ASSESSMENT METHODS FOR SOIL CARBON

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The Determination of Soil C Pool Sizes and Turnover Rates: Biophysical Fractionation and Tracers

E.A. Paul, S.J. Morris and S. Böhm

I. Introduction

The interpretation of soil organic-C (SOC) dynamics in ecosystem functioning, sustainable agriculture, forestry management and global change requires knowledge of the size and fluxes of the SOC pools involved. Accurate assessment of these pools requires analysis of well-taken, representative soil samples of known bulk density to the depth of soil formation. Samples must reflect an understanding of microscale patterns that control SOC dynamics and associated reactions in the field (Robertson and Gross, 1994). Much of the information for global change calculations is interpreted on a regional or global basis. The sites studied must be representative of all areas in the extrapolation. Some soils such as sands have only a limited SOC accumulation capacity; others such as clays have a much higher sequestration capacity.

Soil organic matter is a continuum of a complex of related humic materials. No one fractionation, technique or pool can be expected to adequately characterize the turnover rates of the whole soil. The description of SOC dynamics and modeling has shown that first order kinetics and a three-pool concept can reasonably effectively describe SOC dynamics (Paustian et al., 1992). The ability to estimate soil C is dependent on the accuracy with which C contents of individual layers and horizons can be measured. Total C and N are usually determined with an accuracy of ±2% of the total amount present; this means that management effects cannot usually be determined until after at least 10 years have elapsed. Techniques that measure the more dynamic fractions such as microbial biomass (Horwath et al., 1996), the light fraction (LF) (Janzen et al., 1992), particulate organic matter (POM) (Cambardella and Elliott, 1993) and laboratory incubation (Paul et al., 1999) are more sensitive. Laboratory incubation, especially when combined with C_3-C_4 plant switches, can give turnover estimates for a fast and slow pool after only a few years of management change.

We have used a combination of biological and chemical approaches to analytically determine the pool sizes and their turnover rates (Figure 1). Not all of the approaches in Figure 1 will necessarily be used on a particular sample. The routine determination of pool sizes and fluxes involves acid hydrolysis and incubation. The residue of acid hydrolysis is used to determine the size of the resistant pool (C_r). Carbon dating measures its mean residence time (MRT) (Leavitt et al., 1997). Acid hydrolysis dissolves the polysaccharides and most of the nitrogenous constituents and is known to leave behind the aromatic humics (Martel and Paul, 1974; Scharpenseel and Schiffman, 1977). It will not dissolve modern lignin residues nor does it effectively, as one would hope, separate the effects of treatment such as extended fallow that should result in the loss of all but the resistant fraction. The

