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Supplement of

Molecular characterization of dissolved organic matter from subtropical wetlands: a comparative study through the analysis of optical properties, NMR and FTICR/MS

N. Hertkorn et al.

Correspondence to: R. Jaffé (jaffer@fiu.edu)

Figure Captions:

Fig. S1. ^{1}H NMR spectra of six wetland SPE-DOM (CD_3OD; 500 MHz), acquired with solvent suppression and exclusion regions used in the computation of NMR section integrals and overlay NMR spectra (Fig. 2 and this figure) which denote HD_2COD and residual HDO, with section of unsaturated protons ($\delta_H > 5$ ppm) vertically expanded. Intensities are normalized to 100% total integral in the entire chemical shift range shown ($\delta_H = 0\ldots10$ ppm). Fundamental substructures are indicated from higher to lower field (from right to left), (a) aliphatics, **H**CCC; (b) "acetate-analogue", **H₃**CC(=O)-O-; (c) carboxyl-rich alicyclic materials (CRAM), **H**C(C)-COX; (d) "carbohydrate-like" and methoxy, **H**CO; (e) olefinic, **H**C=C; and (f) aromatic NMR resonances **H**C_{ar} (cf. text). Further division of unsaturated protons provided (f_1) polycyclic and polycarboxylated aromatics as well as six-membered nitrogen heterocycles ($\delta_H > 8$ ppm); (f_2) electron withdrawing substituents (COX; Perdue et al., 2007; $\delta_H \approx 7.3 - 8.0$ ppm); (f_3) electroneutral substituents (alkyl, H, R; $\delta_H \approx 7.0 - 7.3$ ppm); (f_4) electron-donating substituents (OR, OH, phenolics; $\delta_H \approx 6.5 - 7.0$ ppm); (e_1) polarized and conjugated olefins; ($\delta_H \approx 5.5 - 6.5$ ppm); (e_2) isolated olefins ($\delta_H \approx 5.0 - 5.5$ ppm), with conceivable contributions from anomeric protons and ester groups (cf. discussion of 2D NMR spectra).

Fig. S2. ^{1}H NMR spectra of wetland SPE-DOM (CD_3OD; 800 MHz); visual overlay: intensities are normalized to total area in the entire chemical shift range shown (δ_H = 0-10 ppm), with exclusion of residual water and methanol NMR resonances; (left column) entire NMR spectrum (δ_H = 0-10 ppm); (center column) section of unsaturated protons (δ_H = 5 - 10 ppm); (right column) section of aliphatic protons (δ_H = 0 - 5 ppm). Panel A: OKA; panel B: PAN, and panel C: FCE SPE-DOM. Manual overlay according to identical ^{1}H NMR section integral in the respective regions of ^{1}H NMR chemical shift shown. Section of unsaturated protons are denoted as follows (cf. main text): (f1) polycyclic and polycarboxylated aromatics as well as six-membered nitrogen heterocycles ($\delta_H > 8$ ppm); (f2) electron withdrawing substituents (COX; Perdue et al., 2007; ($\delta_H \approx 7.3 - 8.0$ ppm); (f3) electroneutral substituents (alkyl, H, R; $\delta_H \approx 7.0 -$ 7.3 ppm); (f4) electron-donating substituents (OR, OH, phenolics; $\delta_H \approx 6.5 - 7.0$ ppm); (e1) polarized and conjugated olefins; ($\delta_H \approx 5.5 - 6.5$ ppm); (e2) isolated olefins ($\delta_H \approx 5.0 - 5.5$

ppm). Panel D-F: ^{1}H NMR spectra (800 MHz) of three organic matter preparations, acquired in D_2O (blue) and CD_3OD (black, gray): overlay with manually adjusted amplitude for respective sections of chemical shift shown, for improved visual assessment of relative similarity and differences. Panel D: ultrafiltered organic matter FCE-S UDOM (due to the very intense lipid NMR resonance at $\delta_H = 1.28$ ppm, vertical expansions are provided with two intensities to also allow for comparison of non-lipid NMR resonances); panel E: fulvic acid FCE-L FA (panel ΔE shows difference NMR spectra D_2O minus CD_3OD); panel F: SPE-DOM obtained by solid phase extraction with PPL cartridges (FCE-S PPL; panel ΔF shows difference NMR spectra D_2O minus CD_3OD).

Fig. S3. ^{13}C NMR spectra of selected wetland SPE-DOM; full spectra computed with 35 Hz exponential line broadening; insert: section of methoxy peaks (δ_C = 51-59 ppm; computed with 2 Hz line broadening); OKA-L and PAN-S: in $^{12}CD_3OD$ at B_0 = 11.7 T; FCE in CD_3OD at B_0 = 18.8 T.

Fig. S4. ^{1}H, ^{13}C HSQC NMR spectrum of SPE-DOM FCE-S, with regions shown in figures: (A) chemical environments of sp^{3}-hybridized carbon (aliphatic CH_n units; Fig. 6); (B) chemical environments of sp^{2}-hybridized carbon (unsaturated, i. e. olefinic and aromatic CH units; Fig. 5). Sensitivity enhanced apodization is used to emphasize less abundant sp^{2}-hybridized carbon (overall HSQC cross peak integral <4% of aliphatic units) environments at the cost of resolution in case of aliphatic CH_n units ($n = 1 – 3$).

