

# Mineralization rate of $^{14}\text{C}$ -labelled dissolved organic matter from leaf litter in soils of a weathering chronosequence

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## Abstract

During the processes of primary succession and soil development, large stocks of organic C with very long residence times accumulate in many soils. Soluble organic C adsorbed by soils may contribute to the stock of organic C accumulating during soil development. We determined whether the mineralization rate of water-soluble organic C and the insoluble residue from  $^{14}\text{C}$ -labelled leaf litter added to soils from a weathering chronosequence decrease as soil age and adsorption capacity increase. The soils were formed on mudflows of andesitic material deposited about 75, 255, 616 y ago, and another older but undetermined time before this study. The percentage of the DOC adsorbed by the soils increased with age. After 1 year of incubation there were no significant differences in the mineralization rates of DOC added to soils of different ages. The DOC appeared to be comprised of two fractions, one that comprises about 32% of the total that mineralized with a half decay time of 0.02 y (7 d) and a second fraction comprising 68% with a half decay time of about 1.6 y. Consequently, the slowly mineralized fraction of the soluble C contributed to the accumulation of slowly mineralized C in the soil. Both the slowly and rapidly mineralized fractions of the insoluble residue decomposed more slowly than the corresponding fractions in DOC. We found no support for the idea that increased adsorption capacity due to weathering resulted in protection of soluble organic C from microbial mineralization. © 2004 Elsevier Ltd. All rights reserved.

**Keywords:** Decomposition; DOC; Organic matter; Respiration; Microbial; Carbon cycle; Carbon sequestration; Weathering

## 1. Introduction

Many catastrophic disturbances such as volcanism, debris flows, aeolian deposition, fluvial deposition, or tectonic processes initiate primary succession and soil development. Soil development often involves such processes as transformation into finer grained materials, formation of short-range order oxyhydroxides and aluminosilicates, and later, formation of crystalline secondary mineral clays and, in some soils, podzolization (Jenny, 1980). These processes of soil development influence successional changes in vegetation through nutrient and soil water retention. Likewise, the processes of primary succession of vegetation and organic matter production affect soil development through processes such as eluviation

of metals, organic matter accumulation, and organic acid-induced weathering. This interaction between soil development and vegetation is a critical process affecting ecosystem development (Jenny, 1980). Processes of soil weathering that lead to greater adsorption of organic matter might conceivably lead to stabilization of organic matter produced in the ecosystem.

The compounds in dead plant material that form soil organic matter decompose with half times of decay varying from days to a few years. The decomposition rate of lignin, one of the slowest decomposing substances present in the original plant material, has been reported to be  $0.18 \text{ y}^{-1}$  (corresponding to a half time of decay or  $t_{1/2}$  of 3.8 y) in a temperate soil (Lynch, 1991). However,  $^{14}\text{C}$  dating shows the half times of decay of soil organic matter are on the order of hundreds or thousands of years, allowing the accumulation of large amounts of soil C (Paul et al., 1997; Torn et al., 1997). Changes in the accumulation of C in soil are critical in regulating the concentration of  $\text{CO}_2$  in

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the atmosphere, since about twice as much C is stored in soil as is in the atmosphere (Lal et al., 1995).

What causes this sequestration of soil organic C into fractions that are so slow to decompose is not well understood. Three main hypothetical mechanisms are: (1) that humic substances that are formed during decomposition have chemical structures that are inherently difficult for microbes to decompose, (2) that organo-mineral complexes are formed that protect organic matter from enzymatic attack, or (3) that soil organic matter can be shielded from microbial attack in soil pores that are too small for bacteria. These hypotheses are likely not mutually exclusive. For example, adsorption of humic substances to iron oxyhydroxides and subsequent accretion of layers of the complexes might represent a combination of chemical and physical protection from enzymes. The influence of mechanisms 2 and 3 should increase during soil weathering because clays are formed with sub-micrometer pores, and iron and aluminum oxyhydroxides or amorphous aluminosilicates are formed that adsorb organic matter and may protect it from microbial enzymes both physically and chemically (Sollins et al., 1983; Torn et al., 1997).

One way that organic matter can penetrate to lower soil depths, efficiently coat mineral adsorption surfaces, and penetrate sub-micrometer pores is in the form of dissolved organic matter that percolates through the soil. There are other ways that organic matter can be deposited at lower soil depths, such as rhizodeposition, transport through macropores, and bioturbation (including anthropogenic disturbance), but our focus is on dissolved organic matter. Soil materials particularly important in adsorption of natural DOC include Fe, Al oxyhydroxides, short-range-order aluminosilicates (such as allophane), and certain 2:1 layer clays (Greenland, 1971). The most important mechanisms involved in the adsorption of natural DOC include ligand exchange, electrostatic attraction to anion exchange sites, and hydrogen bonding (Parfitt et al., 1977; Qualls, 2000). About 20–30% of senesced leaf litter is soluble in water, and it also contains humic substances that are resistant to microbial degradation even in solution (Qualls et al., 1991; Qualls and Haines, 1992b). We hypothesized that this DOC was more susceptible to sequestration by physical protection in older, more weathered soils because it can be adsorbed onto metal oxyhydroxides or short-range-order aluminosilicates. We also hypothesized that a large fraction (e.g. the humic substance fraction) of the soluble organic C was slow to mineralize.

Our objectives were to evaluate the following hypotheses:

- (1) The mineralization rate of water-soluble organic C from leaf litter added to soil decreases in soil as soil age and adsorption capacity increases;
- (2) The mineralization rate of the insoluble residue does not differ as soil age and adsorption capacity increases;

- (3) The water-soluble fraction of leaf litter consists of two relatively discrete fractions: a very rapidly and a very slowly mineralized fraction;
- (4) The slowly mineralized fractions of both the water-soluble fraction of leaf litter and the insoluble residue both have half decay times on the order of years.

## 2. Methods

### 2.1. The soil weathering chronosequence

The Mt. Shasta mudflow chronosequence is one that has served as a classic example of a soil weathering chronosequence (Dickson and Crocker, 1953a,b; Jenny, 1980; Sollins et al., 1983). During exceptionally warm periods, melting of a glacier on Mt. Shasta has led to catastrophic debris flows of andesitic volcanic material that are then deposited on the toe slopes of Mt. Shasta. These debris flows have not been pyroclastic flows or heated water flows induced by eruption, but have simply been the result of glacial melting. The most recent mudflow, the 'A' flow, occurred in 1925–1926. Another mudflow, the 'B' flow, was dated at about 255 years (age in 2001) by  $^{14}\text{C}$  dating and dendrochronology of a tree buried in the mudflow. The 'D' flow was similarly dated at about 616 y before present by the dendrochronology of the oldest trees. The date of the 'E' flow is uncertain, but is believed by Dickson and Crocker (1953a) and Glauser (1967) to be older than the D flow because it lies below the D flow in an area of overlap. We will arbitrarily refer to the age as  $\sim 1200$  y after Dickson and Crocker (1953a). Jenny (1980) used these mudflows as an example of a chronosequence because the mudflows all originated from the same parent material, and after it was displaced to a lower elevation, it was subjected to the same precipitation and temperature regime. The material was then subjected to much greater leaching by water and higher temperatures, both factors which are important in weathering of volcanic material (Wada and Aomine, 1973).

