Available online at www.sciencedirect.com
SCIENCE DIRECT®
Applied Geochemistry 21 (2006) 1006–1015
Applied Geochemistry
www.elsevier.com/locate/apgeochem

The applicability of accelerated solvent extraction (ASE) to extract lipid biomarkers from soils

Boris Jansen [a,*], Klaas G.J. Nierop [a], Marcel C. Kotte [b], Pim de Voogt [a], Jacobus M. Verstraten [a]

[a] *IBED-Earth Surface Processes and Materials, Universiteit van Amsterdam, Nieuwe Achtergracht 166, NL-1018WV Amsterdam, The Netherlands*
[b] *RIZA Institute for Inland Water Management and Waste Water Treatment, P.O. Box 17, NL-8200 AA Lelystad, The Netherlands*

Received 3 August 2005; accepted 18 February 2006
Editorial handling by R. Jaffe
Available online 6 May 2006

Abstract

We investigated the ability of accelerated solvent extraction (ASE) to extract selected lipid biomarkers (C_{19}–C_{34} *n*-alkanes, *n*-alcohols and *n*-fatty acids as well as dehydroabietic acid and β-sitosterol) from a sandy soil profile under Corsican pine. Two organic layers (moss and F1) as well as two mineral soil horizons (EA and C1) were sampled and extracted with DCM/MeOH (93:7 v/v) by ASE at 75 °C and a pressure of 6.9×10^6 Pa or 17×10^6 Pa. Soxhlet extractions were used as the established reference method. After clean-up and derivatization with BSTFA, the extracts were analyzed on GC/MS.

Using Soxhlet as a reference, we found ASE to extract all compounds adequately. The *n*-alkanes, especially, were found to be extracted very efficiently from all horizons studied. Only the *n*-fatty acids and β-sitosterol from the organic layers seemed to be extracted at a slightly lower efficiency by ASE. In all but two instances the relative abundance of extracted lipids within a component class was the same regardless of the extraction method used.

Using a higher pressure in the ASE extractions significantly increased the extraction efficiency for all component classes in the moss layer, except β-sitosterol. The effect was most pronounced for the *n*-alkanes. In the EA horizon, a higher pressure slightly reduced the extraction efficiency for dehydroabietic acid. The observed differences between ASE and Soxhlet extractions as well as the pressure effect can be explained by a decrease in polarity of the extractant due to the elevated pressure and temperature applied during ASE extractions as compared to Soxhlet extractions. This would mainly increase the extraction efficiency of the least polar biomarkers: the *n*-alkanes as was observed. In addition, a better penetration of still partially water-filled micro pores under elevated pressure and temperature may have played a role.

1. Introduction

Organic matter in soils consists of a wide range of chemical components that originate predominantly from plant litter and the microbial biomass (Kögel-Knabner, 2002). The last decade has seen an increasing scientific interest in the organic chemical composition of soil organic matter (SOM) and changes therein upon soil biogeochemical processes (e.g., Gregorich et al., 1996; Guggenberger and Zech, 1999; Kögel-Knabner, 2002; Nierop et al.,

* Corresponding author.
E-mail address: bjansen@science.uva.nl (B. Jansen).

0883-2927/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.apgeochem.2006.02.021

2001; Wiesenberg et al., 2004b). Extractable lipids constitute a class of organic components in soils that has received particular attention. Reasons for this are amongst others the role of lipids in SOM accumulation and SOM stability (e.g., Naafs et al., 2004b; Poulenard et al., 2004; Rumpel et al., 2004), their significance for terrestrial food-web studies (e.g., Balser et al., 2005; Ruess et al., 2005), their influence on the fate of contaminants in soils (e.g., Chilom et al., 2005) and their use as vegetation tracers (e.g., Ficken et al., 1998; Van Bergen et al., 1997).

The use of lipids as vegetation tracers is based on the principle that plant-specific combinations of lipids are preserved in the soil and can serve as biomarkers to identify past vegetation compositions. Following our previous investigation of organic matter in a Dutch sandy soil under Corsican pine (Nierop and Verstraten, 2004), we considered applying this so-called biomarker technique to determine its past vegetation composition. However, we were faced with the challenge of isolating the selected lipid biomarkers from the soil matrix prior to analysis, as there is no unequivocal technique for this purpose. Instead, a wide range of extraction procedures are applied in contemporary practice, including Soxhlet extraction (e.g., Naafs et al., 2004a; Winkler et al., 2005), sonication (e.g., Dalton et al., 2005; Otto et al., 2005) and even simple shaking with solvent (Quenea et al., 2004). Soxhlet extraction has been used for the purpose of extracting lipids from soils for over 25 years (e.g., Jambu et al., 1978) and forms the basis of EPA method 3540C for the extraction of non-volatile organics from solids such as soils (EPA, 1996). As such, it is the most well-established of the methods mentioned.

