A comparison of methods for determining proximate carbon fractions of forest litter

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Ten forest litters with decomposition state varying from 16.6 to 100% weight remaining were partitioned into subsamples; each subsample was analyzed for proximate carbon fractions using one of two chemical analysis procedures (forage fiber and forest products analyses). Proximate carbon fractions from the simpler forage fiber techniques accurately estimated extractives, cellulose, lignin, and acid-hydrolyzed carbohydrates ($R^2 > 0.83$) determined by the more complex forest products analyses. Decomposition state accounted for most of the residual variance and significantly improved predictive equations for lignin and extractives. The relationship between proximate carbon fractions from the different techniques also varied somewhat among wood, hardwood leaves, and conifer leaves; however, variations were minor relative to the overall trend. Equations developed can be used to extend data availability for developing and validating decomposition models.

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Dix litières forestières ayant un état de décomposition variant de 16,6 à 100% de masse résiduelle ont été partitionnées dans des sous-échantillons; chaque sous-échantillon a été analysé pour les fractions approximatives de carbone en utilisant une des deux procédures analytiques chimiques (les fibres dans les forages et les analyses des produits forestiers). Les fractions approximatives de carbone obtenues par les techniques les plus simples des fibres dans les forages ont estimé de façon précise les extractibles, la cellulose, la lignine et les hydrates de carbone hydrolysés à l'acide ($R^2 > 0{,}83$) déterminés par les analyses plus complexes des produits forestiers. L'état de décomposition a compté pour la majeure partie de la variance résiduelle et a amélioré significativement les équations de prédiction pour la lignine et les extractibles. Les relations entre les fractions approximatives de carbone obtenues à partir des différentes techniques analytiques ont aussi varié quelque peu entre les différents bois, les feuilles de décidus et les feuilles de conifères; toutefois, les variations étaient mineures en relation avec la tendance générale. Les équations développées peuvent être utilisées pour étendre la validité des données pour le développement et la validation des modèles de décomposition.
[Traduit par la revue]

Introduction

The chemical composition of a litter substrate largely determines the rate of its decay (Tenny and Waksman 1929; Cromack 1973; Swift et al. 1979; Berg and Staaf 1980; Melillo et al. 1982; McClaugherty and Berg 1987). Rates of mineralization or immobilization of nitrogen and phosphorus associated with decomposition also vary with carbon chemistry (Swift et al. 1979; Berg and Staaf 1981; Aber and Melillo 1982; Melillo et al. 1983; Berg 1986).

While the precise carbon chemistry of detritus is very complicated, the organic compounds can be placed into three broad categories: (*i*) extractives (nonpolar compounds such as fatty acids and lipids and polar compounds such as sugars and phenolics) (*ii*) polymer carbohydrates or holocellulose (cellulose plus hemicellulose), and (*iii*) acid-insoluble aromatic compounds (lignins). The names for these fractions indicate the major constituents; however, these proximate fractions contain many compounds and the precise organic chemistry likely varies among species and litter types. Because carbon is the major element in these proximate fractions (about 50% of the ash-free material), we refer to proximate carbon fractions. Use of these crude proximate carbon fractions has proved helpful for understanding decomposition of plant material, because each of these proximate carbon fractions decomposes at a different rate and may influence nutrient dynamics separately (Minderman 1968; Berg 1986; McClaugherty and Berg 1987; Melillo et al. 1989).

Quantitative determination of the proximate carbon fractions can be carried out using either the techniques developed for forage fiber analysis (Van Soest 1963; Goering and Van Soest 1970), or with techniques developed for the forest products industry (TAPPI 1969, 1975; Effland 1977) together with techniques developed for analysis of natural wastes, soils, and vegetation (Dubois et al. 1956; Allen et al. 1974). Hereafter, we refer to these two sets of procedures as forage fiber techniques and forest products techniques, respectively.

