

Direct non-invasive ^1H NMR analysis of stream water DOM: Insights into the effects of lyophilization compared to whole water

Supplemental Information

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Running head: Direct ^1H NMR analysis of stream water DOM

Keywords: proton NMR; dissolved organic matter; headwater streams; freeze-dried DOM; whole water analysis, lyophilization, stream water DOM

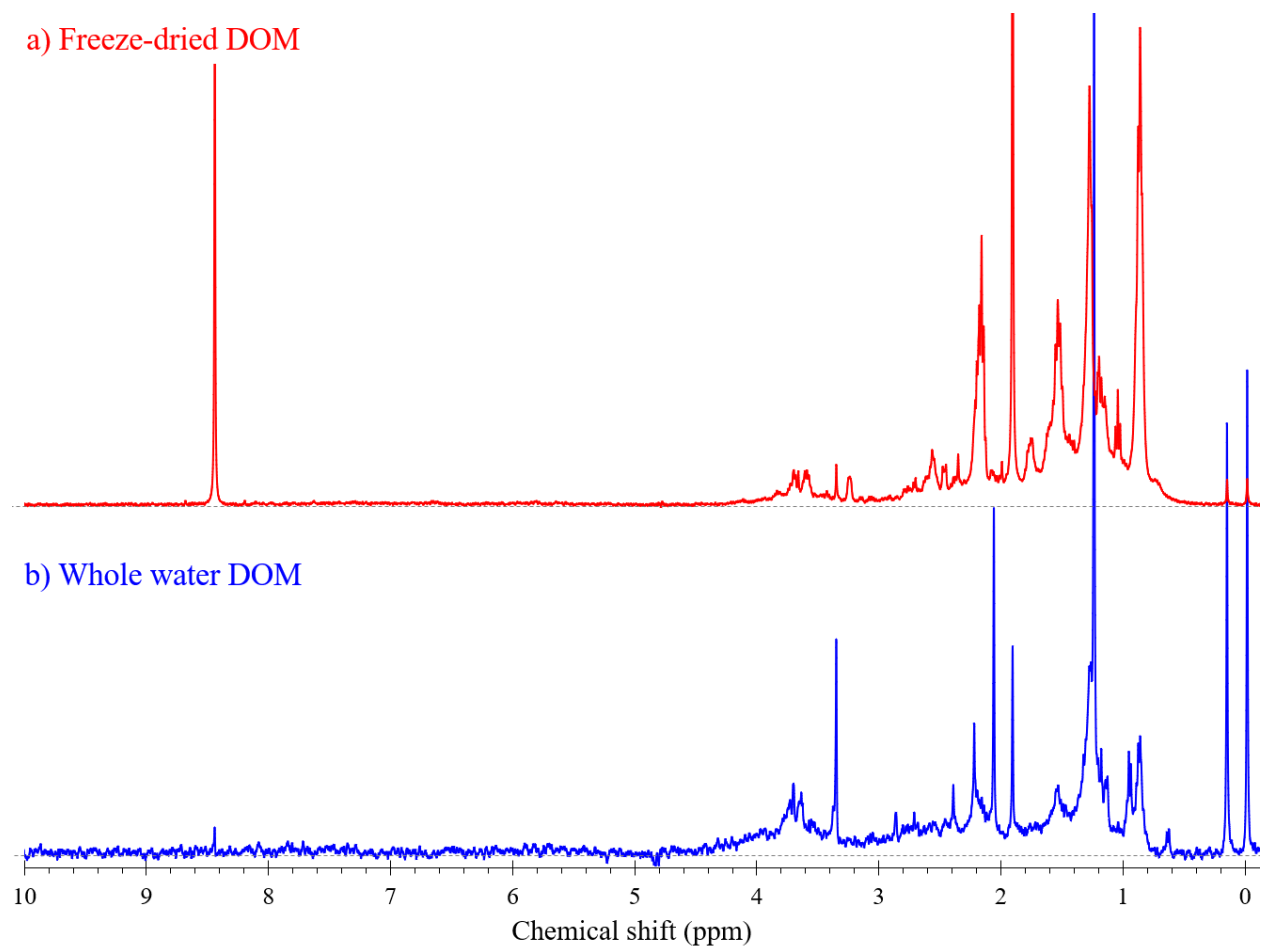


Fig. S1. Full ^1H NMR spectra (0–10 ppm) of stream water DOM, collected from Quebrada Kathia, analyzed after subject to different methods of preparation: a) 10:1 concentration by freeze-drying in $\text{H}_2\text{O}:\text{D}_2\text{O}$, and b) whole water without pre-concentration in $\text{H}_2\text{O}:\text{D}_2\text{O}$. Both spectra have been analyzed under identical instrument parameters and normalized to TSP, the internal standard just before 0.0 ppm

