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Biodegradability of Fractions of Dissolved Organic Carbon Leached from Decomposing Leaf Litter

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Dissolved organic matter leached from decomposing organic matter is important in the leaching of nutrients from the root zone of ecosystems, eluviation of metals, and transport of hydrophobic pollutants. The objective of this study was to compare microbial mineralization rates in intact soil cores of various fractions of water-soluble dissolved organic matter. Uniformly ^{14}C-labeled *Populus fremontii* leaf litter that had decomposed for 1 year was extracted in water and this extract was fractionated into phenolic, humic acid, fulvic acid, hydrophilic acid, and hydrophilic neutral fractions. Fulvic acid comprised 42.1% of C in dissolved organic carbon (DOC) extracted from the litter. These fractions were added to intact cores of soil or sand, and respired $^{14}CO_2$ was collected. The percentage of labeled substrate C mineralized in soil at the end of 1 year was, in order from least to greatest, hydrophilic acid (30.5), fulvic acid (33.8), humic acid (39.0), whole, unfractionated DOC (43.5), unseparated hydrophilic acid and neutral (44.7), phenolic (63.3), glucose (66.4), and hydrophilic neutral (70.2). In acid-washed nutrient-amended sand that was inoculated with soil microbes, mineralization rates of fulvic acid and glucose were lower. The fractionation appeared to separate the DOC into components with widely different rates of mineralization. Results also supported the ideas that the dissolved humic substance and hydrophilic acid fractions are inherently difficult for microbes to mineralize, and this property can contribute to movement of refractory C in soil and into aquatic ecosystems.

Introduction

Natural dissolved organic matter can be transported in water throughout the soil profile, into the smallest pore spaces in soil, and into streams and lakes. The capacity of dissolved organic carbon (DOC) to be transported into the smallest pore spaces and all adsorbing surfaces of the soil matrix means that it can be subject to sequestration if it is resistant to microbial mineralization. Natural dissolved organic matter is composed of a complex mixture of substances that has never been completely characterized. Because it is a complex mixture, it consists of components that have widely different physicochemical properties and may also consist of components that are labile and refractory to microbial decomposition. Some studies of dissolved organic matter from soil solution and from aquatic environments have suggested that it is largely resistant to mineralization while others have suggested that it is not so resistant to mineralization. For example, ^{14}C contents of DOC from some rivers indicate that the DOC is hundreds of years old, which suggests great resistance to mineralization, while that from other rivers indicates recent origin (*1*).

Humic and fulvic acids have been shown to be important components of DOC and have generally been shown to comprise about 50% of DOC in soil solution and streamwater (*2*, *3*). Radiolabeled fungal melanins that resemble humic acids have been prepared from fungal cultures and were found to be resistant to decomposition when incubated in soil (*4*). Jandl and Sollins (*5*) found less than 5% of the C in the hydrophobic acid fraction of a water extract of litter was mineralized in a solution incubation over 110 days.

Other studies suggest that even humic substances are not so resistant to decomposition. For example, an alkaline extract of a forest soil was incubated in liquid culture containing growth medium, and wood-degrading basidiomycetes were able to reduce the color of the solution by 57% in 21 days (*6*). Likewise, a humic acid extracted from forest floor material was incubated in liquid culture with glucose-containing growth medium with manganese, and a litter-degrading basidiomycete was able to reduce the color of the solution by 75% in 42 days (*7*). For aquatic humic substances, up to 27% was removed from solution upon incubation in a glucose-containing nutrient broth, but less than 7% was removed without the full glucose broth (*8*). A humic acid fraction from isolated lake sediments incubated in lake water lost over 60% of C from the solution phase in 95 days (*9*). Beyond these observations, there are few comparisons of decomposition of humic substances and other fractions of natural dissolved organic matter.

A fractionation procedure based on pH-dependent hydrophobic properties and by molecular charge has become perhaps the most common means of characterizing natural dissolved organic matter (*10*), except perhaps the elemental ratios of the material. The fundamental nature of the physicochemical properties that determine this fractionation can be related to the major physical interactions of DOC, such as cation or anion exchange and hydrophobic interactions (*10*). These properties are important in determining adsorption to organic and mineral surfaces and the ability to transport organic and inorganic pollutants (*11*−*13*). However, these physicochemical properties might not necessarily relate to the tendency of C in each of the fractions to be mineralized by microbes. If the fractionation procedure were shown to separate the natural DOC into fractions with widely different rates of microbial mineralization, then it might be concluded that the physicochemical categories also have some biological significance.

The hydrophilic acid fraction isolated by this procedure is generally the second largest fraction of DOC in soil solution and flowing waters (*2*, *3*, *10*). While the previously cited studies have examined microbial decomposition of the humic substance fraction, little is known about the origin or biodegradability of the hydrophilic acid fraction (*2*).

