residue C of plots 205 and 307 (AWF) by 1600 and 2500 years, respectively (average = 2080 years). After 853 days of incubation, hydrolysis increased the ^{14}C age of the residue C of these same two plots by 2960 and 4580 years, respectively (average = 3780 years). Thus, incubation had removed, or made vulnerable to hydrolysis, more of the younger MRT SOC, leaving behind older MRT SOC that was less vulnerable to hydrolysis. Hydrolysis increased the ^{14}C age much more in soils that had been incubated for 853 days by an average of approximately 1700 years. This result can likely be interpreted to indicate that incubation removed some of the recently added plant residue lignin that normally would be retained in the nonhydrolyzable fraction.

$^{13}C/^{12}C$ Isotope Ratios in SOC

The $^{13}C/^{12}C$ ratio, shown as $\delta^{13}C$ in Eq. (2), is expressed in per mill units. Table 5 shows the $\delta^{13}C$ of the SOC 0, 172, 333, and 853 days of incubation. Especially for the Akron plots and for the SP treatment at Sidney, the S.D. of measured $\delta^{13}C$ values narrowed between days 0 and 172. Narrowing of the S.D. likely results from the microbial decomposition of possible remaining plant material not removed during

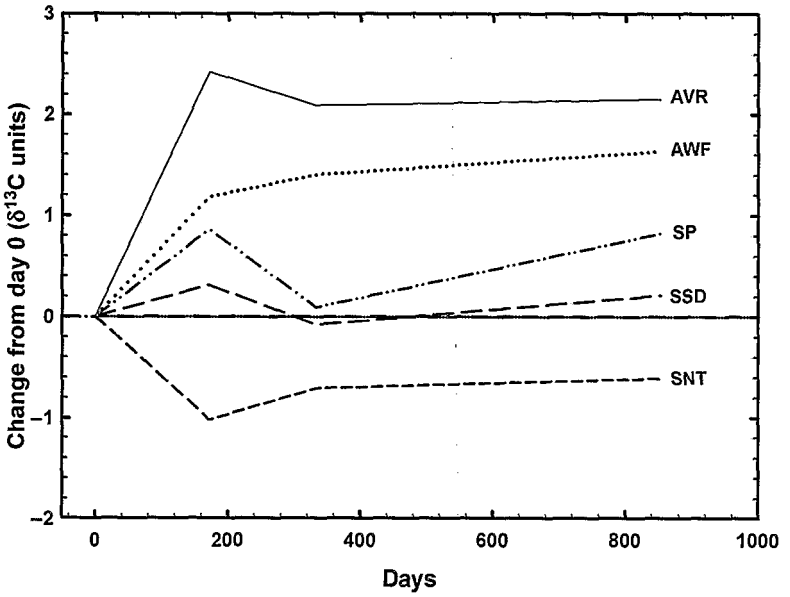

Fig. 3. Change in δ^{13}C in the SOC of incubated soils after 172, 333, and 853 days of incubation as compared by subtraction of the δ^{13}C of the soil at day 0 for the Akron plots, which include AVR and AWF, and the Sidney plots, which include SP, SSD, and SNT.

handpicking (see also Fig. 1). The δ^{13}C values also reflected the crops and whether C3 or C4 plant had been grown on the plots. Of the cultivated plots, only the AVR treatment plots had a C4 plant (corn) growing on them in recent years. The δ^{13}C of SOC from native sites near the Akron and Sidney sites were reported by Follett et al. (1997) to have values of −16.1 and −16.4, respectively. On the SSD plots, Follett et al. (1997) report that the δ^{13}C of their "grab" samples of aboveground plant material averaged −25.74‰, thus resulting in an estimate that about 92.5% of the aboveground biomass was C3 vegetation. Consequently, incubation likely results in the biological consumption of nonrecalcitrant organic matter, recently sequestered SOC, and/or remnants of decomposing plant material. With time of incubation, the δ^{13}C of the soil might be expected to start to approach that of the native soils, and indeed, except for the SNT and SSD treatments, this appears to be the case (Table 5).

