1 **Cultivable, halotolerant ice nucleating bacteria and fungi in coastal precipitation**

2 Charlotte M. Beall*[1], Jennifer M. Michaud*[2], Meredith A. Fish[3], Julie Dinasquet[1], Gavin C.
3 Cornwell[4], M. Dale Stokes[1], Michael D. Burkart[2], Thomas C. Hill[5], Paul J. DeMott[5], and
4 Kimberly A. Prather[†1,2]
5
6 [1]Scripps Institution of Oceanography, La Jolla, CA, 92037, USA
7 [2]Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA
8 92093, USA
9 [3]Department of Earth and Planetary Sciences, Rutgers University, Piscataway, NJ 08854
10 [4]Pacific Northwest National Laboratory, Richland, WA, 99354, USA
11 [5]Department of Atmospheric Science, Colorado State University, Fort Collins, CO, 80523, USA
12
13 *These authors contributed equally.

14 [†]To whom correspondence should be addressed: Kimberly A. Prather (kprather@ucsd.edu)
15
16

18 **Abstract**

19   Ice nucleating particles (INPs) are a rare subset of aerosol particles that initiate cloud

20 droplet freezing at temperatures above the homogenous freezing point of water (-38 °C).

21 Considering that the ocean covers 70% of the earth's surface and represent a large potential

22 source of INPs, it is imperative that the uncertainties in the identities and emissions of ocean INP

23 become better understood. However, the specific underlying drivers of marine INP emissions

24 and their identities remain largely unknown due to limited observations and the challenge

25 involved in isolating exceptionally rare IN forming particles. By generating nascent sea spray

26 aerosol (SSA) over a range of biological conditions, mesocosm studies show that microbes can

27 contribute to marine INPs. Here, we identify 14 (30%) cultivable halotolerant ice nucleating

28 microbes and fungi among 47 total isolates recovered from precipitation and aerosol samples

29 collected in coastal air in Southern California. IN isolates collected in coastal air were found to

30 nucleate ice from extremely warm to moderate freezing temperatures (-2.3 to -18 °C). Air mass

31 trajectory analyses, and cultivability in marine growth media indicate marine origins of these

32   isolates. Further phylogenetic analysis confirmed that at least two of the 14 IN isolates were of

33   marine origin. Moreover, results from cell washing experiments demonstrate that most IN

34   isolates maintained freezing activity in the absence of nutrients and cell growth media. This

35   study provides confirmation of previous studies' findings that implicated microbes as a potential

36   source of marine INPs and additionally demonstrates links between precipitation, marine aerosol

37   and IN microbes.

38   **1 Introduction**

39   Ice nucleating particles (INPs) are rare aerosols (1 in 10^5 or less of total particles in the free

40   troposphere) (Rogers et al., 1998) that induce freezing of cloud droplets at temperatures above the

41   homogenous freezing point of water (-38 °C) and at relative humidities (RH) well below the

42   homogenous freezing RH of aqueous solution droplets. They affect multiple climate-relevant

43   properties of mixed-phase and cold clouds. For example, in-cloud INP distributions can influence

44   the ice-phase partitioning processes that determine a cloud's reflectivity, lifetime and precipitation

45   efficiency (Creamean et al., 2013; DeLeon-Rodriguez et al., 2013; Fröhlich-Nowoisky et al., 2016;

46   Ladino et al., 2016). However, numerical representations of cloud ice processes challenge climate

47   models across all scales (Curry et al., 2000; Furtado and Field, 2017; Kay et al., 2016; Klein et al.,

48   2009; Prenni et al., 2007), and it is believed that the under-characterization of global INP

49   distributions contribute to the relevant uncertainties.

50   Despite recent evidence that sea spray aerosol (SSA) represents a unique source of INPs

51   (DeMott et al., 2016; McCluskey et al., 2016, 2018a, 2018b), that these INPs can contribute

52   significantly to total INP populations (particularly in remote marine regions where terrestrial

53   aerosols are less abundant) (Burrows et al., 2013; Vergara-Temprado et al., 2017; Vergara-

54   Temprado et al., 2018), and that specific parameterization of marine INPs can influence modelled

55   radiative budgets (Wilson et al., 2015), little is known about the actual entities involved in forming

56   marine INPs. (Schnell and Vali, 1975) were the first to associate phytoplankton blooms with raised

57   ice nucleation activity, in seawater sampled shortly after a bloom in Bedford Basin, Nova Scotia.

58   Recent mesocosm studies have linked SSA ice nucleating (IN) activity specifically to the death

59   phase of phytoplankton blooms. (McCluskey et al., 2017) showed that increases in INP emissions

60   corresponded to increased emissions of heterotrophic bacteria and organic species in SSA,

61   implicating microbes and biomolecules as contributors to marine INP populations. Marine

62   microbes were further linked to INPs in (McCluskey et al., 2018a): subsets of INPs in nascent SSA

63   were found to be heat labile, with sizes greater than 0.2 µm, and INP emissions correlated to

64   increased emissions of cells or cellular material. An IN halotolerant strain of *Pseudomonas*

65   *fluorescens* was detected in phytoplankton cultures derived from seawater (Fall and Schnell,

66   1985), and INPs have also been detected in seawater containing marine diatoms, green algae

67   (Alpert et al., 2011; Junge and Swanson, 2007; Ladino et al., 2016; Parker et al., 1985), and sea-

68   ice samples containing marine Antarctic bacteria (Junge and Swanson, 2007; Parker et al., 1985).

69   While indirect evidence indicates marine microbes and other biogenic entities as possible

70   marine INPs, direct observations of any marine IN entity in the atmosphere (i.e. through isolation

71   and identification in an atmospheric sample) were previously nonexistent. Multiple factors make

72   it difficult to determine INP origin, including the low abundance of INPs and the diversity of

73   aerosols with IN ability (e.g. Kanji et al., 2017). Moreover, it is not always possible to differentiate

74   terrestrial and marine air mass influences within the Marine Boundary Layer (MBL). However,

75   cultivable IN microbes have been isolated from clouds and precipitation for decades (e.g. Sands et

76   al., 1982; Failor et al., 2017; Morris et al., 2008), and the origins of IN isolates can be determined

77   by comparing sequences with reference isolates of known origin. There are several caveats to

78   consider when inferring in-cloud INP concentrations or properties from precipitation samples

79   (Petters and Wright, 2015), including "sweep-out" of additional INPs as the hydrometeor traverses

80   the atmosphere below the cloud (Vali, 1974). However, previous studies have derived estimates

81   of in-cloud INP concentrations and origins from the concentrations and identities of IN microbes

82   from ground-level collections (Christner et al., 2008; Failor et al., 2017a; Joyce et al., 2019;

83   Monteil et al., 2014) by assuming that particles in precipitation originate from the cloud rather than

84   the atmospheric column through which the hydrometeor descended. This assumption is supported

85   by (Vali, 1971), which found that subcloud scavenging of aerosol did not affect INPs observed in

86   precipitation collected at the surface in comparisons of INP spectra from surface samples with

87   samples collected at cloud-base. Furthermore, (Wright et al., 2014) estimated that sweep-out

88   contributed between 1.2 and 14% of INPs suspended in a precipitation sample collected at the

89   surface.

90   While evidence exists for relationships between IN microbes and precipitation in terrestrial

91   systems, studies of the relationship between marine INPs, marine microbes, and precipitation

92   remain quite limited. Here we report the identities and freezing temperatures of 14 cultivable

93   halotolerant IN species derived from coastal precipitation and aerosol samples, two of which were

94   marine in origin. Over the course of 11 precipitation events during an EI Niño season, 47 cultivable

95   halotolerant bacteria and fungi were recovered from aerosol and precipitation samples collected in

96   a coastal subtropical climate in southern California. Bacterial and fungal species were isolated,

97   identified, and tested for ice nucleation behavior from 0 to -25 °C using an immersion mode droplet

98   freezing assay technique. Precipitating cloud altitudes and isolate source regions were estimated

99   using the High-Resolution Rapid Refresh atmospheric model (HRRR) and the FLEXible

100 PARTicle dispersion model (FLEXPART)(Stohl et al., 1998), respectively. Finally, the effect of

101 media on the observed IN behavior of isolates was investigated through cell washing experiments.

102 **2 Methods**

103 2.1 *Precipitation and Aerosol Sample Collection Methods*

104 Precipitation and ambient aerosol samples were collected on the Ellen Browning Scripps

105 Memorial Pier at Scripps Institution of Oceanography (SIO) (32.8662 °N, 117.2544 °W) from

106 March 6, 2016 – May 6, 2016. Sampling took place in the surf 8 m above Mean Lower Low Water

107 (MLLW), and samples were only collected during westerly winds. Aerosol samples were collected

108 over 1.5-5 hour periods on polycarbonate filters (45 mm diameter, 0.2 µm pore-size, Whatman®

109 Nuclepore, Chicago, Illinois, USA) placed in open-face Nalgene ® Analytical Filter Units

110 (Waltham, Massachusetts, USA). After collection, aerosol filters were immersed in 12 mL of

111 ultrapure water, and particles were shaken off the filter by hand for 20 minutes. The precipitation

112 samples were collected using a modified Teledyne Isco© Full-Size Portable Sampler (Lincoln,

113 Nebraska, USA), fitted with 24 1-L polypropylene bottles. Prior to sampling, the bottles were

114 immersed in 10 % hydrogen peroxide for 10 minutes, then rinsed three times with ultrapure water.

115 The automated sampler would engage when triggered by precipitation of at least 0.13 cm h^{-1} and

116 would sample using the first of 24 bottles for 30 minutes, and thereafter switch bottles at hourly

117 intervals. Within one to two hours of sample collection, INP concentrations were measured using

118 the SIO-Automated Ice Spectrometer (SIO-AIS) (Beall et al., 2017), an automated offline freezing

119 assay technique for measurement of immersion mode INPs. To decrease the effect of interstitial

120 particle sweep out by falling raindrops on measured INP concentration, precipitation from the first

121 30 minutes was discarded. Sweep out effects have been estimated to contribute between 1.2 and

122 14 % to measured concentrations of INP in a precipitation sample (Wright et al., 2014).

