



Supplement of

The role of surface-active macromolecules in the ice-nucleating ability of lignin, Snomax, and agricultural soil extracts

Kathleen A. Alden et al.

Correspondence to: Kathleen A. Alden (k.a.alden@leeds.ac.uk) and Nadine Borduas-Dedekind (borduas@chem.ubc.ca)

The copyright of individual parts of the supplement might differ from the article licence.

Contents

	S1 Soil Sampling Locations	2
	S2 Details of OCA 15 EC Tensiometer by DataPhysics	3
	S3 TOC Analysis	5
5	S4 High-speed cryo-microscopy	7
	S5 CMC measurements: pyrene fluorescence and conductivity	8
	S6 FINC Calibration	10
	S7 Lignin Monolayer Calculation	12
	S8 Frozen Fraction for Soil Proxies	13
10	S9 Filtering of Snomax Samples	14
	S10Effect of heat tests on surface tension	15



Figure S1. Photographs showing the three different sample locations; (a) University of British Columbia Farm, (b) University of Leeds Farm, and (c) Rothamsted Research (sampled field is situated behind the pictured sampling station) and (d) samples being taken at UBC Farm. Images demonstrate the differences between the three different sampling locations and how the soil samples were taken. All images were taken by Kathleen Thompson and Nicole Link.



Figure S2. Picture of DataPhysics optical contact angle (OCA) 15 EC tensiometer including (a) side view with labelled components, (b) beam path, (c) example image of a pendant droplet. Pictures taken by Paul Bieber.



Figure S3. Average surface tension measurement of MilliQ water droplet measured every 30 seconds over 5 minutes. The error bars represent the standard deviation of three different experiments on three different droplets.



Figure S4. Average surface tension measurement with temperature (as controlled with the temperature control unit) of MilliQ water droplets for two different tests. Compared against literature data from Gittens (1969) and Young and Harkins (1928).

Standard	TIC conc	μ l	TOC conc	1	TC conc	Vol (ml)	
Standard	(ppm)		(ppm)	μ I	(ppm)		
1	1	100	1	100	2	100	
2	2	200	2.5	250	4.5	100	
3	4	400	5	500	9	100	
4	6	600	10	1000	16	100	
5	8	200	25	625	33	25	
6	10	250	50	1250	60	25	
7	12	300	75	1875	87	25	
8	14	350	100	2500	114	25	
9	16	400	120	3000	136	25	
10	18	450	140	3500	158	25	

Table S1. Carbon standards used to calibrate the Multi NC2100S instrument. Completed using 1000ppm TIC and 1000ppm TOC stock solutions purchased from Sigma Aldrich.

Table S2. Total organic carbon (TOC), total inorganic carbon (TIC) and total total carbon (TC) as measured using the Multi NC2100S instrument for our soil extract solutions taken from UBC Farm, UoL Farm and Rothamsted, as well as one handling blank for contamination reference.

Sample	$TOC \ (mgC \ L^{-1})$	$TIC(mgCL^{-1})$	$TC~(mgCL^{-1})$	Dilution	DateTime
UoL Handling Blank	12.6 (±0.0)	19.8 (±0.0)	32.3 (±0.2)	1.00	6/23/2023 19:16
UBC Field C Sample 1	30.0 (±0.0)	$1.2 (\pm 0.0)$	31.3 (±0.1)	1.00	6/23/2023 19:38
UoL Sample 1	$14.1 (\pm 0.0)$	26.3 (±0.6)	$40.4(\pm 1.7)$	1.00	6/23/2023 20:01
Rothamsted Sample 1	$14.7 (\pm 0.0)$	1.5 (±0.0)	16.3 (±0.1)	1.00	6/23/2023 20:28
UBC Field C Sample 1 (Run 2)	30.4 (±0.0)	1.2 (±0.4)	31.5 (±0.4)	1.00	6/23/2023 20:51



Figure S5. TOC measurements for the filtered Snomax sample $(1000 \text{ mg L}^{-1} \text{ stock concentration})$. The calibration curve was generated using standards with 2.00, 5.00, 10.0 and 20.0 mg L⁻¹ TOC. The Snomax sample was diluted with a factor of 10 prior measurements. The resulting calculated concentration was 125 mg L⁻¹ TOC.



