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# Application of $^{13}\text{C}$ NMR spectroscopy to characterize organic chemical components of decomposing coarse woody debris from different climatic regions

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**Abstract.** Solid-state  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy was applied to coarse woody debris (CWD) in different stages of decomposition and collected from forest floor of a subtropical, a cool temperate, and a subalpine forest in Japan. The purpose was to test its applicability to characterize organic chemical composition of CWD of broad-leaved and coniferous trees from different climatic conditions. O-alkyl-C, mainly representing carbohydrates, was the predominant component of CWD at the three sites, accounting for 43.5-58.1% of the NMR spectra. Generally, the relative area under the signals for aromatic-C and phenolic-C, mainly representing lignin, increased, whereas the relative area for O-alkyl-C decreased, as the decay class advanced. The relative area under NMR chemical shift regions was significantly correlated with the chemical properties examined with proximate analyses. That is, O-alkyl-C and di-O-alkyl-C NMR signal areas were positively correlated with the volumetric density of CWD and the content of total carbohydrates. Methoxyl-C, aromatic-C, phenolic-C, carboxyl-C, and carbonyl-C were positively correlated with the contents of acid-unhydrolyzable residues (lignin, tannins, and cutin) and nitrogen. Lignin-C calculated from NMR signals increased, and polysaccharide-C decreased, with the decay class of CWD at the three study sites. A review of previous studies on  $^{13}\text{C}$  NMR spectroscopy for decomposing CWD suggested further needs of its application to broad-leaved trees from tropical and subtropical regions. **Keywords** Carbon-13 nuclear magnetic resonance spectroscopy, coarse woody debris, lignin, decomposition, wood.

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## Introduction

Coarse woody debris (CWD) is a major component of forest biomass, serving as a long-term, large pool of carbon and playing a major role in the maintenance of forest biodiversity (Harmon et al. 1986; Stokland et al. 2012). CWD is characterized by low content of nutrients (Holub et al. 2001, Laiho & Prescott 2004), high content of cell wall polymers such as lignin and holocellulose (Eriksson et al. 1990), and variable decomposition rates (Mackensen et al. 2001, Weedon et al. 2009). Therefore, studying the chemical composition and the decay dynamics of CWD is crucial for understanding and predicting the cycling of carbon and nutrients in forest ecosystems. The composition of organic chemical compounds of CWD has often been loosely determined by proximate analysis. For example, the content of lignin, a dominant organic component that often limits the decomposition (Osono 2007), has been defined as the acid-unhydrolyzable residues (AUR), but AUR may include other recalcitrant compounds such as tannins and cutin (Preston et al. 1997).

Alternatively, recent studies have confirmed the applicability of solid-state  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy for characterizing organic chemical components of CWD in more detail (Wilson 1987, Baldock & Preston 1995).  $^{13}\text{C}$  NMR spectroscopy has been used successfully to understand the decay dynamics of not only CWD (Preston et al. 1990, 1998, 2012, McColl & Powers 1998, Ganjegunte et al. 2004, Strukelj et al. 2012, 2013) but also leaf litter and soil organic matter (Osono et al. 2008, 2014, Ono et al. 2009, 2011, 2013). Despite its potential applicability, a few attempts have been made to investigate with  $^{13}\text{C}$  NMR spectroscopy the organic chemical composition of CWD at different stages of decomposition collected in forests of various climatic conditions.

The purpose of the present study was to characterize the organic chemical composition of

CWD collected from three forest sites in Japan using  $^{13}\text{C}$  NMR spectroscopy. CWD samples (at various stages of decomposition) of dominant broad-leaved trees from a subtropical and a cool temperate forest and of conifers from a subalpine forest were used, as these CWD samples had already been examined for proximate fractions and decomposer fungal assemblages (Fukasawa et al. 2009, 2012, 2014). This allowed comparison of the results of proximate analysis with those of NMR spectroscopy.