Fig. S5. Overlay of ^{1}H, ^{13}C HSQC NMR spectra of SPE-DOM FCE-S (dark blue) and South Atlantic SPE-DOM at fluorescence maximum (48 mg, FMAX; orange: Hertkorn et al., 2013), together with region of HSQC NMR cross peaks accessible for single aromatic rings with full range of electron-withdrawing (COX), electroneutral (R, H) and electron donating substitution (OH, OR), shown in green color (SPARIA: Perdue et al., 2007). Wetland SPE-DOM shows more exhaustive coverage of single aromatic rings from contributions of multiply oxygenated aromatics ($\delta_H < 7$ ppm; $\delta_C < 120$ ppm), likely originating from plant phenolics but also from polycarboxylated aromatics and PAH derivatives ($\delta_H > 8.5$ ppm). In contrast, open ocean SPE-DOM FMAX exhibits a larger abundance as well as overall chemical diversity of α,β unsaturated and C-conjugated olefins, likely originating from marine natural products; for assignment of HSQC cross peaks, see Figs. 4 and 5, and Hertkorn et al., 2013.

Fig. S6. Further evaluation of aliphatic spin systems of wetland SPE-DOM FCE-L. Panel A: overall ^{1}H, ^{1}H JRES NMR spectrum with sections a_1, a_2, a_3, denoting the area of panels B, C, D, which display ^{1}H NMR projections along JRES and ^{1}H, ^{13}C DEPT HSQC NMR spectra (copied from Fig. 6); panel B: section of $\mathrm{OC}\underline{\mathbf{H}}$ aliphatic units, demonstrating (section b_1) presence of intense JRES cross peaks from $\mathrm{O}\underline{\mathbf{CH_3}}$ groups, with absence of J_{HH} splittings; panel C: section of aliphatic $\mathrm{CC}\underline{\mathbf{H}}$ units, with dominance of $\mathrm{HOOC\text{-}C}\underline{\mathbf{H_n}}\mathrm{\text{-}CH_2\text{-}}$ units (triplet J_{HH} splitting; n = 1, 2) over $\mathrm{HOOC\text{-}C}\underline{\mathbf{H_n}}\mathrm{\text{-}CH_{,}\text{-}}$ units (doublet J_{HH} splitting; n = 1, 2) shown in section c_1; section c_2 indicates panel D; panel D: section of aliphatic $\mathrm{CCC}\underline{\mathbf{H}}$ units, showing a remarkable clustering of $\underline{\mathbf{H_3}}\mathrm{C\text{-}CH\text{-}}$ units at $\delta_{\mathbf{H}}$: 1.0 – 1.4 ppm, which indicate pronounced aliphatic branching in section d_1 (doublet splitting from J_{HH}), whereas ethyl groups $\underline{\mathbf{H_3}}\mathrm{C\text{-}CH_2\text{-}}$ dominate the low field section $\delta_H < 1$ ppm (section d_2).

Fig. S7. Mass edited H/C ratios from negative electrospray 12T FTICR mass spectra of Wetlands SPE-DOM: (A) OKA-L; (B) OKA-S; (C) PAN-L; (D) PAN-S; (E) FCE-L; (F) FCE-S. Insert histograms show the number of assigned molecular compositions. Colour code for elemental compositions bearing combinations of C, H, O, N, and S atoms are defined as follows: blue (CHO), orange (CHNO), green (CHOS) and red (CHNOS). Bubble areas reflect the relative intensities of each mass peak.

Fig. S8. Comparative analysis of van Krevelen diagrams derived from negative electrospray 12T FTICR mass spectra derived from four Pantanal and Okavango SPE-OM only. (A) Clustering diagram based on the similarity values between the FTICR mass spectra of these four SPE-DOM; (B) Molecular compositions with rather high abundance in both Okavango SPE-DOM; (C) Molecular compositions with rather high abundance in both Pantanal SPE-DOM, with color code according to molecular series (cf. text). The bright blue triangle denotes aromatic compounds, with aromaticity index AI > 0.5 (Koch and Dittmar, 2006); see Fig. 9 and attendant discussion.

Table S1. ^{1}H NMR section integral (800 MHz) of three different organic matter preparations in two different solvents, namely D_2O and CD_3OD, acquired at 283 K (cf. text).

Table S2. (Top): ^{13}C NMR section integrals (percent of total carbon) and key substructures of wetland SPE-DOM. Bottom: Substructures used for basic NMR-derived reverse mixing model with nominal H/C and O/C ratios given (Hertkorn et al., 2013).

Table S3. Acquisition parameters of NMR spectra, shown according to figures. PK: probeheads used for acquisition of NMR spectra, 8QCI: cryogenic inverse geometry 5 mm z-gradient ^{1}H/^{13}C/^{15}N/^{31}P QCI probe (B_0 = 18.8 T); 8QCO: cryogenic classical geometry 3 mm z-gradient ^{1}H/^{13}C/^{15}N/^{31}P probe (B_0 = 18.8 T); 5TXI: cryogenic inverse geometry 5 mm z-gradient ^{1}H, ^{13}C, ^{15}N probe (B_0 = 11.7 T); 5D: cryogenic classical geometry 5 mm z-gradient ^{13}C, ^{1}H probe (B_0 = 11.7 T); NS: number of scans (for 2D NMR: F2); AQ: acquisition time [ms]; D1: relaxation delay [ms]; NE: number of F1 increments in 2D NMR spectra; WDW1, WDW2: apodization functions in F1/ F2 (EM/GM: line broadening factor [Hz]; QS: shifted square sine bell; SI: sine bell); PR1, PR2: coefficients used for windowing functions WDW1, WDW2, EM/GM are given in [Hz], SI/QS derived functions indicate shift by π/n.