Properties of the soil were summarized by Dickson and Crocker (1953b) and Lilienfein et al. (2003). The 'A' flow is sandy in texture and has not developed an A horizon. The 'B' flow is also sandy in texture, but has developed an A horizon. The 'D' and 'E' flows have much higher concentrations and greater depths of organic matter in the A horizons. At a depth of 0–10 cm, organic carbon concentrations were 4.4, 19, 49, and 45 g kg<sup>-1</sup> in the A, B, D, and E flow soils, respectively from (Lilienfein et al., 2003). The corresponding N concentrations were 0.20, 0.85, 1.84, and 2.00 g kg<sup>-1</sup>, respectively. The corresponding effective cation exchange capacities were low: 14, 65, 39, and 70 mmol<sub>c</sub> kg<sup>-1</sup>, respectively. The specific surface areas were 14, 17, 26, and 25 m<sup>2</sup> g<sup>-1</sup>, in the A, B, D, and E flow soils, respectively. The greater degree of weathering of the older soils was indicated by much greater concentrations of

allophane and oxalate extractable Fe (Lillienfein et al., 2003). Adsorption of DOC derived from the forest floor was greater in the 0–10 cm depth (and other depths) of the two older soils than in the younger soils (Lillienfein et al., 2004). Adsorption isotherm curves are shown in the Lillienfein et al. (2004). These were derived from a conventional adsorption isotherm experiment using a solution to soil ratio of 10:1. X-ray diffraction and differential thermal analyses of the clay fractions indicated small quantities of montmorillonite but no kaolinite (Dickson and Crocker, 1954).

The most recent soils ('A' and 'B' flows) are Typic Haploxerepts (Tephric Regosols in the FAO system), and the older soils ('D' and 'E' flows) are Humic Vitrixerands (Umbric Andosols in the FAO system). Maps of the site are included in Dickson and Crocker (1953a). The A flow is dominated by the pioneer species *Pinus ponderosa* (Doug. Ex Laws), while the other mudflows are dominated by the later successional species *Calocedrus decurrens* (Torr.), *Abies concolor* Gord. and Glend. (Lindl. Ex Hildeb.), *Pseudotsuga menzesei*, and *Quercus kelloggii* Newb.

## 2.2. Preparation of soil cores

Six intact soil cores were taken from the upper 15 cm of the mineral soil, excluding the O horizon, with 3.2 cm inside-diameter plastic tubes in plots located in the A, B, D, and E mudflows. We used one additional core for the B mudflow because we suspected there was more variability in the organic matter of the A horizon, and in fact the standard deviation of the observed data was greatest in the B mudflow cores so use of an extra replicate aided in producing more comparable standard errors across the treatments. In addition, 12 plastic tubes were filled with a mixture of combusted, acid-washed, and neutralized sand containing 10% micron-size silica dust. The micron-size silica dust was added to better mimic the soil texture and to increase water-holding capacity. In the laboratory, we determined the mass of the cores when all soil cores were adjusted to  $-20$  kPa soil matric water potential so that the microflora in all soils would be subjected to the same matric water potential. This soil matric water potential corresponded to a volumetric water content of  $17.9 \pm 2.2$ ,  $16.0 \pm 0.6$ ,  $19.1 \pm 3.8$ , and  $18.9 \pm 1.3\%$  in the A, B, D, E flow cores, respectively. The soil in the cores was left inside the plastic tubes, brought to near saturation, and immediately placed on a bed of diatomaceous earth on a membrane filter apparatus with regulated vacuum of  $-20$  kPa until the mass equilibrated (Qualls and Haines, 1992a). After weighing the cores to determine the 'target mass,' we allowed them to dry from the top until at least 5 ml of water had been lost so that the additions of DOC would not make the soil wetter than the target of  $-20$  kPa. After this drying, the volumetric water content of the soils was  $15.2 \pm 2.1$ ,  $13.3 \pm 0.4$ ,  $16.5 \pm 3.4$ , and  $15.9 \pm 1.0\%$  in the A, B, D, E flow cores, respectively.

Our rationale for using the 0–15 cm depth increment in this study was: (1) the greatest decrease per depth increment in soil solution DOC collected in the field occurred between the forest floor and lysimeters located at 10–20 cm depth (Lillienfein et al., 2004), (2) the adsorption of DOC was greater in the 0–10 and 10–20 cm depth of the older soils despite higher organic C content (Table 1; Lillienfein et al., 2004), (3) and the 0–15 cm depth is the first mineral soil horizon in which the very easily decomposed portion of the DOC is exposed to soil microbial decomposition.

## 2.3. Preparation of $^{14}\text{C}$ -labelled soluble organic matter

Seedlings of *Populus fremontii*, propagated from stem cuttings, were grown for one and a half growing seasons in a sealed growth chamber regulated at 370 ppm  $\text{CO}_2$  and labelled by injections of  $\text{NaH}^{14}\text{CO}_3$  into acid twice a week. Although *Populus fremontii* was a minor component of the community, the very rapid growth rate of this species increased the likelihood of uniform labeling after the second growing season. During July of the second season of  $^{14}\text{C}$  labeling, the seedlings were induced to senesce early by Ethophon, which decomposes to form ethylene, the hormone that induces senescence. After the leaves turned golden brown and were abscised, leaves were air dried only to the point that they could be ground. Subsamples were ground in a coffee grinder and then a ball mill, and 3.5 g of ground leaf material was extracted 5 times in 70 ml deionized water at  $2^\circ\text{C}$  by shaking for 0.5 h. The suspensions were then centrifuged, and the supernatants were poured off, filtered through a  $0.45\text{-}\mu\text{m}$  filter, and frozen at  $-80^\circ\text{C}$  within 2 min, and more water was added to the pelleted solids. Then the solid residue was extracted 4 times at  $46^\circ\text{C}$  for 4 h. The rationale for this extraction was to minimize the decomposition of the organic matter during extraction, while also assuring that the less soluble components were also extracted completely. We reasoned that the most easily decomposed sugars and other hydrophilic neutral substances could be extracted in cold water with minimal decomposition, while the remaining less soluble or less easily desorbed (Qualls, 2000) humic substances could then be extracted at a temperature high enough to inhibit microbial growth. The insoluble residue was air dried and homogenized.

In order to characterize the leaf litter extract, a sample was diluted to  $40\text{ mg L}^{-1}$  DOC and subjected to a fractionation procedure outlined in Qualls and Haines (1991).

