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Changes in the molecular composition of ester-bound aliphatics with depth in an acid andic forest soil

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Abstract

Changes in the molecular composition of ester-linked aliphatic compounds with depth in an acid andic forest soil are studied. Thermally assisted hydrolysis and methylation (THM) using tetramethylammonium hydroxide in combination with gas chromatography/mass spectrometry revealed a dominance of cutin over suberin-derived THM products in the top 5 cm. From 5 to 20 cm, a strong increase in the contribution of suberin-derived THM products was observed. From 20–50 cm, suberin-derived C_{22}, C_{24} (and C_{26}) ω-hydroxy acids were found to decrease relatively strongly with depth compared with other suberin building-blocks. Their decrease with depth in the ester-bound fraction is 'reversely' linked with a strong increase of these compounds in the free extractable lipid fraction which would justify their use as main indicators of a root-derived input to the free extractable lipid fraction. In addition, their relatively strong decrease in the ester-bound fraction suggests that these compounds are more easily released from the suberin structure in soils than other ester-bound aliphatic building-blocks.

Keywords: Suberin; Andisol; (Bio)degradation; Pyrolysis

1. Introduction

Despite the relatively high amounts of soil organic matter (SOM) associated with andic soils (Shoji et al., 1993), studies on the (molecular) composition of SOM in these soils are scarce (Bartoli et al., 2003). Molecular studies enable research to focus on a specific fraction of SOM. Through molecular SOM studies, andic soil processes such as transportation and degradation of SOM can be studied in detail thereby contributing to our understanding of soil processes in andic soils.

Three main sources of plant-derived aliphatic compounds (i.e. $(R–CH_2)_x$, where R is a functional group such as a carboxylic group) have been

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distinguished in soils: (a) free (extractable) lipids, (b) the biopolyesters cutin and suberin and (c) non-hydrolyzable biopolymers, such as cutan and suberan (Nip et al., 1986; Tegelaar et al., 1989, 1995; Augris et al., 1998; Nierop, 1998). In an earlier study, it was shown that aliphatic lipids (nC_6~nC_{32}) linked by ester bonds account for approximately 2.5 wt.% of the TOC in the acid andic forest soil studied (Naafs and van Bergen, 2002). Nierop et al. (2003) found that in acid sandy soils, ester-linked lipids may even account for up to 12.6 wt.% of the TOC. Moreover, it appeared that the contribution of ester-linked aliphatics (e.g. carboxylic acids and ω-hydroxy acids) increases with decreasing soil pH (Nierop et al., 2003).

Through (microbial) hydrolysis, ester-linked aliphatics are released after which they are quickly degraded or become part of the free extractable aliphatic fraction in soils. To date, mainly "refractory" ω-hydroxy acids (and α, ω-dicarboxylic acids) observed in the free extractable lipid fraction have been ascribed to suberin as a predominant source (Bull et al., 2000).

ω-Hydroxy acids have been suggested to be more easily released from the biopolyester structure than other ester-linked aliphatic building-blocks upon base hydrolysis as well as decomposition in soil (Lopes et al., 2000; Nierop et al., 2003). They may form a more accessible part of the biopolyester (Nierop et al., 2003). In addition, di- and tri-hydroxy-carboxylic acids have been suggested to be less susceptible to hydrolysis as being part of the core of the biopolyester structure (Nierop et al., 2003).

During an earlier study, free extractable C_{22}, C_{24} and C_{26} ω-hydroxy acids were observed to increase significantly from the litter layer to a depth of 40 cm (Naafs et al., 2004). In this paper, changes in the molecular composition of ester-linked aliphatics with depth in the same acid andic forest soil profile are studied. In short, soil samples were sieved (<250 μm) to remove roots and remaining basaltic fragments. The sieved soil samples were then Soxhlet extracted to remove free extractable lipids (Naafs et al., 2004) and the residues were subsequently analysed using thermally assisted hydrolysis and methylation (THM) using tetramethylammonium hydroxide (TMAH) in combination with gas chromatography/mass spectrometry (GC/MS). This study will add new information to further decrease the distinct lack of information about soil organic matter chemistry in volcanic soils as recently noticed (Bartoli et al., 2003). In addition, the data obtained provide valuable molecular information on the degradation of suberin (and cutin) in soils.