## Materials and methods

### Study sites and collection of CWD

Samples used in the present study were collected from three sites in Japan: a subtropical forest (ST) in Okinawa, a cool temperate forest (CT) in Kyoto, and a subalpine forest (SA) on Mt. Ontake, Gifu (Table 1). The three sites differed in mean annual temperature but received similar amounts of precipitation annually (Table 1). The major tree species included evergreen broad-leaved trees in ST, deciduous broad-leaved trees in CT, and evergreen coniferous trees in SA. The forest floor mass and its turnover time (as the ratio of forest floor mass to leaf fall mass) were in the order:  $\text{ST} < \text{CT} < \text{SA}$  (Table 1).

Coarse woody debris (CWD) was defined as snags and logs that had diameter  $> 10$  cm at breast height for snags and at the base for logs. The decay rate constant ( $k$ ) of CWD estimated with the input/accumulation ratio method (Harmon et al. 1986) was in the order:  $\text{ST} > \text{CT} > \text{SA}$  (Table 1). In the present study, logs were classified into five decay classes (Sollins 1982), from the least decayed class 1 to the most decayed class 5, and collected at three sites in September and December 2004 and in September 2005 (Table 1). Undecayed wood samples collected from living trees were denoted as the decay class 0. The log samples were then used to determine the density and the

**Table 1** Location, climate, vegetation, and properties of the forest floor and CWD in the study sites in Japan

Site ID	ST	CT	SA
Location <sup>a</sup>			
Latitude N	26°49'	35°18'	35°56'
Longitude E	128°50'	135°43'	137°28'
Elevation (m)	330	660	2050
Site name	Kunigami, Okinawa	Ashiu, Kyoto	Mt. Ontake, Gifu
Climate <sup>a</sup>			
Mean annual temperature (°C)	22	10	2
Annual precipitation (mm)	2456	2495	2500
Climate region	Subtropical	Cool temperate	Subalpine
Vegetation <sup>a</sup>			
Type	Evergreen broad-leaved	Deciduous broad-leaved	Evergreen coniferous
Dominant tree species	<i>Castanopsis sieboldii</i> , <i>Schima wallichii</i>	<i>Fagus crenata</i> , <i>Quercus crispula</i>	<i>Abies mariesii</i> , <i>A. veitchii</i> , <i>Picea jezoensis</i> var. <i>hondoensis</i>
Forest floor <sup>b</sup>			
Forest floor mass (Mg/ha)	12.0	33.3	104.6
Leaf fall mass (Mg/ha/yr)	7.95	3.20	3.59
Turnover time (yr)	1.5	10.4	29.1
Coarse woody debris			
Sampling	December 2004 <sup>c</sup>	September 2005 <sup>d</sup>	September 2004 <sup>e</sup>
CWD mass (Mg/ha)	6.62 <sup>f</sup>	25.34 <sup>f</sup>	42.40 <sup>e</sup>
CWD input (Mg/ha)	0.84 <sup>f</sup>	3.70 <sup>f</sup>	1.54 <sup>e</sup>
Decay rate constant (/yr)	0.146 <sup>f</sup>	0.126 <sup>f</sup>	0.036 <sup>e</sup>

Note. <sup>a</sup>Osono (2015a), <sup>b</sup>Osono (2015b), moder site, <sup>c</sup>Fukasawa et al. (2012), <sup>d</sup>Fukasawa et al. (2009), <sup>e</sup>Fukasawa et al. (2014), <sup>f</sup>Hishinuma et al. (unpublished data)

contents of total carbohydrates (TCH), acid-unhydrolyzable residues (AUR), and nitrogen (Fukasawa et al. 2009, 2012, 2014). Portions of the log samples used for these proximate analyses were combined for each site and each decay class prior to NMR analysis.

### NMR analysis

Cross-polarization (CP) magic angle spinning (MAS) <sup>13</sup>C NMR spectra of the samples were obtained with a Varian Inova 300 spectrometer operating at 75.3 MHz under the following conditions (Gilardi et al. 1995), according to the method described in Osono et al. (2014). The pulse repetition time was 3.2 sec, the CP contact time was 1.5 min, the sweep width was 50 kHz, and the acquisition time was 40

min. MAS was performed at 4.5 kHz in silicon nitride rotors with Torlon caps. The chemical shifts were referenced to the methyl-C signal (17.3 ppm) of hexamethylbenzene.

