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Best practices for precipitation sample storage for offline studies of ice nucleation in marine and coastal environments

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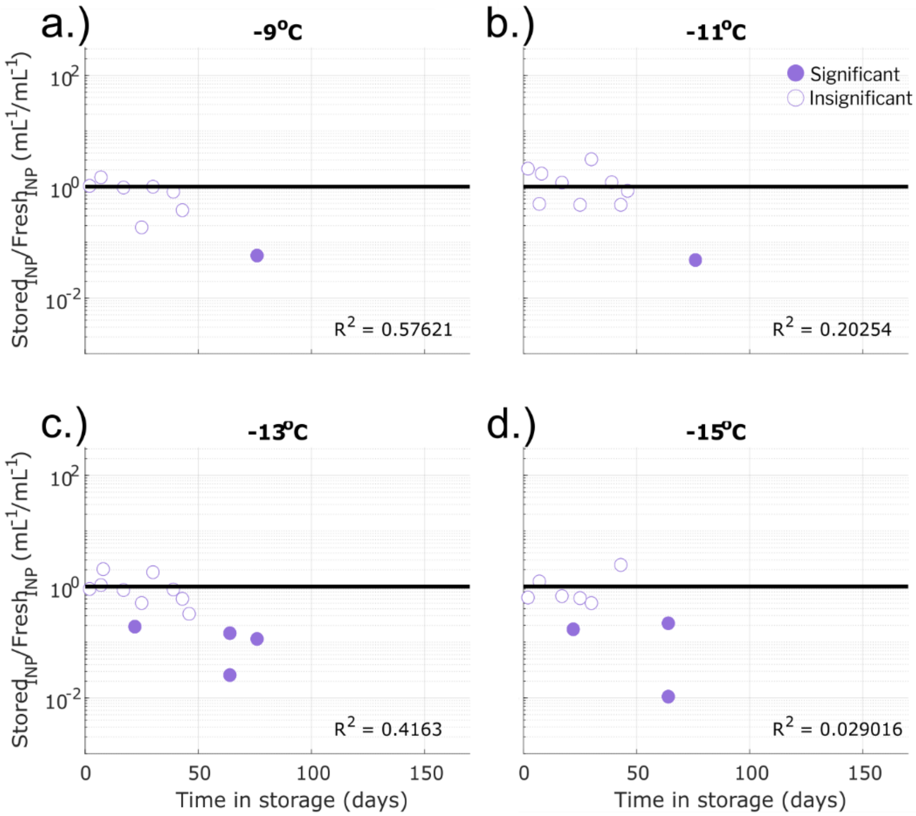

Figure S1: Changes in INP concentration in untreated precipitation samples stored between 1 and 76 days at room temperature (+21-23 °C). Stored sample frozen well fractions that passed Fishers Exact Test ($p < 0.01$) for significant differences from original fresh sample frozen well fractions are indicated with filled markers. R^2 values for linear fits are shown for each INP freezing temperature bin with >3 samples. Results show moderate correlation between storage time interval and INP losses for freezing temperatures >= -9 °C.