2. Materials and methods

2.1. Soil profile

Madeira Island (Portugal) is located in a fully (Atlantic) oceanic domain between 32°38′ and 32°52′N and 16°39′ and 17°16′W, approximately 600 km from the African coast, at the same latitude as Casablanca (Morocco). As part of a larger study, soil samples were taken from three andic profiles located on this island that have been classified as Umbric Andisols (FAO, 1998; Madeira et al., 1994). In this paper, data are shown for one of the profiles in which changes in the free extractable lipid fraction were extensively studied previously (Naafs et al., 2004).

The profile studied is situated on a hill (slope 30°W) near the village of Poiso (altitude 1175 m). The vegetation consisted mainly of grasses (55%), ferns (20%), deciduous trees, i.e., oak and birch (20%), and some mosses (5%). Tree roots and some grass roots were found to a depth of about 80 cm, but most of the intensive "root-activity" was found in the top 1–40 cm consisting mainly of grass fibrils. Three horizons were distinguished; a thin O-horizon (0–2 cm), an A-horizon (2–73 cm) and an E/B-horizon (73–90 cm), the latter being formed by weathered basalt bedrock material. The deeper layer, i.e. C-horizon, was formed by a non-friable, compact layer of weathered basalt, the parent material for this Andisol (Madeira et al., 1994) and therefore not sampled. The O-horizon consisted mainly of litter and vegetation, i.e. grass, moss, twigs, leaves, etc. The A-horizon (colour 4/6 5YR) was characterized by a non-friable structure, clay aggregates with a diameter up to 1 cm and highly weathered basalt fragments up to a diameter of 10 cm. Many roots were found in this horizon, ranging from mainly very fine grass roots in the top 30 cm to coarser roots, up to a diameter of 2 cm at 30–70 cm.

Four samples of approximately 200 g were taken from each depth interval sampled, i.e. 1–5 cm and from 10–50 cm at intervals of 10 cm each for the

andic profile that was focussed on. The top sample is reported to have been taken from a depth of 1–5 cm instead of 2–5 cm to indicate that material from the very thin O-horizon (0–2 cm) may have contributed to the top sample. Due to slight variations in the depth of the O–A-horizon 'boundary', taking a top sample without any O-material is naturally impossible. Directly after sampling, samples from the same horizons were homogenized and subsequently further prepared for analyses (see next section).

2.2. Sample pre-treatment, total organic carbon measurement and solvent extraction

Soil samples were air dried, wrapped in aluminium foil and subsequently oven dried at 60 °C and sieved through a 2 mm and a 250 μm sieve to remove roots and basalt fragments. Root fragments remaining after sieving were removed using a pincet. The pH (H_2O) of the soil was measured in the supernatant suspension of a 1:2.5 (v/v) sieved (<250 μm) sample to water ratio using a Scott Geräte pH meter CG 805. Total organic carbon contents (TOC%) of the dried samples were measured using a Fisons Instruments NA 1500 NCS analyzer, with a cycle time of 180 s, a source temperature of 190 °C and an oxygen flow of ca. 30 l/min. Approximately 15–25 g of the total dried and sieved (<250 μm) sample was Soxhlet extracted to remove extractable lipids using dichloromethane/methanol (DCM/MeOH) (9:1 v/v) for 24 h (for details and analysis of the free extractable lipid fraction see Naafs et al., 2004). The residue was air dried and used for further analyses.