Figure S6. Onset freezing locations for droplets squeezed between two protein-repellent glass slides (a) Lignin (5000 mgC L^{-1}) and (b) literature data for filtered Snomax (125 mgC L^{-1}) from Bieber and Borduas-Dedekind (2024). The mean freezing temperatures were -20.6°C for lignin and -6.3°C for the filtered Snomax sample, which is in the range of the investigated concentrations. Note that the droplet volume was 0.5 µL for these measurements.



Figure S7. Pyrene fluorescence measurements. Pyrene fluorescence 1:3 ratio versus sodium dodecyl sulfate (SDS) concentration (**a**) and lignin concentration (**b**). The I_1/I_3 ratio (y-axes) is the ratio of fluorescent intensities at 373 and 384 nm wavelengths. Markers indicate experimental values in triplicate at each concentration, and curves are a sigmoid fit through the data (only for SDS; lignin measurements have no sigmoid shape). Replicates 1, 2, and 3 (dark to light blue) indicate results of identical solutions pipetted into three different wells. Samples at each concentration contain 2μ M pyrene. Note that the lignin concentrations in mg L⁻¹ on the top x-axis can be converted to mgC L⁻¹ by multiplying by 0.5 and are a different range of concentrations to those used in other measurements.

Table S3. Summary of results of micelle probing measurements for SDS and lignin. Pyrene 1:3 method was performed at 29 °C; numbers are the average \pm standard deviation of three experiments (see Fig. S8). Conductivity reported here were taken at 25 °C; the range is based on two experiments (see Fig. S7).

Compound	Pyrene method CMC (mM)	Conductivity method CMC (mM)
SDS	8.0 ± 0.5	7.60 - 7.95
Lignin	None	None



Figure S8. Conductivity measurements. a) Conductivity versus concentration measurements for sodium dodecyl sulfate (SDS), at 0° C (yellow), 5° C (green), 10° C (light blue), 15° C (medium blue), and 25° C (purple). **b**) Conductivity versus concentration of SDS at 25° C (black) and the first derivative of conductivity-concentration (blue). The first derivative is fit to a sigmoid curve, and the CMC is marked as the inflection point of the sigmoid. **c**) Conductivity versus concentration measurements for lignin, at 0° C (yellow) and 25° C (purple). **d**) Conductivity versus concentration of lignin at 25° C (black) and the first derivative of conductivity-concentration (blue). The first derivative plot shows no sigmoidal shape. Note that the lignin concentrations in mg/L on the top x-axis can be converted to mgC/L by multiplying by 0.5 and are a different range of concentrations to those used in other measurements.

S6 FINC Calibration

20

The average temperature in the well, T_{well} , was calculated from the measured bath temperature, T_{bath} recorded by the Lauda bath thermometer. In this case, samples were analysed at two different time intervals, with some samples analysed in April 2022 and others analysed in March 2023. Therefore, the values of a and b in Eq. 1 were adjusted to account for the change in conditions between the two data analysis periods. So, for the measurements taken in April 2022, a = 0.963 and $b = 0.905 \,^{\circ}\text{C}$ and for measurements taken during March 2023, a = 0.953 and $b = 0.897 \,^{\circ}\text{C}$.

$$T_{\text{well}} = a \cdot T_{\text{bath}} + b$$

(1)



Figure S9. Calibration of the FINC instrument for March 2022, showing the measured temperature inside the PCR tray wells plotted against the temperature reading from the ethanol bath. The plotted equation is the calibration function applied to FINC data collected in March 2022.



Figure S10. Calibration of the FINC instrument for January 2023, showing the measured temperature inside the PCR tray wells plotted against the temperature reading from the ethanol bath. The plotted equation is the calibration function applied to FINC data collected in March 2023.