## 2.4. Addition to soil and sand cores

Four types of treatments were included in the experiment: (1) water extract (DOC) addition to soil cores, (2) nutrient amended water extract addition to sand cores, (3) insoluble residue addition to soil cores, and (4) insoluble residue addition to sand cores. The additions to the sand

cores, which had no non-labelled organic matter, were used to measure the mineralization rate of both radioactivity and added organic C so that we could measure the specific activity of the mineralized substrate over time and correct for any non-uniform  $^{14}\text{C}$  labeling. The nutrient amendments to the sand cores were necessary to remove nutrient limitation, since the sand could supply no nutrients.

The water extracts were thawed and combined, and 5 ml were added to the top of 3 cores of each age of soil. An additional replicate of the B flow soil was also included for a total of 4 cores for the B flow soil. To 9 sand cores, 7 ml of the extract were mixed with a nutrient solution and a microbial inoculum. The nutrient stock was mixed similar to Sanford and Smith (1972), then added at a concentration such that the C/N ratio of the amended extract was 8:1, similar to microbial biomass. The rationale for adding the microbial inoculum was to reduce the effect of any lag phase caused by the lack of a microbial community in the acid washed sand. The microbial inoculum was prepared as in Qualls and Haines (1992b). One hundred and twenty milligrams of the insoluble residue was placed on the top of each of three sand cores and three additional soil cores from each of the four soils. The nutrient amendment and the microbial inoculum were added to the sand cores containing insoluble residue. The insoluble residue was mixed with the upper 2 cm of soil or sand to distribute it in a manner more resembling that of the DOC and to prevent it from drying on the top of the core. The soil cores were placed in Mason jars with two vials filled with 5 ml each of 1 M NaOH. In order to assure that a measurable quantity of  $\text{CO}_2$  was captured in NaOH from the sand cores, we placed three sand cores in each of three Mason jars. Two vials were used to increase the surface area of the NaOH. One sand core with insoluble residue was placed in each of 3 individual Mason jars, since more C was added in each insoluble residue core. A sand core with no added C was added to one Mason jar with 2 vials of NaOH to serve as a blank for scintillation counting and inorganic C analysis. Two wet glass-fiber filters were placed on the sides of all the jars to minimize drying of the soil.

The Mason jars were periodically opened and the NaOH from the 2 vials was removed and combined. The core was weighed and water was added to match the target mass. The jar was fanned to replace oxygen, the glass-fiber filters were moistened, and then fresh NaOH was added. The NaOH was sampled at 1, 2, 3, 5, 6, 8, 10, 12, 14, 16, and 20 days and then at increasing intervals up to 1 year. Analyses of inorganic C respired indicated that depletion of  $\text{O}_2$  in the jars was not significant. The NaOH was diluted in  $\text{CO}_2$ -free water, sealed in an autosampler tube, and analyzed for inorganic C using a Shimadzu 5050 TOC analyzer in inorganic C mode. One millilitre of the NaOH was added to 10 ml of Ecolite scintillation fluid (ICN Corp.) and counted in a Beckman LS60001C liquid scintillation counter (Beckman Corp.) for 10 min. Only disintegrations with energy from 18 to 160 keV were counted to exclude chemiluminescence,

which is an interference with counting  $^{14}\text{C}$  in aqueous alkaline solutions. Beckman Corp. determined counting efficiency using this energy distribution. The disintegrations per minute (DPM) or the inorganic C concentrations of the sand blank were subtracted from all measurements.

Subsamples of the initial water extracts were counted to determine the total radioactivity added to each core ( $7.78 \times 10^4$  DPM in 5 ml extract). In order to determine the initial radioactivity in the insoluble residue added to each core ( $7.17 \times 10^5$  DPM), three 20-mg samples of the insoluble residue were placed in a tin capsule, dropped into the furnace of the Shimadzu 5050 TOC analyzer (Shimadzu Corp., Columbia, MD) at  $670^\circ\text{C}$ , and the effluent gas was trapped in 3 tubes connected in series containing 1 M NaOH. The NaOH solutions were then counted in the liquid scintillation counter. Subsamples of the initial water extract were also analyzed for dissolved organic C using the Shimadzu TOC analyzer, and the added extract was  $976 \text{ mg C L}^{-1}$ . Although, this concentration was higher than the highest concentration in water draining from the forest floor in the same soil chronosequence ( $286 \text{ mg L}^{-1}$ , Lillienfein et al., 2004), it represented a relatively small flux of  $5.4 \text{ g C m}^{-2}$  compared to a flux of  $8.0 \text{ g C m}^{-2}$  leached during one storm alone in autumn (J. Lillienfein, R.G. Qualls, S.D. Bridgman, and S.M. Uelman, unpublished data).

At the conclusion of the experiment, soil in the cores was sliced into depth increments, fresh, moist subsamples were removed, and the C and  $^{14}\text{C}$  in microbial biomass was determined using the chloroform fumigation-direct extraction technique (Vance et al., 1987).

After the experiment was completed, we collected 3 cores from the youngest and 3 from the oldest soils and adjusted water contents using the same method as for the cores used in the experiments. In order to determine the potential distribution of the 5 ml of labelled solution within the soil cores in the absence of adsorption, we added 5 ml of 1 mM NaBr to the cores. We then waited 24 h, divided the cores into upper and lower halves, and extracted the  $\text{Br}^-$  tracer in water. Of the  $\text{Br}^-$  tracer extracted from the youngest soil cores,  $38 \pm 4\%$  was in the lower half of the core, and from the oldest soil,  $34 \pm 7\%$  was in the lower half. Consequently, it appeared that the added solution was distributed through the core by the matric potential gradient.

### 2.5. Estimation of C mineralization rate and correction for non-uniform labeling

In the soil cores, most of the  $\text{CO}_2$  captured in the NaOH originated from the native soil organic matter. Consequently, as is common in  $^{14}\text{C}$  tracer studies, we inferred the mineralization of the added C substrates from radioactivity. However, we used a method to correct for non-uniform radiolabelling of the substrates. In the sand cores, all  $\text{CO}_2$  collected (minus the blank) could be attributed to mineralization of the added C substrates. We plotted the following

function

$$Y = f(X) \quad (1)$$

where  $Y$  = the cumulative  $\text{CO}_2$  collected as a % of  $C$  added (based on inorganic  $C$  analyses of the  $\text{NaOH}$  traps) and  $X$  = cumulative radioactivity collected as a % of  $^{14}\text{C}$  added.

A linear relationship for Eq. (1) with a zero intercept would indicate uniform  $^{14}\text{C}$  specific activity. First, we fit the data from the sand cores to a function that best fit the data as described in Eq. (1). Then, we took the data for the cumulative  $^{14}\text{C}$  radioactivity mineralized from the  $^{14}\text{C}$  DOC additions to soil, and substituted them as  $X$  in Eq. (1) and solved for  $Y$ , the estimated substrate  $C$  mineralized. This was done for each sampling date for the  $^{14}\text{C}$  DOC additions. The implicit assumption was that the  $n$ th percentile of the substrate mineralized from the sand cores had the same specific activity as the  $n$ th percentile of the substrate mineralized from the soil cores. This inference does not presume that the substrate is mineralized at the same rate or even with the same curve shape in the sand and soil cores.

