Interactive comment on “Predicting soil water repellency by hydrophobic organic compounds and their vegetation origin” *by* J. Mao et al.

J. Mao et al.

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Hereby, we thank the anonymous referee#2 for providing detailed comments and constructive suggestions. The remarks and suggestions certainly have helped us to improve the manuscript.

Response to the general comments

This paper is a follow-up of the research by Mao et al. (2014). The soil properties, the sequential extraction procedure and the more detailed information about D, AI and AS fractions used in this manuscript have been introduced and discussed earlier by Mao et al. (2014). We now refer more consistently to this paper and added some of the data if needed in this paper. The present paper focuses on the possible relations between the soil water repellency (SWR)-markers and measured WDPTs to predict soil water repellency level and the link between these SWR-markers and their vegetation origins. However, we agree with the reviewer that adding more detailed information will improve the readability of the paper.

Comment 1: Some data, respectively results are missing and new must be included in the paper to make it suitable for publication.

According to the referee’s comment, we have added soil pH, TN and C/N ratio to *Table 1* (attached to this reply) in the revised manuscript, although we already presented the basic parameters of soils in the previous paper (Mao et al., 2014). Accordingly, we also added more descriptions of the measurements of those soil parameters in ‘Materials and methods’.

Page 5, line 96: (Discussion paper, page 157. section 2.2)

2.2 Soil characteristics measurements

A 1:2.5 (w/w) soil to water ratio was used to determine soil pH value (Metson, 1956), which was measured by using a pH meter (Consort C830). To determine total organic carbon (TOC) and total nitrogen (TN), all soils were decalcified using 1 M HCl to remove inorganic carbon (Van Wesemael, 1955) and ground into fine powder by using planetary ball mills (Pulverisette®5, Fritsch). The TOC and TN contents of the soils were measured using a CNS analyser (Fisons Instruments NA1500).

Comment 2: No enough emphasis has been placed on the other soil properties and characteristics influencing SWR and supporting the discussion section and the conclusions section.

Referring to Mao et al. (2014), we have reported the correlation between the soil characteristics and SWR and discussed about the influence of those characteristics - soil pH value, total organic carbon content (TOC), total nitrogen content (TN) and C/N ratio - on SWR.

Comment 3: No relation has been made between WDPTs of the soil samples as a measure of SWR and the dominant compounds in these samples.

We do not understand this remark of the referee, as the entire paper has discussed the relation between the main soil organic compounds and SWR.

Comment 4: It has not been made clear by the authors why these specific compounds have been chosen as markers, moreover these compound classes are common constituents of TLE of any soil.

We agree with the referee that those compound groups are common to any soil. We have explained in Mao et al. (2014) that after IPA/NH_3 extraction, SWR was eliminated. Those specific compounds were chosen as markers because they are the main components extracted from soils, compared to other compounds, thus they are the most important influencing SWR. We have made clear references to our earlier work.

Comment 5: There are remarks concerning the extraction procedure, quantification procedure and inclusion of new data to support the statements made by the authors. There are already published data providing evidence that SWR can be eliminated by using milder solvents that don't require rupture of ester bonds, even hot water. A mention to these should be made.

We understand the concern of the referee about the procedures supporting our statements. In our paper, we did not break the ester-bounds to eliminate SWR of water repellent soils. We used IPA/NH_3 to extract ester-bound lipids from residual soils, and after the IPA/NH_3 extraction, the soils became wettable. After DCM/MeOH extraction to remove soluble components (the AS fractions), we used BF_3-MeOH to depolymerize the ester bonds involved in the residual IPA/NH_3 extracts (the fractions that was not soluble in DCM-MeOH, i.e. the AI fractions) followed by GC and GC/MS analyses. We have described in great detail the sequential extraction procedures in Mao et al. (2014) and have summarized this in this paper:

Line 129: (Discussion paper, page 158, line 2) The soils became wettable after the IPA/NH_3 extraction. The soluble lipids (AS fraction) were separated from the dried IPA/NH_3 extracts by DCM/MeOH (9:1), and the residues resulted into so-called AI fractions, which involved ester bonds.

