

Decomposition, $\delta^{13}\text{C}$, and the “lignin paradox”

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¹*Pacific Forestry Centre, Natural Resources Canada, Victoria, British Columbia, Canada V8Z 1M5 (e-mail: cpreston@pfc.cfs.nrcan.gc.ca);* ³*Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4. Received 8 March 2005, accepted 24 August 2005.*

Preston, C. M., Trofymow, J. A. and Flanagan, L. B. 2006. **Decomposition, $\delta^{13}\text{C}$, and the “lignin paradox”**. *Can. J. Soil Sci.* **86**: 235–245. The natural abundance of ^{13}C ($\delta^{13}\text{C}$) generally increases with decomposition of organic matter. This is contrary to the expected decrease, as lignin is hypothesized to accumulate relative to isotopically heavier cellulose. Our objective was to test the hypothesis that ^{13}C depletion should be observed for gymnosperm logs that typically develop advanced brown-rot decay with high lignin content. With increasing lignin concentration [previously determined by nuclear magnetic resonance (NMR)], $\delta^{13}\text{C}$ tended to become more negative for samples of *Pseudotsuga menziesii*, *Tsuga heterophylla*, *Thuja plicata*, and unidentified species from Coastal Forest Chronosequence sites of southern Vancouver Island. For a larger sample set without NMR analysis, $\delta^{13}\text{C}$ was significantly more depleted for the highest decay classes, and total C was negatively correlated with $\delta^{13}\text{C}$, consistent with the higher total C of lignin than of cellulose. Relationships of total C and $\delta^{13}\text{C}$ with density were much weaker. We discuss causes for the variability of $\delta^{13}\text{C}$ in coarse woody debris from these sites, and how the apparent paradox in the predicted change of $\delta^{13}\text{C}$ with decomposition is largely due to the confusion of lignin, the biopolymer produced by higher plants, with the acid-unhydrolyzable residue (AUR) of the proximate analysis procedure commonly used to assess litter quality in decomposition studies.

Key words: Coarse woody debris, decomposition, lignin, ^{13}C NMR, $\delta^{13}\text{C}$, proximate analysis

Preston, C. M., Trofymow, J. A. et Flanagan, L. B. 2006. **La décomposition, la teneur en $\delta^{13}\text{C}$ et le « paradoxe de la lignine**. *Can. J. Soil Sci.* **86**: 235–245. La quantité de ^{13}C ($\delta^{13}\text{C}$) présente dans la nature augmente généralement avec la décomposition de la matière organique. Cette constatation contredit la baisse de concentration prévue, l’hypothèse étant que c’est la lignine qui devrait s’accumuler au lieu de l’isotope plus lourd de la cellulose. Les auteurs voulaient savoir si le ^{13}C s’épuise vraiment dans les grumes de gymnospermes qui donnent habituellement une pourriture brune riche en lignine. Avec la hausse de la concentration de lignine (déterminée au préalable par RMN), la teneur en $\delta^{13}\text{C}$ a tendance à devenir négative dans les échantillons de *Pseudotsuga menziesii*, *Tsuga heterophylla*, *Thuja plicata* et des espèces non identifiées venant des chronoséquences de la forêt côtière du sud de l’île Vancouver. Dans une série d’échantillons plus importante n’ayant pas été analysés par RMN, la concentration de $\delta^{13}\text{C}$ était significativement plus faible dans les spécimens à décomposition la plus avancée, et la concentration totale de carbone présentait une corrélation négative avec l’isotope $\delta^{13}\text{C}$, confirmant la concentration supérieure de carbone issu de la lignine plutôt que de la cellulose. Les liens entre la concentration totale de carbone et celle de $\delta^{13}\text{C}$ avec la densité sont nettement plus ténus. Les auteurs parlent des causes de la variabilité de la concentration de $\delta^{13}\text{C}$ dans les débris ligneux grossiers prélevés aux différents sites et du paradoxe apparent quant à l’évolution de la teneur en $\delta^{13}\text{C}$ attribuable à la décomposition. Ce paradoxe résulte, selon eux, d’une confusion entre la lignine, polymère biologique synthétisé par les plantes supérieures, et le résidu acide non hydrolysable laissé par la méthode d’analyse immédiate couramment employée pour évaluer la qualité de l’humus dans les études sur la décomposition.

Mots clés: Débris ligneux grossiers, décomposition, lignine, RMN ^{13}C , $\delta^{13}\text{C}$, analyse immédiate

Decomposition studies often show an inverse relationship of mass loss with litter “lignin”, or “lignin”/nitrogen ratio (e.g., Melillo et al. 1989; Moore et al. 1999; Preston et al. 2000; Prescott et al. 2004). In most cases, this lignin (sometimes called “Klason lignin”) is operationally defined as the acid-unhydrolyzable residue (AUR) from the sequence of non-polar extraction, polar extraction, and acid hydrolysis of the (PA) procedure. However, the AUR fraction also incorporates structures from cutin and condensed tannins (Preston et al. 1997), both of which can make a substantial contribution to the carbon (C) composition of foliar litter from shrubs and trees (Almendros et al. 2000; Lorenz et al. 2000; Preston et al. 2000; Kraus et al. 2003).

By contrast, the Klason lignin fraction of wood (except for those high in tannins) more closely reflects its lignin content (Leary et al. 1986; Love et al. 1992). From this point we use the term “lignin” only for the phenylpropane-based

polymer produced by higher plants, with syringyl, guaiacyl and phenylpropane units having two, one or no methoxyl groups on the aromatic ring. We use AUR for the PA fraction often used to characterize litter in decomposition studies (Trofymow et al. 2002).

