Supplemental Information for

Bomb-radiocarbon signal suggests that soil carbon contributes to chlorophyll *a* in archival oak leaves

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Running head: $\Delta^{14}C$ of chlorophyll *a* in archival oak leaves

Supplemental Text

To identify and characterize impurity carbon in the purified Pheo *a* fractions, three additional assessments based on (i) diode array detector (DAD), (ii) Orbitrap MS, and (iii) GC/MS spectra were performed. Assessment (i) was subject to all eight samples, while assessment (ii) subject to 1952, 1968, 1973, 1982, and 1995 samples and assessment (iii) subject to 1952 and 1968 samples due to availability of leftover materials after CSRA.

(i) DAD spectrum

The DAD spectrum of all samples spanning from 300 to 720 nm absorbance was examined at the time window between 16.5 to 20.5 min when the Pheo *a* allomer, Pheo *a*, and Pheo *a* epimer were collected by fraction collector. The HPLC setting was the same as the second-step separation using Eclipse PAH column. Exogeneous compounds having absorbance at the 300–720 nm range were not found (Figure S1). Based on peak areas at 660 nm of Pheo *a* allomer, Pheo *a*, and Pheo *a* epimer, their relative proportion in each sample was estimated (Figure S2).

(ii) Orbitrap MS spectrum

Selected samples (1952, 1968, 1973, 1982, and 1995 CE) were injected to an UltiMate 3000 and Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer (Thermo Fischer Scientific Inc., Waltham, MA, USA) using the electrospray ionization (ESI) method under an infusion mode. Exogeneous compounds that were ionized by ESI were not found except for Pheo *a* allomers (allomer 1: *m/z* 887.6, $C_{55}H_{74}N_4O_6$, allomer 2: *m/z* 903.6, $C_{55}H_{74}N_4O_7$, allomer 3: *m/z* 919.6, $C_{55}H_{74}N_4O_8$, allomer 4: *m/z* 935.6, $C_{55}H_{74}N_4O_9$) (Figure S3). The addition of oxygen to the Pheo *a* compound likely occurred in sample preparation and/or storage process due to an exposure to ambient air, which does not increase its C/N nor affect

sample purity for CSRA measurements. Based on peak areas at exact *m/z* of Pheo *a* and its allomers, their relative proportion in each sample was estimated (Figure S4). There was a strong positive correlation in proportions of Pheo *a* allomers between DAD-based estimates and Orbitrap MS-based estimates (Figure S5).

(iii) GC/MS spectrum

Selected samples (1952 and 1968CE) were dissolved in *n*-hexane, and loaded on deactivated 1 % H_2O silica gel columns pre-conditioned with hexane. The N-1, N-2, and N-3 fractions were extracted with *n*-hexane, *n*-hexane/dichloromethane (50:50, v/v), and dichloromethane/methanol (90:10, v/v) respectively. After drying with argon gas, these fractions were redissolved in *n*-hexane/dichloromethane and were injected to a gas chromatograph/mass spectrometer (GC/MS) (Agilent 7890A-GC with Agilent 5975C inert XL MSD) equipped with the VF-5ms column (0.25 mm ×30 m, film thickness 0.10 μm) with the electron ionization (EI) method (70eV). The oven temperature was programmed as follows: maintained at 40 °C for 2 min, raised up to 120 °C at 30 °C min^{-1}, then to 320 °C at 6 °C min^{-1}, and maintained at 320 °C for 20 min. Helium was used as the carrier gas with a constant flow are of 1 mL min^{-1}.

For the N-1 and N-2 fractions, no peaks were found. For the N-3 fraction, peaks around 12–13 min appeared both in the in-house Pheo *a* standard and the samples, suggesting that these are ionized fractions of Pheo *a* compound. Exogeneous compounds that were ionized by EI were not found except for pentacyclic triterpenoid compounds (β-amyrin, simiarenol, and their derivatives) eluting around 31–32 min, which were identified by mass fragment patterns (Elgamal et al., 1969; Galeron et al., 2016; Shiojima et al., 1992). (Figures S6–S13).

Supplemental References

Elgamal, M. H. A., Fayez, M. B. E., & Kemp, T. R. (1969). The mass spectra of some triterpenoid dehydration products. *Organic Mass Spectrometry*, *2*(2), 175–194. https://doi.org/10.1002/oms.1210020204

Galeron, M. A., Vaultier, F., & Rontani, J. F. (2016). Oxidation products of α- And β-amyrins: Potential tracers of abiotic degradation of vascular-plant organic matter in aquatic environments. *Environmental Chemistry*, *13*(4), 732–744. https://doi.org/10.1071/EN15237

Shiojima, K., Arai, Y., Masuda, K., Takase, Y., Ageta, T., & Ageta, H. (1992). Mass Spectra of Pentacyclic Triterpenoids. *Chemical and Pharmaceutical Bulletin*, *40*(7), 1683–1690. https://doi.org/10.1248/cpb.40.1683

FIGURE LEGENDS

Figure S1. HPLC/DAD spectrums of Pheo *a* collected through separation using the C18 (1st) and PAH (2nd) columns in (a) 1952 CE, (b) 1965 CE, (c) 1966 CE, (d) 1968 CE, (e) 1973 CE, (f) 1982 CE, (g) 1995 CE, and (h) 2007 CE. Dashed lines indicate fraction collection window (16.5–20.5 min).

Figure S2. DAD peak area (660 nm) percentage of Pheo *a* allomer (16.5–17.8 min), Pheo *a* allomer epimer, Pheo *a* (17.8–19.2 min), and Pheo *a* epimer (19.2–20.5 min).

Figure S3. Orbitrap MS chromatograms of Pheo *a* (*m/z* 871.6, $C_{55}H_{74}N_4O_5$) in (a) an in-house standard, (b) 1952 CE, (c) 1968 CE, (d) 1973 CE, (e) 1982 CE, and (f) 1995 CE.

Figure S4. Orbitrap MS peak area percentage of Pheo *a* and its allomers.

Figure S5. Relationship between DAD peak area (660 nm) percentage and Orbitrap MS peak area percentage of Pheo *a* and its allomers. The strong positive correlation corroborates that the compounds are Pheo *a* derivatives (allomers).

Figure S6. Total ion chromatogram of the N-3 fraction (a) in an in-house standard Pheo *a* and (b) in Pheo *a* extracted from the *Quercus pubescens* leaf collected in 1952 CE and purified for CSRA.

Figure S7. (a) Total ion chromatogram and (b) mass spectrum of the first unknown peak of the N-3 fraction in Pheo *a* in 1952 CE. The asterisk denotes the peak for which the mass spectrum is obtained.

Figure S8. (a) Total ion chromatogram and (b) mass spectrum of the second unknown peak of the N-3 fraction in Pheo *a* in 1952 CE. The asterisk denotes the peak for which the mass spectrum is obtained.

Figure S9. (a) Total ion chromatogram and (b) mass spectrum of the third unknown peak of the N-3 fraction in Pheo *a* in 1952 CE. The asterisk denotes the peak for which the mass spectrum is obtained.

Figure S10. Total ion chromatogram of the N-3 fraction (a) in an in-house standard Pheo *a* and (b) in Pheo *a* extracted from the *Quercus pubescens* leaf collected in 1968 CE and purified for CSRA.

Figure S11. (a) Total ion chromatogram and (b) mass spectrum of the first unknown peak of the N-3 fraction in Pheo *a* in 1968 CE. The asterisk denotes the peak for which the mass spectrum is obtained.

Figure S12. (a) Total ion chromatogram and (b) mass spectrum of the second unknown peak of the N-3 fraction in Pheo *a* in 1968 CE. The asterisk denotes the peak for which the mass spectrum is obtained.

Figure S13. (a) Total ion chromatogram and (b) mass spectrum of the third unknown peak of the N-3 fraction in Pheo *a* in 1968 CE. The asterisk denotes the peak for which the mass spectrum is obtained.

Figure S14. The observed C/N ratios (right axis in red) and blank carbon % (left axis in blue) for Chl *a* purified from *Quercus* leaf samples plotted against collection year CE. Error bars indicate 1σ uncertainties. The dashed red line indicates expected C/N ratios of Chl *a* (11.8). The four older samples collected in 1952, 1965, 1966, and 1968 CE showed significantly higher C/N and blank carbon % than 11.8 and 0, respectively, while the four newer samples collected in 1973, 1982, 1995, and 2007 CE did not.

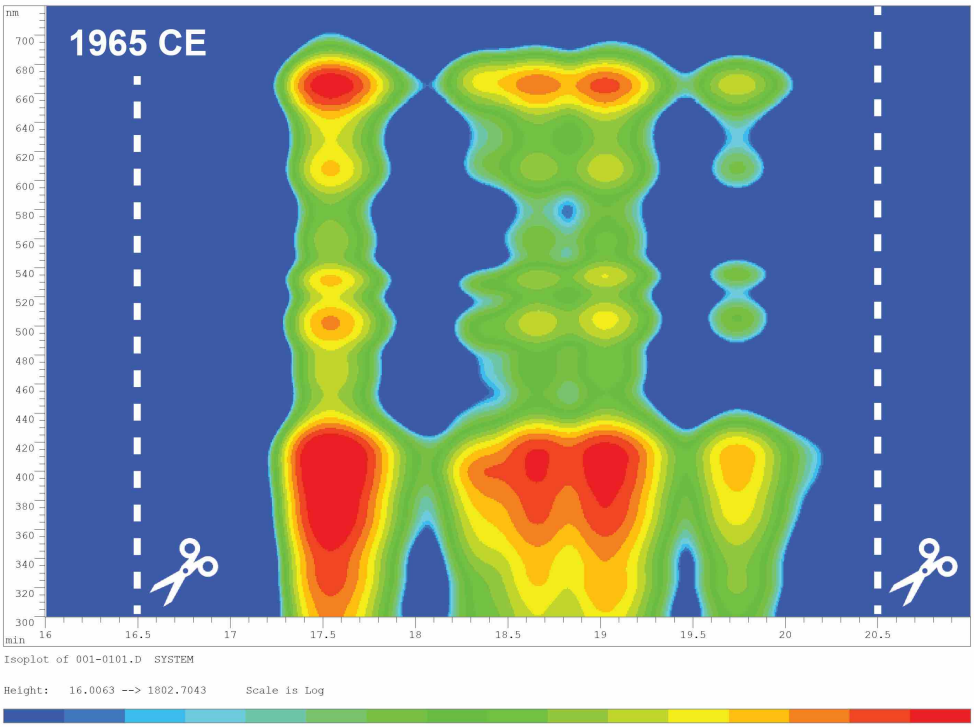
Figure S15. Heatmaps of the difference ($\Delta\Delta^{14}C$) between observed and modelled $\Delta^{14}C_{Chl}$ values on a biplot for soil turnover time (T_s, years) versus soil proportion (P_s, %) for each of the eight samples collected in different years. The $\Delta\Delta^{14}C$ value larger than the 2σ analytical error of CSRA (>16‰) was not considered in this plot. The white arrows denote the smallest $\Delta\Delta^{14}C$ values (i.e., the most plausible models).

