A Mesocosm Double Feature: Insights into the Chemical Makeup of Marine Ice Nucleating Particles

CHRISTINA S. MCCLUSKEY,a,e THOMAS C. J. HILL,a CAMILLE M. SULTANA,b OLGA LASKINA,c,f JONATHAN TRUEBLOOD,c,b MITCHELL V. SANTANDER,b CHARLOTTE M. BEALL,d JENNIFER M. MICHAUD,b SONIA M. KREIDENWEIS,a KIMBERLY A. PRATHER,b,d VICKI GRASSIAN,c,b AND PAUL J. DEMOTTa

a Colorado State University, Fort Collins, Colorado
b University of California, San Diego, La Jolla, California
c The University of Iowa, Iowa City, Iowa
d Scripps Institution of Oceanography, La Jolla, California

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ABSTRACT

The abundance of atmospheric ice nucleating particles (INPs) is a source of uncertainty for numerical representation of ice-phase transitions in mixed-phase clouds. While sea spray aerosol (SSA) exhibits less ice nucleating (IN) ability than terrestrial aerosol, marine INP emissions are linked to oceanic biological activity and are potentially an important source of INPs over remote oceans. Inadequate knowledge of marine INP identity limits the ability to parameterize this complex INP source. A previous manuscript described abundances of marine INPs in relation to several aerosol composition and ocean biology observations during two laboratory mesocosm experiments. In this study, the abundances and chemical and physical properties of INPs found during the same mesocosm experiments were directly probed in SSA, seawater, and surface microlayer samples. Two unique marine INP populations were found: 1) dissolved organic carbon INPs are suggested to be composed of IN-active molecules, and 2) particulate organic carbon INPs are attributed as intact cells or IN-active microbe fragments. Both marine INP types are likely to be emitted into SSA following decay of phytoplankton biomass when 1) the surface microlayer is significantly enriched with exudates and cellular detritus and SSA particles are preferentially coated with IN-active molecules or 2) diatom fragments and bacteria are relatively abundant in seawater and therefore more likely transferred into SSA. These findings inform future efforts for incorporating marine INP emissions into numerical models and motivate future studies to quantify specific marine molecules and isolate phytoplankton, bacteria, and other species that contribute to these marine INP types.

1. Introduction

Ambiguity in the concentrations, sources, composition, and cloud activation properties of naturally occurring aerosol represent a large source of the uncertainty in global model simulations of cloud radiative forcing (Carslaw et al. 2013). This study addresses one critical aspect of aerosol–cloud interactions: characterization of marine ice nucleating particles (INPs). Atmospheric INPs are rare particles (e.g., approximately 1 in $10^5$ aerosol particles in the free troposphere are active as INPs at $-30^\circ$C; Rogers et al. 1998) that are required for heterogeneous ice nucleation, typically via immersion freezing of supercooled liquid cloud droplets. The action of INPs in a cloud, in concert with the Wegener–Bergeron–Findeisen process (Pruppacher and Klett 1997; Verheggen et al. 2007) and other microphysical processes such as riming and aggregation, can alter the precipitation potential, lifetime, and optical properties of mixed-phased clouds. However, these climate-relevant cloud properties in high-latitude oceanic regions are misrepresented in global models in remote
regions like the Southern Ocean (e.g., Franklin et al. 2013; McCoy et al. 2015). Marine INPs may be particularly relevant to ice-phase transitions in remote oceanic regions, such as the Southern Ocean (Burrows et al. 2013), because of a lack of other INP sources (e.g., mineral dust) and a high prevalence of supercooled liquid clouds (Huang et al. 2015). However, few studies have identified the types of sea spray aerosol (SSA) particles that contribute to heterogeneous ice nucleation in these clouds or the mechanisms that control emission rates of INPs from the oceans beneath them.

Number concentrations of INPs and active site densities (number concentration of INPs normalized by total aerosol surface area) associated with SSA generated via wave breaking and bubble bursting at the ocean surface are lower compared to terrestrial sources such as mineral dust (DeMott et al. 2016). Interestingly, laboratory and field studies suggest that the ice nucleating ability of SSA generated from seawater with high biological productivity (e.g., phytoplankton blooms) is greater than that of SSA generated from low biologically productive seawater (e.g., Bigg 1973; Schnell and Vali 1976; DeMott et al. 2016). As such, oceanic biological activity may be an important mechanism for increasing INP number concentrations in remote oceanic regions. Identifying marine INPs is challenging because of their low number concentrations in the marine boundary layer, and thus, the factors that control their release are poorly characterized or parameterized in models.

As a preface to a discussion of the composition of marine INPs, we first summarize findings regarding the chemical composition of SSA. Sea salts, particulate organic matter (e.g., phytoplankton, bacteria) and dissolved organic matter (e.g., organic macromolecules and exudates) are transferred into SSA from the bulk surface seawater (upper 100 m of the ocean) and the sea surface microlayer (SML; upper 50 ± 10 μm of the ocean; Zhang et al. 1998). The transfer of this material to the aerosol phase occurs during wave breaking and subsequent bubble bursting (Blanchard 1963; Blanchard and Woodcock 1957), which is influenced by wind speed (e.g., Gantt et al. 2011; Ovadnevaite et al. 2012). The chemical composition and emissions of SSA are therefore dependent on seawater and SML chemistry, which is closely linked to biological activity. As summarized by Gantt and Meskhidze (2013), organic material makes up large mass fractions in submicron aerosol (Barker and Zeitlin 1972; Oppo et al. 1999; O’Dowd et al. 2004). During periods of enhanced biological productivity, the contribution of non–sea salt sulfate and organic carbon to submicron SSA increases (Hoffman and Duce 1974; O’Dowd et al. 2004; Rinaldi et al. 2009; Miyazaki et al. 2010). Additionally, the abundance of microbes present in seawater and SML increases during phytoplankton blooms, and previous studies indicate that these microbial cells can be transferred into SSA during the bubble-bursting process (e.g., Pósfai et al. 2003; Leck and Bigg 2007; Patterson et al. 2016).