Response to the specific comments

Comment 1: p. 155 line 16 (Atanassova and Doerr, 2010, as well)

We agree with the referee and have added the reference to the revised manuscript:
Line 52: Significantly more organic matter was found in water repellent soils than in wettable soils, but there was no clear correlation between the extracted amounts of organic matter and SWR severity (Atanassova and Doerr, 2010; Mainwaring et al., 2004, 2013).

Comment 2: p. 158 (lines 1-4) Sequential extraction has been previously used by other authors (Franco et al., 2000) and recently Atanassova & Doerr, 2010 where Accelerated Solvent Extraction (ASE) method is used with a similar solvent ratio. A mention of a modification or variation of this method by using conventional Soxhlet should be made.

We agree with the referee that sequential extraction has been used. Referring to section 2.3 of Mao et al. (2014), in which we have mentioned the previous works that have applied a sequential extraction procedure and the modification of this method to our research.

Comment 3: Total yields of extracts in the sequential procedure (D, AI, AS) should be presented and these data involved later in the Discussion section relating SWR (WDPTs) and yields to support statements.

We thank the referee to point presenting total amounts of the extracted fractions. The relative amounts of the main compound groups in the fractions have been shown in Mao et al. (2014). Here, in the present manuscript, we think it would be the best way to present these data by adding the ranges of both absolute and relative amounts of extracted compounds in the D, AI and AS fractions. The following information has been added to the revised manuscript:

Line 272: (Discussion paper, Page 163, line 5) For all soils, the absolute total amounts of the main compound groups in the D, AI and AS fractions ranged from 1.61 to 63.80 mg g^{-1}*soil, from 0.84 to 62.18 mg* g^{-1}*soil and from 0.27 to 40.24 mg* g^{1}*soil, respectively.*

Line 286: (Discussion paper, page 163, line 16) For all soils, the relative total amounts of the main compound groups in the D, AI and AS fractions ranged from 0.74 to 2.74 mg g^{-1}*TOC, from 0.48 to 2.01 mg* g^{-1}*TOC and from 0.24 to 1.43 mg* g^{-1}*TOC, respectively.*

Line 418: (Discussion paper, page 168, line 15, after 'an important SWR fraction') compared to the other two fractions, the AS fraction contained the least amount of extracted SWR-markers.

Comment 4: p.158. l. 24 Quantification has been roughly done or explained in the text. If this standard (squalane) has been used in other studies, it should be explained in detail what RRF to the lipid classes quantified has been used. Approximation and use of peak area integration does not lead to accurate results.

All extracts were analysed on GC-FID first and followed by analysis in GC-MS. As GC-FID produces peaks and their areas that are directly related the carbon content/amount of a compound, quantification using peak areas is quite similar for all types of linear hydrocarbons. Obviously small differences can be expected due to derivatisation of acids and alcohols in the form of methyl esters and TMS ethers, and carbon chain length, but they are small as compared to reproducibility of GC analyses (often 30% difference is considered as quite well reproducibility). Based on GC-MS we experienced for several compounds of interest co-elution with other compounds both derived from plants/soils and contaminants. This hampered a reliable quantification using peaks areas from GC-FID analyses. Instead we used the peaks areas in the TICs obtained from GC-MS analyses in order to correct for co-elution using mass chromatography.

We did not use RRFs to quantify compounds, however, we calculated RRFs of our 5 main types of compounds to check the accuracy of our quantification assuming GC-FID responses to be similar for all compound types. Therefore, RRF = peak area of a given compound upon GC-MS divided by peak area upon GC-FID relative to the squalane standard of which the absolute amount is known are: alkanes: 0.91; alcohols: 0.89; fatty acids: 0.93; ω-hydroxy fatty acids: 0.90; α,ω-dicarboxylic acids:

0.95. Hence, their relative responses were rather similar and thus hardly discriminating between various types of compounds. Therefore, we did not find it necessary to correct for these small differences. Applying the same way of quantification to all compounds/samples will result in possible errors in the accuracy of absolute quantification to be similar for all soils (and plants). However, our aim is not to present absolute and accurate results as such but to compare soils with regard to their SWR marker composition and amounts from which relations between compounds or groups of compounds to SWR can be derived. By doing this, possible errors will be automatically corrected for and thus are negligible. Moreover, in case we used the ratio of two compound groups, i.e. relative value, to predict SWR, possible inaccuracy can be neglected as well.