123   The INP measurement technique is described in detail in (Beall *et al.,* 2017*)*. Briefly, the

124   precipitation samples were distributed in microliter aliquots into a clean 96-well disposable

125   polypropylene sample tray. An equal number and volume of aliquots of ultrapure water accompany

126   each sample in the disposable tray as control for contamination from the loading and/or ultrapure

127   water. The sample trays were then inserted into an aluminum block that is cooled until the samples

128   are frozen. Cumulative INP number concentrations per temperature per volume are calculated

129   using the fraction (*f*) of unfrozen wells per given temperature interval:

130

131    $INP = \dfrac{-ln(f)}{V_d}$ Eq. (1)

132

133   where V_d is the volume of the sample in each well. For aerosol filter samples, cumulative INP

134   number concentrations are calculated using the ratio of the volume used for resuspension of the

135   particles (V_{re}) to the volume of aerosol sampled (V_A) :

136    $INP = \dfrac{-ln(f) \cdot V_{re}}{V_d \cdot V_A}$ Eq. (2)

137

138   The fraction of unfrozen wells (f) is adjusted for contamination by subtracting the number of frozen

139   ultrapure water wells per temperature interval from both the total number of unfrozen wells and

140   total wells of the sample. For this study, 30 x 50 µL droplets were deposited into the droplet assay,

141   yielding a detection limit of 0.675 INP mL^{-1} liquid.

142   Within one to two hours of collection, precipitation and aerosol samples were also

143   inoculated in 5 mL ZoBell growth media (ZoBell, 1947) (5 g peptone, 1 g yeast extract per 1 L of

144   filtered (0.22 µm) autoclaved seawater) and grown under ambient conditions (21 - 24 ˚C). INP

145   concentrations in ZoBell enrichments were measured 1-day post inoculation and for several days

146   thereafter to monitor for sustained IN activity.

147

148   2.2 *Bacterial and fungal isolation and characterization*

149   Precipitation and SSA microorganisms were cultivated using the ZoBell enrichment

150 described above (ZoBell, 1947) (Fisher Scientific, Houston, Texas, USA). Isolation was performed

151 by successive plating on ZoBell agar (BD Bacto™ Agar, Sparks, MD, USA). Liquid cultures were

152 inoculated from single colonies and grown to late exponential phase. DNA was extracted from

153 liquid cultures of isolates after an overnight lysis with proteinaseK (100 µg mL^{-1}) and lysozyme

154 (5 mg mL^{-1}) (MilliporeSigma, Burlington, Massachusetts, USA) (Boström et al., 2004) using a

155 QIAamp® kit (QIAGEN, Hilden, Germany). 16S V4 ribosomal DNA fragments were amplified

156 using the primers 515F (5´ GTGYCAGCMGCCGCGGTAA 3´) and 926R (5´

157 CCGYCAATTCMTTTRAGT 3´)(Walters et al., 2015). PCR products were purified using

158 GenElute^{TM} PCR Clean-up kit (MilliporeSigma). 16S fragment DNA sequences were resolved by

159 Sanger sequencing (Retrogen, San Diego, CA). OTUs were determined from 16S sequences using

160 SINA (Pruesse et al., 2012) and individual sequences were inspected using BLAST

161 (https://www.ncbi.nlm.nih.gov/) for further characterization.

162   To assess for duplicate isolates within the sampling period, 16S sequences were

163 compared. Sequences within the same OTU were adjusted and aligned in

164 DECIPHER(Alignseqs(), AdjustAlignment() with default settings) (Wright, 2015). These

165 sequence alignments were used to generate phylogenetic trees using ClustalW2

166 (UPGMA)(McWilliam et al., 2013) and visualized with iTOL(Letunic and Bork, 2011). Branch

167 distances were used to evaluate sequence similarity. To facilitate comparisons between

168 organisms assigned to the same OTU, identity assignments including divisions at distances > 0.1

169 (e.g. 1, 2, 3…) were further subdivided by distances > 0.01 (e.g. 1a, 1b, 1c…). Nonzero distances

170 < 0.01 were given sub labels (e.g. 1a1, 1a2…). Zero distances were given identical labels.

171 Distances < 0.01 were determined to be possible duplicates if they were collected during the

172 same sampling period unless the organisms had a different phenotype generally indicated by

173 different pigmentation. Each duplicate was tested for its IN ability, and the results are reported in

174 Table S1 and discussed in the main text. Maximum likelihood phylogenetic trees were computed

175 in MEGA7 (Tamura et al., 2013) after ClustalW alignment with reference sequences

176 (https://www.ncbi.nlm.nih.gov/) .

177

178 2.3 *Storm and aerosol source characterization methods*

179   Cloud altitudes at the time of precipitation sample collection were estimated using the

180 High-Resolution Rapid Refresh model (HRRR). The altitudes and pressure levels of clouds were

181 assumed to be located where RH was > 95-100 % in the model. The specific RH criteria applied

182 to each sampling period are provided in Table S2. HRRR model output was compared with surface

183 RH measurements from the SIO pier weather station during sampling periods, and predicted RH

184 was found to agree with observations with an RMSE of < 10 – 15%, which aligns closely with

185 previously reported RH accuracies over the continental US (Benjamin et al., 2016). Three altitudes

186 of the estimated cloud top, middle and bottom were used as release points of FLEXPART 10-day

187 LaGrangian backward trajectories. Back-trajectories were used to identify potential sources of

188 INPs in the precipitation samples, and to indicate potential sources of land-based contamination in

189 aerosol and precipitation samples due to local wind patterns or land-sea breezes. Satellite

190 composites from the National Weather Service Weather Prediction Center's North American

191 Surface Analysis Products were used for synoptic weather analysis to generally characterize

192 meteorology during each rain event (see Table S3).

193   2.4 *Isolate IN activity measurement and controls*

194   To measure the IN activity of each isolate, liquid cultures were grown to late exponential

195   phase. Growth was monitored by optical density (OD) (590 nm). INP concentrations were

196   measured as described in Sect. 2.1 in liquid cultures and compared to a ZoBell blank as a control.

197   Isolate biomass was estimated from OD measurements using the distribution of OD to biomass

198   conversion factors from (Myers et al., 2013). As Myers et al. (2013) found, in a study of 17

199   diverse organisms, OD to biomass conversion factors ranged between 0.35 and 0.65 gDW OD^{-1}

200   L^{-1}; we assume that INP g^{-1} biomass may be estimated from OD within a factor of 2. Thus,

201   isolate INP concentrations, and upper and lower limits of 95% confidence intervals were scaled

202   by $\frac{1}{m}$, where m is the mean, minimum or maximum value of the (Myers et al., 2013) biomass

203   conversion factor distribution, respectively (i.e. 0.5, 0.65 and 0.35 gDW OD^{-1} L^{-1}).

204   To investigate the effect of growth media on IN isolates, a subset of late exponential

205   cultures were washed three times with filtered (0.22 µm) autoclaved seawater (FASW) by

206   successive centrifugation and resuspension. The washing procedure removes everything that is

207   water soluble and whole cells and insoluble molecules pellet upon centrifugation.   INP

208   measurements were taken as described and compared to sterile seawater controls (see Fig. S1b and

209   Fig. S1c).

210   As ZoBell growth media contained INPs at moderate to cold freezing temperatures (-13 to

211   -25 °C, see Fig. S1a), only isolates exhibiting INPs at significantly higher freezing temperatures (-

212   2.3 to -15 °C) or at significantly higher concentrations than their respective ZoBell growth media

213   sample were considered to be IN. The criterion for significance was chosen to be conservative: a

214   data point along an isolate's measured IN spectrum was considered significant if there was no

215   overlap between the 95 % binomial sampling confidence interval of the given data point (Agresti

216   and Coull, 1998) and any ZoBell confidence interval within ± 2.2 °C, the maximum uncertainty in

217   freezing temperature measurement due to heterogeneity in heat transfer rates across the

218   instrument's droplet assay (Beall et al., 2017). This equates to a significance threshold of p < 0.005

219   (Krzywinski and Altman, 2013). The choice of ± 2.2 °C is likely conservative given that in a study

220   of 11 cooling cycles, the average and maximum ΔT observed across the droplet assay when cooling

221   from 0 to -25 °C was 0.38 and 0.98 °C, respectively (and following this study, the addition of a

222   second thermistor under the second sample tray decreased the observed ΔT to within thermistor

223   uncertainty, ± 0.2 °C). The same criterion was applied to isolates washed and suspended in FASW

224   as described above (Figs. S1 b-c). Many isolates were diluted with their respective media (ZoBell

225   or FASW) to decrease opacity such that freezing events could successfully be detected by the

226   camera, so their respective dilution factors were applied to both the INP concentrations measured

227   in the isolate suspension and the INP concentrations measured in the FASW or ZoBell samples for

228   the significance analysis (see Figs. S1 b-c and S2).

229

230   **3 Results and discussion**

231   *3.1 Subtropical coastal storm properties and origins*

232   Aerosol and rain samples were collected from a pier on the coast of La Jolla, CA

233   (32°52'01.4"N 117°15'26.5"W) during an El Niño event spanning 11 precipitation sampling

234   periods March 6 to May 7, 2016. Observations of INPs in precipitation generally fall within bounds

235   of previously reported INP concentrations from precipitation and cloud water samples (Fig. 1, grey

236   shaded region, adapted from Petters and Wright, 2015). AIS measurement uncertainties are