2.3. Thermally assisted hydrolysis and methylation (THM)

Thermally assisted hydrolysis and methylation (THM) analyses were performed by adding a droplet of a 25% solution of tetramethylammonium hydroxide (TMAH) in water to a few milligrams of Soxhlet extracted residue that was pressed onto a ferromagnetic wire with a Curie temperature of 600 °C. The 'wet' sample was dried using a 100 W halogen lamp and subsequently manually inserted into a Horizon Instruments Curie-Point pyrolyser. Samples were heated for 5 s at 600 °C. The pyrolysis unit was connected to a ThermoQuest Trace GC 2000 gas chromatograph. Products were separated by a fused silica column (J and W 30 m×0.32 mm i.d., film thickness 0.25 μm) coated with DB-5. Helium was used as carrier gas at a flow rate of 1.4 ml/min. The GC oven temperature was programmed from 40 °C (1 min) to 320 °C (isothermal for 15 min) at a rate of 7 °C min^{-1}. The GC column was coupled to a Finnigan Trace MS mass spectrometer operating at 70 eV, scanning the range *m/z* 60–600 with a cycling time of 1 s. Identification of the compounds was carried out using their mass spectral data (Table 1) and by interpretation of the spectra and the GC retention times.

Table 1
Characteristic mass fragments of methylated compounds identified in THM total ion current

Compound	Characteristic mass fragments (*m/z*)
ω-Hydroxy alkanoic acids	74, 87, 98, 111, 143, $[M-64]^+$, $[M-47]^+$, $[M-32]^+$, $[M-15]^+$
ω-Hydroxy alkenoic acids	55, 67, 81, 95, 109, 121, 137, $[M-64]^+$, $[M-47]^+$, $[M-32]^+$
C_{16} (9, ω)-Dihydroxy alkanoic acid	71, 109, 173, 201, $[M-32]^+$, $[M-15]^+$
C_{18} (10, ω)-Dihydroxy alkanoic acid	71, 109, 173, 215, $[M-32]^+$, $[M-15]^+$
(9, 10, ω)-Trihydroxy alkanoic acid	71, 81, 187, 201
Unknown (sugar?)	71, 75, 101, 111, 187, 185, 275, 307

3. Results and discussion

Total organic contents decrease from 7.9 wt.% in the top- soil (1–5 cm) to 5.6 wt.% at a depth of 40–50 cm. Soil pH (H_2O) values are all acidic and vary only little between 4.2 and 4.0. Although saponification is a more sensitive method to obtain ester-linked compounds (both aliphatic and/or aromatic) from soils (Naafs and van Bergen, 2002), THM was used in this study as a relatively fast method to get an overview of the main ester-linked compounds. Highly comparable results were obtained in this study using THM (Fig. 1) and an earlier study using saponification of the andic A-horizon from the same profile (Naafs and van Bergen, 2002). Both THM and saponification are therefore considered to yield representative results on ester-linked aliphatic compounds after solvent extraction. However, THM data

