

# **Biogenic Sources of Ice Nucleation Particles at the High Arctic Site Villum Research Station**

## ***Supplementary Information***

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## 1. Methods

### 1.1. Sample collection with high-flow-rate impinger

The samples were collected either into a high-salt sampling solution<sup>1</sup> or into 1 x PBS buffer (containing 0.137 M NaCl, 2.7 mM KCl, 4.29 mM Na<sub>2</sub>HPO<sub>4</sub>, and 1.47 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.2, autoclaved three times for 3 h and 0.1 µm-filter-sterilized). The evaporated sampling liquid was replaced by autoclaved (three times for 3h), filtered (0.1 µm poresize, VacuCap 90 Devices, Pall Corporation, New York) MQ water. The impinger used for sampling into PBS buffer was modified by installing two heaters into the vortex chamber in order to prevent PBS to freeze. The PBS temperature was kept at 1-15°C with a temperature controller. Prior to use, the vortex chamber was cleaned under sterile conditions in a clean lab as described earlier<sup>2</sup> and a blank was collected prior to transporting the chamber to the field. The samples were taken into the PBS buffer from Flyers hut (Figure 1C). Sampling into the high-salt solution using the high-flow-rate impinger was performed upwind of the Villum Research Station at three different sites from a platform 2 m above ground (Figure 1D). The samples were concentrated on Sterivex filters (0.2 µm pore size, Sigma-Aldrich). Two mL of RNA later solution was added to the filtered samples collected into the high-salt sampling solution, which were then stored at room temperature until DNA extraction. The cells collected into the PBS buffer were stored cooled (at 4°C) until the analysis. The cells from these samples were suspended from the Sterivex filters into 3.5 mL of PBS and then used for cultivation and cell quantification by flow cytometry. The concentration of cells ( $c_{\text{cells}}$ ) determined on two occasions by the high-flow-rate impinger and flow cytometry compared well with the  $c_{\text{cells}}$  determined by high-flow-rate filter sampling and microscopy counts considering the different methods used and the different timer resolution covered by the two methods (average  $c_{\text{cells}}$  of  $1.1 \times 10^4 \text{ m}^{-3}$  (StN-PBS-1s and StN-PBS-2s) and average  $c_{\text{cells}}$  of  $2.6 \times 10^4 \text{ m}^{-3}$  (StN-F-4 and StN-F-5)).

### 1.2. Isolation of cultivable microorganisms and screening for ice-nucleation activity

Bacterial and yeast strains were obtained by spreading 100 µl of concentrated snowmelt and PBS sample on R2A medium (cultivation of bacteria<sup>3</sup>) or DG18 medium supplemented with chloramphenicol (cultivation of yeast<sup>4</sup>). Triplicate media were grown at 4°C and at 20°C for two weeks. The colonies were re-plated once observed on the plates and transferred three times or until pure cultures were obtained. All isolates were kept pure throughout this study.

Bacterial DNA was extracted from pure colonies as described previously<sup>5</sup>. The DNA extracts were amplified using primers Bac8f (5'-AGR GTT TGA TCC TGG CTC AG-3') and Bac1492R (5'-CGC CTA CCT TGT TAC GAC TT-3'), using either Red Taq polymerase (Sigma-Aldrich) or HotStar Polymerase (Qiagen) following the instructions of the manufacturer. The amplification program was as follows: a denaturation step of 5 min (RedTaq) or 10 min (Hotstar) at 95°C was followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min and elongation at 72°C for 1 min. A final elongation step of 10 min at 72°C was performed. Once amplified, the PCR products were assessed by gel electrophoresis, using a 1.5% agarose gel, and cleaned using GenElute PCR Cleanup Kit (Sigma-Aldrich) as per the manufacturer's protocol. The cleaned PCR products were then sequenced by Macrogen (Seoul, South Korea). The sequences were quality controlled and trimmed using Geneious version 8.1. The trimmed sequences were then run through Geneious' inbuilt BLAST function.