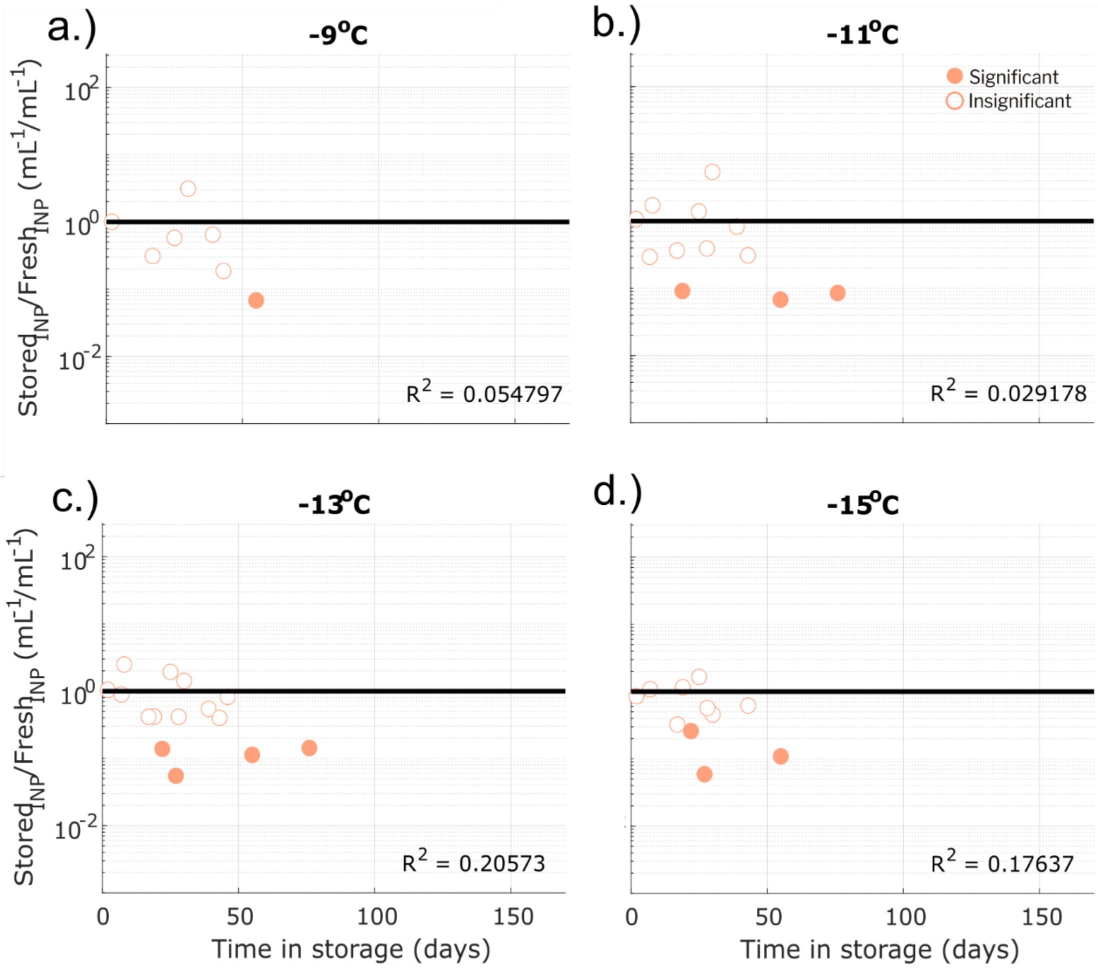

Figure S2: Changes in INP concentration in untreated precipitation samples stored between 1 and 76 days at +4 °C. Stored sample frozen well fractions that passed Fishers Exact Test ($p < 0.01$) for significant differences from original fresh sample frozen well fractions are indicated with filled markers. R^2 values for linear fits are shown for each INP freezing temperature bin with >3 samples. Results show lack of correlation between storage time interval and changes in INP concentrations.

