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QUANTIFYING FINE-ROOT DECOMPOSITION: AN ALTERNATIVE TO BURIED LITTERBAGS

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Abstract. Our understanding of fine-root decay processes is derived almost exclusively from litterbag studies. However, preparation of roots for litterbag studies and their subsequent decay within litterbags represent major departures from in situ conditions. We hypothesized that litterbag studies misrepresent fine-root decay and nutrient release rates during decomposition. To test these hypotheses we developed a new intact-core technique that requires no a priori root processing, retains natural rhizosphere associations, and maintains in situ decay conditions. Using both litterbags and intact cores, we measured annual decay rates and nitrogen release from newly senesced fine roots of silver maple, maize, and winter wheat. After one year, mass loss was 10–23% greater, and nitrogen release was 21–29% higher within intact cores. Differences appeared to result from litterbag-induced alterations to decomposer dynamics and from unavoidable changes to fine-root size-class composition within litterbags. Our results suggest that fine-root decay and nutrient turnover occur significantly faster than estimated from litterbag studies. By minimizing disturbances to roots, soil, and rhizosphere associates prior to root decay, the intact-core technique provides an improved alternative for measuring fine-root decomposition.

Key words: decomposition; fine-root decay; fine roots; intact-core technique; litterbags alter decomposition dynamics; maize; maple; nitrogen release; nutrient release rates; wheat.

INTRODUCTION

Fine-root growth accounts for a large proportion of net primary production in terrestrial ecosystems (Vogt et al. 1996, Gill et al. 2002). Consequently, fine-root turnover represents a major pathway for carbon and nutrient fluxes from plants to soils. However, current difficulties with measuring fine-root decomposition rates hinder our ability to accurately assess the magnitude of this important process.

The litterbag technique is by far the most commonly applied method for measuring fine-root decay (Silver and Miya 2001), and thus has provided us with our current understanding of elemental fluxes through decaying fine roots. However, the appropriateness of litterbag use with fine roots has not been verified (McClaugherty et al. 1982, 1984, Fahey et al. 1988, Ruark 1993, Fahey and Hughes 1994). Fine-root decay rates measured with litterbags appear too low to account for observed fine-root turnover rates obtained using minirhizotrons and other in situ approaches (McClaugherty et al. 1982, 1984, Gholz et al. 1986, Hendrick and Pregitzer 1992, Ruark 1993, Fahey and Arthur 1994). Litterbag preparation typically involves separating roots from soil and rhizosphere communities, washing and drying them, and often incorporates live root material. Thus, data derived from litterbags may misrepresent actual mass loss and nutrient turnover rates. We hypothesized that litterbag use misrepresents in situ decay rates and nitrogen release rates from decaying fine roots.

To test these hypotheses we developed a new intact-core technique that closely mimics in situ fine-root decay conditions. This intact-core method differs from litterbag studies in several important ways. First, because cores are sampled from field soils and are maintained as intact units, the initial mass of roots within each core is unknown. Consequently, mass loss estimates from intact cores are based on changes in population means through time, rather than on changes in individual samples, as is possible with litterbags. This requires that enough cores be processed to accurately quantify the mean root mass at each sampling date. Because fine-root mass varies between locations and vegetation types, the number of cores needed must be determined in advance by each investigator. For this experiment, initial sampling in a maize field (n = 24 cores) gave a mean (95% CI) root biomass of 0.88 (0.68–1.09) g/core. The coefficient of variation did not decline appreciably after 15 samples, so this sample size was applied to all three species, following verification at the time of sampling (see *Methods*, below). Second, because there is no a priori root processing of intact cores, the natural distribution of fine-root size classes is maintained, and rhizosphere associations are kept intact. Therefore, fine roots decompose under more natural conditions than allowed for with litterbags. Intact cores share with litterbags flexibility in experimental design, allowing for reciprocal exchange

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of substrates from one location to another, or for insertion of multiple substrates at a common site. In this study we compared decay and nitrogen release rates from the newly senesced fine roots of *Acer saccharinum* L. (silver maple), *Zea mays* L. (maize), and *Triticum aestivum* L. (winter wheat) using both litterbags and intact cores, all placed at a common site.