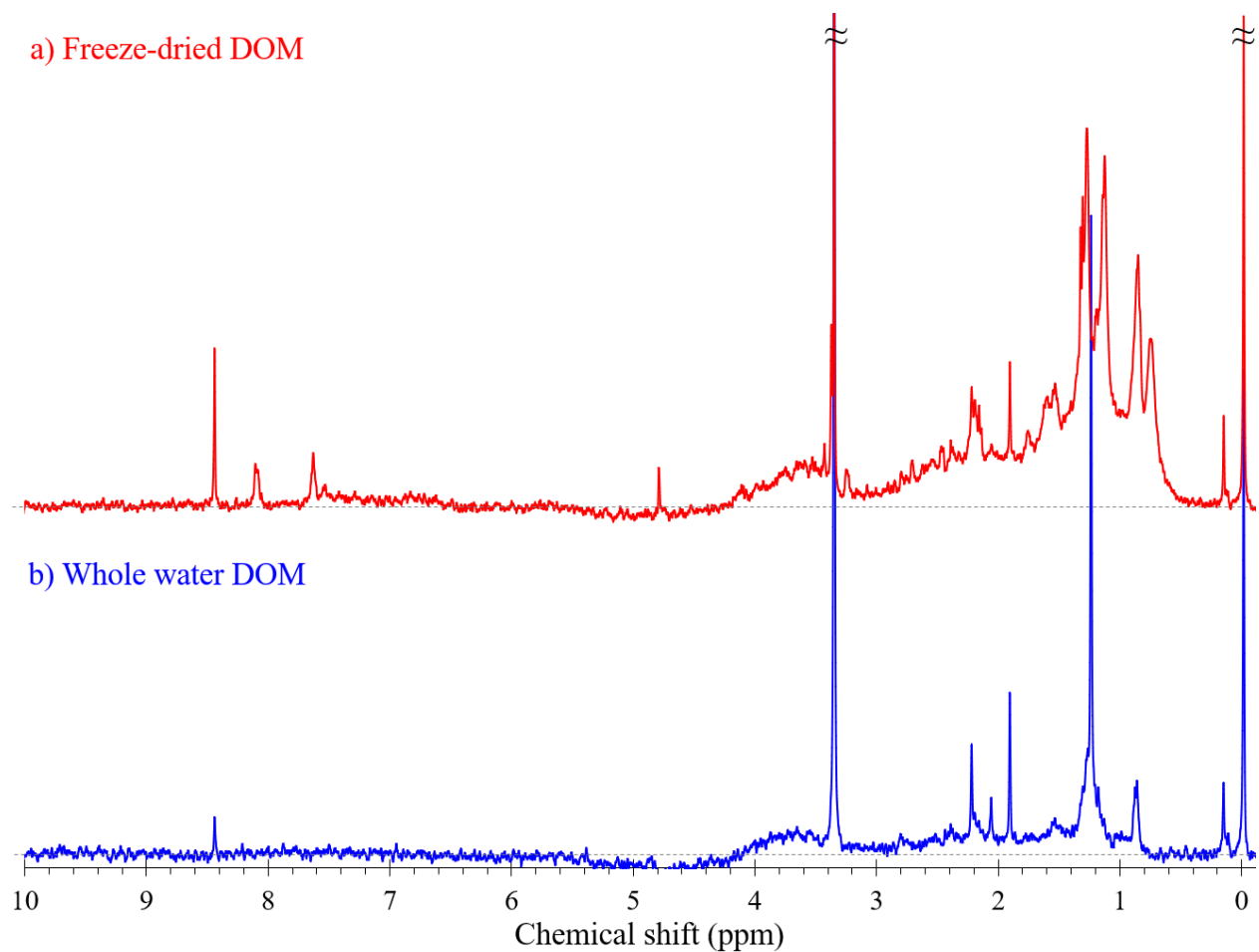
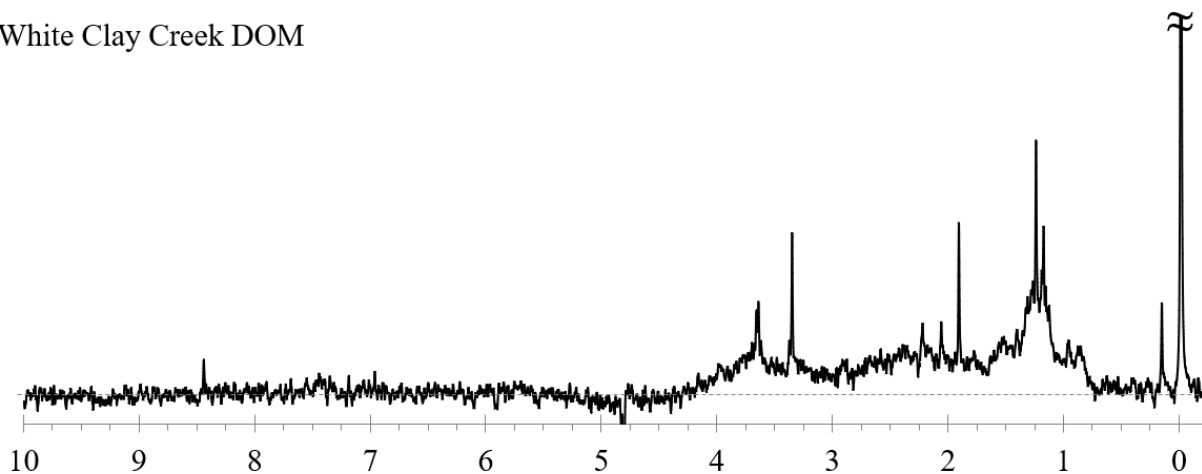


