https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020

Atmospheric Measurement Techniques Discussions

Best practices for precipitation sample storage for offline studies of ice nucleation

Charlotte M. Beall[1], Dolan Lucero[2], Thomas C. Hill[3], Paul J. DeMott[3], M. Dale Stokes[1], Kimberly A. Prather[1,4]

[1]Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, 92037, USA
[2]Department of Earth and Environmental Science, New Mexico Institute of Mining and Technology, Socorro, NM, 87801, USA
[3]Department of Atmospheric Sciences, Colorado State University, Fort Collins, CO, 80523, USA
[4]Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA, 92093, USA

Correspondence to: Kimberly A. Prather (kprather@ucsd.edu)

Abstract. Ice nucleating particles (INPs) are efficiently removed from clouds through precipitation, a convenience of nature for the study of these very rare particles that influence multiple climate-relevant cloud properties including ice crystal concentrations, size distributions, and phase-partitioning processes. INPs suspended in precipitation can be used to estimate in-cloud INP concentrations and to infer their original composition. Offline droplet assays are commonly used to measure INP concentrations in precipitation samples. Heat and filtration "treatments" are also used to probe INP composition and size ranges. Many previous studies report storing samples prior to INP analyses, but little is known about the effects of storage on INP concentration or their sensitivity to treatments. Here, through a study of 15 precipitation samples collected at a coastal location in La Jolla, CA, USA, we found significant changes caused by storage to concentrations of INPs with warm to moderate freezing temperatures (-7 to -19 ºC). We compared four conditions: 1.) storage at room temperature (+ 21-23 ºC), 2.) storage at +4 ºC 3.) storage at -20 ºC, and 4.) flash freezing samples with liquid nitrogen prior to storage at -20 ºC. Results demonstrate that storage can lead to both enhancements and losses of greater than one order of magnitude, with non-heat-labile INPs being generally less sensitive to storage regime, but significant losses of INPs smaller than 0.45 µm in all tested storage protocols. Correlations between total storage time (1-166 days) and changes in INP concentrations were weak across sampling protocols, with the exception of INPs with freezing temperatures >= -9 ºC in samples stored at room temperature or +4 ºC. We provide the following recommendations for preservation of precipitation samples from coastal environments intended for INP analysis: that samples be stored at -20 ºC to minimize storage artifacts, that changes due to storage are likely and an additional uncertainty in INP concentrations, and that filtration treatments be applied only to fresh samples. Average INP losses of 72%, 42%, 25% and 32% were observed for untreated samples stored using the room temperature, +4 ºC, -20 ºC, and flash frozen protocols, respectively. Finally, correction factors are provided so that INP measurements obtained from stored samples may be used to estimate concentrations in fresh samples.

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020


Atmospheric Measurement Techniques Discussions

1. Introduction

In-cloud ice crystals and their formation processes are critical features of Earth's radiative and hydrological balance, affecting multiple climate-relevant cloud properties including cloud lifetime, reflectivity, and precipitation efficiency (DeMott et al., 2010; Lohmann, 2002; Lohmann and Feichter, 2005; Tan et al., 2016; Creamean et al., 2013). Ice nucleating particles (INPs) impact ice crystal concentrations and size distributions in clouds by triggering the freezing of droplets at temperatures above the homogeneous freezing point of water (-38 ºC).

INPs have been sampled in clouds and precipitation for decades (e.g. Rogers et al., 1998; Vali, 1971; Vali, 1966) to measure abundances, probe their compositions and investigate the extent to which they impact the properties of clouds. There are several caveats to consider when inferring in-cloud INP concentrations or properties from precipitation samples (Petters and Wright, 2015a), including "sweep-out" of additional INPs as the hydrometeor traverses the atmosphere below the cloud (Vali, 1974) and heterogeneous chemistry due to adsorption or absorption of gases (Hegg and Hobbs, 1982; Kulmala et al., 1997; Lim et al., 2010). However, assessing the composition of INPs in precipitation samples is more straightforward than cloud particles. Thus, the number of publications reporting measurements of INP concentrations in precipitation has increased significantly over the past decade. Significant insights have been obtained in previous precipitated-based INP studies, including the efficient depletion of INPs relative to other aerosols of similar size in precipitating clouds (Stopelli et al., 2015), constraints on minimum enhancement factors for secondary ice formation processes (Petters and Wright, 2015b), and the identification, characteristics and distribution of various INP populations (e.g. Christner et al., 2008a; Hader et al., 2014; Stopelli et al., 2017). INP concentrations in precipitation have been used to estimate in-cloud concentrations, based on assumptions that the majority of particles (86%) in precipitation originate from the cloud rather than the atmospheric column through which the hydrometeor descended (Wright et al., 2014). Along the same line of reasoning, INPs in precipitation have also been used to infer sources and composition of in-cloud INP populations (e.g. Martin et al., 2019 and Michaud et al., 2014, respectively). Measurements of INPs suspended in precipitation are commonly made offline using a droplet freezing assay technique, and many studies report results from samples stored prior to processing. Storage protocols vary widely, including total storage time, time between collection and storage, and temperature fluctuations between collection, shipment and storage (if these details are provided at all), yet generally samples are stored between + 4 ºC and -20 ºC (see Table S1).

The effects of storage on INPs suspended in precipitation are highly uncertain (Petters and Wright, 2015b), and the understanding of storage effects on INPs collected on filters is similarly lacking (Wex et al., 2019). Stopelli et al. (2014a) studied INP concentrations in a snow sample stored at +4 ºC and observed a decrease in the concentration of INPs active at -10 ºC over 30 days by a factor of ~2. Schnell (1977) reported significant losses in fog and seawater samples after storage at room temperature for short periods (6-11 hours). Several studies have reported on the lability of commercially available dust and biological IN entities in storage above 0 ºC or under freezing conditions, including Arizona Test Dust and SnoMax® (Perkins et al., 2020; Polen et al., 2016; Wex et al., 2015), and similar labilities could affect the INPs of similar composition in precipitation samples (Creamean et al., 2013; Martin et al., 2019). Considering the abundance of precipitation based INP

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020


Atmospheric Measurement Techniques Discussions

studies, the lack of bounds on potential impacts of storage on INP concentration measurements represents a critical uncertainty in conclusions derived from data on stored samples. Furthermore, to determine INP activation mechanisms and composition, previous studies have applied "treatments" to precipitation samples, including heat, filtration, enzymes and peroxide, (e.g. Hill et al., 2014) but it is unknown to what extent storage affects the results of such experiments.

Here we investigate the effects of four storage protocols on INPs using 15 precipitation samples collected between 9/22/2016 and 11/22/2019 at two coastal sites at Scripps Institution of Oceanography, La Jolla, CA, USA: 1.) storage at room temperature (+ 21-23 ºC) , 2.) storage at +4 ºC ("refrigerated"), 3.) storage at -20 ºC ("frozen"), and 4.) flash freezing samples with liquid nitrogen prior to storage at -20 ºC ("flash frozen"). The abundance of previous studies that report storage between +4 ºC and -20 ºC motivated the choice of techniques 2 and 3 (see Table S1). Room temperature storage was chosen to provide context as a "worst-case scenario", and the flash freezing technique was chosen to investigate whether any changes of INP concentrations could be mitigated by instantaneous freezing prior to storage. The 15 precipitation samples in this study were divided into several replicates so that the concentration of INPs could be measured in untreated, heated, and filtered samples when fresh, and again after storage using the 4 techniques described above. Sample replicates were additionally processed at 2 different points in time to investigate the effects of total storage time on INP concentration measurements. Enhancements and losses of INPs according to storage protocol and treatment are reported, as well as recommendations for storage protocols that best preserve INPs in untreated, heated, and filtered precipitation samples from coastal environments.