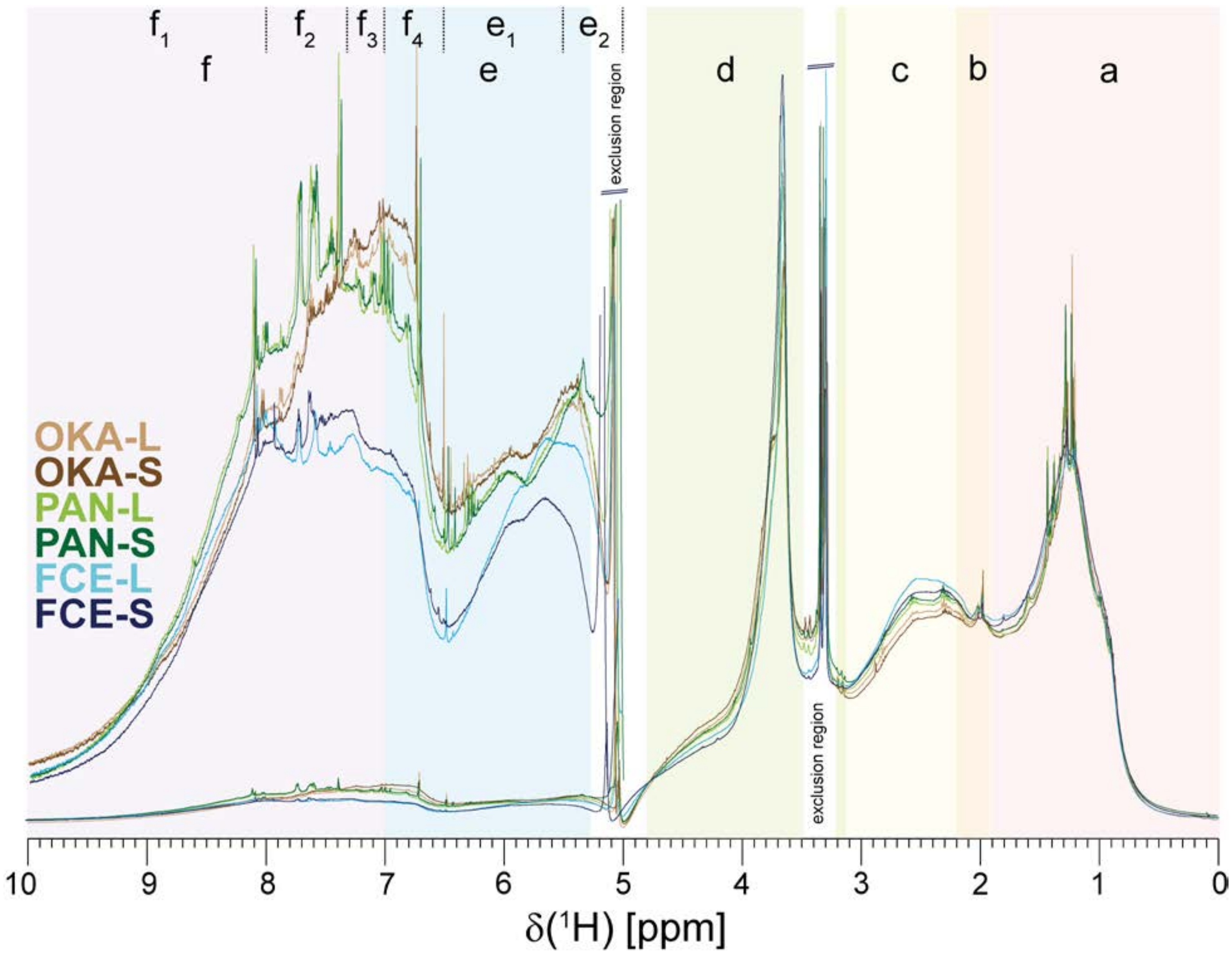
f1
f2
f3
f4
e1
e2
f
e
exclusion region
d
c
b
a
exclusion region
OKA-L
OKA-S
PAN-L
PAN-S
FCE-L
FCE-S
10
9
8
7
6
5
4
3
2
1
0
δ(1H) [ppm]

Fig. S1.

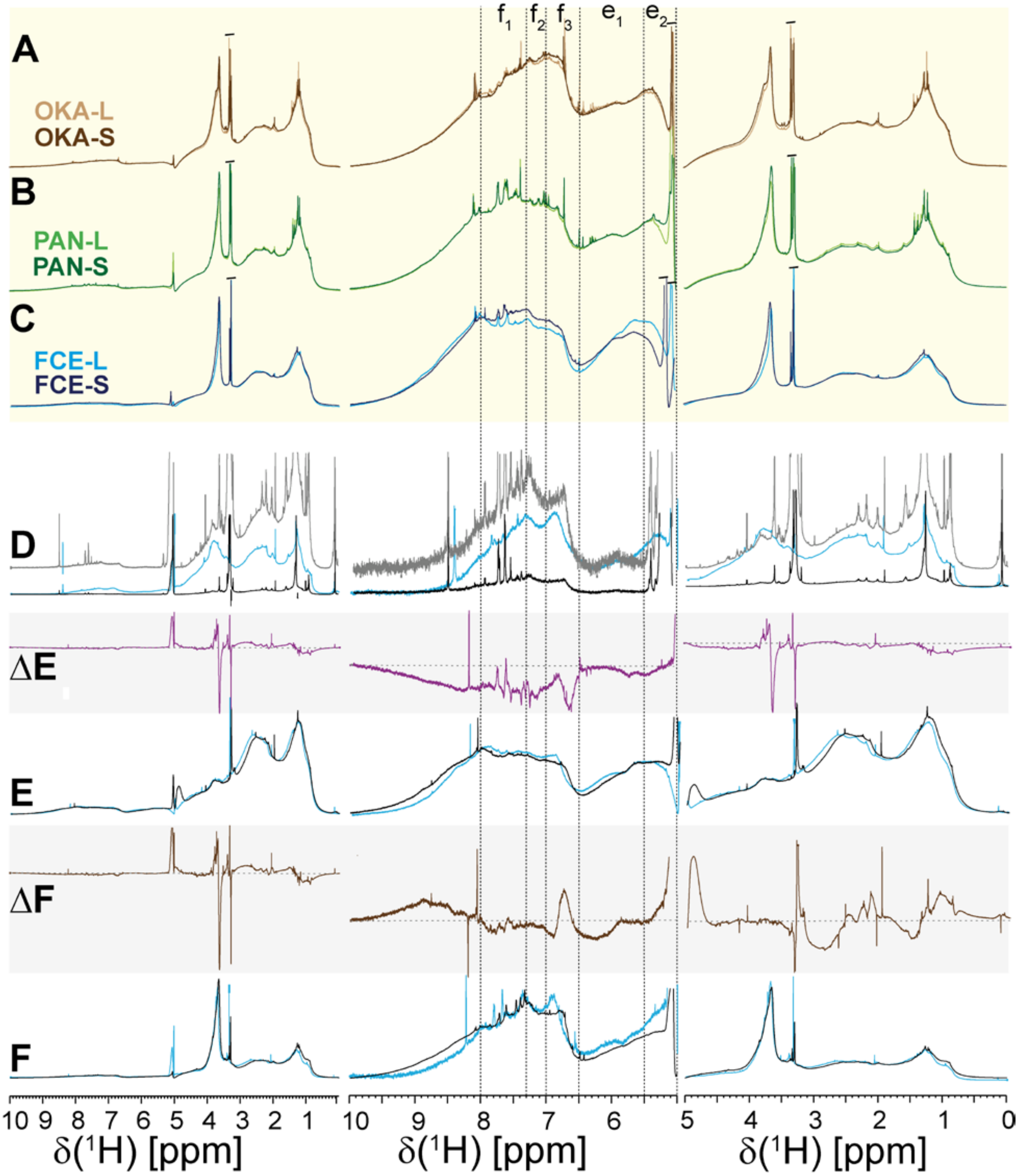
f1
f2
f3
e1
e2
A
OKA-L
OKA-S
B
PAN-L
PAN-S
C
FCE-L
FCE-S
D
ΔE
E
ΔF
F
10 9 8 7 6 5 4 3 2 1
10 9 8 7 6 5
5 4 3 2 1 0
δ(1H) [ppm]
δ(1H) [ppm]
δ(1H) [ppm]

