

The sensitivity of the ice-nucleating ability of minerals to heat and the implications for the heat test for biological ice nucleators

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Supplementary Information

S1 Supplementary table

Table S1: List of past studies in which heat treatments were used to infer the presence of biological INPs in samples of various environmental media.

Study	Sample media	Heat treatment method	Ice nucleation measurement method
Baloh et al., 2019	Snow and surface water	Wet: 95 °C for 20 min	Drop freezing assay: 50 µL droplets in 96-well plates
Barry et al., 2021	Aerosol from wildfire smoke plume	Wet: 95 °C for 20 min	Drop freezing assay: 50 µL droplets in 96-well plates
Boose et al., 2019	Desert dusts from nine worldwide locations	Dry: 300 °C for 10 h	Ice crystal counting by optical particle counter downstream of CFDC*
Christner et al., 2008a and b	Snow and rainwater	Wet: 95 °C for 10 min	Drop freezing assay: 0.25 - 1 mL aliquots in test tubes
Conen et al., 2011	Soils with varying organic content	Wet: 100 °C for 10 min	Drop freezing assay: 50 µL droplets in microfuge tubes
Conen et al., 2016	Aerosol and leaf litter suspension	Wet: 80 °C for 10 min	Drop freezing assay: unstated volume in microfuge tubes
Conen et al., 2017	Aerosol sampled on hillside	Wet: 90 °C for 10 min	Drop freezing assay: filter punches immersed in 100 µL droplets in microfuge tubes
Creamean et al., 2018	Bulk seawater and sea surface microlayer	Wet: 90 °C for 30 min	Drop freezing assay: 2.5 µL droplets on cooling stage
Creamean et al., 2020	Permafrost soil and ice wedge	Wet: 95 °C for 20 min	Drop freezing assay: 50 µL droplets in 96-well plates
D'Souza et al., 2013	Plankton sample from frozen lake	Wet: 45, 65 and 90 °C for 2 h	Drop freezing assay: 80 µL aliquots in microcapillary tubes
Du et al., 2017	Rainwater	Wet: 100 °C for 10 min	Drop freezing assay: 10 µL droplets on cooling stage
Garcia et al., 2012	Aerosol and surface dust collected on a farm	Wet: 98 °C for 20 min	Drop freezing assay: 30 or 50 µL droplets in 96-well plates
Gong et al., 2020	Bulk seawater and sea surface microlayer, cloud water and aerosol	Wet: 95 °C for 1 h	Drop freezing assay: 1 µL droplets on cooling stage and 50 µL droplets in 96-well plates
Hara et al., 2016a	Snow collected from ground	Wet: 40 °C and 90 °C for 1 h	Drop freezing assay: filter punches immersed 0.5 mL in microfuge tubes

Study	Sample media	Heat treatment method	Ice nucleation measurement method
Hara et al., 2016b	Aerosol collected on building top	Wet: 90 °C for 1 h	Drop freezing assay: filter punches immersed 0.5 mL in microfuge tubes
Hartmann et al., 2020	Bulk seawater, sea surface microlayer and fog water	Wet: 95 °C for 1 h	Drop freezing assays: 1 µL droplets on cooling stage and 50 µL droplets in 96-well plates
Henderson-Begg et al., 2009	Lichen samples and aerosol sample in urban location	Wet: 37, 60 and 90 °C for unspecified duration	Not stated
Hill et al., 2014	Vegetation washings and snow and hail from ground	Wet: 60 °C and 90 °C for 10 min	Drop freezing assay: 50 µL droplets in 96-well plates
Hill et al., 2016	Topsoil	Wet: 60 °C and 105 °C for 20 min	Drop freezing assay: 50 µL droplets in 96-well plates
Hiranuma et al., 2020	Aerosol and surface dust sampling on a cattle farm	Dry: 100 °C for 12 h before cloud chamber analysis and drop freezing assays. Wet: 100 °C for 20 min before drop freezing assay only.	Drop freezing assays: 3 µL droplets on cooling stage and 50 µL droplets in 96-well plates; cloud chamber
Irish et al., 2017	Bulk seawater and surface microlayer	Wet: 100 °C for 1 h	Drop freezing assay: 0.6 µL droplets on cooling stage
Iwata et al., 2019	Aerosol collected on building in forest	Dry: 150 °C for 10 min	Visual identification of ice growing on particles on cooling Si substrate
Joly et al., 2014	Cloud water	Wet: 95 °C for 10 min	Drop freezing assay: 20 µL aliquots in microfuge tubes
Joyce et al., 2019	Rainwater, sleet and snow	Wet: 95 °C for 10 min	Drop freezing assay: 200 µL droplets in 96-well plates
Knackstedt et al., 2018	River water and aerosolised river water	Wet: 95 °C for 20 min	Drop freezing assay: 80 µL droplets in 96-well plates
Lu et al., 2016	Rainwater	Wet: 100 °C for 20 min	Drop freezing assay: 10 µL droplets on cooling stage
Martin et al., 2019	Rainwater	Wet: 90 °C for 20 min	Drop freezing assay: 50 µL droplets in 96-well plates
McCluskey et al., 2018a	Aerosol at coastal site	Wet: 95 °C for 20 min	Drop freezing assay: 50 µL droplets in 96-well plates; CFDC*
McCluskey et al., 2018b	Sea spray aerosol, bulk seawater and sea surface microlayer	Wet: 95 °C for 20 min	Drop freezing assay: 50 µL droplets in 96-well plates
Michaud et al., 2014	Hailstones	Wet: 95 °C for 10 min	Drop freezing assay: 50 µL droplets in 96-well plates
Moffett et al., 2018	River water	Wet: 90 °C for 10 min	Differential scanning calorimetry
Moffett et al., 2018	River water	Wet: 95 °C for 20 min	Drop freezing assay: 80-100 µL droplets in 96-well plates
O'Sullivan et al., 2014	Agricultural soils	Wet: 90 °C for 10 min	Drop freezing assay: 1 µL droplets on cooling stage