The CP-MAS <sup>13</sup>C NMR spectra were divided into eight chemical shift ranges according to the procedure of Preston et al. (1998): aliphatic (0-47 ppm), methoxyl (47-60 ppm), O-alkyl (60-95 ppm), di-O-alkyl (95-110 ppm), aromatic (no oxygen attached, 110-140 ppm), phenolic (aromatic carbon with oxygen attached, 140-165 ppm), carboxyl (165-190 ppm), and carbonyl (190-215 ppm). The relative area of these chemical shift regions was calculated for each spectrum as the percentage of total area. Components of TCH and AUR (lignin, tannins, and cutin) examined with the proximate analyses can be assigned into some

NMR chemical shift regions with different proportions (Preston et al. 1997). That is, carbohydrates can be represented by O-alkyl-C, di-O-alkyl-C, and phenolic-C; lignin and tannins can be represented mainly by methoxyl-C, aromatic-C, and phenolic-C but also by O-alkyl-C; cutin can be represented by aliphatic-C, O-alkyl-C, aromatic-C, and carboxyl-C.

### Data analyses

The NMR data were used to calculate lignin-C and polysaccharide-C (Preston et al. 1998) and lignin to polysaccharide (L/P) ratio in CWD:

$$\text{Lignin-C} = \text{phenolic-C} \times 4.5 + \text{methoxyl-C} \quad (1)$$

$$\text{Polysaccharide-C} = 1.2 \times (\text{O-alkyl-C} - \text{phenolic-C} \times 1.5) \quad (2)$$

$$\text{L/P ratio} = \text{Lignin-C} / \text{Polysaccharide-C} \quad (3)$$

The ratio of alkyl-C to O-alkyl-C (A/O-A ratio) provides a sensitive index of the decomposition of soil organic matter (Baldock et al. 1997):

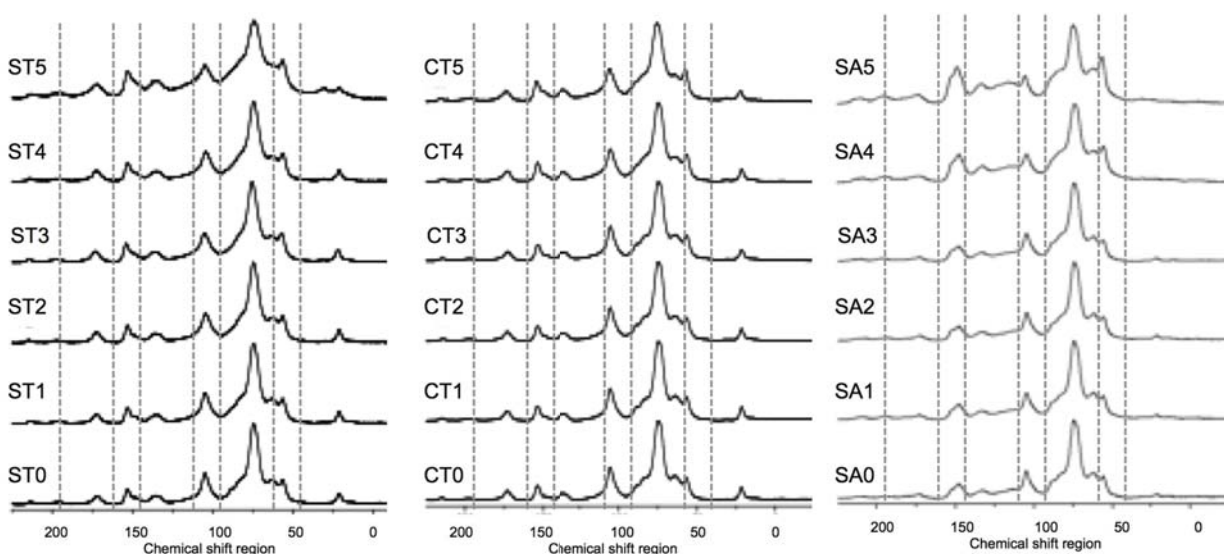
$$\text{A/O-A} = \text{aliphatic-C} / \text{O-alkyl-C} \quad (4)$$

Pearson's correlation coefficients were calculated for linear relationships between the relative area of chemical shift regions and four NMR indices (lignin-C, polysaccharide-C, L/P ratio, and A/O-A) and the density and contents of TCH, AUR, and nitrogen.