Fig. S2.

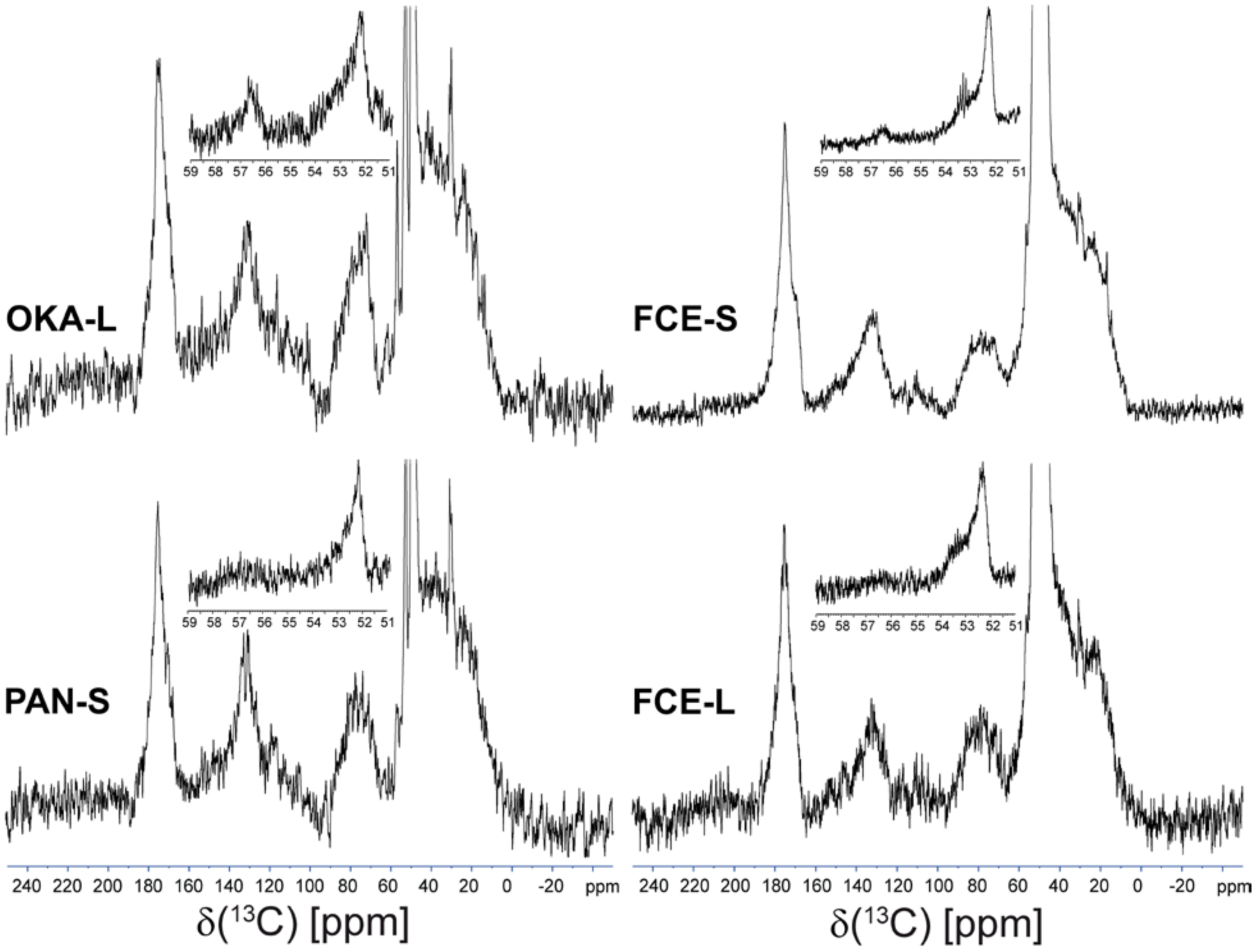
OKA-L
FCE-S
PAN-S
FCE-L
59 58 57 56 55 54 53 52 51
240 220 200 180 160 140 120 100 80 60 40 20 0 -20 ppm
δ(13C) [ppm]

Fig. S3.