To better visualize the changes of the stable isotope C ratios in the SOC, we subtracted the δ^{13}C of the soil at day 0 from the subsequent δ^{13}C values measured after the various times of incubation. As shown in Figure 3, the left side axis is the δ^{13}C at 0, 172, 333, and 853 days of incubation minus the day 0 value. Where the change is positive, then the δ^{13}C of the soil is becoming less negative and moving toward the δ^{13}C of the native soil, and where the difference is negative, the δ^{13}C of the soil is becoming more negative with the time of incubation. Recognizing that soil samples incubated in this study were collected from the 0- to 10-cm depth, the soil material was very much influenced by the field conditions from which it was collected, and the AVR treatment showed a greater influence of C4 plant species than any other treatment. The two treatments where there is minimal or no soil mixing by tillage (SSD and SNT) show the least tendency for the δ^{13}C of the soil to become less negative and, in the case of the SNT, entirely cropped to C3 plants with no tillage, the δ^{13}C of the incubated soil became more negative with time.

CO_2-C Evolution During Incubation

The rate of accumulation of C mineralized from the SOC and then emitted as CO_2 (Figs. 4A, 5A, and 6A) was highest early in the incubation. For plots sampled at both Akron (AVR and AWF) and Sidney, there was a clear separation among the treatments by the end of the study period. The order in which the treatments were separated by the end of the study was in the same order as that of the SOC concentration at the beginning of the study (Table 1). For the AVR treatments, with all plots in no-till, those in continuous corn (306 and 208) for

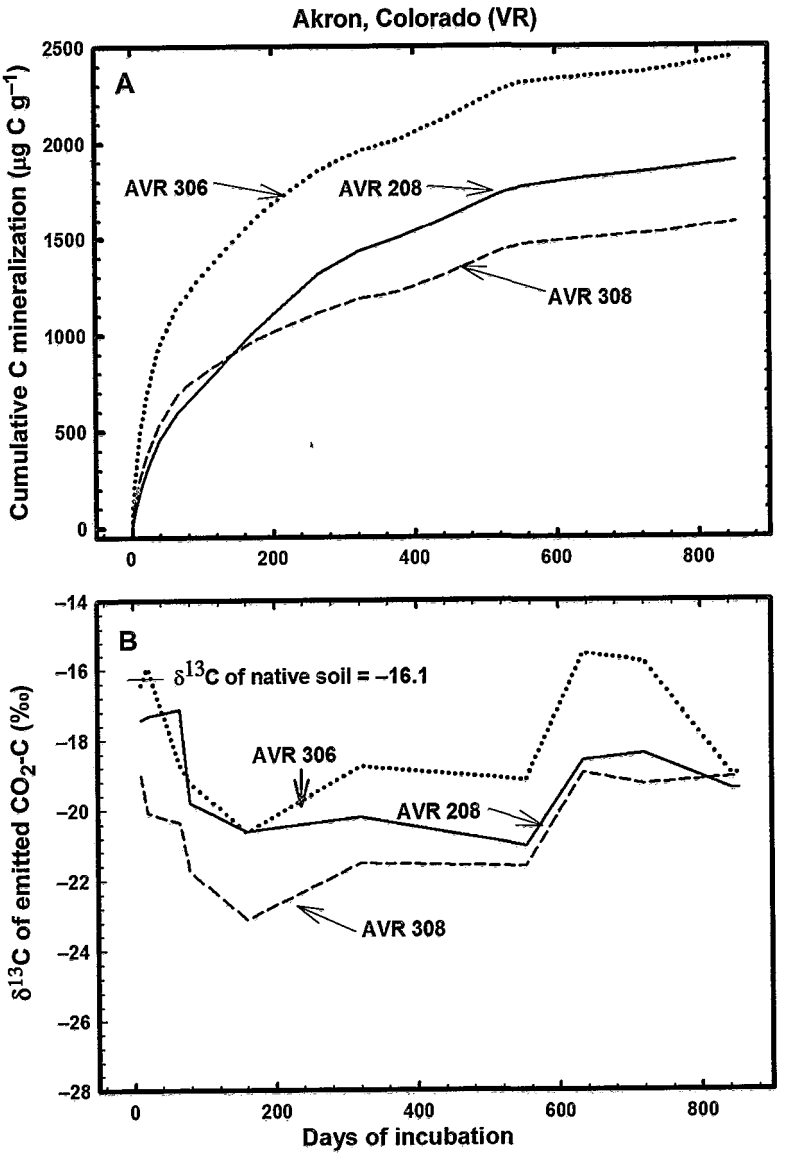

Fig. 4. Soil organic C mineralization (A), measured as cumulative CO_2-C respiration, during 853 days of incubation and (B) the $\delta^{13}C$ of the CO_2-C respired in samples collected at 10, 20, 65, 79, 161, 322, 553, 634, 720, and 842 days for various continuous corn (AVR 208 and 306) and corn-fallow-wheat-corn (AVR 308) plots at Akron, Colorado.