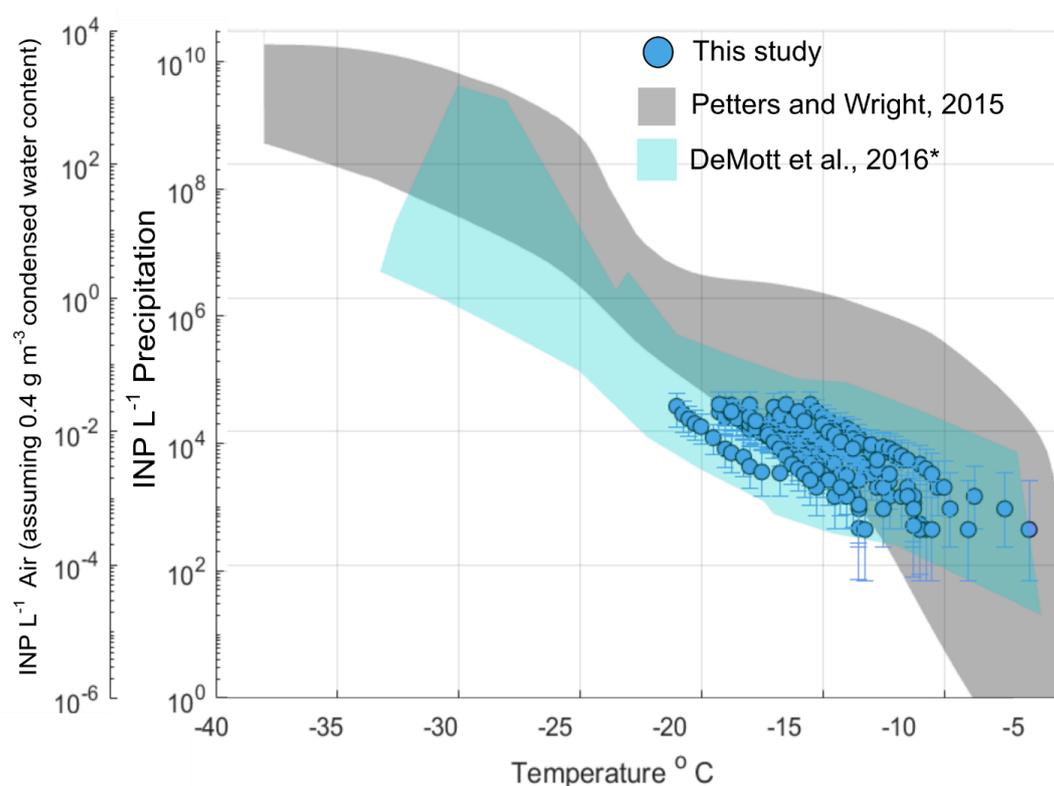
237   represented with 95% binomial sampling intervals (Agresti and Coull, 1998). Observed freezing

238   temperatures ranged from -6.5 to -22.0 ºC, with concentrations up to the limit of testing at 10^5 INP

239   L^{-1} precipitation. Following the assumptions in (Petters and Wright, 2015) to estimate in-cloud

240   INP concentrations from precipitation samples (i.e. condensed water content of 0.4 g m^{-3} air),

241   observations of INP concentrations in fresh precipitation samples are additionally compared to

242   studies of field measurements conducted in marine and coastal environments.

243

244   Figure 1. **INP concentrations per liter precipitation and estimated in-cloud INP**
245   **concentrations per volume of air in 11 precipitation samples collected at Scripps Institution**
246   **of Oceanography Ellen Browning Scripps Memorial Pier (32.8662 °N, 117.2544 °W, La**
247   **Jolla, California, USA) between March and May 2016.** Grey shaded region indicates the
248   spectrum of INP concentrations reported in nine previous studies of precipitation and cloud
249   water samples collected from various seasons and locations worldwide, adapted from Fig. 1 in
250   (Petters and Wright, 2015). The blue shaded region represents the composite spectrum of INP
251   concentrations observed in a range of marine and coastal environments including the Caribbean,
252   East Pacific and Bering Sea as well as laboratory-generated nascent sea spray (DeMott et al.,
253   2016).

254   *DeMott et al., 2016 data has been updated with a completed dataset for the ICE-T study, as
255   shown in (Yang et al., 2020).

256   Figure 1 shows that atmospheric INP concentration estimates compare with INP

257   concentrations observed in a range of marine and coastal environments, including the Caribbean,

258   East Pacific, and Bering Sea, as well as laboratory-generated nascent sea spray aerosol (DeMott et

259   al., 2016). Observations of INPs in aerosol samples are shown in Fig. S3 and are also comparable

260   with those of DeMott et al. (2016).

261   The source regions of aerosols present in precipitating clouds were estimated using 10-day

262   FLEXPART back trajectories (Fig. 2). For each of the 11 sampling periods, back trajectories show

263   that the Pacific Ocean from mid to high latitudes was the primary source region to precipitating

264   cloud layers. Periods 5 – 11 may have been additionally influenced by west coast continental

265   sources (particularly periods 6 and 7). 10-day back trajectory simulations for aerosol samples

266   similarly indicated that marine sources dominated (see Fig. S4). Marine aerosols likely originated

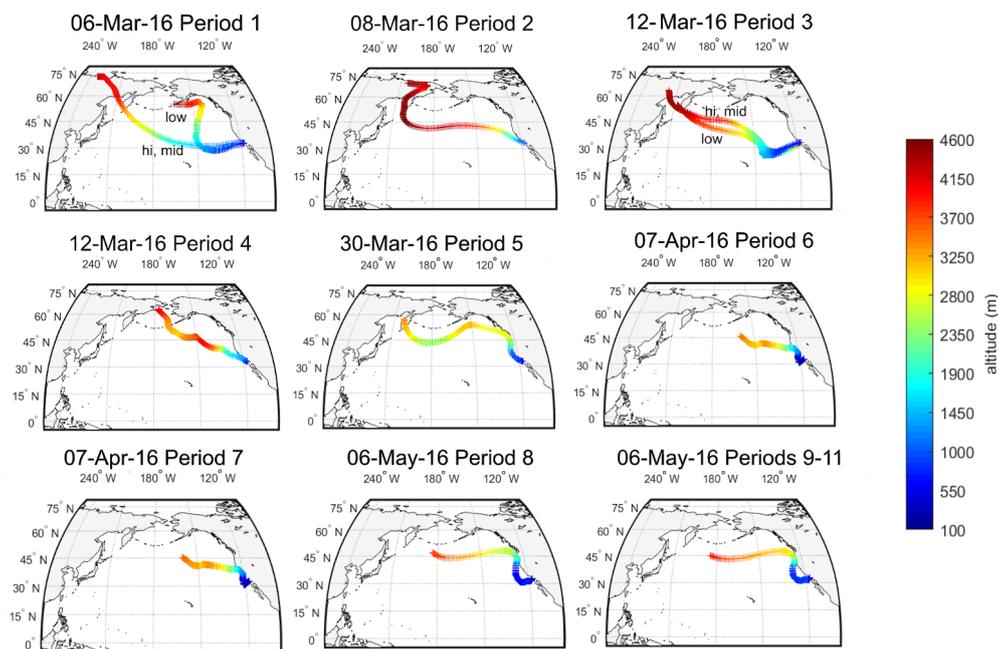
267   from regions near the coast (Periods 2, 4-11, A1, A2, A5) or in the mid Pacific Ocean (Periods 1

268   and 3), where trajectories descended below the marine boundary layer.

269   Cloud bottom and top altitudes were estimated using the High-Resolution Rapid Refresh

270   model (HRRR), defined by the RH criteria in Table S2. Over the 11 precipitation sampling periods,

271   cloud altitude ranged from 950 – 600 mb, bottom to top, or 500 – 4000 m, with temperatures

272   ranging from 265 – 288 K.

273
274
275
276 **Figure 2.** 10-day back-trajectories from cloud base, mid-cloud, and cloud-top during 11
277 precipitation sampling periods at the SIO Pier (32.8662 °N, 117.2544 °W). FLEXPART back-
278 trajectories were used to estimate potential source regions of INPs to the clouds during
279 precipitation events. Shown are the particle centroids of back-trajectories from three release
280 altitudes within each cloud (see Table S2 for details on altitude selection criteria). If trajectories
281 across the three selected release altitudes differentiated, they are labeled "hi" for cloud top, "mid"
282 for halfway between base and top, and "low" for cloud bottom. Origins of particles in the 10-day
283 simulation are shown to range from 4000 m over Russia to 2500 – 3500 m over the Sea of Okhostk,
284 the Bering Sea, and the north Pacific. FLEXPART results suggest a dominance of marine particle
285 sources to clouds for sampling periods 1-11.
286
287 *3.2 Bacterial and fungal taxonomy*

288   Cultivable bacteria and fungi were enriched from rain and aerosol samples in marine

289 bacterial growth media, and strains were further isolated on marine agar. This resulted in 34

290 isolates from rain samples, and 13 isolates from aerosol samples with 29 unique operational

291 taxonomic units (OTUs) as determined by > 97 % sequence identity of 16S sequences (Table

292   S1). Many of the isolates derived from rain and aerosol were highly pigmented, as observed in

293   other studies (Delort et al., 2017; Fahlgren et al., 2010, 2015; Hwang and Cho, 2011; Tong and

294   Lighthart, 1997), presumably aiding their survival under high *uv* exposure (Fig. S5). This

295   pigmentation was especially prevalent in rain samples. The higher number of precipitation-

296   derived isolates compared to aerosol is likely the result of lower aerosol bacterial and fungal

297   loads during rain events. INP concentration decreases in aerosol during precipitation events

298   support this conclusion. For 3 of the 11 precipitation events featured in this study (see Fig. S6),

299   INP concentrations in aerosol were measured immediately before, during, and after precipitation

300   events. In each of the three events, INP concentrations in aerosol decreased below detection

301   levels during precipitation and increased again soon after the end of the precipitation event (in

302   under 24 hours), though not beyond concentrations observed prior to the precipitation event.

303   Interestingly, these features (i.e. the observed decreased INP concentrations during precipitation

304   events and absence of increased INP concentrations within 24 hours of precipitation events) are

305   in opposition to multiple studies of INP concentrations during and after rainfall events in

306   terrestrial systems (Bigg, 1958; Conen et al., 2017; Huffman et al., 2013; Prenni et al., 2013).

307   Additionally, (Levin et al., 2019) observed an increase in INP concentrations after precipitation

308   events in a coastal environment, though this increase may have been related to a shift from

309   marine to terrestrial aerosol sources as indicated by the back trajectories. Thus, results in this

310   study indicate that the positive feedbacks between rainfall and surface INP emissions observed in

311   terrestrial systems (Bigg et al., 2015; Morris et al., 2017) may not always apply to marine

312   environments.

313   The taxonomy of the aerosol and rain isolates show higher diversity in the precipitation

314   samples (Fig. S7 and Table S1), which may be due to artificial biases from low aerosol isolate

315   recovery or sweep out of interstitial particles during raindrop descent. The rain samples had a

316   high proportion of Actinobacteria, whereas in aerosol, Firmicutes and Proteobacteria were more

317   dominant.

318   The microbes isolated in our study are closely related to microbial communities described

319   in other studies, despite being isolated from a warmer climate (Bowers et al., 2009; Fröhlich-

320   Nowoisky et al., 2016; Santl-Temkiv et al., 2015; Vaïtilingom et al., 2012). As (Michaud *et al.*,

321   2018) showed, Actinobacteria, as well as select Proteobacteria and Firmicutes, have an increased

322   ability to aerosolize from seawater, and so SSA emissions may also explain their presence here.

