Evaluation of CuO oxidation parameters for determining the source and stage of lignin degradation in soil

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Abstract. The composition of phenols and other aromatic compounds in organic and mineral soil horizons and their respective source vegetation from different climatic zones of the Canadian Prairies were analyzed using CuO oxidation and gas chromatography-mass spectrometry (GC-MS) to investigate the stage of lignin degradation. Parameters based on the CuO oxidation products were calculated for the soils and corresponding vegetation to determine the lignin sources and to monitor the lignin degradation. In addition to the widely used lignin monomer parameters, parameters resulting from lignin-derived phenolic dimers are used for the first time to assess lignin degradation in soils. The composition of lignin-derived phenols (S/V, C/V) in soil closely matches the composition observed in their respective source plants (grass, Aspen, Pine) reflecting the preservation of characteristic lignin patterns in soils. Degradation parameters based on lignin phenols and benzenes derived from tannins or other phenolic biomolecules indicate a progressive degradation from the vegetation to the soil horizons. In addition to commonly used lignin monomer indicators, parameters based on the lignin dimers are applied. Lignin degradation is found to be lowest in the Pine forest, intermediate in the grassland soils and highest in the Aspen-grassland transition soil. Degradation parameters based on non-lignin aromatic derivatives (3,5-dihydroxybenzoic acid, benzenepolycarboxylic acids) demonstrate a similar trend. The lignin from samples in the cooler climate (Black Chernozems) is observed to be more oxidized than in the soils from the warmer climate (Brown Chernozems) suggesting that abiotic processes may be in involved in the alteration of lignin and other phenolic biomolecules in soils. The results indicate that the comparative analysis of CuO oxidation products of soils and source vegetation is a valuable tool to assess the sources and degradation of lignin in soils.

Introduction

Organic molecules derived from vascular plants are the major source of soil organic matter (SOM) and accounts for approximately 75% of the active soil organic carbon reservoirs (Goñi and Hedges 1992; Hedges 1992). Lignin is one of the most abundant biomolecules of vascular plants and represents a significant input of plant organic matter to soils (Gleixner et al. 2001). Compared to other plant-derived molecules such as cellulose which are rapidly mineralized in soils, lignin decomposition may be relatively slower in cooler climates (Dai et al. 2002) and the accumulation of lignin in some soils has been observed (Lichtfouse et al. 1995; Kögel-Knabner 2002; Dignac et al. 2005). Only a small group of fungi (white-rot and brown-rot fungi) are able to biodegrade lignin in terrestrial environments while bacteria are predominant in the biodegradation of lignin in aquatic systems (Benner et al. 1986; Gleixner et al. 2001). Since the biodegradation of lignin and other constituents of SOM depend on environmental factors such as pH and temperature, changes in climate or land use will alter the SOM decomposition and possibly result in an increased release of carbon dioxide to the atmosphere (Schimel et al. 1994; Trumbore 1997; Franzluebbers et al. 2001; Gleixner et al. 2001). Therefore, the potential for SOM to release elevated amounts of carbon dioxide from increased decomposition rates caused by global warming is of great concern (Trumbore et al. 1996; Schlesinger and Andrews 2000).

The chemical analysis of lignin is difficult due to the intricate structure of the ligno-cellulose complex consisting of the polysaccharide cellulose and the phenol-based network of the lignin macromolecule. However, the alkaline oxidation with CuO and the analysis of the products with gas chromatography-flame ionization detection (GC-FID) or gas chromatography-mass spectrometry (GC-MS) has proven to be a

valuable method for the analyses of lignin and cutin in plants, soils and sediments (Hedges and Ertel 1982; Kögel and Bochter 1985; Goñi et al. 2000; Kögel-Knabner 2000). CuO oxidation does not completely depolymerize lignin and is therefore not quantitative, but the method cleaves aryl ether bonds and releases phenolic monomers and dimers from the outer part of the lignin polymer that are indicative of lignin content and composition (Johansson et al. 1986; Kögel 1986; Goñi and Hedges 1992). Lignin is composed of the derivatives of three basic structural classes: vanillyls, syringyls, and cinnamyls. The composition of these lignin phenols is characteristic of major plant groups such as angiosperms and gymnosperms (Hedges and Mann 1979; Hedges and Ertel 1982). Several parameters for the determination of lignin degradation have been proposed after field observations and laboratory experiments. Laboratory studies such as the incubation of wood with fungi have indicated that the yields of CuO oxidation products decrease with progressing biodegradation while the acid to aldehyde ratios (Ad/Al) of the vanillyl and syringyl type monomers, respectively, increase (Ertel and Hedges 1985; Hedges et al. 1988; Opsahl and Benner 1995). The (Ad/Al) ratios have been applied in numerous studies to determine the degradation stage of SOM and riverine organic matter (OM; e.g., Ertel and Hedges 1984; Hedges et al. 1988; Goñi et al. 1993, 2000). In addition to the lignin monomers, the composition of dimeric lignin phenols was found to be characteristic for the source and degradation of lignin in sediments and several parameters based on lignin dimers were proposed (Goñi and Hedges 1992). In addition, several aromatic compounds such as *p*-hydroxybenzaldehyde and hydroxybenzoic acids are commonly found among the CuO oxidation products of SOM and riverine OM (Hedges and Ertel 1982; Goñi et al. 2000). These benzenes do not solely originate from lignin, but are derived from proteins and/or phenolic plant biomolecules such as tannins (Hedges and Parker 1976; Goñi et al. 2000). Ratios involving benzene derivatives such as the 3,5-dihydroxybenzoic acid/vanillyls ratio were also suggested as degradation parameters for the OM in rivers and soils (Prahl et al. 1994).

Previous studies on the lignin composition using alkaline CuO oxidation focused on the organic matter in river, shelf sediments and soils (e.g., Ertel and Hedges 1985; Kögel and Bochter, 1985; Hedges et al. 1988; Prahl et al. 1994; Goñi et al. 2000; Kögel-Knabner 2000) but this method has not been extensively applied to study the progression of lignin degradation in fresh plant material, leaf litter and SOM. Although the contents of major lignin phenols and the (Ad/Al) ratios for soils with different source vegetation have been reported (e.g., Kögel and Bochter 1985; Ziegler et al. 1986; Sanger et al. 1996; Rumpel et al. 2002), comparative studies of the molecular composition of CuO oxidation products and degradation of lignin in soils and their source plants are not as prevalent (Sanger et al. 1996; van Bergen et al. 1997, 1998; Nierop 2001; Nierop et al. 2001; Nierop and Verstraten 2003). In this study, the CuO oxidation products of organic and mineral soil horizons and their respective source vegetation were analyzed to investigate the lignin degradation in soils at the molecular-level. Parameters for lignin composition and degradation based on the CuO oxidation products were calculated for the fresh and decomposing plants and the soil horizons to determine the lignin sources and to monitor the progressing lignin degradation in soils. Source and degradation parameters based on lignin dimers as previously proposed for riverine OM by Goñi et al. (2000) are applied here to evaluate their applicability and utility to the study of SOM biogeochemistry. The samples originate from the Prairie Ecozone of Western Canada which accounts for 80% of the arable agricultural land in Canada and contains large reserves of soil organic carbon (Janzen et al. 1998).

Materials and methods

Soil and vegetation samples

Soil and vegetation samples were collected from Alberta, Canada in October of 2002. Sample details are listed in Table 1. Samples were collected from a variety of soil environments (grassland, forest-grassland, and pine forest). The grassland sample set (Brown, Dark Brown, and Black Chernozems) includes four different samples that represent soils with similar soil forming factors with the exception of climate. The dominant vegetation, Western Wheatgrass (*Agropyron smithii*), was also collected from some sites and

Table 1. Soil and vegetation sample properties.

Samples	Location	Soil sample depth[a]	pH[b]	Texture	Soil moisture regime[c]
Grassland					
Brown Chernozem	SE of Lethbridge, Alberta	0–15 cm	6.4	Loam	Subarid to Semiarid
Decomposing grass	SE of Lethbridge, Alberta	na	na	na	Subarid to Semiarid
Fresh grass (grass 1)	SE of Lethbridge, Alberta	na	na	na	Subarid to Semiarid
Dark Brown Chernozem Ah	Lethbridge, Alberta	0–15 cm	6.6	Silt Loam	Semiarid
Fresh grass (grass 2)	Lethbridge, Alberta	na	na	na	
Orthic Black Chernozem Ah	Tofield, Alberta	0–15 cm	6.75	Silt Loam	Subhumid
Eluviated Black Chernozem Ah	Ellerslie, Alberta	0–15 cm	6.4	Silt Loam	Subhumid
Aspen-Grassland					
Brown Aspen Leaves	Strathcona county, Alberta	na	na	na	Subhumid
Dark Gray Chernozem Ah	Strathcona county, Alberta	0–10 cm	6.1	Loam	Subhumid
Dark Gray Chernozem Ahe	Strathcona county, Alberta	10–25 cm	6.0	Loam	Subhumid
Pine Forest					
Pine needles	Hinton, Alberta	na	na	na	Subhumid to Humid
Brunisol leaf lifter layer (LFH)	Hinton, Alberta	5–0 cm	5.8	na	Subhumid to Humid
Brunisol O Horizon	Hinton, Alberta	0–20 cm	5.8	na	Subhumid to Humid

na = not applicable.
[a]This is the depth sampled, not the depth of the horizon.
[b]Measured in deionized water.
[c]From Soil Classification Working Group (1998).

analyzed. The mean annual soil temperature varies from 1.7 °C in the Black Chernozemic soil zone to 3.3 °C in the Brown Chernozemic soil zone (Janzen et al. 1998). The annual precipitation is reported to be 452 mm in the Black Chernozemic soil zone and 413 mm in the Brown Chernozemic soil zone (Janzen et al. 1998).

The Dark Gray Chernozem was sampled from the grassland-forest transition zone in Strathcona County, Alberta. Vegetation samples were collected from a stand of Quaking Aspen (*Populus tremula*) in a grassed area and included brown Aspen leaves. Two soil samples were collected from this site. The first is an organic-rich surface horizon (Ah, 0–10 cm) and an eluviated, organic-rich horizon (Ahe, 10–25 cm).

The Eutric Brunisol soil samples (referred to as Brunisol samples) were taken from a pristine conifer forest near Hinton, Alberta, and the vegetation was dominated by Lodgepole Pine (*Pinus contorta*) associated with grasses in the herbaceous layer. Green pine needles, leaf litter consisting mainly of Pine needles and minor amounts of grass and angiosperm leaves, and the organic horizon (O) were sampled. The Brunisolic soil does not have a surface mineral horizon (classified as <17% organic carbon) because the environmental conditions promote the accumulation of organic matter which results in an organic rich (>17% organic carbon) surface horizon (O horizon).

The soil samples were air-dried and stored in glass containers at room temperature. Mineral soil samples were passed through a 2 mm sieve prior to extraction. All plant materials and organic horizons were freeze-dried and kept in glass containers at −20 °C to prevent microbial degradation.