Figure S16. The $\Delta\Delta^{14}C$ heatmap that overlayed all the eight heatmaps in Figure S12. The arithmetic mean of the $\Delta\Delta^{14}C$ values from the eight years are shown. The white arrow denotes the smallest $\Delta\Delta^{14}C$ value (i.e., the most plausible model).

148

149 Figure S1a

150



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152 Figure S1b

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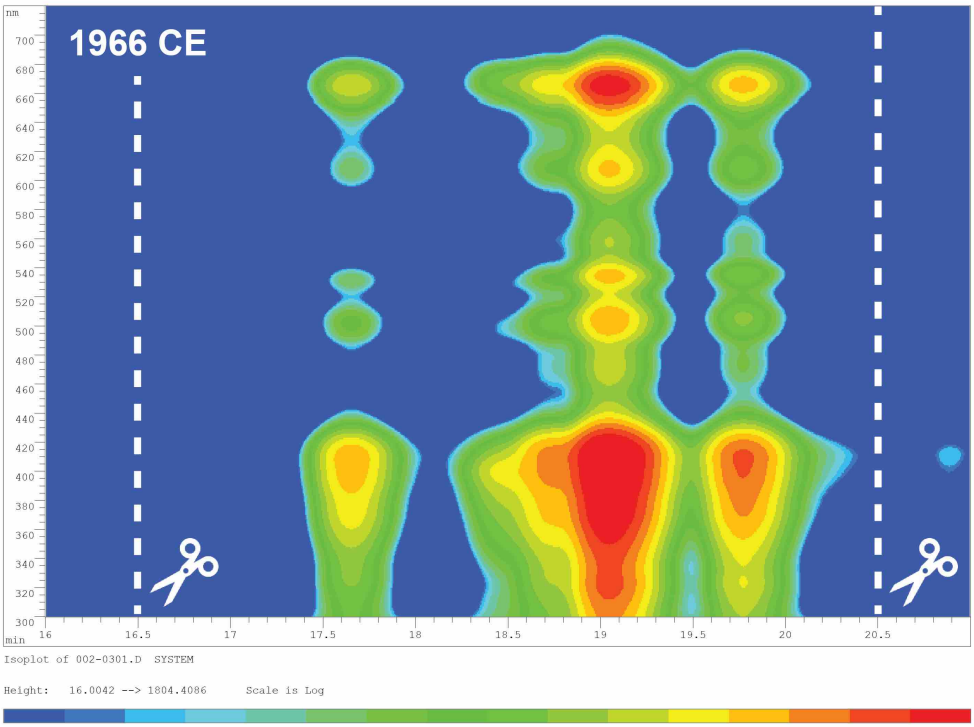

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155 Figure S1c

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158 Figure S1d

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161 Figure S1e

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164 Figure S1f

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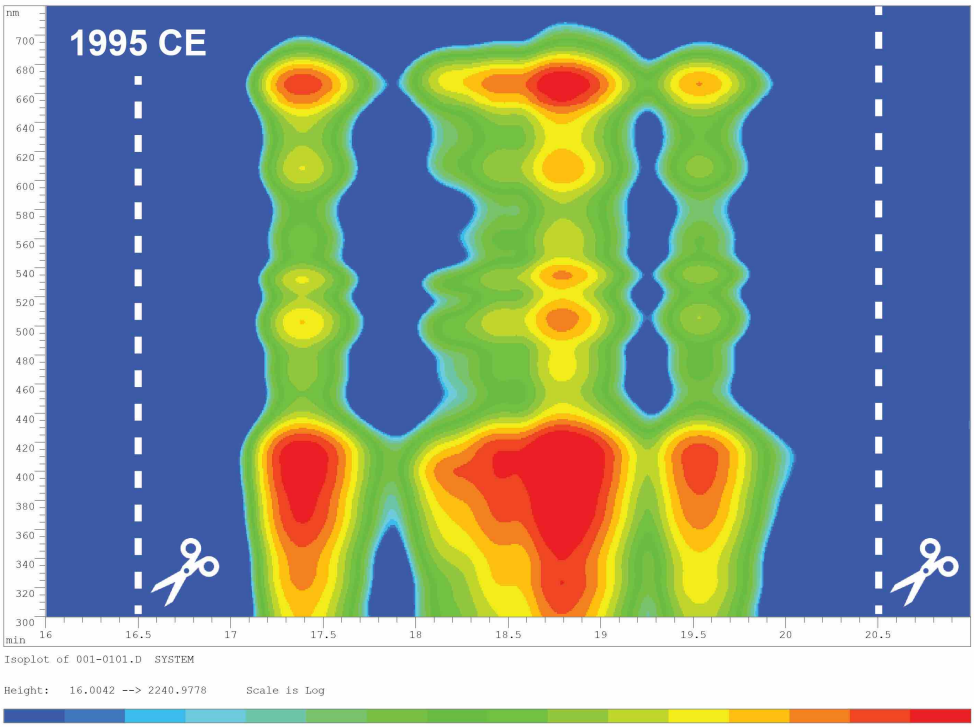

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167 Figure S1g

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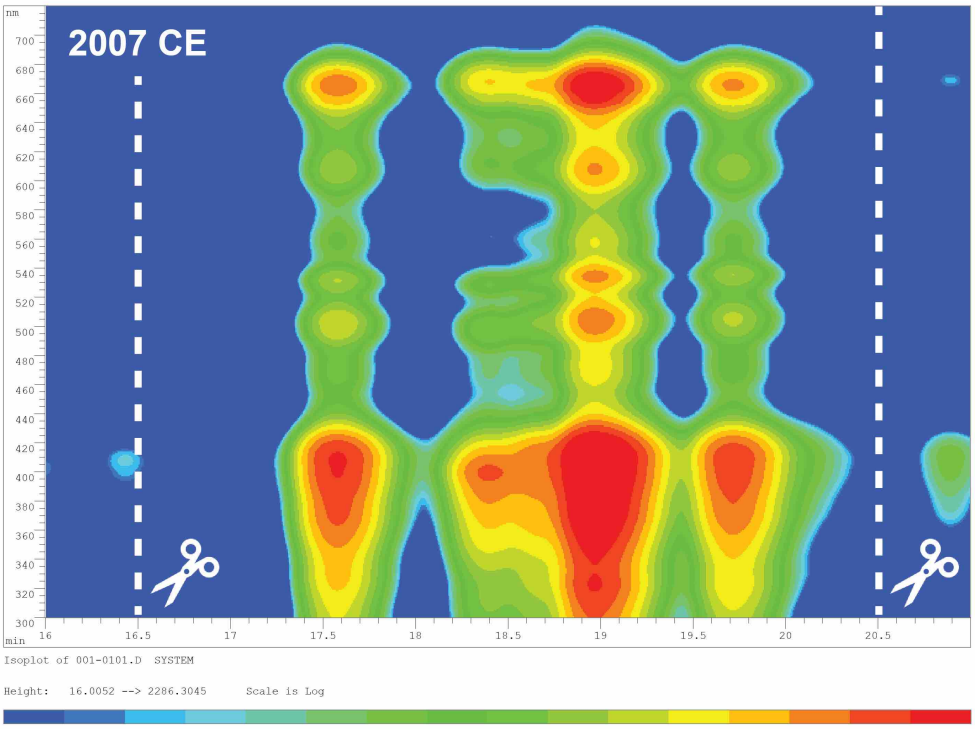

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170 Figure S1h

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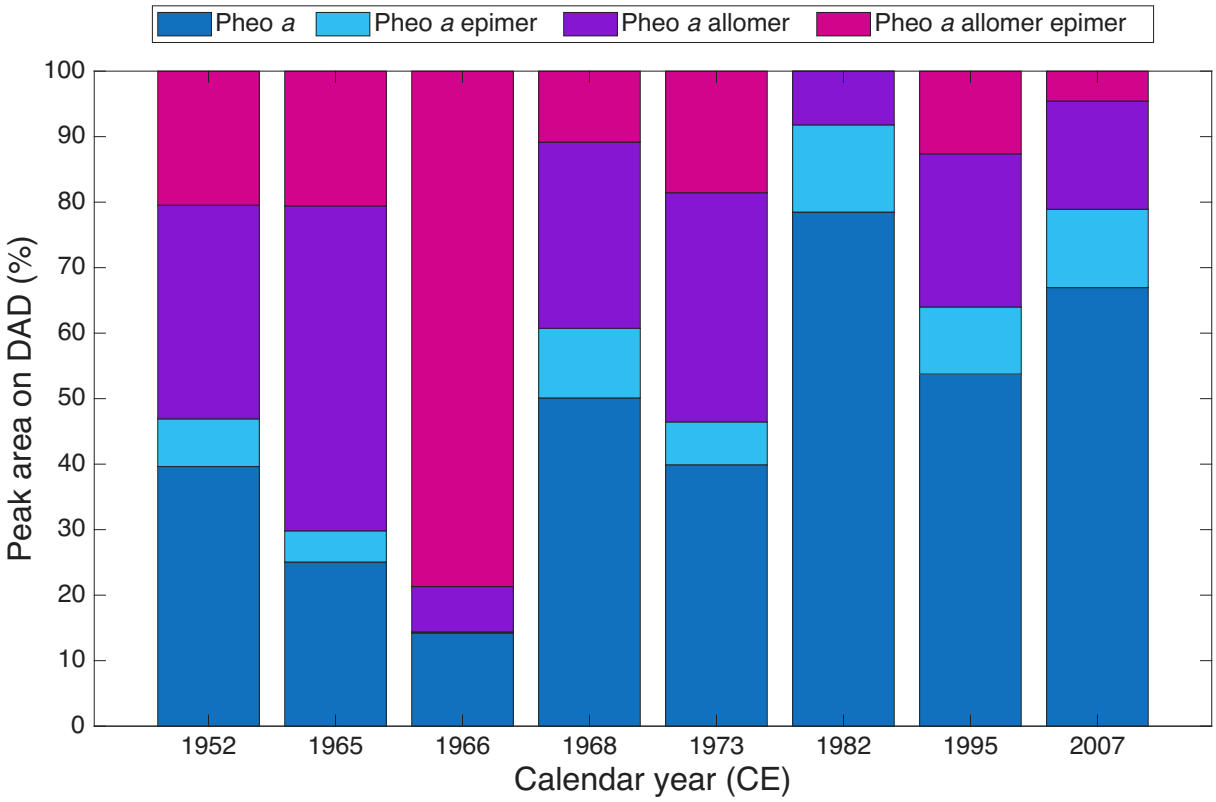

