https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

Similar freezing spectra of particles on plant canopies as in air at a high-altitude site

Annika Einbock[1] and Franz Conen[1]

[1]Department of Environmental Sciences, University of Basel, 4056 Basel, Switzerland

Correspondence to: Annika Einbock (annika.einbock@unibas.ch)

Abstract. Plant canopies are an important source of biological particles aerosolized into the atmosphere. Certain aerosolized microorganisms are able to efficiently freeze slightly supercooled cloud droplets and therefore affect mixed-phase cloud development. Still, spatiotemporal variability of such biological ice nucleating particles (INPs) is currently poorly understood. Here, we study this variability between late summer and leaf shedding on the scale of individual leaves collected about fortnightly from four temperate broadleaf tree species (*Fagus sylvatica*, *Juglans regia*, *Prunus avium* and *Tilia platyphyllos*) on a hillside (Gempen, 650 m a.s.l.) and in a vertical canopy profile of one *Fagus sylvatica* (Hölstein, 550 m a.s.l.) in north-western Switzerland. The cumulative concentration of INPs active at ≥ -10 °C (INP_{-10}) did not vary significantly between the investigated tree species, but as inferred from leaf mass per area and leaf carbon isotopic ratios seemed to be lower on more exposed leaves. Between August and mid-November, median INP_{-10} concentration increased from 4 INPs cm^{-2} leaf area to 38 cm^{-2} leaf area and was positively correlated with mean relative humidity throughout 24 h prior to sampling (Spearman's $r = 0.52$, $p < 0.0001$, $n = 64$). In 53 of the in total 64 samples collected at Gempen, differential INP spectra between -3 °C and -10 °C exhibited clearly discriminable patterns: in 53% of the spectra, the amount of additionally activated INPs increased persistently with each 1 °C decrease in temperature; the remaining spectra displayed significant peaks in differential INP concentration above -9 °C, most frequently in the temperature interval between -8 °C and -9 °C (21%), and between -7 °C and -8 °C (17%). Interestingly, the three most frequent patterns in differential INP spectra on leaves in Gempen were also prevalent in similar fractions in air samples with clearly discriminable patterns at the high-altitude site Jungfraujoch (3580 m a.s.l., Switzerland) collected during summer in the previous year. These findings corroborate the idea that a large fraction of the airborne biological INP population above the Alps during summer originates from plant surfaces. The inquiry into which parameter, or set of parameters, could affect biological INP populations on both scales – upwind airsheds of high-altitude sites as well as individual leaves – is an intriguing question for further exploration. A first guess is that leaf wetness duration plays a role.

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

1 Introduction

Processes at the Earth's surface and in the atmosphere are interrelated. Ice nucleating particles (INPs) emitted from the Earth's surface, for example, can initiate freezing in clouds at temperatures exceeding ~ -38 °C where in their absence droplets remain liquid (Kanji et al., 2017; Murray et al., 2012). The impact on cloud physics critically depends on both, total INP concentration and population properties such as individual freezing temperatures of the present INPs (Hawker et al., 2021). The dynamics of biological INPs at mixed-phase cloud height are poorly understood (Burrows et al., 2022; Cornwell et al., 2023). This is partly due to the large variety and heterogeneity of the involved particles as well as spatiotemporal variations in source activities and drivers thereof. (Burrows et al., 2022; Cornwell et al., 2023).

Soil organic matter (Conen et al., 2011; Hill et al., 2016; O'Sullivan et al., 2014) and living as well as decaying vegetation (Lindemann et al., 1982; Lindow et al., 1978a; Schnell and Vali, 1976) are major sources of biological INPs. Ice nucleating active (INA) microorganisms inhabiting these environments are among the most efficient INPs discovered so far and active at temperatures ≥ -10 °C (INP_{-10}) (Huang et al., 2021). On a global scale, considering land cover (Latham et al., 2014) and leaf area (Vorholt, 2012), plant surfaces provide a giant reservoir of INP_{-10} leaking into the atmosphere. Indication for the large contribution of INA microorganisms emitted from plant canopies to the INP_{-10} population at cloud height above western Europe was revealed through heat treatment of INPs at the High Altitude Research Station Jungfraujoch (JFJ, 46° 32' 53'' N, 07° 59' 02'' E, 3580 m a.s.l.) in Swiss Alps (Conen et al., 2022). During summer and early autumn, admixture of air from the planetary boundary layer at JFJ is enhanced (Griffiths et al., 2014) and most soils in the surrounding temperate region are covered by plants. Under these conditions, the phyllosphere – plant surfaces in contact with the atmosphere (Vorholt, 2012) - in the airshed upwind presumably contribute the majority of INP_{-10} observed at JFJ.

Ice nucleation active microorganisms associated with the phyllosphere include various gram-negative bacteria (Kim et al., 1987; Lindow et al., 1978b; Maki et al., 1974) and fungal species (Morris et al., 2013; Pouleur et al., 1992). The ability of gram-negative bacteria to nucleate ice is rooted in the expression of IN proteins that can aggregate into assemblies of varying sizes. The largest protein clusters make freezing close to 0 °C possible (Govindarajan and Lindow, 1988; Qiu et al., 2019). Recently, such clustering was also discovered in cell-free INPs shed by an ice-nucleation active (INA) fungus (Schwidetzky et al., 2023). Microbial ice-nucleation activity differs between species (Huang et al., 2021) and strains (O'Brien and Lindow, 1988; Yang et al., 2022; Yankofsky et al., 1981) and may allow the most efficient strains to draw water vapour from the atmosphere into zones of a leaf surface that would otherwise remain dry (Einbock and Conen, 2024). In addition, variations in microbial growth and environmental conditions can substantially change freezing characteristics of microbial INA populations (Hirano and Upper, 1989; Lindow et al., 1982; Nemecek-Marshall et al., 1993; O'Brien and Lindow, 1988; Richard et al., 1996; Ruggles et al., 1993; Yang et al., 2022). These variations in expression of ice-nucleation activity, the number of known and perhaps unknown INA species, together with uncertainties concerning their cultivability, make it challenging to predict atmospheric INP dynamics from the assessment of microbial community compositions on leaf surfaces. A complementary approach to understand the link between INPs in phyllosphere and atmosphere is to compare differential INP spectra in both

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

spheres. Here, we investigate INP spectra on leaves of four deciduous tree species from late summer throughout leaf senescence. We discuss trends in cumulative INP_{-10} concentrations and patterns in differential INP spectra. Finally, we compare differential INP spectra on foliage with observations of INP spectra during summer 2022 in air at JFJ.

2 Methods

2.1 Foliage sampling

We sampled leaves of four broadleaf tree species commonly found in temperate forests near Gempen (GEP, 47° 28' 53'' N, 7° 39' 30'' E, ~650 m a.s.l.) and Hölstein (HOL, 47° 26' 17'' N, 7° 46' 37'' E, ~550 m a.s.l.), Switzerland. Both sampling locations are situated on hillsides in the northern Jura mountains at a linear distance of about 10 km from each other. Sampling was conducted between early August and mid-November, 2023. Samples from Gempen were collected approximately fortnightly on eight occasions. In Hölstein, we collected foliage twice, with five weeks in-between (05.09. and 11.10.). Samples were collected between 09.30 am and 11.30 am local time, stored at 5 °C, and analysed for their INP concentration within three to four days.

In Gempen, we sampled leaves of two *Tilia platyphyllos*, *Fagus sylvatica* and *Juglans regia*, respectively, along a 1.5 km path through a small forest surrounded by agricultural land. Additionally, two *Prunus avium* were sampled around 50 m into an adjacent meadow. We sampled trees least overgrown by taller canopies of other species at more open parts of the path and aimed for leaves least likely affected by throughfall from canopies above. Form September 26 onwards, roughly the onset of leaf coloration, the same trees were sampled on successive occasions. The first three sampling occasions, various *T. platyphyllos* (TP1, TP2), *F. sylvatica* (FS2) and *J. regia* (JR2) had been sampled in the same segments of the path as from September 26 onwards. Foliage was cut off trees about 1.5 m to 2.5 m above ground using a pair of flame-sterilized scissors and directly transferred into polyethylene zip bags without further contact. In Hölstein, covering the entire vertical extent of the canopy, foliage of a *F. sylvatica* was collected at about 10 m, 20 m and 30 m above ground. Leaves were accessed from the Swiss Canopy Crane II (Kahmen et al., 2022) and sampled from the outermost canopy parts. At each height, a sample was taken from the south and the north-facing side.