### Results

O-alkyl-C was the predominant component, accounting for 43.5-58.1% of the NMR spectra of CWD at the three study sites, followed by methoxyl-C, di-O-alkyl-C, aromatic-C, and phenolic-C (Fig. 1, Table 2). Generally, the relative area of the signals for aromatic-C and phenolic-C increased, whereas the relative area of O-alkyl-C decreased, with increasing decay class at the three study sites (Fig. 1, Table 2). The relative area of carboxyl-C and carbonyl-C increased, whereas the relative area of di-O-alkyl-C decreased, with increasing decay class at ST and SA (Fig. 1, Table 2). The relative area of methoxyl-C increased with increasing decay class at SA (Fig. 1, Table 2).

Lignin-C content was generally in the order: CT < ST < SA and increased with increasing



**Figure 1**  $^{13}\text{C}$  NMR spectra of CWD in decay classes 0 to 5 at the three sites. ST, subtropical forest; CT, cool temperate forest; SA, subalpine forest. The numbers following the abbreviated site codes indicate the decay class

**Table 2** Relative area (% total area of spectra) of NMR chemical shift regions and NMR indices of CWD

Site	Decay class	Relative area (%)										NMR index <sup>a</sup>			
		Aliphatic (0-47 ppm)	Methoxyl (47-60 ppm)	O-alkyl (60-95 ppm)	di-O-alkyl (95-110 ppm)	Aromatic (110-140 ppm)	Phenolic (140-165 ppm)	Carboxyl (165-190 ppm)	Carbonyl (190-215 ppm)	Lignin-C	Polysaccharide-C	L/P ratio	A/O-A		
ST <sup>a</sup>	0	6.3	9.4	55.1	11.4	6.6	5.8	3.6	1.7	35.5	55.8	0.64	0.115		
	1	6.2	9.2	54.2	11.5	7.0	6.0	4.1	1.9	36.0	54.3	0.66	0.115		
	2	6.9	9.7	53.0	11.4	6.8	6.0	4.4	1.8	36.8	52.8	0.70	0.130		
	3	6.6	9.2	51.9	10.8	7.9	6.9	4.6	2.1	40.3	49.9	0.81	0.126		
	4	5.8	9.5	52.4	11.3	7.7	6.7	4.5	2.1	39.5	50.9	0.78	0.112		
	5	7.8	8.0	44.6	8.9	10.2	9.1	6.8	4.6	49.0	37.1	1.32	0.176		
CT <sup>b</sup>	0	5.4	7.3	58.1	12.7	6.0	5.2	3.8	1.5	30.6	60.4	0.51	0.092		
	1	5.8	8.0	57.9	12.3	5.5	5.1	3.8	1.6	30.9	60.3	0.51	0.101		
	2	5.2	7.6	56.1	12.7	7.5	5.5	3.8	1.6	32.5	57.3	0.57	0.093		
	3	6.2	7.9	52.5	12.3	8.3	6.5	4.2	2.2	37.0	51.4	0.72	0.118		
	4	4.5	7.8	54.0	12.2	8.5	6.7	4.3	2.0	37.9	52.8	0.72	0.084		
	5	5.1	7.7	51.7	11.7	10.3	7.6	4.0	1.9	41.9	48.3	0.87	0.099		
SA <sup>c</sup>	0	2.4	7.8	57.7	10.1	10.1	7.5	2.4	2.0	41.7	55.7	0.75	0.041		
	1	4.0	8.0	53.9	9.8	10.8	7.9	2.9	2.7	43.6	50.5	0.86	0.075		
	2	3.6	8.1	54.5	10.2	10.4	7.6	2.9	2.6	42.5	51.6	0.82	0.067		
	3	1.9	8.3	57.3	10.0	10.0	8.1	2.2	2.2	44.6	54.3	0.82	0.034		
	4	5.7	10.5	45.3	7.6	12.7	11.1	3.4	3.8	60.4	34.4	1.76	0.125		
	5	3.9	10.6	43.5	5.6	15.3	13.1	3.8	4.3	69.3	28.7	2.41	0.090		

Note. <sup>a</sup> Lignin-C - Phenolic x 4.5 + Methoxyl, Polysaccharide-C - 1.2 x (O-alkyl - Phenolic x 1.5), L/P ratio - Lignin-C / Polysaccharide-C, A/O-A - Aliphatic / O-alkyl

decay class (Table 2). Polysaccharide-C was generally in the order: CT > ST, SA and decreased with increasing decay class (Table 2). Consequently, L/P ratio was in the order: CT < ST < SA and increased with increasing decay class (Table 2). A/O-A was generally not related to the decay class (Table 2).