323   Two isolates (one from rain and one from aerosol, 3.5% of total isolates) are related to *Pantoea*

324   sp., strains of which are known to possess IN proteins (e.g., Hill et al., 2014). *Pantoea* sp. and

325   *Psychrobacter* sp. were the only bacterial taxa identified previously known to possess ice

326   nucleation activity (Hill et al., 2014; Ponder et al., 2005). However, both *Psychrobacter sp.* and

327   *Idiomarina* sp. have been shown to be capable of inhibiting ice recrystallization, possibly

328   through the production of antifreeze proteins (AFPs) which can both inhibit freezing at moderate

329   temperatures and serve as INPs at colder temperatures (Wilson and Walker, 2010).

330   The phylogenetic relationships between isolates and reference sequences (Fig. 3)

331   indicate that marine origin is highly likely for two of the 14 IN isolates, *Idiomarina* sp. and

332   *Psychrobacter* sp. 1c2, both of which were derived from coastal aerosol. Additionally,

333   considering the aerosol transport simulation data (Fig. 2), the evidence of marine influence in

334   precipitation INP spectra (Fig. 1), and the use of marine growth media, multiple other IN isolates

335   derived from the precipitation samples are also possibly marine. Furthermore, other IN isolates

336   from precipitation samples cluster closely with marine reference sequences. For example,

337   *Pantoea* sp.1a and *Brevibacterium* sp. show high similarity to reference sequences derived from

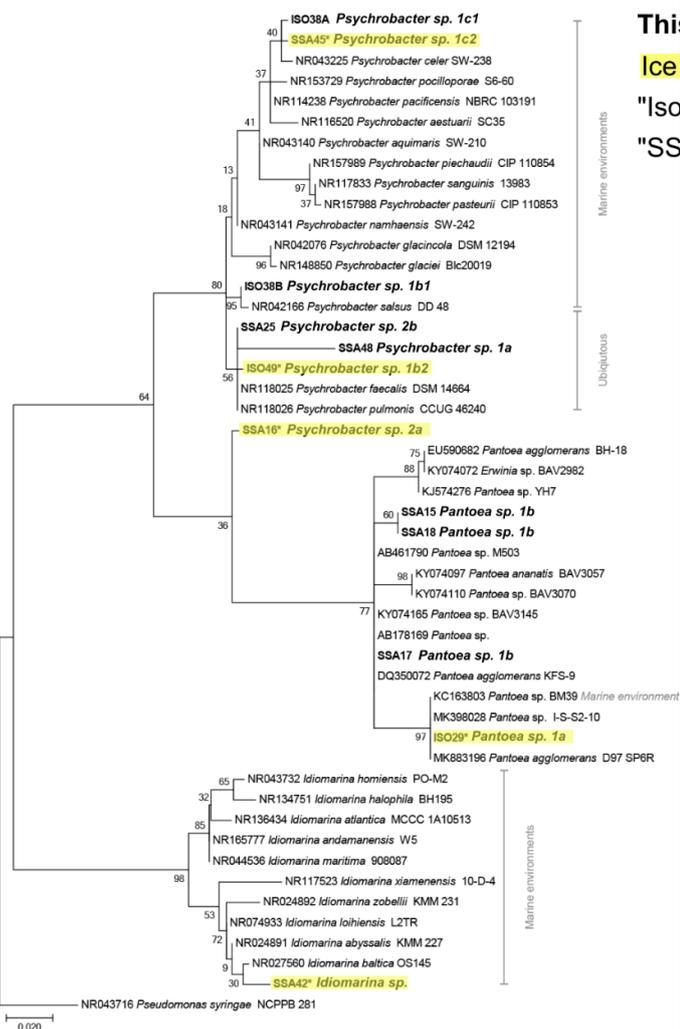
338   marine environments (Fig. 3 and S8). However, several of the species identified in this study are

339   likely more ubiquitous, and closely related to reference isolates found in terrestrial and

340   freshwater systems (Bowers et al., 2009; Fröhlich-Nowoisky et al., 2016; Santl-Temkiv et al.,

341   2015; Vaïtilingom et al., 2012), including two of the IN isolates, *Psychrobacter* sp. 1b2 and

342   *Paenbacillus* sp. 1.

343

344

345 **Figure 3.** Phylogenetic relationships of isolates (in bold) related to Gamma-proteobacteria
346 reference sequences. The environmental source of the reference sequences (based on NCBI
347 metadata) is indicated in grey. Isolates with ice nucleating properties are shaded in yellow;
348 bootstrap values (n=500) are indicated at nodes; scale bar represents changes per positions.
349

350 *3.3 Ice Nucleating Properties of Rain and SSA isolates*

351 Of the 47 total isolates derived from precipitation and aerosol samples, 14 were found to

352 be significantly ice nucleating according to the selection criterion described in Methods Sect. 2.4.

353   Within the technique's temperature and detection limit of 0.675 INP mL^{-1} liquid between 0 and -

354   25 °C, 11 precipitation isolates exhibited freezing temperatures between -2.3 and -24.3 °C, and 3

355   aerosol isolates exhibited freezing temperatures between -14.0 and -24.5 °C (Table 1). Prior to

356   this study, *Lysinibacillus* sp. was the only known gram-positive species found to be capable of

357   ice nucleation (Failor et al., 2017a). Yet several IN isolates identified in this study are also

358   gram-positive, including isolates of *Brevibacterium* sp., *Paenbacillus* sp., *Planococcus* sp.,

359   *Bacillus* sp., *Arthrobacter* sp., and *Cellulosimicrobium* sp.

360   **Table 1**. Identities of 14 cultivable, halotolerant IN bacteria derived from aerosol or precipitation
361   samples (see Table S2 for precipitation and aerosol sample details).
362

IsoID	Isolate	IN Onset Temperature °C	Precipitation or Aerosol Sample Number
Iso2	*Cryptococcus* sp. 1	-9.3	1
Iso10B	*Paenibacillus* sp. 1	-14.8	2
Iso8	*Brevibacterium* sp. 1b	-2.3	4
Iso32B	*Planococcus* sp. 1	-12.3	7
Iso29	*Pantoea* sp. 1a	-17	8
Iso31	*Bacillus* sp.1a1	-14.5	8
Iso21	*Cellulosimicrobium* sp. 1a1	-14	9
Iso23	Unknown *Arthrobacter*	-13.3	9
Iso24A	*Metschikowia* sp.	-16.5	9
Iso27	*Cellulosimicrobium* sp. 1a3	-14.8	10
Iso49	*Psychrobacter* sp. 1b2	-13.8	11
SSA42	*Idiomarina* sp.	-14.3	A1
SSA16	*Psychrobacter* sp. 1c2	-17.5	A2
SSA45	*Psychrobacter* sp. 2b	-14	A5

363

364   Isolate INP spectra are shown in Fig. 4, normalized to biomass, n_m g^{-1} (see Sect. 2.4 for

365   details on biomass estimates). Also plotted in Fig. 4 are observations of a variety of marine and

366   terrestrial bioaerosols from prior studies, including pollens, fungi, lichens, plankton, leaf litter

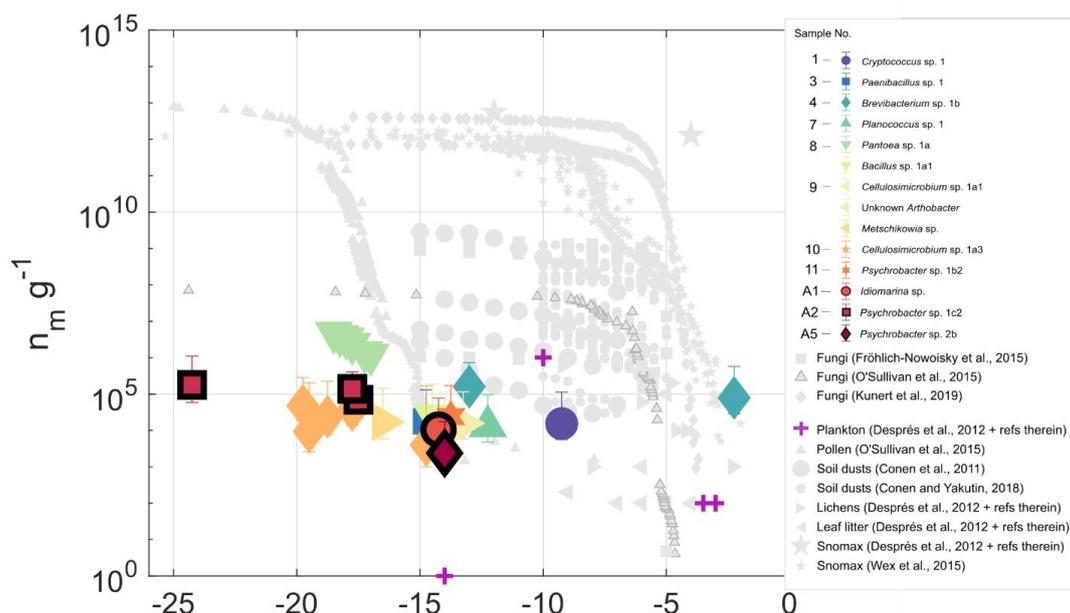
367   and soil dusts (Conen et al., 2011; Conen and Yakutin, 2018; Després et al., 2012; Fröhlich-

368   Nowoisky et al., 2015; Kunert et al., 2019; O'Sullivan et al., 2015; Wex et al., 2015). Results

369   show that with the exception of *Brevibacterium* sp., isolates from this study are generally less

370   efficient than most terrestrial IN biological particles, with lower concentrations and activation

371   temperatures. Concentrations of INP per mL in ZoBell suspension are additionally shown in Fig.