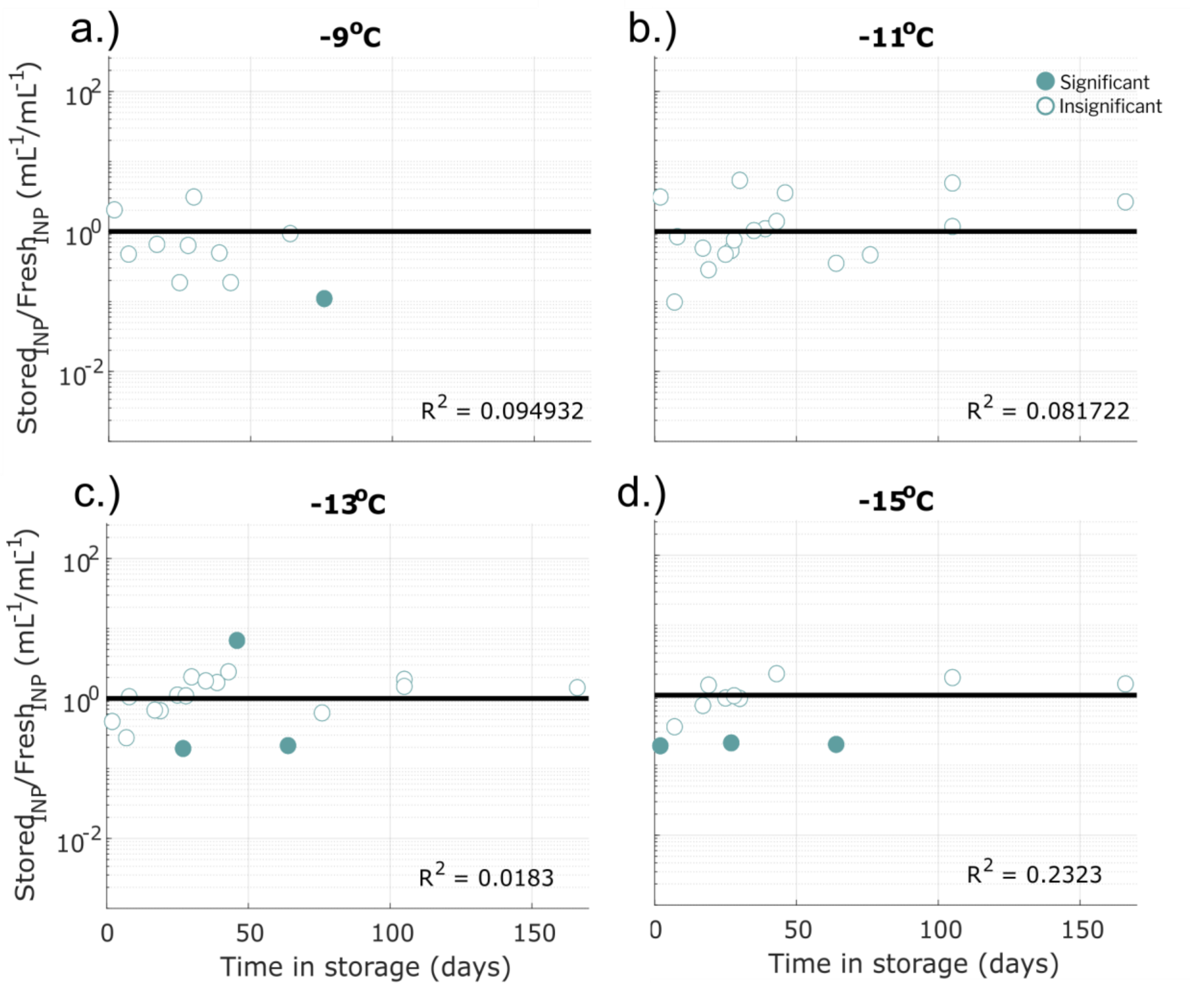

Figure S3: Changes in INP concentration in untreated precipitation samples stored between 1 and 166 days at -20 °C. Stored sample frozen well fractions that passed Fishers Exact Test ($p < 0.01$) for significant differences from original fresh sample frozen well fractions are indicated with filled markers. R^2 values for linear fits are shown for each INP freezing temperature bin with >3 samples. Results show a lack of correlation between storage time interval and INP enhancements or losses.