While being a robust and well-established technique, Soxhlet extraction suffers from three main shortcomings: (i) the necessity of using relatively large extractant volumes of usually 250 mL or more; (ii) long analysis times of typically 16 h per analysis; and (iii) a difficulty to automate. A promising alternative is the relatively new technique of accelerated solvent extraction (ASE) (Richter et al., 1996). In short, ASE extracts samples under elevated temperature, while elevated pressure ensures that volatile extractants remain liquid. ASE can be completely automated, it employs very small extractant volumes (normally 5–30 mL) and has typical extraction times of less than an hour (Richter et al., 1996). As such the technique has the potential to overcome the main shortcoming of Soxhlet extractions. However, while the use of ASE to extract organic contaminants from soils is now reasonably well-established (Giergielewicz-Mozajska et al., 2001), its application to the extraction of soil lipids has received very little attention so far. To our knowledge only two studies have been published to date in which ASE was used to extract lipids from soil samples (Rumpel et al., 2004; Wiesenberg et al., 2004a), and no comparison with other techniques for this purpose has yet been made. Still, such a comparison of ASE with more established techniques is crucial if ASE is to be used in biomarker studies. The reason is that differences in extraction efficiencies for various types of lipids between ASE and other techniques would lead to a difference in the composition of the biomarker signal that is obtained.

Therefore, the purpose of the current study was to examine the efficiency of ASE to extract typical lipid biomarkers from a selection of soil horizons from a Dutch sandy soil under Corsican pine, using Soxhlet extractions as a reference. The biomarkers consisted of a selection from the following component classes: (i) straight-chain lipids; (ii) plant sterols; and (iii) terpenoids.

2. Materials and methods

2.1. Description of site, soil profile and sampling procedures

The selected sampling site is a plot in 'De Schoorlse Duinen', a sand-dune area in The Netherlands near the village of Schoorl. The current vegetation on the study plot consists almost exclusively of Corsican Pine (*Pinus nigra* var. *maritima*) that was planted in 1929 (Nierop and Verstraten, 2004). The only undergrowth present in significant quantities is a moss layer situated between the litter (L) horizon and the F1 horizon. The soil was classified as a Haplic Arenosol (FAO-UNESCO, 1990) and a description of the profile as well as an elemental analysis is provided in Table 1.

From the soil profile we selected 4 horizons for sampling: two from the organic layers (moss layer and F1) and two from the mineral soil (EA and C1). Upon sampling, all samples were air-dried at 30 °C for 24 h. Subsequently, the samples from the organic layers were ground and sieved over 1.0 mm, while the mineral soil samples were sieved over 1.0 mm without prior grinding.

Table 1
Description of the soil profile in De Schoorlse Duinen from which the soil samples (bold) were taken for extraction

Soil horizon	Depth (cm)	Elemental analysis (wt%)		pH[a]
		N	C	
L	+6.8 to +6.5	0.71	52.8	n.d.[b]
Moss layer	+6.5 to +5.5	1.27	47.7	n.d.
F1	+5.5 to +3	1.29	50.0	n.d.
F2 + H	+3–0	0.71	28.5	n.d.
EA	6–8	0.008	0.18	2.8
C1	8– 40	0.006	0.09	3.3
C2	40+	0.005	0.08	3.5

[a] In 0.01 M $CaCl_2$, w/v = 1:2.5.
[b] Not determined.

2.2. Lipid extraction

We extracted between 0.1 and 1.6 g of the material from the organic horizons and 15–130 g of the mineral soil material by Soxhlet or ASE using dichloromethane/methanol (DCM/MeOH) (93:7 v/v) (Wiesenberg et al., 2004a). The extractant volumes were 300 mL in the case of Soxhlet extractions and 11–33 mL for ASE.

Soxhlet extractions were carried out for 24 h using a standard Soxhlet apparatus. As mentioned in the introduction, Soxhlet is an established extraction method for the extraction of lipids from various matrices including soils (e.g., Ehrhardt, 1972; Jambu et al., 1978; Bautista et al., 1999; Zarnowski and Suzuki, 2004). Many studies have shown that Soxhlet extractions of lipids are both quantitative and reproducible. Examples are recent comparisons with extraction of lipids with supercritical CO_2 and by shaking with solvent (Cheung et al., 1998; Bautista et al., 1999; Molkentin et al., 2001). Because of the large body of evidence validating the Soxhlet technique, we decided to perform only single Soxhlet extractions assuming the method to be quantitative and reproducible.

ASE extractions were carried out with a Dionex 200 ASE extractor, using a heating phase of 5 min and a static extraction time of 20 min (Wiesenberg et al., 2004a).

When using ASE, an extraction temperature and pressure need to be selected. The effects of changes in temperature and pressure on the extraction efficiency of ASE for lipids from soil samples have not been studied before. However, increased extraction efficiency of ASE with increasing temperature and pressure has been reported for other components (Richter et al., 1996). Especially an increase in pressure was found to profoundly change extraction efficiencies. For instance the recovery of selected PAHs from water-covered silica increased as much as 155% with an increase in pressure from 6.9×10^6 Pa to 17×10^6 Pa (Richter et al., 1996). To test for similar effects when extracting lipids from soil samples, we decided to apply two pressures of 6.9×10^6 Pa or 17×10^6 Pa, spanning the range used by Richter et al. (1996). To limit the degrees of freedom, we applied a single elevated temperature of 75 °C analogous to the one used by Wiesenberg et al. (2004a) to extract soil lipids. A higher temperature, while possibly increasing extraction efficiency, might cause thermal degradation of the lipid biomarkers under study (Christie, 2003).

Because of the lack of studies testing the applicability of ASE to extract lipids from soils, we felt that in addition to comparing extraction efficiencies with the ones obtained by Soxhlet extractions, we needed to get some indication of the reproducibility of ASE. Therefore, to test the reproducibility of ASE extractions for each of the two chosen pressures they were carried out in triplicate, resulting in a total of 6 ASE extracts per soil horizon.