2. Methods

2.1 Precipitation Sample Collection

Precipitation samples were collected at two coastal locations at Scripps Institution of Oceanography (32.87 N 177.25 W): the rooftop of the Ellen Browning Scripps Memorial Pier laboratory (32.8662 °N, 117.2544 °W) (10 meters above sea level) and the rooftop of a storage container next to Isaacs Hall (32.8698 °N, 117.2522 °W, 58 meters above sea level, 500 m inland). Collection technique varied based on location. At the SIO pier, the Teledyne ISCO model 6712 commercial water sampler (Teledyne ISCO, Inc., US) was used. A plastic funnel, 27 cm in diameter, and Tygon tubing, connected the sampler inlet to the water sampler's distributor arm. The samples were distributed via the distributor arm into one of twenty-four 1-liter polypropylene bottles on an hourly time interval. Bottles were combined when the hourly precipitation volume was insufficient for sample separation and analysis (< 50 mL). At the MESOM Laboratory parking lot, an ISO 6706 plastic graduated cylinder and plastic funnel, 27 cm in diameter, was used for precipitation collection. At both sites, ring stands supported the collection funnels approximately 60 cm above the rooftop. All funnels, tubing, cylinders, and bottles were cleaned with 10% hydrogen peroxide for 10 minutes and rinsed with milli-Q purified water three times immediately before each sampling event.

2.2 Storage Protocols

The following sample storage protocols were used: frozen at -20 ºC, refrigerated at 4 ºC, room temperature (21 - 23 ºC), and flash freezing, or flashing with liquid nitrogen (-196 ºC) before frozen at -20 ºC. All techniques except storage at room temperature are commonly used for offline INP analysis (see Table S1). Excluding the samples that were flash frozen, all

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020


Atmospheric Measurement Techniques Discussions

samples were stored in 50 mL sterile plastic Falcon® tubes (Corning Life Sciences, Corning, NY, USA). Flash frozen samples were stored in polypropylene 5 mL cryovials. Not all samples were stored using all 4 of the storage protocols due to limited volume for some samples. See Tables 2-4 for a summary of the number of samples studied for each storage protocol. Precipitation samples were stored for varying intervals between 1 and 166 days to investigate effects of storage time on INP concentrations. INP measurements were made in two or three time steps: within two hours of collection, and once or twice after storing using one of four storage protocols described in the section above, depending on volume. Stored and fresh samples were analysed in three treatment conditions: 1) raw untreated precipitation, 2) heated over a 95 ºC water bath for 20 minutes and 3) filtered through a 0.45 µm surfactant-free cellulose acetate syringe-filter (Thermo Scientific™ Nalgene™, Waltham, MA, USA).

2.3 INP Analysis

The automated ice spectrometer (AIS) is an offline immersion-mode freezing assay which is described elsewhere (Beall et al., 2017). Briefly, 50 uL aliquots of sample are pipetted into two sterile 96-well polypropylene PCR plates. The plates are inserted into an aluminium block, machined to hold PCR plates, that sits in the coolant bath of a Fisher Scientific Isotemp® Circulator. A thermistor placed atop the left side of the aluminium block, below the PCR plate, recorded temperature. An acrylic plate separated the PCR plates from the ambient lab air. In the headspace between the acrylic plate and the PCR plates, nitrogen gas flowed at a flow rate of 14 Lpm to reduce temperature stratification in the samples (Beall et al., 2017). The nitrogen gas was cooled before emission by passing through the chiller via copper tubing. A 0.5 Megapixel monochrome camera (Point Grey Blackfly 0.5MP Mono GigE POE) performed the image capture. Custom LabView software controlled the camera settings, the rate the chiller cooled, and displayed the temperature of the thermistor.

A control milli-Q water sample is used, typically in the first 30 wells of each sample run, to detect contamination and for subsequent INP concentration calculations. Thirty wells were used per sample to achieve a limit of detection of 0.678 IN mL^{-1}. For each run, the chiller was cooled to -35°C. As the chiller cools the sample plates (1 ˚C/min), the custom LabView virtual instrument records the location and temperature of the freezing event as they occur. Freezing events are detected by the change in pixel intensity of the sample as it changes from liquid to solid.

2.4 Particle Size Distributions

Size distributions of insoluble particles suspended in the fresh and stored precipitation samples were measured using the Multi-sizing Advanced Nanoparticle Tracking Analysis (MANTA) ViewSizer 3000 (Manta Instruments Inc.). The Manta ViewSizer 3000 applies multi-spectral particle tracking analysis (m-PTA) to obtain size distributions of particles of sizes between 10 and 2000 nm with three solid-state lasers with wavelengths of 450 nm, 520 nm and 650 nm. m-PTA has been shown to outperform traditional dynamic light scattering (DLS) techniques when measuring polydisperse particles in suspension (McElfresh et al., 2018). For analysis, 300 videos of the illuminated particles in suspension are recorded, each 10 seconds in length. The software tracks each particle individually, obtaining particle size and number concentration from their Brownian motion and the imaged sample volume.

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020


Atmospheric Measurement Techniques Discussions

3 Results

3.1 INP concentrations in fresh precipitation samples

Figure 1 shows INP concentrations of 15 coastal precipitation samples. Observations generally fall within bounds of previously reported INP concentrations from precipitation and cloud water samples (grey shaded region, adapted from Petters and Wright, 2015b). Observed freezing temperatures ranged from -4.0 to -18.4 ºC, with concentrations up to the limit of testing at 10^5 INP L^{-1} precipitation. AIS measurement uncertainties are represented with 95% binomial sampling intervals (Agresti and Coull, 1998).

3.2 Effects of sample storage on INP concentration measurements

INP concentrations of stored replicate samples are compared with original fresh precipitation samples in Figures 2-4, binned by 2 ºC increments between -7 and -19 ºC. This temperature range was chosen for the analysis because most fresh precipitation samples exhibited significant freezing activity between -7 and -19 ºC. All stored:fresh ratios were calculated from cumulative INP distributions binned by 2 ºC, meaning that the INP concentration in each bin is inclusive of the concentration in the preceding (warmer) temperature bin. The choice of the cumulative distribution was motivated by the fact that it is standard in INP studies to report INP concentrations in terms of the cumulative distribution, and it is important to consider impacts of storage on cumulative INP distributions and any conclusions derived from them. Thus, in this study, significant deviations observed in a stored sample are not necessarily independent, i.e. the sensitivity of INPs to storage in one temperature bin could impact the observed changes in each of the following (colder) temperature bins. For example, in the fresh untreated precipitation samples (see Fig. 1), the contribution of INPs from the preceding 2 ºC bin ranges from 32 to 46% between -9 and -17 ºC.

To investigate correlations between sample storage time and INP enhancements or losses, duplicate samples were archived (when sufficient volume was available) so that each sample could be processed at two distinct points post-collection (see example Fig. S1). For INPs with freezing temperatures >= -9 °C in samples stored at room temperature or +4 °C, time is moderately correlated with changes in INP concentrations (R^2 = 0.62 and 0.53, respectively). Figure S5 shows how losses of warm-freezing INPs in samples stored at +4 °C and room temperature impact the cumulative INP spectra for a select sample. Beyond these exceptions, little to no correlation between storage time and INP enhancements or losses was found for untreated, heated and filtered samples (see Figs S1-S4). This indicates that most of the changes in INPs observed may occur on shorter timescales than those studied here, i.e. < 24 hours.

Figure 2 shows the ratio of stored sample to fresh sample INP concentrations for untreated precipitation samples stored under four conditions: **(a)** room temperature (21 – 23 ºC), **(b)** refrigerated (+ 4 ºC), **(c)** frozen (-20 ºC) and **(d)** flash frozen with liquid nitrogen before storing at -20 ºC. Markers above the 1:1 line indicate enhancements in INP concentration from the fresh sample, while markers below indicate losses. For each temperature bin containing data from at least two sets of replicate samples, the average difference in stored:fresh concentration ratios between replicates are represented with grey bars to indicate measurement variability. Finally, Fisher's Exact Test was applied to frozen and unfrozen well counts between each stored

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020


Atmospheric Measurement Techniques Discussions

sample and its corresponding fresh sample at each of the 2 ºC temperature bins. Stored sample frozen well counts that were significantly different ($p < 0.01$) from fresh sample frozen well fractions as a fraction of total sample wells at each of the 5 temperatures are indicated with filled markers.