the past 4 years emitted more CO_2-C than did plot 308, which had been in a corn-fallow-wheat-corn rotation during the previous 4 years (Fig. 4A). Although plot 208 had a higher beginning SOC concentration than did plot 308, the cumulative C emitted by plot 208 did not begin to exceed that emitted by 308 until approximately day 160 of the study (Fig. 4A). Plot 308 had both a fallow and wheat year between the 2 years of corn grown in this rotation, which may have contributed to a relatively rapid rate of CO_2-C emission, although the SOC concentration at the beginning of the study was lower than for plot 208 (0.87 vs. 1.01%).

At both Akron and Sidney where plots were cropped to a wheat-fallow rotation, tillage type was clearly associated with both the beginning concentrations of SOC and in the amount of CO_2-C emitted during the incubation (Figs. 5A and 6A). Tillage treatments for AWF plots 205, 207, and 307 were no-till, stubble mulch (conventional), and plow tillage for the four preceding years, respectively. The initial rates of

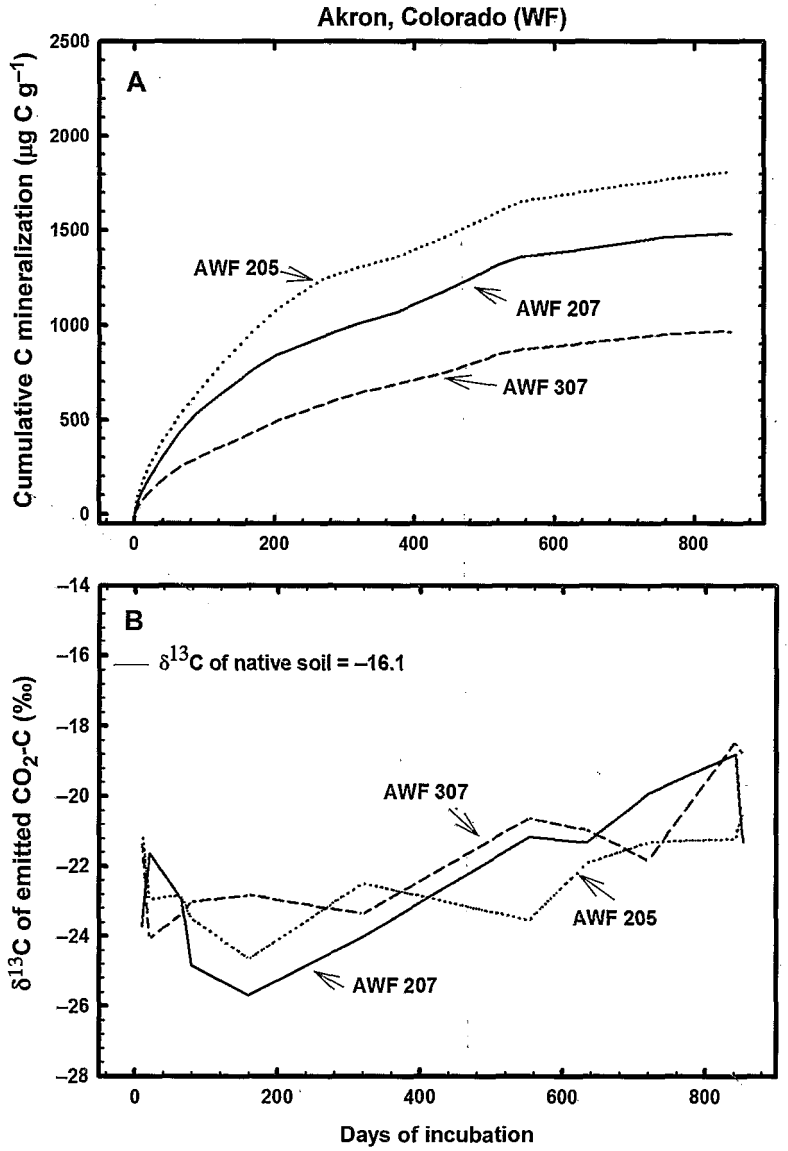

Fig. 5. Soil organic C mineralization (A), measured as cumulative CO_2-C respiration, during 853 days of incubation and (B) the $\delta^{13}C$ of the CO_2-C respired in samples collected at 10, 20, 65, 79, 161, 322, 553, 634, 720, and 842 days for wheat-fallow tillage treatments of plow (AWF 307), stubble-mulch (AWF 205), and no-tillage (AWF 207) treatments at Akron, Colorado.

