

# Workshop on Bioaerosols and Ice Nucleation

Vienna, 22<sup>nd</sup> to 23<sup>rd</sup> April 2023

TUtheSky, Getreidemarkt 9/BC, 1060 Vienna, Austria

organized by AG Grothe



AIRMODUS

22<sup>nd</sup> April 2023 Technical Developments

12:35 – 14:35

## Validation experiments for ice nucleation instruments

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Instruments detecting ice-nucleating particles (INPs) have become more numerous in the last decade and are based on different methods to establish cloud-like conditions. To ensure the accurate quantification of INPs under well-controlled laboratory conditions or in ambient air, these methods need to be validated and intercompared. In light of the ongoing ACTRIS (Aerosol, Clouds and Trace Gases Research Infrastructure) TC CIS (Topical Centre for Cloud In Situ Measurements), we are searching for an ideal experimental set to validate the temperature and counting accuracy of INP measurement techniques across the temperature range relevant for mixed-phase cloud formation.

Here we present results from different laboratory campaigns using the expansion cloud chambers AIDA (Aerosol Interaction and Dynamics in the Atmosphere) and PINE (Portable Ice Nucleation Experiment), the continuous-flow diffusion chamber INKA (Ice Nucleation instrument of the Karlsruhe Institute of Technology, a re-built of the CSU-CFDC (Colorado State University – Continuous Flow Diffusion Chamber), and the filter-based offline analysis method INSEKT (Ice Nucleation Spectrometer of the Karlsruhe Institute of Technology; a re-built of the CSU Ice Spectrometer). The instruments differ not only in their method to create temperature and supersaturated conditions, but also in their sample volume per experiment, and thus their limit of detection.

A set of different experiments were performed using mineral dust samples (Arizona Test Dust, Illite), soil dust samples (South African Soil Dust, Argentina Soil Dust), and proteinaceous particles (Snomax). Better comparability of instruments was observed using the soil dust samples and Snomax, which indicates that organic and biological content of the samples might enhance their suitability for validation experiments. Moreover, the temperature-onset of nucleation using Snomax also enabled to validate the temperature accuracy of the instruments, which is completed by homogeneous freezing experiments.

## **Application of bioaerosol analysis to develop a protocol that can prevent future health problems**

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Bioaerosols may contain parts, whole live, inactive or even dead microorganisms. In addition, many of those still alive or dormant will not withstand the stress forces encountered in air transport and will not be able to continue their life cycle where they are deposited. However, their mere presence can give us a lot of information, for example, about the risk of infection by a pathogen, whose presence in the territory under analysis was previously unknown. Therefore, the study of bioaerosols may be useful, not only to understand the biological composition of the atmosphere, but also to detect those particles that are (potentially) pathogenic for the environment and/or health.

Proof of this is a protocol that has been developed with colleagues at the “Universidad Politécnica de Madrid”, which allows to detect in air samples and in less than 24 hours, the presence of the pathogen that causes pneumonia: *Legionella pneumophila*. This protocol primarily indicates that the presence of this bacterium at detectable (normally undetectable) levels could mean that a potentially dangerous source of infection is forming. Thus, the study of bioaerosols can be approached to develop new prevention techniques for health.

However, much remains to be done, especially in the homogenisation of the techniques and methods used for the study of bioaerosols, at least from a biological point of view. A standardisation of the basic study processes, such as the volume of air collected, the methods used to process the samples and, above all, for the analysis of the data obtained, would allow a good comparison of the results of scientists from different laboratories. Perhaps this is also one of the starting points for discussion.

## Temperature sensitivity of ice nucleating particles in biological and atmospheric samples

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We are presenting the heat sensitivity of some types of INP of biogenic origin, obtained by exposing them to a range of different heating temperatures (60°C, 85°C, and 90°C) for one hour. While the focus of this presentation will be on the laboratory study described below, a short summary will also be presented on heat sensitive INP which we found in long term measurements at different locations on the globe.

Concerning our laboratory study, examined samples included birch pollen (*Betula pendula*), fungi (*Mortierella alpina*, *Fusarium acuminatum*), the bacteria *Pseudomonas syringae* (from a commercially available SNOMAX sample) and aspen leaves (from *Populus tremuloides*) which had been sampled and freeze-dried decades ago.

We additionally compare their heat sensitivity to that of INPs from airborne aerosol samples collected on filters in summer months at Villum Research Station (VRS) in North Greenland, which were exposed to the same heating procedure.

For samples from *F. acuminatum* and *P. syringae*, a continuing decrease in ice activity (expressed as INP per sample mass) was observed for each of the heating steps. For the *B. pendula* sample, highly ice active macromolecules inducing ice nucleation at > -10°C were already destroyed by heating to 60°C, while the signal below -15°C was changed much less by any of the heating steps. The *M. alpina* sample showed no change in ice activity after heating to 60°C, but a strong decrease across the examined temperature range after heating further to 85°C, and some additional decrease (roughly one order of magnitude) after heating to 90°C. The aspen leaf samples showed no noticeable reaction to heating at freezing temperatures below -15°C, but behaved similar to the *M. alpina* sample at -10°C.

Interestingly, for freezing temperatures > -10°C, INP concentrations of VRS summer samples also showed no or only a small decrease in ice activity upon heating to 60°C, similar as the *M. alpina* and aspen leaf samples. Also similar to these two, VRS samples showed a very pronounced decrease upon heating to 85°C and some further decrease upon heating to 90°C. This is interesting in the light that recent research suggested that *M. alpina*, together with the bacteria species *Pantoea ananatis*, are likely sources of the INPs present in an aspen leaf sample of the same batch as the one examined here. Combining these findings, we speculate that *M. alpina* may be of considerable importance as terrestrially sourced atmospheric INPs for regions even including the summer Arctic.