172

173 Figure 2

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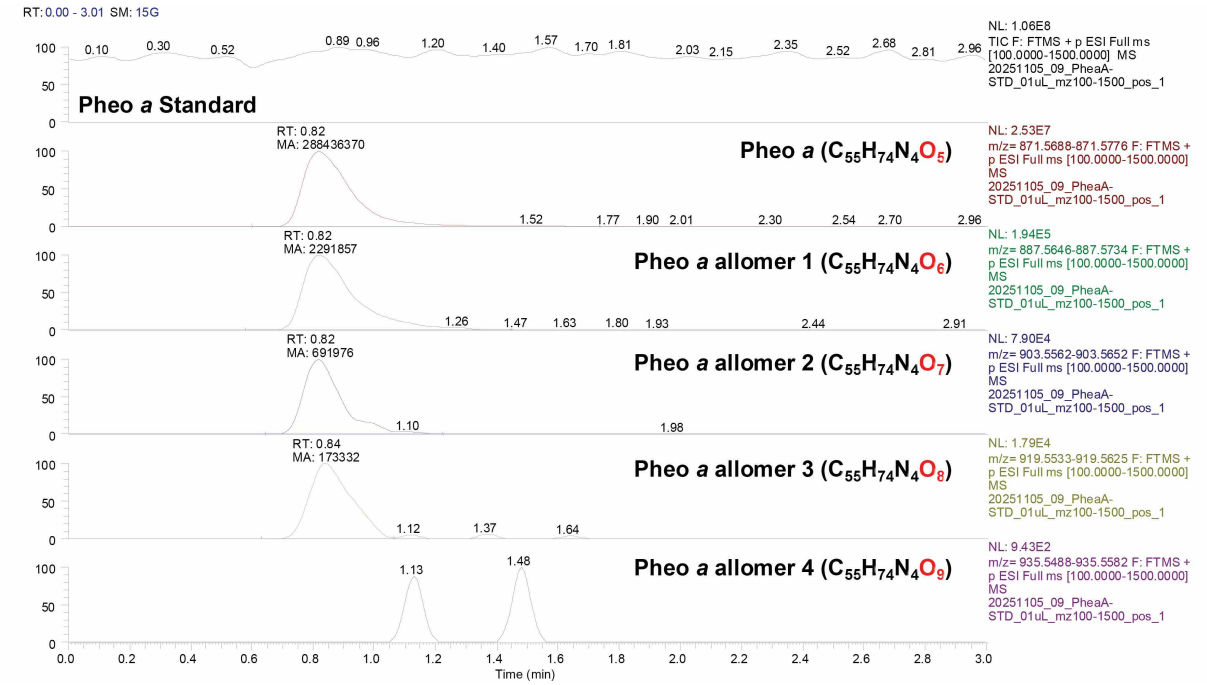
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RT: 0.82
MA: 288436370
Pheo a (C55H74N4O5)
NL: 2.53E7
m/z= 871.5688-871.5776 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_09_PheaA-STD_01uL_mz100-1500_pos_1
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NL: 1.94E5
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NL: 7.90E4
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RT: 0.84
MA: 173332
Pheo a allomer 3 (C55H74N4O8)
NL: 1.79E4
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NL: 9.43E2
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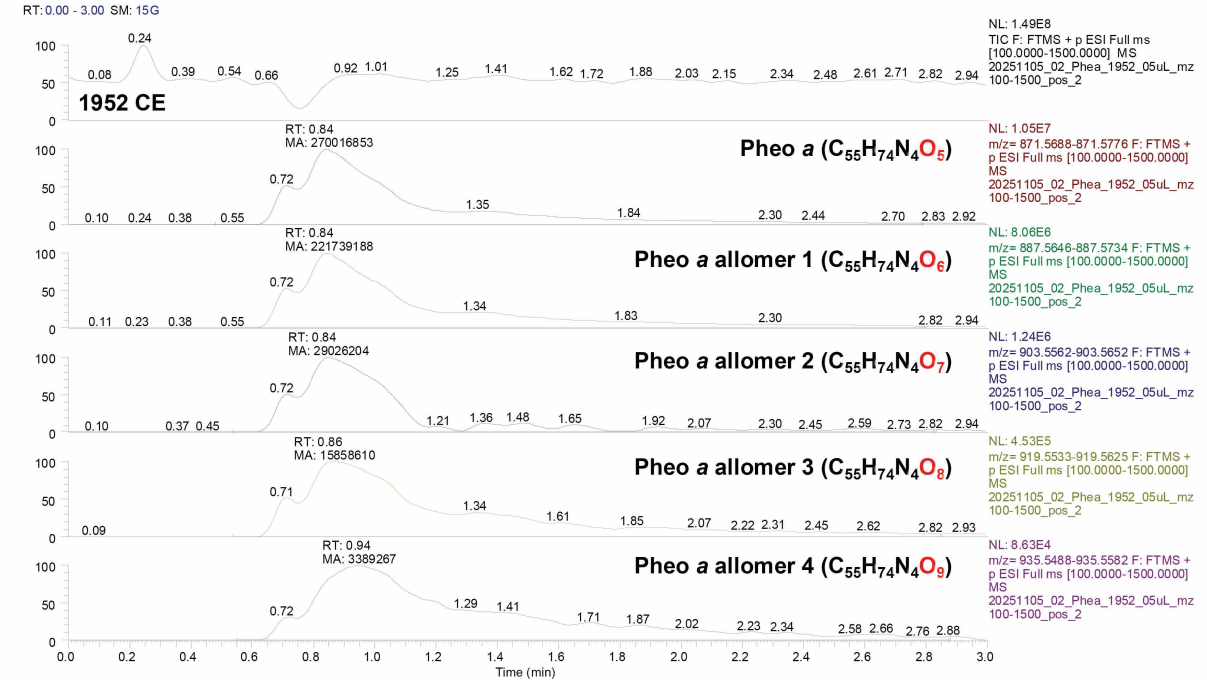
Figure S3a



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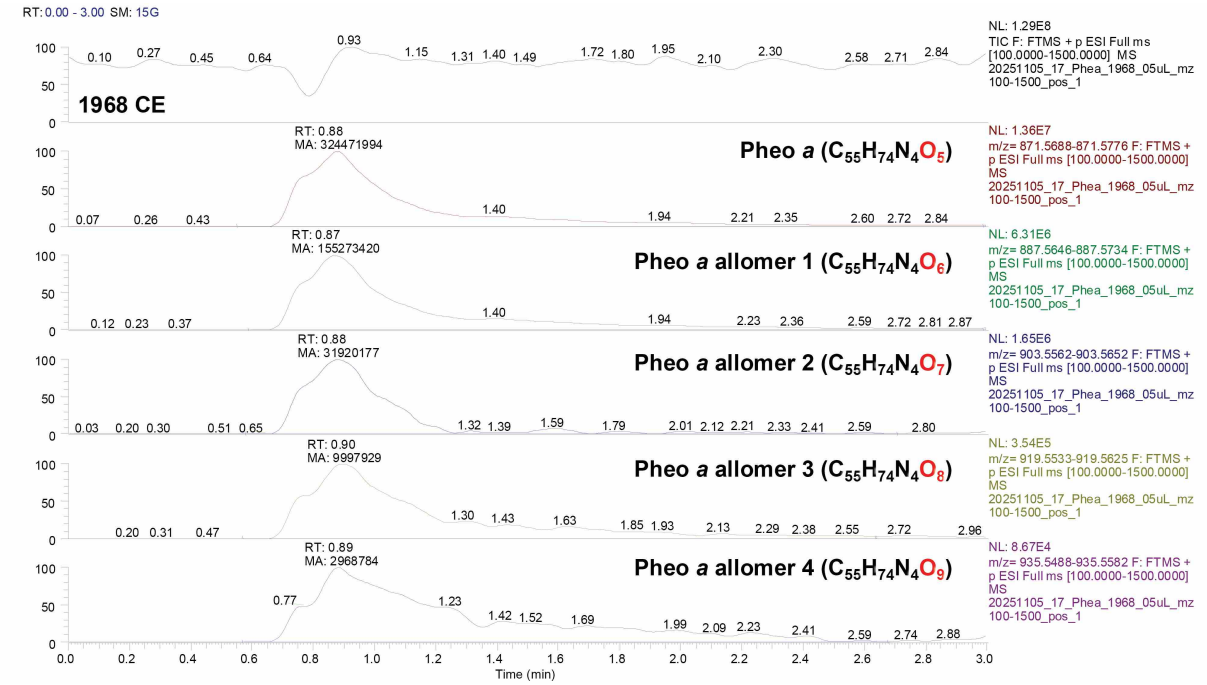
179 Figure S3b