Fig. S2. Full ^1H NMR spectra (0–10 ppm) of stream water DOM, collected from Quebrada Rosa, analyzed after subjected to different methods of preparation: a) freeze-dried but not concentrated in $\text{H}_2\text{O}:\text{D}_2\text{O}$, and b) whole water without pre-concentration in $\text{H}_2\text{O}:\text{D}_2\text{O}$. Both spectra have been analyzed under identical instrument parameters and normalized to TSP, the internal standard just before 0.0 ppm

a) White Clay Creek DOM



b) Great Dismal Swamp DOM

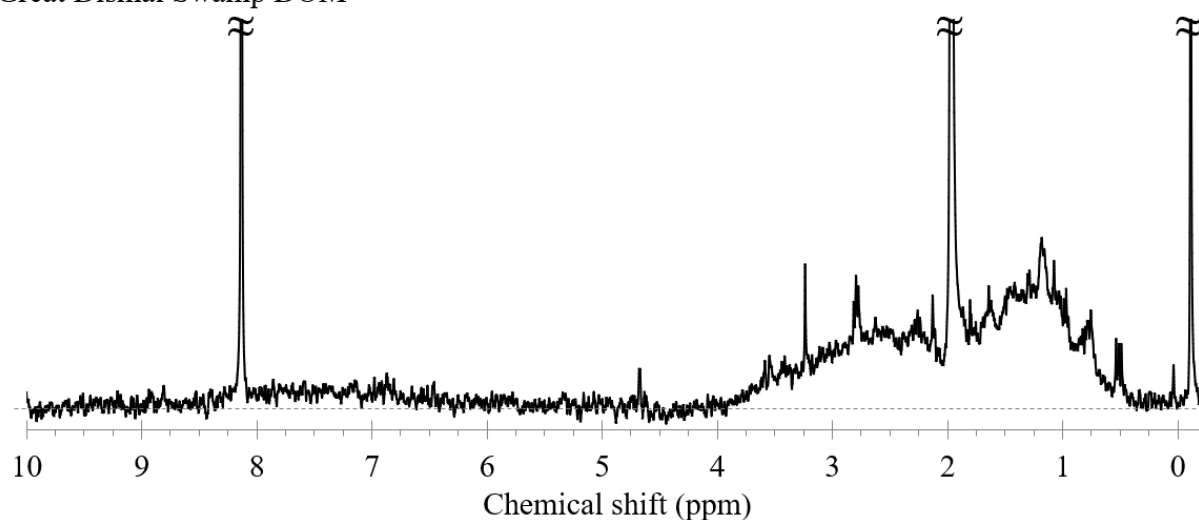


Fig. S3. ¹H NMR spectra (0–10 ppm) of other freshwater DOM acquired using water suppression. Both spectra have sharp peaks indicating peak sharpness is not an artifact from water suppression. Neither sample was optimized for quantification and were run 5 years apart. a) White Clay Creek in Pennsylvania, USA was obtained with 48,000 scans, d1=0.001s, and a DOC value of 142.0 μ M C. b) Great Dismal Swamp in Virginia, USA was obtained with 2,000 scans, d1=2s, and a DOC value of 1.7 mM C.