Because a plot of Eq. (1) for the  $^{14}\text{C}$  insoluble residue added to sand was approximately linear, we inferred that the estimated substrate  $C$  mineralized from the insoluble residue followed the same trend as the radioactivity and that the substrate was approximately uniformly labelled.

## 2.6. % DOC adsorbed in soils

At the end of the experiment, we determined the percentage of  $^{14}\text{C}$  in the initially added DOC that was adsorbed. We dried the soils to eliminate most water that would dilute our added DOC. Then we added a mixture of water and aliquots of the original DOC that had been preserved at  $-20^\circ\text{C}$  to soil samples repacked to the same bulk density in cores in amounts equivalent to the antecedent water content and the amount of DOC added to each gram of soil in the original soil cores. We placed vials of  $\text{NaOH}$  in a bottle with the soil to measure the amount of  $^{14}\text{C}$  mineralized during the adsorption equilibration period. We allowed the soil to equilibrate for 1 h, and then placed the soil in a pressure filtration apparatus (an Ultrafiltration Pressure cell, Millipore Corp., Bedford, MA) fitted with a  $0.45\text{-}\mu\text{m}$  cellulose acetate filter. A set of 6 experiments were also conducted with an incubation time of 8 h to compare the effect of allowing more mineralization during the incubation. An air pressure of 207 kPa was applied so that the interstitial soil solution was expelled. Radioactivity in this soil solution and in the  $\text{NaOH}$  was measured. A control to which only deionized water was added was also treated in the same way as a control. The % of the  $^{14}\text{C}$  radioactivity adsorbed was calculated as

$$[(T - M) - (S - C)] / (T - M) \quad (2)$$

where  $T$  = total radioactivity added to soil,  $S$  = total amount of radioactivity in soil solution,  $M$  = total radioactivity

mineralized, and  $C$  = total amount of radioactivity in control soil solution. The radioactivity in soil solution was calculated so that it included not only the radioactivity in the water expelled from the core, but also that remaining in solution by the following equation

$$S = (RW_{\text{expelled}}) + (RW_{\text{residual}}) \quad (3)$$

where  $R$  is the radioactivity per millilitre water,  $W_{\text{expelled}}$  is the millilitre of water expelled from the core, and  $W_{\text{residual}}$  is the millilitre of water remaining in the core at 207 kPa, determined by weighing the soil in the core before and after drying at  $105^\circ\text{C}$ . Note that this assumes the concentration of radioactivity was the same in the expelled and residual soil solution. Most of the water in the soil was expelled by 207 kPa pressure: 77, 68, 66, and 62% in cores of the A, B, D, and E flow soil cores, respectively, corresponding to volumetric water contents of 4.1, 5.2, 6.4, and 7.1%, respectively.

Note that in Eq. (2), calculation of adsorption is based only on the DOC that is not mineralized during the equilibration period. It could well be that the mineralized DOC was in fact adsorbed first, and that the calculation overestimates the initial adsorption. However, this rapidly mineralized fraction averaged only about 0.6% of the  $^{14}\text{C}$  added over the 1 h period.

## 2.7. Statistical analysis

The cumulative  $^{14}\text{C}$  radioactivity captured in the  $\text{NaOH}$  in each jar was expressed as a percent of the total radioactivity added to the core initially, then plotted as a function of time. The means of the cumulative  $^{14}\text{C}$  or cumulative substrate  $C$  mineralized at 1 year were tested for significant differences by the following ANOVAs: (a) comparing the four soils with DOC added (Hypothesis 1), and (b) comparing the four soils with insoluble residue added (Hypothesis 2). The mineralization of the DOC vs. the insoluble residue was compared using a 2 way ANOVA in which cumulative  $C$  mineralized was the dependent variable, and the independent variables (factors) were: (1)  $C$  fraction (DOC or insoluble residue), and (2) soil age. SAS (SAS Institute, 1999) was used for ANOVA and Tukey's Least Significant Difference tests.

The curves for the cumulative  $C$  mineralized over time were fit to a two-pool (i.e. rapidly and slowly mineralized fractions), first-order model for the accumulation of the product of 2 simultaneous first order reactions (Molina et al., 1980; Bridgham et al., 1998):

$$I_t = M_0 F(1 - e^{-ht}) + M_0(1 - F)(1 - e^{-kt}) \quad (4)$$

where  $I_t$  is the total cumulative mass of mineralized  $C$  at time  $t$ ,  $M_0$  is the total substrate  $C$  initially added,  $F$  is the rapidly mineralized  $C$  pool size,  $1 - F$  is the slowly mineralized  $C$  pool size, and  $h$  and  $k$  are first order rate constants for the rapidly and slowly mineralized fractions, respectively.

The parameters  $F$ ,  $h$ , and  $k$  were determined by nonlinear regression using the interactive web facility at: <http://members.aol.com/johnp71/nonlin.html> by J.C. Pezzullo, Georgetown University, Washington DC, USA. The fit of the model ( $R^2$  and overall  $P$ ) and the significance of the rate constants were used to test hypothesis 3. A comparison of the rate constants ( $k$ ) of the slowly mineralizing component for the DOC and the insoluble residue mineralization was used to test hypothesis 4.

### 3. Results and discussion

#### 3.1. Correction for non-uniform $^{14}\text{C}$ labeling

Our ability to determine the kinetics of the mineralization of the added C substrate depended on our ability to correct for non-uniform labeling. In the sand core to which we added  $^{14}\text{C}$ -labelled DOC, the cumulative radioactivity evolved was not linearly related to the C mineralized (Fig. 1). The most rapidly mineralized  $^{14}\text{C}$  DOC was somewhat higher in specific activity, as indicated by the lower initial slope of the line in Fig. 1. In the insoluble residue, however, the cumulative radioactivity was approximately linearly related to the C mineralized, indicating that the insoluble residue was uniformly labelled (Fig. 1). Because the residues in the sand did not mineralize as rapidly as those on soil, it was necessary to extrapolate the relationship to cover the range of % C mineralized in the soil cores, but since the intercept was nearly 0, it did not appear likely that the remaining material could have had a considerably different specific activity. We also assumed that each percentile of the substrate mineralized from the sand cores has the sample specific activity as the  $n$ th percentile of the substrate mineralized from the soil cores but it is conceivable that different microbial communities in the sand and soil might have mineralized different fractions in different sequences.

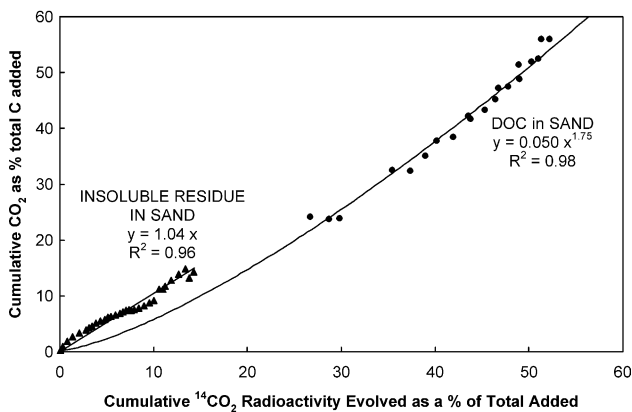


Fig. 1. Cumulative  $\text{CO}_2$  evolved as a % of total C added vs. cumulative  $^{14}\text{CO}_2$  radioactivity evolved as a % of total added to sand cores containing either DOC or insoluble residue of litter.