The above is well-known and commonly not incorporated in papers, and therefore we prefer not to include it as well. We added the following explanation to the revised manuscript:
Page 7, line 151: (Discussion paper, page 158, line 24, at the beginning of the paragraph') Based on GC-FID and GC-MS analyses, the relative response factors of compound groups (alkanes, alcohols, fatty acids, ω-hydroxy fatty acids and α,ω-dicarboxylic acids) were rather similar and hardly discriminating between various types of compounds. Therefore, a known amount of squalane as an internal standard was added to extracts to quantify compounds by peak area integration from GC-MS chromatograms to correct for possible co-eluting compounds.

Comment 5: p. 179 Table 1. WDPTs should be given in antilog instead of log, e.g. -0.48 (0.33 s) for better clarity to the actual water repellency of the samples.

We thank the referee to suggest showing WDPTs also without log scale to be clearer showing the water repellency level of the soils. We added the WDPT values of the soils in second units to *Table 1* (attached to this reply).

Comment 6: p. 159-160 At least two GC TIC chromatograms (of the most and the least water repellent soils and 1-2 of the vegetation) should be presented as evidence in a Figure to see the relative abundances of the compound classes quantified. Are the compound classes quantified in this study the most abundant (dominant) compounds in the extracts? No mention of other abundant compounds is given.

We thank the referee for this suggestion. Referring to Mao et al. (2014) we have shown the GC-TIC chromatograms of the D, AI and AS fractions of the Ah1 horizon under oak, which clearly shows that the most abundant compounds in the extracts are quantified in this study. Therefore, we think it would be better not to show these chromatograms again in this paper. Additionally, we attached the GC chromatograms of extractable lipids of the oak leaves and grasses (leaves combined with roots) with this reply since the chromatograms of the vegetation have not been presented before. For the vegetation extracts and cutin/suberin, a few abundant compounds (such as β-sitosterol, 9,16-dihydroxy-C_{16} acid, 10,16-dihydroxy-C_{16} acid, 9,10,18-trihydroxy-C_{18} acid) have been quantified but were not mentioned in the discussion paper. The reason is that we focus on the link between soil compounds and vegetation preferably comparing the compounds both in the soils and vegetation. However, these above-mentioned compounds exist in the vegetation but are not commonly found in the soil extracts.

According to the referee's suggestion, we added the following information to the revised manuscript:
Page 10, line 197: (Discussion paper, section 3.1.2, page 161, line 14) Besides these three main groups mentioned above, β-sitosterol was abundantly present in all the leaves and roots, but was found in soils with much lower abundance and had an insignificant correlation with SWR, as similar as other sterols (e.g. stigmasterol in mosses). Other typical biomarkers were found in leaves and roots of one or more species but hardly found in all soils, for instance, dehydroabietic acid in black pine needles, in therefore those biomarkers were not taken into account as an SWR marker to predict SWR.

Comment 7: p.161. No references are provided of other authors who identified these compounds in vegetation and soils, e.g. l. 9 (?), l. 13 (?), l. 24 (?) p. 162 l. 10 Ref?

We understand the concern of the referee. We did not provide the authors references who identified compounds in vegetation and soils here in the Result part. However, we provided their references in the Discussion part after line 356 (*Discussion paper, page 171, section 4.4*)

Comment 8: p. 162 n-alkanols were first detected and implicated to influence SWR by Atanassova & Doerr, 2010, citation is required.

n-Alcohols (alkanols) were first found in the extracts of water repellent soils by Mainwaring et al. (2004), and later were also detected in Hansel et al. (2008) and Atanassova and Doerr (2010), which confirms the influence of alcohols on SWR. We added the following information to the new manuscript:
Page 17, line 367: (Discussion paper, page 167, line 11, after 'respectively') In addition, alcohols have been detected in water repellent soils and associate with SWR (Mainwaring et al., 2004; Hansel et al., 2008; Atanassova and Doerr, 2010).