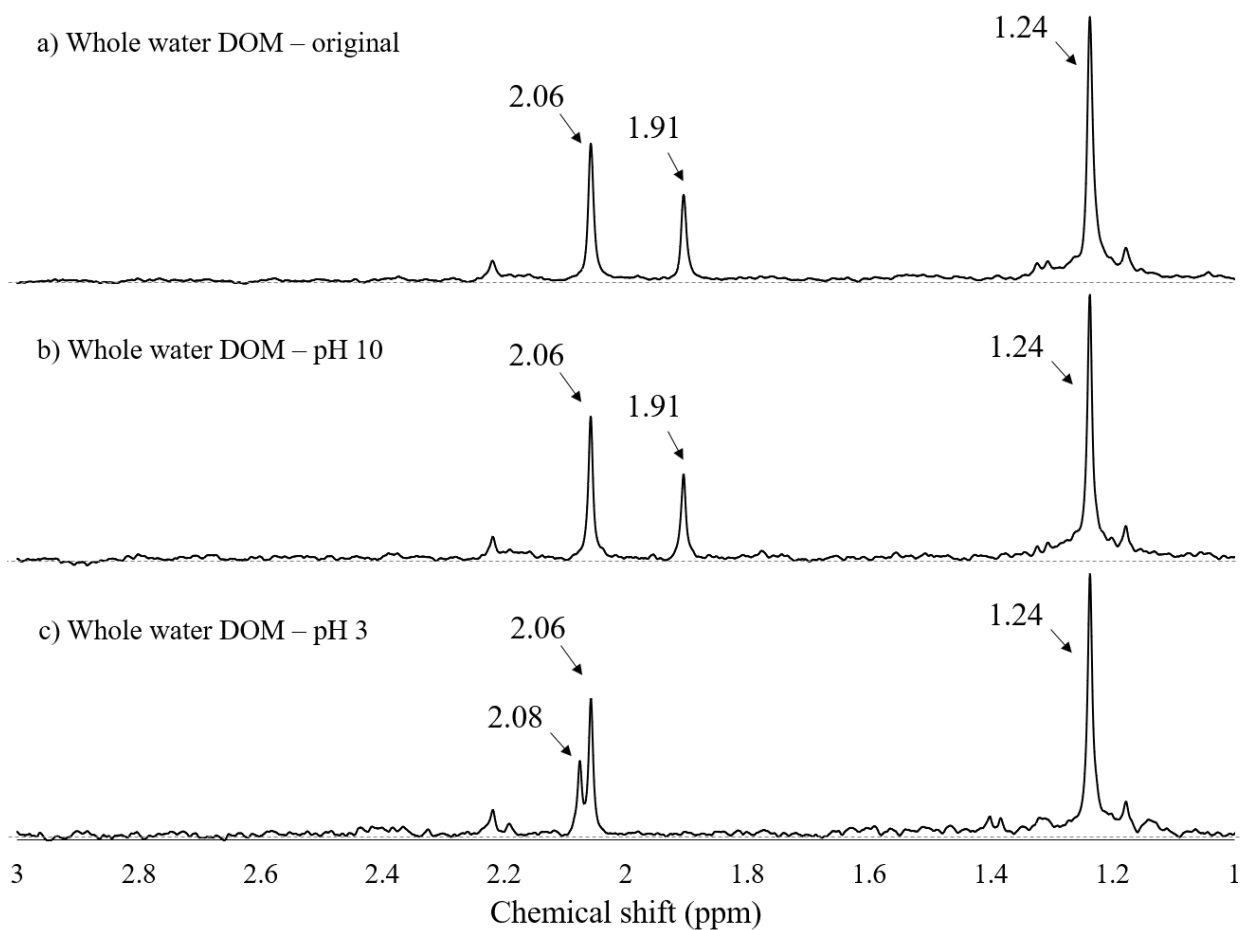


Fig. S4. pH confirmation of acetate at 1.91 ppm. ^1H NMR spectra of a) the original whole water DOM at pH 7.5 and aliquots of the same sample modified to b) pH 10, and c) pH 3. The original stream water DOM was analyzed for a full 20,000 scans while the pH modified samples were only analyzed for 2,000 scans to confirm the peak shift. All spectra have been normalized to the CH_2 peak at 1.24 ppm since the internal standard, TSP, can move with pH.

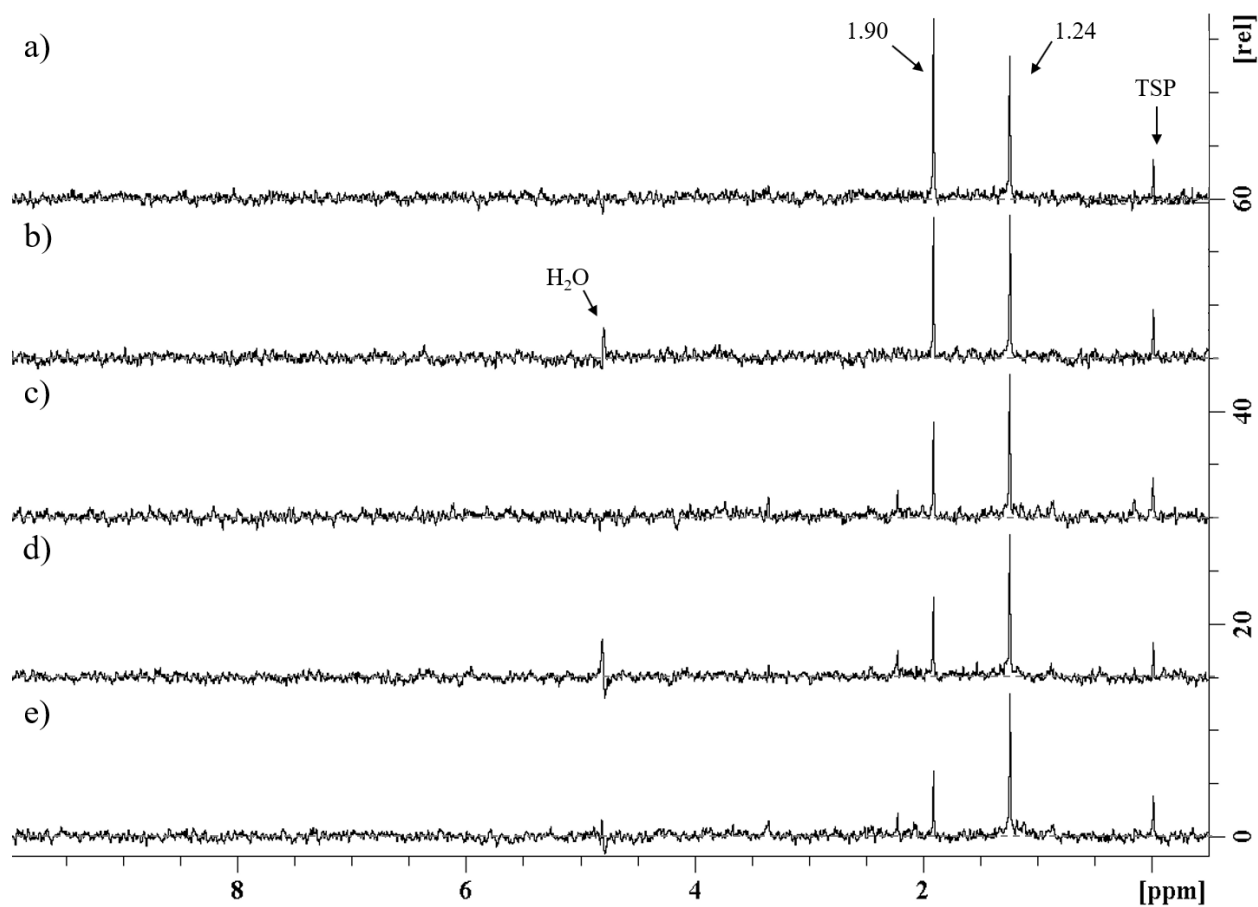


Fig. S5. Standard additions of stream water DOM, collected from Quebrada Kathia, analyzed as whole water by ^1H NMR with water suppression. Spectra shown were analyzed following the same instrumental parameters, except the number of scans ($n_s=600$), described in the methods section of the main manuscript. Spectra are as follows: a) stream water DOM + $4\ \mu\text{M}$ calcium acetate; b) stream water DOM + $3\ \mu\text{M}$ calcium acetate; c) stream water DOM + $1\ \mu\text{M}$ calcium acetate; d) stream water DOM + $0.5\ \mu\text{M}$ calcium acetate; and e) stream water DOM.