Correlation coefficients were calculated for the linear relationships between the relative area of NMR chemical shift regions and NMR indices versus the density and contents of TCH, AUR, and nitrogen of CWD (Table 3). In general, the density and TCH contents were significantly and positively correlated with O-alkyl-C, di-O-alkyl-C, and polysaccharide-C, and significantly and negatively correlated with methoxyl-C, aromatic-C, phenolic-C, carboxyl-C, carbonyl-C, lignin-C, and L/P ratio (Table 3). In contrast, the contents of AUR and nitrogen were significantly and positively correlated with methoxyl-C, aromatic-C, phe-

nolic-C, carboxyl-C, carbonyl-C, lignin-C, and L/P ratio, and significantly and negatively correlated with O-alkyl-C, di-O-alkyl-C, and polysaccharide-C (Table 3).

## Discussion

The relative area of chemical shift regions and the predominance of O-alkyl-C in the NMR spectra of CWD at the three sites (Fig. 1, Table 2) was consistent with previous reports on CWD (reviewed in Table 4), as well as on leaf litter (Osono et al. 2008, 2014, Ono et al. 2009, 2011, 2013). Lower values of relative area of aromatic-C and phenolic-C and higher values of aliphatic-C in broad-leaved than in coniferous trees (Fig. 1, Table 2) were consistent with the previous reports. Those previous studies also showed a decrease in the relative area of O-alkyl-C, a concomitant increase in the rela-

**Table 3** NMR chemical shift regions and indices that were significantly ( $P < 0.05$ ) correlated with the density and the contents of total carbohydrates (TCH), acid-unhydrolyzable residues (AUR), and nitrogen of CWD at least at one of the three study sites. Correlation coefficients for linear relationships not shown in this table were not statistically significant ( $P > 0.05$ ). Numbers indicate the study site where the significant correlation was found: 1, ST; 2, CT; 3, SA

Density	TCH	AUR	Nitrogen
Positive correlation			
O-alkyl <sup>1,2,3</sup>	O-alkyl <sup>1,2,3</sup>	Methoxyl <sup>3</sup>	Methoxyl <sup>3</sup>
di-O-alkyl <sup>3</sup>	di-O-alkyl <sup>1,3</sup>	Aromatic <sup>1,2,3</sup>	Aromatic <sup>1,2,3</sup>
Polysaccharide-C <sup>1,2,3</sup>	Polysaccharide-C <sup>1,2,3</sup>	Phenolic <sup>1,2,3</sup>	Phenolic <sup>1,2,3</sup>
		Carboxyl <sup>1,3</sup>	Carboxyl <sup>1,3</sup>
		Carbonyl <sup>1,3</sup>	Carbonyl <sup>1,3</sup>
		Lignin-C <sup>1,2,3</sup>	Lignin-C <sup>1,2,3</sup>
		L/P ratio <sup>1,2,3</sup>	L/P ratio <sup>1,2,3</sup>
		A/O-A <sup>1</sup>	
Negative correlation			
Methoxyl <sup>3</sup>	Methoxyl <sup>3</sup>		
Aromatic <sup>1,2,3</sup>	Aromatic <sup>1,2,3</sup>		
Phenolic <sup>1,2,3</sup>	Phenolic <sup>1,2,3</sup>		
Carboxyl <sup>1</sup>	Carboxyl <sup>1,3</sup>	O-alkyl <sup>1,2,3</sup>	O-alkyl <sup>1,3</sup>
Carbonyl <sup>3</sup>	Carbonyl <sup>1,3</sup>	di-O-alkyl <sup>1,3</sup>	di-O-alkyl <sup>1,2,3</sup>
Lignin-C <sup>1,2,3</sup>	Lignin-C <sup>1,2,3</sup>	Polysaccharide-C <sup>1,2,3</sup>	Polysaccharide-C <sup>1,2,3</sup>
L/P ratio <sup>1,2,3</sup>	L/P ratio <sup>1,2,3</sup>		
	A/O-A <sup>1</sup>		

tive area of aromatic-C and phenolic-C, and similar patterns of changes in NMR indices according to the decay class or the period of decomposition (Table 4).