CO_2-C emitted for the AWF plots were lower than for the AVR plots, and only plot 205 (no-till) exceeded the amount of CO_2-C emitted by any of the AVR plots. For the Sidney location treatments, the SSD, SNT, and SP treatments were widely separated and emitted 7550, 3450, and 1700 µg CO_2-C g soil^{-1}, respectively, by day 853. The CO_2-C emission from the SP treatment was of similar magnitude to those for AWF treatments. The preceding data show that the most rapid evolution of CO_2 occurs early in the incubation, whereas levels of SMB are largest and likely because of the presence of less recalcitrant SOC and plant materials. By day 79, more than 30% of the CO_2 emission measured during this 853-day study had occurred except for the SP treatment, with about 20% emitted, and the AVR plots, with over 40% emitted. In the case of the SP treatment, the amount of less recalcitrant materials available for use by the SMB would be low. For the AVR plots, presence of corn residues enhanced the rates

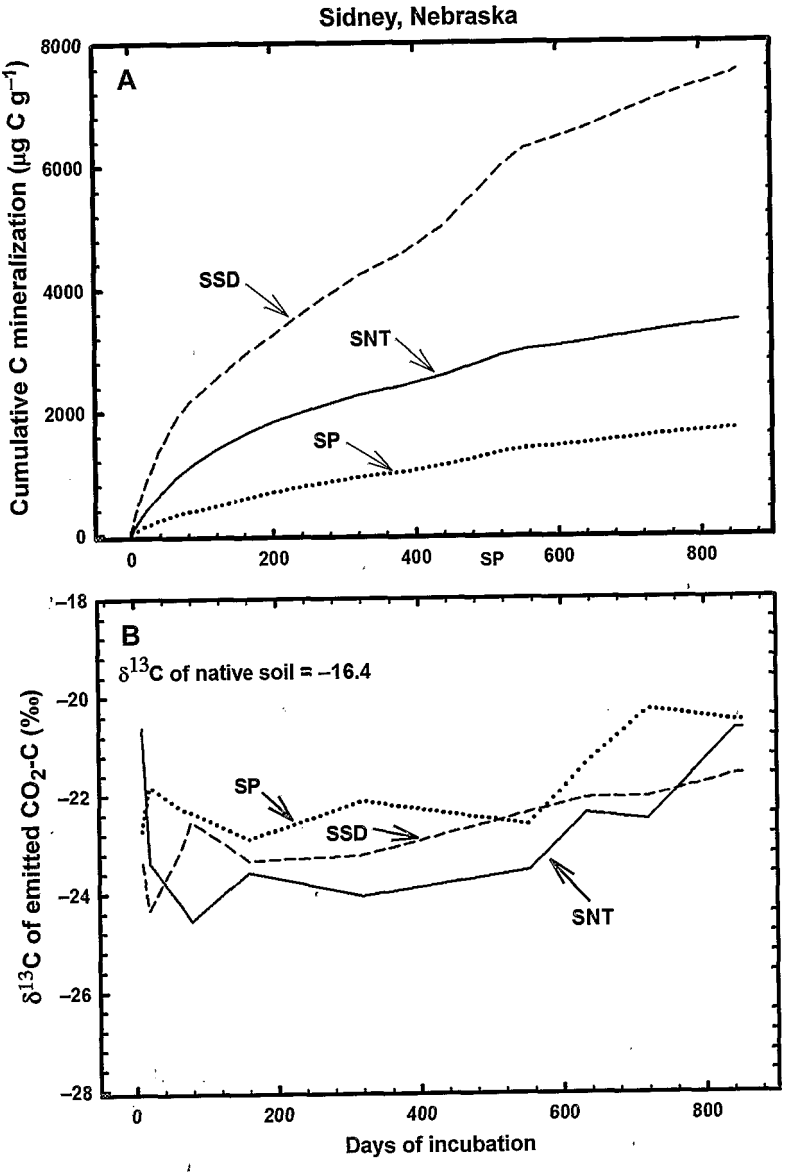

Fig. 6. Soil organic C mineralization (A), measured as cumulative CO_2-C respiration, during 853 days of incubation and (B) the $\delta^{13}C$ of the CO_2-C respired in samples collected at 10, 20, 65, 79, 161, 322, 553, 634, 720, and 842 days for wheat-fallow plow (SP), wheat-fallow no-till (SNT), and sod (SSD) plots at Sidney, Nebraska.

of CO_2 because of their apparent higher availability for microbial decomposition and lower C/N ratio.