## **Exploiting Ultrasonic Levitation to Unravel the Photochemical Processing of Single Ice Crystals and Supercooled Droplets**

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We present the design of an ultrasonic levitation device capable of preparing, levitating, and spectroscopically characterizing low-temperature ice crystals and super-cooled, mergeable droplets. These crystals and droplets can be processed photochemically through exposure to ultraviolet (UV) photons (200–400 nm). Chemical and physical changes are traced spectroscopically via infrared (IR), Raman (Ra), and ultraviolet-visible (UV-VIS) spectroscopy during the photochemical processing. This setup can be also operated in an droplet merging mode exploiting modulation of the amplitude of the carrier wave through a chirped pulse.

22<sup>nd</sup> April 2023 Laboratory Studies

14:35 – 17:05

## **Subpollen particles, other particulate emissions and resuspension of aerosol particles from birch catkins**

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**Abstract.** For decades, the role of birch pollen grains (BPGs) in the atmosphere has been a vital research topic. One significant finding was the ability of BPGs to expel starch granules under humid conditions, referred to as subpollen particles (SPPs). SPPs carry the major birch pollen allergen Bet v 1 and possibly ice-nucleating macromolecules (INMs). They are hypothesized to be the major contributor to the health- and potentially climate-relevant aerosol formed by birch catkins after periods of high relative humidity (RH). However, the exact composition of this aerosol and its behaviour throughout the pollination season still needs to be investigated. In this study, we characterized particulate emissions from birch catkins in terms of morphology, concentration, dependence on RH and elemental composition. Birch catkins were harvested from eight birches at three locations in Vienna at different development stages throughout the pollination season from March 23<sup>rd</sup> to May 5<sup>th</sup>, 2021. We placed the catkins inside an aerosol chamber with two electrical fans. Dispersed particles were collected with a four-stage cascade impactor (Sioutas no. 225-370) to subsequently analyze their morphology and elemental composition with a Scanning Electron Microscope (Zeiss Supra 55 VP) and energy-dispersive x-ray spectroscopy (EDX). We varied the relative humidity in the aerosol chamber with a temperature-controlled humidifier. An Aerodynamic Particle Sizer (TSI APS 3321) with a measuring range from 0.5 to 20  $\mu\text{m}$  recorded particle size distributions and concentrations. We observed particulate emissions at all catkin development stages and at a wide range of RHs. Concentrations of particles with an aerodynamic diameter ranging from 0.5 to 5  $\mu\text{m}$  increased significantly after periods with high RH. Those particles were identified primarily as carbonaceous particles. However, their irregular plate-like morphology did not resemble typical SPPs. We hypothesize that these particles are cuticular waxes dislodged from the surfaces of the catkins' bracts. A considerable amount of ambient aerosol particles was resuspended from birch catkins, with silicon, calcium and iron being the dominant elements, while also containing soot particles. We detected SPPs exclusively on ground samples agglomerated near their parent pollen grains. Our results question the common interpretation that mainly SPPs are emitted from flowering plants and that emissions are triggered only by high RHs.



## Exploring the identification of plant debris by combining fluorescence measurements with holographic images

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Plant debris such as decaying and fragmented plant materials, air-dispersed seeds and fibers and plant waxes is a complex and heterogeneous subgroup of primary biological aerosol particles (PBAPs) that has received little attention so far<sup>1</sup>. Nevertheless, some studies indicate that plant debris is ubiquitous in the atmosphere and can be a predominant component of atmospheric PBAPs in the coarse mode<sup>2-4</sup>. Cellulose, the structural component of plant debris and plant waxes have been shown to act as ice nucleating particles<sup>5,6</sup> and, as with all PBAPs, health effects might also be associated with these particles. However, emission fluxes and emission processes of plant debris are still unclear<sup>7</sup> and large differences in emission estimations of PBAPs (between 50 – 1000 Tg/year)<sup>8</sup> highlight this substantial lack of understanding. Current measurement techniques for plant debris are offline and rely on chemical tracers posing limitations on time resolution and accurate source attributions.

In this study, we use a novel and online state-of-the-art bioaerosols monitor (SwisensPoleno Jupiter)<sup>9</sup> and explore its potential to identify plant fragments from different sources based on fluorescence measurements and holographic images. For each particle, the instrument obtains two images from orthogonal views and measures the fluorescence emission within 5 measurement windows (center wavelength: 357/435/483/562/676nm) triggered by 3 excitation sources (280/365/405nm). In a first step, we evaluate the performance of the instrument by measuring pure biological substances with well-known fluorescence properties (e.g. Tryptophan, Riboflavin, Ergosterol). I will briefly discuss the dependency of the fluorescence signal on particle size and on the position of the particle within the laser beam. In a second step, we investigate the fluorescence characteristics of typical plant fragments from diverse sources: decaying tree leaves, grasses, mosses, seed fibers, lichens and plant waxes. Respective biological materials were collected in the environment and artificially fragmented to obtain particle sizes from about 1 to 200  $\mu\text{m}$ . Classification of the particles is done by means of a machine learning model based on holographic images and/or fluorescence signals. We will discuss to what extent such a classification can be achieved and where ambiguities are expected.