Results in Fig. 2 show that significant enhancements or losses of INPs occurred for all stored samples between -9 and -17 ºC, and that on average, stored samples exhibit INP losses (as indicated by the mean change in each temperature bin). In frozen and flash frozen samples, all enhancements and losses fall within ± 1 order of magnitude, whereas several significant INP losses beyond 1 order of magnitude are shown in room and refrigerated samples. INP concentration changes >= 1 order of magnitude are greater than changes in the ratios of the total insoluble particle population 10 – 2000nm during storage (see Fig. S6). This indicates that the INPs in these samples are more sensitive to storage than the total insoluble particle population. Fig. S5 illustrates the impacts of the 4 storage protocols on the full IN spectra of a select untreated precipitation sample at two time intervals, 27 days and 64 days after collection.

Figure 3 shows the effects of storage on INP observations in heat-treated precipitation samples. Non-heat-labile INPs represented the majority (59% on average) of the total INPs observed in the fresh samples (i.e. 41% of the INPs in fresh samples were heat-labile). Fewer significant losses of non-heat-labile INPs are observed for heat-treated samples stored at room temperature and at 4 °C compared with untreated samples. Again, slightly fewer (2-3) of the total frozen and flash frozen samples exhibit significant losses and enhancements. All observations other than the one significantly enhanced sample in (b) fall within ranges of stored:fresh ratios observed in the total insoluble particle population (see Fig. S7, within an order of magnitude). This demonstrates that non-heat-labile INPs are generally less sensitive to storage than the total INP population (Fig. 2).

Effects of storage protocol on INP concentrations of filtered precipitation samples are shown in Figure 4 (0.45 µm syringe filter, see Sect. 2.3 for details). INPs < 0.45µm represented the majority (69% on average) of total INPs measured at the limit of detection in the fresh precipitation samples. A higher number of filter-treated samples exhibit significant losses across all 4 storage types when compared with the untreated samples. Furthermore, significant losses > 1 order of magnitude are observed across all storage types indicating that INPs < 0.45 µm are generally more sensitive to storage than the total INP population present in precipitation samples.

As the stored:fresh ratios follow a log-normal distribution (one-sample Kolmorgorov-Smirnov test), correction factors and 95% confidence intervals for each storage protocol and sample type (untreated, heat-treated, filtered) were calculated using the geometric mean and standard deviation of ratios of unique samples only between -7 and -17 °C (i.e. omitting any replicates, see Tables 5-7). These correction factors can be applied to measurements from stored samples to estimate the concentrations of INPs in the fresh sample.

4. Discussion

The challenge in selecting a storage protocol for INPs collected *in situ* is that the population composition is unknown, diverse, and the impact of any given technique on the different species may vary. Many types of aerosols can serve as INPs, including

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020


Atmospheric Measurement Techniques Discussions

dusts, metals and metal oxides, organic and glassy aerosols, bioaerosols, organic and mineral soil dust, and combustion products (Kanji et al., 2017). The aim of this study was to identify a storage protocol that best preserves the concentrations and characteristics of the general INP population observed in precipitation samples collected in a coastal environment. To this end, the impacts of 4 storage protocols on 15 untreated, heated, and filtered precipitation samples collected between September 22, 2015 and November 22, 2019 in La Jolla, CA were investigated by comparing measured INP concentrations between fresh and stored replicates. The INPs observed in this study were predominantly small, < 0.45 μm, and insensitive to heat treatment. However, it is worth noting that the degree to which INP sensitivity to storage varies by site or INP source (e.g. with desert or soil-dominant INP populations) remains to be seen.

None of the 4 storage protocols prevented significant losses or enhancements of INP concentrations in all samples, demonstrating that INP concentration measurements on fresh precipitation are superior to measurements on stored samples. However, samples stored under freezing and flash freezing conditions exhibited fewer changes overall compared to refrigerated samples. For example, at the INP activation temperature of -13 °C, in the rain sample that exhibited the greatest losses with storage, over one-fifth of the original concentration was preserved in the frozen sample, whereas $1/20^{th}$ of the original concentration was preserved in the refrigerated sample. These losses are more extreme than those of (Stopelli et al., 2014b), which demonstrated that INP concentrations of a snow sample refrigerated over 30 days decreased only two-fold from 0.027 to 0.013 L^{-1} at -10 ºC.

Non-heat-labile INPs were generally less sensitive to storage than the total INP population, and with the exception of samples stored at room temperature, all techniques yielded similar results with fewer enhancements or losses. Interestingly, INPs < 0.45 μm exhibited more sensitivity to all storage conditions tested than the total INP population, with significant losses observed in several samples leaving between one-fourth and $1/30^{th}$ of the value observed in the original fresh sample. Losses of INPs < 0.45 μm in samples stored at room temperature and +4 °C were comparable to the losses of total INPs in untreated samples and are likely a result of chemical aging in solution. However, losses of INPs < 0.45 micron in samples stored at -20 °C (both frozen and flash frozen) exceeded losses observed in the corresponding untreated samples. This is surprising given that the majority of INPs in this study were resilient to heat treatments of +95 °C. Lacking the identities of INPs observed in this study, a clear mechanism for their losses remains elusive. However, we offer the following points for consideration. It is well known that as a solution freezes, some solute is incorporated into the crystal and some is rejected, leading to enrichment of the solution phase and aggregation of dissolved or colloidal organic matter (Butler, 2002). Thus, as precipitation samples are freezing, small organic INPs may be lost simply due to aggregation in channels of enriched solute. In coastal precipitation samples for example, INPs may be so "lost" as the increased salinity in solution-phase channels destabilizes small suspended particles, allowing them to coagulate and settle (Jackson and Burd, 1998). Another possibility is that as the solution phase is enriched during freezing, smaller INPs may be adsorbing onto the surface of larger particles. The size distributions of total insoluble particles in the frozen samples show that most samples exhibit losses between 0-500 nm after storage and enhancements in sizes > 500 nm (see Fig. S6). This effect is not observed for samples stored at room temperature or at +4 °C.

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020


Atmospheric Measurement Techniques Discussions

These results have implications for the interpretation of heat and filtration treatment experiments. As heat denatures proteins, heat treatments are commonly used to infer contributions of proteinaceous or cellular contributions to INP populations, and filters are commonly applied to identify observed INP size ranges (e.g. McCluskey et al., 2018). For example, a typical analysis involves a comparison of the INP spectrum of an untreated sample to that of the heat-treated or filtered sample, and information about the sizes and biological composition of INPs are derived from this comparison. Our results demonstrate that these treatments may yield significantly different results if treatments are applied to stored samples. Any losses of INPs due to filtering or heat application could be confounded by significant enhancements or losses caused by storage (up to > 1 order of magnitude), resulting in inaccurate conclusions about INP characteristics. In this study, the majority (69%, on average) of INPs observed in fresh precipitation samples were < 0.45 µm. Considering this and that INPs < 0.45 µm exhibit significant losses across all storage types, there is a substantial risk that filter-treatments on stored samples in this study would lead to a false conclusion: that the majority of INPs were > 0.45 µm.

Previous studies on precipitation collected along the California coast have demonstrated the contribution of dust, marine and terrestrial bioparticles to INPs in precipitation (Levin et al., 2019; Martin et al., 2019). Considering that well-characterized IN-active dust and biological standards (Arizona Test Dust and Snomax®, respectively) are sensitive to storage conditions, it is possible that *in situ* dust or biological INPs contributed to the observed INP losses. Perkins et al. (2020) found that the IN-ability of Arizona Test Dust is significantly degraded in most conditions, including aging in deionized water for 1 day, and results from Polen et al. (2016) show that the most efficient (i.e. warmest freezing) components of biological ice nucleators are also the most labile and sensitive to storage. Changes in the total insoluble particle size distribution (see Figs S6 and S7) (± 1 order of magnitude between 10 and 2000 nm) could have additionally contributed to enhancements and losses. For example, the overall losses observed in INPs < 0.45 µm correspond to overall losses of the smallest insoluble particles (10-500 nm) in Fig. S6.

Though non-heat-labile INPs generally exhibit less sensitivity to storage than untreated samples, losses of heat-labile INPs in storage could impact treatment outcomes on stored samples. Assuming negligible effects of storage on the heat-treated sample but significant losses due to storage in the untreated sample (e.g. as was shown to be most likely for untreated samples stored at +4 °C), INP spectra of heat-treated samples could appear to indicate the entire INP population was heat-insensitive. This effect was observed in several samples across storage types (see Fig. S8).