Isolates affiliating to *Pseudomonas*, *Erwinia* and *Xanthomonas* were selected and screened for the presence of the ice nucleation activity gene<sup>6</sup> using the primers INA-3308F (5'-GGC GAT MGV AGC AAA CTS AC-3') and INA-3463R (5'-STG TAV CKT TTN CCG TCCCA-3'). The PCR master mix contained 12.5 µL RedTaq polymerase, 1 µL of each primer, 8.5 µL dH<sub>2</sub>O and the template was 2 µL DNA extract. The PCR program was as follows: A denaturation step at 95°C for 10 minutes followed by 25 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s and

elongation at 72°C for 30 s. A final elongation step was performed at 72°C for 10 min. The products were visualised using gel electrophoresis with a 2% agarose gel.

All yeasts and all bacterial isolates identified as *Pseudomonas*, *Erwinia* or *Xanthomonas* were screened for ice nucleation activity. Liquid bacterial cultures were grown in R2 medium at 20°C for 24 or 48 h on a shaker. The tubes were then transferred to 4°C for 24 h to induce the expression of the ice nucleation activity genes. Four replicates of 240 µL for each culture were transferred to 96-well microtiter plates. The microtiter plates were then placed into the climate chamber (MKT 115, Binder) at -10°C for 100 minutes. Ice formation was assessed visually. If more than two out of four replicates were frozen after 100 min, the strain was considered INA. Yeast cells were scraped off the DG18 medium and suspended in 2 mL of sterile PBS. After cold conditioning at 4°C for >2 h, yeasts were screened for ice nucleation activity at -10°C, -12°C, and -15°C.

### 1.3. SMPS and EDM data

Since 2010 particle number size distributions in the submicrometer range (9-900 nm) have been measured continuously at VRS with a custom-built Scanning Mobility Particle Sizer (SMPS) system. Measurements from this instrument have been used in several previous studies<sup>7-10</sup>. The instrument is described in detail elsewhere<sup>7</sup>. In brief, the SMPS system samples ambient aerosols through a heated particle inlet with no addition aerosol drying, as transition from the cold ambient temperatures into the heated inside of the sample hut ensures sufficient drying. The SMPS utilizes a Vienna-type medium Differential Mobility Analyser (DMA) column with 1 L min<sup>-1</sup> sample flow and 5 L min<sup>-1</sup> sheath flow, and either a TSI model 3010 Condensational Particle Counter (CPC) or a TSI 3772 CPC. Instrument operating parameters, sheath- and sample flow, temperature and relative humidity are monitored and ensured to stay within limits. Inversion of measured raw data is performed off-line, with an algorithm according to previously outlined principles<sup>11</sup>. All inverted measurement data is visually inspected for faulty data or influence from nearby vehicles or other pollution sources. Measurements with faulty data or influence of local pollution are discarded. Measurements of mass concentration of particle smaller than 10 µm (PM10) were made with an Environmental Dust Monitor (EDM) model 107E from GRIMM. Measurements from this instrument were available only during 1. Jan to 28. Apr 2016, making consistent correlation of PM10 with the concentration of INP impossible.

To get an estimate of PM10 concentration outside of the period where EDM measurements were available, measurements of PM10 were correlated with aerosol mass concentrations in the range of 400-900 nm. This was done by transforming the SMPS particle number size distribution into the mass distribution (assumed particle density was 1.5 g cm<sup>-3</sup><sup>12</sup>) and subsequent integration of the particle mass distribution in the range of 400-900 nm. The relationship was found significant using Pearson product-moment correlation ( $R^2 = 0.83$ ,  $df = 2679$ ,  $p < 0.001$ , Figure S1). A linear fit of EDM PM10 as a function of SMPS mass concentration in the range of 400-900 nm ( $M_{SMPS}$ ) yielded:  $PM10 = 5.25 \cdot M_{SMPS} - 0.02$  ( $R^2 = 0.69$ ). This allows us to use  $M_{SMPS}$  as a proxy for PM10. However it is not certain that this correlation is also valid during summer periods, where submicrometer aerosol mass tends to decrease<sup>8</sup>, while PM10 likely is increased due to higher concentrations of wind-blown dust<sup>13</sup>. While this is not a critical parameter for this study, we include it for comparison with the bioaerosol concentrations.