The objective of this study was to compare the microbial mineralization rates of the humic acid, fulvic acid, hydrophilic acid, phenolic, and hydrophilic neutral fractions of DOC extracted from decomposed leaf material. The specific hypotheses were as follows: (1) the fractionation procedure separated components that exhibited widely different rates of mineralization, (2) humic and fulvic acid fractions were mineralized with half decay times of more than 1 year, (3) hydrophilic acid fraction was mineralized at rates similar to that of the fulvic acid fraction, (4) hydrophilic neutral fraction was mineralized more rapidly than other fractions, and (5)

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mineralization of the whole, unfractionated DOC was similar to that of a weighted average of the separated fractions. To evaluate these hypotheses, DOC extracted with water from decomposed ^{14}C-labeled leaf litter was fractionated and these fractions, unfractionated DOC, or glucose were added to intact soil cores so that the DOC substrates were exposed to an intact soil structure and microbial community. Then rates of evolution of $^{14}CO_2$ were measured over 1 year.

Experimental Section

Preparation of Radiolabeled Litter and Fractionation. Seedlings of the tree *Populus fremontii,* propagated from stem cuttings, were grown for 1.5 growing seasons in a sealed growth chamber regulated at 370 ppm CO_2 and labeled by injections of ^{14}C-$NaHCO_3$ into acid twice a week (*14*). After senescence, 5 g of the labeled leaf litter was allowed to decompose for 1 year at 25 °C on top of a freshly gathered sample of the A horizon of a Humic Haploxerand soil 46 cm in area, with a nylon mesh separating the soil and litter so that they could be cleanly separated. Soil was adjusted to a matric water potential of −20 kPa. During this preliminary decomposition, the litter lost 66% of its mass. The rationale for using partially decomposed leaf litter was to allow the process of humification to produce soluble organic matter characteristic of that leaching from the forest floor. Dissolved organic matter has been found to leach slowly from newly fallen litter over periods exceeding 1 year, and the chemical characteristics of natural leachate resemble those of extracts of decomposed litter rather than extracts of fresh litter (*3*, *15*, *16*).

The air-dried decomposed litter was ground with a coffee grinder until it passed a 2 mm sieve. Then 0.8 g dry weight equivalent was extracted by shaking in 30 mL of deionized water for 0.5 h at 3 °C. The supernatant was poured off and the solid residue was then extracted three more times in water at 25 °C for 4 h. The rationale for this extraction was to minimize decomposition of the organic matter during first extraction while also ensuring that the less soluble components were also extracted more completely. I 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) humic substances could then be extracted at higher temperatures. The supernatants were immediately filtered through a prerinsed 0.45 μm cellulose ester membrane filter and immediately frozen.

All extracts were combined, and the water extract was diluted to 125 mL with water and fractionated by Leenheer's (*10*) procedure as modified by Leenheer and Noyes (*17*) (Figure 1). This procedure fractionated the DOC into hydrophilic and hydrophobic fractions, which were further fractionated into acidic, basic, and neutral components as follows. The solution was adjusted to pH 7 and then pumped through 1.9 mL of XAD-8 resin. The "weak hydrophobic acid" (*17*) fraction (i.e., "phenolic" fraction) was eluted with 0.1 M NaOH. The effluent was acidified to pH 2, allowed to sit for 24 h, and then centrifuged to recover the precipitated humic acid fraction. The supernatant was again pumped through XAD-8 resin at pH 2, and the adsorbed fulvic acid fraction was then eluted with 0.1 NaOH. The effluent was then pumped through a cation-exchange column.

The Leenheer (*10*) procedure did not separate the phenolic, fulvic, and humic acid fractions, so the "hydrophobic acid fraction" as used by many other studies should be understood to include all three of these fractions. They were separately assayed in this study because I suspected the phenolic fraction to be different and because a previous study of NaOH-extractable organic matter had found differences in biodegradability between the humic and fulvic acid fractions (*14*).

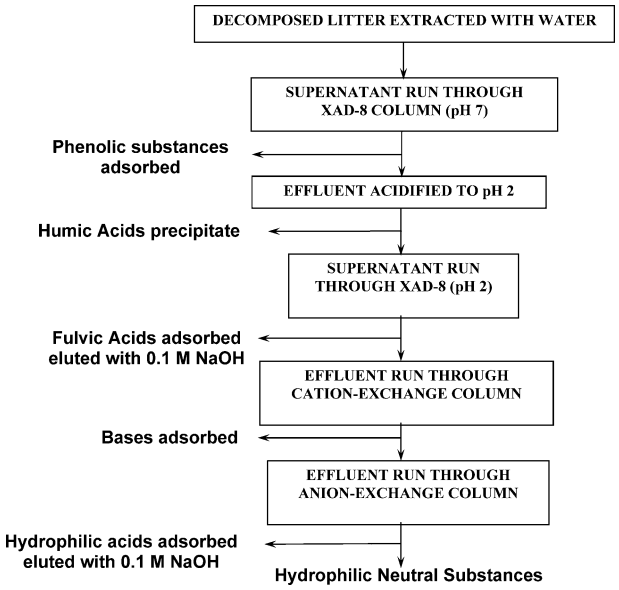

FIGURE 1. Fractionation procedure.