Determination of carbon and nitrogen contents

Carbon and nitrogen contents were determined using an elemental Analyzer Vario EL III (Hanau, Germany) C, H, O, N, S elemental analyzer. Soil samples were ground into a fine powder and milligram quantities were analyzed for carbon and nitrogen contents. Samples were run in duplicate and the average carbon and nitrogen contents are reported in Table 1. The degree of variability between duplicate measurements varied from 0 to 0.5% (data not shown). Inorganic carbon, such as carbonates, was determined by the method of Bundy and Bremner (1972). This entailed acidifying the sample with 2 M HCl, collecting any evolved CO_2 in 5 ml of 2.0 M KOH and then back titrating the KOH to determine the amount of inorganic carbon in the sample. Inorganic carbon was not detected in any of the soil horizons or plants used in this study. Consequently, elemental carbon values represent the amount of organic carbon in these samples.

CuO oxidation

The soil and plant samples were solvent-extracted to remove soluble lipids. The detailed extraction procedure and the composition of extractable lipids were published elsewhere (Otto et al. 2005). Subsamples of the solvent-extracted soil samples were oxidized with CuO to release lignin-derived phenols (modified after Hedges and Ertel 1982). Teflon-lined bombs (20 ml) were loaded with 2 g of dry soil, 1 g CuO (pre-extracted with dichloromethane), 100 mg ammonium iron (II) sulfate hexahydrate $[Fe(NH_4)_2(SO_4)_2 \cdot 6 H_2O]$ and 15 ml of 2 M NaOH. The teflon vessels were purged with nitrogen gas, sealed and heated for 2.5 h at 170 °C. After heating, the bombs were cooled under running water, the liquid was decanted into a teflon centrifuge tube (50 ml), and the residue was washed twice with each 10 ml deionized water using a magnetic stirrer for 10 min. The combined washings were transferred to the centrifuge tube and centrifuged for 30 min at 2500 rpm. The supernatant was decanted into a fresh teflon centrifuge tube, acidified to pH 1 using 6 M HCl and kept for 1 h at room temperature in the dark to prevent reactions of cinnamic acids. After centrifugation (30 min at 2500 rpm) the supernatant was transferred to a separation funnel and liquid–liquid extracted twice with each 50 ml diethyl ether. Anhydrous Na_2SO_4 was added to the combined ether phases to remove any remaining water. The ether extracts were concentrated by rotary evaporation, transferred to 2 ml glass vials and dried under nitrogen gas. The CuO yields were determined by weighing the dry residues. The comparative CuO oxidation of three subsamples (2 g each) of the Orthic Black Chernozem yielded 4.8, 5.0 and 5.5 mg of products, representing 94–108% of the average and demonstrating the reproducibility of the method.

Derivatization of extracts and Gas chromatography – Mass Spectrometry

The CuO oxidation products were redissolved in 500 μl dichloromethane:methanol (1:1; v/v). Aliquots of the extracts (100 μl) were dried in a stream of nitrogen and then converted to trimethylsilyl (TMS) derivatives by reaction with 90 μl N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and 10 μl pyridine for 3 h at 70 °C. After cooling, 100 μl hexane was added to dilute the extracts. GC-MS analyses of the derivatized extracts was performed on an Agilent model 6890N GC coupled to an Agilent model 5973N quadrupole mass selective detector (MSD). Separation was achieved on a HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The GC operating conditions were as follows: temperature hold at 65 °C for 2 min, increase from 65 to 300 °C at a rate of 6 °C/min with a final isothermal hold at 300 °C for 20 min. Helium was used as carrier gas. The sample was injected with a 1:2 split and the injector temperature set at 280 °C. The samples (1 μl) were injected with an Agilent 7683 autosampler. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV ionization energy and scanned from 50 to 650 D. Data were acquired and processed with Agilent Chemstation G1701DA software. Individual compounds were identified by comparison of mass spectra with literature,

Wiley MS library data, comparison with authentic standards, or interpretation of mass spectrometric fragmentation patterns. Vanillic acid (as trimethylsilyl derivative) was used as the external quantification standard.

Lignin composition and degradation parameters

The composition of phenolic lignin compounds obtained after CuO oxidation can be used to calculate parameters for the origin and degradation stage of the lignin. Therefore, the ratios of syringyl to vanillyl (S/V) and cinnamyl to vanillyl type (C/V) monomers according to Ertel and Hedges (1984), the lignin phenol vegetation index (LPVI) proposed by Tareq et al. (2004), and the ratio of lignin dimers vs. monomers (D/M) were calculated to determine sources of lignin in SOM. Parameters for the degradation stage included commonly used parameters such as the total lignin yield after CuO oxidation expressed in mg/g C (VSC), and the ratios of lignin-derived phenolic acids and their corresponding aldehydes (Ad/Al) for vanillyl $(Ad/Al)_v$ and syringyl $(Ad/Al)_s$ units (Ertel and Hedges 1985). In addition, recently proposed degradation parameters based on the lignin dimer composition which have been proposed for the characterization of lignin in sediments (Goñi and Hedges 1992) were applied here for the first time to lignin in soils.

Results and discussion

Carbon and nitrogen contents and CuO oxidation yields

The carbon and nitrogen contents and yields of CuO products are given in Table 2. The carbon contents ranged from 29.7 to 50.9% in the plant samples, 52.0% in the pine leaf litter, 23.2% in the organic (O) horizon and 2.1–14.2% in the mineral horizons (A). The nitrogen contents varied between 0.5–1.4% in the plant samples and 0.2–0.8% in the soil horizons. The atomic carbon to nitrogen (C/N_a) ratios of the plant samples were generally high and decreased in the soil. The carbon content of the roots is lower than the overlying vegetation suggesting that not all of the soil particles were sufficiently removed during sieving.

Table 2. Carbon and nitrogen contents and total extract yields after CuO oxidation of soils and source vegetation from Alberta, Canada.

Samples	Carbon (%)	Nitrogen (%)	C/N_a^1	Yields (mg/g C)
Grassland soils				
Roots of Western Wheatgrass	29.7	0.8	43	301.6
Western Wheatgrass 1(BrCh)	46.0	0.7	77	109.4
Western Wheatgrass 2 (DBrCh)	44.2	0.9	57	125.5
Decomposing Western Wheatgrass	34.8	1.4	29	110.1
Brown Chernozem Ah horizon	2.1	0.2	12	52.8
Dark Brown Chernozem Ah horizon	2.8	0.3	11	57.5
Orthic Black Chernozem Ah horizon	4.4	0.4	13	37.4
Eluviated Black Chernozem Ah horizon	5.3	0.4	16	25.6
Aspen-grassland soil				
Brown leaves of Quaking Aspen	50.9	0.5	119	112.5
Dark Gray Chernozem Ah horizon	14.2	0.2	83	248.0
Dark Gray Chernozem Ahe horizon	5.0	0.3	19	63.4
Pine forest soil				
Green needles of Lodgepole pine	50.5	1.0	59	200.0
Brunisol leaf litter	52.0	0.9	67	135.1
Brunisol O horizon	23.1	0.8	34	252.7

[1] C/N_a = Atomic ratio of carbon to nitrogen.

Fine particles, such as clay minerals, may adhere to the roots making it difficult to obtain a 'clean' root sample. The grass samples yielded 110.1–301.6 mg phenols/g C and the grassland soils 25.2–52.8 phenols mg/g C after CuO oxidation. Despite their higher carbon content, the Black Chernozems yielded less CuO oxidation products than the Brown Chernozems. The plant and soil samples from the aspen-grassland transition soil (Dark Gray Chernozem) yielded 63.4–248.0 mg/g C with the highest amount in the O horizon. The CuO oxidation products extracted from the pine needles, leaf litter and pine forest O horizon varied between 135.1 and 252.7 (mg/gC) with the highest yields from the O horizon.

Composition of CuO oxidation products

The CuO oxidation of plant and soil samples yielded predominantly monomeric and dimeric phenols and benzoic acids (Figure 1). The occurrence and concentrations of individual phenols and benzenes in the samples analyzed are given in Table 3 (grassland soils) and Table 4 (Aspen-grassland soil and Pine forest soil). CuO products observed in all soil and plant samples included benzoic acid, *p*-hydroxybenzaldehyde and *m*- and *p*-hydroxybenzoic acid and 2-carboxypyrrole which were reported as the CuO oxidation products of proteins (Goñi et al. 2000). Another group of benzenes consisting of 3,5-dihydroxybenzoic acid (3,5OH-Bd) and benzenepolycarboxylic acids (BPCAs) was detected in all of the soil samples but was not detected in the grass samples and less abundant in the Aspen leaves and Pine needles. 3,5OH-Bd and BPCAs have been previously identified in numerous soils and soil humic acids after CuO oxidation however, BPCAs are to our knowledge reported here for the first time as CuO oxidation products of plant materials. Within the four grassland soils, the concentrations of the protein-derived benzenes decreased
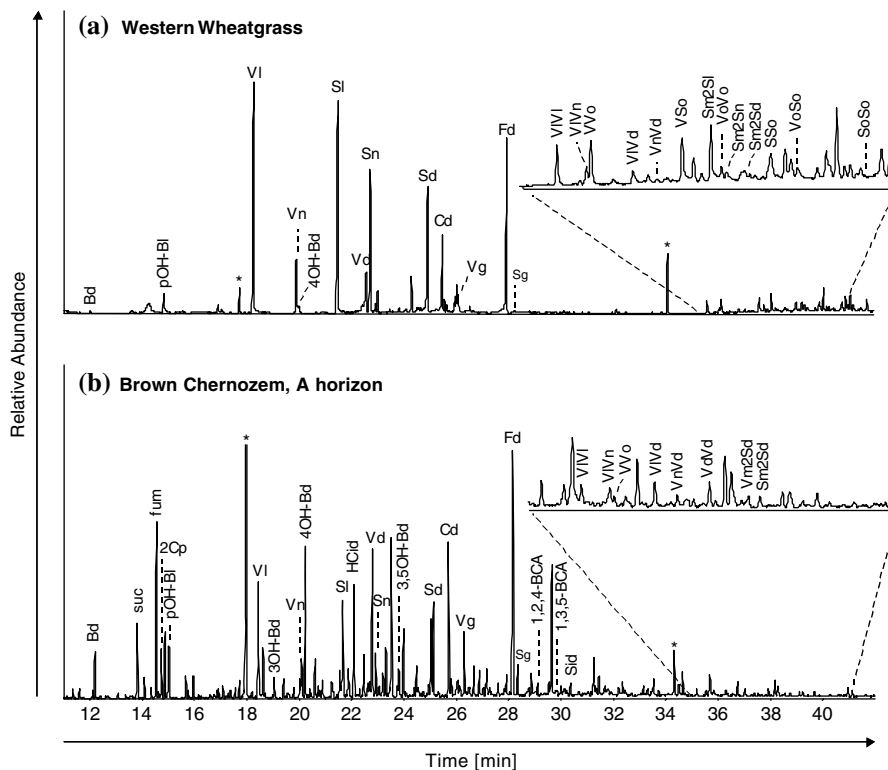

Figure 1. GC-MS chromatograms (TIC) of the silylated CuO oxidation products of (a) Western Wheatgrass and (b) the mineral horizon of a grassland soil (Brown Chernozem) from Alberta, Canada. ☆ = contamination. Peak labels are listed in Table 3.