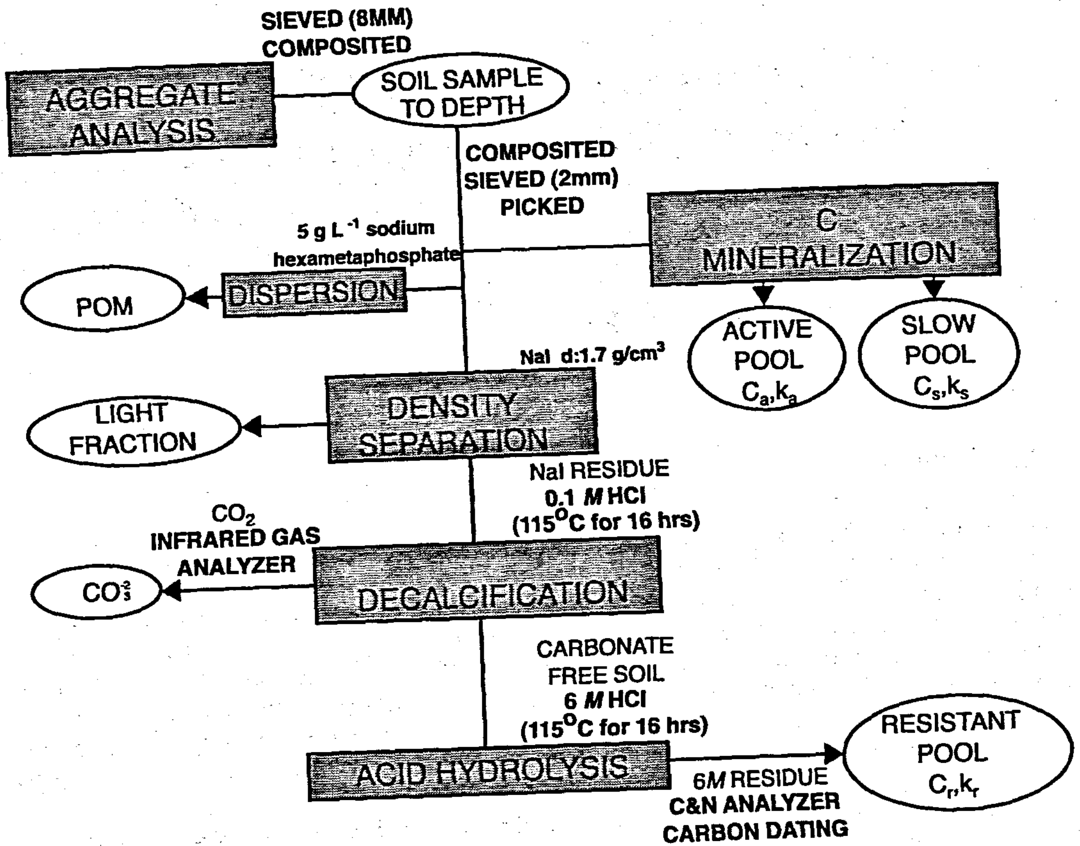

Figure 1. Methods utilized in analytical determination of pool sizes and fluxes.

residue of hydrolysis, however, ranges from 30 to 70% of the SOC and is on average 1400 years older than the total SOC (Leavitt et al., 1996; Paul et al., 1997). The proportion of non-hydrolyzable C is reproducible between samples and is dependent on soil type and parent materials (Collins et al., 1999). Carbon dating is expensive and not readily available. Our calculations show that an MRT of greater than approximately 500 years will not affect the calculations of the pool dynamics of the more bioavailable C_a and C_s pools. The size of the Cr pool however affects the size of the other two and must be known.

Extended incubation utilizes the degradative capacity of the soil biota's enzymes to release the active and slow SOC pools. The CO_2 evolution curves, based on a unit time basis, are used to calculate the dynamics of the active and slow pools. The other approaches shown in Figure 1 provide additional information to that which is determined by acid hydrolysis and incubation. Particulate organic matter and the light fraction are good indicators of turnover (Carter and Stewart, 1996). Soil carbonates can store large amounts of C and result in misinterpretation if not removed or measured separately.

There is a great deal of interest in obtaining some rapid chemical index of the bioavailable fraction. Results from hot water extraction, weak acid hydrolysis, etc., can be correlated to the results from incubation and can be useful in some interpretations (Baker et al., 1998). The results of aggregate

analysis are also useful in measuring the effect of physical protection that controls the dynamics of the C_a and C_s pools. These are described elsewhere (Carter and Stewart, 1996; Smucker et al., 1998).

The ^{13}C present in many agricultural soils, which have recently grown C_4 corn or sorghum on original C_3 forest sites or C_3 wheat on previously C_4 grasslands, provides a useful signal. It is not used in our SOC dynamics calculations directly and therefore can be utilized to validate modeling using the analytically derived data. The ^{13}C signal also provides data on the percent of the SOC derived from recent residues and the proportion of recent residues remaining (Follett et al., 1997). Measurement of the $^{13}CO_2$ produced during incubation can be used to measure the pool sizes and decomposition rate constants of the individual C_3 and C_4 SOC components (Collins et al., 2000).

II. Site Selection and Sampling

Agronomic samples often can be obtained from well-replicated, long-term plots with known histories of climate, management and plant residue inputs (Gregorich et al., 1995; Follett et al., 1997; Paul et al., 1997). We routinely utilize six, 5.4-cm diameter cores taken at 0 to 20, 20 to 50 and 50 to 100 cm depths or from the appropriate soil horizons from each replicate of each treatment. The effects of zero till are best measured by taking additional, near-surface soil samples (0 to 5 cm) and litter samples.

Soil type, history prior to afforestation and present forestry practices affect C storage in forests (Johnson, 1992). Present and former owners and Forest Service and Department of Natural Resources officers can provide management records and oral histories. This information together with topographic maps, county soil surveys, satellite imagery, etc. are utilized for locating afforested, native undisturbed sites and associated current agricultural sites with well-documented histories on equivalent soil type, slope, aspect and vegetation. Accurate measurements of SOC pools in forested sites or among landscapes are especially difficult to achieve. The factors that control SOC turnover rates on these sites are patterned at a number of scales that include differences in regional climate, soil type topography, and vegetation (Morris and Boerner, 1999). Soil characteristics at the base of individual trees have been found to differ from those found 2 m from the base of those trees (Zinke, 1962). The extent of the difference varies with tree species (Finzi et al., 1998 a, b). Measurements of soil C can be impacted by these differences. Soil C was significantly greater downslope than upslope of red oaks in four southern Ohio mixed oak forests (Morris and Boerner, 1999) and drip line estimates would have underestimated downslope organic C by 7% and overestimated upslope organic C by 20%.

