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DISSOLVED ORGANIC CARBON IN OLD FIELD SOILS: COMPOSITIONAL CHANGES DURING THE BIODEGRADATION OF SOIL ORGANIC MATTER

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Summary—The quality of soil dissolved organic carbon (DOC) was examined using the Leenheer DOC fractionation scheme, which separates soluble organic compounds into well-defined functional groups that exhibit similar reactive properties. DOC fractions were measured for five previously cultivated old fields undergoing secondary succession, and an undisturbed oak savanna. Despite differences in field age (time since abandonment), plant community composition, distribution and amounts of phytomass, C and N storage, and potential amounts of CO_2-C and net-N mineralization, the quality of soil DOC did not appear to differ. Nearly all DOC occurred in acid fractions (77%); hydrophilic acids alone constituted 50%. The fractionation procedure was also performed at four different times during a 210-day regulated *in vitro* incubation of the soils. Despite decreasing mineralization response to soil DOC concentrations, the fractional composition of the DOC remained relatively constant throughout the incubation. Although we could not evaluate DOC utilization, the results demonstrated that soil DOC was altered during the decomposition of soil organic matter; both total amounts and the relative N content of the hydrophobic acid fraction increased during the incubation period.

INTRODUCTION

Dissolved organic matter in soil has been used as an indicator of resource quality since it is thought to be readily-available to soil microbes. However, in a companion study, we suggested that not all the DOC in soil is labile (Cook and Allan, 1992). Determination of DOC lability requires characterization techniques that correspond to amounts of biological decomposition and subsequent nutrient release. Fractionation of soluble organic compounds into well-defined, functional groups is considered to be the most logical approach to determine lability. The heterogeneous make-up of soil organic matter makes detection and quantification of individual compounds impractical, and individual identification would not facilitate biodegradation modelling (Hunt, 1978; Leenheer and Huffman, 1976).

In this study, we measured the composition of soil DOC using Leenheer's DOC fractionation scheme to separate hydrophobic and hydrophilic-acids and -neutrals, and total bases (Leenheer and Huffman, 1976; Leenheer, 1981). Although the DOC fractionation scheme may not be the ideal assay for resource quality (Qualls R. G., unpublished Ph.D. thesis, University of Georgia, 1989), we believed it would help explain the relationship between DOC and soil mineralization.

Because different patterns of C and N cycling had been identified in old field and oak savanna soils by Zak *et al.* (1990) and Cook and Allan (1992), we hypothesized that the initial composition of dissolved organic matter would differ among the soils. We expected that these compositional differences would relate to field age (time since agricultural abandonment), plant community composition, or estimates of potentially mineralizable C and N. In addition, we reasoned that large changes would occur in the composition of soil DOC as the soil organic matter was decomposed during a regulated incubation procedure.

MATERIALS AND METHODS

Vegetation sampling and analysis

As described in the companion study (Cook and Allan, 1992) a series of five old fields, aged 12, 32, 37, 55 and 62 yr since abandonment, and an uncultivated oak savanna site were selected at the Cedar Creek Natural History Area. Litter was collected from five sampling areas at each site on 14 and 15 April 1989. Samples consisted of all dead, above-ground plant matter in each sampling area, clipped at the soil surface. Litter was dried at 65°C for 24 h in a forced air drying oven.

Roots and rhizomes that did not pass through a 2 mm sieve were rinsed by brief sonication in 0.1 M NaH_2PO_4 at pH 7 (Gourley *et al.*, 1989). Cleaned roots and rhizomes for each field were dried in a convection oven at 65°C to constant weight. Obviously dead and partially decayed organic material was not included in this weight, but otherwise no attempt was made to distinguish between live and dead roots.

