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Interactive comment on "Dissolved organic matter characteristics of deciduous and coniferous forests with variable management: different at the source, aligned in the soil" *by* Lisa Thieme et al.

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Received and published: 7 February 2019

Dear Malak Tfaily, thank you for your detailed review of our manuscript. In the following, we hope to adequately address your constructive comments and questions.

"Page 5, line 31, did you check if 0.2 μm was enough to eliminate microbial communities originally present in the samples?"

We did not check for complete bacteria removal but filtration through 0.2 μm is a standard procedure for the removal of microorganisms. To our knowledge already a filter pore diameter of < 1 μm would be sufficient to exclude living bacterial cells so that

only even smaller spores could have passed. Please note that the samples were subsequently (re-)inoculated with a microbial community extracted from the soils of the biodiversity exploratory. Therefore, the goal of the filtration was not necessarily a complete sterilization, but a standardization of the microbial community degrading the DOM in the different samples.

"Pages 4-5 what was the total number of samples and how was it distributed in terms of management? Line 16, can you give the break down for the 466 samples?"

We think, the number of analyzed samples per measurement/experiment you are asking for is given in Table 1. Detailed information of pooled samples for FT-ICR-MS and the incubation test regarding management distribution are given in Tables S3 and S4 in the supplement. Moreover, in the supplement (Table S2), detailed numbers of samples per site, plot and ecosystem flux for the 466 fluorescence samples are given.

"Line 16, what do you mean by: To balance uneven sample numbers, we calculated mean EEMs per plot and ecosystem flux resulting in a dataset with 79 samples. Did you collapse the 466 samples into 79 samples to allow for plot versus plot comparison? what was the variability within the same plot? Line 20, how did the optical data look for these samples? "

As is visible in Table S2 the number of available sample numbers per plot and ecosystem flux was not the same for the various sample types (n>10 for TF, SF and LL; n<5 for TOP and SUB). In their tutorial review to PARAFAC, Murphy et al. (2013) caution about unequal numbers of replicated samples in PARAFAC modeling. To avoid this influence, we calculated mean EEMs to use one 'sample' per plot and ecosystem flux to gain a representative model. To give you an impression, Fig.1 shows throughfall (TF) EEM plots for the Schorfheide plot SEW8 (beech unmanaged) for different sampling dates and the resulting mean EEM used in our statistical analysis.

"Do you believe that differences due to management is higher than that between plots within the same forest?"

Detecting differences in fluorescence spectra caused by different management practice versus intra-plot variability depends on the management categories compared. With fluorescence measurements of DOM (please keep in mind that fluorescence measurements address only the portion of DOM able to absorb and emit light) we were able to detect differences due to management decisions like tree species selection. Possible differences between differently managed forests with the same tree species, in our case unmanaged and age-class beech forests, were not statistically distinguishable.

"Page 7, line 14, only six spectra were averaged? Typically, we do at least 100."

Our samples were measured on a FT-ICR-MS Ultra (ThermoFisher equipped with the SIMION optimized ICR cell for more homogeneous magnetic field in contrast to the first ThermoFisher edition, resulting in the specified exactness better than 1ppm). Our own working group-intern improvements led to an exactness better than 500 ppb (as example, mean deviation in this data set is 400 ppb), today. For previous studies in another institute, we used an Apex II Bruker (but of course not for DOM measurements), therefore we know some important technical differences between the ThermoFisher and the Bruker MS, which are important for the comparison of spectra from different instrument types. The need to average large numbers of at least 100 scans is probably related to (I) the use of different mass spectrometers, namely of the suppliers Bruker and Thermo Finnigan, and (II) same term (scan) for different things. The Bruker machine allows to accumulate scans without limit, which means, the longer you measure, the more intensive your mass peaks become (therefore, most people sum up 100-200 scans). In contrast, with Thermo instruments only an accumulation up to 50 so-called μ-scans is noticeable, more μ-scans do not change the signal intensity (kind of included system averaging without any possibility for changing or even excluding by the operator). Each of these 50 μ-scans is one transient, 50 μ-scans together are $\sim$ 3 min measuring time and were combined subsequently to one so-called scan. For further improving spectra quality, we average (no accumulation possible) the data of six such Thermo-scans (with 50 μ-scans each, in total recording time $\sim$ 18 min).