High spatial variability, at even smaller scales, is associated with components of the C and N pools (Starr et al., 1992) and the microorganisms (Morris, 1999) that regulate turnover rates in both agricultural and natural systems. Research on 2 m x 1m plots on soils under a current wheat field and under poplar after 10 years regrowth on previous agricultural land revealed a 5- to 10-fold variation in C and N in both systems (Stoyan et al., 2000). Phosphorus had a 100-fold variation in the wheat and 30-fold in the poplar plot soils. High heterogeneity at the microscale can be minimized through compositing of multiple samples. Hauck et al. (1994) present a concise discussion of field variability and sample collection. Compositing samples does not eliminate heterogeneity at larger scales. Research on site-specific agriculture and the interpretation of processes at the niche level at which they actually occur in ecosystem analysis will require new sampling protocols.

III. Soil Analysis Methodologies

The determination of pool sizes and fluxes involves the partitioning of a composited soil sample by utilizing quartering techniques to ensure adequate subsampling. Soils to be used for C and N analysis and incubation are transported from the field and stored at 4°C. If aggregate analysis (Six et al., 1998; Six et al., 1999) is to be performed, collection and transportation must ensure minimum disturbance

and compaction. Samples are gently separated by hand, sieved through an 8-mm sieve and a subsample for aggregate analysis is dried. Following removal of the subsample for aggregate analysis, the soil is sieved through a 2-mm sieve. Since plant residues are modeled separately in our program, we remove identifiable plant fragments prior to analysis. The subsample to be used in determination of C mineralization rates by the long-term incubation method is then refrigerated. The remaining soil is air-dried.

Inorganic C is a significant component of arid soils and occurs at depth in many humid soils. It can be present in recently limed agricultural soils in sufficient quantities to affect C storage calculations. The turnover of pedogenic carbonates is sufficiently rapid to affect global change calculations. We eliminate inorganic C in agricultural soils for SOC analysis and carbon dating but measure its content in soils used for global change calculations. The soil is treated with 100 ml of 0.25 *M* HCl added to 20 g soil. Care must be taken to remove excess HCl by repeated evaporation. Washing with deionized H_2O after acid treatment can remove soluble C and should be avoided. Alternatively, 100 ml of 0.03*M* H_3PO_4, which does not break down in the C analyzer or mass spectrometers can be used (Follett et al., 1997). The weak phosphoric acid treatment is repeated until the solution remains within 0.2 pH units of the original acid solution. Others have added dilute HCl directly to soil already weighed into silver sampling cups without apparent damage to their instrumentation. The soil inorganic C is measured by difference or by analysis of the CO_2 evolved during acid treatment.

Dried soils, to be utilized for C and N analyses, are ground to pass a 180-µm screen in a roller mill that can handle multiple samples in glass storage containers containing steel bars (Harris and Paul, 1989). The quantity of soil used is corrected for moisture by drying a subsample to 105°C. Samples for C and N and mass spectrometry are weighed to six decimal places on a Cahn balance. Where a Cahn balance is not available, a five-place analytical balance will provide reasonable accuracy. Normal protocols for a Carlo Erba type CHN analyzer are followed (Sollins et al., 1999). Mass spectrometry to measure ^{13}C follows the procedures of Harris and Paul (1989). Appropriate working standards for both SOC and ^{13}C should be inserted in the sampling tray every 12 samples. Working standards for ^{13}C can be corn (−10.75‰) and beet (−25.68‰) sugars standardized against NIST 22 (−29.74‰) or AIEA-C-6, ANU sucrose at −10.43‰. Multiple analysis of the reference samples yields a standard deviation of 0.05‰.

A. Soil Physical and Chemical Analysis

Extensive information is required for interpretation of data gathered on the pool sizes and fluxes of SOC. This includes available P content, pH, cation exchange capacity, soil texture and moisture retention curves. These are conducted according to standardized methods (Page et al., 1982; Carter, 1993). Bulk density can be determined by a number of different methods, but none is without problems (Brady and Weil, 1999). We weigh a number of 5.4-cm-diameter cores obtained either with truck mounted hydraulics or by hand using a hammer driven sampling tube and removal with a portable tripod and associated jack. Alterations of horizon depth as a consequence of management are noted. Root and rock content determinations are made for each profile sampled so that the profile C inventory reflects non-soil particles.

Across-treatment differences in bulk density can impact C content differences among treatments. Corrections for differences in bulk density between sites or treatments involve calculations to equivalent weights to depth among sites (Gregorich et al., 1995). Bulk density differs with horizon, horizon depth differs with treatment and corrections for both must be made to compare equal weights of soil across treatments. A comparison of the effects of bulk density per horizon and horizon depth between a forest site and agricultural site demonstrates how these components differ among soils (Figure 2). Bulk density, weight of soil per horizon and horizon depth differ among all horizons meas-