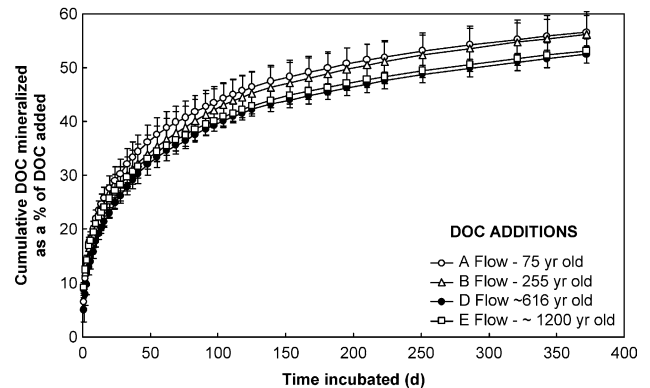


Fig. 2. Cumulative  $^{14}\text{CO}_2$  radioactivity evolved as a % of total added over the time of incubation for the DOC added to cores of soils of different ages. These curves are corrected for variation in specific activity inferred by Fig. 1. Each data point represents 3 replicate cores and error bars represent  $\pm 1$  SEM.

#### 3.2. Mineralization in soils of different age

After the  $^{14}\text{C}$  radioactivity data from the soil cores were corrected for non-uniform labeling using Eq. (1), the mineralization of DOC was not significantly different among the different soil ages (ANOVA,  $P=0.49$ ), (Fig. 2). Consequently, we found no support for hypothesis 1. After 1 year, between 54.5 and 57.6% of the  $^{14}\text{C}$  radioactivity had been mineralized in the soils of four different ages. Although, the means for the A and B flow (57.6 and 57.4%) were slightly higher than those of the D and E flows (54.5 and 55.0%), they were not statistically different. The minimum significant difference that could be detected at  $P=0.05$  was 5.8% using the Tukey's Least Significant Difference Test (SAS Institute, 1999). When we refer to mineralization of  $^{14}\text{C}$  labelled DOC, it should be understood that the carbon could have been mineralized directly from solution, from DOC sorbed to the soil, or from microbial biomass that had assimilated the DOC.

Since the insoluble residue was approximately uniformly labelled, then Fig. 3 was also taken to represent the % C

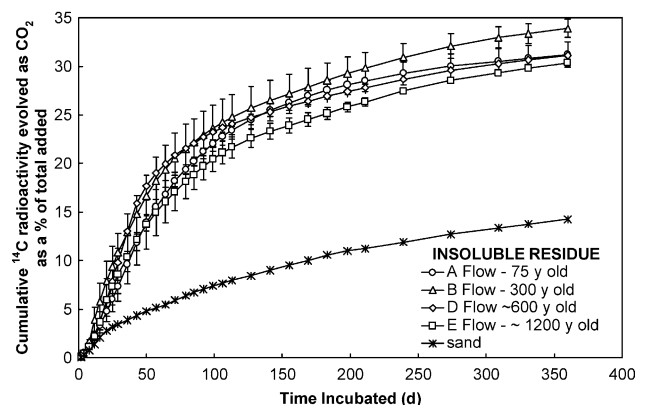


Fig. 3. Cumulative  $^{14}\text{CO}_2$  radioactivity evolved as a % of total added over the time of incubation for the insoluble residue of litter added to cores of soils of different ages and sand cores. Each data point represents 3 replicate cores and error bars represent  $\pm 1$  SEM.

Table 1  
Percentage of added radioactivity remaining in microbial biomass after 1 year of incubation

Form C added	Soil age	% radioactivity released by CH <sub>3</sub> Cl <sup>a</sup>	Total C released by CH <sub>3</sub> Cl fumigation (mg C g <sup>-1</sup> soil) <sup>b</sup>	% C in soil
DOC	A	1.6 ± 0.12	0.02 ± 0.0055	0.6 ± 0.045
	B	0.9 ± 1.7	0.05 ± 0.013	1.8 ± 0.10
	D	4.5 ± 3.8	0.08 ± 0.019	3.4 ± 0.69
	E	5.9 ± 6.0	0.04 ± 0.011	1.4 ± 0.29
Insoluble residue	A	0 ± 0.16	0.09 ± 0.041	1.0 ± 0.19
	B	0 ± 0.01	0.2 ± 0.15	2.5 ± 0.055
	D	0 ± 0.017	0.1 ± 0.035	4.1 ± 0.81
	E	0 ± 0.14	0.1 ± 0.048	2.7 ± 0.78

C in biomass is also expressed in mg C g<sup>-1</sup> soil. Means are of 3 replicates ± 1 SEM (standard error of the mean).

<sup>a</sup> Radioactivity in K<sub>2</sub>SO<sub>4</sub> extracts of CH<sub>3</sub>Cl treated soil-control.

<sup>b</sup> DOC in K<sub>2</sub>SO<sub>4</sub> extracts of CH<sub>3</sub>Cl treated soil-control expressed as mg C g<sup>-1</sup> dry soil.

mineralized from the insoluble residue as a function of time. The cumulative radioactivity mineralized from the insoluble residue also did not differ significantly among the ages of the soils, ranging from 30.4 to 33.9% (ANOVA,  $P=0.35$ ) (Fig. 3). However, in contrast to DOC, the residue in the sand core was mineralized considerably slower than in the soils (Fig. 3). This slower mineralization of the insoluble residue might have been due to nutrient limitation, since the soil cores supplied ambient nutrients in the soil along with those mineralized during the incubation.

The insoluble residue of senesced leaf litter decomposed less rapidly than the DOC over the entire 1-y period (comparing Figs. 2 and 3). The cumulative DOC mineralized at 1 y was 54.6% ± 1.0 s.e. averaged over all the soils, while the cumulative C mineralized from the insoluble residue averaged 31.9% ± 0.8 s.e.. A 2-way ANOVA using C fraction (DOC or insoluble residue) and soil age as independent factors indicated that this difference was significant ( $P<0.001$ ).

