Biogeochemistry (2005) 74: 377–409
DOI 10.1007/s10533-004-5834-8

Degradation and preservation of vascular plant-derived biomarkers in grassland and forest soils from Western Canada

ANGELIKA OTTO and MYRNA J. SIMPSON*
*Department of Physical and Environmental Sciences, Scarborough College, University of Toronto, 1265 Military Trail, Toronto, ON, Canada M1C 1A4; *Author for correspondence (e-mail: myrna.simpson@utoronto.ca; phone: +1-416-287-7234; fax: +1-416-287-7279)*

Received 8 March 2004; accepted in revised form 4 November 2004

Key words: Aliphatic lipids, Cyclic lipids, Soil organic matter, Steroids, Terpenoids

Abstract. The total solvent extracts (TSE) of mineral and organic horizons of selected soils and overlying vegetation were analyzed using gas chromatography–mass spectrometry (GC–MS) to determine the composition of solvent-extractable ('free') lipids in soils and to study the degradation and possible preservation of vascular plant-derived molecular markers (biomarkers) in soils. Major compound classes in the TSE of soils and vegetation included a homologous series of aliphatic lipids (alkanoic acids, alkanols, alkanes), steroids, and terpenoids. Characteristic patterns of aliphatic and cyclic biomarkers derived from the overlying, native vegetation were recognized in the associated soil samples indicating the preservation of lipids from the external waxes of vascular plants in the soil organic matter (SOM). The observed biomarker patterns in the grassland soils (Brown Chernozems) were similar to the compounds identified in their major source vegetation, Western Wheatgrass. A similar composition of biomarkers was observed in Aspen leaves and the soil horizons of the forest–grassland transition soil (Dark Gray Chernozem). The Lodgepole Pine needles yielded a characteristic pattern of diterpenoids that was also detected in leaf litter and the O horizon of the associated forest soil (Brunisol). The results demonstrate that solvent extractable biomarkers derived from vascular plants maintain their characteristic pattern of aliphatic and cyclic lipids despite ongoing degradation processes and are thus valuable molecular markers for the determination of the sources of SOM. Furthermore, the abundance of aliphatic wax lipids in plant material and soils decreased at higher rates than the steroids and terpenoids indicating the preferential degradation of aliphatic over cyclic biomarkers. Most of the plant-derived steroids and terpenoids identified in the soils were unaltered, preserved biomolecules as observed in the source vegetation, but minor amounts of their degradation products were also present. Oxidation products of plant sterols are reported here for the first time in soils. The detected alteration products of steroids and diterpenoids are consistent with the oxidative degradation of free cyclic biomarkers in decomposing plant material and soils.

Introduction

Lipids are common and important constituents of soil organic matter (SOM) (Dinel et al. 1990; Kögel-Knabner 2000, 2002). Aliphatic lipids derived from plant waxes and biopolymers such as suberin and cutin have been proposed as major sources of aliphatic constituents of SOM (Kögel-Knabner 2000).

Contents of bulk lipids and lipid classes (i.e., aliphatic lipids, aromatic lipids, polar lipids, phospholipids) are documented for numerous soils (e.g., Bridson 1985; Ziegler 1989; Dinel et al. 1900; Howard et al. 1998), but reports on the molecular composition of solvent extractable ('free') lipids in soils are rare (Jaffé et al. 1996; van Bergen et al. 1997, 1998; Bull et al. 1998, 2000a, b; Oros et al. 2002). The type of overlying vegetation and the physico-chemical conditions in the soil determines the content and composition of soil lipids (Dinel et al. 1990; van Bergen et al. 1997; Howard et al. 1998). Although the solvent extractable lipids usually comprise less than 10% of SOM (Dinel et al. 1990), they are indicative of the source vegetation because they contain characteristic molecular markers (biomarkers) that provide information about the nature and origin of the SOM (van Bergen et al. 1998). The analysis of solvent extractable biomarkers is thus an important tool to determine the sources of organic matter in soils.

Aliphatic lipids are known to biodegrade in soils. A laboratory study of the degradation of *n*-alkanes in soils revealed that the *n*-alkanes are oxidized to *n*-alkanols and ultimately to their corresponding *n*-alkanoic acids (Amblès et al. 1994b). Fatty acids can be easily metabolized by numerous aerobic bacteria via β-oxidation resulting in the formation of shorter *n*-alkanoic acids (Dinel et al. 1990). Since the products of aliphatic lipid degradation can not be distinguished from the biological precursors, it is difficult to determine the extent of their degradation. Very little is known about the fate of cyclic lipids such as steroids and terpenoids in soils (van Bergen et al. 1997). The incorporation of biosynthesized triterpenols and sterols into humic material via ester and possibly ether bonds has been reported (Gobé et al. 2000), but it is still unclear if degradation products of these biomolecules are formed in the soil or if they are completely mineralized. Lipid analysis in decaying plant material and in contemporary and fossil sediments has indicated that degradation products of steroids and terpenoids are frequently formed in aquatic environments under oxic conditions (e.g., Simoneit, 1986; Simoneit et al. 1986; Killops and Freewin, 1994; Jaffé et al. 1996). Despite the alteration processes, the degradation products still retain their characteristic basic structure and can be used as biomarkers for their biological sources (Streibl and Herout 1969; Tissot and Welte 1984; Simoneit 1986). Furthermore, the type and degree of the alteration provides mechanistic information about the processes that lead to the formation of the degradation products (Tissot and Welte 1984; Killops and Killops 1993). Despite their importance as characteristic biomarkers, the presence of degraded steroids and terpenoids in SOM has not been sufficiently investigated.

In this study, the free lipids from mineral and organic horizons of grassland and forest soils and of their major source plants were analyzed using extraction with medium polar and polar organic solvents and gas chromatography–mass spectrometry (GC–MS) to investigate the preservation and degradation of aliphatic and cyclic lipids originating from the external waxes of the native, overlying vegetation. Total solvent extracts (TSE) without further fractionation were used in this study to identify polar compounds that may contain

characteristic biomarkers (Otto and Simoneit 2001). The objective was to examine relationships between organic matter inputs and the degradation and/or preservation of specific vascular plant biomarkers in SOM.

Material and methods

Samples

The plant material and soil samples were collected from Alberta, Canada. Samples of leaves, decomposing leaves and roots of Western Wheatgrass (*Agropyron smithii*) and the mineral horizons (Ah) of a Brown Chernozem and a Dark Brown Chernozem originated from the grassland prairies near Lethbridge, Alberta (courtesy of Drs. H. Janzen and J. Dormaar). The grass samples were still green, but dry due to the high temperatures in summer. Both Chernozemic soil samples develop in arid to semiarid climate and have a pH range which varies between 6.0 and 6.9 (Soil Classification Working Group 1998). The Dark Gray Chernozem was sampled from the grassland–forest transition zone in Strathcona County, Alberta. The climate regime of the Dark Gray Chernozem is subhumid and the pH of the soil is typically less than that of other Chernozems. The samples were collected from a stand of Quaking Aspen (*Populus tremula*) in a grassed area and included brown Aspen leaves and the organic (O) and mineral horizons (Ah) of the Dark Gray Chernozem. 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) was sampled. The Brunisolic soil does not have a surface mineral horizon because the climatic conditions promote the accumulation of organic matter. 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 averages of the carbon and nitrogen contents are displayed in Table 1.

Table 1. Carbon and nitrogen contents and extract yields of soils and source plants from Alberta, Canada.

Samples	Carbon [%]	Nitrogen [%]	Extract yield [% of C]
Grassland (Brown Chernozem)			
Green Western Wheatgrass	46.0	0.7	5.5
Decomposing Western Wheatgrass	34.8	1.4	18.9
Ah horizon	2.1	0.2	3.8
Grassland (Dark Brown Chernozem)			
Green Western Wheatgrass	44.2	0.9	7.6
Roots of Western Wheatgrass	29.7	0.8	3.2
Ah horizon	2.8	0.3	3.0
Grassland–forest transition (Dark Gray Chernozem)			
Brown leaves of Quaking Aspen	50.9	0.5	13.5
O horizon	14.2	0.2	3.2
Ah horizon	5.0	0.3	1.4
Conifer forest (Brunisol)			
Green needles of Lodgepole pine	50.5	1.0	29.7
Pine leaf litter	52.0	0.9	1.8
O horizon	23.1	0.8	3.7

Extraction and derivatization

The samples (12 g of mineral soil horizons, 1–2.5 g of organic soil horizons and plant material) were sonicated twice for 15 min, each time with 30 ml of de-ionized water to remove highly polar compounds. The water extracted soil residues were then freeze-dried to remove remaining water prior to solvent extraction. The samples were sonicated three times for 15 min with 30 ml of dichloromethane, dichloromethane:methanol (1:1; v/v) and methanol, respectively, to extract non-polar and medium polar components. The combined total solvent extracts (TSE) were filtered through glass fiber filters (Whatman GF/A), concentrated by rotary evaporation, and then dried under nitrogen gas in 2 ml glass vials. The extract yields were determined by weighing the dry extracts.

The TSE were redissolved in 1 ml of a dichloromethane:methanol (1:1; v/v) mixture. Aliquots (100 μl) of the extracts were dried in a stream of nitrogen and then converted to trimethylsilyl derivatives by reaction with 90 μl *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and 10 μl pyridine (9:1; v:v) for 3 h at 70 °C. After cooling, 100 μl hexane was added to dilute the extracts.

Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses of the derivatized TSE was performed on an Agilent model 6890N GC coupled to a Hewlett-Packard model 5973 quadrupole mass selective detector. Separation was achieved on a HP5-MS fused silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness). The GC operating

conditions were as follows: temperature held at 65 °C for 2 min, increase from 65 to 300 °C at a rate of 6 °C min^{-1} with 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 daltons. Data were acquired and processed with the Chemstation G1701DA software. Individual compounds were identified by comparison of mass spectra with literature, NIST and Wiley MS library data, comparison with authentic standards, and interpretation of mass spectrometric fragmentation patterns. Perdeuterated tetracosane ($C_{24}D_{50}$) was used as an external quantification standard and the response factor was assumed to be 1 for all compound classes. Concentrations of individual compounds were calculated from the comparison of peak areas of components and the standard in the total ion current (TIC) and were then normalized to the organic carbon content.

Results

Carbon and nitrogen contents and extract yields

The carbon contents of the plant material and the leaf litter amounted between 29.7% (grass roots) and 52.0% (Pine leaf litter) (Table 1). Organic soil horizons (O) contained 14.2–23.1% and the mineral horizons (Ah) 2.1–5.0% carbon. The nitrogen contents varied between 0.5 and 1.4% with the highest amounts in the decomposing grass and the lowest value in the Aspen leaves. The plant samples and the leaf litter yielded solvent extracts between 3.2% (grass roots) and 29.7% (pine needles) (normalized to carbon content). Extract yields of 1.8 and 3.2% were observed for the organic horizons and 1.4–3.8% for the mineral horizons of the soils.

Composition of extractable components in soils and source vegetation

The applied extraction method with medium polar and polar organic solvents and the analyses of the TSE with GC–MS yields low molecular-weight aliphatic and cyclic lipids and carbohydrates and does not extract larger, polymeric compounds such as phospholipids or polysaccharides. A series of aliphatic lipids (*n*-alkanols, *n*-alkanoic acids, *n*-alkanes, wax esters), steroids, terpenoids, phenols, and carbohydrates (mono- and disaccharides) are the major compound classes in the TSE of all soils and plant materials analyzed (Figures 1–3). The identified steroids (st1–st9) and terpenoids (t1–t7, d1–d8) are listed in Table 2. The quantities of major compound classes identified in the TSE of the vegetation and soil samples are listed in tables as follows: grassland (Table 3), grassland–forest transition (Table 4), and conifer forest (Table 5).