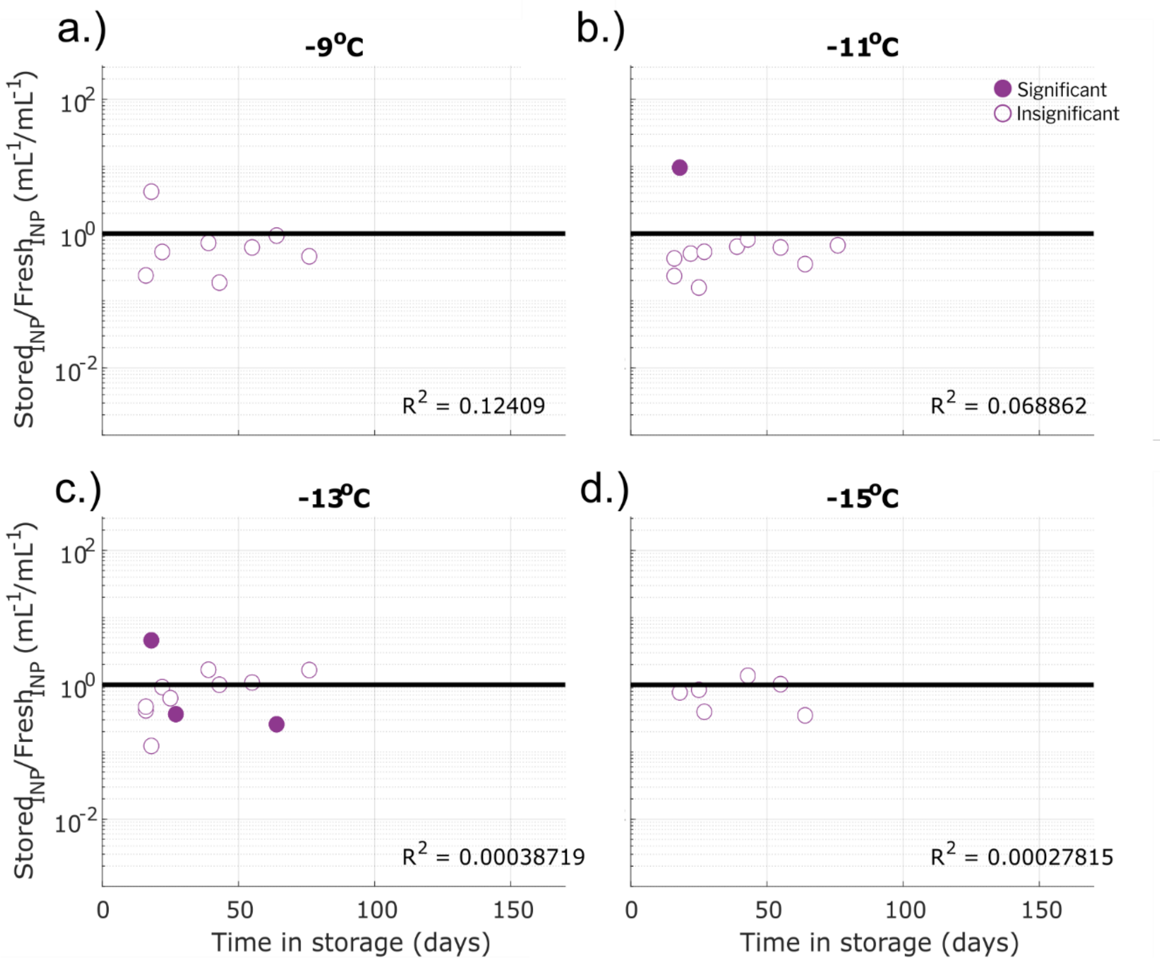

Figure S4: Changes in INP concentration in untreated precipitation samples between 1 and 76 days flash frozen with liquid nitrogen and stored at -20 °C. Stored sample frozen well fractions that passed Fishers Exact Test ($p < 0.01$) for significant differences from original fresh sample frozen well fractions are indicated with filled markers. R^2 values for linear fits are shown for each INP freezing temperature bin with >3 samples. Results show a lack of correlation between storage time interval and INP enhancements or losses.