Comment 9: p.165 l.1-3 correct grammar.

We have corrected the sentence by deleting 'value'; the changed text in the new manuscript is as below:
Page 15, line 324: From the above analysis, individual compound groups in absolute concentrations (μg g^{-1}soil) were in general able to understand the SWR behavior, while using the relative amounts (μg g^{-1}TOC) were not.

Comment 10: p. 166 l. Polar compounds (sugars) and other short chain dicarboxylic acids were shown to be more abundant in less water repellent soils and are also implicated to affect WR (See Atanassova & Doerr, 2010). The higher relative Comp/TOC concentrations of alkanes, alkanols and fatty acids have been implied to affect SWR in the above mentioned study, as well.

We agree with the referee about the importance of alkanes, alkanols and fatty acids influencing SWR and the more abundant short-chain dicarboxylic acids found in less repellent soils. In our paper, the long-chain compounds have a more positive relation with SWR than the short-chain compounds. We added the following information to the new manuscript:
Page 17, line 362: (Discussion paper, page 166, line 11, before 'Mainwaring et al. (2004)' Atanassova and Doerr (2010) also detected more abundant short-chain dicarboxylic acids in less water repellent soils.

Comment 11: p.167 l.5 The phrase: “the positive relations between the absolute amounts of all the compound groups and SWR are most likely?? Following the significant positive relation between TOC and SWR” What does “most likely” imply? What’s the correlation between TOC/SWR of the samples? What is the corr. coeff. R?

We thank the referee to point the phrase ‘most likely’. Referring to Mao et al. (2014) the correlation between TOC/SWR of the soils has been investigated in our previous study. We changed the sentence to make it clearer for the readers to understand the content, see also comment 15:
Page 17, line 379: (Discussion paper, page 167, line 5) For all soils, the positive relations between the absolute amounts of all the compound groups and SWR follow the significant positive relation between TOC and SWR shown by Mao et al. (2014).

Comment 12: p.168 l.19. The fact that there are no alkanes in the AS fraction in a sequential extraction is logical, not unexpected. GC-TIC of a sequential extraction at least of 1 soil (probably the most repellent should be shown).

Referring to the response to comment 6, the GC-TC of the sequential extraction of the topsoil under oak has been presented in Mao et al. (2014). We do not see why it would be logical and unexpected that alkanes are missing from the AS fractions.

Comment 13: p. 171 l. 12-14. That has been shown in other studies. References should be given.

We are the first to describe the relation of the concentrations of α,ω-dicarboxylic acids in the AI fraction between the top- and subsoils; Referring to some textbook knowledge, roots are the main contributors of organic matter to the subsoils. Therefore, we did not provide any reference here.

Comment 14: p.172, l. 1-3 correct grammar. Has this correlation and WDPTs been determined?

We did not mean the statistic correlation between the extractable lipids and soils, moreover, this part emphasizes the plant single compounds in soil, not with soil water repellency. To express it clearer, we have corrected the sentence as followed:
line 510: (Discussion paper, page 172, line 3) Regarding the alcohol group, more alcohols were observed in leaves than in roots and more alcohols were found in the topsoils than in the subsoils, suggesting a large contribution of extractable lipids from plant leaf waxes to the directly underlying (top)soils.

Comment 15: p.172, l. 17-18 The correlation between TOC and SWR should be provided to sustain this statement.

As such a correlation is not the main conclusion from this study, according to the referee’s comment and to sustain our statement, we added more description about the correlation between TOC and SWR to the footnote of Table 1:

Table 1: [c]Soil TOC has a significant positive correlation (r=0.76, p=0.001) with SWR (Mao et al., 2014): $\log_{10}WDPT(s) = 1.96 * \log_{10}TOC + 0.01$

Comment 16: p.172, l. 19 The sentence: “. . .while the long-chain markers rarely have significant positive relations with SWR” . What does “rarely” mean. No correlation coefficient is provided for fatty acids > C22, it means no significant correlation, at all.