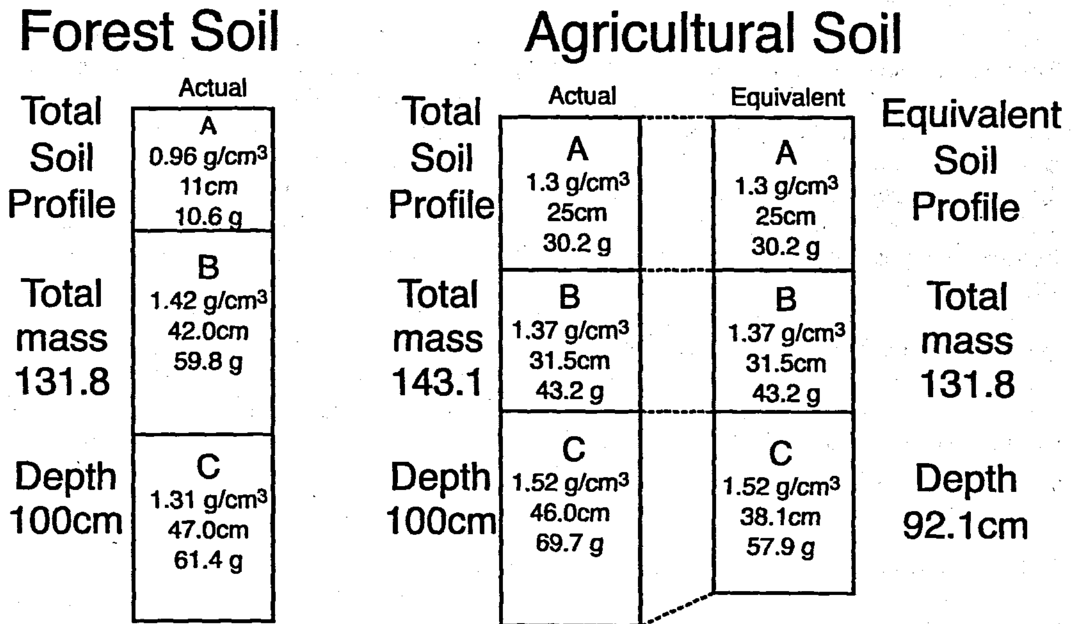

Figure 2. The correction of soil carbon pools on an equivalent weight basis.

ured. The forest soil has a shorter A horizon (11 cm), a lower bulk density (0.96 g cm^{-3}), and less mass of soil (10.6 g) than the A horizon of the agricultural soil (25 cm, 1.3 g cm^{-3}, 30.2 g). A 1 cm x 1cm x 100 cm profile of the forest soil weighs 131.8 g whereas that of the agricultural soil weighs 143.1 g. To compare C contents of these soils equivalent weights must be compared so the agricultural horizon must be shortened to 92.1 cm so that 131.8 g of each soil is compared.

B. Soil C Pools and Fluxes

1. Physical Fractionation

The light fraction (LF), if measured, is separated from the air-dried soil (usually only surface subsamples) by floating 10 g soil in 40 ml NaI solution (SG ~ 1.70 g cm^{-3}) in a 100-ml beaker (Janzen et al., 1992). We use NaI rather than molybdenum polytungstate to lower costs in our multisample analysis. The floating material is washed, finely ground (<180 μm) and analyzed for total C and $\delta^{13}C$. The residual soil is washed three times with 50 ml distilled water to remove NaI.

The measurement of particulate organic matter (POM) involves dispersion of 10 g soil in 30 ml of 5 g L^{-1} sodium hexametaphosphate by shaking for 15 h on a reciprocal shaker (Cambaradella and Elliot, 1992). The slurry is passed through a 53-μm sieve and rinsed thoroughly with water to remove silt and clay. The POM plus sand retained on the sieve is oven dried at 50°C, ground to pass a 180-μm screen and analyzed for C, ^{13}C and N (Harris and Paul, 1989). The POM in this analysis often contains most of the LF unless it is removed prior to analysis. We previously remove all identifiable plant residues.

2. Acid Hydrolysis

The resistant fraction is determined by refluxing 1 g soil in 6 *M* HCl at 115 °C for 16 h. This is most easily done using a temperature-controlled digestion block. The use of reflux condensers fitted with fritted glass joints makes it possible to retain the acid-water mixture. The tubes can be used without condensers if small, glass filter holders or large glass marbles are used to partially enclose the tops. The digestion liquid should condense no more than one half way to the top and the tubes should be shaken occasionally to wash down soil materials that accumulate at the point of condensation. Refluxed samples are washed three times with deionized water, dried at 55°C and ground to pass a 180-μm screen.

Soils to be utilized for C dating are very prone to contamination by the low levels of anthropogenic ^{14}C that are found in many laboratories. We take special precautions when sampling and sieve and dry our samples in an environment, such as a home garage, known not to have been exposed to tracer C and send it to a C dating laboratory (Leavitt et al., 1997) for further processing. The accelerator mass spectrometric analysis allows the use of milligram sized samples that must, however, be representative of the area sampled.