One apparent paradox arising from the confusion of lignin with AUR is the direction of change predicted for the natural abundance of ^{13}C ($\delta^{13}\text{C}$ value) with decomposition. Plants discriminate against ^{13}C during photosynthesis, so that plant tissues are depleted in ^{13}C compared with atmospheric CO_2 (around -8%). C3 plants, which include most

Abbreviations: AUR, acid-unhydrolyzable residue; CFC, Coastal Forest Chronosequence; CWD, coarse woody debris; LOI, loss-on-ignition; NMR, nuclear magnetic resonance; PA, proximate analysis

shrubs and trees, have a mean ^{13}C discrimination against atmosphere (Δ) of 18.3‰ (Pataki et al. 2003), so that $\delta^{13}\text{C}$ values of C3 foliage are typically -25‰ to -28‰. Isotopic fractionation also occurs during subsequent biosynthesis, with depletion generally increasing with the number of steps required for biosynthesis. Thus, lignin of wood is typically depleted by 3.0–4.4‰ relative to cellulose (Benner et al. 1987; Goñi and Eglinton 1996; Spiker and Hatcher 1987; Loader et al. 2003), and plant lipids are even more depleted in ^{13}C (Conte et al. 2003).

The expectation is that lignin, because of its greater recalcitrance, should accumulate with decomposition. This would lead to depletion of $\delta^{13}\text{C}$ in soil organic matter relative to the starting litter inputs, at least in the early stages (e.g., Flanagan et al. 1999; Garten et al. 2000; Quideau et al. 2003). However, numerous studies (e.g., Buchmann et al. 1997; Flanagan et al. 1999; Ehleringer et al. 2000; Feng 2002; Bird et al. 2003; Quideau et al. 2003) have shown that decomposition of organic matter is associated with increasing $\delta^{13}\text{C}$. The $\delta^{13}\text{C}$ value usually increases with soil depth or with decreasing particle-size fraction within a depth range, as soil organic matter becomes increasingly dominated by microbial necromass stabilized by mineral association (Bird et al. 2003).

One reason that ^{13}C depletion is not observed is that decomposition generally proceeds without selective preservation of the aromatic C from lignin and tannins, although the proportion of alkyl C may increase, as shown by solid-state ^{13}C NMR spectroscopy (Baldoek and Preston 1995; Preston 1996; Quideau et al. 2001; Chen and Chiu 2003; Qualls et al. 2003; Sjöberg et al. 2004). One exception occurs with high inputs and slow decomposition of coarse woody debris (CWD), especially from gymnosperms, as found in old-growth coastal rainforests of the Pacific Northwest (Preston et al. 1990, 1998, 2002; Means et al. 1992; deMontigny et al. 1993). The CWD is a conspicuous feature of these forests, and a defining characteristic of old-growth (Sollins et al. 1987; Means et al. 1992; Daniels et al. 1997; Trofymow et al. 2003; Sun et al. 2004).

Previous studies of $\delta^{13}\text{C}$ in archaeological or fossil wood samples showed that $\delta^{13}\text{C}$ decreased as the proportion of lignin increased (Spiker and Hatcher 1987; Schleser et al. 1999; van Bergen and Poole 2002). Studies of decomposition and organic matter in aquatic sediments (Benner et al. 1987; Spiker and Hatcher 1984) similarly concluded that loss of carbohydrates was the primary driver of increasing ^{13}C depletion during early diagenesis. The tendency for increase in alkyl C (lipids) rather than lignin with increasing decomposition, as noted previously, should also cause depletion of ^{13}C .

We investigated whether the hypothesis of ^{13}C depletion of wood with increasing lignin concentration could be verified for a forest landscape with abundant CWD, specifically the Coastal Forest Chronosequence (CFC) sites of Vancouver Island (Trofymow et al. 1997; Trofymow and Porter 1998). A previous study (Preston et al. 1998) showed that total C increased, and density decreased with increasing decay status of CWD. Analysis of 26 samples by solid-state

^{13}C NMR showed that decomposition generally proceeded first by nonselective mass loss, followed by more rapid loss of carbohydrate C and increasing concentration of lignin C, only slightly altered by oxidation and small increase of alkyl (lipid) C. This pattern is consistent with initial decomposition by white-rot fungi, with non-selective mass loss and little change in overall organic composition, or even depletion of lignin. The second stage, with increasing concentration of lignin and colour change to dark reddish-brown, indicates a dominance of brown-rot fungi that have a very limited ability to decompose lignin (Hedges et al. 1988; Jin et al. 1990). In this study, we examined relationships among decay class, density, total C, $\delta^{13}\text{C}$ and NMR-determined lignin for a larger set of CWD samples from the CFC plots and some related samples.

MATERIALS AND METHODS

Sites and Sampling

The eight CFC sites are on southern Vancouver Island, four in Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] dominated forests on the dry leeward east side of the island in very dry variants of the Coastal Western Hemlock zone (CWHxm1&2), and four in western hemlock [*Tsuga heterophylla* (Raf. Sarg)] dominated forests on the wetter windward west side of the island in very wet variants of the zone (CWHvm1). Each site has four stands, one in each of the following seral stages: regeneration (REG, 3–9 yr), immature (IMM, 25–45 yr) and mature (MAT, 65–95 yr) stands resulting from a single harvest, and old-growth (OG, >250 yr). Detailed information is available elsewhere on the CFC project, including site descriptions, sampling protocols, mass of CWD (Trofymow et al. 1997; Wells and Trofymow 1997), and C chemistry of five organic matter pools from forest floor and 10–30 cm depth (Preston et al. 2002).