Meteorological data was obtained from the nearest station operated by the Swiss Federal Office of Meteorology and Climatology (Binningen, 47° 32' N, 7° 35' E, 316 m a.s.l.) 10 km NE of Gempen and the Swiss Federal Institute for Forest, Snow and Landscape Research (Hölstein, 47° 26' N, 7° 47' E, 530 m a.s.l.). Due to the difference in altitude between Binningen and Gempen, temperature was corrected by 0.65 K/ 100 m.

2.1.1 Leaf colour

Leaf colour was determined right after sampling by visually matching adaxial leaf sides to one of 2050 reference colours (NCS Index 2050) provided by the Natural Colour System (NSC). The NCS is a three-dimensional colour model based on human colour perception (Hård and Sivik, 1981) and has been used to assess vegetation colour in the past (Grose, 2014, 2016; Shen

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

et al., 2022; Xing et al., 2019). Briefly, blue, red, yellow and green are considered elementary hues that are represented on a colour circle. Two neighboring elementary hues are mixed in varying proportions to describe the hue of a colour of interest. In addition to a specific hue, each colour is assigned a nuance which depends on its black-/whiteness and chromaticness (Hård and Sivik, 1981).

Colours were assessed on sampling days around noon beneath an east-facing window. All leaves comprising a sample were considered collectively and the main leaf colour identified as colour covering the largest leaf area per sample. Colour determination was performed by one, or two independent observers. Samples of approximately 100 cm^2 leaf area each (81.0 cm^2 to 142.7 cm^2) corresponding to between 1 and 19 leaves were stored in 50 mL polypropylene tubes (Cellstar®, greiner bio-one, Switzerland) at 5 °C until INP analysis. For statistical analysis, NCS hue labels were converted to a length value as described by Xing et al. (2019). For visualization in Fig. 3, Fig S1 and Fig S2, NCS values were converted to HEX colour code using an online tool (https://www.w3schools.com/colors/colors_converter.asp).

2.1.2 INP analysis

Ice nucleating particle concentrations were quantified in leaf washing water. Immediately before each freezing test, the tubes containing the leaf samples were filled up with 50 mL of 0.1% NaCl in Milli-Q® water, sonicated for 1 min (RK 100, Bandelin, Germany) and shaken manually for another 20 s. Leaves were removed and pressed between sheets of thick cellulose paper (Clairefontaine goldline, 300 gm^{-2}, the Netherlands) until further processing. A total of 28.8 mL of leaf washing water was transferred to two subsets of 72 Safe-Lock tubes (Eppendorf, 0.5 mL, Germany), each containing 200 µL of sample. The two subsets were cooled in parallel in two separate cold baths (Lauda RC6, Lauda-Königshofen, Germany) from -3 °C to -10 °C. After every 1 °C step in cooling (rate 0.3 °C min^{-1}), temperature was left unchanged for at least 30 s before the number of frozen droplets was determined visually by one, or two independent observers. When a large fraction of droplets had frozen at -10 °C, additional 1:10 and 1:100 dilutions of the leaf washing water were analysed. Where dilution series overlapped, we selected the data points from the dilution in which the fraction of frozen droplets was closest to 50%. In cases were the pattern of a differential INP spectrum would have changed solely because of a switch between dilution series, this switch was implemented at the next possible temperature interval. Blanks consisting of the utilized 0.1% NaCl Milli-Q® water did not freeze within the investigated temperature range. Differential INP concentrations were quantified according to Eq. 8 in Vali (2019) and cumulative INP concentration were calculated as the sum of differential INP concentrations (Vali, 1971, 2019).

2.1.3 Leaf mass per area, carbon to nitrogen ratio and $\delta^{13}C$ values

Samples were dried collectively at 40 °C until their weight had stabilized. Leaf mass per area (LMA) was calculated as leaf dry weight divided by leaf area as determined with the software WinDIAS 3 (Version 3.3.0.39, Delta-T Devices, United Kingdom). Leaf carbon (C) and nitrogen (N) concentration as well as stable carbon isotope ratio ($\delta^{13}C$, relative to Vienna Peedee Belemnite (VPDB)) was analysed on an elemental analyzer coupled with an isotope ratio mass spectrometer (EA-IRMS, Integra2, Sercon, Crewe, United Kingdom). For that purpose, a number of randomly selected punches (2 mm diameter)

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

were taken from each dried sample and weighed into a tin capsule (0.72 mg to 1.68 mg per sample). For isotopic and size calibration EDTA (lab standard), USGS61, USGS62 and a specific Glycine (Schimmelmann Glycine No. 4) were used. Carbon to nitrogen ratios (C:N ratios) are shown as atomic ratios. According to the analyszed standard substances, the standard deviation for $\delta^{13}C$ is < 0.3‰.

2.2 Aerosol sampling and analysis

In a preceding study at JFJ we had collected on 23 days, between the beginning of July and mid-August 2022, a total of 133 aerosol samples (Einbock, 2023). The observatory JFJ is located at a 3 km higher elevation about 110 km SSE of both foliage sampling sites. About one third of Switzerland is covered by temperate forest and another third is agricultural land (Beyeler et al., 2021). Sampling at JFJ was conducted with a high-flow rate impinger (flow rate 300 L min^{-1}, Coriolis u, Bertin Technologies, France). Ambient aerosol particles were collected into 15 mL of ultrapure water (W4502-1L, Sigma-Aldrich) containing 0.5% NaCl to reduce osmotically induced stress in biological cells (Stopelli et al., 2014). Samples were usually collected throughout 30-min periods consisting of five consecutive 5-min intervals (129 samples). For the remaining four samples, the impinger was operated at three consecutive 5-min intervals. Water lost by evaporation during impinger operation was replenished between the 5-min sampling intervals with NaCl-free ultrapure water. Samples were analysed with the automatic freezing detection apparatus LINDA (Stopelli et al., 2014) immediately after collection in 52 tubes (0.5 mL Eppendorf Safe-Lock, Germany), each containing 200 µL of impinger liquid. All analysed blanks of NaCl-containing and NaCl-free ultrapure water did not contain INPs active above -12 °C. As for the foliage samples, differential INP concentrations were calculated based on Eq. 8 in Vali (2019) and cumulative INP concentrations displayed as sum of differential INP concentrations (Vali, 1971, 2019). Sodium analysis of impinger liquid (940 Professional IC Vario, Metrohm, Switzerland) revealed that its concentration decreased to 70% of its initial concentration during the 25 min the impinger was operated. Therefore, we corrected INP concentrations for potential INP loss with droplets exiting the impinger during collection by multiplying uncorrected INP concentrations with a factor of $\frac{2}{1+0.7}$.

2.3 Statistically significant peaks in differential INP spectra

In immersion freezing experiments, it can be presumed that the number of INPs per droplet activated within each investigated temperature interval is Poisson distributed (Vali, 1971, 2019). Accordingly, the standard deviation for the number of INPs activated within each temperature step equals the square root of the number of freezing events in the assay within this temperature interval (Vali, 2019). We consider a temperature interval (ΔT) in a differential INP spectrum to constitute a significant peak, if the sum of the standard deviations for the number of freezing events in ΔT and $\Delta(T+1)$ is smaller than the difference in the number of freezing events between ΔT and $\Delta(T + 1)$, where $\Delta(T + 1)$ is the next colder temperature interval to ΔT.

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

3 Results and Discussion

3.1 Cumulative INP concentrations

3.1.1 Temporal trends in INP_{-10} concentrations, meteorological parameters and leaf traits

We found a total of 273'295 INP_{-10} on 8'304 cm^2 of leaf area (LA) and 733 INP_{-10} in 968 m^3 of air (local conditions). From the beginning of August until mid-November, median INP_{-10} concentration on leaves collected at GEP increased from 4 cm^{-2} to 38 cm^{-2} LA. Mean relative humidity (RH) throughout the 24 h prior to sampling increased from around 60% to 82% with a transient maximum of 73% on September 26. Mean air temperature throughout the 24 h prior to sampling decreased from about 20 °C in August to approximately 10 °C in mid-October (Fig. 1). Cumulative INP_{-10} concentrations correlated significantly with mean RH (Spearman's $r = 0.52$, $p < 0.0001$, $n = 64$) and mean air temperature (Spearman's $r = -0.34$, $p = 0.006$, $n = 64$), respectively. The correlation between INP_{-10} concentration and mean RH persisted when considering the tree species separately, except for *F. sylvatica*. On the level of individual tree species, the negative correlation between INP_{-10} concentration and mean air temperature was only significant in *P. avium*.

