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

Figure S3: Plot showing $n_i(T)$ 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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