During the 1992 sampling, all CWD samples >12 cm diameter were identified to species where possible, and assigned a decay class in the field according to the system of Sollins (1982): class 1: (logs freshly fallen, bark and all wood sound, current-year twigs attached); class 2: (sapwood decayed but present, bark and heartwood mainly sound, twigs absent); class 3: (logs still support own weight, sapwood decayed but still structurally sound); class 4: (logs do not support own weight, sapwood and bark mainly absent, heartwood not structurally sound, branch stubs can be removed); class 5: (heartwood mainly fragmented, forming ill-defined elongate mounds on the forest floor sometimes invisible from surface). Samples that could not be identified to species were all in decay classes 4 and 5.

For the previous NMR study (Preston et al. 1998), from the large number of CWD samples of >12 cm diameter characterized by decay class, density, and elemental analysis (C, N, P, S), 26 were selected to represent the range of five decay classes for the three main species, Douglas-fir, western hemlock and western redcedar (*Thuja plicata* Donn ex D. Don), and unidentified species of decay classes 4 and 5. These were analyzed by ^{13}C CPMAS NMR at 25 MHz ^{13}C frequency, to estimate percentages of total lignin and carbohydrate C.

For this study we selected 118 CWD samples (basically, as many as possible) from the CFC plots, including those originally analyzed by NMR. Samples were selected as before to cover the range of decay classes 1–5, and the same three species plus unidentified samples. Sample selection was originally restricted to the largest size class (>12 cm diameter), but a few samples of 10–12 cm were included to fill gaps. We also analyzed a sample of fresh heartwood of old-growth western hemlock that was a substrate for the Canadian Intersite Decomposition Experiment (CIDET, Preston et al. 2000; Trofymow et al. 2002), and 15 samples of fresh Douglas-fir heartwood and sapwood obtained from the Shawnigan Lake research site near Victoria, BC, in 1990 (Mitchell et al. 1996).

Additional NMR Data

For this study, we incorporated NMR results for one CWD sample that was run but not used in the original study (an extra Douglas-fir of decay class 2), and added NMR data for the CIDET western hemlock heartwood sample noted above. In Preston et al. (1998), NMR spectra were run with a contact time of 0.5 ms, and the relative areas adjusted using correction factors based on spectra with variation of contact time for one sample. The CIDET wood sample was run on a Bruker MSL 300 (Bruker Instruments Inc., Karlsruhe, Germany) with 1 ms contact time and 4700 Hz magic angle spinning rate (Preston et al. 2000). Because of the higher magnetic field, relative areas for this spectrum were corrected for spinning sidebands. They were then adjusted using correction factors in Davis et al. (1994) developed for wood samples run with 1 ms contact time. A similar approach was used in Preston et al. (1998) to compare results obtained at 0.5 ms contact time with those from a previous NMR study of CWD (Preston et al. 1990) using 1 ms contact time. The quantitative reliability of CP NMR spectra is a complex issue (e.g., Davis et al. 1994; Preston 1996; Smernik and Oades 2000), and the relative areas presented here should not be regarded as absolutely correct, as discussed previously (Preston et al. 1998). However, they are suitable for comparison between samples, and are sufficient to characterize the pathway of changes in organic composition of CWD with decomposition.

Carbon and Ash Analysis

Samples were previously analyzed for total C in 1996 using dry combustion (Preston et al. 1998), but were reanalyzed for this study in 2004 using the same Leco CR–12 instrument with the same protocol. For samples high in total C, the instrument is standardized using a bulk sample of Douglas-fir foliage, collected locally, and standardized against NIST (National Institute of Standards and Technology) SRM 1575 Pine Needles. Standard operating procedure is to run a duplicate and calibration sample for every tenth sample; economic reality precludes routine duplicate or triplicate analysis.

After sampling in 1993, CWD samples had been dried at 70°C, ground in a Wiley mill (typically to 20-mesh), and stored at room temperature. To improve precision for this study, samples were very finely ground using a ball mill (Retsch MM2 Mixer Mill, Retsch GmbH, Haan, Germany),

and redried at 70°C the night before analysis. Very fine grinding was also necessary for the isotope analysis. We determined ash by heating at 600°C for 18 h. Most ash concentrations were too small to measure accurately, so that C concentrations were corrected only when ash was greater than 10 g kg⁻¹. After this, about 12 samples that were outliers on the correlation curve of the two datasets were rerun.

Isotope Analysis

Samples were analyzed for the natural abundance of ¹³C using an elemental analyzer (NC2500, CE Instruments, ThermoQuest Italia, Milan, Italy) coupled to a gas isotope ratio mass spectrometer (Delta Plus, Finnigan Mat, Bremen, Germany) operating in continuous flow mode. A subsample (1–3 mg) was sealed in a tin capsule and loaded into the elemental analyzer for combustion/reduction. Water generated by combustion was removed by a magnesium perchlorate trap. The carbon dioxide and nitrogen gases generated from the combustion/reduction process were separated in the gas chromatograph column of the elemental analyzer and passed directly via a helium gas carrier stream to the inlet of the mass spectrometer for stable isotope analysis. Standard deviations were similar for laboratory standards and for representative samples, typically 0.1‰ or better for $\delta^{13}\text{C}$.

Data Analysis

Regression and Analysis of Variance (AOV) of the density, %C, and $\delta^{13}\text{C}$ data were performed using the general linear model procedure of the SAS package (SAS Institute, Inc. 1999). An initial three-way AOV tested for the effects of decay class, species and zone for a subset of the data (Table 1). Since the species effect was so strong, a three-way AOV testing for the effect of decay class, seral stage, zone, and the two-way interactions of decay class with seral stage or zone was then done for each species including the Unknowns (Table 2). Such analysis was needed as samples of each decay class and species were not available for all seral stages and zones. Where effects were significant, means were separated using the Ryan-Einot-Gabriel-Welsch Multiple F test (SAS Institute, Inc. 1999). A multiple regression (REG procedure) was used to determine the relationship between $\delta^{13}\text{C}$ with C and density.