Study	Sample media	Heat treatment method	Ice nucleation measurement method
O'Sullivan et al., 2015	Woodland soils	Wet: 90 °C for 45 min	Drop freezing assay: 1 µL droplets on cooling stage
O'Sullivan et al., 2018	Aerosol sampling on an arable farm	Wet: 95 °C for 1 hr	Drop freezing assay: 1 µL droplets on cooling stage
Paramonov et al., 2018	Soil and desert dusts	Dry: 300 °C for 2 h	Ice crystal counting by optical particle counter downstream of CFDC*
Šantl-Temkiv et al., 2015	Snow and rainwater	Wet: 95 °C for 10 min	Drop freezing assay: 240 - 300 µL droplets in 96-well plates
Šantl-Temkiv et al., 2019	Aerosol and snow samples	Wet: 100 °C for 10 min	Drop freezing assay: 100 - 200 µL droplets for snow samples and filter punches immersed in 50 µL droplets in 96-well plates
Schneider et al., 2021	Aerosol collected from a boreal forest	Wet: 95 °C for 20 min	Drop freezing assay: 50 µL droplets in 96-well plates
Schnell and Vali, 1976	Leaf litter collected from various locations worldwide and seawater	Wet: 60 – 100 °C for unspecified duration	Drop freezing assay
Steinke et al., 2016	Agricultural soils	Dry: 110 °C for 1 h	Ice crystal concentration by optical particle counter in cloud chamber
Suski et al., 2018	Aerosol and surface dust sampling on an arable farm	Wet: 95°C for 20 min. Dry: 300 °C upstream of CFDC	Drop freezing assay: 50 µL droplets in 96-well plates; ice crystal counting by optical particle counter downstream of CFDC*
Tobo et al., 2020	Aerosol collected from tall TV mast in Tokyo, Japan	Wet: 100°C for 1 h	Drop freezing assay: 5 µL droplets on cooling stage
Tesson and Šantl-Temkiv, 2018	Snow	Wet: 100 °C for 10 min	Drop freezing assay: droplets of unspecified volume on cooling stage
Wilson et al., 2015	Bulk seawater and sea surface microlayer	Wet: 8 temperatures between 20 °C and 100 °C for 10 min	Drop freezing assay: 1 µL droplets on cooling stage
Yadav et al., 2019	Rainwater and desert dust from surface	Wet: 100 °C for 10 min	Drop freezing assay: 1 µL droplets on cooling stage