As an example result for a beech throughfall sample we got 18010 peaks in the averaged spectrum of 6 scans, but it would be only 13003 peaks with 4 scans - all with the same intensive peaks from first scan on. (100 of such scans would imply 300 min = 5 h).

"Can you provide more details regarding formula assignment and the rules that were used? What was the number of unassigned formula? What were the ranges of C, H, N, C, O, S, P? that were used?"

Thank you for pointing out the missing ranges of formula assignments, who will be added to the manuscript.

After manual examinations of included heteroatoms the following constraints were chosen to generate empirical formulae from all peaks: C, H and O unlimited, N and S: 0-3 (without the combination S>1N3), 13C: 0-1 and P=0.

Rules (from organic mass spectrometry): for odd numbered peaks: N=0 or 2 (nitrogen rule), no 13C

For even numbered peaks: no 12CHO compounds; N=1 or 3 or 13C=1, but not in combination

At least O2 incorporated (that means, one COOH group for neg. ions), that means no pure CH compounds

$H/C \leq 2.0$

$O/C \leq 1.4$

Because of only small peaks for CHOSN, we decided to show van Krevelen plots for CHO compounds only, in contrast to other projects. An example of formula numbers of beech throughfall sample can be found in the supporting information to this reply.

"Figure 2, it's hard to see the zoom in but be careful about peak splitting as this can affect your formula assignment. Even though it is hard to see, it appears you had some

peak splitting."

Independent of daily tuning and calibrating, in the course of instrument life time, the peak shapes are changing (quenching of peaks after detections lead to slow adsorption of chemicals on the ICR cell walls too). For best conditions in very complex mixtures like DOM, we have got a (company developed) procedure for adjustment of ICR cell parameters (as result replacing the instrument master file). This procedure is usually not distributed to customers, because of the possibility to misalignments. The procedure is measuring defined standards the first two runs automatically ($\sim$ 1 day), followed by a manual adjustment, especially under consideration of the peak legs (should achieved < 15% peak height). That means, when the peak legs increase (independent of fresh cleaning of the ion source & quadrupole 0), we adjust the cell parameters to prevent peak splitting. You can see randomly selected nominal masses from two different samples as an example in Fig. 2 and Fig.3. In both examples, assignments by our calculation program and manually are highlighted. Also impossible assignments due to multiple possibilities and/or limitation of our constraints or just because of decreasing exactness of peaks due to very small intensities are indicated. As you may see, for all peaks there is at least an explanation – and we found no signs for peak splitting or peaks without sum formula proposal. As you may see, at our Thermo instrument the base line is not visible – obviously base corrected by the manufacturer. We assume, this is the reason for missing peaks bases, which could led to misinterpretations.