372   S10.

373
374 **Figure 4.** INP concentrations (g^{-1} biomass) for 14 halotolerant isolates derived from precipitation
375 and aerosol samples. Also shown are INP observations of various biological particles from
376 published studies. Sample numbers in the legend indicate the precipitation or aerosol sample from
377 which the isolate was derived (see Table S3). Datapoints corresponding to isolates from aerosol
378 are outlined in black. Error bars indicate 95% confidence intervals and uncertainty associated with
379 biomass estimate (see Sect. 3.3 for details). Only freezing activity that was significantly enhanced
380 ($p < 0.005$) above ZoBell growth media is shown. Results show that with the exception of
381 *Brevibacterium* sp., isolates are generally less efficient ice nucleators than most biological INPs
382 of terrestrial origin.
383

384   To examine the IN properties of unique strains within samples, multiple sequence

385   alignment of the 16S sequences was used to identity and remove duplicates. The relationship

386   between 16S sequences of isolates within their OTUs is shown in Fig. S11. Ice nucleating

387   precipitation and aerosol isolates exhibit moderate IN freezing temperatures (< -10 °C) (Fig. 4),

388   with the exception of two warm freezing isolates: a fungal isolate from sampling period 1,

389   *Cryptococcus* sp., which triggered freezing at -9.3 °C, and a bacterial isolate from sampling period

390   4, *Brevibacterium* sp., at an exceptionally warm freezing temperature of -2.3 °C. The freezing

391   temperatures of all but *Brevibacterium* sp. 1b overlap with previously reported freezing

392   temperatures of INPs produced from fresh SSA (-7 to -33 °C), and, in particular, with the freezing

393   temperatures shown to be likely associated with microbes or cellular material in SSA (-8 to -22

394   °C). (DeMott et al., 2016; McCluskey et al., 2017). Isolate freezing temperatures also overlap with

395   INP freezing temperatures in samples of Arctic marine sea surface microlayer (Irish et al., 2017;

396   Wilson et al., 2015).

397   Considering that only IN microbes of continental origins, such as *Pseudomonas syringae*,

398   have been reported with freezing temperatures as high as -2 or -3 °C (e.g. Fröhlich-Nowoisky *et*

399   *al.*, 2016 and references therein), and that SSA is associated with 1000 times fewer ice

400   nucleating active sites per surface area compared to mineral dust (McCluskey et al., 2018b), it

401   would be unexpected to find a marine IN isolate with an extremely warm freezing onset

402   temperature. However, the presence of bacteria closely related to the *Brevibacterium* sp. in

403   marine environments suggests that a marine origin is possible (Fig. S8, see also discussion in

404   Sect. 3.2). Furthermore, the backtrajectory analysis for the sample from which *Brevibacterium*

405   sp. was isolated indicates that North Pacific sources dominated the sampling period.

406   Actinobacteria are common in marine environments (e.g. Bull et al., 2005) and have been

407   identified in nascent SSA (Michaud et al., 2018).

408   To explore the role of the growth media on isolate IN properties, controls were run on nine

409   washed isolates (Fig. S1 and Table S4, see Methods Sect. 2.4). Five of the selected isolates were

410   found to not be significantly IN above sterile ZoBell background, while four were chosen from the

411   subset of significantly IN isolates. Interestingly, the observed INP concentrations of washed

412   isolates above that of the FASW were inconsistently related to activity when grown in ZoBell

413   media, and were generally enhanced. Seven of the nine media-free isolates exhibited significant

414   IN behavior, including 4 isolates that were not IN in ZoBell. Some of the observed differences in

415   ice nucleation above background between isolates suspended in ZoBell and those suspended in

416   FASW could be a result of the differences in the background INP concentrations present in the

417   suspension media (i.e. concentrations of INPs in FASW are less than in ZoBell, thus increasing

418   the temperature range in which IN activity could be detected). Another possibility is that the

419   isolates' IN behavior varied depending on multiple factors, including their viability, environment,

420   stress, and nutrient availability. As washing cells removes soluble molecules, the apparent IN

421   activity of washed suspensions could indicate that the source of IN activity is membrane-

422   associated, or alternatively, that expression of IN activity is sensitive to environmental factors. For

423   example, limited nutrient availability has been shown to enhance IN behavior of both

424   *Lysinibacillus* sp. and *P. syringae* sp. (Failor et al., 2017a; Nemecek-Marshall et al., 1993). The

425   difference in IN activity between ZoBell and FASW suspensions indicates that *in situ*

426   measurements of IN bacteria will be necessary to determine the abundance of active IN microbes

427   in the atmosphere.

428   Another limitation of the cultivation approach is that the concentrations of the IN species

429   in the precipitation samples from which we derived them are unknown. Considering that typically,

430   only a fraction of an IN isolate's cells are actively ice nucleating, even for highly efficient IN

431   microbes such as *P.syringae* (2-4%, Amato et al., 2015), concentrations of active IN microbes in

432   the precipitation samples were likely below the limit of detection (0.675 mL^{-1}, see Sect. 2.1). For

433   example, assuming a high active fraction of 2% and the maximum concentration of cultivable

434   microbes in precipitation observed in (Failor et al., 2017b, ~22,000 L^{-1}), the concentration of

435   actively ice nucleating microbes equal 0.44 mL^{-1}, which is below the limit of detection.

436   One study of note (Failor *et al.*, 2017) used similar cultivation and INP measurement

437   techniques on precipitation samples. While Failor et al. (2017) did not report estimates of source

438   regions or claim marine origin of the IN microbes that were cultivable in marine growth media,

439   they report the presence and IN freezing temperatures of *Pseudomonas* sp. and *Pantoea* sp., both

440   of which were also found here (see also Fall and Schnell, 1985). Additionally, whereas (Failor *et

441   al.*, 2017) reports warm freezing temperatures between -4 and -12 °C for multiple *Pseudomonas*

442   sp. samples, none of the *Pseudomonas* sp. isolated in our study exhibited detectable IN activity.

443   Similarly, pseudomonads were common, but all lacked ice nucleation activity in rain and cloud

444   water samples collected on the coast of Scotland (Ahern et al., 2007). IN observations for *Pantoea*

445   sp. also differ. The *Pantoea* sp. isolate in our study exhibited a moderate IN freezing temperature

446   of -17 °C, but (Failor *et al.*, 2017) reports warm freezing activity between -4 and -10 °C.

447   Interestingly, Failor *et al.'s* (2017) results show discrepancies between IN behavior of

448   isolates directly plated from precipitation samples and those from suspensions of purified strains,

449   supporting our findings that IN behavior can vary between different isolate suspensions. (Failor et

450   al., 2017) suggests that changes in an isolate's IN activity may be explained in part by growth

451   conditions not conducive for the expression of INA, and that INA molecules might generally be

452   produced in higher amounts in oligotrophic conditions, like those found in the atmosphere. In

453   addition to environment-dependent changes in isolate IN activity, the differences between the two

454   studies could also be the result of inherent differences in IN activity between different strains of

455   the same species (Morris et al., 2008). Finally, whereas (Failor *et al.*, 2017) report only IN

456   Gammaproteobateria that were cultivable in marine growth media, we find greater diversity among

457   the IN isolate taxonomies, including Actinobacteria, Bacilli, Saccharomycetes, and

458   Tremellomycetes.

459
460   **4 Conclusions**

461   Through isolation and identification of multiple IN microbes in precipitation and aerosol,

462   this study reveals two specific marine INP identities, *Idiomarina* sp. and *Psychrobacter* sp. 1c2,

463   confirming previous mesocosm studies' implication of marine microbes as INP candidates

464   (McCluskey et al., 2017, 2018a). Furthermore, we isolated six new IN gram-positive bacteria

465   capable of ice-nucleation, as prior to this study, *Lysinibacillus* sp. was the only gram-negative

466   species capable of ice nucleation (Failor et al., 2017). Additionally, through cell washing

467   experiments in which soluble molecules and growth media are eliminated from isolate

468   suspensions, we find that most isolates' IN activities are dependent on growth conditions.

469   Due to the challenge of distinguishing marine from terrestrial INPs in environmental

470   samples, it is impossible to definitively claim marine or terrestrial origins for 10 of the 14 IN

471   isolates featured in this study. In order to survive atmospheric transport and deposition through

472   rainwater, cultivable isolates derived from precipitation must be tolerant of near-freshwater

473   conditions. However, marine origins are possible for the following reasons: aerosol back-

474   trajectories and INP observations during sampling events indicate that marine sources were

475   dominant (Figs. 1-2), multiple isolate sequences show similarity to marine isolation sources in

476   reference sequences (Figs. 3, S8), and isolate freezing temperatures are generally in agreement

477   with previously documented nascent SSA IN freezing temperatures (DeMott et al., 2016;

478   McCluskey et al., 2017, 2018a).

479   While cultivation methods preclude quantification of atmospheric abundance and exclude

480   a large fraction of uncultivable microorganisms, we captured several possible contributors to

481   precipitation IN populations and through isolation maintained the ability to assess their IN

482   activity and other characteristics. Considering the general rarity of atmospheric INPs (1 in 10^5 at

483   -20 °C) (Rogers et al., 1998), the relatively lower concentrations of INPs in marine air masses

484   (DeMott et al., 2016; McCluskey et al., 2018c), and the rarity of cultivable microbes, it is quite

485   surprising that a substantial fraction of the cultivable microbial isolates from precipitation

486   samples were found to be IN at temperatures above -17 °C (11 out of 34 total, or 32%), and

487   suggests that there are more – i.e., a significant fraction – of IN species in aerosols among the

488   substantially larger uncultivable community.

489   Finally, as cultivable populations represent a small fraction of the total microbial

490   community, future studies should combine INP measurements with state-of-the-art sequencing

491   approaches to better identify the putative IN microbes that may not be recovered by cultivation

492   techniques. Further study is also needed to understand the factors, such as atmospheric processing

493   or nutrient limitation, that inhibit or enhance microbe IN behavior, as well as the factors that

494   modulate the emissions of IN bacteria emission from the ocean surface.

495

496 **Acknowledgements**

497 This work was supported by NSF through the NSF Center for Aerosol Impacts on

498 Chemistry of the Environment (CAICE), CHE-1801971, AGS-1451347, and IOS-1516156. This

499 work was additionally supported by grant W912HZ-15-2-0019 from the US Army Corps of

500 Engineers. The authors gratefully acknowledge Kelsey Krug for her assistance with sample

501 archival, and NOAA Earth Systems Research Laboratory (ESRL) for providing the High-

502 Resolution Rapid Refresh model that was used in this study.

503

504 *Data Availability*: The data set supporting this manuscript is hosted by the UCSD Library Digital

505 Collections (https://doi.org/10.6075/J0GQ6W2Z).

506

507 *Supplement Link:* The supplement related to this article is available here:

508

509 *Competing Interests*: The authors declare no conflict of interest.