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## **Investigation of the ice nucleation properties of birch pollen**

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Biological ice nuclei, which occur in pollen, fungal spores or bacteria, enable the freezing of cloud droplets at temperatures above the homogeneous freezing temperature. Although this process is omnipresent, the underlying ice nucleation mechanism, the interaction of the biological material with the water molecules, and their structure are still largely unknown. In this work, the freezing behavior and surface specific properties of birch pollen, more precisely of three fractions of birch pollen washing water, is investigated. We employ three different experimental techniques, namely ice nucleation activity, surface tension and sum frequency generation spectroscopy. We compare the ice nucleation activity of different sized species in the birch pollen washing water obtained after filtering with different pore sizes. These experiments show that the untreated fraction is the most ice nucleating active. Surface tension measurements reveal that all three fractions contain extremely surface active species and lower the surface tension of water by about 20 mN/m. Ice nucleation studies selectively on these surface-active species show that the surface enriched fraction is ice nucleation active. Subsequently, the surface sensitive vibrational spectroscopic method sum frequency generation spectroscopy has been used to characterize the interface. The species enriched at the water-air interface show sugar-like structures. Changes in temperature between -2 to 20 °C do not lead to significant changes of the molecular structure of the surface and the impact of the temperature decrease is comparable to the behavior for pure water in both surface tension and sum frequency spectroscopy experiments. By combining these observations, we aim to contribute to a deeper understanding of the working mechanism behind the ice nucleation process of birch pollen.

**The efflorescence-deliquescence behavior of saliva droplets and its implication  
for viability of airborne microorganisms**

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The aerosol-based transport of viruses and bacteria through the transmission of aerosolized expiratory secretions is one of the main routes for the spreading of infectious diseases such as SARS-CoV-2. A number of studies have confirmed that environmental factors such as temperature and relative humidity can affect the inactivation and transmission of respiratory pathogens. However, there remain significant uncertainties in understanding aerosol micro-physics occurring under different environmental conditions to quantify the survival of microorganisms carried by aerosols. Here we study the size and phase changes of levitated saliva droplets composed of various salts and mucin under well-defined atmospheric conditions. An electrodynamic balance (EDB) is utilized for recording the evaporation and condensation kinetics of single, levitated saliva droplets with a time resolution of seconds. Efflorescence and deliquescence behaviors of droplets are monitored using light scattering and Mie theory. Compared with pure water droplets, a saliva droplet remains stably levitated for hours when the droplet approaches crystallization having reached a final size during evaporation. The morphology of crystallized particles will be imaged using a scanning electron microscope (SEM). The organic-based phase is expected to shield pathogens from inactivation by forming a solid or semisolid shell hindering the diffusion of solutes. This work highlights the importance of accounting for changes in the micro-environment of aerosols undergoing evaporation and condensation in a realistic environment which is needed to study the viability of airborne viruses and other microorganisms.

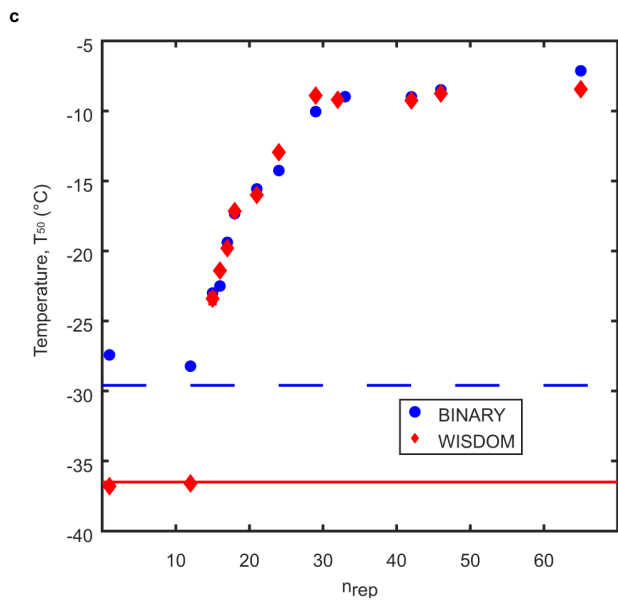
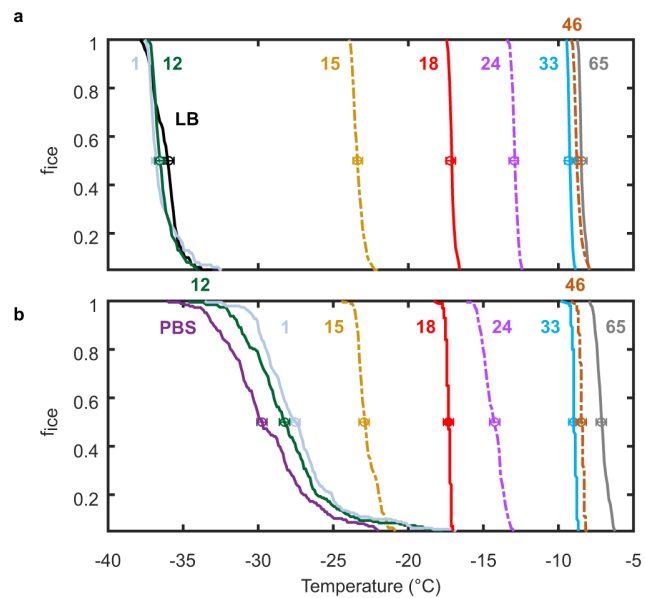
## Water-organizing motif continuity is critical for potent ice nucleation protein activity

**Yinon Rudich**, Thomas Hansen, Jordan Forbes, Akalabya Bissoyi, Lukas Eickhoff, Naama Reicher, Christopher Bon, Virginia K. Walker, Thomas Koop, Ido Braslavsky, Peter L. Davies

Bacterial ice nucleation proteins (INPs) are known as the most effective ice nucleators and can cause frost damage to plants by nucleating ice formation at relatively high temperatures ( $-2\text{ }^{\circ}\text{C}$ ). Airborne INP may affect ice formation in clouds and therefore have an important role in precipitation formation and the hydrological cycle. INPs are thought to self-associate on the outer bacterial membrane, and it was suggested that ice nucleation is probably triggered when many water molecules are organized on one surface in an ice-like manner.