Non the less, the figures included in the discussion manuscript were converted to pdf with the whole document. For the final manuscript all figures will be submitted separately as individual files hopefully avoiding resolution losses.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2018-478/bg-2018-478-AC3-supplement.pdf
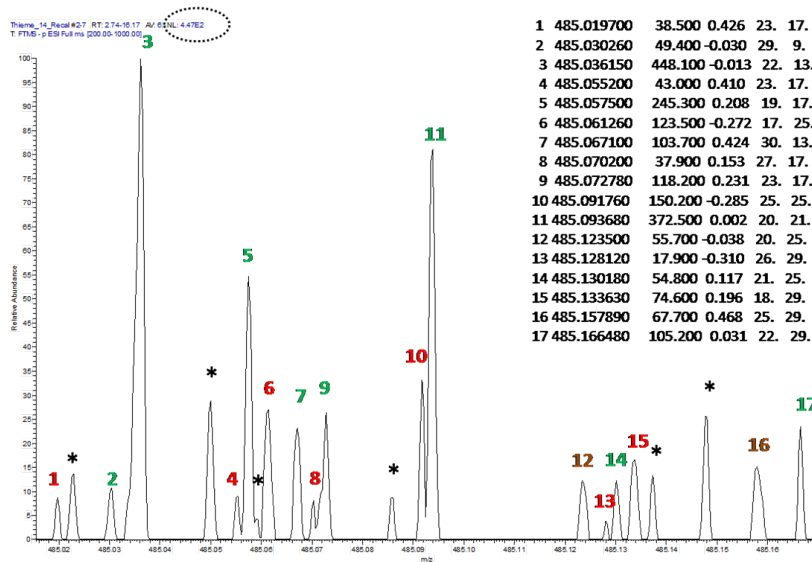
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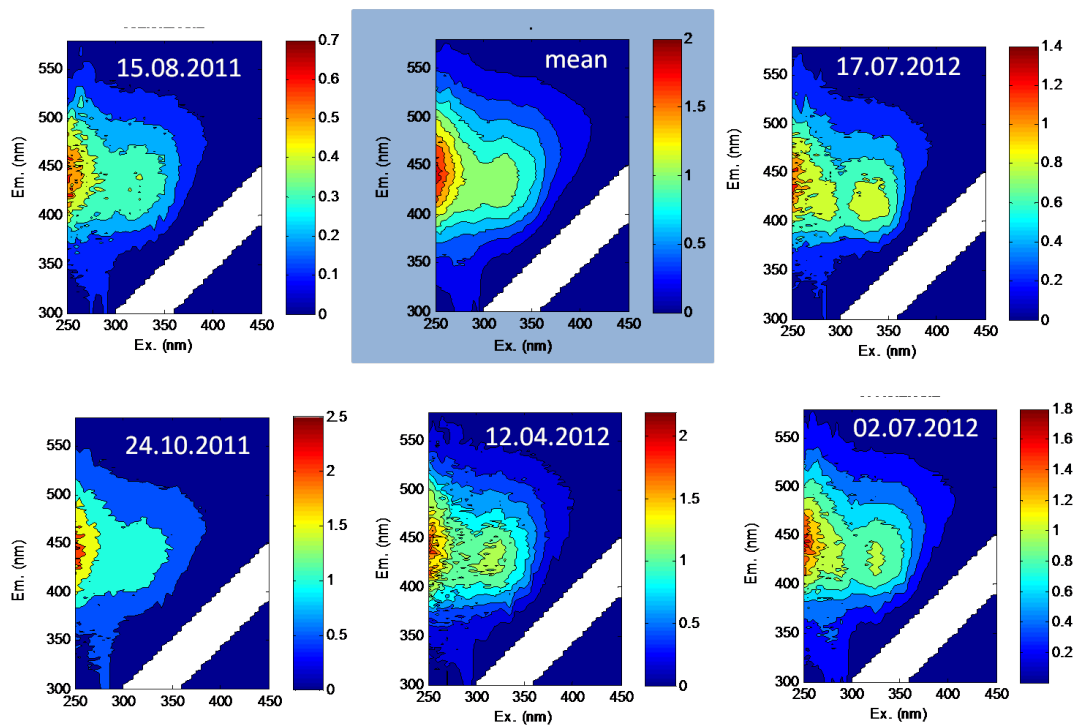
Fig. 1. Fluorescence EEMs plots of different throughfall samples of Schorfheide plot SEW8 (beech unmanaged) for different sampling dates. Mean= mean EEM calculated of all shown measurements