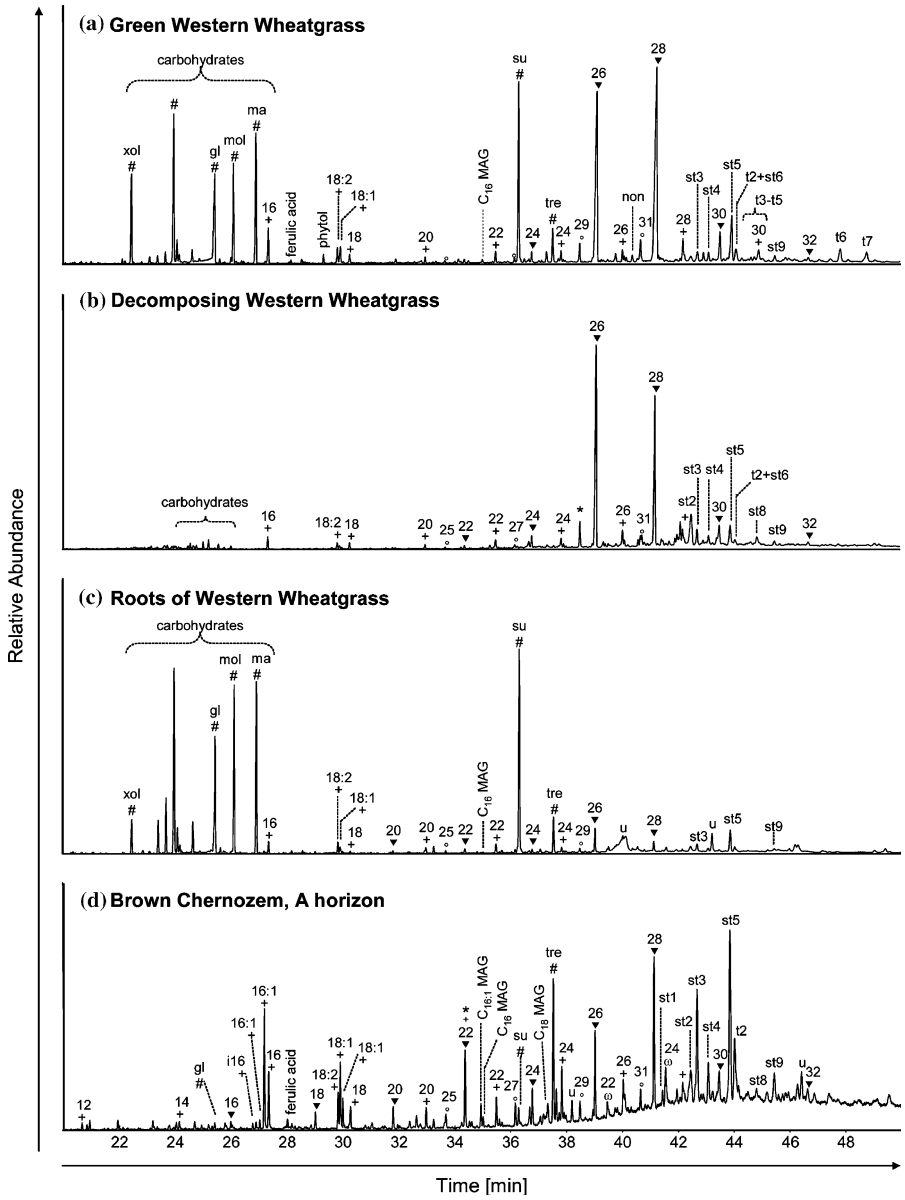

Figure 1. GC–MS chromatograms (TIC) of the free lipids in plant material and the mineral horizon of the grassland soils (Brown and Dark Brown Chernozems). (a) Green Western Wheatgrass, (b) decomposing Western Wheatgrass, (c) roots of Western Wheatgrass, and (d) Brown Chernozem Ah horizon. + = *n*-alkanoic acids, # = carbohydrates (xol = xylitol, gl = glucose, mol = mannitol, ma = mannose, su = sucrose, tre = trehalose), ▼ = *n*-alkanols, ○ = *n*-alkanes, MAG = monoacylglycerides, st1–st8 = steroids, t1–t7 = triterpenoids, u = unknowns, ∗ = contamination. For peak annotation of steroids and triterpenoids see Table 2. Numbers refer to total carbon numbers in aliphatic lipid series.

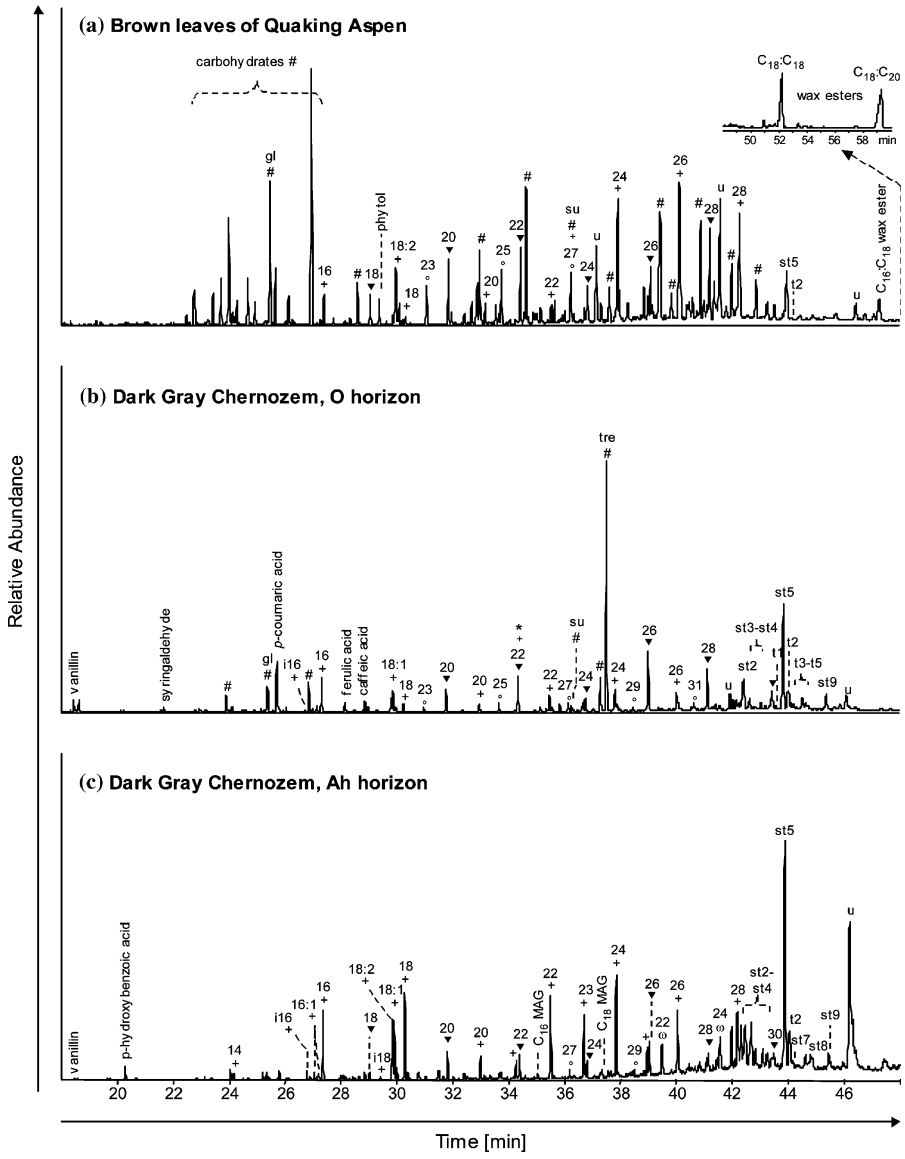

Figure 2. GC–MS chromatograms (TIC) of the free lipids in plant material and two horizons of the grassland–forest transition soil (Dark Gray Chernozem). (a) Brown Aspen leaves, (b) soil O horizon, and (c) soil Ah horizon. + = *n*-alkanoic acids, # = carbohydrates (xol = xylitol, gl = glucose, mol = mannitol, ma = mannose, su = sucrose, tre = trehalose), ▼ = *n*-alkanols, ○ = *n*-alkanes, MAG = monoacylglycerides, st1–st8 = steroids, t1–t7 = triterpenoids, u = unknowns, ∗ = contamination. For peak annotation of steroids and triterpenoids see Table 2. Numbers refer to total carbon numbers in aliphatic lipid series.

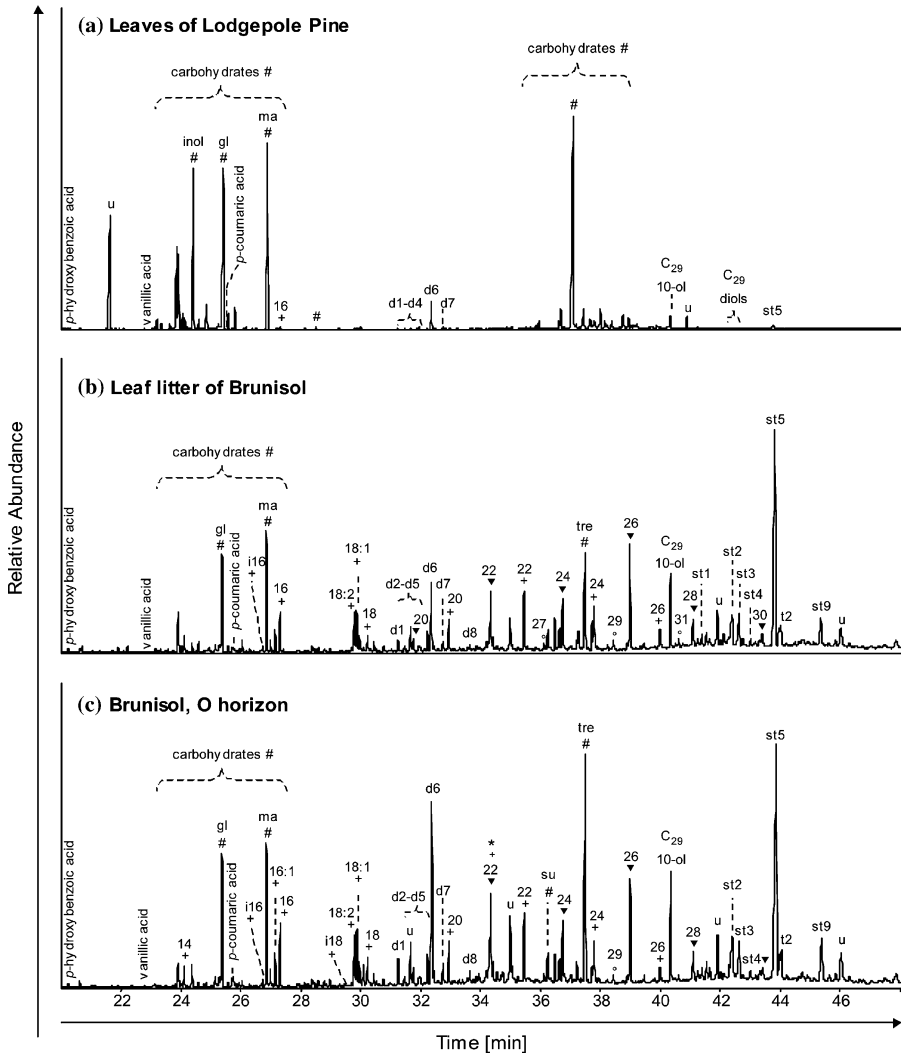

Figure 3. GC–MS chromatograms (TIC) of the free lipids in plant material and two horizons of the Pine forest soil (Brunisol). (a) Pine needles, (b) leaf litter, and (c) soil O horizon. + = *n*-alkanoic acids, # = carbohydrates (xol = xylitol, gl = glucose, mol = mannitol, ma = mannose, su = sucrose, tre = trehalose), ▼ = *n*-alkanols, ○ = *n*-alkanes, MAG = monoacylglycerides, st1–st8 = steroids, d1–d6 = diterpenoids, t1–t7 = triterpenoids, u = unknowns, ∗ = contamination. For peak annotation of steroids and terpenoids see Table 2. Numbers refer to total carbon numbers in aliphatic lipid series.

Grassland vegetation and soils

The TSE of the grassland plant material and the mineral soil horizons contained a series of aliphatic lipids (*n*-alkanols, *n*-alkanoic acids, *n*-alkanes),

Table 2. Steroids and terpenoids identified in the analyzed soils and plants.