2.3. Extract clean-up and derivatization

Upon Soxhlet or ASE extraction, we used an extract clean-up procedure analogous to one described by Naafs et al. (2004a). First, the DCM/MeOH phase was rotary evaporated to complete dryness after which the dry extract was re-dissolved in approximately 2–5 ml DCM/2-propanol (2:1 v/v). Next, the extract was filtered using a Pasteur pipette packed with defatted cotton wool, 0.5 cm Na_2SO_4(s) as a drying agent and 2 cm SiO_2(s) to remove very polar constituents. We added known amounts of an internal standard consisting of deuterated lipids (d_{42}-*n*-C_{20} alkane, d_{41}-*n*-C_{20} alcohol and d_{39}-*n*-C_{20} fatty acid) were added to the filtered extracts, after which we dried them under a gentle stream of N_2(g).

To the dried extracts we added 100 μL of cyclohexane as well as 50 μL of BSTFA (*N*,*O*-bis(trimethylsilyl) trifluoroacetamide) containing 1% TMCS (trimethylchlorosilane). Subsequently, the mixture was heated for 1 h at 70 °C to derivatize all free hydroxyl and carboxylic-acid groups to their corresponding trimethylsilyl (TMS) ethers and esters. After derivatization, we dried the solutions once more under N_2(g) to remove the excess

BSTFA, and subsequently re-dissolved in 200–4000 μL of cyclohexane.

2.4. GC/MS analysis

Gas chromatography–mass spectrometry (GC/MS) analyses were performed on a ThermoQuest Trace GC 2000 gas chromatograph connected to a Finnigan Trace MS quadrupole mass spectrometer. Separation took place by on-column injection of 1.0 μL of the derivatized extracts on a 30 m Restek 5 Sil MS column with an internal diameter of 0.25 mm and film thickness of 0.1 μm, using He as a carrier gas. Temperature programming consisted of an initial temperature of 50 °C for 2 min, heating at 40 °C/min to 80 °C, holding at 80 °C for 2 min, heating at 20 °C/min to 130 °C, immediately followed by heating at 4 °C/min to 350 °C and finally holding at 350 °C for 10 min. The subsequent MS detection in full scan mode used a mass-to-charge ratio (m/z) of 50–650 with a cycle time of 0.65 s and followed electron impact ionization with an ionization energy of 70 eV.

2.5. Interpretation and quantification

From the three component classes of interest (straight-chain lipids, plant sterols and aromatic acids) we selected a set of individual components to be the focus of our study. Specifically, we chose *n*-alkanes, *n*-alcohols and *n*-fatty acids in the range C_{19}–C_{34} as well as β-sitosterol because they represent a signal typical of higher plants (Dinel et al., 1990). In addition, we selected dehydroabietic acid as it is a specific pine-resin signal (Quenea et al., 2004). The selected compounds were identified from the chromatograms based on their mass spectra, using the peak area from selected dominant fragment ions for each class of components. The fragment ions were represented by: m/z 57 for the *n*-alkanes, m/z 75 for the *n*-alcohols, m/z 117 for the *n*-fatty acids, m/z 129 for β-sitosterol and m/z 239 for dehydroabietic acid.

For absolute quantification, we compared the peak areas for each component to the peak areas from the corresponding dominant fragment ions of the deuterated internal standard from the same component class. For this we assumed that for each component class, the response factor for the deuterated standards was equal to that of the selected lipids from that class. The selected fragment ions for the internal standards were represented by: m/z 66 for d_{42}-*n*-C_{20} alkane, m/z 76 for d_{41}-*n*-C_{20} alcohol and m/z 119 for d_{39}-*n*-C_{20} fatty acid. Since deuterated β-sitosterol and dehydroabietic acid were not available, we quantified these two components using d_{41}-*n*-C_{20} alcohol and d_{39}-*n*-C_{20} fatty acid, respectively. While assuming the response factor of these two components to be equal to that of the standards is perhaps speculative, it does not influence the comparison between ASE and Soxhlet extracts since the same quantification procedure was used for both extraction methods.

3. Results and discussion

The concentrations of the selected biomarkers in the ASE and Soxhlet extracts represented as μg/g extracted soil material are presented in Figs. 1–5. The values for the ASE extracts represent the average contents of the various biomarkers in the triplicate extracts with error bars indicating the standard error of the mean. Due to insufficient flushing during the filtering of one of the three low-pressure ASE extracts from the F1 horizon, probably a large fraction of the *n*-fatty acids remained behind on the SiO_2 column resulting in much lower concentrations in the final extract. Consequently, the *n*-fatty acid results for this one extract were discarded. In all other cases, the differences in the three replicates were randomly distributed and could not be contributed to a single systematic source. Most likely the calculated uncertainty was caused by the extensive sample pre-treatment procedure, combined with possible sample inhomogeneity. This conclusion is supported by the observation that the relative abundance of the individual lipids remained constant between replicates.