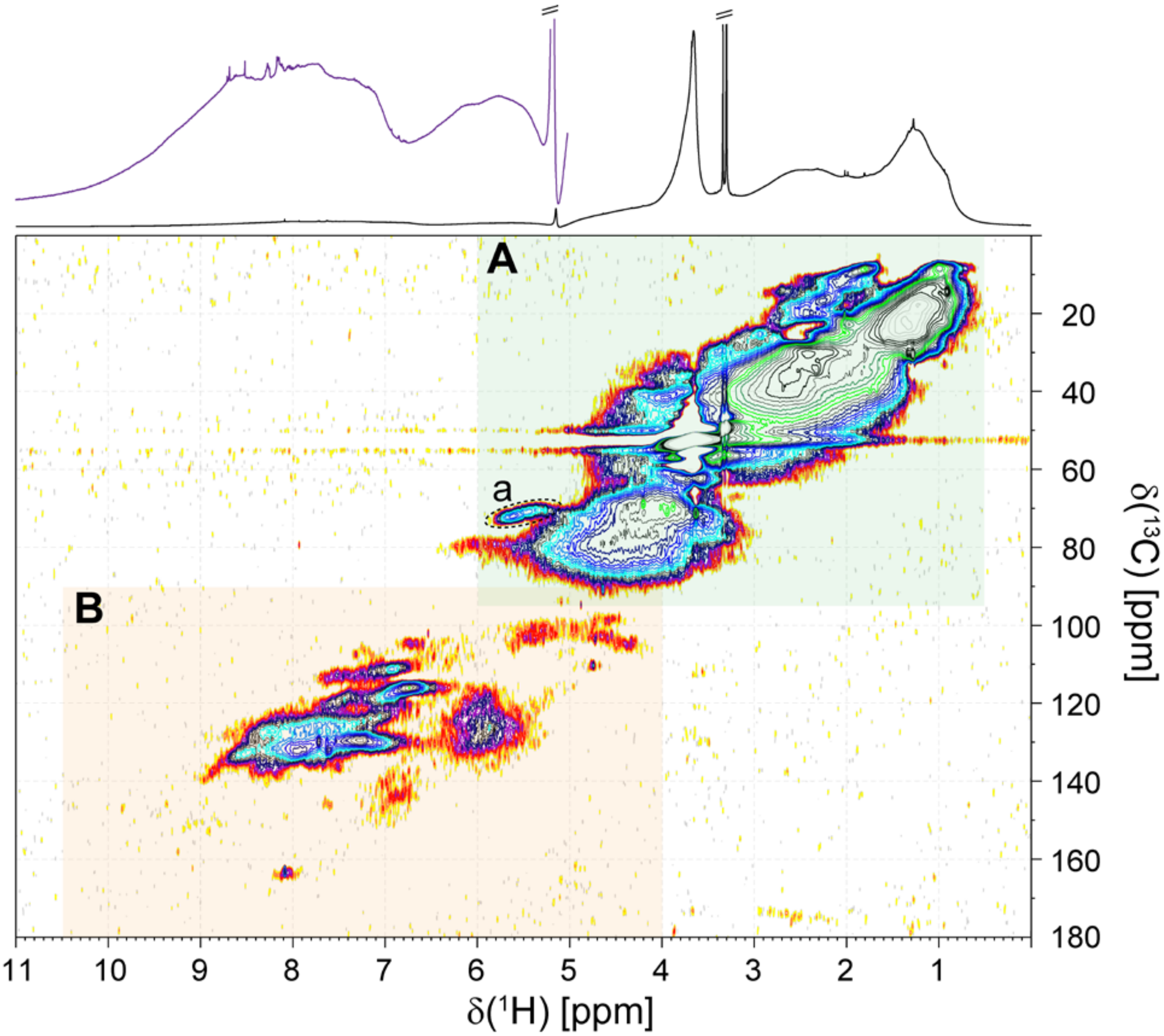
A
B
a
δ(¹³C) [ppm]
20
40
60
80
100
120
140
160
180
11
10
9
8
7
6
5
4
3
2
1
δ(¹H) [ppm]

Fig. S4.