The contents of lignin-C, polysaccharide-C, L/P ratio, and A/O-A of CWD in the present study are within the range found in the previous NMR studies of CWD (Table 4). The lower lignin-C and higher polysaccharide-C in broad-leaved than in coniferous trees (Table 2) generally agreed with those of previous reports (Table 4). The increase of L/P ratio appears a general phenomenon in decomposing CWD across climatic conditions and tree species (Tables 2 and 4). A/O-A has been proposed as a sensitive index of the extent of decomposition of soil organic matter that increases as O-alkyl-C is transformed to alkyl-C (Baldock et al. 1997, Rosenberg et al. 2003). However, A/O-A of CWD in the present study was generally a poor indicator of decomposition, since A/O-A was not significantly related to the decay class or the relative density of wood (Table 3). Previous studies found either variable (Preston et al. 1998; Ganjegunte et al. 2004) or increasing patterns (Strukelj et al. 2012, 2013) of A/O-A with the decomposition of CWD (Table 4).

The relative area under NMR chemical shift regions was generally significantly correlated with the chemical properties examined with proximate analyses, regardless of the study site (Table 3). The general decrease of some components with the decomposition and the concomitant increase in the others indicated the faster net decomposition of the former than the latter and/or the net transformation of the former into the latter. The positive correlations observed here of lignin-C and AUR with the decay class (Table 3) are consistent with the results of Preston et al. (1997) and suggest that lignin is a major component of AUR in wood. The loss of O-alkyl-C and polysaccharide-C was associated with a loss of carbohydrates, such as holocellulose (Wilson et al. 1983). The positive association of lignin-C with nitrogen

content in decaying CWD (Table 3) may be due to the formation of nitrogenous lignin-like substances as secondary compounds, as suggested by Berg (1986) and Takeda and Abe (2001).

The patterns of change in organic chemical composition of decomposing CWD observed in the present study were primarily influenced by three factors: climatic conditions, wood species and quality, and decomposer fungi (Harmon 1986). Of these, the difference in decomposer fungi with different ligninolytic and cellulolytic activities partly accounted for the different patterns of organic chemical components in CWD among the study sites. In fact, CWD of broad-leaved trees at ST and CT harbored predominantly ligninolytic fungi (Fukasawa et al. 2009, 2011, 2012), whereas Fukasawa et al. (2014) encountered both ligninolytic and non-ligninolytic fungi on conifers at SA.

## Conclusions

The present study demonstrates the applicability of solid-state  $^{13}\text{C}$  NMR spectroscopy to CWD at different stages of decomposition collected from forests of different climatic conditions in Japan. The results of  $^{13}\text{C}$  NMR spectroscopy were generally compatible with those of proximate analysis and had a potential to provide useful indicators of long-term decomposition of CWD. The higher lignin content and the higher increment of lignin content with the decay class at SA than at ST and CT may be attributed to wood species (i.e., conifers), the cooler climatic condition, or both. The relative importance of climatic condition and wood species cannot be divided in the present study, however, because of the pseudo-replication of experimental design. The previous studies have tended to be performed on coniferous trees from boreal and temperate forests (Table 4). Thus, further efforts should be paid to the application of  $^{13}\text{C}$  NMR spectroscopy to



**Table 4** A review of relative area (% total area of spectra) of NMR chemical shift regions and NMR indices of CWD. nd, no data. na, Di-O-alkyl-C was combined to O-alkyl-C