## 2. Figures and Tables

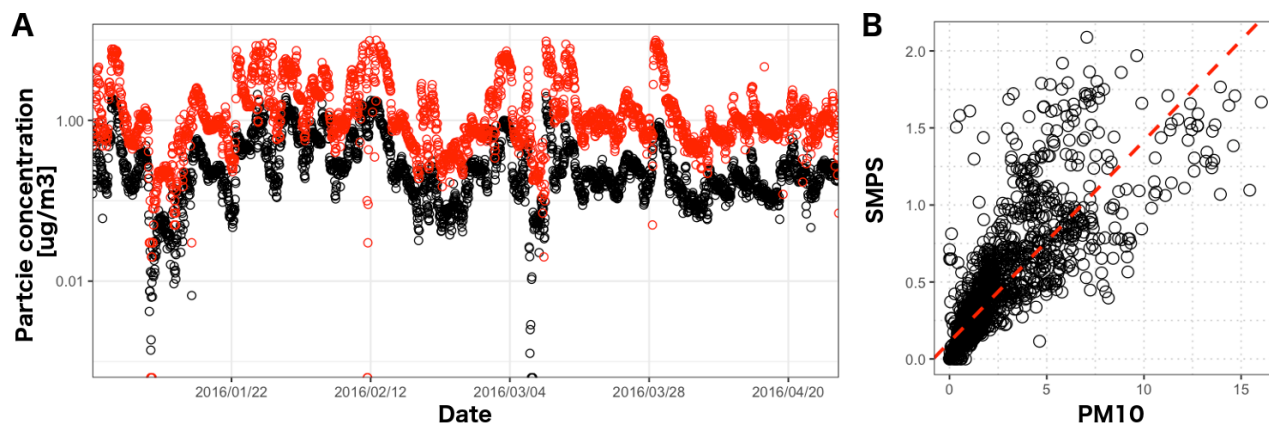


Figure S1: A – Plot showing the mass concentration of particles (averaged ½hourly) over time between Jan 2016 and April 2016. Integrated SMPS mass of the particles with diameters of 400-900 nm (black) and PM10 measured by EDM180 (GRIMM Aerosol) (red). B – Plot showing the relationship between integrated SMPS mass of the particles with diameters of 400-900 nm and the PM10 values. The relationship was found significant using Pearson product-moment correlation ( $R^2 = 0.83$ ,  $df = 2679$ ,  $p < 0.001$ ).