The presence of the plant sterol β-sitosterol and the terpenoid dehydroabietic acid in all horizons (Figs. 4 and 5) confirm the expected higher plant signal (Van Bergen et al., 1997). The same is true for the *n*-alcohols and *n*-fatty acids (Figs. 2 and 3). In higher plants these straight chain lipids originate mainly form plant waxes and usually have carbon numbers in the range of C_{19}–C_{34} in an even-over-odd chain length dominance (Dinel et al., 1990). This arrangement was indeed found in all horizons to the point where in most cases we found only even chain lengths (Figs. 2 and 3). The *n*-alkanes in higher plants also derive mainly from plant waxes and again have chain lengths in the range of C_{19}–C_{34} (Dinel et al., 1990). However, they are usually present in an odd-over-even chain length dominance (Dinel et al., 1990). Such a pattern was found in the moss layer and to

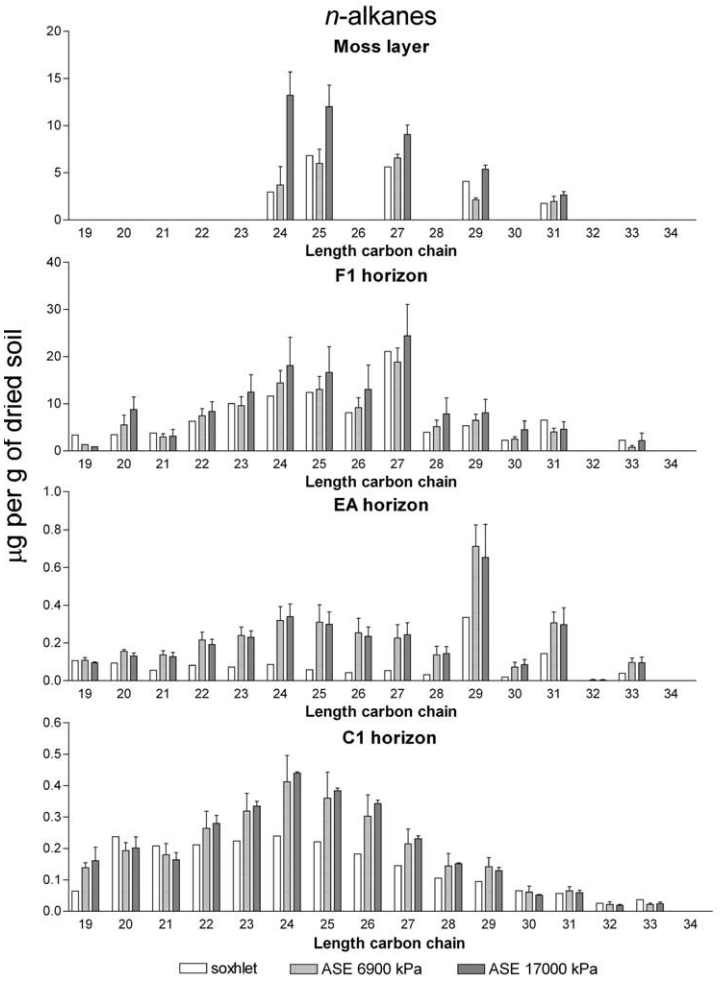

Fig. 1. Concentration of the *n*-alkanes with carbon numbers of C_{19}–C_{34} in the ASE and Soxhlet extracts of the four soil horizons under study.

a lesser extent in the EA horizon (Fig. 1), but in the other horizons significant amounts of *n*-alkanes with even chain lengths were also present. This suggests microbial or fungal input in addition to *n*-alkanes from plant waxes (Van Bergen et al., 1997).

3.1. Performance of ASE: the effect of different pressures

We found significant differences (*t*-test, $\alpha = 0.05$) in the mean extraction efficiency between the two pressures used in the ASE extractions for most lipids from all classes (except β-sitosterol) extracted from the moss layer. In all cases higher pressure resulted in greater extraction efficiency, the effect being most pronounced for the *n*-alkanes (Figs. 1–5). In the other organic layer, the F1 horizon, there also appears to be a trend of slightly larger average extraction efficiencies with increasing pressure for all classes of lipids except the *n*-fatty acids. However, the differences in the means here were too small to be statistically significant. In the mineral soil horizons we only observed a significant effect of pressure on the extraction efficiency for dehydroabietic acid in the EA horizon (Fig. 4), a higher pressure here leading to lower extraction efficiency. For the other lipid classes we could not discern a clear effect of pressure, although there appeared to be a small decrease in extraction efficiency of the *n*-fatty acids upon increasing pressure as well (Fig. 3).

As explained in the introduction section, an increase in extraction efficiency of ASE with increas-