Here we tested the water ordering hypothesis to reveal the mechanism through which INPs trigger ice nucleation. The freezing characterization was carried out by double-blind experiments in both WISDOM (Weizmann Supercooled Droplets Observation on a Microarray (WISDOM) apparatus and BINARY (The Bielefeld Ice Nucleation ARraY). Modeling of *Pseudomonas borealis* INP by AlphaFold predicted that a central domain of 65 tandem 16-residue coils forms a  $\beta$ -solenoid with arrays of outward-pointing threonines and tyrosines, which may organize water molecules into an ice-like pattern. Deletion of sections of the 65 coils found in the INP expressed in *E. coli* Arctic Express have led to a steady loss of ice nucleation activity until a sharper decline in activity was observed at  $\sim 25$  coils and under. Interestingly, mutating the putative water-organizing and dimerization motifs in a subset of these coils decreased the ice nucleation ability even more. Insertion of a bulky domain had the same effect, indicating the importance of the continuity of the water-organizing repeats for the ice nucleation activity. AlphaFold model also suggested that the  $\sim 10$  C-terminal coils have a different function and lack water-organizing motifs, in contrast to the other 55 coils. Deletion of the former eliminated the INP activity.

J. Forbes *et al.*, **Water-organizing motif continuity is critical for potent ice nucleation protein activity**, *Nature Communications*, **13**, 5019 (2022).



22<sup>nd</sup> April 2023 INPs from the field

17:05 – 18:50

# Birch and Pine Forests are Massive Reservoirs of Biological Ice-nucleating Macromolecules

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Silver birch (*Betula pendula*) and Scots pine (*Pinus sylvestris*) are some of the most widespread tree species in the world. They grow in the northern hemisphere and large parts of the boreal forest. Birch trees have been the subject of recent ice nucleation studies and were suggested as an influential emission source of ice-nucleating macromolecules (INMs). The water-soluble INMs were first discovered on various pollen. Later, the same INMs were even found all over the tissue of birch trees. Studies reported that many trees generate INMs as part of a freeze tolerance strategy. If trees release INMs into the atmosphere, forests could be a vast source of INMs.

We collected tissue samples from six Scots pines in urban parks in Vienna, Austria, similar to our previous studies on Silver birches. We investigated the distribution of INMs among three tissue types: bark, branch wood, and needles. INMs from milled and intact tissues were extracted and tested for ice nucleation activity in immersion freezing mode. Additionally, we tested the potential atmospheric release of INMs from the tissue by collecting rainwater directly underneath the trees and measuring its ice nucleation activity.

We found INMs in all samples with freezing onset temperatures between -16°C and -29°C. The bulk samples showed INM concentrations from  $10^5$  to  $10^9$  per mg dry weight active at -25°C and higher. In surface extracts from the intact tissues, the concentrations ranged from  $10^5$  to  $10^8$  INMs per cm<sup>2</sup> of the extracted surface. Most importantly, all rain samples contained INMs with similar freezing onset temperatures to the lab extracts. Additionally, we aimed to quantify the overall INM content per area of *Pinus sylvestris* forest and assumed it is independent of the growing region, as previously reported for birch trees. This allowed us to extrapolate our results to the many locations where Scots pines grow.

Based on our results, we estimate that one square meter of Scots pine stand has the potential to release about  $10^9$  to  $10^{12}$  INMs active at -25°C or higher. Additionally, one square meter of birch stand could release  $10^{13}$  to  $10^{15}$  INMs. Our estimation reveals Scots pines and Silver birches as a massive reservoir of INMs. Therefore, we propose that boreal forests containing large numbers of birch and pine trees must be considered a vast source of atmospheric INMs.

## **The size distribution of ice-nucleating particles in fertile soils from the UK.**

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The ice-nucleating (IN) ability of agricultural soil dust is greater than that of mineral dust due to the presence of ice-active biological materials. However, the distribution of these biological materials across soil dust particles of different sizes is poorly constrained, meaning their atmospheric lifetime is not yet fully understood. For example, if the biological material is mainly associated with the coarse mode particles, its atmospheric lifetime would be shorter than if it is associated with the fine mode particles. This study collected fertile soil samples from the University of Leeds Research Farm, which were air-dried and then aerosolised in a one cubic metre aerosol chamber. The aerosolised sample was size-segregated using an SKC cascade impactor to separate the aerosol into four different size bins. The IN activity of each impactor stage was determined using a droplet freezing assay and normalised by the surface area, as calculated from size distribution measurements. The surface active site density ( $n_s$ ) of the aerosolised soil samples was enhanced at temperatures above  $-15^{\circ}\text{C}$  compared to abiotic mineral dust, indicating the presence of biogenic components within the soil. There was no significant difference in the  $n_s$  over the different size bins, suggesting that the ice-active biological material was evenly distributed throughout the soil dust. This finding is consistent with other evidence which indicates the presence of water-soluble nano-INP in fertile soils which associate with soil dust particles of all sizes when aerosolised.



## **Physicochemical properties of charcoal aerosols derived from biomass pyrolysis affect their ice-nucleating abilities at cirrus and mixed-phase cloud conditions**

**Fabian Mahrt<sup>1,2</sup>, Carolin Rösch<sup>2</sup>, Kunfeng Gao<sup>2</sup>, Christopher H. Dreimol<sup>3</sup>, Maria A. Zawadowicz<sup>4</sup>, and Zamin A. Kanji<sup>1</sup>**

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Aerosol particles play a key role in air pollution and climate. Particles from biomass burning emissions are an important source of ambient aerosols, and are projected to surge in the future as a result of climate and land use changes. Due to the variety of organic fuel materials and combustion types, particles emitted from biomass burning are often complex mixtures of inorganic and organic materials, with soot, ash, and charcoal having been identified as main particle types being emitted. Despite their importance for climate, their ice nucleation activities remain insufficiently understood, in particular for charcoal particles, whose ice nucleation activity has not been reported. Here, we present experiments of the ice nucleation activities of 400 nm size-selected charcoal particles, derived from the pyrolysis of two different biomass fuels, namely a grass charcoal and a wood charcoal.