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299

Table 1. Estimated percent cover (2 October 1989) for live above-ground biomass by vegetation category in Cedar Creek old fields and oak savanna

Vegetation category	Estimated percent cover*					
	Old fields					Oak savanna
	12 yr	32 yr	37 yr	55 yr	62 yr	
Prairie graminoids	<1	42 (3)	68 (13)	29 (17)	61 (8)	42 (7)
Non-prairie graminoids	13 (2)	12 (6)	6 (3)	59 (18)	1 (<1)	17 (5)
Weedy annual/biennial forbs	6 (1)	2 (1)	1 (<1)	1 (<1)	6 (3)	3 (1)
Weedy perennial forbs	0	3 (1)	15 (14)	3 (2)	6 (4)	12 (9)
Aggressive prairie forbs	0	<1	2 (1)	1 (<1)	4 (1)	4 (1)
Less-aggressive prairie forbs	0	0	0	1 (<1)	1 (<1)	1 (<1)
Total live above-ground cover	19 (3)	59 (6)	91 (2)	94 (1)	80 (6)	79 (2)
Dominant vascular plants†	*Aristida basiramea*	*Schizachyrium scoparium* *Poa pratensis*	*Andropogon geradi* *Solidago nemoralis* *P. pratensis*	*P. pratensis* *Bromus inermis* *Schizachyrium scoparium* *Andropogon geradi* *Eragrostis spectabilis* *Quercus ellipsoidalis* *Q. macrocarpa*	*Stipa spartea* *Andropogon geradi* *Carex* spp *Artemisia ludoviciana*	*Schizachyrium scoparium* *Carex* spp *Andropogon geradi* *P. pratensis*

*Mean estimated percent cover with standard error in parentheses ($n = 5$).
†Average cover ($n = 5$) ≥5%; listed in order of decreasing cover.

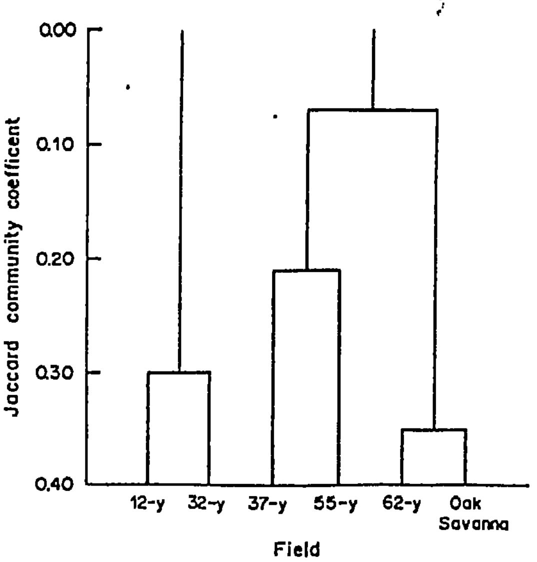

Fig. 1. Plant community similarities based on Jaccard coefficients for Cedar Creek old fields and oak savanna (2 October 1989).

Total Kjeldahl-N in litter and below-ground phytomass was determined by digesting samples with KjelTabs (Fisher Scientific, Pittsburgh, Pa), and analyzing free ammonia in the digests with a Wescan Ammonia Analyzer. Total C was determined by dry combustion in a LECO automatic C analyzer (LECO, St Joseph, Mich.).

Vascular plant species and estimated percentage cover were determined within each sampling area on 2 October 1989. Percent cover by species and bare ground was visually estimated from overhead, considering only two dimensions. To facilitate comparisons between the successional stages, plant species were assigned to six vegetation categories (Table 1). This classification scheme (Jastrow, 1987) is based on the probability of a given species occurring in an undisturbed tallgrass prairie and its ability to invade disturbed sites. Jaccard community coefficients (indices of similarity; Fig. 1) were calculated from species presence and absence data (Goodall, 1978).

DOC fractions

The DOC separation technique was the analytical DOC fractionation scheme developed by Leenheer and Huffman (1979) and Leenheer (1981). Dissolved organic compounds were separated into five, operationally defined categories that combine characteristics of molecular size and surface charge: (1) hydrophobic acids, similar to soil fulvic and humic acids; (2) hydrophilic acids, consisting of humic and non-humic substances with lower molecular size and higher COOH:C ratios (e.g. carboxylic acids); (3) hydrophobic neutrals, primarily composed of hydrocarbons, long-chain fatty acids and carbonyls, alkyl alcohols, and humic substances with few functional groups; (4) hydrophilic neutrals, including simple neutral sugars, alcohols, and non-humic bound polysaccharides; and (5) total bases, comprising proteins, free amino acids and amino sugars, and aromatic amines (Leenheer and Huffman, 1979; Leenheer, 1981; Thurman, 1985; Qualls R. G., unpublished Ph.D. thesis, University of Georgia, 1989).