3. C Mineralization

Mineralizable soil C is measured during extended laboratory incubation at 25°C. Moist, sieved, surface samples (approximately 80 g soil) are placed in tared, 6-oz specimen containers and adjusted to optimum moisture content by adding calculated quantities of H_2O. The optimum moisture content for mineralization can be estimated by placing a known mass of soil into a funnel with a filter paper. The soil, filter paper, and funnel are then weighed together. The funnel, with soil, is then placed into a beaker of water and the water is allowed to move into the soil until the surface of the soil appears shiny. The funnel is then removed from the beaker of water, placed into an airtight container to eliminate evaporation and is allowed to drain. After 24 hours, the funnel containing the drained soil is weighed. Gravimetric water content is determined. If this method is used, the moisture content of the soil to be incubated is raised to 50 to 70% of this value depending on the soil texture. Sandy soils require the lower values so they do not lose their structure. We currently use a method in which 80 g soil, sieved to 2 mm and air dried, is placed into a 5-cm-diameter hollow glass cylinder capped with nylon mesh that is held in place by a rubber band. The cylinder is inverted in 2 mm of water until the water has moved 80% of the way up the soil column (water must not become limiting). The lower, saturated soil is then removed and gravimetric water content is determined on the soil above the saturated soil to just below the layer of dry soil. The samples to be incubated are then raised to this gravimetric water content: 70% of this water content is used when the soil used in the incubation is sandy, highly heterogeneous or comprised of more than one pedogenic horizon to avoid saturating the soil.

The specimen container, with soil, is placed into a quart-size, wide-mouth canning jar containing 20 ml of water to maintain humidity throughout the incubation. When the amount of soil available for testing is limited, smaller jars (i.e. wide-mouth pints or half-pints) may be used in place of the quart jars. The lids of the canning jars are boiled, fitted with septa, placed on the canning jar and sealed with rings. Controls with known CO_2 concentrations (1%) are prepared by injecting CO_2 from sources such as a calibration gas into empty containers. These detect leakage and serve as analytical controls. The samples should be flushed with CO_2 free air to establish that the initial CO_2 concentration is zero. Alternatively if the jar contains ambient CO_2 concentrations at time zero, a baseline CO_2 concentration may be determined with an infrared analyzer or gas chromatography immediately after sealing the jar. Samples are incubated at 25°C in the dark.

Incubations are best conducted with moist field soils as described above. Occasionally it is necessary to use dried, stored soil. Dried soils produce different initial CO_2 evolution curves because of initial recycling of dead biomass and recovery of the biota once water is added. We have found that dried soils produce essentially the same C_s and k_s values as soils utilized immediately in the moist condition (Collins et al., 2000). The decomposition rate constant k_a, however, is higher after air drying. This can be partly corrected for by preincubating dried samples for seven days after wetting.

During incubation, we allow CO_2 to accumulate to some extent between samplings to reduce errors due to improper flushing. Jars should be flushed after four to five samplings or when the CO_2 concentration is expected to exceed 6% before the next sampling period. Two air hoses are inserted into the septa (in from the air source and out to the lab) using small gauge needles. Samples may need to be flushed 20 to 30 min if the concentration of CO_2 is high (>2%) and should be allowed to return to atmospheric pressure following flushing. This is best achieved by placing a water filled syringe with the plunger removed on the out needle. The water in the syringe will bubble if the jar is properly sealed when the jar is being flushed and allow for the pressure to equilibrate with environmental pressure without laboratory CO_2 flowing into the jar when the air source is removed. If the water does not bubble or water flows into the jar rather than maintaining equal pressure with the interior of the jar when the flush air source is removed there is a leak present.

Rather than flushing, samples may be opened and allowed to equilibrate with laboratory air; this compromises seals on jars and laboratory air is very variable in its CO_2 content. Compressed air, from cylinders, may be used with a soda lime scrubber but there may be a 2 to 3°C chilling effect as the compressed air comes in contact with the soil. Decreases in respiration over short measurement increments may result. In-house compressed air may be used with a soda lime scrubber but an oil filter or trap is needed to keep oil from reaching the soil samples. Aquarium pumps may be used in conjunction with appropriate traps but several may need to be used in tandem to achieve pressures great enough to flush several samples at once. Finally, CO_2 concentration should be checked following sample flushing to establish that previous CO_2 has been cleared and to establish a new sample baseline. The new baseline should be established at least 24 h after flushing to allow for a new CO_2 equilibrium to be established within the jar.

The amount of CO_2 evolved is determined, every few days initially, by infrared gas analysis, gas chromatography or by using base traps such as NaOH. If a base trap is used high surface area traps should be replaced and titrated periodically. The time period between CO_2 determination is lengthened at appropriate periods during the incubation. Curve fitting to determine the pool sizes and decomposition rate constants of the active and slow pools requires a minimum of ten values in incubation periods that run from 200 to 800 days. The amount of evolved CO_2 is converted to a rate function by determining headspace CO_2 (usually in ppm or ml CO_2 min^{-1}), converting to moles then grams CO_2-C and dividing by soil weight adjusted for bulk density and gravimetric moisture content and time (see also Robertson et al., 1999).