		MW	Coposition	ID[a]
Steroids				
st1	Cholesterol (cholest-5-en-3β-ol)	386	C27H46O	Knights (1967)
st2	Ergosterol (ergosta-5,7,22-trien-3β-ol)	396	$C_{28}H_{44}O$	Brooks et al. (1968)
st3	Campesterol (ergost-5-en-3β-ol)	400	$C_{28}H_{48}O$	Knights (1967)
st4	Stigmasterol (stigmasta-5,22-dien-3β-ol)	412	$C_{29}H_{48}O$	Knights (1967)
st5	β-Sitosterol (stigmast-5-en-3β-ol)	414	$C_{29}H_{50}O$	S
st6	Stigmastan-3β-ol	416	$C_{29}H_{52}O$	L
st7	Stigmastan-3-one	414	$C_{29}H_{50}O$	L
st8	Stigmasta-3,5-dien-7-one	410	$C_{29}H_{46}O$	L
st9	Sitosterone (stigmast-4-en-3-one)	412	$C_{29}H_{48}O$	L
Triterpenoids				
t1	β-Amyrone (olean-12-en-3-one)	424	$C_{30}H_{48}O$	S
t2	β-Amyrin (olean-12-en-3β-ol)	426	$C_{30}H_{50}O$	S
t3	α-Amyrone (urs-12-en-3-one)	424	$C_{30}H_{48}O$	S
t4	α-Amyrin (urs-12-en-3β-ol)	426	$C_{30}H_{50}O$	S
t5	Lupeol (lup-20(29)-en-3β-ol)	426	$C_{30}H_{50}O$	Tulloch (1982)
t6	Oleanolic acid	456	$C_{30}H_{48}O_3$	Otto and Simoneit (2001)
t7	Ursolic acid	456	$C_{30}H_{48}O_3$	Otto and Simoneit (2001)
Diterpenoids				
d1	Dehydroabietol	286	$C_{20}H_{30}O$	S
d2	Pimaric acid	302	$C_{20}H_{30}O_2$	S
d3	Isopimara-8,15-dienoic acid	302	$C_{20}H_{30}O_2$	L
d4	Isopimaric acid	302	$C_{20}H_{30}O_2$	S
d5	Abieta-6,8,11,13-tetraenoic acid	298	$C_{20}H_{26}O_2$	Otto and Simoneit (2001)
d6	Dehydroabietic acid	300	$C_{20}H_{28}O_2$	S
d7	Abietic acid	302	$C_{20}H_{30}O_2$	S
d8	Abieta-8,11,13,15-tetraenoic acid	298	$C_{20}H_{26}O_2$	Otto and Simoneit (2001)

[a]Alcohols and acids identified as TMS derivatives. S = standard, L = Wiley M.S. library.

Table 3. Major compound classes identified in the total solvent extract of plant material and mineral horizons of the grassland soils (Chernozems).

	Brown Chernozem			Dark Brown Chernozem		
	Green grass	Decomposing grass	Soil Ah horizon	Green grass	Grass roots	Soil Ah horizon
n-Alkanols	C_{24}–C_{32} (C_{26})	C_{22}–C_{32} (C_{26})	C_{16}–C_{32} (C_{28})	C_{24}–C_{30} (C_{28})	C_{20}–C_{28} (C_{26})	C_{16}–C_{32} (C_{28})
Microbial or plant $<C_{20}$ [μg/g C]	–	–	7	–	–	4
Plant $<C_{20}$–C_{32} [μg/g C]	7633	4731	134	3867	518	184
$\sum$ [μg/g C]	7633	4731	141	3867	518	188
n-Alkanoic acids	C_{16}–C_{30} (C_{28})	C_{14}–C_{29} (C_{28})	C_9–C_{28} (C_{24})	C_{16}–C_{30} (C_{28})	C_{16}–C_{24} (C_{22})	C_9–C_{28} (C_{24})
Microbial or plant $<C_{20}$ [μg/g C]	782	249	104	688	352	52
Plant $<C_{20}$–C_{30} [μg/g C]	732	686	58	716	255	52
$\sum$ [μg/g C]	1460	935	162	1404	607	104
n-Alkanes	C_{23}–C_{31} (C_{31})	C_{25}–C_{31} (C_{29})	C_{25}–C_{31} (C_{29})	C_{25}–C_{31} (C_{29})	C_{25}–C_{29} (C_{29})	C_{25}–C_{33} (C_{31})
[μg/g C]	487	165	22	317	106	43
iso-Alkanoic acids	–	–	C_{16}	–	–	C_{16}
[μg/g C]	–	–	2	–	–	1
ω-Hydroxyalkanoic acids	–	C_{22}, C_{24}	C_{22}, C_{24}	–	–	C_{22}, C_{24}
[μg/g C]	–	Traces	22	–	–	18
$\sum$ Aliphatic lipids [μg/g C]	9580	5831	349	5568	1231	354

Steroids	st3–st6, st9 (st5)	st2–st6, st8, st9 (st5)	st1–st5, st8, st9 (st5)	st3–st5 (st5)	st2–st3, st5, st9 (st5)	st1–st5, st8, st9 (st5)
Microbial (st1, st2) [μg/g C]	–	382	25	–	115	37
Plant (st3–st9) [μg/g C]	1120	930	162	929	490	153
∑ [μg/g C]	1120	1312	187	929	605	190
Triterpenoids	t1–t7 (t6)	t2, t4	t2	t2, t4, t6, t7	–	t2
[μg/g C]	808	51	18	163	–	18
∑ Cyclic lipids [μg/g C]	1928	1363	205	1092	605	208
Aliphatic/cyclic lipids	5.0	4.3	1.7	5.1	2.0	1.7
Phenols [μg/g C]	24	–	1	28	–	2
Monoacylglycerides [μg/g C]	30	12	15	75	31	10
∑ Lipids [μg/g C]	11,562	7206	570	6783	1867	574
Carbohydrates [μg/g C]	8413	306	50	20,950	11,791	26
Total [μg/g C]	19,975	7512	620	27,733	13,658	600

Compounds in brackets indicate the predominant molecule within the series. For annotations of steroids and terpenoids see Table 2.

Table 4. Major compound classes identified in the total solvent extracts of plant material and horizons of the grassland–forest transition soil (Dark Gray Chernozem).

	Aspen leaves	Soil O horizon	Soil Ah horizon
n-Alkanols	C_{18}–C_{28} (C_{28})	C_{18}–C_{30} (C_{26})	C_{18}–C_{30} (C_{26})
Microbial or plant $<C_{20}$ [μg/g C]	213	22	4
Plant $<C_{20}$–C_{32} [μg/g C]	3136	918	109
$\sum$ [μg/g C]	3349	940	113
n-Alkanoic acids	C_{16}–C_{28} (C_{26})	C_{15}–C_{28} (C_{24})	C_{14}–C_{28} (C_{24})
Microbial or plant $<C_{20}$ [μg/g C]	907	438	205
Plant $<C_{20}$–C_{30} [μg/g C]	4294	480	334
$\sum$ [μg/g C]	5201	918	539
n-Alkanes	C_{23}–C_{27} (C_{25})	C_{23}–C_{31} (C_{27})	C_{27}–C_{29} (C_{27})
[μg/g C]	1324	160	9
iso-Alkanoic acids	–	C_{16}	C_{16}, C_{18}
[μg/g C]	–	13	8
ω-Hydroxyalkanoic acids	–	–	C_{22}–C_{24}
[μg/g C]	–	–	60
Wax esters	C_{34}, C_{36}, C_{38} (C_{36})	C_{38}	–
[μg/g C]	3779	12	–
$\sum$ Aliphatic lipids [μg/g C]	13,653	2043	729
Steroids	st5	st1–st5, st7–st9 (st5)	st2–st5, st9 (st5)
Microbial (st1, st2) [μg/g C]	–	349	68
Plant (st3–st9) [μg/g C]	682	1066	329
[μg/g C]	682	1451	397
Triterpenoids	t2, t4	t1–t6 (t2)	t2, t6, t7
[μg/g C]	49	277	77
$\sum$ Cyclic lipids [μg/g C]	731	1692	474
Aliphatic/cyclic lipids	18.6	1.2	1.5
Phenols [μg/g C]	–	516	11
Monoacylycerides [μg/g C]	–	–	10
$\sum$ Lipids [μg/g C]	14,384	4251	1224
Carbohydrates [μg/g C]	11,067	1681	7
Total [μg/g C]	25,451	5932	1231

Compounds in brackets indicate the predominant molecule within the series. For annotations of steroids and terpenoids see Table 2.

steroids, triterpenoids, phenols, monoacylglycerides and carbohydrates (Figure 1 and Table 3). The composition of solvent extractable compounds in the leaves of Western Wheatgrass and the soil horizons were similar while the TSE of the grass root biomass illustrated a slight pattern distinction.

Long-chain *n*-alkanols (141–7633 μg/g C) in the range from C_{16} to C_{32} with a strong even-over-odd predominance and a predominant homologue (C_{max}) at C_{26} or C_{28} were prevalent in the vegetation and soil samples. The series of *n*-alkanoic acids (104–1460 μg/g C) included the C_9 acid and predominantly even numbered homologues from C_{14} to C_{30} with C_{max} at C_{28} in the grass, C_{22} in the roots and C_{24} in the soils. Branched alkanoic acids (iso-C_{16} and iso-C_{18}) were detected in trace amounts only in the soils, but not in the plant material. Odd-numbered *n*-alkanes in the range of C_{23} to C_{33} were only minor

Table 5. Major compound classes identified in the total solvent extracts of plant material and horizons of the conifer forest soil (Brunisol).

	Pine needles	Leaf needles	Soil O horizon
Primary *n*-alkanols	–	C_{18}–C_{30} (C_{26})	C_{18}–C_{30} (C_{26})
Microbial or plant $<C_{20}$ [μg/g C]	–	3	13
Plant $<C_{20}$–C_{32} [μg/g C]	–	346	610
$\sum$ [μg/g C]	–	349	623
Secondary *n*-alkanols	C_{29}-10-ol, C_{29} diols	C_{29}-10-ol, C_{29} diols	C_{29}-10-ol, C_{29} diols
[μg/g C]	1248	105	317
n-Alkanoic acids	C_{16}, C_{18} (C_{16})	C_{16}–C_{28} (C_{22})	C_{14}–C_{28} (C_{22})
Microbial or plant $<C_{20}$ [μg/g C]	392	207	551
Plant $<C_{20}$–C_{32} [μg/g C]	–	249	441
$\sum$ [μg/g C]	392	456	992
n-Alkanes	–	C_{27}–C_{31} (C_{29})	C_{29}–C_{31} (C_{31})
[μg/g C]	–	35	40
iso-Alkanoic acids	–	C_{16}–C_{18}	C_{16}–C_{18}
[μg/g C]	–	13	19
$\sum$ Aliphatic lipids [μg/g C]	1640	958	1991
Steroids	st5	st1–st5, st8, st9 (st5)	st1–st5, st8, st9 (st5)
Microbial (st1, st2) [μg/g C]	–	100	258
Plant (st3–st9 [μg/g C])	456	490	1203
$\sum$ [μg/g C]	456	590	1461
Diterpenoids	d1–d4, d6, d7 (d6)	d1–d8 (d6)	d1–d8 (d6)
[μg/g C]	2721	153	603
Triterpenoids	–	t2, t4 (t2)	t2
[μg/g C]	–	20	37
$\sum$ Cyclic lipids [μg/g C]	3177	763	1843
Aliphatic/cyclic lipids	0.5	1.3	1.1
Phenols [μg/g C]	367	11	26
$\sum$ Lipids [μg/g C]	5184	1732	3860
Carbohydrates [μg/g C]	109,042	559	1123
Total [μg/g C]	114,226	2291	4983

Compounds in brackets indicate the predominant molecule within the series. For annotations of steroids terpenoids see Table 2.

components of the plant and soil extracts (22–487 μg/g C). C_{max} of the *n*-alkanes was at C_{29} or C_{31} in all samples. Two ω-hydroxyalkanoic acids (C_{22} and C_{24}) were identified in low amounts (18–22 μg/g C) in both Ah horizons and in trace amounts in the decomposing Western Wheatgrass.

The steroids detected in the grassland samples included eight sterols and steroid ketones (st1–st6, st8, st9) with β-sitosterol (st5) as the predominant steroid in all soil and plant samples. The concentrations of steroids were 187–1312 μg/g C with the lowest amount in the Ah horizon of the Brown Chernozem and the highest value in the decomposing grass from the same location. Campesterol, stigmasterol, β-sitosterol (st3–st5) and sitosterone (st9) were present in the soils and vegetation while ergosterol (st2) was identified in

all samples with the exception of the grass samples. Cholesterol (st1) and stigmastadienone (st8) were observed only in the soil samples while stigmastanol (st6) was found in the grass samples.

Triterpenoids of the oleanane, ursane and lupane classes (t1–t7) were identified in all plant and soil samples with the exception of the grass roots. The major triterpenoid in plants and soils were the oleanane type terpenoids β-amyrin (t2) associated with oleanolic acid (t6) and the ursane α-amyrin (t4), in both grass samples. Compared to the aliphatic lipids and the steroids, the triterpenoids only represented minor components of the TSE (18–808 μg/g C).

The phenolic compound, ferulic acid was detected in low quantities (1–28 μg/g C) in the grass samples and in both Ah horizons of the soils. Three monoacylglycerides with acyl side chains of $C_{16:1}$, C_{16} and C_{18} were identified in all samples (10–75 μg/g C) with the highest abundances in the grasses. The grass and grass roots of Western Wheatgrass yielded a variety of carbohydrates (306–21,025 μg/g C) consisting of mono- and disaccharides with xylitol, glucose, mannitol, mannose and sucrose present as major constituents. Carbohydrates were only minor components in the TSE of the mineral soil horizons (26–50 μg/g C) with the disaccharide trehalose (tre) as the predominant compound.