$\delta^{13}C$ Signature of Emitted CO_2-C

Akron Various-Rotation Plots

The $SrCO_3$ (Harris et al., 1997; Collins et al., 2000) and $BaCl_2$ precipitate techniques (Collins et al., 1992) to determine the $\delta^{13}C$ of the evolved CO_2-C have been used where direct CO_2 injection was not feasible. It measures various management effects upon the oxidation of SOC (Collins et al., 1992; Motavalli et al., 1994; Paul et al., 1999). Measurement of $\delta^{13}C$ of evolved CO_2 allows insight into the biological fractionation of the active C fractions and of a portion of the slow C pool during degradation by soil microorganisms (Collins et al., 2000). The $\delta^{13}C$ signature of emitted CO_2-C for the AVR, AWF, and Sidney plots, discussed previously, is shown in

Figures 4B, 5B, and 6B, respectively. When compared, the pattern for the AVR plots (Fig. 4B) is markedly different from either the AWF or the Sidney plots.

The $\delta^{13}C$ of the wheat and corn was reported as −26.2 and −13.0‰, respectively (Follett et al., 1997). For the AVR plots (Fig. 4B) where corn had been in the rotation, the $\delta^{13}C$ of the evolved CO_2-C during especially days 10, 20, 65, and 79 reflected the microbial decomposition of corn residue, likely from the particulate organic matter fraction. Plots 208 and 306 had been continuously corn plots, whereas plot 308 was in a corn-fallow-wheat-corn rotation for the last 4 years. By day 160, the $\delta^{13}C$ of the evolved CO_2-C was at its most negative values and clearly reflected the microbial decomposition and evolution of C from C3 wheat residue. Through days 322 and 553, there was a slightly less negative or nearly constant $\delta^{13}C$ of the evolved CO_2-C. After day 553 and at days 634 and 720, all three AVR plots evolved CO_2-C with a much less negative $\delta^{13}C$ signal before it became somewhat more negative through day 853 of the incubation. The reason for the less negative $\delta^{13}C$ signal at days 634 and 720 is postulated to result from microbial breakdown of residual pieces of corncobs that were broken up in the flailing process during combining and harvest and that were not removed by screening and picking of the soil during soil preparation for incubation. The potential role of pieces of corncob on the $\delta^{13}C$ signal and the possible timing of C evolution from their decomposition will be discussed subsequently.

It is of also of interest that these $\delta^{13}C$ changes that are found later in the incubation occurred about the same time during the incubation as those resulting from the loss of temperature control and that are shown in Figure 2. Although not as apparent for the AWF and the Sidney plots, this, together with the presence of residual pieces of corncob, might be partly responsible for the observed changes and which are not noted in various other long-term incubation studies.

Akron Wheat-Fallow Plots

By comparison to the AVR plots (Fig. 4B), the AWF plots (Fig. 5B) did not show the large less-negative change in $\delta^{13}C$ at days 634 and 720 discussed previously. Although there was an initial decrease from a less negative to more negative $\delta^{13}C$ signal for the AWF plots, it was more negative than for the AVR plots and remained more negative throughout the entire incubation. Both sets of plots reached their most negative values by day 160, possibly indicating depletion by microbial decomposition of the more accessible C plant C present in the soil, more likely, the plant material from C crops that had been growing on them. After day 160, the CO_2-C emitted from the AWF plots had an increasingly less negative $\delta^{13}C$ signal that changed from an average of −24.4‰ at day 160 to −20.2‰ at day 853. Consistent with data of Follett et al. (1997), we assume that the soil C pools being decomposed after day 160 through day 853 include remnant amounts of SOC from the original prairie soil ($\delta^{13}C$ = −16.1). The authors do not have an explanation for the wide swings in the $\delta^{13}C$ signal of plot 207 between day 700 and day 843.

