Lignin phenols and cutin- and suberin-derived aliphatic monomers as biomarkers for stand history, SOM source, and turnover

Sandra Spielvogel, Jörg Prietzel and Ingrid Kögel-Knabner

Lehrstuhl für Bodenkunde, Technische Universität München, 85350 Freising, Germany, Email spielvogel@wzw.tum.de

Abstract
Each tree species has a unique chemical composition, and also various tree tissues differ in their chemistry. Analysis of lignin phenols and cutin- and suberin-derived aliphatic monomers was employed to investigate wheter their composition can be traced back after decay and transformation into soil organic matter (SOM) to study SOM source, degradation, and stand history.

The composition of bound lipids and lignin compounds in leaves/needles and root material of different tree species and of grasses was analyzed using copper (II) oxide (CuO) oxidation, saponification and subsequent analysis by gas chromatography/mass spectrometry (GC/ MS). The aim was to examine the applicability of these compounds in soils and different density and particle size fractions as biomarkers for the respective tree species and the grass. In contrast to lignin, aliphatic molecules derived from suberins and cutins were preferentially preserved in horizons and soil fractions with mean residence times > 250 years. The pattern of cutin and suberin monomers in the soils and fractions changed with increasing ^{14}C age, but alteration of these aliphatic macromolecules resulted in less degradable structures which are still indicative for the respective plant species.

Key Words
Saponification, CuO oxidation, lipids, forest trees, gas chromatography/mass spectrometry.

Introduction
The composition of lignin components is different for angiosperm, gymnosperm and grass lignin. Leaf cutins and suberin found in barks and roots of different plants are known to exhibit plant specific chemical compositions (Goñi and Hedges 1990). Moreover, laboratory and field degradation studies indicated that degradation processes are not uniform for all lignin, suberin and cutin monomers, but that some constituents are preferentially degraded (Kögel-Knabner *et al.* 1989; Otto and Simpson 2006).
In the present study, the amount and composition of lignin, cutin and suberin in grassland and forest soils stocked with different tree species and selected density and particle size fractions were investigated. The objectives were to analyze, if (i) cutin and suberin monomers are useful biomarkers for the contribution of root-vs. shoot derived OC to SOM in different soil horizons and fractions, (ii) if monomer-specific turnover kinetics during decay and transformation into SOM hinders the identification of plant- and tissue-specific lignin, cutin and suberin signatures or offers additional information about the degradation status of the SOM and (iii) if lignin, cutin and suberin are useful biomarker for vegetation history.

Methods
Study sites, soils and soil sampling
We sampled nine sites at two different study areas in Germany: The National Park Bayerischer Wald, an area with granite, gneiss and quarternary deposits (mainly gneiss debris) parent material, and a pre alpine loess region. A more detailed description of the sites is given in Table 1. Soils were classified according to IUSS Working Group Reference Base 2006. At every site we sampled three randomly distributed soil pits and analyzed the samples from the replicates separately. At each site, leaves or needles, roots, bark and fresh litter were sampled. The forest floor and mineral topsoil horizons of all soil profiles and selected subsoil horizons were collected for chemical analyses.

Table 1. Parent material, soil types, recent and former vegetation of the nine study sites.

Region and Parent material	Soil type	Former vegetation	Recent vegetation
National Park Bayerischer Wald			
Granite	Leptic Entic Podzols (Skeletic)	Norway spruce	Norway spruce
Granite	Leptic Entic Podzols (Skeletic)	Norway spruce	Grass (approx. 25 yr.)
Quarternary deposits	Leptic Cambisols (Dystric, Skeletic)	Norway spruce	Norway spruce
Quarternary deposits	Leptic Cambisols (Dystric, Skeletic)	Norway spruce	Grass (approx. 25 yr.)
Quarternary deposits	Leptic Cambisols (Dystric, Skeletic)	European beech	European beech
Pre alpine loess region			
Loess	Cutanic Alisols (Humic, Siltic)	Norway spruce	Norway spruce
Loess	Cutanic Alisols (Humic, Siltic)	Norway spruce	European beech (approx. 80 yr.)
Loess	Cutanic Alisols (Humic, Siltic)	Norway spruce	Douglas fir (approx. 80 yr.)
Loess	Cutanic Alisols (Humic, Siltic)	Norway spruce	Sessile oak (approx. 80 yr.)

Density and particle size fractionation
Selected A and B horizons were subjected to a two-step density fractionation with Na polytungstate solution with a density of 1.6 g/cm^3 and subsequent particle size fractionation to obtain the free light fraction (fLF) and the occluded light fraction (oLF). After complete dispersion by ultrasonication (450 J/ml), the fractions 2 to 20 µm (silt), and <2 µm (clay) with their respective heavy fraction (HF) were obtained.

Determination of organic carbon (OC) concentration
The concentration of OC was determined for all ground plant samples, bulk soil samples, density, and particle-size separates in two replicates with an Elementar Vario EL analyzer by dry combustion at 950 °C. Since all soil samples were free of carbonate, the measured total C concentration was equivalent to the OC concentration.