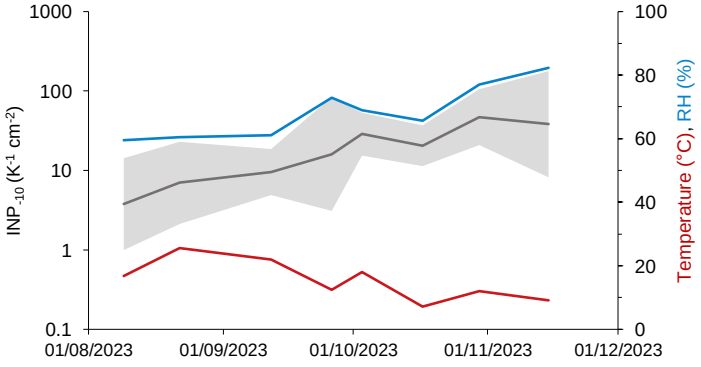


Figure 1: Temporal development of the median INP_{-10} concentration on leaves collected in Gempen (grey line), relative humidity (blue) and air temperature (red). The multiplicative standard deviation (Limpert et al., 2001) of the INP_{-10} concentration is indicated as shaded area.

The activation temperature (≥ -10 °C) of the investigated INPs suggests they are predominantly of biological origin (Huang et al., 2021; Kanji et al., 2017). The analysed sample type (leaf washing water of broadleaf trees) further renders a considerable contribution of INA microorganisms to the investigated INP population likely (Hill et al., 2014; Lindow et al., 1978a). Potential additional sources of INP_{-10} at the sampling locations might include IN molecules derived from pollen (Gute and Abbatt, 2020; Kinney et al., 2024). However, a significant contribution of INA pollen during the studied seasons is unlikely.

Generally, the cumulative INP_{-10} concentration in the bacterial population on foliage is affected by both, population size and the frequency of INPs among cells (nucleation frequency NF) (Lindow et al., 1982). Temperature (Hirano and Upper, 1989; Nemecek-Marshall et al., 1993; Ruggles et al., 1993), RH (Hirano and Upper, 1989; Leben, 1988), nutrient availability (Nemecek-Marshall et al., 1993; Ruggles et al., 1993) and plant genotype (Lindow et al., 1978a; O'Brien and Lindow, 1988), among other factors, have been found to influence population size and NF of epiphytic bacteria, sometimes in an opposing

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

manner. Culture age and variations in growing conditions were further found to influence the ice nucleation activity of different *Fusarium* species (Richard et al., 1996; Yang et al., 2022).

In this study, decreasing air temperatures between late summer and autumn might have triggered an enhanced expression of IN proteins in microbes (Anderson et al., 1982; Hirano and Upper, 1989; Nemecek-Marshall et al., 1993), possibly contributing to the observed increase in INP_{-10} concentrations. The significant correlation between air temperature and INP_{-10} concentration in *P. avium* and the absence of such a correlation in the other species might have been related to the different exposure of the trees. Both *P. avium* were free standing in a meadow and less shielded by forest against radiative cooling compared to the other sampled trees. The *P. avium* trees might have experienced lower leaf temperatures at night, pronouncing the effect of temperature on the observed INP_{-10} concentrations.

Moisture promotes the survival and growth of epiphytic microorganisms (Beattie and Lindow, 1995; Grinberg et al., 2019), affects their spatial distribution within the leaf microhabitat (Doan et al., 2020) and possibly influences the composition of the phyllosphere microbiome (Beattie, 2011). Intense rain events were found to trigger an increase in the population size of the bacterial INA strain *Pseudomonas syringae* pv. syringae on snap bean leaflets (Hirano et al., 1996). Further, high RH fosters the abundance of *Pseudomonas syringae* in the phyllosphere (Leben, 1988) and seems to enhance ice nucleation activity on foliage (Hirano and Upper, 1989). The strong correlation between RH and INP_{-10} concentration observed here indicates an effect of RH on either the abundance of INA microorganisms on foliage, their NF or both, even though relationships cannot fully be disentangled due to the co-occurrence of continuous trends in INP_{-10} concentrations and meteorological parameters. Increasing INP supply in the phyllosphere at elevated RH might, besides differences in emission mechanisms, contribute to enhanced concentration of airborne INPs observed under high RH conditions (Testa et al., 2021; Wright et al., 2014).

On the seasonal scale, changes in climate but also leaf characteristics and plant defence during senescence have been associated with shifts in phyllosphere microbial communities in various ecosystems (Kinkel, 1997; Šigutová et al., 2023; Stone and Jackson, 2021). During senescence, leaf processes change and the nutrient content in foliage decreases considerably (Lim et al., 2007). Here, leaf coloration was mirrored in a distinct shift to larger C:N ratios and a smaller amount of N per leaf dry weight in all sampled trees (Fig. S1). Significant correlations between INP_{-10} concentration and leaf colour NCS code expressed as length value (section 2.1.1) (Spearman's $r = 0.29$, $p = 0.02$, $n = 64$), C:N ratio (Spearman's $r = 0.39$, $p = 0.002$, $n = 64$) and amount of N per leaf dry weight (Spearman's $r = -0.41$, $p = 0.0008$, $n = 64$) were found for the entire GEP data set, but were absent when green and coloured leaves were assessed separately ($p > 0.05$, $n = 38$ and $n = 26$, respectively).

3.1.2 Differences within and between trees

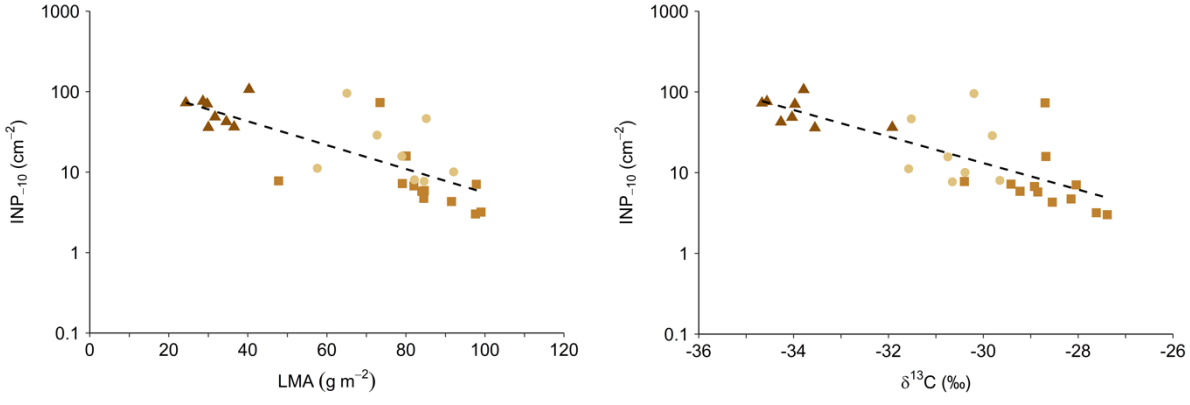
Cumulative INP_{-10} concentrations did not vary significantly between the investigated tree species. Rather, concentrations differed between some individual trees, e.g. between the two *F. sylvatica* and the two *T. platyphyllos* sampled in GEP (Fig S2).

In the vertically sampled *F. sylvatica* (HOL), INP_{-10} concentrations increased from the top (median = 4.0 INP_{-10} cm^{-2} */ 1.5 (multiplicative standard deviation s* (Limpert et al., 2001), n = 4) to the lowest part of the canopy. On the first sampling day,

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

INP_{-10} concentrations were highest in the sample from the lowest position within the canopy and facing a SE direction, with concentrations 10 times (73.1 INP_{-10} cm^{-2}) as high as at the opposite side of the canopy on the same height. Five weeks later, INP concentrations at this position had decreased by a factor of four but were still twice as high as at the opposite canopy side. These differences might be attributed to variations in leaf microclimate or localized plant reactions. As expected from previous findings (Bachofen et al., 2020; Matyssek et al., 2010), LMA was greater at the tree top (94.7 g m^{-2} ± 6.8 g m^{-2}) compared to the lowest part of the canopy (70.1 g m^{-2} ± 15.2 g m^{-2} , n = 4). Likewise, $\delta^{13}C$ values increased with height in the canopy (bottom -29.3‰ ± 0.8‰, top -27.8‰ ± 0.4‰, n = 4, respectively), similar to what has been reported for temperate forests before (Garten and Taylor, 1992; Hanba et al., 1997; Schleser, 1990).