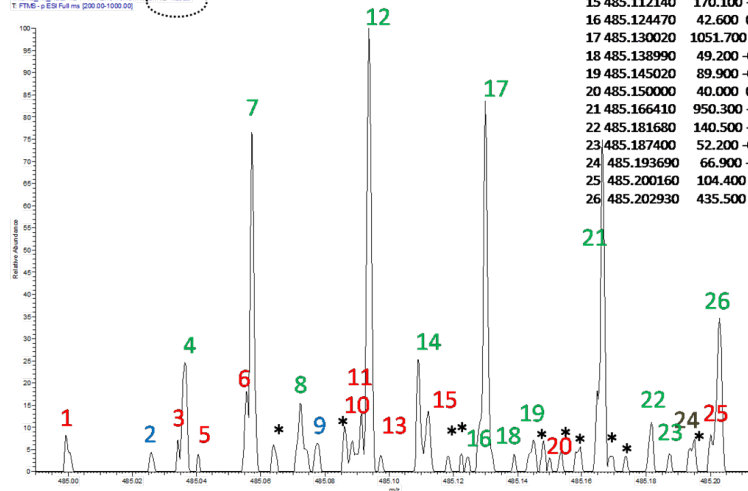**Fig. 2.** Randomly selected nominal mass from beech throughfall sample

pine, litter leachate (m/z 485)

CHO compounds in green
CHOS$_x$ compounds in red
CHON$_y$ compounds in blue
CHON$_y$S$_x$ compounds in brown

* not assigned by limitation of the constraints
(e.g. N>3) or multiple assignments

1	485.000460	55.100	-0.187	22.	13.	9.	0.	2.	0.	17.0	0.4	0.5
2	485.025900	54.100	-0.367	24.	9.	10.	2.	0.	0.	22.0	0.7	0.8
3	485.034190	90.600	-0.340	27.	17.	3.	0.	3.	0.	20.0	-0.2	0.6
4	485.036360	312.000	0.197	22.	13.	13.	0.	0.	0.	17.0	0.6	0.3
5	485.040550	49.200	0.147	20.	21.	8.	0.	3.	0.	11.0	0.0	-0.1
6	485.055610	231.800	-0.049	24.	21.	5.	0.	3.	0.	15.0	-0.2	0.4
7	485.057290	956.800	-0.002	19.	17.	15.	0.	0.	0.	12.0	0.7	-1.0
8	485.072380	198.200	-0.169	23.	17.	12.	0.	0.	0.	16.0	0.3	0.3
9	485.077660	80.300	-0.249	29.	13.	6.	2.	0.	0.	25.0	0.2	0.8
10	485.088510	89.500	-0.164	28.	21.	4.	0.	2.	0.	19.0	-0.3	0.5
11	485.091330	169.000	0.154	24.	21.	9.	0.	1.	0.	15.0	-0.0	0.3
12	485.093670	1255.200	-0.008	20.	21.	14.	0.	0.	0.	11.0	0.4	-0.7
13	485.097370	46.100	0.321	17.	25.	14.	0.	1.	0.	6.0	0.3	-5.0
14	485.109060	327.100	0.126	24.	21.	11.	0.	0.	0.	15.0	0.0	0.2
15	485.112140	170.100	-0.165	21.	25.	11.	0.	1.	0.	10.0	-0.0	-0.3
16	485.124470	42.600	0.280	28.	21.	8.	0.	0.	0.	19.0	-0.2	0.5
17	485.130020	1051.700	-0.043	21.	25.	13.	0.	0.	0.	10.0	0.0	-0.5
18	485.138990	49.200	-0.456	32.	21.	5.	0.	0.	0.	23.0	-0.3	0.6
19	485.145020	89.900	-0.299	25.	25.	10.	0.	0.	0.	14.0	-0.2	0.2
20	485.150000	40.000	0.441	23.	33.	5.	0.	3.	0.	8.0	-0.7	-0.1
21	485.166410	950.300	-0.039	22.	29.	12.	0.	0.	0.	9.0	-0.2	-0.4
22	485.181680	140.500	-0.025	26.	29.	9.	0.	0.	0.	13.0	-0.4	0.2
23	485.187400	52.200	-0.178	19.	33.	14.	0.	0.	0.	4.0	-0.3	-2.2
24	485.193690	66.900	-0.117	26.	33.	3.	2.	2.	0.	12.0	-0.7	0.3
25	485.200160	104.400	-0.172	27.	33.	6.	0.	1.	0.	12.0	-0.7	0.2
26	485.202930	435.500	0.096	23.	33.	11.	0.	0.	0.	8.0	-0.5	-0.3

Fig. 3. Randomly selected nominal mass from pine litter leachate sample