The yields and composition of the solvent extracts of the Western Wheatgrass from the Brown Chernozem and the Dark Brown Chernozem were similar with only slight differences in the C_{max} of the *n*-alkanol at C_{26} or C_{28}, respectively. Both Ah horizons of the grassland Chernozems also yielded similar qualitative patterns of solvent extractable compounds. The TSE yields of the Western Wheatgrass from the Brown Chernozem and the Dark Brown Chernozem are comparable with 19,975 and 27,733 μg/g C. The extract yields for the Ah soil horizons are also similar (620 and 600 μg/g C). The concentrations of total aliphatic and cyclic lipids decreased rapidly from the grass (11,562 and 6783 μg/g C) to the mineral horizons (570 and 574 μg/g C).

Aspen forest–grassland vegetation and soils

The TSE of the brown Aspen (*Populus tremula*) leaves and the organic and mineral horizons of the Dark Gray Chernozem contained a series of aliphatic lipids (*n*-alkanols, *n*-alkanoic acids, *n*-alkanes, wax esters), steroids, terpenoids, phenols and carbohydrates (Table 4 and Figure 2). A similar qualitative pattern of aliphatic lipids and cyclic lipids was observed for the Aspen leaves and the soil horizons. *n*-alkanols that ranged from C_{18} to C_{30} with a strong even-over-odd predominance were observed in all samples (113–3349 μg/g C) and were the prevalent lipid class in the soil O horizon. The maximum homologue (C_{max}) of the *n*-alkanols was at C_{28} in the Aspen leaves and at C_{26} in the soil horizons. The mostly even-numbered *n*-alkanoic acids (539–5201 μg/g C) ranged from C_{14} to C_{28} were major components in all samples and especially abundant in the Aspen leaves and in the Ah horizon. C_{max} of the *n*-alkanoic

acids was C_{26} in the Aspen leaves and C_{24} for both soil horizons. The C_{16} iso-alkanoic acid was the only branched acid detected in the Dark Gray Chernozem (Ah horizon). The *n*-alkanes (9–1324 μg/g C) consisted of odd-numbered homologues in the range of C_{23} to C_{31} with C_{max} at C_{25} in the Aspen leaves and C_{27} in both soil horizons. The Aspen leaves contained a considerable amount (3779 μg/g C) of C_{34}, C_{36} and C_{38} wax esters with C_{max} at C_{36}. Wax esters were found in the O horizon in minor amounts C_{38} (12 μg/g C) and were absent in the Ah horizon.

The plant material and the horizons of the Dark Gray Chernozem contained β-sitosterol (st5) as the main steroid. Additionally, ergosterol (st2), campesterol (st3), stigmasterol (st4), and sitosterone (st9) were detected in both soil horizons. Cholesterol (st1), stigmastan-3-one (st7) and stigmasta-3,5-dien-7-one (st8) were detected only in the O horizon. The total steroid contents were 682 μg/g C in the Aspen leaves, 1415 μg/g C in the O horizon and 397 μg/g C in the Ah horizon.

Triterpenoids were detected only in low concentrations in the Aspen leaves (t2, t4; 49 μg/g C) and in the Ah horizon t2, 77 μg/g C). In contrast, the O horizon contained 277 μg/g C of the oleanane, ursane and lupane type triterpenones and triterpenols (t1–t6) with β-amyrin (t2) as the major constituent. The O horizon contained the phenolic compounds vanillin, syringaldehyde, *p*-coumaric acid, ferulic acid, and caffeic acid (516 μg/g C) whereas only vanillin and *p*-hydroxybenzoic acid were identified in the Ah horizon (11 μg/g C). Phenolics were not detected in the Aspen leaves. The C_{16} and C_{18} monoacylglycerides were identified only in the Ah horizon (10 μg/g C). Carbohydrates were the major constituents in the TSE of the Aspen leaves (11,067 μg/g C) and the O horizon (1681 μg/g C; trehalose predominant) and only minor components in the Ah horizon (7 μg/g C).

The TSE yields decreased from 25,451 μg/g C in the Aspen leaves to 1231 μg/g C in the Ah horizon. A similar trend was observed for the total aliphatic and cyclic lipids. The most significant decrease among the aliphatic lipids was seen for the *n*-alkanes and wax esters. In contrast to the aliphatic lipids, the contents of the steroids and triterpenoids increased from the Aspen leaves to the O horizon and decreased again in the Ah horizon. The highest amounts of phenols were also observed in the O horizon. The content of carbohydrates decreased rapidly from the Aspen leaves to the Ah horizon.

Pine forest vegetation and soils

A homologous series of aliphatic lipids and steroids, diterpenoids, triterpenoids, phenols and carbohydrates represented the major compound classes in the TSE of Lodgepole Pine (*Pinus contorta*) needles and the leaf litter and the O horizon of the Brunisol (Figure 3 and Table 5). A similar compositional pattern of steroids and diterpenoids is observed in both the plant material and the soil horizons. In contrast, the aliphatic series is prominent in the soil horizons,

but occurred in the pine sample only in low amounts. Primary *n*-alkanols in the range from C_{18} to C_{30} with a strong even-over-odd predominance and C_{max} at C_{26} were major components (349 and 623 μg/g C) in the leaf litter and in the O horizon, but were not detectable in the pine needles. All samples contained considerable amounts (105–1248 μg/g C) of secondary C_{29} alkanols that were identified as *n*-nonacosan-10-ol, *n*-nonacosan-5,10-diol, *n*-nonacosan-10,13-diol and *n*-nonacosan-4,10-diol (Hamilton 1995). The *n*-alkanoic acids in the range of C_{14}–C_{28} with C_{max} at C_{22} were prevalent in the TSE of the leaf litter and the O horizon (456 and 992 μg/g C). In contrast, the pine needles contained only the C_{16} and C_{18} *n*-alkanoic acids (392 μg/g C) and long-chain homologues were not observed. Branched iso-alkanoic acids (C_{16} and C_{18}) were detected in low abundance only in the leaf litter and in the O horizon (13 and 19 μg/g C) and were absent in the pine extract. The odd-numbered *n*-alkanes C_{27}–C_{31} with C_{max} at C_{29} or C_{31} were observed only in the soil samples in low concentrations (35 and 40 μg/g C).

The steroids identified in the leaf litter and the O horizon of the Brunisol included five sterols (st1–st5) and two steroid ketones (st8, st9) with β-sitosterol (st5) as the predominant sterol while β-sitosterol was the only steroid detected in the pine needles. The total steroid yields were 456 μg/g C in the pine needles, 590 μg/g C in the leaf litter and 1203 μg/g C in the O horizon. Diterpenoids of the abietane, pimarane, and isopimarane classes were major constituents of the TSE of the plant and soil samples from the pine forest site (153–2721 μg/g C). The pine needles and the soil samples contained six abietane, pimarane and isopimarane type acids and alcohols (d1–d4, d6, d7). Additionally, two abietatetraenoic acids (d5, d8) were identified in both the leaf litter and the O horizon. The triterpenoids β-amyrin (t2) and α-amyrin (t4) were observed as minor components (20 and 37 μg/g C) in the soil samples only.

The phenols *p*-hydroxybenzoic acid, vanillic acid, and *p*-coumaric acid were observed in all three samples of the Brunisol. Additionally, acetovanillone was detected in the pine needles and vanillin in the O horizon. Carbohydrates were the major components in the TSE of the pine needles, leaf litter and the O horizon (559–109,042 μg/g C) with the predominance of glucose and mannose in the pine needles and trehalose in the soil horizons. The concentration of the total extractable components decreased from the Pine needles (114,226 μg/g C) to the leaf litter (2291 μg/g C) and then increased in the O horizon (4983 μg/g C). The same trend was exhibited for the total aliphatic lipids, secondary *n*-alkanols, diterpenoids, total lipids and carbohydrates. In contrast, the abundances of primary *n*-alkanols, *n*-alkanoic acids and steroids increased steadily from the Pine needles to the leaf litter and the O horizon.

Discussion

Homologous series of aliphatic lipids (*n*-alkanols, *n*-alkanoic acids, iso-alkanoic acids, *n*-alkanes, ω-hydroxyalkanoic acids, wax esters), steroids,

terpenoids, phenols and monoacylglycerides represent the classes of free lipids detected in the TSE of the plant and soil samples analyzed. The identified lipids are characteristic biomarkers for distinct classes of organisms and can be used to determine major sources of the SOM (Table 6). The series of long-chain (C_{20}–C_{38}) *n*-alkanols, *n*-alkanoic acids, *n*-alkanes and wax esters observed in the plant materials and the soil samples are characteristic of leaf and suberin-associated surface waxes of vascular plants (Tulloch 1976a; Baker 1982; Kolattukudy and Espelie 1989; Bianchi 1995). In contrast, short-chain ($< C_{20}$) *n*-alkanes, *n*-alkanols and iso-alkanoic acids are characteristic of microorganisms and are consistent with the input of lipids from fungi, bacteria and/or algae (Weete 1976; Harwood and Russell 1984). Short-chain *n*-alkanoic acids (C_{12}–C_{18}) are synthesized by both plants and microorganisms and are therefore not source specific (Weete 1976; Baker 1982; Harwood and Russell 1984). The cyclic lipids encompass steroids and terpenoids that are useful biomarkers. The

Table 6. Sources of aliphatic and cyclic biomarker classes identified in the total solvent extract of grassland and forest soils from Alberta, Canada (after Albro 1976; Tulloch 1976a; Weete 1976; Baker 1982; Harwood and Russell 1984; Kolattukudy and Espelie 1989; Bianchi 1995).

Compound class	Range and preference	Source
Aliphatic lipids		
Primary *n*-alkanols	C_{20}–C_{32} even	Vascular plant waxes
	C_{16}–C_{18}	Microorganisms
Secondary *n*-alkanols	C_{29}-10-ol, C_{29}-diols	Vascular plant waxes
n-Alkanoic acids	C_{20}–C_{32} even	Vascular plant waxes
	C_{14}–C_{18}	Microorganisms
iso-Alkanoic acids	C_{16}, C_{18}	Microorganisms
n-Alkanes	C_{23}–C_{31} odd	Vascular plant waxes
ω-Hydroxyalkanoic acids	C_{22}, C_{24}	Vascular plant waxes, hydrolysis of suberin (vascular plants)
Wax esters	C_{34}, C_{36}, C_{38}	Vascular plant waxes
Monoacylglycerides	$C_{16:1}$, C_{16}, C_{18}	Fats of all organism
Steroids	β-Sitosterol, stigmasterol, campesterol, stigmastano	Plants
	Ergosterol	Fungi
	Cholesterol	Animals, fungi, plants
Triterpenoids	β-amyrin, α-amyrin, lupeol, β-amyrone, α-amyrone, Oleanolic acid, ursolic acid	Angiosperms
Diterpenoids	Dehydroabietol, pimaric acid, isopimara-8, 15-dienoic acid, isopimaric acid, dehydroabietic acid, abietic acid	Conifers
Phenols	Ferulic acid, *p*-hydroxybenzoic acid, vanillin, syringaldehyde, *p*-coumaric acid, ferulic acid, caffeic acid	Suberin-associated waxes and hydrolysis of lignin and suberin (vascular plants)

steroids campesterol, stigmasterol, β-sitosterol, and sitosterone are the most common steroids (phytosterols) in vascular plants (Baker 1982; Harwood and Russell 1984; Bianchi 1995) whereas ergosterol is a specific biomarker for fungi (Weete 1976; Harwood and Russell 1984; Ruzicka et al. 2000). Cholesterol has been reported as a biomarker of soil fauna, fungi, and algae, and it is rarely found in vascular plant waxes (Weete 1976; Harwood and Russell 1984; Noda et al. 1988). Triterpenoids of the oleanane, ursane and lupane type, such as α- and β-amyrin and lupeol, only occur in angiosperms and are characteristic biomarkers for this type of vegetation (Tulloch 1976b; Baker 1982; Bianchi 1995). Typical constituents of conifers are diterpenoid acids of the abietane, pimarane and isopimarane classes (Karrer 1958; Hegnauer 1962, 1992; Karrer et al. 1977; Otto and Wilde 2001).