Hydrophobic- and hydrophilic-bases were not separated because relative amounts of hydrophobic bases are insignificant in natural and soil water (David *et al.*, 1989; Leenheer, 1980; Antweiler and Drever, 1985). Resins were purified as specified by Leenheer (1981).

Soil DOC obtained to measure total amounts (Cook and Allan, 1992) was also used for compositional analysis. Samples were concentrated threefold using a Savant SpeedVac centrifugal vacuum evaporator (Savant Instruments Inc., Farmingdale, N.Y.) so that the concentration of the 225 ml DOC sample fell within the linear range of the XAD-8 adsorption isotherm (Leenheer and Huffman, 1976; Leenheer, 1981). Two of the three water extracts were fractionated for the initial soil sample (0 days) and 700 g samples incubated for 35 and 210 days. Only one replicate was fractionated after 14 and 105 days of incubation. Average values and ranges are reported where two replicates were fractionated. DOC was determined using a standard low temperature Dohrman DC-80 total organic C analyzer (Xertex-Dohrman, Santa Clara, Calif.).

DON was measured in the hydrophobic acid fraction, because it has been reported that a significant portion of N-containing organics and nitrite can be complexed with humic-like substances and polyphenols which may resist degradation (Lytle and Perdue, 1981; Azhar *et al.*, 1986; Brown, 1982; Fog, 1988). DON was determined by subtraction of inorganic-N from total Kjeldahl-N. Inorganic-N was detected with a Wescan Ammonia Analyzer (NO_3^- reduced to NH_4^+ using a Zn column). Kjeldahl-N was determined by digesting samples with permanganate-reduced iron (to reduce free NO_3^- to NH_4^+) and 100:10:1 K_2SO_4:$CuSO_4 5H_2O$:Se in conc. H_2SO_4, and measuring free ammonia with a micro-Kjeldahl distillation apparatus.

RESULTS

Sites at Cedar Creek Natural History Area from which samples were collected are characterized by distinctly different plant communities (Table 1). In general, these communities typify a succession of vascular plant types that occurs at Cedar Creek and in other prairie ecosystems following agricultural disturbance (Inouye *et al.*, 1987; Jastrow, 1987). In the fields used for this study, prairie graminoids and prairie forbs are established by 32 yr, while non-prairie graminoids become less prevalent. Percent cover of weedy forbs appeared to be less dependent on field age, but these species represent only a small portion of the vegetation in the old fields and oak savanna. A slight discontinuity in the gradual succession of vascular plant types occurs in the 55 yr old field, which contains a substantial amount of *Bromus inermis* (a non-prairie graminoid), but overall changes in vegetation types at Cedar Creek seem to primarily depend on field age; thus supporting the findings of Inouye *et al.* (1987).

Jaccard community coefficients are illustrated in Fig. 1 to show similarities among the fields. The 62 yr old field and the oak savanna were most similar in terms of presence or absence of specific plant species, closely followed by the 12 and 32 yr old fields, and the 37 and 55 yr old fields. While the two youngest fields were quite similar to each other, they had very few species in common with the four older fields. We expected these plant community differences to be reflected in the composition of soil DOC.

Total storage (soil + phytomass + litter) of C and N in the Cedar Creek ecosystems increased with time since agricultural disturbance (Table 2). In spring, the majority of organic C and N in the systems was stored in the soil (mean ± SE of 77.0% ± 3.2 and 88.8% ± 1.8, respectively), while the remainder was found in the below-ground phytomass and above-ground litter.