Many decomposition studies estimated proximate carbon fractions using forage fiber techniques (Cromack 1973; Fogel and Cromack 1977; Melillo et al. 1983; Edmonds 1987; MacMillan 1988; Stohlgren 1988). Forage fiber techniques have been useful for identifying the importance of lignin (Cromack 1973; Fogel and Cromack 1977) and initial lignin to nitrogen ratio (Melillo et al. 1983) in controlling mass loss in a wide variety of forest litters. However, several recent studies favor forest products techniques for determining proximate carbon fractions (Berg and Staaf 1980, 1981; McClaugherty and Berg 1987; Melillo et al. 1989). To aid in synthesizing the vast literature on decomposition into general models, we analyzed subsamples of a wide variety of forest detritus using both techniques and compared results. Specifically, we wished to know whether forage fiber analyses could predict proximate carbon fractions as determined by forest products techniques and whether the relationship varied among litter types or with length of decomposition.

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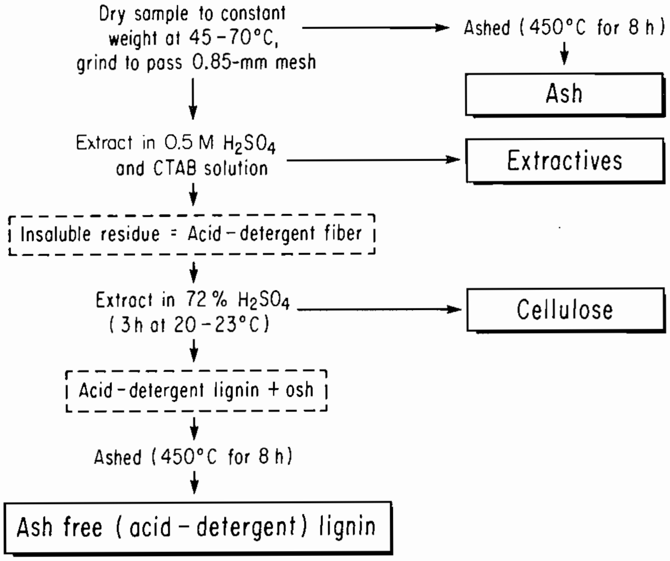

FIG. 1. Flow diagram for forage fiber determination of proximate carbon fractions.

Description of analytical techniques

Forage fiber techniques

The techniques of Van Soest (1963) and Goering and Van Soest (1970) were designed to provide estimates of digestibility and forage quality (related to lignin and lignocellulose). Extractives (hemicelluloses plus other labile carbon fractions) and cellulose are determined by difference (Fig. 1). These techniques have been employed to estimate proximate carbon fractions useful for explaining decay patterns of forest litter (Cromack 1973; Fogel and Cromack 1977; Melillo et al. 1983) and remain widely used (Edmonds 1987; MacMillan 1988; Stohlgren 1988).

Samples were analyzed for acid-detergent fiber (lignocellulose) and acid-detergent lignin using a slight modification of the techniques given by Goering and Van Soest (1970). Acid-detergent fiber was separated from the original material with a mixture of H_2SO_4 and cetyltrimethylammonium bromide. Decahydronaphthalene, used by Goering and Van Soest (1970), reduces foaming but is a toxic irritant. Therefore, digestions were carefully monitored but decahydronaphthalene was omitted from the digestion. The difference between the weight of acid-detergent fiber and the original sample weight estimates extractives, while the difference between acid-detergent lignin and acid-detergent fiber estimates cellulose. The acid-insoluble ash, mostly silica, resides in the lignin fraction. Where substantial amounts of acid-soluble ash occurred (e.g., dogwood foliage), ash in the cellulose fraction was determined by subtracting ash found in lignin from ash in the entire sample. All fractions are presented as percent ash-free dry weight.