RESULTS

Carbon and Ash Analysis

The ash content of most samples was too low to measure with any precision (< 10 g kg⁻¹). Total C was corrected for ash for five samples, one with 84.6% loss-on-ignition (LOI), and four others with LOI between 96.6 and 98.3%. To compare the two sets of total C data for the CFC samples on the same basis, the five high-ash samples were dropped. Results were highly correlated ($C_{1996} = 0.8933 \times C_{2004}$, $R^2 = 0.9986$), but total C values obtained for this study were generally higher than those obtained previously.

Trends in Total C and $\delta^{13}\text{C}$ with Lignin C

Figure 1 shows plots of (a) total C and (b) $\delta^{13}\text{C}$ vs. percentage of lignin C using the slightly expanded set of 28 samples

Table 1. ANOVA testing for effects of decay class (dClass), species (Sp), and zone on wood density (g cm^{-3}), total C (g kg^{-1}), and $\delta^{13}\text{C}$ (‰). Includes data only for species Cw, Fd and Hw, decay-classes 1–4, and low LOI samples. Data for decay class = 0 and Unknown species excluded. Values reported under F value for error is the mean square of the error (MSE). Significant effects are in boldface font ($P < 0.05$) and important trends ($0.05 < P < 0.1$) are underlined

Variable Source	df	Density		1996 C		2004 C		$\delta^{13}\text{C}$	
		F value	P > F	F value	P > F	F value	P > F	F value	P > F
dClass	3	29.72	<.0001	6.72	0.0005	13.92	<.0001	2.49	<u>0.0682</u>
Sp	2	3.51	0.036	6.67	0.0024	7.24	0.0015	10.53	0.0001
zone	1	1.02	0.3164	0.01	0.9172	0.07	0.7949	3.01	<u>0.0879</u>
dClass × Sp	6	0.72	0.6338	0.93	0.4793	0.73	0.6285	1	0.4351
dClass × zone	3	0.36	0.7816	1.59	0.2018	1.47	0.2305	0.23	0.8778
Sp × zone	2	0.15	0.8583	0.57	0.5658	0.84	0.4383	2.66	<u>0.0777</u>
Error (MSE)	62	0.005816		561.6934		474.0192		2.031424	

Table 2. ANOVA testing for effects of decay class (dClass), seral stage (sere), and zone on wood density (g cm^{-3}), total C (g kg^{-1}), and $\delta^{13}\text{C}$ (‰) for each species. Data for decay class = 0 excluded. Values reported under F value for Error is the mean square of the error (MSE). Significant effects are in boldface font ($P < 0.05$) and important trends ($0.05 < P < 0.1$) are underlined

Variable Source	df	Density		1996 C		2004 C		$\delta^{13}\text{C}$	
		F value	P > F	F value	P > F	F value	P > F	F value	P > F
<i>W. redcedar</i>									
dClass	4	13.20	0.001	7.15	0.009	5.09	0.025	0.43	0.782
sere	3	0.52	0.678	1.09	0.408	1.55	0.274	2.50	0.133
zone	1	0.53	0.489	2.22	0.175	7.65	0.024	0.99	0.348
dClass × sere	6	0.56	0.755	1.88	0.201	1.23	0.384	1.92	0.193
dClass × zone	1	0.00	0.993	11.10	0.010	9.29	0.016	0.04	0.848
Error (MSE)	8	0.00411		247		291		1.203	
<i>Douglas-fir</i>									
dClass	4	8.42	0.001	3.73	0.027	9.52	0.001	5.90	0.005
sere	3	0.13	0.941	1.83	0.185	0.16	0.924	0.13	0.942
zone	1	0.01	0.929	1.40	0.256	1.36	0.262	1.93	0.185
dClass × sere	10	0.61	0.780	1.12	0.409	0.81	0.620	0.81	0.621
dClass × zone	1	1.35	0.263	0.40	0.535	0.00	0.954	2.46	0.138
Error (MSE)	15	0.00727		683		449		0.661	
<i>W. hemlock</i>									
dClass	3	5.00	0.023	3.79	0.047	3.34	<u>0.064</u>	0.72	0.562
sere	3	0.41	0.746	0.13	0.939	0.08	0.967	0.48	0.704
zone	1	1.61	0.233	0.19	0.672	0.01	0.939	4.51	<u>0.060</u>
dClass × sere	6	0.53	0.776	0.16	0.983	0.19	0.971	0.94	0.508
dClass × zone	0								
Error (MSE)	10	0.00950		556		856		4.263	
<i>Unknown sp.</i>									
dClass	1	20.43	0.0001	0.61	0.442	5.64	0.026	2.29	0.143
Sere	3	2.35	<u>0.097</u>	2.57	<u>0.077</u>	1.67	0.199	1.11	0.364
Zone	1	0.06	0.804	1.22	0.279	0.36	0.552	8.93	0.006
dClass × sere	3	2.76	<u>0.063</u>	0.99	0.413	1.30	0.296	0.13	0.943
dClass × zone	1	3.08	<u>0.092</u>	0.71	0.406	0.19	0.669	0.01	0.928
Error (MSE)	25	0.001278		791		773		2.986	

(27 CFC plus the CIDET western hemlock wood) and the new total C data. There was a positive correlation of total C with percentage of lignin C (slope 1.36, R^2 0.86, intercept 477). For $\delta^{13}\text{C}$ against lignin, the slope was -0.031 with R^2 of 0.25. The outlier with low $\delta^{13}\text{C}$ and low lignin C is a cedar sample of decay class 1 from the west side. Discarding this point resulted in a slope of -0.036 and R^2 of 0.39. As these qualitative trends were consistent with the hypothesis, we proceeded to investigate relationships among decay class, density, total C and $\delta^{13}\text{C}$ for a wider array of wood and CWD samples.