*CFDC = Continuous Flow Diffusion Chamber

S2 Supplementary figures

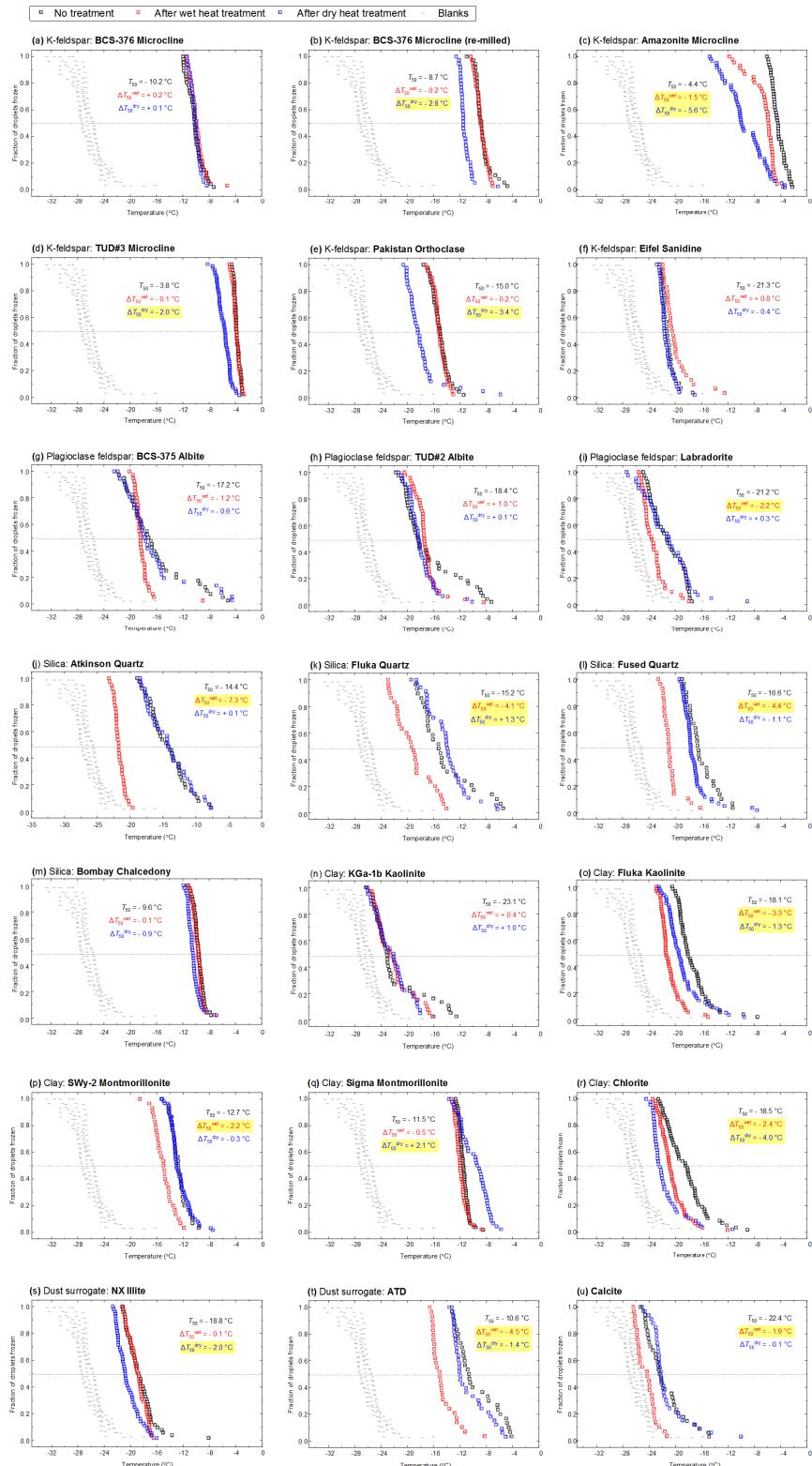


Figure S1: Fraction of droplets frozen ($f_{\text{ice}}(T)$) curves for all mineral-based INP samples. Data for four background runs are shown in each plot. A dotted horizontal line denotes $f_{\text{ice}}(T) = 0.5$, from which T_{50} values were determined. All suspensions were prepared to a concentration of 1 % w/v. Denoted in each panel are T_{50} , $\Delta T_{50}^{\text{wet}}$ and $\Delta T_{50}^{\text{dry}}$ values for the sample, with significant (ΔT_{50} greater than $\pm 1.2^\circ\text{C}$) values highlighted in yellow.