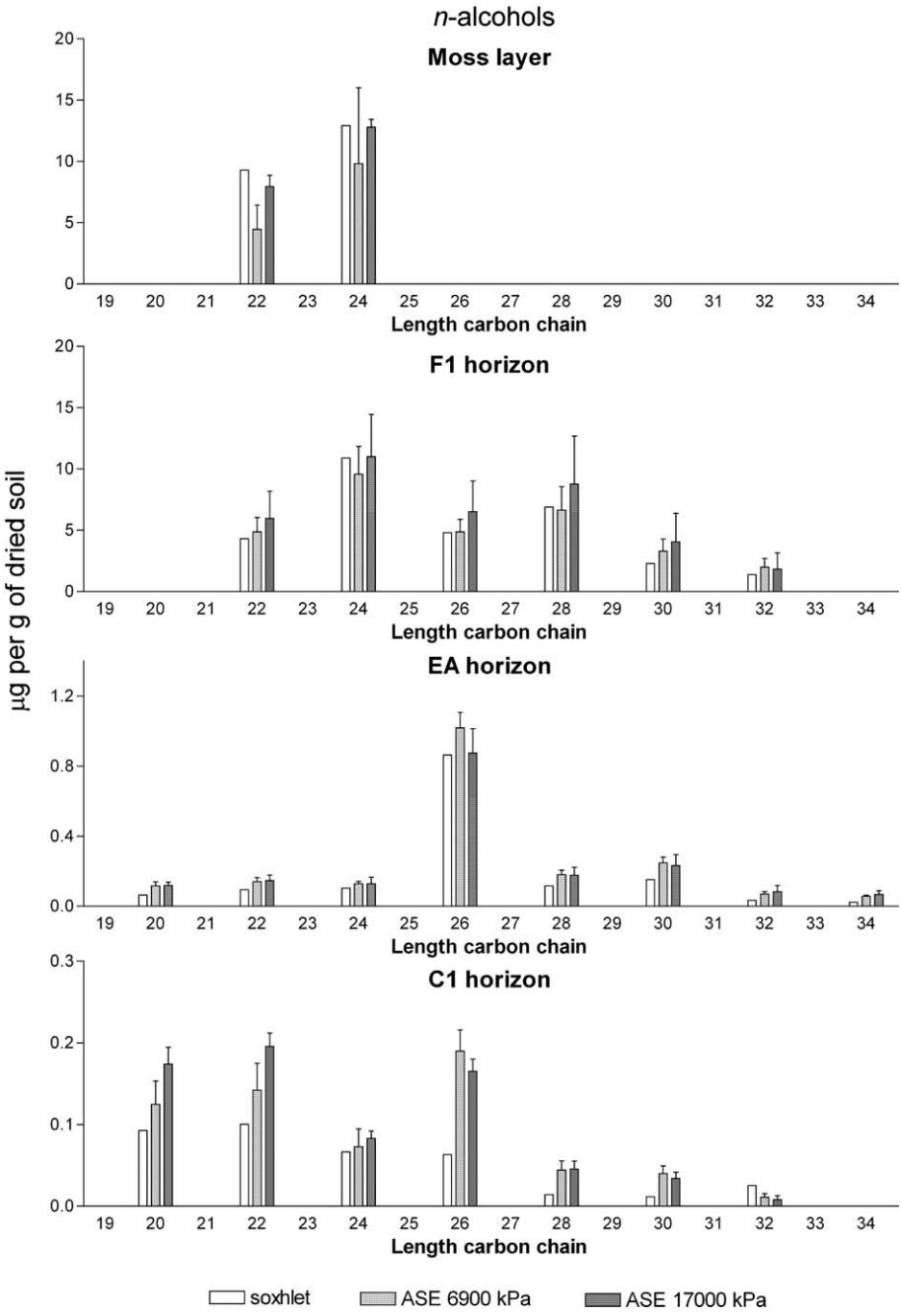

Fig. 2. Concentration of the *n*-alcohols with carbon numbers of C_{19}–C_{34} in the ASE and Soxhlet extracts of the four soil horizons under study.

ing pressure has been reported before for some classes of components, most notably PAHs (Richter et al., 1996). Richter et al. (1996) attributed the increase in extraction efficiency mainly to a better penetration of small water-blocked pores by the extractant. Even though our samples were air-dried, the conditions were quite mild (30 °C), leaving open the possibility of some water remaining especially in the smallest pores. As a consequence, the mechanism hypothesized by Richter et al. (1996) could also have been operating during the extraction of our samples. Because the mineral horizon material is very sandy, most likely it lacks the micro-pore structure that may be present in the abundant