At this point in the fractionation, half of the 125 mL effluent was saved to represent the unseparated acid and hydrophilic neutral fractions. The remaining half of the effluent was then run through 1.9 mL of a weak anion-exchange resin as described by Leenheer (*10*). One reason for saving a portion of the unseparated hydrophilic acid and neutral fractions to evaluate the mineralization rate was because some studies have used the unseparated hydrophilic fractions (*11*, *18*, *19*). A second reason for using an unseparated hydrophilic acid and neutral fraction was because salts cannot be completely removed from the separated hydrophilic acid fraction, and so use of both separated and unseparated hydrophilic fractions in the biodegradation procedure would provide an additional way to evaluate possible artifacts due to the separation procedure. The hydrophilic acid fraction was then eluted with 0.1 M NaOH by the recycling elution procedure described in Leenheer and Noyes (*17*). Subsamples were taken for DOC analysis after each step of the procedure.

With each of the fractions, excess salt was removed immediately after recovery of the fraction and then the fraction was neutralized to pH 7. The humic acid fraction was washed in water adjusted to pH 2 with HCl, and then the suspension was neutralized to pH 7. The phenolic and fulvic acid fractions eluted in 0.1 M NaOH were immediately run though a cation-exchange resin and then the effluent was neutralized. The eluted hydrophilic acid fraction was run though a cation-exchange resin to remove excess Na and then neutralized, which still left excess NaCl. The hydrophilic neutral fraction was run through a cation-exchange column to remove possible contaminants from the anion-exchange resin and was then concentrated by evaporation at 50 °C to increase the amount of radioactivity that could be added to each core. It was free of salts since it had run through cation- and anion-exchange resins. The unseparated portion of the hydrophilic acid and neutral fraction was neutralized to pH 7 and also concentrated by evaporation. All fractions were immediately frozen after neutralization.

Soil Core Preparation and Incubation. The soil used in this study was collected from the oldest soil of the Mt. Shasta mudflow chronosequence, one that has served as a classical example of a soil weathering chronosequence and has often been cited as an example of the rate of accumulation of soil C during soil development (*20*, *21*). The soil was classified as a Humic Haploxerand and was described in Lilienfein et al. (*21*). Intact soil cores were taken from the upper 10 cm

of the A horizon, excluding the O horizon, with 3.2 cm i.d. plastic tubes. In addition, nine plastic tubes were filled with a mixture of combusted, acid-washed, and neutralized sand containing 10% micrometer-size silica dust. The silica dust was added to better mimic the soil texture and increase water holding capacity. All soil and sand cores were adjusted to −20 kPa soil matric water potential so that microflora in all soils would be subjected to the same matric water potential. The soil and sand 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 (*22*). After the cores were weighed to determine the "target mass", they were allowed to dry from the top until at least 4 mL of water had been lost so that additions of solution would not make the soil wetter than the target moisture content.

Aliquots of each of the following fractions were added to three soil cores: (1) unfractionated DOC in water extract, (2) humic acid, (3) phenolic fraction, (4) fulvic acid, (5) hydrophilic acid fraction, (6) hydrophilic neutral fraction, and (7) unseparated hydrophilic acid and neutral fraction. Three soil and three sand cores were incubated without addition of radiolabeled substrate to serve as blanks.

In addition, the fulvic acid fraction was added to three sand cores. The purpose of additions to sand cores was to evaluate mineralization in the absence of adsorption interactions. The fulvic acid fraction was the only one added to sand because it was one likely to adsorb to mineral soil and because there were not sufficient quantities of the other fractions. Because the sand cores were unable to supply exogenous microbial nutrients, aliquots of the extracts were mixed with a nutrient solution and a microbial inoculum. The nutrient stock was mixed like that of Stanford and Smith (*23*) with both N and P and micronutrients, and then added at a concentration such that the C/N ratio of the amended extract was 8, similar to microbial biomass. The microbial inoculum was prepared as in Qualls and Haines (*24*).

To compare mineralization of the natural substrates with a very easily mineralized substrate, a tracer level concentration of uniformly labeled ^{14}C-D-glucose was added to three additional soil and three sand cores. One milliliter of a 2.04 $\times$ 10^{-7} M solution of the uniformly labeled ^{14}C-D-glucose (specific activity 9000 GBq mol^{-1}, Moravek Biochemicals) was added to each of the cores.