We find that the pyrolysis-derived charcoal types investigated do not contribute to ice formation via immersion freezing in mixed-phase clouds. However, we find considerable heterogeneous ice nucleation activity of both charcoal types at cirrus temperatures. Inspection of the ice nucleation results together with dynamic vapor sorption measurements indicates that cirrus ice formation proceeds via pore condensation and freezing. We find wood charcoal to be more ice-active than grass charcoal at cirrus temperatures. We attribute this to the enhanced porosity and water uptake capacity of the wood compared to the grass charcoal. In support of the results, we found a positive correlation of the ice nucleation activity of the wood charcoal particles and their chemical composition, specifically the presence of (inorganic) mineral components, based on single-particle mass spectrometry measurements. Even though correlational in nature, our results corroborate recent findings that ice-active minerals could largely govern the aerosol–cloud interactions of particles emitted from biomass burning emissions.

# The Partitioning Processes of Sea Ice Associated Marine Ice Nucleation Particles Impacting the Arctic Clouds

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The Arctic is warming faster than the rest of the world, making it particularly vulnerable to the impacts of climate change. One consequence of this warming is a decline in multiyear sea ice cover, which results in an increasing open-ocean surface with a much lower albedo therefore leading to positive feedback and enhanced warming. Another factor that plays a role in regulating the temperature in the Arctic is the type and extent of cloud cover. Aerosols that can serve as cloud condensation nuclei or ice nucleating particles (INPs) are key for cloud formation. Some microorganisms are known to produce INPs, but it is not well understood which microorganisms are responsible, which environments they inhabit, and how active they are. In this study, we set out to investigate the partitioning of INPs between the Arctic marine and atmospheric environment by combining in situ measurements with laboratory experiments. First, We used a modified ice-finger to grow sea ice using natural samples from West Greenland and found that INPs concentrate into the ice fraction during sea-ice formation, and that these INPs typically are associated with microorganisms. Next, we wanted to understand the temporal and spatial dynamics of INPs in Arctic sea ice. We collected sea ice cores from the Arctic before and during the spring sea ice phytoplankton bloom and analysed them using cold-stage INP measurements, flow-cytometry, and amplicon sequencing. The results showed that there are between  $<10^5 \cdot \text{L}^{-1}$  (at the top) and  $>10^6 \cdot \text{L}^{-1}$  (at the bottom) INP-10 present in the Arctic sea ice. Finally, we wanted to determine the potential contribution of sea ice to the atmospheric INP pool in the Arctic. We introduced natural samples of sea ice from Nuuk into a temperature-controlled sea spray simulation chamber and quantified the microorganisms and INPs present in the bulk water and air before and after aerosolization. The preliminary results suggests that the microorganisms and highly active INPs are efficiently aerosolized into the atmosphere during bubble-bursting where they may contribute to the formation of ice in clouds.

Overall, this study provides new insight into the role of Arctic sea ice as a reservoir for INPs and the microorganisms that produce them, as well as the mechanisms by which INPs are released into the Arctic atmosphere. This information is important for understanding the impact of climate change on the Arctic region and the potential consequences for the rest of the world.

## Poor ability of bioaerosols to act as cloud-condensation nuclei may suppress their ice nucleation potential in mixed-phase clouds

Tina Šantl-Temkiv<sup>1\*</sup>, Miha Živec<sup>2</sup>, Marie Braad Lund<sup>1</sup>, Mojca Benčina<sup>3</sup>, Samo Stanič<sup>2</sup>, in Griša Močnik<sup>4</sup>

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Bioaerosols are receiving increasing attention due to their ability to nucleate cloud ice at high subzero temperatures, therefore influencing cloud formation, properties and lifetime. The capacity of bioaerosols to act as cloud condensation nuclei (CCN) is a prerequisite to induce ice formation through immersion freezing. Nevertheless, the hygroscopicity of bioaerosols *in situ* is poorly understood. We simultaneously collected bioaerosols from the coarse and interstitial fraction of mixed-phase clouds during autumn 2020 from the Otlica observatory (Slovenia). Using a polarization Raman Lidar we established the presence of both ice particles and liquid droplets in most of the cloud events. The samples were analyzed using Aurora and ID7000 spectral flow cytometers, the PINGUIN cold-stage setup and Illumina MiSeq-facilitated amplicon sequencing to assess bacterial (16S rRNA genes) and fungal (the ITS region) communities. We found that concentrations of both bioaerosols and biogenic ice-nucleating particles (bioINP) were significantly higher in the interstitial phase indicating that the majority were poor CCN unable to compete with more hygroscopic particles. Most taxa were not preferentially partitioning in one of the cloud phases indicating that surface properties generally did not play an important role for CCN. We observed an autofluorescent population of large cells, likely affiliating to *Curtobacterium* sp. and *Cystofilobasidium* sp., which were consistently enriched in cloud particles. Their autofluorescence may be associated with the oxidation of flavins induced by oxidative stress resulting from long atmospheric residence times, which may along with their large size enhance their ability to act as CCN. Overall, we found that both bioaerosols and bioINP were poor CCN *in situ*, which could limit their ability to nucleate cloud ice. We suggest that the link between hygroscopicity and ice-nucleating activity of bioaerosols should be systematically investigated in order to understand their *in situ* effects on cloud formation.