We understand the concern of the referee. We supposed to conclude that the long-chain markers have no significant relations with SWR. To make it clear, we changed the text in the revised manuscript shown as below:
Line 526: (Discussion paper, page 172, line 19) The relative amounts of the most single short-chain SWR-markers negatively relate to SWR, while the long-chain markers have positive but insignificant relations with SWR.

Comment 17: p.184. Figure 2 text on axis should be enlarged

We thank the referee to provide a good suggestion for the figure. In this discussion paper, the orientation is landscape. We believe when the orientation of the page is portrait (like the final publication), the quality will be improved. We will check later in the proof print if changes are needed.

References

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Metson, A. J.: Methods of chemical analysis for soil survey samples, New Zealand Soil Bureau Bulletin 12, Government Printer, Wellington, 22, 1956.

Van Wesemael, J. C. H.: De bepaling van het Calciumcarbonaatgehalte van Gronden, Chemisch Weekblad, 51, 35–36, 1955.

Table 1. Soil profile and vegetation description.

Profile	Sample label	Sampling depth (cm)	Horizon	pH	TOC (mg g^{-1} soil)[c]	TN (mg g^{-1}soil)	C/N ratio	WDPT (s)	$\log_{10}$ WDPT (s)	Repellency class	Vegetation	Vegetation sampled
1	WRC-1[a]	0 – 7	A	8.79	0.76	0.16	4.82	0	-1.00	wettable	*Festuca sp.* (sheep fescue)	Leaves combined with roots
	WRC-2	7 - 14	Ahb[b]	8.33	4.83	0.51	9.54	35	1.55	slight	*Festuca sp.*	
	WRC-3	14 - 20	B	8.72	1.40	0.25	5.66	0.3	-0.48	wettable	*Festuca sp.*	
2	WRC-6	0 – 1	A	8.26	3.47	0.38	9.20	1	0.00	wettable	Algae	None
3	WRC-8	0 – 5	Ah	7.87	5.49	0.49	11.15	148	2.17	strong	*Hypnum Laconosum* (hypmum moss)	Whole moss plants
	WRC-9	5 – 10	B	8.70	1.57	0.25	6.21	2	0.36	wettable	*Hypnum Laconosum*	
4	WRC-10	0 – 10	Ah	6.92	26.80	2.00	13.42	18	1.25	slight	*Hypnum Laconosum*	
5	WRC-13	0 – 16	Ah	5.84	14.98	1.01	14.80	240	2.38	strong	*Pinus nigra* (black pine)	Green needles and roots
6	WRC-14	0 – 9	Ah	7.09	31.08	2.40	12.96	417	2.62	strong	*Crataegus sp.* (hawthorn)	Leaves and roots
	WRC-15	9 – 15	B	7.55	5.02	0.53	9.49	550	2.74	strong	*Crataegus sp.*	
7	WRC-25	0 – 7	Ah	7.66	10.22	0.82	12.47	4786	3.68	extreme	*Hippophae rhamnoides* (sea-buckthorn)	Leaves and roots
	WRC-26	7– 12	B	8.10	4.77	0.45	10.57	331	2.52	strong	*Hippophae rhamnoides*	
8	WRC-30	0 – 2	Ah1	5.76	87.44	6.35	13.77	1905	3.28	severe	*Quercus robur* (common oak)	Leaves and roots
	WRC-31	2 - 4.5	Ah2	5.79	20.71	1.59	13.04	2512	3.40	severe	*Quercus robur*	
	WRC-32	4.5 – 20	B	8.08	2.46	0.27	9.05	14	1.14	slight	*Quercus robur*	

[a] WRC-1 consisted of a top soil, which was formed by wind-blown sand deposition at a grass covered soil.

[b] WRC-2 consisted of a dark brownish Ah horizon with grass roots, which was buried by wind-blown sand deposition.