Changes in Density, Carbon and $\delta^{13}\text{C}$ with Decay Class

Means for density, total C (1996 and 2004 measurements) and $\delta^{13}\text{C}$ by species with decay class are shown in Table 3 (CFC samples only). Comparison of means by species was unbalanced, because there were no samples of western hemlock in decay class 5, and no unknowns in classes 1–3. Means for all species showed that density decreased significantly by decay class except between classes 1 and 2. Although total C values from 2004 were higher, data from 1996 and 2004 were consistent in showing significantly

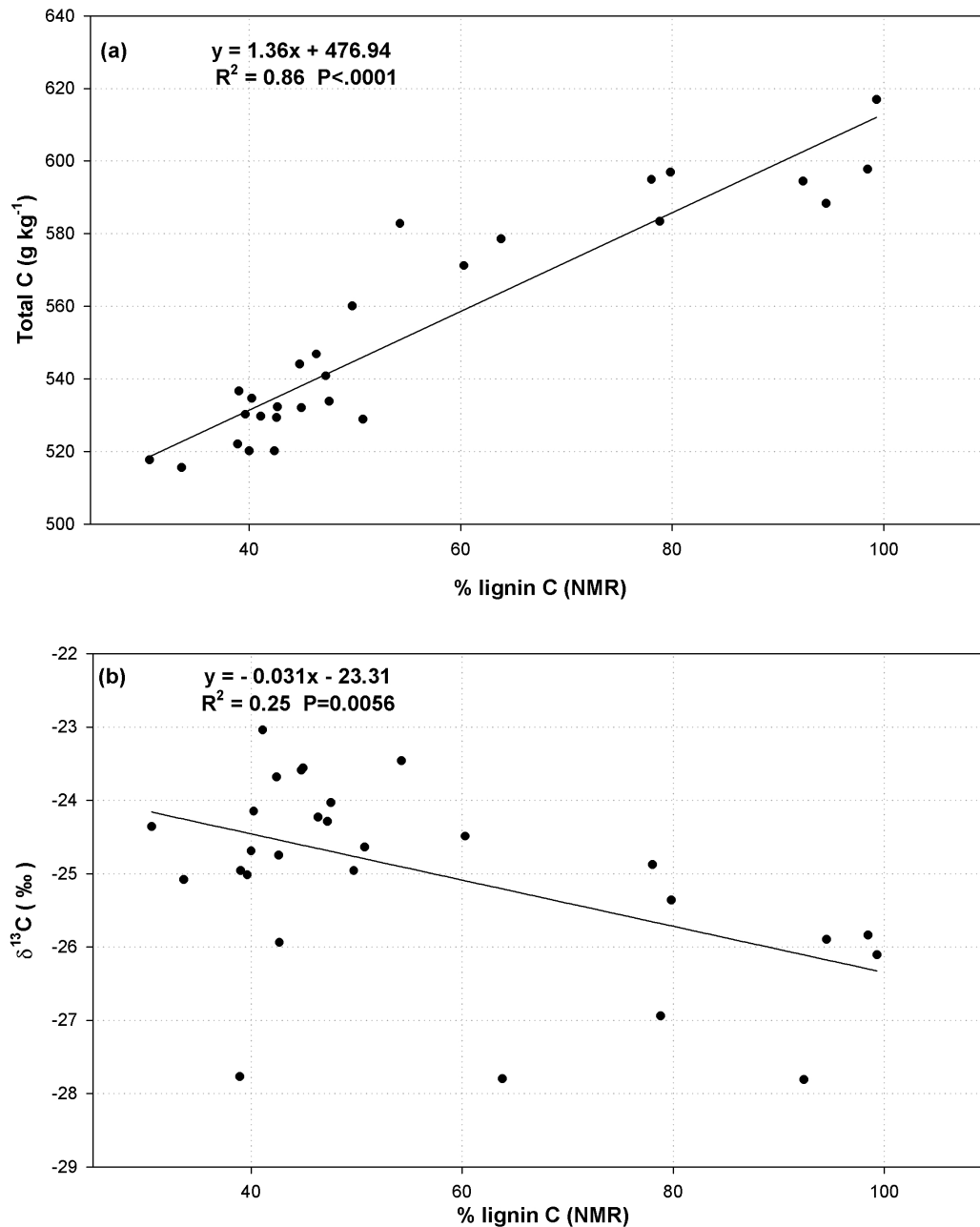


Fig. 1. (a) Total C (2004 data) and (b) $\delta^{13}\text{C}$ vs. percentage of lignin C from NMR, for 27 CFC samples plus CIDET western hemlock wood.

higher total C in decay classes 4–5 than in 1–3. Results for density and total C (1996 values) were of course similar to those reported previously [Table 1 in Preston et al. (1998)], which were based on slightly different sample sets, and with all-species means based on two size classes (7–12 and >12 cm diameter).