Significant enhancements in INP concentrations occurred less frequently than losses. Again, changes in the total particle size distribution could explain some of the observed INP concentration enhancements. Increases in the number concentration of small particles due to breakup of loosely clumped masses of smaller particles could contribute to the increase in INPs. Other possible explanations include the redistribution of dissolved IN-active molecules onto particles (McCluskey et al. 2018), and the release of IN-active content from cells (McCluskey et al. 2018, Wilson et al. 2015) during cell death and lysis post freezing (Mazur et al., 1974). Although some ice nucleating species such as *Pseudomonas syringae* are known to survive unprotected freezing events (Buttner and Amy, 1989), it is worth noting that freezing is lethal for most cells (Mazur, 1984). The fact that cellular INPs are damaged by freezing may have motivated the choice of storage above 0 °C in some studies (see

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020


Atmospheric Measurement Techniques Discussions

Table S1). Non-cellular biological INPs, however, may be conserved in frozen storage. Wright et al., (2013) showed that Snomax® maintains much of its IN-activity even through multiple freeze-thaw cycles, indicating the persistence of large aggregates of the IN-active protein. The limited available research also suggests that freezing will not kill most viruses (Smith et al., 2004), nor will it alter the tertiary structure of gels, vesicles, or cell-free proteins.

5. Conclusions

Based on all observations in this study, we provide the following recommendations for precipitation samples collected in coastal environments for offline INP analyses:

1. Of the 4 storage protocols tested, none prevented changes in INP concentrations across all samples between -7 and -19 ºC. However, whenever processing fresh samples is not possible, our results demonstrate that storage at -20 ºC causes the least changes in INP concentrations.
2. Correction factors and 95% confidence intervals for INP measurements obtained from stored samples are provided (see Tables 5-7).
3. Flash freezing with liquid nitrogen before storing at -20 ºC did not significantly improve conservation of INPs.
4. With the exception of warm-freezing INPs (freezing temperatures >= -9 °C) in samples stored at room temperature or +4 °C, we found little to no correlation between changes in INP concentrations and storage intervals on timescales between 1-166 days, indicating that most enhancements or losses are likely happening during freezing or on timescales < 24 hours.
5. INPs that are insensitive to heat treatments are also less sensitive to storage. However, potential enhancements or losses due to storage (e.g. an average loss of 25% for samples stored at -20 °C) should be treated as additional uncertainty in measurements of INP concentration when comparing heat-treated with untreated INP spectra.
6. Due to the significant losses of INPs < 0.45 μm in storage, regardless of protocol, we recommend applying filtration treatments to fresh samples exclusively.

As measurements of INPs suspended in precipitation samples are used to infer in-cloud INP composition and concentration estimates, they represent important contributions to studies of links between aerosols, cloud processes and precipitation outcomes. This study derives bounds and correction factors for the impacts of storage on INPs and treatment outcomes from changes in INPs observed in coastal precipitation samples, with INPs predominantly insensitive to heat and < 0.45μm in size. However, it remains to be seen how INP sensitivity to storage varies by environment or INP composition. Further studies are needed to bracket storage effects on INP populations with various distributions of terrestrial and marine sources, as well as on heat-labile (biological) INPs, and INPs with colder activation temperatures. These studies could additionally benefit from analysis on how storage impacts differential INP spectra, which could reveal how sensitivity to storage varies by specific freezing temperature ranges. Bounds on the impact of storage will enable more meaningful intercomparisons of datasets and illuminate best practices for preserving INPs for offline analysis.

Data Availability: The data set supporting this manuscript is hosted by the UCSD Library Digital Collections (https://doi.org/10.6075/J0M32T8B).

Supplement Link: The supplement related to this article is available online at:

Author Contributions: CMB wrote the manuscript, prepared figures, led the field campaign and laboratory analysis. DL contributed significantly to the preparation of figures, precipitation sample collection and laboratory analysis. MDS, TCH, PJD and KAP provided feedback on the analyses and manuscript. KAP and PJD are principal investigators on awards CHE-1801971 and AGS-1451347.

Competing Interests: The authors declare no competing interests.

Acknowledgements: This work was supported by NSF through the NSF Center for Aerosol Impacts on Chemistry of the Environment (CAICE) CHE- 1801971 and AGS-1451347.

References

Agresti, A. and Coull, B. A.: Approximate Is Better than "Exact" for Interval Estimation of Binomial Proportions, Am. Stat., 52(2), 119–126, doi:10.2307/2685469, 1998.

Beall, C. M., Stokes, M. D., Hill, T. C., DeMott, P. J., DeWald, J. T., and Prather, K. A.: Automation and heat transfer characterization of immersion mode spectroscopy for analysis of ice nucleating particles, Atmos. Meas. Tech., 10, 2613–2626, doi:10.5194/amt-10-2613-2017, 2017.

Butler, M. F.: Freeze Concentration of Solutes at the Ice/Solution Interface Studied by Optical Interferometry, Cryst. Growth Des., 2(6), 541–548, doi:10.1021/cg025591e, 2002.

Buttner, M. P. and Amy, P. S.: Survival of Ice Nucleation-Active and Genetically Engineered Non-Ice-Nucleating Pseudomonas syringae: Strains after Freezing, Appl. Environ. Microbiol., 55(7), 1690 LP – 1694, 1989.

Christner, B. C., Cai, R., Morris, C. E., McCarter, K. S., Foreman, C. M., Skidmore, M. L., Montross, S. N. and Sands, D. C.: 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., 105(48), 18854–18859, doi:10.1073/pnas.0809816105, 2008a.

Christner, B. C., Morris, C. E., Foreman, C. M., Cai, R. and Sands, D. C.: Ubiquity of Biological Ice Nucleators in Snowfall, Science (80), 319(5867), 1214 LP – 1214, doi:10.1126/science.1149757, 2008b.

Conen, F., Eckhardt, S., Gundersen, H., Stohl, A. and Yttri, K. E.: Rainfall drives atmospheric ice-nucleating particles in the coastal climate of southern Norway, (2013), 11065–11073, 2017.

331 Creamean, J. M., Suski, K. J., Rosenfeld, D., Cazorla, A., DeMott, P. J., Sullivan, R. C., White, A. B., Ralph, F. M., Minnis,
332 P., Comstock, J. M., Tomlinson, J. M. and Prather, K. A.: Dust and biological aerosols from the Sahara and Asia influence
333 precipitation in the Western U.S, Science (80-.)., 340(6127), 1572–1578, doi:10.1126/science.1227279, 2013.
334 Creamean, J. M., Mignani, C., Bukowiecki, N. and Conen, F.: Using freezing spectra characteristics to identify ice-nucleating
335 particle populations during the winter in the Alps, Atmos. Chem. Phys., 19(12), 8123–8140, doi:10.5194/acp-19-8123-2019,
336 2019.
337 DeMott, P. J., Prenni, a J., Liu, X., Kreidenweis, S. M., Petters, M. D., Twohy, C. H., Richardson, M. S., Eidhammer, T. and
338 Rogers, D. C.: Predicting global atmospheric ice nuclei distributions and their impacts on climate., Proc. Natl. Acad. Sci. U.
339 S. A., 107(25), 11217–22, doi:10.1073/pnas.0910818107, 2010.
340 Failor, K. C., Iii, D. G. S., Vinatzer, B. A. and Monteil, C. L.: Ice nucleation active bacteria in precipitation are genetically
341 diverse and nucleate ice by employing different mechanisms, , 1–14, doi:10.1038/ismej.2017.124, 2017.
342 Hader, J. D., Wright, T. P. and Petters, M. D.: Contribution of pollen to atmospheric ice nuclei concentrations, Atmos. Chem.
343 Phys., 14(11), 5433–5449, doi:10.5194/acp-14-5433-2014, 2014.
344 Hegg, D. A. and Hobbs, P. V: Measurements of sulfate production in natural clouds, Atmos. Environ., 16(11), 2663–2668,
345 doi:10.1016/0004-6981(82)90348-1, 1982.
346 Hill, T. C. J., Moffett, B. F., DeMott, P. J., Georgakopoulos, D. G., Stump, W. L. and Franc, G. D.: Measurement of ice
347 nucleation-active bacteria on plants and in precipitation by quantitative PCR, Appl. Environ. Microbiol., 80(4), 1256–1267,
348 doi:10.1128/AEM.02967-13, 2014.
349 Hill, T. C. J., DeMott, P. J., Tobo, Y., Fröhlich-Nowoisky, J., Moffett, B. F., Franc, G. D. and Kreidenweis, S. M.: Sources of
350 organic ice nucleating particles in soils, Atmos. Chem. Phys. Discuss., (January), 1–37, doi:10.5194/acp-2016-1, 2016.
351 Huffman, J. A., Prenni, A. J., Demott, P. J., Pöhlker, C., Mason, R. H., Robinson, N. H., Fröhlich-Nowoisky, J., Tobo, Y.,
352 Després, V. R., Garcia, E., Gochis, D. J., Harris, E., Müller-Germann, I., Ruzene, C., Schmer, B., Sinha, B., Day, D. A.,
353 Andreae, M. O., Jimenez, J. L., Gallagher, M., Kreidenweis, S. M., Bertram, A. K. and Pöschl, U.: High concentrations of
354 biological aerosol particles and ice nuclei during and after rain, Atmos. Chem. Phys., 13(13), 6151–6164, doi:10.5194/acp-13-
355 6151-2013, 2013.
356 Jackson, G. A. and Burd, A. B.: Aggregation in the Marine Environment, Environ. Sci. Technol., 32(19), 2805–2814,
357 doi:10.1021/es980251w, 1998.
358 Kulmala, M., Laaksonen, A., J.Charlson, R. and Korhonen, P.: Clouds without supersaturation, Nature, 388(6640), 336–337,
359 doi:10.1038/41000, 1997.
360 Ladino, L. A., Yakobi-Hancock, J. D., Kilthau, W. P., Mason, R. H., Si, M., Li, J., Miller, L. A., Schiller, C. L., Huffman, J.
361 A., Aller, J. Y., Knopf, D. A., Bertram, A. K. and Abbatt, J. P. D.: Addressing the ice nucleating abilities of marine aerosol: A
362 combination of deposition mode laboratory and field measurements, Atmos. Environ., 132, 1–10,
363 doi:10.1016/j.atmosenv.2016.02.028, 2016.
364 Lim, Y. B., Tan, Y., Perri, M. J., Seitzinger, S. P. and Turpin, B. J.: Aqueous chemistry and its role in secondary organic