Forest products techniques

Litter samples were analyzed with a series of digestions using the procedures outlined in Fig. 2. Proximate carbon fractions, nonpolar extractives (fats, oils, and waxes), polar extractives (simple sugars and polyphenols), acid-soluble products (cellulose), and lignin were determined gravimetrically; simple sugars, phenolics, and hydrolyzed carbohydrates (cellulose plus hemicellulose) were quantified colorimetrically. Materials were dried to a constant weight at 45-70°C, weighed, and ground to pass a 0.85-mm mesh. Dichloromethane removed nonpolar extractives using a slight modification of an extraction procedure presented in TAPPI (1969): 1 g versus 10 g material, 75 mL versus 200 mL dichloromethane, block digestor with condenser versus Soxhlet apparatus. Hot water removed polar extractives from the residue of the dichloromethane extraction (TAPPI 1975). Hot water extracts were analyzed for simple sugars using a phenol - sulfuric acid colorimetric reaction with a glucose standard (Dubois et al. 1956) and for soluble phenolics with the Folin–Denis colorimetric procedure using tannic acid as the standard (Allen et al. 1974). For the Folin–Denis procedure, 1 M Na_2CO_3 (10% (w/v) H_2O) was used instead of 17% (w/v) H_2O.

Residue from the polar and nonpolar extractions was separated into acid-soluble (cellulose) and acid-insoluble (lignin) fractions using a two-stage digestion in 72% followed with 2.5% sulfuric acid (Effland 1977). The acid-soluble fraction was analyzed for hydrolyzed carbohydrates using the phenol - sulfuric acid colorimetric reaction with a glucose standard (Dubois et al. 1956). The difference between the weight of the extractive-free and ash plus lignin residues estimates the acid-soluble or cellulose fraction. Proximate carbon fractions were corrected for ash as in the forage fiber method and presented as ash-free dry weight.

Methods

Subsamples of litter from five separate studies (representing ten different plant materials) were analyzed with both the forage fiber and forest products methods (Table 1). For the sample population, proximate carbon fractions and decomposition states varied widely. Because these samples represent many different studies, we used a variety of compositing and aggregation protocols (Table 1).

Analysis of covariance tested whether the relationship between proximate carbon fractions identified by forage fiber techniques and forest products techniques was significant, and if so, whether it varied with decomposition state or among the wood, deciduous foliage, and coniferous foliage litter types. Testing for differences among litter types is equivalent to testing for equality of slopes in regression (Steele and Torrie 1980). We used ordinary least-squares multiple linear regression to estimate forest products proximate carbon fractions from forage fiber chemistry and decomposition state (represented by percent of original weight remaining). Only coefficients for independent variables that contributed significantly to the regressions are presented in Table 2. Significance level for all tests was $\alpha = 0.05$.

Results and discussion

We developed a set of linear regression equations to estimate the proximate carbon fractions determined by forest products techniques using fractions determined by forage fiber analysis and decomposition state as independent variables. The two methods appeared to be closely related, and the forage fiber techniques estimated the proximate carbon fractions of forest litter remarkably well (Figs. 3-6, Table 2).

The relationship between forage fiber proximate fractions and forest products proximate fractions differed among the coniferous foliage, deciduous foliage, and wood litter types. Litter type significantly influenced regression slopes for lignin ($p = 0.02$), the acid-soluble fraction ($p = 0.01$), and extractable phenolics ($p = 0.02$), but not for hydrolyzed carbohydrates ($p = 0.06$), total extractives ($p = 0.14$),