180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Pheo a (C55H74N4O5)
RT: 0.88
MA: 324471994
NL: 1.36E7
m/z= 871.5688-871.5776 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_17_Phea_1968_05uL_mz 100-1500_pos_1
Pheo a allomer 1 (C55H74N4O6)
RT: 0.87
MA: 155273420
NL: 6.31E6
m/z= 887.5646-887.5734 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_17_Phea_1968_05uL_mz 100-1500_pos_1
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RT: 0.88
MA: 31920177
NL: 1.65E6
m/z= 903.5562-903.5652 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_17_Phea_1968_05uL_mz 100-1500_pos_1
Pheo a allomer 3 (C55H74N4O8)
RT: 0.90
MA: 9997929
NL: 3.54E5
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Pheo a allomer 4 (C55H74N4O9)
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MA: 2968784
NL: 8.67E4
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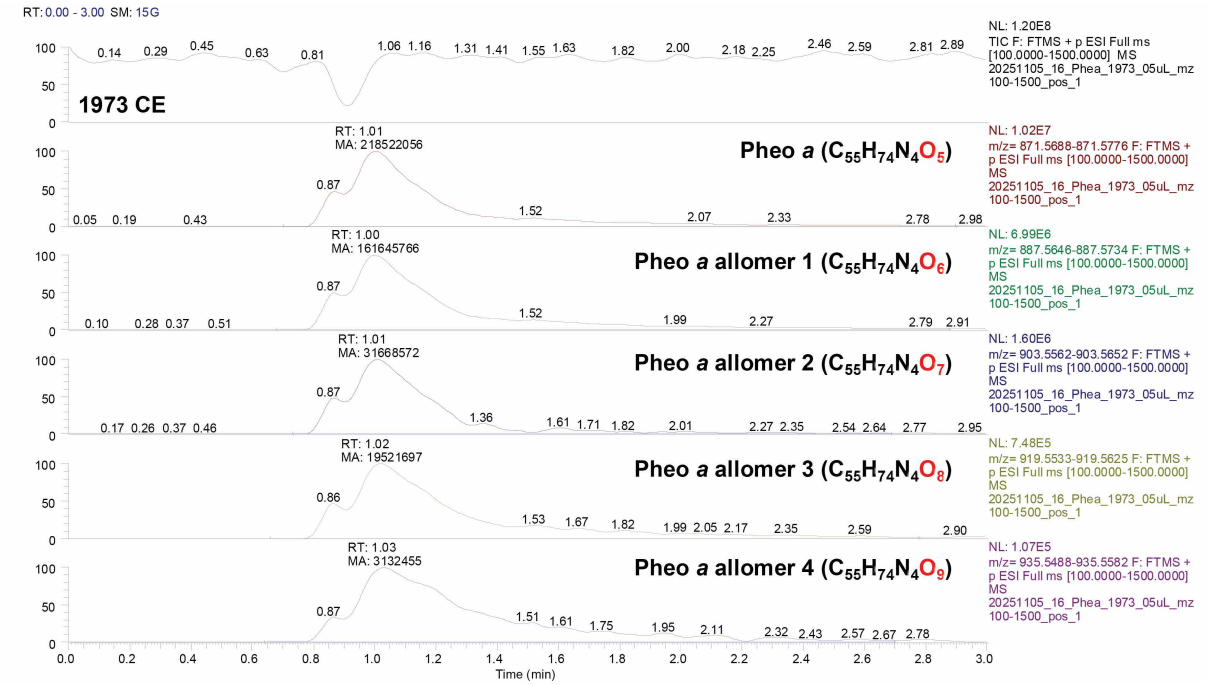
Time (min)

Figure S3c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Pheo a allomer 2 (C55H74N4O7)
Pheo a allomer 3 (C55H74N4O8)
Pheo a allomer 4 (C55H74N4O9)
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MA: 218522056
RT: 1.00
MA: 161645766
RT: 1.01
MA: 31668572
RT: 1.02
MA: 19521697
RT: 1.03
MA: 3132455
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NL: 6.99E6
m/z= 887.5646-887.5734 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_16_Phea_1973_05uL_mz 100-1500_pos_1
NL: 1.60E6
m/z= 903.5562-903.5652 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_16_Phea_1973_05uL_mz 100-1500_pos_1
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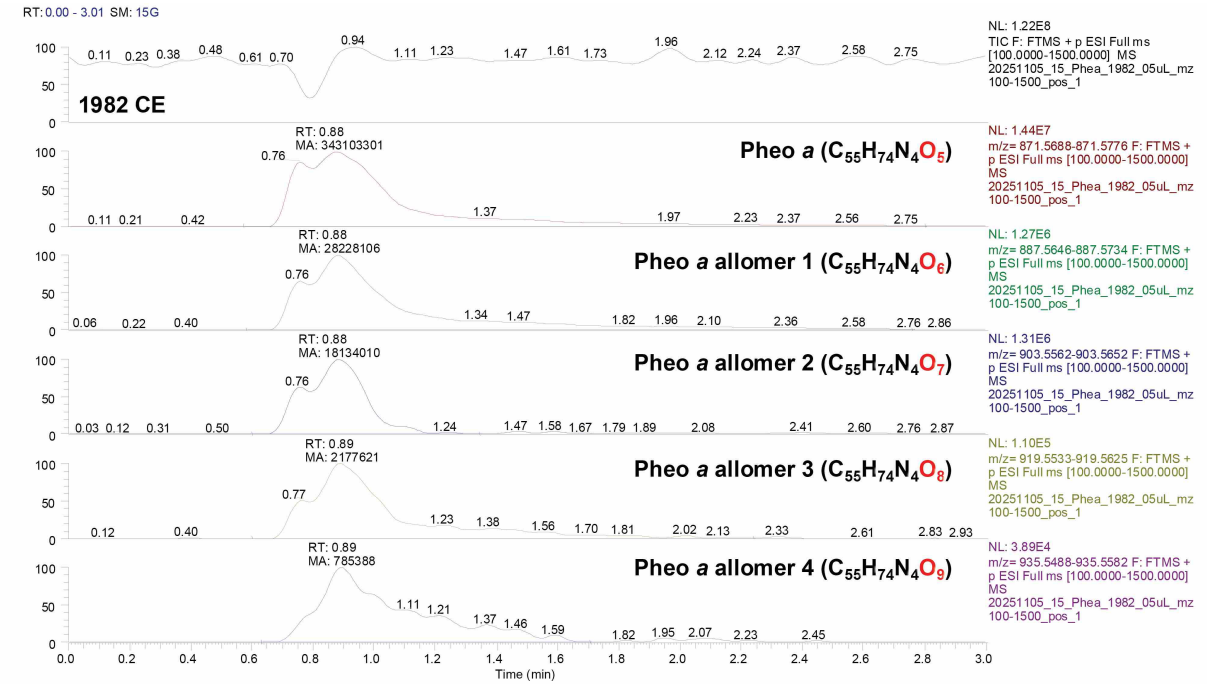
Time (min)

Figure S3d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1982 CE
NL: 1.22E8
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Pheo a (C55H74N4O5)
RT: 0.88
MA: 343103301
NL: 1.44E7
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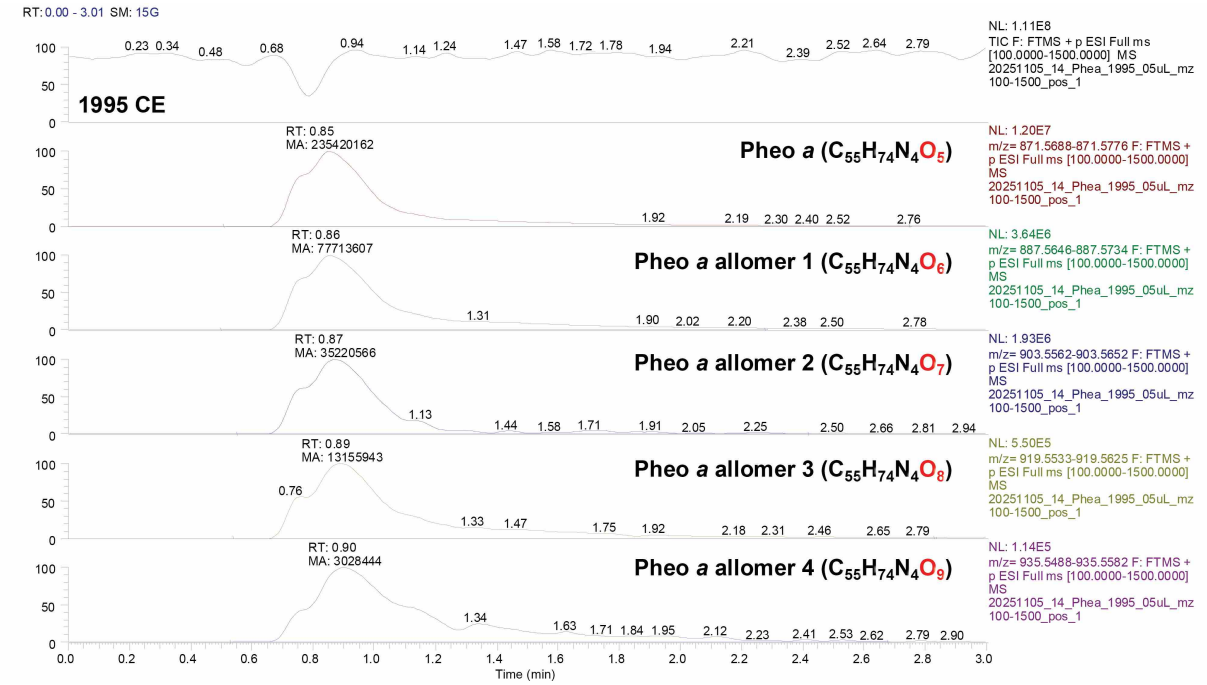
Pheo a allomer 1 (C55H74N4O6)
RT: 0.88
MA: 28228106
NL: 1.27E6
m/z= 887.5646-887.5734 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_15_Phea_1982_05uL_mz 100-1500_pos_1
Pheo a allomer 2 (C55H74N4O7)
RT: 0.88
MA: 18134010
NL: 1.31E6
m/z= 903.5562-903.5652 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_15_Phea_1982_05uL_mz 100-1500_pos_1
Pheo a allomer 3 (C55H74N4O8)
RT: 0.89
MA: 2177621
NL: 1.10E5
m/z= 919.5533-919.5625 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_15_Phea_1982_05uL_mz 100-1500_pos_1
Pheo a allomer 4 (C55H74N4O9)
RT: 0.89
MA: 785388
NL: 3.89E4
m/z= 935.5488-935.5582 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_15_Phea_1982_05uL_mz 100-1500_pos_1
Time (min)