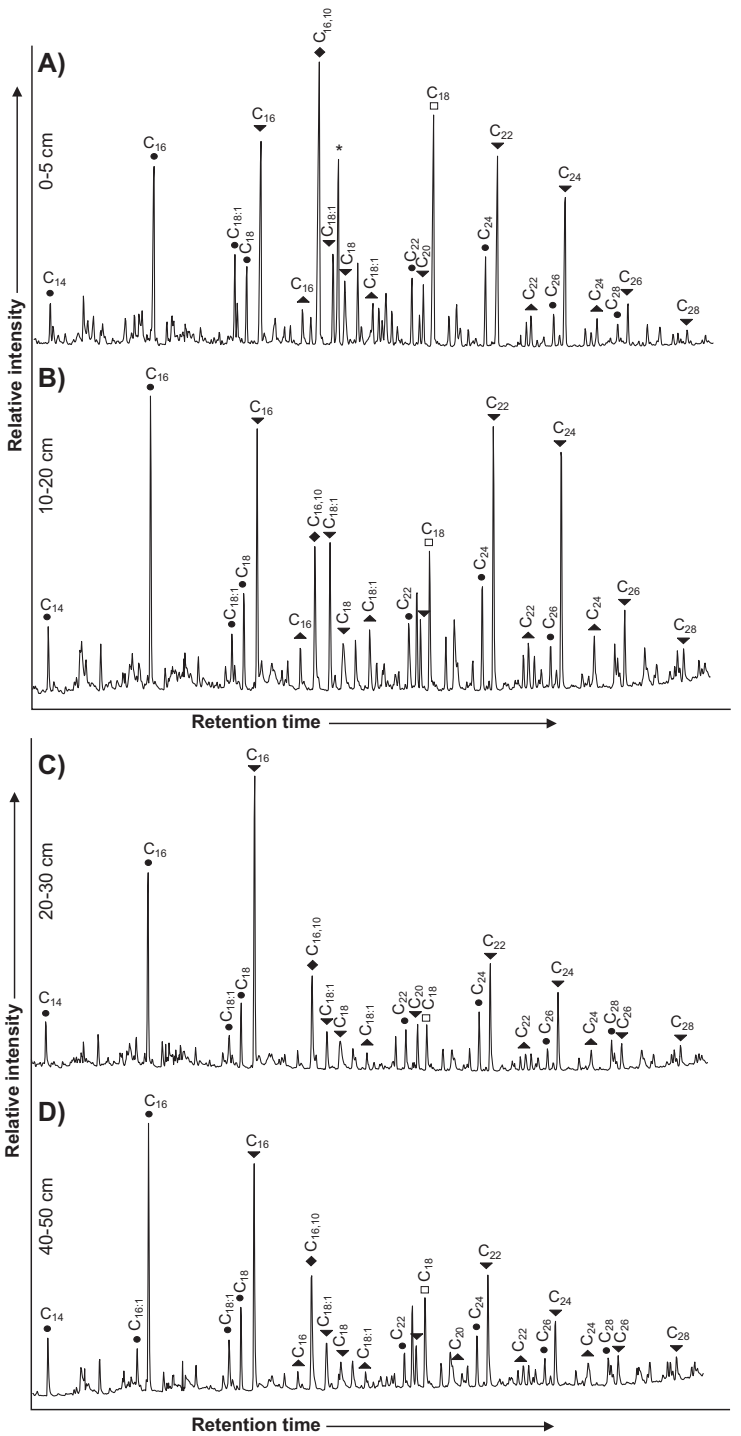

Fig. 1. Partial total ion current of THM products of residues after Soxhlet extraction at depths of: (A) 1–5 cm, (B) 10–20 cm, (C) 20–30 cm and (D) 40–50 cm. Key: ●: *n*-alkanoic acids, ▼: ω-hydroxy acids, ▲: Diacids, ◆: dihydroxy acids, □: trihydroxy acids, *: unknown. C_x above the peaks refers to the total number of carbon atoms. Number after the comma refers to the position of the hydroxy group. Number after the colon refers to the total number of double bonds. Lignin derived THM products eluting prior to the compounds here are not shown.

are less detailed and show lignin-derived compounds (not shown) in addition to aliphatic compounds. All samples analysed by THM show a series of even numbered saturated ω-hydroxy acids ranging from C_{16} to C_{28}, accompanied by C_{18} ω-hydroxyalkenoic acid. In addition, co-eluting C_{16} 10,16-dihydroxy- and C_{16} 9,16-dihydroxy acid (not separately indicated in Fig. 1), C_{18} 9,10,18-trihydroxy acid and series of C_{16} to C_{24} α, ω-alkanedioic acids and C_{14} to C_{28} *n*-alkanoic acids have been identified, both series including unsaturated compounds (see Table 1 for characteristic mass fragments). The sample taken from a depth of 1–5 cm showed a THM product that is classified as unknown but is most likely a sugar (see Table 1 for mass spectral data). Phenolic compounds derived from lignins elute prior to the aliphatic compounds studied and have been left out of consideration. The origin of ester-linked compounds identified in the sub-soil, i.e. 15–50 cm, is discussed in detail in Naafs and van Bergen (2002).