Grassland vegetation and soils

The grassland soil biomarker composition is governed by the input of leaf waxes that are derived from vascular plants, namely angiosperms. The majority of the free lipids are derived from Western Wheatgrass, a common and prevalent native species in the prairie ecozone of Western Canada, and the lipid distribution is consistent with grass/angiosperm vegetative inputs. Lipids originating from microorganisms and soil fauna were also observed but only in minor abundances (Table 3). The most prevalent lipids in the extracts of the grasses and the grassland soils were the C_{26} or C_{28} *n*-alkanols. Leaf waxes from grass species commonly contain *n*-alkanols in the range from C_{22} to C_{32}, but typically display C_{26} and/or C_{28} as the major *n*-alkanol with other homologues present as minor constituents (Tulloch 1976a). The pattern of aliphatic lipids detected in the fresh Western Wheatgrass analyzed in this study is similar to the previously reported composition of this grass species (Tulloch 1976b). The extract yield and lipid composition of the Western Wheatgrass from the Brown and the Dark Brown Chernozem sample sites were similar with only a slight distinction in the Cmax of the *n*-alkanol the (C_{26} vs. C_{28}), which reflects modifications of wax composition due to intraspecific variations or environmental factors such as temperature and humidity (Baker 1982; Bianchi 1995). The patterns of long-chain aliphatic lipids in the Western Wheatgrass and in the Ah horizons of the grassland soils are similar and indicate that native grasses are the major organic matter input in this region (Figure 4). A shorter carbon chain-length of the predominant homologue of alkanols and alkanoic acids was observed in the soil horizons compared to the grass samples. This is likely caused by the degradation of aliphatic lipids to homologues with a shorter chain-length in the soils and/or by the additional input of aliphatic lipids from roots or other plant species. *n*-Alkanols with chain lengths $<C_{20}$ and the branched C_{16} iso-alkanoic acid originating from microorganisms were detected only in the soil extracts and not in the vegetation samples further confirming the source apportionment of these biomarkers. ω-Hydroxyalkanoic

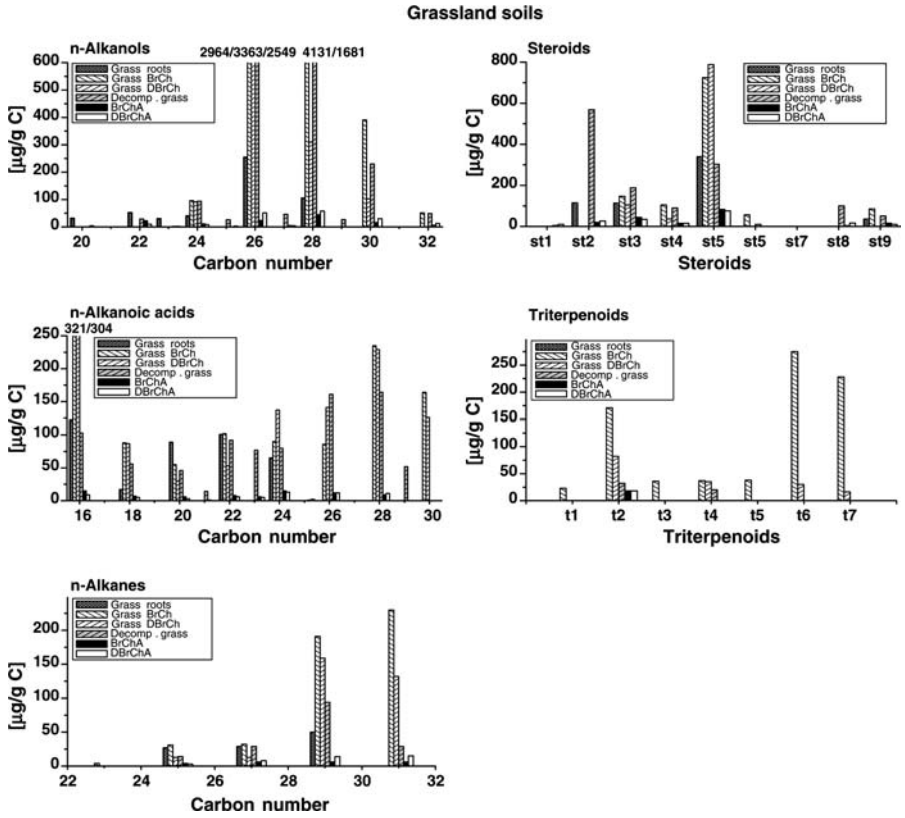

Figure 4. Distribution of aliphatic and cyclic lipids in the vegetation and soil horizons of the grassland soils (BrCh = Brown Chernozem, DBrCh = Dark Brown Chernozem). For peak annotation of steroids and terpenoids see Table 2.

acids are only minor compounds of plant waxes, but are common constituents of suberin, a biopolymer occurring in the bark and roots of plants (Kolattukudy 1981; Kolattukudy and Espelie 1989; Walton 1990). Consequently, the C_{22} and C_{24}-hydroxyalkanoic acids identified in the soil horizons are likely the products of suberin hydrolysis. Suberin exists in a non-extractable, polymeric form in the fresh plant material. Therefore, ω-hydroxyalkanoic acids were not detected in the solvent extract of the fresh grass root biomass. The detected C_{16} and C_{18} monoacylglyderides are unspecific source compounds because they are constituents of fats that are produced by many organisms (Harwood and Russell 1984).

The detection of the steroids (campesterol, stigmasterol, β-sitosterol, and stigmastanol) is consistent with the input of vascular plant material (Baker 1982; Harwood and Russell 1984; Bianchi 1995). Stigmastan-3-one, stigmasta-3,5-dien-7-one, and sitosterone, are the degradation products of both

β-sitosterol and stigmasterol (Mackenzie et al. 1982; Owen et al. 1985) and have thus the same vegetative origin. The occurrence of ergosterol in the decomposing grass and in the soil samples and their absence in the fresh grass confirms the presence and activity of fungi in these soils (Weete 1976; Ruzicka et al. 2000; Harwood and Russell 1984). Cholesterol was detected in the soil samples and indicates the presence and activity of a variety of soil fauna and microorganisms. The detected triterpenoids of the oleanane, ursane and lupane type are consistent with the angiosperm Western Wheatgrass as the major input of organic matter into the soils in this region (Tulloch 1976b; Baker 1982; Bianchi 1995). Minor amounts of lipids originating from other angiosperm species growing in the grassland vegetation may also contribute triterpenoids to the SOM.

The phenolic compound ferulic acid was observed in the undecomposed grass and in the soil samples. Ferulic acid is a degradation product of the biomacromolecules lignin and suberin (Kögel-Knabner 2000), but it has also been reported from suberin-associated waxes, especially those from grasses (Kolattukudy 1981; Kolattukudy and Espelie 1989). Since the applied solvent extraction only removes low molecular-weight lipids and does not affect intact macromolecules in SOM, the ferulic acid is likely a free compound originating from suberin-associated waxes or from the hydrolysis of suberin or lignin by microorganisms in the soil.

Grassland–forest transition vegetation and soils

The aliphatic lipids, plant steroids, and angiosperm-derived triterpenoids detected in the TSE of the Dark Gray Chernozem (Aspen forest–grassland transition soil zone) are characteristic of leaf waxes from angiosperms. Leaves of Aspen and other angiosperms such as grasses and herbaceous vegetation growing in the soil are the probable sources for the majority of the free lipids detected in the Dark Gray Chernozemic soil. Microbial lipids were present only in low quantities indicating a minor contribution of lipids from fungi, bacteria and/or algae. The observed aliphatic lipids are mainly composed of long-chain *n*-alkanols, *n*-alkanoic acids, *n*-alkanes and wax esters typical for vascular plant waxes (Weete 1976; Tulloch 1976a; Baker 1982; Harwood and Russell 1984; Bianchi 1995). The chain-length of the predominant homologue (C_{max}) of the alkanol and alkanoic acid series in the soils is shorter in the soils than in the Aspen leaves as observed for the grass samples (Figure 5) indicating again the degradation of aliphatic lipids and/or the additional input of plant material from other sources such as roots or grasses to the soil. Vascular plant origins are also evident for the suberin-derived C_{22} and C_{24} ω-hydroxyalkanoic acids (Kolattukudy 1981; Kolattukudy and Espelie 1989). Microbial lipid inputs are represented by only minor amounts of C_{16} and C_{18} iso-alkanoic acids and short-chain *n*-alkanols ($<C_{20}$) (Table 4). The non-specific C16 and C_{18} monoacylglyderides were observed in the Ah horizon of the Dark Gray

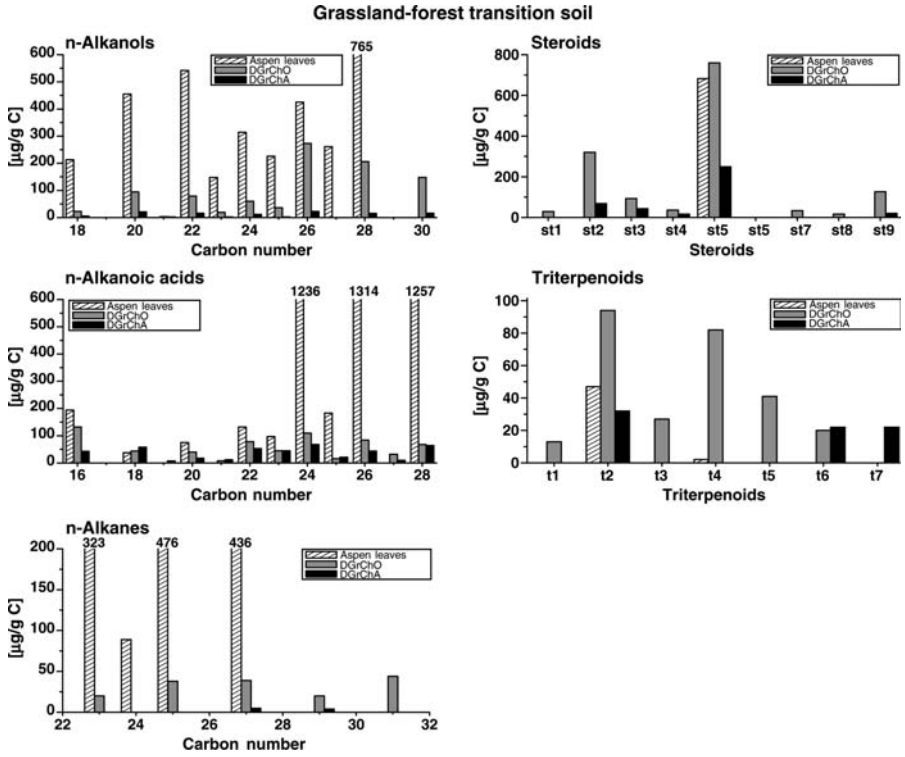

Figure 5. Distribution of aliphatic and cyclic lipids in the vegetation and soil horizons of the grassland–forest transition soil (DGrCh = Dark Gray Chernozem). For peak annotation of steroids and terpenoids see Table 2.

Chernozem indicating the input of fats from various plant, animal and/or microbial sources (Harwood and Russell 1984).

The composition of steroids in the Aspen leaves and the horizons of the Dark Gray Chernozem is similar to the steroids observed in the grassland soils. The phytosterol β-sitosterol was the only steroid detected in the Aspen leaves and the most abundant steroid compound in both soil horizons consistent with its origin from vascular plants (Baker 1982; Harwood and Russell 1984; Bianchi 1995). The detection of ergosterol in the soil organic and mineral horizons and its absence in the Aspen extract indicates that fungi are present and active in the soil horizons. Cholesterol was found only in the O horizon of the soil, representative of small animals (e.g., worms) and/or fungi that are concentrated in the organic-rich horizon (Weete 1976; Harwood and Russell 1984).

The triterpenoids observed in the Aspen leaves and the soil samples of the grassland–forest transition zone were derivatives of the oleanane, ursane and lupane classes characteristic for angiosperms (Tulloch 1976a; Baker 1982;

Bianchi 1995). α-Amyrin, β-amyrin and lupeol were previously reported as constituents of waxes extracted from green leaves of Quaking Aspen (Roshchin et al. 1986). Phenolic compounds such as *p*-hydroxybenzoic acid, vanillin, syringaldehyde, *p*-coumaric acid, and ferulic acid were identified in both soil horizons, but not in the Aspen leaves. The observed phenols are typical monomeric biodegradation products of lignin which is produced only by vascular plants (Hedges and Ertel 1982; Kögel-Knabner 2000). Lignin analysis of major plant groups revealed characteristic compositions for conifer and angiosperm wood, respectively, and for non-woody vascular plant tissues such as leaves, grasses, and conifer needles (Hedges and Mann 1979; Hedges and Ertel 1982). The phenolic compounds observed here include: vanillyl, syringyl and coumaryl, which are characteristic for non-woody angiosperm tissues such as leaves or grass The phenols are thus interpreted as derived from the most prominent non-woody angiosperm materials in the Dark Gray Chernozemic soil zone, the leaves of Aspen and grasses. Only a few fungi species, i.e., white-rot and brown-rot fungi, are known to biodegrade lignin in soils (Hedges et al. 1988; Goni et al. 1993). The observation of solvent extractable lignin-derived phenols in the soils thus provides indirect evidence for the presence of lignin-degrading fungi in the Dark Gray Chernozemic soil zone.