Methods

Field methods

Intact soil cores, 15 cm deep by 5.3 cm in diameter, containing the fine roots of *Acer saccharinum* L. (silver maple), *Triticum aestivum* L. (winter wheat), and *Zea mays* L. (maize) were collected with a steel soil corer into open-ended clear plastic sleeves in 1999. Initial sampling dates for all species were chosen to maximize the proportion of newly senesced fine roots within cores. To sample the senesced roots of silver maple, trees growing in a dense 14-yr-old monoculture were girdled prior to bud break in early spring and roots were sampled following leaf-out in May. Winter wheat was collected immediately following harvest in July, while maize samples were collected following plant senescence, just prior to harvest, in August. By sampling monocultures, we collected cores containing the fine roots of one species only. Cores were sampled nonrandomly at uniform distances from plants to minimize core-to-core variability in fine-root biomass. Cores collected nonrandomly cannot be used to estimate site-level fine-root biomass. During core collection, additional roots were harvested from identical locations and depth increments for use in the litterbag experiment and for tissue chemistry analyses. Following field collection, all samples were stored temporally in a walk-in cooler at 4°C. Determination of per core root masses soon after root sampling verified that variability among cores was quite stable at $n = 15$ cores. More cores would be required from sites where root-mass variability is greater than we encountered.

Prior to core reinsertion into the ground, both ends of each intact core were fitted with 160-μm polyethylene mesh caps. The same mesh was also used to construct 6 × 10 cm litterbags. Roots placed in litterbags were gently washed free from soil, air-dried, and sorted into diameter classes of ≤1-mm and >1-mm but ≤2 mm. Litterbags were filled with ~0.4 g of each diameter class, except for wheat, which lacked roots >1 mm in diameter.

All litterbags and intact cores were inserted into a common site, a streamside meadow dominated by *Agropyron repens* L., *Bromus inermis* Leysser, *Dactylis glomerata* L., *Phalaris arundinaceae* L., and *Poa pratensis* L., in Story County, Iowa, USA (42°11′ N, 93°30′ W). Intact cores were soaked to field capacity, reinserted in their original orientation 5 cm below the soil surface, and covered with soil. Litterbags were also soaked in water and then inserted at an angle of 45° at the same depth as the intact cores. Decay studies of silver maple, winter wheat, and corn roots were initiated in June, August, and November of 1999, respectively. Fifteen intact cores containing the senesced fine roots of each species were systematically removed at the time of their original insertion and in August, November, March, and June for up to one year from decay initiation. In total, ~75 intact cores per species were processed. Litterbag insertion and removal was synchronized with the intact-core schedule, with five litterbags per species removed per collection time, excluding the first collection time. In total, 20 litterbags/species were systematically removed during the experiment.

Soil temperature in the meadow was monitored using thermocouples, with temperature readings taken 12.5 cm deep at 30-min intervals, both inside ($n = 6$ thermocouples) and outside ($n = 10$ thermocouples) of intact cores. Soil moisture was determined gravimetrically at the 5–20 cm soil depth every two weeks for the duration of the experiment. Destructive sampling of extra intact cores was used to determine temporal changes in intact-core soil moisture.

Following field decay, all cores and litterbags were stored at 4°C and sorted within one month from their removal. Prior to processing, all cores were soaked overnight to loosen adhering soil particles and to reduce root fragmentation during sorting. Cores were then gently washed by hand through a series of sieves ranging in mesh size from 4.0 mm to 0.5 mm. All material caught in the screens was rinsed into plastic tubs where organic debris and roots were hand separated. Any tiny root pieces not caught on the 0.5-mm screen were considered particulate soil organic matter and were not collected. Roots were sorted into three size fractions: ''fragments,'' i.e., very small, or broken roots ≤1 mm in diameter; intact roots ≤1 mm in diameter; and roots >1–2 mm in diameter. Initial cores required an average of 2 h to process fully; times were shorter for older cores with fewer roots. Litterbags were processed and analyzed following this same procedure.

Chemical analyses

Roots from initial harvests were used to establish species-specific air-dry to oven-dry (65°C) and oven-dry to ash-free-dry (500°C) mass conversion ratios and for initial tissue chemistry analyses. All mass values reported in this paper refer to ash-free dry masses. Following 65°C drying, all samples were ground to pass through a 70-gauge (212 μm) mesh screen. A modified Van Soest forage fiber technique (Goering and Van Soest 1970, Anonymous 1997) was used to determine initial tissue chemistry (cell soluble, hemi-cellulose, cellulose, and lignin content) for each species. Tissue C and N contents were determined using a Carlo Erba NA 1500 elemental analyzer (CE Instruments, Milan, Italy).