Figure S3e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NL: 1.11E8
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RT: 0.86
MA: 77713607
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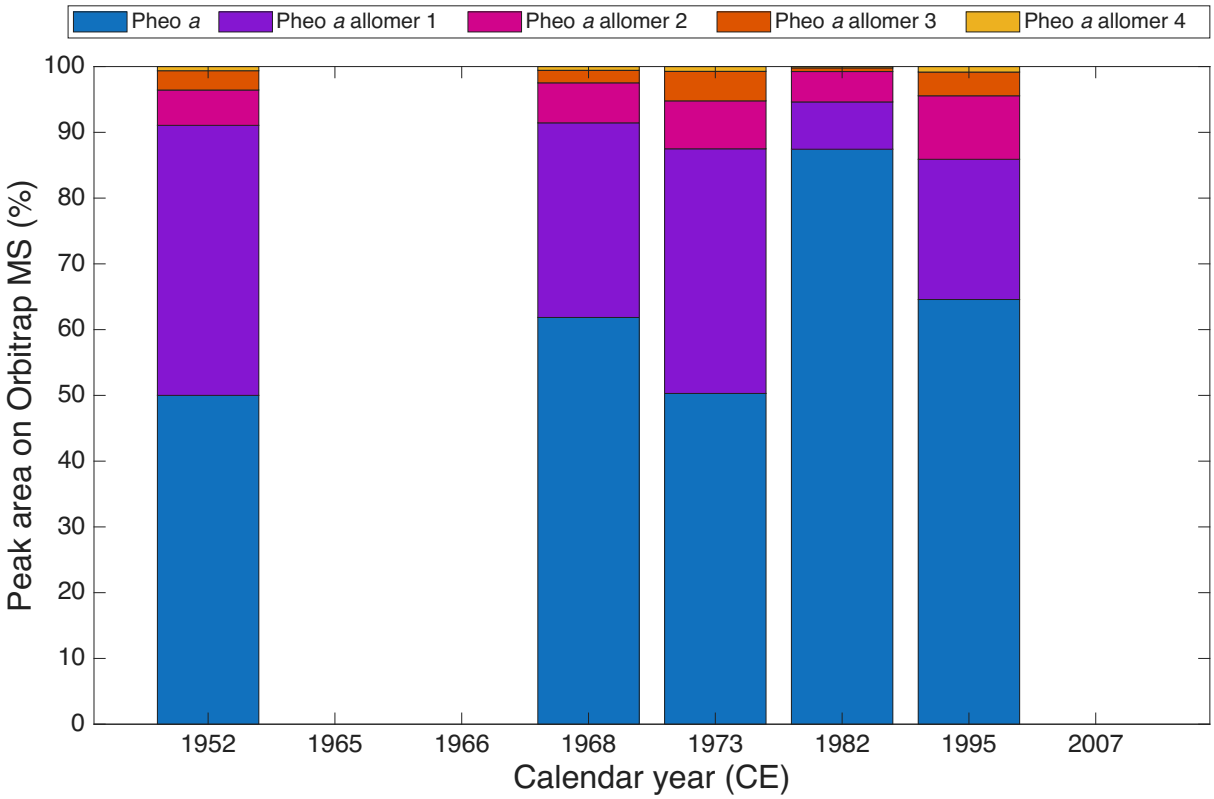
NL: 3.64E6
m/z= 887.5646-887.5734 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_14_Phea_1995_05uL_mz 100-1500_pos_1
RT: 0.87
MA: 35220566
Pheo a allomer 2 (C55H74N4O7)
NL: 1.93E6
m/z= 903.5562-903.5652 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_14_Phea_1995_05uL_mz 100-1500_pos_1
RT: 0.89
MA: 13155943
Pheo a allomer 3 (C55H74N4O8)
NL: 5.50E5
m/z= 919.5533-919.5625 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_14_Phea_1995_05uL_mz 100-1500_pos_1
RT: 0.90
MA: 3028444
Pheo a allomer 4 (C55H74N4O9)
NL: 1.14E5
m/z= 935.5488-935.5582 F: FTMS + p ESI Full ms [100.0000-1500.0000] MS 20251105_14_Phea_1995_05uL_mz 100-1500_pos_1
Time (min)

Figure S3f



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194 Figure S4

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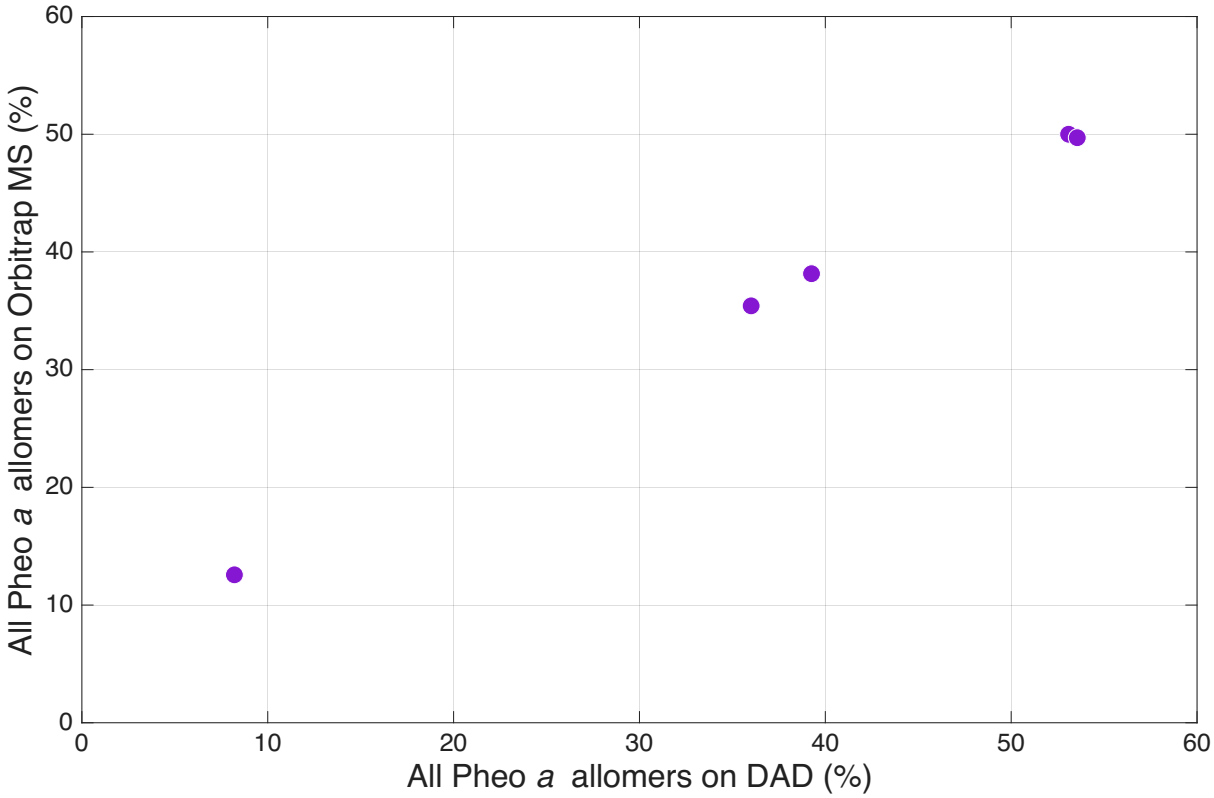

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197 Figure S5

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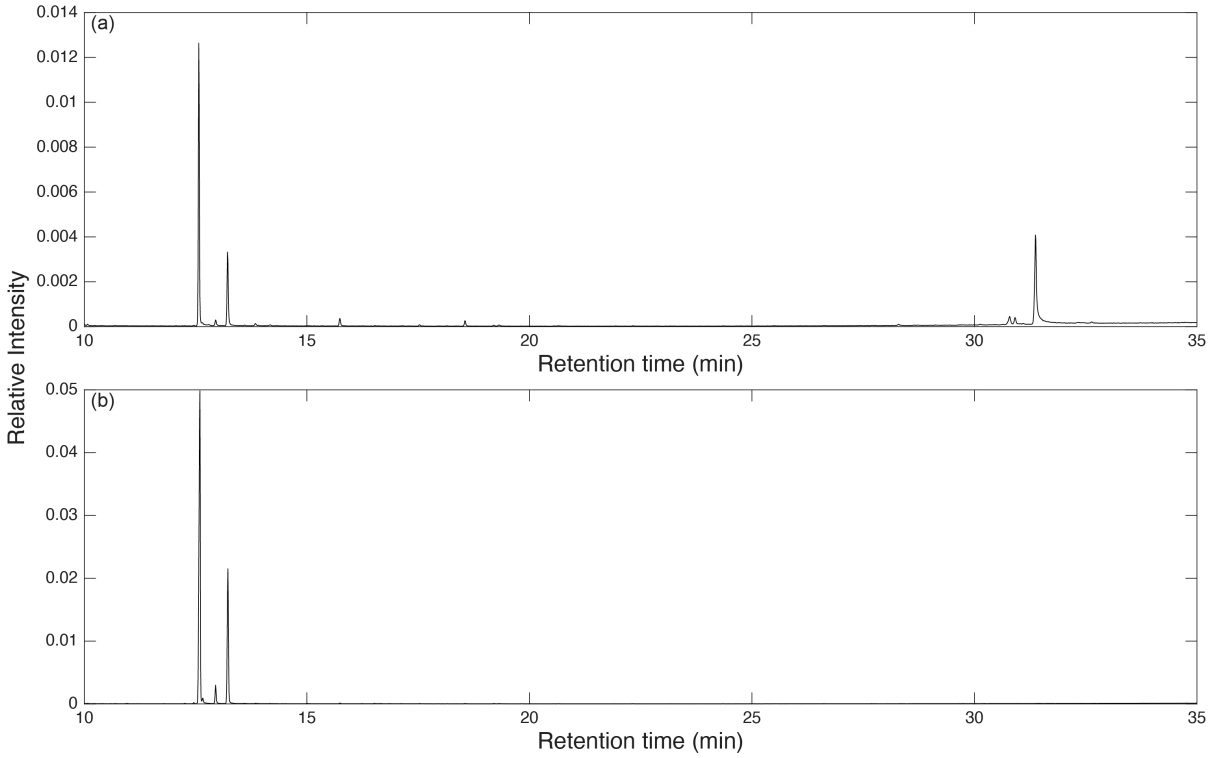
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(b)
Relative Intensity
Retention time (min)
Retention time (min)