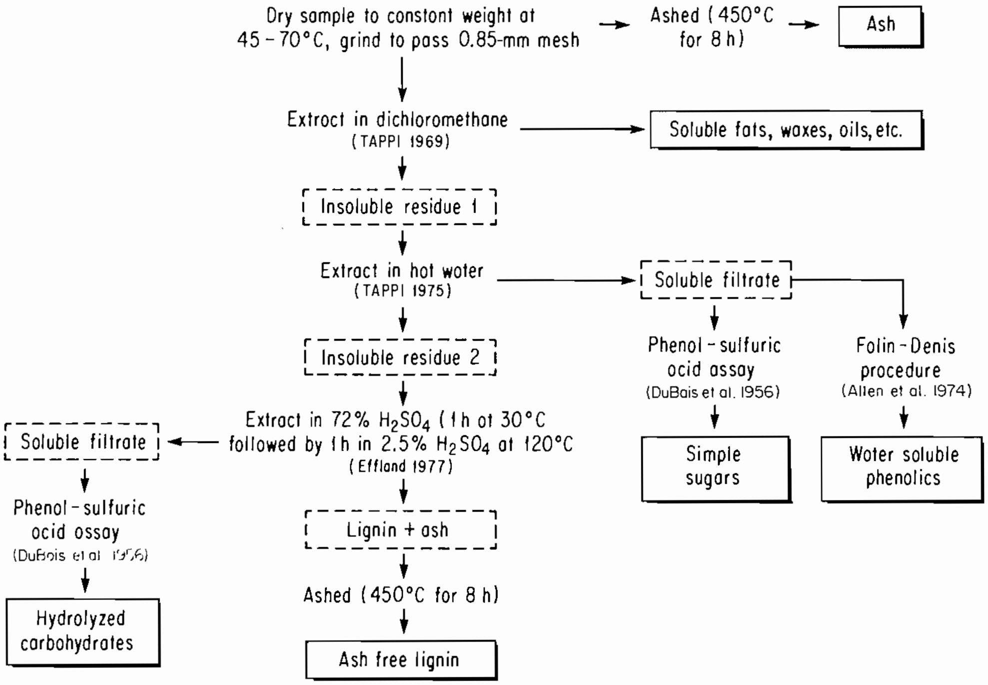

FIG. 2. Flow diagram for forest products determination of proximate carbon fractions.

TABLE 1. Material, length of decomposition, number of pooled samples, type of incubation, compositing protocol, and citation for forest litter used in this study

Material	Length of decomposition (% of weight remaining)	n	Incubation	Compositing protocol		Citation
				Forage fiber	Forest products	
Birch wood (10 logs)	100–15.4	10	Field	1 per sample	1 per sample	Eshleman et al. 1978
Alder, aspen, fir, spruce, and birch wood	100	5	na	1 per sample	1 per sample	Melillo et al. 1983
Senescent red pine needles	100–16.6	11	Field	Mean of 3–5 separate analyses	Composite[a] of 3–5 bags	J.M. Melillo[b]
Fresh red pine needles	100–21.7	8	Field	Mean of 3–5 separate analyses	Composite of 3–5 bags	Melillo et al. 1989
Senescent dogwood leaves[c]	81.3–52.2	4	Lab	Composite of 2–3 samples	Composite of 2–3 samples	A.E. Linkins and J.M. Melillo[b]
Senescent white oak leaves	100–86.8	6	Lab	Mean of 2–3 samples	Composite of 2–3 samples	A.E. Linkins and J.M. Melillo[b]
Senescent red maple leaves	100–76.8	6	Lab	Mean of 2–3 separate samples[d]	Composite of 2–3 samples	A.E. Linkins and J.M. Melillo[b]

[a] All composites assembled by weight.
[b] Personal communication.
[c] Lignin determined with the forage fiber technique for the undecomposed dogwood leaves was used to correct lignin determined by forest products technique for ash present in nonlignin fractions of dogwood leaves; this correction was used for the remaining dogwood samples, and we omitted the undecomposed dogwood litter from this analysis.
[d] Initial only, weeks 1-4 are a composite of 2-3 samples.

polar extractives ($p = 0.08$), nonpolar extractives ($p = 0.95$), and extractable simple sugars ($p = 0.25$). The relationship between the two analytical procedures was the same for coniferous and deciduous foliage except with respect to the nonpolar extractives and phenols. The relationship between the two analytical procedures differed between wood and foliage for all fractions.

Forage fiber techniques predicted forest products cellulose and acid-hydrolyzed carbohydrates very well for wood ($R^2 = 0.93$ and 0.73), while predictions for leaf litter were relatively poor ($R^2 = 0.35$ and 0.68). Trends established for both wood and leaf litter for cellulose and acid-hydrolyzed carbohydrates are consistent (Figs. 5 and 6). Other constituents, nonpolar extractives, water-soluble phenolics, and water-soluble simple sugars generally represented minor components of the litter biomass. Forage fiber carbon fractions estimated these extractive fractions well, but the relationship between the two chemistries also varied somewhat between wood and leaf litter.