TABLE 1. Initial tissue chemistry for each species, categorized by fine-root diameter classes.

Treatment	Cell solubles (%)	Cell wall constituents (%)	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)	N (%)	C (%)
Silver maple							
Fragment	38.5	61.5	7.9	16.2	29.0	1.7 ± 0.04	38.0 ± 1.5
≤1 mm	41.8* ± 0.7	58.2* ± 0.7	7.2 ± 0.5	15.3* ± 0.3	30.9* ± 0.4	1.6* ± 0.01	44.0* ± 0.4
>1 mm, ≤2 mm	38.5 ± 0.5	61.5 ± 0.5	11.4 ± 0.7	24.5 ± 0.8	23.8 ± 1.1	1.1 ± 0.03	48.8 ± 0.2
Maize							
Fragment	46.9	53.1	17.6	13.7	8.4	1.8 ± 0.03	29.2 ± 0.4
≤1 mm	28.1* ± 0.5	71.9* ± 0.5	29.6* ± 0.3	31.0* ± 0.4	6.3 ± 0.3	1.6* ± 0.09	31.5* ± 1.5
>1 mm, ≤2 mm	23.3 ± 0.7	76.7 ± 0.7	32.3 ± 0.6	35.3 ± 0.3	6.9 ± 0.2	1.3 ± 0.02	44.7 ± 1.0
Winter wheat†							
Fragment	40.4	59.6	20.8	13.2	9.0	1.7 ± 0.11	28.0 ± 0.8
≤1 mm	22.9 ± 2.3	77.1 ± 2.3	34.9 ± 1.6	27.6 ± 1.0	6.8 ± 0.1	1.6 ± 0.06	35.5* ± 1.2
>1 mm, ≤2 mm	...	...	...	...	...	...	...

Note: Data are means ± 1 SE.
* $P < 0.05$ (ANOVA; significant differences within species but among diameter classes).
† Winter wheat lacked fine roots >1 mm in diameter.

Data analysis

All decay and nutrient release data were fit to exponential decay models. The effect of methodology (i.e., litterbags vs. intact cores) on fine-root decay was tested using paired t tests of the decay coefficients ($n = 3$ pairs) for both mass loss and N release. Decay and nutrient release rates reported represent composite values for all fine roots ≤2 mm. As such, these values may not reflect values for the smallest, most labile roots. Differences in initial tissue chemistry and C and N concentrations among species, for each root diameter class, were determined using one-way analysis of variance (ANOVA, $n = 3$ samples/species for each diameter class). Differences among root-diameter classes within each species were also determined using ANOVA ($n = 3$ samples/diameter class for each species). Inadequate sample mass prevented the replication of tissue chemistry analyses (i.e., cellulose, lignin, etc.) on fragment roots, thereby preventing statistical comparisons. Fragment root C and N contents were replicated ($n = 3$ samples/species), and statistical comparisons were made.

RESULTS

There were no significant differences between temperatures inside and outside of soil cores. Gravimetric soil moisture contents within the study site never fell below 15% and generally ranged from 20 to 30%. Soil moisture levels inside intact cores were slightly higher than in the meadow soil, but soil moisture changes inside intact cores mimicked soil moisture fluctuations during both soil drying and rewetting periods. Seasonal changes in the moisture content of litterbags were not monitored.

Initial tissue chemistry (Table 1) differed significantly among species within root-diameter classes for all measured variables except initial N concentration in root fragments and ≤1-mm diameter roots (ANOVA, $P < 0.05$). Maple roots had higher concentrations of lignin and cell solubles, while maize and wheat roots contained higher proportions of cellulose, and hemicellulose (Table 1). All species were similar in N composition, but variations in C concentrations produced significantly higher C:N in maple roots than in winter wheat and maize roots (ANOVA, $P < 0.001$). Within species, root-diameter classes differed significantly for most tissue chemistry measures (ANOVA, $P < 0.05$, Table 1).