Whole water process blank

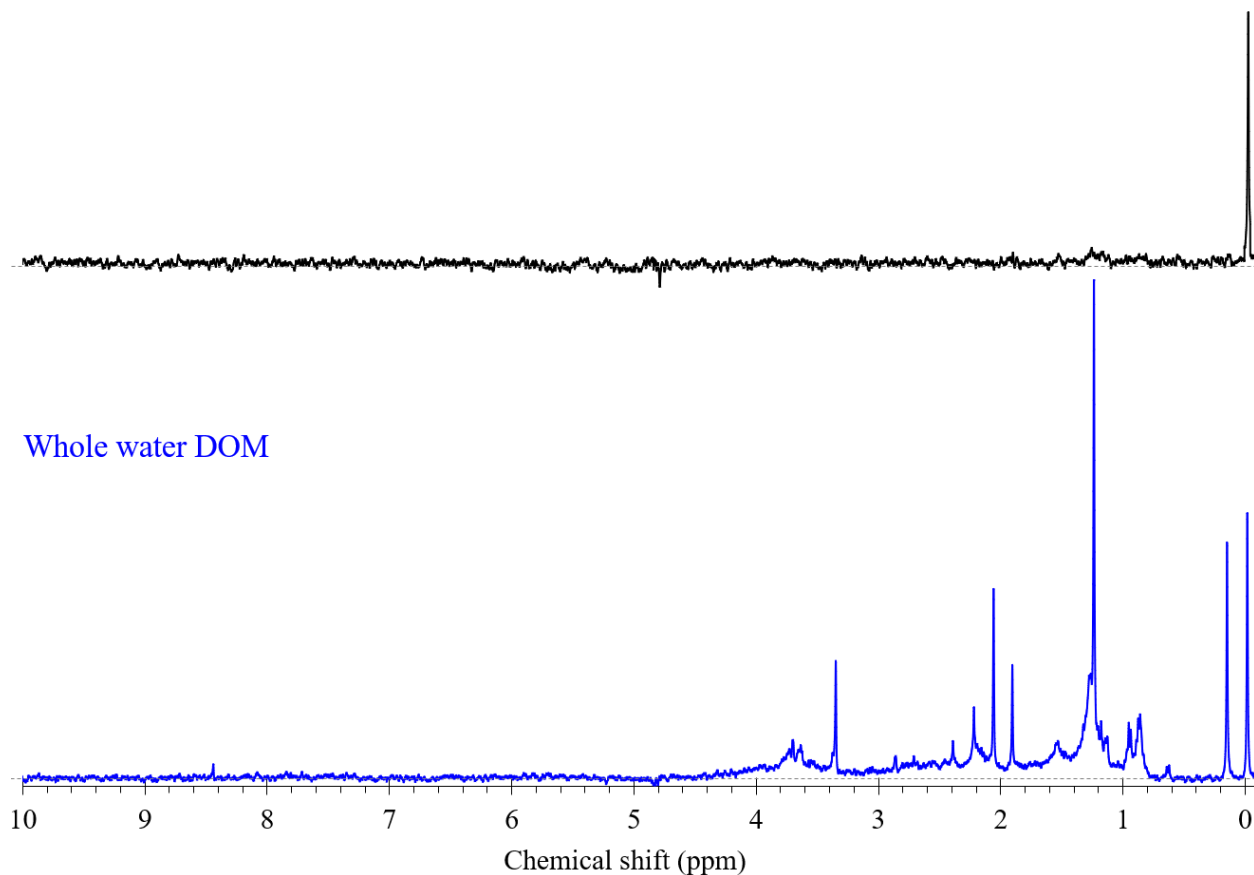


Fig. S6. Full ¹H NMR spectra (0–10 ppm) of the whole water process blank compared to the whole water DOM collected from Quebrada Kathia. The whole water process blank was acquired for 2,000 scans and the whole water DOM was acquired for 20,000 scans. All other instrumental parameters were identical. If any contaminate peaks were present in the whole water process blank, they would have been observed within the first 1,000 scans.