Table 3. Major compounds identified after CuO oxidation of grassland soils and source vegetation from Alberta, Canada.

No.	Compound name	Concentration (µg/g C)								ID[b]
		Roots[a]	Grass 1[a]	Grass 2[a]	Deco.grass[a]	BrCh[a]	DBrCh[a]	BlCh[a]	EBlCh[a]	
Protein-derived products										
Bd	Benzoic acid	301	40	64	99	209	153	89	126	S
pOH-Bl	p-Hydroxybenzaldehyde	883	305	351	433	271	166	80	96	L
3OH-Bd	m-Hydroxybenzoic acid (3-OH)	232	15	28	146	84	69	92	23	L
4OH-Bd	p-Hydroxybenzoic acid (4-OH)	2804	119	345	504	672	439	272	429	L
2Cp	2-Carboxypyrrole	18	16	103	18	210	274	303	317	L
Total		4238	495	891	1200	1446	1101	836	991	
Other benzenes										
3,5OH-Bd	3,5-Dihydroxybenzoic acid	–	–	–	–	154	132	314	350	S
1,4-BCA	1,4-Benzenedicarboxylic acid	–	–	–	–	–	27	31	28	S
1,2,4-BCA	1,2,4-Benzenetricarboxylic acid	–	–	–	–	60	66	226	170	S
1,3,5-BCA	1,3,5-Benzenetricarboxylic acid	–	–	–	–	19	17	170	151	S
1,2,4,5-BCA	1,2,4,5-Benzenetetracarboxylic acid	–								S
Total		0	0	0	0	233	242	741	699	
Lignin monomers										
Vl	Vanillin	14616	12883	13046	4288	572	481	242	293	S
Vn	Acetovanillone	4899	2364	2328	1090	235	239	164	191	A
Vd	Vanillic acid	7602	2499	2664	1962	701	697	427	553	S
Vg	Vanillylglyoxalic acid	2361	803	1359	756	322	305	226	251	B
Total vanillyls		29478	18549	19397	8096	1830	1722	1059	1288	
Sl	Syringaldehyde	8657	9352	9417	3204	551	473	292	240	S
Sn	Acetosyringone	2719	5793	4276	1479	221	263	190	148	B
Sd	Syringic acid	4723	5502	4096	1999	446	502	407	396	S
Sg	Syringylglyoxalic acid	596	21	472	315	146	136	155	179	B
Sid	Sinapic acid	–	–	–	107	63	53	59	81	L
Total syringyls		16695	20668	18261	7104	1427	1427	1103	1044	
Cinnamyls										
HCid	Hydrocinnamic acid	266	–	–	45	523	18	–	–	L
Cd	p-Coumaric acid	4685	3467	2090	764	713	634	946	393	L
Fd	Ferulic acid	8806	8125	7241	1992	1334	898	596	456	L
Total cinnamyls		13757	11592	9331	2801	2570	1550	1542	849	
Total lignin monomers		59930	50809	46989	18001	5827	4699	3704	3181	
Lignin dimers										
Vl-Vl	Dehydrodivanillin	583	517	489	235	39	47	20	28	B
Vl-Vn	Dehydrovanillinacetovanillone	329	126	174	68	26	31	11	15	B
Vl-Vd	Dehydrovanillinvanillic acid	497	249	156	173	48	54	38	58	B

Table 3. Continued.

No.	Compound name	Concentration (µg/g C)								ID[b]
		Roots[a]	Grass 1[a]	Grass 2[a]	Deco.grass[a]	BrCh[a]	DBrCh[a]	BlCh[a]	EBlCh[a]	
Vn-Vd	Dehydroacetovanillonevanillic acid	119	6	–	35	17	24	21	35	B
Vd-Vd	Dehydrodivanillic acid	157	34	66	63	37	45	41	73	B
Total 5,5'-dimers		1685	932	885	574	167	201	131	209	
VVo	Vanillovanillone	420	583	259	146	12	8	10	11	B
VSo	Vanillosyringone	801	607	465	189	9	10	–	–	B
SSo	Syringosyringone	278	–	111	31	–	–	–	–	B
Total α,1-monoketone dimers		1499	1190	835	366	21	18	10	11	
Vm2Sl	2-Vanillylsyringaldehyde	104	–	60	43	21	18	10	11	B
Vm2Sn	2-Vanillylacetosyringone	–	–	–	–	–	18	27	–	B
Vm2Sd	2-Vanillylsyringic acid	–	–	–	–	10	25	–	–	B
Sm2Sl	2-Syringylsyringaldehyde	–	–	–	–	16	21	–	29	B
Sm2Sl	2-Syringylsyringaldehyde	–	258	–	244	–	35	–	–	B
Sm2Sn	2-Syringylacetosyringone	–	43	67	26	–	12	–	–	B
Sm2Sd	2-Syringylsyringic acid	148	51	138	139	25	27	19	43	B
Total α,2-methyl dimers		252	352	265	452	51	138	46	72	
VoVo	Vanillil	308	150	174	44	–	–	–	–	B
VoSo	Vanillosyringil	209	61	149	41	–	–	–	–	B
SoSo	Syringil	62	13	79	13	–	–	–	–	B
Total β,1-diketone dimers		579	224	402	98	0	0	0	0	
Total lignin dimers		4015	2698	2387	1490	239	357	187	292	
Total monomers and dimers		63945	53507	49376	19491	6066	5056	3891	3473	
Total benzenes and phenols		68183	54002	50267	20691	7745	6399	5468	5163	

[a]Roots = roots from Dark Brown Chernozem site, Grass 1 = vegetation from the Brown Chernozem site, Grass 2 = vegetation from the Dark Brown Chernozemic site, Deco. Grass = decomposing grass from the Brown Chernozem site, BrCh = Brown Chernozemic Soil Ah, DBrCh = Dark Brown Chernozemic Soil Ah, BlCh = Black Chernozemic Soil Ah, EBlCh = Eluviated Black Chernozemic Soil Ah.

[b]ID = Identification: A = Morita 1974; B = Goñi and Hedges 1992; I = interpretation of MS fragmentation patterns; L = Wiley MS library; S = standard.

Table 4. Major compounds identified after CuO oxidation of forest soils and source vegetation from Alberta, Canada.

No.	Compound name	Concentration (µg/g C)						ID [b]
		Aspen[a]	DGrCh Ah[a]	DGrCh Ahe[a]	Pine[a]	Brun LFH[a]	Brun O[a]	
Protein-derived products								
Bd	Benzoic acid	102	567	62	202	104	204	S
pOH-Bl	p-Hydroxybenzaldehyde	92	333	8	225	290	145	L
3OH-Bd	m-Hydroxybenzoic acid (3-OH)	265	381	83	799	140	219	L
4OH-Bd	p-Hydroxybenzoic acid (4-OH)	695	1702	548	1082	587	766	L
2Cp	2-Carboxypyrrole	219	488	38	278	203	163	L
Total		1373	3471	739	2586	1324	1497	
Other benzenes								
3,5OH-Bd	3,5-Dihydroxybenzoic acid	510	1160	294	543	265	785	S
1,4-BCA	1,4-Benzenedicarboxylic acid	—	234	104	—	168	97	
1,2,4-BCA	1,2,4-Benzenetricarboxylic acid	110	202	112	57	56	131	S
1,3,5-BCA	1,3,5-Benzenetricarboxylic acid	76	49	28	20	—	30	S
1,2,4,5-BCA	1,2,4,5-Benzenetetracarboxylic acid	—	136	30	—	—	119	S
Total		696	1781	568	620	489	1162	S
Lignin monomers								
Vl	Vanillin	2003	3974	70	5541	5099	2445	S
Vn	Acetovanillone	662	1482	107	1433	1367	1029	A
Vd	Vanillic acid	1096	3181	296	2473	1967	2686	S
Vg	Vanillylglyoxalic acid	530	1281	211	1374	1116	1034	B
Total vanillyls		4291	9918	684	10821	9549	7194	
Sl	Syringaldehyde	2296	2229	52	—	1172	213	S
Sn	Acetosyringone	712	1268	91	—	853	191	B
Sd	Syringic acid	854	1919	183	—	825	363	S
Sg	Syringylglyoxalic acid	439	532	91	—	166	92	B
Sid	Sinapic acid	62	238	22	—	—	—	L
Total syringyls		4363	6186	439	0	3016	859	
HCid	Hydrocinnamic acid	129	73	13	68	55	31	L
Cd	p-Coumaric acid	1112	982	117	323	652	443	L
Fd	Ferulic acid	192	1634	195	471	1368	314	L
Total cinnamyls		1433	2695	325	862	2075	788	
Total lignin monomers		10087	18799	1448	11683	14640	8841	

Table 4. Continued.

No.	Compound name	Concentration (µg/g C)						ID[b]
		Aspen[a]	DGrCh Ah[a]	DGrCh Ahe[a]	Pine[a]	Brun LFH[a]	Brun O[a]	
Lignin dimers								
Vl-Vl	Dehydrodivanillin	71	142	3	271	253	133	B
Vl-Vn	Dehydrovanillinacetovanillone	19	111	–	159	145	143	B
Vl-Vd	Dehydrovanillinvanillic acid	28	142	–	136	135	232	B
Vn-Vd	Dehydroacetovanillonevanillic acid	–	69	–	67	40	129	B
Total 5,5'-dimers		130	533	22	663	603	725	
Vd-Vd	Dehydrodivanillic acid	12	69	19	30	30	88	B
VVo	Vanillovanillone	–	40	1	112	75	26	B
VSo	Vanillosyringone	33	102	–	–	100	–	B
SSo	Syringosyringone	–	–	–	–	–	–	B
Total α,1-monoketone dimers		33	142	1	112	175	26	
Vm2Sl	2-Vanillylsyringaldehyde	–	–	–	30	26	33	B
Vm2Sn	2-Vanillylacetosyringone	–	–	–	–	–	–	B
Vm2Sd	2-Vanillylsyringic acid	–	–	6	–	–	66	B
Sm2Sl	2-Syringylsyringaldehyde	–	–	–	–	–	–	B
Sm2Sn	2-Syringylacetosyringone	109	–	–	–	–	–	B
Sm2Sd	2-Syringylsyringic acid	–	83	13	–	–	–	B
Total α,2-methyl dimers		109	83	19	30	26	99	
VoVo	Vanillil	73	69	6	178	104	99	B
VoSo	Vanillosyringil	31	–	–	–	–	–	B
SoSo	Syringil	35	–	–	–	–	–	B
Total β,1-diketone dimers		139	69	6	178	104	99	
Total lignin dimers		411	827	48	983	908	949	
Total monomers and dimers		10498	19626	1496	12666	15548	9790	
Total benzenes and phenols		12567	24878	2803	15872	17361	12449	

[a]Aspen = aspen vegetation from the Dark Gray Chernozem site, DGrCh Ah = Dark Gray Chernozem soil Ah, DGrCh Ahe = Dark Gray Chernozem soil Ahe, Pine = pine vegetation from the Brunisol site, Brun LFH = Brunisolic soil leaf litter layer, Brun O = Brunisolic soil organic horizon.
[b]ID = Identification: A = Morita, 1974; B = Goñi and Hedges 1992; I = interpretation of MS fragmentation patterns; L = Wiley MS library; S = standard.

from the Brown to the Black Chernozems while the second group of benzenes (3,5OH-Bd, BPCAs) exhibited the opposite trend.