Sidney Plots

The treatments from which soil samples were collected at Sidney, NE (Fig. 6B) included 3 replications of wheat-fallow rotation under no-tillage (SNT) and plow-tillage (SP) and a treatment that was never broken sod (SSD). At the time soil samples were collected, a vegetative change from that of the original native prairie had occurred on the SSD plots and they were growing mostly C3 grasses (soil $\delta^{13}C$ = −20.1‰) (Follett et al., 1997). The adjacent native prairie sites had more C4 grasses present and a soil $\delta^{13}C$ of −16.4‰. The $\delta^{13}C$ of the evolved CO_2-C from the SNT and SP treatments at day 10 was −24.6 and −22.7‰, respectively. After some fluctuations, up even through day 322, the $\delta^{13}C$ of the evolved CO_2-C from the SNT and SP treatments slowly became less negative until they were −20.1 and −20.2‰, respectively, at day 853. The $\delta^{13}C$ of the evolved CO_2-C from the SSD treatment at day 10 was −23.4‰, showed fluctuations through day 160, and then slowly became less negative until day 853 when it was −21.4‰. As at Akron, we assume the explanation for the evolved CO_2-C becoming less negative through day 853 is that remnant amounts of SOC derived from the original prairie soil C pools with their less negative $\delta^{13}C$ are also contributing their signal to that more recent C from wheat and other C3 plants. The $\delta^{13}C$ of the evolved CO_2-C provides unique insights not obtained with other measures and can be especially important to develop

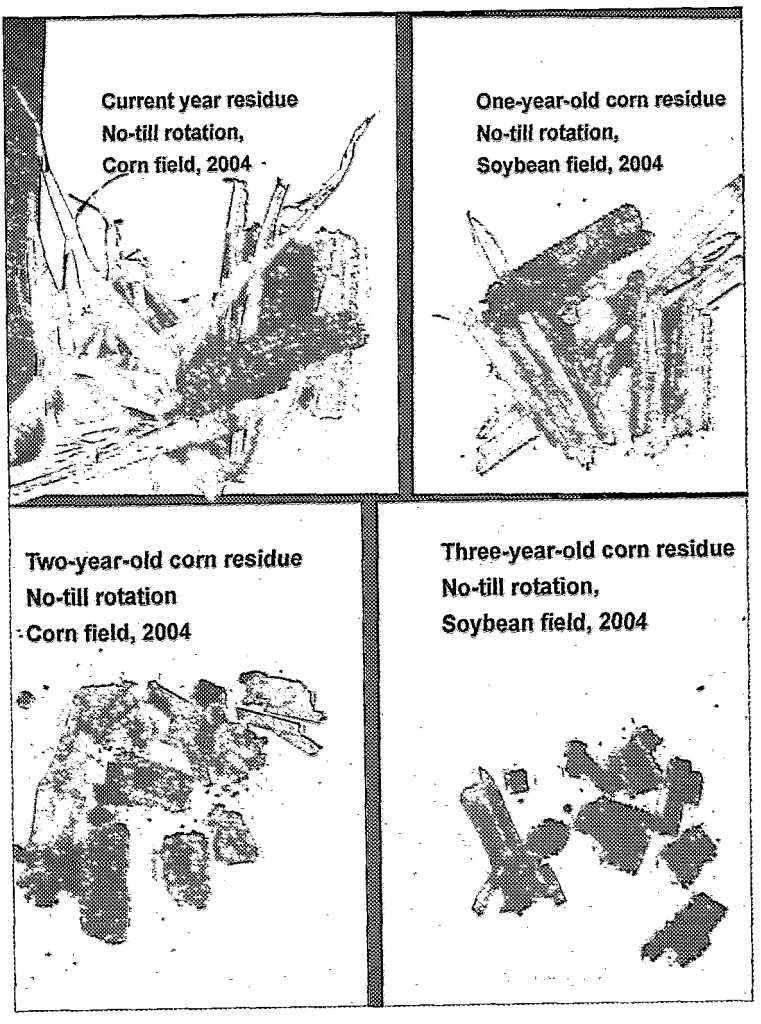

Fig. 7. Pictures of corncobs, stalks, and leaves collected in 2004 under farm-field no-till conditions after 0, 1, 2, and 3 years of weathering and decomposition.

an understanding of the breakdown of various potential C pools over time. The effect of the breakdown of the more available plant material is readily reflected in the $\delta^{13}C$ of the evolved CO_2-C during the earlier part of the incubation (up to 79 days). The steady change in the $\delta^{13}C$ of the evolved CO_2-C toward that associated with SOC from the original native prairie was also detected. The possible effect of remnant pieces of corncob on the $\delta^{13}C$ of the evolved CO_2-C could also be detected to help provide an explanation, along with the additional study of corn residues that follows, of the less negative $\delta^{13}CO_2$ that occurred from days 634 to 720.