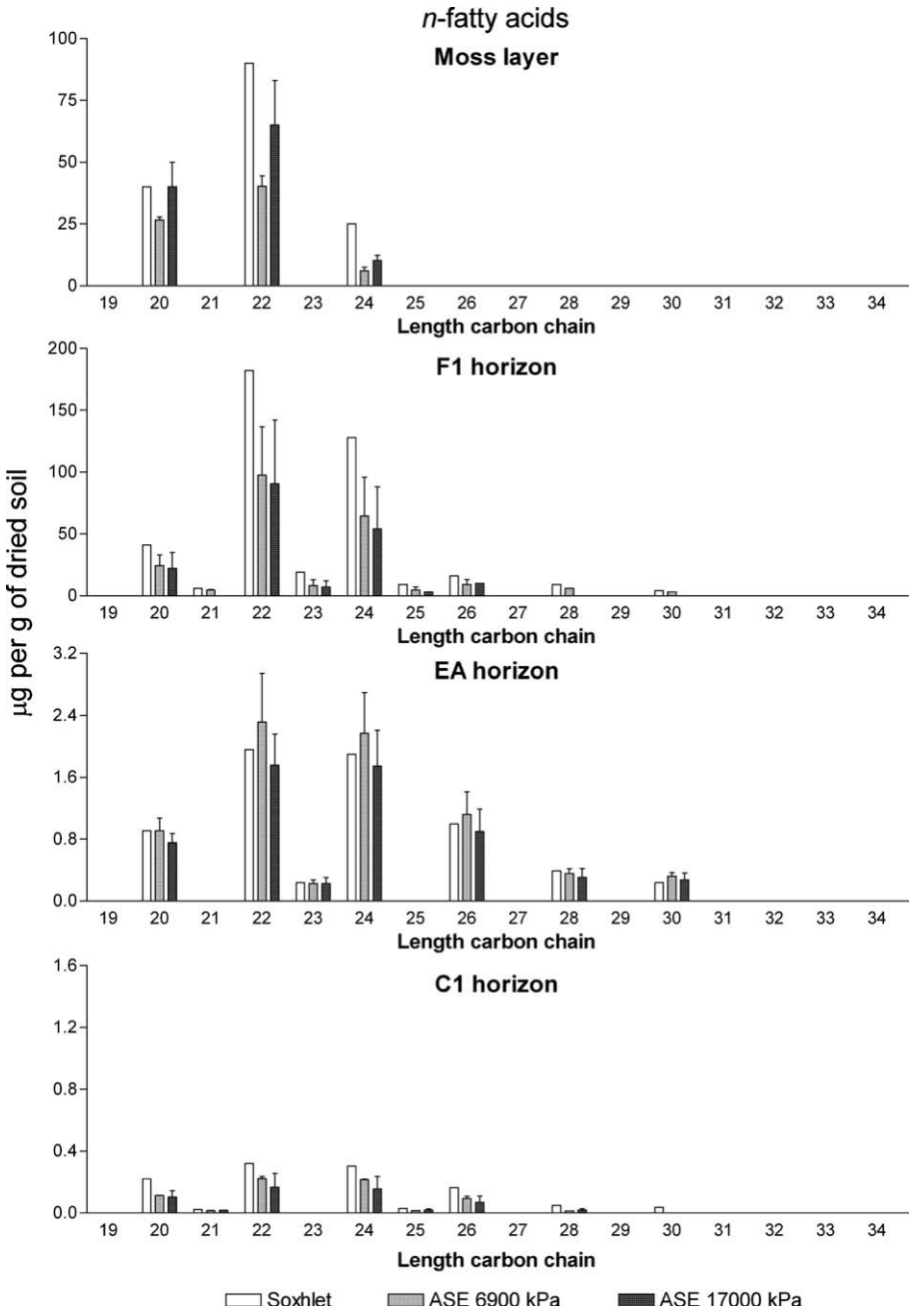

Fig. 3. Concentration of the *n*-fatty acids with carbon numbers of C_{19}–C_{34} in the ASE and Soxhlet extracts of the four soil horizons under study.

organic matter of the organic layers. As such a better penetration of water-blocked pores is unlikely in the mineral horizons, which would explain why no clear pressure effect was observed there for components other than dehydroabietic acid.

However, the effect on dehydroabietic acid is not explained by the above mechanism, nor does it explain the observed differences in the extent of the pressure effect on the various lipid compound classes we examined. For this we propose the following alternative mechanism to have been operating in conjunction with the one just described. The extractant used in the experiments (DCM/MeOH) is commonly applied for lipid extractions (e.g., Naafs et al., 2004a; Nierop et al., 2005; Rumpel et al., 2004; Wiesenberg et al., 2004a). Nevertheless,

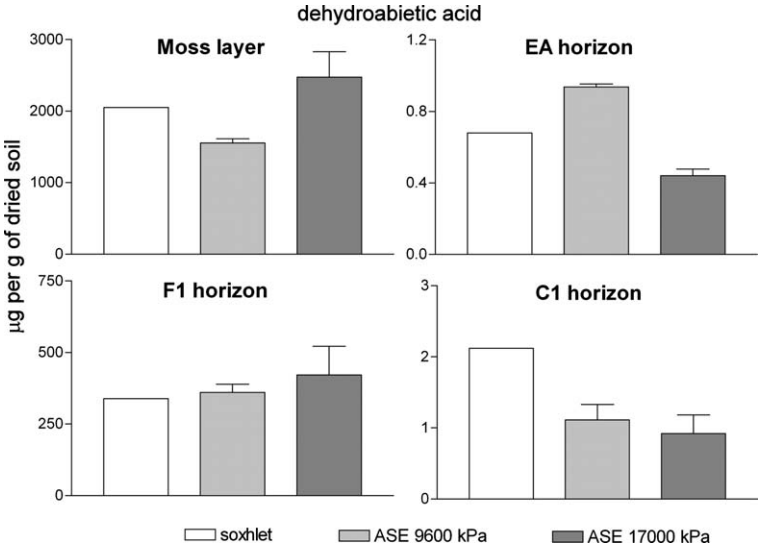

Fig. 4. Concentration of dehydroabietic acid in the ASE and Soxhlet extracts of the four soil horizons under study.

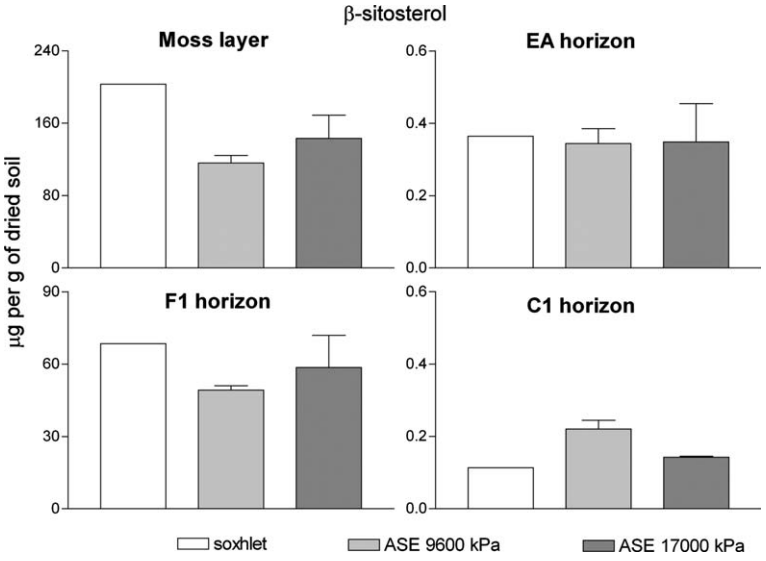

Fig. 5. Concentration of β-sitosterol in the ASE and Soxhlet extracts of the four soil horizons under study.