Both laboratory studies and field measurements have considered many non–sea salt constituents of SSA as possible candidates for heterogeneous immersion-freezing ice nucleation (Burrows et al. 2013). The surface of a marine diatom species, *Thalassiosira pseudonana*, (Knopf et al. 2011), as well as its organic exudates (Wilson et al. 2015), have been shown to promote freezing at conditions relevant for mixed-phase clouds, that is, for temperatures *T* in the range 0° > *T* > –38°C. Regarding the size of INPs, Rosinski et al. (1987) concluded that aerosol particles that were active as ice-forming nuclei were likely carbonaceous, though not cellular, and smaller than 0.3 μm. Similarly, Wilson et al. (2015) concluded that the ice nucleating entities within the SML collected in the North Atlantic were smaller than 0.2 μm. DeMott et al. (2016) found elevated numbers of INPs associated with SSA generated by the frit technique, known to favor production of smaller particle sizes (mode diameter less than 0.1 μm), suggesting that the mode diameter of INPs may be sub-0.1 μm or that the frit technique preferentially transferred smaller ice nucleating material to the aerosol phase. Diverse trends in the abundance of INPs active at different temperatures throughout the duration of laboratory phytoplankton blooms (McCluskey et al. 2017) suggest that there are multiple sources of INPs within SSA. To our knowledge, the identities of marine immersion-freezing INPs have not been directly investigated in captured nascent SSA.

The laboratory setting provides the unique opportunity to sample large volumes of SSA under consistent conditions, which is essential because of the low detection limit required to measure INPs in the marine environment. Additionally, the isolated ocean–atmosphere mesocosm system prevents possible terrestrial interferences that often limit interpretations of ambient data. In this study, we build on previous work (McCluskey et al. 2017) by using a variety of techniques, including online and offline ice nucleation measurements, offline and online chemical measurements of INPs and SSA, and measures of biological productivity, to directly probe the chemical and physical properties of immersion-freezing INPs in laboratory-generated SSA during two phytoplankton bloom experiments.

2. Methods

In McCluskey et al. (2017), data from two mesocosm experiments were used to evaluate changes in abundances
of INPs associated with changes in biological activity, described using chlorophyll-a (Chl a), heterotrophic bacteria (HB) counts, enzyme activities, and total aerosol organic carbon. This manuscript builds on McCluskey et al. (2017) by investigating the composition and characteristics of INPs emitted in SSA during the two phytoplankton blooms. This work includes analyses of two additional phases, bulk seawater and sea surface microlayer, which were not included in McCluskey et al. (2017). Ice crystal residuals were also directly probed for composition and size using Raman microspectroscopy and energy dispersive X-ray spectroscopy (section 2c). Offline treatments, including heating, hydrogen peroxide digestion, and size separation, were used to test the biological and total organic composition and size of ice nucleating material present in aerosol, bulk seawater, and sea surface microlayer samples (section 2c). DNA-based methods were applied to samples collected during one of the studies to screen for ice nucleating active bacteria. Finally, recent advancements in interpretations of single-particle mass spectra (Sultana et al. 2017b,c) were used to assess the abundance of and organic coating present on biological particle types. All of these new advancements in characterization of INPs are presented in the context of the previously published results that described the dynamic trends in INP number concentrations, biological activity, and aerosol composition (McCluskey et al. 2017).

a. Overview of microcosm and mesocosm experiments

Two phytoplankton bloom mesocosms were performed at the University of California, San Diego, and Scripps Institution of Oceanography (SIO). The first experiment utilized a marine aerosol reference tank (MART; Stokes et al. 2013) and occurred from 6 to 27 January 2014 (days 0–6 January 2014). SSA in a MART is generated using a pulsed plunging waterfall technique. Nascent SSA was sampled from an inlet approximately 15 cm from the surface of the seawater. A detailed description of this MART experiment is provided previously (Lee et al. 2015; tank E). The second study discussed here is the Investigation into Marine Particle Chemistry and Transfer Science (IMPACTS) campaign (Wang et al. 2015), which occurred from 3 July to 6 August 2014 (days 0–3 July 2014). For the IMPACTS study, SSA was generated using a wave breaking mechanism (Prather et al. 2013; Collins et al. 2014) in the SIO wave channel. Nascent SSA was sampled from approximately 20 cm above the surface, as described in McCluskey et al. (2017).

Seawater was collected from the Ellen Browning Scripps Memorial Pier (La Jolla, California; 32°52′N, 117°15′24″W) during high-tide periods; collection depths for the MART and IMPACTS studies were 0 and 4 m below the low-tide level (indicated on the pier), respectively, and represent seawater from the upper ocean (McCluskey et al. 2017). Previous studies have shown that these laboratory-generated mechanisms produce representative sea spray aerosol size distributions (Stokes et al. 2013), chemical mixing state (Collins et al. 2014), and number concentrations of INPs (DeMott et al. 2016; McCluskey et al. 2017). Efforts to prevent contamination are described by McCluskey et al. (2017). Microscopy techniques for measuring heterotrophic bacteria counts and Chl a concentrations are described by Lee et al. (2015) and McCluskey et al. (2017). The maximum Chl a concentration during the MART study (39 μg L⁻¹) was higher than that observed during the IMPACTS study (5 μg L⁻¹) because of differences in experimental conditions. Furthermore, phytoplankton and bacteria species are present at elevated concentrations in seawater in the MART study and thus do not represent global ocean conditions. However, both experiments comprise diverse and naturally occurring marine microbial species that participate in realistically complex phytoplankton blooms.

b. Collection and analysis of ice crystal residuals using the continuous flow diffusion chamber

The Colorado State University continuous flow diffusion chamber (CFDC; Rogers et al. 2001; DeMott et al. 2016) was used to measure number concentrations of INPs associated with the SSA generated during the MART and IMPACTS studies. Details of sampling SSA with the CFDC and trends in INP emissions have been previously reported (McCluskey et al. 2017). Briefly, dry particles (aerodynamic particle diameter $D_p < 1.5\,\mu m$ for this study) enter an ice thermal diffusion chamber that is set to expose the aerosol to relative humidity conditions above water saturation, driving both cloud droplet activation and subsequent ice nucleation via condensation and immersion freezing at the aerosol lamina temperature. Number concentrations of activated ice crystals are determined using an optical particle counter, where ice crystals are counted based on their larger sizes (>4 μm geometric diameter) than cloud droplets and liquid aerosols that are evaporated in a sub-water-saturated lower section of the chamber prior to the optical detector. Number concentrations of INPs were monitored daily in both experiments and are reported in McCluskey et al. (2017).