For $\delta^{13}\text{C}$, the means for all species showed that decay class 5 was significantly more depleted in ^{13}C than the lower decay classes. Means for individual species showed that cedar had the highest $\delta^{13}\text{C}$, and the unknown samples the lowest, mainly because the latter were all decay class 4 and

5. For cedar, the data indicate that $\delta^{13}\text{C}$ hardly changed until decay class 5. This may reflect the previous result that cedar was more likely to continue longer with chemically nonselective mass loss, with higher cellulose content, and thus higher ^{13}C persisting to later decay stages. We then explored the influence of seral stage (regeneration to old-growth) and subzone (east vs. west coast) on density, total C, and $\delta^{13}\text{C}$ for all species by decay class, again for the CFC samples only. For density and total C, these effects were small, with decay class the dominating influence, as before (Table 2).

Table 3. Means of density (g cm⁻³), total C (g kg⁻¹) from 1996 and 2004 analyses, and $\delta^{13}\text{C}$ (‰) by decay class for coarse woody debris samples of western redcedar (Cw), Douglas-fir (Fd), western hemlock (Hw) and unknown species (U) from the CFC plots, and cell frequencies by decay class

Decay class	Species				Mean	Same
	Cw	Fd	Hw	U		
Density (g cm⁻³)						
1	0.46	0.40	0.46	.	0.43	A
2	0.33	0.41	0.42	.	0.39	A
3	0.29	0.34	0.33	.	0.32	B
4	0.19	0.21	0.25	0.19	0.21	C
5	0.11	0.20	.	0.13	0.13	D
Mean	0.28	0.33	0.35	0.16	0.28	
Same	B	A	A	C		
1996 C (g kg⁻¹)						
1	476	487	491	.	484	B
2	483	496	476	.	486	B
3	465	488	483	.	480	B
4	488	527	513	519	513	A
5	555	532	.	530	531	A
Mean	483	502	491	525	502	
Same	C	B	BC	A		
2004 C (g kg⁻¹)						
1	533	540	534	.	536	C
2	534	545	531	.	537	C
3	527	546	527	.	537	C
4	553	591	571	583	576	B
5	597	602	.	603	603	A
Mean	541	558	544	593	561	
Same	C	B	BC	A		
$\delta^{13}\text{C}$ (‰)						
1	-24.1	-25.1	-24.0	.	-24.5	A
2	-23.8	-25.5	-26.1	.	-25.3	AB
3	-23.9	-24.0	-25.7	.	-24.3	A
4	-24.0	-25.6	-26.3	-25.9	-25.6	AB
5	-25.4	-25.4	.	-26.8	-26.6	B
Mean	-24.0	-25.1	-25.9	-26.4	-25.4	
Same	A	B	BC	C		
Cell Frequencies						
1	3	4	2	.	9	
2	7	12	10	.	29	
3	5	9	4	.	18	
4	8	8	8	16	40	
5	1	2	.	19	22	
Total	24	35	24	35	118	

For $\delta^{13}\text{C}$ however, there was a zone effect (Table 4), as $\delta^{13}\text{C}$ in cedar was less depleted than in Douglas-fir or western hemlock and did not vary with zone. However, $\delta^{13}\text{C}$ tended to be more depleted in Douglas-fir and western hemlock in the west than in the east zone with the greater effect noticeable in western hemlock (a difference of 2.1 ‰).

Relationships among Total C, $\delta^{13}\text{C}$ and Density

The previous section showed that results for density and total C by decay class for the core CFC samples were similar to those obtained previously, and also demonstrated a decrease in $\delta^{13}\text{C}$ with increasing decay class. Decay class is assessed in the field using bulk properties, is subject to individual bias, and can be quite difficult to assign, especially as the pattern of decay is generally not homogeneous within a

bole (Creed et al. 2004). Furthermore, some highly decayed logs may have little change in chemical composition, or even enrichment in cellulose, compared with sound logs (Preston et al. 1998). In this case, they would not show the changes in total C and $\delta^{13}\text{C}$ expected where the final stages are dominated by brown rot, with increase in lignin concentration. We next examined relationships only among total C (2004 values), $\delta^{13}\text{C}$ and density, using the full sample set including fresh Douglas-fir wood from the Shawnigan Lake biomass sampling, and the CIDET western hemlock wood.

Multiple regression analyses were carried out separately for cedar, hemlock, Douglas-fir and unknown species, for east side only, west side only (except Douglas-fir with too few west-side samples), and both sides, and then for all species combined for east side, west side and both sides. Correlations of total C with density and $\delta^{13}\text{C}$ were all nega-

Table 4. Means of $\delta^{13}\text{C}$ (‰) by decay class and zone for coarse woody debris samples of western redcedar (Cw), Douglas-fir (Fd), western hemlock (Hw) and unknown species (U) from the CFC plots, and cell frequencies by decay class

(a) $\delta^{13}\text{C}$ (‰)									
Species Decay class	Cw Zones		Fd Zones		Hw Zones		U Zones		
	E	W	E	W	E	W	E	W	
1	-23.04	-24.56	-25.09	.	-24.00
2	-24.05	-23.54	-25.28	-26.67	-24.33	-26.51	.	.	.
3	-23.94	.	-24.03	-23.56	-24.75	-25.94	.	.	.
4	-24.18	-23.85	-25.63	.	-24.49	-26.59	-24.81	-26.81	.
5	-25.36	.	-25.37	.	.	.	-25.61	-27.38	.
All	-24.07	-23.91	-25.04	-25.63	-24.32	-26.45	-25.18	-27.15	.