Tree species	Sample	Relative area (%)										NMR index <sup>a</sup>				
		Alkyl	Methoxyl	O-alkyl	Di-O-alkyl	Aromatic	Phenolic	Carbonyl	Lignin-C	Polysaccharide-C	L/P ratio	A/O-A				
<i>Abies magnifica</i> <sup>b</sup>	Year 0	2.0	7.2	65.6	na	16.1	7.7	1.5	41.9	nd	nd	nd	nd	nd	nd	
	Year 17	15.4	7.2	49.7	na	13.0	8.2	6.6	44.1	nd	nd	nd	nd	nd	nd	
<i>Thuja plicata</i> <sup>c</sup>	Decay class 1	3.3	6.7	58.2	9.5	12.3	7.6	2.4	40.9	56.2	0.73	0.057				
	Decay class 2	5.3	7.2	56.7	8.7	12.4	7.4	2.3	40.5	54.7	0.74	0.093				
	Decay class 3	2.0	5.1	64.8	10.9	9.5	5.7	2.0	30.8	67.5	0.46	0.031				
	Decay class 4	3.1	5.5	59.9	9.3	12.1	7.7	2.4	40.2	58.0	0.69	0.052				
	Decay class 5	5.8	10.1	30.5	3.2	23.4	15.5	11.5	79.9	8.7	9.18	0.190				
<i>Pseudotsuga menziesii</i> <sup>c</sup>	Decay class 1	9.4	6.7	55.3	9.4	10.5	7.2	1.5	39.1	53.4	0.73	0.170				
	Decay class 2	8.6	5.3	52.8	8.4	12.3	9.9	2.7	49.9	45.5	1.09	0.163				
	Decay class 3	7.0	7.2	55.4	9.5	11.2	8.4	1.3	45.0	51.4	0.88	0.126				
	Decay class 4	8.2	9.8	35.2	4.1	20.6	15.4	6.7	79.1	14.5	5.45	0.233				
	Decay class 5	4.7	11.3	27.5	2.6	26.3	19.4	8.2	nd	nd	nd	0.171				
<i>Tsuga heterophylla</i> <sup>c</sup>	Decay class 1	3.1	7.1	57.2	8.4	12.1	9.1	3.0	48.1	52.3	0.92	0.054				
	Decay class 2	4.6	7.0	55.9	9.2	12.7	8.8	1.8	46.6	51.2	0.91	0.082				
	Decay class 3	2.4	7.0	59.5	9.9	11.9	7.9	1.4	42.6	57.2	0.74	0.040				
	Decay class 4	6.6	7.4	46.8	7.9	15.7	11.8	3.8	60.5	34.9	1.73	0.141				
<i>Pinus radiata</i> <sup>d</sup>	Year 0	0.7	15.9	46.9	13.1	12.4	7.0	1.1	47.4	43.7	1.09	0.014				
	Year 1	0.7	16.1	46.3	12.4	13.6	7.2	1.8	48.5	42.6	1.14	0.014				
	Year 4	0.7	15.3	46.3	12.7	13.6	7.4	2.1	48.6	42.2	1.15	0.014				
	Year 9	1.1	16.8	35.6	10.6	16.0	9.8	3.4	60.9	25.1	2.43	0.031				
	Year 13	0.7	15.9	44.1	13.0	13.4	8.0	2.7	51.9	38.5	1.35	0.016				
<i>Populus tremuloides</i> <sup>e</sup>	Year 0	6.6	6.0	62.9	14.2	4.8	2.6	3.0	17.8	70.8	0.25	0.104				
	Year 1	6.6	6.3	63.8	14.2	4.3	2.4	2.5	16.9	72.3	0.23	0.103				