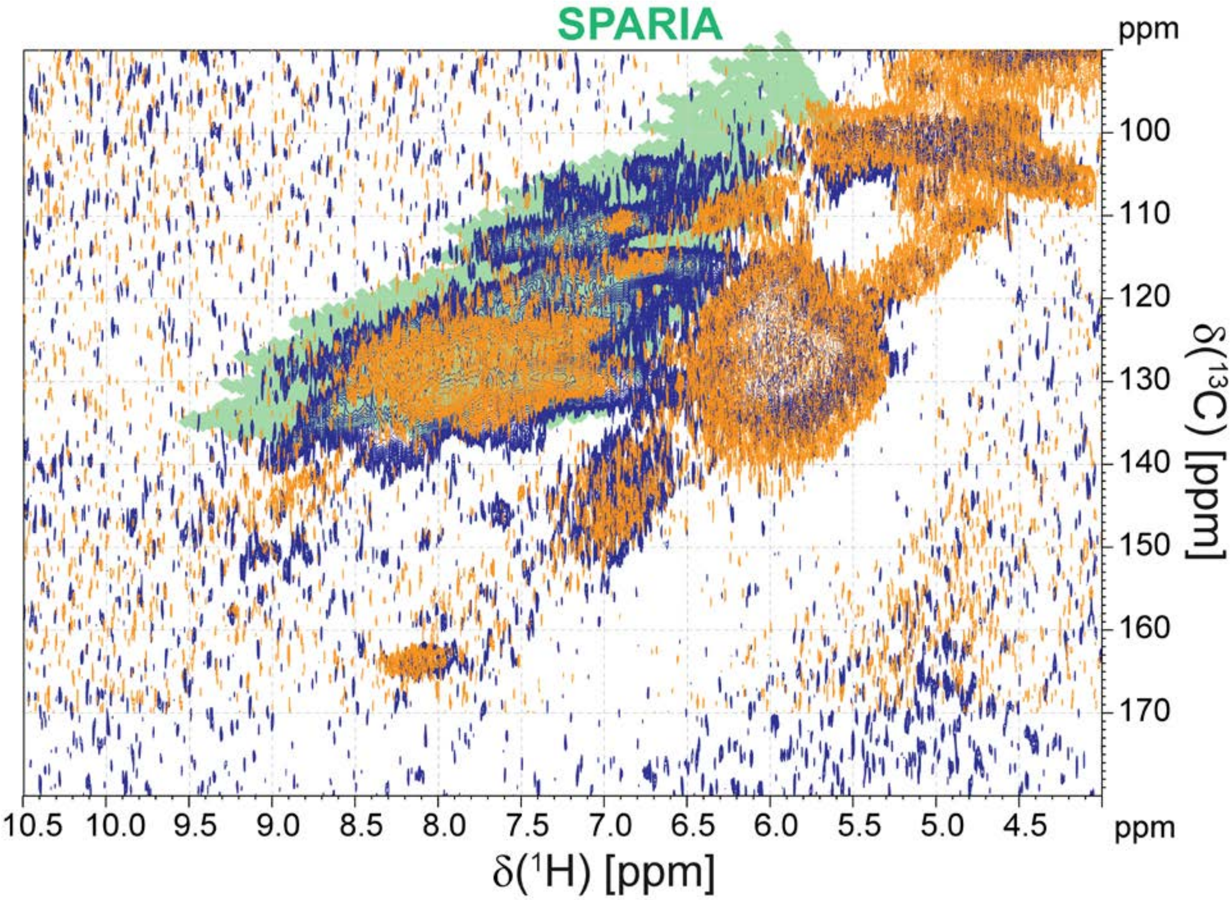
SPARIA
ppm
100
110
120
130
140
150
160
170
δ(^{13}C) [ppm]
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
ppm
δ(^{1}H) [ppm]

Fig. S5.

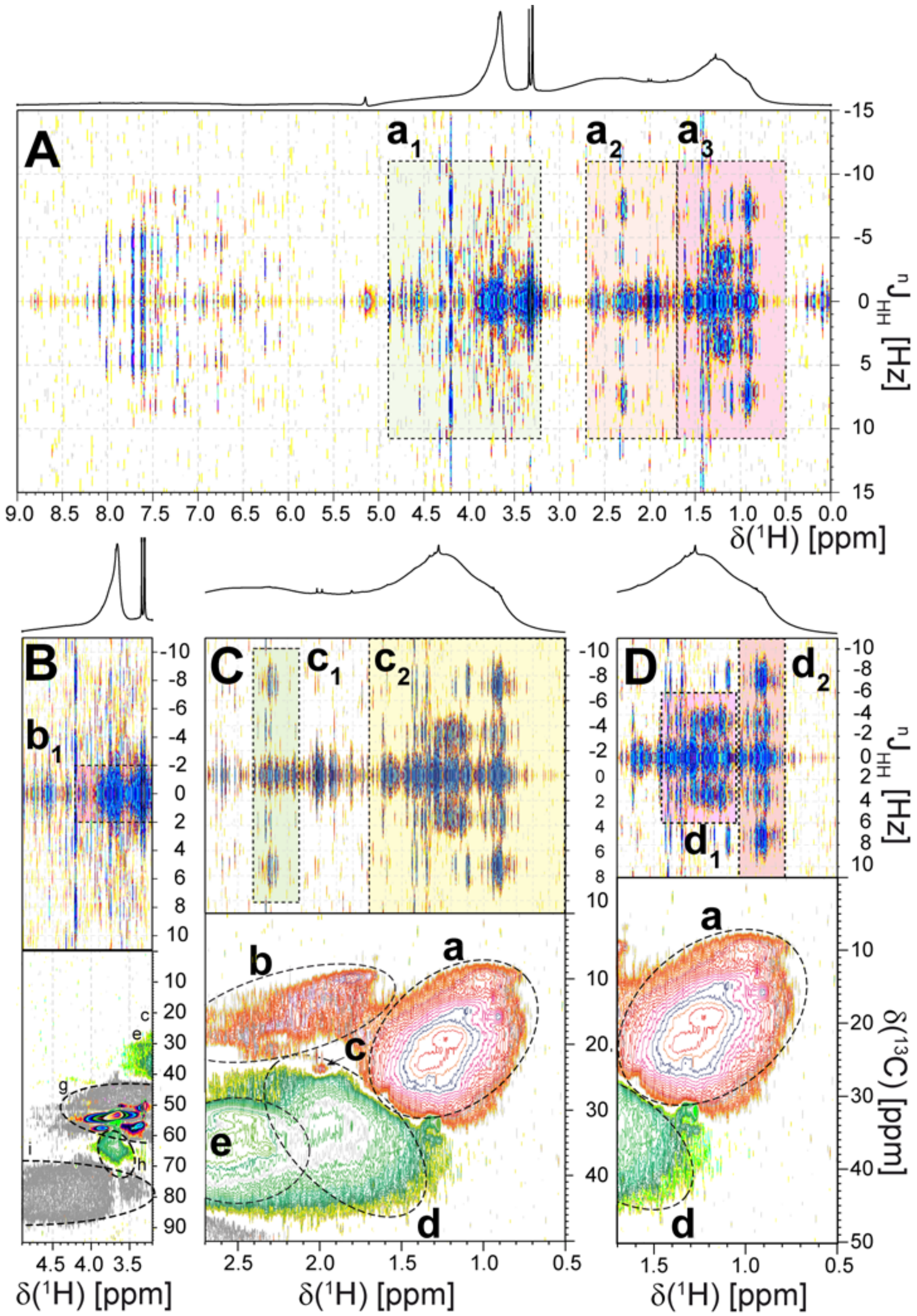
A
a1
a2
a3
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
δ(¹H) [ppm]
-15 -10 -5 0 5 10 15
ⁿJ_HH [Hz]
B
b1
-10 -8 -6 -4 -2 0 2 4 6 8 10
10 20 30 40 50 60 70 80 90
c
e
g
h
i
4.5 4.0 3.5
δ(¹H) [ppm]
C
c1
c2
-10 -8 -6 -4 -2 0 2 4 6 8 10
a
b
c
d
e
10 20 30 40
2.5 2.0 1.5 1.0 0.5
δ(¹H) [ppm]
D
d1
d2
-10 -8 -6 -4 -2 0 2 4 6 8 10
ⁿJ_HH [Hz]
a
d
10 20 30 40 50
δ(¹³C) [ppm]
1.5 1.0 0.5
δ(¹H) [ppm]