In this study, we report results regarding the identity of ice crystal residuals (ICRs) using the same basic approach of Prenni et al. (2009). Ice crystals were collected via use of a single jet impactor (2.9-$\mu$m 50% aerodynamic cutoff diameter; Prenni et al. 2009) at the base of the CFDC. ICRs (i.e., evaporated ice crystals) were
analyzed using scanning electron microscopy (SEM), energy dispersive X-ray (EDX) analyses, and micro-Raman spectroscopy. In each ICR collection period, approximately 5000 ice crystals are collected onto SEM grids [SPI formvar/carbon-coated transmission electron microscopy (TEM) grids, 200 mesh Cu] or onto substrates for application of micro-Raman spectroscopy (precleaned quartz coverslips, Ted Pella, Inc., part number 26016), and a minimum of 50 ICRs were randomly selected and analyzed (only 27 ICRs were analyzed on day 16 of the MART study). These analyses provide direct measures of the composition and size of the material that nucleated ice following condensation of droplets and immersion freezing at $-30^\circ$C (aerodynamic particle diameter $D_a < 1.5 \mu m$ for this study). These collections and analyses were limited to cooler temperatures ($T = -30^\circ$C) because of the low INP number concentrations and limitations of sampling times. Although it is recognized that the statistical confidences will be limited, the analyses will still reveal any major differences in relative contributions between the types of particles that compose the ICRs.

Imagery [Quanta field emission gun (FEG) MK2, SEM] and compositional [Oxford Instruments X-Max energy dispersive X-ray spectroscopy system (EDS)] analyses were performed at the Materials Characterization Laboratory in the Department of Geology and Geophysics (University of Wyoming, Laramie, Wyoming). The spatial resolution of the SEM was 50–100 nm. SEM/EDS analysis was performed on ICRs collected on three separate days (days 9, 16, and 17; indicated in Fig. 1a) during the MART study. ICRs were divided into three categories based on the morphology observed in the SEM imagery: “crystalline with coatings” are particles such as a salt particle with visible significant coating; “heterogeneous” particles are particles that resemble

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**Fig. 1.** Timeline of (a) different tests performed on each day, including heat treatments (red fill), size filtering (blue crosses), and collections of ICRs for Raman (asterisks) and SEM/EDX (hashes) analyses, during the MART study. Also shown are (b) INP number concentrations (McCluskey et al. 2017), (c) Chl a concentrations, (d) counts of HB measured in the SW (blue), SML (green), aerosol (red) (McCluskey et al. 2017), and (e) number fraction of mass spectral types measured during the MART study (Sultana et al. 2017c).
mixtures of constituents with various textures; and “amorphous/unclassified” particles are particles that have indistinct morphology, oftentimes due to low resolution (i.e., \( D_p \) < approximately 100 nm). (These morphology categories are shown in Fig. 6, where they are denoted as A, B, and C classes, respectively.) The maximum dimensions (i.e., length along longest axis) of ICRs were also measured from SEM imagery to estimate size distributions of collected ICRs (as demonstrated and reported in Fig. 6). Particle and background spectra were collected for each ICR and utilized to infer qualitative chemical composition of ICRs. Spectra peaks that were elevated relative to background (open area of grid without particles) spectra were considered important contributors to the ICR spectra. Two ICR types are reported: ICRs containing carbon and oxygen (organics) and ICRs containing sodium chloride (salt). The numbers of ICRs that had these chemical signatures are reported for each morphology class and each ICR sample (Table 3).

Raman microspectroscopy analysis was on ICRs with diameters of approximately 1 \( \mu \)m collected during the MART study on days 20 and 21 and IMPACTS study on days 26 and 27 July 2014 from SSA generated by a MART that contained fresh seawater with no biological treatment (i.e., fresh seawater with no bloom). Submicron aerosol particles collected on day 18 of the MART study following the methods of Cochran et al. (2016) are also shown in this study for comparison. Briefly, spectra were acquired using a LabRam high-resolution (HR) Evolution Raman spectrometer (Horiba) equipped with an Olympus BX41 optical microscope with a 100-times magnifications lens. Spectra in the range from 100 to 4000 cm\(^{-1}\) were acquired by taking the average signal from two exposures of 5–20 s using a 532-nm laser. Compositional information for these particles was based on comparisons to Raman spectra of standards as discussed in detail in Cochran et al. (2016).

c. Investigations of INP composition and size with the ice spectrometer

The Colorado State University (CSU) ice spectrometer was used to investigate the number concentrations of INPs and INEs in aerosol and bulk [i.e., seawater (SW) and SML] samples, respectively. Offline treatments were used to investigate the composition and size of the ice nucleating material, outlined in Fig. S1 in the online supplemental information (SI). During both studies, aerosol was collected onto sterile Nuclepore polycarbonate membrane filters [47-mm diameter; Whatman, General Electric (GE) Healthcare Life Sciences] with pore sizes of 0.2 and 0.05 \( \mu \)m in the MART and IMPACTS studies, respectively. SSA collected for ice spectrometer (IS) analysis during the MART and IMPACTS studies were wet and dry, respectively. During the MART and IMPACTS studies, the volume of air filtered for each sample was 270–431 and 287–876 L, respectively. Collected INPs were analyzed using an offline immersion-freezing technique using the CSU IS (Hiranuma et al. 2015). The details of filter precleaning and collection procedures can be found in McCluskey et al. (2017). Blank filters were analyzed and indicated insignificant background INP concentrations (McCluskey et al. 2017). Briefly, filters were suspended and shaken in 7 mL of filtered, deionized (18 MΩ and filtered through a 0.02-\( \mu \)-m pore Anotop syringe filter; Whatman, GE Healthcare Life Sciences) water and 50-\( \mu \)-L aliquots of the suspension were dispensed into wells (\( n = 24 \) or 32) of two polymerase chain reaction (PCR) trays (96-well plates; \( \mu \)-Cycler) that were then placed into aluminum blocks in the IS. Coolant is fed through heat exchange plates encasing the sides and base of the aluminum blocks in a controlled manner, allowing cooling from 0°C to –25°C. A record of the number of frozen wells at 1°C intervals was used to produce a spectrum of INP number concentrations as a function of temperature, following the methods reported by Vali (1971). Binomial sampling confidence intervals (95%) are used to describe uncertainties associated with the IS (formula 2; Agresti and Coull 1998).