(b) 2004 Cell frequencies									
Species Decay class	Cw Zones		Fd Zones		Hw Zones		U Zones		
	E	W	E	W	E	W	E	W	
1	1	2	4	.	2
2	4	3	10	2	2	8	.	.	.
3	5	.	8	1	1	3	.	.	.
4	4	4	8	.	1	7	7	9	.
5	1	.	2	.	.	.	6	13	.
All	15	9	32	3	6	18	13	22	.

tive, but few significant relationships were found, all between total C and $\delta^{13}\text{C}$. Correlations between $\delta^{13}\text{C}$ and density were weaker, with no significant relationships, and some negative instead of the expected positive slopes. Similar results were found using Douglas-fir plus unknown for the east side and hemlock or cedar plus unknown for the west side. Table 5 shows results from the multiple regression analysis using all species for east side, west side and both zones. In all models the parameter value for C was significantly different from 0 and on its own strongly correlated with $\delta^{13}\text{C}$ (Pearson's $R = -0.5561$). The parameter value for density was not significantly different from 0 and on its own only weakly correlated with $\delta^{13}\text{C}$ (Pearson's $R = 0.1774$). Comparison of slopes for C showed a tendency for west to have greater slope than east, but either zone was not significantly different from the overall model slope for both zones.

DISCUSSION

Sample Total Carbon Concentrations

The reanalysis of total C was done to improve precision of data for this study, but there is recent interest in the C concentration of wood as a factor in C budget modeling (Elias and Potvin 2003; Lamloom and Savidge 2003). Wood biomass is usually multiplied by 0.5 to generate C concentrations, but bolewood C concentrations actually show wide variation. For 32 neotropical tree species, Elias and Potvin (2003) found total C of trunkwood from 444 to 494 g kg⁻¹. Lamloom and Savidge (2003) found 472 to 552 g kg⁻¹ for kiln-dried heartwood of 18 North American conifer species. Their values for Douglas-fir (505 g kg⁻¹), western hemlock (506 g kg⁻¹) and western redcedar (515 g kg⁻¹) are between our 1996 and 2004 values for decay class 1, which should be similar to fresh wood.

Since this was not a methodology study, a detailed discussion is not appropriate, but wood total C values can be

affected by many factors, even when run with appropriate and careful analytical protocols. These include type of sample (core, heartwood, etc.), and degree of grinding, as finer grinding usually improves precision. The very small samples used in some analyzers (e.g., 1 mg) enhance issues related to grinding and representative subsampling. Drying protocols are also an important factor. Lamloom and Savidge (2003) found lower total C after oven-drying at 93°C for 1 wk, presumably to emulate kiln-drying, than after slow desiccation at room temperature under partial vacuum. Our samples were originally dried at 70°C, and for an additional 18–24 h immediately before analysis, which should not have driven off as much volatile C as their kiln-drying protocol.

Our higher values for decay class 1 (2004 values) than in Lamloom and Savidge (2003) may be due to lower drying temperature, and also the nature of the sample. The CWD was sampled as slices of the whole bole, including bark where it was still attached (generally decay classes 1 to 3), with bark being higher in total C than wood (Preston and Forrester 2004). Despite carrying out a number of checks, we cannot account for the discrepancy between our 1996 and 2004 data, which is unlikely to be due solely to the pre-analysis redrying. We have found that despite our best efforts in quality control, that there still remains some variation in repeated analysis. Some further investigation into protocols for total C analysis would be useful, and we also suggest that methods should be reported in more detail, especially the drying procedure.

Testing the $\delta^{13}\text{C}$ Hypothesis

The trends found for the samples previously analyzed for lignin C by NMR are consistent with the hypothesis that $\delta^{13}\text{C}$ of CWD should decrease with increasing lignin concentration of CWD. With all points included, the linear fit in Fig. 1b has a slope of -0.031 , and intercepts corresponding to $\delta^{13}\text{C}$ of -23.3‰ for 0% lignin (100% cellulose) and

Table 5. Results of multiple regression analysis of $\delta^{13}\text{C}$ (‰) on total C (g kg^{-1}), and density (g cm^{-3}) for all species of woody debris in both zones, east zone only and west zone only

Regression model	r^2	$P > F$	Intercept		C		Density	
			B0	(SE B0) ^z	B1	(SE B1)	B2	(SE B2)
Both zones	0.1614	<.0001	-10.57	(3.4000)	-0.025	(0.0055)	-2.29	(1.6059)
East zone	0.3101	<.0001	-14.12	(2.1020)	-0.018	(0.0035)	-1.48	(1.0290)
West zone	0.1123	0.0203	-7.57	(6.9721)	-0.031	(0.0112)	-4.16	(3.1656)

^zSE = standard error.

-26.4‰ for 100% lignin. These are consistent with previous studies showing that lignin was depleted by 1.7–3.3‰ relative to total C of wood, and by 3.0–4.4‰ relative to cellulose (Benner et al. 1987; Goñi and Eglinton 1996; Loader et al. 2003; Spiker and Hatcher 1987). Our slope is therefore within the expected range of -0.030 to -0.044, and the intercepts are also within those reported for cellulose and lignin. For CWD samples ranging from 30% to 100% lignin C, $\delta^{13}\text{C}$ might then be expected to decrease 2.1–3.1‰, or approximately 10%, a small change to detect against natural variation (discussed later).

The fit of total C vs. % lignin (Fig. 1a) is also reasonably consistent with a simple lignin-cellulose model. The y-intercept (477 g kg^{-1}) compares well with the total C for pure cellulose (444 g kg^{-1}). Based on several reported total C values for gymnosperm lignin preparations (Hemmingson and Newman 1985; Brunow et al. 1990; Hatcher 1998; Smernik and Oades 2000), and brown-rot lignin from Douglas-fir (Jin et al. 1990), we estimate lignin C of 610 g kg^{-1} . This compares well with the intercept of 613 g kg^{-1} from Fig. 1a, which also indicates that the range to be expected between fresh wood and lignin is approximately 520 to 620 g kg^{-1} .