While differences among litter types were statistically significant, they were not substantial (Figs. 3–6), except for extractives. Decomposition state, represented by percent

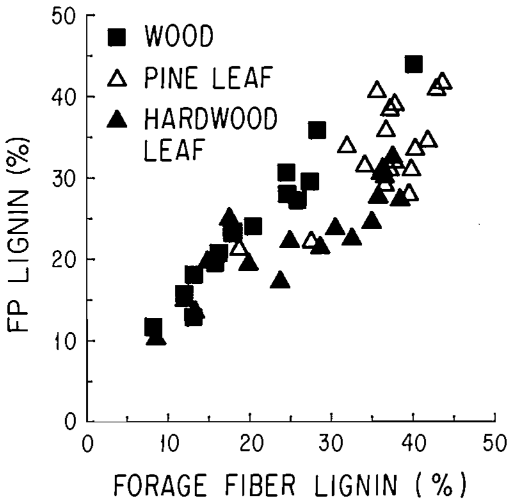

FIG. 3. Lignin determined with forest products (FP) techniques versus lignin determined with forage fiber techniques for three litter types.

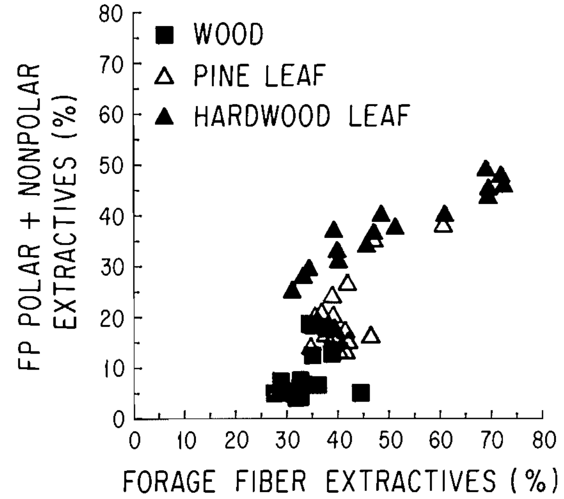

FIG. 4. Polar plus nonpolar extractives determined with forest products (FP) techniques versus extractives determined with forage fiber techniques for three litter types.

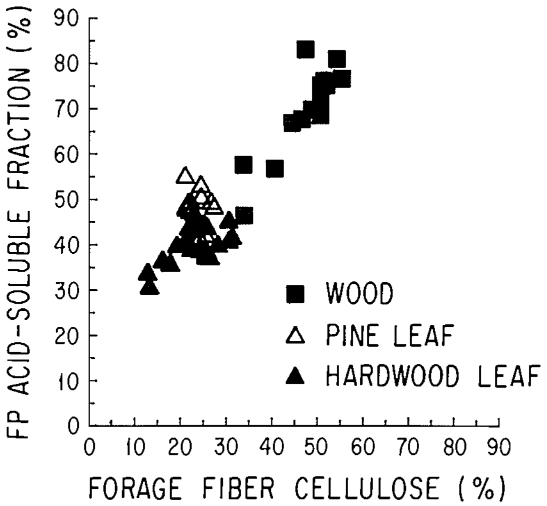

FIG. 5. Acid-soluble or cellulose fraction determined with forest products (FP) techniques versus cellulose determined with forage fiber techniques for three litter types.