because DCM/MeOH is relatively polar, the extraction of *n*-alkanes, which are the least polar lipids investigated, might have been sub-optimal. Because the polarity of a solvent reduces upon increasing pressure at constant temperature, the polarity of the extractant in the ASE extractions at 17×10^6 Pa was lower than at 6.9×10^6 Pa. Lower polarity would explain the increased extraction efficiency for the *n*-alkanes as well as the (slight) decrease in extraction efficiency for dehydroabietic acid and possibly the *n*-fatty acids in the mineral soil horizons.

Overall, because of the sharp increase in extraction efficiency of ASE mainly for the *n*-alkanes, it would appear to be best to use the higher pressure of 17×10^6 Pa when applying ASE to extract the set of lipid biomarkers under study from sandy soil samples.

3.2. Performance of ASE: comparison with Soxhlet extractions

Compared to the Soxhlet extractions, we found the extraction efficiency of ASE for the *n*-alkanes at

its optimal pressure (17×10^6 Pa) to have been very large (Fig. 1). At the same time the extraction efficiency of ASE for the *n*-fatty acids and β-sitosterol from the organic horizons appeared to be slightly sub-optimal (Figs. 3 and 5). For the *n*-alcohols and dehydroabietic acid in all but the C1 horizon, ASE performed adequately, achieving extraction efficiencies similar to Soxhlet extractions (Figs. 2 and 4). Analogous to the pressure effect described in the previous section, a possible explanation for the differences in extraction efficiency between Soxhlet and ASE is the decrease in polarity of the extractant (DCM/MeOH) upon increased temperature and pressure as used during the ASE extractions.

The internal distribution of the biomarkers within a class of compounds in all but two cases (*n*-alkanes in the moss layer and *n*-alcohols in the C1 horizon) was the same for ASE and Soxhlet extractions (Figs. 1–5). Together with the mostly (more than) satisfactory extraction efficiencies we found for ASE extractions, this implies that ASE is a viable method to extract lipids from soils.

4. Conclusions

Altogether, we conclude that when investigating the combination of lipid biomarkers chosen in this study through extraction with the common extractant DCM/MeOH, overall ASE is a viable method to extract lipids from soils. The reasons are: (i) on average better extraction efficiencies, especially for the *n*-alkanes compared to the reference method of Soxhlet extractions; (ii) the reduced volumes of extractant (10–33 mL vs. 300 mL) as well as shorter extraction time (25 min vs. 16–24 h) compared to Soxhlet extractions; and (iii) the possibility of automated extraction of sequences of up to 24 samples. While in some instances the extraction efficiency of ASE for *n*-fatty acids and β-sitosterol appeared to be sub-optimal, the differences were small.

When using ASE, the highest pressure tested (17×10^6 Pa) gave the best results because it strongly increased the extraction efficiency for the *n*-alkanes and dehydroabietic acid in some instances.

The relative abundance of individual lipid biomarkers per component class remained constant in all but two instances, regardless of whether ASE or Soxhlet extractions were used. This enables a correct comparison of the results from investigation using ASE, to those in which Soxhlet extractions were applied.

While our study vindicates the use of ASE to extract the set of lipid biomarkers under study from sandy soils, one should realize that we investigated only a limited set of lipids from one soil type, using one type of extractant. Future investigations comparing multiple extractants with different polarity ranges as well as other classes of lipids and other soil types with a different texture could be helpful in further examining the performance of ASE for this type of investigation.

Acknowledgements

We wish to thank Frans van der Wielen for his extensive experimental support. The Netherlands Foundation for the Advancement of Tropical Research (WOTRO) is gratefully acknowledged for their funding of this project under number WAN 75-406. We thank Fjällräven for their generous sponsoring in the form of clothing and gear.

References

Balser, T.C., Treseder, K.K., Ekenler, M., 2005. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biol. Biochem. 37, 601–604.

Bautista, J.M., González-Vila, F.J., Martín, F., Del Rio, J.C., Gutierrez, A., Verdejo, T., Gustavo Gonzáez, A., 1999. Supercritical-carbon-dioxide extraction of lipids from a contaminated soil. J. Chromatogr. A 845, 365–371.

Cheung, P.C.K., Leung, A.Y.H., Ang, P.O., 1998. Comparison of supercritical carbon dioxide and Soxhlet extraction of lipids from a brown seaweed, *Sargassum hemiphyllum* (turn.) c. Ag. J. Agric. Food Chem. 46, 4228–4232.

Chilom, G., Kohl, S.D., Rice, J.A., 2005. The influence of lipids on the energetics of uptake of polycyclic aromatic hydrocarbons by natural organic matter. J. Environ. Qual. 34, 1055–1062.

Christie, W.W., 2003. Lipid analysis Isolation, Separation, Identification and Structural Analysis of Lipids. The Oily Press, Bridgwater, England, pp. 91–104.