23<sup>rd</sup> April 2023 Bioaerosols from the field

09:00 – 11:15

## Analysis of online measured single aerosol particle fluorescence spectra during the AQABA research cruise around the Arabian Peninsula

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### Abstract

The Middle East represents a hot and arid region characterized by diverse environmental conditions ranging from near-pristine and unpolluted but dusty, to strongly polluted due to areas with high population density. Therefore, a study across the region provides a broad range of atmospheric compositions derived from both natural and anthropogenic sources.

The AQABA (Air Quality and Climate Change in the Arabian BASin) campaign took place around the Arabian Peninsula from June 18<sup>th</sup> to September 3<sup>rd</sup> 2017, on the research ship KOMMANDOR IONA. To address biological and non-biological aerosol load and composition, we used a novel online laser/light-induced fluorescence instrument for real-time analysis of individual bioparticles. The Spectral Intensity Bioaerosol Sensor (SIBS, Droplet Measurement Technologies, Longmont, CO, USA) uses two excitation wavelengths at 285 and 370 nm and resolves fluorescence emission into 16 bins, ranging from 302 to 721 nm.

We will report the first results from this pioneering field campaign and focus on the composition and properties of biological and non-biological coarse particles over a wide variety of environmental conditions. Measurement periods include scenarios such as clean marine air, petrochemical pollution, megacity-influenced air masses, and dust from North Africa. Preliminary data indicate that the SIBS was capable of detecting scenario-specific spectral “fingerprints”, providing a certain level of particle discrimination for upcoming cluster analyses.

## **Rainfall effects on vertical profiles of airborne fungi over a mixed land-use context at the Brazilian Atlantic Forest biodiversity hotspot**

Maurício C. Mantoani<sup>a</sup> et al. 2023. *Agricultural and Forest Meteorology*, 331: 109352. doi.org/10.1016/j.agrformet.2023.109352.

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**Abstract:** Whilst fungi are a large fraction of primary biological aerosol particles (PBAPs) and their impact on global climate has been widely recognised, few studies have empirically assessed fungal vertical profiles and diversity relating those with rainfall. Here, we show the results of fungal PBAPs before and after a rainfall event during a fieldwork campaign using a hot-air balloon over a mixed land-use context at the Brazilian Atlantic Forest biodiversity hotspot. Four flights of *c.* 1 hour each were performed in the early morning from 8th until 11th of March 2022, and data were collected at three sampling heights (0, 150 and 300 m). Rainfall estimation using IMERG data indicated the precipitation event was of 15–20 mm and ERA5/ECMWF data highlighted that most of the airborne samples were taken above the boundary layer height. After the rainfall, the concentration of fungal spores at the ground level remained unchanged, whereas it was reduced to between 2- and 2.5-fold for the 150 and the 300 m heights, respectively. This was also accompanied by a reduction in the number of Pink-CFU, indicating a major drop in fungal PBAPs at higher altitudes associated with the rain. In addition, total spore concentration indicated *Cladosporium* sp. as dominant at all sampling heights, accounting for more than 80% of all spores, whereas *Aspergillus*/*Penicillium*-like represented less than 20%. Our results show the effects of rainfall and altitude on the concentration of fungal PBAPs, indicating how wet removal impacts fungi vertical profiles which has knock-on-effects on cloud and precipitation formation.

**Keywords:** *Aspergillus*/*Penicillium*-like; *Cladosporium*; Cloud formation; Ice nucleation activity; PBAP.

## **Biological Aerosol Particles in the Finnish Sub-Arctic during the PaCE22 campaign**

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This study investigates primary biological aerosol particles (PBAPs) at the Pallas supersite, a pristine site in Finnish Lapland 170 km north of the Arctic circle. We use a Wideband Integrating Bioaerosol Sensor (WIBS 5/NEO – Droplet Measurement Technologies, Longmont, USA) to measure the concentration and size distribution of fluorescent aerosol particles (FAPs). The WIBS detects and sizes particles from 0.5 to 30  $\mu\text{m}$  and measures the autofluorescence of single particles using two excitation wavelengths (280 nm and 370 nm) and two emission bands (310 – 400 nm and 420 – 650 nm), resulting in three fluorescence channels which can further be combined to define 7 types of fluorescent particles. ABC particles for example show fluorescence in all three channels and are therefore a subset of all FAPs (fluorescence detected in at least one channel).

During the campaign (Mid-September to Mid-December), the average total FAP concentration is about  $0.05 \text{ cm}^{-3}$ , a contribution of 11 % to the total aerosol concentration measured with the WIBS. The fraction of FAPs increases with particles size and in the coarse fraction approximately 50 % of all particles fluoresce. From October 24<sup>th</sup> the surrounding land is covered in snow. Snow coverage has a strong influence on ABC-particles: The average concentration of ABC-particles drops from  $0.01 \text{ cm}^{-3}$  during snow-free time to  $0.002 \text{ cm}^{-3}$  during snow coverage, suggesting that most ABC-particles are emitted from local vegetation. FAPs do not follow this trend, as the concentration increases again in December, in a similar manner to the concentration of all particles.



## **Representation of bioaerosols in chemistry transport models**

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Bioaerosols of different types (e.g., pollen, bacteria, marine organic aerosol) are implemented in atmospheric chemistry transport models in increasing complexity since many years. Typical model applications with respect to bioaerosols comprise the atmospheric dispersal of these aerosol particles, e.g., pollen, but also secondary effects such as their impact on cloud formation or chemical aerosol processing.