510

511 *Author Contributions:* CMB and JMM wrote the manuscript, prepared figures, and conducted the

512 field campaign and laboratory measurements. MAF contributed HRRR analysis and

513 characterized meteorology during sampling periods. JD provided phylogenetic analyses and

514 prepared figures. GCC supported FLEXPART simulations. MDS, MDB, TCH, PJD and KAP

515 provided feedback on the study design, analyses and manuscript. KAP and PJD are principal

516 investigators on awards CHE-1801971 and AGS-1451347, and MDB is the principal investigator

517 on award IOS-1516156.

518
519

520 **References**

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

523 Ahern, H. E., Walsh, K. A., Hill, T. C. J. and Moffett, B. F.: Fluorescent pseudomonads isolated
524 from Hebridean cloud and rain water produce biosurfactants but do not cause ice nucleation,
525 Biogeosciences, 4(1), 115–124, doi:10.5194/bg-4-115-2007, 2007.

526 Alpert, P. A., Aller, J. Y. and Knopf, D. A.: Initiation of the ice phase by marine biogenic
527 surfaces in supersaturated gas and supercooled aqueous phases, Phys. Chem. Chem. Phys.,
528 13(44), 19882, doi:10.1039/c1cp21844a, 2011.

529 Amato, P., Joly, M., Schaupp, C., Attard, E., Möhler, O., Morris, C. E., Brunet, Y. and Delort,
530 A.-M.: Survival and ice nucleation activity of bacteria as aerosols in a cloud simulation chamber,
531 Atmos. Chem. Phys., 15(11), 6455–6465, doi:10.5194/acp-15-6455-2015, 2015.

532 Ansmann, A., Tesche, M., Seifert, P., Althausen, D., Engelmann, R., Fruntke, J., Wandinger, U.,
533 Mattis, I. and Müller, D.: Evolution of the ice phase in tropical altocumulus: SAMUM lidar
534 observations over Cape Verde, J. Geophys. Res. Atmos., 114(17), 1–20,
535 doi:10.1029/2008JD011659, 2009.

536 Beall, C. M., Stokes, M. D., Hill, T. C., DeMott, P. J., DeWald, J. T. and Prather, K. A.:
537 Automation and Heat Transfer Characterization of Immersion Mode Spectroscopy for Analysis
538 of Ice Nucleating Particles, Atmos. Meas. Tech., (February), 1–25, doi:10.5194/amt-2016-412,
539 2017.

540 Benjamin, S. G., Weygandt, S. S., Brown, J. M., Hu, M., Alexander, C. R., Smirnova, T. G.,
541 Olson, J. B., James, E. P., Dowell, D. C., Grell, G. A., Lin, H., Peckham, S. E., Smith, T. L.,
542 Moninger, W. R., Kenyon, J. S. and Manikin, G. S.: A North American Hourly Assimilation and
543 Model Forecast Cycle: The Rapid Refresh, Mon. Weather Rev., 144(4), 1669–1694,
544 doi:10.1175/MWR-D-15-0242.1, 2016.

545 Bigg, E. K.: A LONG PERIOD FLUCTUATION IN FREEZING NUCLEUS
546 CONCENTRATIONS, J. Meteorol., doi:10.1175/1520-0469(1958)015<0561:alpfif>2.0.co;2,
547 1958.

548 Bigg, E. K., Soubeyrand, S. and Morris, C. E.: Persistent after-effects of heavy rain on
549 concentrations of ice nuclei and rainfall suggest a biological cause, Atmos. Chem. Phys., 15(5),
550 2313–2326, doi:10.5194/acp-15-2313-2015, 2015.

551 Boström, K. H., Simu, K., Hagström, Å. and Riemann, L.: Optimization of DNA extraction for
552 quantitative marine bacterioplankton community analysis, Limnol. Oceanogr. Methods, 2, 365–
553 373, 2004.

554 Bowers, R. M., Lauber, C. L., Wiedinmyer, C., Hamady, M., Hallar, A. G., Fall, R., Knight, R.
555 and Fierer, N.: Characterization of airborne microbial communities at a high-elevation site and
556 their potential to act as atmospheric ice nuclei, Appl. Environ. Microbiol., 75(15), 5121–5130,
557 doi:10.1128/AEM.00447-09, 2009.

558 Bull, A. T., Stach, J. E. M., Ward, A. C. and Goodfellow, M.: Marine actinobacteria:
559 Perspectives, challenges, future directions, Antonie van Leeuwenhoek, Int. J. Gen. Mol.
560 Microbiol., doi:10.1007/s10482-004-6562-8, 2005.

561   Burrows, S. M., Hoose, C., Pöschl, U. and Lawrence, M. G.: Ice nuclei in marine air: Biogenic
562   particles or dust?, Atmos. Chem. Phys., 13(1), 245–267, doi:10.5194/acp-13-245-2013, 2013.

563   Carro-Calvo, L., Hoose, C., Stengel, M. and Salcedo-Sanz, S.: Cloud glaciation temperature
564   estimation from passive remote sensing data with evolutionary computing, J. Geophys. Res.,
565   121(22), 13,591-13,608, doi:10.1002/2016JD025552, 2016.

566   Christner, B. C., Cai, R., Morris, C. E., McCarter, K. S., Foreman, C. M., Skidmore, M. L.,
567   Montross, S. N. and Sands, D. C.: Geographic, seasonal, and precipitation chemistry influence
568   on the abundance and activity of biological ice nucleators in rain and snow., Proc. Natl. Acad.
569   Sci. U. S. A., 105(48), 18854–18859, doi:10.1073/pnas.0809816105, 2008.

570   Conen, F. and Yakutin, M. V: Soils rich in biological ice-nucleating particles abound in ice-
571   nucleating macromolecules likely produced by fungi, Biogeosciences, 15(14), 4381–4385,
572   doi:10.5194/bg-15-4381-2018, 2018.

573   Conen, F., Morris, C. E., Leifeld, J., Yakutin, M. V and Alewell, C.: Biological residues define
574   the ice nucleation properties of soil dust, Atmos. Chem. Phys., 11(18), 9643–9648,
575   doi:10.5194/acp-11-9643-2011, 2011.

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

578   Creamean, J. M., Suski, K. J., Rosenfeld, D., Cazorla, A., DeMott, P. J., Sullivan, R. C., White,
579   A. B., Ralph, F. M., Minnis, P., Comstock, J. M., Tomlinson, J. M. and Prather, K. A.: Dust and
580   biological aerosols from the Sahara and Asia influence precipitation in the Western U.S, Science
581   (80-.)., 340(6127), 1572–1578, doi:10.1126/science.1227279, 2013.

582   Curry, J. A., Hobbs, P. V., King, M. D., Randall, D. A., Minnis, P., Isaac, G. A., Pinto, J. O.,
583   Uttal, T., Bucholtz, A., Cripe, D. G., Gerber, H., Fairall, C. W., Garrett, T. J., Hudson, J., Intrieri,
584   J. M., Jakob, C., Jensen, T., Lawson, P., Marcotte, D., Nguyen, L., Pilewskie, P., Rangno, A.,
585   Rogers, D. C., Strawbridge, K. B., Valero, F. P. J., Williams, A. G. and Wylie, D.: FIRE arctic
586   clouds experiment, Bull. Am. Meteorol. Soc., 81(1), 5–29, doi:10.1175/1520-
587   0477(2000)081<0005:FACE>2.3.CO;2, 2000.

588   D.C., S., V.E., L., A.L., S. and G., D. S.: The association between bacteria and rain and possible
589   resultant meteorological implications., edited by F. A. O. of the UN, , 86, v.148-152(2-4), 1982.

590   DeLeon-Rodriguez, N., Lathem, T. L., Rodriguez-R, L. M., Barazesh, J. M., Anderson, B. E.,
591   Beyersdorf, A. J., Ziemba, L. D., Bergin, M., Nenes, A. and Konstantinidis, K. T.: Microbiome
592   of the upper troposphere: species composition and prevalence, effects of tropical storms, and
593   atmospheric implications., Proc. Natl. Acad. Sci. U. S. A., 110(7), 2575–80,
594   doi:10.1073/pnas.1212089110, 2013.

595   Delort, A. M., Vaïtilingom, M., Joly, M., Amato, P., Wirgot, N., Lallement, A., Sancelme, M.,
596   Matulova, M. and Deguillaume, L.: Clouds: A Transient and Stressing Habitat for
597   Microorganisms BT - Microbial Ecology of Extreme Environments, edited by C. Chénard and
598   F. M. Lauro, pp. 215–245, Springer International Publishing, Cham., 2017.

599   DeMott, P. J., Hill, T. C. J., McCluskey, C. S., Prather, K. A., Collins, D. B., Sullivan, R. C.,
600   Ruppel, M. J., Mason, R. H., Irish, V. E., Lee, T., Hwang, C. Y., Rhee, T. S., Snider, J. R.,
601   McMeeking, G. R., Dhaniyala, S., Lewis, E. R., Wentzell, J. J. B., Abbatt, J., Lee, C., Sultana, C.
602   M., Ault, A. P., Axson, J. L., Diaz Martinez, M., Venero, I., Santos-Figueroa, G., Stokes, M. D.,

603 Deane, G. B., Mayol-Bracero, O. L., Grassian, V. H., Bertram, T. H., Bertram, A. K., Moffett, B.
604 F. and Franc, G. D.: Sea spray aerosol as a unique source of ice nucleating particles, Proc. Natl.
605 Acad. Sci., 113(21), 5797–5803, doi:10.1073/pnas.1514034112, 2016.

606 Després, V. R., Alex Huffman, J., Burrows, S. M., Hoose, C., Safatov, A. S., Buryak, G.,
607 Fröhlich-Nowoisky, J., Elbert, W., Andreae, M. O., Pöschl, U. and Jaenicke, R.: Primary
608 biological aerosol particles in the atmosphere: A review, Tellus, Ser. B Chem. Phys. Meteorol.,
609 64(1), doi:10.3402/tellusb.v64i0.15598, 2012.

610 Fahlgren, C., Hagström, Å., Nilsson, D. and Zweifel, U. L.: Annual Variations in the Diversity,
611 Viability, and Origin of Airborne Bacteria, Appl. Environ. Microbiol., 76(9), 3015 LP – 3025,
612 doi:10.1128/AEM.02092-09, 2010.

613 Fahlgren, C., Gómez-Consarnau, L., Zábori, J., Lindh, M. V, Krejci, R., Mårtensson, E. M.,
614 Nilsson, D. and Pinhassi, J.: Seawater mesocosm experiments in the Arctic uncover differential
615 transfer of marine bacteria to aerosols, Environ. Microbiol. Rep., 7(3), 460–470,
616 doi:10.1111/1758-2229.12273, 2015.