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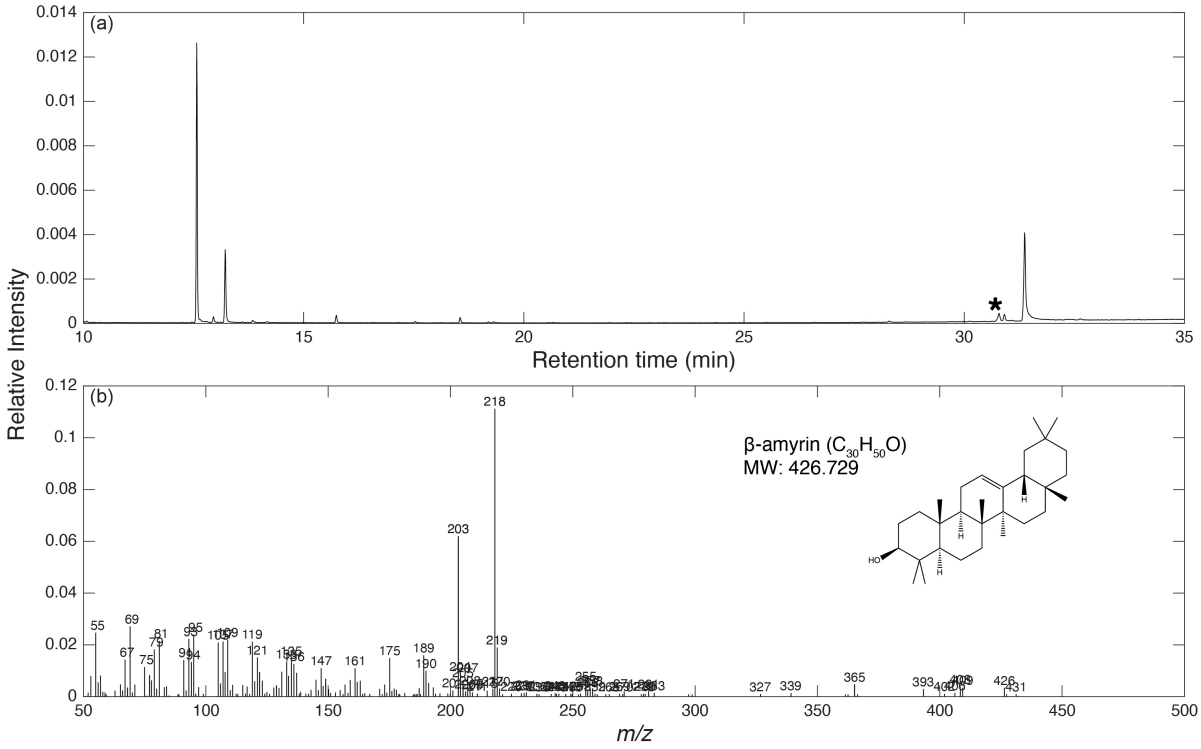
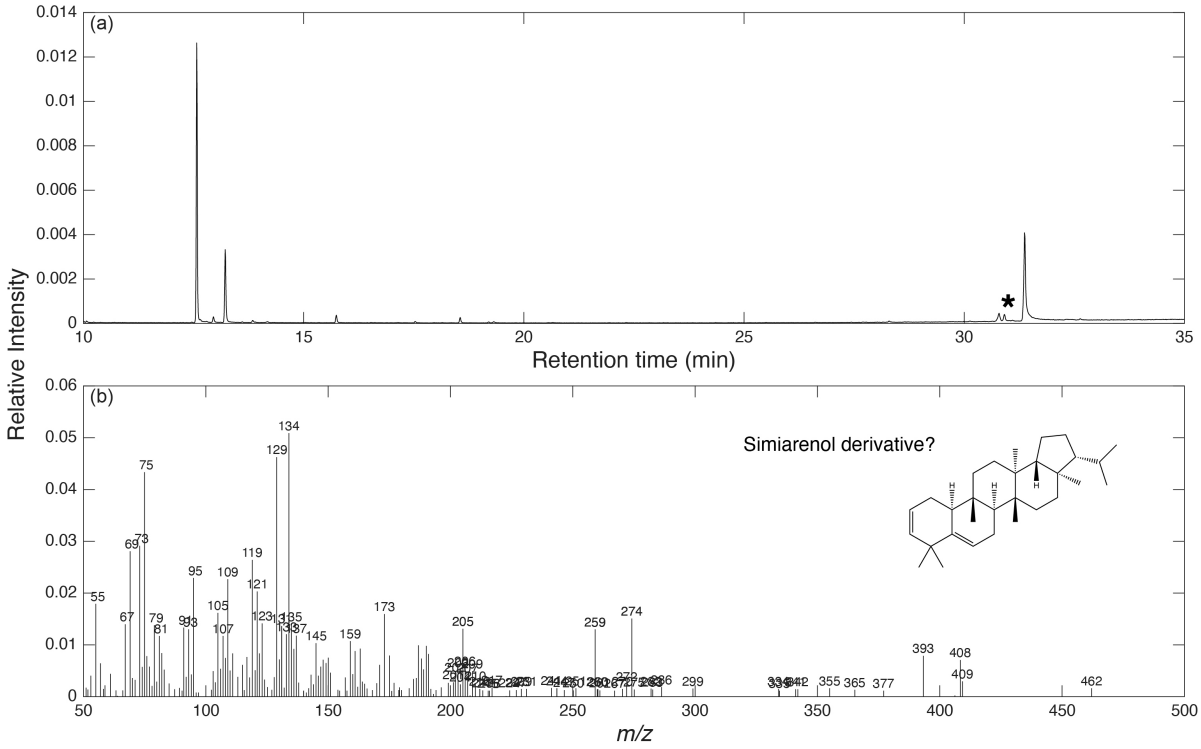

(a)
Relative Intensity
Retention time (min)
*
(b)
m/z
β-amyrin ($C_{30}H_{50}O$)
MW: 426.729
HO
H
218
203
219
69
55
67
75
79
81
95
94
105
109
119
121
147
161
175
189
190
327
339
365
393
426
431