3.1. Cutin vs. suberin input

Cutin is present in cuticles covering all aerial parts of higher plants, while suberin forms both protective and wound-healing layers in barks, woody stems and underground parts, i.e. roots (Kolattukudy, 1980, 1984; Walton, 1990). Comparative studies revealed that cutin and the aliphatic domain of suberin are closely related chemically, since both consist mainly of hydroxy- and epoxy-substituted alkanoic acids (Kolattukudy, 1980; Holloway, 1982, 1983; Walton, 1990; Matzke and Riederer, 1991; Riederer et al., 1993). However, there are a number of differences in their aliphatic monomeric and tertiary structure (Tegelaar et al., 1995). Dihydroxyhexadecanoic acids (i.e. C_{16} family, Kolattukudy, 1980) and 9,10-epoxy-18-hydroxy acids (i.e. C_{18} family, Kolattukudy, 1980) have been identified as major components of plant cutins (Kolattukudy, 1980; Holloway, 1982; Ryser and Holloway, 1985; Matzke and Riederer, 1991). The aliphatic domain (Bernards et al., 1995; Bernards and Lewis, 1998) of most suberins on the contrary is dominated by longer-chain ($>C_{20}$) α, ω-dioic acids and ω-hydroxy acids (Kolattukudy, 1980; Ryser and Holloway, 1985; Matzke and Riederer, 1991; Riederer et al., 1993) with only small amounts of the (ω, 9)-dihydroxyalkanoic acids and (ω, 10)-dihydroxyalkanoic acids, except for some angiosperms (Holloway, 1983). Taking these differences into account, a dominance of cutin- over suberin-derived THM products is clearly reflected in the top-sample (1–5 cm; Fig. 1A) by the dominance of C_{16} 9,16- and C_{16} 10, 16-dihydroxy acid (and C_{18} 9,10,18-trihydroxy acid). The significant dominance of cutin over suberin THM products decreases, however, with depth (Fig. 1B–D). The sub-soil samples (10–50 cm) are dominated by suberin-related, i.e. root (remnants)-derived, THM products reflected by the presence of long-chain ($>C_{20}$) ω-hydroxy acids (and α, ω-alkanedioic acids).

In addition to a change in the cutin/suberin THM product ratio with depth, a change in vegetation input with depth had been observed to cause similar changes in the composition of ester-linked aliphatics with depth (Nierop, 2001). An increase in the input by grass roots over that of tree roots could therefore also cause a relative dominance of C_{16} ω-hydroxy acid over C_{22} and C_{24} hydroxy acids (Nierop, 2001). However, considering the dominance of grasses over trees at the sample site, a diagenetic change in the cutin/suberin ratio is a more likely explanation for the changes observed.

3.2. Changes in molecular composition of root-derived ester-linked lipids with depth

Absolute amounts of pyrolysis may vary simply due to differences in the amount of sample pyrolysed. Still, duplicate pyrolysis-GC-MS analyses of homogeneous soil samples produce data that are highly comparable in terms of relative concentration of pyrolysis products. Therefore, relative concentrations, i.e. ratios, are used in this paper to study changes in molecular composition of ester-linked compounds with depth.

Root material (i.e. suberin) has been considered as a predominant source of aliphatic ω-hydroxy acids in the free extractable lipid fraction in soils (Bull et al., 2000). A (slight) increase in suberin-derived C_{22}, C_{24} (and C_{26}) ω-hydroxy acids vs. cutin-derived C_{16} 9/10-dihydroxy acid was observed from 5 to 20 cm (Fig. 2) related to an increase in the suberin vs. cutin input (see 3.1). However, this trend is not continued through the profile (Fig. 2). From 20–50 cm a relative decrease in the ratios of suberin-derived C_{22}, C_{24} (and C_{26}) ω-hydroxy acids vs. the cutin derived C_{16} 9/10-

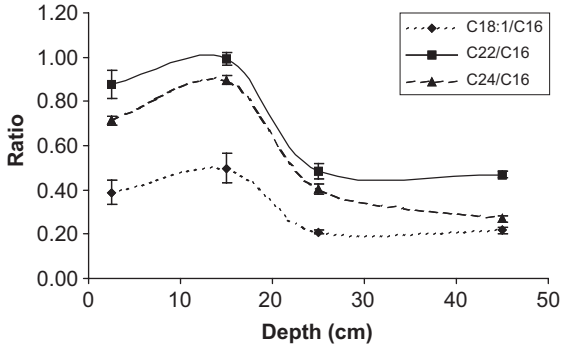

Fig. 2. Intensity ratios of suberin- over cutin-derived THM products vs. depth. Key: C_{22}/C_{16}: C_{22} ω-hydroxy acid/C_{16} 9/10, 16-dihydroxy acid, C_{24}/C_{16}: C_{24} ω-hydroxy acid/C_{16} 9/10, 16-dihydroxy acid, $C_{18:1}/C_{16}$: $C_{18:1}$ ω-hydroxy acid/C_{16} 9/10, 16-dihydroxy acid. I: cutin- over suberin-derived THM product dominance, II: increase in suberin-derived input, III: hydrolysis of $>C_{20}$ ω-hydroxy acids from suberin.