617 Failor, K. C., Schmale, D. G., Vinatzer, B. A. and Monteil, C. L.: Ice nucleation active bacteria
618 in precipitation are genetically diverse and nucleate ice by employing different mechanisms,
619 ISME J., 11(12), 2740–2753, doi:10.1038/ismej.2017.124, 2017a.

620 Failor, K. C., Iii, D. G. S., Vinatzer, B. A. and Monteil, C. L.: Ice nucleation active bacteria in
621 precipitation are genetically diverse and nucleate ice by employing different mechanisms, , 1–14,
622 doi:10.1038/ismej.2017.124, 2017b.

623 Fall, R. and Schnell, R.: Association of an ice-nucleating pseudomonad with cultures of the
624 marine dinoflagellate, Heterocapsa niei., 1985.

625 Fröhlich-Nowoisky, J., Hill, T. C. J., Pummer, B. G., Yordanova, P., Franc, G. D. and Pöschl,
626 U.: Ice nucleation activity in the widespread soil fungus Mortierella alpina, Biogeosciences,
627 12(4), 1057–1071, doi:10.5194/bg-12-1057-2015, 2015.

628 Fröhlich-Nowoisky, J., Kampf, C. J., Weber, B., Huffman, J. A., Pöhlker, C., Andreae, M. O.,
629 Lang-Yona, N., Burrows, S. M., Gunthe, S. S., Elbert, W., Su, H., Hoor, P., Thines, E.,
630 Hoffmann, T., Després, V. R. and P?öschl, U.: Bioaerosols in the Earth system: Climate, health,
631 and ecosystem interactions, Atmos. Res., 182, 346–376, doi:10.1016/j.atmosres.2016.07.018,
632 2016.

633 Furtado, K. and Field, P.: The Role of Ice Microphysics Parametrizations in Determining the
634 Prevalence of Supercooled Liquid Water in High-Resolution Simulations of a Southern Ocean
635 Midlatitude Cyclone, J. Atmos. Sci., 74(6), 2001–2021, doi:10.1175/JAS-D-16-0165.1, 2017.

636 Hill, T. C. J., Moffett, B. F., DeMott, P. J., Georgakopoulos, D. G., Stump, W. L. and Franc, G.
637 D.: Measurement of ice nucleation-active bacteria on plants and in precipitation by quantitative
638 PCR, Appl. Environ. Microbiol., 80(4), 1256–1267, doi:10.1128/AEM.02967-13, 2014.

639 Huffman, J. A., Prenni, A. J., Demott, P. J., Pöhlker, C., Mason, R. H., Robinson, N. H.,
640 Fröhlich-Nowoisky, J., Tobo, Y., Després, V. R., Garcia, E., Gochis, D. J., Harris, E., Müller-
641 Germann, I., Ruzene, C., Schmer, B., Sinha, B., Day, D. A., Andreae, M. O., Jimenez, J. L.,
642 Gallagher, M., Kreidenweis, S. M., Bertram, A. K. and Pöschl, U.: High concentrations of
643 biological aerosol particles and ice nuclei during and after rain, Atmos. Chem. Phys., 13(13),
644 6151–6164, doi:10.5194/acp-13-6151-2013, 2013.

645 Hwang, C. Y. and Cho, B. C.: Prokaryotic abundance and 16S rRNA gene sequences detected in
646 marine aerosols on the East Sea (Korea), FEMS Microbiol. Ecol., 76(2), 327–341,
647 doi:10.1111/j.1574-6941.2011.01053.x, 2011.

648 Irish, V. E., Elizondo, P., Chen, J., Chou, C., Charette, J., Lizotte, M., Ladino, L. A., Wilson, T.
649 W., Gosselin, M., Murray, B. J., Polishchuk, E., Abbatt, J. P. D., Miller, L. A. and Bertram, A.
650 K.: Ice-nucleating particles in Canadian Arctic sea-surface microlayer and bulk seawater, ,
651 10583–10595, 2017.

652 Joyce, R. E., Lavender, H., Farrar, J., Werth, J. T., Weber, C. F., D'Andrilli, J., Vaitilingom, M.
653 and Christner, B. C.: Biological Ice-Nucleating Particles Deposited Year-Round in Subtropical
654 Precipitation, edited by A. J. M. Stams, Appl. Environ. Microbiol., 85(23), e01567-19,
655 doi:10.1128/AEM.01567-19, 2019.

656 Junge, K. and Swanson, B. D.: High-resolution ice nucleation spectra of sea-ice bacteria:
657 implications for cloud formation and life in frozen environments, Biogeosciences Discuss., 4,
658 4261–4282, doi:10.5194/bgd-4-4261-2007, 2007.

659 Kanji, Z. A., Ladino, L. A., Wex, H., Boose, Y., Burkert-Kohn, M., Cziczo, D. J. and Krämer,
660 M.: Overview of Ice Nucleating Particles, Meteorol. Monogr., 58, 1.1-1.33,
661 doi:10.1175/AMSMONOGRAPHS-D-16-0006.1, 2017.

662 Kay, J. E., Wall, C., Yettella, V., Medeiros, B., Hannay, C., Caldwell, P. and Bitz, C.: Global
663 climate impacts of fixing the Southern Ocean shortwave radiation bias in the Community Earth
664 System Model (CESM), J. Clim., 29(12), 4617–4636, doi:10.1175/JCLI-D-15-0358.1, 2016.

665 Klein, S. A., McCoy, R. B., Morrison, H., Ackerman, A. S., Avramov, A., Boer, G. de, Chen,
666 M., Cole, J. N. S., Del Genio, A. D., Falk, M., Foster, M. J., Fridlind, A., Golaz, J.-C., Hashino,
667 T., Harrington, J. Y., Hoose, C., Khairoutdinov, M. F., Larson, V. E., Liu, X., Luo, Y.,
668 McFarquhar, G. M., Menon, S., Neggers, R. A. J., Park, S., Poellot, M. R., Schmidt, J. M.,
669 Sednev, I., Shipway, B. J., Shupe, M. D., Spangenberg, D. A., Sud, Y. C., Turner, D. D., Veron,
670 D. E., Salzen, K. von, Walker, G. K., Wang, Z., Wolf, A. B., Xie, S., Xu, K.-M., Yang, F. and
671 Zhang, G.: Intercomparison of model simulations of mixed-phase clouds observed during the
672 ARM Mixed-Phase Arctic Cloud Experiment. I: single-layer cloud, Q. J. R. Meteorol. Soc.,
673 135(641), 979–1002, doi:10.1002/qj.416, 2009.

674 Knopf, D. A., Alpert, P. A., Wang, B. and Aller, J. Y.: Stimulation of ice nucleation by marine
675 diatoms, Nat. Geosci., 4(2), 88–90, doi:10.1038/ngeo1037, 2011.

676 Krzywinski, M. and Altman, N.: Error bars, Nat. Methods, 10, 921, 2013.

677 Kunert, A. T., Pöhlker, M. L., Tang, K., Krevert, C. S., Wieder, C., Speth, K. R., Hanson, L. E.,
678 Morris, C. E., Schmale III, D. G., Pöschl, U. and Fröhlich-Nowoisky, J.: Macromolecular fungal
679 ice nuclei in \textit{Fusarium}: effects of physical and chemical processing, Biogeosciences,
680 16(23), 4647–4659, doi:10.5194/bg-16-4647-2019, 2019.

681 Ladino, L. A., Yakobi-Hancock, J. D., Kilthau, W. P., Mason, R. H., Si, M., Li, J., Miller, L. A.,
682 Schiller, C. L., Huffman, J. A., Aller, J. Y., Knopf, D. A., Bertram, A. K. and Abbatt, J. P. D.:
683 Addressing the ice nucleating abilities of marine aerosol: A combination of deposition mode
684 laboratory and field measurements, Atmos. Environ., 132, 1–10,
685 doi:10.1016/j.atmosenv.2016.02.028, 2016.

686 Letunic, I. and Bork, P.: Interactive Tree of Life v2: Online annotation and display of

687   phylogenetic trees made easy, Nucleic Acids Res., 39(SUPPL. 2), 475–478,
688   doi:10.1093/nar/gkr201, 2011.

689   McCluskey, C. S., Hill, T. C. J., Malfatti, F., Sultana, C. M., Lee, C., Santander, M. V., Beall, C.
690   M., Moore, K. A., Cornwell, G. C., Collins, D. B., Prather, K. A., Jayarathne, T., Stone, E. A.,
691   Azam, F., Kreidenweis, S. M. and DeMott, P. J.: A dynamic link between ice nucleating particles
692   released in nascent sea spray aerosol and oceanic biological activity during two mesocosm
693   experiments, J. Atmos. Sci., (2015), JAS-D-16-0087.1, doi:10.1175/JAS-D-16-0087.1, 2016.

694   McCluskey, C. S., Hill, T. C. J., Malfatti, F., Sultana, C. M., Lee, C., Santander, M. V., Beall, C.
695   M., Moore, K. A., Cornwell, G. C., Collins, D. B., Prather, K. A., Jayarathne, T., Stone, E. A.,
696   Azam, F., Kreidenweis, S. M. and DeMott, P. J.: A dynamic link between ice nucleating particles
697   released in nascent sea spray aerosol and oceanic biological activity during two mesocosm
698   experiments, J. Atmos. Sci., 74(1), 151–166, doi:10.1175/JAS-D-16-0087.1, 2017.

699   McCluskey, C. S., Hill, T. C. J., Sultana, C. M., Laskina, O., Trueblood, J., Santander, M. V.,
700   Beall, C. M., Michaud, J. M., Kreidenweis, S. M., Prather, K. A., Grassian, V., DeMott, P. J.,
701   McCluskey, C. S., Hill, T. C. J., Sultana, C. M., Laskina, O., Trueblood, J., Santander, M. V.,
702   Beall, C. M., Michaud, J. M., Kreidenweis, S. M., Prather, K. A., Grassian, V. and DeMott, P. J.:
703   A mesocosm double feature: Insights into the chemical make-up of marine ice nucleating
704   particles, J. Atmos. Sci., JAS-D-17-0155.1, doi:10.1175/JAS-D-17-0155.1, 2018a.