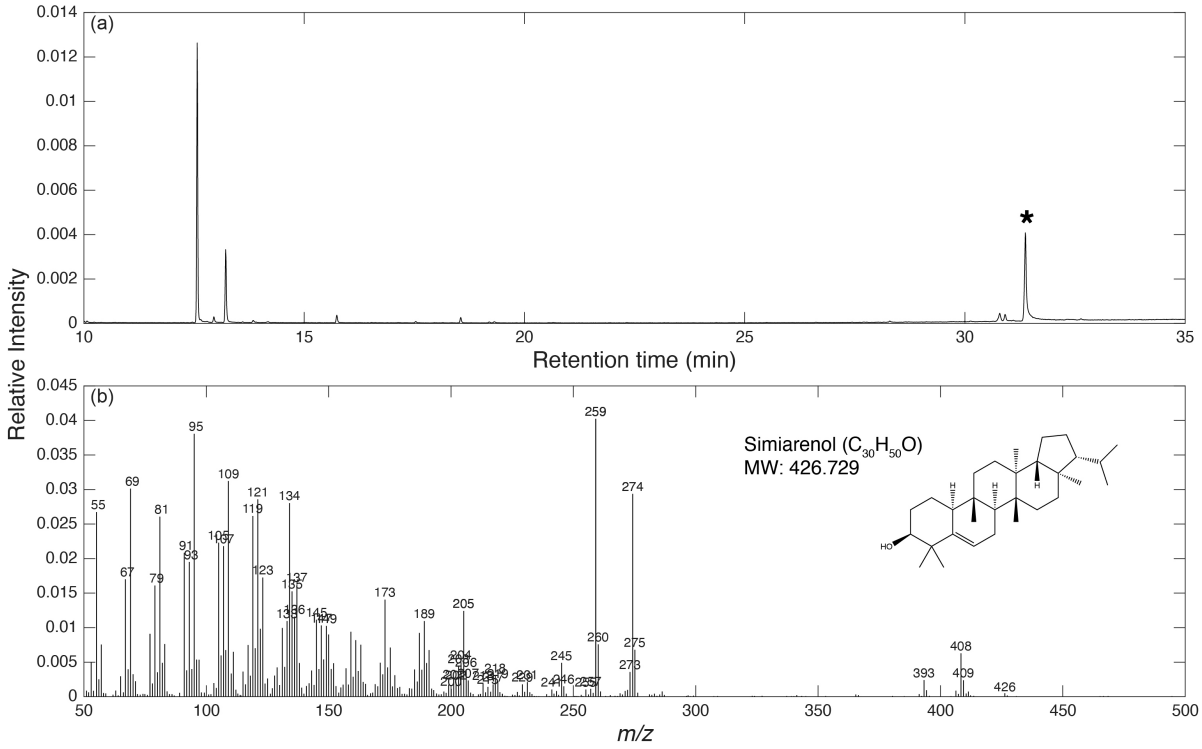
Figure S7



205

206 Figure S8

207



208

209 Figure S9

210

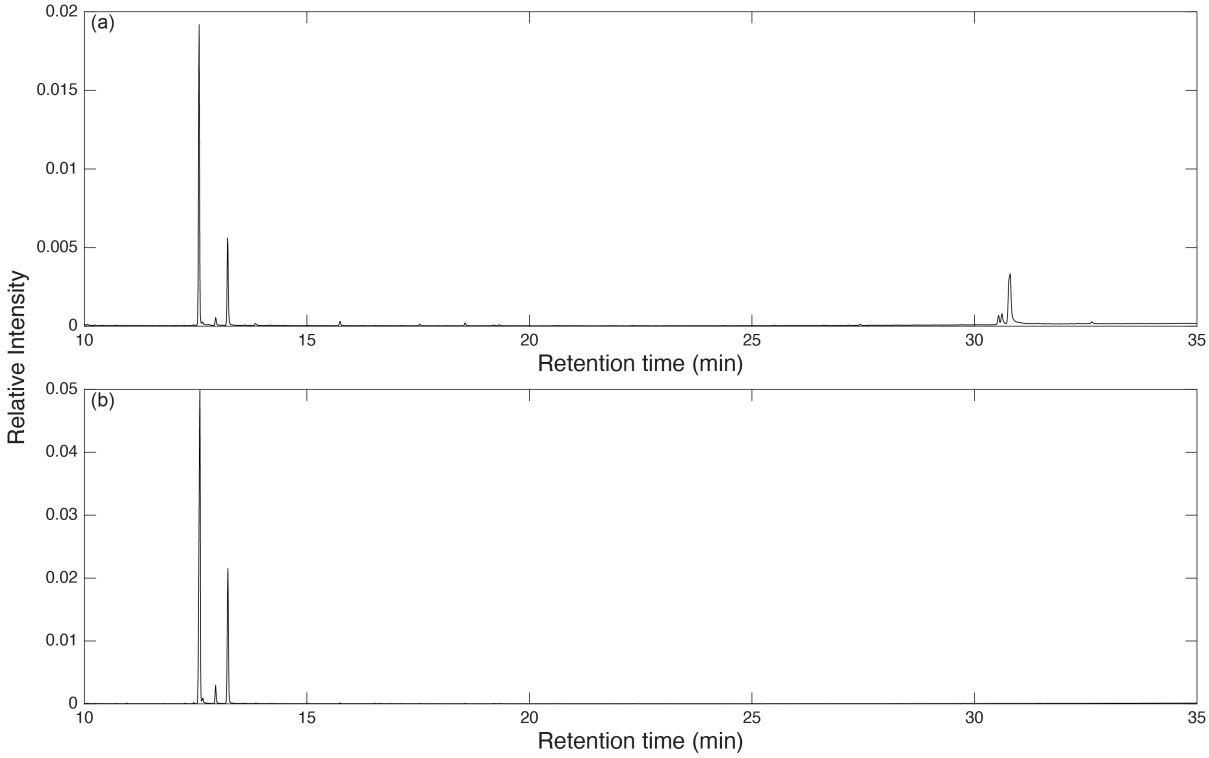
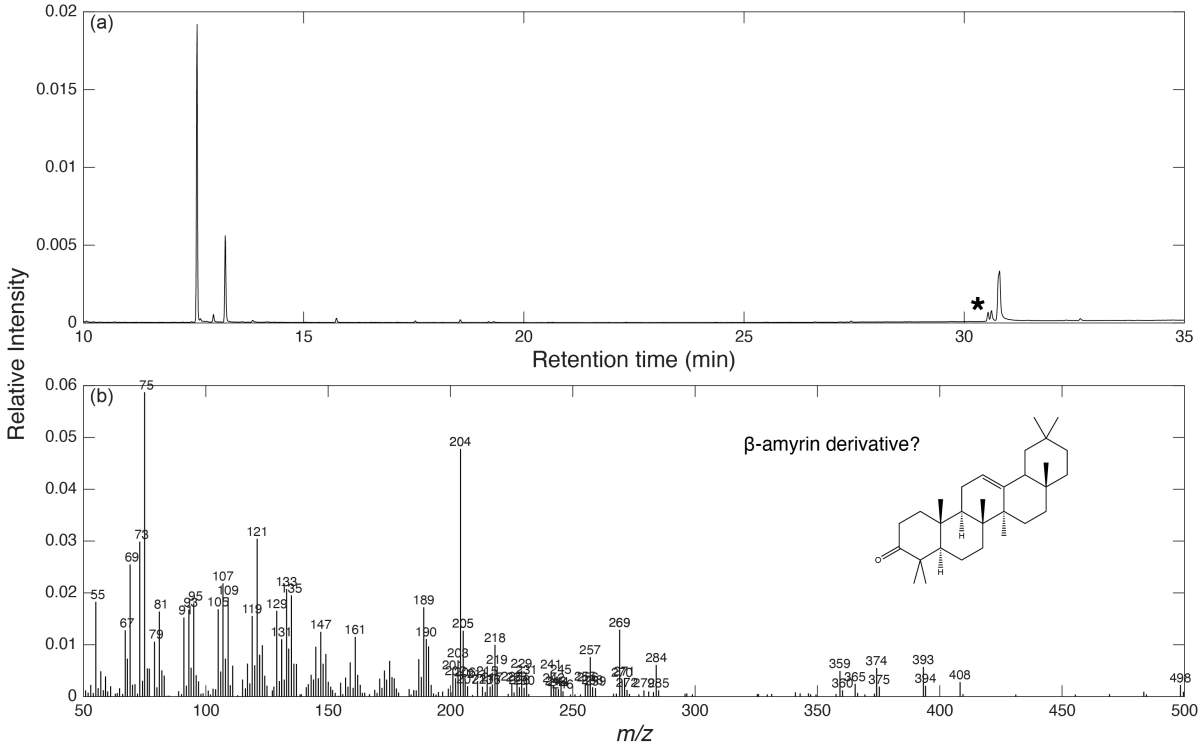

(a)
(b)
Relative Intensity
Retention time (min)
Retention time (min)
0.02
0.015
0.01
0.005
0
0.05
0.04
0.03
0.02
0.01
0
10
15
20
25
30
35

Figure S10



214

215 Figure S11

216

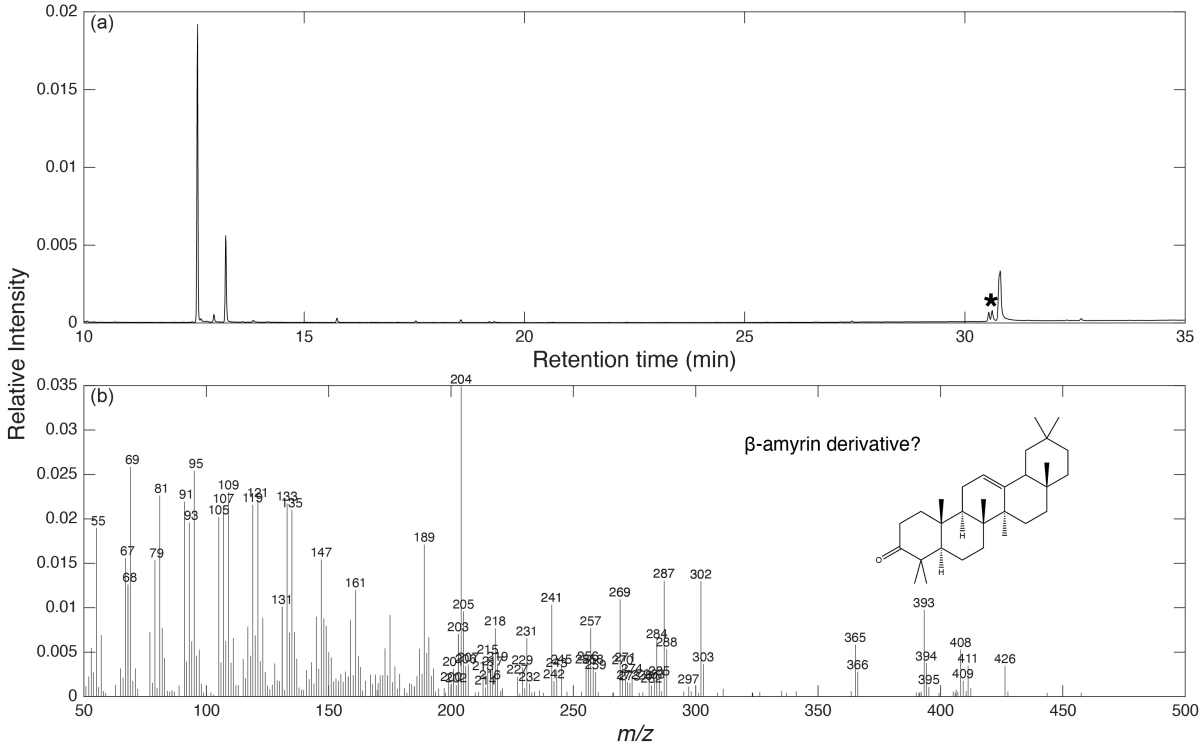

(a)
Relative Intensity
Retention time (min)
*
(b)
β-amyrin derivative?
m/z

Figure S12

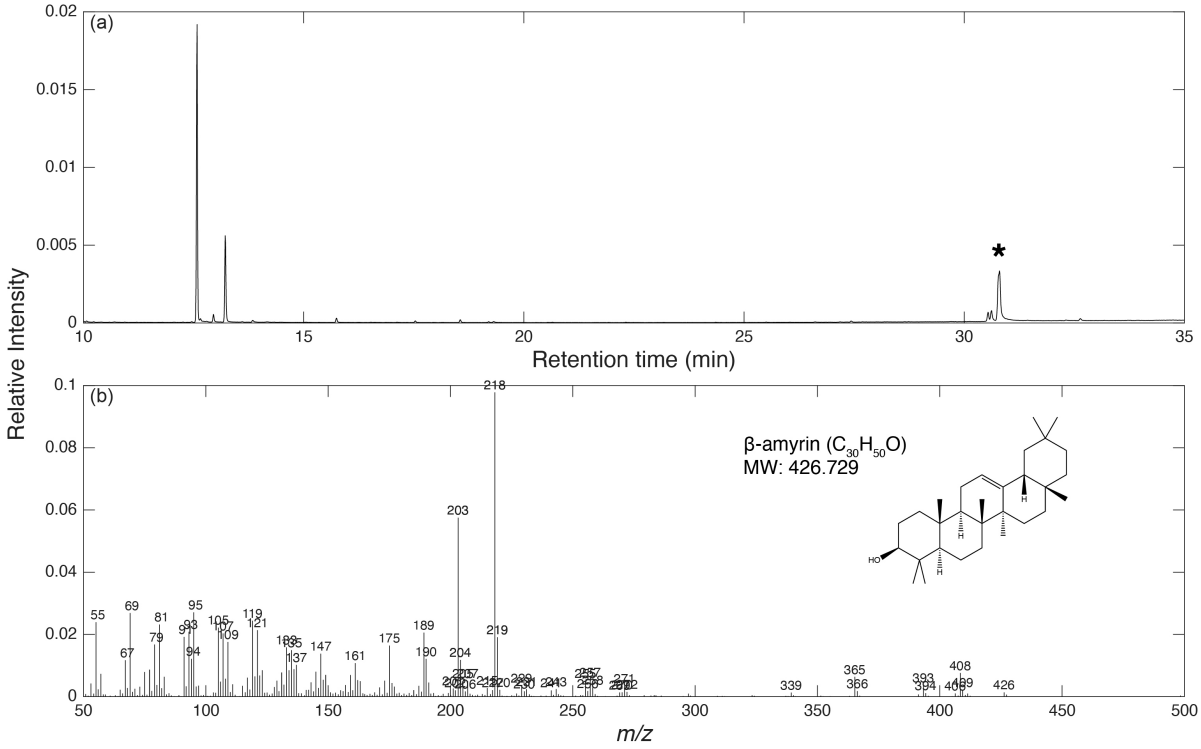

(a)
Relative Intensity
Retention time (min)
*
(b)
β-amyrin ($C_{30}H_{50}O$)
MW: 426.729
m/z