The identified phenolic monomers and dimers of the vanillyl, syringyl and cinnamyl classes are typical CuO oxidation products derived from lignin (Hedges and Mann 1979; Hedges and Ertel 1982; Goñi et al. 2000). Monomers of the vanillyl and cinnamyl type were identified in all soil and plant samples, and syringyl phenols were detected in all samples with the exception of the Pine needles. A series of dimeric phenols was identified according to Goñi and Hedges (1992) and included 5, 5′−, α, 1-monoketone, α, 2-methyl and β, 1-diketone dimers with the 5,5′-dimers as the most abundant class in all soils and plants.

Sources of phenolic and aromatic CuO oxidation products

The composition of lignin-derived phenols is characteristic of major plant groups. Gymnosperm wood contains only vanillyl derivatives while angiosperm wood is composed of approximately equal quantities of vanillyls and syringyls (Hedges and Mann 1979; Hedges and Ertel 1982). In addition to their respective contents of vanillyls or vanillyls/syringyls, non-woody vascular plant tissues of gymnosperms and angiosperms (e.g., conifer needles, grass, angiosperm leaves) contain cinnamyl units that are part of the lignin macromolecule or link carbohydrates and lignin in the ligno-cellulose complex (Iiyama et a1. 1990; Lam et al. 2001). Therefore, the ratios of syringyl to vanillyl (S/V) and cinnamyl to vanillyl (C/V) monomers are indicative of the botanical origin of the lignin and have been used to assess the source of lignin in soils and river sediments (e.g., Ertel and Hedges 1984; Prahl et al. 1994; Goñi et al. 2000). The plotted C/V versus S/V ratios display that the source vegetation and corresponding soil samples lie near each other, indicating the preservation of characteristic lignin patterns in these soils (Figure 2). Soil horizons from the Pine forest soil (Brunisol) are characterized by low S/V ratios (0.1–0.3) due to the absence of syringyl phenols in conifer lignin as documented by the S/V ratio of 0 in the Pine needles (Table 5). Low concentrations of syringyls were detected in the leaf litter and O horizon from the Burnisol site and it is hypothesized that these compounds originate from angiosperm species such as grasses that may be growing in the Pine forest.
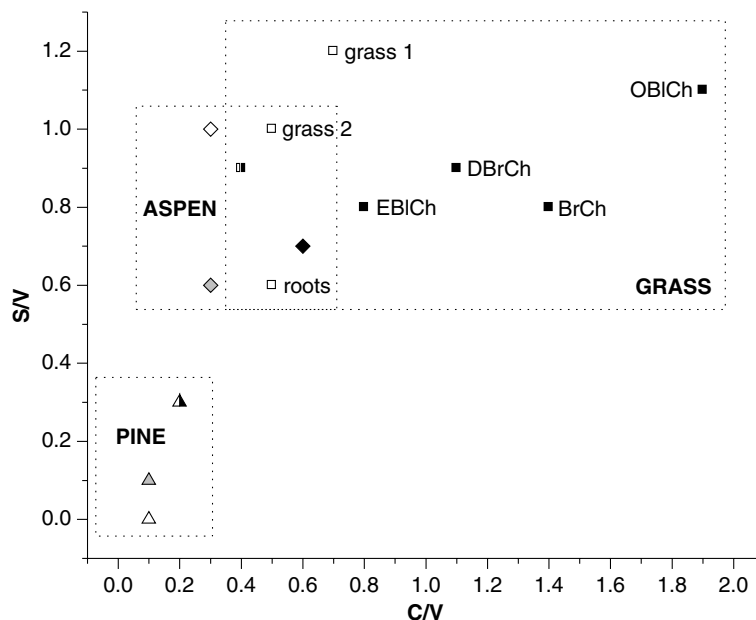

Figure 2. Plot of lignin source parameters for soil and vegetation samples from Alberta, Canada. C/V = cinnamyl/vanillyl phenols, S/V = syringyl/vanillyl phenols. □ = grassland; ◇ = Aspengrassland transition, △ = Pine forest. White = plant samples, black-and-white = leaf litter and decomposing plants, grey = DrGCh upper horizon (Ah), black = soil mineral horizon A.

Table 5. Lignin composition and source parameters of soils and vegetation from Alberta, Canada.

Samples	S/V[a]	C/V[a]	D/M[b]	SR/RR[b]	LPVI[c]
Grassland soils					
Grass roots	0.6	0.5	0.07	1.4	237
Grass (BrCh)	1.2	0.7	0.05	1.9	800
Grass (DBrCh)	1.0	0.5	0.05	1.7	476
Decomposing grass	0.9	0.4	0.08	1.6	276
Brown Chernozem Ah	0.8	1.4	0.05	0.4	1278
Dark Brown Chern. Ah	0.9	1.1	0.08	0.8	1090
Orthic Black Chern. Ah	1.1	1.9	0.05	0.4	2608
Eluv. Black Chern. Ah	0.8	0.8	0.09	0.4	635
Aspen-grassland soil					
Brown Aspen leaves	1.0	0.3	0.04	2.2	285
Dark Gray Chern. Ah	0.6	0.3	0.04	0.6	129
Dark Gray Chern. Ahe	0.7	0.7	0.03	1.2	423
Pine forest soil					
Pine needles	0	0.1	0.08	0.5	2
Brunisol leaf litter	0.3	0.2	0.06	0.5	41
Brunisol O horizon	0.1	0.1	0.11	0.3	5
Literature data					
Gymnosperm needles[d]	0–0.15	0.2–0.6	0.17	–	–
Gymnosperm wood[d]	0–0.15	0–0.05	0.17	–	–
Angiosperm leaves[d]	0.7–2.0	0.3–0.6	0.12	–	–
Angiosperm leaves (grass)[d]	1.0	0.7	0.09	–	–
Angiosperm wood[d]	0.8–3.8	0–0.05	0.04	–	–
Conifer forest soil[e]	0.2	0.2	–	–	–
Deciduous forest soil[e]	0.4	0.08	–	–	–
Gymnosperm wood[f]	–	–	–	–	1
Gymnosperm needles[f]	–	–	–	–	12–27
Angiosperm wood[f]	–	–	–	–	67–415
Angiosperm leaves[f]	–	–	–	–	378–2782

[a]S = syringyl phenols (syringaldehyde, acetosyringone, syringic acid), V = vanillyl phenols (vanillin, acetovanillone, vanillic acid), C = cinnamyl phenols (p-coumaric acid, ferulic acid).

[b]D/M = Dimeric lignin phenols/monomeric lignin phenols. SR/RR: Sidechain-ring dimers/ring-ring dimers = $(\beta,1+\alpha1+\alpha2)/5,5'$-dimers (Goñi and Hedges 1992).

[c]LPVI: Lignin Phenol Vegetation Index. LPVI = $[\{S(S+1)/(V+1)+1\}\times\{C(C+1)/(V+1)+1\}]$. V = vanillin + acetovanillone + vanillic acid; S = syringealdehyde + acetosyringone + syringic acid; C = p-coumaric acid + ferulic acid. V, S and C expressed as % of total VSC (Tareq et al. 2004).

[d]Hedges and Mann 1979; Goñi and Hedges 1992; Goñi et al. 2000.

[e]Prahl et al. 1994.

[f]Tareq et al. 2004.

The S/V ratios of the soils and plants from the grassland and Aspen-grassland soils are between 0.6 and 1.1 and reflect the angiosperm origin of the lignin. The soils and plant samples from the grassland soils had very high C/V ratios (0.4–1.9) due to high cinnamyl contents typical of grasses (Hedges and Mann l979; Goñi et al. 2000). The results are comparable to previously reported C/V and S/V values for conifer forest and grassland soils (e.g., Ugolini et al. 1981; Ertel and Hedges 1984). In addition to the C/V and S/V ratios, the ratio of lignin dimers vs. monomers (D/M) is characteristic for angiosperm or conifer sources (Goñi and Hedges 1992). D/M ratios calculated for the soils and plants analyzed in this study were low in the Aspen-grassland samples (0.03–0.04), intermediate in the grassland soils (0.05–0.09) and the highest in the Pine forest samples (0.06–0.11) (Table 4). This is in accordance with a higher degree of cross-linking predominantly at the 5,5′ position of vanillyls abundant in conifer lignin. Cross-linking at this position is impossible for syringyl units due to methoxy group at this location within the molecule (Goñi and Hedges 1992). The characteristic composition of lignin dimers with a predominance of 5,5′-dimers accompanied by lower amounts of α,1-monoketone, α,2-methyl and β,1-diketone dimers as detected in the plant samples is

preserved in the soil samples and agrees with previously published data on the woody and non-woody tissues of angiosperms and gymnosperms (Goñi and Hedges 1992).

The lignin phenol vegetation index (LPVI) was recently proposed by Tareq et al. (2004) to characterize the lignin composition in peat and is based on the relative abundances of vanillyl V, syringyl S and cinnamyl C type phenols. The formula for this parameter is: $LPVI = [\{S(S+1)/(V+1)+1\} \times \{C(C+1)/(V+1)+1\}$, and results in a value that provides information about the lignin sources. Tareq et al. (2004) stated that the LPVI provides a better resolution than other lignin parameters such as C/V vs. S/V to determine the source vegetation type in complex mixtures such as OM from peat and soils. In contrast to the C/V and S/V ratios, the LPVI yields non-overlapping data for woody and non-woody gymnosperms and angiosperms, respectively (Table 5). The LPVI values for the grassland, Aspen-grassland and Pine forest soils analyzed here are within the ranges reported by Tareq et al. (2004) (Table 5). LPVI values for the soil and plant samples from the grassland (237–2608) and the Aspen-grassland transition are characteristic for non-woody angiosperms (LPVI 378–2782), and the low LPVI for the Pine forest samples (2–41) are typical for predominantly non-woody gymnosperm tissues (LPVI 12–27).