Biomarker extraction and analysis
Previous to isolation of bound lipids, and lignin-derived phenols, solvent extraction was used to remove free lipids and other solvent extractable compounds. (Otto *et al.* 2005). Cutin- and suberin-derived monomers were extracted from the samples using a base hydrolysis method (Otto and Simpson 2006). Lignin phenols were extracted using CuO oxidation as described in Otto and Simpson (2006). Before analysis by Gas Chromatography/Mass Spectrometry (GC/MS), extracts were derivatized to convert compounds to trimethylsilyl derivatives (Otto and Simpson 2006).

Statistical analysis
We tested each lignin, cutin, and suberin monomer, and summarized substance groups and selected compound ratios for its suitability to classify cutins vs. suberins, or to differentiate among different tree species and grass or among fresh and highly degraded SOM. Therefore, the data were subjected to discriminant analyses. For this purpose, a set of cases with known group membership was used for each analysis as a training set in order to select the best discriminating variables. Subsequently, factor analysis was used to group variables with discriminant coefficients > 0.5 and similar information in order to reduce the amount of model variables.

Results

Cutin and suberin monomers as biomarkers for the contribution of aboveground vs. belowground OC input
The base hydrolysis of soil and vegetation samples yielded a series of aliphatic and phenolic compounds corresponding to previously reported compositions of hydrolysates from grassland and forest soils (Kögel-Knabner *et al.* 1989; Otto and Simpson 2007). The sources of some compounds, which are found in animal, plant, and fungal membranes are unspecific (Otto and Simpson 2007). Nevertheless, our statistical analysis identified eight variables which discriminated significantly between cutin and suberin based on their structural units (Table 2). The eight variables where subsequently subjected to a factor analysis which resulted in two factors with an eigenvalue >1 (Figure 1). High loadings for factor a are indicative for cutin/aboveground input, whereas high loadings for factor b are indicative for suberin/belowground input.

Table 2. Variables with discriminant coefficients > 0.5 for discrimination between cutin (aboveground plant input) and suberin (belowground plant input).

Variable	Discrimination coefficient	Occurrence in suberin	Occurrence in cutin
Σ hydrolysable phenols	0.63	Common	-
Σ n-alkan-1-ols	0.64	Common	-
Σ n-alkanoic acids	0.57	Common	-
Σ Long-chain ω-hydroxyalkanoic acids	0.77	Common	-
Σ Long-chain α,ω-diacids	0.63	Common	-
9,10-epoxy-C_{18} α,ω dioic acid	0.56	Common	Rare
Σ Mid-chain hydroxy C_{14}, C_{15}, C_{17} acids	0.77	-	Common
Σ C_{16} Mono- and dihydroxy acids and diacids	0.53	Rare	Common

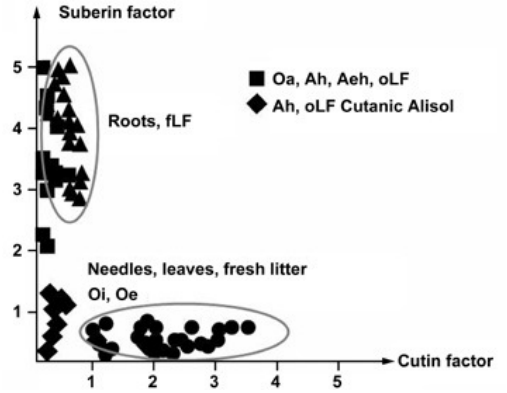

Figure 1. Factor space between factor a (grouping variables indicative for cutin) and factor b (grouping variables indicative for suberin).

Lignin, cutin and suberin monomers as biomarkers for SOC degradation status

Discrimination analysis identified seven variables which discriminated significantly between groups of samples with different degradation status of the OM (Table 3). Most of them are ratios, indicating a preferential degradation of some lignin, cutin and suberin constituents compared to other compounds. High discrimination coefficients of the ω-C_{16}/ΣC_{16} ratio and the ω-C_{18}/ΣC_{18} ratio are consistent with findings of Goñi and Hedges (1990), which reported that cutin acids containing double bonds or more than one hydroxyl group are preferentially degraded compared to ω-hydroxyacids in marine sediments. The ratio between mid-chain-substituted acids to total cutin and suberin acids (ΣMID/ΣSC) generally decreases with increasing degradation status of the SOM, from plant samples to the forest floor horizons.

Increasing lignin degradation is reflected by larger acid/aldehyde ratios for both vanillyl and syringyl units. The faster turnover of syringyl-type compared to vanillyl-type lignin is reflected by the lower discrimination coefficient of the Ac/Al_S ratio compared to the Ac/Al_V ratio.