Pine forest vegetation and soils

The TSE of the pine needles is comprised of large quantities of diterpenoids with low concentrations of aliphatic wax lipids (Table 5 and Figure 6). In contrast, the leaf litter layer and the O horizon of the forest soil contained a mixture of aliphatic lipids and steroids with only a small contribution from diterpenoids. The results indicate that the Brunisolic soil has a mixed input of vascular plant lipids derived from Pine and from angiosperm species such as shrubs or grasses growing in the pine forest. Microbial biomarkers were present only in minor quantities. The aliphatic lipids identified in the pine needles included secondary C_{29} *n*-alkanols and diols, C_{16} and C_{18} *n*-alkanoic acids and only trace amounts of *n*-alkanols. These lipids are common constituents of vascular plant waxes (Tulloch 1976a; Baker 1982; Bianchi 1995). C_{27} and C_{29} alkanediols occur in the waxes of various vascular plants, but they are especially common in the epicuticular waxes of conifers (Bianchi 1995). The aliphatic lipids present in the leaf litter and the O horizon of the Brunisol represent a mixed input of long-chain *n*-alkanols, *n*-alkanoic acids and *n*-alkanes derived from vascular plant waxes and microbial lipids (*n*-alkanols $<C_{20}$, C_{16} and C_{18} iso-alkanoic acids) (Albro 1976; Weete 1976; Baker 1982; Harwood and Russell 1984; Bianchi 1995). The detection of primary *n*-alkanols, long-chain *n*-alkanoic acids and *n*-alkanes in the soil horizons and their absence in the pine needles suggests other sources of lipids. This is in accordance with the observation of partially decomposed leaves of grasses and other angiosperms in the leaf litter derived from herbaceous and shrub vegetation in the pine forest.

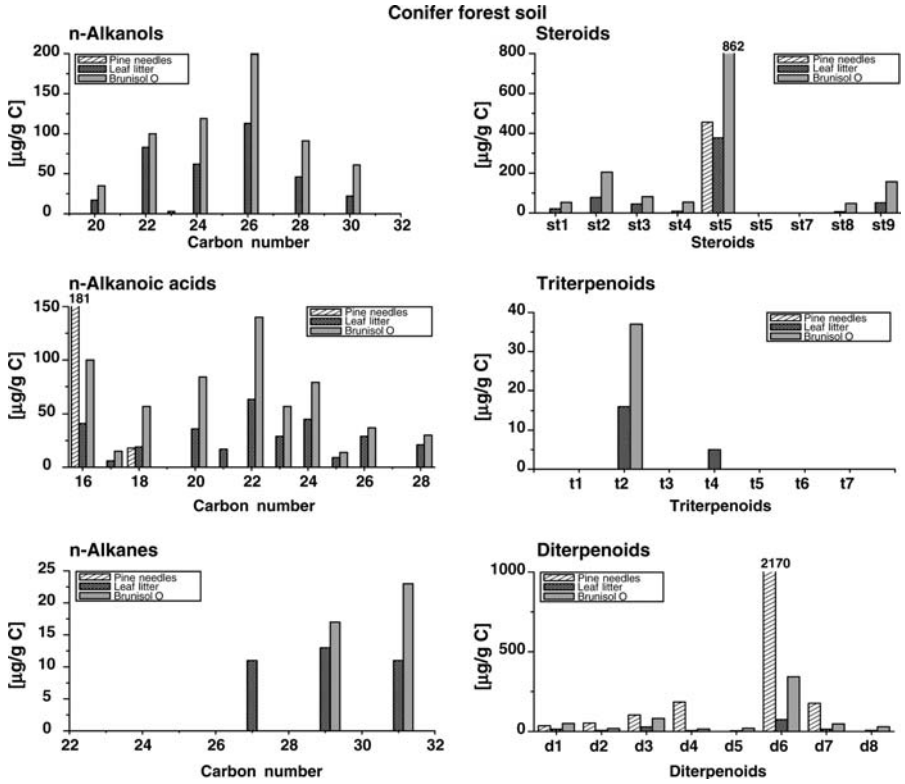

Figure 6. Distribution of aliphatic and cyclic lipids in the vegetation and soil horizons of the conifer forest soil (Brunisol). For peak annotation of steroids and terpenoids see Table 2.

β-Sitosterol is the only steroid detected in the pine needles and is the most common plant sterol (Baker 1982; Harwood and Russell 1984; Bianchi 1995). Phytosterols are also the major steroids in the leaf litter and the O horizon accompanied with minor abundances of cholesterol (microorganisms, animals) and the fungal biomarker ergosterol. Diterpenoids of the abietane, pimarane and isopimarane classes which are characteristic for conifers (Hegnauer 1962, 1992; Karrer et al. 1977; Otto and Wilde 2001) are major components of the pine needles and were detected in both soil horizons. Since Lodgepole Pine is the only conifer present in the Brunisol sample area, the detected diterpenoids are clearly derived from this species. Small abundances of β-amyrin and α-amyrin were observed in the leaf litter and the O horizon, but not in the extract of the pine needles. These triterpenoids are angiosperm biomarkers and do not occur in conifers (Hegnauer 1962, 1992; Otto and Wilde 2001). Therefore, the triterpenoids observed in the Brunisolic soil are derived from angiosperm species growing in the Pine forest (e.g., grass, shrubs).

The composition of the phenols identified in the pine needles and in the Brunisolic soil horizons are pronounced with vanillyl type monomers. The

absence of syringyl units and the presence of coumaryl derivatives indicate that the lignin originates from both woody and non-woody conifer tissues (Hedges and Mann 1979; Hedges and Ertel 1982). The lignin-derived phenols in the Brunisolic samples are thus interpreted as degradation products of the lignin of Lodgepole Pine wood and needles.

Degradation vs. degradation of major lipid classes

The organic compounds present in the source vegetation undergo various degradation processes in the soil horizons and most of the lipid classes and the carbohydrates are rapidly degraded. The concentrations of major biomarker classes derived from vascular plants (*n*-alkanols C_{20}–C_{32}, *n*-alkanoic acids and *n*-alkanes $>C_{18}$, plant steroids st3–st9, triterpenoids, diterpenoids) detected in source plants and soils are displayed in Figure 7. In general, the plant samples contain a higher ratio of aliphatic vs. cyclic lipids than the soil samples. Ratios of aliphatic/cyclic lipids were 4.3 to 5.1 in the fresh and decomposed grasses, 2.0 in the grass roots and 1.7 in both grassland soils (Table 3) while the aliphatic/cyclic lipids ratios were 18.6 in the Aspen leaves and 1.2 and 1.5 in the O horizon and the Ah horizon of the Dark Gray Chernozem, respectively (Table 4). The aliphatic/cyclic lipids ratios are also low in the Brunisol samples with 1.3 in the leaf litter and 1.1 in the O horizon of the Brunisol (Table 5). Due to the very high concentrations of diterpenoids and very low amounts of aliphatic lipids, the ratio of aliphatic/cyclic lipids is 0.5 for the Pine needles. The results indicate that aliphatic lipids are preferentially degraded in the soils compared to the cyclic lipids.

Soil lipids have been described as highly resistant to biodegradation (Dinel et al. 1990; Kögel-Knabner 2000). The extent of degradation depends on microbial activity and physico-chemical soil conditions. Accumulation of lipids has been observed especially in acidic, dry and anaerobic soils due to inhibited microbial activity (Dinel et al. 1990; Bull et al. 2000b). *n*-Alkanoic acids can be rapidly degraded by aerobic bacteria or certain fungi via β-oxidation yielding shorter-chain *n*-alkanoic acids (Dinel et al. 1990). Bull et al. (2000b) reported that the preservation of *n*-alkanes was enhanced in more alkaline soils while *n*-alkanoic acids accumulated in more acidic soils. Since the degradation of aliphatic lipids such as *n*-alkanols, *n*-alkanoic acids and *n*-alkanes results in the formation of the same lipid classes, the products of the degradation can not be distinguished from the original biosynthesized homologues and degradation can only be detected by the decrease or increase of concentrations of lipid classes. But, a shift to a shorter carbon chain-length of the predominant homologue (C_{max}) in the *n*-alkanol and *n*-alkanoic acid series was observed from the source plants to the corresponding soils. The chain-length of the predominant homologue (C_{max}) of the *n*-alkanoic acids was lower in the soil horizons (C_{max} at C_{24} in grassland soil and Aspen soil) than in the respective source plants (C_{max} at C_{28} in Western Wheatgrass and C_{max} at C_{26} in Aspen

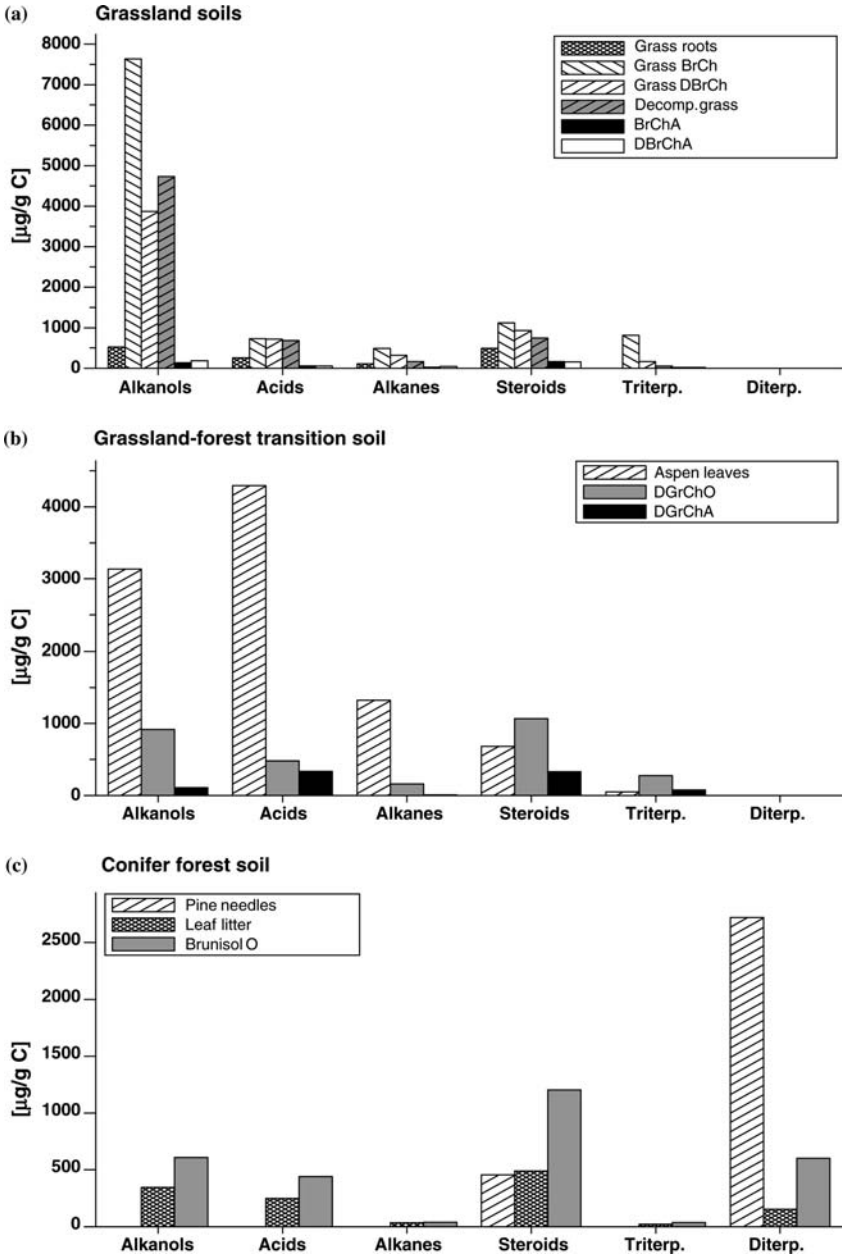

Figure 7. Concentrations of vascular plant biomarkers in the source vegetation and soil horizons of the grassland, grassland–forest transition and conifer forest soils. *n*-Alkanols C_{20}–C_{32}, *n*-alkanoic acids C_{20}–C_{30}, *n*-alkanes C_{23}–C_{31}, plant steroids st3–st9, triterpenoids t1–t7, diterpenoids d1–d8. For peak annotation of steroids and triterpenoids see Table 2.

leaves) suggesting the degradation of long-chain *n*-alkanoic acids to shorter alteration products. However, the input of shorter-chain *n*-alkanoic acids from other plant species exhibiting a different lipid pattern can not be excluded. The same trend of a decrease in C_{max} can be observed for the *n*-alkanols in most of the soil samples analyzed in this study. Since the aliphatic lipids in the Brunisol represent a mixed input from pine and angiosperm species, the interpretation of aliphatic lipid degradation in this soil is difficult. The observation of wax esters in the Aspen leaves and in minor amounts in the O horizon of the Dark Gray Chernozems demonstrates that the hydrolysis of wax esters occurs in the organic horizon of the soil and possibly in the decomposing leaves. Compared to the cyclic lipids identified in the soils, the aliphatic lipids appear to be preferentially degraded.