Leaf mass per area and $\delta^{13}C$ covaried in both, the HOL and GEP data set. The concentration of INP_{-10} tended to be higher on leaves with lower LMA and lower $\delta^{13}C$ values (Fig 2). Both parameters did not correlate with leaf N content. Differences in LMA within broadleaf trees result primarily from variations in light availability (Matyssek et al., 2010). In the absence of variations in the atmospheric $\delta^{13}C$, leaf $\delta^{13}C$ in C_3 plants is largely determined by stomatal aperture and photosynthetic rate (Farquhar et al., 1982). Enhanced $\delta^{13}C$ values are observed when photosynthetic rates are high or stomates closed, e.g. under elevated light intensity or water stress (Farquhar et al., 1982; Schleser, 1990; Waring and Silvester, 1994).



https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

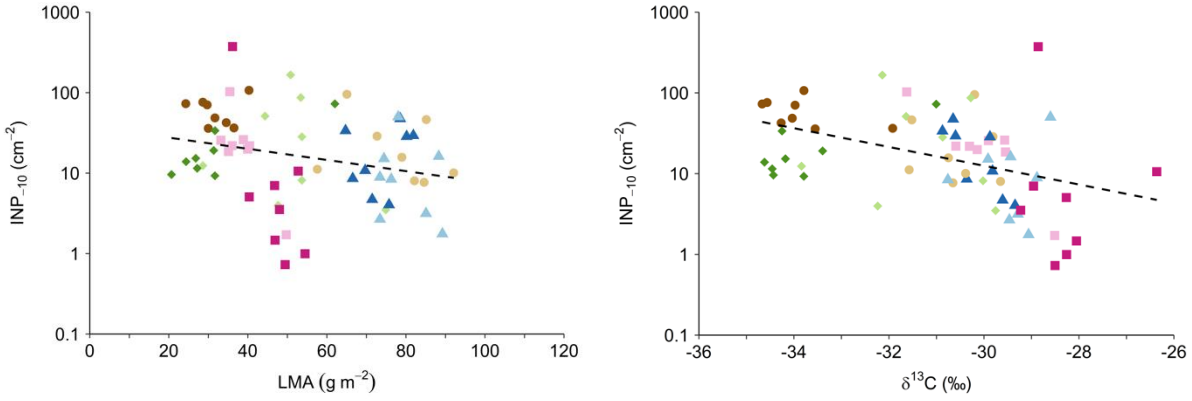


Figure 2: Cumulative INP_{-10} concentration versus leaf mass per area (LMA) (left) and leaf $\delta^{13}C$ values (right) for the GEP data set (bottom) and all *F. sylvatica* samples (top). Colours represent tree species (brown: *F. sylvatica*, blue: *P. avium*, green: *J. regia*, red: *T. platyphyllos*), light colours for first and dark colours for second tree sampled. *F. sylvatica* sampled at Hölstein is represented by squares in the top panels. Results of Spearman correlation analysis from the top left to the bottom right panel are: r = -0.76, p < 0.0001; r = -0.75, p < 0.0001; r = -0.28, p = 0.02; r = -0.51, p < 0.0001).

The change in INP_{-10} concentrations with LMA and $\delta^{13}C$ might be linked to morphological and physiological differences between sun and shade leaves or differences in microhabitat and -climate that are related to variations in LMA and $\delta^{13}C$. For example, our results from Hölstein indicate that INP_{-10} concentrations are lower on more exposed leaves of a canopy, e.g. under increased exposure to solar radiation. Additionally, gradients in parameters such as RH within forest canopies (Zahnd et al., 2023) might lead to decreasing INP_{-10} concentrations towards the canopy top.

3.2 Differential INP concentrations

3.2.1 Spectral types on foliage

Of the 64 foliage samples collected in GEP, 53 displayed two clearly discriminable patterns in differential INP spectra between -3 °C and -10 °C. In 28 samples, differential INP concentrations increased persistently with each 1 °C step in cooling (monotonous spectral type). The remaining 25 samples exhibited one or two significant peaks (section 2.3) at temperatures ranging from -3.5 °C to -8.5 °C (numbers indicate the centre of 1 °C temperature intervals implemented in the assays). Most of these peaks occurred around -8.5 °C (11 peaks) and -7.5 °C (9 peaks). Significant peaks at colder temperatures were more rare (3 peaks each at -6.5 °C and -4.5 °C, 1 peak at -3.5 °C). Generally, discontinuous differential INP spectra indicate that a sample contains distinct INP populations (Vali, 1971). Concurrently, the fraction of INPs among aerosol particles is higher at greater supercooling and the number of airborne INPs was often found to increase roughly exponentially with decreasing temperatures (Fletcher, N. H., 1962; Kanji et al., 2017; Li et al., 2022). This often-described relationship is equivalent to the monotonous spectral type displaying a persistent increase in differential INP concentrations with progressing cooling.

According to their efficiency, efficient biological INP are categorised as either type I, II or III, corresponding to a decrease in nucleation temperature. Type I INPs are active above -5 °C while type III INPs nucleate ice only below -7 °C (Yankofsky et

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

al., 1981). Five of the seven spectra exhibiting peaks at temperatures > -7.5 °C occurred in *J. regia* (Fig. 3). In contrast, such type I and II modes were never found in *F. sylvatica* and only once in *T. platyphyllos* and *P. avium*, respectively. Samples from *F. sylvatica* predominantly featured the monotonous spectral type. This applied to *F. sylvatica* in GEP as well as in HOL. Peaks in differential INP spectra in *P. avium* tended to be at slightly colder temperature and the monotonous spectral type more abundant than in *T. platyphyllos.* These differences indicate that variations in leaf habitat properties between tree species might have contributed variation to the distribution of spectral types among species. Overall, however, the pattern of differential freezing spectra and INP_{-10} concentration differed widely between samples even within the same species. When combined, spectra of all species sampled on a particular day did also show the two discriminable patterns in differential INP spectra (Fig. S3).