Benzene derivatives such as benzoic acid, *p*-hydroxybenzaldehyde and *m*- and *p*-hydroxybenzoic acid and 2-carboxypyrrole were reported as the CuO oxidation products of proteins (Goñi et al. 2000). Since hydroxybenzaldehyde and the hydroxybenzoic acids may also be derived from lignin, their origin from either lignin or proteins cannot be distinguished (Hedges and Parker 1976; Goñi et al. 2000) and they are thus not included in the lignin-derived markers here. Tannins and other flavonoids have been suggested as the sources for 3,5-dihydroxybenzoic acid (Goñi and Hedges 1992, 1995; Prahl et al. 1994). Benzenepolycarboxylic acids (BPCA) have been reported as chemical oxidation products from humic material extracted from coal and paleosols (Schnitzer and Calderoni 1985; Hänninen 1992) and as black carbon markers (Glaser et al. 1998; Glaser and Amelung 2003) and represent highly altered molecules. Although the exact biological sources are unknown, the BPCA are likely derived from condensed aromatic/phenolic biomolecules such as tannins or lignin or from the incomplete combustion or pyrolysis of organic materials.

Degradation parameters

Laboratory studies have demonstrated that the yields of CuO oxidation products from wood decrease with progressing biodegradation by white-rot fungi (Ertel and Hedges 1985; Hedges et al. 1988; Opsahl and Benner 1995). Therefore, the yields of eight major lignin phenols of the vanillyl, syringyl and cinnamyl classes (vanillin, acetovanillone, vanillic acid, syringaldehyde, acetosyringone, syringic acid, *p*-coumaric acid, ferulic acid) expressed in mg/100 mg C (λ8), mg/10 g bulk (Σ8) or mg/g C (VSC) are commonly calculated. The concentrations of major lignin phenols (VSC) obtained from the plant and soils samples analyzed here are comparable to previously reported yields (e.g., Goñi et al. 2000). The VSC yields in plant material are generally higher than in soils (Table 6, Figure 3a). Furthermore, the organic horizons contain higher VSC concentrations than the mineral horizons indicating a progression in lignin degradation along the soil profile. Within the grassland soil series, the VSC yields decreased from the Brown Chernozems to the Black Chernozems suggesting a higher degree of lignin alteration in the Black Chernozems.

The lignin-derived monomeric phenols have been established as valuable parameters for the degradation of OM in soils and river sediments. Biodegradation of lignin by white-rot or brown-rot fungi changes the composition of the lignin (Tien and Kirk 1983; Hedges et al. 1988). Side-chain oxidation, cleavage of C–C bonds and demethylation are the major processes during biodegradation of lignin (Tien and Kirk 1983; ten Have and Teunissen 2001). The decrease of the S/V and C/V ratios with progressing degradation has been observed in the biodegradation of wood (Hedges et al. 1988; Goñi et al. 1993; Opsahl and Benner 1995). Decreasing S/V and C/V ratios are caused by the preferential degradation of syringyls and cinnamyls and the resulting relative enrichment of vanillyls. Changes in the S/V ratios of the soils and their source vegetation analyzed in this study was not observed (Table 5) and may be due to differences in the degradation of woody and other types of plant tissues, such as leaves and needles. The vegetation samples studied were mainly composed of leaves and needles (with the exception of roots) making changes to the

Table 6. Degradation parameters of CuO oxidation products of soils and vegetation from Alberta, Canada.

Samples	VSC[1] (mg/g C)	(Ad/Al)[2]_v	(Ad/Al)[2]_s	(Ad/Al)[3]_vv	5,5'/α,1 Dimers[4]	5,5'/β,1 Dimers[4]	(Ad/Al)[5]_p	$\frac{3,5\text{OH-Bd}^{6}}{V}$	$\frac{\text{BCAs}^{7}}{V}$
Grassland									
Grass roots	56.7	0.5	0.5	0.3	1.1	2.9	3.2	–	–
Grass (BrCh)	50.0	0.2	0.6	0.1	0.8	4.2	0.4	–	–
Grass (DBrCh)	45.2	0.2	0.4	0.1	1.1	2.2	1.0	–	–
Decomp.grass	16.8	0.5	0.6	0.3	1.6	5.9	1.2	–	–
BrCh Ah	4.8	1.2	0.8	0.9	8.0	–	2.5	0.1	0.2
DBrCh Ah	4.2	1.4	1.1	1.0	11.2	–	2.6	0.1	0.2
OBlCh Ah	3.3	1.8	1.4	2.1	13.1	–	3.4	0.4	0.9
EBlCh Ah	2.7	1.9	1.7	2.6	19.0	–	4.5	0.3	0.7
Aspen-grassland									
Aspen leaves	8.9	0.5	0.4	0.2	3.9	0.9	7.6	0.1	0.2
DGrCh Ah	16.7	0.8	0.9	0.5	3.8	7.7	5.1	0.1	0.3
DGrCh Ahe	1.1	4.2	3.5	7.0	22.0	3.7	68.5	0.6	1.2
Pine forest									
Pine needles	10.2	0.4	–	0.1	5.9	3.7	4.8	0.06	0.06
Brun leaf litter	13.3	0.4	0.7	0.1	3.4	5.8	2.0	0.03	0.06
Brunisol O	7.7	1.1	1.7	0.7	27.9	7.3	5.3	0.1	0.2

[1]VSC = Sum of major 8 lignin phenols of the vanillyl V (vanillin,acetovanillone, vanillic acid), syringyl S (syringaldehyde, acetosyringone, syringic acid) and cinnamyl C (*p*-coumaric acid, ferulic acid) type.

[2](Ad/Al)$_v$ = vanillic acid/vanillin; (Ad/Al)$_s$ = syringic acid/syringaldehyde (Ertel and Hedges 1984).

[3](Ad/Al)$_{vv}$ = Dihydrodivanillic acid/dihydrodivanillin (Goñi and Hedges 1992).

[4]5,5'-Dimers: Dehydrodivanillin, dehydrovanillinacetovanillone, dehydrovanillinvanillic acid, dehydroacetovanillonevanillic acid, dehydrodivanillic acid. α,1-Monoketone dimers: Vanillovanillone, vanillosyringone, syringosyringone. β,1-Diketone dimers: Vanillil, vanillosyringil, syringil.

[5](Ad/Al)$_p$ = p-Hydroxybenzoic acid/p-hydroxybenzaldehyde (Hedges et al. 1988).

[6]3,5-OHBd / V = 3,5-dihydroxybenzoic acid/vanillyl phenols (vanillin, acetovanillone, vanillic acid) (Prahl et al. 1994).

[7]BCAs/V = (1,4-benzenedicarboxylic acid + 1,2,4-benzenetricarboxylic acid + 1,3,5-benzenetricarboxylic acid + 1,2,4,5-benzenetetracarboxylic acid)/vanillyl phenols (vanillin, acetovanillone, vanillic acid).
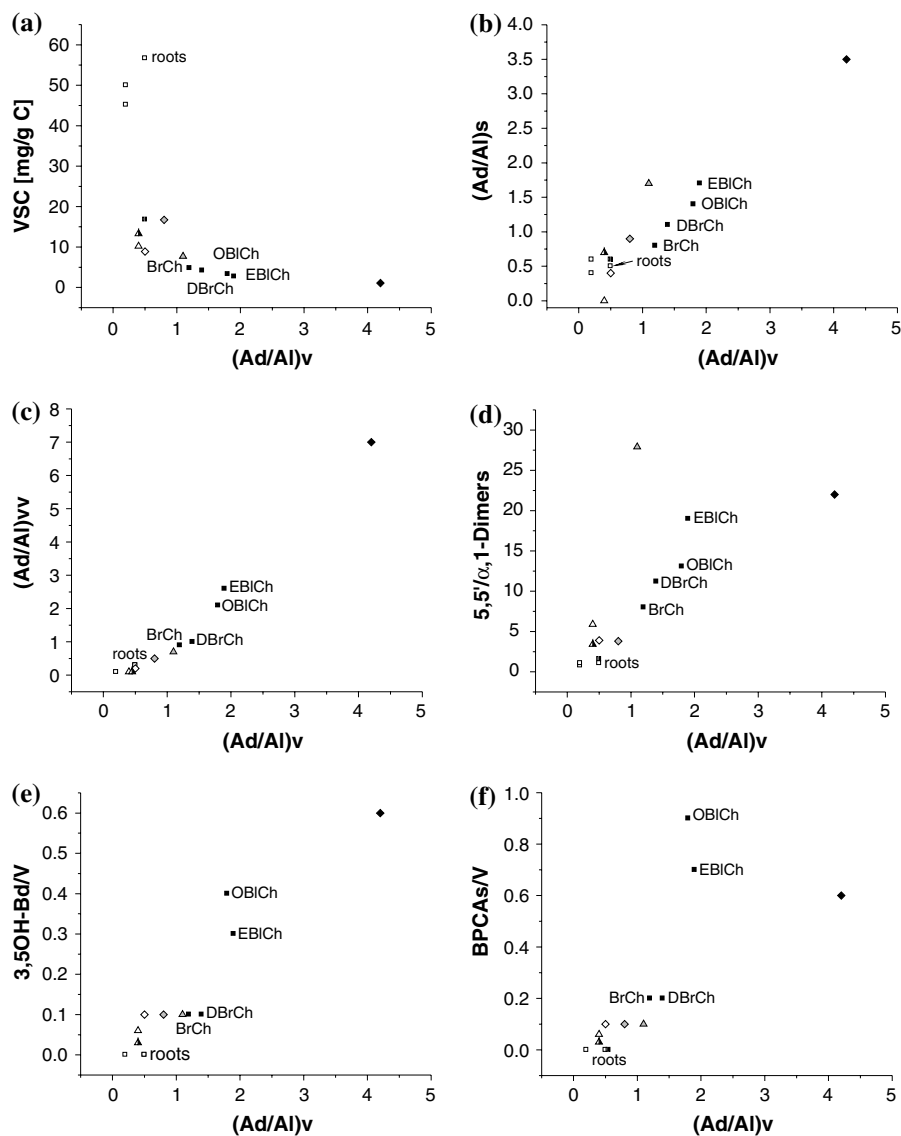
Figure 3. Plots of degradation parameters calculated from the CuO oxidation products from soil and vegetation samples from Alberta, Canada. (a) Yields of 8 major phenols of the vanillyl, syringyl and cinnamyl type (VSC); (b) Acid/aldehyde ratios for vanillyl $(Ad/Al)_v$ and syringyl $(Ad/Al)_s$ monomers; (c) Acid/aldehyde ratios for vanillyl dimers dihydrodivanillic acid/dihydrodivanillin $(Ad/Al)_{vv}$; (d) ratios of lignin dimers 5,5′-dimers/α,1-monoletone dimers; (e) ratios of 3,5-dihydroxybenzoic acid/vanillyls (3,5OH-Bd/V); (f) ratios of benzenepolycarboxylic acids/vanillyls (BPCAs/V). □ = grassland; ◇ = Aspen-grassland transition, △ = Pine forest. White = plant samples, black-and-white = leaf litter and decomposing plants, grey = DrGCh upper horizon (Ah), black = soil mineral horizon A.

S/V ratio difficult to interpret. In contrast, the C/V ratios generally increased from the plants to the soils dominated by angiosperm vegetation indicating the preferential degradation of vanillyls compared cinnamyls.