705   McCluskey, C. S., Ovadnevaite, J., Rinaldi, M., Atkinson, J., Belosi, F., Ceburnis, D., Marullo,
706   S., Hill, T. C. J., Lohmann, U., Kanji, Z. A., O'Dowd, C., Kreidenweis, S. M. and DeMott, P. J.:
707   Marine and Terrestrial Organic Ice-Nucleating Particles in Pristine Marine to Continentally
708   Influenced Northeast Atlantic Air Masses, J. Geophys. Res. Atmos., 123(11), 6196–6212,
709   doi:10.1029/2017JD028033, 2018b.

710   McCluskey, C. S., Hill, T. C. J., Humphries, R. S., Rauker, A. M., Moreau, S., Strutton, P. G.,
711   Chambers, S. D., Williams, A. G., McRobert, I., Ward, J., Keywood, M. D., Harnwell, J.,
712   Ponsonby, W., Loh, Z. M., Krummel, P. B., Protat, A., Kreidenweis, S. M. and DeMott, P. J.:
713   Observations of Ice Nucleating Particles Over Southern Ocean Waters, Geophys. Res. Lett.,
714   45(21), 11,911-989,997, doi:10.1029/2018GL079981, 2018c.

715   McWilliam, H., Li, W., Uludag, M., Squizzato, S., Park, Y. M., Buso, N., Cowley, A. P. and
716   Lopez, R.: Analysis Tool Web Services from the EMBL-EBI., Nucleic Acids Res., 41, 597–600,
717   doi:10.1093/nar/gkt376, 2013.

718   Michaud, J. M., Thompson, L. R., Kaul, D., Espinoza, J. L., Richter, R. A., Xu, Z. Z., Lee, C.,
719   Pham, K. M., Beall, C. M., Malfatti, F., Azam, F., Knight, R., Burkart, M. D., Dupont, C. L. and
720   Prather, K. A.: Taxon-specific aerosolization of bacteria and viruses in an experimental ocean-
721   atmosphere mesocosm, Nat. Commun., 9(1), doi:10.1038/s41467-018-04409-z, 2018.

722   Monteil, C. L., Bardin, M. and Morris, C. E.: Features of air masses associated with the
723   deposition of Pseudomonas syringae and Botrytis cinerea by rain and snowfall, , 8(11), 2290–
724   2304, doi:10.1038/ismej.2014.55, 2014.

725   Morris, C. E., Sands, D. C., Vinatzer, B. A., Glaux, C., Guilbaud, C., Buffière, A., Yan, S.,
726   Dominguez, H. and Thompson, B. M.: The life history of the plant pathogen Pseudomonas
727   syringae is linked to the water cycle, Isme J., 2, 321, 2008.

728   Morris, C. E., Soubeyrand, S., Bigg, E. K., Creamean, J. M. and Sands, D. C.: Mapping rainfall
729   feedback to reveal the potential sensitivity of precipitation to biological aerosols, Bull. Am.

730   Meteorol. Soc., 98(6), 1109–1118, doi:10.1175/BAMS-D-15-00293.1, 2017.

731   Myers, J. A., Curtis, B. S. and Curtis, W. R.: Improving accuracy of cell and chromophore
732   concentration measurements using optical density, BMC Biophys., 6(1), 4, doi:10.1186/2046-
733   1682-6-4, 2013.

734   Nemecek-Marshall, M., LaDuca, R. and Fall, R.: High-level expression of ice nuclei in a
735   Pseudomonas syringae strain is induced by nutrient limitation and low temperature., J. Bacteriol.,
736   175(13), 4062–4070, 1993.

737   O'Sullivan, D., Murray, B. J., Ross, J. F., Whale, T. F., Price, H. C., Atkinson, J. D., Umo, N. S.
738   and Webb, M. E.: The relevance of nanoscale biological fragments for ice nucleation in clouds.,
739   Sci. Rep., 5, 8082, doi:10.1038/srep08082, 2015.

740   Parker, L., Sullivan, C., Forest, T. and Ackley, S.: Ice nucleation activity of antarctic marine
741   microorganisms, Antarct. J., 20, 126–127, 1985.

742   Petters, M. D. and Wright, T. P.: Revisiting ice nucleation from precipitation samples, Geophys.
743   Res. Lett., 42(20), 8758–8766, doi:10.1002/2015GL065733, 2015.

744   Ponder, M. A., Gilmour, S. J., Bergholz, P. W., Mindock, C. A., Hollingsworth, R., Thomashow,
745   M. F. and Tiedje, J. M.: Characterization of potential stress responses in ancient Siberian
746   permafrost psychroactive bacteria, FEMS Microbiol. Ecol., 53(1), 103–115,
747   doi:10.1016/j.femsec.2004.12.003, 2005.

748   Prenni, A. J., Harrington, J. Y., Tjernström, M., DeMott, P. J., Avramov, A., Long, C. N.,
749   Kreidenweis, S. M., Olsson, P. Q. and Verlinde, J.: Can Ice-Nucleating Aerosols Affect Arctic
750   Seasonal Climate?, Bull. Am. Meteorol. Soc., 88(4), 541–550, doi:10.1175/BAMS-88-4-541,
751   2007.

752   Prenni, A. J., Tobo, Y., Garcia, E., DeMott, P. J., Huffman, J. A., McCluskey, C. S.,
753   Kreidenweis, S. M., Prenni, J. E., Pöhlker, C. and Pöschl, U.: The impact of rain on ice nuclei
754   populations at a forested site in Colorado, Geophys. Res. Lett., 40(1), 227–231,
755   doi:doi:10.1029/2012GL053953, 2013.

756   Pruesse, E., Peplies, J. and Glöckner, F. O.: SINA: Accurate high-throughput multiple sequence
757   alignment of ribosomal RNA genes, Bioinformatics, 28(14), 1823–1829,
758   doi:10.1093/bioinformatics/bts252, 2012.

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

761   Sands, D.C., Langhans, V.E., Scharen, A.L., De Smet, G.: The association between bacteria and
762   rain and possible resultant meteorological implications, J. Hungarian Meteorol. Ser., 86, v.148-
763   152(2-4), 1982.

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

768   Schnell, R. C. and Vali, G.: Freezing nuclei in marine waters, Tellus, 27(3), 321–323,
769   doi:10.1111/j.2153-3490.1975.tb01682.x, 1975.

770   Stohl, A., Hittenberger, M. and Wotawa, G.: Validation of the Lagrangian particle dispersion

model FLEXPART against large scale tracer experiment data, Atmos. Environ., 32(24), 4245–4264, 1998.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S.: MEGA6: Molecular Evolutionary Genetics Analysis version 6.0., Mol. Biol. Evol., 30(12), 2725–2729, doi:10.1093/molbev/mst197, 2013.

Tong, Y. and Lighthart, B.: Solar Radiation Is Shown to Select for Pigmented Bacteria in the Ambient Outdoor Atmosphere, Photochem. Photobiol., 65(1), 103–106, doi:10.1111/j.1751-1097.1997.tb01884.x, 1997.

Vaïtilingom, M., Attard, E., Gaiani, N., Sancelme, M., Deguillaume, L., Flossmann, A. I., Amato, P. and Delort, A. M.: Long-term features of cloud microbiology at the puy de Dôme (France), Atmos. Environ., 56, 88–100, doi:10.1016/j.atmosenv.2012.03.072, 2012.

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.

Vergara-Temprado, J. V., Carslaw, K. S., Murray, B. J., Ardon-dryer, K., Bertram, K., Browse, J., Burrows, S. M., Ceburnis, D., Demott, P. J., Mason, R. H., Dowd, C. D. O., Sullivan, D. O., Pringle, K., Rinaldi, M. and Wilson, T. W.: Contribution of feldspar and marine organic aerosols to global ice nucleating particle concentrations, 17, 3637-3658, doi:10.5194/acp-17-3637-2017, 2017.

Vergara-Temprado, J., Miltenberger, A. K., Furtado, K., Grosvenor, D. P., Shipway, B. J., Hill, A. A., Wilkinson, J. M., Field, P. R., Murray, B. J. and Carslaw, K. S.: Strong control of Southern Ocean cloud reflectivity by ice-nucleating particles, Proc. Natl. Acad. Sci., 201721627, doi:10.1073/pnas.1721627115, 2018.

Walters, W., Hyde, E. R., Berg-lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J. a and Jansson, J. K.: Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys, mSystems, 1(1), e0009-15, doi:10.1128/mSystems.00009-15.Editor, 2015.

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.

Wilson, S. L. and Walker, V. K.: Selection of low-temperature resistance in bacteria and potential applications, Environ. Technol., 31(8–9), 943–956, doi:10.1080/09593331003782417, 2010.

Wilson, T. W., Ladino, L. a., Alpert, P. a., Breckels, M. N., Brooks, I. M., Browse, J., Burrows, S. M., Carslaw, K. S., Huffman, J. A., Judd, C., Kilthau, W. P., Mason, R. H., McFiggans, G., Miller, L. a., Nájera, J. J., Polishchuk, E., Rae, S., Schiller, C. L., Si, M., Temprado, J. V., Whale, T. F., Wong, J. P. S., Wurl, O., Yakobi-Hancock, J. D., Abbatt, J. P. D., Aller, J. Y., Bertram, A. K., Knopf, D. a. and Murray, B. J.: A marine biogenic source of atmospheric ice-nucleating particles, Nature, 525(7568), 234–238, doi:10.1038/nature14986, 2015.

814   Wright, E. S.: DECIPHER: harnessing local sequence context to improve protein multiple
815   sequence alignment, BMC Bioinformatics, 16(1), 322, doi:10.1186/s12859-015-0749-z, 2015.

816   Wright, T. P., Hader, J. D., McMeeking, G. R. and Petters, M. D.: High relative humidity as a
817   trigger for widespread release of ice nuclei, Aerosol Sci. Technol., 48(11), i–v,
818   doi:10.1080/02786826.2014.968244, 2014.

819   Yang, J., Wang, Z., Heymsfield, A. J., DeMott, P. J., Twohy, C. H., Suski, K. J. and Toohey, D.
820   W.: High ice concentration observed in tropical maritime stratiform mixed-phase clouds with top
821   temperatures warmer than −8 °C, Atmos. Res., 233, 104719,
822   doi:https://doi.org/10.1016/j.atmosres.2019.104719, 2020.

823   ZoBell, C. E.: Marine Bacteriology, Annu. Rev. Biochem., 16, 565–586, 1947.