Where mass spectrometers with gas handling capability are available for ^{13}C analysis, CO_2 samples can be directly injected (Barrie and Prosser, 1996). If gas-handling facilities are not available or if the samples need to be transported or stored, the CO_2 is trapped in excess NaOH; the amount can be calculated or determined ahead of time. We use 5ml of 2 *M* NaOH for 25 g of soil in the 160-ml jars. Blank jars contain NaOH but no soil. The evolved CO_2 is precipitated as $SrCO_3$ (Harris et al., 1997) using 4 *M* $SrCl_3$: residual NaOH is measured by back titration with 0.3 *M* HCl to pH 7 using an automatic titrator. The $SrCO_3$ is analyzed for ^{13}C content. The calculations involving ^{13}C are shown by Balesdent et al. (1988) or Boutton (1996). The control jars described above also supply useful information on accuracy and leaks.

4. Curve Analysis

The size and turnover rates of the active (C_a) and the slow (C_s) pool are determined by curve analysis of the CO_2 evolved per unit time. The three-pool first order model utilized is

$$C_{(t)} = C_a e^{-kat} + C_s e^{-kst} + C_r e^{-krt}$$

where $C_{(t)}$ is total carbon pool in soil at time t, C_a and k_a represent the active pool and C_s and k_s represent the slow pool. C_r has been previously estimated by acid hydrolysis. Carbon dating that measures the MRT is used to calculate k_r where $k_r = 1/MRT$ as in a first order reaction at steady state. For ease of interpretation, we prefer to show our decomposition kinetics as MRT rather than as the decomposition rate constant k. The laboratory-derived values are scaled to field, mean-annual temperatures (MAT) by assuming a Q_{10} of 2, ($2^{(25-MAT)/10)}$). Where carbon dates are unavailable, MRT is assumed to be 1000 years. Studies of grassland and forest sites (Leavitt et al., 1996; Paul et al., 1997) have shown the non-hydrolyzable, resistant pool to be at least this old in the majority of samples analyzed. The size of the slow pool is defined as $C_s = C_{soc} - C_a - C_r$ with C_{soc} representing the soil C at time 0 (the time of sampling).

The statistical evaluation of the CO_2 evolution data to determine pool sizes and rates using nonlinear regression is relatively straightforward. The parameters C_a, k_a and k_s are calculated using nonlinear regression (NonLIN; Systat, Inc, Evanston IL or PROC NLIN; SAS 1995). Differences in pool sizes or kinetics among several treatments can be determined using a t-test on the asymptotic SE estimates, a sum of squares reduction test or a repeated measures ANOVA (Willson et al., 2000). We have included an example from a Corn Belt soil to highlight each step in the overall analysis from the mineralization data (Table 1). The rate of CO_2 evolution per day (Figure 3) was utilized in the regression analysis to evaluate C_a, k_a, and C_s for each of the depths sampled. We had 18 sampling dates over 500 days for the top 20 cm, 8 sampling dates over 8 dates over 260 days for the 25 to 50 cm depth and 8 sampling dates over 212 days for the 50 to 100 cm depth. SAS PROC NLIN METHOD=MARQUARDT (SAS, 1995) was used in the analysis because it was more robust with our data set than some of the other methods, such as DUD and GAUSSIAN. The choice of specific method is dependent on the data set.

The most important consideration when evaluating pool sizes and rates in this manner is the type of model to fit. Both two- and three-pool models either unconstrained or constrained are available. Our basic model, if carbon dates are available, consists of three pools as follows:

$$dC/dt = C_a * k_a e^{(-ka*days)} + (C_{SOC} - C_r - C_a) * k_s e^{(-ks*days)} + C_r * k_r e^{(-kr*days)}$$

This model utilizes sample SOC, the results of the acid hydrolysis and the age of C_r. Radiocarbon dating of the residue of the acid hydrolysis determines the MRT of C_r. Carbon dating is relatively expensive and therefore C dates are often unavailable. The MRT of C_r is usually so long that the turnover of this pool does not affect the overall calculations of k_a and k_s. This is demonstrated in Table 1 where the measured field MRT was 530 years for the surface soil, 895 years for the 25 to 50 cm depth and 4406 years for the 50 to 100 cm depth. The values for the two more rapid pools are determined originally in laboratory incubation. Use of the Q_{10} equation to establish the equivalent MRT's for incubation under laboratory temperatures results in values of 175, 295 and 1453 years for the three depths. We tested the three pool model by using both the actual measured C dates and by using assumed values of 1000 years as the MRT for the nonhydrolyzable C of these soils (Table 1). The use of 530 years rather than the assumed value of 1000yr resulted in an increase in the MRT of C_s by 1.3 years. The parameters for the dynamics of the fast and slow pools of the lower depths were the same when using either assumed or actual values for the MRT of C_r. These results indicate that