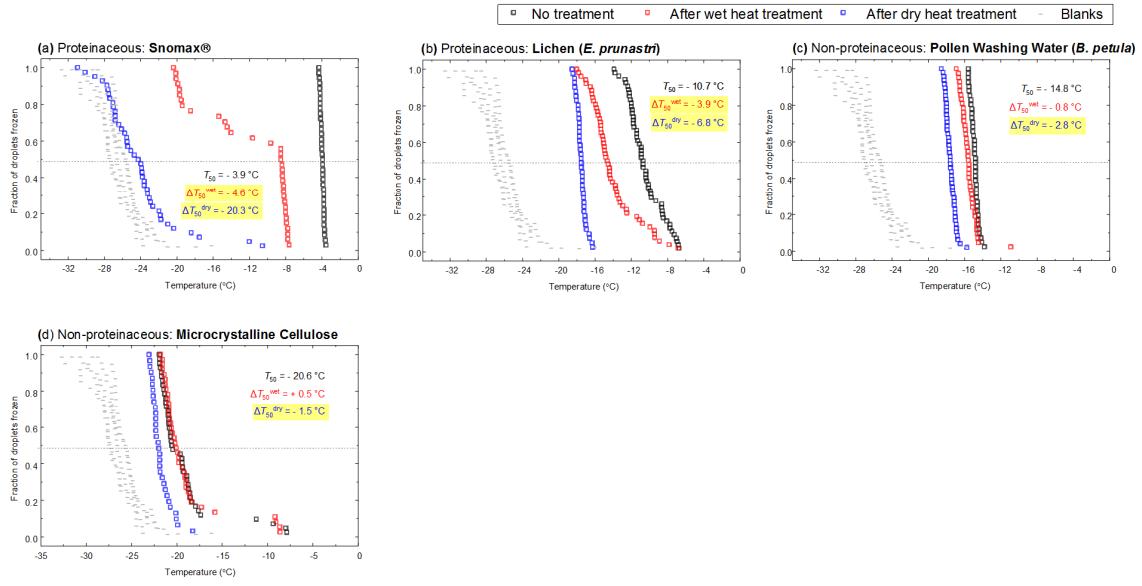


Figure S2: Fraction of droplets frozen ($f_{\text{ice}}(T)$) curves for all biological INP samples. Data for four background runs are shown in each plot. A dotted horizontal line denotes $f_{\text{ice}}(T) = 0.5$, from which T_{50} values were determined. All suspensions were prepared to a concentration of 1 % w/v. Denoted in each panel are T_{50} , $\Delta T_{50}^{\text{wet}}$ and $\Delta T_{50}^{\text{dry}}$ values for the sample, with significant (ΔT_{50} greater than $\pm 1.2^\circ\text{C}$) values highlighted in yellow.

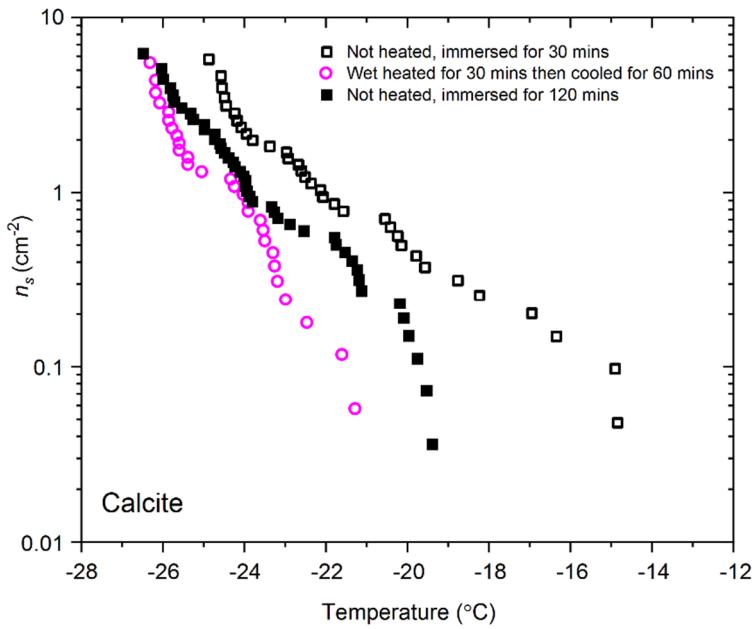


Figure S3: Plot showing $n_s(T)$ data for calcite, illustrating the relative changes in INA before and after wet heating compared to a control suspension (solid squares) that was immersed in room temperature water for the same amount of time as the wet heated sample.

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