365 aerosol (SOA) formation, Atmos. Chem. Phys., 10(21), 10521–10539, doi:10.5194/acp-10-10521-2010, 2010.
366 Lohmann, U.: A glaciation indirect aerosol effect caused by soot aerosols, Geophys. Res. Lett., 29(4), 11–14,
367 doi:10.1029/2001GL014357, 2002.
368 Lohmann, U. and Feichter, J.: Global indirect aerosol effects: a review, Atmos. Chem. Phys., 5(3), 715–737, doi:10.5194/acp-
369 5-715-2005, 2005.
370 Martin, A. C., Cornwell, G., Beall, C. M., Cannon, F., Reilly, S., Schaap, B., Lucero, D., Creamean, J., Ralph, F. M., Mix, H.
371 T. and Prather, K.: Contrasting local and long-range-transported warm ice-nucleating particles during an atmospheric river in
372 coastal California, USA, Atmos. Chem. Phys., 19(7), 4193–4210, doi:10.5194/acp-19-4193-2019, 2019.
373 Mazur, P.: Freezing of living cells: mechanisms and implications, Am. J. Physiol. Physiol., 247(3), C125–C142,
374 doi:10.1152/ajpcell.1984.247.3.C125, 1984.
375 McCluskey, C. S., Hill, T. C. J., Sultana, C. M., Laskina, O., Trueblood, J., Santander, M. V, Beall, C. M., Michaud, J. M.,
376 Kreidenweis, S. M., Prather, K. A., Grassian, V. and DeMott, P. J.: A Mesocosm Double Feature: Insights into the Chemical
377 Makeup of Marine Ice Nucleating Particles, J. Atmos. Sci., 75(7), 2405–2423, doi:10.1175/JAS-D-17-0155.1, 2018.
378 McElfresh, C., Harrington, T. and Vecchio, K. S.: Application of a novel new multispectral nanoparticle tracking technique,
379 Meas. Sci. Technol., 29(6), 65002, doi:10.1088/1361-6501/aab940, 2018.
380 Michaud, A. B., Dore, J. E., Leslie, D., Lyons, W. B., Sands, D. C. and Priscu, J. C.: Biological ice nucleation initiates hailstone
381 formation, J. Geophys. Res. Atmos., 119(21), 12,112-186,197, doi:10.1002/2014JD022004, 2014.
382 Monteil, C. L., Bardin, M. and Morris, C. E.: Features of air masses associated with the deposition of Pseudomonas syringae
383 and Botrytis cinerea by rain and snowfall, , 8(11), 2290–2304, doi:10.1038/ismej.2014.55, 2014.
384 Morris, C. E., Sands, D. C., Vinatzer, B. A., Glaux, C., Guilbaud, C., Buffière, A., Yan, S., Dominguez, H. and Thompson, B.
385 M.: The life history of the plant pathogen Pseudomonas syringae is linked to the water cycle, Isme J., 2, 321, 2008.
386 Morris, C. E., Conen, F., Alex Huffman, J., Phillips, V., Pöschl, U. and Sands, D. C.: Bioprecipitation: A feedback cycle
387 linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere, Glob. Chang.
388 Biol., 20(2), 341–351, doi:10.1111/gcb.12447, 2014.
389 Perkins, R. J., Gillette, S. M., Hill, T. C. J. and DeMott, P. J.: The Labile Nature of Ice Nucleation by Arizona Test Dust, ACS
390 Earth Sp. Chem., 4(1), 133–141, doi:10.1021/acsearthspacechem.9b00304, 2020.
391 Petters, M. D. and Wright, T. P.: Revisiting ice nucleation from precipitation samples, Geophys. Res. Lett., 42(20), 8758–
392 8766, doi:doi:10.1002/2015GL065733, 2015a.
393 Petters, M. D. and Wright, T. P.: Revisiting ice nucleation from precipitation samples, Geophys. Res. Lett., 42(20), 8758–
394 8766, doi:10.1002/2015GL065733, 2015b.
395 Polen, M., Lawlis, E. and Sullivan, R. C.: The unstable ice nucleation properties of Snomax ® bacterial particles, ,
396 doi:10.1002/2016JD025251, 2016.
397 Prenni, A. J., Tobo, Y., Garcia, E., DeMott, P. J., Huffman, J. A., McCluskey, C. S., Kreidenweis, S. M., Prenni, J. E., Pöhlker,
398 C. and Pöschl, U.: The impact of rain on ice nuclei populations at a forested site in Colorado, Geophys. Res. Lett., 40(1), 227–

231, doi:10.1029/2012GL053953, 2013.

Rangel-Alvarado, R. B., Nazarenko, Y. and Ariya, P. A.: Snow-borne nanosized particles: Abundance, distribution, composition, and significance in ice nucleation processes, J. Geophys. Res. Atmos., 120(22), 11,711-760,774, doi:10.1002/2015JD023773, 2015.

Rogers, D. C., DeMott, P. J., Kreidenweis, S. M. and Chen, Y.: Measurements of ice nucleating aerosols during SUCCESS, Geophys. Res. Lett., 25(9), 1383, doi:10.1029/97GL03478, 1998.

Šantl-Temkiv, T., Sahyoun, M., Finster, K., Hartmann, S., Augustin-Bauditz, S., Stratmann, F., Wex, H., Clauss, T., Nielsen, N. W., Sørensen, J. H., Korsholm, U. S., Wick, L. Y. and Karlson, U. G.: Characterization of airborne ice-nucleation-active bacteria and bacterial fragments, Atmos. Environ., 109, 105–117, doi:10.1016/j.atmosenv.2015.02.060, 2015.

Schnell, R. C.: Ice Nuclei in Seawater, Fog Water and Marine Air off the Coast of Nova Scotia: Summer 1975, J. Atmos. Sci., 34(8), 1299–1305, doi:10.1175/1520-0469(1977)034<1299:INISFW>2.0.CO;2, 1977.

Smith, A. W., Skilling, D. E., Castello, J. D. and Rogers, S. O.: Ice as a reservoir for pathogenic human viruses: specifically, caliciviruses, influenza viruses, and enteroviruses, Med. Hypotheses, 63(4), 560–566, doi:10.1016/j.mehy.2004.05.011, 2004.