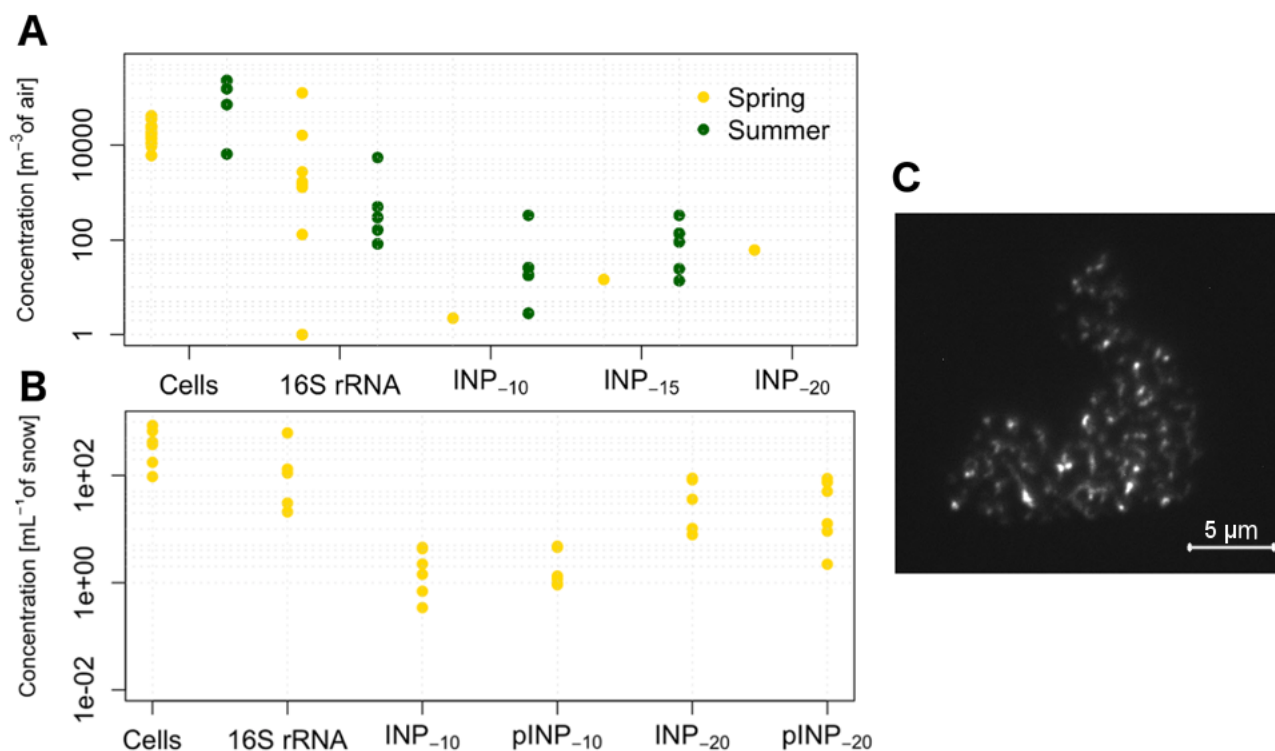


Figure S2: A- Range of concentrations for airborne bacterial cells, 16S rRNA genes, and ice nucleating particles in spring and summer. B- Range of concentrations of wet-deposited bacterial cells, 16S rRNA, and ice nucleating particles in spring, all derived from snowfall. C- A fluorescent microscopy image showing airborne particles containing bacterial cells embedded into an EPS matrix. pINP<sub>-10</sub> – cumulative proteinaceous INP active at  $< -10^\circ\text{C}$ ; pINP<sub>-20</sub> – cumulative proteinaceous INP active at  $< -20^\circ\text{C}$ .

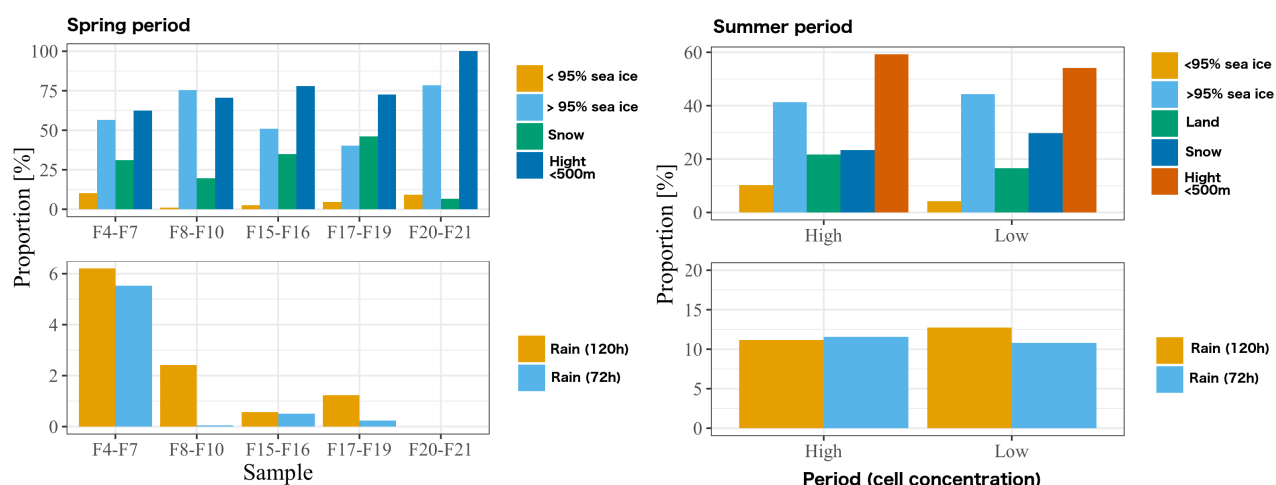


Figure S3: The proportion of time that the backward trajectories spend over regions with different sea-ice and snow coverage, below 500 m height and with rainfall. Rainfall is presented for both 3-day and 5-day backward trajectories. The filter with high bioaerosol concentration in summer: F3, F4, F5 and F7; the filters with low concentration: F1 and F6. Detailed descriptions of the samples can be found in Table S1.