The abundance and characteristics of ice nucleating entities (INEs; Vali et al. 2015) in the seawater and SML was also evaluated. Bulk SW was collected for IS analyses at the beginning (day 0) and end (day 22) of the MART study, and SML samples were collected from the MART using the glass plate method (Cumiliffe et al. 2013) and analyzed on days 4, 10, 14, 16, and 20 (Fig. 1). Because of a limited sample volume, additional seawater was collected from the SIO pier on 7 January 2014 to perform tests; a comparison of the INE spectra from seawater collected from the MART (6 January 2014) and from the SIO pier (7 January 2014) is given in Fig. S2 and shows good agreement. During the IMPACTS study, SW was collected for IS analysis on days 0, 6, 15, and 36 (Fig. 2). SML samples, collected utilizing the same method as the MART study, were collected and analyzed on days 0, 20, 22, 24, 26, and 28 (Fig. 2). Freezing-point-depression effects in SW and SML samples are not accounted for in this study, as discussed in method S1 in the SI.

To determine the size ranges and contribution of biologically material to the INP and INE populations, the filter suspensions (aerosol), bulk seawater, and SML were subjected to heat treatment and filtration. For the heat treatment, the sample solution was heated to 95°C for 20 min, cooled to room temperature, and dispersed into the IS wells for analysis. A reduction in IN activity under heating indicates the contribution of heat-labile
material to the INP population, which represents possible contributions from proteinaceous IN material. Heating will also alter the IN activity of some other classes of macromolecules by unfolding or degrading their structure, as suggested by Pummer et al. (2015). Syringe filters were used to investigate the size range of the INE population in bulk seawater. The sample solution was pushed through a filter with a pore size of 0.2 μm [DISMIC-13 cp; Advantec; prerinsed with deionized (DI) water to remove any INPs in the filter itself], and the filtrate was analyzed in the IS, revealing the contribution of INEs in the <0.2-μm filtrate to the total INE population. This 0.2-μm size cut can be approximately associated with two groups of material in the marine biochemical system, where larger INEs (>0.2 μm) may be grazers, phytoplankton, and bacteria, and smaller INEs (<0.2 μm) may be viruses, vesicles, and dissolved organic carbon such as cellular debris and cell-free molecules (Azam and Malfatti 2007). Method blanks were analyzed and revealed small changes to the INP number concentrations associated with the heating and sizing tests (Fig. S6). Aliquots from three aerosol filter suspensions from the IMPACTS study were also digested with hydrogen peroxide to decompose all organic INPs, as demonstrated by Tobo et al. (2014). One mL of the suspension was combined with 0.5 mL of 30% hydrogen peroxide (H₂O₂; Sigma Aldrich) to achieve a final concentration of 10%; then the mixture was immersed in water, heated to 95°C, for 20 min in a brightly lit laminar-flow cabinet (to generate hydroxyl radicals). To remove residual H₂O₂ (to prevent otherwise significant freezing point depression), catalase (catalase number 100429; MP Biomedicals) was added to the cooled solution. Since catalase itself is decomposed by H₂O₂, while simultaneously catalyzing its disassociation into water and oxygen, the enzyme was added in several 20-μL aliquots, allowing several minutes between each, until no effervescence resulted upon its addition.
Tests of significance between untreated and treated samples used Fisher’s exact test (Fisher 1922) to derive exact p values for the likelihood of the difference in proportions of wells frozen between samples at each temperature. The p value is given by

$$p = \frac{(a + b)(c + d)!((a + c)(b + d)!)}{a!b!c!d!n!},$$

where a and b are the numbers of wells frozen and unfrozen, respectively, in the untreated sample and c and d are the same for the treated sample, at each temperature; n is the combined total number of aliquots being tested in both samples. In this study, values are considered statistically different if they have a p value of less than 0.05.

d. Screening for presence of INA bacteria using DNA-based methods

During the IMPACTS study, standard and quantitative PCR were used to probe samples for the presence of the ice nucleation active (INA) gene, the gene coding for the ice nucleating protein in the known species of IN bacteria (INB; Warren 1995). DNA was extracted from 2 L of bulk seawater and 200 mL of SML, according to the methods of Boström et al. (2004). For standard PCR, using primer pairs 3308f/3463r and 3341fb/3462r1, the reaction composition and PCR cycling conditions were as detailed in Hill et al. (2014) except that reactions did not include EvaGreen and that 40 cycles of amplification were used with annealing and extension at 54°C. The primers used in this study will detect INB from all three families from which they have been recorded (Pseudomonadaceae, Enterobacteriaceae, and Xanthomonadaceae), such as Pseudomonas syringae, Pseudomonas fluorescens, Xanthomonas campestris, and Pantoea agglomerans (Hill et al. 2014). Pseudomonas syringae Cit7 DNA was used as the positive control. Reactions were initiated with 1–70-ng DNA (concentration varied greatly among samples) extracted from bulk water samples on days 0, 8, 15, 19, 24, 28, 34, and 36. For quantitative PCR (qPCR), primer pair 3308f/3463r was used with reaction composition and PCR cycling conditions as detailed in Hill et al. (2014), with annealing and extension at 54°C. Real-time PCR were performed on a Rotor-Gene 3000 (Corbett Research), using Pseudomonas syringae Cit7 DNA for standards.

<table>
<thead>
<tr>
<th>Day of MART</th>
<th>Number concentrations of INEs and INPs (T = −20°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No treatment</td>
</tr>
<tr>
<td></td>
<td>Bulk SW, nINE,SW (mL⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>43 (23–63)</td>
</tr>
<tr>
<td>19</td>
<td>1.7 (0.5–6.0)</td>
</tr>
<tr>
<td>10</td>
<td>22 (11–40)</td>
</tr>
<tr>
<td>14</td>
<td>4.8 (1.3–12)</td>
</tr>
<tr>
<td>16</td>
<td>74 (45–110)</td>
</tr>
<tr>
<td>18</td>
<td>15 (7.3–31)</td>
</tr>
<tr>
<td>20</td>
<td>19 (8.7–37)</td>
</tr>
<tr>
<td>4</td>
<td>BD L</td>
</tr>
<tr>
<td>9</td>
<td>6.1 (3.0–11)</td>
</tr>
<tr>
<td>10</td>
<td>5.6 (2.4–12)</td>
</tr>
<tr>
<td>11</td>
<td>0.95 (1.9–5.1)</td>
</tr>
<tr>
<td>12</td>
<td>0.55 (0.072–3.6)</td>
</tr>
<tr>
<td>13</td>
<td>0.46 (0.4–3)</td>
</tr>
<tr>
<td>14</td>
<td>0.65 (0.13–3.5)</td>
</tr>
<tr>
<td>15</td>
<td>2.7 (1.1–6.8)</td>
</tr>
<tr>
<td>16</td>
<td>3.7 (1.5–8.5)</td>
</tr>
<tr>
<td>17</td>
<td>0.66 (0.13–3.5)</td>
</tr>
<tr>
<td>18</td>
<td>1.3 (0.38–4.5)</td>
</tr>
<tr>
<td>19</td>
<td>0.94 (0.19–3.9)</td>
</tr>
<tr>
<td>20</td>
<td>0.61 (0.12–3.3)</td>
</tr>
</tbody>
</table>
Using 1–35-ng samples of DNA extracted from bulk water on days 24, 34, and 36, and from SML on days 19 and 34, amplicons were assessed using 12 µL of the PCR reaction electrophoresed in 2% MetaPhor agarose gels (Cambrex) in 1 x TAE buffer at 90 V for 45 min, using ethidium bromide for visualization. A 100-bp ladder (Promega) was used for sizing.