Stopelli, E., Conen, F., Zimmermann, L., Alewell, C. and Morris, C. E.: Freezing nucleation apparatus puts new slant on study of biological ice nucleators in precipitation, Atmos. Meas. Tech., 7(1), 129–134, doi:10.5194/amt-7-129-2014, 2014a.

Stopelli, E., Conen, F., Morris, C. E., Herrmann, E., Bukowiecki, N. and Alewell, C.: Ice nucleation active particles are efficiently removed by precipitating clouds, Sci. Rep., 5, 16433, doi:10.1038/srep16433, 2015.

Stopelli, E., Conen, F., Guilbaud, C., Zopfi, J., Alewell, C. and Morris, C. E.: Ice nucleators, bacterial cells and Pseudomonas syringae in precipitation at Jungfraujoch, Biogeosciences, 14(5), 1189–1196, doi:10.5194/bg-14-1189-2017, 2017.

Tan, I., Storelvmo, T. and Zelinka, M. D.: Observational constraints on mixed-phase clouds imply higher climate sensitivity, Science (80), 352(6282), 224 LP – 227, doi:10.1126/science.aad5300, 2016.

Vali, G.: Freezing Nucleus Content of Hail and Rain in Alberta, J. Appl. Meteorol., 10(1), 73–78, 1971.

Vali, G.: Comments on "Freezing Nuclei Derived from Soil Particles," J. Atmos. Sci., 31(5), 1457–1459, doi:10.1175/1520-0469(1974)031<1457:CONDFS>2.0.CO;2, 1974.

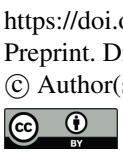
Vali, G.: Sizes of Atmospheric Ice Nuclei, Nature, 212(5060), 384–385, doi:10.1038/212384a0, 1966.

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, Atmos. Chem. Phys., 15(3), 1463–1485, doi:10.5194/acp-15-1463-2015, 2015.

Wex, H., Huang, L., Zhang, W., Hung, H., Traversi, R., Becagli, S., Sheesley, R. J., Moffett, C. E., Barrett, T. E., Bossi, R., Skov, H., Hünerbein, A., Lubitz, J., Löffler, M., Linke, O., Hartmann, M., Herenz, P. and Stratmann, F.: Annual variability of ice-nucleating particle concentrations at different Arctic locations, Atmos. Chem. Phys., 19(7), 5293–5311, doi:10.5194/acp-19-5293-2019, 2019.

Wright, T. P., Hader, J. D., McMeeking, G. R. and Petters, M. D.: High relative humidity as a trigger for widespread release

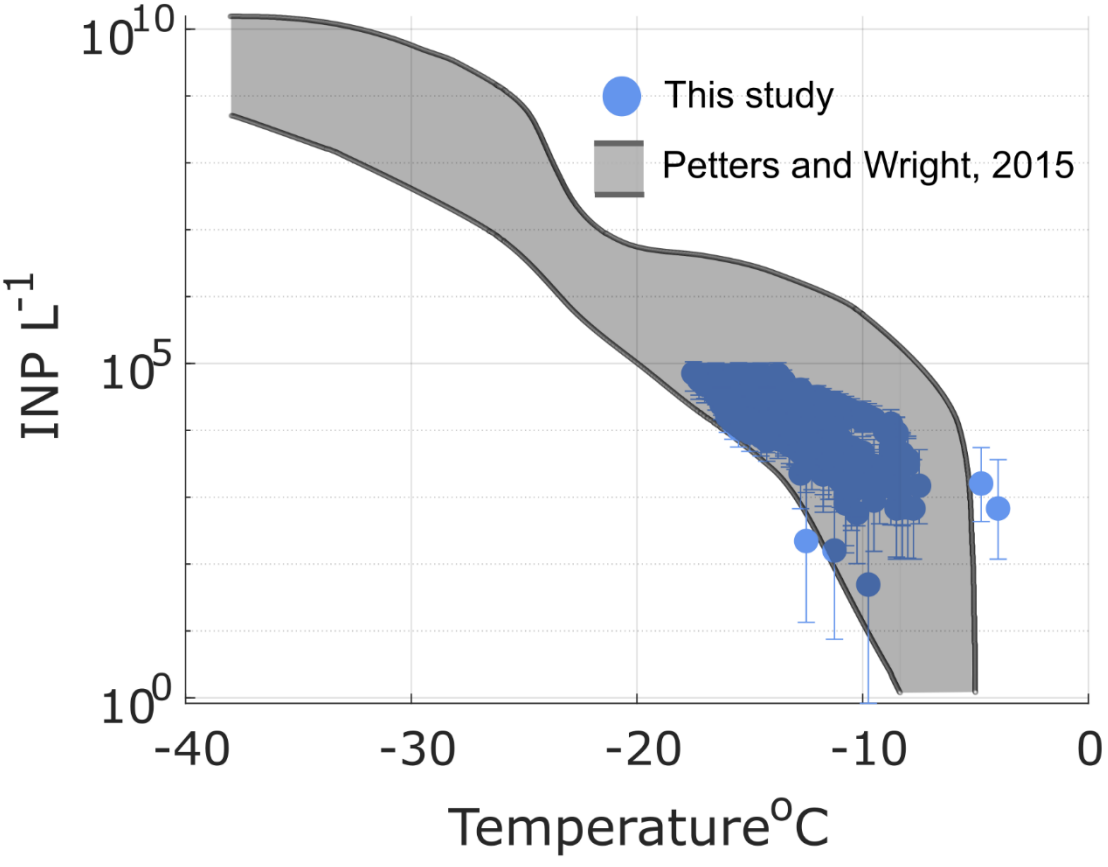
https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020



Atmospheric Measurement Techniques Discussions

433 of ice nuclei, Aerosol Sci. Technol., 48(11), i–v, doi:10.1080/02786826.2014.968244, 2014.

434



435

436 **Figure 1: INP concentrations per liter of precipitation in 15 precipitation samples collected at two coastal sites at**
437 **Scripps Institution of Oceanography (La Jolla, California, USA) between 9/22/2016 and 11/22/2019.** Grey shaded region
438 indicates the spectrum of INP concentrations reported in 9 previous studies of precipitation and cloud water samples collected
439 from various seasons and locations worldwide, adapted from Fig. 1 in (Petters and Wright, 2015b).