The fate of cyclic lipids such as steroids and terpenoids in soils is largely unknown (van Bergen et al. 1997). The decrease of sterols and triterpenols in leaf litter and soils has been previously interpreted as the result of complete mineralization, chemical alteration to degradation products and incorporation of free molecules into the insoluble soil matrix (Amblès et al. 1994a). The incorporation of biosynthesized triterpenols and sterols into humic material via ester and possibly ether bonds has been reported (Gobé et al. 2000). Degradation products of steroids were not detected in several soils analyzed by van Bergen et al. (1997) and the hypothesis that sterols and triterpenols are incorporated into humic substances was favored. The assimilation of *β*-sitosterol by certain insects may also result in the loss of sterols in soil environments (Bull et al. 2000b). Due to the apparent preferential degradation of aliphatic lipids which are prominent in the source plants, the steroids appear to be relatively enriched in the soil horizons. Major steroids identified in the source vegetation and soils were unaltered sterols and *β*-sitosterol frequently was the major single lipid component of the soil extract (e.g., Brown Chernozem Ah horizon, Dark Gray Chernozem Ah horizon, Brunisol leaf litter and O horizon). Besides the unaltered plant sterols, three steroid ketones (st7–st9) were identified in the soils, but not in the plant material. Sitosterone has been reported as a metabolite in the bacterial oxidation of *β*-sitosterol (Owen et al. 1985). Consequently, the detected steroid ketones sitosterone, stigmasta-3,5-dien-7-one and stigmastan-3-one are interpreted as the degradation products of biosynthesized sterols and indicate the oxidative degradation of *β*-sitosterol and stigmasterol (Mackenzie et al. 1982) (Figure 8a). Stigmasta-3,5-dien-7-one and stigmastan-3-one were previously isolated from contemporary and fossil sediments, coals and fossil plants (e.g., Mackenzie et al. 1982; Otto and Simoneit 2001), but were hitherto not reported in soils. The ratios of precursor sterols (*β*-sitosterol, stigmasterol, stigmastanol) to their degradation products (stigmastan-3-one, stigmasta-3,5-dien-7-one, sitosterone) in the grassland soils decrease from the plants to the soil horizons (Table 7) indicating a progressive oxidation of sterols in leaf litter and organic and mineral horizons. Higher ratios in the Ah horizon than in the O horizon could be due to higher level oxidation in the subaerial organic horizon than in the lower Ah horizon. The same trend was also

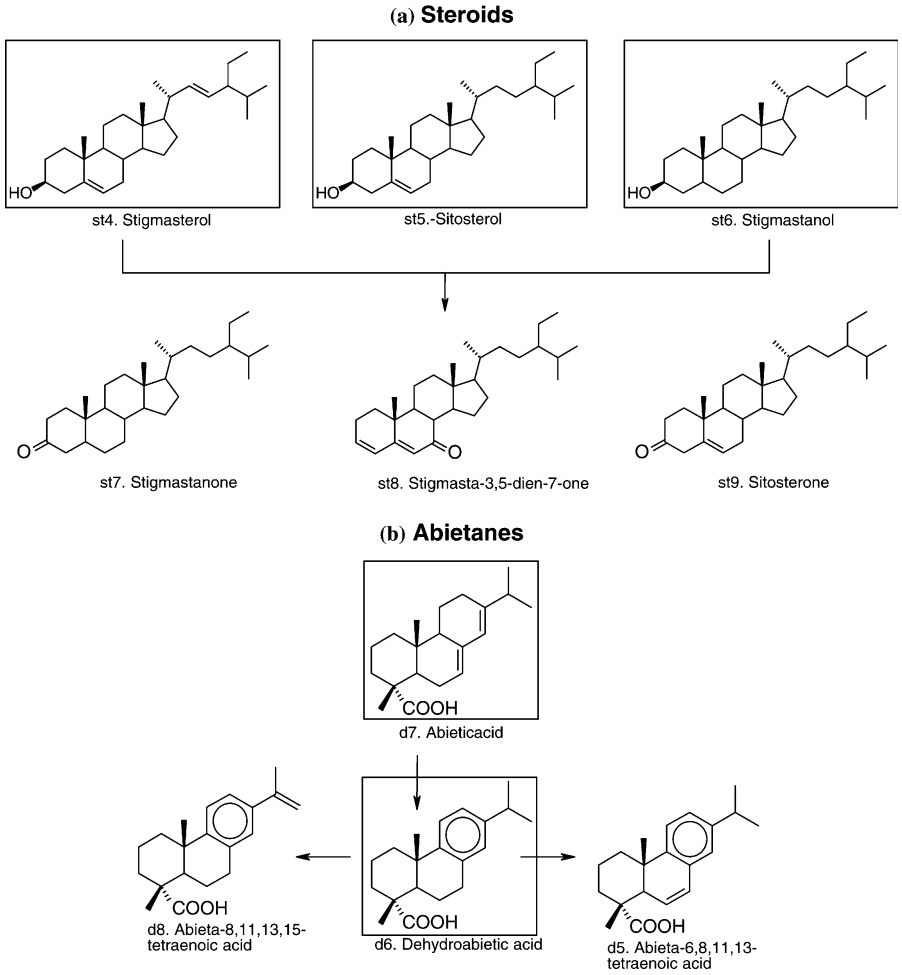

Figure 8. Oxidative degradation of vascular plant biomarkers in soils. (a) Steroids, (b) abietane type diterpenoids. Boxes indicate biological precursors.

observed in the Aspen leaves and the Dark Gray Chernozem where the degraded steroids were only observed in the O horizon. The precursor/product ratio for the steroids in the Brunisol samples decreases from the pine needles to the leaf litter and the O horizon indicating an ongoing degradation of sterols. The results suggest that sterols can be at least partly preserved as free molecules in soils without bonding into the insoluble SOM matrix. The detection of minor amounts of degradation products supports the hypothesis that oxidative degradation processes of steroids occur in soil environments.

Degradation products of triterpenoids of the oleanane, ursane and lupane classes have been reported for numerous contemporary and fossil sediments,

Table 7. Concentrations of vascular plant-derived precursor steroids and their degradation products identified in the plants and soils.

	Precursors[a] [μg/g C]	Degradation products[b] [μg/g C]	Ratio
Grassland soils			
Grass roots	340	36	9.4
Western Wheatgrass BrCh	887	86	10.3
Western Wheatgrass DBrCh	825	–	–
Decomposing Western Wheatgrass	403	150	2.7
Brown Chernozem Ah horizon	98	20	4.9
Dark Brown Chernozem Ah horizon	91	27	3.4
Grassland–forest transition soil			
Aspen leaves	682	0	–
Dark Gray Chernozem O horizon	795	177	4.5
Dark Gray Chernozem Ah horizon	266	20	13.1
Conifer forest soil			
Pine needless	456	0	–
Leaf litter	386	60	6.5
Brunisol O horizon	915	206	4.5

[a]Steroid precursors: β-sitosterol + stigmasterol + stigmastanol.
[b]Steroid degradation products: stigmastan-3-one + stigmasta-3,5-dyen-7-one + sitosterone.

coals and petroleum (e.g., Chaffee et al. 1986; Simoneit 1986; Killops and Killops 1993) and from decaying angiosperm tissues (e.g., Killops and Freewin 1994). Mono- and di-unsaturated, aromatic and ring-A-degraded triterpenoids are the major products of the oxidative degradation of triterpenoids (Chaffee et al. 1986; Simoneit 1986; Killops and Freewin 1994; Jaffé et al. 1996). Although unaltered triterpenoids of the oleanane, ursane and lupane classes were present in the angiosperms and associated soils, degradation products derived from these biomarkers could not be detected. Since the precursor triterpenols and triterpenoic acids were found only in low concentrations in the plants and soils, their degradation products may not have been detected because they were present in concentrations below detection limits.

The degradation (diagenesis) of abietane, pimarane and isopimarane type diterpenoids in contemporary and fossil sediments and in coals is well documented (e.g., Laflamme and Hites 1978, Wakeham et al. 1980; Barnes and Barnes 1983; Simoneit 1986; Otto and Simoneit 2001). The degradation of diterpenoids under oxic conditions leads preferentially to the formation of unsaturated and aromatic products (e.g., simonellit, retene) while reductive processes yield saturated derivatives (Barnes and Barnes 1983; Simoneit 1986). The needles of Lodgepole Pine and the leaf litter and O horizon of the Brunisol contained diterpenoid acids and a diterpenol (dehydroabietol) of the abietane, pimarane and isopimarane type. These diterpenoids are unaltered preserved precursors that are biosynthesized by the living conifer. Despite various degradation processes, these diterpenoids are very stable in the geosphere and were reported from various sediments, coals, peats and fossil conifers (e.g., Chaffee

Table 8. Concentrations of abietane type precursor diterpenoids and their degradation products identified in Lodgepole pine and horizons of the pine forest soil (Brunisol).

	Precursors[a] [μg/g C]	Degradation products[b] [μg/g C]	Ratio
Brunisol			
Lodgepole pine needless	2347	–	–
Leaf litter	88	11	8.0
O horizon	389	49	7.9

[a]Diterpenoid precursors: abietic acid + dehydroabietic acid.
[b]Diterpenoid degradation products: abieta-6,8,11,13-tetraenoic acid + abieta-8,11,13,15-tetraenoic acid.

et al. 1986; Simoneit 1986 and references therein; Simoneit et al. 1986; Lehtonen et al. 2001; Otto and Simoneit 2001). The preservation of the polar diterpenoids in geological samples is in part due to the protection of biomolecules against microbial and abiogenic degradation in the resinous material (Otto et al. 2002). In addition to the unaltered diterpenoids, two abietatetraenoic acids (d5, d8) were observed in the leaf litter and the O horizon of the Brunisol, but not in the extract of the pine needles. These diterpenoids do not occur in living plants and are interpreted as the oxidative degradation products of abietic acid and dehydroabietic acid (Simoneit 1986; Otto and Simoneit 2001). The ratios of abietane type precursors (abietic acid + dehydroabietic acid) and their products (abieta-6,8,11,13-tetraenoic acid + abieta-8,11,13,15-tetraenoic acid) are 8.0 in the leaf litter and 7.9 in the O horizon of the Brunisol indicating the oxidation of abietane precursor acids in both horizons (Table 8). Dehydroabietic acid is a biological precursor occurring in many conifers, but it may also be generated from the oxidation of abietic acid (Simoneit 1986). Therefore, the dehydroabietic acid identified in the soils may be in part a degradation product. However, since dehydroabietic acid and abietic acid were observed in about equal proportions in the soil horizons (5:1 and 7:1) and dehydroabietic acid occurred in even higher abundance in the fresh pine needles (12:1), dehydroabietic acid is considered here as a biosynthesized precursor.

Conclusions

The free lipids identified in the TSE of selected source vegetation and soil horizons are useful biomarkers for the determination of the sources of solvent extractable ('free') soil lipids. Lipid compositions observed in these soils displayed similar signatures observed in their major source plants. The predominant long-chain aliphatic lipids, plant sterols and terpenoids indicate the major input of vascular plant wax lipids into the organic and mineral horizons of the analyzed grassland and forest soils. Individual aliphatic lipids are common in vascular plants and thus non-specific, but characteristic patterns of leaf waxes observed in the source plants were still recognizable in the associated soil horizons. Terpenoid biomarkers typical for angiosperms (triterpenoids:

oleananes, ursanes, lupanes) and conifers (diterpenoids: abietanes, pimaranes, isopimaranes) allow the assignment of SOM to either one of these plant groups and to assess mixed contributions of angiosperms and conifers to the soil. The free lipids in the Brown and Dark Brown Chernozems and in the Dark Gray Chernozem exhibited a lipid composition comparable to the ones observed in their corresponding vegetative inputs, Western Wheatgrass and Quaking Aspen, respectively. The Brunisol horizons contain a mixture of lipids derived from the major conifer species (Logepole Pine) and from herbaceous plants, such as grasses. Lipids derived from microorganisms (short-chain *n*-aliphatic lipids and iso-alkanoic acids, microbial steroids) were detected only in low abundances in the TSE indicating the minor input of low molecular weight microbial lipids into the soils. Similar compositions of free lipids were previously reported for grassland and forest soils (van Bergen et al. 1997, 1998; Bull et al. 1998, 2000a, b; Oros et al. 2002). The results confirm that the major input of solvent extractable soil lipids is derived from vascular plant waxes and microbial lipids represent only minor contributions.