Following one year, fine-root mass loss was greater within intact cores than within litterbags (paired t test, $P < 0.01$). Wheat and maize roots decomposed faster than did silver maple roots within both litterbags and intact cores (Fig. 1a). Maple roots within intact cores lost 23% more mass during the first year than did maple roots within litterbags, whereas wheat and maize fine-root mass loss was 10% and 11% greater within intact cores than in litterbags respectively. This suggests that litterbags may differentially influence measured decay rates among species.

Variability in root mass among litterbags increased through time, while variability in root mass among intact cores remained relatively constant through time (Fig. 2). This effect was more pronounced in the faster decaying wheat and maize roots than in the slower decaying silver maple roots.

N loss rates were significantly slower from roots decaying within litterbags than from roots decaying within intact cores (paired t test, $P < 0.01$). This trend again held true for all three species (Fig. 1b). Following one year, the proportion of original N remaining in intact-core roots was 29% lower in silver maple, 27% lower in maize, and 21% lower in winter wheat than in litterbag roots (Fig. 1b). As with mass loss, species-specific differences in N release between decay methodologies suggests species × methodology interactions. This interaction minimizes the potential for use of cor-

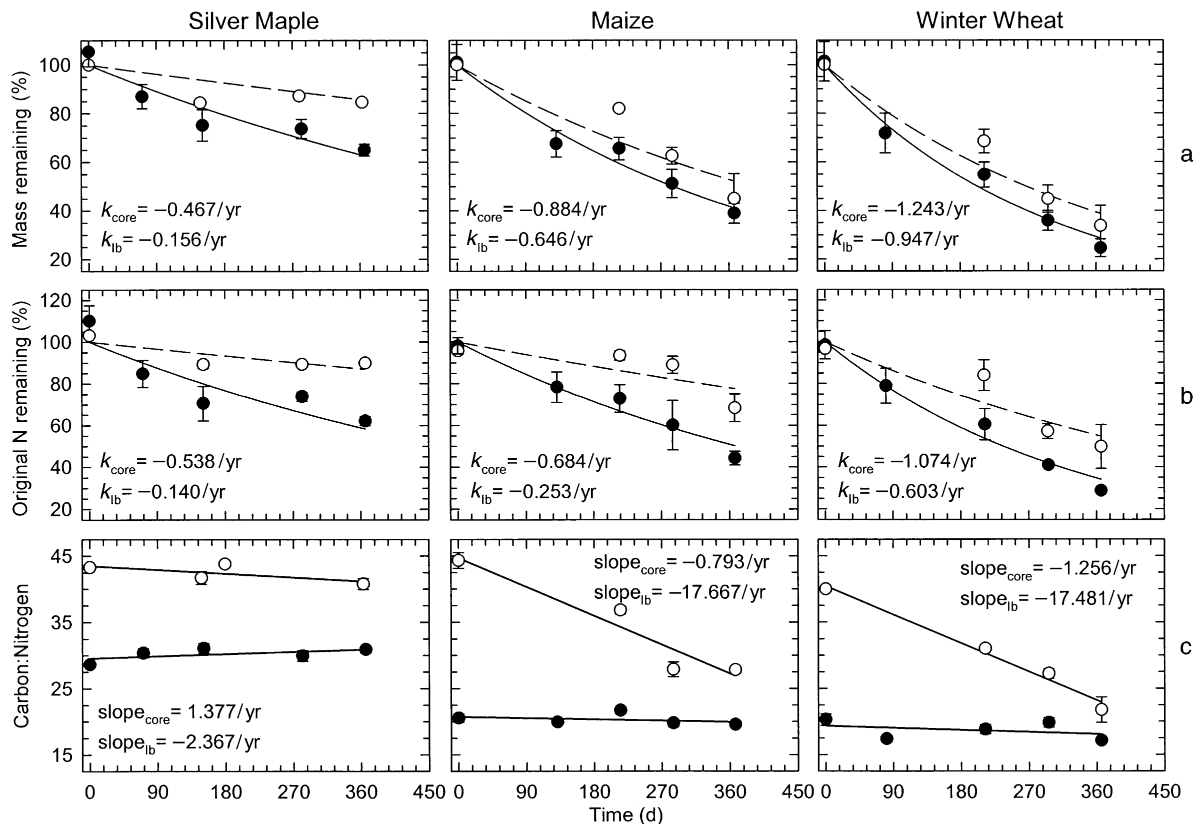

FIG. 1. (a) Mass loss, (b) nitrogen loss, and (c) changes in C:N for fine roots of silver maple, maize, and winter wheat decomposed in a riparian meadow in central Iowa, USA. Solid circles represent intact cores, and open circles represent litterbags (lb); k values are based on exponential decay models. In the upper six panels, the intact-core data are normalized such that 100% equals the $t = 0$ intercept of the exponential decay regression; error bars are ± 1 SE.