Cores were placed in sealed Mason jars and respired $^{14}CO_2$ was collected in two vials, each containing 5 mL of 1 M NaOH. Water was added to each core whenever it was below the target weight to maintain constant soil matric water potential. Cores were incubated for 365 days at 25 ± 0.5 °C.

The NaOH solutions were periodically removed and combined, and 1 mL was added to 10 mL of Ecolite scintillation fluid, which was counted in a Beckman LS60001C liquid scintillation counter for 10 min. Only disintegrations with energy from 6 to 156 keV were counted to exclude chemiluminescence, which interferes with counting ^{14}C in aqueous alkaline solutions. The radioactivity of the soil or sand core blank was subtracted from all measurements.

Analysis of Mineralization Curves. The curves for cumulative C mineralized over time were fit to a two-phase (i.e., labile and refractory), first-order model for the accumulation of the common product of two simultaneous first-order reactions (*25*):

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

where I_t is the cumulative proportion of the total C that had been mineralized at time t, F is the proportion in the labile C pool, $1 - F$ is the proportion in the refractory C pool, and h and k are first-order rate constants for the labile and refractory phases, respectively. Parameters F, h, and k were determined by nonlinear regression with SYSTAT (*26*). Analysis of variance and multiple comparison of means (Tukey's HSD test) for cumulative percent $^{14}CO_2$ evolved after 1 year of incubation were done by after logarithmic transformation of the values expressed as a proportion (*26*). Transformation of the values was necessary because standard deviations tended to be larger for larger proportions. Data were fit to the two-phase first-order model because most mineralization data have been fit to such a model (e.g., refs *14*, *24*, and *25*). It should be realized that the rates of mineralization more likely reflect some distribution of rates rather than two discrete rates and that "labile" and "refractory" are used in a relative sense.

TABLE 1. Percentage of the Total DOC in the Water Extract Present in Each Fraction[a]

fraction	% of DOC	fraction	% of DOC
fulvic acid	42.1	hydrophobic neutral[a]	3.4
hydrophilic acid	23.4	phenolic	2.1
hydrophilic neutral	12.8	hydrophilic base[a]	0.9
humic acid	12.1	total	96.1[b]

[a] Fractions not used in mineralization experiment. [b] The total does not add to 100% due to low recovery of fractions or analytical error.

Results

Initial Content of Each Fraction. The fractions of DOC extracted from the litter were, in order from largest to smallest percentage of the whole DOC extract: fulvic acid, hydrophilic acid, hydrophilic neutral, humic acid, hydrophobic neutral, phenolic, and hydrophilic base (Table 1). The sum of the two humic substance fractions, humic and fulvic acids, was 54.2%. The unseparated hydrophilic acid and neutral fractions represented 36.2%.

Mineralization of Fractions. During the course of 1 year of incubation in soil cores, the fractions were mineralized in the following order from least to greatest percentage of added radioactivity mineralized: hydrophilic acid, fulvic acid, humic acid, whole unfractionated DOC, unseparated hydrophilic acid and neutral fraction, phenolic, and hydrophilic neutral (Figure 2, Table 2). Glucose in soil lost 66.4% of ^{14}C to mineralization. Analysis of variance of the percentages mineralized after 1 year indicated significant differences among the means ($P < 0.05$), supporting hypothesis 1. A multiple comparison of means (Table 2) showed the individual fractions formed two homogeneous groups, one for the hydrophilic, fulvic, and humic acid fractions (supporting hypothesis 3) and another for the more rapidly mineralized fractions, the phenolic fraction, hydrophilic neutral fraction (supporting hypothesis 4), and glucose. The whole unfractionated DOC and the unseparated hydrophilic acid and neutral fractions were intermediate, with means that were not statistically different from the humic and fulvic acid fractions.

All components except glucose exhibited a relatively good fit (r^2 from 0.97 to 0.99) to the two-phase model (eq 1) with a more rapid and a slower phase of mineralization (Table 3, Figure 2). A somewhat more gradual degree of curvature in the transition between the fast and slow phases indicated that the two-component model was somewhat of a simplification. A simple exponential decay model (not shown) did not fit the data well, with the initial phase being too rapid and the latter phase too slow for a good fit. The hydrophilic, fulvic, and humic acid fractions had only a small rapidly mineralized component (parameter F) varying from 17% to 29% (Table 3). The slowly mineralized components of these three fractions comprised over 70% and had half decay times of 3.6−4.9 years (supporting hypothesis 2). Because a majority