The primer and method used for the standard and quantitative PCR analyses in this study had a detection sensitivity of 5 INA gene copies per PCR reaction (Hill et al. 2014). Based on the volume of sample used to extract DNA, the detection limit was estimated to be 0.12 INB mL⁻¹ of seawater and 1.2 INB mL⁻¹ of SML.

e. Online characterization of SSA chemical composition

Size-resolved dual-polarity mass spectra were generated for individual SSA particles utilizing an aerosol time-of-flight mass spectrometer (Gard et al. 1997). Silica gel diffusional driers were utilized to dry the SSA particles before sampling, reducing the relative humidity of the sampled air to less than 15%. A Q-switched neodymium-doped yttrium aluminum garnet (Nd:YAG) laser pulse (266-nm wavelength, 8 ns, 700-µm spot size, 1.1–1.3-mJ pulse energy) desorbed and ionized the chemical components from each particle. Dual-polarity mass spectra were collected [mass-to-charge (m/z) range from −300 to 300] using a reflection time-of-flight mass spectrometer. Details of the aerosol time-of-flight mass spectrometer (ATOFMS) mass spectral analyses are provided in the SI (methods S1).

3. Results

a. Ice nucleating entities in bulk seawater and sea surface microlayer

Number concentrations of INEs active at −20°C in the SW (nINESW) were 43 mL⁻¹ on day 0 and decreased by a factor of 25 to 1.7 mL⁻¹ by day 19 of the MART study (Table 1; Figs. 3a,b). These concentrations are within the range previously reported in bulk seawater from other locations (Schnell and Vali 1974; Schnell 1977) and remain consistent after applying the 2°C freezing-point-depression effect correction used in the previous studies. Filtration tests (0.2-µm filter) revealed that 50%–100% of the INEs in SW active at −20°C were retained in the 0.2-µm filtrate (Table 1; Figs. 3a,b). Number concentrations of INEs in the SML (nINESML) were measured on days 4, 10, 14, 16, 18, and 20 of the MART study (Fig. S3); nINESML active at −20°C were below the detection limit (BDL) at the beginning of the study (day 4) and reached a maximum on day 16 (74 mL⁻¹).

During the IMPACTS study, nINESW was measured on days 0, 6, 15, and 36 and ranged from >54 (day 0) to 2 mL⁻¹ (day 15) at −20°C (Table 2; Figs. 3c–f) throughout the course of the experiment. Filtering tests revealed that over 80% of the SW INEs active at −20°C were larger than 0.2 µm (Table 2; Figs. 3c–f) throughout the course of the experiment. Heat tests revealed that 61%–95% of INEs were heat labile on days 0, 6, and 36. On day 15, seawater INEs were not heat sensitive. IMPACTS SML samples were analyzed on days 0, 20, 22, 24, 26, and 28. The nINESML value at −20°C ranged from 21 (day 26) to 100 mL⁻¹ (day 28) during the study (Fig. S4). Heating tests, performed on SML samples collected on days 0, 20, 24, and 26, showed that a statistically significant portion of the measured SML INEs were heat labile on days 0 and 26 (Fig. S5).

DNA-based tests were used to screen SW and SML for the INA gene during the IMPACTS studies, motivated by previous studies that had isolated up to 0.85 INB mL⁻¹ of seawater collected from the SIO pier (Fall and Schnell 1985). However, the INA gene was not detected in any of the samples tested (i.e., less than 0.12 INB mL⁻¹ of SW and less than 1.2 INB mL⁻¹ of SML). These findings suggest that none of the bacteria contained detectable amounts of known INB strains (e.g., Pseudomonas syringae, Pseudomonas fluorescens, Xanthomonas campestris, and Pantoea agglomerans; Hill et al. 2014) tested with this method. While it is possible that INB strains of tested species were present but possessed alleles of the INA gene that failed to be amplified, the primers used do provide the broadest coverage available short of full metagenomic profiling.

b. Contribution of heat-labile material to aerosol INP populations

Heat treatments were applied to all SSA samples during the MART study, and no significant change in IN activity was observed because of heating for most days (Table 1; Fig. 4). However, during the observed peak periods of INP emissions, on days 14, 15, and 17, heating produced a significant increase (factor of 10–30) in INP number for temperatures from −20°C to −10°C (Fig. 4). An increase in INP activity on exposure to heating has not previously been reported, and some possible mechanisms for this observation are discussed in section 4a.

During the IMPACTS study, smaller aerosol sample volumes were available (shorter sampling times were allowed), and thus, heat treatments were only applied to select samples (Table 2; Fig. 2a). Except for days 25 and 35, where no significant change was observed, heating resulted in a reduction in INP number concentrations at temperatures warmer than −25°C (Fig. 5). The highest contributions of heat-labile INPs (i.e., proteinaceous or thermally unstable IN macromolecules; Pummer et al. 2015; Hill et al. 2014) to the INP population were observed on days 14 and 20. However, on day 16, INPs
were enhanced because of heating, similar to several samples collected during the MART study, which is discussed more in section 4b. The H$_2$O$_2$ treatment, which was used to oxidize and denature all organic matter, was also applied on days 0, 14, and 22, and INP number concentrations were statistically significantly reduced, with the largest impact observed on day 14 (Fig. 5).