However, in contrast to other rather well-known aerosol types, modelling of bioaerosols so far relies on partly vague assumptions for many processes and properties throughout their atmospheric lifetime. One very crucial example are emissions of bioaerosols, which so far insufficiently can account for the diversity of different bioaerosol types. Similar to emission estimates, also available INP-parameterizations do not cover the full complexity and diversity of bioaerosol types.

The aim of the talk is to foster the exchange between observationalists and modellers. Therefore, it is supposed to give a brief overview on the state-of-the-art treatment of bioaerosols in atmospheric chemistry transport models. Emphasis will be put on emission schemes for different bioaerosol types (e.g., land-based and marine aerosols) as well as using the modelled bioaerosol concentrations to derive CCN and INP concentrations as well as effects on clouds. Overall, the presentation will highlight the needs for adequately studying bioaerosols and their impacts from a modelling point of view.

## Bioaerosols in Northern Alaska are Highly Influenced by Ocean and Terrestrial Sources

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Arctic air temperatures are rising up to four times the global rate, rapidly thawing elements of the cryosphere (Rantanen et al. 2022) such as permafrost (*i.e.*, continuously frozen ground for at least two years) and ice wedges (*i.e.*, frozen water accumulated in ground cracks). Thawed permafrost can be introduced into water systems (Matheus Carnevali et al. 2015) by thermokarst lake (TKL) formation and the increasingly common permafrost landslides. Ultimately, these terrestrial elements can be released to the atmosphere through mechanisms such as TKL formation, TKL greenhouse gas bubble-bursting, and bubble-bursting from wind-induced wave action on lakes, lagoons, and the open ocean. Once airborne, terrestrial biological particles can be a source of ice nucleating particles (INPs) active at warm temperatures ( $\geq -10^{\circ}\text{C}$ ), having the potential to alter cloud properties, the radiation budget, and climate in the region. Terrestrial particles from permafrost are known to be highly enriched with INPs compared to other soils (Creamean et al. 2020), highlighting the importance of understanding bioaerosol sources in the region. Arctic mixed-phase clouds (AMPCs) are highly sensitive to the quantity and characteristics of INPs, and hence, INP sources.

Here, we present 16S rRNA gene and INP spectra results from a broad range of samples collected during the ARctic Study of Permafrost Ice Nucleation (ARCSPIN) field campaign in the Utqiagvik region (Alaska, USA) in September 2021. These include air and potential local bioaerosol sources, such as water bodies (*i.e.*, ocean, lagoon, and thermokarst lake), terrestrial (*i.e.*, active layer, permafrost, ice wedge, and sediment), and vegetation samples. Amplicon sequencing beta diversity results show microbial taxa overlap between air and the sample types listed above, suggesting that part of these bioaerosol populations have potential local sources in the region. In addition, microbial source tracking (Sourcetracker2) of bioaerosols collected near different water bodies shows that bioaerosols in Northern Alaska are strongly influenced by seawater and terrestrial elements.

Moreover, air samples collected downwind of thermokarst lakes show these water bodies as a source of bioaerosols, demonstrating the microbial release of particles from these lakes to the atmospheric boundary layer. This is additionally confirmed with INP spectra of upwind and downwind lake air, suggesting that these particular water bodies that cover this Alaskan region have the ability to enrich the atmospheric boundary layer with INPs.

## **Dust particles as a protective environment for bacteria long range transport**

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Airborne microorganisms (bioaerosols), traveling across continents and oceans, can significantly affect aquatic and terrestrial ecology, biogeochemical cycles, and human health. Dust events are a major source of bioaerosols, contributing to their global dispersion. Due to climate change-driven desertification and land-use changes, these events are increasing in intensity and frequency. Long-range transport of microorganisms over dust is therefore expected to become more prominent. Hence, it is essential to understand the role of the dust particle in providing suitable conditions for dust-borne microorganisms, to survive in the atmosphere. Here we will present findings on the impact of dust chemical composition and meteorological conditions on the microbial community composition and bioactivity. Our genomic sequencing results show a distinct difference between day and night dust-borne community composition, with higher richness during nighttime. In addition, we have isolated higher amounts of bacterial and fungal isolates from night-sampled dust, compared to the day-samples. This might relate to the higher relative humidity during night sampling, which allow higher survival rates. We have also found diverse group of spore-forming bacteria such as *Bacillus*, which were isolated from all dust events, indicating their beneficial survival compared to other taxonomic groups. There is a need in further exploration of biofilm formation and survival-related genes in order to understand dust-borne bacterial survival mechanisms.

23<sup>rd</sup> April 2023 Marine Environment

11:15 – 13:15

# Linking Sea Spray, Bioaerosols and Ice-Nucleation Proteins in Arctic Marine Environments

*Christian DF Castenschiold*<sup>1,2,3\*</sup>, *Claudia Mignani*<sup>4</sup>, *Sigurd Christiansen*<sup>6</sup>, *Malin Alsved*<sup>5</sup>, *Sylvie Tesson*<sup>1,7</sup>, *Jakob Löndahl*<sup>5</sup>, *Merete Bilde*<sup>6</sup>, *Luisa Ickes*<sup>8</sup>, *Thomas Bataillon*<sup>9</sup>, *Kai Finster*<sup>1,10</sup> and *Tina Šantl-Temkiv*<sup>1,2,3,10</sup>