S7 Lignin Monolayer Calculation

The droplet volume in our tensiometer measurements, V_d , was used to calculate the average droplet radius r_d using Eq. 2. We then calculated the average surface area of the droplets, A_d , using Eq. 3.

$$r_d = \sqrt[3]{\frac{3V_d}{4\pi}} \tag{2}$$

$$A_d = 4\pi r^2 \tag{3}$$

To estimate the amount of lignin required to cover the surface of these droplets, we first needed to calculate the average 30 cross-sectional area of one molecule of lignin, a_m , using the average radius of one lignin molecule, $r_m = 1.6$ nm, which we stimated using the 10,000 g mol⁻¹ molecular weight and a density of 1 g cm⁻³ as inputs in the Molecular Weight to Size calculator by Nanocomposix (https://nanocomposix.com/pages/molecular-weight-to-size-calculator) (Eq. 4). Next, we used the calculated values of A_d and a_m to calculate the average number of lignin molecules, N, required to cover the surface of the droplet (Eq. 5).

$$35 \quad a_m = \pi r_m^2 \tag{4}$$

$$N = \frac{A_d}{a_m} \tag{5}$$

Next, we were able to calculate the concentration of lignin required to cover the surface of the droplet by converting N into the number of moles of lignin, n, (Eq. 6) and then using the molecular weight of lignin, M, of 10⁴ g mol⁻¹ to calculate the mass of lignin when the surface is saturated (Eq. 7). Finally, the concentration of lignin, C_{lignin} was the calculated mass, m,
divided by the droplet volume, V_d, as shown in Eq. 8, and we arrive at a concentration of 3.46 mg L⁻¹.

$$n = \frac{N}{N_A} \tag{6}$$

$$m = n \times M \tag{7}$$

$$C_{lignin} = \frac{m}{V_d} \tag{8}$$



Figure S11. Fraction frozen $(f_{ice}(T))$ curves as a function of temperature for the two soil subcomponents and their dilutions (a) lignin and (b) Snomax extract.



Figure S12. (a) Fraction frozen as a function of temperature for the filtered (red) and unfiltered Snomax dilution series. (b) Ice-active mass site density (n_m) for the same filtered and unfiltered Snomax dilutions series. For comparison, a parameterization of Snomax as described by Wex et al. (2015) is also plotted.

Surface Tension	UoL Sample 1 (mNm^{-1})				UBC Field C			
Concentration	$10^{6} { m mg} { m L}^{-1}$	$10^5 \mathrm{mg}\mathrm{L}^{-1}$	10^3 mg L ⁻¹	10^3 mg L $^{-1}$	$10^6 \mathrm{mg}\mathrm{L}^{-1}$	$10^5 \mathrm{mg}\mathrm{L}^{-1}$	$10^4 \mathrm{mg} \ \mathrm{L}^{-1}$	10^3 mg L ⁻¹
Before	72.2 ± 0.1	71.2 ± 0.4	71.7 ± 0.1	72.4 ± 0.2	72.2 ± 0.1	72.0 ± 0.3	72.3 ± 0.2	72.3 ± 0.1
After	71.7 ± 0.1	72.0 ± 0.1	72.1 ± 0.2	72.8 ± 0.1	71.9 ± 0.1	72.1 ± 0.2	71.9 ± 0.1	71.7 ± 0.2
Change	-0.5	0.8	0.4	0.4	-0.3	0.1	-0.4	-0.6

Table S4. Surface tension measurements (in mN m⁻¹) taken before and after heating to 95 $^{\circ}$ C for 30 mins.

References

Bieber, P. and Borduas-Dedekind, N.: High-speed cryo-microscopy reveals that ice-nucleating proteins of *Pseudomonas syringae* trigger freezing at hydrophobic interfaces, Science Advances, 10, eadn6606, https://doi.org/10.1126/sciadv.adn6606, 2024.

- 50 Gittens, G. J.: Variation of surface tension of water with temperature, Journal of Colloid and Interface Science, 30, 406–412, https://doi.org/10.1016/0021-9797(69)90409-3, 1969.
- Wex, H., Augustin-Bauditz, S., Boose, Y., Budke, C., Curtius, J., Diehl, K., Dreyer, A., Frank, F., Hartmann, S., Hiranuma, N., Jantsch, E., Kanji, Z. A., Kiselev, A., Koop, T., Möhler, O., Niedermeier, D., Nillius, B., Rösch, M., Rose, D., Schmidt, C., Steinke, I., and Stratmann, F.: Intercomparing different devices for the investigation of ice nucleating particles using Snomax[®] as test substance, Atmospheric Chemistry and Physics, 15, 1463–1485, https://doi.org/10.5194/acp-15-1463-2015, 2015.
- Young, T. and Harkins, W.: Surface Tension Data for Certain Pure Liquids Between 0 and 360 C and For All Types of Solutions at All Temperatures., International Critical Tables, p. 446, 1928.