c. Characteristics of ice crystal residuals

To directly identify the types of particles responsible for ice nucleation from these mesocosm experiments,
nucleated ice crystals were collected downstream of the CFDC, and ICRs were probed with microscopic techniques to determine their morphology and chemical composition. During the MART study, ICRs were collected for SEM/EDS analyses on days 9, 16, and 17, and 27 and 80 ICRs were analyzed for each sample (Table 3). Again, these ICRs are material remaining after ice crystals (collected at approximately $-30^\circ$C) evaporate. The mode diameters of ICRs on days 9, 16, and 17 were 250, 750, and 300 nm, respectively. The morphology analysis (Table 3) revealed many ICRs (10%–30%) were crystalline particles visibly coated (Fig. 6a) and a significant proportion (18%–33%) of ICRs were considered heterogeneous (Fig. 6b). The majority (50%–66%) of the ICRs collected were unclassified because of their indistinct shape or limited resolution of the SEM (Fig. 6c). EDS elemental analyses are summarized in Table 3. All ICRs that were classified as crystalline with coatings contained NaCl, and the majority of these had significant amounts of organic carbon. The primary diameter of these coated crystalline residuals ranged from 200 nm to over 2 $\mu$m, with an average diameter of 0.55–0.62 $\mu$m. We note that the residual material for this morphology type includes a coating that leads to an overestimate of the primary diameter (Fig. 6a). ICRs classified as heterogeneous ranged from 0.22 to 1.8 $\mu$m (mean diameter of 0.56–0.97 $\mu$m), and organic carbon was detected for nearly all ICRs within this morphology type (40 out of 42), while NaCl was detected in only a few of these ICRs. Finally, unclassified ICRs had the smallest sizes (average diameters ranged from 0.3 to 0.48 $\mu$m) because of the contributions from ICRs that were unresolved because of their small size. However, several larger ICRs were also considered unclassified, illustrated by the maximum residual diameters for this morphology type.

The chemical composition of ICRs was also probed via Raman microspectroscopy on days 20 and 21 of the MART study (Fig. 7). These collections occurred following a period of elevated number concentrations of INPs active between $-25^\circ$ and $-15^\circ$C on days 15–18 (Fig. 1a). Raman particle spectra revealed that over 90% ($n = 50$) of the ICR spectral signatures contained long-chain fatty acids (Fig. 7). In contrast, the Raman spectra for the total submicron ($0.56 < D_p < 1 \mu$m) aerosol particle population (shown for day 18, the closest day with available data) were dominated by lipopolysaccharides (indicative of gram-negative bacteria) and long- and short-chain fatty acids.

ICRs collected on day 26 of the IMPACTS study were also analyzed with Raman microspectroscopy. Similar to the ICR samples from the MART experiment, the day 26 IMPACTS SSA sample represents INPs present at the conclusion of a peak in INPs observed at temperatures ranging between $-25^\circ$ and $-15^\circ$C from days 17 to 23 (Fig. 2a). The most common Raman spectra type,
representing over 40% of the ICRs analyzed (n = 165) was siliceous material (Fig. 7), indicative of diatomaceous cellular material. An additional ICR sample was collected from a MART, run coincidentally during the IMPACTS study that contained fresh seawater (collected from SIO on 27 July 2014) and had no nutrient addition. The siliceous material in the ICRs isolated from SSA produced by this MART was similar to those observed in ICRs isolated from SSA on day 26 of the IMPACTS study (Fig. 7). Polysaccharides and long- and short-chain fatty acids were also observed in the IMPACTS ICR populations.

4. Discussion

a. Ice nucleating material during MART study

In the MART study, filtering treatments revealed that a large fraction of the INEs present in the seawater were smaller than 0.2 μm (Table 1; Figs. 3a,b). This finding is consistent with previous investigations that found material smaller than 0.2 μm was a major contributor to INEs in SML samples collected in the North Atlantic and Arctic Oceans (Wilson et al. 2015) and over the Canadian Arctic Ocean region (Irish et al. 2017). INEs in the SML increased toward the end of the study (n_{INE,SW} = 1.7 mL⁻¹ on day 19 and n_{INE,SML} > 20 mL⁻¹ on days 18 and 20). These measurements suggest that, along with other surface-active molecules and constituents, INEs are transferred from the bulk SW to the SML. Higher concentrations of INEs in SML compared to SW have been reported previously (Wilson et al. 2015).

During the MART study, over 80% of the Raman spectra, for ICRs collected at ~30°C, were classified as containing long-chain fatty acids (Fig. 7). The action of long-chain fatty acids as IN-active monolayers or crystal-lites has not been investigated extensively, but previous studies show contradictory results. Gavish et al. (1990)
suggested potential IN activity of carboxylic acid monolayers, warmer than \(-20^\circ\text{C}\), but these observations were made very close to the freezing point of pure water samples in their experimental setup. More recent studies of freezing of palmitic acid (Qiu et al. 2017) and nonadecanoic acid (Knopf and Forrester 2011) monolayers indicate a requirement for much lower temperatures, within several degrees Celsius of the homogeneous freezing limit, consistent with theoretical considerations (Qiu et al. 2017). The SEM analyses showed morphologies including large amounts of crystalline and amorphous materials that included organics (Table 3). We therefore surmise that significant amounts of long-chain fatty acids may be released into SSA and are active as INPs at cooler temperatures \((T < -30^\circ\text{C})\).

INP activity across the range of temperatures did not respond to heat treatments in a manner that would indicate the presence of proteinaceous INPs (Table 1; Fig. 4). Notably, heating resulted in increased IN activity of the collected SSA over a range of temperatures warmer than \(-22^\circ\text{C}\) (by a factor of 10–30 at \(-20^\circ\text{C}\)) on days 14, 15, and 17. This unique heating effect has not been reported previously. As such, we offer a few possible mechanisms that may lead to an increase in

Fig. 5. Temperature spectra of INP number concentrations for SSA samples collected during the IMPACTS study and labeled by day as SSA(day number). Spectra for unperturbed samples are shown in black, spectra for heated samples are shown in red, and spectra for hydrogen peroxide digested samples are shown in yellow. The \(n_{\text{INP}}\) values determined from the treated samples that are statistically significantly different \((p < 0.05)\) from unperturbed sample are shown as filled markers; those that are not statistically different are shown as open markers. Vertical bars are the 95% confidence intervals for the IS measurements.
particulate surface area (i.e., more area available for ice nucleation sites) or that would reveal IN-active surfaces due to heating. Microbial cells may lyse in response to heating, dispersing their membranes and releasing intracellular material, such as viruses, cytoplasm, organelles, and dissolved organic molecules. Some types of diatom membrane surfaces (Knopf et al. 2011) and their contents (Wilson et al. 2015) have been suggested to be IN active in laboratory studies, and their contents may also be released after heating. Junge and Swanson (2008) report no IN activity down to −41°C for a Colwellia infecting phage V9; however, the IN ability of marine viruses and their heat sensitivity is largely unknown. Ice nucleation active molecules from the collected particles may dissolve into the sample solution after degradation from the heat treatment, and upon cooling, these coatings may be redistributed onto particles or even the suspension surface, leading to a higher proportion of particles coated with IN-active organics, or islands of IN-active monolayers on the surface of aliquots tested in the IS. For example, certain alcohols are known to stimulate ice nucleation as monolayers (Gavish et al. 1990; Popovitz-Biro et al. 1991, 1994; Qiu et al. 2017).