Table 3. Variables with discriminant coefficients > 0.5 for discrimination between groups of samples with different degradation status

Variable	Discrimination coefficient	Change during degradation
ω-C_{18}/ΣC_{18}	0.76	Increases with degradation
ω-C_{16}/ΣC_{16}	0.72	Increases with degradation
ΣMID/ΣSC	0.64	Decreases with degradation
Ac/Al_V	0.82	Increases with degradation
Ac/Al_S	0.60	Increases with degradation
S/V	0.64	Decreases with degradation
9,10,18-trihydroxy octadecanoic acid	0.79	Increases with degradation

Lignin, cutin and suberin monomers as biomarkers for recent vegetation and vegetation history

Twelve variables were initially selected by discriminant analysis for the discrimination among the four different tree species and the grass vegetation. However, the first training set, which was applied to choose those variables which significantly contribute to a vegetation-specific signature, only consisted of fresh plant material, topsoil horizons and light fractions with recent ^{14}C ages. Then the twelve variables were subjected to a factor analysis which resulted in three factors with an eigenvalue >1 (Figure 2a). The factor space between factors a and b significantly differentiated between soil samples from sites which are stocked with

different angiosperm species, whereas the factor space between factors b and c differentiated between samples from sites with different gymnosperm species.

Subsequently, we tested the established factors and the discrimination coefficients of the twelve variables for a larger data set, including subsoil horizons and heavy fractions with mean residence times > 250 years. However, only 39% of all cases of this larger test set were classified correctly. Thus, we repeated the discriminant analysis for the larger data set. This time 19 variables were selected by discriminant analysis, including also degradation products of cutin and suberin monomers. Those 19 variables were again grouped into three factors with an eigenvalue >1 (Figure 2b). Two lignin variables (S/V ratio and C/V ratio) were selected by the discriminant analysis with the first data set. In contrast no lignin variables were selected by the discriminant analysis with the second data set including the subsoil horizons and old fractions. This is probably due to the fact that lignin compounds in the subsoil are strongly degraded and have lost source-specific functional groups.

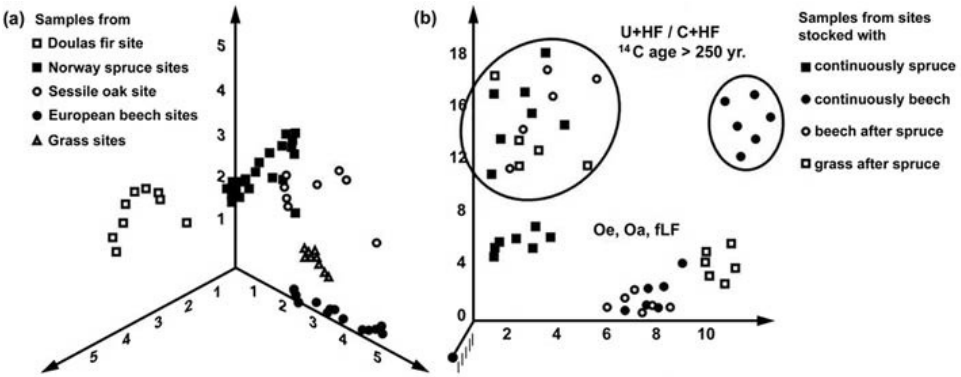

Figure 2. (a) Factor analysis with data set consisting of fresh plant material and soil samples with recent ^{14}C ages; (b) factor analysis with data set including soil samples and fractions with ^{14}C ages > 250 years.

Summary and Conclusion

The analyses of bound lipids and CuO oxidation products of fresh plant material, soils and different density and particle size fractions of sites stocked with Norway spruce, Douglas fir, European beech, Sessile oak or grasses showed that cutin and suberin are useful biomarkers to differentiate between root- vs. shoot input. In combination with lignin, cutin and suberin signatures of the soil and density/particle size fractions also provided important information about the degradation status of the SOM. Sites with different recent vegetation differed in their lignin, cutin and suberin signature of the young SOM and the light fraction; sites with similar former forest stands showed similar cutin and suberin signatures of old SOM and the heavy fraction. In contrast to cutin and suberin, lignin phenols were inappropriate as biomarkers for vegetation history. In summary, this study was able to prove that cutin and suberin are compounds with a high diagnostic value for root- vs. shoot-derived input, recent vegetation and vegetation history.

References

Goñi MA, Hedges JI (1990) The diagenetic behaviour of cutin acids in buried conifer needles and sediments from a coastal marine environment. *Geochimica and Cosmochimica Acta* **54,** 3083-3093.

IUSS Working Group WRB (2006) World reference base for soil resources 2006. World Soil Resources Reports 103. FAO, Rome.

Kögel-Knabner I, Ziegler F, Riederer M, Zech W (1989) Distribution and decomposition pattern of cutin and suberin in forest soils. *Zeitschrift für Pflanzenernährung und Bodenkunde* **152,** 409-413.

Otto A, Simpson MJ (2006) Sources and composition of hydrolysable aliphatic lipids and phenols in soils from western Canada. *Organic Geochemistry* **37,** 385-407.

Otto A, Simpson MJ (2007) Analysis of soil organic matter biomarkers by sequential chemical degradation and gas chromatography – mass spectrometry. *Journal of Separation Science* **30,** 272-282.

Otto A, Shunthirasingham C, Simpson MJ (2005) A comparison of plant and microbial biomarkers in grassland soils from the Prairie Ecozone of Canada. *Organic Geochemistry* **36,** 425-448.