dihydroxy acid is observed (Fig. 2). Directly associated with this decrease, C_{22}, C_{24} (and C_{26}) ω-hydroxy acids were shown to increase with depth (0–40 cm) in the free extractable lipid fraction (Naafs et al., 2004). Assuming an increase in the hydrolysis of biopolyesters with depth and a stable, or even more likely, decreasing cutin input, these data indicate a decrease of C_{22}, C_{24} (and C_{26}) ω-hydroxy acids in the ester-linked suberin fraction from 20–50 cm. Such a decrease in the ester-linked suberin fraction coupled to the increase in the free lipid fraction indicates a 'reverse' link between the occurrence of free extractable- and ester-linked C_{22}, C_{24} (and C_{26}) ω-hydroxy acids. Moreover, such a direct link would justify their use as main indicators of a root-derived input to SOM in the free extractable lipid fraction (Bull et al., 2000; Naafs et al., 2004).

Another rather significant feature is the decrease in relative concentration from 20–50 cm of the ω-hydroxyoctadecenoic acid (Fig. 1). Nierop et al. (2003) showed that this compound is a major building block of oak root suberins. In soils, this compound is quickly degraded (Nierop et al., 2003) as also revealed by its absence in both the free and ester-linked fraction. The only compound observed in the free lipid fraction that might reflect a contribution from hydrolysed $C_{18:1}$ ω-hydroxy acids is C_9 α,ω-alkanedioic acid (Naafs et al., 2004). Within the andic profile, the ester-linked ω-$C_{18:1}$ (hydroxy-) acid is more quickly hydrolysed than most other suberin building blocks as revealed by its relatively strong decrease with depth (Fig. 1). Together with the relatively strong decrease in the ester-linked fraction of C_{22}, C_{24} ω-hydroxy acids and, to a lesser extent, C_{18}–C_{28} α, ω-dioic acids, this again suggests that these long-chain ($>C_{16}$) ω-hydroxy and α, ω-dioic acids are more easily released from the biopolyester structure than other ester-linked aliphatic building-blocks (Lopes et al., 2000; Nierop et al., 2003), including C_{16} ω-hydroxy acid (Fig. 1). They may form a more accessible part of the biopolyester, while di- and tri-hydroxy functionalised acids, as well as C_{16} ω-hydroxy acid (Ray and Stark, 1998), are less susceptible to hydrolysis as being part of the core of the biopolyester structure as suggested earlier (Nierop et al., 2003).

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References

Augris, N., Balesdent, J., Mariotti, A., Derenne, S., Largeau, C., 1998. Structure and origin of insoluble and non-hydrolyzable, aliphatic organic matter in a forest soil. Org. Geochem. 28, 119–124.

Bartoli, F., Buurman, P., Delvaux, B., Madeira, M., 2003. Volcanic soils: properties and processes as a function of soil genesis and land use. Geoderma 117, 183–184.

Bernards, M.A., Lewis, N.G., 1998. The macromolecular aromatic domain in suberized tissue; a changing paradigm. Phytochemistry 47, 915–933.

Bernards, M.A., Lopez, M.L., Zajicek, J., Lewis, N.G., 1995. Hydroxycinnamic acid-derived polymers constitute the polyaromatic domain of suberin. J. Biol. Chem. 270, 7382–7386.

Bull, I.D., Nott, C.J., van Bergen, P.F., Poulton, P.R., Evershed, R.P., 2000. 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.

FAO, 1998. World Reference Base for Soil Resources. World Soil Resources Report 84. Food and Agriculture Organisation of the United Nations, Rome.