Effect of Corn Residues on Incubation Results

Data collected during the incubation of soils collected from the AVR and the $\delta^{13}C$ of the CO_2-C evolving on days 634 and 720 from all three plots in this part of the study were considerably less negative than before day 634 and also less negative than at days 842 and 853 (Fig. 4B). The authors hypothesize that the cause of this anomaly is that pieces of corncob and possibly other corn residues were resistant to microbial attack until well into the incubation because of properties such as the size or shape of the corn particles or high C/N ratio of the material itself. Besides the measured $\delta^{13}C$ data, other measures that may support this hypothesis are shown in Table 4, where ^{14}C dating indicated the MRT of the SOC in the AVR plots is younger and has more recent C present after the 853-day incubation than at day 0. By comparison, ^{14}C dating of the SOC in the AWF plots in which corn was not grown indicated the

TABLE 6

Carbon and N content of corn grain and individual and combined corn residues collected from research and farmer fields in Colorado, Kansas, and Indiana†

Plant part	Location	Time laying in field (months)	N (%)	C (%)	C/N (ratio)
Grain	Fort Collins, Colorado	0	0.93	40.3	43
Cob	Fort Collins, Colorado	4	0.18	41.2	230
Husk	Fort Collins, Colorado	4	0.18	41.9	230
Stalk	Fort Collins, Colorado	4	0.16	42.6	270
Cob	Tribune, Kansas	0	0.18	44.7	253
Combined residues‡	Crawfordsville, Indiana	0	0.43	45	105
Combined residues‡	Crawfordsville, Indiana	12	0.61	41	67
Combined residues‡	Crawfordsville, Indiana	24	0.81	38	47
Combined residues‡	Crawfordsville, Indiana	36	1.39	37	27

†Data points were the average analyses of two samples.
‡See also Figure 7.

MRT was slightly older or unchanged after the 853-day incubation.

As supplemental information to help support the hypothesis about the presence of corn residues resistant to microbial attack, field samples of corn materials were collected and analyzed for N, C, and C/N ratio (Table 6). Compared with that measured for corn grain (C/N ratio of 43), the C/N ratios of corncobs, husks, and stalks were all 230 or more and would be expected to resist microbial breakdown. Based only on the C/N ratio, any of the previously mentioned corn residues might be candidates for the results observed for the AVR plots (Fig. 4B). To provide a visual evaluation of the resistance of corncobs, corn husks, and corn stalks to decomposition in the field, corn residues were collected from two adjacent fields that were cropped to no-till corn or no-till soybean in alternate years, thus allowing the identification and collection of increasingly older corn material by year (Fig. 7). In addition, we analyzed the entire samples shown for C/N ratio to better evaluate the rate of change in C/N ratio with time for samples exposed to weather and decomposition processes in the field for an extended period. The C/N ratios of the combined residues began at 105 and decreased to 67, 47, and 27 after lying on the soil surface (Table 6) for 12, 24, and 36 months, respectively.

CONCLUSIONS

The objective of this long-term study was to determine controls on decomposition and the stability of soil C under various managements that affect pool sizes and turnover rates of SOC fractions. The SMB as a soil-C pool and other SOC pools were evaluated using soil from two locations in the U.S. Great Plains and across various tillage management treatments to provide a range of SMB and soil C amounts and types. The study shows there is a continual breakdown and recycling of soil C into increasingly resistant soil C pools. The measurement of decreases in SOC and SMB-C during an 853-day incubation without added substrate made it possible to determine the rates of loss of both and the role of organisms as a source of mineralizable C. The $\delta^{13}C$ of the evolved CO_2 during incubation made it possible to determine whether the evolved C came from the more recent corn residues or from the soil derived from the original native grassland. Acid hydrolysis and ^{14}C dating before and after the extended incubation characterized the more resistant SOC components. Incubation malfunction between days 500 and day 550 resulted in a 3 to 6.5 °C temperature increase, during which microorganism responded with an approximately 50% increase in respiration rate and seemed to support the possibility that temperature rather than biochemical recalcitrance of the SOC may have been the limiting factor. These results provide insights into the contribution of microbial biomass and changes in isotope ratios in soil that were not previously available in the scientific literature.

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