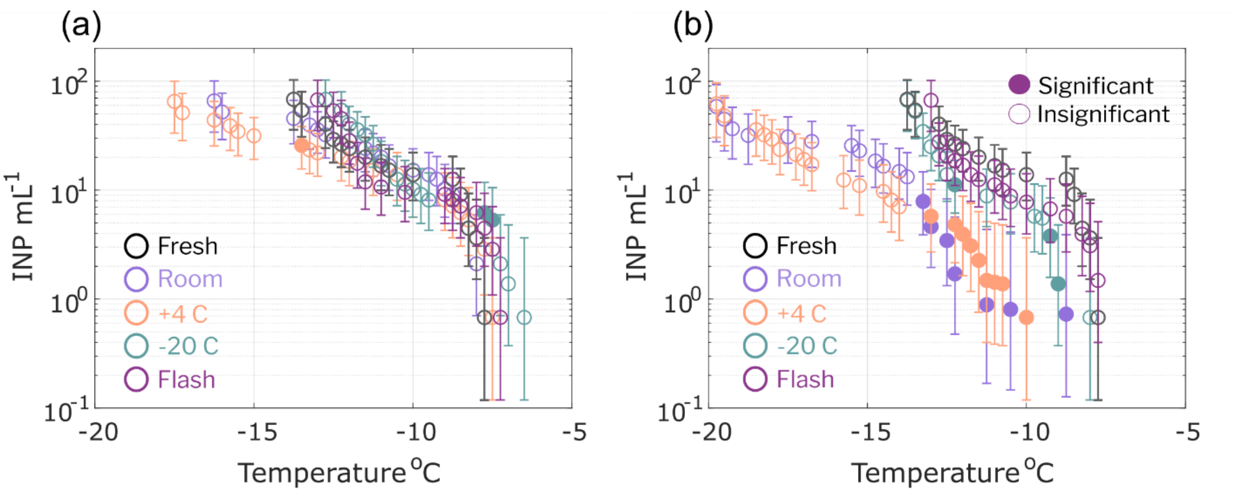

Figure S5: IN spectra of precipitation collected 1/19/2017, fresh and stored samples. IN spectra of stored samples are shown (a) 27 days and (b) 64 days after collection. Stored sample frozen well fractions that passed Fishers Exact Test ($p < 0.01$) for significant differences from original fresh sample frozen well fractions are indicated with filled markers. Results in (a), 27 days after collection, show INPs were generally insensitive to the various storage techniques. IN spectra of samples stored at room temperature and at +4 °C in (b) show significant losses in INP concentrations up to 1 order of magnitude from -10 to -20 ºC, indicating sensitivity to time in storage. Samples stored at -20 ºC or flash frozen were generally insensitive to storage and time interval.