Freeze-dried process blank

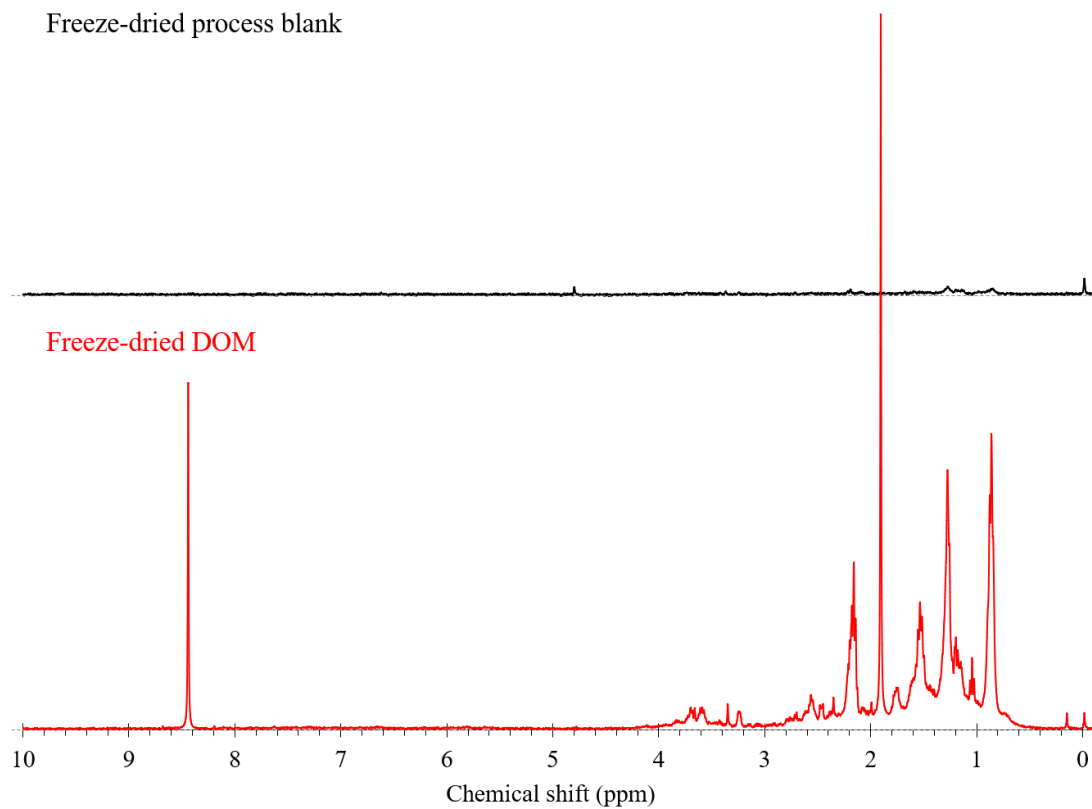


Fig. S7. Full ¹H NMR spectra (0–10 ppm) of freeze-dried process blank compared to the freeze-dried DOM collected from Quebrada Kathia. Both spectra were acquired under identical instrumental parameters and normalized to the internal standard, TSP, just before 0.0 ppm.

Stream	Stream Size	DOC (μM)	pH	Conductivity ($\mu\text{S cm}^{-1}$)
Quebrada Rosa	Smallest	163.9	7.87	356.3
Quebrada Kathia	↓	99.8	7.64	94.4
Rio Tempisquito		59.7	7.94	130.6
Rio Tempisquito Sur.		86.8	7.65	367.7
Confluence	Largest	89.3	7.80	176.4

Table S1. Dissolved organic carbon (DOC), pH, and conductivity measurements for all of the streams sampled in this study.

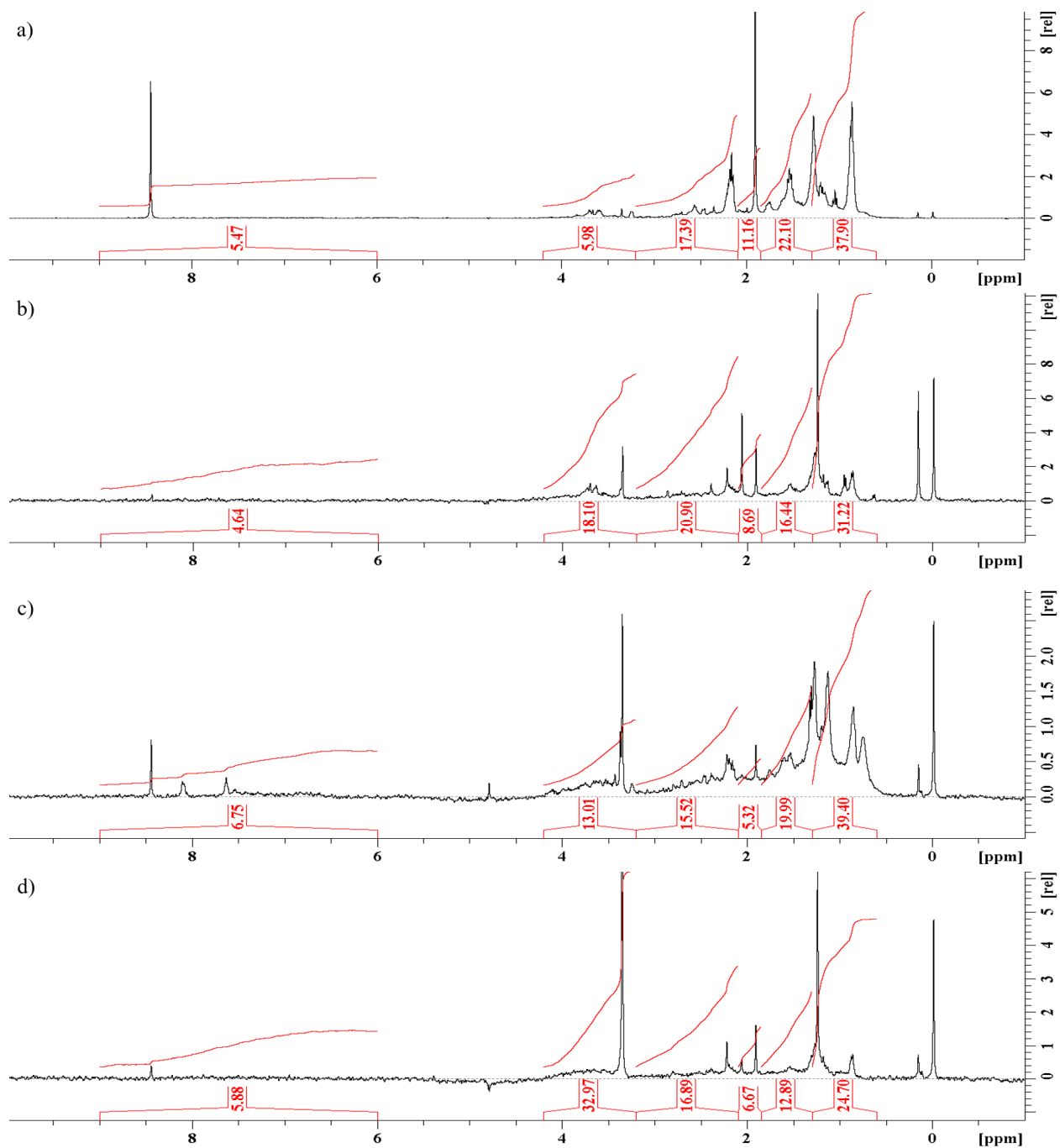


Fig. S8. Integral curves of the ^1H NMR spectra for a) Quebrada Kathia freeze-dried DOM concentrated 10:1; b) Quebrada Kathia whole water DOM; c) Quebrada Rosa freeze-dried DOM not concentrated; d) Quebrada Rosa whole water DOM.