Dalton, C., Birks, H.J.B., Brooks, S.J., Cameron, N.G., Evershed, R.P., Peglar, S.M., Scott, J.A., Thompson, R., 2005. A multi-proxy study of lake-development in response to catchment changes during the Holocene at Lochnagar, north-east Scotland. Palaeogr. Paleaocl. 221, 175–201.

Dinel, H., Schnitzer, M., Meyhus, G.R., 1990. Soil lipids: origin, nature, content, decomposition, and effect on soil physical properties. In: Bollag, J.M., Stotzky, G. (Eds.), Soil Biochemistry. Marcel Dekker, Inc., New York, pp. 397–429.

Ehrhardt, M., 1972. Petroleum hydrocarbons in oysters from Galveston bay. Environ. Pollut. 3, 257–271.

EPA, 1996. METHOD 3450C. Washington, USA.

FAO-UNESCO, 1990. Soil Map of the World Revised Legend. World Soil Resources Report 60, FAO, Rome.

Ficken, K.J., Barber, K.E., Eglinton, G., 1998. Lipid biomarker, $\delta^{13}C$ and plant macrofossil stratigraphy of a Scottish montane

peat bog over the last two millenia. Org. Geochem. 28, 217–237.

Giergielewicz-Mozajska, H., Dabrowski, L., Namiesnik, J., 2001. Accelerated solvent extraction (ASE) in the analysis of environmental solid samples – some aspects of theory and practice. Crit. Rev. Anal. Chem. 31, 149–165.

Gregorich, E.G., Monreal, C.M., Schnitzer, M., Schulten, H.R., 1996. Transformation of plant residues into soil organic matter: chemical characterization of plant tissue, isolated soil fractions, and whole soils. Soil Sci. 161, 680–693.

Guggenberger, G., Zech, W., 1999. Soil organic matter composition under primary forest, pasture, and secondary forest succession, Region Huetar Norte, Costa Rica. Forest Ecol. Manag. 124, 93–104.

Jambu, P., Fustec, E., Jacquesy, R., 1978. Les lipides des sols: nature, origine, evolution, propriétés. Science du Sol. 4, 229–240.

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.

Molkentin, J., Coors, U., Evers, J., Miebs, A., 2001. Comparison of two methods for the direct determination of fat in butter, blended spreads and margarine. Eur. J. Lipid Sci. Technol., 798–803.

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

Naafs, D.F.W., van Bergen, P.F., de Jong, M.A., Oonincx, A., de Leeuw, J.W., 2004b. Total lipid extracts from characteristic soil horizons in a podzol profile. Eur. J. Soil Sci. 55, 657–669.

Nierop, K.G.J., Verstraten, J.M., 2004. Rapid molecular assessment of the bioturbation extent in sandy soil horizons under pine using ester-bound lipids by on-line thermally assisted hydrolysis and methylation-gas chromatography/mass spectrometry. Rapid Commun. Mass Spec. 18, 1081–1088.

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

Nierop, K.G.J., Naafs, D.F.W., Van Bergen, P.F., 2005. Origin, occurrence and fate of extractable lipids in Dutch coastal dune soils along a pH gradient. Org. Geochem. 36, 555–566.

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

Poulenard, J., Michel, J.C., Bartoli, F., Portal, J.M., Podwojewski, P., 2004. Water repellency of volcanic ash soils from Ecuadorian páramo: effect of water content and characteristics of hydrophobic organic matter. Eur. J. Soil Sci. 55, 487–496.

Quenea, K., Derenne, S., Largeau, C., Rumpel, C., Mariotti, A., 2004. Variation in lipid relative abundance and composition among different particle size fractions of a forest soil. Org. Geochem. 35, 1355–1370.

Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N.L., 1996. Accelerated solvent extraction: a technique for sample preparation. Anal. Chem. 68, 1033–1039.

Ruess, L., Tiunov, A., Haubert, D., Richnow, H.H., Haggblom, M.M., Scheu, S., 2005. Carbon stable isotope fractionation and trophic transfer of fatty acids in fungal based soil food chains. Soil Biol. Biochem. 37, 945–953.

Rumpel, C., Seraphin, A., Goebel, M.O., Wiesenberg, G., Gonzales-Vila, F., Bachmann, J., Schwark, L., Michaelis, W., Mariotti, A., Kogel-Knabner, I., 2004. Alkyl C and hydrophobicity in B and C horizons of an acid forest soil. J. Plant Nutr. Soil Sci. 167, 685–692.

Van Bergen, P.F., Bull, I.D., Poulton, P.R., 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. Wild. Org. Geochem. 26, 117–135.

Wiesenberg, G.L.B., Schwark, L., Schmidt, M.W.I., 2004a. Improved automated extraction and separation procedure for soil lipid analyses. Eur. J. Soil Sci. 55, 256–349.

Wiesenberg, G.L.B., Schwarzbauer, J., Schmidt, M.W.I., Schwark, L., 2004b. Source and turnover of organic matter in agricultural soils derived from *n*-alkane/*n*-carboxylic acid compositions and C-isotope signatures. Org. Geochem. 35, 1371–1393.

Winkler, A., Haumaier, L., Zech, W., 2005. Insoluble alkyl carbon components in soils derive mainly from cutin and suberin. Org. Geochem. 36, 519–529.

Zarnowski, R., Suzuki, Y., 2004. Expedient Soxhlet extraction of resorcinolic lipids from wheat grains. J. Food Compos. Anal. 17, 649–663.