Except for the 12 yr old field (with only 19% cover), above-ground litter and percent C and N allocated to this compartment were quite similar among the fields and oak savanna. In contrast, below-ground phytomass allocation (Fig. 2) and percent C and N stored in this compartment strongly reflect the vegetation patterns noted above and in Table 1. Particular reference is made to the 37 and 55 yr old fields, which support a relatively high graminoid cover and allocated a considerably higher proportion of phytomass (both C and N) below-ground. In addition, these sites had significantly

Table 2. C and N storage in Cedar Creek old fields and oak savanna (14 and 15 April 1989)

	Total C				Total Kjeldahl-N			
Field	Total storage*	Soil	Below-ground phytomass	Litter	Total storage*	Soil	Below-ground phytomass	Litter
	$mol\ m^{-2}$	%			$mol\ m^{-2}$	%		
12 yr	75.5	88.7	7.9	3.5	4.76	95.2	3.9	0.9
32 yr	91.5	74.0	14.6	11.3	5.17	88.6	7.4	3.9
37 yr	134	67.5	24.9	7.7	6.46	87.3	9.2	3.4
55 yr	146	70.1	21.6	8.3	8.45	81.8	13.9	4.2
62 yr	195	82.0	9.2	8.7	11.5	89.4	5.1	5.4
Oak savanna	166	80.0	9.5	12.5	8.83	90.7	4.7	4.7

*Litter + below-ground phytomass + soil to 10 cm depth.

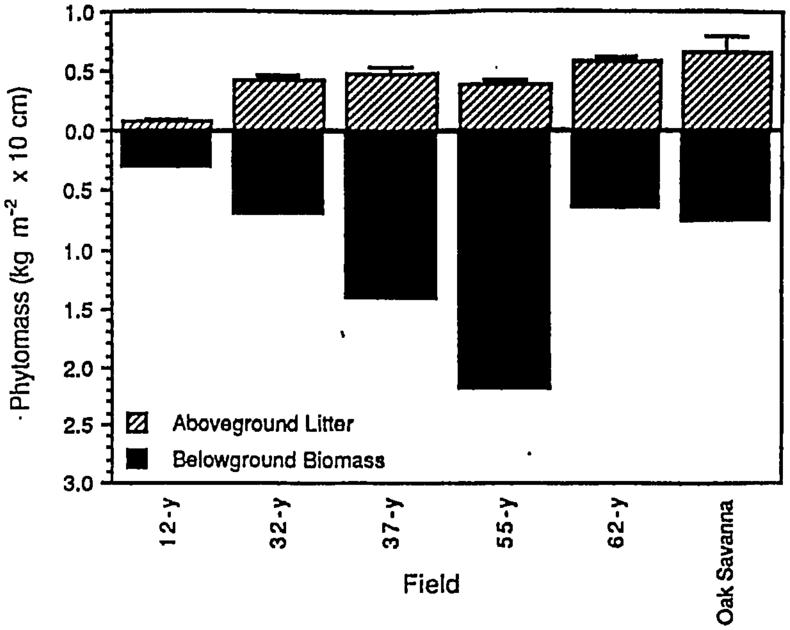

Fig. 2. Above- and below-ground (to 10 cm depth) phytomass allocation for Cedar Creek old fields (standard error bars for above-ground phytomass; $n = 5$) (14 and 15 April 1989).

higher potentially-mineralizable C than the others (Cook and Allan, 1992). This preferential allocation in the graminoid communities could result in increased rhizodeposition or root turnover. In that case, we would expect a similar pattern to be exhibited by the DOC fractions for these fields.

Contrary to our expectations, the composition of soil DOC, as determined by the DOC fractionation scheme, did not differ much among the soils collected from Cedar Creek old fields and oak savanna prior to incubation (Fig. 3). It was also unexpected that while there was a decrease in the mineralization rate response to the size of the DOC pool with duration of incubation (Cook and Allan, 1992), the percentage of DOC in the various fractions remained fairly constant. Nearly all of the DOC was found in the acid fractions (mean ± SE for all 6 soils and replicates was 77.2% ± 1.5%), of which the hydrophilic acids alone composed one-half of the total DOC (50.0% ± 1.4). In decreasing order, the remaining DOC was divided among the hydrophilic neutral, base, and hydrophobic neutral fractions. There was some increase in the base fraction from *ca* 5 to 15–20%. Yet while the proportion of total DOC in the fractions scarcely changed during the course of the incubation, the relative DON content (DON:DOC) in the hydrophobic acid fraction increased dramatically from about 1:12 to 1:3 during the incubation (Fig. 4), with the exception of soils from the 12 and 32 yr old fields at 210 days.