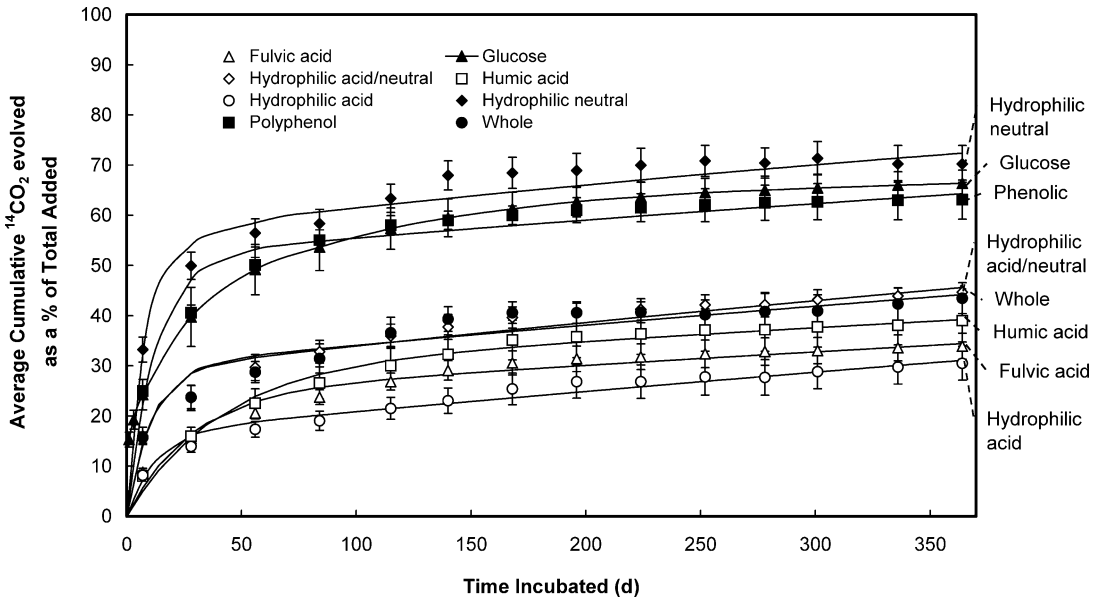

FIGURE 2. Cumulative $^{14}CO_2$ radioactivity evolved as a percentage of total added to soil cores containing various fractions of water-extracted decomposed litter.

TABLE 2. Percentage of ^{14}C Mineralized after 1 Year of Substrates Added to Soil Cores[a]

fraction	% $^{14}CO_2$ evolved
hydrophilic acid	30.5 (2.6) a
fulvic acid	33.8 (2.3) a,b
humic acid	39.0 (4.2) a,b
whole DOC	43.5 (3.0) b,c
hydrophilic acid + neutral	44.7 (1.9) b,c
phenolic	63.3 (1.3) d
glucose	66.4 (2.6) d
hydrophilic neutral	70.2 (3.5) d

[a] Analysis of variance indicated significant differences among means ($P < 0.05$). A Tukey's HSD multiple comparison of means was done, and values followed by the same letter (a–d) were not significantly different.

of the whole unfractionated DOC was composed of the humic substances and hydrophilic acid fractions, it also had a relatively large slowly mineralized component of 70% (parameter $1 - F$) with a very long half decay time of about 3 years.

Discussion

Ability of Fractionation Procedure to Distinguish Fractions of Different Biodegradability. The fractionation procedure did appear to separate the DOC into components of widely different biodegradability (hypothesis 1), at least to the extent that there were two statistically homogeneous groups of individual fractions. However, each fraction was not a simple homogeneous one fitting a single first-order decay function. Still, all fractions were mineralized in the same relative order throughout the entire year's course of mineralization. The statistically different fractions that were relatively rapidly mineralized only initially comprised 14.9% of the DOC, and the procedure was most valuable in distinguishing the more rapidly mineralized hydrophilic neutral fraction that comprised 12.8% of the DOC.

The physicochemical factors that caused fractionation during the procedure were not necessarily those that also directly caused differences in biodegradability. However, certain characteristics that have been hypothesized to contribute to slow biodegradability may be correlated with properties that influence physicochemical behavior during the fractionation. These are discussed for individual fractions in succeeding sections.

Slow Mineralization of Fulvic and Humic Acid Fractions. The humic and fulvic acid fractions together comprised initially comprised 54.6% of the DOC and the large refractory components had half decay times of 4–5 years (Table 3). Even under relatively natural conditions of incubation (intact soil cores with natural microbial communities), most of this natural DOC was very slow to mineralize and could contribute to persistence of organic matter in soil and aquatic environments.

The humic and fulvic acid fraction were mineralized at rates that were not significantly different. However, in another study (*14*) using the same litter that had decomposed for 180 days, the NaOH-extractable humic acid fraction of solid, decomposed litter mineralized more slowly than the NaOH-extractable fulvic acid fraction. Furthermore, the mineralization rate of the humic acid fraction of the NaOH extract was much slower (12.7% ± 2.5% in 1 year) than that of the water-soluble humic acid fraction (39.0% ± 4.2%) in the current study. The NaOH and water-soluble fulvic acid fractions mineralized at more similar rates (29.2% ± 1.2% and 33.8% ± 2.3%, respectively). The NaOH-extractable humic and fulvic acid fractions both comprised a large percentage of the C in the solid decomposed material, 22.7% and 3.9%, respectively, while water-soluble humic and fulvic acids comprised less than 1% of the solid C in the decomposed material. Consequently, the water-soluble humic acid fraction might represent a lower molecular size fraction than the NaOH-soluble humic acid fraction that is much more similar to fulvic acid in biodegradability.