Table 1. Calculation of pool size and rate of turnover for active (C_a, k_a) and slow pools (C_s, k_s) to a depth of 100 cm in an agricultural field at the Kellogg Biological Station (KBS), MI using a three-pool constrained model[a]

Depth (cm)	C_{SOC} mg kg^{-1}	C_r Mg kg^{-1}	k_r day^{-1}	MRT yr laboratory	MRT yr field	C_a mg kg^{-1}	k_a day^{-1}	MRT day laboratory	MRT day field	C_s mg kg^{-1}	k_s day^{-1}	MRT yr laboratory	MRT yr field[b]
Using Measured Values for k_r													
0-20	10700	4800	1.6E-05	175	530	570	5.01E-02	20	60	5330	2.26E-04	12	37
25-50	2600	800	9.3E-06	295	895	132	5.65E-02	18	54	1670	4.55E-04	6	18
50-100	1300	400	6.2E-07	1453	4406	84	3.27E-01	3	9	816	1.24E-03	2	7
Using Assumed Values for k_r													
0-20	10700	4800	8.3E-06	330	1000	569	5.01E-02	20	60	5332	2.34E-04	12	36
25-50	2600	800	8.3E-06	330	1000	132	5.65E-02	18	54	1670	4.55E-04	6	18
50-100	1300	400	8.3E-06	330	1000	84	3.26E-01	3	9	816	1.24E-03	2	7

[a] $C_{(t)} = C_a{*}k_a e^{(-ka{*}days)} + (C_{soc} - C_r - C_a){*}k_s e^{(-ks{*}days)} + C_r{*}k_r e^{(-kr{*}days)}$, $C_{(t)}$=rate of C evolution per unit time ($d(CO_2)\,dt^{-1}$), C_{soc}= SOC measured at time 0, C_r = resistant C (non-hydrolyzable C), k_r = 1/ MRT when using carbon dating or 1/1000 year when using an assumed MRT. C_a = active C, C_s = slow C ($C_{soc} - C_r - C_a$).

[b] Mean annual temperature for KBS is 9.0°C, incubation temperature is 25°C and the Q_{10} correction is $2^{(25-9)/10)}$.

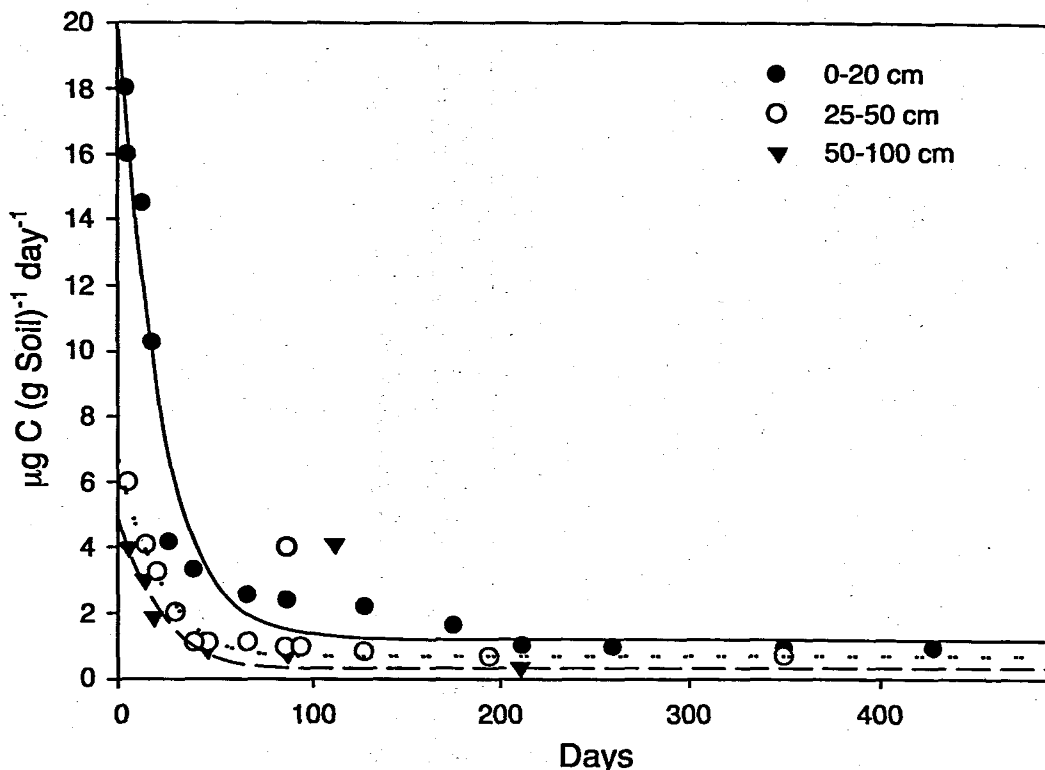

Figure 3. Evolution of CO_2 from three depths of a Michigan field soil expressed on a per unit time basis. Data presented are from Collins et al. (2000).

the three-pool constrained model is robust using either measured or assumed MRT for the C_r pool as long as the size of the C_r pool is known.