[c] Soil TOC had a significant positive correlation (r=0.76, p=0.001) with SWR (Mao et al., 2014): $\log_{10}\mathrm{WDPT(s)} = 1.96^{*} \log_{10}\mathrm{TOC} + 0.01$

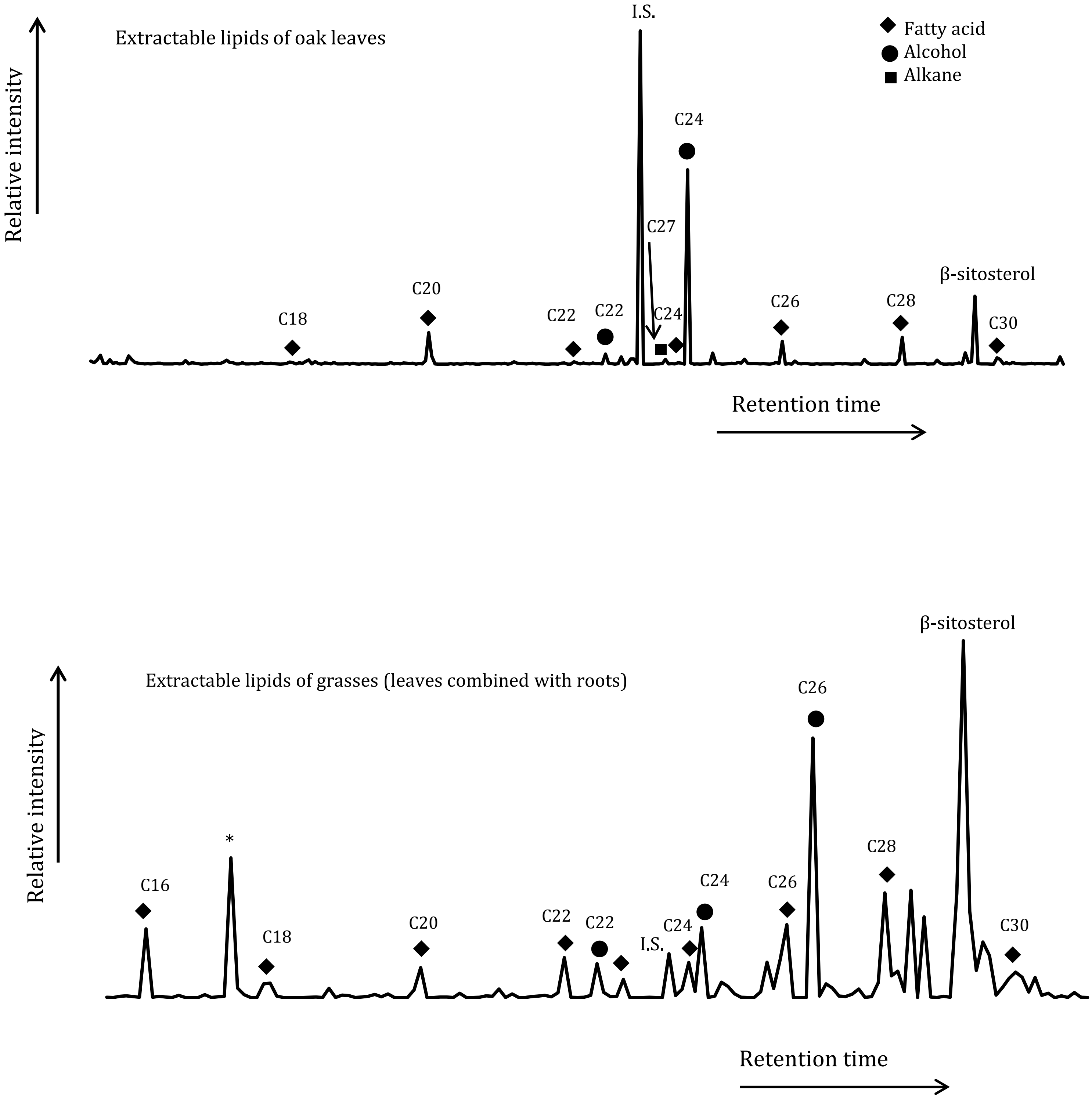

Figure GC chromatograms of the extractable lipids of oak leaves (top) and grasses (bottom).
I.S. : internal standard, *: contaminant, Cn indicates chain length.