Because the study extended over a relatively long period, only a small amount of the radioactivity remained in the living microbial biomass fraction (Table 1). It seems likely that DOC initially assimilated by microorganisms had later been mineralized as these microorganisms died over the long period of incubation. We have simply presented the data directly in terms of chloroform-extractable C, rather than multiplying by a correction factor to include non-lysed microbial biomass C as in Vance et al. (1987). The means of the radioactivity from labelled DOC released by fumigation were all below 5.9%. The means of the two older soils suggest more radioactivity in microbial biomass than in the younger soils, but the variability precluded detecting any significant differences. The <sup>14</sup>C originating from insoluble residue had essentially no radioactivity remaining in microbial biomass. The youngest soil (from the A flow) had the least microbial biomass (total C released by fumigation) and a low soil C content (Table 1). Total C released by fumigation along with the % C in the soil also indicated about 0.2–0.9% of the total C was chloroform

extractable, which would correspond to 0.5–2.4% of the soil C in the form of microbial biomass if we assume an extraction efficiency factor of 0.38 (Vance et al., 1987).

### 3.3. Rates of mineralization

Because there seemed to be a very rapid phase and then a very slow phase of mineralization (Figs. 3 and 4), we fit the data to a model that describes the decomposition of a rapidly and slowly mineralized pool (Table 2). Fig. 4 shows an example of the fit to the data for the DOC in the D flow soil. The fit shows that the assumption of 2 components, especially for the DOC, is a simplification because the data has a somewhat more gradual curvature than the model fit. There are two important parameters to be considered (Table 2): (1) the percentage of total C that is either in the rapidly or slowly decaying fraction ( $F$ ,  $1 - F$ , respectively), and (2) the half times of decay of the rapidly or slowly mineralized fraction ( $t_{1/2}$ ). The rapidly decaying fraction of DOC comprised about 32% of the total and had a half decay time of about 7 d (0.02 y). The slowly decaying fraction

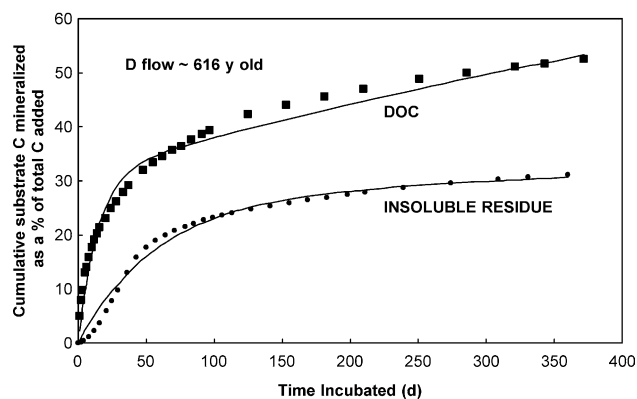


Fig. 4. Fit of data to the two component model (Eq. (3)) for the cumulative <sup>14</sup>CO<sub>2</sub> radioactivity evolved as a % of total added over the time of incubation for the DOC and insoluble residue added to cores of the ~600 years old soil. The parameters of the equations describing the lines, and  $R^2$  values, are indicated in Table 2.

Table 2

Parameters describing the fit of mineralization data to the model  $I_t = M_0F(1 - e^{-ht}) + M_0(1 - F)(1 - e^{-kt})$ , where  $I_t$  is cumulative respired  $\text{CO}_2$  at time  $t$ ,  $M_0$  is the total substrate C initially added,  $F$  is the rapidly mineralized C pool size,  $1 - F$  is the slowly mineralized C pool size, and  $h$  and  $k$  are first order rate constants for two pools, respectively

Mudflow				
Parameter	A	B	D	E
	DOC			
$F$	0.34 (0.010)*	0.31 (0.012)*	0.34 (0.010)*	0.30 (0.010)*
$h$ ( $\text{y}^{-1}$ )	35 (3.2)*	36 (4.1)*	35 (3.2)*	44 (4.9)*
$t_{1/2}$ (y)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)
$1 - F$	0.66 (0.010)	0.69 (0.012)	0.66 (0.010)	0.70 (0.010)
$k$ ( $\text{y}^{-1}$ )	0.44 (0.03)*	0.47 (0.03)*	0.44 (0.03)*	0.42 (0.02)*
$t_{1/2}$ (y)	1.6 (0.09)	1.5 (0.09)	1.6 (0.09)	1.7 (0.10)
$R^2$	0.98*	0.97*	0.98*	0.97*
Insoluble residue				
$F$	0.28 (0.039)*	0.26 (0.011)*	0.27 (0.023)*	0.25 (0.017)*
$h$ ( $\text{y}^{-1}$ )	4.9 (0.39)*	6.4 (0.37)*	6.3 (0.73)*	5.1 (0.41)*
$t_{1/2}$ (y)	0.14 (0.01)	0.11 (0.01)	0.11 (0.01)	0.13 (0.01)
$1 - F$	0.73 (0.039)*	0.74 (0.011)*	0.73 (0.023)*	0.75 (0.017)*
$k$ ( $\text{y}^{-1}$ )	0.06 (0.04)	0.12 (0.02)*	0.06 (0.04)	0.08 (0.026)*
$t_{1/2}$ (y)	11.6 (7.70)	5.7 (0.91)	12.2 (8.7)	8.6 (2.7)
$R^2$	0.99*	0.997*	0.99*	0.9997*

$t_{1/2}$  is the half decay time.

comprised about 68% of the total and had a half decay time of about 1.6 y. Most of the DOC was not rapidly mineralized by microbes.

The slowly decaying fraction of litter DOC decayed slowly enough to contribute to the accumulation of soil organic matter. The fact that we used freshly senesced litter would be expected to maximize the percentage that is in the rapidly decaying fraction. An advantage of this method was that we could observe the entire mineralization curve, including components present only for a short time after senescence as well as those likely to remain in soil solution or in the adsorbed phase for long periods of time. We would expect that litter in the forest floor that had already been decomposed would have less C in the rapidly decaying fraction because it could have been decomposed rapidly in the soil after having been leached. It could even have decomposed in solid phase before sufficient rainfall had occurred to completely leach the soluble material (Qualls and Haines, 1992b). An incubation of the water-soluble fraction of freshly senesced litter in water (as opposed to in soil cores as in the current experiment) from a southeastern US forest gave generally comparable results except that the % in the rapidly decaying fraction differed (Qualls and Haines, 1992b). In that experiment, 59% was in the rapidly decomposing fraction with a half-decay time of 7.5 d, and 42% was in the slowly decaying fraction with a half-decay time of 334 d. The mixture of species was different than in our current experiment and the incubation time was only 133 days, so the half decay time of the slow fraction was determined much less accurately. The half decay time of the slowly decaying fraction in the Oi horizon solution was more accurately determined and was about 501 d ( $1.4 \text{ y}^{-1}$ ) in the experiments of Qualls and Haines (1992b).

The percentage in the relatively rapidly mineralized fraction of DOM extracted from forest Oi, Oe, and maize straw varied from 59 to 88% in water incubations reported by Kalbitz et al. (2003), which is again higher than in our soil core experiments. There might be some doubt as to whether DOC in a water incubation would decompose as rapidly as in soil where a well developed fungal community would be more likely. The fact that leachates incubated in water in the studies of Qualls and Haines and Kalbitz et al. decayed at least as rapidly as ours did in soil or sand suggests otherwise. Since mineralization requires the turnover of DOC taken up into microbial biomass, the longevity of the microbial biomass may influence the percentage that mineralizes rapidly. It is possible that intact soils cores may encourage a larger proportion of DOC to be incorporated by more long-lived fungal biomass, but the effect of the makeup of the microbial community remains a topic to be explored.