Single-particle chemistry data, provided by an ATOFMS, in combination with offline microscopy heterotrophic bacteria counts, were used to indirectly interpret the heat treatment findings. Particles with mass spectra, dominated by potassium, phosphate, and organic nitrogen markers, similar to spectra generated from aerosolized microbes (Fergenson et al. 2004; Czerwieniec et al. 2005), were first identified and reported by Sultana et al. (2017c) and are reported for the MART study here (Fig. 1e). It is maintained that this mass spectral type [biological SSA (BioSS)] is more likely to be generated by cells or cellular fragments desorbed and ionized within the ATOFMS without significant amounts of organic material. If microbes are emitted within droplets that contain significant amounts of dissolved organic material and are therefore already embedded in organic matrices, the ATOFMS is likely to generate mass spectra representative of the thick organic shell of the dried particle and not the embedded microbe within (Sultana et al. 2017c). Sultana et al. demonstrated this behavior through using varied laser power to do “depth profiling” of SSA. Thus, this cellular-like mass spectral type (BioSS) could serve as an indicator for successful transfer of particulate cellular material into the aerosol phase with minimal amounts of extracellular organic material. Elevated number fractions of BioSS were observed on days 11–13 of the MART study followed by a decline in the BioSS mass spectral type (Fig. 1e). In contrast, an offline bulk microscopy technique, which does not account for noncellular organic material, measured highest emissions of total heterotrophic bacteria on days 16 and 18 (Lee et al. 2015). Increasing transfer of noncellular organic material into the aerosol phase could explain the discrepancy between these two. An increase in an organic-rich (Org) mass spectral type and a decrease in mass spectra dominated by sodium chloride ions [sea salt (SS)] followed the phytoplankton bloom (method S2) is suggestive of increasingly thick organic-enriched coatings on dried SSA particles (Sultana et al. 2017a,c) and corresponded to a decrease in the BioSS number fraction and elevated INP number concentrations (days 15–17; Fig. 1). Thus, we hypothesize two possible mechanisms for the heating results: 1) the offline heat treatment of the SSA dissolved organic coatings from the collected particles, which were then redistributed onto all available particles, thereby increasing the surface area of IN-active organic coatings on particles or 2) heating SSA induced bursting of bacteria (or other microbes), thereby augmenting the number of particles with the IN-active dissolved organic carbon (DOC) material.

The contribution of IN-active organic material to the INP population observed during the MART study is further supported by SEM/EDS analysis, revealing that most ICRs contained significant amounts of organic carbon and that >30% of ICRs included visible organic coatings. Since the INEs in the bulk seawater were mostly smaller than 0.2 μm (Figs. 3a,b), the INEs are likely agglomerating into larger particles upon ejection or are attaching to the surfaces of other non IN-active particles such as NaCl.

b. Identity of IN material during IMPACTS study

Here, we observed a significant portion (>80%) of the INEs in the bulk seawater were larger than 0.2 μm and heat labile, indicating that the INEs in the seawater were possibly microbial cells (Table 2; Figs. 3c–f). INEs in the SML were also heat labile. On days of elevated concentrations of INPs active at T = −25°C to −15°C,

\[
\begin{array}{cccc|cccc|cccc}
| & A & B & C & Total & A & B & C & Total & A & B & C & Total \\
|---|---|---|---|-----|---|---|---|-----|---|---|---|-----|
| N | 9 & 18 & 27 & 54 & 8 & 5 & 14 & 27 & 8 & 19 & 53 & 80 \\
| % | 17 & 33 & 50 & 50 & 39 & 19 & 52 & 52 & 10 & 24 & 66 & 66 \\
| N_{org} | 9 & 18 & 41 & 58 & 3 & 5 & 3 & 11 & 8 & 17 & 41 & 66 \\
| N_{NaCl} | 9 & 2 & 7 & 18 & 8 & 4 & 2 & 14 & 8 & 4 & 11 & 23 \\
\end{array}
\]

Table 3. Summary of SEM/EDS results from days 9, 16, and 17 of the MART experiment for the three different morphology categories: crystalline with coatings (type A), heterogeneous (type B), and amorphous/unclassified (type C). Number of ICRs that contained organic carbon N_{org} and sodium chloride N_{NaCl} are also provided for each morphology type. Morphology A is crystalline with coating, B is heterogeneous, and C is unclassified.
(days 14 and 22), the aerosol INPs were degraded by 18%–37% in response to heating (Fig. 5). The H₂O₂ treatments also revealed that the majority of all INPs on days 14 and 22 were organic (i.e., not mineral or refractory material). The fractional contribution of BioSS signatures suggests relatively high transmission of microbes into SSA, with relatively insignificant organic coatings after particle drying, corresponding to higher number concentrations of INPs active at temperatures warmer than −25°C (days 19–24; Fig. 2; Fig. 6. (top) Example SEM images of each morphology group used to classify ICRs collected downstream of the continuous-flow diffusion chamber. Red lines span the primary diameter of each particle to illustrate the measurement of ICR primary diameter. In the bottom panel of (c), the red line is offset to allow visibility of the residual. (bottom) Primary diameters for total ICRs and ICRs in each morphology class. Morphology A is crystalline with organic coating, B is heterogeneous, and C is unclassified. Horizontal line and diamonds represent mode diameter and mean diameter, respectively.)
Silver-rich mass spectra had been previously identified in a wave channel study and were attributed to aerosolized heterotrophic bacteria that had bioaccumulated trace silver material present as contamination in the wave channel (Guasco et al. 2014). In addition, the fractional contribution of mass spectra with silver ion markers (BioAg; method S2) had a similar temporal trend to BioSS and also corresponded well to periods of higher warm INP concentrations. The important contribution of microbes to the INP population during the IMPACTS study was also supported by the Raman analyses, which revealed that over 50% of the ICRs were siliceous, indicating the presence of IN-active microbial material (e.g., diatoms) that dominated INPs even at −30°C. However, qPCR of the INA gene in bulk seawater found no evidence of presently known INB throughout the IMPACTS study. While known INB bacteria were not detected, it is possible that cell-free INEs are produced by one of the bacterial species present, such as has been observed in Pantoea agglomerans (Phelps et al. 1986) and the recently discovered gram-positive IN species from the genus Lysinibacillus (Failor et al. 2017). If an IN bacterium released copious numbers of cell-free INEs, it may contribute significantly to marine INE numbers while remaining undetected among the population. Hence, attribution of specific marine microbes responsible for ice nucleation remains as a topic of research.