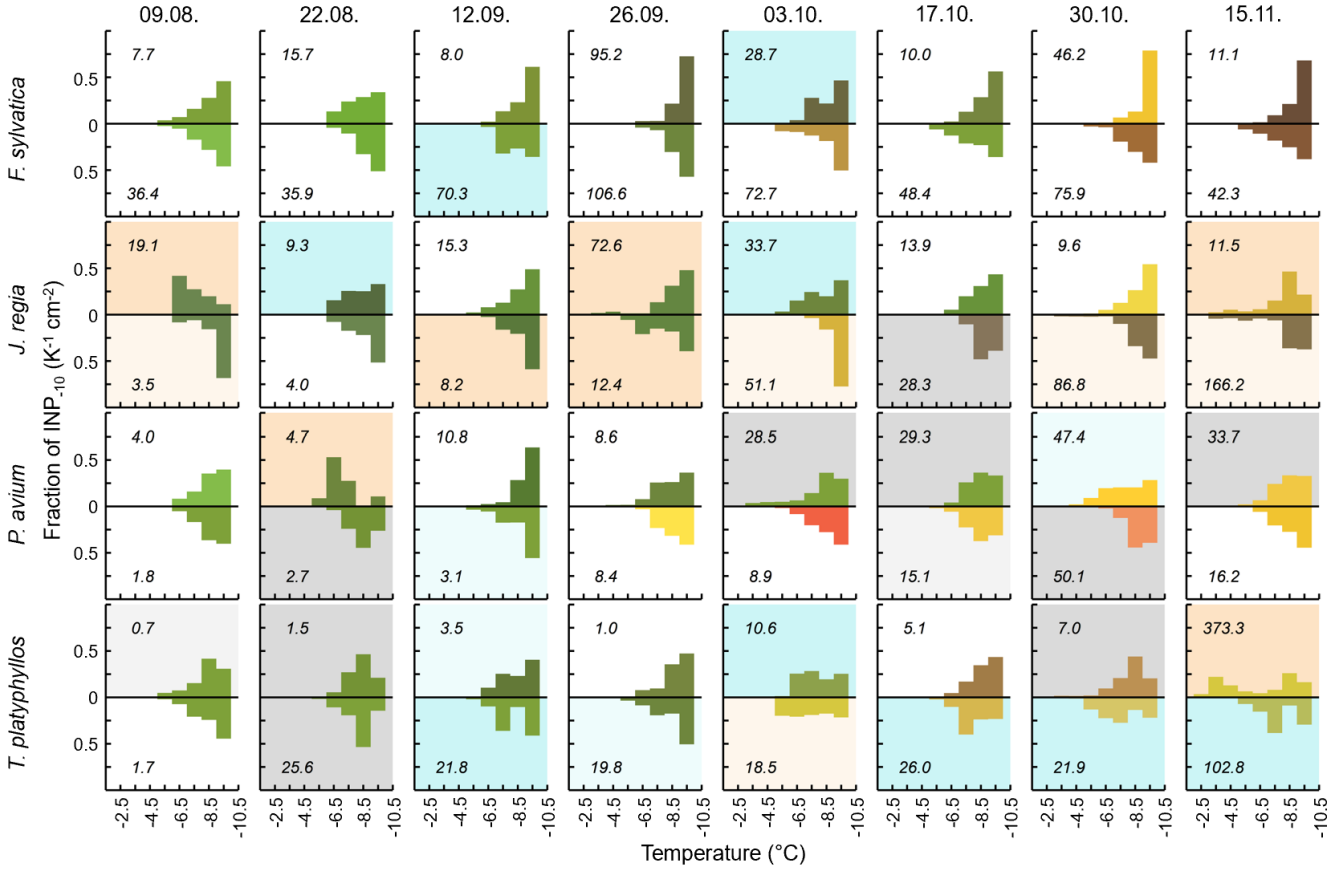


Figure 3: Differential INP concentrations (1 K temperature intervals) for tree species (rows) sampled in Gempen on all sampling days (columns), normalized to cumulative INP_{-10} concentration (numbers in the panels). Both samples per tree species and date are shown (above and below x-axis). Colours reflect leaf colours, panel background indicates the spectral type (white: monotonous spectral type, grey: peak at -8.5 °C, blue: peak at -7.5 °C, red: peak > -7.5 °C). A dark background is for spectra with significant peaks, light colours for spectra with insignificant peaks (section 2.3).

Cumulative INP_{-10} concentrations did not vary systematically between spectral types (Kruskal-Wallis Test, $p > 0.05$). Significant peaks at temperatures > -7.5 °C were observed at total INP_{-10} concentrations as low as 4.7 cm^{-2} leaf area and

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

significant type I peaks at total INP_{-10} concentrations as low as 8.2 cm^{-2} leaf area. This indicates that the occurrence of highly efficient INPs was not solely dependent on the total amount of INP_{-10}.

Studies suggest that the expression and arrangement of IN proteins is substantially influenced by environmental conditions and microhabitat characteristics, including differences between host plant species (Hirano and Upper, 1989; Lindow et al., 1982; O'Brien and Lindow, 1988; Ruggles et al., 1993; Yang et al., 2022). For example, radiation (de Araujo et al., 2019; Govindarajan and Lindow, 1988), desiccation (de Araujo et al., 2019), decreasing pH (Lukas et al., 2022) and high temperatures around 30 °C (Nemecek-Marshall et al., 1993) seem to trigger the dissolution of IN protein complexes, whereas lower temperatures around 15 °C and nutrient limitation can promote their formation (Nemecek-Marshall et al., 1993; Ruggles et al., 1993).

The more efficient an INP, the more sensitive it is to stress (Govindarajan and Lindow, 1988). Once exposed to it, an initially efficient INP will nucleate ice only at colder temperatures (Ruggles et al., 1993). The gradual breakup of larger IN protein clusters under continued stress in unfavorable conditions, or the inhibition of INP cluster formation could be a possible explanation for the development of the monotonous spectral type. This leads us to the conjecture, that conditions for the expression and aggregation of IN proteins were less suitable on *F. sylvatica* as compared to the other investigated tree species.

Overall, none of the investigated leaf traits seems to explain variations in spectral patterns between leaves or tree species. Yet, other differences in microhabitat, for example due to variations in cuticle characteristics, leaf wettability, leaf exudates or leaf topography (Yan et al., 2022), might have contributed to the unequal distribution of spectral patterns between tree species. Additionally, composition and dynamics of the phyllosphere microbiome was found to differ between tree species (Yuan et al., 2023), which might have influenced the composition and INA of microbial species on the investigated leaves. As described above, environmental parameters such as temperature and moisture affect INA microorganisms (Hirano and Upper, 1989; Leben, 1988). These parameters can vary on short spatial scales within single canopies (Batzer et al., 2008; Körner and Hiltbrunner, 2018) and variations in microclimate as well as localized plant reactions might have contributed to variations in spectral patterns between leaves of the same species. In summary, the abundance of INP_{-10} and the pattern of differential freezing spectra seem to be less influenced by intrinsic leaf properties than by external conditions.

3.2.2 Comparison between foliage and air samples in terms of INPs

The median concentration of INP_{-10} observed per cm^{-2} of leaf surface in GEP was equivalent to that in 3.2 m^{-3} of air at Jungfraujoch (16 INP_{-10}). Interestingly, the three most frequent spectral types among the clearly discriminable patterns in GEP - the monotonous spectral type, and the spectral types with significant peaks at -8.5 °C and -7.5 °C - were also prevalent in similar proportions in air samples with clearly defined spectral patterns at Jungfraujoch (Fig. 4). This consistency lends support to the hypothesis that plant surfaces contribute the majority of INP_{-10} to air masses above the Alps, at least during summer and autumn.

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

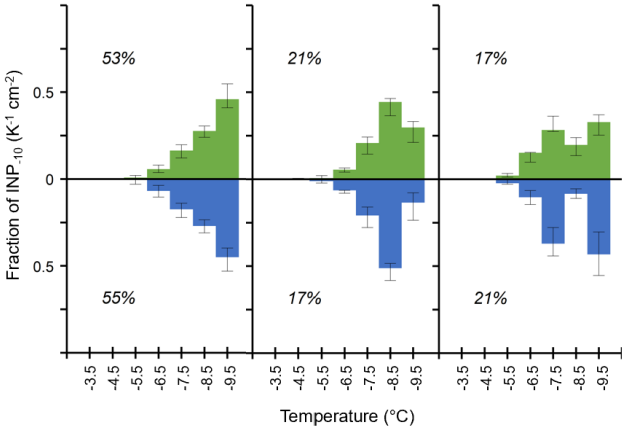


Figure 4: Median of the three most abundant types of differential INP spectra observed on tree leaves (green) in Gempen (650 m a.s.l., n = 53) and in air (blue) at Jungfraujoch (3580 m a.s.l., n = 53), normalized to the cumulative INP_{-10} concentration. Indicated percentages are the relative frequency of each spectral type among spectra with clearly defined patterns found on foliage and in air, respectively, error bars the 1st and 3rd quartile. Note, that the number of analysed droplets per sample is different between Gempen (144) and Jungfraujoch (52).

These findings also suggest that the same parameter, or set of parameters, control the aggregation of IN proteins on the scale of leaves within a canopy as in the wider landscape (i.e., airshed upwind of JFJ). Such a driver could, for example, be leaf wetness duration. It varies within single canopies (Batzer et al., 2008) and within a similar range (< 4 hours to around 10 hours per day) also on the scale of entire landscapes (Asadi and Tian, 2021). Within a canopy, local differences in shading, radiative and convective cooling and wind exposure drive variations in leaf wetness duration (Batzer et al., 2008). Within the landscape, meteorological parameters such as e.g. RH and potential evaporation are good predictors of leaf wetness duration (Asadi and Tian, 2021; Gleason et al., 2008). The effect of RH on INP_{-10} (Fig. 1) hints at moisture not only affecting the size of epiphytic bacterial populations (Caristi et al., 1991), but also INP density in the phyllosphere. Consequently, varying availability of moisture could possibly provide part of the explanation for both, temporal trends (Fig. 1) and differences in INP populations between samples (Fig. 2) observed in this study. Indication for the influence of environmental conditions on biological INP populations aligns with observed differences in the abundance of leaf litter derived INPs between climatic zones (Schnell and Vali, 1976) as well as the influence of meteorological parameters on INA bacteria colonizing crops (Hirano and Upper, 1989; Leben, 1988). The similarity in spectral types and their relative abundance between tree canopies and air at JFJ further suggests a certain consistency in freezing spectra between vegetation types covering a large share of terrestrial surface, e.g. forest, grassland and agricultural crops which also harbour INA microorganisms (Hill et al., 2014; Lindemann et al., 1982; Lindow et al., 1978a). Thus, the similarity of INP abundance and spectra among the four investigated tree species might apply to a broader range of trees and other growth forms.