440

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020

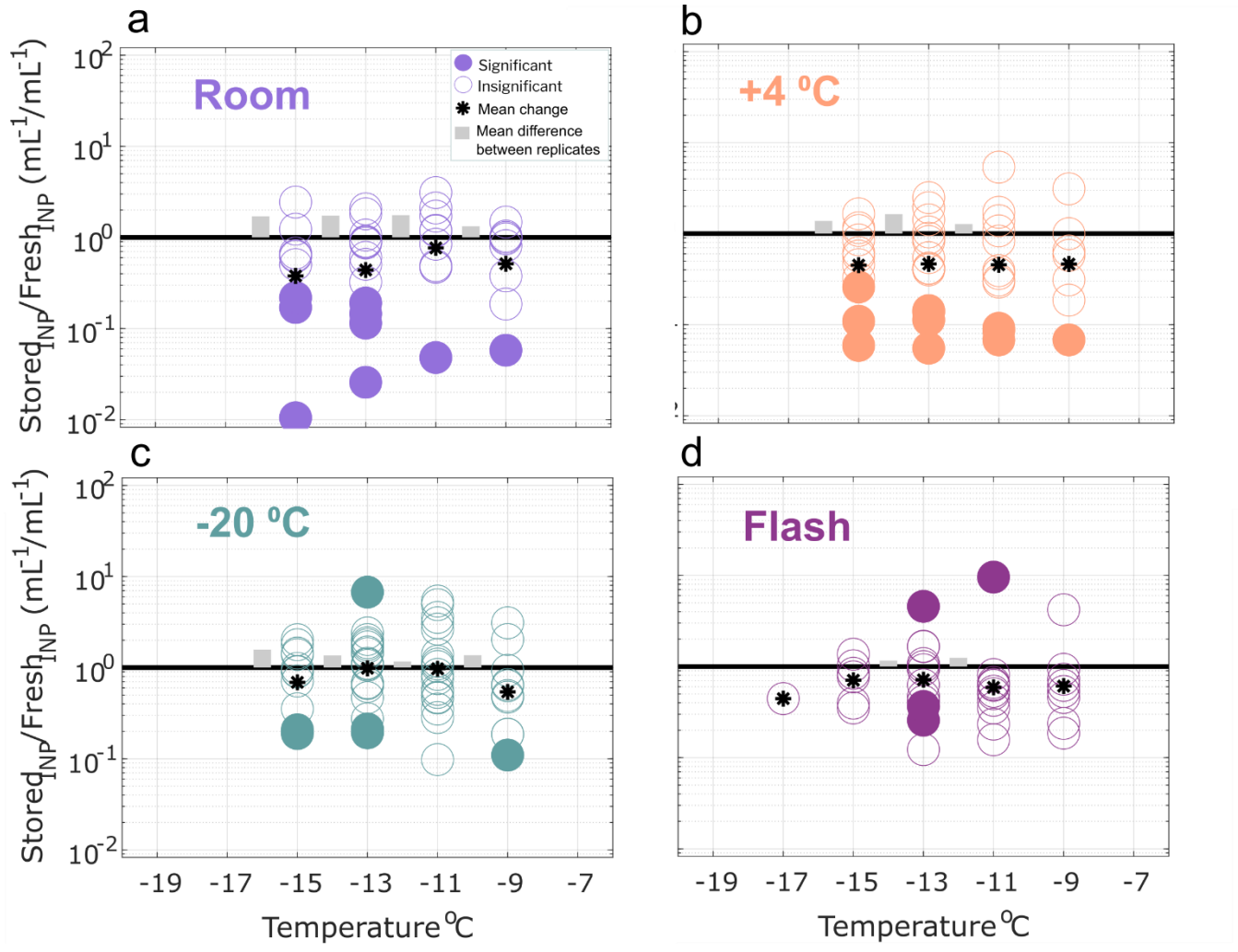


Figure 2: Ratio of INP concentrations measured in untreated precipitation samples (stored:fresh), binned by 2 °C increments between -19 and -7 °C. Four storage protocols were applied: **(a)** room temperature (21-23 °C), **(b)** refrigerated (+4 °C), **(c)** frozen (-20 °C) and **(d)** flash freezing in liquid nitrogen before storing frozen (-20 °C). Eight unique samples are represented in the figure (9 in **(c)**), most of which were processed at two different time intervals between 1 and 166 days post-collection (see Table S1 and Figs S1-S4), and replicates are represented in the figure. Markers above black 1:1 line indicate enhancement of INP concentrations in stored samples, and markers below indicate losses. In temperature bins containing stored:fresh ratios from at least two sets of replicate samples, grey bars represent the average difference between replicates.Stored sample frozen well fractions that passed Fishers Exact Test ($p < 0.01$) for significant differences from original fresh sample frozen well fractions at each of the 5 temperatures are indicated with filled markers, and the mean change in each temperature bin is marked with a star. Results show that on average, INP concentrations decrease in stored samples, and that both room temperature storage and refrigeration result in significant INP losses. Frozen and flash frozen storage show comparable results, with fewer (3-4) of the observations exhibiting significant losses and enhancements in INP concentrations.

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020

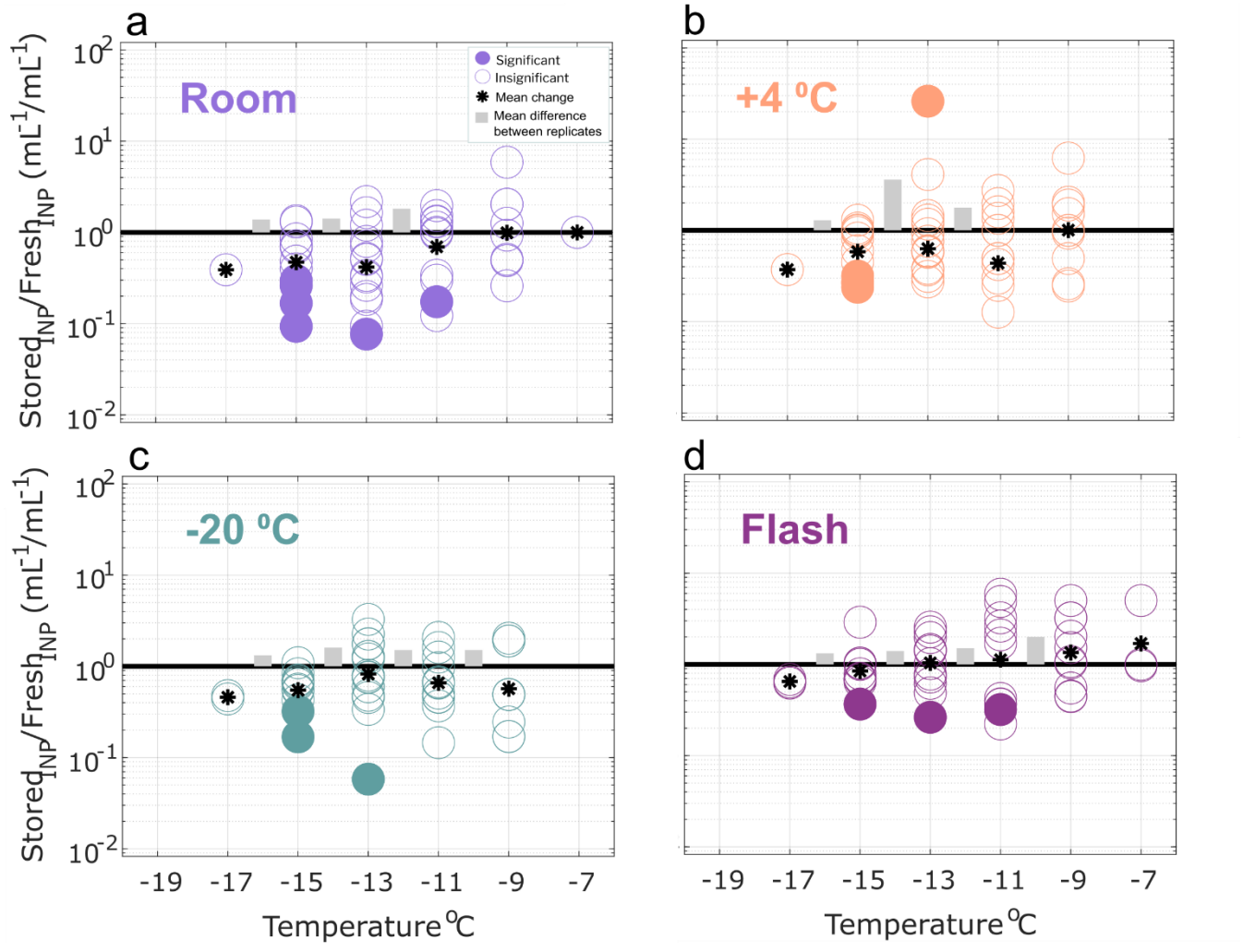


Figure 3: Ratio of INP concentrations measured in heated precipitation samples (stored:fresh), binned by 2 °C increments between -19 and -7 °C. Same samples as shown in Figure 2, but heated to 95 °C for 20 minutes to eliminate heat-labile INPs prior to measurement (see Methods Sect. 2.2 for details). Eight unique samples are represented in the figures, most of which were processed at two different time intervals between 1 and 166 days post-collection (see Table S2), and replicates are represented in the figure. In temperature bins containing stored:fresh ratios from at least two sets of replicate samples, grey bars represent the average difference between replicates. Results show significant losses of INPs in heat-treated samples stored at room temperature. Refrigerated, frozen, and flash frozen samples show comparable results with a few (1-3) samples exhibiting significant losses and enhancements. Non-heat-labile INPs are generally less sensitive to storage protocol than the total INP population in precipitation samples (Fig. 2), with the exception of storage at room temperature.