The progressing lignin degradation is also reflected by elevated ratios of lignin-derived phenolic acids and their corresponding aldehydes (Ad/Al) for both vanillyl and syringyl units (Ertel and Hedges 1985; Hedges et al. 1988; Opsahl and Benner 1995). It was reported that the $(Ad/Al)_v$ ratio increased from 0.15 to 0.5 during biodegradation of wood by white-rot fungi within 12 weeks (Hedges et al. 1988). Therefore, the

$(Ad/Al)_v$ and $(Ad/Al)_s$ ratios are commonly used as indicators of the level of lignin degradation in soils and sediments (Ertel and Hedges 1984; Hedges et al. 1988; Goñi and Hedges 1992; Goñi et al. 1993, 2000; Amelung et al. 1999). Both vanillyls and syringyls typically have (Ad/Al) ratios of 0.1–0.2 for fresh angiosperm and conifer wood, and higher values of 0.2–1.6 were observed for non-woody tissues such as leaves, needles and grasses (Hedges and Mann 1979; Hedges et al. 1988; Benner et al. 1990; da Cunha et al. 2001). For the comparison of data it has to be noted that the (Ad/Al) ratios determined by TMAH thermochemolysis were found to be much higher than those measured by CuO oxidation (Hatcher et al. 1995; Nierop and Verstraten 2003). Increased (Ad/Al) ratios from 0.6 to 7.6 were reported for vanillyl and syringyl monomers obtained from soils and sedimentary plant fragments with the highest values in riverine OM and mineral soil horizons (Ertel and Hedges 1984, 1985; Goñi and Hedges 1992; Nierop and Verstraten 2003). The observed increase of (Ad/Al) ratios with soil depth (e.g., Sanger et al. 1996) suggests that the lignin in the mineral horizons is more degraded than in the organic horizons and in leaf litter layer. $(Ad/Al)_v$, and $(Ad/Al)_s$ ratios for the samples analyzed in this study ranged between 0.2 and 0.5 for the plant samples and 0.8–4.2 for the soils (Table 5). Both ratios increased in parallel ($r = 0.94$) from the plants to the organic horizons and from the organic to the mineral soil horizons (Figure 3b) reflecting the concomitant and progressing oxidation of vanillyl and syringyl units. The (Ad/Al) ratios indicated the highest degree of oxidation in the Aspen-grassland soil. This is in agreement with previous studies and indicates that the lignin degradation in forest soils is higher than in grassland soils due to optimal environmental conditions (pH, temperature, moisture) for the lignin-degrading microorganisms (Paul and Clark 1988). A linear increase was observed within the grassland series from the Brown Chernozem to the Eluviated Black Chernozem (Figure 3b). The same trend of increasing (Ad/Al) ratios with decreasing mean annual temperature (MAT) has also been observed previously in a series of grassland soils from different climatic zones (Amelung et al. 1999). The authors concluded that the lower degree of lignin oxidation in warmer climates is probably caused by inhibited biodegradation due to less availability of the readily metabolized carbohydrates and/or proteins which are needed for co-metabolic biodegradation of lignin. Furthermore, warmer climates may also cause dryer conditions that may also limit the rate of biodegradation.

Lignin phenols have been identified among the CuO oxidation products of lignin, and several degradation parameters based on the lignin dimer composition have been proposed for the characterization of lignin in sediments (Goñi and Hedges 1992 and references therein). The advantage of using dimers is that their origin solely is from lignin while phenol monomers such as vanillin, vanillic acid and ferulic acid are also found in the suberin biomacromolecule in roots and bark (Kolattukudy 1981; Kolattukudy and Espelie 1989; Iiyama et al. 1990; Lam et al. 2001). Analogous to the $(Ad/Al)_v$ ratio for the vanillyl monomers, the ratios of the 5,5′-dimers dihydrodivanillic acid to dihydrodivanillin $(Ad/Al)_{55'vv}$ was calculated for the Canadian soil and vegetation samples (Table 5) and were found to increase proportionally with the $(Ad/Al)_v$ ratios ($r = 0.98$). These data suggest a similar degradation of vanillyl type monomers and dimers in the soils and plants (Figure 3c).

Ratios of the predominant 5,5′-dimers over the $\alpha,1$-dimers $(5,5'/\alpha,1)$ and the $\beta,1$-dimers $(5,5'/\beta,1)$ increase from the plants to the soils indicating the preferential degradation of the side chain-ring dimers ($\alpha,1$ and $\beta,1$ dimers) compared to the more stable ring-ring linked 5,5′-dimers (Table 6). Ratios of the $5,5'/\alpha,1$-dimers do not reveal a consistent trend with the exception of the Pine forest soil (Brunisol) where the $5,5'/\alpha,1$ ratio was found to increase with increasing $(Ad/Al)_v$ ratios (Figure 3d). This difference between the angiosperm (grass, Aspen) and conifer samples is caused by the high abundance of 5,5′-dimers in the undegraded gymnosperms (SR/RR = 0.5).

Although originally used as a degradation parameter for lignin (Hedges and Ertel 1982), the ratio of p-hydroxybenzoic acid to p-hydroxybenzaldehyde $(Ad/Al)_p$ can not be applied for lignin degradation because these compounds are known to have other sources in addition to lignin, namely proteins (Goñi et al. 2000). Despite the mixed sources, the $(Ad/Al)_p$ ratios increase from the plant to the soil samples (Table 6) indicating a greater degree of oxidation in the soils in comparison to the overlying vegetation.

The 3,5-dihydroxybenzoic acid (3,5OH-Bd) identified in the CuO oxidation products is likely derived from polyhydroxyaromatic tannins and has been reported to accumulate in decaying plant cells (Prahl et al. 1994). The ratio of 3,5OH-Bd over lignin-derived vanillyls (3,5OH-Bd/V) increases with increasing

degradation and humification and was proposed as degradation parameter for soils and sedimentary OM (Ugolini et al. 1981; Prahl et al. 1994). In the plant samples analyzed here, 3,5OH-Bd was absent in the grasses, but present in the Aspen leaves and Pine needles (510 and 543 µg/g C) and was detected in all soils in considerable amounts (132-1,160 µg/g C). The absence of 3,5OH-Bd in the CuO oxidation products of the grass samples can be associated with negligible tannin concentrations in grasses compared to higher abundances in leaves of dicotyledons and conifer needles (Hernes and Hedges 2004). 3,5OH-Bd/V ratios calculated for the soils were between 0.1 and 0.6 which is in the range of ratios reported for spruce forest and tundra soils and river sediments (Ugolini et al. 1981; Prahl et al. 1994; Louchouarn et al. 1999; Farella et al. 2001). The highest ratio was observed for the A horizon of the Aspen-grassland soil and the lowest for the Pine forest soil. Higher values for the Black Chernozems than the Brown Chernozems within the grassland soils were observed referring to a higher degree of oxidation in the Black Chernozems. The plot of the 3,5OH-Bd/V vs. (Ad/Al)$_v$ ratios largely matches the degradation stages for the lignin in soils as documented by the lignin phenol parameters (Figure 3b–e) and points toward the concurrent degradation of lignin and tannins in the soils. It is unknown if the generation of the 3,5OH-Bd is governed by microbial activities or through abiotic degradation of tannins or other polyphenolic biomolecules.

The yields of 3,5OH-Bd in the soils and plant samples are comparable to the abundances of the benzenepolycarboxylic acids (BPCAs) detected in the soils, Aspen leaves and Pine needles. Consequently, the BPCAs/V ratios display a similar pattern as the 3,5-DHBA/V (Figure 3e–f) and are therefore suggested as a further degradation parameter. BPCAs have been identified as CuO products from humic material extracted from peat, coal and paleosols (Schnitzer and Calderoni 1985; Hänninen 1992) and were reported as the chemical oxidation products of black carbon (Glaser et al. 1998; Glaser and Amelung 2003). Since the black carbon, paleosols and coal represent highly altered OM, the BPCAs can be interpreted as indicators of a higher stage of OM alteration. BPCAs were obtained from black carbon after harsh chemical oxidation (Glaser et al. 1998; Glaser and Amelung 2003) while the CuO oxidation method used here is comparatively mild. Therefore, the BPCAs detected in the plant and soil samples after CuO oxidation are probably not derived from black carbon oxidation but from another polyaromatic source. Since the BPCAs/V and 3,5OH-Bd ratios are very similar and slightly different from the lignin phenol parameters (Figure 3b–f) it is suggested that the BPCAs and 3,5OH-Bd detected after CuO oxidation have a common source, namely tannins or other polyhydroxyaromatic compounds. BPCAs found in soils after harsh chemical degradation are typically interpreted as black carbon-derived (Glaser et al. 1998). BPCAs have been reported for a few peat and coal humic acids after CuO oxidation, but it is unknown if these samples were subjected to burning (Hänninen 1992). BPCAs identified as the CuO oxidation products of humic and fulvic acids extracted from soils (e.g., Griffith and Schnitzer 1976) may be derived from black carbon generated in a fire or from the oxidation of polyphenolic molecules such as tannins or flavonoids. The presence of 3,5OH-Bd and the BPCAs in the Aspen leaves and the Pine needles and their absence in the grasses suggests that tannins may be the source of these markers because dicotyledons and conifers produce high amounts of tannins while monocotyledons such as grasses yield only trace amounts of tannins (Hernes and Hedges 2004). However, additional studies should focus on the source of BPCAs in vegetation and other pristine samples and determine the mechanisms of BPCA generation with CuO oxidation.

Degradation stages of lignin

Based on the composition of phenols and benzenes yielded after CuO oxidation, the degradation of lignin and, in part, tannin derived biomolecules in soils and their overlying source vegetation was determined. The applied degradation parameters for benzenes and lignin-derived phenols describe the progressing degradation from the plants to the organic and mineral soil horizons of the grassland, Aspen-grassland and Pine forest soils. Although the fungal decomposition is believed to be the main driving force in lignin degradation in soils, abiotic processes such as photochemical alteration or cross-linking might also play an important role in the lignin alteration in soils and riverine OM (Opsahl and Benner 1998; Bertilsson et al. 1999; Otto et al. 2005). Furthermore, abiotic degradation/oxidation may

also be a factor in the generation of highly oxidized benzene derivatives such as polyhydroxybenzoic or BPCAs. Abiotic mechanisms of lignin alteration should be investigated further as they may play a role in lignin transformation in cooler climates.