Fractionation of the DOC in the litter extract yielded the following percentages in each fraction: hydrophobic acids, 38.2%; phenols, 5.5%; hydrophilic acids, 8.2%; hydrophobic neutral, 13.2%; hydrophilic neutral, 36.0%; and bases, 1.0%. The distribution of these fractions was similar to those found in freshly fallen litter of a mixture of species on a deciduous forest watershed, and was also generally similar to water draining from the forest floor just after litter fall (Qualls and Haines, 1991). However, in the study of Qualls et al. (1991), water draining from the forest floor in autumn had a higher content of hydrophilic acids than water extracts of freshly fallen litter because it also contained DOC from older litter. The distribution of fractions in a water extract of the same radiolabelled litter used in this study after 1 year of decomposition (manuscript submitted

for publication) was similar to that of water draining from the forest floor during the spring and summer in the study of Qualls et al. (1991).

### 3.4. Mineralization in soils with different adsorption properties

The older soils of the chronosequence adsorbed much more of the  $^{14}\text{C}$  labelled DOC (Fig. 5). The 1 and 8 h incubation times on a subset of samples yielded similar percentages adsorbed using Eq. (2), suggesting that the assumption that mineralized  $^{14}\text{C}$  during the adsorption experiment was not adsorbed had little effect on the calculation. Despite these differences in the degree of adsorption, there was no significant effect on the mineralization of the added DOC. We had hypothesized that DOC would be protected from enzymatic attack by its ability to penetrate and adsorb on surfaces in very small interstices among clay particles or within the matrix of short-range-order oxyhydroxides and aluminosilicates. There it could be protected from access by bacterial or fungal cells more effectively than could solid particles of organic matter that could not be transported into these interstices.

The most likely sorbant in these soils was allophane. Lilienfein et al. (2003) found a much greater allophane concentration and surface area in the older soils of the D and E flows compared to the younger soils. There were also increases in the concentration of ferrihydrite, but it was present in much lower concentrations than allophane. Allophane is often believed to stabilize organic matter in soils. Andosols have relatively high contents of organic C (Post, 1982). The mean residence time of humus in some allophanic soils was estimated at 2000–5000 y by Wada and Aomine (1973). Along a 4 million year chronosequence of

volcanic soils in Hawaii, the  $\Delta^{14}\text{C}$  of soil organic matter was negatively correlated ( $R^2=0.63$ ) with the content of short-range-order minerals (such as allophane and ferrihydrite), implying lower turnover of soil C with higher short-range-order mineral content (Torn et al., 1997). Our experimental results suggest that these correlative observations do not represent protection of adsorbed dissolved organic matter, at least from leaf leachate.

Saggar et al. (1994) added  $^{14}\text{C}$  glucose to soils that varied in allophane content, and their results have been interpreted to mean that higher allophane contents result in increased stabilization of C (Torn et al., 1997). Over a 28-day incubation period, 65% of the  $^{14}\text{C}$  glucose was mineralized in the soil with the lowest allophane concentration, while 54% was mineralized in the soil with the highest allophane content. However, the sum of the  $^{14}\text{C}$  mineralized plus that in living microbial biomass was 67% in the soil with the highest allophane concentration, and 65% in the soil with the lowest allophane content. Consequently, it appears that microbes that took up the  $^{14}\text{C}$  glucose were longer lived in the soils with higher allophane content for some unknown reason and that the stabilized  $^{14}\text{C}$  (not mineralized or still in live microbial biomass) was similar. Consequently, the findings of Saggar et al. (1994) over a 28-day incubation are not necessarily different than our results.

In non-volcanic soils there are also conflicting indications that C mineralization can be affected by various interactions associated with soil weathering. It is often considered that higher clay content in soils results in more stabilization of organic C. Many studies have found a correlation between organic C content and clay content (Oades, 1988). However, Giardina and Ryan (2000) and Giardina et al. (2001) found no significant correlation of clay content vs. C mineralization per kilogram soil organic C among a number of soils. They also summarized many studies that showed either weak or no correlation of C turnover with clay content (e.g. Motavalli et al., 1995; Sørensen, 1981). On the other hand, Jones and Edwards (1998) found that mineralization of  $^{14}\text{C}$ -labelled citrate was inhibited by additions of clays to a soil slurry. However, mineralization of  $^{14}\text{C}$ -labelled glucose was not influenced by additions of clays to the soil slurry. The mineralization of both citrate and glucose was reduced when  $\text{Fe}(\text{OH})_3$  was added. Nelson et al. (1997) found about a 14% difference in mineralization between ground straw combined with two soils that were mixed to give the same clay content, but one with illite and kaolinite and the other with smectite dominated clays. In a study of ground Eucalyptus litter mixed with either sand, sand mixed with kaolinite, or sandy loam, Skene et al. (1997) concluded the differences in the inorganic matrices had little influence on mineralization. However, in a parallel study using straw, Skene et al. (1996) found differences in mineralization and concluded the inorganic matrix was more likely to influence mineralization of the higher quality substrate in straw. Nelson et al. (1994) reported that weakly adsorbed soluble organic C

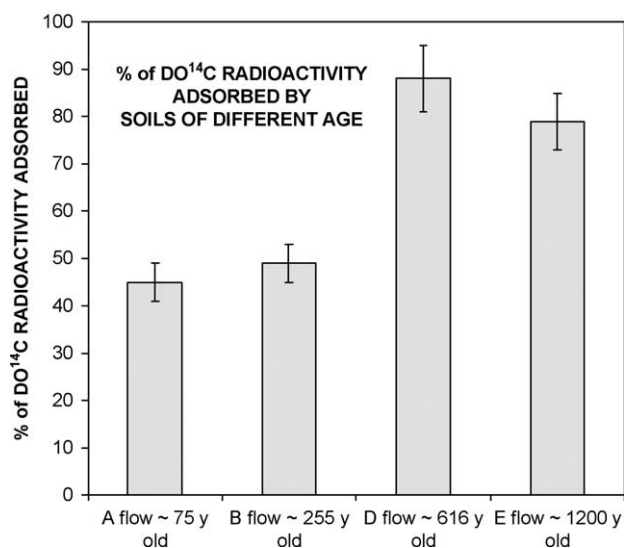


Fig. 5. Percentage of added  $\text{DO}^{14}\text{C}$  radioactivity adsorbed by soils of different ages. Error bars indicate SEM of determinations on 3 replicate cores.

(using phosphate buffer to desorb) was more labile than water extractable organic C in a soil profile that varied in clay content.