Heat-labile and microbe-like INPs were most commonly observed during the IMPACTS study. However, on day 16, heat treatments revealed indication of the DOC INP type that was often observed during the MART study, where a significant increase in IN activity was observed after heating. A change in the heat sensitivity of IN material may be associated with sharp changes in the SSA composition, similar to those investigated by...
Wang et al. (2015), where rapid changes in organic aerosol were attributed to microbial degradation processes that modulate the abundance of organics in the SML. This rapid change in the INP population exemplifies at least a few of the complex processes that govern the relationship between the surface microlayer, biological processes, and the release of marine INPs.

c. Atmospheric implications

In this study, we directly investigated the size and heat sensitivity of material that contributes to the INP population found in nascent sea spray aerosol. Current representation of the emissions of organic aerosol from oceans has indicated that marine INPs may be significant contributors to ice-phase transitions in clouds in oceanic regions that are absent of terrestrial aerosol sources (e.g., Burrows et al. 2013), such as the Southern Ocean. An empirical parameterization was recently developed based on a relationship between INEs and total organic carbon (TOC) measured in SML samples collected in the North Atlantic (Wilson et al. 2015). For laboratory-generated SSA, the Wilson et al. (2015) approach was found to overestimate INP number concentrations compared to measured INP number concentrations (McCluskey et al. 2017). The specific identities of marine INPs and the mechanisms controlling the transfer of INEs from the bulk seawater and surface microlayer to the aerosol phase are important variables that are not represented in current parameterizations for estimating marine INPs.

While these studies were not performed on seawater (and therefore biological species) collected from the Southern Ocean specifically, these studies are some of the first direct investigations on the composition of marine INPs, and knowledge from these studies should be directly applicable to other ocean waters. The findings from this study show that marine INPs consisted of two distinct populations. One population of marine INPs referred to here as “dissolved organic carbon INPs,” are particles that contain INEs that pass through a 0.2-μm filter (i.e., formally defined as marine dissolved organic carbon; Azam and Malfatti 2007); these INPs are not heat labile in these studies and comprise molecules that may occur within or coat particles, embed themselves within exopolymers of colloids, partition into the SML, and even arrange themselves in a favorable lattice structure that catalyzes ice nucleation. DOC INPs may be important contributors to the INP population in cases of dense phytoplankton blooms, where the SML is significantly enriched with IN-active molecules that are transferred into the aerosol phase during the bubble bursting. Additionally, DOC INPs were preferentially detected after the peak in Chl a concentration for the MART phytoplankton bloom, consistent with increased production of organic aerosol that is commonly observed during a bloom’s decay (e.g., O’Dowd et al. 2015; Lee et al. 2015; Wang et al. 2015). Previous observations of DOC INE material (Wilson et al. 2015) and small carbonaceous INPs (Rosinski et al. 1987) fall under the proposed marine DOC INP type. We note that viruses are also possible contributors to the DOC INP population. It is clear that this category possesses subcategories that may be of different chemical makeup and dominate under different biological conditions or under different cloud activation conditions.

The second population of INPs we refer to as “particulate organic carbon (POC) INPs,” which are microbes that are IN active and heat labile and emitted as intact cells or as cell fragments. The contribution of POC INPs depends on the abundance of microbe species that are IN active, which was not quantified for these studies (apart from not detecting the known INB as contributors). Diatoms tested by Knopf et al. (2011) and phytoplankton cultures tested by Schnell and Vali (1974) and Fall and Schnell (1985) are examples of the proposed marine POC INP type.

Both of the proposed marine INP populations were detected in these two laboratory experiments and lagged phytoplankton biomass growth (i.e., Chl a) leading to increases in aerosolized IN-active POC (i.e., bacteria and/or diatom fragments) or to enrichment of IN-active molecules in the SML that coated SSA particles. Both INP types appear to have similar INP temperature regime, where INPs are active at temperatures warmer than −25°C. We propose that future studies should aim to represent marine INPs in a manner that reflects two distinct types of organic matter: IN-active DOC and IN-active POC. Future studies should also aim to quantify the chemical classes of molecules and species of microbes that are IN active over different ocean waters during different seasons. These findings will move us toward a mechanistic understanding of the production of INEs within a phytoplankton bloom life cycle and the transfer of INEs to the aerosol phase mediated by the SML.

5. Summary

Sea spray aerosol serves as an important contributor to INP populations in remote oceanic regions (Burrows et al. 2013; Wilson et al. 2015), yet the mechanisms for the production of IN material and the subsequent release of INPs from the ocean has not been characterized. Previous studies have investigated the identity of marine INPs via correlative analyses with aerosol chemistry and indicators of biological activity in the bulk seawater (e.g., Prather et al. 2013; Wang et al. 2015), through
direct testing of potential INP material in the laboratory (e.g., Knopf et al. 2011; Rosinski et al. 1987), and inferred from surface microlayer samples (Wilson et al. 2015). In this study, during two mesocosm experiments, the identity of marine INPs was directly probed using offline treatments that deactivated biological material (heating or hydrogen peroxide digestion) and isolated the contribution of dissolved organic matter (i.e., material smaller than 0.2 μm) to the overall population of INEs in the bulk seawater. Individual ice crystal residuals were also directly collected and analyzed to determine the chemical composition and size of INPs. Two unique populations of marine INPs were differentiated and are suggested for quantification as separate categories for modeling purposes: 1) DOC INPs are particles that are coated with or contained major masses of IN-active molecules (e.g., exudates) and were more common during the denser phytoplankton bloom that favored the production of organic coatings on particles and 2) POC INPs are IN-active bacteria, diatoms, or grazers, which are represented in SSA as intact cells or cell fragments, that were more prominent following the decline in phytoplankton biomass and absent of significant organic coatings. These findings should motivate future studies to focus on identifying individual molecules and microbes that contribute to both marine INP types, the mechanisms that govern their biochemical production during phytoplankton blooms, and the transfer of these molecules and microbes into SSA.

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