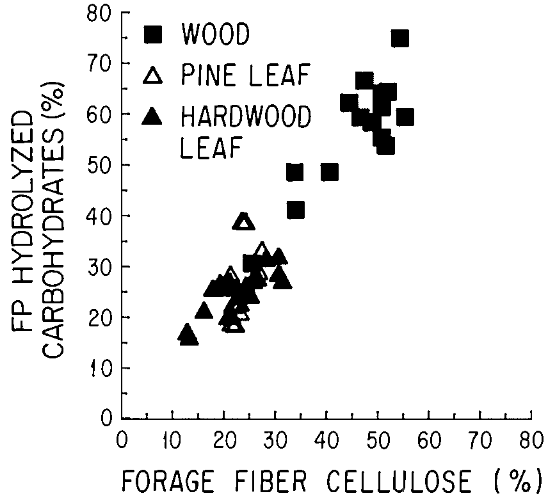

FIG. 6. Hydrolyzed carbohydrates determined with forest products (FP) techniques versus cellulose determined with forage fiber techniques for three litter types.

weight remaining, accounted for most of the important variability among litter types. Therefore, we report results for regressions combined across all litter types (Table 2). Precision of the combined regressions is somewhat lower than the precision of the regressions done separately by litter type. However, we believe that the combined regressions will be more useful for comparing carbon chemistries for a wide range of forest litter.

If the equations in Table 2 are used to reconstruct the entire forest products proximate carbon analysis (polar plus nonpolar extractives, acid-soluble or cellulose fraction, lignin) the total may vary slightly from 100% because each regression was unconstrained. In this case, we recommend estimating lignin and the acid-soluble fraction with the regressions in Table 2 and polar plus nonpolar extractives by difference.

Differences between the carbon fractions identified by the two analytical procedures show some of the benefits of the more complicated forest products procedures, and also how carbon chemistry changes with decomposition. Consistent differences in the relationship between the forage fiber and forest products analyses among litter types and with decomposition state indicate that the forage fiber proximate carbon fractions may not yield as reliable a general estimate of decomposition rate as the more complex forest products chemistry.

Forage fiber lignin analysis may be less sensitive than the forest products lignin analysis to changes that occur during decomposition. For undecomposed leaf litter, the Goering and Van Soest (1970) extraction gives higher lignin values than the procedure of Effland (1977). As the leaf litter decomposes, both the forage fiber and forest products techniques approach similar values for lignin. For woody litter, the forage fiber techniques showed lower lignin values than forest products techniques, but the relationship did not change with decomposition.

The amount of forage fiber extractives for leaf litter changes little with the level of decomposition, while the hot water and dichloromethane extractives determined with the forest products techniques consistently decrease. The forage

TABLE 2. Coefficients for linear regressions estimating forest products proximate carbon fractions from forage fiber chemistry ($n = 50$)

Forest products fraction	Regressor					Statistics	
	Intercept	Forage fiber lignin	Forage fiber cellulose	Forage fiber extractives	% weight remaining	R^2	$S_{y \cdot x}$
Lignin	18.1	0.533	0.0927		−0.131	0.89	2.90
Acid-soluble (cellulose)	19.7		1.04			0.83	5.66
Total extractives	12.4		−0.705	0.435	0.186	0.85	5.35
Hydrolyzed carbohydrates	4.45	−0.170	1.19			0.91	5.02
Nonpolar extractives	8.26		−0.197		0.0340	0.52	2.20
Polar extractives	5.17		−0.522	0.416	0.155	0.81	5.24
Extractable phenolics	2.89		−0.206		0.105	0.53	3.03
Extractable simple sugars	3.41		−0.0952	0.0575	0.0217	0.68	1.16

NOTE: Standard deviations for repeated subsamples of the same material were less than 2 for both analytical techniques for all proximate fractions. $S_{y \cdot x}$, standard error of the estimate.

fiber procedure apparently misses subtle, but important, changes in extractives. Berg (1986) and Melillo et al. (1989) observed that forest products extractives control initial decomposition rate and nitrogen immobilization. Forage fiber techniques miss these changes over time and are not useful for understanding these dynamics.

Conclusions

Results can be used to estimate forest products proximate carbon fractions from Goering and Van Soest (1970) forage fiber techniques. The relationship between the two chemistries varied slightly between wood and leaf litter, and with the state of decomposition, but the differences among litter types were not substantial. Based on this comparison of methods, we would suggest a combined technique: extractions for polar and nonpolar extractives (TAPPI 1969, 1975), and forage fiber for lignin and cellulose.

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