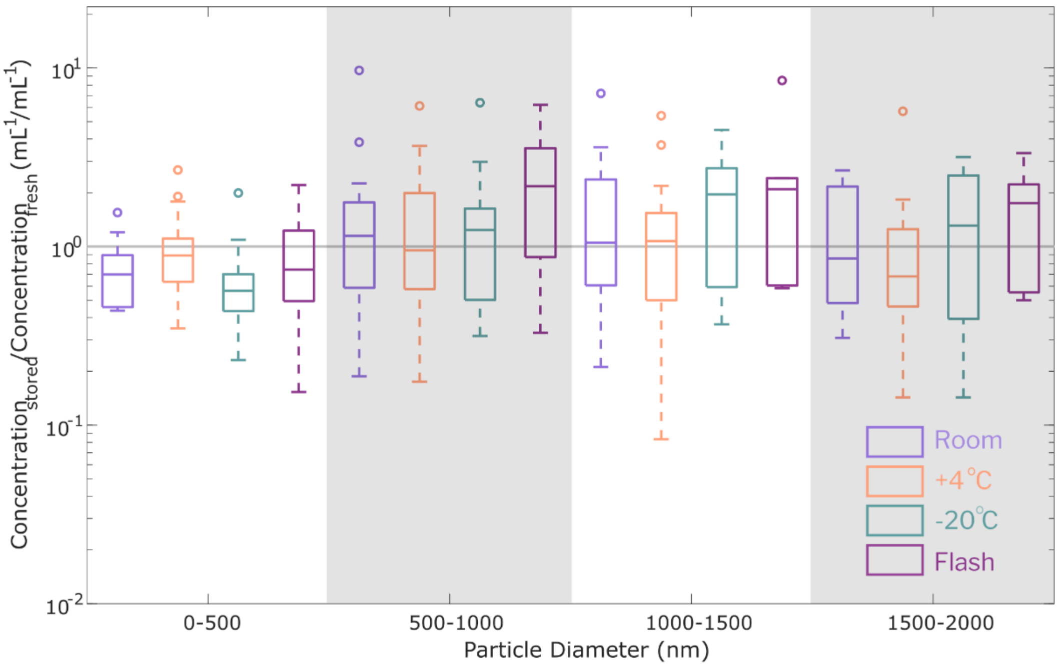

Figure S6: Distribution of total insoluble particle concentration ratios, stored:fresh, for 10 untreated precipitation samples (sampling periods 1-10, see Table 1).

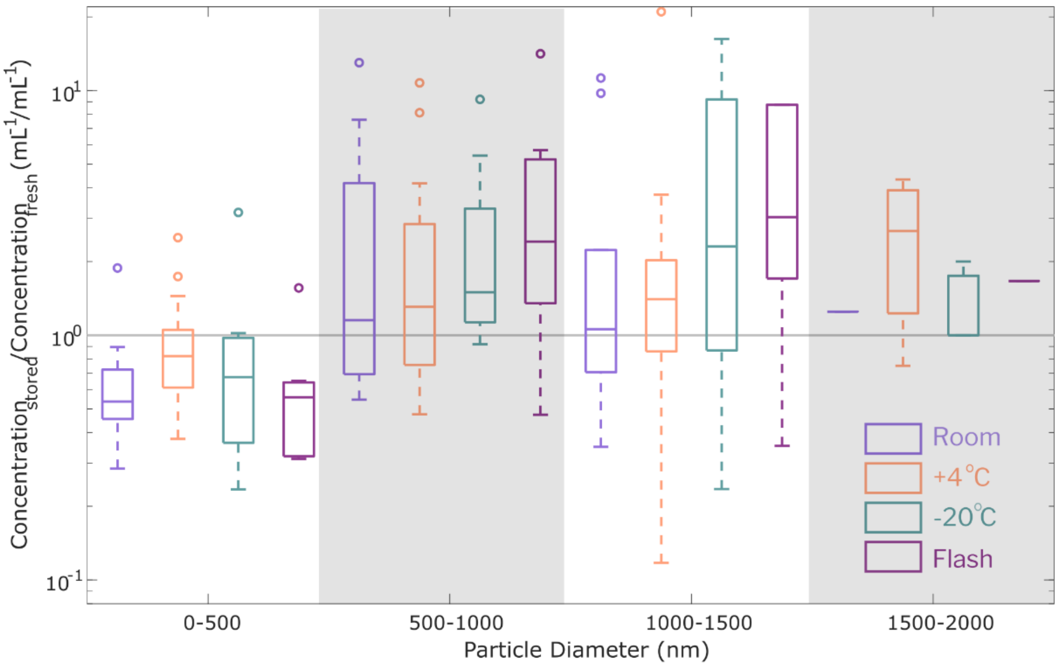

Figure S7: Distribution of total insoluble particle concentration ratios, stored:fresh, for 10 heat-treated precipitation samples (sampling periods 1-10, see Table 1).