Although, there are conflicting reports that changes associated with soil weathering over time can result in ‘protection’ or ‘stabilization’ of soil organic C, we found no evidence of such effects on either the soluble or insoluble fraction of litter in the Mt. Shasta mudflows chronosequence. This was despite a large difference in the degree of adsorption in the soils as they developed (Fig. 5) and an increase in allophane from  $<1.0 \text{ g kg}^{-1}$  soil to  $28 \text{ g kg}^{-1}$  soil (Lilienfein et al., unpublished data). It might be possible that some effects are only manifested at the extremes of humification of the organic matter. In the case of the added DOC, however, the humic substance fraction (hydrophobic acids) already comprised 32% of the fresh leachate (in this study) and one half of older forest floor leachate (Qualls et al., 1991). In addition, the mineralization of the added substrate had proceeded well into the ‘slow mineralization’ stage and still no significant differences had appeared among soils.

### 3.5. Mineralization of soluble vs. insoluble components

The rapidly decaying component of DOC was somewhat larger than that of the insoluble residue in terms of % of total C and also had a half time of decay that was shorter (about 47 d, Table 2). We interpret this phase as the decay of soluble sugars and other carbohydrates in the DOC vs. the decay of cellulose and perhaps insoluble proteins in the insoluble residue. Nykvist (1963) found an average of about 16% of the dry mass of 7 species of litter to be water-extractable mono- or disaccharides and low-molecular-weight aliphatic acids that could be directly taken up by microbes.

The mineralization of the insoluble residue had not proceeded to the point that the  $t_{1/2}$  for the slowly mineralized fraction could be determined accurately from the model, as indicated by the very large standard errors in Table 2. However, the 95% C.I. of the  $k$  did not include a rate greater than 0.66, which corresponded to a  $t_{1/2}$  of less than 1 year. The  $t_{1/2}$  for the slowly decaying fraction of DOC was more tightly constrained at about 1.5 year. Consequently, we found support for hypothesis 4, that the slowly decaying fraction of DOC and the slowly decaying fraction of the insoluble residue were both on the order of years.

In comparing the decomposition of the DOC and the insoluble residues, the effect of the particulate nature of the insoluble residues must be considered. The insoluble residue was ground, so the effect of initial colonization by microbes on surfaces was minimized. We also mixed the residue with the surface layer of soil to make its distribution more similar to that of the DOC. Obviously we could never eliminate the differences between a dissolved substrate and a particulate substrate. We did observe that the insoluble

residue was covered with fungal growth within several days, so it was likely that fungal hyphae could rapidly access the inside of the particles. Swift et al. (1979) reported that grinding oak leaves into several particle sizes from several millimetre to 50–100  $\mu\text{m}$  affected fungal decomposition rate only during the first 14 days, after which there was no difference in  $\text{CO}_2$  loss.

Our impression from the literature is that there is often an assumption that dissolved organic matter or water-extractable C is more labile than the insoluble matter (McCarty and Bremner, 1993). Although, there was a large fraction of labile DOC from the freshly senesced litter that was more labile than the insoluble fraction, more than 60% of the DOC had  $t_{1/2} \geq 1.5$  year.

### 3.6. Decomposition vs. mineralization

The capture of  $\text{CO}_2$  represents mineralization of the original substrate. The decomposition of a C substrate by microbes may be separated into 3 phases: (1) breakdown of macromolecules into forms that can pass through the cell membrane, (2) uptake into the cell, and either (3a) metabolism to  $\text{CO}_2$  (aerobic environments) or (3b) synthesis and production of microbial biomass. Then the microbial biomass may go through the entire cycle of production and decomposition multiple times until it is eventually respired as  $\text{CO}_2$ . Consequently, any transformations that are due to microbial synthesis will affect the rate of mineralization, as the original substrates might be very different in their susceptibility to enzymatic attack. Additions of  $^{14}\text{C}$  glucose to soils often show a large fraction of the substrate being mineralized slowly (Saggar et al., 1994), presumably the result of microbial uptake and re-synthesis. For this reason, some of the fractions that are rapidly broken down might be re-synthesized into less easily mineralized compounds.

In our experiment, only a relatively small amount of radioactivity remained in the chloroform extractable fraction at the end of 1 year. However, microbial biomass that had died but had not been mineralized would contribute to a discrepancy between decomposition and mineralization. In fact, if we consider that the  $^{14}\text{C}$  in living microbial biomass (Table 1) should not be considered as ‘stabilized’ but rather part of a labile fraction, then the small, insignificant difference between soils of different ages in Fig. 2 would be even smaller. The high variability in the % radioactivity released by  $\text{CH}_3\text{Cl}$  fumigation, however, precludes us from making quantitative comparisons between soils involving a few % of the initial  $^{14}\text{C}$  added.

### 3.7. Advantages of the method

Our method of adding  $^{14}\text{C}$ -labelled substrates had several advantages: (1) it could be added to intact soil cores, and the added substrates could be distinguished from native C, (2) it yielded information on the entire decomposition curve, at least up to 1 year, not just the initial rates, and (3) it provided

a way to correct for non-uniform labeling. The fact that the cores were intact was important because the physical soil structure was not disturbed, a factor that might be important in protecting C in interstices and one that was central to our hypothesis that weathering might result in greater degrees of 'protection' from microbial attack. In addition, the microbial community, including fungal mycelia, was intact. We considered it particularly important that fungal mycelia were not disturbed, since they are important in hydrolysis of lignin and humic substances (Stevenson, 1982). One disadvantage was that we cannot compare DOM from a wide variety of sources because it requires labeling over a long period of growth, something much more difficult for perennial plants like trees. It is also not possible to label DOM produced from microbial transformations or from humification reactions.

Other methods for measuring the mineralization rates of natural DOM fall into the following categories: (1) incubation of a liquid solution in which either disappearance of DOC or respiration is measured, (2) passage through a previously colonized column of beads with a very high surface area, and (3) measuring growth rates of bacteria over a short time interval. Although method 1 allows observation of a complete decomposition curve, one concern may be that the microbial community in liquid culture may lack certain functional groups such as lignolytic fungi. Method 2 is also a liquid culture method, but the 'precolonization' of the beads may allow a fully grown microbial community to immediately access the substrate. Method 2, however, does not allow observation of the entire decomposition curve, and sorption to or DOM production in biofilms might be a potential problem. Method 3 gives information on the very small fraction decomposed over the time span of hours. If we had used method 3, we might misinterpret our results and conclude the dissolved organic matter was all very labile.

### 3.8. Summary

1. After 1 year of decomposition, there were no significant differences in the mineralization rates of DOC added to soils of different ages and stages of weathering.
2. After 1 year of decomposition, there were no significant differences in the mineralization rates of the insoluble residue of litter added to soils of different ages and stages of weathering, with a mean of about 32%.
3. The DOC appeared to be comprised of two fractions: one that comprises about 32% of the total that decomposes with a half decay time of 0.02 y (7 d), and a second fraction comprising 68% with a half decay time of about 1.6 y. The slowly decaying fraction likely contributes to the accumulation of slowly mineralized C in soil.
4. The rapidly and slowly mineralized fractions of the insoluble residue both decomposed slower than the corresponding fractions of the DOC.

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