DISCUSSION

Soil DOC in the prairie-oak savanna ecosystems at Cedar Creek Natural History Area was primarily composed of hydrophilic acids. Other studies have also shown that acid fractions dominate total DOC (Qualls R. G., Ph.D. thesis, University of Georgia, 1989; David *et al.*, 1989) although the relative proportion of hydrophobic acids found in the DOC of hardwood forest soils appears to be much greater than in the prairie-oak savanna ecosystems at Cedar Creek. This shift in the type of dissolved acids may result from differences in plant composition and turnover, or dissimilar soil sorption characteristics.

Despite differences in the length of time since abandonment, plant community composition, distribution and amounts of phytomass, soil C and N, and potential amounts of CO_2-C and net-N mineralized, the composition of soil DOC, as determined by the DOC fractionation scheme, did not appear to differ among soils from Cedar Creek old fields and oak savanna. This finding, in conjunction with total DOC measurements from a companion study (Cook and Allan, 1992), indicates that point-time measurements of the amounts and fractions of soluble organic matter in field-moist soils are poor predictors of available resources for decomposition and nutrient release.

One explanation for the poor relationship between DOC concentrations and mineralization rates may be that biodegradation is likely to be highest at adsorption sites rather than in soil solution (Qualls R. G., Ph.D. thesis, University of Georgia, 1989). At these sites, organic matter is concentrated, populations of heterotrophic microorganisms are likely to be highest, and there is direct contact between exoenzymes and substrates. A second explanation is the inability of the fractionation scheme to distinguish recalcitrant macromolecular DOC from more labile monomeric substances complexed with a macromolecular core. In the latter case, a high molecular

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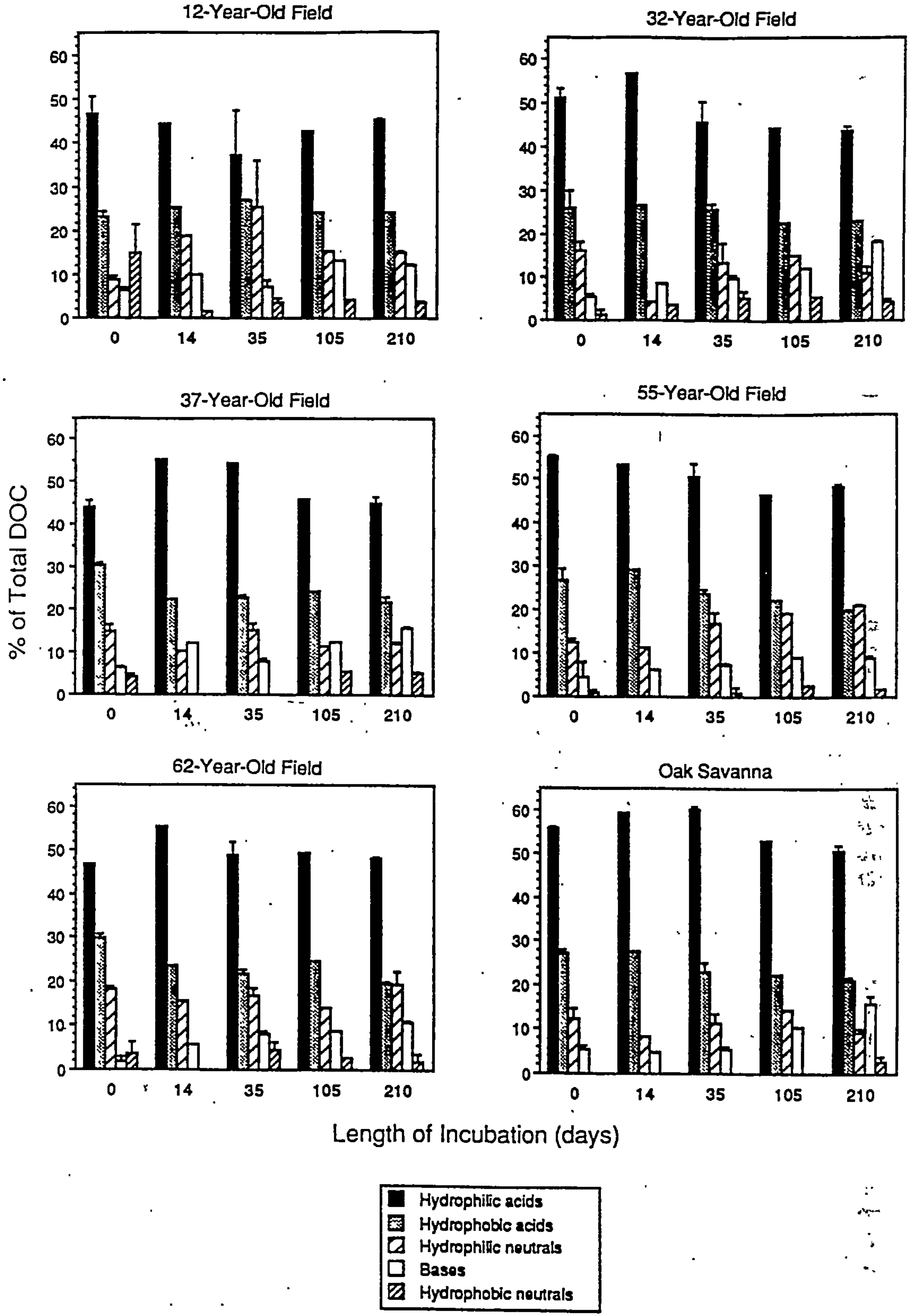