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

4 Conclusion

To conclude, our results indicate that changes in meteorological parameters such as RH and possibly temperature, by affecting the leaf microhabitat, impact the concentration and perhaps activity of INA microorganisms on plant canopies. The similarity in spectral types and their relative abundance between tree canopies upwind of JFJ and in air at JFJ are a novel type of evidence for plant surfaces being a major source of biological INPs at cloud height above the Alps. If increasing RH or decreasing temperature lead to an enhanced INP supply at plant surfaces, the flux of biological INPs from the phyllosphere to the atmosphere might be elevated under these conditions even in the absence of additional emission mechanisms. Therefore, at locations in the atmosphere where mixed-phase clouds can from and INPs originating from the phyllosphere comprise a large part of the biological INP population, changes in meteorological conditions, additionally to e.g. the effect of rainfall (Mignani et al., 2021), in the INP source region cloud impact cloud development. Further exploration and quantification of the effect of meteorological parameters on biological INP populations might reveal interesting insights into their dynamics at mixed-phase cloud height.

Data availability

Leaf data discussed herein is provided in the supplement of this article. Data collected at Jungfraujoch is available upon request from the corresponding author.

Autor contribution

AE and FC designed the study, AE conducted experiments at Jungfraujoch and analysed the data, AE and FC collected and analysed leaf data, AE wrote the manuscript with contributions from FC.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

Sampling a vertical profile of a *Fagus sylvatica* in Hölstein was possible thanks to the Swiss Canopy Crane II, an infrastructure financially supported by the Swiss Federal Office for the Environment FOEN and the University of Basel. We thank the crane operator Niek ten Cate for his support during sampling in Hölstein and Linus Keiser and Jean-Luc Mosimann for their help during the analysis of leaf carbon and nitrogen content. We are grateful to the International Foundation of the High-Altitude Research Stations Jungfraujoch and Gornergrat (HFSJG), 3012 Bern, Switzerland, for providing us the opportunity to work

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

and conduct experiments at the high-altitude observatory Jungfraujoch. We acknowledge financial support for this study by the Swiss National Science Foundation (SNSF), grant no. 200020-212121.

References

Anderson, J. A., Buchanan, D. W., Stall, R. E., and Hall, C. B.: Frost Injury of Tender Plants Increased by *Pseudomonas syringae* van Hall[1], J. Am. Soc. Hortic. Sci., 107, 123–125, https://doi.org/10.21273/JASHS.107.1.123, 1982.

de Araujo, G. G., Rodrigues, F., Gonçalves, F. L. T., and Galante, D.: Survival and ice nucleation activity of *Pseudomonas syringae* strains exposed to simulated high-altitude atmospheric conditions, Sci. Rep., 9, 7768, https://doi.org/10.1038/s41598-019-44283-3, 2019.

Asadi, P. and Tian, D.: Estimating leaf wetness duration with machine learning and climate reanalysis data, Agric. For. Meteorol., 307, 108548, https://doi.org/10.1016/j.agrformet.2021.108548, 2021.

Bachofen, C., D'Odorico, P., and Buchmann, N.: Light and VPD gradients drive foliar nitrogen partitioning and photosynthesis in the canopy of European beech and silver fir, Oecologia, 192, 323–339, 2020.

Batzer, J. C., Gleason, M. L., Taylor, S. E., Koehler, K. J., and Monteiro, J. E. B. A.: Spatial Heterogeneity of Leaf Wetness Duration in Apple Trees and Its Influence on Performance of a Warning System for Sooty Blotch and Flyspeck, Plant Dis., 92, 164–170, https://doi.org/10.1094/PDIS-92-1-0164, 2008.

Beattie, G. A.: Water relations in the interaction of foliar bacterial pathogens with plants, Annu. Rev. Phytopathol., 49, 533–555, https://doi.org/10.1146/annurev-phyto-073009-114436, 2011.

Beattie, G. A. and Lindow, S. E.: The Secret Life of Foliar Bacterial Pathogens on Leaves, Annu. Rev. Phytopathol., 33, 145–172, https://doi.org/10.1146/annurev.py.33.090195.001045, 1995.

Beyeler, A., Douard, R., Jeannet, A., Willi-Tobler, L., and Weibel, F.: Land use in Switzerland - Results of the Swiss land use statistics 2018, Federal Statistical Office (FSO), Neuchâtel, Switzerland, 2021.

Burrows, S. M., McCluskey, C. S., Cornwell, G., Steinke, I., Zhang, K., Zhao, B., Zawadowicz, M., Raman, A., Kulkarni, G., China, S., Zelenyuk, A., and DeMott, P. J.: Ice-Nucleating Particles That Impact Clouds and Climate: Observational and Modeling Research Needs, Rev. Geophys., 60, e2021RG000745, https://doi.org/10.1029/2021RG000745, 2022.

Caristi, J., Sands, D. C., and Georgakopoulos, D. G.: Simulation of epiphytic bacterial growth under field conditions, Simulation, 56, 295–301, https://doi.org/10.1177/003754979105600505, 1991.

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

Conen, F., Einbock, A., Mignani, C., and Hüglin, C.: Measurement report: Ice-nucleating particles active ≥ −15 °C in free tropospheric air over western Europe, Atmos. Chem. Phys., 22, 3433–3444, https://doi.org/10.5194/acp-22-3433-2022, 2022.

Cornwell, G. C., McCluskey, C. S., Hill, T. C. J., Levin, E. T., Rothfuss, N. E., Tai, S.-L., Petters, M. D., DeMott, P. J., Kreidenweis, S., Prather, K. A., and Burrows, S. M.: Bioaerosols are the dominant source of warm-temperature immersion-mode INPs and drive uncertainties in INP predictability, Sci. Adv., 9, eadg3715, https://doi.org/10.1126/sciadv.adg3715, 2023.

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

Doan, H. K., Ngassam, V. N., Gilmore, S. F., Tecon, R., Parikh, A. N., and Leveau, J. H. J.: Topography-Driven Shape, Spread, and Retention of Leaf Surface Water Impacts Microbial Dispersion and Activity in the Phyllosphere, Phytobiomes J., 4, 268–280, https://doi.org/10.1094/PBIOMES-01-20-0006-R, 2020.

Einbock, A.: Quantifying the flux of ice nucleating particles from lowlands to an altitude where primary ice can be formed in clouds, MSc Thesis, University of Basel, Basel, 69 pp., 2023.

Einbock, A. and Conen, F.: Frost-free zone on leaves revisited, Proc. Natl. Acad. Sci. U. S. A., 121, e2407062121, https://doi.org/10.1073/pnas.2407062121, 2024.

Farquhar, G., O'Leary, M. H. O., and Berry, J.: On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves, Aust. J. Plant Physiol., 9, 121–137, 1982.

Fletcher, N. H.: The physics of rainclouds, Cambridge University Press, Cambridge, UK, 410 pp., 1962.

Garten, C. T. and Taylor, G. E.: Foliar $\delta^{13}C$ within a temperate deciduous forest: spatial, temporal, and species sources of variation, Oecologia, 90, 1–7, https://doi.org/10.1007/BF00317801, 1992.

Gleason, M. L., Duttweiler, K. B., Batzer, J. C., Taylor, S. E., Sentelhas, P. C., Monteiro, J. E. B. A., and Gillespie, T. J.: Obtaining weather data for input to crop disease-warning systems: leaf wetness duration as a case study, Sci. agric. (Piracicaba, Braz.), 65, 76–87, https://doi.org/10.1590/S0103-90162008000700013, 2008.

Govindarajan, A. G. and Lindow, S. E.: Size of bacterial ice-nucleation sites measured in situ by radiation inactivation analysis, Proc. Natl. Acad. Sci. U. S. A., 85, 1334–1338, https://doi.org/10.1073/pnas.85.5.1334, 1988.