rection factors to adjust litterbag-determined decay and N release rates to the more realistic intact-core rates. There were no significant changes through time in C:N (Fig. 1c) for any species within intact cores. In contrast, C:N declined significantly through time for maize ($P < 0.05$) and winter wheat ($P < 0.01$) roots within litterbags.

DISCUSSION

The fine roots of all three species decayed faster within intact cores than within litterbags (Fig. 1a). Following one year, there was 23%, 11%, and 10% less root mass remaining in intact cores than in litterbags for silver maple, maize, and winter wheat, respectively. Fine roots within litterbags also released N more slowly than did roots within intact cores (Fig. 1b). In contrast to litterbags, N losses from roots within intact cores closely paralleled mass loss (Fig. 1c). Similar, parallel relationships between mass loss and nutrient release from decaying fine roots (Fig. 1c) have been found with other in situ based approaches, including a trenched-plot experiment in a Puerto Rican subtropical wet forest (Silver and Vogt 1993) and in a clear-cut experiment in a northern hardwood forest (Fahey and Arthur 1994). These findings suggest that litterbag use underestimates fine-root decay rates, and strongly misrepresents N release rates from fine roots decaying in situ.

At the initiation of our study, root-mass variability among samples was greatest within intact cores. However, root-mass variability among litterbags increased through time, while remaining relatively constant within intact cores (Fig. 2). Because litterbags initially had low variability in root mass, increases in variability could only result from differences in litterbag-to-litterbag decay rates. This may have resulted from differences in litterbag-to-litterbag colonization rates by microbial and microarthropod communities, or may have resulted from litterbag-to-litterbag differences in decomposer community composition and subsequent performance (McClaugherty et al. 1984, Ruark 1993). In contrast, the intact cores included their preexisting root–rhizosphere associations within their undisturbed soil matrices, and observed variability in root mass was relatively constant through time. This suggests the presence of a preexisting decomposer community acting immediately upon fine roots within the intact cores. We hypothesize that litterbags decreased fine-root decay rates and increased mass-loss variability through time, in part, by altering the dynamics of the decomposer community.

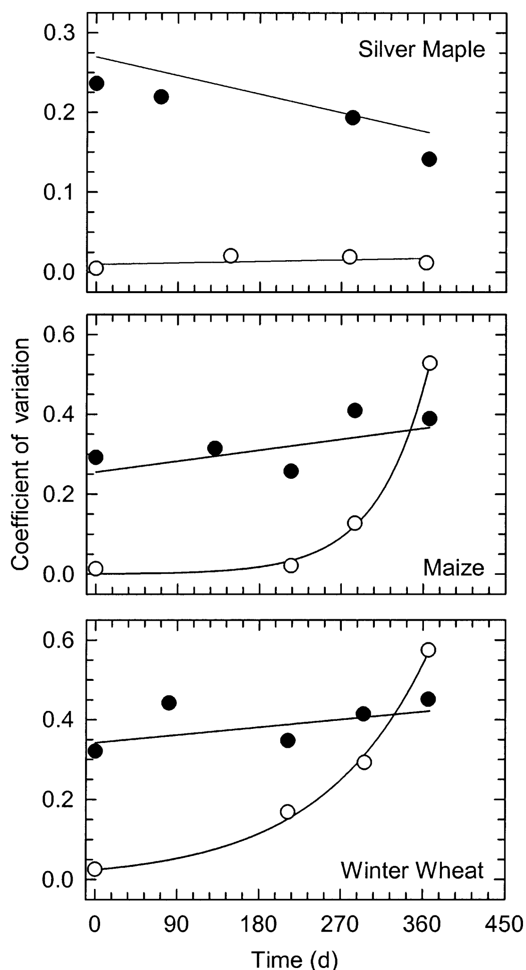

FIG. 2. Coefficients of variation of root biomass (measured in grams) among litterbag and intact-core samples over the first year of decay of silver maple, maize, and winter wheat fine roots. Solid circles represent intact cores, and open circles represent litterbags. Lines of best fit are included only to highlight general trends and are not intended to represent statistical comparisons.