Fig. S6.

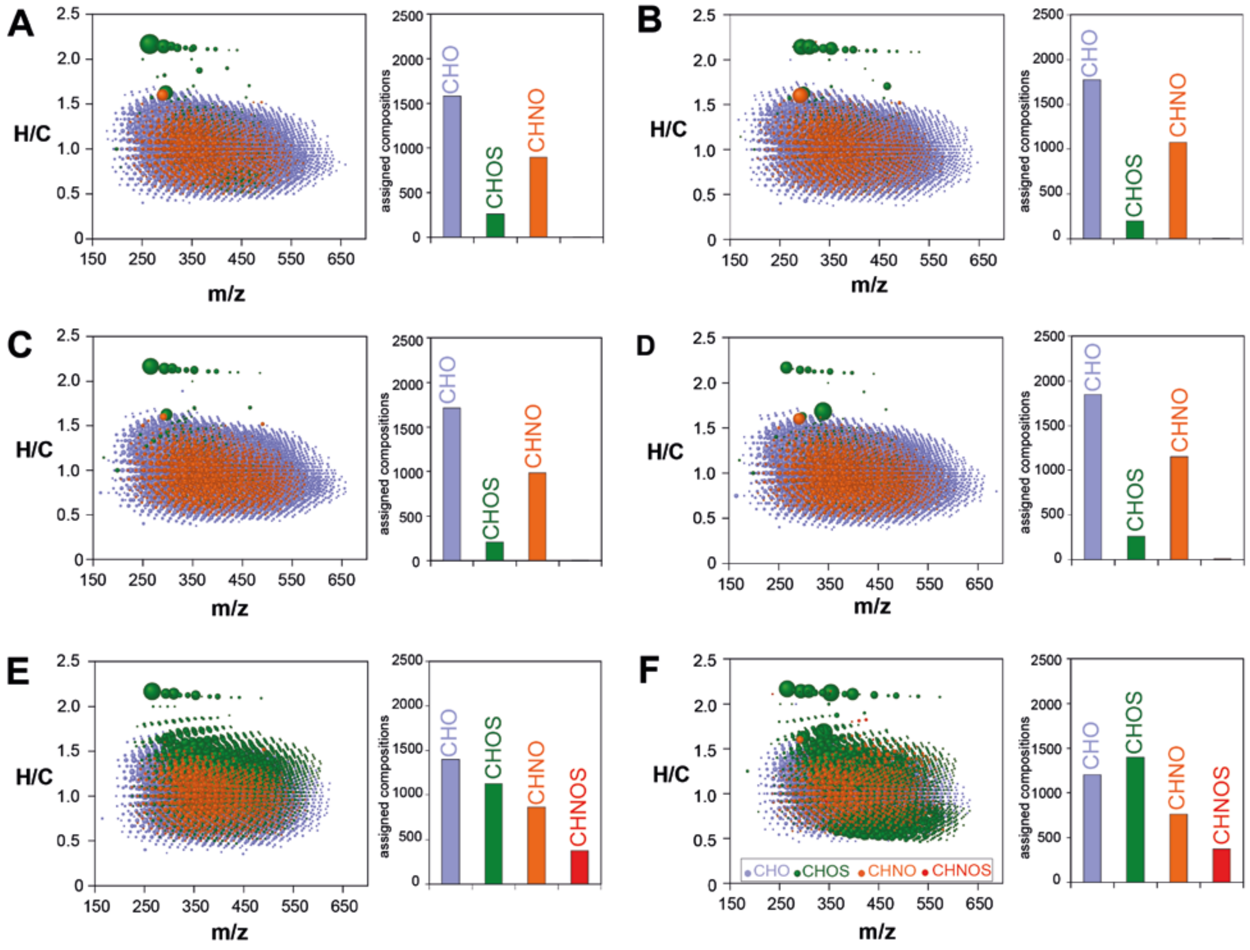
A
B
C
D
E
F
H/C
m/z
2.5
2.0
1.5
1.0
0.5
0
150
250
350
450
550
650
assigned compositions
2500
2000
1500
1000
500
0
CHO
CHOS
CHNO
CHNOS
● CHO ● CHOS ● CHNO ● CHNOS

Fig. S7.