One potential explanation for the slow mineralization of the humic and fulvic acid fractions in the soil might be that they were protected from mineralization by adsorption to mineral soil components. However, in acid-washed sand cores, mineralization rates of the fulvic acid fraction and glucose were even lower than in soil (Figure 3 vs Figure 2). Thus, there was no support for this explanation. A previous study showed that there was no significant difference between mineralization of DOC in the Humic Haploxerand soil used in this study and that in sandier soils of the weathering chronosequence that also had lower degrees of adsorption of the DOC (*27*). The A horizon of the Humic Haploxerand, the same used in this study, adsorbed 80% of the ^{14}C

TABLE 3. Parameters Describing the Fit of Mineralization Data to the Model[a]

	fraction													
parameter	hydrophilic acid		fulvic acid		humic acid		whole DOC		hydrophilic acid + neutral		phenolic		hydrophilic neutral	
F	0.17	(0.01)	0.24	(0.01)	0.29	(0.02)	0.30	(0.01)	0.29	(0.02)	0.52	(0.02)	0.56	(0.02)
h (yr^{-1})	28	(2)	13	(5)	9.3	(1)	32	(4)	36	(5)	29	(3)	43	(5)
$t_{h1/2}$ (yr)	0.024	(0.002)	0.052	(0.020)	0.075	(0.009)	0.022	(0.003)	0.019	(0.003)	0.024	(0.003)	0.016	(0.002)
$1 - F$	0.83	(0.07)	0.76	(0.04)	0.71	(0.04)	0.70	(0.02)	0.71	(0.05)	0.48	(0.02)	0.44	(0.01)
k (yr^{-1})	0.19	(0.02)	0.14	(0.02)	0.15	(0.03)	0.23	(0.01)	0.27	(0.07)	0.30	(0.05)	0.46	(0.03)
$t_{k1/2}$ (yr)	3.6	(0.4)	4.9	(0.8)	4.6	(0.9)	3.0	(0.1)	2.6	(0.7)	2.3	(0.4)	1.5	(0.1)
r^2	0.99		0.98		0.99		0.97		0.98		0.98		0.98	

[a] $I_t = F(1 - e^{-ht}) + (1 - F)(1 - e^{-kt})$, where I_t is cumulative respired CO_2 at time t, F is the labile C pool size (a proportion of 1), $1 - F$ is the recalcitrant C pool size, and h and k are first-order rate constants for the labile and recalcitrant pools, respectively. $t_{h1/2}$ and $t_{k1/2}$ are the half decay times corresponding to the rate constants h and k, respectively. The standard error of the estimate is shown in parentheses. $p < 0.05$ for h_0: that parameter $\neq 0$ for all parameters.

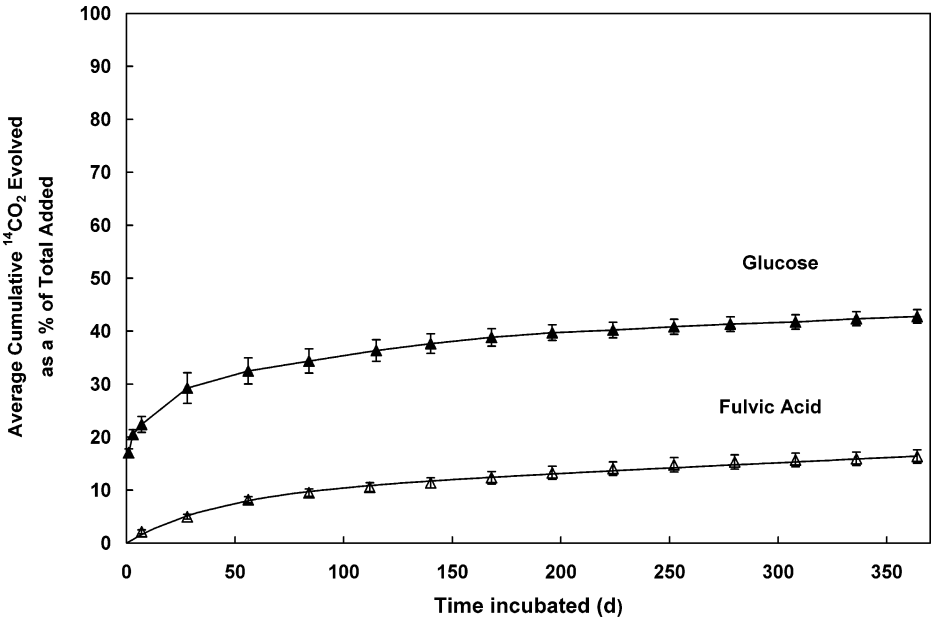

FIGURE 3. Cumulative $^{14}CO_2$ radioactivity evolved as a percentage of total added to sand cores, ^{14}C glucose, and fulvic acid.

radioactivity of a solution of DOC extracted from the newly senesced litter (*27*). Consequently, the inherent recalcitrance due to the molecular structures of the humic and fulvic acid fractions remains as one hypothesis that cannot be rejected in explaining the relative mineralization rates of the fractions.