Litterbags also contained, on average, larger diameter roots than those found within intact cores. This was and is an unavoidable and unintended consequence of litterbag preparation. The fragile nature of the smallest roots precludes their inclusion during litterbag filling. In our study, winter wheat, silver maple, and maize roots within intact cores initially consisted of 60%, 35% and 24% fragment-root component, on a mass basis (Table 2). In contrast, at the initiation of this study litterbags did not include this component at all (Table 2). Whenever litterbags are used there will be an undeniable bias to include larger than average roots, simply because they are more likely to remain as intact pieces. Because tissue chemistry varies with root diameter (Table 1), any shifts in root diameter-class distribution resulting from litterbag preparation will influence measured decay rates. This is not an artifact of our study: data summarized by Silver and Miya (2001: Table 4) show that the fine roots used in litterbag studies contain, on average, less Ca, less N, and more lignin, and decayed more slowly, than did fine roots in other types of decomposition studies. In general, fine roots have higher nutrient concentrations than coarser roots (e.g., Gordon and Jackson 2000). We found that smaller-diameter roots generally had higher N contents, while larger-diameter roots contained more C, cellulose, and hemi-cellulose (Table 1).

In our study, roots within litterbags had almost twice the C:N of roots found within intact cores (Fig. 1c). Initial differences in C:N were long lasting, with final C:N of roots within litterbags never decreasing to the initial C:N of intact core roots, even after one full year of decay. As with changes in variability, methodological differences in C:N through time were most notable for the faster-decaying maize and winter wheat roots (Fig. 1c). For example, in litterbags containing maize or wheat the C:N decreased significantly through time, whereas C:N remained constant through time in the intact cores (Fig. 1c). These differences resulted in dramatically different N release patterns between the two methods. Nitrogen release from decaying roots (per gram of roots per year) was 4.2, 3.5, and 2.1 times greater within intact cores than within litterbags for silver maple, maize, and winter wheat, respectively. Roughly 80% of root decay studies are based on the litterbag method (Silver and Miya 2001). Our data suggest that estimates of fine-root decay and N release derived from litterbag use, and models incorporating those estimates, substantially underestimate fine-root contributions to belowground elemental fluxes.

The intact-core technique that we applied provides an alternative to litterbag studies. By maintaining roots within an intact soil core, this technique preserves the important relationships existing between roots, the soil, and rhizosphere organisms. By doing so, this technique allows roots to decay under far more natural conditions

TABLE 2. Initial proportional composition of fine roots within each diameter class in intact cores and litterbags.

Treatment	Composition (%) Fragment	(≤1 mm)	(>1 mm, ≤2 mm)
Silver maple			
Intact core	34.9* ± 2.1	44.5 ± 2.7	20.6* ± 3.0
Litterbag	0	48.3 ± 0.1	51.7 ± 0.1
Maize			
Intact core	23.8* ± 2.3	60.7* ± 2.8	15.6* ± 2.9
Litterbag	0	48.4 ± 0.2	51.6 ± 0.2
Winter wheat			
Intact core	60.1* ± 3.0	39.9* ± 3.0	0
Litterbag	0	100 ± 0.0	0

Note: Data are means ± 1 SE.

* $P < 0.001$ (*t* test; significant differences within species but between methodologies).

than is possible with litterbags. As a consequence of this precaution, use of this technique does require a greater time investment for sample processing than is required by litterbags. However, we feel that the results of this study show that the benefits far outweigh any costs. Intact cores, like litterbags, provide for flexibility in experimental design by allowing for reciprocal exchange experiments, and for insertion of multiple substrates at a common location. More importantly, however, the intact-core technique overcomes two serious problems of the litterbag approach: bias in root size-class composition and manipulation of the decomposer community. By doing so, we believe that the intact-core technique provides an improved alternative for measuring fine-root decay.

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