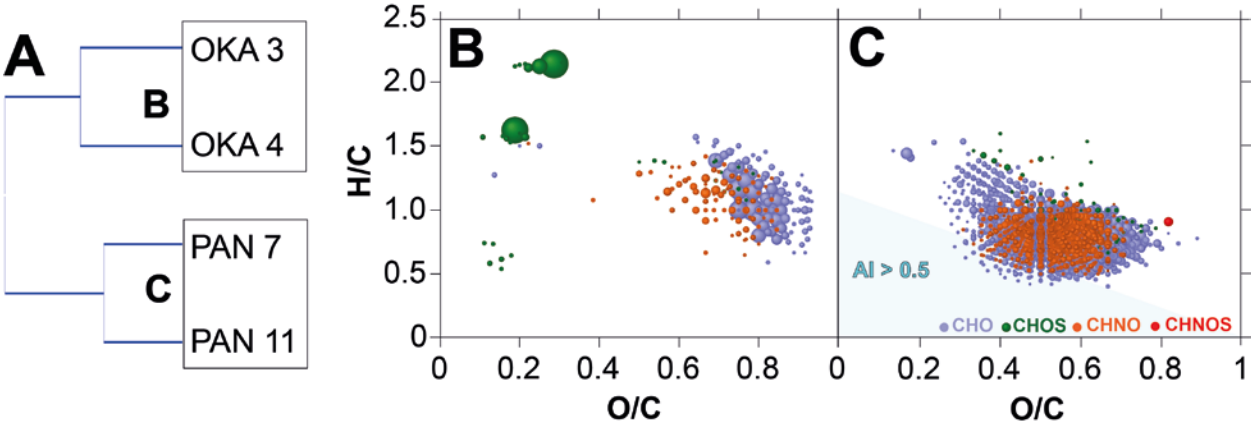
A
OKA 3
B
OKA 4
PAN 7
C
PAN 11
B
C
H/C
2.5
2.0
1.5
1.0
0.5
0
O/C
0
0.2
0.4
0.6
0.8
1
AI > 0.5
CHO
CHOS
CHNO
CHNOS

Fig. S8.

δ (^{1}H) [ppm]	**10.0 - 7.0**	**7.0 - 5.3**	**4.9 - 3.1**	**3.1 - 1.9**	**1.9 - 0.0**
key substructures	$\underline{\mathbf{H}}_{ar}$	$\mathrm{C{=}C\underline{\mathbf{H}}}$, $\mathrm{O_2C\underline{\mathbf{H}}}$	$\mathrm{OC\underline{\mathbf{H}}}$	$\mathrm{XCC\underline{\mathbf{H}}}$	$\mathrm{CCC\underline{\mathbf{H}}}$
D_2O					
FCE-S UDOM	3.6	3.3	35.1	31.8	26.2
FCE-L FA	4.8	3.0	26.5	34.6	31.0
FCE-S PPL	2.7	2.0	43.0	26.5	25.8
CD_3OD					
FCE-S UDOM	0.8	3.3	15.0	29.2	51.6
FCE-L FA	5.2	3.5	18.1	37.6	35.5
FCE-S PPL	3.9	2.3	43.6	23.2	27.1

Table S1.

$\delta(^{13}C)$ ppm	**220-187**	**187-167**	**167-145**	**145-108**	**108-90**	**90-59**	**59-51**	**47-0**	**H/C ratio**	**O/C ratio**
key substructures	$\underline{\mathbf{C}}$=O	$\underline{\mathbf{C}}$OX	$\underline{\mathbf{C}}_{ar}$-O	$\underline{\mathbf{C}}_{ar}$-C,H	$O_2\underline{\mathbf{C}}H$	O$\underline{\mathbf{C}}$H	$O\underline{\mathbf{C}}H_3$	C$\underline{\mathbf{C}}$H		
FCE-S	2.5	13.8	2.5	10.3	2.4	14.2	12.6	41.7	**1.62**	**0.64**
FCE-L	1.6	13.8	2.2	9.5	0.9	11.9	11.6	48.5	**1.70**	**0.57**
OKA-L	2.2	14.8	5.2	17.2	2.4	14.7	7.9	35.6	**1.44**	**0.64**
PAN-S	1.8	14.0	5.0	17.2	2.7	14.5	6.9	37.9	**1.45**	**0.62**
NMR mixing model	**C=O**	**COOH**	**C_{ar}-O**	**C_{ar}-H**	**O_2CH**	**OCH**	**OCH_3**	**CH_2**		
H/C ratio	**0**	**1**	**0**	**1**	**1**	**1**	**3**	**2**		
O/C ratio	**1**	**2**	**1**	**0**	**2**	**1**	**1**	**0**		

Table S2.

spectrum	Figure	PK	NS	AQ [ms]	D1 [ms]	NE	WDW1	WDW2	PR1	PR2	SPE-DOM [mg]
^{1}H NMR	2, S1	5TXI	512-1024	5000	10000	-	-	EM	-	1	3.7 – 9.5 mg
^{1}H NMR	3, S2	8QCI	512-2048	5000	5000	-	-	EM		1	0.3 – 2 mg
^{13}C NMR	S3	5D	74496	1000	14000	-	-	EM	-	35	OKA-L 4.7 mg
^{13}C NMR	S3	5D	44224	1000	14000	-	-	EM	-	35	PAN-S 4.2 mg
^{13}C NMR	S3	8QCO	23420	1000	19000	-	-	EM	-	35	FCE-L 9.5 mg
^{13}C NMR	S3	8QCO	3728	1000	19000	-	-	EM	-	35	FCE-S 9.1 mg
^{1}H, ^{1}H TOCSY	4	5TXI	24	1000	2500	1024	QS	EM	2.5	2.5	see caption
^{1}H, ^{1}H TOCSY	4	8QCI	12	1000	2500	1794	QS	EM	2.5	2.5	FCE-S 9.1 mg
^{1}H,^{13}C DEPT HSQC	5	8QCI	320	250	1250	256	QS	EM	2.5	2.5	FCE-S
^{1}H, ^{1}H JRES	S6	8QCI	3072	1000	500	49	QS	QS	0	0	FCE-S 9.1 mg
^{1}H, ^{13}C HSQC	6, S4, S5	8QCI	1600	250	1250	167	QS	EM	4	7.5	FCE-S

Table S3.