Griffiths, A. D., Conen, F., Weingartner, E., Zimmermann, L., Chambers, S. D., Williams, A. G., and Steinbacher, M.: Surface-to-mountaintop transport characterised by radon observations at the Jungfraujoch, Atmos. Chem. Phys., 14, 12763–12779, https://doi.org/10.5194/acp-14-12763-2014, 2014.

Grinberg, M., Orevi, T., Steinberg, S., and Kashtan, N.: Bacterial survival in microscopic surface wetness, eLife, 8, e48508, https://doi.org/10.7554/eLife.48508, 2019.

Grose, M.: Reading the colours of plants at a finer scale, J. Landsc. Archit., 9, 42–47, https://doi.org/10.1080/18626033.2014.898829, 2014.

Grose, M.: Green leaf colours in a suburban Australian hotspot: Colour differences exist between exotic trees from far afield compared with local species, Landsc. Urban Plan., 146, 20–28, https://doi.org/10.1016/j.landurbplan.2015.10.003, 2016.

Gute, E. and Abbatt, J. P. D.: Ice nucleating behavior of different tree pollen in the immersion mode, Atmos. Environ., 231, 117488, https://doi.org/10.1016/j.atmosenv.2020.117488, 2020.

Hanba, Y. T., Mori, S., Lei, T. T., Koike, T., and Wada, E.: Variations in leaf $\delta^{13}C$ along a vertical profile of irradiance in a temperate Japanese forest, Oecologia, 110, 253–261, https://doi.org/10.1007/s004420050158, 1997.

Hård, A. and Sivik, L.: NCS—Natural Color System: A Swedish Standard for Color Notation, Color Res. Appl., 6, 129–138, https://doi.org/10.1002/col.5080060303, 1981.

Hawker, R. E., Miltenberger, A. K., Wilkinson, J. M., Hill, A. A., Shipway, B. J., Cui, Z., Cotton, R. J., Carslaw, K. S., Field, P. R., and Murray, B. J.: The temperature dependence of ice-nucleating particle concentrations affects the radiative properties of tropical convective cloud systems, Atmos. Chem. Phys., 21, 5439–5461, https://doi.org/10.5194/acp-21-5439-2021, 2021.

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

Hill, T. C. J., Moffett, B. F., DeMott, P. J., Georgakopoulos, D. G., Stump, W. L., and Franc, G. D.: Measurement of Ice Nucleation-Active Bacteria on Plants and in Precipitation by Quantitative PCR, Appl. Environ. Microbiol., 80, 1256–1267, https://doi.org/10.1128/AEM.02967-13, 2014.

Hill, T. C. J., DeMott, P. J., Tobo, Y., Fröhlich-Nowoisky, J., Moffett, B. F., Franc, G. D., and Kreidenweis, S. M.: Sources of organic ice nucleating particles in soils, Atmos. Chem. Phys., 16, 7195–7211, https://doi.org/10.5194/acp-16-7195-2016, 2016.

Hirano, S. S. and Upper, C. D.: Diel Variation in Population Size and Ice Nucleation Activity of *Pseudomonas syringae* on Snap Bean Leaflets, Appl. Environ. Microbiol., 55, 623–630, https://doi.org/10.1128/aem.55.3.623-630.1989, 1989.

Hirano, S. S., Baker, L. S., and Upper, C. D.: Raindrop Momentum Triggers Growth of Leaf-Associated Populations of *Pseudomonas syringae* on Field-Grown Snap Bean Plants, Appl. Environ. Microbiol., 62, 2560–2566, 1996.

Huang, S., Hu, W., Chen, J., Wu, Z., Zhang, D., and Fu, P.: Overview of biological ice nucleating particles in the atmosphere, Environ. Int., 146, 106197, https://doi.org/10.1016/j.envint.2020.106197, 2021.

Kahmen, A., Basler, D., Hoch, G., Link, R. M., Schuldt, B., Zahnd, C., and Arend, M.: Root water uptake depth determines the hydraulic vulnerability of temperate European tree species during the extreme 2018 drought, Plant. Biol., 24, 1224–1239, https://doi.org/10.1111/plb.13476, 2022.

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

Kim, H. K., Orser, C., Lindow, S. E., and Sands, D. C.: *Xanthomonas campestris* pv. translucens Strains Active in Ice Nucleation, Plant Dis., 71, 994–997, 1987.

Kinkel, L. L.: Microbial Population Dynamics on Leaves, Annu. Rev. Phytopathol., 35, 327–347, https://doi.org/10.1146/annurev.phyto.35.1.327, 1997.

Kinney, N. L. H., Hepburn, C. A., Gibson, M. I., Ballesteros, D., and Whale, T. F.: High interspecific variability indicates pollen ice nucleators are incidental, EGUsphere, 1–22, https://doi.org/10.5194/egusphere-2023-2705, 2024.

Körner, C. and Hiltbrunner, E.: The 90 ways to describe plant temperature, Perspect. Plant Ecol. Evol., 30, 16–21, https://doi.org/10.1016/j.ppees.2017.04.004, 2018.

Latham, J. S., Cumani, R., Rosati, I., and Bloise, M.: FAO Global Land Cover (GLC-SHARE) database Beta-Release Version 1.0 Database, Land and Water Division, 2014.

Leben, C.: Relative humidity and the survival of epiphytic bacteria with buds and leaves of cucumber plants, Phytopathology, 78, 179–185, 1988.

Li, G., Wieder, J., Pasquier, J. T., Henneberger, J., and Kanji, Z. A.: Predicting atmospheric background number concentration of ice-nucleating particles in the Arctic, Atmos. Chem. Phys., 22, 14441–14454, https://doi.org/10.5194/acp-22-14441-2022, 2022.

Lim, P. O., Kim, H. J., and Nam, H. G.: Leaf senescence, Annu. Rev. Plant Biol., 58, 115–136, https://doi.org/10.1146/annurev.arplant.57.032905.105316, 2007.

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

Limpert, E., Stahel, W. A., and Abbt, M.: Log-normal Distributions across the Sciences: Keys and Clues, BioScience, 51, 341–352, https://doi.org/10.1641/0006-3568(2001)051[0341:LNDATS]2.0.CO;2, 2001.

Lindemann, J., Constantinidou, H. A., Barchet, W. R., and Upper, C. D.: Plants as Sources of Airborne Bacteria, Including Ice Nucleation-Active Bacteria, Appl. Environ. Microbiol., 44, 1059–1063, 1982.

Lindow, S. E., Arny, D. C., and Upper, C. D.: Distribution of ice nucleation-active bacteria on plants in nature., Appl. Environ. Microbiol., 36, 831–838, 1978a.

Lindow, S. E., Arny, D. C., and Upper, C. D.: Erwinia herbicola: A Bacterial Ice Nucleus Active in Increasing Frost Injury to Corn, Phytopathology, 68, 523–527, https://doi.org/10.1094/Phyto-68-523, 1978b.

Lindow, S. E., Hirano, S. S., Barchet, W. R., Arny, D. C., and Upper, C. D.: Relationship between Ice Nucleation Frequency of Bacteria and Frost Injury, Plant. Physiol., 70, 1090–1093, https://doi.org/10.1104/pp.70.4.1090, 1982.

Lukas, M., Schwidetzky, R., Eufemio, R. J., Bonn, M., and Meister, K.: Toward Understanding Bacterial Ice Nucleation, J. Phys. Chem. B, 126, 1861–1867, https://doi.org/10.1021/acs.jpcb.1c09342, 2022.

Maki, L. R., Galyan, E. L., Chang-Chien, M.-M., and Caldwell, D. R.: Ice Nucleation Induced by *Pseudomonas syringae*[1], Appl. Microbiol., 28, 456–459, 1974.

Matyssek, R., Fromm, J., Rennenberg, H., and Roloff, A.: Biologie der Bäume von der Zelle zur globalen Ebene, 1st ed., Ulmer, Stuttgart, 349 pp., 2010.

Mignani, C., Wieder, J., Sprenger, M. A., Kanji, Z. A., Henneberger, J., Alewell, C., and Conen, F.: Towards parameterising atmospheric concentrations of ice-nucleating particles active at moderate supercooling, Atmos. Chem. Phys., 21, 657–664, https://doi.org/10.5194/acp-21-657-2021, 2021.