Why mineralization rates in sand tended to be lower than in soil was unknown. There was no indication that the lack of an initial microbial community was limiting because there was no obvious lag phase in the initial rates of mineralization in sand (Figure 3). Despite addition of a nutrient amendment sufficient to provide a substrate C/exogenous N ratio of 8, there might still have been some degree of nutrient limitation.

Similarity of Hydrophilic Acid and Fulvic Acid Fractions. The hydrophilic acid fraction typically comprises the second largest fraction next to the humic substances fraction in aquatic and soil solutions (*2*, *3*, *10*). It has implicitly been regarded as more similar to the hydrophilic neutral fraction in some studies in which the hydrophilic acid and neutral fractions were not separated (*11*, *18*, *19*, *28*). Small molecular size organic acids that are likely easily taken up by microbes would be expected to occur in this fraction (*10*), but they might not comprise a large proportion of the fraction. In this study the hydrophilic acid fraction was very slow to mineralize, and the rate was not significantly different from the fulvic and humic acid fractions. In fact, a combination of the hydrophilic acid and neutral fractions combined the slowest and the fastest components to mineralize, and this combination had an intermediate rate of mineralization (Table 2).

In terms of its physicochemical mobility in soil, the hydrophilic acid fraction has also been lumped with the hydrophilic neutral fraction in some adsorption studies (*11*, *28*). While no adsorption studies were done as a part of this study, I suggest that the carboxylic acid functional groups that are likely to cause the negative charge on these substances would also be subject to interactions such as ligand exchange, just as is fulvic acid (*29*). The fact that the unseparated hydrophilic fraction contains both acid and neutral subfractions may be the explanation for the findings of Kaiser et al. (*11*) that the unseparated hydrophilic fraction adsorbed strongly to soil but the hydrophobic fraction adsorbed more strongly. Consequently, it seems likely that the hydrophilic acids may be more similar to fulvic acids in terms of both biodegradability and sorption behavior.

Rapid Mineralization of the Hydrophilic Neutral Fraction. The rapidly mineralized hydrophilic neutral fraction may contain uncharged labile compounds such as carbohydrates and perhaps other uncharged molecules originating from the plant material and microbial biomass. The cumulative percentage mineralized after 1 year was similar to that of glucose (Figure 2).

The mineralization rate of uniformly labeled ^{14}C-D-glucose was included for comparison with mineralization of the DOC

fractions. Glucose, along with the hydrophilic neutral fraction, was mineralized to the greatest extent of all substrates in soil. The mineralization curve for glucose did not fit the two-component model (eq 1) and so it was not fit to a modeled line in Figure 2. While glucose can be taken up directly and easily metabolized by cells, it has been shown in many studies that complete mineralization of a substantial proportion of the C is delayed for periods approaching a year. Amato and Ladd (*32*) added ^{14}C-glucose to 23 soils and at the end of 308 days, 14.8−28.9% of the radioactivity remained in the soil, with 2.1−15.3% remaining in living microbial biomass. Explanations for the persistence of the C originating from glucose have been that a proportion of glucose C is used to form cellular components that are inherently difficult to decompose after death of the cell, that these components are otherwise stabilized by humification or physical protection, or that a fraction remains in living cells. Comparison of the mineralization curves of glucose with the most rapidly mineralized fractions of the water-soluble C from decomposed leaf material suggests that a significant proportion of the C may fairly rapidly undergo the initial stages of decomposition, enzymatic hydrolysis, and cellular uptake while complete mineralization is delayed by its transformation into more refractory cellular components.

Mineralization of Phenolic Fraction. The phenolic fraction was defined as the component of water-soluble C that was hydrophobic at pH 7 but became hydrophilic at alkaline pH (Figure 1). This fraction, which comprised only 2.1% of the DOC, was relatively labile. Using ^{14}C ring-labeled pyrocatechol as a model phenolic compound, Martin and Haider (*30*) found a rate of mineralization of 24% in a soil after 84 days, while in this experiment over 50% the water-soluble phenolic fraction was mineralized. In a previous study, the NaOH-extractable phenolic fraction of decomposed litter lost only 36% ± 4.5% of the C over 1 year (*14*). The polyphenol content of the original undecomposed plant material was analyzed as 2.7% by the Folin Ciocalteau procedure (*31*), but polyphenols require methanol or NaOH to be extracted efficiently. Thus, the relatively small water-soluble phenolic fraction appeared to be more biodegradable than the less water-soluble NaOH-extractable phenolic fraction.