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Clouds have one of the most profound effects on Earth's climate, yet they are still responsible for some of the biggest uncertainties in climate models. Cloud formation, radiative properties, thickness and lifetime are tightly interlinked with the presence of atmospheric particles (aerosols) and the formation of ice. Biological aerosols (bioaerosols) such as ice-nucleation proteins (INpro) produced by microorganisms are most efficient catalysts in the formation of ice and can trigger heterogenous freezing between -1°C and -15°C. Several studies have demonstrated that Arctic environments are a source of airborne INpro. Sea spray is one of the major sources of aerosols, which aside of the sea salt contain large amounts of organic material. These are ejected into the atmosphere through the process of wave breaking and bubble bursting of small bubbles, which eject drops from the sea surface microlayer (SML) to the atmosphere. Here, we present results derived from droplet freezing assays and amplicon sequencing combined with quantitative PCR, targeting the 16S and 18S rRNA genes from sea and aerosol samples collected along a transect from sub- to high Arctic Greenland (Baffin Bay). We demonstrate a positive correlation between INpro concentration and higher latitudes in sea bulk water (SBW). Additionally, we try to link specific taxonomic groups from the microbial communities to INpro production. Last, we aim to investigate if partitioning of specific taxonomic groups can be observed from SBW to SML and from SML to the atmosphere. Finally, we performed laboratorial sea-spray experiments simulating turbulent sea conditions. This study has the potential to help closing the current knowledge gap in understanding the partitioning of microorganisms from the sea to the atmosphere and unravel which microbes are the major contributors to atmospheric INpro and hence cloud formation.

## **Airborne algicidal bacteria may control phytoplankton cell fate in the oceanic blooms**

Authors:

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Ocean microbes perform key functions in biogeochemical cycles triggering nutrient cycling. Recent studies indicated diverse modes of algal-bacterial interactions, including mutualism and pathogenicity. However, the mechanism of bacterial dispersal in the marine ecosystem remained largely underexplored. Here we isolated an airborne algicidal bacterium, *Roseovarius nubinhibens*, emitted to the atmosphere as primary marine aerosol and collected above a coccolithophore bloom in the North Atlantic Ocean. The aerosolized bacteria remained infective and induced lysis of *Emiliana huxleyi* cultures. This suggests that the atmospheric transport of marine bacteria can serve as an effective mechanism of aerial spread of infection over large oceanic regions, signifying its ecological role in regulating cell fate in algal blooms.

## **Spatial Variability and Composition of Ice Nucleating Particles over the Southern Ocean**

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Supercooled liquid clouds are ubiquitous over the Southern Ocean (SO), even down to temperatures of  $-25\text{ }^{\circ}\text{C}$ , and comprise a large fraction of the marine boundary layer clouds. Many Earth System Models and reanalysis products overestimate the occurrence of ice and have insufficient liquid cloud cover in the region, while recent simulations have found that the microphysical representation of ice nucleation and growth has a large impact on these properties. However, measurements of SO ice nucleating particles (INPs) to validate simulated ice nucleation are sparse, and many previous observations were limited to fairly warm temperatures ( $-15$  or  $-20\text{ }^{\circ}\text{C}$ ). Observations of INPs are presented here from two simultaneous field campaigns in the SO during January-March 2018: the Clouds, Aerosols, Precipitation Radiation and atmospheric Composition Over the southern ocean II (CAPRICORN-2) study on the CSIRO R/V Investigator, and the Southern Ocean Cloud Radiation Aerosol Transport Experimental Study (SOCRATES) on the NSF/NCAR G-V aircraft. The SOCRATES campaign is noteworthy for collecting the first in-situ observations in and above cloud in the SO. Measurements of INPs active in the immersion freezing mode were made during both projects in real time with Colorado State University (CSU) Continuous Flow Diffusion Chambers (CFDCs) at temperatures below  $-25\text{ }^{\circ}\text{C}$ , and via offline analyses of aerosol filter and seawater samples using the CSU Ice Spectrometers from  $-10$  to  $-30\text{ }^{\circ}\text{C}$ . INP concentrations were at the lower bound of those from other ocean regions, and much lower than historical measurements in the SO collected prior to the early 1970s. Chemical treatments performed on the filter suspensions were used to infer the fraction of biological, organic, and mineral INPs, which varies with latitude and height, and indicate a variety of sources, including local marine aerosol and dust. Data from G-V overflights of the R/V Investigator were used to investigate the vertical structure of INPs in this region. Electron microscopy analyses of INPs collected from the CFDCs, along with back trajectories and aerosol measurements, provide additional information on INP composition and possible sources.

# **Pronounced Biogenic Sources of Ice Nucleating Particles (INPs) over the Eurasian Arctic Ocean**

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The Arctic region is sensitive to climate change, experiencing an accelerated warming. Cloud radiative properties and related feedback mechanisms on Arctic climate are highly uncertain and dependent on the cloud phase. INPs initiate ice formation in the prevalent Arctic mixed-phase clouds, thereby influencing their persistence and radiative properties. So far, little is known regarding the abundance, variability, and potential sources of INPs in the Arctic owing to the scarcity of observational data, particularly over the remote Arctic Ocean. We study the INP-cloud interactions by improving the knowledge of the abundance and sources of INPs in this region.

We present results from a cruise-based Arctic Century Expedition, which took place from 5 August to 6 September 2021 over the previously unexplored Kara and Laptev Sea in the Eurasian Arctic. Atmospheric INP concentrations and their spatiotemporal variabilities will be presented. Geographical variability of INPs along the ship track are also investigated to assess the influence of possible air masses from different origins, e.g., sea ice, marine or land. Additionally, the associated aerosol physicochemical properties, including particle size distribution, heat lability and biochemical composition will be analyzed with case studies for INP source apportionment. Ultimately, we will report the results from the in-situ sea spray aerosol generation experiments to reveal the phase partitioning of INPs at the sea-air interface, highlighting the importance of the aerosolization mechanisms to the production of marine INPs. To summarize, our results highlight that the marine biogenic INPs, likely originating from the facilitated phytoplankton productivity, dominate the INP populations at warm freezing temperatures ( $T > -15$  °C) over the Arctic Ocean.