Morris, C. E., Sands, D. C., Glaux, C., Samsatly, J., Asaad, S., Moukahel, A. R., Gonçalves, F. L. T., and Bigg, E. K.: Urediospores of rust fungi are ice nucleation active at > −10 °C and harbor ice nucleation active bacteria, Atmos. Chem. Phys., 13, 4223–4233, https://doi.org/10.5194/acp-13-4223-2013, 2013.

Murray, B. J., O'Sullivan, D., Atkinson, J. D., and Webb, M. E.: Ice nucleation by particles immersed in supercooled cloud droplets, Chem. Soc. Rev., 41, 6519–6554, https://doi.org/10.1039/C2CS35200A, 2012.

Nemecek-Marshall, M., LaDuca, R., and Fall, R.: High-level expression of ice nuclei in a *Pseudomonas syringae* strain is induced by nutrient limitation and low temperature, J. Bacteriol., 175, 4062–4070, https://doi.org/10.1128/jb.175.13.4062-4070.1993, 1993.

O'Brien, R. D. and Lindow, S. E.: Effect of Plant Species and Environmental Conditions on Ice Nucleation Activity of *Pseudomonas syringae* on Leaves, Appl. Environ. Microbiol., 54, 2281–2286, https://doi.org/10.1128/aem.54.9.2281-2286.1988, 1988.

O'Sullivan, D., Murray, B. J., Malkin, T. L., Whale, T. F., Umo, N. S., Atkinson, J. D., Price, H. C., Baustian, K. J., Browse, J., and Webb, M. E.: Ice nucleation by fertile soil dusts: relative importance of mineral and biogenic components, Atmos. Chem. Phys., 14, 1853–1867, https://doi.org/10.5194/acp-14-1853-2014, 2014.

Pouleur, S., Richard, C., Martin, J. G., and Antoun, H.: Ice Nucleation Activity in *Fusarium acuminatum* and *Fusarium avenaceum*, Appl. Environ. Microbiol., 58, 2960–2964, https://doi.org/10.1128/aem.58.9.2960-2964.1992, 1992.

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

Qiu, Y., Hudait, A., and Molinero, V.: How Size and Aggregation of Ice-Binding Proteins Control Their Ice Nucleation Efficiency, J. Am. Chem. Soc., 141, 7439–7452, https://doi.org/10.1021/jacs.9b01854, 2019.

Richard, C., Martin, J.-G., and Pouleur, S.: Ice nucleation activity identified in some phytopathogenic *Fusarium* species, Phytoprotection, 77, 83–92, https://doi.org/10.7202/706104ar, 1996.

Ruggles, J. A., Nemecek-Marshall, M., and Fall, R.: Kinetics of appearance and disappearance of classes of bacterial ice nuclei support an aggregation model for ice nucleus assembly., J. Bacteriol., 175, 7216–7221, 1993.

Schleser, G. H.: Investigations of the $\delta^{13}C$ Pattern in Leaves of *Fagus sylvatica* L., J. Exp. Bot., 41, 565–572, 1990.

Schnell, R. C. and Vali, G.: Biogenic Ice Nuclei: Part I. Terrestrial and Marine Sources, J. Atmos. Sci., 33, 1554–1564, https://doi.org/10.1175/1520-0469(1976)033<1554:BINPIT>2.0.CO;2, 1976.

Schwidetzky, R., de Almeida Ribeiro, I., Bothen, N., Backes, A. T., DeVries, A. L., Bonn, M., Fröhlich-Nowoisky, J., Molinero, V., and Meister, K.: Functional aggregation of cell-free proteins enables fungal ice nucleation, Proc. Natl. Acad. Sci. U. S. A., 120, e2303243120, https://doi.org/10.1073/pnas.2303243120, 2023.

Shen, S., Yao, Y., and Li, C.: Quantitative study on landscape colors of plant communities in urban parks based on natural color system and M-S theory in Nanjing, China, Color Res. Appl., 47, 152–163, https://doi.org/10.1002/col.22713, 2022.

Šigutová, H., Šigut, M., Pyszko, P., Kostovčík, M., Kolařík, M., and Drozd, P.: Seasonal Shifts in Bacterial and Fungal Microbiomes of Leaves and Associated Leaf-Mining Larvae Reveal Persistence of Core Taxa Regardless of Diet, Microbiol. Spectr., 11, e03160-22, https://doi.org/10.1128/spectrum.03160-22, 2023.

Stone, B. W. G. and Jackson, C. R.: Seasonal Patterns Contribute More Towards Phyllosphere Bacterial Community Structure than Short-Term Perturbations, Microb. Ecol., 81, 146–156, https://doi.org/10.1007/s00248-020-01564-z, 2021.

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

Testa, B., Hill, T. C. J., Marsden, N. A., Barry, K. R., Hume, C. C., Bian, Q., Uetake, J., Hare, H., Perkins, R. J., Möhler, O., Kreidenweis, S. M., and DeMott, P. J.: Ice Nucleating Particle Connections to Regional Argentinian Land Surface Emissions and Weather During the Cloud, Aerosol, and Complex Terrain Interactions Experiment, J. Geophys. Res. Atmos., 126, e2021JD035186, https://doi.org/10.1029/2021JD035186, 2021.

Vali, G.: Quantitative Evaluation of Experimental Results on the Heterogeneous Freezing Nucleation of Supercooled Liquids, J. Atmos. Sci., 28, 402–409, https://doi.org/10.1175/1520-0469(1971)028<0402:QEOERA>2.0.CO;2, 1971.

Vali, G.: Revisiting the differential freezing nucleus spectra derived from drop-freezing experiments: methods of calculation, applications, and confidence limits, Atmos. Meas. Tech., 12, 1219–1231, https://doi.org/10.5194/amt-12-1219-2019, 2019.

Vorholt, J. A.: Microbial life in the phyllosphere, Nat. Rev. Microbiol., 10, 828–840, https://doi.org/10.1038/nrmicro2910, 2012.

Waring, R. H. and Silvester, W. B.: Variation in foliar $\delta^{13}C$ values within the crowns of *Pinus radiata* trees, Tree Physiol., 14, 1203–1213, https://doi.org/10.1093/treephys/14.11.1203, 1994.

Wright, T. P., Hader, J. D., McMeeking, G. R., and Petters, M. D.: High Relative Humidity as a Trigger for Widespread Release of Ice Nuclei, Aerosol Sci. Technol., 48, i–v, https://doi.org/10.1080/02786826.2014.968244, 2014.

https://doi.org/10.5194/egusphere-2024-2067
Preprint. Discussion started: 22 July 2024

Xing, X., Hao, P., and Dong, L.: Color characteristics of Beijing's regional woody vegetation based on Natural Color System, Color Res. Appl., 44, 595–612, https://doi.org/10.1002/col.22375, 2019.

Yan, K., Han, W., Zhu, Q., Li, C., Dong, Z., and Wang, Y.: Leaf surface microtopography shaping the bacterial community in the phyllosphere: evidence from 11 tree species, Microbiol. Res., 254, 126897, https://doi.org/10.1016/j.micres.2021.126897, 2022.

Yang, S., Rojas, M., Coleman, J. J., and Vinatzer, B. A.: Identification of Candidate Ice Nucleation Activity (INA) Genes in *Fusarium avenaceum* by Combining Phenotypic Characterization with Comparative Genomics and Transcriptomics, J. Fungi, 8, 958, https://doi.org/10.3390/jof8090958, 2022.

Yankofsky, S. A., Levin, Z., Bertold, T., and Sandlerman, N.: Some Basic Characteristics of Bacterial Freezing Nuclei, J. Appl. Meteor. Climatol., 20, 1013–1019, https://doi.org/10.1175/1520-0450(1981)020<1013:SBCOBF>2.0.CO;2, 1981.

Yuan, Z., Ye, J., Lin, F., Wang, X., Yang, T., Bi, B., Mao, Z., Fang, S., Wang, X., Hao, Z., and Ali, A.: Relationships between Phyllosphere Bacterial Communities and Leaf Functional Traits in a Temperate Forest, Plants, 12, 3854, https://doi.org/10.3390/plants12223854, 2023.

Zahnd, C., Arend, M., Kahmen, A., and Hoch, G.: Microclimatic gradients cause phenological variations within temperate tree canopies in autumn but not in spring, Agric. For. Meteorol., 331, 109340, https://doi.org/10.1016/j.agrformet.2023.109340, 2023.