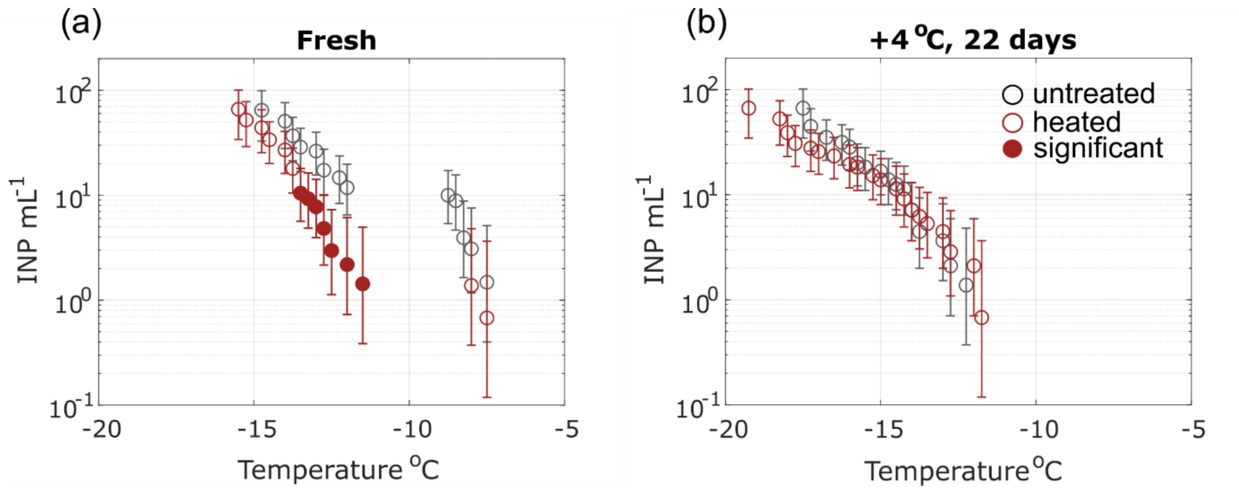

Figure S8: IN spectra of untreated and heat-treated precipitation collected 1/11/2017 for (a) a fresh sample and (b) a replicate stored at +4 °C for 22 days. Significant differences in frozen well fractions between the heat-treated and untreated sample (Fishers Exact Test, $p < 0.01$) are indicated with filled markers. Results in (a) indicate the presence of heat-labile (i.e. proteinaceous or cellular) INPs between -8 and -14 °C. However, results in (b) show a lack of heat-labile INPs and diminished concentrations overall, indicating losses of INPs in storage, including those that are heat-labile.

Table S1: Summary of previously reported precipitation sample storage conditions for offline analysis of INPs

Publication	Storage temperature (ºC)	Total storage time	Other details
1. Vali G. Sizes of Atmospheric Ice Nuclei. Nature. 1966;212(5060):384–5	unspecified	unspecified	
2. Vali G. Freezing Nucleus Content of Hail and Rain in Alberta. J Appl Meteorol. 1971 Jan 23;10(1):73–8.	-80	unspecified	
3. Christner BC, Morris CE, Foreman CM, Cai R, Sands DC. Ubiquity of Biological Ice Nucleators in Snowfall. Science (80-). 2008 Feb 29;319(5867):1214 LP – 1214.	variable	unspecified	Stored snow samples in coolers embedded in snowpack, then retrieved and archived in a -20 ºC freezer, unspecified storage time
4. Christner BC, Cai R, Morris CE, McCarter KS, Foreman CM, Skidmore ML, et al. Geographic, seasonal, and precipitation chemistry influence on the abundance and activity of biological ice nucleators in rain and snow. Proc Natl Acad Sci U S A. 2008;105(48):18854–9.	+4	18 hours	
5. Morris CE, Sands DC, Vinatzer BA, Glaux C, Guilbaud C, Buffière A, et al. The life history of the plant pathogen Pseudomonas syringae is linked to the water cycle. Isme J. 2008 Jan 10;2:321.	unspecified	unspecified	
6. Hill TCJ, Moffett BF, DeMott PJ, Georgakopoulos DG, Stump WL, Franc GD. Measurement of ice nucleation-active bacteria on plants and in precipitation by quantitative PCR. Appl Environ Microbiol. 2014;80(4):1256–67.	-20	unspecified	
7. Hader JD, Wright TP, Petters MD. Contribution of pollen to atmospheric ice nuclei concentrations. Atmos Chem Phys. 2014;14(11):5433–49.	-17	2 weeks	
8. Wright TP, Hader JD, McMeeking GR, Petters MD. High relative humidity as a trigger for widespread release of ice nuclei. Aerosol Sci Technol. 2014;48(11):i–v.	n/a	immediate processing	
9. Stopelli E, Conen F, Zimmermann L, Alewell C, Morris CE. Freezing nucleation apparatus puts new slant on study of biological ice nucleators in precipitation. Atmos Meas Tech. 2014;7(1):129–34.	+4	n/a	storage experiment on snow sample stored at +4 C over 30 days