For the larger dataset, relationships of $\delta^{13}\text{C}$ with density and decay class were weaker. As noted previously, decay class may not correlate well with chemical properties, but the relationships with density were expected to be more robust. However, significant correlations were found between $\delta^{13}\text{C}$ and total C, consistent with an increase in total C as the proportion of lignin increases relative to cellulose and hemicellulose.

Factors Affecting $\delta^{13}\text{C}$ Variation

While producing results not inconsistent with our hypothesis, this study highlights many issues arising from studying this largely natural forest setting. While there are some problems with the decay class system, it is reasonable that significant changes in $\delta^{13}\text{C}$ and total C were only found between decay classes 4 or 5, and the lower decay classes (Table 3). Actual wood decay is not recognized until decay class 2, and the main decay effects occur in classes 3 to 5. In the previous study, Preston et al. (1998) found substantial change in organic composition from decay class 3 to decay class 4 [although the transition was between decay classes 2 and 3 in Preston et al. (1990) for CWD from Washington and Oregon]. Since the proportions of lignin and cellulose hardly changed from decay classes 1 to 3, it is not surprising that bulk total C and $\delta^{13}\text{C}$ only changed significantly for the highest decay classes.

The relationships between total C and $\delta^{13}\text{C}$ for the larger sample set indicate substantial natural variation in $\delta^{13}\text{C}$. In particular, some samples identified as hemlock were highly depleted, especially from the west side. Environmental conditions that reduce photosynthetic discrimination against ^{13}C include moisture stress, bright sunlight, and higher position in the canopy, both within-tree and across species (Buchmann et al. 1997; Flanagan et al. 1999; Bowling et al. 2002; Fessenden and Ehleringer 2002; 2003; Leavitt 2002).

For old-growth forests on the wetter west coast of Vancouver Island, the main natural disturbance is opening of small gaps due to tree death or windthrow (Trofymow et al. 2003). Thus local environments are variable, and may change during the life of a tree. Suppressed hemlock and cedar many grow very slowly for decades in a shaded understory site, then rapidly in a sunlit gap. Trees on the west side experience little moisture stress, and that coupled with shading could enhance isotope discrimination. On the east site, fire was more important historically, with regeneration occurring in drier, more open sites, so that lesser isotope discrimination could be expected.

Recommendations for Further Studies

The CFC project and CWD sampling were designed for more general objectives, including C budget modeling and successional dynamics, and several recommendations could be made to focus on $\delta^{13}\text{C}$ in CWD. More information is needed on the natural range of $\delta^{13}\text{C}$ of wood for these species in coastal forests. Samples were taken as whole discs, including bark if present, but could be taken as smaller portions with homogeneous decay characteristics, and exclusion of fine roots or other non-woody components. Kohzu et al. (2005) recently showed that wood-decomposing fungi were enriched in ^{13}C compared with their woody substrates, beyond what could be attributed to the isotopic variation within wood components. The range of site types could be restricted, for example, to old-growth. Many samples were not identified to species, and even when identified, many were coded as "less certain". It should be possible to identify even highly decomposed logs using biomolecular tools (White et al. 2000). Lignin content of a wider suite of samples should be determined by NMR, or if possible, a faster technique such as Fourier-transform infrared (FTIR) spectroscopy. Determining $\delta^{13}\text{C}$ of isolated cellulose or lignin of some samples could highlight sources of variation. Future studies of detrital organic matter of these forests should consider the possible influence of lignin-rich CWD on forest floor and deeper organic horizons. The over-

all effects may be small, however, as depletion due to lignin-rich woody residues may be compensated by the higher $\delta^{13}\text{C}$ of wood than of foliage (Leavit and Long 1982).

Implications for Decomposition Studies

Much confusion and mal-hypothesizing about decomposition processes arise because of the persistent confusion of the chemical entity lignin, with the AUR from proximate analysis. As an integrated measure of the most chemically resistant, insoluble components, AUR is usually inversely related to decomposition rate, and increases in concentration with decomposition. For most foliage, however, this increase may be due more to the relative increase in alkyl C, whereas NMR or wet-chemical analyses may show a decline or little change of aromatic C, or specific lignin or tannin structures. The ^{13}C depletion found in AUR with respect to total C (Benner et al. 1987; Quideau et al. 2003; Preston, unpublished results) is thus consistent with its incorporation of the most chemically resistant fractions of three biopolymers, cutin, condensed tannins, and lignin.

CONCLUSIONS

The hypothesis that ^{13}C depletion should increase with increasing lignin concentration was tested for CWD in forest sites of Vancouver Island. Samples for which the proportion of lignin C had been previously determined by ^{13}C NMR showed a qualitative trend consistent with this hypothesis, and also with the expected increase in C concentration. For a larger set of CWD samples without NMR analysis, total C increased for the most decayed samples, and significant negative correlations were found between total C and $\delta^{13}\text{C}$, although relationships of total C and $\delta^{13}\text{C}$ with density were very weak. We also found high natural variation in $\delta^{13}\text{C}$ of CWD, especially for hemlock on the wetter west side of Vancouver Island, which made it difficult to establish small changes in $\delta^{13}\text{C}$ with decomposition. Further studies with more appropriate sampling protocol could improve our understanding of the influence of species, growth environment and decay processes on the $\delta^{13}\text{C}$ of CWD. Nonetheless, consistent with previous geochemical studies, our limited results indicate that the natural abundance of ^{13}C in CWD decreases with increasing concentration of chemically defined lignin. This information should help to clarify the "lignin paradox" that has been associated with litter decomposition and misinterpretation of the AUR fraction from proximate analysis.

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