Figure S13

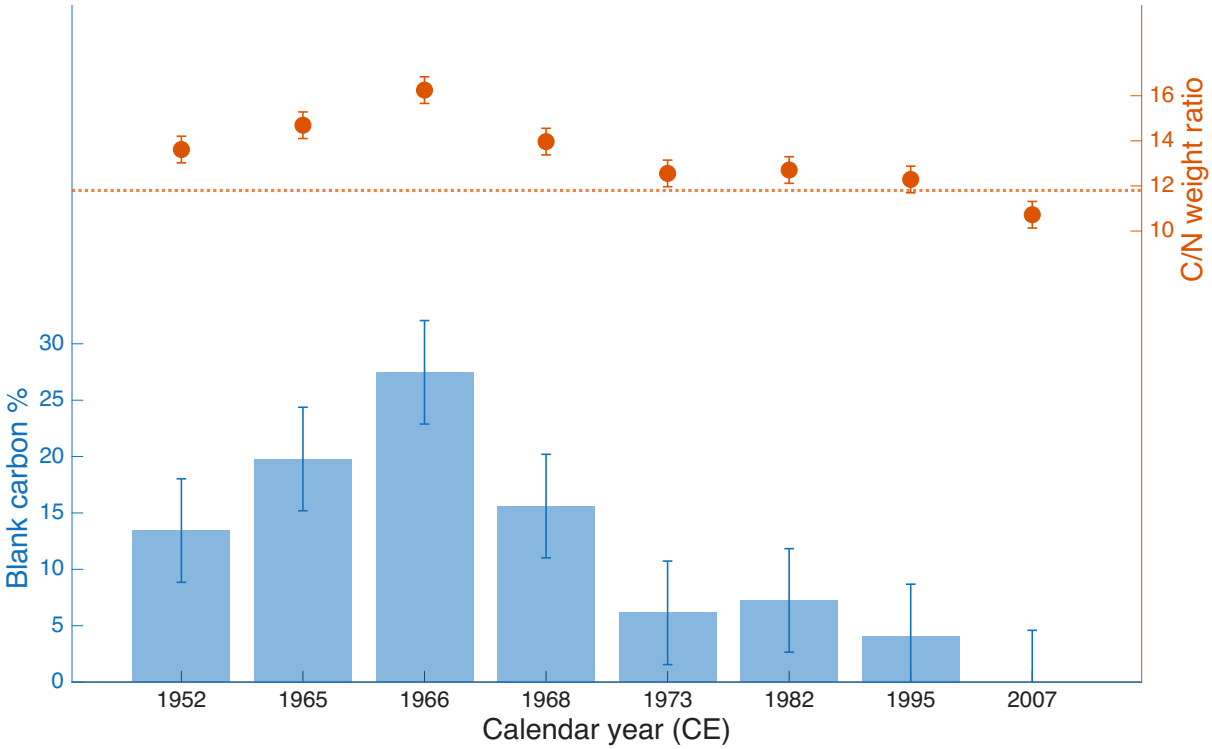

Blank carbon %
0
5
10
15
20
25
30
C/N weight ratio
10
12
14
16
1952
1965
1966
1968
1973
1982
1995
2007
Calendar year (CE)

Figure S14

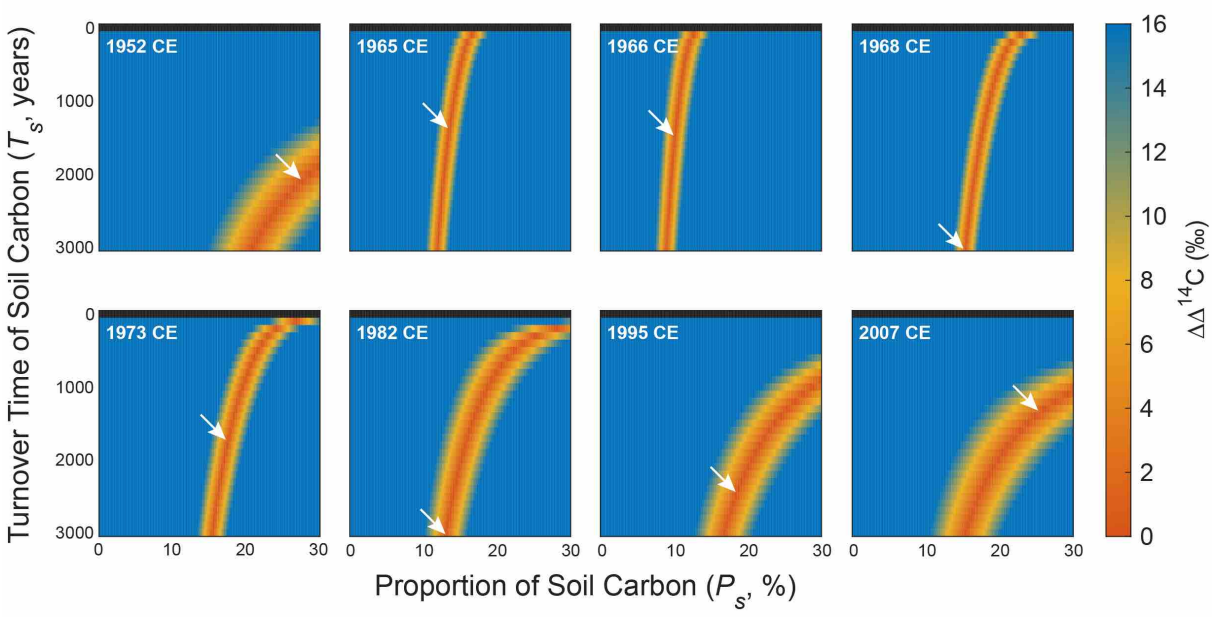
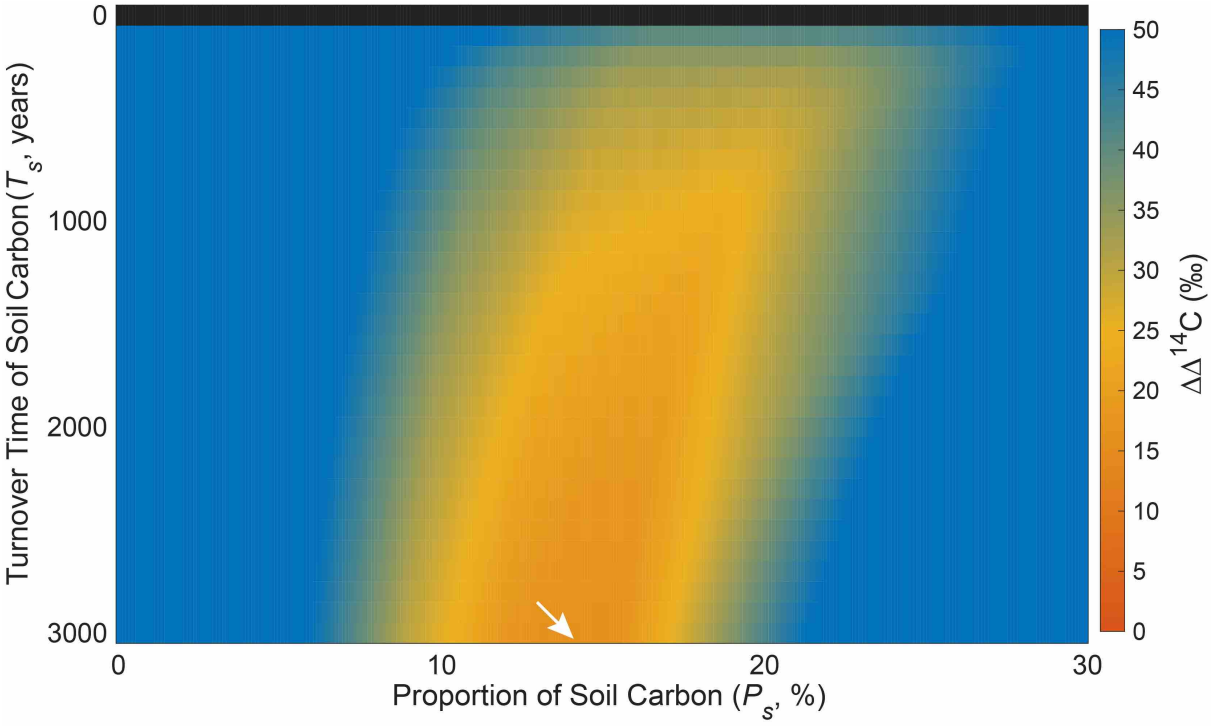

1952 CE
1965 CE
1966 CE
1968 CE
1973 CE
1982 CE
1995 CE
2007 CE
Turnover Time of Soil Carbon (T_s, years)
Proportion of Soil Carbon (P_s, %)
$\Delta\Delta^{14}C$ (‰)
0
1000
2000
3000
10
20
30
16
14
12
10
8
6
4
2

Figure S15



229

230 Figure S16