The progressing degradation of grass lignin is documented by the degradation parameters for the fresh and decomposing grass to the grassland soils. The grass roots exhibited elevated $(Ad/Al)_v$ ratios compared to the grass blades (Table 6). Lignin phenols such as vanillic acid detected in the CuO oxidation products of roots are derived from lignin and non-lignin sources such as suberin (Kolattukudy 1981; Kolattukudy and Espelie 1989) and represent a mixed input. Within the series of the four grassland soils, the lignin in the Black Chernozems is more oxidized than the Brown Chernozems although they have the same major source plant (Western Wheatgrass). While the composition of CuO oxidation products of grasses sampled from the Black and Brown Chernozems are comparable, the total yields individual products differ. The interpretation confirms previously published results of progressing degradation of free and base hydrolyzable lipids in these soil samples (Otto et al. 2005). The soils share the same mineralogy and similar precipitation (Brown Chernozems 413 mm, Black Chernozems 452 mm), but differ in the mean annual temperature (MAT) (Brown Chernozems 3.3 °C, Black Chernozems 1.7 °C) (Dudas and Pawluk 1969; Clayton et al. 1977; Janzen et al. 1998; Salloum et al. 2000). According to the degradation parameters of the CuO oxidation products, the SOM in the Black Chernozems of the colder climate exhibits a higher stage of alteration than the Brown Chernozems of the warmer climate. Amelung et al. (1999) suggested that a lack of carbohydrates which are needed for the co-metabolic biodegradation of lignin then results in a decrease of lignin degradation. But, the comparably higher biodegradation of lignin in the Black Chernozems due to sufficient availability of co-metabolites would result in lower than observed C contents and C/N_a ratios. Oxidation of vanillyl and syringyl aldehydes to their corresponding acids and thus increasing (Ad/Al) ratios could also be affected by abiotic oxidation such as photochemical processes (Opsahl and Benner 1998; Bertilsson et al. 1999). Furthermore, the abiotic cross-linking of lignin and other biomolecules is hypothesized to occur in soils (e.g., Hempfling et al. 1991; Leinweber et al. 1994). Therefore, it is hypothesized that the higher alteration stage may be caused by abiotic alteration of the lignin to a more refractory lignin-based geopolymer through oxidative processes and/or cross-linking. Alteration of aromatic biomolecules such as tannins to less biodegradable or bioavailable structures is supported by the increasing concentrations of 3,5-OH-Bd and BPCAs in the analyzed Chernozems. Derivative thermogravimetry (DTG) and infrared (IR) absorption parameters of the Brown and Black Chernozems also confirmed that the Black Chernozems contained more carboxyl groups indicating a more oxidized stage and were more resistant to thermal decomposition than the Brown Chernozems (Lutwick and Dormaar 1976). The abiotic alteration of lignin to a lignin-based geopolymer possibly inhibits the biodegradation because the enzymes can not attack the altered structures. Consequently, the geomolecule is more refractory and accumulates in the SOM.

The degradation parameters for the plant and soil samples from the Aspen-grassland transition (Dark Gray Chernozem) document a moderate lignin degradation from the Aspen leaves to the O horizon and a rapid increase from the O horizon to the Ah horizon. The Ah horizon exhibited the highest degradation parameters in all soils analyzed here indicating a high level of lignin degradation. Compared to grassland soils, forest soils were found to have higher ratios of fungal over bacterial biomarkers indicating the higher abundance of fungi in the forest soils due to more favorable environmental conditions (Imberger and Chiu 2001). Since the lignin biodegradation is mainly governed by fungi, the high level of lignin degradation in the Aspen-grassland transition soil can be explained by the increased fungal activity which results in lignin biodegradation.

The plant and soil samples from the Pine forest soil (Brunisol) revealed the lowest degree of lignin degradation amongst the soils analyzed. The degradation parameters indicate only minor lignin alteration from the Pine needles to the leaf litter and moderate degradation in the organic soil horizon. The results are in agreement with previous studies reporting the degradation parameters in conifer forest soils are lower than in angiosperm forest soils (e.g., Guggenberger and Zech 1994; Rumpel et al. 2004). Conifer lignin is predominantly composed of vanillyl derivatives which are more resistant to biodegradation compared to cinnamyls and syringyls (Hedges et al. 1988; Goñi et al., 1993; Opsahl and Benner 1995). Furthermore, the

conifer lignin is characterized by high contents of the ring-ring linked 5,5′-dimers (low SR/RR ratio) (Goñi and Hedges 1992) which are more stable against microbial attack than the side-chain linked monomers. As a result of the lignin composition, lignin degradation in conifer forest soils progresses slower than in soils with major input of angiosperms.

Value of lignin phenols as source and degradation parameters for SOM

The CuO oxidation of soils is a quick and reliable method to estimate the sources and degradation stage of lignin. However, source and degradation parameters based on the phenolic CuO oxidation products of soils have to be interpreted with caution, because mixed inputs of biomolecules and degradation processes in the SOM affect the phenol contents in soils. The composition of lignin phenols is characteristic for fresh woody and non-woody angiosperms and gymnosperms, but degradation processes such as the preferential degradation of syringyl and cinnamyl units compared to vanillyl phenols can also affect the value of source parameters calculated for soils. The application of the lignin phenol vegetation index (LPVI) as suggested by Tareq et al. (2004) for complex mixtures such as peat or soils may give a better resolution than the commonly used source parameters S/V vs. C/V and should be tested for other soil samples with a mixed source vegetation. The composition of phenolic CuO oxidation products from soils is further complicated by non-lignin sources for typical lignin phenols such as suberin from roots and bark or the phenolic constituents in wood linking cellulose to the lignin macromolecule (ligno-cellulose complex) also contribute to the phenol pool in soils. Therefore, the use of lignin dimers for source and degradation parameters is advantageous because their origin is solely from lignin. In contrast to the predominantly ether-linked lignin, phenols occurring in suberin and ligno-cellulose are ester-bound and can be removed through alkaline hydrolysis (Kolattukudy 1981; Kolattukudy and Espelie 1989). The CuO oxidation products of soil residues after base hydrolysis exhibited lower degradation parameters than the non-hydrolyzed soils indicating that the hydrolyzable non-lignin phenols affect the "lignin" degradation parameters of the total soil (unpublished data). Therefore, the alkaline hydrolysis of soils prior to CuO oxidation is recommended for more precise information on the lignin sources and degradation in soils.

Conclusions

The comparative analysis of CuO oxidation products from selected grassland and forest soils and their major source plants yielded detailed information about the sources and degradation of lignin and other phenolic biomolecules such as tannins in soils. The composition of lignin-derived phenols detected in the soils matches the phenol patterns observed in the major source plants indicating the preservation of characteristic lignin compositions in the soils. The grassland, Aspen-grassland transition and Pine forest soils can be distinguished by their S/V and C/V ratios, lignin phenol vegetation indices (LPVI), and ratios of lignin dimers and monomers (D/M, SR/RR) characteristic for grasses (monocotyledons), dicotyl angiosperms and conifers. Degradation parameters based on the phenolic and aromatic CuO oxidation products demonstrate the progressing degradation from the plants to the organic and mineral soil horizons. In addition to commonly applied degradation parameters such as the acid to aldehyde (Ad/Al) ratios of vanillyl and syringyl monomers, parameters based on lignin dimers (SR/RR, 5,5′ and benzene derivatives (3,5OH-Bd, BCAs/V) facilitated the more detailed analyses of the degradation of phenolic biomolecules in the soils. Dimeric phenols were previously reported only from wood analyses and sedimentary OM (Goñi and Hedges 1992 and references therein) and are reported here for soils. The use of phenolic dimers to assess lignin degradation is advantageous in comparison to monomers alone, because their only source is lignin while the monomers can also be derived from ester-bound moieties of suberin or the ligno-cellulose complex. Although the exact origin of 3,5-dihydroxybenzoic acid (3,5OH-Bd) and a series of benzene-polycarboxylic acids (BPCAs) is not known, these benzene derivatives are likely not lignin-derived but from polyhydroxybenzenes such as tannins or flavonoids. The degradation parameters of these compounds indicate a common origin and are slightly different from the parameters based on lignin-derived phenols.

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References

Amelung W., Flach K.-W. and Zech W. 1999. Lignin in particle-size fractions of native grassland soils as influenced by climate. Soil Sci. Soc. Am. J. 63: 1222–1228.

Benner R., Moran M.A. and Hodson R.E. 1986. Biogeochemical cycling of lignocellulosic carbon in marine and freshwater ecosystems: relative contributions of prokaryotes and eukaryotes. Limnol. Oceanogr. 31: 89–100.

Benner R., Weliky K. and Hedges J.I. 1990. Early diagenesis of mangrove leaves in a tropical estuary: molecular level analysis of neutral sugars and lignin-derived phenols. Geochim. Cosmochim. Acta 54: 1991–2001.

Bertilsson S.D., Stepanauskas R., Cuadros-Hansson R., Graneli W., Wikner J. and Tranvik L. 1999. Photochemically induced changes in bioavailable carbon and nitrogen pools in a boreal watershed. Aquat. Microb. Ecol. 19: 47–56.

Bundy L.G. and Bremner J.M. 1972. A simple titrimetric method for determination of inorganic carbon in soils. Soil Sci. Soc. Am. Pro. 36: 273–275.

Clayton J.S., Ehrlich W.A., Cann D.B., Day J.H. and Marshall I.B. 1977. Soils of Canada. Canada Department of Agriculture, Ottawa, Ontario.

da Cunha L.C., Serve L., Gadel F. and Blazi J.-L. 2001. Lignin-derived phenolic compounds in the particulate organic matter of a French Mediterranean river: seasonal and spatial variations. Org. Geochem. 32: 305–320.

Dai X.Y., White D. and Ping C.L. 2002. Comparing bioavailability in five Arctic soils by pyrolysis-gas chromatography/mass spectrometry. J. Anal. Appl. Pyrol. 62: 249–258.

Dignac M.-F., Bahri H., Rumpel C., Rasse D.P., Bardoux G., Balesdent J., Girardin C., Chenu C. and Mariotti A. 2005. Carbon-13 natural abundance as a tool to study the dynamics of lignin monomers in soil: an appraisal at the Closeaux experimental field (France). Geoderma 128: 3–17.

Dudas M.J. and Pawluk S. 1969. Chernozem soils of the Alberta Parklands. Geoderma 3: 19–36.

Ertel J.R. and Hedges J.I. 1984. The lignin component of humic substances: distribution among soil and sedimentary humic, fulvic, and base-insoluble fractions. Geochim. Cosmochim. Acta 48: 2065–2074.

Ertel J.R. and Hedges J.I. 1985. Sources of sedimentary humic substances: vascular plant debris. Geochim. Cosmochim. Acta 49: 2097–2107.

Farella N., Lucotte M., Louchouarn P. and Roulet M. 2001. Deforestation modifying terrestrial organic transport in the Rio Trapajos, Brazilian Amazon. Org. Geochem. 32: 1443–1458.

Franzluebbers A.J., Haney R.L., Honeycutt C.W., Arshad M.A., Schomberg H.H. and Hons F.M. 2001. Climatic influences on active fractions of soil organic matter. Soil Biol. Biochem. 33: 1103–1111.

Glaser B. and Amelung W. 2003. Pyrogenic carbon in native grassland soils along a climosequence in North America. Global Biogeochem. Cycles 17: 33/1–33/8.

Glaser B., Haumaier L., Guggenberger G. and Zech W. 1998. Black carbon in soils: the use of benzenecarboxylic acids as specific markers. Org. Geochem. 29: 811–819.

Gleixner G., Czimczik C., Kramer K., Luhker B.M. and Schmidt M.W.I. 2001. Plant compounds and their turnover and stabilization as soil organic matter. In: Schulze E.D., Heimann M., Harrison S., Holland E., Lloyd J., Prentice I.C. and Schimel D. (eds), Global Biogeochemical Cycles in the Climate System, Academic Press, San Diego, pp. 201–215.