Table S1: Overview of all air samples. Site 1: N 81.5901167° W 16.52860°; Site 2: N 81.60958333° W 16.78138333°; Site 3: N 81.6255167° W 16.72255°; Flyers hut: N 81.5816333° W 16.72255°.

| Sample ID        | Method   | Site       | Start date | Start time | End Date   | End time | Duration [h] | Volume of air [m3] |
|------------------|----------|------------|------------|------------|------------|----------|--------------|--------------------|
| StN-F-4          | Filter   | Flyers hut | 21/04/2015 | 15.00      | 22/04/2015 | 15.00    | 24,00        | 23,83              |
| StN-F-5          | Filter   | Flyers hut | 22/04/2015 | 15.05      | 24/04/2015 | 15.25    | 48,42        | 48,08              |
| StN-F-6          | Filter   | Flyers hut | 24/04/2015 | 15.45      | 25/04/2015 | 16.20    | 24,58        | 24,48              |
| StN-F-7          | Filter   | Flyers hut | 25/04/2015 | 17.00      | 27/04/2015 | 17.05    | 48,00        | 48,10              |
| StN-F-8          | Filter   | Flyers hut | 27/04/2015 | 17.05      | 28/04/2015 | 16.05    | 24,00        | 24,05              |
| StN-F-9          | Filter   | Flyers hut | 29/04/2015 | 16.05      | 01/05/2015 | 16.15    | 48,17        | 33,96              |
| StN-F-10         | Filter   | Flyers hut | 01/05/2015 | 16.30      | 05/05/2015 | 10.15    | 89,75        | 67,20              |
| StN-F-15         | Filter   | Flyers hut | 11/05/2015 | 10.00      | 12/05/2015 | 10.15    | 24,25        | 24,74              |
| StN-F-16         | Filter   | Flyers hut | 12/05/2015 | 10.15      | 14/05/2015 | 10.15    | 48,17        | 49,13              |
| StN-F-17         | Filter   | Flyers hut | 14/05/2015 | 10.15      | 15/05/2015 | 10.10    | 23,92        | 24,40              |
| StN-F-18         | Filter   | Flyers hut | 15/05/2015 | 10.10      | 17/05/2015 | 10.10    | 48,00        | 48,96              |
| StN-F-19         | Filter   | Flyers hut | 17/05/2015 | 10.10      | 19/05/2015 | 10.10    | 48,00        | 48,96              |
| StN-F-20         | Filter   | Flyers hut | 19/05/2015 | 10.10      | 20/05/2015 | 10.20    | 24,17        | 24,65              |
| StN-F-21         | Filter   | Flyers hut | 20/05/2015 | 10.20      | 21/05/2015 | 09.40    | 23,33        | 23,80              |
| StN-RNA-1-DNA    | Impinger | 1          | 14/04/2015 | 11.15      | 14/04/2015 | 16.15    | 5,5          | 355,4              |
| StN-RNA-2-DNA    | Impinger | 2          | 16/04/2015 | 12.25      | 16/04/2015 | 17.25    | 5,25         | 339,2              |
| StN-RNA-3-DNA    | Impinger | 2          | 17/04/2015 | 16.00      | 17/04/2015 | 21.00    | 5,17         | 334,1              |
| StN-RNA-4-DNA    | Impinger | 1          | 18/04/2015 | 10.50      | 18/04/2015 | 15.50    | 5            | 323,1              |
| StN-RNA-5-DNA    | Impinger | 1          | 20/04/2015 | 08.35      | 20/04/2015 | 13.35    | 5            | 323,1              |
| StN-RNA-6-DNA    | Impinger | 3          | 21/04/2015 | 20.00      | 22/04/2015 | 00.10    | 4,17         | 269,4              |
| StN-RNA-7-DNA    | Impinger | 3          | 22/04/2015 | 14.10      | 22/04/2015 | 18.05    | 2,25         | 145,4              |
| StN-RNA-8-DNA    | Impinger | 3          | 23/04/2015 | 12.30      | 23/04/2015 | 17.20    | 4,83         | 312,1              |
| StN-RNA-9-DNA    | Impinger | 3          | 24/04/2015 | 14.55      | 24/04/2015 | 19.55    | 5            | 323,1              |
| StN-RNA-10-DNA   | Impinger | 3          | 25/04/2015 | 08.10      | 25/04/2015 | 13.05    | 4,92         | 317,9              |
| StN-RNA-11-DNA   | Impinger | 3          | 27/04/2015 | 11.10      | 27/04/2015 | 16.00    | 4,5          | 290,8              |
| StN-RNA-12-DNA   | Impinger | 3          | 30/04/2015 | 09.15      | 30/04/2015 | 14.15    | 5            | 323,1              |