The series of aliphatic lipids (*n*-alkanols, *n*-alkanoic acids, *n*-alkanes, wax esters) appear to be preferentially degraded compared to the cyclic lipids (steroids and terpenoids) because the decrease of the total aliphatic lipids from the source plants to the soils is more significant than for the cyclic lipids. Since the degradation products of the aliphatic lipids are indistinguishable from the biosynthesized homologues, the degree of degradation can only be inferred from the decrease or increase of concentrations of lipid classes and from the increase of shorter-chain homologues. The shift of chain length to shorter homologues from the source plants (C_{max} at C_{28} or C_{26}) to the associated soil horizons (C_{max} at C_{24}) of the Chernozems is indicative of *n*-alkanoic acid degradation.

The cyclic lipids (steroids and terpenoids) are useful biomarkers for the monitoring of preservation and/or degradation of vascular plant material in soils. The steroids are represented mainly by the unaltered biosynthesized precursors which are relatively enriched in the soil horizons due to the preferential degradation of the aliphatic lipids. β-Sitosterol was observed as the major individual lipid in most of the organic and mineral soil horizons analyzed. In addition to the preserved biosteroids, the soils but not the plant material, contained three degradation products of the plant sterols. The sterol degradation products (steroid ketones) were frequently detected in sediments and coals and are reported here for the first time in soil samples. Decreasing ratios of precursor/product steroids from the source plant to organic and mineral soil horizons indicate a progressive degradation of sterols with the highest degree of degradation in the leaf litter and O horizons. Although triterpenoid precursors of the oleanane, ursane and lupane classes were observed in the grassland and the grassland–forest transition soils, degradation products of triterpenoids were not detected. This may be due to the low concentrations of triterpenoids in the soils and degradation products may be present below detection limits. The diterpenoids detected in the Brunisolic soil

are predominantly unaltered bioterpenoids as observed in the Lodgepole Pine. Low amounts of degradation products derived from abietane type diterpenoids (abietic acid, dehydroabietic acid) indicate a partial oxidative alteration of diterpenoid acids in the Brunisolic soil. The degradation products of steroids and terpenoids still retain their characteristic basic structure and can be assigned back to their biological sources. The degradation products of cyclic lipids such as steroids and terpenoids thus can yield information of the degradation of source specific biomarkers in soils. Future work will concentrate on the selective isolation and concentration of free cyclic lipids from soils to evaluate the presence of further degradation products.

Acknowledgments

We thank Drs. Henry Janzen and John Dormaar of Agriculture and Agri-Food Canada, Lethbridge,for providing the Brown and Dark Brown Chernozem plant and soil samples and Prof. William Kingery of the Department of Plant and Soil Science, Mississippi State University for performing carbon and nitrogen and analysis of the samples. We gratefully acknowledge support of this research from the Canadian Foundation for Climate and Atmospheric Research (GR-327). MJS thanks the Natural Science and Engineering Research Council (NSERC) of Canada for support via an University Faculty Award (UFA). We also thank three anonymous reviewers for their helpful comments which greatly improved the quality of this manuscript.

References

Albro P.W. 1976. Bacterial waxes. In: Kolattukudy P.E. (ed.), Chemistry and Biochemistry of Natural Waxes. Elsevier, Amsterdam, pp. 419–445.

Amblès A., Jambu P., Parlanti E., Joffre J. and Riffe C. 1994a. Incorporation of natural monoacids from plant residues into an hydromorphic forest podzol. Eur. J. Soil Sci. 45: 175–182.

Amblès A., Parlanti E., Jambu P., Mayoungou P. and Jacquesy J.-C. 1994b. *n*-Alkane oxidation in soil: formation of internal monoalkenes. Geoderma 64: 111–124.

Baker E.A. 1982. Chemistry and morphology of plant epicuticular waxes. In: Cutler D.F., Alvin K.L. and Price C.E. (eds), The Plant Cuticle. Linn. Soc. Symp. Ser. 10. Academic Press, London, pp. 139–165.

Barnes M.A. and Barnes W.C. 1983. Oxic and anoxic diagenesis of diterpenes in lacustrine sediments. In: Bjøroy M. (ed.), Organic Geochemistry 1981. Wiley, Chichester, New York, pp. 289–298.

Bianchi G. 1995. Plant waxes. In: Hamilton R.J. (ed.), Waxes: Chemistry, Molecular Biology and Functions. The Oily Press, Dundee, pp. 175–222.

Brooks C.J.W., Horning E.C. and Young J.S. 1968. Characterization of sterols by gas chromatography-mass spectrometry of the trimethylsilyl ethers. Lipids 3: 391–402.

Bridson J.N. 1985. Lipid fraction in forest litter: early stages of decomposition. Soil Biol. Biochem. 17: 285–290.

Bull I.D., Nott C.J., van Bergen P.F., Poulton P.R. and Evershed R.P. 2000a. Organic geochemical studies of soils from the Rothamsted classical experiments. VI. The occurrence and source of organic acids in an experimental grassland soil. Soil Biol. Biochem. 32: 1367–1376.

Bull I.D., van Bergen P.F., Poulton P.R. and Evershed R.P. 1998. Organic geochemical studies of soils from the Rothamsted classical experiments. II. Soils from the Hoosfield Spring Barley Experiment treated with different quantities of manure. Org. Geochem. 28: 11–16.

Bull I.D., van Bergen P.F., Nott C.J., Poulton P.R. and Evershed R.P. 2000b. Organic geochemical studies of soils from the Rothamsted classical experiments. V. The fate of lipids in different long-term experiments. Org. Geochem. 31: 389–408.

Chaffee A.L., Hoover D.S., Johns R.B. and Schweighardt F.K. 1986. Biological markers extractable from coal. In: Johns R.B. (ed.), Biological markers in the Sedimentary Record. Elsevier, Amsterdam, pp. 311–345.

Dinel H., Schnitzer M. and Mehuys G.R. 1990. Soil lipids: origin, nature, content, decomposition, and effect on soil physical properties. In: Bollag J.-M. and Stotzky G. (eds), Soil Biochemistry, Vol. 6. Marcel Dekker, New York, pp. 397–427.

Gobé V., Lemee L. and Amblès A. 2000. Structure elucidation of soil macromolecular lipids by preparative pyrolysis and thermochemolysis. Org. Geochem. 31: 409–419.

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.

Hamilton R.J. 1995. Analysis of waxes. In: Hamilton R.J. (ed.), Waxes: Chemistry, Molecular Biology and Functions. The Oily Press, Dundee, pp. 311–342.

Harwood J.L. and Russell N.J. 1984. Lipids in Plants and Microbes. George Allen and Unwin, London.

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.

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.

Hegnauer R. 1962. Chemotaxonomie der Pflanzen, Vol. 1. Birkhäuser, Basel.

Hegnauer R. 1992. Chemotaxonomie der Pflanzen, Vol. 7. Birkhäuser, Basel.

Howard P.J.A., Howard D.M. and Lowe L.E. 1998. Effects of tree species and soil physicochemical conditions on the nature of soil organic matter. Soil Biol. Biochem. 30: 285–297.

Jaffé R., Elisme T. and Cabrera A.C. 1996. Organic geochemistry of seasonally flooded rain forest soils: molecular composition and early diagenesis of lipid components. Org. Geochem. 25: 9–17.

Karrer W. 1958. Konstitution und Vorkommen der organischen Pflanzenstoffe. Birkhäuser, Basel.

Karrer W., Cherbuliez E. and Eugster C.H. 1977. Konstitution und Vorkommen der organischen Pflanzenstoffe. Ergänzungsband 1. Birkhäuser, Basel.

Killops S.D. and Frewin N.L. 1994. Triterpenoid diagenesis and cuticular preservation. Org. Geochem. 21: 1193–1209.

Killops S.D. and Killops V. 1993. An Introduction to Organic Geochemistry. Longman Scientific and Technical, London.

Knights B.A. 1967. Identification of plant sterols using combined GLC/mass spectrometry. J. Gas Chromatogr. 5: 273–282.

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. Annu. 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.

Laflamme R.E. and Hites R.A. 1978. The global distribution of polycyclic aromatic hydrocarbons in recent sediments. Geochim. Cosmochim. Acta 42: 289–303.
Lehtonen K., Haenninen K. and Ketola M. 2001. Structurally mound lipids in peat humic acids. Org. Geochem. 32: 33–43.
Mackenzie A.S., Brassell S.C., Eglinton G. and Maxwell J.R. 1982. Chemical fossils: the geological fate of steroids. Science 217: 491–504.
Noda M., Tanaka M., Seto Y., Aiba T. and Oku C. 1988. Occurrence of cholesterol as a major sterol component in leaf surface lipids. Lipids 23: 439–444.
Oros D.R., Mazurek M.A, Baham J.E. and Simoneit B.R.T. 2002. Organic tracers from wild fire residues in soils and rain/river wash-out. Water, Air, Soil Pollut. 137: 203–233.
Otto A. and Simoneit B.R.T. 2001. Chemosystematics and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeitz Formation, Saxony, Germany. Geochim. Cosmochim. Acta 65: 3505–3527.
Otto A., White J.D. and Simoneit B.R.T. 2002. Natural product terpenoids in Eocene and Miocene conifer fossils. Science 297: 1543–1544.
Otto A. and Wilde V. 2001. Sesqui-, di-, and triterpenoids as chemosystematic markers in Extant conifers – a review. Bot. Rev. 67: 141–238.
Owen R.W., Mason A.N. and Bilton R.F. 1985. The degradation of β-sitosterol by *Pseudomonas* sp. NCIB 10590 under aerobic conditions. J. Steroid Biochem. 23: 327–332.
Roshchin V.I., Poverinova O.Y., Raldugin V.A. and Pentegova V.A. 1986. Triterpenoid alcohols from *Populus tremula* leaves. Khim. Prir. Soedin. 1986: 516–517.
Ruzicka S., Edgerton D., Norman M. and Hill T. 2000. The utility of ergosterol as a bioindicator of fungi in temperate soils. Soil Biol. Biochem. 32: 989–1005.
Simoneit B.R.T. 1986. Cyclic terpenoids of the geosphere. In: Johns R.B. (ed.), Biological Markers in the Sedimentary Record. Elsevier, Amsterdam, pp. 43–99.
Simoneit B.R.T., Grimalt J.O., Wang T.G., Cox R.E., Hatcher P.G. and Nissenbaum A. 1986. Cyclic terpenoids of contemporary resinous plant detritus and of fossil woods, ambers and coal. Org. Geochem. 10: 877–889.
Soil Classification Working Group. 1998. The Canadian System of Soil Classification Agriculture and Agri-Food Canada. Publication 1646 (Revised), 187 pp.
Streibl M. and Herout V. 1969. Terpenoids – especially oxygenated mono-, sesqui-, di-, and triterpenoids. In: Eglinton F. and Murphy M.T.J. (eds), Organic Geochemistry Methods and Results. Springer, New York, pp. 401–424.
Tissot B.P. and Welte D.H. 1984. Petroleum Formation and Occurrence. Springer, Berlin.
Tulloch A.P. 1976a. Chemistry of waxes of vascular plants. In: Kolattukudy P.E. (ed.), Chemistry and Biochemistry of Natural Waxes. Elsevier, Amsterdam, pp. 235–287.
Tulloch A.P. 1976b. Epicuticular wax of *Agropyron smithii* leaves. Phytochemistry 15: 1153–1156.
Tulloch A.P. 1982. Epicuticular wax of *Eragrostis curvula*. Phytochem. 21: 661–664.
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 dacay. Org. Geochem. 29: 1779–1795.
Wakeham S.G., Schaffner C. and Giger W. 1980. Polycyclic aromatic hydrocarbons in recent lake sediments – II. Compounds derived from biogenic precursors during early diagenesis. Geochim. Cosmochim. Acta 44: 415–429.
Walton T.J. 1990. Waxes, cutin and suberin. Methods Plant Biochem. 4: 105–158.
Weete J.D. 1976. Algal and fungal waxes. In: Kolattukudy P.E. (ed.), Chemistry and Biochemistry of Natural Waxes. Elsevier, Amsterdam, pp. 349–418.
Ziegler F. 1989. Changes of lipid content and lipid composition in forest humus layers derived from Norway Spruce. Soil Biol. Biochem. 21: 237–243.