**Table 4** (continuation)

Tree species	Sample	Relative area (%)										NMR index <sup>a</sup>				
		Alkyl		Methoxyl		O-alkyl		Di-O-alkyl		Aromatic		Phenolic		Carbonyl	Lignin-C	Polysaccharide-C
<i>Picea glauca</i> <sup>e</sup>	Year 3	8.1	7.4	59.0	13.4	5.7	3.2	3.3	3.3	3.2	3.3	21.8	65.0	0.34	0.137	
	Year 6	9.4	7.3	56.6	13.0	6.5	3.5	3.8	3.8	3.5	3.8	22.9	61.7	0.37	0.165	
	Year 0	4.7	6.1	61.9	11.7	9.6	4.1	1.9	1.9	4.1	1.9	24.5	66.9	0.37	0.075	
	Year 1	5.0	6.1	62.0	11.7	9.4	3.8	1.9	1.9	3.8	1.9	23.3	67.6	0.35	0.081	
	Year 6	6.2	6.4	59.9	11.3	9.9	4.0	2.4	2.4	4.0	2.4	24.4	64.7	0.38	0.103	
	Year 0	4.8	6.6	60.3	11.2	10.5	4.4	2.1	2.1	4.4	2.1	26.6	64.4	0.41	0.080	
<i>Abies balsamea</i> <sup>e</sup>	Year 1	4.8	6.6	60.6	11.3	10.4	4.4	1.8	1.8	4.4	1.8	26.3	64.9	0.41	0.080	
	Year 5	5.3	7.2	56.4	10.5	12.6	5.4	2.7	2.7	5.4	2.7	31.5	57.9	0.54	0.093	
	Decay class 1,2	6.8	6.6	61.5	13.4	5.9	3.0	2.8	2.8	3.0	2.8	20.0	68.4	0.29	0.110	
<i>Betula papyrifera</i> <sup>d</sup>	Decay class 5	8.9	6.9	55.4	11.9	8.9	4.2	3.8	3.8	4.2	3.8	25.8	58.9	0.44	0.161	
	Decay class 1,2	7.1	6.6	62.4	13.2	5.7	2.5	2.5	2.5	2.5	2.5	17.8	70.4	0.25	0.114	
<i>Populus tremuloides</i> <sup>f</sup>	Decay class 3,4	7.3	6.4	61.9	13.1	5.7	2.7	2.9	2.9	2.7	2.9	18.5	69.4	0.27	0.119	
	Decay class 5	10.9	7.1	54.7	11.5	7.8	3.6	4.3	4.3	3.6	4.3	23.3	59.1	0.39	0.200	
	Decay class 1,2	5.5	6.7	60.3	10.5	10.8	4.2	1.9	1.9	4.2	1.9	25.7	64.7	0.40	0.092	
<i>Abies balsamea</i> <sup>d</sup>	Decay class 3,4	6.0	6.9	58.4	10.1	11.7	4.6	2.3	2.3	4.6	2.3	27.7	61.7	0.45	0.103	
	Decay class 5	6.5	6.8	57.8	10.1	11.7	4.6	2.5	2.5	4.6	2.5	27.5	61.1	0.45	0.112	
	Decay class 1,2	7.0	6.4	59.6	10.5	11.0	3.9	1.7	1.7	3.9	1.7	23.9	64.5	0.37	0.118	
<i>Pinus banksiana</i> <sup>d</sup>	Decay class 5	14.5	7.0	45.7	7.7	15.6	6.2	3.3	3.3	6.2	3.3	34.7	43.8	0.79	0.318	
	Decay class 1,2	6.0	6.6	59.4	10.4	11.2	4.4	2.1	2.1	4.4	2.1	26.2	63.5	0.41	0.101	
<i>Picea glauca</i> <sup>f</sup>	Decay class 3,4	6.8	7.0	56.3	9.6	13.0	5.0	2.3	2.3	5.0	2.3	29.3	58.6	0.50	0.122	
	Decay class 5	8.3	8.6	47.2	7.5	18.0	7.3	3.2	3.2	7.3	3.2	41.2	43.6	0.94	0.175	

Note. <sup>a</sup> Lignin-C - Phenolic x 4.5 + Methoxyl, Polysaccharide-C -1.2 x (O-alkyl - Phenolic x 1.5), L/P ratio - Lignin-C / Polysaccharide-C, A/O-A - Aliphatic / O-alkyl.

<sup>b</sup>McColl and Powers (1998), California, USA; <sup>c</sup>Preston et al. (1998), British Columbia, Canada, <sup>d</sup>Ganjegunte et al. (2004), New Zealand; <sup>e</sup>Strukelj et al. 2012, Quebec, Canada, <sup>f</sup>Strukelj et al. 2013, Quebec, Canada.

decomposing CWD of broad-leaved trees from tropical and subtropical regions and of the single tree species distributed across different climatic regions.

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