| StN-RNA-13-DNA   | Impinger | 3          | 30/04/2015 | 14.20      | 30/04/2015 | 19.20    | 5            | 323,1              |
| StN-RNA-14-DNA   | Impinger | 3          | 30/04/2015 | 19.25      | 01/05/2015 | 00.25    | 5            | 323,1              |
| StN-RNA-15-DNA   | Impinger | 3          | 06/05/2015 | 16.40      | 06/05/2015 | 21.40    | 5            | 323,1              |
| StN-RNA-16-DNA   | Impinger | 3          | 08/05/2015 | 12.40      | 08/05/2015 | 19.20    | 6,67         | 431,0              |
| StN-RNA-17-DNA   | Impinger | 3          | 12/05/2015 | 17.15      | 12/05/2015 | 22.30    | 5            | 323,1              |
| StN-RNA-18-DNA   | Impinger | 3          | 14/05/2015 | 14.45      | 14/05/2015 | 20.00    | 5,25         | 339,2              |
| StN-PBS-1s       | Impinger | Flyers hut | 21/04/2015 | 15.00      | 21/04/2015 | 17.20    | 2,3          | 404,0              |
| StN-PBS-2s       | Impinger | Flyers hut | 23/04/2015 | 18.20      | 23/04/2015 | 23.10    | 4,5          | 779,3              |
| StN-PBS-3s       | Impinger | Flyers hut | 25/04/2015 | 16.55      | 25/04/2015 | 21.55    | 5            | 323,1              |
| R-16spr-Sample1  | Filter   | Flyers hut | 19/04/2016 | 20.00      | 20/04/2016 | 20.30    | 24,5         | 28,518             |
| R-16spr-Sample2  | Filter   | Flyers hut | 20/04/2016 | 20.30      | 22/04/2016 | 10.30    | 38           | 44,232             |
| R-16spr-Sample3  | Filter   | Flyers hut | 22/04/2016 | 10.30      | 23/04/2016 | 11.30    | 25           | 29,1               |
| R-16spr-Sample4  | Filter   | Flyers hut | 23/04/2016 | 11.30      | 24/04/2016 | 20.15    | 32,75        | 38,121             |
| R-16spr-Sample5  | Filter   | Flyers hut | 25/04/2016 | 11.15      | 26/04/2016 | 12.15    | 25           | 29,1               |
| R-16spr-Sample6  | Filter   | Flyers hut | 26/04/2016 | 12.15      | 27/04/2016 | 12.00    | 23,75        | 27,645             |
| R-16spr-Sample7  | Filter   | Flyers hut | 27/04/2016 | 12.00      | 28/04/2016 | 11.45    | 23,75        | 27,645             |
| R-16spr-Sample8  | Filter   | Flyers hut | 28/04/2016 | 11.45      | 29/04/2016 | 12.30    | 24,75        | 28,809             |
| R-16spr-Sample9  | Filter   | Flyers hut | 29/04/2016 | 12.30      | 30/04/2016 | 14.15    | 25,75        | 29,973             |
| R-16spr-Sample10 | Filter   | Flyers hut | 30/04/2016 | 14.15      | 01/05/2016 | 13.45    | 23,5         | 27,354             |
| R-16spr-Sample11 | Filter   | Flyers hut | 01/05/2016 | 13.45      | 02/05/2016 | 11.15    | 21,5         | 25,026             |
| R-16spr-Sample12 | Filter   | Flyers hut | 02/05/2016 | 11.15      | 03/05/2016 | 15.00    | 27,75        | 32,301             |
| R-16spr-Sample13 | Filter   | Flyers hut | 03/05/2016 | 15.00      | 04/05/2016 | 11.15    | 19,75        | 22,989             |
| R-16spr-Sample14 | Filter   | Flyers hut | 04/05/2016 | 11.15      | 05/05/2016 | 17.15    | 30           | 34,92              |
| R-16sum-Sample 1 | Filter   | Flyers hut | 15/08/2016 | 12.10      | 16/08/2016 | 20.35    | 32,42        | 30,73              |
| R-16sum-Sample 2 | Filter   | Flyers hut | 17/08/2016 | 10.55      | 18/08/2016 | 10.15    | 23,33        | 22,12              |
| R-16sum-Sample 3 | Filter   | Flyers hut | 19/08/2016 | 20.45      | 21/08/2016 | 20.40    | 47,92        | 45,42              |
| R-16sum-Sample 4 | Filter   | Flyers hut | 22/08/2016 | 00.30      | 23/08/2016 | 10.35    | 34,08        | 32,31              |
| R-16sum-Sample 5 | Filter   | Flyers hut | 23/08/2016 | 20.05      | 25/08/2016 | 10.15    | 38,17        | 36,18              |
| R-16sum-Sample 6 | Filter   | Flyers hut | 25/08/2016 | 15.20      | 26/08/2016 | 21.20    | 30,00        | 28,44              |
| R-16sum-Sample 7 | Filter   | Flyers hut | 27/08/2016 | 14.25      | 30/08/2016 | 17.30    | 75,08        | 71,18              |