An alternative to running the three-pool constrained model is the two-pool constrained model:

$$dC/dt = C_a * k_a e^{(-k_a} \quad + (C_{soc} - C_r - C_a) * k_s e^{(-k_s * days)}$$

In this model the size of the C_r pool is used in determining the size of the Cs pool but is not modeled separately. Results from this model differ little from the three-pool model, again suggesting that the size of the C_r pool is important but the age of this pool adds little to the analysis.

A two-pool unconstrained model has also been tested to determine pool sizes and turnover rates. In this model only CO_2 evolution is known and all pools and rates are modeled. The main differences between the results produced by the constrained and unconstrained models are in the size and MRT of the slow pool. In our Corn Belt soil, the unconstrained model underestimated the size of C_s up to 75% and decreased the MRT by 29 years, whereas C_a and k_a were equivalent to the results of the constrained model. Examples of the commands used to determine C_a, k_a, and k_s with the two- and three-pool constrained models, using PROC NLIN (SAS, 1995), are located in Table 2.

The turnover rates produced by the regression analysis can be extrapolated to field conditions by the use of Q_{10} corrections. These adequately describe the temperature dependence of decomposition for soil temperatures of 5 to 35°C (Katterer et al., 1998). Soil moisture has strong effects in arid en-

Table 2. Commands[a] for determining the size and turnover rate of the active (C_a, k_a) soil C pool and turnover rate of the slow (k_s) pool[b] with two- and three-pool constrained models using SAS PROC NLIN (SAS, 1995) for a single soil depth or horizon at a single site

Model	SAS commands

Two pool constrained model	Data;
	Infile 'c:\data.txt;
	Input csoc cr co2 day;
	run;
	proc nlin method=marquardt;
	parameters ka=0.001 ks=0.003 ca=100 to 1000 by 100;
	model co2 = ca*ka*exp(-ka*day) + (csoc-cr-ca)*ks*
	exp(-ks*day);
	der.ca = ka*exp(-ka*day)-ks*exp(-ks*day);
	der.ka=ca*exp(-ka*day)-ca*exp(-ka*day)*ka*day;
	der.ks=(csoc-cr-ca)*exp(-ks*day)-(csoc-cr-ca)*
	exp(-ks*day)*ks*day;
	run;

Three pool constrained model	data;
	infile 'c:\data.txt';
	input csoc cr kr co2 day;
	run;
	proc nlin method=marquardt;
	parameters ka=0.01 ks=0.001 ca=100 to 1000 by 100;
	model co2 = ca*ka*exp(-ka*day) + (csoc-cr-ca)*ks*exp(-ks*day) +
	(cr)*kr*exp(-kr*day);
	der.ca = ka*exp(-ka*day)-ks*exp(-ks*day);
	der.ka=ca*exp(-ka*day)-ca*exp(-ka*day)*ka*day;
	der.ks=(csoc-cr-ca)*exp(-ks*day)-(csoc-cr-ca)*exp(-ks*day)*ks*day;
	run;

[a]Data necessary for this analysis include total soil C (Csoc) determined by combustion, resistant C (Cr) determined with acid hydrolysis, CO_2 evolution rate (CO_2) determined using long-term incubations, day of incubation on which the rate was determined (day) and age of the resistant pool (kr) determined with carbon dating corrected for laboratory incubation temperatures. Derivatives are indicated by der and must be included for the Marquart model used here.
[b]The size of the slow (Cs) pool is constrained to be Csoc-Cr-Ca.

vironments but has been shown to have limited effects on field CO_2 decomposition rates in the Corn Belt and associated forests (Buyanovsky and Wagner, 1998; Paul et al., 1998). Moisture effects are not incorporated into our direct determinations of SOC dynamics of Corn Belt and forest soils. They are used in our general modeling of SOC dynamics (Paustian et al., 1992).

The analytically derived values for the pools sizes and fluxes make it possible to compare management and ecosystem processes on specific soil types. The pool sizes and fluxes vary enough on specific soils that measurements on representative soils need to be made for modeling purposes (Collins et al., 1999, 2000). Our analysis does not include the effects of pool to pool transfers or of the recycling of C during microbial growth. This, as well as calculations on a landscape basis, are best done by using one of the available models. These should be parameterized by the use of analytically derived data such as that described in this chapter.

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