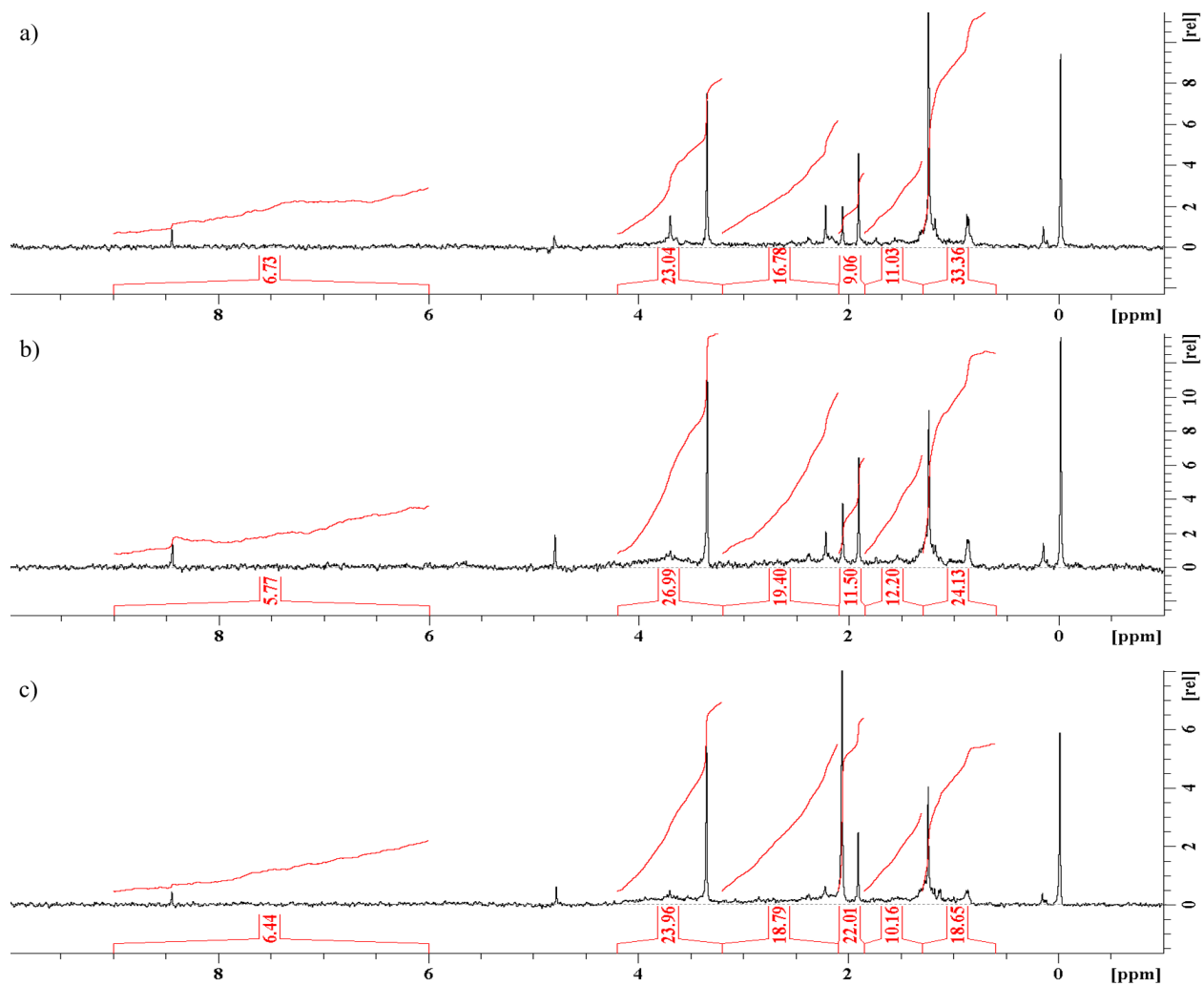
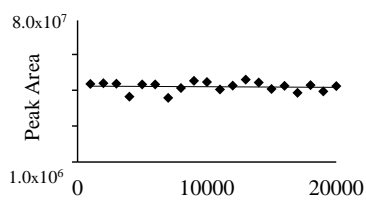
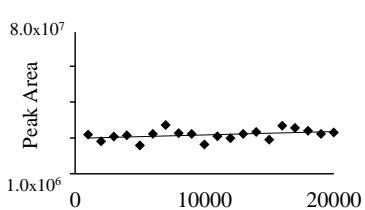


Fig. S9. Integral curves of the ^1H NMR spectra for a) Rio Tempisquito whole water DOM; b) Rio Tempisquito Sur whole water DOM; c) confluence of the Rio Tempisquito and Rio Tempisquito Sur whole water DOM.