Table S2: Spearman's rank correlation coefficient and significance level for the correlation between airborne bioaerosol concentrations, INP concentrations and meteorological parameters. Significant relationships are shown in red. Significance levels: \*  $p < 0.05$ . R – Pearson correlation coefficient,  $M_{SMPS}$  – SMPS mass concentration, Temp – temperature, RH – relative humidity, VD – wind direction, VS – wind speed, RAD – radiation, 16S rRNA – 16S rRNA bacterial marker genes, INP10 – ice nucleating particles active at  $\geq -10^{\circ}\text{C}$ .

|                              | Spring       |          |       |    | Summer   |    |       |    |       |    |
|------------------------------|--------------|----------|-------|----|----------|----|-------|----|-------|----|
|                              | 16S rRNA     |          | Cells |    | 16S rRNA |    | Cells |    | INP10 |    |
|                              | R            | p        | R     | p  | R        | p  | R     | p  | R     | p  |
| <b><math>M_{SMPS}</math></b> | 0,28         | ns       | 0,15  | ns | 0,90     | ns | 0.4   | ns | 0,70  | ns |
| <b>Temp</b>                  | -0,34        | ns       | -0,38 | ns | -0,10    | ns | -0.8  | ns | -0,10 | ns |
| <b>RH</b>                    | <b>-0,58</b> | <b>*</b> | -0,31 | ns | 0,30     | ns | 0.6   | ns | 0,30  | ns |
| <b>VD</b>                    | 0,19         | ns       | -0,07 | ns | -0,30    | ns | 0.4   | ns | -0,30 | ns |
| <b>VS (Mean)</b>             | <b>0,65</b>  | <b>*</b> | -0,32 | ns | 0,00     | ns | 0.4   | ns | 0,00  | ns |
| <b>VS (Max)</b>              | <b>0,59</b>  | <b>*</b> | -0,34 | ns | 0,00     | ns | 0.4   | ns | 0,00  | ns |
| <b>RAD</b>                   | -0,17        | ns       | -0,05 | ns | -0,70    | ns | -1    | ns | -0,70 | ns |
| <b>Pressure</b>              | <b>-0,63</b> | <b>*</b> | -0,12 | ns | -0,50    | ns | 0.8   | ns | -0,50 | ns |

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