Fig. 3. Compositional changes in DOC during the incubation of Cedar Creek soils (mean values, $n = 2$, with range bars for time = 0, 35 and 210 days).

weight fraction would be capable of supporting considerable bacterial growth; otherwise, the high molecular weight fraction may retard bacterial growth (Meyer *et al.*, 1987).

Another unexpected finding of this study was that the DOC fractionation scheme did not measure changes in the DOC "quality" during the biodegradation of finite amounts of soil organic matter. This

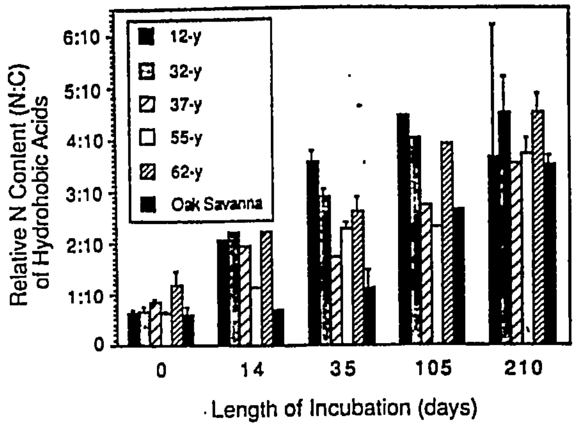

Fig. 4. Relative N content of hydrophobic acids during the incubation of Cedar Creek soils (mean values, $n = 2$, with range bars for time = 0, 35 and 210 days).

does not mean that DOC was not used or altered during the biodegradation of soil organic matter, because total amounts changed (Cook and Allan, 1992) and the relative N content of the hydrophobic acids increased during the incubation (Fig. 4).

Although the amount, size, and surface charge characteristics of DOC may not be useful indices of biodegradability, these parameters may be important for estimating the translocation and export of energy and nutrients in the soil profile. For example, Schoenau and Bettany (1987) demonstrated that soluble, nutrient-enriched fulvic acids in grassland soils are susceptible to deep leaching by percolating water, and may affect turnover dynamics in the surface horizons. McGill and Paul (1976) found that complexation with humic acids accounted for 43% of recently immobilized N. In this study, it was shown that N enrichment of the hydrophobic acid fraction was dramatic (Fig. 4), and occurred within a relatively short time (14 days). While we cannot predict that similar enrichment would occur during biodegradation in the field, a model proposed by Fog (1988) suggests that these nitrogenous substances may be condensation products which are unavailable for biological decomposition, and could represent the potential for significant nutrient losses.

In summary, while the Leenheer fractionation scheme may be useful for comparing different soils or predicting potential C translocation, the fractions do not reflect changes in DOC decomposability or N enrichment over time, nor are they useful indicators of available microbial resources.

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