Holloway, P.J., 1982. The chemical constitution of plant cutins. In: Cutler, D.F., Alvin, K.L., Price, C.E. (Eds.), The Plant Cuticle, Linnean Society Symposium Series, vol. 10. Academic Press, London, pp. 45–85.

Holloway, P.J., 1983. Some variations in the composition of suberin from the cork layers of higher plants. Phytochemistry 22, 495–502.

Kolattukudy, P.E., 1980. Biopolyester membranes of plants: cutin and suberin. Science 208, 990–1000.

Kolattukudy, P.E., 1984. Biochemistry and function of cutin and suberin. Can. J. Bot. 62, 2918–2933.

Lopes, M.H., Gil, A.M., Silvestre, A.J.D., Pascoal Neto, C., 2000. Composition of suberin extracted upon gradual alkaline methanolysis of *Quercus suber* L. cork. J. Agric. Food Chem. 48, 383–391.

Madeira, M., Furtado, A., Jeanroy, E., Herbillon, A.J., 1994. Andisols of Madeira Island (Portugal). Characteristics and classification. Geoderma 62, 363–383.

Matzke, K., Riederer, M., 1991. A comparative study into the chemical constitution of cutins and suberins from *Picea abies* (L.) Karst., *Quercus robur* L., and *Fagus sylvatica* L. Planta 185, 233–245.

Naafs, D.F.W., van Bergen, P.F., 2002. A qualitative study on the chemical composition of ester-bound moieties in an acidic andosolic forest soil. Org. Geochem. 33, 189–199.

Naafs, D.F.W., van Bergen, P.F., Boogert, S.J., de Leeuw, J.W., 2004. Variations with depth and season; solvent extractable lipids in an acid andic forest soil. Soil Biol. Biochem. 36, 297–308.

Nierop, K.G.J., 1998. Origin of aliphatic compounds in a forest soil. Org. Geochem. 29, 1009–1016.

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

Nierop, K.G.J., Naafs, D.F.W., Verstraten, J.M., 2003. Occurrence and distribution of ester-bound lipids in Dutch coastal dune soils along a pH gradient. Org. Geochem. 34, 719–729.

Nip, M., Tegelaar, E.W., Brinkhuis, H., de Leeuw, J.W., Schenk, P.A., Holloway, P.J., 1986. Analysis of modern and fossil plant cuticles by Curie point Py-GC and Curie point Py-GC-MS: recognition of a new, highly aliphatic and resistant biopolymer. Org. Geochem. 10, 769–778.

Ray, A.K., Stark, R.E., 1998. Isolation and molecular structure of an oligomer produced enzymatically from the cuticle of lime fruit. Phytochemistry 48, 1313–1320.

Riederer, M., Matzke, K., Ziegler, F., Kögel-Knabner, I., 1993. Occurrence, distribution and fate of the lipid plant biopolymers cutin and suberin in temperate forest soils. Org. Geochem. 20, 1063–1076.

Ryser, U., Holloway, P.J., 1985. Ultrastructure and chemistry of soluble and polymeric lipids in cell walls from seed coats and fibres of *Gossypium* species. Planta 163, 151–163.

Shoji, S., Nanzyo, M., Dahlgren, R.A., 1993. Volcanic Ash Soils. Genesis, Properties and Utilization. Elsevier, Amsterdam.

Tegelaar, E.W., de Leeuw, J.W., Saiz-Jiminez, C., 1989. Possible origin of aliphatic moieties in humic substances. Sci. Total Environ. 81/82, 1–17.

Tegelaar, E.W., Hollman, G., Vegt van der, P., de Leeuw, J.W., Holloway, P.J., 1995. Chemical characterisation of the periderm tissue of some angiosperm species: recognition of an insoluble, non-hydrolyzable, aliphatic biopolymer (Suberan). Org. Geochem. 23, 239–250.

Walton, T.J., 1990. Waxes, cutin and suberin. In: Harwood, J.L., Bowyer, J.R. (Eds.), Methods in Plant Biochemistry. Academic Press, London, pp. 105–158.