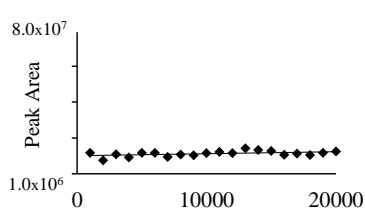
Region 1: 0.6 – 1.3 ppm



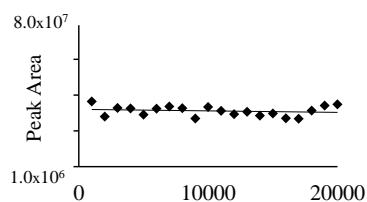
Region 2: 1.3 – 1.85 ppm



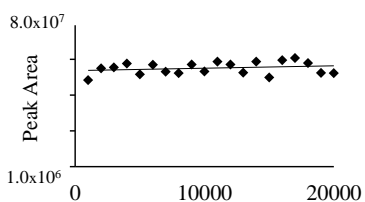
Region 3: 1.85 – 2.1 ppm



Region 4: 2.1 – 3.2 ppm



Region 5: 3.2 – 4.2 ppm



Region 6: 6.0 – 9.0 ppm

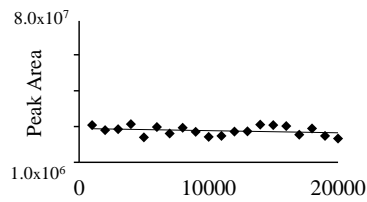


Fig. S10. Area integrations of stream water DOM analyzed as whole water over the course of 49 hours. Spectra were acquired every 1,000 scans for a total of 20,000 scans.

Pulse Sequence

PEW5shapepr

Perfect Echo Watergate Sequence with train of shaped 180 deg pulses on water during relaxation delay. PEW5 described in Ralph W. Adams, Chloe M. Holroyd, Juan A. Aguilar, Mathias Nilsson and Gareth A. Morris Chem. Commun., 2013,49, 358-360

Based on water suppression using watergate W5 pulse sequence with gradients using double echo train of shaped pulses on water during relaxation delay added by Jim Hall at the COSMIC facility.

M. Liu, X. Mao, C. He, H. Huang, J.K. Nicholson & J.C. Lindon, J. Magn. Reson. 132, 125 - 129 (1998)

```
#include <Avance.incl>
```

```
#include <Grad.incl>
```

```
1 ze
```

```
2 30m
```

```
  d1
```

```
3 p20:sp6:f1 ph28
```

```
  4u
```

```
  lo to 3 times l6
```

```
  10u p11:f1
```

```
  p1 ph1
```

```
50u UNBLKGRAD
```

```
  p16:gpl
```

```
  d16 p18:f1
```

```
  p27*0.087 ph3
```

```
  d19*2
```

```
  p27*0.206 ph3
```

```
  d19*2
```

```
  p27*0.413 ph3
```

```
  d19*2
```

```
  p27*0.778 ph3
```

```
  d19*2
```

```
  p27*1.491 ph3
```

```
  d19*2
```

```
  p27*1.491 ph4
```

```
  d19*2
```

```
  p27*0.778 ph4
```

```
  d19*2
```

```
  p27*0.413 ph4
```

```
  d19*2
```

```
  p27*0.206 ph4
```

```
  d19*2
```

```
  p27*0.087 ph4
```

50u
p16:gp1
d16 p11:f1

p1 ph10

50u
p16:gp2
d16 p118:f1
p27*0.087 ph5
d19*2
p27*0.206 ph5
d19*2
p27*0.413 ph5
d19*2
p27*0.778 ph5
d19*2
p27*1.491 ph5
d19*2
p27*1.491 ph6
d19*2
p27*0.778 ph6
d19*2
p27*0.413 ph6
d19*2
p27*0.206 ph6
d19*2
p27*0.087 ph6
p16:gp2
d16
50u BLKGRAD

go=2 ph31
30m mc #0 to 2 F0(zd)
exit

ph1=0 2
ph3=0 0 1 1 2 2 3 3
ph4=2 2 3 3 0 0 1 1
ph5=0 0 0 0 0 0 0 1 1 1 1 1 1 1 1
2 2 2 2 2 2 2 3 3 3 3 3 3 3 3
ph6=2 2 2 2 2 2 2 3 3 3 3 3 3 3 3
0 0 0 0 0 0 0 1 1 1 1 1 1 1 1
ph10=1
ph28=0

ph31=0 2 2 0 0 2 2 0 2 0 0 2 2 0 0 2

;p11 : f1 channel - power level for pulse (default)
;p118: f1 channel - power level for 3-9-19-pulse (watergate)
;p1 : f1 channel - 90 degree high power pulse
;p16: homospoil/gradient pulse
;p20: shaped 180 degree pulse (use 4ms square 100.1000 for most samples; use 2ms square 100.1000 only for very challenging samples)
;p27: f1 channel - 90 degree pulse at p118
;sp6: power level for shape pulse p20
;d1 : relaxation delay; $1-5 * T1$
;d16: delay for homospoil/gradient recovery
;d19: delay for binomial water suppression
;d19 = $(1/(2*d))$, d = distance of next null (in Hz)
;l6: loop counter to define irradiation period. For a 2s period use 500 if your pulse is 4ms.
(i.e. $500 * 4ms = 2s$)
;NS: $8 * n$, total number of scans: $NS * TD0$
;DS: 4

;use gradient ratio: gp 1 : gp 2
; 34 : 22

;for z-only gradients:
;gpz1: 34%
;gpz2: 22%

;use gradient files:
;gpnam1: SINE.100
;gpnam2: SINE.100