10. Michaud AB, Dore JE, Leslie D, Lyons WB, Sands DC, Priscu JC. Biological ice nucleation initiates hailstone formation. J Geophys Res Atmos. 2014;119(21):12,112-186,197.	-30	unspecified	thawing process prior to analysis: 18 hrs at +4 C
11. Rangel-Alvarado RB, Nazarenko Y, Ariya PA. Snow-borne nanosized particles: Abundance, distribution, composition, and significance in ice nucleation processes. J Geophys Res Atmos. 2015;120(22):11,711-760,774.	-10 or -35	unspecified, many within 24 hours	
12. Santl-Temkiv T, Sahyoun M, Finster K, Hartmann S, Augustin-Bauditz S, Stratmann F, et al. Characterization of airborne ice-nucleation-active bacteria and bacterial fragments. Atmos Environ. 2015;109:105–17.	unspecified	unspecified	
13. Petters MD, Wright TP. Revisiting ice nucleation from precipitation samples. Geophys Res Lett. 2015;42(20):8758–66.	+4	0-35 days	
14. Stopelli E, Conen F, Morris CE, Herrmann E, Bukowiecki N, Alewell C. Ice nucleation active particles are efficiently removed by precipitating clouds. Sci Rep. 2015;5:16433.	n/a	processed immediately	
15. Hara K, Maki T, Kakikawa M, Kobayashi F, Matsuki A. Effects of different temperature treatments on biological ice nuclei in snow samples. Atmos Environ. 2016;140:415–9.	-10	unspecified	pre-concentrated samples prior to analysis
16. Stopelli E, Conen F, Guilbaud C, Zopfi J, Alewell C, Morris CE. Ice nucleators, bacterial cells and \textit{Pseudomonas syringae}\hack{\newline} in precipitation at Jungfraujoch. Biogeosciences. 2017;14(5):1189–96.	n/a	processed immediately	
17. Failor KC, Iii DGS, Vinatzer BA, Monteil CL. Ice nucleation active bacteria in precipitation are genetically diverse and nucleate ice by employing different mechanisms. 2017;1–14.	+ 4	unspecified	
18. Martin AC, Cornwell G, Beall CM, Cannon F, Reilly S, Schaap B, et al. Contrasting local and long-range-transported warm ice-nucleating particles during an atmospheric river in coastal California, USA. Atmos Chem Phys. 2019;19(7):4193–210.	-20	4 months	

19. Joyce RE, Lavender H, Farrar J, Werth JT, Weber CF, D'Andrilli J, et al. Biological Ice-Nucleating Particles Deposited Year-Round in Subtropical Precipitation. Stams AJM, editor. Appl Environ Microbiol. 2019 Dec 1;85(23):e01567-19.	+4	0 - 48 hours	
20. Creamean JM, Mignani C, Bukowiecki N, Conen F. Using freezing spectra characteristics to identify ice-nucleating particle populations during the winter in the Alps. Atmos Chem Phys. 2019;19(12):8123–40.	n/a	processed immediately	
21. David RO, Cascajo-Castresana M, Brennan KP, Rösch M, Els N, Werz J, et al. Development of the DRoplet Ice Nuclei Counter Zurich (DRINCZ): validation and application to field-collected snow samples. Atmos Meas Tech. 2019;12(12):6865–88.	-20	unspecified	snow thawed to room temperature (+20 C) before storing at -20 C