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020

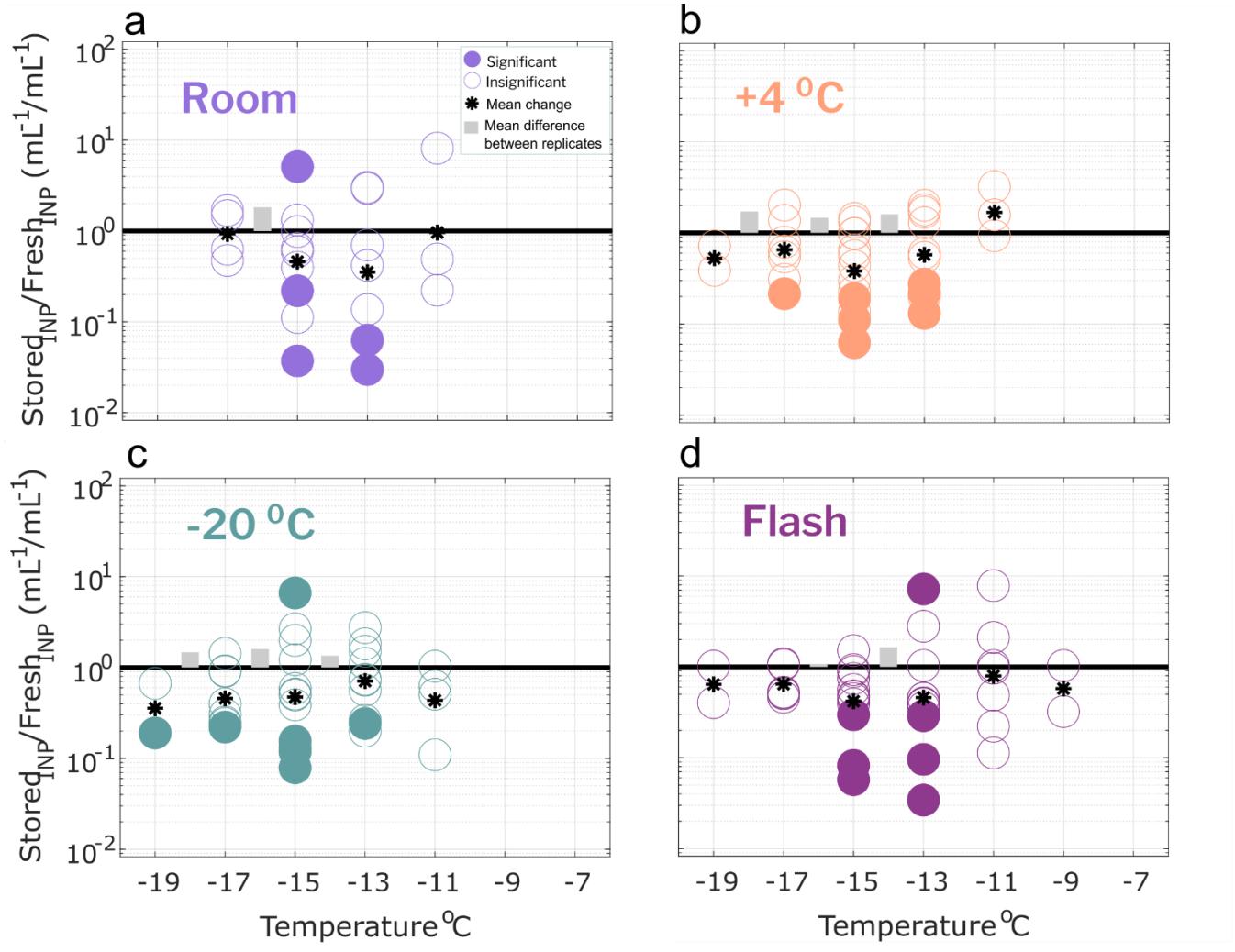


Figure 4: Ratio of INP concentrations measured in filtered (0.45 µm) precipitation samples (stored:fresh), binned by 2 °C increments between -19 and -7 °C. Same samples as in Fig. 2 but filtered with a 0.45 µm syringe filter (see Methods Sect. 2.2 for details). Eight unique samples are represented in the figures (9 in **(c)**), most of which were processed at two different time intervals between 1 and 166 days post-collection (see Table S3), and replicates are represented in the figure. In temperature bins containing stored:fresh ratios from at least two sets of replicate samples, grey bars represent the average difference between replicates. Results show significant losses of INPs in several filtered samples, regardless of storage protocol.

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020


Atmospheric Measurement Techniques Discussions

Table 1. Precipitation sampling periods

UTC Date	UTC time start	UTC time end
9/22/2016	19:20	21:13
9/22/2016	19:42	21:13
12/31/2016	4:53	7:52
1/1/2017	7:53	10:52
1/5/2017	21:02	22:01
1/9/2017	15:51	19:50
1/11/2017	19:00	23:30
1/14/2017	2:03	6:00
1/19/2017	12:30	17:30
1/20/2017	14:15	02:20 (next day)
11/19/2019	22:34	22:45
11/22/2019	4:43	5:42
11/22/2019	6:43	7:42
11/23/2019	7:42	8:41
11/23/2019	8:42	9:41

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020

Atmospheric Measurement Techniques Discussions

Table 2. Summary of unique and replicate untreated precipitation samples used for INP concentration measurements featured in Fig. 2.

Storage protocol	No. of unique samples	No. of stored samples measured at 2 timesteps
Room temperature (+21 - 23 ºC)	8	6
Refrigeration (+4 ºC)	8	8
Freezing (-20 ºC)	9	9
Flash freezing (-20 ºC)	8	4

Table 3. Summary of unique and replicate heat-treated precipitation samples used for INP concentration measurements featured in Fig. 3.

Storage protocol	No. of unique samples	No. of stored samples measured at 2 timesteps
Room temperature (+21 - 23 ºC)	8	6
Refrigeration (+4 ºC)	8	8
Freezing (-20 ºC)	8	7
Flash freezing (-20 ºC)	8	4

Table 4. Summary of unique and replicate filtered (0.45 µm) precipitation samples used for INP concentration measurements featured in Fig. 4.

Storage protocol	No. of unique samples	No. of stored samples measured at 2 timesteps
Room temperature (+21 - 23 ºC)	8	5
Refrigeration (+4 ºC)	8	7
Freezing (-20 ºC)	9	7
Flash freezing (-20 ºC)	8	4

475

https://doi.org/10.5194/amt-2020-183
Preprint. Discussion started: 19 June 2020


Atmospheric Measurement Techniques Discussions

Table 5. Correction factors for INPs with activation temperatures between -7 and -15 °C measured in stored, untreated precipitation samples. Correction factors and confidence intervals were derived from the log-normal distribution of changes observed in INP concentrations due to storage (see Fig. 2 and details in Sect. 3.2).

Storage protocol	Correction factor	95% CI Lower Limit	95% CI Upper Limit
Room temperature (21 - 23 ºC)	x3.59	0.19	69.29
Refrigeration (+4 ºC)	x1.72	0.25	11.27
Freezing (-20 ºC)	x1.34	0.22	8.34
Flash freezing (-20 ºC)	x1.48	0.22	9.88

476

477

478

Table 6. Correction factors for INPs with activation temperatures between -7 and -15 °C measured in stored, heat-treated precipitation samples. Correction factors and confidence intervals were derived from the log-normal distribution of changes observed in INP concentrations due to storage (see Fig. 3 and details in Sect. 3.2).

Storage protocol	Correction factor	95% CI Lower Limit	95% CI Upper Limit
Room temperature (21 - 23 ºC)	x1.41	0.23	8.6
Refrigeration (+4 ºC)	x1.24	0.22	7.04
Freezing (-20 ºC)	x1.05	0.25	4.41
Flash freezing (-20 ºC)	x0.93	0.19	4.4

Table 7. Correction factors for INPs < 0.45 μm with activation temperatures between -9 and -17 °C measured in stored precipitation samples. Correction factors and confidence intervals were derived from the log-normal distribution of changes observed in INP concentrations due to storage (see Fig. 4 and details in Sect. 3.2).

Storage protocol	Correction factor	95% CI Lower Limit	95% CI Upper Limit
Room temperature (21 - 23 ºC)	x2.23	0.15	32.36
Refrigeration (+4 ºC)	x2.37	0.29	19.24
Freezing (-20 ºC)	x1.54	0.19	12.48
Flash freezing (-20 ºC)	x1.82	0.32	10.31

479