Goñi M.A. and Hedges J.I. 1992. Lignin dimers: structures, distribution and geochemical applications. Geochim. Cosmochim. Acta 56: 4025–4043.

Goñi M.A. and Hedges J.I. 1995. Sources and reactivities of marine-derived organic matter in coastal sediments as determined by alkaline CuO oxidation. Geochim. Cosmochim. Acta 59: 2965–2981.

Goñi M.A., Nelson B., Blanchette R.A. and Hedges J.I. 1993. Fungal degradation of wood lignins: geochemical perspectives from CuO-derived phenolic dimers and monomers. Geochim. Cosmochim. Acta 57: 3985–4002.

Goñi M.A., Yunker M.B., Macdonald R.W. and Eglinton T.I. 2000. Distribution and sources of organic biomarkers in arctic sediments from the Mackenzie River and Beaufort Shelf. Mar. Chem. 71: 23–51.

Griffith S.M. and Schnitzer M. 1976. The alkaline cupric oxide oxidation of humic and fulvic acids extracted from tropical volcanic soils. Soil Sci. 122: 191–201.

Guggenberger G. and Zech W. 1994. Composition and dynamics of dissolved carbohydrates and lignin- degradation products in two coniferous forests, N. E. Bavaria, Germany. Soil Biol. Biochem. 26: 19–27.

Hänninen K. 1992. Cupric oxide oxidation of peat and coal humic acids. Sci. Total Environ. 117/118: 75–82.

Hatcher P.G., Nanny M.A., Minard R.D., Dible S.D. and Carson D.M. 1995. Comparison of two thermochemolytic methods for the analysis of lignin in decomposing gymnosperm wood: the CuO oxidation method and the method of thermochemolysis with tetramethylammonium hydroxide (TMAH). Org. Geochem. 23: 881–888.

ten Have R. and Teunissen P.J.M. 2001. Oxidative mechanisms involved in lignin degradation by white-rot fungi. Chem. Rev. 101: 3397–3413.

Hedges J.I. 1992. Global biogeochemical cycles: progress and problems. Mar. Chem. 39: 67–93.

Hedges J.I. and Ertel J.R. 1982. Characterization of lignin by gas capillary chromatography of cupric oxide oxidation products. Anal. Chem. 54: 174–178.

Hedges J.I. and Mann D.C. 1979. The characterization of plant tissues by their lignin oxidation products. Geochim. Cosmochim. Acta 43: 1803–1807.

Hedges J.I. and Parker P.L. 1976. Land derived organic matter in surface sediments from the Gulf of Mexico. Geochim. Cosmochim. Acta 40: 1019–1029.

Hedges J.I., Blanchette R.A., Weliky K. and Devol A.H. 1988. Effects of fungal degradation on the CuO oxidation products of lignin: a controlled laboratory study. Geochim. Cosmochim. Acta 52: 2717–2726.

Hempfling R., Simmleit N. and Schulten H.R. 1991. Characterization and chemodynamics of plant constituents during maturation, senescence and humus genesis in spruce ecosystems. Biogeochemistry 13: 27–60.

Hernes P.J. and Hedges J.I. 2004. Tannin signatures of bark, needles, leaves, cones, and wood at the molecular level. Geochim. Cosmochim. Acta 68: 1293–1307.

Iiyama K., Lam T.B.T. and Stone B.A. 1990. Phenolic acid bridges between polysaccharides and lignin in wheat internodes. Phytochemistry 29: 733–737.

Imberger K.T. and Chiu C.Y. 2001. Spatial changes of soil fungal and bacterial biomass from a sub-alpine coniferous forest to grassland in a humid, sub-tropical region. Biol. Fert. Soils 33: 105–110.

Janzen H.H., Campbell C.A., Izaurralde R.C., Ellert B.H., Juma N., McGill W.B. and Zentner R.P. 1998. Management effects on soil C storage on the Canadian prairies. Soil Till. Res. 47: 181–195.

Johansson M.B., Kögel I. and Zech W. 1986. Changes in the lignin fraction of spruce and pine needle litter during decomposition as studied by some chemical methods. Soil Biol. Biochem. 18: 611–619.

Kögel I. 1986. Estimation and decomposition pattern of the lignin component in forest humus layers. Soil Biol. Biochem. 18: 589–594.

Kögel I. and Bochter R. 1985. Characterization of lignin in forest humus layers by high-performance liquid chromatography of cupric oxide oxidation products. Soil Biol. Biochem. 17: 637–640.

Kögel-Knabner I. 2000. Analytical approaches for characterizing soil organic matter. Org. Geochem. 31: 609–625.

Kögel-Knabner I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol. Biochem. 34: 139–162.

Kolattukudy P.E. 1981. Structure, biosynthesis and biodegradation of cutin and suberin. Ann. Rev. Plant Physiol. 32: 539–567.

Kolattukudy P.E. and Espelie K.E. 1989. Chemistry, biochemistry, and function of suberin and associated waxes. In: Rowe J.W. (ed.), Natural Products of Woody Plants I, Springer, Berlin, pp. 304–367.

Lam T.B.T., Kadoya K. and Iiyama K. 2001. Bonding of hydroxycinnamic acids to lignin: ferulic and *p*-coumaric acids are predominantly linked at the benzyl position of lignin, not the β-position, in grass cell walls. Phytochemistry 57: 987–992.

Leinweber P., Schulten H.R. and Korschens M. 1994. Seasonal variations of soil organic matter in a long- term agricultural experiment. Plant Soil 160: 225–235.

Lichtfouse E., Dou S., Girardin C., Grably M., Balesdent J., Behar F. and Vandenbroucke M. 1995. Unexpected ^{13}C-enrichment of organic components from wheat crop soils: evidence for the *in situ* origin of soil organic matter. Org. Geochem. 23: 865–868.

Louchouarn P., Lucotte M. and Farella N. 1999. Historical and geographical variations of sources and transport of terrigenous organic matter within a large-scale coastal environment. Org. Geochem. 30: 675–699.

Lutwick L.E. and Dormaar J.F. 1976. Relationships between the nature of soil organic matter and root lignins of grasses in a zonal sequence of Chernozemic soils. Can. J. Soil Sci. 56: 363–371.

Morita H. 1974. Persilylation of phenolic ketones. J. Chromatogr. 101: 189–192.

Nierop K.G.J. and Verstraten J.M. 2003. Organic matter formation in sandy subsurface horizons of Dutch coastal dunes in relation to soil acidification. Org. Geochem. 34: 499–513.

Nierop K.G.J. 2001. Temporal and vertical organic matter differentiation along a vegetation succession as revealed by pyrolysis and thermally assisted hydrolysis and methylation. J. Anal. Appl. Pyrol. 61: 111–132.

Nierop K.G.J., van Lagen B. and Buurman P. 2001. Composition of plant tissues and soil organic matter in the first stages of a vegetation succession. Geoderma 100: 1–24.

Opsahl S. and Benner R. 1995. Early diagenesis of vascular plant tissues: lignin and cutin decomposition and biogeochemical implications. Geochim. Cosmochim. Acta 59: 4889–4904.

Opsahl S. and Benner R. 1998. Photochemical reactivity of dissolved lignin in river and ocean waters. Limnol. Oceanogr. 43: 1297–1304.

Otto A., Shunthirasingham C. and Simpson M. 2005. A comparison of plant and microbial biomarkers in grassland soils from the Prairie Ecozone of Canada. Org. Geochem. 36: 425–448.

Paul E.A. and Clark F.E. 1988. Soil microbiology and biochemistry. Academic Press, London, pp. 105–106.

Prahl F.G., Ertel J.R., Goñi M.A., Sparrow M.A. and Eversmeyer B. 1994. Terrestrial organic carbon contributions to sediments on the Washington margin. Geochim. Cosmochim. Acta 58: 3035–3048.

Rumpel C., Eusterhues K. and Kögel-Knabner I. 2004. Location and chemical composition of stabilized organic carbon in topsoil and subsoil horizons of two acid forest soils. Soil Biol. Biochem. 36: 177–190.

Rumpel C., Kögel-Knabner I. and Bruhn F. 2002. Vertical distribution, age, and chemical composition of organic carbon of two forest soils of different pedogenesis. Org. Geochem. 33: 1131–1142.

Salloum M.J., Dudas M.J., McGill W.B. and Murphy S.M. 2000. Surfactant sorption to soil and geologic samples with varying mineralogical and chemical properties. Environ. Toxicol. Chem. 19: 2436–2442.

Sanger L.J., Cox P., Splatt P., Whelan M.J. and Anderson J.M. 1996. Variability in the quality of *Pinus sylvestris* needles and litter from sites with different soil characteristics: lignin and phenylpropanoid signature. Soil Biol. Biochem. 28: 829–835.

Schimel D.S., Braswell B.H., Holland E.A., McKeown R., Ojima D.S., Painter T.H., Parton W.J. and Townsend A.R. 1994. Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. Global Biogeochem. Cycles 8: 279–293.

Schlesinger W.H. and Andrews J.A. 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48: 7–20.

Schnitzer M. and Calderoni G. 1985. Some chemical characteristics of paleosol humic acids. Chem. Geol. 53: 175–184.

Soil Classification Working Group 1998. The Canadian System of Soil Classification (Revised). pp. 1–187. Agriculture and Agri-Food Canada Publication 1646. NRC Research Press, Ottawa, Canada.

Tareq S.M., Tanaka N. and Ohta K. 2004. Biomarker signature in tropical wetland: lignin phenol vegetation index (LPVI) and its implications for reconstructing the paleoenvironment. Sci. Total Environ. 324: 91–103.

Tien M. and Kirk T.K. 1983. Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. Science 221: 661–663.

Trumbore S.E. 1997. Potential responses of soil organic carbon to global environmental change. Proc. Natl. Acad. Sci. USA 94: 8284–8291.

Trumbore S.E., Chadwick O.A. and Amundson R. 1996. Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. Science 272: 393–396.

Ugolini F.C., Reanier R.E., Rau G.H. and Hedges J.I. 1981. Pedological, isotopic, and geochemical investigations of the soils at the boreal forest and alpine tundra transition in northern Alaska. Soil Sci. 131: 359–374.

van Bergen P.F., Bull I.D., Poulton P.R. and Evershed R.P. 1997. Organic geochemical studies of soils from the Rothamsted classical experiments. I. Total lipid extracts, solvent insoluble residues and humic acids from Broadbalk Wilderness. Org. Geochem. 26: 117–135.

van Bergen P.F., Nott C.J., Bull I.D., Poulton P.R. and Evershed R.P. 1998. Organic geochemical studies of soils from the Rothamsted classical experiments. IV. Preliminary results from a study of the effect of soil pH on organic matter decay. Org. Geochem. 29: 1779–1795.

Ziegler F., Kögel I. and Zech W. 1986. Alteration of gymnosperm and angiosperm lignin during decomposition in forest humus layers. Z. Pflanz. Bodenkunde 149: 323–331.