Mineralization of Whole, Unfractionated DOC Compared to That of Constituent Fractions. One way to evaluate whether the method of separation, or the inherent separation of the components, affected mineralization of the individual components is to compare a weighted average of the sum of its constituent fractions to that of the whole unfractionated DOC. The percentage of each constituent fraction in the water extract (from Table 1) was multiplied by the percentage mineralized (from Table 2) and then the product was summed for all fractions. This "weighted average mineralization rate" was 39.4% (± a weighted SE of 1.7%) compared to 43.5% ± 3.0% of the unfractionated DOC that was mineralized. A t-test showed no significant difference between the means (P = 0.33), supporting hypothesis 5. The unseparated hydrophilic acid and neutral fraction was also compared to a weighted average of the separate hydrophilic acid and hydrophilic neutral fractions (Tables 1 and 2). This "weighted average mineralization rate" was 44.5% (± a weighted SE of 2.9%) compared to 44.7% ± 1.9% of the unseparated hydrophilic acid and neutral fraction, with no significant difference between the means as shown by a t-test (P = 0.78).

This "reconstruction" of the mineralization rate of the whole, unfractionated DOC, and also the unseparated hydrophilic fractions, from the mineralization rates of the separated components corresponded reasonably well. A chemical separation of components might have two possible artifacts with respect to their properties in the natural material: (a) elution with 0.1 M NaOH, or precipitation with HCl in the case of humic acid, may produce chemical modifications that affect biodegradability, or (b) intermolecular interactions in the whole material that affect biodegradability are disrupted by separation. Chemical treatment with NaOH might be expected to increase biodegradability if it resulted in extensive hydrolysis. However, the mineralization rate of the humic substance fractions was still very slow. The removal of humic acid or hydrophilic acids that might have inhibitory effects on mineralization of other associated components might also be expected to increase mineralization rates of the isolated components. The humic acid fraction after it was adjusted to pH 7 was a colloidal suspension that was dispersed enough that it did not appear turbid, and so seemed likely to be comparable with the other liquid fractions in terms of physical accessibility. The correspondence of the unseparated fractions with the weighted average of the component fractions suggested that the possible artifacts did not affect the results.

Representativeness of the DOC Used in the Experiment. The DOC from decomposed leaf litter used in this experiment seemed typical in a number of ways of DOC found in soil solution and that leaching into streamwater. In this experiment I used litter that had decomposed for 1 year instead of fresh litter because DOC leaches from litter slowly over very long periods of more than a year (*16*). Adsorption experiments with forest floor material indicated that this prolonged leaching occurred largely because concentrations of DOC in the forest floor often approached the equilibrium for sorption and desorption of DOC (*16*). In addition, the distribution of fractions in fresh litter was different than that of DOC draining from the forest floor over most of the year, with a much larger readily decomposed hydrophilic neutral fraction (*3*, *15*).

In this study the concentration of DOC in the litter extract (50 mg L^{-1}) and the C/N ratio of 32 (not shown) was representative of that draining from the forest floor found in a number of studies. A review of 21 studies (*33*) noted that concentrations of DOC ranged between 21 and 80 mg L^{-1} and that the mean C/N ratio was 25.7 (± SD 9.8, n = 26, excluding three extreme values). The distribution of fractions (Table 1) was also representative of a number of studies of soil solution and streamwater. In a review of six studies that used the Leenheer fractionation procedure including soil solution and streamwater (*2*) and adding studies of Guggenberger and Zech (*3*), Jandl and Sollins, (*5*), and Smolander and Kitunen (*34*), the average percentages of DOC in water leaching from the forest floor were hydrophobic acid (humic and fulvic acids) 54%, hydrophilic acid 29%, and hydrophilic neutral 7%. For mineral soil solutions and streamwaters the corresponding averages were hydrophobic acid 41%, hydrophilic acid 32%, and hydrophilic neutral 13%.

Advantages of the Method. Adding ^{14}C-labeled substrates to evaluate the relative rates of mineralization of different fractions had several advantages: (1) substrates could be added to intact soil cores, with a natural microbial community; (2) added substrates could be distinguished from native C; and (3) the method yielded information on the entire decomposition curve for 1 year, not just initial rates. 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 dissolved or adsorbed C in interstices. In addition, the microbial community, including fungal mycelia, was intact. It was considered particularly important that fungal mycelia were minimally disturbed, since they are important in hydrolysis of lignin and humic substances (*7*, *35*). One disadvantage was that we cannot compare dissolved organic matter from a wide variety of sources because it requires labeling over a long period of growth, something much more difficult for perennial plants such as trees.

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